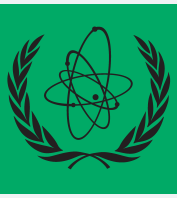
IAEA-TECDOC-1462

Validation of thin-layer chromatographic methods for pesticide residue analysis

Results of the coordinated research projects organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture 1996–2002



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VALIDATION OF THIN-LAYER CHROMATOGRAPHIC METHODS FOR PESTICIDE RESIDUE ANALYSIS

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FOREWORD

Thin-layer chromatography (TLC) has a long history, but has been used only to a limited extent in pesticide residue analytical laboratories since gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) became readily available. Recent developments in the quality of plate coating and detection systems, as well as in extraction and cleanup methods have revived interest in TLC. The combination of these procedures with rigid quality control has created a niche for TLC in laboratories working in compliance with ISO 17025 or GLP.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture recognized the importance of testing pesticide residues, especially in countries with limited resources. A coordinated research programme (CRP) was initiated for investigating the application of TLC detection methods to complement the instrumental techniques in pesticide residue analysis. An initial technical contract provided proof of the concept and elaborated the basic procedures, including a substantial database of retention factors and minimum detectable quantities of pesticides. To satisfy the demands from the eligible laboratories, two similar projects were started in 1997 and 1998. The titles of the projects were as follows:

(i) Validation of Thin-layer Chromatographic Screening Methods for Pesticide Residue Analysis; and

(ii) Alternative Methods to Gas and High Performance Liquid Chromatography for Pesticide Residue Analysis in Grain.

Scientists from 18 countries participated in the above noted two projects. The major tasks of this programme were to adapt the methods, check the repeatability and reproducibility of Rf values, the minimum detectable quantities (MDQ) and apply the methods for determining various pesticide residues in representative matrices. Furthermore, they have extended the methods to other pesticides and commodities of interest in their countries and validated the methods elaborated.

This TECDOC includes the most important results of the CRPs. The Rf and MDQ values reported by the participating scientists are compiled in one table for facilitating the assessment of the repeatability and reproducibility of the results. Since the participants were applying the same basic methodology, described in detail in the first article, these methods are only referred to in the other papers. However, the modifications made by the participants are described in their papers. The purpose of this TECDOC is to provide the readers with comprehensive information on the application of TLC detection methods to complement the instrumental techniques in pesticide residue analysis. Further information on any specific topic may be obtained from the authors.

The participants of the CRPs wish to express their thanks for the opportunity provided by the IAEA to participate in these projects.

A. Ambrus of the Centre for Plant Health and Soil Conservation, Budapest, Hungary, assisted in finalizing this manuscript for publication. The IAEA officers responsible for this publication were D.H. Byron and J. Brodesser of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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CONTENTS

SUMMARY

1. INTRODUCTION

As food safety is among the first priorities in many countries, there is an increasing need for the determination of pesticide residues in various food commodities. The limited resources and constraints in electric power supply limit the range and number of instruments that can be operated in a large number of laboratories. On the other hand, the reliability of the results should be similar regardless of the instrumentation of the laboratories. Consequently, alternative detection and confirmatory methods are required which can complement the analyses performed with gas and liquid chromatography, and can be used in laboratories with limited financial resources and instrumentation.

Thin-layer chromatography (TLC) had been widely used in the 1960s and 1970s for pesticide residue analysis, but has been used only to a limited extent since gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC) became readily available. In recent years, there have been various developments in the quality of plate coating and in detection systems, as well as in extraction and cleanup methods, that made it possible to apply TLC according to current international quality standards [1–3] as an alternative technique for screening for known pesticide residues or for confirmation of tentatively identified compounds [4].

Coordinated research programmes were initiated to study the possibilities of applying TLC detection, in combination with the selected extraction and cleanup methods, for providing an alternative cost effective analytical procedure for detection of pesticide residues in plant commodities, using cabbage, green peas, orange, tomato, maize, rice and wheat as representative sample matrices.

The methods developed are intended for screening and confirmation of pesticide residues in laboratories where the irregular supply of electricity, lack of service, or limited budget do not allow the continuous use of GLC and HPLC techniques, and where the application of mass spectrometric detection is not feasible.

2. OBJECTIVES OF THE COORDINATED RESEARCH PROGRAMMES

The main objectives of the coordinated research programmes (CRPs) were to improve the capability of pesticide residue laboratories in performing the determination of pesticide residues by elaboration of alternative techniques to GC and HPLC detection, and to utilize the analytical methods for risk analysis related to food safety and pesticide management.

The CRPs had the following specific objectives:

- To test the applicability of TLC detection methods for qualitative and quantitative determination of pesticide residues;
- To elaborate extraction and cleanup procedures for determining pesticide residues in cereal grains and vegetables with TLC detection; and
- To validate the methods.

3. METHODS

The method applied for determination of pesticide residues in cereal grains, fruits and vegetables was based on the widely used ethyl acetate extraction. The temperature during extraction was maintained between 25–33°C to obtain good extraction efficiency. It was not allowed to exceed 35°C; otherwise, volatile compounds could be lost or labile compounds could decompose. When deep-frozen samples were processed, the mixture of sample homogenate and the extracting solvent was kept in a water bath at 30°C to reach the specified temperature range.

The extracts were cleaned up on a SX-3 GPC column with ethyl acetate:cyclohexane (1:1) elution followed by a second purification, when necessary, by chromatography on silica gel cartridges or on a florisil column.

The GPC columns were calibrated with the mixture of diazinon and ethion, and the pesticide fraction to be collected from the samples was selected based on the results of the calibration.

LiChrolut® Si 60 500 mg silica gel cartridge was pre-washed with 10 ml toluene:cyclohexane:acetone (60:30:10 v/v) and then with 2 ×. 10 ml toluene:cyclohexane (15:85 v/v). The elution was carried out either with a mixture of toluene:cyclohexane (15:85 v/v), or toluene:cyclohexane:acetone (60:30:10 v/v).

Two g florisil was deactivated with 10% water and the pesticides were eluted with 12 ml dichloromethane:acetone (6:4). The cleaned up extracts were suitable for TLC, GC-NPD and GC-ECD detection.

The residues were primarily separated on silica gel and aluminium oxide layers with ethyl acetate elution. Retention data have been generated and reported to facilitate the separation of the specific groups of pesticides co-eluted by the basic TLC elution systems:

- Silica gel 60 – ethyl acetate
- Silica gel 60 – dichloromethane
- Silica gel 60 – benzene
- Silica gel 60 – cyclohexane:benzene:acetic acid:paraffin oil (200:30:20:1 v/v)
- Silica gel 60 – n-hexane:diethyl ether (1:2 v/v)
- Silica gel (not activated) – petroleum ether:diethyl ether (1:2 v/v)
- Silica gel (not activated) – petroleum ether:diethyl ether (5:1 v/v)
- Silica gel (not activated) – diethyl ether
- Aluminium oxide G (ready made and self prepared) – ethyl acetate
- Aluminium oxide G (ready made) – dichloromethane
- Reversed phase layer RP-18 F-254S – acetone:methanol:water (30:30:30 v/v)

The chromatographic plates were developed in normal tanks saturated with the vapour of the eluent between 20–25^{o}C, which was achieved by immersion of the developing tank into a water bath, to improve the reproducibility of the retention factors of the analytes.

The detectability of the eluted analytes was tested with several methods:

- Method 1 − o-tolidine + potassium iodide [o-TKI]
- Method 2 − p-nitrobenzene-fluoroborate [NBFB]
- Method 3 − p-dimethylamino benzaldehyde [pDB]
- Method 4 − Silver nitrate + UV exposure [AgUV]
- Method 5 − Photosynthesis inhibition (Hill reaction) [Hill]
- Method 6/A − Fungi spore (Aspergillus niger) inhibition [FAN]
- Method 6/B – Fungi spore (Penicillium cyclopium) inhibition (FPC)
- Method 7 − Enzyme inhibition with cow liver extract and β-naphthyl-acetate substrate [EβNA]
- Method 8 − Enzyme inhibition with pig or horse blood serum and acetylthiocholine iodide substrate [EAcI]

The procedures tested before the start of the CRPs and used as basic methods are described in detail by Ambrus et al. [5]. The basic methods were adapted and used by the participants of the CRPs for testing the repeatability and reproducibility of the retention factors (Rf), relative retention factors (RRf), minimum detectable quantities (MDQ), as well as for the determination of limits of detection (LOD), and limits of quantitation (LOQ) of the selected pesticides and their recoveries from several commodities used as representative matrices for validation of the methods. The results of the tests are presented in the following reports making only reference to the basic procedures applied.

4. EVALUATION OF RESULTS

4.1. Extraction and cleanup

The efficiency of ethyl acetate extraction and the applicability of the GPC cleanup were not tested within these projects as they had been proven to be suitable for a very wide range of pesticide-commodity combinations.

The KL SX-3 gel chromatograph operating with constant nitrogen over-pressure of 0.5 bar without electric supply was used in the experiments. The load of the GPC cleanup column was considered acceptable until the minimum detectable quantities of analytes, determined with analytical standards alone, could be seen in the presence of the co-extractives and the Rf value of the analyte was not affected. The gel column containing about 7 g SX-3 gel could be loaded with the concentrated ethyl acetate extract containing a maximum of 30 g sample equivalent of fruits and vegetables and 10 g of cereal grains.

The selection of the pesticide fraction was critical. If the eluent collection was started early (after 8, 9 ml) a substantial portion of the co-extractives remained in the pesticide fraction that might result in interfering spots or peaks and might increase the LOD and LOQ values. On the other hand, if the collection of pesticide fraction was delayed, the recoveries of early eluting compounds might be unacceptably low. The elution patterns of wheat oil and diazinon and triazophos are shown in FIG. 1. In these experiments 94% of wheat oil was eluted in the first 9 ml [6]. The elution pattern of several additional pesticides, corn, rice and wheat co-extractives are shown in Table 1. The critical elution fractions are highlighted in the table.

The co-eluted plant extracts of rice and wheat similarly affected the LOD values. The larger amount of corn co-extractives had significantly decreased the LOD values of some pesticides compared to their detectability (given in brackets) in the presence of rice co-extractives: captan, FAN, 0.04 mg/kg (0.025 mg/kg); oxamyl, EβNA, 0.25 mg/kg, (0.025 mg/kg); dieldrin, AgUV, 0.2 mg/kg, (0.02 mg/kg) [7].

FIG. 2 illustrates the elution patterns of some early eluting pesticides and plant co-extractives [8]. Note that one ml fractions were taken, thus the negative values in the middle of the fraction are artefacts caused by the Excel software algorithm.

These examples clearly indicate that precise calibration of the GPC column is inevitable for obtaining clean eluate and high recoveries, where the elution pattern of co-extractives and analytes overlap. In such cases the collection of pesticide fraction can be started at a half ml, for instance, after 8.5 or 9.5 ml instead of 8, 9 or 10 ml. A somewhat reduced recovery might be well compensated by the lower LOQ.

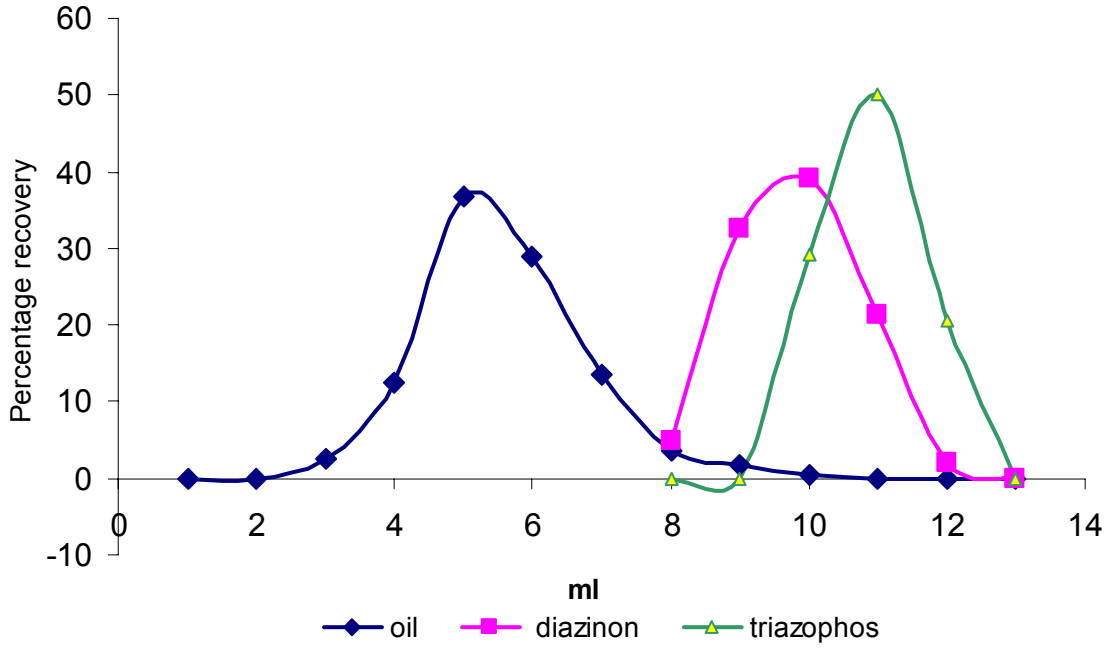


FIG. 1. GPC elution pattern of wheat oil, diazinon and triazophos.

The GPC provided reproducible results if it was calibrated when the column packing was replaced or when the column was used again after a longer period. Note that the packing must always be covered with sufficient amount of eluent.

Where the GPC cannot provide sufficiently clean extracts, a second cleanup step may be necessary. The Lichrolute Si 60 cartridges or florisil column chromatography can be used in combination with GPC cleanup for certain pesticides, but not as a general cleanup procedure [7].

4.2. TLC determination of residues

The elution characteristics and detectability of 234 pesticide compounds were tested with eight elution systems and six detection methods.

TABLE 1. GPC ELUTION PATTERNS OF REPRESENTATIVE CEREALS AND PESTICIDES

Substance	Elution fraction [ml]														Ref.
	5	6	7	8	9	10	11	12	13	14	15	16	17	18-21	
	Percentage of substances recovered														
Corn extract	0	8	18	27	21	15	9	2							7
Rice extract	7	15	20	27	17	10	5	1							7
Wheat extract	7	12	20	26	21	10	5	1							7
	8	25	32	20	9	4	1								8
Atrazine						13	31	35		21					9
Diazinon						5	25	49	12	4	3	1	1	0	7
					4	24	44	13	8	7					8
Dichlorvos					7	41		41		10		1			9
Fenitrothion						14		31		31		1			9
Isoproturon						19	30	33		17					9
Malathion						16		31		30		22		1	9
Metobromuron							18	32		30		21			9
Metribuzin							18	32		25		24			9
Mono-chrotophos					37	63									9
Parathion						6		46		48		1			9
Parathion-me						16		31		30		23		1	9
Pirimiphos-me						18		31		30		21			9
Prometryn							21	30		22		11		15	9
Triazophos						1	6	35	33	14	8	2	1	1	0	7
						4	36	37	16	7						8

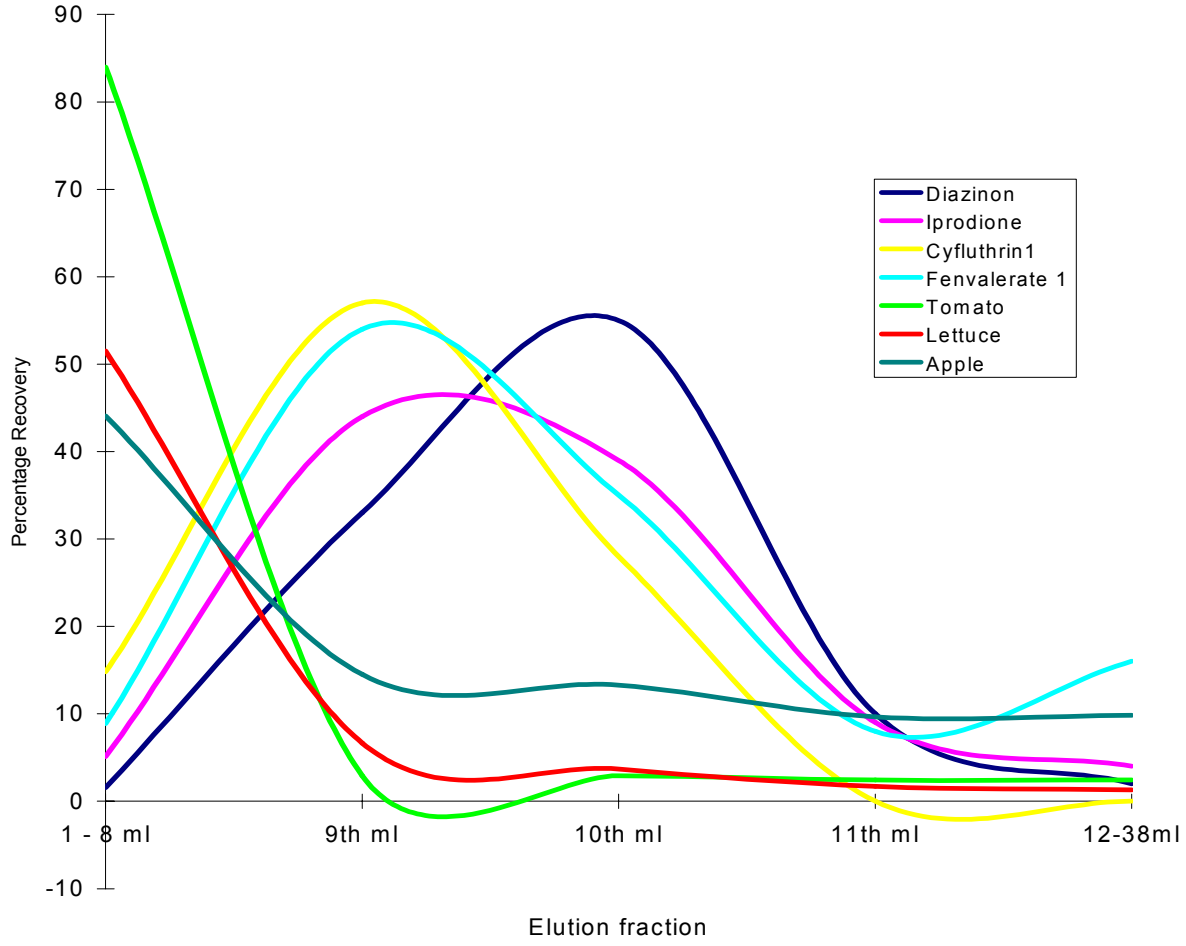


FIG. 2. GPC elution pattern of early eluting pesticides and plant co-extractives.

The effect of the eluent temperature on the Rf values was studied. It was found that temperatures between 20°C and 32°C did not significantly influence the retention of the compounds [5]. Immersion of the developing tank into a water bath reduced the effect of daily variation of the laboratory temperature and improved the reproducibility of the results [7–9, 11].

4.2.1. Silica gel 60 – ethyl acetate elution system

Silica gel 60-ethyl acetate system resulted in a reasonably good spread of Rf values. The majority of compounds eluted between Rf 0.3 and 0.7. In this Rf interval the within-laboratory reproducibility of Rf values was generally good with a relative standard deviation, CV_{wRf}, of < 0.1, the smallest among the systems tested. The average within-laboratory and between-laboratory reproducibility of Rf values were increasing with the decreasing Rf values [5].

The average Rf and CV_r, and CV_R values were calculated for 39 compounds for which results were available from at least four laboratories. The between-laboratory reproducibility of the average Rf values was very good ($CV_{RRf} \leq 11\%$) except diuron (19%), monocrotophos (31%) and prochloraz (17%) [12].

Plates could be loaded with extracts after GPC cleanup representing maximum 600 mg and 500 mg sample equivalents of fruits and vegetables [5, 13] and cereal grains [7], respectively, without affecting the Rf values.

4.3.2. Other elution systems

The Rf value ranges of the majority of compounds and their within-laboratory reproducibility are summarised in Table 2.

TABLE 2. ELUTION CHARACTERISTICS OF PESTICIDES IN VARIOUS ELUTION SYSTEMS

Elution system	Rf range	CV_{wRRf}[1]	Comment
Silica gel 60-dichloromethane	0-0.75	0.05-0.2	About half of Rf values were ≤ 0.2
Silica gel 60-benzene	0-0.70	0.05-0.3	Selectively separates some co-eluting compounds
Silica gel 60-yclohexane:benzene: acetic acid: paraffin oil (200:30:20:1 v/v)	0-0.65	0.05-0.25	Rf values of the majority of compounds were ≤ 0.2
Aluminium oxide G (ready made and self prepared)-ethyl acetate	0-0.91	0-0.15	Most of the Rf were in the 0.7-0.9 range
Aluminium oxide G (ready made)-dichloromethane	0-0.94	0-0.2	Reasonably uniform elution pattern
Reversed phase layer RP-18 F-254S-acetone:methanol:water (30:30:30 v/v)	0-0.78	0.05-0.15	Most uniform elution pattern

(1) CV values obtained from data where Rf values were ≥ 0.2

The between-laboratory reproducibilities of Rf values were very good on the ready-made RP-18 layers. Because the mechanism of elution differs between the reversed phase system and the normal phase, and the retention order on the reversed phase was different from that obtained on the normal phase, separation of suspected compounds on paraffin-coated layers could provide an excellent and cheap confirmation method in the future provided that the sensitive detection of pesticides can be achieved. Further work is required for the elaboration and optimization of the detection conditions with the methods used for reverse phase layers. Until that time the reverse phase layers may be used for confirmation of the identity of residues with UV scanners.

There were insufficient data to estimate the between-laboratory reproducibility of the Rf values obtained with other elution systems.

4.3.3. Evaluation of retention data

The reproducibility of Rf values rapidly improved from Rf 0.05. This is mainly due to the error (the uncertainty is in the order of mm) in the visual observation of the centre of the spots and the deformation of the spots at the start resulting from the large migration velocity of the solvent at the beginning.

The within-plate variability of RRf values was in the same range as that of the Rf values, while the between-plates variability of RRf values was lower than that of Rf values, indicating that the RRf values can be better used for the identification of the compounds possibly present.

The within-laboratory variation of Rf values of various compounds in the presence of plant co-extractives was within the range obtained with analytical standards. The Rf values within one run were only slightly affected by the load of various sample extracts. The within-plate variation caused by sample extracts was smaller than the between plate variation observed when analytical standards were applied alone.

The information on the within-laboratory reproducibility of Rf and RRf values enables the laboratory to establish a "window" (usually $\pm 2*CV_{rtyp}*Rf_{mean}$) within which a pesticide residue may be detected, and select those compounds, taking into account their detectability as well, that may be present in a sample. The retention window may be larger where typical CV_r values, based on the results from several laboratories [12], are not available, and the within-laboratory reproducibility CV_r was determined from ≤ 15 measurements.

Comparison of retention data obtained in the silica gel-ethyl acetate system and in the silica gel-petroleum ether:diethyl ether systems indicated similar advantages in general separation and the latter systems provided selective separation of some compounds. Since the multi-component eluents must be replaced after each plate to keep their composition constant, and taking into consideration the instability of the composition of the solvent mixture caused by the low boiling point of diethyl ether at elevated laboratory temperature, the silica gel-petroleum ether:diethyl ether systems are considered less suitable for general use than the silica gel-ethyl acetate.

The Rf values obtained from individual measurements vary substantially between laboratories, therefore the mean Rf and RRf values obtained on different plates should be reported together with their within-laboratory reproducibility.

Very good correlation ($y = 598x + 0.06$, $R^2 = 0.974$) was found between the Rf values of pesticides measured in two laboratories on freshly activated layers and on those equilibrated to laboratory conditions after elution with the petroleum ether + diethyl ether = 1 + 2 eluent [12]. This finding indicates the possibility of adapting a large Rf data base published in the literature to a given laboratory condition based on the method suggested by Lantos et al. [14]. First the average retention values of a limited number of substances (e.g. 20–25) should be determined, then these values should be correlated with the corresponding published one. The usually polynomial correlation equation obtained can be used to calculate the expectable Rf values of other compounds. The Rf values derived from the correlation equation can only be used as approximate guidance value and must be verified with analytical standards before use.

Summarizing the findings on the elution patterns of pesticides, it is concluded that none of the systems are ideal. In view of the large number of pesticides which can be detected with a given detection method, and the low resolution of TLC plates (maximum 8–10 spots can be separated in an ideal case), the elution of several pesticides with very similar Rf values is inevitable.

In cases of overlapping spots, various solvent mixtures can be used for more selective separation of a few co-eluting compounds. For example, an interesting procedure was applied by Qian and co-workers [9] for the separation of isoproturon, atrazine, metribuzin and prometryn giving 0.61, 1.0, 1.01, 0.97 RRf values, relative to atrazine, in silica-gel-ethyl acetate system. Therefore, the compounds first were eluted with petroleum ether:ethyl acetate

(8:2), then with petroleum ether:ethyl acetate:methanol (8:1:1) in the same direction. The Rf values of isoproturon, metribuzin, atrazine, and prometryn after the second developments were 0.088, 0.35, 0.45, 0.58, respectively, which made their simultaneous detection possible.

4.4. Detection of pesticides

The detectability of pesticides was tested with the following systems:

- UV lamp at 254 nm HF (254) — Silica gel 60 HF_{254}
- o-Tolidine and potassium iodide (o- TKI) — Silica gel 60
- p-nitrobenzene-fluoroborate (NBFB) — Silica gel 60
- p-dimethylamino benzaldehyde (p-DB) — Aluminium oxide G
- Silver nitrate & UV exposition (AgUV) — Aluminium oxide G
- Photosynthesis inhibition (Hill) — Silica gel 60
- Fungi-spore inhibition (*Aspergillus niger*) (FAN) — Silica gel 60
- Fungi-spore inhibition (*Penicillium cyclopium)* (FPC) — Silica gel 60
- Enzyme inhibition with cow liver extract and β-naphthyl acetate substrate (EβNA) — Silica gel 60
- Enzyme inhibition with pig or horse blood serum and acetylthiocholine iodide substrate (EAcI) — Silica gel 60

The *fungi spore inhibition* [FAN] and [FPC] methods provided the best specificity. They sensitively detect only fungicides at the usual concentration level of pesticide residues (0.01–5 mg/kg). Therefore they are very suitable TLC detection methods for residue analysis.

The *Hill reaction* is less specific. It detects ureas and triazines at a limit of quantitation (LOQ) of about 0.002–0.01 mg/kg, but it would also detect some other types of pesticides (not herbicides), for instance, thiabendazol (0.2 mg/kg), azinphos-methyl, triazophos and propham (≥ 1.2 mg/kg).

Enzyme inhibition [EAcI, EβNA] usually sensitively detects phosphate and carbamate type insecticides (LOQ ≥ 0.002–0.01 mg/kg). The enzyme inhibition mechanism is different in the cases of liver extract and blood serum. Consequently, the sensitivity of the detection can be different for some pesticides. For instance, 10–100 fold higher sensitivities were obtained with EβNA than with EAcI in the case of carbofuran, chlorfenvinphos, diazinon, dichlorvos, ethion, monocrotophos, and trifluralin, while the opposite trend (5–10 fold) was observed for 2,4-D, cypermethrin, dithianon, and pentachlorophenol. The latter compounds also indicate that the enzyme inhibition can be caused by non-insecticide pesticides as well. The liver and blood enzymes complement each other, and both should be available in a screening laboratory. The selection of enzyme source depends on the compounds to be analysed.

The *o-tolidine + KI* [o-TKI] *and silver nitrate* [AgUV] are general screening detection methods. They have a medium sensitivity for several compounds. It should be noted, however, that neither of the methods is suitable to detect organochlorine pesticide residues at the current Codex Extraneous Maximum Residue Limits (EMRL). The procedure developed by Pasha et al. may provide solution for the sensitive TLC detection of a wider range of OC and synthetic pyrethroid compounds [15].

It should be noted that the spots of some pesticides rapidly disappear or become faint after reaching optimal intensity (e.g. oxamyl detected with enzyme inhibition methods, all spots detected with Hill reaction), therefore the spots should be marked and evaluated immediately after colour development. Visual observations of interferences, spot, shape and intensity should also be recorded.

Nitrobenzene fluoroborate [NBFB] is a medium sensitive reagent for detecting carbamate type pesticide residues. It can be useful for confirmation of the identity of residues.

Detection with *p-dimethylamino benzaldehyde* has limited use, mainly in confirmation of identity of some residues being present at relatively high concentrations.

The loadability of the layers is limited. The linear range of compounds varied between 1 × MDQ and 10–20 × MDQ concentration range. The calibration lines prepared from the square of the average of minimum and maximum diameters of the spots could be obtained with a regression coefficient of $r \geq 99.95$ [8, 9]. It provided more precise quantitation than the visual comparison of the peak area and intensity [16].

The minimum detectable quantities of analytes [MDQ, ng] were determined with pure analytical standards following their elution and detection. Due to the inherent uncertainty of visualization of spots and the stepwise dilution, two times difference in the reported MDQ values was not considered significant. The MDQ values being ≥5 times smaller or larger than the average were considered atypical.

Generally the between-laboratory reproducibility of MDQ values was not as good as that of the Rf values. This indicates the importance of careful optimization of the detection conditions and checking its proper implementation in case of every plate. Before use, the detectability of each compound to be tested should be verified, and the conditions should be adjusted to achieve at least the typical values reported in this TECDOC.

The bioassay methods (Hill reaction, fungi test, enzyme inhibition) enabled the detection down to 0.1–10 ng, while 20–100 ng could be achieved with the chemical reagents. The detection under UV light with visual observation was not sufficiently sensitive (MDQ ≥ 100–500 ng) for pesticide residue analysis. The sensitivity may be increased by TLC scanner, which makes the detection of a wide range of compounds possible [17].

Taking into account that the TLC plates can be generally loaded with cleaned extracts containing up to 250–300 mg fruits and vegetables sample equivalent, the 1–100 ng MDQ range is required for detection of pesticide residues at around 0.003–0.3 mg/kg concentrations. For cereal grains the maximum load is about 20–40 mg sample equivalent, therefore the lowest LOQ that can be expected is around 0.02 mg/kg.

Analyses of the same extracts with TLC and GC indicated that the TLC determination of known compounds can be performed with comparable accuracy and precision to GC [1, 7–9, 11, 16, 17].

4.5. Application of marker compounds for internal quality control

As the Rf values and the sensitivity of detection may be influenced by many external factors and the quality of the reagents used especially for bioassay methods, the regular monitoring and control of the TLC conditions is essential for obtaining reliable analytical results. The proper elution and detection conditions were checked on each plate by applying a mixture of

analytical standards of properly selected marker compounds at their MDQs. If the marker compounds were well detectable and their Rf values were within the expected range, the analyst could be sure, and could demonstrate at the same time, that the method had been applied properly.

The Rf values of the marker compounds can also be used as reference for the RRf values which greatly facilitates the identification of the spots detected on the plates.

The marker compounds selected should [5]:

- be relatively stable in standard solutions;
- be sensitive for the detection conditions (not appearing on the plate if the conditions are not optimal); and
- have reproducible Rf values.

4.6. Recovery studies

The applicability of the adapted basic methods for the determination of pesticide residues was tested with a number of pesticides in apple, tomato, cocoa, corn, rice and wheat samples which were used as representative matrices according to the principles of single laboratory validation of multi residue procedures.

The result obtained during the validation studies are summarised in Tables 3–7.

The recoveries and their reproducibility were generally within the acceptable range. There was no significant difference between the values obtained with GC and TLC, indicating that TLC determination can be used for the quantitative determination of known substances [8–11].

TABLE 3. SUMMARY OF LOQ VALUES, AND REPEATABILITY OF RECOVERIES OBTAINED WITH Ag-UV

Pesticide	Commodity	Spike level mg/kg	No of tests	LOQ mg/kg	Q%	CV_r%	Ref.
Endosulfan I	Tomato	0.25	5	0.1	78.4	6.0	[13]
		0.5	3		83.3	6.6	
		1	3		92.0	2.1	
					97.7		[18]
Endosulfan II	Tomato	0.25	5	0.1	79.8	4.2	[13]
		0.5	3		90.9	1.1	
		1	3		93.2	3.1	
Lindane	Tomato	0.25	5		79.3	6.3	[13]
		0.5	3		81.4	7.9	
		1	3		83.3	7.9	
					98.6		[18]
Triforine	Wheat	0.1		0.1	88.2	12.4	[7]
		0.2			83.4	9.1	

TABLE 4. SUMMARY OF LOQ VALUES, AND REPEATABILITY AND REPRODUCIBILITY OF RECOVERIES OBTAINED WITH EAcIa

Pesticide	Commodity	Spike mg/kg	No of tests	LOQ mg/kg	Q%	CV_r%	Ref.
Azinphos-methyl	Apple	0.016	5		94		[19]
		0.032	3		89		
Carbaryl	Tomato	0.5	3		79	3.1	[13]
		1	3		83	5.1	
		2	3		85	4.1	
					84		[18]
Chlorpyrifos	Apple	0.002	5		89		[19]
		0.004	3		84		
	Rice	0.01	5	0.01	80	12.5	[4]
		0.05	5		87	9.3	
		0.1	5		89	5.6	
	Tomato	0.002	5		90		[19]
		0.004	3		91		
Diazinon	Rice	0.01	5	0.01	79	9.6	[4]
		0.05	5		85	4.6	
		0.1	5		86	4.4	
Dimethoate	Tomato	1.5			65		[19]
		3			65		
Fenitrothion	Wheat	0.05	5	0.05	85	12.2	[4]
		0.5	5		80	8.3	
		1	5		82	6.5	
Methomyl	Rice	0.29		0.15	69	5	[20]
		0.72			65	16	
Monocrotophos	Apple	0.004	5	0.002	86		[19]
		0.008	5		89		
	Tomato	0.004	5		85		[19]
		0.008	5		88		
					88		[18]
	Rice	0.06		0.03	69	30	[20]
		0.15			66	23	
Oxamyl	Rice	0.01		0.05	93	8.9	[20]
		0.04			73	53	
Parathion-methyl	Rice	0.06		0.02	86	28	[20]
		0.15			78	29	
	Tomato	0.025	5	0.012	76	5.0	[13]
		0.05	3		84	5.1	
		0.1	3		84	4.8	
Phosalone	Rice	0.014		0.05	93	8.89	[20]
		0.035			73	53.6	
		1.4			91	9.1	[20]
		3.6			99	42	
Phosphamidon	Rice	1.428		0.55	91	9.12	[20]
		3.57			100	42.56	
Propoxur[b]	Cocoa	0.25	3	0.3	ND		[11]
		0.50	3		66		
		1.0	3		70		
		0.25+GPC	3		ND		
		0.50+GPC	3		52		
		1.0+GPC	3		64		

Notes: (a) Enzyme source pig blood serum; (b) extracts were analysed directly and after GPC cleanup.

TABLE 5. SUMMARY OF LOQ VALUES, AND REPEATABILITY AND REPRODUCIBILITY OF RECOVERIES OBTAINED WITH EβNA

Pesticide	Commodity	Spike level mg/kg	No of tests	LOQ mg/kg	Q%	CV_r%	Ref.
Azinphos methyl	Apple	0.016	5	0.008	88		[19]
		0.032	5		85		
	Tomato	0.016	5		80	11	[19]
		0.032	5		100	13	
Carbaryl	Apple	2.0	5	0.01		11	[19]
		0.6					
	Tomato	0.5	3		79	3.1	[13]
		1	3		83	5.1	
		2	3		85	4.1	
					99		[18]
Chlorpyrifos	Apple	0.002	5	0.001	99	10	[19]
		0.004	5		100	10	
	Tomato	0.002	5		145	10	[19]
		0.004	5		120	10	
Diazinon	Rice[1]	0.058	5	0.024	93	5.1	[10]
		0.078	5		96	5.7	
		0.20	5		95	8.5	
Dichlorvos	Rice[1]	0.86	5	0.4	88	6.7	[10]
		1.1	5		97	4.3	
		2.9	5		93	8.3	
Dimethoate	Apple	2.0	5	1.50	85	12	[19]
		0.6	5		85		
	Tomato	2.0	5		100	12	[19]
		0.6	5		91		
Monocrotophos	Apple	0.01	5	0.005	75		[19]
		0.04	5		80		
	Tomato	0.01	5	0.005	80		[19]
		0.04	5		81		
Oxamyl	Rice[1]	0.321		0.15	83	12.7	[10]
		0.428			95	9.7	
		1.07			87	12.2	
Parathion-methyl	Tomato	0.025	5		76	5.0	[13]
		0.05	3		84	5.1	
		0.1	3		84	4.8	
		0.032	5	0.015	91	7.3	[10]
		0.042	5		99	7.7	
		0.105	5		93	1.3	
		0.01	5	0.001	94		[19]
Phosalone	Rice[1]	0.16		0.075	95	6.0	[10]
		0.214			94	7.0	
		0.535			95	1.8	

(1) Recoveries from rice and wheat were similar

TABLE 6. SUMMARY OF LOQ VALUES, AND REPEATABILITY AND REPRODUCIBILITY OF RECOVERIES OBTAINED WITH HILL REACTION

Pesticide	Commodity	Spike level mg/kg	No of tests	LOQ mg/kg	Q%	CV_r%	Ref.
Atrazine	Tomato	0.05	5	0.002	92	3.8	[13]
		0.1	3		98	0.7	
		0.2	3		93	3.6	
					93		[18]
		0.12	5	0.003	91		[19]
	Rice	0.1	3		96	14	[9]
	Rice[1]	0.086	5	0.033	94	3.0	[10]
		0.114	5		96	4.6	
		0.285	5		93	3.1	
	Wheat	0.05		0.05	78	9.8	[7]
		0.1		0.05	86	7.5	
		0.2		0.05	85.8	7.2	
		0.1	3	0.03	92	7.8	[9]
	Soil	0.1	3		93	10	[9]
Chlortoluron	Rice[1]	0.1		0.0375	93	4.1	[10]
		0.132			92	3.9	
		0.33			96	7.1	
Isoproturon	Rice	0.1	3	0.03	87	7.2	[9]
	Wheat	0.1	3	0.03	95	4.0	[9]
	Soil	0.1	3		90	6.4	[9]
Linuron	Rice[1]	0.065	5	0.03	96	5.8	[10]
		0.086	5		98	4.5	
		0.215	5		101	4.0	
Metribuzin	Rice	0.1	3		98	3.0	[9]
	Wheat	0.1	3		99	9.8	[9]
	Soil	0.1	3		94	9.2	[9]
Metoxuron	Rice[1]	0.429	5	0.167	96	6.1	[10]
		0.572	5		94	4.7	
		1.43	5		96	7.2	
Prometryn	Rice	0.1	3		92	4.9	[9]
	Wheat	0.1	3		85	3.6	[9]
	Soil	0.1	3		84	7.6	[9]
Simazine	Rice[1]	0.09	5	0.04	96	4.6	[10]
		0.12	5		97	3.5	
		0.30	5		96	4.1	

(1) Recoveries in rice and wheat were similar

TABLE 7. SUMMARY OF LOQ VALUES, AND REPEATABILITY AND REPRODUCIBILITY OF RECOVERIES OBTAINED WITH o-TKI REACTION

Pesticide	Commodity	Spike level mg/kg	No of tests	LOQ mg/kg	Q%	CV_r%	Ref.
Atrazine	Tomato	2.0	5	0.06		2	[19]
		0.6	5			7	
		0.05	5		89	5.6	[13]
		0.1	3		93	3.7	
		0.2	3		92	2.6	
					98		[18]
	Rice[1]	0.54	5	0.25	92	7.3	[10]
		0.72	5		95	8.4	
		1.08	5		101	6.8	
	Rice	1.4	3	0.61	82	12	[20]
Carbaryl	Rice[1]	0.645	5	0.30	96	3.7	[10]
		0.860	5		96	2.5	
		1.290	5		96	5.5	
Chlorbromuron	Rice	5.4	3	0.97	77	20	[20]
Dimethoate	Rice[1]	0.664	5	0.31	102	8.4	[10}
		0.886	5		99	6.6	
		1.326	5		91	5.3	
Diuron	Apple	0.18	5		62	9	[19]
		0.36			67	6	
	Tomato	0.05	3		88	4.2	[13]
		0.1	3		90	9.8	
		0.2	3		89	8.8	
					100		[18]
	Rice[1]	0.96	5	0.45	96	6.8	[10]
		1.28	5		90	6.1	
		1.92	5		95	5.1	
	Rice	1.7	3	0.75	80	9.1	[20]
Linuron	Apple	1.10	5		74	5	[19]
		2.20	5		72	9	
Metoxuron	Apple	0.5	5		74		[19]
		1.0	5		72		
	Tomato	2.0	5	0.28		7	[19]
		0.6	5			8	
Oxamyl	Rice[1]	2.4	5	1.13	95	8.1	[10]
		3.2	5		93	10.3	
		4.8	5		97	7.4	
Teflubenzuron	Rice	2.9	3	1.9	52	37	[20]
		7.2	3	1.4	72	24	
	Rice	2.86	3	1.91	52	37.1	[20]
		7.15	3	1.38	72	24	

4.7. Application of TLC for identification of residues

Two inter-laboratory comparison samples were prepared and sent out to all active participants of the two CRPs and some additional laboratories interested in the programme.

4.7.1. Evaluation of the 1st inter-laboratory comparison study

The aims of the first study were to test the applicability of TLC detection methods for screening unknown pesticide residues in samples and for determining the concentration of pesticides identified.

Institutions were supplied with synthetic test samples containing different pesticides. They were informed that the test samples may contain several pesticide active ingredients from the 210 compounds which had been included in the basic method. Laboratories were advised to identify the kind of active ingredients and to quantify by TLC and/ or GC, both with optional detection techniques. No further information on the nature or number of compounds included in the test samples was given. Thus the study truly represented the situation when a laboratory should screen pesticide residues in samples of unknown origin.

The results of the inter-laboratory comparison test, summarized in Table 8, were submitted in time only by eight participants out of 16.

The average Rf values reported by the participants were in good agreement with the grand average calculated from the method validation results based on known compounds. The between-laboratory reproducibility of Rf values was also within the range expected from method validation data [12]. Triforine was eluted in a different system and its retention values could not be evaluated.

Though the residue concentration reported by the laboratories sometimes substantially varied, the averages of reported values for various compounds were within ±10% and in one case <13% of the assigned value indicating that the detection methods gave unbiased results.

TABLE 8. SUMMARY OF RESULTS OF 1ST INTER-LABORATORY COMPARISON TEST

Compound	Rf[1]	Rf[2]	CV_{Rf}[3]	Sample 1 [µg/ml]		Sample 2 [µg/ml]	
				Assign[4]	Report[5]	Assign[4]	Report[5]
Oxamyl	0.178	*0.17*	0.14	0.5	*0.46*	20	*20.16*
Prochloraz	0.303	*0.29*	0.14	5		--	
Metoxuron	0.312	*0.29*	0.097	1	*1.10*	--	
Methomyl	0.359	*0.38*	0.13	--		20	
Triforine	0.526			--		50	*43.4*
Dichlorvos	0.52	*0.48*	0.146	0.5	*0.45*	--	
Fenarimol	0.513	*0.49*	0.049	10		20	
Captan	0.651	*0.64*	0.031	5	*4.57*	10	*9.23*
Atrazine	0.634	*0.63*	0.045	0.3	*0.34*	5	*4.96*
Dieldrin	0.753	*0.86*	0.058	5	*4.74*	--	
Parathion-methyl	0.691	*0.69*	0.087	0.5	*0.40*	1	*0.93*

1. Assigned value in silica gel EtAc system based on the average of Rfs from method validation (known amples)
2. Average value reported by the inter-laboratory comparison participants
3. CV of reported Rf values from inter-laboratory comparison test
4. Concentration of the compound in the test solution
5. Reported average concentration

The variability of the reported concentration of the pesticide residues was evaluated [21] according to the "IUPAC Harmonized Protocol" [22]. The z-score was calculated from the nominal concentration of the analyte in the test sample, μ, the reported concentration, x_i, and the target standard deviation, σ_t obtained from the Horwitz equation [23].

$$z = \frac{(x_i - \mu)}{\sigma_t}$$

$$\sigma_t = 0.01 RSD\% \mu$$

$$RSD\% = 2^{(1-0.5 \log \mu)}$$

Analysis values within ranges of ±2z with regard to the assigned true value stand for satisfactory laboratory performance, whereas results between ±2z and ±3z would be questionable. Values exceeding ±3z are to be assigned as unsatisfactory. Therefore, a z-score equal to 0 means that the reported value is exactly the same as the assigned value.

It is worth noting that the variability of the results was much smaller, and the frequency of correct identification was much higher for the marker compounds, which were frequently analysed by the participants and consequently they had more experience with those compounds, as was the case for instance with atrazine and parathion-methyl.

For atrazine in sample 1, eight out of 14 results were valid, i.e. within ±2z, two results were between ±2z and ±3z (questionable), four results were outside the ±3z limits and rated "unsatisfactory". For sample 2, 14 out of 18 results were valid, one was between ±2z and ±3z; and three results were "unsatisfactory". In the case of parathion-methyl in sample 1, 13 out of 16 results were valid, and three results were questionable. For sample 2, 10 of 15 results were valid; three between ±2z and ±3z; three results were "unsatisfactory". The distribution of results is illustrated with two examples shown in figures 3 and 4. The results obtained for these compounds are comparable to the findings of other proficiency tests and indicate that even quantitative results can be obtained with TLC by experienced analysts.

4.7.2. Evaluation of the 2nd inter-laboratory comparison study

The second inter-laboratory comparison sample was ground wheat. The participants obtained 300 g ground samples. Each sample was spiked and thoroughly mixed individually to avoid problems from possible inhomogeneity. The samples were sent to 18 laboratories that expressed interest in participating in the exercise. The laboratories were provided with a short list, which indicated the pesticides potentially present. Sixteen laboratories reported the results that are summarised in Table 9.

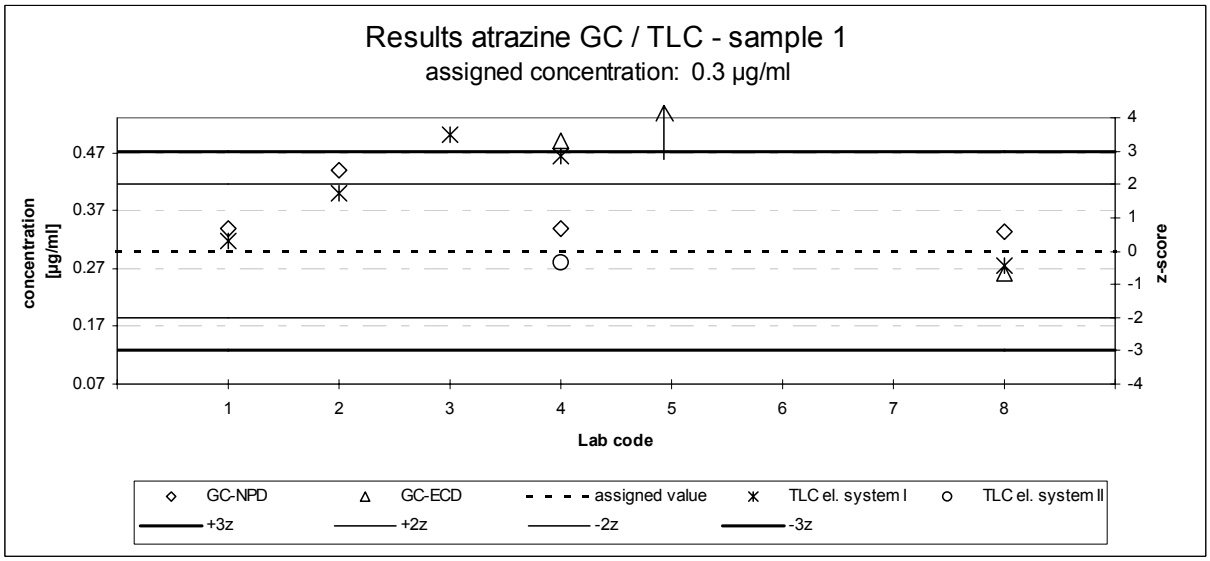
Diuron and fenitrothion were correctly identified by 14 and 12 participants, respectively. Dichlofluanid could be identified only by two participants, but neither of them satisfactorily reported the concentration of the compound. The major problem might be the erroneous standard available in the laboratories.

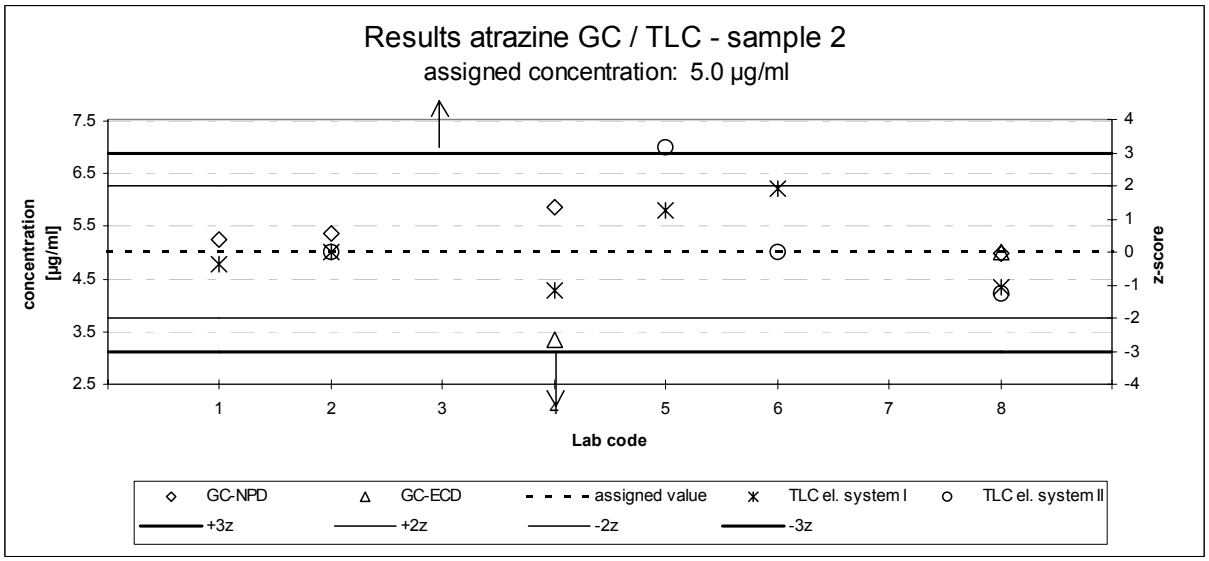
TABLE 9. SUMMARY OF RESULTS OF THE 2nd INTER-LABORATORY COMPARISON EXERCISE

Participant Code	Identity and concentration of residues added to the samples				
	Diuron: 0.51 mg/kg		Fenitrothion 2.01 mg/kg		Dichlofluanid 2.01 mg/kg
	TLC	GLC	TLC	GLC/HPLC	
	Reported concentrations				
1	0.125[a]		0.7[e]		
2	0.45[a]	0.46[d]	1.1	1.12[a]	
3	1.5[e]		2[e]		
4	0.13[a]		1.137		
6	0.058[b]			0.123	0.037[i]
7				3.18[h]	
9	0.19[a]	0.2587[c]	0.82	1.005[b]	
10			0.652[e]		0.084[e]
11	0.022[a]				
12	0.4				
13	0.236		2.54[e]		
14	0.048		0.096[e]		
15	0.654		1.761[e]		
16	0.32[a]		0.41[e]		

Notes: Valid results are highlighted

Mode of detection: a Hill reaction; b: o-TKI; c: NPD;
d: ECD; e: not specified;
f: EbNA; g: EAcI(pig); h: HPLC; i: FAN





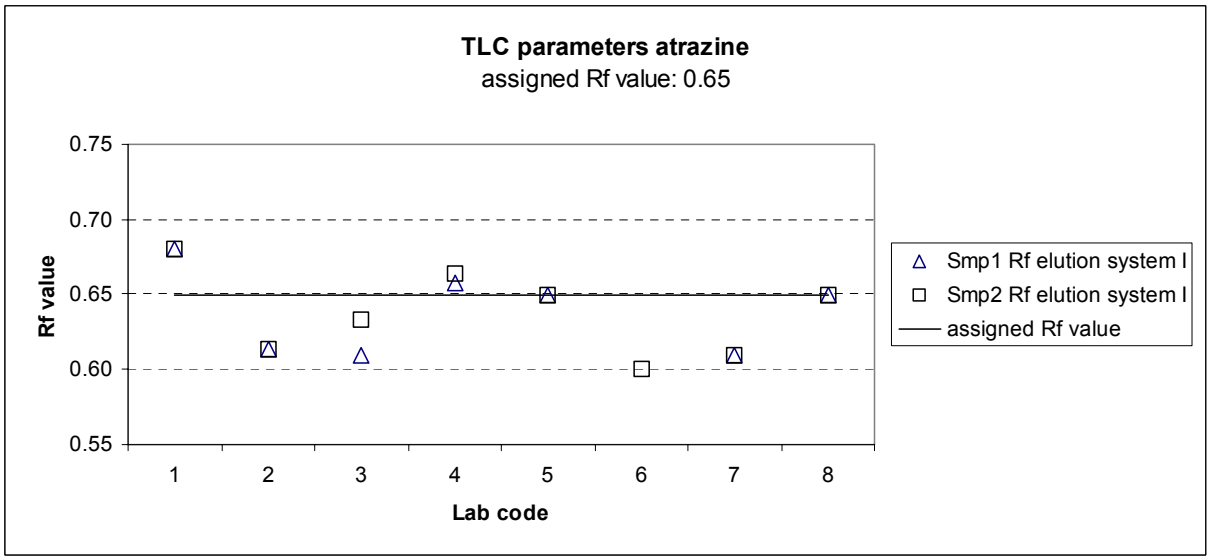
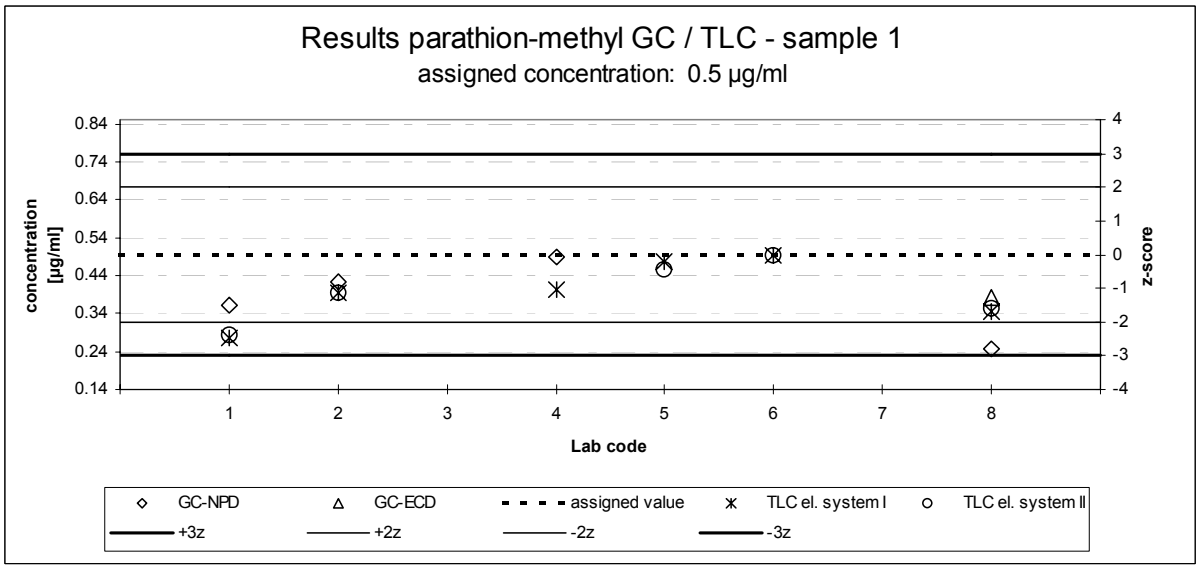
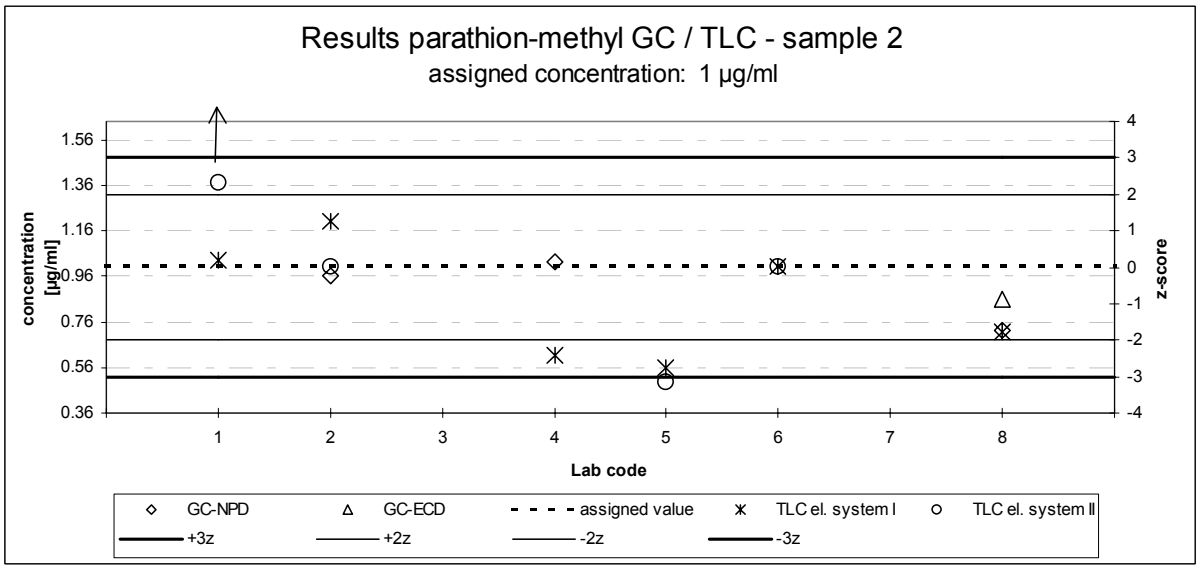


FIG. 3. Results of inter-laboratory comparison test with atrazine.





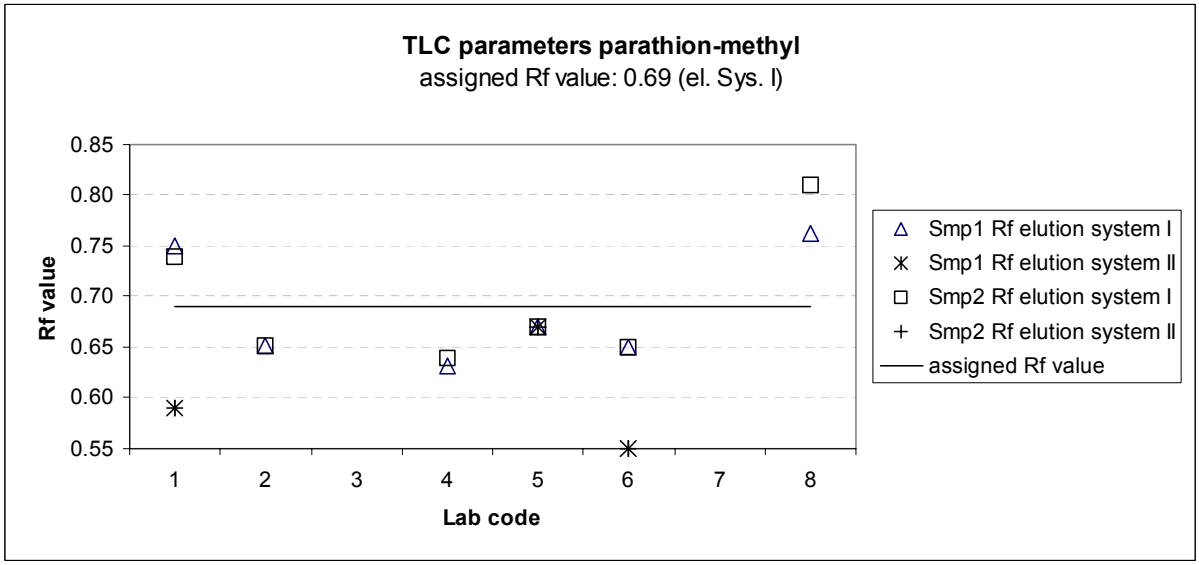


FIG. 4. Results of inter-laboratory comparison test with parathion-methyl.

5. CONCLUSIONS

With CRPs D5.20.33 and D5.20.34 the applicability of TLC detection techniques have been properly explored and demonstrated. Based on the results, it can be concluded that the specific objectives of the projects were achieved:

- Altogether 234 pesticide compounds were included in the programme. Their elution characteristics and detectability were tested with eight elution systems and ten detection methods.
- The within and between laboratories repeatability and reproducibility of retention factors, MDQ, LOQ and recovery values were established.
- Internal quality control procedures were elaborated and applied to demonstrate the reliability of the application of the selected method on each plate.
- The methods were validated in apple, corn, rice, tomato and wheat matrices as representative commodities for cereal grains and fruits of high water content with representative compounds selected from six chemical classes of pesticides.
- The accuracy of the measurements was verified by simultaneous analysis of sample extracts with TLC and GC detection.
- The applicability of the method had been characterised by individual laboratories and through inter-laboratory comparison studies.

The methods elaborated cover a wide range of pesticides, and with *proper experience* they can be used to obtain reliable and valid results. They are suitable for screening for compliance with current MRLs of *specified compounds* including organophosphorus, carbamate insecticides, triazine and urea type of herbicides, benzimidazole and dicarboximide fungicides and several other pesticides of various chemical classes.

However, the TLC based methods should not be used *alone* for determination of pesticide residues in samples of unknown origin, or for screening samples for unspecified compounds.

The confirmation of the identity of the analytes detected is a basic requirement in trace organic analysis. The methods elaborated also make possible the confirmation of compounds which cannot be determined with GC, thus their confirmation would require very expensive LC/MS/MS systems ($200 000 - $300 000). The TLC confirmation of GC amenable compounds is also very important as it eliminates the need of changing columns and time consuming stabilisation of the system. Thus the methods presented improve the capacity of the laboratory without increasing the number of GC and HPLC instruments.

In view of the variability of Rf values and the detection procedures, regular monitoring and control of the TLC conditions is essential for obtaining reliable analytical results. The proper elution and detection conditions can be checked and verified on each plate by applying a mixture of analytical standards of “marker” compounds at their MDQs.

These simple detection methods have a number of limitations too, which must be recognized and taken into account to be used reliably:

- Each person applying the method must have sufficient experience with the technique, which requires more manual skill and attention than modern instruments equipped with auto-samplers. TLC may be used reliably by trained technicians under the supervision of an experienced analyst;

- The laboratory managers should recognize that though the TLC is relatively inexpensive and does not require expensive instrumentation, its reliable application can be expected only from well-trained analysts and the confirmation of residues may require elution in several systems and detection with various methods. Therefore, the analysis of a sample may take a week, and the *management should not expect prompt results*.

6. RECOMMENDATIONS

To facilitate the efficient and reliable application of the TLC methods according to the current quality requirements, the participants of the CRPs made a number of recommendations which are summarized below.

The GPC column must be calibrated when the column packing is replaced or when the column is used again after a longer period. In critical cases the start of pesticide fraction may be selected at half ml between the 8–9 or 9–10 ml eluent (e.g. 8.5 ml) to provide optimal separation.

The elution system should be selected by taking into account the pesticides expected in the samples. The best conditions for both the optimum separation and detection (in terms of linearity, resolution and detection sensitivity) can be achieved with 20 x 20 cm layer when the chromatographic conditions are selected to obtain Rf values ideally between 0.3–0.6 (practically between 0.2–0.7), the sample is applied in spots of 3–4 mm diameter at 2 cm from the edge of the plate within its linear capacity, and the plate is immersed in the developing solvent at 1 cm depth and developed until the solvent front reaches 10 to 15 cm.

The Rf values obtained from individual measurements vary substantially between laboratories, therefore the mean Rf and RRf values obtained on different plates should be reported together with their within-laboratory reproducibility.

Before use, the detectability of each compound to be tested should be verified, and the conditions should be adjusted to achieve at least the typical values reported in this TECDOC.

The between laboratories reproducibility of MDQ and RRf values provide information on the applicability of the TLC detection methods in another laboratory based on the data base generated within these research projects.

The TLC elution and detection can be influenced by a number of factors. Therefore, rigorous internal quality control, including the use of marker compounds on each plate, is required to verify the proper application of the method and reliability of the results.

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COST EFFECTIVE SCREENING METHODS FOR PESTICIDE RESIDUE ANALYSIS IN FRUITS, VEGETABLES AND CEREAL GRAINS[1]

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Abstract

This paper reports the results of studies performed to investigate the potential of applying thin layer chromatography (TLC) detection in combination with selected extraction and cleanup methods, for providing an alternative cost-effective analytical procedure for screening and confirmation of pesticide residues in plant commodities. The extraction was carried out with ethyl acetate and an on-line extraction method applying an acetone-dichloromethane mixture. The extracts were cleaned up with SX-3 gel, an adsorbent mixture of active carbon, magnesia, and diatomaceous earth, and on silica micro cartridges. The Rf values of 118 pesticides were tested in eleven elution systems with UV, and eight biotest methods or and chemical detection reagents. Cabbage, green peas, orange, and tomatoes were selected as representative sample matrices for fruits and vegetables, while maize, rice and wheat represented cereal grains. As an internal quality control measure, marker compounds were applied on each plate to verify the proper elution and detection conditions. The Rf values varied in the different elution systems. The best separation (widest Rf range) was achieved with silica gel (SG)-ethyl acetate (0.05–0.7), SG-benzene, (0.02–0.7) and reverse phase RP-18 F-254S layer with acetone:methanol:water (30:30:30 v/v) (0.1–0.8). The relative standard deviation of Rf values (CV_{Rf}) within-laboratory reproducibility was generally less than 20%, except below 0.2 Rf, where the CV_{Rf} rapidly increased with decreasing Rf values.

The fungi spore inhibition, chloroplast inhibition and enzyme inhibition were found most suitable for detection of pesticides primarily for confirming their identity or screening for known substances. Their use for determination of pesticide residues in samples of unknown origin is not recommended.

1. INTRODUCTION

As food safety is among the first priorities in many countries, there is an increasing need for determination of pesticide residues in various food commodities. The limited resources and constrains in electric power supply limit the range and number of instruments that can be operated in a large number of laboratories. On the other hand, the reliability of the results should be similar regardless the instrumentation of the laboratories. Consequently, alternative detection and confirmatory methods are required which can complement the analyses

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performed with gas and liquid chromatographs, and can be used in laboratories with limited financial resources and instrumentation. The thin layer chromatography, TLC, is one of the alternative techniques which can be used for screening for known pesticide residues or for confirmation of tentatively identified compounds. The analysts should be aware of the limitations of TLC separation and detection in terms of repeatability and reproducibility in order to apply the methods accurately and reliably.

Thin-layer chromatography (TLC) has a long history, but has been used only to a limited extent [1, 2] in pesticide residue analytical laboratories since gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC) became readily available. In recent years, there have been various developments in the quality of plate coating and in detection systems, as well as in extraction and cleanup methods [3]. This study was performed to investigate the possibilities of applying TLC detection in combination with the selected extraction and cleanup methods for providing an alternative cost effective analytical procedure for detection of pesticide residues in plant commodities, using cabbage, green peas, orange, tomato, maize, rice and wheat as representative sample matrices.

The method developed is intended for screening and confirmation of pesticide residues in laboratories where the irregular supply of electricity, lack of service, or a limited budget do not allow the continuous use of GLC and HPLC techniques, and the application of mass spectrometric detection is not feasible.

2. MATERIALS AND EQUIPMENT

2.1. Chemicals and materials

All chemicals used were of analytical grade and they were checked for applicability before use. If interfering spots occurred the solvents were purified or replaced with proper quality product. The chemicals listed can be obtained from any supplier except those where the supplier code is indicated. Other similar products may not give satisfactory results.

2,6-dichlorphenol-indophenol
2,6-dichlorphenol-indophenol Na-salt
Acetic acid
Acetyl thiocholine iodide
Agar-agar
$AgNO_3$
Aluminium oxide G (Merck No.: 1.01090.0500)
Aspergillus Niger spore concentrate
β-naphthyl-acetate
Benzene
Bi-distilled water (collected and stored in Pyrex glass bottle with a ground glass stopper)
Bio-Beads SX-3 200-400 mesh gel (Bio-Rad Cat. No. 152-2750).
Borax
Bromine
Cyclohexane
Dettol™
Dichloromethane
Fast blue BB salt (Echtblau-salt) (Merck Cat.No.1.03191.0025)
Ethanol

Ethyl acetate, EtAc
Ethylene glycol
Glucose
Glycerine
HCl
Hydrogen peroxide
$KMnO_4$
KNO_3
Lichrolut® Si 60 500 mg cartridges (Merck 102024.0001)
Na_2SO_4, anhydrous
$NaHCO_3$
NaOH
o-tolidine
p-dimethylamino-benzaldehyde
p-nitrobenzene diazonium fluoroborate
Paraffin oil
Phenoxy-ethanol,
SX-3 gel, Bio-Rad, Cat. No. 152-2750
TLC plates
- Aluminium oxide G, neutral (Merck 1.01090.6500)
- Aluminium oxide 60 F254 (Merck 1.05713.001)
- Reverse phase TLC plate RP-18 F-254S (Merck No.: 15389
- Silica gel 60 HF254 TLC plate (Merck 1.05715.001)
- Silica gel 60 H, 0.25 mm (Merck 1.05721.001)

Tris (hydroxymethyl) aminomethane
Cotton wool
Gauze, 4 layer

2.2. Equipment

Common laboratory equipment is not listed. All equipment listed can be replaced with other models of similar performance characteristics.

- Analytical balance, 0.0001 g or 0.00001 g sensitivity
- Blender: e.g. Warring blender with 1 litre container or Ultra Turrax UT-25 homogenizer
- Centrifuge, min 4 × 100 ml tubes and 4000 rpm operating speed
- Chopper/mincer suitable for cutting 1–5 kg plant materials into 2–3 mm pieces e.g. Stephan UM-5 Blender, or Hobbart Chopper
- Furnace with operating temperature ≥ 500 ^oC
- Gel column: 450 or 500 mm × 10 mm glass column with adjustable plunger, cap with connecting pipe (e.g. Pharmacia SR 10/50 or Bio-Rad Bio Rex MP gel chromatographic columns)
- GPC apparatus: consisting of a medium pressure pump suitable to deliver constant flow of 1 ml/min, sampler of 1 or 2 ml constant volume, pulse dumpener, or KL SX-3 gel chromatograph operating with constant nitrogen over-pressure of 0.5 att.
- Incubation chamber, operating temperature 37^oC
- Micro syringes with 90^o needle, 10 µl and 20 µl, with 20^o needle 500 µl
- Oven, upper temperature range ≥200 ^oC
- Syringe, 10 ml, glass with solvent resistant barrel
- TLC basic set, e.g. CAMAG, including 254/364 UV lamp, spreader, application guide, atomizer, developing tanks

Top load balance e.g. 200/2000 g range
UV lamp, unfiltered, high intensity (used for air sterilization)
UV lamp 254/365 nm
Vacuum manifold (e.g. Merck 1.19851.0001, or Baker 10 system), optional to hold cartridges and facilitate elution

3. METHODS

3.1. Preparation of portion of sample to be analysed

Systematic studies indicated that it was not easy to obtain a statistically well-mixed sample from certain commodities even if proper chopping equipment (Hobart Chopper, Stephan UM-5 blender) was used. The variability of residues in 30 g analytical test portions, expressed as the coefficient of variation, which could be attributed to the sample processing step was in the range of 3–30% in case of apples, lettuce and tomato depending on the variety of crop and processing device [4, 5]. It was found that the sample material must be cut to reduce the size of sample particles below 2–3 mm in order to obtain a well-mixed sample and to keep the uncertainty derived from sample processing at the minimum. Manual cutting and mixing of plant commodities did not give sufficiently homogeneous analytical sample, and the inhomogeneity might result in unacceptably high variation of the detected residues.

Since the square of the coefficient of variation of the residues (CV) in analytical test portions is inversely proportional to the mass of the test portion, according to the sampling constant $K_s = m \times CV^2$ described by Wallace and Kratochwil [6], the smaller the size of the test portion the larger the random error of the results.

In our experiments the analytical sample was prepared from the total amount of the laboratory sample [7] according to the purpose of the analysis preferably immediately after receipt of the sample. As the purpose of this work was to test the applicability of TLC for regulatory control, the portion of commodity was separated to which the MRL applies [8]. The analytical sample was chopped and minced. The chopped pieces were smaller than 2–3 mm in order to obtain representative portions of the sample. The grain sample was ground to fine powder (≤0.2–03 mm).

The processed sample was well mixed and the required number of 60 g representative portions was taken for analysis. The test portions were extracted as described below or stored individually in double sealed plastic bags in a deep-freezer until extraction.

3.2. Extraction

3.2.1. Extraction of fruits and vegetables

(i) Extraction with ethyl acetate

A 60 g portion of the homogenized laboratory sample was extracted with 120 ml EtAc [9], in the presence of 15 g $NaHCO_3$ and 60 g anhydrous Na_2SO_4 using an Ultra Turrax homogenizer at about 27–30°C. Sixty g Na_2SO_4 was added to remove remaining water. The slurry was well mixed. The sample solvent mixture was kept in a fume hood for about 15 to 30 minutes to let the solvent separate from the solid material. The separated solvent was filtered through a small cotton wool plug into a measuring cylinder to obtain 60 ml filtrate (1 ml = 0.5 g sample). The filtrate was evaporated in a rotary evaporator to about 2–3 ml, the concentrated extract was transferred to a calibrated conical test tube, and the evaporation was continued

with gentle air stream to nearly dryness. About 3 ml of the solvent that was used for the cleanup procedure was added, and finally evaporated to a few tenths of ml. This procedure for changing the solvent was repeated twice. The final volume was adjusted to 1 ml (equivalent to 30 g sample).

The temperature during extraction was maintained between 25–33°C to obtain good extraction efficiency [10] and was not allowed to exceed 35°C otherwise volatile compounds may be lost or labile compounds may decompose. When deep-frozen samples were processed, the mixture of sample homogenate and the extracting solvent was kept in a water bath at 30°C to reach the specified temperature range.

(ii) On-line extraction [11]

Acetone (200 ml), about 30 g sodium chloride, and 150 ml dichloromethane were added to a 100 g portion of the homogenized sample. The content was blended at high speed for two minutes. The organic phase was poured into a 400 ml beaker and dried with anhydrous sodium sulphate. An aliquot of 200 ml of the organic phase was taken, evaporated in a rotary evaporator and the volume was reduced to 2–3 ml. About 5 ml of solvent used for cleanup was added and the evaporation was repeated twice. The final volume was adjusted to 4 ml (equivalent of 57.14 g sample).

3.2.2. Extraction of rice, wheat and corn samples

The sample was allowed to warm up to room temperature and 20 g portion was transferred into a 250 ml extraction vessel. Twenty ml distilled water was added, mixed vigorously and soaked for five minutes. Then 100 ml EtAc, 10 g sodium hydrogen carbonate and 70 g of anhydrous sodium sulphate were added. The vessel was placed on a water bath and the sample solvent mixture was allowed to reach the temperature of about 25^{o}C. The mixture was homogenized with Ultra Turrax (UT-25 homogenizer) at about 25°C for 30 seconds. A 50 ml aliquot was decanted and filtered through a cotton wool plug in a filter funnel. If the extract was turbid, 1 g of sodium hydrogen carbonate was placed on the top of the cotton wool in the filter funnel. The extract was evaporated in a rotary evaporator to nearly dryness. The residue was transferred to a calibrated conical test tube with 2 + 1 + 1 ml ethyl acetate and the solvent was evaporated to nearly dryness. The residue was dissolved in 500 µl mixture of cyclohexane and ethyl acetate (1:1) (representing 10 g sample).

3.3. Cleanup Procedures

The three cleanup procedures tested in this study were: (i) column chromatography on SX-3 gel [12], (ii) mixed adsorbent, and (iii) silica cartridges [13].

(i) SX-3 gel column chromatography

KL SX-3 gel chromatograph with 500 mm x 10 mm glass column (Pharmacia SR 10/50 or Bio-Rad MP gel chromatographic column) packed with Bio-Beads SX-3 200 – 400 mesh gel was applied.

Preparation of column - The gel was soaked in an EtAc:cyclohexane (1+1) mixture and the swollen gel was transferred into the column. The solvent mixture was passed through the column to get a uniform bead.

Calibration of the column – An aliquot of 500 µl EtAc:cyclohexane (1+1) solution containing diazinon and ethion was injected, the pesticides were eluted with ethyl acetate/cyclohexane

(1+1) and the fractions were collected in 1 ml increments up to 30 ml. The pesticide fraction started at the point where about 95% of the pesticides in the test mixture were recovered.

Elution of extracts - The gel chromatographic system was run by injecting 500 µl sample extract, the pesticide fraction were collected as determined earlier during calibration of the system and the system was washed with 30 ml solvent mixture. The system was then ready for the next injection.

The pesticide fraction was evaporated to about 0.3–0.5 ml and made up to a volume of 0.5–1 ml with acetone for direct application on TLC plates. The final volume of the eluate was adjusted to exactly 2 ml before further cleanup on silica cartridge.

The eluate obtained before the pesticide fraction was discarded. The eluates from the washing cycle were collected and combined with the solvent mixture obtained from the evaporation of the pesticide fraction. The ethyl acetate cyclohexane mixture can be regenerated with fractional distillation as an azeotropic mixture (56:44) [14] which can be further used for the GPC cleanup [15].

(ii) Mixed adsorbent

A mixture of 1 g active carbon, 2 g magnesia, 4 g diatomaceous earth (acid-washed) was used. The pre-treatment of the adsorbents was carried out as follows:

Active carbon – A 150 g portion of active carbon in 500 ml 1 N HCl was refluxed for 4 hours. The adsorbent was washed with distilled water until the water contained no chloride ion, and dried at 95–100°C to a constant weight. Care was taken not to increase the temperature above 100°C because adsorptive properties might change. Magnesia was washed in one liter absolute ethanol, filtered, air-dried, and activated at 140°C for four hours. Diatomaceous earth was heated at 400°C for eight hours.

Preparation of the column – Pre-treated adsorbent mixture (7 g) was suspended with 40 ml benzene in cylindrical glass jar. A 18 mm i.d. column was filled with the suspension. The jar and funnel were rinsed with benzene and the later was allowed to flow through column. An aliquot of extract (2–5 ml) equivalent to 20–50 g sample was added and pesticides were eluted with 150 ml dichloromethane. The eluent was evaporated to about 0.3–0.5 ml and its volume was made up to exactly 1 ml with acetone for direct application on TLC plates, or 5 ml cyclohexane was added and the evaporation was repeated twice in order to remove traces of dichloromethane. The final volume of the eluent was adjusted to exactly 2 ml before further cleanup on silica cartridge.

(iii) Silica gel cartridge

LiChrolut Si 60 500 mg cartridges were pre-washed with 10 ml toluene:cyclohexane:acetone (60:30:10 v/v) and then with 2 ×. 10 ml toluene:cyclohexane (15:85 v/v). The concentrated extract was taken up in 2 ml of cyclohexane and transferred onto the column using a10 ml syringe to elute pesticides with one of the elution systems.

The elution option A (for non-polar compounds) consisted of 15 ml toluene:cyclohexane (15:85 v/v), while elution option B comprised of 25 ml toluene:cyclohexane:acetone (60:30:10 v/v). The eluate was concentrated to 1 ml (equivalent to 15 g fruits and vegetables and 10 g grain samples) and was suitable for application on TLC plates or for GC analysis.

3.4. Elution of pesticides on TLC plates

The Rf values were determined in developing tanks kept in water bath held at 20°C in order to reduce the effect of temperature variation in the laboratory. The vapour phase was equilibrated with the eluting solvent by inserting filter paper in the developing tank and waiting for at least 30 minutes before the plates were placed into the tanks. The eluent was allowed to run up to 10 ± 0.5 cm from the origin.

The Rf values were determined in the following TLC systems:

(i) Silica gel 60 F-254 0.25 mm, activated at 105°C for 30 minutes before use, detection under an UV lamp (254/365 nm)

System I. Silica gel 60 F – ethyl acetate

System II. Silica gel 60 F – dichloromethane

System III. Silica gel 60 F – benzene

System IV. Silica gel 60 F – cyclohexane:benzene:acetic acid:paraffin oil (200:30:20:1 v/v)

(ii) Silica gel 60, 0.25 mm (Merck 2299161) activated at 105°C for 30 minutes before use.

This TLC system was used to determine the minimum detectable quantities of pesticides with chemical and bio-assay detection methods.

System IA. Silica gel 60 – ethyl acetate

System VIII. Activated silica-gel – n-hexane:diethyl ether (1:2)

System IX. Silica gel (not activated) – petroleum ether:diethyl ether (1:2)

System X. Silica gel (not activated) – petroleum ether:diethyl ether (5:1)

System XI: Silica gel (not activated) – diethyl ether

(iii) Aluminium oxide G – ethyl acetate, detection under a UV lamp (unfiltered high intensity)

System Va. Self prepared 0.25 mm thick layer with incorporated $AgNO_3$, dried at 80°C for 45 minutes after preparation, and stored over activated silica gel until use.

System Vb. Aluminium oxide G (ready made) – ethyl acetate

System Vc. Aluminium oxide G (ready made) – dichloromethane

(iv) Reversed phase layer RP-18 F-254S (Merck), activated at 120°C for 45 minutes before use, detection under a UV lamp (254/365 nm)

System VI. RP-18 F-254S – acetone:methanol:water (30:30:30 v/v)

(v) Silica gel 60 HF 254 layers were self made and pre-treated with paraffin oil according to Boyce and Millborrow [16].

The plates were dried at room temperature for two hours, and then activated at 105°C for 10 minutes. The activated plates were cooled to room temperature and impregnated by

allowing a 5% solution of liquid paraffin in hexane (v/v) to run to the top of the plate followed by the evaporation of the solvent at 40°C.

System VII. Silica gel 60 HF 254 impregnated with paraffin – acetone:methanol:water (30:30:30 v/v). Detection: under a UV lamp (254/365 nm)

3.5. Detection of Pesticides

3.5.1. Method 1 − o-tolidine + potassium iodide [o-TKI]

The reagent was prepared as follows: o-tolidine (0.5 g) and KI (2 g) were dissolved in 10 ml acetic acid and 2 g in 10 ml distilled water, respectively. These two solutions were mixed and the mixture was diluted to 500 ml with distilled water. The reagent can be stored in refrigerator for two weeks.

Ready-made silica gel plates were used for detection. A 25 ml beaker was placed into the bottom of developing tank, and 8 g $KMnO_4$ and 10 ml concentrated HCl were added into the beaker. The tank was covered with its lid and allowed to stand for few minutes to get the gas phase saturated with chlorine. The eluting solvent was removed from the plate in a fume hood with gentle air stream, and the plate was placed in a developing tank saturated with chlorine for 30 sec. Excess chlorine was removed in a well-ventilated fume hood (about 45 min), and the plate was sprayed with the reagent solution. First the complete removal of chlorine was tested by spraying the upper edge of the plate. (If chlorine is present the sprayed area turns blue!) When no discoloration occurred the entire plate was sprayed.

The detection is not specific. Different substances give blue, lilac or white spots on greyish-white background.

Safety precautions - o-tolidine is classified as a potential carcinogen. Use gloves for handling. Perform chlorination and removal of excess chlorine under a well-ventilated fume hood. Use gloves for placing and removing plates from chlorination tank.

3.5.2. Method 2 − p-nitrobenzene-fluoroborate [NBFB]

The reagent was prepared by dissolving about 0.1 g p-nitrobenzene-diazonium-fluoroborate in a mixture of 2.5 ml ethylene glycol and 22.5 ml ethanol. The solution must be saturated, as indicated by a small portion of non-dissolved material. The reagent solution must be prepared freshly and used within 5–10 minutes.

After elution of samples on ready-made silica-gel plates, the air-dried layer was sprayed with 1.5 M NaOH solution and the plate was placed into an oven at 70°C for 10 minutes. After cooling to room temperature, the plate was sprayed with the fluoroborate reagent solution.

The reagent detects free phenols or phenols derived from the hydrolysis of the compounds with red, lilac, or blue spots occur on white background.

3.5.3. Method 3 − p-dimethylamino benzaldehyde [pDB]

The reagent was freshly prepared before use by dissolving 0.15 g p-dimethylamino-benzaldehyde in the mixture of 47.5 ml ethanol and 2.5 ml HCl. After elution, the air-dried self or ready-made aluminium oxide plates were placed into oven at 160°C for 25 minutes. The plates were cooled to room temperature and sprayed with the reagent.

Compounds which can be hydrolyzed to primary amines (e.g. urea herbicides) can be detected with yellow or (some minutes later) rose spots on white background.

Silica gel plates cannot be used with this detection method as the layer is damaged if heated to 160°C temperature!

3.5.4. Method 4 − Silver nitrate + UV exposure [AgUV]

Method 4a - Al_2O_3 layer sprayed with $AgNO_3$ + phenoxy-ethanol + H_2O_2

$AgNO_3$ (0.1 g) was dissolved in 1 ml freshly prepared double-distilled water, phenoxy-ethanol (20 ml) and 1 drop hydrogen peroxide were added, and the volume was made up to 200 ml with acetone. The reagent can be stored in a brown glass bottle protected from direct UV or sunlight for about a week.

Preparation of TLC plates – 50 g Merck Aluminium oxide G adsorbent was firmly shaken with 55 ml freshly prepared double-distilled water in a 100 ml glass-stoppered Erlenmeyer flask for two minutes. The homogeneous slurry was transferred into the TLC spreader and the plates were drawn without delay. (Calcium sulphate starts to bind immediately) The amount was sufficient to prepare five 20 × 20 cm plates with 0.25 mm thick layer. The uniformity of the layer was checked, and if the layer was not uniform, the plate was carefully shaken horizontally with frequent short strokes immediately after drawing the layer. It usually improved the quality of the layer. The good plates were placed into the storage rack and dried for 15 minutes at room temperature, then at 80°C for 45 minutes. The plates were stored over activated silica gel in a desiccator. The plates with a non-uniform layer were washed and cleaned before the layer got dry.

The self-made aluminium oxide layer was used for elution of samples. After development the layer was dried at room temperature and sprayed uniformly with the reagent solution. The plate was placed under unfiltered intensive UV light until the spots developed. It should be noted that ready-made plates can also be used provided that they do not turn grey when exposed to UV light. Strong bactericide lamps used for air sterilization were found most suitable for general use. However, Pasha and Nayak [17] reported that low intensity UV radiation may be used for selective detection of pesticides containing haloalky moiety.

Specificity: non-specific, detects halogen-containing and several other compounds.

Method 4b − Al_2O_3 incorporated with $AgNO_3$

Merck Aluminium oxide G adsorbent (45 g) was shaken in a 300 ml glass stoppered Erlenmeyer flask with 90 ml 0.2% nitric acid at 250–300 rpm for 15 minutes.

The slurry was transferred into centrifuge tube and centrifuged at 2500 rpm for 10 minutes. The acid layer was decanted and discarded. The Aluminium oxide G was washed three times with 50 ml double-distilled water, and centrifuged after each wash at 2500 rpm for 10 minutes. The water was decanted and discarded. Fifteen ml freshly prepared 1% $AgNO_3$ solution and 15 ml double-distilled water were added to the cleaned aluminium oxide and the slurry was mixed with a glass rod. The homogeneous slurry was poured into the TLC spreader and the plates were drawn without delay at a place protected from sunlight and UV radiation. The procedure was continued as described under Method 4a.

Chlorine containing compounds were detected with greyish-black spots.

Safety precautions: Protective glasses with UV filtration should always be used when checking the development of spots. Skin should not be exposed to UV light for an extended period as strong non-filtered UV radiation can burn the skin.

It should be noted that the time required for colour development depends on the intensity and the spectrum of the UV light source, and the distance between the lamp and the plate. The optimum distance should be determined experimentally. On clear sunny days the plates may be developed under direct sunshine.

Compounds containing chlorine must be kept away from the TLC plates and double-distilled water must be used. If the plates have been exposed to chlorine they turn grey under the UV light. Special attention is required when other TLC detection methods applying HCl or chlorine are used in the laboratory, or the distiller is cleaned with HCl.

3.5.5. Method 5 − Photosynthesis inhibition (Hill reaction) [Hill]

For obtaining chloroplast for the reaction, wheat was grown in pots placed in the window of the laboratory or in a greenhouse. In good quality soil the wheat leaves could be harvested two weeks after seeding. Other leaves with high chlorophyll content, such as spinach, rice or fresh grass can also be used.

Thirty g wheat leaves were cut into 2–4 mm pieces and placed into a mortar. Glycerol (3 ml), double-distilled water (15 ml), and quartz (sea) sand (5 g) were added. The mixture was crushed with a pestle until a fairly homogenous pulp was obtained. Four-layer gauze was put over a beaker, and the homogenate was transferred to the gauze, which was folded up like a knapsack, and the chloroplast suspension was pressed through. The chloroplast suspension was protected from light by wrapping aluminium foil around the beaker, and stored in the refrigerator until use. It was prepared fresh daily before use.

Borax buffer solution was prepared as the mixture of 350 ml 0.05 M borax (9.5 g borax dissolved in 500 ml water) and 150 ml 0.1 mol HCl.

DCPIP reagent was prepared by dissolving 200 mg of 2,6-dichlorphenol-indophenol Na-salt in 500 ml borax buffer solution.

Detecting reagent was made by mixing 20–25 ml wheat pressing with 10 ml DCPIP reagent solution, followed by adding the reagent drop-wise until the colour of the mixture became bluish-green (somewhere between the colours of pH 9–10 on an universal pH paper scale). This amount was enough for two 20×20 cm plates. The reagent must be prepared immediately before spraying the layer. Detection was carried out on a ready-made silica gel plate, which was dried after development and sprayed uniformly with the reagent. The plate was placed about 20 cm below a 60W tungsten lamp (ordinary bulb) for a few minutes. The inhibition should occur within 10 minutes. The spots were usually visible after some minutes and reached optimum after about five minutes. The quantification should be performed immediately after appearance of the spots as they disappear within a few minutes.

Primarily herbicides inhibiting photosynthesis can be detected as blue spots against a greenish background.

3.5.6. Method 6 − Fungi spore (Aspergillus Niger) inhibition [FAN]

Reagents were prepared as follows:

Fungi culture media – About 100 g potato was thoroughly washed in fresh water and disinfected by soaking in 500 ml water containing 5 tablespoons of Detttol. The potatoes were rinsed with freshly distilled water and peeled. Fine pulp was prepared on a sterilized grinder and a 50 g portion was cooked in 250 ml distilled water for an hour. While it is warm the cooked liquid was filtered through one layer of sterile gauze, 5 g glucose and 5 g agar-agar were added. The solution was sterilized in an autoclave at 0.05 MPa (0.5 atm), at 110°C or in a pressure cooker for 60 minutes. Portions of 8–10 ml of the culture medium were poured into sterilized Petri dishes and allowed to cool. The surface of the fresh media was lightly covered with fungal spores with gentle tapping from a developed fungal culture. The Petri dishes were covered with their lids. The Petri dishes were placed in an incubator containing air saturated with water at 25°C. The new culture developed within five days. The Petri dishes were packed in plastic foil and kept in a refrigerator. The culture has to be re-inoculated at least every 4–5 months.

Safety precautions: Handling fungal spore cultures requires special skill and appropriate conditions. If possible, seek the advice of a trained biologist (pathologist) for maintaining the culture. Be careful not to inhale the spores! *Aspergillus Niger* is a toxic fungus!

In order to prepare the suspension of fungi spores, 1.5 g agar was boiled in 70 ml water, 1.5 g glucose, 0.3 g KNO_3 and, with the tip of a glass rod, 1.5 g malt extract were added. The suspension was cooled to 45°C and kept at that temperature. The spores from the fungal culture were removed by adding 30 ml double-distilled water and carefully drawing with a plastic or glass spatula until most spores have loosened. The suspension should be dark grey from the spores. The suspension was added to the agar-agar solution, and the mixture was warmed to, and kept at 40°C. The suspension was filtered through two-layer gauze.

The fungal spore suspension should be used within one hour.

The detection was carried out as follows: After development, the plate was dried with a gentle air stream. The spraying device was warmed up by immersing it in a 40°C de-ionized water bath to prevent the agar from being sticky. The air-dried plate was sprayed with the spore suspension until the layer was thoroughly wet, but avoiding run-off. The plate was immediately placed in an incubator or in an oven saturated with water and held at 37°C for incubation for at least 24 hours. De-ionized water in Petri dishes was placed on every shelf of the oven as it must be pre-saturated with water vapour. The oven used for bio-tests should not be used for other laboratory activities. Incubation for 48 hours gives better growth and the shiny inhibition zones become more visible. The spots can be better observed under narrow angle. It should be noted that this detection method cannot be used in combination with developing solutions containing acetic acid.

The method selectively detects some fungicides. Plant extracts usually do not interfere.

3.5.7. Method 7 − Enzyme inhibition with cow liver extract and β-naphthyl-acetate substrate [EβNA]

The reagents were prepared as follows:

(i) Enzyme solution − Fresh liver was cut into small pieces and 10 g portion was weighed into 90 ml double-distilled water and homogenized with an Ultra Turrax or high speed blender. The homogenate was centrifuged at 4,000 rpm for 10 minutes. The supernatant was collected in 10 or 20 ml portions and placed in a deep-freezer until use. The enzyme solution was diluted three times with bi-distilled water before use.
(ii) β-naphthyl-acetate − 1.25 mg/ml solution in ethanol. It could be stored in refrigerator for an extended period.
(iii) Fast Blue salt − 10 mg salt in 16 ml double-distilled water. It must be prepared freshly for each use.
(iv) Substrate solution − Mixture of 10 ml β-naphthyl-acetate solution and 16 ml Fast Blue-salt solution.

For detection of eluted substances the developed ready-made silica plate was air dried and treated with bromine vapour in a developing tank. For the bromine treatment a 25 ml beaker was placed in the tank and about 0.5–1 ml bromine was transferred with a safety pipette (long measuring pipette with plunger at one end) into the beaker. The tank was covered with its lid. The bromine vapour saturated the tank within a few minutes. The dry plate was placed into the tank for 15 minutes, and then the plate was removed and kept in a well-ventilated fume hood for about 45 min to remove the excess bromine. The plate was sprayed with enzyme solution until it got thoroughly wet and placed into an incubator or oven at 37°C for 30 minutes. The incubator was pre-saturated with water vapour, by placing de-ionized water in Petri dishes on its shelves, in order to ensure that the plate did not dry out during incubation. The excess water was removed from the layer with an air stream after incubation, and then the plate was sprayed with the substrate solution.

Colour reaction – It should be noted that this detection method cannot be used in combination with developing solutions containing acetic acid.

Enzyme inhibiting compounds – especially phosphoric and thio-phosphoric acid esters and carbamate pesticides – were detectable with white spots in a pink (bluish-red) background, Plant extracts usually did not interfere.

3.5.8. Method 8 − Enzyme inhibition with pig or horse blood serum and acetylthiocholine iodide substrate [EAcI]

The reagents were prepared as follows:

(i) 2,6-dichlorphenol-indophenol (0.5 mg/ml solution in distilled water).
(ii) Enzyme solution − The clot (coagulated blood) was broken with a glass rod, transferred into centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. The serum was collected in 10 ml portions and stored in a deep-freezer until use. The cholinesterase activity was determined by the Ellman method. [18]. The serum was diluted with tris-buffer before use to obtain about 140 U/L activity of pig serum, and 570 U/L for horse serum. Where the Ellman test cannot be carried out the enzyme activity (dilution rate) should be determined experimentally to obtain the best sensitivity.
(iii) 0.05 M tris-buffer was prepared by dissolving 3.04 g tris (hydroxymethyl) aminomethane in 500 ml bi-distilled water.
(iv) Substrate solution was prepared by dissolving 1.5 mg/ml acetylthiocholine iodide in water. (It could be stored in a refrigerator at 4°C for up to six weeks.)

The detection was carried out as follows:

The plate was treated with bromine and enzyme solution as described in Method 7. After incubation at 37°C for 30 minutes the excess water was removed with an air stream, the plate was sprayed with substrate solution and incubated again for 15 minutes.

It should be noted that this detection method cannot be used in combination with developing solutions containing acetic acid. Furthermore, the incubation of the plates in Methods 6, 7 and 8 can be very conveniently carried out by inserting the plates in a developing tank saturated with water, and placing the tank into a drying oven set at the appropriate temperature [19]. In this case there is no need for a separate incubator.

The enzyme inhibiting compounds occur as blue spots in white background.

4. RESULTS AND DISCUSSION

4.1. Applicability of Extraction Procedures

Since the extraction efficiency of both the ethyl acetate and the acetone:dichloromethane had been tested extensively by various laboratories and their general efficiency had been demonstrated [20, 21] there was no need to repeat those studies. The applicability of these methods was tested for the amounts of co-extractives obtained from various samples and the load ability of cleanup columns. The colouring spots occurring when extracts representing 80 mg sample without cleanup were eluted in EtAc-Silica gel system are shown in Table 1.

In case of the EtAc extraction, with the exception of green peas, the spots were less intensive or fewer spots were visible than with the on-line extraction. The extracts obtained with the "on-line" extraction procedure were more difficult to clean as well. Regardless the amounts of co-extractives, the micro extraction method described by Steinwandter [22] is not applicable because a 5 g sample is not enough to achieve, the required limits of quantitation for most of the compounds applying TLC detection methods.

TABLE 1. CO-EXTRACTIVES DETECTED UNDER UV LIGHT FROM TOMATO, GREEN PEAS, CABBAGE AND ORANGE SAMPLES

On-line extraction				Ethyl acetate extraction			
Tomato	Green peas	Cabbage	Orange	Tomato	Green peas	Cabbage	Orange
0.34	0.11	0.04	0.02	0.275	0.11	0.04	0.02
0.41	0.54	0.16	0.05	0.35	0.2	0.16	0.06
0.49	0.71	0.26	0.08	0.47	0.24	0.26	0.08
0.58	0.78	0.47	0.31	0.54	0.34	0.36	0.39
0.7		0.55	0.4	0.69	0.52	0.5	0.47
0.8		0.61	0.45	0.77	0.62	0.56	0.58
		0.69	0.56		0.71	0.66	0.67
		0.78	0.65		0.77	0.78	0.75
			0.68				0.81
			0.74				
			0.77				

4.2. Rf Values of pesticides

The activity of the layers, even in the original packing, changes depending on the storage conditions. Therefore, the layers must be freshly reactivated. The differences in the activity of the layer, which can quickly change in very humid atmosphere, and the saturation of the vapour phase of the developing tank can be the major sources of the variation of the Rf values. Therefore, the activation procedures described under 'Elution of pesticides on TLC plates' were strictly followed during the study, and it is recommended to always use freshly activated plates and equilibrated solvent/vapour phases in the thermostated developing tanks to obtain the best reproducibility.

The Rf values were generally measured with 2-7 elutions on different layers at different times. The compounds were detected under UV light or with an appropriate detection method. There was no significant difference between the Rf values obtained in ready-made silica gel HF and H layers. Therefore no distinction was made between these layers in regard to their elution characteristics.

The relative Rf values calculated for atrazine, carbaryl, captan, linuron, and parathion-methyl marker compounds in system I are given in Table 2. The Rf values obtained in systems II–VI together with their calculated within-laboratory reproducibility are summarized in Tables 3–5.

The retention behaviour of some selected pesticides on normal and reversed phase layers is illustrated in Table 6.

TABLE 2. RRf VALUES FOR THE MARKER COMPOUNDS IN SYSTEM I IN INCREASING ORDER ACCORDING TO THE Rf VALUES

Active ingredient	Rf	Detection methods and RRf values calculated for marker compounds				
		o-TKI Hill	o-TKI NBFB	Hill	FAN	EβNA EAcI
		Atrazine	Carbaryl	Captan	Linuron	Parathion-Me
Propamocarb	0.00	0.00	0.00	0.00	0.00	0.00
2,4-D	0.04	0.07	0.07	0.06	0.07	0.06
Haloxyfop	0.05	0.09	0.09	0.08	0.10	0.08
Omethoate	0.06	0.10	0.10	0.09	0.11	0.09
Monocrotophos	0.08	0.13	0.13	0.12	0.14	0.11
Acephate	0.09	0.15	0.15	0.15	0.17	0.14
Ethirimol	0.12	0.20	0.20	0.19	0.22	0.18
Imazalil	0.15	0.25	0.25	0.24	0.27	0.23
Oxamyl	0.19	0.31	0.31	0.30	0.34	0.28
Phosphamidon	0.22	0.37	0.37	0.35	0.40	0.34
Trichlorfon	0.24	0.40	0.40	0.38	0.43	0.36
Dimethoate	0.27	0.45	0.45	0.43	0.49	0.41
Chlordimeform	0.29	0.47	0.47	0.45	0.51	0.43
Carbendazim	0.30	0.49	0.49	0.47	0.53	0.45
Metoxuron	0.30	0.50	0.50	0.47	0.54	0.45

Active ingredient	Rf	Detection methods and RRf values calculated for marker compounds				
		o-TKI Hill	o-TKI NBFB	Hill	FAN	EβNA EAcI
		Atrazine	Carbaryl	Captan	Linuron	Parathion-Me
Benomyl	0.31	0.51	0.51	0.48	0.55	0.46
Tebuconazole	0.33	0.55	0.55	0.53	0.60	0.50
Thiabendazole	0.34	0.55	0.55	0.53	0.60	0.50
Chloroxuron	0.34	0.57	0.57	0.54	0.61	0.52
Oxadixyl	0.36	0.60	0.60	0.57	0.65	0.54
Methomyl	0.36	0.60	0.60	0.57	0.65	0.55
DNOC	0.36	0.60	0.60	0.57	0.65	0.55
Diuron	0.37	0.60	0.60	0.58	0.65	0.55
Pentachlorophenol	0.37	0.60	0.60	0.58	0.65	0.55
Chlortoluron	0.40	0.65	0.65	0.62	0.71	0.59
Methabenzthiazuron	0.41	0.67	0.67	0.64	0.73	0.61
Mevinphos	0.42	0.69	0.69	0.66	0.75	0.63
Fenthion-o	0.42	0.69	0.70	0.66	0.75	0.63
Fenitrothion-o	0.42	0.70	0.70	0.67	0.76	0.64
Pirimicarb	0.45	0.74	0.75	0.71	0.81	0.68
Dioxacarb	0.45	0.75	0.75	0.71	0.81	0.68
Metalaxyl	0.46	0.76	0.76	0.72	0.82	0.69
Nuarimol	0.47	0.77	0.77	0.73	0.83	0.70
Asulam	0.47	0.78	0.78	0.74	0.84	0.71
Fenarimol	0.48	0.79	0.79	0.75	0.85	0.71
Aldicarb	0.48	0.80	0.80	0.76	0.86	0.72
Dichlorvos	0.51	0.83	0.83	0.79	0.90	0.76
Diphenamid	0.52	0.85	0.85	0.81	0.92	0.77
Napropamide	0.52	0.86	0.87	0.82	0.94	0.79
Chlorfenvinphos	0.55	0.90	0.90	0.86	0.98	0.82
3-CO-Carbofuran	0.55	0.91	0.91	0.87	0.99	0.83
Linuron	0.56	0.92	0.92	0.88	1.00	0.84
Bupirimate	0.56	0.92	0.93	0.88	1.00	0.84
Monolinuron	0.56	0.93	0.93	0.88	1.01	0.84
Chlorbromuron	0.57	0.94	0.94	0.89	1.02	0.85
Simazine	0.57	0.94	0.94	0.89	1.02	0.85
Thiophanate-methyl	0.57	0.94	0.94	0.90	1.02	0.86
Metobromuron	0.57	0.95	0.95	0.90	1.03	0.86
Azinphos-methyl	0.58	0.96	0.96	0.92	1.04	0.87

Active ingredient	Rf	Detection methods and RRf values calculated for marker compounds				
		o-TKI Hill	o-TKI NBFB	Hill	FAN	EβNA EAcI
		Atrazine	Carbaryl	Captan	Linuron	Parathion-Me
Carbofuran	0.59	0.98	0.98	0.93	1.06	0.89
Terbutryn	0.60	0.98	0.98	0.94	1.06	0.89
Propachlor	0.60	0.99	0.99	0.94	1.08	0.90
Cyanazine	0.60	1.00	1.00	0.95	1.08	0.91
Carbaryl	0.61	1.00	1.00	0.95	1.08	0.91
Atrazine	0.61	1.00	1.00	0.95	1.08	0.91
Captafol	0.61	1.01	1.01	0.96	1.09	0.92
Dichloran	0.62	1.02	1.03	0.98	1.11	0.93
Metribuzin	0.62	1.03	1.03	0.98	1.11	0.93
Prometryn	0.62	1.03	1.03	0.98	1.11	0.94
Folpet	0.62	1.03	1.03	0.98	1.12	0.94
Methidathion	0.63	1.04	1.04	0.99	1.13	0.95
Aziprotryn	0.63	1.05	1.05	1.00	1.13	0.95
Terbuthylazine	0.63	1.05	1.05	1.00	1.13	0.95
Triazophos	0.63	1.05	1.05	1.00	1.13	0.95
Captan	0.64	1.05	1.05	1.00	1.14	0.95
Iprodione	0.64	1.06	1.06	1.01	1.15	0.96
Lenacil	0.64	1.06	1.06	1.01	1.15	0.96
Malathion	0.64	1.06	1.06	1.01	1.15	0.97
Phenylphenol	0.64	1.06	1.06	1.01	1.15	0.97
Biphenyl	0.64	1.06	1.06	1.01	1.15	0.97
Dichlofluanid	0.65	1.06	1.07	1.01	1.15	0.97
Fenitrothion	0.65	1.07	1.07	1.02	1.16	0.97
Phenmedipham	0.65	1.07	1.07	1.02	1.16	0.97
Ethoxyquin	0.65	1.07	1.07	1.02	1.16	0.97
Etrimfos	0.65	1.07	1.07	1.02	1.16	0.97
Procymidone	0.65	1.07	1.07	1.02	1.16	0.97
Thiometon	0.65	1.07	1.07	1.02	1.16	0.97
Dithianon	0.65	1.07	1.07	1.02	1.16	0.98
Chlorpropham	0.65	1.08	1.08	1.03	1.17	0.98
BCPE	0.65	1.08	1.08	1.03	1.17	0.98
EPTC	0.65	1.08	1.08	1.03	1.17	0.98
Fenthion	0.65	1.08	1.08	1.03	1.17	0.98

Active ingredient	Rf values observed in replicate measurements								
	System II[a]			System III[b]			System IV[c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Cypermethrin	4	0.62	0.040	4	0.002	2.000	2	0.082	0.182
2,4-D	3	0.00		4	0.463	0.097	3	0.344	0.140
p,p'-DDT	4	0.71	0.027	4	0.000		2	0.010	1.414
Deltamethrin	3	0.60	0.001	1	0.144	0.000	1	0.025	0.000
Desmedipham	7	0.09	0.171	3	0.637	0.030	1	0.156	0.000
Diazinon	3	0.01	1.732	3	0.469	0.044	2	0.559	0.006
Dichlofluanid	4	0.51	0.058	3	0.017	0.133	2	0.368	0.079
Dichloran	7	0.56	0.026	4	0.013	2.000	2	0.024	0.059
Dichlorvos	5	0.09	0.229	3	0.062	0.471	2	0.045	0.016
Dieldrin	3	0.57	0.007	1	0.140	0.000	2	0.093	0.046
Dimethoate	5	0.01	0.621	3	0.462	0.070	2	0.134	0.026
Dinobuton	4	0.54	0.054	4	0.293	0.106	1	0.156	0.000
Dioxacarb	5	0.02	0.846	1	0.048	0.000	2	0.272	0.057
Diphenamid	5	0.08	0.397	3	0.036	0.300	2	0.201	0.091
Dithianon	7	0.44	0.042	3	0.442	0.120	3	0.168	0.125
Diuron	5	0.05	0.219	5	0.011	1.144	1	0.160	0.000
DNOC	4	0.32	0.043	4	0.443	0.090	2	0.370	0.044
Endosulfan	3	0.59	0.069	3	0.000		2	0.007	0.544
EPTC	4	0.27	0.101	3	0.012	0.643	3	0.349	0.126
Ethirimol	3	0.00		3	0.328	0.115	2	0.014	0.606
Ethoxyquin	6	0.24	0.139	3	0.005	1.146	2	0.047	0.167
Etrimfos	7	0.30	0.174	4	0.275	0.157	5	0.116	0.138
Fenarimol	5	0.00	2.236	3	0.557	0.042	2	0.008	0.000
Fenitrothion	4	0.55	0.072	4	0.131	0.195	2	0.241	0.041
Fenitrothion-o	4	0.04	0.679	4	0.002	2.000	2	0.493	0.036
Fenpropathrin	4	0.67	0.082	4	0.094	0.290	2	0.304	0.014
Fenthion	3	0.55	0.071	4	0.125	0.096	2	0.014	0.202
Fenthion-o	4	0.06	0.535	2	0.101	0.000	2	0.020	0.109
Folpet	3	0.53	0.011	4	0.394	0.057	2	0.242	0.073
Haloxyfop	4	0.00		3	0.004	1.732	3	0.029	0.282
HCB	3	0.65	0.102	4	0.415	0.066	2	0.231	0.067
Heptachlor	3	0.70	0.019	3	0.454	0.081	2	0.741	0.055
Imazalil	4	0.01	2.000	4	0.018	1.576	3	0.365	0.095
Iprodione	7	0.09	0.135	4	0.264	0.065	2	0.339	0.069

Active ingredient	Rf values observed in replicate measurements								
	System II[a]			System III[b]			System IV[c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Cypermethrin	4	0.62	0.040	4	0.002	2.000	2	0.082	0.182
2,4-D	3	0.00		4	0.463	0.097	3	0.344	0.140
p,p'-DDT	4	0.71	0.027	4	0.000		2	0.010	1.414
Deltamethrin	3	0.60	0.001	1	0.144	0.000	1	0.025	0.000
Desmedipham	7	0.09	0.171	3	0.637	0.030	1	0.156	0.000
Diazinon	3	0.01	1.732	3	0.469	0.044	2	0.559	0.006
Dichlofluanid	4	0.51	0.058	3	0.017	0.133	2	0.368	0.079
Dichloran	7	0.56	0.026	4	0.013	2.000	2	0.024	0.059
Dichlorvos	5	0.09	0.229	3	0.062	0.471	2	0.045	0.016
Dieldrin	3	0.57	0.007	1	0.140	0.000	2	0.093	0.046
Dimethoate	5	0.01	0.621	3	0.462	0.070	2	0.134	0.026
Dinobuton	4	0.54	0.054	4	0.293	0.106	1	0.156	0.000
Dioxacarb	5	0.02	0.846	1	0.048	0.000	2	0.272	0.057
Diphenamid	5	0.08	0.397	3	0.036	0.300	2	0.201	0.091
Dithianon	7	0.44	0.042	3	0.442	0.120	3	0.168	0.125
Diuron	5	0.05	0.219	5	0.011	1.144	1	0.160	0.000
DNOC	4	0.32	0.043	4	0.443	0.090	2	0.370	0.044
Endosulfan	3	0.59	0.069	3	0.000		2	0.007	0.544
EPTC	4	0.27	0.101	3	0.012	0.643	3	0.349	0.126
Ethirimol	3	0.00		3	0.328	0.115	2	0.014	0.606
Ethoxyquin	6	0.24	0.139	3	0.005	1.146	2	0.047	0.167
Etrimfos	7	0.30	0.174	4	0.275	0.157	5	0.116	0.138
Fenarimol	5	0.00	2.236	3	0.557	0.042	2	0.008	0.000
Fenitrothion	4	0.55	0.072	4	0.131	0.195	2	0.241	0.041
Fenitrothion-o	4	0.04	0.679	4	0.002	2.000	2	0.493	0.036
Fenpropathrin	4	0.67	0.082	4	0.094	0.290	2	0.304	0.014
Fenthion	3	0.55	0.071	4	0.125	0.096	2	0.014	0.202
Fenthion-o	4	0.06	0.535	2	0.101	0.000	2	0.020	0.109
Folpet	3	0.53	0.011	4	0.394	0.057	2	0.242	0.073
Haloxyfop	4	0.00		3	0.004	1.732	3	0.029	0.282
HCB	3	0.65	0.102	4	0.415	0.066	2	0.231	0.067
Heptachlor	3	0.70	0.019	3	0.454	0.081	2	0.741	0.055
Imazalil	4	0.01	2.000	4	0.018	1.576	3	0.365	0.095
Iprodione	7	0.09	0.135	4	0.264	0.065	2	0.339	0.069

Active ingredient	Rf values observed in replicate measurements								
	System II[a]			System III[b]			System IV[c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Lenacil (1)	4	0.01	1.238	3	0.004	1.732	3	0.192	0.204
Lenacil (2)	4	0.25	0.141	2	0.591	0.169	2	0.041	0.017
Lindane	4	0.70	0.041	3	0.552	0.249	2	0.615	0.037
Linuron	7	0.25	0.074	3	0.000		1	0.623	0.000
Malathion	6	0.31	0.159	4	0.023	0.189	2	0.000	
Mecarbam	4	0.32	0.055	3	0.119	0.067	3	0.048	0.359
Methabenzthiazuron	3	0.06	0.164	2	0.240	0.133	3	0.071	0.385
Metalaxyl	2	0.02	1.414	2	0.286	0.082	3	0.113	0.137
Methidathion	3	0.31	0.113	3	0.597	0.030	2	0.099	0.814
Methomyl	7	0.03	0.433	5	0.053	0.070	3	0.044	0.404
Metobromuron	4	0.21	0.067	4	0.083	0.186	2	0.148	0.129
Metoxuron	3	0.02	0.377	4	0.087	0.223	2	0.045	0.094
Metribuzin	3	0.14	0.227	4	0.012	0.281	2	0.017	0.643
Mevinphos	4	0.03	0.483	4	0.021	2.000	2	0.136	0.016
Monocrotophos	4	0.00	2.000	3	0.146	0.188	2	0.000	
Monolinuron	5	0.25	0.148	3	0.000		2	0.052	0.163
Napropamide	3	0.06	0.155	3	0.042	0.037	2	0.004	1.414
Nitrofen	4	0.61	0.048	3	0.003	1.732	2	0.053	0.121
Nuarimol	3	0.01	0.868	5	0.030	0.518	2	0.204	0.139
Omethoate	5	0.00		3	0.000		2	0.027	0.262
Oxadiazon	3	0.47	0.041	3	0.000		2	0.000	
Oxadixyl	3	0.02	0.601	3	0.044	0.230	2	0.051	0.014
Oxamyl	4	0.01	0.878	4	0.003	2.000	4	0.079	0.187
Parathion	3	0.59	0.053	3	0.559	0.094	2	0.367	0.095
Parathion-methyl	7	0.56	0.067	4	0.003	1.277	2	0.027	0.262
Pentachlorophenol	3	0.28	0.064	4	0.000		3	0.008	1.231
Phenkapton	6	0.70	0.048	6	0.277	0.126	3	0.274	0.111
Phenylphenol	6	0.44	0.105	3	0.000		3	0.000	
Phenmedipham	4	0.06	0.411	3	0.000		2	0.000	
Phosalone	7	0.55	0.051	4	0.397	0.075	2	0.292	0.010
Phosphamidon	3	0.00	1.732	6	0.384	0.061	3	0.246	0.144
Pirimicarb	7	0.02	1.029	4	0.214	0.140	3	0.191	0.087
Procymidone	4	0.58	0.054	4	0.582	0.060	2	0.480	0.024
Prometryn	3	0.07	0.314	3	0.278	0.037	2	0.176	0.068

Active ingredient	Rf values observed in replicate measurements								
	System II[a]			System III[b]			System IV[c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Propachlor	3	0.15	0.148	3	0.009	0.192	2	0.014	0.202
Propamocarb	5	0.00	1.414	4	0.215	0.023	2	0.179	0.016
Propargite	4	0.52	0.069	4	0.016	2.000	2	0.002	1.414
Propham	7	0.38	0.058	3	0.000		2	0.000	
Prothiofos	4	0.69	0.017	3	0.168	0.076	2	0.155	0.018
Simazine	4	0.05	0.426	4	0.012	0.253	2	0.082	0.276
Tebuconazole	3	0.03	0.341	2	0.039	0.533	2	0.074	0.375
Terbuthylazine	3	0.05	0.145	3	0.000		3	0.000	
Terbutryn	4	0.07	0.377	3	0.300	0.064	3	0.295	0.107
Tetradifon	3	0.57	0.041	6	0.170	0.057	3	0.192	0.140
Tetrasul	3	0.69	0.039	3	0.576	0.067	3	0.562	0.082
Thiabendazole	4	0.02	0.769	3	0.003	1.732	2	0.164	0.134
Thiometon	5	0.55	0.050	3	0.000		2	0.021	0.448
Thiophanate-methyl	2	0.07	0.152	4	0.007	1.350	3	0.197	0.161
Triazophos	7	0.19	0.107	3	0.014	0.277	3	0.049	0.897
Trichlorfon	3	0.29	0.145	3	0.410	0.090	2	0.320	0.042
Trifluralin	3	0.68	0.009	3	0.652	0.036	2	0.585	0.040
Vinclozolin	4	0.59	0.042	3	0.000		2	0.000	

[a] System II: Silica gel - dichloromethane
[b] System III: Silica gel - benzene
[c] System IV: Silica gel – hexane:benzene:acetic acid:paraffin oil system

TABLE 4. AVERAGE Rf AND CVRf VALUES OF PESTICIDES IN ELUTION SYSTEM V

Active ingredient	Rf values observed in replicate measurements								
	System V [a]			System Va [b]			System Vb [c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Acephate	2	0.76	0.020						
Aldicarb	2	0.72	0.008						
Aldrin	3	0.88	0.006						
Asulam	2	0.00		3	0.00	0	3	0.00	0
Atrazine	3	0.80	0.058						
Azinphos-methyl	2	0.81	0.003						
Aziprotryn	3	0.85	0.009						
BCPE	3	0.85	0.014						

Active ingredient	Rf values observed in replicate measurements								
	System V [a]			System Va [b]			System Vb [c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Benefin	2	0.73	0.087	5	0.80	0.005	2	0.761	0.027
Benomyl (1)	3	0.22	0.279						
Benomyl (2)	3	0.65	0.130						
Biphenyl	2	0.86	0.019						
Bromophos-ethyl	2	0.88	0.034						
Bromopropylate	2	0.81	0.036						
Bupirimate	3	0.81	0.006						
Butachlor	3	0.77	0.064						
Butylate	2	0.87	0.020						
Captan	3	0.80	0.058						
Captafol	3	0.77	0.079						
Carbaryl	2	0.77	0.050						
Carbendazim	3	0.23	0.410						
3-CO-Carbofuran	2	0.76	0.040						
Chlorbromuron	2	0.81	0.013	3	0.70	0.020	4	0.558	0.11
Chlordimeform	2	0.84	0.040						
Chlorfenvinphos	2	0.83	0.005						
Chloroxuron	2	0.75	0.008	3	0.60	0.018	3	0.218	0.055
Chloropropylate	2	0.78	0.119						
Chlorpyrifos (1)	2	0.77	0.032						
Chlorpyrifos (2)	2	0.89	0.022						
Chlorpropham	3	0.84	0.039	3	0.75	0.011	3	0.570	0.004
Chlorothalonil	2	0.87	0.012						
Chlortoluron	2	0.74	0.008	3	0.61	0.025	2	0.244	0.055
Cyanazine	2	0.78	0.045						
Cypermethrin (1)	2	0.76	0.052						
Cypermethrin (2)	3	0.88	0.039						
2,4-D	3	0.00							
p,p'-DDT	4	0.86	0.040						
Deltamethrin (1)	2	0.76	0.057						
Deltamethrin (2)	2	0.89	0.031						
Desmedipham	2	0.84	0.015	3	0.72	0.040	2	0.111	0.076
Diazinon (1)	2	0.09	0.546						
Diazinon (2)	3	0.86	0.035						

Active ingredient	Rf values observed in replicate measurements								
	System V [a]			System Va [b]			System Vb [c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Dichlobenil	3	0.82	0.017						
Dichlofluanid (1)	2	0.76	0.068						
Dichlofluanid (2)	3	0.84	0.038						
Dichloran	2	0.79	0.002						
Dichlorvos	2	0.72	0.052						
Dieldrin	5	0.82	0.045						
Dimethoate	2	0.46	0.060						
Dinobuton	2	0.90	0.002						
Diphenamid	2	0.77	0.027						
Dithianon (1)	2	0.77	0.024						
Dithianon (2)	3	0.85	0.011						
Diuron	4	0.68	0.012	5	0.59	0.020	5	0.259	0.055
DNOC	3	0.77	0.020						
Endosulfan	5	0.89	0.019						
EPTC (1)	1	0.78							
EPTC (2)	1	0.88							
Ethirimol	2	0.02	1.414						
Ethoxyquin	2	0.86	0.003						
Etrimfos	1	0.87							
Fenarimol (1)	2	0.58	0.037						
Fenarimol (2)	2	0.75	0.012						
Fenitrothion	2	0.87	0.019						
Fenitrothion-o	1	0.00							
Fenpropathrin	1	0.90	0.000						
Fenthion	3	0.77	0.092						
Fenthion-o	1	0.78							
Folpet	1	0.83							
Haloxyfop	1	0.00							
HCB	5	0.80	0.053						
Heptachlor	2	0.81	0.111						
Imazalil	5	0.36	0.068						
Iprodione	1	0.81							
Lenacil	3	0.72	0.053						
Lindane (1)	5	0.75	0.007						

Active ingredient	Rf values observed in replicate measurements								
	System V [a]			System Va [b]			System Vb [c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Lindane (2)	5	0.87	0.010						
Linuron	3	0.78	0.045	3	0.70	0.030	3	0.561	0.046
Malathion	3	0.85	0.021						
Mecarbam	1	0.87							
Methabenzthiazuron	1	0.76							
Metalaxyl	3	0.73	0.022						
Methidathion	3	0.82	0.014						
Methomyl	1	0.77							
Metobromuron	1	0.80		3	0.70	0.019	2	0.501	0.003
Metoxuron	3	0.59	0.020	5	0.54	0.029	4	0.128	0.057
Metribuzin (1)	1	0.78							
Metribuzin (2)	1	0.82							
Mevinphos	1	0.78							
Monocrotophos	1	0.79							
Monolinuron	3	0.79	0.028	3	0.70	0.017	3	0.519	0.092
Napropamide	1	0.81							
Nitrofen	3	0.77	0.097						
Nuarimol	4	0.55	0.020						
Omethoate	1	0.79							
Oxadiazon	4	0.85	0.044						
Oxadixyl	1	0.32							
Oxamyl	2	0.41	0.074						
Parathion	2	0.87	0.032						
Parathion-methyl	2	0.86	0.023						
Pentachlorophenol	3	0.76	0.046						
Phenkapton	2	0.88	0.017						
Phenylphenol	1	0.75							
Phenmedipham	1	0.76		3	0.70	0.039	2	0.095	0.000
Phosalone	1	0.87							
Phosphamidon	2	0.46	0.026						
Pirimicarb	2	0.78	0.002						
Procymidone	3	0.83	0.039						
Prometryn	3	0.83	0.035						
Propachlor	3	0.79	0.017						

Active ingredient	Rf values observed in replicate measurements								
	System V [a]			System Va [b]			System Vb [c]		
	No.	Average	CV	No.	Average	CV	No.	Average	CV
Propamocarb	3	0.02	1.732						
Propargite	2	0.59	0.131						
Propham	2	0.75	0.008	3	0.70	0.102	2	0.528	0.012
Prothiofos	2	0.80	0.108						
Simazine	2	0.73	0.082						
Tebuconazole	2	0.44	0.072						
Terbuthylazine	2	0.78	0.038						
Terbutryn	2	0.82	0.007						
Tetradifon	2	0.89	0.031						
Tetrasul (1)	2	0.79	0.105						
Tetrasul (2)	2	0.89	0.071						
Thiabendazole	2	0.57	0.025						
Thiometon	4	0.85	0.027						
Thiophanate-methyl (1)	3	0.67	0.051				2	0.772	0.002
Thiophanate-methyl (2)	3	0.86	0.032				2	0.728	0.009

[a] System Va: self made aluminium oxide layer with ethyl acetate elution
[b] System Vb: ready made aluminium oxide layer with ethyl acetate elution
[c] System Vc: ready made aluminium oxide layer with dichloromethane elution

TABLE 5. AVERAGE Rf AND CVrf VALUES OF PESTICIDES IN RP-18 F254S – ACETONE:METHANOL/WATER SYSTEM

Active ingredient	Replicate measurements						MERCK[a]
	1	2	3	4	Average	CV	
Aldicarb	0.51	0.57			0.54	0.073	0.541
Aldrin	0.03	0.02	0.02	0.03	0.02	0.254	
Asulam	0.77	0.78	0.76		0.77	0.010	
Atrazine	0.35	0.33	0.37		0.35	0.051	0.371
Azinphos-methyl	0.29	0.30	0.28		0.29	0.024	
Aziprotryn	0.22	0.23	0.23		0.23	0.022	
BCPE	0.14	0.13	0.13		0.14	0.057	
Benefin	0.06	0.04	0.04	0.05	0.05	0.172	
Benomyl(1)	0.14	0.15	0.14		0.14	0.052	
Benomyl(2)	0.45	0.47	0.46		0.46	0.022	
Biphenyl	0.14	0.14	0.15		0.14	0.043	
Bromophos-ethyl	0.04	0.03	0.03		0.03	0.160	

Active ingredient	Replicate measurements						MERCK[a]
	1	2	3	4	Average	CV	
Bromopropylate	0.07	0.07	0.06		0.07	0.040	
Bupirimate	0.19	0.19	0.20		0.19	0.021	
Butachlor	0.08	0.08	0.08		0.08	0.044	
Captan	0.28	0.27	0.27		0.27	0.014	
Captafol	0.20	0.19	0.19		0.19	0.036	
Carbaryl	0.44	0.43	0.44		0.44	0.005	
Carbendazim	0.45	0.46	0.46		0.46	0.008	
Carbofuran	0.49				0.49		0.510
3-CO-Carbofuran	0.56	0.55	0.56		0.56	0.010	
Chlorbromuron	0.26	0.24	0.22	0.25	0.24	0.067	
Chlordimeform	0.18	0.18	0.16		0.17	0.079	
Chlorfenvinphos	0.18				0.18		0.180
Chloroxuron	0.24	0.19	0.22	0.25	0.22	0.109	0.119
Chloropropylate	0.08	0.08	0.08		0.08	0.029	
Chlorpyrifos	0.60	0.50	0.50		0.53	0.107	
Chlorpropham	0.22				0.22		0.229
Chlorothalonil	0.20	0.19	0.18		0.19	0.054	
Chlortoluron	0.37	0.39	0.36		0.37	0.042	0.391
Cyanazine	0.50				0.50		0.500
Cypermethrin	0.03	0.02	0.02		0.02	0.185	
2,4-D	0.53	0.54			0.53	0.007	0.628
p,p-DDT	0.04	0.04	0.03	0.03	0.04	0.191	
Deltamethrin	0.02	0.02	0.02		0.02	0.029	
Desmedipham	0.35	0.32	0.33		0.33	0.040	
Diazinon	0.14	0.14	0.14		0.14	0.029	
Dichlobenil	0.28	0.27			0.28	0.023	0.257
Dichlofluanid	0.20	0.19	0.19		0.19	0.023	
Dichloran	0.24	0.24	0.23		0.23	0.028	
Dieldrin	0.06	0.05	0.05		0.05	0.110	
Dimethoate	0.64				0.64		0.639
Dinobuton	0.10	0.09	0.08		0.09	0.093	
Dioxacarb	0.64	0.60	0.61		0.62	0.032	
Diphenamid	0.35	0.35	0.35		0.35	0.012	
Dithianon	0.17	0.18	0.17		0.17	0.017	
Diuron	0.33	0.32			0.32	0.020	0.309

Active ingredient	Replicate measurements						MERCK[a]
	1	2	3	4	Average	CV	
DNOC	0.49	0.49	0.50		0.49	0.005	
Endosulfan	0.09	0.07			0.08	0.186	0.061
EPTC	0.20	0.18	0.16		0.18	0.118	
Ethirimol	0.30	0.29	0.29		0.29	0.015	
Ethoxyquin	0.20	0.19	0.19		0.19	0.032	
Etrimfos	0.17	0.15	0.14		0.15	0.077	
Fenarimol	0.23	0.21	0.19		0.21	0.076	
Fenitrothion	0.20	0.20	0.19		0.20	0.032	
Fenitrothion-o	0.44	0.40	0.40		0.41	0.050	
Fenpropathrin	0.04	0.04	0.03		0.04	0.081	
Fenthion	0.16	0.15	0.15		0.15	0.051	
Folpet	0.19	0.19	0.19		0.19	0.021	
Haloxyfop	0.33	0.33	0.33		0.33	0.004	
HCB	0.03	0.02	0.02		0.02	0.289	
Imazalil	0.12	0.14	0.11		0.12	0.093	
Iprodione	0.20	0.17			0.18	0.103	0.180
Lenacil(1)	0.37	0.38	0.37		0.38	0.012	
Lenacil(2)	0.46	0.46	0.47		0.46	0.008	
Linuron	0.26				0.26		0.257
Malathion	0.24	0.23	0.22		0.23	0.037	
Methabenzthiazuron	0.36	0.37			0.36	0.019	0.371
Metalaxyl	0.42	0.37	0.39	0.43	0.40	0.062	
Methidathion	0.29	0.29	0.28		0.28	0.024	
Methomyl	0.70	0.69	0.68		0.69	0.014	
Metobromuron	0.36				0.36		0.350
Metoxuron	0.52				0.52		0.520
Metribuzin	0.46	0.42			0.44	0.069	0.440
Mevinphos	0.59	0.57	0.57		0.58	0.023	
Monocrotophos	0.73	0.72	0.73		0.72	0.005	
Monolinuron	0.36				0.36		0.360
Napropamide	0.22	0.22	0.22		0.22	0.003	
Nitrofen	0.08	0.07	0.07		0.07	0.076	
Nuarimol	0.28	0.27	0.28		0.28	0.022	
Omethoate	0.52	0.51	0.53		0.52	0.022	
Oxadiazon	0.07	0.06	0.07		0.07	0.031	

Active ingredient	Replicate measurements						MERCK[a]
	1	2	3	4	Average	CV	
Oxadixyl	0.56	0.53	0.56		0.55	0.036	
Oxamyl	0.75	0.75	0.74		0.74	0.005	
Parathion	0.15	0.17	0.14		0.16	0.089	0.149
Parathion-methyl	0.25				0.25		0.250
Pentachlorophenol	0.12	0.12	0.14		0.13	0.073	
Phenkapton	0.04	0.03	0.03		0.03	0.179	
Phenylphenol	0.30	0.31	0.28		0.30	0.044	
Phenmedipham	0.32	0.37	0.35		0.35	0.080	0.319
Phosalone	0.12	0.11	0.12		0.11	0.042	
Phosphamidon	0.57	0.55	0.55		0.56	0.015	
Pirimicarb	0.39	0.41	0.37		0.39	0.047	
Procymidone	0.19	0.17			0.18	0.074	0.180
Prometryn	0.22				0.22		0.229
Propachlor	0.33	0.36	0.36	0.33	0.35	0.045	
Propamocarb	0.66	0.68			0.67	0.021	
Propargite	0.05	0.04	0.04		0.04	0.157	
Propham	0.35	0.37			0.36	0.039	0.371
Prothiofos	0.02	0.03	0.03		0.03	0.106	
Simazine	0.46	0.40			0.43	0.094	0.422
Tebuconazole	0.16	0.15	0.16		0.16	0.027	
Terbuthylazine	0.25	0.27			0.26	0.079	0.268
Terbutryn	0.21	0.23	0.21		0.22	0.074	0.229
Tetradifon	0.08	0.07	0.07		0.07	0.042	
Tetrasul	0.01	0.02	0.01		0.01	0.435	
Thiabendazole	0.41	0.42	0.43		0.42	0.020	
Thiometon	0.19	0.19	0.18		0.19	0.030	
Thiophanate-methyl	0.50	0.51	0.47		0.49	0.048	
Triazophos	0.23	0.21	0.21		0.22	0.065	
Trifluralin	0.04				0.04		0.046
Vinclozolin	0.16	0.20	0.17		0.18	0.093	0.149

[a] Rf values reported by Hauck [24]

TABLE 6. COMPARISON OF Rf VALUES OBTAINED IN NORMAL AND REVERSED PHASE SYSTEMS

Active ingredient	**System I Silica gel**	**System V Aluminium oxide**	**System VII Si + paraffin**	**System VI RP-18**
Aldrin	0.67	0.88	0.16	0.33
Aziprotryn	0.63	0.85	0.69	0.25
Chlorpyrifos	0.67	0.77	0.38	0.60
Dithianon	0.65	0.77	0.93	0.24
Methabenzthiazuron	0.62	0.78	0.76	0.36
Monolinuron	0.56	0.79	0.77	0.36
Pirimicarb	0.45	0.78	0.75	0.56
Prometryn	0.62	0.83	0.70	0.22

Since the mechanism of elution differs between the reversed phase system and the normal phase, and the retention order on the reversed phase was different from that obtained on normal phase, separation of suspected compounds on paraffin-coated layers could provide an excellent and cheap confirmation method in the future provided that the sensitive detection of pesticides can be achieved. This aspect was not further investigated within this study. Further work is required for the elaboration and optimization of the detection conditions with the methods used for reverse phase layers. Until that time the reverse phase layers may be used for confirmation of the identity of residues with UV scanners.

In order to provide options for the separation of co-eluting compounds, the Rf values of some pesticides were also tested with hexane:diethyl ether, petroleum diether:ethyl ether and diethyl ether eluents. The results, together with the Rf values in system I, are summarized in Table 7.

TABLE 7. Rf VALUES OF PESTICIDES IN ELUTION SYSTEMS I, VIII, IX, X AND XI

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
2,4,5-T			0.00		
2,4-D	0.040	0.016			
2,4-DB			0.00		
2-Naphthyloxy acetic acid			0.00		
Acephate	0.093		0.00		
Acetochlor	0.637	0.437	0.78		
Acifluorfen-sodium			0.00		
Alachlor	0.635	0.440	0.75		
Aldicarb	0.482		0.25		
Aldrin	0.665		0.99	0.99	
Alpha-cypermethrin	0.679	0.562			
Ametryn			0.49		

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
Amidosulfuron	0.399	0.000			
Amitraz			0.96	0.55	
Atrazine	0.620	0.316	0.57		
Azinphos-methyl	0.581		0.51		
Aziprotryn	0.634		0.87	0.24	
Benfluralin=Benefin	0.682		0.99	0.95	
Bentazone	0.293	0.042	0.14		0.52
Benzoylprop-ethyl			0.68		
Beta cyfluthrin	0.669	0.554			
Beta cypermethrin	0.677	0.572			
Bifenthrin			0.74		
Bitertanol			0.20		
Bromophos			0.99	0.78	
Bromoxynil	0.184	0.050			
Bromuconazole (1)	0.447	0.077			
Bromuconazole (2)	0.236	0.027			
Bupirimate	0.562		0.48		
Butralin			0.99	0.90	
Butylate	0.675	0.586	0.95	0.68	
Captafol	0.612		0.49		
Captan	0.630	0.279	0.49		
Carbaryl	0.598	0.235	0.51		
Carbendazim	0.122	0.017	0.11		0.42
Carbofuran	0.591	0.258	0.47		
Carbondisulfid	0.676	0.550			
Carbosulfan	0.676	0.550			
Carboxin	0.589	0.325	0.63		
Chlorbromuron	0.575	0.249	0.48		
Chlordimeform	0.285		0.55		
Chlorfenvinphos	0.547		0.40		
Chlorfenvinphos	0.547				
Chloridazon			0.12		0.42
Chlormezulon	0.034	0.000			
Chlorothalonil	0.671	0.566			
Chlortoluron	0.398	0.066	0.21		0.51
Chloroxuron	0.341		0.08		
Chlorpropham	0.652		0.91	0.40	

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
Chloropropylate	0.671				
Chlorpyrifos	0.669	0.601	0.99	0.87	
Chlorpyrifos-methyl	0.657	0.587			
Cyproconazole (1)	0.302	0.020			
Cyanazine	0.602	0.211	0.42		0.90
Cycloate			0.93	0.49	
Cymoxanil			0.10		0.41
Cypermethrin	0.673	0.571	0.92		
Cypermethrin	0.673	0.571			
Dalapon-sodium			0.00		
Dazomet			0.12		
Deltamethrin	0.671	0.554			
Desmedipham	0.661		0.65		
Diazinon	0.660	0.446	0.75		
Dicamba	0.034	0.020	0.00		
Dichlorprop	0.043	0.019	0.00		
Dichlorprop-P	0.050	0.021			
Dichlorvos	0.458		0.27		
Diclofop-methyl	0.661	0.527		0.23	
Dieldrin	0.675		0.99	0.99	
Difenoconazole	0.292		0.06		
Difenzoquat methylsulfate	0.000	0.000			
Diflubenzuron	0.619	0.286	0.59	0.09	0.95
Diflufenican	0.630	0.383			
Dimethenamid	0.638	0.323			
Dimethipin	0.429	0.064			
Dimethoate	0.275	0.027	0.06		0.27
Diniconazole	0.459	0.059	0.27		
Dinobuton	0.663		0.95	0.55	
Dinocap			0.95	0.75	
Dinoseb acetate			0.91	0.43	
Dinoterb			0.73	0.22	
Dioxacarb	0.454	0.084	0.17		0.66
Diphenamid	0.515		0.26		
Disulfoton			0.98	0.78	
Ditalimfos			0.73		

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
Dithianon	0.650		0.49		
Diuron	0.368		0.11		0.42
Dodine			0.00		
Endosulfan alpha	0.668	0.604			
Epoxiconazole	0.382	0.043			
EPTC	0.653	0.542	0.90	0.34	
Esfenvalerate	0.680	0.573			
Ethalfluralin	0.682	0.640			
Ethephon	0.000	0.000	0.00		
Ethirimol	0.122		0.00		0.02
Ethoprophos			0.49		
Ethoxyquin	0.647		0.88	0.32	
Ethoxyfen	0.678	0.588			
Etrimfos	0.649	0.545	0.92	0.38	
Fenarimol	0.476		0.25		0.76
Fenchlorazole-ethyl	0.656	0.430			
Fenchlorazole-P-ethyl		0.499			
Fenitrothion	0.644	0.460	0.88	0.32	
Fenoxaprop-P-ethyl	0.663	0.499			
Fenpropimorph	0.572	0.488			
Fenthion	0.654		0.91	0.43	
Fenuron	0.357	0.050			
Fenvalerate			0.95		
Flamprop-M-isopropyl			0.70		
Flurochloridone			0.66		
Fluroxypyr	0.650	0.436			
Flusilazole	0.362	0.030	0.09		
Flutriafol	0.278	0.022			
Folpet	0.624		0.70		
Fonofos			0.95	0.73	
Formothion	0.602	0.182			
Fuberidazole	0.460	0.109			
Glyphosate			0.00		
Haloxyfop	0.054				
HCB	0.692				
Heptachlor	0.673		0.99		
Hexachlorobenzene			0.99		

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
Hexaconazole			0.10		
Hexazinone			0.02		
Hymexazol	0.399	0.156			
Imazalil	0.147	0.000			
Imazametabenz-methyl	0.457	0.088			
Imidacloprid	0.226	0.000			
Iprodione	0.641		0.53		
Isoproturon	0.386	0.055			
Isoxaben	0.618	0.243			
Lambda-cyhalothrin	0.666	0.552			
Lenacil	0.642		0.15		
Lindane	0.672	0.556	0.99		
Linuron	0.559	0.233	0.54		
Malathion	0.643	0.433	0.72	0.17	
MCPA	0.035	0.016	0.00		
Mecarbam	0.670				
Mecoprop	0.065	0.025			
Mecoprop-P	0.086	0.029			
Metalaxyl	0.458		0.13		
Methabenzthiazuron	0.406				
Methamidophos			0.00		
Methidathion	0.631	0.391	0.72		
Methiocarb			0.51		
Methomyl	0.363		0.00		0.01
Methoxychlor			0.99		
Metobromuron	0.573		0.45		0.93
Metolachlor	0.610	0.359	0.72		
Metoxuron	0.308		0.06		0.27
Mevinphos	0.417		0.21		0.60
Molinate	0.621	0.451	0.89	0.33	
Monocrotophos	0.076		0.01		0.04
Monolinuron	0.562		0.45		0.93
Myclobutanil	0.285		0.06		
Napropamide	0.524		0.46		0.95
Nitrothal-isopropyl			0.92	0.46	
Nuarimol	0.466		0.25		0.75

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
o,p-DDT			0.99		
Oxadiazon	0.656		0.99		
Oxamyl	0.185		0.01		0.10
Paclobutrazol			0.25		
Paraquat dichloride			0.00		
Parathion	0.670		0.94	0.51	
Parathion-methyl	0.669	0.478	0.87	0.31	
Penconazole			0.12		
Pendimethalin	0.651	0.567	0.95	0.75	
Permethrin			0.99		
Phenkapton			0.99	0.85	
Phenmedipham	0.646		0.56		
Phenthoate			0.91	0.40	
Phorate	0.666	0.584	0.99	0.82	
Phosalone	0.672		0.72	0.15	
Phosmet			0.71		
Phosphamidon	0.226	0.018	0.03		0.20
Pirimicarb	0.449	0.148	0.32		
Pirimiphos-methyl	0.666	0.528	0.91		
Pirimisulfuron methyl	0.513	0.013			
Prochloraz	0.243	0.028			
Procymidone	0.649		0.71		
Prometryn	0.622		0.67		
Propachlor	0.601	0.302	0.57		
Propham	0.655		0.93	0.38	
Propiconazole (1)	0.314	0.061	0.08		
Propisochlor	0.649	0.489			
Prosulfuron	0.301	0.011			
Prothoate			0.36		0.87
Pyrazophos			0.72	0.10	
Pyridate	0.679	0.563			
Quinalphos	0.631	0.455	0.87	0.26	
Quinclorac	0.060	0.000			
Quinomethionate			0.92	0.62	
Rimsulfuron	0.106	0.000			
Secbumeton			0.75		
Simazine	0.570	0.249	0.49		

Active ingredient	System I[a]	System VII[b]	System IX[c]	System X[d]	System XI[e]
Tebuconazole	0.334	0.022			
Teflubenzuron	0.641	0.333			
Tefluthrin	0.686	0.624			
Terbacil			0.01		0.07
Terbufos			0.99		
Terbuthylazine	0.631		0.61		
Terbutryn	0.603	0.325	0.61		
Tetraconazole	0.371	0.024	0.12		
Tetradifon	0.657		0.97	0.60	
Tetramethrin			0.51		
Tetrasul	0.673		0.99		
Thiabendazole	0.335	0.022	0.20		
Thiobencarb			0.85		
Thiocyclam (hydrogen oxalate)	0.135	0.085			
Thiometon	0.649				
Thiophanate-methyl	0.570	0.133	0.28		
Thiram	0.565	0.251			
Tifensulfuron-methyl	0.217	0.000			
Tralkoxidim	0.659	0.499			
Triadimefon	0.574	0.218	0.48		
Triasulfuron	0.410	0.000			
Triazophos	0.633	0.230	0.46	0.03	0.95
Tribenuron methyl	0.479	0.052			
Trichlorfon	0.242		0.04		0.22
Tridemorph (1)	0.333	0.299			
Tridemorph (2)	0.585	0.527			
Triflumizole			0.10		
Trifluralin	0.682		0.95	0.95	
Triforine	0.492	0.013			
Vernolate			0.72		
Zeta-cypermethrin (1)	0.681	0.572			

[a] System I: Activated silica-gel – ethyl acetate
[b] System VIII: Activated silica-gel – n-hexane:diethyl ether (1:2)
[c] System IX: Silica gel (not activated) – petroleum ether:diethyl ether (1:2)
[d] System X: Silica gel (not activated) – petroleum ether:diethyl ether (5:1)
[e] System XI: Silica gel (not activated) – diethyl ether

The effect of the eluent temperature on the Rf values was studied at 20°C and 32°C in a silica gel-ethyl acetate system. The results, shown in Table 8, indicate that a normal temperature variation in a laboratory without air-conditioning may result in, on an average, around 5% difference in the Rf values which may be critical in case of closely eluting compounds, though its effect is less than the differences caused by relative humidity of the air. Where the change of temperature is large during the day, the conditioning of the temperature of the developing tanks is advisable.

TABLE 8. EFFECT OF TEMPERATURE ON RF VALUES IN SYSTEM I

Active ingredient	Temperature		$Rf_{(32)}/Rf_{(20)}$ %
	20ºC	32ºC	
Aziprotryn	0.63	0.61	95.9
Chlorpyrifos	0.67	0.63	93.9
Dithianon	0.65	0.61	94.2
Fenarimol	0.48	0.45	93.1
Methabenzthiazuron	0.41	0.39	96.6
Monolinuron	0.56	0.53	94.8
Pirimicarb	0.45	0.42	92.9
Prometryn	0.62	0.59	94.1
Tetradifon	0.66	0.64	97.3
Average %			*94.8*

Figures 1–6 show the coefficient of variation of Rf values of pesticides measured in replicate elutions in one laboratory on different days. These Rf values reflect the repeatability and within-laboratory reproducibility of elution and the expected variation of these values. Comparing the figures it can be seen that the coefficient of variation (CV) of the Rf values rapidly increased from $Rf \leq 0.2$. This is mainly due to the error (the uncertainty is in the order of mm) in the visual observation of the centre of the spots and the deformation of the spots at the start resulting from the large migration velocity of the solvent at the beginning [23].

Figures 1–6 provide the easiest and practical way to evaluate the performance and applicability of the elution systems tested.

System I resulted in a reasonably good spread of Rf values in the 0.05–0.7 Rf range. The majority of compounds eluted between 0.3 and 0.7. In this range the CV_{Rf} values were <0.1, the smallest among the systems.

The compounds in System II eluted with Rf from 0 to 0.75. The Rf values of about half of them were ≤0.2 with CV_{Rf} ranging from 0.15 to 2.22.

The elution patterns in System III and System IV were similar with a good spread of the Rf values of the compounds. The CV_{Rf} values were in the same range as that obtained with System II.

The Rf values were highest in System V and, unfortunately, most of them were concentrated in the 0.7–0.9 range. The CV_{Rf} values were ≤0.15 indicating reasonably good reproducibility.

The Rf values on ready made layers were lower than those on self-prepared layers, but the order of elution did not change (Table 3).

System VI showed the most uniform elution pattern. The CV_{Rf} values were ≤0.15 in the 0.1–0.8 Rf range. The reproducibility of Rf values on ready-made RP-18 layers was very good between laboratories. They were in close agreement with the values published by the Merck Company. [24]. The Rf values on self-coated paraffin oil layers (System VII) showed the greatest variation, and for the tested compounds they had the opposite retention order to that obtained on the RP-18 phase.

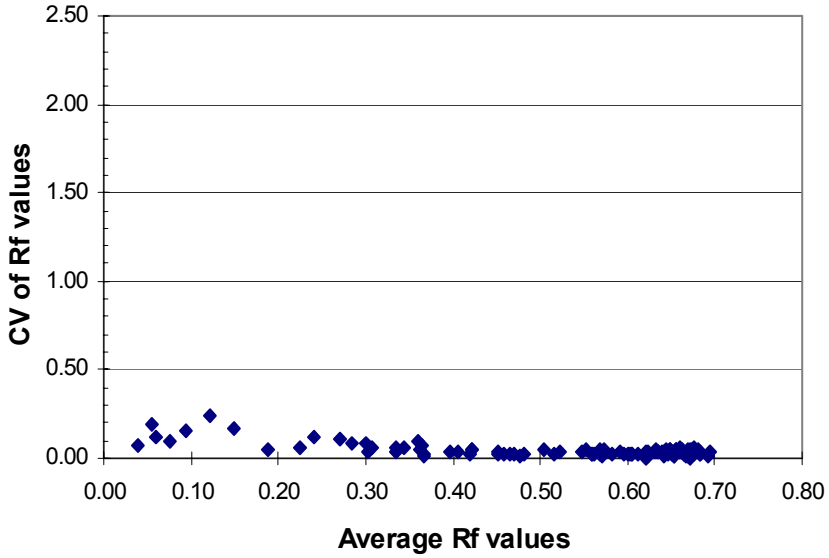

FIG. 1. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System I.

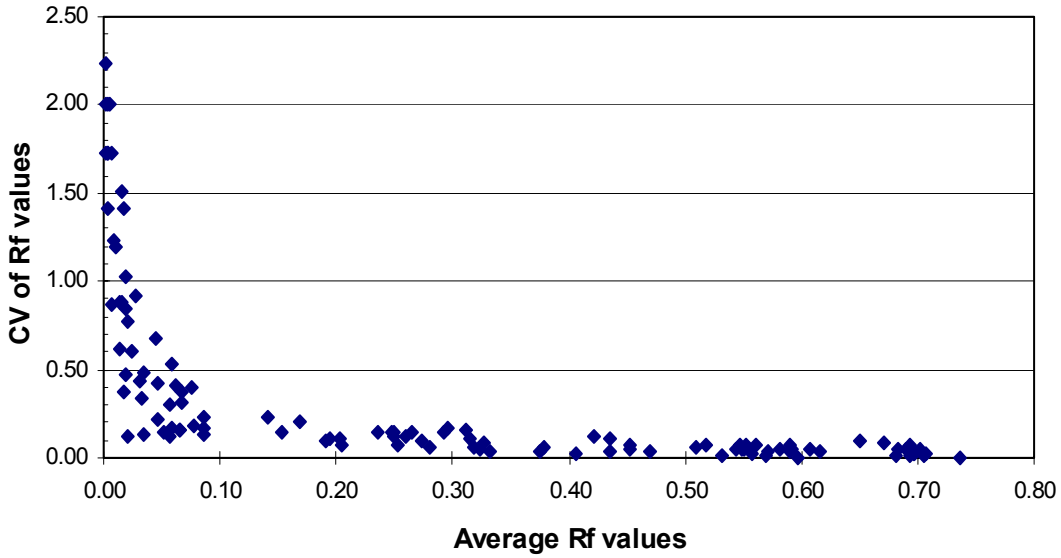

FIG. 2. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System II.

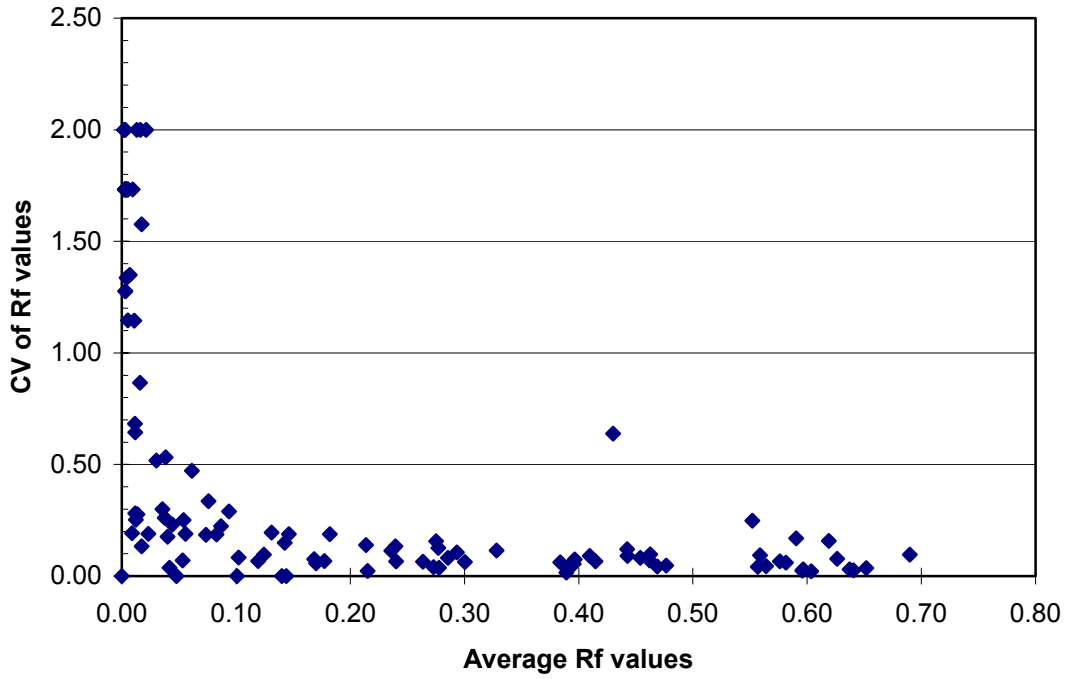

FIG. 3. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System III.

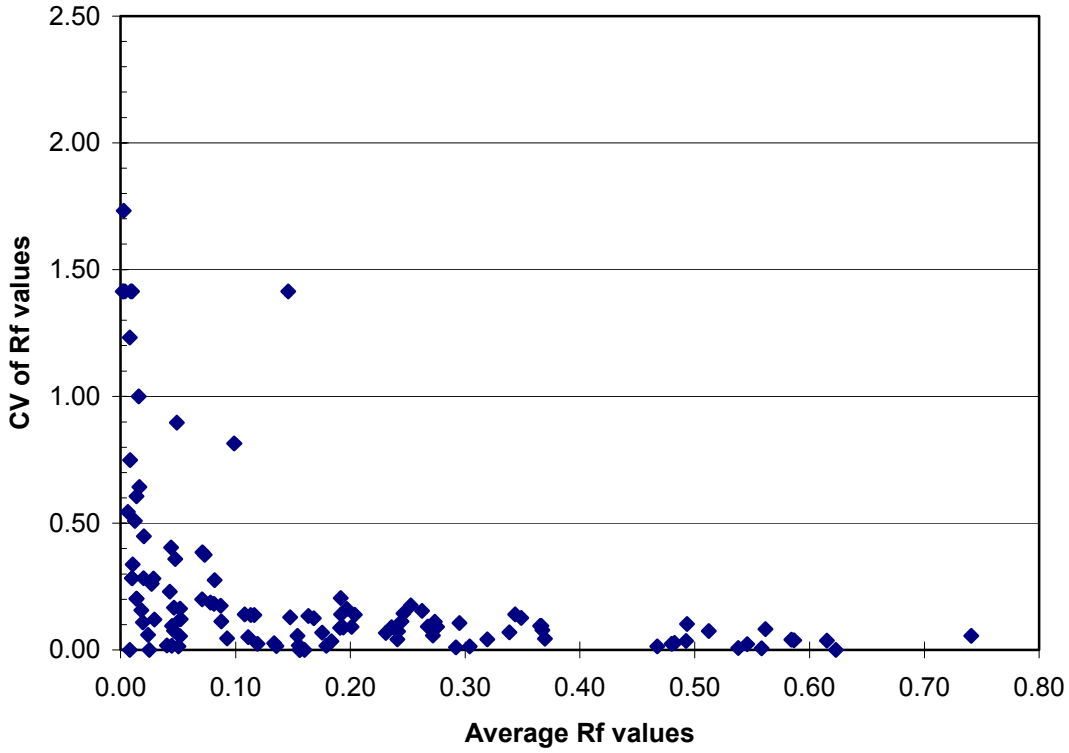

FIG. 4. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System IV.

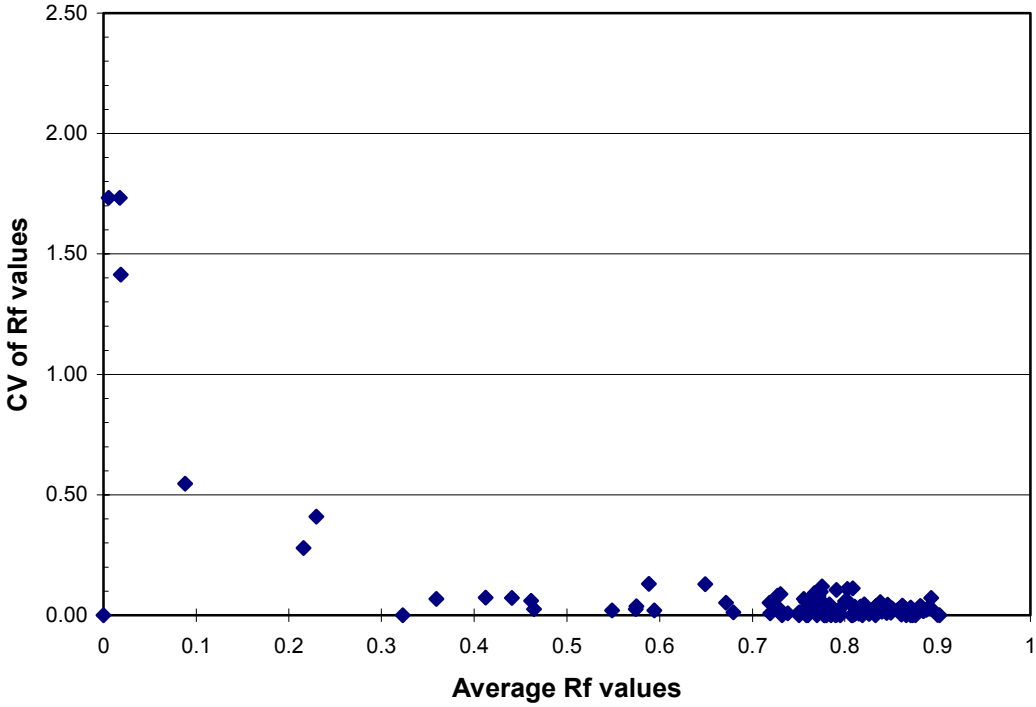

FIG. 5. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System V.

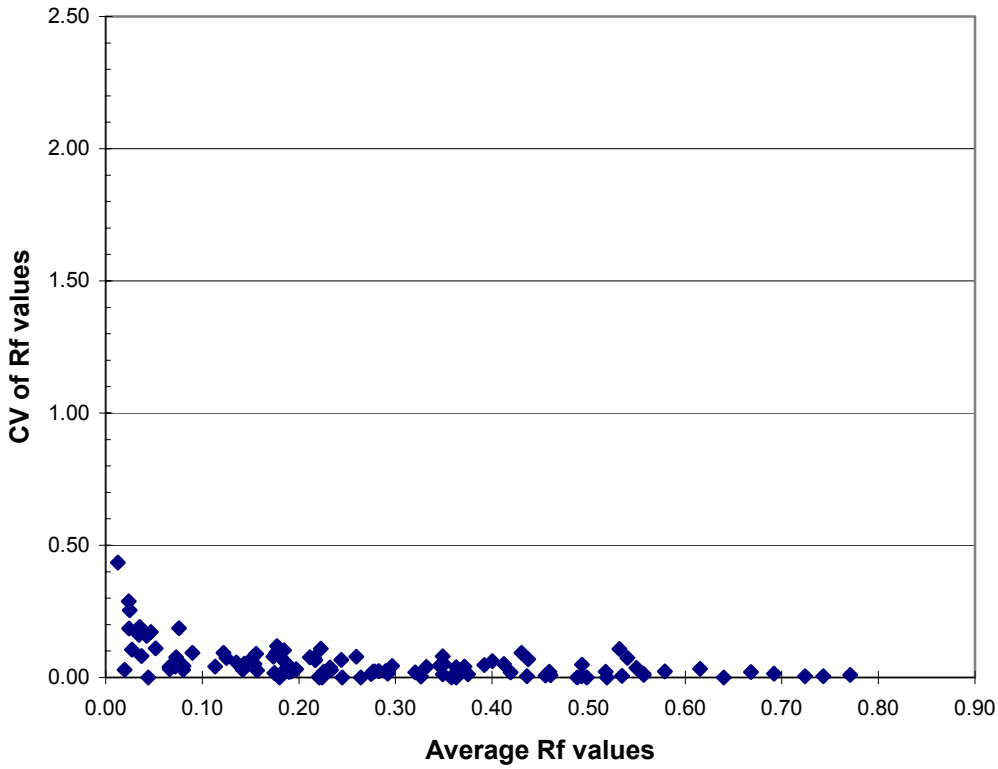

FIG. 6. The coefficient of variation, CV, of Rf values as the function of Rf values in elution System VI.

Comparison of retention data obtained in System I and in the silica gel–petroleum ether:diethyl ether systems [25] (Table 7) indicated similar advantages in general separation and the latter systems provided selective separation of some compounds. Since the multi-component eluents must be replaced after each plate to keep their composition constant, and taking into consideration the instability of the composition of the solvent mixture caused by the low boiling point of diethyl ether at elevated laboratory temperature, the silica gel–petroleum ether:diethyl ether systems are considered less suitable for general use than the silica gel-ethyl acetate.

Summarizing the findings on the elution patterns of pesticides it is concluded that none of the systems is ideal. The elution system should be selected by taking into account the pesticides expected in the samples. Since the Rf values are most reproducible in the Rf range of 0.2–0.8, the compounds to be determined should preferably elute within this range. In view of the large number of pesticides which can be detected with a given detection method, and the low resolution of TLC plates (maximum 8–10 spots can be separated in ideal case), the elution of several pesticides with very similar Rf values is inevitable. In cases of overlapping spots, solvent mixtures can be used for more selective separation.

Where the sensitivity of the detection makes it possible, the appropriate combination of solvent systems for two-dimensional separation would provide a much more selective and efficient separation of the compounds present in the extracts. The increased spread of the spots after the two dimensional elution decreases the minimum detectable quantities (MDQ) of the analytes, and thus limits the applicability of two-dimensional separation for the confirmation of residues.

4.3. Effect of Sample Load on Cleanup Columns, Rf and RRf Values

The different sample equivalents were obtained by extracting 10, 20, 30 60 and 90 g test portions of tomato, cabbage, green peas and orange. The amounts of the ethyl acetate, sodium hydrogen carbonate and sodium sulphate were added in direct proportion to the mass of test portions.

The gel and mixed adsorbent columns were loaded with extracts equivalent to 5, 10, 15, 30 and 45 g plant materials. The cleanup of extracts was carried out according to the methods described above. The pesticide fraction was concentrated to 1 ml in each case. Portions of 20–20 μl of cleaned and concentrated extracts were spotted beside each other on TLC plates representing 100, 200, 300, 600 and 900 mg samples, respectively. On the top of every second spot the mixture of analytical standards was spotted to see the effect of the sample load on the detectability and elution characteristics of the system.

The load of the cleanup column was considered acceptable until the minimum detectable quantities of analytes, determined with analytical standards alone, could be seen in the presence of the co-extractives and the Rf value of the analyte was not affected.

The loadability of cleanup columns was determined with all of the detection methods described before. The chromatograms were visually evaluated immediately after the occurrence of the spots. Some of the plates were scanned with a colour scanner and printed with an inkjet colour printer. Though some of the weak spots seen visually did not occur on the scanned pictures, especially at around the MDQ, it was found that the scanning generally reproduced well the colouration of the plates. During the recent years digital photos were made from the plates instead of scanning. Digital photos truly recorded the plates and could be used for electronic archiving the results of TLC elution and detection.

The gel column containing about 7 g SX-3 gel could be loaded with an ethyl acetate extract containing a maximum of 30 g sample equivalent. The sample load of 600 mg plant extract did not affect the relative Rf values (RRf) values as shown in Table 9.

The extracts with higher sample equivalents could not be properly cleaned by either of the columns.

The within-laboratory variation of Rf values of various compounds in the presence of plant co-extractives was within the expected range. The Rf values within one run were only slightly affected by the load of various sample extracts. The within-plate variation caused by sample extracts was smaller than the between plate variation observed when analytical standards were applied alone. The RRf measured on loaded plates were generally in good agreement between the laboratories, as illustrated in Table 9.

The extracts obtained with the on-line extraction method contained more plant materials and only 10–15 g sample equivalent could be cleaned on the gel column.

The efficiency of mixed adsorbent was not as good as that of the gel column especially for orange extracts. Additional cleanup on the Silica gel cartridge was necessary for all detection methods with chemical reagents.

The Lichrolute Si 60 Cartridges alone could only be loaded with extracts equivalent to 5 g samples. Consequently they were used for an additional cleanup of extracts after the GPC or mixed-adsorbent column.

The applicability of Silica gel cartridges for cleaning the concentrated eluate from the gel column and mixed adsorbent was also tested. The o-TKI and NBFB methods were the most sensitive for co-extractives. The "B" elution method [25 ml toluene:cyclohexane:acetone (60+30+10 v/v)] gave somewhat less clean eluent. Two spots were observed at Rf 0.45 and 0.77 from green peas and one spot (Rf = 0.45) from cabbage. The spots, however, did not influence the detectability of the compounds. There was no spot from the tomato and orange extracts.

The cleanup requirements of sample extracts are summarised in Table 10. The cleaned up extract is also suitable for capillary column - ECD or other GC analyses, and - after filtration through a 0.5 μm filter - for HPLC analysis.

The recovery data of a large number of pesticides on SX-3 gel column and silica gel cartridges were published by several authors [6, 11, 14]. The data indicate that the elution order remains the same but the amount of eluent required for the elution varies depending on the chromatographic system, which needs to be calibrated at regular intervals.

4.4. Detection of pesticides

The minimum detectable quantity (MDQ) of a pesticide is defined as the minimum amount of analytical standard, expressed in nanograms, spotted on the plate which gives a clearly visible spot after elution under average chromatographic conditions. The typical MDQ values of pesticides that could be obtained, when the analytical standards were applied together with the purified sample extracts, with the different detection methods are summarised elsewhere [26]. It should be noted that the spots of some pesticides rapidly disappear or become faint after reaching optimal intensity (e.g. oxamyl detected with enzyme inhibition methods, all spots detected with Hill reaction), therefore the spots should be marked and evaluated immediately after colour development. Visual observations of interferences etc. should also be recorded.

TABLE 9. THE EFFECT OF SAMPLE LOAD ON THE Rf VALUES OF SOME SELECTED PESTICIDES IN ETHYLACETATE SILICA GEL SYSTEM

Active ingredient	Tomato	Orange	Green beans	Cabbage	Mean Rf	RRf[b]	RRf[c]	RRf[d]
o-TKI								
Atrazine	0.73	0.73	0.75	0.75	0.74	1	1	1
Dioxacarb	0.53	0.52	0.55	0.55	0.54	0.73	0.75	0.8
Diuron	0.45	0.45	0.45	0.47	0.46	0.62	0.60	0.72
NBFB								
Dioxacarb	0.52	0.54	0.54	0.54	0.54	0.76	0.75	0.77
Carbaryl	0.74	0.7	0.71	0.7	0.71	1	1	1
pDB								
Chlortoluron	0.5	0.51	0.51	0.51	0.51	0.76	0.71	-
Linuron	0.66	0.67	0.67	0.67	0.67	1	1	
AgUV								
Dieldrin	0.76	0.76	0.75	0.76	0.76	1	1	
Imazalil	0.18	0.18	0.18	0.18	0.18	0.24	0.15	-
Hill								
Atrazine	0.72	0.73	0.73	0.72	0.73	1	1	1
Diuron	0.44	0.45	0.45	0.45	0.45	0.62	0.6	0.72
Chlortoluron	0.5	0.51	0.52	0.51	0.51	0.7	0.65	-
FAN								
Benomyl	0.38	0.35	0.32	0.36	0.35	0.51	0.49	
Captan	0.73	0.68	0.65	0.79	0.69	1	1	1
Thiophanate-methyl	0.68	0.6	0.58	0.6	0.62	0.9	0.9	0.97
EβNA								
Parathion-methyl	0.68	0.68	0.7	0.69	0.69	1	1	1
Mevinphos	0.44	0.44	0.46	0.45	0.45	0.65	0.63	0.87
Oxamyl	0.2	0.2	0.2	0.2	0.2	0.29	0.28	
EAcI								
Parathion-methyl	0.72	0.7	0.7	0.7	0.7	1	1	1
Mevinphos	0.45	0.45	0.45	0.45	0.45	0.63	0.63	0.87
Oxamyl	0.2	0.2	0.2	0.2	0.2	0.28	0.28	

[a] The plates were loaded with 600 mg sample equivalents in extracts purified on gel column.
[b] Relative Rf values measured on loaded plates in the laboratory of JNSz County.
[c] Relative Rf values measured by spotting analytical standards on non-loaded plates in the laboratory of Zala County.
[d] Relative Rf values on non-loaded plates measured during 1975-78 in the laboratories of Plant Protection Service in Hungary [27]. Plates from those batches were not available during this study and the sources of differences in the observed values could not be identified.
- Indicates that the Rf value was not reported.

TABLE 10. CLEANUP REQUIREMENTS OF SAMPLE EXTRACTS WITH VARIOUS DETECTION METHODS

Methods	Tomato		Cabbage		Green peas		Orange	
	300 mg	600 mg	300 mg	600 mg	300 mg	600 mg	300 mg	600 mg
oTKI/SX-3,	yes	yes	yes	yes	yes	yes	yes	yes
oTKI SX-3+ Silica gel	no	no	adv.	yes	no	no	adv.	yes
oTKI/mixed a + Si gel	yes	n.a	yes	n.a.	yes	n.a.	yes	n.a.
NBFB/SX-3 + Si gel	yes	yes	yes	yes	yes	yes	yes	yes
NBFB/mixed a + Si gel	limited	n.a.	limited	n.a.	limited	n.a.	limited	n.a.
AgUV/SX-3	yes	yes	yes	yes	yes	yes	yes	yes
AgUV/SX-3 + Si gel	no	no	no	no	yes	yes	yes	yes
AgUV/mixed a +Si gel	yes	yes	yes	n.a.	yes	n.a.	yes	n.a.
Hill/SX-3	no	yes	no	yes	yes	yes	no	yes
Hill/SX-3 + Si gel	no	no	no	no	no	no	no	no
Hill/mixed a	no	yes	no	yes	yes	yes	no	yes
FAN/SX-3	no	yes	no	yes	no	yes	yes	yes
FAN/SX-3 + Si gel	no	no	no	no	no	no	no	yes
FAN/mixed a	no	yes	no	yes	no	yes	yes	yes
FAN/mixed a + Si gel	no	no	no	no	no	yes	no	yes
E NA/SX-3	yes	yes	yes	yes	yes	yes	yes	yes
E NA/SX-3 + Si gel	no	no	no	no	no	no	no	no
E NA/mixed a	no	yes	no	yes	no	yes	yes	yes
EAcI/SX-3	yes	yes	yes	yes	yes	yes	Yes	yes
EAcI/SX-3 + Si gel	no	no	no	no	no	no	No	no
EAcI/mixed a	no	yes	no	yes	no	yes	Yes	yes

The sample equivalent of fruits and vegetables of high water and chlorophyll content transferred with the concentrated extract into the columns is given under the samples.
yes: further cleanup is necessary
no: further cleanup is not required for TLC or GC ECD detection
adv: further cleanup is recommended
n.a. the cleanup procedure is not suitable for cleaning the extract
Limited: certain sections of the plate contain disturbing spots
Mixed a.: mixed adsorbent

The *fungi spore inhibition* [FAN] method provides the best specificity. It detects only fungicides at the usual concentration level of pesticide residues (0.01–5 mg/kg). It is also sensitive, and therefore one of the most suitable TLC detection method for residue analysis.

The *Hill reaction* is less specific. It detects ureas and triazines at limit of quantitation, LOQ, of about 0.002–0.01 mg/kg, but it would also detect some other types of pesticides (not herbicide), for instance, thiabendazole (0.2 mg/kg), azinphos-methyl, triazophos and propham (≥1.2 mg/kg).

Enzyme inhibition [EAcI, EβNA] usually sensitively detects phosphate and carbamate type insecticides (LOQ ≥ 0.002 – 0.01 mg/kg). The enzyme inhibition mechanism is different in the case of liver extract and blood serum. Consequently the sensitivity of the detection can be different for some pesticides. For instance, 10-100-fold higher sensitivities were obtained with EβNA than with EAcI in the case of carbofuran, chlorfenvinphos, diazinon, dichlorvos, ethion, monocrotophos, and trifluralin, while the opposite trend (5-10-fold) was observed for 2,4-D, cypermethrin, dithianon, and pentachlorphenol. The latter compounds also indicate that the enzyme inhibition can be caused by non-insecticide pesticides as well. The liver and blood enzymes complement each other, and both should be available in a screening laboratory. The selection of the enzyme source depends on the compounds to be analysed.

The *Ortho-tolidine + KI* [o-TKI] *and silver nitrate* [AgUV] are general screening detection methods. They have a medium sensitivity for several compounds. It should be noted, however, that neither of the methods is suitable to detect organochlorine pesticide residues at the current Codex Extraneous Maximum Residue Limits (EMRL).

Nitrobenzene fluoroborate [NBFB] is a medium sensitive reagent for detecting carbamate type pesticide residues. It can be useful for confirmation of the identity of residues.

Detection with *p-dimethylamino benzaldehyde* has limited use, mainly in confirmation of the identity of some residues being present at relatively high concentrations.

4.5. Application of Marker Compounds for Internal Quality Control

In view of the variability of Rf values and the detection procedures, regular monitoring and control of the TLC conditions is essential for obtaining reliable analytical results. The proper elution and detection conditions can be checked on each plate by applying a mixture of analytical standards at their MDQs. If the marker compounds are well detectable and their Rf values are within the expected range, the analyst can be sure and can demonstrate it at the same time that the method was applied properly. The Rf values of the marker compounds can also be used as reference for the RRf values (Table 2) which greatly facilitates the identification of the spots detected on the plates.

The marker compounds selected should [27]:

(i) be relatively stable in standard solutions;
(ii) be sensitive for the detection conditions (not appearing on the plate if the conditions are not optimal); and
(iii) have reproducible Rf values.

The pesticides recommended for quality control of TLC procedures as marker compounds and their Rf and MDQ values are given in Table 11.

TABLE 11. MARKER COMPOUNDS RECOMMENDED FOR INTERNAL QUALITY CONTROL OF TLC DETECTION[a]

Ethyl acetate			Dichloromethane			Benzene			n-hexane:diethyl ether /1:2		
Analyte	ng	Rf	Analyte	ng	Rf	Analyte	ng	Rf	Analyte	ng	Rf
o-TKI											
Atrazine[b]	25	0.61	Dichloran	100	0.56	Endosulfan	100	0.56	Carbosulfan	100	0.55
Diuron	30	0.37	Propham	50	0.38	Pirimiphos-me	500	0.26	Atrazine	25	0.32
Oxamyl	50	0.19	Carbaryl	100	0.19	Carbosulfan	300	0.13	Bensultap	100	0.15
NBFB											
Carbaryl	50	0.61	Phenylphe-nol	50	0.44	***Carbosulfan***	250	0.13	Carbosulfan	50	0.55
Dioxacarb	100	0.45	Carbaryl	50	0.19				Carbaryl	250	0.24
Methomyl	300	0.36									

Ethyl acetate			Dichloromethane			Benzene			n-hexane:diethyl ether /1:2		
Analyte	ng	Rf	Analyte	ng	Rf	Analyte	ng	Rf	Analyte	ng	Rf
pDB											
Benefin	250	0.68	Chlorbromu-ron	50	0.26						
Diuron	50	0.37	Diuron	100	0.05						
Metoxuron	50	0.30	Metoxuron	200	0.02						
AgUV											
Dieldrin	25	0.68	Dieldrin	25	0.57						
Triforine	50	0.48	Linuron	100	0.25						
			Chlorfenvin-phos	200	0.09						
Hill											
Atrazine	1	0.61	Dithianon	100	0.44	Dithianon	100	0.33	Atrazine	1	0.32
Chlortoluron	1	0.40	Linuron	1	0.25	Chlorothalo-nil	250	0.48	Linuron	1	0.23
Metoxuron	5	0.30	Desmedi-pham	1	0.09					1	
FAN											
Captan	20	0.64	Dichloflua-nid	20	0.51	Chlorothalo-nil	20	0.48	Chlorothalo-nil	1000	0.57
Fenarimol	50	0.48	Captan	20	0.33	Folpet	50	0.26	Captan	20	0.28
Prochloraz	10	0.24	Thiram	50	0.24	Captan	20	0.10	Bensultap	50	0.15
EβNA											
Parathion-me	2	0.67	Parathion-me	2	0.56	Pendimetha-lin	2	0.54	Chlorpyrifos-me	1	0.59
Dichlorvos	20	0.50	Etrimfos	5	0.30	Parathion-me	5	0.38	Parathion-me	1	0.48
Oxamyl	10	0.19	Triazophos	1	0.19	Etrimfos	25	0.13	Triazophos	2	0.23
EAcI											
Parathion-me	2	0.67	Parathion-methyl	2	0.56	Chlorpyrifos-me	0.5	0.54	Chlorpyrifos-me	0.2	0.59
Dichlorvos	2	0.50	Etrimfos	10	0.30	Parathion-me	50	0.38	Parathion-me	2	0.48
Methomyl	10	0.19	Triazophos	0.2	0.19	Phosphami-don	2	0.02	Triazophos	0.5	0.23

[a] The layer was silica gel, except for AgUV where aluminium oxide layer was used.
[b] Compounds underlined are used as reference for calculation of RRf values.

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APPLICATION OF TLC FOR CONFIRMATION AND SCREENING OF PESTICIDE RESIDUES IN FRUITS, VEGETABLES AND CEREAL GRAINS: REPEATABILITY AND REPRODUCIBILITY OF Rf AND MDQ VALUES

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Abstract

This paper illustrates the effect of major factors influencing the reproducibility of TLC separation and detection under largely differing environmental and laboratory conditions. The optimum conditions for reproducibility and detection sensitivity was obtained on 20 × 20 cm layer in the retention factor (Rf) range of 0.2–0.7 by applying the sample in spots of 3–4 mm diameter at 2 cm from the edge of the plate. The reproducibility rapidly decreased below Rf = 0.2. Above Rf = 0.2 the within-laboratory reproducibility of 219 pesticides obtained in ethyl acetate silica gel elution system by several laboratories was typically below 10%, while the among-laboratory reproducibility of the average retention factors were generally below 12%. The minimum detectable quantities (MDQ) were determined with nine detection methods. The MDQ values largely varied depending on the mode of detection. Bioassay methods enabled the detection down to 0.1–10 ng, while 20–100 ng could be achieved with the chemical reagents. Higher MDQ values are also reported in this paper to assist the identification of compounds potentially present. The between laboratories reproducibility of MDQ values was typically 1-5 × MDQ_{min}.

1. INTRODUCTION

The objective of this work was to obtain information on the within and among laboratories reproducibility of the TLC separation and detection with the participation of several laboratories [1–18] within the frame of two coordinated research programmes. This report discusses the major factors influencing the reproducibility of TLC separation and detection, presents the retention factors (Rf) and the minimum detectable quantities of 219 pesticide residues measured in some of the participating laboratories, and evaluates their repeatability and reproducibility under largely differing environmental and laboratory conditions.

The thin-layer chromatographic detection methods described in detail in the first paper [19] were used in combination with ethyl acetate extraction, gel permeation chromatographic cleanup and elution on silica gel or alumina layers for the analysis of pesticide residues in cereal grain, fruit and vegetable samples:

1. UV lamp at 254 nm HF (254) — Silica gel 60 HF_{254}
2. o-Tolidine and potassium iodide (o- TKI) — Silica gel 60
3. p-nitrobenezene-fluoroborate (NBFB) — Silica gel 60
4. p-dimethylamino benzaldehyde (p-DB) - Aluminium oxide G
5. Silver nitrate & UV exposition (AgUV) - Aluminium oxide G

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6. Photosynthesis inhibition (Hill) - Silica gel 60
7. Fungi-spore inhibition (*Aspergillus niger*) (FAN) - Silica gel 60
8. Enzyme inhibition with cow liver extract and β-naphthyl acetate substrate (EβNA) - Silica gel 60
9. Enzyme inhibition with pig or horse blood serum and acetylthiocholine iodide substrate (EAcI) - Silica gel 60

2. FACTORS INFLUENCING THE REPRODUCIBILITY OF TLC SEPARATION

The quantitative and sensitive TLC determination of certain pesticide residues in various samples may be achieved under optimum chromatographic conditions. In order to assist the selection of the most suitable conditions and keep the variability of the results at a minimum, the major factors affecting the TLC separation and detection are summarized below and their effects are illustrated with some practical examples.

2.1. Main Parameters Influencing the Values of Theoretical Plate Number (N) or Height Equivalent of Theoretical Plate (H)

The theoretical plate number, N, is affected by [20] the migration velocity of solvent particle size of adsorbent, development length, migration distances of the substances, and linear capacity (loadability) of the layer.

The optimal migration velocity may be achieved by selection of proper elution solvent, or solvent mixture. The pre-coated plates have generally a particle diameter of ~10–15 μm, while the self-made layers are generally prepared from adsorbents of ~30 μm particle size. Longer development lengths are required for optimal separation on layers of larger particle size.

The migration distance and the Rf value of a compound depend on the type and quality of the adsorbent, activity of the layer which is strongly influenced by the humidity of the air and the time during which the activated layer is exposed to the air, mode of saturation of the developing tank, temperature of elution, elution solvent strength, and the analyte.

Different adsorbents of the same type have slightly different elution characteristics. Even the thickness of adsorbent layer influences the migration distance. These effects are illustrated on Figures 1 and 2, where HRf is $100R_f$.

The nearly “ideal” condition for general TLC separation on silica gel is at 11–12% (w/w) water content of the layer [21]. This is achieved when silica gel is at equilibrium with air having a relative humidity of 50% at 20^{o}C.

The activity of the layers, even in the original packing, is changing depending on the storage conditions. The differences in the activity of the layers and the saturation of the vapour phase of the developing tanks can be the major sources of variation of the Rf values. The development of freshly the activated plate results in smaller Rf values of the substances than those on the deactivated one. The Rf values on the less active plate are close to 1.0 for many apolar pesticides, while they are up to 0.65 in case of an active layer.

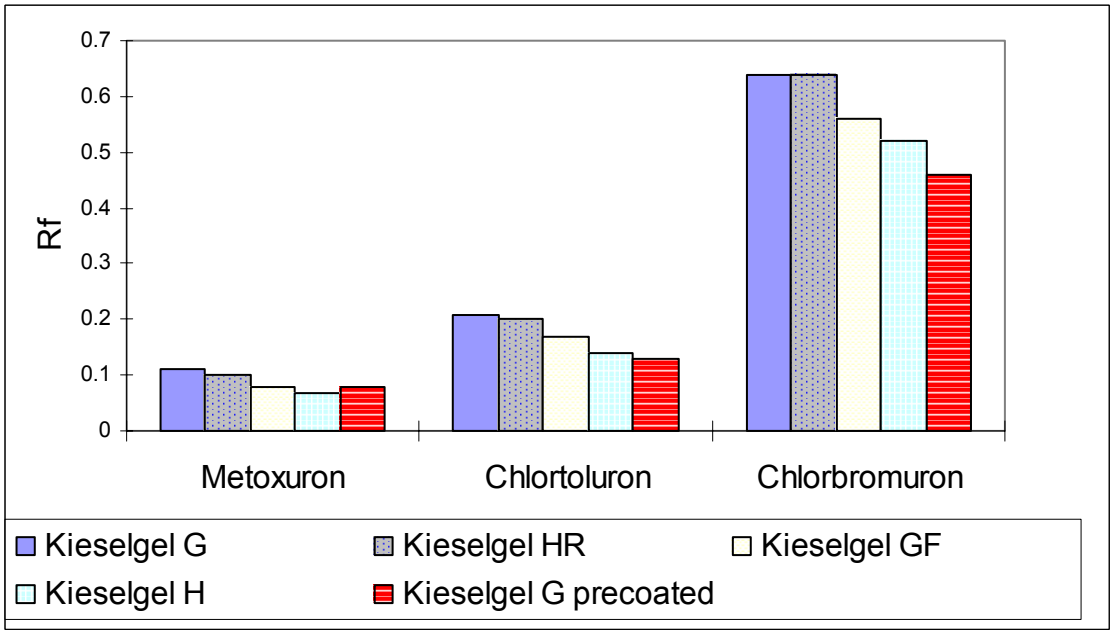

FIG. 1. Effect of the type of silica on Rf with eluent n-hexane+diethylether = 1+2.

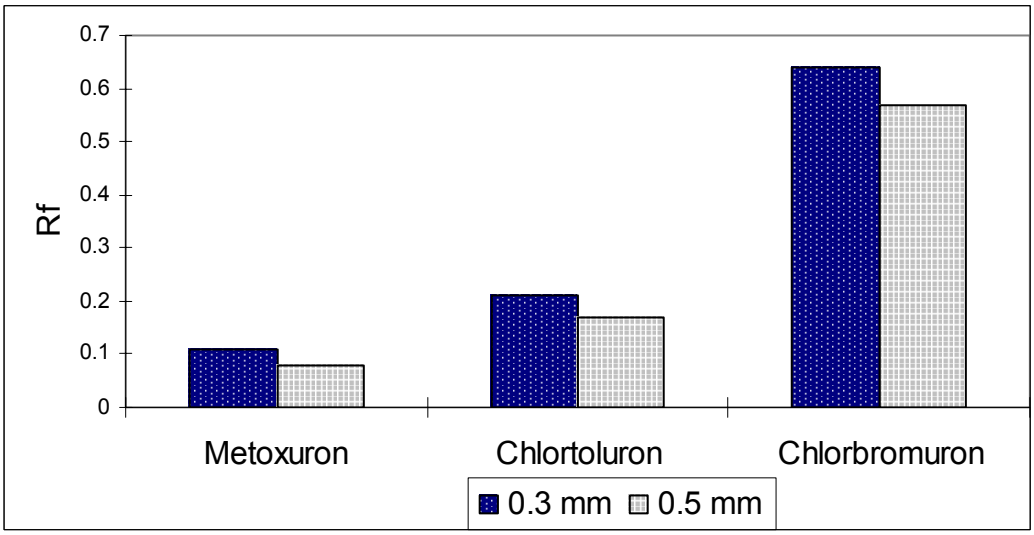

FIG. 2. Effect of thickness of silica-layer on the HRf with eluent n-hexane + diethylether = 1+2.

Though the tests were carried out 15 years apart, very good correlation ($y = 598x + 0.06$, $R^2 = 0.974$) was found, as illustrated on Figure 3, between the Rf values of pesticides measured in two laboratories on freshly activated layers [22] and on those equilibrated to laboratory conditions [23] after elution with the petroleum ether + diethylether = 1 + 2 eluent.

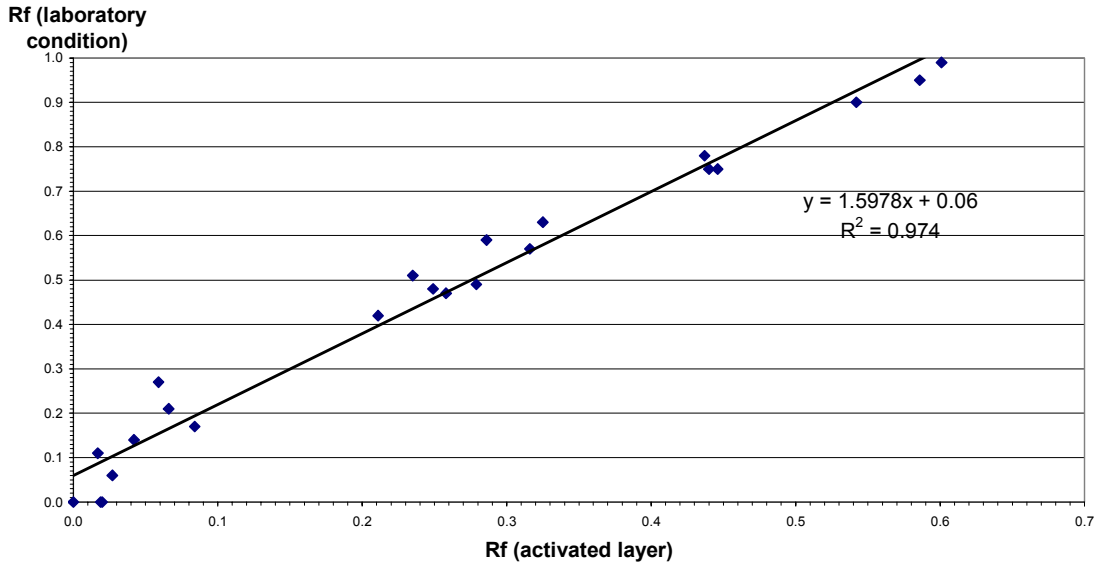

FIG. 3. Comparison of Rf of pesticides on freshly activated layer and on equilibrated one to room conditions. Eluent: petroleum ether + diethylether = 1+2.

It is worth noting that the use of highly active layer results in some loss of separation potential, due to the decreased Rf values even for the apolar compounds. On the other hand, the reproducibility of Rf values is better in the case of freshly activated plates, provided that the plates are not exposed to humid air for an extended period. The effect of relative humidity of air (H%) on the HRf (HRf = 100*Rf) of some pesticides is shown in Figure 4.

In order to obtain the best reproducibility and separation, it is recommended to always use freshly activated plates and equilibrated solvent/vapour phase in the developing tank. When a deactivated layer is required for the separation, the plates should be stored in chambers of defined relative humidity. Saturated solutions of different salts can provide the required relative humidity [24] (Table 1).

The effect of the eluent temperature on the Rf values is less pronounced. A study carried out at 20^{o}C and 32^{o}C in Silica gel – ethyl acetate system [19] indicated, on an average, 5% decrease of the Rf values at the higher temperature.

Snyder [25] established the elution or eluotropic strength of solvents for adsorption chromatography. In this approach the pentane and hexane are the weakest among organic solvents (e_o=0.01), whereas methanol is the strongest one. Benzene or toluene, having medium polarity, has proven to be a good solvent for many separations on silica gel. Elution strength of mixtures may be calculated based on the individual elution strengths and the molar composition of the mixture. The effect of solvent composition on the solvent strength is illustrated on Figure 5 for several solvent mixtures. The figure shows that the stronger solvent has a larger contribution on the elution strength of mixture than the weaker component. By increasing the proportion of the more polar solvent or decreasing the proportion of the less polar solvent the optimal strength of solvent composition may be chosen to get the required Rf-s for the compounds to be separated.

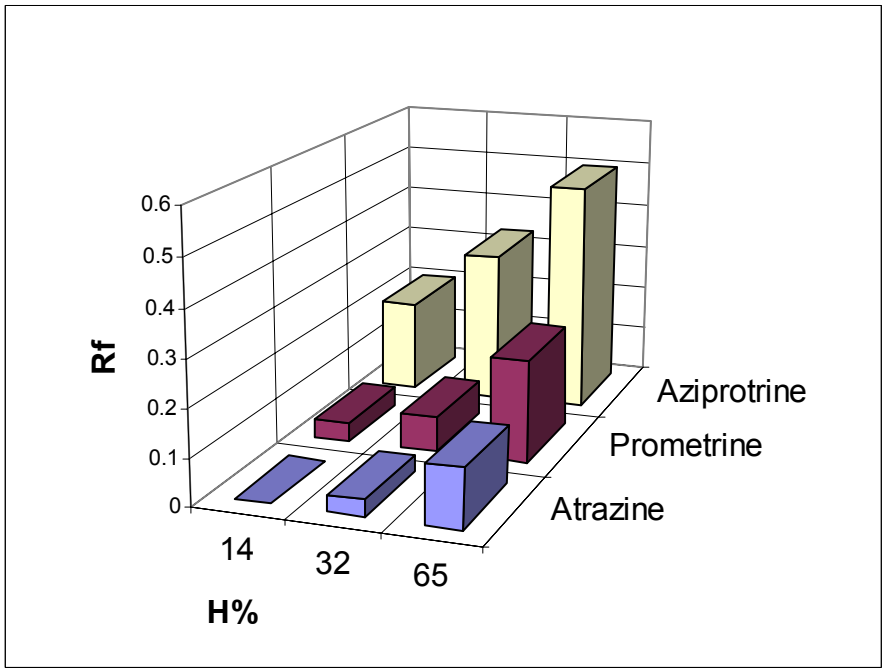

FIG. 4. Effect of relative humidity (H%) on HRf.c b.

TABLE 1. RELATIVE HUMIDITY OVER SATURATED SOLUTIONS OF SALTS AT 20°C

Saturated solution of salt (at 20°C)	Relative humidity over the solution, H%
$Na_2HPO_4.12H_2O$	95
$ZnSO_4.7H_2O$	90
KBr	84
NH_4Cl	79
$NaNO_2$	66
$Mg(NO_3)_2.6H_2O$	56
$Zn(NO_3)_2.6H_2O$	42
$CaCl_2.6H_2O$	31
CH_3COOK	20

A unique feature of TLC is that the separation occurs in a three-phase system, (stationary, mobile and vapour), all of which interact with one another and may or may not be at equilibrium. The mode of saturation has a very important effect on the reproducibility of Rf values. Different developing chambers result in different modes of saturation as well as solvent velocity, which may lead to largely differing average Rf values obtained from 33 replicates as shown in Figure 6.

The resolution (R) of two chromatographic zones is defined [20] as the distance between zone centres (Z_x) divided by the average of the widths (W) of the zones:

$$R = \frac{2\left(R_{f2} - R_{f1}\right)}{W_1 + W_2} \quad \text{equ. 1}$$

The resolution depends on the separation efficiency of the layer (N or H), the Rf values and diameter (W) of the adjacent spots. Resolution is acceptable, if the R value is at least 1.0. The resolution of two adjacent spots, on a layer of 30 μm particle size, has an optimum at about Rf 0.3. Decreasing or increasing the Rf value the resolution will decrease. For practical purposes the Rf range of 0.1–0.6 may be recommended where at least 75% of the optimum resolution can be achieved [20].

The smaller the size of the starting spot (or its width at half height measured with densitometer), the better the separation power. The diameter of the starting spot must be kept at a possible minimum by spotting a very small volume from a concentrated solution (solutions of high viscosity may cause some problems) or by repeated spotting and drying of a diluted solution on the layer. Many devices are developed for accomplishing a proper spotting technique, but the practice of experienced operators is also of great significance.

The linear range of the loadability of the layer may affect both the qualitative and quantitative determination. Beyond its upper limit it can alter the Rf values and make the quantitative determination impossible or false. This is true for both the pure standard compounds and the sample extract containing co-extractives in a large quantity in comparison to the pesticide residues. Since the linear capacity of the layers can be easily exceeded with very concentrated spots and the linear range of detection is also decreased with concentrated spots, the analysts must find a compromise between the optimum separation and detection conditions.

Our experience shows that the best conditions for both the optimum separation and detection (in terms of linearity, resolution and detection sensitivity) can be achieved with 20 × 20 cm layer when the chromatographic conditions are selected to obtain Rf values ideally between 0.3–0.6 (practically between 0.2–0.7), the sample is applied in spots of 3–4 mm diameter at 2 cm from the edge of the plate within its linear capacity, and the plate is immersed in the developing solvent at 1 cm depth and developed until the solvent front reaches 10 to 15 cm.

2.2. Detection of Analytes

When chemical reagents are used to visualize the spots, first the spray should be directed beside the TLC plate for checking that a fine, uniform aerosol mist is produced. Spraying should be continued onto the layer while moving the sprayer carefully back and forth and up and down in a uniform pattern. The reagent is generally sprayed until the layer begins to become translucent. However, the application of the detecting reagent in great excess to the layer should be avoided as it may decrease the minimum detectable quantity (MDQ) of a pesticide or may diminish the visibility of the spot.

The MDQ is defined as the minimum amount of analytical standard, expressed in mass unit, spotted on the plate which gives a clearly visible spot after elution under average chromatographic conditions.

It should be noted that the spots of some pesticides rapidly disappear or become faint after reaching optimum intensity (e.g. oxamyl detected with enzyme inhibition methods, all spots detected with Hill reaction), therefore the spots should be marked and evaluated immediately after colour development.

The linear range of the response of a compound extends from the MDQ value to about 8–10 times MDQ. This range is influenced by the type of the samples too. To eliminate the effects of the co-extractive substances (colouring materials, waxes, oils), cleanup steps are usually indispensable.

3. RESULTS

The Rf values of the selected compounds were generally measured at a minimum of 3–5 replicates by each laboratory. The Rf values, and the coefficient variations (CV) of Rf values of compounds reported by the different laboratories are summarized in Table 2. The reported Rf values were compared to each other and the Dixon outlier test was used to check the suspected values. If the test indicated that the suspected value was an outlier at $\alpha = 0.05$ level, it was considered atypical and was not used for the calculations of typical values. In addition those values were considered atypical where the difference in the consecutive reported average Rf values, organized in rank order, was larger than 0.1. Similarly, the very high CV_r values (e.g. carbaryl 0.355) were not taken into account. These atypical values are listed under the summary line in Table 2.

The average Rf and CV_r, and CV_R values were calculated for 39 compounds for which results from at least four laboratories were available. These average values are given in the summary lines in the table, and they may be considered as typical performance parameters for the TLC elution.

The average within-laboratory reproducibility of Rf values was calculated from the pooled variances (Vp) of the repeated measurements carried out in the individual laboratories:

$$V_p = \frac{\sum \nu_i V_i}{\sum \nu_i} \qquad \text{eq. 2}$$

$$CV_r = \frac{\sqrt{V_p}}{Rf_{mean}} \qquad \text{eq. 3}$$

where ν is the degree of freedom of the calculated variances.

Where the within-laboratory variability, CVr, is much higher than the average, it clearly indicates that the TLC elution conditions were not properly optimized and kept constant during the study. These examples highlight the importance of using optimum conditions and keeping them constant as far as possible.

The reproducibility of the Rf values (CV_R) between-laboratories was calculated from the average Rf_i values reported by the laboratories (n):

$$\hat{Rfi}_{mean} = \frac{\sum Rf_i}{n} \qquad CV_R = \frac{s_{Rfi}}{\hat{Rfi}_{mean}} \qquad \text{eq. 4}$$

As the average Rf values are usually reported, their values (CV_R) were calculated and used to indicate the expectable between-laboratories variation of the measured Rf values.

TABLE 2. RF, CV_1 AND CV_R VALUES OBTAINED IN ETHYLACETATE - SILICA GEL SYSTEM IN 18 LABORATORIES

Active ingredient	No.	Average Rf_i	CV_r	Ref.
2,4-D	4	0.040	0.073	[8]
	4	0.040	0.073	[9]
2-Phenylphenol	3	0.643	0.039	[9]
	4	0.643	0.032	[8]
3-Keto-Carbofuran	3	0.553	0.044	[9]
Acephate	3	0.046	0.330	[6]
	7	0.093	0.151	[9]
Acetochlor	5	0.637	0.032	[8]
Alachlor	4	0.635	0.038	[8]
Aldicarb	8	0.482	0.027	[9]
	3	0.500	0.026	[7]
Aldrin	6	0.665	0.023	[9]
	3	0.670	0.045	[7]
	3	0.700	0.030	[1]
Alphamethrin	3	0.679	0.012	[8]
	3	0.683	0.004	[15]
	3	0.856	0.006	[2]
Ametryn	3	0.610	0.033	[14]
	3	0.610	0.027	[7]
Amidosulfuron	5	0.399	0.044	[8]
Asulam	8	0.471	0.029	[9]
Atrazine	8	0.606	0.019	[9]
	3	0.610	0.011	[14]
	3	0.610	0.003	[7]
	3	0.616	0.037	[2]
	7	0.617	0.009	[8]
	3	0.620	0.040	[1]
	3	0.621	0.020	[5]
	3	0.625	0.014	[6]
	5	0.628	0.017	[13]
	3	0.631	0.006	[15]
	9	0.644	0.018	[4]
	3	0.650	0.024	[12]
	3	0.650	0.015	[3]
	3	0.658	0.017	[17]
	12	0.660	0.031	[18]
	3	0.667	0.054	[11]
	3	0.671	0.013	[16]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Atrazine	**No of labs: 17**	**Rf_{mean}: 0.634**	**CV_r: 0.024**	**CV_R: 0.034**
Atrazine-deisopropyl [c]	3	0.711		[17]
Atrazine-desethyl	3	0.440	0.057	[17]
Azinphos-ethyl	3	0.637	0.009	[3]
	9	0.650	0.032	[4]
Azinphos-methyl	8	0.582	0.020	[9]
	3	0.630	0.040	[1]
Aziprotryn	5	0.634	0.031	[9]
Azoxystrobin	7	0.558	0.029	[8]
BCPE	4	0.653	0.011	[9]
Benefin	5	0.682	0.046	[9]
	5	0.682	0.046	[8]
Benomyl	3	0.651	0.027	[11]
	9	0.657	0.020	[4]
	3	0.660	0.011	[12]
Bensulfuron-methyl	4	0.525	0.062	[8]
Bensultap	4	0.574	0.027	[8]
Bentazone	3	0.190	0.064	[12]
	7	0.293	0.083	[8]
	3	0.304	0.013	[5]
	3	0.340	0.088	[14]
Bentazone	**No of labs: 4**	**Rf_{mean}: 0.312**	**CV_r: 0.074**	**CV_R: 0.078**
Biphenyl	3	0.644	0.045	[9]
Bromophos-ethyl	3	0.660	0.032	[9]
Bromopropylate	3	0.666	0.023	[9]
Bromoxynil	4	0.184	0.157	[8]
	3	0.210	0.000	[12]
Bromuconazole I[a]	5	0.447	0.039	[8]
Bromuconazole II[a]	4	0.236	0.032	[8]
Bupirimate	3	0.540	0.080	[1]
	7	0.560	0.029	[9]
Butachlor	5	0.676	0.041	[9]
Butylate	3	0.675	0.021	[9]
	3	0.675	0.021	[8]
Cadusafos	3	0.677	0.060	[9]
Captafol	5	0.612	0.023	[9]
Captan	3	0.616	0.012	[6]
	15	0.627	0.056	[10]
	3	0.627	0.049	[5]
	3	0.630	0.003	[15]
	7	0.630	0.044	[8]
	7	0.636	0.037	[9]
	3	0.640	0.031	[14]
	3	0.640	0.027	[7]
	9	0.644	0.029	[4]
	5	0.646	0.008	[13]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
	3	0.656	0.018	[18]
	3	0.670	0.031	[12]
	3	0.680	0.030	[1]
	3	0.687	0.017	[3]
	3	0.690	0.072	[17]
	3	0.698	0.012	[16]
Captan	**No of labs: 16**	**Rf_{mean}: 0.651**	**CV_r: 0.038**	**CV_R: 0.040**
Captan [c]	3	0.408	0.191	[11]
Carbaryl	9	0.584	0.017	[4]
	3	0.590	0.014	[7]
	7	0.598	0.031	[8]
	3	0.604	0.020	[6]
	8	0.605	0.026	[9]
	3	0.608	0.081	[18]
	3	0.610	0.011	[12]
	3	0.610	0.066	[14]
	5	0.618	0.018	[13]
	3	0.650	0.000	[3]
	3	0.683	0.060	[11]
	3	0.690	0.080	[1]
Carbaryl	**No of labs: 12**	**Rf_{mean}: 0.621**	**CV_r: 0.039**	**CV_R: 0.056**
Carbaryl [c]	3	0.735		[16]
Carbaryl [c]	3	0.648	0.355	[17]
Carbendazim	15	0.304	0.071	[9]
	6	0.310	0.060	[1]
	7	0.311	0.057	[8]
	10	0.319	0.022	[13]
	9	0.322	0.071	[4]
	6	0.330	0.160	[17]
	3	0.350	0.055	[6]
	3	0.370	0.054	[14]
	3	0.370	0.158	[7]
Carbendazim	**No of labs: 9**	**Rf_{mean}: 0.332**	**CV_r: 0.081**	**CV_R: 0.077**
Carbendazim [c]	3	0.467	0.025	[3]
Carbofuran	3	0.560	0.020	[17]
	3	0.570	0.011	[7]
	3	0.590	0.034	[14]
	7	0.591	0.039	[8]
	8	0.591	0.041	[9]
	5	0.612	0.014	[13]
	3	0.641	0.047	[11]
	3	0.650	0.000	[3]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Carbofuran	**No of labs: 8**	**Rf_{mean}: 0.601**	**CV_r: 0.033**	**CV_R: 0.053**
Carbosulfan	7	0.676	0.018	[8]
Carboxin	7	0.589	0.032	[8]
Chlorbromuron	5	0.568	0.047	[9]
	7	0.575	0.042	[8]
	3	0.575	0.005	[15]
	3	0.655	0.009	[16]
Chlordimeform	3	0.285	0.079	[9]
Chlorfenvinphos	3	0.540	0.002	[15]
Chlorfenvinphos	3	0.547	0.036	[9]
	3	0.547	0.036	[8]
Chloropropylate	3	0.671	0.051	[9]
Chlorothalonil	5	0.668	0.033	[9]
	7	0.671	0.030	[8]
	5	0.674	0.013	[13]
	3	0.680	0.065	[17]
	3	0.700	0.022	[12]
	3	0.703	0.015	[11]
	9	0.713	0.035	[4]
	3	0.730	0.014	[3]
Chlorothalonil	**No of labs: 8**	**Rf_{mean}: 0.692**	**CV_r: 0.032**	**CV_R: 0.033**
Chlortoluron	7	0.391	0.021	[8]
	8	0.396	0.036	[9]
	3	0.398	0.008	[15]
	3	0.412	0.075	[17]
	3	0.419	0.050	[5]
	3	0.424	0.017	[6]
	3	0.448	0.031	[18]
Chlortoluron	**No of labs: 7**	**Rf_{mean}: 0.413**	**CV_r: 0.036**	**CV_R: 0.048**
Chlortoluron [c]	3	0.553	0.019	[16]
Chloroxuron	8	0.343	0.056	[9]
Chloroxuron	3	0.350	0.029	[14]
Chloroxuron	9	0.450	0.033	[3]
Chlorpropham	5	0.652	0.024	[9]
Chlorpyrifos	3	0.633	0.008	[2]
Chlorpyrifos	7	0.669	0.021	[8]
Chlorpyrifos	8	0.669	0.019	[9]
Chlorpyrifos	3	0.670	0.134	[14]
Chlorpyrifos	3	0.670	0.019	[7]
Chlorpyrifos	5	0.672	0.012	[13]
Chlorpyrifos	3	0.713	0.026	[11]
Chlorpyrifos	3	0.720	0.020	[1]
Chlorpyrifos	3	0.730	0.014	[3]
Chlorpyrifos	3	0.739	0.011	[18]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Chlorpyrifos	**No of labs: 10**	**Rf_{mean}: 0.689**	**CV_r: 0.037**	**CV_R: 0.050**
Chlorpyrifos [c]	3	0.760		[16]
Chlorpyrifos-methyl	5	0.657	0.029	[8]
Cyanazine	5	0.602	0.021	[8]
Cyanazine	4	0.604	0.023	[9]
Cyanazine	3	0.659	0.011	[18]
Cyanazine [c]	3	0.490		[17]
Cyfluthrin beta	3	0.669	0.011	[8]
Cyhalothrin	3	0.690	0.041	[7]
Cyhalothrin lambda	3	0.666	0.018	[8]
Cyhalothrin lambda	6	0.670	0.084	[10]
Cypermethrin	4	0.673	0.032	[8]
Cypermethrin	4	0.673	0.032	[9]
Cypermethrin	3	0.807	0.009	[6]
Cypermethrin beta	4	0.677	0.022	[8]
Cypermethrin zeta	4	0.681	0.022	[8]
Cyproconazole (1) [a]	6	0.285	0.117	[8]
Cyproconazole (2) [a]	6	0.358	0.080	[8]
DDT p,p	9	0.666	0.056	[10]
DDT p,p	3	0.672	0.007	[15]
DDT p,p	3	0.680	0.015	[14]
DDT p,p	4	0.684	0.036	[9]
DDT p,p	3	0.815	0.012	[2]
DDT p,p	3	0.851	0.004	[6]
DDT p,p	**No of labs: 6**	**Rf_{mean}: 0.728**	**CV_r: 0.037**	**CV_R: 0.113**
Deltamethrin	4	0.671	0.032	[8]
Deltamethrin	4	0.671	0.032	[9]
Desmedipham	7	0.661	0.031	[8]
Desmedipham	7	0.661	0.031	[9]
Desmedipham	3	0.690	0.010	[12]
Desmedipham [c]	3	0.540		[17]
Desmetryn	3	0.570	0.044	[17]
Desmetryn	9	0.579	0.010	[4]
Diazinon	9	0.633	0.008	[4]
Diazinon	3	0.645	0.028	[6]
Diazinon	6	0.648	0.106	[3]
Diazinon	6	0.660	0.061	[9]
Diazinon	6	0.660	0.061	[8]
Diazinon	3	0.660	0.030	[14]
Diazinon	5	0.664	0.008	[13]
Diazinon	3	0.670	0.014	[7]
Diazinon	3	0.680	0.020	[1]
Diazinon	3	0.680	0.040	[11]
Diazinon	**No of labs: 10**	**Rf_{mean}: 0.660**	**CV_r: 0.052**	**CV_R: 0.023**
Dicamba	5	0.034	0.170	[8]
Dichlofluanid	7	0.632	0.031	[8]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Dichlofluanid	5	0.645	0.033	[9]
Dichlofluanid	3	0.655	0.047	[5]
Dichloran	3	0.621	0.006	[9]
Dichloran	4	0.627	0.017	[8]
Dichlorprop	5	0.043	0.230	[8]
Dichlorprop-P	5	0.050	0.253	[8]
Dichlorvos	7	0.458	0.044	[8]
Dichlorvos	3	0.485	0.031	[6]
Dichlorvos	3	0.499	0.038	[18]
Dichlorvos	3	0.499	0.002	[15]
Dichlorvos	3	0.500	0.016	[14]
Dichlorvos	8	0.505	0.044	[9]
Dichlorvos	8	0.561	0.019	[5]
Dichlorvos	3	0.574	0.085	[11]
Dichlorvos	3	0.610	0.040	[1]
Dichlorvos	**No of labs: 9**	**Rf_{mean}: 0.521**	**CV_r: 0.040**	**CV_R: 0.094**
Dichlorvos [c]	3	0.505		[16]
Diclofop-methyl	3	0.661	0.028	[8]
Dieldrin	3	0.675	0.024	[8]
Dieldrin	3	0.675	0.024	[9]
Dieldrin	3	0.680	0.013	[14]
Dieldrin	3	0.700	0.069	[7]
Dieldrin	5	0.702	0.012	[13]
Dieldrin	3	0.710	0.014	[15]
Dieldrin	3	0.720	0.040	[12]
Dieldrin	3	0.767	0.015	[3]
Dieldrin	3	0.800	0.003	[5]
Dieldrin	3	0.822	0.021	[6]
Dieldrin	3	0.832	0.005	[2]
Dieldrin	10	0.835	0.007	[10]
Dieldrin	3	0.874	0.017	[18]
Dieldrin	**No of labs: 13**	**Rf_{mean}: 0.753**	**CV_r: 0.021**	**CV_R: 0.094**
Difenoconazole	3	0.292	0.092	[8]
Difenzoquat	3	0.000		[8]
Diflubenzuron	4	0.619	0.031	[8]
Diflufenican	7	0.630	0.030	[8]
Dimethametryn	3	0.620	0.038	[7]
Dimethenamid	4	0.638	0.059	[8]
Dimethipin	4	0.429	0.010	[8]
Dimethoate	7	0.272	0.114	[9]
Dimethoate	7	0.275	0.100	[8]
Dimethoate	3	0.276	0.065	[18]
Dimethoate	3	0.289	0.041	[6]
Dimethoate	3	0.290	0.030	[1]
Dimethoate	3	0.290	0.030	[12]
Dimethoate	3	0.290	0.021	[14]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Dimethoate	5	0.314	0.017	[13]
Dimethoate	3	0.340	0.021	[7]
Dimethoate	3	0.342	0.122	[11]
Dimethoate	3	0.380	0.000	[3]
Dimethoate	**No of labs: 11**	**Rf_{mean}: 0.305**	**CV_r: 0.072**	**CV_R: 0.114**
Diniconazole	4	0.459	0.056	[8]
Dinobuton	3	0.662	0.045	[9]
Dioxacarb	3	0.400	0.020	[1]
Dioxacarb	3	0.437	0.054	[18]
Dioxacarb	6	0.453	0.035	[9]
Dioxacarb	7	0.454	0.039	[8]
Dioxacarb	3	0.460	0.009	[7]
Dioxacarb	3	0.460	0.028	[2]
Dioxacarb	3	0.470	0.032	[6]
Dioxacarb	**No of labs: 7**	**Rf_{mean}: 0.448**	**CV_r: 0.035**	**CV_R: 0.052**
Diphenamid	3	0.515	0.020	[9]
Dithianon	7	0.650	0.035	[8]
Dithianon	8	0.650	0.033	[9]
Diuron	3	0.360	0.019	[18]
Diuron	3	0.360	0.070	[1]
Diuron	3	0.362	0.008	[6]
Diuron	8	0.366	0.025	[9]
Diuron	7	0.369	0.015	[8]
Diuron	3	0.380	0.079	[14]
Diuron	3	0.380	0.050	[7]
Diuron	3	0.400	0.040	[12]
Diuron	3	0.403	0.060	[5]
Diuron	9	0.403	0.021	[4]
Diuron	3	0.417	0.005	[15]
Diuron	3	0.418	0.037	[2]
Diuron	5	0.428	0.020	[13]
Diuron	3	0.523	0.017	[16]
Diuron	3	0.533	0.011	[3]
Diuron	3	0.543	0.138	[11]
Diuron	3	0.638	0.041	[17]
Diuron	**No of labs: 17**	**Rf_{mean}: 0.428**	**CV_r: 0.047**	**CV_R: 0.189**
DNOC	7	0.364	0.071	[9]
Dodine	3	0.000		[3]
Endosulfan[b]	4	0.668	0.012	[8]
Endosulfan[b]	3	0.670	0.015	[7]
Endosulfan[b]	3	0.670	0.012	[9]
Endosulfan alpha	9	0.660	0.017	[4]
Endosulfan alpha	3	0.753	0.020	[11]
Endosulfan beta	3	0.638	0.039	[17]
Endosulfan beta	9	0.680	0.019	[4]
Endosulfan beta	3	0.728	0.024	[11]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Endosulfan [c]	3	0.795		[16]
Epoxiconazole	7	0.382	0.034	[8]
Epoxiconazole	3	0.631	0.010	[16]
EPTC	3	0.653	0.006	[9]
EPTC	3	0.653	0.006	[8]
Esfenvalerate	3	0.680	0.014	[8]
Ethalfluralin	4	0.682	0.038	[8]
Ethephon	3	0.000		[8]
Ethirimol	7	0.122	0.238	[9]
Ethoxyfen	3	0.678	0.025	[8]
Ethoxyquin	3	0.647	0.033	[9]
Etrimfos	7	0.649	0.045	[9]
Etrimfos	7	0.649	0.045	[8]
Etrimfos	9	0.693	0.027	[4]
Fenarimol	6	0.476	0.017	[8]
Fenarimol	6	0.476	0.017	[9]
Fenarimol	3	0.479	0.039	[18]
Fenarimol	3	0.496	0.041	[6]
Fenarimol	3	0.498	0.006	[15]
Fenarimol	3	0.503	0.034	[5]
Fenarimol	3	0.508	0.036	[11]
Fenarimol	3	0.510	0.060	[1]
Fenarimol	3	0.520	0.041	[12]
Fenarimol	12	0.589	0.072	[10]
Fenarimol	3	0.590	0.016	[16]
Fenarimol	**No of labs: 11**	**Rf_{mean}: 0.513**	**CV_r: 0.052**	**CV_R: 0.079**
Fenchlorazole-ethyl	5	0.656	0.026	[8]
Fenitrothion	3	0.635	0.001	[5]
Fenitrothion	3	0.643	0.004	[2]
Fenitrothion	7	0.644	0.025	[8]
Fenitrothion	8	0.646	0.025	[9]
Fenitrothion	3	0.650	0.014	[14]
Fenitrothion	5	0.674	0.008	[13]
Fenitrothion	3	0.680	0.020	[7]
Fenitrothion	3	0.720	0.000	[3]
Fenitrothion	3	0.747	0.034	[11]
Fenitrothion	**No of labs: 9**	**Rf_{mean}: 0.671**	**CV_r: 0.020**	**CV_R: 0.058**
Fenitrothion [c]	3	0.684		[16]
Fenitrothion-o	3	0.423	0.046	[9]
Fenoxaprop-P-ethyl	3	0.663	0.047	[8]
Fenpropimorph	3	0.572	0.021	[8]
Fenthion	6	0.654	0.042	[9]
Fenthion	3	0.660	0.015	[14]
Fenthion	3	0.710	0.022	[6]
Fenthion-o	3	0.421	0.044	[9]
Fenuron	3	0.357	0.036	[8]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Fluroxypyr	5	0.650	0.015	[8]
Flusilazole	4	0.362	0.057	[8]
Flutriafol	7	0.278	0.096	[8]
Folpet	7	0.624	0.007	[8]
Folpet	8	0.624	0.007	[9]
Folpet	3	0.680	0.015	[3]
Formothion	7	0.602	0.029	[8]
Fuberidazole	5	0.460	0.083	[8]
Furathiocarb	3	0.650	0.031	[14]
Haloxyflop	4	0.054	0.195	[9]
HCB	2	0.692	0.016	[9]
Heptachlor	3	0.673	0.017	[9]
Heptenophos	9	0.494	0.046	[4]
Hymexazol	4	0.399	0.075	[8]
Imazalil	3	0.140	0.071	[3]
Imazalil	6	0.147	0.135	[8]
Imazalil	8	0.149	0.162	[9]
Imazalil	9	0.153	0.065	[4]
Imazalil	3	0.160	0.140	[1]
Imazalil	**No of labs: 5**	**Rf_{mean}: 0.150**	**CV_r: 0.123**	**CV_R: 0.050**
Imazalil [c]	3	0.076	0.039	[17]
Imazalil [c]	3	0.270	0.020	[12]
Imazalil [c]	3	0.429	0.015	[16]
Imazamethabenz-methyl	6	0.457	0.039	[8]
Imidacloprid	3	0.226	0.035	[8]
Ioxynil	3	0.300	0.041	[12]
Iprodione	6	0.641	0.034	[9]
Iprodione	9	0.647	0.019	[4]
Iprodione	3	0.680	0.030	[12]
Isazofos	3	0.640	0.031	[14]
Isazofos	3	0.690		[7]
Isoproturon	3	0.376	0.005	[15]
Isoproturon	7	0.386	0.034	[8]
Isoxaben	5	0.618	0.023	[8]
Lenacil	3	0.440	0.016	[12]
Lenacil	4	0.642	0.013	[9]
Lindane	3	0.672	0.005	[9]
Lindane	3	0.672	0.005	[8]
Lindane	3	0.700	0.014	[14]
Lindane	3	0.700	0.071	[7]
Lindane	**No of labs: 4**	**Rf_{mean}: 0.686**	**CV_r: 0.037**	**CV_R: 0.023**
Linuron	3	0.542	0.017	[17]
Linuron	5	0.559	0.027	[9]
Linuron	5	0.568	0.006	[8]
Linuron	3	0.570	0.020	[1]
Linuron	3	0.587	0.018	[6]
Linuron	3	0.591	0.011	[2]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Linuron	**No of labs: 6**	**Rf_{mean}: 0.569**	**CV_r: 0.019**	**CV_R: 0.032**
Linuron [c]	3	0.609		[16]
Malathion	8	0.643	0.024	[9]
Malathion	7	0.643	0.025	[8]
Malathion	9	0.647	0.019	[4]
Malathion	5	0.664	0.008	[13]
Malathion	3	0.700	0.007	[7]
Malathion	3	0.703	0.008	[3]
Malathion	3	0.750	0.019	[11]
Malathion	**No of labs: 7**	**Rf_{mean}: 0.679**	**CV_r: 0.019**	**CV_R: 0.060**
MCPA	7	0.035	0.262	[8]
MCPA [c]	3	0.000		[1]
Mecarbam	4	0.670	0.049	[9]
Mecoprop	6	0.065	0.277	[8]
Mecoprop-P	3	0.086	0.134	[8]
Metalaxyl	3	0.458	0.021	[9]
Metalaxyl	3	0.460	0.015	[12]
Metalaxyl	3	0.460	0.043	[14]
Methabenzthiazuron	7	0.406	0.033	[9]
Methamidophos	3	0.160	0.000	[3]
Methidathion	3	0.620	0.050	[1]
Methidathion	3	0.622	0.015	[6]
Methidathion	6	0.631	0.042	[9]
Methidathion	6	0.631	0.042	[8]
Methidathion	3	0.650	0.062	[14]
Methidathion	**No of labs: 5**	**Rf_{mean}: 0.631**	**CV_r: 0.045**	**CV_R: 0.019**
Methomyl	3	0.322	0.023	[18]
Methomyl	3	0.330	0.058	[17]
Methomyl	3	0.351	0.046	[16]
Methomyl	3	0.356	0.006	[15]
Methomyl	8	0.363	0.043	[9]
Methomyl	7	0.366	0.062	[8]
Methomyl	3	0.370	0.038	[14]
Methomyl	3	0.372	0.059	[6]
Methomyl	5	0.406	0.013	[13]
Methomyl	**No of labs: 9**	**Rf_{mean}: 0.359**	**CV_r: 0.045**	**CV_R: 0.068**
Methoxychlor	3	0.817	0.002	[2]
Metobromuron	3	0.570	0.018	[14]
Metobromuron	4	0.573	0.045	[9]
Metobromuron	3	0.590	0.046	[7]
Metolachlor	5	0.610	0.035	[8]
Metoxuron	3	0.291	0.007	[15]
Metoxuron	3	0.297	0.028	[6]
Metoxuron	3	0.300	0.010	[1]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Metoxuron	8	0.302	0.032	[9]
Metoxuron	3	0.316	0.068	[5]
Metoxuron	7	0.334	0.015	[8]
Metoxuron	3	0.344	0.029	[18]
Metoxuron	**No of labs: 7**	**Rf_{mean}: 0.312**	**CV_r: 0.031**	**CV_R: 0.064**
Metoxuron [c]	3	0.476	0.012	[16]
Metribuzin	3	0.590	0.085	[17]
Metribuzin	5	0.622	0.042	[9]
Metribuzin	9	0.644	0.018	[4]
Metribuzin	3	0.650	0.041	[12]
Metribuzin	3	0.687	0.022	[3]
Metribuzin	**No of labs: 5**	**Rf_{mean}: 0.639**	**CV_r: 0.038**	**CV_R: 0.056**
Mevinphos	8	0.419	0.029	[9]
Mevinphos	5	0.426	0.013	[13]
Mevinphos	3	0.512	0.053	[11]
Mevinphos	3	0.520	0.100	[1]
Mevinphos	3	0.520	0.000	[3]
Mevinphos	**No of labs: 5**	**Rf_{mean}: 0.479**	**CV_r: 0.046**	**CV_R: 0.109**
Molinate	4	0.621	0.028	[8]
Monocrotophos	3	0.044	0.259	[6]
Monocrotophos	3	0.050	0.280	[1]
Monocrotophos	6	0.076	0.098	[9]
Monocrotophos	3	0.080	0.100	[14]
Monocrotophos	3	0.095	0.218	[11]
Monocrotophos	3	0.100	0.144	[7]
Monocrotophos	5	0.106	0.084	[13]
Monocrotophos	**No of labs: 7**	**Rf_{mean}: 0.079**	**CV_r: 0.150**	**CV_R: 0.307**
Monolinuron	7	0.562	0.026	[9]
Myclobutanil	5	0.285	0.051	[8]
Napropamide	4	0.524	0.035	[9]
Nitrofen	3	0.666	0.041	[9]
Nuarimol	8	0.466	0.028	[9]
Omethoate	6	0.060	0.118	[9]
Oxadiazon	3	0.656	0.031	[9]
Oxadiazon	3	0.680	0.029	[14]
Oxadixyl	3	0.361	0.097	[9]
Oxamyl	9	0.146	0.094	[18]
Oxamyl	3	0.170	0.051	[12]
Oxamyl	3	0.173	0.059	[5]
Oxamyl	3	0.181	0.107	[6]
Oxamyl	7	0.185	0.056	[8]
Oxamyl	3	0.186	0.022	[15]
Oxamyl	8	0.188	0.050	[9]
Oxamyl	3	0.192	0.149	[11]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Oxamyl	**No of labs: 8**	**Rf_{mean}: 0.178**	**CV_r: 0.076**	**CV_R: 0.083**
Oxamyl [c]	9	0.293	0.104	[3]
Oxamyl [c]	3	0.315	0.026	[16]
Parathion	8	0.669	0.030	[9]
Parathion	3	0.740	0.034	[5]
Parathion-methyl	3	0.636	0.008	[2]
Parathion-methyl	3	0.666	0.004	[15]
Parathion-methyl	8	0.666	0.028	[9]
Parathion-methyl	5	0.668	0.013	[13]
Parathion-methyl	7	0.669	0.029	[8]
Parathion-methyl	3	0.670	0.017	[7]
Parathion-methyl	9	0.674	0.013	[4]
Parathion-methyl	3	0.680	0.056	[12]
Parathion-methyl	3	0.680	0.015	[14]
Parathion-methyl	3	0.680	0.015	[3]
Parathion-methyl	3	0.680	0.020	[6]
Parathion-methyl	3	0.696	0.047	[11]
Parathion-methyl	9	0.711	0.041	[18]
Parathion-methyl	3	0.750	0.030	[1]
Parathion-methyl	3	0.759	0.037	[5]
Parathion-methyl	3	0.771	0.017	[16]
Parathion-methyl	**No of labs: 16**	**Rf_{mean}: 0.691**	**CV_r: 0.028**	**CV_R: 0.055**
Penconazole	3	0.360	0.042	[12]
Penconazole	5	0.246	0.134	[8]
Pendimethalin	7	0.651	0.031	[8]
Pentachlorophenol	3	0.366	0.009	[9]
Phenkapton	8	0.683	0.020	[9]
Phenkapton	7	0.683	0.022	[8]
Phenmedipham	8	0.646	0.038	[9]
Phorate	7	0.666	0.033	[8]
Phosalone	3	0.671	0.009	[6]
Phosalone	8	0.671	0.024	[9]
Phosalone	3	0.690	0.023	[1]
Phosalone	3	0.690	0.023	[12]
Phosalone	9	0.707	0.029	[4]
Phosalone	**No of labs: 5**	**Rf_{mean}: 0.686**	**CV_r: 0.025**	**CV_R: 0.022**
Phosalone [c]	3	0.531	0.010	[16]
Phosphamidon	8	0.224	0.066	[9]
Phosphamidon	7	0.226	0.067	[8]
Phosphamidon	3	0.230	0.035	[14]
Phosphamidon [c]	3	0.400	0.019	[16]
Pirimicarb	3	0.440	0.000	[1]
Pirimicarb	3	0.440	0.000	[12]
Pirimicarb	7	0.449	0.031	[8]
Pirimicarb	8	0.451	0.029	[9]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Pirimicarb	3	0.473	0.004	[17]
Pirimicarb	9	0.478	0.027	[4]
Pirimicarb	3	0.516	0.052	[11]
Pirimicarb	3	0.520	0.022	[6]
Pirimicarb	9	0.554	0.056	[3]
Pirimicarb	**No of labs: 9**	**Rf_{mean}: 0.480**	**CV_r: 0.038**	**CV_R: 0.085**
Pirimiphos-methyl	5	0.666	0.035	[8]
Pirimiphos-methyl	9	0.683	0.010	[4]
Pirimiphos-methyl	3	0.710	0.013	[7]
Pirimiphos-methyl [c]	3	0.700		[17]
Pirimiphos-methyl [c]	3	0.730		[16]
Pirimisulfuron-methyl	4	0.513	0.068	[8]
Prochloraz	7	0.243	0.128	[8]
Prochloraz	3	0.282	0.044	[18]
Prochloraz	3	0.295	0.033	[6]
Prochloraz	3	0.312	0.010	[15]
Prochloraz	3	0.380	0.038	[5]
Prochloraz	**No of labs: 5**	**Rf_{mean}: 0.303**	**CV_r: 0.073**	**CV_R: 0.166**
Prochloraz [c]	13	0.486	0.135	[10]
Prochloraz [c]	3	0.537	0.013	[16]
Procymidone	6	0.649	0.048	[9]
Procymidone	9	0.687	0.016	[4]
Prometryn	8	0.623	0.033	[9]
Prometryn	3	0.630	0.014	[14]
Prometryn	3	0.650	0.024	[1]
Prometryn	3	0.650	0.024	[12]
Prometryn	9	0.664	0.028	[4]
Prometryn	3	0.686	0.016	[17]
Prometryn	**No of labs: 6**	**Rf_{mean}: 0.651**	**CV_r: 0.027**	**CV_R: 0.035**
Propachlor	4	0.601	0.028	[8]
Propachlor	4	0.601	0.028	[9]
Propanil	3	0.550	0.018	[14]
Propargite	5	0.671	0.023	[9]
Propham	5	0.655	0.047	[8]
Propham	5	0.655	0.047	[9]
Propiconazole (1)	5	0.314	0.075	[8]
Propiconazole (2)	6	0.390	0.059	[8]
Propisochlor	3	0.649	0.010	[8]
Propoxur	3	0.580	0.011	[7]
Prosulfuron	5	0.301	0.092	[8]
Prothiofos	4	0.660	0.041	[9]
Pyridate	7	0.679	0.021	[8]
Quinalphos	3	0.563	0.007	[15]
Quinalphos	3	0.631	0.059	[8]
Quinclorac	6	0.060	0.240	[8]
Rimsulfuron	4	0.106	0.145	[8]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Simazine	3	0.555	0.021	[5]
Simazine	4	0.569	0.026	[9]
Simazine	7	0.570	0.025	[8]
Simazine	3	0.570	0.018	[14]
Simazine	9	0.572	0.023	[4]
Simazine	3	0.580	0.010	[1]
Simazine	3	0.592	0.013	[6]
Simazine	**No of labs: 7**	**Rf_{mean}: 0.572**	**CV_r: 0.022**	**CV_R: 0.020**
Sulcotrione	5	0.034	0.287	[8]
Tebuconazole	6	0.324	0.077	[8]
Tebuconazole	3	0.334	0.060	[9]
Tebuconazole	3	0.337	0.038	[5]
Teflubenzuron	7	0.641	0.044	[8]
Teflubenzuron	3	0.672	0.009	[16]
Tefluthrin	3	0.686	0.018	[8]
Terbuthylazine	3	0.537	0.037	[17]
Terbuthylazine	6	0.634	0.045	[9]
Terbuthylazine	3	0.640	0.031	[14]
Terbutryn	3	0.589	0.010	[17]
Terbutryn	3	0.595	0.025	[9]
Terbutryn	6	0.603	0.024	[8]
Tetraconazole	3	0.371	0.077	[8]
Tetradifon	4	0.657	0.043	[9]
Tetrasul	3	0.673	0.044	[9]
Thiabendazole	7	0.335	0.040	[9]
Thiabendazole	7	0.335	0.040	[8]
Thiabendazole	3	0.370	0.026	[18]
Thiabendazole	3	0.370	0.030	[1]
Thiabendazole	3	0.370	0.040	[6]
Thiabendazole	**No of labs: 5**	**Rf_{mean}: 0.356**	**CV_r: 0.037**	**CV_R: 0.053**
Thiabendazole [c]		0.710	0.012	[16]
Thiocyclam	5	0.135	0.085	[8]
Thiometon	3	0.649	0.032	[9]
Thiophanate-methyl	5	0.556	0.010	[13]
Thiophanate-methyl	7	0.570	0.017	[8]
Thiophanate-methyl	8	0.571	0.016	[9]
Thiophanate-methyl	3	0.591	0.019	[6]
Thiophanate-methyl	3	0.596	0.030	[11]
Thiophanate-methyl	**No of labs: 5**	**Rf_{mean}: 0.577**	**CV_r: 0.018**	**CV_R: 0.028**
Thiram	6	0.565	0.017	[8]
Tifensulfuron-methyl	4	0.217	0.099	[8]
Tralkoxydim	5	0.659	0.022	[8]
Triadimefon	3	0.574	0.025	[8]
Triasulfuron	4	0.410	0.097	[8]
Triazophos	7	0.633	0.039	[8]

Active ingredient	No.	Average Rf_i	CV_r	Ref.
Triazophos	8	0.634	0.036	[9]
Triazophos	3	0.740	0.020	[1]
Triazophos [c]	3	0.470		[16]
Tribenuron-methyl	3	0.479	0.032	[8]
Trichlorfon	3	0.240	0.083	[14]
Trichlorfon	5	0.242	0.120	[9]
Tridemorph (1)	5	0.333	0.083	[8]
Tridemorph (2)	5	0.585	0.015	[8]
Trifluralin	5	0.682	0.020	[9]
Trifluralin	3	0.690	0.023	[1]
Trifluralin	3	0.690	0.023	[12]
Trifluralin	6	0.703	0.007	[3]
Trifluralin	9	0.738	0.011	[4]
Trifluralin	**No of labs: 5**	**Rf_{mean}: 0.701**	**CV_r: 0.015**	**CV_R: 0.032**
Triforine	7	0.492	0.056	[8]
Triforine	3	0.500	0.008	[5]
Triforine	3	0.524	0.092	[18]
Triforine	3	0.530	0.008	[15]
Triforine	3	0.586	0.009	[6]
Triforine	**No of labs: 5**	**Rf_{mean}: 0.526**	**CV_r: 0.049**	**CV_R: 0.070**
Triforine [c]	41	0.583	0.362	[10]
Vinclozolin	3	0.667	0.016	[9]

[a] I, II: or 1, 2: either two separate standards for the isomers of the active ingredient, or a second unidentified spot occurred in the analytical standard of the compound.
[b] The alpha and beta isomers cannot be separated by the elution system applied.
[c] The reported values were not incorporated in the calculations as either the R_f or CV_r values were considered atypical.

There was no correlation between the within-plate, CV_{wRf}, and between plates, CV_{bRf}, variability of Rf or RRf values obtained for various compounds in different laboratories as shown in Figures 7 and 8. The corresponding CV_w and CV_b pairs were measured in one laboratory in both cases.

The within-plate variability of RRf values was in the same range as that of the Rf values, while the between plates variability of RRf values was lower than that of Rf values, indicating that the RRf values can be better used for the identification of the compounds possibly present.

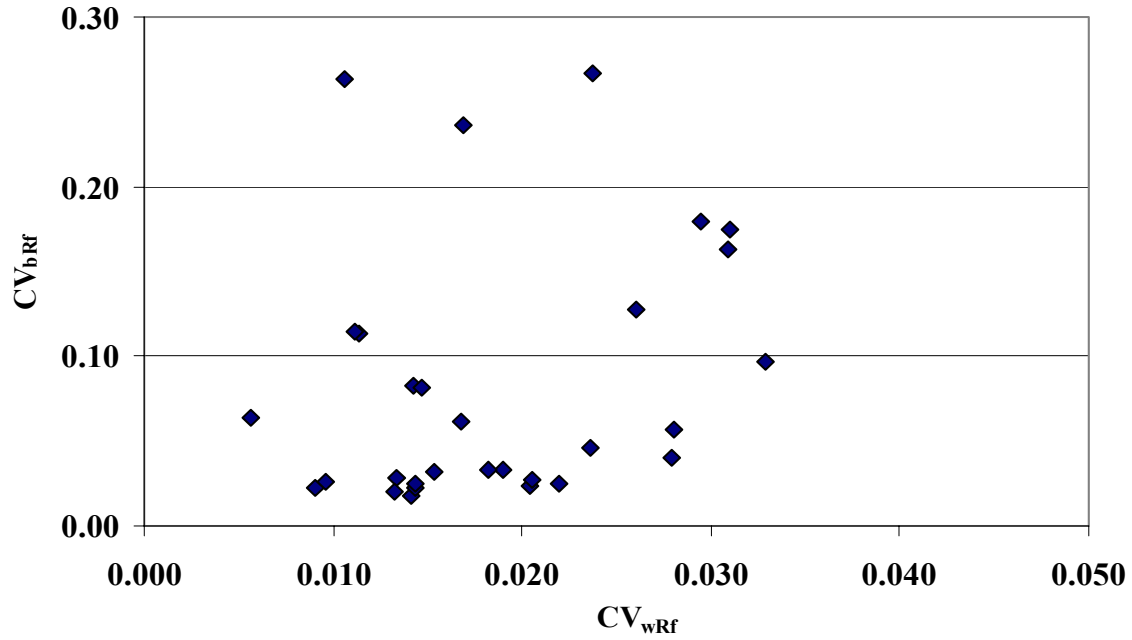

FIG. 7. Relation of within and between plates CV values of Rfs in one laboratory.

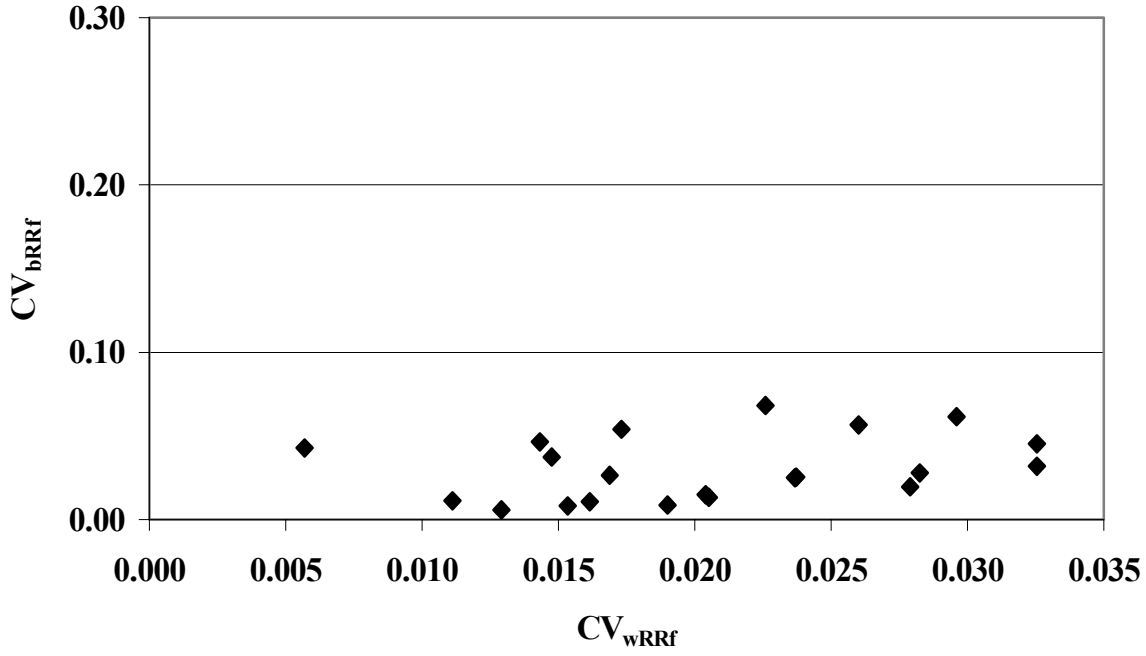

FIG. 8. Relation of within and between plates CV values of RRfs in one laboratory.

The within-laboratory reproducibility of Rf values, CVr, was on an average 2.3 times lower than the between laboratories reproducibility CV_R. Their correlation is shown on Figure 9. The relatively small difference is due to the fact that the CV_R was calculated from the average

Rf values obtained by the different laboratories, while the CV_r reflects the average variability of individual measurements in one laboratory. Taking into account that the Rf_{imean} values were obtained, on an average, from 3–5 replicate measurements, the between-laboratories coefficient of variation of Rf values of individual measurements is expected to be generally between √ 5 and √ 3 times the reported CV_R.

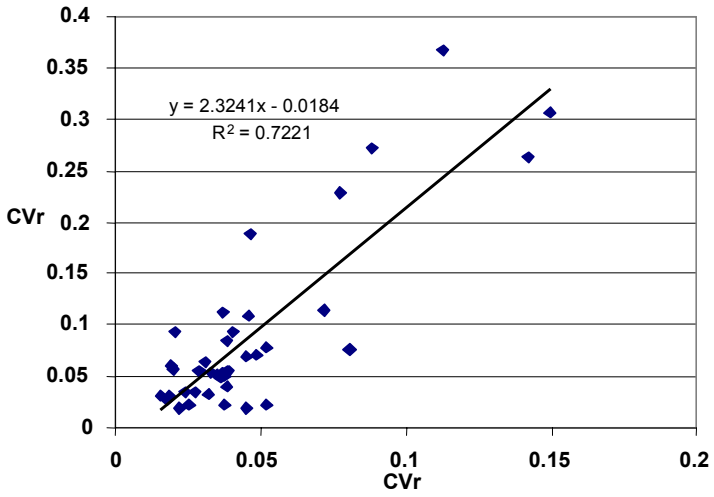

FIG. 9. Relation of between-laboratories CV_R and within-laboratory CVr of Rfs values.

The average within-laboratory and between-laboratories reproducibility of Rf values are increasing with the decreasing Rf values (Figures 10 and 11). The CVr values were below 6% above Rf = 0.3, and between 6 and 10% below Rf = 0.2. This finding is in agreement with the general tendency of within-laboratory variability reported earlier [19]. The CV_R values sowed similar tendency. Above Rf = 0.2 they were generally less than 12% with a few exceptions.

The minimum detectable quantities of analytes [MDQ, ng] were determined with pure analytical standards following their elution and detection. The dilutions of the analytical standards around the expected MDQ were usually made to provide relative concentrations of 1.3, 1, 0.7, 0.5. Due to the inherent uncertainty of visualization of spots and the stepwise dilution, two times difference in the reported MDQ values is not considered significant.

The reported MDQ values are summarized in Table 3. Where at least three comparable results were available from different laboratories for two detection methods, the generally expectable average MDQ range is indicated with bold faced letters in the Table.

The MDQ values being ≥ 5 times smaller or larger than the average were considered atypical. Such cases are highlighted in Table 3 to indicate the large variation in the sensitivity of detection and the importance of proper selection of detection conditions.

The bioassay methods (Hill reaction, fungi test, enzyme inhibition) enabled the detection down to 0.1–10 ng, while 20–100 ng could be achieved with the chemical reagents. The detection under UV light with visual observation was not sufficiently sensitive (MDQ ≥ 100–500 ng) for pesticide residue analysis. The sensitivity may be increased by a TLC scanner which makes the detection of wide range of compounds possible [26].

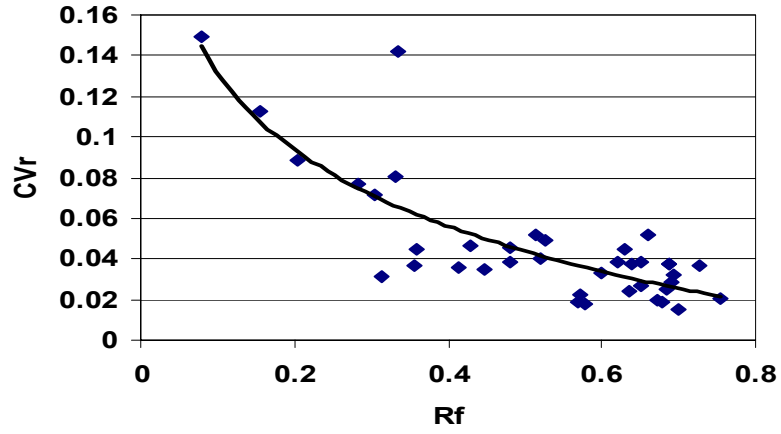

FIG. 10. Relation of within-laboratory typical CVr of Rf values and Rf.

Taking into account that the TLC plates can be generally loaded up to 300 mg sample equivalent [19], the 1–100 ng MDQ range is required for detection of pesticide residues at around 0.003–0.3 mg/kg concentrations. However, the information on non-detectability or detectability at or above 3000 ng (≥ 10 mg/kg) is useful as those substances can usually be excluded from the list of potentially detected compounds based on their Rf values, and hereby facilitates the identification of residues present. Therefore, the high MDQ values or the highest amounts which did not give visible spots are also included in the Table.

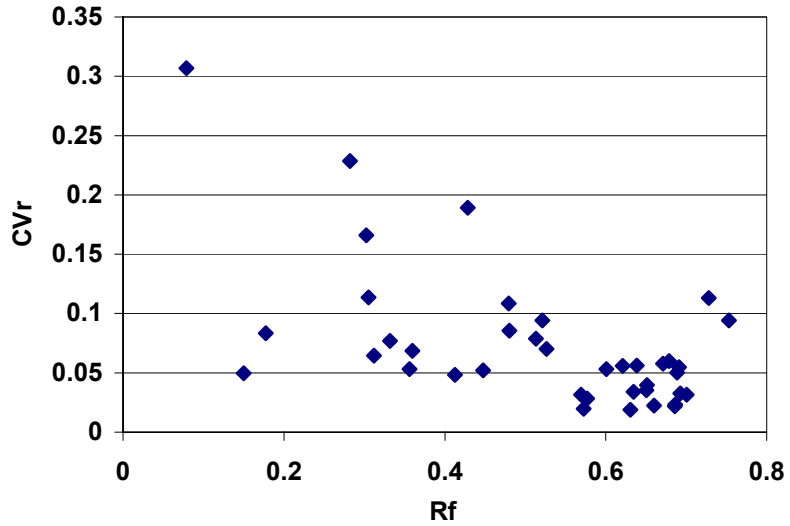

FIG. 11. Relation of between-laboratories CV_R of Rf values and the Rf.

TABLE 3. MINIMUM DETECTABLE QUANTITIES OF PESTICIDES WITH NINE DETECTION METHODS

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
2,4-D	5000	5000	>5000	500	>5000	>5000	1000	500	5000	[8]
	5000	5000	>5000	500	>5000		1000	500	5000	[9]
2-Phenylphenol	500	250	50	5000	>5000	500	1000	500	500	[9]
	500	250	50	>5000	>5000	500	1000	500	500	[8]
3-Keto-Carbofuran	500	50	100	5000	>5000	>5000	>5000	500	50	[9]
Acephate									25	[6]
	5000	200	>5000	5000	>5000	>5000	5000	2500	10	[9]
Acetochlor	1000	1000	>5000	100	>5000	5000	2000	5000	2000	[8]
Alachlor	500	500	>5000	100	>5000	>5000	1000	2500	1000	[8]
Aldicarb	1500	100	>5000	1000	>5000	>5000	>5000	500	3	[9]
		100						500	3	[7]
Aldrin	5000	2000	>5000	50	>5000		1000	5000	>5000	[9]
		2000								[7]
										[1]
Alphamethrin	100	2500	>5000	100	>5000	>5000	5000	1000	2500	[8]
				45						[15]
				200						[2]
Ametryn	50	15	>5000	40			0.6	3000	1000	[14]
Amidosulfuron	100	10	>5000	>5000	500	5000	>5000	1000	500	[8]
Asulam	300	100	5000	1000	100	>5000	1000	500	1000	[9]
Atrazine	500	25	5000	240	>5000		1	1000	5000	[9]
	300	20	>5000	200			0.6	1000	5000	[14]
		25		400			0.25		1500	[7]
		25								[2]
	500	25	>5000	250	>5000	>5000	1	1000	5000	[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		25			>1000	>1000	1	>1000	>1000	[1]
		25					1			[5]
		12.5					1			[6]
		30	5000				5			[13]
		20					0.5			[15]
		20		200		>5000	0.6	800	5000	[4]
		20	5000	200	4000	4000	0.5	2000	1000	[12]
		12.5	>10000	50	>5000	>5000	0.7	>5000	>5000	[3]
		20					1			[17]
		12.5					0.5			[18]
		12.5	>5000	125	>5000		0.25	500	>5000	[11]
		25					0.5			[16]
Atrazine	**300-500**	**12.5-25**	**≥ 5000**	**125-250**	**≥ 4000**	**≥ 4000**	**0.5-1**	**500-800**	**≥ 1000**	
Azinphos-ethyl		1250	>10000	5000	1800	>5000	5000	50	5	[3]
		1500		5000		5000	5000	5	20	[4]
Azinphos-me	750	2500	>5000	1000	5000		500	2	10	[9]
					>1000	>1000	500	2	10	[1]
Aziprotryn	250	100	>5000	100	>5000		100	1000	1000	[9]
Azoxystrobin						2				[8]
BCPE	2000	3000	>5000	100	>5000		1000	5000	2500	[9]
Benefin	500	1000	>5000	5000	250	>5000	>5000	>5000	2500	[9]
	500	1000	>5000	>5000	250	>5000	>5000	>5000	2500	[8]
Benomyl		500	>5000	500	>5000	20	250	1000	2000	[11]
		300	5000	100	1000	40	4000	5000	5000	[12]
Bensulfuron-me	100	25	>5000	>5000	500	1000	>5000	1000	100	[8]
Bensultap	500	100	>5000	>5000	>5000	1000	>5000	5000	1000	[8]
Bentazone		100			2000	4000	1	1000	2500	[12]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
	250	100	>5000	>5000	1000	>5000	5	5	1000	[8]
							8			[5]
	100	20	>5000		>5000		2000	800	500	[14]
Bentazone	**100-250**	**100**	**>5000**		**1000-2000**	**≥ 4000**	**5-8**	**800-1000**	**500-1000**	
Biphenyl	500	5000	>5000	5000	>5000	>5000	5000	>5000	>5000	[9]
Bromophos-ethyl	5000	500	>5000	1000	>5000	5000	>5000	1	0.5	[9]
Bromopropylate	1000	5000	>5000	1000	>5000		>5000	5000	2500	[9]
Bromoxynil	100	1000	>5000	>5000	5000	2500	10	1000	2500	[8]
		300			5000	3000	1	1000	2000	[12]
Bromuconazole I and II*	5000	1000	>5000	250	>5000	20	500	5000	1000	[8]
Bupirimate	800	50	>5000	250	>5000	500	1000	2500	1000	[9]
Butachlor	5000	5000	>5000	100	>5000	5000	1000	1000	2500	[9]
Butylate	5000	300	>5000	5000	>5000		1000	1000	1000	[9]
	5000	300	>5000	>5000	>5000	>5000	1000	1000	1000	[8]
Cadusafos	>250	>250	>250	>250	>250	>250	>250	>250	100	[9]
Captafol	5000	1000	>5000	100	>5000	10	>5000	5000	1000	[9]
Captan						15				[6]
				10						[10]
						20				[5]
						20				[15]
	1000	2000	>5000	50	>5000	20	5000	500	500	[8]
	1000	2000	>5000	100	>5000	20	5000	500	500	[9]
	1000	1000	>5000	100		50	2000	500	500	[14]
		5000		100				500	500	[7]
		2000		80		16	4000	400	500	[4]
								550		[13]
						10				[18]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		2000	5000	100	4000	20	4000	300	400	[12]
		2000			>1000	20	>1000	700	700	[1]
		1000	>10000		>5000	350	1000	1000	500	[3]
						50				[17]
			>5000	100	>5000	20	>5000	300	1000	[11]
						20				[16]
Captan	**1000**	**1000-2000**	**≥ 5000**	**50-100**	**4000-5000**	**10-20**	**1000-2000**	**300-500**	**500**	
Carbaryl		80	50	4000	5000	5000	4000	2	8	[4]
		100	37.5					2	10	[7]
	1000	100	50	>5000	>5000	2500	>5000	10	10	[8]
		75								[6]
	1000	100	50	5000	>5000		>5000	2	10	[9]
								5		[18]
		100	50	5000	4000	3000	4000	2	50	[12]
	1000	50	50	3000	4000	1100	4000	2	10	[14]
		100	50		3090			2		[13]
		33.3	60	2500	2750	>5000	2500	100	10	[3]
		100	100	2500	1000	>5000	>5000	20	20	[11]
		100			>1000	>1000	>1000	2	10	[1]
Carbaryl	**1000**	**50-100**	**50**	**2500**	**2000-3000**	**1000-2500**	**2500-4000**	**2**	**10**	
Carbendazim	2000	1000	>5000	200	>5000	20	1000	500	500	[9]
		500			>1000	50	>1000	>1000	>1000	[1]
				50		20		10		[17]
	1000	300	5000	>5000	1000	10	>5000	>5000	500	[8]
							5000	510		[13]
		1000			>1000	50	>1000	700	700	[1]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		300						>5000		[13]
		300	5000	100	>5000	40	4000	5000	500	[4]
						40				[6]
				50		50				[17]
	2000		>5000	200		20	1300	500	500	[14]
		333	>10000	>5000	>5000	75		1200	500	[3]
		300		100					500	[7]
Carbendazim	**1000-2000**	**300-500**	**≥ 5000**	**50-100**	**≥ 1000**	**10-20**	**1000-1300**	**500**	**500**	
Carbofuran			50	75				100		[17]
		500	30					500	100	[7]
	2000	300		5000	3000		>5000	1000	50	[14]
	2000	500	50	>5000	5000	5000	>5000	1000	50	[8]
	2000	500	50	>5000	5000		>5000	1000	50	[9]
		600	60					>5000		[13]
		200	100	>5000	>5000	>5000	>5000		1500	[11]
		100	70	5000	>5000	>5000	2500	2500	50	[3]
Carbofuran	**2000**	**200-300**	**50**	**≥ 5000**	**3000-5000**	**5000**	**≥ 2500**	**100-500**	**50**	
Carbosulfan	500	100	250	>5000	>5000	5000	>5000	500	15	[8]
Carboxin	100	100	>5000	>5000	500	1000	10	250	1000	[8]
Chlorbromuron	250	50	>5000	500	50		1	5000	1000	[9]
	250	50	>5000	100	50	2000	1	5000	500	[8]
		37.5								[16]
Chlordimeform	500	300	>5000	100	5000	5000	>5000	500	1000	[9]
Chlorfenvinphos									20	[15]
Chloropropylate	5000	1000	>5000	200	>5000	5000	>5000	5000	2500	[9]
Chlorothalonil	700	5000	>5000	100	>5000	50	250	>5000	>5000	[9]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
	700	5000	>5000	100	>5000	25	250	>5000	>5000	[8]
							1400	>5000		[13]
		100				50				[17]
		5000	5000	100	5000	50	200	4000	200	[12]
		>5000	>5000	80	>5000	50	250	1000	>5000	[11]
		5000		80		40	250	5000	5000	[4]
		6000	>10000	40	>5000	24	>5000	5000	5000	[3]
Chlorothalonil	**700**	**5000**	**≥ 5000**	**80-100**	**≥ 5000**	**25-50**	**200-250**	**4000-5000**	**5000**	
Chlortoluron	500	100	>5000	200	50	1000	1	2500	1000	[8]
	500	100	>5000	200	50		1	2500	1000	[9]
							2			[15]
				75			1			[17]
							1			[5]
							0.5			[16]
							0.75			[6]
							0.5			[18]
Chlortoluron	**500**	**100**	**>5000**	**75-200**	**50**		**0.5-1**	**2500**	**1000**	
Chloroxuron	500	100	>5000	100	50		1	1000	1000	[9]
	500	50	3000	50	50	1000	0.6	1000	1000	[14]
		33.3	>10000	50	75	4000	0.5	1000	1000	[3]
Chloroxuron		**50**	**3000-5000**	**50-100**	**50-75**		**0.5-1**	**1000**	**1000**	
Chlorpropham	2000	100	>5000	150	100		250	5000	1000	[9]
Chlorpyrifos								1		[2]
	2000	1000	>5000	25	>5000	>5000	3000	0.5	0.2	[8]
	2000	1000	>5000	250	>5000		5000	0.5	0.5	[9]
	2000	1000		200	4000	5000	2500	0.5	0.25	[14]
		>600		250				1	50	[7]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
								0.5		[13]
			>5000		>5000		250	0.5	1	[11]
					>1000	>1000	>1000	1.5	0.2	[1]
		6000	>10000		>5000	>5000		0.5	0.5	[3]
								0.25		[18]
Chlorpyrifos	**2000**	**1000**	**>5000**	**200-250**	**4000-5000**	**≥ 5000**	**2500-3000**	**0.5**	**0.2-0.5**	
Chlorpyrifos-me	5000	5000	>5000	500	>5000	5000	1000	1	0.5	[8]
Cyanazine	1000	100	>5000	200	>5000	>5000	1	5000	1000	[8]
	1000	100	>5000	250	>5000		1	5000	1000	[9]
							0.5			[18]
		100					1			[17]
Cyanazine		**100**					**0.5-1**			
Cyfluthrin beta	5000	1000	>5000	100	>5000	>5000	2500	1000	5000	[8]
Cyhalothrin lambda	5000	5000	>5000	1000	>5000	>5000	>5000	5000	5000	[8]
				50						[10]
Cypermethrin[a]	2000	5000	5000	100	>5000	>5000	5000	500	2500	[8]
	2000	5000	5000	250	>5000	>5000	>5000	500	2500	[9]
Cypermethrin β	5000	2500	>5000	100	>5000	>5000	>5000	1000	5000	[8]
Cypermethrin ζ	500	5000	>5000	100	>5000	>5000	500	1000	1000	[8]
				250						[6]
Cypermethrin		**2500-5000**	**≥ 5000**	**100**	**>5000**	**>5000**		**500-1000**	**1000-2500**	
Cyproconazole	2500	5000	>5000	2500	>5000	20	>5000	2500	500	[8]
DDT p,p				10						[10]
	5000	3000		50			2000	5000	>5000	[14]
	5000	5000	>5000	50	>5000		5000	5000	>5000	[9]
				250						[2]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
				37.5						[6]
DDT p,p	**5000**	**3000-5000**		**40-50**				**5000**	**>5000**	
Deltamethrin	1000	5000	5000	250	>5000	5000	2000	5000	5000	[8]
	1000	5000	5000	500	>5000		>5000	5000	5000	[9]
Desmedipham	1400	50	50	>5000	100	5000	1	200	500	[8]
	1400	50	50	5000	100		1	200	500	[9]
		50	50	300	100	4000	1	100	400	[12]
		50	50				1			[17]
Desmedipham		**50**	**50**		**100**	**4-5000**	**1**	**100-200**	**400-500**	
Desmetryn		50		50			1			[17]
		20		250		>5000	1	800	5000	[4]
Diazinon		4000		1000		>5000	5000	400	0.5	[4]
								2		[6]
		2500	>10000	>5000	>5000	>5000	>5000	500	0.2	[3]
	1000	5000	>5000	1000	>5000		5000	500	0.2	[9]
	1000	5000	>5000	>5000	>5000	>5000	5000	2	0.2	[8]
	600	3000		1000	4000		2600	1	0.25	[14]
		5000					2650	540		[13]
		300						300	0.2	[7]
		>1000			>1000	>1000	>1000	50	0.2	[1]
		500	>5000	500	>5000		500	10	5	[11]
Diazinon	**600-1000**	**300-500**	**>5000**	**500-1000**	**4000-5000**	**>5000**	**2500-5000**	**1-2**	**0.2**	
Dicamba	1000	2000	5000	500	>5000	>5000	1000	1000	5000	[8]
Dichlofluanid	2500	3000	5000	100	5000	20	>5000	500	2500	[8]
	2500	3000	5000	100	5000	100	>5000	500	2500	[9]
						100				[5]
Dichloran	1000	100	>5000	100	5000	5000	>5000	>5000	>5000	[9]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
	1000	100	>5000	50	5000	5000	>5000	>5000	>5000	[8]
Dichlorprop	2500	1000	>5000	100	>5000	>5000	>5000	500	5000	[8]
Dichlorprop-P	5000	1000	>5000	500	>5000	>5000	>5000	1000	1000	[8]
Dichlorvos	>5000	>5000	>5000	250	5000	>5000	>5000	20	2	[8]
								20		[6]
								10		[18]
								10		[15]
	>5000	4000					5000	20	2.5	[14]
	>5000	>5000	>5000	500	5000		>5000	20	2	[9]
								20		[5]
		>5000	>5000	500	>5000		>5000	50	5	[11]
		>1000			>1000	>1000		20	10	[1]
Dichlorvos	**>5000**	**>5000**	**>5000**	**250-500**	**5000**	**>5000**	**≥ 5000**	**10-20**	**2-2.5**	
Diclofop-me	500	5000	>5000	500	>5000	5000	1000	100	1000	[8]
Dieldrin	5000	1500	>5000	25	>5000	>5000	>5000	>5000	>5000	[8]
	5000	1500	>5000	75	>5000		>5000	>5000	>5000	[9]
	5000	1000		70			2000	>5000	5000	[14]
				75						[7]
				35						[15]
		1500	5000	75	5000	300	4000	4000	4000	[12]
			>10000	75	>5000	5000	5000	>5000	>5000	[3]
				100						[5]
				18.75						[6]
				500						[2]
				12.5						[18]
Dieldrin	**5000**	**1000-1500**	**≥ 5000**	**20-35**	**≥ 5000**	**≥ 5000**	**≥ 5000**	**≥ 5000**	**≥ 5000**	
Difenoconazole						20				[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Difenzoquat	50	5000	>5000	>5000	>5000	>5000	2500	5000	2500	[8]
Diflubenzuron	100	100	>5000	500	100	>5000	>5000	200	>5000	[8]
Diflufenican	200	100	>5000	>5000	2500	>5000	5000	500	500	[8]
Dimethametryn							0.13			[7]
Dimethenamid	1000	5000	>5000	500	>5000	5000	1000	1000	1000	[8]
Dimethipin	>5000	>5000	>5000	>5000	>5000	>5000	1000	>5000	>5000	[8]
Dimethoate	5000	100	>5000	500	>5000		>5000	500	1000	[9]
	5000	100	>5000	>5000	>5000	>5000	>5000	500	1000	[8]
		50								[18]
		100								[6]
		>1000			>1000		>1000		1000	[1]
		100	5000	500	5000	4000	4000	500	1500	[12]
	3000	50	4000	500	>5000		3200	500	500	[14]
		100						510		[13]
		100		500				500	1000	[7]
		180	5000	500	>5000		5000			[11]
		50	>10000	>5000	>5000	>5000	5000	5000	1000	[3]
Dimethoate	**3000-5000**	**50-100**	**4000-5000**	**500**	**>5000**	**4000-5000**	**3000-4000**	**500**	**500-1000**	
Diniconazole	100	500	>5000	5000	>5000	100	>5000	5000	5000	[8]
Dinobuton	500	1000	500	5000	>5000	500	1000	>5000	5000	[9]
Dioxacarb		50			>1000		>1000		300	[1]
		12.5								[18]
	5000	25	100	>5000	5000		>5000	500	100	[9]
	5000	25	100	>5000	5000	>5000	>5000	500	100	[8]
		25	100					500	100	[7]
		37.5								[2]
		50								[6]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Dioxacarb	**5000**	**25-50**	**100**	**>5000**	**5000**	**>5000**	**>5000**	**500**	**100**	
Diphenamid	2500	3000	>5000	5000	>5000		5000	>5000	5000	[9]
Dithianon	250	3000	1000	>5000	5000	100	100	500	5000	[8]
	250	3000	1000	100	5000	100	100	500	5000	[9]
Diuron		15								[18]
		50			50		1	>1000	>1000	[1]
		30								[6]
	300	30	>5000	100	50		1	2500	1000	[9]
	300	30	>5000	100	50	5000	1	2500	1000	[8]
	150	20		100	30	>5000	0.6	300	1000	[14]
		30		100	50		0.25			[7]
		30	5000	100	50	50	0.5	2500	1000	[12]
		30								[5]
		30	>5000	80	50	>5000	1	2500	2000	[4]
		30								[15]
		60								[2]
		40			40		6.5	2005		[13]
		30								[16]
		30	>10000	50	50	4500	0.5	5000	2000	[3]
			>5000	100	50					[11]
		20					1			[17]
Diuron	**150-300**	**15-20**	**≥ 5000**	**100**	**30-50**	**4500-5000**	**0.5-1**	**2000-2500**	**1000**	
DNOC	500	3000	5000	5000	5000	1500	20	>5000	5000	[9]
Dodine		30	>10000	1500	>5000	>5000	300	5000		[3]
Endosulfan				50						[17]
		300		40		>5000	3000	3000	3000	[4]
	5000	300	>5000	50	>5000	5000	5000	>5000	>5000	[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		300		50						[7]
	5000	300	>5000	50	>5000		>5000	5000	>5000	[9]
		300		40		>5000	3000	4000	3000	[4]
			>5000		>5000	5000	250	>5000	>5000	[11]
			>5000		>5000	5000	250	>5000	>5000	[11]
Endosulfan	**5000**	**300**	**>5000**	**40-50**	**>5000**	**≥ 5000**	**3000**	**4000-5000**	**3000**	
Epoxiconazole	5000	1000	>5000	2500	>5000	50	>5000	5000	>5000	[8]
						25				[16]
EPTC	5000	300	>5000	5000	>5000		5000	500	500	[9]
	5000	300	>5000	>5000	>5000	>5000	5000	500	500	[8]
Esfenvalerate	2500	1000	>5000	500	>5000	>5000	>5000	1000	>5000	[8]
Ethalfluralin	100	>5000	5000	>5000	100	>5000	500	1000	>5000	[8]
Ethephon	5000	5000	>5000	5000	>5000	>5000	5000	5000	1000	[8]
Ethirimol	1000	100	>5000	1000	>5000	5000	5000	1000	2500	[9]
Ethoxyfen	500	>5000	>5000	100	>5000	>5000	500	5000	1000	[8]
Ethoxyquin	500	500	5000	500	5000	5000	1000	500	500	[9]
Etrimfos	2500	500	>5000	5000	>5000		5000	5	10	[9]
	2500	500	>5000	>5000	>5000	>5000	5000	5	10	[8]
		400	>5000	5000	>5000	>5000	4000	5	10	[4]
Fenarimol	150	200	>5000	250	>5000	50	5000	>5000	500	[8]
	150	200	>5000	500	>5000	50	5000	>5000	500	[9]
						25				[18]
						50				[6]
						1				[15]
						50				[5]
			>5000	200	>5000	50		>5000		[11]
		200			>1000	100	>1000	>1000	>1000	[1]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		20	5000	500	5000	75	4000	4000	500	[12]
										[10]
						75				[16]
Fenarimol	**150**	**200**	**≥ 5000**	**200-250**	**≥ 5000**	**25-50**	**4000-5000**	**4000-5000**	**500**	
Fenchlorazole-ethyl	500	5000	>5000	100	250	>5000	1000	1000	500	[8]
Fenitrothion								10		[5]
								5		[2]
	1000	5000	>5000	>5000	>5000	2500	2000	2.5	2.5	[8]
	1000	5000	>5000	1000	>5000		>5000	5	5	[9]
	600	3000		1000	>5000		3000	5	10	[14]
					5000			6		[13]
					150			15	5	[7]
		3333.3	>10000	1000	>5000	5000	2500	5	5	[3]
		>5000	>5000	2500	5000		>5000	5	5	[11]
Fenitrothion	**600-1000**	**3000**	**>5000**	**1000**	**≥ 5000**		**2000-2500**	**5**	**2.5-5**	
Fenitrothion-o	>125	50	>125	125	>125	125	>100	10	5	[9]
Fenoxaprop-P-ethyl	250	5000	500	500	250	5000	5000	1000	1000	[8]
Fenpropimorph	5000	100	>5000	>5000	>5000	2500	>5000	500	1000	[8]
Fenthion	1000	5000	>5000	100	>5000		>5000	50	5	[9]
	600	5000		50			2000	50		[14]
									5	[6]
Fenthion-o	>12.5	>10	>12.5	12-May	>12.5	>12.5	>10	>12.5	10	[9]
Fenuron	100	100	>5000	>5000	100	>5000	2	2500	2500	[8]
Fluroxypyr	500	100	>5000	100	>5000	5000	250	1000	500	[8]
Flusilazole	5000	500	>5000	>5000	>5000	50	5000	>5000	>5000	[8]
Flutriafol	2500	5000	>5000	>5000	>5000	25	5000	5000	2500	[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Folpet	2500	1500	>5000	100	>5000	20	>5000	500	500	[8]
	2500	1500	>5000	200	>5000	30	>5000	500	500	[9]
		3000	>10000	100	>5000	120	1800	5000	600	[3]
Formothion	5000	1000	>5000	>5000	>5000	>5000	5000	500	1000	[8]
Fuberidazole	100	5000	>5000	>5000	>5000	500	100	250	500	[8]
Furathiocarb	200	3000	200	1500	5000		0.75	200	1000	[14]
Haloxyflop	5000	1000	>5000	5000	>5000	5000	1000	500	1000	[9]
HCB	5000	2500	>5000	50	>5000	1000	>5000	>5000	>5000	[9]
Heptachlor	>5000	5000	>5000	100	>5000	>5000	5000	5000	>5000	[9]
Heptenophos		4000		1000		>5000	3000	30	100	[4]
Hymexazol	2500	>5000	>5000	>5000	>5000	2000	>5000	1000	5000	[8]
Imazalil		100				100				[17]
		33.3	>10000	250	>5000	200	4000	>5000	2000	[3]
	2500	100	>5000	100	>5000	15	1000	1000	1000	[8]
	2500	100	>5000	100	>5000	100	1000	1000	1000	[9]
		60	>5000	100	>5000	80	1000	1000	1000	[4]
		100			>1000	100	>1000			[1]
		100		100	5000	75	1000	1000	1000	[12]
Imazalil	**2500**	**30-60**	**>5000**	**100**	**≥ 5000**	**75-80**	**1000**	**1000**	**1000**	
Imazamethabenz-me	100	100	>5000	>5000	>5000	>5000	>5000	>5000	1000	[8]
Imidacloprid	100	100	>5000	1000	2500	>5000	>5000	>5000	5000	[8]
Ioxynil		50					1		500	[12]
Iprodione	5000	3000	>5000	1000	5000	300	2500	5000	2500	[9]
		2400		1000		240	2500	4000	3000	[4]
		3000	2500	1000	5000	300	250	4000	1000	[12]
Isazofos	1000	500		5000	5000		4000	5	500	[14]
		3000						20		[7]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Isoproturon							2			[15]
	250	100	>5000	>5000	>5000	5000	1	5	500	[8]
Isoxaben	1000	100	>5000	>5000	>5000	5000	2000	500	500	[8]
Lenacil		200	5000	1000	5000	4000	1	2000	3000	[12]
	1500	200	2500	500	>5000		1	1000	5000	[9]
Lindane	5000	500	>5000	50	>5000		1000	>5000		[9]
	5000	500	>5000	25	>5000	>5000	1000	>5000	>5000	[8]
	5000	550		50				3000	5000	[14]
		500		5						[7]
Lindane	**5000**	**500**	**>5000**	**25-50**	**>5000**		**1000**	**≥ 3000**	**≥ 5000**	
Linuron							1			[17]
							1			[16]
	250	300	>5000	50	100		1	5000	5000	[9]
	250	300	>5000	100	100	1000	1	5000	5000	[8]
										[1]
							0.75			[6]
		300								[2]
Linuron		**300**					**0.75-1**			
Malathion	5000	1000	>5000	50	>5000		1000	10	10	[9]
	5000	100	>5000	>5000	>5000	>5000	1000	10	10	[8]
		1000	>5000	50	>5000	>5000	800	10	8	[4]
							2200			[13]
		1500						10	10	[7]
		1000	>10000	>5000	>5000	5000	>5000	20	10	[3]
		1500	>5000		>5000	10	500	30	10	[11]
Malathion		**1000-1500**	**>5000**		**>5000**	**≥ 5000**	**500-1000**	**10-20**	**8-10**	
MCPA	1000	500	>5000	500	>5000	>5000	500	500	1000	[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Mecarbam	>250	200	>250	250	>250	>250	250	100	10	[9]
Mecoprop	2500	500	>5000	500	>5000	>5000	5000	250	500	[8]
Mecoprop-P	5000	2500	>5000	500	>5000	>5000	>5000	1000	1000	[8]
Metalaxyl	5000	1000	>5000	500	>5000		>5000	5000	5000	[9]
		1000	5000	500	5000		4000	5000	2500	[12]
	5000	800		500			>5000	5000	5000	[14]
Methabenzthiazuron	250	200	>5000	5000	5000		5	5000	2500	[9]
Methamidophos		10	2500	2500	>5000	>5000	>5000	2500		[3]
Methidathion								56		[6]
	1000	5000	>5000	50	5000		>5000	75	100	[9]
	1000	5000	>5000	>5000	5000	5000	2000	75	100	[8]
	1000	5000		50		>5000	3000	75	100	[14]
Methidathion	**1000**	**5000**						**75**	**100**	
Methomyl									5	[18]
									5	[16]
								40		[15]
	1500	100	300	5000	>5000		>5000	100	10	[9]
	1500	100	300	>5000	>5000		>5000	100	10	[8]
	1500	100		3000			4500	100	15	[14]
		50							5	[6]
		100	300					110		[13]
Methomyl	**1500**	**50-100**	**300**	**3000-5000**			**4500-5000**	**100**	**5-10**	
Methoxychlor				50						[2]
Metobromuron	300	300	4000	100	100	>5000	0.5	1000	1000	[14]
	480	300	>5000	200	100		1	5000	1000	[9]
		200					0.5			[7]
Metobromuron		**200-300**					**0.5-1**			

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Metolachlor	1000	>5000	>5000	100	>5000	>5000	3000	1000	1000	[8]
Metoxuron							2			[15]
							2.5			[16]
							2.5			[6]
		100			50	>1000				[1]
	480	100	>5000	100	50		10	2000	1000	[9]
							5			[5]
	480	100	>5000	200	50	>5000	5	2000	1000	[8]
							2.5			[18]
Metoxuron		**100**			**50**		**2.5-5**			
Metribuzin		20					1			[17]
	1000	400	5000	5000	5000		1	5000	2500	[9]
		400		5000		>5000	0.8	4000	2500	[4]
		400	5000	5000	5000		1	3000	2000	[12]
		3000	>10000	5000	4000	5000	0.05	>5000	3000	[3]
Metribuzin		**400**		**5000**	**4000-5000**		**0.05-1**	**3000-5000**	**2000-3000**	
Mevinphos	2500	5000	2500	5000	>5000		>5000	5	10	[9]
			5000							[13]
			>5000	>5000	>5000		5000			[11]
		>1000			>1000	>1000	>1000		10	[1]
		5000	>10000		>5000	5000	>5000	1000	10	[3]
Mevinphos					**>5000**		**≥ 5000**		**10**	
Molinate	5000	100	>5000	>5000	>5000	>5000	2000	1000	1000	[8]
Monocrotophos									15	[6]
		>1000			>1000	>1000	>1000		5	[1]
	2000	100	5000	5000	>5000		>5000	250	5	[9]
	1500	80	3000	3000	>5000		3700	250		[14]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		300	5000	>5000	>5000		>5000			[11]
		300						250	5	[7]
		150	5000		5220					[13]
Monocrotophos		**80-150**	**3000-5000**	**3000-5000**	**>5000**		**3700-5000**	**250**	**5**	
Monolinuron	250	300	5000	50	100		5	5000	5000	[9]
Myclobutanil						50				[8]
Napropamide	1000	1000	>5000	5000	>5000	5000	1000	5000	1000	[9]
Nitrofen	1000	5000	>5000	100	5000	5000	2500	5000	5000	[9]
Nuarimol	600	500	5000	100	>5000	100	>5000	5000	5000	[9]
Omethoate	5000	500	5000	5000	5000		>5000	2500	1000	[9]
Oxadiazon	1000	500	>5000	100	>5000		1000	5000	5000	[9]
	500	300		50	5000		700	2000	3500	[14]
Oxadixyl	2500	2500	>5000	5000	5000	>5000	>5000	>5000	5000	[9]
Oxamyl		50						2	2	[18]
		50	5000	5000	5000	5000	4000	10	2	[12]
		100						10		[5]
		75						7.5	1	[6]
	2000	100	5000	>5000	5000	>5000	>5000	10	2	[8]
		90		25				10		[15]
	2000	100	5000	5000	5000		>5000	10	2	[9]
		100	>5000	5000	>5000		>5000			[11]
		66.7	>10000	2500	> 5000	>5000	>5000	10	2	[3]
		100						50	1	[16]
Oxamyl		**50-100**	**5000**	**2500-5000**	**5000**	**≥ 5000**	**4000-5000**	**7.5-10**	**1-2**	
Parathion	1000	5000	>5000	1000	>5000		1000	0.5	1	[9]
								2		[5]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Parathion-me								2		[2]
								1	2	[15]
	1500	5000	>5000	1000	>5000		>5000	1	2	[9]
					5000					[13]
	1500	5000	>5000	>5000	>5000	5000	>5000	2	2	[8]
		5000			150			40	4	[7]
		5000		1000		5000	5000	1	2	[4]
		5000	5000	1000	5000	5000	4000	1	1	[12]
	1500	4000	5000	1000	>5000	>5000	2600	0.5	1	[14]
		5000	>10000		>5000	5000	>5000	1	2	[3]
								0.75	1	[6]
			5000	1000	>5000	1	5000	5		[11]
								10	1	[18]
		>1000			>1000	>1000	>1000	1	2	[1]
								2		[5]
									1	[16]
Parathion-me	**1500**	**4000-5000**	**≥ 5000**	**1000**	**≥ 5000**	**≥ 5000**	**2600-4000**	**0.5-2**	**1-2**	
Penconazole		5000		5000			6000	5000	1000	[12]
						25				[8]
Pendimethalin	250	100	5000	>5000	250	>5000	>5000	25	250	[8]
Pentachlorophenol	1000	1000	>5000	500	>5000	1000	100	500	5000	[9]
Phenkapton	1000	5000	>5000	200	>5000		>5000	5	5	[9]
	1000	5000	>5000	500	>5000		>5000	5	5	[8]
Phenmedipham	1000	100	50	5000	100		1	100	50	[9]
Phorate	5000	1000	>5000	>5000	>5000	>5000	5000	10	10	[8]
Phosalone								3.75		[6]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
	2500	5000	500	1000	>5000		>5000	5	5	[9]
		>1000			>1000	>1000	>1000		2	[1]
		5000	5000	1000	4000	2000	4000	5	100	[12]
									2.5	[16]
		5000		1000		>5000	4000	5	5	[4]
Phosalone		**5000**		**1000**	**4000-5000**		**4000**	**4-5**	**2-5**	
Phosphamidon	2500	5000	>5000	500	>5000		>5000	250	50	[9]
	2500	5000	>5000	250	>5000	>5000	>5000	250	50	[8]
	2500	4000		500	5000		4000	250	50	[14]
									25	[16]
Phosphamidon	**2500**	**4000-5000**		**250-500**	**≥ 5000**		**4000-5000**	**250**	**25-50**	
Pirimicarb		300	50	500	5000	4000	5000	100	50	[12]
	500	300	50	>5000	5000	>5000	5000	100	100	[8]
	500	300	50	1000	5000		5000	100	100	[9]
			50					100		[17]
		300		800		>5000	4000	100	80	[4]
		300	500		1000		>5000			[11]
									125	[6]
		300		5000	>5000	5000	1800	5000	100	[3]
Pirimicarb		**300**	**50**	**500-1000**	**≥ 5000**	**4000-5000**	**4000-5000**	**100**	**50-100**	
Pirimiphos-me	100	500	5000	>5000	>5000	>5000	1000	50	100	[8]
		4000	4000	1000	>5000	5000	5000	50	5	[4]
		500						200	8	[7]
		500								[17]
								50		[16]
Pirimiphos-me		**500**						**50**		

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Pirimisulfuron-me	100	25	>5000	>5000	2500	>5000	5000	>5000	500	[8]
Prochloraz	5000	100	>5000	500	>5000	10	5000	1000	1000	[8]
						13				[18]
						19				[6]
						25				[16]
						16				[15]
						25				[5]
Prochloraz						**10-25**				
Procymidone	2000	5000	>5000	250	5000	500	>5000	>5000	5000	[9]
		4000		250		400	>5000	>5000	5000	[4]
Prometryn	500	100	5000	500	>5000		1	>5000	1500	[9]
	200	50	5000	500	5000		0.6	>5000	1500	[14]
										[1]
		75	5000	100	5000		1	5000	1000	[12]
		80		400		>5000	0.8	5000	1500	[4]
		20					1			[17]
Prometryn		**50-80**	**5000**	**400-500**	**5000**		**0.6-1**	**5000**	**1000-1500**	
Propachlor	2500	5000	>5000	250	>5000	5000	5000	>5000	5000	[8]
	2500	5000	>5000	100	5000		>5000	>5000	5000	[9]
Propanil	100	300		150	100	5000	0.75	4	1500	[14]
Propargite	2500	5000	5000	5000	>5000		>5000	1000	5000	[9]
Propham	2000	50	5000	>5000	500		500	>5000	2500	[8]
	2000	50	5000	100	500		500	>5000	2500	[9]
Propiconazole (1)	2500	1000	>5000	1000	>5000	50	500	2500	1000	[8]
Propisochlor	2000	5000	>5000	250	>5000	>5000	1000	5000	1000	[8]
Propoxur		250	>300	300				200	5	[7]
Prosulfuron	2500	10	>5000	>5000	>5000	>5000	5000	1000	>5000	[8]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
Prothiofos	5000	2500	>5000	100	>5000	5000	1000	200	500	[9]
Pyridate	200	5000	>5000	>5000	>5000	5000	200	1000	500	[8]
Quinalphos									5	[15]
	500	>5000	>5000	>5000	>5000	1000	>5000	0.5	0.5	[8]
Quinchlorac	1000	1000	>5000	>5000	>5000	>5000	1000	500	5000	[8]
Rimsulfuron	500	100	>5000	>5000	2500	>5000	5000	5000	1000	[8]
Simazine							3			[5]
	800	50	5000	250	>5000		1	>5000	5000	[9]
	800	50	5000	250	>5000	>5000	1	>5000	5000	[8]
	1000	25		200			0.6	2500	5000	[14]
		25		200		>5000	0.8	>5000	>5000	[4]
		50			>1000	>1000	1		>1000	[1]
							1			[6]
Simazine	**800-1000**	**25-50**		**200-250**	**>5000**	**>5000**	**0.6-1**	**≥ 5000**	**≥ 5000**	
Sulcotrione	100	5000	>5000	2500	>5000	>5000	5000	500	1000	[8]
Tebuconazole	5000	2500	>5000	250	>5000	50	1000	5000	5000	[8]
	5000	2500	>5000	1000	>5000		1000	5000	5000	[9]
						50				[5]
Teflubenzuron	100	100	>5000	500	1000	>5000	>5000	100	1000	[8]
		50								[16]
Tefluthrin	5000	>5000	>5000	500	>5000	>5000	5000	1000	5000	[8]
Terbuthylazine		20					1			[17]
	500	50	5000	250	>5000		1	5000	1000	[9]
	500	25		250			0.6	5000	2000	[14]
Terbuthylazine		**20-50**					**0.6-1**			
Terbutryn		50					1			[17]
	800	100	5000	500	>5000		1	>5000	>5000	[9]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
	800	100	5000	>5000	>5000	5000	1	>5000	2500	[8]
Terbutryn		**50-100**					**1**			
Tetraconazole	5000	5000	>5000	500	>5000	500	5000	2500	1000	[8]
Tetradifon	250	5000	>5000	200	>5000		5000	5000	>5000	[9]
Tetrasul	500	5000	>5000	100	>5000	5000	>5000	5000	>5000	[9]
Thiabendazole	250	1000	>5000	50	>5000	200	100	5000	5000	[9]
	250	1000	>5000	>5000	>5000	200	100	5000	5000	[8]
							50			[18]
		1000			>1000		>1000	250		[1]
							150			[16]
							150			[6]
Thiabendazole		**1000**			**>5000**		**50-150**			
Tifensulfuron-me	1000	50	>5000	>5000	>5000	>5000	5000	5000	>5000	[8]
Thiocyclam	1000	100	>5000	>5000	>5000	250	>5000	1000	2500	[8]
Thiometon	5000	5000	>5000	50	5000	5000	1000	100	5000	[9]
Thiophanate-me			5000		5000		>5000	1000		[13]
	250	50	5000	>5000	>5000	100	5000	1000	500	[8]
	250	200	5000	200	5000	100	5000	1000	500	[9]
		50				150				[6]
		400	5000		5000	100	>5000			[11]
Thiophanate-me					**≥ 5000**	**100-150**	**5000**	**1000**		
Thiram	100	5000	5000	>5000	>5000	50	>5000	250	1000	[8]
Tralkoxydim	1000	1000	>5000	>5000	5000	>5000	50	1000	500	[8]
Triadimefon	5000	500	>5000	>5000	>5000	2500	>5000	2500	500	[8]
Triasulfuron	1000	100	>5000	>5000	>5000	>5000	>5000	2500	>5000	[8]
Triazophos	500	1000	>5000	>5000	>5000	5000	500	1	0.2	[8]
	500	1000	>5000	200	>5000		500	1	0.2	[9]

	MDQ (ng)									
Active ingredient	$HF_{(254)}$	o-TKI	NBFB	AgUV	p-DB	FAN	Hill	EßNA	EAcI	Ref.
		1000			>1000		>1000		0.2	[1]
								2		[16]
Triazophos		**1000**			**>5000**			**1-2**	**0.2**	
Tribenuron-me	2500	500	>5000	>5000	>5000	5000	2500	>5000	>5000	[8]
Trichlorfon	5000	>5000		80			>5000	500	100	[14]
	5000	>5000	5000	100	>5000		>5000	500	100	[9]
Tridemorph (1)	>5000	500	>5000	>5000	>5000	5000	>5000	1000	2500	[8]
Trifluralin	800	500	5000	1000	250		1000	2500	50	[9]
		500			500	>1000	>1000	>1000	1000	[1]
		500		1000	300	1500	1000	1000	100	[12]
		500	>10000	>5000	250	5000	1000	1000	50	[3]
		400		1000		>5000	1000	2000	100	[4]
Trifluralin		**400-500**		**1000**	**250-300**		**1000**	**1000-2000**	**50-100**	
Triforine	5000	100	>5000	50	>5000	100	>5000	5000	>5000	[8]
				160						[5]
				50						[18]
				20						[15]
				250						[6]
Triforine				**20-50**						
Vinclozolin	5000	1000	>5000	500	500	600	>5000	5000	500	[9]

(a) The α,β and ζ isomers cannot be separated in this elution system. The MDQ range given includes the values reported either for single isomers, where available, or those obtained with mixture of isomers. For practical purposes there is no need to distinguish them.

4. EVALUATION OF THE RESULTS

The within-laboratory reproducibility of Rf and RRf values enables the laboratory to establish a "window" (usually $\pm 2*CV_r*Rf_{mean}$) within which a pesticide residue may be detected, and select those compounds, taking into account their detectability as well, that may be present in a sample.

The between-laboratories reproducibility of MDQ and RRf values provide information on the applicability of the TLC detection methods in another laboratory based on the data base generated within this research project.

The Rf values obtained from individual measurements vary substantially between laboratories, therefore the mean Rf and RRf values obtained on different plates should be reported together with their *within-laboratory* reproducibility.

The very good correlation of Rf values obtained in two laboratories (Figure 3) ($R^2 = 0.974$) indicates the possibility of transformation of the data base of average retentions obtained in one laboratory to another laboratory operating under different environmental conditions. However, the actual Rf values must be verified in the laboratory at the time of the identification/confirmation of suspected compounds.

The TLC separation and detection of various pesticide residues can be carried out by experienced analysts in one laboratory with appropriate reproducibility that enables the use of the TLC methods described for cost effective screening large number of samples for specified compounds, or for the confirmation of the analytes identified tentatively with GC and HPLC detection.

TLC is especially useful for the confirmation of compounds which cannot be detected with GC and a HPLC/MS (preferably MS/MS) instrument is not available.

The drawback of the simplicity of the technique is that it may be necessary to develop several plates and apply different detection methods for the confirmation of an analyte in a complex mixture, which is time consuming and labour intensive. Further, it is not applicable for all compounds.

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DETERMINATION OF PESTICIDE RESIDUES IN CEREAL GRAINS

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Abstract

The applicability of the TLC for determination of pesticide residues in cereal grains was studied using corn, rice and wheat as representative commodities and atrazine, captan, chlorpyrifos, chlortoluron, diazinon, diuron, fenitrothion, metoxuron, prochloraz, triforine as representative compounds. Following the extraction with ethyl acetate the efficiency of extraction was tested with Bio-Rad SX-3 gel, GPC, silica gel, florisil and RP-18 reverse phase silica cartridge. The GPC alone or in combination with silica or florisil cleanup were the most suitable for cleanup of the extracts. The TLC elution characteristics of 131 pesticide active ingredients were tested with eight elution systems. The detectability of the selected compounds was determined with six detection methods including two chemical and four bioassay procedures. In addition to the basic methods, the non-toxic *Penicillium cyclopium* fungi spore inhibition was introduced and it was found very sensitive for some fungicide compounds. The minimum detectable quantities of the tested compounds ranged from 1 ng to 100 ng. The average recoveries from rice and wheat ranged from 78% to 89%, and the limits of quantitation, LOQ, were between 0.01 and 0.2 mg/kg for the selected ten compounds.

1. INTRODUCTION

The gas-liquid chromatographic (GLC) or high-pressure liquid chromatographic (HPLC) determinations of the pesticide residues alone do not provide their selective separation and detection in order to confirm the identity of the analytes present in the sample. The identity of the residues must be confirmed with additional methods. The mass spectrometric (MS) detection would provide the most convenient tools for confirmation, but this technology, especially LC/MS, is not available in many laboratories or the laboratory infrastructure does not make possible their continuous use.

The objective of this work was to investigate the applicability of TLC detection technique for the determination of pesticide residues in cereal grains. The basic methods described by Ambrus et al. [1] were used in combination with additional cleanup procedures and GC determination of the residues. In our experiments corn, rice and wheat were used as representative commodities and 38 pesticides of 12 chemical classes were selected as representative compounds.

2. MATERIALS AND EQUIPMENT

The chemicals, materials and equipment required for the basic procedures are described elsewhere [1]. The additional ones are listed below.

Acetone, LiChrosolv
Citric acid
Magnesium sulphate

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Potato dextrose agar
Sodium chloride
Penicillium cyclopium spore concentrate
LiChrolut Si (500 mg) cartridges
LiChrolut RP-18 (500 mg) cartridges
Mashed potato powder
Para film

Pesticide analytical standards were obtained from the manufacturers with quality certificate or they were purchased from Dr. Ehrernstorfer GmbH, Germany. All chemicals were analytical grade and checked for their purity before use. If interfering spots occurred the solvents were purified or replaced with a proper quality product.

Gas chromatograph

The gas chromatograph used was a Packard 428 GC with ECD and NPD detectors. The columns used were: 9.5 m × 0.25 mm CP Sil 5 CB, df = 0.12 μm and 14.5 m × 0.53 mm CP Sil 8 CB, df = 1.5 μm. Injector and detector temperatures were 230^{o}C and 290^{o}C, respectively. The column was operated isothermally at 170 or 210^{o}C; or programmed from 70^{o} at the rate of 10^{o}C/min to 205 or 260^{o}C. The injected volume of the sample was 1 μl.

3. METHODS

The extraction of samples, the GPC and Si-cartridge cleanup, the TLC elution and detection were carried out as described in the basic method [1].

3.1. Cleanup

We studied the loadability of GPC columns. The applicability of Si-cartridge, different types of florisil, neutral alumina and RP-18 reversed phase cartridge were also examined for use as second cleanup after the GPC.

3.1.1. Loadability of GPC column

The KL SX-3 gel chromatograph with 200 mm ×. 10 mm glass column packed with Bio-Beads SX-3 200-400 mesh gel was used in our experiments. In order to establish the elution profile, the amount of oil and dry materials in corn, rice and wheat samples were determined. Twenty grams of samples were extracted with ethyl acetate according to the basic procedure [1]. The extracts were evaporated with a rotary evaporator to oil. The amount of oil and dry material was determined by weighing.

For determining the loadability of the column, sample portions of 10, 20, 30 and 40 g were extracted. The amounts of ethyl acetate, sodium sulphate and sodium hydrogen carbonate were increased in direct proportion to the sample portion weight. The filtered extract was concentrated to 500 μl and transferred to the GPC column. The first 9 ml eluate (cyclohexane: ethyl acetate 1:1) was discarded (first fraction). The pesticide active ingredients were eluted in the second (pesticide) fraction (10-20 ml). The eluate was evaporated to almost dryness and taken up in one ml acetone for TLC or GC analysis. The load of the column was acceptable until it did not affect the limit of detection, LOD, of the compounds.

3.1.2. Silica gel cartridge

The evaporated pesticide fraction from the GPC cleanup was dissolved in cyclohexane and 10 g sample equivalent was transferred into the 500 mg Silica (LiChrolut Si) cartridge and eluted with 15 ml toluene:cyclohexane 15:85 v/v (1st fraction) followed by 25 ml toluene:cyclohexane:acetone 60:30:10 v/v (2nd fraction).

3.1.3. Florisil column

The following combinations were tested:

I 4 g super active florisil, with an eluent: 50 ml dichloromethane:acetone (8:2).

II 5 g super active florisil, with an eluent: 80 ml n-hexane:dichloromethane (6:4).

III 2 g deactivated florisil (with 5% water), with two eluents. Eluent I.: 20 ml n-hexane: dichloromethane (7:3) discarded. Eluent II. 20 ml dichloromethane:acetone (95:5).

IV 2 g deactivated florisil (with 10 % water) with an eluent: 12 ml dichloromethane: acetone (6:4).

The chromatographic column (10 mm i.d.) was filled with florisil and topped with anhydrous sodium sulphate (1 cm). One ml mixture of the selected active ingredients was transferred into the column in n-hexane:dichloromethane (7:3). The pesticides were eluted. Each eluate was evaporated and the residue was dissolved in acetone:n-hexane (1:1) for analysis.

3.1.4 LiChrolut RP-18 (C-18) cartridge

The cartridge was conditioned with 2 × 3 ml n-hexane. The mixture of the selected compounds was transferred to the cartridge in 1 ml mixture of n-hexane:acetone (1:1). The solvents were passed through the column. One ml n-hexane was transferred to the column. After a two-minute stabilization period, the elution was started with a flow rate of 1 drop/sec, and continued with another 1 ml n-hexane (1st fraction). The column was dried under vacuum. One ml methanol was transferred to the column. After two minutes the elution was started with a flow rate of one drop/sec, and continued with another 1 ml methanol (2nd fraction). The fractions were evaporated under vacuum to near dryness and the residues were dissolved in acetone for the GC and TLC analysis.

3.2. TLC Separation

A spotting plan was prepared and recorded in the lab-book in advance with the amounts of substances to be applied in the order of spots numbered from 1–11 starting at the left corner of the plate. Using a micro-syringe and spotting guide 10–20 µl solutions were applied in spots of 3–4 mm diameter along the line at 2 cm from the bottom edge of the plate. This was considered as a compromise for the optimum for both qualitative and quantitative determination. The same volume from the extracts as well as from the standard solutions was applied carefully to avoid damaging of the layer with the needle.

A filter paper was placed into the developing tanks for 30 min before eluting the plates to obtain saturated vapour phase in the tank. The tank was filled with the developing solvent to obtain one cm immersion depth for the plate. The developing tank was placed into a water basin for improving the reproducibility of retention values. By following this procedure the

temperature of the developing tank could be kept within ± 2°C during one day. The plates were eluted up to 11 ± 0.5 cm from the start point. The volume of the mobile phase was adjusted after each elution. If solvent mixture was used, the mobile phase was replaced after each elution to keep its composition constant.

The Rf values were determined in the following chromatographic systems:

Layer: Silica gel 60, 0.25 mm (Merck: 1.05713.0001), activated at 105°C for 30 minutes before use.

System I	Silica gel 60 - Ethyl acetate (Si-E)
System II	Silica gel 60 – Dichloromethane (Si-D)
System III	Silica gel 60 – Benzene (Si-B)
System IV	Silica gel 60 - n-Hexane:Diethyl ether (1:2) (Si-H/De)

Layer: Aluminium oxide 60 G neutral (Merck: 1.01090.0500), self prepared 0.25 mm layer, dried at 75°C for 45 minutes after preparation and stored over activated silica gel until use.

System V	self-made Aluminium oxide - Ethyl acetate (s.m. A-E)
System VI	self-made Aluminium oxide - Dichloromethane (s.m. A-D)

Layer: Aluminium oxide 60 F_{254}, 0.25 mm (Merck: 1.05713.0001) activated at 75°C for 15 minutes before use.

System VII	Aluminium oxide 60 F_{254}-Ethyl acetate (A-E)
System VIII	Aluminium oxide 60 F_{254}-Dichlormethane(A-D)

3.3. Detection of the analytes

The analytes were detected with:

o-Tolidine + potassium iodide [o-TKI],
Photosynthesis inhibition [Hill],
Fungi spore inhibition [FAN],
Enzyme inhibition (with cow liver extract) [EβNA],
Enzyme inhibition (with pig serum) [EAcI], and
Silver nitrate + UV exposition [AgUV].

In addition to the *Aspergillus niger,* the applicability of fungi spore *Penicillium cyclopium* was also tested.

Method 6/B Fungi spore (*Penicillium cyclopium)* inhibition (FPC).

Specificity: selectively detects some fungicides, plant extracts usually do not interfere.

Reagents: Fungi culture media; boil 5 g mashed potato powder in 250 ml water for one hour. Add 5 g glucose and 5 g potato dextrose agar to the solution and boil it for 15 minutes. Adjust the pH of the solution to 3.5–4.5 with 5% of citric acid. Pour 8–10 ml of culture media to sterilized Petri dishes and cool them to room temperature. Cover them with another Petri dish containing fungi culture and with gentle hitting transfer some fungi spores to the surface of fresh media. Cover the Petri dish with its own lid. Place the Petri dishes in an incubator containing air saturated with water vapour at 25°C. The new culture develops within five days. Pack the Petri dishes in plastic foil and keep them in refrigerator. The culture has to be re-inoculated at least in every 4–5 months.

Suspension of fungi spores: dissolve 7 g potassium dihydrogen phosphate, 3 g disodium hydrogen phosphate, 4 g potassium nitrate, 1 g magnesium sulphate and 1 g sodium chloride in one litre distilled water. Add 2.5 g glucose to 50 ml portion of the solution and wash off the spores from the fungi culture, being in the Petri dish, with the solution. This suspension is enough for two plates. Incubate the plates as described under Method 6 [FAN] [1].

Note: *Penicillium cyclopium* is not toxic fungi.

3.3.1. Testing the Limit of Detection

The minimum detectable quantities of analytes [MDQ, ng], defined as the minimum amount of analytical standard which gives a distinct spot on the TLC plate, following their elution and detection.

The limit of detection [LOD, mg pure analyte/kg sample] is defined as the lowest amount of the analyte, which can be detected on the plate in the presence of the co-extractives from the sample matrix. The procedure is illustrated in Figure 1 for the determination of the LOD for atrazine.

Atrazine	**MDQ** (HILL): 1 ng				
Spotted sample equivalent [mg]	5	10	20	40	100
	⇩	⇩	⇩	⇩	⇩
Spotted atrazine [ng]	1	1	1	1	1
Visibility of analyte	yes	yes	yes	no	no
LOD, [mg/kg]	0.2	0.1	**0.05**	**--**	**--**

FIG. 1. Illustration of the determination of limit of detection of atrazine.

Twenty µl of the concentrated extracts, containing 5, 10, 20, 40, and 100 mg sample equivalent, were spotted to the TLC layer. Five µl of atrazine standard solution (1 ng atrazine) were spotted over the spots of the samples. The plates were developed with ethyl acetate. After drying the plates, the compounds were detected with Hill reaction. The spot of one nanogram atrazine was visible in the presence of 5, 10 and 20 mg sample. Because the spot of 1 ng atrazine could not be seen where 40 and 100 mg sample matrix were applied, the limit of detection (LOD) was estimated to be 0.05 mg/kg.

3.3.2. Determination of limit of quantitation [LOQ, mg/kg]

Untreated rice and wheat samples were spiked with 10 compounds chosen from those which can be sensitively detected with various methods. The levels of the fortification were normally the LOQ, maximum residue limit (MRL) and 2 x MRL. (e.g. atrazine, chlorbromuron, chlorpyrifos, diazinon, fenitrothion and prochloraz). For captan and metoxuron the fortification levels were LOQ, 2 x LOQ and 5 LOQ, because they did not have MRLs in the tested grains. For diuron the fortification levels were LOQ, 5 x LOQ and 10 x LOQ. Spiking with triforine was carried out at LOQ and 2 x LOQ, because its MRL was the same as its LOQ.

4. RESULTS AND DISCUSSION

4.1. Loadability of GPC Column

The oily dry materials calculated for 10 g sample equivalent were: corn 234 mg, rice 34 mg and wheat 111 mg. The gel column was loaded with 100 mg co-extractives, obtained from the sample materials, dissolved in 0.5 ml cyclohexane:ethyl acetate 1:1. The average elution patterns based on three replicate measurements are shown in Table 1.

TABLE 1. DISTRIBUTION OF THE OIL AND DRY MATERIALS OF CORN, RICE, AND WHEAT SAMPLES, AND DIAZINON AND TRIAZOPHOS ON BIO BEADS SX-3 GEL COLUMN

Eluents (ml)	Rice 100 mg	Wheat 100 mg	Corn 100 mg	Diazinon 10 μg	Triazophos 10 μg
2	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0
5	6.5	6.9	**0.3**	0.0	0.0
6	15.0	11.9	**8.2**	0.0	0.0
7	19.8	20.0	**18.4**	0.0	0.0
8	26.7	25.7	**26.7**	0.0	0.0
9	16.5	20.7	**20.8**	0.0	0.0
10	10.3	9.5	**15.0**	5.2	0.5
11	4.6	4.8	**9.0**	25.3	6.0
12	0.5	0.5	**1.6**	49.0	35.1
13	0.0	0.0	0.0	12.3	33.2
14	0.0	0.0	0.0	4.2	13.7
15	0.0	0.0	0.0	3.0	7.8
16	0.0	0.0	0.0	0.5	1.9
17	0.0	0.0	0.0	0.5	1.1
18	0.0	0.0	0.0	0.0	0.8
19	0.0	0.0	0.0	0.0	0.0
Sum	*99.9*	*100*	*100*	*100*	*100*

The results indicate that the pesticide fraction was between 10th and 17th ml of the eluent. However, 25.6%, 15.4%, 14.8% of co-extractives remained in the pesticide fraction from corn, rice, and wheat, respectively.

The LOD values of marker compounds obtained after the GPC cleanup are shown Table 2.

The loadability of the GPC column varied depending on the detection method and the analyte. We concluded that the column could be generally loaded with the concentrated ethyl acetate extract of 10 g sample equivalent. For detection with Hill reaction and FAN, a load of 20 g sample equivalent was possible.

After GPC cleanup, the LOD values of some compounds did not allow the detection of residues at the Codex or Hungarian MRL values, therefore the possibility of the inclusion of a second cleanup step in the procedure was examined.

TABLE 2. THE LOD VALUES OF MARKER COMPOUNDS OBTAINED AFTER THE GPC CLEANUP

Compounds	TLC Method	MDQ [ng]	LOD [mg/kg] after GPC		
			Rice	Wheat	Corn
Atrazine	o-TKI	25	0.2	0.1	0.1
Diuron		30	0.25	0.1	0.25
Oxamyl		100	0.2	0.2	0.2
Atrazine	Hill	1	0.05	0.02	0.05
Chlortoluron		1	0.05	0.02	0.02
Metoxuron		5	0.1	0.1	0.1
Captan	FAN	20	0.025	0.025	0.04
Fenarimol		50	0.1	0.1	0.1
Prochloraz		10	0.03	0.03	0.05
Parathion-methyl	EβNA	2	0.05	0.05	0.025
Dichlorvos		20	0.025	0.025	0.025
Oxamyl		10	0.025	0.05	0.25
Parathion-methyl	EAcI pig	2	0.2	0.2	0.2
Methomyl		10	0.125	0.125	0.125
Oxamyl		2	0.025	0.025	0.025
Dieldrin	AgUV	25	0.02	0.05	0.2
Triforine		50	0.04	0.04	0.04

4.2. Recoveries and LODs of pesticides on silica gel cartridges

The recoveries of marker compounds obtained with four replicates on silica gel cartridge are shown in Table 3. In the first fraction only dieldrin could be recovered, while in the second fraction good recoveries were obtained for atrazine, diuron, chlortoluron, metoxuron, captan, fenarimol, prochloraz and triforine. The recoveries of parathion-methyl were about 50%. The dichlorvos, methomyl and oxamyl could not be recovered.

The LOD values of the selected compounds obtained with the combined GPC and silica gel cleanup are shown in Table 4.

The LOD values of atrazine, chlortoluron, metoxuron (Hill reaction), captan, fenarimol and prochloraz (FAN) and parathion-methyl (Enzyme inhibition) were decreased. The detectability of other compounds was not improved.

The co-eluted plant extracts of rice and wheat similarly affected the LOD values. The larger amount of corn co-extractives had significantly decreased the LOD values of some pesticides (Table 4) compared to their detectability (given in brackets) in the presence of corn co-extractives: atrazine, Hill, 0.05 mg/kg (0.01 mg/kg), captan, FAN, 0.04 mg/kg (0.025 mg/kg); prochloraz, FAN 0.05 mg/kg (0.02 mg/kg). Acceptable recoveries were obtained in four replicates only on the florisil column (IV). The results summarized in Table 5.

The LOD values of the 13 compounds were checked only on that column. The rounded average LOD values after the combined GPC and florisil (IV) cleanup are shown in Table 6.

TABLE 3. RECOVERIES OF SELECTED PESTICIDES ON SILICA GEL CARTRIDGE

Compounds	TLC detection method	Load of column (μg)	Recovery (%) in fractions	
			1	2
Atrazine	o-TKI	10	0	85.2
	Hill	0.4	0	79.8
Diuron	o-TKI	12	0	91.0
Oxamyl	o-TKI	40	0	11.8
	EβNA	8	0	0
	EAcI pig	0.8	0	0
Chlortoluron	Hill	0.4	0	86.80
Metoxuron	Hill	2	0	77.00
Captan	FAN	8	0	96.70
Fenarimol	FAN	20	0	96.00
Prochloraz	FAN	10	0	102.20
Dichlorvos	EβNA	8	0	0
Methomyl	EAcI pig	2	0	0
Parathion-methyl	EAcI pig	0.8	0	56.80
	EβNA	0.8	0	51.30
Dieldrin	AgUV	5	83.3	0
Triforine	AgUV	10	0	80.10

TABLE 4. LOD VALUES OBTAINED WITH THE COMBINATION OF GPC AND SILICA CARTRIDGE CLEANUP

Compounds	Detection method	MDQ (ng)	LOD [mg/kg] after					
			GPC			GPC+ SiO_2		
			Rice	Wheat	Corn	Rice	Wheat	Corn
Atrazine	o-TKI	25	0.2	0.1	0.1	0.2	0.1	0.1
Diuron		30	0.25	0.1	0.25	0.25	0.1	0.25
Atrazine	Hill	1	0.05	0.02	0.05	0.05	0.01	0.01
Chlortoluron		1	0.05	0.02	0.02	0.025	0.01	0.02
Metoxuron		5	0.1	0.1	0.1	0.05	0.05	0.05
Captan	FAN	20	0.025	0.025	0.04	0.02	0.025	0.025
Fenarimol		50	0.1	0.1	0.1	0.04	0.04	0.1
Prochloraz		25	0.03	0.03	0.05	0.02	0.02	0.02
Parathion-methyl	EβNA	2	0.05	0.05	0.025	0.025	0.05	0.025
Parathion-methyl	EAcI pig	2	0.2	0.2	0.2	0.05	0.2	0.2
Dieldrin	AgUV	25	0.02	0.05	0.2	0.02	0.05	0.2
Triforine		50	0.04	0.04	0.04	0.04	0.04	0.04

4.3. Recoveries and LOD of pesticides on florisil columns

The recoveries on florisil columns are shown in Table 5.

TABLE 5. RECOVERIES OF PESTICIDES ON FLORISIL COLUMNS

Active ingredients	Added (μg)	Recoveries (%)			
		Column I	Column II	Column III	Column IV
Atrazine	0.4	114.8	0	0	91.9
Chlortoluron	0.4	93.0	0	0	90.7
Dichlorvos	8	0	0	0.6	44.6
Dieldrin	5	94.5	31.2	1.9	103.4
Methomyl	2	0	0	41.0	82.9
Oxamyl	8	5.9	0	67.9	87.0
Parathion-methyl	0.8	107.0	0	1.4	84.8

The LOD values of atrazine, chlortoluron, metoxuron, captan, fenarimol, prochloraz and parathion-methyl were decreased with Hill, FAN and Enzyme inhibition detection methods (Table 6). The decrease was similar to silica gel cartridge cleanup. The second cleanup did not decrease the detectability of selected compounds with o-TKI and AgUV.

TABLE 6. LOD VALUES OBTAINED AFER THE COMBINED GPC AND FLORISIL CLEANUP

Compound	Detection method	MDQ (ng)	LOD [mg/kg] after					
			GPC			GPC + Florisil		
			Rice	Wheat	Corn	Rice	Wheat	Corn
Atrazine	o-TKI	25	0.2	0.1	0.1	0.2	0.1	0.1
Diuron		30	0.25	0.1	0.25	0.25	0.1	0.25
Oxamyl		100	0.2	0.2	0.2	0.2	0.2	0.2
Atrazine	Hill	1	0.05	0.02	0.05	0.01	0.02	0.05
Chlortoluron		1	0.05	0.02	0.02	0.01	0.02	0.01
Metoxuron		5	0.1	0.1	0.1	0.05	0.05	0.05
Captan	FAN	20	0.025	0.025	0.04	0.02	0.025	0.04
Fenarimol		50	0.1	0.1	0.1	0.05	0.05	0.1
Prochloraz		25	0.03	0.03	0.05	0.01	0.01	0.01
Parathion-methyl	EβNA	2	0.05	0.05	0.025	0.025	0.05	0.025
Parathion-methyl	EAcI pig	2	0.2	0.2	0.2	0.1	0.1	0.05
Methomyl		10	0.125	0.125	0.125	0.125	0.125	0.125
Oxamyl		2	0.025	0.025	0.025	0.025	0.025	0.025
Dieldrin	AgUV	25	0.02	0.05	0.2	0.02	0.05	0.2
Triforine		50	0.04	0.04	0.04	0.04	0.04	0.04

4.4. Recoveries of pesticides on RP-18 column

The recoveries (Table 7) of selected compounds were generally low on the RP-18 cartridge. Therefore this cleanup procedure was not tested for its applicability to improve the detectability of compounds.

TABLE 7. RECOVERIES OF SELECTED COMPOUNDS ON RP-18 CARTRIDGE

Marker compounds	TLC detection method	Load of column (µg)	Average recovery of 3 replicates (%)	
			Eluate I	Eluate II
Atrazine	o-TKI	10	44.7	1.5
Diuron		12	47.7	4.1
Oxamyl		40	57.9	0
Chlortoluron	Hill	0.4	59.1	15.2
Metoxuron		2	65.4	6.7
Captan	FAN	8	40.5	0
Fenarimol		20	46.8	4.4
Prochloraz		10	72.2	0
Dichlorvos	EβNA	8	52.7	0
Methomyl	EAcI pig	2	59.6	0
Parathion-methyl	EAcI pig EβNA	0.8	59.1	0
Dieldrin	AgUV	5	44.1	0.4
Triforine		10	46.1	0

4.5. TLC Separation and Detection

The average Rf values based on 3 to 7 replicate measurements are summarized in Table 8.

TABLE 8. AVERAGE RF VALUES OF PESTICIDES IN I-VIII ELUTION SYSTEMS

Active ingredient	Si-E	Si-D	Si-B	Si-H/De	s.m. A-E	s.m. A-D	A-E	A-D
Acetochlor	0.637	0.199	0.051	0.437	0.850	0.570	-	-
Alachlor	0.635	0.184	0.038	0.440	0.889	0.531	-	-
Alphamethrin	0.679	0.609	0.495	0.562	0.887	0.885	-	-
Amidosulfuron	0.399	0.000	0.000	0.000	-	-	0.629	0.118
Atrazine	0.620	0.035	0.000	0.316	0.781	0.260	-	-
Azinphos-methyl	-	0.206	-	-	-	-	-	-
Azoxystrobin	0.558	-	-	-	-	-	-	-
Benefin	0.682	0.694	0.626	-	-	-	0.798	0.776
Benomyl	0.311	0.169	0.028	0.017	-	-		
Bensulfuron-methyl	0.525	0.000	0.000	0.030	-	-	0.625	0.115
Bensultap	0.574	0.126	0.015	0.154	-	-		
Bentazone	0.293	0.013	0.000	0.042	-	-	0.000	0.000
Beta-cyfluthrin	0.669	0.588	0.437	0.554	0.893	0.877	-	-
Beta-cypermethrin (1)	0.677	0.612	0.460	0.572	0.889	0.876	-	-
Beta-cypermethrin (2)	-	-	0.498	-	-	-	-	-
Bromophos-ethyl	-	0.686	-	-	-	-	-	-
Bromoxynil	0.184	0.133	0.076	0.050	-	-	-	-
Bromuconazole I	0.447	0.013	0.000	0.077	0.429	0.023	-	-
Bromuconazole II	0.236	0.011	0.000	0.027	0.203	0.024	-	-
Butylate	0.675	0.341	0.202	0.586	-	-	-	-
Captan	0.630	0.331	0.103	0.279	0.814	0.000	-	-
Carbaryl	0.598	0.193	0.029	0.235	-	-	-	-
Carbendazim	0.122	0.000	0.000	0.017	-	-	-	-
Carbofuran	0.591	0.056	0.018	0.258	-	-	-	-
Carbosulfan	0.676	0.342	0.128	0.550	-	-	-	-
Carboxin	0.589	0.203	0.059	0.325	-	-	0.691	0.508
Chlorbromuron	0.575	0.254	0.057	0.249	0.773	0.785	0.697	0.567
Chlorfenvinphos	0.547	0.078	0.000		0.796	0.372	-	-

Active ingredient	Si-E	Si-D	Si-B	Si-H/De	s.m. A-E	s.m. A-D	A-E	A-D
Chlormezulon	0.034	0.000	0.000	0.000	0.000	0.000	-	-
Chlorothalonil	0.671	0.000	0.475	0.566	0.860	0.899	-	-
Chlortoluron	0.398	0.024	0.000	0.066	0.716	0.341	0.617	0.221
Chlorpyrifos	0.669	0.682	0.567	0.601	0.753	0.000	-	-
Chlorpyrifos-methyl	0.657	0.629	0.544	0.587	0.000	0.000	-	-
Cyanazine	0.602	0.020	0.000	0.211	0.741	0.194	-	-
Cypermethrin	0.673	0.614	0.473	0.571	0.872	0.864	-	-
Cyproconazole (1)	0.285	0.010	0.000	0.020	0.157	0.010	-	-
Cyproconazole (2)	0.358	-	-	-	0.240		-	-
2,4-D	0.040	0.000	0.000	0.016	0.000	0.000	-	-
Deltamethrin	0.671	0.598	0.486	0.554	0.873	0.918	-	-
Desmedipham	0.661	0.083	0.017	-	-	-	0.716	0.111
Diazinon	0.660	0.162	0.000	0.446	-	-	-	-
Dicamba	0.034	0.000	0.000	0.020	0.000	0.000	-	-
Dichlofluanid	0.632	0.505	0.296	-	0.827	0.923	-	-
Dichloran	0.627	0.557	0.396	-	0.781	0.792	-	-
Dichlorprop	0.043	0.000	0.000	0.019	0.000	0.000	-	-
Dichlorprop-P	0.050	0.000	0.000	0.021	0.000	0.000	-	-
Dichlorvos	0.458	0.079	0.036		0.021	0.000	-	-
Diclofop-methyl	0.661	0.476	0.271	0.527	0.875	0.900	-	-
Dieldrin	0.675	0.569	0.449	-	0.892	0.882	-	-
Difenoconazole	0.292	-	-	-	-	-	-	-
Difenzoquat	0.000	0.000	0.000	0.000	-	-	-	-
Diflubenzuron	0.619	0.201	0.041	0.286	0.831	0.197	0.693	0.231
Diflufenican	0.630	0.295	0.109	0.383	-	-	0.731	0.664
Dimethenamid	0.638	0.083	0.015	0.323	0.835	0.369	-	-
Dimethoate	0.275	0.018	0.012	0.027	-	-	-	-
Dimethipin	0.429	0.000	0.000	0.064	-	-	-	-
Diniconazole	0.459	0.014	0.000	0.059	0.377	0.022	-	-
Dioxacarb	0.454	0.024	0.000	0.084	-	-	-	-
Dithianon	0.650	0.435	0.328	-	-	-	-	-
Diuron	0.368	0.047	0.005	-	0.686	0.248	0.592	0.259

Active ingredient	Si-E	Si-D	Si-B	Si-H/De	s.m. A-E	s.m. A-D	A-E	A-D
Endosulfan (I)	0.668	0.574	0.557	0.604	0.872	0.829	-	-
Endosulfan (II)	-	-	-	-	-	0.907	-	-
Epoxiconazole	0.382	0.027	0.000	0.043	0.405	0.061	-	-
EPTC	0.653	0.270	0.118	0.542	-	-	-	-
Esfenvalerate	0.680	0.601	0.459	0.573	0.892	0.935	-	-
Ethalfluralin	0.682	0.678	0.599	0.640	-	-	0.791	0.780
Ethephon	0.000	0.000	0.000	0.000	0.000	0.000	-	-
Ethoxifen	0.678	0.450	0.298	0.588	0.913	0.886	-	-
Etrimfos	0.649	0.287	0.128	0.545	-	-	-	-
Fenarimol	0.476	0.000	0.002		0.575	0.051	-	-
Fenconazole	0.246	-	-	-	-	-	-	-
Fenchlorazol-ethyl	0.656	0.351	0.101	0.430	0.868	0.840	-	-
Fenitrothion	0.644	0.548	0.400	0.460	-	-	-	-
Fenoxaprop-P-ethyl	0.663	0.291	0.066	0.499	0.867	0.806	0.776	0.640
Fenpropimorph	0.572	0.000	0.009	0.488	-	-	-	-
Fenuron	0.357	0.024	0.000	0.050	-	-	0.566	0.179
Fluroxypyr	0.650	0.224	0.050	0.436	0.861	0.684	-	-
Flusilazole	0.362	0.017	0.000	0.030	-	-	-	-
Flutriafol	0.278	0.009	0.000	0.022	-	-	-	-
Folpet	0.624	0.532	0.259		0.805	0.823	-	-
Formothion	0.602	0.161	0.042	0.182	-	-	-	-
Fuberidazole	0.460	0.027	0.000	0.109	-	-	-	-
Hymexazol	0.399	0.008	0.000	0.156	-	-	-	-
Imazalil	0.147	0.000	0.000	0.000	0.102	0.000	-	-
Imazamethabenz-methyl	0.457	0.000	0.000	0.088	-	-	-	-
Imidacloprid	0.226	0.000	0.000	0.000	0.388	0.029	0.388	0.047
Isoproturon	0.386	0.026	0.000	0.055	-	-	-	-
Isoxaben	0.618	0.121	0.020	0.243	-	-	-	-
Lambda-cyhalothrin	0.666	0.637	0.512	0.552	0.876	0.898	-	-
Lindane	0.672	0.697	0.597	0.556	0.871	0.926	-	-
Linuron	0.559	0.254	0.055	0.233	0.760	0.673	0.697	0.561
Malathion	0.643	0.294	0.086	0.433	-	-	-	-

Active ingredient	Si-E	Si-D	Si-B	Si-H/De	s.m. A-E	s.m. A-D	A-E	A-D
MCPA	0.035	0.000	0.000	0.016	0.000	0.000	-	-
Mecoprop	0.065	0.000	0.000	0.025	0.000	0.000	-	-
Mecoprop-P	0.086	0.000	0.000	0.029	0.000	0.000	-	-
Methidathion	0.631	0.306	0.159	0.391	-	-	-	-
Methomyl	0.366	0.031	0.000	-	-	-	-	-
Metolachlor	0.610	0.062	0.019	0.359	0.849	0.392	-	-
Metoxuron	0.308	0.018	0.003	-	0.594	0.136	0.529	0.126
Molinate	0.621	0.268	0.106	0.451	-	-	-	-
Myclobutanil	0.285	-	-	-	-	-	-	-
Oxamyl	0.185	0.018	0.000	-	-	-	-	-
Parathion-methyl	0.669	0.561	0.384	0.478	-	-	-	-
Pendimethalin	0.651	0.631	0.535	0.567	-	-	0.778	0.784
Phenkapton	0.683	0.699	0.582	-	0.885	0.894		
Phenylphenol	0.643	0.452	0.278	-	-	-	-	-
Phorate	0.666	0.617	0.485	0.584	-	-	-	-
Phosphamidon	0.226	0.000	0.000	0.018	0.448	0.017	-	-
Pirimicarb	0.449	0.018	0.000	0.148	-	-	-	-
Pirimiphos-methyl	0.666	0.366	0.255	0.528	-	-	-	-
Pirimisulfuron-methyl	0.513	0.012	0.000	0.013	-	-	0.000	0.499
Prochloraz	0.243	0.000	0.000	0.028	0.361	0.008	-	-
Propachlor	0.601	0.146	0.039	0.302	0.783	0.389	-	-
Propham	0.655	0.378	0.170	-	-	-	0.714	0.528
Propiconazole (1)	0.314	0.000	0.000	0.061	0.187	0.062	-	-
Propiconazole (2)	0.390	-	-	-	0.294	-	-	-
Propisochlor	0.649	0.271	0.070	0.489	0.875	0.579	-	-
Prosulfuron	0.301	0.000	0.000	0.011	-	-	-	-
Pyridate	0.679	0.270	0.090	0.563	-	-	-	-
Quinalphos	0.631	0.272	0.120	0.455	-	-	-	-
Quinchlorac	0.060	0.000	0.000	0.000	-	-	-	-
Rimsulfuron	0.106	0.000	0.000	0.000	-	-	0.145	0.000
Simazine	0.570	0.040	0.000	0.249	0.716	0.194	-	-
Tebuconazole	0.324	0.034	0.000	0.022	0.160	0.019	-	-

Active ingredient	Si-E	Si-D	Si-B	Si-H/De	s.m. A-E	s.m. A-D	A-E	A-D
Teflubenzuron	0.641	0.269	0.073	0.333	0.816	0.447	0.679	0.327
Tefluthrin	0.686	0.678	0.602	0.624	0.886	0.923	-	-
Terbutryn	0.603	0.057	0.013	0.325	-	-	-	-
Tetraconazole	0.371	0.023	0.000	0.024	0.555	0.062	-	-
Thiabendazole	0.335	0.015	0.000	0.022	-	-	-	-
Thiocyclam	0.135	0.016	0.000	0.085	-	-	-	-
Thiophanate-methyl	0.570	0.061	0.000	0.133	-	-	-	-
Thiram	0.565	0.244	0.071	0.251	-	-	-	-
Tifensulfuron-methyl	0.217	0.000	0.000	0.000	-	-	-	-
Tralkoxydim	0.659	0.242	0.090	0.499	-	-	0.189	0.036
Triadimefon	0.574	0.029	0.018	0.218	-	-	-	-
Triasulfuron	0.410	0.000	0.000	0.000	-	-	-	-
Triazophos	0.633	0.197	0.040	0.230	-	-	-	-
Tribenuron-methyl	0.479	0.022	0.000	0.052	-	-	-	-
Tridemorph (1)	0.333	0.000	0.008	0.299	-	-	-	-
Tridemorph (2)	0.585	-	-	0.527	-	-	-	-
Triforine	0.492	0.000	0.000	0.013	0.487	0.000	-	-
Zeta-cypermethrin (1)	0.681	0.618	0.472	0.572	0.889	0.908	-	-
Zeta-cypermethrin (2)	-	-	0.511	-	-	-	-	-

The range of Rf and CV values in various systems were (first Rf then CV values):

	Rf range	CV range
System I (Si-E)	0.000 and 0.686	0.005-0.29
System II (Si-D)	0.000 and 0.699	0.001-0.59
System III (Si-B)	0.000 and 0.626	0.005-1.73
System IV (Si-H/DE)	0.000 and 0.64	0.000-0.50
System V (s.m.A-E)	0.000 and 0.913	0.007-0.36
System VI (s.m. A-D)	0.000 and 0.935	0.000-0.41
System VII (A-E)	0.000 and 0.798	0.005-0.12
System VIII (A-D)	0.000 and 0.784	0.002-0.25

In System III many Rf values were below 0.05. For the other compounds it provided a selective separation compared to Systems I and II. The high CV values were observed at Rf values below 0.05–0.1. The distribution of CV values as a function of Rf values were similar to those found in our previous study [1].

4.6. Minimum Detectable Quantities (MDQ) of Pesticides

The MDQ values determined with elution Systems I and V and with o-TKI, Hill, FAN, EβNA, EAcI and AgUV detection methods are published elsewhere [2]. The *Penicillium cyclopium* fungi spore inhibition is very sensitive for some fungicide compounds (Table 9). For instance one ng azoxystrobin or 5 ng carbendazim could be detected.

TABLE 9. DETECTABILITY OF FUNGICIDES WITH PENICILLUM CYCLOPIUM (FPC)

Compound	MDQ[a] (ng)
Azoxystrobin	1
Carbendazim	5
Captan	20
Chlorothalonil	10
Dichlofluanid	20
Fenarimol	50
Folpet	20
Imazalil	10
Prochloraz	10
Thiabendazole	50
Thiophanate-methyl	10

[a] Rounded results of 3 replicates

The typical TLC chromatograms for the detectability of marker compounds and loadability of layers are shown in Figures 2–7.

4.7. Limit of Quantitation and Recovery Values

The recoveries of selected compounds were determined in five replicates at 2 or 3 spiking levels. The residues were detected with TLC and where it was possible with GLC. The results, summarized in Table 10, indicate that the mean recoveries determined at different spiking levels did not differ significantly. There was no significant difference between the average recoveries obtained with GC and TLC detection methods. The average recoveries ranged between 76.8% and 92.8%.

The reproducibility of the whole procedure was very good. The CV values of individual recoveries obtained at each fortification level ranged from 2.8% to 12.5%, while the average CV values for pesticide-commodity pairs ranged from 4.0% to 10.8%.

Both the recovery and the reproducibility of the whole procedure were within the acceptable limits specified by the Good Laboratory Practice in Pesticide Residue Analysis [3].

Materials spotted:

1 Solvent blank
2 Extract of 120 mg control wheat
3 120 mg extract of wheat, fortified: atrazine 0.21, diuron 0.25, oxamyl 0.83 mg/kg
4 Extract of 500 mg control wheat
5 500 mg extract of wheat, fortified: atrazine 0.05, diuron 0.06, oxamyl 0.20 mg/kg
6 Marker: atrazine "A" (25 ng), diuron "B" (30 ng) and oxamyl "C" (100 ng)
7 Extract of 60 mg control wheat
8 60 mg extract of wheat, fortified: atrazine 0.42, diuron 0.5, oxamyl 1.67 mg/kg
9 Extract of 250 mg control wheat
10 250 mg extract of wheat, fortified: atrazine 0.10, diuron 0.12, oxamyl 0.40 mg/kg
11 Solvent blank

A

B

C

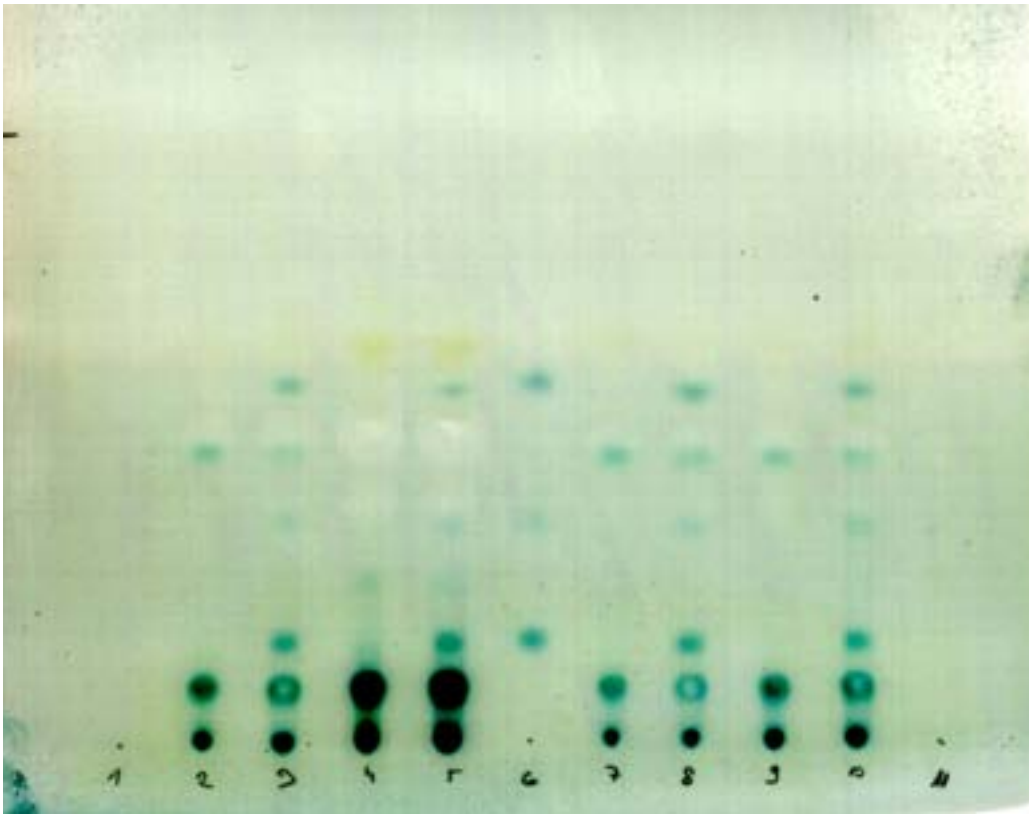

FIG. 2. LOD with o-tolidine and potassium iodide (o-TKI) after GPC cleanup.

Materials spotted:

1 Extract of 5 mg control corn
2 5 mg extract of corn, fortified: atrazine 0.2, chlortoluron 0.2, metoxuron 1 mg/kg
3 Extract of 10 mg control corn
4 10 mg extract of corn, fortified: atrazine 0.1, chlortoluron 0.1, metoxuron 0.5 mg/kg
5 Extract of 20 mg control corn
6 20 mg extract of corn, fortified: atrazine 0.05, chlortoluron 0.05, metoxuron 0.25 mg/kg
7 Marker: atrazine "A" (1 ng), chlortoluron "B" (1 ng) and metoxuron "C" (5 ng)
8 Extract of 50 mg control corn
9 50 mg extract of corn, fortified: atrazine 0.02, chlortoluron 0.02, metoxuron 0.1 mg/kg
10 2.5 mg extract of corn, fortified: atrazine 0.4, chlortoluron 0.4, metoxuron 2 mg/kg
11 Extract of 2.5 mg control corn

A

B

C

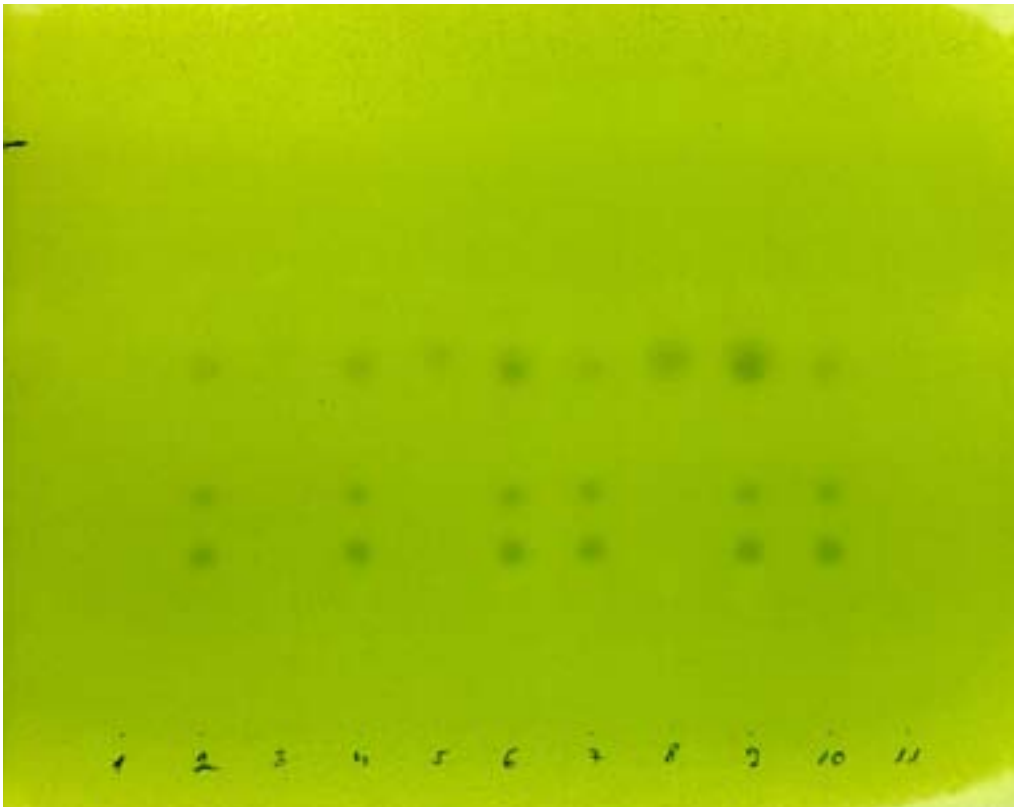

FIG. 3. LOD with photosynthesis inhibition (Hill) after GPC cleanup.

Materials spotted:

1 Marker: captan (20 ng), fenarimol (50 ng) and prochloraz (25 ng)
2 Extract of 800 mg control wheat
3 800 mg extract of wheat, fortified: captan 0.025, fenarimol 0.062, prochloraz 0.031 mg/kg
4 Extract of 400 mg control wheat
5 400 mg extract of wheat, fortified: captan 0.05, fenarimol 0.125, prochloraz 0.062 mg/kg
6 Marker: captan "A" (20 ng), fenarimol "B" (50 ng) and prochloraz "C" (25 ng)
7 Extract of 800 mg control rice
8 800 mg extract of rice, fortified: captan 0.025, fenarimol 0.062, prochloraz 0.031 mg/kg
9 Extract of 400 mg control rice
10 400 mg extract of rice, fortified: captan 0.05, fenarimol 0.125, prochloraz 0.062 mg/kg
11 Marker: captan (20 ng), fenarimol (50 ng) and prochloraz (25 ng)

A

B

C

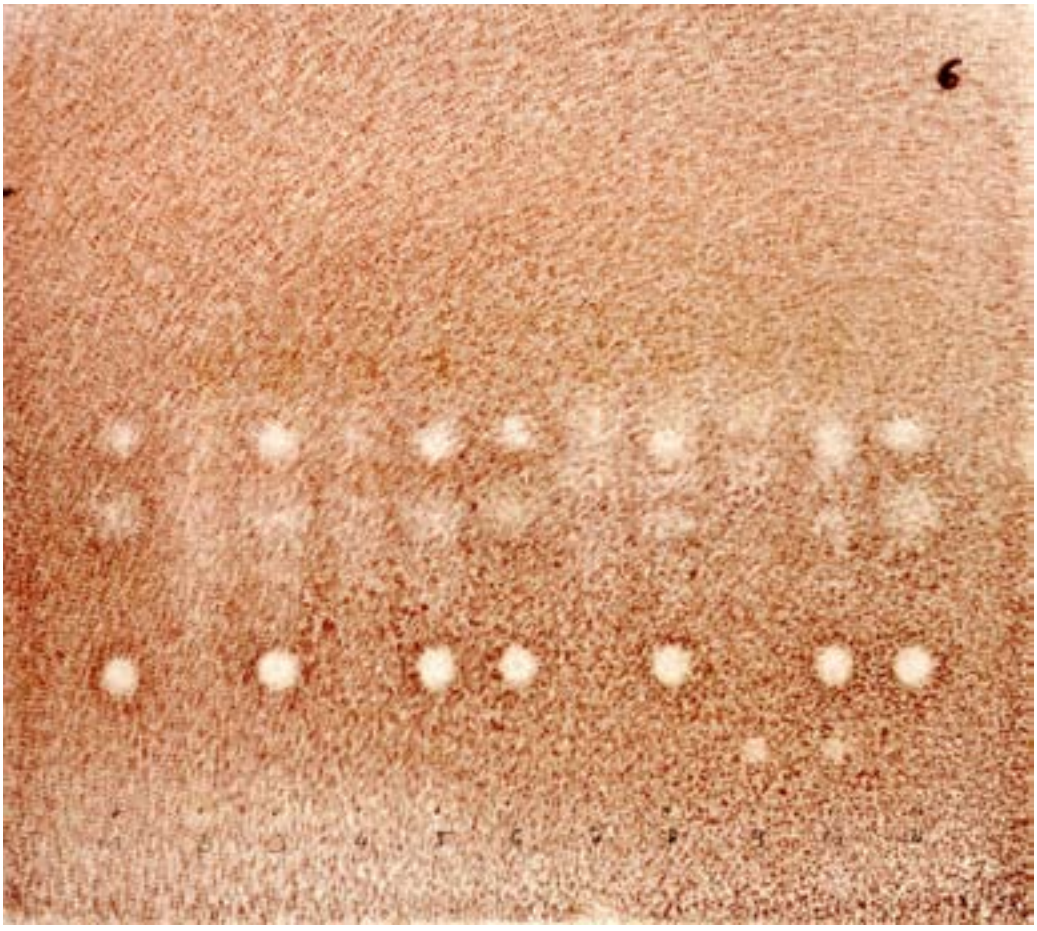

FIG. 4. LOD with fungi spore inhibition (FAN) after GPC cleanup.

Materials spotted:

1 Extract of 4 mg control rice
2 4 mg extract of rice, fortified: parathion-methyl 0.5, dichlorvos 5 and oxamyl 5 mg/kg
3 Extract of 40 mg control rice
4 40 mg extract of rice, fortified: parathion-methyl 0.05, dichlorvos 0.5 and oxamyl 0.5 mg/kg
5 Extract of 400 mg control rice
6 400 mg extract of rice, fortified: parathion-methyl 0.005, dichlorvos 0.05 and oxamyl 0.05 mg/kg
7 Marker: parathion-methyl "A" (2 ng), dichlorvos "B" (20 ng) and oxamyl "C" (20 ng)
8 Extract of 80 mg control rice
9 80 mg extract of rice, fortified: parathion-methyl 0.025, dichlorvos 0.25 and oxamyl 0.25 mg/kg
10 Extract of 800 mg control rice
11 800 mg extract of rice, fortified: parathion-methyl 0.0025, dichlorvos 0.025 and oxamyl 0.025 mg/kg

A

B

C

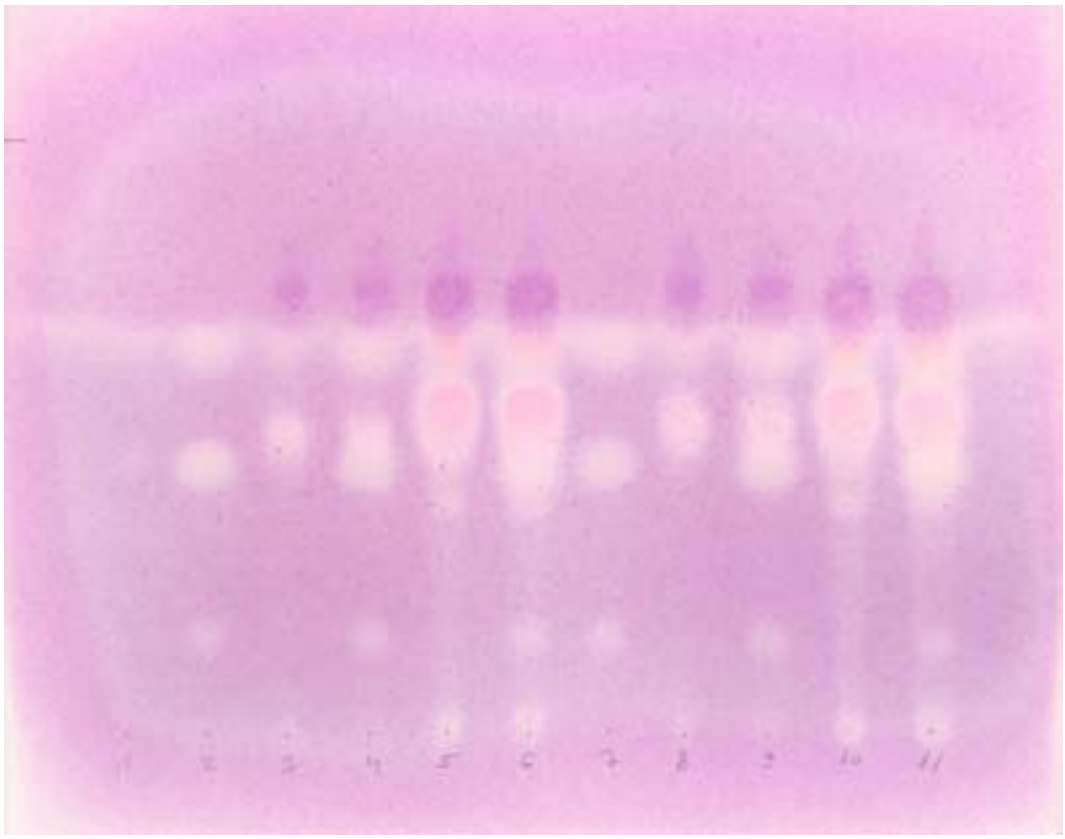

FIG. 5. LOD with cow liver extract enzyme inhibition and β-naphthyl-acetate substrate (EβNA) after GPC cleanup.

Materials spotted:

1 Marker: parathion-methyl “A” (2 ng), dichlorvos “B” (2 ng), methomyl “C” (10 ng) and oxamyl “D” (2 ng)
2 Extract of 10 mg control wheat
3 10 mg extract of wheat, fortified: parathion-methyl 0.2, dichlorvos 0.2 methomyl 1 and oxamyl 0.2 mg/kg
4 Extract of 20 mg control wheat
5 20 mg extract of wheat, fortified: parathion-methyl 0.1, dichlorvos 0.1, methomyl 0.5 and oxamyl 0.1 mg/kg
6 Marker: parathion-methyl “A” (2 ng), dichlorvos “B” (2 ng), methomyl “C” (10 ng) and oxamyl “D” (2 ng)
7 Extract of 40 mg control wheat
8 40 mg extract of wheat, fortified: parathion-methyl 0.05, dichlorvos 0.05, methomyl 0.25 and oxamyl 0.05 mg/kg
9 Extract of 80 mg control wheat
10 80 mg extract of wheat, fortified: parathion-methyl 0.025, dichlorvos 0.025, methomyl 0.125 and oxamyl 0.025 mg/kg
11 Marker: parathion-methyl “A” (4 ng), dichlorvos “B” (4 ng), methomyl “C” (20 ng) and oxamyl “D” (4 ng)

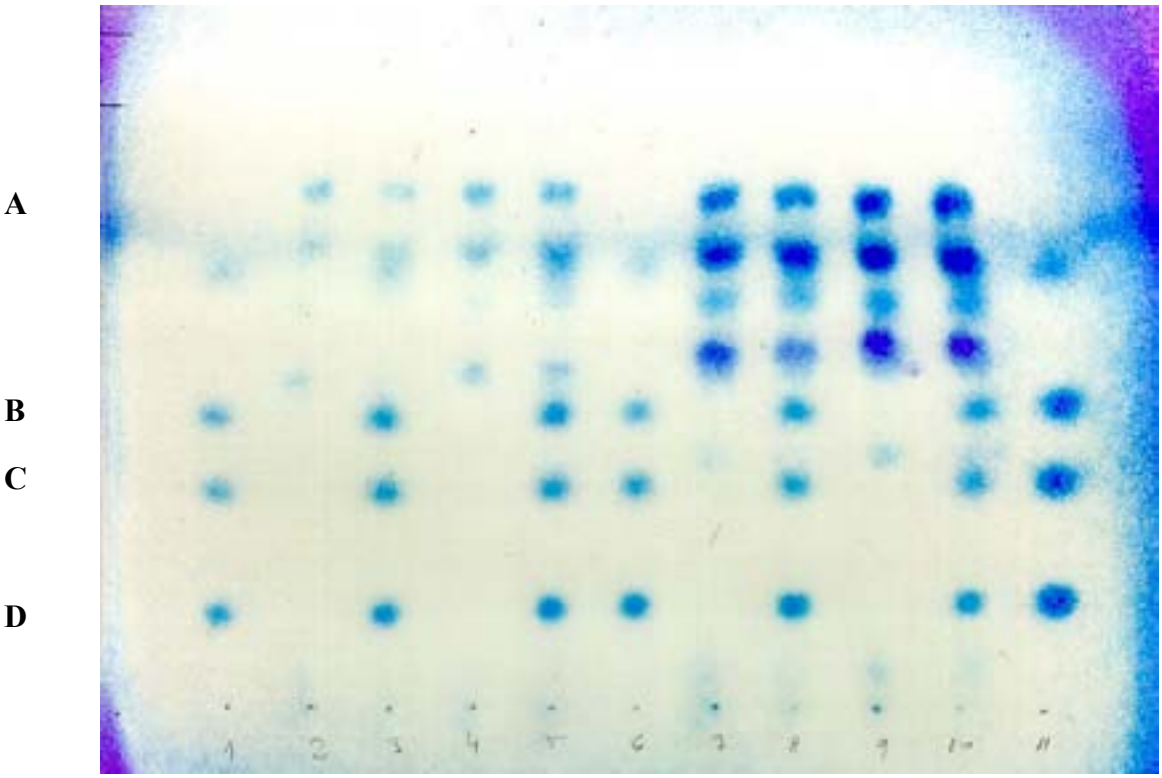

FIG. 6. LOD with pig blood serum enzyme inhibition and acethylthiocholine iodide substrat (EAcI pig) after GPC cleanup.

Materials spotted:

1 Extract of 250 mg control rice
2 250 mg extract of rice, fortified: dieldrin 0.1, triforine 0.2 mg/kg
3 Extract of 500 mg control rice
4 500 mg extract of rice, fortified: dieldrin 0.05, triforine 0.1 mg/kg
5 Extract of 625 mg control rice
6 625 mg extract of rice, fortified: dieldrin 0.04, triforine 0.08 mg/kg
7 Marker: dieldrin "A" (25 ng), triforine "B" (50 ng)
8 Extract of 1250 mg control rice
9 1250 mg extract of rice, fortified: dieldrin 0.02, triforine 0.04 mg/kg
10 Extract of 1000 mg control rice
11 1000 mg extract of rice, fortified: dieldrin 0.025, triforine 0.05 mg/kg

A

B

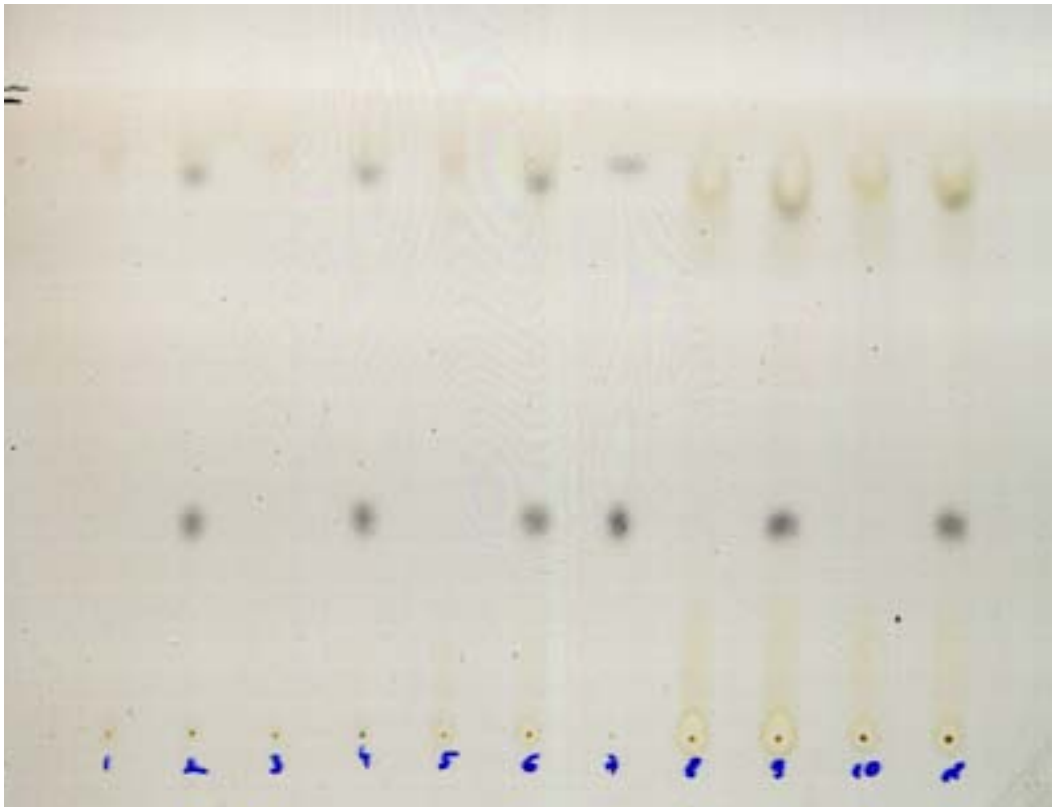

FIG. 7. LOD with silver nitrate (AgUV) reaction after GPC cleanup.

TABLE 10. RECOVERIES OF MARKER COMPOUNDS FROM RICE AND WHEAT SAMPLES

Matrix/compound	Spike mg/kg	TLC			GLC		
		Detection	Q%	CV%	Detection	Q%	CV%
Rice							
Chlorpyrifos	0.01	EAcI	80.0	12.5	NPD	84.0	13.6
	0.05	EAcI	86.8	9.3	NPD	88.4	7.6
	0.1	EAcI	89.2	5.6	NPD	84.4	6.1
Diazinon	0.01	EAcI	79.0	9.6	NPD	86.0	10.4
	0.05	EAcI	85.2	4.6	NPD	82.4	6.5
	0.1	EAcI	86.2	4.4	NPD	82.4	9.1
Diuron	0.1	Hill	80.2	10.3	-	-	-
	0.5	Hill	85.9	6.8	-	-	-
	1	Hill	86.7	3.0	-	-	-
Wheat							
Atrazine	0.05	Hill	78.00	9.8	NPD	78.4	7.1
	0.1	Hill	85.6	7.5	NPD	87.8	5.4
	0.2	Hill	85.8	7.2	NPD	88.6	2.9
Chlortoluron	0.05	Hill	79.2	9.0	-	-	-
	0.1	Hill	82.6	6.0	-	-	-
	0.2	Hill	83.6	5.5	-	-	-
Metoxuron	0.25	Hill	77.6	7.0	-	-	-
	0.5	Hill	78.4	7.3	-	-	-
	1.25	Hill	81.2	5.0	-	-	-
Fenitrothion	0.05	EAcI	84.8	12.2	NPD	82.8	10.3
	0.5	EAcI	80.0	8.3	NPD	81.8	6.0
	1	EAcI	81.6	6.5	NPD	81.1	6.3
Captan	0.2	FAN	76.8	8.5	ECD	78.3	7.7
	0.4	FAN	92.8	5.7	ECD	86.1	5.6
	1	FAN	87.4	4.0	ECD	87.3	3.2
Prochloraz	0.05	FAN	87.5	5.5	ECD	84.0	11.0
	0.5	FAN	86.0	2.8	ECD	86.4	6.5
	1	FAN	88.3	3.2	ECD	86.6	4.5
Triforine	0.1	AgUV	88.2	12.4	-	-	-
	0.2	AgUV	83.4	9.1	-	-	-

The limits of quantitation obtained with the selected compounds are given in Table 11.

In view of the wide range of chemical classes of the selected compounds, it can be concluded that TLC detection can be a useful tool in confirmation of the identity of residues or screening samples for specified compounds at or above 0.01–0.1 mg/kg level. Taking into account the MDQ values, the compounds and detection methods can be selected. Their applicability must be verified in each case before the use of the method.

TABLE 11. LOQ AND MRL VALUES OF THE SELECTED COMPOUNDS IN RICE AND WHEAT

Compound name	LOQ mg/kg	MRL[a] [mg/kg]	Detection method
Atrazine	0.05	0.1	Hill
Captan	0.2	-	FAN
Chlortoluron	0.05	0.1	Hill
Chlorpyrifos	0.01	0.05**	EAcI pig
Diazinon	0.01	0.05**	EAcI pig
Diuron	0.1	-	Hill
Metoxuron	0.25	-	Hill
Fenitrothion	0.05	0.5	EAcI pig
Prochloraz	0.05	0.5*	FAN
Triforine	0.1	0.1*	AgUV

(a) Hungarian MRLs. The Codex MRLs are indicated with:
* Codex MRLs
** MRLs established by the European Commission

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A THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF PESTICIDES CONTAINING HALOALKYL GROUP

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Abstract

Pesticides containing haloalkyl group were analysed by a simple thin-layer chromatographic (TLC) method. Pre-coated alumina plates incorporated with silver nitrate were exposed to low intensity UV radiation at 254 nm. Pesticides appeared as black spots on a colourless background and the chromatogram was stable for several days. The method was suitable for pesticides containing haloalkyl groups, e.g. triforine, DDT, its metabolites and analogues, captan, captafol and synthetic pyrethroids. The minimum detectable quantity (MDQ) of the pesticides was 4 to 50 ng. Endosulfan, aldrin, endrin, and HCH did not respond but dieldrin appeared as a spot at 50 ng MDQ level.

1. INTRODUCTION

Organochlorine compounds (OC) are ubiquitous, persistent, toxic and bioaccumulate in nature. The unique properties of such compounds make them environmentally persistent with a global distribution and they are, thus, exerting chronic-toxic effects on wildlife and humans [1]. Chlorinated pesticide residues are analysed by gas chromatography using an electron capture detector with a high sensitivity [2]. The method involves meticulous cleanup of the extract containing the pesticide with a low sample through-put, and requires trained personnel and expensive equipment that is not available in all laboratories in developing countries. Simple and rapid methods are therefore required to analyze a large number of samples. TLC methods reported in the literature [3, 4] for the analysis of chlorinated pesticides use o-tolidine and the sensitivity is also low.

TLC method using silica gel layers incorporated with silver nitrate with subsequent exposure to UV light has been used earlier [5]. In this procedure, alumina G is washed with 0.2% HNO_3 and then centrifuged to remove the supernatant. The alumina is then washed three times with water and centrifuged each time. Finally, silver nitrate solution is added to the slurry and coated onto glass plates and dried. After spotting with pesticides and elution with appropriate solvent(s), the plates are exposed to UV light at 254 nm from a powerful sterilizing lamp. This method is elaborate and tedious and has been reported for the detection of aldrin, dieldrin, endrin, lindane, DDT, DDE and other unspecified organochlorine pesticides. Moreover, layers of self-made plates have a varying thickness and therefore consistent and reproducible Rf values cannot be obtained. A similar method has been reported for the detection of halogenated pyrethroids [6]. This paper reports a method in which pre-coated alumina plates were sprayed with silver nitrate solution and dried in an oven and exposed to UV radiation at 254 nm. The method is simple and specific for pesticides containing haloalkyl group(s).

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2. MATERIALS AND METHODS

Pre-coated neutral alumina layer on aluminium sheets of size 20 × 20 cm and 0.2 mm thickness were purchased from E. Merck, Germany (Catalog No. 1.05550). Silver nitrate (99.9% pure) was supplied by Qualigens, Mumbai. Double distilled water was used throughout the experiment.

The UV lamp was purchased from CAMAG (Muttenz, Switzerland) with the following specifications: CE: 12 VDC/VAC 50/60 MHz 19 VA (Catalog No. 0229120) with an Intronic adapter: AG P/N 2: 101 979 AC input: 230 V, 50 Hz, 150 mA; output: 12 V, 1.6 A, 19.2 VA. Range: 254 and 386 nm.

The UV chamber had a base measuring 45 × 26 cm and height of ca. 15 cm. It had a rectangular opening 23 × 5 cm on the top over which the UV lamp was placed so that UV radiation fell directly into the chamber. The chamber had a sliding door and a viewing glass filter.

The TLC developing chamber was purchased from Sigma-Aldrich, USA. It was internally lined with Whatman No. 1 filter paper. The eluent (160 ml) was poured into the tank, the internally lined filter paper was wetted with the eluent and the tank was allowed to saturate with the solvent vapour for a minimum period of 3 hours.

2.1. Preparation of TLC Plates and Analysis

A precoated alumina plate was sprayed heavily and uniformly with freshly prepared 1% silver nitrate solution such that the plate was thoroughly wet. The plate was air-dried and heated in an oven at 45ºC for 20 min, cooled and stored in dark. Pesticide solutions were spotted 1.8 cm from the bottom of the plate with a minimum distance of 1.5 cm between the spots. The plate was eluted with ethyl acetate in the developing tank. After the eluent had travelled about 3/4 of the plate, the solvent front was marked and the plate was taken out and air-dried. Subsequently the plate was irradiated with UV radiation at 254 nm in the chamber for 30 min. Black coloured spots appeared on a colourless or light-grey background. The colour of the spots improved when plates were stored outside the UV chamber overnight. The chromatogram was stable for several days.

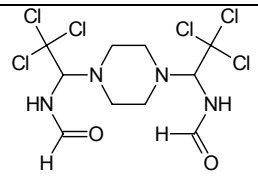
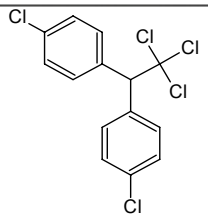
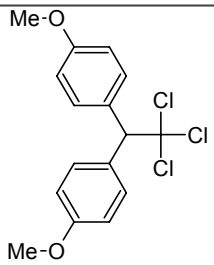
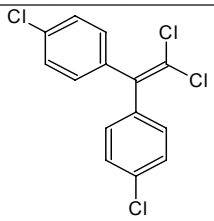
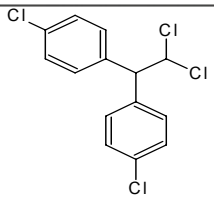
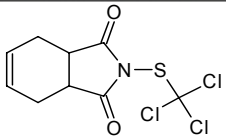
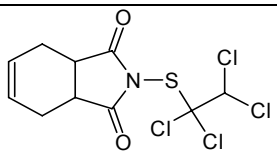
3. RESULTS AND DISCUSSION

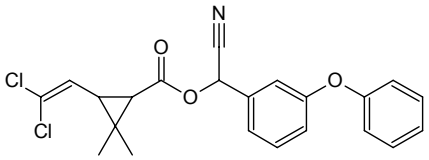
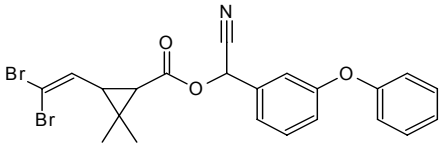
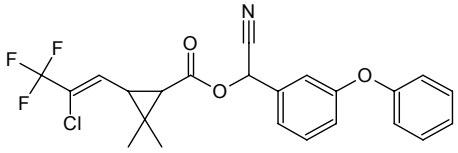
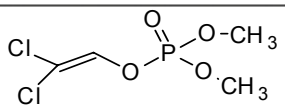
It is well known that commercially available pre-coated plates have uniform thickness and the Rf values are more consistent between trials when compared with self-made plates. The present method employs the pre-coated plates and a simple method of incorporation of silver nitrate into the alumina layers. Rf values of the pesticides in different eluents have already been reported in an earlier method [6]. We have shown that this method is specific to pesticides containing haloalkyl group, and it does not respond to dieldrin, HCH isomers and other cyclodiens including endosulfan. The MDQ values are presented in Table 1. Triforine containing two trichloromethyl groups was found to be twice as sensitive as DDT that contains only one group (Table. 1). We were able to detect captan and captafol containing S-trichloromethyl and S-tetrachloroethyl groups. Synthetic pyrethroids containing haloalkenyl groups, e.g., dihalovinyl and triflourochloropropenyl groups were detected at a higher level, and our results were similar to the earlier method of detection of pyrethroids wherein self-made plates were used [6]. Dichlorvos was detected at a higher level probably due to evaporation of dichlorvos from the TLC plate due to its low boiling point. The limit of detection (LOD) for DDT was 0.25 mg/kg and the limit of quantitation (LOQ) after gel permeation chromatography was 0.54 mg/kg in wheat samples.

4. CONCLUSION

The TLC method developed using silver nitrate incorporated alumina plates and low intensity UV radiation at 254 nm can be used to detect pesticides specifically containing haloalkyl and haloalkenyl groups. The test does not respond to dieldrin, HCH isomers and other cyclodiens tested.

TABLE 1. MDQ VALUES OF THE HALOALKYL PESTICIDES

Name	Systemic name	Structure	MDQ
Triforine	2,2,2-Trichloro-1-{4-[2,2,2-trichloro-1-(formylamino) ethyl]piperazin-1-yl} ethylformamide		4 ng
p,p-DDT	1-Chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl] benzene		10 ng
Methoxychlor	1-Methoxy-4-[2,2,2-trichloro-1-(4-methoxyphenyl) ethyl]benzene		10 ng
p,p-DDE	1-Chloro-4-[2,2-dichloro-1-(4-chlorophenyl)vinyl] benzene		10 ng
p,p-DDD	1-Chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl] benzene		10 ng
Captan	2-[(trichloromethyl)thio]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione		10 ng
Captafol	2-[(1,1,2,2-tetrachloroethyl) thio]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione		20 ng

Name	Systemic name	Structure	MDQ
Cypermethrin	Cyano(3-phenoxyphenyl) methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate		40 ng
Deltamethrin	Cyano(3-phenoxyphenyl) methyl 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-carboxylate		40 ng
λ-Cyhalothrin	Cyano(3-phenoxyphenyl) methyl 3-[(1Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-carboxylate		50 ng
Dichlorvos	2,2-Dichlorovinyl dimethyl phosphate		1 μg

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USE OF MULTI-RESIDUE EXTRACTION ASSOCIATED WITH TLC SEPARATION AND BIOLOGICAL DETECTION IN FRUIT AND TOMATO

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Abstract

A multi-residue method with ethyl acetate as the solvent was applied for extracting pesticides residues from apples and tomatoes. The identification of the pesticides was carried out by thin layer chromatographic (TLC) techniques, which was also used as a semi-quantitative method. Analytical characteristics such as recovery, reproducibility, repeatability, linearity range and limit of determination were obtained in order to validate the methodology. Spiked samples with a mixture of pesticides in the concentration range in accordance with the Codex maximum residues limits were quantified comparatively both by TLC and gas chromatographic/high pressure liquid chromatographic (GC/HPLC) procedures.

1. INTRODUCTION

The analysis of pesticides in food on a regular basis is necessary to ensure proper use of the chemicals and to protect the consumers and the environment. Analysis of fruits and vegetables for pesticide residues is complex and requires both sophisticated equipments (GC or HPLC) and trained personnel. However, there is some other versatile and low cost options for these purposes like the TLC methodology that have recently been used only occasionally [1–3]. The validation of TLC detection methods for pesticide residue analysis was initiated by IAEA in order to communicate the knowledge on the benefits of TLC separation associated with biological/colorimetric detection and provide constructive information on the quality of data produced by several laboratories using this technique in different countries.

One of the main objectives in the validation of this new methodology was to use a multi-residue procedure to determine as many pesticides as possible [4–6] and to reach detection limit values adequate for checking the maximum residue limits (MRLs) established by the CODEX Alimentarius [7]. Thus, much attention was paid to the minimum detectable quantity (MDQ) and the limit of quantitation (LOQ) calculation, the estimation of within-laboratory precision and the comparison of the performance of TLC detection with GC and HPLC.

Ethyl acetate extraction combined with gel permeation chromatography on SX-3 and TLC separation/on-plate quantification has been demonstrated to be robust, precise and sensitive, and could be successfully used for assessing pesticide residues in vegetables and fruits.

2. MATERIALS AND METHODS

The analysis was carried out according to methodology described by Ambrus et al. [8]. Standard solutions were prepared in acetone from Ehrenstorfer pesticides standards. A homogenized portion of a laboratory sample of apple and/or tomato was extracted with ethyl acetate in the presence of $NaHCO_3$ and anhydrous Na_2SO_4, applying Ultra Turrax homogenizer. The supernatant was separated from the solid material by filtration, concentrated by a rotary evaporator and with a slight nitrogen flow.

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For recovery studies, analytical standards solutions were added to the portions of laboratory sample before extraction. The cleanup was carried out by gel permeation chromatography (GPC), with Bio beads SX-3, as the stationary phase, and dichloromethane:cyclohexane (1:1) as the mobile phase. The system was previously calibrated with a solution containing dimethoate, chlorpyrifos, methidathion and azinphos-methyl. The first 9 ml were discarded and the next 20 ml were collected in 2 ml portions for analysis. The elution profile of test compounds is illustrated in Figure 1.

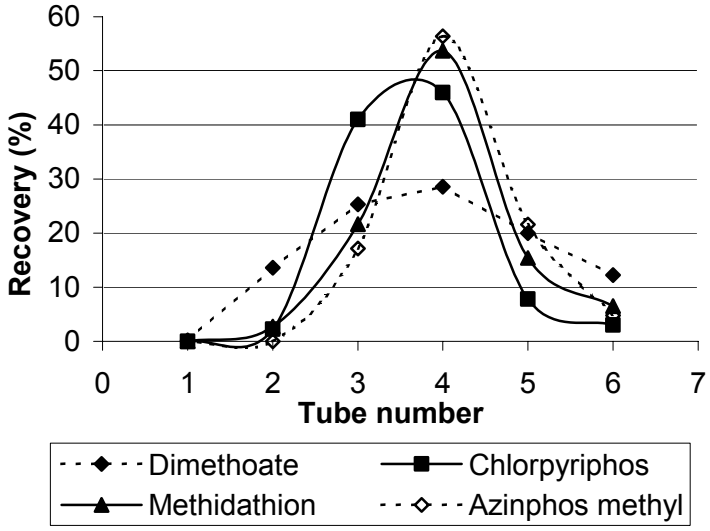

FIG. 1. Calibration of GPC system.

Samples and standards were spotted on freshly reactivated TLC plates. The plates were developed in equilibrated solvent/vapour phases tanks. The solvent used was ethyl acetate. The chemical, biological and enzymatic detection methods used were the followings: o-tolidine + potassium iodide, non specific (o-TKI), photosynthesis inhibition, specific for herbicides inhibiting the photosynthesis (Hill), fungi spore inhibition (*Aspergillus niger*) selectively detects some fungicides (FAN), enzyme inhibition with cow liver extract and β-naphtyl acetate substrate, specific for cholinesterase inhibiting compounds (EβNA), enzyme inhibition with pig blood serum and acetylthiocholine iodide acetate substrate, specific for cholinesterase inhibiting compounds (EAcI). Also GC, and HPLC with NPD and UV detection were used for the comparative procedure.

3. RESULTS AND DISCUSSION

Pesticides studied included herbicides, organophosphorus, carbamate insecticides and fungicides representing several chemical classes of compounds widely used in the Northern Patagonic Region (Argentine). Herbicides are used mainly in corn production but may also be used in orchards where the fruits can be contaminated by spray-drift, while the other families of pesticides included in this study are used in fruit production and horticulture.

3.1. Analytical Characteristics

Considering the quantitative requirements of the procedure and the need of validation of analytical methods, several experiments were carried out to obtain the analytical characteristics of the methods including linearity range, precision, reproducibility and limit of determination (LOD).

The linearity of the method was tested over a range between 0.04 and 0.15 mg/kg for atrazine and diuron and 0.10 and 0.80 mg/kg for metoxuron and linuron with the o-TKI detection method; between 0.05 and 1.00 mg/kg for fungicides with FAN detection method and between 0.0005 and 0.005 mg/kg for organophosphates and carbamates with EβNA. Dimethoate was tested over a range between 0.16 and 2.5 mg/kg. Five concentration levels were used for this purpose and each level was analysed in triplicate. After plotting the spot area versus sample concentration to generate the calibration curves, a linear statistical regression model was applied to obtain the corresponding values for slope and intercept for each compounds. Coefficients of determination R^2 were higher than 0.994 for most compounds.

The repeatability of the method was determined by analyzing five spiked samples at two concentration levels each. The results obtained (CV% repeatability) are shown in Tables 1 and 2.

Within-laboratory reproducibility was also studied by obtaining coefficients of variation of the analysis of three replicates of apple samples spiked at 0.6 mg/kg for herbicides and fungicides and 0.1 mg/kg for organophosphates (except dimethoate, which had 2.5 mg/kg) and carbaryl on three different days. Values obtained are presented in Table 1 and were lower than 15% for all compounds studied except for dimethoate.

The limit of determination (LOD) showed in Table 1 was obtained from the TLC plates corresponding to the ethyl acetate extraction and gel permeation chromatography on SX-3 followed by direct quantification on the plates with biological (FAN and EβNA) or colorimetric (o-TKI) detection methods. Samples were fortified at a level corresponding to the minimum detectable spot area on the plates. Similar results were obtained with tomato (Table 2). As can be seen in Tables 1 and 2 the experimental LODs obtained are at the range suitable for the CODEX Alimentarius requirements for some compounds, mainly organophosphates and fungicides.

3.2. Recovery Experiments

In Table 3 the mean recoveries obtained with the multi-residue method using apple and tomato at two spike levels (corresponding to 2 and 4 LOD) are presented. In most cases the fungicides and organophosphates pesticides have been recovered consistently and reliably in both matrix. The average recovery range varied from 70–113% which was in agreement with those reported [9]. Exceptions were chlorpyrifos (120–145%), dimethoate and the herbicides metoxuron, diuron and linuron. The organophosphate dimethoate could not be reliably determined with the pig serum EAcI method. The detection of herbicides mentioned with Hill method was not reproducible at the higher fortification level. No reason could be found.

The LOQ values not included in Tables 1 and 2 were for fenarimol 0.3 mg/kg and for monocrotophos 1.5 mg/kg.

Losses of pesticide residues during sample processing and extraction have been demonstrated in several matrices [10, 11]. The validation of these steps of the procedure has not been the objective of the present work. However, we attempted to evaluate whether during the analysis the use of the GPC procedure for cleanup introduces losses of pesticide residues in apple and tomato matrices. For that purpose we compared the recovery obtained with and without cleanup with different quantification methods.

TABLE 1. PRECISION EXPRESSED AS REPEATABILITY AND REPRODUCIBILITY OF THE RECOMENDED PROCEDURE APPLIED TO APPLE SAMPLES

Pesticide	Repeatability (CV%)		Reproducibility (CV%)	LOQ mg/kg	Codex MRL mg/kg
	Spike level 2.0 mg/kg	Spike level 0.6 mg/kg	Spike level 0.6 mg/kg		
Herbicides					
Atrazine	3 [a]	7 [a]	8 [a]	0.06 [a] 0.002 [b]	—
Diuron	9 [a]	6 [a]	6 [a]	0.09 [a] 0.002 [b]	—
Metoxuron	7 [a]	9 [a]	10 [a]	0.25 [a] 0.003 [b]	—
Linuron	5 [a]	9 [a]	9 [a]	0.55 [a] 0.002 [b]	—
Fungicides					
Benomyl	7 [c]	12 [c]	12 [c]	0.15 [c]	—
Captan	10 [c]	9 [c]	10 [c]	0.05 [c]	25
Thiabendazol	8 [c]	9 [c]	15 [c]	0.42 [c]	10
Organophosphates and carbamates					
Azinphos methyl	11 [d]	13 [d]	7 [d]	0.008 [d]	2
Chlorpyrifos	10 [d]	10 [d]	8 [d]	0.001 [d]	1
Dimethoate	12 [d]	—	18 [d]	1.50 [d]	1
Parathion	8 [d]	10 [d]	8 [d]	0.001 [d]	0.05
Carbaryl	11 [d]	—	11 [d]	0.008 [d]	5

[a] detection: o-TKI; [b] detection: Hill; [c] detection: FAN; [d] detection: EβNA

TABLE 2. PRECISION EXPRESSED AS REPEATABILITY AND REPRODUCIBILITY OF THE RECOMMENDED PROCEDURE APPLIED TO TOMATOE SAMPLES

Pesticide	Repeatability (CV%)		Reproducibility (CV%)	LOD (mg/kg)	Codex MRL (mg/kg)
	Spike level 2.0 mg/kg	Spike level 0.6 mg/kg	Spike level 0.6 mg/kg		
Herbicides					
Atrazine	2 [a]	7 [a]	7 [a]	0.06 [a] 0.003 [b]	0.5
Diuron	7 [a]	8 [a]	7 [a]	0.09 [a] 0.003 [b]	—
Metoxuron	7 [a]	8 [a]	10 [a]	0.28 [a] 0.008 [b]	—
Linuron	5 [a]	7 [a]	9 [a]	0.65 [a] 0.004 [b]	—
Fungicides					
Benomyl	5 [c]	10 [c]	10 [c]	0.15 [c]	0.2-3
Captan	10 [c]	9 [c]	10 [c]	0.07 [c]	15
Thiabendazole	8 [c]	9 [c]	14 [c]	0.45 [c]	2
Organophosphates and carbamates					
Azinphos methyl	11 [d]	13 [d]	7 [d]	0.008 [d]	0.5
Chlorpyrifos	10 [d]	10 [d]	8 [d]	0.001 [d]	0.5
Dimethoate	12 [d]	—	18 [d]	1.50 [d]	1
Parathion	8 [d]	10 [d]	8 [d]	0.001 [d]	0.07
Carbaryl	11 [d]	—	11 [d]	0.01 [d]	—

[a] detection: o-TKI; [b] detection: Hill; [c] detection: FAN, [d] detection: EβNA

TABLE 3. RECOVERIES OF VARIOUS PESTICIDES FROM DIFFERENT MATRICES

Compound	Matrix	Method	Mean recovery[a]	
			Spike: 2LOD	Spike: 4LOD
Captan	Tomato	FAN	99	113
	Apple		97	99
Benomyl	Tomato	FAN	88	95
	Apple		93	100
Fenarimol	Tomato	FAN	89	91
	Apple		85	96
Azinphos methyl	Tomato	EβNA	80	100
		Hill	95	92
	Apple	EβNA	88	85
		EAcI	94	89
Dimethoate	Tomato	EβNA	100	91
		EAcI	65	65
	Apple	EβNA	85	85
		EAcI	----	38
Chlorpyrifos	Tomato	EβNA	145	120
		EAcI	90	91
	Apple	EβNA	99	100
		EAcI	89	84
Monocrotophos	Tomato	EβNA	80	81
		EAcI	85	88
	Apple	EβNA	75	80
		EAcI	86	89
Metoxuron	Tomato	Hill	88	----
	Apple	o-TKI	74	72
Diuron	Tomato	Hill	83	---
	Apple	o-TKI	62	67
Compound	Matrix	Method	Mean recovery[a]	
			Spike: 2LOD	Spike: 4LOD
Linuron	Apple	Hill	74	72
	Tomato	Hill	85	----
Atrazine	Tomato	Hill	91	----

[a] based on three replicate measurements

We included for comparison some GLC analysis with organophosphates. Results presented in Figure 2 indicated that the use of SX-3 gel column for cleanup did not introduce differences in the recovery of the pesticides analysed. However, some differences according to the method of quantification became evident. The results presented indicated that recoveries with TLC procedures were larger than with GC. This apparently better performance may be produced by the subjective estimation of the spot size on the TLC plate by measuring the diameter of the spot.

To fully validate the multi-residue extraction/TLC-based procedure, its performance was compared to that of quantification with GC or HPLC. The procedure was applied to two separate spiked apple samples, one with a mixture of organophosphates and the other with a mixture of herbicides. Results are presented in Table 4. Using paired t-test, it was observed that the concentration calculated with both procedures were comparable at a significance level of 5%.

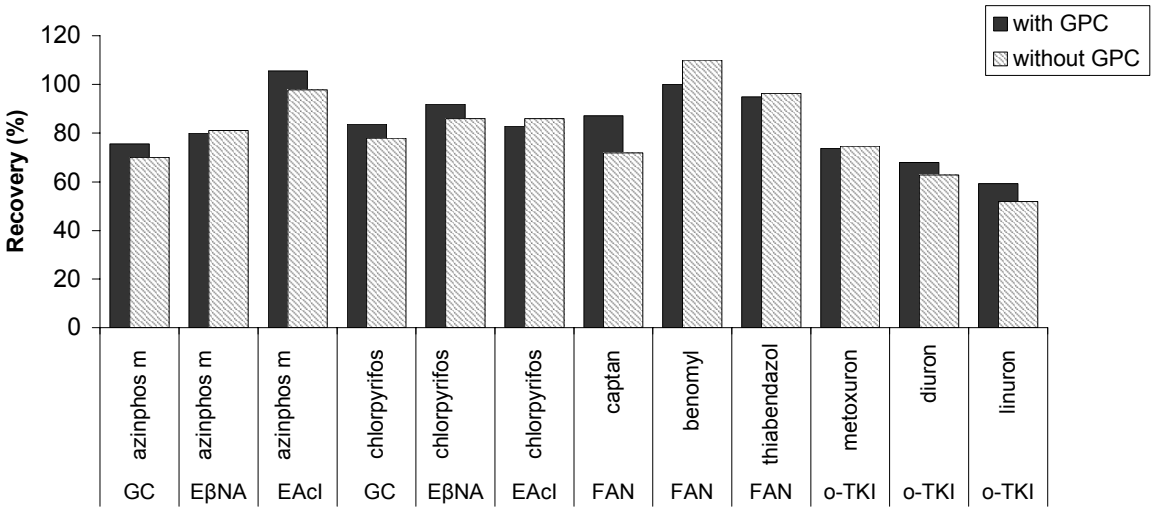

FIG. 2. Recoveries obtained by different quantification methods with and without cleanup.

TABLE 4. COMPARISON OF GC AND TLC - EACI PROCEDURES FOR PESTICIDE DETERMINATION IN APPLES SAMPLES

Compound	Concentration (mg/kg)	
	GC	TLC - EAcI
Azinphos methyl [a]	0.093 + 0.002	0.09 + 0.06
Chlorpyrifos [a]	0.08 + 0.06	0.09 + 0.03
Monocrotophos [b]	2.43 + 0.23	2.31 + 0.18
	HPLC	**TLC - Hill**
Diuron [c]	0.069 + 0.008	0.072 + 0.004
Linuron [c]	0.066 + 0.005	0.062 + 0.004

[a] spiking level: 0.1 mg/kg
[b] spiking level: 2.50 mg/kg
[c] spiking level: 0.08 mg/kg

4. CONCLUSION

The suitability of the multi-residue analytical method for analysis of pesticides on apple and tomatoes using TLC separation with biological/colorimetric detection was investigated. The extraction and cleanup procedure described as well as the TLC separation and quantitation performed is suitable for multi-residue screening of apple and tomato for a wide range of pesticides at CODEX MRL, except for a few unstable or highly polar compounds. This method does not need sophisticated equipment and may be implemented with trained personnel with limited facilities.

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ADAPTATION AND VALIDATION OF THE TLC DETECTION METHODS IN DETERMINATION OF PESTICIDE RESIDUES IN GRAIN

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Abstract

This work was performed to investigate the possibilities of applying prescribed TLC detection methods in combination with GPC cleanup procedure in grains as alternative methods to gas and high performance liquid chromatography for pesticide residue analyses. The MDQ, RF and RRf values of marker and selected compounds were close to those reported in the basic method. The recoveries and reproducibility of the method obtained with wheat and rice samples in our laboratory were within the limits specified by the Codex GLs on method validation. The recoveries determined by GC analyses were practically the same which confirms the accuracy of TLC detection.

1. INTRODUCTION

Thin layer chromatography (TLC) has been used in pesticide residue analysis for gaining semi-quantitative information on the concentration of pesticide residues in the sample or for confirmation of pesticides identified by gas liquid chromatography (GLC) or high pressure liquid chromatography (HPLC) [1]. This study was performed to investigate the possibilities of applying various TLC detection methods in combination with ethyl acetate extraction and GPC cleanup procedures in grains as alternative methods to gas and high performance liquid chromatography for pesticide residue analyses. Further, the purpose of the study was to establish the within-laboratory reproducibility of the MDQ, Rf and RRf values of selected pesticides with the elution and detection methods, and to determine the limit of quantitation of residues in wheat and rice samples.

2. MATERIALS AND EQUIPMENT

2.1. Chemicals and reagents

The materials required for the basic methodology and the preparation of reagents are described by Ambrus et al. [2]. The alterations made in our laboratory are indicated under the methods. All chemicals used were of analytical grade and obtained from Merck Co, Germany. Analytical standards of pesticides were obtained from Dr. Ehrenstorfer GmbH, Germany.

2.2. Equipment

Gas Chromatograph: Varian 3300 with ECD and FPD
Column: SPB 5, 30 m × 0.25 mm, film thickness 0.25 μm
Carrier gas: nitrogen flow rate of 1.2 ml/min
Detector temperature: 300ºC
Injector temperature: 230ºC
Column temperature: 120ºC, 1 min, 3ºC /min to 240ºC

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3. METHODS

3.1. Extraction and cleanup

Ten g of wheat was extracted with 50 ml ethyl acetate in the presence of 35g anhydrous sodium sulphate and 5g sodium carbonate. After filtration, remnants in the flask were washed three times with three ml of ethyl acetate.

Extracts were cleaned on Bio-Beads SX-3 packed in 200 mm × 10mm glass column in semi-automatic gel chromatograph (KL-SX-3). Calibration was performed in three replicates with injection of 500µl ethyl acetate:cyclohexane 1:1 solution containing 0.108 g wheat extract and of 250 ng diazinon and 125 ng triazophos.

3.2. TLC separation and detection methods

The detection and elution methods used and pesticides detected in this study are listed in Table 1. The commercially available silica gel 60, 0.25 mm plates were activated at 105°C for 30 minutes before use. The self-made aluminium oxide G incorporated with silver nitrate layers, 0.25 mm, were dried at room temperature and then activated at 75°C for 45min. The activated plates were stored over activated silica gel until use. Ethyl acetate was used for developing the plates in the normal developing tank, which was kept in a water bath at 20–24°C in order to reduce the effect of temperature variation in the laboratory. The eluent was equilibrated with the vapour phase by inserting filter paper in the developing tank and waiting for a minimum of 30 minutes before the plates were placed into the tank. All standards and samples were spotted in equal volumes (20 µl), in order to ensure comparable starting spot sizes [1].

TABLE 1. DETECTION AND ELUTION METHODS USED AND PESTICIDES DETECTED IN THIS STUDY

Detection method	Elution	Pesticides tested
o-TKI	pre-coated silica gel plate with ethyl acetate	atrazine, diuron, oxamyl, carbaryl and dimethoate
AgUV	self made Aluminium oxide layer with ethyl acetate	dieldrin and triforine
Hill	pre-coated silica gel plate with ethyl acetate	atrazine, chlortoluron, metoxuron, simazine and linuron
FAN	pre-coated silica gel plate with ethyl acetate	captan, fenarimol and prochloraz
EβNA	pre-coated silica gel plate with ethyl acetate	dichlorvos, parathion-methyl, oxamyl, diazinon and phosalone
EacI	pre-coated silica gel plate with ethyl acetate	oxamyl, parathion-methyl and methomyl

We modified the procedure of photosynthesis inhibition (Hill reaction) by using fresh spinach instead of rice or wheat leaves for making the reagent for visualization. Spinach had a higher amount of chlorophyll than wheat or rice, and we needed much more DCPIP solution to

obtain a bluish-green mixture. We took 30 g of spinach leaves and cut them into 2–4 mm pieces, then 3 ml glycerol, 15 ml double distilled water and 5 g quartz sand were added and the mixture was smashed with a pestle until a fairly homogenous pulp was obtained. The homogenate was transferred to four layers of gauze, folded up like a knapsack, and the chloroplast suspension was pressed through the gauze. We obtained approximately 20 ml of chloroplast suspension and stored it in the refrigerator until use. It could be prepared one day before use. Borax buffer solution and DCPIP reagents were prepared according to the basic method [2]. The detecting reagent for visualization was prepared, immediately before spraying the plate, by adding 18–20 ml of DCPIP solution to the chloroplast suspension, until the colour of the mixture became bluish-green (somewhere between the colours of pH 9 and 10 on an universal pH paper scale).

3.3. Limit of detection of the compounds

The limit of detection (LOD) is the concentration derived from the smallest response that can be detected with reasonable certainty for given analytical procedure [3]. It was determined in three replicates by adding the analyte in varying amounts to the blank extract of rice and wheat and spotting the mixture on the TLC plates. The LOD value was the minimum concentration of the compound which gave a distinct spot on the plate in the presence of co-extractives from the cleaned-up sample [4].

The LOD characterizes the detectability of compounds in the presence of co-extractives. It was calculated according to equation (1):

$$\text{LOD [mg/kg]} = \text{MDQ (ng)} / \text{amount of sample equivalent (mg)} \quad (1)$$

3.4. Determination of performance characteristics of the method

The detection methods applied were: o-TKI, Hill and EβNA. Recovery tests were done for all marker and selected compounds in the presence of wheat (for o-TKI and EβNA methods) and rice extracts (for Hill method).

The fortification levels of samples were selected taking into account the LOD and MRL of tested compounds. The estimated limit of quantification (LOQ) was calculated from the LOD and a minimum recovery, Q, of 70%.

LOQ was calculated as:

$$\text{LOQ} = \text{LOD} / \text{Q} \quad (2)$$

Marker compounds were included in the tests as representative compounds regardless of their MRLs. The actual recovery tests for the other compounds were only performed if the calculated LOQ with the detection method used was less than or equal to the national MRL. Where the LOD was larger than the MRL, the detection method was not considered suitable for the purpose of the analysis. Therefore, the method validation process was not continued. The same principle can be applied for new compounds which are intended to be analysed with one of the detection methods taking into account the MDQ values reported [5].

In our experiments a minimum of five analytical portions were spiked at 1.5 LOQ, 2 LOQ and three or five LOQ levels and the recovered amounts of the analytes were determined applying a minimum of three-point calibration. The calibrated range was selected to be within the linear range of TLC detection. The recovery studies were performed in two days including all spiking levels in the sets on each day. The individual recoveries, their average, standard deviation and CV were calculated.

Determination of the residues in the spiked and blank sample extracts was also performed with GC, based on five points of calibration, to confirm the accuracy of TLC detection. The goodness of TLC and GC calibration was demonstrated with the adjusted coefficient of determination (R'^2), obtained with Excel linear regression tool, and the standard deviation of relative residuals ($S_{\Delta y/\hat{y}}$) calculated according to eq. 3.

$$S_{\Delta y/\hat{y}} = \sqrt{\frac{\sum (Y_i - \overline{Y})^2}{nk - 2}} \quad (3)$$

where: $\Delta y = y_i - \hat{y}$; $Y = \Delta y/\hat{y}$ and $\hat{y}$ is the response predicted from the linear regression, and k and n are the number of replicate injections and calibration points, respectively. The $S_{\Delta y/\hat{y}}$ is calculated with nk-2 degrees of freedom. The TLC and GLC calibration was accepted if $S_{\Delta y/\hat{y}}$ was less than 0.1.

4. RESULTS AND DISCUSSION

4.1. Calibration of GPC

Table 2 shows the average distribution of wheat oil and test mixture of pesticides. During the first 9 ml, 93.8% of oil was eluted. Diazinon started to occur in the 9th ml and its elution was finished in the next 11 ml with an average recovery of 85%. Since the triazophos elution was also practically completed with the first 20 ml (86%), the pesticide fraction for analysis was established between the 10 and 20 ml.

TABLE 2. DISTRIBUTION OF WHEAT OIL, DIAZINON AND TRIAZOPHOS ON BIO-BEADS SX-3 WITH ETHYL-ACETATE:CYCLOHEXANE (1:1)

Eluent	Elution of wheat oil		Elution of diazinon		Elution of triazophos	
Ml	mg	%	ng	%	ng	%
0-3	0	0	-	-	-	-
4	1.07	1.0	-	-	-	-
5	7.43	6.93	-	-	-	-
6	26.73	25.0	-	-	-	-
7	31.4	32.43	-	-	-	-
8	21.9	20.47	-	-	-	-
9	9.46	8.83	8.01	3.7	-	-
10	4.6	4.3	51.74	23.9	4.4	4.1
11	1.13	1.03	92.27	43.93	38.43	35.87
12	-	-	28.64	13.23	39.45	36.83
13	-	-	16.59	7.67	16.82	15.7
14-20	-	-	14.22	6.57	7.77	7.27
21-30	-	-	-	-	0.25	0.23
Total	*107.07*	*99.12*	*216.47*	*86.58*	*107.11*	*85.69*

TABLE 3. THE AVERAGE RF AND RRf VALUES OF MARKER AND SELECTED COMPOUNDS AND THEIR WITHIN LABORATORY REPRODUCIBILITY (CV%)

Detection method/ Active ingredient	Rf[1]	Rf[2]	CV (%)	RRf[1]	RRf[2]	CV (%)
o-TKI						
Atrazine	0.620	0.635	1.89	1	1	0
Diuron	0.368	0.362	0.830	0.60	0.587	1.90
Oxamyl	0.189	0.178	7.30	0.30	0.280	9.18
Dimethoate	0.275	0.289	4.10	0.44	0.465	4.51
Carbaryl	0.59	0.604	2.01	0.95	0.97	1.50
AgUV						
Dieldrin	0.834	0.820	3.27	1	1	0
Triforin	0.608	0.586	0.870	0.729	0.7147	0.580
Hill						
Atrazine	0.620	0.618	1.96	1	1	0
Chlortoluron	0.398	0.390	2.11	0.642	0.627	0.431
Metoxuron	0.303	0.300	6.67	0.489	0.480	1.96
Simazine	0.57	0.588	1.02	0.92	0.949	3.30
Linuron	0.559	0.582	1.98	0.90	0.934	2.60
FAN						
Captan	0.630	0.616	1.23	1	1	0
Fenarimol	0.476	0.471	3.93	0.755	0.762	3.31
Procloraz	0.314	0.295	3.32	0.498	0.479	3.13
EβNA						
Parathion-methyl	0.655	0.669	2.68	1	1	0
Dichlorvos	0.505	0.485	3.05	0.755	0.725	0.412
Oxamyl	0.189	0.178	9.16	0.283	0.265	13.5
Diazinon	0.66	0.645	2.82	0.987	0.9793	2.03
Phosalone	0.671	0.671	0.89	1.003	0.995	0.811
EAcI						
Parathion-methyl	0.669	0.687	1.76	1	1	0
Oxamyl	0.189	0.186	14.14	0.283	0.270	8.93
Methomyl	0.363	0.363	5.92	0.543	0.527	2.05

Notes: (1) From reference 3, (2) Measured in our laboratory

We realized that it would be necessary to discard the 10th ml (with 4.3% of oil), to avoid colouring spots on the plate. However, it must be pointed out that discarding the 10th ml of the eluent results in about 24% loss of diazinon and its low recovery (61%) was not acceptable (<70%) [6]. The recovery of some larger pesticide molecules eluting earlier (such as fipronil, pyrethroids etc.) may be much lower under such elution conditions.

4.2. MDQ, RF and RRf values for Marker and Selected Compounds

All detection methods were applied in three runs for each marker and selected compounds. The MDQ values of marker and selected compounds are reported elsewhere [6]. They were half or about ¾ of those reported in the basic method [7]. The within-laboratory reproducibility of Rf and RRf values are shown in Table 3. There was no significant difference between the reported and measured values, indicating the good reproducibility of the elution procedure.

4.3. LOD and Recoveries of Compounds

MDQ and LOD values obtained and the corresponding MRLs are summarized in Table 4. The recoveries of the compounds were determined on different days with three detection methods at three fortification levels, in five replicate fortified samples and duplicate spotting of the sample extracts on each plate. The untreated samples were spiked at 1.5 estimated LOQ; 2 LOQ and 3 or 5 times LOQ levels.

TABLE 4. MDQ, LOD AND MRL FOR THE MARKER AND SELECTED COMPOUNDS

Detection	Active ingredient	MDQ (ng)	LOD[1] (mg/kg)	MRL[(2)] (mg/kg)
o-TKI	Atrazine	25	0.25	0.1
	Diuron	45	0.45	0.1
	Oxamyl	113	1.125	0.05
	Dimethoate	100	0.31	0.2
	Carbaryl	75	0.30	0.2
Hill	Atrazine	1	0.033	0.1
	Chlortoluron	1.1	0.0375	0.1
	Metoxuron	5	0.167	0.1
	Simazine	1	0.04	0.1
	Linuron	0.75	0.03	0.1
EβNA	Parathion-methyl	1.12	0.015	0.1
	Dichlorvos	30	0.40	2
	Oxamyl	11.2	0.15	0.05
	Diazinon	2	0.027	0.2
	Phosalone	3.8	0.075	0.2

(1) The LOD values were practically the same in the presence of wheat and rice extracts.
(2) National MRLs.

The recoveries ranged between 87.4–102% and the details are given in Tables 5–7.

There were no significant differences in the recoveries obtained on different days. The within-laboratory reproducibility of the measurements, characterized by the CV value of the recoveries, was between 1.3 and 13% and it concords with the expected performance parameters of analytical methods for pesticide residues [6].

Standard concentrations of calibration mixtures embraced all fortification levels and were spotted on the TLC plates in duplicate. The linear regression equations, the corresponding regression coefficients and the relative residual standard deviations of three-level calibrations are presented in Table 8. The calibration was accepted if the standard deviation of relative residuals were less than 0.1.

TABLE 5. AVERAGE RECOVERIES OF MARKERS AND SELECTED COMPOUNDS FROM RICE AND WHEAT WITH O-TKI DETECTION

Active ingredient	Fortification (mg/kg)	Found[1] (mg/kg)	Q[1] (%)	CV (%)
Atrazine	0.54	0.499	92.44	7.3
	0.72	0.687	95.42	8.4
	1.08	1.09	100.89	6.8
Diuron	0.96	0.924	95.87	6.8
	1.28	1.15	89.95	6.1
	1.92	1.82	94.84	5.1
Oxamyl	2.4	2.28	94.91	8.1
	3.2	2.99	93.31	10.3
	4.8	4.63	96.52	7.4
Dimethoate	0.664	0.677	101.96	8.4
	0.886	0.874	98.67	6.6
	1.326	1.20	90.87	5.3
Carbaryl	0.645	0.621	96.28	3.7
	0.860	0.825	95.91	2.5
	1.290	1.23	95.77	5.5

(1) Average of 5 replicate measurements

TABLE 6. AVERAGE RECOVERIES OF MARKER AND SELECTED COMPOUNDS FROM RICE AND WHEAT WITH HILL REACTION

Active ingredient	Fortification (mg/kg)	Found[1] (mg/kg)	Q[1] (%)	CV (%)
Atrazine	0.086	0.081	93.95	3.0
	0.114	0.109	95.96	4.6
	0.285	0.264	92.77	3.1
Chlortoluron	0.1	0.093	92.80	4.1
	0.132	0.122	92.42	3.9
	0.33	0.318	96.36	7.1
Metoxuron	0.429	0.411	95.85	6.1
	0.572	0.536	93.67	4.7
	1.43	1.38	96.42	7.2
Simazine	0.09	0.087	96.22	4.6
	0.12	0.116	97.00	3.5
	0.30	0.279	95.87	4.1
Linuron	0.065	0.063	96.31	5.8
	0.086	0.084	97.91	4.5
	0.215	0.217	100.93	4.0

(1) Average of 5 replicate measurements

TABLE 7. AVERAGE RECOVERIES OF MARKER AND SELECTED COMPOUNDS FROM RICE AND WHEAT WITH EßNA DETECTION

Active ingredient	Fortification (mg/kg)	Found[1] (mg/kg)	Q[1] (%)	CV (%)
Parathion-methyl	0.032	0.029	90.62	7.3
	0.042	0.041	98.57	7.7
	0.105	0.097	92.57	1.3
Dichlorvos	0.857	0.757	88.38	6.7
	1.142	1.10	96.71	4.3
	2.855	2.16	93.12	8.3
Oxamyl	0.321	0.266	82.93	12.7
	0.428	0.410	95.48	9.7
	1.07	0.935	87.40	12.2
Diazinon	0.0585	0.054	92.65	5.1
	0.078	0.075	96.41	5.7
	0.195	0.184	94.56	8.5
Phosalone	0.16	0.152	95.25	6.0
	0.214	0.200	93.64	7.0
	0.535	0.508	94.88	1.8

(1) Average of 5 replicate measurements

TABLE 8. CALIBRATION PARAMETERS FOR MARKERS AND SELECTED COMPOUNDS FOR TLC METHODS WITH THREE LEVEL CALIBRATION

Active ingredient	Regression equation Y=ax + b	Correlation coefficient (R^2)	Relative residual SD ($S_{\Delta y/y}$)
Atrazine $_{OTKI}$	Y=0.019x + 4.091	0.9932	0.02945
Atrazine$_{Hill}$	Y=0.683x + 4.5	0.9921	0.02231
Carbaryl$_{OTKI}$	Y=0.0089x + 3.03	0.9932	0.01641
Chlortoluron$_{Hill}$	Y=0.4368x + 3.95	0.9980	0.00468
Diazinon$_{EBNA}$	Y=0.1643x + 3.85	0.9699	0.02827
Dichlorvos$_{EBNA}$	Y=0.012x + 3.88	0.9954	0.00811
Dimethoate $_{OTKI}$	Y=0.0038x + 4.03	0.9997	0.01616
Diuron $_{OTKI}$	Y=0.0108x + 2.985	0.9999	0.00152
Linuron $_{Hill}$	Y=0.6x + 4.383	0.9919	0.01335
Simazine $_{Hill}$	Y=0.683x + 4	0.9991	0.00514
Oxamyl $_{OTKI}$	Y=0.005x + 2.77	0.9905	0.01840
Oxamyl$_{EBNA}$	Y=0.0136x + 4.15	0.975	0.01439
Parathion-me$_{EBNA}$	Y=0.305x + 4.5	0.9942	0.01005
Phosalone$_{EBNA}$	Y=0.098x + 3.325	0.9936	0.01434
Metoxuron$_{Hill}$	Y=0.07x + 3.86	0.9986	0.0047

The same sample extracts, which were applied on TLC plates were checked with GC analyzes. Recoveries were between 93.6 and 98.5% with CVs of 1.75–4.35%. The recovery results of GC analyses are presented in Table 9. Calculations of standard deviations of relative residuals, that is the measure of relative precision of the system [8], and coefficient of correlations (R^2) which show how well the experimental points fit to a straight line, are presented in Table 10. Standard deviations of relative residuals were between 0.0211–0.0998, which was lower than the acceptability limit (0.1).

4.4. Results of reproducibility between laboratories

Inter-comparison test samples were dispatched two times for TLC analysis. Our results are presented in Tables 11 and 12. The results indicate the identification of compounds in complex mixture could not be performed satisfactorily and the determination of identified compounds could only be considered semi-quantitative.

TABLE 9. AVERAGE RECOVERIES OF MARKER AND SELECTED COMPOUNDS FROM RICE AND WHEAT OBTAINED WITH GC

Active Ingredient	Fortification (mg/kg)	Found[1] (mg/kg)	Q[1] (%)	CV (%)
Atrazine	0.54	0.505	93.59	3.0
	0.72	0.683	94.89	4.3
	1.08	1.06	98.52	1.7
Dimethoate	0.664	0.636	95.95	2.1
	0.886	0.847	95.60	3.7
	1.326	1.25	94.00	3.7
Atrazine	0.086	0.083	96.28	3.1
	0.114	0.112	97.89	1.8
	0.285	0.269	94.59	2.4
Simazine	0.09	0.087	96.67	2.7
	0.12	0.117	97.17	3.1
	0.30	0.285	94.93	3.8
Parathion-methyl	0.032	0.030	92.5	3.8
	0.042	0.041	97.62	3.0
	0.105	0.100	95.62	1.3
Dichlorvos	0.857	0.784	91.48	4.5
	1.142	1.12	98.49	2.2
	2.855	2.73	95.75	2.7
Diazinon	0.0585	0.056	95.73	4.2
	0.078	0.074	94.87	2.7
	0.195	0.184	94.56	5.6
Phosalone	0.16	0.154	96.12	1.2
	0.214	0.205	95.89	4.2
	0.535	0.519	94.05	1.1

(1) Average of 5 measurements.

TABLE 10. CALIBRATION PARAMETERS FOR MARKER AND SELECTED COMPOUNDS FOR GC METHODS, WITH FIVE LEVEL CALIBRATION

Active ingredients	Regression equation Y = ax + b	Correlation coefficient (R^2)	Relative residual SD* ($S_{\Delta y/y}$)
Atrazine	Y = 46806x + 1962	0.9980	0.0998
Diazinon	Y = 8584x + 960	0.9994	0.0802
Dichlorvos	Y = 9820 x + 2774	0.9999	0.0273
Dimethoate	Y = 2526x + 4581	0.9995	0.0278
Parathion-methyl	Y = 25554x + 1855	0.9999	0.0493
Phosalone	Y = 34829x + 2588	0.9998	0.0800
Simazine	Y = 9837x + 1932	0.9989	0.0805

TABLE 11. TLC RESULTS OF FIRST INTER-COMPARISON TEST SAMPLES

Compound/ Method	Contents of test ample 1(μg/ml)		Contents of test sample 2(μg/ml)	
	Reported	Found	Reported	Found
Parathion-methyl/EaCI	0.5	0.50	1.0	1.0
Dieldrin/AgUV	5.0	1.50	-	-
Metoxuron/Hill	1.0	1.25	-	-
Triforine/ o-TKI	-	6.25	50.0	50.0
Prochloraz/FAN	5.0	2.50	-	2.0
Captan/FAN	5.0	2.50	10.0	5.0
Oxamyl/EbNA	0.5	-	20.0	10.0
Methomyl/EbNA	-	-	20.0	12.5
Atrazine/Hill	0.3	-	5.0	5.0
Fenarimol/FAN	10.0	-	20.0	9.4
Dichlorvos	0.5	-	-	-

TABLE 12. RESULTS OF SECOND INTER-COMPARISON TEST SAMPLES

Compound/Method	Amount of active ingredients (mg/kg)	
	Reported*	Found
Diuron/Hill	0.507	0.125
Fenitrothion/EbNA	2.01	0.7

5. CONCLUSIONS

Since there are many factors which influence the TLC detection, one must strictly follow the prescribed conditions to get good results. The prevalence of proper elution and detection conditions should be verified by spotting a marker compound on each plate. When the tested TLC methods were carried out under controlled conditions (saturation of the chamber, limited variation of temperature, freshly activated plates etc.) the MDQ, RF and RRf values of marker and selected compounds were well reproducible within the laboratory and between the laboratories. The simultaneous GC analysis confirmed the accuracy of the results.

The methods could be used for semi-quantitative determination of pesticide residues.

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COMPARISON OF THIN LAYER AND GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF HERBICIDE RESIDUES IN GRAIN AND SOIL

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Abstract

Multi-residue analysis method of herbicides in wheat, rice and soil was studied. Semi-automatic gel permeation chromatography (GPC) equipment was used to clean up the extracts, and the residues were analysed by TLC-Hill reaction and GC-NPD methods. The Rf values, the relative Rf values to atrazine (RRf) and the minimum detectable quantities (MDQs) in silica gel-ethyl acetate system were determined. The average recoveries were 84.4%–98.7% with CV of 3.0%–14% by TLC at 0.1 mg/kg fortification levels and, with the exception of isoproturon, 80%–110% with a CV of 0.67%-13% by GC at three different levels. The performance of these two methods was compared.

1. INTRODUCTION

Urea and triazine herbicides are used extensively to protect a large number of crops, such as wheat, maize and vegetables against weeds. Monitoring of their residues in grain and soil is important to protect public health and control agricultural production. Most published methods for separation, detection and quantitation of urea and triazine herbicides in grain, soil and water are based on gas chromatography (GC) and liquid chromatography (LC) [1]. However, GC determination of urea herbicides without prior derivatization is difficult because of their low response and thermal instability.

The determination of herbicide residues in rice, wheat and soil by TLC [2, 3] and GC-NPD was studied in this paper. The samples were extracted with ethyl acetate, and cleaned up with GPC according to DFG method S19 [4]. The TLC chromatographic separation and detection was performed on silica gel plates with a photosynthesis inhibition bio-assay (TLC-Hill reaction) detection method [5].

The minimum detectable quantities (MDQs), Rf and RRf values and recoveries of residues of five selected urea and triazine herbicides (isoproturon, atrazine, metobromuron, metribuzin and prometryn) were determined in rice and wheat grains and soil samples.

2. MATERIALS

Isoproturon (97.0%), atrazine (99.9%), metobromuron (99.4%), metribuzin (99.5%), prometryn (98.1%) and diuron (99.0%) analytical standards were obtained from the Institute for Control of Agrochemicals of Ministry of Agriculture.

Wheat leaves were used to obtain chloroplast for the Hill reaction according to the basic procedure [5].

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3. METHODS

3.1. Extraction and GPC cleanup

Weigh 20 g of ground wheat (or rice) or soil sample into the Erlenmeyer flask. Add 20 ml distilled water and stir vigorously. Add 15 g NaCl, 80 ml acetone and 50 ml cyclohexane and ethyl acetate (1:1). Homogenize the mixture in a Warring Blender. Add 30 g of anhydrous sodium sulphate to the sample, shake for 30 minutes. Decant and filter 65 ml aliquot (equal to 10 g sample). Evaporate the extract to dryness. Dissolve the residue in 1 ml mixture of cyclohexane and ethyl acetate (1:1) for GPC cleanup. Fill the 200 x 10 mm glass column with 8 g swollen Bio-Rad SX-3 gel. Calibrate new GPC columns before use. Inject 1 ml sample extract into the column and elute the sample with cyclohexane and ethyl acetate (1:1). Collect the pesticide fraction, evaporate it to nearly dryness and take it up in 1–2 ml ethyl acetate.

3.2. Development of TLC plate

Use freshly activated 20 × 20 cm silica gel TLC plates. Prepare a spotting plan in advance and spot 10–20 µl pesticide solution in order of the spots numbered from 1–11 starting from the left side of the plate. Apply the similar volume from the plant extracts as well as from the standard solutions on the plate. Fill the tank with acetyl acetate to obtain 1 cm immersion depth for the plate. Place filter paper into developing tanks for 30 min before eluting the plates to obtain saturated vapour phase in the tank. Place the developing tank into a water basin and keep the temperature within ±2°C between 20°C and 30°C for improving reproducibility of retention values. Elute plates up to 11–12 cm from the origin. Adjust the volume of the mobile phase after each elution. In order to obtain good separation of the spots for the determination of residues in rice, wheat and soil samples, develop the plate in the same direction with two different developing solvents: first with petroleum ether:ethyl acetate (8:2), then with petroleum ether:ethyl acetate:methanol (8:1:1).

3.3. TLC detection

Air dry the developed plate and spray it uniformly with the detecting reagent prepared according the basic procedure [5]. Place the plate about 20 cm below a 60 W Tungsten lamp (ordinary bulb) for a few minutes. The inhibition should occur resulting in bluish spots in greenish background within 10 minutes. The spots are usually visible after some minutes and reach optimum after about five minutes. The quantitation should be performed immediately after the appearance of the spots as they disappear within a few minutes.

3.4. GLC detection

The GLC determination was carried out under the following conditions:

GLC:	VARIAN-3800 GC-NPD	
Column:	HP-5/MS fused silica capillary column, 30 m×0.25 mm×0.25 µm	
Temperature:	Injection port:	250ºC
	Detector:	300ºC
	Column:	80ºC (1 min) $\frac{10ºC/min}{}$ 160ºC $\frac{3ºC/min}{}$ 180ºC $\frac{10ºC/min}{}$ 220ºC (5 min)
Flow rate:	H_2:	4.0 ml/min
	Air:	175 ml/min
	N_2:	1.0 ml/min
	N_2:	25 ml/min (make up gas)
Volume of injection:		0.5 µl (splitless injection)

4. RESULTS AND DISCUSSION

4.1. GC Separation and detection

Retention time (Rt), linear range of standard curve and limit of detection (LOD) of five herbicides obtained with GC-NPD method are listed in Table 1. The results showed that they could be separated under the experimental conditions described. The regression coefficients of the linear calibration range were higher than 0.9946. The sensitivity of atrazine, metribuzin, prometryn and metobromuron was high and their LODs were in the range of 3×10^{-11}–5×10^{-10} g but the sensitivity of isoproturon was low and its LOD was 6×10^{-9} g.

TABLE 1. RETENTION TIME, LINEARITY, LIMIT OF DETECTION AND LIMIT OF QUANTITATION OF FIVE HERBICIDES BY GC-NPD METHOD

Pesticide	Rt (min)	Linear Range (ng)	Linear Equation	r	Limit of Detection (g)	LOQ (mg/kg)	
						grain	soil
Isoproturon	5.022	6–60	Y = 0.0083x + 0.0174	0.9946	6×10^{-9}	—*	—
Atrazine	10.955	0.4–10	Y = 0.0567x + 0.0056	0.9966	4×10^{-11}	0.03	0.01
Metobromuron	12.778	4–40	Y = 0.0123x – 0.0004	0.9953	5×10^{-10}	0.3	0.06
Metribuzin	13.344	3–30	Y = 0.02x + 0.0101	0.9971	4×10^{-11}	0.05	0.08
Prometryn	13.991	1–13	Y = 0.053x + 0.0061	0.9997	3×10^{-11}	0.03	0.08

* Note: The recoveries of isoproturon were very high because of the interference from the matrices. Its LOQ could not be determined.

4.2. Elution pattern of herbicides by GPC

The amount of herbicides was determined by GC method described above. Results of the elution of herbicides by GPC (Table 2) showed that pesticides were not eluted at the first 9 ml, but nearly all of the co-extracted materials were eluted in this fraction. Consequently, when the sample extracts were eluted the first 9 ml of the eluent was discarded and the 10^{th}-25^{th} ml was collected for further determination.

TABLE 2. THE ELUTION PATTERN OF FIVE HERBICIDES FROM GPC

Fraction No.	Elution Volume (ml)	Isoproturon (%)	Atrazine (%)	Metobromuron (%)	Metribuzin (%)	Prometryn (%)
1	1–7	—	—	—	—	—
2	8	—	—	—	—	—
3	9	—	—	—	—	—
4	10	19.35	12.54	—	—	—
5	11	30.47	31.10	17.57	18.36	21.40
6	12–13	32.69	35.05	31.58	31.84	30.39
7	14–15	17.49	21.30	29.73	25.34	22.40
8	16–17	—	—	21.12	24.46	10.99
9	18–19	—	—	—	—	8.75
10	20–21	—	—	—	—	6.25
11	22–23	—	—	—	—	—

4.3. Recovery of herbicides from fortified samples by GC method

The test portions of rice, wheat and soil samples were fortified with the herbicides at three different levels, from 0.1 to 5 mg/kg in three replicates at each level. The samples were extracted and cleaned up by the method described. The results of three replicate analyses (Table 3) showed that the average recoveries of atrazine, prometryn, metribuzin and metobromuron in rice, wheat and soil were in the range of 80.2%–110% with CV 0.67%–13%. The recoveries of isoproturon were high because of the interference from the impurities. The limit of quantification (LOQ) of atrazine, prometryn and metribuzin in rice, wheat and soil samples were lower than 0.08 mg/kg. LOQ of metobromuron in rice and wheat samples was 0.3 mg/kg. The determination of LOQ of isoproturon was not possible in these samples because of the interferences from the matrices.

TABLE 3. THE RECOVERY OF HERBICIDES IN FORTIFIED SAMPLES BY GC-NPD METHOD

Pesticide	Spike level (mg/kg)	Rice		Wheat		Soil	
		Average Recovery (%)	CV (%)	Average Recovery (%)	CV (%)	Average Recovery (%)	CV (%)
Isoproturon	5	93.7	15	101	18	88	5.7
	1	126	4.0	130	7.7	140	4.3
	0.1	151	2.7	150	5.7	141	1.1
Atrazine	4	104	2.2	92.7	6.9	84.3	2.7
	1.5	87.9	2.2	103	2.5	99.6	0.67
	0.1	80.2	7.8	101	13	85.6	2.8
Metobromuron	5	87.0	3.4	91.8	6.9	84.0	3.8
	1	88.9	11	83.6	10	87.8	3.0
	0.1	ND	—	ND	—	89.2	4.6
Metribuzin	5	110	5.2	101	4.3	85.1	3.7
	1	93.3	7.8	107	1.4	98.2	5.5
	0.1	89.7	8.0	108	3.2	98.0	5.1
Prometryn	5	103	2.7	81.0	8.0	81.2	0.93
	1.5	86.0	2.8	88.1	5.6	101	1.4
	0.1	81.8	4.3	102	1.7	102	4.4

4.4. TLC Detectability and recovery of compounds

The RRf values for atrazine, linearity, MDQs and LOQs of five herbicides obtained with Hill reaction after elution in silica gel ethyl acetate system are listed in Table 4. The results showed that the RRf values of metobromuron, metribuzin, prometryn and atrazine were very close and they could not be separated on one plate after developing in ethyl acetate. The RRf values of isoproturon, atrazine, metribuzin and prometryn in silica-gel-ethyl acetate system were 0.61, 1.0, 1.013, 0.97. Therefore, first with petroleum ether:ethyl acetate (8:2), then with petroleum ether:ethyl acetate:methanol (8:1:1) elution was performed in the same direction.

The Rf values of isoproturon, metribuzin, atrazine, and prometryn after the second development were 0.088, 0.353, 0.449, 0.581, respectively, which made their simultaneous detection possible.

The detection sensitivity of isoproturon, atrazine, metribuzin and prometryn was high and their MDQs were in the range of 1–3 ng. The sensitivity of metobromuron by TLC-Hill reaction method was low (MDQ 20 ng).

TABLE 4. THE RRf, LINEAR RANGE, MDQ AND LOQ OF HERBICIDES BY TLC METHOD

Pesticide	RRf	Linear Range (ng)	Linear Equation	r	MDQ (ng)	LOQ (mg/kg)
Isoproturon	0.61	1–100	Y=0.4123x + 0.2357	0.9890	1	0.03
Atrazine	1	1–40	Y=0.2760x + 0.6422	0.9834	1	0.03
Metobromuron	0.94	20–150	Y=0.8158x - 0.4124	0.9779	20	0.6
Metribuzin	1.013	2–100	Y=0.6581x + 0.1759	0.9750	2	0.06
Prometryn	0.97	3–100	Y=0.3368x + 0.3903	0.9918	3	0.1

The replicate recoveries of four herbicides in rice, wheat and soil samples at 0.1 mg/kg spiking level are listed in Table 5. Metobromuron was not detectable at 0.1 mg/kg fortification level.

TABLE 5. THE RECOVERIES OF TESTED PESTICIDES FROM FORTIFIED SAMPLES BY TLC METHOD

Sample	Pesticide	Spike (mg/kg)	Recovery (%)			Average Recovery (%)	CV %
			1	**2**	**3**		
Rice	Isoproturon	0.1	87	81	93	86.8	7.2
	Atrazine	0.1	105	103	80	95.9	14
	Metobromuron	0.1	ND	ND	ND	—	—
	Prometryn	0.1	96	91	88	91.6	4.9
	Metribuzin	0.1	102	97	96	98.5	3.0
Wheat	Isoproturon	0.1	95	91	98	94.7	4.0
	Atrazine	0.1	96	96	84	91.8	7.8
	Metobromuron	0.1	ND	ND	ND	—	—
	Prometryn	0.1	88	82	86	85.2	3.6
	Metribuzin	0.1	93	110	93	98.7	9.8
Soil	Isoproturon	0.1	94	92	83	89.8	6.4
	Atrazine	0.1	103	92	84	92.9	10
	Metobromuron	0.1	ND	ND	ND	—	—
	Prometryn	0.1	89	77	86	84.4	7.6
	Metribuzin	0.1	101	97	84	93.9	9.2

The average recoveries of four herbicides in rice, wheat and soil samples were in the range of 84.4%–98.7% with CV 3.0%–14%. The limit of quantitation of isoproturon, atrazine, metribuzin and prometryn in rice, wheat and soil samples were in the range of 0.03 mg/kg–0.1 mg/kg, the limit of quantitation in rice, wheat and soil samples of metobromuron was 0.6 mg/kg (Table 4).

4.5. Comparison of TLC and GC Methods

Urea and triazine herbicides in wheat, rice and soil were detected with GC-NPD and TLC methods. The results (Tables 3 and 5) showed that the recoveries of herbicides from the samples spiked at 0.1 mg/kg level were nearly the same with GC and TLC except urea herbicides, isoproturon and metobromuron. For the latter compounds, the largely different sensitivity of detection did not allow their determination at 0.1 mg/kg spike level with GLC and TLC, respectively.

The results showed that the TLC-Hill method was sensitive and reproducible for most of the compounds tested. It could be used by monitoring laboratories to carry out the preliminary screening of samples in order to complement instrumental analyses.

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VALIDATION OF THIN LAYER CHROMATOGRAPHIC SCREENING METHODS FOR PESTICIDE RESIDUE ANALYSIS

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Abstract

Thin layer chromatographic (TLC) separation and detection methods were tested as a cost efficient alternative for identification and quantitative determination of numerous pesticide residues. Over 80% reproducibility was obtained for Rf and MDQ values compared to those previously reported. The applicability of the methods resulted in recovery values between 75 and 100%. The sample load up to 600 mg sample equivalent did not affect the Rf values significantly.

1. INTRODUCTION

Pesticides play an important role in agriculture for increasing the food production and protection of harvested crops. There are hundreds of active ingredients in different kinds of formulations. Since pesticides are toxic substances, it is necessary to have reliable data concerning their residues in food. In many developing countries analytical laboratories cannot be supplied adequately with expensive equipment, such as gas chromatograph (GC), high performance liquid chromatograph (HPLC) or mass spectrometer (MS). There is a need for analytical methods, which can be used as an alternative to these detection methods. Thin layer chromatography (TLC) is a relatively low-cost technique that is appropriate for many routine screening operations in pesticide residue monitoring.

In this article some results of the validation of thin layer chromatographic methods for pesticide residue analysis are discussed.

2. MATERIALS AND METHODS

Pesticide reference standards were supplied by Dr. Ehrenstorfer (Germany). Stock standards solutions (1 mg/ml) were prepared in acetone. Working solutions were prepared from the stock solutions by dilution. All chemicals were supplied by Merk Co, Germany. Aspergillus Niger spore concentrate were obtained locally. Chloroplast, cow liver extract and pig serum were prepared in the laboratory.

The extraction with ethyl acetate, the GPC cleanup on SX-3 gel, the TLC separation and detection procedures, including o-Tolidine + potassium iodide (o-TKI), Photosynthesis inhibition, (Hill), Fungi spore inhibition (FAN), Enzyme inhibition with cow liver extract and β-naphtyl acetate substrate (EβNA), Enzyme inhibition with pig blood serum and acetylthiocholine iodide acetate substrate, (EAcI), were applied without any change as they are described in detail in this TECDOC [1].

The GC determination was performed with an NPD detector on a Tsvet 550 GC, and with ECD detector on a HP 5890 GC. A 1 m × 3 mm column packed with 5% SE-30 on Chromaton N-AW-DMCS or N-AW-HMDS(0,16–0,20) was used. An HP 1100 LC equipped

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UV and DAD detectors and a Hypersil ODS, 125 × 2mm (particle size – 3 μm) column was used for the liquid chromatographic measurements.

3. RESULTS AND DISCUSSION

The work carried out within this programme included:

- Checking the reproducibility of Rf values obtained in various elution systems under different laboratory conditions;
- Determination of the minimum detectable quantities (MDQ) for various pesticides with different TLC detection methods, and testing their repeatability and between laboratories reproducibility;
- Determining the recoveries of selected compounds from tomato at three spiking levels applying the ethyl acetate extraction and six detection procedures.

The Rf values were measured in nine replicates by spotting each compound three times on one plate and repeating the test on three plates on different days. Standard deviations and coefficients of variation were calculated within plates and between plates. The within plate variations were smaller than between plates variations. Based on our results silica gel - ethyl acetate system is considered suitable for the purpose of screening pesticides residues. About 80% of Rf values measured in silica gel - ethyl acetate system did not differ significantly from those reported earlier [1]. The Rf values measured in this system are summarized in Table 1.

The effect of sample load on Rf values was studied. The extracts of tomato, cabbage and potato were cleaned on the gel column. The TLC plates were loaded with 300 mg and 600 mg sample equivalent. The results revealed (Table 2) that the sample load had no significant effect on the Rf values.

The minimum detectable quantity (MDQ) of a pesticide is defined as the minimum amount of analytical standard, expressed in nanogram, spotted on the plate, which gives clearly visible spots after elution with the specified system. The MDQ values of pesticides found with different detection methods were the same or lower than previously reported [1]. The sensitivity of detection can be influenced by several factors. In order to get reliable MDQ values, it is necessary to strictly follow the described analytical procedure. The proper elution and detection conditions should be checked on each plate by applying a mixture of marker compounds at their MDQ's.

The applicability of the method was tested with a number of compounds representing various chemical classes of pesticides. The recoveries were determined by spotting the equivalents of 600 mg of tomato, potato and cabbage samples. The recoveries typically ranged from 75% to 100% with different TLC detection methods. The results obtained with tomato are shown in Table 3.

4. CONCLUSIONS

The Rf and MDQ values reported by the developer's laboratory could be reproduced over 80% of the pesticide residues tested, which confirmed the reproducibility of the TLC procedures applied.

The TLC separation and detection method can be used for identification and quantitative determination of a large number of pesticide residues by an experienced analyst. The method has certain limitations, which should always be taken into account when it is applied. There

may be cases where the identity of compounds present cannot be decided based on TLC detection alone, or the LOD of the compounds is not low enough for screening residues which are present in low concentrations. In such cases more selective and sensitive methods should be used.

TABLE 1. RF VALUES OF PESTICIE IN SILICA GEL 60 F254 - ETHYLACETATE SYSTEM

Pesticide	Plate 1		Plate 2		Plate 3		Among plates	
	Rf[a]	CV_r	Rf[a]	CV_r	Rf[a]	CV_r	Rf[b]	CV_R
Atrazine	0.643	0.9	0.633	0.9	0.657	0.9	0.644	1.8
Azinphos-ethyl	0.673	0.9	0.650	0	0.627	0.9	0.650	3.2
Benomyl	0.647	0.9	0.650	0	0.673	0.9	0.657	2.0
Captan	0.643	2.4	0.660	3.0	0.630	1.6	0.644	2.9
Carbaryl	0.597	1.0	0.577	1.0	0.580	0	0.584	1.7
Carbendazim	0.330	0	0.343	1.7	0.293	2.0	0.322	7.1
Chlorothalonil	0.710	0	0.743	0.8	0.687	0.8	0.713	3.5
Desmetryn	0.573	1.0	0.580	0	0.583	1.0	0.579	1.0
Diazinon	0.630	0	0.630	0	0.640	0	0.633	0.8
Diuron	0.413	1.4	0.400	0	0.397	1.5	0.403	2.1
Endosulfan I	0.660	1.5	0.653	0.9	0.667	2.3	0.660	1.7
Endosulfan II	0.673	0.9	0.673	2.3	0.693	0.8	0.680	1.9
Etrimfos	0.687	0.8	0.717	0.8	0.677	0.9	0.693	2.7
Heptenophos	0.497	6.2	0.477	3.2	0.510	2.0	0.494	4.6
Imazalil	0.157	9.8	0.157	3.7	0.147	3.9	0.153	6.5
Iprodione	0.643	0.9	0.660	1.5	0.637	0.9	0.647	1.9
Malathion	0.643	0.9	0.660	1.5	0.637	0.9	0.647	1.9
Metribuzin	0.630	0	0.653	0.9	0.650	0	0.644	1.8
Parathion-methyl	0.667	0.9	0.683	0.8	0.673	0.9	0.674	1.3
Phosalone	0.717	0.8	0.723	0.8	0.680	0	0.707	2.9
Pirimicarb	0.493	1.2	0.470	0	0.470	2.1	0.478	2.7
Pirimiphos-methyl	0.680	1.5	0.690	0	0.680	0	0.683	1.0
Procymidone	0.677	0.9	0.683	0.8	0.700	0	0.687	1.6
Prometryn	0.680	0	0.673	0.9	0.640	0	0.664	2.8
Simazine	0.573	1.0	0.560	1.8	0.583	2.0	0.572	2.3
Trifluralin	0.737	0.8	0.747	0.8	0.730	0	0.738	1.1

a) average of 3 values from one plate
b) average of Rf values obtained on 3 different plates on different days

TABLE 2. THE EFFECT OF SAMPLE LOAD ON THE Rf VALUES OF SOME SELECTED PESTICIDES

Method	Active Ingredient	Rf values of compounds on silica gel plates loaded with sample equivalents							
		300 mg				600 mg			
		Tomato	Cabbage	Potato	Mean	Tomato	Cabbage	Potato	Mean
o-TKI	Atrazine	0.64	0.65	0.64	**0.64**	0.64	0.64	0.63	**0.64**
	Diuron	0.41	0.39	0.40	**0.40**	0.40	0.40	0.42	**0.41**
	Simazine	0.56	0.56	0.57	**0.56**	0.58	0.57	0.58	**0.58**
NBFB	Carbaryl	0.57	0.60	0.59	**0.59**	0.56	0.59	0.58	**0.58**
	Pirimicarb	0.46	0.47	0.46	**0.46**	0.46	0.48	0.47	**0.47**
pDB	Diuron	0.64	0.64	0.64	**0.64**	0.63	0.65	0.63	**0.64**
AgUV	Captan	0.76	0.74	0.74	**0.75**	0.76	0.75	0.74	**0.75**
	Dieldrin	0.79	0.80	0.80	**0.80**	0.78	0.79	0.81	**0.79**
Hill	Atrazine	0.65	0.66	0.63	**0.65**	0.64	0.64	0.63	**0.64**
	Diuron	0.39	0.39	0.39	**0.39**	0.40	0.41	0.37	**0.39**
	Simazine	0.57	0.58	0.57	**0.57**	0.57	0.57	0.58	**0.57**
FAN	Captan	0.76	0.76	0.76	**0.76**	0.78	0.75	0.76	**0.76**
	Chlorothlonil	0.74	0.72	0.75	**0.74**	0.72	0.73	0.75	**0.73**
EβNA	Parathion-methyl	0.67	0.67	0.67	**0.67**	0.67	0.66	0.68	**0.67**
	Etrimfos	0.72	0.66	0.71	**0.70**	0.70	0.69	0.70	**0.70**
EAcl	Parathion-methyl	0.68	0.68	0.67	**0.68**	0.67	0.67	0.67	**0.67**
	Pirimiphos-methyl	0.69	0.68	0.68	**0.68**	0.69	0.69	0.68	**0.69**

TABLE 3. RECOVERIES OF SELECTED COMPOUNDS FROM TOMATOE SAMPLES WITH TLC AND GC DETECTION

Method	Pesticides	Levels mg/kg	Replicate measurements					Average	SD	CV %
			1	2	3	4	5			
o-TKI	Atrazine	0.05	90.1	85.9	89.1	82.3	95.7	88.62	5.00	5.6
		0.1	92.3	90.5	97.2			93.33	3.47	3.7
		0.2	90.2	91.8	95			92.33	2.44	2.6
	Diuron	0.05	81.7	89.5	90.1	86.1	90.5	87.58	3.72	4.2
		0.1	95.7	95.3	80.1			90.37	8.89	9.8
		0.2	90.3	80.3	95.7			88.77	7.81	8.8
GLC - NPD	Atrazine	0.05	84.9	85.9	76.3	80.1	90.4	83.52	5.45	6.5
		0.1	87.1	97.2	95.7			93.33	5.45	5.8
		0.2	93.1	92.8	85.5			90.47	4.30	4.8

Method	Pesticides	Levels mg/kg	Replicate measurements					Average	SD	CV %
			1	2	3	4	5			
GLC - ECD	Diuron	0.05	91.7	85.5	91.1	87.1	89.5	88.98	2.64	3.0
		0.1	92.7	90.3	82.3			88.43	5.45	6.2
		0.2	94.6	90.3	87.7			90.87	3.48	3.8
Ag-UV	Endosulfan I	0.25	85.1	75.2	80.1	73	78.4	78.36	4.67	6.0
		0.5	78.1	89.1	82.7			83.30	5.52	6.6
		1	91.5	94.1	90.3			91.97	1.94	2.1
	Endosulfan II	0.25	80.1	75.8	79.1	85.1	78.9	79.80	3.37	4.2
		0.5	92.1	90.4	90.3			90.93	1.01	1.1
		1	95.7	93.8	90.1			93.20	2.85	3.1
	Lindane	0.25	75.6	78.5	87.4	74.9	80.3	79.34	5.01	6.3
		0.5	82.4	74.5	87.3			81.40	6.46	7.9
		1	84.2	76.3	89.3			83.27	6.55	7.9
GLC - ECD	Endosulfan I	0.25	91.1	86.7	95.1	92	84.4	89.86	4.28	4.8
		0.5	88.1	89.1	83.7			86.97	2.87	3.3
		1	97.5	94.6	90.8			94.30	3.36	3.6
	Endosulfan II	0.25	88.1	85.8	79.8	95.9	98.9	89.70	7.73	8.6
		0.5	94.1	85.4	95.3			91.60	5.40	5.9
		1	96.8	91.8	89.4			92.67	3.78	4.1
	Lindane	0.25	95.6	98.5	98.4	94.9	91.3	95.74	2.96	3.1
		0.5	97.5	101.7	95.8			98.33	3.04	3.1
		1	99.8	97.8	96.6			98.07	1.62	1.6
FAN	Captan	0.25	80.3	70.5	70.1	81.3	89	78.24	7.99	10.2
		0.5	81.3	78.5	80.1			79.97	1.40	1.8
		1	80.1	87.1	90.3			85.83	5.22	6.1
HPLC - UV - DAD	Captan	0.25	83.3	87.5	80.1	90.3	90	86.24	4.43	5.1
		0.5	91.8	88.6	89.9			90.10	1.61	1.8
		1	90.7	87.5	85.3			87.83	2.72	3.1
Hill	Atrazine	0.05	95.6	90.1	89.5	88.3	95.4	91.78	3.46	3.8
		0.1	97.1	98.1	98.5			97.90	0.72	0.7
		0.2	89	95.3	94.2			92.83	3.37	3.6
	Diuron	0.05	93.2	87.5	95.8	101	97.1	94.92	5.01	5.3
		0.1	99.1	98.4	95.6			97.70	1.85	1.9
		0.2	95.1	94.2	90.3			93.20	2.55	2.7
GLC - NPD	Atrazine	0.05	84.9	85.9	76.3	80.1	90.4	83.52	5.45	6.5
		0.1	87.1	97.2	95.7			93.33	5.45	5.8
		0.2	93.1	92.8	85.5			90.47	4.30	4.8

Method	Pesticides	Levels mg/kg	Replicate measurements					Average	SD	CV %
			1	2	3	4	5			
GLC - ECD	Diuron	0.05	91.7	85.5	91.1	87.1	89.5	88.98	2.64	3.0
		0.1	92.7	90.3	82.3			88.43	5.45	6.2
		0.2	94.6	90.3	87.7			90.87	3.48	3.8
EβNA	Parathion-methyl	0.025	75.1	72.8	82.4	77.1	73.8	76.24	3.80	5.0
		0.05	79.6	85.1	88.1			84.27	4.31	5.1
		0.1	82.3	88.5	80.9			83.90	4.04	4.8
	Carbaryl	0.5	78.4	76.9	81.7			79.00	2.46	3.1
		1	81.6	79.4	87.6			82.87	4.24	5.1
		2	81.3	88.2	84.5			84.67	3.45	4.1
GLC - NPD	Parathion-methyl	0.025	89.1	80.4	90.5	83.3	91.8	87.02	4.92	5.7
		0.05	91.2	93.9	88.7			91.27	2.60	2.8
		0.1	88.6	96.3	91.8			92.23	3.87	4.2
HPLC - UV - DAD	Carbaryl	0.5	89.2	87.1	87.5	89.8	85.9	87.90	1.59	1.8
		1	89.7	90.7	95.6			92.00	3.16	3.4
		2	94.9	94	96.7			95.20	1.37	1.4
EacI pig	Parathion-methyl	0.025	75.1	72.8	82.4	77.1	73.8	76.24	3.80	5.0
		0.05	79.6	85.1	88.1			84.27	4.31	5.1
		0.1	82.3	88.5	80.9			83.90	4.04	4.8
	Carbaryl	0.5	78.4	76.9	81.7			79.00	2.46	3.1
		1	81.6	79.4	87.6			82.87	4.24	5.1
		2	81.3	88.2	84.5			84.67	3.45	4.1
GLC - NPD	Parathion-methyl	0.025	87.7	82.3	91.8	94.6	84.8	88.24	5.01	5.7
		0.05	89.7	94.1	88.8			90.87	2.84	3.1
		0.1	87.6	89.3	90.7			89.20	1.55	1.7
HPLC - UV - DAD	Carbaryl	0.5	88.6	86.8	90.5	87.3	92.4	89.12	2.33	2.6
		1	88.7	85.4	90.9			88.33	2.77	3.1
		2	89.5	95.7	94.6			93.27	3.31	3.5

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DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN GRAIN BY TLC

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Abstract

Multi-residue analysis of organophosphorus pesticides (OPs) in rice was studied. Semi-automatic gel permeation chromatography (GPC) was used to cleanup the extracts. The OPs were analysed by TLC- cholinesterase inhibition method and GC-FPD methods. The minimum detectable quantities (MDQ), Rf and RRf values of pesticides by TLC were determined. The average recoveries of OPs from fortified rice samples at three different levels were 75%–88% by TLC and 82%–93% by GC. Comparison of the results with GC showed that TLC method was less sensitive than GC, but it could be used by monitoring laboratories to carry out the preliminary screening of samples in order to supplement instrumental analyses.

1. INTRODUCTION

Organophosphorus pesticides (OPs) are widely used in plant protection in China. The residues of these pesticides in grains used for food are of great public concern. In this study the determination of OPs in rice by thin layer chromatograph (TLC) [1] and verification by gas chromatography (GC) [2] were carried out. The principle of TLC detection method is based on the inhibition of cholinesterase enzyme by OPs. [3] This method is specific for phosphoric and thio-phosphoric acid esters and carbamate pesticides, plant extracts usually do not interfere. The extraction and cleanup of rice samples were carried out with ethyl acetate and gel permeation chromatography (GPC) [4].

2. MATERIALS

Analytical standards (purity ≥98%) of dichlorvos, monocrotophos, parathion- methyl, fenitrothion, pirimiphos-methyl, malathion and parathion were obtained from the Institute of Control of Agrochemicals of Ministry of Agriculture. Stock solutions (1 mg/ml) for each pesticide were prepared in 50 ml volumetric flasks with ethyl acetate. Portions of stock solutions were diluted to 25 ml with ethyl acetate to make an intermediate mixed standard solution.

Cow liver extract was used as enzyme source. The reagents were prepared and applied according to the procedures described by Ambrus et al. [5].

3. METHODS

3.1. Sample Extraction and GPC Cleanup

Weigh 20 g of ground rice sample into the Erlenmeyer flask. Add 5 ml ethyl acetate and 20 ml distilled water and stir vigorously. Then add 95 ml ethyl acetate, 10 g $NaHCO_3$ and 70 g anhydrous Na_2SO_4 into the flask. Homogenize the mixture with a Waring blender. Filter

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and decant 50 ml aliquot (equal to 10 g sample). Evaporate the extract to dryness. Dissolve the residue in 0.5–1 ml mixture of cyclohexane and ethyl acetate (1:1) for GPC cleanup. Fill the 10 × 200 mm glass column with 7–8 g swelled bio-bead SX-3 gel. Inject 0.5 ml concentrated sample extract into the column and elute the sample with cyclohexane and ethyl acetate (1:1). Discard the first 8 ml eluate. Collect fraction of 9 to 25 ml. Evaporate it nearly to dryness and take it up in 1 ml acetone for determination of pesticide residues.

3.2. TLC separation and detection

Use freshly activated 20 × 20 cm TLC plate. Prepare a spotting plan for the plate in advance and spot 10–20 µl of the pesticide solution in order of spots numbered 1 to 11 from the left edge of the plate. Apply the same volume from the plant extracts as well as from the standard solutions on the plate. Fill the tank with acetyl acetate to obtain 1 cm immersion depth for the plate. Place filter paper into developing tanks for 30 min before eluting the plates to obtain saturated vapour phase in the tank. Place the developing tanks into water basin and keep the temperature within 25 ± 2°C for improving reproducibility of retention values. Elute plates up to 10–12 cm from the original points of spotting. Adjust the volume of the mobile phase after each elution. Air-dry the commercially available silica gel plate and treat the silica layer with bromine vapour before detection.

Spray the plate with enzyme solution until it gets thoroughly wet, and place it into an incubator or oven saturated with water vapour at 37^{o}C for 30 minutes. Make sure that the plate does not get dry during incubation. Spray the plate with the substrate solution. The white spots occur in pink (bluish-red) background. Evaluate the plates immediately after the full colour development. Mark the position (boundary) of the spots and measure the Rf values.

3.3. GC determination

GC model: Shimadzu GC – 14A FPD (P)
GC condition:

Column:	OV-101 fused silica capillary column, 10 m × 0.25 mm	
Temperature:	Injection port:	210ºC
	Detector:	250ºC
	Column:	100ºC (1 min) $\xrightarrow{10ºC/min}$ 150ºC (15 min)
Attenuation:	2^3	
Chart speed:	10 mm/min	
Sensitivity:	10^1	
Flow rate:	H_2:	1.0 kg/cm^2
	Air:	0.6 kg/cm^2
	N_2:	1.0 ml/min
	N_2:	30 ml/min (make up gas)
Split ratio:	30:1	

Volume of injection: 1 µl or 2 µl

4. RESULTS AND DISCUSSION

4.1. GC Separation

Retention time (Rt), calibrated range of standard curve and limit of detection (LOD) of seven OP compounds by GC method are listed in Table 1. The results showed that pesticides could be separated under the experimental conditions described according to their retention times. The correlation coefficients of the linear range were higher than 0.9845 and the MDQs were

in the range of 1-4 × 10^{-10} g (0.1–0.4 ng). The LOQs of these pesticides in rice samples were in the range of 0.005 mg/kg–0.02 mg/kg.

4.2. Elution pattern of OPs on GPC

The amount of OPs was determined by GC method described above. The results from the eluting OPs showed, the pesticides were not eluted at the first 8 ml, but all of them were eluted between the 9th and 19th ml. When sample extracts were eluted the first 8 ml was discarded and 9–25 ml fraction was collected for further determination. The results are shown in Table 2.

TABLE 1. RETENTION TIME, LINEARITY, LIMIT OF DETECTION AND LIMIT OF QUANTITATION OF SEVEN OPS IN RICE BY GC METHOD

Pesticide	Rt (min)	Calibrated Range (ng)	Linear Equation	γ^2	MDQ (g)	LOQ (mg/kg)
Dichlorvos	2.202	0.1 - 6	Y=12218x–2343.8	0.9858	1 × 10^{-10}	0.005
Monocrotophos	7.317	1 - 6	Y=4476.2x–354.5	0.9845	4 × 10^{-10}	0.02
Parathion-methyl	12.312	0.1 - 6	Y=9008x–135.2	0.9922	2 × 10^{-10}	0.005
Fenitrothion	14.833	0.1 - 6	Y=9193.7x–413.7	0.9885	2 × 10^{-10}	0.005
Pirimiphos-methyl	16.353	0.1 - 6	Y=11092x1–130	0.9959	2 × 10^{-10}	0.005
Malathion	17.075	0.4 - 6	Y=12648x1–199.8	0.9977	2 × 10^{-10}	0.005
Parathion	17.762	0.1 - 6	Y=46328x–1298.6	0.9990	2 × 10^{-10}	0.005

TABLE 2. THE ELUTION PATTERN OF SEVEN OPs BY GPC

Fraction No.	GPC Elution Volume Range (ml)	Dichlorvos (%)	Monocro-tophos (%)	Parathion-methyl (%)	Fenitro-thion (%)	Pirimiphos-methyl (%)	Malathion (%)	Parathion (%)
1	1–7	—	—	—	—	—	—	—
2	7–8	—	—	—	—	—	—	—
3	8–9	7.0	36.9	—	—	—	—	—
4	9–11	40.7	63.1	15.8	14.1	17.9	16.0	5.8
5	11–13	41.2	—	31.0	31.2	30.8	31.0	45.5
6	13–15	10.1	—	30.0	31.0	30.1	30.2	47.6
7	15–17	1.0	—	22.7	0.6	21.2	22.1	1.1
8	17–19	—	—	0.5	—	—	0.7	—
9	19–21	—	—	—	—	—	—	—

4.3. Recovery of OPs from fortified rice samples by GC Method

The rice samples were fortified with OPs at three different levels, 0.05, 0.1 and 0.5 mg/kg, in three replicates. The samples were extracted, cleaned up and determined by GC with the methods described above. The results (Table 3) showed that the average recoveries of seven OPs were in the range of 82.3%–93.1% with CV 1.6%–8.9%.

4.4. TLC determination

The results of TLC determination are listed in Table 4. The Rf values of parathion methyl, fenitrothion and parathion in ethyl acetate system were very close to each other and their RRf values were 1, 1.006 and 0.996. Thus, they could not be separated on one plate after development. In TLC experiments these three pesticides had to be detected separately. The correlation coefficients of the linear range were higher than 0.9561, which are not as good as GC. The MDQs of seven OP compounds were in the range of 2×10^{-9}g–10^{-7}g, which were 10–100 times higher than those obtained with GC. The LOQs of these pesticides in rice samples were 0.043 mg/kg-0.214 mg/kg.

TABLE 3. THE RECOVERY OF THE OPs IN FORTIFIED RICE BY GC METHOD

Pesticide	Fortification (mg/kg)	Recovery (%)			Average Recovery (%)	CV %
		1	**2**	**3**		
Dichlorvos	0.05	80	81	86	82.3	3.9
	0.1	82	85	88	85.3	3.6
	0.5	91	87	79	85.5	7.4
Monocrotophos	0.05	93	95	89	92.6	3.4
	0.1	85	91	84	86.9	4.3
	0.5	84	94	79	85.9	8.9
Parathion-methyl	0.05	92	89	92	91.2	1.9
	0.1	87	95	90	90.7	4.1
	0.5	86	88	83	85.5	2.5
Fenitrothion	0.05	90	86	92	89.3	3.6
	0.1	96	90	87	91.1	4.9
	0.5	92	87	92	90.4	2.9
Pirimiphos-methyl	0.05	83	87	85	85.3	2.3
	0.1	88	84	86	86.2	2.3
	0.5	94	90	86	89.8	4.5
Malathion	0.05	85	91	85	86.8	3.8
	0.1	91	85	95	90.5	5.3
	0.5	88	84	92	88.0	4.6
Parathion	0.05	89	87	91	89.2	2.6
	0.1	93	95	92	93.1	1.6
	0.5	84	91	87	87.0	4.2

TABLE 4. Rf, LINEARITY, MDQ AND LOQ OF SEVEN OPs BY TLC METHOD

Pesticide	Rf	RRf	Linear Range (ng)	Linear Equation	γ^2	MDQ (ng)	LOQ (mg/kg)
Dichlorvos	0.438	0.653	20–100	Y=7.9115x – 6.121	0.9803	20	0.2
Monocrotophos	0.11	0.164	120–600	Y=3.2789x – 2.4022	0.9848	200	—
Parathion-methyl	0.671	1.000	2–40	Y=2.5338x + 3.3983	0.9831	2	0.04
Fenitrothion	0.675	1.006	10–150	Y=7.0343x + 2.196	0.9703	10	0.2
Pirimiphos-methyl	0.615	0.917	3–40	Y=5.4579x + 2.202	0.9857	3	—
Malathion	0.645	0.961	200–600	Y=14.752x – 28.964	0.9561	200	—
Parathion	0.668	0.996	2–40	Y=0.5672x + 1.5699	0.9708	2	0.04

4.5. Recovery of OPs from fortified rice samples with TLC method

The rice samples were fortified with four OPs at three different levels, 1.5 LOQ, MRL and 2 MRL, respectively with five replicates. The samples were extracted, cleaned up and determined by TLC with the method described above. The fortification levels [mg/kg] and the recovery values are shown in Table 5. The average recoveries of four OPs were in the range of 75.4%–88.1% with CV 3.1%–15%.

TABLE 5. THE RECOVERIES OF TESTED PESTICIDES IN FORTIFIED RICE SAMPLES BY TLC METHOD

Pesticide	Spike level (mg/kg)	Recovery (%)					Average Recovery (%)	CV (%)
		1	**2**	**3**	**4**	**5**		
Parathion-methyl	0.043	70	95	70	77	71	76.6	14
	0.1	79	89	88	75	68	79.8	11
	0.2	74	73	79	76	75	75.4	3.1
Dichlorvos	0.1	ND	ND	ND	ND	ND	—	—
	0.2	88	97	86	81	84	87.3	6.9
	0.43	94	90	85	78	77	84.7	8.7
Fenitrothion	0.214	90	85	91	80	75	84.1	7.8
	0.5	87	102	92	82	77	88.1	11
	1.0	80	84	82	71	68	76.8	9.0
Parathion	0.043	76	78	86	73	76	77.7	6.2
	0.1	87	92	93	69	68	82.0	15
	0.2	86	82	76	78	75	79.2	6.0

4.6. Comparison of the results of GC and TLC methods

Seven OPs could be separated under the GC conditions described, but in the TLC experiments, especially by using only ethyl acetate as developing solvent, several OPs, such as parathion methyl, fenitrothion, parathion, malathion and pirimiphos-methyl, could not be separated on one plate. The correlation coefficients of the linear range were higher than 0.9845 and 0.9561 for GC and TLC, respectively. The MDQs were in the range of $1–4 \times 10^{-10}$ g for GC and the 2×10^{-9} g–10^{-7} g for TLC. The LOQs of these pesticides in rice samples were 0.005 mg/kg–0.02 mg/kg and 0.04 mg/kg–0.2 mg/kg for GC and TLC, respectively. The average recoveries of OPs were in the range of 82.3%–93.1% with CVs of 1.6%–8.9% and 75.4%–88.1% with CVs of 3.1%–15% by GC and TLC, respectively. In the TLC study the LOQ of dichlorvos was 0.2 mg/kg, this method could not detect the maximum residue limit (0.1 mg/kg) of dichlorvos in grain, but the sensitivity of parathion methyl and parathion was very high, their MDQs were 2 ng and LOQs were 0.04 mg/kg. In general TLC methods can be used for specified compounds to carry out the preliminary screening of samples in order to supplement instrumental analyses.

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ADAPTATION OF TLC DETECTION METHOD FOR THE DETERMINATION OF PESTICIDE RESIDUES IN GRAINS

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Abstract

In this study the retention factor (Rf), relative retention factor (RRf) and minimum detectable quantities (MDQ) of some pesticide active ingredients were determined with various TLC detection methods. The MDQs of the compounds were verified with grain extracts after subjecting them to cleanup procedure with the KL-SX-3 GPC gel chromatograph. The limits of detection (LOD) of compounds were calculated.

1. INTRODUCTION

Thin layer chromatography (TLC) is used in analytical laboratories for the separation of soluble mixtures containing known and unknown compounds and for the identification and quantitative determination of known compounds. The acceptance and importance of TLC is mainly due to its simplicity, speed and low cost. Although Rf values are affected by various factors such as temperature, layer thickness, saturation of the developing tank, eluent and activation of the plate, when standardized conditions are applied, reproducible Rf and RRf values can be obtained and TLC can be used for screening pesticide residues in samples of unknown origin [1-3].

This study was performed to investigate the possibilities of applying TLC detection in combination with the widely used ethyl acetate extraction and GPC SX-3 cleanup methods for providing an alternative cost effective analytical procedure for screening pesticide residues in cereal grains. We tested the applicability of the following detection methods: o-tolidine plus KI, $AgNO_3$ plus UV radiation, photosynthesis inhibition, bioassay with fungi spores, and enzyme inhibition. To verify the appropriate performance of the elution and detection procedures we applied marker compounds as suggested by Ambrus et al [4].

2. MATERIALS AND METHODS

The following pesticide active ingredients, obtained from Dr. Ehrenstorfer Laboratories GmbH, Germany, were used in the project: acephate, atrazine, captan, carbaryl, carbandezim, chlorpyrifos, chlortoluron, cyanazine, diazinon, dichlorvos, dichlofluanid, dioxacarb, dieldrin, dimethoate, diuron, ethion, fenarimol, fenitrothion, fenthion, linuron, malathion, methomyl, metoxuron, monocrotophos, oxamyl, parathion-methyl, pirimicarb, pirimphos-methyl, prochloraz, thiabendazole, thiophanat-methyl, triazophos, and triforine.

2.1. Chemicals and reagents

All chemicals used were analytical grade and solvents were reagent grade (Merck). The preparation of reagents was carried out as described by Ambrus et al. [4]. Instead of double

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distilled water, sterilized water was used for making spore suspension of *Aspergillus niger* to avoid the infection of the fungi culture with other saprophyte fungi.

2.2. Extraction

The basic extraction procedure applied within the CRP was followed [4]. Fifty ml ethyl acetate was added to the sample and reagents blank. For recovery studies 49 ml ethyl acetate was added to the spiked samples. Blank samples were fortified with 1 ml standard mixture at the level of 0.1 mg/kg diazinon and 0.05 mg/kg triazophos. The final extracts were taken up with about 250 µl of ethyl acetate-cyclohexane (1:1), corresponding to 5 g sample equivalent, for injecting to GPC.

2.3. Cleanup

2.3.1. Calibration of GPC column

Gel permeation chromatography columns (200 mm × 10 mm glass column) were filled with 8 g SX-3 gel as described in the operation manual. The KL-SX-3 GPC gel chromatograph was operated with constant nitrogen over-pressure of 0.5 bar providing a constant flow rate of approximately 1 ml/min for the ethyl acetate/cyclohexane (1:1) eluting solvent mixture.

The calibration of the GPC column were performed with diazinon and triazophos and wheat oil in triplicate. The standard mixture in 5 ml ethyl acetate:cyclohexane (1:1) containing approximately 1 µg:ml of diazinon and 0.5 µg/ml triazophos was prepared. An aliquot (250 µl) of standard mixture was injected (correspond 0.25 µg diazinon, 0.125 µg triazophos) into the stabilized GPC column. The first 7 ml was collected and discarded. Then the eluent was collected in 1 ml increments until 13 ml. The next 6 ml (fraction 14–20 ml) and the fraction of 21–30 ml were collected together. A total of 30 ml eluent was obtained. The eight different fractions were evaporated to nearly dryness and re-dissolved in acetone to 1 ml for the GC analysis.

To get grain oil, 200 g corn or wheat flour was soaked in 200 ml water for 30 min and extracted with 400 ml ethyl acetate. The organic phase was decanted, filtered, and evaporated to dryness and the oily material collected in a test tube. The dry extract was weighed and an ethyl acetate:cyclohexane (1:1) solution was prepared from it to obtain about 400 mg/ml solution. An aliquot (250 µl) of the solution was injected into GPC column (wheat oil extract injected corresponded to 100 mg of the concentrated plant extract). The appropriate numbers of calibrated and graduated test tubes were prepared. Their weight was determined with ≤ 0.0001g accuracy. The first 7 ml were discarded, then the eluent was collected in 1 ml increments until a total volume of 13 ml was eluted. The eluent was evaporated to dryness (until constant weight) and the eluted dry material was weighed. The test was performed in three replicates.

2.3.2. Cleanup of the extracts

The concentrated extract representing 5 g rice or wheat sample was injected into the column and allowed to run at the eluent flow rate described above. The flow rate was checked during the elution. The first 9 ml eluate was discarded and the next 20 ml eluate was collected as pesticide fraction. The eluent was evaporated to nearly dryness and dissolved in acetone for application on the TLC plates and also for GC-NPD analysis to determine the recovery.

2.4. TLC detections

The TLC elution of pesticides were performed with Silica gel 60, 0.25 mm (Merck:1.05721): ethyl acetate system and aluminium oxide F254 60, 0.2 mm (Merck:1.05550): ethyl acetate system.

The Rf values were determined in developing tanks kept in water bath adjusted to 23ºC in order to reduce the effect of temperature variation in the laboratory. Eighty ml eluent was poured in the tank. The eluent was equilibrated with the vapour phase by inserting filter paper in the developing tank and waiting for minimum 30 minutes before the plates were placed into the tank (Note: The tank must be protected from sunlight). The silica gel plates were activated at 105ºC for 30 minutes before use. Twenty µl extract and/or standard solution was applied in uniform spots of about 4–6 mm on the plate 2 cm from the bottom edge. The plate was placed into the eluting solvent at 1 cm depth in the saturated developing tank. The eluent was allowed to run up to 11 cm from the origin.

Visualization of the spots on the plate was performed with the following chemical reagent and bioassay techniques [4].

Method 1. o-Tolidine + potassium iodide (o-TKI)
Method 2. Photosynthesis inhibition: Hill reaction (Hill)
Method 3. Fungi spore (*Aspergillus niger*) inhibition (FAN)
Method 4. Enzyme inhibition with cow liver extract and ß-naphthyl-acetate substrate (EßNA)
Method 5. Enzyme inhibition with horse blood serum and acetyl-thiocholine iodide substrate (EAcI horse)
Method 6. Aluminium oxide G incorporated with silver nitrate + UV exposition (AgUV)

The modifications and improvements introduced to detection methods were the following:

a) The sprayer was connected with a pipe to the exhaust of the vacuum pump, so that spraying of the reagent was done effectively.

b) Hill method: For detecting reagent, wheat pressing was diluted with DCPIP at the ratio of 1:3 to 1:5 (v/v), depending on chlorophyll content of the leaves, until the colour of the mixture becomes bluish-green.

c) FAN method: Instead of self-made fungi culture media, ready-made fungi culture media (Microbiology Potato dextrose agar for microbiology, Merck 1.10130) was used. During the spraying of fungi spore suspension (since spraying solution may cool down and the solid particles can block the atomizer), another sprayer was held in hot water in case it was needed. To obtain a well saturated tank and preventing other saprophyte fungi development on the plate, the developing tank (used for incubation) was filled with sterilized hot water and kept for about 15 minutes, then the excess water was poured out keeping about 2 cm water in the tank which was covered and cooled down to 37ºC.

d) Ag-UV method: Reagent: dissolve 0.15 g $AgNO_3$ in 15 ml freshly prepared bi-distilled water. Detection: instead of self-made plate, we used pre-coated neutral alumina layer on aluminium sheet 20 ×20 cm, 0.2 mm thickness (aluminiumoxide F_{254} 60 Merck, 1.05550). The plate was sprayed with reagent solution uniformly (no gaps

and lines of liquid flowing down the plate), dried with a fan and kept in an oven at 60ºC for 20 min. The pesticide solution was spotted to the plate and the plate was developed with ethyl acetate solvent. The plate was air-dried and kept in the UV chamber at 254 nm for 20–30 min. Grey coloured spots appeared on a colourless or light-grey background. The colour of the spots improved sometimes when plates were stored outside.

2.5. GLC detections

Hewlett Packard (HP6890) GC equipped with a NPD was used under the following conditions: capillary column (30.0 × 250 µm × 0.25 µm nominal film thickness, HP 19091S-433, HP-5MS 5% phenyl methyl siloxane); carrier gas nitrogen 1.3 ml/min (for diazinon and triazophos 1.1 ml/min); hydrogen 2.0 ml/min; air 60 ml/min. Operating conditions; column temperature: 140–250ºC; initial time: 1 min; rise: 5ºC/min; final time: 3 min; run time: 26 min; detector: 280ºC; injector: 270ºC (splitless).

The ECD was operated with the same type of column at temperatures: column: 200ºC, detector: 270ºC; injector: 230ºC (splitless).

3. RESULTS AND DISCUSSION

3.1. Calibration of the GPC Column

Figure 1 shows the average of three replicates of elution profiles of wheat oil and standard mixture. In the first 9 ml 94.14% of wheat oil was eluted through the column. Diazinon and triazophos were eluted in 10–20 ml and 12–20 ml fractions, respectively.

3.2. Recovery Study

The performance of the cleanup method was checked with recovery experiments by fortifying blank sample portions with 1 ml standard mixture at the level of 0.1 mg/kg diazinon and 0.05 mg/kg triazophos in triplicate. An aliquot of (250 µl) concentrated extract (representing 5 g wheat sample) 500 ng diazinon and 250 ng triazophos was injected to GPC. The percentage of recovery for diazinon and triazophos were 85.5% and 100.4%, respectively (Table 1).

3.3. Detectability of marker and selected compounds

Under the specific conditions described for the individual methods, the retention factors (Rf) were measured with three runs on different plates for each marker compound and selected compounds. The Rf, RRf, and their CV's determined with o-TKI, Hill, FAN, EβNA, EAcI-horse, and AgUV detection methods are given in Table 2. The Rf and RRf values were in close agreement with those reported earlier, except oxamyl. The MDQ values determined with the same detection methods are given in Table 3.

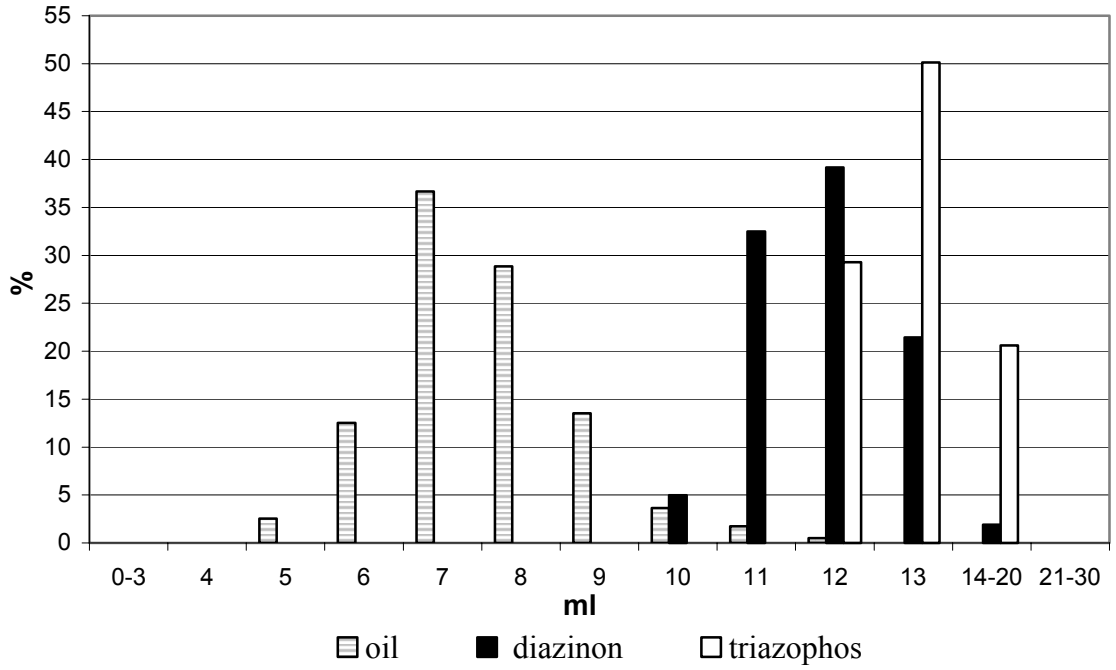

FIG. 1. Distribution of wheat oil, diazinon and triazophos on Bio-Beads SX-3 gel with ethyl acetate:cyclohexane (1:1).

TABLE 1. RECOVERY OF DIAZINON AND TRIAZOPHOS FROM FORTIFIED WHEAT SAMPLES WITH GC - NP DETECTION AT 0.1mg/kg AND 0.05mg/kg SPIKING LEVELS, RESPECTIVELY

	Recovery (%)			Average Recovery (%)	CV %
	1	**2**	**3**		
Diazinon	94.2	78.56	83.8	85.53	9.39
Triazophos	102.9	89.35	108.9	100.38	9.97

3.4. Verification of MDQ of the compounds in the presence of cleaned up plant extract

The purpose of this study was to test the detectability of compounds in the presence of co-extractives being in the cleaned up extracts. If the co-extractives substantially affect the detectability of the compounds then the sample equivalent spotted onto the TLC plates should be reduced or further cleanup may be necessary to achieve the required sensitivity. The tests were carried out in three replicates with the blank extracts of wheat and rice after the GPC cleanup. The amounts of extracts that could be spotted on to the TLC plate was determined experimentally. We found that, 10 mg sample equivalent applied in 20 µl did not affect the detectability of the compounds with the detection procedures except in the case of detection with AgUV method where only 5 mg sample equivalent in 20 µl could be spotted.

The LOD was calculated according to equation (1).

$$LOD = \frac{MDQ[ng]}{M[mg]} \quad (1)$$

where M is the sample equivalent applied on the layer. The LOD values calculated are summarized in Table 3.

TABLE 2. SUMMARY OF RF AND RRf DETERMINED WITH VARIOUS DETECTION METHODS

Method	Compound	Rf values					RRf values		Reported[a]	
		1	2	3	Mean	CV%	Mean	CV%	Rf	RRf
o-TKI	Atrazine	0.61	0.62	0.60	0.61	1.98	1	0	0.62	1
	Diuron	0.36	0.36	0.35	0.36	1.87	0.587	1.06	0.368	0.594
	Oxamyl	0.15	0.16	0.14	0.15	5.82	0.247	4.29	0.189	0.305
	Dimethoate	0.27	0.26	0.30	0.276	6.48	0.440	3.43	0.275	0.443
	Dioxacarb	0.42	0.42	0.46	0.44	5.44	0.695	8.10	0.454	0.732
Hill	Atrazine	0.62	0.66	0.68	0.65	4.55	1	0	0.62	1
	Chlortoluron	0.43	0.47	0.45	0.45	3.87	0.691	3.49	0.398	0.642
	Metoxuron	0.34	0.37	0.33	0.35	5.43	0.533	6.19	0.303	0.489
	Cyanazin	0.58	0.59	0.59	0.59	1.38	0.979	0.47	0.602	0.971
	Thiabendazole	0.39	0.36	0.36	0.37	4.60	0.618	5.46	0.335	0.540
FAN	Captan	0.66	0.64	0.67	0.66	1.78	1	0	0.63	1
	Fenarimol	0.46	0.48	0.50	0.48	3.86	0.730	3.63	0.476	0.756
	Prochloraz	0.27	0.28	0.30	0.28	4.39	0.430	3.29	0.314	0.498
EβNA	Parathion-M	0.75	0.70	0.71	0.72	3.81	1	0	0.669	1
	Dichlorvos	0.52	0.49	0.49	0.50	3.83	0.691	0.34	0.505	0.755
	Oxamyl	0.16	0.16	0.15	0.16	2.46	0.217	4.22	0.189	0.283
	Carbaryl	0.63	0.63	0.65	0.64	1.43	0.887	7.75	0.598	0.894
	Chlorpyrifos	0.74	0.74	0.75	0.74	1.11	1.011	0.20	0.669	1
EAcI horse	Parathion-M	0.68	0.66	0.70	0.68	3.12	1	0	0.669	1
	Oxamyl	0.12	0.13	0.13	0.13	3.71	0.189	3.08	0.189	0.283
	Methomyl	0.32	0.33	0.31	0.32	2.30	0.472	5.42	0.363	0.543
AgUV	Dieldrin	0.89	0.86	0.87	0.87	1.72	1	0	0.834	1
	Triforine	0.54	0.56	0.47	0.52	9.15	0.600	9.08	0.788	0.729

[a] Reported Rf by Ambrus et al. [4]

3.5. Inter-comparison test sample result

Inter-comparison test samples were prepared by the Agrochemicals Unit, IAEA Laboratories for analysis with TLC and optionally by GC. The results obtained in our laboratory with TLC and GC, are shown in Table 4. There were some differences in marker compound results. Valid results were obtained with TLC detection in fifty-six percent of the cases. The second inter-comparison test sample was ground wheat. The residues found with TLC and GLC detection are shown in Table 5. Dichlofluanid could not be found in the second inter-comparison test sample.

TABLE 3. MDQ AND LOD OF THE MARKER AND SELECTED COMPOUNDS IN THE WHEAT AND RICE EXTRACTS

Method	Compound		MDQ (ng)	LOD (ng/mg)	
			Found	Wheat extract	Rice extract
o-TKI	Marker	Atrazine	12.5	1.25	1.25
		Diuron	15	1.5	1.5
		Oxamyl	50	5	5
	Selected	Dimethoate	50	5	-
		Dioxacarb	12.5	1.25	-
Hill	Marker	Atrazine	0.5	0.05	0.05
		Chlortoluron	0.5	0.05	0.05
		Metoxuron	2.5	0.25	0.25
	Selected	Cyanazin	50	5	-
		Thiabendazole	0.5	0.05	-
FAN	Marker	Captan	10	1	-
		Fenarimol	25	2.5	-
		Prochloraz	12.5	1.25	-
EβNA	Marker	Parathion-M	0.5	0.05	0.05
		Dichlorvos	10	1	1
		Oxamyl	10	1	1
	Selected	Carbaryl	5	0.5	-
		Chlorpyrifos	0.25	0.025	-
EAcI horse	Marker	Parathion-M	1	0.05	0.05
		Oxamyl	2	1	1
		Methomyl	5	1	1
AgUV	Marker	Dieldrin	12.5	2.5	-
		Triforine	50	10	-

4. CONCLUSIONS

To ensure the reproducibility of the Rf values and the required separation efficiency, the experimental conditions must be controlled properly. The use of the marker compounds have proven to be very satisfactory for this purpose. The MDQ of the marker compound, specific for a detection procedure, should be spotted on each plate on which qualitative and quantitative determinations are carried out.

The Rf, RRf and MDQ values of marker and selected compounds determined with various detection method were in agreement with those reported earlier [3] except oxamyl.

The MDQ values in some cases were less than those previously reported [3]. The MDQ's values were also verified in the presence of cleaned-up extracts. The elution profiles of wheat oil diazinon and triazophos showed that KL-SX-3 GPC gel chromatograph is a very useful tool for cleaning up of plant extracts.

TABLE 4. TLC AND GC ANALYSIS OF FIRST INTERCOMPARISON TEST SAMPLES

Compound	Test 1 sample, µg/ml				Test 2 sample, µg/ml			
	Rep[1]	Found			Rep[1]	Found		
		TLC	NPD	ECD		TLC	NPD	ECD
Oxamyl	0.5	0.626^{EBNA}	0.545	-	20	**10.7^{EBNA}**	**56.4**	-
Prochloraz	5	**26.83^{OTKI}**		**44**	-			**65.9**
Metoxuron	1	1.1^{HILL}	0.896	-	-			
Methomyl	-				20	**96.8 ^{OTKI}**		**60**
Triforine	-				50	41.28^{AgUV}		38.05
Dichlorvos	0.5	0.459^{EBNA}	0.447		-			
Fenarimol	10	12.99^{OTKI}	9.3		20	**26.83**^{OTKI}	18.4	
Captan	5	**nd**[2]	4.06	**6.84**	10	**nd**[2]	7.87	10.4
Atrazine	0.3	**0.463**^{OTKI}	0.338	0.49	5	4.28^{OTKI}	5.86	**3.35**
Dieldrin	5	**nd**[2]	6.3	**7.2**	-		**6.12**	**0.043**
ParathionM	0.5	0.407^{EBNA}	0.494	-	1	0.61^{EBNA}	1.02	

[1] Assigned value
[2] not found
Bold face: outside the ±3S range

TABLE 5. TLC AND GC ANALYSİS OF SECOND INTERCOMPARİSON TEST SAMPLES

Compound	Rep μg/g[1]	Found	
		TLC	GLC
Dichlofluanid	2.01	nd	nd
Diuron	0.235	0.19	0.26
Fenitrothion	0.863	0.82	1.0

[1] Assigned value

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THIN LAYER CHROMATOGRAPHY AS AN ALTERNATIVE METHOD FOR PESTICIDE ANALYSIS IN RICE GRAINS

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Abstract

The applicability of thin layer chromatography for the analysis of pesticide residues in grains as an alternative method to gas and high performance liquid chromatography was evaluated. Recoveries of six selected pesticides representing different pesticide classes as well as marker compounds for the four TLC detection methods employed ranged from 60 to 102%. The CVs of most of the pesticides analysed were within the range of CV indicated excepted for pesticide residue analysis. Thin layer chromatography can be a useful tool to analyze pesticide residues in rice grains.

1. INTRODUCTION

Thin layer chromatography (TLC) has been used for the qualitative and quantitative analysis of a wide variety of compounds [1]. It is a quick and efficient technique for the detection and determination of many different types of compounds. When standardized conditions are used, TLC can be used for screening pesticide residues in samples of unknown origin [2].

In the Philippines, very few laboratories, both private and government owned, are dedicated to studying pesticide residues. This may be partly due to the high cost associated with putting up an operational pesticide residue laboratory. The need to quantify residues in foods using cheap and fast but reliable techniques becomes more relevant especially for laboratories where the irregular supply of electricity, lack of service or limited budget do not allow continuous use of gas chromatographic (GC) and high pressure liquid chromatographic (HPLC) techniques. This paper reports the results of validation study on the application of TLC for pesticide residue analysis in rice grains.

2. MATERIALS AND METHODS

2.1. Materials

The pesticides, solvents and reagents used were of analytical grade quality and were obtained from Merck Co. and Ehrernstorfer Ltd, Germany.

The basic TLC separation and detection methods elaborated for use within the coordinated research programme [3] were applied for the validation study. The changes made in the procedures during the method adaptation are described hereunder.

Chloroplast was obtained from rice seedlings instead of wheat. Fifty grams of finely cut leaves was used with the same proportion of chemicals as described earlier for the Hill reaction [3]. *Aspergillus niger* spores were obtained from laboratory cultured specimen. Pig's blood serum was obtained from a freshly slaughtered animal and was frozen until use. Ten ml of the serum was diluted with 7 ml tris buffer prior to use.

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In the absence of an incubator, a TLC developing tank with a ground glass lid was used for the development of the fungi culture for FAN detection method. The tank lined with filter paper was saturated by adding about 2 cm water. The upper 3 cm of the adsorbent layer was removed from the plate. The plate was placed in the tank with the scraped part in the water and was covered with the glass lid [4]. The tank was kept for 48 hours in a laboratory oven at 37°C.

2.2. Extraction, cleanup and TLC detection

Ten grams of previously ground-milled rice was soaked with 10 ml distilled water for 30 minutes. The temperature of the slurry was maintained at 28ºC. The rice grains were fortified with the selected pesticides at two fortification levels: twice the limit of quantification (LOQ), at the recommended maximum residue limits, MRL, and five times the LOQ in the absence of MRL. The following reagents were added to the test portion: 50 ml ethyl acetate, 5 g sodium hydrogen carbonate and 35 g, anhydrous sodium sulphate. The mixture was homogenized with Ultra Turrax for 30 seconds. The organic phase was collected and filtered through a cotton wool plug to obtain a 25 ml extract. The extract was evaporated in a rotary evaporator to approximately 1 ml, quantitatively transferred to a test tube and the final volume was adjusted to 1 ml. An aliquot of 250 µl was taken for cleanup in a gel permeation chromatograph. It was eluted with ethyl acetate:cyclohexane (1:1) at a flow rate of approximately 1 ml/min. The first 8 ml eluate was discarded and the next 22 ml eluate was collected. It was evaporated to nearly dryness and taken up in 1 ml acetone for direct application to TLC plates.

Commercially available adsorbent silica gel TLC plates were reactivated in an oven at 110°C for 30 minutes and kept in a desiccator for 30 minutes before use. The plates were exposed to high humidity and temperature during application of the samples. This could last between 30 min to an hour. Temperatures ranged from 25-30°C and relative humidity was between 81–98%.

Detection of the pesticides was achieved using the following TLC detection methods: o-tolidine+potassium iodide (o-TKI), bioassay with fungi spores (FAN), enzyme inhibition with pig blood serum (EAcI) and photosynthesis inhibition (Hill Reaction).

3. RESULTS AND DISCUSSION

The minimum detectable quantity (MDQ) of the selected pesticides was determined (Table 1) with tests performed on three separate TLC plates.

TABLE 1. Rf AND MINIMUM DETECTABLE QUANTITY (MDQ) OF SELECTED COMPOUNDS

Pesticide	Rf			Average Rf	CV	MDQ (ng)
	1	2	3			
Epoxyconazole	0.561	0.566	0.761	0.629	18	25
Imazalil	0.425	0.42	0.442	0.429	2.7	13
Chlorbromuron	0.656	0.661	0.649	0.655	0.92	38
Teflubenzuron	0.658	0.678	0.68	0.672	1.8	50
Phosphamidon	0.39	0.39	0.42	0.40	4.3	25
Phosalone	0.7	0.72	0.7	0.71	1.6	3

The applicability of thin layer chromatography to detect pesticide residues in rice grains was tested initially with the marker compounds for each of the TLC detection method. The use of these marker compounds was appropriate for controlling the proper conditions of the detection method.

Results of validation study on rice grains fortified with marker compounds for the different TLC detection methods are presented in Table 2. There were differences in the recovery at the two fortification levels used with the EAcI method. This reflects the day-to-day variation in temperature and relative humidity among others. The relative humidity varied from 81-98% during the course of the study. The obtained limit of quantification (LOQ) was comparable with the estimated LOQ computed at 70% recovery.

TABLE 2. RECOVERIES AND LOQ VALUES OF MARLKER COMPOUNDS FROM RICE

Method	Pesticide	Applied Amount (μg/g)	Recovery (%)	CV	LOQ[a] (mg/kg)	LOQ[b] (mg/kg)
o-TKI	Oxamyl	5.7	60	4.2	2.9	3.3
	Diuron	1.7	80	9.1	0.86	0.75
	Atrazine	1.4	82	12	0.71	0.61
	Chlorbromuron	5.4	77	20	1.1	0.97
	Teflubenzuron	2.9	52	37	1.4	1.9
		7.2	72	24	1.4	1.4
EAcI	Methomyl	0.29	69	5	0.14	0.15
		0.72	65	16	0.14	0.16
	Oxamyl	0.06	69	30	0.03	0.03
		0.15	66	23	0.03	0.03
	Parathion-methyl	0.06	86	28	0.03	0.02
		0.15	78	29	0.03	0.03
	Phosphamidon	1.4	91	9.1	0.71	0.55
		3.6	99	42	0.71	0.5
	Phosalone	0.01	93	8.9	0.07	0.05
		0.04	73	53	0.07	0.07

[a] estimated based on the MDQ values
[b] verified experimentally

Validation of the method with the selected pesticides gave good recovery except teflubenzuron (Table 3). As with the marker compounds, there were differences in recovery between the two fortification levels.

Quantitation was achieved by comparing spot area with that of the standard pesticide solutions. This method has its own limitations since spot area can differ from plate to plate

and it is also influenced by the shape of the spot and distortion caused by tailing [5] which was observed in the results obtained in the laboratory.

Day-to-day variation in climatic conditions especially high relative humidity, which is fairly common especially in the area where the research was conducted, might have an effect on the repeatability and reproducibility of the results. The observed difference in spot area may also be influenced by the difference in the migration rates of the mobile phase at any particular time.

TABLE 3. RECOVERIES AND LOQ VALUES OF SELECTED PESTICIDES FROM RICE

Method	Pesticide	Applied Amount (ug/gm)	Recovery (%)	SD	CV	Estimated LOQ	Obtained LOQ
FAN	Imazalil	0.72	106.4	7.97	7.48	0.36	0.24
		1.8	63.85	6.8	10.65	0.36	0.39
	Epoxyconazole	1.428	73.5	28.29	38.49	0.714	0.68
		3.57	102.33	8.37	8.18	0.714	0.49
EAcI	Phosphamidon	1.428	91.2	8.31	9.12	0.714	0.548
		3.57	99.8	42.49	42.56	0.714	0.50
	Phosalone	0.014	92.85	8.25	8.89	0.07	0.05
		0.035	73.14	39.2	53.61	0.07	0.068
o-TKI	Chlorbromuron	5.35	77.38	15.27	19.74	1.07	0.97
	Teflubenzuron	2.86	52.44	19.46	37.1	1.43	1.91
		7.15	72.26	17.36	24.02	1.43	1.38

The CV values give an indication of the reproducibility of the methods employed. The CVs of most of the pesticides analysed were within the range of CV indicated by Lantos [1]. Variation in CV among the pesticides analysed indicated the importance of several factors in order to achieve repeatability of results. Some of these are the following: plate to plate variation of the results [6] due to difference in climatic conditions, saturation of the chamber [1] and sample load. The spots may become distorted and the Rf may change if the layer is overloaded with the extracted material [7].

The results of this study demonstrate that TLC can be used as an alternative method to analyze pesticide residues in grains. This is very helpful for laboratories especially in developing countries where budgetary constraints limit their ability to do pesticide residue research.

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THIN LAYER AND GAS CHROMATOGRAPHIC STUDY OF THE PERSISTENCE OF ATRAZINE IN TROPICAL SOILS

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Abstract

Evidence is presented to show that atrazine is completely lost from the topsoil in a maize plantation within five weeks. Considering the copious rainfall experienced during the period under study, the loss of atrazine from the topsoil has been attributed to either degradation or leaching. Thin layer chromatographic (TLC) determination of atrazine after cleanup has been demonstrated to give comparable residue data to that obtained by gas chromatographic (GC) analysis.

1. INTRODUCTION

Atrazine is extensively used in Ghana to control weeds in pineapple, maize and tomatoes. It is slightly to moderately toxic to humans and other animals [1]. Atrazine is persistent in soil and has been demonstrated to persist for longer than one year under dry or cold conditions [2]. It is also moderately or highly mobile in soils with low clay or organic matter content. Because atrazine does not adsorb strongly to soil particles and has a lengthy half-life (60 to >100 days), it has a high potential for contamination of groundwater despite its moderate solubility in water [3]. It is no wonder then that atrazine is the second most common pesticide found in private wells and in community wells [4]. Trace amounts have been found in drinking water samples and in groundwater samples in a number of states in the United States [5, 2]. A five-year survey of drinking water wells detected atrazine in an estimated 1.7% of community water systems and 0.7% of rural domestic wells nationwide in the USA [5]. The magnitude of use of atrazine in Ghana therefore calls for a study on persistence of atrazine in tropical soils.

The currently used methods for detecting and quantifying herbicides are gas chromatography (GC) and high performance liquid chromatography (HPLC). These techniques have the disadvantage of being expensive, consume expensive high purity gases and solvents and require a high level of expertise for their operation and maintenance [6]. Using the inhibiting property of herbicides to photosynthesis reaction, Ambrus et. al. [7] successfully analysed a broad range of herbicides by TLC.

The objective of this study is to investigate the persistence of atrazine in tropical soils using TLC and GC.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Ethyl acetate, dichloromethane and benzene were analytical grade and obtained from Fluka Chemie AG, Buchs, Switzerland. Atrazine standard was purchased from Dr. Ehrenstorfer

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GmbH, Germany. All other chemicals and TLC plates [20×20cm, Silica gel 60 F 254 (5721, 5715) and Aluminium oxide G (5713)] were obtained from Merck KgaA, Darmstadt, Germany.

Preparation of standard solutions: 1.0 mg/ml primary standard stock solution of atrazine used in this study was prepared by dissolving approximately 0.05 g pure pesticide (corrected for standard purity, e.g.: for % purity of 94% 0.05 × 100/94 g was weighed) in 50 ml acetone.

Spinach leaf pressing: 30 g spinach leaves were weighed into a mortar. Three ml glycerol, 15 ml double distilled water and 5 g acid washed sand were added. The mixture was ground into a homogenous pulp. The homogenate was transferred into cheesecloth in a funnel and pressed through. The filtrate was protected from sunlight by wrapping the container in aluminium foil, and was stored in a refrigerator until use. Fresh filtrate was prepared daily.

Borax (di-sodium tetra borate) buffer solution: 350 ml of 0.05M borax solution (9.5 g Borax dissolved in 500 ml water) was mixed with 150 ml 0.1M HCL.

DCPIP reagent: 200 mg of 2,6 -dichlorophenol-indophenol Na-salt was dissolved in 500 ml borax buffer solution.

2.2. Apparatus

TLC basic set including application guide, atomizer and developing tank were obtained from CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland. Micro syringes (10 µl) with needle (Hamilton) were obtained from Supelco Inc., Supelco Park, Bellefork, Pa., U.S.A.

Gas chromatograph: Varian Star 3400CX GC equipped with an ECD ^{63}Ni and a 15 m (0.53 µm i.d. × 1.5 µm film) J & W DB5 capillary column.

2.3. Herbicide Application

The experiment was conducted on a 1.5 ha. plot. Four sub-plots each measuring 50 m × 50 m were marked out. The commercial product investigated was a powdered formulation containing 200 g-atrazine active ingredients per kilogram of sample. The spray solution was prepared by suspending 2 kg of atrazine powder in 300 litre of water. This was used to spray one hectare of plot in accordance with the manufacturer's recommendations. Topsoil samples were taken at the depth of 0-15 cm from the four sub-plots in a maize plantation. Two diagonally opposite plots were treated with atrazine whilst the other two were kept as control.

2.4. Sampling

Sampling was done at weekly intervals for five weeks post application of herbicide. Eight cores of soils were sampled at random from each sub-plot using a soil auger (2.5 cm in diameter and 20 cm deep). The cores were weighed before and after removal of stones and ground to pass through a 2.5 cm sieve. The ground soil was mixed thoroughly and sub-samples in triplicate were taken for residue analysis at stated intervals.

2.5. Determination of residues

Triplicate soil samples (50 g), which had been sieved through 2 mm mesh, were Soxhlet extracted for 5 hours with 250 ml methanol. An aliquot (5 µl) was taken for TLC analysis using Hills reaction detection method [8]. A further 50 ml aliquot of the methanol extract was

evaporated to dryness in a rotary evaporator and the residue taken up in 1 ml methanol/water mixture (1:2.5). The diluted extract was passed through a reconditioned C-18 solid phase extraction (SPE) column at a flow rate of 2 ml/min. The column was vacuum dried for 15 minutes. The pesticide eluted with 2 ml ethyl acetate. An aliquot (5 µl) was injected into the GC, whist 10 µl aliquot was analysed by TLC.

Gas chromatographic analysis was performed with a 15 m (0.53 µm i.d. × 1.5 µm film) J & W DB5 capillary column. The nitrogen gas flow rate was 8 ml/min with the following operating conditions: injector temperature: 230ºC; column temperature: 200ºC and detector temperature: 200ºC.

The extracts and standard solutions (10–20 µl) were transferred manually using a syringe with 90º cut needle tip onto the freshly activated TLC plates. Standards and samples were applied in spots of the same size. This was achieved by spotting the same volume and using the same solvent. The spots were applied with a spotting guide, they were allowed to dry before spotting the next portion again until the whole volume has been applied. Care was taken to include a marker compound on each plate.

The spotted TLC plates were placed in developing tanks kept in a water bath thermostated at 24ºC in order to reduce the effects of temperature variation in the laboratory. The eluent was equilibrated with the vapour phase by inserting filter paper in the developing tank and waiting for 30 minutes before the plates were placed into the tanks. The ethyl acetate eluent was allowed to run up to 10 ± 0.5 cm from the origin.

The plates were air-dried and uniformly sprayed with a freshly prepared detecting reagent made from a mixture of 10 ml spinach leaf pressing and 13.5 ml DCPIP solution. The plates were immediately placed about 20 cm below a 60W wolfram lamp. The inhibition occurred within 10 minutes and dark bluish-green spots appeared against a light green background. Spots were however unstable and disappeared within a few minutes.

2.6. Recovery Experiments

Fifty gram of soil was spiked with various amounts of atrazine standard as described in Table 1. Samples were extracted and cleaned up, and subsequently analysed by GC and TLC as described in earlier. Fortification of soil with atrazine gave recoveries of 70% and 85% for gas chromatographic analysis.

TABLE 1. RECOVERY OF ATRAZINE FROM SOIL BY TLC/HILL'S REACTION

Fortification levels (mg/kg)	n	Residue measured (mg/kg)	Recovery %	±SD
0.06 (approx. 1 × MDQ)	3	ND	ND	—
0.12 (2 × MDQ)	3	0.08	67	12
0.24 (4 × MDQ)	3	0.18	75	6
0.30 (5 × MDQ)	3	0.23	77	5
0.12 + SPE	3	0.05	41.6	8
0.24 + SPE	3	0.16	67	15
0.30 + SPE	3	0.22	73	11

SD: standard deviation

For quantitative analysis by TLC, a calibration curve was prepared based on the average diameter of standard spots as a function of applied amount [μg]. The concentration of the sample was then interpolated from the curve.

3. RESULTS AND DISCUSSION

Table 2 and Fig. 1 compare the atrazine residue levels obtained from cleaned and uncleaned extracts from the soil samples using GLC and TLC detection. The results of both GC and TLC analyses show that atrazine concentration in the soil was below detection level five weeks after the treatment of the plots.

TABLE 2. RESIDUE LEVELS OF ATRAZINE IN SOIL

Day of Sampling	Atrazine residues (mg/kg)		
	TLC*	TLC**	GLC
Before treatment	N.D.	N.D.	N.D.
7th	27	23	25
21st	9.7 ± 0.8	5.7 ± 0.6	6.5 ± 0.1
28th	0.050	0.030	0.024
35th	N.D.	N.D.	N.D.

* Results without cleanup
** Results with cleanup
N.D. Not detected

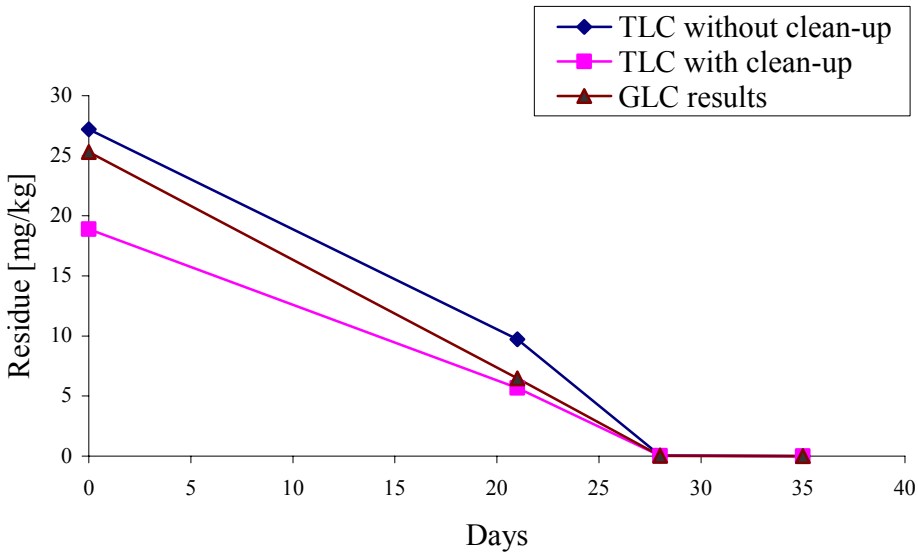

FIG. 1. Change in residue levels of atrazine in soil with time after application.

Atrazine is moderately soluble in water. Thus, considering the copious rainfall experienced during the period under study (Table 3), the loss of atrazine from the topsoil may be attributed to either or combination of the following processes: degradation, leaching and run-off from the field.

TABLE 3. MONTHLY TOTAL RAINFALL AND AVERAGE TEMPERATURE DURING THE EXPERIMENT

Month	Total Rainfall (mm)	Ave. Temperature (ºC)
February	98.0	27.2
March	252.7	27.8
April	86.2	27.8
May	224.6	27.0

Chemical hydrolysis, followed by biodegradation, is considered the most important route of disappearance from aquatic environments; whilst bio-concentration and volatilization of atrazine are not considered environmentally important [5].

A comparison of the results from the TLC with that of the GLC, it appears that the method of estimation by taking the diameter of the spots on the TLC achieves an accuracy and reproducibility in the range 10-20%. This degree of accuracy is adequate in most situations in environmental monitoring. It can be deduced therefore, that TLC determination of atrazine after cleanup of extract as demonstrated in this study, is comparable to analysis by GC.

4. CONCLUSION

Results obtained in this study show that atrazine concentration decreased below the limit of detection in the topsoil in a maize plantation within five weeks. Considering the copious rainfall experienced during the period under study, the loss of atrazine from the topsoil may be attributed to degradation and leaching. TLC determination of atrazine after cleanup of extract as demonstrated in this study, gives comparable residue data to that obtained by GC.

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COMPARISON OF THIN LAYER CHROMATOGRAPHIC AND GAS CHROMATOGRAPHIC DETERMINATION OF PROPOXUR RESIDUES IN A COCOA ECOSYSTEM

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Abstract

The fate of propoxur in a cocoa ecosystem has been studied using thin layer chromatographic (TLC) and gas chromatographic (GC) methods. Residues of propoxur as determined by both TLC and GC were not significantly different. TLC analysis of propoxur residues in soil, cocoa leaves and pods did not require any rigorous cleanup since residues measured from cleaned extracts and without cleanup were not significantly different. The residue levels of propoxur in the soil were found to decrease rapidly and, by the 21st day, none was detected in the topsoil (0-15 cm). Evidence of leaching of propoxur residues in the soil has also been demonstrated. The amount left in the top soil after the first seven days were 27%, 23% and 24% of the initial one as determined by the TLC without cleanup, TLC with cleanup and GLC, respectively. No propoxur residue was detected in topsoil 21 days after spraying. About 38% of pesticides detected on the cocoa pod on the day of treatment remained on the pod seven days after treatment. The residue detected on the leaves on the day of treatment was higher than that in or on the soil. This decreased rapidly to 1.7% in 21 days compared to 16% for the soil and 23% for the pod.

1. INTRODUCTION

Cocoa is usually cultivated in a multi-cropping system in Ghana. Propoxur is one of two pesticides that is registered and recommended for the control of capsids in cocoa. The application is carried out four times a year on prophylactic basis (i.e. calendar spray) in August, September, October and December. Propoxur is a toxic compound, with moderate to low persistence with reported field half-lives in the soil environment of 14 to 50 days [1]. Propoxur can also enter the roots of a plant and travel to the leaves, where it can then poison insects that feed on the leaves. It was shown to have residual activity of up to one month when applied to plant surfaces [2].

The pesticide residues including propoxur are generally determined by GC or HPLC [3]. However, TLC appears to be gaining popularity again as an important analytical tool for analysis of pesticides. This is due to the fact that TLC offers the opportunity to undertake analysis where the combination of factors including, inaccessibility to instrumental service facilities, spare parts and continuous electricity supply, makes instrumental analysis almost impossible.

The objectives of this study are to determine the fate of propoxur residues in cocoa ecosystem using GC and TLC and to compare the efficiency of recovery of the two methods.

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2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Ethyl acetate, dichloromethane and benzene were analytical grade and obtained from Fluka Chemie AG, Buchs, Switzerland. Pesticide standards were purchased from Dr Ehrenstorfer GmbH, Ausberg, Germany, and had purity ranging from 94.0% to 99.5%. All other chemicals and TLC plates (20 × 20 cm) Silica gel 60 F 254 (5721, 5715) and Aluminium oxide G (5713) were purchased from Merck (E) GmbH, Germany.

2.2. Preparation of standard and reagent solutions

Primary standard stock solution (1.0 mg/ml) of propoxur used in this study was prepared by dissolving approximately 0.05 g pure pesticide (corrected for standard purity, e.g for % purity of 94, 0.05 × 100/94 g was weighed) in 50 ml acetone.

Pig blood serum was collected from the University of Ghana Research Farm at Nungua, and congealed at 2–3ºC for one hour. The separated blood serum was centrifuged for 10 minutes at 4000 rpm. Serum was collected in 10 ml portions and kept frozen until use. Cholinesterase activity was adjusted to 140 U/l by dilution with tris buffer before use. 2,6-dichloro-indophenol Na-salt: 50 mg of the salt was dissolved in 50 mg distilled water to give 1 mg/ml solution.

The other chemicals and reagents were prepared as described in the basic method [4].

2.3. Apparatus

A TLC basic set including application guide, atomizer and developing tank were obtained from CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland. Micro syringes (10 µl) with needle (Hamilton) were obtained from Supelco Inc. US.

Gas chromatograph: Varian Star 3400 CX GC equipped with a thermo-ionic detector (TSD) and a 15 m (0.53 µm i.d. × 1.5 µm film) J & W DB5 capillary column.

2.4. Pesticide Treatment

The experiment was conducted on a 6-hectare cocoa plantation situated at the Cocoa Research Institute, Tafo, in the Eastern Region of Ghana. Four plots, each measuring 15 m × 15 m were marked out. The plot that had been earmarked for pesticide treatment was sprayed with a water emulsion of commercial propoxur of 50 g/litre of water. The spraying was done from two sides of a tree trunk into the canopy, as has been the normal practice (T2 method) to achieve adequate coverage. In line with the recommended practice, application of pesticide was repeated three weeks after the first application. The pod, leaves and soil samples were taken on the day of treatment and subsequently, at weekly intervals for two months for analysis.

Soil samples were taken at depths of 0–15 cm and 15–30 cm respectively for analysis. Eight cores of soils were sampled at random from each sub-plot using a soil auger (2.5 cm in diameter and 20 cm deep). The cores were weighed before and after removal of stones and ground to pass through a 2.5 cm sieve. The ground soil was mixed thoroughly and sub-samples in triplicate were taken for residue analysis at stated intervals. Propoxur residues were determined by TLC and GLC.

2.5. Extraction and Cleanup

2.5.1. Leaves and pod

Leaf tissue (60 g) or the peeled skin of the pods (60 g) was treated as follows: The sample was homogenized with 60 ml ethyl acetate in the presence of 50 g $NaHCO_3$ and 50 g anhydrous sodium sulphate. The extract was kept in a fume hood for 30 minutes to allow separation of the solvent from the solid material. The decanted solvent was filtered through a small cotton wool plug into a measuring cylinder to obtain a 30 ml filtrate (1 ml = 1 g sample). The filtrate was evaporated to 3 ml in a rotary evaporator and transferred into a calibrated conical test tube. The evaporation was continued to near dryness with a gentle stream of nitrogen. The final volume was adjusted to 2 ml with ethyl acetate for cleanup using GPC.

SX-3 bead, previously soaked for six hours in cyclohexane:ethyl acetate (1:1) was packed into a 10 mm i.d. × 20 cm × 1.2 mm thick Pyrex glass gel column under nitrogen pressure. Cyclohexane:ethyl acetate (1:1) solvent mixture was pumped under pressure from a solvent reservoir by means of nitrogen gas through the column. Flow rate was adjusted to 1.5 ml/min following calibration with standard propoxur solution. 200 µl of the extract were injected unto the column and the fractions collected were concentrated to 100 µl under nitrogen for direct spotting of 10 µl of resultant extract on TLC plates. The extract was also made up to 2 ml and 5 µl used for gas chromatographic determination.

2.5.2. Soil

Triplicate soil samples (50 g), which had been sieved through 2 mm mesh, were Soxhlet extracted for five hours with 250 ml methanol. A 50 ml aliquot of methanol extract was evaporated to dryness in a rotary evaporator and the residue taken up in 1 ml methanol/water mixture (1:2.5). The diluted extract was passed through a reconditioned C-18 solid phase extraction (SPE) column at a flow rate of 2 ml/min. The column was vacuum dried for 15 minutes, and then the pesticide was eluted with 2 ml toluene. 5 µl was injected onto the GC, whilst 10 µl aliquot was analysed by TLC using the enzyme inhibition method of detection.

2.6. Recovery Experiments

Leaves and pods: 60 g each of cocoa pods and leaves were spiked with various amounts of propoxur standard as indicated in Table 1. Samples were homogenized, extracted and cleaned up as described above, and subsequently analysed by GC and TLC. The recovery of propoxur from fortified leaves and pods was 75% and 80% with gas chromatographic analysis.

Freshly activated plates were always used for TLC separation. Spotting was done manually. 10 µl of the extracts (cleaned or uncleaned) was applied to the adsorbent layer with a syringe, with a 90º cut needle tip. Standards were applied in spots of the same size; this was achieved by spotting the same volume, using the same solvent. The spots were applied with a spotting guide and allowed to dry before the next portion was spotted until the whole volume had been transferred. Care was taken to include a marker compound on each plate.

Pesticide standard (10 to 20 µl) was spotted on chromatographic plate. The lower edge of the plate was placed in 1 cm ethyl acetate eluting solvent in saturated developing tank. The plate was eluted with ethyl acetate until the solvent front moved 10 ± 0.5 cm from the origin.

TABLE 1. RECOVERY OF PROPOXUR FROM COCOA LEAVES BY TLC EAcI METHOD

Fortification levels (mg/kg)	n	Residue measured (mg/kg)	Recovery %	±SD
0.25 (1 × MDQ)	3	N.D.	N.D.	—
0.50 (2 × MDQ)	3	0.36	66	10
1.0 (4 × MDQ)	3	0.70	70	4
0.25 + GPC	3	N.D.	N.D.	—
0.50 + GPC	3	0.26	52	20
1.0 + GPC	3	0.64	64	7

SD: standard deviation
N.D.: not detected

The enzyme inhibition method applying *pig blood serum and acetylthiocholine iodide substrate,* EAcI [4] was used to detect propoxur residues. Air-dried plate was treated with bromine and enzyme solution. After incubation at 37ºC for 30 minutes, excess water was removed with air steam. Substrate solution was sprayed on the plate and the latter was subsequently incubated for another 15 minutes. The reagent solution was then sprayed on the plate. Propoxur appeared as blue spots on a white background.

For quantitative analysis by TLC, a number of standards were spotted on the same plate with the samples. After development and visualization, the sample spots were visually compared with the standard spots. The concentration of the sample was taken as that of the standard spot for which the intensity and size were about the same.

The GC analysis was performed with Gas a 15 m (0.53 µm i.d. × 1.5 µm film) J & W DB5 capillary column. The nitrogen gas flow rate was 9 ml/min with the following operating conditions: injector temperature: 230ºC; detector temperature: 300ºC; column 60ºC for 2 min and programmed at 30ºC /minute to 200ºC for 12 minutes.

3. RESULTS AND DISCUSSIONS

The averages of the residue levels of propoxur in soils from treated plots are presented in Table 2. The propoxur residues in the soil decreased rapidly from an initial level of about 0.7 mg/kg, and by the 21st day no residue was detected in the topsoil (0–15 cm). The amount left in the top soil after the first seven days were 0.21, 0.16, and 0.18 mg/kg representing 27%, 23% and 24% of initial residue as determined by the TLC without cleanup, TLC with cleanup and GLC, respectively. The results of the analysis from the various methods of analysis were in very good agreement.

No propoxur residues were detected in the deep soil (15–30 cm) on the day of treatment. Thereafter, residue levels in the deep soil started accumulating and reached a maximum of 0.98 mg/kg within 14 days, thereafter it decreased and none was detected 21 days after the first treatment.

TABLE 2. RESIDUE LEVELS OF PROPOXUR IN SOIL

Day of Sampling	Sampling Depth (cm)	Propoxur residue [mg/kg]		
		TLC*	TLC**	GLC
Pretreatment	0-15	N.D.	N.D.	N.D.
	15-30	N.D.	N.D.	N.D.
After 1st treatment				
1st	0-15	0.79 ± 0.21	0.70 ± 0.09	0.76 ± 0.12
	15-30	N.D.	N.D.	N.D.
7th	0-15	0.21 ± 0.09	0.16 ± 0.05	0.18 ± 0.04
	15-30	0.81 ± 0.04	0.58 ± 0.18	0.64 ± 0.01
14th	0-15	0.53 ± 0.11	0.79 ± 0.16	0.46 ± 0.03
	15-30	0.98 ± 0.05	0.90 ± 0.08	0.10 ± 0.05
21st	0-15	N.D.	N.D.	N.D.
	15-30	0.13 ± 0.09	0.11 ± 0.07	0.12 ± 0.06
After 2nd treatment				
7th	0-15	0.69 ± 0.10	0.51 ± 0.09	0.68 ± 0.03
	15-30	N.D.	N.D.	N.D.
14th	0-15	0.46 ± 0.11	0.28 ± 0.08	0.41 ± 0.04
	15-30	N.D.	N.D.	N.D.
21st	0-15	N.D.	N.D.	N.D.
	15-30	N.D.	N.D.	N.D.

TLC* Results without cleanup
TLC** Results with cleanup
N.D. Not detected

The total amount of pesticide residues measured with TLC within the top 0-30 cm of soil on the seventh and fourteenth day of application was about double the amount detected in the top 0-15 cm of soil on the first day of application. This might be due to the sampling uncertainty and the fact that the heavy rainfall washed down pesticides from the canopy of leaves and pods onto the soil after the first application.

The initial accumulation in the deeper soil layer could be attributed to leaching from the topsoil considering the heavy rainfall during the study. The rapid dissipation of propoxur from the soil between the 14th and 21st day after application, however, could be attributed to the high temperatures during the experimental period (30 ± 3ºC). Increasing temperature is known to increase the rate of loss of pesticides from soils as well as microbiological degradation.

About 38% (0.26 and 0.33 mg/kg depending on method of analysis) of propoxur residues detected on the pod on the day of treatment remained seven days after treatment (Table 3).

Though the residue levels of propoxur measured on the pod on the day of treatment were somewhat higher than that in the soil, taking into account the sampled depth of soil (15 cm), the surface/mass ratio of soil is much smaller than that of the pod indicating that the spraying was not efficient and large amount of spray reached the soil surface.

TABLE 3. RESIDUE LEVELS OF PROPOXUR ON COCOA POD

Day of Sampling	Propoxur residue [mg/kg]	
	TLC**	GLC
Pretreatment	N.D.	N.D.
After 1st treatment		
1st	0.92 ± 0.17	0.86 ± 0.14
7th	0.26 ± 0.08	0.33 ± 0.11
14th	0.19 ± 0.06	0.15 ± 0.03
21st	0.09 ± 0.04	0.20 ± 0.10
After 2nd treatment		
7th	0.25 ± 0.06	0.38 ± 0.02
14th	0.07 ± 0.03	0.09 ± 0.01
21st	N.D.	N.D.

TLC* Results with cleanup
N.D. Not detected

The average of the results of leaf analysis obtained from the four subplots of the cocoa trees are shown on Table 4.

The initial residue found on the leaf was higher than that in/on the pod and the soil. Forty-five percent of the initial propoxur residue detected on the day of treatment remained seven days after treatment. This decreased rapidly to 1.7% in 21 days compared to 16% for the soil and 23% for the pod. The dissipation rate was therefore higher in the leaves. This can be attributed to the fact that the leaves are more exposed to the direct sunlight, wind and rainfall which causes dissipation and washing of surface easily compared to the soil which was virtually shaded from the sun by the canopy of the trees. The pods, also hiding in the canopy are somehow shielded. The TLC methodology could not detect any residue on the 21st day after the first treatment for the leaf because it was below its detection limit.

TABLE 4. RESIDUE LEVELS OF PROPOXUR ON COCOA LEAVES

Day of Sampling	Propoxur residue [mg/kg]	
	TLC*	GLC
Pretreatment	N.D.	N.D.
After 1st treatment		
1st	1.67 ± 0.42	1.75 ± 0.12
7th	0.65 ± 0.33	0.76 ± 0.20
14th	0.07 ± 0.01	0.05 ± 0.01
21st	N.D.	0.03 ± 0.01
After 2nd treatment		
7th	0.93 ± 0.06	1.02 ± 0.11
14th	0.39 ± 0.10	0.46 ± 0.15
21st	0.05 ± 0.02	0.08 ± 0.01
28th	N.D.	0.01 ± 0.01

TLC* Results with cleanup
N.D. Not detected

4. CONCLUSION

No propoxur residues could be detected in the cocoa pods and leaves 28 days after spraying. The rate of dissipation was faster in the leaves than in the soils or the pods.

Residues of propoxur determined by both TLC and GC were not significantly different. TLC analysis of propoxur residues in soil, cocoa leaves and pods may not need any rigorous cleanup since residues measured from cleaned and uncleaned extracts were not significantly different. The results presented show the potential of using TLC to cut down cost.

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GLOSSARY OF TERMS AND ABBREVIATIONS

Analyte	The chemical substance sought or determined in a sample.
Analytical portion	A representative quantity of material removed from the analytical sample of proper size for measurement of the residue concentration.
Confirmatory method	Methods that provide complete or complementary information enabling the analyte to be identified with an acceptable degree of certainty [at the Accepted Limit or level of interest]. As far as possible, confirmatory methods provide information on the chemical character of the analyte, preferably using spectrometric techniques. If a single technique lacks sufficient specificity, then confirmation may be achieved by additional procedures consisting of suitable combinations of cleanup, chromatographic separation(s) and selective detection. Bioassays can also provide some confirmatory data. In addition to the confirmation of the identity of an analyte, its concentration shall also be confirmed. This may be accomplished by analysis of a second test portion and/or re-analysis of the initial test portion with an appropriate alternative method (e.g. different column and/or detector). The qualitative and quantitative confirmation may also be carried out by the same method, when appropriate.
CV_r	Relative precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on replicate analytical portions in the same laboratory by the same operator using the same equipment within short intervals of time. It is expressed as a fraction or percentage of the ratio of repeatability standard deviation and the mean of test results.
CV_R	Relative closeness of agreement between results obtained with the same method on replicate analytical portions with different operators and using different equipment (within-laboratory reproducibility). Similarly, when the tests are performed in different laboratories the inter-laboratory reproducibility is obtained. It is expressed as a fraction or percentage of the ratio of reproducibility standard deviation and the mean of test results.
ECD	Electron capture detector
FPD	Flame photometric detector
GLC, GC	Gas liquid chromatography, Gas chromotography
GPC	Gel permeation chromatography
HRf	100 x Rf
Laboratory sample	The sample as received at the laboratory (not including the packaging).
Limit of detection, LOD	The smallest concentration of the analyte that can be detected on the TLC plate with reasonable certainty in the presence of coextracted substances from the sample. It is expressed in mg analyte/kg sample.

Limit of quantitation, LOQ	The smallest concentration of the analyte in the test sample that can be determined with acceptable precision (repeatability). It is expressed in mg analyte/kg sample [mg/kg].
Method	The series of procedures from receipt of a sample for analysis through to the production of the final result.
Method validation	Process of verifying that a method is fit for purpose.
Minimum Detectable Quantity, MDQ	The minimum amount of analytical standard spotted on the plate which gives a clearly visible spot after elution under average chromatographic conditions. It is usually expressed in nanograms [ng].
MRL	Maximum Residue Limit expressed in mg residue/kg sample.
Multi-residue Method, MRM	Method that is suitable for the identification and quantitation of a range of analytes, usually in a number of different matrices.
NPD	Nitrogen phosphor specific thermo-ionic detector.
Performance verification	Sets of quality control data generated during the analysis of batches of samples to support the validity of on-going analyses. The data can be used to refine the performance parameters of the method.
Reagent blank	Complete analysis made without the inclusion of sample materials for QC purpose.
Recovery, Q	A fraction or percentage of an analyte recovered following extraction and analysis of a blank sample to which the analyte has been added at a known concentration (spiked sample or reference material).
Representative analyte	Analyte chosen to represent a group of analytes that are likely to be similar in their behaviour through a multi-residue analytical method, as judged by their physico-chemical properties e.g. structure, water solubility, K_{ow}, polarity, volatility, hydrolytic stability, pKa etc.
Representative commodity	Single food or feed used to represent a commodity group for method validation purposes. A commodity may be considered representative on the basis of proximate sample composition, such as water, fat/oil, acid, sugar and chlorophyll contents, or biological similarities of tissues etc.
Resolution, R	The distance between the centres of two chromatographic zones divided by the average of the widths of the zones.
Retention factor, Rf	Retention factor of the analyte on the TLC plate is the ratio of the migration distances of the analyte and the eluent, (dimensionless quantity).
Relative retention factor, RRf	Relative retention factor of the analyte on the TLC plate is the ratio of the migration distances of the analyte and the eluent, related to the migration distance of the marker compound (RRf = Rf of target compound/ Rf of marker compound).
Sample preparation	The procedure used, if required, to convert the laboratory sample into the analytical sample, by removal of parts (soil, stones, bones, etc.) not to be included in the analysis.

Sample processing	The procedure(s) (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution, prior to removal of the analytical portion. The processing element of preparation must be designed to avoid inducing changes in the concentration of the analyte.
Screening method	A method used to detect the presence of an analyte or class of analytes at or above the minimum concentration of interest. It should be designed to avoid false negative results at a specified probability level (generally $\beta = 5\%$). Qualitative positive results may be required to be confirmed by confirmatory or reference methods.
Specificity	Extent to which a method provides responses from the detection system which can be considered exclusively characteristic of the analyte.
TLC	Thin layer chromotography

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