Method development for pesticide residues analysis in Herbs, to support regulatory aspects of food safety and public health within the MERCOSUR countries

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MERCOSUR

60% of the population consume medicinal plants regularly

Mercosur population(mill)	
	40
	200
	6
	3
	30

Where do the consumed medicinal plants in MERCOSUR came from?

Medicinal plants and derivatives production

Conventional : Uses pesticides like any agricultural production

PESTICIDE RESIDUES EXPECTED

Organic/biological/ecological: No pesticides allowed, secondary metabolism enhaced

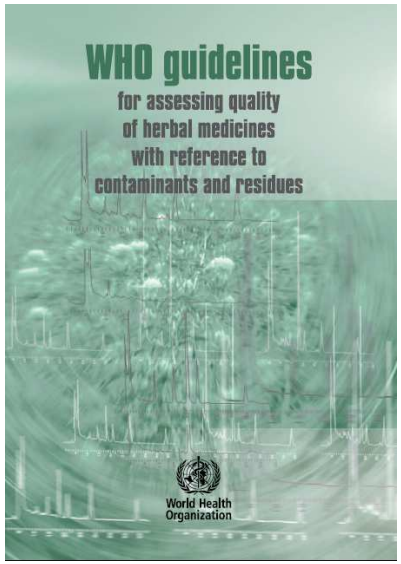
ABSCENCE OF PESTICIDE RESIDUES MUST BE CONFIRMED

When pesticide are used in MAPs production?

Residues					
General classification	Group	Subgroup	Specific examples	Possible sources	Stage of production at which detectable[a]
Agrochemical residues	Pesticides	Insecticides	Carbamate, chlorinated hydrocarbons, organophosphorus	Air, soil, water, during cultivation/growth, post-harvest processing	1,2,3,4
		Herbicides	2,4-D, 2,4,5-T	Air, soil, water, during cultivation/growth, post-harvest processing	1,2,3,4
		Fungicides	Dithiocarbamate	Air, soil, water, during cultivation/growth	1,2,3,4
	Fumigants	Chemical agents	Ethylene oxide, phosphine, methyl bromide, sulfur dioxide	Post-harvest processing	2,3,4
	Disease control agents	Antiviral agents	Thiamethoxam	During cultivation	1,2,3,4
Residual solvents		Organic solvents	Acetone, methanol, ethanol, butanol	Manufacturing process	3,4

[a] Stage of production at which detectable: 1, medicinal plants; 2, herbal materials; 3, herbal preparations; 4, finished herbal products.

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues



Medicinal plants could contain pesticide residues which accumualate as a consequence of agricultural practices such as fumigations, during pre or post harvest or soil treatments.
It is strongly adviced that each Medicinal Plants producer or commerzialer country should have at least one laboratory capable of performig these determinations

Medicinal plants could be ruled by the general specifications developed for foods, particuarly those included in the ***Codex Alimentarius***

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues

Codex Alimentarius used matched combinations pesticide-food product

Table 4. The list of approved pesticides for spices[a] and their maximum residue limits (MRLs) (Codex Alimentarius Commission, 2005)[b]

Pesticide (CCPR-number)	Group or sub-group of spices	MRL (mg/kg)
Acephate (095)	Entire group 028[c]	0.2 (*)
Azinphos-methyl (002)	Entire group 028[c]	0.5 (*)
Chlorpyrifos (017)	Seeds	5
	Fruits or berries	1
	Roots or rhizomes	1
Chlorpyrifos-methy1 (090)	Seeds	1
	Fruits	0.3
	Roots or rhizomes	5
Cypermethrin (118)	Fruits or berries	0.1
	Roots or rhizomes	0.2
Diazinon (22)	Seeds	5
	Fruits	0.1 (*)
	Roots or rhizomes	0.5
Dichlorvos (025)	Entire group 028[c]	0.1 (*)
Dicofol (026)	Seeds	0.05 (*)
	Fruits or berries	0.1
	Roots or rhizomes	0.1

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues

Pesticides residues in medicinal plants

- analytical challenges:

  MAPs are complex matrices containing different amounts of secondary metabolites (1-20%) with similar physicochemical properties to the most common pesticides employed for crop protection, either before or post harvest

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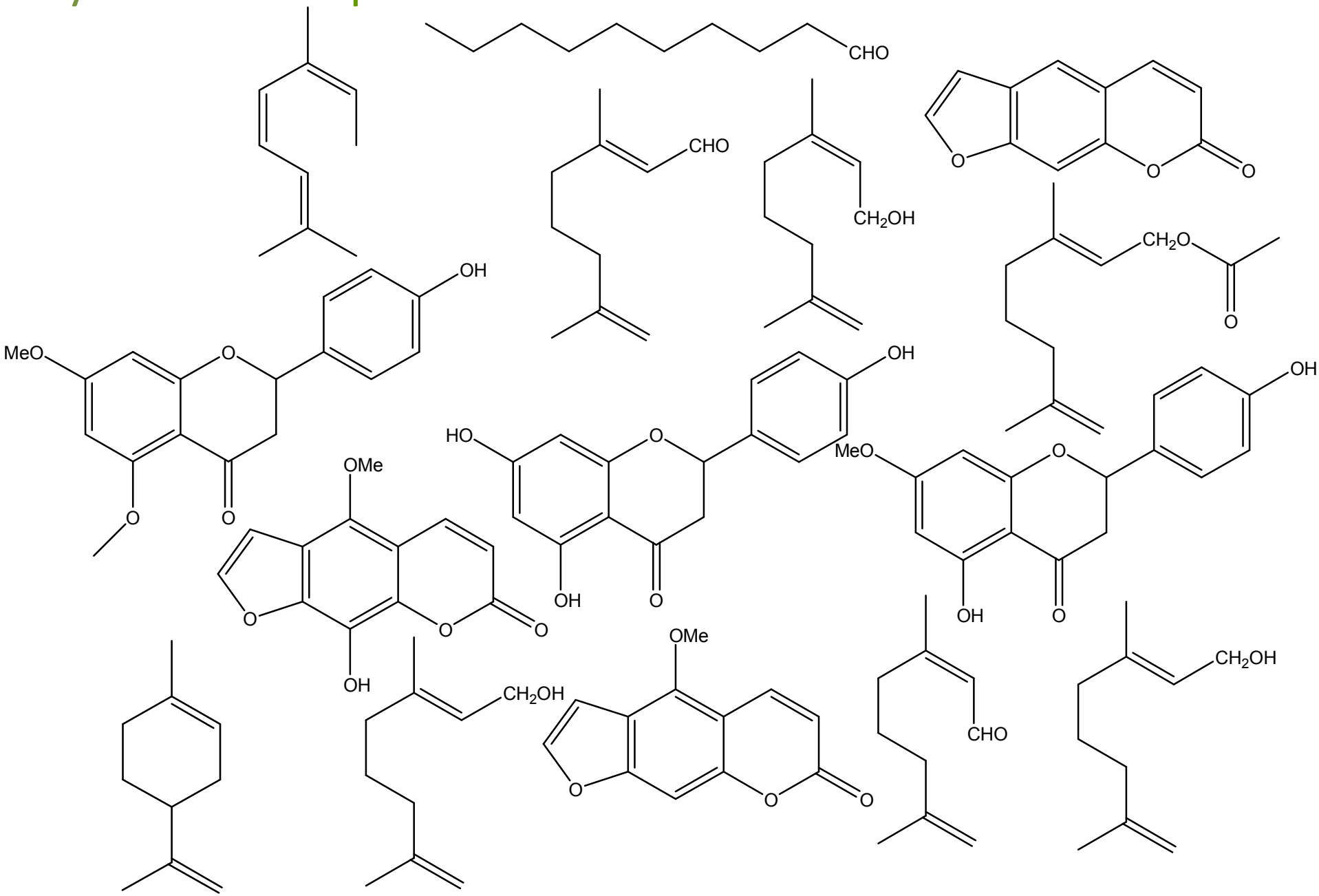
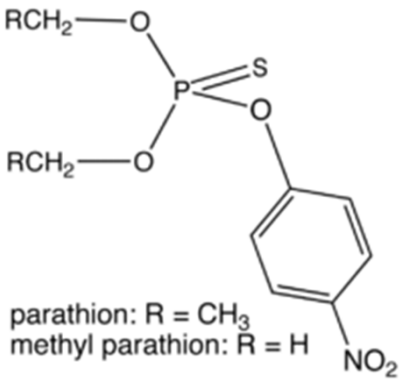
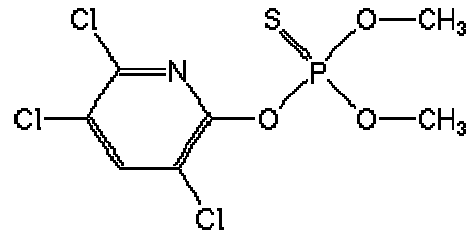
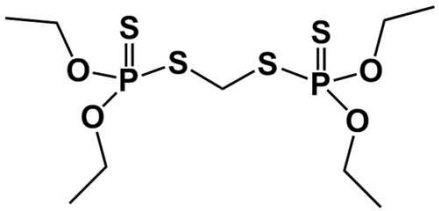
Phytochemicals present in citrus essential oils

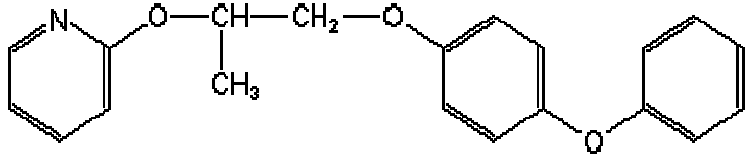


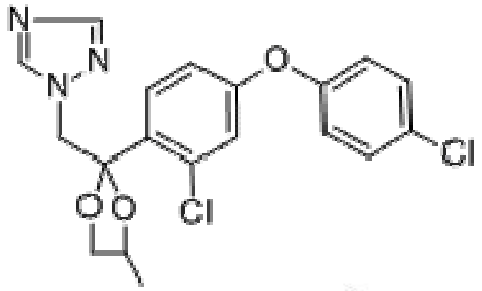
Common pesticides employed in crop protection

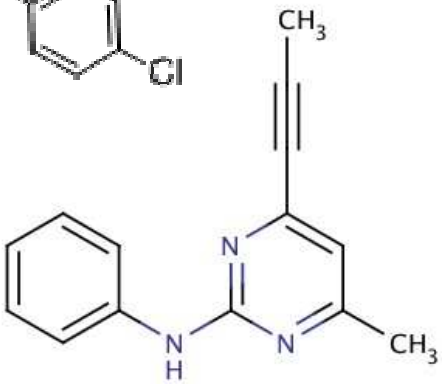


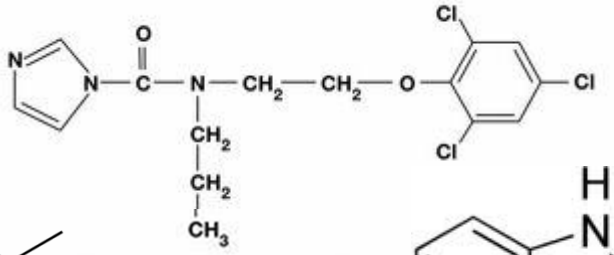


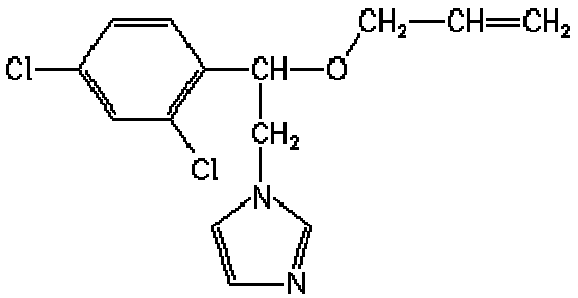


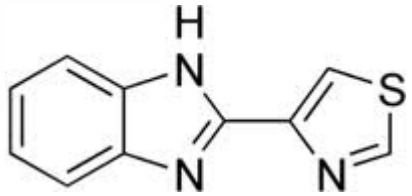


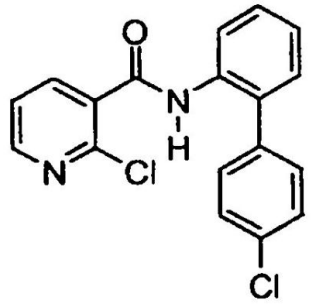


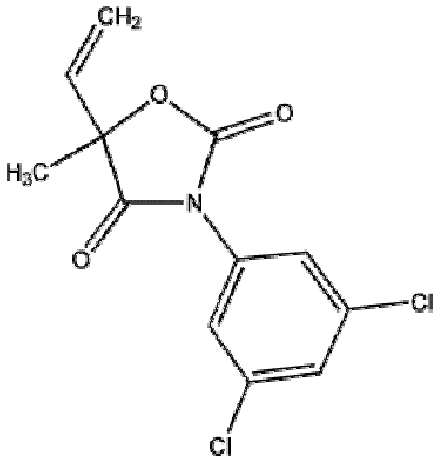


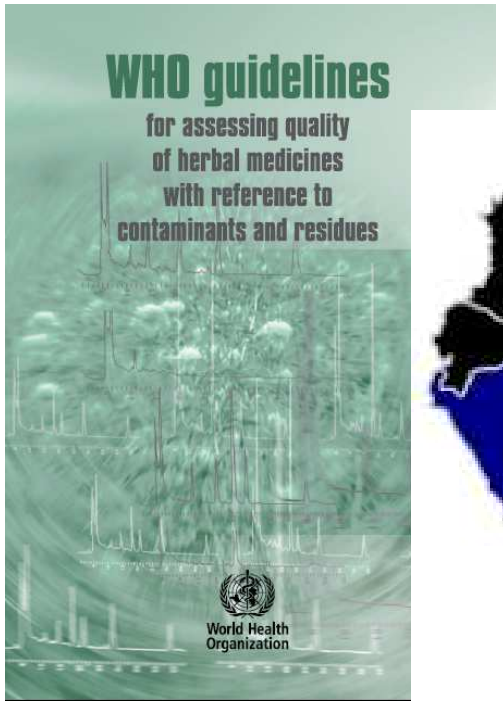








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with reference to
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World Health
Organization


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(MERCOSUL)


OPEAN
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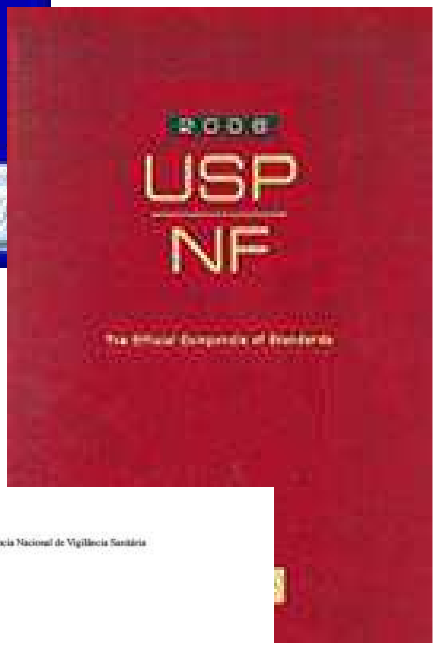
7.0


2008
USP
NF


2011

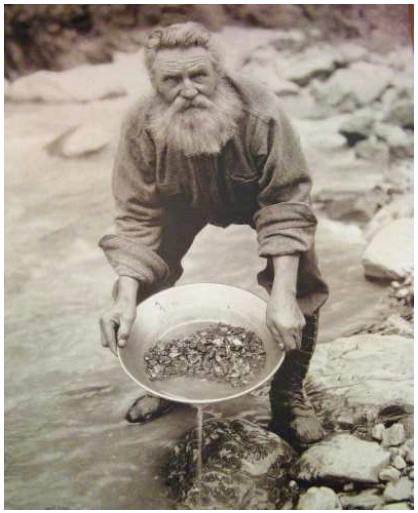

FARMACOPEA
NACIONAL
ARGENTINA


Agência Nacional de Vigilância Sanitária
Farmacopeia
Brasileira
Volume 2
5ª edição
Brasília
2010

Characteristics of Pharmacopeical protocols

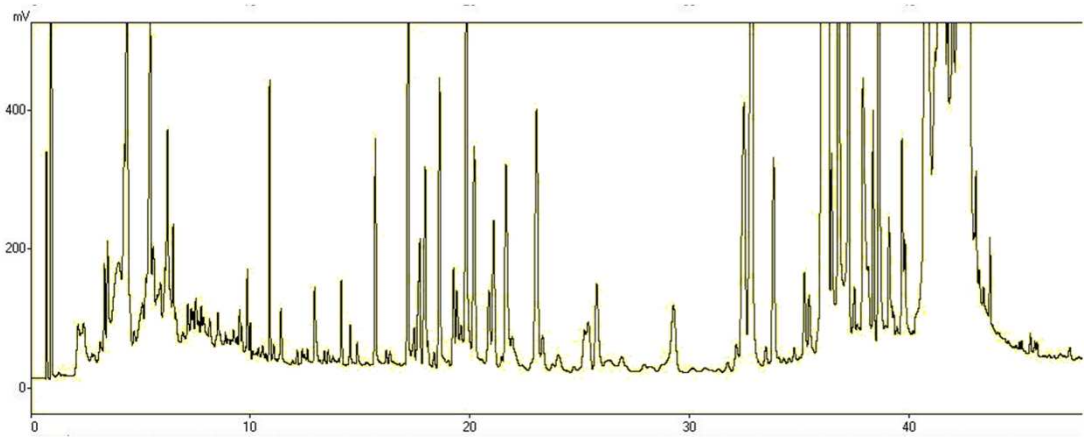
There is always a reference substance (that is provided by the pharmacopeia´s board)

…what about for pesticides in natural products or medicinal plants?



the concept :
Pesticide residues is a group of compounds to be determined by a general procedure, as ashes or moisture

Exhaustive sample preparation for the pesticide residue analysis was the solution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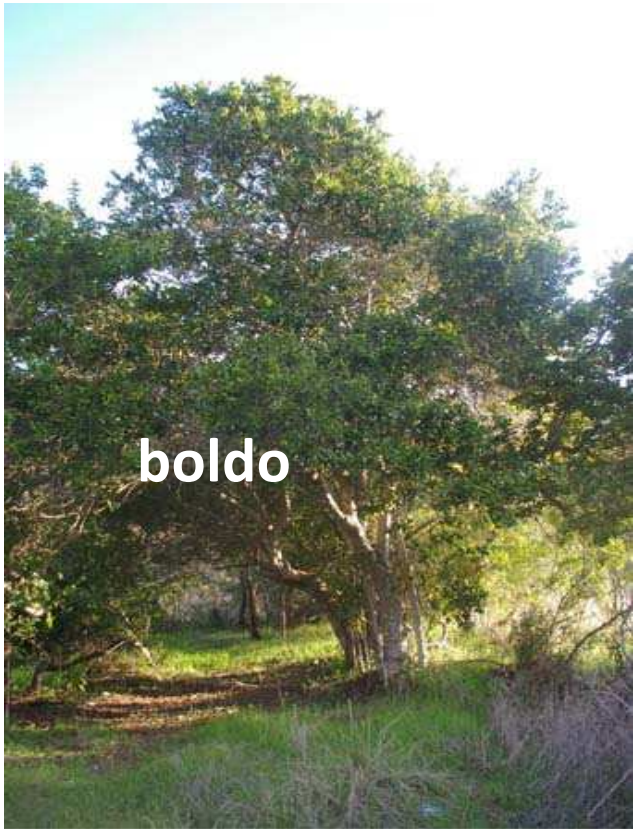
Matrix effects


Calendula


marcela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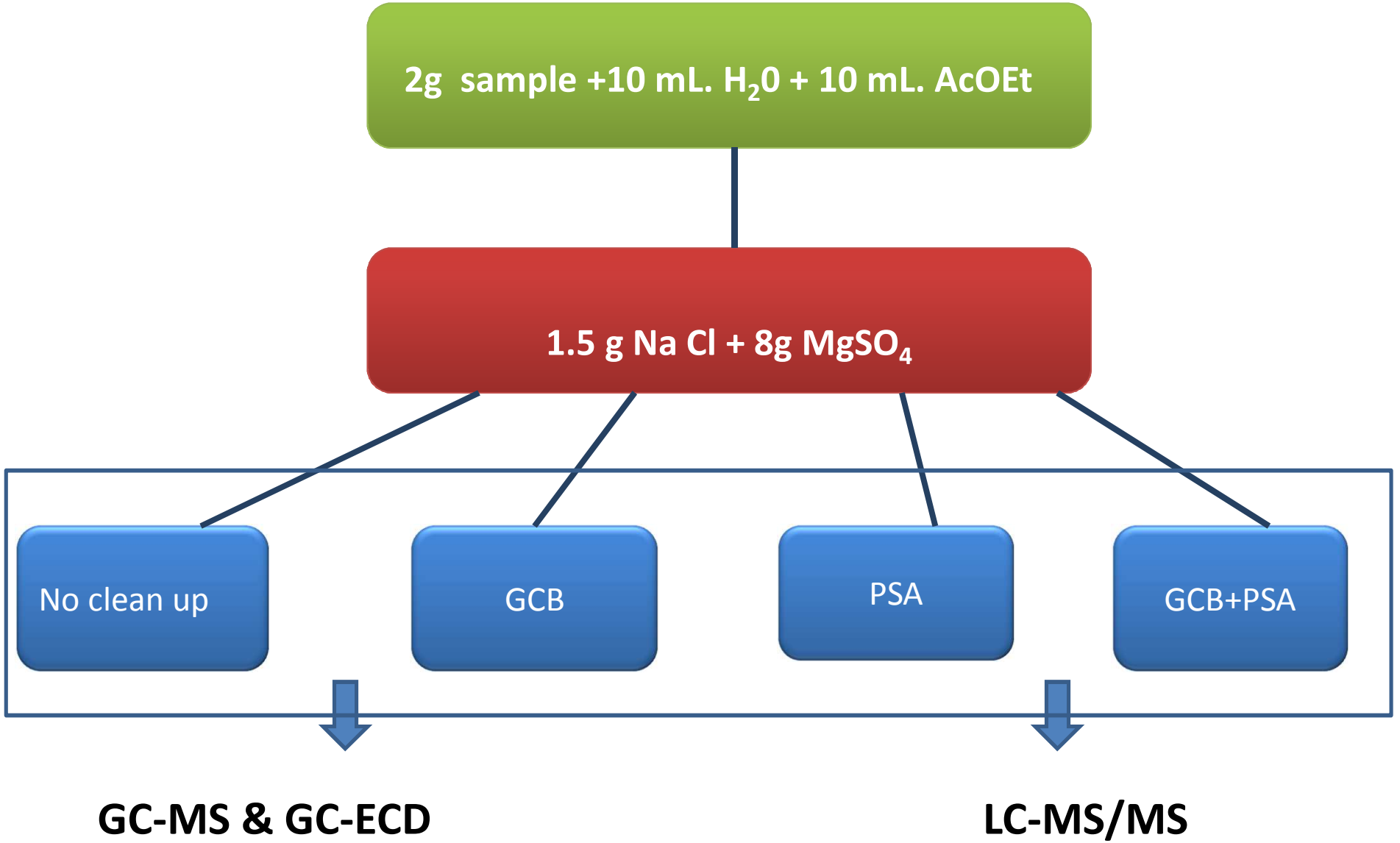
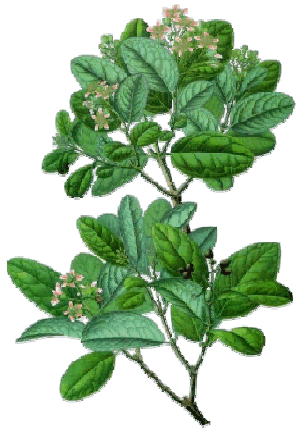
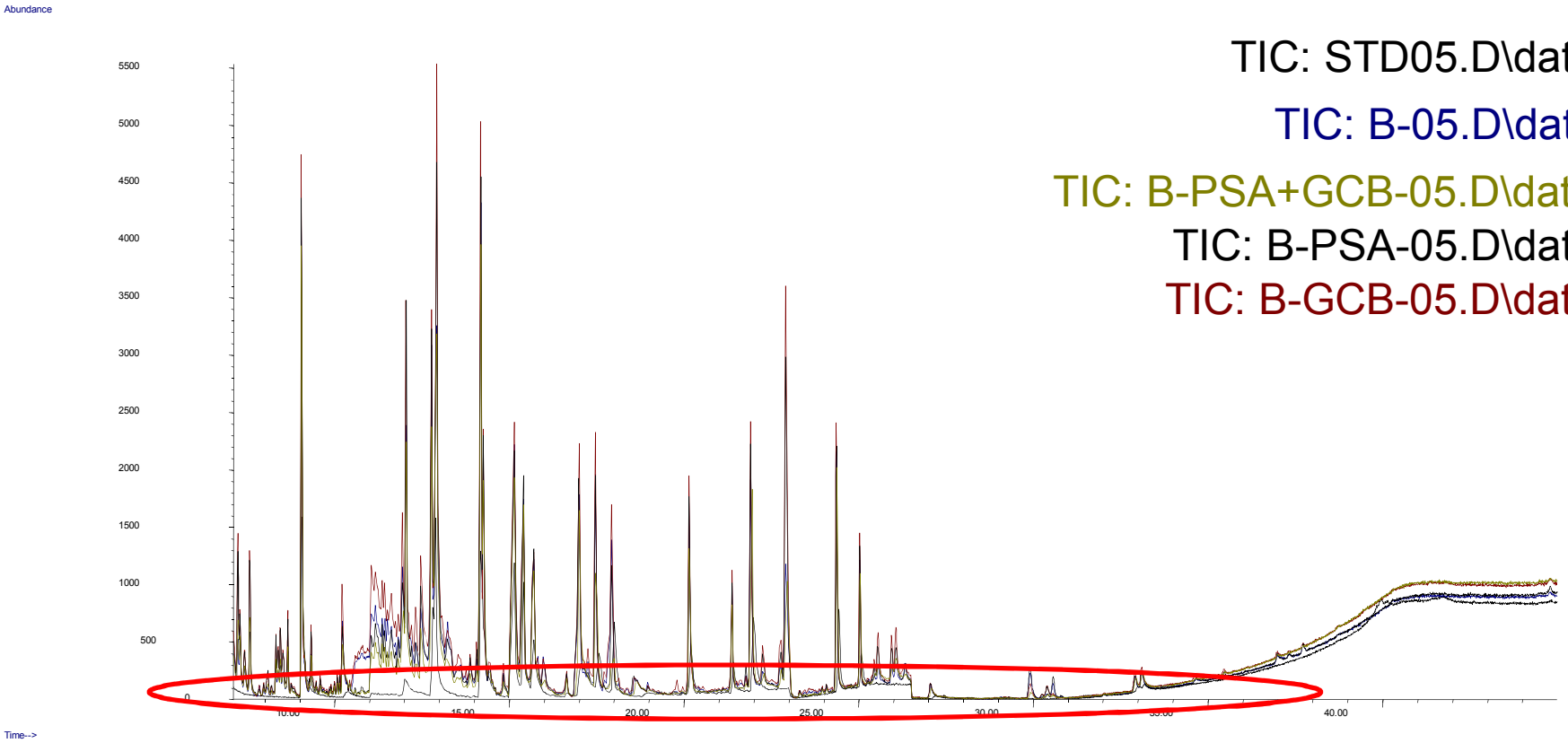
hiperico


2g sample +10 mL. H_20 + 10 mL. AcOEt
1.5 g Na Cl + 8g $MgSO_4$
No clean up
GCB
PSA
GCB+PSA
GC-MS & GC-ECD
LC-MS/MS

Boldo





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TIC: B-05.D\data.ms

TIC: B-PSA+GCB-05.D\data.ms

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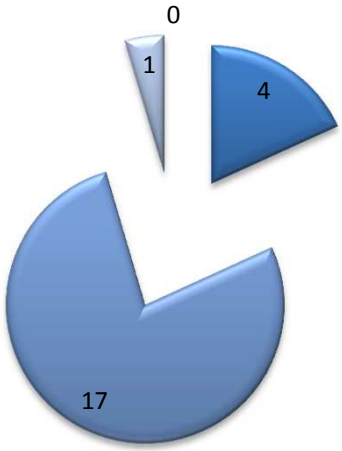
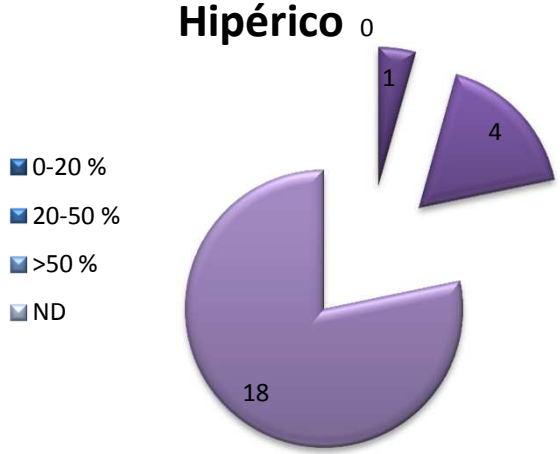
TIC: B-GCB-05.D\data.ms

Matrix effect in GC-MS for some common medicinal plants

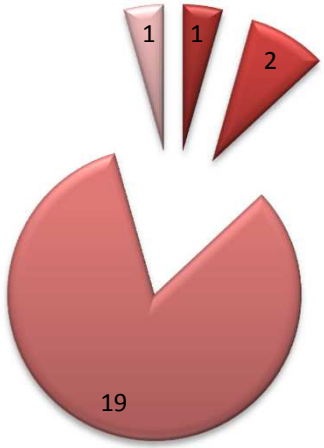
Boldo



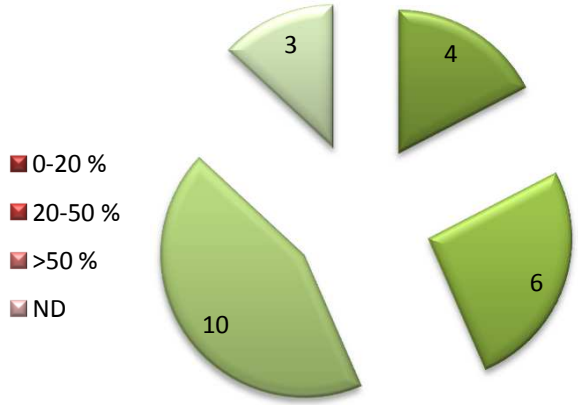
Hipérico



Caléndula



Marcela



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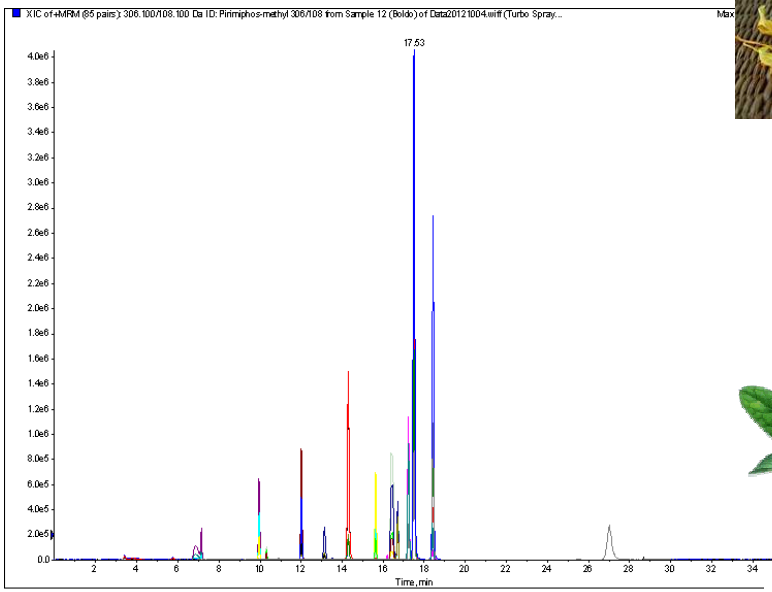
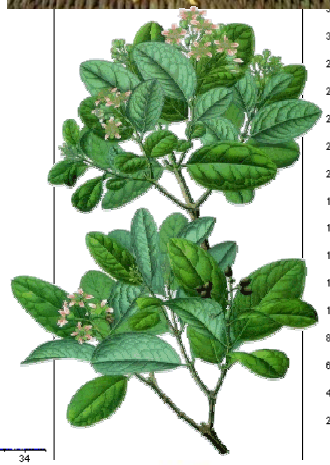
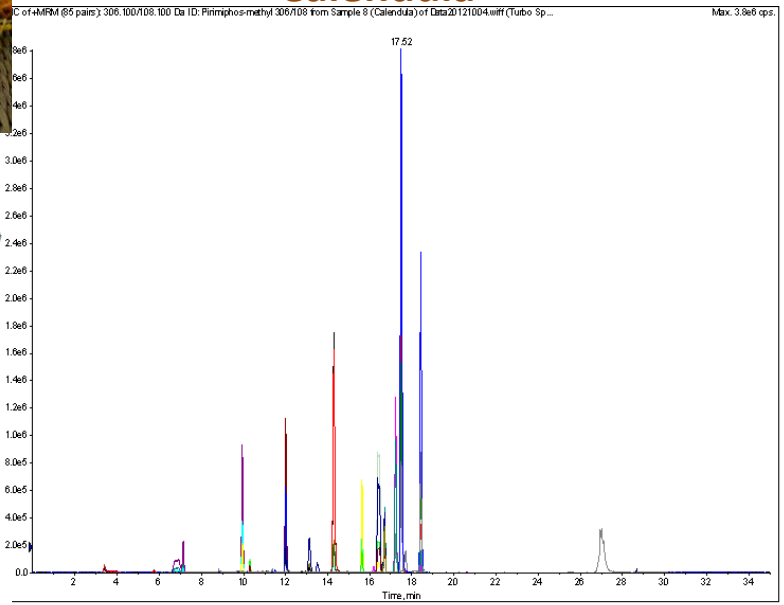




Calendula



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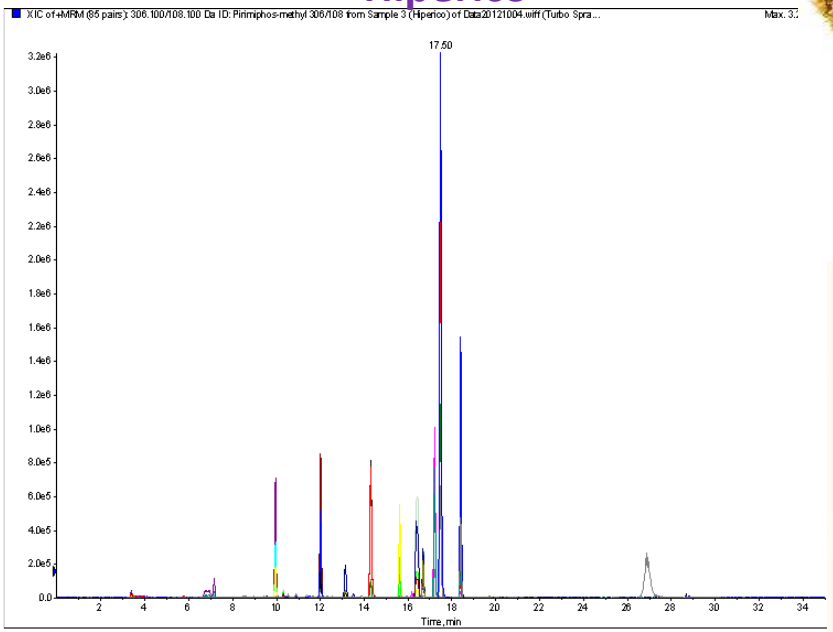

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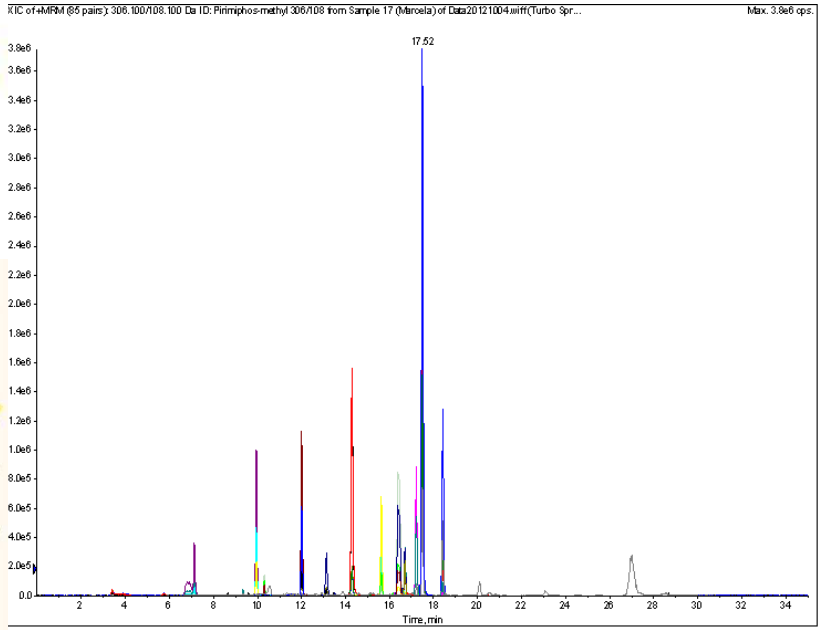
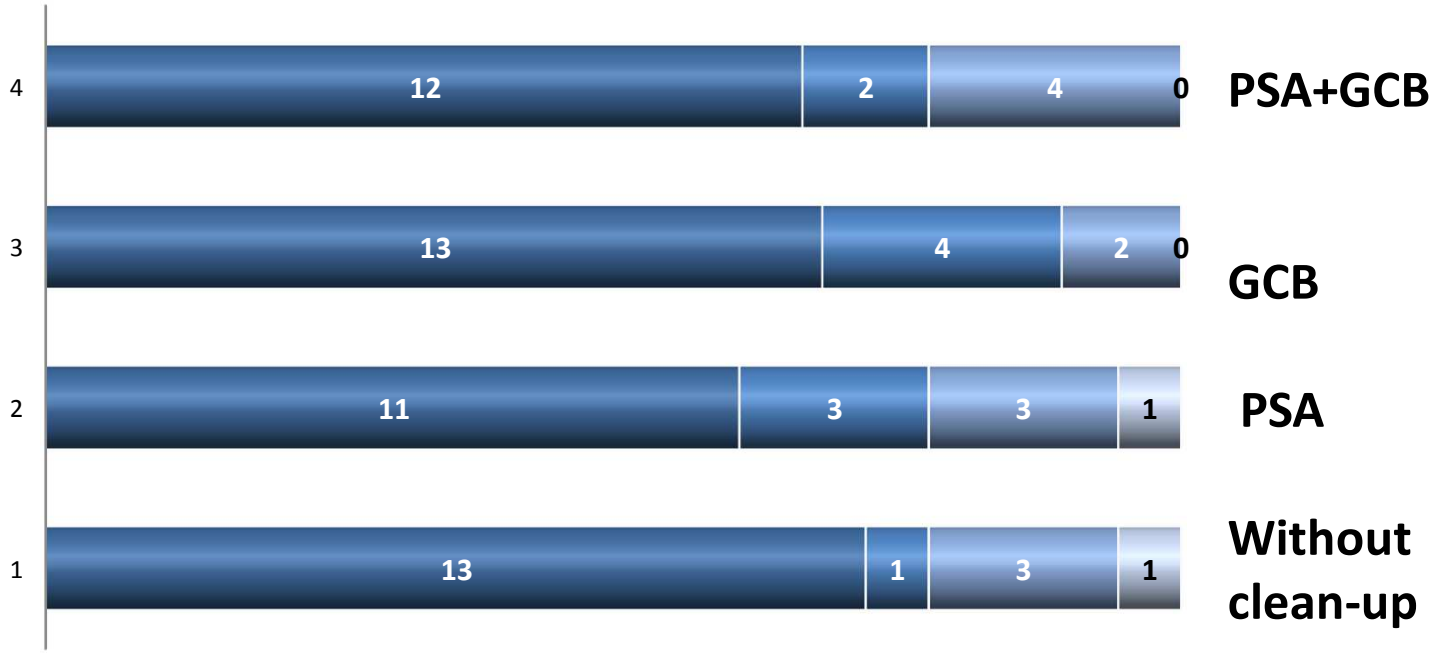


Boldo: Matrix Effect LC-MS/MS



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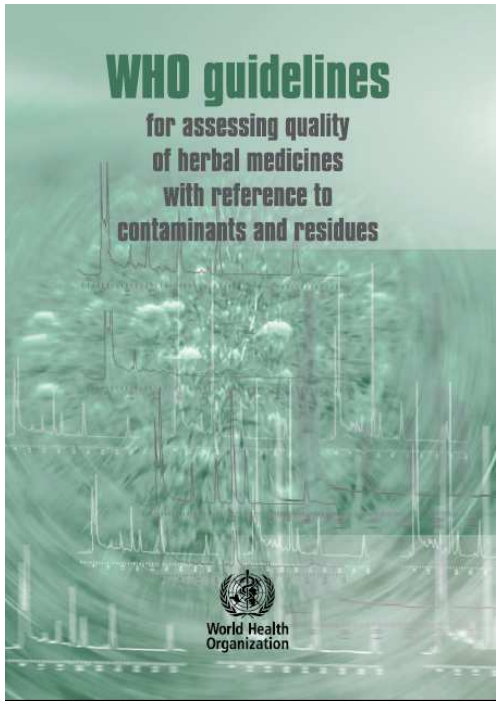
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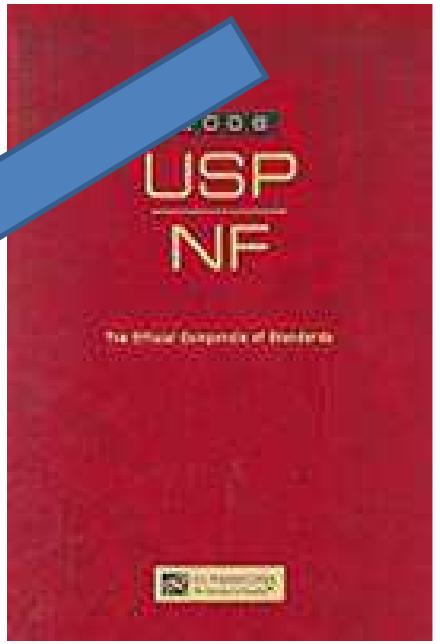
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2011

A NEW CONCEPT

07/2008:20813

2.8.13. PESTICIDE RESIDUES

Definition. For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs. The term includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops, either before or after harvest, to protect the commodity from deterioration during storage and transport. Pesticide residues can be present and are controlled in herbal drugs and herbal drug preparations.

Limits. Unless otherwise indicated in the monograph, the herbal drug to be examined at least complies with the limits indicated in Table 2.8.13.-1. The limits applying to pesticides that are not listed in Table 2.8.13.-1 and whose presence is suspected for any reason comply with the limits (levels) cross referred to by Regulation (EC) No. 396/2005, including annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 nor in European Union texts are calculated using the following expression:

$$\frac{ADI \times M}{MDD_{HD} \times 100}$$

ADI – acceptable daily intake, as published by FAO-WHO, in milligrams per kilogram of body mass,

M – body mass in kilograms (60 kg),

MDD_{HD} – daily dose of the herbal drug, in kilograms.

The limits for pesticides in herbal drug preparations are calculated using the following expressions:

If DER ≤ 10:

$$MRL_{HD} \times DER$$

If DER > 10:

$$\frac{ADI \times M}{MDD_{HD} \times 100}$$

MRL_{HD} – maximum residue limit of the pesticide in the herbal drug as given in Table 2.8.13.-1 or in EU texts or calculated using the expression

Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1
Bromide, inorganic (calculated as bromide ion)	50
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Bromopropylate	2
Chlordane (sum of *cis*-, *trans*- and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin (sum of)	0.1
λ-Cyhalothrin	1
Cypermethrin and isomers (sum of)	1
DDT (sum of *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-TDE and *p,p'*-TDE)	1
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2
Endosulfan (sum of isomers and endosulfan sulfate)	2
Endrin	0.05
Ethion	2
Etrimphos	0.05
Fenchlorphos (sum of fenchlorphos and fenchlorphos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.05
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxonsulfon and fensulfothion-sulfon)	0.05

Substance	Limit (mg/kg)
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon-sulfon, fenthion-oxon-sulfoxid, fenthion-sulfon and fenthion-sulfoxid)	0.05
Fenvalerate	1.5
Flucythrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ- and ε-)	0.2
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
Parathion-ethyl and Paraoxon-ethyl (sum of)	0.5
Parathion-methyl and Paraoxon-methyl (sum of)	0.2
Pendimethalin	0.1
Pentachloranisol	0.01
Permethrin and isomers (sum of)	1
Phosalone	0.1
Phosmet	0.05
Piperonyl butoxide	3
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I and pyrethrin II)	3
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline and methyl pentachlorphenyl sulfide)	1
S421	0.02
Tecnazene	0.05
Tetradifon	0.2
Vinclozolin	0.4

Sampling of herbal drugs. Sampling is done according to the general chapter *2.8.20. Herbal drugs: sampling and sample preparation.*

Qualitative and quantitative analysis of pesticide residues. The analytical procedures used are validated (e.g. according to Document N° SANCO/10232/2006). In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, is suitable for the combination pesticide residue/substance to be examined, and not susceptible to interference from co-extractives;
- natural occurrence of some constituents is considered in the interpretation of results (e.g. disulfide from Cruciferaceae);
- the concentration of test and reference solutions and the setting of the apparatus are such that the responses used for quantification of the pesticide residues are within the dynamic range of the detector. Test solutions containing pesticide residues at a level outside the dynamic range, may be diluted within the calibration range, provided that the concentration of the matrix in the solution is adjusted in the case where the calibration solutions must be matrix-matched;
- between 70 per cent to 110 per cent of each pesticide is recovered;
- repeatability of the method: RSD is not greater than the values indicated in Table 2.8.13.-2;
- reproducibility of the method: RSD is not greater than the values indicated in Table 2.8.13.-2.

Table 2.8.13.-2

Concentration range of the pesticide (mg/kg)	Repeatability (RSD) (per cent)	Reproducibility (RSD) (per cent)
0.001 - 0.01	20	60
> 0.01 - 0.1	20	40
> 0.1 - 1	15	30
> 1	10	20

01/2008:20814

2.8.14. DETERMINATION OF TANNINS IN HERBAL DRUGS

Carry out all the extraction and dilution operations protected from light.

In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) (*2.9.12*) or the extract in a 250 mL round-bottomed flask add 150 mL of *water R*. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 mL volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 mL with *water R*. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 mL with *water R*. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

Total polyphenols. Dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_1), using *water R* as the compensation liquid.

Polyphenols not adsorbed by hide powder. To 10.0 mL of the filtrate, add 0.10 g of *hide powder CRS* and shake vigorously for 60 min. Filter and dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_2), using *water R* as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_3), using *water R* as the compensation liquid.

Introduce into the flask the prescribed quantity of the drug and continue the distillation as described above for the time and at the rate prescribed. Stop the heating and after 10 min read the volume of liquid collected in the graduated tube and subtract the volume of xylene previously noted. The difference represents the quantity of essential oil in the mass of the drug taken. Calculate the result as millilitres per kilogram of drug.

When the essential oil is to be used for other analytical purposes, the water-free mixture of xylene and essential oil may be recovered as follows: remove the stopper *K'* and introduce 0.1 mL of a 1 g/L solution of *sodium fluoresceinate R* and 0.5 mL of *water R*. Lower the mixture of xylene and essential oil into the bulb-shaped swelling *L* by means of the three-way tap, allow to stand for 5 min and lower the mixture slowly until it just reaches the level of the tap *M*. Open the tap anti-clockwise so that the water flows out of the connecting tube *BM*. Wash the tube with *acetone R* and with a little *toluene R* introduced through the filling funnel *N*. Turn the tap anti-clockwise in order to recover the mixture of xylene and essential oil in an appropriate flask.

07/2008:20813

2.8.13. PESTICIDE RESIDUES

Definition. For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs. The term includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops, either before or after harvest, to protect the commodity from deterioration during storage and transport. Pesticide residues can be present and are controlled in herbal drugs and herbal drug preparations.

Limits. Unless otherwise indicated in the monograph, the herbal drug to be examined at least complies with the limits indicated in Table 2.8.13.-1. The limits applying to pesticides that are not listed in Table 2.8.13.-1 and whose presence is suspected for any reason comply with the limits (levels) cross referred to by Regulation (EC) No. 396/2005, including annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 nor in European Union texts are calculated using the following expression:

$$\frac{ADI \times M}{MDD_{HD} \times 100}$$

ADI – acceptable daily intake, as published by FAO-WHO, in milligrams per kilogram of body mass,

M – body mass in kilograms (60 kg),

MDD_{HD} – daily dose of the herbal drug, in kilograms.

The limits for pesticides in herbal drug preparations are calculated using the following expressions:

If DER ≤ 10:

$$MRL_{HD} \times DER$$

If DER > 10:

$$\frac{ADI \times M}{MDD_{HP} \times 100}$$

MRL_{HD} – maximum residue limit of the pesticide in the herbal drug as given in Table 2.8.13.-1 or in EU texts or calculated using the expression mentioned above;

DER – drug/extract ratio, i.e. the ratio between the quantity of herbal drug used in the manufacture of a herbal drug preparation and the quantity of herbal drug preparation obtained;

MDD_{HP} – daily dose of the herbal drug preparation, in kilograms.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).

Table 2.8.13.-1

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1
Bromide, inorganic (calculated as b	
Bromophos-ethyl	
Bromophos-methyl	
Bromopropylate	
Chlordane (sum of *cis*-, *trans*- and	
Chlorfenvinphos	
Chlorpyriphos-ethyl	
Chlorpyriphos-methyl	
Chlorthal-dimethyl	0.01
Cyfluthrin (sum of)	0.1
λ-Cyhalothrin	1
Cypermethrin and isomers (sum of)	1
DDT (sum of *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-TDE and *p,p'*-TDE)	1
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS_2)	2
Endosulfan (sum of isomers and endosulfan sulfate)	2
Endrin	0.05
Ethion	2
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.05
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxonsulfon and fensulfothion-sulfon)	0.05

Substance	Limit (mg/kg)
Penthion (sum of fenthion, fenthion-oxon, fenthion-oxon-sulfon, fenthion-oxon-sulfoxid, fenthion-sulfon and fenthion-sulfoxid)	0.05
Penvalerate	1.5
Phacytinate	0.05
τ-Fluvalinate	0.05
Phosphos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide and *trans*-heptachlorepoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ- and ε-)	0.2
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and maloxon (sum of)	1
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
[illegible]rason-ethyl (sum of)	0.5
[illegible]rason-methyl (sum of)	0.2
	0.1
	0.01
[illegible]t (sum of)	1
	0.1
	0.05
	2
	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I and pyrethrin II)	2
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorphenyl sulfide)	1
S-421	0.02
Tecnazene	0.05
Tetradifon	0.2
Vinclozolin	0.4

Sampling of herbal drugs. Sampling is done according to the general chapter *2.8.20. Herbal drugs: sampling and sample preparation.*

Qualitative and quantitative analysis of pesticide residues. The analytical procedures used are validated (e.g. according to Document N° SANCO/10232/2006). In particular, they satisfy the following criteria:

- the chosen method, especially the purification step, is suitable for the combination pesticide residue/substance to be examined, and not susceptible to interference from co-extractives;
- natural occurrence of some constituents is considered in the interpretation of results (e.g. disulfide from Cruciferaceae);
- the concentration of test and reference solutions and the setting of the apparatus are such that the responses used for quantification of the pesticide residues are within the dynamic range of the detector. Test solutions containing pesticide residues at a level outside the dynamic range, may be diluted within the calibration range, provided that the concentration of the matrix in the solution is adjusted in the case where the calibration solutions must be matrix-matched;
- between 70 per cent to 110 per cent of each pesticide is recovered;
- repeatability of the method: RSD is not greater than the values indicated in Table 2.8.13.-2;
- reproducibility of the method: RSD is not greater than the values indicated in Table 2.8.13.-2.

Table 2.8.13.-2

Concentration range of the pesticide (mg/kg)	Repeatability (RSD) (per cent)	Reproducibility (RSD) (per cent)
0.001 - 0.01	30	60
> 0.01 - 0.1	20	40
> 0.1 - 1	15	30
> 1	10	20

01/2008:20814

2.8.14. DETERMINATION OF TANNINS IN HERBAL DRUGS

Carry out all the extraction and dilution operations protected from light.

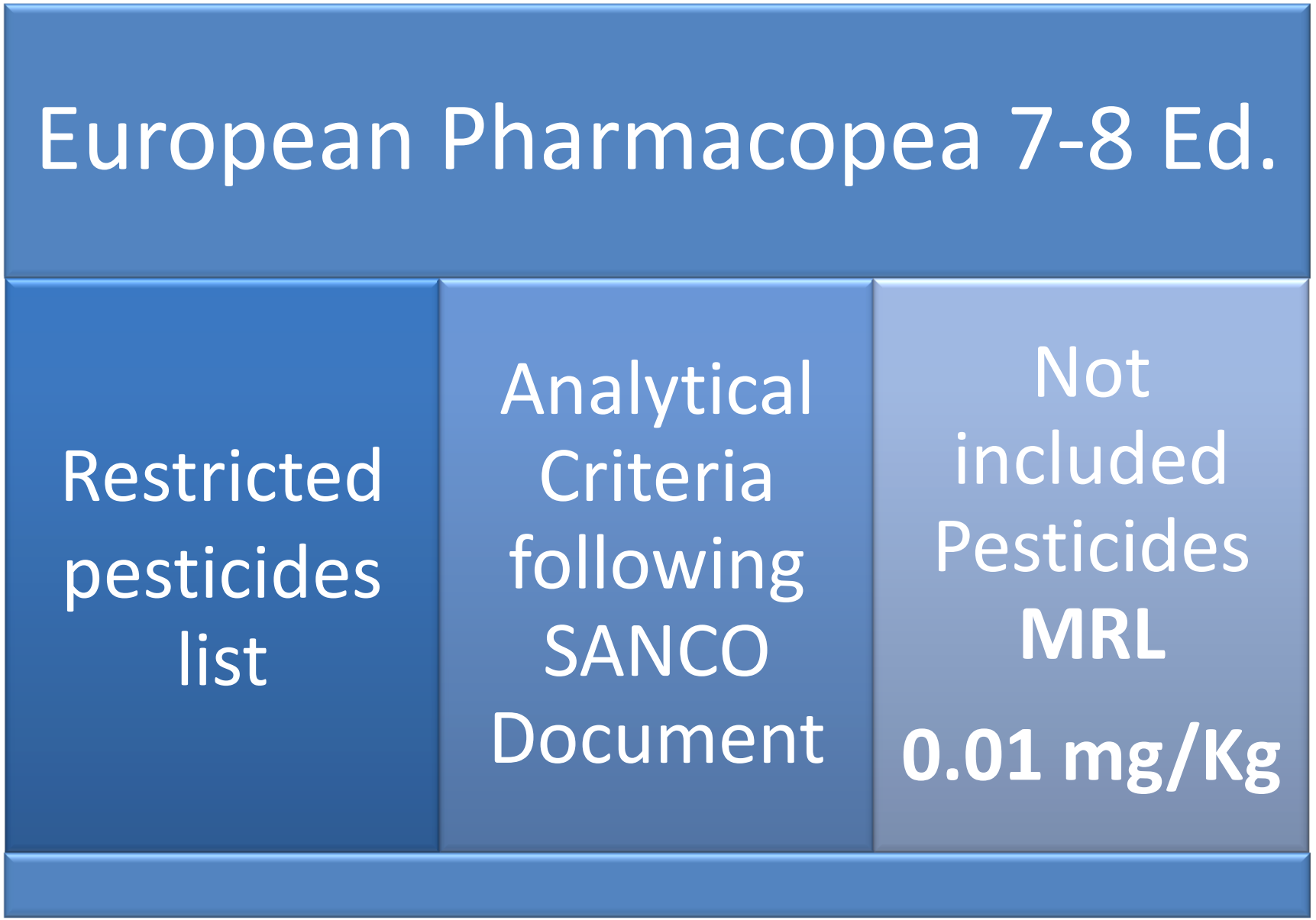
In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) (*2.9.12*) or the extract in a 250 mL round-bottomed flask add 150 mL of *water R*. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 mL volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 mL with *water R*. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 mL with *water R*. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

Total polyphenols. Dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_1), using *water R* as the compensation liquid.

Polyphenols not adsorbed by hide powder. To 10.0 mL of the filtrate, add 0.10 g of *hide powder CRS* and shake vigorously for 60 min. Filter and dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_2), using *water R* as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_3), using *water R* as the compensation liquid.


European Pharmacopea 7-8 Ed.
Restricted pesticides list
Analytical Criteria following SANCO Document
Not included Pesticides
MRL
0.01 mg/Kg

How is the fitting between the pharmacopeia & the food regulation?

The case of Ginger

Pesticides	EU-MRLs		EUPharm
Acephate	0.20	✖	0.10
Alachlor	0.01	✖	0.05
Aldrin & Dieldrin	0.10	✖	0.25
Azimphos ethyl	0.05	✖	0.10
Azimphos methyl	0.5	✖	1.00
Azoxystrobin	0.1		**EUMRLs**

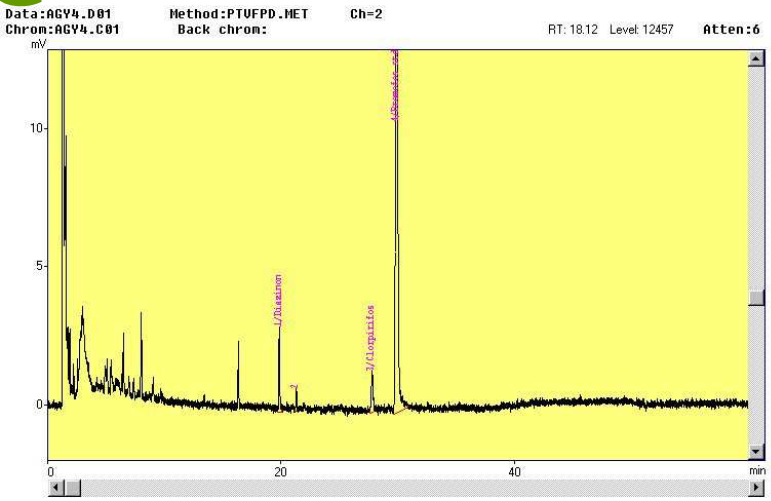
10 more pesticides with MRL >0.01 begining with a

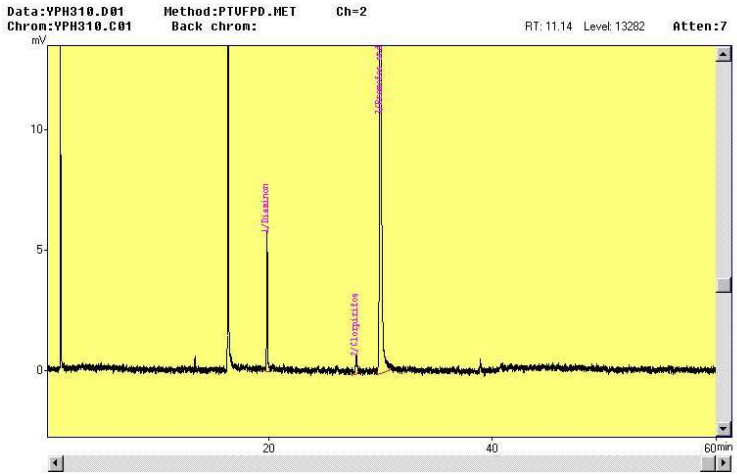
What is needed for registration?

Toxicological evaluation of the pesticide residues in herbs

Validated analytical procedure for the specific medicinal plant

Yerba mate





- Daily intake for uruguayan population of 21.5 g,

Argentina 18.5g

Brasil 5.3g

* That means aprox 8Kg/person /y

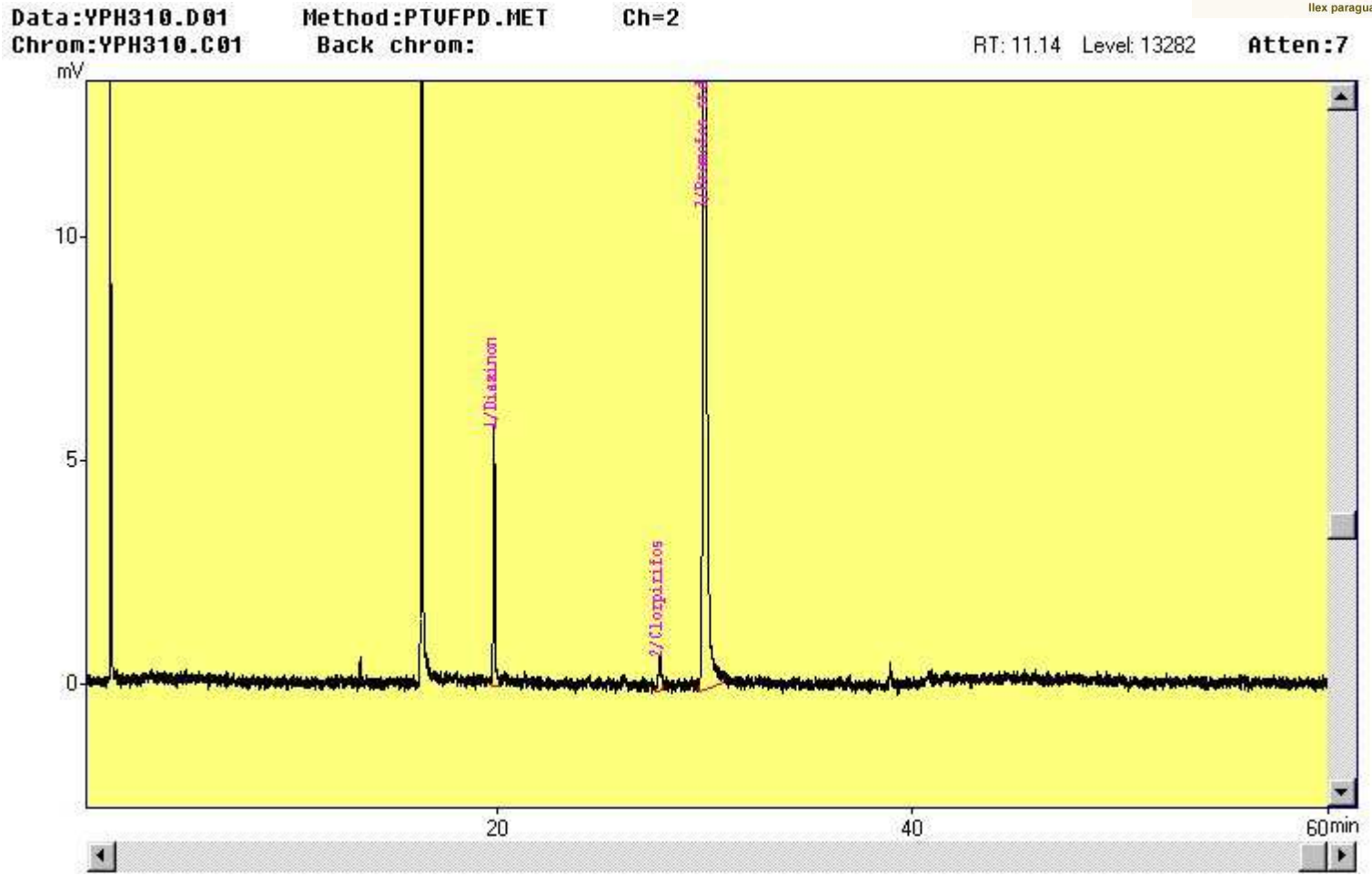
FAO Stat, 2007

FARMACOGNOSIA y PRODUCTOS NATURALES
Montevideo
Universidad de la República

Yerba mate

Mini Luke Extract

1)Acetone 2) Clean up: Extraction (DCM;Hex 1:1), Florisil



Pesticide residues in yerba mate

30% of the samples do not have any pesticide residue

Sample	ΣPesticides (ppb, μg/Kg)
1	66
2	16
3	10
4	5
5	105

Diazinon

Metidathion

Fenitrothion

Endosulfan

Endoslfan sulphate

Ethion

Malathion

Parathion(ethyl/methyl)

Chlorpirifos

Cypermethrin

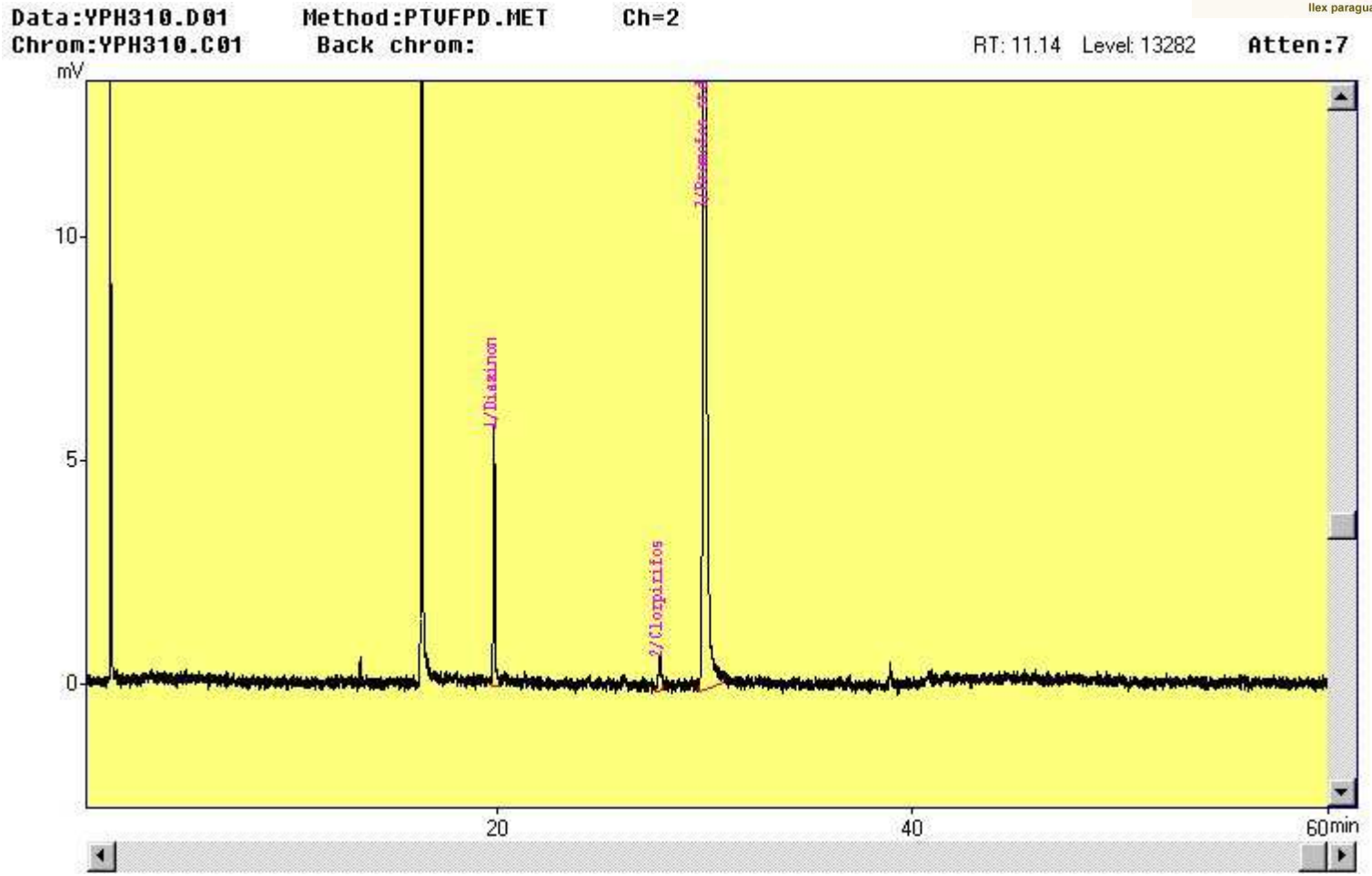
Deltamethrin

FARMACOGNOSIA y PRODUCTOS NATURALES
Montevideo
Universidad de la República

Yerba mate

Mini Luke Extract

1)Acetone 2) Clean up: DCM;Hex 1:1 extr., Florisil



Transference of pesticide residues to mate brew

Experimental design

- Pesticide Spiked Yerba mate

Fortified

- Infusión collection

Simulation of mate drinking

- L-L (H_2O:CH_2Cl_2) y SPE

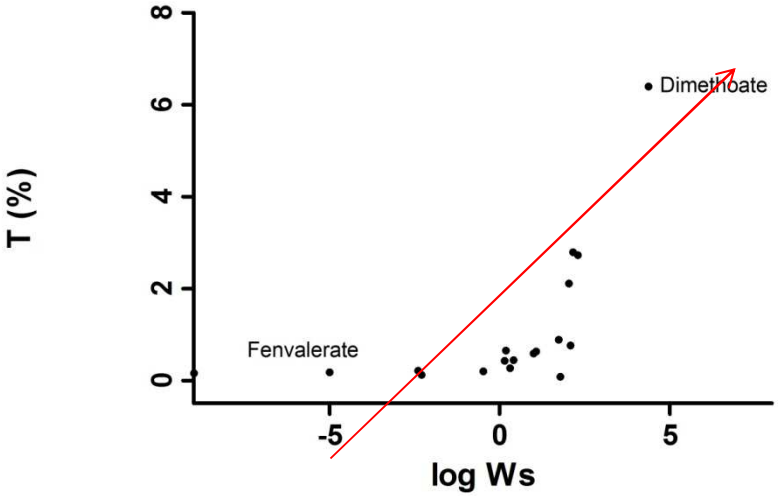
Análisis GC-ECD/FPD

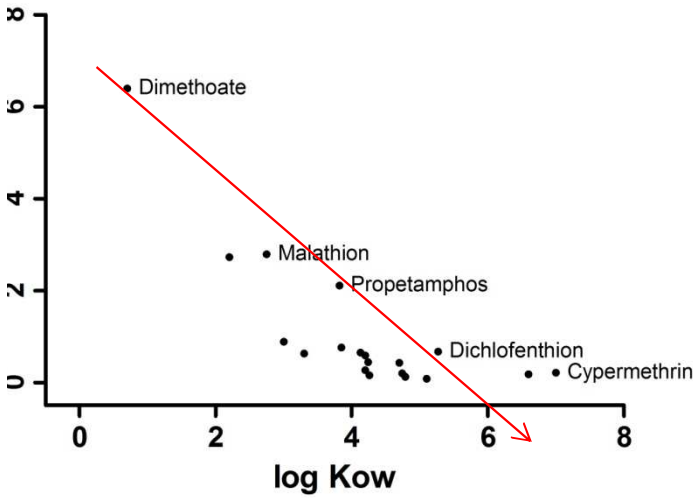
	Recuperación (%)	DSR (%)	GC-FPD	GC-ECD	$T_{R,R,}$
(Z+E) Clorfenvinfos	89,4	9,2	†		1,07
Clorpirifos	101,1	4,7	†		0,98
Clorpirifos-metil	83,4	7,1	†		0,88
Coumafos	78,2	7,3	†		1,41
λ-Cihalotrina	***124,5***	***22,8***		†	1,48/1,52
Cipermetrina	***140,2***	***25,7***		†	1,77/1,78
Deltametrina	***178,3***	18,4		†	1,86/1,88
Diazinon	84,2	14,3	†		0,78
Diclofention	94,3	2,2	†		0,91
Dimetoato	64,2	15,6	†		0,80
α+β Endosulfan	112,5	14,2		†	1,04/1,43
Etion	84,5	2,4	†		1,24
Fenvalerato	116,3	14,7		†	1,83/1,84
Malation	83,4	7,1	†		0,97
Metidatin	***128,2***	14,7	†		1,11
Paration-metil	118,2	17,6	†		0,93
Pirimifos-metil	87,6	13,2	†		0,94
Propetamfos	74,3	8,1	†		0,85
τ-Fluvalinato	105,7	13,2		†	1,85/1,85

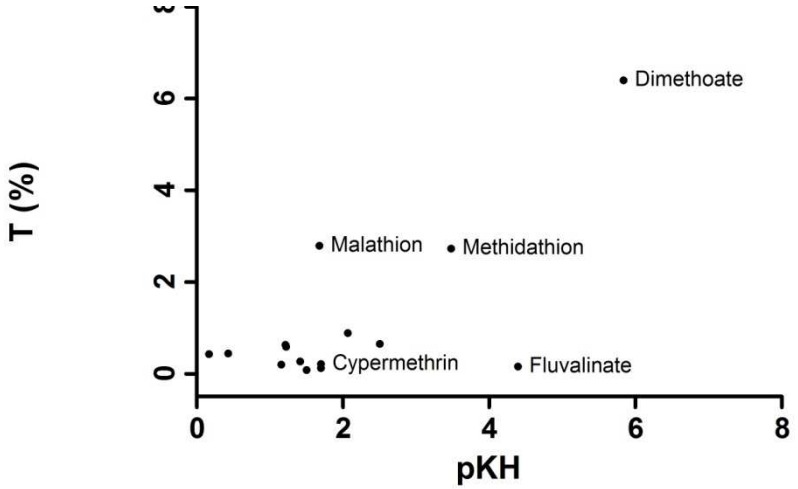
Pérez-Parada, A.; González, J.; Pareja, L.; Geis-Asteggiante, L.; Colazzo, M.; Niell, S.; Besil, N.; González, G.; Cesio, V.; Heinzen, H. Transfer of pesticides to the brew during mate drinking process and their relationship with physicochemical properties *Journal of Environmental Science and Health Part B (2010) 45, 830–837*

Transference of pesticide residues to mate brew

Transference (T(%)) vs physicochemical properties







$T \propto W_s$

$T \propto 1/K_{ow}$

$T \propto 1/H$?

Pérez-Parada, A.; González, J.; Pareja, L.; Geis-Asteggiante, L.; Colazzo, M.; Niell, S.; Besil, N.; González, G.; Cesio, V.; Heinzen, H. *Journal of Environmental Science and Health Part B (2010) 45, 830–837*

Pesticida	T (%)	ADI (mg/kg pc)*	DSR (%)	ARLs Estimados (mg/kg)
(Z+E) Clorfenvinfos	0,76		18,6	
Clorpirifos	0,43		6,9	
(Z+E) Clorfenvinfos		0,0005		0,0081
Clorpirifos-metil	0,44		5,8	
Clorpirifos		0,0100		0,0921
Coumafos	0,65		17,7	
Clorpirifos-metil		0,0100		0,0943
λ-Cihalotrina	0,12		18,2	
Coumafos		0,0350		0,4875
Cipermetrina	0,21		24,3	
λ-Cihalotrina		0,0020		0,0051
Deltametrina	0,08		15,4	
Cipermetrina		0,0500		0,2250
Diazinon	0,63		18,3	
Deltametrina		0,0100		0,0171
Diclofention	0,67		4,0	
Diazinon		0,0002		0,0027
Dimetoato	6,40	—	21,7	—
Diclofention				
α+β Endosulfan	0,20		24,2	
Dimetoato		0,0020		0,2743
Etion	0,27		5,4	
α+β Endosulfan		0,0060		0,0257
Fenvalerato	0,18		11,3	
Etion		0,0020		0,0116
Malation	2,79		2,3	
Fenvalerato		0,0200		0,0771
Metidation	2,73		4,0	
Malation		0,3000		17,936
Paration-metil	0,89		8,7	
Metidation		0,0010		0,0585
Pirimifos-metil	0,59		6,1	
Paration-metil		0,0030		0,0572
Propetamfos	2,11		6,8	
Pirimifos-metil		0,0300		0,3793
τ-Fluvalinato	0,16		18,3	
Propetamfos		—		—
τ-Fluvalinato		0,0100		0,0343

Transference to the brew & ARLs

$$ARL = \frac{ADI \times E \times P}{100 \times MDI}$$

ARL= Acceptable residue leve(mg/kg vegetal material)
ADI= Accepted Daily Intake (mg/kg bw)
E= transference factor the infusion
MDI= Average intake of the plant material(kg)
P= Body weight (kg), 60 kg

Maximum limit of pesticide residues for medicinal plant materials. Quality control methods for medicinal plant materials. World Health Organization, Geneva, 1998, 54-57

Final objetive

To estimate the total pesticide daily intake

$$MTDI=\Sigma MRL_i \times F_i$$

TMDI Maximal Theoretical Diary Ingest

Fi National commodity consum per person (Kg)

What is needed for registration?

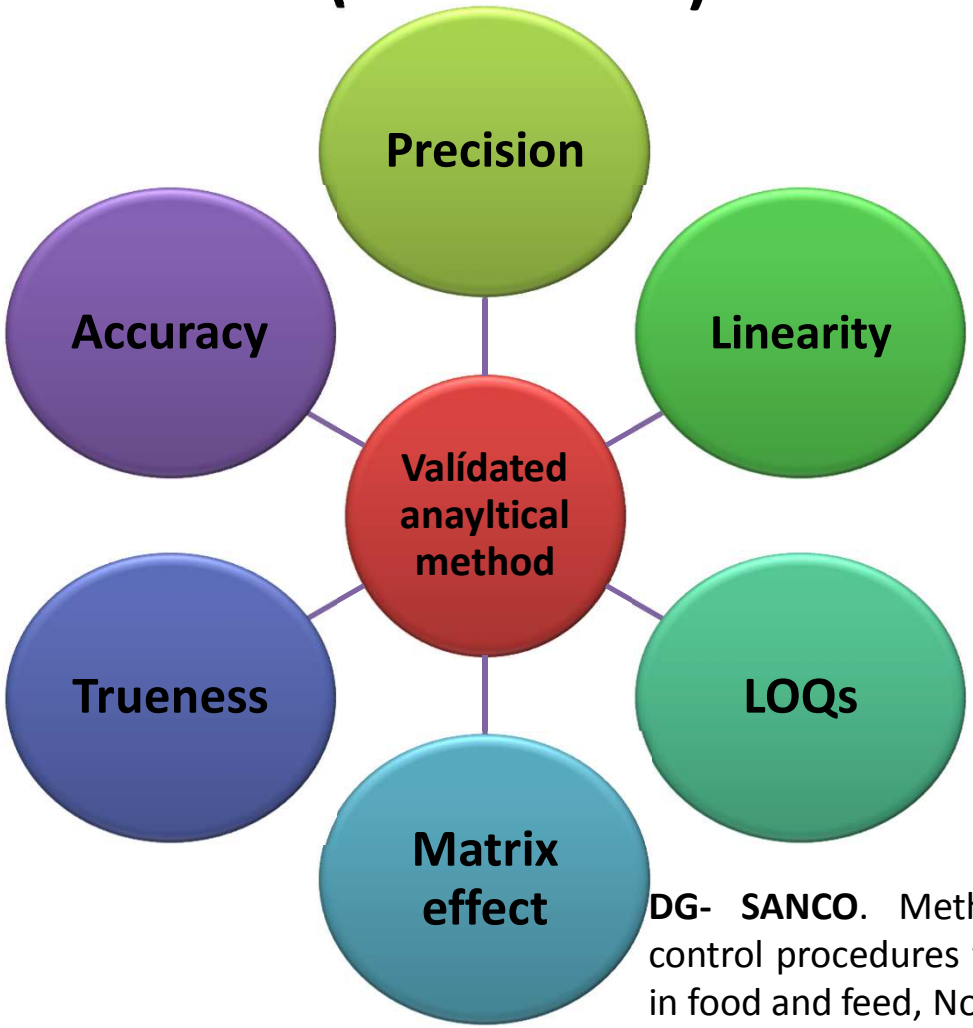
Toxicological evaluation of the pesticide residues in herbs

Validated analytical procedure for the specific medicinal plant

Validation parameters

Directorate General for Health & Consumers

(DG-SANCO)



DG- SANCO. Method validation and quality control procedures for pesticide residue analysis in food and feed, No. SANCO/12495/2011

Why to look after organochlorines?

OC are persistent compounds in soils, can contaminate plants growing in such soils, most dramatically rihzomes or roots .

How to look after organochlorines?

10g grated fresh ginger root + 3,5ml H_2O are shaken vigorously for 1' then 500mg $Al_2(SO_4)_3$ are added and then shaken for 1'

Extraction

10ml ethyl acetate are added and shaken vigorously for 3' then 8g $MgSO_4$+ 1,5g NaCl are added and shaken 4' and centrifuged 3' at 4000rpm

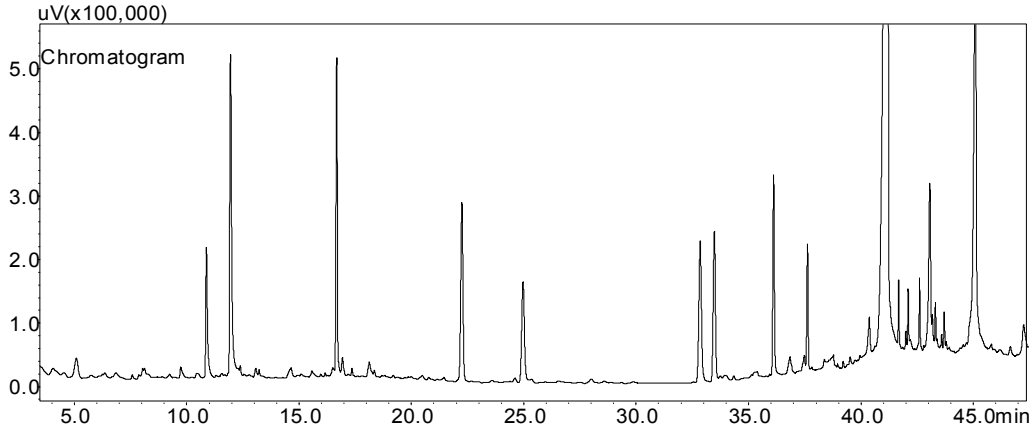
3ml aliquot of supernatant is taken, 450mg of $MgSO_4$+ 75mg of PSA are added, vortexed 1' and centrifuged 3' at 4000rpm

d-SPE clean-up

GC-ECD analysis

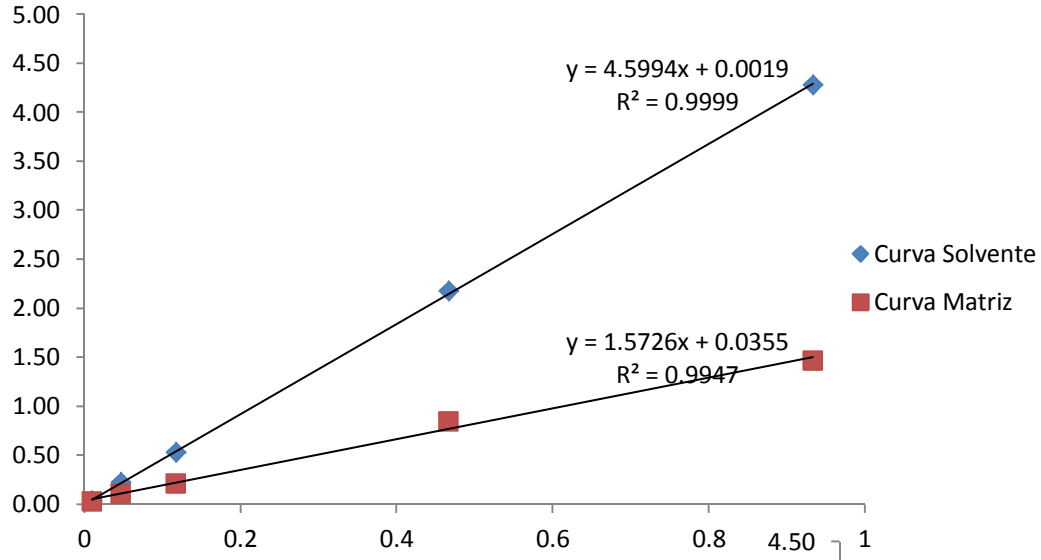
2ml aliquot of supernatant is taken and dried under flow of N_2 then re-dissolved in 1ml Bromophos-methyl 1mg/L (Internal Standard) followed by direct analysis via GC-ECD.

PESTICIDE	250 µg/L		125 µg/L		62,5 µg/L	
	R(%)	RSD(%)	R(%)	RSD(%)	R(%)	RSD(%)
Lindane	**79,0**	**6,7**	**77,0**	**3,5**	**74,9**	**6,5**
Aldrin	100,6	7,7	106,4	7,3	100,4	9,1
Dieldrin	**82,5**	**4,3**	**94,2**	**5,4**	**83,4**	**12,8**
p,p´-DDE	105,4	14,4	95,8	13,3	107,9	7,5
p,p´-DDD	**96,5**	**11,4**	**89,1**	**6,3**	**98,2**	**12,9**
p,p´-DDT	94,0	8,9	95,2	4,6	84,4	4,9



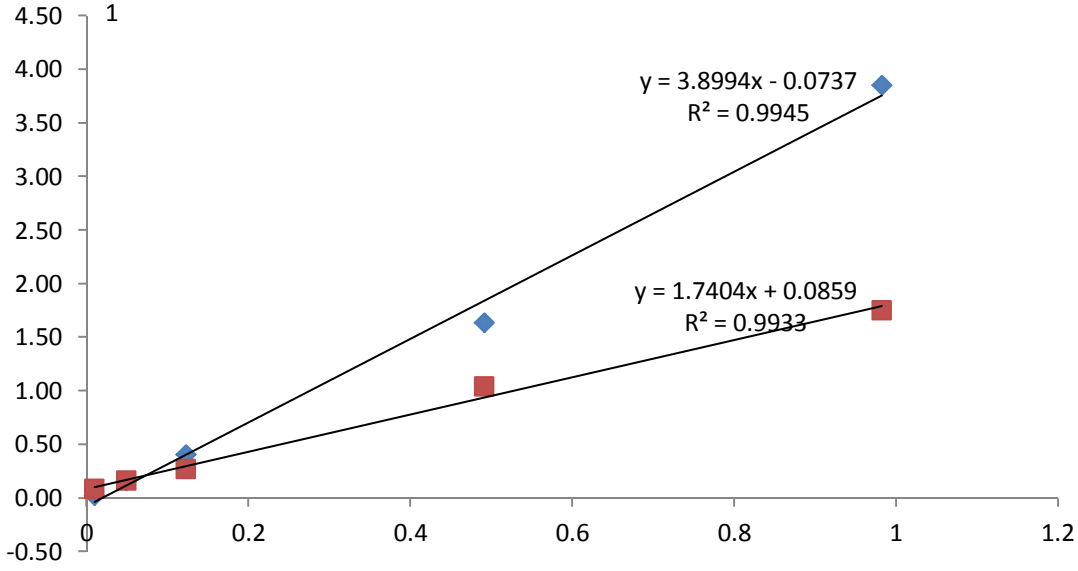
GC-ECD Ginger

Matrix Efect

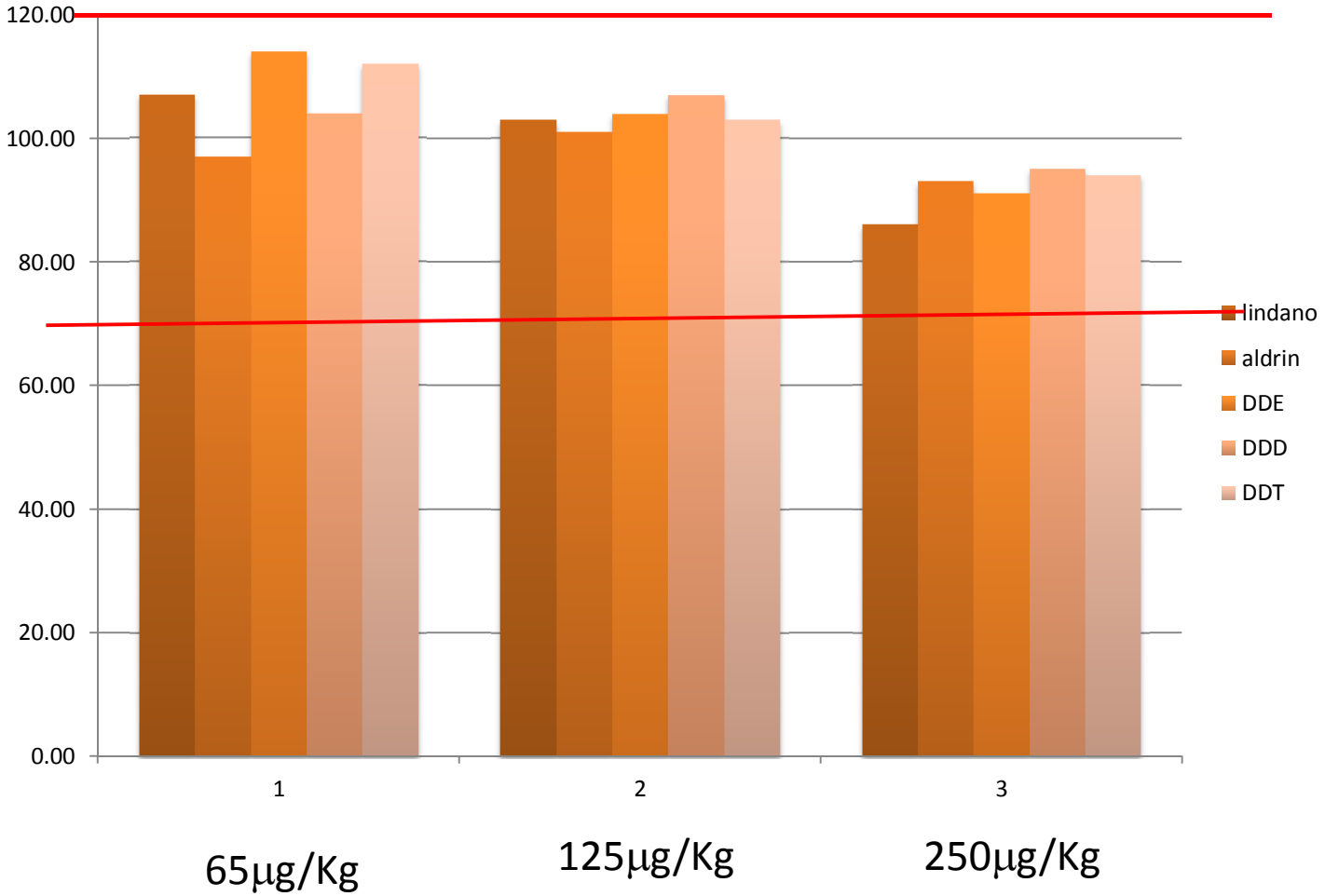


Aldrin

lindane



Ginger: OC Recoveries



GC-ECD Ginger

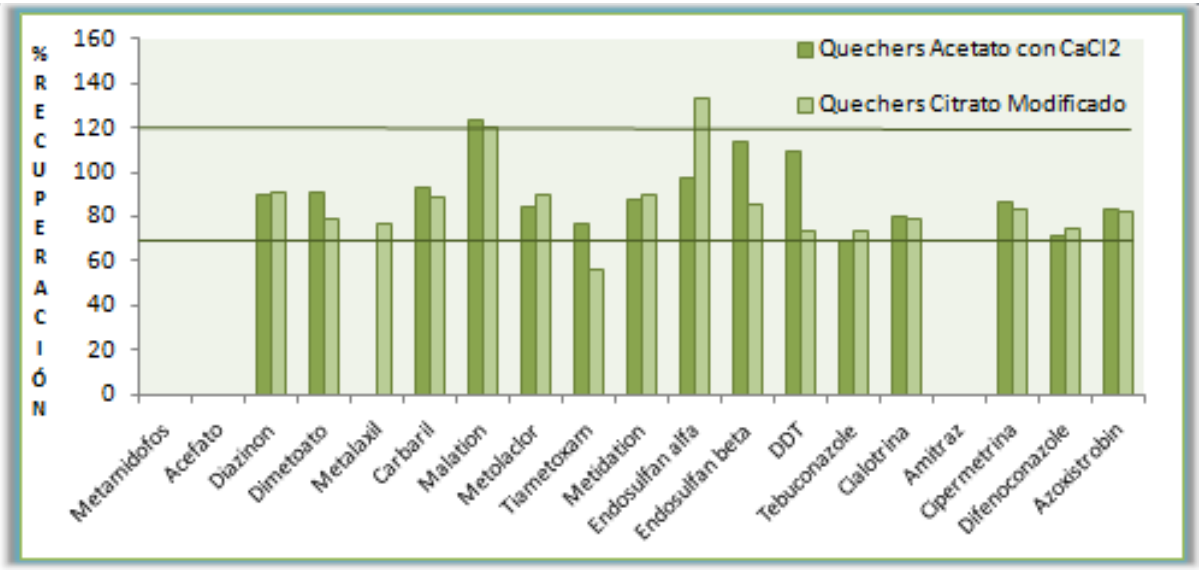
Reproductibility intra-lab

OC	Recovery (%)	RSDwr
Lindane	97	4
Aldrin	107	3
DDE	103	3
DDD	87	2
DDT	118	8

Repeteability

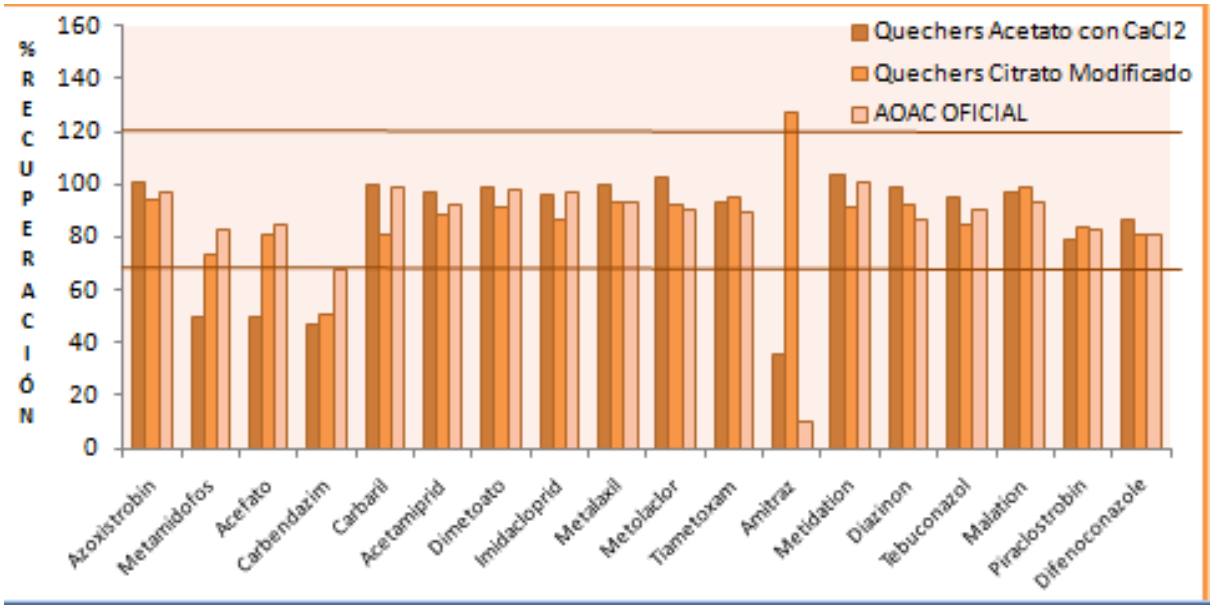
OC	Recovery (%)	RSDwr
Lindane	90	7
Aldrin	97	6
DDE	95	5
DDD	94	7
DDT	104	12

Recoveries for model pesticides in *Peumus boldus* using QuECheRS approaches



GC/MS

LC-MS/MS



Pesticides in medicinal & aromatics plants

Chamomille

Hawthorn

Artichoke leaves

Hypericum perforatum

FARMACOGNOSIA y PRODUCTOS NATURALES
Montevideo
Universidad de la República

Summary

➢Analytical methods form Pharmacopoeias such as the USP do not consider MAPs as individual matrices. They propose an exhaustive clean up procedure, consuming huge amounts of time and reagents, with an intermediate sensitivity.

➢In the 7th Ed. the European Pharmacopeia introduced a new concept, **that it is being implemented with minor adjustments by the coming MERCOSUR Pharmacopeia**

➢ it seems preferably to stablish general parameters for method validation and toxicological evaluation , that should be adjusted for each case.

➢It is neccessary to develop new analytical methodologies as well toxicological criteria for the different latin american medicinal herbs.


http://www.yannarthusbertra

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