

## On the Quantification of Atorvastatin and Celecoxib Active Ingredients in Commercial Solid Drugs using the TT-PIXE and TT-PIGE Techniques

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**Abstract.** The quantification of the active ingredient (AI) in drugs is a crucial and important step in the drug quality control process. This is usually performed by using wet chemical techniques like HPLC, LC-MS/MS, UV spectrophotometry and other appropriate organic analytical methods. In the case of an active ingredient contains specific heteroatoms (F, S, Cl, Br,...), elemental IBA technique can be explored for molecular quantification. IBA techniques permit the analysis of the sample under solid form, without any laborious sample preparation. This is an advantage when the number of sample is relatively large. In this work, we demonstrate the ability of the Thick target PIXE (TT-PIXE) and the TT-PIGE techniques for rapid and accurate quantification of celecoxib and atorvastatin in commercial solid drugs. The experimental aspects related to the quantification validity are presented and discussed.

### 1. Introduction

Commercial drugs contain one or more active ingredients (AI) mixed with a variety of passive substances (excipient). The determination of the active ingredient concentration in commercial drugs is of a great interest in the drug quality control processes. This step aims to ensure, among others, the administration of the drug to the patient at the most effective and appropriate dose. For this, the drug must contain, practically the amount of active ingredients stated on its label. The limited available literature concerning the analysis of drugs under solid form is dealing, in general, with qualitative analysis using among others Nuclear Magnetic Resonance, Raman spectroscopy and powder X-ray diffraction for controlling the stability of the AI during the manufacturing process and to identify several AI simultaneously in a solid pharmaceutical forms [1-3]. Generally, the determination of AI concentration in solid drug is routinely done by using universal techniques, where the sample should be under liquid form, such as chromatography [4,5], UV spectrophotometry [6], micellar electrokinetic capillary electrophoresis and stripping voltametric [7]. Using the above cited techniques, the sample preparation steps, especially when a large number of samples are to be analyzed, are laborious and time consuming. However, The PIGE and specially the PIXE techniques were extensively used for the determination of trace elements in various biological samples [8,9]. Recently the micro-PIXE technique was used to monitor drug release from specific polymer matrix [10]. If the chemical structure of the active ingredient contains at least one specific heteroatom (i.e. Li, F, S, Cl, Br, etc...), the quantification of the AI, in commercial solid drugs, become possible, via the quantification of the heteroatom of interest, by using appropriate ion beam elemental analysis like the PIXE and the PIGE techniques. The first can be applied when the AI contains heavy or semi heavy heteroatom ( $Z > 11$ ) when the second one can be applied for the determination of light heteroatoms. In this case, the advantage of such IBA techniques is

not only their possibilities to analyze the ‘as received’ solid drugs without any sophisticated sample preparation, but for their accurate and rapid analysis within few minutes acquisition time per sample.

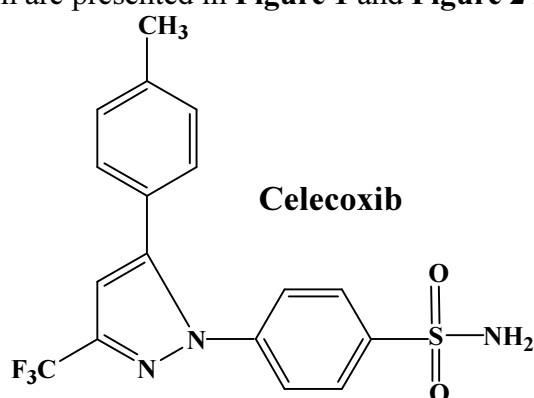
In this work we demonstrate, the reliability of the TT-PIXE (Thick Target PIXE) and the TT-PIGE techniques, via the quantification of sulfur and fluorine respectively, for rapid quantification of the Celecoxib and Atorvastatin AI molecules in various solid drugs, commercialized by different pharmaceutical companies.

Some practical aspects concerning the stability of the sample under irradiation, matrix effect, quantification procedures and accuracy of the analysis, will be discussed.

## 2. Experimental

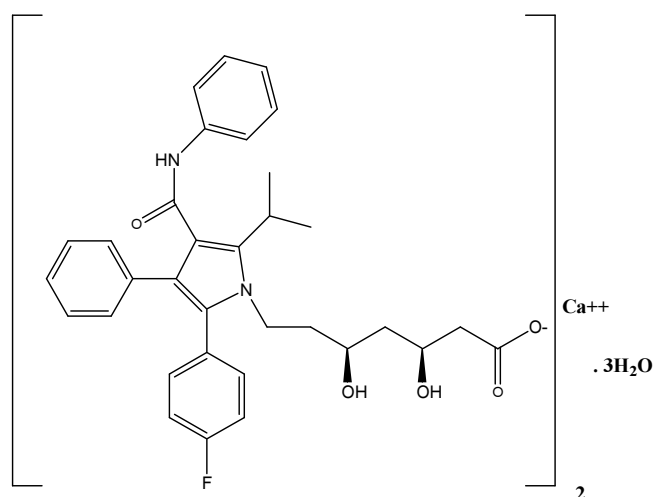
### 2.1 Samples

Two active ingredients were characterized during this study the first one is the 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide or Celecoxib which is a non steroidal anti-inflammatory active ingredient and the 7-[2-(4-fluororophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-yl-pyrol-1-dihydroxy-heptanoic acid or Atorvastatin which is an anti- hyperlipidemic active ingredient. The molecular structures of Celecoxib and Atorvastatin are presented in **Figure 1** and **Figure 2** respectively.



**Figure 1:** Molecular structure of celecoxib.

To assign the active ingredients, a pure celecoxib and a pure atorvastatin used as external standards (synthesized and characterized in our university laboratories). The standard was available in powdered form 100% pure (its purity was checked using H-NMR and <sup>13</sup>C-NMR). Celecoxib was quantified in two different commercial dosage forms (Celebrex<sup>®</sup> 200mg, Pfizer and Celex<sup>®</sup> 100mg, Alpha). Atorvastatin was quantified in three different commercial brand names (Storvas<sup>®</sup> 10 mg, Lipitor<sup>®</sup> 10 mg and Lipinorm<sup>®</sup> 10 mg). The commercial dosage forms were available as capsules and tablets. The excipients of Celex and Celebrex were: Sodium carboxyl methyl cellulose, gelatin, lactose monohydrate, magnesium stearate, polyrinylpyrrolidin-2-one, lactose, cellulose, sodium aryl sulfate and titanium oxide. The excipients used for drugs formulation are always fluorine free.



**Figure 2:** Molecular structure of atorvastatin.

### 2.2 Sample preparation

The drug (capsulated fine white powder or pellets) was pulled out from the capsules, if applicable, and weighted with an analytical balance. For the commercial drugs and for the external standards, two kinds of samples were prepared: (i) the drug or the standard (0.6 g) was mixed with 20% ultra pure graphite. The mixture was pulverized and homogenized then pressed into pellet using boric acid as external binder via  $\sim 1 \text{ ton/cm}^2$  press. (ii) The samples were prepared without graphite, using 0.5g to 0.8g of powder drug, preset into pellets using external binder. In this case, a thin layer of carbon was deposited onto the surface of the sample in order to ensure good surface conductivity as required for in vacuum ion beam analysis techniques.

The diameter of the internal part of the pellet (drug) was  $\sim 5 \text{ mm}$ .

### 2.3 Experimental set-up

The PIGE and the PIXE experiments were carried out by using 3 MeV proton beam delivered by the NEC 1.7 MV 5-SDH tandem accelerator at the Lebanese Atomic Energy Commission. The beam ( $\sim 3 \text{ mm}$  diameter) hit the target at 0 degree. The emitted gamma rays were detected, at  $45^\circ$  referring to the target normal, by an HPGe detector with 40% relative efficiency and FWHM  $\sim 1.9 \text{ keV}$  at 1332 keV, properly shielded with lead. X-ray emission from targets was detected using a Si(Li) detector with  $12.7 \mu\text{m}$  thick Be window and 165 eV measured FWHM energy resolution at 5.9 keV, situated at  $135^\circ$  referring to the beam direction.  $131 \mu\text{m}$  thick Kapton<sup>®</sup> filter was inserted between the sample and the detector. For RBS measurements, using 2 MeV proton beam, a silicon PIPS detector situated at  $165^\circ$  referring to the beam direction, was used. In order to have an accurate charge measurement, secondary electrons suppression was realized by putting the target between two appropriate aluminum wires biased at -400 V. A detailed description of our experimental set-up has been reported elsewhere [11].

The PIXE and RBS spectra were treated using GUPIX and SIMNRA codes respectively. The PIGE spectra were treated (determination of the peak area with accurate background subtraction) by using the SPECTR computer code.

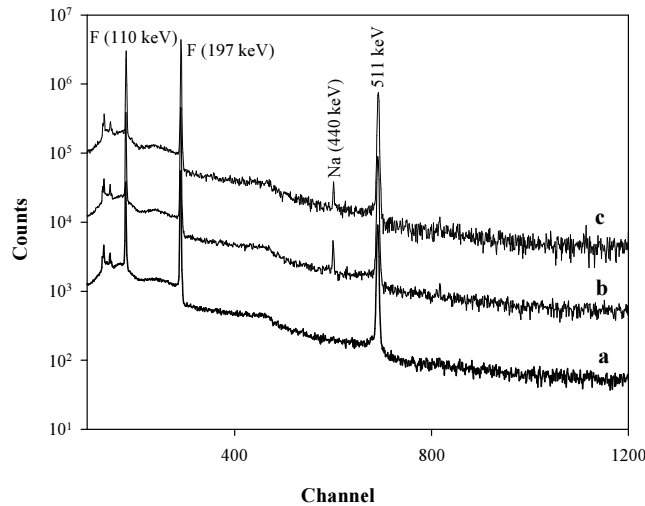
### 3. Results and discussion

#### 3.1 Stability under ion irradiation

The analysis of thick target biological sample, using ion beam, should take into account some practical aspects like the stability of the matrix and eventually the loss of volatile elements under ion irradiation. It is known that, the addition of pure graphite to biological samples, prepared under pellet form, decreases drastically the irradiation effect [12]. Although beam induced target damage is mostly radiation dose dependant. Since that the concentration of sulfur and fluorine in the analyzed samples were in the percent level, the use of a beam current of 0.2 to 1 nA was totally enough to get elegant spectra within few minutes. In spite of this particularity in our measurements, a systematic study was undertaken to ensure the trueness of the results. The pellets, with 20% graphite and that graphite free, were analyzed under 3 MeV proton beam bombardment, using different beam currents (0.1nA, 2nA and 5nA) and different acquisition time, for dose and dose rate effect assessment. The total accumulated charge was between 0.5 and 5  $\mu\text{C}$ . Under these conditions, for the two kind of samples cited above, the number of counts of F and S per  $\mu\text{C}$  versus accumulated charge was practically the same. Consequently, for practical considerations, the analysis of celecoxib and atorvastatin in drugs was performed using graphite free pellets.

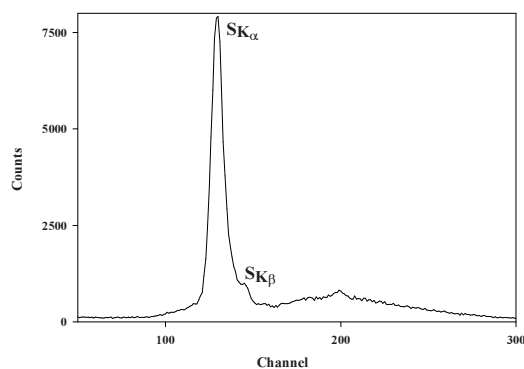
#### 3.2 Celecoxib quantification by PIGE and PIXE

For the quantification of celecoxib in commercial drugs, a pure celecoxib sample was used as external standard. Celecoxib contains S and F as interesting heteroatoms, for PIXE and PIGE analysis. For the quantification of celecoxib, by PIGE via the fluorine atom, the 197 keV gamma ray emitted via the  $^{19}\text{F}(p,p'\gamma)^{19}\text{F}$  nuclear reaction was used. In fact, this gamma ray is specific to fluorine (no interference) and has high cross section [13]. In **Figure 3** is presented the PIGE spectra of celecoxib standard, Celex-Alpha and Celebrex-Pfeizer commercial drugs. The sodium signal at 440 keV, observed for Celex and Celebrex, is originated from the sodium aryl sulfate (excipient). The concentration of sodium is in ppm range. For quantification of celecoxib, via F or S atoms, one can note that the excipients of the analyzed commercial drugs were fluorine free. In contrast, among others, the excipients contain traces of sulfur, originated from the sodium aryl sulfate compound. However, in the PIXE spectra, the contribution of S-containing sodium aryl sulfate is totally negligible compared to S-containing active ingredient contribution. In fact, the concentration of S-containing active ingredient, in the studied drugs, was in the percent level.



**Figure 3:** PIGE spectra of three celecoxib thick target samples: a) pure celecoxib standard, b) celebrex-Pfizer 200 mg et c) celex-Alpha 100mg.

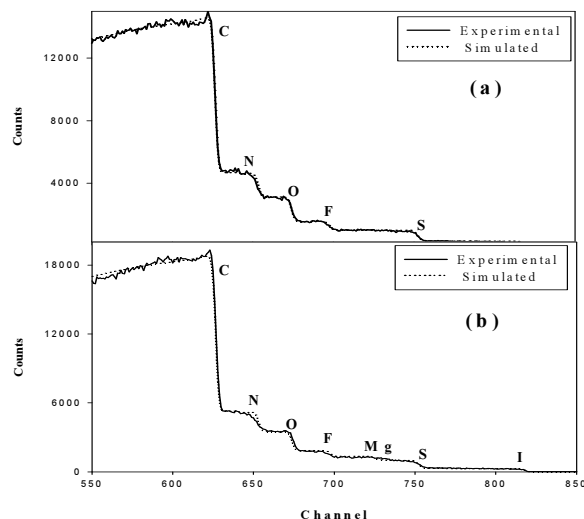
In **Figure 4** is presented a typical PIXE spectrum of a drug sample bombarded by 3 MeV proton beam. For the quantification of celecoxib in the analyzed drug samples, using pure celecoxib as external standard, one can ask about the matrix correction needed for accurate quantification of the Al. The matrix of the standard can be considered very similar to the one of celecoxib based drugs. In fact, the analyzed drugs contain a quantity of excipient not exceeding  $\sim 30\%$  of the mass of the drug. In addition, the excipient is mostly composed by hydrogen, carbon and oxygen atoms. In order to check our assumption, the compositions of the celecoxib standard material and for one commercial celecoxib based drug, studied in this work, were determined by using the RBS technique.



**Figure 4:** Typical PIXE spectrum of a thick target celecoxib based drug.

In **Figure 5** are presented the experimental and the simulated RBS spectra of the celecoxib standard and the Celex-Alpha, using 2 MeV proton beam. In **table 1** is presented the composition of the analyzed samples. The excellent agreement between the theoretical and the

experimental composition, for the celecoxib standard material, demonstrates the reliability of our RBS experimental setup for such measurements. The main logical difference between the composition of the standard and the composition of the analyzed drugs was the high percentage of hydrogen, in the drug forms, due to the chemical composition of the used excipient and to the presence of new heteroatoms, with low concentration, originated essentially from the excipient itself (i.e. Mg).



**Figure 4:** Experimental and simulated RBS spectra of a thick target of (a): pure celecoxib standard ( $I \sim 5\text{nA}$ , acquisition time 2.5 hours using 2 MeV proton beam) and (b): Celex-Alpha ( $I \sim 5\text{nA}$ , acquisition time 3.3 hours using 2 MeV proton beam).

Using the measured compositions, one can calculate, via the Bragg law, the energy loss for different proton energies. It turned out that, the difference of the energy loss, between the celecoxib standard material and the celecoxib based drug form, does not exceed in any case 10 %. This result can practically confirm the high similarity between the matrices of the celecoxib standard and the celecoxib based drug form.

Elemental Composition (%)														
	$C_t$	$C_m$	$H_t$	$H_m$	$F_t$	$F_m$	$N_t$	$N_m$	$O_t$	$O_m$	$S_t$	$S_m$	$Mg_t$	$Mg_m$
Celecoxib standard	53.54	55.12	3.67	3.98	14.96	15.6	11.02	7.49	8.39	8.64	8.39	9.07	---	---
Celex-Alpha	---	54.78	---	6.05	---	9.94	---	7.36	---	9.28	---	7.21	---	4.51

**Table 1:** Calculated ( $C_t$ ) and measured ( $C_m$ ), using RBS, elemental composition of a pure celecoxib sample. The measured elemental composition for a celecoxib based drug (Celex-Alpha) is also presented; the RBS reveals that the sample contains  $\sim 1\%$  iodine (The drug synthesis and formulation processes might be a possible origin of this element).

Consequently, by using the PIXE and the PIGE techniques, the calculation of the concentration of the celecoxib in the analyzed drugs was done without matrix correction. In this way the mass of the active ingredient in the drug can be expressed as :

$$m_{AI} = m_{drug} \times Y_{drug}/Y_{stand} \quad (1)$$

$m_{drug}$  is the mass of the drug,  $Y_{drug}$  and  $Y_{stand}$  are the yield (counts/ $\mu$ C) of fluorine (in the case of PIGE) or sulfur (in the case of PIXE) in the drug and in the standard respectively.

In **table 2** is presented the measured and the labeled celecoxib concentrations. The obtained results show a good agreement with the labeled concentrations. This can demonstrate the ability of the PIXE and PIGE techniques for a rapid ( $\sim 10$  minutes acquisition time) and accurate determination of celecoxib active ingredient in commercial drugs.

	Celecoxib standard	Celebrex-Pfizer		Celex-Alpha	
		Labeled	Measured	Labeled	Measured
F (PIGE)	100 %	72.5%	72% $\pm$ 0.5	68.9%	68.2 % $\pm$ 0.5
S (PIXE)	100 %	72.5%	74.7% $\pm$ 0.8	68.9%	68.2 % $\pm$ 0.8

**Table 2:** Comparison between labeled and measured concentrations for celecoxib, in different commercial drug samples, using PIXE and PIGE techniques.

Finally, it can be noted that the direct quantification of celecoxib in drugs, by using the RBS technique, was possible in this case. In fact, the heteroatoms of interest (F and S) are in high concentrations in the sample (percent level). For example the obtained results, via the F signal, were in excellent agreement with those obtained by PIXE and PIGE. In contrast, the relatively long acquisition time ( $\sim 2$ -3 hours/sample) needed, for optimized RBS setup, to have results with acceptable precision was a serious limitation.

### 3.3 Atorvastatin quantification by PIGE

For the quantification of atorvastatin in Lipitor, Lipinorm and Storvas commercial solid drugs, a pure atorvastatin sample was used as external standard. Atorvastatin contains F as interesting heteroatom, for PIGE analysis. The 197 keV gamma ray emitted via the  $^{19}\text{F}(p,p'\gamma)^{19}\text{F}$  nuclear reaction was used.

When quantification was done directly in relative to the external standard without any matrix correction, high discrepancy (20-25%) was systematically observed between the experimental results and the labeled values of the three analysed commercial brand names. The RBS technique, using 2 MeV proton beam, was used to determine the elemental composition of Storvas, Lipitor and Lipinorm samples. We present in **table 3** the elemental composition of the Lipitor sample, obtained by RBS, and the theoretical and experimental elemental compositions of the atorvastatin external standard. One can see clearly the difference between the elemental composition of the external standard and the commercial atorvastatin based commercial drug (Lipitor is presented as example). In fact, the significant difference was in the carbon, oxygen and calcium composition.

Elemental composition %												
	Ct	Cm	Ht	Hm	Ft	Fm	Nt	Nm	Ot	Om	Cat	Cam
<b>Atorvastatin</b>	<b>68.61</b>	<b>69.42</b>	<b>5.93</b>	<b>6.00</b>	<b>3.29</b>	<b>3.29</b>	<b>4.85</b>	<b>4.78</b>	<b>13.85</b>	<b>12.82</b>	<b>3.47</b>	<b>3.69</b>
<b>Lipitor®</b>		<b>40.3</b>		<b>6.76</b>		<b>0.25</b>		<b>4.16</b>		<b>35.6</b>		<b>12.9</b>

**Table 3:** Calculated ( $C_l$ ) and measured ( $C_m$ ), using RBS, elemental composition of a pure atorvastatin sample. The measured elemental composition for an atorvastatin based drug (Lipitor) is also presented.

Consequently, for reliable analysis a matrix correction is needed. In this way the mass of the active ingredient in the drug can be expressed as

$m_{AI} = m_{drug} \times (Y_{drug}/Y_{stand}) \times (S_{drug}/S_{stand})$  where S is the stopping power at the so called ( $E_{1/2}$ ) energy [14]. The ( $E_{1/2}$ ) energy can be obtained via the 197 keV gamma excitation function. The Detailed procedure concerning the quantification using the PIGE technique by using external standard can be found elsewhere [15].

In this way, when the matrix correction was taken into account, the discrepancy between the labeled and the measured values decrease to  $\sim 10\%$ . Furthermore, appropriate classical wet chemical analytical technique (i.e. UV spectrophotometry) was used for comparison. **Table 4** shows the results of the mass of atorvastatin active ingredient in Lipinorm, Lipitor and Storvas by using the PIGE technique with and without matrix correction as well as the results obtained by UV spectrophotometry. One can see the good agreement between the results obtained by the PIGE technique, when matrix correction was applied, and those obtained by the UV technique.

Atorvastatin Active Ingredient (mg)								
Storvas® 10 mg			Lipitor® 10 mg			Lipinorm® 10 mg		
Measured relatively/std	measured with UV correction	UV	Measured relatively/std	measured with UV correction	UV	Measured relatively/std	measured with UV correction	UV
12.5±0.3	11.2±0.3	10.9±0.4	11.9±0.3	11.0±0.3	10.8±0.2	12.1±0.2	11.0±0.3	11.0±0.1

**Table 4:** Comparison between labeled, measured concentrations for atorvastatin using PIGE with and without matrix correction and those obtained by UV spectrophotometry technique in three commercial drug samples.

#### 4. Conclusion

In this work it was demonstrated that the TT-PIXE and the TT-PIGE techniques are powerful for rapid and accurate quantification of celecoxib and atorvastatin in different commercial solid drug forms, via the detection of interesting heteroatoms. The stability of the thick target drug samples was carefully checked in order to ensure results with high analytical trueness. The analysis using an external standard without or with matrix correction was validated. The obtained results were in excellent agreement with the labeled concentrations, checked by a classical wet chemical technique (i.e. UV spectrophotometer). The accuracy and rapidity of the analysis (few minutes per sample) and data treatment offers a new overview for the PIXE and the PIGE technique in the field of drug quality.



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