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A simple protocol for the characterization of fraudulent erectile dysfunction medicines using ion beam analytical techniques

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ABSTRACT

In this work we developed a simple and fast protocol based solely on ion beam analytical techniques for the analysis of erectile dysfunction medicines containing sildenafil and vardenafil as active ingredients. To that end, RBS (Rutherford Backscattering Spectrometry), PIXE (Particle-Induced X-ray Emission) and MeV-SIMS (Secondary Ion Mass Spectrometry) were employed in the analysis of sildenafil citrate (Viagra®), vardenafil hydrochloride (Levitra®) and tadalafil (Cialis®) among other medications. The RBS and PIXE techniques provide information on the concentrations of major and trace elements present in the tablets respectively, while the MeV-SIMS technique was used for the determination of the molecular profile of the samples. The protocol is based on the detection of sulfur present in the tablets through the PIXE technique, while its validation relies on the detection of the active ingredients with the MeV-SIMS technique.

The results confirm that the protocol is quite suitable for the discrimination of different sildenafil- and vardenafil-based medicines. On the other hand, this protocol cannot be used for the study of tadalafil-based medicines since no detectable element by PIXE is present in its active ingredient.

1. Introduction

Sildenafil, vardenafil and tadalafil are strong type-5 phosphodiesterase (PDE-5) inhibitors that prevent the neutralization of intracellular cyclic guanosine monophosphate by PDE-5. Despite the PDE-5 inhibitor was initially considered a good candidate for the treatment of cardiovascular disorders [1], it soon became clear that it could also be used as an oral therapy for male erectile dysfunction [1,2]. Sildenafil became known worldwide in 1998 when Pfizer started marketing Viagra® tablets as an oral therapy for erectile dysfunction [2,3]. Soon after, the United States Food and Drug Administration approved Levitra® from Bayer Pharmaceuticals and Cialis® from Eli Lilly for the US market [4–6]. Despite these medicines are used for the treatment of erectile dysfunction, they differ somewhat in some respects like the persistence in the human body and the onset of action among others [5,7,8]. Some technical information of these pharmaceuticals is provided in Table 1.

Due to their high market value, these pharmaceuticals attracted the attention of several companies and already during the first years of the new millennium the global market was flooded with substandard products including food supplements containing the analogues of sildenafil, vardenafil and tadalafil [7,9]. As far as the drug industry is concerned, analogues are those molecules that have similar composition and structure of well-known molecules whose efficacy and safety profile were certified and approved for human consumption [7]. Despite the resemblance, analogues may not perform as the original molecule [10], which poses serious health risks for those consuming such drugs [7].

Just a few years after the debut of Viagra® in the retail market worldwide, the perils of advertisement and marketing of counterfeit sildenafil-based products and aphrodisiac supplements over the internet raised the awareness of the scientific community [9]. As the e-commerce progressed at an astonishing pace, the selling of fraudulent erectile dysfunction medicines among others reached unprecedented levels [11], fostering crackdown operations led by international law enforcement institutions [12–15]. Educational material on counterfeit

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Table 1
Stoichiometry and molar masses (in daltons) of the active ingredients (AI) and of the respective pharmaceutical formulations (PF).

Brand Name	Active Ingredient (AI)	Stoichiometry and Mass (AI)	Pharmaceutical Formulation (PF)	Stoichiometry and Mass (PF)
Viagra®	Sildenafil	$C_{22}H_{30}N_6O_4S$ 474.6 Da	Sildenafil Citrate	$C_{28}H_{38}N_6O_{11}S$ 666.7 Da
Levitra®	Vardenafil	$C_{23}H_{32}N_6O_4S$ 488.6 Da	Vardenafil Hydrochloride	$C_{23}H_{33}ClN_6O_4S$ 525.1 Da
Cialis®	Tadalafil	$C_{22}H_{19}N_3O_4$ 389.4 Da	Tadalafil	Tadalafil

medicines including topics like how to identify fake medicines and on the potential dangers they pose to human health became common place on the internet [16–18].

Different analytical techniques have been used to characterize and identify legitimate and fake erectile dysfunction medicines at distinct complexity levels, starting from just an inspection of the physical profile of the tablets [19] up to more sophisticated techniques like the Tandem Mass Spectrometry (MS/MS) requiring at least two stages of analysis [20]. Although other techniques like Nuclear Magnetic Resonance (NMR), Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and Raman spectrometry have been employed in the study of erectile dysfunction medicines as well [9,21,22], mass spectrometry and its variants have been the techniques of choice as far as molecular analysis is concerned [3,7,9,23,24,25]. For elemental analysis, Instrumental Neutron Activation Analysis (INAA) and Inductive Coupled Plasma (ICP) techniques have proved to be valuable tools for forensic purposes [25,26].

Facilities around the world working with MeV ion accelerators employ techniques like Particle-Induced X-ray Emission (PIXE), Rutherford Backscattering Spectrometry (RBS) and Secondary Ion Mass Spectrometry with MeV ions (MeV-SIMS) in order to characterize a wide range of materials from the elemental and molecular points of view [27–29]. In general, ion beam analytical techniques are relatively fast, usually requiring little sample handling and, most importantly, are non-destructive, meaning that the same sample can be measured as many times as needed. The combination of different ion beam analytical techniques has shown the potentialities of such techniques for a variety of applications including forensic studies [30–32]. As far as sildenafil is concerned, PIXE proved to be a valuable tool for the analysis of authentic Viagra® and other sildenafil-based illegal medicines and supplements through the quantification of sulfur present in the tablets [31].

The aim of the present work is to provide a simple and fast PIXE protocol for the detection of fraudulent sildenafil- and vardenafil-based erectile dysfunction medicines. To that end, RBS experiments will be used to determine the major elements of these medicines. PIXE will be employed as the main quantitative elemental tool while MeV-SIMS is used to validate the protocol. It is important to note that these medicines are characterized by the presence of one sulfur atom in their active ingredients (see Table 1), which makes them very attractive for PIXE analysis. For a broader perspective of the results, other medicines and one supplement were studied as well.

2. Experimental procedure

2.1. Samples preparation protocols

Seven different brands of medicines were chosen for the present study: five sildenafil-based medicines; one tadalafil-based medicine; and one vardenafil-based medicine. Among the sildenafil-based medicines are Viagra® (PFZ), the Brazilian generics from Empresa Brasileira de Medicamentos® (EMS) and Neo-Química® (NQM), and finally the generic Pramil® (PRM) sold in Paraguay. One compounded medicine from Biostévi Pharma® (BTV) was analyzed as well. Other medicines like Levitra® (LVT), a vardenafil-based medicine and Cialis® (CLS), a tadalafil-based medicine, were analyzed for comparison purposes. Finally, one supplement, Ativo – Ultra® (ATV), supposedly based on maca root was studied in the present work. Maca is extracted from a plant (*Lepidium meyenii*) grown in the Peruvian Andes mountains with aphrodisiac properties [33].

Nine samples of each medicine were selected from three different batches for analysis. Each of the samples was measured 4 times. Table 2 shows important parameters of the main pharmaceuticals studied in this work. Table 3 shows the excipients used in the medicines as provided by the manufacturers.

For the PIXE and RBS experiments, the tablets' surfaces were scratched with a file in order to remove the tablets' thin coating films. Despite its simplicity, this procedure is of utmost importance since PIXE is focused on the detection of sulfur present in the pharmaceutical's active ingredient (Table 1). However, Table 3 reveals that some excipients used as medicine coatings like indigo carmine and aluminum lake blue have sulfur in their formulas. In this way, the removal of the coatings prevents any interference of sulfur coming from the excipients. For the capsules, the powder was extracted from the outer shell, homogenized and pressed into 2 mm thick pellets. All samples were placed in target holders inside the reaction chambers kept under a pressure of about 10^{-5} mbar for the irradiation sessions.

For the MeV-SIMS experiments, thick targets cannot be used since the sample's stage is polarized to 2 kV in order to promote the ejection of secondary compounds and molecules towards the time-of-flight spectrometer. Therefore, a simple protocol was developed for the preparation of all samples. The medicines and supplements were dissolved in ethylic alcohol in the proportion of 1 mg/ml. Subsequently, the solution was dropped on a clean < 100 > Si wafer and left to dry for 1 h. Finally, the samples were accommodated in the samples' stage inside the reaction chamber kept under a pressure better than $2x10^{-6}$ mbar.

2.2. RBS experiments

The RBS technique [34] is quite suitable for the detection of major constituents in organic materials where elements like carbon, nitrogen and oxygen are abundant. A 3 MV Tandetron accelerator provided beams of 1.2 MeV He^+ ions for the backscattering experiments. One sample of each medicine was irradiated during 30 min. Typical ion currents were in the range of 5 nA to 10 nA. The ions backscattered by the atoms of the sample were detected by a Si surface barrier detector placed at 15° with respect to the beam direction. The energy resolution of the detector is around 20 keV. The RBS spectra were analyzed with the SIMNRA software [35].

2.3. PIXE experiments

PIXE [34] relies on the excitation of the target atoms through the

Table 2
Pharmaceuticals studied in this work. The masses of the active ingredient per pill were provided by the manufacturers. NA: Not Available.

Brand Name	Active Ingredient/Mass per Pill	Pill Style	Pill Mass (mg)
Viagra® (PFZ)	Sildenafil/50 mg	Tablet	318 ± 1
EMS® (EMS)	Sildenafil/50 mg	Tablet	360 ± 1
Neo-Química® (NQM)	Sildenafil/50 mg	Tablet	321 ± 1
Biostévi® (BTV)	Sildenafil/50 mg	Capsule	137 ± 2
Pramil® (PRM)	Sildenafil/50 mg	Tablet	124 ± 1
Levitra® (LVT)	Vardenafil/20 mg	Tablet	187 ± 1
Cialis® (CLS)	Tadalafil/20 mg	Tablet	363 ± 1
Ativo-Ultra® (ATV)	Maca Root/NA	Capsule	508 ± 6

Table 3
Excipients of the medicines according to the manufacturers. No information of the excipients was found for the compounded medicine BTV and the supplement ATV.

Excipient	Stoichiometry	Molar Mass	Medicines
Cellulose	$C_6H_{10}O_5$	162.1 Da	PFZ, EMS, NQM, CLS, LVT, PRM
Calcium Phosphate	$Ca_3(PO_4)_2$	310.2 Da	PFZ, NQM, PRM
Carmellose Sodium	$C_8H_{16}NaO_8$	263.2 Da	PFZ, EMS, NQM, CLS, PRM
Magnesium Stearate	$Mg(C_{18}H_{35}O_2)_2$	591.2 Da	PFZ, EMS, NQM, CLS, LVT, PRM
Hypromellose	$C_{56}H_{108}O_{30}$	1261.4 Da	PFZ, EMS, NQM, CLS, LVT, PRM
Lactose	$C_{12}H_{22}O_{11}$	342.3 Da	PFZ, EMS, CLS, PRM
Triacetin	$C_9H_{14}O_6$	218.2 Da	PFZ, CLS, PRM
Indigo Carmine	$C_{16}H_8N_2Na_2O_8S_2$	466.4 Da	PFZ, PRM
Titanium Dioxide	TiO_2	79.9 Da	PFZ, EMS, NQM, CLS, LVT, PRM
Aluminum Lake Blue	$C_{48}H_{24}Al_2N_6O_{24}S_6$	1315.1 Da	PFZ, EMS, NQM, PRM
Silicon Dioxide	SiO_2	60.1 Da	EMS, NQM, LVT
Macrogol	$C_{2n}H_{4n+2}O_{n+1}$	(44.0n + 18.0) Da	EMS, NQM, LVT
Talcum	$Mg_3Si_4O_{10}(OH)_2$	379.3 Da	NQM, CLS
Ferric Oxide Yellow	H_3FeO_3	106.9 Da	CLS, LVT
Sodium Lauryl Sulfate	$NaSO_4C_{12}H_{25}$	288.4 Da	CLS
Hyprolose	C_3H_7O	59.1 Da	CLS
Crospovidone	C_6H_9NO	111.1 Da	LVT
Ferric Oxide Red	Fe_2O_3	160.0 Da	LVT

removal of inner shell electrons. If the decay is radiative, then characteristic X-rays are produced. In this way, PIXE is suitable for the quantification of elements, usually with atomic number greater than 10. For the irradiations, 2 MeV H^+ ions provided by the Tandetron accelerator were employed in all experiments. All nine samples from each medicine were irradiated at 4 different points of the sample with currents of about 1 nA during 30 s each. The characteristic X-rays were detected by a Si(Li) detector placed at 45° with respect to the beam direction. The energy resolution of the detector is around 150 eV at 5.9 keV. The X-ray spectra were analyzed with the GUPIXWIN software [36].

2.4. MeV-SIMS experiments

The MeV-SIMS setup consists of a reaction chamber coupled with a time-of-flight spectrometer. The samples were irradiated with 6 MeV Cu^{4+} ions during 2 min each. Typical currents were about 500 pA at the target. The target holder was biased with 2 kV pulses of 10 kHz in order to extract the secondary ions sputtered from the sample's surface. Secondary ions were time-analyzed by a Kore Time-of-Flight Mass Spectrometer [37] equipped with reflectron electrodes and a microchannel plate system for the detection of the secondary ions. The mass spectra were analyzed with the GRAMS software [38].

3. Results and discussion

A typical RBS spectrum is shown in Fig. 1. The edges of C and those corresponding to other elements are discernible. The relatively high background at low energies indicates the contribution of multiple scattering effects. The RBS results for the medicines are shown in Table 4. The matrix of light elements not detected by PIXE consists basically of carbon, oxygen and nitrogen. For the majority of the medicines, carbon corresponds to about 68 % of the matrix content, followed by oxygen (about 24 %) and nitrogen (about 9 %). On the other hand, BTV is characterized by the presence of oxygen and silicon only, while ATV has carbon and oxygen as major matrix elements. It is important to stress that this analysis neglected the presence of hydrogen in the matrix.

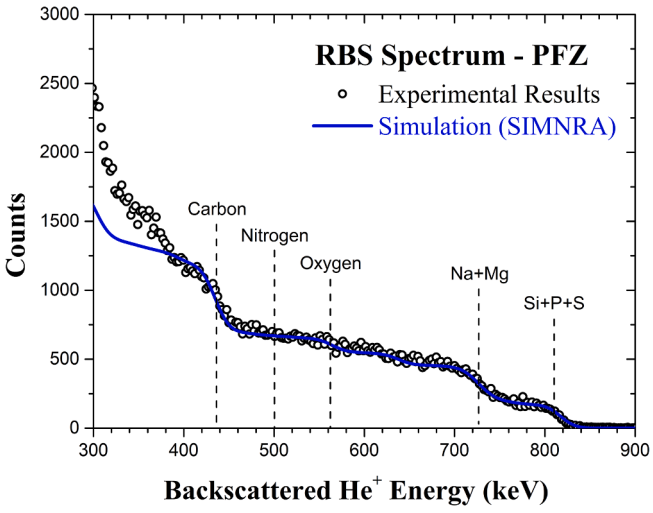

Fig. 1. RBS spectrum of Viagra® (PFZ). The primary He^+ energy is 1.2 MeV. Dashed lines indicate the approximate edge position of the corresponding elements. The blue curve represents the spectrum simulation with SIMNRA [35].

Table 4
Matrix elements of the medicines studied in this work. All concentrations were normalized to 100%. All values are given as dry weight percentage. See text for further details.

Element	PFZ	EMS	NQM	BTV	PRM	CLS	LVT	ATV
C	69 %	68 %	65 %	–	66 %	70 %	69 %	79 %
O	23 %	23 %	28 %	75 %	19 %	25 %	23 %	21 %
N	8 %	9 %	7 %	–	15 %	5 %	8 %	–
Si	–	–	–	25 %	–	–	–	–

Indeed, matrix effects like X-ray self-absorption within the target and secondary fluorescence are negligible as far as H content is concerned. The impact of H on the elemental concentrations is estimated to be of the order of 1 %. Finally, the data shown in Table 4 serve as an input for the GUPIXWIN software [36] in order to calculate corrections in the elemental concentrations due to matrix effects.

Fig. 2 shows typical X-ray spectra obtained for the PFZ, CLS and LVT medicines. These qualitative PIXE results already reveal interesting features. First of all, the elemental profile is quite distinct for these medicines. For instance, LVT is characterized by the presence of

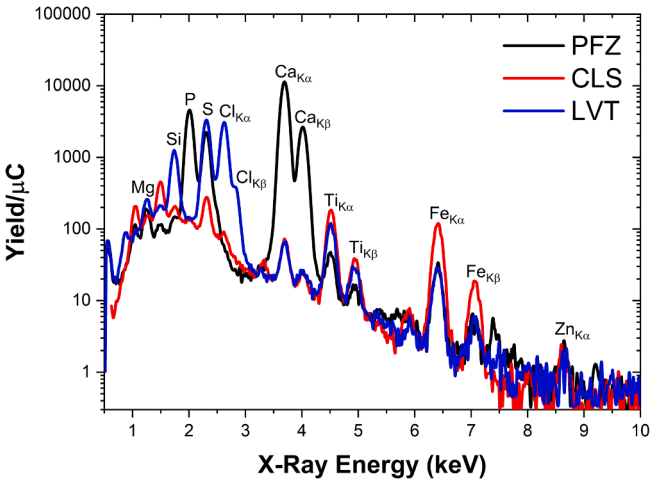

Fig. 2. X-ray spectra of PFZ (black curve), CLS (red curve) and LVT (green curve) as a function of the X-ray energy. The yields were normalized by the charge accumulated during the experiments.

chlorine, which is compatible with its pharmaceutical formulation (Table 1). Moreover, PFZ and LVT have a substantial concentration sulfur, while a much less amount of it is found in CLS. This result is corroborated by the presence of sulfur in the active ingredients of both PFZ and LVT (Table 1).

The elemental concentrations of all medicines studied in this work are shown in Table 5. Among the sildenafil-based medicines, PFZ and the generics EMS and NQM sold in the Brazilian market have approximately the same sulfur concentrations (around 10000 ppm). Conversely, the generic PRM has about 2 times more sulfur, while BTV has the largest concentration of this element among those sildenafil-based medicines followed by PRM.

As expected, LVT has substantial concentrations of sulfur and chlorine due to its active ingredient and pharmaceutical formulation (Table 1). On the other hand, CLS has the lowest sulfur concentration among all medicines, most likely coming from the excipient sodium lauryl sulfate (Table 3). Finally, ATV is characterized by relatively large amounts of silicon, sulfur, manganese and copper.

The expected sulfur mass to be found in the medicines can be calculated from the stoichiometry (Table 1) and the masses (Table 2) of the active ingredients in the tablets. The values of (3.38 ± 0.34) mg and (1.31 ± 0.13) mg of sulfur are expected to be found in the sildenafil- and vardenafil-based medicines respectively. An uncertainty of 10 % [39] was assumed for the amount of the active ingredients per tablet. The results of the sulfur concentrations shown in Table 5 can be converted to the expected sulfur mass using the tablets' masses displayed in Table 2. These results are summarized in Fig. 3. The medicines PFZ and its generics EMS, NQM and PRM have sulfur masses compatible with the expected value of sildenafil-based medicines. On the other hand, BTV has about 59 % more sulfur than expected, thus indicating a substantial deviation from the other sildenafil-based medicines.

The LVT result reveals that its sulfur content is compatible with the value of 1.31 mg, thus agreeing with a vardenafil-based medicine. On the other hand, the sulfur masses of CLS and ATV are compatible neither with sildenafil- nor with vardenafil-based medicines.

In order to check the validity of the protocol developed for the characterization of sildenafil and vardenafil, MeV-SIMS experiments were carried out for all medicines studied in this work. Fig. 4 shows the mass spectra of the sildenafil-based medicines. It can be clearly seen that all the spectra are dominated by a prominent peak at 475 Da, thus confirming that the sildenafil compound is present in the medicines. Other peaks at 283 Da, 311 Da and 377 Da have already been identified as fragments of sildenafil [3,23] and show up in the spectra as well. Worth to mention that the 475 Da peak of BTV had to be halved in order to preserve a common scale for all spectra.

Fig. 5 shows the MeV-SIMS spectra of LVT and CLS medicines and the ATV supplement. The LVT spectrum is dominated by the presence of vardenafil at 489 Da. Other peaks at 312 Da and 256 Da are visible in the spectrum and correspond to the fragmentation of the vardenafil

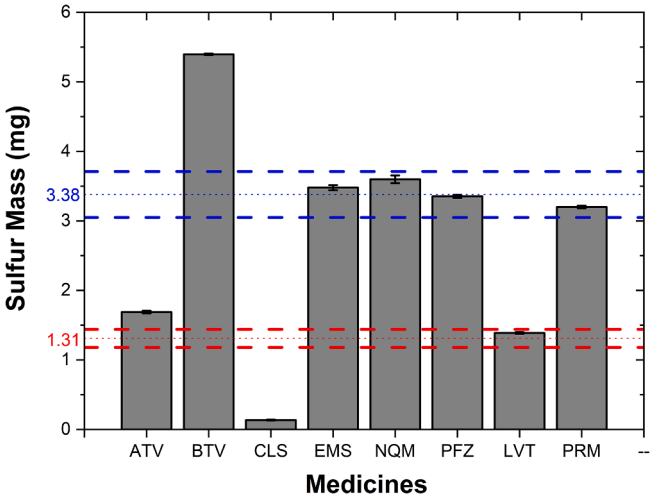

Fig. 3. Sulfur contents of the medicines studied in this work. The thin blue and red dotted lines represent the expected sulfur masses to be found in the sildenafil- (3.38 mg) and vardenafil-based (1.31 mg) medicines respectively. The regions between the blue and red dashed lines represent the 10 % uncertainty [39] assumed for the amount of active ingredients per tablet. The uncertainties are given by the standard deviation of the mean.

molecule [23]. The CLS spectrum is characterized by a strong peak at 366 Da followed by the tadalafil molecule at 389 Da. Despite the peak at 366 Da has already been identified as a fragment of the tadalafil analogue *n*-octylnortadalafil [40], the mass of the intact analogue molecule (488 Da) is not present in the spectrum. On the other hand, the peak located at 262 Da has been identified as a tadalafil fragment as well [23].

Finally, the most surprising result is the mass spectrum of ATV revealing the presence of sildenafil (475 Da) and tadalafil (389 Da) in minute amounts. Other peaks at lower masses are visible as well, thus indicating that a large variety of substances make part of this supplement. Only one peak located at 116 Da (not shown in Fig. 5) was already reported as being characteristic of the Peruvian maca [41].

4. Concluding Remarks

In this work a simple and relatively fast protocol based solely on the PIXE technique was developed for the discrimination of sildenafil- and vardenafil-based medicines. The validation of the protocol was carried out with the MeV-SIMS technique. According to this protocol, the sildenafil-based medicines PFZ and its generics EMS and NQM from Brazil and PRM from Paraguay do comply with the amount of sildenafil present in the active ingredient as informed by the manufacturers. On the other hand, the sulfur concentration found in BTV is much higher

Table 5
Elemental concentrations of all medicines studied in this work. The values are given in milligrams of element per kilogram of tablet, i.e. in parts per million (ppm).

	PFZ	EMS	NQM	BTV	PRM	CLS	LVT	ATV
Na	2546 ± 189	1049 ± 289	2356 ± 371	–	3214 ± 203	2519 ± 141	–	–
Mg	1270 ± 36	675 ± 77	3981 ± 2466	72889 ± 509	4387 ± 219	206 ± 33	661 ± 34	–
Al	62 ± 6	108 ± 22	138 ± 15	–	–	1208 ± 127	246 ± 60	526 ± 66
Si	344 ± 26	9684 ± 678	5998 ± 278	114121 ± 470	8538 ± 176	156 ± 33	2781 ± 95	7203 ± 246
P	7343 ± 218	–	6406 ± 231	–	–	–	–	–
S	10602 ± 88	9831 ± 142	11648 ± 149	38710 ± 269	25596 ± 208	397 ± 16	7427 ± 68	3338 ± 49
Cl	–	–	–	–	–	–	6895 ± 60	705 ± 33
K	–	–	–	–	28 ± 3	26 ± 5	–	634 ± 10
Ca	37304 ± 429	34 ± 5	18539 ± 1612	–	188 ± 108	49 ± 9	70 ± 15	395 ± 39
Ti	60 ± 18	34 ± 2	26 ± 16	78 ± 5	21 ± 4	236 ± 121	79 ± 38	219 ± 43
Mn	–	1.0 ± 0.2	–	–	–	3.5 ± 0.3	–	2607 ± 123
Fe	51 ± 8	31 ± 7	69 ± 6	1052 ± 10	22.5 ± 0.2	153 ± 71	21 ± 11	98 ± 20
Cu	–	–	–	–	–	–	–	874 ± 85
Zn	–	–	–	–	–	2.8 ± 0.7	–	–

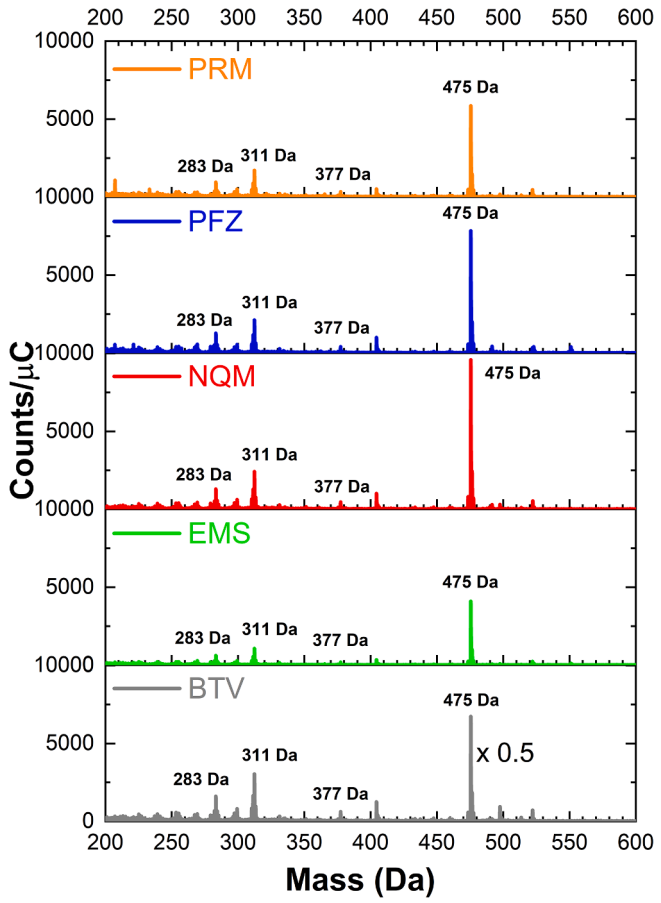

Fig. 4. MeV-SIMS spectra of the sildenafil-based medicines. All spectra were normalized by the charge accumulated during the experiments. For the sake of clarity, the intensity of the 475 Da peak of the BTV was divided by 2.

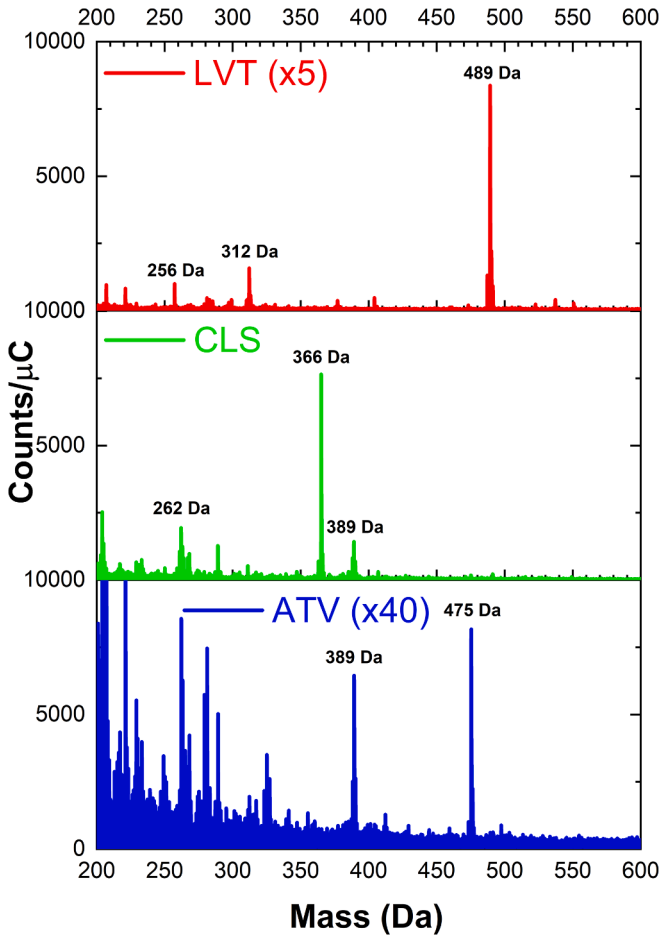

Fig. 5. MeV-SIMS spectra of LVT, CLS and ATV. All spectra were normalized by the charge accumulated during the experiments. For the sake of clarity, the LVT and ATV spectra were multiplied by 5 and 40 respectively.

than the expected concentration of this element. According to the manufacturer, each BTV capsule should have 50 mg of sildenafil. If we assume that all sulfur content is related exclusively to the active ingredient (sildenafil), then our result is compatible with 79.8 mg of sildenafil per capsule. This supposition is corroborated by the MeV-SIMS results shown in Fig. 4. Another possible explanation is that there is indeed 50 mg of sildenafil per capsule, while the remaining excess of sulfur comes from the excipient used in the formulation. In this case, no definite conclusion can be drawn since no information on the excipients used in this compounded medicine was found.

The results obtained for the vardenafil-based LVT based on the sulfur mass agree with the information provided by the manufacturer on the amount of active ingredient present per tablet. Conversely, the sulfur mass of CLS indicates that its formulation is related neither to sildenafil nor to vardenafil.

The results obtained for sildenafil and vardenafil based on the sulfur mass present in the tablets were corroborated by the MeV-SIMS experiments. On the other hand, a tadalafil fragment but not the intact molecule was identified by MeV-SIMS. An interesting case was observed for the ATV supplement where MeV-SIMS results revealed that this supplement has tiny amounts of sildenafil and tadalafil. Unfortunately, there is no information at all on the exact formulation of this supplement. According to the nutritional facts provided by the manufacturer, there should be substantial amounts of magnesium and zinc. Our PIXE results show that the concentrations of Mg and Zn are below the limit of detection (67 ppm and 3 ppm respectively), thus in clear disagreement with the information provided for this supplement.

The protocol developed in this work shows how techniques like RBS, PIXE and MeV-SIMS can work synergistically for different applications including forensics and can be employed by any facility working with ion beam analytical techniques. In this protocol, RBS and PIXE were employed for the detection of major and trace elements in the medicines respectively, while MeV-SIMS was used to validate the protocol. This protocol can be applied to sildenafil- and vardenafil-based medicines and supplements since it relies on the detection of sulfur present in the active ingredients of these medicines. It is important that the excipients of the medicines under study do not have sulfur in their formulation to avoid misinterpretations.

CRediT authorship contribution statement

G.M.S. Souza: Software, Validation, Formal analysis, Investigation, Methodology, Conceptualization. **R. Debastiani:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization. **P. Chytry:** Validation, Methodology, Investigation, Conceptualization. **M. Knebel:** Methodology, Investigation. **R. Thomaz:** Methodology, Conceptualization. **L. Amaral:** Methodology, Investigation, Conceptualization. **J.F. Dias:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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