Research Article

Ion beam analysis (IBA) and instrumental neutron activation analysis (INAA) for forensic characterisation of authentic Viagra® and of sildenafil-based illegal products

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Highlights

- **First combined approach using INAA, PIXE and MeV-SIMS for sildenafil-based products analysis**
- **PIXE provides elemental profiles of tablets with no sample preparation.**
- **MeV-SIMS provides mass spectra of sildenafil with minimum sample preparation.**
- **Both IBA and INAA discriminate between authentic Viagra® and illegal products.**
- **An inter-laboratory classification system based on shared INAA results is possible.**

Abstract

Illegal trafficking of pharmaceutical products by criminal organisations is a global threat for public health. Drugs for erectile dysfunction such as phosphodiesterase type 5 inhibitors are the most commonly counterfeited medicines in Europe. The search of possible toxic chemical substances in seized products is needed to provide early warning for public health. Furthermore, the elemental profile of the seized products can be useful in criminal investigations. For the first time an ion beam analysis (IBA) procedure to characterise authentic Viagra® tablets and sildenafil-based illegal products is described. Moreover, results are compared with the ones obtained by instrumental neutron activation analysis (INAA) on authentic Viagra® tablets in two reactors. IBA results showed that a combination of particle-induced X-ray emission (PIXE) and secondary ion mass spectrometry using primary ions with energies in the range of several MeV (MeV-SIMS) is a powerful tool to characterise different products in a straightforward manner, allowing discrimination between legal and illegal products. INAA allowed accurate elemental quantification and also showed a great potential for the future implementation of an inter-laboratory classification system.

Keywords

Illegal pharmaceutical products, counterfeit pharmaceutical products, dietary supplements, neutron activation analysis, ion beam analysis, forensic analysis, sildenafil, Viagra®.

Introduction

Operation Pangea XIII, conducted in March 2020, saw a rise in fake medical products related to the outbreak of the COVID-19 disease. Participating authorities seized 4.4 million units of counterfeit facemasks, substandard hand sanitizers and unauthorized antiviral medication for an overall value corresponding to USD 14 million. During the operation 121 people were arrested and more than 2500 websites were taken offline [1]. This is just the latest example of illegal trafficking of pharmaceutical products by criminal organisations, endangering lives worldwide with substandard, counterfeit and possibly toxic products, often misusing the Internet [2]. Illicit markets for counterfeit pharmaceuticals are attractive for counterfeiters, given their high profit margins, low risks of detection and prosecution, weak penalties, and the ease with which consumers can be deceived into believing that the counterfeit products are genuine [3]. To protect public health and to allow effective criminal investigation in the field, analytical tools are needed to a proper forensic characterisation of such products. The analysis is needed to detect possible toxic chemical substances, and to demonstrate the illegal nature of the product and support inferences about the source of seized material [4]. Drugs for erectile dysfunction (ED) are the most commonly counterfeited medicines in Europe and their active pharmaceutical ingredients are also found in dietary supplements [5-6]. US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) approved sildenafil (Viagra® by Pfizer) in 1998. It was the first oral pharmaceutical product to treat men with ED by inhibiting the type-5 phosphodiesterase (PDE_5) enzyme [7]. Forensic characterization of counterfeit Viagra® can be carried out by both image processing [8] and analytical techniques [9]. The analytical approaches tested include liquid chromatography-mass spectrometry (LC-MS) [10-15], nuclear magnetic resonance (NMR) [16-17], Fourier transform infrared spectroscopy (FTIR) [18] and Raman micro-spectroscopy [19]. Recently Romolo et al. have proposed a combined use of instrumental neutron activation analysis (INAA) and liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) to characterize sildenafil-based products seized on the Italian illegal market [20]. A similar approach has been proposed by Tandoh et al. to study amoxicillin drugs in Ghana, using both LC and particle-induced X-ray emission (PIXE) [21].

Besides PIXE, other accelerator-based techniques like secondary ion mass spectrometry using primary ions with energies in the range of several MeV (MeV-SIMS) [22] are important tools for quantitative and qualitative analysis of materials in the context of forensic sciences. PIXE and MeV-SIMS belongs to the group of tools called ion beam analysis (IBA). Despite IBA techniques require the use of MeV ion accelerators and ancilliary equipment, they provide important advantages over e.g. ICP-based techniques. Indeed, the power of IBA techniques resides in their intrinsic feature

of non-destructiveness, which preserves the samples for cross-checking procedures and allows several measurements of the same sample if needed. Moreover, these techniques require either little or no sample preparation at all and measurements are carried out in a relatively short time, usually of the order of a couple of minutes. Most PIXE setups can detect elements above Z = 11 with limits of detection (LOD) in the range of parts per million. On the other hand, MeV-SIMS detects compounds and agregates ejected from the samples under MeV ions bombardment, thus probing molecules present in the samples in a straightforward manner. Finally the IBA tools (MeV-SIMS and PIXE) allow both qualitative and quantitative analysis.

The aim of the present research was to further study and develop the approach based on INAA to characterise both the authentic and illegal pharmaceuticals containing sildenafil seized in Italy and to provide quantitative elemental data for forensic purposes. Moreover, it is proposed for the first time a combined approach based on IBA (both PIXE and MeV-SIMS) and INAA, allowing characterisation of both authentic and illegal pharmaceuticals containing sildenafil. The specific role of IBA and INAA in this research was to obtain information on the presence of both toxic elements and trace elements. The former elements are useful to be detected to protect public health while the latter elements allow inferring about the possible common origin of confiscated material. Statistical evaluation was also carried out to study the possibility of implementing an inter-laboratory classification system based on a shared database of INAA results.

Experimental

Chemicals and reagents

The PIXE system was calibrated with several NIST reference materials including apple leaves standard (NIST 1515) and Buffalo river sediments (NIST 8704) with recovery values varying from 2% for Cu up to 7% for Fe [23].

The MeV-SIMS spectrometer was calibrated with a thin film of polystyrene deposited over a silicon wafer grown in the <100> direction (C_8H_8/Si). The film was deposited through a standard spin coating procedure. Polystyrene was chosen because of its well know fragmentation and its prominent peak at around 91 Da corresponding to the C_7H_7 fragment. Finally, ethyl alcohol was used in order to dissolve all powder samples while silicon wafers grown in the <100> direction was used as substrate for the samples.

A standard reference material NIST 1577C (Bovine Liver) and a blank (empty sample capsule) have been prepared and analysed simultaneously with the samples as a part of internal quality control for verification of the whole INAA analysis protocol. Flux monitors containing 60 mg Zn foil have been used to determine the neutron flux during the irradiations for the short-lived radionuclides at the SBP facility. Each sample was irradiated together with one of these flux monitors. Flux monitors containing 1 mg Zn (homemade standard solution pipetted on a filter paper in a capsule) have been used to determine the neutron flux during the BP3 facility irradiations of the samples. Each sample was sandwiched between two flux monitors.

Samples analysed by IBA

Five illegal products containing sildenafil, four 50 mg Viagra® (Pfizer) tablets and a powder of pure sildenafil were submitted to PIXE analysis. The illegal products sold through the net and confiscated were called: Blue (one capsule), Cenforce (one tablet), Silagra (one tablet), Vigora (one tablet) and a supplement called "Golden Root" (one capsule containing sildenafil and thiosildenafil).

Samples analysed by INAA

Tablets of Viagra® 25 mg and Viagra® 100 mg were analysed by INAA in two different laboratories. In one of them also two illegal products containing sildenafil, named "Kamagra gold" (1) and "Cenforce" (2), sold through the net and confiscated were analysed.

IBA: PIXE and MeV-SIMS equipment, conditions and sample preparation

The PIXE and MeV-SIMS experiments were carried out at the Ion Implantation Laboratory of the Institute of Physics (Federal University of Rio Grande do Sul). A 3 megavolts Tandetron accelerator was used in all experiments. For the PIXE experiments, all samples in the form of tablets were fixed in the target holder for the irradiations without any extra handling. On the other hand, sildenafil powder was deposited over carbon tape and fixed in the target holder for the measurements. Other powdered samples including X-ray standards were homogenized with a mortar and pestle and finally pressed into 2 mm thick pellets. The target holder was positioned inside the reaction chamber using an electromechanical apparatus and monitored through CCD camera. The pressure inside the reaction chamber was of the order of 10^{-6} mbar. Two MeV protons irradiated all samples

during 1000 seconds with an average current of 0.5 nA. The X-rays induced in the samples through the proton bombardment were detected by a Si(Li) detector placed at 135° with respect to the proton beam. The energy resolution of the Si(Li) detector was 150 eV at 5.9 keV. The analysis of the X-ray spectra was carried out using the GUPIXWIN software developed at the University of Guelph (Canada) [24-26].

For the MeV-SIMS measurements, the pills and powder were diluted in ethyl alcohol in the proportion of 5 mg of material per 1 mL of ethyl alcohol. The solution was dropped on crystal silicon grown in the <100> direction. After drying, the samples were placed in the target holder inside the MeV-SIMS reaction chamber kept at a pressure of the order of 10^{-7} mbar. The samples were bombarded with 6 MeV Cu^{4+} ions during 300 seconds. Typical currents were about 10 nA. The aggregates ejected from the samples were directed to the Kore Technologies (UK) time-of-flight spectrometer. The time-of-flight was converted to mass through a proper calibration of the system using a thin polystyrene film deposited over crystal silicon.

INAA equipment, conditions and sample preparation

Neutron activation analysis (NAA) is an isotope specific analytical technique for the qualitative and quantitative measurement of chemical elements [27]. For Viagra® 25mg, the whole tablet (approx. 160 mg) has been analysed. This tablet fitted in the INAA sample capsule of 0.6 cc. For Kamagra Gold, Cenforce and Viagra® 100 mg a subsample of approx. 300 mg has been taken from a pulverised whole tablet for the analysis. This is the maximum that fits the INAA sample capsule. A whole tablet weighed between 500 mg – 630 mg. So about a half tablet has been subsampled and used for analysis.

The irradiation was performed in 2 different pneumatic irradiation facilities of the Hoger Onderwijs Reactor of the Interfaculty Reactor Institute. The SBP irradiation facility for short-lived radionuclides and the BP3 irradiation facility for the mid-lived and long-lived radionuclides. The irradiation end of both facilities are located aside the reactor core in the water reflector.

The Thermal neutron flux in the SBP facility is of the order of 1.5×10^{13} $cm^{-2}s^{-1}$ and an irradiation time of 30 s was applied. After about 15 minutes decay time, the gamma-ray spectra of the irradiated samples were measured during 3 minutes on a coaxial Ortec Ge(Li) detector, absolute photopeak efficiency 2.1×10^{-3} for the 1332 keV photopeak of Co-60, in a horizontal dipstick configuration. Dead time during measurement was corrected with the pulser method. The dead times were 25.1% or lower for the samples.

Thermal neutron flux in the BP3 facility is of the order of 5 x 10^{12} $cm^{-2}s^{-1}$ and an irradiation time of 1 hour was applied. After about 3 days decay time, the gamma-ray spectra of the irradiated samples were measured during 1 hour on a coaxial Ortec Ge(Li) detector, absolute photopeak efficiency 6.3 x 10^{-3} for the 1332 keV photopeak of Co-60, in a vertical dipstick configuration. Dead time during measurement was corrected with the pulser method. The dead times were 19% or lower for the samples.

After about 3 weeks decay time, the gamma-ray spectra of the irradiated samples were measured again during 1 hour in a well-type Canberra Ge(Li) detector, absolute photopeak efficiency 4.2 x 10^{-2} for the 1332 keV photopeak of Co-60. Dead time during measurement was corrected with the pulser method. The dead times were 6.3% or lower for the samples.

The INAA software from the Reactor Institute Delft was used for gamma-ray spectrum analysis and interpretation.

Authentic Viagra® tablets were also analysed in the nuclear analytical laboratory of the International Centre for Environmental and Nuclear Sciences (ICENS) using the JM-1 SLOWPOKE-2 research reactor. For the Viagra® 25 mg tablets, samples were pulverized and then pelletized in Whatman® Grade 41, Ashless Filter Paper after accurately weighing out an approximate mass of 150 mg. The pellet was sealed in acid washed polyethylene bags and then encapsulated in an acid washed Polyvials™ EP290 NAA neutron activation analysis small lab vial prior to irradiation. For Viagra® 100 mg tablets the same sample preparation and packaging procedure was followed. Blanks were also prepared to correct for the possible contribution from the filter paper and irradiation vials. In addition to this protocol, a sample of Viagra® 100 mg was pulverised and a mass of approximately 1.2 g accurately weighed out into an acid-washed Polyvials™ EP338 NAA neutron activation analysis small lab vial and then heat sealed before being encapsulated in an acid-washed Polyvials™ EP290 NAA neutron activation analysis small lab vial. This sample was only irradiated for intermediate and long-lived radionuclides. All samples were irradiated in the inner sites of the JM-1 SLOWPOKE-2 reactor located in the annular beryllium reflector. For irradiation and analysis of short-lived radionuclides both Viagra® 25 and 100 mg samples were irradiated at a thermal neutron flux of 5 X 10^{11} $cm^{-2}s^{-1}$ for 3 minutes. After a decay period of approximately 10 minutes samples were measured for 5 minutes and then measured again after a decay period of approximately 60 minutes samples for 10 minutes. During measurement samples had dead times of less than 5%.

For intermediate and long-lived radionuclides, the samples were irradiated for 8 hours at a thermal neutron flux of 1 X 10^{12} $cm^{-2}s^{-1}$. The samples underwent a decay time of 4 days before being measured for approximately 1 hour and then a decay period of about 2 weeks and measured for 4

hours. The additional sample of Viagra® 100 mg was irradiated for 4 hours at a thermal neutron flux of 1 X 10^{12} $cm^{-2}s^{-1}$. The decay period and measurement times remained the same. During measurement samples had dead times of less than 10%. All samples were measured on an Ortec high purity germanium (HPGe) detector with a relative photopeak efficiency of 71% and a resolution of 1.9 keV at the Co-60-line of 1332 keV, in a vertical dipstick configuration. Analysis and quantification of the gamma-ray spectrum was carried out using in-house INAA software.

Statistical evaluation

ANOVA One Way and Tukey's Post hoc test (5%) statistical analyses were carried out in order to check whether there are differences in elemental concentrations among the tablets. Microcal Origin 9 Professional software was employed for the statistical evaluation of the data.

INAA data were used for further investigating the selectivity of the chemical profiles and the possibility to discriminate between Viagra® and illegal pills. In this regard, principal component analysis (PCA) has initially been carried out on centred and scaled data for overall data mining. Then a random forest (RF) model has been applied, in order to infer suitable classification rules. For this, the number of trees to grow in the forest (ntree) has been set to 1000 and the number of input variables randomly sampled as candidates at each nodes (mtry) has been set to 2. Trees generated through this procedure have finally been inspected to manually find the most accurate classification model. All calculations were carried out with R statistical software. PCA and RF analysis were performed using the "stats" and "party" packages, respectively.

Results

IBA: PIXE and MeV-SIMS results

PIXE analysis of original 50 mg Viagra® (Pfizer) tablets gave peaks of Na, Mg, Si, P, S, Ca, Ti, Fe, Ni and Zn. One PIXE spectrum obtained during the research is reported in Figure 1. The sulphur peak is explained by the presence of S in the molecular formula of sildenafil: $C_{22}H_{30}N_6O_4S$.

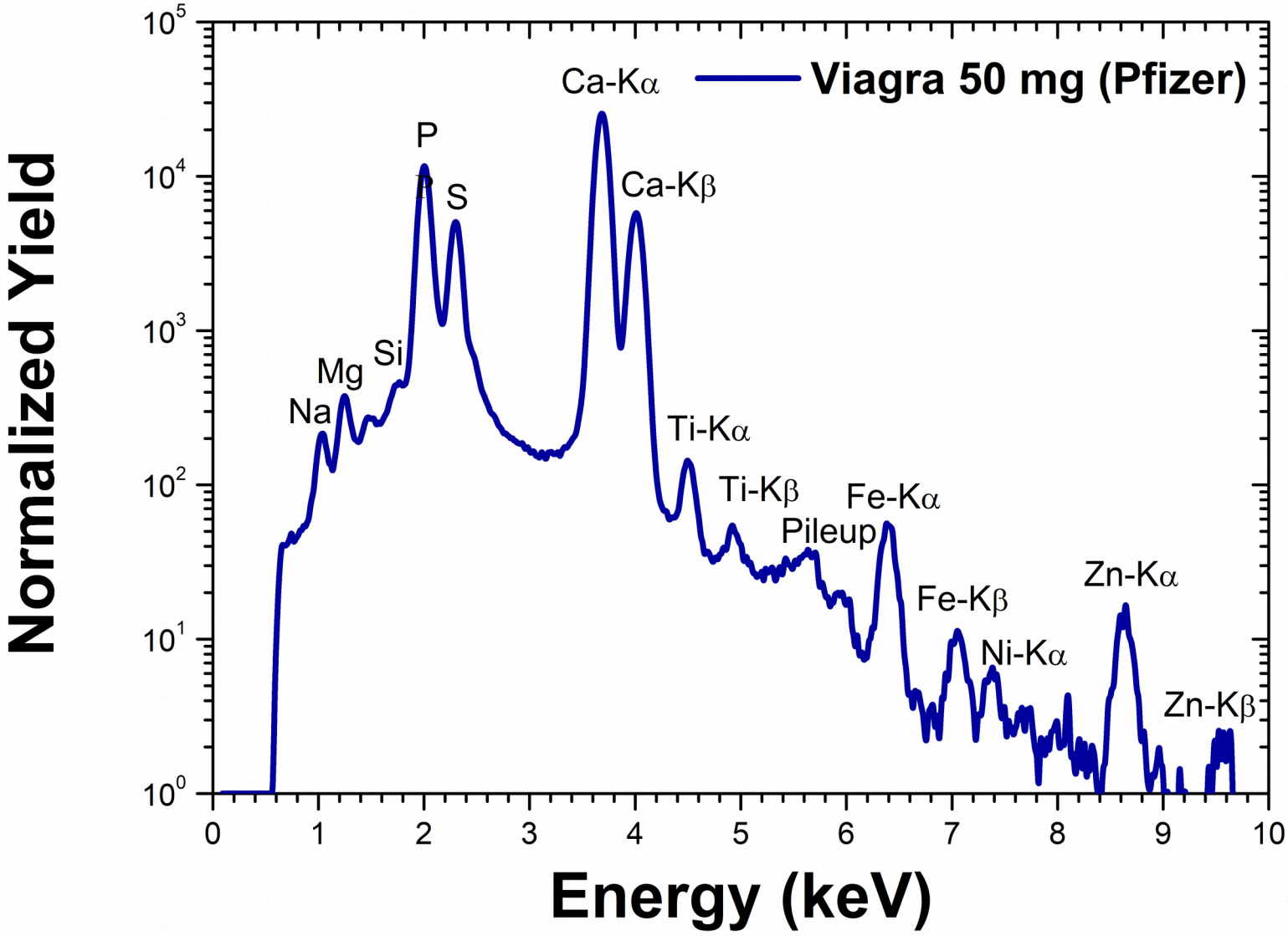
Normalized Yield
Energy (keV)
Viagra 50 mg (Pfizer)
Na
Mg
Si
P
S
Ca-Kα
Ca-Kβ
Ti-Kα
Ti-Kβ
Pileup
Fe-Kα
Fe-Kβ
Ni-Kα
Zn-Kα
Zn-Kβ
10^0
10^1
10^2
10^3
10^4
10^5
0
1
2
3
4
5
6
7
8
9
10

Figure 1. X-ray yield as a function of the X-ray energy obtained for one original 50 mg Viagra® (Pfizer) tablet. The spectrum was normalized by the charge accumulated during the experiment.

The PIXE spectra obtained during the research from some pure sildenafil and from illegal products containing sildenafil gave peaks of Na, Mg, Si, P, S, Cl, K, Ca, Ti, Mn, Fe and Zn. PIXE spectra are reported in Figure 2.

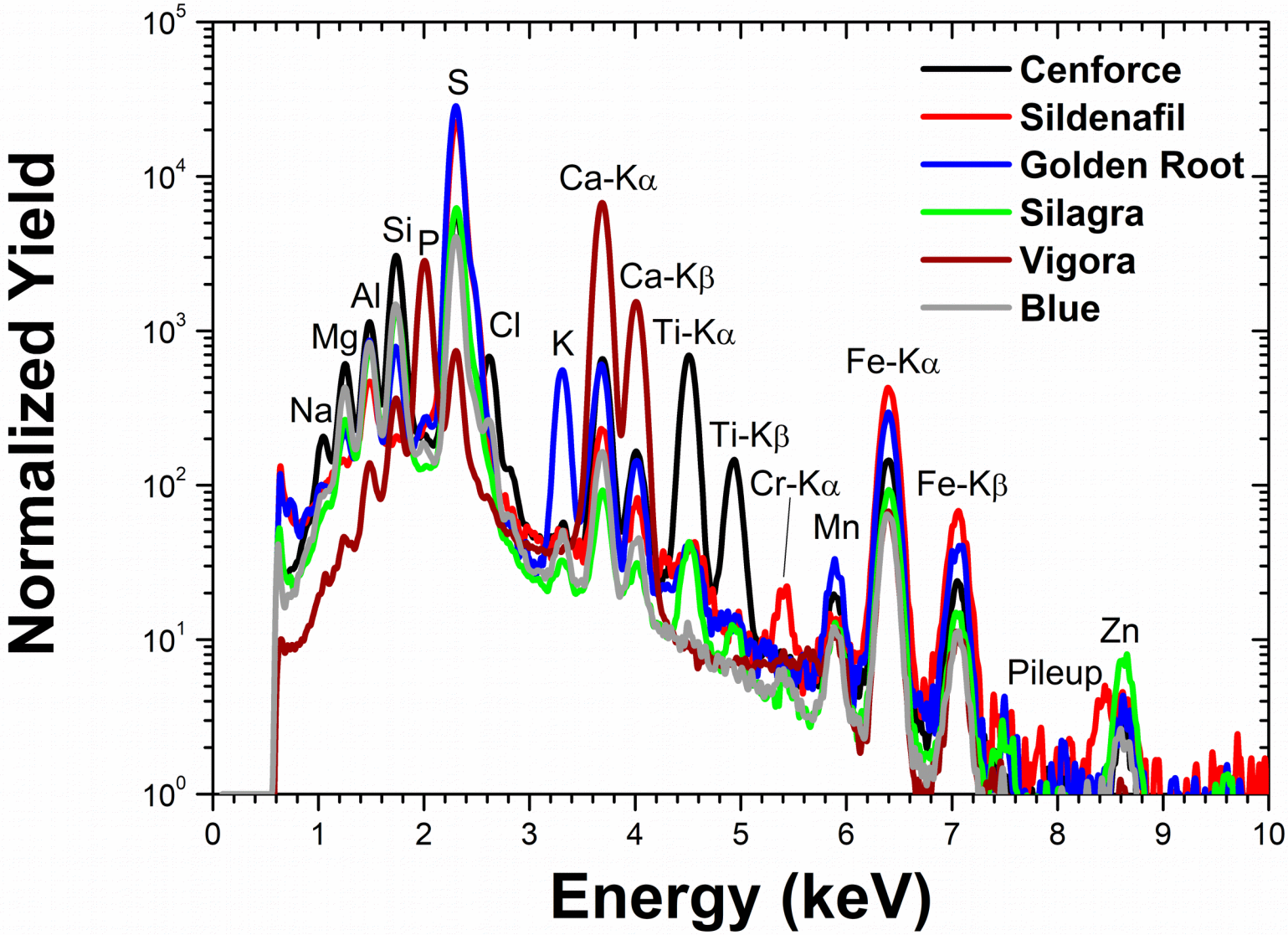
Normalized Yield
Energy (keV)
Cenforce
Sildenafil
Golden Root
Silagra
Vigora
Blue
Na
Mg
Al
Si
P
S
Cl
K
Ca-Kα
Ca-Kβ
Ti-Kα
Ti-Kβ
Cr-Kα
Mn
Fe-Kα
Fe-Kβ
Pileup
Zn

Figure 2. X-ray yield as a function of the X-ray energy obtained from some pure sildenafil and from illegal products containing sildenafil. The spectra were normalized by the charge accumulated during the experiments.

Table 1 reports the analytical findings from both authentic Viagra®, pure sildenafil and illegal sildenafil-based products related to the following elements Na, Mg, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Zn, and Br. For authentic Viagra® the following 11 elements were identified and quantified: Na, Mg, Si, P, S, Ca, Ti, Mn, Fe, Zn, and Br. For illegal sildenafil-based products the following 13 elements were identified and quantified in at least one specimen: Na, Mg, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Zn, and Br. The superscript letters (a-e) in Table 1 indicate within each row that the elemental concentrations are statistically the same ($p < 0.05$).

Secondary ion mass spectrometry (MeV-SIMS) spectra obtained during the research are reported in Figure 3. They clearly show a peak close to the m/z of the protonated sildenafil molecule (molecular weight: 474.6 g/mol). In reference 20 the sildenafil exact mass $[M+H]^+ = 475.2122$ is reported.

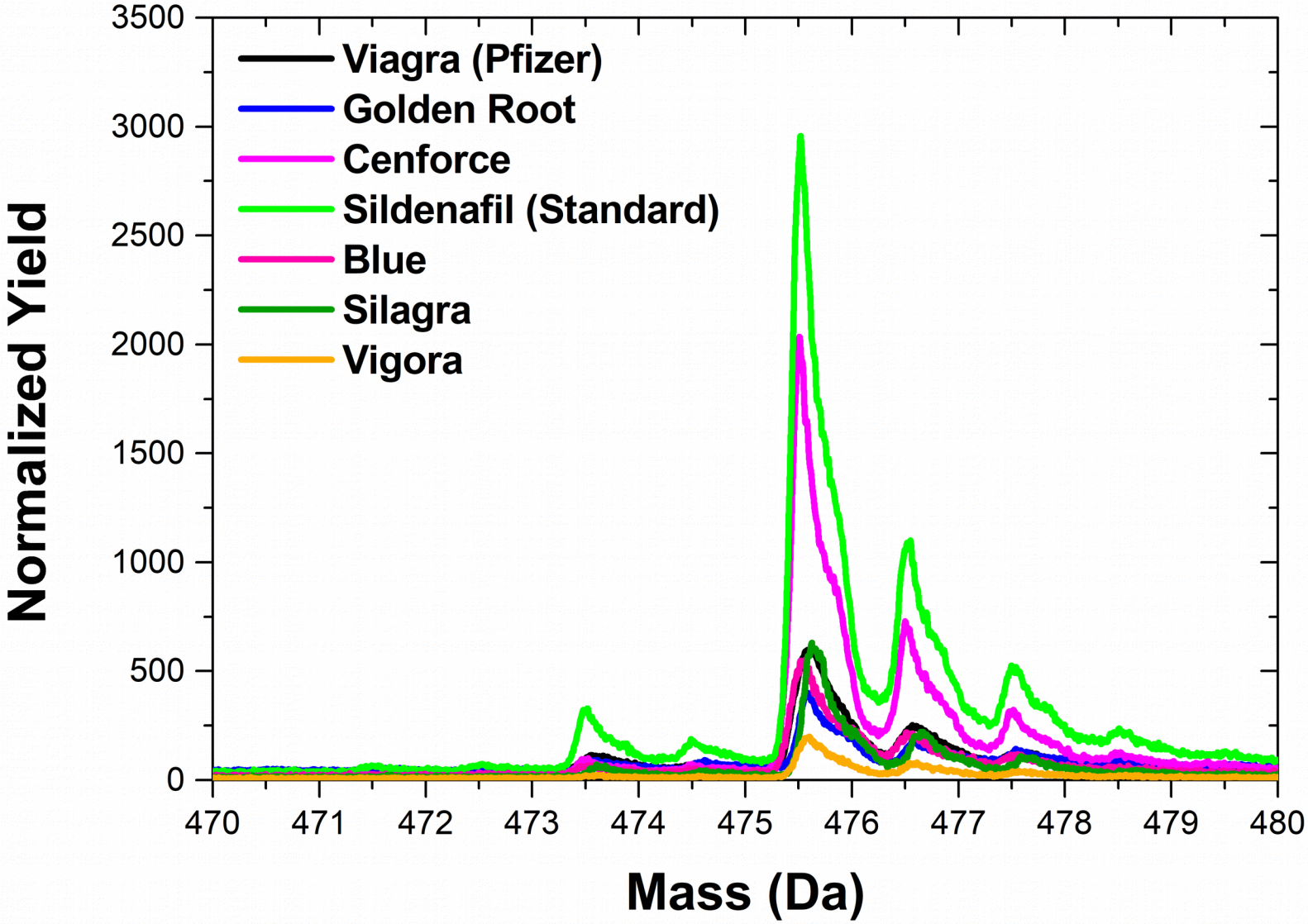
Normalized Yield
Mass (Da)
Viagra (Pfizer)
Golden Root
Cenforce
Sildenafil (Standard)
Blue
Silagra
Vigora
0
500
1000
1500
2000
2500
3000
3500
470
471
472
473
474
475
476
477
478
479
480

Figure 3. MeV-SIMS spectra obtained after analysis of original Viagra® (Pfizer) and illegal products containing sildenafil. The yields were normalized by the charge accumulated during the experiments. See text for further explanation.

INAA results

Table 2 shows the INAA results obtained in the Reactor Institute of the University of Technology Delft (RID) and the International Centre for Environmental and Nuclear Sciences (ICENS). The following 19 elements were identified and quantified: Na, Mg, Al, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Zn, Br, Sb, La, Sm, Hf, and Ta.

Statistical evaluation

The primary aim of forensic analysis when applied to the investigation of pharmaceutical products is that to provide evidence to help in-charge law enforcement agencies to determine whether a new questioned sample submitted to analysis is original or counterfeit. This procedure can largely be seen as a classification problem that, consequently, relies on some fundamental assumptions. The most important of them are (1) that the different classes (i.e., "original" and "counterfeit") are actually characterised by differentiable elemental concentrations (high inter-variability criterion), but also (2) that these elemental concentrations are sufficiently reproducible within the same class (low intra-variability criterion). For inter-laboratory applications, as the one investigated here, the systematic biases between the different laboratories are a major factor affecting the intra-variability of elemental concentrations (in this case, the observed counts for elements) and highly correlated measures are preferred.

For IBA results, ANOVA analysis was carried out, mainly in order to study inter-variability between data. This allowed finding statistically significant differences between the samples ($p < 0.05$) that may be helpful for the purpose of forensic applications. For each element (row), observed groups are represented with subscript letter in Table 1. It is, however, also evident from these results that some samples presented not statistically differentiable measures. A more in-depth analysis was carried out on the INAA results, as these data allowed a more meaningful comparison thanks to the measures taken on Viagra® by different laboratories. In Figure 4, an inter-laboratory comparison for the measured concentration of the 5 main elements (Na, Ca, Br, La and Cl) is presented. It was thus found that results provided by the Dutch and Jamaican

laboratories were very consistent. Considered that no inter-laboratory validation was performed before this study, this was considered a noteworthy achievement and very promising for future implementations in forensic analysis. Related RSDs were also relatively low (< 20 %) for most of the elements (except Br).

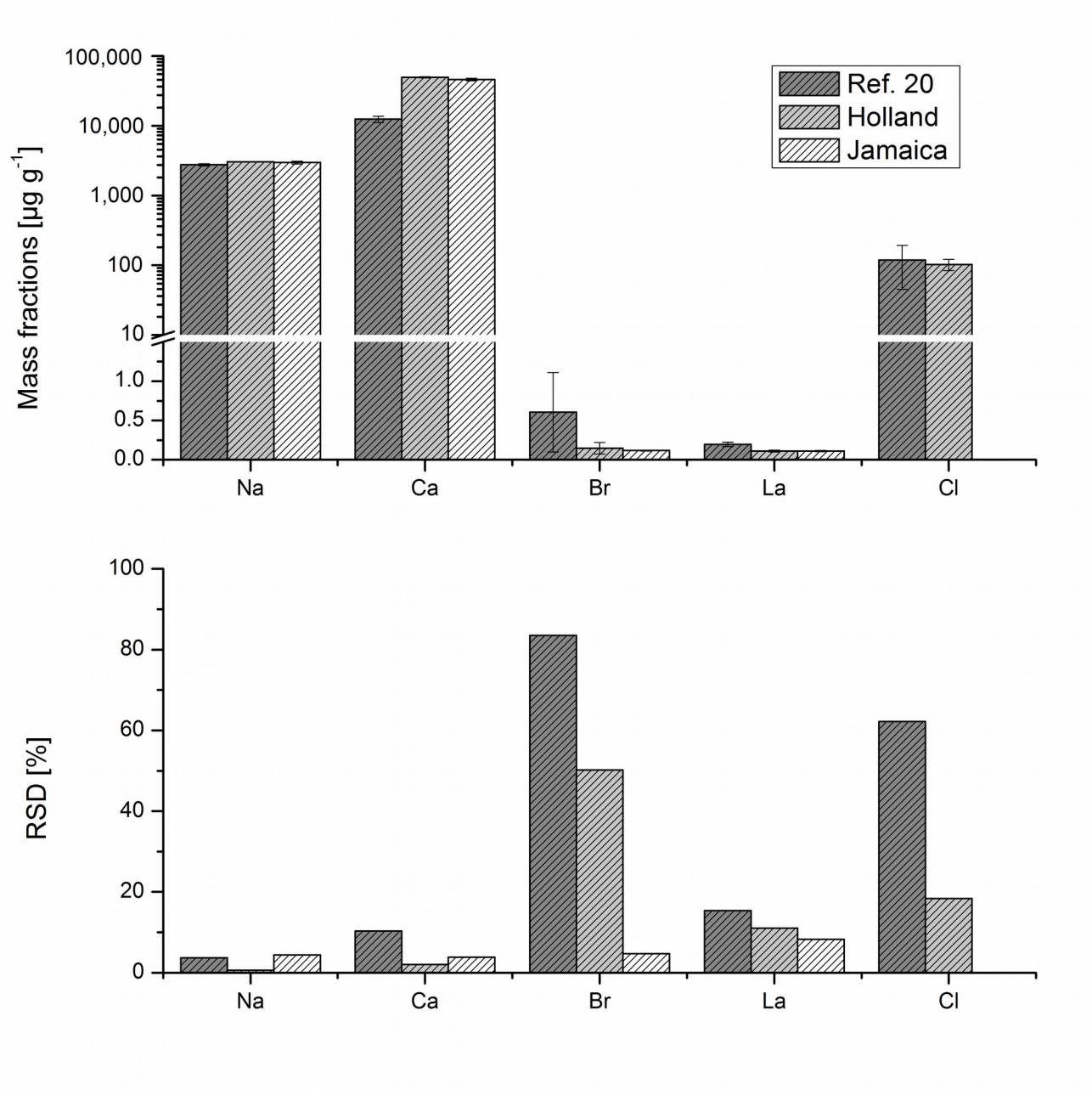
Mass fractions [µg g-1]
100,000
10,000
1,000
100
10
1.0
0.5
0.0
Ref. 20
Holland
Jamaica
Na
Ca
Br
La
Cl
RSD [%]
100
80
60
40
20
0
Na
Ca
Br
La
Cl

Figure 4. Inter-laboratory comparison of the observed elemental concentrations and related measurement RSDs for the main five elements in tablets from [20] the Reactor Institute of the University of Technology Delft (RID) and the International Centre for Environmental and Nuclear Sciences (ICENS).

An unsupervised approach, i.e. principal component analysis (PCA) was applied to INAA data, in order to carry out initial data mining and further investigate class intra- and inter-variability. Again, only the 5 main elements (Na, Ca, Br, La and Cl) were considered as they were the only one measured across all the labs. The related score plot is shown in Figure 5. It could thus be observed that all Viagra® measurements showed a visible and compact cluster (yellow ellipsis), which was not superposed to any other measurements taken on the counterfeit pills (blue points). This proved that Viagra® tablets were actually characterised by relatively selective profiles compared to counterfeit pills and were likely composed by discriminant elemental concentrations that could be exploited for the purpose of classification. An analysis of the PCA loadings (arrows) confirmed this conclusion and, especially, that Viagra® was generally characterised by high concentrations of Ca, and low concentrations of Br and Cl.

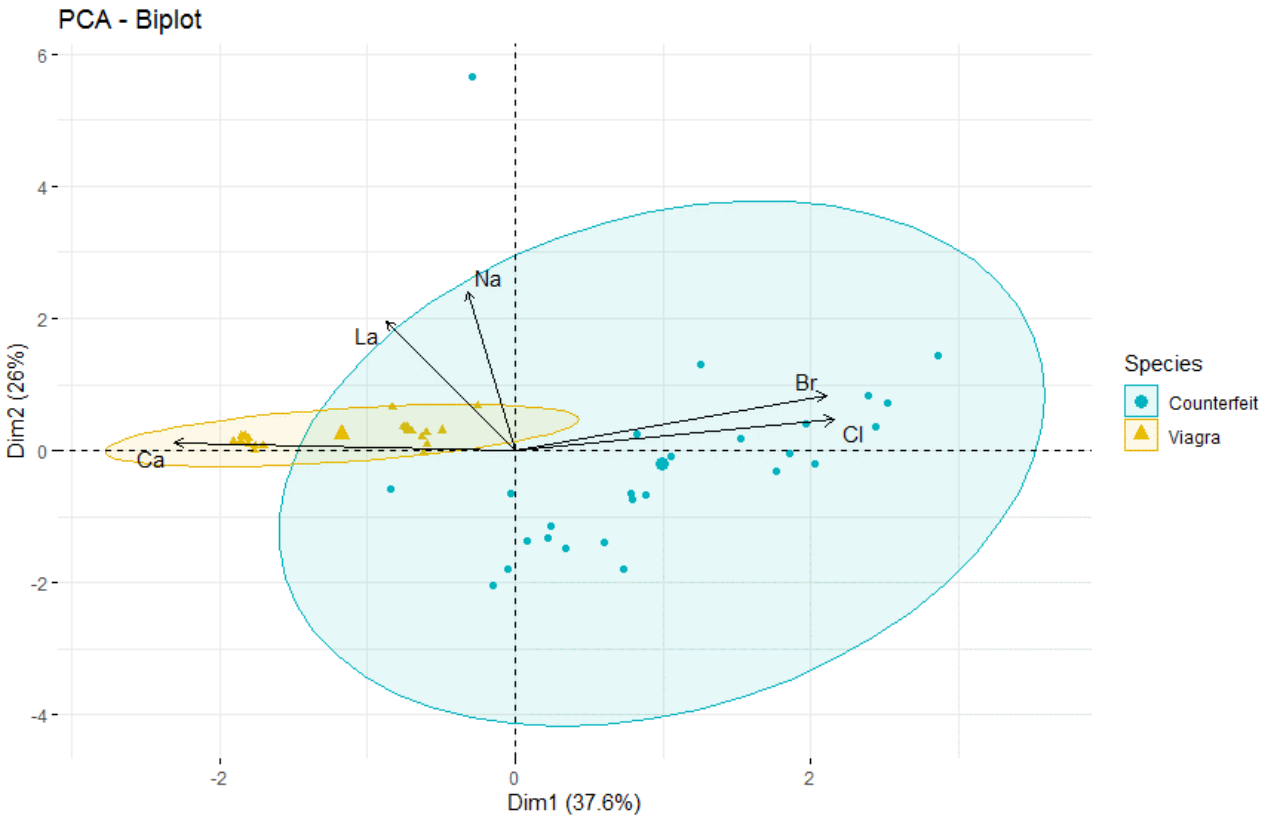

Figure 5. Score plot of the first two principal components after PCA analysis of the INAA data. Arrows represent the PCA loadings. It can be noticed that the Viagra® measurement cluster (yellow ellipsis) doesn't superpose to any counterfeit measurement (blue points), but the inverse is true.

The data showed selective features. This was good from the point of the view of their discrimination and implementation of an inter-laboratory classification approach for forensic applications, i.e. a decision-making approach that is meant to help forensic scientists in different laboratories to classify a newly analysed sample as being original Viagra® or a counterfeit alternative. However, this is unlikely to be done using simple, linear classifiers, due to the really variable composition of the counterfeit pills. This is evident in the PCA score plot from the big confidence interval of

counterfeit data (blue ellipsis), which partially overlap with Viagra® data (despite no blue point is in the confidence interval of the Viagra® measurements). As a consequence, a non-linear classifier would potentially be more suitable for the purpose and should at least be considered in future applications. It is also evident from the PCA loadings that La and Na, as well Br and Cl, were strongly correlated between them (i.e., collinear), and they could thus provide redundant information. A further feature selection could also be done.

No systematic analysis of classification performances has been carried out here, due the low number of data that would not allow a robust validation of any trained model. However, as a preliminary investigation for the implementation of an inter-laboratory classification approach, a simple multi-boundary method was tested through the application of a supervised tree-based model (i.e., random forest, RF), which is an intrinsically non-linear classifier. Really promising results were obtained. Indeed, a series of "rules" could be inferred from data, allowing the observation of a perfect discrimination between all the INAA analysis collected during this work (see tree model in Figure 6). These are summarised through the following set of decisional conditions:

1. if Na $\leq$2,120 μg g^{-1}: the product is likely a counterfeit Viagra® tablet,
2. if Na > 2,120 μg g^{-1} and Ca $\leq$ 5,000 μg g^{-1}: the product is likely a counterfeit Viagra® tablet,
3. if Na > 2,120 μg g^{-1} and Ca > 5,000 μg g^{-1}: the product is likely an original Viagra® tablet.

Further validation is needed to assess if this approach would be generalisable in forensic casework and what would be the associated hit/error rates, but it seems a viable and simple solution.

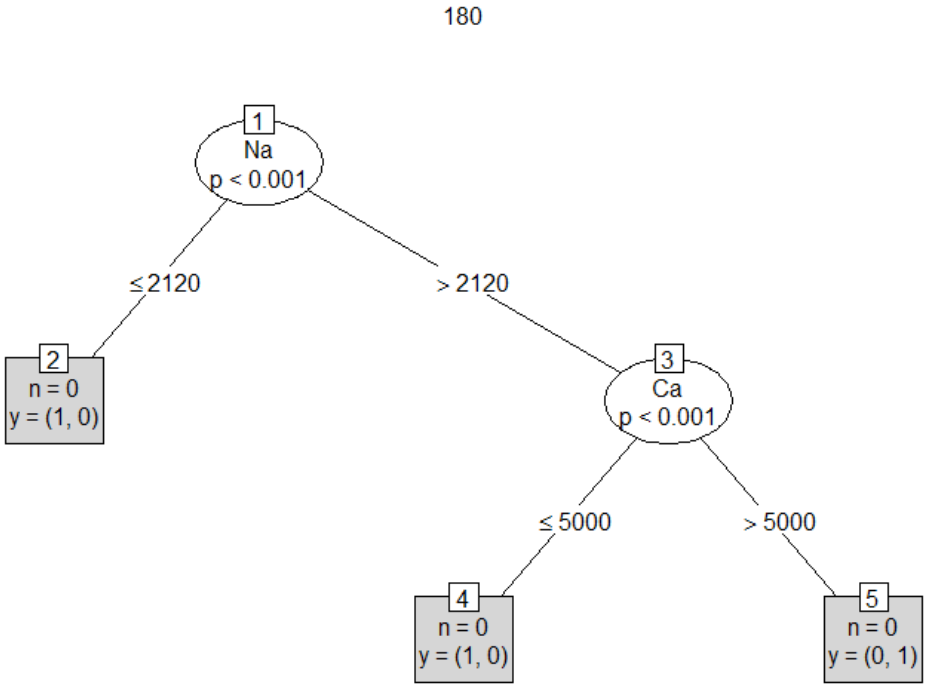

Figure 6. Best classification rules for the INAA dataset to achieve discrimination between Viagra® and counterfeit classes obtained after analysis by random forest. See the text for a summary of the decisional conditions extracted from this graphs.

Discussion

IBA techniques demonstrated to provide both elemental profiles of tablets and mass spectrometric analysis of the sildenafil protonated molecule with no sample preparation. From the qualitative point of view, it is interesting to notice that PIXE easily provides in the spectra the peak produced by S, element not detected by INAA in the experimental setting reported in reference 20. The spectrum in Figure 1, showing the X-ray results from a 50 mg Viagra® tablet, is dominated by the presence of Ca, P and S. On the other hand, common elements like Cl and K are completely absent from

this spectrum. In Figure 2 it is possible to identify different profiles for each particular product. For instance, Cenforce is characterized by the presence of Mg, Al, Si, Cl and Ti while Blue is completely depleted of Ti. Moreover, sildenafil standard and Golden Root have S as highest peak while Vigora has Ca as highest peak and no K in its spectrum.

A better characterisation of samples is based on the elemental concentrations shown in Table 1. The first striking feature displayed by this table is that the elemental concentrations are not uniform across the products. In particular, it is interesting to compare Viagra® with sildenafil standard. In Viagra® tablets there is a mixture of sildenafil and other excipients and therefore there are several other elements besides the ones in sildenafil formula ($C_{22}H_{30}N_6O_4S$) such as P and Ca. However, the presence of other elements like Ca and Fe might be due to the interaction of the proton beam with the carbon tape used as a substrate to analyse the sildenafil powder sample.

Another interesting result concerns the relatively large concentration of S present in all samples. This fact could be an indicative that these products are indeed based on sildenafil, since this molecule bears one atom of sulphur. However, there is no direct correlation of the amount of S and the presence of sildenafil since this element could belong to another chemical compound in the sample. MeV-SIMS experiments allowed further characterisation, showing a clear peak around 474.5 Da, which corresponds to the molecular mass of protonated sildenafil (see Figure 3). Moreover, several protonated fragments showed up, further confirming the presence of sildenafil in the analysed products. It is important to mention that the MeV-SIMS system used in the present work was tested with several organic and inorganic standards, including sildenafil. The peak at 474.5 Da showed up only for the sildenafil standard and the sildenafil-based products and did not show up with blank samples.

If we compare IBA performance with INAA, the first comment is about sample preparation and representativeness. If ion beam analyses are carried out on tablets without sample preparation, INAA sample preparation is extremely simple: for Kamagra Gold, Cenforce and Viagra® 100 mg a whole tablet can be divided in 2 subsamples of approx. 300 mg so that the whole tablet can be analysed. IBA results could be theoretically affected by surface contamination of items (a reasonable hypothesis when dealing with illegal products), INAA avoids representative sub-sampling and sample preparation difficulties, providing results from the bulk of the material. IBA is perfectly suitable for discriminating between authentic Viagra® and illegal sildenafil-based products and surface contaminants makes this job easier but when collecting information for chemical profiling, the INAA bulk approach is definitely an added value for forensic analysis, as expected based on already published research [28].

It is also very interesting that INAA results from two different reactors in two different countries showed a very good agreement. The statistical evaluation not only supported to visualise the discrimination between authentic Viagra® and illegal sildenafil-based products but open the door to the development of a database very useful to fight illegal trades thanks to chemical profiling, based on further research in the field.

Conclusion

Both IBA and INAA are effective to discriminate between authentic Viagra® and illegal sildenafil-based products.

Concerning ion-based techniques, PIXE and MeV-SIMS are non-destructive. Therefore, they may play an important role in forensics when preserving the integrity of the samples is mandatory. PIXE requires no sample preparation and can identify suitable markers for different products in analysis of a few minutes only. The drawback of this technique is that it is sensitive to elements only. In this case, MeV-SIMS can provide molecular information of the samples under study, providing evidence of the compounds making up the sample with minimum sample handling. Therefore, it has been shown that a combination of PIXE and MeV-SIMS is a powerful tool to discriminate different products in a straightforward manner.

Results of analysis by INAA of authentic Viagra® and of sildenafil-based illegal products were already reported but this is the first time that the INAA approach is compared with the IBA approach. It is also the first time that INAA results obtained in two different reactors on the same samples are compared. The data reported show accurate elemental quantification, showing a great potential for the future implementation of an inter-laboratory classification system.

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Figure Captions

Figure 1. X-ray yield as a function of the X-ray energy obtained for one original 50 mg Viagra® (Pfizer) tablet. The spectrum was normalized by the charge accumulated during the experiment.

Figure 2. X-ray yield as a function of the X-ray energy obtained from some pure sildenafil and from illegal products containing sildenafil. The spectra were normalized by the charge accumulated during the experiments.

Figure 3. MeV-SIMS spectra obtained after analysis of original Viagra® (Pfizer) and illegal products containing sildenafil. The yields were normalized by the charge accumulated during the experiments. See text for further explanation.

Figure 4. Inter-laboratory comparison of the observed elemental concentrations and related measurement RSDs for the main five elements in tablets from [20] the Reactor Institute of the University of Technology Delft (RID) and the International Centre for Environmental and Nuclear Sciences (ICENS).

Figure 5. Score plot of the first two principal components after PCA analysis of the INAA data. Arrows represent the PCA loadings. It can be noticed that the Viagra® measurement cluster (yellow ellipsis) doesn't superpose to any counterfeit measurement (blue points), but the inverse is true.

Figure 6. Best classification rules for the INAA dataset to achieve discrimination between Viagra® and counterfeit classes obtained after analysis by random forest. See the text for a summary of the decisional conditions extracted from this graphs.