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## Research Article

### **Ion beam analysis (IBA) and instrumental neutron activation analysis (INAA) for forensic characterisation of sildenafil based products**

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## Abstract

The diffusion of illegal medicinal products on internet is a serious criminal problem threatening public health worldwide.

Drugs for erectile dysfunction such as phosphodiesterase type 5 inhibitor are the most commonly counterfeited medicines in Europe. The search of possible toxic chemical substances in seized products is needed. Moreover, the profiling of the material can be the source of relevant forensic information. For the first time a combined approach based on liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) and instrumental neutron activation analysis (INAA) is proposed and tested, allowing characterisation of both authentic and illegal pharmaceuticals containing sildenafil seized in Italy. LC-HRMS allowed the detection and identification of unknown impurities not reported on labels of illegal products and the quantitation of the sildenafil. INAA showed to be suitable to provide both qualitative and quantitative information for forensic purposes on 23 elements, allowing discrimination between legal and illegal products.

## Keywords

**Illegal pharmaceutical products, neutron activation analysis, ion beam analysis, forensic analysis, sildenafil.**

## Introduction

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Criminals have found many opportunities to misuse the Internet, including the illegal trafficking of pharmaceutical products endangering lives worldwide with substandard, counterfeit and possibly toxic products [1-3]. 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, aiming 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 principles 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<sub>5</sub>) 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 transformed 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 ion-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). These analytical approach is non-destructive, thus preserving the original samples for further analysis if needed. On one hand, most PIXE setups can detect elements above  $Z = 11$  with limits of detection in the range of parts per million. On the other hand, MeV-SIMS detects compounds and aggregates ejected from the samples under MeV ions bombardment, thus probing molecules present in the samples in a straightforward manner. Finally the IBA tools allow quantitative analysis of elements analysed by PIXE.

The aim of the present research was to further study and develop the approach based on INAA to characterize both the authentic and illegal pharmaceuticals containing sildenafil seized in Italy and to provide quantitative elemental data for forensic purposes. The specific role of IBA and INAA and 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.

## Experimental

### Neutron activation analysis

Neutron activation analysis (NAA) is an isotope specific analytical technique for the qualitative and quantitative measurement of chemical elements. The method is based upon the conversion of stable atomic nuclei into radioactive nuclei by irradiation with neutrons and the subsequent detection of the radiation emitted during the decay of these radioactive nuclei. All of the stable elements have properties suitable for production of radioactive isotopes albeit at strongly different reaction rates. Each radionuclide is uniquely characterized by its decay constant—the probability for the nuclear decay in unit time- and the type and energy of the emitted radiation. Amongst the several types of radiation that can be emitted, gamma-radiation offers the best characteristics for the selective and simultaneous detection of radionuclides and thus of elements.

Bode, Peter. (2017). Neutron Activation Analysis (NAA). 10.1007/978-3-319-33163-8\_10.

### Chemicals and reagents

A standard reference material NIST 1577C (Bovine Liver) and a blank (empty sample capsule) have been similarly prepared and analysed simultaneously with the samples. Both as a part of internal quality control for verification of the whole analysis protocol.

Flux monitors containing 60 mg Zn foil have been used to determine the neutron flux during the SBP facility irradiations of the samples. 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

4 Samples of illegal products containing sildenafil sold through the net ... confiscated by Carabinieri.

(1) Kamagra Gold, (2) Cenforce, (3) Viagra 100mg and (4) Viagra 25mg.

3 tablets of each product have been analysed.

figures 1-

### Sample preparation

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 100mg 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.

### Sample irradiation and measurements

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  $1.5 \times 10^{17} \text{ s}^{-1}\text{m}^{-2}$  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  $2.1 \times 10^{-3}$  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  $5 \times 10^{16} \text{ s}^{-1}\text{m}^{-2}$  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  $6.3 \times 10^{-3}$  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  $4.2 \times 10^{-2}$  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.

### Spectrum analysis and interpretation

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

## **Results**

### **Discussion**

For Kamagra Gold, Cenforce and Viagra 100mg a whole tablet can be divided in 2 subsamples of approx. 300 mg so that the whole tablet can be analysed. This to avoid the representability question of a subsample taken from a whole tablet.

## **Conclusion**

## Aknowledgements

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**Figure Captions**