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Quantitative and qualitative analysis of polycyclic aromatic hydrocarbons in urine samples using a non-separative method based on mass spectrometry

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ABSTRACT

In this work, a method for the quantitative and qualitative analysis of 11 polycyclic aromatic hydrocarbons (PAHs) in urine samples is reported. The method is based on the coupling of a programmed temperature vaporizer (PTV) with a quadrupole mass spectrometer (qMS), via a deactivated fused silica tubing. Before the PTV-qMS analysis, the samples were subjected to a liquid-liquid extraction (LLE).

The method was rapid since no chromatographic separation was performed. The samples were introduced directly into the PTV, and the analytes were trapped in the Tenax-TA® packed liner while the solvent was purged. After that, all the compounds reached the mass spectrometer, obtaining the fingerprint of the analysed samples.

Urine samples free of PAHs and the same samples spiked with the compounds were analysed. The resulting profile signals were used to quantify the analytes using multivariate calibration, and to classify the samples according to the presence or absence of the PAHs. In the latter task, non-supervised and supervised pattern recognition techniques were employed. The calibration models worked satisfactorily and errors lower or equal to 15% were obtained, in most cases, when an external validation set was analysed. Regarding the classification of the samples, most of the supervised pattern recognition techniques provided excellent results (100% success), where all of the samples were classified correctly.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that consist of, at least, two fused aromatic rings. These ubiquitous contaminants are released into the atmosphere by incomplete combustion from both natural (forest fires, volcanic eruptions) and anthropogenic (vehicle emissions, cigarette smoke, cooking) sources. Since these processes are present in many industries, PAHs have been considered as exposure markers where higher levels of these compounds can be detected, for example, in different types of workers such as coke oven workers [1–5], firefighters [6,7], aluminium workers [8], and those workers exposed to diesel exhaust [9]. These compounds have also been detected depending on diet [10,11] and smoking habits [12].

Several PAHs have been classified by the International Agency for Research on Cancer (IARC) as possible or probable human carcinogens [13], raising great health concerns all over the world. For this reason, many studies are aimed at associating the risk of cancer [14,15] and the presence of other adverse health effects [16–18] with the concentration of PAHs found in people exposed to these compounds. In addition, the European Commission has established maximum levels for PAHs in several matrixes, for instance in food [19] and primary smoke products [20]. The maximum levels permitted are in the range of μg kg^{-1}.

Once PAHs have entered the human body by the inhalation of contaminated air, ingestion or dermal absorption, they can be subjected to successive metabolic biotransformations, including oxidation, hydroxylation and hydration, and generate derivatives of the corresponding PAHs. This is why most studies report the simultaneous quantification of hydroxylated metabolites [2,4–7,10,12]. However, the determination of unmetabolized PAHs is less explored. Very few applications have been found in the literature for determining unmetabolized parent compounds in urine [1,3,4,8,9,11]. The concentrations of PAHs for people exposed to these analytes found in literature have been reported to be mostly in the range of μg L^{-1} [1,3,4,21–24]. In addition, the analysis of PAHs has been performed in other matrices during the last few years, including hair [25], blood and plasma [26], edible vegetable oil [27], water [28], smoked fish [29], milk [30] and gasoline [31].

Because these compounds are present at trace concentrations, they must be extracted from the matrix and preconcentrated before analysis.

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The issue of extracting PAHs from urine has been approached by using headspace-solid phase microextraction (HS-SPME) [1,4,9,21–23], dispersive liquid-liquid microextraction (DLLME) [32], solid phase microextraction (SPME) [3] and solid phase extraction (SPE) [8,24,33].

A number of analytical methods, including those based on gas chromatography-mass spectrometry (GC-MS) [1,3,4,9,22–24,33], gas chromatography-flame ionization detection (GC-FID) [21,32] and high performance liquid chromatography-fluorescence detection (HPLC-F) [8], have been developed to analyse PAHs in urine samples.

An interesting alternative that has not been particularly explored, to date, is the use of mass spectrometry detection without chromatographic separation for the analysis of PAHs in urine samples. Some examples of this approach include the analysis of the hydroxylated metabolites of polycyclic aromatic hydrocarbons using solid phase extraction-electrospray ionization tandem mass spectrometry (SPE-ESI-MS/MS) [34] and solid phase microextraction-glass capillary nanoelectrospray ionization with a hybrid triple quadrupole/linear ion trap mass spectrometer (SPME-nanoESI-MS) [35]. Although good results have been obtained with these techniques, they are expensive and not available in all laboratories.

In this study, a rapid, simple and non-separative method for the analysis of 11 PAHs in urine samples is proposed. The aim of this work was to reduce analysis time, as well as to obtain low detection limits that allow the determination of the PAHs in the urine of people exposed to these analytes in the common concentration range. The method is based on liquid-liquid extraction (LLE) and subsequent analysis with a programmed temperature vaporizer and a quadrupole mass spectrometer (PTV-qMS) followed by chemometric techniques. In order to assess the potential of the proposed method, the study was divided into two different tasks with the aim to obtain both quantitative and qualitative information. To this end, the method was used to determine the concentration of 11 PAHs in urine samples (quantitative analysis) and to discriminate those samples with and without PAHs (qualitative analysis). To the best of our knowledge, this is the first time a programmed temperature vaporizer coupled with quadrupole mass spectrometer has been used to analyse unmetabolized PAHs in urine samples.

2. Experimental

2.1. Reagents and stock solutions

The standards of naphthalene, 2-methylnaphthalene, biphenyl, 4-phenyltoluene, fluorene, phenanthrene and fluoranthene were supplied by Acros Organics (Geel, Belgium). The standards of acenaphthylene, acenaphthene, chrysene, benzo(k)fluoranthene and methanol were supplied by Sigma-Aldrich (Steinheim, Germany). N-hexane was purchased from Scharlab (Barcelona, Spain). The purity of all the reagents was at least 96%.

Stock solutions of 100 mg L^{-1} of each analyte were prepared in methanol, except for chrysene, which was prepared in acetone. Individual stock solutions of each analyte (6–20 mg L^{-1}) for subsequent dilutions were prepared in methanol. All the solutions were stored at 4 °C.

2.2. Urine samples

Human urine samples were collected from 27 adults (13 women, 14 men), aged between 27 and 83 years, in a disposable sterile specimen collection cup. The samples were frozen and stored at −20 °C in the dark prior to use. The pH range varied from 4.6 to 8.1. A GC-qMS analysis confirmed the absence of the studied analytes in the samples. These 27 urine samples were spiked at different concentration levels (1.09–58.05 μg L^{-1}) by adding 225 μL of the corresponding solution containing the 11 PAHs of study in order to obtain the spiked urine group.

Before liquid-liquid extraction, the urine samples were thawed at room temperature and transferred to a 12-mL polypropylene tube (Scharlab). The urines were centrifuged at 1811 × *g* for 10 min and after that, the sediment was eliminated.

Written informed consent was obtained from each volunteer.

2.3. Liquid-liquid extraction

Liquid-liquid extraction of the urine samples was carried out by transferring 6 mL of urine, 225 μL of methanol (non-spiked samples) or 225 μL of a solution with the PAHs (spiked samples) and 1 mL of hexane to a 15-mL glass centrifuge tube with a PTFE screw cap (Scharlab). After vortexing for 2 min (maximum setting), the sample was centrifuged at 2415 × *g* for 5 min to separate the organic and aqueous phases. The organic extract was collected and placed in a GC vial (Scharlab).

2.4. PTV-qMS conditions

The vial that contained the organic extract was placed in a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) equipped with two trays, each with 21 positions for holding the samples. The injection of the sample was carried out with a programmed temperature vaporizer (PTV) inlet (CIS-4, Gerstel, Baltimore, MD) using a liner (71 mm x 2 mm) packed with Tenax-TA®. The injection volume was 30 μL. The operating mode selected was solvent vent. The injector was set at 115 °C for 0.46 min, with a vent flow of 150 mL min^{-1} at 6.00 psi. After eliminating the solvent, the split valve was closed and the liner was heated (12 °C s^{-1}) until 340 °C (injection time: 1.75 min) for desorbing and transferring the analytes to the column. Then, the split valve was opened and the final temperature was held for 2.5 min for cleaning the system. A purge flow of 150 mL min^{-1} was used. The cooling of the PTV system was accomplished with liquid CO_2.

An Agilent 6890 GC device was equipped with an ultimate plus deactivated fused silica tubing (30 m x 0.250 mm) from J&W Scientific (Folsom, CA, USA) as the interface between the PTV inlet and the qMS, which was maintained at 340 °C throughout the signal recording time of 3.70 min. Thus, the analytes reached the detector without separation. Additionally, approximately 2.20 min were needed to re-establish the initial conditions of the PTV inlet; therefore, the analysis time per sample was in the region of 6 min. The carrier gas was helium N50 (99.999% pure, Air Liquide).

The detector used was a quadrupole mass spectrometer (HP 5973 N) equipped with an inert ion source. It was operated in electron-ionization mode (ionization voltage: 70 eV). Ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Data acquisition was performed in full scan mode (0.71 scan s^{-1}). A solvent delay of 1.10 min was established. The *m/z* range was 35–300 amu.

2.5. PTV-GC-qMS conditions

This method was used to check the urine samples for the presence or absence of the PAHs included in the study.

The experimental conditions for the PTV inlet were the same as those described for the non-separative methodology. To perform the GC-qMS measurements, the GC device was equipped with a HP5-MS UI capillary column (30 m x 0.250 mm x 0.25 μm) from J&W Scientific (Folsom, CA, USA). The initial oven temperature was 60 °C (0.5 min). This was increased at a rate of 60 °C min^{-1} to 175 °C and then further increased at 45 °C min^{-1} to 325 °C. This temperature was held for 2.5 min. The total chromatographic run time was 8.25 min. Additionally, about 8 min were needed to achieve the initial conditions of the programmed temperature vaporizer and gas chromatograph; therefore, the time between sample runs was 17 min.

The analyses were performed in a synchronous SIM/scan mode, allowing the collection of both SIM and full scan data in a single run. A