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Analytical Methods

On-line MSPD-SPE-HPLC/FLD analysis of polycyclic aromatic hydrocarbons in bovine tissues

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ABSTRACT

A fast method was optimized and validated for simultaneous trace determination of four polycyclic aromatic hydrocarbons: benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene in bovine tissues. The determination was performed by matrix solid-phase dispersion (MSPD) coupled on-line to solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with fluorescence detection (FLD). The sample was dispersed on C_{18} silica sorbent and then the on-line MSPD-SPE-HPLC/FLD method was applied. Several parameters were optimized: cleaning and elution sequences applied to the MSPD cartridge, the flow rate and dilution of extract used for SPE loading. The on-line method was validated over a concentration range of 0.1–0.6 ng g^{-1} obtaining good linearity ($r \geqslant 0.998$) and precision (RSD) $\leqslant$ 10%. Recovery ranged from 96 to 99% and the limits of detection were 0.012 ng g^{-1}. This methodology was applied to liver samples from unhealthy animals. The results demonstrate that MSDP-SPE-HPLC/FLD method provides reliable, sensitive, accurate and fast data to the food control.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more simple aromatic rings without heteroatoms or substituents. PAHs are formed and released to the environment through natural and anthropogenic sources. These pollutants are toxic, some of them carcinogenic (Gdula et al., 2015), persistent and bioaccumulable in lipid tissues of higher organisms, including humans (Thing, Chuen, Yew, & Siang, 2011). Intake of contaminated food is the principal way of human exposure to PAHs, accounting for >90% if compared to other ways such as inhalation and dermal contact (Gdula et al., 2015).

PAHs are significantly present in aquatic organisms and meat due to heat processes such as smoking, grilling and smoke drying. Moreover, all kind of food can be contaminated via packaging material or additives. The presence of PAHs in fish, crabs, bivalves, mussels, tea, uncooked food such as vegetables, algae, seeds and grains have been demonstrated (Alomirah et al., 2011; Duedahl-Olesen et al., 2015; Garcia, Reynoso, & Resnik, 2015). Although meat, specially smoked meat, has been extensively monitored for PAHs by using extraction techniques such as Solid-Liquid Extraction (SLE) and Solid-Phase Extraction (SPE) for clean-up, research on method development of PAHs for different bovine tissues is scarce (Plaza, Garrido, & Martínez, 2010). In relation to the extraction techniques, it is remarkable the fact that the conventional techniques such as soxhlet (solid matrices) and SLE are still widely used, although the application of less-solvent-consuming techniques, such as microextraction techniques, has been recently reported (Plaza et al., 2010).

The International Agency for Research on Cancer (*IARC*) has listed 10 PAHs, including benzo[*a*]pyrene (BaP), benz[*a*]anthracene (BaA) and benzo[*k*]fluoranthene (BkF), as possible human carcinogens (Pratt et al., 2007). Then also, the Environmental Protection Agency (US-EPA) selected 16 PAHs as priority pollutants and they are commonly used to characterize the PAH content from different samples (EPA Method 61040 CFR Part 136, 2004).

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For this reason, the development of analytical methods to determine these compounds in animal matrices and animal-derived food products has recently become a very important field. Liquid chromatography (LC) coupled to fluorescence detector (FLD) and mass spectrometry (MSD) detector are systems commonly used for the trace-levels analysis of PAHs from complex samples. For instance, fish, algae, shrimp, vegetables oils and other seafood were analyzed by LC coupled to FLD reaching detection limits between 0.00010 and 0.3 μg kg^{-1} (Ciecierska & Obiedzinski, 2013; Purcaro, Moret, & Conte, 2013; Zhang, Xue, & Dai, 2010). Nevertheless, at present the PAHs extraction from bovine tissues followed by chromatographic analysis is still limited because there are many problems associated to sample pre-treatment, clean up and sensibility of the methods. Thus, classical methods based on soxhlet, solid-liquid and supercritical fluid extraction are considered as standard methods for pre-treatment of solid samples containing PAHs (Amezcua, Ávila, Trejo, & Meléndez, 2012; Kalachova et al., 2011). In recent years, the availability of new extraction techniques has increased; their main aim is to provide support for the replacement of conventional methods by simplest, faster and sensitive protocols. The techniques successfully used for analysis of PAHs are: accelerated solvent extraction (ASE) (Suranová, Semanová, S kláršová, & Simko, 2015), ultrasonic assisted extraction (UAE) (Liu, Qi, Yan, Jia, & Yu, 2011; Sanz, Bocanegra, Ortiz, & Cámara, 2010), microwave assisted extraction (MAE) (Ghasemzadeh, Mohammadi, Hashemi, Khaksar, & Haratian, 2012), even the more recent techniques such as in-tube solid phase microextraction (IT-SPME) and vesicular supramolecular solvent-based microextraction (López, Ballesteros, & Rubio, 2014). However, some of these methodologies require expensive equipment but the sorbent-based techniques such as the Matrix Solid Phase Dispersion (MSPD) which was introduced by Barker in 1989 (Barker, 2000), provide a quick, simple and economic possibility to obtain analyzable extracts by liquid and gas chromatography with high recoveries and minimized interferences from matrix (Barker, 2007; García, Cela, Lorenzo, & Carro, 2012; Shen et al., 2011; Zhang et al., 2012). In addition, this technique integrates sampling, extraction and preconcentration in a simple step procedure.

It is well known that, MSPD has been successfully applied to the extraction of a wide range of drugs, PAHs, pesticides, naturally occurring constituents and other compounds from a wide variety of complex plant and animal samples (Capriotti et al., 2015; Rallis, Sakkas, Boumba, Vougiouklakis, & Albanis, 2012; Shen et al., 2011; Wen et al., 2012; Xu et al., 2013).

Most of the reported MSPD extraction methods are used off-line, meaning that extraction and analysis are performed separately. However, frequently, risks of sample loss and contamination are major problems in multi-step sample off-line pre-treatment methods. These systems should nevertheless be integrated as an on-line system, where the whole analytical procedure takes place in a closed, usually automated system. In this way, many of the problems associated with the traditional approaches could be avoided (Hyotylainen & Riekkola, 2004). However, only few works reported the use of an on-line MSPD method: Sulfonamides in carp tissues and chloramphenicol in soft-shelled turtle tissues by MSPD-ultra fast-LC-MS/MS (Lu et al., 2012) and a MSPD-SPE-LC/DAD coupling for analysis of organophosphorus pesticides in bovine tissue (Gutiérrez-Valencia & García de Llasera, 2011). In our work, the last mentioned method was modified for the analysis of the hydrophobic PAHs (BaA, BbF, BkF and BaP) in different bovine tissues (muscle, liver and lung); the optimization and validation of the MSPD-SPE-HPLC/FLD method was performed with liver samples and described in detail. Subsequently, the method was applied to the analysis of PAHs in some bovine samples that presented pathological lesions and were collected from dead animals having an unknown disease.

2. Materials and methods

2.1. Chemical and materials

Methanol and acetonitrile were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). The PAHs standards (benzo[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkA) and benzo[*a*]pyrene (BaP)) were from Chem Service (West Chester, PA, USA) with a certified purities > 99%. Water (18.2 MΩ cm^{-1} resistivity) was obtained from a Millipore deionizer Simplicity UV (Bedford, MA, USA). A standard solution containing the four analytes (25 mg L^{-1} each) was prepared in methanol and stored in the dark at 4.0 °C. From this solution, working standard solutions were prepared by appropriate dilution in methanol at different concentration levels (0.25–1.50 μg L^{-1}). Silica LC-18-PAH (particle diameter 45 μm) from CHROMABOND (Bethlehem, PA, USA) and silica LC-Si (particle diameter 40 μm) from SUPELCO (Bellefonte, PA, USA) were used as dispersing and clean-up sorbents respectively, to perform MSPD. Nucleosil silica C_{18} sorbent (particle diameter 10 μm) purchased from Phenomenex (San Francisco, CA, USA) was used to pack the SPE precolumn (20 mm × 2.0 mm I.D.) in the MSPD-SPE-HPLC/FLD system. One milliliter disposable plastic syringe barrels were obtained from Varian (Palo Alto, CA, USA).

2.2. Sample preparation

The bovine tissue samples (liver, muscle and lung) used for the validation of the on-line MSPD-SPE-HPLC/FLD method were obtained in supermarkets from Mexico City. PAHs free samples checked by our laboratory were used as blank samples for recovery studies and calibration experiments. Ten grams of bovine tissue were homogenized with an Ultraturrax apparatus (IKLLabortechnik, Staufen, Germany) and stored at −4.0 °C until analysis. The bovine liver tissue samples used for application of the validated method were obtained from the Animal Pathology Laboratory of the *Facultad de Estudios Superiores FES-Cuautitlán*, UNAM (Mexico). The samples collected from animals suspected of death by intoxication were immediately stored at −20 °C until analysis.

The on-line device for the extraction and analysis of PAHs is show in Fig. 1 (Gutiérrez-Valencia & García de Llasera, 2011) and the preparation of the MSPD cartridge is briefly described here: 200 mg of C_{18}-sorbent, that had been previously washed with 1 mL of methanol by each gram of sorbent and vacuum dried, were

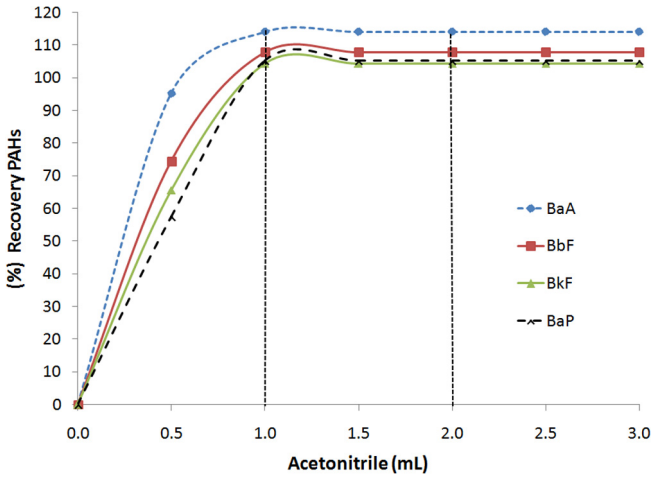

Fig. 1. Elution profile of all PAHs. (%) Recoveries of PAHs as a function of acetonitrile volume. Previous washing step: 5.0 mL water + 5.0 mL of 30:70 v/v acetonitrile–water + 4 mL of 40:60 v/v acetonitrile–water. MSPD was performed with 0.200 g of C_{18} and 0.050 g of liver.