



# Extraction of polycyclic aromatic hydrocarbons from polyhydroxyalkanoates before gas chromatography/mass spectrometry analysis

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## ABSTRACT

Among the organic contaminants that could pass from waste to polyhydroxyalkanoates (PHAs), there are the polycyclic aromatic hydrocarbons (PAHs). For this reason, we have developed a rapid analytical method for the determination of sixteen PAHs in PHAs. PAHs were extracted by *n*-hexane, after matrix dispersion and crumbling into sand; the extract was purified by solid phase extraction using florisil as adsorbent. Recoveries in the range of 89–101% were obtained for the deuterated analytes, except for the two with the lowest molecular weight. Trueness between 92% and 108% and within-laboratory precision (expressed as relative standard deviation)  $\leq 18\%$  were estimated for all the analytes. Gas chromatography/mass spectrometry was used for analyte determination. Method limits of quantification were suitable to assure that PAH presence in PHA biopolymers is much below the limits set by European law for plastic materials. Indeed, analysis of two different PHA samples showed that contamination is limited to few compounds at non-concerning levels.

## 1. Introduction

Nowadays, in line with the Circular Economy approach, great effort is being devoted to reduce waste amount by reusing it and recovering high value substances. For example, it is possible to transform organic waste via anaerobic fermentation in volatile fatty acids (VFAs) [1], which can be used as a low cost external carbon source for the production of environmentally friendly products, such as polyhydroxyalkanoates (PHAs). PHAs are completely biodegradable and biocompatible polyesters with mechanical properties comparable to those of conventional oil based polymers [2]. The employment of organic fraction of municipal solid waste (OFMSW) as a new feedstock for microorganism-based PHA production is very attractive to reduce PHA high cost and increase their sustainability [3]. Nevertheless, it is

necessary to guarantee suitable quality standards and the absence of incorporated chemical contaminants in the final product.

Polycyclic aromatic hydrocarbons (PAHs), constituted by two or more polycondensed aromatic rings, are mainly formed during the incomplete combustion of materials containing saturated hydrocarbons. PAH physicochemical properties (i.e., low vapor pressure and low solubility in water for high molecular weight PAHs, see [Supplementary material Table S1](#)), make them highly persistent and ubiquitous in the environment. PAHs can also reach the food chain, and their presence in the OFMSW has been reported [4–7].

The PAH adverse effects on human health are well known [8] and, accordingly to the International Agency for Research on Cancer (IARC) [9], benzo[a]pyrene is classified as carcinogenic to humans (group 1), whereas other PAHs as probably or possibly carcinogenic to humans

**Abbreviations:** ACY, acenaphthylene; ACY-d8, acenaphthylene-d8; ACE, acenaphthene; ACE-d10, acenaphthene-d10; ANT, anthracene; BaA, benz[a]anthracene; BaA-d12, benz[a]anthracene-d12; BaP, benzo[a]pyrene; BaP-d12, benzo[a]pyrene-d12; BbF, benzo[b]fluoranthene; BbF-d12, benzo[b]fluoranthene-d12; BeP-d12, benzo[e]pyrene-d12; BkF, benzo[k]fluoranthene; BkF-d12, benzo[k]fluoranthene-d12; BPE, benzo[ghi]perylene; BPE-d12, benzo[g,h,i]perylene-d12; CHR, chrysene; CHR-d12, chrysene-d12; DBaA, dibenz[a,h]anthracene; DBaA-d14, dibenz[a,h]anthracene-d14; FLT, fluoranthene; FLT-d10, fluoranthene-d10; FLU, fluorine; IARC, International Agency for Research on Cancer; IPY, indeno[1,2,3-cd]pyrene; IPY-d12, indeno[1,2,3-c,d]pyrene-d12; NAP, naphthalene; NAP-d8, naphthalene-d8; OFMSW, organic fraction of municipal solid waste; PAH, polycyclic aromatic hydrocarbons; PER-d12, perylene-d12; PHA, polyhydroxyalkanoate; PHE, phenanthrene; PHE-d10, phenanthrene-d10; PTV, programmed-temperature vaporizing; PYR, pyrene; PYR-d10, pyrene-d10; US EPA, United States Environment Protection Agency; VFAs, volatile fatty acids

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(group 2A and 2B, respectively) (see [Supplementary material Table S1](#)).

The United States Environment Protection Agency (US EPA) has identified a list of sixteen PAHs as priority compounds for monitoring. Also, the European Union has issued directives and regulations aimed to reduce PAH presence into the environment [10,11] and food [12]; in various articles (e.g. bicycles, household utensils, trolleys, clothing, footwear etc.) and toys, eight PAHs must have concentrations < 1 and 0.5 mg kg<sup>-1</sup> each, respectively (see [Supplementary material Table S1](#) [11]).

Several procedures are available for extraction of PAHs from various matrices [13,14], whereas for their determination gas chromatography-mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) with UV or fluorescence detection are commonly used.

To the authors' best knowledge, determination of PAHs in PHA biopolymers has not been reported in the literature, yet. In the present work, a procedure to extract the sixteen priority PAHs from PHAs was optimized. The critical step was to select a solvent able to extract the target analytes without dissolving the PHA. Then, the extract was cleaned up by solid phase extraction (SPE), and PAHs were quantified by GC/MS with isotopic dilution method. The method was finally applied to two PHAs obtained by using the OFMSW as feedstock (PHA<sub>OFMSW</sub>) and a synthetic VFA mixture (PHA<sub>synth</sub>).

## 2. Experimental

### 2.1. Materials and reagents

Sand (SiO<sub>2</sub>; purum per analysis; acid purified; 40–200 mesh) was purchased from Sigma-Aldrich (Milan, Italy). Before use, the sand was washed with 1 mol L<sup>-1</sup> hydrochloric acid, then with ultrapure water (five times) and finally with methanol (three times) to remove residual impurity.

Florisil (MgO<sub>3</sub>Si; 60–100 mesh) was obtained from Carlo Erba (Milan, Italy). Florisil cartridges were prepared by filling 6 mL polypropylene tubes (Supelco, Bellefonte, PA, USA) with 500 mg of the adsorbent placed between two polyethylene frits (Supelco).

Dichloromethane and *n*-hexane were of HPLC or GC grade and were purchased from Sigma-Aldrich (Milan, Italy), as well as hydrochloric acid. Acetonitrile of HPLC grade was purchased from VWR International (Milan, Italy). Ultrapure water (resistivity 18.2 MΩ cm) was obtained by an Arium 611 VF system from Sartorius (Göttingen, Germany).

### 2.2. Standard solutions

A standard mixture containing the US EPA sixteen priority PAHs (PAH calibration mix, TraceCERT<sup>®</sup> grade, 10 μg mL<sup>-1</sup> each component in acetonitrile) was purchased from Supelco. The sixteen PAH mixture included naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IPY), dibenz[a,h]anthracene (DBahA), and benzo[ghi]perylene (BPE). Structures are reported in the [Supplementary material Fig. S1](#).

A mixture of fourteen perdeuterated PAHs (chemical purities > 98%, isotopic purities ≥ 98%), concentration 100 μg mL<sup>-1</sup> in iso-octane/toluene 75:25 (v/v), was obtained from Wellington laboratories (Guelph, ON, Canada). The deuterated internal standard (d-IS) mixture included naphthalene-d8 (NAP-d8), 2-methylnaphthalene-d10, acenaphthylene-d8 (ACY-d8), phenanthrene-d10 (PHE-d10), fluoranthene-d10 (FLT-d10), benz[a]anthracene-d12 (BaA-d12), chrysene-d12 (CHR-d12), benzo[b]fluoranthene-d12 (BbF-d12), benzo[k]fluoranthene-d12 (BkF-d12), benzo[a]pyrene-d12 (BaP-d12), perylene-d12 (PER-d12), indeno[1,2,3-c,d]pyrene-d12 (IPY-d12), dibenz[a,h]anthracene-d14 (DBahA-d14), and benzo[g,h,i]perylene-d12 (BPE-d12).

Recovery standard stock solution L429-RS, containing 100 ng μL<sup>-1</sup> (1.2 mL in iso-octane) of acenaphthene-d10 (ACE-d10), pyrene-d10 (PYR-d10) and benzo[e]pyrene-d12 (BeP-d12) was obtained from Wellington Laboratories.

### 2.3. PHA samples

The two PHA freeze-dried samples were kindly provided by Prof. Mauro Majone from the Chemistry Department of University of Rome “La Sapienza”. Details on their production are reported in the [Supplementary material](#). The PHA<sub>OFMSW</sub> and PHA<sub>synth</sub> polymers were provided at 90% and 80% (w/w) purity, respectively.

### 2.4. Instrumental analysis

For preliminary experiments, a Shimadzu (Kyoto, Japan) HPLC system was used, which included two Nexera XR LC-20 AD dual-plunger parallel flow pumps, a CBM-20A controller, a Prominence SPD-M20A diode array detector, a DGU-20A3 online degasser, and a CTO-20A column oven. The chromatographic column was a 250 mm × 4.6 mm i.d. (5 μm particle size) BEH C18 (Waters, Milford, MA, USA). Mobile phase was water/acetonitrile; the gradient profile, referring to acetonitrile percentage, was from 60% to 100% in 30 min, followed by an equilibration step at 60% for 15 min. Flow-rate was 0.5 mL min<sup>-1</sup>; injection volume was 10 μL. UV detection was carried out at 220 and 254 nm wavelengths.

PAH determination was carried out using a gas chromatograph coupled with a mass spectrometer, namely an ISQ<sup>™</sup> Series Single Quadrupole GC/MS (Thermo Fisher Scientific, St. Peters, MO, USA) equipped with a SLB<sup>™</sup>-5 ms (Supelco, Milan, Italy) fused silica capillary GC column, poly(5% diphenyl/95% dimethyl siloxane) phase, 30 m × 0.25 mm i.d., 0.25 μm film thickness. He (99.9995% purity) was used as carrier gas. The injector was a programmed-temperature vaporizing (PTV) inlet; the injection volume was 1 μL. Temperature programming was set as follows: the initial oven temperature was 40 °C, kept constant for 5 min, then increased to 290 °C with 12 °C min<sup>-1</sup> and kept constant for 6 min; finally, the temperature was increased to 325 °C with 20 °C min<sup>-1</sup> and kept constant for 15 min. The temperature of transfer line was 270 °C. MS was operated in electron ionization mode (EI, 70 eV), and acquisition was performed using single ion monitoring (SIM) at the molecular ion of each PAH at the time windows corresponding to their retention times. Each PAH was quantified using its corresponding perdeuterated isotopologue or a structural omologue perdeuterated isotopologue as IS (see [Supplementary material Table S2](#)).

### 2.5. Sample preparation

#### 2.5.1. Sample extraction

A 100 mg-PHA sample aliquot was cut into small pieces (ca. 2 mm large), dispersed with 800 mg of washed sand in a ceramic mortar by blending with a pestle by the aid of liquid nitrogen. After about 10 min, a fine, dry and homogenous powder was obtained; then, the powder was transferred into a beaker. At this point, 3 mL acetonitrile was added to the powder to increase its wettability before spiking with the d-IS solution mix and, for recovery experiments, with the analyte calibration mix solution too. Solvents were allowed to evaporate for 2 h under the laboratory fume hood.

After that, the powder (ca. 900 mg constituted by polymer and sand) was added with 10 mL *n*-hexane and placed in an ultrasonic bath for 40 min. After centrifugation at 2000g for 20 min (to remove residual sand), the supernatant was collected; the procedure was repeated once. The supernatants (total volume < 20 mL) were mixed and cleaned up by SPE.

#### 2.5.2. Sample clean-up

Immediately prior to use, the SPE cartridge packed with florisil was

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