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Evaluation of the stability of selected hydroxy derivatives of polycyclic aromatic hydrocarbons in environmental samples

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ABSTRACT

In the conducted research, a methodology for the determination of three hydroxy derivatives of polycyclic aromatic hydrocarbons (OH-PAHs) (1-hydroxypyrene, 2-hydroxyfluorene, 2-hydroxynaphthalene) was developed by using a liquid chromatography equipped with a spectrophotometric detector and a Kinetex C18 column. Solid-phase extraction efficiency tests were carried out for cartridges filled with various sorbents. The column filled with silica gel modified with octadecyl groups showed the highest recovery (74%–92%). The last stage of the research was to assess the stability of selected OH-PAHs in order to determine whether any transformation is possible during their transport to the environment from the wastewater treatment plants. It was decided to simulate the conditions in the sewage system during the summer and winter periods by conducting an experiment at a temperature of 22°C and 4°C. In addition, the analyses were carried out in parallel on two matrices with the addition of analytes standards: deionized water and real wastewater. The stability test that was performed demonstrated that within 72 h, regardless of the prevailing conditions, all analysed OH-PAHs remain stable, which means that no hydraulic retention occurs in the analysed period. Furthermore, no differences between the used matrices were noticed, which proves that the chemical diversity of wastewater does not accelerate their degradation in the sewage system.

Keywords: Hydroxy derivatives of polycyclic aromatic hydrocarbons; Biomarker; Stability; Wastewater

1. Introduction

Relevant epidemiological information on the lifestyle or public health of the population can be obtained by chemical analysis of the wastewater. Selected products of human metabolism, that is, biomarkers, are identified in the wastewater as indicators of exposure of the population inhabiting the area of a particular wastewater treatment plant [1–4]. Biomarkers are products of the transformation of almost everything that a person is exposed to, they are excreted from the body along with urine and stools and end up in the sewage system [5].

Among the air pollutants, polycyclic aromatic hydrocarbons (PAHs), which arise from incomplete combustion of organic matter, should be distinguished [6–8]. PAHs constitute a large group of organic compounds (approx. 10,000) containing from a few to a dozen aromatic rings, which have always been present in the environment, but the civilization and technological development has resulted in an increase in exposure to these compounds [9]. These substances strongly adsorb on solid particles, which get into the human body with them and subsequently contribute to the formation of neoplastic changes in the cells of the body [10,11]. In the human body, PAHs are rapidly metabolized to

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hydroxy derivatives (OH-PAHs) and leave the body within a few days [12]. OH-PAHs with a lower molecular weight are excreted mainly in the urine, while those with a higher molecular weight are mainly excreted in the bile with the stool [13]. Due to the ease of release of OH-PAH into human excreta, they can be considered biomarkers of PAH exposure. OH-PAHs go to a wastewater treatment plant along with wastewater, from where they are released together with treated wastewater into the water environment [14]. As carcinogenic and mutagenic substances, OH-PAHs manifest a more harmful effect on the human body than their starting compounds [15].

One of the most important features of biomarkers, next to a sufficiently high concentration to enable detection in wastewater, is their stability. It is important that the concentration of the test substance does not change from the moment of exit, that is, the toilet, to the time of transport to the wastewater treatment plant, and during collection and analysis [16].

The conducted research included the development of analytical methodology enabling the determination of selected hydroxy derivatives of PAHs in wastewater with the use of high-performance liquid chromatography with the spectrophotometric detector (HPLC/UV-Vis). This involved, firstly, chromatographic separation of selected compounds and method validation, and secondly, check the efficiency of the solid phase extraction (SPE) process depending on the fill sorbent application. Then, the stability of OH-PAH in wastewater was assessed depending on the conditions simulating temperatures in the sewage system corresponding to winter and summer periods. Information on the stability of these biomarkers in treated wastewater is important as they are used to transport OH-PAHs to the environment.

2. Experiment-related part

2.1. Selection of analytes

The analyzes were conducted for selected OH-PAHs, which are 1-hydroxypyrene (1-OH-PYR), 2-hydroxyfluorene (2-OH-FL), 2-hydroxynaphthalene (2-OH-NP). Their characteristics are presented in Table 1.

1-OH-PYR has been considered a marker of occupational exposure to PAHs since the 1980s, while a decade later it was used to assess exposure to PAH from the environment [17,18]. Further 2-OH-FL and 2-OH-NP are considered to be markers of PAH exposure, especially their concentration in urine is associated with air pollution-related to industrial development [19–22].

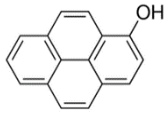
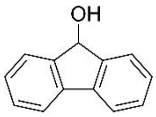
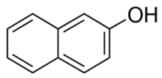
2.2. Chemicals and materials

Each of the analytical standards with purity above 98% was purchased from Sigma-Aldrich (Poland). The analytical solvents used for the research, methanol and acetonitrile, of analytical purity, came from POCH (Gliwice, Poland). Deionized water (<0.07 S/cm) used for SPE and as HPLC mobile phase was obtained from the HLP5 system (Hydrolab, Gdańsk). The OH-PAH standard solutions and their mixtures were prepared in methanol and stored at subzero temperature, protected from light. The concentrations of the stock solutions of standards were as follows: 1-OH-PYR 111 μg/mL, 2-OH-FL 133 μg/mL, 116 2-OH-NP μg/mL. For the efficiency test of the SPE process, four cartridges with different packing sorbents were used: C18 (3 mL/500 mg) from J. T. Backer (Netherlands), Drug (3 mL/200 mg) and Easy (3 mL/200 mg) from Macherey-Nagel, Oasis HLB (3 mL/60 mg) from waters. Temporarily treated wastewater obtained from the Płaszów Wastewater Treatment Plant in Kraków (Poland) was filtered on GF-1 and GF-5 glass microfiber filters bought from Macherey-Nagel.

2.3. Analytical method

A method for the separation of individual compounds by means of the HPLC/UV-Vis technique was developed: Varian Star liquid chromatograph equipped with a spectrophotometric detector and a Kinetex C18 column (length 7.5 cm, diameter 4.6 mm, filling 2.6 μm in the core-shell technology). Gradient elution was used, where the mobile phases were deionized water acidified with acetic acid to pH = 3.5 and acetonitrile. The analysis was started with the following eluent ratios: 25% deionized water, 75% acetonitrile. By the sixth minute, the proportion of

Table 1
Primary characteristics of test compounds

Name of compounds	Formula	CAS No.	Water solubility (mg/L)	Molecular weight (g/mol)	Number of rings
1-hydroxypyrene (1-OH-PYR)		5315-79-7	0.017	218.255	4
2-hydroxyfluorene (2-OH-FL)		2443-58-5	0.05	182.218	3
2-hydroxynaphthalene (2-OH-NP)		135-19-3	0.74	144.173	2

acetonitrile increased to 100%. This phase arrangement was maintained until the end of the analysis, that is, for 15 min. For the next 10 min, the system was recalibrated. The wavelength was set at 210 nm and the flow at 0.8 mL/min. The injection volume was 10 µL. Calibration curves were prepared for eight points, and then the validation parameters of the method were determined on their basis. The obtained results are presented in Table 2. All three calibration curves are characterized by a high regression coefficient ($R^2 > 0.993$), while the coefficient of variation was below 5%. According to ISO standards, when R^2 exceeds 0.98, the method is considered to be linear in a given range, that is, the obtained measurement results are directly proportional to the concentration (content) of the substance in the sample in the given range [23].

2.4. Solid-phase extraction efficiency tests

Cartridges with different fillers were used to test the efficiency of the SPE process. Prior to use, the cartridges were conditioned with 2 mL of methanol and 2 mL of deionized water. Samples of model wastewater, in the form of deionized water with the addition of standards, were dosed using a 12-position SPE system by J. T. Backer (Netherlands) in a set with a diaphragm pump. Then, the filter on which the analytes were retained was dried for about 20 min and eluted. 4 mL of methanol was used to wash out the desired components. The collected extracts were evaporated to dryness at 35°C and in the presence of argon and redissolved in 0.2 mL of methanol. The prepared solutions were analysed by HPLC/UV-Vis. For each type of cartridge, the described test was performed three times. The efficiency of the SPE process, presented as a percentage of recovery, was calculated from the formula:

$$\frac{x_i}{\mu}\cdot 100\%, \tag{1}$$

where x_i is the amount of analyte determined in the test sample; µ is the known amount of analyte in the test sample [23].

The test carried out in this way made it possible to select the extraction column that was used for further stages of the research, that is, the evaluation of the stability of selected OH-PAHs.

2.5. OH-PAHs stability testing

The stability of selected hydroxy derivatives of PAHs was assessed when used as a matrix of treated wastewater and deionized water. 2 L of wastewater (previously filtered on GF-1 and GF-5 glass filters) and 2 L of water were prepared, which were enriched with the standard 44.4 µg 1-OH-PYR, 53.2 µg 2-OH-FL and 46.4 µg 2-OH-FL then was homogenized by shaking. The samples prepared in this way were divided into 200 mL portions and placed in glass bottles with a volume of 250 mL. In order to simulate the conditions in the sewage system in winter, the samples were placed in a refrigerator (4°C), and in the case of representing summer conditions, the samples were left at room temperature (22°C). In both cases, the samples were stored in the dark. The concentrations of individual compounds were tested after 2, 4, 6, 24, 48 and 72 h by means of HPLC/UV-Vis analyses according to the described procedure, preceded by the SPE process. The analysis was performed four times for each of the samples.

3. Results and discussion

3.1. Efficiency of the SPE

The SPE process efficiency results for the four columns: C18, Oasis, Easy and Drug are shown in Fig. 1. The bar graph shows the average recovery values for the individual compounds. There are also error bars that show the standard deviation values.

The extraction column labeled Drug is filled with modified silica. It is intended to enrich urine medications. It is intended to enrich drugs from urine samples. During the tests, it showed a different value of recoveries for individual compounds. The disadvantage of this type of sorbent was the low repeatability of the results, as evidenced by high values of the standard deviation for 2-OH-NP (from 6% to 45%) and low recovery for 2-OH-FL (12%). The Easy extraction column is filled with polystyrene-divinylbenzene copolymer. The efficiency values obtained for this cartridge showed the smallest differences between the individual compounds than the Drug column. The effectiveness of Easy was small, as it was between 40% and 50%. The HLB extraction columns with universal polymeric reversed-phase packing had similar recovery values to the C18 column with sorbent filling, octadecyl-modified silica gel. No statistically significant differences were observed after the *t*-student test for $p = 0.05$. However, it was decided to use the C18 columns in further studies, due to higher mean recoveries for 2-OH-NP and 1-OH-PYR as well as smaller values of standard deviations for 2-OH-FL. The C18 column showed the following mean recoveries: 74% for 2-OH-FL, 77% for 2-OH-NP and as much as 92% for 1-OH-PYR. Also in the literature, there is information indicating the C18 columns as the most appropriate for

Table 2
Method validation parameters

Compounds	Retention time (min)	R^2	Range of linearity (µg/mL)	Limit of quantification (LOQ) (ng/mL)	Limit of detection (LOD) (ng/mL)
2-OH-NP	4.989	0.9958	0.2–29.0	75.5	25.2
2-OH-FL	7.160	0.9948	0.3–33.3	86.6	28.9
1-OH-PYR	10.919	0.9938	0.9–27.8	289.0	96.3

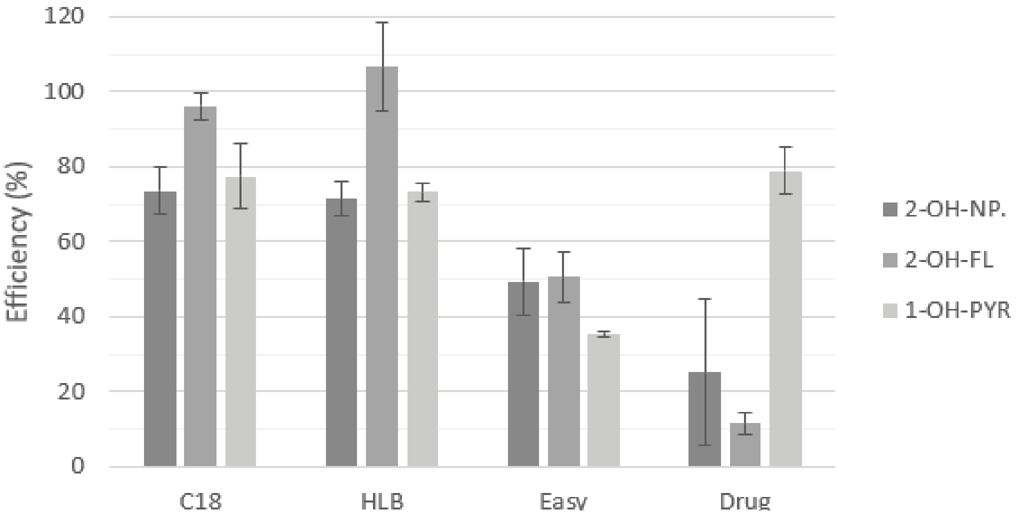


Fig. 1. The efficiency of the extraction process to the solid phase.

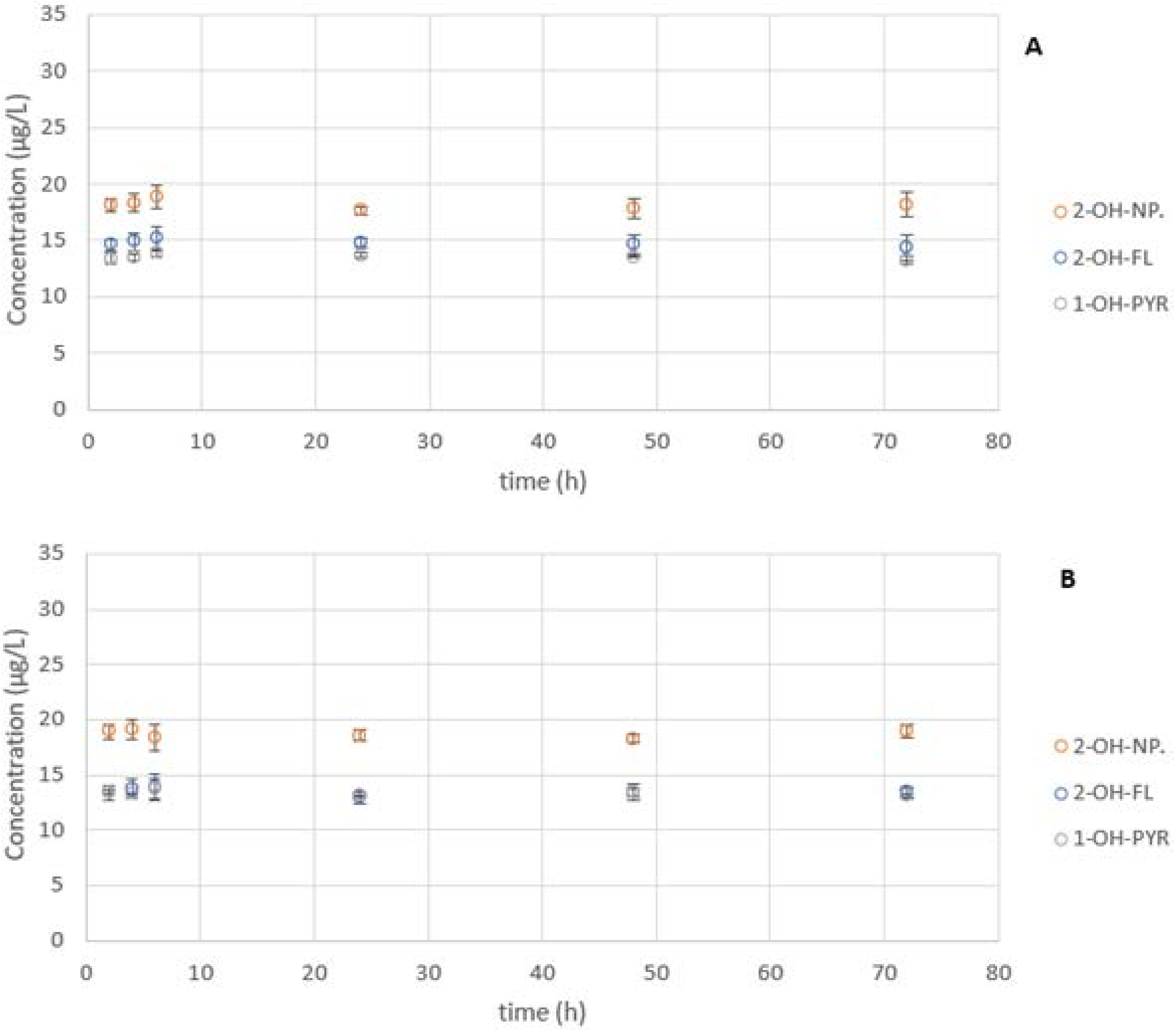


Fig. 2. The stability of selected OH-PAHs in deionized water at 22°C (A) and 4°C (B).

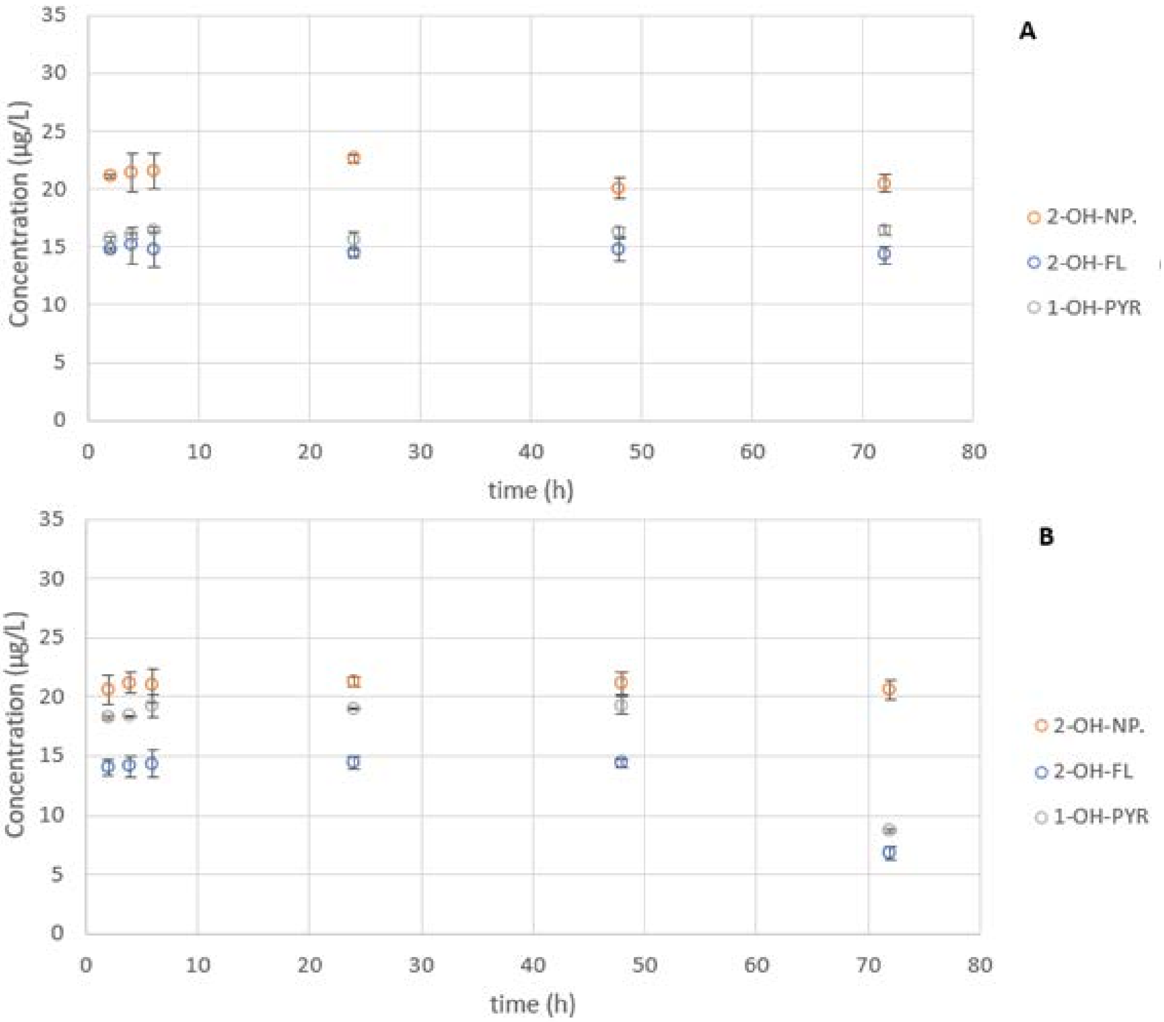


Fig. 3. The stability of selected OH-PAHs in treated wastewater at 22°C (A) and 4°C (B).

the OH-PAH analysis, while the recoveries obtained in the test performed were similar to those described in the literature [19,24,25].

3.2. Stability of biomarkers

The results of the experiment, aimed at assessing the stability of OH-PAH over time, depending on the temperature conditions and the matrices used, are shown in Figs. 2 and 3. The graphs show how the concentration of the analyte changes with the storage time of the sample. For each analysed sample, the graphs include error bars in the form of standard deviation.

The presented stability curves for both matrices, deionized water and treated wastewater show the character of a stable straight line for all compounds except 2-OH-FL and 1-OH-PYR in wastewater. The mass balance analysis for these compounds in wastewater at 4°C showed their decrease after 48 h by 52%. The concentrations changes of all compounds after 48 h were within the error margins of the analytical method and cannot be considered significant. Regardless of the temperature at which the experiment took place, the compounds are invariably stable. No temperature influence means that during 48 h the atmospheric conditions do not cause changes in the concentration of OH-PAH.

Currently, there are no literature reports on the stability of OH-PAHs in wastewater. There are only studies that have been performed to determine the elimination half-life of the PAH metabolites from the human body. The estimated half-life of OH-PAHs in the human body is on average several hours [26]. 1-OH-PYR exhibits a mean half-life from 29 (6.4–128) h for the exposure source of diesel exhaust [27] to 3.9 (3.0–5.7) h where the exposure was eating barbecued chicken [26]. In the studies of Sobus et al. [28], urine collected from asphalt pavers was analysed. The average half-lives calculated for the sum of 1- and 2-OH-NP were 26 (14–116) h. In the case of exposure to PAHs resulting from smoking, the average half-life for 2-OH-NP was 9.4 (4.9–12.24) h, while for 2-OH-FL 4.1 (2.5–5.0) h [29].

The above information and the experiment carried out indicate that the OH-PAHs in question are more stable in wastewater, where they are not subjected to additional processes taking place in the human body. Their concentrations do not change over 48 h, while in the human body the concentrations would decrease several times during this period of time. During the transport of wastewater from the toilet to the wastewater treatment plant, there should be no change in OH-PAH concentrations, taking into account the matrices in which they are located. This was confirmed by the tests carried out, because both in deionized water and in treated wastewater, selected biomarkers are equally stable. The chemical diversity of wastewater, the multitude of compounds contained in it, do not affect the degradation rate of OH-PAHs. Additionally, regardless of the prevailing temperature in the sewage system, there is no hydraulic retention, stoppage or reduction in the amount of OH-PAHs.

4. Conclusion

The developed analytical method is used to determine selected OH-PAHs by means of HPLC/UV-Vis. In the conducted research in addition to the method validation, an efficiency test of the SPE process was performed. C18 extraction columns were selected as the most appropriate for the separation of OH-PAHs from the wastewater. On the basis of the experiment assessing the stability of OH-PAHs, it can be concluded that these compounds are stable in wastewater for a period of at least 72 h. This means that the analyzes of environmental samples to determine the presence of the discussed analytes should be performed within three days from the moment they leave the human body with urine and feces.

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