

Optimization and Validation of HPLC–UV–DAD and HPLC–APCI–MS Methodologies for the Determination of Selected PAHs in Water Samples

Guilherme M. Titato and Fernando M. Lanças*

University of São Paulo, Institute of Chemistry at São Carlos, Laboratory of Chromatography, P.O. Box 780, CEP 13566-590, São Carlos – SP, Brazil

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants resulting from emissions of a variety of sources including industrial combustion, discharge of fossil fuels, and residential heating. Because of their mutagenic and carcinogenic properties, the study of PAHs in environmental matrices is of great importance. In this work, the extraction of 9 out of the 16 PAH priority pollutants according to the U.S. Environmental Protection Agency is carried out through liquid–liquid extraction (LLE) and solid-phase extraction (SPE). The determination of PAHs is made by high-performance liquid chromatography with diode-array detection and liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Between the extraction techniques used, LLE is revealed to be efficient in the extraction of the higher molecular weight PAHs, though SPE is adequate for the extraction of all PAHs. In the real water samples analyzed, no PAH is detected under the analysis conditions used.

Introduction

Currently, the quality maintenance of water is a very important goal being researched by the governments of the world. For two decades, considerable attention has been given to the possible presence of organic pollutant materials in trace levels in drinking waters (1).

Because there are diverse types of environmental samples that are analyzed with the goal of the positive identification of organic micropollutants (1–4), it can be imagined that the same procedure could easily be applied in the case of drinking waters. However, the extraction of pollutants from drinking water samples at trace levels is not an easy procedure because there is no singular technique capable of effectively analyzing all organic pollutants that can be present in this matrix. For quantitative

analysis of water samples it is necessary to achieve detection limits (LOD) lower than those required for other types of environmental samples. For this reason, the extraction and quantitation of organic pollutants have been the object of extensive work under development (5).

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants resulting from emissions of a variety of sources including industrial combustion and discharge of fossil fuels and residential heating. Because of their mutagenic and carcinogenic properties, the study of PAHs in environmental matrices including air, water, and soil are of great importance. PAHs are usually present in environmental samples as extremely complex mixtures that contain many isomeric structures and alkylated isomers. These compounds can be introduced into aqueous medium in several ways, including sewer waters from industry and particulate materials carried by wind or rainwater (6).

Because of their nonpolar nature and high molecular weight, PAHs present low solubility in water ($\sim \mu\text{g/L}$). Drinking water must contain only 1 to 10 ng/L of each PAH, and the maximum limit (adding all compounds) cannot exceed 100 ng/L.

Since its inception in the early 1970's, high-performance liquid chromatography (HPLC) has been used for the separation of PAHs. Since Schmit's (7) report, reversed-phase on chemically bonded C18 phases has become the most popular HPLC mode for the separation of PAHs.

Combined liquid chromatography (LC) with mass spectrometry (MS) (HPLC–MS) can be considered as one of the most important techniques of analysis of the last decade, becoming preferred in the pharmaceutical industry and also presenting an important role in environmental analyses (8,9).

Different methods of coupling LC to MS and some commercial interfaces are available. For 80 years, thermospray was the most used technique. With the advent of atmospheric pressure ionization (API) techniques for sample introduction into an MS, HPLC–MS had an increase in popularity. Because API is a soft and efficient ionization method, it is adequate for polar, ionic, and high-molecular mass compound analyses. The most common

* Author to whom correspondence should be addressed: email flancas@iqsc.usp.br.

interfaces based on API are the electrospray (ESI) and its modification—named ionspray (ISP), both interfaces in which the ionization process occurs in liquid phase. Another API interface, in which the ion-molecule reactions occur in gaseous phase, is termed atmospheric pressure chemical ionization (APCI) (10).

The literature includes several reports describing the extraction and analysis of PAHs in environmental samples, including water (11–17). Wise, Sander, and May (11) had made the determination of the 16 PAHs priority pollutants in environmental samples by HPLC using octadecylsilane columns of several manufacturers. They had verified differences of selectivity between the columns because of the type of synthesis of the C18 phase (monomeric or polymeric). Sargenti and McNair (12) had published a paper in which they present a comparison of efficiency between processes of extraction [liquid–liquid extraction (LLE), solid-phase extraction (SPE), and supercritical fluid extraction (SFE)] of the 16 priority pollutants PAHs in drinking water [determination made by gas chromatography (GC)–MS]. These studies have shown that for SPE, the C18 phase is revealed to be most efficient in the recovery of the volatile PAHs. Coupling SPE and SFE was also carried out, showing good recovery for all compounds. In the analysis using only SFE, the recovery of the compounds was less than that obtained by coupling SPE and SFE. LLE [U.S. Environmental Protection Agency (EPA) official technique] presented the lowest values of recovery and reproducibility when compared with the other investigated methods.

Chen studied some PAHs in water samples by HPLC with fluorescence detection after extraction by solid-phase microextraction (SPME). The separation and detection of five selected PAHs were run within 25 min with an analytical C18 column. Compared with conventional methods, SPME presented higher recovery (> 88%) (18).

Although the study and characterization of PAHs by LC–MS has appeared only more recently (13), methods involving HPLC–APCI–MS in PAHs analyses of different matrices have already been studied (14–16). Barceló and Pérez (17) had made the determination of PAHs in residual water samples using an APCI interface. They compared the results with those obtained using a more conventional method (GC–MS). The results obtained with HPLC–APCI–MS were very similar when compared with GC–MS, with detection limits in the range of 0.05–0.22 mg/kg.

The agricultural activity has an important role in the Brazilian economy. Consequently, large land areas are destined for food production, with prominence for the cultures of oranges, soybean, and sugarcane. The latter is widely cultivated in the region that this work was developed in and, therefore, the monitoring of pollutants emission from this culture to the environment is very important to the maintenance of the health of the people that live in proximity to these areas. It is well known that the sugarcane burning process (essential for the harvest process) can produce some toxic compounds, among them PAHs, which can be eventually introduced into the river waters around the cultivation area. Thus, the monitoring of the PAH presence in these waters was the main motivation for the development of the present methodology.

Experimental

Chemicals

The solvents used in this work included acetonitrile and methanol (HPLC grade) from Mallinckrodt (Paris, KY) and dichloromethane from Synth (Diadema, São Paulo, Brazil) and Mallinckrodt. SPE cartridges (octadecylsilane) were obtained from Supelco (Bellefonte, NJ). HPLC-grade water was obtained in a Milli-Q system (Millipore, São Paulo, Brazil).

PAHs analytical standards [naphthalene, acenaphthylene, acenaphthene, fenantrene, anthracene, fluoranthene, pyrene, chrysene, and dibenz(a,h)anthracene] were supplied by Supelco and PolyScience (Niles, IL). The individual stock solutions were prepared by dissolving a certain mass of each PAH in acetonitrile. Standard working solutions at various concentrations (10, 7, 5, 2, 1, 0.1, 0.05, 0.01, and 0.001 µg/mL) were prepared by appropriate dilution of aliquots of the stock solution in acetonitrile, in order to prepare the calibration curve and to calculate the LOD and quantitation limits (LOQ) for all compounds.

Extraction methods

Liquid–liquid extraction

The following LLE procedure was applied to the extraction of water samples: Milli-Q grade water (100 mL) was spiked with a standard working solution of PAHs in the concentration of 10 µg/mL of each compound. The water sample was then transferred to a 250-mL glass funnel, where it was extracted with three portions of 30 mL of dichloromethane. The dichloromethane extracts were combined and dried under anhydrous sodium sulphate. The dichloromethane fraction was then evaporated to dryness by passing a controlled and gentle flow of nitrogen, and the extracted PAHs were redissolved in acetonitrile in order to get a PAH mixture in the concentration of 5 µg/mL of each compound.

SPE

Three different quantities of octadecylsilane phase (100, 200, and 300 mg) were evaluated. The following procedure was used: 100 mL of Milli-Q-grade water was spiked with a standard working solution of PAHs in the concentration of 10 µg/mL of each compound. After that, the C18 cartridges were conditioned with 5 mL of methanol and 5 mL of Milli-Q water. After conditioning, the water samples were passed through the SPE cartridges using a Visiprep DL system from Supelco. The next step was to dry the solid phase (using a vacuum system) for approximately 10 min to eliminate the excess water. Acetonitrile was used to elute the compounds, in order to get a PAH mixture in the concentration of 5 µg/mL of each compound.

Determination methods

HPLC–UV–diode-array detection

The optimized conditions included the use of an LC 10-AD series HPLC system (Shimadzu, Kyoto, Japan). A Supelcosil LC-18 (25 cm × 4.6 mm, 5 µm) column was used (Supelco). The mobile phase consisted of acetonitrile–water (70:30, v/v). The flow rate was 0.8 mL/min. The analysis was performed at room temperature (~ 30°C). A Shimadzu SPD M10A UV–diode-array detector (DAD) was used as the detector. The wavelength was 220

and 254 nm [compounds detected at 220 nm: naphthalene, acenaphthylene, acenaphthene, and dibenz(a,h)anthracene; compounds at 254 nm: phenanthrene, anthracene, fluoranthene, pyrene, and chrysene]. The injection volume was 20 μ L, and the elution made was isocratic.

HPLC-APCI-MS

A Platform LC-MS system was used (Micromass, Manchester, U.K.). The HPLC system consisted of a syringe pump from CE Instruments (Milan, Italy) directly coupled to the MS interface. An ODS column (15 cm \times 2.1 mm, 5 μ m) from Shimadzu was used. The mobile phase consisted of acetonitrile-water (80:20, v/v) with 0.15 trifluoroacetic acid (Mallinckrodt). The conditions also included a 120- μ L/min flow rate; 10- μ L injection volume; room temperature (\sim 30°C) analysis temperature; 5000 V capillary voltage; 20 V cone voltage; 150°C source temperature; 450°C APCI temperature; and 250 L/h drying gas flow rate (N_2).

With the goal of a quantitative analysis inside of the reliable limits of the method used, all injections involving the different extracts were repeated three times, and the average value of the areas was used for the compounds quantitation.

Results and Discussion

Method validation

Determination of the LOD, LOQ, and linearity range of the method

The LODs were calculated as being three times the average level of the baseline noise (measured from the injection of standard solutions containing individual PAHs), and the LOQs were calculated as 10 times this same level (19) (Table I). It was verified that the LOD and LOQ values obtained were lower for HPLC-UV, probably because of the good sensitivity detection that this detection technique provides to the PAHs. Analysis of these compounds by HPLC-MS presented lower sensitivity (higher LOD and LOQ values) because the high chemical stability of the PAHs makes the protonation of these molecules difficult (in the positive ionization mode).

All PAHs presented excellent linearity inside the studied con-

centration range (0.1 to 10 μ g/mL), using the HPLC-UV-DAD technique; in HPLC-APCI-MS, linearity was studied from LOQ values of each compound up to a concentration of 10 μ g/mL for all PAHs. In both techniques the correlation coefficients were higher than 0.998.

PAH qualitative analysis

PAH identification by HPLC-UV-DAD was made through the retention times and UV spectrum of individually injected PAH

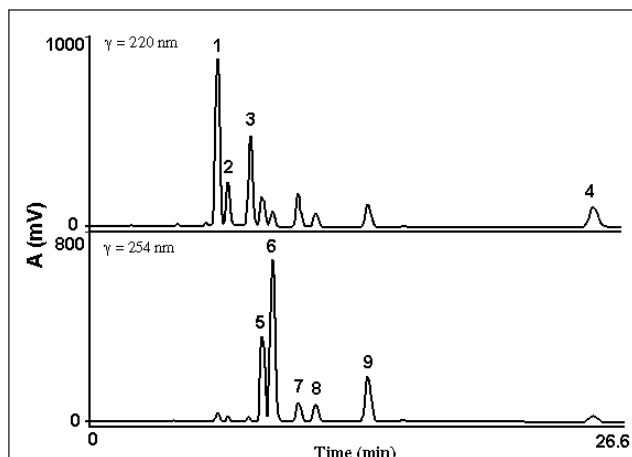


Figure 1. HPLC-UV-DAD chromatogram from a standard mixture containing selected PAHs (10 μ g/mL). Peak identity: naphthalene (1), acenaphthylene (2), acenaphthene (3), dibenz(a,h)anthracene (4), phenanthrene (5), anthracene (6), fluoranthene (7), pyrene (8), and chrysene (9).

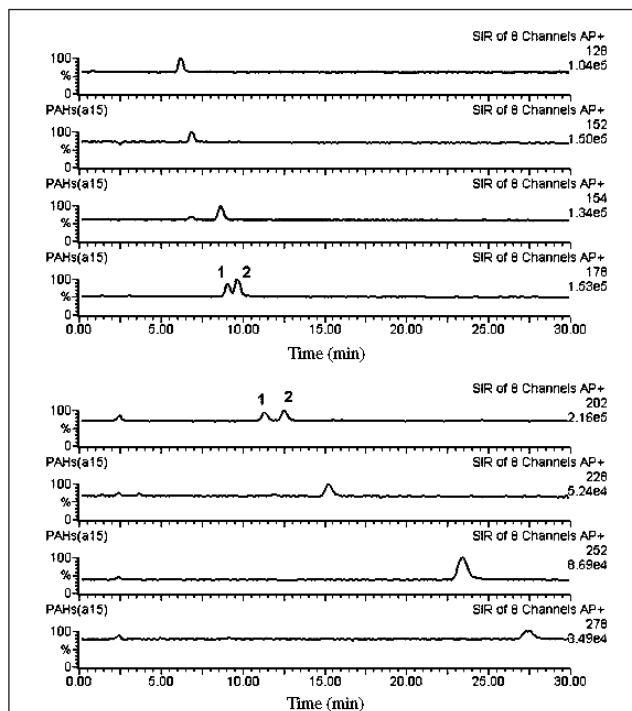


Figure 2. HPLC-APCI-MS chromatogram from a standard mixture containing selected PAHs (1 μ g/mL). Peak identity: naphthalene (m/z 128); acenaphthylene (m/z 152); acenaphthene (m/z 154); phenanthrene [m/z 178 (1)]; anthracene [m/z 178 (2)]; fluoranthene [m/z 202 (1)]; pyrene [m/z 202 (2)]; chrysene (m/z 228); benzo(a)pyrene (m/z 252); and dibenz(a,h)anthracene (m/z 278).

Table I. LODs and LOQs for the Selected PAHs

Compound	LOD		LOQ	
	HPLC (DAD) (μ g/mL)	LC-APCI-MS (μ g/mL)	HPLC (DAD) (μ g/mL)	LC-APCI-MS (μ g/mL)
Naphthalene	0.0010	0.05	0.0033	0.165
Acenaphthylene	0.0100	0.05	0.0333	0.165
Acenaphthene	0.0010	0.05	0.0033	0.165
Phenanthrene	0.0010	0.05	0.0033	0.165
Anthracene	0.0008	0.05	0.0026	0.165
Fluoranthene	0.0100	0.08	0.0333	0.264
Pyrene	0.0100	0.08	0.0333	0.264
Chrysene	0.0050	0.05	0.0166	0.165
Dibenz(a,h)-anthracene	0.0300	0.12	0.0999	0.396

standard solutions under the optimized analytical conditions. Figure 1 shows the chromatogram of a PAH standard mixture (10 µg/mL of each compound) employing two different wavelengths.

When using the HPLC–APCI–MS technique (operating in the positive mode), the compounds are usually identified through their quasimolecular ion, $[M+H]^+$. However, as already explained in the previous section of this paper, because of their chemical stability, PAH protonation becomes difficult. For this reason, the identification was made from the intact molecular mass of the compounds (Figure 2).

PAH recovery from aqueous samples

Just as the analysis by HPLC–UV showed higher sensitivity (lower LOD values) than that obtained by HPLC–MS for the selected PAHs, the studies of PAH recovery from spiked water samples performed using LLE and SPE were accomplished only by the HPLC–UV method.

Table II shows the PAH recovery and their relative standard deviations (RSDs) obtained by LLE (concentration of 5 µg/mL) using the HPLC–UV–DAD method. It can be observed that this technique was revealed to be efficient for the extraction of the high-molecular-weight PAHs (recoveries > 80%), though for the

most volatile PAHs (naphthalene, acenaphthylene, and acenaphthene), even so detected, their concentrations were found to be below their LOQs, which prevented their quantitation. The absence of these compounds could be attributable to losses during the LLE process because this technique involves some stages that may cause volatilization of these PAHs.

In the SPE method, three different quantities of C18 phase (100, 200, and 300 mg) were tested. The best extraction performance was reached using 200 and 300 mg of octadecylsilane phase (concentration of 5 mg/L of each PAH), as shown in Table III. The results obtained with 100 mg of C18 phase were unsatisfactory and, for this reason, discarded. Recovery data, as well as in the LLE method, were accomplished by HPLC–UV–DAD because this technique presented better LOD results than HPLC–APCI–MS method.

It was observed that SPE was an ideal technique for PAH extraction, from the most volatile to the high-molecular-weight compounds, which were shown to be more efficient when compared with LLE. One of the factors that may explain these results is the lower number of stages involved in the SPE method.

River water sample analysis

In this stage, after the optimization of all extraction and determination conditions using Milli-Q-grade water spiked with analytical standards, analysis of the Araraquara city surface river water (water collections were made in the basins of the Ribeirão das Cruzes, Córrego do Paiol and Córrego das Anhumas, Brazil) was carried out with the goal of identification and quantitation of the investigated PAHs.

Preliminary tests revealed that matrix effects were not present (if present, they were considered negligible) in the real samples. For this reason, the validation developed and reported using purified water was directly transferred to the real samples.

Compound	Recovery (%)	RSD (%) ⁿ⁼³
Naphthalene	n.q. [†]	–
Acenaphthylene	n.q.	–
Acenaphthene	n.q.	–
Phenanthrene	85.0	8.0
Anthracene	91.0	8.0
Fluoranthene	95.0	8.5
Pyrene	92.0	8.5
Chrysene	98.0	8.0
Dibenz(a,h)anthracene	94.0	8.0

* Final concentration of 5 µg/mL.
† n.q. = compound not quantitated.

Compound	200 mg of C18		300 mg of C18	
	Recov. (%)	RSD [†] (%)	Recov. (%)	RSD [†] (%)
Naphthalene	82.0	3.0	86.0	5.0
Acenaphthylene	91.0	3.0	91.0	5.0
Acenaphthene	91.0	3.0	87.0	5.0
Phenanthrene	91.0	3.0	91.0	5.0
Anthracene	57.0	3.0	66.0	5.0
Fluoranthene	88.0	3.0	91.0	5.0
Pyrene	89.0	3.0	90.0	5.0
Chrysene	79.0	5.0	82.0	7.0
Dibenz(a,h)anthracene	82.0	5.0	85.0	9.0

* Final concentration of 5 µg/mL.
† n = 3.

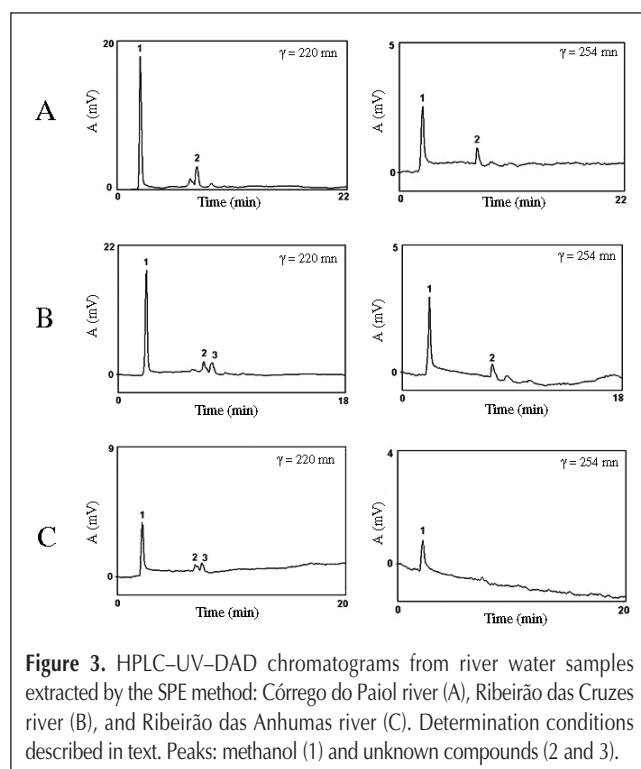


Figure 3. HPLC–UV–DAD chromatograms from river water samples extracted by the SPE method: Córrego do Paiol river (A), Ribeirão das Cruzes river (B), and Ribeirão das Anhumas river (C). Determination conditions described in text. Peaks: methanol (1) and unknown compounds (2 and 3).

SPE was chosen as the extraction method for the real samples because of its simplicity, excellent recovery results obtained from the spiked water samples, and good data reproducibility. The SPE procedure applied to the real samples was identical to that described for the spiked samples (shown in the Experimental section).

Typical chromatograms obtained by HPLC–UV–DAD from river water samples are shown in Figure 3. As can be observed, none of the PAHs was found under the analytical conditions used. However, some peaks are present, such as methanol, used in the C18 cartridge conditioning (denoted by number 1 in all chromatograms). Other peaks of lower intensity (such as those denoted by numbers 2 and 3 in the chromatograms corresponding to the Córrego das Anhumas water samples) showed retention time next to the acenaphthylene analytical standard. However, this hypothesis was discarded because its UV spectrum did not show any similarity with the UV spectrum obtained using a standard solution of this compound.

Figure 4 shows a chromatogram obtained by HPLC–APCI–MS from Córrego do Paiol extracted water sample. As shown in this chromatogram, a peak with a retention time of 5.16 min was detected in water samples of both Córrego do Paiol and Córrego das Anhumas, at m/z 252 [corresponding to benzo(a)pyrene]. However, the identity of this compound was not confirmed because, in accordance with the developed method, benzo(a)pyrene elutes in a retention time of approximately 23 min (see Figure 2). All other water samples analyzed by this technique showed the same chromatographic profile and, for this reason, were not presented in this paper.

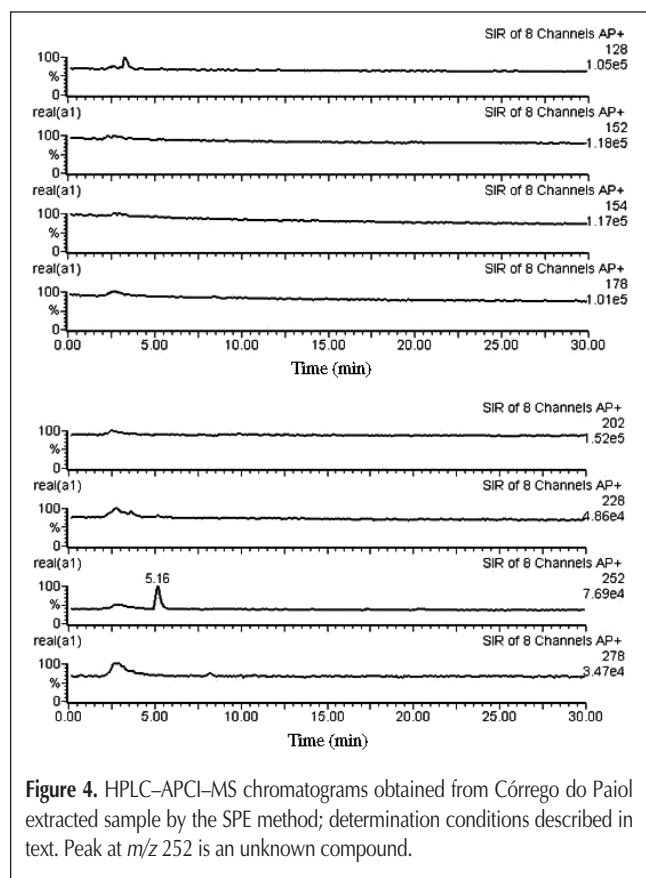


Figure 4. HPLC–APCI–MS chromatograms obtained from Córrego do Paiol extracted sample by the SPE method; determination conditions described in text. Peak at m/z 252 is an unknown compound.

Conclusion

The developed method showed that, between the extraction techniques used, LLE was revealed to be more adequate for extraction of the high-molecular-weight PAHs, though SPE proved to be efficient for extraction of all compounds. The best LOQ values were obtained using HPLC–UV–DAD because of the characteristics of the PAH molecules (presence of chromospheres groups) that make the UV technique very sensitive for this class of compounds. However, MS detection shows some advantages in relation to UV detection, such as structural information about compounds, which is very important in order to prevent false positives with relation to the identity of compounds in the real sample.

In the analysis of the river water samples from Araraquara by HPLC–UV and HPLC–MS, no PAH was found using the developed and optimized methodology for these compounds.

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