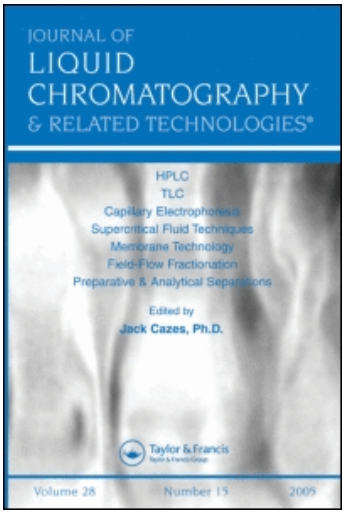

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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY HPLC WITH SPECTROFLUORIMETRIC DETECTION AND WAVELENGTH PROGRAMMING

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ABSTRACT

The sixteen polycyclic aromatic hydrocarbons (PAH) classified as priority pollutants by the EPA have been determined by HPLC, in isocratic conditions, with spectrofluorimetric detection and wavelength programming. The possibility of selecting the optimum wavelengths for each PAH gives the advantages of increased sensitivity - reaching the low pg level - and of improved selectivity, because it is possible to carry out an independent determination of coeluting compounds, provided that their excitation and emission spectra are different enough.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are of anthropogenic and natural origin and can be found in a wide range of concentrations in all kinds of environmental samples.[1] As their carcinogenic and mutagenic properties are well known, it is of the utmost importance to develop fast, selective and sensitive procedures for their identification and detection in all kinds of samples.

The most widely used procedures for their analysis are gas chromatography with either FID or MS detection[2-3] and high performance liquid chromatography with UV-Visible or fluorimetric detection.[4-7] One of the methods offering better promises is that of HPLC with fluorescent detection (HPLC-FI). The possibility of selecting both the excitation and emission wavelengths significantly increases the selectivity and sensitivity of this technique over those obtained by UV-Visible detection. Moreover, its already good performances can be widely improved by the use of the so-called wavelength-programming method, in which, instead of recording the whole chromatogram at fixed settings, the excitation and emission wavelengths are changed as a function of time, in order to use the optimum conditions for each compound to be determined.[8-10]

According to the data available in the literature, separation and determination of PAH is better carried out making use of C_{18} columns with a high polymerization degree[9] and with an acetonitrile-water mobile phase. In most cases results are improved by the use of an elution gradient.[11-12]

In the present paper an isocratic elution procedure has been used, because of the limitations of the chromatographic system. In these conditions separation is not perfect, as two of the PAH (benzo[a]pyrene and crysene) coelute and others (acenaphthene, anthracene, phenanthrene and fluorene, or benzo[ghi]perylene and indene [1,2,3-cd]pyrene) have closely similar retention times. However, the individual determination of all compounds studied - with the exception of acenaphthylene, whose fluorescence is virtually nil - is made possible by the use of wavelength programming and, eventually, the recording of two consecutive chromatograms. Moreover, the use of the optimum excitation and emission wavelengths for each compound has led to a significant improvement of the detection limits, which have been lowered to the low-pg level.

This method has been used for the determination of PAH in a reference material (marine sediment HS-3, NRCC) with satisfactory results.

MATERIALS

Stock standard solutions (about 200 $\mu g.mL^{-1}$) of acenaphthene, anthracene, bez[a]anthracene, benzo[a]pyrene, crysene, dibenzo[a,h]anthracene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene were prepared by dissolving the pure solid (Supelco) in either methanol or acetonitrile, depending on its solubility. Solutions of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene and indene[1,2,3-cd]pyrene in either acetonitrile or methylene chloride (all at about 200 $\mu g.mL^{-1}$), as well as a standard solution containing the sixteen PAH classified as

primary pollutants by the EPA, were purchased from Supelco. Working standards were prepared by dilution of the stock solutions with acetonitrile.

A certified reference material (marine sediment HS-3, National Research Council of Canada) was used to test the validity of the method.

Acetonitrile, methanol and methylene chloride were of HPLC quality (Merck).

Doubly distilled water (Culligan Ultrapure GS) was used in the mobile phase.

The mobile phase consisted of 80/20 acetonitrile/water and, before use, it was filtered through a 0.22 μm membrane filter and degasified with an helium stream.

The chromatographic system consisted of a twin-piston Gynkotek 480 HPLC pump, a Gynkotek MSV6 automatic injector, with a 20 μL injection loop, and a 125 mm LiChrospher 100 RP-18 column (Merck, Darmstadt), with 4 mm internal diameter and 5 μm particle size. An isocratic elution procedure was used throughout.

An Aminco Bowman Series 2 spectrofluorimeter, equipped with a 25 μl flow cell (Hellma 176.752) was used for detection.

RESULTS AND DISCUSSION

Standard solutions of all the individual compounds were used to determine the optimum excitation and emission wavelengths and the actual retention time for all of them in the experimental conditions used. Results are given in Table 1.

The standard solutions containing all 16 PAH was then injected. As the isocratic elution procedure used did not allow a complete separation of all compounds, two consecutive chromatograms were recorded. The spectrofluorimeter was programmed to shift excitation and emission wavelengths in order to detect alternatively eluting compounds in their optimum conditions. Therefore, the first chromatogram was used for the determination of naphthalene, fluorene, phenanthrene, fluoranthene, crysene, benzo[a]pyrene, benzo[k]fluoranthene and indene [1,2,3-cd]pyrene, while the second was used for the determination of acenaphthene, anthracene, pyrene, benzo[a]anthracene, dibenzo[a,h]anthracene, benzo[b]fluoranthene and benzo[ghi]perylene.

The wavelength programming allowed the independent determination of

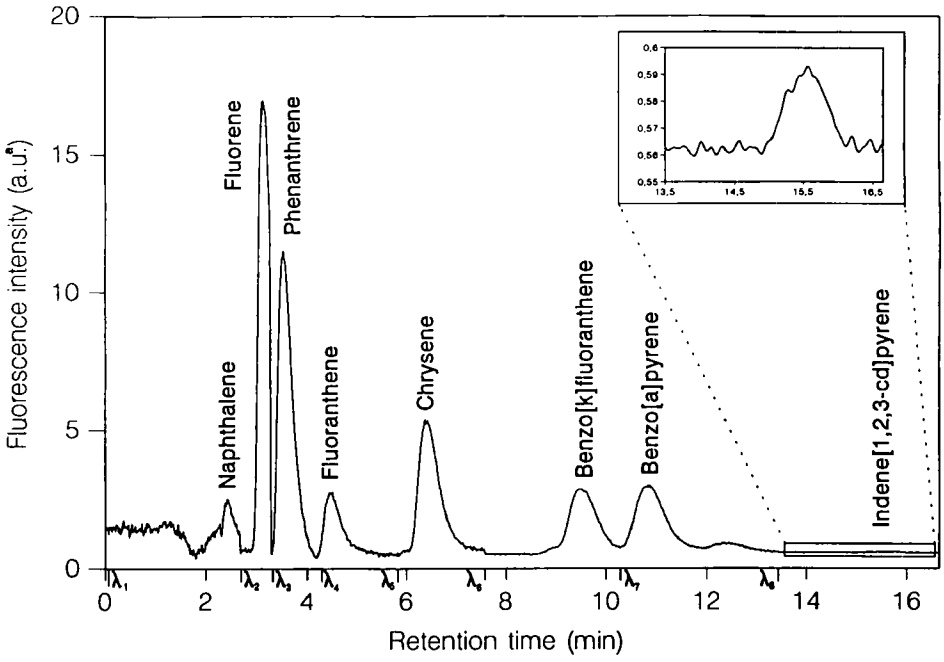

Figure 1. Chromatogram of a solution containing sixteen PAHs obtained with detector conditions set to detect eight of them. [a] Arbitrary units.

compounds which, in fact, could not be separated in the elution conditions used. This is shown in Figure 1 and Figure 2. Some overlapping can still be observed in some cases, but this did not affect the results, as the retention times were extremely reproducible and the wavelengths were shifted before the maximum of each peak was reached. This fact allowed the peak height to be measured, even if in some cases the whole peak could not be recorded.

The fact that wavelengths programming virtually eliminated the interference from coeluting compounds is shown in Fig. 3. In Fig. 3a, the detector was set at the optimum conditions for acenaphthene, and only fluorene did show a low fluorescence intensity, which did not interfere. In Fig. 3b and 3c the detector was set at the optimum conditions for anthracene and fluorene, respectively, and at these conditions the intensities of the other compounds were virtually nil. Only in the case of phenanthrene (Fig. 3d) had other compounds (acenaphthene and fluorene, concretely) significant fluorescence, but their retention times were different enough to allow the peak height of phenanthrene to be measured.

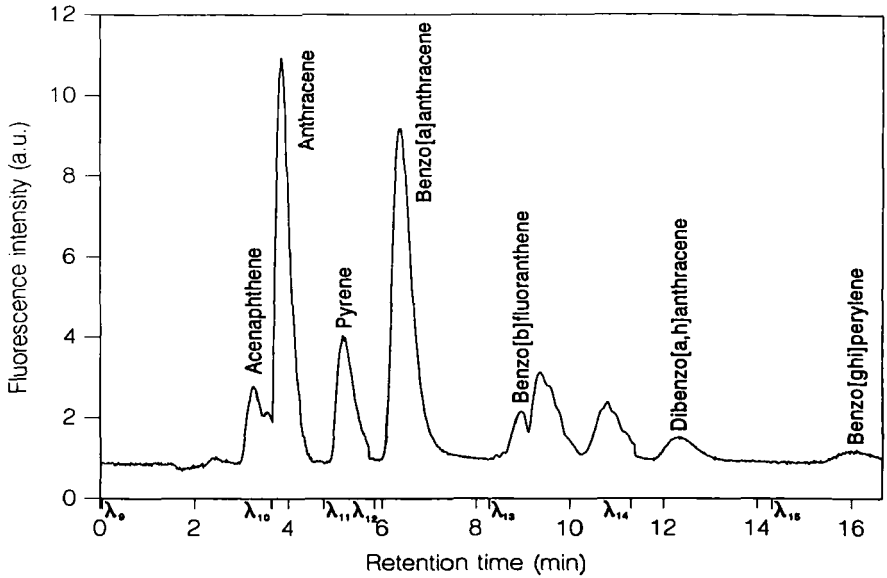

Figure 2. Chromatogram of a solution containing sixteen PAHs obtained with detector conditions set to detect the remaining seven of them (Acenaphthylene is not fluorescent).

After testing the correctness of the proposed method, the quality parameters were determined. In Table 2, detection limits (calculated as three times the standard deviation of the blank), the linear range and the precision, both of the peak height and of the retention time, for each compound are shown. The precision was determined at an amount of PAHs equal to 10-fold its detection limit. It can be observed that the possibility of always using the optimum excitation and emission wavelengths has led to very good detection limits, better, in some cases, than the lowest given in the literature.

The linear range given for each compound was found at the experimental conditions used to determine its detection limit. This means that the detector is set to detect very low amounts of the compound and that it can be easily saturated if the PAH is very fluorescent. Even with this difficulty, the linear ranges go from a minimum level of between 3 and 170 pg of injected PAH to a maximum level of between 500 and 5000 pg (Table 2). These limits could be increased by changing the detection conditions.

Precision for retention times ranged between 0.5 % and 1.5 % RSD, while for

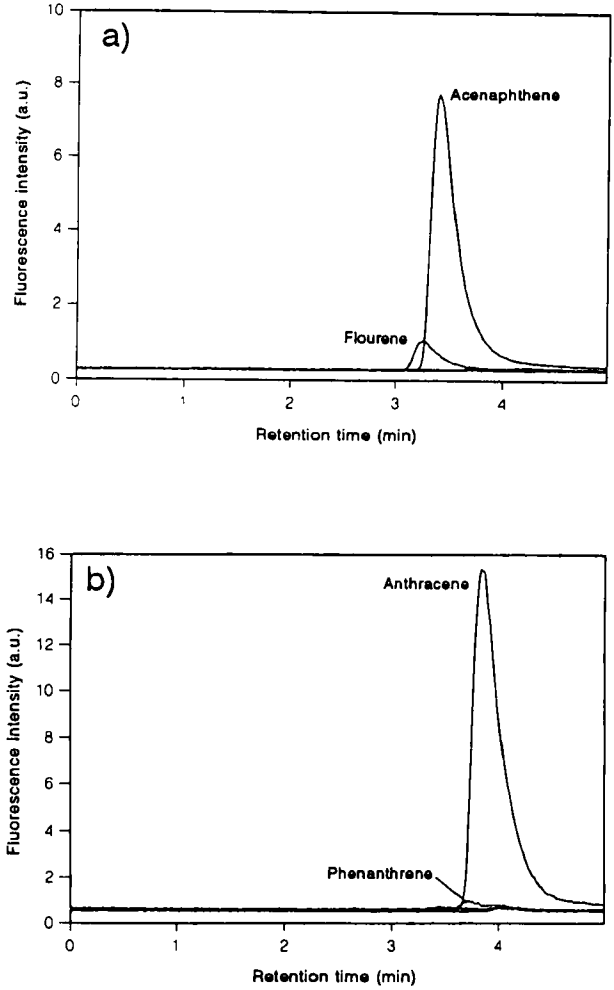

Figure 3. Determination of co-eluting PAHs (acenaphthene, anthracene, fluorene and phenanthrene) with wavelength programming.
a) Detector set at the conditions for acenaphthene. b) Detector set at the conditions for anthracene. c) Detector set at the conditions for fluorene. d) Detector set at the conditions for phenanthrene.

peak heights RSD increased from 1.9 % to 10% as the retention time increased.

When the detection limits were determined, the calculated values did not agree

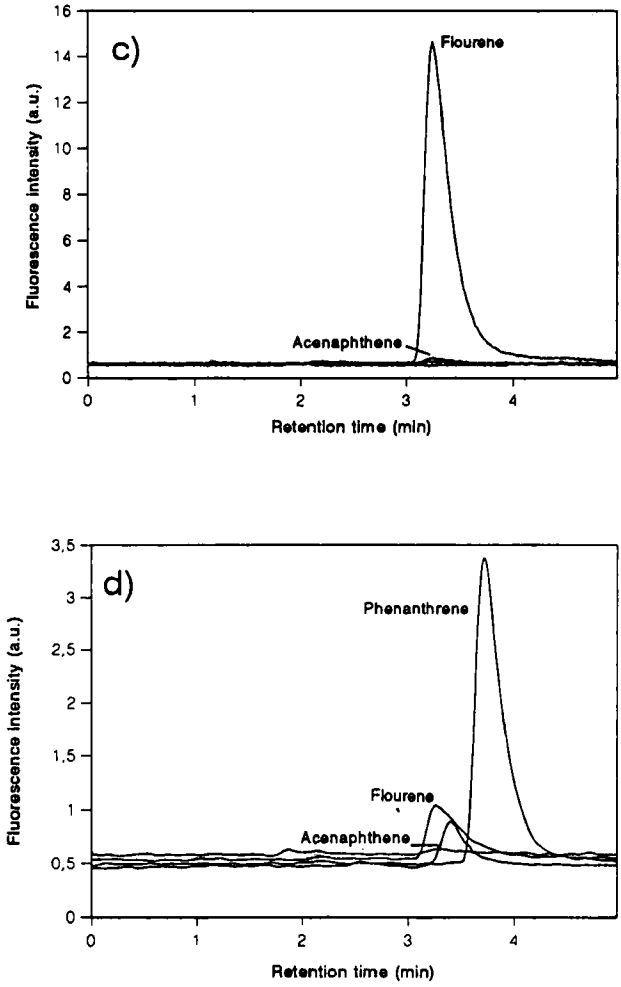

Figure 3. (continued). See previous page for details.

with those that could be observed from the experimental data. The difficulty lay in the fact that an increase in the voltage applied to the photomultiplier meant an increased signal, but also an increased background noise. In order to improve the signal-to-noise ratio, the chromatograms were smoothed by the fast Fourier transform algorithm (11 points).[13] This method significantly reduced the background noise but left the peak unchanged. In Table 3 the detection limits found

Table 1

Optimal Wavelengths and Retention Times for Each PAH

PAH	λ_{ex} (nm)	λ_{em} (nm)	t_R (min)
Acenaphthene	290	323	3.35
Anthracene	251	402	3.83
Benz[*a*]anthracene	287	388	6.32
Benzo[*b*]fluoranthene	254	437	9.97
Benzo[*k*]fluoranthene	304	411	9.60
Benzo[*ghi*]perylene	291	409	16.15
Benzo[*a*]pyrene	295	406	10.65
Chrysene	267	363	6.28
Dibenzo[*a,h*]anthracene	297	396	12.28
Fluoranthene	286	464	4.40
Fluorene	265	304	3.13
Indene[1,2,3-*cd*]pyrene	295	496	15.67
Naphthalene	221	323	2.42
Phenanthrene	250	348	3.50
Pyrene	275	374	5.08

by this procedure are shown. These limits, moreover, coincide with those that can be observed in practice.

This method was used to determine the PAHs of a certified reference material (marine sediment HS-3, NRCC). Two extraction procedures (sonication and Soxhlet), two solvents (acetonitrile and methylene chloride) and several extraction times were tested.

In the sonication method, about 1 g of sample was extracted in an ultrasonic bath with 25 mL of the solvent. No increase in recovery was detected with extraction times longer than 45 minutes. A second extraction increased the recovery only slightly (about 3%). Therefore, a single extraction of 45 minutes was used in all further research. The solution was filtered through a membrane filter before use. If methylene chloride was used for extraction, acetonitrile was then added and the methylene chloride was evaporated in a nitrogen stream. In Table 4 the recovery values are given.

In the Soxhlet extraction method, about 2 g of sample were extracted with 250

Table 2

Figures of Merit for the Method

PAH	Detection limit (pg)	Linear Range (pg)[a]	RSD(%)[b] t_R	RSD(%)[b] Peak Ht
Acenaphthene	12.4	41 - 2000	1.0	3.4
Anthracene	3.7	12 - 2000	1.8	1.9
Benz[*a*]anthracene	2.5	8 - 1000	1.2	3.5
Benzo[*b*]fluoranthene	16.0	53 - 2000	0.7	7.8
Benzo[*k*]fluoranthene	0.8	3 - 500	0.7	8.8
Benzo[*gh*]perylene	42.5	142 - 4500	0.6	3.1
Benzo[*a*]pyrene	2.3	8 - 1000	1.3	6.6
Chrysene	7.7	26 - 4000	1.2	5.8
Dibenzo[*a,h*]anthracene	3.2	11 - 2500	1.1	7.0
Fluoranthene	41.3	138 - 3000	0.9	3.7
Fluorene	5.0	17 - 1250	0.6	2.9
Indene[1,2,3-*cd*]pyrene	28.0	93 - 5000	1.0	3.0
Naphthalene	51.0	170 - 5000	0.7	3.8
Phenanthrene	21.7	72 - 5000	1.1	4.4
Pyrene	29.1	97 - 5000	1.1	4.9

[a]At the same conditions used for the detection limits
[b]Mean of six independent replicates at a concentration ten times the detection limit

mL of methylene chloride for 12 hours at 4-6 cycles per hour. The extract was filtered and the solvent evaporated as before. As shown in Table 4, no significant differences in recoveries were found between this method and the sonication procedure, in concordance with literature data.[14]

Recoveries, calculated from the certified mean values, are of about 60%. It must be noticed, however, that in some cases the certified values have very wide confidence intervals.

It was decided not to spike the sample, because, according to literature data,[15] there are significant differences in the recoveries of native and spiked PAHs.

Table 3

Detection Limits after FFT Smoothing of the Chromatograms

PAH	Detection Limit (pg) After FFT Smoothing
Acenaphthene	1.7
Anthracene	1.7
Benz[*a*]anthracene	0.8
Benzo[*b*]fluoranthene	4.2
Benzo[*k*]fluoranthene	0.2
Benzo[*ghi*]perylene	3.4
Benzo[*a*]pyrene	0.7
Chrysene	2.4
Dibenzo[*a,h*]anthracene	0.7
Fluoranthene	9.9
Fluorene	1.0
Indene[1,2,3-*cd*]pyrene	8.4
Naphthalene	22.9
Phenanthrene	1.8
Pyrene	6.8

Table 4

Recoveries of PAHs in a Marine Sediment Sample (HS-3, NRCC)

	HS-3 Certified Value ($mg \cdot kg^{-1}$)	Recovery[a] Sonication With Acetonitrile		Recovery[a] Sonication With Dichloromethane		Recovery[a] Soxhlet With Dichloromethane	
		$mg \cdot kg^{-1}$[b]	%[c]	$mg \cdot kg^{-1}$[b]	%[c]	$mg \cdot kg^{-1}$[b]	%[c]
Acenaphthene	4.5 ± 1.5	2.6 ± 0.2	57.8 (2.2)	3.3 ± 0.7	73.3 (6.7)	3.6 ± 0.2	80.0 (2.2)
Anthracene	13.4 ± 0.5	3.9 ± 0.2	29.1 (0.7)	4.0 ± 0.5	29.9 (1.5)	3.4 ± 1.2	25.4 (3.7)
Benz[*a*]-anthracene	14.6 ± 2	10.9 ± 2.5	74.7 (6.8)	8.6 ± 1.2	58.9 (3.4)	10.5 ± 0.2	71.9 (0.7)
Benzo[*b*]-fluoranthene	7.7 ± 1.2	9.4± 1.7	122.1 (9.1)	11.1 ± 0.5	144.2 (2.6)	10.8 ± 0.7	140.3 (2.8)
Benzo[*k*]-fluoranthene	2..8 ± 2	3.8 ± 0.2	135.7 (3.6)	3.6 ± 0.5	135.7 (3.6)	4.0 ± 0.2	142.9 (3.6)
Benzo[*ghi*]-perylene	5 ± 2	3.0 ± 0.7	60.0 (6.0)	3.4 ± 0.5	68.0 (4.0)	2.9 ± 0.2	58.0 (2.0)
Benzo[*a*]pyrene	7.4 ± 3.6	5.4 ± 1.2	73.0 (6.8)	4.2 ± 0.5	56.8 (2.7)	3.3 ± 2.2	44.6 (12.1)
Chrysene	14.1 ± 2	8.8 ± 2.2	62.4 (6.4)	8.1 ± 0.7	57.4 (2.1)	6.4 ± 1.5	45.4 (4.3)
Dibenzo[a,h]-anthracene	1.3 ± 0.5	1.0 ± 0.2	76.9 (7.7)	1.0 ± 0.2	76.9 (7.7)	0.8 ± 0.2	61.5 (7.7)

Table 4. (Continued)

Recoveries of PAHs in a Marine Sediment Sample (HS-3, NRCC)

	HS-3 Certified Value ($mg \cdot kg^{-1}$)	Recovery[a] Sonication With Acetonitrile		Recovery[a] Sonication With Dichloromethane		Recovery[a] Soxhlet With Dichloromethane	
		$mg \cdot kg^{-1}$[b]	%[c]	$mg \cdot kg^{-1}$[b]	%[c]	$mg \cdot kg^{-1}$[b]	%[c]
Fluoranthene	60 ± 9	54.5 ± 3.2	90.8 (2.2)	77.0 ± 8.7	128.3 (5.8)	60.9 ± 4.0	101.5 (2.7)
Fluorene	13.3 ± 3.1	7.3 ± 0.2	54.8 (0.8)	7.5 ± 1.0	56.4 (3.0)	7.4 ± 0.7	55.6 (2.2)
Indene[1,2,3-*cd*]-pyrene	5.4 ± 1.3	3.9 ± 1.2	72.2 (9.3)	6.0 ± 0.5	111.1 (3.7)	3.5 ± 0.5	64.8 (3.7)
Naphthalene	9.0 ± 0.7	8.0 ± 0.7	80.8 (3.0)	9.9 ± 0.7	110.3 (3.0)	4.7 ± 0.5	52.2 (2.2)
Phenanthrene	85 ± 20	53.6 ± 5.7	63.1 (2.7)	57.9 ± 14.1	68.1 (6.7)	50.7 ± 6.9	59.6 (3.3)
Pyrene	39 ± 9	27.3 ± 1.7	70.0 (1.8)	33.7 ± 5.5	86.4 (5.6)	33.8 ± 3.5	86.7 (3.6)

[a]Mean of three independent determinations
[b]Confidence intervals at 0.05 significance level
[c]The relative standard deviation of recoveries are given in parentheses

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