

Liquid Chromatography

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Analysis of PAHs in Marine Plastic Debris from North Atlantic Gyre by UHPLC and Fluorescence Detection

Introduction

Accumulation of microplastic debris in the marine environment is a cause of

societal concern due to the large amount, the persistence and the lack of knowledge about the impact on the marine ecosystem and food chain. An additional worry however is the potential for microplastic particles to attract and concentrate persistent organic pollutants (POPs) on the particle surface to levels much higher than in the surrounding water. These concentrated POPs can be potentially harmful to bio-organisms in the ocean. One example is polyaromatic hydrocarbons (PAHs) which can lead to toxic or chronic effects.¹⁻³ Consequently, the 16 most relevant PAHs in term of environmental pollution have become intensively studied pollutants in plastic debris. Standard and official methods for their analysis are available in guidelines for air, water, solid waste and food analysis. These methods generally specify UHPLC, usually with UV and fluorescence detection, with run times more than 30 min. To attain better selectivity, stationary phases specifically designed for PAHs analyses are required also as more powerful UHPLC systems. Ultra-performance chromatography systems allow working at pressures up to 800 bar and therefore with columns packed with small particles (sub-2 μm particle sized). The aim of this study was to investigate the resolution limit and analysis time for 15 PAHs using a PerkinElmer UHPLC system in combination with the very sensitive fluorescence detection according to the NF T90-210 standard in order to analyze very low concentrations of PAHs (in the range of ppb) in plastic debris. In this application note you will learn about a rapid UHPLC gradient method for simultaneous determination of 15 polyaromatic hydrocarbons (PAHs) according to NF standard T 90-210 (v. 05/2009 (5.2)).⁴ A baseline resolution for all 15 target analytes in less than 18 minutes could be developed using an Agilent Zorbax Eclipse PAH 1.8 μm column. Fluorescence detection with the powerful PerkinElmer UHPLC system allows limits of quantification below 5 ppb for all PAHs, except acenaphthene, phenanthrene fluoranthene benzo(b) fluoranthene and benzo(k)fluoranthene with a lower LOQ (2.5 ppb), and fluorene, anthracene benzo(a) anthracene, benzo(a)pyrene and pyrene with the lowest ones (0.5 to 1 ppb).

Experimental

Hardware/Software

A PerkinElmer UHPLC system was used, including the Solvent Delivery Module (quaternary pump), Sample Module and Column Module (PerkinElmer, Shelton, CT, USA). An Agilent Zorbax Eclipse PAH 1.8 μm 150 x 2.1 mm column (Agilent Technologies, Les Ulis, France) was used for all separations. Detection was accomplished with PerkinElmer UHPLC Solvent Delivery Module, UV detector and FL detectors (PerkinElmer, Shelton, CT, USA). All instrument control, analysis and data processing was performed via associated data handling software.

Method Parameters

LC method parameters are gathered in the following Tables. In Table 1, general conditions and mobile phase gradient with eluent A as acetonitrile and eluent B as water and in Table 2, fluorescence detector program.

Table 1. LC conditions and mobile phase gradient.

LC Conditions																													
Column	Agilent Zorbax Eclipse PAH 1.8 μm 150 x 2.1 mm ID																												
Flow Rate	0.35 mL min ⁻¹																												
Eluent A	ACN																												
Eluent B	H ₂ O																												
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%A</th> <th>%B</th> <th>Curve</th> </tr> </thead> <tbody> <tr> <td>Initial</td> <td>40</td> <td>60</td> <td>Initial</td> </tr> <tr> <td>1</td> <td>40</td> <td>60</td> <td>6</td> </tr> <tr> <td>15</td> <td>100</td> <td>0</td> <td>6</td> </tr> <tr> <td>22</td> <td>100</td> <td>0</td> <td>6</td> </tr> <tr> <td>23</td> <td>40</td> <td>60</td> <td>6</td> </tr> <tr> <td>28</td> <td>40</td> <td>60</td> <td>6</td> </tr> </tbody> </table>	Time (min)	%A	%B	Curve	Initial	40	60	Initial	1	40	60	6	15	100	0	6	22	100	0	6	23	40	60	6	28	40	60	6
	Time (min)	%A	%B	Curve																									
	Initial	40	60	Initial																									
	1	40	60	6																									
	15	100	0	6																									
	22	100	0	6																									
23	40	60	6																										
28	40	60	6																										
Oven Temperature	40 °C																												
Injection Volume	2 μL																												
UV Detection Wavelength	229 and 320 nm																												
Sampling (Data) Rate	1 pt.s ⁻¹																												
Run Time	20 min (injection-to-injection)																												

The gain for FL detector was fixed as a function of the concentration range and was equal to 100 for concentration in the range 0.5-10 ppb respectively. Four channels (A, B, C and D) method with different excitation and emission wavelength (λ_{ex} and λ_{em} respectively) was developed and is described in Table 2.

Table 2. FLD program.

Time (min)	λ (nm)	Channel A	Channel B	Channel C	Channel D
Initial	λ_{ex}	248	270	270	280
	λ_{em}	375	324	385	462
10	λ_{ex}		290		
	λ_{em}		430		
14	λ_{ex}	300			
	λ_{em}	466			

Detection of PAHs was undertaken according to the following detection conditions gathered in Table 3.

Table 3. PAHs detection conditions.

Fluorescence Channel				
PAH	A	B	C	D
Naphtalene		×		
Acenaphtene		×		
Fluorene		×		
Phenanthrene	×			
Anthracene	×			
Fluoranthene				×
Pyrene			×	
Benzo(a)anthracene			×	
Chrysene			×	
Benzo(b)fluoranthene		×		
Benzo(k)fluoranthene		×		
Benzo(a)pyrene				×
diBenzo(ah)anthracene		×		
Benzo(ghi)perylene		×		
Indeno(1,2,3-cd)pyrene	×			

Solvents, Standards and Samples

The water and acetonitrile used for UHPLC analysis were Optima[®] LC/MS grade provided by Fisher Chemical SAS. A stock PAH standard mixture was obtained from Supelco (Sigma Aldrich[®], Saint Quentin Fallavier, France). The stock mixture contained: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(j)fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and ideno(1,2,3-cd)pyrene at 10 ppm in acetonitrile. In a 10-mL volumetric flask, a stock PAH standard solution was prepared in acetonitrile to obtain the final concentration of 1000 ppb. Solutions were further diluted in acetonitrile to 0.5 to 10 ppb for fluorescence detection to determine linearity at ppb levels. Plastic debris was collected from North Atlantic gyre in May 2015 by French Expedition 7^{ème} Continent. PAHs were extracted by solvent extraction using a binary mixture of heptane and dechlorinated methane and mechanical stirring (24 h twice). Before complete evaporation, 50 μL of DMF was added and after a few minutes 200 μL of acetonitrile. All samples were filtered on 0.2 μm PTFE filters of 4 mm in diameter provided by Thermo Scientific[®] (Chromacol[®] 4-SF-02(T)) before injection.

Method Validation

This method has been validated according to the AFNOR NF T90-210 (2009) French guideline for water physicochemical analysis. This standard is used to validate analytical methods in the field of water analysis. Conventionally, the limit of quantification and the linearity of an analytical method is calculated by ten times analysis of the standard deviation (and a minimum of five times). Before the "A test" of the NF T90-210 standard, Grubbs statistical test was applied to eliminate outliers in a univariate data set. The validation of linearity was based on five times analysis (once a day during five consecutive days) of standard solutions prepared each day. This test is based on the comparison of the observed model with the experimental error via statistical test (Fisher) of adequacy.

The linearity is validated if the error associated to the model is significantly negligible compared to the experimental error. Therefore, to validate the linearity, the calibration range with six concentration levels (0.5, 1, 2.5, 5, 7 and 10 µg/L) between 0.5-10 µg/L was analyzed in conditions of repeatability. The "B test" of the NF T90-210 standard presents the advantage of being able to test and validate experimentally a presupposed

LOQ, which is a more realistic manner than that based on the standard deviation. Preliminary tests were conducted in order to estimate the LOQ; then, the validation was performed by analyzing two standards at presumed LOQ, in triplicates on five consecutive days. As fixed by the NF T90-210 standard, LOQ is validated if uncertainty is below 60%. The accuracy of the method was then verified with the "D test" of the NF T90-210 standard. This test aids the evaluation of the intermediate precision and the observed bias in comparison to prefixed references. References correspond to the maximum acceptable deviation (MAD) fixed by the laboratory (MAD = 60%). The validation of the accuracy requires the five times preparation and analysis of three standards at different concentrations in conditions of intermediate precision.

Results and Considerations

Figure 1 shows the peak profile of the 15 PAHs at a concentration of 5 ppb. Only 15 of the 16 PAHs are detected because acenaphthylene does not fluoresce. Chromatograms on Figure 1 were collected by fluorescence. Separation for the 15 PAHs was achieved in less than 17 minutes and most were quantifiable down to ppb levels, as specified in Table 3.

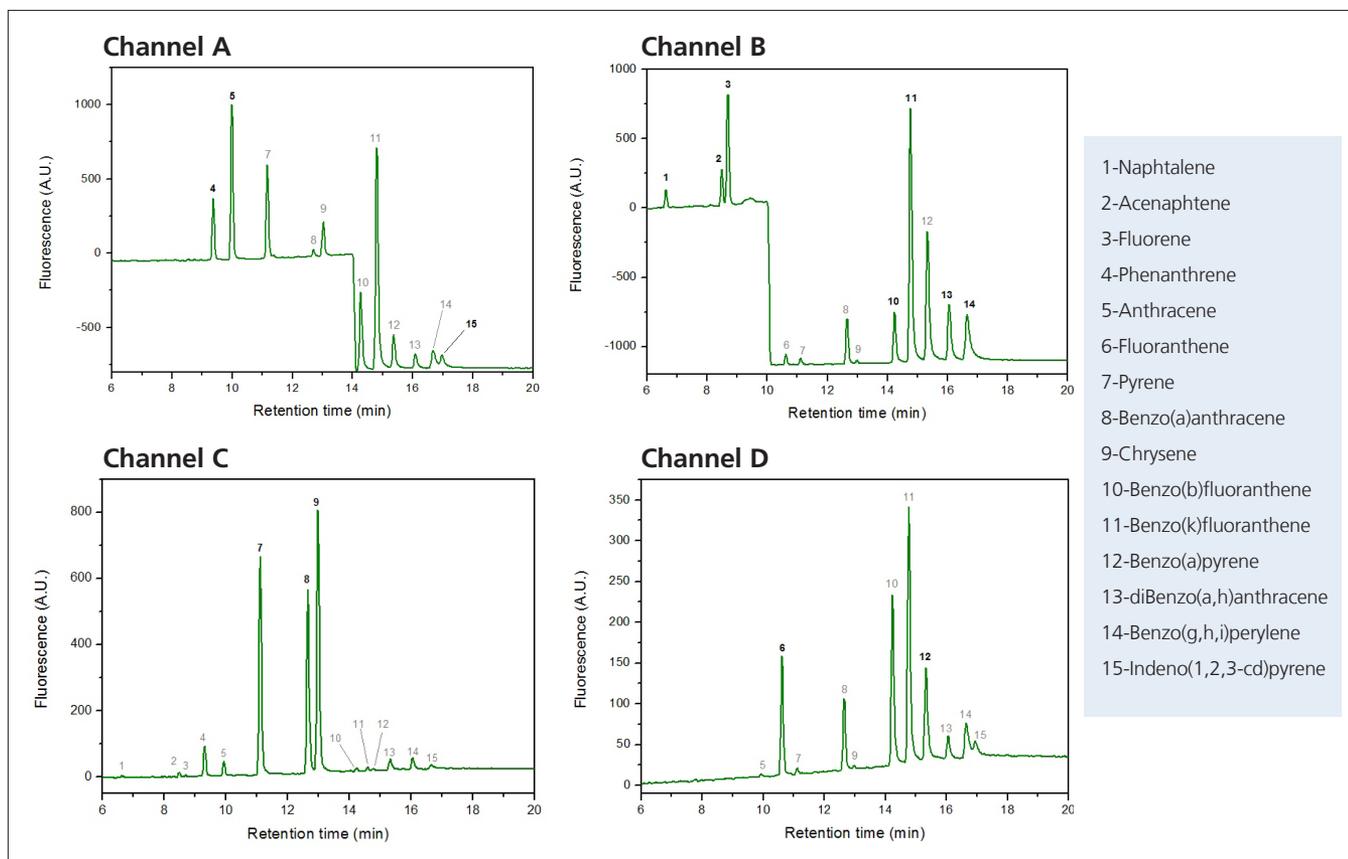


Figure 1. Fluorescence trace of the PAHs separation (7 µg/L).

Calibration

Calibration was done according to NF T 90-120 and the detection and quantification limit (LOQ) under repeatability were evaluated according to the same method. We chose to evaluate peak areas with the aim to reach a determination limit of a few µg/L for the quantification of PAHs with FL detector (FLD) E Calibration curves examples are shown in Figure 2 for the fluorescence detected substances Naphthalene, Phenanthrene, Fluoranthene and Pyrene and represented by peaks 1, 3, 4 and 6 in the FLD chromatogram shown above. The confidence interval according to EN ISO 17993 is also shown in red.

We demonstrate linearity for PAHs by fluorescence detection (Ex/Em wavelengths as specified in the NFT 90-120 method). Some calibration curves are presented in Figure 3 in the range 1-10 ppb. Using the NFT 90-120 method, correlation coefficients are indicated in Table 4 considering linear fitting ($y = ax + b$, y being the area and x PAH concentration) as well as limit of quantification (using a Maximum Acceptable Deviation of 15%). Linear fittings and regression coefficient (R^2) were automatically determined by NFT 90-120 method between LOQ and 10 ppb. One can note that for all PAHs, correlation coefficients higher than 0.999 were obtained.

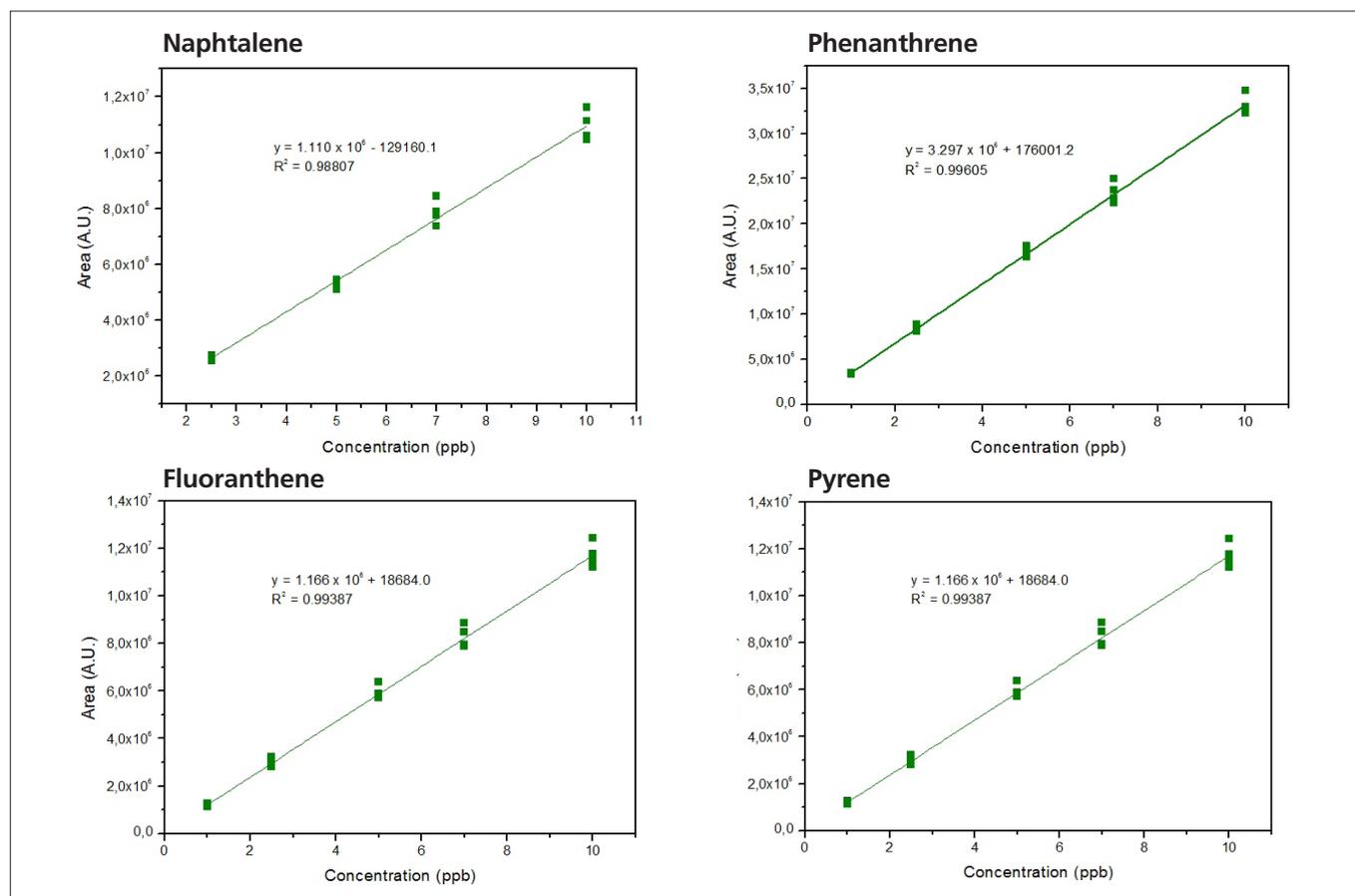


Figure 2. Calibration curve for pyrene, phenanthrene and naphthalene for the first calibration range (1-10 ppb) detected with FLD (all experimental points are plotted).

Table 4. Retention time, limit of quantification and correlation coefficient (R^2) of 16 PAHs.

Fluorescence Channel	Retention Time (min)	LOQ (ppb)	$a \times 10^7$	b	R^2
Naphtalene	6,67	5	9.0145	1.1643×10^{-1}	0.99881
Acenaphthylene*	7,43	10	3655.2	1.6127×10^{-1}	0.99993
Acenaphtene	8,54	2.5	4.9146	9.2652×10^{-3}	0.99890
Fluorene	8,74	1	1.5747	-6.5695×10^{-3}	0.99996
Phenanthrene	9,37	2.5	3.0334	-5.3388×10^{-2}	0.99994
Anthracene	10,00	1	1.1873	1.8462×10^{-2}	0.99992
Fluoranthene	10,67	2.5	8.5764	-4.2598×10^{-2}	0.99996
Pyrene	11,16	0.5	1.8317	-2.4191×10^{-2}	0.99992
Benzo(a)anthracene	12,70	1	2.1172	-3.8712×10^{-2}	0.99996
Chrysene	13,03	1	1.4742	-4.0780×10^{-2}	0.99995
Benzo(b)fluoranthene	14,29	2.5	2.9070	4.3395×10^{-2}	0.99996
Benzo(k)fluoranthene	14,83	2.5	0.5696	5.4586×10^{-2}	0.99998
Benzo(a)pyrene	15,39	1	8.3745	-3.8785×10^{-2}	0.99969
diBenzo(a,h)anthracene	16,11	5	12.2717	-3.8785×10^{-2}	0.99990
Benzo(g,h,i)perylene	16,71	5	1.9880	6.6110×10^{-2}	0.99982
Indeno(1,2,3-cd)pyrene	17,01	5	7.5081	6.6123×10^{-2}	0.99987

* UV absorption at 320 nm

Method Performance

Limit of Detection	≤ 5 ppb (detected with FLD) < 5 ppb (Acenaphthylene; detected with UV)
Linearity (r ²)	> 0.999 for all PAH
Linearity Range	1 – 10 ppb for the calibration range

As can be observed in Figure 3, quantifiable PAHs were found in plastic debris. The total amount of 15 PAHs was determined to be equal to around 20 ng/g of polymer considering the quantification of 4 of them (naphtalene, fluorene, phenanthrene and pyrene) as gathered in Table 2.

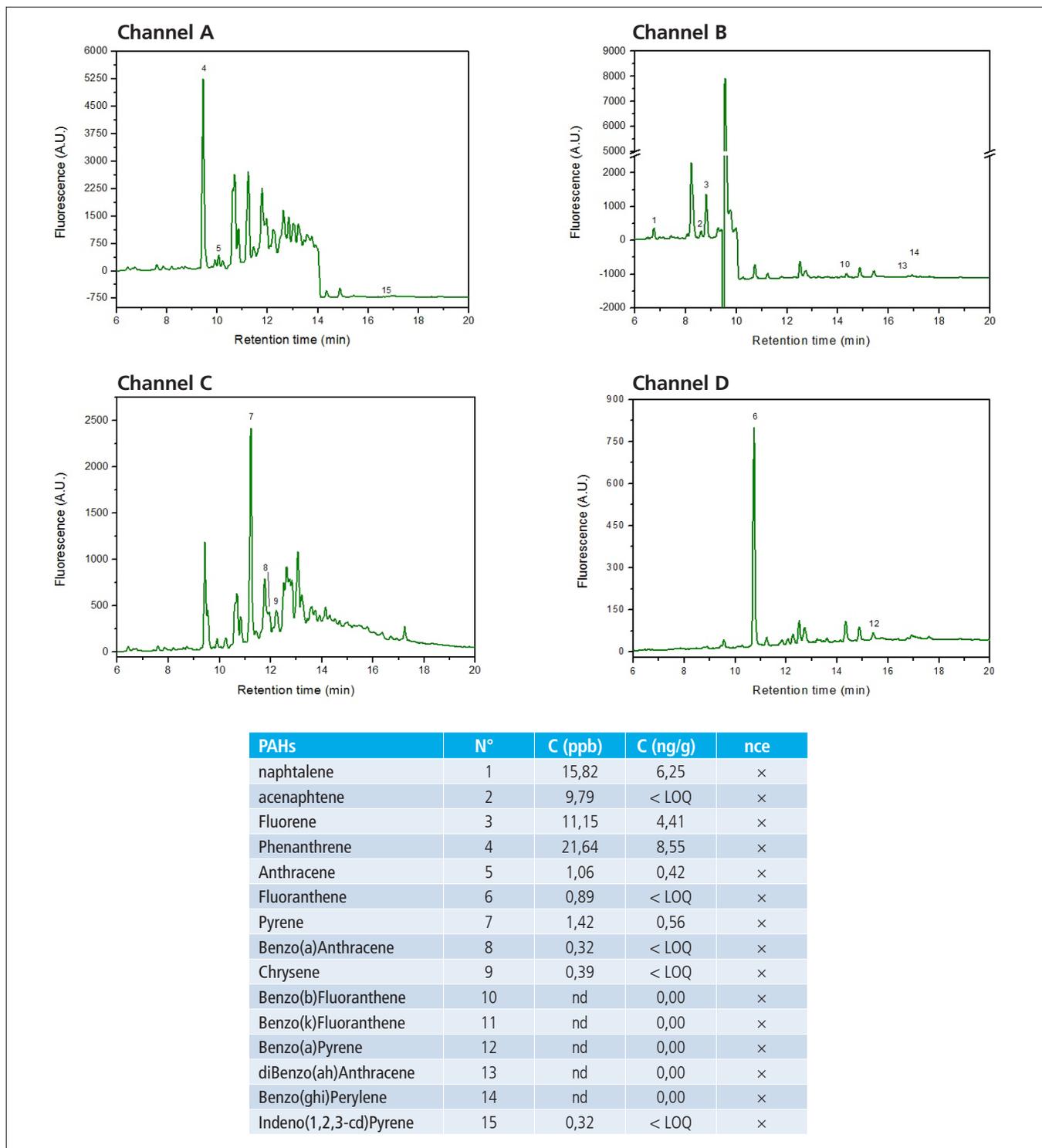


Figure 3. Fluorescence trace of a plastic debris.

Conclusions

The goal of this study was to measure the levels of PAHs concentrated on the surface of microplastic particles in order to begin to understand the risk to the marine biological ecosystem. This work has demonstrated the fast, effective chromatographic separation of 16 PAHs using a PerkinElmer UHPLC system with FL and UV absorption detector. The results exhibited exceptional linearity for each PAH over the tested concentration ranges (10-0.5 ppb).

The analyzed plastic debris showed detectable amount of PAHs and demonstrated the ability of Solvent Delivery Module FL detectors to detect PAHs at ppb levels.

The study has shown that the PerkinElmer UHPLC with fluorescence detection is a sensitive and linear approach allowing vital understanding of the potential for microplastic particles to act as a vector for PAH pollutants.

Reference

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