Validation of routine polycyclic aromatic hydrocarbons analysis in waters

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse organic compounds composed of two or more aromatic rings. Some have been classified as carcinogens. Their presence in surface water or groundwater is an indication of a source of pollution. Due to their low solubility in water, direct PAH analysis in water remains a challenge. Many methods have been developed to ensure PAH analysis, and gas chromatography-mass spectrometry (GC-MS) is the gold standard for PAH determination in complex matrices. However, the required system for direct PAH analysis in water must be more sensitive than GC-MS. Therefore, the fluorescence detector has been coupled to the latest Thermo Scientific™ Vanquish™ UHPLC system. This analysis has been developed in a routine laboratory focused on production and reliability. The recommended analytical procedures are documented in United States Environmental Protection Agency (U.S. EPA) Method 610[1] or International Organization for Standardization (ISO) 17993.[2]

The presented method is a compromise of speed, resolution, and robustness required for separation and quantification at very low levels of eleven PAHs. This method has been implemented in a compliant laboratory according to COFRAC regulation.[3] Analytical methods must be validated before production. We present a validated workflow, including sample preparation according to NFT 90-210 referential.[4] Three matrices were validated: tap water, mineral water, including carbogaseous water, and wastewater.

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Experimental

PAHs extraction

Three water types were evaluated for PAHs content during this study: tap water, mineral water, carbonated water, and wastewater. For each water type, PAHs extraction is based on liquid-liquid extraction (LLE).

For tap water and carbonated water, the same protocol has been performed:

1. Add 25 mL of hexane to the sampling container containing 1000 mL of the water sample. Shake the container.
2. Pour the entire volume into a separatory funnel. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for ten minutes with periodic venting to release pressure excess. Allow the organic layer to separate from the water phase for a minimum of 5 min.
3. Collect the aqueous layer in sample container.
4. Collect the organic layer extract (hexane) in a 250 mL Erlenmeyer flask.
5. For all samples, repeat this extraction with new hexane twice.
6. In addition, if the sample presents some visible particles, repeat this extraction with new hexane twice.
7. Remove water from the organic solution (minimum 3 × 25 mL) using anhydrous sodium sulfate (5 g) for 15 min.
8. Transfer the organic extraction solution into a tube for evaporation.
9. Rinse the Erlenmeyer flask with 10 mL hexane and add 40 µL of 1-octanol.
10. To concentrate the sample, use a low temperature (35 °C max.) evaporating system. Evaporation must be done after 20 min.
11. Add 1 mL of ethanol in the evaporating tube containing dry extract and put the tube in an ultrasonic bath for 30 s.
12. Fill the sample vial with ethanolic solution after filtration using an Acrodisc™ Teflon™ filter (0.45 µm).

Note: For carbonated waters, before extraction, shake the sample solution vigorously and use an ultrasonic bath for 45 min to achieve an efficient degassing.

For wastewater, the same approach has been used but the protocol is slightly different:

1. Add 100 mL of sample and 900 mL Evian™ water as a diluent into a separatory funnel.
2. Extract the PAHs by shaking thoroughly.
3. Add 25 mL of hexane to the sampling container containing 1000 mL of the water sample. Shake the separatory funnel for 10 min.
4. Allow the organic layer to separate from the water phase for a minimum of 5 min and up to 15 min depending on the water quality.
5. Collect the aqueous layer in a 1 L bottle.
6. Collect the organic layer extract (hexane) in a 250 mL Erlenmeyer flask.

   For this kind of sample repeat this extraction with new hexane four times.
7. Remove water from the organic solution (5 × 25 mL) using anhydrous sodium sulfate (5 g) for 15 min.
8. Transfer the organic extraction solution into a tube for evaporation.
9. Finish the extraction as described above for fresh water and carbonated water samples.

Using this LLE process, PAHs will be concentrated one thousand times. The liquid chromatography fluorescence detector (LC-FLD) PAHs analysis will be achieved using a simple direct injection without solid phase extraction (SPE) pre-concentration.

LC analysis

The Thermo Scientific™ Vanquish™ Flex UHPLC system has been used for this study. The complete setup is outlined below:

- Quaternary pump F (P/N VF-P20-A) equipped with standard mixer volume
- Split sampler FT (P/N VF-A10-A)
- Column compartment H (P/N VH-C10-A) equipped with 1 µL passive preheater
- Diode array detector FG (P/N VF-D11-A) equipped with a standard flow cell, 13 µL, SST (P/N 6083.0510)
- Fluorescence detector F (P/N VF-D50-A) equipped with a standard flow cell, 8 µL, biocompatible (P/N 6079.4230)

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.10

LC column and conditions

Parameter	Value
LC column	Thermo Scientific™ Hypersil™ Green PAH, 100 × 4.6 mm, 3 µm (P/N 31103-104630)
Mobile phase A	Water
Mobile phase B	Acetonitrile
Flow rate	1.5 ml/min
Gradient	See Table 1
Column oven	20 °C forced air mode, fan speed 5
Injection volume	20 µL
Sampler wash solution	Acetonitrile/isopropanol 50/50 v/v
Fluorescence detector settings	See Table 2

Table 1. Gradient details

Time (min)	Flow rate (mL/min)	% A	%B
0	1.5	60	40
2	1.5	60	40
8	1.5	25	75
10	1.5	25	75
11	1.5	0	100
16.5	1.5	0	100
16.5	1.5	60	40
20	1.5	60	40

The Hypersil Green PAH column features a specially tailored alkyl-bonded silica with high carbon loading. This column has been designed specifically for the separation of PAHs and optimized for the published EPA or ISO method.[1, 2] Separation of PAHs using simple eluents like water and acetonitrile will be very easy to implement in a routine laboratory.

Also, to simplify the method and improve the response stability, a common excitation/emission setting was used for compounds eluted between 10 and 14.25 minutes (Table 2).

Table 2. FLD settings

Time (min)	FLD λex (nm)	FLD λem (nm)	Sensitivity	Filter wheel (nm)	Lamp mode
0	265	350	6	280	High power
8.75	260	410	5	370	High power
9.4	290	470	6	463	High power
10	270	410	6	370	High power
11.7	270	410	3	370	High power
13.1	270	410	3	370	High power
14.25	270	410	6	370	High power
15.55	305	500	8	370	High power

Results and discussion

PAHs separation, detection, and confirmation

Separation of 17 PAHs has been performed using an acetonitrile gradient in 16 min. Additional time, up to 20 min, is required to equilibrate the column before the next injection. Figure 1 illustrates the resolving power of the Hypersil Green PAH column after injection of 20 µL of 50 µg/L standard solution and at the minimum validated level 1 µg/L. This case study focuses on 11 PAHs for complete validation; other PAHs are already validated in the laboratory using another technique. All compounds of interest are baseline resolved even at high concentration and a very high retention time stability is ensured by the Vanquish UHPLC system. Relative standard deviation of retention remains very low for all compounds of interest and stays below 0.1% (data not shown). This facilitates automated reprocessing actions and allows automated actions using Chromeleon CDS software, which includes an automated Intelligent Run Control (IRC) tool as part of the sequence acquisition and processing. Using Chromeleon CDS software, all calculations and unconditional tests are automatically performed by the software. With the IRC tool, the system can react to modify the sample list after the analysis. Based on user-specified criteria, the software can determine if PAH is detected in the sample and launch the dedicated action. In this case, the system automatically inserts a new line in the initial sequence.

A decision diagram, a sample insertion in the sequence, and tracking in the audit trail are shown in Figure 2. The IRC tool is very useful in a routine laboratory to make the right decision without the user's presence. In Figure 2, sample re-injection has been done overnight, so the laboratory productivity increased drastically. Confirmation of PAHs detected, according to the regulation, is performed only if saving time and reducing analysis cost is required. Two-dimensional data are acquired from the

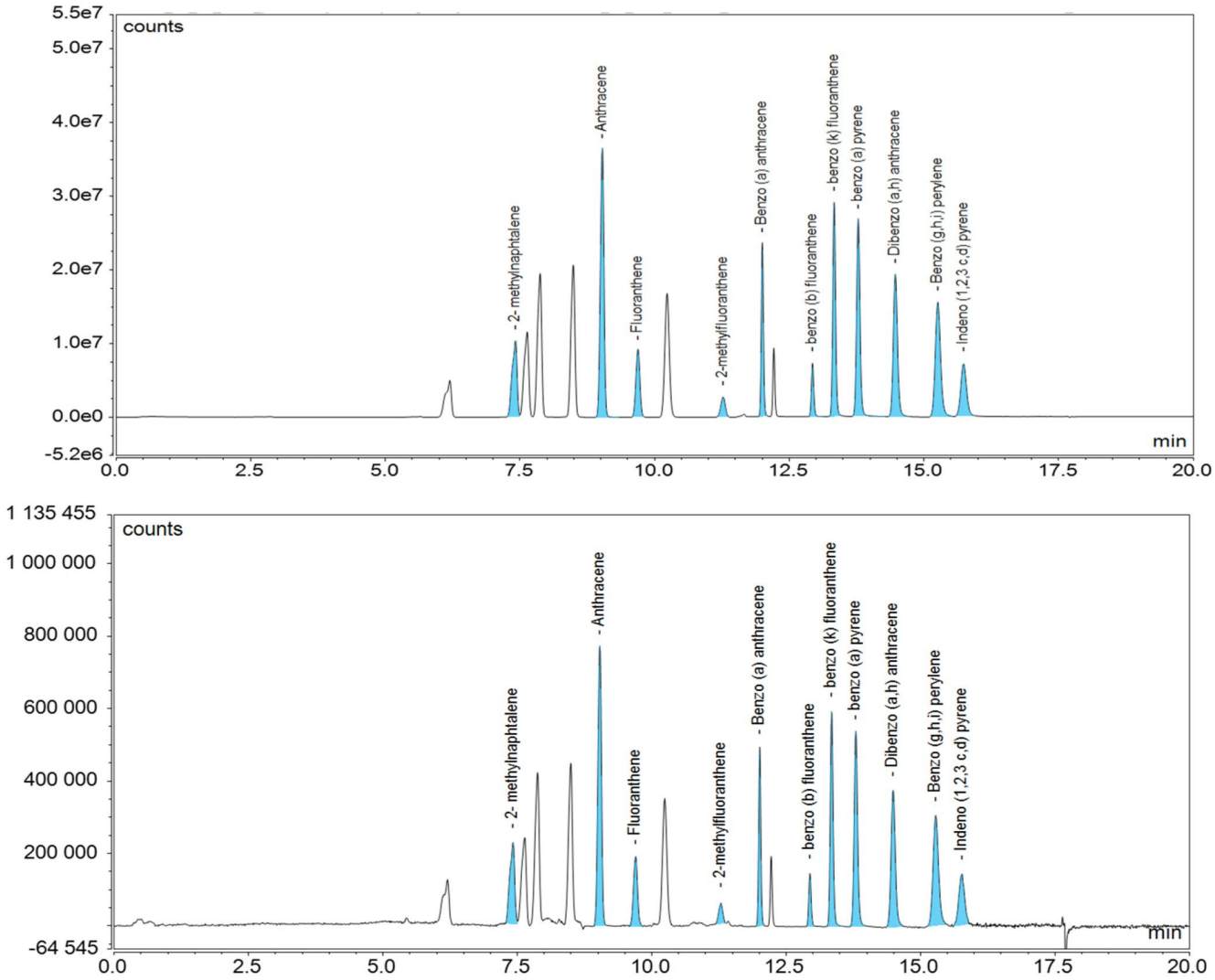

Figure 1. Chromatograms obtained after 20 µL direct injection of commercial standard solutions of 17 PAHs at 50 µg/L (upper trace) and at 1 µg/L (lower trace)

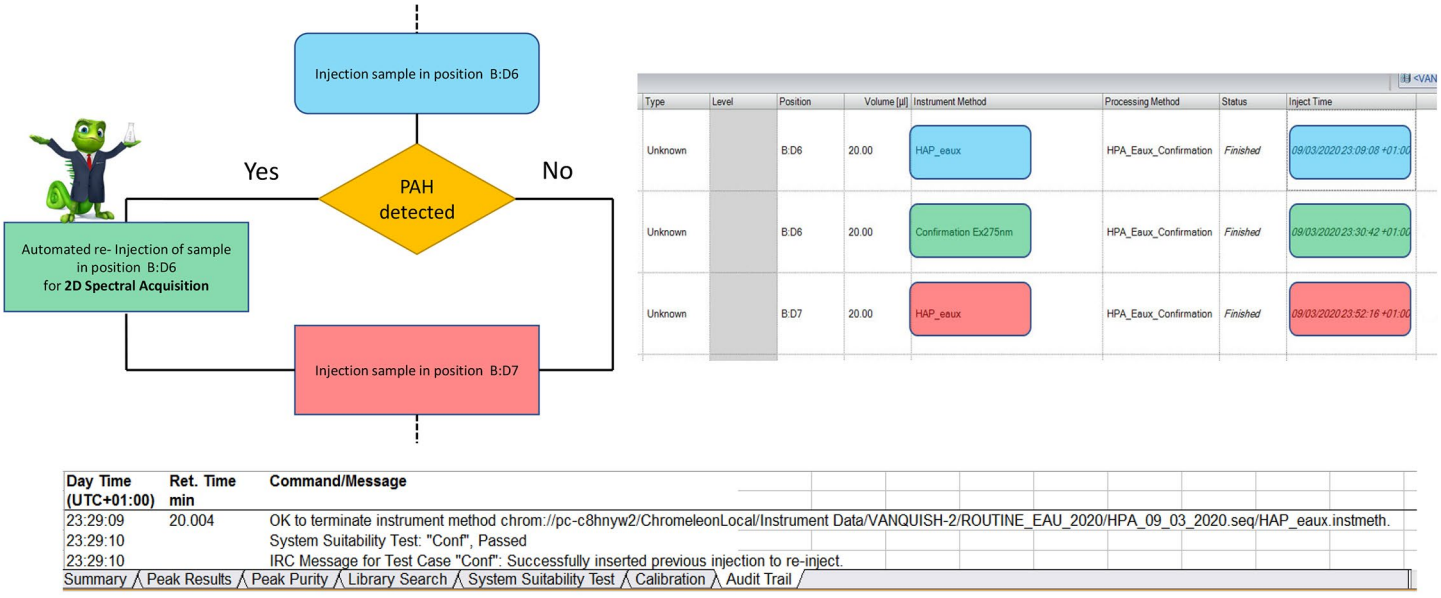

Figure 2. Decision diagram used after unknown samples analysis and illustration of automated insertion of sample in the initial sequence if PAH is detected. Sample re-injection is tracked in the sample audit trail.

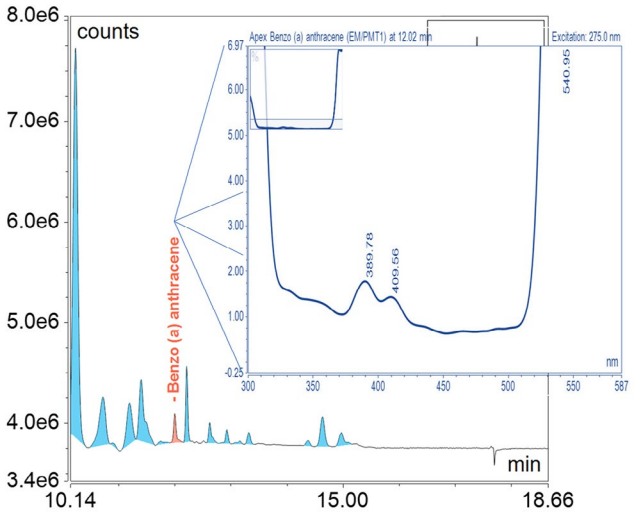

Figure 3. Benzo(*a*)anthracene 2D spectrum acquired for PAH presence confirmation: fixed excitation wavelength 275 nm and variable emission wavelength from 300 up to 587 nm

sample re-injection. Figure 3 shows a fluorescence field acquired at fixed excitation wavelength (Ex. @ 275 nm) for benzo(*a*)anthracene. Characteristic optima appear at 389.78 and 409.56 nm. Comparison with the fluorescence field previously acquired using a standard solution allows confirmation of the PAH presence.

Method validation

To validate this method, the NF T 90-210 referential was used. The first step of this validation process is an evaluation of calibration function in a calibration range by a comparison with the maximum allowed deviation. In the first time, five inter-day independent calibrations were performed. Linearity in the complete calibration range was tested. Figure 4 represents calibration linear models based on these injections' batches. The range of concentration is

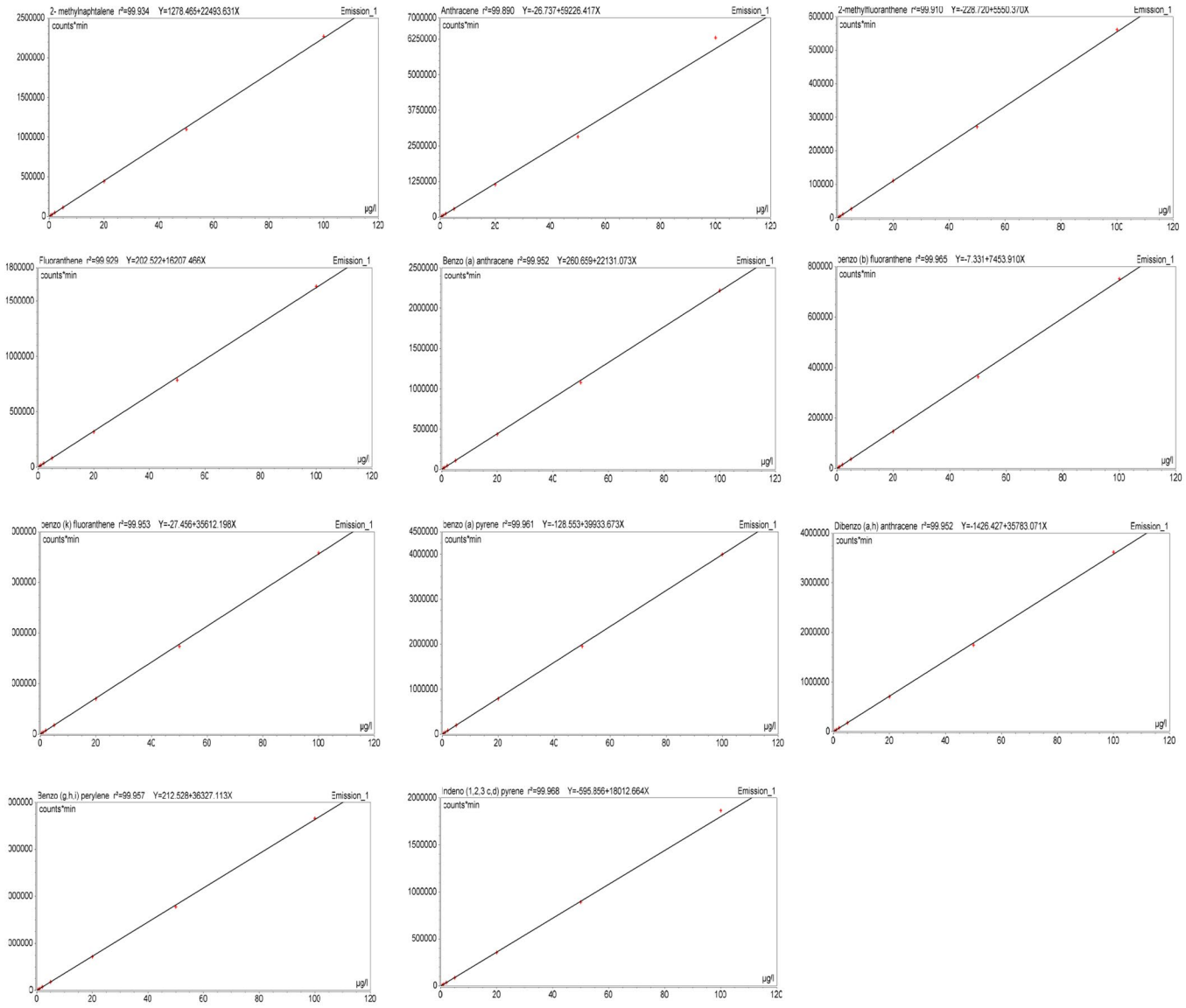

Figure 4. Calibration data for 11 PAH detected using the fluorescence detector. Calibration has been performed from 0.5 up to 100 µg/L using a commercial standard solution.

between 0.5 and 100 µg/L. The coefficient of determination for each curve is upper 0.999 (Table 3). Compilation of these data allows the evaluation of the bias in comparison with a user-fixed maximum allowed deviation. In this case, maximum allowed deviation is fixed to 20%, and each calibration level has been tested to ensure that the bias remains below the absolute targeted 20%. Figure 5 illustrates each bias for each PAH at each calibration level. The main distortion between theorical and experimental is observed at the extrema: 0.5 and 100 µg/L. However, for all compounds, the required first validation step is achieved.

Figure 6 shows the different calculated relative bias in each matrix (tap water, mineral water and wastewater) at each tested quantification limit: 1, 20, and 100 ng/L. In this test, the goal was to verify if a quantification limit is acceptable in a considered matrix. At each concentration level tested, calculated bias or relative bias from average plus standard deviation must stay lower than 60% for 1 ng/L or 30% for 20 and 100 ng/L. Data shown in Figure 6 validate all quantification limits for 2-methlufluoranthene except for 1 ng/L in wastewater matrix. In this case, method precision was ensured from 20 ng/L. Using the same process, each limit of quantification (LOQ) was determined and reported (Tables 3 and 4).

The next validation step consists of a sample preparation impact evaluation on the PAHs recovery percentage (average of five yields using three amounts 1, 20, and 100 ng/L). The recovery range was from 81 to 90%, demonstrating that this LC-FLD method provides good selectivity and suitability for the determination of PAHs in tap water and mineral water samples (Table 3). The wastewater recovery range is between 83 and 86% (Table 4), which is in accordance with limits (recovery yield allowed between 70 and 110%) set by the laboratory.

Figure 7 represents a chromatogram comparison obtained after injection of different water matrices spiked with the PAHs standard mix solution. In each matrix, separation remains stable. This method conserves resolution and peak intensity for each PAH analyzed. However, due to interfering peaks in some wastewater samples, the LOQ has been increased from 10 to 200 ng/L for 2 methylfluoranthene, benzo(*g,h,i*)perylene, dibenzo(*a,h*)anthracene, and fluoranthene (Table 4).

Table 3. Performance summary for each PAH in tap water and mineral water

Compound	Plan A maximum bias calculated over the concentration range	Plan B LOQ purposed	Amount range (ng/L)	Plan C % recovery	Plan D interferers evaluation	Method validation NF T 90-210
2-methylfluoranthene	5.46%	1	1 to 100	90%	-	✓
2-methylnaphtalene	8.05%	20	20 to 100	90%	!	✓*
Anthracene	6.58%	1	1 to 100	81%	-	✓
Benzo(*a*)anthracene	7.95%	1	1 to 100	83%	-	✓
Benzo(*a*)pyrene	-4.78%	1	1 to 100	88%	-	✓
Benzo(*b*)fluoranthene	-4.87%	1	1 to 100	88%	-	✓
Benzo(*g,h,i*)perylene	6.57%	1	1 to 100	89%	-	✓
Benzo(*k*)fluoranthene	-4.89%	1	1 to 100	86%	-	✓
Dibenzo(*a,h*)anthracene	8.90%	1	1 to 100	90%	-	✓
Fluoranthene	10.70%	1	1 to 100	84%	-	✓
Indeno(1,2,3-*c,d*)pyrene	-4.95%	1	1 to 100	87%	-	✓

! interferer detected in carbogaseous water preventing 2-methylnaphtalene quantification at 1 ng/L

- no interferer reported ✓ compound validated

* LOQ was raised to 20 ng/L to be NF 90-210 compliant

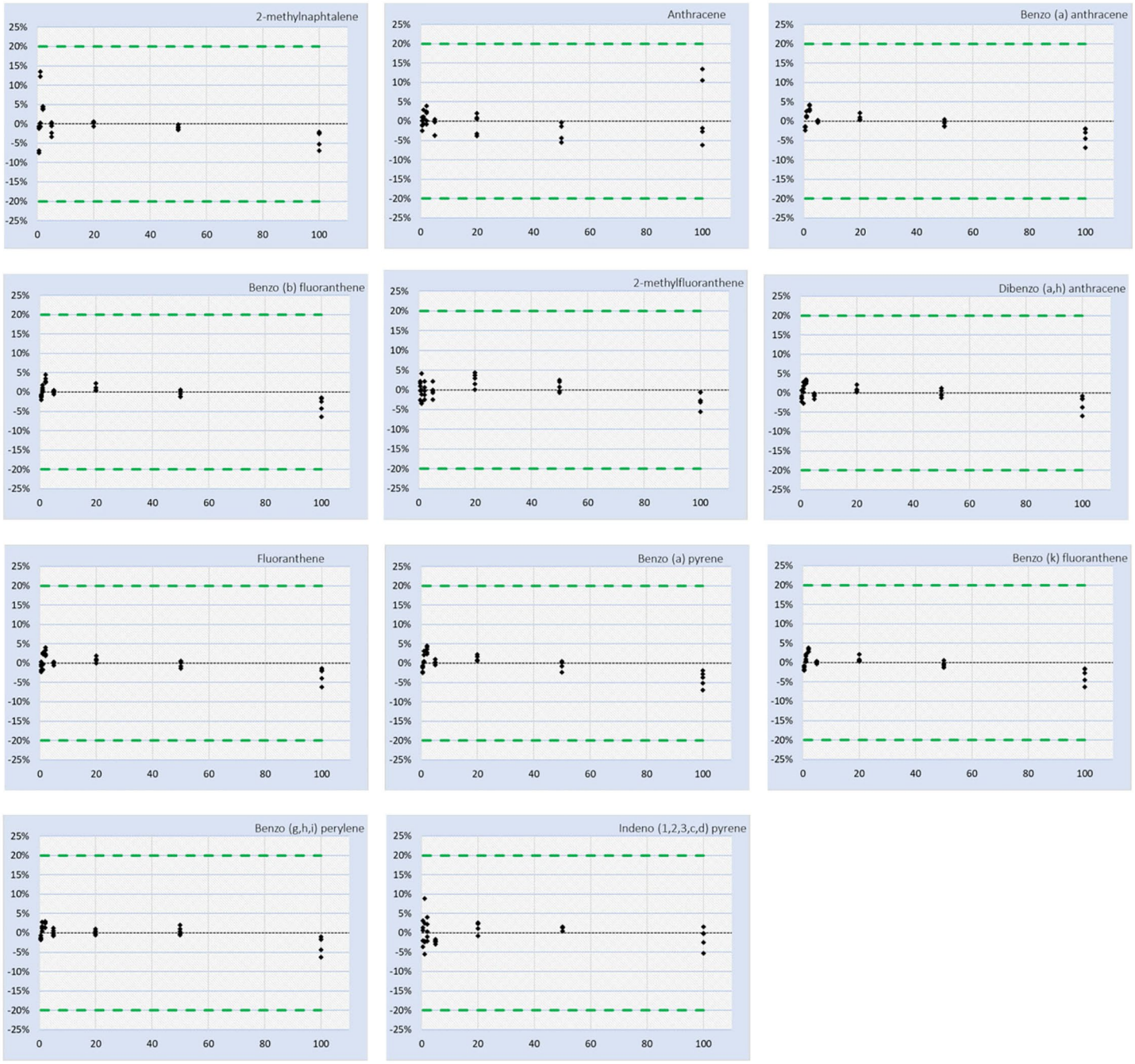

Figure 5. Graphical representation of relative bias for each PAH at each calibration level (horizontal axis expressed in ng/L)

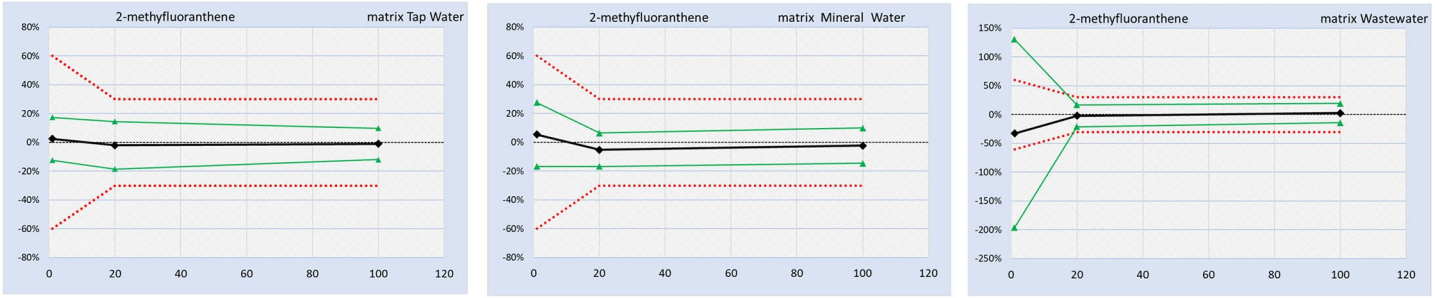

Figure 6. Graphical representation of calculated relative bias (black trace), relative bias from average ±2 SD (green traces), and allowed relative bias (dotted red traces) for 2-methylfluoranthene. Three concentrations were the purpose for evaluation: 1, 20, and 100 ng/L (horizontal axis) in three matrices (from left to right): tap water, mineral water, and wastewater.

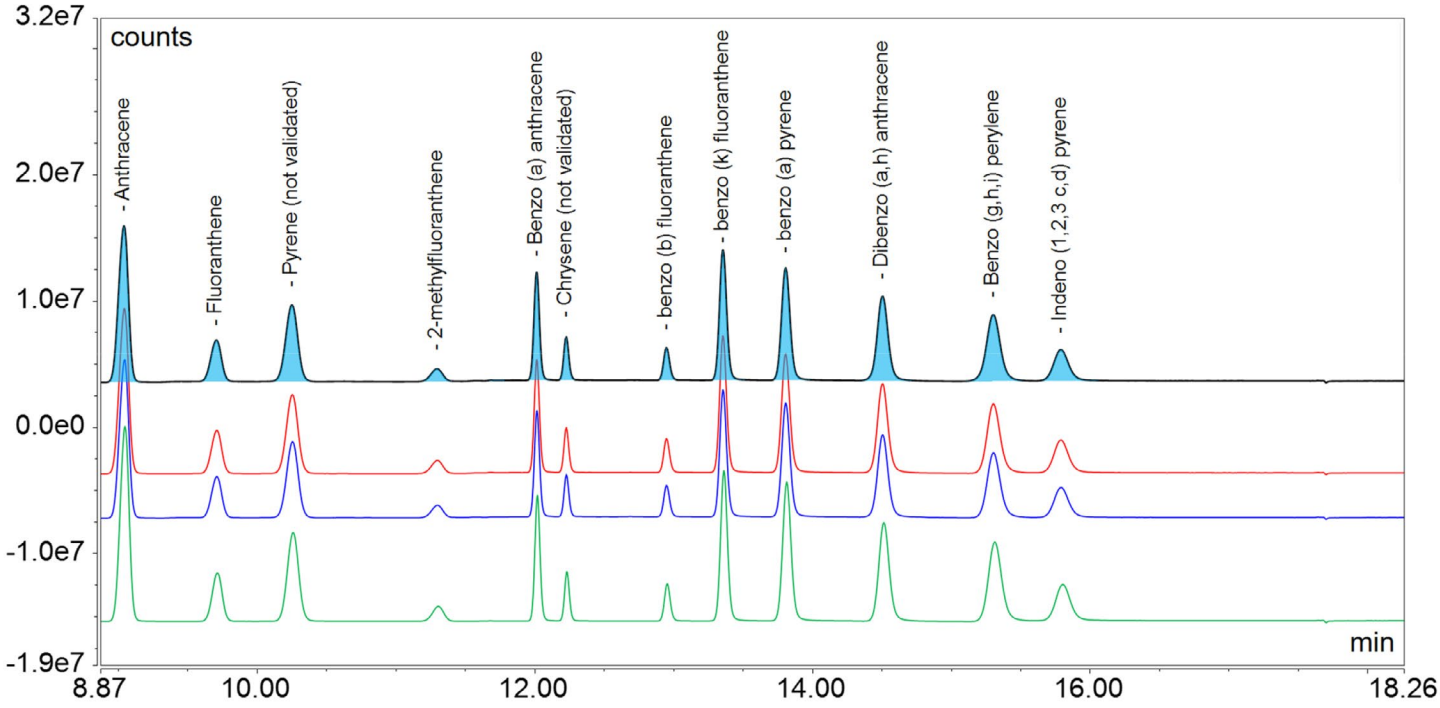

Figure 7. Overlay of chromatograms obtained after 20 µL direct injection of water samples spiked with PAHs 20 µg/L. Zoom on baseline between 8.87 and 18.26 min (black trace = PAH standard solution 20 µg/L, red trace = tap water spiked with PAH standard solution 20 µg/L, blue trace = carbogaseous mineral water spiked with PAH standard solution 20 µg/L, and green trace = wastewater spiked with PAH standard solution 20 µg/L.

Table 4. Performance summary for each PAH in wastewater

Compound	Plan A maximum bias calculated over the concentration range	Plan B LOQ purposed	Amount range (ng/L)	Plan C % recovery	Plan D interferers evaluation	Method validation NF T 90-210
2-methylfluoranthene	2.71%	200	200 to 1000	84%	!	✓*
2-methynaphtalene	n/a	n/a	n/a	n/a	!	×
Anthracene	6.58%	10	10 to 1000	83%	-	✓
Benzo(*a*)anthracene	3.57%	10	10 to 1000	84%	-	✓
Benzo(*a*)pyrene	7.09%	10	10 to 1000	80%	-	✓
Benzo(*b*)fluoranthene	9.87%	10	10 to 1000	86%	-	✓
Benzo(*g,h,i*)perylene	0.43%	200	200 to 1000	82%	!	✓*
Benzo(*k*)fluoranthene	5.58%	10	10 to 1000	85%	-	✓
Dibenzo(*a,h*)anthracene	6.92%	200	200 to 1000	85%	!	✓*
Fluoranthene	-0.34%	200	200 to 1000	86%	!	✓*
Indeno(1,2,3-*c,d*)pyrene	-5.6%	10	10 to 1000	86%	-	✓

! interferer detected in wastewater preventing 2-methylnaphtalene, benzo(g,*h*,i)perylene, and dibenzo(*a,h*)anthracene quantification at 10 ng/L

- no interferer reported ✓ compound validated × compound not validated in this wastewater matrix

* LOQ was raised to 200 ng/L to be NF 90-210 compliant

An illustration of PAH detection in tap water is shown in Figure 8. Detection of 3.75 ng/L of fluoranthene has been performed. Data processing is very easy using Chromeleon 7 CDS software with the SmartPeaks™ Integration Assistant tool for automated peak detection and integration. After automatic peak detection and quantification, the confirmation process is automatically launched. Chromeleon 7 CDS and 2D spectra were recorded by re-injection of the same sample. Separated injections for quantitation and confirmation allows conservation of very low detection levels for quantitation aspect. High sensitivity levels in the confirmation mode allows to increase discriminating results in comparison with baseline spectrum. This confirmation process launches only if required to ensure this method is compliant with regulation, as EN ISO 17993 regulation.

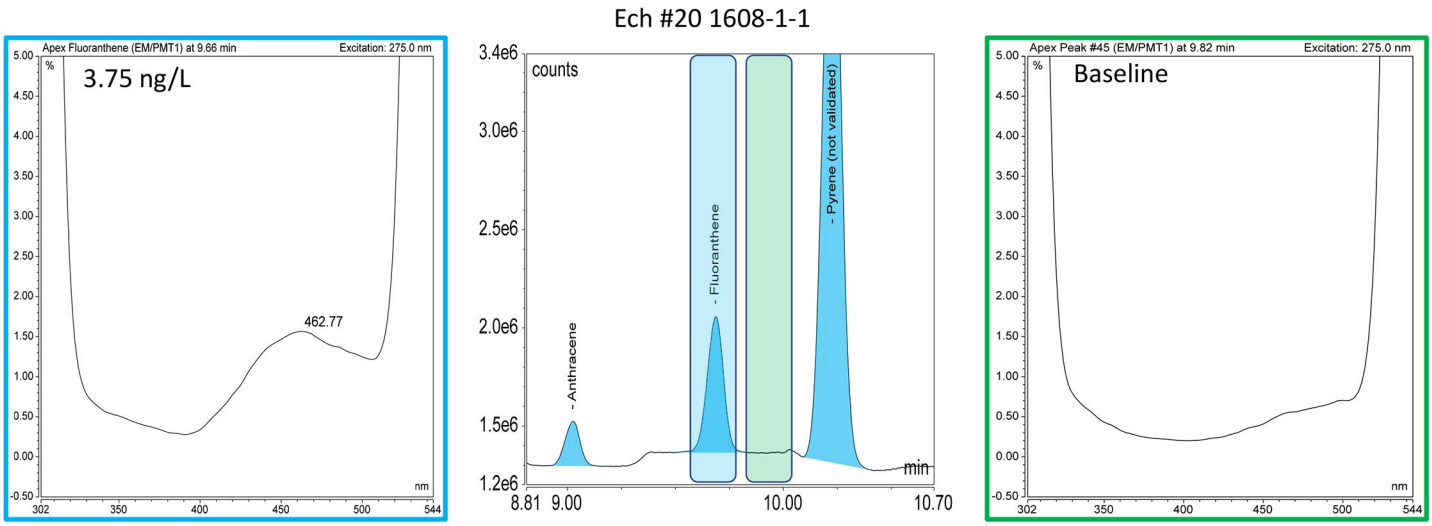

Figure 8. Chromatogram obtained after 20 µL direct injection of tap water sample. The zoom is on the fluoranthene 2D spectrum acquired for confirmation (blue box) and baseline 2D spectrum (green box).

Conclusion

This work describes an HPLC method validation with fluorescence detection for rapid and sensitive determination of eleven PAHs in different water matrices. The determination is performed using a Vanquish UHPLC system controlled by Chromeleon CDS software and combined with a dedicated Hypersil Green PAH analytical column. This method setup is fully validated and compliant with NF T 90-210 guidelines. Implementation in routines is easy and the analytical process suggested allows production of faster results in comparison with other techniques or multi-technique combinations.

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