AKADÉMIAI KIADÓ

Acta Chromatographica

DOI:
10.1556/1326.2023.01118
© 2023 The Author(s)

ORIGINAL RESEARCH PAPER

# Optimization of excitation and emission wavelengths for the UHPLC fluorescence detector for priority polycyclic aromatic hydrocarbons (PAHs)

BELGİN İZGİ and SELMAN KANDER*

Department of Chemistry, Faculty of Art & Science, Bursa Uludag University, Bursa, Turkey

Received: November 24, 2022 • Accepted: January 12, 2023

**ABSTRACT**

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants (POPs) that are widely distributed in the environment and cause significant environmental damage. Furthermore, they endanger human health by polluting food from the natural environment and food processing. Therefore, it is necessary to accurately detect PAHs in various sample matrices, which requires precise, practical, and rapid detection methods. The purpose of this research is to develop a high sensitivity analysis method by analyzing the optimum excitation and emission wavelengths of EPA's 15 priority polyaromatic hydrocarbons in the UHPLC fluorescence detector (Acenaphthene, Anthracene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[ghi]perylene, Benzo[a]pyrene, Chrysene, Dibenzo[a,h]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-cd]pyrene, Naphthalene, Phenanthrene, and Pyrene). An average of 17–25 analyses were performed for each polyaromatic hydrocarbon, and optimized excitation and emission wavelengths were obtained. LOD levels between 2 and 90 ppt were obtained with the method created in this direction. It is worth mentioning that the limits achieved for some PAH parameters are lower than those reported in the literature after pre-concentration steps.

**KEYWORDS**

UHPLC, fluorescence, PAHs, optimization

## 1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants, more succinctly termed PAHs or polyarenes [1]. Primarily due to forest fires, volcanic eruptions, residential wood burning, and vehicle exhaust. It can also enter water bodies through effluents from factories, sewage treatment plants and into the soil of hazardous waste landfills through effluents from storage containers. In the marine compartment, petroleum inputs are significant due to river discharges, accidental crude oil spills, ballast operations of ships, sewage disposal, offshore production, and transport [2].

PAHs which are listed as priority pollutants by the US Environmental Protection Agency (EPA) and the European Union [3–5]. The International Agency for Research on Cancer (IARC) has identified 16 PAHs [Benzo[g,h,i]perylene (BghiP), acenaphthene (Act), naphthalene (Nap), phenanthrene (Phe), anthracene (Ant), benz[a]anthracene (BaA), chrysene (Chr), fluorene (Fln), benz[b]fluoranthene (BbF), indeno[1,2,3-cd]pyrene (IcdP), carbazole (Crb), benzo[k]fluoranthene (BkF), pyrene (Py), dibenz[a,h]anthracene (DahA) benzo[a]pyrene (BaP), fluoranthene (Flt) including 6 of the 16 EPA regulated PAHs, as potential carcinogens [6, 7].

Determination of PAHs in water samples, especially drinking water, is difficult because of their low solubility, which results in low concentrations [8]. Methods currently

*Corresponding author.
E-mail: skander@buski.gov.tr

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

used to detect PAHs require chromatographic separation by liquid or gas chromatography, followed by detection using mass spectrometry or fluorescence spectroscopy. Even though these methods are precision, they require significant time and resources, limiting the ability to conduct high throughput assays of large populations to measure toxicant exposure [9–13]. The selectivity is based on the fact that relatively few compounds show intrinsic fluorescence (fl) and that emission intensity depends on two variables: excitation (ex) and emission (em) wavelengths. Therefore, fluorescent compounds can be determined by their excitation, emission, or synchronous spectroscopy [14]. PAHs have good fluorescence activity, and their maximum fluorescence occurs at different Ex/Em wavelengths. Changing the Ex/Em wavelength is necessary to obtain the best detection sensitivity [15]. Programmed fluorescence switching (switching to the maximum Ex/Em wavelength of each PAH when the PAH peak crosses the fluorescence detector) is required to obtain the best sensitivity for each PAH In this study, all ex and em PAH wavelengths in the literature were compiled, and optimum ex/em wavelengths were determined for 15 Pahs. In this way, much lower limit of detection (LOD) and limit of quantification (LOQ) levels have been obtained, and method sensitivity has been improved.

## 2. MATERIALS AND METHODS

### 2.1. Reagents and chemicals

Analytical standard PAHs from Dr. Ehrenstorfer (Augsburg, Germany) and High Performance Liquid Chromatography (HPLC) grade solvents: acetonitrile, and water gradient grade for liquid chromatography (LC) from Merck; (München, Germany) were used. Monitor the blank to guarantee that the reagents do not contain PAH in detectable concentrations. Stock solutions contain 10 µg $L^{-1}$ of individual standards. Prepare at least five calibration solutions by appropriately diluting the stock solution using acetonitrile as the solvent. The diluted solutions were stored in amber vials at 4 °C to avoid photo-degradation of PAHs.

### 2.2. Instrumentation

Analyses were performed using a Perkin Elmer Ultra High Performance Liquid Chromatography (UHPLC) system (Waltham, Massachusetts, USA) consisting of an FX-20 Quaternary pump, Flexar FX UHPLC, Autosampler with degasser, Flexar LC Column Oven and Flexar Fluorescence LC Detector. The data was collected and analyzed using Chromera Speciation Software.

### 2.3. Chromatographic conditions

The analytical column used was a UHPLC column: Brownlee Analytical PAH, 150 × 3.2 mm, 5 µm particle size, Perkin Elmer (Waltham, MA, USA); This column has been specifically designed for the analysis of priority polluting PAHs. The analytical column was kept at 25 °C during the analysis. An isocratic elution (30/70 v/v, mobile phase A and mobile phase B) was utilized. Mobile phase A consisted of water, and mobile phase B consisted of acetonitrile flow rate of 0.8 mL $min^{-1}$ was used. The injection volume was 20 µL. Sampling rate is the frequency at which snapshots of an analog signal are recorded. Thus the more snapshots per second, the higher the sample rate and the better the quality. Sampling rate 5 pts/s was preferred. Selecting the baseline mode "autozero" prevents the detector from cutting peak areas at wavelength transitions. Chromatographic conditions used in optimization studies Fig. 1.

Wavelength detection studies were carried out with "medium" detector sensitivity. The reason for working at medium level is to prevent the peaks from being overloaded. In this way, all peaks were correctly observed. In addition, the energy level increases significantly in fluorescence detectors' high sensitivity and concentration work. The detector turns itself off to avoid damage. This event was observed at the data level in the study performed at ex/em 250/500 nm and given Fig. 2.

LOD and LOQ runs at this level have been changed to "super high". The reason for working with super high sensitivity in LOD and LOQ work is to see the full performance of the equipment. Quantitative determination was carried out using an external calibration curve method. The calibration curves for all compounds were duplicated with a relative standard deviation (RSD) < 3%. The abundances of the selected compounds were calculated by comparing the areas of the peaks on the calibration curve with the of the peaks of the individual PAHs obtained from the HPLC fluorescence detector chromatograms. Peak identification was carried out by comparison of retention times with standards.

### 2.4. HPLC conditions and method optimization studies

The selection of the Ex/Em wavelength pair is essential for optimizing the sensitivity of a fluorescence detection assay. A wavelength change should be made when the fluorescence is low. At high fluorescence, wavelength changes can lead to a displacement of the baseline. Readjusting the baseline after changing the wavelength may interfere with integration and quantification. Changing the wavelengths and damping may be necessary simultaneously to obtain constant peak heights. To develop the peak lengths and areas of polyaromatic hydrocarbons, it is necessary to determine the most suitable ex and em wavelengths. Standard wavelengths used in the literature have been identified and listed for 15 PAHs. For each PAH, between 17 and 25 different ex and em values were determined from the literature, and the data in the study were obtained by analyzing them. In our study, the best analytical values for 15 PAHs were determined simultaneously with ex and em values among the values in the table. The validation study was also carried out with

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

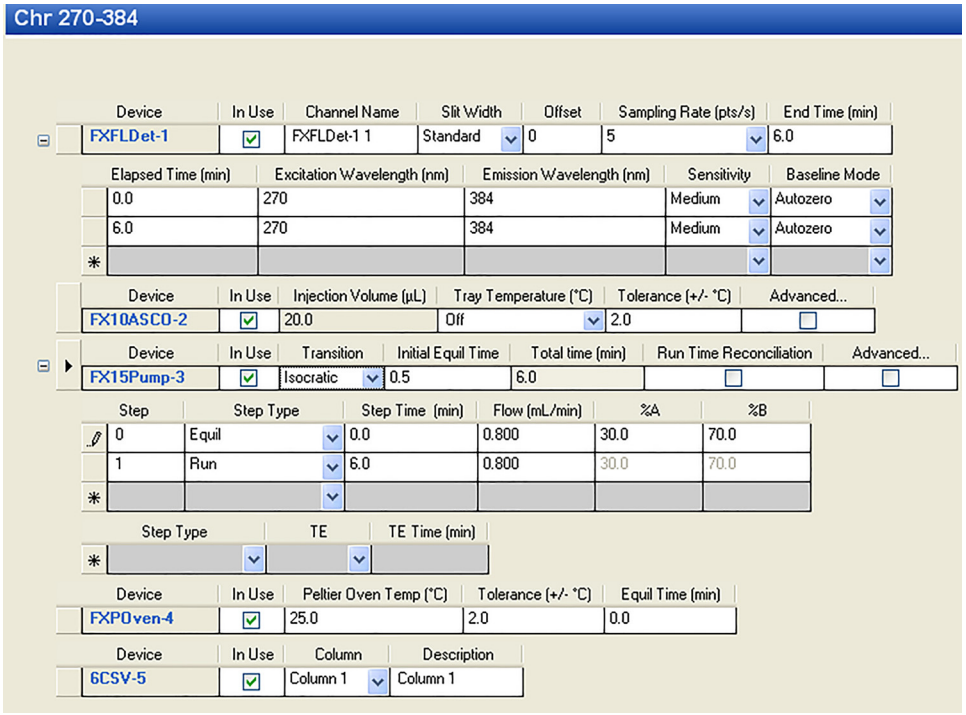


*Fig. 1.* Exemplary procedure of analysis used in optimization studies

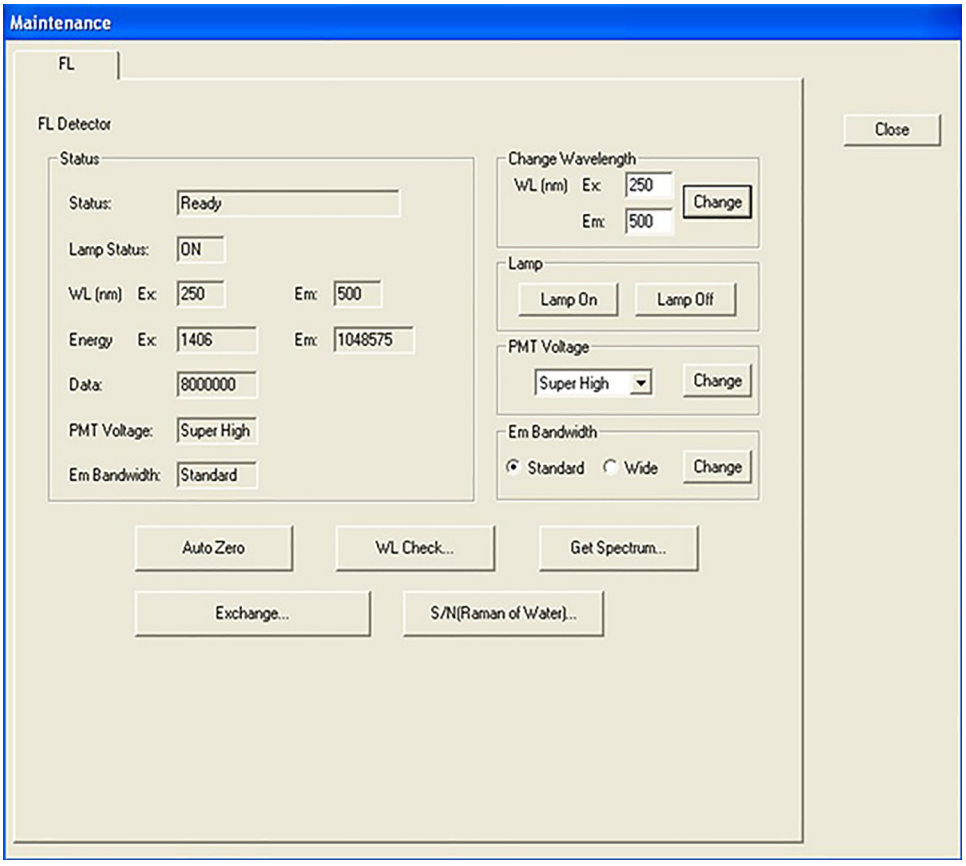


*Fig. 2.* Energy level test of fluorescence detector

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

these data. All results in HPLC analyzes are shown by determining the maximum peak heights of the analytes in flu unit. The flu unit is the response expression of the analyte's fluorescence measurement. On the other hand, it can be considered the equivalent of the absorbance value in the HPLC system. Depending on the concentration, flu values of the analyte were evaluated according to the peak height and area. The maximum value was obtained for calculating the maximum peak heights and the area used in the quantification. As a result of the analyses carried out in the high sensitivity mode of the fluorescence detector, the peak heights in the flu unit were obtained. The optimum wavelength was determined by the highest peak area received. A calibration chart was created according to the optimally determined ex, and em values and repeatability studies were carried out.

LOD and LOQ were calculated according to the data obtained from these studies. Particularly in analyses with low legal limits, the sensitive detection limit is critical to approach these sensitive detection limits in chromatography, it is necessary to have good peak separations and high peak areas. In this sense, many studies conducted under the HPLC fluorescence detector, regardless of matrix difference, examined and verified the wavelengths used.

The obtained chromatograms were overlapped and the wavelength pair with the highest peak length was determined. The chromatograms are marked in different colors to distinguish them. There is a slight shift in the retention time of the peaks. The increase in the number of injections slightly decreased the retention in the column. Example overlapped chromatogram are given in Fig. 3.

### 2.5. Data description and research methodology

Since certified reference materials were used in all experimental studies, the matrix difference was ignored in detecting ex/em pairs in the literature. In this way, ex/em pairs belonging to almost every matrix were detected, the overlapping pairs were eliminated and lists were created. Studies of ex/em couples whose mobile carrier phases are acn/water were preferred not to reflect the solvent effect on the analysis results.

## 3. RESULTS AND DISCUSSION

Analyses were performed according to the ex/em wavelength pairs given in Table 1. With these analyzes, the changes of ex and em wavelengths on fluorescence were determined. Line structure PAHs showed better fluorescence than branched structure PAHs. The chromatograms were overlapped, and the effect of wavelength changes on the peak lengths in fluorescence (flu) was observed. The overlaid chromatograms of all PAHs are given in Fig. 4.

In this sense, it has been determined that the excitation value of PAHs is more decisive in fluorescence analysis. The relevant analyses results are given in Table 2.

In the wavelength studies, no peak was detected in 9 wavelength pairs. These wavelength pairs belong to FL and IcdP. FL and IcdP did not peak at their respective wavelengths. In particular, the increase in the difference between emission and excitation wavelengths of IcdP resulted in a decrease in fluorescence. While BaA and BaP provide a balanced distribution regarding flu results, the highest flu value belongs to FLN. When all wavelength pairs were examined, the lowest flu values were determined to belong to IcdP. Different fluorescence radiations were detected in very close ex/em wavelength pairs. When the results of the analyzes performed at the 275/330 and 276/330 wavelengths of the FLN are examined, it is seen that a 1 nm change in the excitation value causes a 32-unit difference in flu. In the analysis results of BaA at 270/384 and 270/385 wavelengths, it was seen that a 1 nm change in the emission

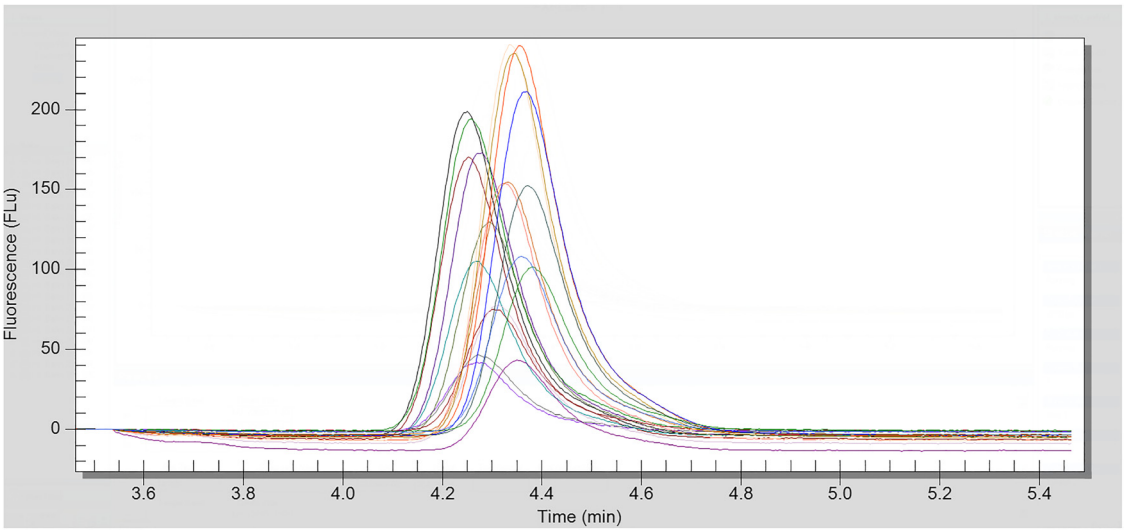


*Fig. 3.* Example overlapped chromatogram

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

*Table 1.* Excitation and emission wavelengths [15–45]

| NAP | ACT | FLN | PHE | ANT | FL | PY | BaA | CHR | BbF | BkF | BaP | DahA | BghiP | IcdP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 215/330 | 220/325 | 263/310 | 247/364 | 247/401 | 280/460 | 236/389 | 275/389 | 260/381 | 256/446 | 295/410 | 260/408 | 290/398 | 290/415 | 248/484 |
| 219/330 | 224/320 | 265/310 | 250/365 | 248/405 | 280/450 | 237/385 | 281/391 | 264/381 | 258/442 | 290/412 | 288/406 | 285/396 | 290/410 | 290/499 |
| 220/330 | 220/320 | 275/315 | 250/366 | 250/402 | 281/453 | 238/398 | 277/393 | 265/380 | 254/451 | 290/410 | 281/407 | 294/398 | 290/418 | 290/500 |
| 217/338 | 276/330 | 270/323 | 247/357 | 250/406 | 232/445 | 270/390 | 284/390 | 260/370 | 249/443 | 296/426 | 290/410 | 285/404 | 292/415 | 250/470 |
| 221/337 | 275/330 | 279/306 | 250/368 | 250/380 | 237/460 | 240/386 | 270/390 | 267/385 | 280/438 | 243/412 | 280/410 | 290/418 | 290/420 | 293/498 |
| 222/329 | 280/324 | 280/324 | 246/370 | 252/402 | 270/450 | 332/378 | 270/410 | 260/390 | 255/420 | 302/431 | 295/405 | 290/420 | 294/425 | 300/500 |
| 224/330 | 280/330 | 275/330 | 244/370 | 252/400 | 270/440 | 254/390 | 270/384 | 270/384 | 266/425 | 294/425 | 295/410 | 290/415 | 295/410 | 302/500 |
| 224/320 | 225/315 | 276/330 | 252/365 | 250/408 | 270/470 | 240/400 | 270/385 | 270/385 | 290/430 | 303/432 | 266/415 | 289/422 | 285/416 | 274/507 |
| 267/330 | 227/315 | 234/320 | 240/360 | 251/378 | 284/467 | 246/375 | 268/398 | 270/367 | 260/420 | 290/430 | 290/430 | 290/410 | 289/422 | 293/493 |
| 275/330 | 235/332 | 280/330 | 252/370 | 248/375 | 237/440 | 334/371 | 267/385 | 269/361 | 294/425 | 300/440 | 266/425 | 298/398 | 295/425 | 302/510 |
| 277/330 | 290/337 | 225/315 | 252/372 | 250/375 | 285/465 | 252/400 | 287/386 | 254/390 | 298/436 | 288/406 | 297/405 | 295/405 | 295/405 | 300/470 |
| 270/323 | 270/323 | 250/341 | 246/375 | 254/402 | 280/420 | 248/375 | 260/390 | 270/390 | 300/440 | 307/413 | 260/420 | 296/404 | 290/430 | 305/480 |
| 277/337 | 292/322 | 227/315 | 248/375 | 250/420 | 288/450 | 276/391 | 290/395 | 268/398 | 300/445 | 255/420 | 298/407 | 295/410 | 296/406 | 300/466 |
| 276/323 | 234/320 | 224/320 | 250/380 | 255/380 | 238/418 | 238/418 | 254/390 | 277/376 | 302/452 | 266/415 | 250/400 | 295/425 | 285/404 | 300/464 |
| 280/330 | 275/350 | 220/325 | 254/375 | 238/418 | 290/447 | 250/420 | 290/404 | 270/410 | 290/410 | 260/420 | 255/420 | 300/400 | 296/404 | 300/465 |
| 278/322 | 280/355 | 290/337 | 275/350 | 240/430 | 337/440 | 237/440 | 265/380 | 260/420 | 250/400 | 266/425 | 298/404 | 268/398 | 300/415 | 268/398 |
| 280/324 | 275/315 | 275/350 | 280/355 | 244/370 | 260/420 | 270/440 | 260/420 | 277/393 | 268/398 | 250/400 | 268/398 | 300/415 | 260/420 | 296/404 |
| 275/350 | 265/360 | 265/360 | 240/400 | 252/372 | 250/420 | - | 240/400 | 240/400 | - | 256/446 | 294/425 | 290/430 | 302/419 | 300/440 |
| 280/355 | 250/341 | 280/355 | 252/400 | 250/368 | 365/462 | - | 277/376 | 238/398 | - | 260/460 | 296/406 | 260/420 | 300/440 | 250/495 |
| 248/375 | 248/375 | 240/368 | 294/347 | 260/420 | 240/400 | - | 238/398 | 290/404 | - | 268/398 | 256/446 | 300/440 | 268/398 | 245/500 |
| - | - | 248/375 | 297/367 | - | 252/402 | - | - | - | - | - | 260/460 | 234/420 | 305/420 | 250/500 |
| - | - |  | - | - | 252/400 | - | - | - | - | - | 300/440 | 300/469 | 234/420 | 246/503 |
| - | - | - | - | - | 248/375 | - | - | - | - | - |  | 300/470 | 300/465 | 251/510 |
| - | - | - | - | - | - | - | - | - | - | - | - | - | 300/470 | - |
| - | - | - | - | - | - | - | - | - | - | - | - | - | 302/500 | - |

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

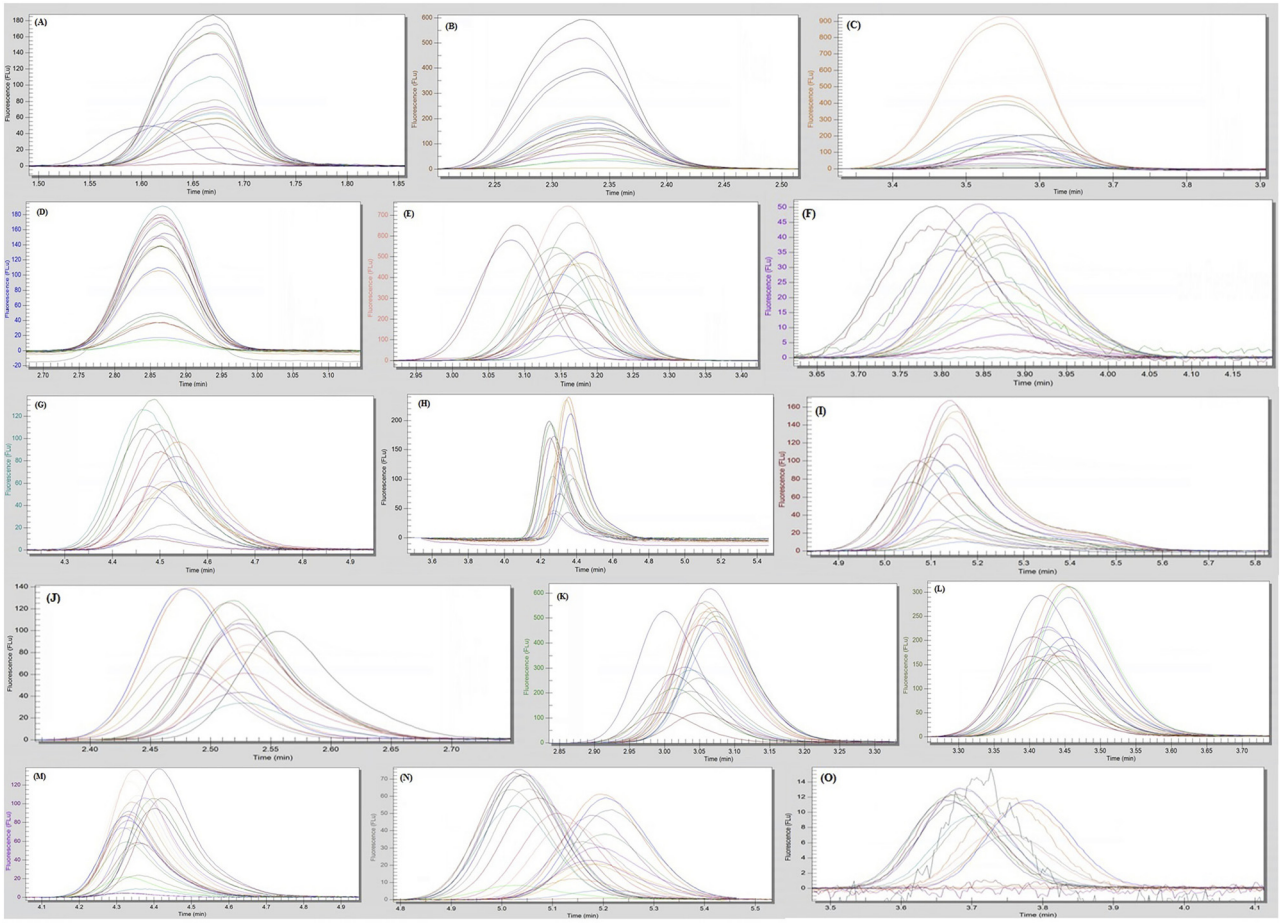

*Fig. 4.* Chromatograms of analyses at different ex/em wavelengths A: Naphthalene, B: Acenaphthene, C: Fluorene, D: Phenanthrene, E: Anthracene, F: Fluoranthene, G: Pyrene, H: Benzo[a]anthracene, I: Chrysene, J: Benzo[b]fluoranthene, K: Benzo[k]fluoranthene, L: Benzo[a]pyrene, M: Dibenzo[ah]anthracene, N: Benzo[ghi]perylene, O: Indeno[1,2,3cd]pyrene

value caused a 3-unit difference in flu. It was observed that BkF, ANT and BaP showed good fluorescence at all detected wavelength pairs. According to the flu values taken for each PAH given in Table 2, ex and em values with the best analytical performance were accepted as optimum operating conditions.

## 3.1. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) are terms used to describe the smallest concentration of a measurand that can be reliably measured by an analytical procedure. LOD for each analyte were calculated as being three times the average level of the baseline noise (analyzed from the injection of individual PAH containing standard solutions), and the LOQ was calculated as ten times this same level.

It is worth mentioning that the limits so far achieved for some PAH parameters are lower than those reported in the literature after pre-concentration steps. UHPLC floresence method was proposed for the analysis of fifteen PAH, and validated the developed method, optimum ex/em wavelength pairs, optimum peak height, the calibration curve equations, linear ranges, linearity coefficients ($R^2$), limits of detection (LODs) and limits of quantification (LOQs) are summarized in Table 3.

The method developed chromatographic separation and detection, was used inside an inter-laboratory study aimed at the determination of the analytes BaP, BbF, BkF, IcdIP and BghiP in drinking water samples. The Inter-laboratory test (named Water Chemistry (Aquacheck)) was organized and managed by LGC. The composition of the samples is reported in Table 4 together with the z-scores obtained. Absolute values of z-scores (|z| values) are used for assessing the acceptability of the results as follows:

$|z| \leq 2$ acceptable result; $2<|z|<3$ doubtful result; $|z| \geq 3$ unacceptable result. From the data shown in Table 4, it can be observed that since |z| values are included between 0.05 and 1.60 the method developed performs satisfactorily. Accuracy of the UHPLC method through z-score values inside an inter-laboratory study given Table 4. The method optimized is currently routinely used for the determination of PAHs by the laboratory in charge of the monitoring of the drinking water quality for the city of Bursa.

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

*Table 2.* PAH analysis results

| PAH analysis results | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAP | Flu | ACT | Flu | FLN | Flu | PHE | Flu | ANT | Flu | FL | Flu | PY | Flu | BaA | Flu |
| 215/330 | 185 | 220/325 | 518 | 263/310 | 930 | 247/364 | 191 | 247/401 | 745 | 280/460 | 51 | 236/389 | 135 | 275/389 | 241 |
| 219/330 | 176 | 224/320 | 400 | 265/310 | 886 | 250/365 | 180 | 248/405 | 663 | 280/450 | 50 | 237/385 | 126 | 281/391 | 240 |
| 220/330 | 166 | 220/320 | 385 | 275/315 | 443 | 250/366 | 178 | 250/402 | 653 | 281/453 | 48 | 238/398 | 113 | 277/393 | 235 |
| 217/338 | 164 | 276/330 | 210 | 270/323 | 415 | 247/357 | 176 | 250/406 | 580 | 232/445 | 44 | 270/390 | 109 | 284/390 | 211 |
| 221/337 | 139 | 275/330 | 205 | 279/306 | 391 | 250/368 | 173 | 250/380 | 544 | 237/460 | 43 | 240/386 | 108 | 270/390 | 199 |
| 222/329 | 137 | 280/324 | 198 | 280/324 | 207 | 246/370 | 172 | 252/402 | 524 | 270/450 | 43 | 332/378 | 97 | 270/410 | 194 |
| 224/330 | 110 | 280/330 | 185 | 275/330 | 200 | 244/370 | 168 | 252/400 | 520 | 270/440 | 41 | 254/390 | 89 | 270/384 | 173 |
| 224/320 | 82 | 225/315 | 183 | 276/330 | 168 | 252/365 | 166 | 250/408 | 518 | 270/470 | 41 | 240/400 | 88 | 270/385 | 170 |
| 267/330 | 73 | 227/315 | 162 | 234/320 | 135 | 240/360 | 155 | 251/378 | 468 | 284/467 | 38 | 246/375 | 62 | 268/398 | 155 |
| 275/330 | 71 | 235/332 | 155 | 280/330 | 134 | 252/370 | 149 | 248/375 | 462 | 237/440 | 36 | 334/371 | 61 | 267/385 | 154 |
| 277/330 | 66 | 290/337 | 155 | 225/315 | 115 | 252/372 | 139 | 250/375 | 415 | 285/465 | 35 | 252/400 | 59 | 287/386 | 152 |
| 270/323 | 65 | 270/323 | 142 | 250/341 | 113 | 246/375 | 138 | 254/402 | 410 | 280/420 | 25 | 248/375 | 58 | 260/390 | 120 |
| 277/337 | 60 | 292/322 | 140 | 227/315 | 105 | 248/375 | 138 | 250/420 | 326 | 288/450 | 25 | 276/391 | 57 | 290/395 | 108 |
| 276/323 | 59 | 234/320 | 128 | 224/320 | 92 | 250/380 | 110 | 255/380 | 297 | 238/418 | 18 | 238/418 | 47 | 254/390 | 105 |
| 280/330 | 56 | 275/350 | 109 | 220/325 | 85 | 254/375 | 110 | 238/418 | 267 | 290/447 | 18 | 250/420 | 23 | 290/404 | 101 |
| 278/322 | 53 | 280/355 | 95 | 290/337 | 66 | 275/350 | 50 | 240/430 | 255 | 337/440 | 15 | 237/440 | 12 | 265/380 | 77 |
| 280/324 | 50 | 275/315 | 63 | 275/350 | 34 | 280/355 | 47 | 244/370 | 229 | 260/420 | 14 | 270/440 | 10 | 260/420 | 75 |
| 275/350 | 36 | 265/360 | 40 | 265/360 | 24 | 240/400 | 37 | 252/372 | 228 | 250/420 | 13 | - | - | 240/400 | 46 |
| 280/355 | 22 | 250/341 | 34 | 280/355 | 16 | 252/400 | 37 | 250/368 | 120 | 365/462 | 8 | - | - | 277/376 | 43 |
| 248/375 | 3 | 248/375 | 4 | 240/368 | 7 | 294/347 | 17 | 260/420 | 61 | 240/400 | 4 | - | - | 238/398 | 42 |
| - | - | - | - | 248/375 | 5 | 297/367 | 14 | - | - | 252/402 | 4 | - | - | - | - |
| - | - | - | - | - | - | - | - | - | - | 252/400 | 3 | - | - | - | - |
| - | - | - | - | - | - | - | - | - | - | 248/375 | nd | - | - | - | - |
| - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| PAH analysis results | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHR | Flu | BbF | Flu | BkF | Flu | BaP | Flu | DahA | Flu | BghiP | Flu | IcdP | Flu |
| 260/381 | 167 | 256/446 | 139 | 295/410 | 617 | 260/408 | 317 | 290/398 | 137 | 290/415 | 76 | 248/484 | 16 |
| 264/381 | 163 | 258/442 | 138 | 290/412 | 566 | 288/406 | 312 | 285/396 | 136 | 290/410 | 74 | 290/499 | 13 |
| 265/380 | 155 | 254/451 | 127 | 290/410 | 560 | 281/407 | 311 | 294/398 | 124 | 290/418 | 73 | 290/500 | 13 |
| 260/370 | 148 | 249/443 | 125 | 296/426 | 541 | 290/410 | 294 | 285/404 | 109 | 292/415 | 73 | 250/470 | 12 |
| 267/385 | 130 | 280/438 | 111 | 243/412 | 528 | 280/410 | 289 | 290/418 | 106 | 290/420 | 72 | 293/498 | 12 |
| 260/390 | 118 | 255/420 | 106 | 302/431 | 527 | 295/405 | 228 | 290/420 | 106 | 294/425 | 64 | 300/500 | 12 |
| 270/384 | 104 | 266/425 | 106 | 294/425 | 526 | 295/410 | 222 | 290/415 | 105 | 295/410 | 64 | 302/500 | 12 |
| 270/385 | 101 | 290/430 | 102 | 303/432 | 508 | 266/415 | 207 | 289/422 | 102 | 285/416 | 61 | 274/507 | 12 |
| 270/367 | 100 | 260/420 | 99 | 290/430 | 501 | 290/430 | 207 | 290/410 | 95 | 289/422 | 59 | 293/493 | 11 |
| 269/361 | 95 | 294/425 | 87 | 300/440 | 486 | 266/425 | 189 | 298/398 | 92 | 295/425 | 59 | 302/510 | 11 |
| 254/390 | 87 | 298/436 | 81 | 288/406 | 471 | 297/405 | 187 | 295/405 | 88 | 295/405 | 54 | 300/470 | 10 |
| 270/390 | 77 | 300/440 | 76 | 307/413 | 441 | 260/420 | 180 | 296/404 | 87 | 290/430 | 52 | 305/480 | 10 |
| 268/398 | 64 | 300/445 | 75 | 255/420 | 304 | 298/407 | 171 | 295/410 | 82 | 296/406 | 50 | 300/466 | 8 |
| 277/376 | 40 | 302/452 | 62 | 266/415 | 292 | 250/400 | 170 | 295/425 | 74 | 285/404 | 49 | 300/464 | 7 |
| 270/410 | 35 | 290/410 | 61 | 260/420 | 275 | 255/420 | 168 | 300/400 | 74 | 296/404 | 45 | 300/465 | 7 |
| 260/420 | 27 | 250/400 | 44 | 266/425 | 260 | 298/404 | 166 | 268/398 | 59 | 300/415 | 38 | 268/398 | nd |
| 277/393 | 26 | 268/398 | 34 | 250/400 | 219 | 268/398 | 160 | 300/415 | 59 | 260/420 | 34 | 296/404 | nd |
| 240/400 | 16 | - | - | 256/446 | 207 | 294/425 | 160 | 290/430 | 58 | 302/419 | 31 | 300/440 | nd |
| 238/398 | 15 | - | - | 260/460 | 122 | 296/406 | 122 | 260/420 | 24 | 300/440 | 23 | 250/495 | nd |
| 290/404 | 10 | - | - | 268/398 | 120 | 256/446 | 70 | 300/440 | 22 | 268/398 | 21 | 245/500 | nd |
| - | - | - | - | - | - | 260/460 | 53 | 234/420 | 9 | 305/420 | 21 | 250/500 | nd |
| - | - | - | - | - | - | 300/440 | 48 | 300/469 | 5 | 234/420 | 12 | 246/503 | nd |
| - | - | - | - | - | - | - | - | 300/470 | 4 | 300/465 | 8 | 251/510 | nd |
| - | - | - | - | - | - | - | - | - | - | 300/470 | 5 | - | - |
| - | - | - | - | - | - | - | - | - | - | 302/500 | 1 | - | - |

*nd: not detected.

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

*Table 3.* Regression equation, linear range, LODs and LOQs ($n = 7$)

| | Ex/Em | Flu | $R^2$ | Regression equation | Linear range (ng $L^{-1}$) | LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) |
|---|---|---|---|---|---|---|---|
| NAP | 215/330 | 185 | 0.999860 | $y = 13.28x + 28.16$ | 0.1–10 | 0.003 | 0.010 |
| ACT | 222/329 | 593 | 0.999803 | $y = 16.34x + 45.11$ | 0.1–10 | 0.002 | 0.007 |
| FLN | 263/310 | 930 | 0.999919 | $y = 5.32x + 17.07$ | 0.1–10 | 0.002 | 0.007 |
| PHE | 247/364 | 191 | 0.999733 | $y = 12.5x + 29.10$ | 0.1–10 | 0.009 | 0.030 |
| ANT | 247/401 | 617 | 0.999938 | $y = 31.45x + 124.4$ | 0.1–10 | 0.005 | 0.007 |
| FL | 280/460 | 51 | 0.999865 | $y = 7.1x + 14.30$ | 0.1–10 | 0.009 | 0.030 |
| PY | 236/389 | 135 | 0.999887 | $y = 3.112x + 6.98$ | 0.1–10 | 0.015 | 0.050 |
| BaA | 275/389 | 241 | 0.999910 | $y = 7.45x + 14.20$ | 0.1–10 | 0.015 | 0.050 |
| CHR | 260/381 | 167 | 0.999954 | $y = 11.35x + 28.15$ | 0.1–10 | 0.015 | 0.050 |
| BbF | 256/446 | 139 | 0.999877 | $y = 17.10x + 37.11$ | 0.1–10 | 0.015 | 0.050 |
| BkF | 295/410 | 617 | 0.999915 | $y = 5.31x + 10.55$ | 0.1–10 | 0.006 | 0.020 |
| BaP | 260/408 | 317 | 0.999888 | $y = 3.35x+6.55$ | 0.1–10 | 0.009 | 0.030 |
| DahA | 290/398 | 137 | 0.999944 | $y = 6.06x+8.35$ | 0.1–10 | 0.020 | 0.007 |
| BghiP | 290/415 | 76 | 0.999013 | $y = 12.2x+10.56$ | 0.1–10 | 0.025 | 0.083 |
| IcdP | 248/484 | 16 | 0.999801 | $y = 7.28x+14.30$ | 0.1–10 | 0.090 | 0.300 |

*Table 4.* Accuracy of the UHPLC method through z-score values inside an inter-laboratory study

| Analyte | Results | Units | Z Score | Assigned Value | Lab. Numbers | Roboust SD | SD |
|---|---|---|---|---|---|---|---|
| FL | 20.7 | ng $L^{-1}$ | 0.05 | 20.6 | 39 | 1.93 | 3.37 |
| BbF | 8.60 | ng $L^{-1}$ | 0.52 | 7.55 | 49 | 1.053 | 1.232 |
| BkF | 8.21 | ng $L^{-1}$ | 0.14 | 7.93 | 49 | 0.971 | 1.633 |
| BaP | 3.81 | ng $L^{-1}$ | 0.18 | 3.72 | 49 | 0.830 | 0.786 |
| BghiP | 20.80 | ng $L^{-1}$ | 1.60 | 17.60 | 48 | 2.669 | 3.076 |
| IcdP | 11.24 | ng $L^{-1}$ | 0.79 | 9.67 | 49 | 1.550 | 1.816 |

## 4. CONCLUSION

An optimization study was performed to improve the sensitivity to find the specific emission and excitation wavelengths for each analyte. This task was accomplished by optimizing the excitation and emission wavelengths during the chromatographic run in correspondence with the elution of the analytes. Optimum wavelength pairs defined for PAHs as NAP 215/330, ACT 22/329, FLN 263/310, PHE 247/364, ANT 247/401, FL 2840/460, PY 236/389, BaA 275/389, CHR 260/381, BbF 256/446, BkF 295/410, BaP 260/408, DahA 290/398, BghiP 290/415 and IcdP 248/484. Improvements to the method have significantly improved its sensitivity, allowing the determination of low concentration levels associated with such a sample. It was verified that the obtained LOD and LOQ were lower for HPLC Fl, possibly due to the excellent sensitivity of this technique to detect PAH. The optimum ex and em values obtained can be studied for 15 PAHs in a wide range of samples.

*Author contributions:* Bİ carried out designing of the current study and coordination of the manuscript. SK carried out the experimental work and drafted the manuscript and participated in the design Bİ and SK reviewed the manuscript. All authors read and approved the final manuscript.

*Funding:* The authors did not receive support from any organization for the submitted work.

*Availability of data and materials:* All data and materials are available upon request.

## DECLARATIONS

*Conflict of interest:* The authors declare that they have no conflict of interest.

*Research involving human participants and/or animals:* This research did not involve human participants or animals.

*Informed consent:* Informed consent obtaining for this type of study is not required.

## ACKNOWLEDGEMENTS

The author Selman Kander is thankful to Bursa Water and Sewerage Administration General Directorate (BUSKİ), for providing facilities to perform this research work.

## REFERENCES

1. Nielsen, T. Reactivity of polycyclic aromatic hydrocarbons toward nitrating species. *Environ. Sci. Technol.* **1984**, *18*, 157–63. https://doi.org/10.1021/es00121a005.

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

2. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Washington, D.C., *Vol. 18*, **1985**, 141–7, https://doi.org/10.3109/15569529909037564.
3. Busetti, F.; Heitz, A.; Cuomo, M.; Badoer, S.; Traverso, P. Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant. *J. Chromatogr. A*. **2006**, *1102*, 104–15. https://doi.org/10.1016/j.chroma.2005.10.013.
4. Disposal and Recycling Routes for Sewage Sludge Part 4: Economic Report. European Commission, Brussels, Belguim, **2002**.
5. Manoli, E.; Samara, C. The removal of polycyclic aromatic hydrocarbons in the wastewater treatment process: experimental calculations and model predictions. *Environ. Pollut.* **2008**, *151*, 477–85. https://doi.org/10.1016/j.envpol.2007.04.009.
6. Zhang, K.; Zhang, B. Z.; Li, S. M.; Wong, C. S.; Zeng, E. Y. Calculated respiratory exposure to indoor size-fractioned polycyclic aromatic hydrocarbons in an urban environment. *Sci. Total Environ.* **2012**, *431*, 245–51. https://doi.org/10.1016/j.scitotenv.2012.05.059.
7. Guo, W.; He, M.; Yang, Z.; Lin, C.; Quan, X.; Wang, H. Distribution of polycyclic aromatic hydrocarbons in water, suspended particulate matter and sediment from Daliao River Watershed, China. *Chemosphere* **2007**, *68*, 93–104. https://doi.org/10.1016/j.chemosphere.2006.12.072.
8. Wolska, L. Determination (Monitoring) of PAHs in surface waters: why an operationally defined procedure is needed. *Anal. Bioanal. Chem.* **2008**, *391*, 2647–52. https://doi.org/10.1007/s00216-008-2173-y.
9. Nieva-Cano, M. J.; Rubio-Barroso, S.; Santos-Delgado, M. J. Determination of PAH in food samples by HPLC with fluorimetric detection following sonication extraction without sample clean-up. *Analyst* **2001**, *126*, 1326–31. https://doi.org/10.1039/b102546p.
10. Khalili, N. R.; Scheff, P. A.; Holsen, T. M. PAH source fingerprints for coke ovens, diesel and, gasoline engines, highway tunnels, and wood combustion emissions. *Atmos. Environ.* **1995**, *29*, 533–42. https://doi.org/10.1016/1352-2310(94)00275-P.
11. Aiken, A. C.; DeCarlo, P. F.; Jimenez, J. L. Elemental analysis of aerosol organic nitrates with electron ionization high-resolution mass spectrometry. *Anal. Chem.* **2007**, *79*, 8350–8. https://doi.org/10.5194/amt-3-301-2010.
12. Delgado, B.; Pino, V.; Ayala, J. H.; González, V.; Afonso, A. M. Nonionic surfactant mixtures: a new cloud-point extraction approach for the determination of PAHs in seawater using HPLC with fluorimetric detection. *Anal. Chim. Acta* **2004**, *518*, 165–72. https://doi.org/10.1016/j.aca.2004.05.005.
13. Frysinger, G. S.; Gaines, R. B.; Xu, L. Resolving the unresolved complex mixture in petroleum-contaminated sediments. *Environ. Sci. Technol.* **2003**, *37*, 1653–62. https://doi.org/10.1021/es020742n.
14. Lloyd, J. B. F. Synchronized excitation of fluorescence emission spectra. *Nat. Phys. Sci.* **1971**, *231*, 64–5. https://doi.org/10.1038/physci231064a0.
15. Jing, C.; Zhenyu, D.; Qun, X.; Lina, L.; Rohrer, J. Sensitive and rapid determination of polycyclic aromatic hydrocarbons in tap water. *Thermoscientific - Appl. Note* **2017**, 70923.
16. ISO 17993 Water Quality, Determination of 15 Polycyclic Aromatic Hydrocarbons (PAH) in Water by HPLC with Fluorescence Detection after Liquid-Liquid Extraction. International Organization for Standardization, Geneva, Switzerland, **2002**.
17. Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection. Waters, Milford, Massachusetts, USA, **2008**.
18. Bruzzoniti, M. C.; Fungi, M.; Sarzanini, C. Determination of EPA's priority pollutant polycyclic aromatic hydrocarbons in drinking waters by solid phase extraction-HPLC. *Anal. Methods* **2010**, *2*, 739–45. https://doi.org/10.1039/b9ay00203k.
19. Williamson, K. S.; Petty, J. D.; Huckins, J. N.; Lebo, J. A.; Kaiser, E. M. HPLC-PFD determination of priority pollutant PAHs in water, sediment, and semipermeable membrane devices. *Chemosphere* **2002**, *49*, 703–15. https://doi.org/10.1016/S0045-6535(02)00394-6.
20. Delhomme, O.; Rieb, E.; Millet, M. Solid-phase extraction and LC with fluorescence detection for analysis of PAHs in rainwater, **2007**, 163–71. https://doi.org/10.1365/s10337-006-0144-z.
21. Kayali-Sayadi, M. N.; Rubio-Barroso, S.; Beceiro-Roldan, C.; Polo-Diez, L. M. Rapid determination of PAHs in drinking water samples using solid-phase extraction and HPLC with programmed fluorescence detection. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 3135–46. https://doi.org/10.1080/10826079608015813.
22. Automated Stir Bar Sorptive Extraction (SBSE) in Combination with HPLC - Fluorescence Detection for the Determination of Polycyclic Aromatic Hydrocarbons in Water AppNote. Gerstel, **2002**.
23. Silva, S. A. da; Sampaio, G. R.; Torres, E. A. F. da S. Optimization and validation of a method using UHPLC-fluorescence for the analysis of polycyclic aromatic hydrocarbons in cold-pressed vegetable oils. *Food Chem.* **2017**, *221*, 809–14. https://doi.org/10.1016/j.foodchem.2016.11.098.
24. Berset, J. D.; Ejem, M.; Holzer, R.; Lischer, P. Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. *Anal. Chim. Acta* **1999**, *383*, 263–75. https://doi.org/10.1016/S0003-2670(98)00817-4.
25. Pagliuca, G.; Gazzotti, T.; Zironi, E.; Serrazanetti, G. P.; Mollica, D.; Rosmini, R. Determination of high molecular mass polycyclic aromatic hydrocarbons in a typical Italian smoked cheese by HPLC-FL. *J. Agric. Food Chem.* **2003**, *51*, 5111–5. https://doi.org/10.1021/jf034305j.
26. Okuda, T.; Naoi, D.; Tenmoku, M.; Tanaka, S.; He, K.; Ma, Y.; Yang, F.; Lei, Y.; Jia, Y.; Zhang, D. Polycyclic aromatic hydrocarbons (PAHs) in the aerosol in Beijing, China, measured by aminopropylsilane chemically-bonded stationary-phase column chromatography and HPLC/fluorescence detection. *Chemosphere* **2006**, *65*, 427–35. https://doi.org/10.1016/j.chemosphere.2006.01.064.
27. Pensado, L.; Blanco, E.; Casais, M. C.; Mejuto, M. C.; Martinez, E.; Carro, A. M.; Cela, R. Strategic sample composition in the screening of polycyclic aromatic hydrocarbons in drinking water samples using liquid chromatography with fluorimetric detection **2004**, *1056*, 121–30. https://doi.org/10.1016/j.chroma.2004.04.066.
28. Zhang, H.; Xue, M.; Dai, Z. Determination of polycyclic aromatic hydrocarbons in aquatic products by HPLC-fluorescence. *J. Food Compos. Anal.* **2010**, *23*, 469–74. https://doi.org/10.1016/j.jfca.2009.12.016.


Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC

29. Ishizaki, A.; Saito, K.; Hanioka, N.; Narimatsu, S.; Kataoka, H. Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection. *J. Chromatogr. A.* **2010**, *1217*, 5555–63. https://doi.org/10.1016/j.chroma.2010.06.068.
30. Fernández, M.; Clavijo, S.; Forteza, R.; Cerdà, V. Determination of polycyclic aromatic hydrocarbons using lab on valve dispersive liquid-liquid microextraction coupled to high performance chromatography. *Talanta* **2015**, *138*, 190–5. https://doi.org/10.1016/j.talanta.2015.02.007.
31. Yang, Y.; Dong, X.; Jin, M.; Ren, Q. Rapid determination of polycyclic aromatic hydrocarbons in natural tocopherols by high-performance liquid chromatography with fluorescence detection. *Food Chem.* **2008**, *110*, 226–32. https://doi.org/10.1016/j.foodchem.2008.01.062.
32. Application Note 95, Polycyclic Aromatic Hydrocarbon Determination by Reversed-phase High-Performance Liquid Chromatography. Thermo Fisher Scientific, Waltham, Massachusetts, USA, **1994**.
33. Veiga, L. L. A.; Amorim, H.; Moraes, J.; Silva, M. C.; Raices, R. S. L.; Quiterio, S. L. Quantification of polycyclic aromatic hydrocarbons in toasted guaraná (Paullinia Cupana) by high-performance liquid chromatography with a fluorescence detector. *Food Chem.* **2014**, *152*, 612–8. https://doi.org/10.1016/j.foodchem.2013.11.154.
34. Denis, E. H.; Toney, J. L.; Tarozo, R.; Scott Anderson, R.; Roach, L. D.; Huang, Y. Polycyclic aromatic hydrocarbons (PAHs) in lake sediments record historic fire events: validation using HPLC-fluorescence detection. *Org. Geochem.* **2012**, *45*, 7–17. https://doi.org/10.1016/j.orggeochem.2012.01.005.
35. Huang, Y.; Wei, J.; Song, J.; Chen, M.; Luo, Y. Determination of low levels of polycyclic aromatic hydrocarbons in soil by high performance liquid chromatography with tandem fluorescence and diode-array detectors. *Chemosphere* **2013**, *92*, 1010–6. https://doi.org/10.1016/j.chemosphere.2013.03.035.
36. Windal, I.; Boxus, L.; Hanot, V. Validation of the analysis of the 15+1 European-priority polycyclic aromatic hydrocarbons by donnor–acceptor complex chromatography and high-performance liquid chromatography–ultraviolet/fluorescence detection. *J. Chromatogr. A.* **2008**, *1212*, 16–22. https://doi.org/10.1016/j.chroma.2008.09.104.
37. Sikalos, T. I.; Paleologos, E. K.; Karayannis, M. I. Monitoring of time variation and effect of some meteorological parameters in polynuclear aromatic hydrocarbons in Ioannina, Greece with the aid of HPLC-fluorescence analysis. *Talanta* **2002**, *58*, 497–510. https://doi.org/10.1016/S0039-9140(02)00287-4.
38. Ishizaki, A.; Sito, K.; Kataoka, H. Analysis of contaminant polycyclic aromatic hydrocarbons in tea products and crude drugs. *Anal. Methods* **2011**, *3*, 299–305. https://doi.org/10.1039/c0ay00423e.
39. Jing, C.; Zhenyu, D.; Qun, X.; Lina, L.; Rohrer, J. Sensitive and rapid determination of polycyclic aromatic hydrocarbons in tap water. *Thermoscientific – Appl. Note* **2014**, *1085*, 1–8.
40. Jánská, M.; Tomaniová, M.; Hajšlová, J.; Kocourek, V. Optimization of the procedure for the determination of polycyclic aromatic hydrocarbons and their derivatives in fish tissue: estimation of measurements uncertainty. *Food Addit. Contam.* **2006**, *23*, 309–25. https://doi.org/10.1080/02652030500401207.
41. Application Note 7, HPLC Detector Options for the Determination of Polynuclear Aromatic Hydrocarbons. Varian, Palo Alto, CA, USA, **2009**.
42. Węgrzyn, E.; Grześkiewicz, S.; Popławska, W.; Głód, B. K. Modified analytical method for polycyclic aromatic hydrocarbons, using SEC for sample preparation and RP-HPLC with fluorecent Detection.Application to different food samples. *ACTA Chromatogr.* **2006**, 233–49.
43. Capelo, J. L.; Galesio, M. M.; Felisberto, G. M.; Vaz, C.; Pessoa, J. C. Micro-focused ultrasonic solid-liquid extraction (MFUSLE) combined with HPLC and fluorescence detection for PAHs determination in sediments: optimization and linking with the analytical minimalism concept. *Talanta* **2005**, *66*, 1272–80. https://doi.org/10.1016/j.talanta.2005.01.046.
44. Williams, R.; Meares, J.; Brooks, L.; Watts, R.; Lemieux, P. Priority pollutant PAH analysis of incinerator emission particles using HPLC and optimized fluorescence detection. *Int. J. Environ. Anal. Chem.* **1994**, *54*, 299–314. https://doi.org/10.1080/03067319408034096.
45. Wang, H.; Campiglia, A. D. Determination of polycyclic aromatic hydrocarbons in drinking water samples by solid-phase nanoextraction and high-performance liquid chromatography. *Anal. Chem.* **2008**, *80*, 8202–9. https://doi.org/10.1021/ac8014824.


**Open Access.** This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes – if any – are indicated.

Brought to you by Uludag University | Unauthenticated | Downloaded 03/10/23 10:05 PM UTC