ENVIRONMENTAL ANALYSIS

QUANTIFICATION OF COMPLEX POLYCYCLIC AROMATIC HYDROCARBONS OR PETROLEUM OILS IN WATER WITH CARY ECLIPSE FLUORESCENCE SPECTROPHOTOMETER ACCORDING TO ASTM D 5412-93 (2000)

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Abstract

The aim of the work was to apply the procedure for the determination of polyaromatic hydrocarbons (PAH) according to **ASTM D 5412 – 93 (Reapproved 2000) "Standard Test Method for Quantification of Complex Polycyclic Aromatic Hydrocarbon Mixtures or Petroleum Oils in Water"** (from this point on it will be referred to as D 5412-93) (1) using the Agilent Cary Eclipse Fluorescence Spectrophotometer.

As test substances we selected naphthalene, benzo[a]pyrene and anthracene. Benzo[a]pyrene was selected, as the most common PAH required for the determination, as it is a component of most PAH mixtures and a procarcinogen, and is usually determined as an individual target PAH substance (http://water.epa.gov/drink/contaminants/basicinformation/benzo-a-pyrene.cfm). Naphthalene and anthracene are selected as instrumental standards recommended by D 5412-93 (1). In these tests, we restricted ourselves with the test of neat solutions according to Section 11 of (1).

Materials and methods

Equipment

Fluorimetric apparatus

We used an Agilent Cary Eclipse Fluorescence Spectrophotometer with a standard photometric cell accessory and software. It fully complies with the requirements for the apparatus for the Test Method (Sections 7.1, 7.2 and 7.4 of D 5412 -93 (1)) According to the requirement for fluorescence cells (Section 7.3 of D 5412 -93 (1)) we used quartz fluorimetric cells with the path length of 1 cm and the height of 5 cm. The preparation of the apparatus for work was made according to Section 10 of D 5412 -93 (1) and the manual of the Agilent Cary Eclipse Fluorescence Spectrophotometer.

Auxiliary equipment

An Ohaus Explorer Pro analytical balance (Ohaus, Switzerland; max weight 210 g, d = 0.1 mg) was used for weighing dry powder samples.

Glassware and plasticware

A-class volumetric flasks («ISOLAB», Germany) from borosilicate glass with volume (50.000 ±0.060) mL, (100.00 ±0.10) mL and (500.00 ±0.60) mL were used for diluted mercury solutions preparation. 4-Dram Amber Glass Vials (Specialty Bottle, 3434 4th Avenue S, Seattle, WA 98134, USA) were used for sample and calibration preparations. Automatic Eppendorf Research® pipettes («Eppendorf International», Germany) with Eppendorf Biopur® pipette tips were used throughout. Hamilton microliter syringes of the 900 series for dispensing volumes from 0.5 µL up to 10 µL (Hamilton Company USA, 4970 Energy Way, Reno, Nevada 89502) were used. Kimble volumetric pipets (Class A) of 0.5 mL were also used.

Reagents and solvent

Deionized water (18.2 MΩ·cm) from a Milli-Q Academic system (Millipore, France) was used throughout for the solution preparation. Cyclohexane (CAS Number 110-82-7), HPLC grade, ≥99.9% from Sigma-Aldrich was used throughout. Naphthalene (CAS Number 91-20-3), analytical reagent 99.7% from Fluka and benzo[a]pyrene (CAS Number 50-32-8), ≥96% (HPLC) from Sigma–Aldrich were used throughout. Also, anthracene (CAS Number 120-12-7), analytical standard (99.0%) for environmental analysis from Fluka was used for preparing artificial mixtures of PAHs in cyclohexane. Nitric Acid (1 + 1) was used as in Section 8.5 of of D 5412 -93 (1) One volume of concentrated analytical grade HNO_3 (Reakhim, Moscow; sp. gr. 1.42) was added to one volume of deionized water. All other reagents and solvents were selected as required (Section 8 of D 5412 -93 (1)). Standard solutions were kept no more than 3 days in the refrigerator in amber-glass vials away from light.

Procedures

All the procedures were made according to Section 11 of D 5412 -93 (1). Certain conditions for individual substances are shown below. The only change in the procedure is the use of plastic pipette tips for solution preparation as the experiments showed insignificant differences of the blank signal and signals compared to PAHs made using glass pipettes, glass syringes and pipette tips. All the calibrations were made using three replicate measurements according to the Section 12 of D 5412 -93 (1) starting at 100 µg/mL in the cyclohexane and diluting down. The standard solutions spanned a range from 5 µg/mL to 0.2 µg/mL for naphthalene and to 10 ng/mL for benzo[a]pyrene.

Data treatment

The measurement results are presented in accordance with the requirements of ISO/IEC 17025:2005 (2). The limits of detection (**LOD**) and quantification (**LOQ**) were calculated as 3σ- and 10σ-criteria (in CZE by peak heights) according to the IUPAC 1998 recommendations for the presentation of the results of chemical analysis (3). Simple variance analysis, the separation of the total standard deviation σ into the repeatability (deviation between replicate measurements) and temporal (day-to-day deviation) portions, was done according to the approach proposed in (4). All the calibrations and determinations fully comply with the Section 12 "Quality Control Measures" of D 5412 -93 (1). Although solvent blanks were found low (see below) for all the concentrations of PAHs analyzed solvent blanks were subtracted out of the spectra for more accurate quantification. All the data obtained within Agilent Cary Eclipse Fluorescence Spectrophotometer software were exported as text files into Microsoft Excel 2013 and treated in this software.

Results and Discussion

Sample Preparation

Solvent blank

The fluorescence solvent blank was tested according to Section 8.4 of D 5412 -93 (1) For wavelengths 380–450 nm for benzo[a]pyrene and for 300–480 nm for naphthalene the intensity of solvent blank was (1.3 ± 0.5)% from the intensity of the maximum emission peak for the lowest concentration of the PAH analyzed, which satisfies the requirement of less than 5 % of Section 8.4 of D 5412 -93 (1).

Water purity

According to the requirements of Section 8.3 of D 5412 -93 (1), the purity of the water used was checked by analyzing a water blank using the same instrumental conditions as for the solvent blank. The results showed the negligible differences between water and solvent blanks.

Pipette usage

For the example of naphthalene, three variants of solution preparation (*i*) glass micropipet, 10 to 50-µL capacity—recommended in Section 7.5 of D 5412 -93 (1); (*ii*) Hamilton microsyringes; and (*iii*) plastic tips of Eppendorf pipettes. It was found that the negligent use of any of these variants of solution treatment affected the total error of the measurements. As a result, we used Eppendorf pipettes for sample preparation throughout the results from this point on.

Naphthalene

Naphthalene was selected as the most common PAH with technogenic and as human uses and the recommended instrumental standard for this Test Method (1). Fluorescence spectra (Figure 1) correspond to the existing data with distinct peak maxima at 324 and 338 nm and a shoulder peak at 350–352 nm (5). The excitation wavelength of 250 nm was selected by recording the intensity of the maximum peak heigh in the

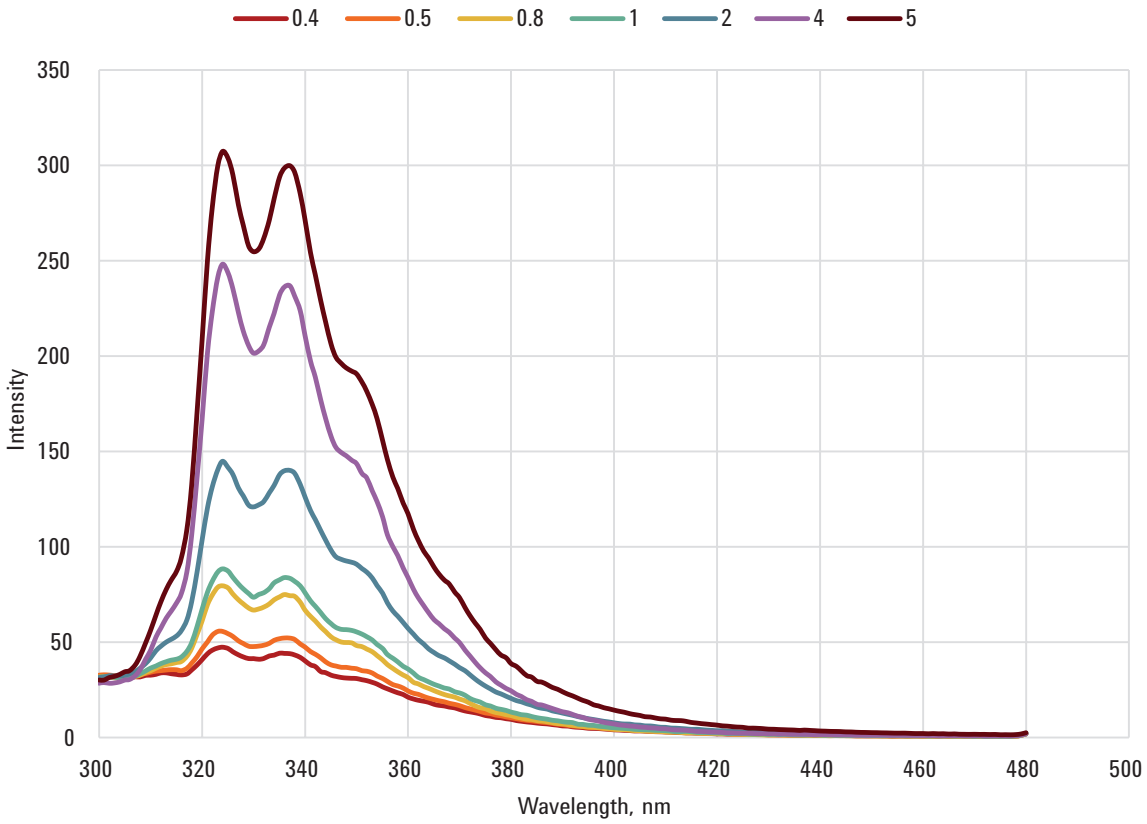


Figure 1: Fluorescence spectra of naphthalene, Ex. Wavelength 250 nm, Ex. Slit 10 nm, Em. Slit 5 nm. Figures in the legend denote final concentrations in µg/mL.

emission spectra (324 nm) as a function of the excitation wavelength. Short-term stability of the measurements was tested by measuring the relative standard deviation (RSD) for three replicate measurements in the information-bearing region of 300–400 nm. The RSD in this region (Figure 2a) is no higher than 0.1 (for low concentrations), 0.05 for medium concentrations and around 0.01–0.02 in the vicinity of the analytical fluorescence maximum. This is within the limits for the precision and bias of the method according to Section 13 of D 5412 -93 (1). The long-term precision was determined by triplicately measuring the spectra for the same three levels of concentrations, on a weekly basis, in accordance with Sections 12 and 13 of D 5412 -93 (1). It was found that the data comply with the precision and bias requirements of the D 5412 -93.

Performance parameters

Calibration plots were built from freshly prepared solutions. The calibration equation is described with an equation (Figure 3)

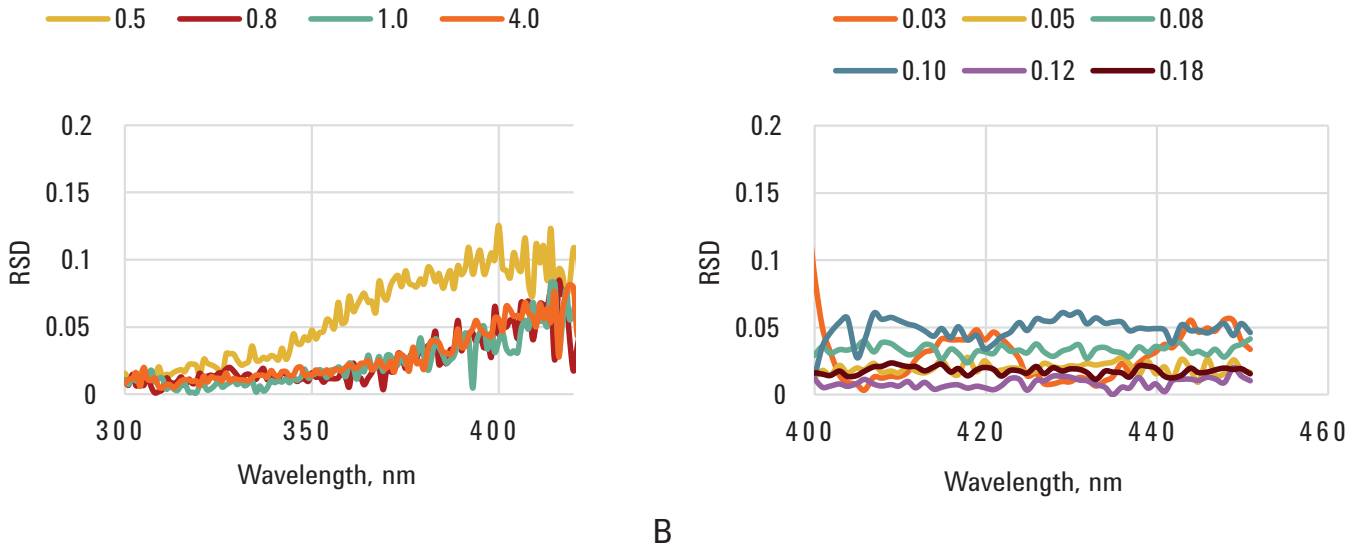


Figure 2: Relative standard deviations of measurements of three replicates of test solutions of (A) naphthalene (Ex. Wavelength 250 nm, Ex. Slit 10 nm, Em. Slit 5 nm) and (B) benzo[a]pyrene (Ex. Wavelength 268 nm, Ex. Slit 10 nm, Em. Slit 5 nm.). Figures in the legend denote final concentrations in µg/mL.

$I=(55.2\pm0.9)c + (30\pm2)$, $P=0.95$, $n=20$, $r=0.9985$

where I is fluorescence intensity (arb. units) and c is the concentration of naphthalene (in µg/mL). The limit of detection of naphthalene is 0.13 µg/mL. LOQ is 0.5 µg/mL

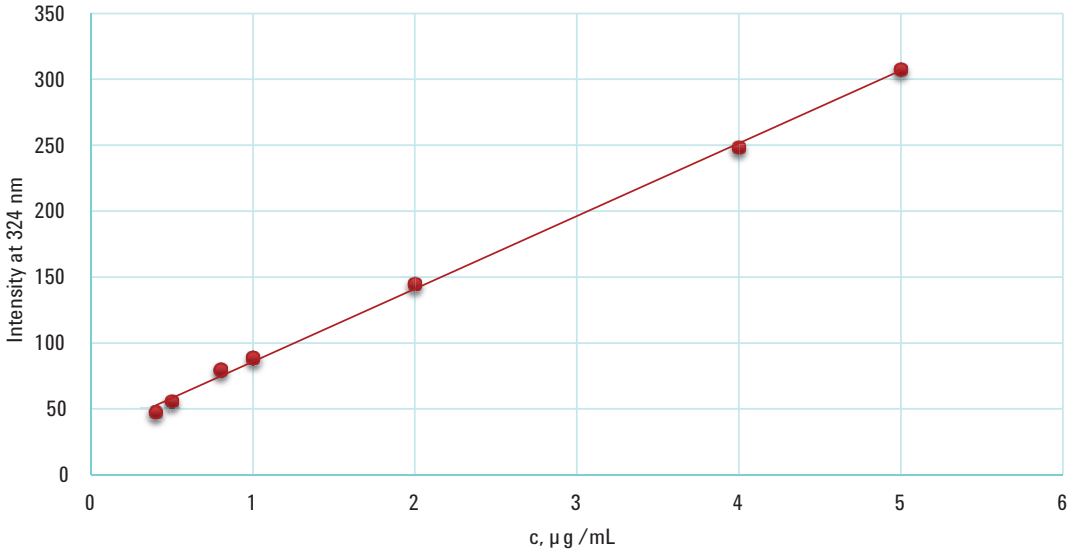


Figure 3. Calibration plot (points for the same concentration are averaged) for the fluorimetric determination of naphthalene at 324 nm, Ex. Wavelength 250 nm, Ex. Slit 10 nm, Em. Slit 5 nm.

For the compatibility of measurement conditions for benzo[a] pyrene (see below) we tested the performance parameters for naphthalene for Ex. Wavelength 268 nm, Ex. Slit 10 nm, Em. Slit 5 nm. The calibration parameters slightly degraded compared to the above equation, retaining a high coeffcient of correlation;

$I=(44\pm2)c+(32\pm3)$, $P=0.95$, $n=20$, $r=0.9902$

where I is fluorescence intensity (arb. units) and c is the concentration of naphthalene (in µg/mL). The limit of detection of naphthalene under these conditions is 0.5 µg/mL. Thus, a decrease in the sensitivity of determination, although significant, is within the same order of values as above for the optimum conditions for naphthalene. The recoveries for the neat samples of naphthalene were found in concordance with the conditions discussed above and according to Sections 12 and 13 of D 5412-93 (1), Table 1. All the values are within the required range.

Amount added, µg/mL	Amount found, µg/mL	Bias, %
0.40	0.38	-4.6
0.50	0.52	3.2
0.80	0.78	-2.1
1.00	1.01	0.9
2.00	2.03	1.7
4.00	4.07	1.9
5.00	5.00	0.001

Table 1. Recoveries of known amounts of naphthalene.

Thus, the conditions of the fluorimetric determination of naphthalene fully comply with the requirements of D 5412-93 (1)

Benzo[a]pyrene

Benzo[a]pyrene was selected as a very significant procarcinogen, which is commonly found since it is formed as a result of the incomplete combustion of organic materials. As with the case of naphthalene, fluorescence spectra (Figure 4) correspond to the existing data with distinct peak maxima at 405 and 429 nm and a shoulder peak at 434–435 nm (6). The excitation wavelength of 268 nm was selected by recording the intensity of the maximum peak heigh in the emission spectra (405 nm) as a function of the excitation wavelength.

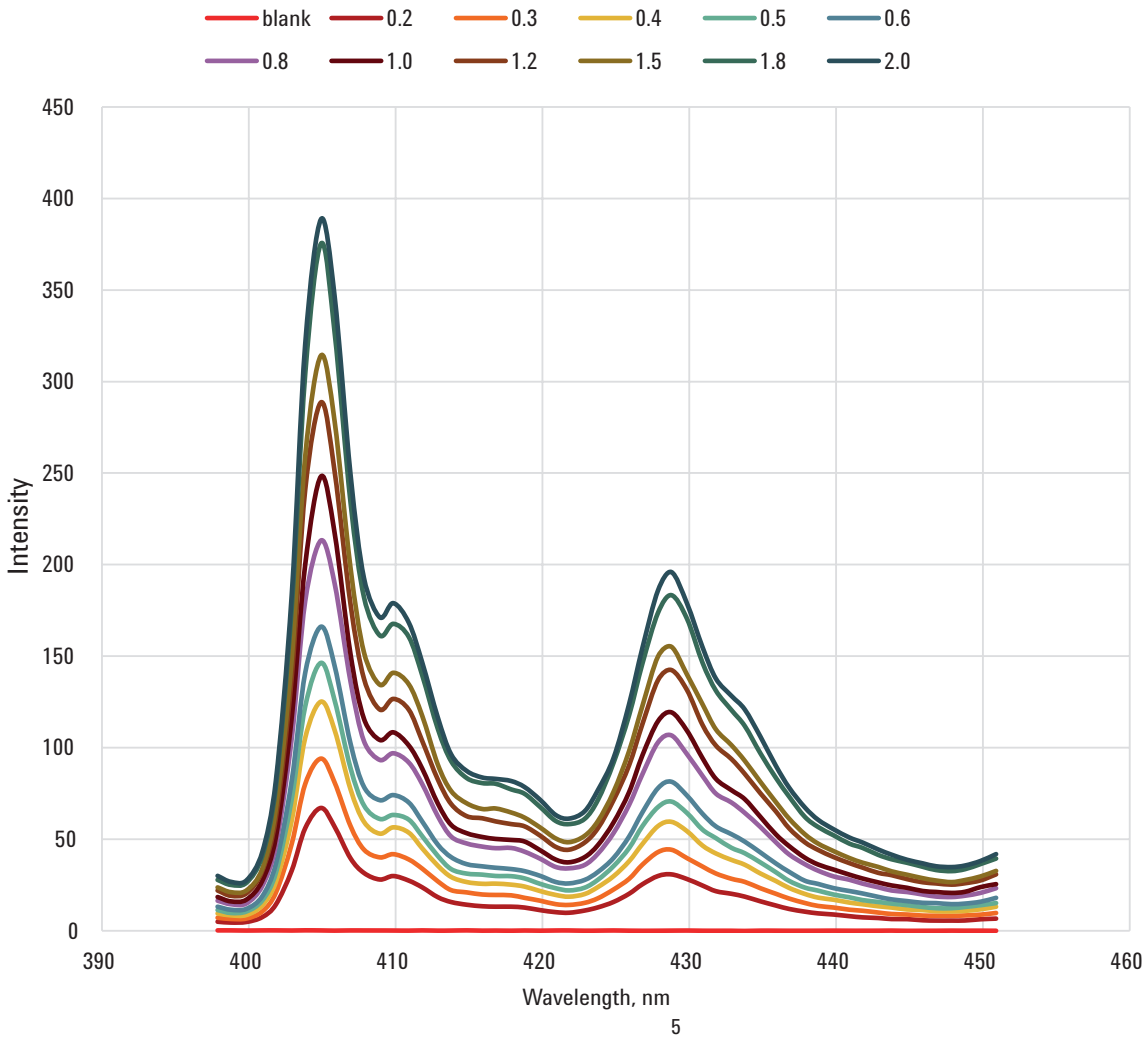


Figure 4: Fluorescence spectra of benzo[a] pyrene, Ex. Wavelength 268 nm, Ex. Slit 5 nm, Em. Slit 2,5 nm. Figures in the legend denote final concentrations in µg/mL.

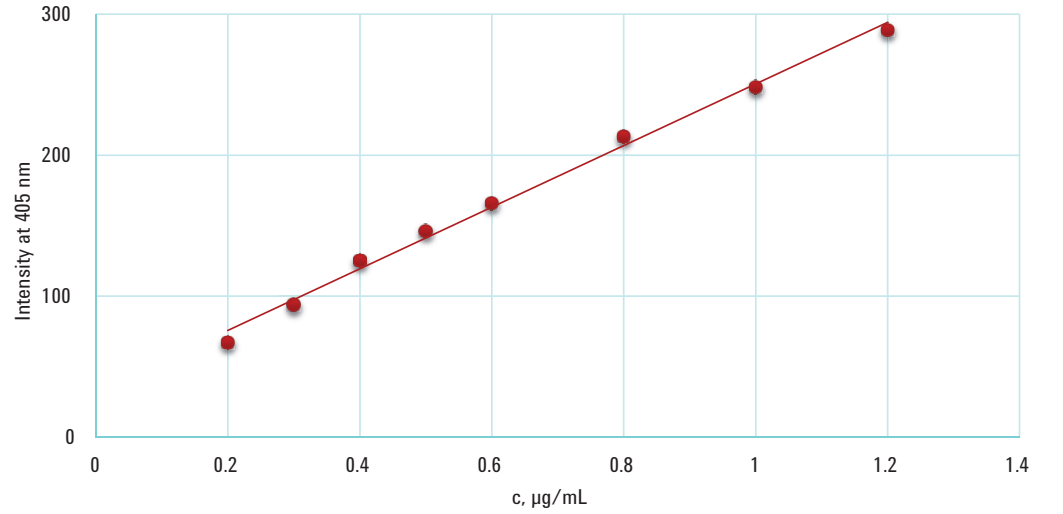
Short-term stability of the measurements of benzo[a]pyrene was tested by measuring the relative standard deviation (RSD) for three replicate measurements in the information-bearing region of 400–450 nm (Figure 2b). For submicrogram concentrations, the RSD in this region is no higher than 0,15 and 0.05 for medium concentrations, and does not depend on the closeness of the wavelength to the analytical fluorescence maximum. As for naphthalene, this is within the limits for the precision and bias of the method according to Section 13 of D 5412 -93 (1). The long-term precision was determined by triplicately measuring the spectra for the same three levels of concentrations, on a weekly basis, in accordance with Sections 12 and 13 of D 5412 -93 (1). It was found that, similarly to naphthalene, the data comply with the precision and bias requirements of the D 5412 -93.

Performance parameters

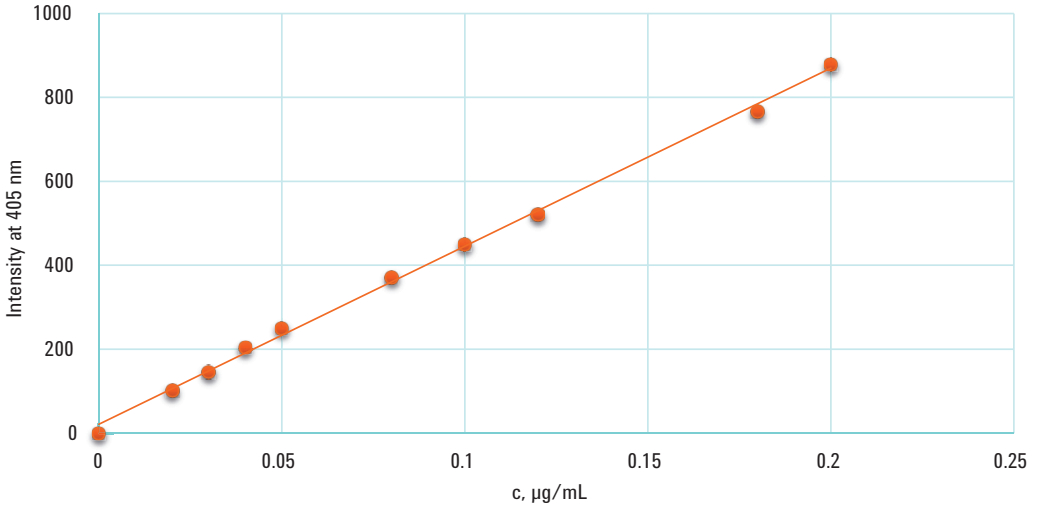
Calibration plots were built from freshly prepared solutions. The calibration equation is described with an equation (for Ex. Slit 5 nm, Em. Slit 2.5 nm, see Figure 5A)

$I=(218\pm6)c+(32\pm4)$, $P=0.95$, $n=11$, $r=0.9944$

where I is fluorescence intensity (arb. units) and c is the concentration of naphthalene (in µg/mL). The limit of detection of naphthalene is 0.06 µg/mL. It is noteworthy that the intercept of the calibration curve differs insignificantly from the value above for naphthalene, and the coefficient of correlation around 0.995.



A



B

Figure 5. Calibration plots for the fluorimetric determination of benzo[a]pyrene at 405 nm plot (points for the same concentration are averaged), Ex. Wavelength 268 nm, (A) Ex. Slit 5 nm, Em. Slit 2.5 nm; (B) Ex. Slit 10 nm, Em. Slit 5 nm.

By increasing the widths of the excitation and emission slits (Ex. Slit 5 nm, Em. Slit 2.5 nm), it is possible to increase the sensitivity of measurements (Figure 5B). The equation is

$I=(4190\pm60)c+(30\pm7)$, $P=0.95$, $n=22$, $r=0.9984$

where I is fluorescence intensity (arb. units) and c is the concentration of naphthalene (in µg/mL). The limit of detection of naphthalene is 0.005 µg/mL. Thus, the limit of detection is decreased by an order with the same intercept and very good coefficient of correlation. The recoveries for the neat samples of benzo[a]pyrene were found in concordance with the conditions discussed above and according to Sections 12 and 13 of D 5412-93 (1), Table 2. All the values are within the required range.

Amount added, µg/mL	Amount found, µg/mL	Bias, %
0.030	0.030	0.9
0.040	0.039	-3.4
0.050	0.052	-0.03
0.080	0.084	5.0
0.100	0.100	-0.4
0.120	0.118	-1.4
0.180	0.176	-1.7

Table 2. Recoveries of known amounts of benzo[a]pyrene.

Mixtures

Mixtures of naphthalene, benzo[a]pyrene, and anthracene, each of 0.5 µg/mL, were prepared and diluted giving the range of total PAHs concentrations of 0.03–0.5 µg/mL, which is within the range of calibration of benzo[a]pyrene, see the previous section. Under the conditions for benzo[a]pyrene, these solutions were tested (Table 3).

Total PAH amount added, µg/mL	Total PAH amount found, µg/mL	Bias, %
0.03	0.034	13
0.05	0.048	-4
0.08	0.095	19
0.1	0.014	40
0.3	0.33	10
0.4	0.42	5
0.5	0.57	14

Table 3. Recoveries of known amounts of mixtures of naphthalene, benzo[a] pyrene, and anthracene.

The recoveries are Sections 12 and 13 of D 5412-93 (1). All the values are within the required range.

Conclusion

Thus, we found that the conditions and requirements of the standard test method for quantification of complex polycyclic aromatic hydrocarbon mixtures (ASTM D 5412 – 93 (Reapproved 2000)) are readily met using an Agilent Cary Eclipse Fluorescence Spectrophotometer with standard accessories and software.

For naphthalene and benzo[a]pyrene, good performance parameters and submicrogram limits of detections are achieved (5 ng/mL for benzo[a]pyrene), the recoveries of test solutions for the microgram amounts of selected PAHs are within the required levels according to the ASTM D 5412 – 93 (Reapproved 2000). For artificial mixtures, the recoveries of total PAHs determined as benzo[a]pyrene are also within the required level of precision.

References

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