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RESEARCH PAPER

Determination of heavy polycyclic aromatic hydrocarbons of concern in edible oils via excitation–emission fluorescence spectroscopy on nylon membranes coupled to unfolded partial least-squares/residual bilinearization

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Abstract Seven heavy polycyclic aromatic hydrocarbons (PAHs) of concern on the US Environmental Protection Agency priority pollutant list (benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*c*,*d*]-pyrene) were simultaneously analyzed in extra virgin olive oil. The analysis is based on the measurement of excitation–emission matrices on nylon membrane and processing of data using unfolded partial least-squares regression with residual bilinearization (U-PLS/RBL). The conditions needed to retain the PAHs present in the oil matrix on the nylon membrane were evaluated. The limit of detection for the proposed method ranged from 0.29 to 1.0 μg kg^{-1}, with recoveries between 64 and 78 %. The predicted U-PLS/RBL concentrations compared favorably with those measured using high-performance liquid chromatography with fluorescence detection. The proposed method was applied to ten samples of edible oil, two of which presented PAHs ranging from 0.35 to 0.63 μg kg^{-1}. The principal advantages of the proposed analytical method are that it provides a significant reduction in time and solvent consumption with a similar limit of detection as compared with chromatography.

Keywords Polycyclic aromatic hydrocarbons · Edible oils · Fluorescence excitation–emission on nylon · Multivariate calibration

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large family of organic compounds that contain two or more fused aromatic rings that are composed of carbon and hydrogen atoms [1]. These are ubiquitous compounds, and those with high molecular weight (five to six fused aromatic rings) have lipophilic character and are primarily of interest because of their carcinogenic and mutagenic characteristics. PAHs are mainly produced by the incomplete combustion of organic matter and are continuously released into the atmosphere from natural and anthropogenic sources [2].

Humans are primarily exposed to PAHs through the direct inhalation of polluted air or tobacco smoke; direct skin contact with polluted soils, soot or tars; and ingestion of contaminated water or food, especially fatty food (animal or vegetable) [3]. Accordingly, several heavy PAHs have been detected in edible oils, including olive and sunflower oils (SO) [4].

The occurrence of PAHs in edible oils can primarily be attributed to environmental contamination of raw vegetable materials and to the contamination that occurs during their processing, wherein processes such as seed drying, solvent extraction, soil burning, packaging of materials, and mineral oils used to lubricate the machinery for extracting oil from plants represent possible contamination sources and pathways [4, 5].

The carcinogenic and mutagenic characteristics of the heavier PAHs on the priority pollutant list of the US Environmental Protection Agency (US-EPA) justify the careful analytical

control of their presence in foods, specifically fatty foods, and make the development of unambiguous and uniform regulations necessary. In July 2001, Spain passed a legislation that limits the concentration of eight heavy PAHs in olive pomace oils, including benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), indeno[1,2,3-*c*,*d*]-pyrene (IP), dibenz [*a*,*h*]anthracene (DBahA), and benzo[*g*,*h*,*i*]perylene (BghiP). A maximum limit of 2 $\mu g\ kg^{-1}$ for each single PAH and 5 $\mu g\ kg^{-1}$ for the sum of the eight heavy PAHs was established [6]. More recently, the European Food Safety Authority published a new regulation that establishes a limit of 2 $\mu g\ kg^{-1}$ for BaP and 10 $\mu g\ kg^{-1}$ for the sum of four heavy PAHs (BaP, crisene, BaA, and BbF) [7]. In 2003, Chile modified its Sanitary Decree no. 977 of 1996 and established the same maximum limits as the Spanish legislation for edible oils and fats in general [8].

The two major problems associated with the determination of PAHs in complex matrices, such as vegetable oils and fats, are the diversity of potential interferences and low analyte levels [5]. The proposed methods for determination of PAHs in oils include extraction and purification for the partial or complete removal of lipophilic components co-extracted with the target compound followed by a chromatographic determination [1, 4]. All of these methodologies are laborious, time consuming, and expensive, not only for the sample pretreatment but also for the analytical determination. One alternative to chromatographic analysis is fluorescence spectroscopy. Molecular fluorescence measurements can be rapidly and inexpensively performed. Many environmentally important hydrocarbon contaminants are naturally fluorescent and detectable at the microgram-per-kilogram level. Unfortunately, their broad fluorescence bands and the considerable number of naturally fluorescent compounds prevent complete analyte selectivity with both excitation- and emission-based measurements [9, 10]. An alternative approach to improve the selectivity of this analytical method is the use of advanced chemometric tools, such as second-order multivariate calibration methods. Some second-order methods allow for the direct determination of concentrations and the estimation of spectral profiles of sample components in the presence of many unknown constituents. These properties, termed the second-order advantage, remove the need to physically eliminate interfering compounds or to construct large and diverse calibration sets [11–14].

At the very low concentrations detected in edible oil samples, the quantification of PAHs requires a pre-concentration step. Some techniques developed for this purpose include liquid–liquid and solid-phase extraction (SPE), solid-phase microextraction and head-space solid-phase microextraction. A newer approach is the adsorption of PAHs dissolved in water samples on octadecyl (C_{18}) or nylon membranes with a subsequent determination of analytes by phosphorescence or fluorescence spectroscopy on the solid surface [15–17]. Thus, extraction membranes serve the dual purpose of pre-concentrating samples and acting as solid surfaces for fluorescence measurements. Preconcentration on membranes and excitation–emission fluorescence spectroscopy, associated to different chemometrics algorithms, have been proposed for the simultaneous determination of BaP and DBahA in water [13] and monohydroxy-PAHs in urine samples [18]. However, the suitability of this analytical approach has not been investigated in more complex samples such as edible oils.

The present work explores the possibility of applying excitation–emission fluorescence spectroscopy on nylon membranes, using unfolded partial least-squares regression coupled to residual bilinearization (U-PLS/RBL), for the simultaneous determination of seven heavy PAHs—including BaA, BbF, BkF, BaP, DBahA, BghiP, and IP from the US-EPA list—in edible oils. This group of PAHs coincides with the Spanish legislation. BeP was not considered in the present study since has not been included in the group of carcinogenic PAHs [19]. Microwave-assisted liquid–liquid extraction coupled to SPE with silica was required as a previous sample preparation step. The predicted U-PLS/RBL concentrations were compared with those obtained using high-performance liquid chromatography (HPLC) with fluorescence detection as the reference method.

Theory

Unfolded partial least squares regression with residual bilinearization (U-PLS/RBL).

The U-PLS method is a variant of the classical partial least squares (PLS) that was proposed for second-order data where three-way data are unfolded into vectors before two-way PLS calibration [20]. If the calibration was exact, the regression coefficients, **v**, could be employed to estimate the analyte concentrations in an unknown specimen using Eq. (1),

$$y_u = \mathbf{t^T}_{\mathbf{u}}\mathbf{v} \tag{1}$$

where $\mathbf{t}_{\mathrm{u}}$ is the test sample score, which is obtained by projection of the vectorized (unfolded) data for the test sample $\mathbf{X}_{\mathrm{u}}$ onto the space of the A latent factors, as indicated in Eq. (2),

$$\mathbf{t}_{\mathrm{u}} = \left(\mathbf{W}^T\mathbf{P}\right)^{-1}\mathbf{W}^T\mathrm{vec}(\mathbf{X}_{\mathrm{u}}) \tag{2}$$

where **P** and **W** are the matrix of loadings and weight loadings, respectively; vec(.) implies the vectorization operator and ***T*** the transposition operator.

When unexpected constituents occur in $\mathbf{X}_{\mathrm{u}}$, the sample scores given in Eq. (1) are unsuitable for analyte prediction

and the U-PLS method must be coupled to RBL to achieve the second-order advantage. RBL is a post-calibration procedure that is based on principal component analysis (PCA) to model the presence of unexpected constituents in a sample [13, 21–23].

The RBL procedure consists maintaining the matrix of loadings P constant at the calibration values and varying $\mathbf{t}_u$ to minimize the norm of residual error. The standard deviation (s_{RBL}) of the residuals can be used as a measure of the goodness of fit for the RBL procedure and to estimate the number of unexpected constituents according to Bortolato et al. [13]. In this approach the s_{RBL} is assumed to stabilize at a value compatible with the instrumental noise when the correct value of RBL components is reached [13, 21].

Experimental

Reagents and solutions

Acenaphthylene, anthracene, phenanthrene, fluoranthene, fluorene, BbF, BkF, and IP were purchased from Accustan dard (New Haven, CT, USA); acenaphthene, pyrene, and chrysene were obtained from Supelco (Bellefonte, PA, USA); naphthalene, BaA, and BghiP were purchased from Aldrich (Steinheim, Germany); and BaP and DBahA were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All reagents were of high-purity grade and used as received.

Acetonitrile, *n*-hexane and 2-propanol were purchased form Merck (Darmstadt, Germany), and dichloromethane was obtained from Mallinckrodt Chemicals (Phillipsburg, PH, USA). All of these reagents were of HPLC grade and used as received.

Stock solutions of pure analytes (100 μg mL^{-1}) were prepared in acetonitrile. From these solutions, more dilute solutions (100 μg L^{-1}) in *n*-hexane were obtained at appropriate volumes, evaporating them under a nitrogen stream and diluting with *n*-hexane. All of the solutions were stored in silaned amber vials at 4 °C in darkness. The solutions were stable for almost six months. The PAH reagents were handled with extreme caution, including the use of gloves and protective clothing.

Apparatus and software

A Milestone Microwave Laboratory System (Sorisole, BG, Italy) equipped with a high-performance microwave digestion unit (model mls-1200 Mega), an exhaust module (model EM-45/A), a terminal Mega-240, and a ten-position rotor was used for sample preparation.

A Varian Cary-Eclipse luminescence spectrometer (Mulgrave, Australia) equipped with a xenon flash lamp was used to obtain the excitation–emission fluorescent matrices (EEFMs). The EEFMs on the nylon surfaces were recorded at 600 nm min^{-1}, for a λ_{exc} range of 250–400 nm at 5-nm increments and for a λ_{em} range of 370–550 nm every 2 nm. The widths of the excitation and emission slits were 5 and 2.5 nm, respectively. The spectra were saved in ASCII format and transferred to a computer for subsequent manipulation. The routines for data pretreatment used to eliminate Rayleigh and Raman scattering peaks from the EEFMs [24], and subsequent data processing were implemented in MATLAB [25]. The routines employed for U-PLS/RBL are available on the internet [26]. All of the algorithms were implemented using the graphical interface of the MVC2 toolbox [27], which is also available on the internet [28].

HPLC with fluorescence detector (HPLC-FLD) analysis was performed on a liquid chromatograph equipped with a Waters 600 HPLC pump, a Waters 2475 fluorescence detector and a Waters 717 auto sampler (Milford, MA, USA). The column was an Inertsil HPLC ODS-P (250 x 4.6 mm ID, 5 μm particle size) purchased from GL Sciences (Tokyo, Japan). The mobile phase was a mixture of acetonitrile (A) and water (B) at a flow rate of 1.4 mL min^{-1}. The following gradient program was used: 0–0.1 min 70 % A isocratic; 0.1–10 min linear gradient 90 % A; 10–15 min 90 % A isocratic; 15–20 min linear gradient 100 % A; 20–32 min 100 % A isocratic. Then, back to the initial condition: 32–35 min linear gradient 70 % A; 35–38 min 70 % A isocratic. An injection volume of 20 μL was employed. Four channels were used to define the excitation and emission wavelengths (λ_{exc}/λ_{em}) in the fluorescence detector as follows: channel A 220/330; channel B 292/410; channel C 292/426; and channel D 300/500.

Microwave-assisted liquid–liquid extraction coupled to SPE

A glass system previously designed in our laboratory was used during the microwave-assisted extraction (MAE) [29]. An aliquot of 1.00±0.01 g of oil was carefully weighed and transferred to a 50-mL Erlenmeyer flask equipped with a ground-glass joint. Then 30 mL of acetonitrile was added and an air-cooled condenser was attached to the ground-glass joint of the flask. The glass system was heated in a microwave oven for 19 min at 150 W. Only eight of the ten positions available in the rotor were used. After cooling, the inner wall of the condenser was rinsed with a few milliliters of acetonitrile and then removed from the flask. The top layer was carefully transferred with a Pasteur pipette into a 50-mL round-bottom flask, and the extract was concentrated to dryness in a vacuum rotary evaporator equipped with a 65 °C water bath. Then, the extract was dissolved in 1 mL of *n*-hexane.

Solid phase extraction cleanup was performed using a 2-g silica SPE cartridge obtained from Supelco (Bellefonte, PA, USA). The 2-g silica cartridge was previously washed with 5 mL of dichloromethane and conditioned with 5 mL of *n*-hexane. Then, 1 mL of the dissolved extract was loaded onto the cartridge (0.5 mL for SO), and the PAHs were eluted with

a 15-mL mixture of *n*-hexane:dichloromethane 80:20 (*v*/*v*). All of the eluate was collected in a 22-mL amber vial and concentrated under a nitrogen stream. The residue was dissolved in 1 mL of 2-propanol, and then 4 mL of water were added. This solution was filtered through a nylon membrane for sorption and to obtain the EEFMs in the luminescence spectrometer. For test samples, half of the 2-propanol solution underwent this procedure; the remaining 0.5 mL was used for HPLC-FLD analysis.

Sorption and fluorescence spectroscopy on a nylon surface

The 0.20-μm pore-size nylon membranes (Supelco, Bellefonte, PA) were divided into 13 mm disks and placed into a 13-mm stainless steel syringe filter (Advantec, Dublin, CA). To concentrate the analytes onto a restricted area of the nylon surface, a polytetrafluoroethylene (PTFE) ring was fitted over the membrane before the extraction. Thus, a nylon surface with an effective diameter of 7 mm was exposed to each flowing solution. A 5.0-mL glass syringe with a PTFE plunger tip (Hamilton, Reno, NV) was coupled to the filter holder, and positive pressure was used to force solutions through the nylon disk. The time involved in this step was about 5 min/sample. Following analyte sorption, the membrane was partially dried by forcing 50 mL of air through the disk using a 50-mL syringe (Hamilton, Reno, NV) and was completely dried on a heating plate for 3 min at about 50 °C. For EEFM measurements, the disk was kept on a holder with the aid of double-sided adhesive tape, and it was placed into the spectrofluorimeter in such a way that the angle formed between the excitation and emission beams was 90°, with an incident angle of 45°.

Calibration set samples

Nineteen samples of a commercial organic extra-virgin olive oil (EVOO) were used to prepare the calibration set. These samples were processed using the sample preparation procedure described above and spiked with different volumes (on the order of microliters) of dilute solutions of the seven selected PAHs prior to SPE on silica. The quantitative recovery of the SPE on silica was studied and previously established [30]. Twelve samples of the set correspond to the mass provided by a Plackett-Burman design. The tested masses were in the range of 0.25–4.00 ng for BaA, BbF, BaP, DBahA, and BghiP; 0.16–1.0 ng for BkF; and 1.0–5.0 ng for IP. The remaining seven samples corresponded to a blank solution, a solution that contained all of the studied PAHs at an average mass (2.12 ng for BaA, BbF, BaP, DBahA, and BghiP; 0.58 ng for BkF; and 3.0 ng for IP) and five additional solutions that contained BbF (1.0–5.0 ng), BkF (0.2–1.0 ng), BghiP (1.0–4.0 ng), and IP (2.0–6.0 ng). Following analyte sorption on the nylon membranes, the EEFMs were then read and subjected to second-order data analysis.

Validation set samples

A test set of thirteen validation samples, different from the calibration ones, was prepared and processed in a similar way to the calibration solutions. The mass of each analyte was selected at random from their corresponding calibration ranges.

Test set samples

To test the versatility of the investigated method, different brands and types of edible oils were analyzed. Five brands of EVOO and two brands of common SO were purchased at a local supermarket. Because these samples either did not contain PAHs or their concentrations were lower than the detection limits of the studied methods, a recovery and predictive capacity study was performed; wherein these samples were spiked with the seven studied PAHs at the beginning of the process with masses different than those used for calibration, following a random selection from the corresponding calibration ranges. These samples were subsequently processed using the sample preparation procedure described above, and the EEFMs were read. A total of 12 spiked samples were prepared for this purpose, including 10 samples from the five different brands of EVOO and 2 samples from the two brands of SO. These samples were also analyzed using HPLC-FLD as a reference method [1].

Real samples

A set of ten samples corresponding to different brands of EVOO was purchased at a local supermarket in November 2012 and analyzed using the proposed method.

Results and discussion

The overlap of the emission fluorescence spectra for the seven studied PAHs is a well-known fact. Consequently, simultaneous fluorimetric determination of these compounds represents a significant analytical challenge. Furthermore, we have demonstrated that oil matrices complicate the determination of PAHs because of the presence of pigments (primarily pheophytin and chlorophyll) and tocopherols that produce inner filter phenomena and partial overlap with the bands of PAHs at short wavelengths [30]. As a result, even when second-order methods are used, a sample preparation step is hard to avoid. The proposed sample preparation method, combining microwave-assisted liquid–liquid extraction with SPE on silica, permits the analytes to be extracted and eliminates the primary causes of interference for the unimpeded detection of PAHs in edible oils.

Sorption and fluorescence spectroscopy on nylon surface

The ability of nylon to retain PAHs after a solid phase extraction procedure from water samples containing these compounds has been demonstrated by Bortolato et al. [17]. This fact permitted the authors to develop a method for the determination of BaP and DBahA in water samples based on sorption to nylon surfaces and on chemometrics-assisted, excitation–emission fluorescence spectroscopy [13]. As can be observed in Fig. 1, the adsorption efficiency of PAHs onto the nylon membrane depends of the polarity of the solvent. In a mixture of water-acetonitrile, with water content equal to or higher than 70 % (*v*/*v*), retention of the compounds is quantitative.

However, in the case of the oil samples, the residues obtained after the MAE-SPE step were not dissolved in the solvent (observed as turbidity), making their sorption onto the nylon membranes impossible. Considering its capacity to both dissolve oil and mix with water, 2-propanol is a solvent frequently used as an injection vehicle in the analysis of oil samples by HPLC reversed-phase methods. Thus, a water–2-propanol 80:20 mixture was used to dissolve the oil residues and as a medium for the sorption of PAHs onto nylon membranes. We estimated not necessary to perform a detailed study with water-propanol mixtures since the oil residue was soluble only in this mixture and with this medium (80:20) the retention on the membrane was quantitative. Figure 2 presents a photograph taken under UV light of the nylon membranes, showing the PAH retention that results from using the water-2-propanol 80:20 mixture as a solvent. Alternately, Fig. 3 shows the emissions spectra of PAHs on nylon after passing a standard solution and spiked oil extract through the nylon medium. Although the sorption of PAHs was quantitative in both cases (the fluorescence signals of the solutions before and after the extraction were compared being the second value less than 1 % of the first), the emission intensity of the oily sample was lower than that of the standard solution. Hence, owing to this matrix effect on the fluorescence, the calibration step was completed in the presence of the oily matrix. These spectra were obtained with slit widths of 5 and 2.5 nm for excitation and emission, respectively. Wider slits results in an increased baseline and saturation of the detector. Decreased slit width selected could potentially improve the selectivity of the technique.

Second-order multivariate calibration

Validation set samples

To construct the second-order calibration models, EEFMs were recorded over a wide spectral range that included the fluorescence signals of all of the analytes studied. The broad, featureless emission bands from Rayleigh and Raman scattering were eliminated from the EEFMs using routines for data pre-treatment [24]. A typical corrected excitation–emission matrix is shown in Fig. 4a. In Fig. 4b, the luminescence of PAHs between 250 and 400 nm for excitation and 370 and 550 nm for emission can be observed. In general, a significant baseline is observed in blank, standard, spiked and real samples (Fig. 4), most likely due to membrane background emissions and solvent impurities. Accordingly, an additional algorithm for baseline correction was investigated. However, this correction produced a bias in estimated concentrations, thus only Rayleigh and Raman scattering corrections were applied for further analysis.

U-PLS/RBL was applied to the three-way data calibration set unfolded into vectors. This algorithm has shown better performance than PARAFAC for predicting the concentration of PAHs in oil samples [31]. Moreover, PLS-based method appears to be more flexible and provide better figures of merit than their competitors [21, 32]. The selection of the optimum spectral range and the optimum number of factors was performed using

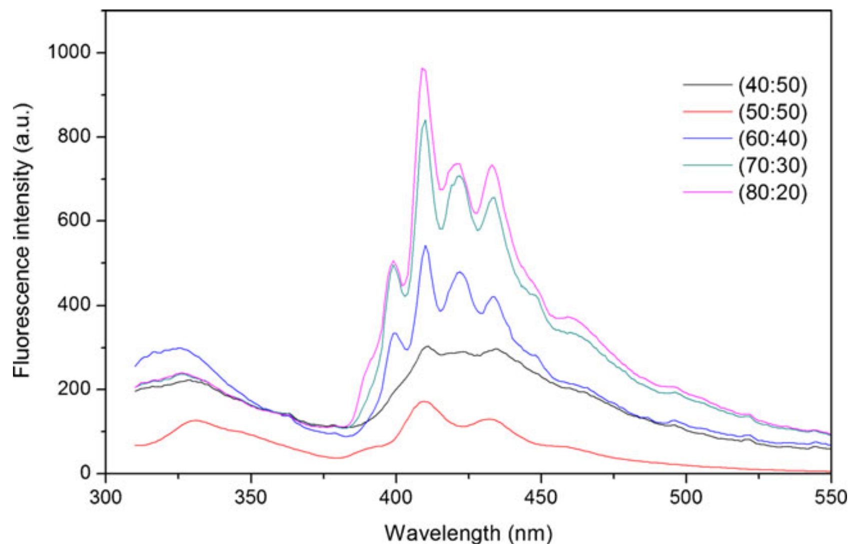


Fig. 1 Emission spectra of the PAH mixture on nylon membranes treated with 74 ng of total mass dissolved in 5 mL of a water–acetonitrile solvent with increasing water content

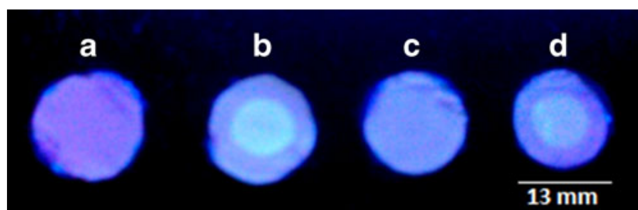


Fig. 2 Photograph of nylon membranes irradiated with a UV lamp (254 nm) after treatment with 5 mL of water-2-propanol 80:20 as solvent: (*a*) blank, (*b*) solution with 25 ng of the PAH mixture, (*c*) solution with oil residue after MAE-SPE, and (*d*) solution with oil residue after MAE-SPE, fortified with 10 ng of the PAH mixture

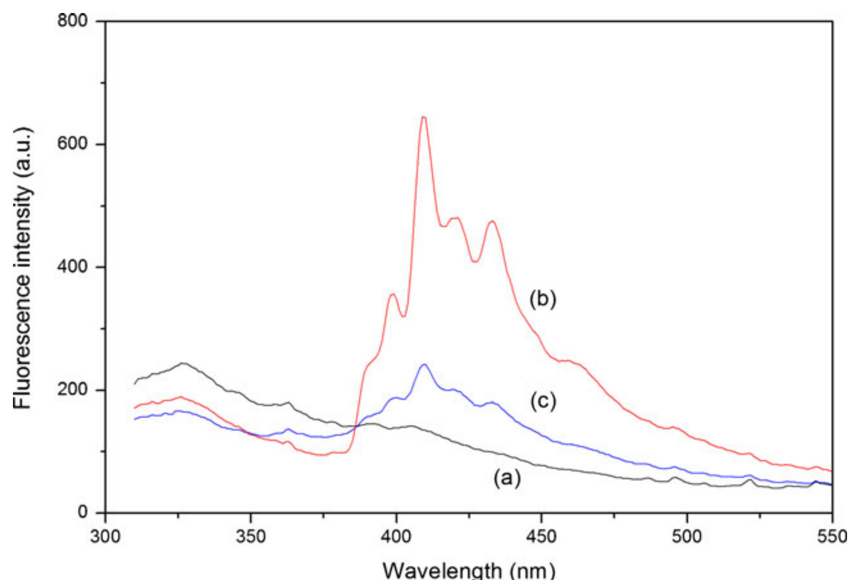
the cross-validation method described by Haaland and Thomas [22]. This optimum number is estimated by calculating the ratio $F(A)$=PRESS(A<A*)/PRESS(A*), where PRESS is the predicted error sum of squares, defined as PRESS=$\Sigma_I^I(y_{\text{nominal}}-y_{\text{predicted}})^2$; A is a trial number of factors; and A^* corresponds to the minimum PRESS. The number of optimum factors was selected as that leading to a probability of less than 75 % and F>1. Note that RBL is not required for calibration samples because they did not include unexpected components.

Table 1 [33, 34] presents the number of factors and the final excitation and emission spectral ranges selected for each analyte when U-PLS was applied. The optimum spectral ranges for each analyte were similar, with the exception of IP, which showed emissions at longer wavelengths. The estimated number of factors ranged from 3 to 8, representing latent variables (abstract loadings and regression coefficients) that do not have any physical interpretation and that only indicate the adequate fit of the sample signal to the calibration model.

U-PLS/RBL was then applied to predict the analyte concentration in the validation samples. In the first instance, RBL was not included for validation samples because a calibration step was previously completed in the presence of the oil matrix. However, high differences between nominal and predicted values were obtained. A visual inspection of the nylon membranes after sorption showed a difference in color between the calibration and validation samples. Both sample sets were treated in the same way, except for the batch of silica cartridges used for SPE. Thus, one of the principal sources of error is related to the irreproducibility of the sorbent material in the cartridges of silica used during this step. This characteristic produced differences in the material adsorbed on the nylon and consequently slight differences in the EEFMs. Figure 4b, c presents three-dimensional plots of the EEFMs on nylon surfaces for a calibration and validation sample, respectively. Note that RBL was required for the validation samples because they contained unexpected components. The number of RBL factors, which were estimated according to the procedure described above [13], ranged from 0 to 3, depending on the analyzed PAH and the corresponding spectral range.

The principal analytical characteristics for the determination of the seven studied PAHs in the validation samples using U-PLS/RBL are shown in Table 1. This algorithm yielded good predictions for PAHs with a relative error (REP) equal to or less than 22 %, except in the case of BbF, which presented a REP of 27 %. The limits of detection (LODs) obtained were less than or equal to 1.0 μg kg^{-1}. These values were lower than those previously obtained in solution, except for BbF and BkF [31]. In the case of BghiP and IP, reductions of four and three times in their LODs were observed. Furthermore, the LODs obtained using the U-PLS/RBL are on the order of those reported for HPLC-FLD methods [2, 4, 5, 35].

Fig. 3 Emission spectra of the PAH mixture on nylon membranes treated with 5 mL of water-2-propanol 80:20 as solvent: (*a*) blank, (*b*) solution with 25 ng of the PAH mixture, and (*c*) solution with oil residue after MAE-SPE, fortified with 25 ng of the PAH mixture



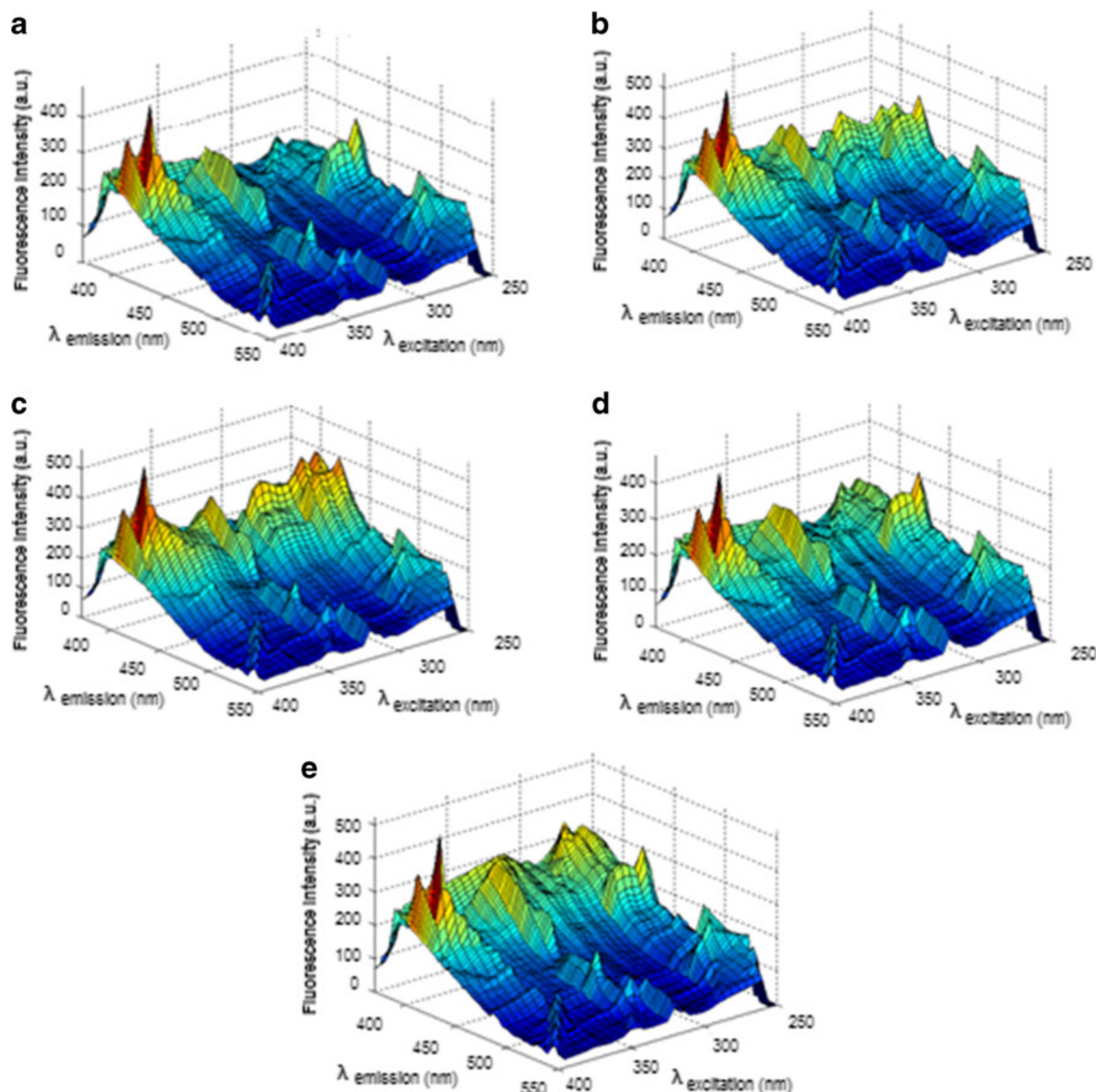


Fig. 4 Three-dimensional plots for the excitation–emission fluorescence spectra on nylon membranes treated with (**a**) solvent, (**b**) a calibration sample containing 21.4 ng of the PAH mixture (4.00 ng of BaA, BbF, BaP and DBahA; 0.16 ng of BkF; 0.25 ng of BghiP and 5.00 ng of IP), (**c**) a validation sample containing 17.5 ng of the PAH mixture (1.10 ng of BaA, 1.80 ng of BbF, 0.80 ng of BkF, 3.10 ng of BaP, 3.10 ng of DBahA, 2.80 ng of BghiP and 4.80 ng of IP) (**d**) EVOO sample containing 0.35 ng of DBahA, (**e**) EVOO sample containing 0.63 ng of BaA, 0.43 ng of BaP and 0.40 ng of DBahA

Test samples of spiked edible oils

The predictive capacity of the calibration model using U-PLS/RBL was evaluated with different brands and types of edible oils. A total of 12 spiked samples of edible oils were analyzed, including 10 samples of five different brands of EVOO and 2 samples of two brands of SO. For this sample set, the number of RBL factors ranged from 1 to 3, depending on the analyzed PAH and the corresponding spectral range. Table 2 presents the mean recovery and standard deviation results obtained for the determination of the seven PAHs studied in these samples.

As can be observed, the mean recoveries obtained using U-PLS/RBL ranged from 64 to 78 % and were in agreement with the values previously reported for this sample preparation method [31]. Under similar experimental conditions for microwave-assisted extraction of PAHs from airborne particulate matter, degradation of these compounds was not observed [36]. Moreover, fluorescence spectra similar in intensity and shape were obtained before and after heating a diluted solution of the PAHs in acetonitrile in the selected experimental conditions (results not shown). Thus, degradation of PAHs during the extraction was discarded. Conversely, no significant differences between the concentrations

Table 1 Excitation–emission spectral ranges and number of factors obtained from the calibration step (U-PLS)

	U-PLS/RBL						
	BaA	BbF	BkF	BaP	DBahA	BghiP	IP
λ excitation (nm)	270–350	305–315	300–325	250–350	260–340	285–315	300–325
λ emission (nm)	370–418	416–506	376–418	406–440	370–428	400–458	428–520
A	5	6	7	3	8	4	7
RMSEP (μg kg^{-1})[a]	0.32	0.77	0.14	0.30	0.20	0.38	0.56
REP (%)[b]	17	27	22	15	10	17	21
γ^{-1} (μg kg^{-1})[c]	0.08	0.22	0.10	0.08	0.07	0.12	0.11
LOD (μg kg^{-})[d]	0.31	1.0	0.37	0.29	0.30	0.43	0.62

Analytical characteristics obtained from the validation step (U-PLS/RBL)

[a] Root mean square error of prediction, RMSEP=$[(1/I)\Sigma_1^I\ (c_{nominal}-c_{predicted})^2\,]^{1/2}$, where I is the number of prediction samples and $c_{nominal}$ and $c_{predicted}$ are the actual and predicted concentrations, respectively

[b] Relative error of prediction, REP=100×RMSEP/c_{mean} where c_{mean} is the mean calibration concentration

[c] Inverse of the analytical sensitivity (γ), $\gamma^{-1}=s_x/\mathrm{SEN}_n$ where s_x is the instrumental noise and SEN_n is the sensitivity [33]. The s_x and SEN_n values are averages of the values corresponding to 11 validation samples

[d] Limit of detection, LOD=3.3 γ^{-1} [34]

predicted by U-PLS/RBL and the concentrations obtained from HPLC-FLD were observed. Consequently, the theoretical (1,0) points are included within or are close to the borders of the elliptical joint regions. Figure 5 presents the plots of the U-PLS/RBL predicted concentrations as a function of the values obtained by HPLC-FLD and the corresponding elliptical joint regions (at a 95 % confidence level) for BaP, DBahA, and IP as representative compounds. In addition, the mean recoveries were compared using a mean *t* test, and no significant differences between the methods were observed, with the exception of IP, which was overestimated by U-PLS/RBL (18 % relative error; Table 2).

Conversely, except for BkF, DBahA and IP, the standard deviation was larger for U-PLS/RBL than HPLC-FLD (Table 2). As was previously discussed, one of the sources of error is the irreproducibility of the sorbent material in the cartridges of silica used during the SPE. Moreover, filtration through the nylon membrane, its position over the holder and the position of the holder in the luminescence spectrometer all affect the precision. Nevertheless, the values were satisfactory, especially considering the complexity of the matrix and the low concentration levels of the analytes. Therefore, based on the obtained results, the proposed method using EEFMs on nylon membranes coupled with U-PLS/RBL compares favorably with the reference HPLC-FLD method. Furthermore, U-PLS/RBL provides a significant reduction in time and solvent consumption. Considering the analysis of ten samples, this method requires 20 ml of hexane and of 2 h, whereas HPLC requires 10 mL of 2-propanol, 500 mL of acetonitrile, and 6.5 h. However, the present method is limited to only seven PAHs, whereas HPLC can be used with a higher number of PAHs.

Table 2 Mean recovery and standard deviation for seven studied PAHs in spiked samples of different types and brands of edible oil samples using U-PLS/RBL and HPLC-FLD as reference method

	HPLC-FLD		U-PLS/RBL		*p* values	
	Recovery	SD	Recovery	SD	Recovery	SD
BaA	73	6	78	16	0.34	0.005*
BbF	74	8	76	17	0.74	0.02*
BkF	65	12	66	13	0.86	0.93
BaP	66	7	72	14	0.21	0.03*
DBahA	71	9	76	15	0.33	0.06
BghiP	62	6	64	11	0.62	0.02*
IP	62	7	73	10	0.006*	0.18

*Significant differences at a 95 % confidence level

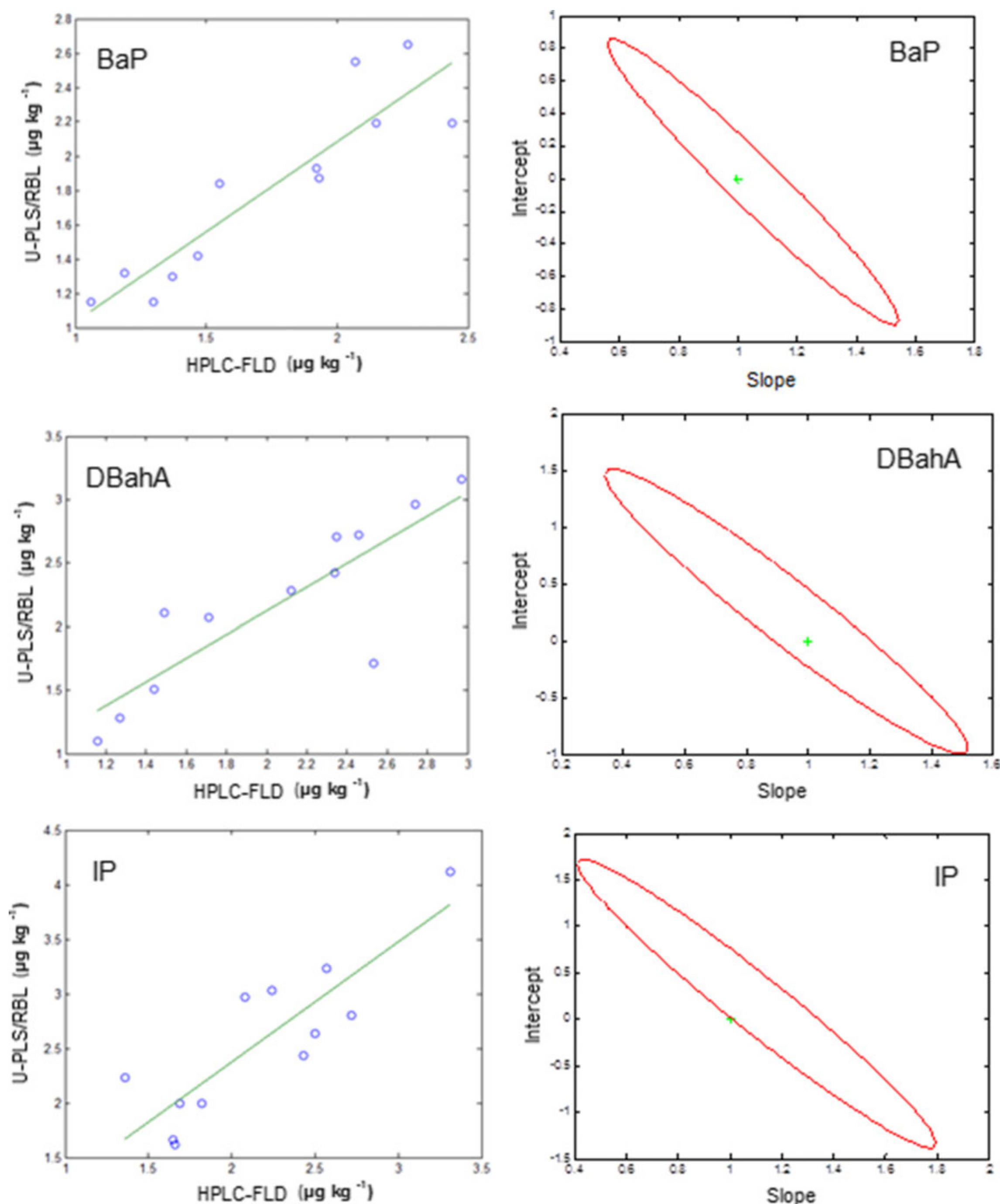


Fig. 5 Plots for U-PLS/RBL predicted concentrations as a function of the obtained HPLC-FLD values for BaP, DBahA and IP, and the corresponding elliptical joint regions (at a 95 % confidence level) for the slopes and intercepts of the regression on the U-PLS/RBL versus HPLC-FLD plots. The black circle in the elliptical plots marks the theoretical point (intercept: 0, slope: 1)

Real samples

A set of ten samples corresponding to ten brands of EVOO was analyzed in duplicate using the proposed method. In one sample, DBahA was detected at 0.35 (0.04) μg kg^{-1} and in another, BaP and DBahA were detected at 0.63 (0.01), 0.43 (0.07), and 0.40 (0.04) μg kg^{-1}, respectively. None of these samples had a total content of PAHs higher than the maximum limit of 10 μg kg^{-1} for the sum of all heavy PAHs [7, 8]. Moreover, these samples showed better precision than the test samples. Figure 4d, e presents three-dimensional plots of the EEFMs on the nylon surface for both real samples.

Conclusions

Fluorescence excitation–emission spectroscopy on nylon membranes, associated with U-PLS/RBL, has been demonstrated to be a useful tool for resolving a mixture of heavy US-EPA PAHs in the presence of a very complex matrix such as that of edible oil. It has been demonstrated that PAHs immersed in this type of matrix can be adsorbed onto nylon membranes and show emission of light. However, the complexity of the oily matrix—with native compounds that present spectral overlap with heavy PAHs and that can produce inner filter effects—necessitates a sample pre-treatment procedure (microwave-assisted liquid–liquid extraction with SPE on silica) for adsorption onto nylon and for the selective detection of PAHs. After pretreatment, U-PLS/RBL can effectively resolve a mixture of analytes in the presence of unknown compounds. The accuracy of the proposed method was tested by predicting the selected PAHs in different brands and types of edible oils. The U-PLS/RBL predicted concentrations were compared with the values obtained using HPLC-FLD and, with the exception of IP, no significant differences between them were observed. Furthermore, the LODs obtained using the proposed methods are comparable to those reported using HPLC methods. Thus, the proposed method using EEFMs on nylon membranes coupled with U-PLS/RBL are comparable and provide a suitable alternative to the chromatographic method.

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