JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Combination of Liquid Chromatography with Multivariate Curve Resolution-Alternating Least-Squares (MCR-ALS) in the Quantitation of Polycyclic Aromatic Hydrocarbons Present in Paprika Samples

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Supporting Information

ABSTRACT: This work presents a strategy for quantitating polycyclic aromatic hydrocarbons (PAHs) in smoked paprika samples. For this, a liquid chromatographic method with fluorimetric detection (HPLC-FLD) was optimized. To resolve some interference co-eluting with the target analytes, the second-order multivariate curve resolution-alternating least-squares (MCR-ALS) algorithm has been employed combined with this liquid chromatographic method. Among the eight PAHs quantified (fluorene, phenanthrene, anthracene, pyrene, chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene) by HPLC-FLD, only in the case of fluorene, pyrene, and benzo[*b*]fluoranthene was it necessary to apply the second-order algorithm for their resolution. Limits of detection and quantitation were between 0.015 and 0.45 mg/kg and between 0.15 and 1.5 mg/kg, respectively. Good recovery results (>80%) for paprika were obtained via the complete extraction procedure, consisting of an extraction from the matrix and the cleanup of the extract by means of silica cartridges. Higher concentrations of chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene were found in the paprika samples, with respect to the maximal amounts allowed for other spices that are under European Regulation (EU) N° 2015/1933.

KEYWORDS: *smoked paprika, PAHs, MCR-ALS, HPLC, fluorescence detection*

INTRODUCTION

Paprika is a product obtained from dehydrated and milled fruits of certain varieties of red peppers (*Capsicum annum* L.). This product is interesting because of its antioxidant properties and other properties that provide health benefits, and it is commonly used for culinary and industrial purposes.[1] In Spain, two areas are characterized by the production of paprika, which are La Vera (Extremadura) and Murcia, both recognized under the Protected Designation of Origin (PDO) by the European Union.

In the La Vera region, a characteristic system is employed to obtain paprika from dried peppers. In this way, peppers are smoked−dried (oak or holm wood fire), while in other Spanish areas or in other countries, previously to the production of paprika, peppers are dried with hot air or sun.[2] Perfect dehydration of the fruits is obtained with this smoking system, and it confers on paprika its three fundamental characteristics: flavor, color stability, and aroma.[3] However, this product can contain polycyclic aromatic hydrocarbons (PAHs) because of this drying process.

In the case of smoked foods, the PAH content can be influenced by different parameters, for example, wood temperature attained during combustion, moisture of wood, and oxygen concentration in the combustion chamber. In addition, the nature of the wood can be another parameter that influences the production of PAHs. Some studies recommend to use hardwoods, instead of softwoods, to reduce the level of PAHs in smoke and, consequently, in smoked foods. However, some authors do not agree with this finding because of the fact that some studies show that concentrations of PAHs in smoke are very similar for both woods, softwood and hardwood.[4,5]

The World Health Organization (WHO),[6] the International Agency for Research on Cancer (IARC),[7] the European Food Safety Authority (EFSA),[8] and the U.S. Environmental Protection Agency (EPA)[9] have reported the carcinogenic, mutagenic, and bioaccumulative capacities of PAHs. In this sense, PAHs have been classified as carcinogenic (1) (benzo[*a*]pyrene), probably carcinogenic (2A) [dibenz[*a*,*h*]anthracene], possibly carcinogenic (2B) [benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, indeno[1,2,3-*cd*]pyrene, and naphthalene], and not classifiable [anthracene, benzo[*g*,*h*,*i*]perylene, fluoranthene, fluorene, phenanthrene, and pyrene]. The three main routes of human exposure to these compounds are inhalation, ingestion, or skin contact.[10,11]

Priority PAHs subjected to control are listed in European regulations. Hence, in accordance with EC regulation 1881/2006, later modified by EC regulation 835/2011, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene are to be controlled in oils, smoked meat and fish products, and components of baby food.[12,13] In addition, another

Received: August 31, 2016
Revised: October 7, 2016
Accepted: October 7, 2016
Published: October 7, 2016

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

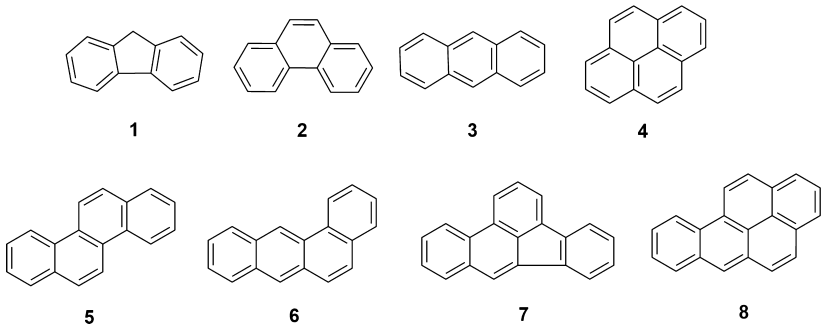

Figure 1. Structures of each of the examined polycyclic hydrocarbons: fluorene (**1**), phenanthrene (**2**), anthracene (**3**), pyrene (**4**), chrysene (**5**), benzo[*a*]anthracene (**6**), benzo[*b*]fluoranthene (**7**), and benzo[*a*]pyrene (**8**).

modification has been included by EC regulation 2015/1933 in the case of the maximal content of PAHs in cocoa fiber, banana chips, food supplements, dried herbs, and dried spices, which does not include paprika.[14]

Recently, liquid chromatography coupled to fluorescence detection (HPLC-FLD) has been commonly employed in the determination of PAHs in foods.[11,15−20] Gas chromatography (GC) has also been widely used to determine PAHs, for example, in tea infusion[21] or chorizo samples.[22] However, in the case of paprika samples or other matrices related to them, such as peppers, only one study referring to peppers[23] and another to smoked paprika[20] have been found. In the first study mentioned, the analytes studied are not the same as those in our work, and in the second study, the chromatographic conditions are difficult to follow because they describe them as a combination of different methods.

When a chromatographic experiment is performed, a good separation is expected. However, on some occasions, the separation is not complete and some peaks are overlapping. In these cases, selectivity can be achieved by multivariate analysis of the generated three-way data sets. The obtained second-order signals, conveniently decomposed, allow the identification of the analyte of interest, and this can be performed even in the presence of interference or unexpected components not modeled in the calibration stage. This property is usually known as the second-order advantage.[24] The advantages and drawbacks associated with combining multivariate calibration and chromatography have already been discussed.[25,26] To date, few literature references concern chromatograpy with fluorimetric detection in combination with different second-order algorithms, in this context, the pioneering work of Appellof and Davidson[27] using a video fluorimeter as a chromatographic detector and some applications for PAHs and naphthalene derivative resolution.[28−30] In particular, with respect to the use of the MCR-ALS (multivariate curve resolution-alternating least-squares) algorithm with these second-order data, recently, very few references like the work of Bortolato et al.[31] have been found. Today, the frequency of use of chemometric tools is increasing in the analytical determination of minor components in food. In this sense, separation techniques coupled to MCR-ALS have been employed by several authors to quantitate phenolic acids in virgin olive oil[32,33] and pesticides in water[34] or food.[35]

The production of pepper employed to produce paprika has increased in Spain, and this could indicate that the consumption of paprika is increasing. Hitherto, PAHs are not usually controlled in paprika. In our opinion, it is important that we start to do it, taking into account the fact that their use can increase in many areas, such as cooking and as additives in other foods. With this background, the objective of this work was to quantitate PAHs by HPLC-FLD in paprika samples divided into two groups, one of them obtained by means of a smoking process, and to evaluate the content of these according to other regulated spices. Chemometric tools were employed to determine matrix interference as necessary.

MATERIALS AND METHODS

Chemical Reagents and Samples. The PAHs studied, fluorene (**1**), phenanthrene (**2**), anthracene (**3**), pyrene (**4**), chrysene (**5**), benzo[*a*]anthracene (**6**), benzo[*b*]fluoranthene (**7**), and benzo[*a*]-pyrene (**8**) (Figure 1), all >99%, were purchased from Sigma-Aldrich Química, S.A. (Madrid, Spain). Stock solutions of each individual analyte were prepared in acetonitrile (MeCN) and stored at 4 °C until they were used.

LC-grade acetonitrile solvent was obtained from Sigma. LC-grade iso-hexane and diethyl ether were from Panreac Química, S.A.U. (Barcelona, Spain). A Milli-Q water system (Millipore S.A.S., Molsheim, France) was employed for high-purity water. Sep-Pak Plus silica cartridges (690 mg) were provided from Waters Corp. (Milford, MA).

The paprika samples were obtained from different origins, the Regulatory Council of the Designation of Origin "Pimentón de La Vera", and local markets. The origin of the Spanish Protected Designation of Origin (PDO) "Pimentón de La Vera" samples can be ensured. However, the origin of the samples that did not belong to the Spanish PDO is not available, although it is reported in their label that they have been packaged in Spain.

Instrumentation and Software. The liquid chromatographic system used was a LC instrument, model 1100 (Agilent Technologies, Palo Alto, CA), equipped with a degasser, a quaternary pump, a column oven, an autosampler (Agilent 1260 infinity), an UV/vis diode array detector (DAD), and a fluorescence detector (FLD). OpenLAB LC ChemStation software, version A.01.04, was used to control the instrument and for data acquisition and data analysis. A 100 mm × 4.6 mm (inside diameter), 1.8 μm, Zorbax Eclipse XDB-C18 column (Agilent Technologies) was utilized.

Calibration curves for the chromatographic analysis and analytical figures of merit, including limits of detection and quantitation according to the Long and Winefordner criterion, were obtained by means of the ACOC program developed by this group.[36]

The software package The Unscrambler, version 6.11 (CAMO ASA, Trondheim, Norway), was used for the experimental design.

Second-order data analysis were performed using MatLab R2008a, version 7.6 (The Mathworks, Natick, MA), and the MVC2 routine developed by Olivieri et al.[37]

Chromatographic Conditions. The mobile phase used consisted of H_2O (solvent A) and acetonitrile (solvent B). An isocratic elution (35:65 A:B) and a constant flow rate of 0.8 mL min^{-1} were employed for the analysis of PAHs. The injection volume was 20 μL. FLD

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

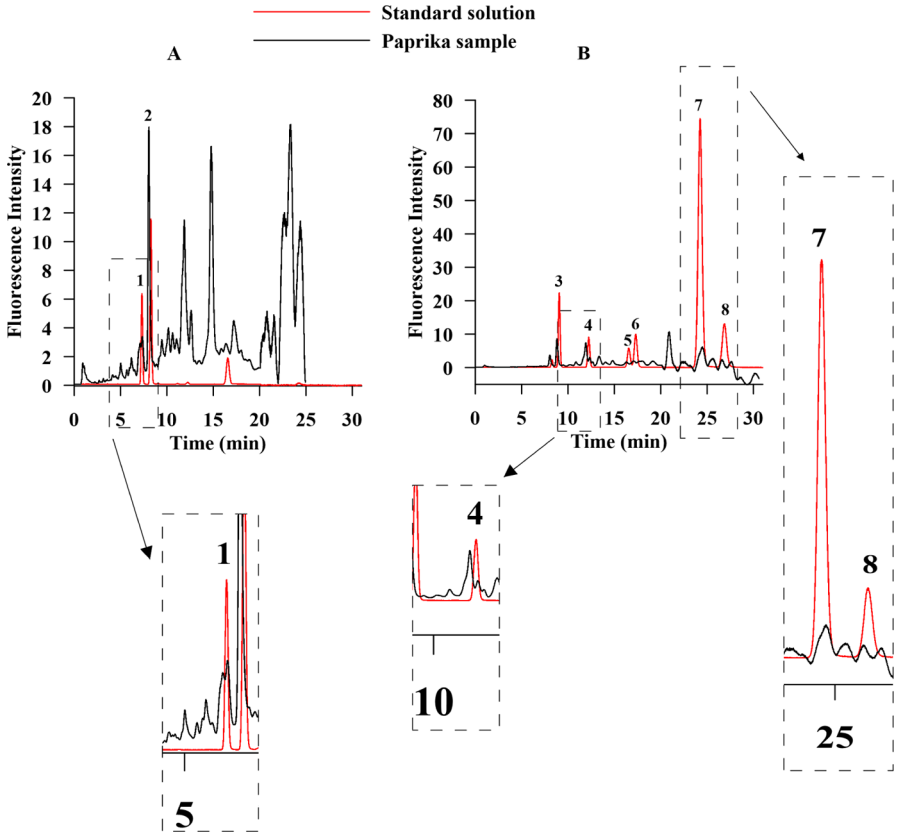

Figure 2. Chromatograms corresponding to a standard solution (red line) and a PDO paprika sample (black line) obtained with the final conditions employed: (A) λ_{exc} and λ_{em} values of 260 and 352 nm, respectively, and (B) λ_{exc} and λ_{em} values of 260 and 420 nm, respectively.

detection was performed at 260 nm for the excitation wavelength and 352 and 420 nm for the emission wavelengths.

Calibration Samples for Univariate Analysis. To obtain the univariate calibration curves for each analyte, standard solutions containing mixtures of the eight PAHs (**1**–**8**) were prepared in acetonitrile from more concentrated stock solutions in acetonitrile. The concentration ranges utilized were 10–150 μg/L for fluorene, 20–350 μg/L for phenanthrene, 20–250 μg/L for anthracene, chrysene, and pyrene, 3–100 μg/L for benzo[*a*]anthracene, 1–90 μg/L for benzo[*b*]fluoranthene, and 0.1–10 μg/L for benzo[*a*]pyrene. The Chemstation package was used to measure the peak area values under the different detection conditions.

Calibration, Validation, and Spiked Samples for MCR-ALS Analysis. The solutions containing mixtures of the eight PAHs employed in the univariate calibration curves were used as a calibration set for univariate analysis of phenanthrene, anthracene, chrysene, benzo[*a*]anthracene, and benzo[*a*]pyrene and for MCR-ALS analysis of fluorene, pyrene, and benzo[*b*]fluoranthene. A validation set containing **1**–**7** in the range of 20–100 μg/L and **8** in the range of 1–8 μg/L was also prepared in acetonitrile. A spiked sample set was prepared by fortifying paprika with known concentrations of these analytes to validate the developed methodology. Because analytes can be lost during the extraction stages, in the event that full extraction does not take place, the fortification of paprika was performed after the extraction procedure.

Data matrices, obtained in the chromatographic system with a fast scanning fluorescence detector (FSFD), were collected every 6.5 s using wavelengths from 300 to 460 nm in steps of 1 nm, setting the excitation wavelength to 260 nm. Second-order HPLC-FLD matrices of size 161 × 283 (number of spectroscopic data points × time) were obtained and used for the following analysis of the data. MCR-ALS analysis of fluorene, pyrene, and benzo[*b*]fluoranthene was performed in the time regions described below.

Real Samples. To extract the analytes from paprika samples, a 0.2 g precisely weighed aliquot of this product was extracted with 10 mL of diethyl ether for 10 min in an ultrasonic bath. The extract solution was centrifuged for 10 min and evaporated to dryness. The residue was suspended in 5 mL of iso-hexane and loaded on a silica cartridge without preconditioning, and then the PAHs were eluted from the cartridge with 7 mL of iso-hexane. This extract and the 5 mL fraction initially percolated were combined, to identify retained and unretained analytes, evaporated to dryness, and reconstituted in 5 mL of acetonitrile for its chromatographic analysis. A dilution factor of 1–2 was employed before the injection of the extracts.

Chemometric Algorithm (MCR-ALS). MCR-ALS is the algorithm of choice in chromatography because MCR-ALS can handle data sets deviating from trilinearity, which is a common situation in chromatographic data sets. Elution time shifts or peak shape changes of the different analytes, occurring from sample to sample, are the reason for the trilinearity deviations. In this algorithm, an augmented data matrix is created from the test data matrices and the calibration data matrices.[38] The augmentation was performed in the row direction (time elution). The bilinear decomposition of augmented matrix **D** is given according to the expression

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E} \quad (1)$$

In this equation, **D** (size $J \times K$) is the matrix of experimental data. In this matrix, J is the number of elution time data points (number of rows of each data matrix) and K is the number of emission wavelengths (number of columns of each data matrix). **C** (size $J \times N$) is the matrix that contains the concentration profiles of the N components present in the samples (columns); $\mathbf{S}^T$ is the matrix that contains the component spectra (rows), and **E** (size $J \times K$) is a matrix of residuals not fitted by the model.

The first step in MCR-ALS studies is to obtain a rough estimation of the number of components, which can be simply performed by visual inspection of singular values or principal component analysis

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254–8262

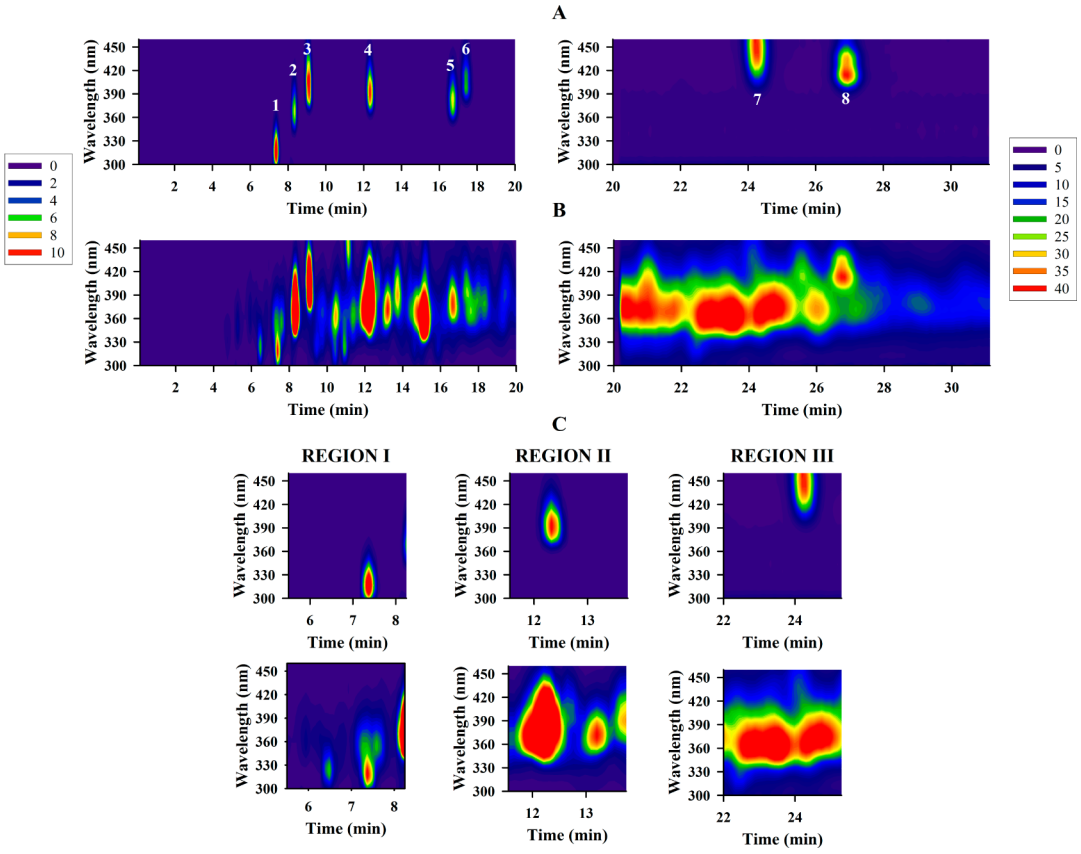

Figure 3. Two-dimensional contour plots for a standard solution of (A) the eight PAHs studied and (B) an extract of paprika belonging to the PDO. (C) Regions chosen for the quantitation of fluorene, pyrene, and benzo[*b*]fluoranthene.

(PCA). The resolution is accomplished using an iterative ALS procedure and requires initialization with parameters as close as possible to the final results. Several methods can be used for this purpose.[35,39] In this work, the estimation of the spectra of species was performed from the analysis of the so-called "purest" spectra, applying a multivariate algorithm that extracts pure component spectra from a series of spectra of mixtures of varying composition.[40−42]

Once MCR-ALS decomposition is performed and compounds are identified, the MCR-ALS scores are calculated per analyte and sample as the integrated area under the related resolved profile:

$$a(i, n) = \sum_{j=1+(i-1)J}^{iJ} C(j, n) \quad (2)$$

where $a(i,n)$ is the score for analyte n in sample i and $C(j,n)$ is the element of the analyte profile in the augmented mode. For the calibration samples, a regression of the scores of a particular analyte against nominal concentration values is performed to build a calibration curve. Afterward, this calibration curve can be used for concentration prediction in unknown samples by interpolation.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions. First, the optimization of the chromatographic conditions was performed. As described in the literature, in most cases, a gradient elution is employed to analyze these compounds in food.[4,11,15,16,18,19] In the case presented here, both gradient elution and several isocratic modes were applied, with similar results: some analytes (fluorene, pyrene, and benzo[*b*]-fluoranthene) co-eluted with matrix interference in the real paprika samples, even after the clean-up step. Therefore, to avoid the time needed to stabilize and condition the chromatographic column, one of the isocratic modes was chosen [35:65 (v/v) H_2O/MeCN]. The analysis time required was similar to those of previous studies.[16,19]

Another inconvenience in this analysis was the different analyte concentrations found in the samples, for example, between phenanthrene and benzo[*a*]pyrene. The first was on the order of milligrams per kilogram, and the second was on the order of micrograms per kilogram. To deal with this, a change in the gain of the fluorescence detector in the chromatographic system was programmed. The gain was changed from 12 to 16 over 20 min, and this allowed all analytes to be determined with a single injection. Figure 2 shows a chromatogram of a standard solution and a paprika sample with these final conditions selected. It can be appreciated in Figure 2 that some analytes present matrix interference that co-elutes with fluorene, pyrene, and benzo[*b*]fluoranthene. For this reason, it was necessary to employ a second-order algorithm (MCR-ALS) to quantitate these analytes.

Analytical Parameters for the External Standard Methodology. To validate this method, linearity, precision, and accuracy, limits of detection (LODs) and limits of quantitation (LOQs) were calculated. The calibration curves of each compound were constructed, and the analytical figures of merit were obtained employing the peak areas (PAs) in the FLD. The linearity was very good for all PAHs with correlation coefficients (r^2) of >0.99. Limits of detection[43] were between 0.015 and 0.45 mg/kg, and limits of quantitation were between 0.050 and 1.5 mg/kg.

The precision (inter- and intraday) was evaluated by analyzing several standard solutions on the same day (intraday

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

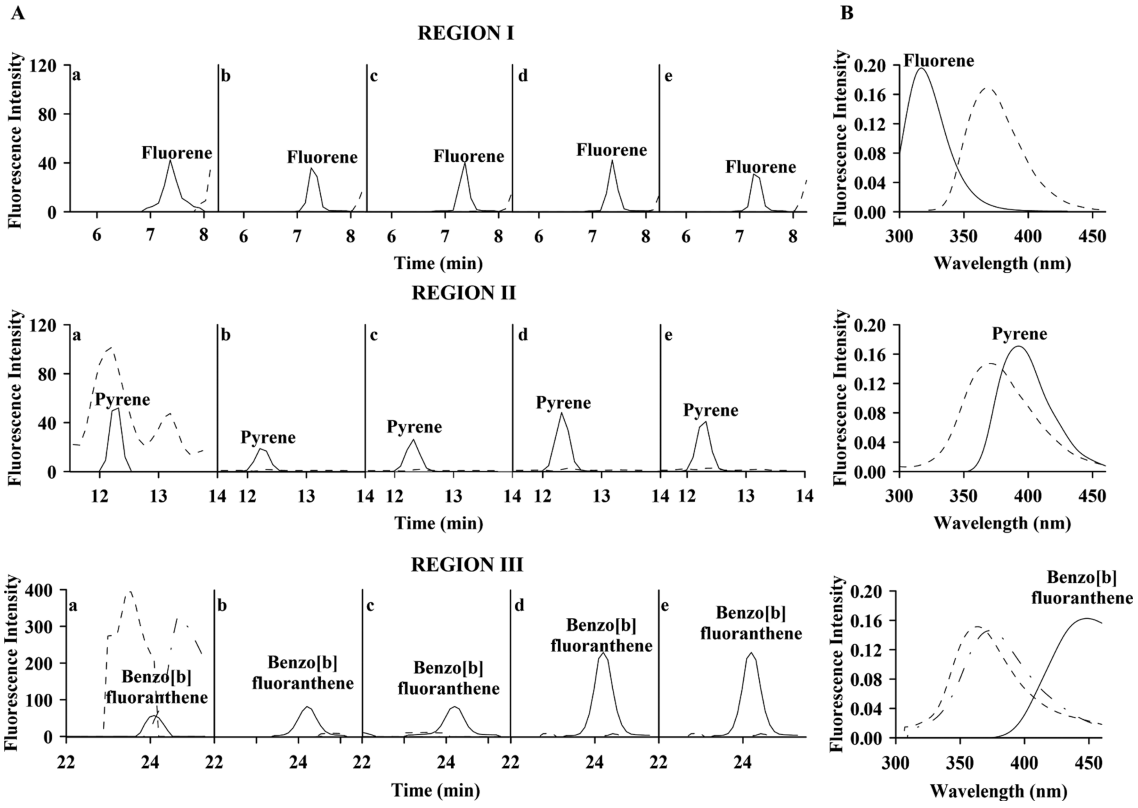

Figure 4. (A) Elution profiles retrieved by MCR-ALS analysis for (a) each region of a paprika sample and (b−e) several standard solutions. (B) Emission spectra produced by MCR-ALS analysis for each region. Dashed lines corresponding to elution profiles and emission spectra retrieved by MCR-ALS for unknown compounds.

precision; n = 8) and on different days over a period of 7 days (interday precision), These solutions were prepared at two different concentrations, containing each compound (30 μg/L) except in the case of benzo[a]pyrene (8 μg/L) or containing each compound (15 μg/L) except in the case of benzo[b]-fluoranthene and benzo[a]pyrene (0.1 μg/L). The relative standard deviation (RSD) values of peak areas and retention times (t_R) were determined for each compound. In all cases, the precision was better than 7.5%, being between 0.1 and 5.6% (RSD values) for the intraday precision and between 0.5 and 7.5% (RSD values) for the interday precision.

MCR-ALS Analysis. To quantitate the three analytes that presented interference in their chromatographic elution (fluorene, pyrene, and benzo[b]fluoranthene), MCR-ALS data processing was employed. This algorithm allows processing of second-order data that are not trilinear because of the presence of elution time shift from run to run.

The first step in MCR-ALS analysis is to obtain the second-order data, in this case matrices $X \times Y$ (number of spectral data points × time). Thus, Figure 3 shows second-order data matrices of size 161 × 283 (number of spectral data points × time), obtained in the chromatographic system, of a standard solution containing the eight PAHs quantified and a paprika sample belonging to the PDO. The presence of matrix interference in the case of the paprika sample should be noted.

For the analysis of data, each chromatographic data matrix was divided into different time regions following a strategy similar to those of other authors:[32,34,35,44,45] region I (5.5−8.25 min), region II (11.55−13.75 min), and region III (22.0−25.3 min). Region I includes the first analyte eluted, between those investigated in this section (fluorene); region II includes the second analyte (pyrene), and region III includes the third analyte (benzo[b]fluoranthene). When the emission wavelength was being recorded, the complete range of wavelengths was used.

Augmented matrices are necessary to apply the MCR-ALS algorithm. The algorithm was applied, for each time region, to augmented matrices in the elution time direction, corresponding to the simultaneous analysis of the HPLC-FLD data matrices for the calibration set of samples. The number of components in each augmented matrix was estimated by principal component analysis (PCA) and justified taking into account the presence of the corresponding analytes, possible interference, and background signals. Non-negativity restriction was applied in both modes, spectroscopic spectral data and time, and unimodality restriction was applied in the elution time mode only to the signals corresponding to the analytes and not to the background signal. After ALS optimization for each sample, and with the aid of the corresponding pseudounivariate calibration curves, the constituents were identified and quantified. Analytical figures of merit corresponding to linear regression of scores versus the corresponding nominal concentrations were calculated. First, the methodology was validated. Thus, on one hand, validation samples consisted of standard solutions with contents of fluorene, pyrene, and benzo[b]fluoranthene within the range of the calibration set. In this set, the number of principal component analysis was found to be 1 in the case of fluorene and pyrene and 2 in the case of benzo[b]fluoranthene. On the other hand, a set of fortified paprika samples with known concentrations of these analytes was also employed to validate the methodology. This addition was made after the extraction procedure to avoid

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

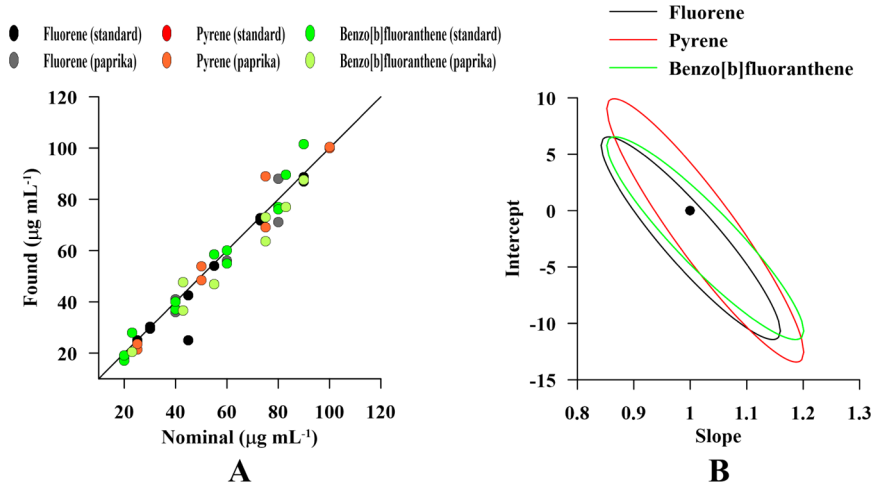

Figure 5. (A) Plots of predicted concentrations of fluorene, pyrene, and benzo[*b*]fluoranthene as a function of the nominal values. Black and gray symbols correspond to data for the fluorene standard and fluorene paprika fortification, respectively; red and orange symbols correspond to data for the pyrene standard and pyrene paprika fortification, respectively, and green and light green symbols correspond to data for the benzo[*b*]fluoranthene standard and benzo[*b*]fluoranthene paprika fortification, respectively. (B) Corresponding elliptical joint regions (at the 95% confidence level) for the slopes and intercepts of the regressions. The theoretical point (intercept = 0; slope = 1) is marked in the figure by the black point.

recovery loss during this stage. The concentrations found in fortified paprika samples were calculated taking into account the analyte concentrations, predicted by the algorithm, in the sample without fortification.

In the case of paprika samples, the number of principal component analysis found was 2 in the case of fluorene, 2 in the case of pyrene, and 4 in the case of benzo[*b*]fluoranthene. Figure 4 shows the elution time profiles produced by MCR-ALS analysis for each region of a paprika sample and different standard samples. Also, the emission spectra retrieved for each region are shown in Figure 4.

Figure 5 displays the good recovery results in validation samples (standard solutions and fortified paprika samples, data combined in the same figure) in addition to the elliptical joint confidence region (EJCR)[46] for the slope and intercept of the plot corresponding to each analyte. The theoretically expected values of 1 and 0 for the slope and intercept, respectively, are included in all ellipses. This fact shows the accuracy of the applied methodology for these compounds in validation samples.

Analysis of Real Paprika Samples. *Treatment of the Sample.* To quantitate PAHs in paprika samples, first, the analytes were extracted from paprika. In the clean-up and concentration step, we tested whether when the extract containing the PAHs was loaded in a silica cartridge, the analytes were not completely retained. For this, we decided to employ the minimal volume of iso-hexane to elute the PAHs from the cartridge with the aim of retaining other types of interference present in the matrix of paprika such as higher-polarity fluorescent compounds, for example, capsaicinoids, flavonoids, tocopherols, etc. This volume was 7 mL, in addition to an additional 5 mL of the initial percolate.

This procedure was assayed with a 5 mL standard solution containing the eight PAHs studied, and the recovery results, corresponding to triplicate analysis, were better than 80% in all cases.

The effectiveness of the complete procedure of extraction and clean-up was probed by means of a recovery study ($n = 6$). Known amounts of each analyte were added to a paprika sample in the same range that could occur in this kind of sample. The extraction described above was employed, and the recovery results were better than 82% in all cases. The repeatability was analyzed in this assay, and the RSD values in all cases were <7%.

Taking into account all of these results, we can conclude that the extraction procedure was effective in terms of repeatability and recovery extraction. This is a simple and quick method for extraction of these compounds from the paprika matrix.

Quantitation of Real Samples. As indicated throughout this paper, fluorene, pyrene, and benzo[*b*]fluoranthene have been quantified by means of MCR-ALS and the rest of the studied PAHs have been quantified by means of a conventional external standard methodology (Figure 5). Two groups of samples have been established according to their belonging or not to the Spanish Protected Designation of Origin (PDO) "Pimentón de La Vera" because the latter are smoked−dried. Table 1 shows the results obtained for different paprika samples as well as their standard deviation calculated using the method of Miller and Miller.[47]

We can observe that paprika samples that are smoked−dried present higher values of PAHs, the mean total content being between 17.1 and 35.2 mg/kg. With regard to the contents of four of the PAHs (chrysene, benzo[*a*]anthracene, benzo[*b*]-fluoranthene, and benzo[*a*]pyrene), whose limits are fixed in EC 2015[14] for other dried spices, it is noticeable that these are higher than the established limits. However, some paprika samples that do not belong to the PDO and, consequently, were not produced by the smoked system also contained these compounds, but their content was lower. In this case, the presence of PAHs could be due to some of the drying steps, in which an increase in temperature is produced, although in smaller amounts. However, this fact cannot be considered to be dangerous given the small amounts of this spice usually utilized, which is reflected in the lack of regulations about the PAH contents of paprika.

Results presented in this work are similar to those obtained by Fasano et al.,[20] the only previous quantitation of these compounds in smoked paprika samples. However, chromato-

DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

Table 1. Results of the Analysis of PAHs in Real Paprika Samples[a]

	concentration ± SD (mg/kg)							
sample	fluorene	phenanthrene	anthracene	pyrene	chrysene	benzo[*a*]anthracene	benzo[*b*]fluoranthene	benzo[*a*]pyrene
				PDO				
1	1.91 ± 0.08	11.01 ± 0.06	2.47 ± 0.05	2.3 ± 0.1	0.8 ± 0.1	0.35 ± 0.08	ND	0.032 ± 0.009
2	2.01 ± 0.08	11.81 ± 0.06	2.64 ± 0.05	1.5 ± 0.1	0.9 ± 0.1	0.41 ± 0.08	ND	0.040 ± 0.009
3	2.95 ± 0.08	16.69 ± 0.07	4.14 ± 0.05	3.2 ± 0.1	1.2 ± 0.1	0.39 ± 0.08	ND	0.037 ± 0.009
4	3.48 ± 0.08	13.04 ± 0.06	2.95 ± 0.05	2.3 ± 0.1	1.7 ± 0.1	0.48 ± 0.08	0.19 ± 0.04	0.061 ± 0.009
5	2.09 ± 0.08	10.41 ± 0.06	2.37 ± 0.05	1.5 ± 0.1	0.9 ± 0.1	0.35 ± 0.08	ND	0.046 ± 0.009
6	1.83 ± 0.08	11.27 ± 0.06	2.54 ± 0.05	2.2 ± 0.1	1.1 ± 0.1	0.53 ± 0.08	ND	0.041 ± 0.009
7	2.70 ± 0.08	16.50 ± 0.07	4.23 ± 0.05	2.2 ± 0.1	1.2 ± 0.1	0.36 ± 0.08	ND	0.032 ± 0.009
8	2.51 ± 0.08	16.63 ± 0.07	4.29 ± 0.05	2.4 ± 0.1	1.2 ± 0.1	0.44 ± 0.08	ND	0.034 ± 0.009
9	2.52 ± 0.08	14.97 ± 0.07	3.13 ± 0.05	2.2 ± 0.1	1.2 ± 0.1	0.58 ± 0.08	ND	0.150 ± 0.009
10	2.17 ± 0.08	12.16 ± 0.06	2.83 ± 0.05	2.8 ± 0.1	1.1 ± 0.1	0.55 ± 0.08	ND	0.065 ± 0.009
11	1.77 ± 0.08	9.80 ± 0.06	2.30 ± 0.05	1.8 ± 0.1	0.9 ± 0.1	0.42 ± 0.08	ND	0.041 ± 0.009
12	2.29 ± 0.08	18.89 ± 0.08	4.33 ± 0.05	6.3 ± 0.1	1.6 ± 0.1	1.22 ± 0.08	0.32 ± 0.04	0.289 ± 0.009
13	1.57 ± 0.08	11.48 ± 0.06	2.44 ± 0.05	3.1 ± 0.1	1.0 ± 0.1	0.58 ± 0.08	ND	0.122 ± 0.009
14	1.78 ± 0.08	12.10 ± 0.06	2.74 ± 0.05	2.3 ± 0.1	1.3 ± 0.1	0.49 ± 0.08	0.21 ± 0.04	0.054 ± 0.009
15	1.98 ± 0.08	12.50 ± 0.06	2.79 ± 0.05	2.3 ± 0.1	1.2 ± 0.1	0.54 ± 0.08	0.19 ± 0.04	0.061 ± 0.009
16	1.86 ± 0.08	10.92 ± 0.06	2.37 ± 0.05	1.9 ± 0.1	0.9 ± 0.1	0.36 ± 0.08	ND	0.053 ± 0.009
17	2.63 ± 0.08	10.00 ± 0.06	2.06 ± 0.05	3.9 ± 0.1	0.7 ± 0.1	0.32 ± 0.08	ND	0.022 ± 0.009
18	2.26 ± 0.08	18.56 ± 0.08	4.36 ± 0.05	1.5 ± 0.1	1.4 ± 0.1	0.69 ± 0.08	ND	0.060 ± 0.009
19	2.30 ± 0.08	17.27 ± 0.07	4.00 ± 0.05	3.5 ± 0.1	1.3 ± 0.1	0.65 ± 0.08	0.19 ± 0.04	0.064 ± 0.009
20	1.43 ± 0.08	13.53 ± 0.07	3.14 ± 0.05	2.4 ± 0.1	1.0 ± 0.1	0.51 ± 0.08	0.21 ± 0.04	0.030 ± 0.009
21	2.22 ± 0.08	14.76 ± 0.07	3.32 ± 0.05	3.2 ± 0.1	1.2 ± 0.1	0.56 ± 0.08	0.20 ± 0.04	0.066 ± 0.009
				No PDO				
22	0.60 ± 0.08	0.10 ± 0.05	0.03 ± 0.05	ND	ND	ND	ND	ND
23	0.16 ± 0.09	0.68 ± 0.05	0.17 ± 0.05	NQ	NQ	ND	ND	NQ
24	0.08 ± 0.09	0.18 ± 0.05	0.04 ± 0.05	ND	ND	ND	ND	0.044 ± 0.009
25	0.12 ± 0.09	0.11 ± 0.06	0.04 ± 0.05	ND	ND	ND	ND	0.013 ± 0.009
26	0.24 ± 0.09	0.19 ± 0.05	0.05 ± 0.05	ND	NQ	NQ	0.06 ± 0.04	0.032 ± 0.009
27	0.11 ± 0.09	NQ	ND	ND	ND	ND	ND	NQ
28	0.04 ± 0.09	NQ	ND	ND	ND	ND	ND	ND
29	0.08 ± 0.09	NQ	NQ	ND	ND	ND	ND	ND
30	0.98 ± 0.08	2.29 ± 0.05	0.65 ± 0.05	0.4 ± 0.1	0.2 ± 0.1	NQ	0.04 ± 0.04	0.060 ± 0.009
31	0.06 ± 0.09	0.10 ± 0.05	0.03 ± 0.05	ND	ND	ND	ND	NQ
32	0.17 ± 0.09	0.50 ± 0.05	0.12 ± 0.05	ND	NQ	ND	ND	NQ
33	0.04 ± 0.09	NQ	NQ	ND	NQ	ND	ND	NQ
34	0.41 ± 0.09	2.11 ± 0.05	0.55 ± 0.05	0.3 ± 0.1	0.2 ± 0.1	NQ	ND	0.011 ± 0.009
35	0.07 ± 0.09	0.18 ± 0.05	0.05 ± 0.05	ND	NQ	ND	ND	NQ
36	0.42 ± 0.08	0.94 ± 0.05	0.27 ± 0.05	0.1 ± 0.1	NQ	ND	ND	0.025 ± 0.009
37	0.02 ± 0.09	0.14 ± 0.05	0.04 ± 0.05	ND	ND	ND	ND	NQ
38	0.30 ± 0.09	1.56 ± 0.05	0.40 ± 0.05	0.2 ± 0.1	0.1 ± 0.1	NQ	ND	NQ
39	0.07 ± 0.09	0.12 ± 0.06	0.04 ± 0.05	ND	ND	ND	ND	NQ
40	0.49 ± 0.08	1.11 ± 0.05	0.30 ± 0.05	ND	NQ	ND	ND	0.028 ± 0.009
41	0.20 ± 0.09	0.82 ± 0.05	0.18 ± 0.05	NQ	0.1 ± 0.1	ND	ND	0.011 ± 0.009
42	1.41 ± 0.09	7.86 ± 0.06	1.88 ± 0.05	1.6 ± 0.1	0.7 ± 0.1	0.34 ± 0.08	ND	0.039 ± 0.009

[a]Abbreviations: SD, standard deviation, calculated as SD = $S_r/b \times [1/m + 1/n + (y_c - y)^2/b^2 S_{xx}]^{1/2}$; ND, not detectable (signal not detected); NQ, not quantifiable (signal detected below the LOQ).

graphic conditions and the shape of the chromatograms cannot be compared because no chromatogram is shown in this article, as they report that the analysis was performed by a combination of several determination methods.[4,17,23,48,49]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b03852.

Analytical figures of merit for the chromatographic external standard methodology (Table S1), analytical figures of merit corresponding to the linear regression of scores versus the corresponding nominal concentrations (Table S2), and relative standard deviation (RSD) values of peak area and retention times (t_R), obtained in the evaluation of the precision of the chromatographic method (Table S3) (PDF)

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DOI: 10.1021/acs.jafc.6b03852
J. Agric. Food Chem. 2016, 64, 8254−8262

Funding

Financial support was provided by the Ministerio de Economía y Competitividad of Spain (Project CTQ2014-52309-P) and the Gobierno de Extremadura (GR15090-Research Group FQM003), both co-financed by the European FEDER funds. O.M.-M. is grateful to the Ministerio de Educación, Cultura y Deporte of Spain for a FPU grant (Resolución de 18 de noviembre de 2013, de la Secretaría de Estado de Educacíon, Formación Profesional y Universidades, BOE no 279, de 21/11/13, reference number FPU13/02249). R.L.P. is grateful to Universidad Nacional de Rosario and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

PAHs, polycyclic aromatic hydrocarbons; MCR-ALS, multivariate curve resolution-alternative least-squares; PDO, Protected Designation of Origin; PCA, principal component analysis

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