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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Abstract

This work presents a strategy for quantitating polycyclic aromatic hydrocarbons (PAHs) in smoked paprika samples. For this, a liquid chromatographic method with fluorescence detection (HPLC-FLD) has been optimized. In order to resolve some interferences 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%) were obtained in the complete extraction procedure from the paprika, consisting in an extraction from the matrix and the clean-up of the extract by means of silica cartridges. Higher concentrations of chrysene, benzo[a]anthracene, benzo[*b*]fluoranthene and benzo[*a*]pyrene were found at the paprika samples, with respect to the maxima amounts allowed for other spices that are under European Regulation (EU) Nº 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 according to its antioxidant and other positive properties for the health, and it is commonly used for culinary and industrial purposes.[1] In Spain, two areas are characterized by production of paprika, which are La Vera (Extremadura) and Murcia, both recognized under Protected Designation of Origin (PDO) by the European Union.

La Vera paprika is obtained from dried peppers by means of a characteristic system. Thus, La Vera peppers are smoked-dried (oak or holm wood fire) while the rest of peppers produced in other Spanish areas or in other countries are sun dried or hot air dried.[2] This smoking system provides the necessary heat for the perfect dehydration of the fruits. It is a slow process, lasting ten to fifteen days, and it confers on the paprika its three fundamental characteristics: aroma, flavour and colour stability.[3] However, this product can contain polycyclic aromatic hydrocarbons (PAHs) because of this drying process.

In the case of smoked foods, the PAHs content depends on parameters such as moisture content of the wood used for smoking, temperature attained by the wood during combustion and concentration of oxygen in the combustion chamber. Another factor considered to be related to the production of PAHs is the nature of the wood itself. The use of hardwoods, instead of softwoods, has been recommended to reduce the presence the PAHs in smoke and, consequently, in smoked foods. However, some authors do not agree with this aspect due to the fact that in some studies the PAH concentrations found in smoke coming from softwood and from hardwood are very similar.[4, 5]

The World Health Organisation (WHO),[6] International Agency for Research on Cancer (IARC),[7] European Food Safety Authority (EFSA)[8] and US Environmental Protection Agency (EPA)[9] have reported the carcinogenic, mutagenic and bio accumulative 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). Humans can be exposed to PAHs through three main routes: inhalation, skin contact and ingestion.[10, 11]

Priority PAHs subjected to control are listed in European regulations. Hence, in accordance with EC regulation 1881/2006, later modified by the 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 modification has been included by the EC regulation 2015/1933 in the case of the maximum content of PAHs in cocoa fibre, 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 it, 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 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, in some occasions, it is not possible and overlapping peaks occur. In these cases, multivariate data analysis can be used for achieving selectivity by mathematical means. The information provided by the second-order signals, together with an adequate decomposition of the generated three-way data sets, enables one to identify the analyte, even in the presence of interferences not modelled in the calibration stage. This is known as the second-order advantage. This second-order multivariate calibration can be performed when full selectivity in the chromatographic separation is not achieved, even in the presence of unexpected components.[24] In some reports, the advantages and drawbacks associated with the combination of multivariate calibration and chromatography have been discussed.[25, 26] It is interesting to note that few literature works concern HPLC-FLD in combination with different second-order algorithms. Early references are the pioneering work of Appellof and Davidson[27] using a video fluorimeter as liquid chromatographic detector and some applications for PAHs and naphthalene

derivatives resolution.[28-30] Particularly, with respect to the use of MCR-ALS (multivariate curve resolution-alternating least squares) algorithm with these second-order data, recently, very few references as the work of Bortolato et al.[31] have been found. Nowadays, the use of chemometric tools is increasing in the analytical determination of minor components in food. In this sense, separative techniques coupled to MCR-ALS have been employed by several authors to quantitate phenolic acids in virgin olive oil,[32, 33] 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 to start to do it, taking into account 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 in two groups, one of them obtained by means of a smoking process, and evaluating the content of these according to other spices regulated. Chemometric tools were employed to solve matrix interferences 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) >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 ºC until use.

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

Paprika is produced from dried peppers whose stem and seeds are eliminated in later stages before milling. In this case, samples of paprika belonging to different origins, the Spanish Protected Designation of Origin (PDO) "*Pimentón de La Vera*" and other different producers, were obtained from

Regulatory Council of the Designation of Origin "*Pimentón de La Vera*" and from local market, respectively. The origin of the samples which did not belong to the Spanish PDO is not available although in their label it is reported that they have been packaged in Spain.

Instrumentation and software

The chromatographic studies were performed with a model 1100 LC instrument (Agilent Technologies, Palo Alto, CA), equipped with degasser, quaternary pump, column oven, autosampler Agilent 1260 infinity, UV/Vis diode array detector (DAD) and fluorescence-detector (FLD). The OpenLAB LC ChemStation software, ver. A.01.04, was used to control the instrument, data acquisition and data analysis. The column used was a 100 mm x 4.6 mm i.d., 1.8 μm, Zorbax Eclipse XDB-C18 (Agilent Technologies).

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

The software package The Unscrambler v6. 11 (CAMO ASA, Trondheim, Norway) was used for the experimental design.

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

Chromatographic conditions

All chromatographic analysis were performed by using a mobile phase consisting in H_2O (solvent A) and acetonitrile (solvent B). The isocratic elution was employed for the PAHs analysis and consisted of 35:65 A:B. The flow rate was set constant at 0.8 mL min^{-1} and the injection volume was 20 μL. The FLD detection was 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, taking the corresponding volumes of more concentrated stock solutions in acetonitrile. The concentrations employed were in the ranges 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 in 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 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 20 – 100 µg/L and **8** in the range 1 – 8 µg/L was also prepared in acetonitrile. A spiked sample set was prepared by fortifying paprika with known concentration of these analytes to validate the developed methodology. Because loss of analytes can be produced in 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 300-460 nm in steps of 1 nm, setting the excitation wavelength at 260 nm. Second order HPLC-FLD matrices of size 161 x 283 (spectroscopic data points x 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

In order to extract the analytes from paprika samples, 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, then the PAHs were eluted from the cartridge

157 with 7 mL of iso-hexane. This extract together with the 5 mL fraction initially percolated were
158 combined, in order to obtain retained and unretained analytes, evaporated to dryness and reconstituted
159 in 5 mL of acetonitrile for its chromatographic analysis. A dilution factor of 1 to 2 was employed before
160 the injection of the extracts.

161 **Chemometric algorithm**

162 **MCR-ALS**

163 MCR-ALS is an algorithm capable of handling data sets deviating from trilinearity, i.e. in which elution
164 time shifts or peak shape changes occur for analytes from sample to sample. In this method, an
165 augmented data matrix is created from the test data matrices and the calibration data matrices.[38] The
166 augmentation was performed in the row direction (time elution). The bilinear decomposition of the
167 augmented matrix D is performed according to the expression:

168 $$D = C\,S^T + E \qquad \text{(Eqn. 1)}$$

169 In this expression D (size J x K) is the matrix of experimental data. In this matrix, J is the number of
170 elution time data points (number of rows of each data matrix) and K is the number of emission
171 wavelengths (number of columns of each data matrix). C (size J x N) is the matrix which contains the
172 concentration profiles of the N components present in the samples (columns), S^T is the matrix which
173 contains the component spectra (rows) and E (size J x K) is a matrix of residuals not fitted by the model.

174 The first step in MCR-ALS studies is to obtain a rough estimation of the number of components, which
175 can be simply performed by visual inspection of singular values or principal component analysis (PCA).
176 The resolution is accomplished using an iterative ALS procedure and requires initialization with
177 parameters as close as possible to the final results. Several methods can be used for this purpose.[35, 39] In
178 this work, the species spectra were estimated from the analysis of the so-called 'purest' spectra, applying
179 a multivariate algorithm which extracts pure component spectra from a series of spectra of mixtures of
180 varying composition.[40-42]

Once MCR-ALS results are obtained and compounds are identified, the MCR-ALS scores are obtained per analyte and sample as the integrated area under the related resolved profile:

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

Where $a(i,n)$ is the score for the analyte n in the sample i, and $C(j,n)$ is the element of the analyte profile in the augmented mode. The scores of a particular analyte for the calibration samples are then regressed against nominal concentration values to build a calibration curve that can be used afterwards for concentration prediction in unknown samples by interpolation.

Results and discussion

Optimization of the chromatographic conditions

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

Another inconvenience in this analysis was the different values of analytes concentration found in the samples, for example, between phenanthrene and benzo[*a*]pyrene. The first one was in the order of mg/kg and the second one was in the order of μg kg^{-1}. 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 from 20 min and this allowed determining all analytes with a single injection. Figure 1 shows a chromatogram of a standard solution and a paprika sample with these final conditions selected. It can be appreciated in the Figure 1 that some analytes present matrix interferences which co-elute 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.

206 **Analytical parameters for the external standard methodology**

207 The validation of the method was carried out in terms of linearity, precision and accuracy, limits of
208 detection (LOD) and quantitation (LOQ). The calibration curves of each compound were constructed,
209 and the analytical figures of merit were calculated employing the peak areas (PA) in the FLD detector.
210 The linearity was very good for all PAHs with correlation coefficients (r^2) higher than 0.99. Limits of
211 detection[43] were between 0.015 mg/kg and 0.45 mg/kg and limits of quantitation were between 0.050
212 mg/kg and 1.5 mg/kg.

213 The evaluation of the precision was performed by carrying out the analysis of several standard solutions
214 containing 30 μg/L of each compound except in the case of benzo[*a*]pyrene (8 μg/L) in the same day
215 (intra-day precision, n = 8), and different days during 7 days (inter-day precision). The precision was
216 also examined for several standard solutions containing 15 μg/L of each compound except in the case
217 of benzo[*b*]fluoranthene and benzo[*a*]pyrene (0.1 μg/L) in the same day (intra-day precision, n = 8) and
218 different days during 7 days (inter-day precision). The relative standard deviation (RSD) values of peak
219 area and retention times (t_R) were determined for each compound. In all cases, the precision was better
220 than 7.5%, being between 0.1 and 5.6% (RSD values) in the intra-day precision and between 0.5 and
221 7.5% (RSD values) in the inter-day precision.

222 **MCR-ALS analysis**

223 In order to quantitate the three analytes which presented interferences in their chromatographic elution
224 (fluorene, pyrene and benzo[*b*]fluoranthene), MCR-ALS data processing was employed. This algorithm
225 allows processing of second-order data which are not trilinear because of the presence of elution time
226 shift from run to run.
227 The first step to carry out the MCR-ALS analysis is to obtain the second-order data, in this case matrices
228 X x Y (spectral data points x time). Thus, Figure 2 shows second-order data matrices of size 161 x 283
229 (spectral data points x time), obtained in the chromatographic system, of a standard solution containing
230 the eight PAHs quantified and a paprika sample belonging to the PDO. The presence of matrix
231 interferences in the case of paprika sample can be noted.

For the analysis of data, each chromatographic data matrix was divided into different time regions following a strategy similar to 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). In the case of the emission wavelength recording, the complete range of wavelengths was used.

For applying the MCR-ALS algorithm, augmented matrices are necessary. For each time region, MCR-ALS algorithm was applied 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 interferences 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 but not to the background signal. After ALS optimization for each sample, the constituents were identified and the quantitation was carried out with the aid of the corresponding pseudo-univariate calibration curves. Analytical figures of merit corresponding to linear regression of scores versus the corresponding nominal concentrations were calculated. Firstly, the validation of the methodology was performed. Thus, on the one hand, validation samples consisted of standard solutions with content of fluorene, pyrene and benzo[*b*]fluoranthene within the range of the calibration set. In this set, the number of principal component analysis found was 1 in the case of fluorene and pyrene and 2 in case of benzo[*b*]fluoranthene. On the other hand, a set of fortified paprika samples with known concentration of these analytes was also employed to validate the methodology. This addition was made after extraction procedure in order to avoid recovery loss in this stage. The concentration found in fortified paprika samples were calculated taking into account the analytes concentrations, predicted by the algorithm, in the sample without fortifying.

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 3 shows the elution

time profiles retrieved 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 the Figure 3.

Figure 4 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. Because all ellipses include the theoretically expected values of (1, 0) for the slope and intercept, respectively, the accuracy of the applied methodology for these compounds in validation samples can be claimed.

Analysis of real paprika samples

Treatment of the sample

In order to quantitate PAHs in paprika samples, firstly, the analytes were extracted from paprika. In the clean-up and concentration step, it was tested whether, when the extract containing the PAHs was loaded in a silica cartridge, the analytes were not completely retained. For this, it was decided to employ the minimal volume of iso-hexane to elute the PAHs from the cartridge with the aim of retaining other interferences present in the matrix of paprika such as fluorescent compounds of higher polarity, for example, capsaicinoids, flavonoids, tocopherols, etc... This volume was 7 mL, in addition to another 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 analysis in triplicate, 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 recoveries results were better than 82% in all cases. The repeatability was analysed in this assay and the RSD (%) values in all cases were lower than 7%.

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

Quantitation of real samples

As it has been indicated throughout the entire study, 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 conventional external standard methodology. 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 according Miller and Miller.[47]

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

Results presented in this work are similar to those obtained by Fasano et al.,[20] the only previous study found in the literature about the quantitation of these compounds in smoked paprika samples. However, chromatographic 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 the combination of several determination methods.[4, 17, 23, 48, 49]

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

309 **Acknowledgments**

310 Financial support was provided by the Ministerio de Economía y Competitividad of Spain (Project
311 CTQ2014-52309-P) and the Gobierno de Extremadura (GR15090-Research Group FQM003), both co-
312 financed by the European FEDER funds. Olga Monago Maraña is grateful to the Ministerio de
313 Educación, Cultura y Deporte of Spain for a FPU grant (Resolución de 18 de noviembre de 2013, de la
314 Secretaría de Estado de Educación, Formación Profesional y Universidades, BOE n◦ 279, de 21/11/13,
315 reference number FPU13/02249). Rocio Laura Perez is grateful to Universidad Nacional de Rosario and
316 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

317 **Conflict of interest**

318 The authors declare that they have no conflict of interest.

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320 **Supporting Information**

321 This material is available free of charge via the Internet at http://pubs.acs.org.

322 Table S1: Analytical figures of merit for the chromatographic external standard methodology

323 Table S2: Analytical figures of merit corresponding to linear regression of scores versus the
324 corresponding nominal concentrations

325 Table S2: Relative standard deviation (RSD) values of peak area and retention times (t_R), obtained in
326 the evaluation of the precision of chromatographic method

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Figure captions

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**.

Figure 2. Chromatograms corresponding to a standard solution (red line) and a PDO paprika sample (black line) obtained with the final conditions employed (A) $\lambda_{exc}/\lambda_{em}$ = 260/352 nm and (B) $\lambda_{exc}/\lambda_{em}$ = 260/420 nm.

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.

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

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

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

Sample	Concentration ± SD (mg/kg)							
PDO	**Fluorene**	**Phenanthrene**	**Anthracene**	**Pyrene**	**Chrysene**	**Benzo[*a*]anthracene**	**Benzo[*b*]fluoranthene**	**Benzo[*a*]pyrene**
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

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

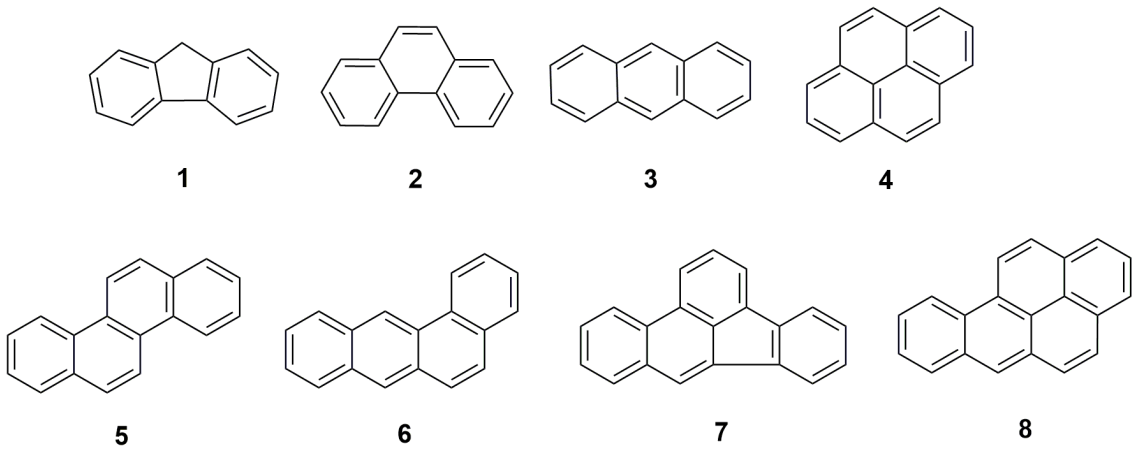

Figure 1

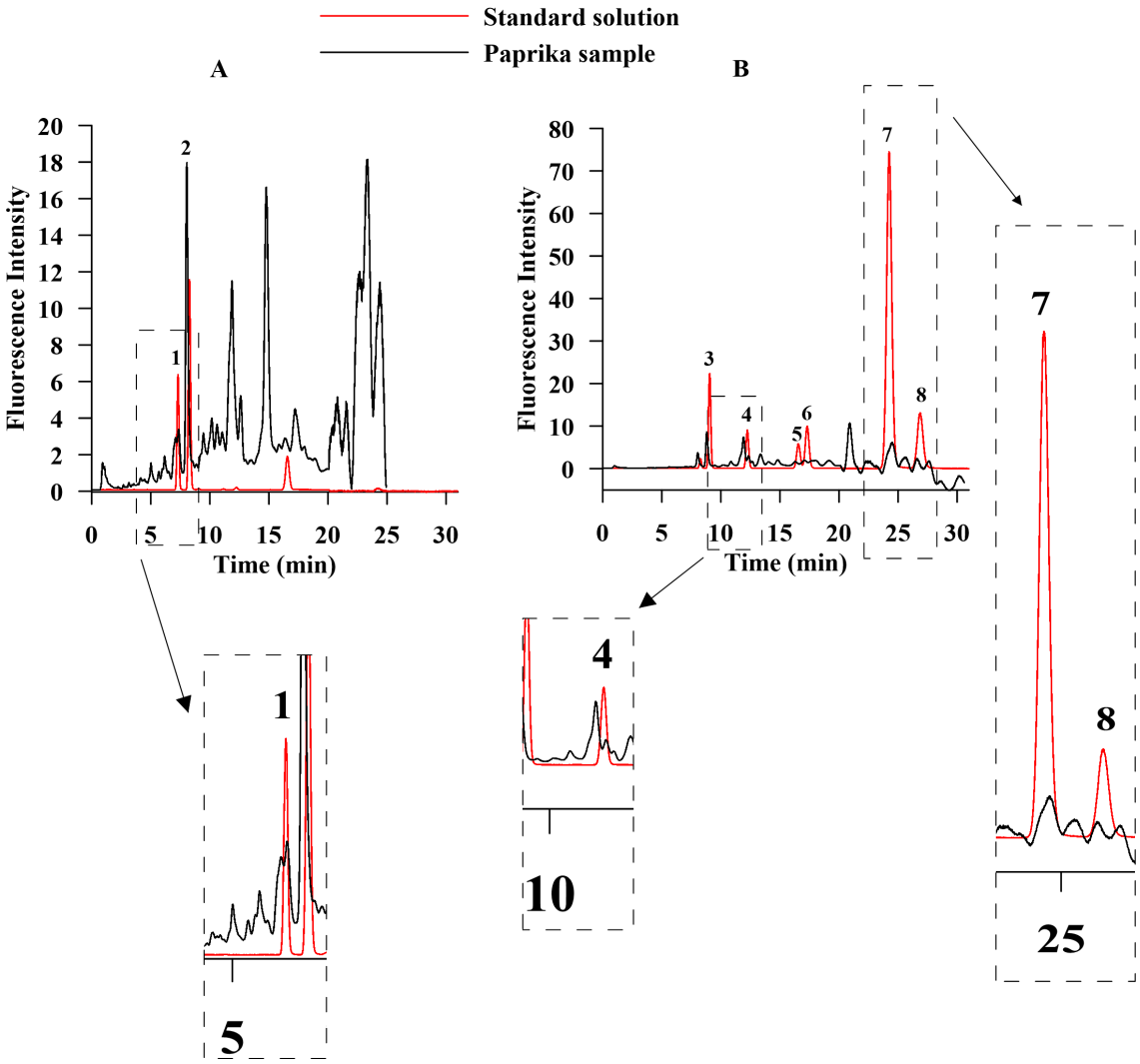
Standard solution
Paprika sample
A
B
Fluorescence Intensity
Time (min)
1
2
3
4
5
6
7
8
10
5
25

Figure 2

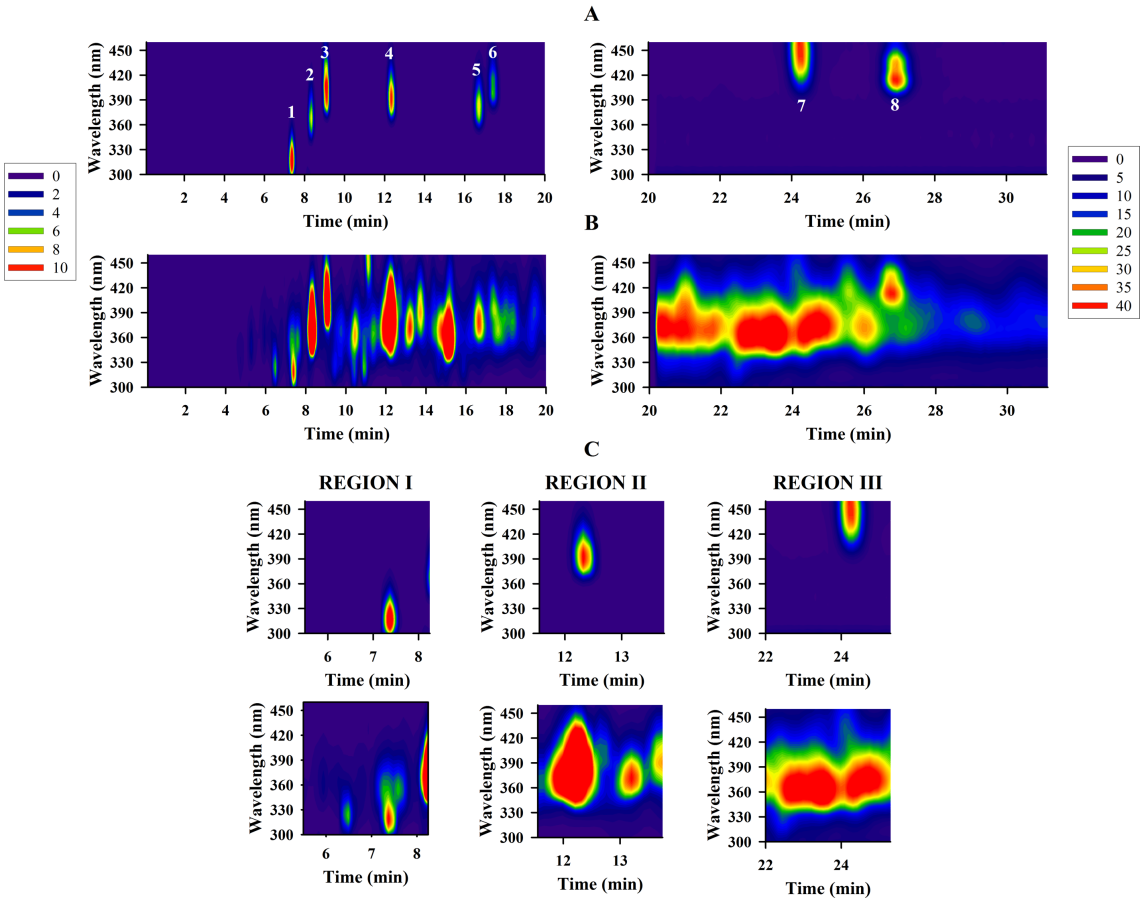
A
B
C
Wavelength (nm)
Time (min)
1
2
3
4
5
6
7
8
REGION I
REGION II
REGION III
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40

Figure 3

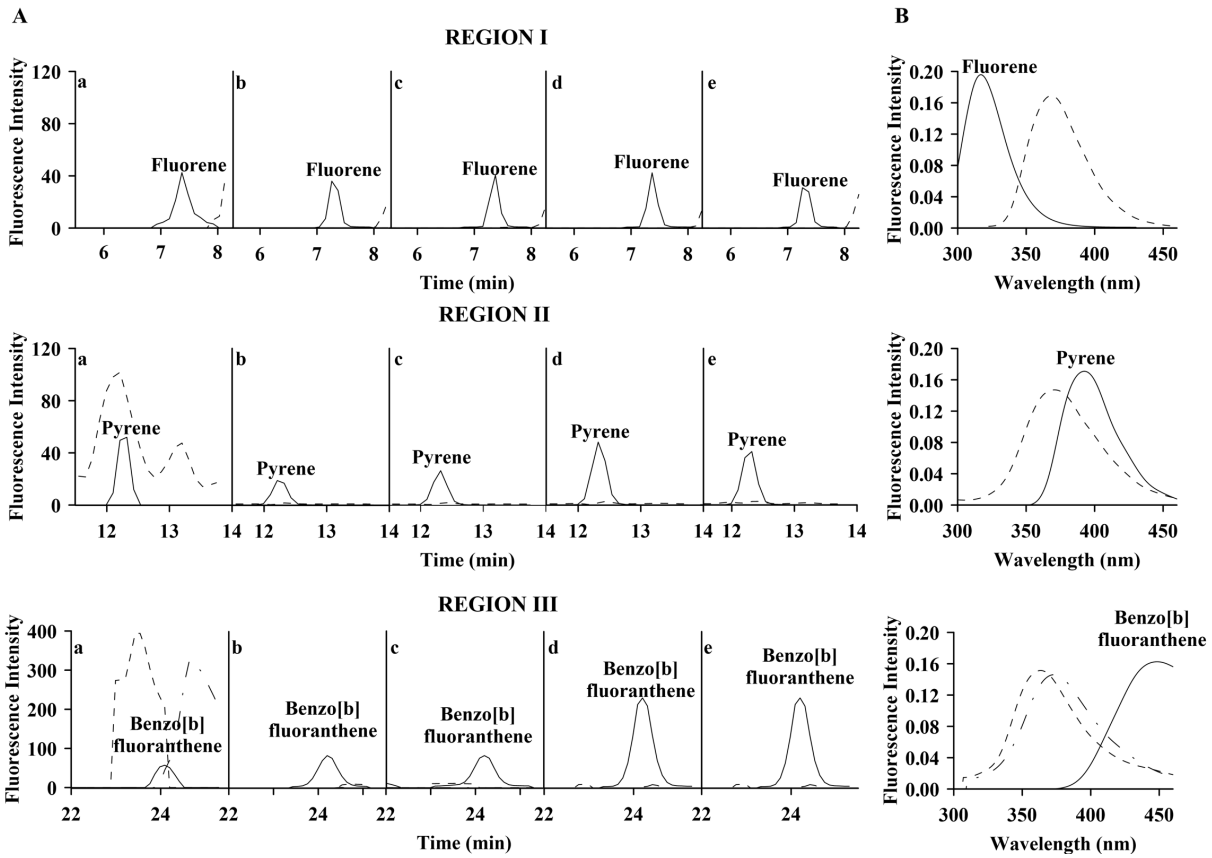
A
B
REGION I
REGION II
REGION III
Fluorescence Intensity
Time (min)
Wavelength (nm)
a
b
c
d
e
Fluorene
Pyrene
Benzo[b] fluoranthene

Figure 4

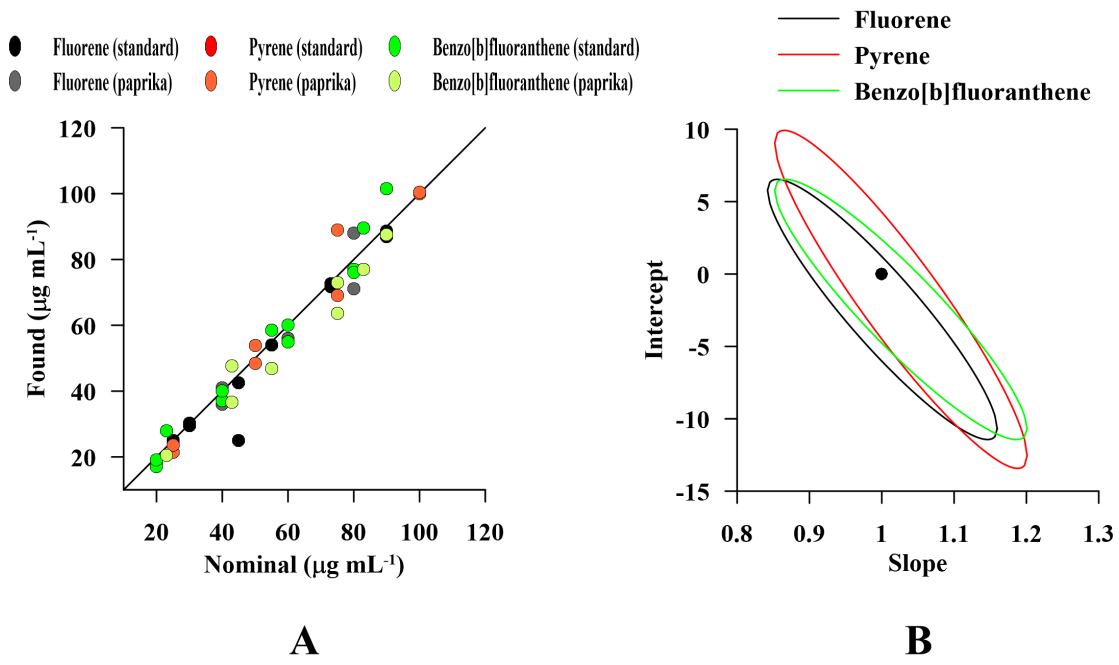
Fluorene (standard)
Pyrene (standard)
Benzo[b]fluoranthene (standard)
Fluorene (paprika)
Pyrene (paprika)
Benzo[b]fluoranthene (paprika)
Found (µg mL-1)
Nominal (µg mL-1)
A
Fluorene
Pyrene
Benzo[b]fluoranthene
Intercept
Slope
B

Figure 5

Table of Contents Graphic