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Original Paper

Multivariate optimization of a GC–MS method for determination of sixteen priority polycyclic aromatic hydrocarbons in environmental samples

This paper describes the development and optimization, by using multivariate analysis, of a GC–MS-SIM method for evaluation of the 16 polyaromatic hydrocarbons considered as priority pollutants in atmospheric particulate material by the US EPA. In order to assure an adequate separation in the shortest analysis time, a multivariate design was used to set the conditions of the oven temperature program. The optimization process was carried out using factorial fractional design and Box–Behnken design. The following factors were evaluated: initial temperature, temperature rate #1, intermediary temperature, temperature rate #2, and final temperature. The optimized conditions were set at: 70°C (2 min) → 200°C (30°C/min, 5 min) → 300°C (5°C/min, 1.67 min). Moreover, we have also optimized the injector temperature as 310°C and sampling time as 0.8 min. The total analysis time was 33 min. Validation of GC–MS-SIM yielded satisfactory results for repetitivity of the detector response and retention times, and linearity of calibration curves. LOD were established as 0.13–0.34 ng/mL (peak area) and 0.18–0.72 ng/mL (peak height). The method has been shown to be appropriate for the analysis of samples of atmospheric particulate material and/or other environmental matrices.

Keywords: Atmospheric aerosols / GC–MS / Multivariate design / PAHs

Received: November 6, 2007; revised: January 23, 2008; accepted: February 7, 2008

DOI 10.1002/jssc.200700573

1 Introduction

The environmental analysis of polycyclic aromatic hydrocarbons (PAHs) has been considered of importance for decades due to their potent mutagenic and carcinogenic properties and comparatively long lifetime in the environment [1, 2]. PAHs are formed either by incomplete combustion or pyrolysis of organic matter containing carbon and hydrogen. They are ubiquitous and abundant pollutants that are emitted from several natural or anthropogenic sources (the former being more relevant in remote sites and the latter more important in urban sites) and may be present in the atmosphere, hydrosphere, and lithosphere. Major sources include emissions from fossil fuel combustion, forest fires, industrial fumes, oil spills, and road construction materials [1, 3–10]. Moreover, in urban environments, diesel and gasoline engine exhausts are important sources of PAHs. Special attention has been paid to atmospheric particles emitted by diesel engines, due to the numerous *in vivo* and *in vitro* studies that have established their adverse effects on human health [3–5, 11, 12]. The determination of PAHs in air is therefore of great importance for air quality studies and for prediction of human exposure to this class of compounds. Several different analytical methods have been developed to determine PAHs in environmental samples and other types of matrices [3, 5, 8, 9, 13–18].

Measuring PAHs by means of chemical analyses of particulate matter is often time-consuming because of the great diversity of these compounds at low concentrations in ambient air. It is an analytical challenge to be able to identify most of the PAH compounds in atmospheric samples and achieve low detection and quantification limits. Studies by different authors at different locations

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Abbreviations: PAH, polycyclic aromatic hydrocarbon

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have shown that average values for the 16 EPA PAHs obtained in environmental samples range between 0.04 and 15.30 ng/m^3. Sample treatment is usually performed manually; hence it is tedious [19, 20] and consumes large amounts of many different solvents if classical PAHs extraction methods are used [20].

Atmospheric samples contain a large number of PAH compounds that differ little in structure and molecular weight; and thus require high resolution techniques for separation and analysis. Reliable data regarding the composition and concentration of PAHs in particulate matter can be obtained through different gas or liquid chromatographic methods. The GC–MS methodology provides extensive information on sample composition and enables better compound identification on the basis of structural information. This system records a specified ion at a selected time and confers both better selectivity and better sensitivity [19, 20]. Some weak points of this separation and detection technique in PAH determination are mainly co-elution of isomers or structurally similar compounds as well as long chromatographic runs (about 45–90 min of total analysis time). In order to minimize these undesired aspects in PAHs analysis, we propose a multivariate optimization approach to developing a GC–MS methodology for PAH determination, achieving good improvements in resolution by both avoiding co-elution of isomers and reducing the total time of analysis.

Optimization of the chromatographic conditions may be carried out by a traditional univariate approach, in which each factor is studied separately, or by multivariate experimental design strategy, which allows for simultaneous variation of all evaluated factors, making it possible to distinguish interactions among them that would not be detectable by classical univariate experimental design [21, 22]. Multivariate optimization design also allows a reduction in the number of required experiments, without loss of information.

The optimization of chromatographic conditions has been carried out using multivariate strategies such as factorial design and response surface methodology type central composite design, and Box–Behnken design [23–27]. Moreover, since the aim of this study was to assure an adequate separation of the 16 PAHs, in the shortest analysis time, a multivariate design was used to set the conditions of the oven temperature programming (column heating). The optimization process was carried out using both factorial fractional and Box–Behnken design. The evaluated parameters used to attain reliable chromatographic conditions for separation of PAH were: initial temperature (°C), temperature rate #1 (°C/min), intermediate temperature (°C), temperature rate #2 (°C/min), and final temperature (°C), in order to attain a reliable improvement in the chromatographic separation of the priority PAH.

2 Experimental

In this study, we employed 16 PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene) standard solution (AccuStandard Inc., USA), acetonitrile of chromatographic and spectroscopic grade (J. T. Baker) for dilution of standard solutions, a GCMS-QP2010 gas chromatograph–mass spectrometer system model with AOC-20i autosampler (Shimadzu, Japan), an Elite5MS GC column (30 m × 0.25 mm id × 0.25 μm film thickness) (Perkin Elmer, USA), and a NanoPure Diamond (USA) water purifying system.

Chromatographic analyses were carried out starting with procedures already described in the literature [28–32] as well as acquired expertise of this research group in PAH analysis by GC–MS [23, 33]. Briefly, our starting column oven program, set in a univariate manner, was: (i) oven: 60°C (1 min) → 280°C (5°C/min) → 280°C (15 min); (ii) injector: 270°C, splitless mode; (iii) transfer line: 270°C, (iv) ion source: 230°C, (v) analyzer: 150°C; electron impact energy: 70 eV [23, 33]. Although it is possible to acquire reliable data for a part of the sixteen PAHs considered using this procedure, there are some problems: (i) it is not possible to attain completely satisfactory resolution for the following pairs of isomers: phenanthrene/anthracene, benzo[*a*]anthracene/chrysene, benzo[*b*]fluoranthene/benzo[*k*]fluoranthene, and indeno[1,2,3-*c,d*]pyrene/dibenz[*a,h*]anthracene; (ii) the lower the vapor pressure of the PAH, the less sharp is its peak; and (iii) the proposed temperature program takes 60 min for each sample to be completely eluted from column. In the face of all these points, we decide to improve our experimental conditions. Therefore, taking into account the chromatographic column specifications and considering the following parameters: (a) multivariate design for defining of temperature programming of the oven/column; (b) injector temperature effect; and (c) sampling time effect, we have developed an optimized gas GC–MS-SIM method by multivariate strategies such as factorial design and response surface methodology type central composite design, and Box–Behnken design.

2.1 Multivariate design

Multivariate design was employed as a strategy to attain higher peak resolutions in shorter total analysis time. The following parameters related to oven temperature programming were evaluated: initial temperature (°C), temperature rate #1 (°C/min), intermediate temperature (°C), temperature rate #2 (°C/min), and final temperature

Table 1. List of PAHs studied and selected ions (*m/z*) for each PAH.

PAH	Abbreviation	Base ion	Reference ion
Naphthalene	NAP	128	102
Acenaphthylene	ACY	152	76
Acenaphthene	ACE	153	76
Fluorene	FLU	166	82
Phenanthrene	PHE	178	152
Anthracene	ANT	178	89
Fluoranthene	FLT	202	101
Pyrene	PYR	202	101
Benzo[*a*]anthracene	BaA	228	114
Chrysene	CRY	228	113
Benzo[*b*]fluoranthene	BbF	252	126
Benzo[*k*]fluoranthene	BkF	252	126
Benzo[*a*]pyrene	BaP	252	126
Indeno[1,2,3-*c,d*]pyrene	IND	276	138
Dibenz[*a,h*]anthracene	DBA	278	139
Benzo[*g,h,i*]perylene	BgP	276	138

(°C). Statistical calculations were performed using the *Statistica 6.0* package (Statsoft, USA).

First, in order to obtain qualitative information about analytes, all multivariate optimization experiments were carried out in SCAN MODE by using a 4 μg/mL standard solution of the 16 priority PAHs, specified as follows: injector temperature: 280°C; splitless time: 0.40 min; helium flow controlling mode: linear velocity; helium linear velocity: 40 cm/s; column flow: 1.19 mL/min; pressure: 123.2 kPa. Mass spectrometer conditions were: scan range: 40 – 350 amu, ionization chamber temperature: 250°C and transfer line temperature: 280°C. Once the best conditions for the studied parameters were reached by multivariate design, we set a SIM mode method to be used in quantitative determinations of PAHs. In this optimized quantitative PAH method, calibration plots were obtained from external standard solutions, prepared by dilution of two PAH stock solutions of 0.1 mg/mL and 2.0 mg/mL (100 μg/mL and 2000 μg/mL) respectively in MeOH/CH_2Cl_2 (1:1) to the range of concentrations expected for samples (1 to 5000 ng/mL). In quantitative analysis, samples were run in the SIM mode with reference ions (*m/z*) for each of the 16 PAHs, with tolerance limit of 30%, according to Table 1. In order to obtain a more reliable analyte identification procedure, we decided to monitor two *m/z* signals for each PAH, trying to quantify unequivocally our target compound present in the samples and to avoid some possible "ghost" or interferent peaks.

At this stage, the response equation was based on retention times (*tA*) and resolution (*R*) for the following PAHs pairs: PHE and ANT; BaA and CRY; BbF and BkF; IND and DBA.

$$\text{Response} = (R_{p1}/t_{R1} + R_{p2}/t_{R2} + R_{p3}/t_{R3} + R_{p4}/t_{R4})/4 \quad (1)$$

where: R_{pn} is the resolution for the n^{th} evaluated pair and t_{Rn} is the retention time for the second peak in each corresponding pair.

2.2 Injector temperature effect study

Studying the injector temperature effect, nine experiments were carried out in SCAN MODE by using a 4 μg/mL standard solution of the 16 priority PAHs, in the temperature range of 260 – 340°C by measuring either peak area or peak height.

2.3 Sampling time effect study

The time interval between sample injection and split valve may influence the detector signal of analytes. In this regard, experiments were performed in the SIM mode with an 80 ng/mL standard solution, varying the split valve opening time from 0.3 to 1.0 min. Evaluation of detector response was carried out by measuring either peak area or peak height of the 16 PAH.

2.4 Retention time, peak area, and peak height repetitivity

Inter-day repetitivity was evaluated by injection of 1.0 μL of standard solutions of 10, 20, 40, and 100 ng/mL (five replicates of each). We then calculated the mean of these quintuplicates of each concentration. Inter-day precision was estimated by coefficient of variation (CV%).

2.5 Limit of detection (LOD) and limit of quantification (LOQ)

An external analytical curve was plotted for each PAH, ranging from 1 to 10 ng/mL (three replicates of each) in the SIM mode, for calculation of LOD and LOQ. Limit of detection and limit of quantification were calculated as follows [34, 35].

$$\text{LOD} = 3.0 \times \frac{s}{a} \quad (2)$$

$$\text{LOQ} = 10 \times \frac{s}{a} \quad (3)$$

where *s* is the standard deviation of linear coefficient from the analytical curve and *a* is the angular coefficient from the analytical curve.

3 Results and discussion

3.1 Multivariate design

Optimization of the chromatographic conditions was carried out using multivariate strategies, initially with

Table 2. Detailed description of the factorial design #1 and design #2.

Factor [Abbreviation]	Minimum level (−)	Medium level (0)	Maximum level (+)
Design #1			
Initial temperature (°C) [IT]	50	70	90
Temperature rate #1 (°C/min) [R1]	5	10	15
Intermediary temperature (°C) [MT]	140	160	180
Temperature rate #2 (°C/min) [R2]	4	8	12
Final temperature (°C) [FT]	280	300	320
Design #2			
Initial temperature (°C) [IT]	60	70	80
Temperature rate #1 (°C/min) [R1]	10	20	30
Intermediary temperature (°C) [MT]	160	180	200
Temperature rate #2 (°C/min) [R2]	4	6	8

factorial design (2^{5-2}), using the response defined by Eq. (1) in order to evaluate the factors listed in Table 2a.

The first factorial fractional design was carried out using 2^{5-2} design with 3 replicates in the central point, resulting in 11 experiments. The results of the first experiment run with the factorial design showed that R1, IT, MT, and R2 were significant at 95% confidence level (Pareto's chart, Fig. 1a). Positive values for IT (14.55), R1 (33.3), and MT (9.51) indicate that increasing these parameters would result in response increment, and hence in better results. A negative value for R2 (−5.11) means that decreasing temperature rate R2 would also contribute to an improved response. Final temperature (FT) was not statistically significant at 95% confidence level so we could use any value in the studied range. The final temperature was selected as 300°C in the following experiments.

It is important to stress that in the experiments with IT = 90°C and R2 = 15°C/min, the naphthalene signal was lost since this is the most volatile species among the sixteen studied PAHs. In view of this observation, although Pareto's chart indicates improving response with rising IT, it was opted to narrow the IT range in the second experiment (from 50 − 90°C to 60 − 80°C). Furthermore, factorial design #1 showed consistent results since slow temperature rates in the second ramp (R2) could result in better separations of the heaviest PAHs (R2 coincides with the region corresponding to the more difficult conditions of chromatographic separation). On the other hand, the necessity of increasing R1, IT, and MT suggested by Pareto's chart could be reflected in reduction of the total analysis time with no effect on peak resolution at the beginning of the chromatogram. Taking these details into account, a further factorial fractional design using a 2^{4-2} matrix with higher values of R1, IT, and MT as well as lower values of R2 was carried out (Table 2b and Fig. 1b). This 2^{4-2} factorial fractional design with three replicates at the central point resulted in 11 experiments, with minimum (−1), medium (0), and maximum (+1) levels for each studied factor.

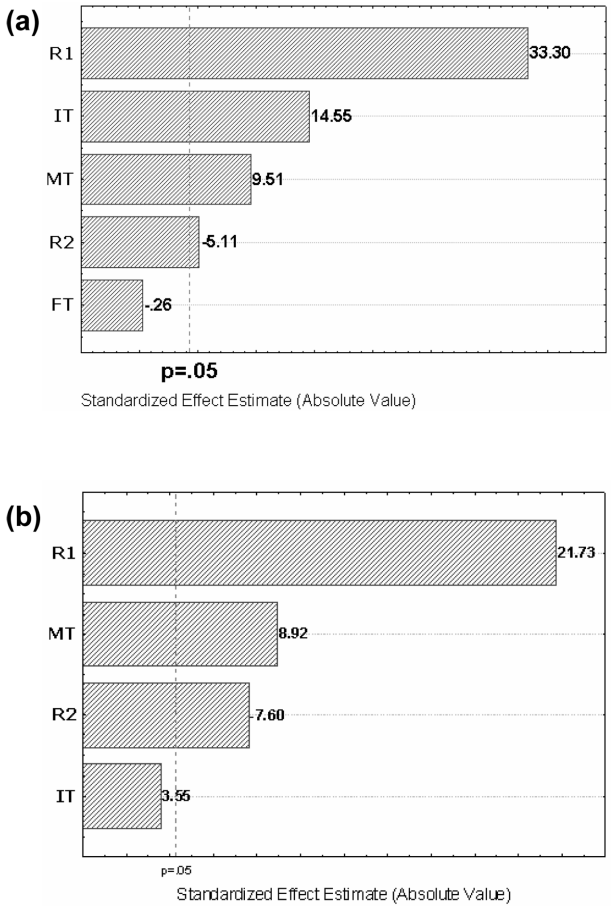

Figure 1. (a) Pareto's chart of the studied parameters in factorial design #1; (b) Pareto's chart of the effect studied in factorial design #2. (R1 = temperature rate #1 (°C/min); R2 = temperature rate #2 (°C/min); IT = initial temperature (°C); FT = final temperature (°C); MT= intermediary temperature (°C).

Pareto's chart in Fig. 1b shows that R1, MT, and R2 are statistically significant at 95% confidence level. Positive values for R1 and MT indicate that their increase could result in increases of response. A negative value of R2 means that the response would be raised if R2 were decreased. This was concordant with the previous experimental planning, according to which higher response would be obtained on increasing R1 and MT as well as reducing R2. Thus it was verified that IT was now not statistically significant and was therefore set at 70°C.

Since the number of significant variables was reduced to three and those are close to ideal (good resolution and short analysis time), Box–Behnken design was performed for estimating the response surface in order to find its maxima (which correspond to critical points or optima) using a quadratic equation. In Table 3, factors and levels

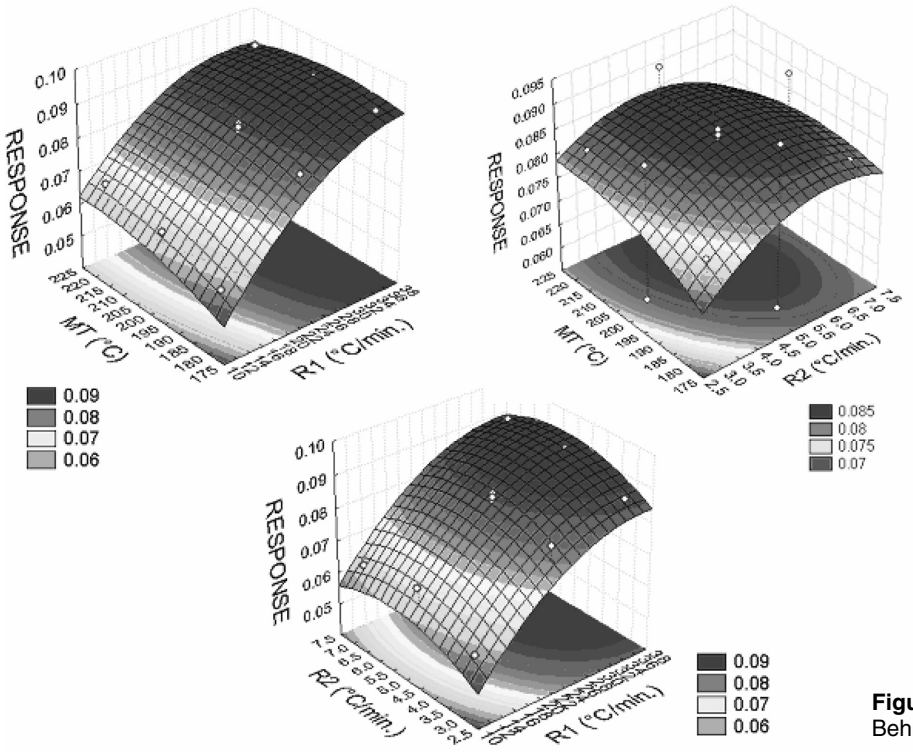

Figure 2. Response surfaces type Box–Behnken design.

Table 3. Detailed description of the Box–Behnken design.

Factor [Abbreviation]	Minimum level (−)	Medium level (0)	Maximum level (+)
Temperature rate #1 (°C/min) [R1]	12	24	36
Intermediary temperature (°C) [MT]	180	200	220
Temperature rate #2 (°C/min) [R2]	3	5	7

Table 4. Critical values obtained by Box–Behnken design: (a) central body method; (b) Derringer and Suich method

Factor [Abbreviation]	Critical value
(a)	
Temperature rate #1 (°C/min) [R1]	37.6
Intermediary temperature (°C) [MT]	192.7
Temperature rate #2 (°C/min) [R2]	5.6
(b)	
Temperature rate #1 (°C/min) [R1]	29.1
Intermediary temperature (°C) [MT]	194.8
Temperature rate #2 (°C/min) [R2]	5.2

used in the construction of the Box–Behnken design are described.

In this manner, experimental design of the Box–Behnken type was used in association with the methodology of the response surface for optimization of the chromatographic conditions, resulting in 18 experiments with six replicates at the central point.

The response surface (Fig. 2) enables us to conclude that the estimated optimum point is out of our working range of studied variables. This implies that it is still necessary to perform more experiments aiming to cover the critical point region and/or defining a response that brings both resolution improvement and analysis time reduction.

The chromatogram in Fig. 3 was acquired in an experiment with the highest R1 (36°C/min). In that chromatogram, phenanthrene (peak 5, t_r = 9.41 min) and anthracene (t_r = 9.53 min) are too close, enabling interference in signal integration. One possible explanation is that using the retention time (t_r) of the last peak of each pair in the denominator of the response equation could have contributed to an excessive reduction of analysis time to the detriment of analyte separations since t_r is of higher magnitude than the corresponding resolution. This fact would imply a greater weighting of analysis time, resulting in a poorer separation. We therefore consider that Eq. (6) does not reach completely the desired goals and should be reassessed and improved. More specifically, R1 should be fitted between 12 and 36°C to have a maximum in the response surface (optimum point) (Table 4a).

In this way, we decide to construct a new equation which could represent the response to be optimized by using the same significant variables as are found in the factorial design #2: R1, MT and R2. Thus, a new response equation was developed based on the method of Der-

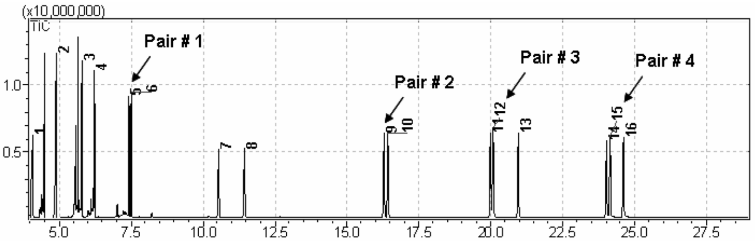

Figure 3. A GC–MS-SCAN chromatogram of a 4 μg/mL 16 PAH standard (1 – naphthalene; 2 – acenaphthylene; 3 – acenaphthene; 4 – fluorene; 5 – phenanthrene; 6 – anthracene; 7 – fluoranthene; 8 – pyrene; 9 – benzo[*a*]anthracene; 10 – chrysene; 11 – benzo[*b*]fluoranthene; 12 – benzo[*k*]fluoranthene; 13 – benzo[*a*]pyrene; 14 – indeno[1,2,3-*c,d*]pyrene; 15 – dibenz[*a,h*]-anthracene; 16 – benzo[*g,h,i*]perylene). Chromatographic conditions: (i) *injector temperature:* 280°C; (ii) *injection mode:* splitless; (iii) *oven*: 70°C (2 min) → 200°C (36°C/min) → 200°C (5 min) → 300°C (7°C/min) → 300°C (5 min); (iv) *transfer line*: 280°C; (v) *ion source*: 250°C; (vi) *electron impact energy*: 70 eV.

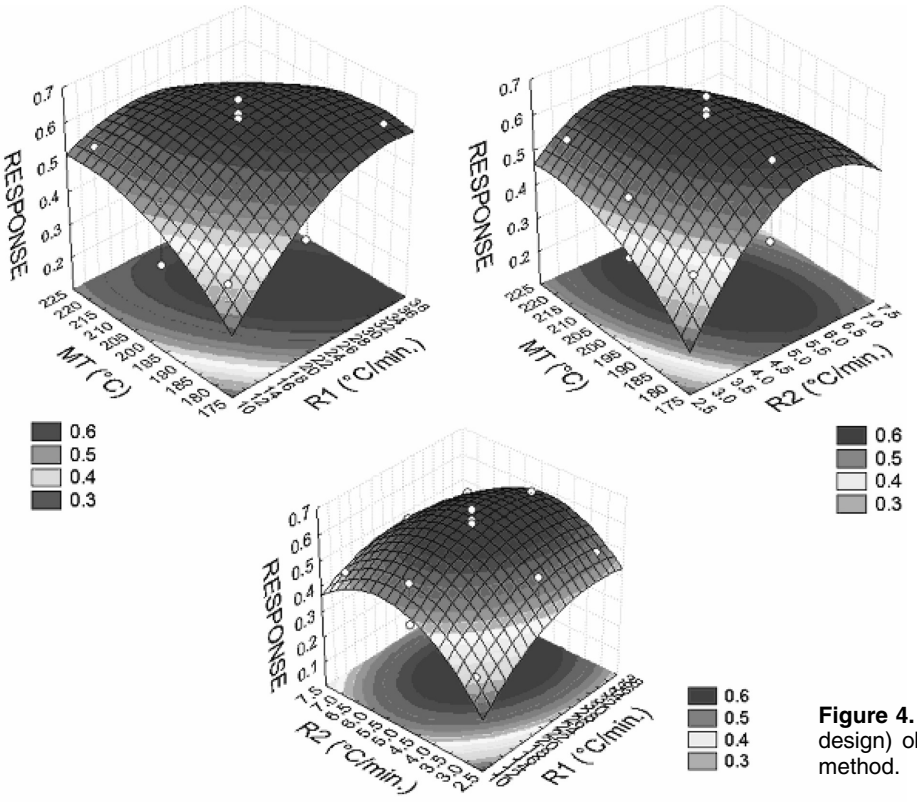

Figure 4. Response surfaces (Box–Behnken design) obtained by the Derringer and Suich method.

ringer and Suich [28, 29, 36, 37] allowing equalization of the magnitudes of analysis time and resolution averages of the four pairs studied, avoiding that one of them represent an excess weight in relation to the others.

The codification of both analysis time and resolution average was carried out in order to adjust the values on a scale from 0 to 1, both of them being of the same order of magnitude. The employed maxima and minima values in the codification were based on experimental results of resolution average between studied pairs, both for the observed analysis time as well as for the main objective, *i.e.* best resolution in the shortest analysis time. The analysis time was therefore established in the range 20 – 50 min (50 ⇒ 0 and 20 ⇒ 1); the shorter the analysis time, the closer to unity the codified analysis time value would be. Resolution was defined from 1.0 – 1.6 (1.0 ⇒ 0 and 1.6 ⇒ 1); the higher the resolution, the closer to unity the codified resolution would be. In this context, codification was performed in keeping with the following equations:

$$tA_{cod} = -(tA - 50)/(50 - 20) \quad (4)$$

$$R_{cod} = (R - 1.0)/(1.6 - 1.0) \quad (5)$$

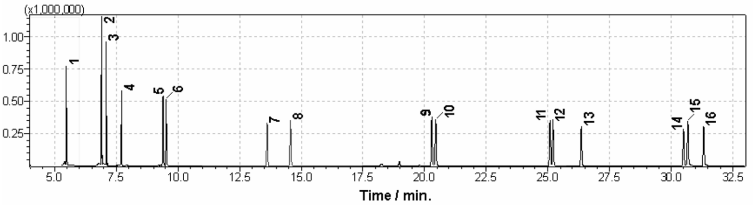

Figure 5. A GC–MS-SIM chromatogram of a 0.4 μg/mL PAH standard after optimization (1 – naphthalene; 2 – acenaphthylene; 3 – acenaphthene; 4 – fluorene; 5 – phenantrene; 6 – anthracene; 7 – fluoranthene; 8 – pyrene; 9 – benzo[*a*]anthracene; 10 – chrysene; 11 – benzo[*b*]fluoranthene; 12 – benzo[*k*]fluoranthene; 13 – benzo[*a*]pyrene; 14 – indeno[1,2,3-*c,d*]pyrene; 15 – dibenz[*a,h*]anthracene; 16 – benzo[*g,h,i*]perylene).Chromatographic conditions: (i) *injector*: 310°C, (ii) *injection mode*: splitless; (iii) *oven*: 70°C (2 min) → 200°C (30°C/min) → 200°C (5 min) → 300°C (5.0°C/min) → 300°C (1.67 min); (iv) *transfer line*: 280°C; (v) *ion source*: 250°C; (vi) *electron impact energy*: 70 eV.

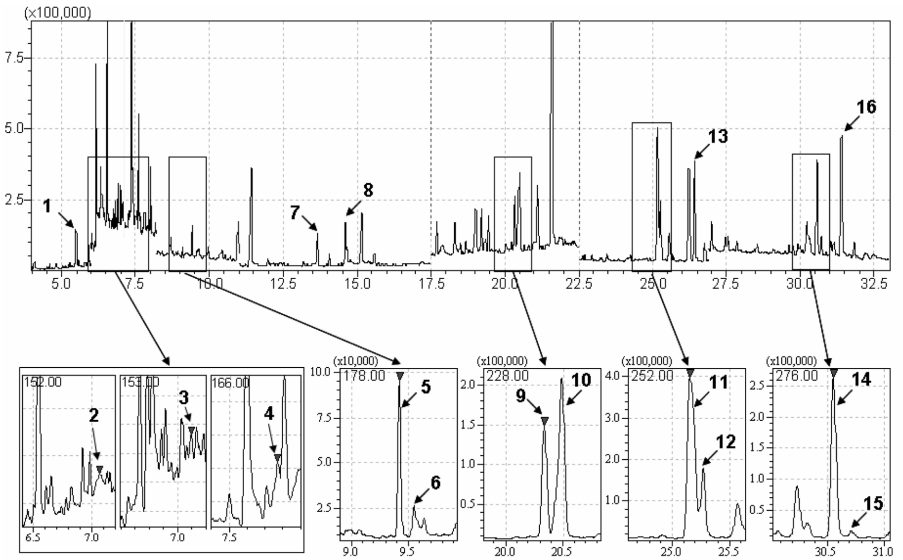

Figure 6. GC–MS-SIM chromatogram of a real sample after optimization (1 – naphthalene; 2 – acenaphthylene; 3 – acenaphthene; 4 – fluorene; 5 – phenanthrene; 6 – anthracene; 7 – fluoranthene; 8 – pyrene; 9 – benzo[*a*]anthracene; 10 – chrysene; 11 – benzo[*b*]fluoranthene; 12 – benzo[*k*]fluoranthene; 13 – benzo[*a*]pyrene; 14 – indeno[1,2,3-*c,d*]pyrene; 15 – dibenz[*a,h*]-anthracene; 16 – benzo[*g,h,i*]perylene). Chromatographic conditions: (i) *injector*: 310°C, (ii) *injection mode*: splitless; (iii) *oven*: 70°C (2 min) → 200°C (30°C/min) → 200°C (5 min) → 300°C (5.0°C/min) → 300°C (1.67 min); (iv) *transfer line*: 280°C; (v) *ion source*: 250°C; (vi) *electron impact energy*: 70 eV.

where tA_{cod} is the codified analysis time; tA is the analysis time (considered as the retention time of the last peak + 2 min); R_{cod} is the codified resolution average; and R is the average of resolution evaluated peak pairs.

After performing the codification of resolution and analysis time, the new response was defined as the geometric average between codified values of analysis time and resolution:

$$\text{Response} = \sqrt{tA_{cod} \cdot R_{cod}} \quad (6)$$

The response surfaces obtained by Eq. (6) allowed location of maxima in the range of investigated variables (Fig. 4). Critical values are showed in Table 4b.

Comparing the new critical values (calculated with Eq. (6)) (Table 4b) with those from Table 4a, it is observed that the main difference is that R1 has changed from 37.6 to 29.1°C/min (*i.e.*, in the established range of 12 and 36°C).

According to multivariate designs, the following optimized chromatographic conditions were found: temperature oven: (i) *injector*: 310°C, (ii) *injection mode*: splitless; (iii) *oven*: 70°C (2 min) → 200°C (30°C/min) → 200°C (5 min) → 300°C (5.0°C/min) → 300°C (1.67 min); (iv) *transfer line*: 280°C; v) *ion source*: 250°C; (vi) *electron impact energy*: 70 eV. Figure 5 shows a PAH standard chromatogram, acquired under optimized conditions which permitted excellent separation of the 16 PAH in a total analysis time of 33 min. Figure 6 depicts the chromatogram of a real sample of atmospheric aerosol which shows good resolution of all 16 PAHs with neither co-elutions nor interferences. It is worthwhile mentioning that the four PAHs pairs: PHE and ANT; BaA and CRY; BbF and

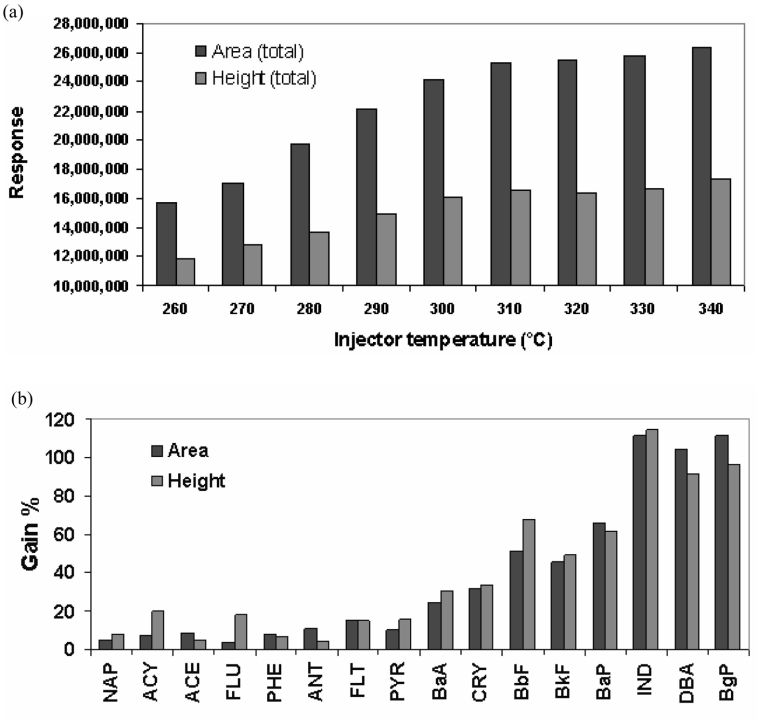

Figure 7. (a) Detector response as a function of injector temperature; (b) percent increment of detector response for each target PAH. Temperature range: 280 to 310°C.

BkF; IND and DBA, used as references in the multivariate design, were quite well separated (Fig. 6).

3.2 Injector temperature effect study

This study was carried out in nine experiments using a 5 μg/mL PAH analytical solution. Variations of the injector temperature were applied in the range between 260 and 340°C. Results are shown in the Fig. 7a as the sum of either peak area or peak height of the 16 PAH. An increase of the detector response was observed when the temperature was raised from 260 to 310°C. This represented a 61% and 39% rise in the peak area and peak height, respectively, of all PAH. Inside this interval a more pronounced signal increase for the heaviest PAH (thus with a low vapor pressure and a high molar mass) was noted, and this could be utilized as an advantage of this method in the analysis of particulate matter or other complex environmental matrices (Fig. 7b).

Since thermal decomposition of the PAHs is more likely to occur at higher temperatures and there was no significant gain in sensitivity above 310°C, this temperature was set as the critical value (optimum) for the following experiments.

3.3 Sampling time effect study

On using the splitless injection mode it is necessary to adjust the time interval between sample injection and flow splitting valve since this parameter influences the quantity of sample introduced into the column. This study was performed in the SIM mode using a 100 ng/mL PAH standard solution and 1.0 μL of sample. The time of valve opening ranged from 0.3 to 1.0 min and the detector response was evaluated as peak area or peak height of 16 PAH (Fig. 6). In this figure it is evident that there is a consistent detector response gain until 0.8 min. Thus 0.8 min was considered as the best sampling time (Fig. 8).

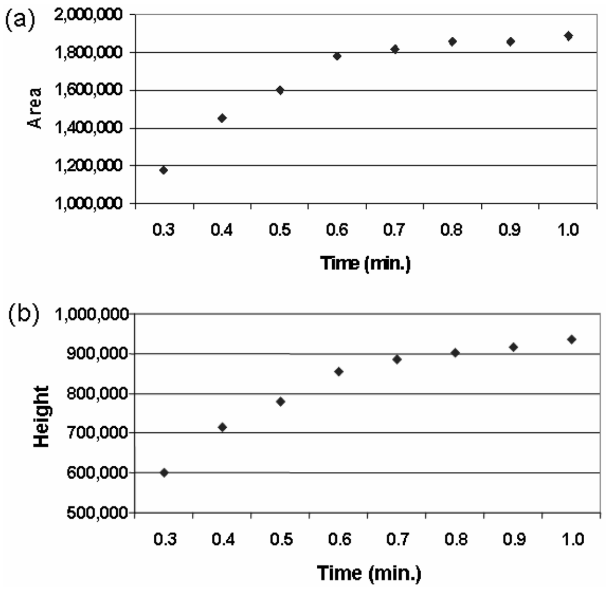

Figure 8. Detector response as a function of sampling time. (a) Summation of peak area; (b) summation of peak height.

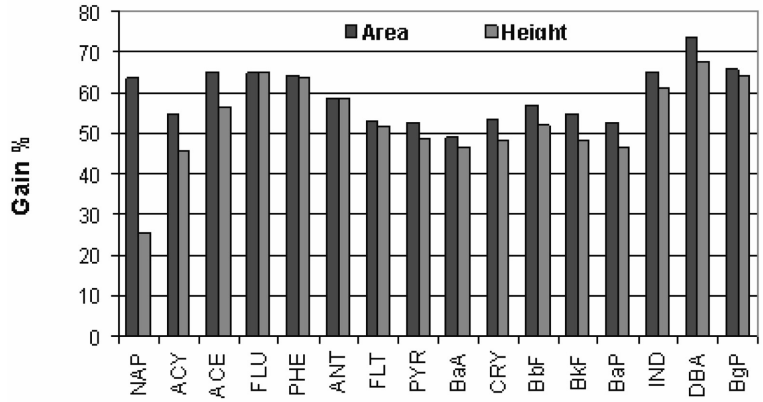

Figure 9. Detector response gain for each PAH as a function of sampling time of 0.8 min.

Table 5. Repetitivity of retention time.

Peak	PAH	t_R (min)*	*s*	CV%
1	Naphthalene	5.485	0.002	0.032
2	Acenaphthylene	6.918	0.001	0.019
3	Acenaphthene	7.095	0.001	0.016
4	Fluorene	7.703	0.001	0.017
5	Phenanthrene	9.410	0.002	0.022
6	Anthracene	9.534	0.002	0.024
7	Fluoranthene	13.627	0.003	0.024
8	Pyrene	14.583	0.003	0.019
9	Benzo[*a*]anthracene	20.299	0.002	0.012
10	Chrysene	20.453	0.003	0.014
11	Benzo[*b*]fluoranthene	25.101	0.003	0.012
12	Benzo[*k*]fluoranthene	25.220	0.002	0.009
13	Benzo[*a*]pyrene	26.370	0.003	0.012
14	Indeno[1,2,3-*c*,*d*]pyrene	30.508	0.003	0.009
15	Dibenz[*a*,*h*]anthracene	30.680	0.002	0.007
16	Benzo[*g*,*h*,*i*]perylene	31.338	0.003	0.009

* Refers to average of two standards, from 10 inter-day determinations.
t_R = retention time.
s = standard deviation.
CV = coefficient of variation.

The analysis of detector response for each PAH showed that there is an increment of 48.92% (CRY) to 73.61% (DBA) of PAH area and 25.48% (NAP) to 67.81% of PAH height (Fig. 9).

3.4 Repetitivity of detector response and retention time

Repetitivity of detector response was studied for both peak area and peak height, carried out in five inter-day replicates by injecting of 1.0 µL of each of the 10, 20, 40, and 100 ng/mL standard solutions. The obtained results showed coefficients of variation (CV%) between 0.28 and 7.55% for peak area and between 0.53 and 11.34% for peak height.

Repetitivity of retention time was evaluated in 10 inter-day replicates with 40 and 100 ng/mL standard solution of 16 PAH. Results are listed in Table 5.

Table 6. Correlation coefficient (r^2), LOD and LOQ for GC-MS-SIM.

Peak	PAH	Area			Height		
		LOD (ng/mL)	LOQ (ng/mL)	r^2	LOD (ng/mL)	LOQ (ng/mL)	r^2
1	NAP	0.19	0.58	0.9994	0.72	2.39	0.9893
2	ACY	0.22	0.67	0.9991	0.20	0.65	0.9992
3	ACE	0.30	0.90	0.9985	0.26	0.86	0.9986
4	FLU	0.13	0.38	0.9997	0.18	0.61	0.9993
5	PHE	0.13	0.39	0.9997	0.24	0.79	0.9988
6	ANT	0.13	0.38	0.9997	0.18	0.62	0.9993
7	FLT	0.14	0.44	0.9996	0.21	0.71	0.9991
8	PYR	0.17	0.51	0.9995	0.24	0.80	0.9988
9	BaA	0.14	0.43	0.9996	0.23	0.78	0.9988
10	CRY	0.16	0.49	0.9995	0.23	0.77	0.9989
11	BbF	0.19	0.59	0.9993	0.23	0.76	0.9989
12	BkF	0.20	0.61	0.9993	0.21	0.69	0.9991
13	BaP	0.19	0.58	0.9994	0.26	0.86	0.9986
14	IND	0.34	1.04	0.9980	0.40	1.35	0.9966
15	DBA	0.33	1.00	0.9981	0.46	1.52	0.9957
16	BgP	0.19	0.75	0.9989	0.31	1.04	0.9980

3.5 Limits of detection (LOD) and limits of quantification (LOQ)

The LOD and LOQ determinations were performed as already described in the experimental section. Results are compiled in Table 6.

LOD were 0.13 to 0.34 ng/mL and 0.18 to 0.72 ng/mL for peak area and peak height, respectively. LOQ were calculated to be 0.38 to 1.04 ng/mL for peak area and 0.61 to 2.39 ng/mL for peak height. These findings are satisfactory and consistent with those found in the literature.

4 Concluding remarks

The multivariate design was adequate to assure the separation of the 16 US-EPA priority PAHs with the best resolution in the shortest analysis time, reducing the total chromatographic run from 45 – 60 to 33 min. The method has also been shown to be appropriate for the analysis of environmental samples and other matrices.

The authors wish to thank Brazilian agencies who have funded this work: CNPq, FAPESB, CAPES, FINEP, ANEEL, PRONEX, and RECOMBIO.

The authors declared no conflict of interest.

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