ELSEVIER

Journal of Chromatography A, 976 (2002) 301–307

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of *N*-nitrosamines in latex by sequential supercritical fluid extraction and derivatization

F. Reche[a], M.C. Garrigós[a], M.L. Marín[b], A. Jiménez*[,a]

[a]*Analytical Chemistry Department, University of Alicante, P.O. Box 99, 03080 Alicante, Spain*

[b]*Institute of Toys Technology (AIJU), Av. Industria 23, 03440, Ibi, Alicante, Spain*

Abstract

A new method to determine *N*-nitrosamines in latex products has been developed by combination of supercritical fluids and chemical derivatization. A new design for a liquid trap has been introduced. A factorial fractional design was used in order to evaluate the influence of the different factors affecting the process. Factors such as pressure, temperature, static and dynamic time, restrictor temperature and volume of an hydrobromic acid–acetic anhydride mixture (1:10, v/v) were included in the design. CO_2 was used as the extraction fluid. Gas chromatography with nitrogen and phosphorus sensitive detection was employed to achieve good sensitivity attending to the molecular structure of these compounds (*N*-nitrosamines and their corresponding secondary amines). The obtained results have shown to be useful to increase selectivity and reduce sample handling.

Keywords: Latex; Supercritical fluid extraction; Factorial design; Derivatization, GC; Nitrosamines

1. Introduction

The use of *N*-nitrosamines precursors as preservatives in latex products for children, such as teethers or pacifiers, has been usual in the last decades. But, even traditional studies have noted the mutagenicity and carcinogenic effects of most of the *N*-nitrosamines [1]. The toxicity of these compounds can be observed even at low levels (μg/kg) [2]. *N*-Nitrosamines have been determined in a wide variety of samples, such as drinking water [4], drug formulations [5,6], foods [3,7–10], tobacco [11–14] or rubber products [15–19], where these compounds appear when alkylamines derivatives are used as accelerators and stabilizers in the vulcanization process [18]. Due to the high toxicity of *N*-nitrosamines and the complexity of these matrices, the search of reliable analytical methods with minimal sample handling is advisable. Confirmation of results by several means as GC–MS or UV decomposition is normally required [20].

Supercritical fluid extraction (SFE) has been successfully used to determine a lot of different analytes, such as semivolatile compounds [21], polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from environmental samples [22] phthalates in poly(vinyl chloride) (PVC) [23] and aromatic amines in finger-paints [24]. The use of SFE in removing *N*-nitrosamines from different samples including food [8–10], tobacco [12–14], and others has been reported. SFE combines several advantages as minimizing sample

*Corresponding author. Tel.: +34-96-590-3777; fax: +34-96-590-3781.

E-mail address: alfjimenez@ua.es (A. Jiménez).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01157-3

handling, providing fairly clean extracts, expediting sample preparation and reducing the use of environmentally toxic solvents [25,26]. Moreover, supercritical fluids offer the possibility of developing chemical derivatization under controlled conditions of pressure and temperature. The supercritical fluid derivatization and extraction (SFDE) is not employed for a high number of applications, but some work in organometallics from sediments and soils [21], caffeine in coffee beans [22], phenol in wood soot leachate [21], alkylbenzenesulfonates in wastewater sludge [24] or formaldehyde in finger-paints [27] has been reported in literature.

This study has two main aims. The first one is reducing sample handling and consequently the risk of exposure for the analyst in the chemical laboratory, and the second one is making easier the identification and determination of the *N*-nitrosamines between the large number of coextracted compounds with minimum experimental efforts. It is well known that *N*-nitrosamines can be denitrosated to secondary amines in the presence of hydrobromic acid and acetic anhydride [30–32]. The application of this reaction in supercritical conditions forms the basic goal of this work.

2. Experimental

2.1. Materials and chemicals

Four *N*-nitrosamines were chosen among those normally found in latex products, two of them aliphatic [*N*-nitrosodimethylamine (NDMA)] and *N*-nitrosodiethylamine (NDEA)] and two alicyclic [*N*-nitrosopyrrolidine (NPYR) and *N*-nitrosopiperidine (NPIP)]. All *N*-nitrosamines and the corresponding secondary amines were obtained from Sigma (St. Louis, MO, USA). Stock solutions (3000 ppm) of each compound were prepared in methylene chloride (analytical grade) purchased from Normapur (Prolabo, Barcelona, Spain). Laboratory made probes were prepared using polyisoprene (Revultex® Lan 960) as polymer matrix and a solution of cyclohexylamine in ethanol as coagulant, both provided by a toy producer, Nuky Baby (Alicante, Spain). The probes were prepared by pouring alternating thin layers of the liquid polymer and the coagulant solution into a 6-cm diameter aluminium vessel. After the coagulant solution layer, the adequate quantity of the stock solution of each *N*-nitrosamine was added. This procedure was repeated twice, finishing with a liquid polymer and coagulant solution layer. Finally, the probes were cured at 60 °C for 7 h, resulting in a final concentration of 100 ppm for each analyte. Then, the probes were cut in small pieces (2×2 mm) to be used later.

A 1:10 (v/v) mixture of hydrobromic acid (48%, Sigma) and acetic anhydride (97%, Panreac Química, Barcelona, Spain) were used to prepare the denitrosation reagent [30].

2.2. Supercritical fluid extraction

SFDE was performed (off-line mode) using an ISCO Model SFX-220 extraction system (ISCO, Lincoln, NE, USA) consisting of an SFX-220 extractor, a SFX-200 controller and a 100DX-syringe pump. Supercritical grade CO_2 was obtained from Abelló Linde (Valencia, Spain). A total of 0.10 g of each probe was introduced in a stainless steel cartridge (internal volume, 2.5 ml). The denitrosation

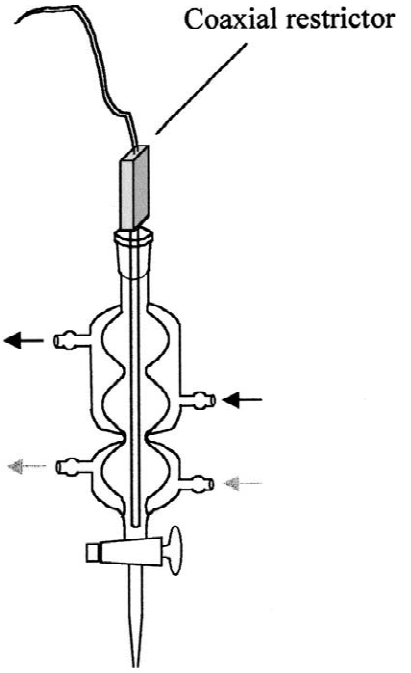

Fig. 1. Scheme of the liquid trap for collection in off-line SFDE.

reagent was added directly to the cartridge before extraction, with a small amount of quartz wool, which helps to minimize the dead volume of the cartridge. The collection system was a capillary restrictor coaxially heated. The outlet of the restrictor was introduced in a liquid trap consisting of a piece of glass with three bulbous sections (approximately 10 ml each) surrounded by two separate temperature jackets, one of them for the bulb on the base and the other one for the two superior bulbs. The selection of the length of this piece was according to the length of the coaxial restrictor employed with the supercritical fluid (approximately 15 cm). The collection device has a female clear-seal joint and a burette stopcock and tip (see Fig. 1). All extractions were carried out in the static/dynamic mode, with the use of the selected static and dynamic extraction times while the upper jacket was cooled with an ethylene glycol–water (1:1) mixture at −10 °C in order to avoid the loss of analytes. Finally, the extract was transferred into a 10-ml volumetric flask. The solvent used was methylene chloride.

2.3. Gas chromatography

Analysis of extracts was carried out by using a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) equipped with a Shimadzu AOC-20i autoinjector, a TR-WAX.DB capillary column (60 m× 0.25 mm I.D. and 0.2 μm film thickness) (Teknokroma, Barcelona, Spain), a split–splitless injector and a nitrogen–phosphorus detection (NPD) system. Helium was used as the carrier gas, with a linear velocity of 19 cm/s and a head pressure of 125 kPa. The injector temperature was 225 °C and the detector temperature was 300 °C with a 50 pA current. Column temperature was programmed in four steps from 80 °C (hold 2 min) to 155 °C (hold 0 min) at 5 °C/min, to 175 °C (hold 0 min) at 20 °C/min, to 195 °C (hold 0 min) at 3 °C/min, to 215 °C (hold 18 min) at 20 °C/min. A 2-μl sample was injected in the splitless mode (1.5 min splitless time). Quantification of each *N*-nitrosamine and secondary amine was performed by comparing their chromatographic peak areas for sample extracts with those of standards in the same concentration range.

2.4. Design of experiments

A fractional factorial design 2^{6-3} for the fortified probes was carried out to distinguish the significant factors affecting the supercritical process. All statistical calculations were developed with Statgraphics Plus for Windows v. 4.0 (Statistical Graphics, Rockville, MD, USA).

3. Results and discussion

The use of conventional SFE traps was difficult for the extraction of *N*-nitrosamines from latex samples and recoveries were non-acceptable, as they were too low. Therefore, the development of a new liquid trap with several requirements was necessary in order to enhance extraction recoveries. First of all, an efficient refrigeration device should be coupled to the selected trap in order to avoid the throwing of these toxic compounds to the atmosphere. In addition, the efficiency of the trap can be increased by using bulbous sections to permit the movement of the solvent when the supercritical fluid is decompressed along the restrictor and, additionally increasing the contact between CO_2 with analytes and the collection solvent in the narrowing between bulbs. Moreover, a second jacket could permit to heat the collection solvent in order to increase its solvent power or to develop secondary reactions. Finally, a

Table 1
Codification of factors

Levels	*P* (MPa)	*T* (°C)	*S* (min)	*D* (min)	*R* (°C)	*H* (μl)
−2	13.8	50	2	2	150	0
−1	24.1	68	6.5	6.5	170	25
0	34.5	85	11	11	190	50
1	44.8	102	15.5	15.5	210	75
2	55.2	120	20	20	225	100

Table 2
List of experiments in the fractional factorial design

Experiment	*P*	*T*	*S*	*D*	*R*	*H*
FFD	−1	−1	−1	1	1	1
FFD-2	1	−1	−1	−l	−1	1
FFD-3	−1	1	−1	−l	1	−1
FFD-4	1	1	−1	1	−1	−1
FFD-5	−1	−1	1	1	−1	−1
FFD-6	1	−1	1	−1	1	−l
FFD-7	−1	1	1	−1	−1	1
FFD-8	1	1	1	1	1	1
FFD-Central	0	0	0	0	0	0

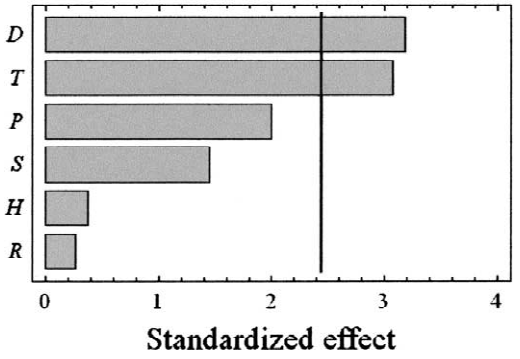

Fig. 2. Pareto chart for $Y = (\%W)^{1.18}$.

female clear-seal joint and the burette stopcock and tip was added to permit liquid–liquid extractions.

Six factors were considered in the study of SFDE for *N*-nitrosamines in latex products, i.e., CO_2 pressure (*P*), extraction temperature (*T*), static and dynamic extraction times (*S* and *D*), restrictor temperature (*R*) and volume of denitrosation reagent (*H*). In order to separate each factor of the measure unit, the low and high values for each one were selected according to the experimental limitations and codified to be −2 and +2 from the centre of the design (0 for each factor). Table 1 list the values of each factor and their corresponding codified value. The effect of the six factors considered was studied by a fractional factorial design with two levels (−1 and +1). This design requires eight experiments, performed randomized. It is assumed that the main factors are those significant to the process [28,29]. An extra experiment (five replicates) was included to have a rough estimation of the standard error and the response in the centre of the design. The evaluated responses were mass loss (% *W*), which includes all those coextracted components and peak area (*A*) for each secondary amine. The conditions of SFDE in this design are presented in Table 2.

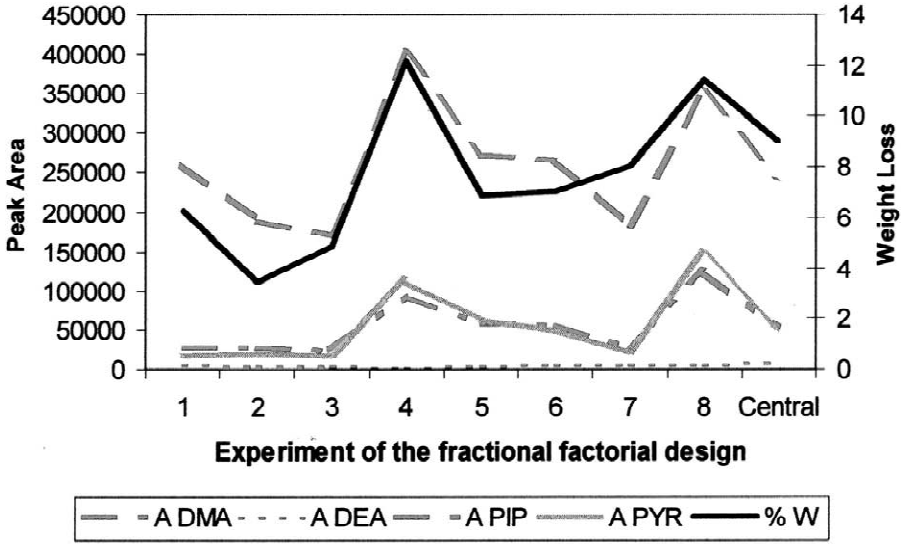

Fig. 3. Results of the fractional factorial design.

In order to stabilize the variance of results, an appropriate power transformation ($Y = y^{\lambda}$) of either response ($y = \%W$ or $y = A$) was carried out before the analysis of results. A suitable transformation of the response is recommended when large differences between the values of response are observed [29]. The best transformation is achieved when the sum of squares of residuals is the lowest as a function of the exponent X. The optimum transformation for mass loss ($Y = (\%W)^{\lambda}$) was obtained for $\lambda = 1.18$. The significant factors were identified by using Pareto charts. Fig. 2 shows the Pareto chart for $Y =$

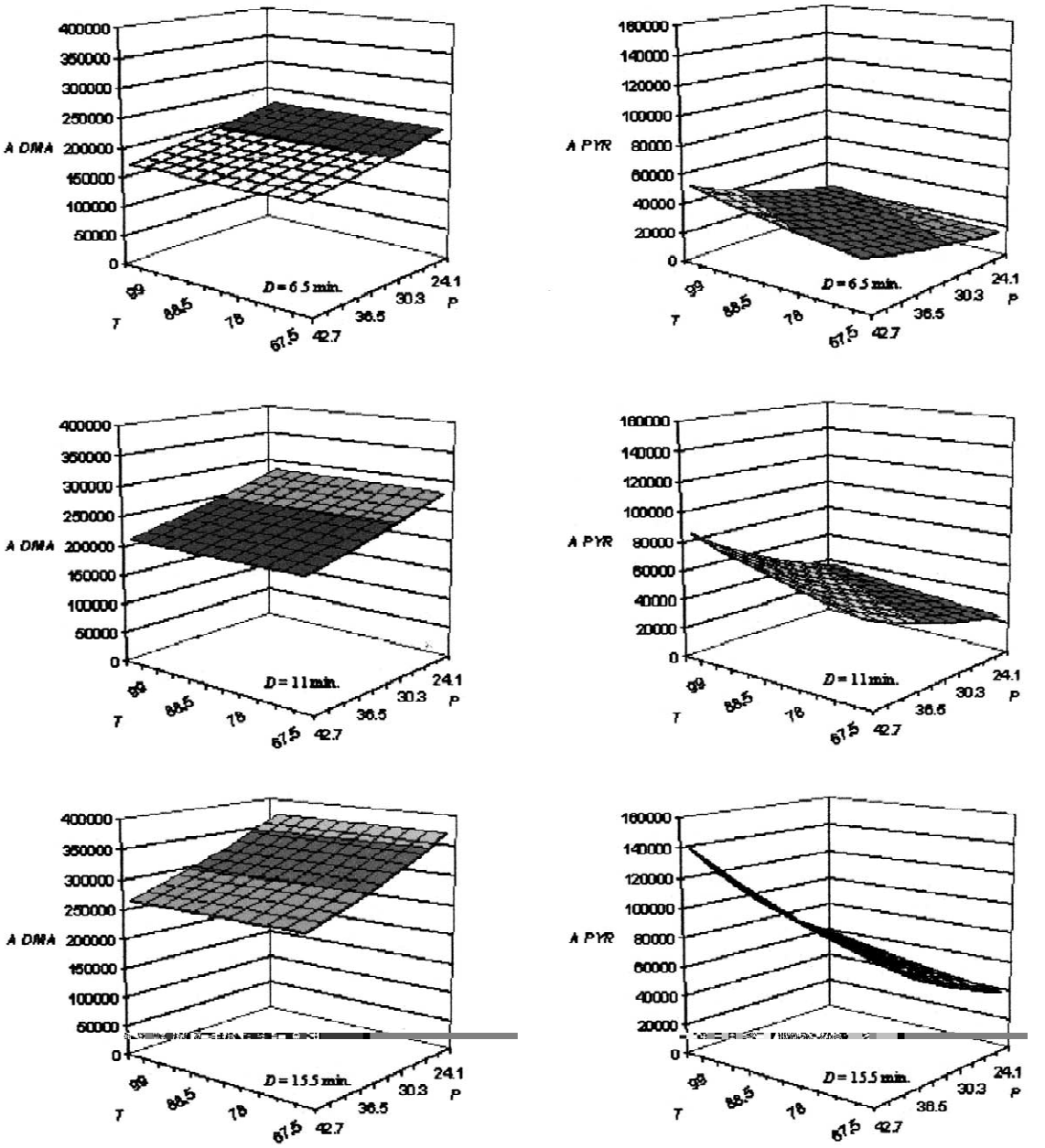

Fig. 4. Example of response surfaces for alicyclic and cyclic secondary amines.

$(\%W)^{1.18}$ showing two significant factors, *D* and *T*, with positive effects, meaning %*W* can be improved by an increase of these factors, although this conclusion should be taken with caution because interactions between factors are not considered in this fractional factorial design. On the other hand, Pareto charts for peak area of the secondary amines showed differences in the significant factors, making difficult a global view of results. According to Fig. 3, a similar behaviour between peak area and mass loss for each secondary amine can be observed, except for DEA that presents a large decrease in sensitivity. This trend led to choose *T* and *D* for special analysis. Moreover, *P* was included because, due to the generators selected for define the design ($+PT$, $+PS$ and $+TS$), the main factor *P* is confounded with the *TD* and *SR* interactions. The calculation of the theoretical responses according to the model assumed for each secondary amine was carried out by maintaining those factors as significant. For alicyclic and cyclic amines, two different types of response surfaces were observed, one example being presented in Fig. 4. In the case of alicyclic amines *T* has a low influence and *P* has an important negative effect increased when *D* is higher. However, cyclic amines increase their response when *P* and *T* are higher and this effect is reinforced for a high value of *D*. This trend is similar to the behaviour of %*W*. An intermediate option should be selected when several *N*-nitrosamines are present in the sample. According to Fig. 3, conditions of the experiment 4 ($P = 44.8$ MPa, $T = 102$ °C, $S = 6.5$ min, $D = 15.5$ min, $R = 170$ °C and $H = 25$ μl) can give good results.

A complete explanation of the opposite behaviour for alicyclic and cyclic *N*-nitrosamines is not evident because several considerations should be taken into account. Due to the polarity of *N*-nitrosamines and the corresponding secondary amines, equilibrium should occur between the time *N*-nitrosamines are put in contact with the supercritical fluid, and the adequate extraction conditions for the secondary amines formed into the supercritical fluid extractor.

4. Conclusions

Derivatization in supercritical conditions of *N*-nitrosamines proposed as the main goal of this work is possible. The addition of a denitrosation reagent into the extractor combined with an adequate liquid trap permits to elucidate the presence of *N*-nitrosamines in latex samples as their potential precursors. It also permits the analysis with no manipulation of analytes and a considerably reduction in time, avoiding sources of error and a prolonged contact with these toxic compounds. SFE is a extraction technique that permits the reduction in solvent waste and provides clean extracts, therefore the detection with G–NPD can be used practically without interferences.

On the other hand, the performance of this new liquid trap can permit the development of coupled derivatization reactions without any transfer at the necessary temperature and minimizing the losses of analytes thanks to the use of the most convenient refrigeration.

References

[1] P.N. Magee, J. Barnes, J. Cancer 10 (1956) 114.
[2] R.E. Kirk, D.F. Othmer, in: Encyclopedia of Chemical Technology, Wiley, New York, 1978.
[3] A.R. Tricker, R. Preussmann, Mutat. Res. 259 (1991) 277.
[4] W.I. Komoto, C.J. Dooley, W. Fiddler, Water Res. 15 (1981) 1099.
[5] B.A. Dawsoh, R. C Lawrence, J. Assoc. Off. Anal. Chem. 70 (1987) 554.
[6] L.B. Niclsen, S. Lings, Med. Hypoth. 42 (1994) 265.
[7] K. Takatsuki, T. Kikuchi, J. Chromatogr. 508 (1990) 357.
[8] R.J. Maxwell, J.W. Pensabene, W. Fiddler, J. Chromatogr. 31 (1993) 212.
[9] J.W. Pensabene, W. Fiddler, R.J. Maxwell, A.R. Lightfield, J.W. Hampson, J. AOAC Int. 78 (1995) 744.
[10] W. Fiddler, J.W. Pensabene, J. AOAC Int. 79 (1996) 895.
[11] C. Ruhl, J.D. Adams, D. Hoffman, J. Anal. Toxicol. 4 (1980) 255.
[12] B. Prokopczyk, D. Hoffmann, J.E. Cox, V. Djordjevic, K.D. Brunnemann, Chem. Res. Toxicol. 5 (1992) 336.
[13] B. Prokopczyk, M. Wu, J.E. Cox, S. Amin, D. Desai, A.M. Idris, D. Hoffmann, J. Agric. Food Chem. 43 (1995) 916.
[14] S. Song, D.L. Ashley, Anal. Chem. 71 (1999) 1303.
[15] J.I. Gray, M.A. Stachiw, J. Assoc. Off. Anal. Chem. 70 (1987) 64.
[16] B.G. Österdahl, Food. Chem. Toxicol. 21 (1983) 755.
[17] S.M. Billedean, H.C. Thompson, B.J. Miller, M.L. Wind, J. Assoc. Off Anal. Chem. 69 (1986) 31.
[18] H.C. Thompson, S.M. Billedeau, B.J. Miller, E.B. Hansen, J.P. Freeman, M.L. Wind, J. Toxicol. Environ. Health 13 (1984) 615.

[19] N.P. Sen, S.W. Seaman, S.C. Kushwaha, J. Chromatogr. 463 (1989) 419.
[20] CEN/Technical Committee 252, European Standard EN 12868, Child Use and Care Articles–Methods for Determining the Release of *N*-Nitrosamines and *N*-Nitrosables Substances from Elastomer or Rubber Teats and Soothers, September 1999.
[21] J.J. Langenfeld, M.D. Buford, S.B. Hawthorne, D.J. Miller, J. Chromatogr. 594 (1992) 297.
[22] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 66 (1994) 909.
[23] M.L. Marín, A. Jiménez, J. López, J. Vilaplana, J. Chromatogr. A 750 (1996) 183.
[24] M.C. Garrigós, F. Reche, K. Pemías, A. Sánchez, A. Jiménez, J. Chromatogr. A 819 (1998) 259.
[25] L.T. Taylor, Introduction to Supercritical Fluid Extraction, R&D Magazine, USA, 1995.
[26] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A.
[27] F. Reche, M.C. Garrigós, A. Sánchez, A. Jiménez, J. Chromatogr. A 896 (2000) 51.
[28] D.C. Montgomery, in: Design and Analysis of Experiments, Wiley, New York, 1976.
[29] G.E.P. Box, N.R. Draper, in: Empirical Model-Building and Response Surfaces, Wiley, New York, 1987.
[30] M. Zheng, C. Fu, H. Xu, Analyst 118 (1993) 269.
[31] H. Kataoka, S. Shindoh, M. Makita, J. Chromatogr. A 723 (1996) 93.
[32] L. Cárdenes, I.H. Ayala, V. Gonzalez, A.M. Afonso, J. Chromatogr. A 946 (2002) 133.