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Use of solid-phase microextraction coupled with gas chromatography for the determination of residual solvents in pharmaceutical products

Stéphanie Legrand, José Dugay, Jérôme Vial*

Laboratoire Environnement et Chimie Analytique, UMR 7121, Ecole Supérieure de Physique et Chimie Industrielles de la Ville de Paris, 10 Rue Vauquelin, 75005 Paris, France

Abstract

The aim of this work was to prove that solid-phase microextraction coupled with gas chromatography could be used for the determination and quantification of residual solvents in drugs. Four solvents were selected for the experiments: ethanol, cyclohexane, triethylamine and pyridine, together with a model powdered drug substance. Several kinds of fibers, together with the extraction mode, were evaluated to determine the most appropriate one for the simultaneous extraction of the four solvents. The most promising conditions were obtained with the Carboxen-polydimethylsiloxane fiber in the headspace of the aqueous solution that contained the dissolved powder. A concentrated phosphate buffer was added to the aqueous solution to set the pH at 9.6 in order to enable good extraction of triethylamine, and the optimum extraction time was experimentally determined. A multi-criteria optimization was also carried out by means of design of experiments to optimize remaining parameters: the extraction temperature was set at 40 °C, the ionic strength at 1.77 mol l^{-1} and the volume of the aqueous solution at 7.2 ml. The method of standard additions was used for quantitative analysis. Its performance was evaluated and validated: the pooled RSD was around 15%, the limits of detection were all of the ppb level and the method was both accurate and linear.

Keywords: Solid-phase microextraction; Headspace analysis; Pharmaceutical analysis; Experimental design; Ethanol; Cyclohexane; Triethylamine; Pyridine

1. Introduction

Solid-phase microextraction (SPME) was introduced by Pawliszyn and co-workers [1,2] and represented an advance as a solvent-free alternative for the extraction of organic compounds from water samples [3–6]. In SPME, the analytes are extracted into a stationary phase attached to a length of fused-silica fiber. Though exhaustive extraction does not occur, an equilibrium is established, as analytes partition between the stationary phase and the aqueous phase or its headspace phase [7]. SPME has been applied for the extraction of a wide range of organic compounds from various matrices [8–12]. In the pharmaceutical industry, it is a major concern to detect and quantify residual solvents in drug substances or drug products, because some of them can be highly toxic even at trace levels. The most commonly used technique for the analysis of residual solvents is conventional gas chromatography (GC). To achieve acceptable detectability, large amounts of

*Corresponding author.
E-mail address: jerome.vial@espci.fr (J. Vial).

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the product need to be injected, which generally leads to clogging of the injector and requires frequent changes of the insert. Headspace injection is an alternative technique, but is rather limited in terms of optimization possibilities with respect to its selectivity. Consequently, as suggested by recent articles [13–17], we tried to use SPME–GC to determine and quantify residual solvents in drugs. The originality of our work lies in the comprehensive approach we chose for developing and optimizing the extraction method, from the choice of the fiber to the quantitative determination of the amounts of solvents. Experimentally, a model drug powder soluble in water was chosen and residues of four solvents, ethanol, cyclohexane, triethylamine and pyridine were investigated.

2. Experimental

2.1. Products and reagents

The model pharmaceutical product, denoted drug A, was a powder easily soluble in water, sampled from a batch of industrial production. The solution obtained after dissolution was weakly acidic. Because of confidentiality it is not possible to give further information about this product. The four residual solvents investigated were all involved in the synthesis of drug A. Ethanol was the purification/crystallization solvent, cyclohexane was used to denature ethanol, triethylamine was a synthesis reactant and pyridine was the extracting solvent. Pure standards of these four residual solvents were purchased from Sigma–Aldrich (L'isle d'Abeau Chesne, France) and were all of analytical grade. Deionized water was obtained from a Milli-Q water system (Millipore, St. Quentin-en-Yvelines, France). Disodium hydrogenphosphate was used to adjust the pH and sodium chloride (purity >99.5%) was purchased from VWR International (Fontenay-Sous-Bois, France).

2.2. SPME fiber

Five kinds of SPME fibers, purchased from Supelco (St. Quentin Fallavier, France) were evaluated: polydimethylsiloxane (PDMS) 100 μm, polydimethylsiloxane–divinylbenzene (PDMS–DVB) 65 μm, carbowax-divinylbenzene (CW-DVB) 65 μm, polyacrylate (PA) 85 μm and carboxen-polydimethylsiloxane (CAR-PDMS) 75 μm. All fibers were conditioned in the hot injector port of the gas chromatograph according to instructions provided by the supplier. To make a comparison possible between the different fibers, standard conditions were used to carry out the experiments. The mass of drug A was 100 mg, extraction vial volume was 11 ml (when drug A was dissolved, the water volume was 9 ml), extraction time was 20 min, extraction temperature was 60 °C, desorption time was 5 min and the desorption temperature was 300 °C. No significant fiber carry-over was observed thanks to both the high volatility of the solvents analyzed and the long desorption time.

2.3. Instrumentation and GC method

Analyses were carried out on a Varian 3800 CX system connected to Varian 8200 CX AutoSampler for SPME (Varian, Les Ulis, France). The chromatograph was equipped with a 1078 split/splitless injector and a flame ionization detection (FID) system. The injector temperature was set at 300 °C. It worked in splitless mode for 1 min and then a split ratio of 40 was applied for the rest of the analysis. Detector temperature was set at 270 °C, make-up flow-rate at 25 ml min^{-1}, hydrogen flow at 30 ml min^{-1} and air flow at 300 ml min^{-1}. Helium was used as carrier gas at 1.5 ml min^{-1}. Analytes were separated using a CP-Select 624 CB column (Chrompack, Les Ulis, France), 30 m×0.25 mm ID, with a phase thickness of 1.8 μm, using the following temperature program: 40 °C held for 3 min, then increase by 3 °C min^{-1} to 100 °C, finally increase by 20 °C min^{-1} to 250 °C and hold for 5 min.

3. Results and discussion

The GC method was considered optimized. All our efforts were focused on the development and optimization of the extraction process by SPME.

3.1. Fiber and mode

The first step consisted of choosing the best fiber/extraction mode combination. Three different modes were possible. In the direct mode, the fiber was dipped in an aqueous solution containing the dissolved powder. In the headspace-over-the-liquid mode, the fiber was placed in the headspace above the aqueous solution that contained the dissolved powder. In the headspace-over-the-powder mode, the fiber was placed in the headspace above the powder. Comparison between the results obtained with the 15 fiber/extraction mode combinations was carried out under conditions detailed in the Experimental section. Results are given in Fig. 1.

Extraction efficiencies with the first three fibers (PA, CW-DVB and PDMS) were globally lower than with the other fibers, regardless of the mode used. The PDMS–DVB fiber gave better extraction efficiencies in all three modes but still two to three times less than the CAR-PDMS fiber. With the CAR-PDMS fiber, the amount of solvent extracted was maximum in the headspace-over-the-liquid mode. However, triethylamine was not detected at all, unlike in the headspace-over-the-powder mode. In fact, because of its basic properties, triethylamine was poorly extracted from the water solution. When 100 mg of drug A were diluted in 9 ml of water, the measured pH value was around 5. At this pH, triethylamine (with a pK_a of 9.6) was nearly totally protonated. Consequently its volatility was negligible, which explained why this solvent was not extracted by the fiber. To overcome this problem, the pH of the sample solution was set at basic values by the addition of 0.02 mol l^{-1} Na_2HPO_4 and concentrated sodium hydroxide. Two experiments were carried out at pH 9.6 and pH 12. At pH 9.6, triethylamine was half protonated, half neutral, while at pH 12 the neutral form was predominant (results are presented in Fig. 2). As expected, a higher pH led to improved extraction of triethylamine. At pH 12, triethylamine extraction was so strong that other solvents, especially ethanol and cyclohexane, were less extracted because of the competitive sorption of triethylamine on the fiber. Finally, a pH value of 9.6 was chosen as the best compromise for a ''balanced'' extraction of all solvents.

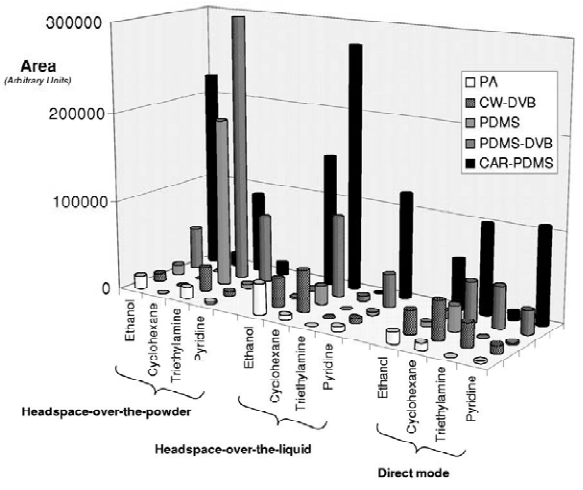

Fig. 1. Comparison of the five fibers evaluated in each of the three modes.

3.2. Extraction time

Extraction isotherms were obtained for the four solvents to find the optimal extraction time using the CAR-PDMS fiber and the headspace-over-the-liquid mode, with a pH adjusted to 9.6. All other conditions were the same as those described in the Experimental section (results are given in Fig. 3). After 10 min, the curves for cyclohexane and ethanol reached a plateau. For triethylamine, the plateau was reached after 20 min. However, for pyridine, the signal was increasing even after 60 min, indicating that equilibrium was not reached. Consequently, 30 min was chosen as a compromise because pyridine extraction was sufficient. However, special care must be taken

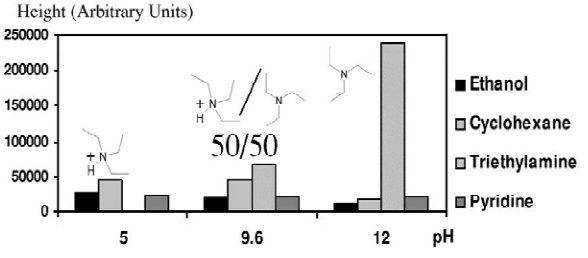

Fig. 2. Influence of the pH of the dissolution solution in the headspace-over-the-liquid mode on the CAR-PDMS fiber [triethylamine = $N(C_2H_5)_3$].

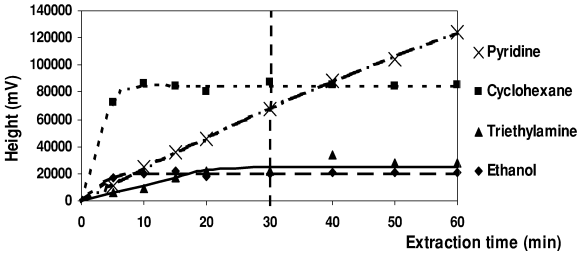

Fig. 3. Extraction isotherms for the four solvents in the headspace-over-the-liquid mode (pH 9.6) on the CAR-PDMS fiber.

to respect the timing to avoid any variability in the amount of pyridine extracted.

3.3. Final optimization by a 2^3 experimental design

The final aim of this work was to get a ''balanced'' chromatogram with approximately all the peaks of the same height. It meant that rather than a selective extraction for a specific solvent, a simultaneous and balanced extraction of all four solvents was sought. Peak heights were preferred to peak areas because another study showed that reproducibility was better with peak heights in the low concentration range [18]. After the choice of the fiber, the mode and the extraction time, the remaining parameters were optimized by experimental design [19,20]. The influence of three factors was studied:

- The temperature of the vials during extraction, denoted temperature.
- The salt effect controlled by the concentration of sodium chloride added to the aqueous solution, denoted [NaCl].
- The volume of water in the vial used to dissolve 100 mg of drug A, denoted volume.

Other parameters remained fixed at values determined previously. The experimental design chosen was a repeated two-level, full-factorial design with five center points. Center points and repetitions were used to estimate the repeatability of the experiments and to determine the significance of the calculated effects. Repetitions were true ones, meaning that they corresponded to the complete analysis cycle and not only to repeated injections of the same preparation. Experimental levels for the factors are given in Table 1.

Table 1
Values of the factors used for the experimental design

Level	Low	Center	High
Temperature	40 °C	50 °C	60 °C
[NaCl]	0	2 mol l^{-1}	4 mol l^{-1}
Volume	5 ml	7 ml	9 ml

Experiments were carried out in a randomized order. The four responses measured were the heights of the peaks of the four solvents. Both principal effects and interactions were evaluated. To judge if the model was adapted, the lack-of-fit test was applied: for all responses no significant lack-of-fit was noticed. The relative standard deviations for peak height were 14.6% for ethanol, 12.4% for cyclohexane, 9.0% for triethylamine and 8.5% for pyridine. Calculated effects, along with their significance, expressed as the probability to get a value higher than that observed, are given in Table 2. X_1 represents the factor Temperature, X_2 the factor [NaCl], X_3 the factor Volume and X_iX_j represents the interaction between X_i and X_j.

A positive value indicated that an increase in the factor or interaction level produced an increase in the considered peak height and vice versa. For ethanol and cyclohexane, only main effects were significant while for triethylamine and pyridine first order interactions were also significant and their intensity could not be neglected compared to those of main effects, indicating that the choice of a full factorial design rather than a saturated design was justified a posteriori. Interestingly, the signs of effects varied depending on the solvent considered, e.g. [NaCl] had a positive effect for ethanol, triethylamine and pyridine, but a negative effect for cyclohexane. This phenomenon illustrated the complexity of the mechanisms involved in headspace SPME, mechanisms governed by distribution constants of several compounds between different phases. Consequently, finding an optimum required more modeling. For that, we defined a response function, $F = \Sigma_{solvents}(H_i - \overline{H})^2$, minimum of which corresponded to the criterion of a balanced chromatogram where H_i represents the height of the peak of solvent i and $\overline{H}$

Table 2
Significant factors and interactions

Parameter	Ethanol peak height		Cyclohexane peak height		Triethylamine peak height		Pyridine peak height	
	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|
Temperature (X_1)	−1810	34.1%	**−5140**	**1.1%**	**46 062**	**<0.1%**	**43 349**	**<0.1%**
[NaCl] (X_2)	**21 931**	**<0.1%**	**−9658**	**<0.1%**	**80 021**	**<0.1%**	**50 810**	**<0.1%**
Volume (X_3)	**−11 436**	**<0.1%**	**5736**	**0.6%**	**−11 452**	**<0.1%**	**−32191**	**<0.1%**
X_1X_2	−2676	16.8%	−1264	47.8%	**24 647**	**<0.1%**	**15 451**	**<0.1%**
X_1X_3	146	93.8%	319	85.7%	**−7822**	**1.2%**	**−13 569**	**<0.1%**
X_2X_3	−2309	23.0%	1963	27.7%	**−7084**	**2.0%**	**−9959**	**0.2%**
$X_1X_2X_3$	1691	37.3%	1180	50.8%	−1886	49.2%	−2357	36.0%

Significant values (α set at five chances out of 100) are given in bold.

represents the average height. To minimize *F*, a numerical method (the solver function of Excel) was used with the constraint of remaining inside the limits of the experimental domain. Optimal conditions were reached when Temperature was set at 40 °C, [NaCl] at 1.77 mol l^{-1} and Volume at 7.2 ml. These conditions were validated by an additional experiment carried out under these conditions. As can be seen in Fig. 4, conditions were satisfactory and could be used to select the method of quantification.

3.4. Quantitative analysis

Several approaches were possible to quantify the amount of the four solvents. The simplest one, external standard, was known to provide poor results in SPME when a real matrix was used [21]. Consequently, two methods, standard additions [22] and successive extractions, were considered to cope with the matrix effects. The method of successive extractions, calculating the initial quantity of product from the results of a few consecutive extractions by a mathematical model, was applied to the four residual solvents. Nine successive extraction experiments were carried out, yielding the Ln(H_j) versus the injection number lines shown in Fig. 5. This method of quantification could not be applied, except for cyclohexane, because for the three other solvents the fraction taken at each extraction was too low.

The method of standard additions was then considered. Negligible volumes (that did not include significant dilution) of pure solvents were added to aqueous solutions containing 100 mg of drug A. Samples were then analyzed by the SPME–GC method under the optimal conditions using a single extraction. The performance of the standard addition method was evaluated using basic validation requirements. Selectivity of the method was satisfactory. As shown in Fig. 4, the peaks of all four solvents were fully resolved and no interfering peak was detected. Precision was calculated from the intercept of the confidence curves [23] of the standard additions regression line with the *x*-axis. This approach was a bit more complicated than the one based on repeated determinations, but yielded more realistic results.

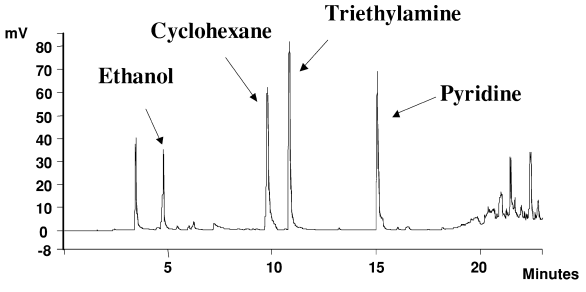

Fig. 4. Chromatogram obtained under the optimal conditions.

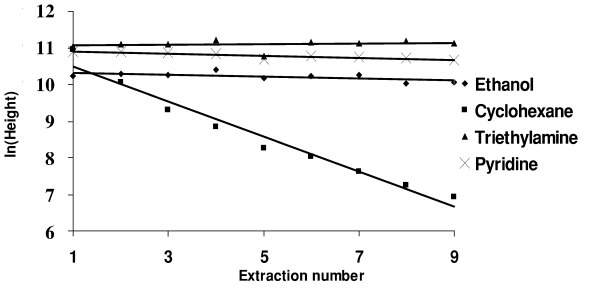

Fig. 5. Results of the successive extractions method.

Since three independent sets of data obtained on three different days were used to get the standard addition lines, the reported dispersion corresponds to day-to-day intermediate precision. Values observed for each solvent are given in Table 3; the pooled RSD is around 15%, which complies with the precision required for residual solvent determination. Linearity was indicated by the residuals of the standard additions curves: as shown by the example of pyridine in Fig. 6—similar behavior was observed for the three other solvents—no discrepancies were observed. Adequate accuracy was observed with spiked real samples (Table 3). The limits of detection (LODs) were evaluated at a signal-to-noise ratio of 3 and are shown in Table 3. All LODs were below ppb levels, which underlined the ability of the developed SPME–GC method to obtain a simultaneous extraction of all four solvents. The limits of quantitation were evaluated at a signal-to-noise ratio of 10 and are reported in Table 3. The method of standard additions was suitable for all four solvents.

Table 4 gives a comparison between SMPE values obtained by the method of standard additions and ''usual'' values, i.e. values obtained by the usual method requiring injection of a large amount of the sample directly into the GC system. For ethanol and pyridine, the SPME values were very close, taking into account the precision of the measurement, to the ''usual'' values. For cyclohexane, the SPME value was higher but remained low compared with other solvents. For this compound, SPME seemed to present better detectability than the ''usual'' method. For triethylamine, the SPME value was twice as high as the ''usual'' value. This surprising result could come from the fact that the ''usual'' method tended to underestimate the triethylamine amount. This is quite understandable considering that in the ''usual'' method a solid is transferred inside the insert that regularly causes clogging. It is likely that this solid could include some triethylamine. Thus, the SPME–GC method seems to be more powerful than the ''usual'' one.

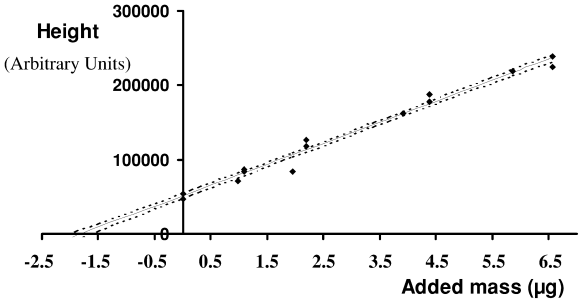

Fig. 6. Results of the standard addition method for pyridine. Dashed lines represent the confidence curves of the regression line (full line).

3.5. Summary of the optimum conditions

The parameter values corresponding to the optimum conditions for the simultaneous determination and quantification of ethanol, cyclohexane, triethylamine and pyridine in drug A are listed in Table 5.

4. Conclusion

Through an example it has been demonstrated that SPME–GC could appear as an elegant alternative for the determination and quantification of residual solvents in drugs. In our case, the best conditions were obtained by combining reasoning based on the chemical properties of the solvents concerned (e.g. pH) and a systematic approach based on experimental design. Quantification, which is usually the weakness of SPME, was not limiting as indicated by the confidence intervals of the results for standard additions. This study suggests that SPME–GC can be used in the pharmaceutical industry for solvent

Table 3
Characteristics the SMPE–GC method

	Precision (RSD, %)	Recovery (%)	LOD (pg)	LOQ (pg)
Ethanol	16.5	94	50	170
Cyclohexane	14.6	114	1	4
Triethylamine	18.7	103	10	40
Pyridine	10.9	98	1	4

Table 4
Comparison of the results for quantitative analysis (values in ppm)

	Standard additions	Multiple extractions	''Usual'' value (usual GC–FID method)
Ethanol	341 [291–397]	na	300
Cyclohexane	10.8 [9.5–12.5]	2.3 [2.0–2.7]	Non-detected (<2)
Triethylamine	192 [163–228]	na	80
Pyridine	18.3 [16.4–20.2]	na	20

na, non-applicable, value in brackets corresponds to ±SD deduced from the confidence curves of the standard addition line.

Table 5
Optimum conditions for the SPME–GC method

Parameter	Value
Fiber	CAR-PDMS
Extraction mode	Headspace-over-the-liquid
Water dissolution volume	7.2 ml
Headspace volume	3.8 ml
pH of the dissolution solution	9.6 (Na_2HPO_4 0.02 mol l^{-1})
[NaCl]	1.77 mol l^{-1}
Extraction temperature	40 °C
Extraction time	30 min
Desorption time	5 min
Desorption temperature	300 °C
Method of quantification	Standard additions
GC method	See Experimental section

residue determination and represents a clean approach compared with the direct injection of large amounts of sample into the GC system. Henceforth, SPME–GC should be evaluated for other kinds of pharmaceutical formulations, such as creams, patches or suppositories, to determine if the presence of excipients causes problems or not.

References

[1] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[2] D. Louch, S. Motlagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[3] D.W. Potter, J. Pawliszyn, J. Chromatogr. 625 (1992) 247.
[4] K.D. Bucholz, J. Pawliszyn, Environ. Sci. Technol. 27 (1993) 2844.
[5] J. Horng, S. Huang, J. Chromatogr. A 678 (1994) 313.
[6] D.W. Potter, J. Pawliszyn, Environ. Sci. Technol. 28 (1994) 298.
[7] J. Pawliszyn, in: Solid Phase Microextraction—Theory and Practice, Wiley–VCH, New York, 1997, p. 43.
[8] I. Koide, O. Noguchi, K. Okada et al., J. Chromatogr. A 707 (1998) 99.
[9] J. Dugay, C. Miège, M.-C. Hennion, J. Chromatogr. A 795 (1998) 27.
[10] A.L. Simplicio, L.V. Boas, J. Chromatogr. A 833 (1999) 35.
[11] W.J. Havenga, E.R. Rohwer, J. Chromatogr. A 848 (1999) 279.
[12] M. Mestres, M.P. Marti, O. Busto, J. Guasch, J. Chromatogr. A 849 (1999) 293.
[13] C.C. Camarasu, M. Mezei-Szucs, G.B. Varga, J. Pharm. Biomed. Anal. 18 (1998) 623.
[14] C.C. Camarasu, J. Pharm. Biomed. Anal. 23 (2000) 197.
[15] S.A. Coran, V. Giannellini, S. Furlanetto, M. Bambagiotti-Albertini, S. Pinzauti, J. Chromatogr. A 915 (2001) 209.
[16] M. Chiarotti, R. Marsili, A. Moreda-Pineiro, J. Chromatogr. B 772 (2002) 249.
[17] A.R. Raghani, J. Pharm. Biomed. Anal. 29 (2002) 507.
[18] J. Vial, K. Le Mapihan, A. Jardy, Chromatographia, (2003) in press.
[19] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, in: Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997, p. 659.
[20] J. Goupy, La Méthode des Plans d'Expériences, Dunod, Paris, 1988.
[21] J. Ai, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Letchworth, 1999, p. 22.
[22] J. Vial, A. Jardy, in: J. Cazes (Ed.), Encyclopedia of Chromatography, Marcel Dekker, New York, 2001, p. 696.
[23] J.N. Miller, J.C. Miller, in: Statistics and Chemometrics for Analytical Chemistry, Prentice Hall, Harlow, 2000, p. 118.