ELSEVIER

Journal of Chromatography A 819 (1998) 259–266

JOURNAL OF
CHROMATOGRAPHY A

Determination of some aromatic amines in finger-paints for children's use by supercritical fluid extraction combined with gas chromatography

M.C. Garrigós[a,*], F. Reche[a], K. Pernías[b], A. Sánchez[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 test method based on supercritical fluid extraction (SFE) and gas chromatography has been developed for some aromatic amines, such as 4-chloro-*o*-toluidine, β-naphthylamine and 4-aminobiphenyl. A two-level factor design was used as the optimization procedure. Four main variables were considered: CO_2 pressure, extraction temperature, static extraction time and volume of modifier (methanol). Results obtained for 4-chloro-*o*-toluidine, indicated that the volume of modifier was the variable with the most important influence on extraction, CO_2 pressure had a negative effect and temperature and time were less significant. For the other amines, static time was the most important variable in both cases, followed by CO_2 pressure and volume of modifier, with no influence of temperature. SFE was compared with Soxhlet extraction, and was found to give higher recoveries in all cases. Other commercial finger-paints were tested for the presence of aromatic amines.

Keywords: Extraction methods; Factorial design; Optimization; Amines, aromatic; Chlorotoluidine; Naphthylamine; Aminobiphenyl

1. Introduction

Aromatic amines are toxic and may have a mutagenic impact and carcinogenic activity (both in animals and humans) even at low concentrations [1]. The most common among these are 2-naphthylamine, 4-chloro-*o*-toluidine and 4-aminobiphenyls [2]. These amines are used in finger-paints for children use as colorant precursors, and their use is going to be limited in the future. Finger-paints present significant risks for children, with the possibility of ingestion of the paint and intensive skin contact. However, an analytical methodology to get a fast, reproducible and efficient determination of these compounds is lacking at the present time. A provisional method elaborated by the Swiss Food Regulation (SFR), permits the determination of carcinogenic aromatic amines in painting and drawing materials. The analytes are extracted by a solid–liquid extraction and determined by HPLC, but some previous separation and purification steps are necessary, resulting in a slow and tedious method. Therefore, the development of a fast and reproducible test method for this kind of samples is necessary.

Finger-paints are paste and/or jelly-like coloured preparations specially designed for children, and they are directly applicable to suitable surfaces with fingers and hands, but also suitable for painting. They essentially consist of, in addition to water,

---

[*] Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00432-4

colouring agents, fillers, moisturizers, binders, preservatives and detergents.

The interest in supercritical fluid extraction (SFE) has been growing rapidly during recent years. SFE minimizes sample handling, provides fairly clean extracts, expedites sample preparation and reduces the use of environmentally aggressive solvents [3]. Examples of SFE applications include polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from environmental samples [4], semivolatile compounds [5] and N-nitrosamines in food [6], and phthalate plasticisers in PVC samples [7].

The application of SFE to the extraction of amines, however, has been quite limited. Ashraf-Khorassani et al. evaluated SFE and chromatography of primary, secondary, and tertiary aliphatic amines and aromatic amines using both carbon dioxide and nitrous oxide in Celite, soil and a silica matrix [8]. The use of nitrous oxide modified with an adequate amine significantly improved the SFE of aromatic amines from soils [9]. Oostdyck et al. reported the optimization of a SFE method for the determination of primary aromatic amines in soil matrices using N_2O [10]. Parameters investigated included the use of chemical modifiers, modifier concentration, modifier volume, pressure, temperature, static extraction time, extraction volume and modifier addition cycles. The optimization was carried out in the classical parameter-to-parameter method. Nitrous oxide and the addition of a small amount of 1,6-hexanediamine in methanol (5%) directly to the extraction chamber provided the best recoveries for aromatic amines. However, the recoveries for some aromatic amines were still poor. An additional problem of this method was the use of nitrous oxide, a dangerous and highly explosive fluid. Janda et al. showed that aromatic amines are extractable with pure supercritical CO_2 [11]. Recoveries depend on the matrix type; the highest recoveries were obtained for inert matrices and the more acidic the matrix, the lower recoveries. However, CO_2 polarity is low and it may be necessary to develop an appropriate strategy to enhance supercritical extraction efficiencies of polar compounds with the use of polar modifiers [12]. Unfortunately, highly basic primary amines cannot be used as CO_2 polarity modifiers because of the carbamate formation [8].

When developing a SFE method, formal optimization methods are generally preferred. Factor designs have the advantage of obtaining more information with as few runs as possible by varying several factors at once. Therefore, factor experiment detects and estimates any interaction, which classical experiment cannot do [13]. Factorial designs have been used for the simultaneous determination of various analytical SFE parameters in different samples [13–18].

The work presented here includes investigation and optimization of a method based on SFE and gas chromatography (GC) for the analysis of primary aromatic amines from finger-paints.

2. Experimental

2.1. Chemicals

Reagent grade standards of 4-chloro-*o*-toluidine, β-naphthylamine and 4-aminobiphenyl were obtained from Sigma (St. Louis, MO, USA). Stock solutions (20 μg/g) of each compound were prepared in methylene chloride. Analytical grade methylene chloride and methanol were obtained from Normapur (Prolabo, Barcelona, Spain).

2.2. Supercritical extraction

SFE was performed (off-line mode) using an ISCO Model SFX-220 extraction system (Lincoln, NE, USA) consisting of a SFX-220 extractor, a SFX 200 controller and a 100DX-syringe pump. Supercritical grade CO_2 was obtained from Abelló Linde (Valencia, Spain). Approximately 0.2 g of dry sample were introduced in the stainless steel cartridge (internal volume, 2.5 ml). The modifier (methanol) 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 capillary restrictor was coaxially heated and the temperature was programmed to 90°C for the experiments at 13.8 MPa and 105°C for the 55.2 MPa measurements. The outlet of the restrictor was immersed in a graduate dark vial with 10 ml of methylene chloride to trap the extracted analytes. All extractions were carried out in the static/dynamic mode, with an adequate static time followed by a 15 min dynamic extraction.

2.3. Optimisation

A full 2^4 factorial design for a white commercial paint fortified with 200 μg of each amine was carried out in order to optimize the SFE parameters. Approximately 0.6 g of wet paint were fortified with 0.25 ml from a 1000 ppm solution of each amine prepared in methanol (equivalent to 200 μg of each amine). The sample was extended onto an inert surface and dried at 40°C for 30 min, immediately prior to extraction, so these samples can be considered fresh spikes. This procedure results in approximately 0.2 g of dry paint which was totally transferred to the cartridge. Samples were cut in thin sheets (3 mm width) after drying.

SFE results were compared with those obtained for a Soxhlet extraction in which 0.8 g of paint were extracted with 70 ml of methylene chloride in a reflux for 5 h. The final extract was filled to the mark of a 100-ml graduated flask. Additional SFE extractions were performed using different finger-paints in order to determine the amine contents of these samples, using the optimal extraction conditions previously established.

2.4. Gas chromatography

Analysis of the extracts was carried out by GC, using a Shimadzu GC-17A (Kyoto, Japan) gas chromatograph equipped with a Shimadzu AOC-20i auto-injector, a TRB-5 capillary column (30 m×0.32 mm I.D. with 0.5 μm film thickness) (Supelco, Bellefonte, PA, USA), a split-splitless injector and a flame ionization detection system (FID). Helium was used as the carrier gas, with a linear velocity of 70 cm/s and a head pressure of 153 KPa. The injector and detector temperatures were 300°C, and the column temperature was programmed from 70°C (hold 1.5 min) to 230°C (hold 3.5 min) at a 12°C/min heating rate. A 2-μl volume of sample was injected in the splitless mode (1.5 min splitless-

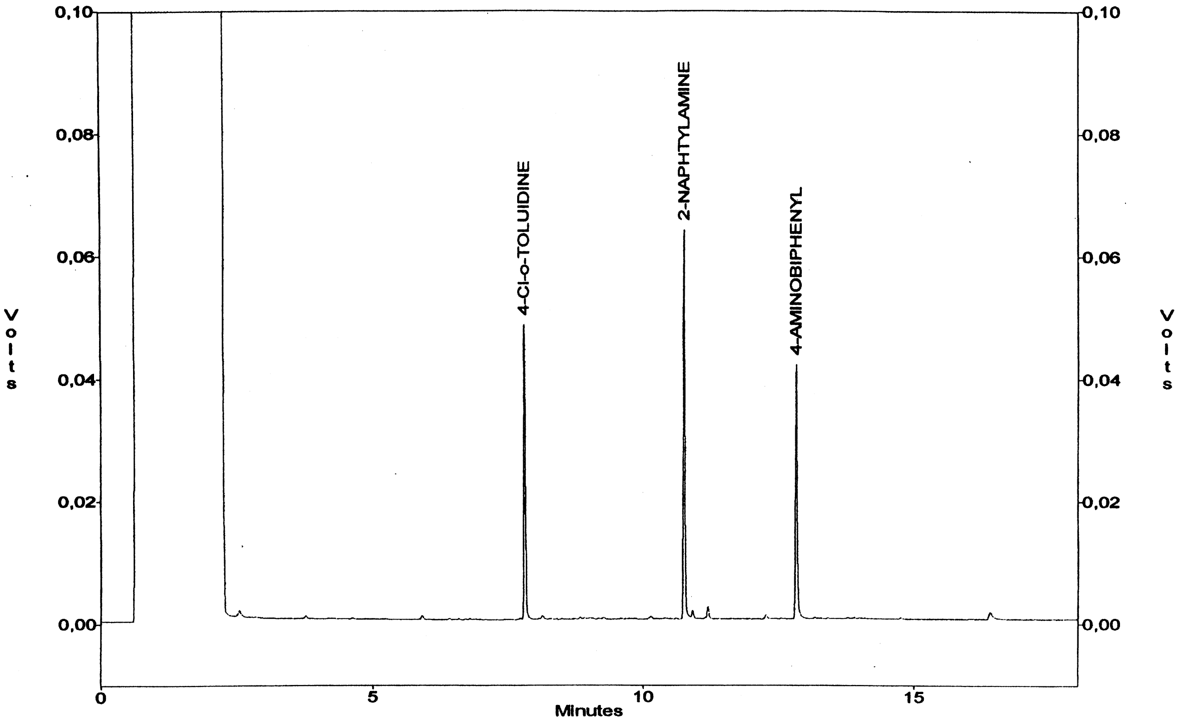


Fig. 1. GC separation of amines.

period). Quantitation of the amines was performed by comparing chromatographic peak areas for sample extracts with those of standards in the same concentration range. An example of the GC separation is shown in Fig. 1.

3. Results and discussion

Calibration curves were run at five concentration levels using appropriately diluted standards. Each concentration level was injected in triplicate, and chromatographic peak areas were fitted by linear regression. The linearity range was 0.5–20 μg/g with a correlation coefficient of 0.999.

In order to obtain the optimal conditions for the extraction, a white commercial paint was fortified with 200 μg of every analyte. The effect of different variables affecting supercritical extraction of aromatic amines in finger-paints was studied by a four-factor design with two levels for each factor (low and high). This design requires 16 experiments plus one in the middle. Experiments were performed in duplicate and were run in a randomized fashion. Table 1 lists the upper and lower values for each factor which were selected according to the instrumental limitations. The investigated parameters were: CO_2 pressure (P), extraction temperature (T), static time (t) and volume of modifier (methanol) (V). The highest recoveries obtained for the amines were: 66.4% for 4-chloro-*o*-toluidine, 57.3% for β-naphthylamine and 56.7% for 4-aminobiphenyl. Fig. 2 compares the recoveries obtained for the different experiments.

A statistical analysis was carried out to evaluate the effects of the experimental variables resulting in

Table 1
Variables and design of four factors at two levels

(a) Two-level factors

Variables	Range
P=CO_2 pressure (MPa)	13.8–55.2
T=Extraction temperature (°C)	50–120
t=Extraction static time (min)	2–15
V=Methanol volume (μl)	0–80
Factor coding	(−)–(+)

(b) Two-level factorial design

Run	Coded[a]				Actual			
	P	T	t	V	P	T	t	V
1	−	−	−	−	13.8	50	2	0
2	+	−	−	−	55.2	50	2	0
3	−	+	−	−	13.8	120	2	0
4	+	+	−	−	55.2	120	2	0
5	−	−	+	−	13.8	50	15	0
6	+	−	+	−	55.2	50	15	0
7	−	+	+	−	13.8	120	15	0
8	+	+	+	−	55.2	120	15	0
9	−	−	−	+	13.8	50	2	80
10	+	−	−	+	55.2	50	2	80
11	−	+	−	+	13.8	120	2	80
12	+	+	−	+	55.2	120	2	80
13	−	−	+	+	13.8	50	15	80
14	+	−	+	+	55.2	50	15	80
15	−	+	+	+	13.8	120	15	80
16	+	+	+	+	55.2	120	15	80
17	0	0	0	0	34.5	85	8	40

[a] −=low level, +=high level, 0=middle level.

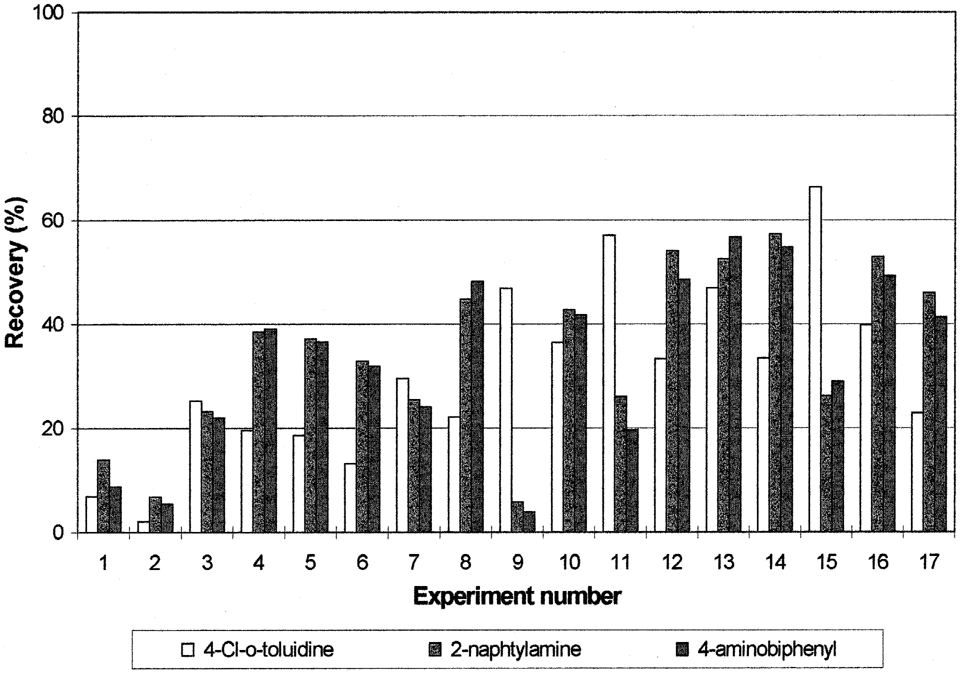


Fig. 2. Comparison between experiments in a 2^4 factorial design for SFE of the three amines.

the standardized effect Pareto charts shown in Figs. 3–5. Vertical lines indicate the statistical significance bound for the effects at a 95% probability. Recoveries for 4-chloro-*o*-toluidine are maximum when conditions are 13.8 MPa, 120°C, 15 min and 80 μl of methanol (experiment 15). The volume of modifier is

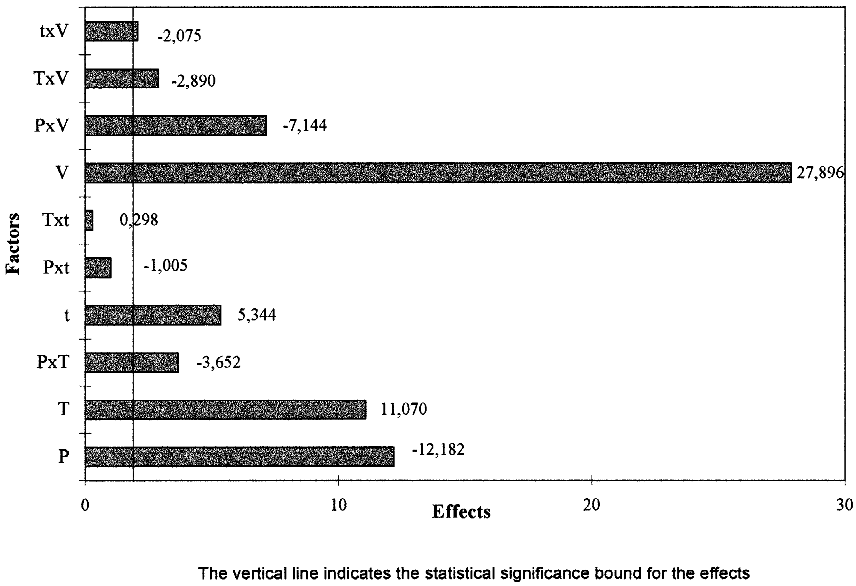
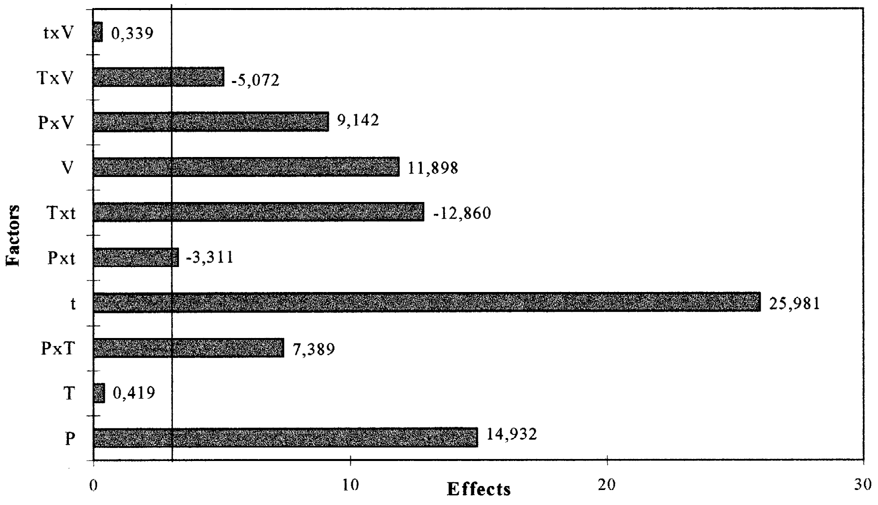


Fig. 3. Pareto chart for standardized effects of 4-chloro-*o*-toluidine.

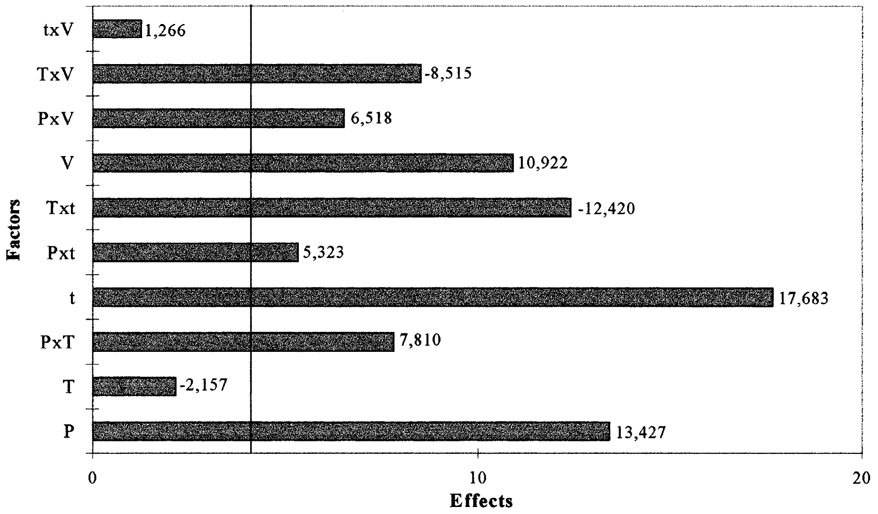


The vertical line indicates the statistical significance bound for the effects

Fig. 4. Pareto chart for standardized effects of β-naphthylamine.

the variable with the most important statistic influence on extraction, CO_2 pressure (with a negative effect), temperature and time being less significant. Thus, it can be observed in Fig. 2 that recoveries for 4-chloro-*o*-toluidine are clearly higher in the experiments where methanol has been added. In addition, some significant negative interactions between pressure and volume of modifier as well as between pressure and extraction temperature can be observed.

Methanol is currently the most common modifier for supercritical CO_2 [19]. The improvement of the extraction yields observed with the addition of modifier must therefore be due to the increase in the diffusion rate of the solutes when the paint particles



The vertical line indicates the statistical significance bound for the effects

Fig. 5. Pareto chart for standardized effects of 4-aminobyphenyl.

swell by the uptake of modifier. Modifiers not only swell the polymer matrix, but also increase the supercritical fluid density and polarity.

The effect of the temperature in SFE has been also studied. Thus, high temperatures increase diffusion coefficients of the solutes in the paint, whereas the density and the solvent strength of the supercritical fluid decrease. An increase in the temperature speeds up the diffusion of the solutes in the material increasing the extraction rate.

β-Naphthylamine and 4-aminobiphenyl show a maximum recovery for 55.2 MPa, 50°C, 15 min and 80 μl of methanol (experiment 14). The most important variable in both cases was static time, followed by CO_2 pressure and volume of modifier, with no influence for the extraction temperature. Pareto charts for these two amines are presented in Figs. 4 and 5. Some significant positive interactions between pressure and volume of modifier as well as between pressure and extraction temperature can be observed. Moreover, significant negative interactions between extraction temperature and static time as well as between extraction temperature and volume of modifier are seen.

The time the modifier remains in the extraction cell is an important factor for the extraction process, because a long static time permits the modifier to be more in contact with the polymer. At high pressures and low temperatures the density of the supercritical fluid is high. As the diffusion coefficients of the analytes in the polymer matrix are relatively small, molecules on the surface are rapidly removed out of the extraction cell by the flow of the high density carbon dioxide. The extraction rate is hence determined by the diffusion of the solutes in the polymer matrix. As the solubility of the analytes in the supercritical fluid is not the rate-limiting parameter, the improvement of the SFE recovery upon the addition of the modifier must be mainly due to the swelling of the polymer [20].

Some additional experiments near the point of maximum recovery were carried out in order to try to improve the recoveries for the amines. For 4-chloro-*o*-toluidine, the volume of methanol was increased to 200 μl, while for the other two amines the static time was increased to 30 min. No improvement was observed in any case.

Soxhlet extractions were carried out for the same spiked sample in order to compare the new SFE method with a traditional extraction. Recoveries obtained were: 26.6% for 4-chloro-*o*-toluidine, 28.8% for β-naphthylamine and 26.4% for 4-aminobiphenyl. A comparison between recoveries obtained for SFE and Soxhlet extraction is presented in Fig. 6. SFE seems to be a better extraction method for the determination of aromatic amines in finger-paints, as it increases recoveries, diminishes the presence of coextractive components and reduces both solvent waste and time.

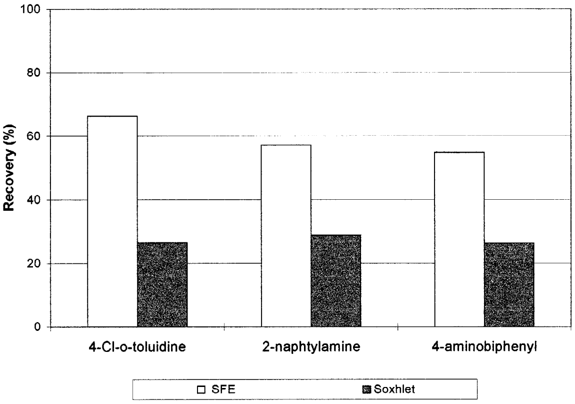


Fig. 6. Comparison of results for SFE and Soxhlet extraction for the amines.

The proposed method was validated by application to some other commercial finger-paints to find if these toxic compounds are really used in formulations. The analysis revealed that 4-chloro-*o*-toluidine was present in only one of the three paints tested. The concentration of this amine in the paint was $(3.34\pm0.38)\cdot10^{-4}$ μg analyte/g dry paint ($n=4$ replicates and t for a 95% probability).

4. Conclusions

A SFE–GC method has been developed for the extraction and determination of 4-chloro-*o*-toluidine, β-naphthylamine and 4-aminobiphenyl from finger-paints. A factorial design has shown the relative importance of the variables affecting the recovery when SFE is used. Parameters investigated were: CO_2 pressure, extraction temperature, static extraction time and volume of modifier (methanol). The highest recoveries obtained for the amines were: 66.4% for 4-chloro-*o*-toluidine, 57.3% for β-naphthylamine and 56.7% for 4-aminobiphenyl. Recoveries for 4-chloro-*o*-toluidine were maximum at 13.5 MPa, 120°C, 15 min and 80 μl of methanol. The volume of modifier is the variable which presents the most important influence in extraction, CO_2 pressure (with a negative effect), temperature and time being less significant. β-Naphthylamine and 4-aminobiphenyl present a maximum recovery at 55.2 MPa, 50°C, 15 min and 80 μl of methanol. The static time was the most important variable in both cases, followed by CO_2 pressure and volume of modifier, with no influence for the temperature. SFE has proved to be a reproducible and effective method to get a fast analysis. If compared with Soxhlet extraction, this method yields higher recoveries for the three amines and the reduction of the amount of solvent waste and time. The application of the proposed method to other commercial finger-paints revealed that only one of the three paints tested had an aromatic amine in its formulation (4-chloro-*o*-toluidine). The concentration of this amine in the paint was $(3.34\pm0.38).10^{-4}$ μg analyte/g dry paint ($n=4$ replicates and t for a 95% probability).

Acknowledgements

The authors wish to express their appreciation to the CICYT (SPAIN) (Projects PETRI 95-0059-CT and PETRI-95-0160-CT) for their financial support.

References

[1] B. Gawdzik, U. Czerwinska-Bil, Chromatographia 32 (1991) 167.
[2] S.P. Gupta, Ind. J. Chromatogr. 30B (1991) 122.
[3] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A.
[4] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 66 (1994) 909.
[5] J.J. Langenfeld, M.D. Buford, S.B. Hawthorne, D.J. Miller, J. Chromatogr. 594 (1992) 297.
[6] W. Fiddler, J.W. Pensabane, J. AOAC Int. 79 (1996) 895.
[7] M.L. Marín, A. Jiménez, J. López, J. Vilaplana, J. Chromatogr. A 750 (1996) 183.
[8] M. Ashraf-Khorassani, L.T. Taylor, P. Zimmerman, Anal. Chem. 62 (1990) 1177.
[9] T.S. Oostdyk, R.L. Grob, J.L. Snyder, M.E. Mcnally, Anal. Chem. 65 (1993) 596.
[10] T.S. Oostdyk, R.L. Grob, J.L. Snyder, M.E. Mcnally, J. Chromatogr. Sci. 31 (1993) 177.
[11] V. Janda, J. Kriz, J. Vejrosta, K.D. Bartle, J. Chromatogr. A 669 (1994) 241.
[12] M.D. Luque de Castro, M.T. Tena, Trends Anal. Chem. 15 (1996) 32.
[13] J.S. Ho, P.H. Tang, J. Chromatogr. Sci. 30 (1992) 344.
[14] K. Li, C.P. Ong, S.F.Y. Li, J. Chromatogr. Sci. 32 (1994) 53.
[15] M.P. Llompart, R.A. Lorenzo, R. Cela, J. Chromatogr. A 723 (1996) 123.
[16] R. Cela-Torrijos, M. Miguens-Rodríguez, A.M. Carro-Díaz, R.A. Lorenzo-Ferreira, J. Chromatogr. A 750 (1996) 199.
[17] M.P. Llompart, R.A. Lorenzo, R. Cela, J. Chromatogr. Sci. 34 (1996) 43.
[18] M. Kane, J.R. Dean, S.M. Hitchen, C.J. Dowle, R.L. Tranter, Anal. Chim. Acta 271 (1993) 83.
[19] L.T. Taylor, R & D Mag., (1995) 1.
[20] X. Lou, H-G. Janssen, C.A. Cramers, J. Microcol. Sep. 7 (1995) 303.