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Optimization of parameters for the analysis of aromatic amines in finger-paints

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Abstract

A study for the optimisation of the supercritical fluid extraction (SFE) of some aromatic amines (4-chloro-*o*-toluidine, 2-naphthylamine, 4-aminobiphenyl and benzidine) in finger-paints was conducted. The influence of different variables related to the technique on recoveries was investigated. The analytes were subsequently analysed by gas chromatography after SFE. The study allowed the estimation of four main factors (temperature, pressure, static time and volume of modifier) on recoveries by the use of a two-level factor design, where most significant parameters as well as second- and third-order interactions were identified. Other factors, such as type and volume of modifier and time of contact between the spiker solution and the sample prior to extraction, were also studied. The influence of matrix on extraction recovery was also evaluated by applying the method to different finger-paints, and recoveries were similar or even higher in some cases. The drying process of samples was also studied, while classical drying in an oven and microwaves were compared, with similar efficiencies in both methods. The method was validated by extracting the aromatic amines from some commercial finger-paints.

Keywords: Extraction methods; Factorial design; Optimization; Amines, aromatic; Aminobiphenyl; Benzidine; Chloro-toluidine; Naphthylamine

1. Introduction

Finger-paints are paste and/or jelly-like, coloured preparations directly applicable to suitable surfaces with fingers and hands and specially designed for children. They essentially consist of, in addition to water, colouring agents, fillers, binders, humectants, preservatives, surfactants and embittering agents. Finger-paints present possibilities for significant risks for children, as the ingestion of paint material and the possibility of prolonged skin contact.

Primary aromatic amines, such as 4-chloro-*o*-toluidine, 2-naphthylamine, 4-aminobiphenyl and benzidine, are of particular concern as a result of their toxicity and prevalent use as colorant precursors in finger-paints [1]. As a result of the potential toxicity of such compounds, some requirements for colouring agents are willing to be set in European Standard EN71-7 for finger-paints. However, a fast, reproducible and efficient analytical method for the determination of these compounds is lacking at the present time.

Supercritical fluid extraction (SFE) has shown much potential skills for the isolation of organic compounds from various samples [2]. SFE minimises sample handling, provides fairly clean extracts, expedites sample preparation and reduces the use and disposal of environmentally aggressive solvents. Additionally, in many cases, SFE provides recoveries

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even better than those of conventional solvent extraction techniques [3]. Supercritical CO_2 is by far the fluid most commonly used in SFE. Many different samples have been extracted with supercritical CO_2, e.g., cholesterol in noodles [4], polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from environmental samples [5], semivolatile compounds [6], *N*-nitrosamines in food [7], and phthalate plasticizers [8,9] and phenol [10] in PVC samples. However, one of the limitations for the use of CO_2 is its low polarity. Therefore it may be necessary to enhance supercritical extraction efficiencies of polar compounds with the use of modifiers [11].

On the other hand, not many SFE methods applied to the analysis of amines have been reported. Ashraf-Khorassani et al. evaluated the supercritical fluid extraction and chromatography of primary, secondary, and tertiary aliphatic and aromatic amines using both carbon dioxide and nitrous oxide in Celite, soil and a silica matrix [12]. The use of N_2O modified with an adequate amine that improved significantly the SFE of aromatic amines from soils was also reported [13,14]. However, the recoveries for some aromatic amines were still poor, and the use of nitrous oxide, a dangerous and highly explosive fluid, was considered an additional problem. Janda et al. showed that aromatic amines are extractable with pure supercritical CO_2 [15]. Highest recoveries were obtained for inert matrices and the more acidic the matrix, the lower recoveries obtained.

Factorial designs can be considered as an effective tool to simultaneously study the influence of several parameters with a reduced number of experiments. They also detect and estimate any interaction which classical experiments cannot do [16]. Factorial designs have been used for the simultaneous determination of various analytical SFE parameters in different samples [17–19]. We have reported an SFE-GC method for some aromatic amines used in finger-paints and some preliminary results were presented [20]. A 2^4 factorial design was used to study some SFE parameters and the results were compared with those obtained with Soxhlet extraction. However, benzidine (known as a very toxic amine) was not included in the study. In addition, the drying process was achieved in a classical oven, leading to a waste of time in the preparation step for SFE.

The work presented here includes an extensive investigation and optimisation of a SFE method for extraction of the above-indicated primary aromatic amines from finger-paints. The main parameters investigated here by using a two-level factorial design were temperature, pressure, static time and volume of modifier. Other important factors, such as type and volume of modifier, time of contact between spiker solution and sample prior to extraction and matrix influence were also investigated. Sample preparation was also optimised by the use of microwave heating.

2. Experimental

2.1. Materials and Instrumentation

Reagent grade standards of 4-chloro-*o*-toluidine, β-naphthylamine, 4-aminobiphenyl and benzidine were obtained from Sigma (St. Louis, MO, USA). Stock solutions of each compound were prepared in methylene chloride (20 μg/g). Analytical-grade methylene chloride and methanol were obtained from Normapur (Prolabo, Barcelona, Spain) and supercritical grade CO_2 was supplied by Abelló Linde (Valencia, Spain).

Finger-paint samples were selected from those commercially available in toyshops. Some tests for sample characterisation were carried out in order to determine the main components of these finger-paints. In this way, the polymeric base was found to be poly(vinyl alcohol) or poly(vinyl acetate). Some phthalate plasticizers were also found in some paints, as well as different mineral fillers (talcum, calcium carbonate).

SFE was carried out by using an ISCO SFX-220 extraction system (Lincoln, NE, USA). Methylene chloride was used as collection fluid, and the restrictor temperature was maintained at 90°C for the experiments at 13.8 MPa and at 105°C for the 55.2 MPa measurements. All extractions with supercritical CO_2 were carried out with an adequate static time followed by a 15-min dynamic extraction. The analysis of extracts was carried out using a Shimadzu GC-17A (Kyoto, Japan) gas chromatograph, as indicated in a previous work [20].

Table 1
Comparison between microwave drying and conventional oven drying for some finger-paints (3 replicates)

Paint	% Oven mass loss[a]	% Microwave mass loss[b]
White	55.2±1.8	59.4±0.7
Red	30.8±1.3	31.3±0.9
Blue	52.8±0.9	61.2±1.4
Magenta	35.6±1.1	39.2±0.7

[a] 40°C and 30 min.
[b] 760 W and 1.5 min.

2.2. Sample preparation and optimisation procedure

A full 2^4 factorial design for a white commercial paint was carried out in order to optimise the SFE parameters. Approximately 0.6 g of wet paint were fortified with 200 μg of each amine (0.25 ml from a 1000-ppm solution of amines in methanol). The sample was extended onto an inert surface and dried, immediately prior to extraction. This procedure results in approximately 0.2 g of dry paint, which was totally transferred to the cartridge, and the modifier (methanol) was added directly to the cartridge before extraction. Samples were cut in sheets (3 mm width) after drying.

Two procedures for drying the sample were compared: classical drying in an oven at 40°C for 30 min [20], and microwave heating at 80% of maximum power for 1.5 min. An MSP-1000 (CEM, NC, USA) microwave oven was employed with (950±50 W) maximum power. Table 1 compares both drying procedures for some finger-paints. Drying efficiencies were comparable for both methods (no significant differences were found at a 95% probability), showing microwave drying as a more convenient method in terms of time. Microwave heating was already successfully used in this kind of samples as drying method [21].

3. Results and discussion

The effect of four main variables on SFE of aromatic amines in finger-paints was simultaneously studied using a four-factor design, with two levels for each factor (low and high). The selected factors were supercritical CO_2 pressure (P), extraction temperature (T), static time (t) and volume of methanol (V). The 16 experiments plus one in the middle were performed in duplicate and were run randomised. Table 2 lists the design matrix for experiments, as well as the values given to each factor, which were selected according to the instrumental limitations.

Maximum recovery for 4-chloro-*o*-toluidine was found at experiment 15 (13.8 MPa, 120°C, 15 min and 80 μl of methanol), and for the other three amines at experiment 16 (55.2 MPa, 120°C, 15 min and 80 μl of methanol). When results obtained here are compared to those previously obtained [20], it must be noted that a general improvement in recoveries is observed for the three amines studied. Thus, the maximum recovery for 4-chloro-*o*-toluidine increases from 66.4 to 81.5%, while similar improvements are observed for the other two analytes. These results indicate that the new extraction

Table 2
Factor levels and design matrix in the 2^4 factorial design

Factor					Units			Levels								
Variable		Key						Low (−)			High (+)			Middle (0)		
Pressure		*P*			MPa			13.8			55.2			34.5		
Temperature		*T*			°C			50			120			85		
Static time		*t*			min			2			15			8		
Methanol volume		*V*			μl			0			80			40		
Run																
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
P	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	0
T	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+	+	0
t	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+	0
V	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	0

conditions here proposed are more efficient and the overall method presents better analytical parameters. A chromatogram of the white spiked paint at 55.2 MPa, 120°C, 15 min and 80 μl methanol is shown in Fig. 1. The peaks corresponding to the analytes are clearly observed and can be easily quantified in any case. Some additional peaks probably corresponding to some impurities of the commercial paint extracted together to the amines are also observed in Fig. 1.

A statistical analysis of results was performed considering all possible interactions resulting in Fig. 2, where only the statistical significant effects are presented (95% probability). The most significant parameters as well as second- and third-order interactions were identified, getting to some general conclusions. In this way, all main effects are significant for all the amines. A high temperature is needed, indicating that diffusion of amines will be an important step in the extraction process. In terms of solubility, a low pressure and the presence of modifier seem to be necessary for the extraction of 4-Chloro-*o*-toluidine, while a high pressure is needed for the other amines. *P–T* and *P–V* interactions suggest that a compromise between an increase in CO_2 polarity and diffusion coefficients of the analytes must exist, to get an effective increase in the analytes recovery [22].

3.1. Effect of the spiker time

Some experiments were conducted at conditions of maximum recovery, in which time between the addition of spiked analyte and the SFE using CO_2 and methanol was varied. In this way, samples were spiked with the amines and stored from 2 to 24 h. Then the SFE after the addition of methanol was carried out. Recoveries decrease from the 0-min experiment to the 2-h experiment, and a further decrease for the 24-h experiment is noted, as can be seen in Table 3. This fact must be due to the adsorption of the basic amines into the paint matrix.

3.2. Effect of modifiers

Modifiers increase the polarity of supercritical CO_2, and consequently an increase in solubility of the highly polar aromatic amines should be expected. Methanol is currently the most common modifier for

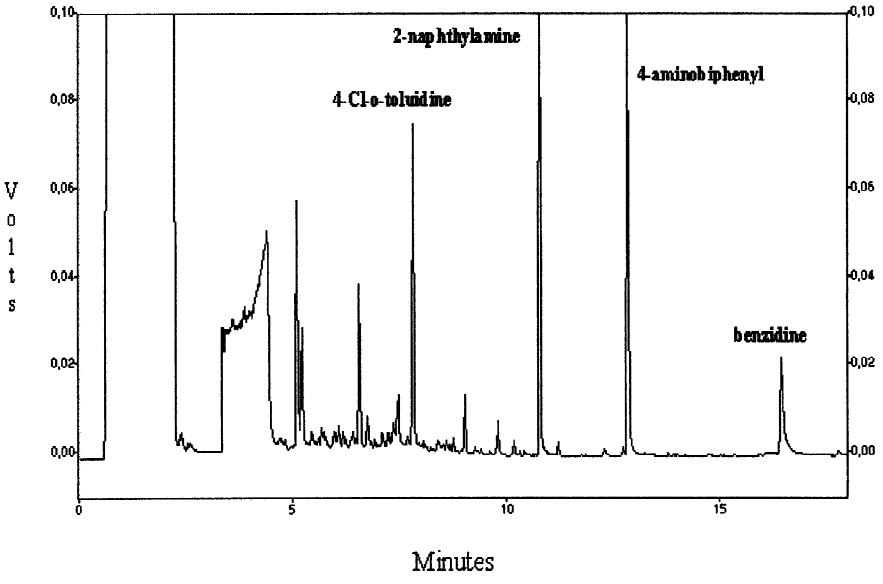

Fig. 1. Chromatogram of the white spiked paint at 55.2 MPa, 120°C, 15 min and 80 μl methanol.

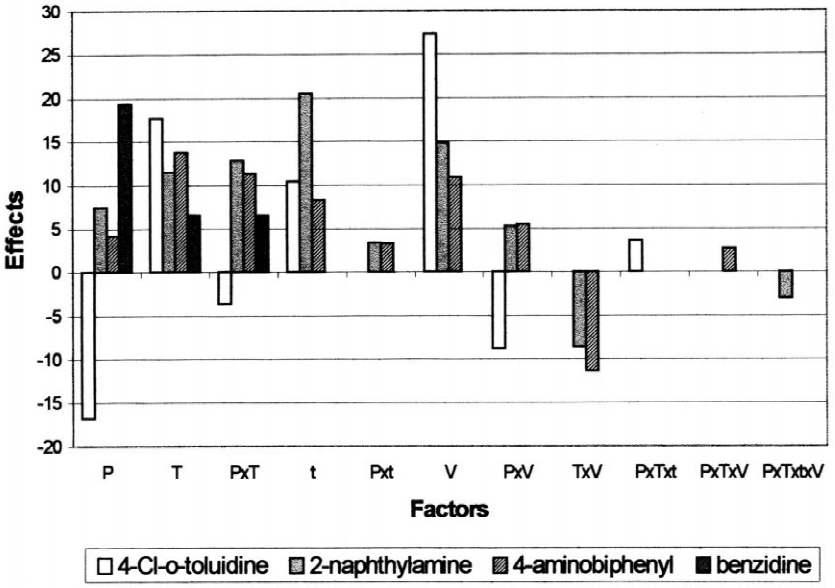

Fig. 2. Standardised significant effects for the amines.

supercritical CO_2 [23]. Nevertheless, the effect of other alternative modifiers at the optimum conditions previously found, was evaluated. The recovery data for the extraction of the amines using these modifiers, as well as the results found for methanol are presented in Fig. 3. As can be observed, methanol is the most effective modifier, as the recoveries are higher in all cases. In the case of benzidine the differences are not very significant, but recoveries with methanol are still higher.

As the effect of the volume of methanol added to the extraction vessel is clearly significant in the extraction of 4-chloro-*o*-toluidine, and taking into account that it only interacts with pressure (in a negative form), extractions at 13.8 MPa (120°C and 15 min static time) were performed with 200 and 500 μl of methanol. Overall recoveries increased with volume of methanol at 200 μl getting a maximum value for 4-chloro-*o*-toluidine of 91.4%, and remained practically constant at 500 μl.

Therefore, it can be concluded that the final maximum recoveries and conditions found for the spiked finger-paint used in the present study are those presented in Table 4.

Table 3
Influence of spiker time on recovery for aromatic amines

Compound	Recovery (%, $n=2$)		
	0 min	1 h	24 h
4-Chloro-*o*-toluidine	81.5	67.9	61.1
2-Naphthylamine	69.2	40.8	30.0
4-Aminobiphenyl	60.2	34.5	26.8
Benzidine	33.2	23.9	14.6

3.3. Effect of matrix

In order to study the influence of the matrix on the recovery of amines, some other finger-paints were selected and the method proposed here was applied. Thus, three other paints were spiked with the same quantity of aromatic amines and recoveries obtained were compared to those found for the white paint, as shown in Table 5. The first two paints are poly(vinyl alcohol)-based and the other two are poly(vinyl acetate)-based. Higher or similar recoveries were found for the amines, indicating that amine recovery can change depending on matrix components. In the

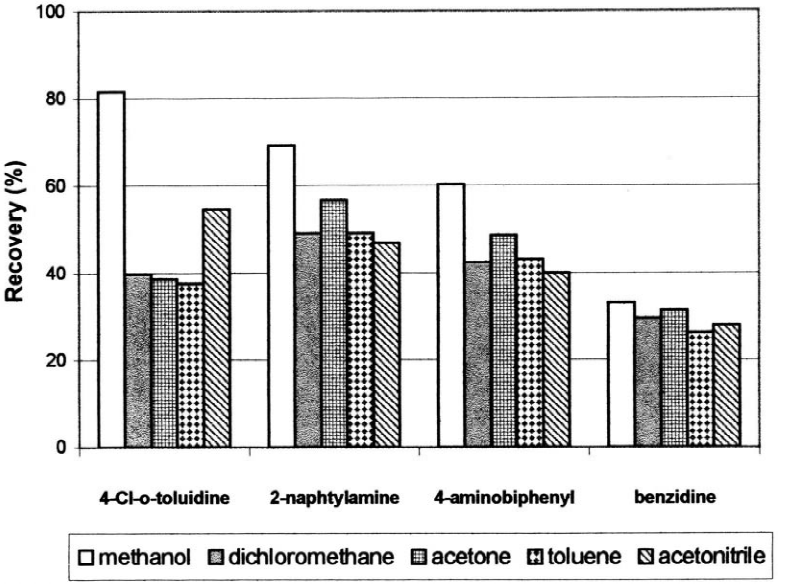

Fig. 3. Comparison between different modifiers for the aromatic amines.

Table 4
Final recoveries and conditions for the amines

Amine	Max. recovery (%)[a]	Run	Conditions			
			P (MPa)	*T* (°C)	*V* (μl)	*t* (min)
4-Chloro-*o*-toluidine	91.4	15	13.8	120	200	15
2-Naphthylamine	69.2	16	55.2	120	80	15
4-Aminobiphenyl	60.2	16	55.2	120	80	15
Benzidine	33.2	16	55.2	120	80	15

[a] Two replicates.

case of benzidine, recoveries are comparable in the four paints analysed, suggesting that the most influential factor in extraction should be the CO_2 solubility instead of the analyte–matrix interaction.

3.4. Determination of aromatic amines in real samples

In order to validate the proposed method of

Table 5
Recoveries for different real spiked paints tested

Paint	Recovery (%, $n=3$)			
	4-Chloro-*o*-toluidine	2-Naphthylamine	4-Aminobiphenyl	Benzidine
White	91.4±3.1	69.2±5.2	60.1±4.5	33.2±1.9
Black	87.0±2.9	82.3±4.4	74.4±3.9	32.9±2.1
Red	80.1±2.6	81.0±4.1	83.3±2.7	34.9±2.3
Magenta	76.2±1.3	79.6±3.8	76.3±2.9	34.4±3.1

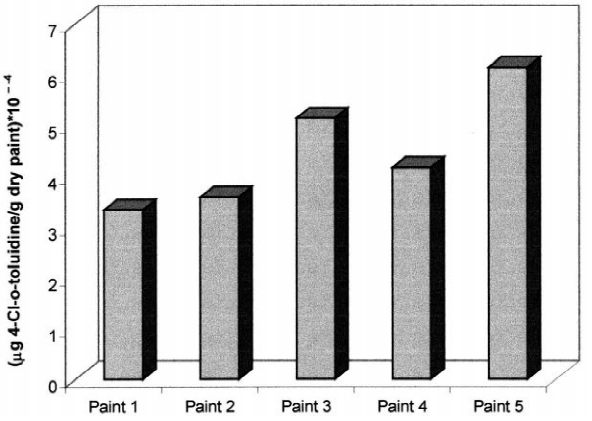

Fig. 4. 4-Chloro-*o*-toluidine extracted in different real finger-paints.

extraction for the aromatic amines, some real samples were selected and analysed. The amines were extracted under the optimum conditions and determined by GC–flame ionization detection (FID). The results obtained from four replicates are shown in Fig. 4. As can be seen, different quantities of 4-Chloro-*o*-toluidine are present in five of the samples tested, with no evidence of the other amines.

4. Conclusions

A thorough optimisation of a supercritical extraction method for the determination of primary aromatic amines in finger-paints is presented here. Parameters investigated included CO_2 pressure, extraction temperature, static extraction time and volume of modifier (methanol). The effect of these factors on recovery of aromatic amines was evaluated by a 2^4 factorial design and some second- and high-order interactions were identified. Microwave heating if compared with conventional drying in an oven, yields comparable efficiencies but a considerable reduction of time is an important advantage of this drying technique. Aged samples showed a decrease on recovery probably due to the adsorption of amines in the paint matrix. The use of other modifiers did not improve recoveries, but in the case of 4-chloro-*o*-toluidine the addition of 200 μl of methanol raised the recovery of this amine significantly. Maximum final recoveries in the white spiked paint for the amines were 91.4% for 4-chloro-*o*-toluidine, 69.2% for 2-naphthylamine, 60.2% for 4-aminobiphenyl and 33.2% for benzidine. Final conditions for maximum recovery were 13.8 MPa, 120°C, 15 min and 200 μl of methanol for 4-chloro-*o*-toluidine and 55.2 MPa, 120°C, 15 min and 80 μl of methanol for the other three amines. The influence of matrix on the extraction recovery was also studied by applying the method to different finger-paints, with similar or higher recoveries in some cases. The method was validated by extracting the aromatic amines from other commercial finger-paints, and different amounts of 4-Chloro-*o*-toluidine were found in some of the paints analysed.

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