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# Microextraction by packed sorbent and salting-out-assisted liquid–liquid extraction for the determination of aromatic amines formed from azo dyes in textiles

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ABSTRACT

EU legislation prohibits the use of certain azo dyes which, on reduction, form any of 22 aromatic amines listed in Regulation (EC) 1907/2006 at concentrations above the threshold limit of 30 mg $Kg^{-1}$.

Two different extraction techniques for the determination of aromatic amines formed from azo dyes in textiles in combination with gas chromatography–mass spectrometry (GC–MS) are described. The first one is based on microextraction by packed sorbent (MEPS) and the other approach involves salting-out-assisted liquid–liquid extraction (SALLE). The influence of several parameters on the efficiency of the extraction using MEPS (sorbent material, sample volume, elution solvent, elution volume and washing steps, among others) and SALLE (extraction volume and amount of salt) were investigated. In addition, chromatographic separation was optimized and quadrupole mass spectrometry was evaluated using the synchronous SIM/scan data acquisition mode. The repeatability ($n=8$, $S/N=3$) of the methods, calculated as the relative standard deviation (RSD) was below 15 and 11% for all compounds when MEPS and SALLE were used, respectively.

Standard additions procedure was used to quantify the aromatic amines in the textil samples. The detection limits in the samples for both methods were lower than the maximum value allowed by legislation. The results obtained in the analysis of textiles revealed the presence of o-anisidine, p-chloroaniline, 4-chloro-o-toluidine, 2-naphthylamine and 3,3′-dimethoxybenzidine in some of them.



## 1. Introduction

Azo dyes are synthetic organic colorants commonly prepared by coupling a diazonium compound with a phenol or an aromatic amine and they are used in many areas such as nutrition, cosmetics, and the paper, pharmaceutical, printing ink, textile and tanning industries, among others.

These compounds are of concern regarding human health owing to the possibility of the reduction and formation of mutagenic and carcinogenic aromatic amines. Some azo dyes may react with substances excreted from human bodies [1,2] (e.g., metabolites, sweat) to generate carcinogenic aromatic amines, causing high risks to human health and the environment. The main routes of consumer exposure to azo dyes and their degradation products [3,4] are oral ingestion (e.g., young children sucking on toys that contain dyed textile or leather garments) and absorption through the skin. In addition, under standard conditions azo dyes resist biodegradation and are therefore difficult to remove from the ecosystem.

Restrictions on the use of azo dyes are laid down in Annex XVII of the EU chemical Regulation (EC) 1907/2006 [5]. This Regulation promotes a single integrated system for the registration, evaluation and authorization of chemicals (REACH). It has been effective since 1st June 2009 and replaced Directive 76/769/EEC on the marketing and use of dangerous substances and preparations. EU legislation specifically prohibits the use of certain azo dyes which, upon reduction, form any of 22 listed amines [5] at concentrations above the threshold limit of 30 mg $Kg^{-1}$ (each amine separately). It is applicable to all textile and leather products that may come into direct and prolonged contact with the human skin or mouth. All parts of the product should comply with this limit and it is not permitted to refer to the average concentration of a certain amine in a given complete product.

Azo dyes in textiles are usually determined indirectly [6–13] by measuring the corresponding amines formed after chemical reduction. The general process comprises four steps: pre-treatment; reduction, where the azo dyes react with a reducing agent; extraction, where the amines formed are collected, and finally

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determination of the aromatic amines with analytical techniques such as high performance liquid chromatography with ultraviolet detection (HPLC–UV) [6,7], tandem mass spectrometry detection (HPLC–MS) [8] or diode array detection (HPLC–DAD) [9], gas chromatography with mass spectrometry detection (GC–MS) [10] and pyrolysis–GC–MS [11,12], which do not involve the chemical reduction of azo dyes. Solid-phase microextraction (SPME) [10], liquid–liquid extraction (LLE) [8], microwave-assisted extraction (MAE) [7,9] and supercritical fluid extraction (SFE) [6,9] are the four extraction techniques most frequently used. Non-separative methods based on infrared spectroscopy (IR) [13] and desorption electrospray ionization tandem mass spectrometry (DESI-MS) [2] have also been developed for the analysis of aromatic amines in textiles.

The official method listed in the EU legislation to detect the use of certain azo dyes employs conventional liquid–liquid extraction, which is time consuming, requires large consumption of hazardous organic solvents and it involves solvent evaporation steps.

To overcome these drawbacks, we describe the development and validation of two different methods based on microextraction by packed sorbent (MEPS) and salting-out-assisted liquid–liquid extraction (SALLE), which reduce the analysis time and the amount of organic solvent used. The first one [14,15] is based on the miniaturization of conventional solid-phase extraction (SPE). A small amount of packed sorbent inside a cartridge is placed in a syringe, and sample extraction is achieved in the packed bed. MEPS can be connected on-line to the analytical instrument for automated methods or it can be used for on-site sampling. This extraction technique has mainly been used in bioanalysis [16–18] and environmental water analysis [19–21]. SALLE is a technique based on liquid–liquid extraction in which an appropriate concentration of salt is added to achieve the separation of the aqueous phase from the partially miscible organic phase. It is highly compatible with different analytical techniques, such as GC and HPLC and it has been used above all in biological [22–24] and environmental water samples [25,26]. In this work, the organic extracts from MEPS or SALLE were analyzed using capillary gas chromatography coupled to mass spectrometry in synchronous SIM/scan data acquisition mode.

19 of the 22 amines aromatic covered by EU legislation were studied. The three other compounds (o-aminoazotoluene, 5-nitro-o-toluidine and 4-aminoazobenzene) were not considered because they have azo and nitro groups, which would be reduced in the presence of the reducing agent. Moreover, two of them (o-aminoazotoluene and 5-nitro-o-toluidine) form the reduction products o-toluidine and 2,4-diaminotoluene, both included in the EU legislation and in this work.

## 2. Experimental

### 2.1. Reagents

The 19 aromatic amines shown in Table 1 (o-toluidine, o-anisidine, p-chloroaniline, p-cresidine, 2,4,5-trimethylaniline, 4-chloro-o-toluidine, 2,4-diaminotoluene, 2,4-diaminoanisole, 2-naphthylamine, 4-aminobiphenyl, 4,4'-oxydianiline, 4,4'-methylenedianiline, benzidine, 4,4'-methylenedi-o-toluidine, 3,3'-dimethylbenzidine, 4,4'-thiodianiline, 2,2'-dichloro-4,4'-methylenedianiline, 3,3'-dichlorobenzidine and 3,3'-dimethoxybenzidine) and the textile dyes Direct Blue 15 and Chlorazol Black were supplied by Sigma-Aldrich (Steinheim, Germany). The aromatic amines were analytical standards $\geq$99% pure, except for 4-chloro-o-toluidine, 2,4-diaminotoluene, 4,4'-methylenedianiline, 2,2'-dichloro-4,4'-methylenedianiline ($\geq$98%) and 4,4'-methylenedi-o-toluidine (95%). The textile dye purity was 40 and 45% for the Direct Blue 15 and Chlorazol Black, respectively. Sodium dithionite (85%) was purchased from Acros Organics (Geel, Belgium). The solvents used were methanol from Merck (Darmstadt, Germany) and ethyl acetate and 1-propanol from Sigma-Aldrich. All the solvents were HPLC grade ($\geq$99.9). Ultrapure water was obtained using a Wasserlab water purification system (Noain, Spain).

### 2.2. Stock solutions

Stock solutions (1000 mg $L^{-1}$) of the aromatic amines were prepared in methanol and stored at 4 °C. Working solutions containing the 19 compounds studied were prepared by appropriate dilutions of the stock solutions in a citrate buffer solution (pH 6.0) containing sodium dithionite (200 mg $mL^{-1}$). Working solutions were subjected to the SALLE or MEPS process as described below and were employed to obtain the calibration curves and the detection and quantification limits.

### 2.3. Samples

3 different cotton textile samples (samples 1, 2 and 3) were analyzed. Small pieces of about 25 $mm^2$ of the textile material were cut until a weight of 0.20 g was reached.

**Table 1**
Log $K_{ow}$, p$K_a$, retention time, peak width at half height and $m/z$ ratios selected in SIM mode of the compounds studied.

| Analyte | log $K_{ow}$ | p$K_a$ | $t_R$ (min) | $W_{1/2}$ (s) | SIM group and dwell time | Quantitation ion | Qualifier ion |
|---|---|---|---|---|---|---|---|
| o-Toluidine (No. 1) | 1.32 | 4.5 | 3.218 | 0.54 | 1 (10 ms) | 106 | 107, 77 |
| o-Anisidine (No. 2) | 0.37 | 4.5 | 3.541 | 0.54 | 2 (10 ms) | 108 | 123, 80 |
| p-Chloroaniline (No. 3) | 1.83 | 4.0 | 3.639 | 0.60 | | 127 | 129, 65 |
| p-Cresidine (No. 4) | 0.81 | 4.7 | 3.854 | 0.60 | 3 (10 ms) | 122 | 137, 94 |
| 2,4,5-Trimethylaniline (No. 5) | 2.33 | 5.0 | 3.941 | 0.60 | | 120 | 135, 134 |
| 4-Chloro-o-toluidine (No. 6) | 2.18 | 3.8 | 3.969 | 0.60 | | 141 | 106, 140 |
| 2,4-Diaminotoluene (No. 7) | −0.41 | 5.1 | 4.228 | 0.60 | 4 (10 ms) | 122 | 121, 94 |
| 2,4-Diaminoanisole (No. 8) | −0.80 | 5.3 | 4.473 | 0.66 | | 123 | 138, 95 |
| 2-Naphthylamine (No. 9) | 2.61 | 4.2 | 4.820 | 0.78 | 5 (10 ms) | 143 | 115, 116 |
| 4-Aminobiphenyl (No. 10) | 3.27 | 4.3 | 5.558 | 1.14 | 6 (10 ms) | 169 | 168, 170 |
| 4,4'-Oxydianiline (No. 11) | 1.35 | 5.5 | 8.052 | 2.46 | 7 (30 ms) | 200 | 108, 171 |
| 4,4'-Methylenedianiline (No. 12) | 2.51 | 5.3 | 8.240 | 2.22 | | 198 | 197, 106 |
| Benzidine (No. 13) | 2.10 | 4.7 | 8.299 | 2.34 | | 184 | 185, 92 |
| 4,4'-Methylenedi-o-toluidine (No. 14) | 3.29 | 5.2 | 10.441 | 2.88 | 8 (100 ms) | 226 | 211, 225 |
| 3,3'-Dimethylbenzidine (No. 15) | 2.91 | 4.6 | 11.237 | 3.24 | | 212 | 196, 213 |
| 4,4'-Thiodianiline (No. 16) | 1.58 | 4.6 | 11.972 | 3.66 | | 216 | 184, 217 |
| 2,2'-Dichloro-4,4'-Methylenedianiline (No. 17) | 4.05 | 3.3 | 14.272 | 4.74 | 9 (100 ms) | 231 | 266, 140 |
| 3,3'-Dichlorobenzidine (No. 18) | 3.81 | 2.7 | 14.515 | 4.68 | | 252 | 254, 253 |
| 3,3'-Dimethoxybenzidine (No. 19) | 1.01 | 4.7 | 14.758 | 4.98 | | 244 | 201, 229 |

### 2.4. Sample preparation: reductive cleavage of the azo group

0.20 g of textile sample, 4.25 mL of citrate buffer solution (pH 6.0, preheated to 70 °C) and 0.75 mL of sodium dithionite solution (200 mg $mL^{-1}$, freshly prepared) were added to a 15-mL glass centrifuge tube with a screw cap (Scharlau, Spain). After shaking at 3000 rpm for 1 min with a Vortex device, the tube was kept at 70 °C for 30 min in an ultrasonic bath (all textile samples were submerged in the solution). Then, the tube was cooled to room temperature.

### 2.5. MEPS conditions

In the MEPS procedure, the analytes were retained in a packed sorbent inside a cartridge placed directly in a syringe. The assembly is called Barrel Insert and Needle (BIN). It was provided by SGE Analytical Science (Griesheim, Germany) and contains 4 mg of a highly porous polystyrene DVB material, HyperSep™ Retain™ PEP (polar enhanced polymer), modified with urea functional groups to give a balanced retention of polar and non-polar analytes. Particle size is 40–60 μm and pore size 60 Å. A hand-held automated analytical syringe coupled to a 500 μL MEPS syringe (SGE Analytical Science) was employed. With this, 10 different flow rates can be selected, ranging between 18 μL $s^{-1}$ and 300 μL $s^{-1}$.

The optimum conditions were as follows: the sorbent was first conditioned with two cycles, each containing 500 μL of 1-propanol and 500 μL of ultrapure water at the lowest allowed flow rate (18 μL $s^{-1}$). After shaking (3000 rpm for 1 min) and filtering the sample through a 0.45 μm PTFE filter (Scharlau, Spain), the analytes were extracted by drawing and discarding 3.0 mL of the sample (6 cycles of 500 μL at a flow rate of 18 μL $s^{-1}$) after chemical reduction. Then, the sorbent was washed with 250 μL of ultrapure water to remove possible interfering substances and the cartridge was dried by pumping air through it (10 × 500 μL) at the highest flow rate (300 μL $s^{-1}$). The analytes were eluted with 60 μL of 1-propanol (flow rate 18 μL $s^{-1}$); this volume was pumped through the cartridge and placed in the GC vial (Scharlau, Spain). After elution, the cartridge was washed with three cycles, each containing 500 μL of 1-propanol and 500 μL of ultrapure water. This latter process was performed to wash out the sorbent and prevent memory effect problems. The time needed for each MEPS extraction was about 22 min. All MEPS steps were carried out manually.

### 2.6. SALLE conditions

After chemical reduction, 750 μL of ethyl acetate (extraction solvent) was added to the glass centrifuge tube (containing the textile sample) and the mixture was shaken at 3000 rpm for 1 min. Finally, the tube was centrifuged at 4000 rpm for 5 min, after which the organic extract was filtered through a 0.45 μm PTFE filter and placed in a GC vial. The high salt concentration in the samples (buffer and reducer) was sufficient to cause the formation of two phases without the addition of any additional amount of salt. The time needed for each SALLE extraction was about 7 min. The centrifuge had 12 positions for simultaneous work.

### 2.7. GC–MS conditions

The vial containing the organic extract was placed in a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) which was equipped with two trays, each of them with 21 positions for samples. 1 μL of sample was injected in the injection port (CIS-4, Gerstel, Baltimore, MD) using the hot split mode (1:10) at 275 °C.

Gas chromatography was performed on a low-polarity DB-VRX capillary column (20 m × 0.18 mm × 1 μm) from Agilent Technologies using an Agilent 6890 gas chromatograph. The initial oven temperature was 70 °C for 1.0 min; this was increased at a rate of 60 °C $min^{-1}$ to 175 °C and then further increased at 45 °C $min^{-1}$ to 250 °C and held for 12.50 min. The total chromatographic runtime was 16.92 min. Additionally, about 4 min were necessary to re-establish the initial conditions, so the analysis time per sample was in the region of 21 min.The carrier gas was helium N50 (99.995% pure, Air Liquide), and the flow rate was 2.0 mL $min^{-1}$.

The detector was a quadrupole mass spectrometer (HP5973N) equipped with an inert ion source. It was operated in electron ionization mode using an ionization voltage of 70 eV. The ion source temperature was 230 °C, and the quadrupole was set at 150 °C. The analyses were performed in synchronous SIM/scan mode, which allowed the collection of both SIM and full scan data in a single run. Two full scan groups – 45 to 170 *m/z* from 2.50 to 7.00 min (sampling rate 1) and 45 to 270 *m/z* from 7.00 to 16.92 min (sampling rate 8) – were used for compound identification by comparison of the experimental spectra with those of the NIST'08 database (NIST/EPA/NIH Mass Spectral Library, version 2.0). Selected ion-monitoring (SIM) was used for quantification, choosing the characteristic ions in each case (Table 1), with a dwell time of 10, 30 and 100 ms for analytes No. 1–10, 11–13 and 14–19, respectively.

### 2.8. Data analysis

Data collection was performed with the Enhanced ChemStation from Agilent Technologies [27].

## 3. Results and discussion

### 3.1. Preliminary study of methods

#### 3.1.1. MEPS parameters

C18 (SGE Analytical Science), Hypercarb™ PGC (porous graphitic carbon, Thermo Scientific) and HyperSep™ Retain™ PEP (polar enhanced polymer, SGE Analytical Science) were tested as sorbent material for MEPS. The first one is a highly retentive alkyl-bonded phase for non-polar to moderately polar compounds; the second one is a material for the retention of polar compounds, and the last one is a polymeric material for polar and non-polar analytes. Beside these sorbents, four elution solvents were studied for each material: methanol, acetone, 1-propanol and a mixture of ethyl acetate and methanol (1:4, v/v). Although more apolar solvents were assayed (methyl tert-butyl ether and dichloromethane, among others), these were not used owing to the formation of two phases in the sample elution process. The high concentration of salts in the sample decreased the solubility of water in the elution solvent, meaning that despite the drying step the formation of the two steps was favoured, leading to considerable irreproducibility in the chromatographic injection. This effect was also observed when the volume of air passed through the cartridge was increased 2-, 3- and 4-fold.

The strongest signals (peak areas of the compounds in the chromatograms) were obtained when 1-propanol was used for all the sorbents tested. Accordingly, this was selected as the elution solvent.

Fig. 1 shows the chromatograms obtained when a laboratory-prepared sample spiked with the aromatic amines was subjected to the MEPS process using the different sorbent materials and 1-propanol as the elution solvent. The strongest signals were obtained when HyperSep™ Retain™ PEP was used. Accordingly, this was chosen as the sorbent material for further experiments

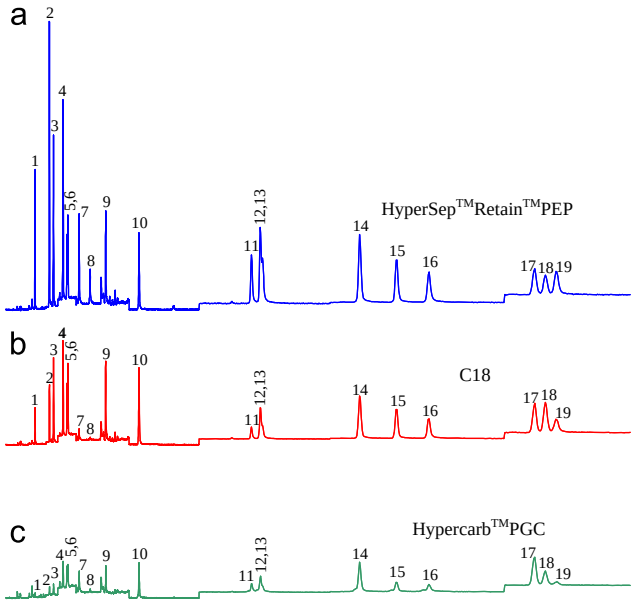


**Fig. 1.** Total ion chromatograms (SIM mode) for a laboratory-prepared solution, containing the 19 aromatic amines when different sorbent materials were tested in the MEPS procedure. The sorbents were: (a) HyperSep™ Retain™ PEP (polar enhanced polymer), (b) C18 and (c) Hypercarb™ PGC (porous graphitic carbon). All chromatograms are shown at the same scale.

due to its strong retention properties for both polar and non-polar analytes.

Elution volumes between 30 and 140 μL were studied. Not only one but also two, three and four portions were tested and each portion was analyzed separately. The sum of the analytical signals of the four portions was considered as 100%. Based on this, the percentage corresponding to each portion was calculated and in each case the sum of the first three portions of the elution solvent represented more than 90%. Fig. 2 shows the desorbed percentage obtained for o-anisidine (No. 2) and 2,2'-dichloro-4,4'-methylenedianiline (No. 17) for the different portions of solvent. The compounds are labeled with numbers according to Fig. 1 and Table 1. The percentage of compound desorbed increased with increasing elution volumes, up to 80 μL for the first nine compounds (Fig. 2a) and up to 120 μL for the last ten compounds (Fig. 2b). However, the analytical signal decreased for all the compounds when the volume was higher than 60 μL (Fig. 2c and d). In view of these observations, the percentage of desorption and the analytical signal, a final volume of 60 μL was chosen. Elution in two portions (2 × 30 μL) was also evaluated, but no improvement was achieved. With this volume (60 μL), a portion of the analytes remained retained in the MEPS BIN. Carry-over was tested by injecting a blank after the highest concentration level used in the calibrations (Table 2). To eliminate the memory effect, the sorbent was washed with one, two and three cycles, each containing 500 μL of 1-propanol and 500 μL of ultrapure water. The carry-over was

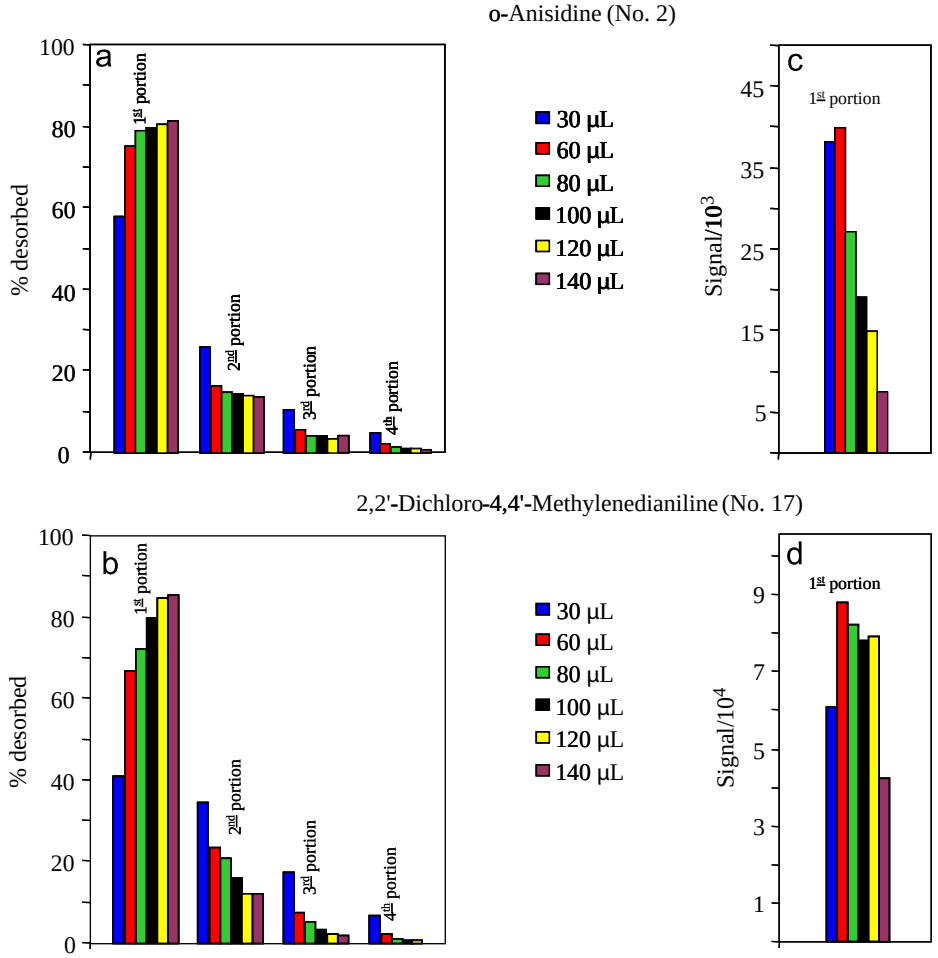


**Fig. 2.** Desorbed percentage corresponding to the first four portions of the elution solvent and analytical signal for the first portion of the elution solvent for o-anisidine (a and c) and 2,2'-dichloro-4,4'-methylenedianiline (b and d) in the MEPS procedure.

**Table 2**
Analytical characteristics of the two methods studied in ultrapure water.

| Analyte | MEPS–GC–MS | | | | SALLE–GC–MS | | |
|---|---|---|---|---|---|---|---|
| | Linearity | | Carryover % | Recovery % | Linearity | | Recovery % |
| | Range (μg $L^{-1}$) | $R^2$ | | | Range (μg $L^{-1}$) | $R^2$ | |
| 1 | DL-2.55 | 0.9994 | < 0.01 | 33 | DL-16.5 | 0.9989 | 92 |
| 2 | DL-7.67 | 0.9981 | < 0.01 | 33 | DL-17.8 | 0.9989 | 75 |
| 3 | DL-6.15 | 0.9995 | 0.09 | 72 | DL-16.1 | 0.9990 | 98 |
| 4 | DL-6.18 | 0.9994 | < 0.01 | 78 | DL-16.7 | 0.9993 | 84 |
| 5 | DL-4.92 | 0.9993 | 0.11 | 87 | DL-16.5 | 0.9989 | 89 |
| 6 | DL-5.03 | 0.9992 | < 0.01 | 96 | DL-16.0 | 0.9993 | 96 |
| 7 | DL-497 | 0.9999 | 0.80 | 2 | DL-50.4 | 0.9988 | 37 |
| 8 | DL-989 | 0.9991 | 0.82 | 1 | DL-249 | 0.9983 | 26 |
| 9 | DL-5.98 | 0.9992 | 0.04 | 72 | DL-16.3 | 0.9995 | 98 |
| 10 | DL-6.13 | 0.9996 | < 0.01 | 78 | DL-26.2 | 0.9994 | 97 |
| 11 | DL-38.2 | 0.9976 | 0.28 | 25 | DL-249 | 0.9984 | 97 |
| 12 | DL-38.4 | 0.9983 | 0.20 | 32 | DL-124 | 0.9998 | 98 |
| 13 | DL-16.0 | 0.9993 | < 0.01 | 32 | DL-63.7 | 0.9995 | 92 |
| 14 | DL-38.6 | 0.9988 | < 0.01 | 71 | DL-239 | 0.9971 | 99 |
| 15 | DL-16.0 | 0.9996 | < 0.01 | 57 | DL-253 | 0.9988 | 95 |
| 16 | DL-31.7 | 1.0000 | < 0.01 | 39 | DL-258 | 0.9983 | 84 |
| 17 | DL-123 | 0.9975 | < 0.01 | 59 | DL-260 | 0.9976 | 77 |
| 18 | DL-60.7 | 0.9991 | < 0.01 | 52 | DL-250 | 0.9982 | 68 |
| 19 | DL-96.9 | 0.9972 | < 0.01 | 44 | DL-246 | 0.9966 | 94 |

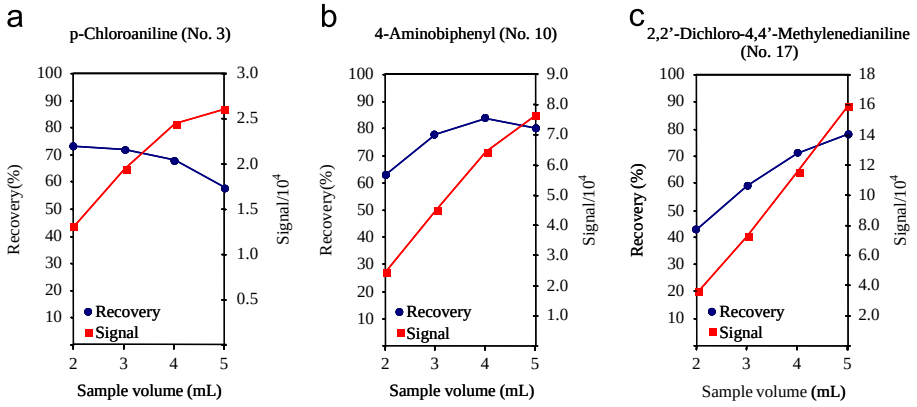


**Fig. 3.** Recoveries and signals obtained by extracting increasing sample volumes with a HyperSep™ Retain™ PEP sorbent for p-chloroaniline (a), 4-aminobiphenyl (b) and 2,2'-dichloro-4,4'-methylenedianiline (c).

higher than 1.0% for 2,4-diaminotoluene (2.1%), 2,4-diaminoanisole (2.7%) and 4,4'-oxydianiline (1.2%) when one cycle was used. When two cycles were used, only 2,4-diaminotoluene (1.1%), and 2,4-diaminoanisole (1.2%) provided values greater than 1.0%. In the case of three cycles, the carry-over was less than or equal to 0.28% for all the compounds studied (Table 2), except for 2,4-diaminotoluene (0.80%) and 2,4-diaminoanisole (0.82%). Carry-over did not decrease upon performing more than three cycles and this value was chosen for further experiments.

For extraction, the sample was pumped up once and discarded [20]. The effect of sample volume was studied using increasing volumes (2.0–5.0 mL) of a laboratory-prepared sample spiked with the aromatic amines. Recoveries were determined by comparison of the signals (peak areas) obtained by MEPS versus the signals obtained for a standard in 1-propanol injected directly into the GC–MS system at an equivalent concentration. Fig. 3 shows the recovery and the signal for p-chloroaniline (No. 3), 4-aminobiphenyl (No. 10) and 2,2'-dichloro-4,4'-methylenedianiline (No. 17) when the sample volume was increased. Assuming that the use of larger volumes could improve the detection limits, 3.0 mL was chosen as the sample volume since an acceptable recovery was achieved and the time needed for each MEPS extraction increased with the volume. As may be seen in Fig. 3, recovery decreased as the volume was increased from 3.0 to 4.0 mL for p-chloroaniline; it increased slightly in the case of 4-aminobiphenyl, and increased further in the case of 2,2'-dichloro-4,4'-methylenedianiline. The recoveries (Table 2) ranged between 25 and 96% for all the compounds except for 2,4-diaminotoluene (2%), and 2,4-diaminoanisole (1%), which were poorly extracted in the sorbent. The differences in recoveries are due to the different polarity of the analytes and, therefore, different retention in the MEPS cartridge.

### 3.1.2. SALLE parameters

The variables studied were the extraction volume and the amount of salt (NaCl). Different volumes of ethyl acetate (between 750 μL and 1000 μL) were added to the 5-mL laboratory-prepared sample spiked with the aromatic amines, observing that the strongest analytical signals were obtained with 750 μL. The use of smaller volumes hindered suitable sampling of this phase for later injection into the GC–MS system. Accordingly, that volume was chosen.

Owing to the high salt concentration in the samples (buffer and reducer) salting-out-assisted liquid–liquid extraction was tested in three different situations: without adding NaCl and adding

additional amounts of NaCl of 0.25 and 0.50 g. In all three cases, suitable phase separation was achieved and the same analytical signal was observed for all the compounds. The amount of salt in the samples was sufficient to cause the formation of two phases without the addition of any additional amount, such that it was decided not to add salt in future experiments.

Recoveries were obtained in the same way as indicated previously and are shown in Table 2. The values ranged between 68 and 99% for all the compounds except for 2,4-diaminotoluene (37%) and 2,4-diaminoanisole (26%), whose log $K_{ow}$ values are −0.41 and −0.80, respectively. This method achieved greater recoveries than the MEPS method for all the analytes studied.

### 3.1.3. GC parameters

In order to perform the separation of aromatic amines by gas chromatography, the maximum temperature ramps permitted by the oven of the chromatograph and the capillary column were chosen. Under these conditions, the initial column temperature was optimized. Values ranging between 50 and 90 °C were studied and it was set to 70 °C, affording adequate separation of the analytes without prolonging the analysis time excessively. As may be seen in Fig. 1a, 15 out of the 19 compounds appeared completely separated from the others, and the other 4 were seen in 2 partially overlapping pairs. The partial overlapping of these compounds did not prevent their individual chromatographic quantification using the extracted ion chromatograms, as may be seen for the 2,4,5-trimethylaniline/4-chloro-o-toluidine (see Fig. 4a) and 4,4'-methylendianiline/benzidine pairs of compounds (see Fig. 4b).

All the peaks had widths at half height ($W_{1/2}$) of less than 3 s (ranging from 0.54 s for o-toluidine to 2.88 s for 4,4'-methylenedi-o-toluidine), except for peaks No. 15–19 (ranging from 3.24 s for 3,3'-dimethylbenzidine to 4.98 s for 3,3'-dimethoxybenzidine). In fast GC, the usual value [28] for peak widths at half height is 0.2–3 s. Thus, this case represented a fast GC application for 14 of the 19 compounds. Table 1 shows the retention times, and the peak widths at half height.

### 3.1.4. MS parameters

Two full scan groups – 45 to 170 $m/z$ from 2.50 to 7.00 min and 45 to 270 $m/z$ from 7.00 to 16.92 min – were used. Different sampling rates were assayed (1, 2, 4 and 8). This value is related to the number of times the abundance of each mass is recorded before going on to the next mass. In the first group the peak width at half height ranged between 0.54 and 1.14 s and it became necessary to use the highest number of cycles per second permitted by the quadrupole (sampling rate 1) for the peaks to be defined suitably. In the case of the second group, the peak widths were greater (2.22–4.98 s) and this allowed suitable definition of the peaks with a lower number of scans, thus increasing the $S/N$ ratio. Sampling rate 8 was selected for scan group 2.

Different dwell times (1–100 ms) were assayed in SIM mode. The extracted ion chromatogram for o-anisidine (No. 2) and 3,3'-dichlorobenzidine (No. 18) are shown in Fig. 5. For analytes No. 1–10, a dwell time of 100 ms afforded poor peak definition owing to the few points defining it. A dwell time of 1 ms provided better peak definition than 100 ms but the noise increased. A compromise for both parameters was found when a dwell time of 10 ms was used. In the case of analytes No. 11–13 and 14–19, 30 and 100 ms were used.

## 3.2. Evaluation of the methods

For each of the methods studied, ultrapure aqueous solutions of the aromatic amines (with the same proportion of reducing agent and buffer solution as the samples) were prepared at 6 different concentrations (Table 2). Calibration samples were not subjected to the heating process (70 °C, 30 min), because the same analytical signals were obtained in both situations, as expected. MEPS and SALLE were carried out and the organic extract was analyzed using GC–MS (three replicates per calibration standard). The analytical signals used for the calibration curves were the peak areas of the compounds in the extracted ion chromatograms (SIM mode) for the quantitation of the ions shown in Table 1. All calibrations showed good linear behavior and the values of the coefficient of determination ($R^2$) were satisfactory for all the compounds using both methods, as shown in Table 2. The models did not show any lack of fit. The limits of detection and quantification were calculated as 3 and 10 times, respectively, the standard deviation of a standard solution ($n=8$), which provided an $S/N$ ratio of approximately 3, divided by the slope of the calibration straight line. The limits of detection in ultrapure water (Table 3) were within the 0.040 and 42 μg $L^{-1}$ range for the method in which MEPS was used and ranged between 0.10 and 3.7 μg $L^{-1}$ for the method based on SALLE. The improvement in the detection limits ranged between 2- and 14-fold for 10 of the compounds (analytes No. 4–6, 9, 10, 14, 15, 17–19) when MEPS was used in comparison to SALLE. Although recovery was higher when SALLE was used, the improvement in the limits of detection was due to the increase in the preconcentration with MEPS on using a lower extraction volume (60 μL) than with SALLE (750 μL). In the case of 2,4-diaminotoluene (No. 7) and 2,4-diaminoanisole (No. 8), the detection limits for SALLE were 10 and 23 times lower, respectively, than those found with MEPS since they were poorly extracted in the sorbent. For the other 7 compounds (analytes No. 1–3, 11–13,16), the limits were similar. Repeatability and reproducibility were determined using a standard solution, which provided an $S/N$ ratio of approximately 3. Repeatability was

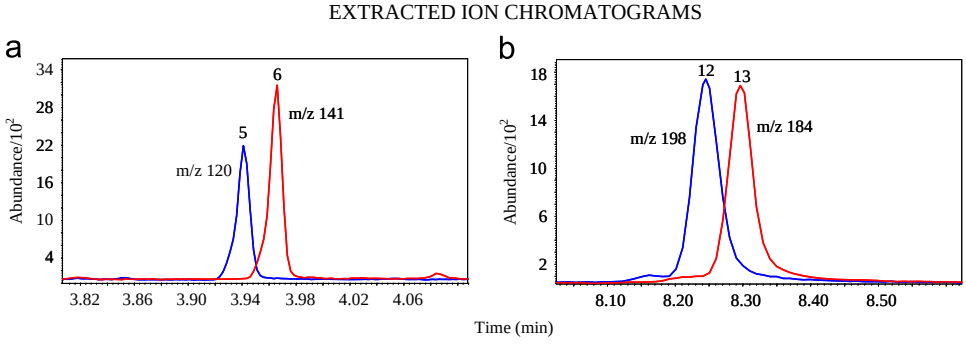


**Fig. 4.** Extracted ion chromatograms (SIM mode) for a laboratory-prepared solution, containing the 19 aromatic amines, corresponding to the pairs of compounds (a) 2,4,5-trimethylaniline ($m/z$ 120)/4-chloro-o-toluidine ($m/z$ 141), and (b) 4,4'-methylenedianiline ($m/z$ 198)/benzidine ($m/z$ 184). The compounds are labeled with numbers as in Fig. 1.

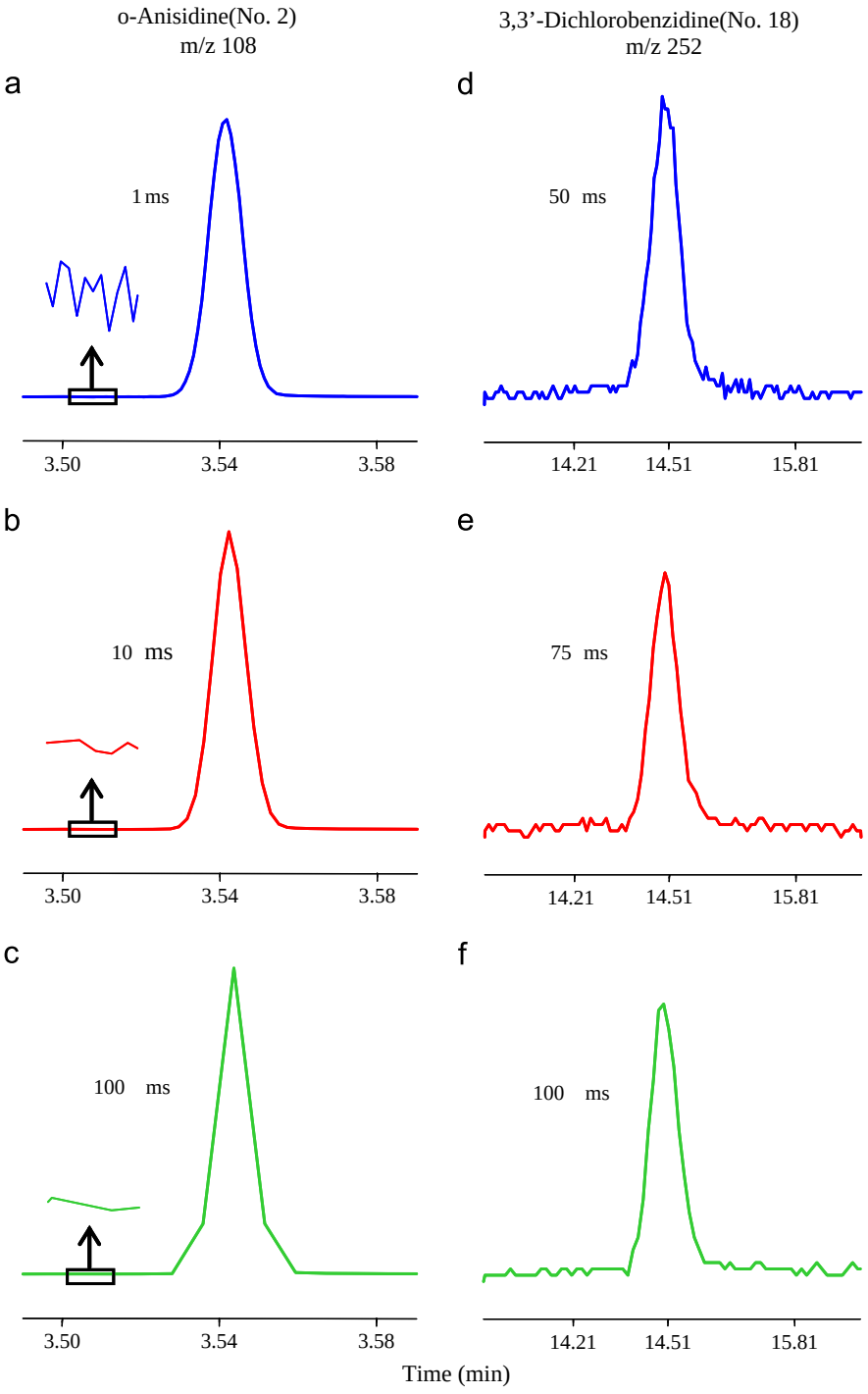


**Fig. 5.** Extracted ion chromatograms (SIM mode) for a laboratory-prepared solution corresponding to o-anisidine (a–c) and 3,3'-dichlorobenzidine (d,–f) when different dwell times were tested.

evaluated by performing extraction and injection into GC–MS 8 times on the same day. The relative standard deviation (RSD) was lower than or equal to 15% for both methods (Table 3), indicating good precision. To determine reproducibility, extraction and injection were performed 8 times per day on 3 days. In all cases, the RSD was lower than or equal to 22% (Table 3), indicating the acceptable reproducibility of the methods.

The proposed methods have some advantages over those reported in the literature. In particular, methods based on HPLC [6–9] have longer chromatographic run times (97, 97, 29.5 and 55 min taking into account the chromatographic run time and the post-run time for equilibration of the column) than the proposed method (21 min). The processes of the extraction of the aromatic amines in these four methods were MAE [6,7,9], SFE [9] and liquid–liquid extraction [8]. The extraction times for the methods based on MAE (30 min) and SFE (45 min) were longer than that described for the present working method with SALLE (7 min) and MEPS (22 min). In some investigations [6,7,9] the time spent in reducing the azo groups was shorter than that used here (5–10 as compared with 30 min). The volume of extractant used in the

**Table 3**
Repeatability, reproducibility and detection (DL) and quantification limits (QL) in ultrapure water for the two methods studied.

| Analyte | MEPS–GC–MS | | | | SALLE–GC–MS | | | |
|---|---|---|---|---|---|---|---|---|
| | Repeatability (RSD %) | Reproducibility (RSD %) | DL ($\mu g\ L^{-1}$) | QL ($\mu g\ L^{-1}$) | Repeatability (RSD %) | Reproducibility (RSD %) | DL ($\mu g\ L^{-1}$) | QL ($\mu g\ L^{-1}$) |
| 1 | 4 | 9 | 0.089 | 0.30 | 4 | 16 | 0.10 | 0.35 |
| 2 | 8 | 9 | 0.17 | 0.57 | 9 | 15 | 0.22 | 0.73 |
| 3 | 8 | 10 | 0.19 | 0.63 | 5 | 13 | 0.15 | 0.49 |
| 4 | 6 | 11 | 0.040 | 0.13 | 7 | 12 | 0.19 | 0.65 |
| 5 | 9 | 9 | 0.060 | 0.20 | 8 | 13 | 0.24 | 0.79 |
| 6 | 4 | 9 | 0.051 | 0.17 | 3 | 15 | 0.15 | 0.50 |
| 7 | 14 | 20 | 4.6 | 15 | 6 | 9 | 0.47 | 1.5 |
| 8 | 15 | 22 | 42 | 139 | 5 | 18 | 1.8 | 5.9 |
| 9 | 2 | 9 | 0.074 | 0.25 | 6 | 11 | 0.16 | 0.54 |
| 10 | 7 | 6 | 0.13 | 0.42 | 6 | 7 | 0.24 | 0.81 |
| 11 | 5 | 16 | 1.1 | 3.8 | 6 | 5 | 1.4 | 4.6 |
| 12 | 5 | 15 | 1.1 | 3.6 | 5 | 9 | 1.2 | 3.9 |
| 13 | 7 | 17 | 0.34 | 1.1 | 3 | 9 | 0.36 | 1.2 |
| 14 | 5 | 15 | 0.76 | 2.5 | 5 | 7 | 1.7 | 5.8 |
| 15 | 4 | 15 | 0.47 | 1.6 | 6 | 9 | 1.6 | 5.4 |
| 16 | 4 | 15 | 1.4 | 4.7 | 4 | 8 | 1.1 | 3.7 |
| 17 | 7 | 11 | 0.80 | 2.7 | 7 | 9 | 3.7 | 12 |
| 18 | 2 | 10 | 0.20 | 0.67 | 5 | 14 | 2.7 | 9.1 |
| 19 | 3 | 16 | 1.0 | 3.4 | 11 | 17 | 1.8 | 6.1 |

proposed SALLE method (750 μL of ethyl acetate) was lower than that used in the liquid–liquid extraction method (40 mL, methyl tert-butyl ether) cited previously [8].

Regarding the methods reported in the literature based on GC [10–12], the chromatographic run times (25, 12 and 12 min) were similar to that used here. The extraction of compounds was based on SPME in the first work [10] and on thermal extraction by pyrolysis in the other two. In the SPME method, the detection limits for 2,4-diaminotoluene and 2,4-diaminoanisole were higher (75 mg $Kg^{-1}$) than that permitted by the legislation (30 mg $Kg^{-1}$) owing to the low extraction efficiency of the fiber (CW-DVB). Pyrolysis–GC has been used for the qualitative analysis of aromatic amines, allowing a reduction in the analysis time because no sample preparation (chemical reduction treatment) is required. However, false negatives have been reported [11,12], because some aromatic amines are not released from the dye by thermal degradation of the azo bonds without chemical reduction treatment.

### 3.3. Reductive cleavage of the azo group in textiles

Initially we tested a method [10] that includes the following steps: 0.20 g of textile sample and 4.25 mL of citrate buffer solution (pH 6.0, preheated to 70 °C) were added to a 15-mL glass centrifuge tube with a screw cap. After shaking at 3000 rpm for 1 min, the tube was kept at 70 °C for 30 min. Then, the tube was opened and 0.75 mL of sodium dithionite solution (200 mg $mL^{-1}$) was added. The tube, after shaking at 3000 rpm for 1 min is re-maintained at 70 °C for 30 min in an ultrasonic bath. Following this, the tube was cooled to room temperature. The results obtained with this method were compared with those of another in which the heating step was omitted prior to the addition of the reducing agent. The analytical signals of the aromatic amines formed were similar, such that it was decided to remove this step in order to reduce the time of analysis. Additionally, it was decided to submerge the glass centrifuge tube in an ultrasonic bath during the reduction step to favor the reaction.

To eliminate suspended particles in the samples, they were filtered after chemical reduction, as detailed in the experimental section. PTFE and Nylon filters, both 0.45 μm pore size, were assayed. Because 2,2'-dichloro-4,4'-methylenedianiline and 3,3'-dichlorobenzidine were adsorbed in the Nylon filter, a PTFE filter was used in all the experiments.

### 3.4. Determination of aromatic amines in different textiles and azo dyes

The determination of aromatic amines using MEPS–GC–MS and SALLE–GC–MS was carried out with three different textile samples. In order to check the possible existence of a matrix effect, a study was undertaken in which the signals obtained in two different samples were compared. The first was a solution of the 19 compounds in ultrapure water (with the same proportion of reducing agent and buffer solution as the samples) and the second was the textile sample spiked with all the analytes that were not detected in the sample at the same proportions as above. If no matrix effect occurs, both signals should be similar. After chemical reduction, the samples were subjected to the MEPS and SALLE process and then to GC–MS analysis. The samples containing the textile always provided lower signals than those corresponding to the ultrapure water. These results were confirmed at different concentrations and a matrix effect was found in all the samples. In view of this, quantification was performed with the standard additions method. Although the limits of detection depend on the type of sample, the values found for the three samples studied with MEPS and SALLE ranged between 0.0034 and 1.2 mg $Kg^{-1}$ for all the analytes with the exception of 2,4-diaminotoluene (0.30 and 5.1 mg $Kg^{-1}$ with SALLE and MEPS, respectively) and 2,4-diaminoanisole (1.2 and 16 mg $Kg^{-1}$ with SALLE and MEPS, respectively). As expected, the limits of detection in the samples were higher than those obtained in aqueous solution without a textile sample (Table 3), although in all cases the values were lower than the maximum permitted by current legislation.

The following compounds were found in the textile samples: o-anisidine (sample No. 1), p-chloroaniline (samples No. 2 and 3), 4-chloro-o-toluidine (sample No. 1), 2-naphthylamine (sample No. 3) and 3,3'-dimethoxybenzidine (sample No. 3).

Fig. 6 shows the chromatograms obtained for the sample No. 3 when the MEPS–GC–MS and SALLE–GC–MS methods were used. The synchronous SIM/scan detector mode allowed the collection of both SIM and full scan data in a single run. Fig. 6a, c, e and g corresponds to chromatograms of all the ions both in scan mode (Fig. 6a and e) and in SIM mode (Fig. 6c and g). Fig. 6b, d, f and h are plots of the chromatograms of the extracted ions in scan (Fig. 6b and f) and SIM (Fig. 6d and h) mode. In the scan mode chromatograms, with the database it was possible to identify the

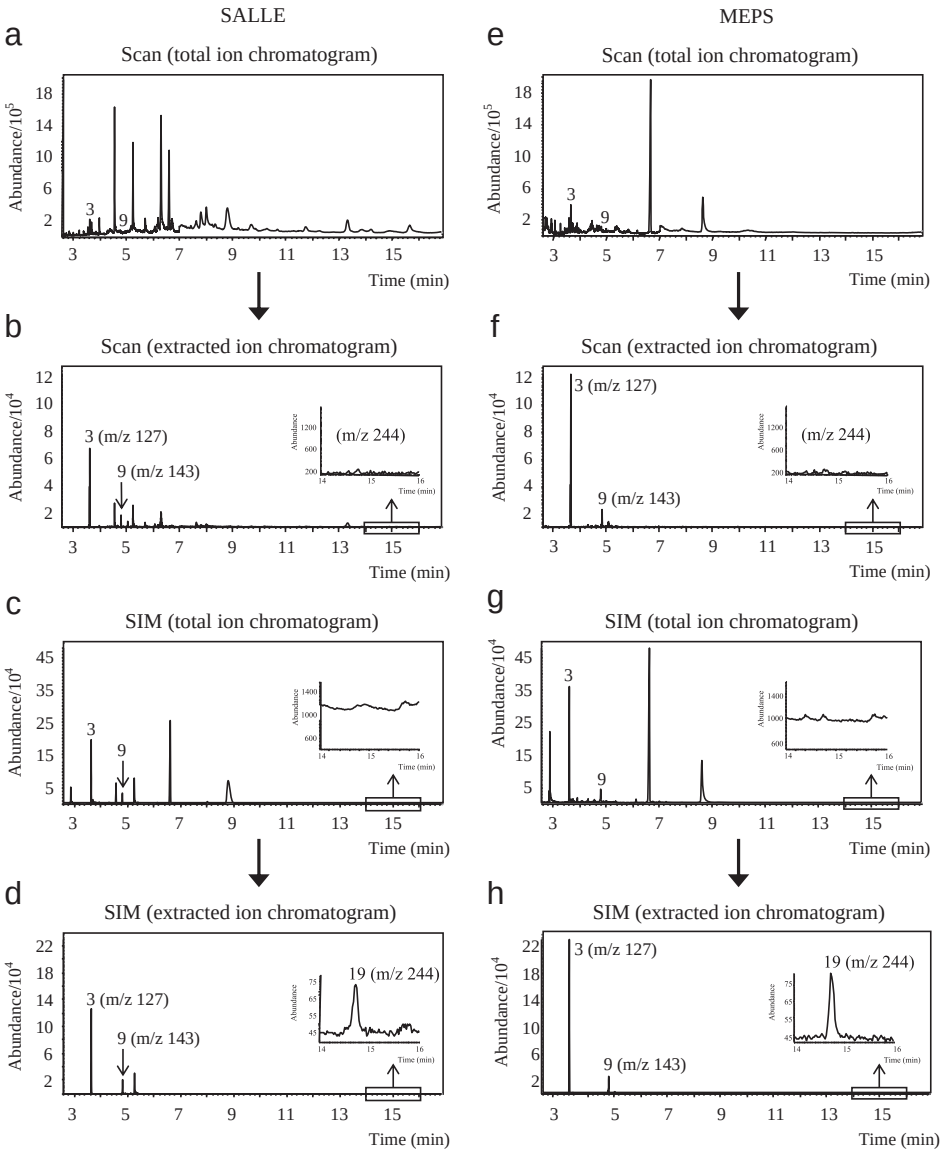


**Fig. 6.** Chromatograms for textile sample No. 3 in scan and SIM mode for the SALLE–GC–MS (a–d) and MEPS–GC–MS (e–h) methods. b and f corresponds to extracted ion chromatograms in scan mode. d and h corresponds to extracted ion chromatograms in SIM mode.

analytes p-chloroaniline (No. 3) and 2-naphthylamine (No. 9). However, it was not possible to identify 3,3'-dimethoxybenzidine (No. 19) owing to its low abundance. Similarly, this analyte was not detected in the extracted ion chromatograms (quantitation ion) in scan mode. According to the chromatograms of all the ions in scan mode (Fig. 6a and e), the organic extract obtained after SALLE extraction contained a higher number of co-extracted matrix compounds than that obtained with MEPS, as expected. The most abundant analytes identified in Fig. 6a were undecanoic, dodecanoic and tridecanoic acids. These compounds may sometimes interfere in the determination of the analytes of interest, but the use of mass spectrometry can reduce this problem via the extracted ion chromatograms. Moreover, in the cases with an important overlapping of peaks it could be interesting to perform extraction with MEPS, since this is less affected by interferents. The chromatograms obtained in SIM mode were used for the quantification of the analytes. In the extracted ion chromatograms in SIM mode (Fig. 6d and h) 3,3'-dimethoxybenzidine (No. 19) was found. It was possible to assign this compound correctly because its $t_R$ and the abundance ratios of the $m/z$ recorded (quantifying and qualifying ions) coincide with those of the standard.

**Table 4**
Concentration range (mg $Kg^{-1}$) for the standard additions, predicted concentration (mg $Kg^{-1}$) and confidence interval (95% probability) for the compounds in the textile samples with the two methods studied.

| Analyte | Method | Standard additions | Textile No. 1 | Textile No. 2 | Textile No. 3 |
|---|---|---|---|---|---|
| 2 | MEPS | 0–0.27 | $0.070 \pm 0.005$ | | |
| | SALLE | | $0.072 \pm 0.007$ | | |
| 3 | MEPS | 0–0.30 | | $0.27 \pm 0.02$ | $11 \pm 1$[a] |
| | SALLE | | | $0.27 \pm 0.02$ | $11 \pm 1$[a] |
| 6 | MEPS | 0–0.10 | $0.040 \pm 0.002$ | | |
| | SALLE | | $0.042 \pm 0.005$ | | |
| 9 | MEPS | 0–0.15 | | | $2.5 \pm 0.2$[a] |
| | SALLE | | | | $2.5 \pm 0.2$[a] |
| 19 | MEPS | 0–0.50 | | | $0.37 \pm 0.05$ |
| | SALLE | | | | $0.37 \pm 0.05$ |

[a] The sample was diluted 50-fold for quantification of the analyte.

Table 4 shows the different analytes found in the samples, the concentration range for the standard additions set (five concentration levels), the concentrations obtained using MEPS and SALLE,

and their confidence intervals (95% probability). The results obtained in both cases were similar for all the samples and they were lower than the maximum value allowed by the legislation.

To check the possibilities of the methods for compounds that were not detected, the samples were spiked with them in a concentration range between 0.040 and 4.85 mg $Kg^{-1}$. Accuracy was determined by comparison of the amount added and the predicted value using the standard additions method. It ranged between 81–118% and 80–110% for MEPS and SALLE, respectively. These results highlight the applicability of the proposed methods for the quantification of these compounds in textiles.

In addition, two azo dyes were analyzed (Chlorazol Black and Direct Blue 15). Ultrapure water solutions spiked with the dyes (500 μg $L^{-1}$) were prepared and were subjected to the chemical reduction process. Following this, they were analyzed using the MEPS–GC–MS and SALLE–GC–MS methods. As expected, the aromatic amines benzidine and 3,3′-dimethoxybenzidine were released from the dyes Chlorazol Black and Direct Blue 15, respectively. Similar concentrations were obtained with the MEPS and SALLE methods for both analytes and the results pointed to a reduction efficiency of 47 and 23% for Chlorazol Black and Direct Blue 15, respectively. In order to approximate the process of the chemical reduction to biological reduction in humans, the reduction step must be carried out under conditions no more drastic than here to ensure that the amine will be formed as a result of the reduction of azo groups and not through other decomposition reactions [13].

## 4. Conclusions

Two new methods for the determination of 19 aromatic amines formed from azo dyes in textiles have been implemented based on extraction and preconcentration by MEPS and SALLE. In both methods, gas chromatography with mass spectrometry detection (synchronous SIM/scan data acquisition mode) was used. In the method based on MEPS, lower detection limits were obtained in comparison to SALLE. In addition, samples after MEPS procedure contained less interfering compounds from the matrix. However, the best recoveries were obtained with SALLE. Furthermore, the time required for extraction and preconcentration of the analytes is lower with SALLE in comparison to MEPS

Good results were achieved for all the compounds in terms of linearity, repeatability and reproducibility. Only one HyperSep™ Retain™ PEP MEPS BIN was used in this work and it continues to work properly; this can be considered proof of the good robustness and stability of the MEPS procedure. Because there was no significant variability in either sample preparation or in sample injection, no internal standard was used.

After the chemical reduction of azo dyes from textiles, aromatic amines were successfully quantified using the standard additions procedure since a matrix effect was observed. The detection limits in the samples of all the compounds were lower than the maximum permitted value by EU legislation (30 mg $Kg^{-1}$).

The proposed methods reduce the time of analysis in both the analyte extraction step [6,7,9], and in chromatographic separation [6–10].

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