Talanta 119 (2014) 375–384

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

Miguel del Nogal Sánchez, Patricia Martín Santos, Cristina Pérez Sappó, José Luis Pérez Pavón*, Bernardo Moreno Cordero

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, Universidad de Salamanca, 37008 Salamanca, Spain

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
8 November 2013
Accepted 14 November 2013
Available online 21 November 2013

Keywords:
Aromatic amines
Microextraction by packed sorbent
Salting-out-assisted liquid–liquid extraction
Mass spectrometry

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

---

* Corresponding author. Fax: +34 923 294483.
E-mail address: jlpp@usal.es (J.L.P. Pavón).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.041

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}, pK_a, retention time, peak width at half height and m/z ratios selected in SIM mode of the compounds studied.

Analyte	log K_{ow}	pK_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