AN ULTRASOUND-ASSISTED EXTRACTION PROCEDURE FOR THE DETERMINATION OF BANNED AZO DYES IN LEATHER

by

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ABSTRACT

The potential risk for consumers to be exposed to banned azo dyes in leather is strong motivation for the development of an analytical test procedure with improved accuracy. The currently most accepted analytical procedure for this application, the German standard DIN 53316 method, suffers from major drawbacks including low accuracy and the usage of hazardous organic solvents. As a consequence, an alternative procedure based on ultrasound-assisted extraction (UAE) was developed for the determination of banned azo dyes in leather. The optimized UAE procedure was applied to bovine, sheep, and goat leather. These results were compared with those obtained by employing the DIN 53316 method or microwave-assisted extraction (MAE). Usually, the performance of UAE was better than the DIN 53316 method and comparable to MAE. Furthermore, the UAE procedure utilizes acidified water as extraction solvent rather than methyl *tert.*-butyl ether. Because of its simplicity and the inexpensive equipment required, the proposed procedure has the potential to become the preferred alternative for analyzing banned azo dyes in leather on a routine basis.

ABSTRACTO

El riesgo potencial para los consumidores que están expuestos a colorantes azoicos prohibidos redunda en una fuerte motivación para desarrollar un procedimiento de evaluación analítica con una exactitud mejorada. El procedimiento analítico más aceptado actualmente para esta aplicación, el método standard alemán DIN 53316, adolece de grandes desventajas como poca exactitud y el uso de solventes orgánicos peligrosos. Como consecuencia, se desarrolló un procedimiento alternativo basado en una extracción asistida con ultrasonido (UAE) para detectar colorantes azoicos prohibidos en los cueros. El método UAE mejorado fue aplicado a cueros bovinos, ovinos y caprinos. Estos resultados fueron comparados con los obtenidos al emplear el método DIN 53316 o la extracción asistida por microondas (MAE). Usualmente, el rendimiento de la UAE fue mejor que el método DIN 53316 y comparable con la MAE. Más aún, los procedimientos de la UAE utilizan agua acidificada como solvente de extracción en vez del metil *tert*-butil éter. Debido a su simplicidad y al equipo de bajo costo requerido, el procedimiento propuesto tiene el poder de transformarse en la alternativa preferida para analizar colorantes azoicos prohibidos en cueros en forma rutinaria.

INTRODUCTION

The discovery that human skin bacteria can reduce azo dyes, forming carcinogenic aromatic amines[1], has raised concern within the European Union (EU) about the health risk to consumers, exposed to these harmful substances through wearing leather products. The resulting EU ban[2] on such azo dyes, which may form any of 22 listed amines after reduction, motivates the development of accurate and easily applied analytical test procedures.

Determination of banned azo dyes in leather usually comprises four steps: (1) degreasing to facilitate sample wetting, (2) reduction of the dyes, (3) extraction of the amines formed, and finally (4) determination of the amines with an analytical technique.[3] The German DIN 53316 method[4] is currently the most widely accepted analytical method for this application. Unfortunately, this method is time-consuming, requires use of hazardous organic solvents, and is characterized by relatively low accuracy.

In a previous study[5], we optimized experimental conditions in different steps (i.e. degreasing, reduction, and extraction) of an analytical procedure reported by Eskilsson et al.[6] for the determination of banned azo dyes in different types of leather. In this procedure, degreasing was performed by use of supercritical-fluid extraction (SFE), whereas reduction and extractions were performed by use of microwave-assisted extraction (MAE). Even though substantial improvements in accuracy were achieved, the recoveries obtained were still unacceptably low (<60 percent) for some of the dyes. It was recently shown that by applying standard addition

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methodology, using the corresponding azo dyes as spiking agents, instead of external standard calibration, nearly 100 percent recovery for most of the investigated dyes could be achieved.[7] However, an analytical procedure based on SFE and MAE is not feasible for use on a routine basis, mainly due to high equipment costs and the need for skilled operators.

Use of ultrasound to enhance extraction and digestion in sample preparation is a growing trend.[8] The principal devices used in sonochemistry are baths and probes, the former being predominant. Ultrasound-assisted extraction (UAE) is a simple and inexpensive technique that has proven to be useful in various applications, for example in the determination of pesticides[9-11] and metals[12,13] in soil and for isolation of natural compounds from plants.[14-16] In some applications, the performance of UAE is even comparable to that of SFE and MAE.[17-18]

The aim of the present study was to investigate the possibility of developing a simple UAE procedure, which would be more accurate and environmentally friendly than the prevailing DIN 53316 method[4] while in addition providing results in good agreement with the equipment-intensive MAE procedures.[5,7]

EXPERIMENTAL

Samples

Samples of bovine, sheep, and goat leather, processed at the Research Center for Leather and Artificial Leather (FILK, Freiberg, Germany), were used in this study. Processing steps prior to analysis consisted of dyeing, waterproofing, and grinding to a particle size no greater than 4 mm. The dyes considered were Acid Red 035, Acid Orange 031, Acid Black 077, Acid Black 209, Direct Blue 015, Direct Red 061, and Acid Red 4. The aromatic amines formed after reduction of these dyes are *o*-toluidine, 4-chloroaniline, benzidine, 3,3-dimethylbenzidine, 3,3-dimethoxybenzidine, 3,3-dichlorobenzidine, and 2-methoxyaniline, respectively. Sample information is given in Table I.

Chemicals

Methanol, cyclohexane, methyl tert.-butyl ether (LiChroSolv gradient grade), hydrochloric acid (HCl, 37 percent, pro analysis), and citric acid/sodium hydroxide buffer (CertiPUR, pH 6 at 20°C) were purchased from Merck (Darmstadt, Germany). Water was produced by a Milli-Q Ultra-pure water-purification system (Millipore, Bedford, MA, USA). Sodium dithionite (85 percent, Acros Organics, Geel, Belgium) dissolved in Milli-Q water was used as reducing agent. Carbon dioxide (99.998 percent purity, AGA Gas, Sundbyberg, Sweden) was used as extraction fluid throughout the SFE experiments. Benzidine (≥98 percent), 3,3'-dimethoxybenzidine (≥97 percent), and 3,3'-dimethylbenzidine (≥ 98 percent) were purchased from Fluka (Buchs, Switzerland), *o*-toluidine (>99 percent) and 3,3'-dichlorobenzidine (>99 percent) from Sigma-Aldrich (Steinheim, Germany), and finally 4-chloroaniline (Pestanal) from Riedel-de Haën (Seelze, Germany). Mixed stock solutions of the dyes were prepared by dissolution in methanol. These solutions were then further diluted to appropriate concentrations as needed. Stock solutions of each amine were prepared in methanol at a concentration of 500 µg mL^{-1}. Mixed standard solutions of amines were prepared by mixing the stock solutions and then diluting these to appropriate concentrations with methanol.

TABLE I
Sample Information

Leather	Banned azo dyes		Amines	
	Name	Concentration (mg kg^{-1} leather)	Name	Concentration (mg kg^{-1} leather) ± c.i.
Bovine	Acid Red 4	267	2-Methoxyaniline	32 ± 42
	Acid Red 035	381	*o*-Toluidine	40 ± 14
	Acid Black 077	811	Benzidine	73 ± 56
	Acid Orange 031	218	4-Chloroaniline	44 ± 10
	Direct Blue 015	400	3,3′-Dimethoxybenzidine	64 ± 19
	Acid Black 209	590	3,3′-Dimethylbenzidine	53 ± 50
	Direct Red 061	290	3,3′-Dichlorobenzidine	58 ± 20
Sheep	Acid Black 077	500	Benzidine	45 ± 56
	Acid Black 209	367	3,3′-Dimethylbenzidine	33 ± 50
Goat	Acid Red 035	276	*o*-Toluidine	29 ± 14
	Direct Blue 015	256	3,3′-Dimethoxybenzidine	41 ± 19

c.i. confidence interval (%) at 95% level

Equipment

SFE was performed with a Hewlett-Packard (Wilmington, DE, USA, HP) 7680T supercritical-fluid extractor equipped with standard 7-mL stainless steel extraction thimbles. The extracted compounds were collected on an HP standard solid-phase trap packed with octadecyl silica (ODS).

UAE was performed by use of two different ultrasonication baths: a Branson 3200 (50 kHz, Branson Ultrasonics Corporation, Danbury, USA), equipped with digital timer (0-99 min) and a digital heat control (0-65°C), and a Sonorex Super RK 100 H (35 kHz, Bandelin Electronic, Berlin, Germany), equipped with a manual timer (1-15 min or continuous) and manual heat control (30-80°C).

High-performance liquid chromatography (HPLC) analysis was performed with an HP (Palo Alto, CA, USA) modular system which consisted of an 1100 Series quaternary pump, an 1100 Series vacuum degasser, an 1100 Series autosampler, and a 1050 Series multiple wavelength detector. Data acquisition was performed using HP ChemStation software (Rev. A.04.01).

Analytical procedures

In this study, two UAE procedures, differing in the quantification method applied, were developed. Quantification was accomplished either by external standard calibration or by standard addition of azo dyes. The UAE procedures were compared to procedures based on the DIN 53316 method[4] or MAE.[5,7] A brief description of the different procedures considered follows below.

All samples were degreased with SFE to enable an equitable comparison of the reduction and extraction efficiencies of the different analytical procedures. SFE was performed in the dynamic mode at a pressure of 13.8 MPa and a temperature of 40°C for 30 min using neat carbon dioxide, delivered at a flow-rate of 4 mL min^{-1}.

In all procedures, final determination of the dyes was performed indirectly by measuring the corresponding amines, formed after reduction, in sample extracts by means of HPLC.

Ultrasound-assisted extraction

Degreased leather samples (1 g) were weighed and quantitatively transferred into 50-mL glass flasks. Determinations based on external standard calibration were performed using non-spiked samples whereas standard additions were performed by spiking degreased leather samples with 1 mL of azo dye solution at four different levels: 0, 0.25, 1, and 4 times the concentrations of dyes present in the samples (Table I). After spiking, the solution was allowed to penetrate the matrix for about 10 min. An amount of 20 mL buffer (pH 6) and 1 mL of freshly prepared dithionite solution (0.3 g mL^{-1}) were then added to the samples for the reductive step. The flasks were sealed with screw caps, shaken briefly to ensure wetting of the entire sample, and then placed in the ultrasonication bath. The reduction process was performed by use of UAE under conditions selected. After reduction, during which time extraction is also initiated, the resulting solutions were filtered through 1.2 µm glass microfiber filters (GF/C, Whatman, Maidstone, UK) and collected in 50-mL volumetric flasks. Three consecutive further extractions of the leather matrix were performed by UAE with 8 mL of 1 M HCl each to release the amines from the matrix. After each extraction, the extracts were filtered and collected using the same filters and volumetric flasks as used in the reduction step. On completion of the extraction procedure, the pH of the extracts obtained was adjusted to 6 using ammonia and the extracts were diluted to 50 mL with buffer.

DIN 53316

Quantities of 17 mL of pre-heated buffer (70 ± 5°C, pH 6) were added to 50-mL glass flasks, each containing 1 g of degreased leather sample, which were then sealed and kept in a water bath at 70°C for 30 min. The samples were then treated twice with 1.5 mL of aqueous dithionite (0.2 g mL^{-1}) at 70°C for a total heating time of 20 min. The amines formed were transferred to a methyl tert.-butyl ether (MTBE) phase by means of liquid-liquid extraction (25-30 min) using kiselguhr columns (Extrelut® NT 20, Merck). The MTBE extracts (40 mL) were then concentrated under mild conditions and the residue was dissolved in methanol prior to chromatographic analysis. Further details regarding experimental conditions are given elsewhere.[4]

Microwave-assisted extraction

Degreased leather samples (1 g) were weighed and transferred quantitatively into 100-mL MAE vessels. In accordance with the UAE procedure, determinations based on external standard calibration were performed using non-spiked samples, whereas standard additions were performed by spiking degreased leather samples at four different concentration levels. An amount of 20 mL buffer (pH 6) and 1 mL freshly prepared dithionite solution were added to the samples. The vessels were then placed in the microwave oven and the reduction process was initiated under selected conditions. After reduction, the samples were filtered and subsequently extracted by MAE three times at 40°C for 10 min each using 8 mL extraction solvent. Further details regarding experimental conditions of the MAE procedures are given elsewhere.[5,7]

Chromatographic analysis

The extracts were analyzed by injecting 10 µL aliquots into the HPLC system. Separation of the amines was accomplished by gradient elution at ambient temperature on a LiChrospher® 60 RP- Select B column (Merck, 250 mm x 4 mm ID, 5 µm particle size), preceded by a guard-column (4 mm x 4 mm) of the same packing material. The mobile phase was composed of phosphate buffer (3 mM, pH 7) (eluent A) and methanol (eluent B). Linear gradient elution was performed from 25 to 80 percent of eluent B in A within 70 min at a flow-rate of 0.3 mL min^{-1}. The system then returned to its initial conditions within 2 min and was re-equilibrated for 20 min, giving a total analysis time of 92 min. Benzidine, 3,3-dimethoxybenzidine, 3,3-dimethylbenzidine, and 3,3-dichlorobenzidine were detected at 280 nm, and 2-methoxyaniline, 4-chloroaniline, and *o*-toluidine were detected at 240 nm. Quantification was based on peak height and identification was based on the

retention times of amine standards. Calibration curves for the amines were constructed after analysis of external standards at five concentration levels. Each level was analyzed in triplicate, and chromatographic peak heights were fitted by linear least-squares regression. The corresponding regression coefficients (R^2) were always higher than 0.999 for the dynamic range studied (1-50 ppm).

RESULTS AND DISCUSSION

Degreasing

Degreasing of leather samples by SFE results in better selectivity, with respect to co-extracted azo dyes, than UAE.[6] However, for laboratories without access to expensive SFE equipment, efficient degreasing of the leather samples can be accomplished by ultrasonication for 2 x 20 min at 40°C using 20 mL of *n*-hexane (as in the DIN 53316 method[4]).

Optimization of UAE

Bovine leather was chosen for the optimization of UAE conditions because it was believed to be the most complex matrix of those available (i.e. bovine, sheep, and goat). All experiments were performed by employing the Branson equipment.

Reduction

Because the optimum dithionite concentration (0.3 g g^{-1} sample) for the leather samples used in this study had previously been established[5], the influence of reaction time and temperature on the reduction by use of ultrasound treatment was initially investigated. Accordingly, azo dyes in samples of bovine leather were reduced in the ultrasonication bath for different times (5 or 10 min) and at different temperatures (40 or 55°C). The harmful amines, formed after reduction, were determined by direct injection of aliquots into the HPLC using external standard calibration for quantification. It should be mentioned that the extraction steps were not performed before the quantification. Recovery rates were estimated as the ratio of found/theoretical concentrations of amines and expressed as a percentage. The results from this investigation are presented in Table II.

As can be seen in Table II, the recoveries obtained were usually very low, especially for benzidine and its analogues. This indicates the occurrence of strong matrix-analyte interactions between the leather and amines, including the amines formed after reduction, thus underscoring the need for subsequent extraction. 3,3′-Dichlorobenzidine was not even detectable because of its poor water solubility at pH 6. Recoveries obtained for 2-methoxyaniline and 4-chloroaniline were slightly higher when the reduction was performed at 55°C in comparison with 40°C. For all the other amines considered, however, neither the temperature nor the reaction time had a strong effect on the reduction. A reaction time of 5 min and a temperature of 55°C were therefore chosen for all further experiments.

Extraction

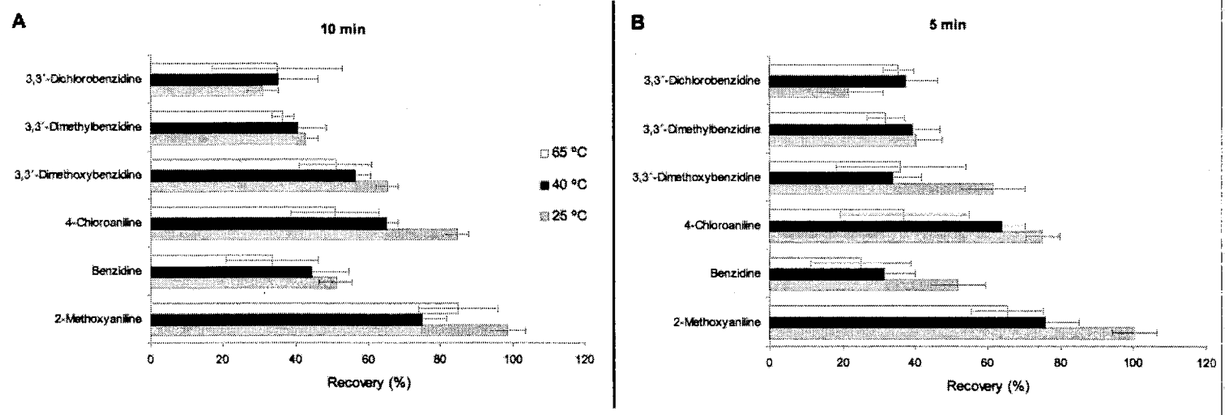
The aim of this study was to investigate the influence of time and temperature on the extraction when 1 M HCl was used as solvent. Azo dyes in samples of bovine leather were reduced and the amines formed were subsequently extracted by UAE for different times (5 or 10 min) and at different temperatures (25, 40, or 65°C). The amines were quantitatively determined by means of HPLC and external standard calibration. The results are illustrated in Figure 1.

Usually, decreasing the extraction temperature resulted in higher recoveries and better repeatability, considering the relative standard deviation (RSD). For 3,3′-dichlorobenzidine, however, the recoveries obtained were highest at 40°C, an intermediate temperature.

TABLE II
Recoveries Obtained After Reduction of Azo Dyes in Bovine Leather by Use of UAE for Different Times and at Different Temperatures.

Amines	Recovery (%) (i) 40°C 5 min	40°C 10 min	55°C 5 min	55°C 10 min
2-Methoxyaniline	51 (11)	51 (6)	55 (1)	53 (7)
o-Toluidine	n.q.	n.q.	n.q.	n.q.
Benzidine	5 (16)	6 (4)	6 (6)	6 (5)
4-Chloroaniline	17 (12)	18 (5)	20 (9)	19 (6)
3,3′-Dimethoxybenzidine	6 (9)	6 (3)	5 (13)	6 (5)
3,3′-Dimethylbenzidine	3 (8)	3 (4)	3 (15)	3 (7)
3,3′-Dichlorobenzidine	n.d.	n.d.	n.d.	n.d.

(i) Data are presented as means (n=3) and RSD values (%) are shown within the brackets.
n.q. not quantifiable because of co-elution of unknown compounds.
n.d. not detectable.



Recoveries (percent, n=3) obtained from the analysis of bovine leather by use of UAE under different extraction conditions. Samples were reduced (5 min, 55°C, 0.3 g dithionite g^{-1} sample) and extracted three times for 10 min (A) or 5 min (B) at 25, 40, and 65°C. Error bars represent percent relative standard deviation (RSD).

TABLE III
Comparison between different analytical procedures for the determination of banned azo dyes in bovine, sheep, and goat leather.

Leather	Amines	Recovery (%) External standard calibration DIN 53316	MAE (i)	UAE Branson	Standard addition UAE Sonorex	MAE (ii)	UAE Sonorex
Bovine	2-Methoxyaniline	75 (14)	79 (3)	99 (5)	110 (4)	107 (9)	121 (4)
	o-Toluidine	57 (5)	82 (3)	n.q.	104 (3)	104 (19)	115 (14)
	Benzidine	3 (3)	39 (4)	51 (5)	61 (7)	78 (12)	113 (13)
	4-Chloroaniline	34 (2)	62 (3)	85 (3)	68 (5)	100 (10)	102 (11)
	3,3′-Dimethoxybenzidine	10 (1)	57 (3)	65 (3)	60 (9)	86 (11)	90 (14)
	3,3′-Dimethylbenzidine	11 (3)	37 (1)	43 (4)	47 (7)	110 (3)	94 (12)
	3,3′-Dichlorobenzidine	38 (5)	29 (2)	31 (4)	47 (2)	105 (iii)	79 (10)
Sheep	Benzidine	13 (8)	46 (6)	59 (12)	36 (7)	90 (4)	66 (12)
	3,3′-Dimethylbenzidine	20 (5)	36 (8)	41 (9)	42 (3)	86 (3)	70 (10)
Goat	*o*-Toluidine	47 (14)	73 (4)	74 (4)	115 (8)	104 (7)	121 (16)
	3,3′-Dimethoxybenzidine	10 (11)	39 (5)	64 (5)	58 (3)	81 (7)	94 (20)

Recoveries are presented as means (n=3) and RSD (%) values are shown within the brackets. Standard addition was performed at four different concentration levels, with triplicate determinations for each level.
(i) Data from 5.
(ii) Data from 7.
(iii) One outlier excluded from the data set.
n.q. not quantifiable because of co-elution of unknown compounds.

Using an extraction temperature of 25°C, recoveries for 4-chloroaniline and 3,3′-dichlorobenzidine increased by *ca* 10 percent when the extractions were performed for 10 min instead of 5 min, whereas for the other amines extraction time was not found to be important with respect to recovery. From these results, an extraction time and temperature of 10 min and 25°C, respectively, were considered to provide optimum performance and were therefore used in the subsequent UAE experiments.

Comparison of analytical procedures

The optimized UAE method was applied to bovine, sheep, and goat leather. Two different ultrasonication baths were employed to enable a statistical comparison of the precision between digital (Branson) and manual (Sonorex) control of time and temperature in UAE. Quantification was accomplished using external standard calibration as well as standard addition of the investigated azo dyes at four different levels. These results were compared with those obtained on the same type of leather samples by employing the DIN 53316 method[4] and with results previously obtained by use of MAE.[5,7] The results are presented in Table III.

The recoveries obtained by employing the DIN 53316 method were usually very low. For benzidine and 3-3′dimethoxy benzidine, recoveries were below 20 percent in all leather substrates. This can probably be attributed to the poor extraction efficiency of MTBE in this particular method. Regardless of the equipment used, the UAE procedure with external standard calibration resulted in about 25 to 50 percent higher recoveries than those obtained with the DIN 53316 method, with the exception of 3,3′-dichlorobenzidine. Furthermore, this UAE procedure appeared not to be as matrix dependent as the DIN 53316 method. It should be mentioned that the origin of the unknown compound, co-eluting with *o*-toluidine when analysing bovine leather by use of the Branson UAE equipment, is still not clear. However, this compound is probably not related to the leather composition since the same type of leather samples were processed in the other procedures without this problem.

The recoveries obtained using the MAE[5] and UAE procedures based on external standard calibration were usually comparable. Even though the difference in performance of these two procedures was thus only marginal, UAE is far simpler and the equipment cost is about 10-15 times lower than that of MAE.

To investigate whether digital control of time and temperature exhibits better precision than manual control in UAE, an F-test (one-tailed) was applied to the standard deviations of the mean recoveries obtained. However, the variance ratio F-test values calculated for $P = 0.05$ and $v_1 = v_2 = 2$ (degrees of freedom) did not exceed the tabulated value of $F = 19.00$, indicating insignificant difference between the precision of the two baths utilized. As a consequence, the less expensive Sonorex equipment was employed in the subsequent UAE experiments.

As stated previously[7] and also confirmed in this study (see Table III), it is difficult to achieve acceptable recoveries for benzidine and its analogues using quantification by means of external standard calibration. However, standard addition of azo dyes has proven to be a successful way to improve quantification[7]. Therefore, a previously developed standard addition methodology[7] was applied in the optimized UAE procedure. Standard additions were performed by spiking degreased leather samples with azo dye solution at four different concentration levels. The samples were then treated according to the optimized UAE procedure. Standard addition curves were obtained by linear least-squares regression of amine peak heights (y) versus added amount of analytes (x), with regard to amines. Extrapolation of the curves to the x-intercept indicated the concentration of amine present in the samples. The linearity of the standard addition curves was usually good, with regression coefficients (R^2) higher than 0.998 except for 3,3′-dimethylbenzidine (0.995) from sheep leather. The recoveries obtained using this standard addition approach are also presented in Table III. In addition, previously published MAE results[7] were included in the Table for comparative purposes.

In Table III, it is shown that the obtained recoveries were in the generally acceptable range of about 70-120 percent. For sheep leather, however, the recoveries obtained were slightly lower than those obtained for bovine and goat leather. However, this leather material showed a higher tendency to float on the surface of the buffer solution than the other two leather types, even though the glass flasks were vigorously shaken. This might have affected the completeness of the reduction step and consequently the recovery. Even so, the recoveries were still 50 percent higher than those obtained for sheep leather when employing the DIN 53316 method.[4] From the standard addition results in Table III, it is not possible to state which procedure, UAE or MAE[7], provides the best accuracy. The UAE procedure, however, usually resulted in higher RSD values. It has been demonstrated that, due to formation of standing waves, the local sound intensity in a flask fixed in an ultrasonic bath is strongly susceptible to changes in experimental conditions, thus affecting the precision.[8] Utilization of probe devices instead of baths might improve the repeatability. Nevertheless, taking into account the complexity of the matrix together with that of the multi-step UAE procedure, RSD values ≤ 20 percent must be considered to be acceptable.

Concerning sample throughput, it should be possible for one person to analyze at least four samples (i.e. 16 replicate determinations) in six or seven hours by employing the proposed UAE procedure and standard additions at four levels. The number of reductions and extractions that can be performed simultaneously is basically only limited by the size of the ultrasonication baths utilized. Access to an autosampler enables HPLC analysis for a 24-hour period without supervision. Furthermore, the HPLC conditions employed in this study are merely recommendations and should thus be optimized for each new matrix considered.

CONCLUSIONS

An analytical procedure for the determination of banned azo dyes in leather has been developed based on ultrasound-assisted extraction (UAE) in sample preparation and quantification using standard addition of the corresponding dyes. This procedure gave recovery values in the range of 70-120 percent around the theoretical target values supplied by the leather producer. The accuracy was much better than the standard DIN 53316 method.[4] The proposed procedure was straightforward and simple to perform. In addition, it did not require any expensive equipment, provided that the degreasing step is performed according to the DIN 53316 method[4].

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REFERENCES

1. Platzek T, Lang C, Grohmann G, Gi US, Baltes W, Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria in vitro, *Hum Exp Toxicol* **18**(9), 552-559, 1999.
2. European Commission, Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azo colourants), *Off J Eur Commun L* **243**, 15-18, 2002.
3. Ahlstrom L-H, Eskilsson CS, Bjorklund E, Determination of banned azo dyes in consumer goods, *Trends Anal Chem* **24**, 49-56, 2005.
4. DIN 53316, Nachweis Bestimmter Azofarbstoffe in Leder, *Deutsches Institut für Normung*, 1997.
5. Ahlstrom L-H, Björklund E, Mathiasson L, Optimization of an analytical procedure for the determination of banned azo dyes in leather, *Anal Bioanal Chem* **382**, 1320-1327, and 2005.
6. Eskilsson CS, Davidsson R, Mathiasson L, Harmful azo colorants in leather -Determination based on their cleavage and extraction of corresponding carcinogenic aromatic amines using modern extraction techniques, *J Chromatogr A* **955**, 215-227, 2002.
7. Ahlstrom, L-H, Raab, J, Mathiasson, L, Application of standard addition methodology for the determination of banned azo dyes in different leather types, *Anal Chim Acta* **552**, 76-80, 2005.
8. Priego-Capote F, Luque de Castro MD, Analytical uses of ultrasound - I. Sample preparation, *Trends Anal Chem* **23**, 644-653, 2004.
9. Rosales-Conrado N, León-González ME, Pérez-Arribas LV, Polo-Díez LM, Determination of chlorophenoxy acid herbicides and their esters in soil by capillary high performance liquid chromatography with ultraviolet detection, using large volume injection and temperature gradient, *Anal Chim Acta* **470**, 147-154, 2002.
10. Sanchez-Brunete C, Miguel E, Tadeo JL, Multiresidue analysis of fungicides in soil by sonication-assisted extraction in small columns and gas chromatography, *J Chromatogr A* **976**, 319-327, 2002.
11. Sanchez-Brunete C, Rodriguez A, Tadeo JL, Multiresidue analysis of carbamate pesticides in soil by sonication-assisted extraction in small columns and liquid chromatography, *J Chromatogr A* **1007**, 85-91, 2003.
12. Al-Merey R, Al-Masri MS, Bozou R, Cold ultrasonic acid extraction of copper, lead and zinc from soil samples, *Anal Chim Acta* **452**, 143-148, 2002.
13. Vaisanen A, Suontamo R, Silvonen J, Rintala J, Ultrasound-assisted extraction in the determination of arsenic, cadmium, copper, lead, and silver in contaminated soil samples by inductively coupled plasma atomic emission spectrometry, *Anal Bioanal Chem* **373**, 93-97, 2002.
14. Wu J, Lin L, Chau F, Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells, *Ultrason Sonochem* **8**, 347-352, 2001.
15. Rostagno MA, Palma M, Barroso CG, Ultrasound-assisted extraction of soy isoflavones, *J Chromatogr A* **1012**, 119-128, 2003.
16. Caldeira I, Pereira R, Clímaco, MC, Belchior AP, Bruno de Sousa R, Improved method for extraction of aroma compounds in aged brandies and aqueous alcoholic wood extracts using ultrasound, *Anal Chim Acta* **513**, 125-134, 2004.
17. Luque-García JL, Luque de Castro MD, Ultrasound: a powerful tool for leaching, *Trends Anal Chem* **22**, 41-47, 2003.
18. Pastor A, Vazquez E, Ciscar R, de la Guardia M, Efficiency of the microwave-assisted extraction of hydrocarbons and pesticides from sediments, *Anal Chim Acta* **344**, 241-249, 1997.