Determination of Dimethylfumarate in Leather and Footwear by Solid-Phase Micro Extraction and Gas Chromatography-Mass Spectrometry

by

R. M. Cuadros,* J. Font, M. Reyes, LL. Ollé and A. Marsal[a]
Igualada Engineering School (EEI). Universitat Politècnica de Catalunya (UPC).
Plaça del Rei, 15, 08700 Igualada, Spain.
[a]*Department of Chemical and Surfactants Technology, IQAC, CSIC*
J. Girona, 18-26, 08034 Barcelona, Spain

Abstract

In the last two years, Europe has experienced a rise in skin allergy and dermatitis due to goods of an Asian provenance that have been treated with dimethylfumarate (DMFU). Accordingly, laboratories in the leather and footwear sectors have been obliged to develop analytical methods to determine the presence of this substance given the absence of an official method. The ban on DMFU as laid down in Decision 2009/251 of the European Union establishes a maximum concentration of DMFU in products of 0.1 mg/kg. A simple non-destructive rapid method based on manual headspace solid-phase micro extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) is proposed to detect DMFU in leather and footwear. Thereafter, the samples in which DMFU is detected are analysed by a solid-liquid extraction (SLE) with acetone after which DMFU is quantitatively determined by GC-MS. The quantitative method is validated in terms of linearity, precision, sensitivity and recovery; demonstrating its reliability. Quantification is performed using naphthalene-D8 as internal standard. The detection limits are 0.005 mg/kg and 0.03 mg/kg for the HS-SPME-GC-MS and SLE-GC-MS methods, respectively. Given that these limits are below the maximum limit of 0.1 mg/kg imposed by the European Union, the proposed methods are suitable for determining DMFU content in real samples.

Resumen

Durante los dos últimos años, en Europa se han detectado casos de alergias dérmicas y dermatitis debido al contacto con mercancías de origen asiático que habían sido tratadas con dimetilfumarato (DMFU). En consecuencia, los laboratorios de los sectores del cuero y calzado se han visto obligados a desarrollar métodos analíticos para determinar la presencia de esta substancia dada la inexistencia de un método oficial. En la Resolución 2009/251 de la Unión Europea se estableció en 0.1 mg/kg la máxima concentración de DMFU en productos de consumo. Para detectar DMFU en cuero y calzado se ha propuesto un método sencillo, rápido y no destructivo basado en la cromatografía de gases con detector de masas (GC-MS) y la técnica de preparación de muestra de espacio de cabeza y microextracción en fase sólida (HS-SPME). En el caso de identificación positiva en DMFU mediante este método, se procede a una extracción sólido-líquido (SLE) con acetona y el DMFU se determina cuantitativamente por GC-MS. El método cuantitativo se ha validado mediante la determinación de su linealidad, precisión, sensibilidad y recuperación, demostrando su confiabilidad. El método de cuantificación ha utilizado naftaleno-D8 como patrón interno. Los límites de detección para los métodos HS-SPME-GC-MS y SLE-GC-MS han sido respectivamente 0.005 mg/kg y 0.03 mg/kg. Como estos límites son inferiores al límite máximo de 0.1 mg/kg impuesto por la Unión Europea, los métodos propuestos son aptos para la determinación de DMFU en muestras reales.

*Corresponding author e-mail: rosa.cuadros@eei.upc.edu.
Manuscript received March 23, 2010, accepted for publication June 18, 2010.

INTRODUCTION

Dimethylfumarate (DMFU) has been successfully used to treat psoriasis. DMFU is also used by producers as a biocide to kill moulds that may cause furnishings or shoe leather to deteriorate during storage and transportation in a humid climate. Placed in sachets and in some cases mixed with silicagel that are fixed to the furniture or added to the footwear boxes, DMFU evaporates and impregnates the leather, protecting it from moulds. In other cases, DMFU is applied for fumigation of containers that export footwear to Europe. However, in 2008 it was found that products containing DMFU could adversely affect consumers. DMFU penetrated the clothes to the skin of many users of sofas and footwear in Europe causing skin itching, irritation, redness, and pain and even dermatitis that was difficult to treat.[1]

In March of 2009, Member States of the European Union voted in favour of banning the anti-fungal agent DMFU in consumer products.[2] Such products already present on the market will have to be recalled and withdrawn without delay (Decision 2009/251). The ban of DMFU establishes a maximum concentration of DMFU in products of 0.1 mg/kg. This is considered to be well below the concentration of 1 mg/kg, which showed a strong reaction in a clinical study carried out on humans.[1] Accordingly, the analytical method employed must reliably quantify 0.1 mg DMFU per kg of product or part of the product. This means that the quantification limit of the method should be 0.1 mg/kg or less.

Until 2009, there was very little information about DMFU assay. Determination of DMFU in some samples, such as antimicrobial preservatives and biological matrices has been carried out by high performance liquid chromatography.[3,4] In 2008, analysing the samples by a headspace-GC-MS semi quantitative method, Rantanen[1] established for the first time a relationship between contact dermatitis and presence of DMFU in leather goods. In 2009, Lamas et al. developed a method for the determination of DMFU in desiccant and anti-mould sachets based on solid-liquid extraction (SLE) and gas chromatography with electron-capture[5] or with mass spectroscopy detection.[6] Narizzano et al. have recommended two methods by GC-MS. One of them is based on the extraction with acetone, whereas the other is based on the headspace solid-phase micro extraction. The authors applied the latter to samples that cause difficulties to the solid-liquid extraction.[7] The European Committee of Standardization is currently preparing a draft GC-MS method to determine DMFU in footwear. Homogenous textile and leather samples of the shoes are cut in pieces and then extracted with methanol for one hour in an ultrasonic bath.[8]

All of these procedures of sample preparation are time consuming. However, given the large number of batches to be checked, it is necessary to have at our disposal a rapid screening method that, without losing time in footwear sample preparation, significantly reduces the duration of the analysis and the cost of chemicals and, at the same time, complies with the detection limit established by the European Commission. Solid phase micro extraction (SPME), developed by Pawliszyn and co-workers,[9,10] is a method that integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. SPME has been widely used in the environmental, biological, pharmaceutical, food, and on-site analyses fields.[11-14]

In this work, a qualitative method based on headspace solid-phase micro extraction (HS-SPME) and a quantitative method based on conventional solid liquid extraction (SLE) are developed for the determination of DMFU in leather and footwear. To our knowledge, this is the second work, apart from that of Narizzano,[7] in which an application of SPME in leather sampling and analyses field is reported in any scientific journal. Unlike the work of Narizzano, the determination of DMFU in the emissions of the whole shoe by HS-SPME GC-MS is proposed in the present method. This first analysis, which is the qualitative determination, is carried out with all the footwear batches to be checked. Thereafter, those shoes in which DMFU is detected are quantitatively analysed by the SLE-GC-MS method. Given that most of the footwear received from Asia during the second half of 2009 is free of DMFU, only a few samples will need to be quantitatively analysed. This method enables us therefore to analyse many footwear batches in a very short time. SPME has three main advantages: a marked increase in sensitivity and selectivity with respect to SL extraction, the possibility of performing the extraction directly from the whole shoe, obviating the need for cutting and separating components, and a considerable saving in time, chemicals and laboratory wastes.

EXPERIMENTAL

Materials

All solvents used were of pesticide analysis grade. The DMFU 97% and Naphthalene-D8, used as internal standard (IS), were purchased from Sigma-Aldrich and Supelco, respectively. Portable manual samplers of SPME with an extraction fiber (coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm were purchased from Supelco. DMFU and Naphthalene-D8 were dissolved in acetone to prepare stock solutions at concentrations of 200 mg/L and 500 mg/L, respectively.

Instrumentation

All analyses were performed using a gas chromatograph (Konik HRGC 4000B, Spain) and a fused silica capillary column (TRB-5MS 30m×0.25mm i.d.×0.25μm thick Teknokroma, Spain) equipped with a quadrupole mass spectrometer (Konik MS Q12, Spain). The GC injector temperature was 260°C for both methods. The column oven

temperature started at 70°C, was held at this temperature for 1min, heated at 5°C/min to 200°C and then maintained for 1min. Helium was used as carrier gas with a flow-rate of 1.0 mL/min and a 1:30 split ratio. Electron impact at 70 eV was selected as the ionization mode for the mass spectrometer. The temperature of the transfer line was 200°C, and the source heating was at 150°C. The mass spectrometer was tuned with perfluorotributylamine (PFTBA) each day on start up.

A split/splitless injection mode of 20 seconds was used for HS-SPME analysis. The injection mode was splitless for SL extraction analysis. A volume of 1 μL of sample was injected by an auto sampler (Konik Robokrom, Spain). A 1.5 μL volume was injected instead of 1 μL in the samples in which DMFU was detected at very low concentrations. Mass spectrum was used for qualitative confirmation of DMFU. The total mass scanning range was 50–150m/z. DMFU was identified by retention time and comparison with the mass spectrum provided by the National Institute of Standard and Technology (NIST05- Mass Spectral Library, USA). DMFU was quantified in selected ion monitoring (SIM) mode. The target ions for quantification and confirmation were 113 and 85 m/z, respectively. The target ion of naphthalene-D8 for quantification was 136 m/z. Fig. 1 shows the mass spectrum of DMFU. A sonicator (Estmon JPC 350, Spain) was used for SLE method.

Samples

Forty-eight shoes and fifteen pig and lamb hides for lining were analyzed. Footwear samples were purchased in shops in Spain from March to December of 2009. The samples of lining leather were provided by Spanish traders of footwear supplies in 2009. All of the samples analyzed were made in China.

Procedure

Qualitative SPME method: A whole shoe was placed inside a vacuum desiccator of approximately 9L of capacity adapted for head space sampling purposes by fitting a reducing-adapter piece with a hole cap PTFE/silicone septum, as shown in Fig. 2. Samples in the adapted desiccator were heated at 60°C in an oven with an equilibration time of 25 min. Next, the potential DMFU was extracted for 15 min by the fiber coated with polydimethylsiloxane/divinylbenzene. The desorption time of the fiber in the GC injector was 20 seconds. The set sampler–fiber was discarded after it was employed for 50 determinations. At the beginning of every working session, a blank and a sample of a blank leather fortified to 1 mg/kg of DMFU were analyzed as a control of the whole HS-SPME-GC-MS process.

Quantitative SLE method: Samples were cut into small pieces. A test portion of about 1 g was weighted in a 25 mL glass screw top bottle, and 20 mL of acetone were added. The bottle was closed and the sample was sonicated for 20 min at 30°C. The supernatant was transferred into a vial and naphthalene-D8 was added as internal standard. The extract was concentrated to approximately 2 mL using a rotary evaporator in the samples in which DMFU had been detected at very low concentrations. Thereafter, the extract was filtered through a 0.45 µm PVDF filter. The use of a surrogate was avoided by analysing the fortified samples in each shoe or leather sample in which DMFU was detected.

RESULTS AND DISCUSSION

Initial experiments were carried out to verify the possibility of proposing a SPME GC-MS method for the quantitative determination of DMFU. Five different fibers were checked and all of them showed capacity for DMFU extraction. However, two of them, those coated with polydimethylsiloxane (PDMS) of 100μm and with Carboxen/polydimethylsiloxane (Carboxen/PDMS) of 75μm, were discarded for having a lower

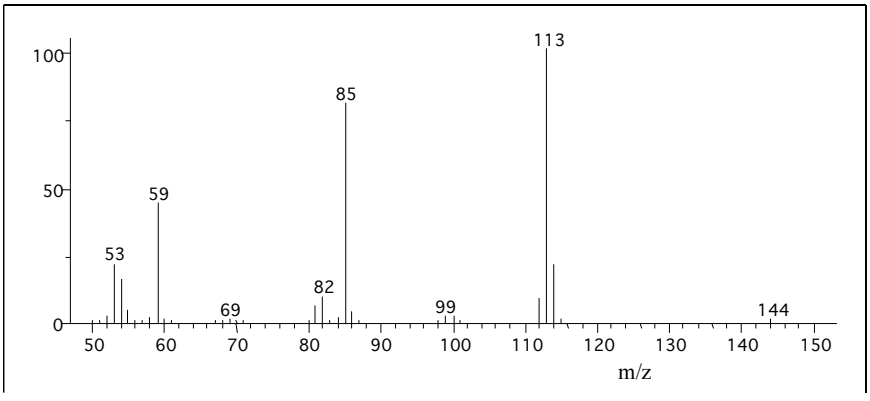

Figure 1. Mass spectrum of DMFU obtained from the Total Ion Current chromatogram of one real shoe sample analyzed by HS-SPME-GC-MS.

Figure 2. An adapted desiccator placed in an oven was used as extraction chamber for the HS-SPME method.

TABLE I

Mean recovery and intraday Relative Standard Deviation (RSD) for the SLE method.

	Chromium leather		Vegetable leather	
Amount spiked	**Mean recovery (%)**	**% RSD (n=5)**	**Mean recovery (%)**	**% RSD (n=5)**
1.2 mg/kg	91.6	± 6.3	89.0	± 6.6
12.0 mg/kg	96.6	± 3.6	98.7	± 1.0
120 mg/kg	101.9	± 5.1	97.2	± 5.8

sensitivity. These fibers are suitable for extracting compounds of higher volatility. The Carbowax/divinylbenzene (CW/DVB) fiber of 75μm showed a good performance but was discarded because of supply difficulties. Supelco finally eliminated this fiber from its catalogue. The divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber of 50/30 μm and the polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber of 65 μm had the best performance for extracting DMFU from all the fibers commercially available. Of all the fibers, the PDMS/DVB fiber showed the highest sensitivity in the extraction conditions described in the procedure section

The recovery obtained by HS-SPME in some of the leather-fortified samples was surprisingly low. This recovery was lower than 20% for some vegetable tanned skins, in contrast to recoveries higher than 80% obtained with other leather samples. It seems that DMFU is firmly retained by certain leathers resulting in a significant diminution of its volatility. A hydrolysis or other type of decomposition of the DMFU molecule absorbed by the leather fibers was discarded as a possible reason of poor recovery since the recovery by the SLE method was always in the range 85–110% with the same samples of leather. The generic designation of "leather" includes all the materials obtained by collagen tanning. There are many types of tanning procedures (chrome, chrome-aluminium, aluminium, aluminium-vegetable tannin, chrome-vegetable tannin, catechin type vegetable tannin, gallic type vegetable tannin, aldehyde-vegetable tannin, wet-white, etc….) and the number of different possibilities of retanning, fatliquoring, dyeing and finishing processes is even higher.[15] All of this results in a large number of matrixes.

Needless to say, to establish a correlation between all potential leather matrixes and effective volatility of the DMFU would neither be feasible nor productive. Because of the influence exerted by the matrix of some leathers on the recovery of DMFU by HS-SPME, the authors propose to use this technique only for the qualitative determination of this compound. The HS-SPME-GC-MS technique is perfectly suitable for the preliminary screening function of identifying the samples that contain traces of DMFU, which should be quantitatively analyzed by SL-GC-MS, and the samples that are free of DMFU, which can be put directly on the market.

Solvent selection

Trials of extraction of DMFU from leather were carried out with dichloromethane and hexane. These solvents are routinely used for the solid-liquid extraction of fat soluble substances in leather in accordance with the Standards ISO 4048:2008 and ASTM D3495, respectively. However, low recoveries were obtained, below 60%. New extraction trials were carried out with a polar solvent. Very good recoveries were achieved with acetone, as can be seen in Table 1. Another polar solvent like acetonitrile, which is used in the determination of preservatives in leather[16] could be also a good choice, but it was not tested because of its toxicity and high cost. The methods of Lamas[5], Narizzano[7] and CEN[8] use ethyl acetate, acetone and methanol, respectively. However, it is known that methanol attacks and partially dissolves silica of the capillary columns, which could cause problems.

Limits of detection and quantification

Limits of detection (LOD), defined for a signal-to-noise ratio of 3 (S/N=3), were estimated for both extraction methods. A limit of detection six times lower than that of the SLE method was achieved using the HS-SPME method despite the fact that the SLE extract was concentrated from the initial 20 mL to 2 mL. The limit of quantification (LOQ), defined for a signal-to-noise ratio of 10 (S/N=10), was also estimated for the SLE method. Results are given in Table 2.

Linearity, recovery and precision of the SLE method

A stock standard solution prepared in acetone was diluted to obtain solutions of DMFU at five different concentrations ranging from 0.05 to 1.2 mg/L. IS was added to obtain a concentration of 1.0 mg/L in each solution. The peak areas

TABLE II

Limit of detection and limit of quantification of DMFU.

	HS-SPME method	SLE method
LOD	0.005 mg/kg	0.03 mg/kg
LOQ	–	0.10 mg/kg

LOD: limit of detection, S/N=3

LOQ: limit of quantification, S/N=10

were measured to construct calibration curves. Linearity was verified over the entire working range. A correlation coefficient of 0.9994 was found. The recovery study was performed at three levels: 1.2, 12, and 120 mg/kg. Two kinds of leather were used: a sample of pure chrome tanned lamb leather and a sample of pure vegetable tanned calf leather that were previously analyzed to confirm the absence of DMFU. Both samples were cut into small pieces. Portions of 1 g were transferred to 25 mL screw top bottles and were spiked with 1 mL of a standard solution of DMFU in acetone. The solvent was allowed to evaporate at 23°C for 24 hours. The average recoveries were greater than 85% in all cases, as shown in Table 1.

TABLE III

Concentration of DMFU in four shoes purchased in shops in Spain in March of 2009. DMFU was not detected in other 44 footwear and 15 lining leather samples acquired between March and December 2009.

Sample	Average concentration of DMFU in mg/kg	% RSD (n=3)
S1	215	± 6.0
S2	51.1	± 7.0
S3	22.0	± 7.3
S4	45.7	± 4.9

The intraday precision of the method was evaluated by calculating the relative standard deviation (RSD) of replicated analysis (n=5) of the recovery study. Results are included in Table 1. RSD values were lower than ±7%. The interday precision was estimated from day 1 to day 5 for a chromium leather sample spiked with 12 mg/kg of DMFU. A relative standard deviation of ±6.5% was found. The developed HS-SPME method was used to detect DMFU in 48 footwear and 15 lining leather samples imported from China. DMFU was identified only in 4 shoe samples. The signals were strong enough to repeat the test in TIC conditions. The positive comparison with the NIST spectrum of DMFU allowed the identification to be confirmed. Therefore, the SLE method was applied in these samples. Fig. 3 shows the HS-SPME and SLE SIM chromatograms of one of the real shoe samples. Concentrations ranging from 20 mg/kg to 215 mg/kg were found. Results are reported in Table 3. Recovery of DMFU varied between 88 to 103%.

CONCLUSIONS

A method for the determination of DMFU in leather and footwear has been developed. This method is based on HS-SPME GC-MS for the qualitative detection of DMFU, which is subsequently quantified by SLE GC-MS. The HS-SPME GC-MS allows the rapid and very sensitive detection of DMFU without any sample preparation. In the case of footwear, the whole shoe is analysed. In addition, it avoids the use of hazardous materials like solvents and does not generate significant laboratory wastes.

DMFU was determined in 48 samples of Chinese footwear and in 15 samples of lining leather imported from China. Given that no lining leather samples contained DMFU and it was detected in

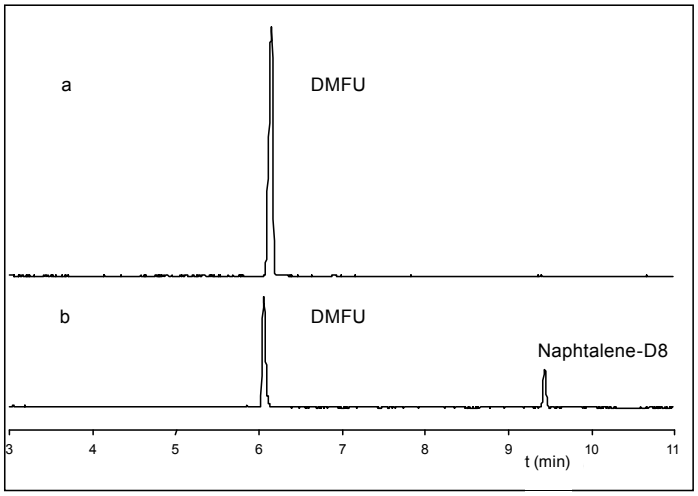

Figure 3. HS-SPME (a) and SLE (b) SIM chromatograms of one real shoe sample that contained 215 mg/kg of DMFU. The DMFU signal obtained by HS-SPME is stronger than the signal obtained by SLE. Experimental conditions are explained in the Instrumentation section.

no more than four shoe samples, only few samples will need to be quantitatively analysed. The HS-SPME method enables us therefore to analyse a large number of footwear batches in a very short time. However, low recovery rates were obtained by HS-SPME in some leather samples so that this technique could not be used for DMFU quantification. Consequently, a validated solid-liquid extraction method with acetone for the quantitative determination of DMFU is provided. This method has been successfully applied to footwear and leather samples in which DMFU was detected by HS-SPME.

In chromium tanned leather, the fortifications of 1.2, 12 and 120 mg/kg yielded average DMFU recoveries of 91.6%, 96.6% and 101.9%, respectively. For vegetable leather, the same fortifications yielded recoveries of 89.0%, 98.7% and 97.2%, respectively. A very innovative method based on SPME technique for leather sampling and analysis is presented in this paper. The application of SPME technique in this field is hardly reported in bibliography.

ACKNOWLEDGMENTS

The authors are indebted to the *Agència Catalana de Consum* for supplying the footwear samples for analysis and for permission to publish a summary of the results obtained and to José Castro for its assistance in the graphic design. The authors would also like to acknowledge the Spanish Ministry of Science and Innovation for the funding obtained through the CTQ 2009-08347 Project.

REFERENCES

1. Rantanen, T.; The cause of the Chinese sofa/chair dermatitis epidemic is likely to be contact allergy to dimethylfumarate, a novel potent contact sensitizer. *British Journal of Dermatology* **159,** 218–221, 2008.
2. European Directive. Commission Decision of 17 March 2009, Official Journal of the European Union, L74/32–34, March 2009.
3. Lui, S. and Su, K.; Determination of antimicrobial preservative dimethyl fumarate by high performance liquid chromatography. *College of Chemistry and Chemical Engineering* **16**, 180–181, 1998.
4. Jirovski, D. and Wiegrebe, W.; HPLC-Analysis of fumarates in biological matrices. *Monatshefte für Chemie*. **135**, 1563–1568, 2004.
5. Lamas, J.P., Sanchez-Prado, L., Garcia-Jares, C. and Llompart, M.; Determination of dimethyl fumarate in desiccant and mouldproof agents using ultrasound-assisted extraction gas chromatography with electron-capture detection. *Journal of Chromatography A*. **1216**, 5755–5758, 2009.
6. Lamas, J.P., Sanchez-Prado, L., Regueiro, J., Llompart, M. and Garcia-Jares, C.; Determination of dimethyl fumarate and other potential allergens in desiccant and antimould sachets. *Anal Bioanal. Chem*. **394**, 2231–2239, 2009.
7. Narizzano, R., Risso, F., Venturelli, G., Devia, C., Carlini, E. and Maggiolo, S.; Gas-chromatography-mass spectrometry analysis of dimethyl fumarate in consumer products. *Journal of Chromatography A*. **1216**, 6762–6766, 2009.
8. Draft method CEN/TC 309 WG 2 N 163. Draft method to determine the concentration of Dimethylfumarate (DMFU) in footwear materials. Central Secretariat of the European Committee of Standardization, Brussels, Belgium, 2009.
9. Belardi, R.G. and Pawliszyn, J.; The application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns. *Water Pollution Research Journal of Canada* **24**, 179, 1989.
10. Arthur, C.L. and Pawliszyn, J.; Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anaytical Chemistry* **62**, 2145, 1990.
11. Ouyang, G. and Pawliszyn, J.; SPME in environmental analysis. *Analytical and bioanalytical chemistry J. Anal Bioanal. Chem*. **386**, 1059–1073, 2006.
12. Kataoka, H., Ishizakia, A., Nonakaa, Y. and Saito, K.; Developments and applications of capillary microextraction techniques: A review. *Analytica Chimica Acta* **655**, 8–29, 2009.
13. Ouyang, G. and Pawliszyn, J.; Recent developments in SPME for on-site analysis and monitoring *Trends in Analytical Chemistry* **25**, 692–703, 2006.
14. Díez, S. and Bayona, J.M.; Determination of Hg and organomercury species following SPME: A review. *Talanta* **77**, 21–27, 2008.
15. Fundamentals of Leather Manufacturing. E. Heidemann Roether KG. Darmstadt, 1993.
16. Draft ISO method prEN ISO 13365–Determination of preservative content (TCMTB-OPP-CMK-OIT) in leather. International Standard Organization, Geneva, Switzerland, 2009.