Characterization of the Volatile Organic Compounds by HS-SPME-CG-MS in the Leather Sector

by

R. M. Cuadros,[1]* A. Marsal[2] L. Ollé,[1] A. Bacardit[1] and J. Font[1]

[1]*Igualada Leather Technology School, EEI, A3 Chair in Leather Innovation UPC,*
Plaça del Rei, 15, 08700 Igualada, Spain.

[2]*Department of Chemical and Surfactants Technology, IQAC, CSIC. J.*
Girona, 18-26, 08034 Barcelona, Spain

Abstract

Current European legislation demands a reduction in the amount of volatile organic compounds (VOCs) used in industrial processes. The presence of these compounds in the leather industry arises from the chemicals used in the various stages of the leather manufacturing process. An important aim of tanners is to reduce or eliminate VOCs, without lowering the quality of their leather products. The HS-SPME-GC-MS method is an innovation in leather analysis. The solid phase micro extraction (SPME) is a sample preparation technique used for the extraction of volatile and semi-volatile organic compounds and is becoming widely accepted as the technique of choice for many applications and that can be connected to gas chromatography. The main advantages of this technique are speed, sensitivity, and the fact that it requires no sample handling or solvent extraction. Other advantages include the fact that the concentration and the extraction are reached simultaneously and that it enables on-site extraction of the analyte, even without the prior destruction of the sample. This paper shows the development of a new HS-SPME-GC-MS method with a deuterated internal standard for the detection and identification of volatile organic compounds emitted by leather. This method enables us to carry out a simple and rapid determination of the qualitative and semi-quantitative composition of the organic compounds in the samples.

Introduction

Current European legislation demands that companies reduce the amount of volatile organic compounds used in industrial processes. The tannery industry, in the leather manufacturing process, uses products containing volatile organic compounds especially in post-tanning and finishing processes.

Gas chromatography and its coupling with mass spectrometry (GC-MS) are the most used techniques to separate, identify and quantify the volatile and semi-volatile compounds from complex mixtures.[1] The most commonly used techniques for the extraction of organic compounds from a sample are the purge and trap technique (P&T), headspace methods (HS) for concentration of volatile compounds, liquid-liquid extraction and solid phase extraction. However, these methods have several disadvantages, such as the high cost and excessive preparation time.[2] The standards developed by car companies such as the PV 3341 Standard of the Volkswagen Group or the GMW 8081 Standard of General Motors establish methods of analysis based on headspace coupled with gas chromatography (GC) and FID to define standards of quality for upholstery leather in relation to volatile organic compounds.[3]

*Corresponding author e-mail: rosa.cuadros@eei.upc.edu.
Manuscript received May 16, 2013; accepted for publication August 8, 2013

A number of efforts have been devoted in recent years to improving sensitivity and rapidity of the analytical methods. The solid phase micro extraction (SPME) is an adsorption/desorption technique of sample preparation that was developed by J. Pawliszyn to increase speed in this aspect and avoid the use of solvents. The SPME is a rapid technique that does not require sample handling and achieves the "in situ" extraction of the analyte and its simultaneous concentration.[4,5] In SPME, equilibria are established among the concentrations of an analyte in the sample, in the headspace above the sample, and in the polymer coating on the fused silica fiber.[7]

The SPME requires no equipment investment providing a significant economic advantage over the HS and P&T techniques. The same chromatograph can be used for conventional applications with automatic injection and also for manual injections with SPME. Some of the volatile substances used in tannery processes, such as N,N-dimethylformamide, N,N-dimethylacetamide, ethylene glycol, dimethyl ether, 2-ethoxyethyl acetate, 2-methoxyethanol, are regulated and classified as *Substances of Very High Concern* (SVHC) by the *REACH* regulation on chemicals.[6] Moreover, if the Restricted Substances Lists (RSL) of large firms finally incorporate volatile compounds, these lists will be of banned specific molecules, which will be necessary to search and identify in a simple, rapid and safe way. These requirements, regardless of the chosen extraction procedure, can only be met by gas chromatography with mass spectroscopy as the determination method.

OBJECTIVE

The general objective is to develop a method to determine volatile organic compounds in hides/skins by gas chromatography with the MS detector and with SPME as sample preparation technique. This method should help to improve the sensitivity in the detection of these compounds with respect to the currently existing procedures and should allow the identification of these substances. This is essential because it will allow the tanner to know the origin of these molecules in the skin it sells. The method developed should be essentially qualitative but also with capacity to provide results at least at a semi quantitative level.

The main objective is to identify which substances emit the hides/skins and then quantify them as far as possible. Exact quantification is not an essential objective because the concentration of VOC in skins/hides is not stable and tends to decrease with time because of volatilization losses.

EXPERIMENTAL

Materials

All solvents used were of pesticide analysis grade. The 1,4-dioxane-d_8, used as internal standard (IS), was supplied by Sigma-Aldrich. Portable manual samplers of SPME with an extraction fiber coated with carboxene/polydimethylsiloxane (PDMS) 75µm, polydimethylsiloxane (PDMS) 100µm, polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65µm and divinylbenzene/carboxene/polydimethylsiloxane 50/30µm were supplied by Supelco (USA) (Figure 1).

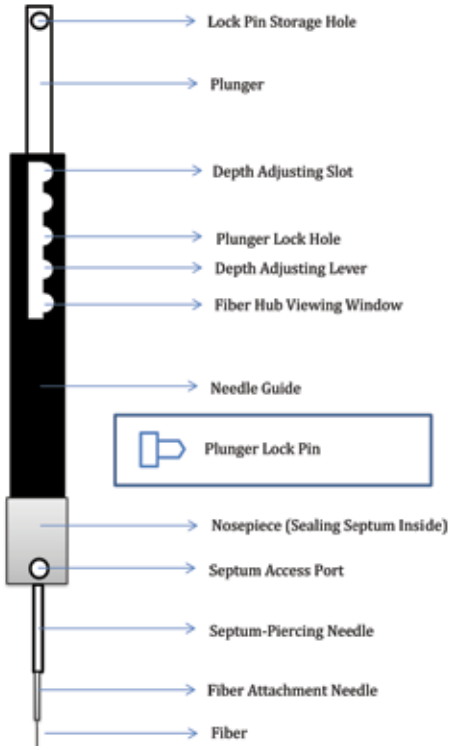


Figure 1. Description of the portable manual samplers of SPME.

Instrumentation

All analyses were performed using a gas chromatograph (Konik HRGC 4000B, Spain) and a fused silica capillary column (TRB-624 of 60m x 0.32mm x 1.8µm film Teknokroma, Spain) equipped with a quadrupole mass spectrometer (Konik MS Q12, Spain).

The GC injector temperature was 280°C . The column oven temperature started at 55°C , was held at this temperature for 1min, heated at 6°C /min to 180°C and heated at 15°C /min to 230°C then maintained for 3min. Helium was used as carrier gas with a flow-rate of 2.0 mL/min and a 1:25 split ratio. Electron impact at 70 eV was selected as the ionization mode for the mass spectrometer. The temperature of the transfer line was 230°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 50 seconds was used for HS-SPME analysis.

Mass spectrum was used for qualitative confirmation of VOCs. The total mass scanning range was 30–300m/z. VOCs were identified by retention time and comparison with the mass spectra provided by the National Institute of Standard and Technology (NIST05- Mass Spectral Library, USA).

Samples

A mixture of volatile and semi-volatile organic compounds currently employed in the leather manufacturing industry was used in this study. Substances used to calibrate the system (standard VOCs reference solution) are 2-butanone, 2-propanol, toluene, 1-butanol, n-butyl acetate, 2-ethoxyethanol, diisobutylketone, butylglycol, butyl glycol acetate, gamma-butyrolactone and isooctane and were supplied by Sigma-Aldrich. The standard VOCs reference solution was prepared by mixing 1 g of each substance and by weighing 1.2 g of this mixture and diluting to 1 liter with ultrapure water.

A ground wet blue hide, which was completely dehydrated by heating at 102°C in an oven for 24 h, and kept in desiccator, was used as a leather blank sample to develop the analytical method with the SPME technique.

Procedure

Leather samples were cut in small pieces. A sample of 0.7000 g ± 0.1000 g was added to a 20 mL vial capped with a penetrable septum. The capped vial was heated for 15 minutes

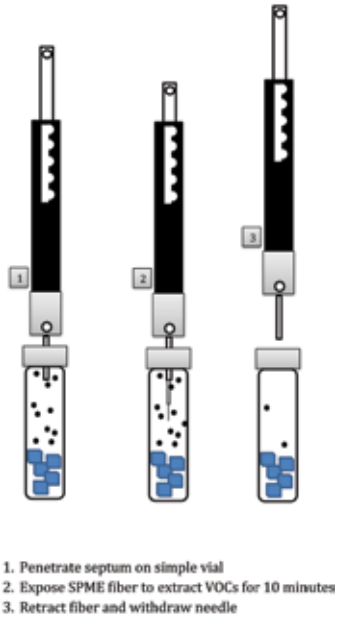


Figure 2. Description of HS-SPME extraction process.

Figure 3. Image of HS-SPME extraction process.

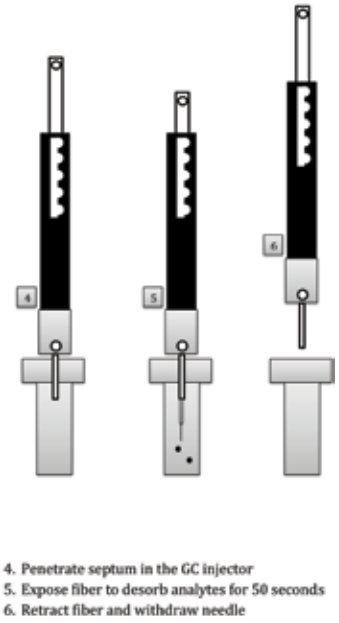


Figure 4. Description of HS-SPME desorption process.

Figure 5. Image of HS-SPME desorption process in the GC injector.

at 65°C to reach solid-vapor equilibrium in the headspace of the vial afterwards, the SPME fiber was introduced through the septum of the vial for 10 minutes while maintaining the temperature at 65°C (Figures 2 and 3). Subsequently, the fiber was removed and injected into the gas chromatograph for 50 seconds at 280°C (Figures 4 and 5). The fiber was heated for 10 minutes at 280°C to prepare it for the next extraction.

RESULTS AND DISCUSSION

Selection of the Fiber

Fiber selection was based on the recommendations of the authors who have used this technique.[7,8] Four commercial fibers, which were, in principle, suitable for determining VOCs in hides were selected.

Four aliquots of the leather blank sample were spiked with the standard VOCs reference solution to a concentration of 160 µg/g of total VOCs. Each spiked sample was extracted with one different fiber. The sensitivity of the response was taken into consideration when selecting the SPME fiber, which were the most suitable one for the larger number of analytes (Table 1).

The highest chromatographic responses were obtained with the carboxen/PDMS 75µm fiber. Consequently, this fiber was chosen to develop the method to determine VOC in hides with the SPME technique

The desorption temperature and desorption time are two variables that were set in accordance with literature.[8,9] The other two parameters, extraction temperature and extraction time were optimized experimentally in an interval from 35°C to 80°C and from 5 to 20 minutes, respectively.

The most suitable extraction temperature for VOC mixtures of composition similar to that of the standard VOC reference solution was 65°C. The best time for the extraction of a solution of volatile organic compounds similar to that proposed in this study was set at 10 minutes.

Limits of Detection

The limit of detection (LOD) is defined as the signal to noise ratio equal to 3 (S / N = 3) and was determined by the method described in section 3.4 by adding a known concentration of the standard VOCs reference solution to the blank leather sample (3.3). The values obtained are shown in Table 2.

Deuterated Internal Standard

The internal standard calibration method compares the areas of the peaks of the components of the sample to the area of the peak of a pure compound of which we added a known quantity. The possible instability of the mass detector is largely offset by the use of an internal standard with properties and extraction similar to the analyte.

The disadvantage of using conventional internal standards is that they are substances that may be present in commercial

TABLE I
Values of ΣArea of the chromatograms of the four studied fibers.

Fiber	ΣMean Area (arbitrary units) at 65°C	ΣMean Area (arbitrary units) at 80°C
Carboxen/ Polydimethylsiloxane (CAR/PDMS) 75 µm	3652	2406
Polydimethylsiloxane (PDMS) 100 µm	219	437
PDMS/Divinylbenzene (DVB) 65 µm	529	543
DVB/Carboxen/PDMS 50/30 µm	962	567

TABLE II
Limit of detection and Relative Standard Deviation (RSD) of VOCs.

Chemical	LOD (µg/g hide)	RSD ±%
Isopropyl alcohol	0.002	13
Methyl Ethyl Ketone	0.1	11
Isooctane	3.9	24
1-Butanol	0.3	18
2-Ethoxyethanol	0.01	29
Toluene	0.002	29
n-Butyl Acetate	0.001	5.2
Butyl Glycol	0.002	5.1
Diisobutyl Ketone	0.004	3.4
γ-Butyrolactone	0.002	7.7
Butyl Glycol Acetate	0.002	22

samples and can interfere with the determination of volatile organic compounds of the hides. The possible interferences of the standards with the analytes are avoided by using deuterated internal standards, which are not present in commercial samples.

The internal standard 1,4-dioxane-d_8 from Sigma-Aldrich was chosen for this work. This chemical was selected because of its similarity in polarity and volatility of the studied analytes. It is soluble in water, of high purity, which is necessary to perform a quantitative calibration and it is commercially available at a reasonable price. Acetone-d_6 was discarded as internal standard due to its excessive volatility, which is unrepresentative of the volatility of the analytes normally detected in hide/skin samples.

Linearity

Calibration with internal standard, 1,4-dioxane-d_8 from Sigma-Aldrich, was prepared. The extraction was performed by applying the same procedure that was applied to real samples (section 3.4). An alcohol, a ketone and two esters, one of which was a glycol ester, were selected as analytes (see Table 3).

The calibration plots were obtained for each of the four VOCs from multi-component standards. Four standards of different concentrations were prepared. Table 3 shows the concentration range of the solutions used as calibration standards. Internal standard concentration was 85 µg/g in each sample.

TABLE III
Linearity and correlation coefficient of the four studied chemicals.

Chemical	Concentration range µg analyte/g hide	Correlation Coefficient (r)
Acetone	15 a 750	0.9999
2-Butanol	15 a 750	0.9990
n-Butyl Acetate	2 a 85	0.9910
Butyl Glycol Acetate	2 a 85	0.9977

Recovery and Precision

The precision and recovery of the method were studied for the same four chemicals: acetone, 2-butanol, n-butyl acetate and butyl glycol acetate.

This study was performed for different levels of concentration of the four analytes. The study was carried out within the same week as calibration. Each chemical was previously put together with the hide/skin to reach equilibrium. Thus, the recovery study was performed in the same way and under the same conditions as the calibration analysis. The developed method should be classified as semi-quantitative one given that recoveries ranged between 92 and 150%.

The precision of the results obtained in replicates performed within a period of five days was assessed by calculating the relative standard deviation (RSD) of the replicates (n = 4) of the recovery study. The value of RSD varied from ± 3 to ± 30%.

Analysis of Commercial Samples

The method was applied to recently finished hides of Spanish manufacture and to the analysis of leather articles (bags and shoes).

Hides were analyzed by HS-SPME-GC-MS according to the procedure described in section 3.4., adding 75 µg of internal standard (1,4-dioxane-d_8). The qualitative composition of the samples was determined by identifying the peaks by means of the NIST05 library spectra. The most relevant chemicals were quantified in several of the samples examined. Table 4 shows the chemicals identified. Concentrations ranging from 15 µg n-butyl acetate/g hide to 200 µg of butyl glycol acetate/g hide were obtained. Some of the most frequently detected VOCs arose from the use of nitrocellulose lacquers, even those that are emulsifiable in water. The concentration of these VOCs decreased rapidly with time. The highest values corresponded to samples taken at the exit of the drying tunnels of the finishing lines. Some of the results are shown in chromatograms 1, 2 and 3.

Figure 6. An adapted desiccator placed in an oven was used as an extraction chamber for the HS-SPME method.

TABLE IV
Chemicals detected in commercial samples.

Chemical identified	Number of commercial samples in which the chemicals were detected
Acetone	Detected in 4 of 20 samples
n-Butyl Acetate	Detected in 6 of 20 samples
Butyl Glycol	Detected in 7 of 20 samples
Butyl Glycol Acetate	Detected in 7 of 20 samples
1-Methoxi-2-propanol	Detected in 2 of 20 samples
Toluene	Detected in 2 of 20 samples
Xylene	Detected in 3 of 20 samples
2-Butanone	Detected in 3 of 20 samples
Dodecane	Detected in 3 of 20 samples
Undecane	Detected in 2 of 20 samples
Tridecane	Detected in 3 of 20 samples
Dimethylformamide	Detected in 2 of 20 samples
1-Butanol	Detected in 1 of 20 samples

Chromatogram 1 corresponds to a hide tanned with vegetable extracts for insole manufacture. These articles have a very light finish, which is sufficient to achieve better values of perspiration fastness. A concentration of 15 µg of butyl glycol acetate / g hide was obtained. Analysis was performed after 6 days.

Chromatogram 2 corresponds to a sample of engraved nappa for leather goods. In this case, 200 µg of butyl glycol acetate / g skin were quantified. The sample was taken several minutes after the completion of the finishing process.

Chromatogram 3 corresponds to the analysis of a handbag sample. There are no accurate data for the time that elapsed between manufacture and analysis, but can be assured that it was at least four weeks. This example illustrates the ability of the method to analyze a wide range of leather articles.[10]

A leather handbag 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 Figure 6. Samples in the adapted desiccator were heated at 65°C in an oven with an equilibration time of 15 min. Next, the potential VOCs were extracted for 10 min by the fiber coated with carboxen/polydimethylsiloxane. The desorption time of the fiber in the GC injector was 50 seconds.

The method affords a rapid detection, sensitive and without destruction or preparation of the sample. It is also an example of that in some skins the organic volatile compounds show a high persistence since they are still detected despite the time elapsed. Some of these VOCs are included in the *REACH* list as *Substances of Very High Concern (SVHC)*, as in the case of dimethylformamide.[6]

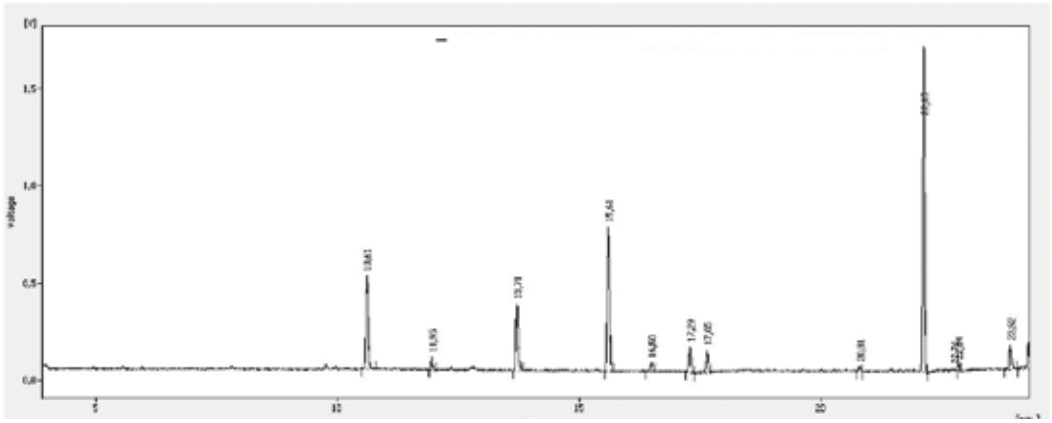
Using the results obtained in the analyses of commercial samples, a table of the relative retention time for more than 30 different chemicals was built with 1,4-dioxane-d_8 as an internal standard. Table 4 shows some of the chemicals detected.

CONCLUSIONS

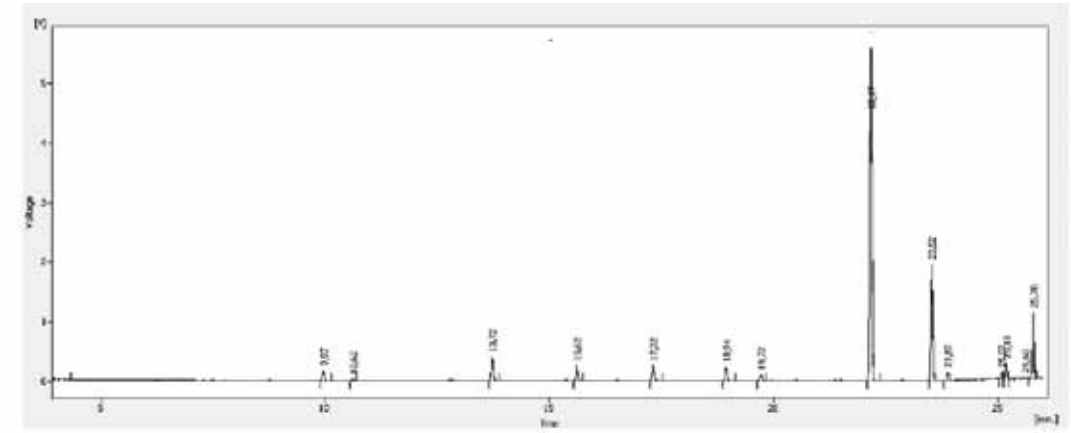
A new sample preparation technique, the solid phase micro extraction (HS-SPME), which may be applied to hides/skin samples, has been developed. This technique significantly improves the sensitivity, rapidity and especially the quantity and quality of the information obtained from other analytical methods. Simplicity and economy are two important advantages of this technique since investment in hardware is not necessary.

The carboxen/PDMS fiber of 75 µm was selected to determine VOCs in hides/skins by the SPME sample preparation technique given that it yielded a response in area much greater than the other studied fibers. The extraction based on HS-SPME and on the quantification with GC-MS gives responses that are unstable over time. The calibration curves are not valid for use over prolonged periods of time. As regards quantitative analysis, the calibration curve should be determined during the working session in which the problem samples are analyzed. However, the HS-SPME/GC-MS method allows a rapid determination of the qualitative and semi-quantitative composition of the volatile organic compounds present in the problem samples.

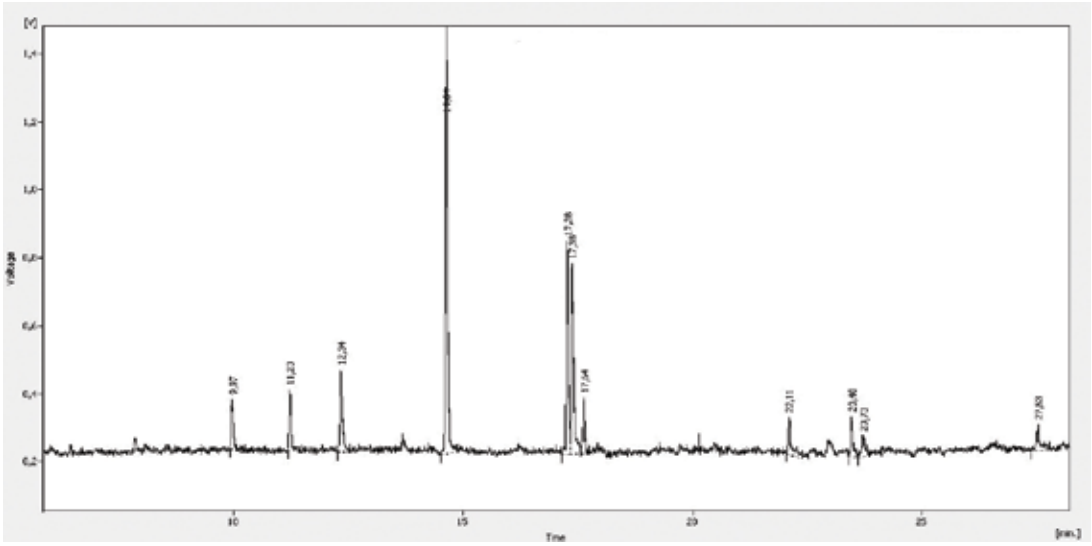
The exact quantification is not an essential objective because the concentration of VOCs in hides/skins is not stable and tends to decrease over time due to volatilization losses. The repeatability of the HS-SPME/GC-MS method, RSD values from ± 3 to ± 30%, is similar to that obtained in the repeatability study performed with direct injection GC-MS. These values demonstrate that the analytical results are not



Chromatogram 1. Results of the VOC analysis in vegetable leather for insoles. Peaks: n-Butyl Acetate (13.71 min), 1-Methoxi-2-Propyl Acetate (15.61 min), Butyl Glycol (17.29 min), Cyclohexanone (17.65 min) and Butyl Glycol Acetate (22.13 min).



Chromatogram 2. Results of the VOC analysis in nappa for leather goods. Peaks: n-Butyl Acetate(13.72 min), p-Xylene (15.62 min), Butyl Glycol (17.32 min), 2,4-Dimethylhexane (18.94 min), Butyl Glycol Acetate (22.17 min), 2-Ethylhexyl Acetate (23.52 min) and Tridecane (25.78 min).



Chromatogram 3. Results of the VOC analysis in the handbag. Peaks: Methoxipropanol (9.97 min), Dimethylformamide (14.64 min), Butyl Glycol (17.28 min), Dimethylacetamide (17.38 min), Cyclohexanone (17.64 min) and Butyl Glycol Acetate (22.11 min)

worse by working with fibers at least in the range of concentrations studied. The qualitative determination of VOCs in commercial samples demonstrates that the HS-SPME/GC-MS method affords rapid and sensitive VOCs detection without destruction or preparation of the sample. This method has been utilized with all leather types: shoes, handbags and leather goods. The results obtained for each sample enable the producer of leather articles to identify the volatile organic compounds and the manufacturing process in which originate and help resolve the problem by influencing on its source.

ACKNOWLEDGEMENTS

This work was funded by the Spanish Ministry of Science and Innovation through the CTQ2009-08347 Project.

REFERENCES

1. Allwood, J. W., Goodacre, R.; An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. *Phytochemical Analysis* **21**(1), 33-47, 2010.
2. Pawliszyn, J.; Handbook of Solid Phase Microextraction. Chemical Industry Press, 2009. ISBN 978-7-122-04701-4.
3. Schulz, H. Volatile matters released by leather. Methods, sources, substances. Leder & Häutemarkt **3**, 25 – 34, 2002.
4. Pawliszyn, J.; Solid Phase Microextraction (SPME). The Chemical Educator **2**, Nº4. New York: Springer-Verlag, Inc., 1997.
5. Pawliszyn, J.; Solid Phase Microextraction Theory and Practice. New York: Wiley-VCH. Inc., 1997. ISBN 0-471-19034-9.
6. Commission Regulation (EC) No 552/2009 of 22 June 2009, amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) as regards Annex XVII. Official Journal of the European Union of 26 June 2009, L 164/7
7. Supelco Bulletin 923. Solid Phase Microextraction: Theory and Optimization of Conditions. Sigma-Aldrich Co, 1998.
8. Miyazawa, M., Kimura, M., Yabe, Y., Tsukamoto, D., Sakamoto, M., Horibe, I., Okuno, Y.; Use of Solid Phase Microextraction (SPME) for profiling the volatile metabolites produced by glomerella cingulata. *Journal of Oleo Science* **57** (11), 585-590, 2008
9. Sousa, E. T., Rodrigues, F. D., Martins, C. C., de Oliveira, F.S., Pereira, P.A. D., de Andrade, J.B.; Multivariate optimization and HS-SPME/GC-MS analysis of VOC in red, yellow and purple varieties of Capsicum chinense sp. Peppers. *Microchemical Journal* **82**, 142-149, 2006.
10. Cuadros, R.M., Font, J., Reyes. M., Ollé. Ll., Marsal, A.; Determination of dimethylfumarate in leather and footwear by solid-phase microextraction and gas chromatography-mass spectrometry. *JALCA* **105**, 395-400, 2010.