INTER-LABORATORY STUDY ON FORMALDEHYDE DETERMINATION BY HPLC

by

J. FONT[1], S. VIERA[1], T. RIUS[1], M. REYES[1], M. JORBA[2], E. VERDÚ[3], M.A.JUÁREZ[4], S. CUADROS[5], A. MARSAL[5]

[1] *Igualada Leather Technology School (EUETII)*
PLAÇA DEL REI 15, 08700 IGUALADA, SPAIN

[2] *Research Association of the Leather Industries and Annexes (AIICA)*
AVDA. PLA DE LA MASSA S/N, 08700 IGUALADA, SPAIN

[3] *Technological Institute for Footwear and Related Industries (INESCOP)*
POL. IND. CAMPO ALTO, 03600 ELDA, SPAIN

[4] *Center of Applied Innovation in Competitive Technologies (CIATEC)*
OMEGA 201, 37545 LEÓN, MÉXICO

[5] *Chemical and Environmental Research Institute of Barcelona (CID-CSIC)*
JORDI GIRONA 18-26, 08034 BARCELONA, SPAIN

ABSTRACT

The colorimetric determination of formaldehyde in a water extract of a leather sample is a procedure that has been criticized because of possible interferences of other aldehydes and coloured substances that could interfere in the spectrophotometric detection. The measurement by liquid chromatography HPLC is an alternative method that was developed some years ago. This method is more sophisticated but more selective and free of the aforementioned interferences. It is not sensitive to coloured extracts. With the implementation of HPLC equipment in many laboratories, this choice has become feasible in our sector.

The process is selective. Formaldehyde is separated and quantified as a derivative from other aldehydes and ketones by liquid chromatography. The free-formaldehyde and formaldehyde which is hydrolysed during extraction to yield free-formaldehyde are detected by this method. The sample is eluted with water at 40°C. The eluate is mixed with 2,4 dinitrophenylhydrazine, whereby aldehydes and ketones react to yield the respective hydrazones. These are separated by means of a reversed-phase HPLC method, detected at 350 nm and quantified.

The aim of this work is to present a collaborative inter-laboratory study coordinated by the Igualada Leather Technology School and carried out with four other laboratories that had previously implemented the HPLC method or that were planning to do this. Determination of formaldehyde content in leather was carried out in each laboratory in accordance with prEN ISO 17226:2005 - HPLC Standard, developed by the Committee CEN/TC 289. Part 2 of the ISO 5725 Standard (Basic method for the determination of repeatability and reproducibility of a standard measurement method) was applied to examine the results.

The study proved successful. The HPLC method achieved very reproducible results between laboratories. This work has also demonstrated that other aldehydes, glutaraldehyde included, do not interfere in the chromatographic method. The use of a PDA detector increases the confidence of the detection of formaldehyde in leather samples.

RESUMEN

La determinación de formaldehído por colorimetría en el extracto acuoso de la piel es un procedimiento que ha obtenido muchas críticas por posibles interferencias de otros aldehídos y por la presencia de sustancias coloreadas en los extractos que entorpecen la medida espectrofotométrica. La alternativa desarrollada desde hace pocos años es la medición por cromatografía líquida HPLC, un método más sofisticado pero a la vez mucho más selectivo y por tanto libre de las interferencias antes comentadas. A medida que los laboratorios se han ido equipando con instrumentos HPLC esta alternativa se ha convertido en una realidad factible.

El objetivo de este trabajo es presentar un estudio colaborativo ínter laboratorios coordinado por la EUETII con la participación de otros 4 laboratorios que, o bien ya tenían en funcionamiento el método

* Corresponding author - E-mail: ammeco@iiqab.cics.com
Manuscript received June 1, 2007, and accepted for publication October 18, 2007

HPLC, o a su vez tenían la intención de implantarlo. La determinación del contenido de formaldehído de la piel se ha realizado por el procedimiento basado en la cromatografía líquida HPLC descrito en la Norma Europea prEN ISO 17226:2005.

Este procedimiento está basado en la cuantificación del formaldehído derivatizado con dinitrofenilhidracina, separándolo de otros posibles aldehídos y acetonas con ayuda de la cromatografía líquida. El formaldehído que se detecta es el formaldehído libre y el formaldehído hidrolizado durante la extracción. Se realizó también un estudio correspondiente a la posible interferencia de otros aldehídos.

Los resultados se estudiaron mediante la aplicación de la norma ISO 5725 para la "*Validación de un método en estudios ínter laboratorios* ". El estudio ha procedido con éxito, obteniéndose unos resultados robustos y bastante repetibles para cada muestra de piel dentro de cada laboratorio y muy reproducibles entre laboratorios.

INTRODUCTION

The Leather Technology School of Igualada, together with other four laboratories, carried out a collaborative study to test the new pr EN/ISO 17226-1 method developed by the CEN/TC 289 Committee, to determine the formaldehyde content in leather by HPLC.

In the study, three different skins or hides were analyzed, with five replicates of each. Likewise, the recovery rate of at least two of the replicates was determined. Once the formaldehyde concentration on each sample was obtained, the results were studied by the ISO 5725 Standard to validate the method through inter laboratory study. In this way, the achievement of reproducible results with this new method by laboratories specialized in the leather sector was verified. The possible interference of other aldehydes, mainly acetaldehyde and glutaraldehyde, on the results provided by HPLC, was also studied.

Background

Formaldehyde can be incorporated into leather at different stages of the manufacturing process1. It can be used in the wetting step in furriery, as a fixing agent for casein finishes, and for wool ironing in double face skins. Some pretanning, tanning and retanning agents may be other sources of formaldehyde[1,2].

Nowadays, ecological and toxicological issues have assumed increasing importance in leather commercialization. Residual monomers in leather have become the chief concern of both leather manufacturers and consumers[3]. This concern had its origin in the automobile industry and subsequently spread to the footwear and clothing industries. There are, currently, no legal limits in Europe on the restriction of formaldehyde in leather. However, the main eco-labels, such as Oko-Tex[4] and the European Eco-label for footwear[5] as well as the quality specifications of some important international purchasers of finished skins/hides, have their own restrictions concerning the amount of free formaldehyde in leather. Therefore, the laboratories of the leather sector are often requested to determine the free formaldehyde content in leather.

For many years, analytical methods to determine the formaldehyde content in leather had not been unified because of theoretical and practical aspects. Different analytical methods were therefore established[1]. The colorimetric method based on the reaction with acetylacetone constitutes the classic procedure to determine the content of free formaldehyde in the leather extract. However, although this method is suitable for colourless extracts or those with a slight coloration, it interferes with strongly coloured leather. Another drawback of this method is the possible interference caused by other aldehydes, such as acetaldehyde and glutaraldehyde[6].

The automobile companies have their own test methods, which are not affected by these problems. For example in the German VDA 275 Standard[8], a test leather sample of 4 cm x 10 cm is suspended for 3 hours in a sealed flask at 60ºC. The released formaldehyde vapors reach equilibrium between the gas phase and a liquid phase of 50 mL of water, which is placed in the bottom of the flask without contact with the leather sample. In this way, the formaldehyde solution obtained is always colourless regardless of the leather colour. The released formaldehyde is then determined by the acetylacetone colorimetric method without any significant interference. This technique, known as the *gas phase method*, measures only the free formaldehyde which is present in leather[1].

However, although the VDA 275 Standard normally provides very good results in the testing of upholstery leather samples, the application of this method to other skins and hides - in particular double-face and furry skins- gives rise to very irreproducible results[9].

Origin of the HPLC chromatographic method

Hydrazine reagents, in particular the 2,4-dinitrophenylhydrazine (2,4-DNPH), are the most widely used group of derivative agents for carbonyl compounds. They have been used for several decades for identification purposes[10]. However, the development of HPLC made their application to quantitative analysis[11] possible.

The reaction of 2,4-DNPH with aldehydes and ketones results in the formation of the respective hydrazones. The hydrazones are typically detected by UV spectroscopy after HPLC separation. As the chromophoric properties of the 2,4-DNPH reagent are very similar to those of the derivatives, the HPLC step is required to obtain quantitative information on the analyte concentration[11].

A reversed-phase stationary phase allows the separation of the remaining 2,4-DNPH reagent from the aldehyde derivatives. The first peak corresponds to the reagent, whereas the aldehyde derivatives appear later on in the chromatogram. Figures 2 and

3 show that the higher the number of carbon atoms of each aldehyde derivative, the greater the retention time. Chemically bonded octadecylsilane (C18) is the most frequently used stationary phase. Separations are easily performed using binary eluents, normally consisting of water and one organic solvent such as acetonitrile or methanol[12].

The aim of this study is to demonstrate that the determination of formaldehyde content in leather by HPLC constitutes a valid approach to solving all the interferences of the colorimetric method, even in the analyses of difficult samples such as strongly dyed double-face skins.

New analytical Standard

Until 2005, the DIN 53315 Standard was the conventional method to determine the formaldehyde content in leather[1,6].

In 2005, the new prEN ISO 17226 Standard[7], based on the former DIN 53315, was prepared by the IUC of the IULTCS Commission in collaboration with the CEN/TC 289 committee to determine the formaldehyde content in leather. Consequently, the DIN 53315 Standard was replaced by the prEN ISO 17226 not only in Germany, but in the whole European Union. Moreover, this ISO International Standard is applied in the whole world.

Subsequently, this Standard was split into two Parts as a function of the analytical technique used to determine the formaldehyde content in leather: the EN ISO 17226-1 by means of liquid chromatography (HPLC) and the EN ISO 17226-2 by colorimetric analysis. Both parts are technically equivalent to IUC 19-1 and IUC 19-2, respectively.

EXPERIMENTAL

Description of the EN ISO 17226-1 Standard

Principle

Formaldehyde is separated and quantified as a derivative from other aldehydes and ketones by liquid chromatography. The free-formaldehyde and formaldehyde which is hydrolysed during extraction to yield free-formaldehyde is detected by this method.

Extraction and reaction with 2,4-dinitrophenylhydrazine

2g ± 0.1g of ground leather are weighed into a 100 mL Erlenmeyer flash with a glass stopper. 50 mL of a detergent solution (0.1 % sodium dodecyl sulphate) are added together with a magnetic stirrer. The flask is closed with a glass stopper. The content of the flask is stirred smoothly at 40 ± 0.5ºC in a water bath for 60 ± 2 min.

Thereafter, the warm extract solution is immediately filtered through a glass fibre filter of 0.45 µm. The filtrate, in a closed flask, is cooled down to room temperature.

It is important that the leather/solution ratio is not modified. Extraction and analysis should be performed on the same working day

In a 20 mL volumetric flask, add 8 mL of acetonitrile and 10 mL of the filtrate (if the expected content is <100 mg/kg) or 5 mL (if the expected content is >100 mg/kg) and homogenize. Add 1.0 mL of 2,4 - dinitrophenylhydrazine and fill with ultra pure water up to the mark.

Allow to react for at least 60 min, but not more than 180 min. The samples are filtered through a polyamide membrane filter and they are analysed using HPLC liquid chromatography.

HPLC Conditions

The conditions used were the following:

* *Flow rate*:	1.0 mL/min
* *Mobile phase*:	acetonitrile / water (60:40)
* *Separation column*:	A C18 reversed phase column with 1cm precolumn. For example, in the EUETII lab: Waters Xterra MS C18; 5 µm, 3.9 x 150 mm.
* *UV detection wavelength*:	350 nm. In the EUETII lab, a PDA detector in the range 280 - 450 nm, using the 350 nm wavelength for quantitative purposes.
* *Injection volume*:	20 µL

Interlaboratory collaborative study

The aim of this work is to carry out an inter laboratory study to determine the formaldehyde content in leather by applying the EN ISO 17226-1 Standard. The results of each sample obtained by the laboratories taking part in the study will be analysed.

Besides the analytical laboratory from the Leather Technology School of Igualada (**EUETII**), four laboratories took part in this Project: Research Association of the Leather Industries and Annexes (**AIICA**), from Igualada; Center of Applied Innovation in Competitive Technologies (**CIATEC**), from León, Guanajuato (Méjico); Chemical and Environmental Research Institute of Barcelona - Spanish Council for Scientific Research (**IIQAB - CSIC**), from Barcelona, and Technological Institute for Footwear and Related Industries (**INESCOP**), from Elda (Alicante).

The results were analysed by applying the ISO 5726 International Standard "Validation of a method in inter-laboratory studies" .The analysis was based on Part II of the Standard "Determination of repeatability and reproducibility for a Standard test by inter-laboratory tests".

Samples

Each participant laboratory analysed three different skin/hides. The skin/hides were the same for all the laboratories. Five replicates of each skin/hide were carried out by the laboratories. Likewise, the recovery rate of at least two replicates was determined.

The three different skin/hides were prepared by the EUETII students and randomly named:

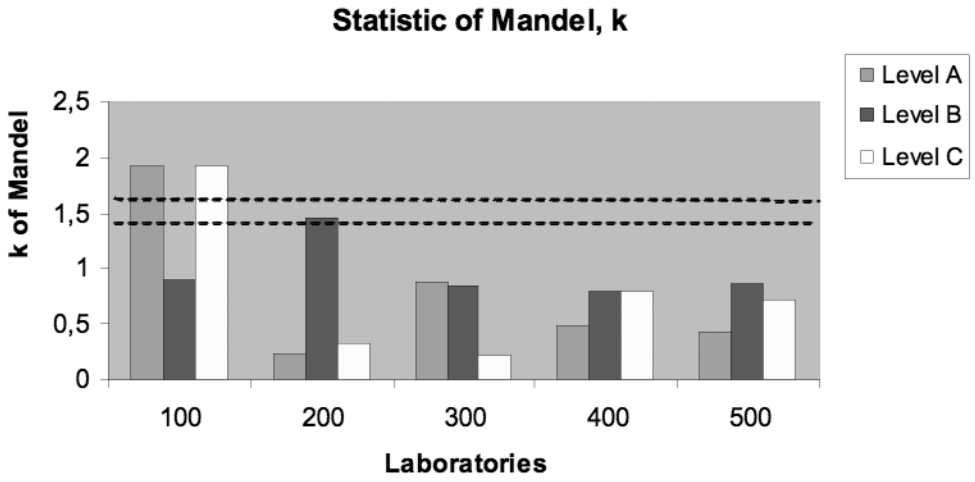

Figure 1: k statistic of Mandel

- Hide A: bovine hide for upper leather coloured black and casein cross linked finishing.
- Skin B: nappa of sheepskin coloured brown
- Skin C: double-face of sheepskin coloured black

Fourteen g. of ground sample were sent to each participant. Together with the samples, a letter describing the method to be applied as well as the period of time necessary for the analyses and the deadline for sending the results was sent. A table for result presentation was also submitted. This table included the codes for laboratory identification.

The analyses were made over the same time period (interval of two weeks) in order to minimise variability in conditions and results. On receipt of the samples, each laboratory commenced work separately.

The slope of the calibration curve must be known to determine the formaldehyde content in the analysed skins/hides. Each laboratory performed its own calibration in accordance with the conditions of the EN ISO 17226 Standard.

RESULTS

Once the analyses were performed, each laboratory sent the corresponding table of results to EUETII in order to determine the repeatability and the reproducibility of a standard method of measurement. The five laboratories are identified by a code assigned at random as 100, 200, 300, 400 and 500.

Tables I, II and III show the results obtained in the inter laboratory study.

After receiving all the results, the data were analysed in accordance with the international ISO 5725 Standard to validate the new method[13].

Application of the ISO 5725 Standard

It was applied the second part of the Standard ISO 5725 - 2 to determine the repeatability and the reproducibility of a method of measurement.

The data analysis was carried out by considering the following steps:

- o A critical examination of the data to identify outliers and other irregularities
- o Calculation of the preliminary values of precision and mean for each level independently
- o Establishment of final values of precision and averages including a relation between precision and the m level when the analyses indicate that such a relation can exist

The ideal case is $\boldsymbol{p}$ laboratories ($i = 1,2\ldots p$) each one at $\boldsymbol{q}$ levels of concentration ($j = 1, 2\ldots q$) with n replicates at each level, yielding a total of pqn results of the test

Data analysis (ISO 5725 - 2)

Analytical method: Determination of formaldehyde in skin/hides by HPLC liquid chromatography.

Reference: EN ISO 17226-1 Standard

Description: Five laboratories took part in the study. Each laboratory analysed the same three skin/hides performing five replicates of each and the recovery rate of, at least, two of the replicates in accordance with the method described in the above reference.

All the analyses were made over the same period time (from 19 to 28 April 2006).

Once the study was applied, a report was drawn up.

Summary of the report

a) Outlier laboratories.

Although the two outlier values that were found corresponded to the same laboratory, it was decided not to regard this laboratory as an outlier one.

b) Outlier or straggler values

By applying the Cochran test[13] two outlier values were found:

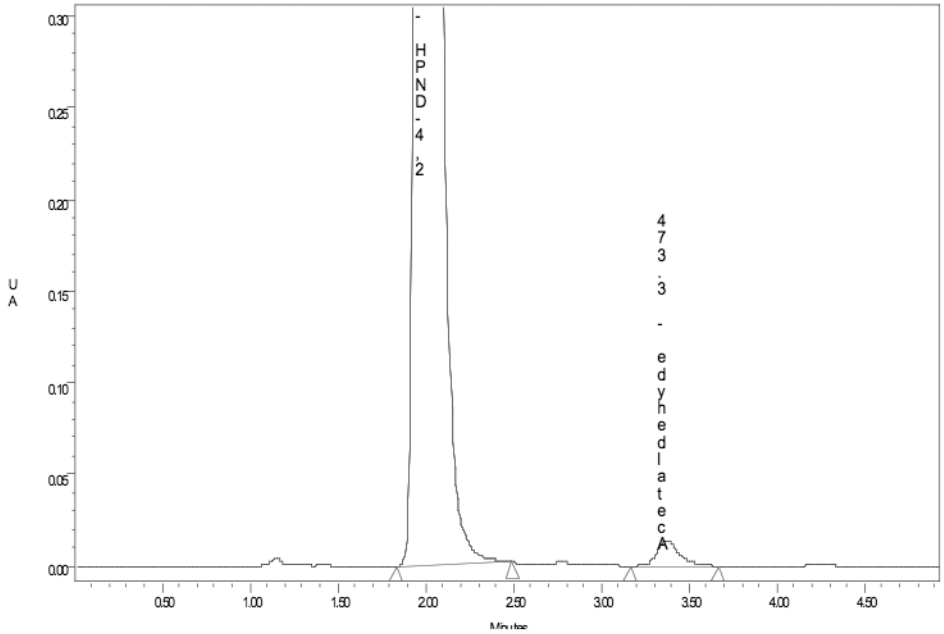

Figure 2: Chromatogram of acetaldehyde in accordance with the *prEN ISO 17226-1* Standard.

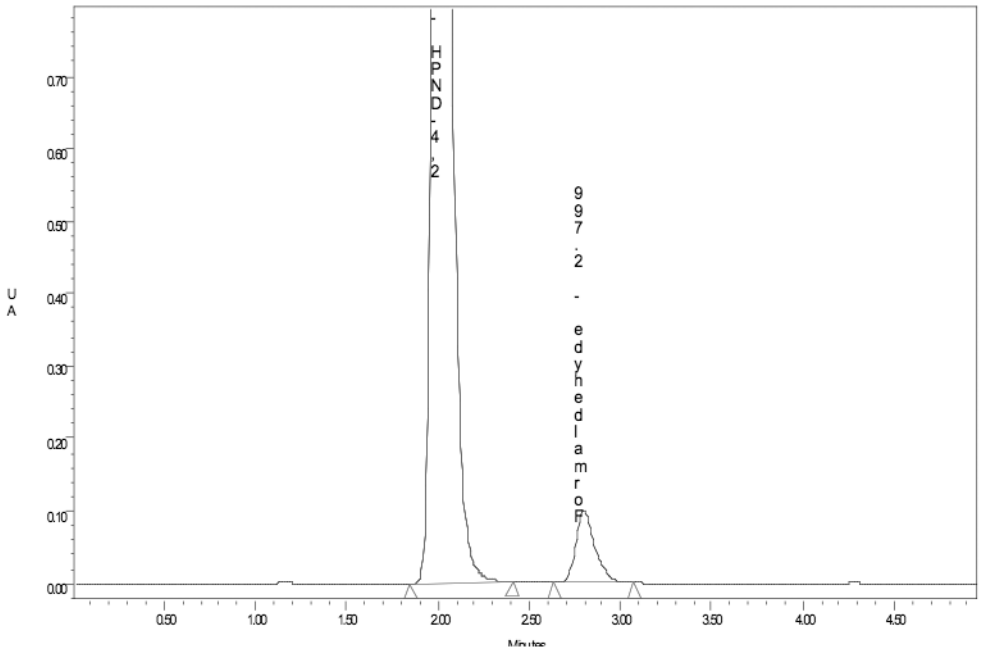

Figure 3: Chromatogram of formaldehyde in accordance with the *prEN ISO 17226-1* Standard

laboratory 100 for hide A and laboratory 100 for skin C. Given the big difference between these two values from the rest, they were separated. On the application of the statistic of Mandel (see Figure 1), two stragglers or outliers were suspected. The suspicion was subsequently confirmed with the Cochran test.

No explanation was found for these values so that, in accordance with the Standard, they were excluded.

c) Results obtained: average, repeatability and reproducibility

Table IV shows the results of average and variations obtained once the divergent values of laboratory 100 (for hide A and skin C) were eliminated.

d) Modified forms A and B

Tables V and VI show the forms A and B, respectively, once the divergent values of laboratory 100 were excluded.

e) Questions

The ISO 5725-2 Standard raises a series of questions about the results obtained. The answers to these questions in our case were the following:

Were the results divergent, outlier or straggler? If so, were they due to the description of the Standard for the new method?

The outliers found were not related to an error of the method since the description of it was very detailed. It has to be

TABLE I
Formaldehyde Content in mg/kg for Hide A in the Inter Laboratory Study

Skin B	1 mg/kg	2 mg/kg	3 mg/kg	4 mg/kg	5 mg/kg	Averge mg/kg	R.S.D. %
100	680.69	674.55	761.13	804.14	782.75	740.7	± 8.0
200	533.49	542.22	539.79	549.35	551.13	543.2	± 1.3
300	632.2	659.0	702.8	670.7	642.3	661.4	± 4.2
400	767.6	742.1	738.4	741.5	768.3	751.6	± 2.0
500	765.0	766.1	787.3	778.2	752.1	769.7	± 1.8
Average value of all participants						693.3	

TABLE II
Formaldehyde Content in mg/kg for Skin B in the Inter Laboratory Study

Skin B	1 mg/kg	2 mg/kg	3 mg/kg	4 mg/kg	5 mg/kg	Averge mg/kg	R.S.D. %
100	27.77	27.91	30.07	28.24	28.97	28.6	± 3.3
200	35.89	34.49	32.98	36.68	36.38	35.3	± 4.4
300	30.6	30.3	32.6	31.5	31.1	31.2	± 2.9
400	32.5	31.1	31.3	32.8	32.8	32.1	± 2.6
500	32.9	32.6	32.2	30.7	32.8	32.2	± 2.8
Average value of all participants						31.9	

TABLE III
Formaldehyde Content in mg/kg for Skin C in the Inter Laboratory Study

Skin C	1 mg/kg	2 mg/kg	3 mg/kg	4 mg/kg	5 mg/kg	Averge mg/kg	R.S.D. %
100	205.65	139.39	170.61	145.12	141.57	160.5	± 17.6
200	191.27	199.46	191.53	195.0	187.11	192.9	± 2.4
300	135.1	140.1	138.7	143.4	141.8	139.8	± 2.3
400	133.4	157.9	152.5	164.3	153.0	152.2	± 7.6
500	180.9	168.0	183.1	159.1	164.7	171.2	± 6.1
Average value of all participants						163.3	

borne in mind that formaldehyde is a gas which is not retained and in some replicates loss due to volatilization could occur.

What action was taken with respect to the outlier laboratory?

No action was taken since there was no laboratory considered as outlier.

Did the results of the outlier laboratories or any comment received suggest improvements to the new method?

No. Clearly, this is a very reproducible method and we therefore believe that it does not need any improvement. No comments were received from any laboratory in this connection.

Study of possible interferences due to other aldehydes

Along this study it has been mentioned that the reaction of derivatization with acetylacetone, which is the basis of the colorimetric method, is not absolutely selective for formaldehyde since acetylacetone can also react with other aldehydes. This is due to the fact that all aldehydes present their maximum absorbance at a wavelength of approximately 410 nm, giving rise to possible false positive results.

These possible interferences due to other aldehydes, which can be observed in the colorimetric method, are completely avoided in the method based on HPLC liquid chromatography, since this method is absolutely selective for formaldehyde.

TABLE IV
Results Obtained: Average and Variations

Level	p_j	$\bar{m}_J$	s_{rj}	S_{RJ}
A	4	681.5	17.4	102.4
B	5	31.9	1.1	2.6
C	4	164.0	8.3	24.3

p_j = number of laboratories contributing
m_j = mean;
s_{rj} = repeatability standard deviation
S_{RJ} = reproducibility standard deviation

In order to corroborate the above affirmation, a solution of the following aldehydes, which could cause interference in the formaldehyde determination, was prepared: acetaldehyde, glutaraldehyde and benzaldehyde.

An aliquot of each of the prepared solutions was reacted with 2,4 - dinitrophenylhydrazine and subjected to HPLC liquid chromatography in accordance with the pr EN ISO 17226-1 Standard.

As observed in the chromatogram, there is a peak corresponding to acetaldehyde with a retention time of 3.37 min. The retention time for the formaldehyde is approximately 2.80 minutes (figure 3), which is considerably different from that found for the acetaldehyde. Consequently, interference due to acetaldehyde is not possible.

The chromatogram shown in figure 4 confirms the lack of the signal for glutaraldehyde. In the same way, any peak is observed between 2.5 and 5.0 minutes in the chromatogram corresponding to the benzaldehyde sample. Consequently, the interference due to these two aldehydes in formaldehyde determination is not possible.

Because of their higher molecular weight, it is very probable that the retention time for these two compounds be higher than 5 minutes[11], which is the time established in the chromatographic method.

Possibilities of the photo diode array detector

With the PDA detector it is possible to obtain the UV spectra of each peak during the chromatogram of an actual sample. These spectra are then compared with those of standards of the main aldehydes (see figure 5), which are included in a previously created library.

Although there is a certain similarity between the spectra, the algorithm of comparison included in the PDA software allows the identification of the formaldehyde peak with a high level of confidence. In addition, given that the other aldehydes of interest have very different retention times, the identification and quantification of formaldehyde is carried out without any significant interference.

TABLE V
Form A: Collection of Original Data

	Level		
Laboratory	**A**	**B**	**C**
100	---	27.8	---
	---	27.9	---
	---	30.1	---
	---	28.2	---
	---	29.0	---
200	533.5	35.9	191.3
	542.2	34.5	199.5
	539.8	33.0	191.5
	549.4	36.7	195.0
	551.1	36.4	187.1
300	632.3	30.6	135.1
	659.0	30.3	140.1
	702.8	32.6	138.7
	670.7	31.5	143.4
	642.3	31.1	141.8
400	767.6	32.5	133.4
	742.1	31.1	157.9
	738.4	31.3	152.5
	741.5	32.8	164.3
	768.3	32.8	153.0
500	765.0	32.9	180.9
	766.1	32.6	168.0
	787.3	32.2	183.1
	778.2	30.7	159.1
	752.1	32.8	164.7

TABLE VI
Form B: Collection of all the Mean Values

	Level		
Laboratory	**A**	**B**	**C**
100	---	28.6	---
200	543.2	35.3	192.9
300	661.4	31.2	139.8
400	751.6	32.1	152.2
500	769.7	32.2	171.2
Average of all the laboratories	681.5	31.9	164.0

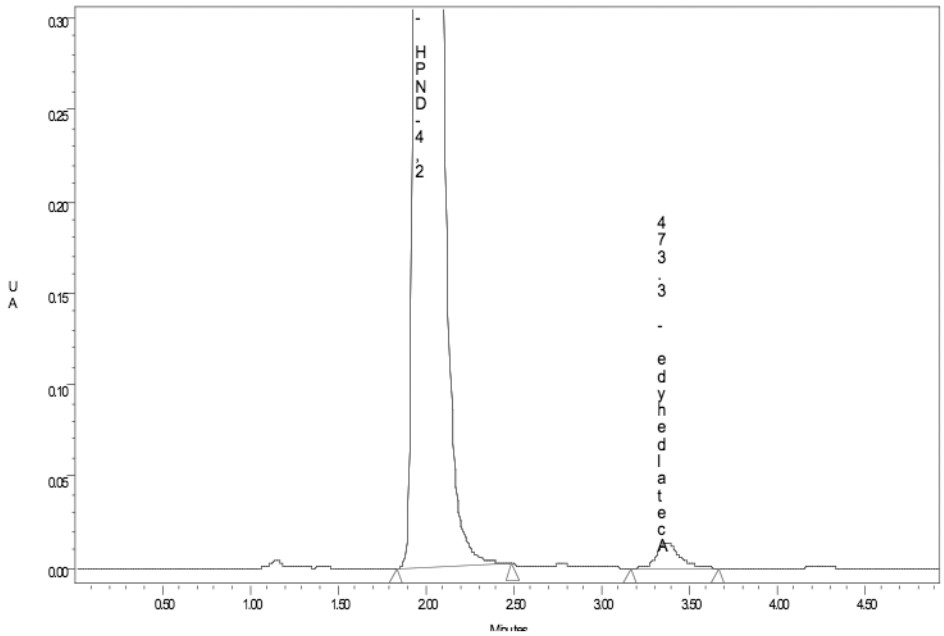

Figure 4: Chromatogram corresponding to the absence of glutaraldehyde signal in accordance with the *prEN ISO 17226-1* Standard..

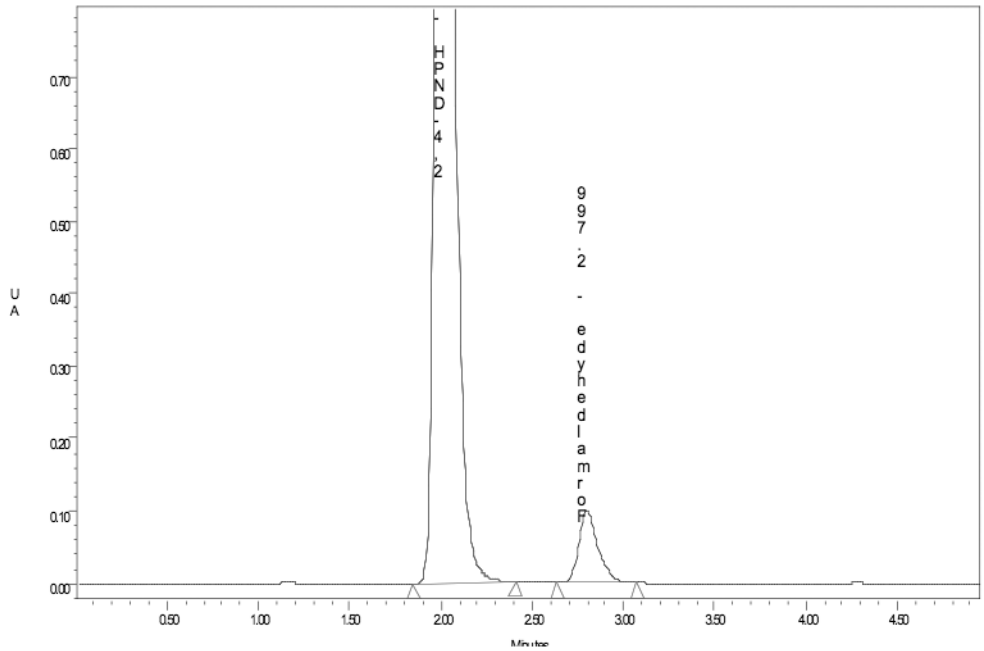

Figure 5: The UV spectra recorded by the PDA detector of the hidrazone derivatives of formaldehyde (solid line) and acetaldehyde (dashed line) are compared.

CONCLUSIONS

The colorimetric method based on the reaction with acetylacetone was the conventional procedure for the determination of the formaldehyde content in a leather extract. However, because of the difficulties arising from this method, priority has been given to HPLC.

It should also be noted that in case where the results differ, in accordance with the ISO International Organization the chromatographic method (EN ISO 17226-1) should be used in precedence to the colorimetric method (EN ISO 17226-2).

In order to assess the capacity of obtaining reproducible results with the EN ISO 17226-1 Standard, a collaborative study, which apart from the EUETII laboratory, counted on the participation of three Spanish laboratories and one Mexican laboratory, was undertaken. The five laboratories analyzed the same three skins and the results were studied jointly applying the criteria proposed by the ISO 5725 Standard to validate a standard method of measurement.

Once the results from the five participating laboratories were obtained and studied following the application of the ISO Standard, it may be observed that this method achieved some very reproducible results. Despite the complexity of the

procedure and bearing in mind that loss of formaldehyde for volatilization could occur, only two out of fifteen results obtained from the laboratories were divergent.

These two divergent results were confirmed by the statistical test that measures the variability within the same laboratory, not by the test that measures the statistical coherence between laboratories.

It was also confirmed that HPLC liquid chromatography to determine formaldehyde content in skins/hides is a very selective method since the other aldehydes in the extracts do not interfere in this determination.

It is very important to emphasize that this work also demonstrated that the interference of glutaraldehyde in the results was null.

Thus, despite the heterogeneity of the skins/hides and bearing in mind that formaldehyde is a very volatile substance that could result in a decrease of its content during sample transport, the inter laboratory collaboration was a success.

REFERENCES

1. G. Wolff, S. Hüffer; *JALCA* **97**, 456-464 (2002).
2. T.F. Allsop; Scavenging of analyzable formaldehyde in wool-skins tanned with phosphonium compounds, XXVI IULTCS Congress, Cape Town, South Africa, 2001.
3. B. Naviglio, G. Calvanese, G. Tortora, L. Cipollaro, G. Pierri; Characterization of tannery chemicals: Retanning agents. IULTCS II Eurocongress. Istambul, 24-27 May, 2006.
4. Öko-Tex Standard 100 issued by the Internationale Gemeinschaft für Forschung und Prüfung auf dem Gebiet der Textilökologie.
5. Commission Decision of 18th March 2002 establishing the revised ecological criteria for the award of the Community eco-label to footwear. Decision number C (2002) 1015, Official Journal of the European Community of 20 March 2002, L 77/50 to L 77/56.
6. J. Font; Análisis y ensayos en la industria del curtido. Tannery School, Igualada, Spain 2007.
7. prEN ISO 17226:2005. Leather - Chemical tests - Determination of formaldehyde content in leather (ISO/FDIS 17226:2005)
8. VDA stands for "Verband der Automobilindustrie": Association of the automobile industry.
9. Trials carried out in the Laboratories of the Igualada Leather Technology School which have not been published.
10. C.F. Allen, J.H. Richmond, J.; *Org. Chem.* **2**, 222-226,1937.
11. M. Vogel, A. Büldt, U. Karst,; *Fresenius J. Anal. Chem.* **366**, 781-791,2000.
12. V.R. Meyer. Practical High-Performance Liquid Chromatography. John Wiley & Sons, Chichester, 1996. Pages 144-148.
13. ISO 5725-2, Accuracy (trueness and precision) of measurement methods and results - Part 2:Basic method for the determination of repeatability and reproducibility of a standard measurement method.