HPLC versus Spectrophotometry for the Quantitation of Trace Amounts of Formaldehyde in Leathers

by

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Abstract

Several countries recently tightened their regulation concerning the formaldehyde content in leather samples. The assay is commonly done following the instructions of the standard CEN ISO TS 17226-2003. The norm proposes two methods applied after aqueous micellar extraction of the shredded leather sample: a colorimetric method and a chromatographic method. 110 leather samples were assayed for formaldehyde content over a two year period. The two recommended methods were used in parallel. The results are compared showing a high coherence in trend. The differences in absolute values can be as high as 300% in the high (300 mg/kg) as well as in the low (3 mg/kg) concentration levels. The chromatographic method working with a diode array detector (DAD) was found three to four times more sensitive than the spectrophotometric method reaching a limit of quantification (LOQ) of 2.5 mg/kg. The chromatographic limit of detection (LOD) was found to be 0.5 mg/kg. 19 samples were assayed using a third method: liquid chromatography with mass spectrometry detection (MS). The LOD and LOQ values were lowered using a tandem quadrupole MS that has also a much better selectivity working in the secondary ion MS/MS mode. Such an expensive detector may not be justified at the present for formaldehyde evaluation. A fluorimetric detector is recommended.

Resumen

Varios países recientemente han hecho más estrictas sus normas regulatorias sobre contenido del formaldehído en muestras de cuero. La estimación del formol comúnmente se hace siguiendo la norma CEN ISO TS 17226-2003. La norma propone dos métodos aplicables luego de una extracción acuosa de una micela proveniente de una muestra de cuero triturado: un método colorimétrico y otro cromatográfico. 110 muestras de cuero fueron examinadas por su contenido de formaldehído durante un período de dos años. Los dos métodos recomendados se utilizaron paralelamente. Los resultados se comparan demostrando alta coherencia en tendencia. Las diferencias entre valores absolutos pueden ser de hasta 300% en los altos niveles de concentración (300mg/Kg.) como en los bajos (3 mg/Kg.) niveles de concentración. El método cromatográfico utilizando un conjunto de diodos detectores (DAD) resultó ser de tres a cuatro veces más sensitivo que el método espectrofotométrico, alcanzando un límite inferior de detección cuantitativa (LOQ) de 2.5 mg/Kg. El límite cromatográfico de detección (LOD) se encontró ser 0.5mg/Kg. 19 probetas fueron evaluadas por un tercer método: cromatografía liquida con detección por medio de espectrometría de masa (MS). Los valores tanto de LOD como LOQ fueron reducidos utilizando un espectrómetro de masas con un conjunto tándem de cuatro electrodos [Massenfilter] con selectividad aumentada por operar en una modalidad MS/MS de ion secundario. Un detector tan caro pueda que no se justifique hoy en día para estimar el contenido de formaldehído. Un detector fluorimétrico es recomendado.

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INTRODUCTION

Formaldehyde, (CH_2O, Mw=30) is a gas at room temperature (b.p. -21°C). It is highly soluble in water and most polar solvents and also very reactive, easy to oxidize in formic acid and to polymerize. It is a very useful compound used in the production of resins, wood products, plastics, fertilizers, foam insulation and, in leather industry, it is an excellent disinfectant, sterilizing and tanning agent.[1] The problem is that formaldehyde is known for a long time to have carcinogenic and allergenic properties (especially in case of oral or skin exposition).[2]

Recently (October 2006), the People's Republic of China issued the Chinese Standard GB 20400-2006 for leather and fur that limited the formaldehyde content to less than 300 mg/kg (300 ppm) for Class C leather products (clothing, tapestry, decoration with no direct skin contact). The levels are down to less than 75 mg/kg for Class B leather products (all clothing without lining allowing direct skin contact) and less than 20 mg/kg for Class A products (same as Class B but involving products for babies).[3] These levels are consistent with the limits established by the European Union and Japan.

Formaldehyde has been routinely analyzed for years. The protocol of the current analytical procedure for formaldehyde assessment is extensively detailed by the standard CEN ISO TS 17226-2003. Russia uses Norm GOST 16704-71 and the Chinese norm is referred as GB/T 2912.1-1998. These norms are roughly equivalent proposing two methods for the quantification of formaldehyde in leather products: a spectrophotometric method and a chromatographic method. In both methods, formaldehyde contained in the leather sample is first extracted and reacted with a chemical producing a colored derivative that will be either directly quantified using a spectrophotometer or separated by a chromatographic column in the reversed phase mode and quantified with e.g. a diode array detector (DAD). In Europe, the standard CEN ISO TS 17226 is becoming EN ISO 17226 part 1 for the chromatographic method, separated from EN ISO 17226 part 2 for the spectrophotometric method. The chromatographic part 1 will be made the official method opposable in case of litigation.

With the tightening of the international regulations concerning formaldehyde content in leather products, it was decided to revisit the routinely used methods.[5] This paper compares the results obtain in the formaldehyde determination of more than one hundred different samples of various origins using the two methods. The results are discussed in term of coherence, sensitivity and experimental simplicity. A few determinations were also done using a recent mass spectrometer (MS) as the detector in liquid chromatography (LC) and compared with the classical DAD chromatographic results.

EXPERIMENTAL

Leather samples

The Lyon's facility of the French Centre Technique Cuir Chaussure Maroquinerie (CTC, Technical Center for Leather, footwear and leather good) has a recognized expertise in the analysis and control of leather samples. It routinely receives on a normal working day about ten different samples with different analyses requested. 110 formaldehyde determinations were requested by the CTC customers and the results were saved over a two year period. The results given to the customer correspond to a coherent average value of three assays made with the same method. Since the two methods were used, the two average values were compared. They are globally presented here. For obvious confidentiality reasons, the names and/or affiliations of our customers cannot be given.

The leather samples were classified in two groups: 6 vegetable-tanned samples making about 5% of the total set and 105 most commonly chromium-tanned samples making the remaining 95%. All samples were manually cut in pieces making few grams. A grinder was used to shred the leather pieces in particles not greater than 4 mm.

Chemicals

Water was produced by a Milli-Q Ultra-pure water purification system (Millipore, Bedford, MA, USA). The chromatographic reversed phase method needed acetonitrile and water. Acetonitrile (HiperSolv gradient grade) was obtained from Merck (Darmstadt, Germany). The extracting salt, sodium dodecylsulfate (GPR Rectapur grade) and the buffer chemicals, ammonium acetate and glacial acid acetic (Normapur grade) were also purchased from Merck. The derivatizing reagent 5,5'-dimethyl-1,3-cyclohexanedione (CAS : 126-81-8, common name : Dimedone) was obtained from Fluka (Sigma-Aldrich group, L'Isle d'Abeau, France). The other derivatizing agent, pentane-2,4-dione (CAS : 123-54-6, common name : acetylacetone) was supplied by Carlo Erba (Carlo Erba-SDS, Peypin, France).

A certified aqueous 63 mM formaldehyde solution (Fluka) was used for the calibration of the spectrophotometric method. It corresponds to a concentration of formaldehyde of 1.9 g/L. The chromatographic method was calibrated using a formaldehyde-2,4-dinitrophenyl¬hydrazone (DNPH) derivative standard solution obtained from Supelco (Sigma-Aldrich group, L'Isle d'Abeau, France). The solution corresponds to a concentration of formaldehyde of 100 mg/L. Standard solutions of formaldehyde or formaldehyde derivative were prepared by diluting the relevant reference solution with water (spectrophotometric method) or acetonitrile (chromatographic method).

Equipment

Colorimetric method

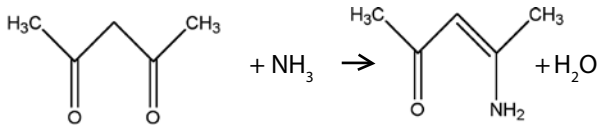
The spectrophotometric method was performed using a SAFASmc² double beam spectrophotometer (Monaco, France) working with a tungsten lamp. The pathlength is 20 mm.

Liquid chromatography/Diode array detector method

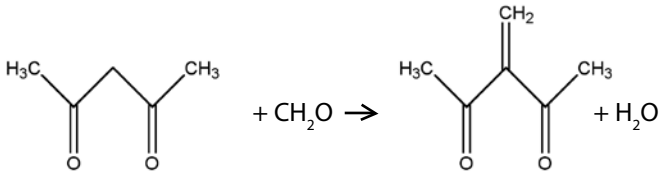
Series Agilent system (Palo Alto, CA, USA) consisting in a 1100 S quaternary pump, a vacuum degasser, an autosampler and a 1100 diode array detector (DAD). All data acquisition was performed using a Hewlett Packard pavilion a340 computer running the Agilent Chemstation Software (Rev A09.03).

Liquid chromatography/Mass spectrometry method

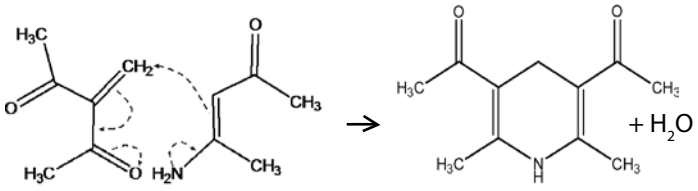
A triple quadripole mass spectrometer Agilent 6410 equipped with the 6490B multimode source operating in the Atmospheric Pressure Chemical Ionization (APCI) mode was available for coupling with a second Agilent 1200 modular system. Data acquisition for LC/MS was performed using a Dell Vostro 400 running the Agilent Mass Hunter software (Rev B01.03).



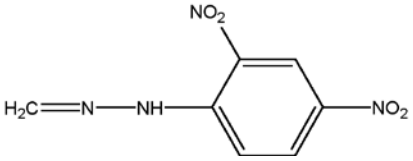
Solution A (acetylacetone in excess)



Intermediate reaction with formaldehyde



Formation of 3,5-diacety1-4,4'-dihydrogeno lutidine



Formaldehyde 2,4-dinitrophenylhydrazone

Figure 1: Chemistry involoved in the colorimetric detection of formaldehyde.

ANALYTICAL PROCEDURE

Extraction

A similar extraction procedure was used for both methods. Two grams of shredded leather were extracted by 50 ml of a 0.1% sodium dodecylsulfate micellar solution. The mixture was gently shaken at 40° ± 0.5°C in a water bath for 60 min ± 2 min. The warm extract solution is immediately filtered on a glass fiber filter (pore size is between 40 to 100 μm) and then cooled down to room temperature. The cooled filtrate is immediately tested for formaldehyde content by the colorimetric method and, simultaneously, by the chromatographic method. In numerous occasions, it was not possible to perform the two formaldehyde determination simultaneously. Then, a fresh filtrated extract was prepared the same day just before determination.

Colorimetric method

Chemical derivatization

The full procedure for the determination of formaldehyde is fully detailed in Part 5 of the norm CEN ISO TS 17226-2003. Two molecules of the betadiketone acetylacetone are needed for one formaldehyde molecule. The first molecule reacts with one molecule of ammonia to form an intermediate imine. The second acetylacetone molecule react with formaldehyde to form an adduct that can combine with the imine to make the bright yellow colored and fluorescent derivatized lutidine (Fig. 1) [4]. The method involves the use of three solutions:

Solution A: The derivatizing Solution A contains 150 g of ammonium acetate, 2 mL of glacial acid acetic and 2 mL of acetylacetone.

Solution B: The blank Solution B contains 150 g of ammonium acetate and 2 mL of acetic acid. It is the same as Solution A without the derivatizing agent, acetylacetone.

Solution C: is used to test for compounds other than formaldehyde that could cause coloring with acetylacetone. Solution C is made by 5 g of dimedone dissolved in one liter of water. Dimedone is a beta diketone similar to acetylacetone able to react with formaldehyde blocking it for further reaction with acetylacetone. Dimedone will not react rapidly with other aldehydes.

Protocol

5 mL of the cooled filtrate are mixed with 5 mL of Solution A in a first test tube. After 30 min of stirring at 40°C, all formaldehyde contained in the filtrate should be derivatized forming the lutidine yellow colored derivative, Fig. 1. Another 5 mL portion of the cooled filtrate is mixed with 5 mL of Solution B in a second test tube and stirred for 30 min at 40°C. It will serve as the blank reference tube. The first test tube is placed in the sample beam of the spectrophotometer. The second test tube is placed in the reference beam of

the spectrophotometer. The absorbance is measured at 412 nm. Using a calibration curve, the measured absorbance value allows calculating the formaldehyde content in the leather sample.

The calibration curve is obtained measuring the absorbance of five known solutions prepared using the certified 1.9 g/L solution. The five selected concentrations are 3.2 and 1.6 mg/L, 800, 600 and 400 µg/L prepared with 5 mL of Solution A. The blank solution is prepared with five milliliters of Solution A mixed with five milliliters of the extracting micellar solution. The calibration curve should be linear with regression coefficient (R^2) higher than 0.998 for the dynamic range studied (400-3200 μg/L). It corresponds to a formaldehyde content in the initial solid leather sample lying between 20 and 160 mg/kg.

The absence of interfering aldehydes is checked on the filtrate. 1 mL of Solution C is mixed with 5 mL of filtrate and stirred for 10 min at 40°C. Next, 5 mL of Solution A is added and the total 11 mL mixture is stirred for half an hour at 40°C. The reference solution is made in parallel with 1 mL of Solution C, 5 mL of filtrate and 5 mL of solution B. The absorbance is measured at 412 nm and must be close to 0 to ensure that no interfering aldehyde is present in the filtrate.

The absence of formaldehyde in the extraction reagents is checked by incubating for 30 min at 40°C 5 mL of the sodium dodecylsulfate micellar solution mixed with 5 mL Solution A. The absorbance is checked at 412 nm versus a 5 mL micellar solution plus 5 mL distilled water.

TABLE I
Mobile phase composition used for the chromatographic analysis of the DNPH derivatives

Flow rate : 0.5 ml/min	Time min	acetonitrile % v/v
Injection time	0	50
Gradient at 4% /3 min	12	66
Gradient at 8% /min	15	90
	17	90
Back to initial conditions	19	50
Column equilibration	32	50

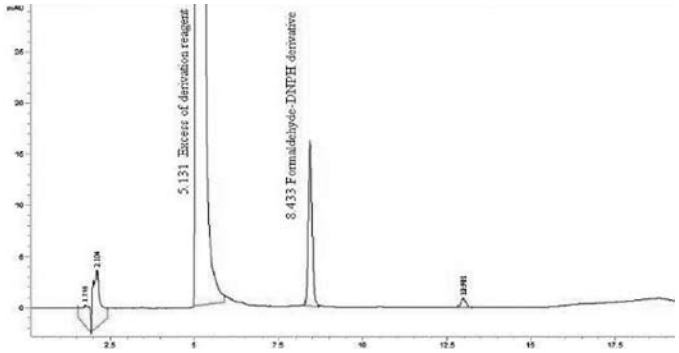


Figure 2: Chromatogram of a Quality Control sample spiking with 190 µg/l of formaldehyde corresponding to a content of 9.5 mg/kg in leather

TABLE II
Mass spectrometer settings (APCI source).

Parameter	Value
APCI ionization mode	negative
Drying gas temperature	300°C
Vaporization temperature	250°C
Drying gas flow-rate	5 L/min
Nebulisation pressure	140 kPa or 20 psi
Capillary voltage	2500 V
Corona current	5 µA
Formaldehyde detection	
Mass of selected fragment	209.1
Fragmentor voltage for MS/MS	100 V
Secondary mass for quantitation	132.8
Collision Energy	15 a.u
Secondary mass for quantitation	76.2
Collision Energy	15 a.u

a.u : arbitrary unit

LC/DAD method

Solution for sample derivatization

Formaldehyde contained in the filtrate is derived by 2,4-dinitrophenyl¬hydrazine (DNPH) for easy and sensitive detection. A chromatographic column is used to separate the formaldehyde-DNPH derivative from other aldehyde and ketone-DNPH. The derivative solution is prepared dissolving 60 mg ± 2 mg of DNPH in 20 mL of concentrated phosphoric acid (85% w/w) to give a 0.3% DNPH acidic solution.

TABLE III
Repartition of the samples according to their formaldehyde content.

Formaldehyde content mg/kg	Colorimetric method Number of samples	LC/DAD method Number of samples
$[CH_2O] < 10$	26	24
$10 \leq [CH_2O] < 75$	63	66
$75 \leq [CH_2O] < 150$	8	9
$150 \leq [CH_2O] < 300$	9	10
$[CH_2O] \geq 300$	4	1
$[CH_2O]_{HPLC}/[CH_2O]_{colorimetric}$ ratio	Percentage of samples	
$0.12 < R < 0.4$	11	
$0.4 \leq R < 0.8$	32	
$0.8 \leq R < 1.2$	36	
$1.2 \leq R < 2.5$	14	
$2.5 \leq R < 3.9$	7	

Derivatization procedure
5 mL of the filtrate are mixed in a 10 mL volumetric flask with 4 ml acetonitrile and 500 µL of the derivative solution and the flask is filled up to the mark with water. The mixture is left standing for at least 60 min to have the formaldehyde-DNPH fully developed (Fig. 1, bottom). It is immediately filtered and chromatographed.

Chromatographic analysis
Reversed phase liquid chromatography (RPLC) works with a polar aqueous mobile phase and an apolar stationary phase. The stationary phase was an octadecyl (C18) bonded silica contained in a 25 cm x 3 mm ID Purosphered® Star C18 column (Merck (Darmstadt, Germany)). 10 μL of filtrate were directly injected in the column and an acetonitrile-water gradient elution was run as described in Table 1. The temperature was regulated at 30°C. This gradient gave a total analysis time of 32 min. A chromatogram of the analysis is shown in figure 2.

The formaldehyde-DNPH derivative was detected at its strong UV absorbance maximum at 360 nm. The background noise was taken in the visible transparent region of the DNPH derivative spectrum at 540 nm. The DAD detector was able to record and to store on the computer hard disk a full 200-500 nm spectrum every second. Quantification is based on peak area and identification is based on both retention time and spectral comparison.

Standards were prepared using a formaldehyde-DNPH certified solution of 100 µg/mL or ppm. The calibration curve was constructed injecting five concentration levels. The five respective peak areas were fitted by linear regression. In all cases, the regression coefficients (R^2) were higher than 0.997 for the dynamic range studied (50-1000 μg/L) corresponding to an initial formaldehyde content in the solid leather between 2.5 and 50 mg/kg.

LC/MS method
This alternative LC/MS chromatographic method works with a different detector compared to the LC/DAD method. Since the used MS detector was very sensitive, the filtrate was diluted five fold with acetonitrile/water 50/50 v/v prior to injection. All other chemical and chromatographic conditions were the same for the DAD detection and MS detection.

The formaldehyde-DNPH derivative is detected with APCI operating in negative mode. APCI is able to work with a relatively high flow-rate (compared to MS with an electrospray ionization source). The 0.5 mL/min used with the LC/DAD method is compatible with the LC/MS method with an APCI source. The MS apparatus used allowed to work on a particular selected fragment using the very specific and sensitive MS/MS mode with no major modifications of the LC conditions. It was however required to divert to waste the first five min of elution in order to avoid pollution of the ionization source with excess unreacted and unretained DNPH. Table 2 lists the full set of MS settings.

The formaldehyde derivative was detected using the primary ion obtained at a fragment mass of 205 according Multiple Reaction Monitoring. The secondary fragment at 76.2 was used for formaldehyde qualification and the MS/MS fragment at 132.8 was used for quantification (Table 2). The calibration curve was constructed using another certified formaldehyde-DNPH solution with five concentrations taken in the 10-500 μg/L range. The peak areas were plotted versus the calibrated concentrations giving a straight line with a regression coefficient (R^2) higher than 0.997.

Results and Discussion

Spectrophotometric versus DAD chromatographic method

Comparing results

Fig. 3 compares the results obtained for the 111 different samples evaluated for formaldehyde content by the two methods. The log-log representation was selected given the wide range of concentration obtained. The straight line is the regression line with the simple equation: *log ([CH_2O] spectrophotometry) = 1.0263 x log ([CH_2O] LC DAD)* with an acceptable R^2 regression coefficient of 0.74. The slope of the regression line is remarkably close to unity demonstrating a clear coherence of results obtained with the two methods. The norm CEN ISO TS17226-2003 states that "the two methods should give similar trends but not necessarily the same absolute results," which was exactly what was observed with our set of data.

No difference was observed between the vegetable and chromium tanned samples possibly because the set of vegetable tanned samples was too small (5% of the total sample set).

The highest formaldehyde concentration was 500 mg/kg obtained by spectrophotometry with a colorimetric determination of 10 mg/L three times higher than the maximum concentration (3.2 mg/L) of the calibration curve. It was obtained for a brightly white colored leather sample. The corresponding chromatographic result was 221 mg/kg corresponding to only 44% of the colorimetry result. This value was four times higher than the maximum concentration (50 mg/kg) of the chromatographic calibration curve. It is seen that the two methods do give the same trend.

The highest formaldehyde concentration obtained with the chromatographic method was 334 mg/kg corresponding to a filtrate DNPH concentration of 6.7 mg/L almost seven times higher than the maximum concentration of the calibration curve. It was obtained for a native leather sample. The corresponding spectrophotometric result was 316 mg/kg completely coherent with the chromatographic value.

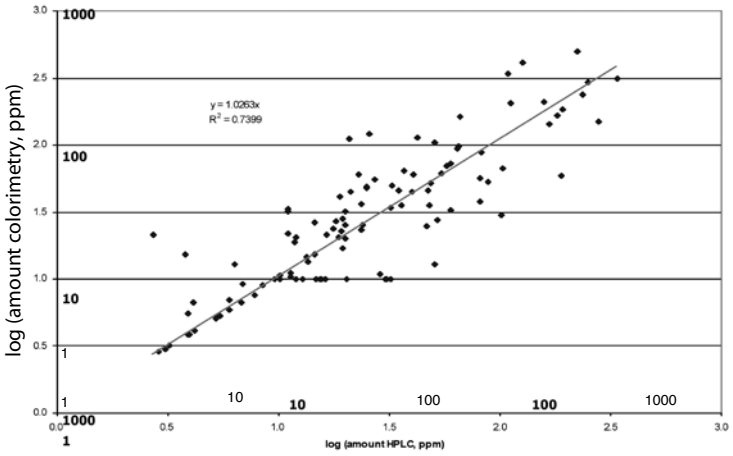


Figure 3: Comparison of the formaldehyde content obtained with spectrophotometric method versus chromatographic DAD method. The concentrations are expressed in mg/kg or ppm of solid leather in a log-log scale.

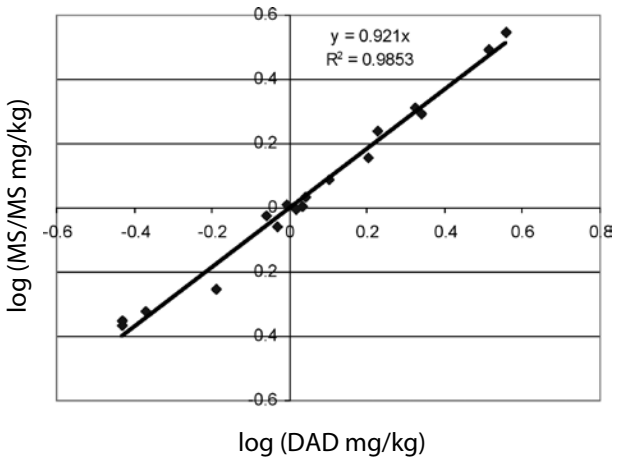


Figure 4: Comparison of the formaldehyde content obtained with the MS chromatographic method versus DAD chromatographic method for 19 leather samples. The concentrations are expressed in mg/kg or ppm of solid leather in a log-log scale.

The maximum discrepancy between the two methods was obtained with a vegetable-tanned leather sample also white colored. The results were 22 mg/kg and 2.7 mg/kg obtained respectively by spectrophotometry and chromatography. The colorimetric result is eight times higher than the chromatographic result, both results being well within the calibration curves. The opposite situation was observed with a black colored leather sample. The respective results were 13 mg/kg and 50 mg/kg. The chromatographic result is 4 times higher than the colorimetric result.

Table 3 lists the results sorted by increasing formaldehyde content and by the determination methods. The coherence of the results obtained using the two methods is seen one more time for the whole range of concentrations. The bottom of

Table 3 compares the ratios of the results obtained by the two methods for the same sample. 82% of the samples gave results by the two methods that were coherent the HPLC over spectrophotometric result ratio being between 0.4 and 2.5. For more than one sample over three (36%), the two methods gave similar results with ratio falling between 0.8 and 1.2 (Table 3).

Method performances

Clear differences can be seen on method performances. The chromatographic method is significantly more sensitive than the spectrophotometric method. A limit of quantification (LOQ) of 50 μg/L (corresponding to 2.5 mg/kg of solid leather) was estimated according the XPT-90210 standard that needs at least 25 different measurements to be significant. The corresponding LOQ, given by the CEN ISO TS 17226 norm for the spectroscopic method, is about four times higher being 0.2 mg/L or 10 mg/kg.

The DAD LC limit of detection (LOD) was estimated taking the formaldehyde concentration producing a signal corresponding to three times the average noise level (signal to noise ratio = 3). The chromatographic LOD value is 10 µg/L (or 10 ppb) corresponding to 0.5 mg/kg of leather or 0.5 ppm. This LOD compares well with the 3 µg/L LOD recently obtained with a sensitive UV detector in the determination of formaldehyde by flow injection analysis of wastewaters [5].

The spectrophotometric LOD value is not given by the CEN ISO TS 17226 norm. It is estimated to be around 40 µg/L (or 40 ppb) or 2 mg/kg of leather. Indeed, Fig. 3 shows 16 spectroscopic measurements that were below the given 10 mg/kg LOQ value going as low as 3 mg/kg corresponding to a filtrate concentration of 60 µg/L. All 16 spectroscopic measurements were corroborated by the corresponding chromatographic measurements.

Stability of the Formaldehyde-DNPH derivative

The CEN ISO TS 17726 norm requests that the DNPH formaldehyde derivatization be conducted for at least 60 min but no more than 180 min, suggesting that the DNPH derivative may not be stable. Then the stability of the derivative was investigated.

Three samples, two standard solutions containing 0.19 and 0.48 mg/L formaldehyde and one real sample were analyzed preparing the DNPH derivatives early morning. The derivatives were analyzed four times over a working day keeping the solution at room temperature in the dark. No differences were observed between the first early morning results and the late day results after 7 hours (less than 0.3% variation for the three samples).

Use of a MS detector

The MS detector is more sensitive than the DAD detector. 17 leather samples containing very low amounts of formaldehyde were analyzed using the MS detector as described in the experimental section. Fig. 3 compares the results obtained with the DAD detector and the MS detector on a log-log scale similar to that of Fig. 1. The slope of the regression line is very close to unity with a regression coefficient (R^2) of 0.985 demonstrating the excellent agreement between the results obtained with the two detectors.

The advantages of the MS/MS detection are an excellent selectivity obtained working with a secondary ion. This selectivity is associated with a greater sensitivity compared to the DAD detector. Recall that all MS measurements were done with five times diluted filtrate solutions. The drawback of the method is its cost. The problem of interfering aldehydes was not encountered with the samples treated. Obviously MS detection would be far superior to DAD detection in the case of a compound co-eluting with the formaldehyde derivative.

CONCLUSIONS

The spectrophotometric and the chromatographic reference methods give coherent results when applied to the same sample. Modern automated HPLC equipments render the ease of use of the chromatographic method comparable to the spectrophotometric method. The cost of the equipment is still very much in favor of the spectrophotometric method. However, since the present trend is irreversibly to lower the tolerated formaldehyde level in leathers, the chromatographic method is likely to become the preferred method for this assay. Not surprisingly, the use of a triple quad MS detector improved the method sensitivity and especially its selectivity. However the cost of the equipment does not justify its use for formaldehyde determination in leather at the levels investigated today. Since the DNPH formaldehyde derivative formed in the chromatographic procedure is fluorescent, the use of a fluorimetric detector, orders of magnitude more sensitive than a DAD detector, would be recommended in the case of very low concentration determinations.

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