Determination of Fungicides in Residual Tanning Floats Using Solid Phase Micro Extraction

by

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Abstract

Solid-phase microextraction (SPME) was optimized for extraction of the leather preservative agents 2-(thiocyanomethylthio)-benzothiazole (TCMTB), 4-chloro-3-methylphenol (PCMC), 2-phenylphenol (OPP), 2-Octyl-3(2H)-isothiazolone (OIT), 2-mercaptobenzothiazol (MBT) and 3-iodo-2-propynyl-butylcarbamate (IPBC) in spent tanning floats. Determination was carried out by high performance liquid chromatography (HPLC) with photo diode array detection (PDA). The following parameters were studied to achieve the maximum efficiency in extraction: fiber type, adsorption conditions (extraction time, ionic strength, temperature) and desorption parameters (time, temperature and composition of the desorption solvent). Results indicate that SPME using a 60 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber is appropriate for the extraction of these types of compounds. Recoveries ranged from 82% to 116% with RSDs between ±8% and ±12% and limits of detection below 1 mg/L except for IPBC. The optimized procedure was successfully applied for the determination of leather preservatives in eleven residual tanning floats taken from different companies. This method enables us to determine quantitatively the fungicides contained in the residual floats. Consequently, it will constitute a very useful tool to improve the preservative uptake in leather manufacturing processes.

Resumen

Micro extracción de fase-sólida (SPME) fue optimizada para la extracción de seis agentes químicos preservantes para cueros en los baños agotados de curtición. La determinación se efectuó por medio de cromatografía líquida de alto rendimiento (HPLC) por medio de un conjunto detector de fotodiodos (PDA). Los siguientes parámetros fueron controlados hasta alcanzar una máxima eficiencia en extracción: tipo de fibra, condiciones de adsorción (tiempo de extracción, tensión iónica, temperatura) así como los parámetros de desorción (tiempo, temperatura, composición del solvente de desorción). Los resultados indican que una SPME utilizando 60μm polidimetilsiloxano/divinilbenceno (PDMS/DVB) como fibra es apropiada para la extracción de estos tipos de compuestos. Recuperaciones del orden de 82%-116% con RSD's de ±8% a ±12% con límites de detección inferiores a 1mg/L. exceptuando el caso del fungicida 3-yodo-2-propinil-butilcarbamato (IPBC). El procedimiento optimizado fue exitósamente aplicado para la determinación de agentes conservantes en los once baños residuales de curtición obtenidos de diferentes compañías. Este método entonces nos permite determinar cuantitativamente los fungicidas contenidos en los baños residuales. Consecuentemente, esto constituirá una herramienta muy útil para mejorar la incorporación de conservantes en la curtición del cuero.

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Introduction

TCMTB is the most widely used preservative for controlling fungi in leather industry, but PCMC, OPP, OIT and MBT are also being employed. It is very common to use combinations of two or more molecules.[1,2] Figures 1 to 6 of a recent paper of this Journal show the chemical structure of these fungicides.[2] However, TCMTB and other fungicides used in the leather sector are not effective against many types of fungi. Moreover, their fungicidal capacity for vegetable or wet white leather is lower than for chrome leather. For this reason, the potential use of other molecules such as 3-iodo-2-propynyl-butylcarbamate (IPBC) in the leather sector is currently being investigated. The use of mixtures of three components, like PCMC, OPP and 2-benzyl-4-chlorophenol, has also been proposed.[3]

Determination of TCMTB was carried out by liquid chromatography (HPLC) rather than by GC to avoid the risk of thermal decomposition. The leather sample is ground in a mill and is extracted with a mixture of water/acetonitrile with the aid of ultrasonic waves for one hour. The filtered extract is analyzed by reversed phase HPLC with UV detection.[4] In a recent paper, we demonstrated that this method enables the simultaneous determination of eight fungicides in leather samples, including some promising preservatives under research studies that are not yet routinely used in this industry. The quantitative method was successfully validated in terms of linearity, precision, sensitivity and recovery.[2]

Nevertheless, no method for the simultaneous determination of different commercial preservatives in leather manufacturing waste waters has been reported. Analyses of floats before and after leather processes are useful to give an indication of the effectiveness of the treatment as measured by the uptake.

Parbery et. al. determined methylene bis thiocyanate and TCMTB in leather process liquors by liquid-liquid extraction (LLE) with CH_2Cl_2 and HPLC separation and quantification.[5] Two more papers have been published, but for only TCMTB determination. Meneses et al. proposed electroreduction of TCMTB and then electroanalytical determination [6] and Hinojosa et al. suggested indirect extraction-spectrophotometric determination after breakdown to 2-mercaptobenzothiazole[7]. Neither of these methods has been applied on a large scale, because the leather sector demands the simultaneous determination of different preservative compounds. In fact, very little is known about the residual content of fungicides in the effluent of a chrome tanning bath process. Parbery et al. found between 3 and 20 mg/L of TCMTB at the end of different industrial processes, and Hinojosa et al. found 42 to 116 mg/L in the industrial liquors analyzed in their research. Meneses et al. did not report any TCMTB concentration in real industrial wastewaters. The lack of data is due to the difficulty of analyzing such unclean and highly concentrated tanning wastewaters. In leather manufacture, a portion of the trivalent chromium offer remains in the exhausted bath after the tanning process, with large amounts of substances such as sodium salts, proteins, aminoacids, carboxylic acids and fats among others. This constitutes a complex analytical matrix for the quantitative determination of the residual content of fungicide at the level of ppms.

The methods developed for the determination of TCMTB, MBT and other benzimidazoles in tannery industrial wastewaters are also very limited. Fiehn et al.[8] and Reemtsma[9] used reversed phase HPLC coupled to UV detection and mass spectrometry, respectively. Although applied in the tanning process, TCMTB has not been detected in any of the analyzed tannery wastewaters owing to the known hydrolysis of TCMTB under alkaline conditions, such as in tannery effluents. Therefore, analyses for TCMTB uptake determination must be carried out in the residual tanning floats before mixing them with other waste waters.

The aim of this paper is to describe a rapid and simple method for the simultaneous determination of TCMTB and other fungicides in residual tanning floats and to evaluate the parameters of its validation. The waste bath sample is diluted, filtered, and the analytes are extracted by means of SPME technique. Determination is carried out by HPLC-PDA after desorption of the fiber. The extraction step of the developed method is absolutely innovative in the field of leather sector analysis.

The HPLC step of the method is the same as that previously validated in our earlier paper.[2] HPLC-PDA is the selected method because it is suitable for the simultaneous determination of the different fungicides used in the leather industry and also for other molecules that are currently being investigated. The sensitivity and selectivity of the method is enhanced by the selection of a specific optimal detection wavelength for each molecule. Moreover, the UV spectrum obtained from PDA detection allows the reliable confirmation of analyte identity. This is a robust method for the large number of variations that real samples may present i.e. variations that result from the origin of these samples (residual floats of chrome, wet-white or vegetable tanning process) and from the characteristics of the final leather articles to be manufactured. Finally, given that it is the same technique used in the ISO Official Standard for the determination of preservatives in hides and leather,[2,4] the laboratories of leather companies already have the necessary equipment.

The classic LLE method involves three sequential extractions of the sample with dichloromethane. The layers are separated by centrifugation[5]. Other authors use ethyl acetate (twice) and toluene.[8] The remaining organic solvent must be removed by evaporation to be replaced by methanol, acetonitrile or another

compatible solvent with the HPLC-reverse phase system. In the analyses of some real spent process liquors, emulsified layers are very often formed. These emulsions are difficult to break and, therefore, the quantitative recovery of the preservatives is not easy. LLE is time-consuming, uses hazardous organic solvents and is not cost-effective.

Very sensitive methods based on solid-phase extraction have been developed for determining some antifouling pesticides in water.[10, 11] However, of the fungicides used in the leather industry, only TCMTB has been included in such studies. In addition, these methods have been developed for environmental water samples with a matrix, which is much cleaner than that found in the residual floats of a tannery.

SPME has been used in the determination of both phenolic and benzimidazole fungicides, but not simultaneously. This SPME technique has two possible approaches: headspace exposure and direct immersion of the fiber in the aqueous sample. However, SPME extraction of fungicides from the headspace exposure would be limited to only volatile compounds, excluding molecules like OPP, TCMTB and OIT.

López et al.[12] indicated that SPME using a 75 µm carboxen–polydimethylsiloxane (CAR/PDMS) fiber is suitable for extraction of benzimidazole fungicides in environmental water samples. However, Hu et al.[13] selected the 60 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB) fiber because of its higher sensitivity in the determination of benzimidazole fungicides in aqueous apple extracts.

Phenolic preservatives have been widely extracted from environmental water samples by direct immersion SPME. Polar fibers, such as polyacrylate (PA), PDMS-DVB, and carbowax-templated resin (CW-TPR), have been successfully tested to extract phenolic compounds from water samples. However, when using PA fiber in SPME-HPLC, phenolic compounds are not totally desorbed from the fiber during the desorption step. [14-17]

The novelty of this paper lies in the extraction step of the method. It is based on the SPME technique. It has clear advantages over LLE: a marked increase in sensitivity; the simplicity of performing the extraction in only one cycle of absorption and desorption, and a considerable saving in time, chemicals and laboratory wastes.The method has been validated and applied in the analyses of ten residual tanning floats from four companies that produce different kinds of leather articles.

Experimental

Materials

Formic acid (~98%) for mass spectroscopy was obtained from Fluka. Analytical standards of fungicides were obtained from Supelco (TCMTB, PCMC), from Fluka (OIT, OPP, TCP), and from Aldrich (IPBC). Qualitative filter papers were supplied by Filtros Anoia (Spain). Acetonitrile as solvent was of HPLC-gradient grade from Panreac (Spain). Milli-Q ultrapure water (Millipore, USA) was used in the mobile phase and for reagents preparation. 2,4,6-Trichlorophenol from Fluka was used as Internal Standard (IS). IS stock solution was 6.25 mg/L in acetonitrile. Sodium chloride for ionic strength buffer solution (30% w/v NaCl and 0.20% v/v formic acid) was obtained from Panreac (Spain). Portable manual samplers of SPME with an extraction fiber coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm were purchased from Supelco (USA) (Product number 57359-U).

Instrumentation

HPLC-PDA system: Alliance 2695 Separation Module (Waters Corporation, USA) fitted with a 2998 PDA Detector. A Mediterranean Sea_{18} 15 x 0.46 cm 3μm column (Teknokroma, Spain) packed with C18 reversed-phase was used. UV scanning detection was performed between 190 and 380 nm. The mobile phase was 0.1% formic acid in water (A): 0.1% formic acid in acetonitrile (B). Gradient: 60% B, 6 min isocratic, then programmed linear to 95% B in 9 min. Flow was 0.9 mL/min. The oven temperature was held at 30ºC.

Samples

Eleven samples of industrial residual floats were analyzed. Seven were from chromium tanning floats, two were from wet white tanning baths, one was a residual float of a retanning process, and the last one was a residual bath of the fatliquoring process of vegetable leather production.

Procedure

A sample of approximately 20 mL of the float is filtered through a qualitative filter paper. 1.00 mL of the filtered float is pipetted into a beaker, which contains 10.0 mL of milli-Q water. From this solution, 1.00 mL (or 5.00 mL for low concentration floats) is transferred into a 50.0 mL volumetric flask, which contains 25.0 mL of ionic strength buffer and 1.00 mL of internal standard solution and is subsequently filled with milli-Q water to the mark.

8.0 mL of the former solution are then transferred to a 10mL vial. A magnetic stirring bar of 2 mm diameter and 8 mm long is added and the vial is capped.

The capped vial is preheated at 30ºC in a water bath for five minutes. The stirring speed is adjusted at 900 rpm. Then, the septum-piercing needle of the SPME portable sampler is introduced through the septum of the vial. The fiber is extended and immersed into the solution for 70 minutes at 30ºC (Figures 1 and 2).

Once the extraction step is completed, the fiber is introduced into a glass screw neck vial that contains 0.400 mL of a

Figure 1. Picture of extraction process.

Figure 2. Detailed picture of extraction process.

solution of 60% v/v acetonitrile in water for the static desorption step. The vial is held at 45ºC for 20 minutes. Subsequently, the fiber is retracted and the vial is homogenized. A 25-µL or 50-µL volume of this solution is injected into the HPLC system. After each analysis, the fiber is washed with Milli-Q water to remove the residues of NaCl.

The fiber can be reused until we detect that the sensitivity of the method decreases, i.e., when the area of the IS peak decreases substantially. From our experience, the fiber can be reused 75 – 85 times.

Analyte peak identity is determined by matching up the retention time with that obtained from the injection of analytical standards, and confirmed by comparing the UV spectrum obtained from each compound peak with that obtained from the standards. Detected preservatives are quantified using the calibration plots previously prepared with solutions of a given concentration of the standard fungicides extracted by SPME in the same manner as the samples.

The sensitivity and selectivity of the method is enhanced by the selection of a specific optimal detection wavelength for each molecule. The use of a PDA detector enables us to determine each fungicide at its own optimal wavelength. In accordance with the results of our previous paper [2], the best detection wavelengths are the following: for IPBC, 193 nm; for MBT, 324 nm; for OIT, 279 nm; for TCP, 290 nm; for OPP, 246 nm; for PCMC, 228 nm and for TCMTB 223 nm.

Results and Discussion

The development of a SPME method involves the selection of the fiber and the optimization of desorption and adsorption conditions.

Fiber Choice and Development of Desorption Conditions

The performance of three different commercial fibers was compared: PDMS-DVB (60 µm), CAR-PDMS (75 µm) and PDMS (100 µm). The mixture acetonitrile/water, which was recommended as mobile phase in the ISO: 13365 Standard, was used as desorption solution. The use of acetonitrile alone is not advisable since this shortens the fiber lifetime[15]. The influence of temperature between 25ºC and 45ºC on fungicide recovery was investigated. A temperature of 45ºC provided the best results. Moreover, the acidification of the desorption solution with 0.1% of formic acid impaired the efficiency of the process for all fibers under study.

Given that the PDMS fiber did not have sufficient affinity for the analytes, it was discarded. Comparative studies of desorption with the two other fibers at different times and temperatures were carried out. It was concluded that the PDMS-DVB fiber allowed a total desorption after 20 min at 45ºC. This total recovery was not achieved with the CAR-PDMS fiber even though the desorption time was prolonged.

Consequently, the PDMS-DVB fiber was selected for the study.

Adsorption Conditions

There are some variables that should be adjusted in SPME to achieve good extraction efficiencies. These parameters include time and temperature of adsorption, sample volume, stirring conditions, and ionic strength of the sample.

The volume of the sample was fixed at 8.0 mL so that the fiber was completely immersed but maintaining a safe distance with the stirring bar. A lower volume is not recommended to avoid the risk of breaking the fiber.

For many organic compounds, aqueous solubilities decrease in the presence of large amounts of salt due to salting-out effect [18]. Two tests with different concentrations of NaCL (5 and 15%), both with 0.1% of formic acid, were carried out to evaluate the effect of the ionic strength.

Higher salt concentrations can damage the fiber [19]. The results obtained showed that for all the fungicides, except for TCMTB, the recoveries substantially improved with the highest salt concentration (15% of NaCl). By contrast, TCMTB adsorption was independent of salt concentration.

The investigated temperatures were 30, 45, 60 and. 75ºC. For all the fungicides, the highest adsorption was achieved at 30ºC.

The SPME technique is based on the equilibrium established between the aqueous medium and the fiber coating of the analyte concentration. The time necessary to reach this equilibrium may vary from a few minutes to several hours.

The studied interval was 30, 50, 70, 90 and 120 min for each analyte in the presence of all other target compounds. Shaking was kept constant at 900 rpm with the same type of magnetic bar. The results showed that 120 min were not sufficient to reach equilibrium.

Jiu Ai [20] demonstrated that if adsorption time and shaking conditions are kept constant, it is not necessary to reach equilibrium to carry out a quantitative analysis in reproducible conditions. He termed this as non-equilibrium conditions.

The robustness of the quantitative results under non-equilibrium conditions at 70 min and for three concentration levels, between 0.1 and 1 mg/L, was investigated. The best level for calibration corresponded to the lowest concentration.

Validation

Linearity

In order to compensate for the possible diminution in efficiency of the fiber during its lifetime, a calibration with 2,4,6-trichlorophenol as internal standard was preferred. Calibration was carried out by the same SPME treatment as real samples.

The calibration plots were obtained for each of the six fungicides from multi-component standard solutions that contain all the analytes. Four standards of different concentration and a blank were prepared. Table 1 shows the concentration range of the extraction solutions used as calibration standards. Internal standard concentration was 125μg/L in every solution. Each point on the calibration plot was the arithmetic mean from two independent chromatographic injections.

Recovery and Precision

Synthetic samples were prepared from a residual float of a tanning process free from fungicides and spiked with given amounts of the analytes. To measure the accuracy and the precision of the analytical method, the samples were analysed each day during one week. The results obtained (average of the Recovery as well as the Relative Standard Deviation) are listed in Table 2.

TABLE I
Range and linearity of the calibration carried out for each analyte.

Fungicide	Concentration range	Correlation coefficient (r)
TCMTB	48 - 244 μg/L	0.9992
PCMC	27 - 136 μg/L	0.9999
OPP	14 – 71 μg/L	0.9999
IPBC	199 - 995 μg/L	0.998
MBT	18 - 91 μg/L	0.998
OIT	21 - 108 μg/L	0.9999

TABLE II
Recovery and precision.

Fungicide	Concentration	Recovery (%, n=5)	RSD (%, n=5)
TCMTB	2.0 mg/L	95.0	± 8.6
PCMC	2.0 mg/L	116	± 12
OPP	1.7 mg/L	88.0	± 7.7
IPBC	14 mg/L	84.8	± 9.1
MBT	1.4 mg/L	81.9	± 12
OIT	1.9 mg/L	99.4	± 12

Limits of Detection and Limits of Quantification

Detection limits (LOD) were calculated for a signal-to-noise ratio of 3. Limits of quantification (LOQ) defined for a signal-to-noise ratio of 10 were also estimated. These values are calculated for 50-μL injection volumes and are shown in Table 3.

Robustness of Calibration

Four months later, a new calibration was carried out. New standard solutions were prepared but employing the same fiber, which had been used in approximately 70 absorption-desorption processes during this period. The Student's t-test was used to compare the differences between the results of the same samples calculated with both calibrations. No significant difference was observed in spite of the ageing of the fiber and the possible dispersion introduced as a consequence of the preparation of new solutions of standards or reactants. Moreover, small deviations in the shaking speed or in the control of temperature and absorption-desorption time made no difference.

Analyses of Real Samples

The method was applied in the analyses of eleven residual tanning floats from four companies. The results are presented in table 4. As can be seen, residual floats 1 to 6 have higher concentrations than floats 7 to 11. This can be explained because samples 1 to 6 are residual floats from two tanneries that produce wet blue and wet white for storage purposes. By contrast, samples 7 to 11 are residual floats from two tanneries that employ a lower amount of fungicide because leather is produced in a follow-on process. Some floats contained more than one fungicide molecule. In the recipe of sample number eleven, PCMC and OPP were applied in the tanning process and TCMTB in the fatliquoring one. The fatliquoring residual float contained TCMTB and the remains of PCMC and OPP released from the tanned leather to the waste bath. There was no fiber damage or poor chromatographic resolution or any problem in the analyses of such real samples from diverse sources containing complex matrixes.

CONCLUSIONS

A very sensitive and highly specific method based on solid-phase microextraction and liquid chromatography-photo diodes array detection was developed and validated to determine residual amounts of preservatives in wasted floats. This method avoids the use of hazardous materials like solvents and does not generate significant laboratory wastes. Sample preparation consists of a 2-hour extraction, filtration, and injection. The UV spectrum from PDA detection allows us to reliably confirm analyte identity. The wavelength used for quantitative determination is specific for each fungicide.

The analyses of real samples showed residual amounts of TCMTB ranging from 28 to 80 mg/L in wasted tanning floats

TABLE III
Limits of detection and quantification.

Fungicide	LOD (mg/L)	LOQ (mg/L)
TCMTB	0.25	0.83
PCMC	0.14	0.47
OPP	0.06	0.20
IPBC	6	17
MBT	0.05	0.17
OIT	0.07	0.25

TABLE IV
Results obtained in the analyses of eleven residual floats from different sources.

Sample	Fungicide detected	Concentration found
Residual float of wet blue production. Company 1- wb1.	TCMTB	78.5 mg/L
Residual float of wet blue production. Company 1- wb2.	TCMTB	77.7 mg/L
Residual float of wet blue production. Company 1- wb3.	TCMTB	47.2 mg/L
Residual float of wet white production. Company 1- ww4.	TCMTB	18.7 mg/L
Residual float of wet white production. Company 1- ww5.	TCMTB	20.2 mg/L
Residual float of wet blue production. Company 2- wb6.	TCMTB	28.2 mg/L
Residual float of tanning process. Company 3- tan7.	TCMTB	6.0 mg/L
Residual float of tanning process. Company 3- tan8.	TCMTB	5.7 mg/L
Residual float of tanning process. Company 3- tan9.	TCMTB	5.3 mg/L
Residual float of retanning process. Company 3- ret10.	TCMTB PCMC OIT	4.7 mg/L 2.4 mg/L 2.1 mg/L
Residual float of fatliquoring process in vegetable leather production. Company 4- veg11.	TCMTB PCMC OPP	2.7 mg/L 7.0 mg/L 2.2 mg/L

for wet blue for storage production and from 5 to 6 mg/L for wet blue to be shaved and retanned in a short period of time. Wet white residual floats contained a lower amount of TCMTB. This was due to a better yield of the process, because the amount of TCMTB, the length of the process and the volume of the float were the same as in wet blue production. Furthermore, the residual amounts of TCMTB in the wet white leathers were higher than in the wet blue ones.

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