Improved Method for Accurate and Efficient Analysis of Chlorophenols in Leather Compared with Conventional Steam Distillation Operation Specified by ISO 17070:2015

by
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Abstract

An improved analytical method was built based on the conventional standard method ISO 17070-2015 for the determination of 19 chlorophenols in leather. This developed method involved ultrasonic assisted extraction using methanol containing formic acid, derivatization using acetic anhydride, solid-phase extraction (SPE) cleanup with silica-gel cartridge. Final analysis of the chlorophenols compounds was performed by Gas Chromatography - Mass Spectrometry (GC-MS). Optimum conditions for sample extraction, such as time, temperature and formic acid content in methanol were studied. Satisfactory recoveries in the range of 92-105% (RSD, 2.1-7.3%) for the targets were obtained, and detection limits were in the range of 0.09-0.15 mg/kg. The developed procedure was evaluated and compared with ISO 17070-2015 which uses steam distillation for sample extraction. The method was successfully applied to determine 19 chlorophenols in series of leather samples from different origin. This study demonstrated that the ultrasonic assisted extraction, followed by acetyl derivatization and silica-gel cleanup coupled with GC-MS, can be used as an alternative to ISO 17070-2015, for detecting chlorophenols in leather.

Introduction

Chlorophenols (CPs) are well known ubiquitous pollutants in the environment for many decades.[1] Generally, CPs consist of three monochlorophenols (MCPs), six dichlorophenols (DCPs), six tri- chlorophenols (TrCPs), three tetrachlorophenols (TeCPs), and pentachlorophenol (PCP). Due to their antimicrobiological properties, CPs are used as herbicides, insecticides and fungicides in agriculture, and also as wood preservatives and intermediates in paper and leather industries.[2] CPs are persistent toxic substances which cause histopathological changes and mutations in aquatic life, and some are probable human carcinogens, e.g., PCP.[3] CPs have been classified by the International Agency for Research on Cancer (IARC) as possible carcinogenic agents.[4] Meanwhile, the negative effect of CPs on human health has led to their categorization and inclusion by the US Environmental Protection Agency (EPA) and the Commission of the European Communities in the lists of priority pollutants.[5-6]

Although the use of CPs has been officially restricted, they can still be found in many different matrices like food, water, sludge, soil, wood, textile and leather.[7-8] The presence of CPs in leather presents a health risk if there is an exposure to skin especially at a high temperature, for example, in car seats or sofa because of their lipophilic character.[1] There is no doubt that their analysis is of great importance in controlling the quality of leather. Therefore, providing an appropriate method is essential to screen residues of CPs in leather samples. Though, their analysis in leather has not been studied enough and few articles are available in the literatures.

The current standard method for analyzing 19 CPs in leather samples is ISO 17070-2015, which was totally transformed from the earlier edition ISO 17070-2006 specified for PCP determination. This method uses a steam distillation extraction, followed by acetyl derivatization and GC analysis. The methodology was initially used for analyzing CPs in soils and wood samples.[9] Steam distillation extraction gives relatively clean extracts, although the process takes a few hours or longer. To our knowledge, the validation of this procedure for leather has not yet been carried out. This method is time consuming, making it unsuitable when testing large numbers of leather samples. In addition, thermal decomposition of some halogenic dyes to release chlorophenol (as 4-MCP) due to the high temperature during the steam distillation, which might give false results.[10-11]

To solve the time consuming problem associated with steam distillation in ISO 17070-2015, a direct method has been reported based on Accelerated Solvent Extraction (ASE) technique followed by solid-phase extraction (SPE) cleanup and HPLC analysis.[8] Although this procedure presented a quick and efficient operation, the equipment of ASE was seldom employed in normal laboratory compared with Soxhlet or ultrasonic apparatus. Besides, the high pressure and temperature (100°C) might easily cause the release of CPs from certain halogenic dyes.[11] Comparatively, ultrasonic assisted solvent extraction is commonly preferred due to its high efficiency, simplicity and low extraction temperature. Ultrasonic assisted extraction has been officially recommended and applied for solid sample extraction as rubber, textile and leather in the normal laboratory (EPA 3550C, ASTM G136).

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Although HPLC might be desirable for directly detecting CPs without further derivatization, peak resolution of CPs has often been poor because of the co-elution of isomers as well as the interference of co-extractives.[12] LC-MS has been investigated for screening CPs and presented satisfactory results,[11-13] but the procedure is rarely employed in the normal laboratory due to the high cost of the equipment. Comparatively, method including acetyl derivatization based on GC is still predominant for routine analysis of CPs due to GC popular use, as well as its high resolution and sensitivity,[14] even if the acetylation operation requires extra time.

The aim of this work is to develop an improved procedure based on ISO 17070-2015, for determining CPs in leather with higher efficiency. The procedure is characterized by accuracy, adaptability, and reduced execution time. To improve extraction efficiency, extraction by ultrasonic assisted methanol containing formic acid is applied. The extracted CPs are further derivatized with acetic anhydride, purified and enriched by SPE, and detected by GC-MS. A validation procedure has been proposed and the optimized method can be applied to determine the 19 CPs (as listed in Table I) in real leather samples. The characteristics of the approach were compared with those obtained by ISO 17070-2015.

Experimental

Reagents

Acetone, n-hexane and methanol used to dissolve standards, and to prepare the extracts were of pesticide-grade obtained from Fisher Scientific (Shanghai, China). Acetic anhydride, anhydrous potassium carbonate (K_2CO_3), formic acid (88%), triethylamine and anhydrous sodium sulfate (Na_2SO_4) were of analytical grade from Anpel Co. Ltd (Shanghai, China). Na_2SO_4 was heated at 150°C for 24 h prior to use.

Reference standards of 19 CPs (in Table I), and internal standard Tetrachloro-o-Methoxyphenol (TCG), were obtained from Sigma-Aldrich (Shanghai, China), and their stock solutions (1000 mg/L) were prepared by dissolving appropriate amounts of the commercial products in acetone and stored in glass-stoppered bottles at 4°C. Appropriate volumes of the stock solutions were diluted to prepare solutions containing chlorophenols at 0.2-10 mg/L by n-hexane/acetone (2:1, v/v). Table I lists the CPs with numbers and abbreviations identifying the compounds.

Apparatus

The following apparatus were used in the experiment: Shaking machine designed especially for separating funnel (Haode in Hunan, China), votex for mixing (NuoMi in Changzhou, China), ultrasonic water-bath with ultrasonic frequency 45/60/100 kHz and power 200 W (Kunshan, China), steam generator with power 4 kW (Sespur in Foshan, China), PTFE membrane filter of 0.45 μm ×10 mm (Shanghai, China), and Solid Phase Extraction (SPE) system, with Silica-gel cartridge 0.5 g/6 mL (Agela in Tianjin, China).

Sample preparation

Eighty-five leather samples were analyzed, comprising shoe leather (25), sofa leather (32), garment leather (23) and car seat leather (5). These samples were obtained from the market located in Haining China-Leather City. Leather samples with moisture of 9-13% w/w, were suitably prepared by cutting into pieces (~3 mm × 3 mm) and sealed in bags lined with aluminum foil, which were ready for use.

The negative leather samples were prepared by the following operation. Sample pieces were immersed in ~10-fold methanol containing formic acid (0.5%) and treated by ultrasonic for 60 min at 45-55°C. Then the leather pieces were filtered and retreated twice under the same conditions. Further, the leather pieces were immersed in distilled water for 5 min. After filtering, the pieces were left standing at standard atmosphere for at least 7 days until the moisture content was at the range of 9-13% w/w.

The spiked samples were prepared by adding aliquots of CPs stock solutions to the negative leather pieces of 2 g. The stock solution was added to the sample slowly while verifying that the solution was absorbed by the pieces completely. Then the samples were conditioned in a sealed glass bottle for at least 7 days at room temperature, to allow the full adsorption of the CPs by leather fiber.

Sample extraction

Leather samples (2 g) were placed in a 50 mL screw-capped glass bottle. Methanol (20 mL) containing formic acid (0.5%, w/w) was added, the bottle was then sealed and immersed into the ultrasonic water bath (45 kHz) and treated continuously for 30 min at 45-55°C. After cooling to room temperature, the methanol was retrieved and the leather pieces were re-extracted with another 20 mL methanol containing formic acid (0.5%, w/w) with ultrasonic for 10 min. The extracts were then pooled into a pear-shaped flask and basified with 2 mL K_2CO_3 solution (1 mol/L) to prevent the loss of CPs. The solution was then concentrated at ~50°Cwith a rotary evaporator under reduced pressure, to yield residues about ~2 mL.

Derivatization

The derivatization of CPs was according to the routine operation described in ISO 17070-2015 and other references,[16-17] with minor modifications. Briefly, the residue in the flask was dissolved in 20 mL K_2CO_3 solution (0.1 mol/L) and transferred into a separating funnel. Then 4 mL aliquot of n-hexane, 0.1 mL triethylamine and 0.3 mL acetic anhydride was introduced into the funnel in that

order. The funnel was then fixed onto a shaking machine. It was initially shaken manually until the evolution of CO_2 subsided. Afterwards, it was shaken vigorously for 30 min, to yield acetylated CPs. The n-hexane extracts were then retrieved and the remaining water solution was re-extracted with another 4 mL n-hexane. The n-hexane extracts were pooled and dehydrated by Na_2SO_4, in readiness for cleanup.

Cleanup

The commonly used silica-gel cartridge (0.5 g/6 mL) was chosen for cleanup.[15,18] The cartridges were first cleaned and conditioned with 2 × 3 mL of n-hexane, then the dehydrated n-hexane extracts (~30 mL) was transferred into the cartridge. The solution was allowed to run through the cartridge freely at a flow rate of approximately one drop per second. The cartridge was then instantly washed using 5 mL of n-hexane, followed a gentle purge by a rubber pipette bulb, or short pulse of vacuum, to remove excess of washing solution. Elution was performed with 1 × 1 mL mixture of n-hexane/acetone (2:1, v/v), and eluants were pooled into a 2.0 mL volumetric flask and filled with n-hexane to the mark. The solution was then filtered on a 0.45 μm PTFE filter, which was now ready for GC-MS analysis.

A flowchart for the GC-MS analysis of CPs in leather is shown in Figure 1.

Sample test according to ISO 17070-2015

For method comparison, samples were tested according to the description in ISO 17070-2015. The samples (1 g) were first extracted with steam distillation and the eluted CPs were captured by K_2CO_3 (5 g), which were finally made up to 500 mL using distilled water. Then 100 mL of the target solution was transferred into a separating funnel for acetyl derivatization. For analytical tool, GC-MS was used because of the insensitivity of MCP/DiCP by Electron Capture Detector (ECD). Besides, cleanup after acetyl derivatization, were also included to improve the detection limits. The final solution of 2.0 mL was collected and filtered for GC-MS analysis.

GC-MS analysis

Agilent 8890 gas chromatograph equipped with 5977A mass detector operated in the positive electron impact mode (EI+) was used. The separation was performed on a fused silica capillary column (DB-5 ms; film thickness, 0.25 μm; 30 m × 0.25 m i.d. (J&W Science)). Splitless injection (1 μL) with purge time 0.8 min was made by an autosampler. The temperature of the injector was 260°C. The initial oven temperature was 60°C, and it was programmed to 100°C at 15°C/min and then to 220°C at 8°C/min, followed by a 50°C/min to 270°C with 5 min hold. The MS parameters were as follows: carrier gas, helium; flow rate, 1.0 mL/min; transfer line temperature, 260°C; solvent delay time, 2 min; ion source temperature, 150°C; quadrupole temperature, 230°C.

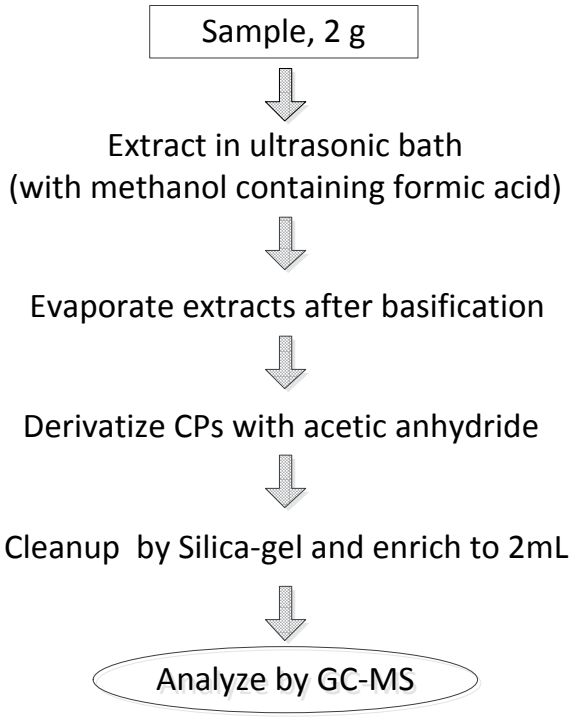

Figure 1. General flowchart of analytical procedure developed in present work

Table I

Target CPs with their CAS No., pK_a, retention time and detection ions

No	Compound	CAS. No	pK_a [a)]	Retention time (min)	Selected ions (m/z)	
					Quantitation ion	Confirmation ion
1	2-MCP	95-57-8	8.56	5.22	128	130
2	3-MCP	108-43-0	-	5.56	128	130
3	4-MCP	106-48-9	9.18	5.67	128	130
4	2,6-DiCP	87-65-0	6.79	6.97	162	164
5	2,4-DiCP	120-83-2	7.89	7.27	162	164
6	2,5-DiCP	583-78-8	-	7.27	162	164
7	3,5-DiCP	591-35-5	-	7.45	162	164
8	2,3-DiCP	576-24-9	-	7.73	162	164
9	3,4-DiCP	95-77-2	-	8.10	162	164
10	2,4,6-TrCP	88-06-2	6.23	8.65	196	198, 200
11	2,3,6-TrCP	933-75-5	5.80	9.30	196	198, 200
12	2,3,5-TrCP	933-78-8	-	9.43	196	198, 200
13	2,4,5-TrCP	95-95-4	7.40	9.51	196	198, 200
14	2,3,4-TrCP	15950-66-0	-	10.14	196	198, 200
15	3,4,5-TrCP	609-19-8	7.84	10.34	196	198, 200
16	2,3,5,6-TeCP	935-95-5	5.22	11.25	232	230, 234
17	2,3,4,6-TeCP	58-90-2	6.35	11.34	232	230, 234
18	2,3,4,5-TeCP	4901-51-3	5.14	12.13	232	230, 234
19	PCP	87-86-5	4.70	13.75	266	268, 264
20	TCG	2539-17-5	6.16	14.13	262	260, 247

[a)] pK_a Acid-base dissociation constant.[15,19]

Results and Discussion

Analysis of CPs with GC-MS

The standards of 19 CPs and TCG (internal standard) were analyzed with GC-MS at the concentration of 1.0 mg/L. Identity of each analyte peak was carried out by comparing GC retention time and the confirmation-to-quantification ion ratio to reference standards. The selected ions and the retention time for the analytes were summarized in Table I. For quantitation, chromatograms were registered using selective ion monitoring (SIM) of the main characteristic fragment ions for each of the analytes. It was noted that 2,4-DiCP and 2,5-DiCP were co-eluted as one peak (7.27 min) due to their closely characteristics, and the two were presumed one analyte in the investigation.

Optimization of extraction process

For determining CPs in leather, the first step is to isolate them from the solid matrix. In this work, ultrasonic assisted solvent extraction was selected due to its ease of operation and simplicity. For the extraction solvent, methanol was chosen because of its relatively low toxicity, availability, as well as the compatibility with the wide range of CPs polarity (3.2-4.8, $LogK_{ow}$).[19] Furthermore, methanol was desirable for eluting targets in leather (see ISO 16189-2013, and ISO 19070-2016) due to its less solubility for the matric substances as oils and polymers which co-exist in the leather fiber, thus, to reduce interfering species in the final solution for GC analysis. On the other hand, the wide range of CPs acidity (4.1-9.2, pK_a as listed in Table I) requires the extraction solvent to be acidified, to ensure all CPs were in their neutral form, thus, to increase their solubility in the methanol and enhance the extraction efficiency. Based on this consideration, a portion of formic acid (0.1-1.0% w/w) was introduced into the methanol used in the experiments. The evaluation of all the results was based on the 19 targets recoveries which should be at the range of 90-110%. With the systematically investigation of spiked samples with all CPs concentrations of 1.0 mg/kg, it was found the optimized extraction temperature for the total CPs was 45-55°C with extraction times of 2 × 30 min under ultrasonic frequency 45 kHz, and suitable proportion of formic

Table II

Optimized parameters of the extraction conditions

Variable	Parameters tested	Optimal
Ultrasonic frequency (kHz)	45, 60, 100	45
Extraction temperature (°C)	25, 35, 40, 45, 50, 55	45-55
Extraction time (min)	2 × 20, 2 × 30, 2 × 40	2 × 30
Formic acid concentration (w/w)	0.1%, 0.3%, 0.5%, 0.7%, 1.0%	0.5%

acid in methanol is 0.5%. The optimized parameters were listed in Table II.

The release of mono chlorophenol (as 4-MCP) from halogenated AZO dyestuff in high temperature has been reported,[10-11] and it was thought that the reason lies in the break of C-N bond of the AZO dye during the distillation process. It was apparent that the optimized extraction temperature (listed in Table II) was significantly lower than the steam temperature used in ISO 17070-2015, indicating these extraction conditions are quite gentle, and as such would not lead to breakdown of dyestuffs in the matrix, especially the release of MCP from certain halogenated AZO dyestuffs. In fact, it was noted that leathers are commonly dyed at the temperature of 50-55°C or higher in the tannery,[20] and these dyestuffs would easily be abandoned, if they can be broken at 45-55°C.

Effects of methanol on derivatization

In this work, concentration of sample extracts was performed prior to acetyl derivatization. Notably, methanol might remain in the residues if the evaporation is not carried out thoroughly, which might affect the derivatization process. Therefore, the influence of methanol on the acetyl derivatization was investigated. The experiments were performed by addition of methanol (0.2-1.8 mL) into 20 mL K_2CO_3 solution (0.1 mol/L) containing the 19 CPs standards. CPs in this solutions were acetylated [16] after mixed with hexane (4 mL), triethylamine and (0.1 mL) and acetic anhydride (0.3 mL). The recovery of each CP was calculated and compared based on their peak area, to evaluate the derivatization efficiency. As presented in Figure 2, when the volume of methanol is less than 0.6 mL in the system, the recoveries of the CPs remained relatively constant (90-105%), indicating limited or no unfavorable effects of methanol on the derivatization process. However, higher volume as 0.6 mL of methanol began to cause an obvious decline in the recoveries, especially for MCP and DiCP with lower chlorine contents, demonstrating that methanol more than 0.6 mL brought adverse effect on their derivatization step. The reason might be that extra methanol (> 0.6 mL) changes the interface behavior between n-haxane and water,[16-17] slowing down or preventing the acetylation reaction. Hence, the methanol volume in the evaporation residues should be controlled smaller than 0.6 mL. In fact, this problem could be easily resolved by extending evaporation time. Practical operation indicates that 11 min evaporation (-0.07 MPa, 50°C) is

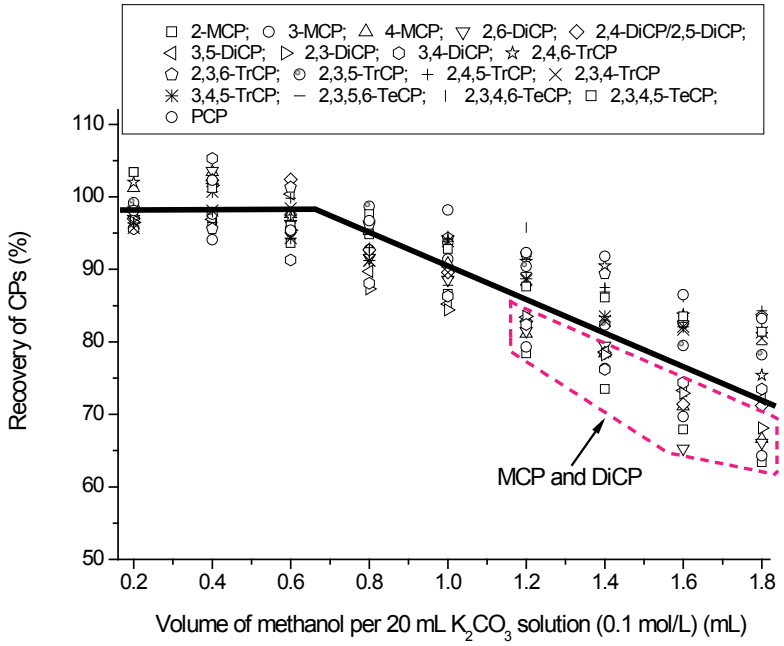

Figure 2. Effects of methanol on the acetyl derivatization efficiency

enough to remove the methanol in the mixture, leaving ~2 mL aqueous residues which was mainly from the 2 mL K_2CO_3 solution introduced prior to evaporation.

Cleanup with silica-gel cartridge

Purification of extracts to reduce matrix disturbance plays a key role for ensuring sensitivity of a method. It is especially important for samples containing complex organics (oils, polymers, dyestuff) such as leather. In this investigation, Silica-gel cartridges (0.5 g/6 mL) were selected according to the references,[15] and applied to clean up the n-hexane extracts isolated from the derivatization system. Tests showed that acetylated CPs (0-30 μg) in n-hexane extracts (~8 mL) are totally absorbed by the silica-gel frits. These acetylated CPs cannot be eluted during the washing process using ~5 mL of n-hexane for removing the disturbance, but they can be totally eluted by using a small volume (1 × 1 mL) of n-hexane/acetone (2:1, v/v) in the next elution operation. Thus, it could be considered that this cleanup technique can effectively reduce the matrix interference indicated by the circle box in Figure 3A, as well as pre-concentrate the acetylated CPs. The representative chromatogram of the extracts after silica-gel cleanup was shown in Figure 3B, which was in definite contrast with the original extracts presented in Figure 3A.

Method validation

The developed method was validated by determining the linearity, sensitivity and reproducibility. These characteristics were also compared with these obtained by ISO 17070-2015, as listed in Table III.

Linearity

The linearity of the method was determined by performing internal calibration curves with negative leather sample spiked with increasing concentrations of the 19 CPs standard mixtures, as well as a constant concentration of TCG internal standard. The spiking levels were at 0.2-10 mg/kg range for the 19 CPs. With the developed method, the linear range of CPs was at 0.4-10 mg/kg range with 1 mg/kg for TCG. While with ISO 17070-2015, the linear range of CPs was changed to 2-10 mg/kg range with 3 mg/kg for TCG, revealing a wider test concentration range of this improved procedure. In all cases, the relative responses of CPs were linear at the range of concentrations studied and the correlation coefficients (r^2) were all more than 0.993. However, based on the known amounts of CPs spiked, the range of the mean recoveries for the CPs were at 92-105% for this developed method, and 70-107% for ISO 17070-2015, as summarized in Table III. The data indicated a relatively low recovery for ISO 17070-2015, especially for TeCP and PCP with high boiling points.

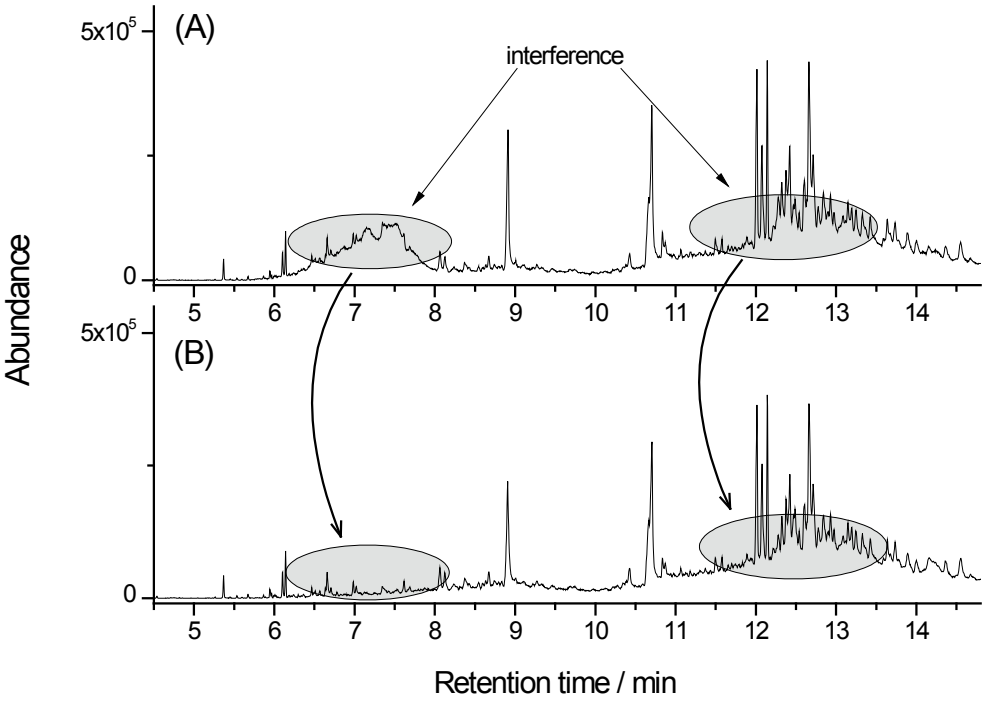

Figure 3. Total ion chromatograms of Sample extract **(A)** before and **(B)** after clean-up with Silica-gel cartridge

Reproducibility

Precision of the methods was assayed by repeatability studies for the peak area measurements of the negative samples with spiking level 1 mg/kg for each CPs (n = 6). In each case, reproducibility, expressed as relative standard deviation (RSD) based on the peak area of acetylated CPs, was obtained at 2.1-7.3% with this developed method, and 6.6-22.5% with ISO 17070-2015. The results indicated the robustness of the developed method due to the ultrasonic assisted extraction operation which is more reliable than steam distillation specified in ISO 17070-2015.

Limits of detection (LOD)

Negative samples spiked with mixed standards of CPs were used for the measurements of LOD, which were determined according to the instrumental detection limits, as well as weight of the sample and volume of the final extracts. LOD for each CPs determined by considering signal-to-noise of 3:1, ranged from 0.09 mg/kg (MCP) to 0.15 mg/kg (PCP) with the developed method, and from 0.5 mg/kg (MCP) to 1.5 mg/kg (PCP) with ISO 17070-2015 with GC-MS. This indicated that LODs obtained in the developed method were enhanced by a factor ranging between 5-10 with respect to ISO 17070-2015. These results demonstrated a greater sensitivity for this investigated method with ultrasonic extraction and SPE cleanup.

Comparison with ISO 17070-2015

Besides the contrasts in Table III, this developed procedure was further compared with ISO 17070-2015 with respect to extraction time, solvent use, handling, cost and batch test, as listed in Table IV. As discussed above, the major disadvantages of ISO 17070-2015 are long time extraction and failure for batch test. Although this proposed approach is relatively costly due to the silica-gel cartridge and solvent use, it provides a more sensitive and highly efficient way of testing CPs in leather, to reach the increasingly stringent requirements by European Union (EU) REACH regulation, National Compulsory Standards of China, or by Organizations such

Table III

Analytical parameters for this investigated procedure and ISO 17050-2015 with GC-MS

	Method of this work (GC-MS)				ISO 17070-2015 (GC-MS)			
Analyte	Pretreatment efficiency (%)[a)]	Linear range (mg/kg)	LOD (mg/kg)	RSD (%)[b)]	Pretreatment efficiency (%)[a)]	Linear range (mg/kg)	LOD (mg/kg)	RSD (%)[b)]
2-MCP	104.1	0.4-10	0.10	3.1	103.3	2-10	0.50	9.8
3-MCP	93.5	0.4-10	0.10	4.2	102.3	2-10	0.50	12.3
4-MCP	95.7	0.4-10	0.10	3.9	103.6	2-10	0.50	10.1
2,6-DiCP	97.4	0.4-10	0.10	4.3	93.2	2-10	0.50	6.6
2,4-DiCP/2,5-DiCP	95.7	0.4-10	0.10	2.9	106.6	2-10	0.50	7.5
3,5-DiCP	96.6	0.4-10	0.10	2.1	97.1	2-10	0.50	8.3
2,3-DiCP	99.1	0.4-10	0.10	6.7	102.6	2-10	0.60	9.2
3,4-DiCP	102.3	0.4-10	0.09	5.5	96.3	2-10	0.60	10.1
2,4,6-TrCP	98.2	0.4-10	0.10	3.9	93.9	2-10	0.60	13.5
2,3,6-TrCP	104.7	0.4-10	0.10	3.8	87.6	2-10	0.60	14.8
2,3,5-TrCP	95.3	0.4-10	0.10	5.5	83.5	2-10	0.60	16.7
2,4,5-TrCP	93.2	0.4-10	0.10	7.3	88.9	2-10	0.60	8.8
2,3,4-TrCP	102.5	0.4-10	0.09	6.1	86.4	2-10	0.60	12.1
3,4,5-TrCP	101.7	0.4-10	0.10	5.2	85.5	3-10	0.80	13.6
2,3,5,6-TeCP	93.2	0.5-10	0.15	4.6	73.3	4-10	1.2	15.9
2,3,4,6-TeCP	98.7	0.4-10	0.10	5.8	76.5	3-10	1.0	21.3
2,3,4,5-TeCP	92.6	0.4-10	0.10	6.7	77.9	3-10	1.0	22.5
PCP	93.5	0.5-10	0.15	6.4	70.1	4-10	1.5	19.7

[a)] Expressed as mean recoveries of n = 6.
[b)] Relative standard deviations.

Table IV

Comparison of the investigated method with ISO 17070-2015

Subjects	Method of this work	ISO 17070-2015
Extraction time	60-70 min	Up to 120 min
Batch test	Yes	No
Handling	Easy	Relatively easy
Solvent use	Methanol (40 mL), hexane (15 mL)	Hexane (40 mL)
Cost	Relatively costly	Relatively low

as OEKO-TEX® Association, AAFA (American Apparel & Footwear Association), among others.

This developed procedure was not further compared with other related methods as reported with ASE extraction and HPLC analysis,[8] because mono-chlorophenols were not included in the investigation, and the qualification assurance of method with HPLC-DAD is generally inferior to GC-MS. Although the quantification assurance with HPLC-DAD is superior to GC-MS, the internal calibration in this method can sufficiently make the test results reliable.

Analysis of real samples

The developed procedure was applied to determine the studied CPs in series of leather samples of different origin, including sheep-skin, goat-skin and cattle-hide (designed for garments, sofa, shoes and car seat) to confirm its applicability and feasibility. Figure 4 shows the typical GC-MS chromatograms obtained from cattle-hide sofa leather. The peak of 2,4,6-TrCP was easily identified by retention time and mass spectrum. The SIM detection (Figure 4B) allowed to further disregard the matrix observed in the total ion current (TIC) chromatograms (Figure 4A) and helped the quantitation using area response. This demonstrated the high selectivity and reliability of the method.

A total of 85 different samples comprising shoe leather (25), sofa leather (32), garment leather (23), car seat leather (5) were analyzed in this study. Six samples were found containing DiCP or TrCP, indicating the ratio of positive samples were about 7%. The other CPs were not detected. DiCP was detected in sheep-skin garment leather with concentration of 3.8 mg/kg, and TrCP was found in 5

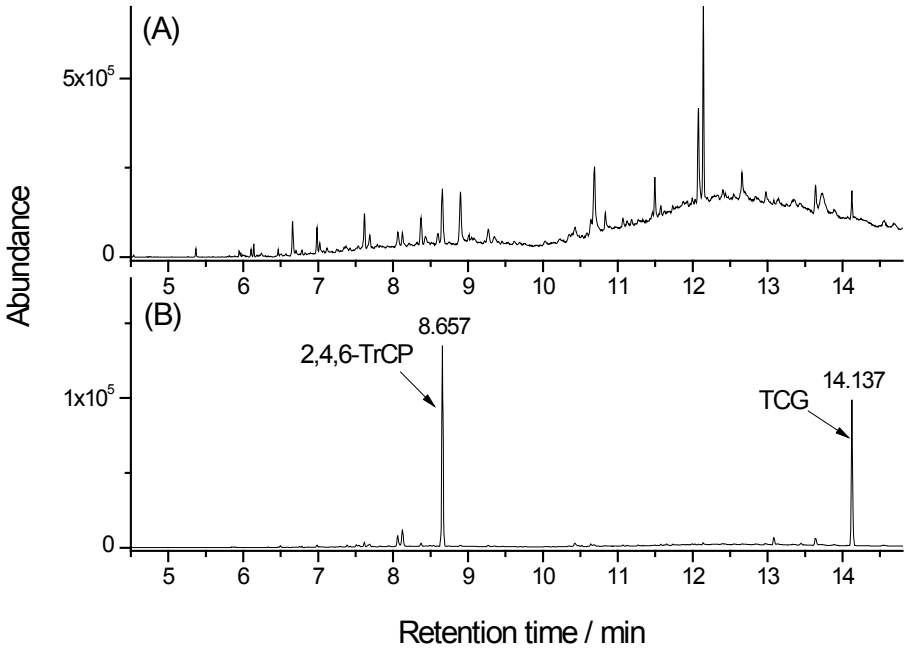

Figure 4. Total ion current **(A)** and selective ion monitoring **(B)** chromatograms of TrCP in the extracts of cattle-hide shoe leather (concentration of 0.88 mg/kg)

Table V

Levels of the CPs found in leather samples

Compounds	Number of positive Leather sample detected.				Conc. Range (mg/kg)
	Shoe	Sofa	Garment	Car seat	
2,4-DiCP	0	0	1	0	3.8
2,4,6-TrCP	1	2	2	0	0.88-14.6

samples with concentration range at 0.88-14.6 mg/kg, as shown in Table V. These results revealed the current levels of CPs in leather, and proved the occurrence of 2,4,6-TrCP in leather.

Conclusions

An improved method for detecting 19 chlorophenols in leather was investigated to solve the problems of time consuming and failure of batch test associated with ISO 17070-2015. The developed procedure consists of ultrasonic assisted extraction, acetyl derivatization and SPE cleanup followed by GC-MS detection. The ultrasonic assisted extraction with methanol containing formic acid (0.5%) presents high extraction efficiency, and this extraction technique is especially suitable for batch operation. The cleanup with silica-gel cartridge is effective to remove matrix disturbance, as well as enrich the analytes in the final solution for GC-MS analysis. The optimized method was validated and presented desirable recovery with lower RSD and higher sensitivity. The obtained results were compared with ISO 17070-2015, and indicated the method could be a good alternative to the steam distillation method in the analysis of 19 chlorophenols in leather. Finally, the procedure was applied to series of practical leather, and the preliminary results from 85 samples demonstrated current occurrence and concentrations of these targets, as well as the potential presence of 2,4,6-TrCP.

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