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Determination of organochlorine pesticides in leather by solid-phase microextraction and gas chromatography-mass spectrometry

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Abstract

An analytical procedure based on the solid-phase microextraction technique (SPME) combined with gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS) for the determination of organochlorine pesticides (OCPs) extracted directly from leather samples is proposed. The extraction conditions for the headspace SPME (HS-SPME) procedure were optimized using experimental designs. The fibers and the ionic strength were optimized by the univariate procedure, while a Doehlert matrix was used to optimize the extraction time and temperature. The best extraction conditions were obtained using a PDMS/DVB fiber with extraction time and temperature of 35 min and 80 °C, respectively. No salt addition was necessary. Detection limits ranging from 3.3 to 11 ng g^{-1} and relative standard deviations (RSD, n = 5) lower than 14.2% were obtained. The recovery was studied at three concentration levels by adding different amounts of organochlorine pesticides to the leather samples (600, 900 and 1500 ng g^{-1}) and excellent recoveries ranging from 93.7 to 107.3% were obtained.

Keywords: organochlorine pesticides, HS-SPME, GC-MS, leather.

1. Introduction

Leather quality is dependent on several factors, including careful breeding of the herd, pest control, and appropriate ways to identify, confine and transport the animals. After the slaughter, degradation of the animal hide through the action of microorganisms must be avoided, allowing efficient processing and the obtainment of leathers and skins with very high quality. The quality of the leathers is also dependent on appropriate manipulation, conservation and storing of the hide. When the time between the slaughter and hide processing is short (6 to 12 h) the hide can be stored without pretreatment, if the temperature conditions are appropriate. However, if the hide will be subjected to a long period of storage and/or transportation, especially at high temperatures, it must go through a pretreatment called tanning. In general, for the preservation of leathers the hides are stacked and layers of salt are placed between the hides. The hides can also be immersed in brine before stacking. By applying this procedure the hides can be stored for several months before they are processed. The hides can also be preserved by refrigeration or air drying, which is used for small-scale processing. Skins containing salt show good resistance against microorganisms; however, the salt can cause dehydration, eliminating water and partially the soluble proteins. Besides the use of salt, some leather suppliers use insecticides to repel insects and/or biocides to help preserve the leather during storage and transportation[1].

The use of dichlorodiphenyltrichloroethane (DDT) and lindane was first reported in 1954, mainly to protect raw hides from insect damage during the leather drying process[2]. Dieldrin, another organochlorine pesticide (OCP), was introduced to protect the leather against ectoparasites[2]. Lindane was one of the most commonly used insecticides for protecting hides and skins until 1990[2]. However, the use of DDT and other OCPs has been prohibited in many countries since the 1970s, mainly because of the widespread environmental contamination due to their persistence, bioaccumulation and high toxicity[2]. The persistence of these compounds in the environment is due to their low degradation by biotic and abiotic process, leading to a long half-live time, which can be years or even decades[3]. Because OCPs are fat-soluble they can be absorbed by living organisms through alimentation, breathing and the skin. After absorption, these compounds are distributed to many types of tissue including blood[4]. The toxicity of these contaminants is very complex and is specific to each compound. Therefore, multiple toxic responses can occur according to the species, gender and organ affected[5].

In spite of the great importance of monitoring OCP residues in leather and leather goods, very little research has been carried out on sample preparation to quantify OCPs in skins and leathers. To the best of our knowledge, there is only one study reported in the literature for the determination of OCPs in a leather matrix. The OCPs were extracted from skins and leather in a Soxhlet extractor using hexane and the concentration and cleaning of the extract was performed in a Florisil column[2]. The use of microextraction techniques like SPME to determine contaminants in leather was proposed by Carasek et al.[6] to quantify phenols and chlorophenols.

The aim of this study was to develop a method for the simultaneous determination of eleven OCPs in leather samples employing HS-SPME and gas chromatography-mass spectrometry (GC-MS). The fibers PDMS 100 μm, PDMS 30 μm and PDMS/DVB 65 μm were selected to study the effectiveness for the extraction of the target compounds. A Doehlert design was used to select the best extraction conditions. The effect of the ionic strength on the extraction efficiency was determined using the univariate procedure. Finally, the optimized method was applied for the determination of OCPs in leather.

2. Materials and Methods

2.1. Chemicals and reagents

Certified standards of pesticides (α-BHC, β-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, 4,4' DDE, dieldrin, 4,4' DDD, 4,4' DDT and methoxychlor) were obtained at concentrations of 250 mg L^{-1} in methanol, with the exception of methoxychlor for which the concentration was 1000 mg L^{-1} in toluene/hexane 50:50 (Supelco, Bellefonte, USA). Working solutions were prepared by diluting stock solutions with methanol (Sigma Aldrich, Steinheim, Germany). Sodium chloride (Vetec, Rio de Janeiro, Brazil) was used to study the ionic strength.

2.2. Instrumentation

All of the chromatography analysis was performed using a Shimadzu GC-2010 Plus gas chromatograph coupled to a mass spectrometer. An Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 µm) manufactured by Restek (Bellefonte, PA, USA) was used for the GC separation. Helium (99.999%) was used as the carrier gas at a constant pressure of 100 kPA. The injections were performed in the splitless mode. The initial oven temperature was 100 °C (held for 2 min) and increased to 180 °C at a rate of 6 °C min^{-1}, and increased again at a rate of 5 °C min^{-1} to 220 °C (0 min), and finally to 250 °C at a rate of 3 °C min^{-1}. The injector temperature was 260 ºC and the fiber desorption time was 5 min. Desorption conditions were fixed so that no memory effects were observed. The transfer line and the ion source temperatures were set at 200 ºC and 210 ºC, respectively. The acquisition of the ionization was carried out in SIM mode to avoid overlapping of the sample peaks with those of the target analytes. The selected ions used for quantification of the target analytes (m/z ratio) were: 183-181-219 (α-BHC), 219-181-109 (β-BHC), 100-272-274 (heptachlor), 66-263-79 (aldrin), 81-353-355 (heptachlor epoxido), 241-239-195 (endosulfan I), 246-318-248 (4,4' DDE), 79-81-82 (dieldrin), 235-237-165 (4,4' DDD and 4,4' DDT) and 227-228-152 (methoxychlor).

2.3. SPME procedure

The pesticides were extracted by SPME from the headspace of the matrix. The HS-SPME was performed using commercial fiber with a manual holder (Supelco, Bellefonte, USA). The extractions were carried out using a PDMS/DVB fiber with 65 µm thickness (Supelco, Bellefonte, PA, USA). The fibers were conditioned according to the temperature and time provided by the manufacturer before use.

2.4. Leather sample preparation

"Wet blue" leather samples were supplied by the Brazilian Institute for the Technology of Leather and Artifacts (*Instituto Brasileiro de Tecnologia do Couro e Artefatos*, Novo Hamburgo, Rio Grande do Sul, Brazil). Leather samples weighing 100 mg, free from the target analytes, were cut into cubes of approximately 2 mm^2 and placed in 4 mL vials. The samples were spiked with pesticide standard and stored for 24 hours at -18 °C to allow its interaction with the sample matrix. At the time of extraction 1 ml of distilled water was added and a magnetic stir bar was used for stirring. For all of the optimization steps the samples were spiked with 30 µL of the pesticide standard at a concentration of 3 mg L^{-1}, with the exception of methoxychlor for which the concentration was 12 mg L^{-1}.

2.5. Selection of the coated fiber for HS-SPME procedure

The following polymeric fibers were studied: PDMS 100 µm, PMDS 30 µm and PDMS/DVB 65 µm, all purchased from Supelco (Bellefonte, PA, USA). The pesticide extractions were performed using 100 mg of leather prepared as described in section 2.4. The different fiber coatings were evaluated in the headspace mode at 75 °C applying a time of 35 min with maximum sample agitation.

2.6. Optimization of extraction conditions

The influence of the ionic strength was investigated using saturated sodium chloride solution and distilled water. The extraction procedure was carried

out at 75 °C applying a time of 35 min with maximum sample agitation. A multivariate optimization procedure to select the best extraction time (10 to 40 min) and temperature (40 to 85 °C) was performed using a Doehlert matrix. The optimization of the extraction conditions was carried out with PMDS/DVB fiber in the sodium chloride solution. The experimental design and data treatment were carried out using the software Statistica 7.0.®

3. Results and Discussion

3.1. Selection of the coated fiber for HS-SPME procedure

Figure 1 shows the chromatograms obtained for each SPME fiber used in this study. It can be observed that the PDMS/DVB fiber showed better extraction efficiency than the other fibers for all target compounds and this fiber was therefore selected to continue this study.

3.2. Optimization of extraction conditions

The effect of the ionic strength on the extraction efficiency was studied using a univariate design, adding distilled water or saturated sodium chloride solution to the vials containing the leather samples. The results of this study, shown in Figure 2, indicate a reduction in the extraction efficiency of the OCPs when the ionic strength was increased. This finding is in agreement with that reported by Dong[7], Prates[8] and Pawliszyn[9,10,11] who studied the salting out effect on the extraction of OCPs. Therefore, no salt was added to the vials for the next steps of this study.

In order to determine the best extraction time and temperature for the extraction of the OCPs from the leather matrix, a Doehlert design was used. The aforementioned ranges for these variables were studied. Figure 3 shows the response surface obtained using the geometric mean of the set of peak areas observed for the target compounds as the response. Figure 3 shows that the temperatures should be higher than those studied and that extraction times of 30 - 40 min should be selected to obtain the maximum extraction of the OCPs from the leather. However, due to the exothermic nature of the SPME process temperatures higher than 85 ºC were not applied in this study. In conclusion, the optimized conditions were extraction time of 35 min, 80 °C as the extraction temperature and no salt addition.

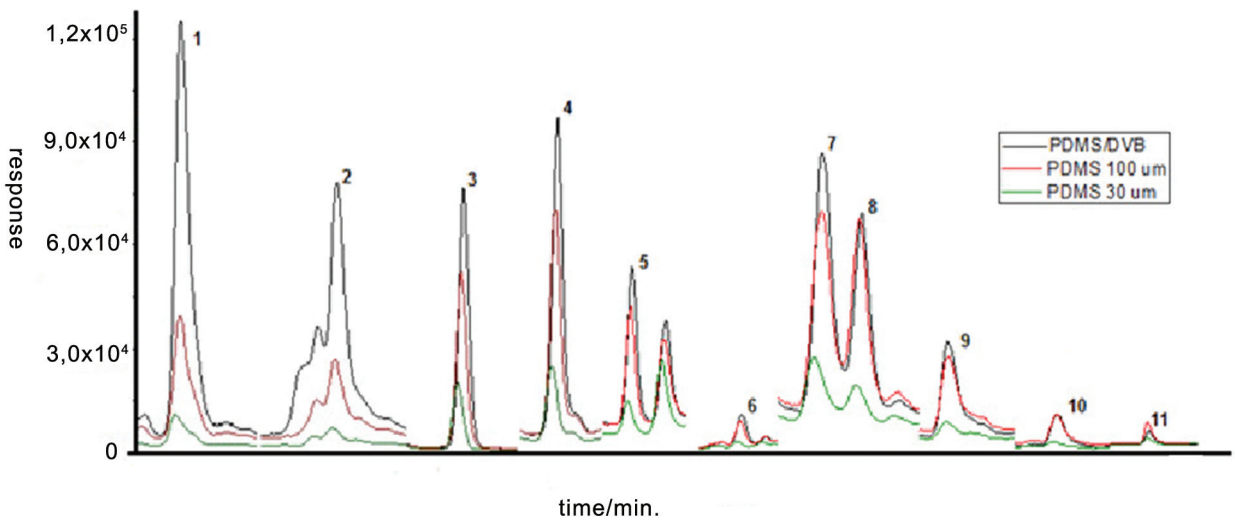

Figure 1. Chromatograms in SIM mode for three different SPME fibers for the OCP peak identification: 1 = α-BHC, 2 = β-BHC, 3 = heptachloro, 4 = aldrin, 5 = heptachloro epoxide, 6 = endosulfan I, 7 = DDE, 8 = dieldrin, 9 = DDD, 10 = DDT, 11 = methoxychlor.

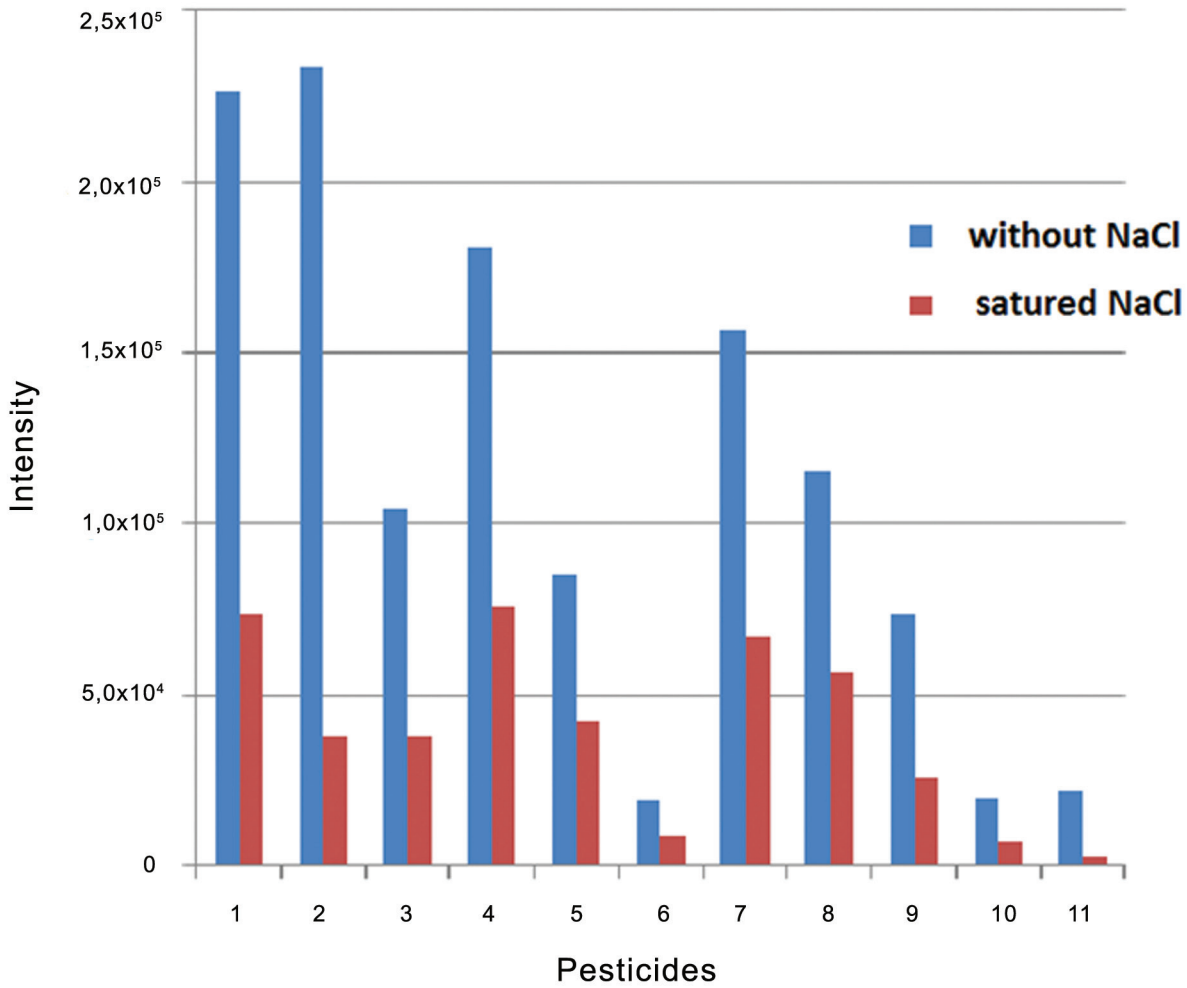

Figure 2. Influence of ionic strength with NaCl and without NaCl (1 = α-BHC, 2 = β-BHC, 3 = heptachloro, 4 = aldrin, 5 = heptachloro epoxide, 6 = endosulfan I, 7 = DDE, 8 = dieldrin, 9 = DDD, 10 = DDT, 11 = methoxychlor).

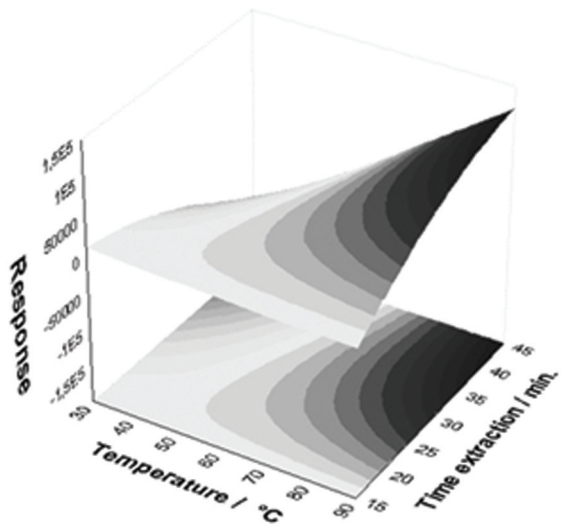

Figure 3. Response surfaces obtained from the Doehlert design for two variables – extraction time and temperature – for the HS-SPME of OCPs in leather.

3.3. Analytical figures of merit

The performance of the method was verified through some analytical figures of merit using the optimized conditions. The analytical curve was built spiking 100 mg of leather sample with 5, 10, 20, 30, 40 and 50 µL of standard pesticide solution (each pesticide at 3 mg L^{-1} with the exception of methoxychlor at 12 mg L^{-1}). The limit of detection was calculated as three times the standard deviation of the intercept of the calibration curve divided by the angular coefficient of the calibration curve. The limit of detection for all pesticides ranged from 3.3 ng g^{-1} to 11.0 ng g^{-1} (with the exception of methoxychlor, the value for which was 107.6 ng g^{-1}). The limit of quantification (LOQ) was calculated as 3.3 fold the LOD. The linear range varied from 10.9 to

Table 1. Linear range, linear correlation coefficients, relative standard deviations and detection limits for the optimized method.

Analytes	Linear range (ng g^{-1})	R	LOD (ng g^{-1})	LOQ (ng g^{-1})	RSD%
α-BHC	10.94 - 1500	0.9993	3.28	9.84	7.8
β-BHC	20.3 - 1500	0.9975	6.22	20.53	9.8
Heptachloro	25.36 - 1500	0.9963	7.59	22.77	10.8
Aldrin	25.20 - 1500	0.9963	7.56	24,95	9.2
Heptachloro epox.	18.02 - 1500	0.9974	5.41	17,85	6.1
Endosulfan I	25.36 - 1500	0.9962	7.61	25.11	6.5
4'4 DDE	21.37 - 1500	0.9964	6.41	21.15	10.9
Dieldrin	22.14 - 1500	0.9980	6.64	21.91	6.8
4'4 DDD	36.60 - 1500	0.9946	10.98	36.23	13.9
4'4 DDT	34.65 - 1500	0.9950	10.39	34.29	13.1
Methoxychlor	32.27 - 6000	0.9970	10.56	34.85	14.3

R = correlation coefficients; LOD = Limit of detection; RSD = relative standard deviation (450 ng g^{-1}; n = 5).

Table 2. Repeatability study using three different concentration levels.

	Level 1 (600 ng g^{-1})	Level 2 (900 ng g^{-1})	Level 3 (1500 ng g^{-1})
Analytes	**Recovery (%)**		
α-BHC	97.1 ± 5.3	99.7 ± 4.7	99.6 ± 3.9
β-BHC	103.0 ± 6.1	97.9 ± 3.5	101.6 ± 2.9
Heptachloro	94.7 ± 6.5	101.8 ± 4.2	98.9 ± 5.2
Aldrin	95.7 ± 4.8	98.2 ± 3.6	102.7 ± 4.2
Heptachloro epox.	100.7 ± 7.2	97.7 ± 8.6	100.2 ± 7.3
Endosulfan I	101.7 ± 4.6	100.0 ± 7.1	101.9 ± 6.5
4'4 DDE	93.2 ± 4.9	98.9 ± 2.2	101.0 ± 3.2
Dieldrin	102.5 ± 3.2	99.1 ± 6.2	98.9 ± 5.6
4'4 DDD	95.0 ± 8.1	105.4 ± 7.8	98.8 ± 9.2
4'4 DDT	107.8 ± 7.7	105.4 ± 5.5	106.9 ± 8.1
	Level 1 (2400 ng g^{-1})	**Level 2 (3600 ng g^{-1})**	**Level 3 (6000 ng g^{-1})**
		Recovery (%)	
Methoxychlor	107.3 ± 10.2	96.8 ± 9.9	100.4 ± 7.4

n = 3.

1500 ng g^{-1} for all target pesticides (with the exception of methoxychlor with a range of 32.3 and 6000 ng g^{-1}) and the linear correlations ranged from 0.9946 to 0.9993. The method repeatability was estimated by calculating the relative standard deviation (RSD%) of five replicates after spiking the leather with a concentration of 450 ng g^{-1} (with the exception of methoxychlor for which the concentration was 1800 ng g^{-1}). Excellent values in the range of 6.1–14.3% were obtained. The individual results for each target compound can be found in Table 1 which shows similar or better results that those reported by Font and Marsal[2]. However, the SPME procedure showed some important advantages including shorter extraction time (35 min compared with over 24 h), less

sample required (100 mg compared with 5 g) and less sample manipulation.

3.4. Method application and recovery tests

The newly developed method was applied to determine OCPs in leather acquired from tanneries in Novo Hamburgo, Rio Grande do Sul State, Brazil. However, the leather samples were free of the target compounds or they were present below the limit of detection of the proposed method. This may be due to restrictions regarding the use of these compounds for leather treatment in Brazil, in place since 2006. In order to verify the accuracy of the method developed, the leather samples were spiked using three concentration levels, 600, 900 and 1500 ng g^{-1} (with the exception of methoxychlor for which the concentrations were 2400, 3600 and 6000 ng g^{-1}), and the pesticide recovery was determined for each sample. The results obtained can be seen in Table 2. This study showed that the method has an excellent recovery ranging between 93.7 and 107.3%, indicating that the method is reliable for the determination of these compounds in leather samples using the matrix-matched strategy for the calibration.

4. Conclusions

The method using HS-SPME extraction followed by GC-SIM-MS was shown to be very sensitive, precise and accurate. Furthermore, this is a simple and relatively fast method requiring very small amounts of sample and no pre-treatment of the sample prior to the HS-SPME is necessary. These findings are of particular significance given the complexity of leather samples.

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