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Evaluation of solid-phase microextraction conditions for the determination of chlorophenols in honey samples using gas chromatography

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Abstract

A rapid and solvent-free method for the determination of nine chlorophenol compounds (CPs) in honey samples using headspace solid-phase microextraction (HS-SPME) and gas chromatography with atomic emission detection (GC-AED) is developed. The different factors affecting the efficiency of the extraction and derivatization steps were carefully optimized. The polydimethylsiloxane-divinylbenzene (PDMS/DVB) fiber was the most suitable for preconcentrating the analytes from the headspace of an aqueous solution containing the dissolved honey samples where the chlorophenols had been submitted to acetylation. When the matrix effect was evaluated for different samples, it was concluded that standard addition calibration was required for quantification purposes. Detection limits roughly ranged from 0.1 to 2.4 ng g^{-1}, depending on the compound and the honey sample analyzed, with a fiber time exposure of only 15 min at 75 °C. The optimized method was successfully applied to different samples, some of the studied chlorophenols being detected in some of the analyzed honeys at concentration levels 0.6-9.4 ng g^{-1}.

Keywords: Chlorophenols (CPs); Honey; Headspace solid-phase microextraction (HS-SPME); Gas Chromatography – Atomic Emission Detection (GC-AED).

1. Introduction

The antimicrobiological properties of chlorophenols (CPs) have led to their use as disinfectants, in agriculture as herbicides, insecticides and fungicides, and also as wood preservatives. Although the use of CPs has been restricted since 1984 [1], because of their high resistence to biodegradation, they can still be found in honey, being transported by bees when travelling to collect nectar or even because of the treatment of wooden beehives.

The negative effect of CPs for human health has led to their categorisation and inclusion by the US Environmental Protection Agency and the Commission of the European Communities (Directive 76/464/EC) in the lists of priority pollutants [2]. Nevertheless, although the European Union (EU) included in the 1221/97/CE directive [3] the need to improve the treatment conditions of beehives, maximum residue limits (MRLs) for CPs in honey have never been established, and there is no doubt that their analysis is of great importance for controlling the quality of honey. The toxicity of CPs depends on the pH and the total number of chlorine atoms in the molecule, pentachlorophenol being the most toxic of the 19 members of this family [4].

A wide number of procedures have been described for chlorophenol analysis in environmental samples, but no references has been found to honey analysis. Gas chromatography (GC) is a popular technique for this purpose and derivatization of polar compounds is advisable to improve peak shape and sensitivity of the method. Acetylation has been the most widely used

derivatization method [5-10] because it permits analytes to be derivatized directly in the aqueous phase, although other reagents [11-14] have also been used. Mass spectrometry (MS) [12-19] is the most commonly used detection system for GC chlorophenol analysis, although electron-capture detection (ECD) [10,11,20,21] and, to a lesser extent, flame ionization detection (FID) [22,23] and atomic emission detection [24-26] have also been used.

The predictable low concentration levels of chlorophenols in honey samples, as well as its complex matrix, mean that trace enrichment and clean-up steps must be introduced. CPs have been preconcentrated by liquid-liquid extraction (LLE) [6,7,11] and solid-phase extraction (SPE) [4,12,18,24,25]. However, conventional procedures present the inherent disadvantages of being time consuming, labour intensive and hazardous to human health because of the use of organic solvents. Purge-and-trap (PT) [22,26] represents an alternative for low-substituted chlorophenols. With stir bar sorptive extraction (SBSE) [27] and solid-phase microextraction (SPME) [5,8,10,15,17,19-21,28,29] many of the problems associated with conventional procedures can be avoided. The solubility of honey in water means that solvent extraction of the polar analytes from the food matrix is not necessary, making SPME suitable for this analysis.

This paper describes a method for the analysis of nine chlorophenols in honey samples. The acetylated analytes are preconcentrated by SPME in the headspace mode, gas chromatographied and detected with the selective atomic emission detector.

2. Experimental

2.1. Chemicals

2-Chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were purchased from Aldrich (Steinheim, Germany). 4-Chlorophenol (4-CP), 2,6-dichlorophenol (2,6-DCP), 2,4,5-trichlorophenol (2,4,5-TCP) and 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). A 2000 μg ml^{-1} methanolic solution of 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP) was purchased from Supelco (Bellefonte, PA, USA). All compounds were of 98-99.9% purity. Individual stock solutions of the solid compounds were prepared using HPLC grade methanol, as a solvent (1000 μg ml^{-1}). Aqueous standard solutions were freshly prepared to spike samples in order to optimize the analysis procedure. Acetic anhydride and anhydrous potassium carbonate were purchased from Fluka (Buchs, Switzerland) and sodium chloride of 99.5% purity was from Sigma (St. Louis, MO, USA).

The plasma gas and carrier gas used for GC was helium. The reagent gas for the AED was oxygen. Nitrogen was used for purging the AED system. All the gases were supplied by Air Liquide (Madrid, Spain).

2.2. Instrumentation

The SPME device for manual sampling consisted of a holder assembly and several replaceable fibers, all obtained from Supelco. SPME fibers coated with non-bonded polydimethylsiloxane (PDMS) of 100 µm thickness, bonded polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 µm, bonded Carboxen/polydimethylsiloxane (CAR/PDMS) of 75 µm and bonded polyacrylate (PA) of 85 µm, were obtained from Supelco. The fibers were conditioned prior to use by heating in the injection port of the chromatographic system under the conditions recommended by the manufacturer for each fiber coating. Whenever needed, the conditioning step was repeated for fiber cleanup. All analyses were performed in 15 ml clear glass vials and the solutions were stirred with a magnetic stirrer (RH KT/C IKA-werke, Germany) using PTFE-coated magnetic stir bars (10 mm x 6 mm O.D.). To prevent analyte evaporation, vials sealed with hole-caps and PTFE/silicone septa were used. A home made system was used to control temperature.

An Agilent 6890 gas chromatograph was directly coupled by a transfer line to a G2350A microwave-induced plasma atomic emission detector (Agilent). Updated G2070AA ChemStation application with the G2360AA GC-AED software was used to control and automate many features of the GC and AED systems, and for data acquisition and treatment. The chromatograph was fitted with a 30 m x 0.32 mm I.D. HP-5, 5% diphenyl 95% dimethyl polysiloxane capillary column from Agilent with a 0.25 µm film thickness. Desorption of the fibers into the injection port was carried out in the splitless mode at 200 °C for 1

min. The internal volume of the inlet liner was 900 µl. The temperature program used was as follows: rising temperature from 40 °C to 180 °C at 15 °C min^{-1} and held for 4 min. Helium was used as the carrier gas and as AED make-up gas at 4 and 40 ml min^{-1}, respectively. Oxygen was used as the only scavenger gas at 20 psi. Solvent venting was switched on immediately after starting the desorption step and switched off 3 min later. The transfer line and the cavity temperatures were set at the same value as recommended by the manufacturer, 325 °C. Filter and backamount adjustment in the AED were set according to Agilent default specifications. The spectrometer was purged with a nitrogen gas flow rate of 2.5 l min^{-1}. All compounds were quantified in the chlorine 479 nm emission line, using peak area as the analytical parameter. Since the retention time for the most retained compound was 12 min and taking into account the time value of 15 min adopted for the SPME adsorption step, the analysis of the nine chlorophenols studied can be performed in 30 min.

2.3. Samples. SPME and in situ derivatization

Eight different honey samples were obtained from different suppliers. These samples had been labelled as rosemary (samples 1-4), heather (samples 5-6), orange blossom (sample 7) and eucalyptus (sample 8), but no verification of the floral origin was made.

To carry out the extractions, 1 g of a honey sample were weighed into a 15 ml SPME glass vial and 2 g of sodium chloride and 6.7 ml of a 0.75% (w/v)

potassium carbonate solution were added, checking that pH values close to 11 were attained. Then 280 µl of acetic anhydride were added and the vial immediately sealed with the cap after introducing the magnetic stir bar. The mixture was homogenized by inserting the vial in the home-made heating block previously programmed at 75 °C and maintaining the stirring speed at maximum power for 1 min. After this simultaneous homogenization and derivatization step the fiber was exposed to the headspace for 15 min over the aqueous mixture, which was continuously stirred at 1600 rpm and thermostated at 75 °C. Subsequently the fiber was retracted into the needle and transferred to the injection port of the GC with the split valve closed at 200 °C for 1 min. Each sampling was performed in triplicate.

2.4. Recovery assays

Since no reference materials are available for the validation of the method, spiked samples were prepared. Honey samples were spiked as follows: 0.1 ml of a working methanolic solution, containing the analytes at concentration levels ranging from 5 ng ml^{-1} to 0.5 µg ml^{-1}, were added to 1 g of honey placed in a 15 ml SPME vial, corresponding to fortification levels of approximately 0.5 and 50 ng g^{-1}, respectively. The spiked samples were set aside for 60 min at room temperature to let the methanol evaporate before sample analysis as described above. The fortification procedure was applied to three different honey samples at two concentration levels and three replicates were analyzed in each case, corresponding to three aliquots of each sample independently fortified and analyzed.

3. Results and discussion

3.1. Chromatographic and AED parameters

Before optimizing the chromatographic and detection parameters, preliminary experiments were carried out to ascertain whether or not it was necessary to derivatize the analytes. In an attempt to avoid using a derivatization step, free chlorophenols were adsorbed onto the PA fiber in the headspace and immersion extraction modes, from 0.5 g of spiked honey dissolved in 7 ml water. As expected, greater sensitivity was attained when the fiber was immersed in the solution, but the chromatograms obtained showed peak tailings and no significant improvement in sensitivity was obtained by adding acid and/or sodium salt to the extraction medium, at different temperatures and at different times on the adsorption stage. Contrary to the literature [5], a substantial increase in sensitivity was observed when the analogous non-polar compounds were adsorbed on the PA fiber, both in the headspace and immersion modes. This effect could be attributed to the complex nature of the honey matrix, which blocks the stationary phase of the fiber and prevents the chlorophenols from being adsorbed. Derivatization with acetic anhydride was selected because extraction into organic solvents prior to the addition of the derivatizing reagent is thus avoided [5,26,27].

To proceed with the GC-AED optimization, we carried out several experiments with 0.5 g of previously fortified honey dissolved in 7 ml of water, with the concentration of the studied compounds in the solution ranging from 30

to 100 ng ml^{-1}, depending on the compound, which, after derivatization, were submitted to HS-SPME using a PDMS/DVB fiber. The oven temperature was increased from the initial temperature of 40 °C to 180 °C, thus permitting the mono-, di- and trisubstituted chlorocompounds to elute. When the oven was maintained at 180 °C, the two tetrasubstituted compounds and PCP were eluted. Separation was carried out using different constant flow-rates of between 1 and 4 ml min^{-1}. The value selected was 4 ml min^{-1}, since this reduced the analysis time needed and increased the sensitivity compared with lower flow rates.

Reagent gas pressure and make-up gas flow-rate were the parameters optimized for AED. The helium make-up flow was varied between 30 and 45 ml min^{-1}, being measured with the window purge gas flow on. No overlapping peaks were observed with any of the flow-rates assayed, and even though the greatest sensitivity was attained using the lowest flow-rate, 40 ml min^{-1} was adopted as a compromise value because the baseline noise increased with lower helium flow-rates. The sensitivity of the studied compounds was affected by oxygen pressure, which was studied in the interval 15-25 psi, an increase in oxygen pressure leading to a decrease in peak area. A pressure of 20 psi was adopted to avoid accumulation of elemental carbon in the AED discharge tube.

3.2. Optimization of the derivatization and SPME stages

Although the acetylation reaction has been widely studied [5,25], preliminary experiments were carried out to select the optimum conditions for

maximum SPME efficiency with a PDMS/DVB fiber, in the headspace mode, in the presence of honey. The effect of different concentrations of potassium carbonate added to the extraction medium before the derivatizing agent was studied, as a way of counteracting the decrease in pH caused by the acetic acid produced in the acetylation reaction. The pH of the reaction medium was varied by diluting 0.5 g of honey Sample 5 in 7 ml of aqueous solutions containing concentrations of potassium carbonate ranging between 0 and 2% (w/v), which correspond to pH values of 4 to 12, higher concentrations leading to a substantial decrease in sensitivity, probably due to the destruction of the reactive. An acetic anhydride concentration of 4% (v/v) was used. Best sensitivity was attained for all compounds when the carbonate concentration was 0.75% (w/v), which resulted in a pH value of 11.1. Solutions of 0.5-1 g of honey of different floral origin and visual aspect in 7 ml of the optimized carbonate concentration provided pH values ranging between 11 and 11.5. With respect to the derivatizing reagent, concentrations ranging from 1 to 15% (v/v) were assayed, the best signals being obtained for a 4% (v/v) concentration. Note that signals were almost lost for mono-, di- and trichlorinated compounds when the acetic anhydride was higher than 10% (v/v).

Once the conditions for the derivatizing stage had been selected, the influence of changing the ionic strength of the matrix was studied by adding different masses of sodium chloride ranging from 0 to 2.8 g, to 7 ml of solution containing 0.5 g of honey in the conditions optimized for derivatizing the analytes. As expected, sodium chloride enhanced the chlorophenol amount extracted by the fiber. The salting out effect is compound-dependent, and

signals increased with salt concentration in the studied range for the mono-, di- and trichlorocompounds. For the rest of the compounds, sensitivity increased up to a 15% (w/v) salt concentration, and then slightly decreased. A value of 28% (w/v) sodium chloride concentration was selected. No significant differences were obtained when using potassium chloride or ammonium sulphate.

3.2.1. Selection of the fiber coating and extraction mode

Four fiber coatings (CAR/PDMS, PA, PDMS and PDMS/DVB) of different polarities and retention powers were assayed to preconcentrate the acetylated chlorophenols from 0.5 g of a honey sample dissolved in a 7-ml volume solution, which was previously fortified at concentration levels ranging from 0.1 to 0.5 μg g^{-1}, depending on the compound. 15 min and 90 °C were the conditions used in the extraction stage. Temperatures 20 °C lower than those recommended by the manufacturer as the maximum for each particular fiber coating were applied as the desorption temperature. The carboxen/PDMS coating was discarded because of the great memory effect observed, even when desorption times of 10 min were applied. Figure 1 shows the results obtained comparing the other three fiber coatings assayed in headspace and direct immersion modes for five of the studied compounds, where the maximum peak area for each compound obtained from the overall experiences was assigned as the 100% relative peak area. As can be observed, the mixed coating fiber of PDMS containing the copolymer DVB retained all the analytes studied to a greater extent in the headspace extraction mode. Of note is the fact that only in the immersion mode and for the tri-, tetra- and pentachlorophenol

compounds was the mixed fiber coating not the best choice, showing great sensitivity the PDMS fiber. On the other hand, no differences were attained in using the PDMS coating in the headspace or immersion modes. The other four compounds not represented in Figure 1 showed similar behaviour to their corresponding n-substituted compounds. The headspace mode was selected, not only because it provided best results, but because reproducibility and fiber life-time were higher, thus protecting the fiber from damage by high molecular mass and non-volatile compounds in the honey sample matrix.

3.2.2. Absorption time and temperature

The absorption time for the acetylated chlorophenols was studied by increasing the time of the PDMS/DVB fiber exposure from 10 to 60 min, and maintaining the vial at 90 °C. A compound-dependent behaviour was obtained, as can be observed in Fig. 2A, where the influence of this parameter is expressed by reference to the maximum extraction efficiency obtained for each analyte in particular. Although maximum sensitivity was attained at 30 min for all compounds except for monochlorinated compounds, for practical purposes 15 min was the value adopted. On the other hand, the influence of the absorption temperature was studied between 60 and 95 °C. As shown in Fig. 2B, sensitivity increased with the temperature for the two tetrasubstituted compounds and for PCP. Nevertheless, 75 °C provided the highest signal for the rest of compounds. Therefore, 15 min and 75 ° C were the adopted conditions.

Since PA fiber appeared to be a possible alternative, its adsorption time up to 90 min as well as its adsorption temperature were further studied, the

extraction efficiency not improving by more than 60% in any case. Therefore the PDMS/DVB fiber was finally selected, since it provided the best results probably due to the copolymer DVB, which favours the extraction of aromatic compounds [20].

3.2.3. Extraction solution volume

The volume of the solution optimized for the sample dissolution and analyte derivatization was varied between 3 and 7 ml, higher volumes leading to partial immersion of the fiber in the solution [30]. For mono-, di- and trichlorinated compounds, the lower the headspace volume the higher the extraction, whereas this parameter did not affect tetrachlorophenols. Pentachlorophenol was the only compound whose behaviour reflected that reported by Llompart et all. [8], a slight decrease in sensitivity being observed with a decrease in the headspace volume. All these experiments were carried out in the presence of 0.5 g of previously fortified honey and the effect of the sample concentration taken into account. For further experiments, the honey samples were dissolved in 7 ml of the aqueous derivatization solution.

3.2.4. Desorption parameters

The PDMS/DVB fiber was submitted to different temperatures in the injection port of the GC ranging between 200 and 250 °C. Low-substituted compounds (mono- and di-) increased their sensitivity by about a 10% with the temperature in the studied range, and the signal did not vary with this parameter for the trichlorophenols studied, whereas the sensitivity of tetrasubstituted compounds and pentachlorophenol decreased by about a 25% when desorption

temperature increased. Taking into account that these latter compounds did not attain their maximum extraction efficiency with the extraction time and temperature selected, 200 °C was adopted as desorption temperature. As regards desorption time, although 0.5 min was sufficient to desorb the trapped analytes, the fiber was maintained for 1 min in the injection port.

3.2.5. Sample mass

In order to select the mass sample to be submitted to the SPME procedure, a given mass of each chlorophenol compound was submitted to the SPME optimized procedure in the presence of different masses of honey Sample 2, which had previously been seen to be analytes free. Honey masses ranging between 0.5 and 2 g were assayed. The results appear in Figure 3, where the extraction efficiency refers to the values obtained in the absence of honey. As can be observed, for each one of the sample masses studied, the matrix effect increased with the number of chlorine atoms present in the molecule. In the case of low level chlorinated compounds, increasing the sample mass decreased sensitivity to a greater extent than was the case for high chlorinated compounds. A sample mass of 1 g was finally selected, taking into account the slopes of the standard addition calibration graphs obtained with different sample masses, to obtain the lowest detection limits.

3*.3. Analytical characteristics of the method*

The matrix effect was studied by comparing the slopes of aqueous standards and standard additions calibration graphs for three samples of

different visual aspect and floral origin, obtained by plotting concentration (at six different levels) against peak area and following linear regression analysis. Table 1 shows the data obtained. A paired t-test was applied being the “p” values obtained less than 0.05 in all cases except for 4-CP and in the case of honey Sample 8 for 2-CP and 2,6-DCP. As a consequence, to obtain a general reliable procedure, the standard addition method is recommended for quantification purposes. It is interesting that both in the presence and in the absence of honey, the two trichlorophenols studied showed the highest sensitivity.

The analytical characteristics of the method were calculated for the eucalyptus honey (Sample 8), taking into account that the data obtained differ owing to the sample analyzed. The correlation coefficients obtained demonstrated a directly proportional relationship between the amount of analyte extracted and its concentration in the sample. The repeatability was calculated by using the relative standard deviation from a series of ten HS-SPME consecutive analyses of 1 g honey Sample 8 fortified at two concentration levels, the results obtained appearing in Table 2. Detection limits lower than 2.4 ng g^{-1} were obtained by using a signal-to-noise ratio of three (Table 2). Taking into account that aliquots of 1 g of honey sample were submitted to the analysis procedure, the absolute detection limits obtained ranged from 0.1 to 2.4 ng for 2,4,6-TCP and 4-CP, respectively. The quantification limits (calculated from a signal-to-noise ratio of ten) varied between 0.33 to 8 ng g^{-1} for 2,4,6-TCP and 4-CP, respectively.

3.4. Real samples and validation of the method

The optimized procedure was applied to eight different honey samples. Three honey samples appeared to be absolutely free of the studied compounds. Two samples provided signal only for one of the analytes studied. Table 3 shows the results obtained. As no reference materials are available, recovery studies were carried out in order to check the accuracy of the proposed method, fortifying honey samples 6, 7 and 8 at two concentration levels with the nine chlorophenols. The data obtained appear in Table 4, where an average recovery ± SD (n=54) of 100.0 ± 9.04 was obtained, taking into account the known analyte contents for these samples. Figure 4 shows the elution profile obtained from honey sample 4, non-spiked and spiked with a standard mixture of chlorophenols, under the selected conditions, the compounds eluting in the following order: 2-CP (6.19 min), 4-CP (6.5 min), 2,6-DCP (7.35 min), 2,4-DCP (7.53 min), 2,4,6-TCP (8.36 min), 2,4,5-TCP (8.84 min), 2,3,4,6-TeCP (9.9 min), 2,3,4,5-TeCP (10.47 min) and PCP (12.0 min).

4. Conclusion

Solid phase microextraction appears in the optimized procedure as an interesting preconcentration system which avoids the use of organic solvents and permits high extraction efficiencies of chlorophenols to be attained from the headspace in only 15 min. The life-time of the fibers is also lengthened. Furthermore, the excellent selectivity of the atomic emission detector provides

nearly specific chromatograms. Nevertheless, the complexity of this type of matrix makes it necessary to quantify the analytes by means of the standard additions method. The analytical characteristics of the proposed method make it a useful tool for the routine monitoring of chlorophenols in honey samples.

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FIGURES

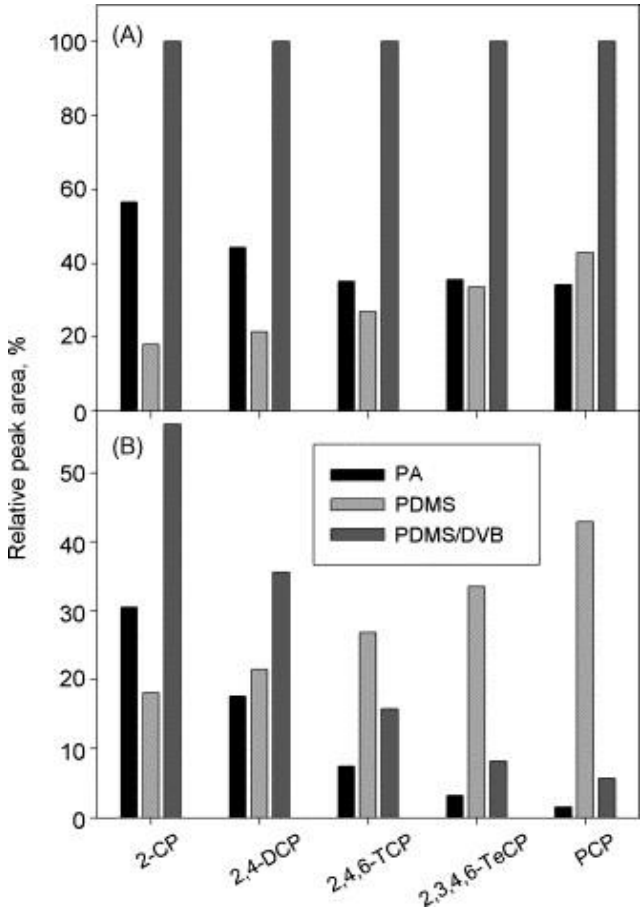

Fig. 1. Influence of type of fiber coating on the extraction efficiency of five selected compounds in the (A) headspace and (B) direct immersion extraction modes.

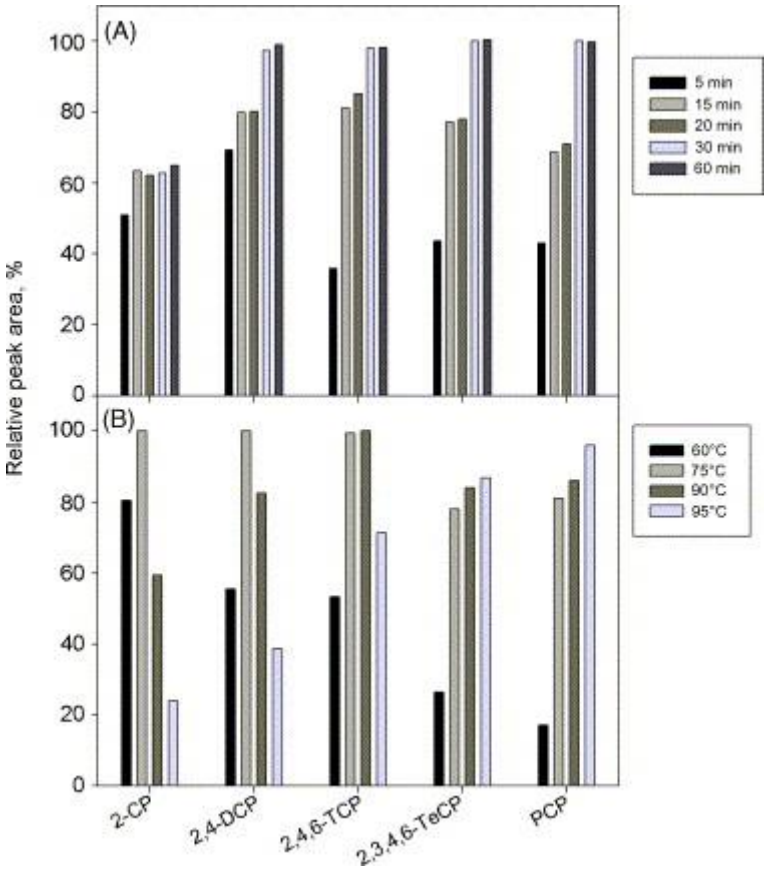

Fig. 2. Effect of the extraction time (A) and extraction temperature (B) when using PDMS/DVB fiber in the headspace mode, on the extraction efficiency using 0.5 g of a fortified honey sample diluted with 7 ml aqueous solution.

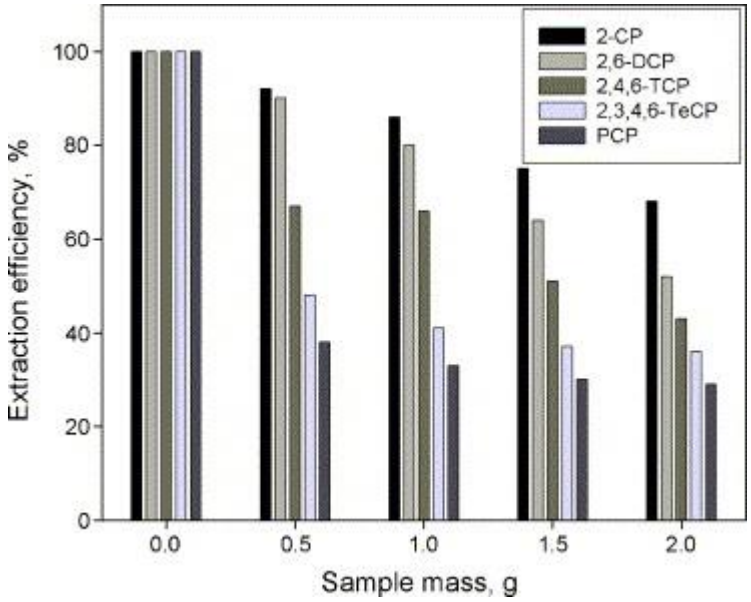

Fig. 3. Influence of the presence of different sample masses of honey on the SPME extraction efficiency of the analytes.

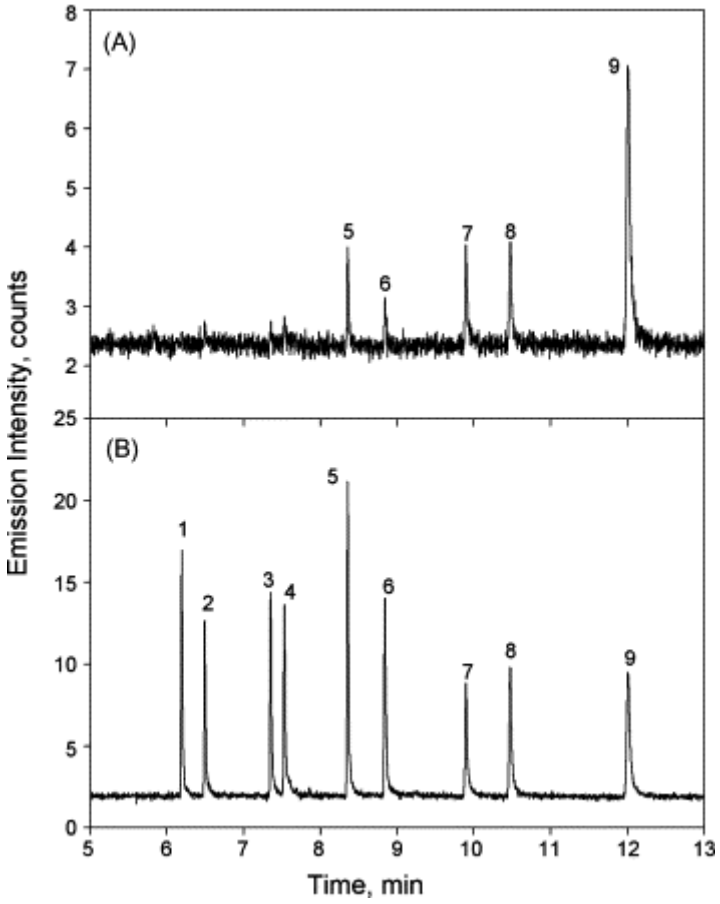

Fig. 4. SPME-GC-AED chromatograms obtained from honey sample 6 unfortified (A) and fortified (B) with a standard mixture of the chlorophenols at concentration levels of: (1) 2-CP, 100 ng g^{-1}; (2) 4-CP, 33.5 ng g^{-1}; (3) 2,6-DCP, 9.2 ng g^{-1}; (4) 2,4-DCP, 10.9 ng g^{-1}; (5) 2,4,6-TCP, 3.4 ng g^{-1}; (6) 2,4,5-TCP, 12.2 ng g^{-1}; (7) 2,3,4,6-TeCP, 4.3 ng g^{-1}; (8) 2,3,4,5-TeCP, 12.2 ng g^{-1}; (9) PCP, 11.1 ng g^{-1}.

Table 1. Calibration slopes for different honey samples under the optimized conditions

Analyte	Slope[a] (mean value ± standard deviation), ml ng^{-1}			
	Aqueous standard	Honey 6	Honey 7	Honey 8
2-CP	3.572 ± 0.166	1.569 ± 0.123	2.236 ± 0.146	3.143 ± 0.280
4-CP	3.420 ± 0.129	3.177 ± 0.208	3.147 ± 0.250	2.949 ± 0.221
2,6-DCP	17.43 ± 1.23	11.53 ± 0.59	10.76 ± 0.33	14.80 ± 0.54
2,4-DCP	25.14 ± 1.71	14.46 ± 0.82	14.12 ± 0.32	17.58 ± 0.83
2,4,6-TCP	103.6 ± 5.6	61.73 ± 1.26	73.79 ± 2.41	50.95 ± 2.24
2,4,5-TCP	57.71 ± 3.35	14.67 ± 0.35	19.73 ± 0.68	26.18 ± 0.96
2,3,4,6-TeCP	47.57 ± 2.72	23.66 ± 0.78	20.93 ± 0.75	34.66 ± 1.95
2,3,4,5-TeCP	44.86 ± 2.05	11.96 ± 0.31	19.73 ± 0.85	16.83 ± 0.82
PCP	39.31 ± 1.12	16.98 ± 0.77	23.85 ± 1.11	16.54 ± 0.72

[a] (n=2).

Table 2. Calibration characteristics obtained for Honey 8

Compound	Intercept[a]	Correlation coefficient	Linearity range, (ng g^{-1})	Limit of detection (ng g^{-1})	RSD[b] (%)	RSD[b] (%)
2-CP	-0.802 ± 1.125	0.991	5 – 100	2.2	11.6 (20)	7.02 (80)
4-CP	2.857 ± 0.989	0.993	5 – 100	2.4	7.33 (20)	6.64 (80)
2,6-DCP	3.341 ± 0.912	0.997	1 – 125	0.5	11.6 (5)	8.76 (50)
2,4-DCP	3.372 ± 1.885	0.996	0,5 – 125	0.4	7.14 (5)	6.90 (50)
2,4,6-TCP	1.061 ± 0.984	0.995	0,2 – 50	0.1	5.57 (1)	5.40 (20)
2,4,5-TCP	2.012 ± 1.036	0.997	0,6 – 50	0.3	9.08 (5)	7.03 (20)
2,3,4,6-TeCP	2.964 ± 0.754	0.995	0,4 – 50	0.2	8.96 (5)	7.50 (20)
2,3,4,5-TeCP	4.980 ± 1.782	0.996	0,6 – 100	0.4	8.25 (5)	7.25 (20)
PCP	1.862 ± 1.458	0.996	0,6 – 100	0.4	11.7 (5)	7.41 (20)

[a] Mean value ± standard deviation (n=2).

[b] n=10. Values in brackets are concentrations in ng g^{-1}.

Table 3. Results for the analysis of honey samples

Compound	Content[a] (ng g^{-1})				
	Honey 1	Honey 4	Honey 6	Honey 7	Honey 8
2,4-DCP	ND	ND	ND	ND	2.8 ± 0.9
2,4,6-TCP	ND	0.7 ± 0.3	0.4 ± 0.2	0.9 ± 1.1	1.0 ± 0.8
2,4,5-TCP	ND	ND	0.6 ± 0.2	ND	1.9 ± 0.8
2,3,4,6-TeCP	ND	2.2 ± 0.9	1.2 ± 0.4	ND	1.2 ± 0.7
2,3,4,5-TeCP	ND	3.7 ± 1.0	3.6 ± 0.6	ND	ND
PCP	2.1 ± 0.5	2.3 ± 0.8	9.4 ± 0.5	ND	2.5 ± 1.1

[a] Mean value ± standard deviation (n=3).

ND means non-detected.

Table 4. Mean recovery efficiencies in fortified samples using the proposed method

Compound	Spike level, (ng g^{-1})	Recovery[a] (%)		
		Honey 6	Honey 7	Honey 8
2-CP	25	84.9	92.5	110.2
	50	89.1	105.6	97.8
4-CP	25	93.5	90.9	107.5
	50	98.6	106.9	100.6
2,6-DCP	10	89.0	83.8	102.7
	20	106.7	107.8	91.6
2,4-DCP	10	84.0	96.9	108.4
	20	98.1	95.4	90.7
2,4,6-TCP	0.5	85.7	107.5	104.8
	5	107.7	93.1	85.4
2,4,5-TCP	5	100.3	97.5	114.4
	20	104.9	108.0	104.2
2,3,4,6-TeCP	5	93.8	106.5	94.5
	20	120.0	113.2	100.5
2,3,4,5-TeCP	10	103.6	85.3	115.2
	20	96.5	108.8	100.4
PCP	10	116.4	106.8	100.5
	20	94.1	99.1	99.8

[a] Mean value (n=3).

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