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A sensitive procedure based on solid-phase microextraction and gas chromatography for determining pentachlorophenol in different foodstuffs

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A sensitive procedure based on solid-phase microextraction and gas chromatography for determining pentachlorophenol in different foodstuffs

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Abstract

A new method for the determination of pentachlorophenol (PCP) in different foods was developed using capillary gas chromatography (GC) and microwave induced-plasma atomic emission spectrometry (MIP-AED) for detection. The analyte is first derivatized and then extracted and pre-concentrated by solid-phase microextraction (SPME) in headspace (HS) mode. A clear matrix effect was found for the different samples under study, so that standard addition was required for quantification. Detection limits ranging from 0.03 to 6.0 ng g⁻¹ were obtained, depending on the sample analyzed. The method gave recoveries from spiked samples in the 81-109% range. Concentration levels of PCP ranging from 0.3 to 1.5 ng g⁻¹ were found in honey, but no PCP was detected in other samples.

Keywords: Honey, Gelatine, Pentachlorophenol, Headspace Solid-Phase Microextraction (HS-SPME), Gas Chromatography – Atomic Emission Detection (GC-AED).

Introduction

Despite restrictions concerning the use of pentachlorophenol (PCP), its widespread use in the past together with its high resistance to biodegradation has led to its detection in the environment. PCP may be present in honey and related foods, having been transported by bees when travelling to collect nectar or even because of the treatment of wooden beehives. On the other hand, the

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1 presence of PCP in sap syrups and gelatines can be attributed to its use in
2 agriculture or to prevent mould and fungal growth in animal hides used for
3 gelatine manufacture.
4
5 Pentachlorophenol is included in the most relevant priority pollutant lists (Pérez-
6 Bendito and Rubio 1999) and has also been classified by the World Health
7 Organisation (WHO) as a possible carcinogenic agent to humans (Guidelines
8 for drinking-water quality 1998). Although the European Union (EU) considered
9 the need to improve the treatment conditions of beehives (Commission
10 Directive 97/1221/EC), no maximum residue limit (MRL) for PCP has been
11 established for honey or for edible gelatine.
12
13 Several techniques have been used for PCP analysis, including liquid
14 chromatography (LC) (Han et al. 2005), supercritical fluid chromatography
15 (SFC) (Bernal et al. 1997) and capillary electrophoresis (CE) (Martínez et al.
16 1996, Zhou et al. 2004); however, gas chromatography (GC) is the most widely
17 used method since it provides the best sensitivity. Mass spectrometry (MS)
18 (Crespín et al. 1999, Diserens 2001) and electron capture detector (ECD) (Insa
19 et al. 2004, Domeño et al. 2005) are the most commonly used detectors for
20 pentachlorophenol determination by GC. On the other hand, atomic emission
21 detection (AED) provides selective information which cannot be obtained with
22 other detectors. Compared to GC-MS the instrumentation used in GC-AED is
23 easy to operate, and its chromatograms can be interpreted by a semi-skilled
24 analyst.
25

1 Solid-phase microextraction (SPME) (Buchholz and Pawliszyn 1994, Bianchi et
2 al. 2002, Llompарт et al. 2002, Ribeiro et al. 2002, Li et al. 2004, Martínez-
3 Uruñuela et al. 2004, Bagheri et al. 2005, Domeño et al. 2005) appears as an
4 interesting alternative to conventional liquid-liquid extraction (LLE)
5 (Hoogerbrugge et al. 1999, Diserens 2001) and solid-phase extraction (SPE)
6 (Muiño and Lozano 1991, Rodríguez et al. 1996, Crespín et al. 1999, Lacorte et
7 al. 2000, Oliveira et al. 2002) because extraction can be made rapidly and
8 directly, without organic solvent, and can easily be automated.

9
10 In spite of the importance of controlling the PCP content of foods, the number of
11 articles dealing with its determination is relatively low. As far as we know, only
12 three publications deal with honey samples (Muiño et al. 1991, Sherma and
13 McGinnis 1995, Campillo et al. 2006) and another three with gelatine samples
14 (Borsetti and Thurston 1984, Yip 1985, AOAC Official Methods of Analysis
15 1990). The procedures proposed for gelatines are based on tedious sample
16 treatment involving acid-hydrolysis, base partition, cleanup and LLE. Despite the
17 advantages of using the SPME approach with the samples under study in the
18 present work, only one previous report (Campillo et al. 2006) applies this
19 methodology to PCP analysis in honey. This paper is an extension of that
20 analytical procedure. The sensitivity is here enhanced and the optimized
21 procedure applied to other samples for which the literature describes laborious
22 and time-consuming procedures. If comparing the proposed method with
23 relevant articles involving the use of SPME (Ribeiro et al. 2002, Domeño et al.
24 2005) two advantages are achieved by means of the approach here reported,
25 namely a saving of time as well as an enhancement of the sensitivity.

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Materials and methods

Chemicals

Pentachlorophenol (PCP) was purchased from Aldrich (Steinheim, Germany) with 99% purity. A stock solution of 1000 µg ml⁻¹ was prepared in HPLC grade methanol. Aqueous standard solutions of 100 ng ml⁻¹ were freshly prepared for spiking 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 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).

Instrumentation

The SPME device for manual sampling consisted of a holder assembly and several replaceable fibers, obtained from Supelco (Bellefonte, PA, USA). SPME fibers of six different coating materials were obtained from Supelco: polydimethylsiloxane (PDMS) of 100 µm thickness, polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 µm, carboxen/polydimethylsiloxane (CAR/PDMS) of 75 µm, polyacrylate (PA) of 85 µm, divinylbenzene/carboxen/polydimethylsiloxane (DBV/CAR/PDMS) of 50/30 µm and carbowax/divinylbenzene (CW/DVB) of 70 µm. Prior to use the fibers were conditioned 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 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 built in the Central Laboratory Service of the University of Murcia and consisting of a drilled block provided with an electronic temperature control system was used for heating.

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 non-polar 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 $^{\circ}\text{C}$ for 1 min. The oven temperature program simply consisted of rising temperature from 80 $^{\circ}\text{C}$ to 180 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C min}^{-1}$ and holding for 4 min. Helium was used as the carrier gas and as AED make-up gas, being set 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 3.5 min later. The transfer line and the cavity temperatures were set at the same value as recommended by the manufacturer, 325 $^{\circ}\text{C}$. Filter and backamount adjustment in the AED were set according to Agilent default

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specifications. The spectrometer was purged with a nitrogen gas flow rate of 2.5 l min⁻¹. The element monitorized was chlorine at the 479.45 nm line. Taking into account the time of 15 min adopted for the SPME adsorption step, and since the analyte eluted with a retention time of 6.09 min, the analysis of each sample lasts about 22 min.

Samples. SPME and in situ derivatization procedure

Ten different honey samples labelled as eucalyptus (samples 1-3), *ericaceous* (sample 4), rosemary (samples 5-7), orange blossom (samples 8-9) and heather (sample 10) were obtained from a local supermarket. No indication of the country of origin was provided on the label. Two royal jelly samples (fresh and lyophilized), palm honey and a maple syrup samples were obtained from a local dietetic shop. Three powdered mixtures containing approximately 15% (w/w) of gelatine of different flavours (samples 1-3) were obtained from a local supermarket and four pure gelatines (samples 4-7) used for sweets production were provided by a local manufacturer.

To carry out the extraction, samples of 1.5 g (0.5 g for gelatine samples) were weighed into a 15 ml SPME glass vial and 4 ml of a solution containing 4 and 0.75% (w/v) of sodium chloride and potassium carbonate, respectively, were added. Then 160 µl of acetic anhydride were added, the vial was immediately sealed with the cap after introducing the magnetic stir bar and the mixture was homogenized by inserting the vial in the home-made heating block previously programmed at 95 °C and maintaining the stirring at maximum power for 1 min. After this simultaneous homogenization and derivatization step, the fiber was

exposed for 15 min to the headspace over the aqueous mixture, which was continuously stirred at 1400 rpm and thermostated at 95 °C. Subsequently the PDMS-DVB 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.

Recovery assays

Since no reference materials are available for the validation of the method, spiked samples were prepared. Samples were spiked as follows: 0.1 ml of a working methanolic solution, containing pentachlorophenol at concentration levels ranging from 3.5 to 300 ng ml⁻¹, were added to 1.5 g of sample (0.5 g of gelatine) placed in a 15 ml SPME-vial, corresponding roughly to fortification levels of 0.25 and 20 ng g⁻¹, respectively. Fortification levels ranged between 0.5 and 5 ng g⁻¹ in the case of the gelatine samples. The spiked samples were set aside for 60 min at room temperature to let the methanol evaporate before being analyzed as described above. The fortification procedure was applied to two different honey samples, to the fresh royal jelly, the palm honey and a gelatine sample at four concentration levels and three replicates corresponding to three aliquots of each sample independently fortified and analyzed, were analyzed in each case.

Results and discussion

Using the previously optimized derivatization conditions (Campillo et al. 2006), the program temperature eluted the acetylated pentachlorophenol at 6.09 min using a carrier gas flow-rate of 4 ml min⁻¹, while the oven temperature was

1 maintained at 180 °C. Higher flow-rates are not recommended because
2 problems in the stationary phase appear.

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11 4 *Optimization of the SPME stage*

12 All the optimization experiments were carried out in parallel for honey and
13 gelatine, in both cases dissolving 0.5 g of fortified samples in 7 ml of aqueous
14 solution containing the appropriate chemicals for the derivatization step.

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22 9 *Selection of the extraction mode and the type of fiber.*

23 The extraction efficiency of six different fiber coatings was checked using both
24 the headspace and the immersion mode. These experiments were carried out
25 by exposing the fibers to the sample for 15 min at 90 °C, and desorbing the
26 analyte in the GC injection port for 1 min at a temperature 20 °C below that
27 recommended by the manufacturer for each coating. The carboxen/PDMS fiber
28 showed a marked memory effect, which persisted despite applying desorption
29 times as long as 10 min, for which reason its use was discarded. The results
30 obtained for the other five fiber coatings appear in Figure 1, where the influence
31 of this parameter is expressed by reference to the maximum extraction
32 efficiency, which was obtained with the PDMS/DVB fiber in the headspace
33 mode. Except for the PDMS coating, the headspace mode provided better
34 extraction efficiencies than the immersion mode. Since the HS extraction mode
35 is also advisable for prolonging the life time of the fiber coatings, this extraction
36 mode was adopted. The extraction efficiency did not exceed 50% for any
37 coating except in the case of the PDMS/DVB fiber.

[insert Figure 1 about here]

Addition of salt.

The effect of the ionic strength on the extraction efficiency of the acetylated analyte was studied by changing the sodium chloride concentration between 0 and 40% (w/v). In the case of the honey sample, the sensitivity increased up to a concentration of 4% (w/v) and then remained practically constant at concentrations up to 15% (w/v), while higher salt concentrations led to a slight decrease in the signal. Probably owing to the high salt content of the products manufactured with animal tissues, the addition of sodium chloride did not change the sensitivity for PCP in the gelatine matrix. A 4% (w/v) sodium salt concentration was finally selected for all the samples.

Extraction temperature.

The influence of the sample temperature was examined from 55 to 98 °C. The extraction efficiency was significantly enhanced by increasing the temperature up to 95 °C. While at 98 °C losses of the acetylated PCP were observed, owing to a decomposition process of the analyte, providing a chromatographic peak with a retention time of 5.2 min. Thus, 95 °C was selected as the optimum temperature.

Extraction time.

The extraction time was studied in the 5-60 min range with 95 °C as the sample-vial temperature, equilibrium being reached in 15 min. Of note is the fact that the signal decreased at 60 min, probably as a consequence of the

decomposition of the analyte. An adsorption time of 15 min was adopted, for the maximum sensitivity. Desorption parameters of 200 °C and 1 min were used, as has been previously recommended (Campillo et al. 2006).

Agitation speed.

The stirring speed was varied between 0 to 2000 rpm, although sensitivity practically did not vary between 1000 and 2000 rpm. Therefore, 1400 rpm was adopted because it provided the best repeatability. Moreover, the peak areas obtained in the selected stirring conditions and without stirring indicated that stirring resulted in a 1.5 fold increase in the extraction efficiency of the acetylated pentachlorophenol.

Extraction solution volume and sample mass.

Extraction volumes of 4, 5, 6 and 7 ml were assayed, resulting that the lower the extraction solution volume the higher the extraction efficiency. A solution volume of 4 ml was finally selected, the headspace volume being 11 ml. Volumes below 4 ml are not recommended because of poor homogenization of the samples.

The influence of the sample mass was checked by pre-concentrating 4 ml of a 5 ng ml⁻¹ PCP solution in the presence of different masses of honey or gelatine in the 0.1-2 g range. The results obtained showed a clear matrix effect in both cases. Sample masses of 0.5 g were found to be suitable for gelatine samples and 1.5 g for honey and the rest of the samples considered. When higher amounts were assayed, the repeatability of the extraction step worsened.

Analytical characteristics of the method

The matrix effect was studied by comparing the slopes of aqueous standards and standard additions calibration graphs for two honey samples of different visual aspect and floral origin, two gelatines and the other four samples under study. Different values for the calibration slopes were obtained when standard addition calibration graphs were constructed, the values depending on the sample used. The slopes obtained for the different samples under the optimized conditions appear in [Figure 2](#). In the case of the lyophilized royal jelly, 0.1 g of sample was submitted to analysis, which corresponds roughly to 1.5 g of the fresh royal jelly. In fact, if the total content of the lyophilized sample capsule was submitted to the whole procedure, the matrix effect was so high that the signal did not increase even after adding 100 ng PCP per gram of sample. In all cases, six spiking levels were preconcentrated and measured, peak areas being used for calibration purposes in all cases. The results clearly showed that the standard additions method is required for quantification. Correlation coefficients (r^2) higher than 0.9989 were obtained in all cases for the standard additions graphs. Taking into account that the procedure involves derivatization of the analyte and SPME preconcentration, this good correlation is of note.

[insert [Figure 2](#) about here]

The detection limit for PCP using a signal-to-noise ratio of three and considering the slope of the calibration graph obtained for 1.5 g of honey sample 4 was calculated to be 0.075 ng g⁻¹. The quantification limit for the same honey

sample using a signal-to-noise ratio of ten was calculated to be 0.25 ng g⁻¹. These values should be considered as orientative as they are dependent on the sample analyzed. Thus, the detection limits obtained for palm honey and lyophilized royal jelly (which correspond to the samples with the lowest and the highest matrix effect, respectively) were 0.03 and 6.0 ng g⁻¹, respectively. The repeatability was calculated using the relative standard deviation for ten successive injections of the honey sample 4 fortified at two concentration levels, the obtained values being 9.8 and 11.8% for 3.5 and 10 ng g⁻¹, respectively.

Real samples and validation of the method

The optimized procedure was applied to ten different honey samples, to four different related food samples, as well as to seven gelatine samples. Table I shows the results obtained. All the honeys and one of the gelatine samples analyzed provided signals for PCP which corresponded to very low concentration levels in the 0.3-1.5 ng g⁻¹ range. PCP was not detected in the other samples analyzed. Note that a 25 g-daily intake of the samples appearing in Table II is far from what considered as dangerous for human health. It should be noted that, taking into consideration the maximum admissible concentration in drinking water of 1.0 ng ml⁻¹ established by the US EPA (<http://www.epa.gov>), a daily intake of 1.4 kg of honey sample 2, would provide the same dose of PCP contained in 2 l of a drinking water containing the maximum residue limit permitted (1.0 ng ml⁻¹). Obviously, such a honey intake is not viable, so that the low PCP value found does not represent a risk for the consumer.

[insert Table I about here]

As no reference materials were available, recovery studies were carried out in order to check the accuracy of the proposed method, fortifying honey samples 2 and 6, gelatine sample 6, as well as the fresh royal jelly and the palm honey at four concentration levels with PCP as described above. The data of PCP found for honey samples 2 and 6 have been calculated by subtracting the corresponding analyte contents. The data obtained appear in Table II, providing recovery values ranging from 81 to 109%, with an average recovery $\pm$ SD (n=60) of 96.4 ± 8.2 .

[insert Table II about here]

Conclusion

The SPME-GC-AED combination provides a rapid and sensitive procedure that can be useful in the routine control of PCP residues in honey as well as in several other foodstuffs. The high pre-concentration power of solid phase microextraction under the selected conditions and the excellent sensitivity of the atomic emission detector provide nearly specific chromatograms, and detection limits for the samples analyzed never before attained, applying low extraction times and disposable commercial fibers. Nevertheless, the complexity of the matrix of the samples under study makes it necessary to quantify the analyte by means of the standard additions method.

Acknowledgements

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LEGENDS FOR THE FIGURES

Figure 1. Influence of the type of fiber coating and the extraction mode on the extraction efficiency of pentachlorophenol for 0.5 g of honey fortified at the concentration level of 20 ng g⁻¹, dissolved in 7 ml water and acetyled under the adopted conditions.

Figure 2. Slopes obtained when using aqueous calibration and standard addition method to different samples applying the finally selected conditions. Vertical bars indicate standard deviations from n=3.

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242x243mm (150 x 150 DPI)

209x251mm (150 x 150 DPI)

Table I

Results of pentachlorophenol for the honey samples analyzed using the proposed SPME/GC-AED method

Sample	Content*, ng g ⁻¹
Honey 1 (eucalyptus)	1.34 ± 0.27
Honey 2 (eucalyptus)	1.46 ± 0.21
Honey 3 (eucalyptus)	0.83 ± 0.20
Honey 4 (<i>ericaceous</i>)	0.61 ± 0.12
Honey 5 (rosemary)	0.51 ± 0.10
Honey 6 (rosemary)	0.89 ± 0.11
Honey 7 (rosemary)	0.92 ± 0.12
Honey 8 (orange blossom)	0.61 ± 0.20
Honey 9 (orange blossom)	0.32 ± 0.05
Honey 10 (heather)	1.1 ± 0.11
Gelatine 1 (orange flavour)	0.30 ± 0.09

* Mean value ± standard deviation (n=3).

Table II

Determination results of the recovery study in several samples for the optimized procedure

Honey 2		Honey 6		Fresh royal jelly		Palm honey		Gelatine 6	
Added*	Found ^{*,†}	Added*	Found ^{*,†}	Added*	Found ^{*,†}	Added*	Found ^{*,†}	Added*	Found ^{*,†}
0.98	0.80 ± 0.35	0.62	0.56 ± 0.13	2.02	1.74 ± 0.91	0.25	0.23 ± 0.09	0.50	0.42 ± 0.10
1.5	1.61 ± 0.22	1.04	0.96 ± 0.19	5.10	4.87 ± 0.35	1.04	0.91 ± 0.21	2.20	1.60 ± 0.11
5.02	5.22 ± 0.15	2.37	2.25 ± 0.15	10.25	11.09 ± 0.22	2.51	2.63 ± 0.14	2.98	2.09 ± 0.20
10.4	10.7 ± 0.30	5.02	4.85 ± 0.13	18.39	19.41 ± 0.24	5.09	4.74 ± 0.29	4.98	4.89 ± 0.24
* Concentration in ng g ⁻¹ .									
† Mean value ± standard deviation (n=3).									