



HAL
open science

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

Manuel Hernandez-Cordoba, Natalia Campillo, Rosa Peñalver

► **To cite this version:**

Manuel Hernandez-Cordoba, Natalia Campillo, Rosa Peñalver. A sensitive procedure based on solid-phase microextraction and gas chromatography for determining pentachlorophenol in different foodstuffs. *Food Additives and Contaminants*, 2007, 24 (07), pp.777-783. 10.1080/02652030701216735 . hal-00577286

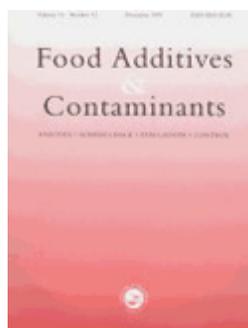
HAL Id: hal-00577286

<https://hal.science/hal-00577286>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

| | |
|-------------------------------|--|
| Journal: | <i>Food Additives and Contaminants</i> |
| Manuscript ID: | TFAC-2006-338.R1 |
| Manuscript Type: | Original Research Paper |
| Date Submitted by the Author: | 12-Jan-2007 |
| Complete List of Authors: | Hernandez-Cordoba, Manuel; University of Murcia, Department of Analytical Chemistry Campillo, Natalia; University of Murcia, Department of Analytical Chemistry Peñalver, Rosa; University of Murcia, Department of Analytical Chemistry |
| Methods/Techniques: | Chromatography - GC, Clean-up - SPME, Headspace |
| Additives/Contaminants: | Pesticides - organochlorine |
| Food Types: | Honey |
| | |

SCHOLARONE™
Manuscripts

1
2
3 1
4
5 2 **A sensitive procedure based on solid-phase microextraction**
6 **and gas chromatography for determining pentachlorophenol in**
7 **different foodstuffs**
8
9 4
10
11 5
12
13 6

14 7 **Natalia Campillo, Rosa Peñalver, Manuel Hernández-Córdoba***
15
16 8

17
18 9 *Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia,*
19 *E-30071 Murcia, Spain*
20
21
22 11
23
24 12
25
26 13

27 14 *Corresponding author:
28

29 15 Prof. Manuel Hernández-Córdoba

30 16 Department of Analytical Chemistry

31 17 Faculty of Chemistry

32 18 University of Murcia

33 19 E-30071 Murcia

34 20 SPAIN
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

21
22 Tel.: +34 968 367406

23 FAX: +34 968 364148

24 e-mail: hcordoba@um.es
25

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

1
2
3 1 Solid-phase microextraction (SPME) (Buchholz and Pawliszyn 1994, Bianchi et
4
5 al. 2002, Llompарт et al. 2002, Ribeiro et al. 2002, Li et al. 2004, Martínez-
6
7 Uruñuela et al. 2004, Bagheri et al. 2005, Domeño et al. 2005) appears as an
8
9 interesting alternative to conventional liquid-liquid extraction (LLE)
10
11 (Hoogerbrugge et al. 1999, Diserens 2001) and solid-phase extraction (SPE)
12
13 (Muiño and Lozano 1991, Rodríguez et al. 1996, Crespín et al. 1999, Lacorte et
14
15 al. 2000, Oliveira et al. 2002) because extraction can be made rapidly and
16
17 directly, without organic solvent, and can easily be automated.
18
19
20
21
22
23

24
25 10 In spite of the importance of controlling the PCP content of foods, the number of
26
27 11 articles dealing with its determination is relatively low. As far as we know, only
28
29 12 three publications deal with honey samples (Muiño et al.1991, Sherma and
30
31 13 McGinnis 1995, Campillo et al. 2006) and another three with gelatine samples
32
33 14 (Borsetti and Thurston 1984, Yip 1985, AOAC Official Methods of Analysis
34
35 15 1990). The procedures proposed for gelatines are based on tedious sample
36
37 16 treatment involving acid-hydrolysis, base partition, cleanup and LLE. Despite the
38
39 17 advantages of using the SPME approach with the samples under study in the
40
41 18 present work, only one previous report (Campillo et al. 2006) applies this
42
43 19 methodology to PCP analysis in honey. This paper is an extension of that
44
45 20 analytical procedure. The sensitivity is here enhanced and the optimized
46
47 21 procedure applied to other samples for which the literature describes laborious
48
49 22 and time-consuming procedures. If comparing the proposed method with
50
51 23 relevant articles involving the use of SPME (Ribeiro et al. 2002, Domeño et al.
52
53 24 2005) two advantages are achieved by means of the approach here reported,
54
55 25 namely a saving of time as well as an enhancement of the sensitivity.
56
57
58
59
60

1
2
3 1
4
5
6 2

Materials and methods

Chemicals

4 Pentachlorophenol (PCP) was purchased from Aldrich (Steinheim, Germany)
5 with 99% purity. A stock solution of 1000 $\mu\text{g ml}^{-1}$ was prepared in HPLC grade
6 methanol. Aqueous standard solutions of 100 ng ml^{-1} were freshly prepared for
7 spiking samples in order to optimize the analysis procedure. Acetic anhydride
8 and anhydrous potassium carbonate were purchased from Fluka (Buchs,
9 Switzerland) and sodium chloride of 99.5% purity from Sigma (St. Louis, MO,
10 USA).

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

Instrumentation

17 The SPME device for manual sampling consisted of a holder assembly and
18 several replaceable fibers, obtained from Supelco (Bellefonte, PA, USA).
19 SPME fibers of six different coating materials were obtained from Supelco:
20 polydimethylsiloxane (PDMS) of 100 μm thickness,
21 polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 μm ,
22 carboxen/polydimethylsiloxane (CAR/PDMS) of 75 μm , polyacrylate (PA) of 85
23 μm , divinylbenzene/carboxen/polydimethylsiloxane (DBV/CAR/PDMS) of 50/30
24 μm and carbowax/divinylbenzene (CW/DVB) of 70 μm . Prior to use the fibers
25 were conditioned by heating in the injection port of the chromatographic system

1 under the conditions recommended by the manufacturer for each fiber coating.
2 Whenever needed, the conditioning step was repeated for fiber cleanup. All
3 analyses were performed in 15 ml clear glass vials and the solutions were
4 stirred using PTFE-coated magnetic stir bars (10 mm x 6 mm o.d.). To prevent
5 analyte evaporation, vials sealed with hole-caps and PTFE/silicone septa were
6 used. A home-made system built in the Central Laboratory Service of the
7 University of Murcia and consisting of a drilled block provided with an electronic
8 temperature control system was used for heating.

9
10 An Agilent 6890 gas chromatograph was directly coupled by a transfer line to a
11 G2350A microwave-induced plasma atomic emission detector (Agilent).
12 Updated G2070AA ChemStation application with the G2360AA GC-AED
13 software was used to control and automate many features of the GC and AED
14 systems, and for data acquisition and treatment. The chromatograph was fitted
15 with a 30 m x 0.32 mm i.d. HP-5, 5% diphenyl 95% dimethyl polysiloxane non-
16 polar capillary column from Agilent with a 0.25 μm film thickness. Desorption of
17 the fibers into the injection port was carried out in the splitless mode at 200 $^{\circ}\text{C}$
18 for 1 min. The oven temperature program simply consisted of rising temperature
19 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
20 the carrier gas and as AED make-up gas, being set at 4 and 40 ml min^{-1} ,
21 respectively. Oxygen was used as the only scavenger gas at 20 psi. Solvent
22 venting was switched on immediately after starting the desorption step and
23 switched off 3.5 min later. The transfer line and the cavity temperatures were
24 set at the same value as recommended by the manufacturer, 325 $^{\circ}\text{C}$. Filter and
25 backamount adjustment in the AED were set according to Agilent default

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

6 7 *Samples. SPME and in situ derivatization procedure*

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

17
18 To carry out the extraction, samples of 1.5 g (0.5 g for gelatine samples) were
19 weighed into a 15 ml SPME glass vial and 4 ml of a solution containing 4 and
20 0.75% (w/v) of sodium chloride and potassium carbonate, respectively, were
21 added. Then 160 µl of acetic anhydride were added, the vial was immediately
22 sealed with the cap after introducing the magnetic stir bar and the mixture was
23 homogenized by inserting the vial in the home-made heating block previously
24 programmed at 95 °C and maintaining the stirring at maximum power for 1 min.
25 After this simultaneous homogenization and derivatization step, the fiber was

1 exposed for 15 min to the headspace over the aqueous mixture, which was
2 continuously stirred at 1400 rpm and thermostated at 95 °C. Subsequently the
3 PDMS-DVB fiber was retracted into the needle and transferred to the injection
4 port of the GC with the split valve closed at 200 °C for 1 min. Each sampling
5 was performed in triplicate.

6 7 *Recovery assays*

8 Since no reference materials are available for the validation of the method,
9 spiked samples were prepared. Samples were spiked as follows: 0.1 ml of a
10 working methanolic solution, containing pentachlorophenol at concentration
11 levels ranging from 3.5 to 300 ng ml⁻¹, were added to 1.5 g of sample (0.5 g of
12 gelatine) placed in a 15 ml SPME-vial, corresponding roughly to fortification
13 levels of 0.25 and 20 ng g⁻¹, respectively. Fortification levels ranged between
14 0.5 and 5 ng g⁻¹ in the case of the gelatine samples. The spiked samples were
15 set aside for 60 min at room temperature to let the methanol evaporate before
16 being analyzed as described above. The fortification procedure was applied to
17 two different honey samples, to the fresh royal jelly, the palm honey and a
18 gelatine sample at four concentration levels and three replicates corresponding
19 to three aliquots of each sample independently fortified and analyzed, were
20 analyzed in each case.

21 22 **Results and discussion**

23 Using the previously optimized derivatization conditions (Campillo et al. 2006),
24 the program temperature eluted the acetylated pentachlorophenol at 6.09 min
25 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.

3

4 *Optimization of the SPME stage*

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

8

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

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

25

1
2
3
4 [insert Figure 1 about here]
5
6
7

8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3 *Addition of salt.*

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

13
14 *Extraction temperature.*

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

21
22 *Extraction time.*

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

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

4 *Agitation speed.*

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

11 *Extraction solution volume and sample mass.*

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

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

1

2 *Analytical characteristics of the method*

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

20

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

22

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

1 sample using a signal-to-noise ratio of ten was calculated to be 0.25 ng g^{-1} .
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 sample using a signal-to-noise ratio of ten was calculated to be 0.25 ng g^{-1} .
2 These values should be considered as orientative as they are dependent on the
3 sample analyzed. Thus, the detection limits obtained for palm honey and
4 lyophilized royal jelly (which correspond to the samples with the lowest and the
5 highest matrix effect, respectively) were 0.03 and 6.0 ng g^{-1} , respectively. The
6 repeatability was calculated using the relative standard deviation for ten
7 successive injections of the honey sample 4 fortified at two concentration levels,
8 the obtained values being 9.8 and 11.8% for 3.5 and 10 ng g^{-1} , respectively.
9

10 *Real samples and validation of the method*

11 The optimized procedure was applied to ten different honey samples, to four
12 different related food samples, as well as to seven gelatine samples. Table I
13 shows the results obtained. All the honeys and one of the gelatine samples
14 analyzed provided signals for PCP which corresponded to very low
15 concentration levels in the $0.3\text{-}1.5 \text{ ng g}^{-1}$ range. PCP was not detected in the
16 other samples analyzed. Note that a 25 g -daily intake of the samples appearing
17 in Table II is far from what considered as dangerous for human health. It should
18 be noted that, taking into consideration the maximum admissible concentration
19 in drinking water of 1.0 ng ml^{-1} established by the US EPA (<http://www.epa.gov>),
20 a daily intake of 1.4 kg of honey sample 2, would provide the same dose of PCP
21 contained in 2 l of a drinking water containing the maximum residue limit
22 permitted (1.0 ng ml^{-1}). Obviously, such a honey intake is not viable, so that the
23 low PCP value found does not represent a risk for the consumer.
24
25

[insert Table I about here]

1
2
3 1
4
5 2 As no reference materials were available, recovery studies were carried out in
6
7
8 3 order to check the accuracy of the proposed method, fortifying honey samples 2
9
10 4 and 6, gelatine sample 6, as well as the fresh royal jelly and the palm honey at
11
12 5 four concentration levels with PCP as described above. The data of PCP found
13
14 6 for honey samples 2 and 6 have been calculated by subtracting the
15
16 7 corresponding analyte contents. The data obtained appear in Table II, providing
17
18 8 recovery values ranging from 81 to 109%, with an average recovery \pm SD
19
20 9 (n=60) of 96.4 ± 8.2 .

21
22
23
24
25 [insert Table II about here]
26
27
28
29
30
31
32

33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

13 Conclusion

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

24 Acknowledgements

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 The authors are grateful to the Spanish Ministerio de Educacion y Ciencia, MEC
2 (Project CTQ2006-08037/BQU) for financial support. R. Peñalver acknowledges
3 a fellowship from MEC.
4

For Peer Review Only

1 LEGENDS FOR THE FIGURES

2
3
4
5
6
7
8 Figure 1. Influence of the type of fiber coating and the extraction mode on the
9 extraction efficiency of pentachlorophenol for 0.5 g of honey fortified at the
10 concentration level of 20 ng g⁻¹, dissolved in 7 ml water and acetyled under the
11 adopted conditions.
12
13
14
15
16
17

18
19
20 Figure 2. Slopes obtained when using aqueous calibration and standard
21 addition method to different samples applying the finally selected conditions.
22
23
24 Vertical bars indicate standard deviations from n=3.
25
26
27

28 References

- 29
30
31 Association of Official Analytical Chemists 1990. Pentachlorophenol in gelatine,
32 gas chromatographic method. Official Methods of Analysis, 15th Ed. p.
33 308. Kenneth Helrich, ed. Virginia: USA.
34
35
36
37
38 Bagheri H, Mir A, Babanezhad E. 2005. An electropolymerized aniline-base
39 fiber coating for solid phase microextraction of phenols from water.
40
41 Analytica Chimica Acta 532:89-95.
42
43
44
45
46 Bernal JL, Nozal MJ, Toribio L, Serna ML, Borrull F, Marce RM, Pocurull E.
47
48 1997. Determination of phenolic compounds in water samples by online
49 solid-phase extraction-supercritical-fluid chromatography with diode-array
50 detection. Chromatographia 46:295-300.
51
52
53
54
55
56
57 Bianchi F, Careri M, Mucchino C, Musci M. 2002. Improved determination of
58 chlorophenols in water by solid-phase microextraction followed by
59
60

- 1
2
3 1 benzoylation and gas chromatography with electron capture detection.
4
5
6 2 Chromatographia :595-600.
7
8 3 Borsetti AP, Thurston LS. 1984. Gas chromatographic determination of
9
10 4 pentachlorophenol in gelatin. Journal of Association of Official Analytical
11
12 5 Chemistry 67:275-277.
13
14
15 6 Buchholz KD, Pawliszyn J. 1994. Optimization of solid-phase microextraction
16
17 7 conditions for determination of phenols. Analytical Chemistry 66:160-167.
18
19
20 8 Campillo N, Peñalver R, Hernández-Córdoba M. 2006. Evaluation of solid-
21
22 9 phase microextraction conditions for the determination of chlorophenols
23
24 10 in honey samples using gas chromatography. Journal of
25
26 11 Chromatography A 1125:31-37.
27
28
29 12 Crespín MA, Cárdenas S, Gallego M, Valcárcel M. 1999. Discrimination of
30
31 13 structural isomers of chlorinated phenols in waters using gas
32
33 14 chromatography-mass spectrometry in the negative chemical ionization
34
35 15 mode. Journal of Chromatography A 830:165-174.
36
37
38 16 Commission Directive 97/1221/CE of 25 June 1997. Official Journal of the
39
40 17 European Communities. European Community, Brussels.
41
42
43 18 Diserens JM. 2001. Rapid determination of nineteen chlorophenols in wood,
44
45 19 paper, cardboard, fruits, and fruit juices by gas chromatography/mass
46
47 20 spectrometry. Journal of AOAC International 84:853-860.
48
49
50 21 Domeño C, Munizza G, Nerín C. 2005. Development of a solid-phase
51
52 22 microextraction method for direct determination of pentachlorophenol in
53
54 23 paper and board samples: comparison with conventional extraction.
55
56 24 Journal of Chromatography A 1095:8-15.
57
58
59
60

- 1
2
3 1 Guidelines for drinking-water quality, 2nd ed. Vol.2 Health criteria and other
4 supporting information 1996, 940 and Addendum to vol. 2 1998, 281;
5
6 2
7 Geneva, World Health Organization.
8
9
10 4 Han DM, Fang GZ, Yan XP. 2005. Preparation and evaluation of a molecularly
11 imprinted sol-gel material for on-line solid-phase extraction coupled with
12 high performance liquid chromatography for the determination of trace
13 pentachlorophenol in water samples. *Journal of Chromatography A*
14 1100:131-136.
15
16
17 9 Hoogerbrugge RH, Gort SM, van der Velde Piet van Zoonen. 1999. Multi- and
18 univariate interpretation of the interlaboratory validation of PrEN 12673;
19 GC determination of chlorophenols in water. *Analytica Chimica Acta*
20 388:119-135.
21
22
23 13 Insa S, Salvadó V, Anticó E. 2004. Development of solid-phase extraction and
24 solid-phase microextraction methods for the determination of
25 chlorophenols in cork macerate and wine samples. *Journal of*
26 *Chromatography A* 1047:15-20.
27
28
29 17 Lacorte S, Guiffard I, Fraisse D, Barceló D. 2000. Broad spectrum analysis of
30 109 priority compounds listed in the 76/464/CEE council directive using
31 solid-phase extraction and GC/EI/MS. *Analytical Chemistry* 72:1430-
32 1440.
33
34
35 21 Li X, Zeng Z, Zhou J. 2004. High thermal-stable sol-gel-coated calyx[4]arene
36 fiber for solid-phase microextraction of chlorophenols. *Analytica Chimica*
37 *Acta* 509:27-37.
38
39
40 24 Llompart M, Lourido M, Landín P, García-Jares C, Cela R. 2002. Optimization
41 of a derivatization-solid-phase microextraction method for the analysis of
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

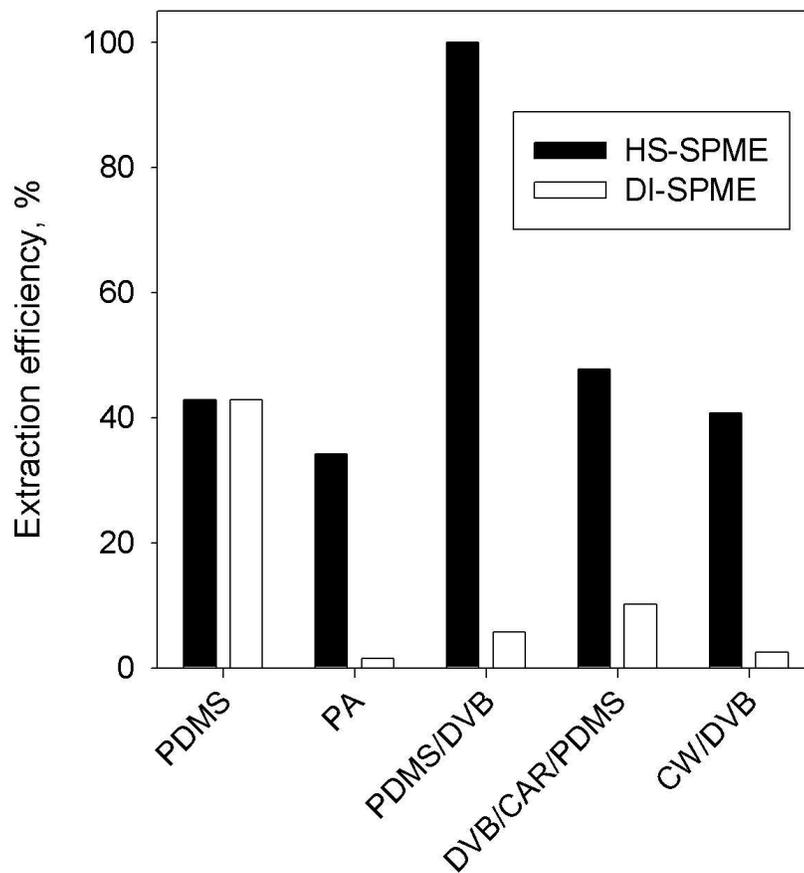
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1 thirty phenolic pollutants in water samples. *Journal of Chromatography A*
- 2 963:137-148.
- 3 Martínez D, Pocurull E, Marce RM, Borrull F, Calull M. 1996. Comparative study
- 4 of the use of high-performance liquid chromatography and capillary
- 5 electrophoresis of phenolic compounds in water. *Chromatographia*
- 6 43:619-624.
- 7 Martínez-Uruñuela A, González-Sáiz JM, Pizarro C. 2004. Optimisation of the
- 8 derivatization reaction and subsequent headspace solid-phase
- 9 microextraction method for the direct determination of chlorophenols in
- 10 red wine. *Journal of Chromatography A* 1048:141-151.
- 11 Muiño MAF, Lozano JS. 1991. Mass spectrometric determination of
- 12 pentachlorophenol in honey. *Analytica Chimica Acta* 247:121-123.
- 13 Oliveira EC, Ruaro Peralba M, Bastos Caramão E. 2002. Solid phase extraction
- 14 applied to chlorinated phenolics present in the effluent from a pulp mill.
- 15 *Journal of Separation Science* 25:356-360.
- 16 Pérez-Bendito D, Rubio S. 1999. *Environmental Analytical Chemistry*. In: Weber
- 17 SG. *Comprehensive Analytical Chemistry*. Vol. XXXII. Amsterdam:
- 18 Elsevier. p 568.
- 19 Ribeiro A, Neves MH, Almeida MF, Alves A, Santos L. 2002. Direct
- 20 determination of chlorophenols in landfill leachates by solid-phase micro-
- 21 extraction-gas chromatography-mass spectrometry. *Journal of*
- 22 *Chromatography A* 975:267-274.
- 23 Rodríguez I, Turnes MI, Mejuto MC, Cela R. 1996. Determination of
- 24 chlorophenols at the sub-ppb level in tap water using derivatization, solid-

- 1
2
3 1 phase microextraction and gas chromatography with plasma atomic
4
5 2 emission detection. Journal of Chromatography A 721:297-304.
6
7
8 3 Sherma J, McGinnis SC. 1995. Determination of pentachlorophenol and
9
10 4 cymiazole in water and honey by C-18 solid-phase extraction and
11
12 5 quantitative HPTLC. Journal of Liquid Chromatography 18:755-761.
13
14
15 6 US Environmental Protection Agency [internet]. Available from:
16
17 7 <http://www.epa.gov/safewater/mcl.htm>. Accessed 2006 November 6.
18
19
20 8 Yip G. 1985. Gas chromatographic determination of pentachlorophenol in
21
22 9 gelatin: collaborative study. Journal of Association of Official Analytical
23
24 10 Chemistry 68:419-421.
25
26
27 11 Zhou QX, Liu JF, Jiang GB, Liu GG, Cai YQ. 2004. Sensitivity enhancement of
28
29 12 chlorinated phenols by continuous flow liquid membrane extraction
30
31 13 followed by capillary electrophoresis. Journal of Separation Science
32
33 14 27:576-58
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

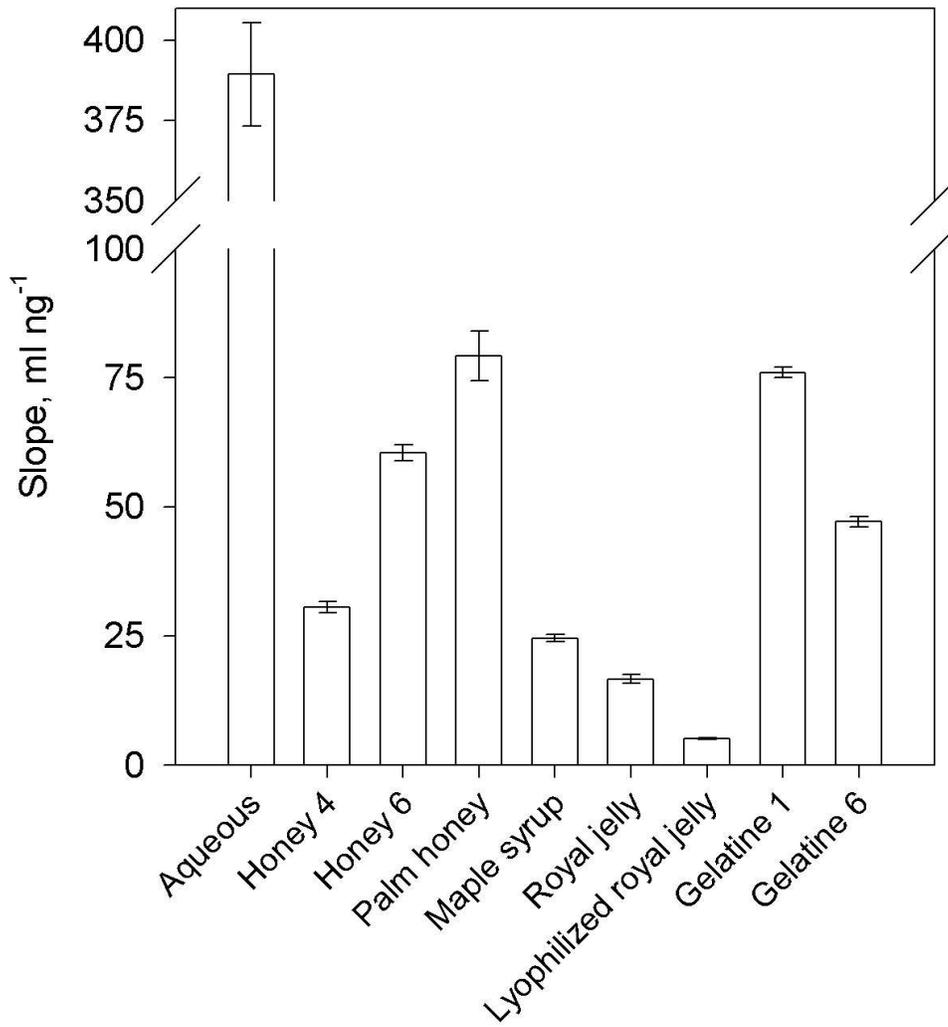
For Peer Review Only



242x243mm (150 x 150 DPI)

only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



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).

1
2
3
4
5
6
7
8
9
10

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).