ELSEVIER

Journal of Chromatography A, 829 (1998) 269–277

JOURNAL OF CHROMATOGRAPHY A

# Solid-phase microextraction applied to the analysis of pesticide residues in honey using gas chromatography with electron-capture detection[1]

J.J. Jiménez*, J.L. Bernal, M.J. del Nozal, M.T. Martín, A.L. Mayorga

*Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Prado de la Magdalena s/n, 47005 Valladolid, Spain*

Received 28 July 1998; received in revised form 7 October 1998; accepted 9 October 1998

**Abstract**

The possibilities of using solid-phase microextraction to determine residues of pesticides in honey have been examined. For this purpose, three types of fiber have been assayed: polyacrylate of 85 μm thickness, and polydimethylsiloxane of 7 and 100 μm thickness. They have been applied to the extraction of 21 pesticides of different chemical families. The effects of the temperature, extraction time and ionic strength on the microextraction have been studied, proposing the most adequate for each fiber. Under optimized conditions, precision, intervals of linearity and detection limits were evaluated. 

*Keywords:* Honey; Extraction methods; Solid-phase microextraction; Food analysis; Pesticides

## 1. Introduction

Mainly as a consequence of chemi-therapic treatments on beehives it is possible that residues of certain pesticides could appear in apiarian products, so it is convenient to evaluate them in order to maintain the characteristics that as a natural product the honey is supposed to have. Being a complex and very assorted matrix according to the botanical origin, the determination of these residues often includes an isolation–concentration stage. Thus, liquid–liquid extraction [1,2], solid-phase extraction on octadecylsilane and Florisil [2–5], and supercritical extraction [6] have been applied successfully; advantages and disadvantages are found in terms of rapidity, simplicity and economy for each type of extraction.

With the recent introduction of solid-phase microextraction (SPME), and its successful application to the determination of pesticide residues in aqueous samples [7–12], we considered the possibility of applying the technique to more complex samples. In this work, we studied the viability of three SPME fibers, based on polydimethylsiloxane and polyacrylate, which are usually employed for aqueous media, in the analysis of pesticide residues in raw honey, including compounds of different chemical

*Corresponding author.

[1]Presented at the 27th Scientific Meeting of the Group of Chromatography and Related Techniques of the Spanish Royal Society of Chemistry, Lugo, 8–10 July 1998.

0021-9673/98/$ – see front matter 
PII: S0021-9673(98)00826-7

family in order to assess the real possibilities of this technique.

For each fiber, the most adequate conditions for the extraction have been examined and chosen with the aim of enhancing the extraction efficiency, so parameters, affecting the distribution equilibrium of the analytes between fiber and solution, such as temperature, ionic strength (salting out effect) and the time necessary to achieve the equilibrium, have been optimized. For this, analytical characteristics such as reproducibility, linearity range and detection limits, have been examined for each fiber tested. At the same time, the possible influence of the matrix effect derived from the different honey samples has also been considered. The extraction and desorption of the SPME fibers have been monitored using gas chromatography with electron-capture detection (GC–ECD).

## 2. Experimental

### 2.1. Chemicals and apparatus

Pesticide certified standards were obtained from Promochem (Wesel, Germany) and Chemservice (West Chester, PA, USA). Residue analysis grade methanol, *n*-hexane and acetone were provided by Labscan (Dublin, Ireland). Stock solutions of pesticides were made in acetone and *n*-hexane. Sodium chloride (analysis grade) was supplied by Panreac and ultrapure water was obtained from a Milli-Q Plus apparatus from Millipore (Milford, MA, USA).

An SPME fiber holder for manual use, part 5-7330, and a kit of manual fiber assemblies (part 5-7306) were provided by Supelco (Bellefonte, PA, USA). The fiber coatings assayed were as follows: non-bonded 100 μm polydimethylsiloxane, bonded 7 μm polydimethylsiloxane and partially crosslinked 85 μm polyacrylate. An ultrasonic water bath and a ceramic-top stirring hotplate were supplied by Selecta (Barcelona, Spain).

SPME fibers were conditioned before use as follows. The 100 μm polydimethylsiloxane fiber was heated at 260°C for 1 h. The 7 μm polydimethylsiloxane fiber was conditioned at 320°C for 2 h and the 85 μm polyacrylate fiber was heated at 300°C for 2 h.

### 2.2. Solid-phase microextraction

To carry out the extractions, the honey was always diluted with water in a proportion 1:5 (e.g., 5 g of honey were made up to 25 ml of water). To study the influence of the ionic strength, 5 g of NaCl was added and dissolved with the aid of an ultrasonic water bath; amounts greater than 5 g presented problems for their total dissolution. Aliquots of 3 ml of the honey–water solution were placed in 4-ml vials. Then, 50 μl of a solution in methanol containing the pesticides in a known concentration were added to the vial, introducing a microbar for stirring. The vial was sealed with a PTFE septum and put into a water bath to obtain the adequate temperature. The agitation system was started and then the fiber was introduced in the vial.

The same procedure was followed for the three types of fibers, varying the temperature of the bath from 20 to 70°C, and the extraction times from 30 to 180 min.

### 2.3. Gas chromatography–electron-capture detection

A Hewlett-Packard (Avondale, PA, USA) 5890 gas chromatograph fitted with an electron-capture detector and a 60 m×0.25 mm capillary column coated with a 0.25 μm thick film of 50% phenylmethylpolysiloxane (Quadrex Scientific, Surrey, UK) was used. Desorption of the 7 μm polydimethylsiloxane and 85 μm polyacrylate fibers into the injection port was carried out at 270°C for 4 min while the 100 μm polydimethylsiloxane fiber was desorbed at 260°C for 4 min. The oven temperature programme was as follows: initial temperature 50°C, held for 5 min, a 25°C/min ramp to 160°C, a 1.2°C/min ramp to 260°C, and finally a 20°C/min ramp to 275°C, held for 34 min. The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. The split valve was opened after 5 min. Argon–methane (90:10) was used as auxiliary gas for ECD. The detector temperature was 300°C.

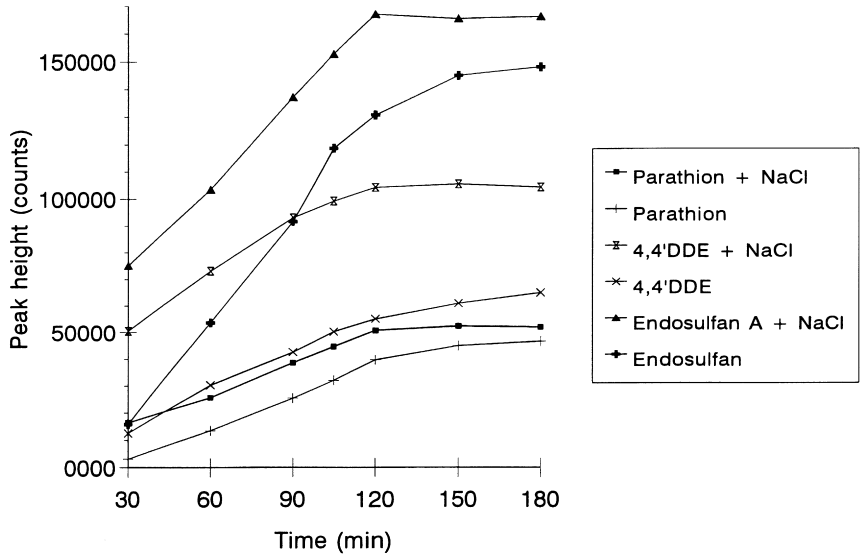


Fig. 1. Variation of the peak height with extraction time and with NaCl addition. Temperature: 30°C. Concentration of each pesticide: 0.2 mg/kg. 7 μm polydimethylsiloxane fiber. $n=7$.

## 3. Results and discussion

### *3.1. Polydimethylsiloxane fiber (7 μm), bonded*

In Fig. 1, the height of the chromatographic signal obtained for different extraction times when this fiber was employed in the extraction of samples with and without previous addition of NaCl is shown. It can be observed that the presence of NaCl helps the extraction; peak heights increased from 10 to 100% depending on the pesticide. This effect tends to be more pronounced for smaller extraction times. It could be also observed that the signal increased with the extraction time until reaching a virtually constant value, these times ranged from 100 to 120 min for most of the compounds, except for ethion and lindane, which required times greater than 150 min to reach constant values. To shorten the analysis time, an extraction time of 120 min was chosen, previously NaCl had been added to the samples.

In relation to the influence of temperature it could be observed that the extraction supplied chromatographic peaks whose maximum heights were in the 25–35°C interval. The peak height varied considerably according to the compounds, with the maximum obtained for 4,4′-DDE whose peak height increased nearly 400% whereas the minimum increase was found for lindane and α-HCH, approximately 80%. Fig. 2 shows an example for four of the pesticides studied. For the following experiments a temperature of 30°C was selected.

Concerning the reproducibility obtained in the extraction from a honey sample spiked with 0.1 mg/kg of each pesticide, the relative standard deviation (R.S.D.) of the peak height varied over the range 8–25%, after seven consecutive sample preparations from the same honey sample, excepting α-HCH whose R.S.D. was 2.5%.

To determine the interval of linearity, honey samples spiked with increasing amounts of pesticides were used. The linearity was practically a decade and ranged between 30 and 500 ng at the most, and correlation coefficients, $r^2$, of the order of 0.900 to 0.990 were obtained, except for the fluvalinate, 0.999. The detection limits (signal-to-noise ratio about 10) varied from concentrations of about 0.001 mg/kg for the organochlorine pesticides to 0.06 mg/kg for the organophosphorus compounds.

In Fig. 3, a chromatogram of a spiked honey extract containing all the pesticides is shown. As can be seen, the chromatogram presents a great front with a tail that decreases progressively until disappearing at approximately 50 min. No interferences were encountered for the compounds of interest. For

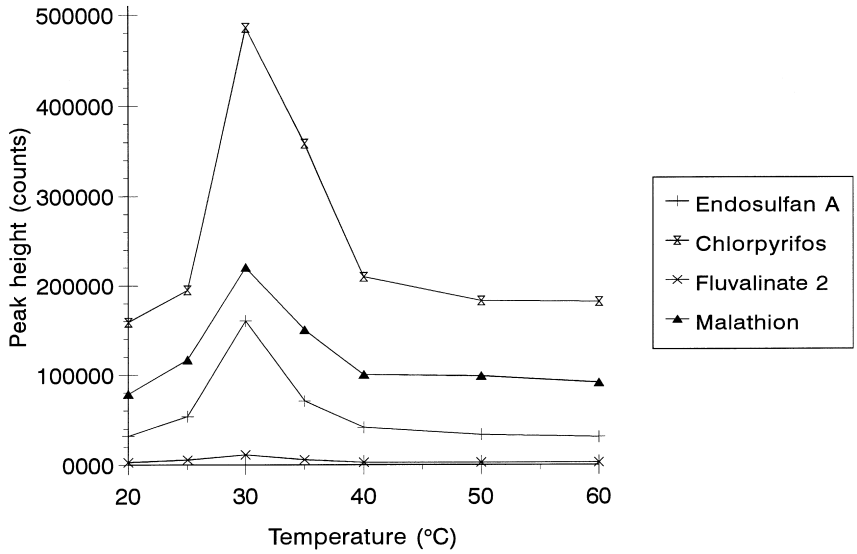


Fig. 2. Variation of the peak height with extraction temperature. Extraction time: 120 min. NaCl added. Concentration of each pesticide: 0.2 mg/kg. 7 μm polydimethylsiloxane fiber. $n=7$.

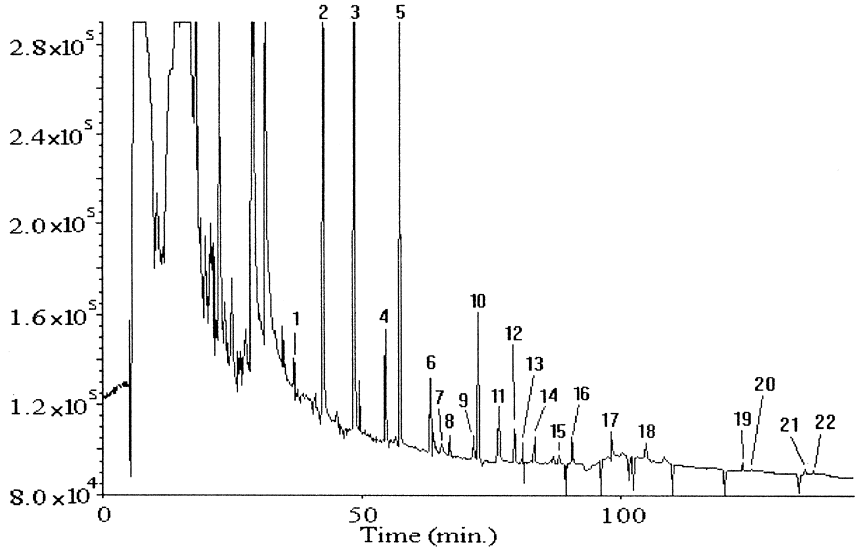


Fig. 3. Chromatogram obtained by using the procedure proposed for the 7 μm polydimethylsiloxane fiber on a spiked sample. See Table 1 for peak identification.

extracts obtained without the addition of salt, the front was somewhat smaller.

### 3.2. Polydimethylsiloxane fiber (100 μm), non-bonded

In this case, and repeating a similar study to the previous one, it could be seen that the addition of NaCl generally decreased the height of the chromatographic peaks of the pesticides by a percentage that varied approximately between 40 and 400%, according to the assays made on honey containing 0.1 mg/kg of each compound at a temperature of 50°C and during a 2 h extraction. In the presence of NaCl, fluvalinate was not detected in the sample at all.

As regards the extraction temperature, a very different behaviour from that of the previous fiber was observed. Chromatographic response increased at higher temperatures for most of the compounds; at low extraction temperatures (i.e., 30–50°C) response was less than at higher temperatures (50–70°C), probably due to the enhanced transfer of the analytes towards the fiber. Nevertheless, for some compounds, such as malathion, vinclozolin, lindane, α-HCH and parathion the signal remained virtually constant (Fig. 4). A temperature of 70°C was selected to carry out the analysis.

The influence of the extraction time was similar to that of the previous fiber. The chromatographic signal increased gradually with the extraction time and stabilized at about 60 min for most of the compounds. This indicated that a state of equilibrium had been reached in the partition process. The addition of salt did not affect the time needed to reach equilibrium.

The reproducibility (R.S.D.) calculated under similar conditions to the those above-mentioned varied between 1.5% for acrinathrin and parathion and 18% for aldrin ($n=7$). When the vial of extraction was saturated with NaCl, the R.S.D. was multiplied at least by two, with values ranging from 5% to 45%. Intervals of linearity about two decades were obtained for this fiber (Fig. 5), ranging from concentrations of 6.5 to 500 ng for several of the studied compounds, and with correlation coefficients of 0.990–0.999. Detection limits ranged from 0.03 mg/kg for fluvalinate to 0.0001 mg/kg for compounds such as α-HCH, lindane, endosulfan A and chlorpyrifos. The chromatograms of the extracts were very similar to those recorded with the 7 μm polydimethylpolysiloxane fiber.

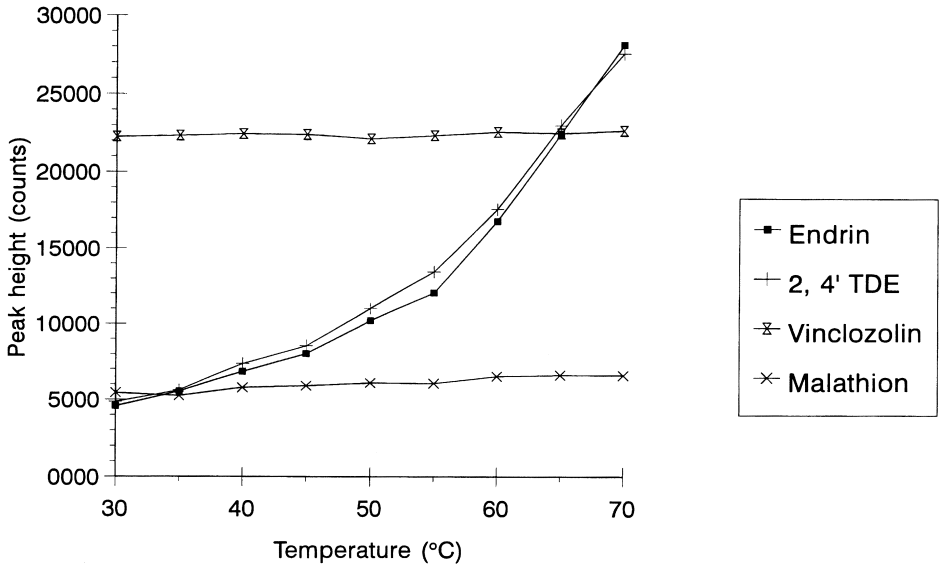


Fig. 4. Variation of the peak height with extraction temperature. Extraction time: 120 min. Without NaCl. Concentration of each pesticide: 0.1 mg/kg. 100 μm polydimethylsiloxane fiber. $n=7$.

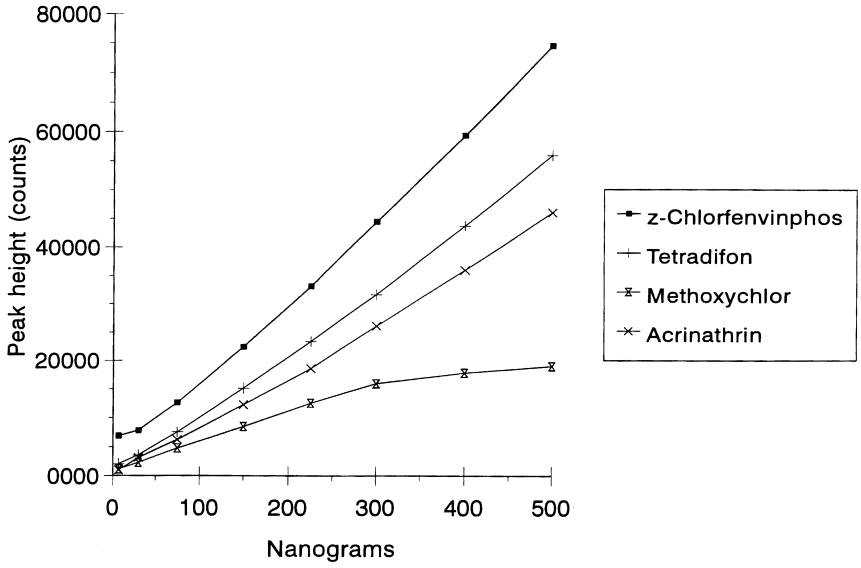


Fig. 5. Calibration graphs for the 100 μm polydimethylsiloxane fiber. Extraction time: 60 min. Without NaCl. Temperature: 70°C. $n=7$.

### *3.3. Polyacrylate fiber (85 μm), partially crosslinked*

With this fiber, the addition of salt did not generally increase the extraction performance. In the trials without salt addition, the peak height of the pyrethroids was multiplied by 5 in comparison with those containing salt. The following experiments were conducted without the addition of salt.

In relation to the extraction temperature, it could be observed that when the temperature increased above 45°C the signal increased gradually until reaching a constant value for a temperature of 60°C, so this temperature was considered suitable for this type of fiber. At 60°C the peak height was three- to five-times higher in relation to that one obtained at 30°C. In this case, the equilibrium state was reached at approximately 105 min for the different compounds.

For this fiber, the R.S.D. was of the order of 7–24% ($n=7$), with a small interval of linearity, frequently between 30–300 or 30–400 ng, and with correlation coefficients between 0.900 and 0.990. The detection limits were similar to those obtained for the 7 μm polydimethylsiloxane fiber.

The chromatograms achieved present a huge-intense front and a greater number of substances coextracted in comparison with the previous fibers. The addition of salt complicated the chromatograms even more.

### *3.4. Comparison between the three microextraction fibers*

From the comparative study of the results supplied by the three fibers on spiked honey samples it can be appreciated that the 100 μm polydimethylsiloxane fiber was the most adequate for various reasons: the reproducibility was greater, with an obtained average from the data of reproducibility of 9.3% compared with 14.5% for the 7 μm and 16.2% for the polyacrylate fibers; the detection limits were clearly lower, with a mean value of 0.004 mg/kg compared with a value of 0.02 mg/kg for the other fibers; the interval of linearity was also wider, and extended towards lower concentrations of pesticides, and the correlation coefficients were higher. Furthermore, the time necessary to perform the extraction was also less (60 min), and the chromatograms obtained were simpler. Consequently the 100 μm polydimethylsiloxane fiber was used for application studies.

### 3.5. Application of the 100 μm polydimethylsiloxane fiber to honey analysis

It is known that the sample matrix can affect the efficiency of the extraction and also the desorption and transfer of pesticides from the injection port towards the chromatographic column. Because of this it has been necessary to verify if the performance of calibrations with a type of honey influences the quantification of pesticides present in other types of honey. For this purpose, a calibration curve was made with the honey used in the above-mentioned experiments and the pesticides added to honeys of various botanical origins were measured. For this purpose, seven multifloral honeys and six honeys from different botanical origins (*Thymus* spp., *Lavandula stoechas*, *Erica* spp., *Calluna vulgaris*, *Quercus* spp., *Rosmarinus officinalis*) were selected. Table 1 shows, as an example, the results obtained on a spiked multifloral honey for three fortification levels, with five replicates for each level. The recoveries deviated slightly from 100% with reproducibilities similar to those already mentioned for this fiber.

Depending on the type of honey analyzed, background noise and the intensity of the chromatographic front are notably different. Chromatograms similar to that shown in Fig. 3 are found between the most complex of the obtained ones. In Fig. 6 a simpler chromatogram of a non-spiked multifloral honey sample where two organochlorine compounds are detected is shown.

In an attempt to reduce the number of compounds coextracted and to obtain simpler chromatograms, several experiments using 0.5 g of honey in 25 ml of water, instead of 5 g, were also performed. The profile of the chromatograms was similar in both cases, without substantial improvements. Neverthe-

Table 1
Recoveries obtained in the extraction of pesticides with the 100 μm polydimethylsiloxane fiber on spiked honey ($n=5$)

| | Pesticide | 0.08 mg/kg | | 0.2 mg/kg | | 0.5 mg/kg | |
|---|---|---|---|---|---|---|---|
| | | Recovery (%) | R.S.D. (%) | Recovery (%) | R.S.D. (%) | Recovery (%) | R.S.D. (%) |
| 1 | Demeton-*S*-methyl | 84 | 19.0 | 88 | 17.4 | 82 | 13.0 |
| 2 | α-HCH | 97 | 8.4 | 105 | 10.2 | 99 | 7.8 |
| 3 | Lindane | 110 | 8.5 | 102 | 10.2 | 108 | 10.8 |
| 4 | Vinclozolin | 104 | 5.2 | 105 | 4.7 | 98 | 6.0 |
| 5 | Aldrin | 85 | 19.8 | 102 | 16.4 | 95 | 14.0 |
| 6 | Chlorpyrifos | 100 | 9.9 | 92 | 9.3 | 89 | 10.0 |
| 7 | Malathion | 88 | 6.5 | 97 | 7.0 | 108 | 6.1 |
| 8 | Parathion | 115 | 2.5 | 104 | 3.0 | 106 | 2.9 |
| 9 | Chlorfenvinphos (Z isomer) | 98 | 5.2 | 107 | 3.8 | 104 | 4.7 |
| 10 | Endosulfan A | 84 | 11.0 | 87 | 9.4 | 95 | 11.9 |
| 11 | 4,4′-DDE | 92 | 10.7 | 88 | 8.9 | 83 | 11.5 |
| 12 | Captan | 95 | 16.0 | 91 | 17.7 | 89 | 18.2 |
| 13 | 2,4′-TDE | 89 | 17.4 | 86 | 18.2 | 81 | 16.5 |
| 14 | Endrin | 115 | 8.4 | 94 | 8.0 | 91 | 6.5 |
| 15 | Ethion | 115 | 10.2 | 97 | 11.5 | 87 | 11.0 |
| 16 | 4,4′-DDT | 104 | 11.9 | 100 | 12.0 | 117 | 14.0 |
| 17 | Acrinathrin[a] | 111 | 3.2 | 101 | 2.9 | 99 | 3.8 |
| 18 | Methoxychlor | 89 | 9.8 | 92 | 8.7 | 94 | 10.8 |
| 19 | Tetradifon | 87 | 14.5 | 94 | 16.0 | 103 | 12.0 |
| 20 | Phosalone | 118 | 8.4 | 112 | 7.6 | 103 | 8.0 |
| 21 | Fluvalinate 1 | 101 | 10.0 | 98 | 8.6 | 105 | 9.5 |
| 22 | Fluvalinate 2 | 99 | 9.4 | 95 | 11.5 | 103 | 10.0 |

[a] Most abundant isomer.

R.S.D.: Relative standard deviation.

α-HCH: α-Hexachlorocyclohexane, 4,4′-DDE: 1,1′-(2,2-dichloroethenylidene)bis(4-chlorobenzene), 2,4′-TDE: 1,1′-(2,2-dichloroethylidene)bis(4-chlorobenzene), 4,4′-DDT: 1,1′-(2,2,2-trichloroethylidene)bis(4-chlorobenzene).

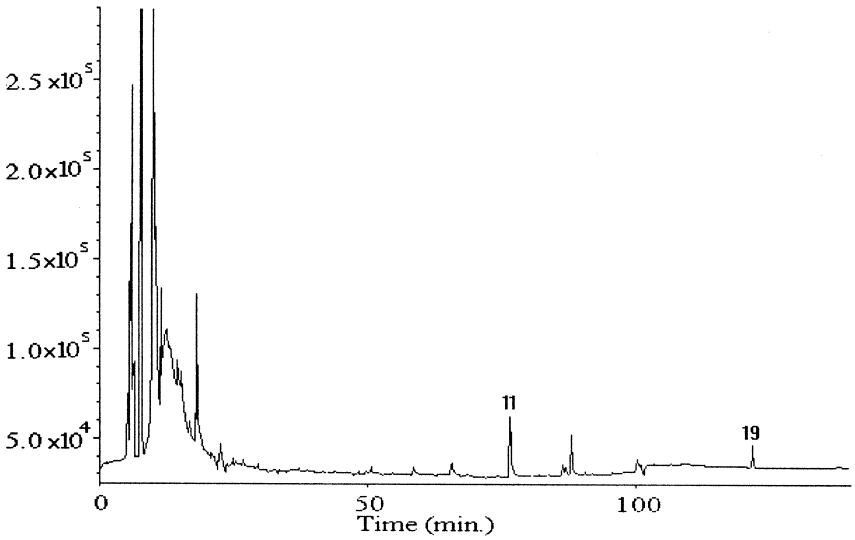


Fig. 6. Chromatogram obtained by using the procedure proposed for the 100 μm polydimethylsiloxane fiber on a non-spiked sample. See Table 1 for peak identification.

less, it was observed that the chromatographic response of the pesticides was increased in comparison to the previous experiments, likely by the reduced adsorption of artifacts originated from the matrix. However, the greater dilution of the sample did not imply an improvement of the detection limits of the procedure since the greater extraction of the pesticides did not compensate the reduction of honey sample, therefore the limits of detection expressed in mg/kg were higher than the ones already mentioned.

Finally, it is necessary to point out that after performing about 60 analyses, it was found that the height of the chromatographic peaks of the pesticides extracted was reduced by approximately 10% as a consequence of the deterioration of the fiber.

### *3.6. Comparison with other extraction procedures*

SPME applied to honey analysis presents advantages in comparison to conventional methods referring to the organic solvent saving and operation simplicity, even though the manual SPME, used here, is very tedious and time consuming. Concerning the quantitative results, the detection limits reached using SPME–GC–ECD are basically of the same magnitude as those achieved after an extraction with organic solvents or in solid-phase, while the reproducibility obtained in SPME is clearly lower in relation to the mentioned methods [1,2,5]. We recommend that SPME be used to accomplish semiquantitative screening. Once compounds are identified, a conventional extraction procedure could be used to quantitate. On the other hand, the matrix effects derived from the use of calibrations with different types of samples seem to slightly affect the quantitative analysis.

## 4. Conclusions

SPME is useful for the analysis of pesticide residues in honey, however, the low reproducibility obtained suggests its use as a semiquantitative technique. It is also interesting to point out the duration of the process in relation to other extraction procedures, such as solid-phase extraction, which can limit seriously its application to large quantities of samples. Of the fibers examined, the 100 μm polysiloxane fiber led to better results. It must be noted that it is always required to examine its response in relation to the extraction conditions (time, temperature, stationary phase), the type of compounds to evaluate and the different matrix (botanical origin) in order to achieve some analytically useful results.

## Acknowledgements

We would like to thank Teknokroma Ltd. (Barcelona, Spain) for letting us the holders for the fiber assays.

## References

[1] J.J. Jiménez, J.L. Bernal, J. Atienza, Chromatographia 42 (1996) 130.
[2] J.L. Bernal, M[a].J. del Nozal, J.M[a]. Rivera, J.J. Jiménez, J. Atienza, J. Chromatogr. A 754 (1996) 507.
[3] M.B. Taccheo, M. de Paoli, C. Spessotto, Pestic. Sci. 25 (1989) 11.
[4] M.B. Taccheo, M. de Paoli, A. Valentino, Pestic. Sci. 28 (1990) 197.
[5] J.J. Jiménez, J.L. Bernal, M[a].J. del Nozal, L. Toribio, M[a].T. Martín, J. Chromatogr. A, 823 (1998) 381.
[6] J.J. Jiménez, J. Atienza, J.L. Bernal, J. High. Resolut. Chromatogr. 18 (1995) 367.
[7] T.K. Choudhury, K.O. Gerhardt, T.P. Mawhinney, Environ. Sci. Technol. 30 (1996) 3259.
[8] I.J. Barnabas, J.R. Dean, I.A. Fowlis, S.P. Owen, J. Chromatogr. A 705 (1995) 302.
[9] R. Young, V. López Avila, W.F. Beckert, J. High Resolut. Chromatogr. 19 (1996) 247.
[10] R. Eisert, K. Levsen, G. Wunsch, J. Chromatogr. A 683 (1994) 175.
[11] R. Eisert, K. Levsen, Fresenius J. Anal. Chem. 351 (1995) 143.
[12] T. Gorecki, R. Mindrup, P. Pawliszyn, Analyst 121 (1996) 1381.