Apidologie 33 (2002) 545–552
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DOI: 10.1051/apido:2002038

Original article

Application of solid phase microextraction to evaluate traces of thymol in honey

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(Received 20 October 2001; revised 7 November 2001; accepted 5 April 2002)

Abstract – The solid phase microextraction technique was tested for thymol evaluation in honey. Thymol can be present in honey as a residue of treatments against *Varroa destructor* Honey was sampled from apiaries treated with anti-Varroa products whose active ingredient is thymol. Thymol evaluation was done using the internal standard method; benzophenone and carvacrol were tested as internal standards/ the best results were obtained using benzophenone. The application of an alkaline hydrolysis was important for obtaining quantitative recoveries.

honey / solid phase microextraction / thymol / *Varroa destructor* / gas chromatography

1. INTRODUCTION

Thymol is present as a natural compound in lime tree (*Tilia* spp.) honey, but it can also be found in other types of honey as a residue of treatments against *Varroa destructor*.

The use of acaricides often involves an accumulation of toxic molecules derived from their active ingredients in beehive products. Experiments are currently underway to define the conditions of use of "natural" substances so as to limit negative effects as far as possible. It has been demonstrated that, unlike other chemical acaricides, thymol does not accumulate in the honey or the wax, even after long-term use, if the instructions for its correct use are followed (Bogdanov, 1998).

Thymol levels found in honey are generally low. For example, in lime honey thymol values have been measured in the range of 0.02 to 0.16 mg/kg (Bogdanov et al., 1998) and are within the limits of health safety standards (LD50 orally in rats/ 980 mg/kg, cited by Merck Index, 9540 12th edition, 1996). However, if thymol is not used properly, it can reach levels in the honey that can alter the flavour of the product (Bogdanov et al., 1999) which is not in accordance with the regulations that govern the characteristics of honey.

Thymol-based acaricides are widely used in Italy to control *V. destructor*; the evaluation of the thymol content is therefore of great interest in food product control. In Switzerland, a limit of 0.8 mg/kg has been set, while within the EU,

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according to EC Reg. 1742/96, no limit has been fixed.

Solid phase microextraction (SPME), which was introduced relatively recently, was conceived and proposed as an alternative technique to traditional solid phase extraction (SPE) by researchers at the University of Waterloo (Arthur and Pawliszyn, 1990; Zhang and Pawliszyn, 1993a, 1993b). It solves most of the problems associated with the commonly-used extraction methods, allows rapid extraction without solvents of volatile and semi-volatile organic compounds, is simple to use, is inexpensive, and can be automated. This technique can extract and concentrate the organic molecules of compounds in water samples or in the headspace through their adsorption on a fused silica fibre coated with an organic phase. The fibre is then put into the injector of a gas chromatograph, where the adsorbed molecules are heat-desorbed and transferred directly onto the column.

SPME is an equilibrium technique, so the compounds are not completely extracted from the matrix. The measurable limits are in the order of 1 μg/kg (Penton, 1996). The adsorption process is driven by the repartition equilibrium between the sample and the organic phase which is chemically linked to the fibre (or between sample, headspace and organic phase, in the case of a headspace analysis). Some factors that influence the solubility of the molecules or their presence in the headspace (pH, ionic strength, temperature, etc.) consequently also affect the adsorption on the fibre and the sensitivity of the method. The availability of fibres with different adsorption, lipophilic and polarity characteristics allows the most suitable fibre to be chosen for the class or group of compounds being studied.

The aim of this research was to test the SPME analysis technique to evaluate the residues of thymol treatment. Because of its simplicity, it could represent an easier alternative to the SPE system currently used, and it is a improvement over gas chromatography/mass spectrometry (GC/MS) or high performance liquid chromatography (HPLC) systems. The results obtained with SPME were compared to those of a traditional evaluation method, based on adsorption on an SPE filter followed by gas chromatography evaluation (Bogdanov et al., 1998).

2. MATERIALS AND METHODS

2.1. Sampling

In this work calibration was carried out by adding known amounts of thymol to one sample of honey produced in Friuli Venezia Giulia that resulted in thymol free honey on the basis of previous analysis.

Eight authentic samples were supplied by the Swiss Apiculture Research Centre in Bern from beehives that had been treated for different lengths of time with a thymol-based anti-Varroa product (Bogdanov et al., 1999). The thymol was evaluated by SPE analysis and gas chromatography (Bogdanov et al., 1998).

The method for evaluating thymol using SPE has been described in detail in a previous publication (Bogdanov et al., 1998). In summary, 10 g honey are solubilised in 20 mL distilled water/ethyl alcohol (80/20 v/v); this solution is passed through an SPE extraction column (C18 solid phase). After washing with water and drying the SPE column, the thymol is eluted with 2 mL acetone; this solution is then injected in gas chromatography.

The thymol content is calculated using the external standard method; the sensitivity of the method is 0.02 mg/kg and the measurable limit is 0.1 mg/kg.

This method requires the introduction of a correction factor that takes into account the fact that recovery values below 0.75 mg/kg do not supply reliable data. The recovery values at different concentration levels and precision, expressed as percentage coefficients of variation, are reported in Table I.

Table I. Values of recovery and relative standard deviation recorded for the different thymol concentrations added to a honey sample through SPE-GC according to Bogdanov et al., 1998.

mg/kg added	Recovery %	RSD %	Number of replicates
0.10	66.6	10.0	6
0.25	60.6	4.1	6
0.50	61.2	8.3	6
0.75	87.5	6.0	6
1.00	93.5	9.9	6
2.00	94.1	7.7	6

2.2. Instruments

An SPME device (Supelco Co., Belefonte, PA) was equipped with a fibre assembly coated with polyacrylate (Pac, thickness 85 μm).

A Carlo Erba 5300 gas chromatograph equipped with a Model SSL71 split/splitless injector (temperature/ 250 ^{o}C) and a flame ionisation detector (FID; temperature/ 250 ^{o}C) was used. The injector port was equipped with a narrow bore (0.75 mm i.d.) glass liner to minimise peak broadering. Separation was carried out on a HP-INNOWax column 30 m × 0.32 mm i.d., 0.5 μm film thickness (Hewlett Packard). The column temperature was held at 50 ^{o}C for 4 min, then increased to 230 ^{o}C (at 10 ^{o}C/min), then held at 230 ^{o}C for 10 min and finally increased to 250 ^{o}C (at 10 ^{o}C/min). The carrier gas (helium) flow rate was 1.5 mL/min.

Injections were carried out by means of splitless system/ the split valve was opened 3 min after injection (split ratio 50/ 1).

2.3. Reagents

Anhydrous sodium sulphate (BDH Laboratory Supplies, Poole, England) was pre-treated overnight in an oven at 80 ^{o}C and left to cool in a dessicator before use to avoid any contamination by volatile substances in the environment and to standardise the water level.

Benzophenone solution (BDH Laboratory Supplies, Poole, England; 99.9% purity) was obtained by dissolving 15 mg benzophenone in 8 mL acetone (BDH Laboratory Supplies, Poole, England) and adjusting the volume to 25 mL with deionised water.

Carvacrol solution (Fluka, Buchs, Switzerland; purity 99.9%) was obtained by dissolving 9.5 mg carvacrol in 1.5 mL ethanol and adjusting the volume to 25 mL with deionised water.

Sodium hydroxide solution was 1 M (Carlo Erba, Milan, Italy).

2.4. SPME operating conditions

Precisely 3 ± 0.001 g honey and 0.5 g anhydrous sodium sulphate were weighed in a 10 mL glass vial. The amount of sodium sulphate was sufficient to absorb the water contained in the honey.

The vial, hermetically sealed by the silicon and Teflon septum (Alltech, Milan, Italy) and metal ring (Alltech, Milan, Italy) was placed in a thermostatic bath at 70 ^{o}C for 30 minutes for the conditioning phase. During this phase, the sample was shaken manually twice.

The fibre for SPME was then inserted in the headspace of the vial maintained at 70 ^{o}C for 20 minutes. At this point, the fibre, retracted in the needle was extracted from the vial, inserted in the gas chromatograph injector at 250 ^{o}C immediately after closing the splitting valve, and the analysis began.

The vials and septa were pre-treated overnight in an oven at 80 ^{o}C and left to cool in a dryer before use to avoid any contamination by volatile substances in the environment.

2.5. Evaluation of thymol response factors

To evaluate thymol-related response factors, increasing concentrations (0.06 mg/kg; 0.12 mg/kg; 0.51 mg/kg; 0.9 mg/kg) of thymol were added to a chestnut honey which was previously analysed by SPME to check for the absence of thymol in the experimental conditions adopted. The thymol stock solution was prepared by dissolving 15 mg thymol (Fluka, Buchs, Switzerland, purity 99.9%) in 25 mL water. The sample, composed of 20 g honey, 3.3 g anhydrous sodium sulphate, with 3.5 μL of internal standard solution added (benzophenone) and different concentrations of thymol solution, was placed in a 25 mL vial, sealed and manually shaken, carefully, for 0.5 minutes. To ensure that added thymol reached an equilibrium inside honey, samples were analysed after one week/ 3 g honey were drawn from each vial and analysed using the method described above.

From the areas of the peaks, the response factor (K) was calculated using the formula/

$$K = (p_{SI} \times A_x)/p_x \times A_{SI}$$

where K = response factor;

p_{SI} = internal standard (benzophenone) weight in mg;

p_x = thymol weight in mg;

A_x = thymol area;

A_{SI} = internal standard area (benzophenone).

2.6. Evaluation of thymol content

The thymol content in the honey was evaluated by SPME and GC. Either 0.5 μL benzophenone solution or 0.5 μL carvacrol solution were added to the sample as internal standards. For the samples with a high thymol concentration (≥ 0.48 mg/kg), it was considered appropriate to weigh 1 g honey and 0.2 g anhydrous sodium sulphate. For the other samples with concentrations of ≤ 0.17 mg/kg, 3 g honey and 0.5 g salt were weighed. The above-described

headspace analysis method with SPME was applied to these samples.

2.7. Hydrolysis tests

Chestnut honey with 0.51 mg/kg thymol added (see Sect. 2.5) used for method calibration was analysed again after 3 months. The observation of lower results ($\Delta = -15\%$ of thymol) suggested that thymol could perhaps bind to some honey compound, maybe because of the presence of an hydroxy group in its molecule.

With the aim of maximising the recovery of thymol from the honey, it was considered appropriate to subject the honey to alkaline hydrolysis to free any esterified thymol. Evaluation of the required amount of NaOH was obtained by selecting two samples, one with low and one with high thymol content (TH 7 with ≤0.02 mg/kg and TH 1 with 0.91 mg/kg). Both were subjected to the following tests:

TEST (a)

1 g honey sample;

0.3 g anhydrous sodium sulphate;

0.15 mL NaOH 1 M;

0.5 μL benzophenone solution.

TEST (b)

1 g honey sample;

0.4 g anhydrous sodium sulphate;

0.30 mL NaOH 1 M;

0.5 μL benzophenone solution.

The sample preparation and analysis were carried out as described previously. Test (b) was then applied to the other honey samples using both 0.5 μL carvacrol solution as internal standard and 0.5 μL benzophenone solution.

3. RESULTS

3.1. Internal standard/ thymol and carvacrol

The response factor, calculated using benzophenone as an internal standard, was 1.96; the thymol concentration range between 0.90 and 0.06 mg/kg used for this evaluation was chosen because this interval includes the thymol contents found on average in the sample that was analysed by SPE within the framework of the present research.

Carvacrol was the second compound taken into consideration as internal standard. This molecule differs from thymol only by the position of an hydroxyl on the aromatic ring. Given the close chemical similarity between the molecules of carvacrol and thymol, it is possible to hypothesise a behaviour similar to flame ionisation detector (FID) and therefore the same response factor. The response factor, using this internal standard, was therefore assumed to be equal to 1.

The repeatability of the method was calculated using both carvacrol and benzophenone. Five replications were done on the same honey sample (TH 3) with a known amount of thymol (0.68 mg/kg).

The data relating to the repeatability are reported in Table II. As demonstrated by the data in this table, the relative standard deviation (RSD%) is much higher (22.6%) when carvacrol was used as internal standard. The relative standard deviation (14%) obtained using benzophenone as internal standard was lower and this compound was then chosen as the internal standard.

Contrary to what had been expected, the choice of carvacrol was demonstrated as being

Table II. Repeatability tests data for thymol evaluation in honey sample TH 3 with SPME method. Two internal standards was compared.

SAMPLE TH 3	R1	R2	R3	R4	R5	mean	Std. dev.	RSD %
Thymol (mg/kg)[a]	0.35	0.25	0.34	0.28	0.35	0.32	0.04	14
Thymol (mg/kg)[b]	0.47	0.49	0.58	0.36	0.33	0.44	0.10	22.6

[a] Internal standard: benzophenone.
[b] Internal standard: carvacrol.
R Replicate.

unsuitable. Although carvacrol, by its chemical nature, could be considered better as internal standard, the complexity of the chromatogram and the retention time of carvacrol itself being very close to that of other interferers mean that the errors of integration on the carvacrol peak are higher than those of the benzophenone peak. The latter in fact eluted in an area of the chromatogram that was free of other interfering peaks (see Fig. 1).

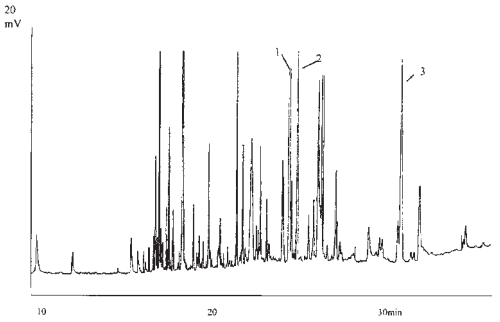

Figure 1. SPME-GC profile of honey sample TH 7. 1: carvacrol (I.S.), 2: thymol; 3: benzophenone (I.S.).

3.2. Thymol content

The thymol content was then evaluated in eight honey samples in which thymol, as evaluated by the SPE-GC method, ranged between 0.02 mg/kg and 0.91 mg/kg. The results of this evaluation are given in Table III. As the table shows, the experiments done on all the samples allow smaller amounts of thymol to be measured than those evaluated using SPE, except for the samples with amounts of 0.07 and (0.02 mg/kg. The observed differences between SPE and SPME could depend on the fact that SPME allowed for a more efficient trapping and concentration of thymol and furthermore SPME analyses were performed only on these samples after several months. The fact that samples submitted to hydrolysis showed higher amounts of thymol suggested that thymol could be bound to honey compounds (esterification could be improved by acid pH of honey).

Given this, an alkaline hydrolysis was carried out to maximise the recovery of thymol from the honey.

3.3. Hydrolysis test

The preliminary experiment to establish the optimal quantity of NaOH was evaluated for two samples containing the highest and lowest thymol concentrations, using benzophenone as an internal standard. Table IV, which gives the results of this experiment, shows that the addition of 0.15 mL NaOH (1 M) to 1 g of honey was not able to free all the thymol present in esterified form, while, by adding 0.30 mL, very high recoveries were obtained for both samples. The values of the thymol concentrations measured in this latter case are in fact very close to those initially obtained with the SPE method. It was therefore decided to add this quantity of sodium hydroxide to each sample.

3.4. Method repeatability

The repeatability of the method was evaluated on the TH 3 honey sample with sodium hydroxide added (0.30 mL). The results are reported in Table V.

A simple comparison of the repeatability data before and after hydrolysis shows that the coefficients of variation obtained after hydrolysis were lower. The relative standard deviation (RSD%) found using benzophenone as an internal standard was 6.56% compared to 14%

Table III. Thymol content data of honey using two evaluation methods. SPE and SPME. For SPME analysis two internal standard were compared.

SAMPLE	TH 1	TH 2	TH 3	TH 4	TH 5	TH 6	TH 7	TH 8
SPE: Thymol (mg/kg)	0.91	0.83	0.68	0.48	0.17	0.07	≤0.02	≤0.02
SPME: Thymol (mg/kg)[a]	0.65	0.38	0.36	0.46	0.06	0.09	0.03	0.03
SPME: Thymol (mg/kg)[b]	0.76	0.59	0.50	0.46	0.05	0.06	0.05	0.03

[a] Internal standard: benzophenone.
[b] Internal standard: carvacrol.

Table IV. Thymol evaluation data of honey samples TH 1, TH 7 by SPME method with and without hydrolysis of honey. Recoveries by SPME method were compared with recoveries by SPE method.

SAMPLE	SPE: Thymol (mg/kg)	SPME: Thymol (mg/kg)		
		Before hydrolysis	After hydrolysis	
			with 0.15 mL NaOH 1 M	with 0.30 mL NaOH 1 M
TH 1	0.91	0.65	0.72	0.89
TH 7	≤0.02	0.03	0.04	0.05

Table V. Repeatability tests data for thymol evaluation in honey sample TH 3 with SPME method after hydrolysis. Two internal standards were compared.

SAMPLE TH 3 after hydrolysis	R1	R2	R3	R4	R5	Mean	Std dev.	RSD %
Thymol (mg/kg)[a]	0.64	0.6	0.71	0.63	0.66	0.65	0.04	6.56
Thymol (mg/kg)[b]	1.14	0.98	1.07	0.72	0.96	0.97	0.16	16.4

[a] Internal standard: benzophenone.
[b] Internal standard: carvacrol.
R Replicate.

found in the test without hydrolysis; the RSD% found using carvacrol as internal standard was 16.4%, compared to 22.6% found in the test without hydrolysis.

The RSD% for evaluations made on a honey sample with 0.75 mg/kg added experimentally using the SPE GC method was 6% (Tab. I). In practice, the RSD% values of the two methods were comparable, but the recovery values of the SPME method were higher after alkaline hydrolysis.

3.5. Thymol content after hydrolysis

All the other honey samples were analysed under the specific hydrolysis conditions reported in the experimental section. The data are reported in Table VI and reveal that the use of hydrolysis allows, in most cases, recovery of higher quantities of thymol that are closer to those initially obtained with the SPE technique (Fig. 2).

Sample TH 5 behave in a different way than the other types of honey with both standards/ the thymol concentration after hydrolysis declined instead of increased.

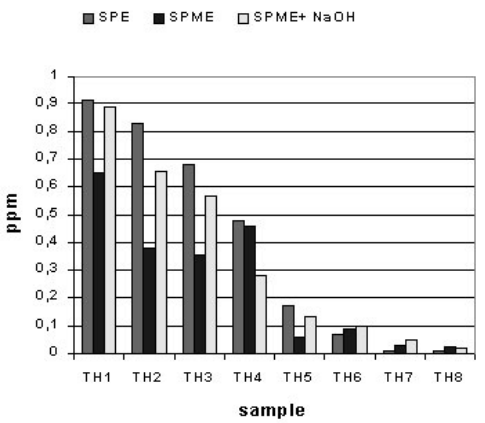

Figure 2. Representation of the data from the thymol evaluation of honey obtained using SPE method, SPME method and SPME method alkaline hydrolysis of sample (SPME+NaOH).

4. CONCLUSIONS

The best results were obtained using benzophenone as the internal standard, since in the case of carvacrol, higher recoveries values were recorded than those using the SPE-GC

Table VI. Thymol content data of honey using two evaluation methods: SPE and SPME after hydrolysis. For SPME analysis two internal standard were compared.

SAMPLE	TH 1	TH 2	TH 3	TH 4	TH 5	TH 6	TH 7	TH 8
SPE-GC: Thymol (mg/kg)	0.91	0.83	0.68	0.48	0.17	0.07	≤0.02	≤0.02
SPME after hydrolysis: Thymol (mg/kg)[a]	0.89	0.65	0.56	0.28	0.13	0.10	0.05	0.02
SPME after hydrolysis: Thymol (mg/kg)[b]	1.14	1.01	0.85	0.44	0.11	0.12	0.07	0.02

[a] Internal standard: benzophenone.
[b] Internal standard: carvacrol.

method, especially in the samples with the highest thymol concentrations.

The poor efficacy of hydrolysis at the lowest concentrations could also be due to a problem linked to the kinetics of ester formation/ it is probable that higher thymol concentrations favour the formation of esters.

These results demonstrate that SPME coupled with hydrolysis is a promising method for a rapid and economic evaluation of thymol in honey. The proposed method ensures good quantification of the thymol in honey at the concentrations of interest and is faster and less laborious than the SPE method currently used.

ACKNOWLEDGEMENTS

This work was carried out within the framework of the project "A.M.A." with financial support of Italian Ministry of Agriculture, paper No. 144.

Résumé – Application de la microextraction en phase solide à l'évaluation des traces de thymol dans le miel. Le thymol est un composé naturel du miel de tilleul mais peut être aussi trouvé dans d'autres types de miel en tant que résidu des traitements contre l'acarien *Varroa destructor*. Les niveaux de thymol trouvés dans le miel sont généralement faibles et dans les limites des normes de sécurité alimentaire (pour la santé). Néanmoins, si le thymol n'est pas utilisé correctement, il peut atteindre des niveaux susceptibles d'altérer le goût du miel (Bogdanov et al., 1999). Les acaricides à base de thymol sont largement utilisés en Italie dans la lutte contre *V. destructor*. C'est pourquoi l'évaluation de la teneur en thymol est importante pour le contrôle des produits alimentaires. En Suisse il a été établi une limite de 0,8 mg/kg, mais au sein de l'Union Européenne, selon le règlement EC Reg. 1742/96, aucune limite n'a été fixée. La mise au point d'une méthode pour déterminer la teneur en thymol des miels constitue un champ d'application intéressant de la chimie analytique. Comme le thymol est une substance assez volatile, il peut être déterminé en analysant « l'head space » du miel, espace situé au-dessus de la surface du miel et qui renferme la plupart des composés volatils. Ces dernières années un certain nombre de méthodes analytiques dédiées à l'head space ont été mises au point, dont la microextraction en phase solide (SPME). La SPME est une méthode assez simple qui utilise une fibre optique enduite d'un polymère pour absorber des molécules volatiles. Une fois les molécules absorbées, elles peuvent être désorbées dans un injecteur de chromatographie en phase gazeuse simplement grâce à la température. La méthode mise au point permet de quantifier le thymol en une heure environ. Dans cette étude, la SPME couplée à l'hydrolyse a été utilisée pour évaluer la teneur en thymol de huit miels. Un calibrage avait d'abord été fait en ajoutant une quantité connue de thymol à un échantillon de miel. La répétabilité des résultats est tout à fait satisfaisante (moins de 10 % de différence entre deux mesures) et la méthode présente une bonne sensibilité (moins d'un mg/kg, Tab. VI).

miel / thymol / microextraction en phase solide / chromatographie phase gazeuse / résidu / *Varroa destructor*

Zusammenfassung – Anwendung der Festphasen-Mikroextraktion zur Bestimmung von geringen Thymolrückständen im Honig. Thymol ist ein natürlicher Bestandteil im Honig von Lindenbäumen, aber es kommt auch in anderen Honigen als Rückstand von Behandlungen der Varroosis vor. Die Thymolrückstände sind im allgemeinen niedrig und unterhalb der Grenzen der zugelassenen Höchstmenge. Bei unsachgemäßer Thymolbehandlung können die Rückstände jedoch eine Konzentration erreichen, die zu einem veränderten Geschmack des

Honigs führt (Bogdanov et al., 1999). Dies entspricht nicht den Vorschriften über Eigenschaften des Honigs. Akarizide auf der Basis von Thymol werden in Italien häufig zur Varroosis Therapie benutzt. Deshalb ist die Bestimmung des Thymolgehalts für die Lebensmittelkontrolle wichtig. In der Schweiz wurde ein Grenze von 0,8 mg/kg festgesetzt. In der EU dagegen wurden nach EC Reg.1742/96 keine Grenzwerte bestimmt. Die Entwicklung eines Testes für Thymol ist ein interessantes Gebiet für die analytische Chemie: da es sich bei Thymol um eine recht flüchtige Substanz handelt, kann sie im "head space" des Honigs bestimmt werden. Unter "head space" versteht man den Raum dicht über der Oberfläche des Honigs, in dem fast alle flüchtigen Substanzen vorhanden sind. Der "head space" wird auch untersucht, um die botanische Herkunft des Honigs zu bestimmen. In den letzten Jahren sind verschiedene Methoden zur Analyse des "head space" entwickelt worden, darunter auch die Festphasen Mikorextraktion (SPME). SPME ist ein recht einfaches Verfahren, das auf der Absorption der flüchtige Moleküle einer mit einem Polymer beschichtete Glasfaser beruht. Sind die Moleküle absorbiert, können sie durch einfache Erwärmung in einen Gaschromatographen übertragen werden. Die hier beschriebene Methode, bei der SPME gekoppelt mit einer Hydrolyse zur Thymolbestimmung benutzt wird, quantifiziert das Thymol innerhalb einer Stunde. In dieser Untersuchung wurde der Thymolgehalt von 8 Honigen bestimmt. Für die Eichung war zuvor eine definierte Menge Thymol der Honigprobe beigefügt worden. Die Ergebnisse zeigten eine zufriedenstellende Wiederholbarkeit (weniger als 10% Unterschied zwischen 2 Messungen, Tab. V) und eine ausreichende Empfindlichkeit (weniger als 1 mg/kg, Tab. VI).

Honig / Festphasen-Mikroextraktion / Thymol / *Varroa destructor* / Gaschromatographie

REFERENCES

Bogdanov S., Imdorf A., Kilchenmann V. (1998) Residues in wax and honey after Apilife VAR treatment, Apidologie 29**,** 513–524.

Bogdanov S., Imdorf A., Kilchenmann V., Fluri P. (1999) Telaini al timolo Frakno per la lotta contro la *Varroa jacobsoni*, L'Ape 82, 6–13.

Arthur A.C., Pawliszyn J. (1990) Solid Phase Microextraction with thermal desorption using fused silica optical fibers, Anal. Chem. 62**,** 2145–2148.

Penton Z. (1996) Sample preparation for gas chromatography with solid phase extraction and microextraction, Adv. Chromatogr. 37, 205–236.

Zhang Z., Pawliszyn J. (1993) Headspace solid-phase microextraction, Anal. Chem. 65, 1843–1852.

Zhang Z., Pawliszyn J. (1993) Analysis of organic compounds in environmental samples by headspace solid phase microextraction, J. High Res. Chromatogr. 16, 689–692.