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Gas chromatographic determination of acrinathrine and 3-phenoxybenzaldehyde residues in honey

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Abstract

A procedure involving an extraction step and further gas chromatographic analysis with flame ionization detection to determine residues of acrinathrine and its main metabolite, 3-phenoxybenzaldehyde, in honey is proposed. Residues can be isolated from the matrix by means of liquid–liquid extraction with a mixture of benzene–isopropanol, by solid-phase extraction with octadecylsilane cartridges or Florisil packed columns, the latter method giving higher recoveries. Assays on spiked honey samples are carried out to test the procedures that are afterwards applied to honey samples from treated beehives.

Keywords: Honey; Food analysis; Extraction methods; Acrinathrine; Phenoxybenzaldehyde; Pesticides

1. Introduction

To protect honeybees from the mite *Varroa Jacobsoni Oud* apiarists usually apply the widespread acaricide, fluvalinate; however it is nowadays known that the mite has become increasingly resistant to this product, because of this the efficiency of alternative compounds is being tested. Among the proposed products another pyrethroid, acrinathrine, seems to be quite useful, so it is necessary not only to prove its effectiveness but also to know if any treatment residues can appear in the honey. As acrinathrine is quite a new product, there are only a few publications related to its determination, usually carried out by high-performance liquid chromatography [1,2]. We included the determination of its residues in honey in two multiresidue methods involving a solid-phase microextraction [3] and a solid-phase extraction on Florisil packed columns [4]. Acrinathrine is quickly degraded in honey [5], appearing firstly as its main metabolite, 3-phenoxybenzaldehyde (PBA), because of that and based on our previous experience, in this work we have tried to adapt a method that allows simultaneous determination of both compounds. So, extraction procedures for the analysis of acrinathrine and PBA by gas chromatography with flame ionization detection (GC–FID) are studied. For this purpose, three extraction procedures have been assayed: a solvent extraction with benzene–isopropanol, useful to extract a similar pyrethroid – fluvalinate – [6] and a solid-phase extraction with the above mentioned Florisil columns or using octadecylsilane (ODS) cartridges. The extraction procedures are tested on

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laboratory-spiked honeys and then applied to raw-honey samples collected from beehives treated with the commercial product.

2. Experimental

2.1. Reagents and apparatus

Residue analysis-grade methanol, benzene, *n*-hexane, dichloromethane, isopropanol and acetone were supplied by Scharlau (Barcelona, Spain) and Lab-Scan (Dublin, Ireland). Acrinathrine and PBA reference standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and the commercial product Rufast (15% acrinathrine) from AgrEvo (Hanxton, Cambridge, UK). Stock solutions were prepared in acetone (1000 mg/l). Ultrapure water was obtained from a Milli-Q apparatus (Millipore, Milford, MA, USA).

ODS Sep-Pak cartridges of 500 mg were purchased from Waters (Milford, MA, USA) and Florisil of 60–100 mesh was obtained from Baker (Deventer, The Netherlands). Centrifuges and shakers were supplied by Selecta (Barcelona, Spain). A rotary evaporator equipped with a thermostated water bath and a vacuum system was supplied by Büchi (Plawil, Switzerland). Disposable PTFE syringe filter units, 0.50 μm pore size, were purchased from Microfiltration Systems (Dublin, CA, USA).

2.2. Gas chromatography

A Hewlett-Packard (Avondale, PA, USA) Model 5890 Series II gas chromatograph equipped with an HP7673 autosampler, a flame ionization detector, and a 25 m×0.25 mm capillary column coated with a 0.25 μm thick film of phenyl–methylpolysiloxane (Sugelabor, Madrid, Spain) was used. The oven temperature programme was as follows: initial temperature 50°C, held for 1 min, a 8°C/min ramp to 100°C, and finally a 4°C/min ramp to 275°C, held for 15 min. The carrier gas (He) flow-rate was constant during the run-time (0.7 ml/min, measured at 50°C). Splitless injection (4 μl) was carried out at 225°C, the purge valve was on at 1 min. Hydrogen (30 ml/min), air (300 ml/min) and helium (25 ml/min) were used as auxiliary gases for the flame ionization detector. The detector temperature was 300°C.

2.3. Sample preparation

Raw honey that usually contains extraneous matter was first stirred at room temperature and passed through a 0.5 mm glass plate. Crystallized honey was directly passed through the plate. In both cases it was very helpful to press the sample with a spatula.

Afterwards, a 50 g amount of honey was heated at 35°C for 15 min and spiked with 0.5 ml of an acetone solution containing the standards. The mixture was vigorously shaken to achieve a good homogenization and stored at 4°C in darkness prior to analysis. Each spiked sample was used for a maximum of 7 days.

2.4. Solvent extraction

Honey (1 g) was mixed with 5 ml of water and extracted with 25 ml of a benzene–isopropanol (1:1, v/v) mixture, by mechanical shaking for 20 min. The organic layer was separated by centrifugation at 5000 *g* for 15 min and collected. The aqueous phase was extracted twice more, according to the above-mentioned procedure; then, the three organic portions were combined and evaporated to dryness in a rotary evaporator at 30°C under a gentle vacuum. Finally, the residue was dissolved in 2 ml of acetone and filtered through a 0.50-μm pore-size PTFE filter.

2.5. Solid-phase extraction on ODS cartridges

A 1-g amount of honey was mixed with 30 ml of water and the mixture was homogenized by mechanical shaking for 5 min. Simultaneously, ODS cartridges were conditioned by successive elution of 10 ml of methanol and 10 ml of water, using a gentle suction with the aid of a pump. Then, the 30-ml sample was eluted at a rate of about 5 ml/min and the cartridge dried with nitrogen for about 45 min. Finally, the cartridge was eluted by gravity with 2 ml of acetone after a soaking time of 4 min.

2.6. Solid-phase extraction on Florisil packed columns

The extraction procedure, and the preparation of the packed columns, are described in Ref. [4]. Briefly: 1 g of honey was mixed with 2 ml of methanol and the mixture was poured onto the Florisil column. The column was eluted with 30 ml of hexane–dichloromethane (1:1), and the eluate was evaporated in a rotary evaporator at 30°C. The residue was dissolved in 2 ml of acetone for GC analysis.

A matrix-standard calibration must be performed in combination with this sample preparation to quantify the target analytes in the extracts correctly.

3. Results and discussion

3.1. Analysis procedures

In the GC analysis when the acrinathrine standard is injected two chromatographic peaks belonging to two isomers in very different proportion always appear; we have used the predominant isomer peak to carry out the quantitative study. Table 1 shows the recovery and precision achieved in the analysis of honey samples spiked with different acrinathrine and PBA amounts. As can be observed recoveries of solvent extraction were higher than 90% for the three concentrations assayed, 0.1, 1 and 10 mg/kg. The recovery slightly decreases when the concentration increases. The relative standard deviation (RSD) varies between 4.5 and 5.9% ($n=5$), obtaining the worst precision for the lowest concentration, 0.1 mg/kg.

As regards the solid-phase extraction on ODS cartridges, the recovery of acrinathrine is always very low, lower than 25%, especially for the highest concentrations. The recovery of PBA is also low, 58% for 0.1 mg/kg, decreasing gradually when the concentration increases. The low recovery of pyrethroids in aqueous samples has been generally attributed to their low solubility in water, which

Table 1
Recovery and precision (RSD) obtained in the analysis of acrinathrine and 3-phenoxybenzaldehyde according to the extraction procedure used ($n=5$)

Concentration spiked (mg/kg)		Recovery (%)	Relative standard deviation (%)
Solvent extraction			
0.1	Acrinathrine	97	5.7
0.1	PBA	95	5.9
1	Acrinathrine	93	4.5
1	PBA	93	4.9
10	Acrinathrine	92	4.0
10	PBA	91	4.5
Solid-phase extraction on ODS cartridge			
0.1	Acrinathrine	24	4.5
0.1	PBA	58	6.0
1	Acrinathrine	13	5.2
1	PBA	42	6.4
10	Acrinathrine	12	7.1
10	PBA	29	8.0
Solid-phase extraction on Florisil packed column			
0.1	Acrinathrine	99	3.6
0.1	PBA	101	3.5
1	Acrinathrine	99	3.4
1	PBA	100	3.3
10	Acrinathrine	99	3.4
10	PBA	100	3.2

makes the isolation of the analytes from the solid matrix more difficult.

The recoveries obtained with solid-phase extraction on Florisil are close to 100%, favored by the application of a matrix-standard calibration to avoid the quantitation errors arising from the honey matrix [4]. The precision obtained by this procedure is the best in comparison to the previous one, ranging from 3.2 to 3.6% for the three concentrations assayed.

Calibration graphs were obtained with concentrations in the 0.025–10 mg/l range. The coefficient of correlation (r^2) of the linear fitting made with standards solved in acetone was always higher than 0.9990, for the two compounds, while for the matrix-standard calibration was, at least, 0.990.

The detection limits were estimated by spiking the extracts from acaricide-free honey. Only the extraction by organic solvents and by Florisil were considered owing to their good recoveries. No differences between the two extraction procedures were observed. Considering a signal-to-noise ratio of 5, the detection limit was 0.001 mg/kg for acrinathrine and about 0.01 mg/kg for PBA. These concentrations were perfectly detected in the five experiments made with different honeys. The chromatogram of an extract from a honey sample spiked with 0.005 mg/kg of achrinathrine, cleaned-up on a Florisil column, is shown in Fig. 1.

Fig. 2 shows the chromatograms obtained after the sample preparation. The extraction on Florisil and

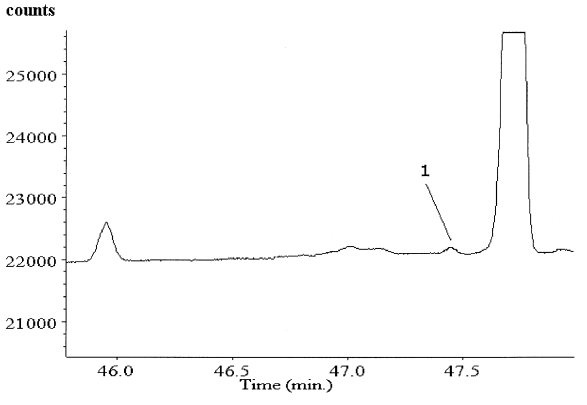

Fig. 1. Chromatogram obtained from a honey sample spiked with 0.005 mg/kg of acrinathrine, after applying solid-phase extraction on Florisil.

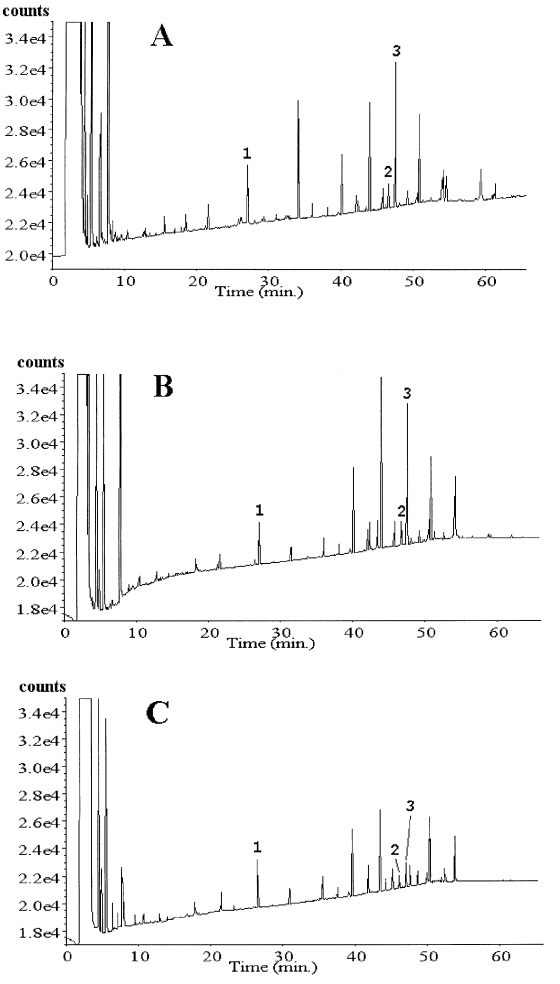

Fig. 2. Chromatograms obtained after applying the extraction procedures to a spiked honey sample: (a) solvent extraction; (b) solid-phase extraction on Florisil; (c) solid-phase extraction on ODS. 1: PBA, 2: minor isomer, 3: predominant isomer.

ODS provided the simpler chromatograms in which the acrinathrine and PBA peaks are well resolved and free from interferences. The chromatograms from the solvent extraction were characterized by a high front and higher number of peaks.

In consequence both procedures (solvent extraction and Florisil columns) compounds can be used to isolate the compounds. From a practical point of view we recommended solid-phase extraction because it gives the cleanest chromatograms, saves time and also avoids the use of benzene.

Table 2
Acrinathrine and PBA concentrations (in mg/kg) found in honey from 10 treated beehives

Honey	Acrinathrine	PBA
1	<LOD	1.3
2	<LOD	0.9
3	<LOD	1.1
4	<LOD	0.7
5	<LOD	2.4
6	<LOD	2.1
7	<LOD	0.5
8	<LOD	1.0
9	<LOD	1.1
10	<LOD	0.8

<LOD: Below detection limit.

3.2. Application to real samples

Beehives infected with the mite were treated with the commercial formulation Rufast. Honey samples were taken and submitted to the procedure based on the use of Florisil columns.

For this purpose, two small boards were immersed in a 1:19 (v/v) solution of Rufast in water for 24 h. After that, they were placed into each beehive for 6 weeks. The honey yielded during the 6 weeks was collected and analyzed.

The results and a chromatogram are shown in Table 2 and Fig. 3. As can be seen acrinathrine does not appear in quantities over the detection limit while PBA is detected in a range of concentrations that vary between 0.5 and 2.4 mg/kg.

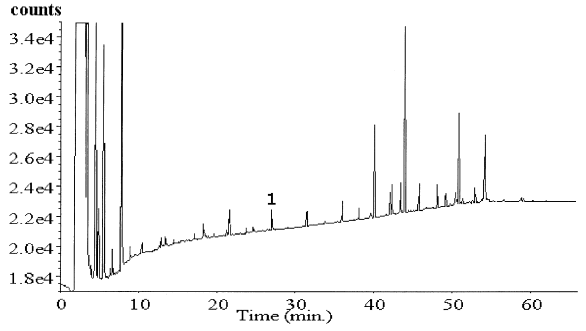

Fig. 3. Chromatogram of a raw-honey sample from treated beehives. Clean-up on Florisil. 1: PBA.

4. Conclusions

Solid-phase extraction on Florisil packed columns and the extraction with a benzene–isopropanol (1:1) mixture make possible the reliable and precise determination of acrinathrine, and its degradation product, PBA, in honey by GC–FID. Nevertheless, it is more advisable to use of the solid-phase extraction procedure because the chromatograms obtained are cleaner and the time of the overall procedure is shortened; furthermore the use of benzene is avoided.

A detection limit of about 0.01 mg/kg, is obtained for the degradation product of the acrinathrine, PBA.

The probability of finding those compounds in honey from correctly treated beehives is very low, and in that situation it is much better to consider the degradation product.

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