J Sci Food Agric 1998, **77**, 149–152

A Rapid and Low Cost Monitoring Method for Fluvalinate Determination in Honey

Stefan M Waliszewski,[1]* Violeta T Pardio,[1] Krzysztof N Waliszewski,[2] Angelica Ochoa[2] and Rosa M Infanzon[1]

[1] Pesticide Research Laboratory, Institute of Forensic Medicine, Veracruz University, Juan Pablo II esq. Reyes Heroles s/n, 91950 Veracruz, Veracruz, PO Box 1380, Mexico
[2] Instituto Tecnológico de Veracruz, Departamento de Ingeniería Química y Bioquímica, Avenida Cirunvalación 2777, 91870 Veracruz, Veracruz, PO Box 1420, Mexico

(Received 14 April 1997; revised version received 23 June 1997; accepted 23 September 1997)

Abstract: A simple analytical method for determining Fluvalinate residues in honey is described. Analyses were carried out by gas chromatography–electron capture detector (ECD), using a borosilicate glass column packed with 3% SP-2100. Fluvalinate residues were extracted from honey samples with *n*-hexane and acetic acid. Mean recoveries ranged from 98·1 ± 6·9 to 101·9 ± 7·6% with SD < 10 after standard addition of 20, 50 and 500 µg. No interferences of other pesticides were detected. ECD responses were linear within the range studied of 10–50 pg of Fluvalinate with a coefficient of determination 0·994, and a detection limit of 3 mg kg^{-1} was established. The use of a packed column allowed the exclusion of an expensive clean-up step. This fast, low-cost analytical method is adequate for monitoring studies of honey samples. © 1998 SCI.

J Sci Food Agric **77**, 149–152 (1998)

Key words: fluvalinate; residues; honey

INTRODUCTION

In 1995, honey bee production exported from Mexico reached 27 685 tons, representing the second largest exporter worldwide (INEGI 1995). However, since 1992 the widespread diffusion of the ectoparasitic mite *Varroa jacobsoni* has caused serious weakening of bee colonies, resulting in large honey production losses and a decrease in exports from 37% in 1977 to 13·2% in 1993 (Rodríguez and Moro 1982; Perea 1994; INEGI 1995).

A large number of chemical substances have been used to prevent and control this ectoparasitic mite in honeybees. The most frequently used are Amitraz, Bromopropylate, Coumaphos, Cymiazole, Fluvalinate and Malathion (Fernández-Muiño *et al* 1995).

Fluvalinate is currently one of the most widely used acaricides. This compound is a synthetic pyrethroid non-toxic and non-repellent to honeybees, easy to use and has relatively low mammalian oral toxicity with a LD_{50} of 3000 mg kg^{-1} for rats (Taccheo *et al* 1990; Worthing and Hance 1991; Nakamura *et al* 1993). It has been recognised by the Mexican government as an approved acaricide for treatment of beehives. A tolerance level was set for Fluvalinate residues of 0·05 mg kg^{-1} in honey, according to US Environmental Protection Agency (Anon 1990; CICOPLAFEST 1994). The use of this acaricide to treat beehives implies a risk of direct contamination of honey, pollen and wax. Thus, several analytical methods for the gas chromatographic determination of Fluvalinate residues in honey with different extraction procedures and detection system have been developed (Fernández-Muiño *et al* 1995; García *et al* 1996).

The aim of this paper is to describe a simple, low-cost method for determining Fluvalinate residues in honey. The proposed method uses *n*-hexane and acetic acid to extract Fluvalinate residues. The purified extracts are analysed using a packed column GC with electron capture detection. This analytical technique could be

* To whom correspondence should be addressed.

 J Sci Food Agric 0022–5142/98/$17.50. Printed in Great Britain

applied for screening Fluvalinate residues during monitoring studies as a means of routine quality control of honey.

MATERIALS AND METHODS

Reagents

The analytical reagents—acetone, *n*-hexane and glacial acetic acid solvents and anhydrous sodium sulphate powder heated at 650°C for 16 h before use—were purchased from JT Baker, Mexico City, Mexico. Fluvalinate analytical reference standard, purity 93·7%, Batch No TDA-5094 was supplied by Zoecon Corporation (Palo Alto, CA, USA).

Gas chromatography

A VARIAN 3300 chromatograph (Palo Alto, CA, USA) equipped with a Ni-63 ECD and a borosilicate glass column, 100 cm × 2 mm id packed with 3% SP-2100 on Supelcoport 80/100 mesh (Supelco USA), and an integrator model VARIAN 4400 was used. The temperature conditions, were as follows: column 230°C, detector 300°C and injector port 250°C. The flow-rate for carrier gas (Nitrogen) was 25 ml min^{-1}. The direct injection volume was 1 µl.

Quality control

To determine method quality a recovery study was performed on 10 replicates of blank honey samples. These blanks were spiked to achieve three final concentrations of Fluvalinate: 20, 50 and 500 mg kg^{-1}. The fortification studies were performed considering the binding processes of pesticides occurring in biological matrices. Spiked samples were prepared as follows: 1 ml of Fluvalinate solution in hexane (20, 50 and 500 mg kg^{-1}) was added to a 100 ml beaker. The solvent was left to evaporate under ambient conditions. Then, 10 g of honey, free of Fluvalinate residues, was added and sonificated in order to ensure homogeneity and the adequate incorporation of Fluvalinate into the honey. Once spiked, the fortified samples were kept at 8°C, covered with aluminium foil, until the next day (approximately 16 h) for analysis. The results were analysed statistically using Minitab 10.1 for Macintosh.

Detection limit

The detection limit, defined as the concentration of Fluvalinate giving a signal five times the noise was determined using the integrator with ECD attenuation × 16 and range 10, by the analyses of 20 honey samples collected from treated hives which presented ranges of 3–7 µg kg^{-1} of Fluvalinate, and 20 honey samples non-treated with Fluvalinate.

Analytical procedure

A 10 g honey sample was weighed into a 200 ml beaker and diluted twice with 100 ml of distilled water and transferred to a separatory funnel of 500 ml. The diluted sample was extracted with a mixture of 50 ml of *n*-hexane plus 25 ml of glacial acetic acid. The content was shaken vigorously for approximately 30 s and left for 5–10 min to phase separation. The aqueous phase was transferred to another separatory funnel and the residues were extracted twice more with 50 ml of *n*-hexane each time. The combined hexane extracts were left for 10 min in order to improve the phase separation. Then, the hexane extract was transferred to an Erlenmeyer flask and the emulsion was broken down by adding sodium sulphate. The hexane extract was dried by filtration through an anhydrous sulphate layer and then rotary evaporated to dryness at 65°C water bath temperature. The dried residues were diluted with *n*-hexane and quantitatively transferred to a 10 ml calibrated tube and the final volume was adjusted to 10·0 ml.

RESULTS AND DISCUSSION

Fortification levels, mean recoveries, standard deviations and coefficients of variation of spiked honey samples are shown in Table 1. Mean recoveries ranged from 98·1 to 101·9% with standard deviations and coefficients of variation below 10%, indicating excellent repeatibility of the method. The mixture of two solvents, *n*-hexane and glacial acetic acid, increased the solvent polarity and decreased the co-extraction of interfering compounds. Moreover, acidifying the aqueous phase, the extraction of fluvalinate into the hexane phase is facilitated. No interferences of other pesticides, like organochlorines, were observed during the gas chromatographic analyses, since they were eluted more rapidly from the column with retention times below 1·5 min, compared with the retention time of 6·68 min for Fluvalinate. Figure 1 illustrates the gas chromatograms of a standard solution of Fluvalinate

TABLE 1
Fortification levels (mg kg^{-1}), mean recoveries (X), standard deviations (SD) and coefficients of variation (V%) in honey samples* fortified with Fluvalinate ($n = 10$)

Fortification levels	$X \pm SD$	V %
20	98·1 ± 6·9	7·2
50	98·9 ± 5·4	5·7
500	101·9 ± 7·6	8·0

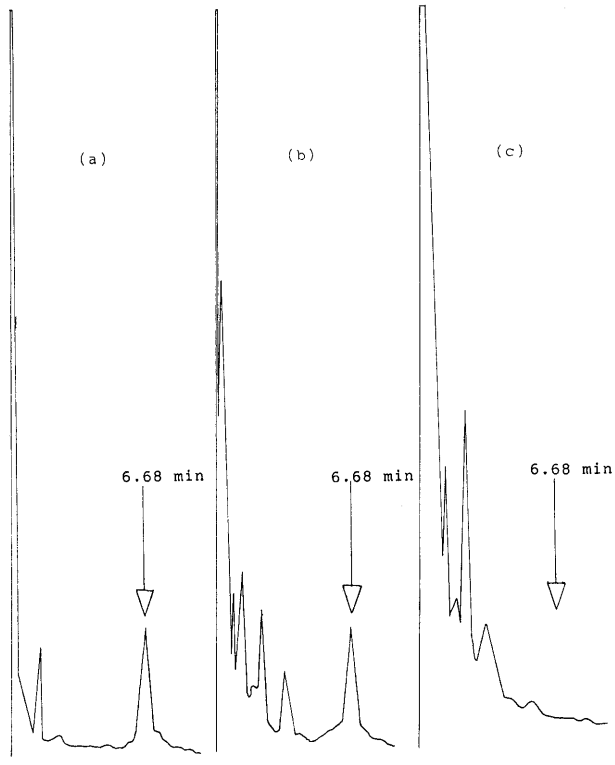

Fig 1. Gas chromatograms: (a) standard solution of Fluvalinate (50 mg kg^{-1}), (b) honey extract with 50 mg kg^{-1} of Fluvalinate and (c) honey sample without Fluvalinate.

(50 mg kg^{-1}), a honey sample with 50 mg kg^{-1} of Fluvalinate and a honey sample without Fluvalinate.

To evaluate the linear regression of ECD responses with respect to Fluvalinate, 1 ml aliquots of Fluvalinate solutions with 10, 20, 30, 40 and 50 pg were used. The detector responses to the sum of Fluvalinate isomers was linear for a range from 10 to 50 pg with a coefficient of determination 0·994.

The detection limit for this method was established at 3 mg kg^{-1} which is 10 times less than the Mexican tolerance level (CICOPLAFEST 1994). The detection limit and recoveries of this method are compared to other procedures in Table 2.

In this method a clean-up step was not necessary due to the use of a GC column packed with a non-polar phase which retained the interfering compounds from the sample. Moreover, this phase permitted the obtaining of only one peak of Fluvalinate without separating its tautomers.

CONCLUSIONS

Compared with other methods employed for pesticide residue determination in honey (Drescher and Fiedler 1983; Taccheo *et al* 1990) and multiresidue methods (Specht and Tillkes 1980; Nakamura *et al* 1993; Pang *et al* 1994; Tsumura *et al* 1994), this analytical method excludes the costly adsorbents used in the clean-up step, is less time consuming and is suitable to perform as a prior screening of Fluvalinate residues during monitoring studies of honey samples.

REFERENCES

Anon 1990 *Chemical News and Toxic Chemical News* 6.

Atienza J, Jiménez J J, Bernal J L, Martín M T 1993 Supercritical fluid extraction of Fluvalinate residues in honey. Determination by high performance liquid chromatography. *J Chromatogr A* **655** 95–99.

Balayannis P G, Santas L A 1992 Dissipation of malathion and Fluvalinate residues from honey. *J Apic Res* **31** (2) 70–76.

CICOPLAFEST 1994 *Catálogo Oficial de Plaguicidas.* Comisión Intersecretarial para el Control de Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Tóxicas, Mexico.

Drescher W, Fiedler L 1983 Methode zum Nachweis von Rückständen der Insektizide Acephat, Dimethoat, Methamidophos und Omethoat in kleinen Nektarmengen. *Chemosphere* **12** 11 1605–1610.

Fernández M A, Simal L J 1993 Gas chromatographic–mass spectrometric method for the simultaneous determination of

TABLE 2
Comparison of analytical methods for Fluvalinate determination in honey

Method[a]	*Recovery* (%)	*Detection limit* (*mg* kg^{-1})	*Reference*
GC-ECD	NM	8	Stricker *et al* (1989)
GC-ECD	85	10	Taccheo *et al* (1990)
GC-ECD	NM	9	Sancho *et al* (1992)
GC-ECD	90	1	Neri *et al* (1992)
GC-ECD	90	5	Balayannis and Santas (1992)
GC-MS	90	10	Fernández and Simal (1993)
HPLC	94	20	Atienza *et al* (1993)
GC-ECD	99	3	This method

[a] GC-ECD, gas chromatography-electron capture detector; GC-MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography.
[b] NM, not mentioned.

Amitraz, Bromopropylate, Coumaphos, Cymiazole and Fluvalinate residues in honey. *Analyst* **118** 1519–1522.

Fernández-Muiño M A, Sancho M T, Muniategui S, Huidobro F J, Simal L J 1995 Acaricide residues in honey: analytical methods and levels found. *J Food Prot* **58** (4) 449–454.

García M A, Fernández M I, Herrero C, Melgar M J 1996 Acaricide residue determination in honey. *Bull Environ Contam Toxicol* **56** 881–887.

INEGI 1995 *El Sector Alimentario en México*. Instituto Nacional de Estadística, Geografía e Informática, México.

Nakamura Y, Tonogai Y, Tsumura Y, Ito Y 1993 Determination of pyrethroid residues in vegetables, fruits, grains, beans and green tea leaves: Applications to pyrethroid residue monitoring studies. *J AOAC Int* **76** (6) 1348–1361.

Neri B, Ubaldi A, Barchi D, Di Llullo A, Czzani R 1992 Determination of fluvalinate residues in honey. *Ind Aliment* **31** (307) 748–750.

Pang G F, Fan Ch L, Chao Y Z 1994 Packed-column gas chromatographic method for the simultaneous determination of 10 pyrethroid insecticide residues in fruits, vegetables and grains. *J AOAC Int* **77** (3) 738–747.

Perea M R 1994 Factores que hacen competitiva a la miel mexicana en el mercado de exportación, con respecto a otras apiculturas internacionales. Tesis Ingeniería Química, UNAM, Mexico.

Rodríquez D S R, Moro M J 1992 Varroa found in México. *Apic Res* **132** 728–729.

Sancho M T, Muniategui S, Huidobro J F, Lozano J S (1992) Aging of honey. *J Agric Food Chem* **40** (1) 134–138.

Specht W, Tillkes M 1980 Gas-chromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie un Mini-Kieselgel-Säulen-Chromatographie. *Fresenius Z Anal Chem* **301** 300–307.

Stricker O, Gierschner K, Vorwohl G 1989 Gaschromatographische bestimmung von Brompropylat, 4,4′-Dibrobenzophenon, Coumaphos und Fluvalinat in Honing. *Deutsche Lebensmittel-Rundschau* **85** (3) 72–75.

Taccheo B M, De Paoli M, Valentino A 1990 Determination of tau-Fluvalinate residues in honey. *Pest Sci* **28** 197–202.

Tsumura Y, Wada Y, Fujiwara Y, Nakamura Y, Tonogai Y, Ito Y 1994 Simultaneous determination of 13 synthetic pyrethroids and their metabolite, 3-phenoxybenzoic acid, in tea by gas chromatography. *J Agric Food Chem* **42** (12) 2922–2925.

Worthing C R, Hance R V 1991 *The Pesticide Manual* (9th edn). British Crop Protection Council.