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Gas chromatography with electron-capture and nitrogen–phosphorus detection in the analysis of pesticides in honey after elution from a Florisil column
Influence of the honey matrix on the quantitative results

J.J. Jiménez*, J.L. Bernal, M[a].J. del Nozal, L. Toribio, M[a].T. Martín

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

Abstract

A modified procedure to extract pesticides from honey samples that involves loading the honey onto a Florisil packed column and subsequently eluting it with an *n*-hexane–dichloromethane mixture is proposed. Anomalous high gas chromatographic responses and subsequently very high recoveries for the pesticides in the extracts were obtained by a conventional calibration with pesticide solutions in organic solvent. This effect was attributed to the honey matrix and can be circumvented by using spiked honey extracts as calibration standards.

Keywords: Honey; Sample preparation; Matrix effects; Food analysis; Pesticides

1. Introduction

Pesticides in honey are usually extracted by treating the sample with an organic solvent [1–3], or in solid-phase by passage through octadecylsilane cartridges [4–6], after dilution of the honey sample with water. Then, the extract can be subjected to a clean-up step by an octadecylsilane or Florisil column, liquid–liquid partitioning or thin-layer chromatography [1,2,4,7,8]. For residues, the extract is commonly analyzed by gas chromatography (GC) or high-performance liquid chromatography (HPLC), according to the nature and number of the pesticides to be determined [9–13].

In the present work, a modified, fast and simple method, to analyze pesticides in honey is proposed. The procedure involves loading the honey sample on a Florisil packed-column, which is subsequently eluted with an *n*-hexane–dichloromethane (1:1, v/v) mixture, thus combining extraction and clean-up in a single step. The performance of the procedure was verified on unrefined multifloral honeys sampled directly from the combs.

For the analysis of pesticides, a GC procedure with electron-capture (ECD) and nitrogen–phosphorus detection (NPD) was used. Anomalous results were observed in the pesticide recovery analysis by conventional calibration, and ascribed to the occurrence of honey matrix residues in the extracts, proposing a solution for their correct quantitation.

2. Experimental

2.1. Reagents

Pesticide standards (99% minimum purity) were

*Corresponding author.

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obtained from Riedel-de Haën (Hannover, Germany) and Promochem (Wessel, Germany). Residue analysis grade methanol, dichloromethane, acetone and *n*-hexane were supplied by Lab-Scan (Dublin, Ireland). Florisil of 60–100 mesh was purchased from Baker (Deventer, Netherlands).

Pesticide stock solutions were made in acetone or *n*-hexane, according to their solubility. Mixtures of these pesticide solutions were used to carry out the assays; for this purpose, dilutions were made with acetone.

2.2. Preparation of spiked samples

Raw honey containing extraneous matter was first stirred at room temperature and passed through an 0.5-mm glass plate. Crystallized honey was gently pressed with a spatula through the plate. Then, 50 g of honey were heated at 35°C for 15 min and spiked with 0.5 ml of a solution in acetone containing the pesticides. The mixture was homogenized by vigorous shaking and stored at 4°C in darkness prior to analysis. Each spiked sample was used for a maximum of 7 days, after which it was discarded.

2.3. Preparation of Florisil packed-columns

Florisil was conditioned by heating at 120°C for 4 h before use. A glass column, 10 cm×1 cm I.D., was prepared from Florisil slurry in *n*-hexane–dichloromethane (1:1, v/v) and compacted with a rod. Care was taken to prevent the column from drying.

2.4. Extraction/clean-up procedure

Honey (1 g) was mixed with methanol (2 ml) and homogenized by shaking to reduce its viscosity and facilitate handling. Then, the 2 ml sample was poured onto the Florisil column, and percolated. The column was eluted by gravity with 30 ml of *n*-hexane–dichloromethane (1:1, v/v). The eluate was evaporated in a rotary evaporator from Büchs (Plawil, Switzerland) at 30°C and the residue dissolved in acetone (1 ml) for GC analysis.

2.5. GC–ECD/NPD analysis

A Hewlett-Packard (Avondale, PA, USA) 5890 gas chromatograph equipped with an HP7673 autosampler, two detectors (electron-capture and nitrogen–phosphorus) and a 60 m×0.25 mm capillary column coated with an 0.25 μm thick film of 50% phenylmethylpolysiloxane, named 007-17, from Quadrex Scientific (Surrey, UK) was used. The oven temperature programme was as follows: initial temperature 50°C, held for 1 min; 25°C/min ramp to 160°C; then an 1.2°C/min ramp to 260°C; and finally a 20°C/min ramp to 275°C, held for 60 min. The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. Splitless injection of a 2 μl volume was performed at 200°C and the purge valve was on at 1 min. Hydrogen, air and helium were used as auxiliary gases for NPD, and argon–methane (90:10, v/v) for ECD. Both detector temperatures were 300°C.

3. Results and discussion

3.1. Conventional calibration

Table 1 shows the recoveries obtained by a conventional external standard calibration on honeys spiked with different amounts of each pesticide studied. Recoveries were much higher than expected, which was attributed to the so-called 'matrix-effect' previously described in the GC analysis of other types of matrix [14–16]. Recoveries decreased and approached 100% as the pesticide concentration was raised from 0.025 to 2.5 mg/kg, indicating that the matrix-effect was more marked at low analyte concentrations. Recoveries of up to 1000% were even obtained for a concentration of 0.025 mg/kg.

3.2. Matrix–standard calibration

In order to determine reliably the pesticide concentration of unknown samples and avoid the quantitative errors arising from the matrix effect, an 1:1 matrix–standard calibration (mixture of honey extract and standard in solution, 1:1) was assayed. Table 2 shows the recoveries determined by an 1:1 matrix–standard calibration on solvent–honey ex-

Table 1
Recovery (%) of pesticides obtained by a conventional solvent-calibration, and a 1:1 matrix–standard calibration (in parentheses), on honeys spiked at different concentrations ($n=7$)

Pesticide	Concentration level (mg/kg)				
	0.025	0.125	0.25	1	2.5
Demeton-*S*-methyl	563	372	167	128	94
	(100)	(95)	(97)	(99)	(97)
Phorate	682	431	236	186	141
	(102)	(99)	(99)	(97)	(99)
α-Benzene hexachloride (HCH)	552	212	179	165	151
	(105)	(105)	(102)	(98)	(97)
Diazinon	759	279	244	191	163
	(134)	(125)	(123)	(110)	(97)
Lindane	391	197	151	138	164
	(100)	(102)	(100)	(97)	(96)
Heptachlor	596	377	289	203	182
	(98)	(98)	(99)	(98)	(97)
Vinclozolin	647	335	250	209	171
	(101)	(99)	(97)	(95)	(94)
δ-HCH	835	272	225	214	201
	(100)	(104)	(102)	(99)	(98)
Aldrin	246	170	165	161	150
	(99)	(99)	(96)	(95)	(97)
Chlorpyrifos	589	317	230	231	182
	(101)	(99)	(99)	(96)	(94)
Malathion	405	381	290	221	174
	(115)	(104)	(103)	(98)	(96)
Parathion	7397	631	419	359	269
	(130)	(109)	(104)	(95)	(90)
Dicofol	1256	981	413	404	207
	(105)	(100)	(99)	(98)	(95)
trans-Heptachlor	559	217	176	165	166
epoxide	(99)	(99)	(99)	(98)	(97)
Chlorfenvinphos E	487	292	182	118	97
	(104)	(99)	(99)	(99)	(95)
cis-Heptachlor	593	219	179	161	163
epoxide	(100)	(98)	(99)	(99)	(96)
Chlorfenvinphos Z	420	300	201	150	105
	(99)	(99)	(97)	(97)	(95)
2,4′-DDE	445	241	221	212	195
	(100)	(100)	(95)	(96)	(97)
Endosulfan A	230	191	193	187	172
	(98)	(99)	(98)	(96)	(95)
Quinalphos	421	279	183	115	100
	(110)	(101)	(100)	(98)	(92)
4,4′-DDE	740	276	231	222	209
	(100)	(102)	(99)	(99)	(99)
Captan	1028	329	165	99	99
	(109)	(105)	(103)	(94)	(94)
Folpet	2380	321	164	108	107
	(98)	(98)	(97)	(96)	(94)
2,4′-TDE	561	254	226	221	210
	(97)	(98)	(99)	(99)	(98)
Endrin	573	251	222	212	210
	(98)	(97)	(98)	(97)	(96)

(*Continued overleaf*)

Table 1. Continued

Pesticide	Concentration level (mg/kg)				
	0.025	0.125	0.25	1	2.5
2,4′-DDT	735	257	211	198	195
	(101)	(99)	(98)	(95)	(96)
4,4′-TDE	764	270	223	221	219
	(96)	(94)	(95)	(99)	(96)
Ethion	567	264	171	115	91
	(104)	(98)	(102)	(100)	(98)
Endosulfan B	420	220	205	198	180
	(100)	(100)	(98)	(99)	(97)
4,4′-DDT	913	269	205	190	198
	(98)	(99)	(98)	(96)	(96)
Acrinathrin	548	321	203	133	124
	(109)	(105)	(104)	(99)	(99)
Iprodione	948	405	350	259	174
	(112)	(106)	(102)	(97)	(92)
Methoxichlor	799	252	199	181	179
	(108)	(105)	(104)	(99)	(97)
Tetradifon	626	234	196	182	165
	(98)	(99)	(97)	(96)	(94)
Phosalone	482	312	183	120	116
	(102)	(98)	(99)	(95)	(93)
Fluvalinate 1	650	311	176	112	102
	(108)	(106)	(102)	(100)	(98)
Fluvalinate 2	604	202	167	122	110
	(105)	(107)	(101)	(99)	(98)

tract mixtures in various ratios, and always spiked with the same amount of each pesticide, 0.5 mg/kg. Not all the pesticides behave in the same way. However, in many cases a tendency to higher recoveries was observed for the mixtures with higher honey content. In fact, pure honey extracts (0:100 ratio) provided recoveries about 110–120% for some pesticides, particularly organophosphorus compounds such as phosalone, parathion, malathion, phorate and diazinon.

Table 1 also shows the results (in parentheses) obtained in the analysis of honey samples spiked with different pesticide concentrations after carrying out an 1:1 matrix–standard calibration. Though results vary for each pesticide, generally the recoveries are higher for decreasing concentrations in the sample. For instance, diazinon, parathion, captan and iprodione recoveries increased from 97% to 134%, 90% to 130%, 94% to 109% and 92 to 112%, respectively, on decreasing the spiked concentration from 2.5 to 0.025 mg/kg.

Based on the above results, the use of an 1:1 matrix–standard calibration is not effective enough to overcome the quantitative errors arising from the matrix effect. As an alternative, a calibration performed with honey samples spiked at different concentration levels and subjected to the proposed extraction/clean-up process was considered in this work. Using this calibration, samples spiked with concentrations from 0.125 to 2.5 mg/kg gave average recoveries in the range 97–102% for all the pesticides. Unlike the previous calibration procedure, the pesticide concentration did not influence the results. The relative standard deviation was about 3.4–6.4% ($n=7$) depending on the particular pesticide.

The main analytical characteristics of the procedure that involves the use of spiked honey extracts as calibration standards are shown in Table 3. The detection limits were calculated on the basis of a signal-to-noise ratio of 3 by spiking at low concentrations the honey samples and subjecting them to the sample preparation.

This procedure decreases the number of vials to be

Table 2
Recovery of pesticides obtained by a matrix–standard (1:1) calibration on solvent–matrix mixtures in different proportion and containing 0.5 mg/kg of each one ($n=7$)

Pesticide	Recovery (%) at different solvent–honey ratios			
	0:100	25:75	50:50	75:25
Demeton-*S*-methyl	120	103	102	99
Phorate	110	100	102	97
α-HCH	107	101	100	95
Diazinon	132	100	98	99
Lindane	103	98	98	96
Heptachlor	103	100	97	97
Vinclozolin	110	98	99	99
δ-HCH	99	97	97	96
Aldrin	99	100	98	97
Chlorpyrifos	115	100	100	94
Malathion	130	102	99	100
Parathion	125	100	100	96
Dicofol	105	98	99	99
trans-Heptachlor epoxide	103	99	98	95
Chlorfenvinphos E	101	99	98	97
cis-Heptachlor epoxide	98	98	97	96
Chlorfenvinphos Z	99	98	98	97
2,4′-DDE	102	103	98	96
Endosulfan A	104	97	93	94
Quinalphos	106	103	101	99
4,4′-DDE	104	104	101	97
Captan	117	103	101	100
Folpet	111	100	99	97
2,4′-TDE	99	100	98	95
Endrin	99	98	97	97
2,4′-DDT	98	97	96	94
4,4′-TDE	96	95	95	96
Ethion	119	99	99	100
Endosulfan B	101	98	98	97
4,4′-DDT	97	98	97	96
Acrinathrin	103	101	99	97
Iprodione	100	99	97	94
Methoxichlor	106	105	98	99
Tetradifon	100	101	99	97
Phosalone	112	100	104	99
Fluvalinate 1	103	99	102	99
Fluvalinate 2	105	99	101	100

injected in the GC equipment in comparison with a standard addition method, which requires the addition of increasing amounts of pesticides to each extract to avoid the matrix-effect.

Fig. 1 shows the chromatograms for the honey extracts as obtained by GC–ECD and GC–NPD. Simple chromatograms with good baselines were achieved in both cases. The presence of chromatographic peaks from the honey matrix that might introduce errors in the qualitative or quantitative analyses was minimum.

3.3. Comparison with other analysis procedures

Solid-phase extraction (SPE) on Florisil packed columns makes the isolation of pesticides from the honey matrix easier and cheaper than the commonly used procedures. Furthermore, it is not necessary to

Table 3
Retention time, detection limit and linearity for the calibration standards treated as the samples ($n=7$)

	Pesticide	Retention time (min)	Detection	Detection limit (μg/kg)	Linear dynamic range (mg/l)	Correlation coefficient
1	Demeton-*S*-methyl	36.75	NPD	5	0.15–2.0	0.991
2	Phorate	37.92	NPD	1	0.15–2.0	0.992
3	α-HCH	39.58	ECD	0.05	0.1–1.3	0.992
4	Diazinon	43.42	NPD	1	0.15–1.8	0.997
5	Lindane	46.01	ECD	0.05	0.1–1.3	0.995
6	Heptachlor	49.84	ECD	0.025	0.1–1.3	0.994
7	Vinclozolin	51.41	ECD	0.25	0.1–1.6	0.995
8	δ-HCH	53.71	ECD	0.05	0.1–1.3	0.990
9	Aldrin	54.67	ECD	0.025	0.1–1.5	0.994
10	Chlorpyrifos	60.31	NPD	0.5	0.15–2.0	0.998
11	Malathion	61.33	NPD	0.5	0.15–2.0	0.992
12	Parathion	62.28	NPD	1	0.15–2.0	0.992
13	Dicofol	63.04	ECD	1	0.2–1.8	0.991
14	*trans*-Heptachlor epoxide	64.25	ECD	0.025	0.1–1.2	0.990
15	Chlorfenvinphos E	65.23	NPD	1	0.15–2.0	0.994
16	*cis*-Heptachlor epoxide	65.89	ECD	0.025	0.1–1.2	0.994
17	Chlorfenvinphos Z	68.78	NPD	1	0.15–2.0	0.991
18	2,4′-DDE	69.73	ECD	0.075	0.1–1.3	0.995
19	Endosulfan A	70.02	ECD	0.075	0.1–1.6	0.995
20	Quinalphos	70.82	NPD	1	0.15–2.0	0.995
21	4,4′-DDE	73.79	ECD	0.075	0.1–1.3	0.994
22	Captan	75.87	ECD	50	0.2–1.0	0.985
23	Folpet	77.23	ECD	25	0.2–1.0	0.981
24	2-4′-TDE	78.14	ECD	0.05	0.1–1.3	0.990
25	Endrin	80.94	ECD	0.025	0.1–1.2	0.994
26	2,4′-DDT	82.58	ECD	0.075	0.1–1.3	0.997
27	4,4′-TDE	83.05	ECD	0.05	0.1–1.3	0.995
28	Ethion	85.01	NPD	1	0.15–2.0	0.994
29	Endosulfan B	85.09	ECD	0.2	0.1–1.5	0.991
30	4,4′-DDT	87.96	ECD	0.1	0.1–1.3	0.993
31	Acrinathrin	94.81	ECD	25	0.1–1.5	0.990
32	Iprodione	95.19	NPD	75	0.25–1.7	0.995
33	Methoxichlor	100.65	ECD	0.1	0.1–1.2	0.992
34	Tetradifon	104.61	ECD	0.01	0.1–1.2	0.994
35	Phosalone	106.09	NPD	0.5	0.15–2.0	0.997
36	Fluvalinate 1	132.80	ECD	25	0.1–1.5	0.990
37	Fluvalinate 2	134.57	ECD	25	0.1–1.5	0.990

correct the concentration results according to the recoveries supplied by the method, this is possible because the calibrations performed consider the influence of the matrix.

The chromatograms from the extracts obtained by Florisil-SPE are very simple in comparison with those obtained by other procedures [1,4], mainly if raw honey is analyzed. This is important for a multiresidue analysis of pesticides at trace levels. The detection limits were of the order of micrograms per kilogram, which are similar or lower than those achieved by the usual procedures. The linearity of the calibration graphs is also comparable: the coefficients of correlation are 0.990 or better [1,4,7].

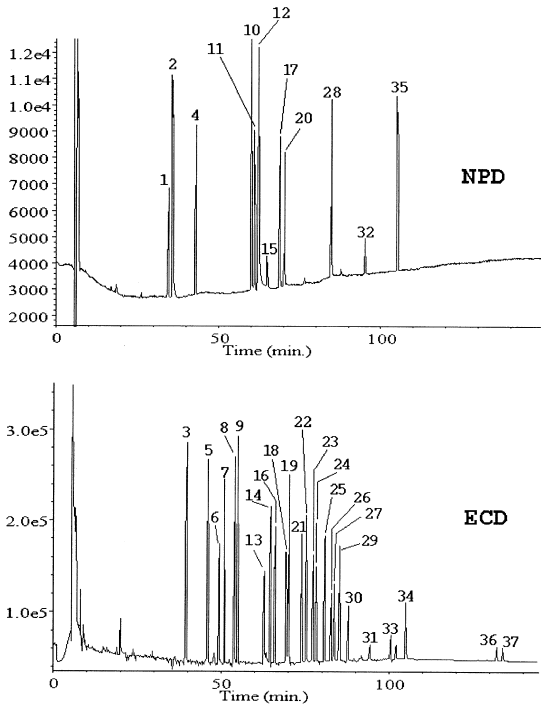

Fig. 1. Chromatograms of honey-extracts obtained by GC–NPD (top) and GC–ECD (bottom). See Table 3 for identification of peaks.

4. Conclusions

The simultaneous extraction/clean-up procedure proposed for the determination of pesticide residues in raw honey using a Florisil packed column is a quick, reproducible and reliable alternative to the normally used methods, moreover it provides simple chromatograms by ECD and NPD.

A matrix–standard calibration is required for the quantitative analysis of the pesticides in honey extracts to reduce the influence of the matrix on the concentrations calculated, particularly for low concentrations. For this purpose, the use of calibration standards consisting of spiked honey extracts is preferred.

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