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Confirmation method for the identification and determination of some organophosphorus and organochlorine pesticides in cocoa beans by gas chromatography–mass spectrometry

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ABSTRACT

The main objective of this work is to develop a routine quality control method for pesticide residues in cocoa beans, using gas chromatography–mass spectrometry. The investigated pesticides, which are used to control pests in the growing of cacao, are: Acephate, Propoxur, HCH, Heptachlor, Fenitrothion, Pirimiphos-methyl, Aldrin, Dieldrin, pp'-DDE, op-DDE and DDT. Two extraction methods were tested. The first was based on strong attack by concentrated sulphuric acid and later extraction with *n*-hexane: the investigated residues were Acephate, HCH, Fenitrothion and DDT; recoveries were 68–95% and the detection limits 0.5–10 ppb. The second extraction method was based on the Universal Trace Residue Extractor (UNITREX), which consists of a distillation system for organophosphorus and organochlorine pesticides in fatty samples. The investigated residues were Heptachlor, Pirimiphos-methyl, Aldrin, Propoxur, Dieldrin, op-DDE and pp'-DDE; recoveries were 67–88% and the detection limits 1–10 ppb.

INTRODUCTION

Pesticide residues in cocoa beans can be determined by multiresidual analytical methods [1–5]. This paper describes two extraction methods for use in such methods. Both methods are suitable for cocoa bean samples with a high fat content (*ca.* 60%), and the fat-soluble characteristics of the pesticide residues cause no interference. The extraction mode is selected by the nature of the residues to be determinated.

Extracts are injected and chromatographied in a gas chromatographic (GC) system with double capillary columns, and electron–capture and nitrogen–phosphorus detectors. The results are confirmed by quadrupole mass spectrometry (MS) in electron-impact (EI) mode. Selected-ion monitoring (SIM) is used to obtain increased sensitivity and specificity [6]. The confirmation by MS is suggest-

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ed to be essential for the validation of the results, from electron-capture detection (ECD) and nitrogen–phosphorus detection (NPD).

EXPERIMENTAL

Chemicals

Standards of 99–100% purity were obtained from Riedel de Haën (Hannover, F.R.G.). Acetone *n*-hexane, diethyl ether and sulphuric acid (98%) were reagents for analysis from Carlo Erba (Milan, Italy). Anhydrous sodium sulphate was also from Carlo Erba. Magnesium silicate for chromatography (Florisil) was S.D.S. from Serva (Heidelberg, F.R.G.).

Apparatus

A Büchi R-111 evaporator (Switzerland) and a Universal Trace Residue Extractor (UNITREX) [Scientific Glass Engineering (SGE), Australia] were used.

GC–ECD/NPD equipment. The GC–ECD/NPD instrumentation was a Model 5890 A gas chromatograph with a single injector and double capillary columns (ultra performance capillary column, Hewlett-Packard, packed with cross-linked 5% phenylmethyl silicone; 25 m × 0.2 mm I.D. and equipped with an electron-capture detector and a nitrogen–phosphorus detector from Hewlett-Packard. The automatic sampler (Hewlett-Packard 7673 was supplied with a tray holding 100 samples. An HP Model 5895 data station was used.

This was a multidimensional system in parallel, with a split injector to which two capillary columns and two detectors were fitted. Working conditions were the same for all the pesticides studied [7]: carrier gas, helium; total flow-rate, 20 ml/min; gas linear speed for the column, 20 ml/s. For NPD: auxiliary gas, helium (30 ml/min), hydrogen (3.5 ml/min), air (95 ml/min). For ECD: auxiliary gas, helium (60 ml/min). The temperature was initially held for 5 min at 125°C, then increased at 2.5°C/min to 270°C, where it was held for 15 min. The injection volume was 2 μl.

GC–MS. The GC–MS system consisted of a Model 5890 A gas chromatograph with a single injector and splitless, double capillary columns (ultra performance capillary column, Hewlett-Packard, packed with cross-linked 5% phenylmethyl silicone; 25 m × 0.2 mm I.D. and equipped with a mass spectrometric detector (MSD) (quadrupole EI type) from Hewlett-Packard. The automatic sampler (Hewlett-Packard 7673 A) was supplied with a tray holding 100 samples. An HP model 59970 MS Chemstation data station was used.

Working conditions were: carrier gas, helium; total flow-rate 20 ml/min; column flow, 0.5–0.6 ml/min; MS auxiliary gas, helium at 30 ml/min.

The temperature programme and sample volume were the same as for ECD/NPD.

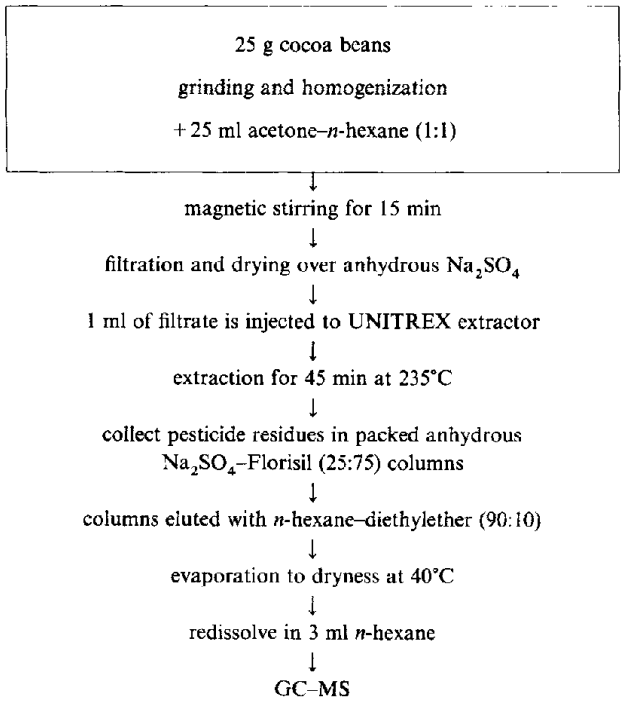

Fig. 1. UNITREX extraction.

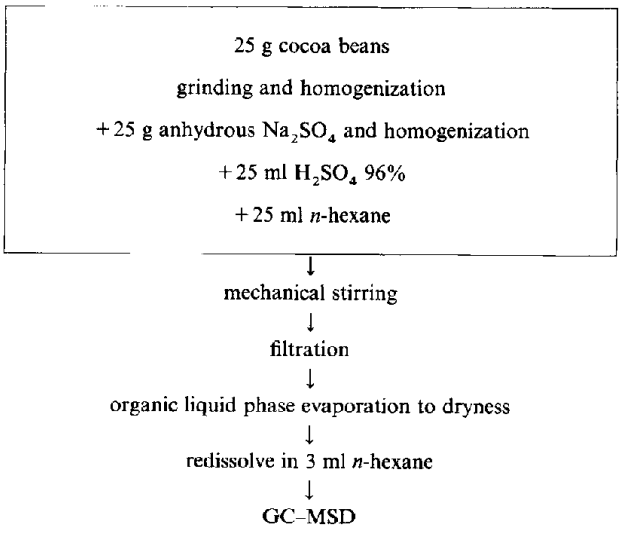

Fig. 2. Sulphuric acid extraction.

Extraction methods

We tested two extraction methods: UNITREX extraction [8,9] and sulphuric acid extraction [10].

UNITREX extraction (Fig. 1). UNITREX is a suitable system for extraction and concentration of pesticide residues in fatty samples. It consists of a cylindrical aluminium block with a heating system and pneumatic control of the carrier gas (nitrogen). This block can work simultaneously with ten extraction columns filled with small diameter glass pellets. The system is thermostated at 235°C. Distillation draws the pesticide residues to the end of the column, which is connected to a small column filled with anhydrous sodium sulphate–florisil (25:75) where residues are trapped. The final extracts are completely clear, which means that this extraction and clean-up process is suitable for cocoa bean samples.

The pesticide residues investigated by this process were: Heptachlor, Pirimiphos-methyl, Aldrin, Propoxur, Dieldrin, op-DDE and pp'-DDE.

Sulphuric acid extraction (Fig. 2). This method used concentrated sulphuric acid to break down organic matter, followed by extraction of pesticides with *n*-hexane.

It is suitable for the analysis of Accphate, HCH, Fenitrothion and DDT.

RESULTS AND DISCUSSION

GC–ECD/NPD

The extracts obtained by using either UNITREX or sulphuric acid extraction were chromatographed by GC–ECD/NPD. This equipment, with two capillary columns and both detectors in parallel, allows the simultaneous determination of halogenated and phosphorus- or nitrogen-containing compounds, and is very useful in multiresidue analysis: the high sensitivity gives limits of detection of less than 1 ppm. However, the chromatograms from cocoa bean samples reveal many peaks that force a confirmation of results by GC–MS.

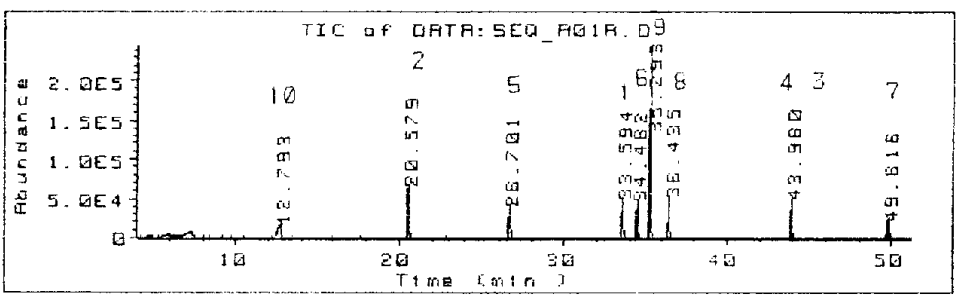

Fig. 3. Typical SCAN chromatogram from the standards. Peaks: 1 = Heptachlor; 2 = Propoxur; 3 = pp'-DDE; 4 = Dieldrin; 5 = HCH; 6 = Fenitrothion; 7 = DDT; 8 = Aldrin; 9 = Pirimiphos-methyl; 10 = Acephate.

TABLE I

IONS MONITORED IN THE SIM MODE

Compound	*m/z*
Acephate	43, 136, 94
HCH	181, 103, 109
Fenitrothion	125, 109, 79
DDT	235, 237, 165
Heptachlor	100, 272, 274
Pirimiphos-methyl	290, 276, 125
Aldrin	66, 91, 79
Propoxur	110, 152, 43
Dieldrin	79, 82, 81
op-DDE	176, 245, 317
pp'-DDE	246, 105, 318

GC–MSD

In order to confirm the ECD/NPD results, MS detection uses two different parameters: retention time and the mass spectrum of each peak in the chromatogram.

By using pesticide standards we found retention times and mass spectra of each pesticide in the SCAN mode (Fig. 3). Working conditions were checked in order to ensure that the chromatographic separation and three characteristic *m/z* values were selected. These three *m/z* values for each pesticide were used in the

TABLE II

DETECTION LIMITS

Pesticide	Detection limit (ppb)
UNITREX extraction	
Heptachlor	10
Pirimiphos-methyl	10
Aldrin	1
Propoxur	5
Dieldrin	5
op-DDE	5
pp'-DDE	5
H_2SO_4 extraction	
Acephate	10
HCH	0.5
Fenitrothion	1
DDT	1

SIM mode, and their values are listed in Table I. In order to identify a peak we checked its retention time and the presence of the three characteristic m/z ions, and confirm that their relative proportions were similar to the standard.

Detection limits and linearity were investigated for each pesticide. Both parameters are suitable for our purposes. Detection limits range between 0.1 and 10 ppb; individual values are listed in Table II. Correlation coefficients for linearity range between 0.98 and 0.99. The accuracies for individual pesticides are listed in Table III.

TABLE III

ACCURACY DATA FOR PESTICIDES IN COCOA BEANS

Compound	Spiked (ppm)	Found (ppm)	Coefficient of variation (%)
Acephate	1.5	1.2	6.4
	2.4	1.9	3.2
	5.6	4.4	2.1
HCH	1.3	1.4	7.2
	2.2	2.0	6.3
	5.4	5.1	3.1
Fenitrothion	1.6	1.1	8.1
	2.3	1.8	3.2
	5.3	4.2	2.4
DDT	1.4	1.1	7.2
	2.4	1.7	5.1
	5.4	3.8	2.0
Heptachlor	1.5	1.3	8.3
	2.4	2.1	4.2
	5.3	4.9	2.4
Pirimiphos-methyl	1.2	1.0	6.3
	2.4	1.6	3.1
	5.4	3.2	2.1
Aldrin	1.3	1.2	7.4
	2.5	1.9	3.6
	5.3	4.3	1.7
Propoxur	1.2	1.1	9.5
	2.4	1.8	7.2
	5.3	4.8	3.3
Dieldrin	1.5	1.1	9.1
	2.4	1.8	8.1
	5.6	4.2	4.3
op-DDE	1.5	1.2	10.5
	2.3	1.9	3.2
	5.3	4.0	7.4
pp'-DDE	1.3	0.9	8.3
	2.4	1.6	3.1
	5.6	3.8	9.7

CONCLUSIONS

Our work shows the suitability of GC–MS for the confirmation of GC–ECD/NPD results in the analysis of pesticide residues in cocoa beans. Detection limits are sufficient for the tested pesticide residues, in accordance with legislation. Correlation coefficients of the calibration graph are, in all cases, between 0.99 and 0.98. The present method is suggested for routine analysis when the pesticide residues are defined and concrete, and when a good chromatographic separation is obtained. With this method we avoid the false-positive and false-negative results that can be obtained using GC–ECD/NPD alone.

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