Diatomaceous earth-assisted extraction for the multiresidue determination of pesticides

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Abstract

An effective multiresidue diatomaceous earth-assisted extraction and gas chromatographic–electron capture detection and thermionic sensitive detection of over 90 pesticides belonging to several chemical families is described. The homogeneous vegetal sample pulp was mixed with diatomaceous earth to obtain a free-flowing powder which was extracted with ethyl acetate. Recoveries of several pesticides including apolar and polar ones were tested in 10 replicates on apple material and the analysis performed on two GLC systems, each one equipped with two columns of different polarities and two detectors. The recovery of pesticide residues belonging to different chemical classes on the matrix studied was satisfactory (72–116%) for the pesticides studied and no further clean-up was required for subsequent gas chromatographic analysis. Results are discussed according to the combination column–detector used.

Keywords: Diatomaceous earth; Extraction methods; Food analysis; Pesticides

1. Introduction

The control of pesticide residues in food both for regulatory and commercial purposes, involves large numbers of samples. Pesticide residue analyses are usually costly and time consuming. Moreover the remarkable number of pesticides available to protect crops from pests and weeds require reliable methods of extraction and adequate instrumental methodology. The instrumental analysis often requires an adequate clean-up of the extracts which may result in losses.

Multiresidue (MR) methods were firstly developed to improve the cost-effectiveness without sacrificing the reliability of results. MR methods were usually performed by an acetone extraction and a partition in

a solvent of different polarity (methylene chloride or light petroleum) [1] or by a solid-pahse extraction (SPE) as an alternative to the liquid–liquid partition, or by direct extractions with ethyl acetate [2]. Matrix solid-phase dispersion (MSPD) were chosen in order to reduce the requirements for solvent, SPE cartridges and time. Different matrixes and different solvents have been used in MSPD for the extraction: organophosphorous compounds were extracted with light petroleum from plant material mixed with silica gel with recoveries of 75% or above [3]; organochlorine compounds were extracted with light petroleum–methylene chloride (4:1) from some leguminosae and solanaceae families mixed with Florisil with recoveries above 80% [4]; pesticide residues belonging to different chemical families were extracted from fruit and vegetables mixed with Florisil and ethyl acetate or methylene chloride–

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acetone (9:1) [5] or mixed with diatomaceous earth and extracted with methylene chloride [6], showing good recoveries from different matrixes. In this study an attempt was made to extend the work of multiresidue extraction and develop methodology that would combine a single extraction of non-thermally labile pesticide of different chemical classes with the detection and confirmation by two gas chromatographs equipped with two columns and two detectors published elsewhere [7].

2. Experimental

2.1. Apparatus

Two Varian gas chromatographs were set up each with two columns of different polarities connected by a Y connection to a precolumn of de-activated silica which was in turn connected to the injector. Each column was connected to an electron-capture detection (ECD) system in one instrument and a thermionic sensitive detection (TSD) system in the other. Details on columns, injectors, detectors, equipments used, etc., are reported elsewhere [7]. Temperature program is as follows: column 60°C, hold 3 min, increase to 150°C at 20°C/min, no hold time, increase to 280°C at 3°C/min, hold time 5 min.

Statistical analyses were performed using the SAS software (SAS release 6.08) [8]. Analysis of variance (ANOVA) for unbalanced data was performed using as classification variables the combination column–detector with the recovery as the dependent variable. The analysis was carried out for each and every pesticide.

2.2. Solvents and reagents

Ethyl acetate Suprasolv (Merck), acetone DAB-BP (Merck), sodium sulphate anhydrous analytical-reagent grade ACS-ISO (Merck), were obtained from Bracco (Milan, Italy). Diatomaceous earth Hydromatrix was obtained from Varian (Leinì, Italy).

2.3. Reference material

Pesticide standards were certified with purities ranging from 92 to 99.9% (Ehrenstorfer, Germany;

Krasiejko, Poland) and purchased from Lab-Service Analitica (Anzola Emilia, Bologna, Italy). Stock solutions were prepared dissolving each pesticide in pure acetone (DAB-BP).

Different aliquots of stock solution in acetone containing each pesticide at 0.4–0.5 mg/ml were carefully taken by a micropipette (Transfpettor, 20–100 μl) to create pooled mixed solutions in the working range of 1–2 μg/g. Each pooled solution contained about 10 pesticides that were chosen according to their retention time and to the detector used for quantitation. Each solution also contained ethion that was used as a reference peak to calculate the relative retention time (RRT) of the corresponding pesticides and help identify them on the four columns used.

2.4. Preparation of sample extract

A 500 g amount of vegetal sample was chopped in a food chopper (Braun ZK 100) and 25-g of chopped and homogeneously mixed sample were weighed into a 250 ml beaker.

For the preparation of fortified samples, 5 ml of each solution and 25 g of diatomaceous earth were added to a blank apple sample of known origin and carefully mixed. 150 ml of ethyl acetate was then added and the diatomaceous earth–vegetable mixture was homogenised in Ultra-Turrax (Janke and Kunkel, Germany) for 10 min. The bulk mass was filtered through buchner with glass microfibre filter (Whatman GF/A) and the filtrate collected into a 500 ml round-bottomed flask. The diatomaceous earth–vegetal mixture was extracted again with 100 ml of ethyl acetate for 5 min and filtered on the same buchner. The extracts collected were concentrated using a rotary vacuum evaporator (Buchi R-114, Germany) (P=150 mbar; T=40°C) and finally with a stream of nitrogen to dryness.

The final volume of eluate was adjusted at 5 ml with acetone and subject to gas-chromatographic analysis. Ten replicates were run for most of the compounds studied.

Quantification of recoveries were performed extracting the blank apple in the same way as reported above. The final volume of eluate was adjusted at 5 ml by adding alternatively each of the pooled solutions used to fortify the samples. The calibration

Table 1

Spiking level, number of replicates, mean percentage recovery, standard deviation and detection limit (with the corresponding column) for the pesticides studied in fortified apple matrix according to each column–TSD combination

Compound	Conc (µg/g)	DB-1701–TSD			DB-5–TSD			LOD (µg/g)	Column	$p>F$
		n	Mean recovery (%)	S.D.	n	Mean recovery (%)	S.D.			
Acephate	1.84	8	88	6	8	76	10	0.001	1701	**
Aldicarb	1.59	9	94	13	4	97	15	0.05	1701	
Azinphos-ethyl	1.424	10	92	7	10	89	5	0.005	1701	
Azinphos-methyl	1.568	9	95	12	10	108	7	0.01	5	
Benalaxyl	1.488		NC		10	85	11	0.05	5	
Bitertanolo	2.912	10	83	6		NC		0.01	1701	
Carbaryl	1.408	8	99	8	6	97	13	0.05	both	
Carbofuran	1.509	9	101	8	9	104	7	0.01	1701	
Chlorfenvinphos1	1.432	10	85	10	9	75	9	0.01	1701	**
Chlorfenvinphos2	1.432	10	87	10	10	84	7	0.001	1701	**
Chlorpyriphos-ethyl	1.654	10	101	9	10	99	7	0.005	1701	***
Chlorpyriphos-methyl	1.68	10	100	9	10	98	7	0.001	1701	***
Cyproconazole	1.532	10	87	6		NC		0.01	5	
Cymoxanil	1.496		NC		10	76	12	0.05	5	
Diazinon	1.696	10	104	10	10	100	8	0.001	1701	**
Dimethoate	1.836	10	90	12	10	95	8	0.001	1701	*
Etaconazole1	2.016	10	72	11		NC		0.05	5	
Etaconazole2	2.016	10	80	7		NC		0.05	1701	
Ethiofencarb	1.936	7	72	12	9	88	18	0.05	5	*
Ethion	1	39	95	7	30	90	11	0.001	both	
Ethoprophos	1.456	10	108	10	10	78	5	0.001	1701	***
Fenamiphos	1.646	10	90	31	5	102	10	0.005	1701	
Fenithrothion	1.9	10	101	9	10	99	7	0.001	1701	
Fenoxycarb	1.604	9	101	7	4	107	4	0.05	1701	
Flusilazol	1.632	9	75	8	10	78	11	0.01	5	*
Flutriafol	1.612	10	86	5		NC		0.01	1701	
Fonofos	1.616	10	101	10	10	97	7	0.001	1701	**
Formothion	1.496	9	103	10	10	100	8	0.005	1701	**
Furalaxyl	1.616	10	86	10		NC		0.05	1701	
Furathiocarb	1.756	4	104	6	1	101		0.05	5	
Heptenofos	1.626	10	87	9	10	96	7	0.005	1701	**
Hexaconazole	1.756	10	91	6		NC		0.01	1701	
Isofenphos	1.596	10	90	9	10	83	6	0.005	1701	**
Malathion	1.844	10	101	10	10	101	8	0.005	1701	
Metalaxyl	1.728	9	88	11	10	87	8	0.05	1701	
Metamidophos	1.44	9	55	9	8	89	7	0.001	1701	***
Methidathion	1788	10	101	14	10	103	9	0.005	1701	
Methiocarb	1.488	9	97	8	6	102	14	0.05	1701	
Omethoate	1.456	8	63	7		NC		0.005	1701	
Oxadixyl	1.742	9	84	10		NC		0.01	1701	
Parathion-ethyl	1.66	10	85	10	10	88	7	0.001	1701	*
Parathion-methyl	1.708	10	86	14	9	91	10	0.005	1701	*
Penconazole	1.956	10	89	6		NC		0.01	1701	
Phenthoate	1.68	10	103	10	10	102	8	0.005	1701	
Phorate	1.61	10	81	9	10	81	12	0.001	1701	
Phosalone	1.664	9	100	16	9	106	11	0.005	1701	
Phosphamidon1	1.696	10	97	13	10	96	6	0.01	1701	
Phosphamidon2	1.696	8	93	23	10	95	8	0.01	5	
Pirimicarb	1.6	8	98	14	8	100	14	0.01	1701	*

Table 1 (continued)

Compound	Conc (µg/g)	DB-1701–TSD			DB-5–TSD			LOD (µg/g)	Column	$p > F$
		n	Mean recovery (%)	S.D.	n	Mean recovery (%)	S.D.			
Pirimiphos-ethyl	1.452	10	84	10		NC		0.01	1701	
Pirimiphos-methyl	1.852	10	87	9	10	90	6	0.001	1701	*
Profenofos	1.932	10	86	15	10	95	8	0.001	1701	
Propiconazole1	1.656	10	85	6		NC		0.05	1701	
Propiconazole2	1.656	10	87	6		NC		0.05	1701	
Propoxur	1.442	7	96	9	7	101	17	0.05	1701	
Pyrazophos	1.82	9	106	12	9	106	11	0.001	1701	
Quinalphos	1.82	10	92	12	10	72	9	0.001	5	***
Tebuconazole	1.864	10	84	6		NC		0.01	1701	
Tolclophos-methyl	1.78	10	87	9	10	92	5	0.001	1701	*
Triadimefon	1.536	10	96	6		NC		0.01	1701	
Triadimenol	1.44	10	91	6		NC		0.01	1701	
Trichlorfon1	1.806	10	80	8		NC		0.05	1701	
Trichlorfon4	1.806	9	111	11		NC		0.05	1701	
Vamidothion	1.692	9	77	23		NC		0.005	1701	

NC=not computed.
n=number of replicates.
$p > F$=significance probability level associated with the F value (mean square for the model/mean square for the error; it tests how well the model as a whole (adjusted for the mean) accounts for the dependent variable behaviour).
$p > F$: *< 0.05; **< 0.01; ***< 0.001.

solutions so obtained were used to quantify the fortified samples. Three injections for each replicate as well as for the calibration solutions were performed in order to increase the confidence of the measurements. The linearity of both ECD systems and TSD systems had been tested before and were consistent over the pesticide concentrations used.

3. Results and discussion

Tables 1 and 2 report the spiking level concentrations, percentage recoveries and detection limits (LODs) for the pesticides studied in fortified apple matrix according to each column–detector combination. LODs were measured on both columns and may be different for each combination; Tables 1 and 2 report detection limits for the more sensitive combination.

The use of diatomaceous earth as polar phase mixed with vegetal material and extracted with ethyl acetate allowed us to determine more than 90 pesticides belonging to different chemical classes. With the method used, recoveries were between 75 and 116% for the compounds studied. The reproducibility expressed as relative standard deviation was between 6 and 15% for most compounds. The gas chromatographic configuration used allowed us to choose the best column on which to determine and quantify the numerous pesticides under investigation. The mixtures of standard solutions used to quantify the fortified samples were added to blank apple extracts to avoid erroneous quantifications due to matrix effects and solute discrimination previously reported [9].

Representative GC chromatograms from the control, standard and fortified sample are shown in Figs. 1–3.

The extraction method used in this study differs from MSPD methods in which the sample itself is dispersed over the large surface of a solid-phase and packed inside a column before extraction. Preferential flow and vegetal material not carefully mixed are the principal drawbacks that can result in poor recovery efficiency in column extraction methods. Besides, with some samples, it is difficult to homogenise the vegetal mass so the resulting material has a smaller specific area to interact with the

Table 2

Spiking level, number of replicates, mean percentage recovery, standard deviation and detection limit (with the corresponding column) for the pesticides studied in fortified apple matrix according to each column–ECD combination

Compound	Conc (μg/g)	DB-1701–ECD			DB-5–ECD			LOD (μg/g)	Column	$p > F$
		n	Mean recovery (%)	S.D.	n	Mean recovery (%)	S.D.			
Acrinatrine	1.376		NC		8	100	12	0.005	1701	
Aldrin	1.712	9	95	9	10	97	6	0.005	1701	
Alphametrine	1.288	10	86	20		NC		0.001	1701	
Bifentrin	1.552	9	99	10		NC		0.01	1701	
Captafol	1.504		NC		10	93	10	0.01	1701	
Captan	1.6	9	89	10	4	98	25	0.01	1701	
Chlorotalonil	1.792	10	101	10		NC		0.005	5	
Chlozolinate	1.82	10	98	6	10	94	4	0.005	5	
Cifluthrin1	1.62		NC		9	105	9	0.01	1701	
Cifluthrin3	1.62		NC		9	107	10	0.01	5	
Cypermethrine1	1.556		NC		9	106	8	0.01	1701	
Cypermethrine2	1.556		NC		9	109	10	0.05	5	
Cypermethrine3	1.556		NC		6	110	9	0.05	5	
Deltamethrin	1.648		NC		9	98	5	0.005	1701	
o-p'-DDT1	1.616		NC		7	106	16	0.01	1701	
o-p'-DDT2	1.616		NC		8	88	13	0.005	1701	
p-p'DDT1	1.372	9	102	15		NC		0.01	1701	
p-p'DDT2	1.372	8	93	16		NC		0.01		
Dichlofluanide	1.804	5	97	26	10	91	10	0.001	5	
Dicloran	1.62	9	116	10	10	100	9	0.001	1701	**
Dieldrin	1.312	9	99	2	10	100	7	0.001	1701	
Endosulfan1	2.124	6	98	15	10	98	4	0.01	5	
Endosulfan2	2.124	9	98	3	8	93	11	0.005	1701	
Endrin	1.507	9	99	2	10	101	7	0.005	1701	
Endrin2	1.507	8	82	42	7	89	11	0.005	1701	*
Esfenvalerate	1.648	9	101	2		NC		0.001	5	
Ethion	1	29	92	5	35	95	15	0.001	both	
Fenarimol	1.756	10	98	4	10	91	8	0.001	5	**
Fenpropatrin	1.736	8	101	8		NC		0.01	1701	
Fenvalerate1	1.872	10	96	3	10	80	15	0.005	1701	***
Fenvalerate2	1.872	10	95	5	10	81	14	0.005	1701	***
Flucitrinate1	1.704	9	93	4	10	82	14	0.005	1701	***
Flucitrinate2	1.704	10	96	6	10	81	14	0.005	1701	***
Fluvalinate	1.788		NC		9	108	9	0.005	1701	
Folpet1	1.664	9	62	10	7	12	5	0.01	1701	***
Folpet2	1.664		NC		5	101	25	0.05	5	
Heptachlor	1.648	7	105	12	10	96	4	0.005	1701	
Iprodione	1.664	6	84	12	10	91	15	0.005	1701	**
Lindane	1.661	9	99	4	10	101	8	0.005	1701	
Myclobutanil	1.592	10	87	17	10	91	14	0.005	5	**
Nuarimol	1.56	10	100	3	10	86	16	0.001	1701	**
Permethrin	1.596	9	101	8		NC		0.05	1701	
Procimidone	1.648	5	91	28	10	94	9	0.01	1701	
Vinclozolin	2.032	7	102	4	10	98	7	0.01	1701	

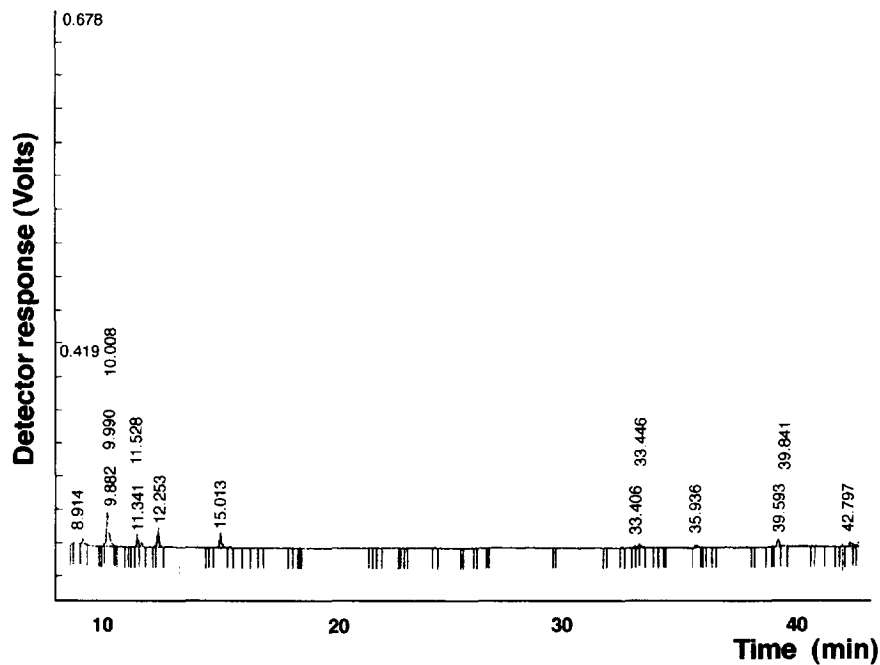Symbols and abbreviations as reported in Table 1.

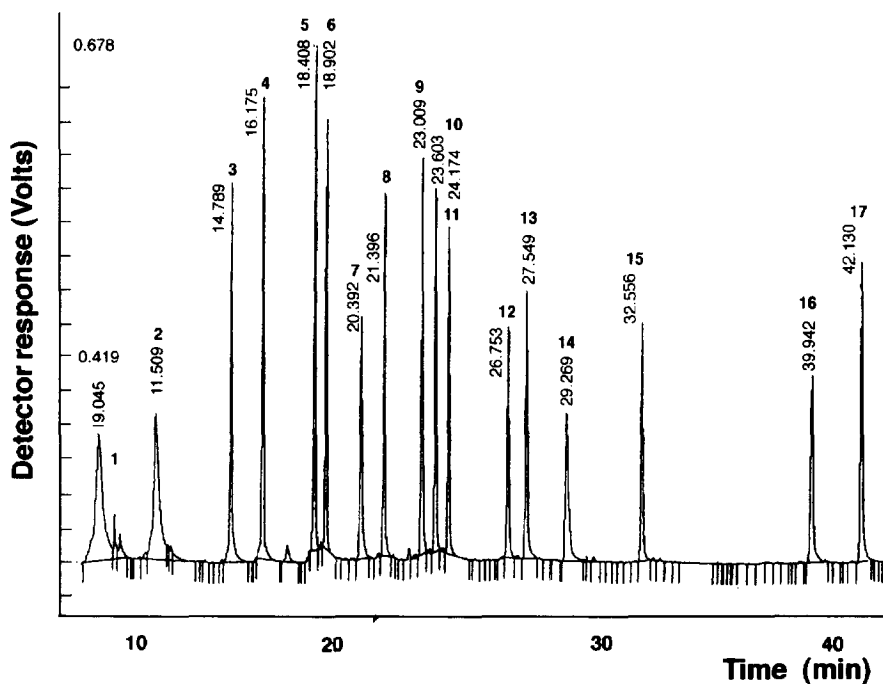Fig. 1. Chromatogram of a blank sample on DB-5–TSD system.

Fig. 2. Chromatogram of a standard mix on DB-5–TSD system. 1=metamidophos, 2=acephate, 3=ethoprophos, 4=phorate, 5=fonofos, 6=diazinon, 7=formothion, 8=chlorpyriphos-methyl, 9=fenithrothion, 10=malathion, 11=chlorpyriphos-ethyl, 12=phenthoate, 13= methidathion, 14=fenamiphos, 15=ethion, 16=phosalone, 17=pyrazophos.
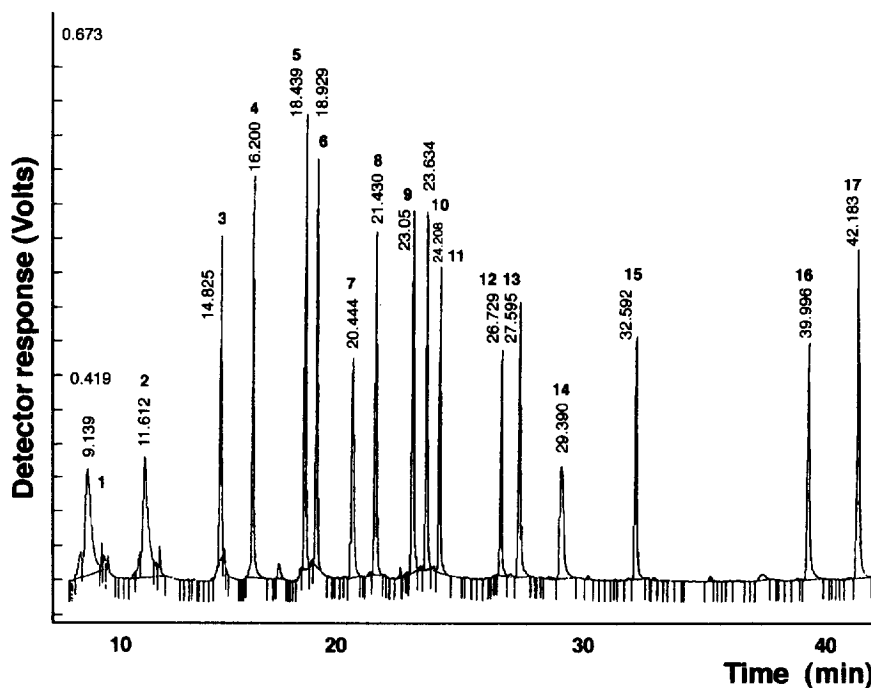
Fig. 3. Chromatogram of a fortified[a] apple sample on DB-5–TSD system (same standard mix as Fig. 2). 1=metamidophos, 2=acephate, 3=ethoprophos, 4=phorate, 5=fonofos, 6=diazinon, 7=formothion, 8=chlorpyriphos-methyl, 9=fenithrothion, 10=malathion, 11= chlorpyriphos-ethyl, 12=phenthoate, 13=methidathion, 14=fenamiphos, 15=ethion, 16=phosalone, 17=pyrazophos. [a] Spiking level as reported in Table 2 for the corresponding compound.

solvents used in extractions. This problem is easily overcome with the Ultra-Turrax homogenisation. Ethyl acetate proved to be a good solvent to use because more effective to extract pesticides from different chemical classes (data not shown). The concentrated extract can be directly analysed chromatographically without additional purification as previously reported [6].

Other recovery studies are being carried out on different vegetal matrices such as potato and peach and the extracts generally need not an additional purification and can be analysed using both ECD and TSD. However for matrices which contain electron-capturing material such as onion the analysis using ECD is virtually impossible. For such matrices additional purification is required.

When the pesticides are considered together the ANOVA analysis showed that the recovery is influenced by the columns used ($p > F = 0.04$). The precision of the extraction method depends on the different combination column–detector used (Table

3). The precision is similar on both the columns coupled to ECD systems while the recoveries quantified on the DB-5–TSD system are less variable than those quantified on the DB-1701–TSD system.

When the ANOVA was carried out by each pesticide the precision of recovery for some compounds is independent from the column used while for others there is a significant difference between the column DB-5 or DB-1701 used in the quantitation process (Tables 1 and 2). For these compounds

Table 3
Overall recovery efficiencies of every pesticide according to each column–detector combination

Column–detector	d.f.	R.S.D.	Root MSE
DB-1701–ECD	248	14.8	14.1
DB-1701–TSD	632	58.1	57.5
DB-5–ECD	399	18	17
DB-5–TSD	420	13.4	12.4

d.f.=degree of freedom.
MSE=mean square error.

the column on which the recovery is more efficient should be used after controlling that the reproducibility of extractions among the replicates is consistent.

The experience with various high-resolution gas chromatography (HRGC) injectors lead to the hypothesis that a short residence time in the injection region is more beneficial than a low temperature for the stability of the majority of pesticides [10]. Coextractives also build-up in this reactive region and in the head of the column leading to longer residence time which causes losses and degradation. The use of an uncoated precolumn of de-activated silica distributes the co-extractives in a less concentrated bands [11] as well as acting as a retention gap and thus reduce the build-up of the less volatile components which leads to progressive, and sometimes rapid, deterioration of chromatographic performance (resolution and sensitivity) for pesticides in food extracts [12].

The temperature program, the use of a split/splitless injector with low volume insert interfaced to an uncoated, deactivated pre-column allowed to achieve excellent results by replacement of the precolumn after 100–150 analyses.

4. Conclusions

The diatomaceous earth as solid-phase to assist the extraction method is effective for the multiresidue determinations of more than 90 pesticides belonging to different chemical families. Mean percentage recoveries and reproducibility of extractions were good for most of the compounds studied.

The method requires less glassware and it is faster than conventional extraction techniques such as liquid–liquid partition and SPE but the sample size requires a relative large amount of ethyl acetate. Experiments are being carried out in order to optimize these parameters and reduce the overall cost of analysis.

The use of the diatomaceous earth eliminates the necessity to purify the extracts of the matrix studied, at least for apples, and furnishes extracts which can be directly analysed chromatographically. The gas chromatographic configuration used allows us to determine and quantify the numerous pesticides under investigation.

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