Determination of Multi-pesticide Residues in Rapeseed by GC×GC-mass Spectrometry

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Abstract

(Background) Cold pressed rapeseed oil put forward to requirement of raw rapeseed resource with as low as possible contaminants such as chemical pesticides. Actually, for rapeseed, maximum pesticide residue levels have been set by the European Union and the Codex Alimentarius Commission of the Agriculture Organization of the United Nations (FAO). Therefore, it is necessary to monitor their residues regularly through multi-residue analytic methods with high accuracy and sensitivity.

(Objective) The aim of the study was to establish an analytical method for acurate measurement of multi-pesticide residues in rapeseeds by comprehensive two-dimensional gas chromatography (GC×GC) employing a recently introduced high-speed time of flight mass spectrometric detector (TOF MS).

(Method) In this research, liquid-liquid extraction (LLE) method was used to extract and purify pesticide residues from rapeseeds with high oil content. Qualitative and quantitative analyses was carried out by GC×GC-TOF/MS.

(Result) Adding standard materials to blank sample of rapeseed for the test of recovery, the good recoveries were found. The result of variance analysis showed that the method could be well used in determination of pesticide residues in rapeseeds.

(Conclusion)LLE coupled with GC×GC/TOF MS, can be considered an accurate alternative method for multi-residues measuring semivolatile pesticide residues potentially occurring in rapeseed. In terms of sensitivity, the developed method showed detection limits as low as μg kg^{-1} levels.

Keywords: rapeseed; liquid-liquid extraction (LLE); comprehensive two-dimensional gas chromatography-flight mass spectrometric detector (GC×GC-TOF/MS).

1. Introduction

Cold pressed rapeseed oil put forward to requirement of raw rapeseed resource with few contaminants such as chemical pesticides. Organophosphorus pesticides (OPPs) are among the most commonly employed pesticides world wide for rapeseed. OPPs are very toxic when absorbed by human organisms because of acetyl-cholinesterase de-activation [1]. Actually, for rapeseed, maximum OPPs levels have been set by the European Union and the Codex Alimentarius Commission of the Agriculture Organization of the United Nations (FAO). Therefore, it is necessary to monitor their residues regularly through multi-residue analytic methods with high accuracy and sensitivity. Recently introduced technique, the comprehensive two-dimensional gas chromatography (GC×GC) brings the separation potential superior to any conventional gas chromatographic separation. In GC×GC, two columns of different selectivity are serially coupled via a modulation device, which cuts small portions (typically 2–10 s) of the effluent from the first column, refocuses them and samples onto the second column. Each pulse generates its own very fast chromatogram. The principles and instrumentation of comprehensive two-dimensional gas chromatography have been recently reviewed in several papers [2–6]. Very promising results of the coupling of GC×GC with a TOF MS for the trace-level determination of pesticides in vegetables [7] have been reported. Very recently, the first fully-integrated GC×GC–TOF MS instrument has been introduced [8].In our study, the LECO Pegasus 4D GC×GC–TOF MS system was evaluated for the analysis of OPPs in rapeseed samples. Special focus was laid on the potential of the technique to reliably identify the OPPs at levels 1 μg/kg. Also, we aimed to evaluate the quantitative performance characteristics of a developed GC×GC–TOF-MS method.

2. Experimental

2.1. Reagents and materials

Acetone, n-Hexane, and anhydrous Na_2SO_4 (analytical reagent grade) were obtained from Fisher Scientific (Loughborough UK). Florisil (100-200 mesh), aminopropyl and neutral alumina were purchased from hebao Scientific (shanghai China). Standards of pesticide residues (purity>98.0%) (Table 1) were purchased from Chinese CRM/RM Information Center (Beijin, China). The black-seeded rapeseeds obtained at a local market.

2.2. Apparatus

Pegasus IV GC×GC–TOFMS system (Leco, St. Joseph, MI, USA) including an Agilent 7890N GC system (Agilent Technologies, USA) and equipped with a CTC Combipal (CTC Analytics, Switzerland) autosampler was used for analysis. Dry nitrogen gas (INOX Air Product, Mumbai, India), liquid nitrogen and compressed air were provided for modulation. Ultra-pure grade helium was used as the carrier gas.

2.4. Sample preparation

0.5 g of comminuted rapeseed sample were extracted with 10 mL acetone–hexane (20/80, v/v) for 15 min in an ultrasonic bath at room temperature. After addition of 5 g of Na_2SO_4, the mixture was filtered under vacuum and the filtrate was evaporated. The eluting solution was collected in 25 mL graduated glass tubes and then concentrated to 1 mL with a gentle stream of nitrogen. The extract was collected in a vial, directly subjected to GC×GC TOF-MS analysis.

2.5. GC×GC TOF-MS conditions

The GC×GC separation was performed by injecting 2μL (splitless) on a DB-5MS capillary column (5% phenyl polysilphenylenesiloxane; 30m×0.25mm, 0.25μm) connected in series to a DB-17ht capillary column ((50%-Phenyl)-methylpolysiloxane; 1.5m×0.10mm, 0.10μm) as the secondary column. Helium was used as the carrier at the corrected constantflowrate of 2 mL/min. The injector port was set at 290℃. Transfer line temperature was maintained at 280 ℃. Electron impact ionization was achieved at 70 eV and the ion source temperature was set at 250 ℃. The detector voltage was set at −1700V and the data acquisition was carried out within the mass range of 50-550 m/z at acquisition rate of 100 spectra/s at 2-D mode.

3. Results and discussion

3.1. Selection of the column set

A narrow bore capillary column with a non-polar phase (DB-5 MS) was selected as the first dimension to emphasize separation according to the boiling points of the compounds. A column of 0.25mm i.d. and 0.25 μm film thickness was selected to produce sufficiently broad peaks to give the appropriate number of chromatographic slices after modulation. This was important because we wanted to keep a constant temperature difference between the two columns, with the secondary column operating at a significantly higher temperature. Higher temperatures on the second dimension microbore columns were used to reduce analyte peak widths. Different “classical” secondary dimension phases were studied. A mid-polar phase

Table 1 Retention time under the experimental conditions used, *m/z* ions selected for quantification and identification of the pesticide residues investigated by GC×GC–TOF/MS

peak	Name	Similarity	t_{R1}(s)	t_{R1}(s)	Quant Masses	Weight	Area
1	Dichlorvos	921	342	0.7	109	220	31153178
2	Isoprocarb	952	435	1.01	121	193	46154477
3	Ethoprophos	909	474	1.12	158	242	6726316
4	Sulfotep	963	486	1.22	322	322	2976684
5	Phorate	913	501	1.28	121	260	7136970
6	Terbufos	860	546	1.42	57	288	16374043
7	Diazinon	939	546	1.45	179	304	33052195
8	Phosphamidon (Z)	820	552	1.76	127	299	2477624
9	Fonofos	967	555	1.66	109	246	35735957
10	Pirimicarb	957	579	2	166	238	14050781
11	Phosphamidon (Z)	947	606	2.11	127	299	5724914
12	Chlorpyrifos-methyl	935	621	2.12	47	321	42401652
13	Alachlor	857	630	2	160	269	6355406
14	Methyl parathion	951	636	2.23	109	263	7406319
15	Carbaryl	945	654	2.7	144	201	17648242
16	Pirimiphos methyl	916	663	2.26	125	305	6126297
17	Fenitrothion	950	678	2.39	109	277	5075001
18	Fenthion	954	711	2.38	278	278	6511439
19	Isocarbophos	943	729	2.44	136	289	9235176
20	Methidathion	921	819	2.42	145	302	5839387
21	Endosulfan I	950	846	1.9	170	404	1085155
22	Oxadiazon	942	873	1.65	175	344	10872668
23	Endosulfan I	893	942	2.26	195	404	643313
24	Ethion	929	945	2.14	153	384	72504337
25	Triazophos	973	975	2.8	161	313	3874629
26	Phosalone	952	1197	2.43	182	367	30715154

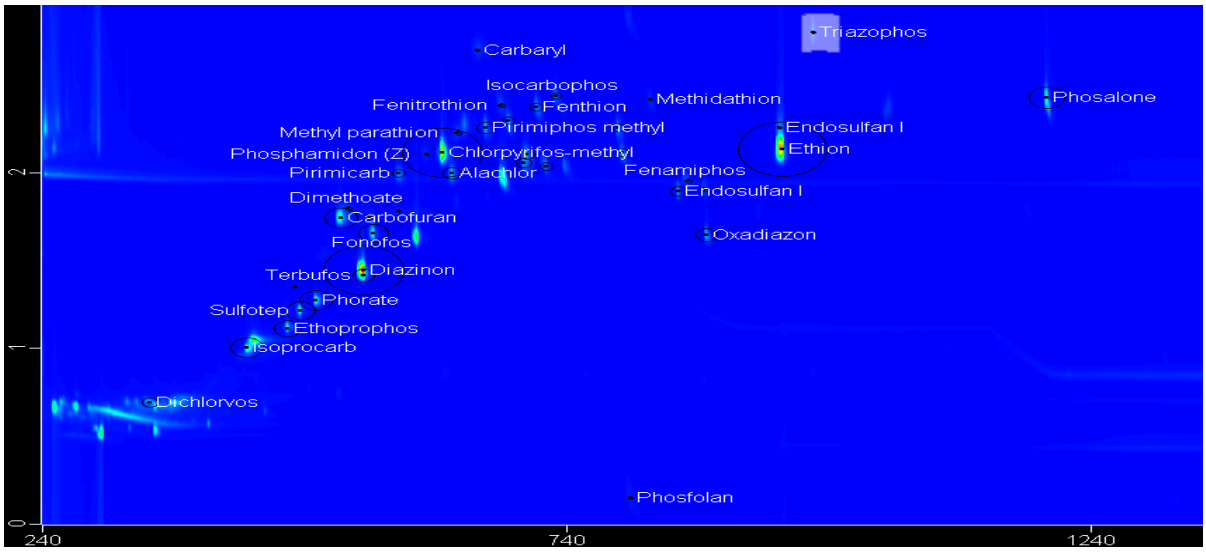

Table 1 Retention time under the experimental conditions used, *m/z* ions selected for quantification and identification of the pesticide residues investigated by GC×GC–TOF/MS
(DB-17ht) was investigated and gave a good separation of the OPPs. All of the OPPs were baseline separated.

3.3. Improvement of chromatographic separation

The application of GC×GC in OPPs analysis resulted in improved chromatographic resolution both in terms of: (i) separation of individual OPPs from each other; and (ii) separation of OPPs from matrix components. Fig. 1 shows the contour plot of the OPPs standard at a concentration of 1 µg/mL. For the identification of individual OPPs in this figure see Table 1.

3.5. Analytical Performances

3.5.1. Calibration curves and limits of detection

To investigate potential limitations in OPPs were prepared in the range from 1 to 100 ng/mL and analysed. The excellent linearity was still obtained for the majority of the target compounds. Using a representative set of rapeseed samples, the level at which the OPPs were identified through automatic screening by the software using the similarity threshold of 800 was assessed.

3.5.2. Method accuracy and precision

The overall method accuracy and precision were determined by analyzing fortified rapeseed samples in fivefold at two levels (10 and 100 µg/kg). From these data, the average recovery for the determination of OPPs ranged from 85 to 105%.

3.5.3. Monitoring real life samples

The ability of the GC×GC/TOF-MS to determine OPPs in real life samples was tested by analyzing black-seeded rapeseeds obtained at a local market. OPPs were detected using a non-target search enabled by ChromaTOF software. The content of particular OPPs was calculated (n=5) for fonofos 5µg/kg (R.S.D. = 9%) and for fenthion 8µg/kg (R.S.D. = 11%).

4. Conclusion

This work demonstrated that a method based on GC×GC/TOF-MS was suitable for both qualitative screening and quantitative determination of a wide range of pesticide residues including OPPs in rapeseed samples at µg/kg levels.

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Reference

[1] H. Tsoukali, M. Tsoungas, Vet. Human Toxicol. 38 (1996) 366.
[2] W. Bertsch, J. High Resolut. Chromatogr. 23 (2000) 167–181.
[3] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331–347.
[4] P.J. Marriott, R. Shellie, Trends Anal. Chem. 21 (2002) 573–583.
[5] M. Pursch, K. Sun, B. Winniford, H. Cortes, A. Weber, T. McCabe, J. Luong, Anal. Bioanal. Chem. 373 (2002) 356–367.
[6] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69–108.
[7] J. Dallüge, M. van Rijn, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 965 (2002) 207–217.
[8] Four dimensions of analytical resolution for enhanced analysis of complex mixtures, Looking Beyond the Peaks . . . Pegasus 4D®, Pegasus 4D Specification Note, LECO St. Joseph, MI, USA.