SFE APPLICATION NOTE 03-09

Supercritical Fluid Extraction of Residual Pesticides in Spinach

Introduction

In Japan, on the 29th of May 2006 the Ministry of Health, Labor and Welfare (MHLW) promulgated the Positive List System for residual pesticides, food additives, and veterinary medicines remaining in foods, following the revision of the Food Hygiene Law. In this list approximately 800 kinds of those agricultural chemicals were registered. This system is to prohibit the distribution of foods that contain more than 0.01 ppm of each chemical.

The extraction of residual pesticides in foods has been performed by the solvent extraction method. This method, however, takes about 4 - 5 hours for each extraction, and needs large quantities of organic solvent. In recent years, supercritical fluid extraction (SFE) using supercritical carbon dioxide has attracted much attention as an alternative method to the solvent extraction method.

We have developed a fully automated residual pesticide extraction system, and applied this system to the analysis of a coffee bean sample. Extracted components were analyzed by GC-MS/MS.

Experimental

The newly developed fully automated residual pesticide extraction system was used throughout the experiment. The schematic diagram of this system is shown in Figure 1.

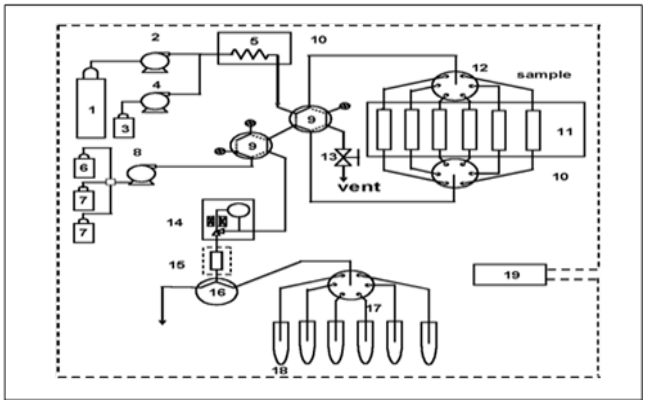


Figure 1. Schematic Diagram of the fully automated system for supercritical fluid extraction of residual pesticides.
System configuration: 1 = carbon dioxide cylinder, 2 = liquefied carbon dioxide delivery pump, 3 = modifier, 4 = modifier delivery pump, 5 = preheating coil, 6 = solvent for trap elution, 7 = rinse solution for trap column, 8 = solvent delivery pump, 9 = switching valve for flow line, 10 = oven, 11 = extraction vessels, 12 = 6-vessel changer, 13 = release valve, 14 = automatic back pressure regulator, 15 = trap column, 16 = 3-way valve, 17 = 6-way flow line switching valve, 18 = collection tubes, 19 = system controller.
Supercritical CO_2 delivered by pump 2 passes through one of vessels 11 in which the sample is loaded and then pesticides are extracted. The extracted pesticides are concentrated by a trap column, is eluted by acetonitrile (2 mL) delivered by pump 8, and is collected in one of collection tubes 18. This system is automatically controlled by 19, system controller.

Supercritical fluid extraction conditions: extraction tube : 10 mL (10 mm x 127 mm), supercritical fluid : CO_2, back pressure = 15 MPa, extraction time : 30 min, flow rate : 2 mL/min, trap column : ODS (4.6 mm x 50 mm, 30 µm), solvent for trap elution : acetonitrile 2 mL (flow rate : 2 mL/min).

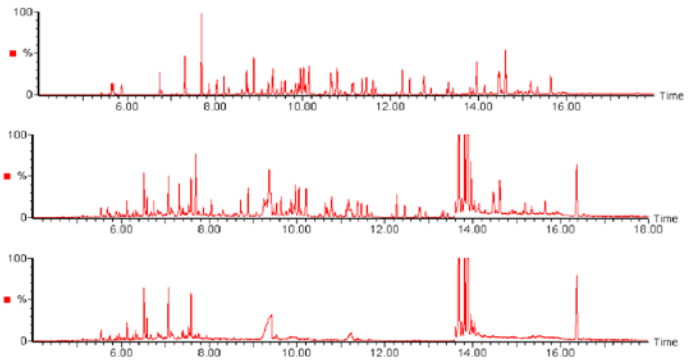
As an analytical sample, spinach was selected. Sixty-eight kinds of pesticides were added to the spinach to be a concentration of 0.1 ppm for each pesticide except captan, 1 ppm and acetamiprid, 0.5 ppm. Two grams of the spinach and 2 g of Hydromatrix (a dehydrating agent) were loaded in each extraction vessel; SFE was applied at an extraction pressure of 15 MPa, at an extraction temperature of 40°C, for an extraction time of 30 min; the extracted components were adsorbed on a trap column; the trapped components were eluted with acetonitrile; the acetonitrile solution was evaporated to dryness with nitrogen gas; and the residue was dissolved in 3 mL of acetone containing 0.05% of PEG200 and PEG400. A portion of this solution was injected onto the GC.

GC Conditions:
Instrument : Quattro micro GC (Waters micromass)
Ionization method : EI
Measurement mode : MRM, SIM
Ionization source temperature : 280°C
Interface temperature : 280°C
GC : 6890N(Agilent)
Injection method : Splitless
Injection volume : 1 µL, Inlet temperature : 250°C
Column : DB-5MS(30 m x 0.25 mm)
Column temperature : 50°C (0 min)—50°C (1 min) — 200°C (7 min) — 250°C (9 min) — 300°C (11 min).

Results and Discussion

Chromatograms of the standard mixture (upper), the sample added with the standard (middle), and the blank (lower) are shown in Figure 2.

Figure 2. GC chromatograms of spinach sample.
Upper : standard mixture (68 components)
Middle : sample added with standard mixture
Lower : blank Measurement conditions



Standard mixture solution contains 68 components as below. : 1: Acephate, 2: Acetamiprid, 3: Bendiocarb, 4: Bitertanol, 5: Butylate, 6: Captan, 7: Carbaryl 8: Chinomethionat, 9: Chlorfenvinphos, 10: Chlorpyriphos, 11: Cyfluthrin, 12: Cypermethrin, 13: Deltamethrin, 14: Diazinon, 15: Dichlofluanid, 16: Dichlorvos, 17: Diethofencarb, 18: Dimethylvinphos, 19: EPN, 20: Esprocarb, 21: Ethiofencarb, 22: Ethoprophos, 23: Fenarimol, 24: Fenitrothin, 25: Fenobucarb, 26: Fensulfothion, 27: Fenvalerate, 28: Flucythrinate, 29: Flusilazole, 30: Flutolanil, 31: Fluvalinate, 32: Imibenconazole, 33: Iprodione, 34: Isofenphos , 35: Isofenphos P=O, 36: Isoprocarb, 37: Lenacil, 38: Malathion, 39: Mefenacet, 40: Mepronil, 41: Methamidophos, 42: Metolaclior, 43: p,p'-DDE, 44: Paclobutrazol, 45: Pencycuron, 46: Pendimethalin, 47: Permethalin, 48: Phenthoate, 49: Phosalone, 50: Pirimifos-methyl, 51: p,p'-DDD, 52: Pretilachlor, 53: Propiconazole, 54: Pyraclofos, 55: Pyridaben, 56: Pyridaphenthion, 57: Pyrimidifen, 58: Quinalphos, 59: Tefluthrin, 60: Terbucarb, 61: Terbufos, 62: Thenylchlor, 63: Tolclofos-methyl, 64: Triadimenol, 65: α-BHC, 66: β-BHC, 67: γ-BHC, 68: δ-BHC .

As shown in Table 1, among the 68 pesticides, 47 exhibited more than 70% recovery, and 61 more than 50% recovery. The recovery of acetamiprid and butylate was 20% and 45%, respectively. Acephate, captan, dichlofluanid, and methamidophos exhibited less than 10% recovery; their high hydrophilicity and adsorption on the dehydrating agent seemed to be responsible for a poor recovery in SFE.

Table 1 Recovery of Pesticides.

No.	Pesticide	Recovery (%)	No.	Pesticide	Recovery (%)
1	**Acephate**	**9.2**	35	Isofenphos P=O	98.0
2	**Acetamiprid**	**19.8**	36	Isoprocarb	89.1
3	Bendiocarb	113.0	37	Lenacil	79.9
4	Bitertanol	64.0	38	Malathion	91.8
5	**Butylate**	**45.1**	39	Mefenacet	87.4
6	**Captan**	**3.8**	40	Mepronil	75.7
7	Carbaryl	130.5	41	**Methamidophos**	**4.7**
8	Chinomethionat	97.0	42	Metolachlor	97.7
9	Chlorfenvinphos	76.1	43	p,p'-DDE	71.7
10	Chlorpyriphos	84.0	44	Paclobutrazol	111.6
11	Cyfluthrin	59.1	45	Pencycuron	59.9
12	Cypermethrin	86.7	46	Pendimethalin	95.6
13	Deltamethrin	57.8	47	Permethalin	57.3
14	Diazinon	78.6	48	Phenthoate	77.0
15	**Dichlofluanid**	**2.9**	49	Phosalone	73.5
16	Dichlorvos	60.1	50	Pirimifos-methyl	90.2
17	Diethofencarb	101.5	51	p,p'-DDD	81.8
18	Dimethylvinphos	90.9	52	Pretilachlor	88.9
19	EPN	75.0	53	Propiconazole	76.1
20	Esprocarb	79.1	54	Pyraclofos	76.6
21	Ethiofencarb	44.1	55	Pyridaben	68.6
22	Ethoprophos	95.2	56	Pyridaphenthion	92.4
23	Fenarimol	73.0	57	Pyrimidifen	77.0
24	Fenitrothion	107.8	58	Quinalphos	82.9
25	Fenobucarb	89.0	59	Tefluthrin	64.5
26	Fensulfothion	101.6	60	Terbucarb	89.3
27	Fenvalerate	64.5	61	Terbufos	52.2
28	Flucythrinate	56.9	62	Thenylchlor	96.4
29	Flusilazole	86.5	62	Tolclofos-methyl	83.5
30	Flutolanil	92.1	64	Triadimenol	93.4
31	Fluvalinate	50.7	65	α-BHC	92.0
32	Imibenconazole	63.2	66	β-BHC	92.7
33	Iprodione	105.4	67	γ-BHC	88.1
34	Isofenphos	57.1	68	δ-BHC	106.4

References:

1) Ministry of Health, Labor and Welfare Official Gazette No. 498
2) Ministry of Health, Labor and Welfare Official Gazette No. 497

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