SFE APPLICATION NOTE 03-09

Supercritical Fluid Extraction of Residual Pesticides in Coffee Beans

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.

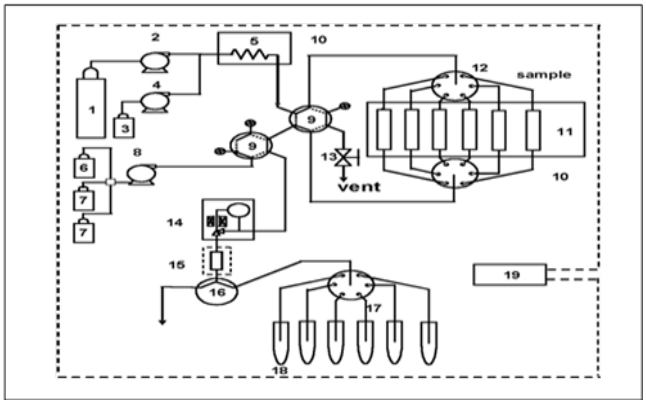


Figure 1. Schematic Diagram of the fully automated system for supercritical fluid extraction of residual pesticides.
System configuration: 1 = carbon dioxide cylinder, 2 = liquefied carbon dioxide delivery pump, 3 = modifier, 4 = modifier delivery pump, 5 = preheating coil, 6 = solvent for trap elution, 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).

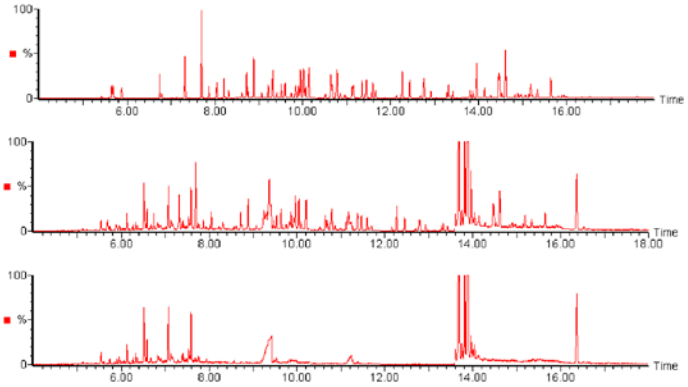
A sample of coffee beans was selected for analysis. Sixty-eight kinds of pesticides were added to the coffee beans at a concentration of 0.1 ppm for each pesticide except captan, 1 ppm and acetamiprid, 0.5 ppm. Three grams of the coffee beans were loaded into each extraction vessel; SFE was applied at 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 wheat flour 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, 58 exhibited more than 70% recovery, and 66 more than 50% recovery. The recovery of acetamiprid and pencycuron was 42% and 14%, respectively. Acetamiprid, due to its high hydrophilicity, indicated low solubility in supercritical carbon dioxide, resulting in a poor recovery in SFE. Low recovery of pencycuron seemed to be ascribed to the sample matrix.

Table 1. Recovery of Pesticides.

No.	Pesticide	Recovery (%)	No.	Pesticide	Recovery (%)
1	Acephate	95.4	35	Isofenphos P=O	79.5
2	**Acetamiprid**	**42.2**	36	Isoprocarb	70.8
3	Bendiocarb	84.2	37	Lenacil	63.8
4	Bitertanol	81.8	38	Malathion	91.9
5	Butylate	62.6	39	Mefenacet	85.4
6	Captan	54.6	40	Mepronil	75.7
7	Carbaryl	99.1	41	Methamidophos	65.4
8	Chinomethionat	94.6	42	Metolachlor	73.5
9	Chlorfenvinphos	73.0	43	p,p'-DDE	70.0
10	Chlorpyriphos	80.5	44	Paclobutrazol	112.7
11	Cyfluthrin	85.6	45	**Pencycuron**	**14.3**
12	Cypermethrin	78.4	46	Pendimethalin	74.4
13	Deltamethrin	97.3	47	Permethalin	73.3
14	Diazinon	72.9	48	Phenthoate	67.6
15	Dichlofluanid	85.6	49	Phosalone	90.2
16	Dichlorvos	61.6	50	Pirimifos-methyl	78.7
17	Diethofencarb	80.6	51	p,p'-DDD	76.2
18	Dimethylvinphos	71.9	52	Pretilachlor	75.6
19	EPN	71.2	53	Propiconazole	73.5
20	Esprocarb	109.5	54	Pyraclofos	76.9
21	Ethiofencarb	104.4	55	Pyridaben	72.1
22	Ethoprophos	74.4	56	Pyridaphenthion	85.0
23	Fenarimol	74.3	57	Pyrimidifen	71.0
24	Fenitrothion	91.8	58	Quinalphos	72.0
25	Fenobucarb	77.6	59	Tefluthrin	70.2
26	Fensulfothion	88.9	60	Terbucarb	102.1
27	Fenvalerate	79.6	61	Terbufos	65.6
28	Flucythrinate	83.3	62	Thenylchlor	73.3
29	Flusilazole	73.5	62	Tolclofos-methyl	73.9
30	Flutolanil	101.3	64	Triadimenol	71.3
31	Fluvalinate	88.7	65	α-BHC	74.5
32	Imibenconazole	70.3	66	β-BHC	73.3
33	Iprodione	92.0	67	γ-BHC	70.9
34	Isofenphos	57.5	68	δ-BHC	78.1

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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