SFE APPLICATION NOTE 03-09

Supercritical Fluid Extraction of Residual Pesticides in Wheat Flour

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 wheat flour 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.

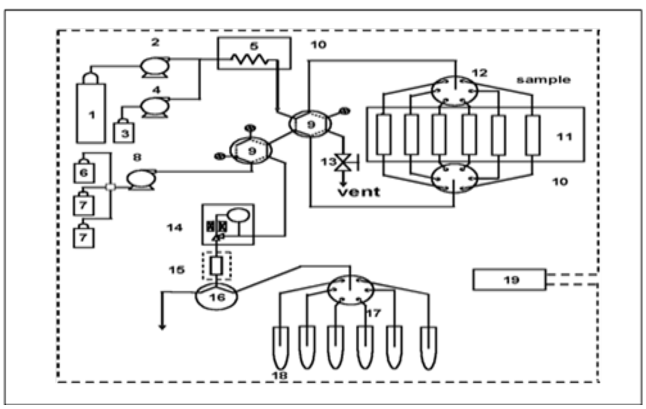


Figure 1. Schematic Diagram of the fully automated system for supercritical fluid extraction of residual pesticides.
System configuration: 1 = carbon dioxide cylinder, 2 = liquefied carbon dioxide delivery pump, 3 = modifier, 4 = modifier delivery pump, 5 = preheating coil, 6 = solvent for trap elution,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

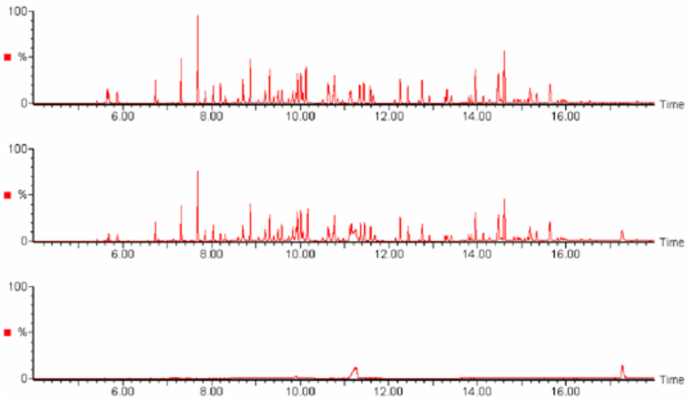
As an analytical sample, wheat flour was selected. Sixty-eight kinds of pesticides were added to the flour to a concentration of 0.1 ppm for each pesticide except captan, 1 ppm and acetamiprid, 0.5 ppm. Four grams of the wheat flower and 2 g of Hydromatrix (a dehydrating agent) were loaded in each extraction 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 wheat flour.
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, 61 exhibited more than 70% recovery, and 64 more than 50% recovery. The recovery of acetamiprid and methamidophos was as low as 39.4 and 15.6%, respectively. Acetamiprid and methamidophos, due to their high hydrophilicity, indicated low solubility in supercritical carbon dioxide, resulting in a poor recovery in SFE.

Table 1. Recovery of each pesticide.

No.	Pesticide	Recovery (%)	No.	Pesticide	Recovery (%)
1	Acephate	57.1	35	Isofenphos P=O	90.7
2	**Acetamiprid**	**39.4**	36	Isoprocarb	96.7
3	Bendiocarb	98.7	37	Lenacil	80.9
4	Bitertanol	91.4	38	Malathion	94.7
5	Butylate	94.0	39	Mefenacet	93.3
6	Captan	90.2	40	Mepronil	97.3
7	Carbaryl	98.6	41	**Methamidophos**	**15.6**
8	Chinomethionat	103.7	42	Metolachlor	94.4
9	Chlorfenvinphos	108.5	43	p,p'-DDE	100.0
10	Chlorpyriphos	102.7	44	Paclobutrazol	87.5
11	Cyfluthrin	105.1	45	Pencycuron	88.5
12	Cypermethrin	99.0	46	Pendimethalin	110.8
13	Deltamethrin	85.3	47	Permethalin	97.8
14	Diazinon	92.5	48	Phenthoate	102.6
15	Dichlofluanid	85.6	49	Phosalone	101.0
16	Dichlorvos	87.2	50	Pirimifos-methyl	97.0
17	Diethofencarb	99.0	51	p,p'-DDD	101.6
18	Dimethylvinphos	96.8	52	Pretilachlor	97.5
19	EPN	89.9	53	Propiconazole	103.0
20	Esprocarb	102.0	54	Pyraclofos	112.5
21	Ethiofencarb	93.4	55	Pyridaben	91.9
22	Ethoprophos	100.9	56	Pyridaphenthion	92.1
23	Fenarimol	117.9	57	Pyrimidifen	113.2
24	Fenitrothion	110.8	58	Quinalphos	97.0
25	Fenobucarb	94.8	59	Tefluthrin	103.7
26	Fensulfothion	95.8	60	Terbucarb	97.6
27	Fenvalerate	103.7	61	Terbufos	99.6
28	Flucythrinate	103.9	62	Thenylchlor	91.0
29	Flusilazole	101.6	62	Tolclofos-methyl	103.9
30	Flutolanil	96.4	64	Triadimenol	86.5
31	Fluvalinate	114.4	65	α-BHC	99.3
32	Imibenconazole	101.0	66	β-BHC	96.9
33	Iprodione	109.3	67	γ-BHC	101.5
34	Isofenphos	99.5	68	δ-BHC	94.6

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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