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 requires a large volume 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 analysis of spinach 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.

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. The residue was dissolved in 2 mL of acetone containing 0.05% of PEG200 and PEG400. A portion of this solution was injected onto the GC.

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.

As shown in Table 1, among 68 components of the pesticides, 47 components exhibited more than 70% recovery, and 61 components more than 50% recovery. The recovery of acetamiprid and butylate was as low as 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.

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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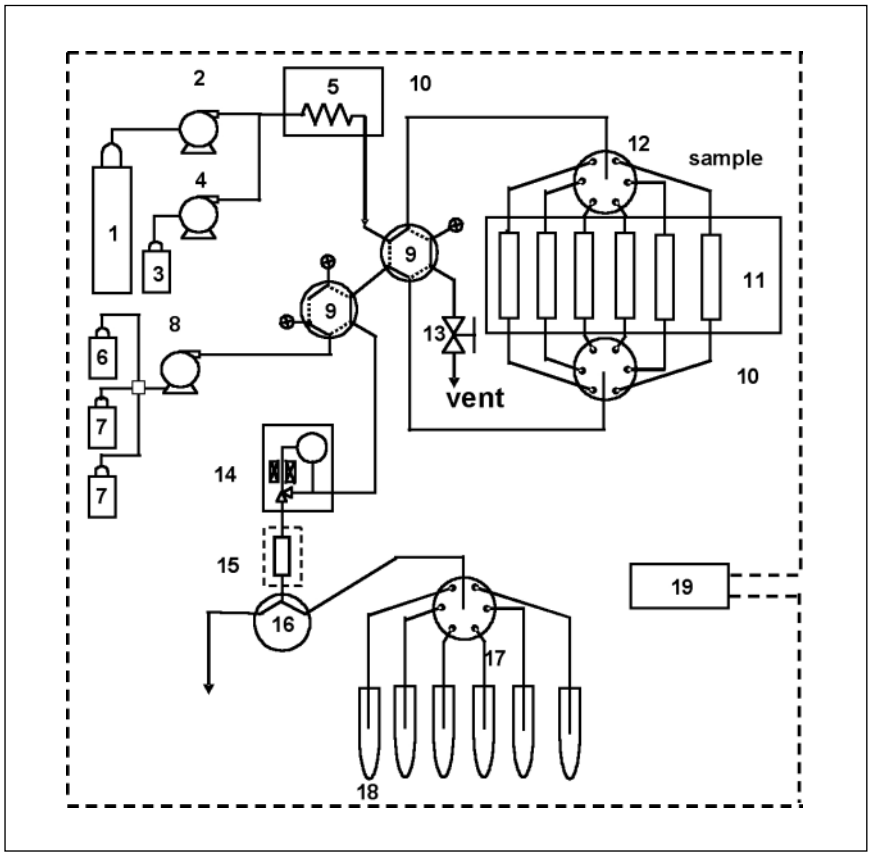

Figure 1 Schematic Diagram of fully automated system for supercritical fluid extraction of residual pesticides
System configuration: 1 = carbon dioxide cylinder, 2 = liquefied carbon dioxide delivery pump, 3 = modifier, 4 = modifier delivery pump, 5 = preheating coil, 6 = solvent for trap elution, 7 = 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 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 = 2mL/min).
Supercritical CO_2 delivered by pump 2 passes through one of the vessels 11 in which the sample is loaded and then pesticides are extracted. The extracted pesticides are concentrated by trap column and 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.

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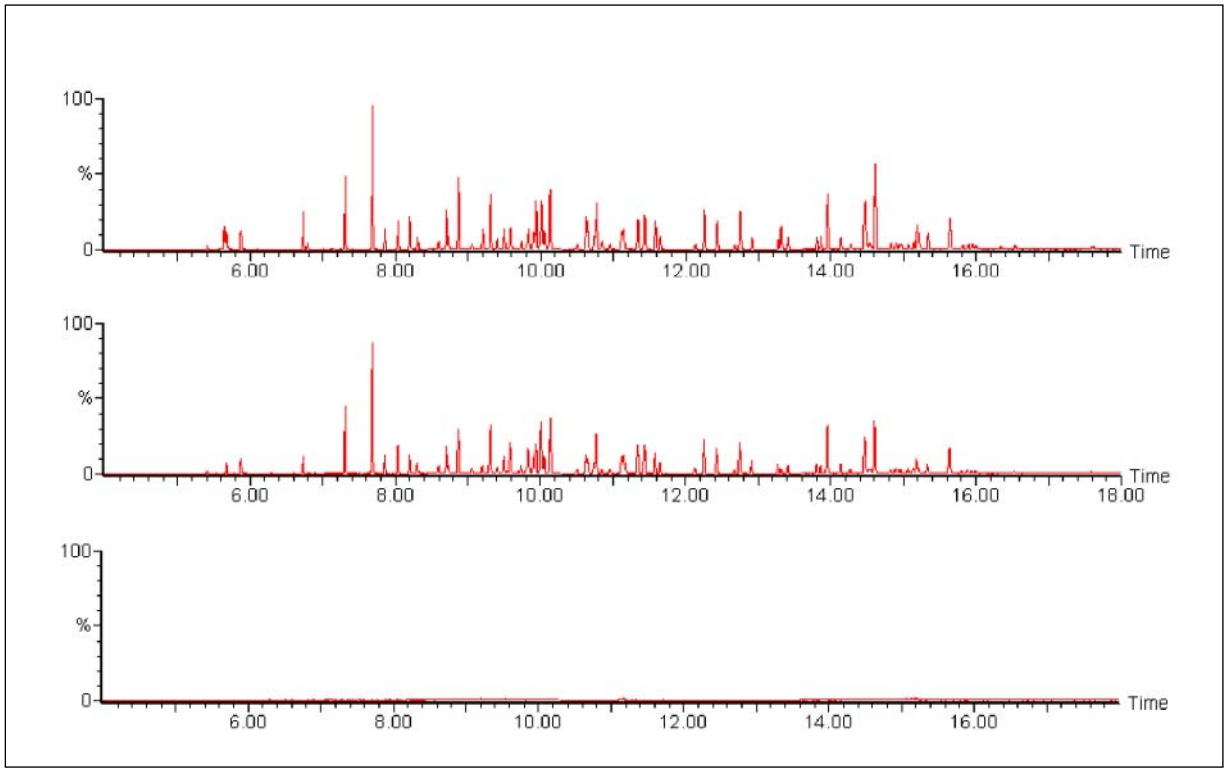

Figure 2 GC chromatograms of spinach sample. Upper：standard mixture (68 components), Middle： sample added with standard mixture ,and Lower：blank.
Measurement conditions：Instrument=Quattro micro GC (Waters micromass), Ionization method = EI, Measurement mode=MRM, SIM, Ionization source temperature=280 ºC, Interface temperature=280 ºC , GC=6890N(Agilent), Injection method=Splitless, Injection volume=1μL, Inlet temperature=250 ºC, Column＝DB-5MS(30m x 0.25mm), Column temperature=50 ºC(0 min) —50 ºC(1 min) — 200 ºC(7 min) — 250 ºC(9 min) — 300 ºC (11 min).
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: Flutoranil, 32: Imibenconazole, 33: Iprodione, 34: Isofenphos , 35: Isofenphos P=O, 36: Isoprocarb, 37: Lenacil, 38: Malathion, 39: Mefenacet, 40: Mepronil, 41: Methamidophos, 42: Metolachior, 43: p,p'-DDE, 44: Paclobutrazol, 45: Pencycuron, 46: Pendimethalin, 47: Permethalin, 48: Phenthoate, 49: Phosalone, 50: Pirimifos-methyl, 51: p,p'-DDD, 52: Pretilachior, 52: Pretilachlor, 53: Propiconazole, 54: Pyraclofos, 55: Pyridaben, 56: Pyridaphenthion, 57: Pyrimidifen, 58: Quinalphos, 59: Tefluthrin, 60: Terbucarb, 62: Thenylchlor,

63: Tolclofos-methyl, 64: Triadimenol, 65: α-BHC, 66: β-BHC, 67: γ-BHC, 68: δ-BHC .

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Table 1 The recovery of each pesticide

No	Pesticide	Recovery (%)	No	Pesticide	Recovery (%)	No	Pesticide	Recovery (%)
1	**Acephate**	**9.2**	24	Fenitrothion	107.8	47	Permethrin	57.3
2	**Acetamiprid**	**19.8**	25	Fenobucarb	89.0	48	Phenthoate	77.0
3	Bendiocarb	113.0	26	Fensulfothion	101.6	49	Phosalone	73.5
4	Bitertanol	64.0	27	Fenvalerate	64.5	50	Pirimifos-methyl	90.2
5	**Butylate**	**45.1**	28	Flucythrinate	56.9	51	p,p'-DDD	81.8
6	**Captan**	**3.8**	29	Flusilazole	86.5	52	Pretilachlor	88.9
7	Carbaryl	130.5	30	Flutolanil	92.1	53	Propiconazole	76.1
8	Chinomethionat	97.0	31	Fluvalinate	50.7	54	Pyraclofos	76.6
9	Chlorfenvinphos	76.1	32	Imibenconazole	63.2	55	Pyridaben	68.6
10	Chlorpyriphone	84.0	33	Iprodione	105.4	56	Pyridaphenthion	92.4
11	Cyfluthrin	59.1	34	isofenphos	57.1	57	Pyrimidifen	77.0
12	Cypermethrin	86.7	35	Isofenphos P=O	98.0	58	Quinalphos	82.9
13	Deltamethrin	57.8	36	Isoprocarb	89.1	59	Tefluthrin	64.5
14	Diazinon	78.6	37	Lenacil	79.9	60	Terbucarb	89.3
15	**Dichlofluanid**	**2.9**	38	Malathion	91.8	61	Terbufos	52.2
16	Dichlorvos	60.1	39	Mefenacet	87.4	62	Thenylchlor	96.4
17	Diethofencarb	101.5	40	Mepronil	75.7	63	Tolclofos-methyl	83.5
18	Dimethylvinphos	90.9	**41**	**Methamidophos**	**4.7**	64	Triadimenol	93.4
19	EPN	75.0	42	Metolachlor	97.7	65	α-BHC	92.0
20	Esprocarb	79.1	43	p,p'-DDE	71.7	66	β-BHC	92.7
21	Ethiofencarb	44.1	44	Paclobutrazol	111.6	67	γ-BHC	88.1
22	Ethoprophos	95.2	45	Pencycuron	59.9	68	δ-BHC	106.4
23	Fenarimol	73.0	46	Pendimethalin	95.6			

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