

Solid Phase Extraction of Pesticides from Fruits and Vegetables, for Analysis by GC or HPLC

Recovery rates for polar analytes, including carbamate and thiourea pesticides, are higher and less variable with ENVI-Carb carbon-based solid phase extraction tubes than with C8- or C18- silica-based tubes. Independent investigators found ENVI-Carb tubes to be an effective part of sample cleanup for more than 200 organochlorine, organophosphorus, nitrogen-containing, and carbamate pesticides, from many fruits and vegetables. ENVI-Carb tubes enabled these investigators to recover several pesticides they could not recover from charcoal/Celite minicolumns, and eliminated their labor-intensive minicolumn preparation process. Analysis for most of these pesticides is by GC; 10 carbamates are analyzed by HPLC.

Key Words:

- pesticides ● organochlorine pesticides
- organophosphorus pesticides
- nitrogen-containing pesticides ● carbamate pesticides
- sample preparation ● solid phase extraction

Concern over pesticide residues in fruits and vegetables has led to the development of many methods for monitoring these compounds. At the same time, regulatory agencies and concerned analysts are attempting to reduce the amounts of organic solvents used in sample preparation. Solid phase extraction (SPE) has proven very effective for cleaning, extracting and concentrating pollutants in analyses of environmental samples. Currently, many SPE methods for extracting pollutants from aqueous environmental samples employ octyl (C8) or octadecyl (C18) phases bonded to a silica support. Using these materials, nonpolar analytes can be recovered at high rates and with good reproducibility. Often, however, acidic, basic, and other polar analytes (e.g., carbamate and thiourea pesticides) are recovered at low rates when typical reversed phase extraction conditions are used.

Relative to traditional liquid-liquid extraction or SPE with C8 or C18 silica-based packings, SPE tubes containing ENVI™-Carb carbon-based packing provide superior, more uniform recovery of polar analytes, and comparable results for less polar compounds. Typical results for polar pesticides are summarized in Table 1.

Physical characteristics of ENVI-Carb carbon and C8- or C18-modified silica are listed in Table 2. Because the carbon-based packing is nonporous, samples can be processed rapidly – adsorption does not require dispersion of analytes into porous regions. Furthermore, although the surface area of the nonporous carbon is smaller than that of the porous silica (measured by

nitrogen BET), the carbon's capacity for pesticides is not compromised. The bed weight typically required is only one half that needed with the silica-based packings.

Table 1. Pesticides Recovery Is Highest Using ENVI-Carb SPE Tubes

Analyte	Recovery (mean % ± standard deviation)		
	Solid Phase Extraction ENVI-Carb [■] n=5	C8/C18 Silica* n=4	Liquid/Liquid Extraction* n=4
Oxamyl	95 ± 5	53 ± 1	55 ± 16
Methomyl	97 ± 5	43 ± 1	74 ± 8
Aldicarb	96 ± 3	67 ± 8	88 ± 8
Monuron	97 ± 4	90 ± 6	90 ± 4
Carbaryl	98 ± 5	74 ± 15	102 ± 13
Diuron	98 ± 6	90 ± 6	94 ± 3

1 liter water samples, HPLC/UV analyses

■ Data from Supelco laboratories.

* Data from B.E. Goodby, *Environmental Laboratory*, June/July 1990, pp 19-58.

Table 2. Physical Characteristics of ENVI-Carb Carbon and Silica-Based SPE Packings

ENVI-Carb Carbon	C8- & C18-Modified Silica
graphitized carbon black hydrophobic irregular particles, 40-100µm nonporous surface area: 100m ² /g	silane phase-modified silica gel hydrophobic irregular particles, 40-60µm porous (60-300Å) surface area: 400-600m ² /g

Investigators at Agriculture and Agri-Food Canada (Ottawa, Ontario) have developed a multiple-residue cleanup and analysis for monitoring more than 200 organochlorine, organophosphorus, nitrogen-containing, and carbamate pesticides in fruits and vegetables. Their flexible system allows rapid screening of "rush" samples (Table 3) as well as thorough cleanup of complex (high background) samples. Initially, these investigators used an extraction procedure that included extraction minicolumns containing a mixture of charcoal and Celite® (1), but ENVI-Carb tubes enabled them to recover several pesticides that they could not recover from the charcoal/Celite adsorbent. This led them to replace the charcoal/Celite minicolumns with ENVI-Carb SPE tubes, eliminating the labor-intensive process of preparing the minicolumns. The Canadian investigators' data in Table 4 summarize a comparison of pesticide recoveries from ENVI-Carb tubes and charcoal/Celite minicolumns, and include typical recovery rates from pesticide-spiked fruits and vegetables, using ENVI-Carb tubes. Analytes that could not be recovered from charcoal/Celite minicolumns are in bold print. A typical analysis, using C18, ENVI-Carb, and aminopropyl SPE tubes, is shown in Figure A.

Table 3. Extraction of Pesticides from Fruits and Vegetables ("Rush" Samples)

1. Homogenize 50g chopped sample with 100mL acetonitrile (e.g., Omni-mixer, half-speed, 5 min).
2. Add 10g sodium chloride (= 8mL in a graduated cylinder). Homogenize 5 min.[□]
3. Transfer ~13mL of acetonitrile (top) layer to 15mL graduated centrifuge tube.
4. Add ~3g sodium sulfate (liquid level to 15mL mark), cap, shake well to remove water.
5. Centrifuge at high speed for 5 min.
6. Transfer 10mL aliquot (= 5g of sample) to a clean 15mL tube.[▲] Evaporate to 0.5mL under **clean** nitrogen (water bath, 35°C).
7. Transfer to ENVI-Carb SPE tube (6mL tube, 500mg packing).[▼]
8. Elute pesticides with 20mL acetonitrile/toluene (3:1).[◆]
9. Using a rotary evaporator, concentrate sample to ~2mL. Add 2 x 10mL acetone, concentrating the material to ~2mL after each addition, to make a solvent exchange to acetone.
10. Transfer quantitatively to a clean 15mL tube. Add 50µL internal standard (50ng/µL cis-chlordane in acetone), then bring volume to 2.5mL with acetone (final concentrations = 2g/mL extract, 1.0ng/µL cis-chlordane).

GC/mass-specific detection (for organochlorine, organophosphorus, nitrogen-containing pesticides)

1. Set aside 0.5mL final extract for GC/MSD analysis. For chromatography, see reference 1.

HPLC/postcolumn derivatization/fluorescence detection (for carbamates)

1. Concentrate remaining 2.0mL final extract to 0.2mL.
2. Add 20µL internal standard (40ng/µL isoproc carb in methanol), then bring volume to 0.8mL with water (pH 3.0 with 36.5–38% HCl/water, 1:4), filter with 0.45µm pore filter (final concentration = 1.0ng/µL isoproc carb). For chromatography, see reference 2.

[□] The routine procedure includes a C18 clean-up step after step 2. Condition a 6mL/500mg Bond Elut C18 tube with acetonitrile followed by ~2mL aliquot of the acetonitrile layer (discard), then elute ~13mL of the acetonitrile layer into a 15mL graduated centrifuge tube. Proceed to step 4.

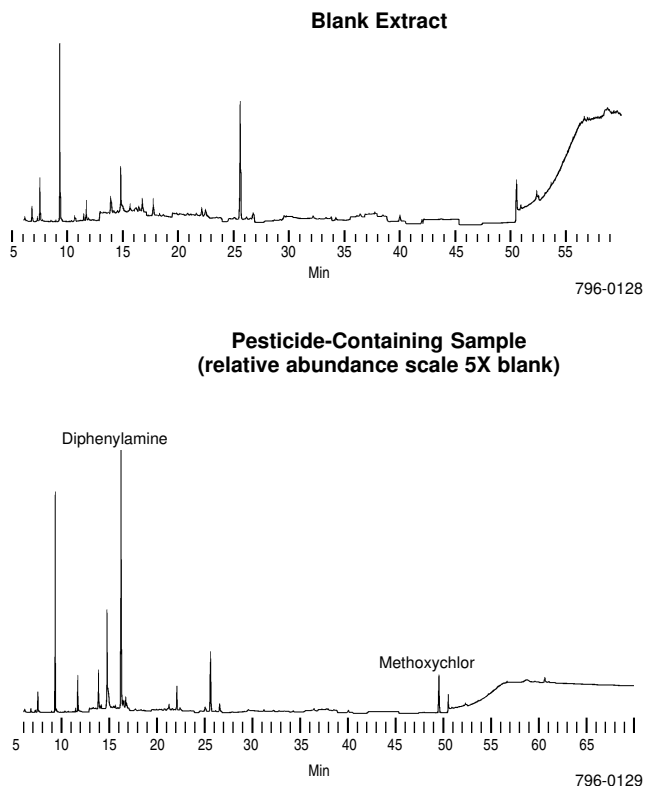
[▲] For rush samples, when analysis of carbamates is not required, use a 4mL aliquot.

[▼] In the routine procedure an aminopropyl Sep-Pak cartridge is coupled to the outlet of the ENVI-Carb tube.

[◆] If samples do not contain any of the following: acephate, bromophos-ethyl, Captan, chlorthiophos, Coumaphos, dichlofluanid, hexachlorobenzene, iodofenphos, leptophos, pyrazophos, quintozone, tolyfluanid, the volume of elution solvent can be reduced to 5mL.

In the preparation of a fruit or vegetable extract for multiple-residue analysis, additional purification may be necessary to reduce background in the chromatogram. Remove nonpolar interferences from the sample matrix by adding a C18 clean-up step (see footnotes to Table 3). Except for Mirex, recoveries are similar to those obtained by using an ENVI-Carb tube alone. Remove polar interferences by coupling an SPE tube containing an aminopropyl-silica adsorbent to the ENVI-Carb tube (see footnotes to Table 3). Except for Folpet, pesticide recovery is equal to recovery from an ENVI-Carb tube alone.

Figure A. Pesticides in Apple (Total Ion Chromatograms)



Chromatograms provided by J. Fillion, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario.

In the Canadian group's sample-screening applications, the analysis for organochlorine, organophosphorus, and nitrogen-containing pesticides (more than 200 compounds) is by capillary gas chromatography with mass-specific detection; residues are identified by retention time and ion ratios. Analysis for 10 carbamates is by HPLC with UV detection. Analytes monitored in each GC run (approximately 115 compounds and 80 compounds, respectively) are listed, along with recovery rates and limits of detection, in (1). Postcolumn derivatization and HPLC columns and conditions are described in (2).

Solid phase extraction of environmental samples on ENVI-Carb tubes offers several significant advantages over liquid/liquid extraction or solid phase extraction on silica-based packings. Relative to liquid/liquid extractions, SPE eliminates the need for expensive glassware, large volumes of solvent, and long preparation times. Visiprep™ vacuum manifolds automate the technique, for processing 1-12 or 1-24 samples simultaneously. Relative to SPE on silica-based SPE packings, ENVI-Carb tubes offer superior, more consistent recovery for a wide range of organic pollutants.

Table 4. Recovery of Pesticides, Using ENVI-Carb SPE Tubes

Analyte	Level (ppm)	Extraction Media Compared				Typical Recovery from ENVI-Carb Tubes (mean %)		
		ENVI-Carb Tube*		Charcoal/Celite**		Pineapple	Sweet Potato	Green Pea
		(mean % ± % C.V.)		(mean % ± % C.V.)				
Acephate	0.25	70	8	67	42	77	87	78
Alachlor	0.1	95	11	97	11	97	106	93
Aldrin	0.1	82	11	88	13	95	99	85
Allethrin	0.1	NA	NA	87	8	—	—	—
Ametryn	0.1	103	4	88	6	101	104	99
Aspon	0.1	82	17	91	16	100	104	88
Atrazine	0.1	91	9	98	16	83	109	100
Atrazine de-ethyl	0.1	85	11	93	14	75	105	101
Azinphos-ethyl	0.1	95	12	99	55	80	105	105
Azinphos-methyl	0.25	108	16	76	8	88	110	135
Benfluralin	0.1	81	14	82	12	95	101	88
Benzoylprop-ethyl	0.1	NA	NA	90	6	—	—	—
□-BHC	0.1	87	9	85	11	94	100	90
□-BHC	0.1	92	10	93	12	96	105	97
Bifenox	0.1	NA	NA	87	7	—	—	—
Bromacil	0.5	105	5	85	7	104	106	101
Bromophos	0.1	93	11	72	24	98	95	104
Bromophos-ethyl	0.1	94	12	95	13	101	105	90
Bromopropylate	0.1	100	9	89	6	96	85	100
Butralin	0.1	NA	NA	88	8	—	—	—
Butylate	0.1	67	11	59	25	66	84	68
Captafol	0.5	103	7	87	10	114	107	111
Captan	0.5	87	9	71	69	69	103	ND
Carbophenothion	0.1	95	13	93	16	97	113	89
Carboxin	0.1	80	21	53	36	91	95	40
Chlorbenside	0.1	90	15	75	20	97	102	90
trans-Chlordane	0.1	86	11	95	11	102	102	89
Chlordimeform	0.1	98	3	79	5	95	98	92
Chlorfenson	0.1	97	13	97	17	—	—	—
Chlorfenvinphos (e)	0.1	106	19	99	16	100	107	98
Chlorfenvinphos (z)	0.1	108	21	99	14	99	109	98
Chlorflurenol-methyl	0.1	105	5	80	6	105	104	104
Chlormephos	0.1	70	10	61	14	69	87	76
Chlorobenzilate	0.1	106	4	89	6	108	102	101
Chlorobromuron	0.25	96	7	120	17	ND	98	107
Chlorothalonil	0.5	87	12	NA	NA	99	109	ND
Chlorpropham	0.1	90	9	94	14	95	105	95
Chlorpyrifos	0.1	90	10	93	13	99	106	93
Chlorpyrifos-methyl	0.1	91	9	80	11	99	104	90
Chlorthal-dimethyl	0.1	94	11	98	12	99	107	97
Chlorthiophos	0.1	95	12	96	12	100	105	90
Chlozolinate	0.1	NA	NA	84	7	—	—	—
Clomazone	0.1	99	5	86	7	105	104	96
Coumaphos	0.25	107	9	79	6	96	101	113
Crotoxypfos	0.1	106	12	83	8	102	104	105
Crufomate	0.25	109	11	88	6	105	107	111
Cyanazine	0.1	87	8	96	14	98	108	89
Cyanophos	0.1	91	11	89	14	98	106	88
Cycloate	0.1	84	11	70	15	103	98	84
Cyfluthrin 1	0.5	98	3	83	7	108	108	86
Cyfluthrin 4	0.5	97	9	81	6	99	106	85
Cypermethrin 1	0.5	100	5	83	7	106	105	92
Cypermethrin 4	0.5	97	7	82	6	106	106	90
Cyprazine	0.1	104	4	90	7	99	104	101
4,4'-DDD	0.1	103	7	89	6	—	—	—
4,4'-DDE	0.1	90	13	94	14	100	105	85
2,4'-DDT	0.1	93	11	92	13	99	104	81
4,4'-DDT	0.1	89	13	91	17	97	106	79
Deltamethrin	0.5	99	13	79	7	101	124	88
Demeton	0.1	58	40	65	31	70	89	65
Demeton-S-methyl	0.1	91	4	72	15	102	104	73
Desmetryn	0.1	93	10	92	14	98	109	100
Dialifos	0.1	103	4	90	6	102	96	107
Di-allate 1	0.1	89	9	84	9	92	99	90
Di-allate 2	0.1	90	10	85	19	94	101	91
Diazinon	0.1	91	10	93	25	95	107	96
Dichlobenil	0.1	68	10	59	26	66	88	73
Dichlofenthion	0.1	89	10	92	10	98	105	94
Dichlofluanid	0.1	100	13	69	79	101	95	ND
Dichlormid	0.1	78	11	68	12	79	98	74

Table 4. Recovery of Pesticides, Using ENVI-Carb SPE Tubes (contd.)

Analyte	Level (ppm)	Extraction Media Compared				Typical Recovery from ENVI-Carb Tubes (mean %)		
		ENVI-Carb Tube* (mean % ± % C.V.)		Charcoal/Celite** (mean % ± % C.V.)		Pineapple	Sweet Potato	Green Pea
Dichlorvos-Naled	0.1	78	10	61	15	76	89	69
Dicloran	0.1	86	12	ND	ND	93	101?	103
Dicofol	0.1	95	15	82	17	100	106	95
Dicrotophos	0.1	90	11	93	27	100	106	100
Dieldrin	0.1	93	11	97	15	—	—	—
Dimethachlor	0.1	103	3	90	6	—	—	—
Dimethoate	0.1	89	8	95	17	100	104	100
Dinitramine	0.1	104	7	86	9	91	93	85
Dioxathion	0.1	88	8	92	10	96	106	93
Diphenamid	0.1	104	5	90	5	106	104	102
Diphenylamine	0.1	74	16	ND	ND	81	99	91
Disulfoton	0.1	64	35	76	13	94	92	74
Endosulfan-I	0.1	91	10	98	14	98	111	89
Endosulfan-II	0.1	96	13	99	13	99	105	90
Endosulfan sulfate	0.1	93	10	93	18	98	110	84
Endrin	0.1	97	12	97	14	91	103	91
EPN	0.1	101	13	96	22	96	111	94
EPTC	0.1	66	10	55	28	62	84	69
Erbon	0.1	101	9	76	9	104	69	90
Ethalfuralin	0.1	82	11	85	14	92	97	82
Ethion	0.1	99	14	95	19	98	107	89
Ethoprophos	0.1	96	7	84	9	107	104	91
Ethylan	0.1	101	8	88	7	106	102	92
Etridiazol	0.1	NA	NA	48	25	—	—	—
Etrimfos	0.1	102	3	86	8	106	104	93
Fenamiphos	0.1	106	14	79	11	102	105	96
Fenarimol	0.1	98	5	87	6	103	102	99
Fenchlorphos	0.1	90	10	72	20	98	104	86
Fenitrothion	0.1	102	16	76	39	102	110	92
Fenson	0.1	102	2	88	6	107	108	100
Fensulfothion	0.25	111	13	85	7	108	108	106
Fenthion	0.1	79	23	87	14	99	104	89
Fenvalerate 1	0.1	96	8	79	7	98	115	92
Fenvalerate 2	0.1	97	12	77	7	106	105	86
Flamprop-methyl	0.1	NA	NA	89	6	—	—	—
Fluchloralin	0.1	103	10	86	9	105	102	89
Folpet	0.5	91	12	73	81	92	108	ND
Fonofos	0.1	89	11	88	11	97	105	93
□-HCH	0.1	101	5	82	9	—	—	—
Heptachlor	0.1	83	11	83	19	94	101	86
Heptachlor epoxide	0.25	100	6	89	6	106	102	95
Heptanofos	0.1	95	5	82	9	104	103	91
Hexachlorobenzene	0.1	56	24	ND	ND	55	68	65
Hexazinone	0.1	97	12	101	21	90	104	101
Iodofenphos	0.1	103	16	69	32	95	105	80
Iprodione	0.1	105	6	78	7	109	113	83
Isofenphos	0.1	101	16	96	16	99	105	96
Leptophos	0.1	100	5	69	6	102	101	92
□-BHC (Lindane)	0.1	89	10	89	10	98	104	92
Linuron	0.5	100	8	106	12	102	94	105
Malaaxon	0.1	102	18	101	49	106	119	91
Malathion	0.1	100	17	92	22	101	109	93
Metalaxyl	0.1	95	10	99	13	98	105	101
Metazachlor	0.1	104	6	88	6	109	104	99
Methamidophos	0.5	73	2	62	7	72	79	65
Methidathion	0.1	107	22	90	18	94	107	90
Methoprotryn	0.1	108	7	85	6	102	104	102
Methoxychlor	0.1	95	12	97	15	98	107	91
Methyl Trithion	0.1	106	7	78	8	101	105	99
Metobromuron	0.1	105	9	95	10	114	109	102
Metolachlor	0.1	95	11	98	14	101	108	101
Metribuzin	0.1	91	9	65	34	74	98	91
cis-Mevinphos	0.1	89	9	88	18	98	104	89
trans-Mevinphos	0.1	91	9	91	16	98	107	90
Mirex	0.1	84	17	94	15	98	104	78
Monocrotophos	0.1	94	8	81	7	105	99	106
Monolinuron	0.1	101	7	94	9	101	106	95
Myclobutanil	0.1	105	6	87	6	105	104	102
Nitralin	0.1	104	11	101	8	104	91	98
Nitrapyrin	0.1	NA	NA	52	23	—	—	—

Table 4. Recovery of Pesticides, Using ENVI-Carb SPE Tubes (contd.)

Analyte	Level (ppm)	Extraction Media Compared				Typical Recovery from ENVI-Carb Tubes (mean %)		
		ENVI-Carb Tube* (mean % ± % C.V.)	Charcoal/Celite** (mean % ± % C.V.)		Pineapple	Sweet Potato	Green Pea	
Nitrofen	0.1	113	22	93	29	98	108	93
Nitrothal-isopropyl	0.1	NA	NA	86	8	—	—	—
Norflurazon	0.1	105	7	83	7	104	106	108
Omethoate	0.25	69	12	74	84	93	96	93
Oxadiazon	0.1	101	6	89	6	92	96	99
Oxycarboxin	0.1	93	27	87	5	101	93	100
Oxychlorthane	0.25	97	8	89	7	105	103	92
Oxyfluorfen	0.1	110	10	87	8	112	105	92
Paraoxon	0.25	107	8	84	7	111	109	98
Parathion	0.1	111	23	89	28	99	112	100
Parathion-methyl	0.1	93	15	73	14	96	110	91
Pebulate	0.1	69	18	53	24	93	89	76
Pendimethalin	0.1	106	8	88	8	—	—	—
cis-Permethrin	0.1	86	20	97	18	98	104	87
trans-Permethrin	0.1	87	19	97	20	95	104	87
Phenthoate	0.1	104	7	85	8	105	108	95
Phorate	0.1	75	21	77	11	88	102	85
Phosalone	0.1	94	12	98	17	95	109	93
Phosmet	0.1	92	12	88	26	93	111?	93
Phosphamidon	0.1	92	10	102	15	90	113	100
Pirimicarb	0.1	92	10	95	11	64	107	93
Pirimiphos-ethyl	0.1	93	11	95	21	98	107	97
Pirimiphos-methyl	0.1	93	11	94	15	94	107	94
Procymidone	0.1	98	15	99	13	99	105	95
Profenofos	0.1	109	21	97	19	100	115	97
Profluralin	0.1	82	12	87	23	95	103	90
Prometon	0.1	104	3	86	6	81	104	99
Prometryn	0.1	95	11	96	14	100	107	98
Propanil	0.1	103	5	ND	ND	134	107	103
Propargite	0.1	98	4	90	6	105	100	91
Propazine	0.1	90	9	98	18	87	107	97
Propetamphos	0.1	102	7	88	7	108	103	98
Propiconazole 1	0.1	104	6	85	7	107	103	104
Propiconazole 2	0.1	103	4	86	7	105	104	104
Propyzamide	0.1	91	10	93	9	94	108	100
Prothiofos	0.25	100	6	88	6	108	102	92
Pyrazophos	0.1	96	11	87	25	93	105	97
Quinalophos	0.1	107	19	97	13	98	108	99
Quintozene	0.1	85	9	82	15	91	103	91
Schradan	0.25	NA	NA	56	21	—	—	—
Simazine	0.1	100	16	95	2	80	110	103
Sulfotep	0.1	88	11	88	19	93	105	98
TCMTB	0.1	NA	NA	72	9	—	—	—
Tecnazene	0.1	80	10	71	18	81	98	84
Terbacil	0.25	104	4	87	6	97	106	101
Terbufos	0.1	82	11	84	9	93	101	89
Terbutryn	0.1	92	13	95	12	94	106	98
Terbutylazine	0.1	92	9	97	13	86	107	98
Tetrachlorvinphos	0.1	103	17	98	16	97	111	90
Tetradifon	0.1	95	12	100	19	98	104	97
Tetramethrin 1	0.1	104	6	86	6	101	103	103
Tetramethrin 2	0.1	103	6	87	7	105	103	100
Tolyfluanid	0.1	100	12	73	36	99	105	ND
Triadimefon	0.1	101	15	97	12	—	—	—
Triadimenol	0.1	103	7	86	6	107	101	103
Triallate	0.1	90	9	90	11	98	100	91
Triazophos	0.1	104	7	86	6	104	106	109
Trifluralin	0.1	81	13	84	16	96	103	87
Vernolate	0.1	67	16	52	23	96	89	76
Vinclozolin	0.1	93	11	97	12	101	104	97

Extraction procedure per Table 3, except steps 7 and 8 (ENVI-Carb vs charcoal/Celite):

*6mL ENVI-Carb SPE Tube (500 mg packing); eluant: 20mL acetonitrile/toluene 3:1; matrices: banana, pear; n = 6.

**2g charcoal/Celite (1:4); eluant: 50mL acetonitrile/toluene 3:1; matrices: banana, pear, carrot; n = 9 or 12.

NA – data not available

ND – not detected

Ordering Information:

Description	Cat. No.
ENVI-Carb Solid Phase Extraction Tubes	
3mL, 250mg packing, pk. of 54	57088
6mL, 250mg packing, pk. of 30	57092
6mL, 500mg packing, pk. of 30	57094
AutoTrace SPE Tube Adapters	
For using ENVI-Carb tubes with Zymark® AutoTrace unit.	
3mL, pk. of 6	57123
6mL, pk. of 6	57126
Visiprep Vacuum Manifolds[▲]	
For processing 1-12 or 1-24 samples simultaneously. For details, and descriptions of other models, refer to the current Supelco catalog and request publication 495121.	
12-Port model	57030-U
24-Port model	57250-U
12-Port model with disposable valve liners	57044
24-Port model with disposable valve liners	57265

[▲]US Pat. Nos. D. 289,861; 4,810,471; other patents pending.

For our complete list of solid phase extraction tubes, request publication 412130.

References

1. Fillion, J. R. Hindle, M. Lacroix, and J. Selwyn, *Journal AOAC International*, **78**: 1252-1266 (1995).
 2. Chaput, D., *J. Assoc. Official Analytical Chemists*, **71**: 542-546 (1988).
- References not available from Supelco.

Acknowledgment

The extraction process in Table 3 and data in Table 4 were provided by J. Fillion and J.C. Selwyn, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario. The chromatograms in Figure A were provided by J. Fillion.

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