Matrix Behavior during Sample Preparation Using Metabolomics Analysis Approach for Pesticide Residue Analysis by GC-MS in Agricultural Products

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ABSTRACT: The detailed matrices and their behaviors during pesticide residue analyses were clarified using a metabolomics analysis approach. The matrix profile was investigated using two different extraction solvents, acetone and acetonitrile. Acetone extracted the matrix components with a wide range of log $P_{O/W}$ values. Components with log $P_{O/W}$ values >10, such as sterols and tocopherols, and components with log $P_{O/W}$ values <3.2 were more extracted by acetone than by acetonitrile. In contrast, components with log $P_{O/W}$ values in the range from 3.2 to 10 were extracted by both acetone and acetonitrile at the same concentration level. The study also examined the difference in the column cleanup efficiency using a solid phase extraction (SPE). Florisil, silica gel, NH$_2$, PSA, and GCB were selected as representative columns for pesticide residue analysis, and acetone extraction of brown rice was selected in this experiment. Most of the matrix components were removed by either column, whereas monoacylglycerols, which are the components causing the matrix effect, were not removed by any column. Understanding such a detailed matrix behavior helps to develop a better analytical method for pesticide analysis using GC-MS.

KEYWORDS: pesticide residue analysis, matrix component, agricultural products, metabolomics, GC-MS

■ INTRODUCTION

An analytical method for pesticide residues in foods comprising sampling, extraction, and column cleanup steps has been established over the years.[1] Acetone, acetonitrile, or methanol has been used as the extraction solvent because these solvents are easily miscible with the agricultural products and penetrate the tissue of the samples. Liquid/liquid extraction (e.g., n-hexane, ethyl acetate, methylene chloride/water) is used to extract the pesticides and remove the polar matrices such as sugars. n-Hexane/acetonitrile extraction is used to remove lipids. Many types of columns can be selected on the basis of the characteristics of the measured compounds and sample matrices. Florisil and silica gel columns have been widely used in the individual method by the Japanese Ministry of Health, Labour and Welfare (MHLW)[2] to remove polar matrices. Sometimes, other characteristic columns, such as a silver nitrate containing column to remove sulfur compounds from onion or garlic, have been used.[1] Since around 2000, GC-MS has been widely used, because it provides the simultaneous determination and confirmation of a large number of pesticides instead of using different types of GC detectors. At the same time, the miniaturization of the sample preparation using a minicolumn proceeded. Cairn et al. reported that an octadecyl (C18) column, anion-exchange column, and aminopropyl (NH$_2$) column removed the majority of hydrocarbon-like molecules, colored compounds and flavors, and sugars, respectively.[3] Fillion et al. also applied C18 and NH$_2$ columns for 251 pesticides.[4] Akiyama et al. demonstrated that an ethylenediamine-N-propylsilanized silica gel column (PSA) and NH$_2$

column removed fatty acid and chlorophyll. They adopted the PSA column instead of the NH$_2$ column because the recovery rates of some pesticides, having an acid–amide bond in their structure, were low.[5] Ueno et al. used gel permeation chromatography (GPC) before the solid phase extraction (SPE) cleanup because GPC separated the compounds by molecular weight.[6] Recently, the QuEChERS method that was developed by Anastassiades et al. has been widely used. They reported that dispersive-SPE with PSA was used to remove organic acids, polar pigments, and sugars,[7] and this method was validated for 229 pesticides by Lehotay et al.[8] Okihashi et al. developed a modified QuEChERS method and used the SPE column cleanup instead of the dispersive-SPE.[9]

In all of the studies, the important things were how the multiresidue analysis was performed, how the limit of detection (LOD) and the limit of quantitation (LOQ) were reduced, and how the sample preparation was simplified. Although they mentioned which matrices were removed by which cleanup, they did not indicate the specific component's name. They saw the disappearance of the color using graphite carbon black (GCB) or a decrease in the peak of the fatty acid as a chromatographic interference. They might also estimate the

Table 1. Characteristics of Each SPE

name	filler	filler wt (mg)	reaction	common elute solvent
C18	octadecyl	1000	reverse phase and distribution	acetonitrile
silica gel	silica gel	690	normal phase and adsorption	acetone/n-hexane, ethyl acetate, etc.
Florisil	magnesium silicate	910	normal phase and adsorption	acetone/n-hexane, ethyl acetate, etc.
NH$_2$	aminopropylsilanized silica gel	360	ion exchange, normal phase, and distribution	acetone, ethyl acetate, etc.
PSA	ethylenediamine-N-propylsilanized silica gel	500	ion exchange, normal phase, and distribution	acetone, ethyl acetate, etc.
GCB	graphite carbon	500	adsorption	acetonitrile/toluene, acetone, etc.

removal matrices theoretically on the basis of their physical and chemical properties.

In the multiresidue analysis of pesticides, large matrix interference on the chromatographic peaks and matrix enhancement effect[10−18] are problems. The matrix enhancement effect means that the response of pesticides in foods is higher than that in the matrix-free standard solution. These phenomena cause difficulty in the accurate quantification of the pesticides. Although GC-MS/MS has been widely used for its selectivity in the past few years, the matrix enhancement effect remains.[19,20] The matrix effect is caused by the adsorption of the pesticides on the active sites in the injection port,[11,13] column, and ion source.[21]

Meanwhile, a metabolomics analysis is the metabolic profiling of metabolites from polar compounds, such as sugars, organic acids, and amino acids, to mid-low polar compounds, such as fatty acids and sterols in cells of all organisms (e.g., plants, humans, microbes). For all of these compounds, GC-MS is one of the most popular techniques because GC-MS is a robust and highly sensitive method with many databases.[22−26] The peak detection software, such as the Automated Mass Spectral Deconvolution and Identification System (AMDIS) by the National Institute of Standards and Technology (NIST), accelerated the multitarget metabolic profiling analysis using GC-MS for complex biological samples. Fiehn et al. identified the metabolites in *Arabidopsis* using GC-MS and AMDIS.[23] The general metabolic profiling analysis procedure[22] is as follows: (1) The samples are extracted with a mixture of methanol, water, and chloroform or with a mixture of acetonitrile, water, and isopropanol, etc. (2) The dried samples are methoxyaminated, followed by trimethylsilylation. (3) GC-MS analysis is performed followed by (4) data analysis.

If the detailed matrices, which interfere with the pesticide chromatographic peaks or which cause the matrix effect, are determined, we can efficiently remove them. In the present study, we focused on a metabolomics analysis approach to identify the matrix components in each step of the sample preparation.

We first studied the matrix profiling of sample solutions extracted by two different popular solvents, acetone and acetonitrile, using three different types of agricultural products, that is, spinach, orange, and brown rice. These samples are representative agricultural products based on the "the validation guideline for pesticide residue analysis in foods" by the Ministry of Health, Labour and Welfare (MHLW), Japan.[27] Acetone is used in the method[28] by the U.S. Food and Drug Administration (FDA) and in the Notification method by the MHLW.[2] These methods are based on the reports by Luke et al.[29,30] On the other hand, acetonitrile was adopted by the California Department of Food and Agriculture (CFDA)[28] and has been used in the Japanese Positive List System (PLS) for multiresidue analysis.[31] These methods are based on the report by Fillion et al.[4,32] According to *The Merck Index*,[33] acetone is a

solvent for extracting fats, oils, waxes, resins, rubber, plastics, etc. Acetonitrile is miscible with water, methanol, methyl acetate, ethyl acetate, and acetone but immiscible in many saturated hydrocarbons. This means that acetone easily dissolves many polar to nonpolar compounds, whereas acetonitrile dissolves most compounds except for the nonpolar compounds.

The next experiment was the column cleanup efficiency using different types of SPE cartridge columns, that is, Florisil, silica gel, NH$_2$, PSA, and GCB columns, which are commonly used for pesticide residue analyses. The brown rice extract by acetone was selected in this experiment.

The purpose of this study was to investigate whether the metabolomics analysis approach could be applied to identify the matrix components and to know the matrix behavior during the sample preparation using common solvents and SPE columns.

■ EXPERIMENTAL METHODS

Reagents and Apparatus. Acetone and acetonitrile, high-purity grades for pesticide residue analysis, were obtained from Wako Pure Chemical Industries (Osaka, Japan). Methoxyamine hydrochloride and pyridine were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Thermo Fisher Scientific (Rockford, IL, USA).

The Sep-Pak plus silica gel column (filler weight = 690 mg), Florisil column (910 mg), and NH$_2$ column (360 mg) were obtained from Waters (Milford, MA, USA). The Bond elute C18 column (filler weight/column size = 1 g/6 mL), PSA column (500 mg/6 mL), and GCB column (500 mg/6 mL) were obtained from Agilent Technologies (Lake Forest, CA, USA). The characteristics of each SPE are listed in Table 1.

The spinach and orange were purchased from local food stores in Japan. The brown rice was harvested in Ibaraki prefecture, Japan.

Sample Preparation for Experiment 1. We selected acetone and acetonitrile as the representative extraction solvents for the pesticide residue analysis. The sample solution after extraction by acetone is usually evaporated to dryness. On the other hand, the sample solution after being extracted by acetonitrile is easily separated from the water layer by adding sodium chloride.[31] In this experiment, because the purpose was to compare the difference between the two extractive solvents, the same analytical method was performed, and the C18 cartridge column was used instead of liquid−liquid extraction.[34,35] Twenty gram (wet weight) aliquots of homogenized spinach and orange were extracted with 100 mL of acetone or acetonitrile using a homogenizer for 3 min. A 10 g (dry weight) sample of homogenized brown rice was extracted with 100 mL of acetone and acetonitrile after soaking in 20 mL of water for 15 min. For the dried samples, the samples are soaked in water to efficiently extract the pesticides.[31] The mixture was then filtered by vacuum suction. The residual cake was washed with 50 mL of the solvent and filtered. The filtrates were combined and concentrated by a rotary evaporator in a water bath below 40 °C. After a C18 cartridge column had been conditioned with 5 mL of acetonitrile and 5 mL of water, the concentrated sample solution (adjusted to 20 g by adding water) was loaded on the column. The inside of the flask was washed with 5 mL of water/acetonitrile

Table 2. Matrix Components in Spinach Extraction

compound	mol formula	CAS Registry No.	log $P_{O/W}$	group	concentration[a] acetone	concentration[a] acetonitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
myristic acid	$C_{14}H_{28}O_2$	544-63-8	5.79	fatty acid	B	B
pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	6.30	fatty acid	B	B
palmitoleic acid	$C_{16}H_{30}O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	A	A
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.96	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
stearic acid	$C_{18}H_{36}O_2$	57-11-4	8.22	fatty acid	C	C
palmiteladic acid	$C_{16}H_{30}O_2$		fatty acid		B	B
11-eicosenoic acid	$C_{20}H_{38}O_2$	2462-94-4	8.44	fatty acid	C	C
linolenic acid, methyl ester	$C_{19}H_{32}O_2$	301-00-8	6.96	fatty acid, ester	C	C
myristin, 1-mono-	$C_{17}H_{30}O4$	589-68-4	5.05	monoacylglycerol	C	C
linolenin, 1-mono-	$C_{21}H_{36}O_4$	18465-99-1	5.41	monoacylglycerol	B	B
palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	B	B
palmitin, 1-mono-	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	A	A
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	A	A
olein, 2-mono-	$C_{21}H_{40}O_4$	3443-84-3	6.94	monoacylglycerol	A	A
stearin, 2-mono-	$C_{21}H_{42}O_4$	621-61-4	7.46	monoacylglycerol	B	B
4-vinylguaiacol	$C_9H_{10}O_2$	7786-61-0	1.93	terpenoid	B	C
phytol	$C_{12}H_{40}O$	150-86-7	8.23	terpenoid	B	B
β-tocopherol	$C_{28}H_{48}O_2$	148-03-8	10.72	tocopherol	<1	<1
α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	B	ND
stigmasterol	$C_{29}H_{48}O$	83-48-7	10.07	sterol	C	C
glucose	$C_6H_{12}O_6$	50-99-7	−2.49	sugar	B	B
fructose	$C_6H_{12}O_6$	57-48-7	−1.47	sugar	B	B
coumaran	C_8H_8O	496-16-2	2.14		C	<1

[a]A, ≥1000 mg/kg; B, ≥10 mg/kg, <1000 mg/kg; C, ≥1 mg/kg, < 10 mg/kg; <1, <1 mg/kg.

(80:20, v/v), and this rinse was also passed through the column and then discarded. The column was vacuum-dried for 1 min. A 10 mL aliquot of acetonitrile was passed through and collected. The eluate was evaporated to dryness under 40 °C, and the residue was dissolved in 1 mL of acetone. These sample solutions were then used for the next step, derivatization.

Sample Preparation for Experiment 2. A 25 g aliquot of homogenized brown rice was extracted with 250 mL of acetone after soaking in 50 mL of water for 15 min. The mixture then was filtered by vacuum suction. The residue was extracted again with 50 mL of acetone. The residual cake was washed with 100 mL of acetone and filtered. Acetone was added to the filtrates to make a 500 mL solution, and then 80 mL (equivalent to a 4 g sample) was measured for the column cleanup experiment. Each sample solution was concentrated by rotary evaporation in a water bath below 40 °C. The procedure for the C18 column was the same as for experiment 1. After passing through the C18 column, the eluate was evaporated to dryness under 40 °C, and the residue was applied to a cleanup test using each SPE. The residue, which was passed only through the C18 cartridge column, was the control sample. The samples treated with each column cleanup were compared to the control samples.

After the silica gel and Florisil columns were rinsed with 5 mL of n-hexane, the residues were loaded. SPE was done as follows: fraction-1 (Fr-1), n-hexane, 5 mL; Fr-2, acetone/n-hexane (5:95, v/v); Fr-3, acetone/n-hexane (15:85, v/v); Fr-4, acetone/n-hexane (50:50, v/v). As for the NH$_2$ and PSA columns, the columns were rinsed with 5 mL of acetone, and then the residues were applied and eluted with 5 mL of acetone. As for the GCB column, the column was rinsed with 10 mL of acetonitrile/toluene (75:25, v/v), and then the residue was applied and eluted with 10 mL of acetonitrile/toluene (75:25, v/v). All of the eluates were evaporated to dryness under 40 °C, and then the residues

were dissolved in 200 μL of acetone. These sample solutions were then followed by derivatization.

To calculate the cleanup efficiency, the samples (n = 2) treated by each column were compared to the control samples (passed only through the C18 column). The formula for the elution rate from each column is as follows:

$$\text{elution rate } (\%) = (\text{mean intensity value of eluted matrix}$$
$$\text{component from each column}$$
$$/\text{mean intensity value of components passed}$$
$$\text{through the C18 column}) \times 100$$

Derivatization. All samples were analyzed by GC-MS with derivatization. The derivatization procedure was applied using a metabolomics technique.[22,36] Methoxyamination was performed prior to the trimethylsilylation, and this was done to protect the carbonyl groups because α-keto acids tend to undergo chemical loss of carboxyl groups as carbon dioxide if the keto group is left unprotected. The hydrophilic functional groups (e.g., carboxyl, hydroxyl, amino, immino, or sulfuryl groups) are trimethylsilylated to remove the hydrogen bond formations to increase the volatility. This also reduces any interaction with the column phase that can cause tailing peaks, a poor sensitivity, and poor chromatographic separation. For derivatization, 100 μL of the sample solution was dried by a centrifugal concentrator. The residue was then methoxyaminated using 10 μL of methoxyamine hydrochloride in pyridine (40 mg/mL) and stored at 30 °C for 90 min. For the trimethylsilylation, after the addition of 90 μL of N-methyl-N-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane (MSTFA + 1% TMCS) to the methoxyaminated samples, the mixture was stored at 37 °C for 30 min.

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Table 3. Matrix Components in Orange Extraction

compound	mol formula	CAS Registry No.	log $P_{O/W}$	group	concentration[a] acetone	concentration[a] acet0nitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
myristic acid	$C_{16}H_{32}O_2$	544-63-8	5.79	fatty acid	B	B
pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	6.30	fatty acid	C	C
palmitoleic acid	$C_{16}H_{30}O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	A	A
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.81	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
oleic acid	$C_{18}H_{34}O_2$	112-80-1	7.42	fatty acid	A	A
decanal	$C_{10}H_{20}O$	112-31-2	4.09	aliphatic aldehyde	C	C
dodecanal	$C_{12}H_{24}O$	112-54-9	5.16	aliphatic aldehyde	C	C
p-coumaric acid	$C_9H_8O_3$	7400-08-0	1.01	aromatic carboxylic acid	A	B
benzoic acid	$C_7H_6O_2$	65-85-0	1.56	aromatic carboxylic acid	B	C
4-vinylguaiacol	$C_9H_{10}O_2$	7786-61-0	1.93	aromatic carboxylic acid	B	C
3′,5′-dimethoxyacetophenone	$C_{10}H_{12}O_3$	39151-19-4	1.9	aromatic ether, ester	C	C
4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$		0.92	aromatic alcohol, ether	B	C
trans-ferulic acid	$C_{10}H_{10}O_4$	537-98-4	0.96	aromatic alcohol, ether	A	<1
(−)-cis-carane	$C_{10}H_{18}$	2778-68-9	4.77	hydrocarbon	<1	C
3,3′,4′,5,5′,7,8-heptamethoxyflavone	$C_{22}H_{24}O_9$		1.35	flavonoid	A	A
flavone, 3,3′,4,5,5′,7-hexamethoxy-	$C_{21}H_{22}O_8$	14813-27-5	2.49	flavonoid	A	A
4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-	$C_{20}H_{20}O_8$	479-90-3	2.55	flavonoid	B	B
hesperetin	$C_{16}H_{14}O_6$	520-33-2	2.90	flavonoid	B	B
4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-	$C_{20}H_{20}O_7$	2306-27-6	3.08	flavonoid	B	C
naringenin	$C_{15}H_{12}O_5$	480-41-1	3.19	flavonoid	B	<1
flavone, 4′,5,6,7-tetramethoxy-	$C_{19}H_{18}O_6$	1168-42-9	3.26	flavonoid	C	<1
5,5′-dimethoxy-3,3′-dimethyl-2,2′-binaphthalene-1,1′,4,4′-tetrone	$C_{24}H_{18}O_6$	54215-49-5	4.70	flavonoid	B	<1
4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-	$C_{20}H_{20}O_7$	2306-27-6		flavonoid	B	C
myristin, 1-mono-	$C_{17}H_{30}O4$	589-68-4	5.05	monoacylglycerol	C	C
linolenin, 1-mono-	$C_{21}H_{36}O_4$	18465-99-1	5.41	monoacylglycerol	C	C
palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	C	C
palmitin, 1-mono-	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	B	B
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	B	B
olein, 2-mono-	$C_{21}H_{40}O_4$	3443-84-3	6.94	monoacylglycerol	B	B
limonin	$C_{26}H_{30}O_8$	1180-71-8	1.66	terpenoid	B	B
2-cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	$C_{10}H_{14}O$	16750-82-6	2.15	terpenoid	C	<1
limonene oxide, trans-	$C_{10}H_{16}O$	6909-30-4	2.43	terpenoid	C	C
4-terpinenol	$C_{10}H_{18}O$	562-74-3	2.54	terpenoid	C	<1
p-mentha-1(7),8(10)-dien-9-ol	$C_{10}H_{16}O$	29548-13-8	2.65	terpenoid	C	C
perilla aldehyde	$C_{10}H_{14}O$	2111-75-3	2.68	terpenoid	C	C
α-terpineol	$C_{10}H_{18}O$	98-55-5	2.79	terpenoid	B	C
bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	$C_{15}H_{24}O_2$		2.88	terpenoid	C	C
α-citral	$C_{10}H_{16}O$	141-27-5	3.17	terpenoid	B	B
linalool	$C_{10}H_{18}O$	78-70-6	3.28	terpenoid	B	B
citronellol	$C_{10}H_{20}O$	26489-01-0	3.38	terpenoid	B	C
(R)-(+)-citronellal	$C_{10}H_{18}O$	2385-77-5	3.48	terpenoid	C	B
3,4-2H-coumarin, 4,4,5,6,8-pentamethyl-	$C_{14}H_{18}O_2$		3.74	terpenoid	C	C
nootkatone	$C_{15}H_{22}O$	4674-50-4	3.84	terpenoid	B	B
2-hexenoic acid, butyl ester, (E)-	$C_{10}H_{18}O_2$	54411-16-4	3.97	terpenoid	B	C
nerol acetate	$C_{12}H_{20}O_2$	141-12-8	4.1	terpenoid	C	C
geraniol acetate	$C_{12}H_{20}O_2$	105-87-3	4.1	terpenoid	C	C
limonene	$C_{10}H_{16}$	138-86-3	4.45	terpenoid	B	B
β-eudesmol	$C_{15}H_{26}O$	473-15-4	4.68	terpenoid	B	B

Table 3. continued

compound	mol formula	CAS Registry No.	log $P_{O/W}$	group	concentration[a] acetone	concentration[a] acet0nitrile
α-sinenasal	$C_{15}H_{22}O$	4955-32-2	4.86	terpenoid	C	C
farnesol	$C_{15}H_{26}O$	4602-84-0	5.31	terpenoid	B	B
trans,trans-farnesol	$C_{15}H_{26}O$	106-28-5	5.31	terpenoid	B	B
farnesol, acetate	$C_{17}H_{28}O_2$		6.14	terpenoid	C	C
$(-)$-α-panasinsen	$C_{15}H_{24}$	56633-28-4	6.36	terpenoid	C	C
valencene	$C_{15}H_{24}$	4630-07-3	6.49	terpenoid	B	A
β-elemene, $(-)$-	$C_{15}H_{24}$	110823-68-2	6.63	terpenoid	C	B
caryophyllene	$C_{15}H_{24}$	87-44-5	6.78	terpenoid	C	C
δ-cadinene, $(+)$-	$C_{15}H_{24}$	483-76-1	6.83	terpenoid	C	B
eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	473-04-1		terpenoid	C	C
β-cuvebene	$C_{15}H_{24}$	13744-15-5		terpenoid	C	C
β-tocopherol	$C_{28}H_{48}O_2$	148-03-8	10.72	tocopherol	C	C
α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	C	C
sucrose	$C_{12}H_{22}O_{11}$	57-50-1	−4.49	sugar	B	B
glucose	$C_6H_{12}O_6$	50-99-7	−2.49	sugar	B	B
fructose	$C_6H_{12}O_6$	57-48-7	−1.47	sugar	B	B
4,10-(methanoxymethano)-10*H*-cyclopenta[*a*]phenanthren-3(4*H*)-one, 17-(acetyloxy)-1,2,7,8,9,11,12,13,14,15,16,17-dodecahydro-13-methyl-, [4*S*-(4α,8α,9β,10α,13α,14β,17α]-	$C_{22}H_{30}O_4$	56786-53-9	−1.47		B	C
obacunone	$C_{26}H_{30}O_7$	751-03-1	2.91		C	<1
hedycaryol	$C_{15}H_{26}O$	21657-90-9	5.20		C	C
chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	**2921-88-2**	**4.77**	**pesticide**	**C**	**C**

[a]A, ≥1000 mg/kg; B, ≥10 mg/kg, <1000 mg/kg; C, ≥1 mg/kg, <10 mg/kg; <1, <1 mg/kg.

GC-MS Conditions. A GC-MS analysis was performed using an Agilent 7890A GC system coupled to an Agilent 5975C TAD mass spectrometer (Little Falls, DE, USA). The sample solutions were injected with 1 μL in the split mode (split ratio was set at 10:1) by an Agilent 7693 autoinjector. The inlet temperature was 250 °C. An Agilent fused silica capillary column, DB-5 msDG (5% phenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, with a 10 m nonpolar deactivated precolumn directly connected to the DB-5 ms (Folsom, CA, USA)) was used. After the sample injection, the oven temperature was maintained at 60 °C for 1 min and then ramped to 325 at 10 °C/min. The quadrupole was operated in the scan mode (*m/z* 50−600). The transfer line, ion source, and quadrupole were set at 290, 250, and 150 °C, respectively. Myristic acid-d_{27} was locked at 16.752 min to use the Fiehn metabolomics library (Agilent Technologies, Inc.).[36]

Identification. All data were deconvoluted by AMDIS and automatically identified the deconvoluted spectra by Fiehn metabolomics library.[36] The Fiehn metabolomics library was created by Professor Oliver Fiehn[37] and includes around 1000 metabolites with both mass spectra (derivatized by methoxyamination and trimethylsililation) and "RI Calibration Data". The "RI Calibration Data" is the calibration file between the RI of the fatty acid methyl ester (FAME) and retention time (RT). If the matching scores of the target compounds were low or there were identified compounds, a library search was done by a NIST search directly from AMDIS for more identification. W9N08 (combined library Wiley9 and NIST08, Agilent Technologies, Inc.) and the free database from the Max Planck Institute of Molecular Plant Physiology[38] were added to the NIST search program. Although Wiley9 and NIST08 contain many derivatized compounds, the registered name is the trimethylsilylated name (e.g., hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester). We drew the structure without derivatization and confirmed it again by its formula in the NIST program. The "Chemistry of Organic Natural Resources" was also used for more confirmation.[39]

■ **RESULTS AND DISCUSSION**

Experiment 1. Difference between Two Extractive Solvents, Acetone and Acetonitrile. Highly concentrated

compounds, such as fatty acids, flavonoids, sterols, and terpenoids, were found in the samples. The mean semi-quantified values of five replicates (*n* = 5) were calculated by the area of myristic acid-d_{27}. All results are shown in Tables 2−4.

Some fatty acids, such as palmitic acid and linoleic acid, were extracted at several hundred milligrams per kilogram from all samples. According to the standard tables of food composition in Japan,[40] palmitic acid, oleic acid, and linoleic acid are contained at 5200, 8000, and 8600 mg/kg, respectively, in the case of brown rice. Although around 90% of the fatty acids was removed by solvent extraction and a C18 column, excess fatty acids remained at high concentrations in the extracted solutions.

Although pesticides have a wide variety of properties and cannot be completely explained by their *n*-octanol/water partition coefficient (log $P_{O/W}$) value, the log $P_{O/W}$ value is sometimes used to help characterize the pesticides.[35,41] Because the matrix components have a wide range of properties as well as pesticides, the polarity of the components, the log $P_{O/W}$ value, was used as one of the indicators. The relationship between the matrices and the log $P_{O/W}$ value is shown in Figure 1. The matrix components with log $P_{O/W}$ values >10, such as sterols, were more extracted by acetone than by acetonitrile. The different extraction ability of low polar components influenced the next cleanup. The C18 column is not only used in place of the liquid−liquid extraction but also used for removing low polar compounds. Therefore, the combination of low extracting power of fat by acetonitrile and the removal ability of low polar components by the C18 column enabled the efficient removal of sterols from the samples. However, when acetone was used as the extraction solvent, excess sterols could not be completely removed by the C18 column. Compounds with log $P_{O/W}$ values <3.2, such as benzoic acid, 4-vinylguaiacol, *p*-coumaric acid, and some flavonoids, were more extracted by

Table 4. Matrix Components in Brown Rice Extraction

compound	mol formula	CAS Registry No.	log $P_{O/W}$	group	concentration[a] qcetone	concentration[a] acetonitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
miristic acid	$C_{16}H_{32}O_2$	57-10-3	5.79	fatty acid	A	A
pentadecanoic acid	$C_{15}H_{34}O_2$	1002-84-2	6.30	fatty acid	C	C
palmitoleic acid	$C_{16}H30O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	B	B
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.81	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecenoic acid	$C_{17}H_{32}O_2$	26265-99-6	7.28	fatty acid	C	C
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
oleic acid	$C_{18}H_{42}O_2$	112-80-1	7.70	fatty acid	A	A
stearic acid	$C_{18}H_{36}O_2$	57-11-4	8.22	fatty acid	B	B
palmitelaidic acid	$C_{16}H_{30}O_2$		8.22	fatty acid	C	C
11-eicosenoic acid	$C_{20}H_{38}O_2$	2462-94-4	8.44	fatty acid	C	C
arachidic acid	$C_{20}H_{40}O_2$	506-30-9	8.85	fatty acid	C	C
behenic acid	$C_{22}H_{44}O_2$	112-85-6	9.87	fatty acid	<1	<1
lignoceric acid	$C_{24}H_{48}O_2$	557-59-5	10.89	fatty acid	<1	<1
13-docosenamide, (Z)-	$C_{22}H_{43}NO$	112-84-5	8.87	aliphatic amide	B	<1
butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$		9.24	fatty acid, ester	B	<1
myristin, 1-mono-	$C_{17}H_{30}O_4$	589-68-4	5.05	monoacylglycerol	B	B
myristin, 2-mono-	$C_{17}H_{34}O_4$	3443-83-2	5.33	monoacylglycerol	B	B
palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	C	C
palmitin, 1-mono	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	B	B
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	B	B
stigmasterol	$C_{29}H_{48}O$	83-48-7	10.07	sterol	C	<1
campesterol	$C_{28}H_{48}O$	474-62-4	10.20	sterol	B	<1
9,19-cyclolanost-24-en-3-ol, (3β-	$C_{30}H_{50}O$	469-38-5	10.31	sterol	B	C
9,19-cyclolanostan-3-ol, 24-methylene-, (3β-	$C_{31}H_{52}O$	1449-09-8	10.66	sterol	B	<1
β-sitosterol	$C_{29}H_{50}O$	83-46-5	10.73	sterol	B	C
α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	B	C
γ-tocopherol	$C_{28}H_{48}O_2$	119-13-1	11.44	tocopherol	C	<1
squalene	$C_{30}H_{50}$	7683-64-9	12.19	terpenoid	B	<1
ferulic acid	$C_{10}H_{10}O_4$	1135-24-6	1.64	aromatic carboxylic acid	C	C
p-coumaric acid	$C_9H_8O_3$	7400-08-0	1.88	aromatic carboxylic acid	C	C
sucrose	$C_{12}H_{22}O_{11}$	57-50-1	−4.49	sugar	B	B
glucose	$C_6H_{12}O_6$	50-99-7	−2.49	sugar	C	C

[a]A, ≥1000 mg/kg; B, ≥10 mg/kg, <1000 mg/kg; C, ≥1 mg/kg, <10 mg/kg; <1, <1 mg/kg.
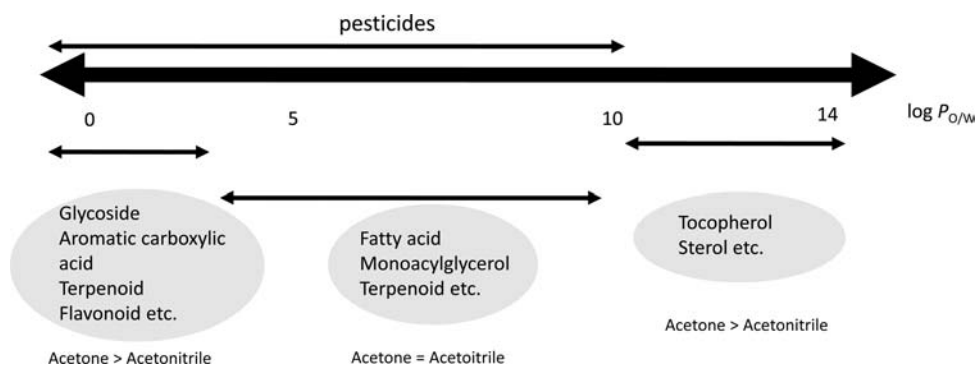

Figure 1. Relationship between each component and log $P_{O/W}$.

acetone than by acetonitrile. For these components, additional interactions, such as hydrogen bonds, might have worked, because both acetone and acetonitrile were high polar solvents. Many kinds of terpenoids, which are characteristic compounds in orange, were extracted at the same concentration level by both acetone and acetonitrile, and their log $P_{O/W}$ values were in the range from 3.5 to 7. The log $P_{O/W}$ values of the monoacylglycerols, which are common compounds in all pf the samples, are in the range from 5 to 7.5, and they were also extracted at the same concentration level by both acetone and acetonitrile. Glucose and fructose have two different structures, that is, a cyclic structure and a chain structure, and are in

Table 5. Elution Rate (Percent)[a] of Matrix Components from Each Column

	silica gel				Florisil						
	Fr-1[b]	Fr-2[c]	Fr-3[d]	Fr-4[e]	Fr-1[b]	Fr-2[c]	Fr-3[d]	Fr-4[e]	NH$_2$	PSA	GCB
fatty acid											
lauric acid	0.0	0.0	103	7.9	0.0	0.3	0.4	0.3	5.6	0.4	61.6
myristic acid	0.1	0.1	93.0	5.4	0.1	0.1	0.1	0.1	6.3	0.1	65.8
pentadecanoic acid	0.4	1.0	93.7	5.4	0.3	0.8	0.6	0.9	6.2	0.6	55.2
palmitoleic acid	0.1	0.0	93.2	5.8	0.0	0.1	0.0	0.2	6.0	0.0	62.6
palmitic acid	0.1	0.3	113	4.4	0.1	0.4	0.3	0.2	8.9	0.2	65.5
heptadecanoic acid	0.0	0.0	97.7	3.0	0.0	0.0	0.0	0.0	2.6	0.0	50.8
heptadecenoic acid	0.0	1.8	93.7	4.4	0.3	1.9	2.0	1.1	8.6	0.0	50.2
linoleic acid	0.0	0.1	99.4	13.9	0.0	0.1	0.1	0.0	13.9	0.1	88.6
oleic acid	0.1	0.3	112	5.1	0.0	0.4	0.3	0.1	11.2	0.3	88.5
linolenic acid	0.0	0.0	111	5.3	0.0	0.0	0.0	0.0	10.4	0.0	83.0
stearic acid	0.5	1.3	94.8	3.4	0.4	1.3	1.4	0.9	11.2	0.9	39.0
11-eicosenoic acid	0.0	0.2	90.0	3.2	0.0	0.3	0.3	0.4	8.2	0.3	50.2
arachidic acid	0.5	1.1	95.5	3.5	0.3	1.2	1.3	1.2	9.7	1.5	1.3
behenic acid	0.0	0.5	86.0	2.6	0.1	0.4	0.5	0.0	10.5	0.0	1.4
lignoceric acid	0.0	0.3	97.8	2.0	0.0	0.7	0.6	0.8	14.9	1.4	0.8
sugar											
glucose	0.0	0.0	0.0	11.6	0.0	0.0	0.0	0.0	0.0	0.0	76.3
sucrose	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.0	1.3	0.1	73.2
monoacylglycerol											
myristin, 1-mono-	0.0	0.0	0.2	63.1	0.0	0.0	0.1	62.7	52.7	50.6	55.7
palmitin, 2-mono-	0.0	0.0	0.0	62.1	0.0	0.0	0.0	46.8	43.9	39.9	46.4
palmitin, 1-mono-	0.0	0.0	0.2	68.0	0.0	0.0	0.2	62.3	47.1	45.6	41.7
linolein, 2-mono-	0.0	0.0	0.0	68.5	0.0	0.0	0.0	47.2	49.8	44.9	46.4
linolein, 1-mono-	0.0	0.0	0.2	73.9	0.0	0.0	0.1	74.0	54.2	54.3	55.8
tocopherol											
α-tocopherol	0.0	97.0	6.6	0.1	0.0	85.2	2.5	0.1	63.2	80.2	0.1
γ-tocopherol	0.0	0.0	88.4	0.0	0.0	13.0	31.8	0.0	55.5	56.9	0.0
carboxylic acid											
p-coumaric acid	4.0	0.9	2.4	5.3	0.4	0.0	0.9	1.6	10.9	1.7	2.1
ferulic acid	5.2	4.8	72.2	46.1	5.0	5.9	7.6	85.6	99.1	51.7	12.7
squalene											
squalene	15.1	32.0	1.9	0.2	1.7	81.6	0.9	0.3	72.9	69.1	8.3
sterol											
campesterol	0.0	0.7	73.2	0.8	0.0	8.3	74.2	0.8	82.7	59.4	0.0
β-sitosterol	0.0	0.4	76.0	0.2	0.0	7.7	75.3	0.3	84.0	59.8	0.0
stigmasterol	0.0	0.0	79.2	0.0	0.0	4.3	72.5	0.0	74.3	54.1	0.0
9,19-cyclolanost-24-en-3-ol, (3β)-	0.0	50.6	28.1	0.0	0.0	63.4	18.7	0.0	75.3	58.3	0.0
9,19-cyclolanostan-3-ol, 24-methylene-, (3β)-	0.0	51.9	25.5	0.0	0.0	66.5	16.2	0.0	79.7	63.3	0.0

[a](Mean value of the intensity of eluted matrix component from each column/mean value of the intensity of the components passed through the C18 column) × 100. [b]Fr-1, n-hexane. [c]Fr-2, acetone/n-hexane (5:95, v/v). [d]Fr-3, acetone/n-hexane (15:85, v/v). [e]Fr-4, acetone/n-hexane (50:50, v/v).

equilibrium in an aqueous solution. Most of the glucose and fructose exist as cyclic structures in an aqueous solution,[42] but no data on their equilibrium states in an organic solvent were found. These cyclic sugars were extracted by both solvents at similar levels. However, the chain structure was found, and they were more extracted by acetonitrile than by acetone. In addition, dozens of unknown peaks of sugar-like components were found in the orange sample. Although most sugar-like components are not listed in the database, they have the characteristic mass spectra of sugars; m/z 204, 217, 361, etc. These sugar-like components might be glycosides, which are bonded to other compounds or functional groups. Some of them were more extracted by acetone than by acetonitrile, whereas some of them were extracted by both solvents at the same level. The solvent, which easily dissolved these glycosides, might be dependent on the binding compounds.

On the basis of these results, acetone extracted the matrix components with a wide range of log $P_{O/W}$ values. In contrast, acetonitrile extracted the matrix components having log $P_{O/W}$ values in the range from 3.2 to 10. The log $P_{O/W}$ values of the pesticides analyzed by GC-MS are mostly <10. When acetonitrile was used as the extraction solvent, fewer low polar matrix components were extracted. Because both acetone and acetonitrile are high polar solvents, the polar matrix components should be dissolved in both solvents. However, some polar matrix components were more significantly extracted by acetone than by acetonitrile. There might be other interactions as described above. Therefore, acetone dissolved much of the polar matrix components. In fact, for many of the polar pesticides with log $P_{O/W}$ values <3.2, a good recovery rate of the multiresidue analysis using acetonitrile was proved by many previous studies.[4−9]

Experiment 2. Cleanup Efficiency by Solid Phase Extraction (SPE). In experiment 2, we examined the column cleanup efficiency using several kinds of SPE columns. Because orange contained too many matrices, and it was biased to glycosides, the brown rice sample extracted using acetone was selected as the model sample in this experiment. The reason for using acetone extraction was that it was suitable to evaluate the column efficiency due to the many types of matrix components from the result of experiment 1. All of the results are shown in Table 5.

Fatty acids, which are the main matrix components in the brown rice, have both a nonpolar hydrocarbon group and polar carboxyl group. Fatty acids were eluted from the silica gel column with n-hexane/acetone (15:85, v/v), but removed by a Florisil column because the polar interactions of Florisil are greater than that of the silica gel column. Both the NH$_2$ and PSA columns are used to exclude fats, but the PSA column showed a higher cleanup efficiency than the NH$_2$ column, and this result agreed with the report by Okihashi et al.[9]

Sugars contain many hydroxyl groups (−OH) and water-soluble components. Sugars were removed by all columns except the GCB column. In addition, sugars do not move to the organic solvent layer when liquid−liquid extraction is applied during the general method of pesticide residue analysis.

Two carboxylic acids, p-coumaric acid and ferulic acid, were found in the brown rice extraction, and their structures were similar; both contain a phenolic hydroxyl group and a carboxyl group. However, the removal rate of p-coumaric acid was higher than that of ferulic acid by any column.

α-Tocopherol, γ-tocopherol, and squalene are low polar compnents and were eluted from the silica gel and Florisil columns by acetone/n-hexane (5:95−15:85 v/v). The NH$_2$ and PSA columns did not effectively remove them. Only the GCB column removed them.

Monoacylglycerols are fat decomposition compounds and contain both a nonpolar hydrocarbon group and a polar hydroxyl group. Apporoximately 45−75% of the monoacylglycerols are eluted from any column. This result agreed with our other study.[43] We demonstrated that monoacylglycerols are the compounds that cause the matrix enhancement effect and remain at about 100 mg/kg in the sample solution when using the multiresidue method of the PLS, which adopts the combination of the GCB and NH$_2$ column cleanup. Because monoacylglycerols are midpolar components and their molecular weights are around 300−360, they might behave in the same manner as some pesticides. Although the Florisil and silica gel columns are rarely used for the multiresidue analysis because of their strong adsorption, there is the ability to adjust the proper ratio of the solvent mixture to remove the monoacylglycerols. In fact, Iijima et al. demonstrated the use of the silica gel column for multiresidue analysis, and most pesticides were eluted with acetone/n-hexane(15:85, v/v).[44] Otherwise, searching for a suitable column to remove them is required.

Because sterols are low polar components, they were eluted from the Florisil and silica gel columns. The NH$_2$ and PSA columns could not sufficiently remove them, whereas the GCB column removed them because of their flat structures. In addition, a C18 column is used in the PLS method (used for grains, seeds, and beans) to remove fats.

Although the aim of study was not to determine the recommended sample preparation, some interesting facts were revealed. Although 90% of the fatty acids were removed by C18, fatty acids were still in the main matrices. Most matrices were removed by either column. However, the monoacylglycerols, which are the components causing matrix enhancement effect, remained in the common multiresidue analysis. Thus, the detailed matrix behaviors during the sample preparation for the pesticide residue analysis were clarified using the metabolomics analysis approach. This approach can be helpful in designing the extraction and cleanup procedures (e.g., types of columns or elution solvent). This approach is also helpful for evaluating the method for other agricultural products (or biological samples) and to develop a better analytical method.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

GC-MS, gas chromatography−mass spectrometry; SPE, solid phase extraction; C18, octadecyl minicolumn; NH$_2$, aminopropylsilanized silica gel minicolumn; PSA, ethylenediamine-N-propylsilanized silica gel minicolumn; GCB, graphite carbon black minicolumn; PLS, Positive List System.

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Reference 41 was incorrectly cited, and references 4 and 6 were updated in the version of this paper published October 1, 2012. The correct version published October 4, 2012.