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Application Note

No.29

Simple Analysis of Pesticides Using AOC-MEPS System

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1. Introduction

MEPS (Micro Extraction by Packed Sorbent), a solid phase micro extraction system developed by SGE, consists of a solid-phase extraction cartridge that is integrated in a syringe needle. Analytes of interest can be trapped on the solid phase by simply aspirating the sample, and can accommodate volumes ranging from microliters to milliliters. The analytes that are bound to the sorbent material in the cartridge are dissolved by aspirating elution solvent (volumes from as little as a few to hundreds of microliters), and then injecting directly into the GC or GC/MS for analysis. MEPS provides easy use, and unlike conventional solid phase extraction cartridges, repeat use is possible. The AOC-MEPS system, which consists of the MEPS attached to Shimadzu's general-purpose AOC-20i autoinjector, permits automated operation from pretreatment to analysis. Thus, extraction and concentration of trace components in the sample for analysis is now possible using the AOC-MEPS system.

In this Application Note, we introduce examples of analysis of pesticide residues in crude food extracts and in water using the AOC-MEPS system.

Fig. 1-1 MEPS Syringe and MEPS Needle

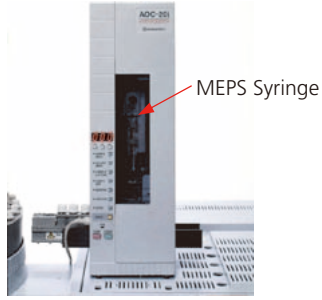

Fig. 1-2 AOC-MEPS System

Table of Contents

2. Analysis of Pesticides in Crude Food Extract Using MEPS

2-1. Introduction

Analysis of pesticide residues in crops by GC and GC/MS is typically subject to tedious and time-consuming extraction and cleanup operations. These operations are often conducted using a solid phase extraction cartridge, and involve consumption of large amounts of solvents. Furthermore, as these solid phase extraction cartridges are almost always discarded after one use, this process becomes quite costly. MEPS, on the other hand, not only offers very simple operation, it can also be used repeatedly. Here, we investigated the use of the AOC-MEPS system in the analysis of pesticide residues in crude food extracts, and report on the excellent results obtained.

2-2. Experimental

The investigation was conducted using the Shimadzu GC 2010 Plus gas chromatograph and the AOC-MEPS system, which utilizes the MEPS-supported Shimadzu AOC-20i autoinjector. Data processing was conducted using the Shimadzu GCsolution workstation. The MEPS cartridge, which houses a copolymer such as polystyrene divinylbenzene (PDVB) as the sorbent bed, was used to retain and concentrate the analytes from the sample extract. The investigation was conducted using the large sample injection method with a PTV GC injection unit. For the detector, the FPD-2010 Plus was used.

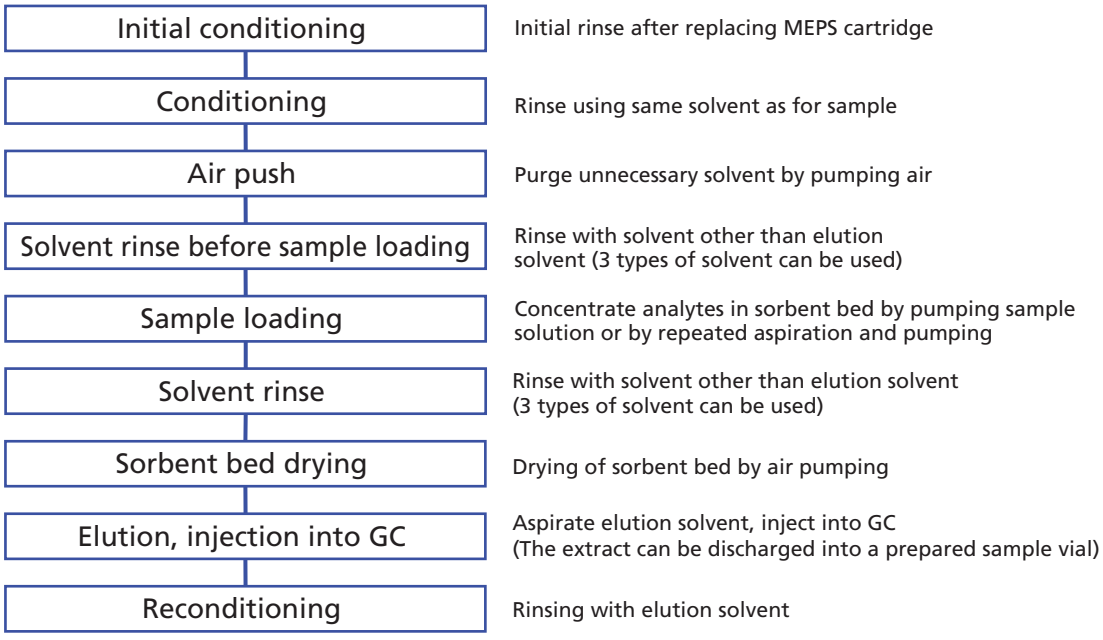

Fig. 2-1 Possible Sequence for AOC-MEPS

When setting up analysis using the AOC-MEPS system, each step of the sequence can be arbitrarily set as shown in the example of Fig. 2-1, with separate settings possible for aspiration volume, aspiration frequency, and aspiration / discharge speed. Unlike the typical solid phase cartridge, the MEPS cartridge is reusable, thereby facilitating automation of processing for multiple samples using the same cartridge. Typically, after a large amount of sample solution is loaded onto the sorbent bed to retain the desired analytes, elution is conducted with a smaller volume of solvent, and the eluate is subsequently injected into the GC. Since desorption from the sorbent bed requires more than 20 or 30 µL of solvent, it is effective to introduce a large volume of sample using the PTV for sample injection. Since a large volume of solvent is discharged from the split vent, sample injection was conducted at a low temperature so that only the solvent would be vaporized; a high split ratio is used during the solvent evaporation phase of the injection. After the discharge of the solvent, the split ratio was lowered (1:1), and the PTV temperature was increased to transfer the analyte compounds to the analytical column (Fig. 2-2).

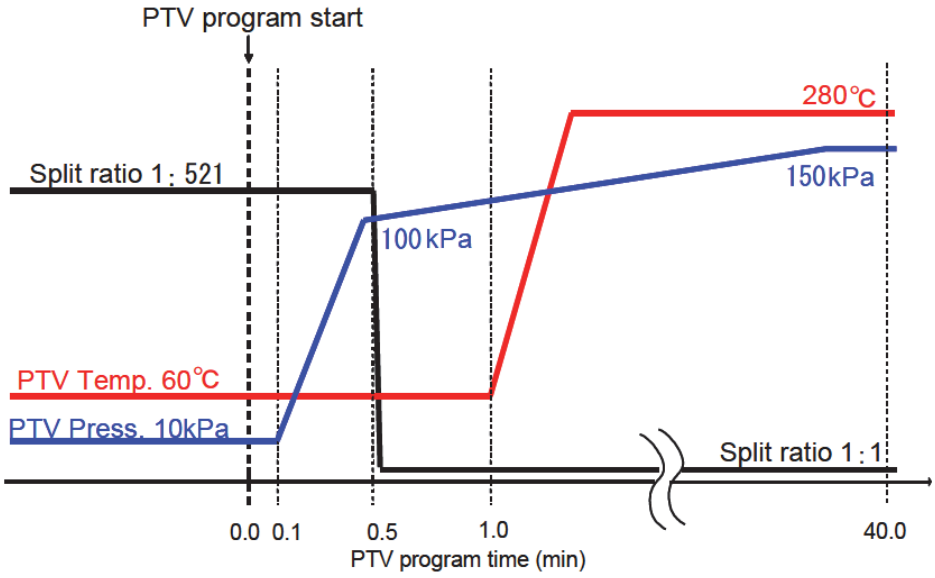

Fig. 2-2 PTV Analysis Conditions

We investigated the extraction and concentration of phosphorus pesticides in a crude extract solution obtained by a QuEChERS-like simple extraction method using the AOC-MEPS system. At the same time, we investigated the method used for washing the sorbent bed after the sample is loaded onto the MEPS cartridge in order to remove interfering contaminants that could adversely affect the GC-FPD chromatogram. Shown below (Fig. 2-3) is the QuEChERS method operation flow that was used.

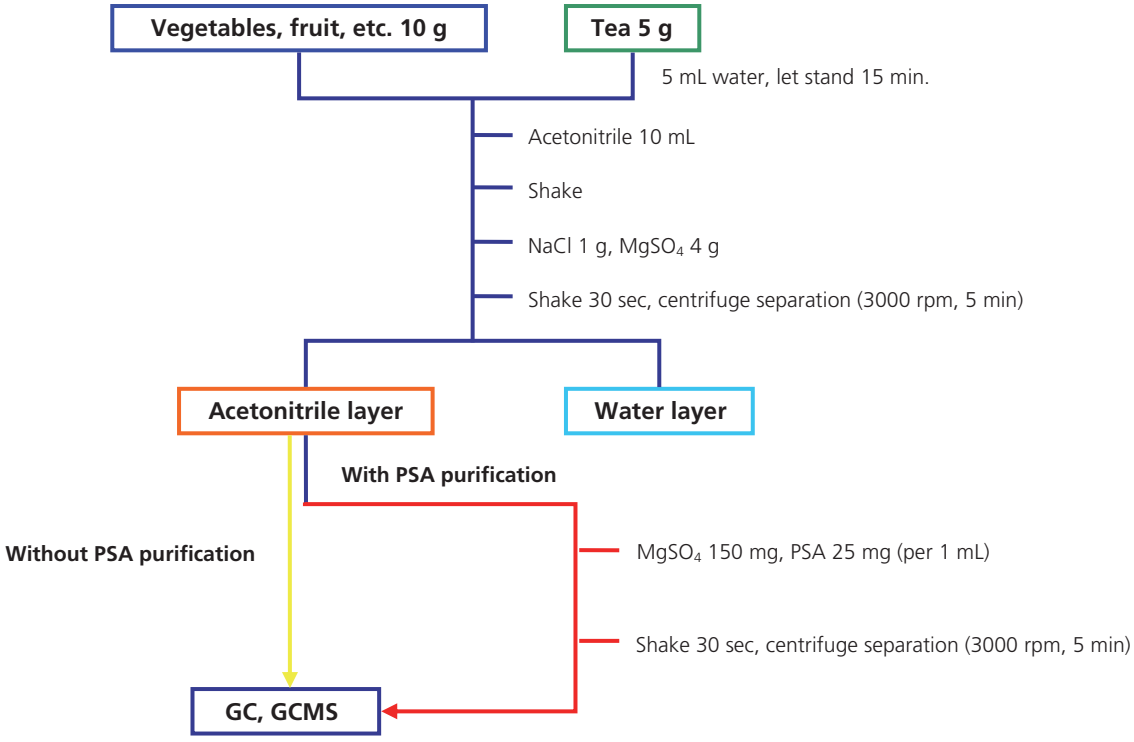

Fig. 2-3 QuEChERS Method Operation Flow

2-3. Analysis of Pesticide Standard Solution Containing 22 Phosphate Compounds

When the same acetonitrile solution that was used in the QuEChERS extract solution was loaded onto the PDVB sorbent material in the MEPS cartridge, pesticide retention was almost non-existent, and this lack of pesticide concentration persisted even after increasing the sample load quantity. However, after diluting the standard solution with water, it was found that nearly all the pesticides were retained and concentrated.

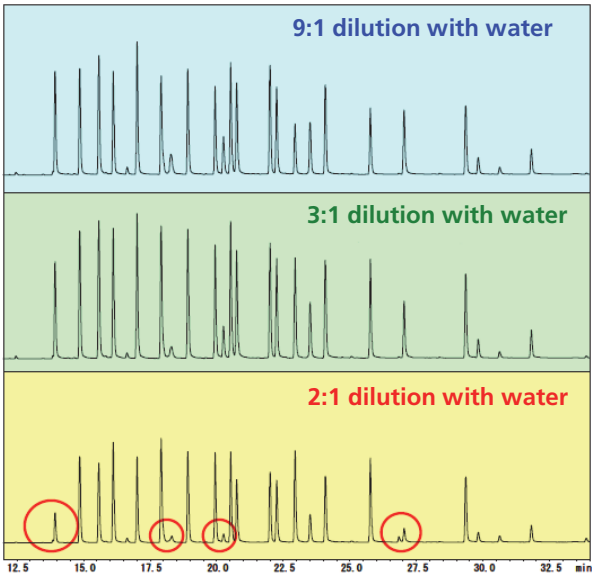

Fig. 2-4 Comparison Using Different Dilution Ratios

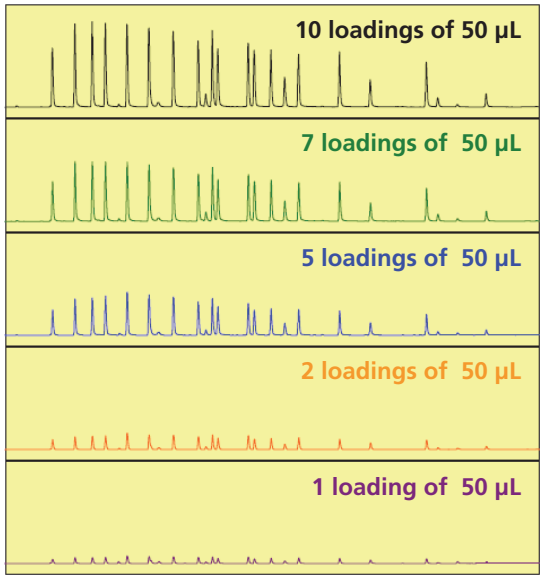

Fig. 2-5 Comparison Based on Number of Loadings (Quantity)

Even compounds that show low recovery using a 1:1 dilution with water showed improved recovery using a 3:1 dilution. The pattern difference between the 9:1 and 3:1 dilution with water was slight, so from the standpoint of concentration efficiency, subsequent studies were conducted using a 3:1 dilution with water. Since a correlation was obtained between the loading frequency (quantity) using the 3:1 diluted sample and the area value, the sample loading volume was 250 µL based on 5 times loading of 50 µL, to ensure that each peak of 10 ppb could be sufficiently identified in the chromatogram. Elution of 50 µL (acetone) was used to transfer the analytes into the GC.

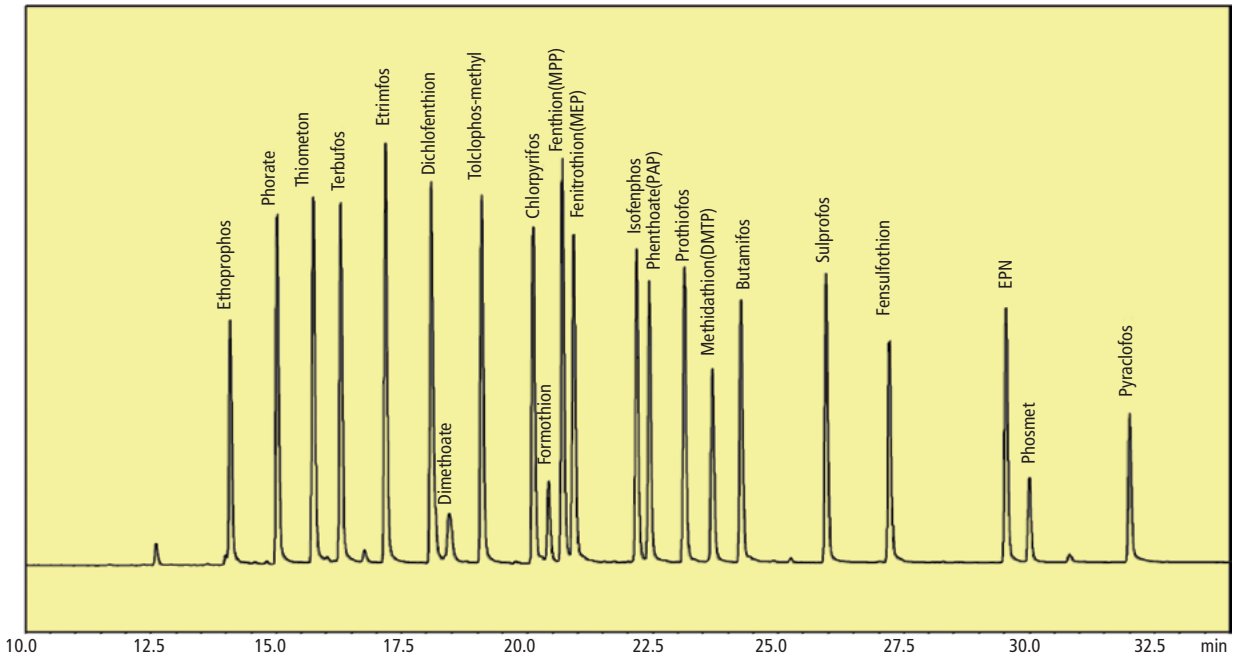

Fig. 2-6 Chromatogram of Standard Solution of 22 Phosphate Pesticides (40 ppb each)

Table 2-1 GC Analytical Conditions

Instrument	: GC-2010 Plus + AOC-MEPS system
Column	: DB-1701 (30 mL. × 0.25 mmI.D., 0.25 µm)
PTV temperature	: 60 °C (1 min) – 250 °C/min – 280 °C (40 min)
PTV pressure	: 10 kPa (0.1 min) – 400 kPa/min – 100 kPa – 1.5 kPa/min – 150 kPa (7 min)
Injection mode	: Split 1:521 (0.5 min) – 1:1
Column temperature	: 50 °C (2 min) – 25 °C /min – 150 °C – 5 °C /min – 270 °C (10 min)
Carrier gas	: He
Detector	: FPD-2010 Plus H_2 : 62.5 mL/min Air : 90 mL/min
Injection volume	: 50 µL (PTV large volume sample introduction)

2-4. Calibration Curves and Linearity

Phosphate pesticide standard solutions were prepared using acetonitrile solvent to obtain concentrations of 4, 10, 40, 100 and 200 ppb, respectively, and these were each diluted 4:1 using water. They were then loaded on MEPS cartridges using a total volume of 250 µL (50 µL × 5), and then eluted with 50 µL of acetone. The results of analysis by PTV large volume sample introduction yielded excellent linearity (Fig. 2-7). In addition, excellent repeatability of detector response was obtained for 18 of the pesticides, except for Dimethoate, Formothion, Phosmet, and Pyraclofos, using 3 continuous measurements of the 40 ppb standard solution (Table 2-2).

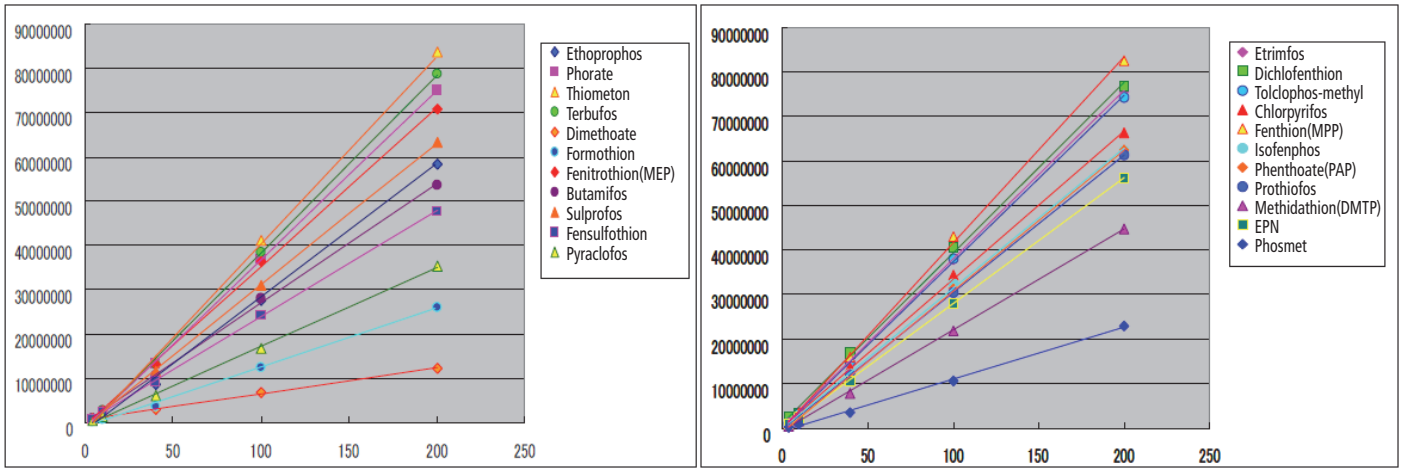

Fig. 2-7 Calibration Curves

Table 2-2 Repeatability of Area Values

	1	2	3	AVERAGE	C.V.%
Ethoprophos	8147703	7767045	7688837	7867861.7	3.12
Phorate	12360073	11802682	11970729	12044494.7	2.37
Thiometon	12811219	12441850	12459312	12570793.7	1.66
Terbufos	12438131	11746601	11870154	12018295.3	3.07
Etrimfos	15303635	15139680	15111542	15184952.3	0.68
Dichlofenthion	16912623	16361640	15863700	16379321.0	3.20
Dimethoate	3105261	2944642	2163236	2737713.0	18.41
Tolclophos-methyl	14413398	14056246	14022202	14163948.7	1.53
Chlorpyrifos	13191049	13040585	12740884	12990839.3	1.76
Formothion	1586174	1231507	836834	1218171.7	30.77
Fenthion(MPP)	15457424	15243827	14660557	15120602.7	2.73

	1	2	3	AVERAGE	C.V.%
Fenitrothion(MEP)	12265071	12077099	11563524	11968564.7	3.03
Isofenphos	11890568	12075960	11651575	11872701.0	1.79
Phenth oate(PAP)	10016391	10134952	9832163	9994502.0	1.53
Prothiofos	11366531	11400734	10848739	11205334.7	2.76
Methi dathion(DMTP)	6676671	6639973	6349097	6555247.0	2.74
Butamifos	10064702	10167573	9878495	10036923.3	1.46
Sulprofos	10746249	10800627	10685668	10744181.3	0.54
Fensulfothion	8784169	8726718	8202845	8571244.0	3.74
EPN	9974250	10005808	9812549	9930869.0	1.04
Phosmet	1972192	1631491	1551771	1718484.7	12.99
Pyraclofos	4259372	4049841	3745695	4018302.7	6.43

2-5. Analysis of Tea Leaves

After diluting a QuEChERS extract solution of tea leaves 3:1 in the same way as with the standard sample, it was possible to conduct pretreatment and analysis using a PDVB MEPS cartridge. However, at the low concentrations, caffeine and other contaminants appeared as interfering peaks in the chromatogram. Therefore, after loading the extract solution on the MEPS cartridge, water and a rinse solution consisting of 1:1 methanol / water was used to reduce the interference due to caffeine and other contaminants while the pesticides were retained on the cartridge. The pretreatment flow diagram is shown in Fig. 2-8. The region of caffeine elution is compared in the chromatograms of Fig. 2-9, and a chromatogram of all the pesticide peaks is shown in Fig. 2-10.

Conditioning	Rinse using water Aspirate 80 µL 10 times at 5 µL/sec, discharge at 5 µL/sec
Sample loading	Aspirate sample solution (3:1 dilution with water), discharge into waste bottle Aspirate 50 µL 5 times at 5 µL/sec, discharge at 5 µL/sec
Solvent rinse 1	Rinse with water (rinse solvent 1) Aspirate 80 µL 10 times at 5 µL/sec, discharge at 5 µL/sec
Solvent rinse 2	Rinse with methanol / water 1:1 (rinse solvent 2) Aspirate 80 µL 5 times at 5 µL/sec, discharge at 5 µL/sec
Sorbent bed drying	Dry sorbent bed by air pumping from air vial Aspirate 80 µL 20 times at 10 µL/sec, discharge at 50 µL/sec
Elution, injection into GC	Aspirate 50 µL acetone (elution solvent), inject into GC Aspiration: 3 µL/sec, Injection: 1 µL/sec
Reconditioning	Rinse with acetone (elution solvent) Aspirate 80 µL 5 times at 10 µL/sec, discharge at 10 µL/sec

Fig. 2-8 MEPS Pretreatment Flow for Tea Leaves

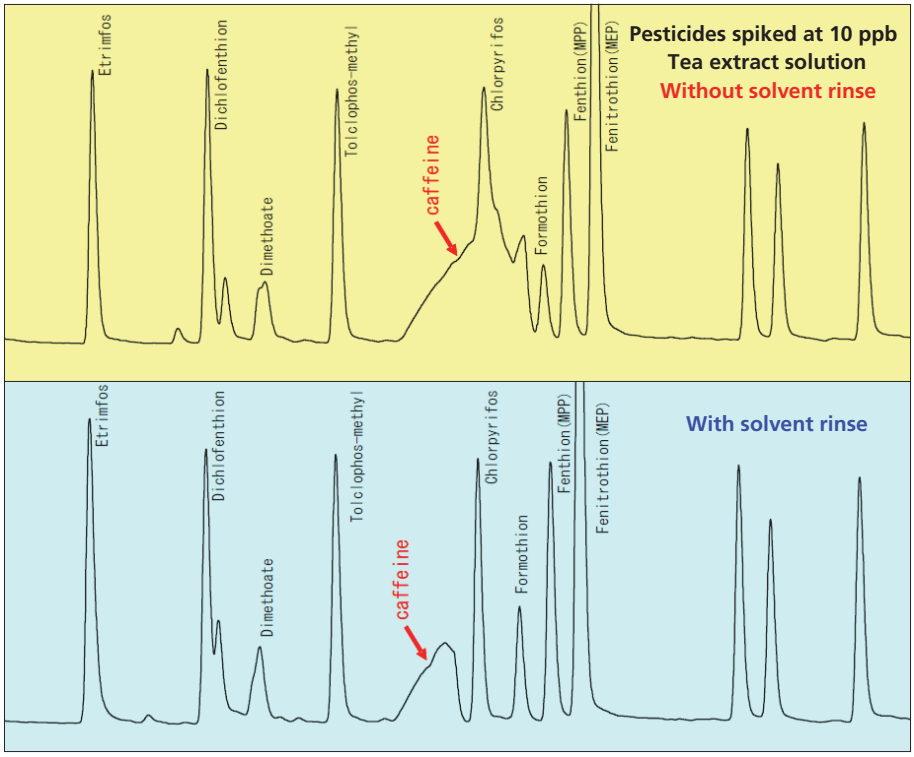

Fig. 2-9 Caffeine Removal Using Solvent Rinse

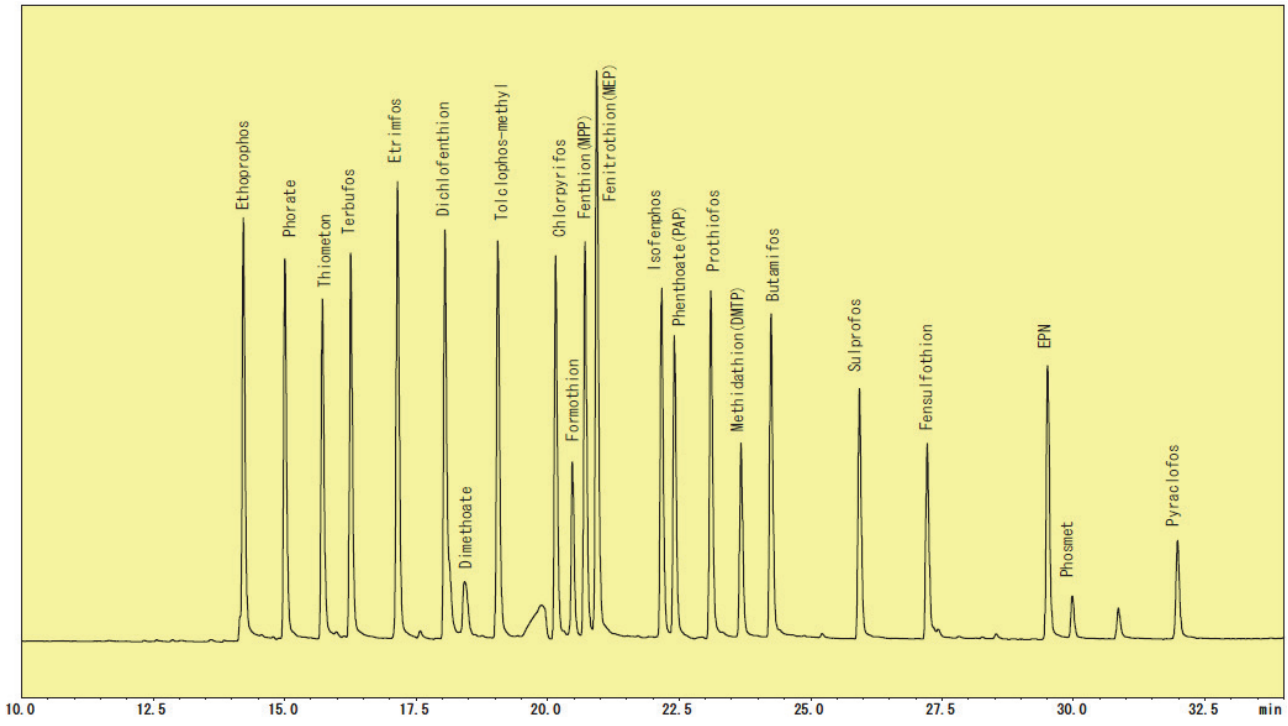

Fig. 2-10 Chromatogram of Tea Leaf Extract Solution Spiked with Pesticides at 40 ppb

2-6. Stability of Sample Extract Diluted with Water

A QuEChERS extract solution was diluted 3:1 with water and then analyzed in order to conduct pretreatment using the PDVB MEPS cartridge. But when the diluted solution was set aside for an extended period of time prior to analysis, a remarkable decrease in the response of low-intensity components was observed. Fig. 2-11 shows an example of the reduction in response after 8 hours following dilution. This demonstrates the importance of conducting analysis as soon as possible after sample dilution to mitigate the effects of degradation, etc. that might be related to aqueous dilution.

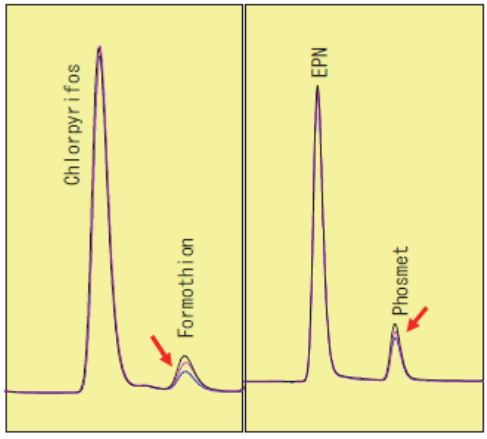

Fig. 2-11 Significant Degradation of Compounds (Formothion and Phosmet)

2-7. Analysis of Daikon Radish

Pretreatment and analysis of daikon radish were also possible using the PDVB MEPS cartridge by diluting the daikon extract solution 3:1 with water. MEPS pretreatment using solvent rinsing with water and a 1:1 methanol / water solution is unnecessary for a sample such as daikon, which generates few interfering chromatographic peaks. Analysis was possible even for the sample that was not subjected to PSA purification using the QuEChERS method (Fig. 2-12, 2-13).

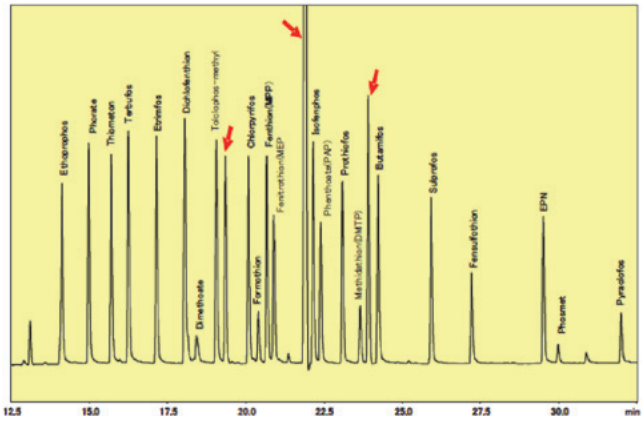

Fig. 2-12 Analysis of Crude Daikon Radish Extract Solution (Without PSA Purification)

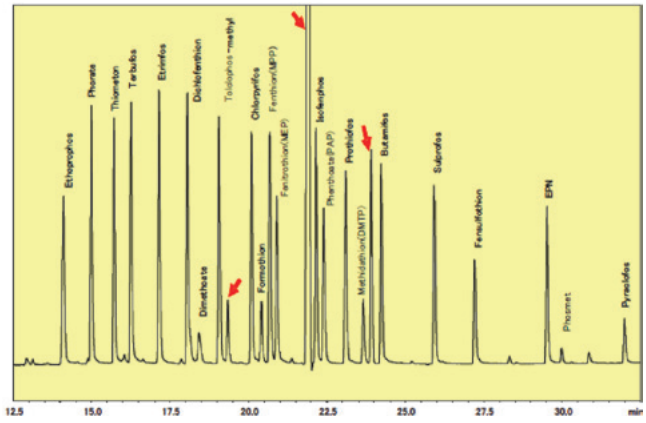

Fig. 2-13 Analysis of Daikon Radish Extract Solution (With PSA Purification)

2-8. Durability of MEPS Cartridge

Fig. 2-14 shows a chromatogram obtained from analysis of a 40 ppb standard sample following 40 measurements of an actual sample and more than 60 measurements of a standard sample. Some of the peaks generated due to contaminant residues in the MEPS cartridge are seen to overlap analyte peaks. Therefore, rinsing was conducted by repeatedly aspirating and discharging a few hundred microliters of dichloromethane to remove contaminants remaining in the MEPS cartridge. Fig. 2-15 shows that the 40 ppb standard solution chromatogram obtained after the rinsing process is relatively free from interfering contaminants. The chromatographic pattern for the analyte compounds was almost unchanged before and after rinsing of the MEPS cartridge.

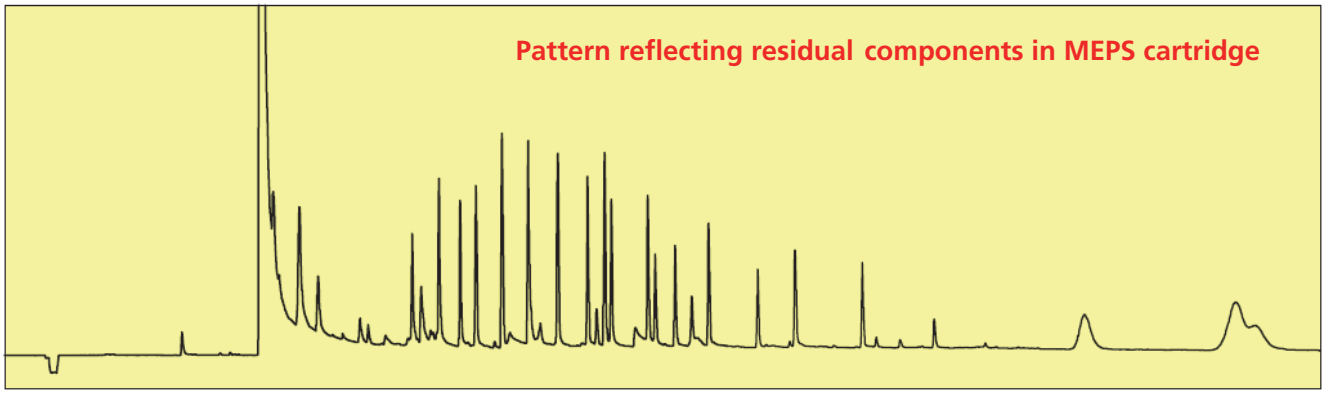

Fig. 2-14 Chromatogram of 40 ppb Standard Sample (After 40 Repeat Measurements of Actual Sample)

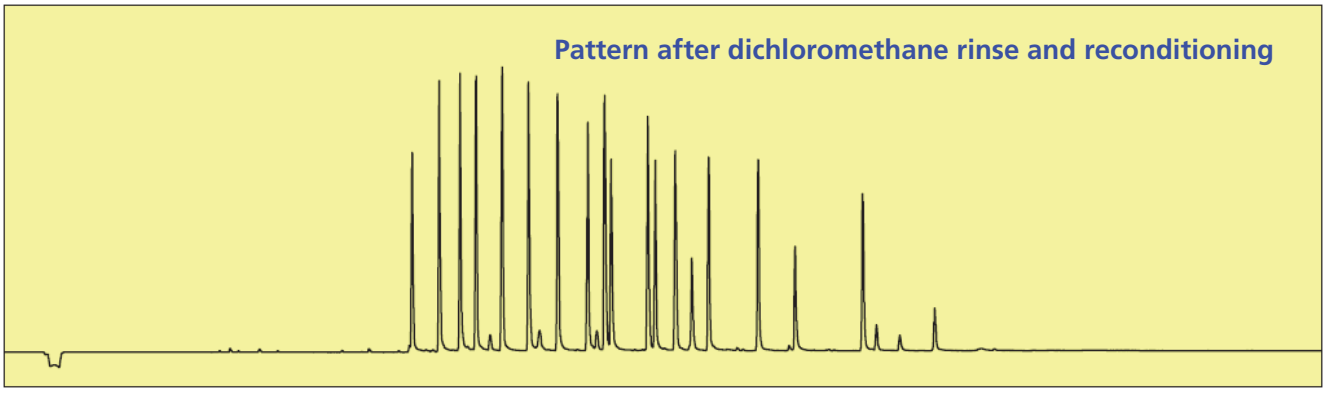

Fig. 2-15 Chromatogram of 40 ppb Standard Sample (After Reconditioning)

2-9. Summary

The AOC-MEPS system permits easy analysis of QuEChERS extract solutions, and can be considered to be an effective method for screening analysis. In cases where the MEPS cartridge was used for pretreatment of QuEChERS extracts of samples such as tea leaves that contain a large amount of caffeine, it was found to be possible to reduce interfering peaks due to impurities such as caffeine by optimizing the rinse solvent. The MEPS cartridge was able to be used more than a hundred times by applying the pretreatment conditions introduced here.

3. Simple Analysis of Pesticides in Water Using MEPS

3-1. Introduction

Analysis of pesticide residues in water by GC or GC/MS requires cumbersome pretreatment operations using a solid phase extraction cartridge, whereas using the AOC-MEPS system, this pretreatment process can be automated to greatly reduce the effort involved in the analysis. Here we report on the excellent results obtained for 222 of pesticide residues from the simple analysis (screening) using the AOC-MEPS system.

3-2. Instrument

The system used for this study included the Shimadzu AOC-MEPS system, the Shimadzu GCMS-QP2010 Ultra gas chromatograph mass spectrometer, and the Shimadzu GCMSsolution workstation. For the MEPS sorbent bed, polystyrene divinylbenzene polymer (PDVB) and octadecyl (C18) were used, and measurement was conducted by the large sample injection method using a PTV GC injection unit.

3-3. Investigation of Large Volume Sample Introduction Method

When sample pretreatment is automated using the AOC-MEPS system, the sample injection volume is much greater (from tens to hundreds of microliters) than that with a typical injection because the elution solvent is introduced without further concentration from the MEPS into the gas chromatograph. Therefore, a large-volume sample introduction mechanism is required to eliminate the solvent in the injection unit to permit concentration of the analyte components. Thus, we performed a study of the various analytical conditions used in the Programmable Temperature Vaporization (PTV) injector. A special MEPS insert with wool was used for the measurements. The PTV initial temperature was set to a temperature that would permit vaporization of the solvent only, allowing collection of the analyte components immediately following sample injection. After removal of the solvent, the split ratio was reduced, the PTV temperature was raised, and the analyte components collected on the wool were vaporized and introduced into the column. A schematic diagram of the PTV unit is shown in Fig. 3-1, and the PTV analytical conditions are shown in Fig. 3-2.

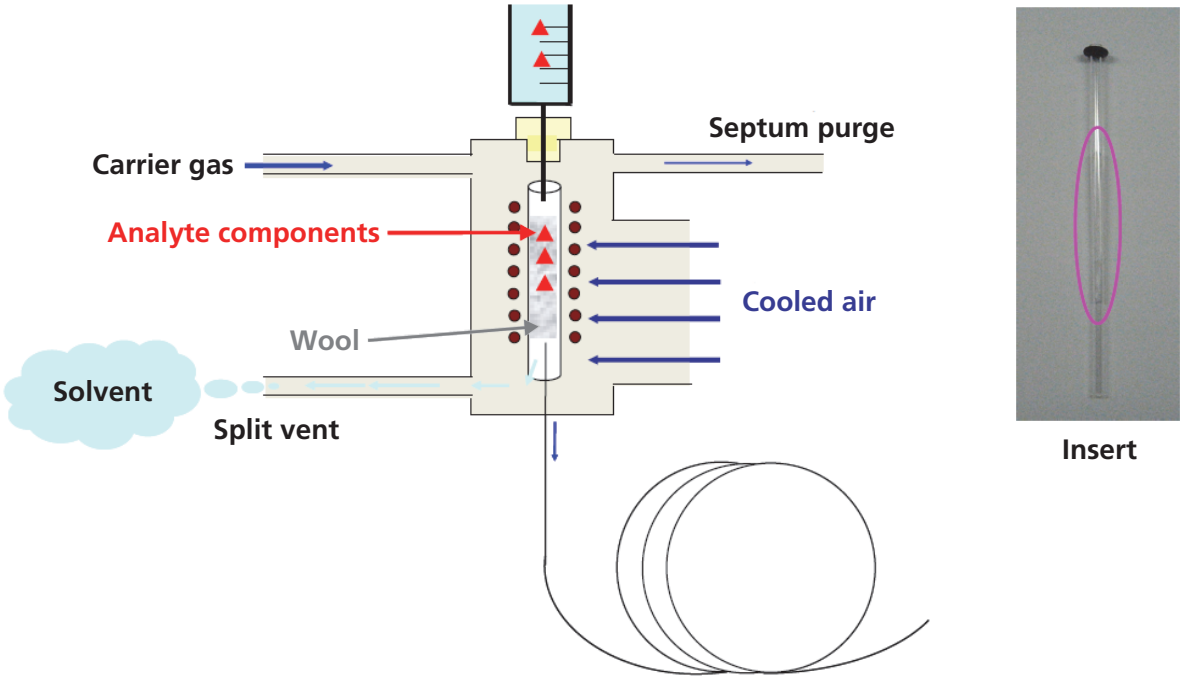

Fig. 3-1 Programmable Temperature Vaporization Injector

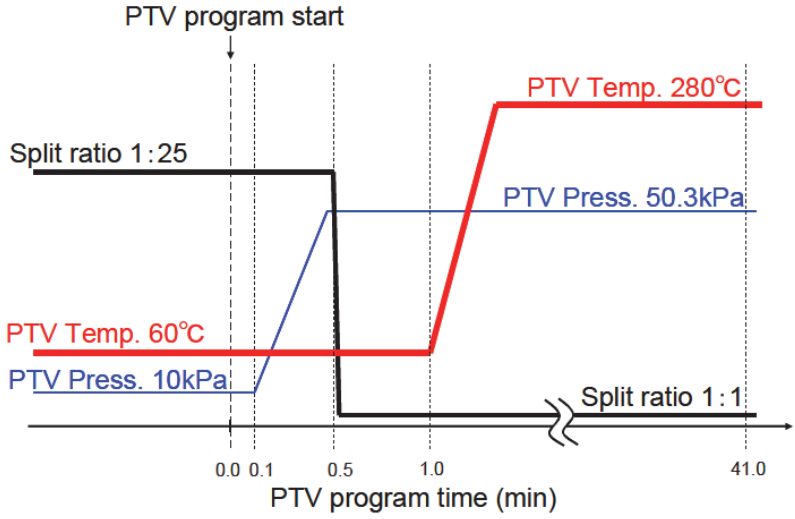

Fig. 3-2 PTV Analytical Conditions

Increasing the injection volume to improve sensitivity and recovery could allow incomplete retention of the analyte components in the injection port insert, allowing them to easily break through and be lost. It is generally assumed that relatively low-boiling point compounds are more susceptible to this loss. Thus, we investigated the effects of a range of injection volumes (specifically, 50, 100, 150, and 200 µL) on such analyte loss. Using a GC/MS equipped with a PTV, area values equivalent to 2 ng for each analyte (with a split ratio of 1:1) were determined, and the area % with respect to each of the injection volumes was subsequently determined. To ensure that the same absolute amount (2 ng) was injected into the GC/MS, a 40 pg/µL solution was used for the 50 µL injection, a 20 pg/µL solution for the 100 µL injection, a 13.3 pg/µL solution for the 150 µL injection, and a 10 pg/µL solution was used for the 200 µL injection. Using acetone as the solvent, the area % was the mean value of two measurements. The area % values for the 50 compounds with early retention times (low boiling points) are shown (Fig. 3-3). Almost no relative loss of early-eluting analytes was seen with the 50 µL injections, but as the injection volume was increased, the loss became greater.

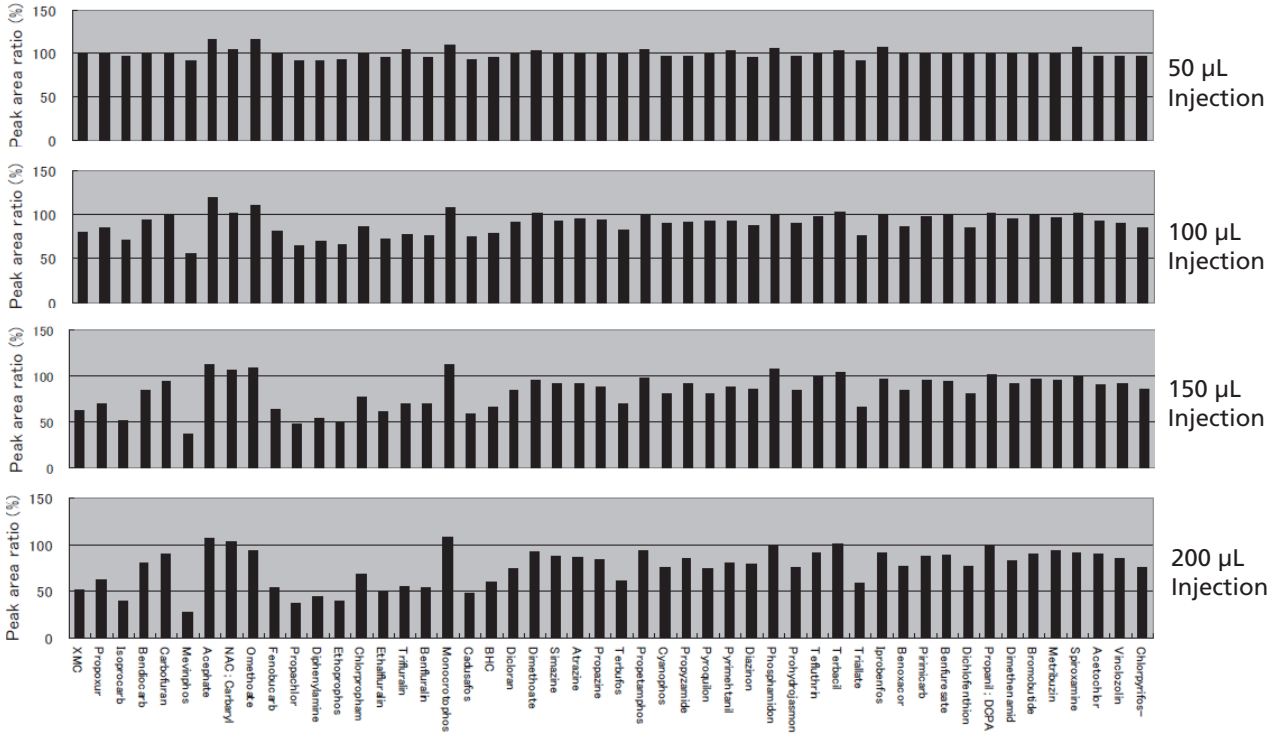

Fig. 3-3 Results of Analyte Loss Effect Study

3-4. Investigation of Elution Solvent

Various solvents, including acetone, dichloromethane, acetone / hexane mixture (1:1), and ethanol, were investigated to determine which would be appropriate for eluting the pesticides retained in the MEPS. Each of the pesticides were added to purified water at a concentration of 2 ng/mL, and after extracting the pesticides using PDVB and C18 (50 µL × 20 repetitions = 1 mL), elution and injection were conducted using 50 µL of each solvent. The elution performance for each pesticide was determined, repeating the elution 3 – 4 times using the same cartridge. The mean value of 3 measurements was used in the case of acetone, and the mean value of 2 measurements was used for the other solvents. The pretreatment flow diagram is shown in Fig. 3-4, and the analytical conditions and target pesticides are shown in Table 3-1.

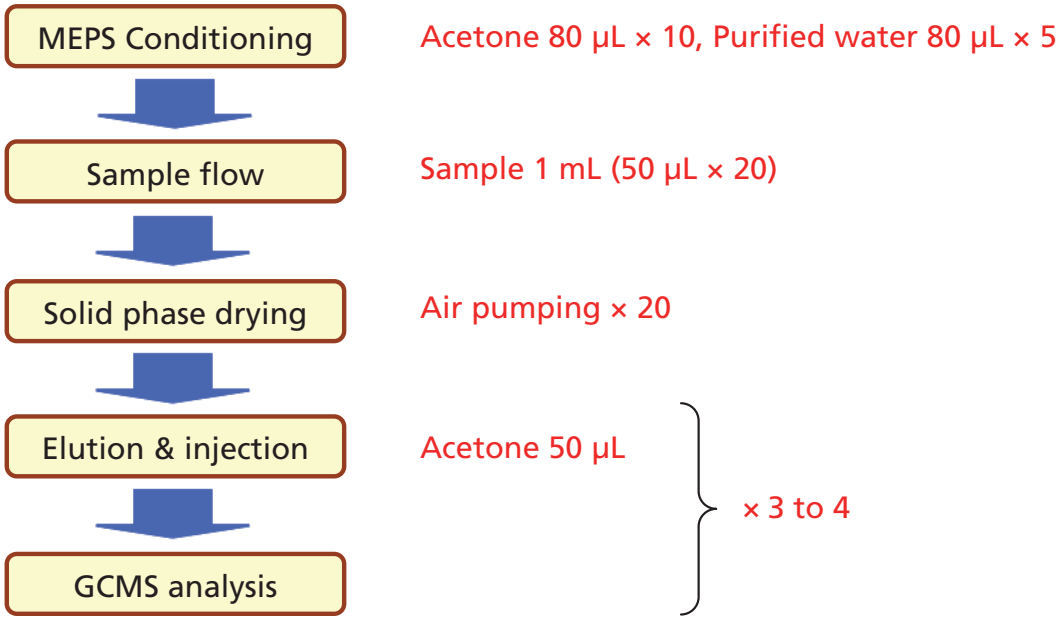

Fig. 3-4 AOC-MEPS System Pretreatment Flow

Table 3-1 Analytical Conditions and Target Pesticides

Instrument	: Shimadzu AOC-MEPS system + Shimadzu GC/MS QP-2010 Ultra
Injection port	: Programmable Temperature Vaporization injector OCI/PTV-2010 Plus
Injection port temperature	: 60 °C (1.0 min) – 250 °C/min – 280 °C (40 min)
Injection port pressure	: 10 kPa (0.1 min) – 400 kPa/min – 50.3 kPa (41 min)
Split ratio	: 25 (0.5 min) → 1
Column	: Rxi-5 ms (30 mL. × 0.32 mmI.D., 0.25 µm)
Column temperature	: 80 °C (2 min) – 20 °C /min – 180 °C – 5 °C /min – 300 °C (10 min)
Detector	: MS
Ion source temperature	: 230 °C
Interface temperature	: 300 °C
Measurement mode	: Scan mode
Measurement *m/z* range	: 29 – 700
Target pesticides	: Wako Pure Chemical Industries – PL-1-1, 2-1, 3-1, 4-1, 5-1, 6-1, 9-1, 10-1, 11-1 (222 substances excluding 8 substances that decompose or break through easily)

The results of the investigation of the PDVB elution solvent are shown in Fig. 3-5, and those of the C18 elution solvent are shown in Fig. 3-6. The pesticides were detected up to the third elution using each solvent, and no great differences in elution efficiency were seen. With acetone, most of the pesticide substances were eluted up to the second elution using both PDVB and C18, but less than 3 % of the pesticides were eluted at the third elution, and almost no elution was seen at the 4th elution. Therefore, it was confirmed that 150 µL is sufficient for use as an elution solvent. With the AOC-MEPS system, drying of the solid phase is conducted by pumping the syringe plunger in air, but because the air flow is not one-directional, it is difficult to achieve complete drying. Thus, a slight amount of water remains in the solid phase. It is therefore presumed that if a hydrophobic solvent, which is likely to possess high elution strength, is used as the elution solvent, the residual water would interfere with the contact between the collected pesticide and the elution solvent, thereby reducing the elution efficiency. Acetone was chosen as the elution solvent because of its hydrophilicity, as well as its low toxicity. Despite its relatively weak elution strength, good elution efficiency was obtained using acetone.

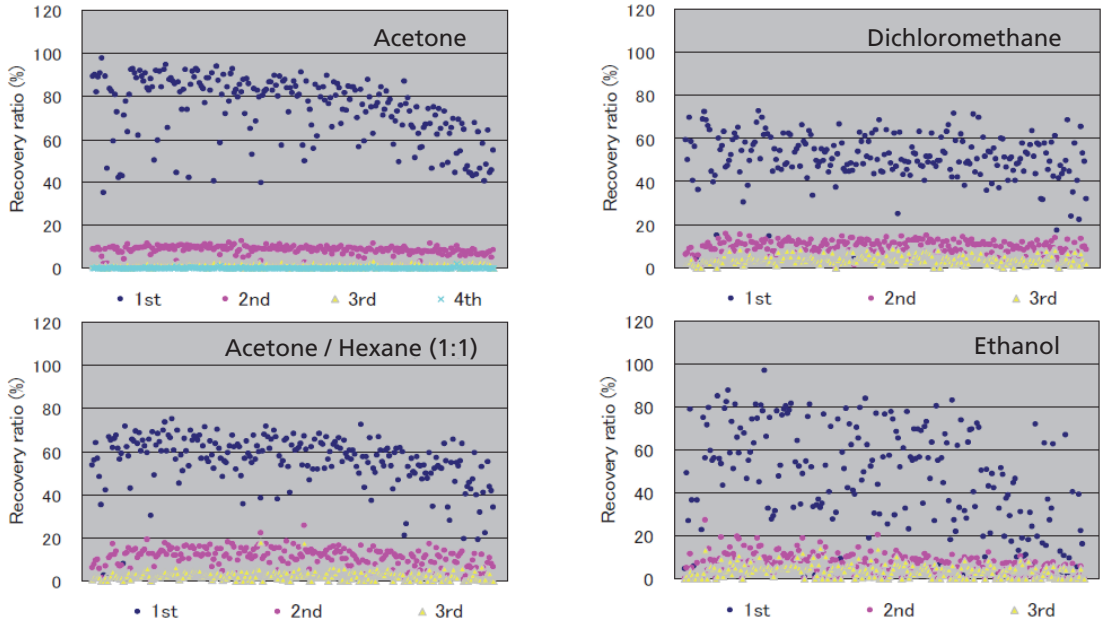

Fig. 3-5 Results of Investigation of Elution Solvent using PDVB

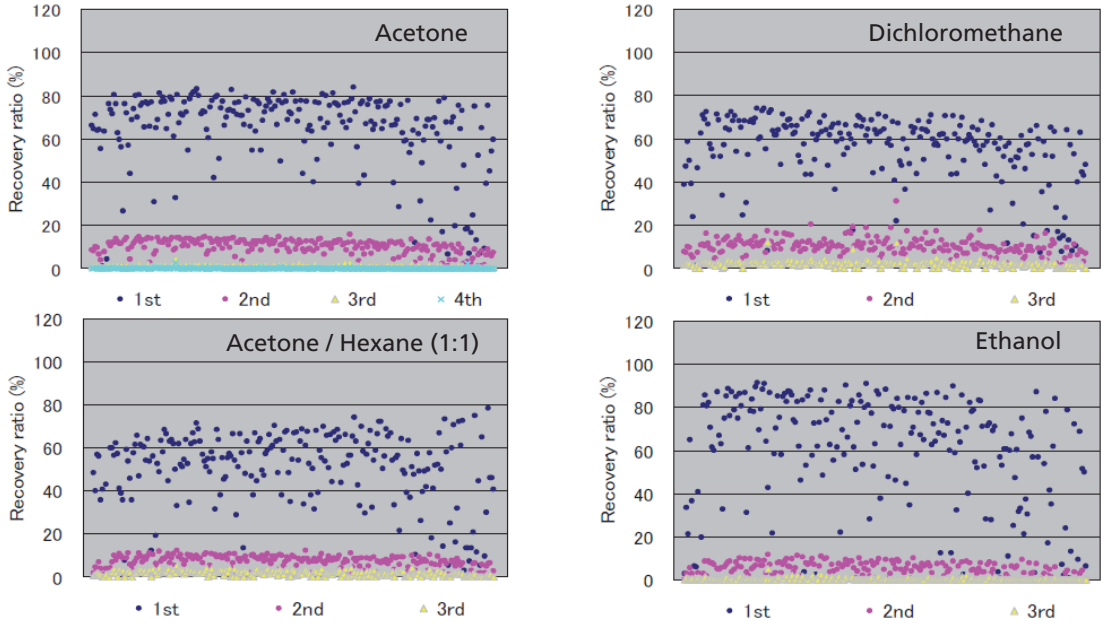

Fig. 3-6 Results of Investigation of Elution Solvent using C18

3-5. Spike – Recovery Test

After transferring 4 mL of purified water to a vial, 4 µL of an acetone solution spiked with each pesticide at a concentration of 2 ng/µL (2 ppm) was then transferred to the vial (the concentration of each pesticide present in the spiked sample was 2 ng/mL (2 ppb)). PDVB and C18 were used as sorbent bed materials in the MEPS cartridges. 1 mL of sample water (50 µL × 20 repetitions) was passed through the MEPS to extract the pesticides from the sample water, and after the solid phase was dried by air pumping, elution and injection were repeated 4 times using 50 µL of acetone. The recovery rate was determined by summing the quantitation values of the 4 injections. The mean value of three repeat measurements was taken as the spike recovery test result. The chromatogram of the standard sample is shown in Fig. 3-7, and the recovery rates are shown in Fig. 3-8.

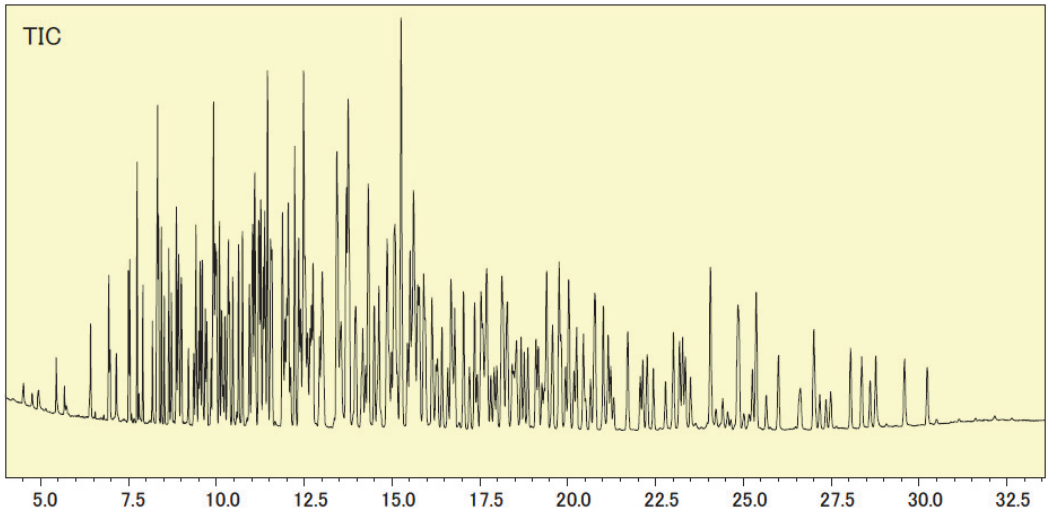

Fig. 3-7 TIC Chromatogram Obtained Using 50 µL Injection of 40 ng/mL Standard Solution (Corresponds to 2 ng/mL Concentration in Water)

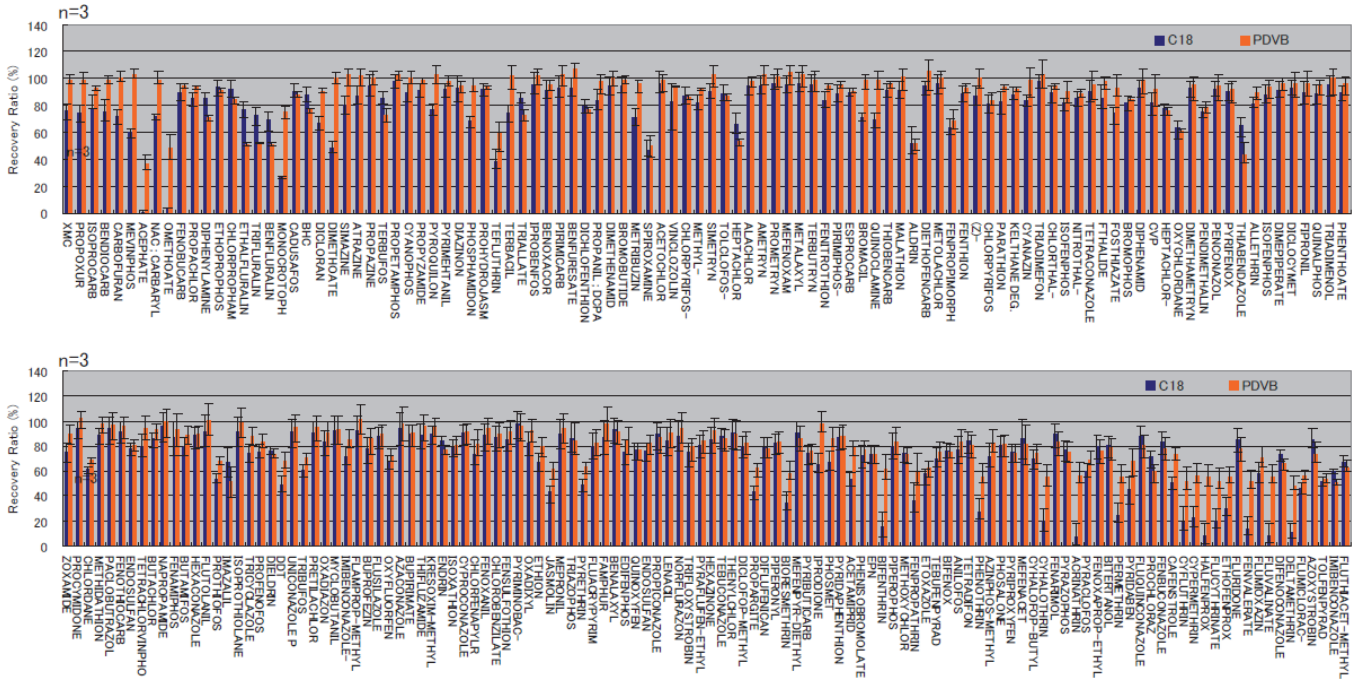

Fig. 3-8 Spike and Recovery Test Results Using Purified Water (Comparison of C18 and PDVB)

Substances which displayed low recoveries included analytes with extremely high water-solubility (Acephate, Omethoate, etc.) and highly hydrophobic analytes (Acrinathrin, Fluvalinate, Halfenprox, etc.). Those in which recoveries were less than 50 % included 28 substances when using C18, and 5 substances when using PDVB. Overall, however, recovery rates were relatively good when using PDVB. In particular, there was a noticeable trend in which many Carbamate and Pyrethroid pesticides showed recoveries more than 20 % higher when using PDVB rather than C18.

Spike recovery testing was then conducted using river water. Here, PDVB was used exclusively as the MEPS sorbent bed material. Recoveries were determined using the same procedure as that for the purified water. Fig. 3-9 shows a comparison of the spike and recovery test results obtained for the river water and the purified water.

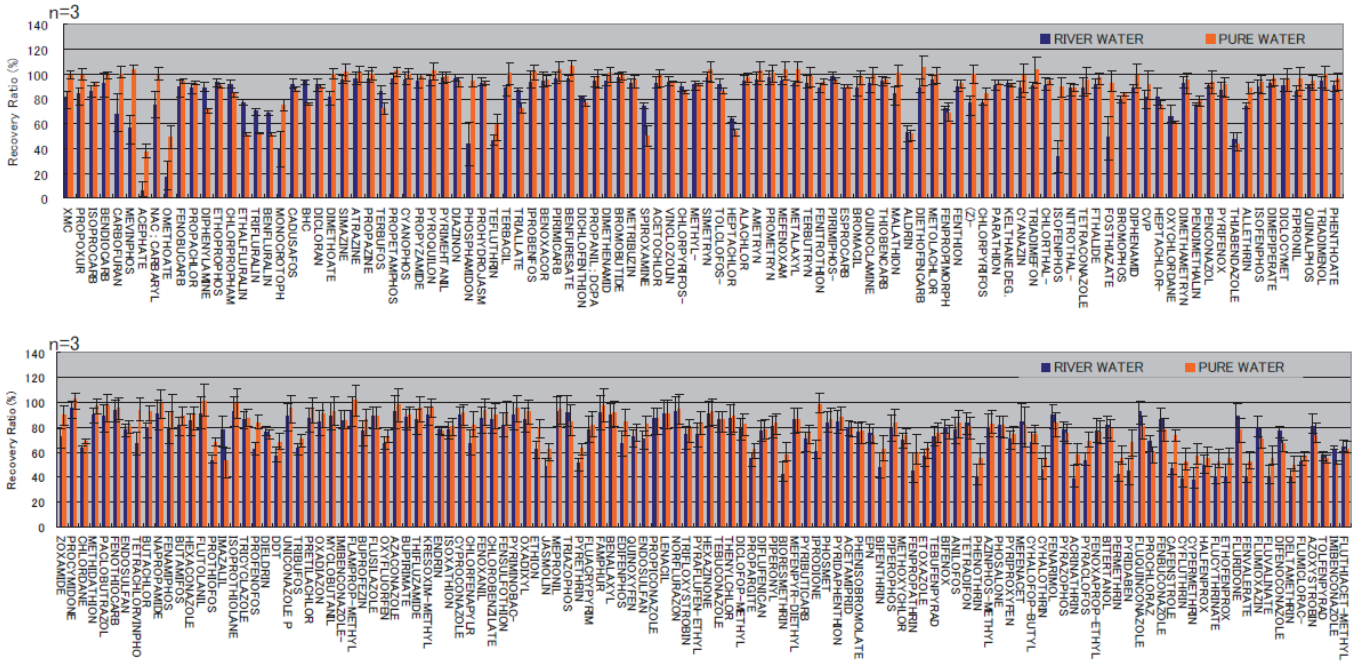

Fig. 3-9 Spike and Recovery Test Results for River Water Using PDVB (Comparison of Results Using River Water and Purified Water)

Although several components displayed varying behavior when using river water, generally excellent recovery rates were obtained.

3-6. Summary

Using the AOC-MEPS system, it was possible to automate the processes from sample pretreatment to injection in the analysis of pesticide residues in water. When spike and recovery testing was conducted for purified water and river water using PDVB, excellent recovery rates were obtained for most of the pesticide components. Based on these results, we believe that this method may be extremely effective for screening of pesticide residues in water.

4. Product Details

4-1. AOC-MEPS System

The AOC-MEPS system consists of an autoinjector, the MEPS syringe kit for the AOC-20i, and the PTV injection unit, etc.

Part Name	Part Number	Remarks
AOC-20i Autoinjector	221-72315-XX	Set includes injector, built-in power supply, installation parts, and standard accessories
MEPS syringe kit for AOC-20i	221-73836-41	Includes: For long turret MEPS (P/N 221-73837-41) Long turret (P/N 221-49976) 1 piece Case for 4 mL vials (P/N 221-32949-01) 8 pieces MEPS label (P/N 221-73838) 1 piece Solvent warning label (P/N 221-45257) 1 piece Protective guide label (P/N 221-49359) 1 piece Pack of 50 4 mL sample vials (P/N 221-34269-91) Set includes vials, caps, and septa
OCT/PTV-2010 (100 – 120 V)	221-71042-91	When high-power oven GC is used, OCT/PTV-2010 (220 – 240 V): P/N 221-71042-34 is required

• The MEPS syringe kit supports AOC-20i ROM Ver. 3.0 or later.

4-2. MEPS Consumables

Part Name	Part Number	Remarks
MEPS Syringe	533-M0156259	For AOC-20i
MEPS BIN PDVB	533-M2900607SH	5 pieces included (Sorbent material specific to Shimadzu AOC)
MEPS BIN C18	533-M2900601SH	5 pieces included
MEPS BIN Silica	533-M2900602SH	5 pieces included
MEPS BIN C8 + SCX	533-M2900603SH	5 pieces included
MEPS BIN C2	533-M2900604SH	5 pieces included
MEPS BIN C8	533-M2900606SH	5 pieces included
Method Development Kit	533-M2900605SH	Includes 1 each of C18, C8, C2, Silica, C8 + SCX
PTV Insert for MEPS	533-M092291SH	Filled with glass wool, 5 pieces included, 2.5 mmI.D.

• In Japan, these parts are handled by Shimadzu GLC Ltd.
• Customers outside Japan should order these parts from the Shimadzu subsidiary for that region.
• Any other maintenance or consumable parts are the same as those for the AOC-20 Series. For further details, please refer to the AOC-20 Series Instruction Manual.

Note : This material is based on information available at the time of its publication, and may be modified without prior notice.

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