Simultaneous Analytical Method I for Agricultural Chemicals using LC/MS (Agricultural Products)

Since May 2003, when the Positive List System was enacted, the Ministry of Health, Labor and Welfare has announced various methods for conducting simultaneous analyses using GC/MS, LC/MS, and HPLC. These include "Simultaneous Analytical Method I for Agricultural Chemicals using LC/MS (Agricultural Products)" which is used to analyze 42 components, and "Simultaneous Analytical Method II for Agricultural Chemicals using LC/MS (Agricultural Products)" used for 25 components. In October, 2003, new agricultural chemicals were added to Simultaneous Analytical Methods I and II, and "Simultaneous Analytical Method for Agricultural Chemicals using LC/MS (Livestock and Seafood Products)" was also published.

Introduced in this report are examples of simultaneous analyses of the 42 components described in “Simultaneous Analytical Method I for Agricultural Chemicals using LC/MS (Agricultural Products).” Figure 1 shows an MRM chromatogram of agricultural chemical reference standards (0.01mg/L). Each reference standard was dissolved and mixed in acetonitrile, then diluted with methanol to prepare sample solutions for analysis. Table 1 lists ions selected for MRM for each of the substances analyzed. Forty ions were detected with ESI (+) and 2 ions with ESI (–).

Additional recovery tests of substances analyzed were also conducted. The pretreatment process, performed in accordance with Simultaneous Analytical Method I, consisted of extraction, salting out, dehydration and purification by a mini-column. Figure 2 shows MRM chromatograms of a sample solution prepared by conducting pretreatment and purification procedures using a cucumber with no additions, as well as a sample solution prepared following these pretreatment and purification procedures after agricultural chemicals were added to the cucumber (concentrations in final solution: 0.01mg/L). Peak shapes of the analytes were good, with no interference from foreign components, and good recovery of ≥90% were obtained.

Table 1: List of monitoring selected ions

Pesticides	Ionization	Monitoring ion (m/z)	Pesticides	Ionization	Monitoring ion (m/z)
Azamethiphos	ESI(+)	325.03/182.60	Thiabendazole	ESI(+)	201.92/174.70
Azinphos-methyl	ESI(+)	318.05/159.70	Thiamethoxam	ESI(+)	292.06/210.70
Anilofos	ESI(+)	368.07/198.70	Tralkoxydim-1	ESI(-)	328.40/254.00
Abamectin B1a	ESI(+)	890.70/305.10	Tralkoxydim-2	ESI(+)	330.30/137.60
Isoxaflutole	ESI(+)	359.90/250.80	Triticonazole	ESI(+)	318.10/69.60
Iprovalicarb	ESI(+)	321.29/118.70	Tridemorph-1	ESI(+)	298.38/129.70
Imidacloprid	ESI(+)	256.02/208.90	Tridemorph-2	ESI(+)	298.38/129.70
Indoxacarb	ESI(+)	528.25/149.60	Naproanilide	ESI(+)	292.20/170.60
Oxycarboxin	ESI(+)	268.06/174.70	Pyrazolynate	ESI(+)	439.17/90.60
Oryzalin	ESI(-)	345.30/280.90	Pyriftalid	ESI(+)	318.90/138.60
Quizalofop-p-tefuryl	ESI(+)	429.10/298.90	Fenoxycarb	ESI(+)	302.20/115.50
Cloquintocet-mexyl	ESI(+)	336.00/237.80	Ferimzone(E)	ESI(+)	255.14/131.60
Clothianidin	ESI(+)	249.96/168.70	Ferimzone(Z)	ESI(+)	255.14/131.60
Chromafenozide	ESI(+)	395.36/174.70	Phenmedipham	ESI(+)	318.20/135.60
Clomeprop	ESI(+)	324.00/119.60	Butafenacil	ESI(+)	492.27/330.80
Chloridazon	ESI(+)	221.96/91.60	Furathiocarb	ESI(+)	383.24/251.80
Cyazofamid	ESI(+)	325.14/107.40	Benzofenap	ESI(+)	431.24/104.50
Cyflufenamide	ESI(+)	413.24/294.90	Milbemectin A3	ESI(+)	511.30/457.20
Simeconazole	ESI(+)	294.19/69.60	Milbemectin A4	ESI(+)	525.30/108.70
Dimethirimol	ESI(+)	210.05/70.50	Methoxyfenozide	ESI(+)	369.31/148.70
Thiacloprid	ESI(+)	253.00/125.60	Lactofen	ESI(+)	479.24/343.80

Table 2: Analytical conditions

Column:	**TSKgel ODS-100V, 3μm, 2.0mm ID × 15cm**
Mobile phase A:	5mmol/L ammonium acetate in water
Mobile phase B:	5mmol/L ammonium acetate in methanol
Gradient:	0 min (15%B) →1 min (40%B) →3.5 min (40%B) → 6 min (50%B) →8 min (55%B) → 17.5 min (95%B) →30 min (95%B)
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	5μL
Detection:	Quattro Premier™ XE (Waters)

Figure 1: MRM chromatogram of agricultural chemical reference standards (0.01mg/L)

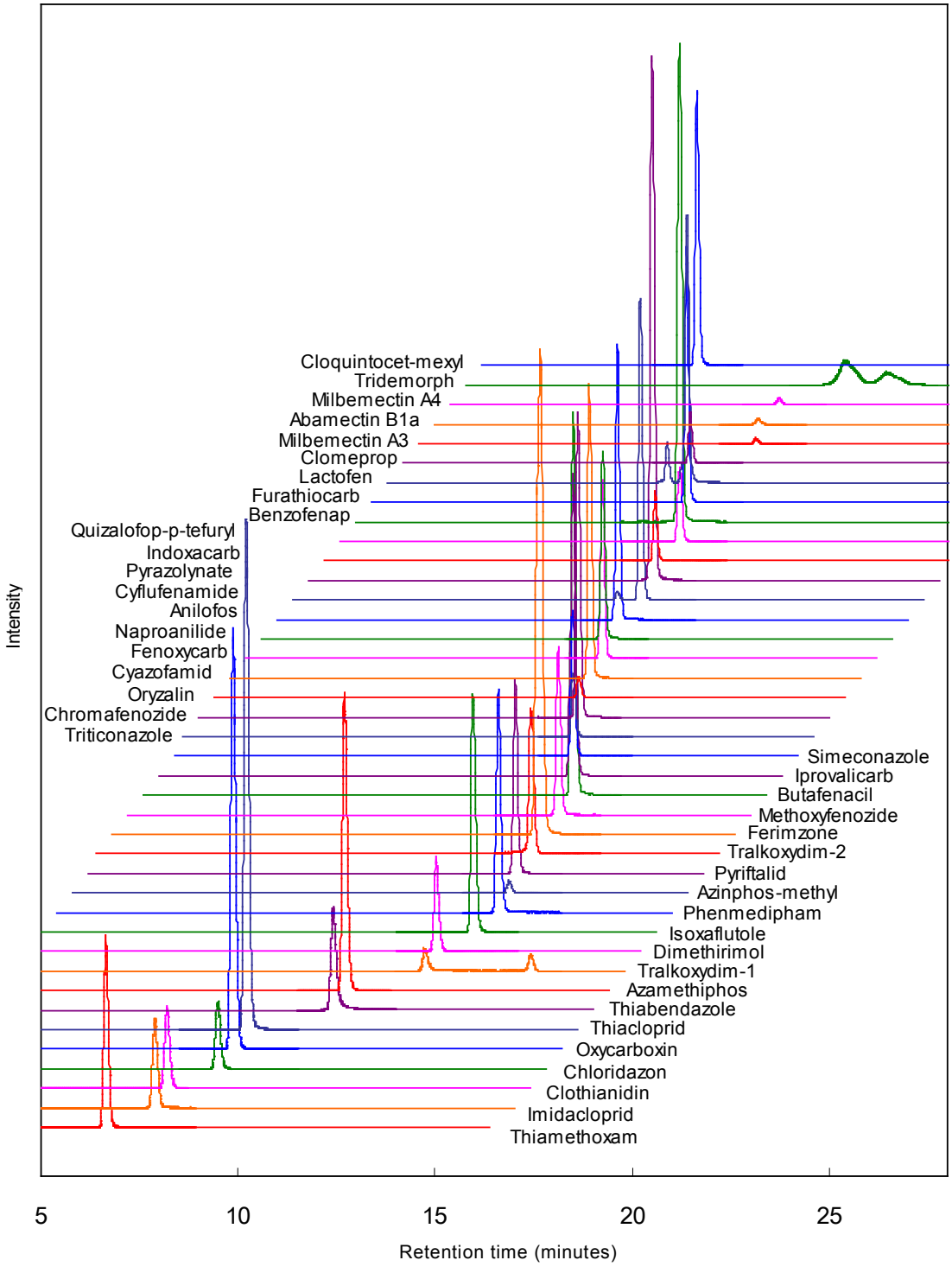
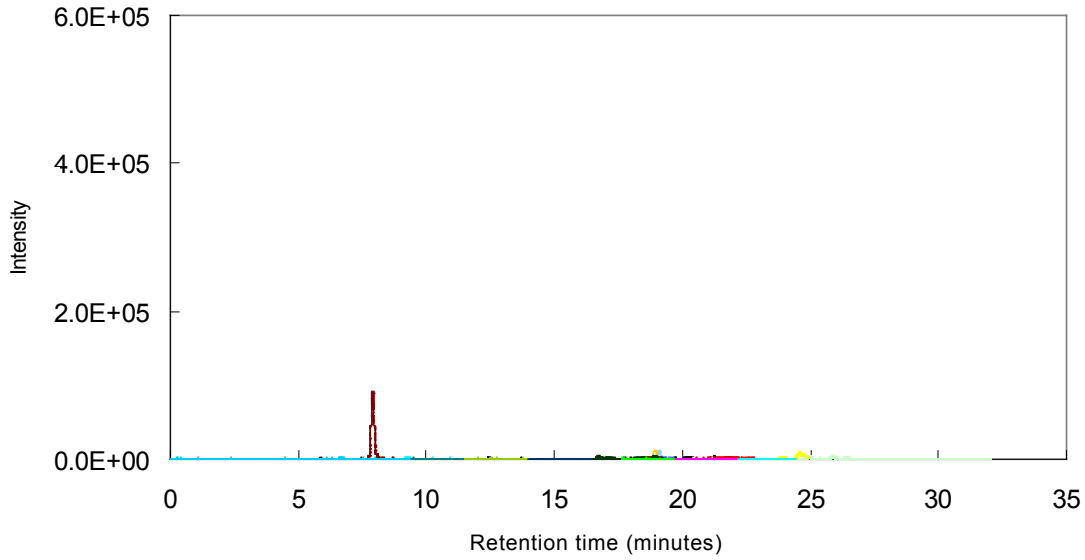
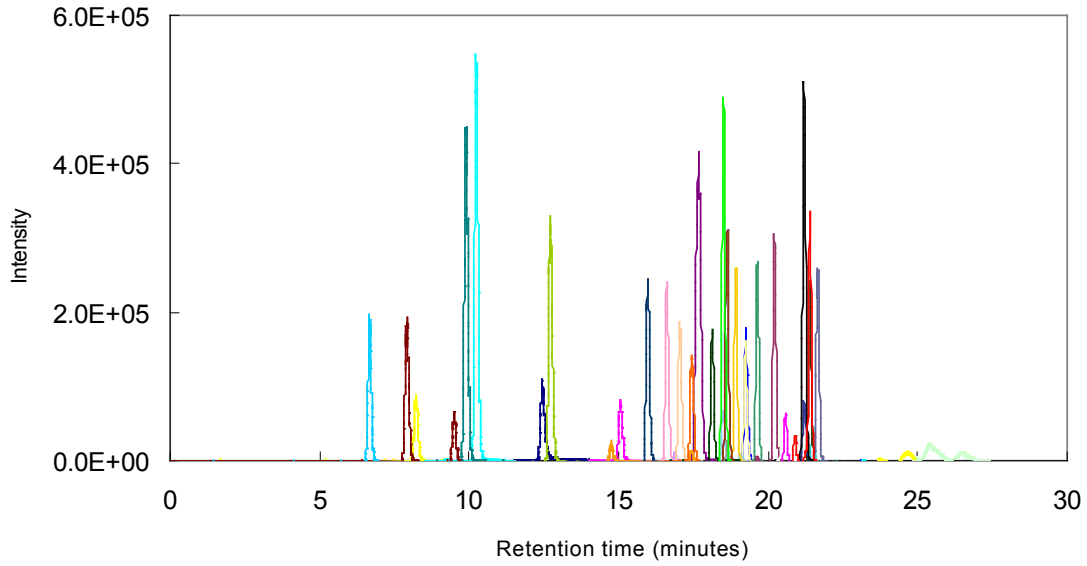


Figure 2: MRM chromatograms



*Test solution prepared by pretreatment and purification of cucumber with no added chemicals.



*Test solution prepared by pretreatment and purification of cucumber after adding agricultural chemicals.

Table 3. Method for preparing test solutions

1) Extraction

Weigh out an aliquot (20.0g) of the sample. Add 20mL of water and allow to stand for 15 minutes. Next, add 50mL of acetonitrile and homogenize, followed by suction filtration. Then, add 20mL of acetonitrile to the residue on the filter paper, homogenize, and collect the filtrate by suction filtration. Combine the resulting filtrates and bring to 100mL with acetonitrile.

To 20mL of the extract, add 10g of sodium chloride and 20mL of 0.5mol/L phosphate buffer, pH 7.0, and agitate. After allowing the mixture to stand, discard the aqueous layer. Next, add anhydrous sodium sulfate to dehydrate the acetonitrile layer by adding and filtering out anhydrous sodium sulfate. Concentrate the filtrate at ≤40°C to extract the solvent. Dissolve the residue with 2mL of a 3:1 acetonitrile:toluene mixture.

2) Purification

Next, inject 10mL of a mixed solution of acetonitrile and toluene (3:1) to a graphite carbon/aminopropyl silylation silica-gel packed mini-column (Envicarb/NH2; Supelco, 500mg/500mg, 20mL), and discard the effluent. After injecting the solution obtained in step 1) into this column, inject 20mL of a 3:1 acetonitrile:toluene solution , and condense the entire eluate to ≤1 mL at ≤40 °C. Next, add 10mL of acetone, further condense to ≤1mL at ≤40 °C, and add an additional 5mL of acetone to condense and remove the solvent.

Dissolve the residue in methanol and bring to 4.0mL to prepare the test solution.

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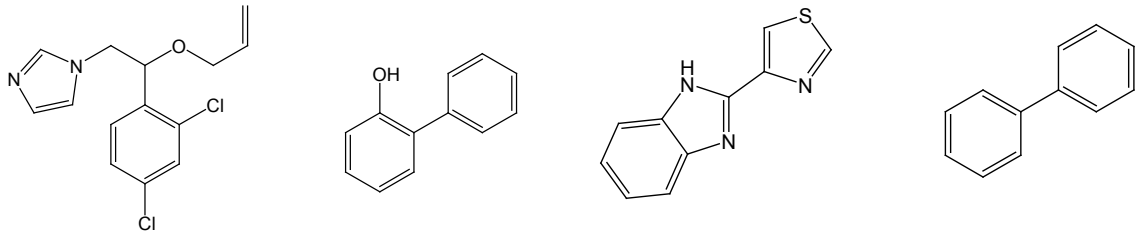
Analysis of Fungicides in Disposable Chopsticks by HPLC

Fungicides are used as post-harvest agricultural chemicals for long-term preservation of fruits and vegetables. Four types of fungicides are approved for use in Japan: imazalil (IMZ), orthophenylphenol (OPP), thiabendazole (TBZ), and diphenyl (DP). Residue standards have been established for these fungicides on citrus fruits such as lemons and grapefruit, and bananas. In addition, in Notification No. 1113001 of the Inspection and Safety Division, and No. 1113001 of the Standards and Evaluation Division of the Department of Food Safety (Nov. 13, 2007) of the Ministry of Health, Labour and Welfare (MHLW), it is stipulated that limits for these 4 types of fungicides in disposable chopsticks should be "not detected."

Presented in this report, based on the regulations cited above, are examples of individual analyses of imzalil and simultaneous analyses of orthophenylphenol, thiabendazole, and diphenyl in disposable chopsticks. Various types of disposable chopsticks were analyzed (bamboo, wood, domestically produced, produced in China), but no fungicides were detected in any samples.

Figure 1. Fungicides



IMZ OPP TBZ DP

Table 1. Conditions for individual analysis of Imazalil

Column:	**TSKgel ODS-100V, 5µm, 4.6mm ID x 25cm**
Mobile phase:	methanol/water=75/25
Flow rate:	1.0mL/min
Detection:	UV@230nm
Temperature:	40°C
Injection vol.:	20µL

Table 2. Conditions for simultaneous analysis of orthophenylphenol, thiabendazole, and diphenyl

Column:	**TSKgel ODS-100V, 5µm, 4.6mm ID x 25cm**
Mobile phase:	10mmol/L SDS in methanol/acetonitrile/water=60/5/35, pH 2.70 adjusted with phosphoric acid
Flow rate:	1.0mL/min
Detection:	FL (Ex: 285nm, Em: 325nm)
Temperature:	40°C
Injection vol.:	20µL

Figure 2. Chromatograms of IMZ reference standard, disposable chopstick (bamboo) extract, disposable chopstick (wood) extract, and disposable chopstick (bamboo) extract (added sample)

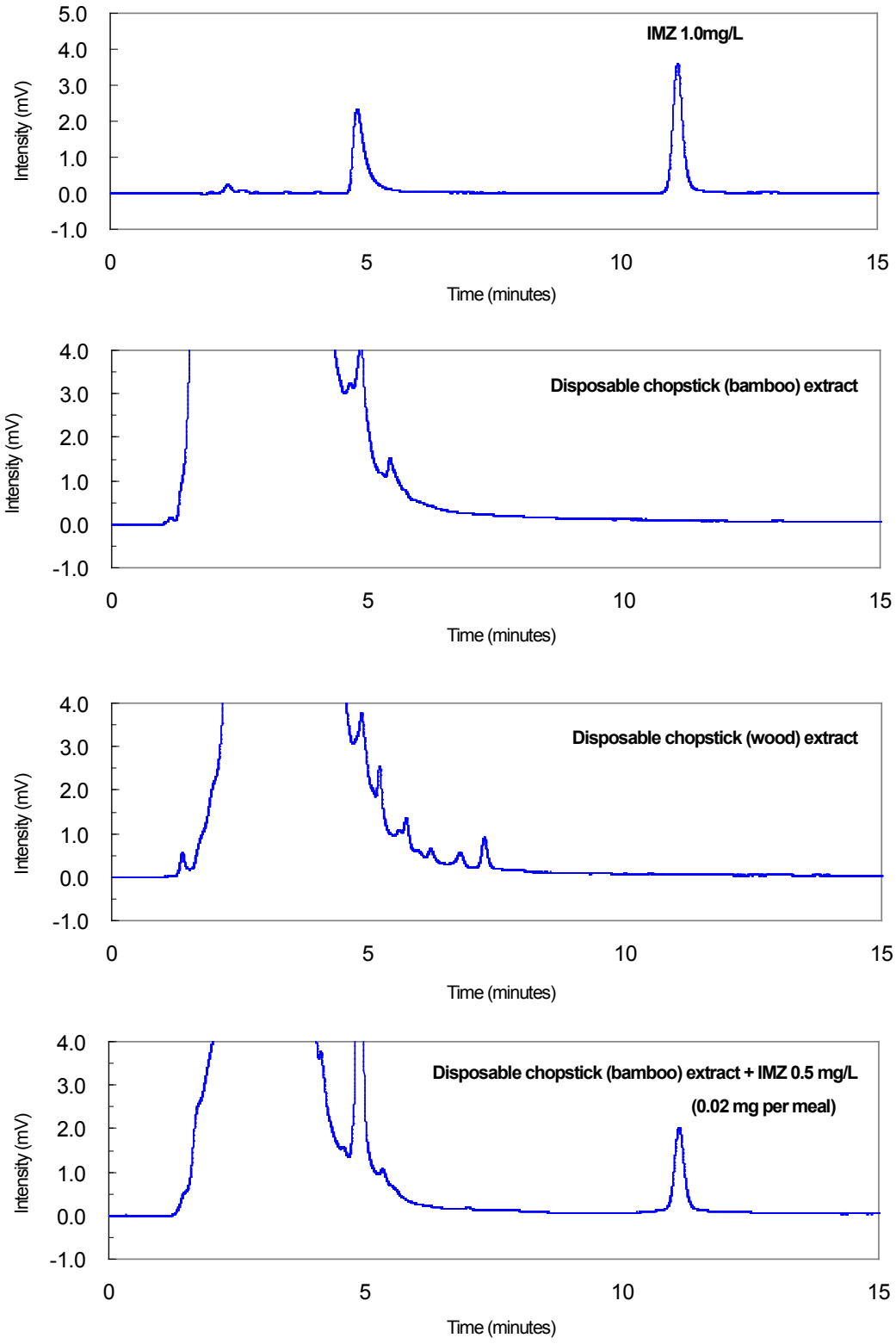


Figure 3. Chromatograms of reference standard (OPP, TBZ, DP), disposable chopstick (bamboo) extract, disposable chopstick (wood) extract, and disposable chopstick (bamboo) extract (added sample)

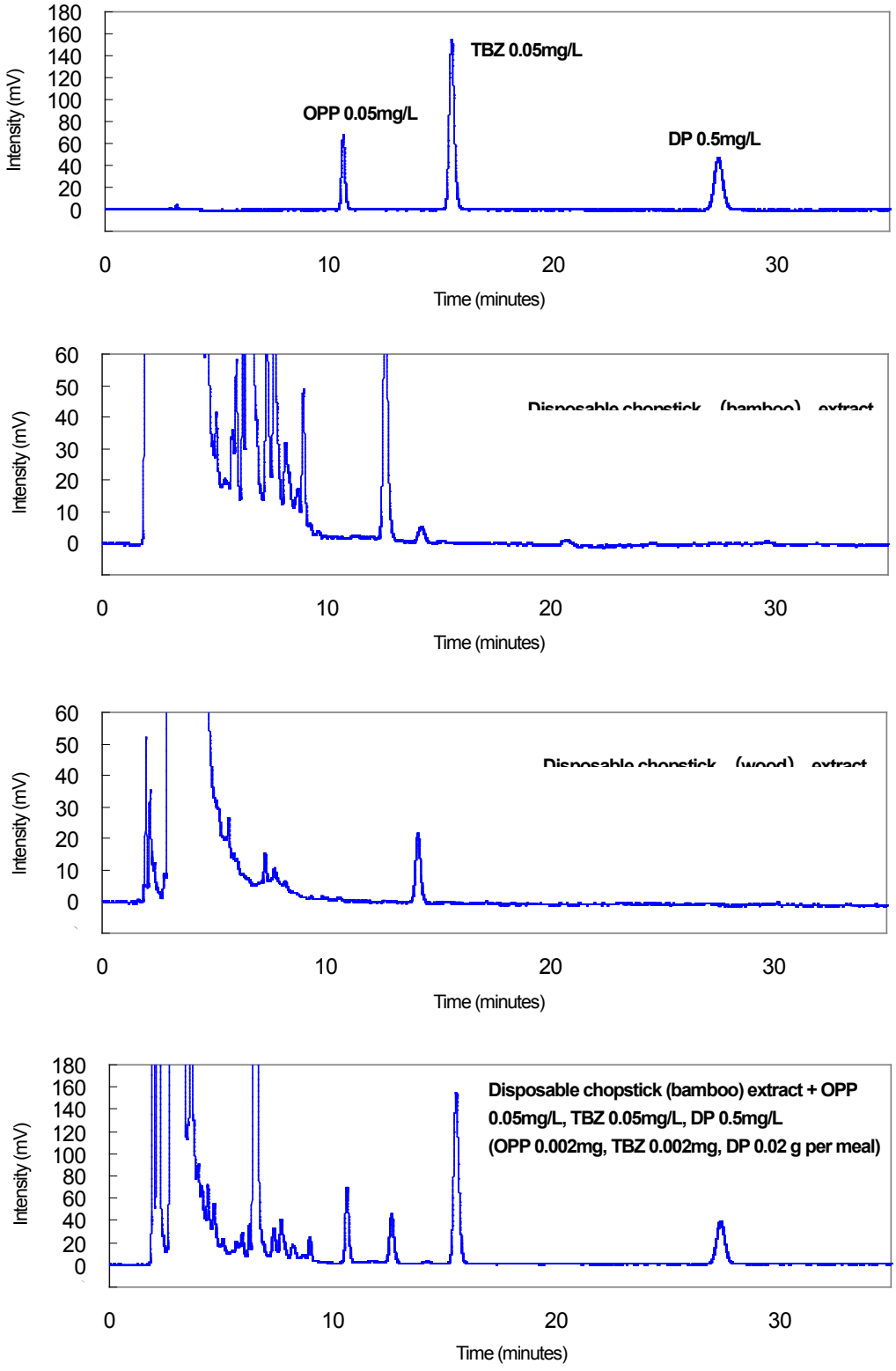


Figure 4. Procedure for extracting fungicides from disposable chopsticks

Place 20mL of aqueous solution of 20% ethanol in 25mL stoppered test tubes and heat at 60°C.

↓

Break disposable chopstick in two and immerse.

↓

Let stand at 60°C for 30 minutes.

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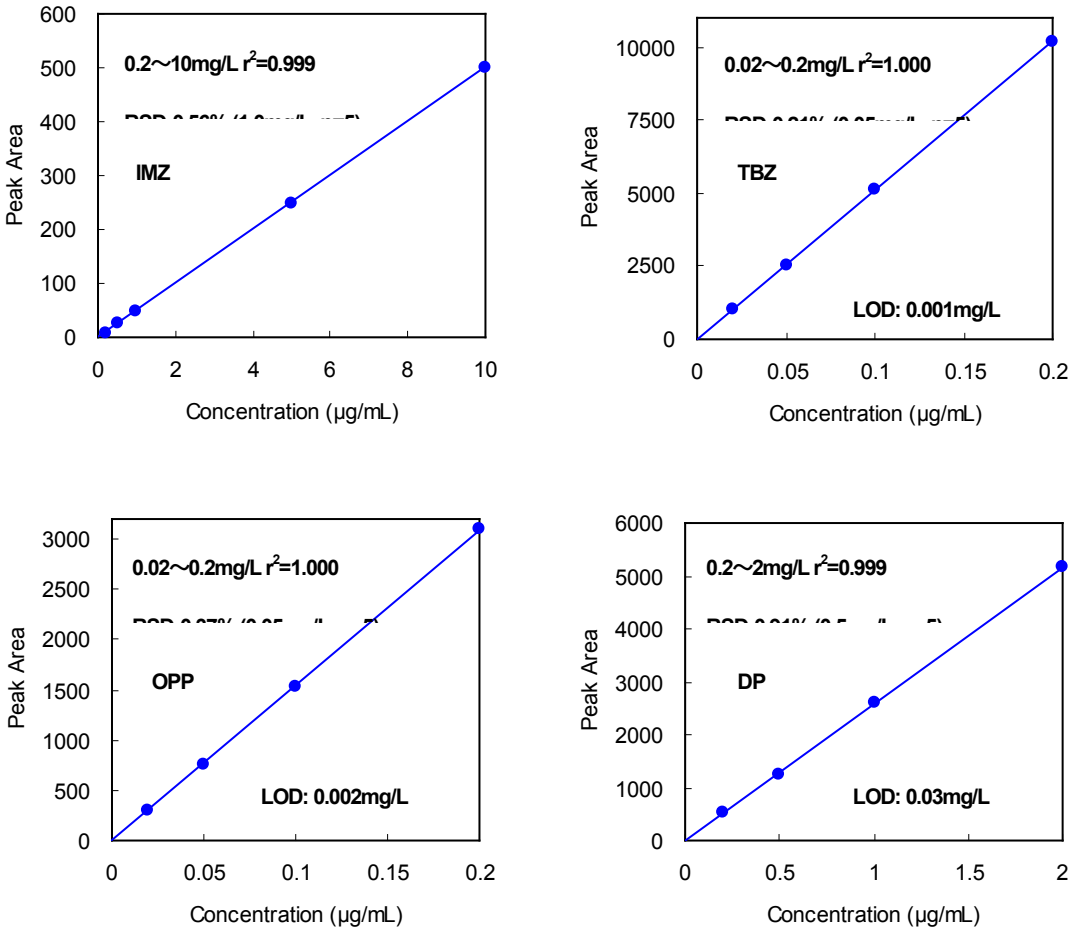
Remove disposable chopstick and filter residual liquid (pore size: 0.5μm) to prepare samples for analysis.

Figure 5. Calibration curves, repeatability and LOD

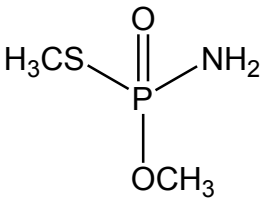


Analysis of Methamidophos and Acephate in Tea by LC-MS/MS

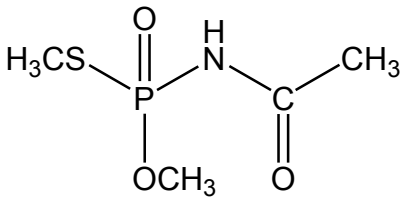
Methamidophos is an organophosphate insecticide that has been banned for use in Japan. Through the Positive List System, the Ministry of Health Labour and Welfare has advised that GC should be used as the method for analyzing methamidophos. However, as methamidophos has a polar structure similar to acephate, this compound is not readily applicable to GC analysis.

Here, methamidophos and acephate were simultaneously analyzed using a simulated sample created by adding standard substances to tea. Analysis was performed after first removing highly hydrophobic foreign matter in a two-stage solid-phase extraction process conducted as a pretreatment process. In this process, recovery rates of ≥75% were achieved for added analytes. Both components showed good linearity in a concentration range of 0.5 to 50ppb.

Figure 1. Structural formulas



Methamidophos



Acephate

Table 1. Analytical conditions

Column:	TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Mobile phase:	A: water,
	B: acetonitrile
	A/B = 95/5
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	5µL
Instrument:	Agilent 1200SL series
	QTRAP (MDS SCIEX)
Ion Source:	ESI (Positive)
	m/z – 142>94 (Methamidophos), 184>143 (Acephate)

Figure 2. Chromatogram of methamidophos and acephate (5ppb)

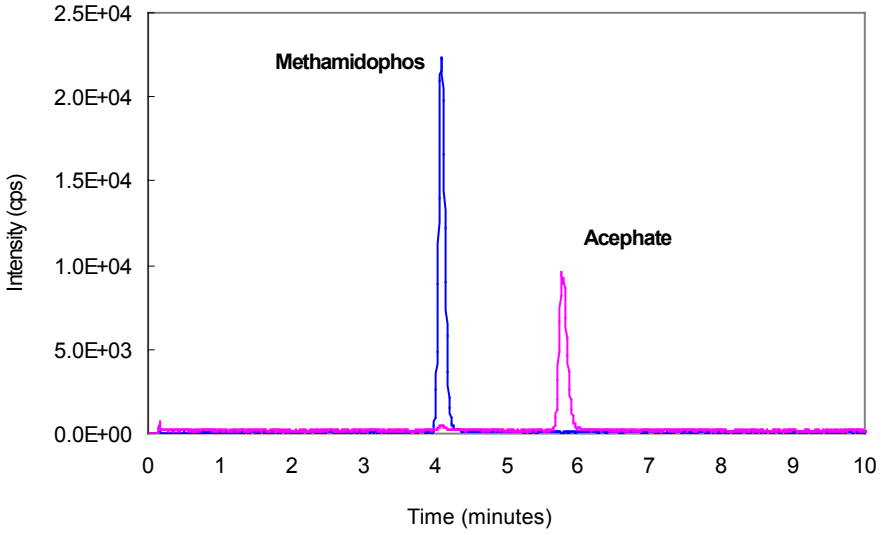


Figure 3. Pretreatment of samples

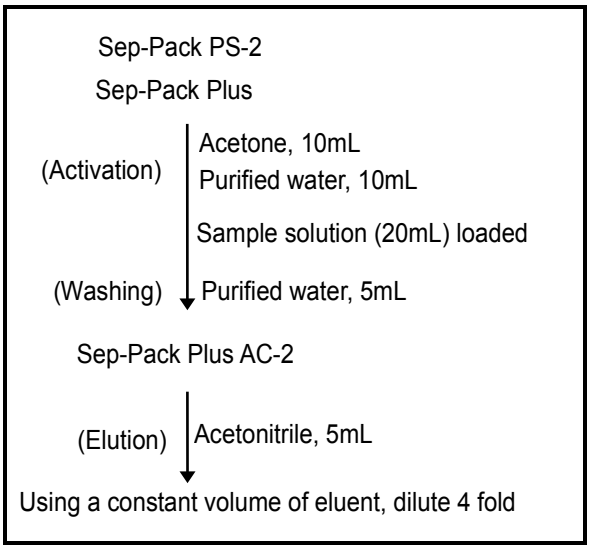
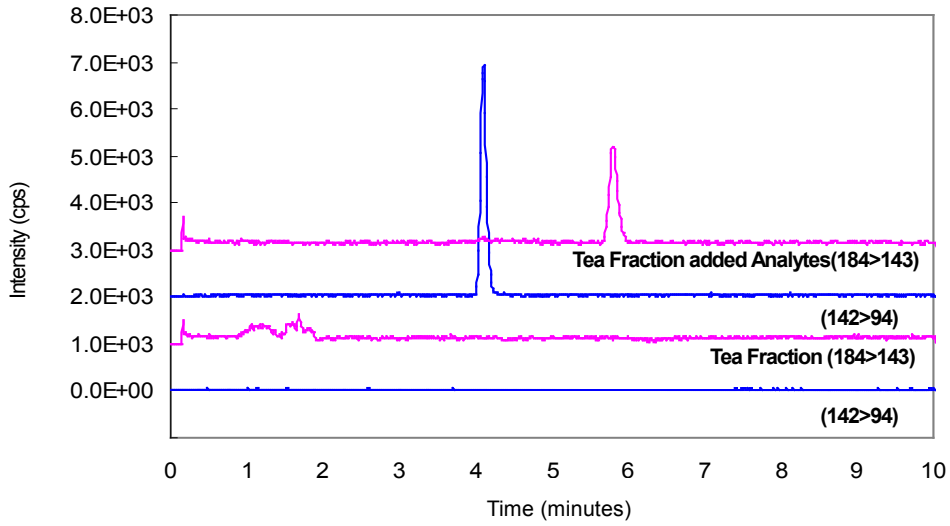


Figure 4. Chromatograms of tea and tea with added analytes (1ppb)



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