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)

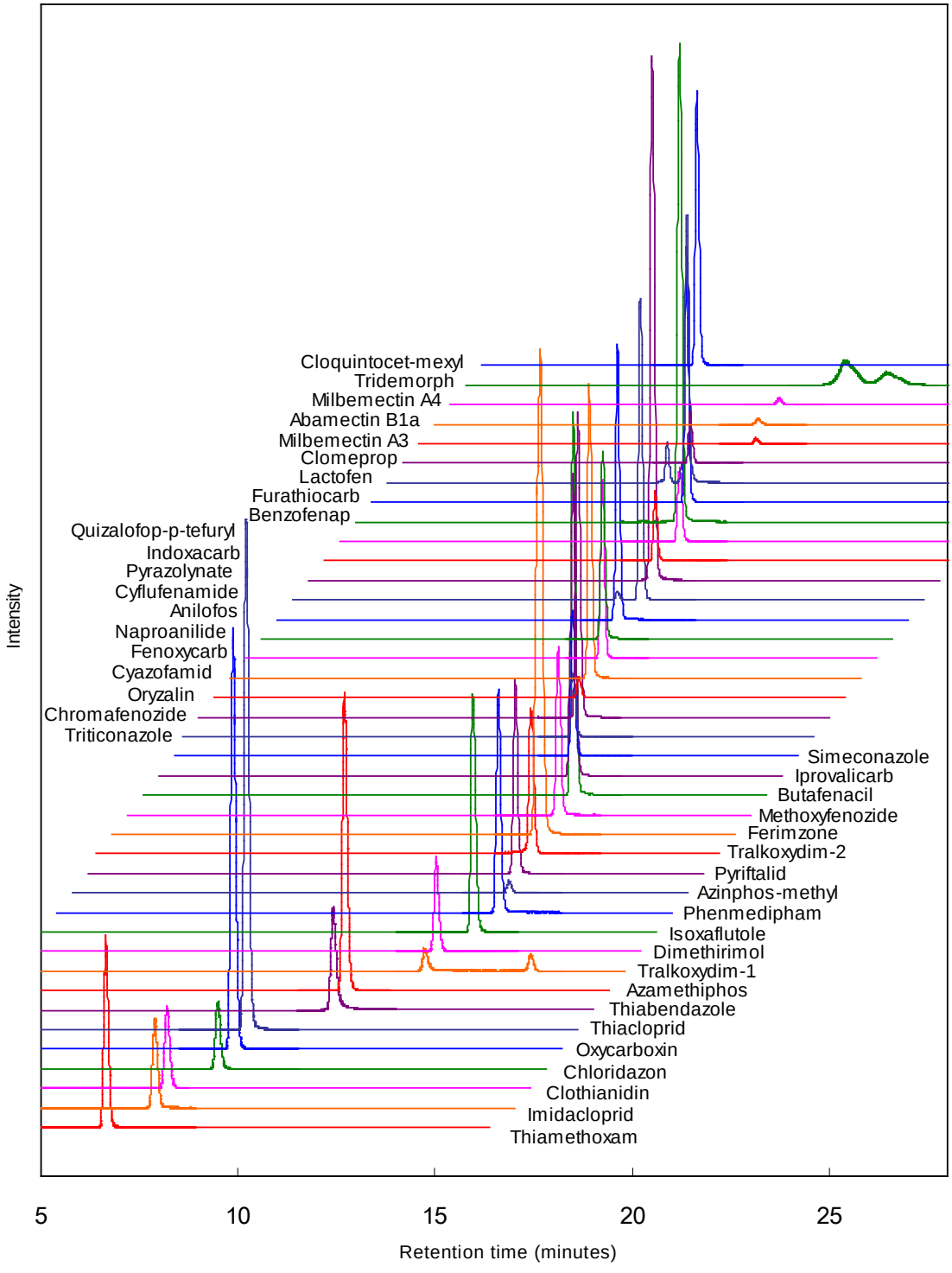
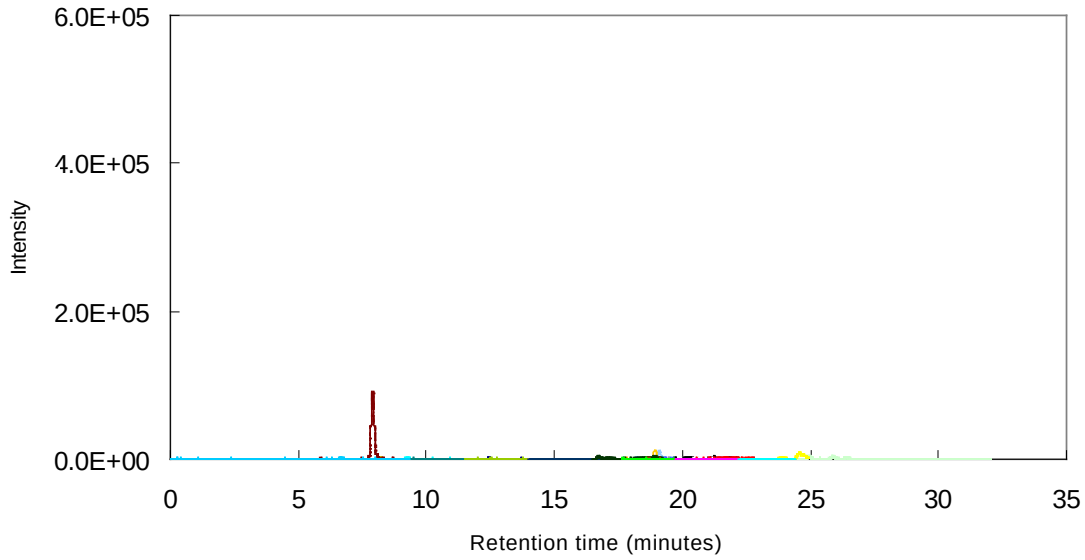
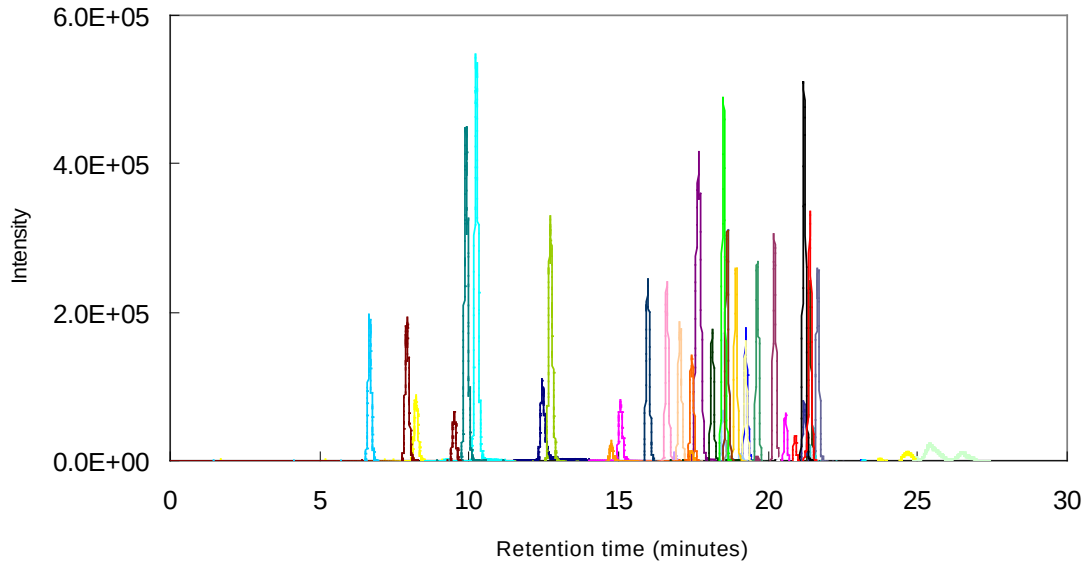


Figure 2: MRM chromatograms



*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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