

Application News

No.L480

High Performance Liquid Chromatography

Simultaneous Determination of Asulam, Thiophanate-Methyl, Siduron, Iprodione by Prominence-i

A ministerial ordinance (Ministry of Health, Labour and Welfare Ordinance No. 101) concerning new water quality standards was promulgated in Japan on May 30, 2003, and in conjunction with this ordinance, "complementary parameters for setting water quality management targets (27 items)" were announced on October 10, 2003 via the Ministry of Health, Labor and Welfare; Health Policy Bureau Ordinance No. 1010004. Of these water quality management parameters, 102 pesticides were selected as target components, and 11 of these are specified to be inspected using high-performance liquid chromatography. Further, among these, the "Separate Method 9" pesticide

test method has been specified for analysis of four substances, asulam, thiophanate-methyl, siduron, and iprodione, which are widely used as golf course pesticides. Golf courses are often located in upstream river areas, and because of the potential impact on public waters, use of these substances has generated concern, attracting considerable social attention. Here, simultaneous analysis of these four pesticides in a standard sample was conducted in compliance with the "Separate Method 9" using the Prominence-i integrated high-performance liquid chromatograph, and the measurement accuracy and linearity were then evaluated.

■ Analysis of Standard Mixture

Fig. 1 shows the structural formulas of the four substances. The target values for these pesticides are set to 0.2 mg/L for asulam, and 0.3 mg/L for the other three substances, and the target value for measurement accuracy is set to 1/100 (0.003 mg/L, and for asulam, 0.002 mg/L), with a coefficient of variation within 20 %. As the test water must be concentrated 500-fold using solid-phase extraction before conducting analysis as specified in "Separate Method 9", the sample concentration will be 1.5 mg/L (1.0 mg/L in the case of asulam,) when conducting HPLC analysis. Fig. 2 shows the chromatograms obtained using a mixture of standard samples, and Table 1 shows the analytical conditions. The sample concentrations were 1.0 mg/L for asulam, and 1.5 mg/L for thiophanate-methyl, siduron, and iprodione, respectively. The 50 mM KH_2PO_4 that was used for the mobile phase was prepared by dissolving 6.8 g potassium dihydrogen phosphate in 1 L ultra-pure water, and phosphoric acid was added to obtain acidity of pH 3. The method specifies that measurement be conducted using wavelengths of 270 nm for asulam, and 230 nm for the other three substances, but analysis was conducted using the dual-wavelength monitoring mode of the Prominence-i. As a result, all of the components were separated and detected with high sensitivity at 1/100 of the target value.

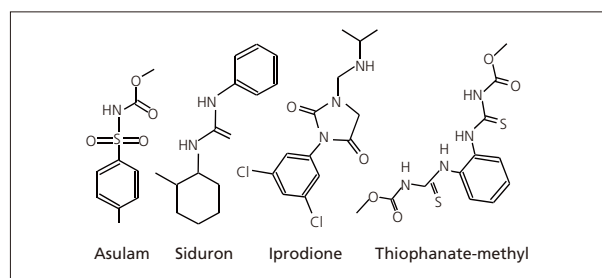


Fig. 1 Structural Formulas

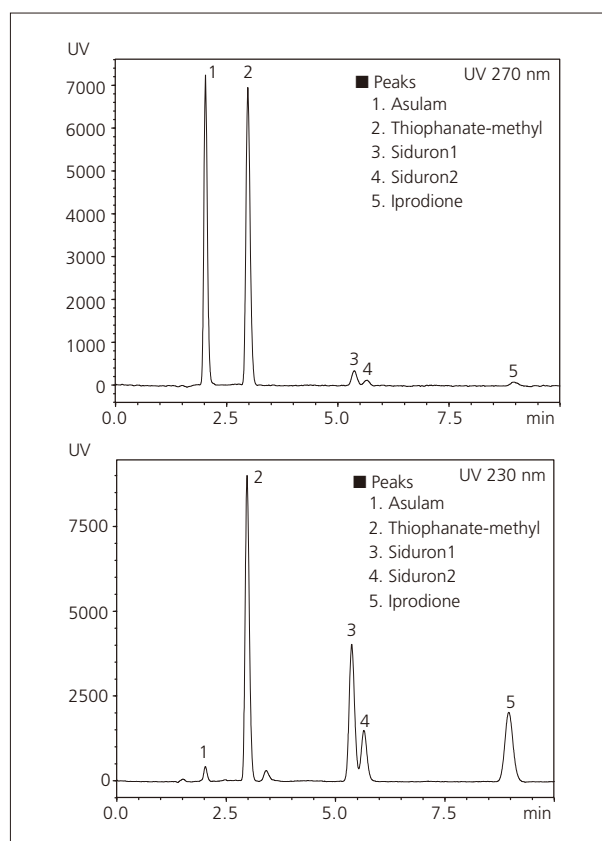


Fig. 2 Chromatogram of Standard Sample Analyzed Using Dual-Wavelength Monitoring

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D.)
Mobile Phase	: 50 mM KH_2PO_4 (pH 3.0) / Acetonitrile = 55 / 45 (v/v%)
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Detection	: Prominence-i UV at 230 nm, 270 nm
Inject Vol.	: 10 μL

Names of Compounds*

1. Asulam (1.0 mg/L)
2. Thiophanate-methyl (1.5 mg/L)
3. Siduron1 (1.5 mg/L)
4. Siduron2 (1.5 mg/L)
5. Iprodione (1.5 mg/L)

* Numbered according to numbers on chromatogram

■ Repeatability

Using the Prominence-i, repeatability of retention time and area value of the four pesticides were checked based on five repeat measurements (n=5) using their respective target concentrations. The obtained %RSD values for the respective retention times and area values are shown in Table 2.

Table 2 Repeatability of Peak Area and Retention Time

	Retention Time %RSD	Peak Area %RSD
1. Asulam	0.031	0.33
2. Thiophanate-methyl	0.018	0.26
3. Siduron 1	0.019	0.50
4. Siduron 2	0.026	0.16
5. Iprodione	0.035	0.34

■ Linearity

Fig. 3 shows the calibration curves generated using standard mixture solutions prepared using acetonitrile to obtain component concentrations ranging from 0.08 to 5 mg/L. Since two peaks of Siduron are detected using the analytical conditions in Table 1, the calibration curve was generated using the sum of the peak areas of Siduron 1 and Siduron 2. The coefficient of determination (R^2) obtained with respect to all of the concentrations was greater than 0.9999, indicating excellent linearity.

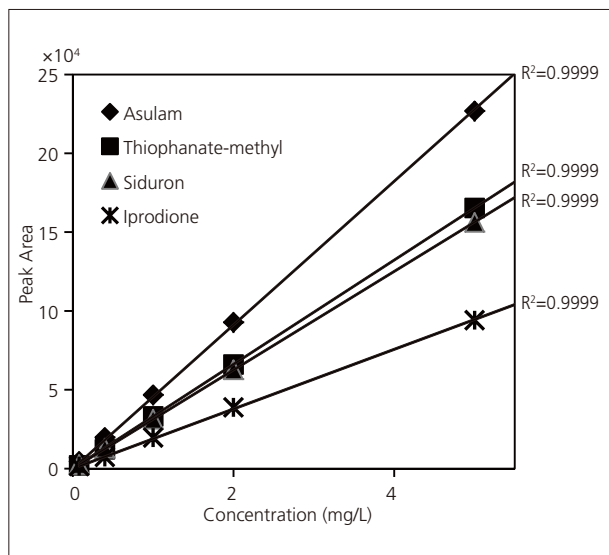


Fig. 3 Calibration Curves for Golf Course Pesticides (0.08 – 5 mg/L)

■ Analysis of Four Pesticides at High Sensitivity

The analysis in which the chromatogram of Fig. 4 was obtained using a concentration of 0.08 mg/L for each of the constituent pesticides was repeated six times (n=6), and the repeatability results of these measurements are shown in Table 3. The results indicate that good repeatability can be obtained even at a concentration less than 1/1000 of the reference values.

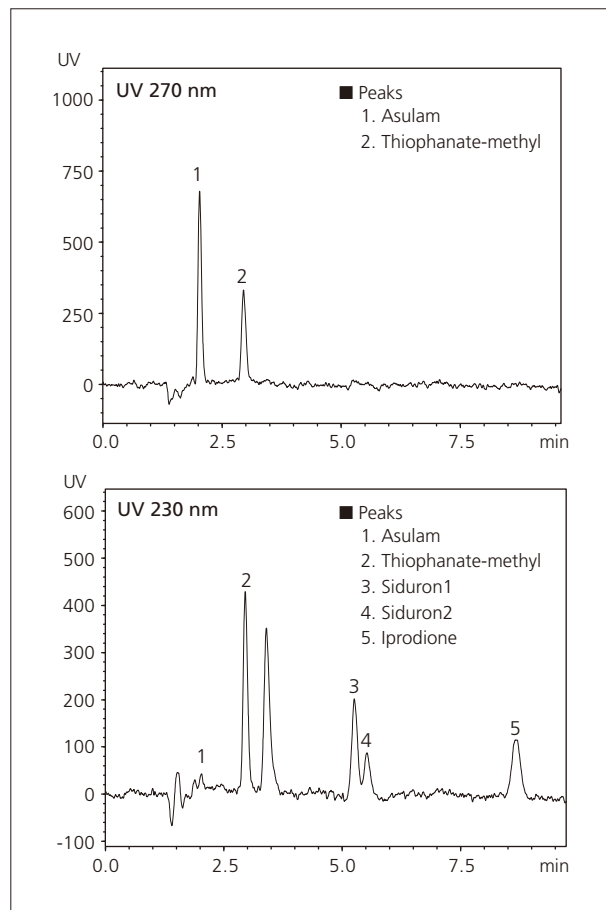


Fig. 4 Chromatogram of Four Golf Course Pesticides (each 0.08 mg/L)

Table 3 Repeatability of Peak Area at Concentration of 0.08 mg/L

	Peak Area %RSD
1. Asulam	1.22
2. Thiophanate-methyl	2.65
3. Siduron 1	1.94
4. Siduron 2	3.71
5. Iprodione	2.22

Note: Numbers correspond to those above the chromatogram of Fig. 4