Detecting and Positively Identifying Sub-ppb Level Pesticides Using a New HPLC System with Mass Spectral Detection

GIVING ENVIRONMENTAL CHEMISTS THE POWER TO SEPARATE AND IDENTIFY PESTICIDES IN WATER

Waters Integrity™ System, the only integrated, high performance benchtop HPLC system combining both photodiode array (PDA) and mass spectral (MS) detection, rapidly screens samples for the presence of a wide variety of pesticides in water. It was designed for the chemist who is not an expert in mass spectrometry, but is interested in identifying the constituents of HPLC samples. With a single injection, the Integrity System will separate, measure the concentrations of, and identify sample compounds by comparing their mass spectra to the mass spectra of several hundred thousand compounds stored on the system's desktop computer. By combining this technology with the sample enrichment capabilities of solid phase extraction, the analysis and reliable identification of pesticides at ppt levels all in one injection becomes practical. This capability is essential for compliance with European Economic Community drinking water directives.

The Integrity System is capable of giving you the following information:
- Peak purity verification
- Quantitation of sample constituents
- Positive peak identification

Waters Integrity System consists of a solvent delivery system, autosampler, column bypass module, photodiode array detector, mass spectral detector, and Millennium® 2010 Chromatography Workstation. All instrument functions are controlled from the keyboard of the workstation as is all data processing and spectral library matching.

DETECTING PESTICIDES IN DRINKING WATER USING BENCHTOP LC/MS

To evaluate the Integrity System for pesticide screening, parallel studies were performed in Waters U.S. and European environmental methods laboratories. The LC/MS method incorporated a solid phase extraction step using Waters C_{18} Environmental Plus Sep-Pak® Cartridge to achieve sub-ppb pesticide detection and identification in drinking water. The representative set of pesticides used in this study are listed in Table I, together with their chemical class, application and characteristic ions for identification. Good practice in compound confirmation by MS is to monitor for both the primary and secondary ion of the target analyte, expressed in m/z or Atomic Mass Units (AMU). They represent the major intensities of the unique mass spectrum of the individual compound. If both ions are detected after monitoring at the specified AMU values, confirmation of peak identity is obtained.

In preparation for the study, the HPLC separation was refined by developing a gradient analysis with a maximum concentration of 85% water and a volatile ammonium acetate buffer. The Integrity System's mass detector has been used with as much as 95% water in the mobile phase.

The chromatogram in Figure 1 was generated by the Integrity System's photodiode array detector using the detector's MaxPlot feature. In this mode, the detector scans the entire 190 – 800 nm wavelength range and plots a maximum absorbance chromatogram for each peak it finds. The power of the photodiode array detector to determine peak purity is useful for detecting coeluting compounds and indicating when it is necessary to modify the HPLC separation so that only homogenous peaks enter the mass detector interface. In designing the Integrity System, Waters design engineers took great care to ensure that the "look and feel" of the results processing and reporting routines of both the photodiode array and mass spectral detectors are very similar.

A LOOK AT THE RESULTS

The ThermaBeam™ mass detector is a high sensitivity mass detector designed specifically for use with HPLC. It produces library searchable electron impact ionization spectra of molecules as large as 1000 MW. Each molecule's mass spectra is unique and is the universal "signature" of a compound. Spectra are searched against either the optional Wiley Registry of mass spectral data or a user-built library to identify target analytes. Automated library matching routines for this purpose are provided in Waters Millennium Chromatography Manager software.

Table I: Pesticides Used in this Study
Pesticide Analysis by HPLC with PDA/MS Detection

Pesticide	Action	Family	Primary & Secondary Ions (m/z)
2,4-D	Herbicide	Chlorinated Phenoxy	162 & 220
Warfarin	Rodenticide	Hydroxycoumarin	265 & 308
Atrazine	Herbicide	Triazine	200 & 215
Carbaryl	Insecticide	Carbamate	144 & 115
Diuron	Herbicide	Substituted Urea	232 & 187
Linuron	Herbicide	Substituted Urea	248 & 160

Figure 1: Photodiode Array Chromatogram Pesticide Analysis by LC/MS

System: Waters Integrity™ System
Column: Waters Nova-Pak® C₁₈ 15 x 2.0 mm
Eluent A: 10 mM Ammonium Acetate
Eluent B: 100% Acetonitrile
Linear Gradient: 85% A/15% B, hold 2 min., then
 to 35% 17 min, hold 3 min. then to
 100% B, 22 min., hold 3 min., back to
 initial conditions, 3 min.
Flow Rate: 0.35 mL/min.
Detection: Waters 996 PDA MaxPlot Display
 (210 nm to 310 nm)
Amount: 300 ng on column

1. 2,4-D
2. Warfarin
3. Dinoseb
4. Atrazine
5. Carbaryl
6. Diuron
7. Linuron

(AU vs Minutes chromatogram)

Figure 2: Total Ion Current Chromatogram Produced by the Integrity System's Mass Detector

Detection: ThermaBeam™ Mass Detector
Scan: MaxPlot 140 m/z to 320 m/z
Smoothed: by 31 point Savitsky-Golay
EI Source: 220°C

1. 2,4-D
2. Warfarin
3. Atrazine
4. Carbaryl
5. Diuron
6. Linuron

(Intensity vs Minutes chromatogram)

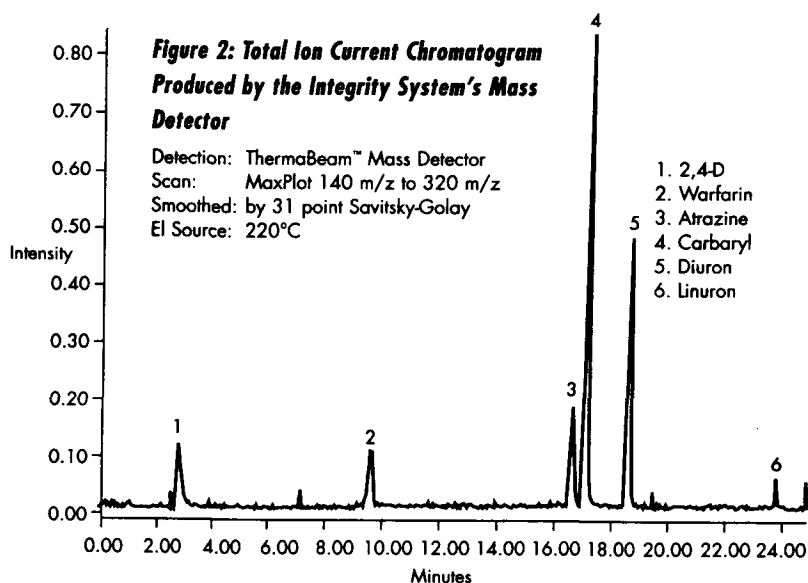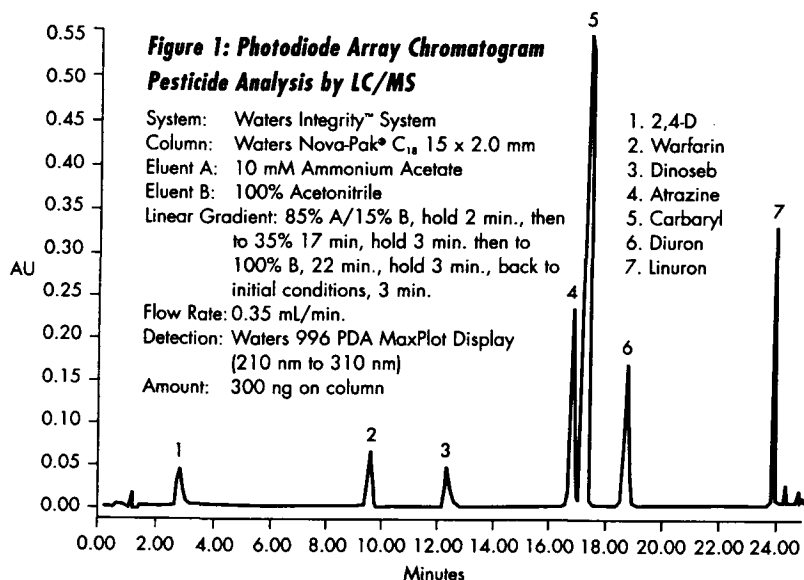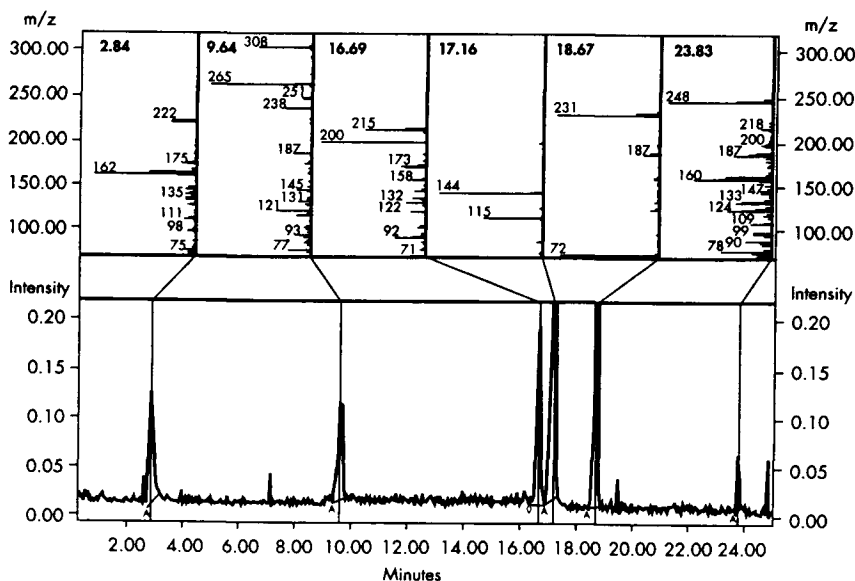## Figure 3: The Mass Spectrum Index Plot

(Mass Spectrum Index Plot: m/z vs Minutes and Intensity vs Minutes)

The low dispersion characteristics and minimal delay volume of the Integrity System are demonstrated by comparing Figures 1 and 2. Figure 1 shows the output of the Integrity System's photodiode array detector. Figure 2 shows the chromatogram produced by the system's mass spectral detector. As the sample passes from the PDA detector to the MS detector there is no loss of resolution between closely eluting atrazine and carbaryl analytes. Peak retention times are virtually identical. The spectra for Dinoseb (peak 3 in the PDA chromatogram) was not observed in the total ion current chromatogram, probably due to thermal degradation under the conditions used to ionize the compounds in the mass detector interface.

In Figure 2, the Integrity System's mass detector produces a molecular ion for each compound in the HPLC sample and generates a Total Ion Current Chromatogram which closely resembles the chromatogram in Figure 1. While operating the mass detector in scan mode, the MaxPlot function displays the maximum intensity for each peak.

Figure 3 is a Mass Spectrum Index Plot of the pesticides in the HPLC sample and is obtained in the same way as a Spectrum Index Plot is obtained using a Waters PDA detector. The mass spectra are extracted from the raw data and displayed for each chromatographic peak. It is possible to confirm or identify the peaks by operating the MS detector in Selected Ion Monitoring Mode (SIM) by programming the MS detector to search for the primary and secondary ions listed in Table I.

In Figure 3 the Mass Spectrum Index Plot (top), over the preselected AMU range, is displayed for each chromatographic peak (bottom).

The Integrity System is capable of giving you peak purity verification, quantitation of sample constituents, and positive peak identification.
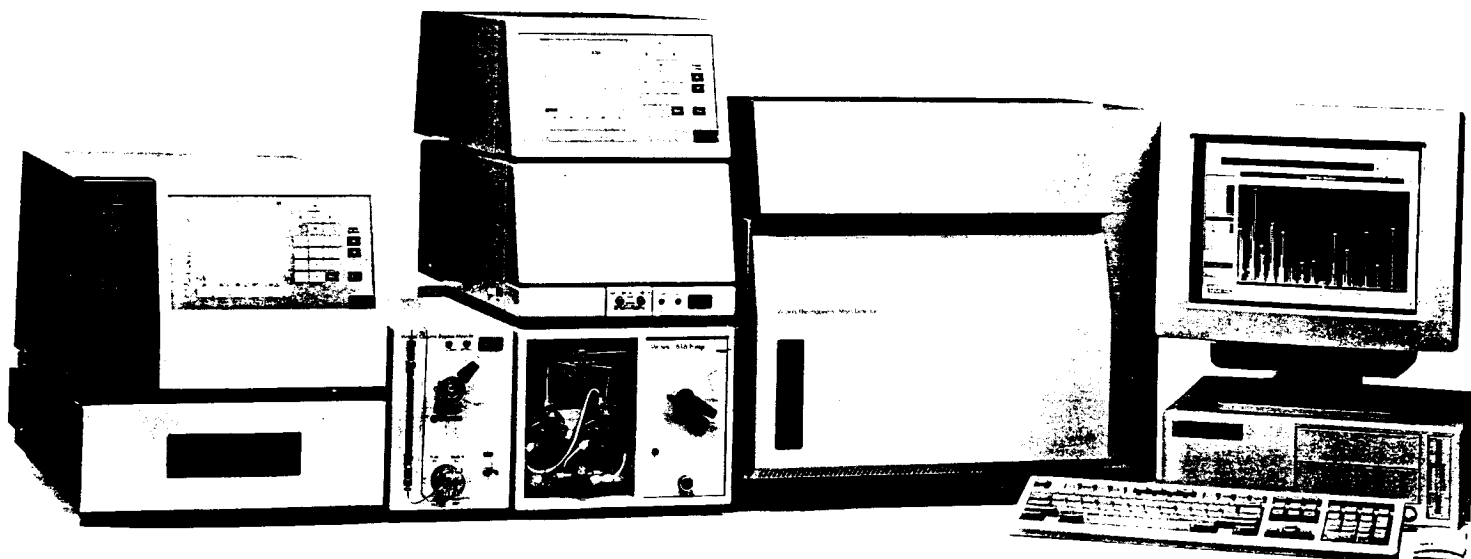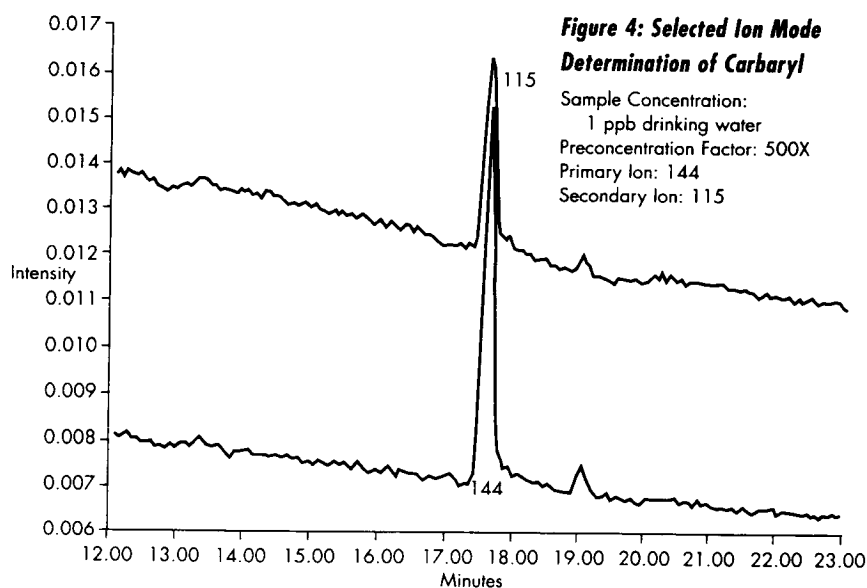

TRACE LEVEL CARBARYL DETECTION

Positive compound identification of 1 ppb carbaryl in drinking water was confirmed by the MS detector operating in the Selected Ion Mode (SIM), monitoring the primary and secondary ions at 144 and 115 AMU respectively (Figure 4). Excellent signal was achieved, indicating detection limits well below 1 ppb. For samples containing multiple components, it is possible to program and monitor up to 31 channels simultaneously in the Selected Ion Mode.

The exceptional sub-ppb sensitivity achieved by the Integrity System corresponds to 5 ng on column. The results shown are part of a more extensive study.

Estimated detection limits for various pesticides spiked in laboratory water are shown in Table II. In drinking water, matrix effects such as the presence of humic acids might raise UV detection limits by an order of magnitude. MS limits in SIM mode should not be affected by humic acids.

Table II Pesticide Detection Limits (ng on column)

	Detector: PDA	MS SIM
Bentazone	0.3	2
Metamitron	0.3	2
Chloridazon	0.3	2
2,4-D	0.3	2
MCPA	0.3	2
Mecoprop	0.3	2
Dichlorprop	0.3	2
Simazine	0.9	1
Atrazine	0.9	3
Diuron	0.9	1
Propazine	0.9	3
Linuron	0.9	3

Figure 4: Selected Ion Mode Determination of Carbaryl

Sample Concentration:
  1 ppb drinking water
Preconcentration Factor: 500X
Primary Ion: 144
Secondary Ion: 115

WATERS INTEGRITY SYSTEM—GIVING ENVIRONMENTAL CHEMISTS A POWERFUL TOOL FOR PESTICIDE IDENTIFICATION

Waters Integrity System is a powerful tool for those whose task is to identify specific pesticides in water. The Integrity System provides positive peak identification and confirmation at sensitivity levels mandated by European Economic Community drinking water directives when used with solid phase extraction for sample preconcentration. This is especially valuable for screening samples in which multiple pesticides may be present.