



## Project Summary

# The Application of High Performance Liquid Chromatography/Mass Spectrometry to Environmental Analysis

L. D. Betowski and T. L. Jones

High performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is described for the analysis of samples for environmental pollutants. During the course of this work different techniques were used to increase both the sensitivity and specificity of HPLC/MS. These included the utilization of two different interfaces for the mass spectrometer - the thermospray and the particle beam systems. Refinements to the thermospray system were made with the use of a wire-repeller to enhance sensitivity and also to increase the structural information obtained from mass spectra. The operation of the instrument in the discharge negative ionization mode further increased the specificity of these techniques as did the use of a triple quadrupole mass spectrometer in the HPLC/MS system.

Two different sets of compounds were used in this study. Organic dyes were used to test the improvements in the system. Since work was performed with dyes previously and since dye compounds encompass a variety of structural functionalities, the dyes made good test compounds. Chlorinated herbicides were studied in an effort to analyze for these compounds directly without having to prepare derivatives before separation.

This report covers a period from October 1, 1988, to May 31, 1989, and work was completed as of July 31, 1989.

*This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

### Introduction

There has been recent interest in the use of high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) for environmental analysis. For reasons of non-volatility or thermal lability, an estimated 80 percent of organic compounds are not amenable to analysis by gas chromatography (GC) methods. The two most widely used versions of HPLC/MS are the thermospray interface and the particle beam interface. Thermospray HPLC/MS is an introduction technique, but with the aid of a volatile buffer can also be an ionization technique. By a process of ion evaporation and ion-molecule reactions, the thermospray interface has been successful in analyzing for polar compounds at good sensitivities. The drawback to thermospray ionization is the

lack of structural information for many compounds. Mass spectra under thermospray ionization are characterized by one or two ions per compound. The particle beam interface, on the other hand, allows electron impact (EI) mass spectra to be generated on the compound of choice. This allows structural interpretation and library searches to identify compounds.

This report examines some refinements that were made to a thermospray HPLC/MS system to increase both the sensitivity and the specificity of the technique. In addition, a particle beam system has been evaluated and comparisons with the thermospray system have been made.

Two sets of compounds were chosen for study in this report. Organic dyes were chosen to test various modifications of the HPLC/MS systems. Dyes offer a wide range of structural functionalities and are often non-volatile. The methods for testing for chlorinated herbicides are labor-intensive and require the use of dangerous reagents to convert the chlorinated acids to their corresponding methyl esters for analysis by GC methods. Methods based on HPLC/MS provide the opportunity for analyzing these compounds directly without the need of hydrolysis and derivatization steps.

## Procedure

The instrument used in these experiments was a triple quadrupole mass spectrometer (Finnigan MAT TSQ™ 45) equipped with either a thermospray interface and ion source (Vestec Corp.) or a particle beam interface (Extrel ThermaBeam™). A modification was made in the thermospray ion source and consisted of the addition of a wire-repeller. A hole was drilled exactly opposite the ion-extraction funnel and a copper wire, insulated with a ceramic tube, was introduced. This wire-repeller was operated in two configurations. In the first configuration, which was devised to maximize the sensitivity of thermospray ionization, the wire-repeller was flush to the base of the ion chamber and was operated at 200-250 V. In the other configuration, which was meant to increase fragmentation, the wire-repeller was inserted 4 mm into the ion chamber and was operated at 400 V.

The thermospray HPLC/MS was operated in four modes:

- (1) positive ion single quadrupole scan with the wire-repeller in the enhancement mode.
- (2) positive ion single quadrupole scan in the repeller-induced fragmentation mode.

- (3) positive ion daughter ion scan mode in which collision activated dissociation experiments were performed on the parent ions.
- (4) negative ion discharge mode.

The particle beam interface was attached to the triple quadrupole mass spectrometer by means of a machined adapter which was heatable to 250°C.

## Results and Discussion

The limits of detection of some commercial dyes are reported on Table 1. The limits of detection were obtained by recording the ion traces at characteristic masses of the dyes under full-mass scan with a signal-to-noise ratio of at least three. Also included in this table are limits of detection for six of the dyes that were previously obtained under thermospray ionization with an ion source without a repeller. Enhancement factors of between 10 and 400 were observed for the configuration with the wire-repeller. The comparison of thermospray with repeller and the particle beam system shows differences in limits of detection between the two of between 20 and 16,000 in favor of thermospray ionization. While these numbers may have some meaning about the intrinsic sensitivities of the two systems, part of the differences can be explained as differences in

**Table 1.** Detection Limits for Commercial Dyes

Dye	Structure	NW	Dye Content (%)	TSP (w/o repeller) Detection Limit* (ng)	TSP (with repeller) Detection Limit* (ng)	PB LC/MS Detection Limit** (ng)
Disperse Yellow 5	1	324	C.30	-	5	500
Disperse Orange 13	2	352	C.15	150-200	20	5500
Solvent Red 3	3	292	100	-	10	200
Disperse Orange 3	4	242	C.20	-	2.5	50
Disperse Red 13	5	348	C.25	-	10	4400
Solvent Red 23	6	352	C.85	50	5	500
Disperse Brown 1	7	432	C.25	-	10	2700
Disperse Red 1	8	314	C.30	-	0.1	250
Disperse Orange 25	9	323	C.20	-	10	500
Disperse Blue 79	10	624	100	250-500	10	300
Basic Green 4	17	329	C.98	20	0.67	700
Disperse Blue 3	18	296	C.20	20	0.05	800
Fluorescent Brightener 236	19	389	100	25-50	4	600
Solvent Red 49	20	442	C.97	-	1	500

\*Flow injection analysis.

\*\*On column injection.

experimental design. For example, because of the different kind of interface and mass spectrometer it was difficult to optimize the performance of the particle beam on the triple quadrupole MS.

The number of fragment ions and, therefore, structural information was much greater for the particle beam system than in thermospray ionization with single quadrupole scanning. However, the use of CAD experiments on the protonated molecule gave approximately the same degree of fragmentation as what was produced under electron impact conditions in the particle beam system. In addition, the use of the wire-repeller to produce CAD spectra in the ion source and the use of the discharge negative-ion mode (for compounds with high electron affinities)

both resulted in significant increases in the amount of fragmentation.

## **Conclusions and Recommendations**

The use of HPLC methods to introduce compounds into a massspectrometer offers the potential to regulate pollutants that have been outside the scope of traditional analytical methods. Two important features that should characterize a mass spectral-based method for the analysis of environmental samples are sensitivity and specificity. This work demonstrated the successful application of a wire-repeller to improve sensitivity in the positive ion thermospray mode. Several techniques were applied to increase the specificity of HPLC/MS. The use of the

discharge negative ion mode was shown to give much fragmentation at good sensitivities for compounds with high electron affinities. The use of the wire-repeller to effect fragmentation was successful, but at a reduced level of sensitivity. Further work is needed on improving limits of detection in this mode. The use of a triple quadrupole mass spectrometer, when available, proved to be a good option in obtaining structural information from compounds introduced under thermospray ionization. Finally, the particle beam HPLC/MS system showed good specificity. However, the limits of detection for a series of dyes could be improved. Work is needed both in this area and in adding to the electron impact mass spectral data base for compounds that must be analyzed by HPLC methods.