



## Project Summary

# Performance Evaluation of Particle Beam Liquid Chromatography/Mass Spectrometry for the Measurement of Acid Herbicides

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**Particle beam liquid chromatography/mass spectrometry (LC/MS) was evaluated for the measurement of acid herbicides. An acetic acid/ammonium acetate/methanol solvent system with a C-8 reversed phase column gave baseline resolution of all target analytes. Detection limits in the full scan mode were 100 ng to 500 ng for most of the target analytes. Dalapon and dinoseb were not detected. Response curves over the range 200 ng to 2000 ng were non-linear for most of the analytes. Response factors tended to increase with increasing analyte concentration. Mass spectra were variable and exhibited abundant ions corresponding to "thermal" decomposition mechanisms. Spectral appearance was dependent on analyte concentration, source conditions, and source temperature. Only spectra acquired at high concentration were library matchable. Therefore, a rugged and reliable method to identify and quantify acid herbicides in environmental samples based on particle beam LC/MS technology does not appear feasible at this time.**

**This report covers the period from May 1, 1989 to March 30, 1990, and work was completed as of June 15, 1990.**

***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

Particle beam (PB) high performance liquid chromatography/mass spectrometry (HPLC/MS) is a technique capable of producing liquid chromatographic separation and electron ionization (EI) mass spectra for polar nonvolatile and/or thermally labile organic compounds. The PB interface is only one of several HPLC/MS interfaces that have been developed. The thermospray (TS) HPLC/MS interface is probably the most widely utilized today. However, the lack of spectral information produced by this soft ionization technique has limited the use of TS HPLC/MS in the identification of unknown organic compounds. The development of the PB HPLC/MS has led to an interface capable of removing a large portion of the mobile phase from the HPLC effluent. Once most of the mobile phase has been removed the analyte particles enter the mass spectrometer ion source where they are vaporized and subsequently ionized under electron bombardment. The EI spectra generated in such a process contain substantial structural information.

The phenoxyacid herbicides are one class of compounds for which an HPLC/MS method for identification and quantification would be very useful. Presently, samples are analyzed for these compounds under methods 8150 and 8151 of the SW-846. These methods involve hydrolysis and derivatization with diazomethane before analysis by gas chromatography (GC) with an electron capture detector (ECD). The derivatization step is both time consuming



and dangerous; a method eliminating its use would be advantageous. Therefore, a thermospray HPLC/MS method for the direct analysis of these compounds has been proposed (EPA/600/X-89/176 July 1989, Liquid Chromatography/Mass Spectrometry Performance Evaluation of Chlorinated Herbicides and Their Esters). Likewise, this report describes the application of PB HPLC/MS to the analysis of phenoxyacid herbicides. Factors affecting both the chromatography and the response characteristics were investigated.

## Procedure

The initial chromatographic separations were developed on a Hewlett-Packard 1090L liquid chromatograph equipped with an autosampler and a diode array detector. The HPLC system was controlled by a Hewlett-Packard HPLC ChemStation. The PB HPLC/MS consisted of a 1090L liquid chromatograph equipped with an auto injector and filter photometric detector. The HPLC was connected to a Hewlett-Packard 59980A particle beam interface. The HPLC was controlled by a local user-interface while the mass spectrometer was controlled by a Hewlett-Packard 59970 MS ChemStation.

HPLC flow rates of either 0.25 mL/min or 0.4 mL/min were used. Several mobile phases and HPLC columns were considered. Routine PB parameters were: nebulizer setting of 12, nebulization helium pressure of 30-50 psi, desolvation chamber temperature of 45° to 55°C, and PB probe distance to the source of 0.5 mm. The PB desolvation chamber vacuum pressure was estimated at 200 torr by the instrument manufacturer. The pressure in the first stage of the momentum separator was typically 10 torr and that of the second stage was typically 0.5 torr as measured by Hastings-Raydist gauges. The mass spectrometer ion source for these studies was slightly modified. First, a stainless steel plug was inserted into the GC inlet of the source. Second, the particle beam inlet was drilled to a larger diameter by the instrument manufacturer. Except for one set of experiments the ion source was operated at 250°C. A typical MS operating pressure of  $1.2 \times 10^{-5}$  torr was measured by a Bayard-Alpert ion gauge tube. The mass spectrometer was run in the EI mode (except for one experiment) with a filament emission current of 300  $\mu$ A and an electron energy of 70 eV. The MS electron multiplier was a Galileo channeltron and was typically operated at 2200 V. The MS system was tuned to maximize the m/z 219 ion of PFTBA introduced through a reservoir on the PB transfer tube.

The acid herbicide standards were pure compounds (>97%) obtained from the U.S. EPA Repository (Research Triangle Park, North Carolina). The compounds were subsequently diluted with acetonitrile.

## Results and Discussion

Before any mass spectrometric work was carried out, experiments were performed on an HPLC/UV system to optimize the chromatographic separations for the phenoxyacid herbicides. After such a separation was identified, these conditions were tried on the HPLC coupled with the PB interface. The system was further modified and the final chromatographic conditions that were used for all subsequent studies are listed in Table 1.

**Table 1.** Chromatographic Conditions for the PB HPLC/MS System

Column: Spherisorb S3C-8 5 $\mu$ m 2 x 100 mm		
Flow: 0.25 mL/min		Temperature: 50°C
Gradient:		
Time(min)	1% Acetic Acid + 0.01 M NH <sub>4</sub> OAc	CH <sub>3</sub> OH (1% HOAc)
0	75%	25%
2	75%	25%
25	40%	60%

**Table 2.** Estimated Limits of Detection

Compound	Limit of Detection (ng)
Dalapon	ND
Dicamba	520
2,4-D	130
MCPA	260
2,4,5-T	320
Dichloroprop	130
MCPP	400
Silvex	130
2,4-DB	120
Dinoseb	>2500
2,4-D butoxy ethyl ester	50
2,4,5-T butoxy ethyl ester	50

ND Not detected

Response curves were prepared for each analyte over a range of 200 ng to 2000 ng. Dicamba and MCPA exhibited near linear response over this range, but the response to silvex and 2,4-DB was distinctly nonlinear. Response to the other analytes was intermediate with relative standard deviations ranging from 15 to 25 percent. Examination of the response curve data reveals two trends. First, response curves for most of the target analytes were nonlinear over the range 200 ng to 2000 ng. Secondly, response factors tend to increase with increasing analyte amount.

The flow through the column had been 0.4 mL/min, but because of the high initial aqueous content of the mobile phase a substantial loss of sensitivity for the acid herbicides through the PB interface was observed. To compensate for this loss of sensitivity, the flow rate was reduced to 0.25 mL/min and the nebulizer helium pressure was increased to 50 psi.

The limits of detection for the phenoxyacid herbicides are listed in Table 2. These data were estimated from a full scan total ion chromatogram at a concentration near these limits. The individual peaks were extrapolated to give a signal to noise ratio of three. The addition of ammonium acetate to the mobile phase appears to slightly improve the limits of detection for these compounds.

The single day, single concentration level (1000 ng) reproducibility was generally less than 10 percent for the phenoxyacid herbicides. However, the variation in day to day response at a single level (1000 ng) was between 43 and 51 percent. These data were recorded after every attempt was made to return the instrument to the same operating condition each day.

An experiment was designed to examine matrix effects on phenoxyacid herbicide response. Instrument response to a mixed herbicide standard solution (200 ng/ $\mu$ L) was compared to the response observed for

acid herbicides in a soil extract and a soil extract containing phenolic compounds. The results of this experiment indicate the matrix components from the extract and the soil extract plus phenols do not affect the response characteristics by a significant amount.

There has been evidence of variation in the quality of the EI spectra from the PB introduction of the phenoxyacid herbicides. Several ions which appear in the spectra of these compounds under certain conditions are characteristic of thermal decomposition in the ion source. Experiments designed to study this phenomenon indicate that the particle beam spectra of the phenoxyacid herbicides are variable and depend on source cleanliness, quality of the water in the mobile phase, source temperature, and analyte concentration. Preliminary results suggest a surface catalyzed decomposition may be occurring inside the ion source.

### **Conclusions and Recommendations**

A rugged and reliable method to identify and quantify acid herbicides in environmental samples based on PB LC/MS technology does not appear feasible at this time. A number of fundamental issues need to be addressed before a reliable method can be developed. Among these issues are poor

sensitivity, non-linear response, response drift, and variation in spectral quality.

Based on SW-846 extraction procedures and assuming 10  $\mu$ L injection volumes, the observed detection limits for the analytes except dalapon and dinoseb which were not detected result in method detection limits of 50 to 250  $\mu$ g/L. These values are comparable to Method 8150 detection limits for MCPA and MCPP, but are considerably higher than the method detection limits for the remaining acid herbicides. Selected ion monitoring of the acid herbicides improved detection limits by 10 to 50 times, but identification based on full scan mass spectra is lost. Some improvement in detection limits may be achieved by summing over all isotopic ions associated with the base peak rather than quantifying on the base peak alone. Further enhancements may be achieved by incorporating specialized injection techniques. One of these techniques, well suited to liquid chromatography, is on column preconcentration used in conjunction with a switching valve. In addition, larger sample size or more extensive concentration may be incorporated into the sample preparation to improve overall method detection limits.

Response curves for most of the acid herbicides exhibited non-linear behavior over the range 200 ng to 2000 ng. Re-

sponse factors tended to increase with increasing concentration. Inaccuracy resulting from inappropriate calibration may be minimized by operating over a narrower calibration range. For these compounds, linear calibration models may be used provided the calibration range is less than a factor of 10. For an extended range, a non-linear calibration must be employed to maintain acceptable accuracy.

Spectral quality was found to be dependant on the amount of analyte reaching the ion source of the mass spectrometer. At high levels of sample (2 $\mu$ g), the EI mass spectra are of library matchable quality. However, at lower sample amounts the mass spectra show an increase in the relative abundance of thermal decomposition ions. Investigation into the cause of thermal decomposition ions leads us to the hypothesis that these ions are formed through interactions of analyte particles with the ion source surface resulting in decomposition to a more thermally stable and volatile species followed by desorption and subsequent ionization. The extent to which these events occur are variable; depending on analyte concentration, source temperature, and source cleanliness, and may be the principal cause of non-linear response over an extended range and response variation at low concentrations.

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*The complete report, entitled "Performance Evaluation of Particle Beam Liquid Chromatography/Mass Spectrometry for the Measurement of Acid Herbicides," (Order No.*

*PB90-270 547/AS; Cost: \$17.00, cost subject to change) will be available only from:*

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