Development of Analytical Methods for Non-Volatiles Using Liquid Chromatography/ Particle Beam/ Mass Spectrometry

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ABSTRACT

High performance liquid chromatography (HPLC)/ particle beam (PB)/ mass spectrometry (MS) was studied to determine its suitability as a major component of a general purpose, broad spectrum analytical method for the determination of non-volatile organic compounds in environmental matrices. A reverse phase gradient elution HPLC separation was developed for a test mixture of compounds that are insufficiently volatile for gas chromatography (GC). These compounds included aromatic diamines, urea herbicides, carbamate pesticides, and chlorinated acid herbicides. Three different commercial PB interfaces were used and performance factors measured included instrument detection limits and short term ion stabilities.

INTRODUCTION

Willoughby and Browner have described an improved HPLC/MS interface consisting of an aerosol generator, a desolvation chamber, and a PB momentum separator (1). The design was originally named MAGIC (monodisperse aerosol generation interface for combining liquid chromatography with mass spectrometry), but with the development of several commercial variations, it has become known simply as a particle beam interface. Because these interfaces make it possible to obtain conventional electron impact (EI) mass spectra, a new approach to the development of a broad spectrum HPLC/MS method for the simultaneous identification and measurement of toxic organic environmental pollutants, not amenable to capillary column GC/MS (non-volatiles), is now possible. Two critical issues for an LC/MS method are the stabilities of integrated ion intensities over a minimum eight-hour period, and the relative stabilities and reproducibilities of the EI mass spectra over a similar time period. Ion intensity stability over a minimum eight-hour period is required so that concentration calibrations maintain validity for a reasonable number of samples. Similarly, EI spectra must be relatively stable to permit automatic peak identifications via reverse search algorithms and spectra from reference data bases.

Factors which affect the overall response of an analyte are the operating parameters of the interface and properties of the analyte itself. Adjustable operating parameters for particle beam interfaces include: the position of the capillary transfer line with respect to the entrance to the

desolvation chamber, the temperature of the desolvation chamber, the temperature of the nebulizer, the flow rate of the nebulization gas, and the composition of the mobile phase. Optimization of these parameters has been described elsewhere (2). This report describes results from efforts to improve method precision and instrument detection limits by the addition of method controls such as proper column conditioning and mobile phase composition modification through post column addition of nonaqueous solvents.

EXPERIMENTAL

Experiments were performed on three different HPLC/PB/MS systems: (A) a Hewlett-Packard (HP) system consisting of an HP model 1090 HPLC, an HP 59980A particle beam interface, and an HP model 5988 mass spectrometer; (B) a system consisting of a Waters PB 600 MS HPLC, an Extrel Thermabeam™ interface, and an Extrel ELQ 400-1 mass spectrometer; and (C) a system consisting of a Waters 600 MS HPLC, and a Vestec Model 101 mass spectrometer with a Universal LC-EI interface. All operating conditions were as similar as possible on all three systems. The HPLC column was a Waters C18 Novapak 15 cm x 2 mm (id) packed with 4 micron particles. The mobile phase was a mixture of acetonitrile and water containing 0.01 M ammonium acetate. The solvent composition was held for one minute at 25% acetonitrile, then linear programmed to 70% acetonitrile in 29 minutes (0.15 % acetic acid was added when analyzing acids and the gradient was from 15% acetonitrile to 70% acetonitrile in 45 minutes). Injection size was 3-5 µl with autoinjection and the flow rate was 0.3ml/min through the column. Post column addition of acetonitrile was done on all 3 three systems (0.1ml/min on System A and B; 0.7ml/min on System C).

RESULTS AND DISCUSSION

Overall system performance was found to improve with the addition of acetonitrile added post column. This allows the interface to operate under more ideal conditions by increasing the percentage of organic component in the mobile phase to at least 30-40%. Not only does this result in better stability, but also increases the working range for HPLC gradients and, for some compounds, increases sensitivity. Ammonium acetate has been found to cause serious column bleeding on some C18 columns. This column bleed can contaminate the particle beam interface, and ion source, causing response to degrade over time and therefore, poor precision in integrated ion intensities. Overnight flushing of the HPLC columns with acetonitrile also improved stability by eliminating column bleed (dimethyloctadecylsilanol). For most compounds, mean relative standard deviations (RSD) of signal intensities are now in the range of 2-15% over an eight hour period (see Table). Estimated detection limits, in nanograms injected, for a 3:1 signal to noise ratio of the quantitation ion are also given and are in the range necessary for environmental analysis.

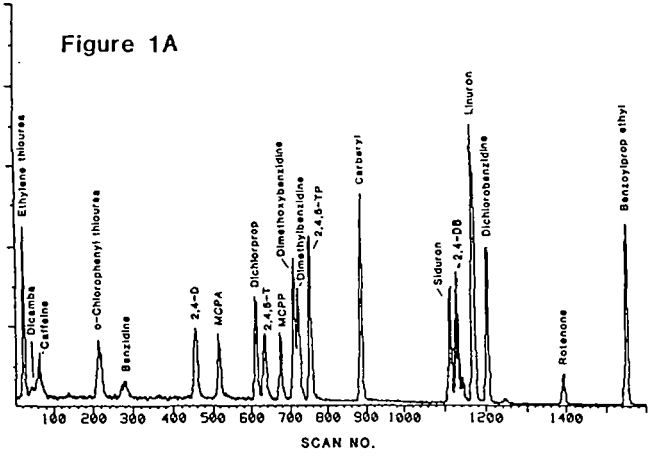
	SYSTEM A		SYSTEM B		SYSTEM C	
	RSD (%)	DET LIM (ng)	RSD (%)	DET LIM (ng)	RSD (%)	DET LIM (ng)
Ethylene thiourea	2.2	6	5.9	4	3.6	5
Caffeine	3.2	1	5.7	2	4.8	5
O-Cl-phenylthiourea	4.0	7	6.7	30	12.2	60
Benzidine	6.2	4	7.8	3	7.0	20
Dimethoxybenzidine	6.6	6	7.3	20	4.9	10
Dimethylbenzidine	5.9	2	7.0	10	5.6	10
Carbaryl	4.9	30	4.6	20	5.0	100
Siduron	2.7	2	6.3	8	3.1	1
Linuron	7.6	130	4.6	40	4.4	100
Dichlorobenzidine	3.1	3	3.2	3	7.7	5
Rotenone	10.0	70	7.2	40	5.3	5
Benzoylprop ethyl	2.4	4	2.7	7	8.7	5

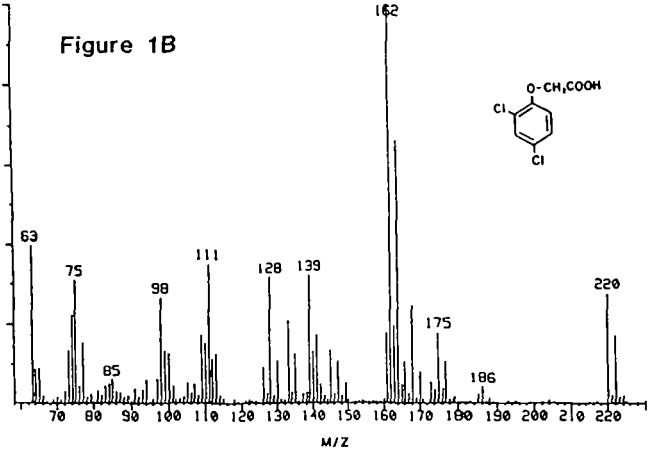
This technique is also amenable to carboxylic acids such as phenoxy acid herbicides. By adding acetic acid to the mobile phase, these compounds can be chromatographed, along with the above compounds, as free acids without the need for the derivitization required by GC analysis (see Figure 1A). Figure 1B shows the EI mass spectrum for 2,4-D. Preliminary data indicates that precision (10-20%) and detection limits (10-100ng) are also in the range required for environmental analysis.

While studying the performance characteristics of the three particle beam interfaces, enhanced positive ion abundances were observed for coeluting compounds and with the addition of ammonium acetate to the mobile phase. The ammonium acetate enhancements are attributed to both improved chromatographic efficiency for basic compounds, such as benzidine, and to a particle beam carrier process (3). This carrier process enhances sensitivity in HPLC/PB/MS by improving analyte transport efficiency through the interface. However, coeluting substances may cause strong positive bias which may adversely impact quantitative analysis. Efforts to control this positive bias are currently under study.

CONCLUSIONS

HPLC/particle beam/MS shows promise as a broad spectrum analytical technique applicable to the determination of a variety of non-volatile compounds in environmental samples. The combination of precise retention times, EI mass spectra, and isotope distribution patterns from compounds containing naturally occurring isotopes gives excellent information for the unequivocal identification of target and unexpected analytes. The precision and instrument detection limits obtained with HPLC/PB/MS are encouraging, and suggest that simultaneous quantitative analysis will be possible.





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