Interlaboratory Comparison of Thermospray and Particle Beam Liquid Chromatography/Mass Spectrometry Interfaces: Evaluation of a Chlorinated Phenoxy Acid Herbicide Liquid Chromatography/Mass Spectrometry Analysis Method

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■ Seven laboratories participated in an interlaboratory evaluation of a liquid chromatography/mass spectrometry (LC/MS) method for the analysis of 10 chlorinated phenoxy acid herbicides. The focus of this evaluation was to test the intercomparability of LC/MS data obtained from two types of LC/MS interfaces [i.e., thermospray (TS) and particle beam (PB)]. Eight simulated sample extracts were sent to each laboratory for LC/MS analysis. There were statistically significant differences between interfaces in the quantitative data for all analytes except 2-(2,4,5-trichlorophenoxy)propanoic acid (silvex). Particle beam exhibited a high positive bias and a low relative standard deviation at the highest sample extract concentration range, 500 μg/mL, while TS showed a low bias and a low relative standard deviation at the lowest sample extract concentration range, 5 μg/mL. Another factor of this study was to look for any performance differences between interfaces of the same type, but differing manufacturers. A statistical difference was observed, between PB interfaces, for 2-(1-methylpropyl)-4,6-dinitrophenol (dinoseb).

Introduction

A number of compounds of environmental interest, including many on the U.S. Environmental Protection Agency's (U.S. EPA) Resource Conservation and Recovery Act (RCRA) Appendix IX list, are polar, nonvolatile, and/or thermally labile. Thus they are not amenable to conventional gas chromatography (GC) analysis. To address this problem efforts are now underway within the U.S. EPA to develop suitable techniques for these compounds.

Examples of environmental organic contaminants that can be analyzed by liquid chromatography/mass spectrometry (LC/MS) methods are organophosphorus pesticides (*1*), triazine herbicides (*2*), and the chlorinated phenoxy acid herbicides (*3*). This latter group of compounds can be analyzed directly as the free acids, as well as the esters, by LC/MS. The chlorinated acid herbicides generally have a low mammalian toxicity, but impurities and high dosages may cause teratogenic effects in rodents (*4*).

Currently, U.S. EPA RCRA Solid Waste-846 (SW-846) methods 8150 and 8151 (*5*) are approved by the U.S. EPA for the analysis of chlorinated herbicides in solid waste under the Resource Conservation and Recovery Act. These methods specify quantitation by GC with electron capture detector (ECD) and optional GC/MS confirmation. Also required are the use of hydrolysis and subsequent esterification of the sample extracts prior to analysis to convert the herbicides to gas chromatographable esters. Disadvantages with this method are that the hydrolysis step is time-consuming, and not always quantitative, and the usual esterification reagent, diazomethane, is potentially carcinogenic and explosive. The analysis of several chlorinated herbicides [specifically (2,4-dichlorophenoxy)acetic

0013-936X/91/0925-1880$02.50/0

Table I. Precision and Accuracy of Interlaboratory Data

analytes	particle beam mean % recovery[a]			thermospray mean % recovery[a]		
	$\bar{X}$ (% RSD)[b]	$\bar{X}$ (% RSD)[c]	$\bar{X}$ (% RSD)[d]	$\bar{X}$ (% RSD)[b]	$\bar{X}$ (% RSD)[c]	$\bar{X}$ (% RSD)[d]
2,4,5-T	109 (14)	63 (33)	223 (2.1)	90 (23)	62 (68)	90 (28)
butoxy 2,4,5-T	135 (25)	79 (39)	240 (28)	90 (29)	85 (9)	99 (17)
2,4-D	111 (14)	85 (36)	270 (30)	86 (17)	64 (80)	103 (31)
2,4-DB	120 (13)	72 (30)	207 (14)	95 (22)	104 (28)	96 (21)
dalapon	ND	ND	ND	83 (13)	121 (99)	150 (4)
dicamba	95 (24)	73 (89)	ND	77 (25)	90 (23)	105 (12)
dichlorprop	111 (13)	101 (24)	323 (19)	84 (20)	96 (15)	102 (22)
dinoseb	63 (13)	30 (3)	ND	78 (15)	86 (57)	108 (30)
MCPA	111 (20)	106 (25)	280 (10)	89 (11)	96 (20)	94 (18)
MCPP	107 (17)	101 (37)	290 (8.3)	86 (12)	76 (74)	98 (15)
silvex	122 (20)	72 (45)	220 (6.5)	96 (27)	65 (71)	87 (15)

[a] ND, not detected. [b] 500 μg/mL. [c] 50 μg/mL. [d] 5 μg/mL.

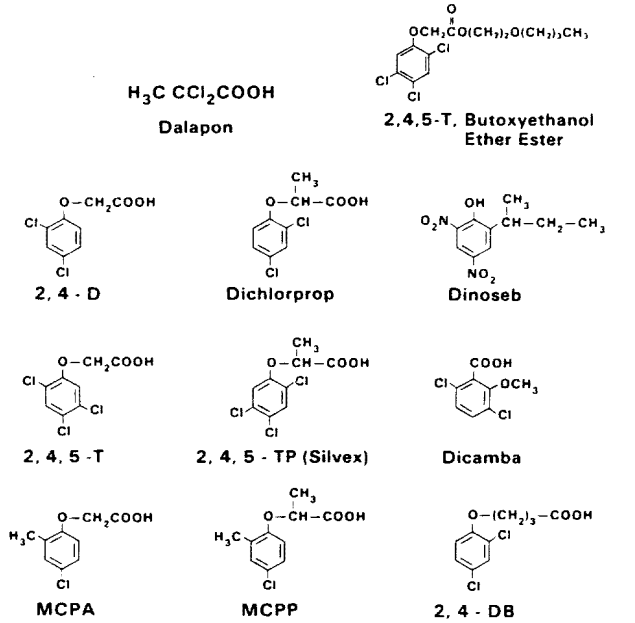

Figure 1. Structures of ten chlorinated phenoxy acid herbicides and one ester.

acid (2,4-D), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP)] by reverse-phase high-performance liquid chromatography with ultraviolet (HPLC/UV) detection has been reported in the literature (*6*). A multilaboratory collaborative study of the HPLC/UV method has been conducted and published (*7*). The use of LC/MS not only eliminates the need for the hydrolysis and esterification steps, but also provides a single-step analysis with selective mass spectral detection.

Recent developments in LC/MS interfaces led to the initiation of this project. The purpose of this study was to compare and evaluate LC/MS interface devices [e.g., thermospray (TS) and particle beam (PB)] for their applicability to the analysis of acid herbicides and their esters. The TS (*8*) interface allows all of the LC effluent to enter into the mass spectrometer source, where ionization occurs by ion evaporation (buffer assisted) in the positive ionization mode or with the assistance of a discharge electrode in the negative ionization mode. The PB (*9*) interface relies on the principle of particle separation; the LC effluent enters into a nebulization chamber and then a desolvation chamber, where the lighter solvents are pumped away, and the heavier analytes are condensed into a analyte-enriched particle beam that enters directly into the MS source. Once inside the MS source "traditional" electron ionization occurs, i.e., filament-assisted ionization. Two types of PB interfaces were examined in this study; one PB interface has a heated nebulization chamber, while the other does not.

Experimental Section

A concentrated stock standard was prepared containing 11 analytes, listed in Table I, each at 1000 μg/mL, in acetonitrile. One milliliter of the stock standard solution was sent with each sample set for instrument calibration and analyte quantitation. Because methanol can methylate free acid herbicides, or tranesterify herbicide esters, all standards and extracts were prepared in acetonitrile.

Each laboratory was provided with eight simulated sample extracts and one blank. The simulated sample extracts were prepared by dilution from the stock standard solution and sealed in 1.6-mL screw-cap, Teflon septa sealed, glass vials. The stock standard was used for preparation of simulated sample extracts, so that all chemicals used were traceable to a single original source. The simulated sample extracts consisted of duplicate extracts, 1 mL each, at four different concentration levels. Each laboratory was required to perform triplicate analysis on the duplicate sample extracts.

The laboratories involved utilized three TS-LC/MS (one Finnigan, one Hewlett-Packard, and one Vestec) and four PB-LC/MS instruments (two Extrel and two Hewlett-Packard).

A method blank extract was shipped with each sample set. The blank was prepared by extracting tap water, using the same extraction procedure as outlined in SW-846 method 8150.

Because of the complex nature of LC/MS operation, specific operating parameters were not given to the participants concerning instrument (interface and MS) tuning and calibration. The laboratories were advised to follow the instrument manufacturer's specifications for optimal interface performance. Instructions, in the form of a simplified version of the TS-LC/MS protocol for the analysis of chlorinated phenoxy acid herbicides, were sent to each participant. It was recommended that those laboratories using TS utilize the negative ionization mode for detection and quantitation (*3*). Quantitation for both interfaces was achieved by using the external standard method.

Recommended analytical column: 15 cm × 2.1 mm i.d., C-18 reverse phase, 0.5-μm particle size, and use of a guard column. HPLC gradient elution conditions: time 0 min, 50% water (with 1% acetic acid)/50% methanol (with 1% acetic acid); hold for 2 min; time 12 min, 40% water/60% methanol; time 18 min, 100% methanol; hold for 10 min; return to 50% water/50% methanol in 10 min; hold for 5 min before starting next analysis. It is necessary to have 1% acetic acid in the mobile phases in order to keep the acid analytes stabilized in their acid form.

Separate HPLC column flow rates were recommended for the different LC column to interface plumbing configurations: for those laboratories using TS interfaces and postcolumn additions of 0.1 M ammonium acetate, column flow rates of 0.4–0.6 mL/min, with 0.8 mL/min postcolumn flow; for laboratories with TS interfaces, but without postcolumn addition, total column flow rates of 1.0–1.2 mL/min; and for those laboratories with PB interfaces, total column flow rates of 0.4–0.6 mL/min.

Results and Discussion

Data were collected from four laboratories using the PB interface and from three laboratories using the TS interface. The data collected were used to compare and evaluate the two LC/MS interface devices for their relative strengths and weaknesses for herbicide acid and ester quantitation.

A Statistical Analysis Systems (SAS) software program was employed to statistically treat the laboratory data. A probability (P) value (*10*) was calculated to determine if significant differences existed within the different data sets. For the data sets to be significantly different, with 95% confidence, the P value must be less than 0.05.

Initially, duplicate extracts at the same concentration level were evaluated separately to determine if there were statistical differences in the results between the two samples. Statistical analysis on the triplicate LC/MS data from the same laboratory from the duplicate extracts indicated no significant difference. This was not unexpected, because these extracts were identically prepared. It was important, however, to demonstrate that statistically the procedure showed no within-laboratory bias between the duplicate extracts.

Because the data indicated no within-laboratory bias, the analyte recovery data from each laboratory was pooled. However, there was an indication of a sample preparation error for the concentration level 250 μg/mL; therefore, the data collected from this concentration level were considered as outliers and were not used.

Two different statistical approaches were used to examine the remaining data for similarities and/or differences between the PB and TS interfaces. First, a probability (P) value was calculated by pooling the mean percent recovery from each interface type at each concentration level (i.e., 500, 50, and 5 μg/mL) and comparing the interface results. As an example, the analyte (2,4-dichlorophenoxy)acetic acid (2,4-D) exhibits a statistical difference between PB and TS at the theoretical concentration of 500 μg/mL. The calculated P value is 0.0433, indicating a significant statistical difference between the two sets of pooled data at that concentration level. At the lower individual 2,4-D concentration levels (e.g., 50 and 5 μg/mL), there was no indication of a statistical difference between the two interfaces (PB and TS). Figure 2 is a bar graph showing the differences between the PB and TS, using normalized P values, P', (where $P' = P - 0.05$, and $P' < 0$ indicates a significant statistical difference) at the individual concentration levels. No other analytes exhibited a significant statistical difference between the interfaces at the individual concentration levels. It should be noted that, while not giving a P value less that 0.05, the analytes 2,2-dichloropropanoic acid (dalapon), 2-(1-methylpropyl)-4,6-dinitrophenol (dinoseb), and 3,6-dichloro-2-methoxybenzoic acid (dicamba) were not detectable by the PB interfaces at the low concentration level, 5 μg/mL. 2,2-Dichloropropanoic acid (dalapon), an aliphatic acid, was not detected at any concentration level by the PB interfaces. This is probably due to its high volatility; it is either vaporized along with the solvent in the desolvation chamber and pumped away or it does not form particles after the solvent is removed.

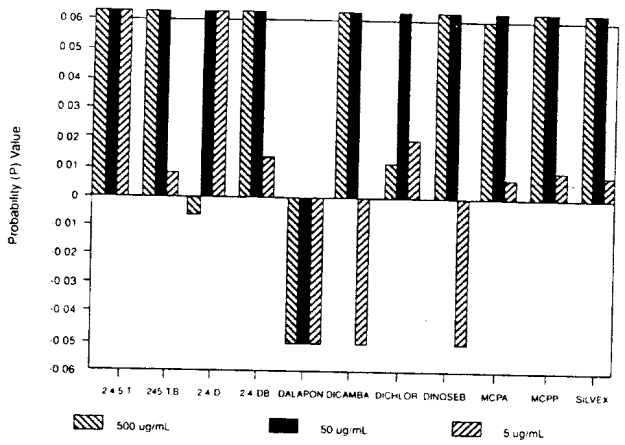

Figure 2. Probability values for individual concentrations, PB vs TS.

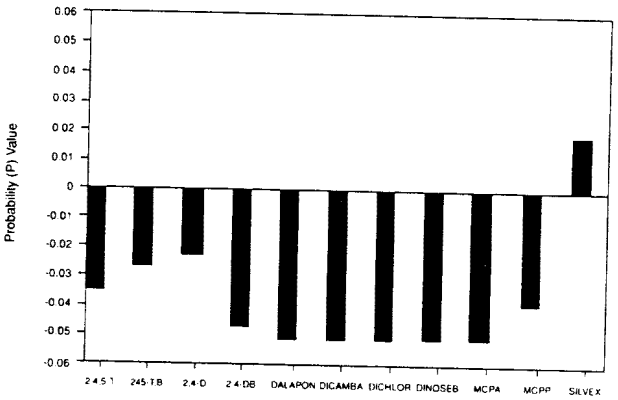

Figure 3. Probability values for mean percent recovery, PB vs TS.

The second method for testing the data was to examine the pooled mean recoveries from each interface over all the concentration levels and examine the results for a difference between the interfaces. This difference between the interfaces over all the concentration levels gave more interesting results than the first statistical method. What was surprising is that while most compounds did not exhibit a statistical difference at the individual concentration levels, the combined concentration data gave an indication of fundamental differences between the interfaces (PB and TS). Figure 3 is another bar graph showing the differences between the PB and TS with normalized P values, P', ($P' = P - 0.05$, and $P' < 0$ is considered a significant statistical difference) over all the concentration levels.

As an example, the analyte (2,4,5-trichlorophenoxy)-acetic acid (2,4,5-T) gives no indication at the individual concentration levels that there is a statistical difference between the interfaces. However, when the P value from overall concentration levels is examined, there is an indication of a difference between the two interfaces (i.e., $P' = -0.0353 < 0$). What is occurring is that at 500 and 50 μg/mL the two interfaces behave similarly; however, at 5 μg/mL the PB interface gives a very high mean percent recovery, 223%, while the TS interface has a mean percent recovery of 90%. This overall difference gives the low P value. All of the analytes show this same PB vs TS deviation, where the two interfaces behave similarly at the high and medium concentration levels, but depart dramatically at the low concentration level, giving the low P values.

Another objective of this study was to compare the performance of the differing manufacture types of LC/MS interface devices to each other (i.e., PB to PB and TS to

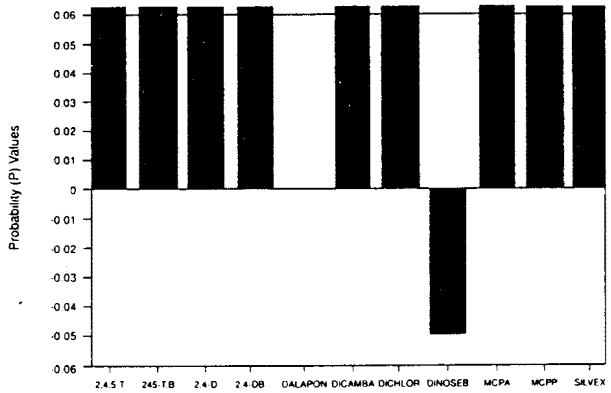

Figure 4. Probability values for combined concentrations (PB): manufacturer A vs manufacturer B.

TS). The experimental design for making such an evaluation is influenced by the number of participating laboratories and their instrumentation configurations.

There were only three laboratories with TS interfaces and each was from a differing manufacturer; therefore no statistical evaluation was possible.

Four sets of PB data, produced by using interfaces made by two different instrument manufacturers (A and B), were received. This allowed for a limited comparison of the PB results. The *P* test was applied, as previously discussed, by combining the data from the two laboratories using PB from manufacturer A and comparing it with the combined data from the two laboratories using manufacturer B. There was no indication of significant statistical differences between the two data sets; see Figure 4. However, 2-(1-methylpropyl)-4,6-dinitrophenol (dinoseb) was not detected at any level when analyzed by interface A and only by one laboratory using interface B.

A comparison of the overall precision and accuracy between PB and TS is shown in Table I. PB has a tendency to give bias high results, at 500 μg/mL (average percent mean bias is +8%), and TS tends to give bias low results (average percent mean bias is −13%) when compared to the true value. The tabulated results for the precision data indicate that PB, at 500 μg/mL, gives slightly better precision (average % RSD = 17%) than TS (average % RSD = 19%).

At the medium concentration level, 50 μg/mL, there was no clear difference between the results obtained from PB and TS, although both are biased low compared to the true value and both exhibit poor precision, average % RSD is for PB 36% and 49% for TS.

Only one PB laboratory could detect analytes at the 5 μg/mL concentration level. The results reported by this laboratory were nearly twice as high as the true value. This strongly indicates that the detection limit for these compounds by PB is above 5 μg/mL. Figure 5 compares the percent recovery of 2,4,5-T at each concentration level for the four PB-LC/MS interfaces. Two of the three laboratories using TS reported values for the 5 μg/mL concentration level, and the results show a very low bias (average percent bias is 3%) and an average % RSD of 19%; see Table I. This is a strong indication that TS provides better sensitivity, with greater accuracy, than PB in detecting low levels of these compounds, although with moderate precision. An example of the percent recoveries for 2,4,5-T at each concentration level for the three TS interfaces is shown in Figure 6.

Conclusion

Although the data collected were from a limited data set (i.e., seven laboratories, and one type of extract) the statistical data showed some interesting and informative results.

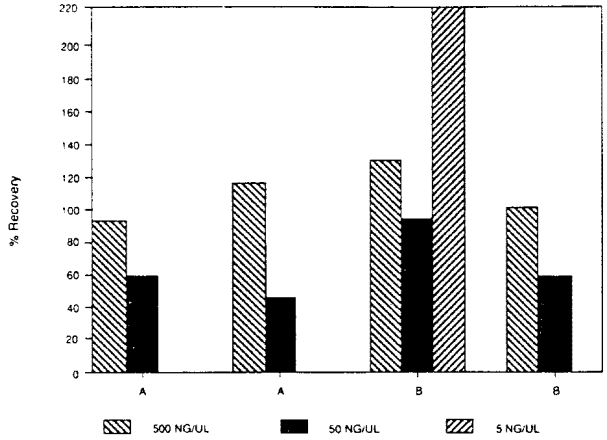

Figure 5. Comparison of the percent recovery of 2,4,5-T between four PB-LC/MS interfaces.

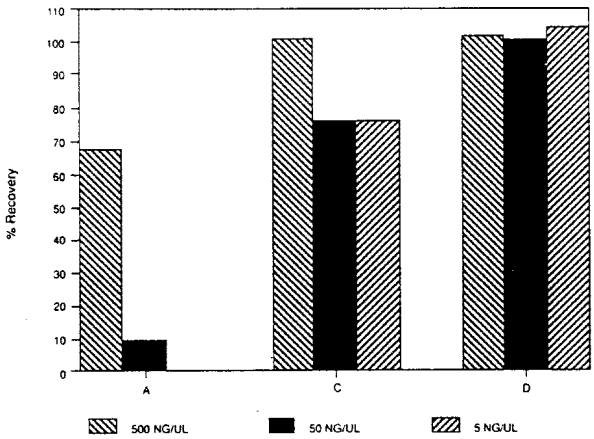

Figure 6. Comparison of the percent recovery of 2,4,5-T between three TS-LC/MS interfaces.

A low molecular weight chlorinated aliphatic acid, 2,2-dichloropropanoic acid, was not detected by PB, presumably because it is too volatile to be transmitted through the PB interface. The substituted phenol, 2-(1-methylpropyl)-4,6-dinitrophenol, responds poorly to PB; even at the highest concentration level, 500 μg/mL, only one of the four PB laboratories reported values for 2-(1-methylpropyl)-4,6-dinitrophenol. 3,6-Dichloro-2-methoxybenzoic acid did not respond well with PB, especially at the lower concentration levels.

PB generally gives better precision than TS, particularly at the high concentration level (500 μg/mL). This is indicated by the lower % RSD values, shown in Table I. Since TS is more sensitive in detecting the target analytes, the extracts often had to be diluted prior to injection in order to be within the linear response calibration range of the instrument. Therefore, dilution errors may have contributed to the poorer % RSD observed for TS. However, it can be assumed that part of the precision difference is due to the fundamental differences in the operating principles of the two interface systems.

The choice of TS or PB interfaces will depend on the type of analytes and the analytical requirements of the data user. From this study one can conclude that for the analysis of low-level samples, TS, with negative ion detection, would be preferred for phenoxy acid herbicides. For the analysis of high-level samples in which identification of the analytes is essential, PB, with electron ionization, might be preferred. Further work should delineate the general applicability, if any, of these conclusions.

Acknowledgments

We thank the individuals and laboratories who volunteered their time and efforts to this study: Dr. Robert Betham; Dr. Mark Brown, California Department of Environmental Services; ENSECO; Dr. Thomas Behymer, U.S. EPA, EMSL-Cincinnati; Dr. Paul Goodley, Hewlett-Packard Corp.; Dr. Mark Roby and Chris Pace, Lockheed Engineering and Sciences Co.; and Dr. Jack Northington, West Coast Analytical Services.

Registry No. 2,4-D, 94-75-7; 2,4,5-T, 93-76-5; MCPA, 94-74-6; MCPP, 7085-19-0; 2,4,5-TP, 93-72-1; 2,4-DP, 94-82-6; dalapon, 75-99-0; dichloroprop, 120-36-5; 2,4,5-T butoxyethanol ether ester, 2545-59-7; dinoseb, 88-85-7; dicamba, 1918-00-9.

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Received for review October 24, 1990. Revised manuscript received April 5, 1991. Accepted April 10, 1991. Notice: Although the research described in this article has been supported by the United States Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement nor recommendation for use.