Development of Mass Spectrometry Compatible Liquid Chromatography Mobile Phases

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Introduction

Liquid chromatography coupled with mass spectrometry (LC/MS) provides a powerful tool for compound and identification studies. Unfortunately many commonly used HPLC mobile phases are not compatible with mass spectrometry. Modifiers such as phosphate buffers and hexane sulfonic acid were originally chosen by chromatographers strictly for their chromatographic benefits. Experience indicates more volatile buffers are required to maintain performance of the mass spectrometer (1-3). Modifiers can be chosen, as presented in this work, that preserve the chromatographic benefits and develop a method amenable to LC/MS use.
Background

Chromatographers use various criteria when choosing materials compatible with their separation needs:
- Extinction coefficient
- Solvent wavelength cutoff
- Buffer capacity
- Solubility
- Selectivity

Mass spectrometrists have concerns for introduction of a sample into the source of the instrument:
- Maximizing sensitivity performance
- Background ions present in the spectra
- Heat impact on EI traceable spectra
Problem:

- Convert an established method from a non-volatile buffer to one compatible with LC/MS.
- Maintain chromatographic resolution and wide range of selectivity with near 100% aqueous to high organic gradient conditions.
- Deliver good quality electron-impact (EI) library searchable mass spectra.
Methods and Materials

A method for determining chlorinated phenoxy acids in water by high performance liquid chromatography using a photodiode array detector, proposed by the US EPA environmental monitoring systems laboratory, is used to illustrate a method of adapting existing separations to LC/MS use.\(^4\)
Instrumentation

- Waters Integrity LC/MS System controlled via Millennium 2010 Chromatography Manager Software
  - Model 717 plus Autosampler
  - Model 616 Low Dispersion HPLC Pump
  - Model 996 Photodiode Array UV Detector
  - Thermabeam Mass Detector
- HPLC Conditions
  - 100 mM Ammonium Acetate: ACN (pH 6.9)
  - 0.5% TEA:ACN (pH 7.3)
  - 95% aqueous held until 2 minutes followed by a linear gradient to 60% organic at 10 minutes.
  - Flow rate - 0.250 ml/min
  - 2mm x 15cm NovaPAK C18 Reverse-phase column
- Acquisition Parameters
  - Photodiode array - 210-310 nm at 1.2 nm resolution and 1 spectra/sec.
  - Mass spectrometer - EI source 250 C with acquisition from 42 - 260 amu at 1 spectra/sec.
Experimental

- Separations such as those presented here may simply require substituting the salt of a weak acid and weak base as a direct replacement of the original less volatile modifier. Using the Henderson-Hasselbalch equation buffer capacities can be determined based on pH.
- Additionally, the effect of the mobile phase choice on the resulting spectra and the effectiveness of library matching using the Palisades Probability-based Matching algorithm must be considered.
- Some attempts to develop methods for LC/MS determination (5) of phenoxy acid herbicides have been found to yield poor chromatographic resolution and poor spectral quality among other problems.
Buffer Capacity as a Function of pH
(A.L.L Duchateau, et. al., JChrom., 552, 1991)

\[ \beta_{\text{in mM}} = \text{buffer capacity} \]

(A) 0.05 \( M \) ammonium formate solution; (B) 0.05 \( M \) ammonium acetate solution; (C) 0.05 \( M \) ammonium bicarbonate solution. pH adjustments done with acetic acid.
Original Method

- Eight chlorinated phenoxy acid herbicides (including 5-hydroxydicamba)
- Dual column (primary column and confirmation column)
- 0.025 M phosphate and acetonitrile gradient - 40 minutes plus 10-20 minutes to re-establish initial conditions.
- Photodiode array only - no mass spectrometry.
Conversion Method A

- 100mM ammonium acetate:acetonitrile gradient
- Single 2mm NovaPAK C18 column
- Photodiode array and particle beam mass spectrometer in-line.
- Method A and Method B provided good chromatographic resolution and reduce run time from 40 minutes to 15 minutes.
Conversion Method B

- 0.5% triethyl amine:acetonitrile gradient (same profile as Method A)
- Single 2mm NovaPAK C18 column
- Photodiode array and particle beam mass spectrometer in-line.
- Method A and Method B provided good chromatographic resolution and reduced run time from 40 minutes to 15 minutes.
Results

- Examination of the mass spectra from each run shows a preference for Method A due to the reduced background interference and the enhanced library match capability.
Results
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Photodiode array was used to examine the standards for homogeneity which can be used to corroborate ions found in the mass spectra that do not appear to be coherent with the compound found.
Results

Mass spectrometry was used for positive compound identification since the change in chemistry resulted in the two hydrophilic peaks (Picloram and Chloramben) eluting in reverse order.
Conclusion

- The addition of mass spectrometry to proposed EPA Method 555 for the determination of chlorinated phenoxy acid herbicides in water achieves:
  - Simplification by reducing the system from two columns to a single analytical column.
  - The same level of specificity as a GC/MS method by using LC/MS to produce EI library-searchable spectra.
  - Shortened run times and higher throughput at lower narrow-bore flow rates (40 minutes acquisition to 15 minutes).
References


