

## ION-PAIRING CHROMATOGRAPHY OF ALKALOIDS AND RELATED COMPOUNDS

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### ABSTRACT

While liquid chromatographic methods would seem to be ideal for the analysis of alkaloids because of the mild conditions of separation, some problems have been encountered. Separations utilizing normal- or reverse-phase systems with conventional solvents are frequently accompanied by poor peak symmetry and low column efficiency. Improved separations were obtained by chromatography on reverse-phase columns using buffered solutions of various salts with methanol or acetonitrile. Column efficiency, peak symmetry, and retention characteristics were examined as a function of the counter-ion used, pH, ion concentration, and organic solvent used. This separation system is very versatile for the analysis of alkaloids, other nitrogen heterocycles, and some quaternary nitrogen compounds.

### INTRODUCTION

Physiological and toxicological studies of alkaloids and other organic nitrogen compounds require selective, sensitive methods of analysis. Liquid chromatography is often the method of choice due to its mild separation conditions. However, conventional adsorption chromatography and many reverse-phase systems tended to result in poor separations, with excessive retention and distorted peak shape, as well as low column efficiency. Organic bases may be chromatographed in alkaline media to suppress protonation, but this practice is not compatible with the silica gel-based stationary phases usually used. However, protonated alkaloids and quaternary ammonium compounds may be chromatographed in the presence of suitable counter-ions which form neutral ion pairs with the cation. This separation may be achieved at moderate pH values on conventional nonpolar (reverse-phase) stationary phases. Factors influencing the separation include the type and concentration of the counter-ion, pH, and the organic modifier used.

### MATERIALS AND METHODS

The liquid chromatograph used consisted of a Model 6000A pump with the U6K injector (Waters Associates, Milford, MA) coupled with the Beckman Model 153 ultraviolet absorbance detector (Beckman Instruments, Fullerton, CA). The column was

a reverse-phase C<sub>18</sub> type with 10 micron packing, 25 cm long and 4.6 mm I.D. obtained from Alltech Associates (Deerfield, IL).

The mobile phase was prepared by mixing 1:1 (v/v) solutions of methanol or acetonitrile with buffer. The buffer was prepared by adding triethylamine to 0.01 to 0.1 M perchloric, sulfuric, or phosphoric acid. The buffer was brought to a pH of 3.5, 4.5, or 5.5 for alkaloid separations. Other buffers used included ammonium sulfate solutions adjusted to the desired pH and sodium dodecyl sulfate solutions.

Chromatographic conditions were a flow rate of 1.0 mL/min with monitoring at 254 nm at a sensitivity of 0.16 absorbance unit full scale. Microgram quantities of various alkaloids were injected, and the chromatograms were examined for retention characteristics, number of theoretical plates, and peak symmetry.

### RESULTS AND DISCUSSION

The solute retention, column efficiency, and peak symmetry factors were calculated from a large amount of data. The effect of experimental parameters on these may be expressed as follows:

- (1) Solute Retention
  - (a) Retention times and capacity ratio decrease as the counter-ion concentration increased, though not linearly.
  - (b) Retention of the alkaloids increased as the buffer pH increased (quaternary ammonium compounds are only slightly affected).
  - (c) Retention, as a function of counter-ion concentration, was far more variable when ammonium ion replaced triethylammonium ion.
- (2) Column Efficiency
  - (a) Column efficiency is better in acetonitrile than in methanol, sometimes by a factor of two or three.
  - (b) The use of triethylammonium ion gave better column efficiency than when ammonium ion was used, even though these cations are not the counter-ions for the alkaloids.
- (3) Peak Symmetry
  - (a) Peak symmetry was generally better as the counter-ion concentration increased from 0.01 to 0.10 M. All counter-ions used in this study gave reasonable peak symmetry.
  - (b) When ammonium ion replaced triethylammonium ion in the buffer, peak asymmetry increased.

While satisfactory chromatograms could be obtained under a wide variety of conditions, counter-ion concentrations in the range of 0.05-0.10 M were found to be best for a variety of compounds. When sodium dodecyl sulfate was used to provide the counter-ion, however, only acetonitrile performed well as the organic solvent. Methanol-dodecyl sulfate systems were not satisfactory. When using ion-pairing chromatography, it is important to give the system enough time to equilibrate before samples and standards are chromatographed.

The basic theory of ion-pairing techniques has been discussed (Eksborg and Schill, 1973), but exact mechanisms apparently have not been established. One postulated mechanism is that of ion-pair formation in solution followed by partition into the stationary phase (Gloor and Johnson, 1977). The other suggests that the counter-ions are adsorbed onto the stationary phase, where they then act as a dynamic ion exchanger. The organic solvent itself is partitioned onto the stationary phase (McCormick and Karger, 1980). The observation that both small hydrophilic ions (sulfate, perchlorate, and phosphate) and an ion with a hydrophobic group (dodecyl sulfate) can be used effectively suggests that both mechanisms may be applicable.

#### LITERATURE CITED

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