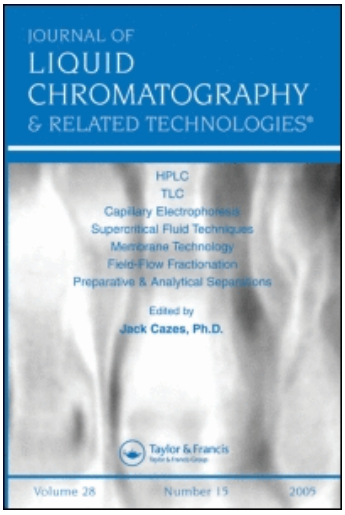


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Preparative Scale Reverse Phase HPLC Separation of Epimeric Alkaloids Using Camphorsulfonic Acid as an Ion Pairing Reagent

Paul S. Salva[a]; Gilbert J. Hite[a]; James G. Henkel[a]
[a] U-92 University of Connecticut Storrs, Section of Medicinal Chemistry and Pharmacognosy School of Pharmacy, CT, USA

To cite this Article Salva, Paul S. , Hite, Gilbert J. and Henkel, James G.(1982) 'The Preparative Scale Reverse Phase HPLC Separation of Epimeric Alkaloids Using Camphorsulfonic Acid as an Ion Pairing Reagent', Journal of Liquid Chromatography & Related Technologies, 5: 2, 305 — 312
To link to this Article: DOI: 10.1080/01483918208069072
URL: http://dx.doi.org/10.1080/01483918208069072

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(2), 305-312 (1982)

THE PREPARATIVE SCALE REVERSE PHASE HPLC SEPARATION OF EPIMERIC ALKALOIDS USING CAMPHORSULFONIC ACID AS AN ION PAIRING REAGENT

Paul S. Salva, Gilbert J. Hite, and James G. Henkel*
Section of Medicinal Chemistry and Pharmacognosy
School of Pharmacy, U-92
University of Connecticut
Storrs, CT 06268 USA

ABSTRACT

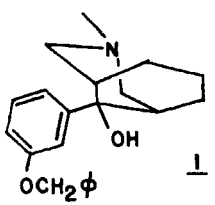
A reverse phase paired ion HPLC procedure is described for the separation of multigram quantities of epimeric alkaloids using camphorsulfonic acid as the ion-pairing reagent.

INTRODUCTION

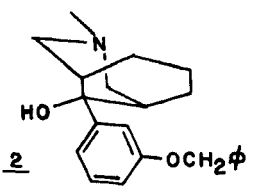
In medicinal chemical research it is often necessary to separate relatively large amounts of epimeric compounds for subsequent structure-activity studies. With the advent of preparative HPLC, multigram separations of a great many classes of compounds have become feasible(1-4). However, there are limitations imposed on this technology by the paucity of stationary phases available in commercial preparative scale cartridges, i. e., silica gel normal phase and C_{18} reverse phase. While many classes of compounds are successfully separated using these stationary phases, there has been a notable lack of success with alkaloids. Normal phase chromatography has been

generally unsatisfactory due to the highly polar and acidic nature of the adsorbant. While reverse phase HPLC offers some advantage(5-7), alkaloids tend to exhibit nonideal behavior, resulting in streaking and poor resolution. Also, the usual reverse phase procedure is of limited value on the preparative scale due to the rapid deactivation of the stationary phase that occurs at the high pH required for successful separations. More recently, ion-paired reverse phase techniques have been particularly successful for the separation of mixtures of alkaloids on the analytical scale(8-12). Unfortunately, the very high cost of the long-chain sulfonic acids used as ion-pairing reagents makes their use on the preparative scale economically unfeasible.

In order to overcome this disadvantage, we have investigated the use of commercially available racemic camphor-10-sulfonic acid (CSA) as an ion-pairing reagent on the preparative scale for the separation of mixtures of alkaloids. While CSA has proven to be useful on the analytical scale(13,14), no preparative applications have been reported. Preliminary trials on reverse phase TLC using ion-pair techniques showed no measurable differences in separation with either CSA or the long-chain alkyl sulfonic acids. As representative alkaloids we have chosen the 9-disubstituted alpha- and beta-3-azabicyclo[3.3.1]nonanes 1 and 2, which occur in a 70:30 ratio as the products of a Grignard reaction.



1



2

EXPERIMENTAL

Reagents:

The reverse phase TLC plates were Whatman KC18F. Methanol and ethyl acetate were obtained from J.T.Baker and distilled from glass. The water was doubly distilled from a glass apparatus. The CSA was obtained from Aldrich Chemical Co. (Metuchen, NJ) and was purified by treatment with decolorizing carbon (Norite A) followed by recrystallization from ethyl acetate. The purified CSA was then dissolved in water and passed through a flash chromatography column filled with bondapak C_{18} (Waters Associates, Milford, MA) to remove nonpolar impurities. The solution was lyophilized and the solid was recrystallized from ethyl acetate again before use.

Preparation of Mobile Phase:

Two mobile phases were used. One was 60 volume % aqueous buffered methanol, and the other was 20 volume % aqueous buffered methanol. Both contained 10 mM CSA, 2% acetic acid, and were buffered to pH 2.5 with sodium acetate trihydrate. Each was prepared by dissolving the necessary amounts of CSA, glacial acetic acid, and sodium acetate trihydrate in the aqueous portions of the mobile phases. These aqueous solutions and the methanol were filtered separately through 0.45 micron Millipore filters immediately prior to use and were then mixed to produce the final mobile phases stated above.

The Apparatus:

The HPLC apparatus used was a Prep/LC 500 unit equipped with a refractive index detector (Waters Associates, Milford, MA). The column was a cartridge containing 500 g of bondapak C_{18} reverse phase packing material also obtained from Waters Associates.

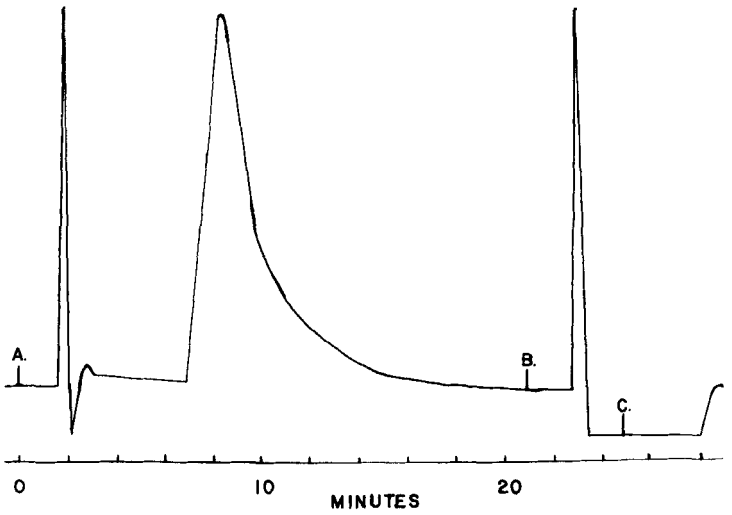


Figure 1. Detector output for the separation of 1 and 2. The chart speed was 2 min/cm at a flow rate of 250 mL/min. Legends: A. Injection of eluent; B. Change to 20 volume % aqueous buffered methanol; C. Restore 60 volume % aqueous buffered methanol.

RESULTS AND DISCUSSION

A typical chromatographic separation of 1 and 2 is illustrated in Figure 1. The best results were obtained using a 60 volume % aqueous buffered methanol mobile phase. After allowing the stationary and mobile phases to equilibrate, the alkaloid mixture was introduced in 10 gram quantities as the CSA salts in a minimum amount of methanol (final volume 25mL). Under these conditions, the alpha epimer eluted first, followed by the beta epimer. We subsequently found that the time and solvent volume needed for elution of the beta epimer could be substantially reduced by converting in one step to 20 volume % aqueous buffered methanol immediately after the alpha epimer had eluted. All of the beta epimer was eluted after two column volumes under these conditions. Using this procedure, four or five successive separations

could be performed before deterioration of the baseline on the refractive index detector occurred. A stable baseline was restored by flushing the system with 6 - 8 liters of water to remove the pairing reagent and buffers, then with 2 liters of 50 volume % aqueous methanol followed by 2 liters of 100 % methanol. The column was then re-equilibrated by treatment with 2 liters of 50 volume % aqueous methanol again, and finally with 60 volume % aqueous buffered methanol. Failure to remove the 100% methanol from the column before the buffered mobile phase is introduced results in precipitation of the salts on the column and subsequent difficulty in restoring it to the functional state. The column can be used indefinitely without loss of performance under these conditions.

The alkaloids were recovered by combining homogeneous fractions and alkalinizing them to pH 10 with sodium hydroxide pellets. The methanol was evaporated in vacuo and the resulting alkaline aqueous suspension was subjected to overnight continuous extraction with chloroform. The organic phase from the extraction was then dried over anhydrous sodium sulfate and evaporated to dryness, producing essentially quantitative recovery of the free bases.

The chromatographic efficiency of the preparative scale cartridge described herein did not even closely approach that exhibited by the microparticulate HPLC columns presently available for analytical separations. In fact, $\underset{\sim}{1}$ and $\underset{\sim}{2}$ were easily separable under a variety of simple conditions using a 10-micron C_{18} analytical column. Because of this large difference in efficiencies, it is quite difficult to find the optimal preparative scale chromatographic parameters using an analytical HPLC system. We have found that for scouting purposes, commercially available reverse phase TLC plates more realistically approximate the chromatographic behavior exhibited on the preparative scale. Moreover,

the epimeric purity of the resulting eluent fractions may easily be checked by the same technique.

The success of the chromatographic separations using this technique is apparently highly sensitive to the composition of the mobile phase. For example, the concentration of ion pairing reagent affects the chromatographic behavior, with higher concentrations of CSA increasing the efficiency of the column toward the eluents, at least over a limited range. Thus, mobile phase that was 10 mM in CSA produced much sharper peaks and improved resolution over mobile phase that was 5 mM in CSA. While still higher concentrations of CSA may further improve chromatographic behavior in these systems, they were not investigated. Using higher concentrations of pairing reagent requires the use of a buffer, since the lower pH of the more concentrated CSA solution causes column degradation by loss of covalently bonded stationary phase. In this study, sodium acetate trihydrate was used to obtain the proper pH for the mobile phase. Also, the presence of acetic acid was found to be necessary for a successful separation. However, there were no differences in the quality of the separations over the range of acetic acid concentrations investigated (1%-3.5%).

We briefly investigated the influence of pH upon separation. As one would expect, pH strongly influences chromatographic behavior in this system. Increasing the pH of the mobile phase from pH 2.5 to 3.0 (or even to pH 2.75) significantly degrades chromatographic performance. On this basis we may state that at least to a first approximation, the best chromatographic performance in systems of this kind may be obtained at the highest millimolar concentration of CSA and the lowest pH of the mobile phase consistent with column and eluent stability.

We feel that this method offers several advantages for the separation of alkaloids on the preparative scale. The separation is

complete and reproducible, recovery of the alkaloids is facile and quantitative, and perhaps most importantly, the reagents used are inexpensive and readily available. A singular disadvantage is the expense of the C_{18} stationary phase. While this expense necessitates the rigorous purification of the reagents used in order to insure column longevity, with proper care the column remains functional indefinitely. We are presently investigating the general applicability of this procedure to other classes of alkaloids.

ACKNOWLEDGEMENTS

This work was supported by grant No. DA-01612 (to GJH) from the National Institute of Drug Abuse, Department of Health and Human Services. We also thank Dr. C. M. Ireland for helpful discussions.

REFERENCES

1. Yoshida, T., Shu, C.-K., and Theimer, E. T., J. Chromatogr., 137, 461 (1977).

2. Krupcik, J., Kuz, J., Prusova, D., Suchanek, P., and Cervenka, Z., J. Chromatogr., 142, 797 (1977).

3. Pettei, M. J., Pilkiewicz, F. G., and Nakanishi, K., Tetr.Lett., 1977, 2083.

4. Jones, B. B., Clark, B. C., and Iacobuzzi, G. A., J. Chromatogr., 178, 575 (1979).

5. Poochikian, G. K. and Cradock, J. C., J. Chromatogr., 171, 371 (1979).

6. Sasse, F., Hammer, J., and Berlin, J., J. Chromatogr., 194, 234 (1980).

7. Hanna, S., Insler, M., Zapata, R., and Lachman, L., J. Chromatogr., 200, 277 (1980).

8. Muzard, G. and LePecq, J. B., J. Chromatogr., 169, 446 (1979).

9. Bannister, S. J., Stevens, J., Musson, D., and Sternson, L. A., J. Chromatogr., 176, 381 (1979).

10. Mourot, D., Delepine, B., Boiseau, J., and Gayot, G., J. Chromatogr., 190, 486 (1980).

11. Lokke, H., J. Chromatogr., 200, 234 (1980).

12. Brown, N. D., Scovill, J. P., Sleeman, H. K., and Doctor, B. P., J. Chromatogr., 200, 267 (1980).

13. Gloor, R. and Johnson, E. L., J. Chromatogr. Sci., 15, 413 (1977).

14. Landis, J. B., Grant, M. E., and Nelson, S. A., J. Chromatogr., 202, 99 (1980).