Journal of Chromatography, 176 (1979) 470–472

CHROM. 11,951

Note

Effect of zinc ions on the reversed-phase separation and quantification of trace isomeric aminobenzoic acids in aqueous solution by high-performance liquid chromatography

V. WALTERS and N. V. RAGHAVAN
Radiation Laboratory, University of Notre Dame, Notre Dame, Ind. 46556 (U.S.A.)
(First received April 5th, 1979; revised manuscript received May 2nd, 1979)

During the course of our investigation on the reactivity of amino radical with benzoic acid, it was necessary to develop a sensitive method for the detection and quantification of isomeric aminobenzoic acids from benzoic acid in aqueous solution. Separations were attempted on μBondapak C_{18}, anion-exchange and cation-exchange columns. The best separation was achieved on μBondapak C_{18} column under reversed-phase conditions. The addition of metal ions either in the mobile phase or chemically bonded to the stationary phase, has been applied for selective separation recently[1]. The effect of the addition of Zn^{2+} in the mobile phase has resulted in increasing the resolution in the separation of isomeric aminobenzoic acids.

EXPERIMENTAL

The main features of the liquid chromatograph used have previously been described[2,3]. It consists of the following components: Water Assoc. Milford, Mass., U.S.A. Model 6000A pump, a 100-μl six-port injection value. The chromatographic columns used were μBondapak C_{18} obtained from Waters Assoc., cation- and anion-exchange columns obtained from Vydac, Los Angeles, Calif., U.S.A. The detector was a Vari-Chrom UV–Vis variable-wavelength detector obtained from Varian. In all of our studies reported here, the photometric range of the detector was set at 0.02. The flow-rate was 1.8 ml/min at an operating pressure of 1000 p.s.i. The detection was carried out at 230 nm, where aminobenzoic acids have maximum absorbance. The UV absorption spectra were taken using a Cary 219 UV–visible spectrophotometer.

RESULTS AND DISCUSSION

Using a Zipax anion-exchange column, Klein *et al.*[4] have separated isomeric hydroxybenzoic acids with considerable difficulty. The *ortho* component was retained in the column for a longer time. Initially the separation of isomeric aminobenzoic acids was attempted using a Vydac anion-exchange column with 0.03 *M* K_2HPO_4 at pH 6.5. Both *ortho*-aminobenzoic acid and benzoic acid eluted together with long retention times (60 min). Varying the pH of the mboile phase to 7.5 did not improve the separation. Using 0.02 *M* borate buffer at pH 9.0 as the mobile phase, the separa-

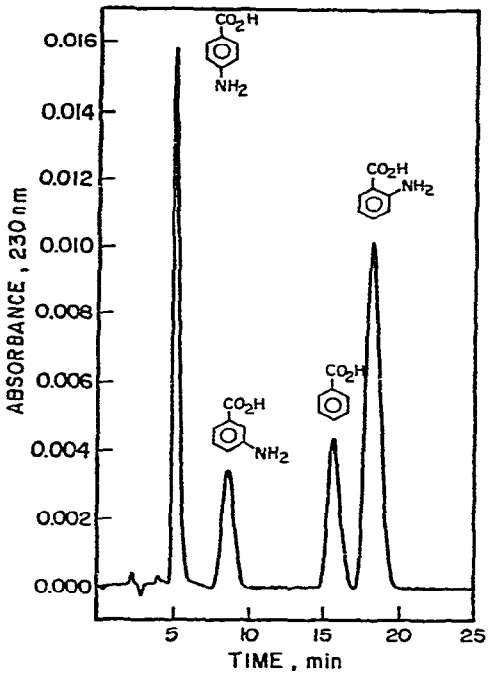

Fig. 1. Separation of isomeric aminobenzoic acids (10 μM each) from benzoic acid (10 μM) on μBondapak C_{18} column with 0.01 M NaH_2PO_4 and water–methanol (96:4) as the mobile phase. The detection was carried out at 230 nm.

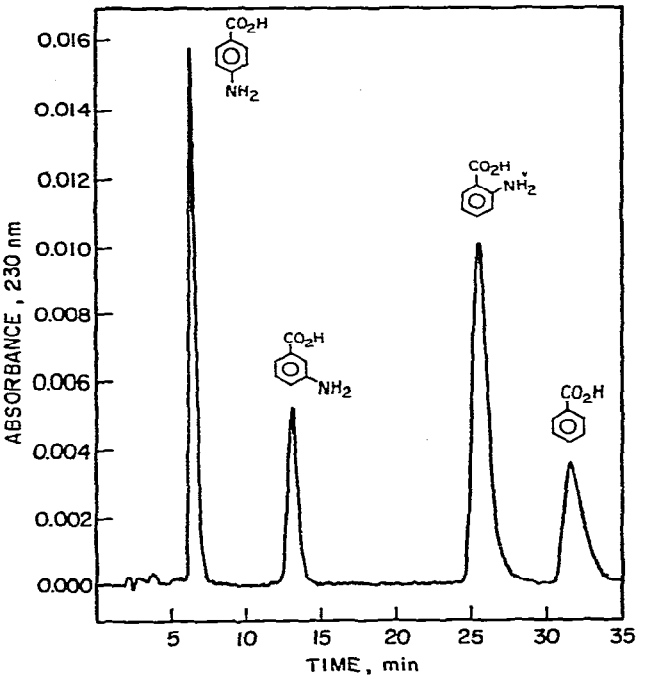

Fig. 2. Separation of isomeric aminobenzoic acids from benzoic acid. Experimental conditions are the same as in Fig. 1, except 0.002 M $ZnCl_2$ was added to the mobile phase.

tion of *ortho* component from benzoic acid was not achieved. Separation was also attempted on Vydac cation-exchange resin using sodium acetate at pH 4.0 as the mobile phase. All the components were not very well resolved.

The separation was achieved on μBondapak C_{18} column using water–methanol (96:4) and 0.01 *M* NaH_2PO_4 and the chromatogram obtained at $\lambda = 230$ nm is shown in Fig. 1. The separation of *ortho*-aminobenzoic acid from benzoic acid is not very well resolved. Since the formation constant of Zn^{2+} with *ortho*-aminobenzoate is much larger than the other components[5], it was hoped that the addition of Zn^{2+} in the mobile phase would show some effect on the chromatogram. The chromatogram obtained on adding 0.002 *M* of Zn^{2+} to the mobile phase is shown in Fig. 2. The pH of the mobile phase was decreased by 0.2 upon the addition of Zn^{2+}. As can be seen from Fig. 2, the *ortho*-aminobenzoic acid and benzoic acid are well separated. Moreover the *ortho* isomer has a shorter retention time which may be due to the formation of labile either mono- or bidentate Zn(II) complex. Very low concentration of Zn^{2+}, $1.0 \cdot 10^{-4}$ *M*, did not show any appreciable effect on the separation. It was possible to detect 2 μM of each of the components with an accuracy of $\pm$ 5%.

ACKNOWLEDGEMENT

The research described herein was supported by the Office of Basic Energy Sciences of the Department of Energy; document No. NDRL-1976, the Notre Dame Radiation Laboratory.

REFERENCES

1 N. H. C. Cooke, R. L. Viavattene, R. Eksteen, W. S. Wong, G. Davies and B. L. Karger, *J. Chromatogr.*, 149 (1978) 391.
2 K. Bhatia and R. H. Schuler, *J. Phys. Chem.*, 77 (1973) 1356.
3 N. V. Raghavan, *J. Chromatogr.*, 168 (1979) 523.
4 G. W. Klein, K. Bhatia, V. Madhavan and R. H. Schuler, *J. Phys. Chem.*, 79 (1975) 1767.
5 L. G. Sillen and A. E. Martell, *Spec. Publ. Chem. Soc.*, No. 17 (1964) 533, and 25 (1971) 547.