Journal of Chromatography, 466 (1989) 438–441
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 245

Note

Separation of simple guanidines on cation-exchange columns using indirect ultraviolet detection

THOMAS A. WALKER
Chemical Products Discovery, The NutraSweet Co., 601 E. Kensington Road, Mt. Prospect, IL 60056 (U.S.A.)

(First received December 6th, 1988; revised manuscript received December 28th, 1988)

Indirect photometric or "vacancy" chromatography (IPC) is an analytical technique where ionic analytes are separated on an ion-exchange column and are then detected through a photometric process[1,2]. IPC, which was described by Small and Miller, Jr. in 1982[1], consists of an UV-absorbing counterion in the mobile phase that competes with UV-transparent, injected analyte ions for the ion-exchange sites. As the UV-transparent analyte elutes off the column, it replaces the UV-absorbing counterion in the effluent. This replacement leads to a decrease in absorbance at the detector and produces a negative peak.

IPC has become a commonly used method for the analysis of inorganic and organic UV-transparent ions where a strong cation- or anion-exchange column is used[3–6]. Indirect UV detection has also been extended to reversed-phase[7] and ion-interaction chromatographic separations[8,9] as well as for separations where low-capacity ion-exchange columns are used[10,11]. The separation and indirect UV detection of organic analyte cations on low-capacity cation-exchange columns, however, has not been studied to the extent that other chromatographic systems have.

This paper describes the separation and indirect UV detection of several simple guanidines on a low-capacity polymeric cation-exchange column and on a silica-based strong cation-exchange column. Quantitation of the guanidines was also studied on the low-capacity cation-exchange column.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile was obtained from Fisher Scientific (Fairlawn, NJ, U.S.A). HPLC-grade water was obtained by passing deionized water through a Millipore water purification unit. Benzyltrimethylammonium chloride and the guanidines were obtained from Aldrich (Milwaukee, WI, U.S.A). All chemicals were reagent grade.

Apparatus

The liquid chromatographic (LC) apparatus used consisted of a WISP Model 710B Autosampler, Waters Model 590 high-performance liquid chromatography

0021-9673/89/$03.50

(HPLC) pump, a Kratos Model 783 variable-wavelength UV detector, and a Linear Model 500 strip-chart recorder. The columns used in this study were: a 150 × 4.1 mm I.D. Hamilton PRP-X200 low-capacity cation-exchange column available from Hamilton (Reno, NV, U.S.A.) and a 250 × 4.6 mm I.D. Whatman Partisil 10 SCX (Cherry Hill, NJ, U.S.A.). The PRP-X200 column is a spherical, 10 μm poly(styrene–divinylbenzene) sulfonate packing with a cation-exchange capacity of 35 μequiv./g. Flow-rates of 1.0 ml/min were used, unless noted otherwise. Aqueous analyte samples of approximately 1 mg/ml were used. Sample aliquots of 10 μl were used. Inlet pressures of 500–600 p.s.i. were observed.

RESULTS AND DISCUSSION

Several groups have used low-capacity cation-exchange columns for the separation of inorganic and organic analyte cations[10–15]. Cantwell and co-workers[12–14] has shown that a dual retention mechanism of cation exchange and adsorption account for the retention of organic analytes that contain both a fixed charge site and a hydrophobic center. Differences in elution orders for organic cations have been observed when comparing separations on polymer-based low-capacity cation-exchange columns and silica-based strong cation-exchange columns[10]. These changes in the elution order can be attributed to adsorption that takes place between the hydrophobic center of the analyte and the adsorption sites on the low-capacity cation-exchange column. Changes in the elution order of organic cations on the low-capacity cation-exchange column can be accomplished by adjusting the amount of added organic modifier and/or by manipulating the mobile phase ionic strength[10].

In IPC, the added UV-absorbing countercation has the dual role of: (1) displacement of an analyte cation from the cation-exchange column and (2) detection of an UV-transparent analyte cation as a dip or trough in the baseline absorbance. When a low-capacity cation-exchange column is used for separating organic analyte cations, the UV-absorbing countercation will be involved in the detection of the organic analyte cation and will compete for the cationic exchange sites. If the UV-transparent analyte is retained predominantly by adsorption, then the UV-absorbing countercations role is just the indirect UV detection of the organic analyte.

Fig. 1 shows the separation and indirect UV detection of several guanidines on a silica-based strong cation-exchange column (I) and on the PRP-X200 column (II). Elution orders were found to be almost reversed when the two columns were compared for this separation. It should be noted that the guanidine separations on the two columns were optimized so that the resulting chromatograms could be directly compared.

It was observed during this study that the elution order of the guanidines on the PRP-X200 column could be changed by adjusting the amount of added acetonitrile or by adjusting the mobile phase ionic strength. If the amount of acetonitrile in the mobile phase was increased, the retention of 1-ethylguanidine (F) was reduced. However, the retention of guanidine (E) does not change. This shows that the adsorption mechanisms plays a more predominant role in the retention of 1-ethylguanidine whereas guanidine is retained exclusively by a cation-exchange mechanism. A combination of adsorption/ion-exchange mechanisms accounts for the retention of the other guanidines.

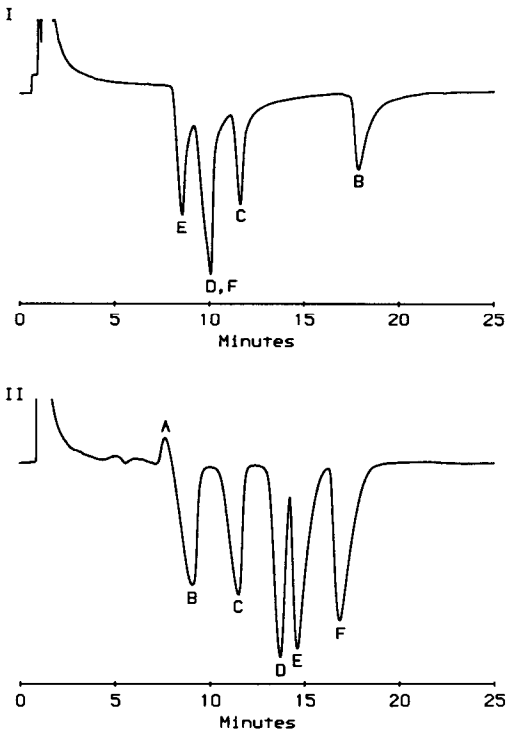

Fig. 1. Chromatogram of (A) system peak, (B) 1,1,3,3-tetramethylguanidine, (C) 1,1-dimethylguanidine, (D) 1-methylguanidine, (E) guanidine, (F) 1-ethylguanidine. Conditions: mobile phase, (I) 0.0025 *M* benzyltrimethylammonium chloride in acetonitrile–water (20:80) (II) 0.0008 *M* benzyltrimethylammonium chloride in acetonitrile–water (20:80); columns, (I) Whatman Partisil 10 SCX (250 × 4.6 mm I.D.), (II) PRP-X200 (150 × 4.1 mm I.D., 10 mm); flow-rate, (I) 2.0 ml/min, (II) 1.0 ml/min; detector, 0.04 a.u.f.s. at 268 nm.

Quantitation was done for the guanidines on the PRP-X200 column. Calibration curves for all of the guanidines were found to be linear over a range of 5 to 800 ppm with a detection limit of 1–3 ppm. Several samples with a known amount of 1,1,3,3-tetramethylguanidine, 1,1-dimethylguanidine, 1-methylguanidine, guanidine and 1-ethylguanidine were prepared and analyzed. Average recoveries of 96.4, 98.5, 98.8, 100.9 and 99.0% with relative standard deviations of 5.6, 1.5, 3.3, 1.9 and 2.4%, respectively, were found for each analyte.

REFERENCES

1 H. Small and T. E. Miller, Jr., *Anal. Chem.*, 54 (1982) 462.
2 H. Small and T. E. Miller, Jr., *U.S. Pat.*, 4414842.
3 J. R. Larson, *J. Chromatogr.*, 356 (1986) 379.
4 B. P. Downey and D. R. Jenke, *J. Chromatogr., Sci.*, 25 (1987) 519.
5 J. R. Larson and C. D. Pfeiffer, *J. Chromatogr.*, 259 (1983) 519.
6 Z. Iskandarani and T. E. Miller, Jr., *Anal. Chem.*, 57 (1985) 1591.
7 G. A. Benson and M. Lennon, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 109.
8 P. G. Rigas and D. J. Pietrzyk, *Anal. Chem.*, 58 (1986) 2226.
9 W. F. Barber and P. W. Carr, *J. Chromatogr.*, 316 (1984) 211.

10 T. A. Walker, *J. Liq. Chromatogr.*, 11 (1988) 1513.
11 R. C. L. Foley and P. R. Haddad, *J. Chromatogr.*, 366 (1986) 13.
12 F. F. Cantwell, in J. A. Marinsky and Y. Marcus (Editors), *Ion Exchange and Solvent Extraction*, Vol 9, Marcel Dekker, New York, 1985, p. 339.
13 R. A. Hux and F. F. Cantwell, *Anal. Chem.*, 56 (1984) 1258.
14 S. Afrashtehfar and F. F. Cantwell, *Anal. Chem.*, 54 (1982) 2422.
15 D. J. Pietrzyk, Z. Iskandarani and G. L. Schmitt, *J. Liq. Chromatogr.*, 9 (1986) 2633.