Journal of Chromatography, 502 (1990) 241–242
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 053

Letter to the Editor

Fast separation of dihydro ergot alkaloids on an octylsilica column

Sir,

For the separation of the three components [dihydroergocornine (I), dihydroergocristine (III), dihydro-($\alpha+\beta$)-ergocryptine (II, IV), silica-based hydrocarbon stationary phase (octylsilica, octadecylsilica) and methanol[1] and acetonitrile[2–5] containing aqueous buffer (pH ≈ 7) as eluents are used. As buffers, phosphate[1], ammonium carbonate[2–5], triethanolamine–citric acid or triethanolamine–sodium acetate mixtures[5] are used. A reasonable separation of the four components, including the α- and β-isomers of dihydroergocryptine, was achieved only in alkaline eluents containing diethylamine[5] or triethylamine[6–8]. The developed method suggests the use of tetrabutylammonium hydroxide (TBAOH) rather than toxic and environmentally objectionable di- or triethylamine.

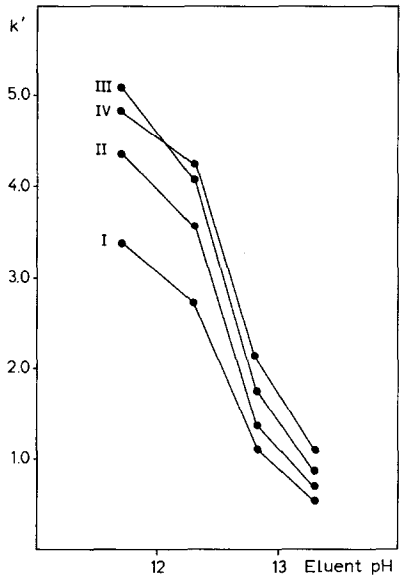
The analytical column (150 × 4.0 mm I.D.) was packed with 5-μm octylsilica (BST SI-100-S5 C_8; Bioseparation Technologies, Budapest, Hungary). In order to avoid rapid degradation of the stationary phase in analytical column, a short protecting column (30 × 4.0 mm I.D.) filled with octylsilica was inserted between the separation column and the injector and a silica column (100 × 4.0 mm I.D.) (10-μm LiChrosorb SI-100; Merck, Darmstadt, F.R.G.) was fitted between the pump and the injector. Before starting the measurements the analytical column was washed with acetonitrile–5 m*M* aqueous TBAOH (47:53) until the pH of the effluent reached the alkaline range (50–60 ml).

After the measurements, the column was washed to neutral with acetonitrile–water (47:53). Under these conditions, the analytical column could be used for 3 months (approximately 350–400 injections) without deterioration.

The separation of the four dihydro ergot alkaloids was studied as a function of eluent pH. At a constant acetonitrile concentration (47%), the concentration of the TBAOH was varied between 1 and 10 m*M* (pH 11.8–13.4). As Fig. 1 shows, the capacity factor (k') of the alkaloids decreases rapidly at pH > 12, and the elution sequence of dihydroergocristine (III) and dihydro-β-ergocryptine (IV) also changed. At pH > 12.4 there was a significant increase in selectivity. Without affecting the selectivity, the retention of the alkaloids changed when the acetonitrile concentation was varied in the range 40–55% at a constant (5 m*M*) TBAOH concentration.

With an eluent containing 45% acetonitrile, complete baseline separation is possible (Fig. 2) in 8 min. Although this separation time is twice that achieved by

0021-9673/90/$03.50



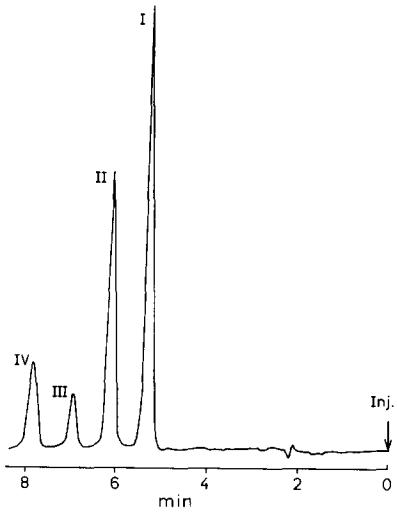


Fig. 1. Change in the capacity factor of the dihydro ergot alkaloids as a function of the eluent pH. Stationary phase, octylsilica; mobile phase, acetonitrile–10^{-2}–10^{-3} *M* aqueous TBAOH (47:53). Samples: I = dihydroergocornine; II = dihydro-α-ergocryptine; III = dihydroergocristine; IV = dihydro-β-ergocryptine.

Fig. 2. Chromatograms of the dihydro ergot alkaloids. Mobile phase; acetonitrile–$5 \cdot 10^{-3}$ *M* aqueous TBAOH (45:55); flow-rate, 0.8 ml/min; chart speed, 1.5 cm/min; injected sample, 1.56 μg of dihydro ergot alkaloids in 10 μl; λ = 280 nm, 0.2 a.u.f.s. Compounds as in Fig. 1.

high-performance liquid chromatography (HPLC)[8] at a flow-rate of 2.2 ml/min, it is half that obtained with the usual LC methods[6].

Research Group for Analytical Chemistry of the Hungarian Academy of Sciences, P.O. Box 158, H-8201 Veszprém (Hungary)

ELISABETH PAPP

1 M. Popl, Le Duy Ky and J. Strnadová, *J. Chromatogr. Sci.*, 23 (1985) 95.
2 R. V. Vivilecchia, R. L. Cotter, R. J. Limpert, N. Z. Thimot and J. N. Little, *J. Chromatogr.*, 99 (1974) 407.
3 J. Dolinar, *Chromatographia*, 10 (1977) 364.
4 L. Szepesy, I. Fehér, G. Szepesi and M. Gazdag, *Magy. Kém. Foly.*, 84 (1978) 375.
5 S. L. Ali and T. Strittmatter, *Int. J. Pharm.*, 4 (1979) 111.
6 V. Hartmann, M. Rödiger, W. Ableidinger and H. Bethke, *J. Pharm. Sci.*, 67 (1978) 98.
7 P. Spiegl and H. Viernstein, *J. Chromatogr.*, 294 (1984) 452.
8 J. P. Chervet and D. Plas, *J. Chromatogr.*, 295 (1984) 282.

(First received July 10th, 1989; revised manuscript received September 11th, 1989)