Journal of Chromatography, 508 (1990) 229–231
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 294

Note

Separation of spermidine derivatives by reversed-phase high-performance liquid chromatography using a UV-absorbing counter ion

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(First received September 20th, 1989; revised manuscript received December 18th, 1989)

Polyamines are cationic substances with a wide distribution in living organisms. Although on the cellular level their functions are, at least in part, still obscure[1], they are essential for the growth, proliferation and differentiation of cells[1,2]. *In vitro* they are known to interact with DNA.

In the course of our synthetic studies on spermidines[3,4], we required the simplest method possible for establishing the purity of the final products. This paper describes a convenient isocratic chromatographic system that is particularly useful for the analysis of acetylated spermidines. It exploits a UV-absorbing counter ion and indirect detection[5]. Consequently, no derivatization is involved.

Several sensitive procedures are already available for the analysis of spermidine and its acetylated derivatives in physiological samples[6]. A well known procedure[7,8] exploits *n*-octanesulphonate as the ion-pairing agent and *o*-phthalaldehyde for post-column derivatization. UV-absorbing counter ions have been widely used for the separation and detection of amines. However, to our knowledge they have not been applied to polyamines, probably because detection based on fluorescence gives a higher sensitivity. In the present instance, however, simplicity was more important than very high sensitivity.

EXPERIMENTAL

The acetylated spermidines originated from our laboratory[4]. The ethyl derivatives were obtained by reduction with Red-Al of the corresponding acetyl compounds. All other amines were commercial samples.

The isocratic mobile phases used were phosphate buffers prepared from orthophosphoric acid and sodium dihydrogenphosphate (ionic strength 0.01 *M*, pH 2.0), with ethanol as modifier. 2-Naphthalenesulphonate was added at a concentration of 0.0004 *M*.

The liquid chromatographic system used consisted of two Constametric pumps

0021-9673/90/$03.50

(one should be sufficient), an M 1601 Gradient Master, a Spectromonitor III and a Rheodyne Model 7125 injector.

The columns (150 × 4.6 mm I.D.) were obtained from Polymer Labs. (PLRP-S, 100 Å, 5 μm) and Phase Separations (Spherisorb, ODS-2, 10 μm).

The solvent reservoirs, the injection device and the separation columns were carefully thermostated at 25.0 ± 0.1°C in a water-bath.

RESULTS AND DISCUSSION

The separation of three acetylated spermidines on the PLRP-S column is shown in Fig. 1. The three peaks appear with k' values between 2 and 4 and α values of about 1.3. The baseline is satisfactory but careful thermostating is essential. Qualitatively similar results could be obtained on the Spherisorb column but in this case the selectivity was lower ($\alpha < 1.2$). The compounds appeared in the same order. On this column less ethanol was required (2.5%).

The separation of N^1- and N^8-ethylspermidine proved much more difficult than that of the acetylated derivatives. At higher ethanol concentrations with the compounds eluting before the system peaks, no separation of these isomers was obtained on either column. Spermidine itself was, however, easily separated from these ethyl analogues and appeared earlier in the chromatograms. At lower ethanol concentrations with the compounds eluting after the system peaks, separation was only obtained on the Spherisorb column (0.9% ethanol, $k'_{SP} = 12.6$, $k' = 19.7$–21.5, $\alpha = 1.08$). In this way we could prove that both of the ethylspermidines were pure isomers.

As an additional demonstration of the usefulness and convenience of the described procedure, the separation of the underivatized homologous diamines putrescine, cadaverine and hexamethylenediamine was undertaken. On a C_{18} column the peaks appeared in order of increasing hydrophobicity, as demonstrated in Fig. 2.

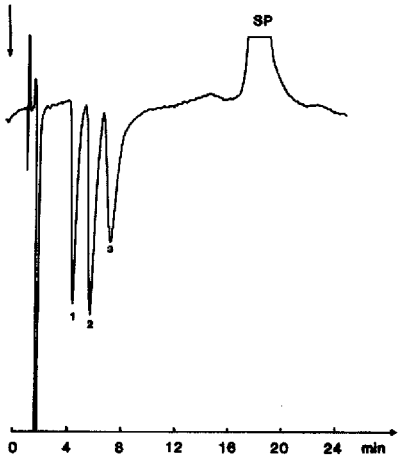

Fig. 1. Separation of three monoacetylated spermidines. Mobile phase, 0.01 *M* phosphate buffer (pH 2.0)–ethanol (94:6); counter ion, 4×10^{-4} *M* 2-naphthalenesulphonate; support, PLRP-S, 100 Å, 5 μm; flow-rate, 1.0 ml/min; detection wavelength, 254 nm at 0.1 a.u.f.s.; temperature, 25.0°C. Peaks: 1 = N^4-acetyl-, 2 = N^1-acetyl- and 3 = N^8-acetylspermidine; SP = system peak.

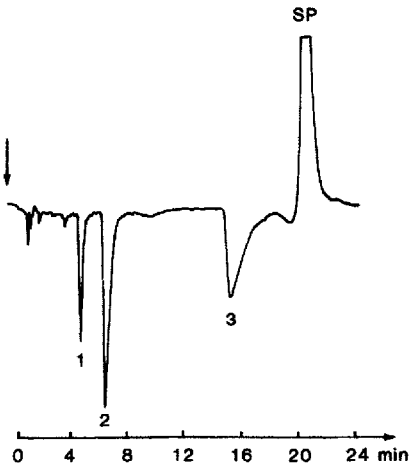

Fig. 2. Separation of (1) putrescine, (2) cadaverine and (3) hexamethylenediamine. Mobile phase, 0.01 *M* phosphate buffer (pH 2.0) alone; counter ion, 4×10^{-4} *M* 2-naphthalenesulphonate; support, Spherisorb C_{18}, ODS-2, 10 μm; flow-rate, detection wavelength and temperature as in Fig. 1. SP = system peak.

The procedure described is suitable for the detection of a variety of di- and polyamines and our aim was to keep it as simple as possible. It is primarily intended for qualitative work when extreme sensitivity is not required. With respect to the sensitivity of the system, it can be stated that 30–50 pmol of N-acetyl- or N-ethylspermidine are easily detected, which is satisfactory in a synthetic context. The detection limit, however, is lower.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Swedish Natural Science Research Council and by a scholarship (to M.L.S.A.) from the Swedish Institute. M.L.S.A. also acknowledges leave of absence from the University of Porto, Portugal.

REFERENCES

1 C. W. Tabor and H. Tabor, *Annu. Rev. Biochem.*, 53 (1984) 749.
2 O. Heby, *News Physiol. Sci.*, 1 (1986) 12.
3 M. Lurdes S. Almeida, L. Grehn and U. Ragnarsson, *J. Chem. Soc., Perkin Trans. 1*, (1988) 1905.
4 M. Lurdes S. Almeida, L. Grehn and U. Ragnarsson, *Acta Chem. Scand.*, 43 (1989) 990.
5 G. Schill and J. Crommen, *Trends Anal. Chem.*, 6 (1987) 111.
6 N. Seiler, *J. Chromatogr.*, 379 (1986) 157.
7 N. Seiler and B. Knödgen, *J. Chromatogr.*, 221 (1980) 227.
8 C. Löser, U. Wunderlich and U. R. Fölsch, *J. Chromatogr.*, 430 (1988) 249.