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Note

High-speed liquid chromatography of alkaloids. II

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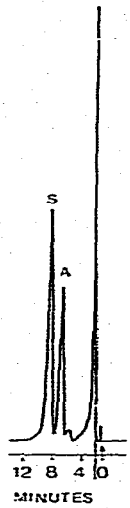
In Part I[1], we described the separation and identification of a number of high-molecular-weight alkaloids by means of high-speed liquid chromatography in neutral solvent systems on a silica gel (5 μm) column. Two pairs of the alkaloids investigated, serpentine–alstonine and strychnine–brucine, however, were not eluted within a reasonable time or were not separated. For serpentine–alstonine, we were not able to obtain a satisfactory separation by means of either thin-layer or gas–liquid chromatography, in the latter case because of the instability of the alkaloids. Because of the anhydronium character of these two alkaloids, tailing was observed in neutral solvent systems in high-speed liquid chromatography. The addition of 1% of diethylamine to diethyl ether–methanol (7 + 3) solvent, however, reduced the tailing and led to a good separation (Fig. 1).

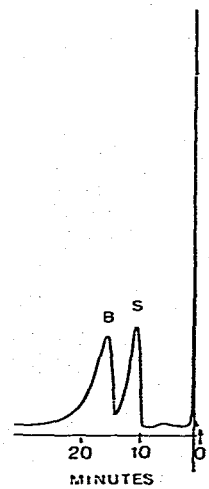
Strychnine and brucine were eluted with reasonable retention times in solvent systems containing chloroform and methanol, but were not separated. In systems containing diethyl ether and methanol, a separation was obtained, but with high retention times and much tailing (Fig. 2). The addition of 1% of diethylamine to diethyl ether–methanol (9 + 1) reduced the tailing considerably and the alkaloids were eluted much faster (Fig. 3).

The addition of diethylamine to neutral solvent systems in the high-speed liquid chromatographic analysis of alkaloids such as strychnine–brucine and serpentine–alstonine results in better separations by reducing the tailing and shortening the retention times. Further, for the liquid chromatographic analysis of other alkaloids, the addition of alkali to the solvent systems may be advisable.

EXPERIMENTAL

The analyses were carried out on a Packard Model 8200 liquid chromatograph equipped with a UV detector for the wavelengths 254 nm and 280 nm. A stainless-steel column, 30 cm × 2 mm I.D., filled with Merckosorb Si 60 (5 μm), was used. The oven temperature was maintained at 20°. Strychnine and brucine were analyzed at a flow-rate of 1.36 ml/min (at a pressure of 165 kg/cm^2), the detection being carried out at a wavelength of 254 nm. The solvent system was diethyl ether–methanol (9 + 1) containing 1% of diethylamine (all pro analysi grade chemicals). The retention times were 1.5 min for strychnine and 2.4 min for brucine. Alstonine and serpentine were analyzed at a flow-rate of 1 ml/min (at a pressure of 175 kg/cm^2), the de-





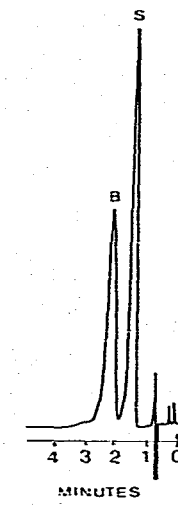


Fig. 1. Liquid chromatogram of alstonine (A) and serpentine (S) on Merckosorb Si 60 (5 μm) using diethyl ether–methanol (7 + 3) containing 1% of diethylamine. Flow-rate, 1 ml/min; detection, UV at 254 nm.

Fig. 2. Liquid chromatogram of strychnine (S) and brucine (B) on Merckosorb Si 60 (5 μm) using diethyl ether–methanol (1 + 1). Flow-rate, 1.15 ml/min; detection, UV at 254 nm.

Fig. 3. Liquid chromatogram of strychnine (S) and brucine (B) on Merckosorb Si 60 (5 μm) using diethyl ether–methanol (9 + 1) containing 1% of diethylamine. Flow-rate, 1.36 ml/min; detection, UV at 254 nm.

tection being carried out at a wavelength of 254 nm. The solvent system was diethyl ether–methanol (7 + 3) containing 1% of diethylamine. The retention times were 6.3 min for alstonine and 8.2 min for serpentine.

REFERENCES

1 R. Verpoorte and A. Baerheim Svendsen, *J. Chromatogr.*, 100 (1974) 227.