Journal of Chromatography, 238 (1982) 250–252
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,614

Note

Ready separation of ergocornine, α-and β-ergocryptine by high-performance liquid chromatography

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(Received December 9th, 1981)

In recent years there has been increasing interest in the therapeutic use of ergot alkaloids. This has posed serious analytical problems and high-performance liquid chromatography (HPLC) has played an important rôle in solving them. One of the most delicate analytical problems in this field is the separation of the components of the ergotoxine and dihydroergotoxine groups, respectively: ergocornine (I), α and β-ergocryptine (II, III) and ergocristine (IV) or their dihydro derivatives (Ia–IVa).

Most workers dealing in this area have used reversed-phase HPLC systems: chemically bonded C_{18} or C_8 phases with buffered mixtures of water and acetonitrile or methanol. With slightly alkaline or neutral eluents, only partial separation of the components has been achieved. Vivilecchia *et al.*[1] separated Ia, IIa + IIIa and IVa using a mixture of acetonitrile and 0.01 *M* aqueous ammonium carbonate, while Dolinar[2], Szepesy *et al.*[3] and Fankel and Slad[4], using similar mixtures, and Hartman *et al.*[5], using a mixture of methanol and aqueous ammonium acetate, have achieved partial separation of the isomeric ergocryptines. The complete separation of the four components has been achieved by Hartmann *et al.*[5] and Ali and Strittmatter[6] with the aid of strongly-alkaline eluents (mixtures of water, acetonitrile and tri- or diethylamine with pH values above 12). Szepesi *et al.*[7] successfully used chromatography on silica for the separation of several ergot alkaloids, among them the above mentioned derivatives.

The aim of this paper is to describe a reversed-phase HPLC system enabling the separation of ergocornine, α- and β-ergocryptine by using a neutral eluent.

EXPERIMENTAL

A Hewlett-Packard 1010B high-pressure liquid chromatograph was used equipped with a 1030B variable-wavelength UV detector and Valco loop injector.

The separation was carried out at ambient temperature, using a column (25 cm × 4.6 mm) of LiChrosorb RP-18, 10 μm (Chrompak, Middelburg, The Netherlands), and tetrahydrofuran–0.01 *M* aqueous ammonium acetate (4:6) as the eluent at a flow-rate of 1.5 ml/min. The chromatograms were monitored at 280 nm in the case of the dihydroergotoxine series and at 322 nm in the case of the ergotoxine series.

The samples (methanesulphonates, free bases or dried extracts of fermentation liquors) were dissolved in the eluent and 5-μl portions of the solutions thus obtained were injected into the chromatograph.

0021-9673/82/0000–0000/$02.75

TABLE I

SEPARATION OF ERGOTOXINES AND DIHYDROERGOTOXINES

Compound		*Capacity factor, k'*
I	Ergocornine	4.22
Ia	Dihydroergocornine	4.38
II	α-Ergocryptine	5.00
IIa	Dihydro-α-ergocryptine	5.44
III	β-Ergocryptine	5.78
IIIa	Dihydro-β-ergocryptine	5.91

RESULTS AND DISCUSSION

Although the strongly alkaline eluent described for the separation of the four components of ergotoxines[5] has been claimed not to be as corrosive as might have been expected[8] and the method based on it seems to be generally accepted, it is still reasonable to search for neutral eluents assuring longer column lifetimes. After having tried a number of eluents we have found that the alkaline eluent of Hartmann

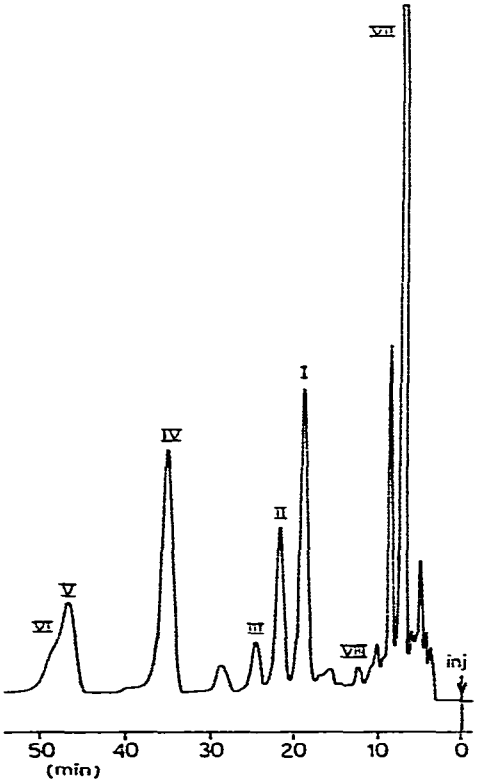

Fig. 1. Chromatogram of the fermentation liquor in the production of ergocornine–ergocryptine. For details see Experimental. Peaks: I = ergocornine; II = α-ergocryptine; III = β-ergocryptine; IV = ergocorninine; V = α-ergocryptinine; VI = β-ergocryptinine; VII = ergometrine; VIII = ergometrinine.

et al.[5] really affords the only possibility to separate the four components. However, tetrahydrofuran–0.01 *M* aqueous ammonium acetate (4:6) is suitable for the complete separation of ergocornine, α and β-ergocryptine (ergocristine is eluted together with β-ergocryptine). As is seen from the data of Table I, this system is suitable for the solution of any analytical problems where the task is the separation of I, II and III or Ia, IIa and IIIa.

We have routinely used this system for the estimation of I, II and III in fermentation liquors where IV (ergocristine) is not present. Fig. 1 shows the chromatogram of an extract of a typical fermentation liquor.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. K. Zalai for valuable discussions and Mrs. M. Melegh for technical assistance.

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