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SEPARATION OF ERGOTOXINE ALKALOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON SILICA

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SUMMARY

Separation of ergot alkaloids on silica packing was studied, using different mixtures of hexane, chloroform and acetonitrile as eluent. It was found that an improved separation of ergocornine, α- and β-ergocryptine as well as of their "inine" isomers can be achieved by using an appropriate eluent composition. The four stereoisomers of ergocristine (ergocristinine, aci-ergocristinine, ergocristine and aci-ergocristine) can also be separated. Because the "inine" isomers are eluted first and the elution order of ergotoxine alkaloids is opposite to that obtained in reversed-phase systems, the application of the method gives an easy quantitation of "inine" isomers and α- and β-ergocryptine in plant extracts and fermentation products. Increasing the polarity of the eluent by adding methanol results in a rapid and simple method for the group separation of ergot alkaloids.

INTRODUCTION

In the last few years, high-performance liquid chromatography (HPLC) has become the basic method for the analysis of ergot alkaloids. Great efforts have been made to find the most suitable system for complete separation of all ergot and dihydroergot alkaloids. Both partition and adsorption chromatography have been investigated for this purpose, but an incomplete separation was found for ergotoxine and dihydroergotoxine alkaloids on silica packings[1,2], and in our earlier paper a similar conclusion was reported[3]. Thus reversed-phase chromatography is preferred, and the chemically bonded octadecyl silica stationary phase with acetonitrile–aqueous 0.01 *M* ammonium carbonate as eluent has been generally applied[3–10]. Considerable progress was achieved by Hartmann *et al.*[11] using a microparticulate reversed phase and water–acetonitrile–triethylamine (32:8:1) eluent mixture for the separation of four components of dihydroergotoxine (dihydroergocristine, dihydroergocornine, dihydro-α-ergocryptine and dihydro-β-ergocryptine).

In this paper we report the use of a microparticulate silica and hexane–chloroform–acetonitrile eluent mixture for the separation of ergot alkaloids. The aim of our work was the complete separation of stereo- and structural isomers of ergotoxine alkaloids on silica and to elaborate the optimal conditions for their determination in plant extracts and fermentation products.

EXPERIMENTAL

Chromatography

Liquid chromatography was performed on a Varian Model 8500 liquid chromatograph. It was operated with a stop-flow injector and a variable-wavelength Variscan 635 UV spectrophotometric detector at 320 nm. The columns (25 cm × 4.6 mm I.D.) were packed with LiChrosorb SI-60 (5 μm and 10 μm) (Pierce, Rotterdam, The Netherlands).

Reagents

All solvents used were of HPLC grade and were obtained from E. Merck (Darmstadt, G.F.R.). The compounds to be separated were prepared at the Chemical Works of Gedeon Richter (Budapest, Hungary) and were considered to be of the highest available purity.

RESULTS AND DISCUSSION

Recently, adsorption chromatography had been considered to be ineffective for the separation of ergotoxine alkaloids (ergocristine, ergocornine, α-ergocryptine, β-ergocryptine), because of their virtually identical retentions in the systems previously investigated. Although their stereoisomers (so-called "inine" isomers) are well separated from the active compounds, there was no separation between the individual isomers. This limitation of adsorption chromatography can be eliminated.

The analytical tasks can be divided into three parts; the separation of the individual components of ergotoxine alkaloids as well as that of their "inine" isomers; the determination of the optimal conditions for their estimation in plant extracts and fermentation products; and the achievement of an improved separation for those cases where group separation is required (separation of ergotoxine alkaloids from more polar compounds than ergotamine and ergometrine).

The investigations were carried out with hexane–chloroform–acetonitrile eluent mixtures, LiChrosorb SI-60 stationary phase. To determine the optimum separation system, the effects of the silica particle size and of the eluent composition on the retention behaviour of ergot alkaloids were examined. Table I shows the capacity ratios, k', and separation factors, α measured. It can be seen that ergocornine, α-ergocryptine and β-ergocryptine are satisfactorily separated using hexane–chloroform–acetonitrile (56:22:22). A partial separation can be achieved for ergocornine and ergocristine. This fact, however, is of little importance, because ergocornine does not occur together with ergocristine in analytical practice.

From the results obtained it can be concluded, that both 5-μm and 10-μm silica packings can be used well for the separation. The separation of ergocornine, α- and β-ergocryptine as well as of their "inine" isomers is illustrated in Fig. 1.

Using the above optimal eluent composition for the separation, the percentage of the active compounds and contaminants in plant extracts and fermentation products as well as in intermediates and pharmaceutical products can be determined. Fig. 2A shows the separation of ergotoxine alkaloids in "ergocristine-rich" plant extract. Not only the α-ergocryptine impurity but also β-ergocryptine in trace amount can be recognized. Fig. 2B shows the composition of a raw fermented product. In

TABLE I

CAPACITY RATIOS (k') AND SEPARATION FACTORS (α) FOR ERGOT ALKALOIDS ON SILICA PACKINGS WITH DIFFERENT ELUENTS

Eluents: A = hexane–chloroform–acetonitrile (60:25:15); B = hexane–chloroform–acetonitrile (56:22:22); C = hexane–chloroform–acetonitrile (55:20:25); D = hexane–chloroform–acetonitrile–methanol (55:20:25:3).

Column, LiChrosorb SI-60 (5 μm); flow-rate, 100 cm³/h.

Substance	*A*		*B*		*C*		*D*	
	k'	α	k'	α	k'	α	k'	α
β-Ergocryptinine	2.04	1.24	1.00	1.08	0.95	1.05	0.77	
α-Ergocryptinine	2.54		1.08	1.22	1.00		0.77	
Ergocristinine		1.28	1.32	1.06		1.22	0.77	
Ergocorninine	3.24		1.40	1.99	1.22		0.77	1.31
Ergotaminine		2.31	2.78	1.18		2.25	1.01	1.20
β-Ergocryptine	7.48	1.24	3.29	1.12	2.75	1.12	1.21	
α-Ergocryptine	9.28		3.67		3.09		1.21	
Ergocristine		1.16	4.78	1.20		1.15	1.21	
Ergocornine	10.80		4.40		3.54		1.21	1.74
Ergometrinine				4.67			2.11	1.19
Ergotamine			20.54				2.52	2.32
Ergometrine							5.84	

Column, LiChrosorb SI-60 (10 μm); flow-rate, 60 cm³/h.

Substance	*A*		*B*		*C*	
	k'	α	k'	α	k'	α
β-Ergocryptinine	2.59	1.13	0.88	1.14	0.86	1.09
α-Ergocryptinine	2.93		1.00	1.14	0.94	
Ergocristinine		1.31	1.14	1.12		1.32
Ergocorninine	3.85		1.28	1.45	1.25	
Ergotaminine		2.64	1.86	1.40		2.03
β-Ergocryptine	10.20	1.13	2.60	1.15	2.54	1.06
α-Ergocryptine	11.51		2.98	1.11	2.68	
Ergocristine		1.19	3.32	1.05		1.16
Ergocornine	13.67		3.50		3.34	

Fig. 3 the separation of ergotoxine alkaloids in "ergocornine–ergocryptine-rich" plant extracts is shown. These plant extracts contain ergocornine, α- and β-ergocryptine in different ratios depending on the origin of the sample. In order to obtain a better separation of "inine" isomers from the impurities, the composition of the eluent was a slightly modified, to hexane–chloroform–acetonitrile (55:20:25).

In connection with the separation problem illustrated in Figs. 2 and 3, it must be noted that the ergotoxine alkaloids should be accompanied by more polar ergot alkaloids. However, because ergotamine usually does not occur in the sample and ergometrine cannot be eluted with the eluent used, these compounds not cause unfavourable baseline shift. Nevertheless, from time to time, the column has to be washed with a more polar solvent to remove the strongly retarded components from the stationary phase.

The long known "inine" isomerization of ergot alkaloids, which takes place

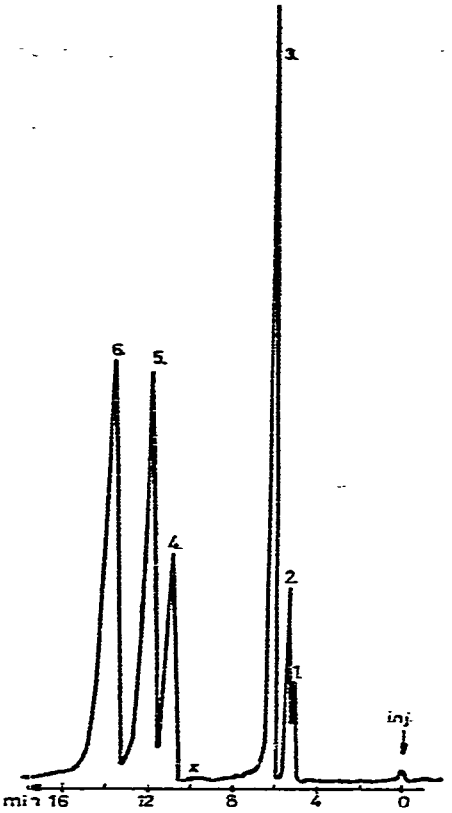


Fig. 1. Separation of ergotoxine alkaloids on silica. Column: LiChrosorb SI-60 (5 μm), 250 × 4.6 mm I.D. Detector: UV, 320 nm. Eluent: hexane–chloroform–acetonitrile (56:22:22), flow-rate 100 cm^3/h. Peaks: 1 = β-ergocryptinine; 2 = α-ergocryptinine; 3 = ergocorninine; 4 = β-ergocryptine; 5 = α-ergocryptine; 6 = ergocornine; x = unknown.

at the C-8 position of the lysergic acid portion, is not the only isomerisation reaction observed. In an acidic medium another reversible acid-catalyzed isomerization reaction of ergot alkaloids, so-called "aci-isomerisation", was described[12]. It represents an epimerization at the C-2′ position of the peptide portion.

Fig. 4 depicts the structures of the four isomers of ergocristine and their separation is shown in Fig. 5.

As in Figs. 1–3, only a partial separation can be achieved for α- and β-ergocryptinine. When the "inine" isomers of ergocornine, α- and β-ergocryptine are to be separated, hexane–chloroform–acetonitrile (60:25:15) can be used as eluent. The complete separation of the three "inine" isomers is illustrated in Fig. 6.

In our earlier paper[3] we published a method for the group separation of all ergot alkaloids. To improve this separation system we attempted to apply the eluent composition used for the separation of ergotoxine alkaloids. Increasing the polarity of the eluent by adding methanol resulted in a rapid and simple separation of ergotoxine alkaloids and more polar ergot alkaloids (Fig. 7). It can be seen that

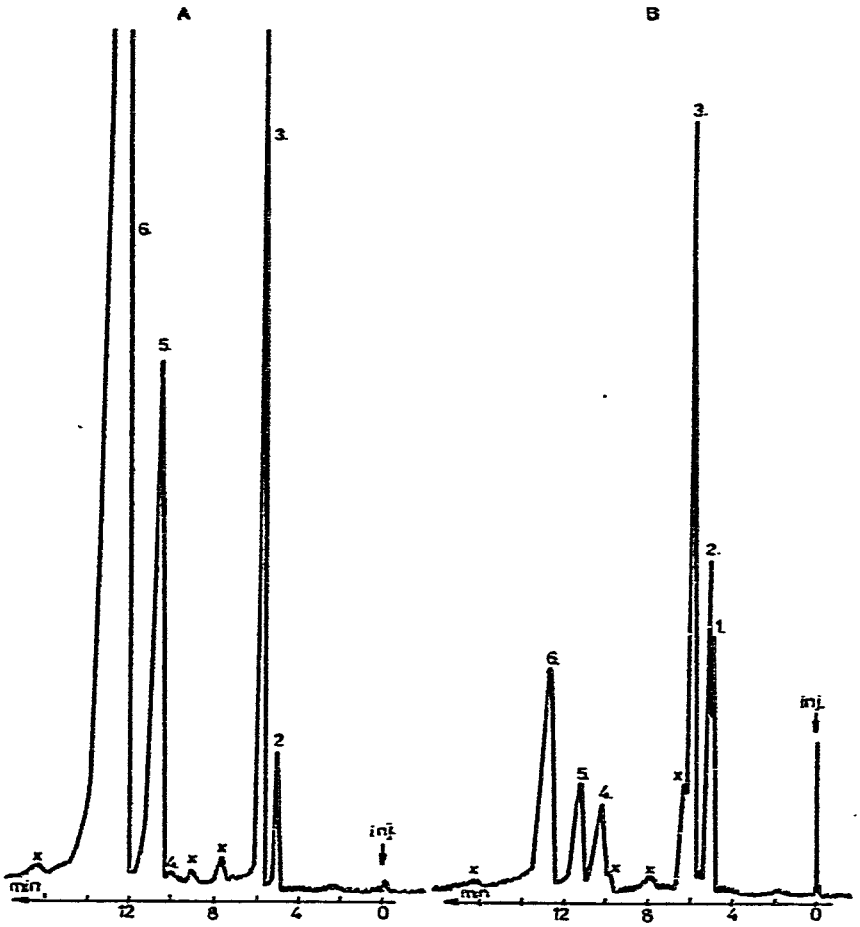


Fig. 2. Chromatograms of "ergocristine-rich" plant extract (A) and raw fermented product (B). Conditions as in Fig. 1. Peaks: A, 2 = α-ergocryptinine; 3 = ergocristinine; 4 = β-ergocryptine, 5 = α-ergocryptine; 6 = ergocristine; x = unknown; B, 1 = β-ergocryptinine; 3 = α-ergocryptinine; 3 = ergocorninine; 4 = β-ergocryptine; 5 = α-ergocryptine; 6 = ergocornine; x = unknown.

the ergotoxine alkaloids (ergocornine, ergocristine, α- and β-ergocryptine) are eluted with virtually identical retentions, hence their total amount can be determined. The stereoisomers of ergotoxine alkaloids (ergocorninine, ergocristinine, α- and β-ergocryptinine) are well separated from the active compounds and again their total amount can be determined. Ergotamine and ergometrine are also well separated from each other, from their "inine" isomers and from ergotoxine and ergotoxinine alkaloids.

CONCLUSIONS

According to our results, the hexane–chloroform–acetonitrile eluent system can be applied for the separation of the individual components of ergotoxine

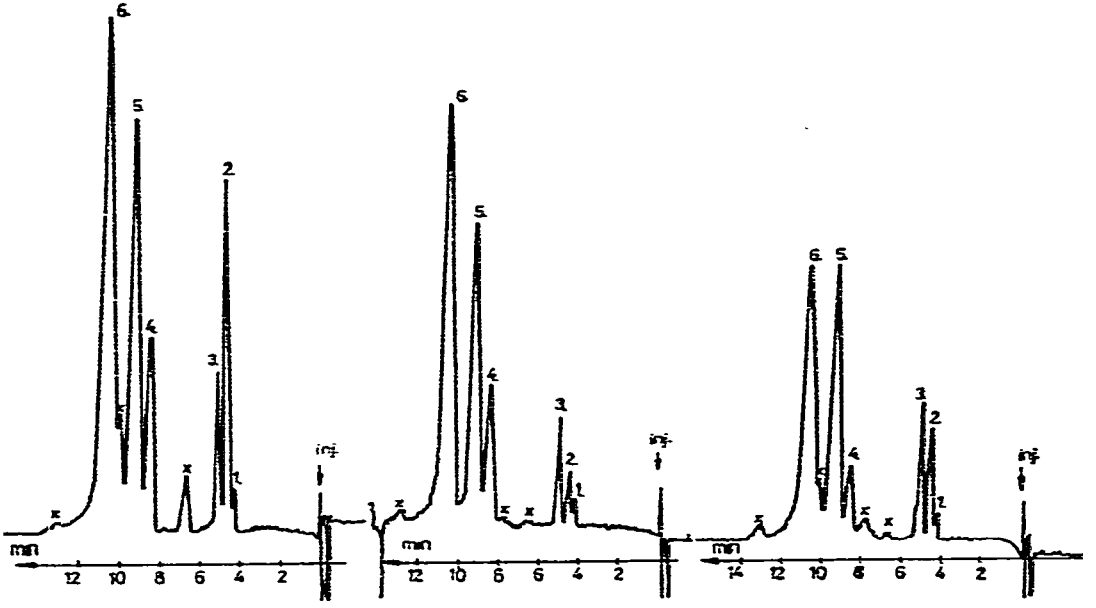


Fig. 3. Chromatograms of "ergocornine–ergocryptine-rich" plant extracts. Eluent: hexane–chloroform–acetonitrile (55:20:25), flow-rate 100 cm³/h. Other Conditions, and identified compounds, as in Fig. 1.

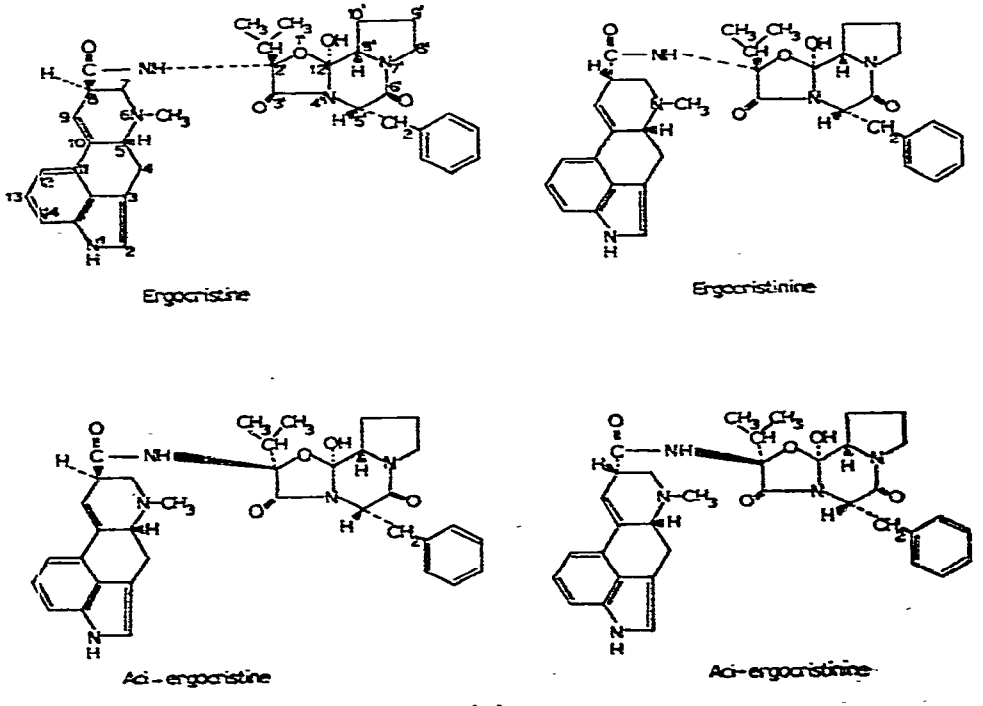


Fig. 4. Structures of four isomers of ergocristine.

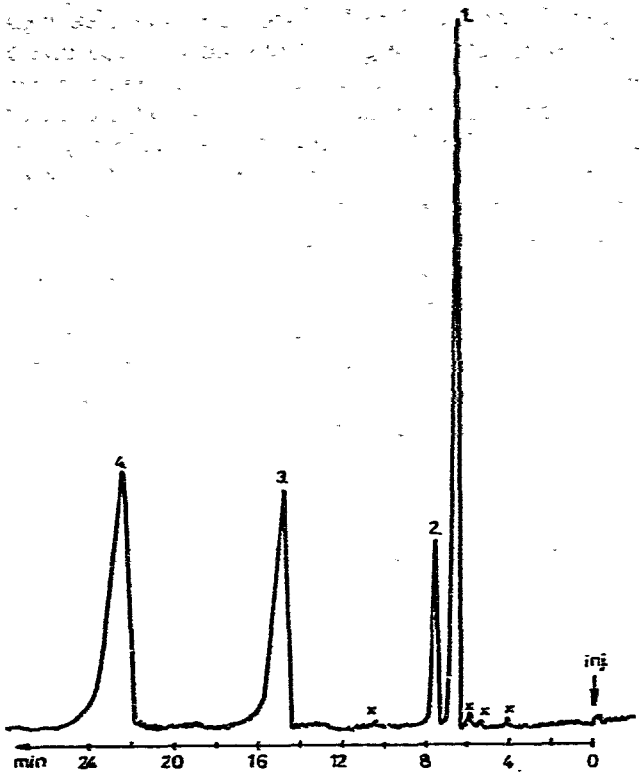
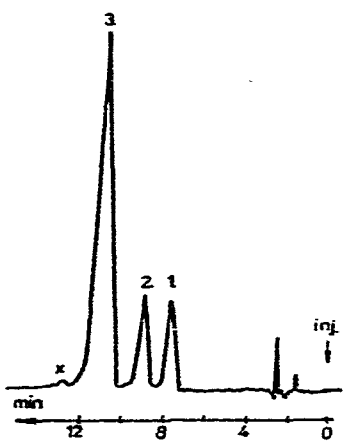


Fig. 5. Separation of four isomers of ergocristine. Conditions as in Fig. 1. Peaks: 1 = ergocristinine; 2 = aci-ergocristinine; 3 = ergocristine; 4 = aci-ergocristine; x = unknown.



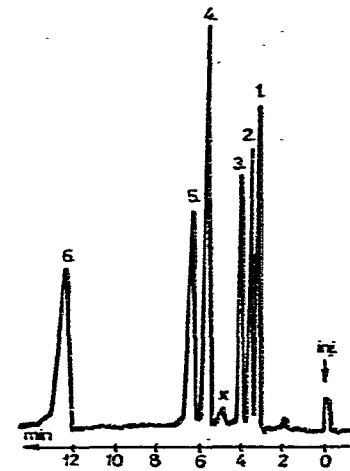


Fig. 6. Separation of "inine" isomers of ergotoxine alkaloids. Eluent: hexane–chloroform–acetonitrile (60:25:15), flow-rate 100 cm³/h. Other conditions as in Fig. 1. Peaks: 1 = β-ergocryptinine; 2 = α-ergocryptinine; 3 = ergocorninine; x = unknown.

Fig. 7. Group separation of ergot alkaloids. Eluent: hexane–chloroform–acetonitrile–methanol (55:20:25:3), flow-rate 100 cm³/h. Other conditions as in Fig. 1. Peaks: 1 = ergocristinine + ergocorninine + α- and β-ergocryptinine; 2 = ergotaminine; 3 = ergocristine + ergocornine + α- and β-ergocryptine; 4 = ergometrinine; 5 = ergotamine; 6 = ergometrine; x = unknown.

alkaloids as well as that of their "inine" isomers. The "aci-isomers" are also well separated. This method has some advantages over the generally used reversed-phase chromatographic systems. First, the "inine" isomers, which are the possible impurities in intermediates, plant extracts and fermentation products, are eluted. This enables their easy quantitation at low concentrations. The change in elution order compared to the reversed-phase system also permits the quantitative determination of β-ergocryptine, because it occurs in lowest concentration in the ergotoxine mixture. Secondly, the chromatographic system is simple and of high stability, as supported by its routine use in our laboratory for years; more than 1000 analyses have been carried out on the same column without any loss in separation efficiency.

However, this method has a limitation. It cannot be used for the analysis of dihydroergotoxine alkaloids. Only a partial separation can be achieved for dihydroergocornine, dihydroergocristine and dihydroergocryptine and there is no separation between dihydro-α-ergocryptine and dihydro-β-ergocryptine.

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