Journal of Chromatography, 149 (1978) 271–280

CHROM. 10,866

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ERGOT ALKALOIDS

LÁSZLÓ SZEPESY and ISTVÁN FEHÉR
Hungarian Oil and Gas Research Institute, Veszprém (Hungary)
and
GÁBOR SZEPESI and MÁRIA GAZDAG
Chemical Works of G. Richter Ltd., Budapest (Hungary)

SUMMARY

In order to develop a general method for the analysis of ergot alkaloids, the application of high-performance liquid chromatography has been investigated.

The different analytical tasks involved can be solved unambiguously on two columns containing different packings (silica and a reversed-phase packing) of high efficiency. The silica packing serves for group separation and the analysis of contaminants. A rapid and simple method is presented for the screening of plant extracts. Separation of the active compounds was accomplished on reversed-phase packings and the influence of the bonded hydrocarbon groups was investigated. By using a high-efficiency microparticulate reversed-phase packing, baseline separation can be achieved under isocratic conditions for the active compounds and also the stereo- and structural isomers can be separated.

INTRODUCTION

The qualitative and quantitative analysis of ergot alkaloids and hydrogenated ergot alkaloids has been an important task in pharmaceutical analysis for several years, and considerable interest has been shown recently in improving the efficiency and accuracy of these analyses.

In the past, paper and thin-layer chromatography were used for the separation of ergot alkaloids[1–9]. Quantitative analysis of the separated alkaloids was accomplished by UV or visible-light spectrophotometry, densitometry, fluorimetry or fluorodensitometry. These planar chromatographic techniques, however, cannot meet the current requirements of separation efficiency and accuracy. For some of the compounds involved only poor separations can be achieved, and the separation of the non-hydrogenated and hydrogenated ergot alkaloids is also inadequate. These techniques are time consuming, and the quantitative analysis can be regarded as satisfactory only when densitometry is used.

Recently, methods have been developed for the analysis of ergot alkaloids by gas chromatography[10,11]. Because of the thermal instability and low vapour pressure

of the ergot alkaloids, direct gas chromatography cannot be applied. However, it was found that the ergot–peptide alkaloids would undergo thermal degradation in a reproducible manner in the injection port of the gas chromatograph and, by separating the peptide degradation products, the parent alkaloids can be identified. This method can be applied, of course, only to peptide alkaloids that contain peptides with different structures.

Considering the characteristics and structures of the ergot alkaloids, high-performance liquid chromatography (HPLC) seems to be the most appropriate method for their separation. The first publications in this field appeared only a few years ago[12–16]. In some of the methods described silica packing[12–13] and in others reversed-phase packings[14–16] were used for the separation. On the packings used and under the operating conditions applied, however, only partial separations of the ergot alkaloids investigated were achieved.

An excellent separation for ergotamine and its closely related derivatives can be achieved on reversed-phase packing too, by using isocratic and gradient systems[17,18].

After the preparation of this paper, an HPLC method was published by Dolinar[9] for the analysis of ergot alkaloids on reversed-phase packings. Our results and conclusions obtained on reversed-phase packings are very similar to those described by Dolinar. Dolinar, however, did not include in his investigation the separation of ergot alkaloids on a silica packing, which can be used for a group separation and the analysis of contaminants.

In the course of the production of pharmaceuticals containing ergot alkaloids, the composition of the feedstocks, intermediates and final pharmaceutical products must be determined.

Ergot alkaloids are produced either by the extraction of plants (*Secale cornutum*) or by a fermentation process. Extraction of plants furnishes ergotamine and ergocristine, whereas the fermentation process furnishes ergocornine, ergocryptine and ergometrine. Both the plant extract and the fermented product contain various active compounds and also many different contaminants (stereo and structural isomers). In Fig. 1 a scheme of the production of ergot alkaloids is shown, which provides a basis for establishing the analytical tasks.

The main task of our investigation was to develop HPLC methods for the general and uniform analysis of ergot alkaloids.

EXPERIMENTAL

Thin-layer chromatography

Thin-layer chromatography was performed on Polygram Sil G_{254} sheets (Macherey, Nagel & Co., Düren, G.F.R.) with the solvent system acetone–0.1 *M* ammonium carbonate solution–ethanol (32.5 : 67.5 : 1).

The spots were located under 254-nm UV light. Quantitative evaluation was carried out by scraping off the spots and performing a specific colour reaction, followed by spectrophotometric determination of the colour intensity[20].

High-performance liquid chromatography

Liquid chromatography was performed using a Varian Model 8500 and a home-built liquid chromatograph.

The Varian Model 8500 was operated with a stop-flow injector and a variable-wavelength Variscan 635 UV spectrophotometric detector.

The home-built apparatus consisted of a Orlita Type M3 S4/4 pump, a Waters U6K injector and a variable-wavelength (200–400 nm) Cecil CE-212 UV detector.

Columns

The columns employed were home-made, of length 250 mm and I.D. 2 and 4 mm.

Packing of the columns was carried out by a modified slurry technique developed in our laboratory[21]. The packings investigated were LiChrosorb SI 60, 10 μm (Merck, Darmstadt, G.F.R.), Silica SI 60, 10 μm (University of Saarbrücken, Saarbrücken, G.F.R.), LiChrosorb RP-2, 5 μm, and LiChrosorb RP-8, 10 μm (Merck), and silica RP-18, 12 and 10 μm (University of Saarbrücken).

Reagents

The chemicals and solvents used were of analytical-reagent grade (Reanal, Budapest, Hungary). All solvents were freshly distilled in glass apparatus, then degassed in an ultrasonic bath before use.

RESULTS AND DISCUSSION

On the basis of Fig. 1, the analytical tasks can be divided into two main groups, one of which is the determination of the percentage of the active compounds and the contaminants in the plant extracts and the fermented products, as well as in the intermediates and pharmaceutical products where a group separation is required.

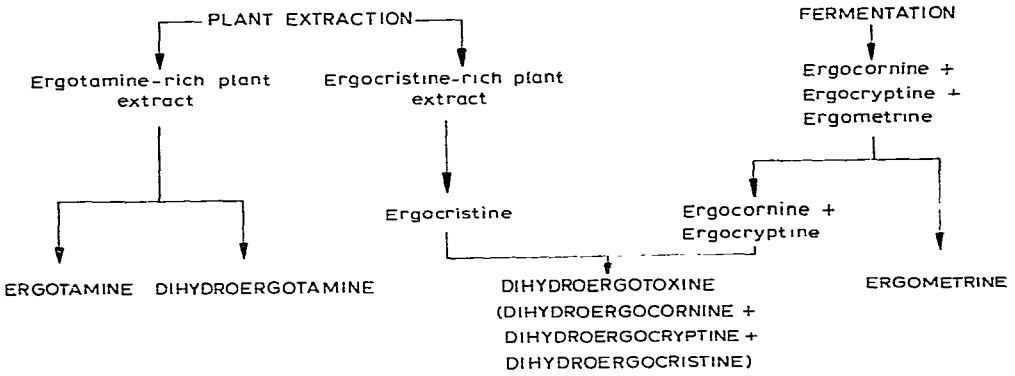

Fig. 1. Scheme of the production of ergot alkaloids.

For this type of separation, silica was found to be a suitable packing. The investigations were carried out with two different silica packings, with chloroform–ethanol (95:5), chloroform–*n*-hexane–ethanol (40:40:10) and chloroform–methanol (95:5) as eluents.

In Table I the capacity ratios (k') measured for the compounds investigated are given.

TABLE I

CAPACITY RATIOS (k') OF ERGOT ALKALOIDS ON SILICA PACKINGS WITH DIFFERENT ELUENTS

Substance	*LiChrosorb SI 60 (n-hexane–chloroform–ethanol, 40:40:10)*	*Silica SI 60 (chloroform–methanol, 95:5)*	*LiChrosorb SI 60 (chloroform–ethanol, 95:5)*
Ergometrine maleate	17.41	—	—
Ergometrinine	10.00	—	—
Ergotamine tartarate	6.27	—	1.80
Ergocornine	1.90	0.60	0.50
Ergocryptine	1.90	0.60	0.50
Ergocristine	1.90	0.60	0.50
Ergotaminine	1.40	0.37	0.28
Ergocorninine	0.93	0.25	0.13
Ergocryptinine	0.93	0.25	0.13
Ergocristinine	0.93	0.25	0.13
Dihydroergocornine methanesulphonate	—	1.75	—
Dihydroergocryptine methanesulphonate	—	1.65	—
Dihydroergocristine methanesulphonate	—	1.50	—

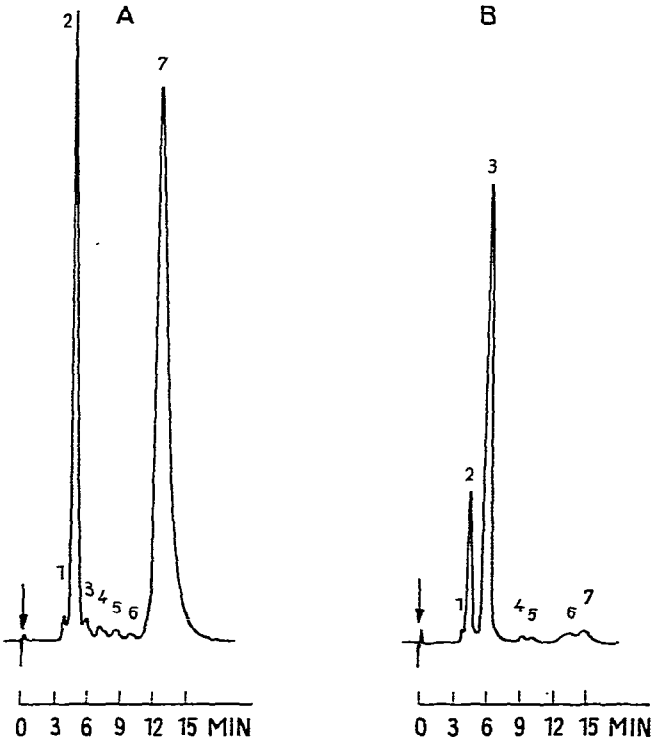

Fig. 2. Chromatograms of two different plant extracts. (A) Ergotamine-rich plant: 1 and 3–6 = impurities; 2 = ergotaminine; 7 = ergotamine. (B) Ergocristine-rich plant: 1 and 4–7 = impurities; 2 = ergocristinine; 3 = ergocristine. Varian Series 8500 liquid chromatograph; detector, UV, 320 nm; column, 250 × 2 mm, LiChrosorb SI 60 (10 μm); eluent, chloroform–ethanol (95:5); flow-rate, 10 $cm^3 \cdot h^{-1}$.

It can be seen that the ergotoxine alkaloids (ergocornine, ergocryptine and ergocristine) are eluted with virtually identical retentions in the systems investigated, *i.e.*, their total amount can be determined. The stereoisomers of the ergotoxine alkaloids (ergocorninine, ergocryptinine and ergocristinine) 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 and from their inin isomers.

The analysis on a silica column can be used for the determination of the active compounds in plant extracts and in the intermediates. In Fig. 2, chromatograms of two different plant extracts are presented.

The quantitative evaluation of the chromatograms was carried out by the internal standard method. In order to achieve good separation and accuracy, for the analysis of ergotamine-containing samples 1-phenyl-2,3-dimethyl-4-dimethylamino-5-pyrazolone and for the analysis of ergocristine-containing samples 2-propylthiocarbamoyl-4-pyridine were used as internal standards. Chromatograms of plant extracts diluted with the internal standard stock solutions are shown in Fig. 3.

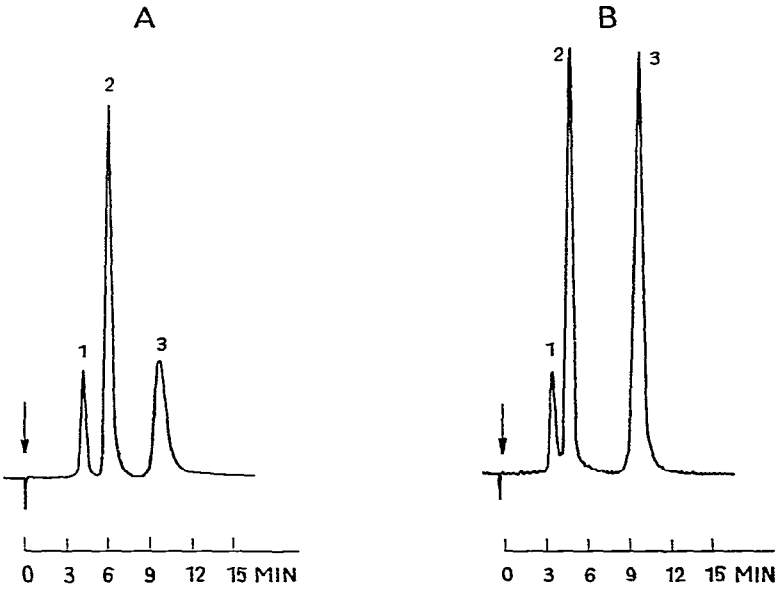

Fig. 3. Analysis of plant extracts by the internal standard method. (A) Ergotamine-rich plant: 1 = ergotaminine; 2 = internal standard; 3 = ergotamine. (B) Ergocristine-rich plant: 1 = ergocristinine; 2 = ergocristine; 3 = internal standard. Operating conditions as in Fig. 2; flow-rate, 15 $cm^3 \cdot h^{-1}$.

A similar analytical task is the determination of ergocornine and ergocryptine, and ergometrine, in the fermented product. The chromatogram of a raw fermented product is shown in Fig. 4.

From the second column in Table I it can also be seen that the hydrogenated ergotoxine alkaloids are well separated from the non-hydrogenated alkaloids and are eluted with virtually identical retentions. Therefore, the contaminants of the intermediates and the final products can also be determined by separation on a silica column.

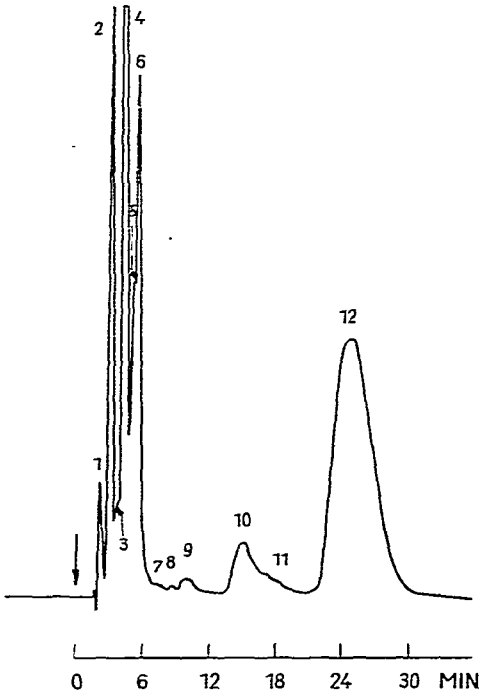

Fig. 4. Chromatogram of a fermentation raw product: 1, 5–8 and 11 = *impurities*; 2 = ergocorninine + ergocryptinine; 3 = ergotaminine; 4 = ergocornine + ergocryptine; 10 = ergometrinine; 12 = ergometrine. Operating conditions as in Fig. 2. Eluent, *n*-hexane–chloroform–ethanol (40:40:10); flow-rate, 20 $cm^3 \cdot h^{-1}$.

In order to separate the individual ergotoxine alkaloids and to determine their highly polar contaminants, reversed-phase packings were used. By using bonded-phase packings containing C_2, C_8 and C_{18} alkyl groups, the effect of chain length on the separation characteristics was investigated.

The results obtained for the reversed-phase packings are given in Table II. It can be seen that within a given group of compounds, the individual compounds are well separated, even the ergocryptine–ergocristine and dihydroergocryptine–dihydroergocristine pairs. The relative amounts of the individual components were determined at 320 nm in the ergotoxine samples and at 280 nm in the dihydroergotoxine samples. Only a partial separation between the ergotoxine and dihydroergotoxine alkaloids can be achieved, and for this reason the determination of ergotoxine contaminants in dihydroergotoxine products can be accomplished more precisely on a silica column.

The results in Table II show that by increasing the chain length of the bonded alkyl group on the silica surface, the capacity ratio and to some extent also the selectivity of the packing increase for the compounds investigated. The best resolution was achieved on the RP-18 packing for both the ergotoxine and dihydroergotoxine samples. The separation of the ergotoxine alkaloids is shown in Fig. 5 and that of the dihydroergotoxine alkaloids in Fig. 7c. It can be seen that under the operating

TABLE II

CAPACITY RATIOS (k') AND SEPARATION FACTORS (α) FOR ERGOT ALKALOIDS ON REVERSED-PHASE PACKINGS

Eluent: acetonitrile–0.01 *M* ammonium carbonate solution (2:3).

Substance	*LiChrosorb RP-2*		*LiChrosorb RP-8*		*Silica RP-18*	
	k'	α	k'	α	k'	α
Lysergic acid	0.16		0.20		0.10	
Ergometrine maleate	1.54	1.47	1.11	2.11	0.81	2.27
Ergometrinine	2.27	2.35	2.34	3.21	1.84	3.95
Ergotamine tartarate	5.34	1.17	7.51	1.46	7.27	1.63
Ergocornine	6.24	1.25	11.00	1.37	11.86	1.57
Ergocryptine	7.8	1.08	15.14	1.10	18.65	1.10
Ergocristine	8.45	1.53	16.66	1.75	20.50	1.86
Ergotaminine	9.87	1.12	—	1.47	29.6	1.58
Ergocorninine	12.91		29.17		38.2	
Ergocryptinine	14.50		43.06		60.6	
Dihydroergotamine tartarate	—		—		7.94	
Dihydroergocornine methanesulphonate	7.15	1.27	9.43	1.44	9.7	1.50
Dihydroergocryptine methanesulphonate	9.11	1.08	13.60	1.10	14.6	1.20
Dihydroergocristine methanesulphonate	9.82		14.91		17.60	

conditions used, baseline separation can be accomplished for all components under isocratic operation.

A special task emerged in connection with the ergocornine–ergocryptine samples obtained from the fermented product, involving the separation of α- and β-ergocryptine. A thin-layer chromatographic technique was formerly developed for this task and furnished well separated spots of the α- and β-isomers on a silica layer[20]. Experiments were carried out to transfer this separation with the same solvent system to a column, but without success. It seems that the separation on the layer proceeds according to a liquid–liquid partition mechanism as the silica layer is covered with

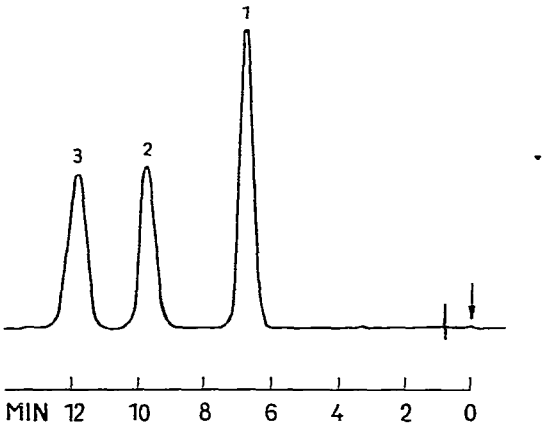

Fig. 5. Separation of ergotoxine alkaloids: 1 = ergocornine; 2 = ergocryptine; 3 = ergocristine. Apparatus, home-made; detector, UV, 320 nm; Column, 250 × 4 mm, silica RP-18 (10 μm); eluent, 40 vol.-% acetonitrile in 0.01 *M* ammonium carbonate solution; flow-rate, 130 $cm^3 \cdot h^{-1}$; P = 105 atm.

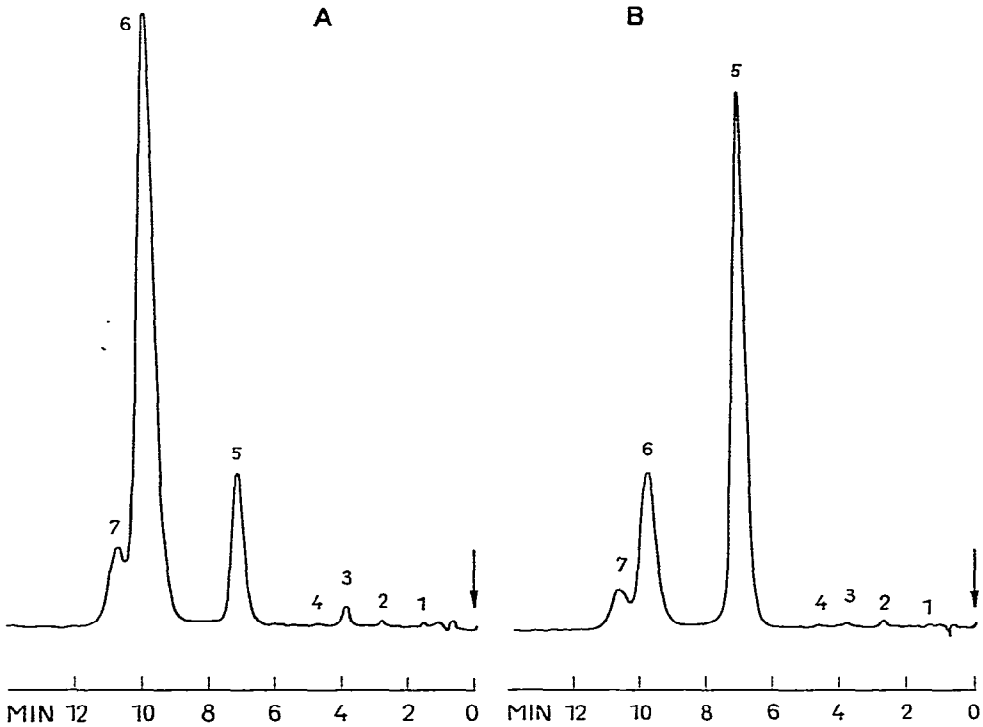

Fig. 6. Separation of α- and β-ergocryptine. (A) Ergocryptine-rich sample: 1–4 = impurities (1.0%); 5 = ergocornine (15.0%); 6 = α-ergocryptine (72.8%); 7 = β-ergocryptine (11.2%). (B) Ergocornine-rich sample: 1–4 = impurities (0.8%); 5 = ergocornine (68.2%); 6 = α-ergocryptine (25.7%); 7 = β-ergocryptine (5.3%). Operating conditions as in Fig. 5.

TABLE III

COMPARISON OF DIFFERENT CHROMATOGRAPHIC TECHNIQUES FOR THE ANALYSIS OF DIHYDROERGOTOXINE METHANESULPHONATE

Substance	*TLC*		*GLC*		*HPLC*	
	R_F value	*Composition (%)*	*Retention time (min)*	*Composition (%)*	*Retention time (min)*	*Composition (%)*
Dihydroergocornine methanesulphonate	0.55	36.66	9.0	36.82	4.9	36.86
Dihydroergocryptine methanesulphonate	0.47	31.00	10.0	30.95	7.2	30.74
Dihydroergocristine methanesulphonate	0.38	32.34	14.6	32.23	8.5	32.39
Relative standard deviation (%)		±3.5		±1.5		±1.3
Analysis time (min)		180		20		15
Sample required (μg)		100		45		25

the most volatile component of the solvent system, *i.e.*, acetone, through the vapour space. In the column, however, the silica packing is in equilibrium with the water–acetone solvent system and no separation can be achieved. This indicates at the same time that, contrary to some statements in the literature, thin-layer chromatographic separations cannot always be transferred to columns, at least when multi-component solvent systems are used.

It was found that by using a well packed high-efficiency column, the α- and β-ergocryptines can be separated on a reversed-phase RP-18 packing. By using more efficient columns (smaller particle size) even better separations can be achieved. The separation of two samples of different compositions is shown in Fig. 6.

Finally, the results obtained for the determination of the composition of a dihydroergotoxine methanesulphonate product by using different chromatographic techniques are shown in Table III. It can be seen that the analysis of the product investigated can be accomplished most quickly and with the highest precision by HPLC. The chromatograms obtained by the three different techniques are presented in Fig. 7.

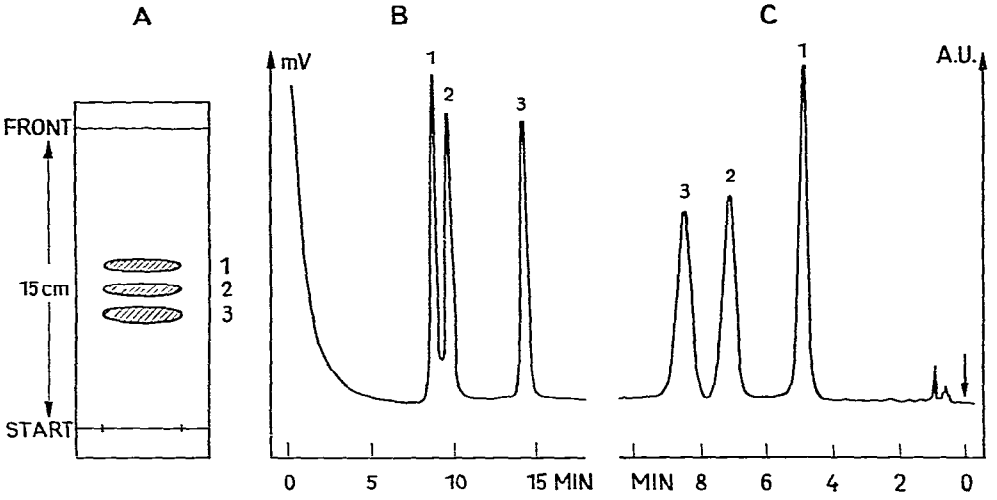

Fig. 7. Analysis of dihydroergotoxine methanesulphonate by different chromatographic techniques. 1 = Dihydroergocornine methanesulphonate; 2 = dihydroergocryptine methanesulphonate; 3 = dihydroergocristine methanesulphonate. (A) TLC: Polygram Sil G sheet; eluent, acetone–0.1 *M* ammonium carbonate solution–ethanol (32.5:67.5:1). (B) GLC: column, 1 m × 3.2 mm, 2% Dexsil 300 on Gas-Chrom Q; carrier gas, N_2, 11.5 $cm^3 \cdot min^{-1}$; temperature, 180 to 280° at 5°/min[10]. (C) HPLC: operating conditions as in Fig. 5; detector, UV, 280 nm; flow-rate, 190 $cm^3 \cdot h^{-1}$; P = 135 atm.

CONCLUSIONS

The analytical tasks involved in the production of ergot alkaloid pharmaceuticals can be accomplished in more simply, more quickly and with higher precision by HPLC than by the thin-layer and gas chromatographic methods used previously.

All of the analytical tasks that have arisen can be solved under isocratic conditions by using two different types of packings (silica and reversed-phase RP-18).

On the silica column group separations and the determination of contaminants, and on the reversed-phase column the separation of the active compounds and their stereo- and structural isomers, can be accomplished.

ACKNOWLEDGEMENTS

We are grateful to Prof. I. Halász and Prof. H. Engelhardt for gifts of silica and reversed-phase packings developed at the University of Saarbrücken.

REFERENCES

1 J. Kolsek, *Microchim. Acta*, 10 (1956) 1500.
2 J. L. McLaughlin, J. E. Goyan and A. G. Paul, *J. Pharm. Sci.*, 53 (1964) 306.
3 K. Genest, *J. Chromatogr.*, 19 (1965) 531.
4 L. A. dal Cortivo, J. R. Broich, A. Dihrberg and B. Newman, *Anal. Chem.*, 38 (1966) 1959.
5 S. Keipert and R. Voigt, *J. Chromatogr.*, 64 (1972) 327.
6 R. Fowler, P. J. Gomm and D. A. Patterson, *J. Chromatogr.*, 72 (1972) 351.
7 J. Reichelt and S. Kudrnáč, *J. Chromatogr.*, 87 (1973) 433.
8 E. Eckart and S. Walter, *Planta Med.*, 27 (1975) 58.
9 M. Prosek, E. Kucan, M. Katic and M. Bano, *Chromatographia*, 9 (1976) 273 and 325; 10 (1977) 147.
10 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 122 (1976) 479.
11 F. J. W. van Mansvelt, J. E. Greving and R. A. Zeeuw, *J. Chromatogr.*, in press.
12 R. A. Heacock, K. R. Langille, J. D. MacNeil and R. W. Frei, *J. Chromatogr.*, 77 (1973) 425.
13 J. D. Wittwer, Jr. and J. H. Kluckholm, *J. Chromatogr. Sci.*, 11 (1973) 1.
14 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
15 R. V. Vivilecchia, R. L. Cotter, R. J. Limpert, N. Z. Thimot and J. N. Little, *J. Chromatogr.*, 99 (1974) 407.
16 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 120 (1976) 496.
17 H. Bethke, B. Delz and K. Stich, *J. Chromatogr.*, 123 (1976) 193.
18 F. Erni, R. W. Frei and W. Lindner, *J. Chromatogr.*, 125 (1976) 265.
19 J. Dolinar, *Chromatographia*, 10 (1977) 364.
20 G. Szepesi and M. Gazdag, in preparation.
21 L. Szepesy, M. Kraxner and I. Fehér, *1st Danube Symposium on Chromatography, 27–30th Sept. 1976, Szeged, Hungary*, Abstracts, Hungarian Chemical Society, Budapest, 1976, p. 2.