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DETERMINATION OF DIHYDROERGOTOXINE ALKALOIDS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A gas–liquid chromatographic (GLC) method has been developed for the separation and determination of dihydroergotoxine alkaloids (dihydroergocristine, dihydroergokryptine and dihydroergocornine), involving quantitative decomposition of the compounds catalysed by a metal surface. GLC was carried out with 2% Dexsil 300 on Gas-Chrom Q as stationary phase, with temperature programming. For the quantitation, phenylbutazone was used as the internal standard.

INTRODUCTION

There has been considerable interest in recent years in sensitive and specific analytical methods for the ergot and dihydroergot alkaloids. Many thin-layer and paper chromatographic methods for the determination of ergot alkaloids have been based on the Van Urk reaction or the natural fluorescence of the separated spots[1–8]. These methods, however, have the disadvantages that they are time consuming and their accuracy and precision are unsatisfactory. High-pressure liquid chromatography offers the most attractive possibility with respect to both separation and quantitation[9–12]. However, it does not seem to have been used for routine work. For this reason, a rapid gas–liquid chromatographic (GLC) method was developed for the above purpose. The method is based on the quantitative decomposition of the compounds, catalysed by a metal surface, and the different migration rates of the peptide moieties formed from various dihydroergotoxine alkaloids during the decomposition.

EXPERIMENTAL

The strongly basic anion exchanger Dowex 1-X2 (100–200 mesh; Fluka, Buchs, Switzerland) was used for the liberation of the free base forms of dihydroxyergotoxine alkaloids from their methanesulphonic acid salt forms in a methanolic medium. The anion exchanger was converted into the OH form with 1 *N* sodium hydroxide solution, washed with water until the eluate was neutral and stored under methanol before use.

The sample investigated was dissolved in 5 ml of a mixture of methanol and acetonitrile (1:1), the final concentration of each component being about 0.5%. After

applying suitable amounts of the anion exchanger, this solution was filtered into a 10-ml volumetric flask, then 2 ml of 1.5% phenylbutazone solution (prepared with a mixture of methanol and acetonitrile) was pipetted into the flask and the volume was made up to the mark with the solvent mixture. A 2-μl volume of this solution was injected into the chromatograph using a metal vaporizer (see below).

All experiments were carried out on a Carlo Erba Fractovap Linea G gas chromatograph. Spiral stainless-steel columns (1 m × 3.2 mm) packed with 2% Dexsil 300 (Applied Science Labs., State College, Pa., U.S.A.) on 80–100 mesh Gas-Chrom Q were employed. The metal vaporizer used is part of the standard injection system fitted to the Fractovap Linea G chromatograph. It consists of a stainless-steel tube surrounded by an electrically heated metal block (Fig. 1).

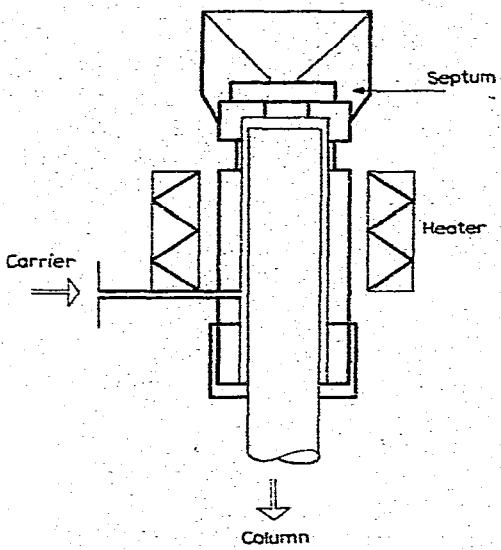


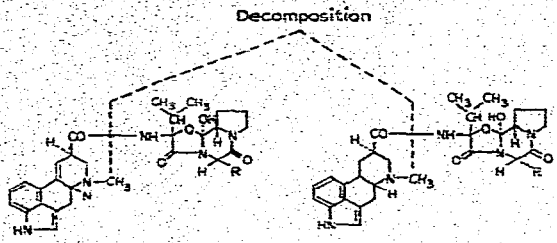
Fig. 1. Fractovap injector.

The compounds to be separated were prepared at the Chemical Works of G. Richter Ltd. (Budapest, Hungary) and were considered to be of the highest available purity. All chemicals used were of reagent grade and were obtained from Reanal (Budapest, Hungary).

RESULTS

The structures of ergotoxine and dihydroergotoxine alkaloids are shown in Fig. 2. Neither compound can be determined by GLC in the salt form owing to their low volatility, and in the base form no separation can be achieved without significant decomposition of the compounds. The only possibility for effecting their GLC measurement is if this decomposition is complete. Only one type of reaction product is formed, and this process is very reproducible.

GLC conditions have been found such that the molecules of ergotoxine and



R	A Ergotoxine group	B Dihydroergotoxine group
Isopropyl	Ergocornine	Dihydroergocornine (I)
Isobutyl	Ergokryptine	Dihydroergokryptine (II)
Benzyl	Ergocristine	Dihydroergocristine (III)

Fig. 2. Structures of ergotoxine and dihydroergotoxine alkaloids investigated.

dihydroergotoxine alkaloids are each split into two components. The decomposition is assumed to proceed as shown in Fig. 2. (This paper deals only with the application and optimization of the method; the proof of the above assumption by mass spectrometry is being studied and will be published later.) The peptide moieties of the compounds are different, resulting in peaks with different retention times on the chromatogram.

In order to determine the optimal conditions for the decomposition, the dependence of the GLC separation of the compounds on the temperature of the vaporizer was investigated.

Fig. 3a and b shows that when the temperature of the vaporizer is lower than 210°, the peak shape and the resolution are not satisfactory. When the temperature is higher than 260° (Fig. 3d), the appearence of new peaks on the chromatogram indicates that the decomposition of the compounds results in several products. There is no significant difference when the temperature of the vaporizer is between 230° and 250°. In further investigations a temperature of 235° was chosen (Fig. 3c).

Temperature-programmed analysis proved to be most suitable for the separation. Figs. 4 and 5 show a comparison of the GLC separations of a dihydroergotoxine sample using isothermal and temperature-programmed analysis. When the separation was started under isothermal conditions, a complete separation of the three components could be achieved, but the analysis time was too long. When the analysis was performed with temperature programming, the analysis time was much shorter, with no significant decrease in resolution.

It should be emphasized that this method, as already mentioned, is based on the separation of the peptide moiety of the molecule. Therefore, compounds that have the same peptide moieties and differ only in the lysergic acid part of the molecule (hydrogenated or non-hydrogenated, ergotoxine or stereoisomeric ergotinine) cannot be separated from each other by this method. However, all impurities of the sample that have different structures in the peptide moiety appear as separate peaks on the chromatogram, so that the retention times and mass spectrometric data may be very

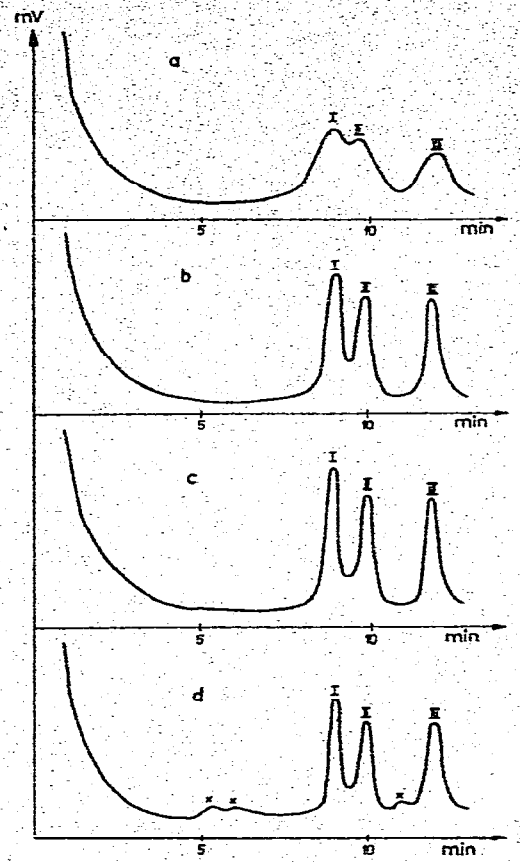


Fig. 3. Dependence of chromatographic separation of compounds on the temperature of the vaporizer. Operating conditions: 1 m column packed with 2% Dexsil 300; temperature programmed from 180 to 280° at 5°/min; nitrogen carrier gas flow-rate 11.5 ml/min. Compounds: I, dihydroergocornine (17.61 μg per 2 μl); II, dihydroergokryptine (14.34 μg per 2 μl); III, dihydroergocristine (16.08 μg per 2 μl). Temperature of vaporizer: (a) 195°; (b) 210°; (c) 235°; (d) 290°. × = Unknown.

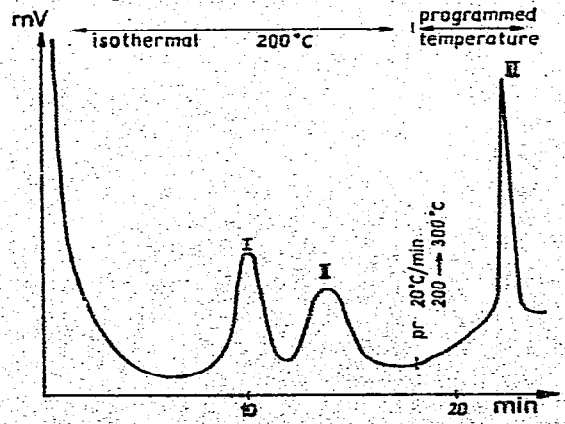


Fig. 4. Separation of compounds I, II and III (see Fig. 3). Operating conditions: Column as in Fig. 3. Temperature: 200° for 18 min, then temperature programmed from 200 to 300° at 20°/min. Temperature of vaporizer: 235°.

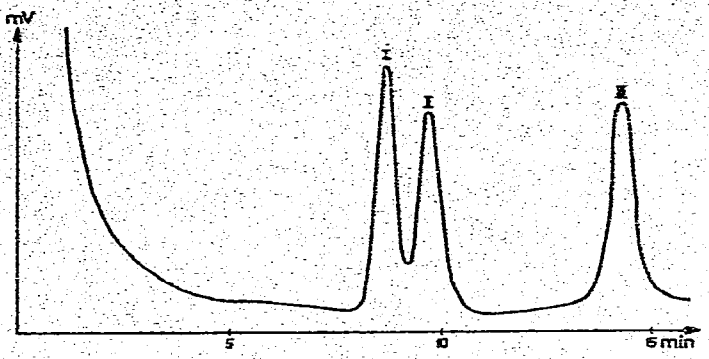


Fig. 5. Separation of compounds I, II and III (see Fig. 3) using temperature-programmed analysis. Column as in Fig. 3. Temperature of vaporizer: 235°. Initial temperature, programming rate and final temperature as in Fig. 3.

valuable for characterization purposes. The retention times of ergot and dihydroergot alkaloids investigated using the optimal GLC conditions are presented in Table I.

For the quantitation, phenylbutazone, which is satisfactorily separated from the sample components, was used as an internal standard. Fig. 6 shows the chromatogram of dihydroergotoxine alkaloids and phenylbutazone. The calibration graph for the three components using the internal standard method is shown in Fig. 7.

TABLE I

RETENTION TIMES OF THE ALKALOIDS INVESTIGATED

Operating conditions as in Fig. 4.

Compound	*Retention time (min)*	*Column temperature (°C)*
Ergocornine Ergocorninine Dihydroergocornine Dihydroergocorninine	9.01	225
Ergokryptine Ergokryptinine Dihydroergokryptine Dihydroergokryptinine	10.03	230
Ergocristine Ergocristinine Dihydroergocristine Dihydroergocristinine	14.6	253
Phenylbutazone	11.92	239

CONCLUSION

It has been found that the application of a strongly basic anion exchanger for the conversion of the methanesulphonic acid salts of dihydroergotoxine alkaloids into their free base forms offers a very good method of avoiding the decomposition

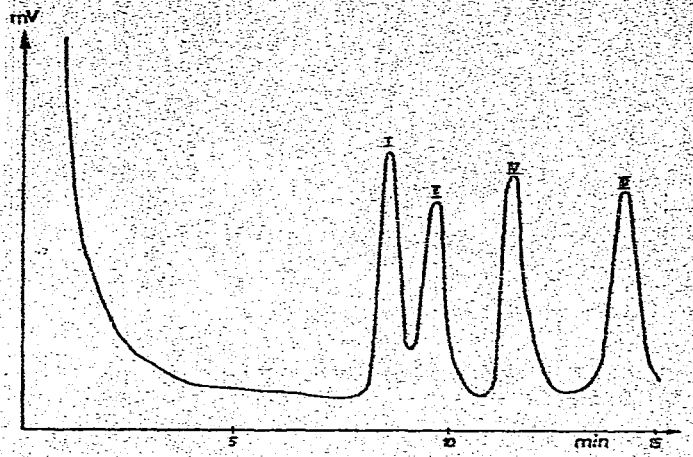


Fig. 6. Separation of compounds I, II and III (see Fig. 3) and phenylbutazone (IV). Column as in Fig. 3.

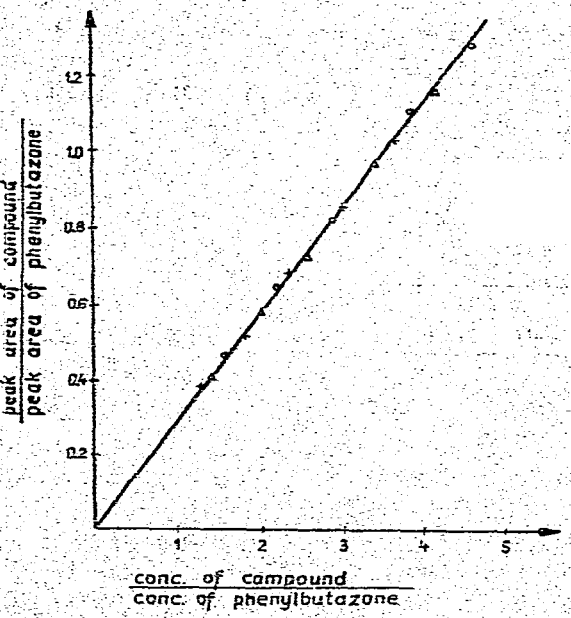


Fig. 7. Calibration graph for compounds using the internal standard method for the quantitation. ○ = I; + = II; △ = III.

and isomerization of the compounds before chromatography. A great advantage of Dowex 1 from the viewpoint of preparation of stock solutions for chromatography is that it can be used in a methanolic medium.

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