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Short Communication

Two-dimensional gas chromatography for the enantiomeric separation of ephedrine and phenaminum and their metabolites

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Abstract

Two-dimensional gas chromatography was used for enantiomeric separation of ephedrine, phenaminum and their metabolites in human urine. The main column was coated with a 2,6-di-O-pentyl-3-O-trifluoroacetylated-α-cyclodextrin and an SE-54 phase column was used as precolumn. The results showed that two-dimensional chromatography could greatly simplify the sample preparation, eliminate the interference of sample matrices, and improve the resolution of the enantiomers of interest.

1. Introduction

More and more attention is paid to drugs with optical activity. The reasons are that (1) the asymmetry of a molecule has strong influence on its biological activity due to the stereospecificity of the enzymatic reaction. Consequently, only one of the configurations of the enantiomer produces physiological activity; (2) study of the stereochemistry of drugs is beneficial to the industry in increasing the yields and saving raw materials; and (3) high purity chiral drugs may be less harmful to the organism owing to the lower amount of drugs required.

Ephedrine and amphetamine, commonly used as excitants, are chiral drugs with nerves sympathetics chemotropism. The efficacy of the dextrorotatory isomer of ephedrine is inferior to that of the levorotatory one. On the other hand, the excitability of central nerves by the dextrorotatory configuration of amphetamine is three to four times as high as that by the levorotatory one. The enantiomeric separation is thus of importance for the study of drug absorption, attribution, metabolism and excretion in the human body. One of the effective methods used for enantiomer resolution of drugs is gas chromatography with columns coated with chiral stationary phases. Frank and co-workers [1] have carried out successfully the stereo differentiation of ephedrine on a column coated with a chirasil-val stationary phase. Konig *et al.* [3] have also shown the enantiomer separation of phendic α and β-receptor active drugs with the chiral stationary phase XE-60-L-valilne-(*R*)-α-phenylethylamide.

In the last few years, an important breakthrough in the gas chromatographic separation of enantiomers has been achieved by using modi-

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fied cyclodextrins as chiral stationary phase in high resolution capillary columns [2]. This new chiral stationary phase can resolve a great number of enantiomers from polar to nonpolar compounds.

The aim of this paper is to show the possibility of multi-dimensional gas chromatography (MDGC) with a modified α-cyclodextrin phase as second column for the enantiomeric separation of ephedrine, amphetamine and their metabolites and to develop a simple, accurate and highly sensitive method for the stereoanalysis of drugs without the need of tedious sample pretreatment.

2. Experimental

2.1. Instrumentation

A Siemens Sichromat-2 gas chromatograph with a double oven and two flame ionization detectors was used. Hydrogen was used as carrier gas with an inlet pressure of 1.35 bar and a middle pressure of 0.95 bar. The injection temperature and detection temperature were both 260°C. The amount injected was 0.5 μl, with a splitting ratio of 1:30. A cross-linked SE 54 glass column was used for preseparation (25 m × 0.25 mm I.D.). A glass capillary column (38 m × 0.27 mm I.D.) coated with 2,6-di-O-pentyl-3-O-trifluoroacetylated-α-cyclodextrin phase was used for chiral separation. Both capillary columns were prepared in our laboratory [4]. A Live-T-piece, connected with the SE-54 column and the chiral column, was used to achieve heart-cutting. After the sample was injected into the preseparation column, the desired component was transferred onto the chiral column using the Live-T-piece. The other compounds were excluded from the chiral column by backflushing.

2.2. Chemicals

D-Ephedrine, L-norephedrine, (+)- and (−)-amphetamine were used as optically pure references. Ephedrine hydrochloride and amphetamine tablets were commercially available. All other chemicals used were of analytical grade.

2.3. Sample preparation

Seven tablets (210 mg) of ephedrine hydrochloride were dissolved in distilled water, alkalinized to pH 8 with sodium hydroxide and then twice extracted with 5 ml of diethyl ether. The extract was then dried with a stream of nitrogen and the residue was used for trifluoroacetylation. The final preparations were made up with hexane to a final volume of 1 ml. The same handling procedure was applied to phenaminum.

To urine samples (50 ml) collected over a 30-h period after oral intake of the tablets were added 5 ml aqueous solution of sodium hydroxide (5 *N*) and 10 g of sodium chloride. The samples were extracted twice with 5 ml diethyl ether. The organic phase was dried with anhydrous sodium sulfate, filtered, and dried with a stream of nitrogen. The residue was trifluoroacetylized and made up to a final volume of 1 ml with hexane.

3. Results and discussion

A major problem usually encountered in clinical drug analysis is the interference caused by the complex matrix. A tedious purification procedure is often required. In a typical chromatographic analysis of alkaline drugs with a single column, the sample preparation steps generally include sample extraction, acidic back-extraction, alkaline wash, derivatization and removal of excess reagent. By using MDGC analysis in the present experiment, the preparation procedure could be simplified to two steps, *i.e.* after derivatization of the extract the sample could be directly analyzed by MDGC. This is accomplished by transferring only the fraction of interest onto the chiral column for further separation by means of the heart-cutting technique, thereby excluding most of the interfering components from the separation column. Thus the interference of the sample matrix could be eliminated to a great extent and fewer purification steps are

necessary. Another obvious advantage is that the chiral column life-time could be prolonged. For a cyclodextrin derivative phase, the upper temperature limit is *ca.* 200°C. If the less volatile components can not be removed from the chiral column by heating to 200°C, contamination of the chiral column often occurs in the analysis of samples with complex matrices. By using a heart-cutting and backflushing technique, this contamination could be avoided.

The chromatograms of the enantiomeric separation of two chiral drugs are shown in Figs. 1 and 2, respectively. The results show that the levorotatory isomer (−) is predominant in the prototype drug ephedrine whereas amphetamine is composed of a nearly racemic mixture.

Frank *et al.* [1] reported the enantiomeric separation of ephedrine by using a single chirasil-val phase column. In comparison with the result

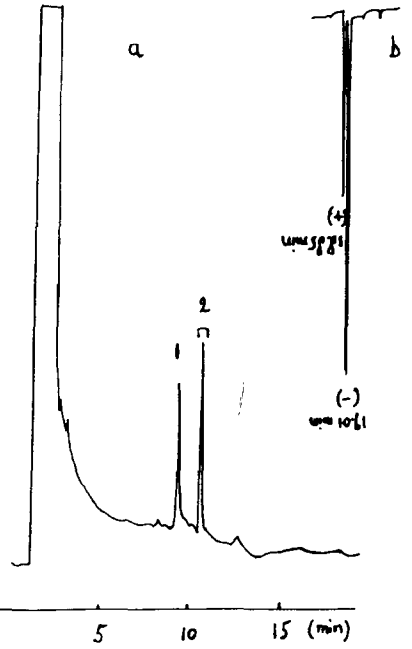

Fig. 1. The enantiomer resolution of the prototype drug ephedrine by MDGC. Peaks: 1. unknown, 2. ephedrine. (a) Preseparation on a 25 m × 0.25 mm I.D. SE-54 glass column; temperature program: 120°C to 280°C at 5°C/min. (b) Enantiomer separation on a 38 m × 0.27 mm I.D. glass modified α-cyclodextrin; temperature program: 120°C to 200°C at 3°C/min. Carrier gas: H_2, $P_A = 1.35$ bar, $P_M = 0.95$ bar.

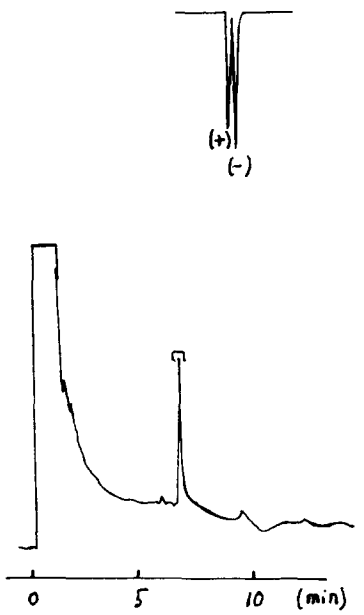

Fig. 2. The enantiomer resolution of the prototype drug amphetamine by MDGC. Conditions: see Fig. 1.

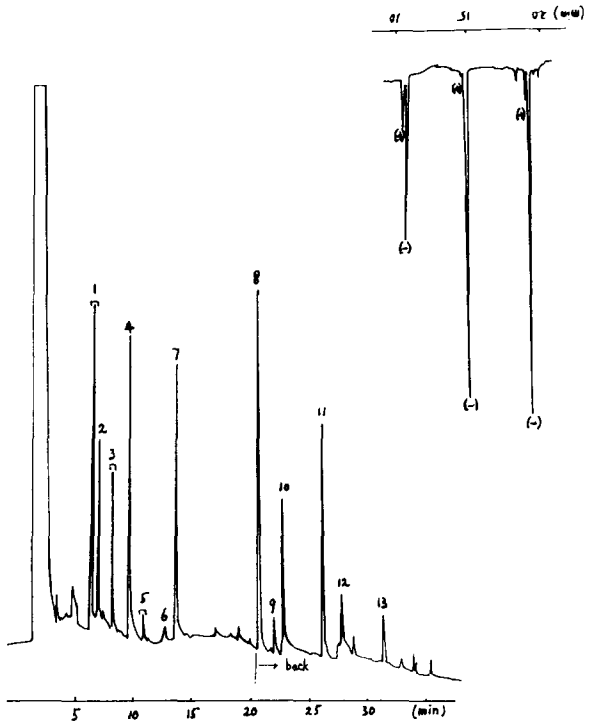

Fig. 3. The enantiomer resolution of the metabolites of ephedrine and amphetamine in human urine by MDGC. Conditions: see Fig. 1. Peaks: 1. amphetamine, 3. norephedrine, 5. ephedrine; others unknown.

reported here, we found that better resolution could be achieved on the α-cyclodextrin derivative phase than on the chirasil-val phase. Therefore the α-cyclodextrin derivative phase column is an ideal choice for the main column in MDGC.

The metabolites of ephedrine hydrochloride and phenaminum in a urine sample were also determined by using the heart-cutting technique. Fig. 3 shows that norephedrine is the main metabolite of ephedrine and that both norephedrine and amphetamine predominantly occur in the levorotatory configuration. The great difference in the rotation of the drug before and after metabolism also proves the selective absorption of the drugs by the human body.

4. Conclusion

MDGC using a second chiral column is an effective method for the enantiomeric analysis of chiral drugs and their metabolites. By using the heat-cutting technique, sample preparation could be greatly simplified, sample loss is eliminated and the analysis time is reduced.

5. Acknowledgements

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6. References

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