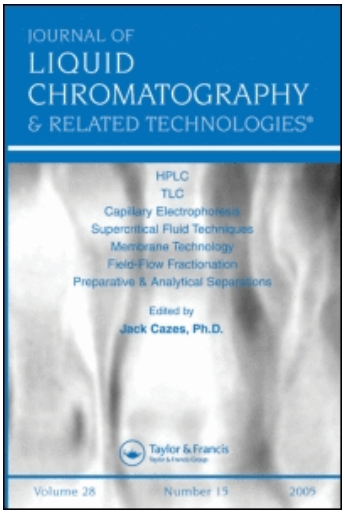

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Direct Chiral Liquid Chromatographic Separation of Tocainide Enantiomers on a Crownpak (Cr) Column and its Application to Pharmaceutical Formulations and Biological Fluids

Hassan Y. Aboul-enein[a]; S. A. Bakr[a]; M. R. Islam[a]; R. Rothchild[b]
[a] Drug Development Laboratory, Radionuclide & Cyclotron Operations Department, King Faisal Specialist Hospital & Research Centre, Riyadh, Kingdom of Saudi Arabia [b] Department of Science, The City University of New York John Jay College of Criminal Justice, New York

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DIRECT CHIRAL LIQUID CHROMATOGRAPHIC SEPARATION OF TOCAINIDE ENANTIOMERS ON A CROWNPAK (CR) COLUMN AND ITS APPLICATION TO PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS*

HASSAN Y. ABOUL-ENEIN[1]**, S. A. BAKR[1],
M. R. ISLAM[1], AND R. ROTHCHILD[2]
[1]*Drug Development Laboratory*
Radionuclide & Cyclotron Operations Department
King Faisal Specialist Hospital & Research Centre
P.O. Box 3354
Riyadh 11211, Kingdom of Saudi Arabia
[2]*The City University of New York*
John Jay College of Criminal Justice
Department of Science
445 West 59th Street
New York, New York 10019-1199

ABSTRACT

Direct chiral liquid chromatographic resolution of racemic tocainide was accomplished without any derivatization using a crownpak CR(+) column as a chiral stationary phase. The stereochemical resolution (R) with symmetrical peaks was 0.97 and a separation factor (α) of 1.44 were obtained. Optimization of separation was achieved using different concentrations of perchloric acid.

INTRODUCTION

Tocainide (2-amino-N-(2,6-dimethylphenyl)-propanamide, Fig. (1), is a primary amine analog of lidocaine[1] and is classified as a 1B antiarrhythmic agent(2). The racemic drug is used clinically for the treatment of life-threatening arrhythmias.

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** Author to whom correspondence should be addressed.

Several studies reported the stereoselective disposition of tocainide in healthy young volunteers, in patients with acute ventricular arrhythmias and in rats and mice(3). It was found that the R(-)-enantiomer was eliminated more rapidly from plasma(4,5). The pharmacology and toxicity of tocainide display important enantiomeric differences; R(-)-tocainide is about three times more potent than the S(+)-enantiomer. Also, the margin of safety is about three times greater for the R(-) than the S(+)-tocainide in the treatment of cardiac arrhythmias(6). Several liquid chromatographic (LC) and gas chromatographic (GC) analyses for for racemic tocainide in biological samples have been performed[5]. Furthermore, several GC and LC methods were reported for the separation of tocainide enantiomers, all of which required derivatization(4,5,7,8,9). Schill et al,(10) reported a direct stereoselective separation of tocainide on alpha-1 acid glycoprotein chiral stationary phase.

The present communication describes an isocratic, simple, direct LC method for the resolution of tocainide enantiomers using a Crownpak CR (+) column, and 0.1N perchloric acid as a mobile phase. Maximum stereochemical resolution (R) of 0.97 and stereochemical separation factor (α) of 1.44 are obtained. The method was successfully applied for the separation of enantiomers and optical purity determination of the drug in pharmaceutical dosage forms and in urine.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Waters model M-45 pump and U6K injector. A Lambda-Max model 480 LC UV detector operated at 254 nm. A Crownpak CR(+) analytical column (25cm X 4.6mm ID, particle size 10µm) (Daicel Chemical Industries, Tokyo, Japan) was used.

Chemicals

Racemic tocainide (Lot No. 25-237-10), (-)-R-tocainide (H181/69), (+)-S-tocainide (H181/68), and Tonocard 400mg tablets (Batch No. 0K227) were kindly supplied by Astra, Sodertalje, Sweden. Perchloric acid 70% was obtained from BDH Chemical Ltd., Poole, England.

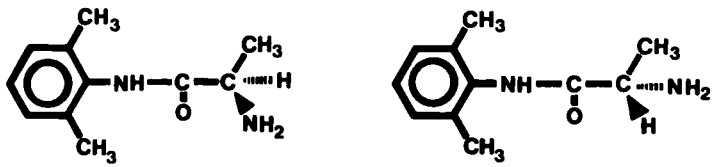

Figure 1. The absoloute configuration of (+)-S and (-)-R-tocainide

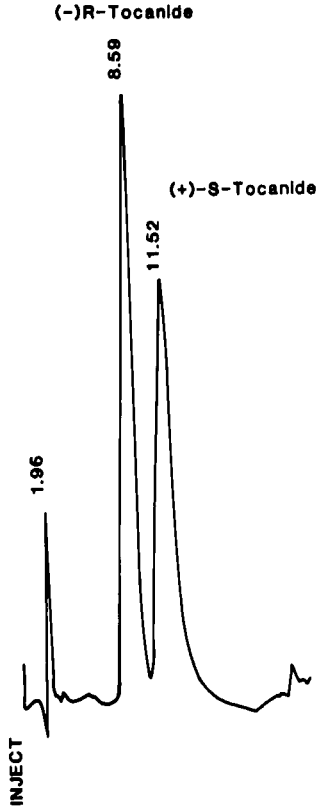

Figure 2. LC separation of racemic tocainide. Column: Crownpak CR(+) (250 x 4.6 mm I.D.); mobile phase: 0.1N perchloric acid; flow rate: 1.0 ml/min.; chart speed: 0.3 cm/min.; temperature: 24°C; pressure: 500 psi; detector: UV254 nm; sensitivity range: 0.01 AUFS.

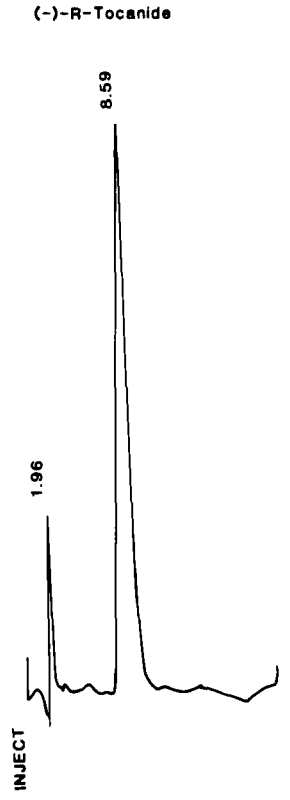

Figure 3. Chromatogram of (-)-R-tocainide. Conditions were the same as Fig. 1, except sample amount was 5 nmole.

CHROMATOGRAPHIC CONDITIONS

The maximum and symmetrical stereochemical resolution of tocainide was obtained using 0.1N perchloric acid as mobile phase. Flow rate was 1ml/min and chart speed was 0.30 cm/min. Temperature was maintained at 24°C and pressure was kept at 500 psi throughout the experiment. Detection was obtained at UV 254nm with sensitivity range 0.01 AUFS. Sample amount injected was 10 nmole for racemic tocainide and 5 nmole for (-)-R-tocainide and (+)-S-tocainide enantiomers.

DETERMINATION OF ENANTIOMERIC ELUTION ORDER

The enantiomeric elution order was determined by chromatographing the separate enantiomers under the same conditions. Thus the peak that eluted with a lower

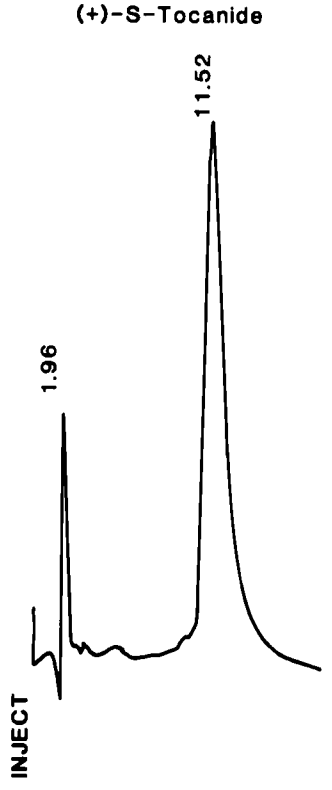

Figure 4. Chromatogram of (+)-S-tocainide. Conditions were the same as Fig. 1, except sample amount was 5 nmole.

capacity factor was identified as (-)-R-tocainide while the peak with higher capacity factor was identified as (+)-S-tocainide.

RESULTS AND DISCUSSIONS

Although several GC and LC chromatographic methods for the separation and assay of tocainide enantiomers in biological fluids have been reported, these method were indirect and required derivatization of the drug enantiomers to a mixture of their corresponding diastereomers by using suitable chiral derivatising agents(4,5,7-9). The main disadvantages of the indirect method for enantiomeric excess determination are the possibility of: a) racemization of

one enantiomer to the other during the derivatization reaction, b) preferential conversion of one enantiomer to the derivative, and c) preferential loss or fractionation of one diastereomer during workup. Finally, an indirect method renders the analysis lengthy since it usually requires sample extraction and entails an extra step, and also presupposes that a suitable derivatising agent is available with known (high) enantiomeric excess. Schill et al (10) reported the only direct enantiomeric separation of tocainide using α_1-acid glycoprotein column.

We have reported here the detailed description of a liquid chromatographic method for the direct separation of tocainide enantiomers using the commercially available crownpak CR(+) column. Different concentrations of perchloric acid were used as mobile phase to optimize the separation. The chromatogram of the enantiomeric separation of racemic tocainide is shown in Figure 2 for comparison with the chromatograms of samples of (-)-R-tocainide (Figure 3) and (+)-S-tocainide (Figure 4). The maximum and symmetrical stereochemical resolution (R) of 0.97 and stereochemical separation factor (α) of 1.44 was obtained using 0.1 N perchloric acid as mobile phase. The method was successfully applied for the separation of enantiomers and opti cal purity determination of the drug in pharmaceutical dosage forms Tonocard 400 mg tablets and in urine samples after extraction with methylene chloride. Details are being submitted for separate publication. This method can also be applied for preparative chromatography for the isolation of large quantities of the respective enantiomers.

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