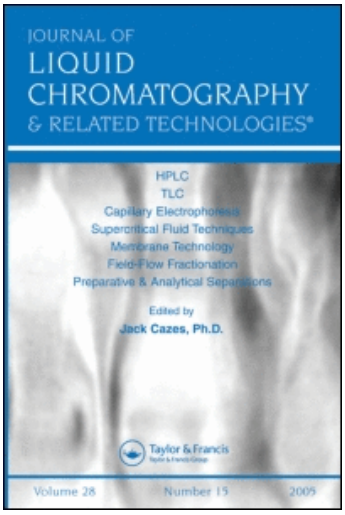

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DIRECT HPLC RESOLUTION OF RACEMIC NOMIFENSINE HYDROGEN MALEATE USING A CHIRAL BETA-CYCLODEXTRIN-BONDED STATIONARY PHASE

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ABSTRACT

A direct liquid chromatographic method was developed for the optimization of separation of racemic S,R-nomifensine hydrogen maleate, a psychotropic drug, this was achieved without any derivatization, using a β-cyclodextrin-bonded phase column. A separation factor of 1.46 and resolution of 4.30 were obtained for the enantiomers of nomifensine hydrogen maleate. The effect of pH, temperature and methanol content in mobile phase on retention and enantioselectivity of nomifensine hydrogen maleate was also demonstrated.

INTRODUCTION

Nomifensine (8-amino-1,2,3,4-tetrahydro-2-methyl-4-phenyl isoquinoline) maleate is used as a psychotropic agent for the treatment of depression. Studies done by Poncelet et. al. (1) showed that the stimulatory and anti-depressant type effects of S,R-nomifensine were caused by the S-enantiomer only. Schacht and Leven (2) also

reported that S-nomifensine was 1.8-2.6 times more potent than the racemate and R-nomifensine was ≥300 times less potent than the racemate in terms of catecholamine uptake inhibition in rat brain synaptosome. Recently, Kruse (3) evaluated the S- and R-enantiomers of nomifensine for psychopharmacological effects and anti-depression activity in mice and rats against the S,R-nomifensine hydrogen maleate (the racemic mixture). It was found that S-enantiomer was on average 1.7 and 2.8 times more potent than the racemate with intraperitoneal (i.p.) and oral route administrations respectively. The R-enantiomer displayed slight activity only when injected i.p. and was on average ≥30 times less potent than the racemate. Furthermore, the R-enantiomer devoid of stimulant or stereotypic behavioural activity and had a rather sedative effect.

From the above cited pharmacological results, it is obvious that S-nomifensine enantiomer is responsible for the observed pharmacological activity. In order to predict the therapeutic activity of a mixture nomifensine hydrogen maleate enantiomers, it is desirable to use an efficient method for their chromatographic separation to determine their proportion.

The absolute configurations of S- and R-nomifensine enantiomers were recently established (Fig. 1) by X-ray analysis and comparative CD spectra (4). Several chromatographic methods including thin layer, gas-liquid and high performance liquid chromatographic techniques (5-10) were reported for the detection and quantitation of the S,R-nomifensine hydrogen maleate (the racemate) and its metabolites in various body fluids. Kunstmann et. al. (4) recently reported a HPLC analysis to establish the enantiomeric purity of S- and R-nomifensine which were chemically separated. The analysis of the enantiomers was obtained through derivatization with (-)-2-methoxy-2-(trifluoromethyl) phenylacetic acid chloride using Lichrosorb Si 60 column.

This paper reports a direct resolution and optimization of the separation of enantiomers of nomifensine hydrogen maleate on a

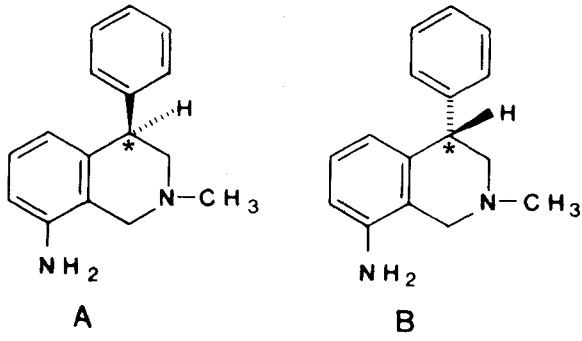

Fig. 1. The absolute configuration of S- and R-nomifensine.

(a) S-nomifensine (b) R-nomifensine

β-cyclodextrin column without any derivatizations. Cyclodextrins are chiral, toroidal-shaped molecules containing from six to twelve glucose units bonded through α-(1,4) linkages. The structure of the cyclodextrin resembles a truncated cone with both ends open. The ability of cyclodextrins to form inclusion complexes with variety of chiral molecules make it particularly useful for separation (11, 12). The effect of pH, methanol content in the mobile phase as an organic modifier and temperature on the retention and enantioselectivity of nomifensine hydrogen maleate was studied.

EXPERIMENTAL

Apparatus:

The liquid chromatography system consisted of a Waters Model M-45 pump, a Waters U6K injector and Waters Lamda-Max Model 480LC spectrophotometer UV detector operated at 239 nm. The column (250 x 4.6 mm i.d.) is a Cyclobond I with the beta form consisting of 7 glucopyranose unit coupled to high purity 5 μ silica gel and was purchased from Astec, (N.J., U.S.A).

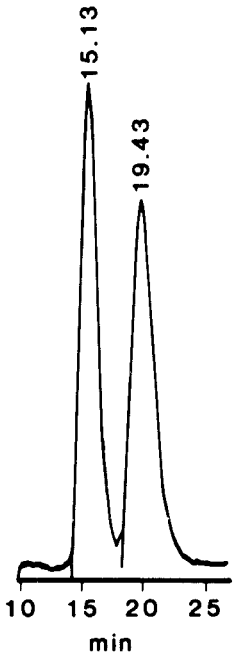

Fig. 2. Liquid chromatographic separation of S,R-nomifensine hydrogen maleate. Mobile phase: TEAA buffer (pH 3.5), 20% MeOH; Flow-rate: 0.5ml/min.; Temperature: 8°C. Column: β-cyclodextrin silica (250 x 4.6 mm, i.d.); UV 239; Sample amount: 3 nmol.

Chemicals:

Racemic nomifensine hydrogen maleate, its S-enantiomer and R-enantiomer (Op. E 206) were kindly supplied by Hoechst AG, West Germany. Triethylamine was obtained from Fisher Scientific and Allied Company (New Jersey, U.S.A.). Glacial acetic acid was obtained from Matheson Coleman and Bell Manufacturing Chemists (Ohio, U.S.A.) HPLC grade methanol was obtained from Alltech Associates, Inc. Appled Science Labs (Illinois, U.S.A.).

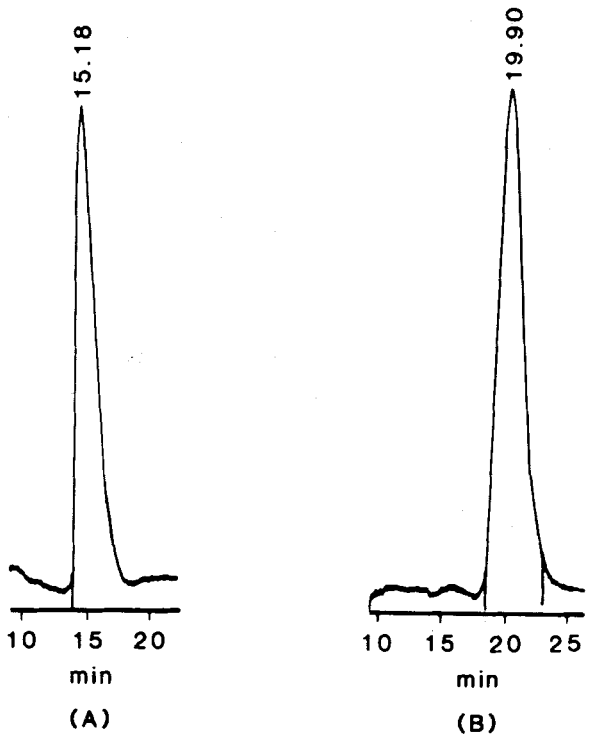

Fig. 3. Chromatogram of (a) S-nomifensine and (b) R-nomifensine. Conditions same as fig. 2. Sample amount: 2 nmol.

RESULTS AND DISCUSSIONS

Although a HPLC analysis was performed by Kunstmann (4) to establish the enantiomeric purity of S- and R-nomifensine through derivatization, but was proved to be lengthy and time-consuming. However, no work has been reported on the direct resolution and optimization of separation of racemic nomifensine hydrogen maleate. This paper describes the separation of enantiomers of nomifensine hydrogen maleate using a β-cyclodextrin column. The mobile phase consist of methanol and triethylammonium acetate (TEAA) buffer [0.1% Triethylamine in water] and the pH

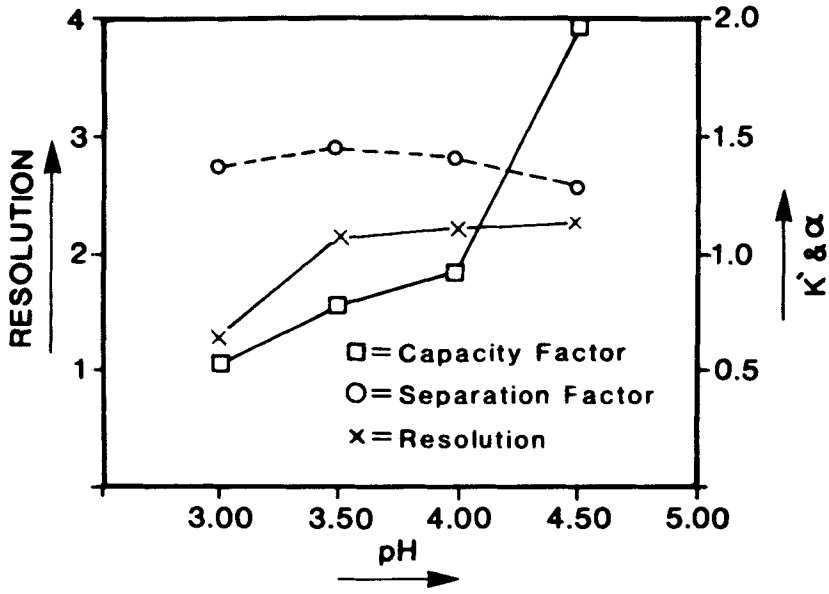

Fig. 4. Influence of pH on capacity factor, separation factor & resolution of S,R-nomifensine hydrogen maleate. Mobile phase: TEAA buffer (pH3-4.5); Temperature: 23°C. Other conditions same as fig. 2.

was adjusted using glacial acetic acid. The best separation was obtained using 20% methanol and TEAA buffer (pH 3.5) at 8°C (Fig. 2). Comparing the retention time and capacity factor of the chromatogram of S-nomifensine (Fig. 3a) and chromatogram of R-nomifensine (Fig. 3b), the peak eluted with a lower capacity factor was identified as S-nomifensine while the peak eluted with a higher capacity factor was identified as R-nomifensine.

The optimization of the separation was achieved using different solvent systems at different temperatures. It was found that the cyclodextrin matrix is very sensitive to any small changes in temperature, pH and concentration of the organic modifier. The effect of ionic strength on resolution was not observed when 0.1 M NH_4Cl was added to the mobile phase. An increase in pH caused an increase in the capacity

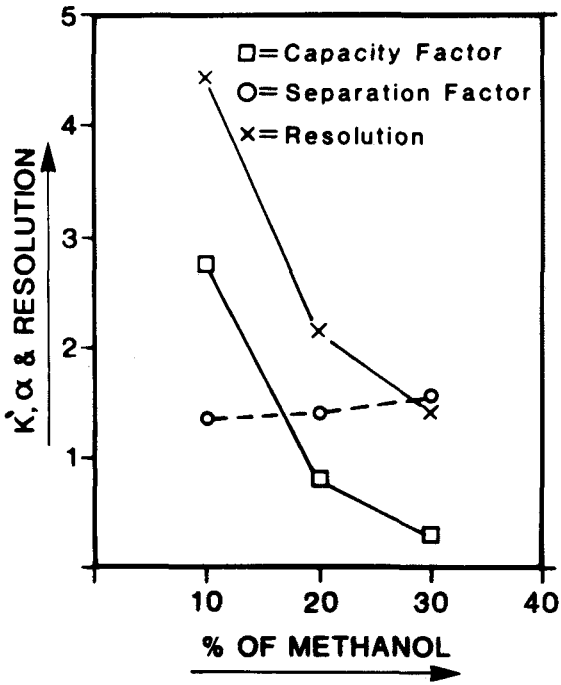

Fig. 5. Influence of methanol concentration on capacity factor, separation factor & resolution of S,R-nomifensine hydrogen maleate. Mobile phase: MeOH (10 - 30%); Temperature: 23°C. Other conditions same as fig. 2.

factor (k') and resolution. But the best separation factor (α) was obtained at pH 3.5 (Fig. 4).

Concentration of organic modifier is of major importance in reversed phase chromatography. This effect was found to be very pronounced in the separation of racemic nomifensine on a β-cyclodextrin column (Fig. 5). A small change in concentration of methanol in the mobile phase causes a relatively large change in capacity factor (k') and resolution. They increased with decreasing concentration of methanol. But the separation factor (α) was found to decrease with the decreasing concentration of methanol. A high capacity factor (k') caused a decrease in the

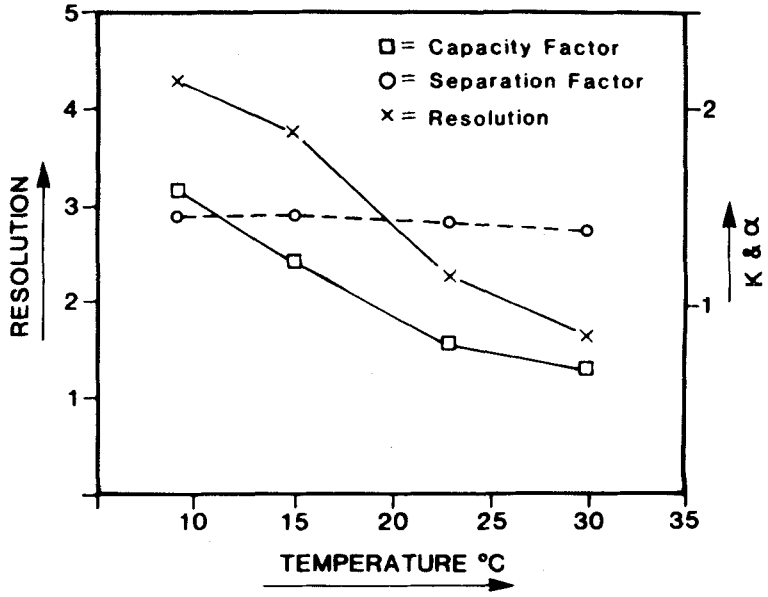

Fig. 6.: Influence of temperature on capacity factor, separation factor & resolution of S,R-nomifensine hydrogen maleate. Temperature: 8 - 30°C. Other conditions same as fig. 2.

separation factor (α) but improved the resolution. We found 20% methanol in the mobile phase to be optimal for separation.

Both resolution and capacity factor (k') were increased at 8°C with baseline separation (Fig.6). The effect of temperature on the separation factor (α) was not prominent.

In summary, a direct and simple method for optimizing the separation of the enantiomers of R,S-nomifensine hydrogen maleate was achieved using a commercially available β-cyclodextrin column.

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