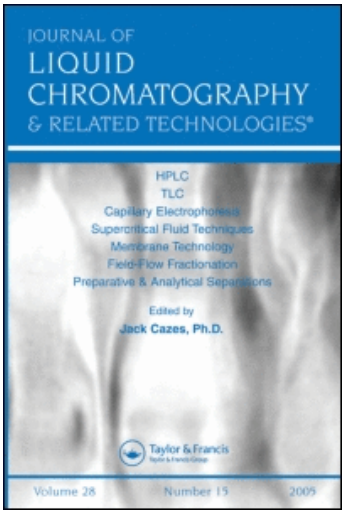

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ENANTIOMERIC SEPARATION OF SUBSTITUTED QUINUCLIDINES AND AZA-NORBORNANES BY HPLC USING AN ACETYLATED β-CYCLODEXTRIN BONDED STATIONARY PHASE

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ABSTRACT

A method for the HPLC enantiomeric separation of substituted aza-bicycles (quinuclidines and aza-norbornanes) has been developed, after borane complexation with borane-THF, using an acetylated β-cyclodextrin column. The procedure is relatively simple and has been applied to the determination of the enantiomeric purity of several synthetic compounds of potential pharmaceutical interest.

INTRODUCTION

Quinuclidines and aza-norbornanes substituted in the C3 position with heterocycles such as oxadiazoles and oxazoles (table 1) are extremely potent

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muscarinic agonists and may have some therapeutic use in the treatment of Alzheimers Disease. All these compounds possess a chiral centre at the site of substitution. In order to gain more information for structure-activity relationships and to assess the potential pharmacological differences of each enantiomer, classical resolution by recrystallisation of diastereomeric tartrate salts was attempted. An assay was subsequently required to check efficiency of the resolution and to determine final enantiomeric purity.

High performance liquid chromatographic methods, both by direct and indirect means, have been increasingly applied to the separation of enantiomers due to inherent accuracy, sensitivity and speed. Indirect methods involve derivatisation with an optically pure reagent to form diastereomers which can be separated on an achiral column. However, care must be taken to ensure the chiral reagent is enantiomerically pure, and that there is no kinetic discrimination or racemisation during the reaction. Direct methods, utilising chiral stationary phases, are usually preferable due to elimination of the need for chiral derivatisation. A large number of chiral stationary phases are now commercially available, however few of these are suitable for enantiomeric separation of relatively polar, basic amines.

The α_1-acid glycoprotein bonded phase (Enantiopac, LKB, Sweden) was initially tried; however lack of retention was a consistent problem and resolution was poor as a result. Chiral derivatisation of L-660,863 was attempted using 1-methyl phenyl isocyanate, but no separation of diastereomers was obtained on reverse or normal phase columns, probably due to the large distance between chiral centres. Chiral derivatisation of the remaining compounds was not feasible.

Unmodified and derivatised β and α-cyclodextrin bonded phases have been extensively used for enantiomeric separations (1-8). Solute structural requirements for separation are a hydrophobic moiety (usually one or more

aromatic rings) which can include into the cyclodextrin cavity and one or more hydrogen bonding groups which can interact with the secondary hydroxyl groups on the rim of the cavity. Both of these structural features should be in close proximity to the chiral centre. Attempts at enantiomeric separation, using either acetylated or underivatised α or β-cyclodextrin, proved fruitless, again due to the lack of retention of the analytes.

Borane complexation has been used for neutralising and increasing lipophilicity of tertiary amines (9) but this technique has not previously been used to facilitate enantiomeric resolution using a chiral stationary phase. Borane complexation takes place between a relatively basic tertiary amine and a reactive borane complex such as borane.THF by donation of the nitrogen lone pair of electrons to the electron-deficient boron atom forming a dative covalent bond.

Very few examples of enantiomeric resolution of aliphatic racemates using cyclodextrin-based systems have been reported although Armstrong *et al* (10) has had recent success using derivatised cyclodextrin-based phases for GC. Furthermore, to our knowledge, there are only two examples (2,8) of enhanced separation by acetylated β-cyclodextrin over underivatised cyclodextrin bonded phases. In this paper we describe the enantiomeric separation of substituted aza-bicyclic racemates on an acetylated β-cyclodextrin bonded stationary phase after borane complexation.

EXPERIMENTAL

Materials

All racemates were synthesized 'in house' and identity and purity checked by MS, NMR, HPLC and elemental analysis. Borane-THF and

diethyl ether (analytical grade) was obtained from Aldrich. All HPLC solvents were of HPLC grade and obtained from Fisons. Water was of MilliQ (Millipore) quality.

Complexation procedure

To a solution of the amine (0.1mmol) in THF (0.5ml) at -78°C was added BH_3. THF complex (0.15ml of a 1M solution in THF) with mixing. Immediately, the solution was warmed to 0°C, then water (5ml) and diethyl ether (5ml) were added and mixed. The ether layer was removed, dried with anhydrous sodium sulphate and concentrated to dryness. The complex was redissolved in methanol prior to HPLC injection.

High Pressure Liquid Chromatography

All separations were performed at ambient temperature (23°C) with a Hewlett Packard 1090 liquid chromatograph, equipped with a linear diode-array detector (Hewlett Packard, Avondale USA) set at 210nm. The chromatograph was equipped with an autoinjector comprising a Rheodyne 7010 injection valve and a 25µl loop. 5µl, typically, was injected. A flow rate of 1ml/min was used throughout.

Acetylated α and β and underivatised cyclodextrin bonded phase columns, (250 x 4.6mm i.d., 5µm particle size, manufactured by Astec, Whippany, USA), were obtained from Technicol (Stockport, UK).

All solvents were filtered through a 0.22µm filter before use and were continuously degassed during use by helium sparging.

RESULTS AND DISCUSSION

The derivatisation procedure was rapid at low temperatures. Care was needed to avoid excess warming which resulted in reduction of the heteroaromatic ring. Under these conditions borane complexation took place only on the quinuclidine nitrogen. Once the reaction had been quenched, the complex was stable and used for a period of weeks. No racemisation was observed.

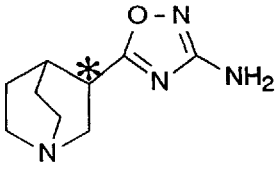
Initial attempts at enantiomeric separation of L-660,863-borane using both underivatised and acetylated α-cyclodextrin columns were totally unsuccessful ($\alpha = 1$). Underivatised β-cyclodextrin only gave a very small amount of separation ($\alpha = 1.04$ Rs = 0.35). The acetylated β-cyclodextrin bonded phase gave a much improved separation and this was subsequently used for all further investigations.

Table 1 lists the enantiomeric separation data for the three compounds investigated.

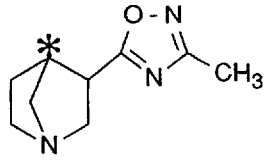
Studies were performed to determine the effect on resolution of altering mobile phase and temperature. In all cases, use of acetonitrile instead of methanol as the organic modifier resulted in loss of separation. Addition of salts (ammonium acetate, ammonium nitrate and triethylamine acetate between pH's 4-7 did not significantly change resolution. Also, despite a large increase in retention, no improvement in resolution was seen on reduction of the temperature to 4°C. No detailed optimisation of methanol content in the mobile phase was carried out, the resolutions obtained being sufficient for the purposes of the assay (accurate determination of enantiomeric purity up to 98%). Figure 1 shows a typical chromatogram of a partially resolved sample of L-661,320 giving an enantiomeric purity of 95.7%.

TABLE 1

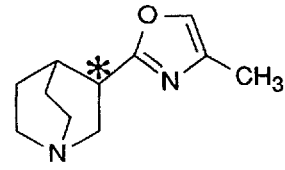
Enantiomeric Separation of Substituted Aza-Bicycles after Borane Complexation.



L-660,863



L-670,548



L-661,320

Compound	Structure	R.T. @	α	R_s	Mobile phase
L-660,863		23.0	1.12	0.91	40% MeOH
L-661,320		28.0	1.15	1.15	40% MeOH
L-670,548		29.7	1.12	0.96	25% MeOH

* Enantiomers arising from this chiral centre.

@ Retention time of first eluted enantiomer (mins).

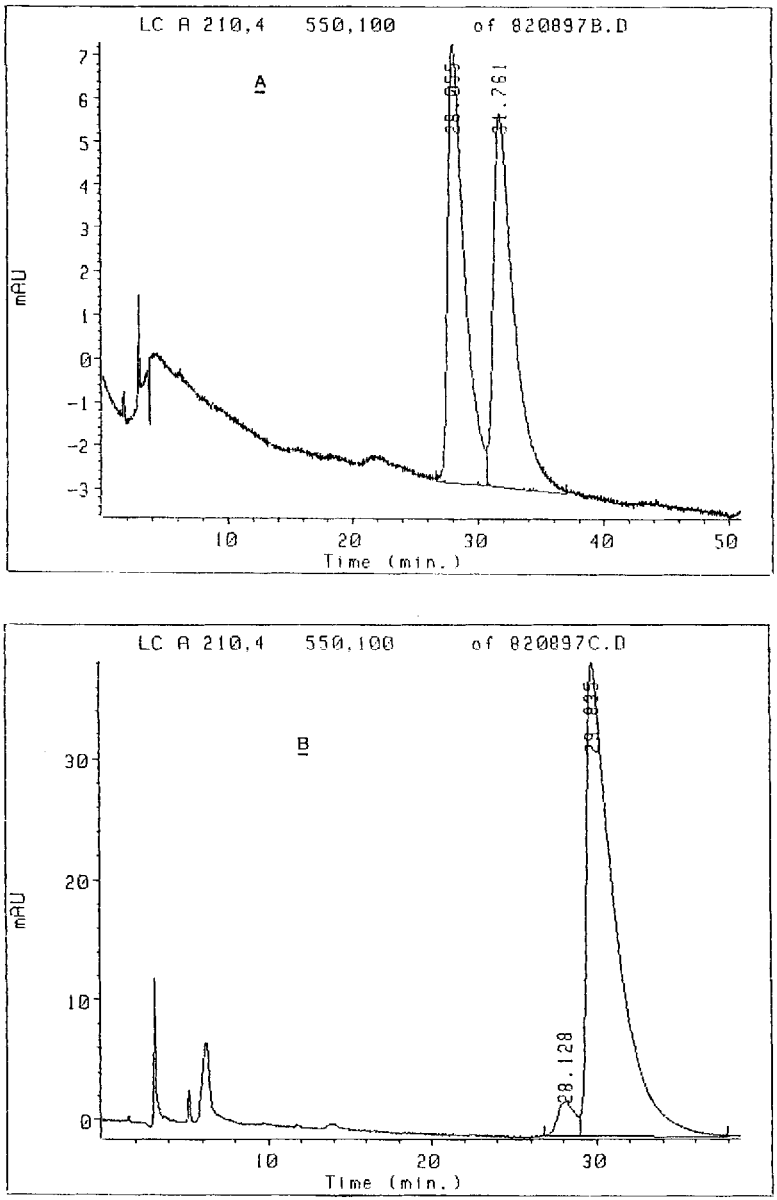


FIGURE 1. Chromatogram showing separation of the enantiomers of L661320 as the borane complex (A) racemic sample (B) partially resolved sample possessing enantiomeric purity of 95.7%.

The putative mechanism for these separations is that borane complexation considerably increases the lipophilicity of the aza-bicycle enabling increased stability of inclusion complexation, and also increases its size, so that a much better 'fit' into the cyclodextrin cavity is produced. Chiral discrimination then occurs because of enantiomeric differences in the hydrogen bonding between the heterocycle and the per-acetylated secondary hydroxyl groups on the outer rim of the cavity. Inclusion of the heterocyclic ring into the cyclodextrin cavity may occur, but will not influence enantiomeric resolution because of its much smaller size, and consequently poorer fit, and lack of hydrogen bonding groups of the borane complexed aza-bicycle. Of interest is the reduced methanol content needed to obtain similar retention times of L-670548, containing one less methylene group in the aza-bicylic ring, compared to the substituted quinuclidines. This suggests a less 'tight fit' of the aza-bicyclic ring in the cyclodextrin cavity.

The reason for enhanced enantiomeric resolution using the per-acetylated cyclodextrin is uncertain. Possibly the extension of hydrogen bonding groups away from the cavity causes a more optimal spatial interaction with the hydrogen bonding groups on the heterocycle. Alternatively, a dipole stacking interaction could be taking place between the C = N group nearest the chiral centre of the analyte and the carbonyl group of the peracetylated secondary hydroxyl groups.

It was clear, during this work that the nature and age of the column were important in the degree of enantiomeric separation obtained. Variations from column to column were observed, and might be due to differences in the degree of acetylation or to the stability of the cyclodextrin derivative.

The technique is being applied to other substituted aza-bicycles and also applied to preparative enantiomeric resolution of these compounds.

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