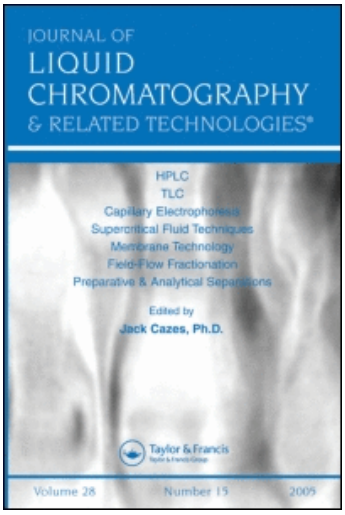

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ENANTIOMERIC SEPARATION OF SUBSTITUTED QUINUCLIDINYL-PYRAZINES BY HPLC USING AN ACETYLATED β-CYCLODEXTRIN CHIRAL STATIONARY PHASE

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ABSTRACT

A method is described in which the enantiomers of substituted quinuclidinyl-pyrazines are separated using an acetylated β-cyclodextrin chiral stationary phase following facile derivatisation with borane-THF complex. This simple protocol has been applied to the analysis of enantiomeric purity of a number of derivatives following resolution by formation of diastereomeric salts. The effects of substituent, mobile phase composition and column age are discussed.

INTRODUCTION

The cholinergic hypothesis of Alzheimer's disease (**1-3**) has led to the belief that by enhancing muscarinic cholinergic transmission within the cerebral cortex, a beneficial effect would be observed in the management of the learning and memory

disorders commonly associated with the disease. As part of a study into muscarinic agonists designed to act directly on the post-synaptic muscarinic receptors in the cerebral cortex, a series of quinuclidinyl-pyridine derivatives were synthesised and biochemically evaluated, initially as the racemates (**4**). More detailed *in-vitro* and *in-vivo* evaluations of the individual enantiomers required resolution of the compounds of interest hence an assay was required to monitor the progress of resolution after crystallisation of diastereomeric salts and determine enantiomeric purity of final compounds.

The use of native cyclodextrin bonded chiral stationary phases (CSP's) for the separation of enantiomers is well documented (**5-11**) whilst the development and application of bonded derivatised cyclodextrins to provide additional chiral recognition properties is a more recent phenomenon (**12-15**). The general requirement for chiral recognition in these systems is the formation of an inclusion complex by the analyte in the hydrophobic cyclodextrin cavity plus hydrogen bonding interactions to the secondary hydroxyls at the mouth of the cavity. Derivatisation of these hydroxyl groups, as in the case of acetylated ß-cyclodextrin, can extend the chiral recognition outwards from the cavity thereby providing greater enantiomeric discrimination.

In this study borane complexation, to form a boron-nitrogen dative covalent bond, was used as this had been shown to neutralise the basicity of tertiary amines (**17**) thereby enhancing the hydrophobicity and facilitating the enantiomeric separation by improvement of the tendency of such molecules to include in a ß-cyclodextrin (**16**). Since of the pyrazine moiety is resistant to reduction with borane, complexation is facile and does not have to be carried out at reduced temperature. This allows the procedure to be performed *in-situ* on a small scale, in contrast to methodology described previously (**16**).

The effect of changing substituent at the 6-position of the pyrazine was investigated to see if a correlation existed between substituent size and resolution. The effect of mobile phase composition, particularly the effect of organic modifier concentration was investigated and note was made of the variability in resolution between column batches which may be related to column age.

EXPERIMENTAL

Materials

All racemates described were synthesised in-house with identity and purity confirmed by NMR, MS, HPLC and elemental analysis. Borane-THF complex was obtained from Aldrich. HPLC grade methanol and all other reagents were obtained from Fisons and water was of Millipore MilliQ grade.

Instrumentation

An HP1090M series high performance liquid chromatograph was used (Hewlett Packard, Avondale, USA). The system comprises of an autoinjector, consisting of a Rheodyne 7010 injection valve fitted with a 250 µl loop, an autosampler and a DR-5 solvent delivery system. Detection was by UV using a built-in linear photodiode array detector and data was processed using a 79994A PASCAL workstation.

Chromatographic Conditions

HPLC analysis was performed using columns containing acetylated β-cyclodextrin or native β-cyclodextrin bonded to silica (Astec, Whippany, USA) supplied by Technicol (Stockport, UK) with identical dimensions (250 x 4.6mm i.d.) and silica particle size (5 µm). Mobile phases were methanol-water mixtures and the flow rate was 0.8 ml/min. The photodiode-array detector was set to the λ_{max} for the particular analyte with a bandwidth of 10nm. The reference wavelength was 550nm with a bandwidth of 100nm. All analyses were performed at ambient temperature.

Derivatisation Procedure

The free base of the sample was prepared by taking the compound (1-4mg), dissolving in 100mM potassium carbonate (ca. 0.5ml) and extracting into either dichloromethane or ethyl acetate (4ml). The organic layer was removed and

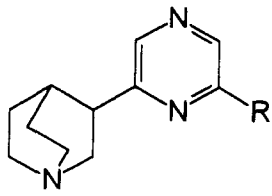

FIGURE 1. STRUCTURE OF 6-SUBSTITUTED 3-(PYRAZIN-2-YL)-QUINUCLIDINE

evaporated to dryness under a stream of dry nitrogen. The sample was then treated directly with an excess of borane-THF complex (0.3ml of a 1M solution in THF) at room temperature. After ca. 20 seconds, the reaction was quenched by addition of a 50% methanol-water mixture (0.7ml). This solution was then amenable to direct injection onto the HPLC system described above.

RESULTS AND DISCUSSION

The borane derivatisation was virtually instantaneous at room temperature with no observed reduction of the pyrazine ring observed. However, care was needed in the analysis to ensure that only mono-borane was present as some complexation to the pyrazine nitrogen was observed to form a bis-borane complex. This could easily be distinguished from parent compound and mono-borane by its UV characteristics as determined by HPLC-diode-array; structural identity was confirmed by NMR analysis. The bis-borane was however unstable and would decompose to the mono-borane on gentle warming (40 degrees C, 15 minutes) or prolonged standing (room temperature, 2 hours). It was ensured that this procedure induced no racemisation by removing and reforming the borane complex of a single enantiomer and confirming that the enantiomeric excess was identical both before and after.

The effect of varying the 6-substituent of the quinuclidinyl pyrazine derivatives under investigation (Figure 1) was investigated using an acetylated β-cyclodextrin column, and the results for separation and resolution are summarised

TABLE 1. EFFECT OF 6-SUBSTITUENT ON SEPARATION AND RESOLUTION. Column: acetylated β-cyclodextrin. Mobile phase: 50% MeOH/water. Flow: 0.8 ml/min.

6-Substituent	∝	R_S
H	1.06	0.72
Cl	1.18	2.40
Br	1.12	1.51
Et	1.19	2.25
OEt	1.14	1.84
OMe	1.17	1.80
Me	1.29	3.94
SMe	1.09	0.98

in Table 1. Previous work describing the separation of substituted quinuclidinyl and aza-norbornyl systems (**16**) suggested that this would be the CSP of choice as both native ∝- and β-cyclodextrin columns gave little or no separation. Attempts to resolve the quinuclidinyl-pyrazine derivatives on the ∝-cyclodextrin all proved unsuccessful; the results for a direct comparison using the native β-cyclodextrin column are summarised in Table 2.

As can be seen from Table 1, the substituent appears to have a pronounced effect on both separation and resolution. The 6-hydrogen substituted compound shows the lowest resolution, yet a simple increase to methyl group appears to give the maximal resolution of the compounds studied. 6-Chloro and 6-ethyl are comparable, then the general trend appears to be that increasing the size of the substituent results in a reduction of both separation and resolution. It is suggested that increasing substituent size exerts a steric effect by reduction of the degree of insertion of the aza-bicycle into the cyclodextrin cavity. This causes the stereospecific interactions to be non-optimal although the change in electronics may also influence such interactions. In all cases, as can be seen from Table 2, changing from an acetylated to a native β-cyclodextrin column results in a reduction and in some cases a complete loss of separation. The fact that some

TABLE 2. EFFECT OF 6-SUBSTITUENT ON SEPARATION. Column: Native β-cyclodextrin. Mobile phase: 25% MeOH/water. Flow: 1.0 ml/min.

6-Substituent	∝
Cl	1.09
Br	1.00
Et	1.06
OEt	1.06
OMe	1.09
Me	1.15
SMe	1.00

TABLE 3. EFFECT OF MOBILE-PHASE COMPOSITION ON SEPARATION AND RESOLUTION FOR 3-(6-METHYLPYRAZINE-2-YL) QUINUCLIDINE. Column: acetylated β-cyclodextrin. Flow: 0.8 ml/min.

% MeOH	∝	R_S
90	1.26	1.30
80	1.29	1.92
70	1.30	2.34
60	1.29	3.38
50	1.29	3.94
40	1.29	5.54
30	1.28	3.80

chiral recognition is observed leads us to hypothesise that the effect of acetylation of the cyclodextrin is to open the mouth of the cavity slightly and thereby provide a better inclusion complex and extend the chiral recognition away from the ring.

As maximal separation was seen with the 6-methyl and 6-chloro derivatives, these compounds were investigated further to examine the effect of mobile phase composition on resolution. As can be seen from Tables 3 and 4, the

TABLE 4. EFFECT OF MOBILE-PHASE COMPOSITION ON SEPARATION AND RESOLUTION FOR 3-(6-CHLOROPYRAZINE-2-YL) QUINUCLIDINE. Column: acetylated β-cyclodextrin. Flow: 0.8 ml/min.

% MeOH	∝	R_S
80	1.14	0.97
70	1.15	1.16
60	1.17	1.79
50	1.17	2.33
40	1.19	2.88
30	1.19	2.65

TABLE 5. EFFECT OF DIFFERING COLUMN BATCH OF ACETYLATED β-CYCLODEXTRIN ON SEPARATION AND RESOLUTION FOR 3-(6-METHYLPYRAZINE-2-YL) QUINUCLIDINE. Mobile phase: 50% MeOH/water. Flow: 0.8 ml/min.

Column #	∝	R_S
1	1.29	3.94
2	1.29	2.99
3	1.30	2.30
4	1.29	1.82

effect on ∝-value is very small but the effect on resolution is more pronounced, with a maximal effect observable at 40% MeOH. At lower proportions of methanol the resolution decreases as increased retention causes increased broadening of the peaks. The optimum percentage of methanol for our studies was determined to be 50% as this combined a good separation efficiency with a reasonable retention time to allow an adequate number of samples to be processed.

A brief study confirmed our assessment that the age of the column is important for these separations. Table 5 shows the effect of keeping all parameters

constant and examining the effect of changing column. Full records of the number of injections each column had been exposed to was not available but the clear trend was that α was not affected whereas resolution was. The oldest column, column #1, gave the best resolution and column #4, which was brand new, gave the lowest. This observation cannot adequately be explained by column ageing which might be expected, by hydrolytic processes, to expose more free silanols and reduce rather than increase efficiency.

From these results and those reported previously (**16**) it can be seen that borane complexation offers a convenient method for the enantiomeric analysis of substituted aza-bicyclic systems.

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