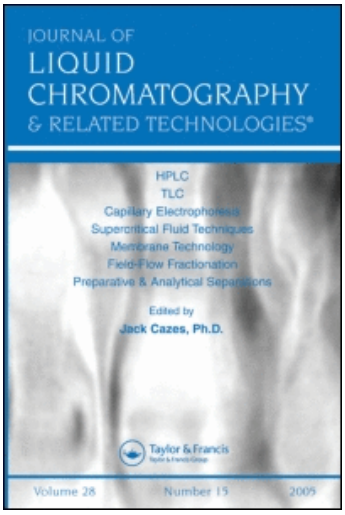

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An Investigation of Factors Influencing the Resolution of Chiral Alkyl Aromatic Amines on a Dinitrobenzoyl-(S)-Leucine HPLC Column

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AN INVESTIGATION OF FACTORS INFLUENCING THE RESOLUTION OF CHIRAL ALKYL AROMATIC AMINES ON A DINITROBENZOYL-(S)-LEUCINE HPLC COLUMN

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ABSTRACT

Normal phase HPLC methodology was utilised to obtain separation of (±)-1-(4-bromophenyl)ethylamine and (±)-1-(1-Naphthyl)ethylamine on a 3,5- dinitrobenzoyl-(S)-Leucine Pirkle type chiral stationary phase (CSP). A proposed retention mechanism (based on a three point interaction model) is outlined where the key interaction is steric attraction/repulsion between aryl groups on the solutes and the (S) Leucine group on the CSP.

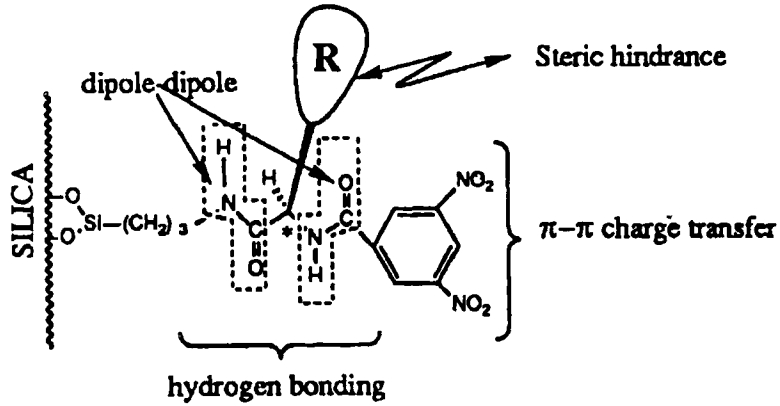


Figure 1. Interaction sites on π acidic Pirkle type chiral stationary phases.

INTRODUCTION

Over the last 15 years, the technique of choice for the determination of the enantiomeric composition in mixtures of enantiomers has been HPLC. There are many types of chiral stationary phases (CSP's) available and perhaps the most versatile is the brush type CSP, pioneered by the Pirkle group.[1] The brush type (also known as Pirkle type) stationary phases rely on a series of designed multiple interactions between the CSP and the solute enantiomers. Chiral separation occurs when one enantiomer can interact simultaneously at three points along the CSP. This three point interaction can occur by a number of different interaction modes including (a) π-π stacking, (b) dipole–dipole interactions, (c) hydrogen bonding, (d) steric attraction/repulsion (see Figure 1).

The principal interaction with brush-type columns is the interaction between a π acidic functionality and a π basic functionality, one of which is present on the CSP, the other being present or introduced onto the chiral analytes. The π acidic Pirkle bases usually consist of 3,5–dinitrobenzamides of α–amino acids, the most common examples being 3,5–dinitrobenzoyl-(D)-phenylglycine[2a] and (S)–N–3, 5–dinitrobenzoylleucine.[2b] The π acidic stationery phases have proved extremely useful in the monitoring of asymmetric synthesis[3] and in the analysis of commercial drugs such as Naproxen[4] and Nadolol.[5]

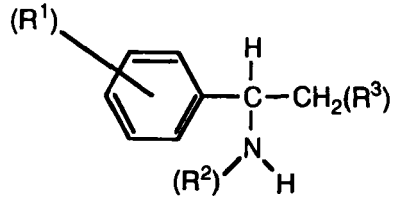


Figure 2. General structure of the alkyl aromatic amines.

The basis of chiral discrimination with brush–type columns is to generate diasteriomeric complexes between the CSP and the enantiomers. The stability of the diasterioisomers is influenced by attractive as well as repulsive interactions between the enantiomers and the CSP. The extent of the enantioselectivity on Pirkle type CSP's will be determined by the differences in stability between the diasterioisomers with the most stable diasterioisomeric complex being retained the longest.

In order to introduce π acid / π base charge transfer between π acidic CSP's and analytes, it is necessary to derivatize the analytes (if they do not already possess a π basic functionality) and introduce an N-aryl π basic group. Numerous acyl chlorides can be used to achieve this goal, the most commonly used reagent being α– naphthoyl chloride,[6,7] partly because of its strong π basicity and partly because it possesses a strong chromophore in the UV region and thus eases detection. The π basic group stacks on top of the π acidic group on the chiral stationery phase and holds the enantiomers in place to allow the other chiral interactions (hydrogen bonding, dipole/dipole interactions, steric attraction/repulsion) to effect enantioseparation.

The nature of Pirkle columns is such that they perform best in the normal phase mode, usually utilising n-hexane modified by a polar alcohol. It has been observed that in general less polar mobile phase systems lead to greater resolution using Pirkle CSP's and conversely that increasing the mobile phase polarity decreases retention and chiral discrimination between enantiomers.[8] It is also found that the nature of the alcohol modifier is important in effecting resolution of enantiomers, with 2-propanol being the modifier of choice for most applications.[5]

The purpose of this study is to observe the effects of hydrogen bonding and steric attraction/repulsion on the enantioselectivity of the DNBLeucine CSP. We studied a range of alkyl aromatic amines of the general structure shown in Figure 2. By varying the nature of the R groups, we can observe the effect of changing the R groups and determine the importance of hydrogen bonding and steric attraction/repulsion in effecting enantioseparation. In order to ensure that a π acidic/π basic interaction is present, we derivatized the amine using 3-toluoyl chloride because the analytes were judged to be sufficiently chromophoric for UV detection.

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using a Shimadzu LC-4A HPLC system fitted with a Rheodyne model 7125 injector with a 20 μL sample loop, a Shimadzu SPD-2AS UV detector set at 254 nm and a Shimadzu CR3A Chromatopac integrating recorder.

Materials

All solvents were of HPLC grade. 4M sodium hydroxide was prepared using reagent grade sodium hydroxide pellets (BDH Chemicals, Poole, Dorset, England). 3-toluoyl chloride (> 99% purity) was obtained from Aldrich (Gillingham, Dorset, England). All chiral amines were obtained from Arran Chemical Co. Ltd.. The HPLC column used (Bakerbond Chiral Phase DNBLeucine (covalent) 5 μm, 4.6 x 250 mm) was obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA).

Derivatization of Enantiomers

All enantiomers were derivatized as follows prior to HPLC analysis: Approximately 15 mg of the racemic analyte was weighed out accurately into a test tube. To this was added 1.5 mL of dichloromethane and 0.5 mL of 4M sodium hydroxide. The test tube was placed on a vortex mixer and vortexed for 30 seconds. To this was added 30 μL of 3–toluoyl chloride. Again the test tube

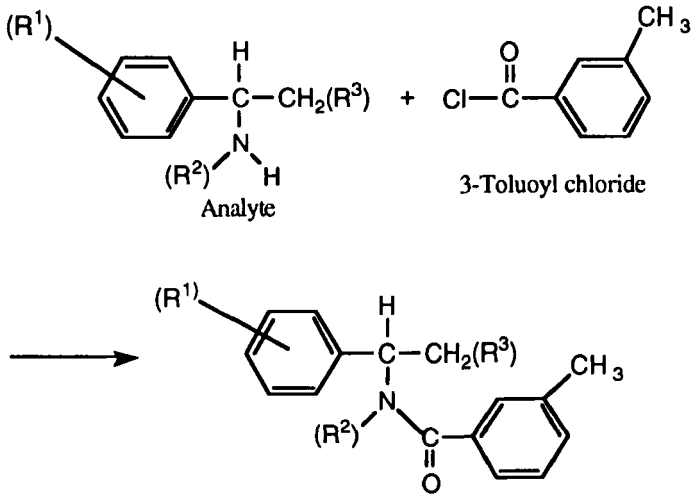


Figure 3. Derivatizing reaction between analytes and 3-toluoyl chloride.
was placed on the mixer and vortexed for at least 30 seconds (derivatization reaction is shown in Figure 3).

After 30 seconds two layers separated. 100 μL of the lower (organic) layer was transferred to a 25 mL volumetric flask. The flask was filled to volume using 2-propanol. This solution was filtered through a 0.45 μm membrane filter prior to analysis by HPLC. Linearity testing on (±)-1-(4-bromophenyl)ethylamine involved weighing different amounts of racemate and then following the derivatization procedure. Limits of detection involved looking at different weight ratios of (R)-(+) and (S)-(-)-1-(4-bromophenyl)ethylamine prior to carrying out derivatization procedure.

Important points to note for this procedure include;

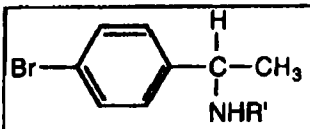
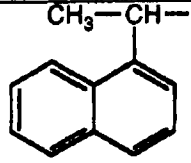
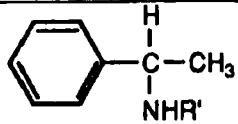
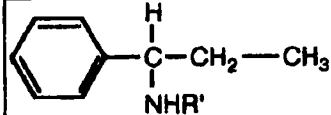
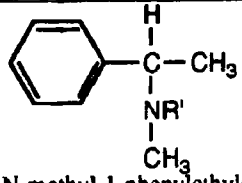
(i) All enantiomers were weighed out under nitrogen to prevent reaction with atmospheric carbon dioxide.

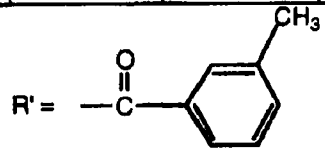
(ii) 3–Toluoyl chloride was also weighed out under nitrogen owing to its moisture sensitivity and its lacrymatory properties.

(iii) To ensure complete derivatization of the analyte, 30 seconds mixing at each stage of vortex mixing is essential.

Table 1

Summary of Results for the Range of Alkyl Aryl Amines

Compound	Capacity Factor (k)	Separation Factor (α)	Resolution
 1-(4-bromophenyl)ethylamine	4.17 (+)	1.21	1.81
 1-(1-naphthyl)ethylamine	5.06 (+)	1.2	2.05
 1-phenylethylamine	4.03 (±)	–	–
 1-phenylpropylamine	4.16 (±)	–	–
 N-methyl-1-phenylethylamine	2.31 (±)	–	–



Conditions: Flow rate = 0.5 mL / min; mobile phase 6% 2-propanol in hexane.

HPLC Conditions

The following HPLC conditions were used throughout . Column : DNBleucine (covalent); Mobile phase: 94 : 6 hexane - 2-propanol; Flow rate : 0.5 mL/min; Injection volume : 20μL; Detector : UV at 254 nm; Absorbance range : 1.0 AUFS; Attenuation : 6.

RESULTS

The chromatograms obtained were evaluated in terms of retention, separation (α values) and resolution. Retention is reported in terms of capacity factors (k) of the first eluted enantiomer. Where no separation has been effected, retention of the racemate is reported as the capacity factor for the racemate. Each sample was chromatographed under the same conditions of mobile phase, stationary phase and integrator conditions so that the data obtained could be evaluated in terms of enantiomeric structure and chromatographic behaviour under similar conditions. Table 1 gives a summary of results.

(±)-1-(4-Bromophenyl)ethylamine is resolved into its (R) and (S) forms under these chromatographic conditions (see Figure 4). The chromatogram shows two peaks with separation α =1.21, a resolution of 1.81 and for the first eluted isomer (R)-(+), a capacity factor of k = 4.17. The method is reproducible (Coefficient of Variation Area (R)-(+) = 3.01%, Coefficient of Variation Area (S)-(-) = 1.93%), linear in the range of 0 - 25 mg (see Figure 5) of sample derivatized as per procedure and has a limit of detection of 99.7 : 0.3 (R) : (S) using the method outlined.

Similarly resolution was obtained for (±)-1-(1-naphthyl)ethylamine which gave a capacity factor for the first eluted isomer (R)-(+) of k = 5.06. Separation (a =1.20) and resolution (= 2.05) were along the same magnitude of values for (±)-1-(4-bromophenyl)ethylamine.

However no resolution was obtained for (±)-1-phenylethylamine (k (±)= 4.03), (±)1-phenylpropylamine (k(±) = 4.16) or (±)-N-methyl-1-phenylethylamine (k(±) = 2.31) under the conditions stated.

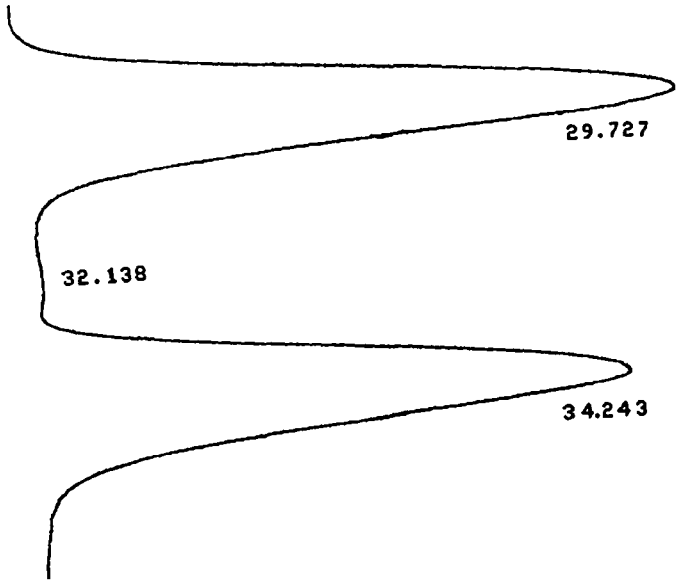


Figure 4. Chromatogram of resolved (±)-1-(4-bromophenyl)ethylamine, (R)-(+) isomer eluting first.

DISCUSSION

As can be seen from the results section, two out of the five racemic mixtures studied were resolved under the listed chromatographic conditions. Possible reasons for this are discussed below.

Separation of (±)-1-(4-bromophenyl)ethylamine and (±)-1-(1-naphthyl)-ethylamine

Working on the basis of the three point interaction model to enforce enantiomeric separation, the proposed interactions common to the separation of (±)-1-(4-bromophenyl)ethylamine and (±)-1-(1-naphthyl)ethylamine are shown in Figure 6. Figure 6 outlines the diasterioisomer formed between the CSP and (R)-(+)-1-(4-bromophenyl)ethylamine. As can be seen, the three points of interaction are (I) π-π stacking between the dinitrobenzoyl group on the

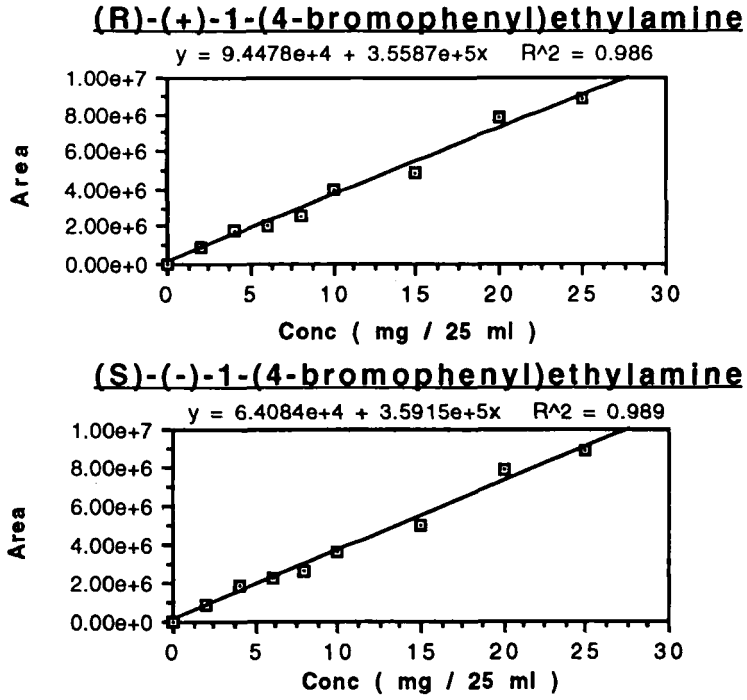


Figure 5. Linearity graphs for (±)-1-(4-bromophenyl)ethylamine.

stationary phase and the 3-toluoyl group on the analyte, (ii) Hydrogen bonding between the aminyl hydrogen on the analyte and the carboxyl oxygen on the CSP (with possible additional hydrogen bonding between the carboxyl oxygen on the analyte and the aminyl hydrogen on the CSP) and (iii) The steric interactions associated with the relative geometry and size of the methyl group and the 4-bromophenyl group on the chiral carbon of the analyte and the (S)-Leucine group on the chiral stationary phase. It can be clearly seen that if the geometry of the methyl and 4-bromophenyl groups are reversed (as is the case in (S)-(-)-1-(4-bromophenyl)ethylamine then different steric interactions will occur between the analyte and the (S)-Leucine group on the CSP.

A similar argument can be applied to (±)-1-(1-naphthyl)ethylamine, where the resolution of the (R)-(+) and (S)-(-) enantiomers is slightly greater.

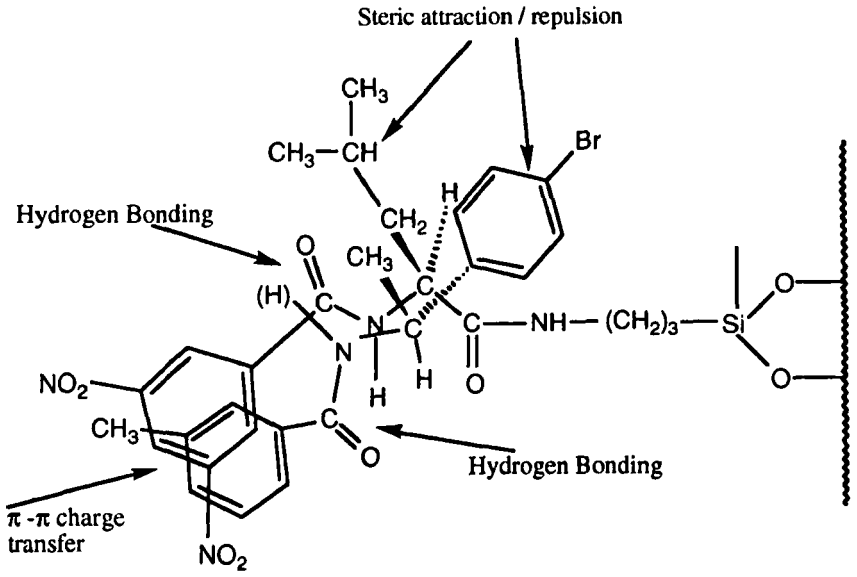


Figure 6. Resolution of (R)-(+)-1-(4-bromophenyl)ethylamine on DNB-(S)-Leucine. The key interactions between the stationary phase and analyte are highlighted.

Again the principle interactions are (i) π - π stacking between the dinitrobenzoyl and 3-toluoyl groups, (ii) hydrogen bonding between aminyl hydrogens and carboxyl oxygens and (iii) steric attraction and repulsion relating to the stereochemical configuration of the (R)-(+) and (S)-(-) enantiomers. The increase in resolution relative to (±)-1-(4-bromophenyl)ethylamine seems to be due to the increased size of the naphthyl group over the 4-bromophenyl group. Thus it seems possible that the size of the aryl group is influencing separation, a point which is further discussed in later sections.

One problem which was initially encountered during the separation of (±)-1-(4-bromophenyl)ethylamine was a lack of reproducibility in relation to replicate injections. Complete baseline resolution between the enantiomers was achieved in moving from an initial mobile phase composition of 88 : 12 hexane / 2-propanol to 94 : 6 hexane / 2-propanol, which, although it lengthened retention times, made for good reproducibility for the separation method. This

point highlights the importance of achieving the correct balance between resolution and retention on CSP's, which will depend on the application of the method. The linear range (0 -25 mg) and limit of detection (99.7 : 0.3) for (±)-1-(4-bromophenyl)ethylamine make this separation method very applicable to quality control testing of the enantiomeric composition of 1-(4-bromophenyl)ethylamine in batch manufacturing.

(±)-1-Phenylethylamine and (±)-1-Phenylpropylamine

In order to investigate the magnitude of the steric attraction / repulsion interaction on the enantioselectivity of the DNBLeucine stationary phase, both of the above compounds were investigated under the same chromatographic conditions as used for (±)-1-(4-bromophenyl)ethylamine and (±)-1-(1-naphthyl)ethylamine. (±)-1-Phenylethylamine possesses the same functionality's as the resolved enantiomers except for the size of the aryl group. The fact that it lacks a bulky moiety on the phenyl ring, and the fact that it has not been resolved on DNBLeucine under the stated chromatographic conditions is further evidence to suggest that the size of the aryl group in the aryl alkyl amines could be the key to their separating ability on the DNBLeucine stationary phase. The fact that (±)-1-phenylpropylamine has not been resolved suggests that it is primarily the aryl group and not the alkyl group which is the discriminatory factor in enantioseparation on this stationary phase. It is interesting to note that the extent of retention of (±)-1-phenylethylamine and (±)-1-phenylpropylamine on this CSP is similar, which is in good correlation with their chemical structure. (±)-1-Phenylpropylamine is retained longer because of its extra methylene group. The results seem to suggest that the phenyl group on the amines is not sufficient alone to provide chiral discrimination. This situation can be highlighted in Figure 7.

Figure 7(a) shows the case where the phenyl group attached to the chiral centre of the analyte is not substituted. The two methyl groups attached to the methyne group on the end of the leucine side chain on the CSP have unrestricted rotation around the methyne group. This is the case both (a) where the alkyl group on the analyte is in the same plane of space as the (S)-leucine side chain on the CSP and (b) where the phenyl group on the analyte is in the configuration shown in Figure 7(a).

The situation where the aryl group on the analyte is larger (e.g. 4-bromophenyl) is shown in Figure 7(b). When the alkyl group on the analyte is

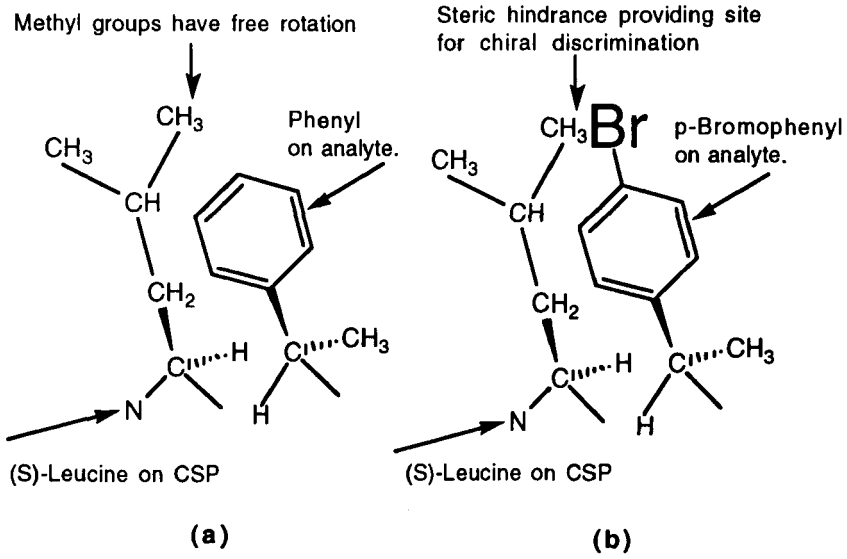


Figure 7. (a) Interaction when the phenyl group on the analyte is in the same plane as the (S)-Leucine on the CSP, (b) Interaction when the p-Bromophenyl group on the analyte is in the same plane as the (S)-Leucine group on the CSP.

in the same spatial plane as the (S)-leucine group, the methyl groups again have free rotation. However, in the case of the substituted phenyl and the (S)-leucine being in the same plane (as in Figure 7(b)), it can be clearly seen that the methyl groups have restricted rotation around the methyne group due to the bulky bromo group present on the phenyl ring of the analyte.

It is possible that here is the basis for chiral discrimination. The enantiomeric configuration for substituted aryl alkyl amines shown in Figure 7(b) will be the least retained enantiomer because it forms the least stable diasterioisomer with the DNBLeucine stationary phase.

The most retained enantiomer will be that which allows free rotation of the two methyl groups around the methyne group on the (S)-leucine portion of the CSP.

(±) N-Methyl-1-phenylethylamine

As seen, no separation of (±) N-methyl-1-phenylethylamine has occurred under the given chromatographic conditions. This is again most probably due to the lack of substitution on the phenyl ring. With this racemate there is limited hydrogen bonding capacity. The only site for hydrogen bonding is between the carboxyl oxygen on the analyte and the aminyl hydrogen on the chiral stationary phase. This limited hydrogen bonding capability is highlighted in the capacity factor obtained for this racemate (2.31) as compared to the capacity factor obtained for (±)-1-phenylethylamine (4.03), where hydrogen bonding occurs between the aminyl hydrogen on the analyte and the carboxyl oxygen on the stationary phase.

Overall, the experimental data highlights the three point interaction criteria which must be met in order to achieve enantioseparation. With regards to our choice of analytes and stationary phase, the three interactions are

(i) the π basic site on the analyte stacking on the π acidic site of the CSP,

(ii) hydrogen bonding between aminyl hydrogens and carboxyl oxygens and

(iii) steric interactions between substituted phenyls on the analyte and the (S) leucine group on the stationary phase.

The latter interaction is indicated as the key point in distinguishing between enantiomers.

ACKNOWLEDGEMENTS

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