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# Enantiomeric resolution of derivatized DL-amino acids by high-performance liquid chromatography using a β-cyclodextrin chiral stationary phase: A comparison between derivatization labels

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**Abstract**

A chiral stationary phase with immobilized β-cyclodextrin was used for the liquid chromatographic separation of amino acid derivatives into their enantiomers. Several reagents differing in size, structure and linking group (i.e. the isothiocyanates PITC, NITC, DNITC and DABITC, the activated ester AQC, and the sulfonyl chlorides DNS-Cl and DABS-Cl) were used to derivatize the amino groups of α-amino acids prior to separation. A compilation of the selectivity data obtained with the different labels is given as guideline in selecting appropriate labels for a particular separation problem.

## 1. Introduction

The analysis of amino acid enantiomers belongs to the very early examples of chromatographic separations of enantiomers and still plays an important role. The amount of certain D-amino acids in various types of samples has found to be of relevance for many aspects of biochemical and molecular biological research [1,2], in the control of food and beverages [3,4], as well as in age determination [5–7]. Various chiral separation methods are available for the enantiomeric resolution of amino acids. Methods based on chiral ligand-exchange [8–11] and on the formation of inclusion complexes with cyclodextrins (CD) [12–14] have received the most attention. Cyclodextrins were employed in their native form or after modification by methylation, acetylation or by binding other structural moieties to the hydroxyl groups of the glucose [15–17]. Cyclodextrin systems, especially when using stationary phases with immobilized cyclodextrins, are easy to use, and separations can be carried out in a rather simple, and when employing aqueous/alcoholic mixed eluents, rather inexpensive way. This method turned out to be of particular interest for many separation problems. Furthermore, it can easily be combined with a non-chiral preseparation in a two-column set-up [18–21].

Bonded β-CD stationary phases are commercial available. These phases are not necessarily

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identical in their separation characteristics, as they might use different linking groups between spacer and selector and exhibit different selector densities. The enantioselectivity coefficients may vary to some extent when using $\beta$-CD stationary phases from different suppliers. Recently, a new material has become available from the E. Merck company under the trade name ChiraDex [22]. This stationary phase is used for the present investigation.

Amino acids are usually analyzed after pre-column derivatization in order to allow more sensitive detection and/or to make them more suitable for the chromatographic separation. The chiral separability of the derivatives is generally dependent on the type of derivatization, especially when using chiral selectors operating in a host–guest mechanism. Separations of amino acids as dansyl derivatives and OPA derivatives by means of $\beta$-CD-chiral stationary phases (CSPs) have been reported [12,14,15,21,23]. The popular FMOC derivatives cannot be separated by $\beta$-CD systems, with a few exceptions; they are, however, readily separable in $\gamma$-CD systems [24].

There are a number of other derivatization reagents available which can be utilized for the enantioseparation of amino acids in $\beta$-CD systems, and the present paper deals with an investigation of the enantioseparation attainable by means of some of these derivatizations. The investigation covers the following derivatization reagents: 5-dimethylaminonaphthalene-1-sulfonyl chloride (DNS), 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DABS), phenyl isothiocyanate (PITC), 1-naphthyl isothiocyanate (NITC), 4-dimethylaminoazobenzene-4′-isothiocyanate (DABITC), 4-dimethylamino-1-naphthyl isothiocyanate (DNITC), and the newly introduced 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) [17,25]. The structures of the amino acid derivatives are shown in Fig. 1. With the DNS and DABS reactions, the labels are linked by a sulfonamide group, with the others either by a thiourea (PITC, NITC, DNITC, DABITC) or an urea group (AQC). Many of these derivatives offer specific advantages (e.g. high absorbance, strong fluorescence, fast reaction rate, stable products, minimum peak interferences caused by the reagent) and suffer from specific limitations (e.g. limited stability, limited degree of reaction, side reactions, slow reaction rates, etc.). The aim of the present paper is to offer some guidelines for selecting the labels appropriate for a particular amino acid separation problem, and, secondly, to contribute to the discussion of the effect of the derivatization labels on chiral recognition by $\beta$-CD host–guest complexation.

## 2. Experimental

### 2.1. Apparatus

The liquid chromatographic system consisted of a pump (L-6200, Intelligent Pump, Merck-Hitachi, Tokyo, Japan), a syringe valve injector (Rheodyne 7125, Cotati, CA, USA) equipped with a 20-$\mu$l loop and an UV detector (L-4000, Merck-Hitachi) connected to an integrator (D-2000, Chromatointegrator, Merck-Hitachi). Column temperature was controlled by a water jacket around the column using a thermostat (LTD, 6, Grant, Herts, UK).

### 2.2. Columns

A stainless-steel column (250 × 4.0 mm I.D.) prepacked with immobilized $\beta$-CD, mean particle diameter 5 $\mu$m, (Chiradex, E. Merck, Darmstadt, Germany) was used.

### 2.3. Chemicals

Methanol (MeOH) and acetonitrile of LiChrosolv grade and triethylamine (TEA) of pro analysi grade were obtained from E. Merck. Water was twice distilled from a quartz apparatus and processed through an Elgastat UHQ apparatus (Elga, High Wycombe Bucks, UK). The eluents were premixed and degassed in an ultrasonic bath before use. Standard samples of free amino acids as well as DNS amino acids

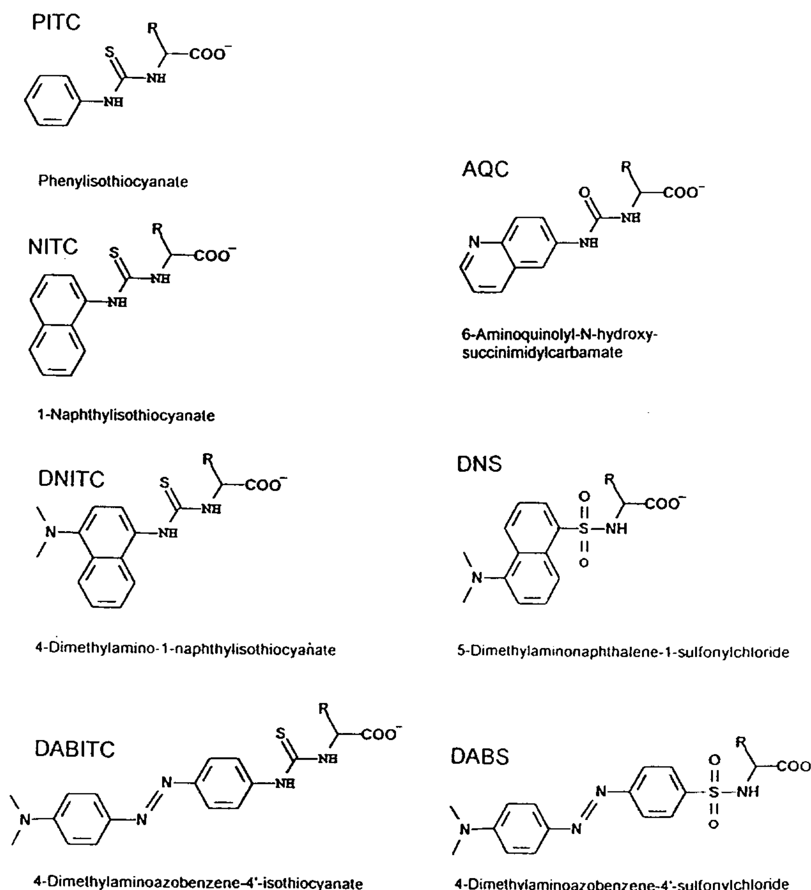


Fig. 1. Chemical structures of the amino acid derivatives investigated, R = amino acid side chain. Abbreviations refer to the reagents employed.

were obtained from Sigma (Deisenhofen, Germany).

The derivatization reagents 5-dimethylaminonaphthalene-1-sulfonyl chloride (DNS-Cl), PITC, and NITC were purchased from Sigma. DNITC, DABITC and 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl) were purchased from Fluka (Buchs, Switzerland). AQC with the trade name AccQ-Fluor$^{TM}$ was obtained from Waters (Bedford, MA, USA).

Boric acid ($H_3BO_3$), ammonium acetate ($NH_4Ac$), sodium hydroxide (NaOH), disodium hydrogenphosphate ($Na_2HPO_4$), sodium dihydrogenphosphate ($NaH_2PO_4$), pyridine, acetone, and hydrochloric were obtained from E. Merck in pro analysi grade.

### 2.4. Derivatization procedures

*DNS derivatives [26]*

Approximately 5 mg of the amino acids were dissolved in 460 $\mu$l borate buffer (0.1 *M* boric acid, adjusted to pH 9 by addition of NaOH) and 300 $\mu$l of 0.1 *M* DNS-Cl dissolved in acetone were added. The sample was kept in the dark for 2 h at room temperature to complete the reaction. Then the sample was dried by vacuum centrifugation, washed once by adding 500 $\mu$l of a mixture of acetone–1 *M* HCl (19:1, v/v) and centrifuged for 5 min to eliminate the unreacted reagent and insoluble salts. The liquid was again dried by vacuum centrifugation. After resolubilization of the derivatized amino acids in the

mobile phase the samples were ready for injection.

*DABS derivatives [27]*

Approximately 5 mg of the amino acid were dissolved in phosphate buffer (0.1 *M* $Na_2HPO_4$ adjusted with 0.1 *M* $NaH_2PO_4$ to pH 7), and 100 μl reagent (100 mg DABS-Cl in 10 ml acetone) were added. The sample was kept at 65°C for 30 min to complete the reaction and then dried by vacuum centrifugation. Resolubilization as above.

*PITC-, NITC-, DABITC- and DNITC derivatives [28]*

A buffer solution was prepared by mixing 5 ml methanol, 2.5 ml pyridine, 1 ml triethylamine and 1.5 ml water. A 500-μl volume of 0.1 *M* solution of the reagents dissolved in acetonitrile was added to the buffer. A 200-μl aliquot of this resulting reagent solution was added to a few milligrams of the amino acids. To complete the reaction, samples were kept at room temperature for 5–10 min under protection from light. The samples were dried by vacuum centrifugation and aliquots were resolubilized in the mobile phase.

*AQC derivatives [29,30]*

"AccQ.Fluor Reagent" was completely reconstituted in 1 ml "AccQ.Fluor Reagent Diluent", yielding approximately 10 m*M* 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) in acetonitrile. The sample was reconstituted in 70 μl "AccQ.Fluor Borate Buffer" and 20 μl "AccQ.Fluor Reagent" was added. After vortexing, the samples were allowed to stand for one min at room temperature and then heated in an oven for 10 min at 55°C.

### 2.5. Chromatographic conditions

The mobile phases consisted of aqueous buffer solutions (0.1 *M* ammonium acetate and 0.1% v/v TEA and adjusted to pH 5.5–6.5 with acetic acid) which were mixed with methanol. The methanol concentrations were kept constant within the series of the various types of derivatives. They are specified in the tables and figures given. Temperature was carefully maintained at 20 ± 0.1°C. Flow-rates of 0.5 ml/min were used, unless indicated otherwise. Amounts of injected amino acid derivatives ranged between 20 and 200 ng. The detection wavelength was 254 nm for all derivatives except for DABS, where 456 nm was used.

Plate number calculation was based on peak-width data measured at 0.607 of the peak hight.

## 3. Results and discussion

### 3.1. Selection of derivatization reagent

Enantioselectivity data (separation factors) and capacity factors of the derivatized amino acids measured under the chosen experimental conditions (water–methanol mixed mobile phases, modifier content between 40 and 90% v/v as specified, temperature of 20°C) are listed in the Tables 1 and 2. The experimental conditions were kept constant for a series of amino acids carrying an identical derivatization label. Methanol concentrations were chosen in order to obtain rather short retention times, i.e. 10 to 20 min for most compounds applying a flow-rate of 0.5 ml/min. A compilation of those amino acid derivatives which, under the chosen conditions, can completely or almost completely be resolved to baseline is given in Table 3. Appropriate excitation and emission wavelengths for the fluorescence detection are shown in Table 4. Tables 1–3 are aimed to provide a guideline facilitating the selection of derivatization labels well suited for the solution of a given separation problem. The following résumé can be drawn from the given data.

(i) Amino acids carrying a dansyl-label are enantioseparated in all instances, except for DNS-DL-Trp. Baseline separation is achieved under the chosen experimental conditions if the separation factor is higher than ca. 1.18. Under these conditions the column exhibits approximately 3000–4000 theoretical plates per 25 cm. Due to the high methanol concentration rather low capacity factors are obtained (between 1 and

Table 1
Enantioselectivity coefficients, $\alpha = k'_2/k'_1$[a], for differently labeled DL-amino acids at 20°C

| Amino acid | DNS | DABS | PITC | NITC | DNITC | DABITC | AQC |
|---|---|---|---|---|---|---|---|
| Ala | 1.08 | 1.14 | 1.17 | 1.06 | 1.00 | 1.00 | 1.08 |
| Val | 1.22 | 1.64 | 1.30 | 1.10 | 1.00 | 1.00 | 1.00 |
| Leu | 1.75 | 1.19 | 1.00 | 1.14 | 1.40 | 1.43 | 1.39 |
| Ile | 1.32 | | 1.21 | 1.00 | 1.08 | 1.10 | 1.13 |
| Pro | 1.10 | 1.00 | 1.14 | 1.22 | 1.10 | 1.44 | 1.10 |
| Met | 1.24 | 1.31 | 1.06 | 1.08 | 1.18 | 1.19 | 1.08 |
| Cys | 1.15 | 1.00 | 1.82 | 1.07 | 1.00 | 1.33 | 1.08 |
| Ser | 1.16 | 1.15 | 1.11 | 1.07 | 1.00 | 1.00 | 1.07 |
| Thr | 1.29 | 1.13 | 1.25 | 1.06 | 1.00 | 1.00 | 1.07 |
| Lys | 1.10 | 1.20 | 1.28 | 1.14 | 1.11 | 1.12 | 1.16 |
| Lys[b] | | 1.25 | | | 1.32 | | 1.07 |
| Arg | 1.16 | 1.22 | 1.24 | 1.13 | 1.13 | 1.14 | 1.13 |
| Asn | 1.11 | 1.10 | 1.25 | | 1.17 | 1.18 | 1.11 |
| Gln | 1.14 | 1.16 | 1.16 | | 1.04 | 1.07 | 1.06 |
| Asp | 1.13 | | 1.24 | | 1.10 | | 1.20 |
| Glu | 1.15 | | 1.14 | 1.04 | 1.00 | 1.05 | 1.00 |
| Phe | 1.43 | 1.13 | 1.21 | 1.13 | 1.14 | 1.16 | 1.13 |
| Trp | 1.00 | 1.19 | 1.24 | 1.35 | 1.00 | 1.00 | 1.04 |
| His | 1.07 | 1.14 | 1.18 | 1.12 | 1.08 | 1.09 | 1.06 |
| Tyr | 1.25 | 1.20 | 1.06 | 1.00 | 1.10 | 1.11 | 1.25 |

Chromatographic conditions: immobilized $\beta$-cyclodextrin stationary phase (ChiraDex). Eluent composition: DNS-derivatives, aqueous buffer [(0.1 *M* $NH_4Ac$, 0.1% TEA, pH 5.5)–MeOH (30:70, v/v)]; DABS-derivatives, aqueous buffer [(0.1 *M* $NH_4Ac$, 0.1% TEA, pH 5.5)–MeOH (10:90, v/v)]; PITC-, NITC-, DABITC-, DNITC-derivatives, aqueous buffer [(0.1 *M* $NH_4Ac$, pH 6.5)–MeOH (60:40, v/v)]; AQC-derivatives, aqueous buffer [(0.1 *M* $NH_4Ac$, 0.1% TEA; pH 6.5)–MeOH (50:50, v/v)].

[a] $k'_1$ and $k'_2$ are the capacity factors of the first and second enantiomer, respectively. The hold up time was obtained by assuming a column porosity of 0.75.

[b] $N^{\alpha}$,$N^{\epsilon}$ derivatized compound.

3), except for the two acidic amino acids Asp and Glu.

(ii) Phenylisothiocyanate-derivatized amino acids are enantioseparated in all instances, except for PITC-DL-Leu. Again, the capacity factor values are low under the selected mobile phase conditions allowing analysis times of ca. 20 min at the given flow; the only exceptions were Asp and Glu and the two aromatic amino acids Phe and Trp. The highest enantioselectivity coefficients for a particular amino acid are usually achieved by using either PITC or DNS. PITC turned out to be the best derivatization agent for Ala and Glu, the two amino acids which are of special interest in the context of D-amino acid analysis in bacterial cell membranes. Separations of a few PITC amino acids are shown in Fig. 2.

(iii) DABS-labeled amino acids are enantioseparated in many instances. For DL-Met and DL-Val the best separations have been achieved using this label. As general drawback, these derivatives are eluted with long retention times, and the large capacity factors could not be reduced by variation of the eluent composition.

(iv) NITC-, DNITC- and DABITC-derivatized amino acids, all of them exhibiting a thiourea linkage group, are resolved with separation factors generally lower than those of the corresponding PITC analytes, with the exceptions of Leu, Pro, Met, Tyr and Trp. NITC-DL-Trp and DABITC-DL-Pro were separated with the highest separation factors achievable amongst all the labels investigated. Nevertheless, except for the analytes mentioned, there is no observed advantage in the use of these types of derivatizations over PITC with respect to separation selectivity. Clearly, however, the fluorescence activity of the NITC, DNITC and DABITC moiety allows one

Table 2
Capacity factors of the second eluted enantiomer, $k'_2$, of differently labeled amino acids, at 20°C

| Amino acid | DNS | DABS | PITC | NITC | DNITC | DABITC | AQC |
|---|---|---|---|---|---|---|---|
| Ala | 2.52 | 9.57 | 3.01 | 2.93 | 1.98 | 3.18 | 2.45 |
| Val | 2.03 | 12.34 | 3.34 | 2.79 | 2.19 | 2.72 | 2.15 |
| Leu | 2.54 | 14.05 | 5.00 | 4.90 | 4.58 | 6.86 | 4.89 |
| Ile | 1.68 | | 4.22 | 3.32 | 2.84 | 4.19 | 3.16 |
| Pro | 1.40 | 18.21 | 3.13 | 3.81 | 3.79 | 6.92 | 2.51 |
| Met | 1.33 | 11.37 | 3.86 | 3.94 | 3.32 | 4.89 | 2.90 |
| Cys | 2.99 | 8.32 | 6.68 | 4.91 | 3.58 | 5.58 | 2.40 |
| Ser | 1.37 | 13.39 | 3.11 | 3.17 | 2.28 | 3.39 | 2.50 |
| Thr | 1.46 | 10.10 | 2.93 | 2.77 | 2.14 | 3.00 | 2.29 |
| Lys | 1.22 | 5.23 | 1.61 | 1.48 | 1.63 | 1.86 | 4.86 |
| Lys[a] | | 21.28 | | | 4.97 | | |
| Arg | 0.84 | 3.68 | 1.42 | 1.42 | 1.60 | 1.85 | 1.44 |
| Asn | 1.68 | 19.89 | 3.68 | | 3.06 | 4.65 | 2.46 |
| Gln | 1.38 | 12.84 | 2.69 | | | 3.39 | 2.05 |
| Asp | 18.03 | | 29.69 | | 19.26 | | 16.64 |
| Glu | 9.17 | >30.00 | 17.65 | 15.82 | 11.66 | 20.44 | 10.08 |
| Phe | 2.42 | 13.23 | 8.11 | 8.54 | 4.66 | 7.07 | 7.06 |
| Trp | 1.63 | 14.65 | 7.87 | 10.34 | 5.10 | 7.75 | 4.82 |
| His | 1.66 | 14.75 | 3.00 | 3.12 | 2.96 | 3.64 | 2.50 |
| Tyr | 2.67 | 15.30 | 5.33 | 4.94 | 2.95 | 4.37 | 5.19 |

Chromatographic conditions as in Table 1.
[a] $N^{\alpha},N^{\epsilon}$ derivatized compound.

Table 3
Guidelines for the separation of differently labeled amino acids by use of the $\beta$-cyclodextrin chiral stationary phase

| Amino acid | DNS | DABS | PITC | NITC | DNITC | DABITC | AQC |
|---|---|---|---|---|---|---|---|
| Ala | (+) | + | + + | | | | (+) |
| Val | + | + + | + | (+) | | | |
| Leu | + + | (+) | | (+) | + | + + | + |
| Pro | (+) | | (+) | + | | + + | |
| Met | + | + + | | | (+) | + | |
| Cys | | | + + | | | + | |
| Ser | + [a] | + | (+) | | | | |
| Thr | + [a] | (+) | + | | | | |
| Lys | (+) | + | + + | | + | | |
| Arg | + | + + | + + | | (+) | (+) | (+) |
| Asn | (+) | (+) | + | | (+) | + | |
| Gln | (+) | (+) | + + | | | | |
| Asp | (+) | | + | | (+) | | + + |
| Glu | + | | + + | | | | |
| Phe | + + | (+) | + | + | (+) | + | (+) |
| Trp | | + | + + | + | | | |
| His | | (+) | + + [a] | (+) | | | |
| Tyr | + + | + | | | | (+) | + + |

+ = complete resolution; (+) = nearly baseline resolution; + + = best separation achieved for a particular amino acid. Chromatographic conditions as given in the Tables 1 and 2.
[a] Lower methanol concentration recommended than given in Table 1.

Table 4
Excitation and emission wavelengths to be considered for the various fluorescent derivatization labels

| Derivatization | Wavelength (nm) | |
|---|---|---|
| | Excitation | Emission |
| DNS | 325 | 350[a] |
| NITC | 250 | 410 |
| DNITC | 240 | 490 |
| DABITC | 240 | 480 |
| AQC | 250 | 395[b] |

[a] Ref. [31].
[b] Ref. [17].

to take advantage of improved detection sensitivity.

(v) AQC derivatives, exhibiting an urea linkage, are separated with separation factors very similar to those obtained with the thiourea-linked labels NITC, DNITC and DABITC. This means that in most instances the selectivity coefficients achieved are not sufficiently high to achieve baseline separation of the enantiomers under the given conditions (cf. Table 3). It might be of interest that enantioseparation of AQC amino acids in the reversed-phase mode (using eluent mixtures of methanol and aqueous buffer) is reported for Leu only [17] when using a different type of stationary phase where native $\beta$-CD is immobilized via epoxyalkylsiloxanes.

Examples for amino acid separations readily achievable after DABS-, NITC- and AQC derivatization are illustrated by the chromatograms in Fig. 3.

### 3.2. Influence of eluent composition

All data given in the Tables are collected at constant eluent composition within a series of analytes. It can be assumed that the chosen separation conditions were not necessarily optimum for all analytes. A certain, though limited, improvement of the resolution can still be expected in several instances when optimizing

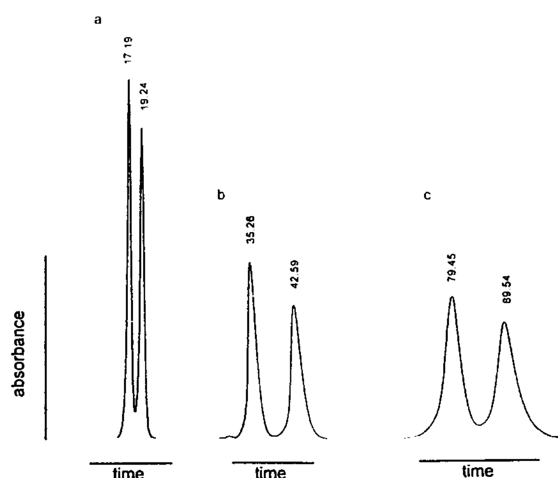


Fig. 2. Section of chromatograms of PITC-derivatized amino acids (a) DL-alanine, (b) DL-tryptophane, (c) DL-glutamic acid. Retention times in minutes. Flow-rate: 0.5 ml/min; eluent composition, aqueous buffer [0.1 $M$ $NH_4Ac$ pH 6.5–MeOH (60:40, v/v)]. Other chromatographic conditions as specified in Experimental.

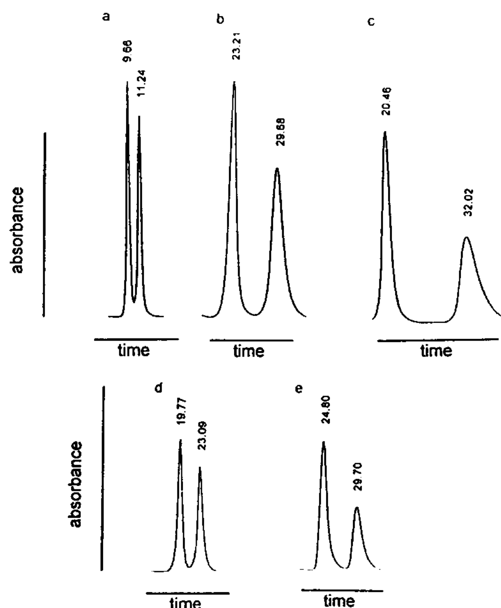


Fig. 3. Sections of chromatograms of selected DABS-, AQC- and NITC-derivatized amino acids readily separable under the chosen eluent and temperature conditions. (a) DABS-DL-arginine, (b) DABS-DL-methionine, (c) DABS-DL-valine, (d) NITC-DL-proline, and (e) AQC-DL-tyrosine. Eluent conditions: (a,b,c) aqueous buffer [0.1 $M$ $NH_4Ac$ pH 5.5–methanol (10:90, v/v)]; (d,e) aqueous buffer [0.1 $M$ $NH_4Ac$ pH 6.5–methanol (60:40 and 50:50, v/v)], respectively. All other conditions as specified in Experimental.

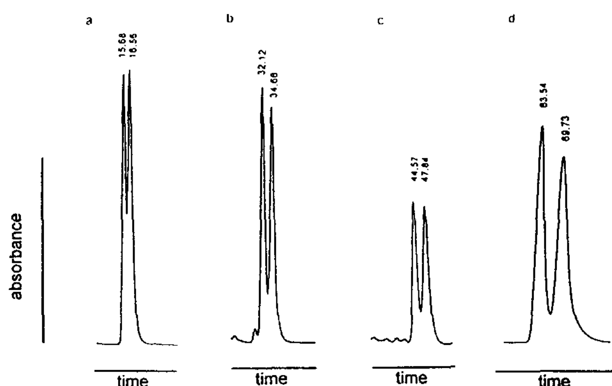


Fig. 4. Section of chromatograms of AQC-DL-alanine using the following chromatographic conditions: (a) 50% (v/v) methanol, 20°C, 0.5 ml/min; (b) 20% (v/v) methanol, 20°C, 0.7 ml/min; (c) 10% (v/v) methanol, 20°C, 0.8 ml/min; (d) 20% (v/v) methanol, 10°C, 0.5 ml/min.

the eluent composition and exploiting temperature effects. An example of the influence of modifier concentration and temperature is given in Fig. 4 for AQC-DL-alanine.

Analytes with sulfonamide linked labels, i.e. DNS- and DABS amino acids, are found to require a higher modifier concentration in order to be eluted with retention times comparable to analytes carrying the corresponding thiourea linked labels. The two acidic amino acids Glu and Asp exhibit very high capacity factors, $k'$, in all instances. For these analytes faster analysis will be obtained by using $\beta$-cyclodextrin as mobile phase additive [21].

## 4. Conclusions

The chromatographic resolution which can be achieved for a given pair of enantiomers is found to be significantly dependent on the derivatization label used. For the selection of the derivatization labels most appropriate for the enantiomeric resolution of particular amino acids, the selectivity and capacity factor data in the Tables 1–3 can serve as a guideline.

All derivatizations discussed here, except PITC derivatization, yield fluorescent derivatives, thus enabling the use of the significantly higher sensitivity and better selectivity of fluorescence detection if required. Another advantage of the AQC reagent is that its hydrolysis products are only weakly fluorescent and do not cause peak interferences in the chromatogram. Concerning the effort and time requirements for the derivatization procedure, there is a significant advantage in using the AQC reagent, as the whole derivatization procedure is completed in a few minutes and is easy to handle. Considering the stability of the products, the thiourea and urea derivatives obtained by the various isothiocyanate and the AccQ-Tag reagents respectively, are stable for weeks, even in solution. The stability of DNS amino acids in solution is much worse and cooling at −20°C is required for longer storage.

No simple rules can be given regarding the relationship between the structural features of the analytes and the achieved enantioselectivity. This relationship is apparently complex for separations based on a host–guest mechanism. Small changes in the analyte structure often result in major changes in the enantioselectivity coefficients. Therefore, only a few remarks are made on the given results: (i) Sulfonamide and thiourea linking groups are not comparable at all with respect to their effect on chiral discrimination. This is probably due to both electronic and steric reasons. Large differences are seen in the pattern of the selectivity data when comparing the series of DNS derivatives with that of the DNITC derivatives although these compounds have very similar fluorescent moieties; this also holds for a comparison between the series of DABS- with that of DABITC derivatives. In the majority of cases the derivatives carrying a sulfonamidic linking group exhibit the higher separation factors. (ii) The separation factors obtained for the DABITC- and DNITC derivatives of the same amino acid are very similar although their fluorescent moieties are very different with respect to size and shape. These similarities are also found for the AQC derivatives. In these cases thiourea and urea linking groups are comparable with respect to chiral recognition. On the other hand, the data of the PITC derivatives show very few similarities to those of the other thiourea compounds. We

hypothesize that the global orientation adopted by most of the amino acid derivatives in their interactions with the β-CD selector is similar for the DNITC-, DABITC- and AQC derivatives, but different for the PITC derivatives.

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