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Titel der Diplomarbeit

„Characterisation and Synthesis of
chiral zwitterionic stationary phases by HPLC"

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„Charakterisierung und Synthese von
chiralen zwitterionischen stationären Phasen mittels HPLC"

Verfasser

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1. Introduction

1.1. Biological aspects of chirality

The importance of enantioselective analysis in organic chemistry, food chemistry, toxicology, agrochemical industry and in many other fields is rising. Nowadays these analyses are inevitable for research and development of chiral compounds. In pharmaceutical industry it is important to determine if there is an enantiomeric impurity of the chiral drug, also in organic synthesis, when enantiomeric compounds are used as chiral synthons. Enantiomers have the same constitution but another spatial orientation of atoms or of atom-groups. Enantiomers are not superimposable with their mirror images. They have the same physical (except for the ability to rotate) and chemical properties.

Speaking of chiral molecules, chirality plays a decisive role in biological systems. Chiral compounds have different activity and kinetic profiles of the stereoselective binding to chiral targets like receptors, enzymes, ion channels or drug transporters[1]. Therefore, enantiomeric drugs can have different therapeutic effects on human beings. Enantiomeric drugs that show positive effects on human beings are called eutomers and the other one which have no or a negative effect (toxic) are called distomers.

One enantiomer can exhibit undesirable side-effects or can act as a competitive antagonist of the other and the drug would be less potent in the human body. Therefore, such a substance must be separated from each other when used as a drug.

There are also many pharmacokinetical differences like in protein binding, metabolizing and also pharmacodynamical differences. Single enantiomers of chiral active pharmaceutical ingredients may differ in adsorption, distribution, metabolism and excretion. Furthermore, pharmacological or toxicological effects play a role. For instance, in the case of α-methyl DOPA there is different pharmacological activity. (S)-α-methyl DOPA has an antihypertensive activity and (R)-α-methyl DOPA is inactive (an isomeric ballast). Furthermore, enantiomers can have similar pharmacological activity, but different quantitative potenties (e.g. as β-adrenetic blocking agents do).

A lot of flavouring agents are chiral. Their flavour and taste depend on the configuration of the corresponding enantiomer. For instance, limonene is used as a flavor. The (R)-enantiomer has an orange odour whereas the (S)-enantiomer has turpentine odor. Giving another example, the (R)-enantiomer of asparagine has a sweet taste and the (S)-enantiomer has a bitter taste.

To sum up, there are many effects that can occur if the drugs and biological compounds are not enantiomerically pure. Therefore, to avoid such effects to happen, the FDA (American Food and Drug administration) claims that there should be invented stricter levels of optical purity (>99.99)[2].

Chiral building blocks of drugs are in many cases amino acids. Amino acids consist of a carboxyl group and an amino group. Amino acids are amphoteric species, which means they can act as an acid or as a base. Besides, they are zwitterions or molecules that have both a positive and a negative charge under distinct pH-conditions (dependent on the pI of the amino acid). Moreover, single amino acids build up peptides via a peptide bond and peptide units build up proteins[3]. There exist twenty proteinogenic amino acids, which are classified into essential and non essential amino acids. Essential amino acids are amino acids which are indispensable for human organism but cannot be produced by the human organism itself.

All naturally occurring amino acids that make up proteins are in the L-configuration[4]. However, there exist also D-amino acids in the human body for example D-alanine and D-aspartic acid[5, 6].

1.2. Enantioseparation – enantioselectice HPLC

Enantioseparation of racemic compounds is of great interest in many fields of research, including biomedical research and food production[7].

The requirements concerning chiral separation are very high. There should be high sensitivity and good reproducibility. Further requirements are that only low amounts of substance are needed and simple sample preparation. There exist many techniques and methods to separate racemic mixtures such as crystallization[8], capillary electrophoresis[9], simulated moving bed technology[10] and enantioselective liquid chromatography. High performance liquid chromatography (HPLC) can be used for chiral separations very effectively.

The main application of using HPLC in this area is the preparative separation of racemic drug compounds and for analytical determination of enantiomeric purity[11, 12, 13].

As mentioned before, enantiomers have the same physicochemical properties. Thus, they can not be separated using stationary phases with an achiral environment. One possibility is to convert enantiomers in diastereomers or diastereomeric associates, which differ in their physicochemical properties and therefore can be separated on achiral stationary phases.
There are two modes that can be applied in enantioselective chromatography: the indirect and the direct approach.

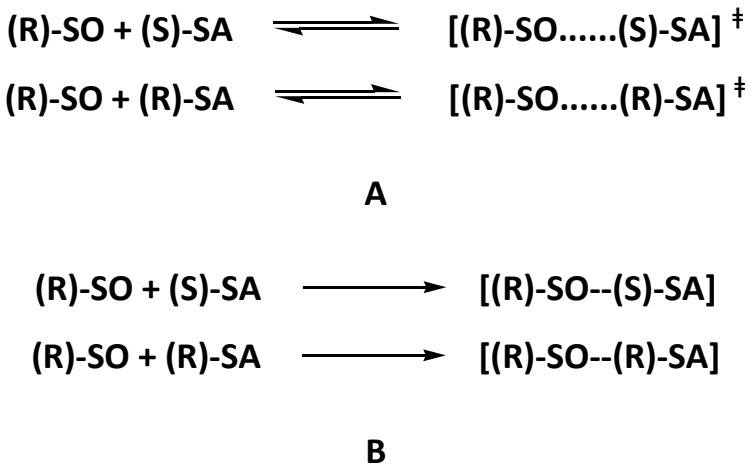


Figure 1.1 A*: scheme of direct method (associated),* ***B:*** *scheme of indirect method (covalent bounded).*

In the indirect approach a diastereomer is formed by formation of a covalent bond between a chiral selector (SO) and the enantiomers of the analyte, the so called selectand (SA). Then the formed diastereomers can be separated by achiral chromatography (e. g. reversed phase column). These formed diastereomers can be separated then by achiral chromatography or by electrophoretic methods[14]. However, there are several prerequisites for the chiral derivatizing agent (CDA). The CDA has to be of high enantiomeric purity. If not undesirable side-products (= diastereomers formed by the enantiomeric impurity) can be built. Other important requirement is that kinetic racemate resolution has to be avoided. This phenomenon occurs when the rate constants k_R and k_S of the (R)- and (S)-enantiomers for the derivatization reaction

are different and the reaction is stopped before completion. The enantiomer ratio will depart from the racemate ratio (50:50). Thus, to avoid this phenomenon the reaction has to be completed and CDA should be available in excess.

In contrary, the direct approach relies on the reversible formation of diastereomeric molecule associates. The SO interacts with each of the two enantiomers and forms diastereomeric associates. The difference is in the different binding strength of the complexes. The direct approach can be carried out in two ways:

In the first case, the chiral SO is covalently linked or physically adsorbed onto support material (e.g. silica). The mobile phase is free of chiral additives. As mentioned before, the diastereomeric associate is formed between the chiral SA and the chiral SO on the surface of the CSP. The enantiomer which forms the "stronger" (= energetically higher) diastereomeric complex will elute later, the weaker diastereomeric complex will elute first. This method is easily to carry out in practical work.

The second way is to use a chiral mobile phase additive (CPMA). An achiral stationary phase combined with a mobile phase containing a chiral selector. Diastereomeric complexes are formed between the enantiomers of the racemate and the SO than in the mobile phase. One enantiomer interacts stronger with the SO as the other enantiomer. Thus, they are eluted at different elution times[14].

1.2.1. Chiral Recognition - Three-point attachment

In enantioselective liquid chromatography chiral recognition takes place between the SO and the SA. Some models were developed to explain the recognition. One of them is the three-point-rule from Dalgliesh[15]. Later on, Pirkle expressed the three-point rule in a more general way: *"Chiral recognition requires a minimum of three simultaneous interactions between the CSP and at least one of the enantiomers, with at least one of these interactions being stereo chemically dependent."*[15]

Not all three interaction must be attractive, in many cases repulsive steric interaction are involved.

The general principle of chiral recognition was initiated and further developed by Davankov[16] . This model makes it easy to show that the sign of enantioselectivity inverts when one of the three interaction is repulsive and not attractive. The three point interaction rule differs from the three point attachment model from Dalgliesh. According to Dalgliesh, repulsion is seen as productive, as an attractive interaction. In **figure 1.2** the three-point-interaction-model is shown. The classical three-point interaction model is often represented by amino acids in ligand exchange mode.

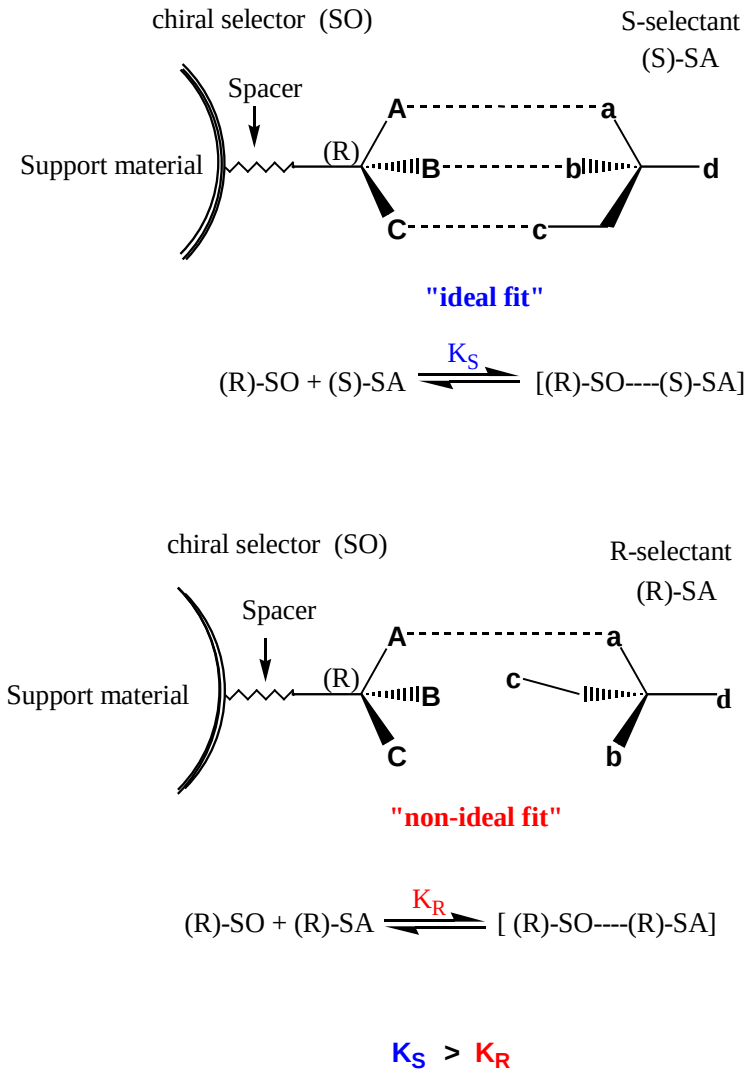


Figure 1.2: *three-point interaction model*

From a thermodynamical point of view enantioseparation is based on the difference in Gibbs energy (Δ G) in formation of two diastereomeric complexes (**equation 1.1**) $\Delta G°_R$ and $\Delta G°_S$

express the affinity of the enantiomers to the chiral selector and are related to the equilibrium constant K_R and K_S (**equation 1.2**).

$$\Delta G°_S \neq \Delta G°_R \qquad [1.1]$$

$$\Delta G°_S = -RT \ln K_S \qquad \Delta G°_R = -RT \ln K_R \qquad [1.2]$$

T … temperature in K

R … gas constant

The equilibrium constants K_R and K_S are related to the retention factors k_R and k_S by **equation 1.3**. The phase ratio is represented by Φ.

$$k_S = K_S \cdot \Phi \qquad k_R = K_R \cdot \Phi \qquad [1.3]$$

The selectivity coefficient α depends on the ratio of the retention factors of the enantiomers, where the retention factor of the more retained enantiomer is divided by the retention factor of the less retained enantiomer ($k_S > k_R$). This relation is combined with the **equation 1.3**.

$$\alpha = \frac{k_S}{k_R} = \frac{K_S \cdot \Phi}{K_R \cdot \Phi} \qquad [1.4]$$

When combining **equation 1.2** and **1.4**, the relation between selectivity coefficient α and the Gibbs energy $\Delta\Delta G°_{R,S}$. $\Delta\Delta G°_{R,S}$ expresses the difference in the free energy changes between the (R)- and the (S)-enantiomers. The relation is expressed in **equation 1.5**.

$$\Delta\Delta G°_{R,S} = \Delta G°_S - \Delta G°_R = -RT \frac{K_S}{K_R} = -RT \ln\alpha \qquad [1.5]$$

The free energy is also connected to the enthalphy and to the entropy which is expressed in the so called Gibbs-Helmholtz-equation:

$$\Delta\Delta G^{\circ}_{R,S} = \Delta\Delta H^{\circ}_{R,S} - T\Delta\Delta S^{\circ}_{R,S} \qquad [1.6]$$

When combining **equation 1.5** with **equation 1.6** a correlation between the relation of the enthalphic ($\Delta\Delta H^{\circ}_{R,S}$) and entropic ($\Delta\Delta S^{\circ}_{R,S}$) contributions to the selectivity coefficient α can be established.

$$\ln \alpha_{R,S} = -\frac{\Delta\Delta H^{\circ}_{R,S}}{RT} + \frac{\Delta\Delta S^{\circ}_{R,S}}{R} \qquad [1.7.]$$

Using the van't Hoff plot (ln α versus 1/T) where $\Delta\Delta H^{\circ}_{R,S}$ and $\Delta\Delta S^{\circ}_{R,S}$ can be determined by the slope and by the intercept, respectively, this thermodynamic analysis enables to find out whether separation is dominated by enthalphic or entropic contributions. The enthalpic values are mostly negative according to **equation 1.7**.

Experimental observations showed that the chiral recognition is usually enthalpically controlled. A difference in Gibbs free energy between both diastereomeric complexes is essential to achieve enantioseparation. The principal forces are non-covalent bonds such as ionic interaction (electrostatic interactions between positively and negatively charged molecule groups), hydrogen bonding (between hydrogen-donor and hydrogen-acceptor groups), dipole-dipole-interactions, π- π-interactions and hydrophobic interactions between lipophilic moieties of SA and SO. Regarding mobile phase aspects, one can say that the higher the polarity of the eluent the lower the strength of the electrostatic interactions. When using an aqueous mobile phase hydrophobic interactions come to the fore.[17]

1.3. chiral stationary phases (CSPs)

Every class of chiral stationary phase has strengths and weaknesses in regard to selectivity, stability and loadability. A plethora of chiral stationary phases can be used for different separation problems. **Figure 1.3** gives an overview of commonly used CSPs.

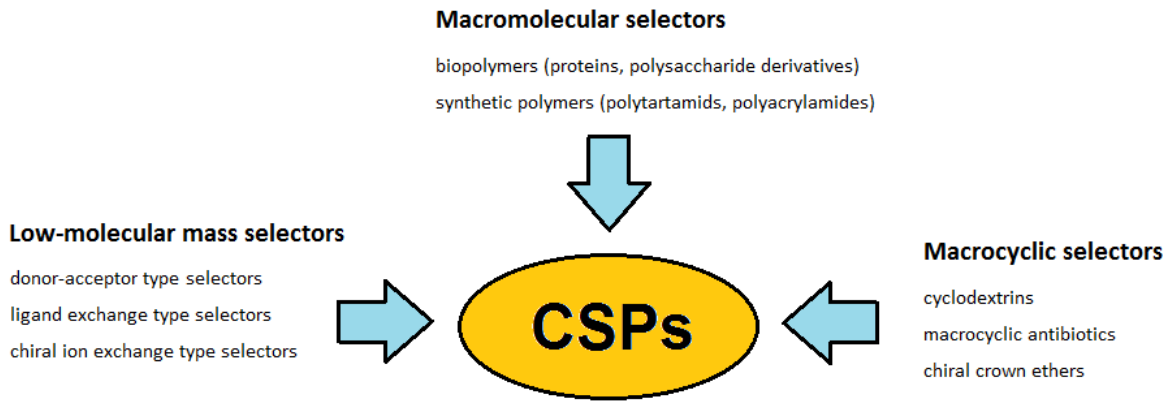


Figure 1.3: *Overview: usual classification of CSPs*

1.4. chiral ionizable stationary phases

Certain CSPs are ionizable, which will be closely discussed in the following chapters. Their behavior to analytes and their interaction mechanism are different to these of other CSP. In **figure 1.4** the main representatives of ionizable CSPs are shown.

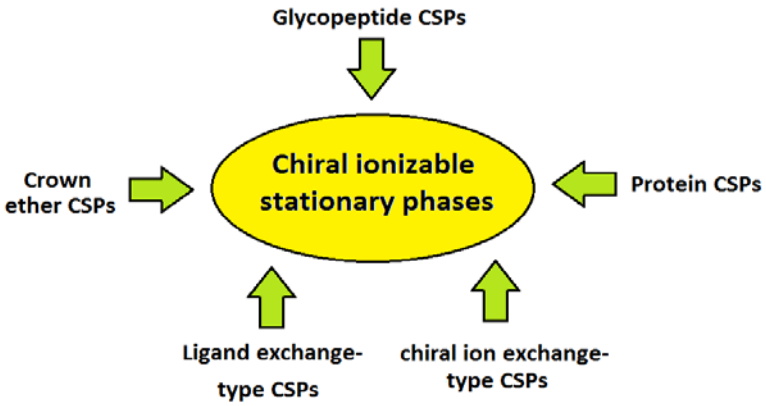


Figure 1.4*: Overview of chiral ionizable stationary phases*

1.4.1. Glycopeptide-type CSPs

The CSPs based on glycopeptide antibiotics were introduced by Armstrong[18].

In general, the used selectors have molecular weights between 600 and 2200 Daltons. The commercialized CSPs are based on vancomycin, teicoplanin, teicoplanin aglycon and ristocetin. Their structure is highly complex and they have different degrees of O-glycosylation. Armstrong studied the impact of chemical modification on chiral recognition capabilities of vancomycin and other glycopeptides[19].

Glycopeptide antibiotics have multiple functional group assemblies that may act as potential enantioselective binding sites for several chiral acids and bases. The chiral recognition mechanism is based on the combining of hydrophobic inclusion and ionic interactions and strong hydrogen bonding[12].

These CSPs can be used in both reversed-phase (RP) and normal-phase (NP) conditions showing different enantioselectivities in each mobile phase. They also can be used in polar organic (PO) mode.

It has been reported that acidic, basic, neutral and zwitterionic analytes can be enantioseparated with these CSPs[1, 17].

The glycopeptide phases are suitable with mass spectrometry (MS). Occasionally MS can be used only in RP- and PO-mode. For preparative liquid chromatography glycopeptide CSPs are not very suitable, because of limited sample loading capacity. Another disadvantage is that enantiomeric CSPs are not available.

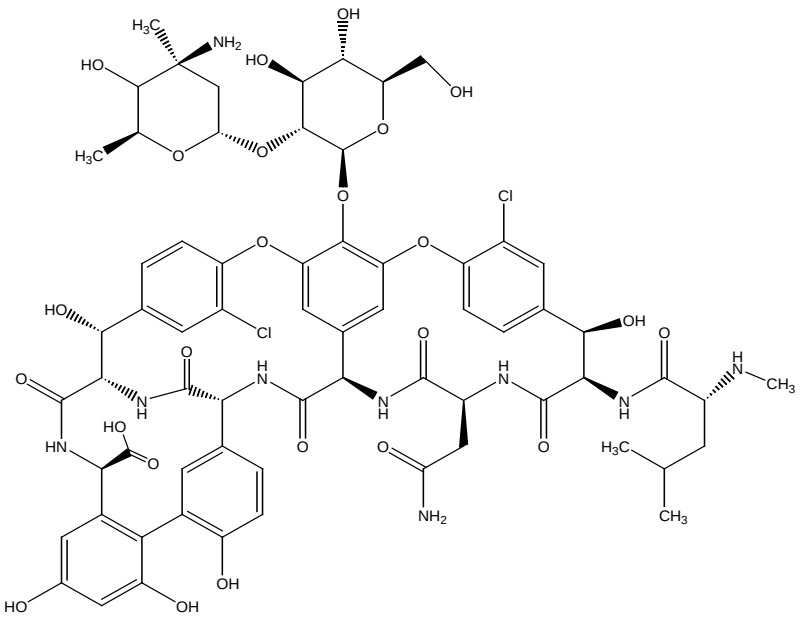


Figure 1.5: *Structure of vancomycin*

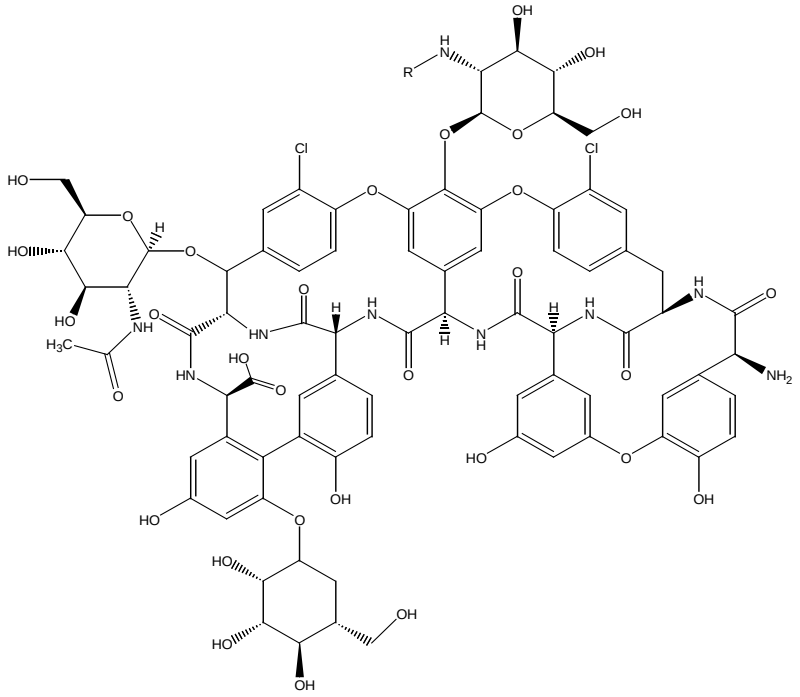


Figure 1.6: *Structure of teicoplanin*

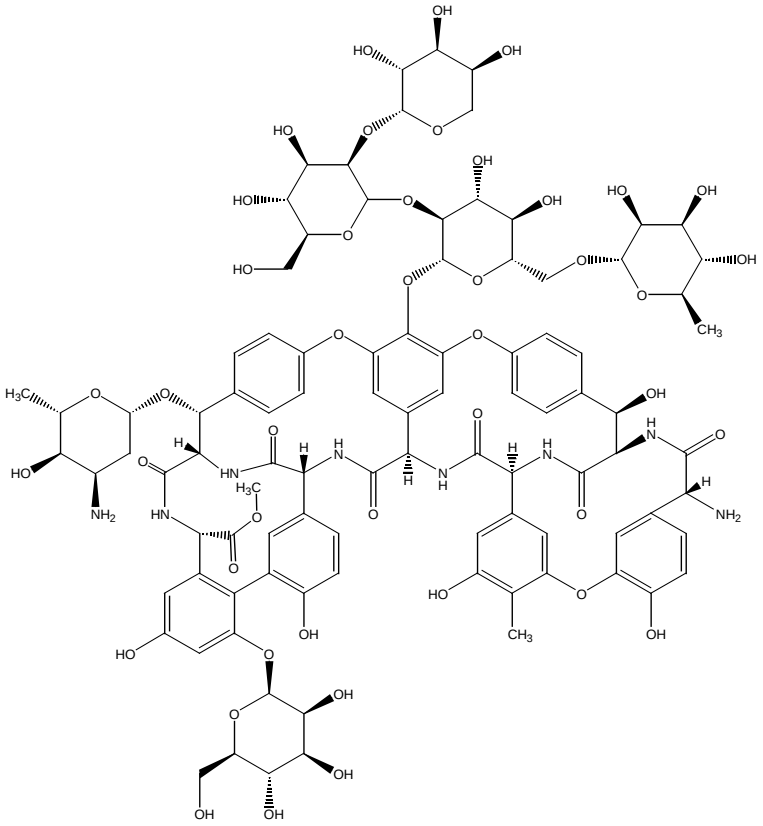


Figure 1.7: *Structure of ristocetin A*

1.4.2. Crown Ethers

Another important types of macrocyclic CSPs are chiral crown ethers, introduced by Cram and coworkers[20,21].

Crown ethers are cyclic compounds with repeating units of (-O-C_2H_4-). They were first synthesized by Peterson[22]. Such Crown ethers contain a hydrophobic exterior and hydrophilic cavities. The hydrophilic character comes from the oxygen atoms and therefore a strong affinity to cations is available. When used as a CSP, the ethylene-bridges of such 18-crown-6 ether are substituted by a chiral moiety (e.g. enantiomeric binaphtyl derivatives). Chiral 18-crown-6-ether selectors can be coated on reversed phase stationary phases[23, 24]. They are commercially available under the name Crownpak CR (+)® from Chiral Technologies. Further chiral crown ethers are 18-crown-6-ethers with tartaric acid moeities. This SO is immobilized onto silica gel and the corresponding CSPs is available under the trade name Chirosil RCA (+)® and Chirosil RCA (-)®, both from Regis. Chiral crown ether type based CSPs are illustrated in **figure 1.8**.

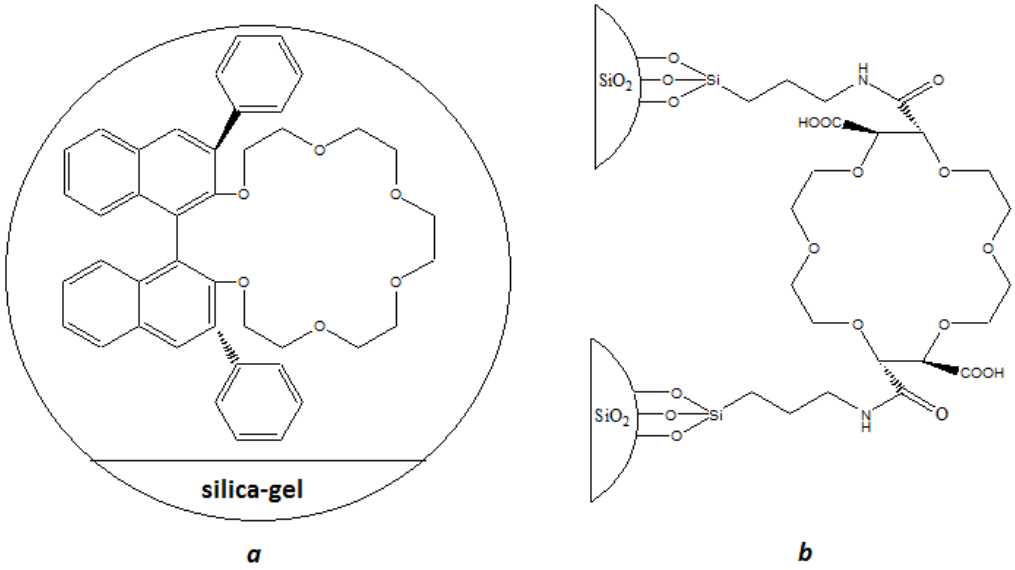


Figure 1.8.: ***a****: crownpak ®,* ***b:*** *crown ether Chiro Sil ®*

The chiral recognition is based on the formation of multiple hydrogen bonds (triple hydrogen bond) between a protonated primary amino group of the analyte and the ether-oxygens of the crown framework. The size and charge of the guest cation and the size of the cavity affect the strength of the interactions[25].

Such 18-crown-6 ethers are used in the reversed phase mode. These CSPs can be used under aqueous condition with addition of small amounts of organic modifier such as methanol and acetonitrile. The eluent has to be strongly acidic (pH between 1 and 3.5)[26].

The enantioselectivity of these chiral crown ethers for primary amines (amino acids and amino alcohols) is controlled by the steric factors of the substituents attached to the chiral part. Chirosil RCA (+)® is as well able to separate secondary amines[27]. Good resolution for separation of enantiomers could be achieved with β-blocker-type analytes [13].

Both enantiomeric forms of the chiral crown ether are available.

1.4.3. Protein-based CSPs

For enantioseparation of ionizable chiral analytes also proteins as chiral selectors can be used. However, only a limited number of proteins are suitable as for HPLC CSPs. Different types of proteins were investigated, including albumins such as human serum albumin (HSA) by Allenmark[28] and bovine serum albumine (BSA) by Domenici[29], glycoproteins such as α_1-acid glycoprotein (AGP) by Hermansson[30], ovomucoid from chicken egg whites (OMCHI) by Miwa [31] or enzymes such as Trypsin[32] or cellobiohydrolase I (CHB I)[33]. HSA is commercially available as Chiral OVM®, AGP is commercially available as chiral AGP® and OMCHI as ES-OVM®.
There are two methods for preparation of protein-based CSPs. The protein can be physically adsorbed or covalently bound onto the supporting material. For supporting materials agarose, silica gel and polymers are used.

HSA is structurally related to BSA and the characteristics of stereoselective binding are similar. On HSA-based CSPs warfarin was enantioseparated[28]. AGP consists of a single peptide chain. Information about the chiral recognition sites and mechanism of AGP is not fully discovered. CHB I is an acidic glycoprotein and has a binding domain connected to the rest of the enzyme. The core is enzymatically active. Good enantioselectivity is obtained for the separation of β-blocking agents such as propranolol.

Enantioseparation on protein-based CSPs was achieved for when using acidic, basic and neutral analytes. Hydrophobic, electrostatic and hydrogen bonding interaction are responsible for chiral recognition. Protein phases are always used in aqueous or hydroorganic mobile phases. Parameters such as pH-value of the mobile phase, type and concentration of organic modifier, type and strength of the buffer and temperature are important variables adjust in retention and enantioselectivity.

Compared to other CSPs Protein-CSPs have a low loading capacity. Moreover, they show low efficiency because of slow desorption kinetics. Protein CSPs are not useful for preparative separations. A certain number of drug protein binding studies were done[34]. Besides, protein CSPs denature in organic mobile phases. Protein CSPs have to be stored in aqueous mobile phases. The exact mechanism of by chiral recognition is not resolved completely.

1.4.4. Ligand Exchange – type CSP

Enantioselective ligand exchange chromatography (CLEC) was developed by Davankov and Rogozhin in 1971[35, 36].

This CSP is based on rigid cyclic amino acids such as L-proline and L-hydroxyproline analoga which function as chelating selectors. For supporting the chelating process certain metal-ions such as Cu^{2+} or Ni^{2+} are added to the mobile phase. These metal-chelating functionalities are crucial for formation of SO-SA complexes in both SO and the analytes. CLEC invokes the presence of multicomponent complexes containing a central metal ion complexed by two chelating chiral bifunctional molecules. A diastereomeric ternary chelate complex is built [15].

Spatial factors are decisive for the difference of the stability in the diastereomeric complex, which leads to different retention times. Ligand exchange is based to reversible contact of the selector and analyte species. Immobilized CLEC-selectors are commercially available as CHIRALPAK® MA, from Chiral Technology. Target analytes for CLEC-CSPs are α-amino acids, 1,2-amino alcohols or α-hydroxy acids.

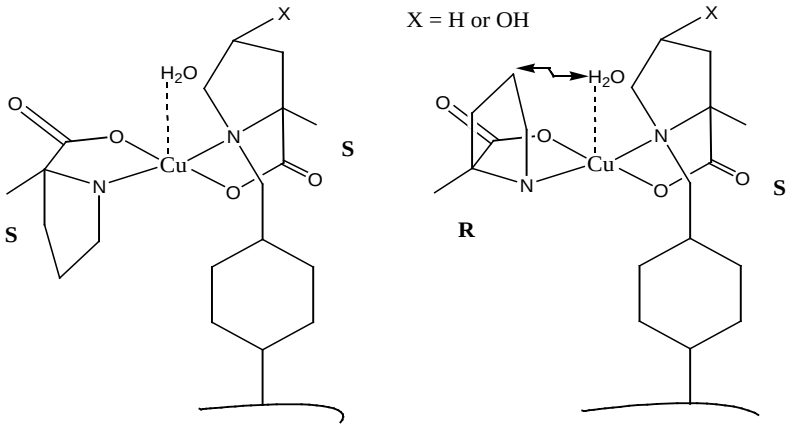


Figure 1.9.: *ligand exchange phases (polystyrene support)*

Nowadays CLEC is not in widespread use anymore. It can only be applied on a small range of analytes, the toxic metal ions in the mobile phase disturb the detection and it is hard to purify the channels of the HPLC instruments. Moreover, the loading capacity is also limited and is not suited for preparative separations.

1.4.5. Ion Exchange-type CSPs

A diverse chiral ion exchange-type CSPs was developed for enantioseparation of ionizable chiral molecules. For instance, chiral anion-exchange phases based on cinchona alkaloids are used for separation of chiral acids[37], chiral cation-exchanger based on chiral amino sulfonic acids or carboxylic acids are in use for separation of bases[38] . Recently, zwitterionic ion-exchangers were developed for the separation of chiral acids, bases and chiral zwitterionic compounds such as amino acids and peptides[39]. Commercially available ion exchange-type CSPs are Chiralpak QN-AX® and Chiralpak QD-AX® both from Chiral Technologies.

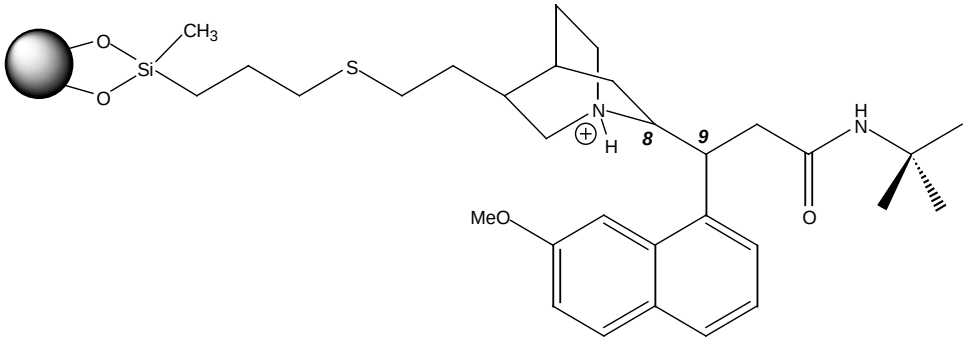


Figure 1.10: *Chiralpak QN-AX (8S, 9R) and Chiralpak QD-AX (8R, 9S)*

Useful anion exchange chiral groups are primary, secondary tertiary or quaternary amino groups. As cation exchange groups carboxylic-, sulfonic-, sulfinic-, phosphoric-, phosphonic- or phosphinic acids can be used.

The prerequisites for a successful enantioseparation are ionizable analytes under corresponding mobile phase pH-conditions. These CSPs have a high loading capacity and thus they are well suited for semipreparative and preparative separations. They are suitable for MS-hyphenation. There exist several types of chiral ion exchange CSPs, which are discussed in the following chapters.

1.4.5.1. ergot-alkaloid-based CSPs

One group of chiral anion exchangers are semisynthetic ergot-alkaloid derivatives (e.g. 1-(3-aminopropyl)-(5*R*, 8*S*, 10*R*)-terguide). They were introduced and intensively studied by Sinibaldi[40]. In **figure 1.11** a characteristic ergot-alkaloid-based CSP is shown.

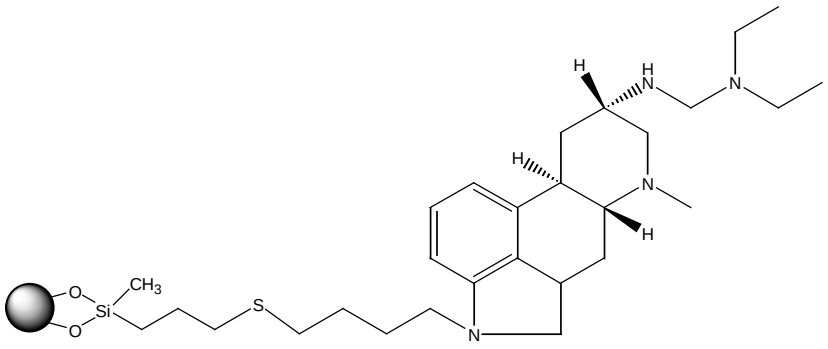


Figure 1.11: *chiral selector on silica gel*

Ergot-alkaloid-based CSPs have two or three asymmetric carbons, as an indole ring, a proton acceptor, tertiary nitrogens as electrostatic interaction sites and a sterically hindering group. The interaction between the analytes and the selector is mainly driven by hydrophobic and ion-exchange effect[40].

Ergot alkaloid-based CSPs show good enantioselectivity for some carboxylic group-containing racemates [40, 41].

When using reversed phase mode, where the amount of organic modifier was increased, a decrease in retention time was seen. These CSPs have a low molecular mass and their production costs are low.

1.4.5.2. Cinchona alkaloid-based CSPs – anion exchange CSPs

Cinchona alkaloid based CSPs were mentioned before briefly. They were applied first by Rosini[42] and commercially available Cinchona alkaloid based CSPs were developed by Lämmerhofer and Lindner[43].

These chiral stationary phases are derived from QN, QD and cinchonidine. QN and QD are available as cheap natural sources for stereodiscriminating auxiliars (selectors, SO) and they are also used as resolving agents for chiral acids[43]. Cinchonidine has the same structure like QN but without the methoxy-group on the six position of the quinoline moiety. The structure of QN and QD is built up by the planar quinoline and a rigid quinuclidine ring, which are connected by a secondary methyl alcohol bridge (see **figure 1.12**). The molecules can be seen as a semirigid framework and thus as promising SO molecules[43].

In detail, QN and QD have five stereogenic centers and they differ only in absolute configuration at C_8 and C_9 and they also behave as "pseudo-enantiomerics". QN and QD are diastereomers to each other, but they show an enantiomeric behavior. The stereoselectivity is under control of the stereogenic centers C_8 and C_9.

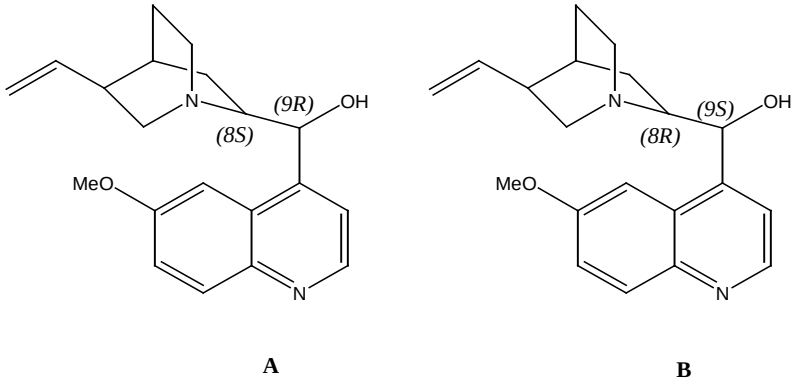


Figure 1.12.: *A: quinine (QN), B: quinidine (QD)*

Different QN and QD derivatives were tested. Good results in enanatioseparation achieved new QN and QD carbamate-based CSPs, especially tert-butyl-carbamoylated-QN and QD CSPs. They were synthesized by Salvadori[44] earlier and tested in anion-exchange mode by Lämmerhofer and Lindner[43].

There are several sites fore intermolecular interactions like the basic aliphatic nitrogen group of the quinuclidine ring for electrostatic interactions (ion-pairing), the hydrogen-donor-acceptor site of the carbamate group for hydrogen binding and/or dipole-dipole interaction, the π-basic quinoline ring for intermolecular π-π interaction, the bulk quinuclidine group and the planar quinoline ring for steric interaction (attraction or repulsion). The tertiary amine from the quinuclidine forms an ion-pair with acidic analytes and thus can be classified as a weak ion exchanger (WAX). Intermolecular ion-pairing can be seen as a primary attractive interaction force.

The alkaloid residue is anchored to the γ–mercaptopropyl silica gel by a radical reaction attack of the thiolgroup to the vinyl doublebound of the alkaloid.

Comparing such QN and QD-based CSPs with each other they show similar enantioselectivity for the same analyte but exhibiting opposite elution orders. Experiments have also shown that when using QN-based WAX-CSP (D)-amino acids elute first and when using QD-based WAX-CSP (L)-amino acids elute first. This can be of interest when doing preparative separations or quantifications of enantiomeric impurities.

In buffered hydro-organic mobile phase these CSPs have been turned out as successful for separation of N-derivatized α- (especially for N-3,5-dinitrobenzoyl α-amino acid derivatives), β- and γ−amino acids[45]. Acidic analytes like carboxylic, sulfonic, phosphoric and phosphonic acids are deprotoned to a high degree under corresponding mobile phases with certain pH-conditions. The retention and enantioselectivity can be controlled by the concentration and type of buffer, the organic modifier, the type and the concentration of the counterion and by the temperature[43]. The cinchona alkaloid-based ion exchange CSPs are preferentially used in the polar organic or RP-mode.

Derivatization of diverse functional groups of the cinchona alkaloids selector backbone opens a modification to optimize stereoselectivity[43,45]. In particular the carbamate function on the C_9 position can be further derivatized (e.g. to N-methyl tert-butyl carbamoylated quinine SO).

1.4.5.3. sulfonic acid-based CSPs – cation exchange CSPs

To the before introduced anion exchange CSP there exist as well a contra part – the cation exchange CSP.

Strong cation exchange (SCX)-CSPs were developed by Hoffmann and Lindner in 2007[46].

For the SO of the SCX-CSP β-amino sulfonic acids were used, which had two chiral centers.

(1S,2S)-2-aminocyclohexanesulfonic acid was used as it is pictured in **figure. 1.13.**

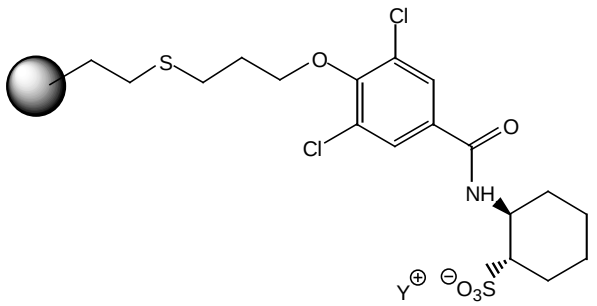


***Fig. 1.13**.: SCX-CSP being of (1S, 2S)-configuration.*

Furthermore, the SO consists of a π-acidic aromatic moiety which can take part in π-π-interactions and the existing amide group can serve for H-bonding.

This "β-aminocyclohexanesulfonic acid" (ACHSA) SO is covalently attached to a chromatographic support. The anionic sulfonate group of the SO provides ion pairing with the chiral basic molecules which are protonated under the used mobile phase conditions. Basic molecules as bunitrolol, salbutamol or methoxamine were enantioseparated on these cation-exchange CSPs. Furhtermore, the SOs have been proven to be sucessful for use in capillary electrophoresis [47,48] and in HPLC[46].

Chromatographic evaluation of these SCX-CSPs was carried out in a non aqueous polar organic mobile phase. It was observed that with increasing ACN-content selectivity and resolution increased. These CSPs are used in weakly acidic mobile phases. However, their application is defined on the separation of basic analytes.

1.4.5.4. Zwitterionic CSPs based on cinchona alkaloids

The earlier proposed weak anion exchanger (WAX) based on cinchona alkaloids are limited in their application range. To overcome this limited application the WAX-SO has to be combined with an opposite charged moiety. Aminosulfonic acid-based chiral strong cation exchangers (SCX) fulfill this profile. With the help of a synthesis approach a chiral WAX-selector and a SCX-selector can be fused into a promising zwitterionic selector[49].

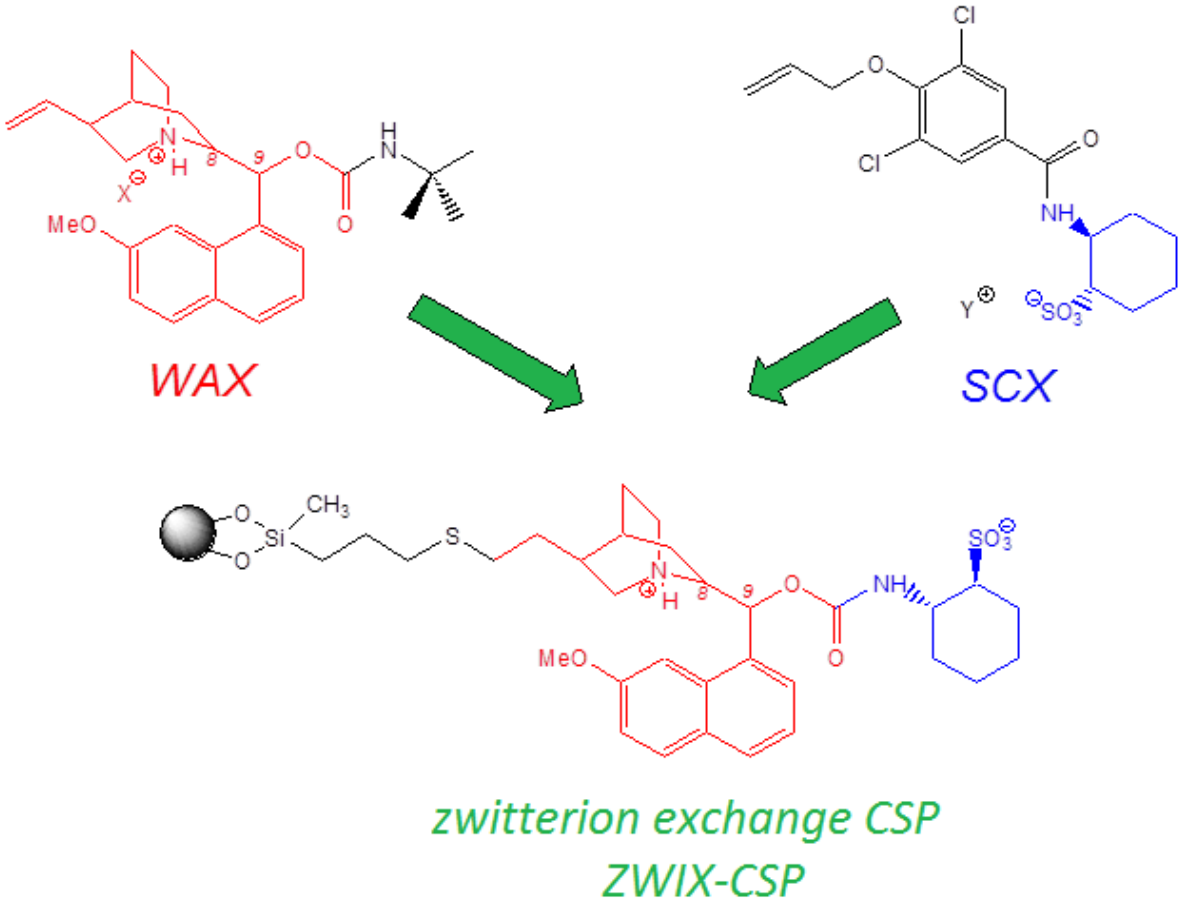


Figure: *fusion of WAX and SCX into a ZWIX*

The ZWIX-selector is anchored to silica gel by a radical reaction attack of the thiolgroup to the vinyldoublebound of the alkaloid.

In the zwitterionic-exchange process there are two ionizable groups on the SO. These groups can also be characterized as an intramolecular counter-ion (IMCI). The two intramolecularly ionizable groups are not only responsible for ion pairing, they also act as counter-ions in the

ion-exchange-processes. Therefore, retention is only weakly affected by changes of the counter-ion concentration in the mobile phase.

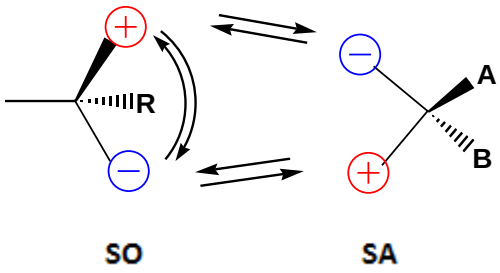


Figure 1.4.5.2.: *show IMCI-effect*

The IMCI feature influences on the use of acidic and basic additives and allows faster separation with a lower amount of buffer salts in the mobile phase. Compared to the AX-type CSPs under the same elution conditions the retention time of the chiral acidic analytes analytes is shortened. This effect can be attributed to the contribution of the IMCI group. When the counter-ion in the mobile phase is low, the IMCI effect is a dominant factor for retention. For separation of zwitterionic analytes a double ion pairing process takes place[50].

The ZWIX-CSPs are normally used in polar organic mode with MeOH as bulk solvent. Addition of low amounts of can particulate improved enantioselectivity and increased retention. Acidic and basic additives in an acidic to base ratio of 2:1 has proven to be the best compromise between good and fast separations.

The parentchiral anion and chiral cation exchanger are limited to analytes which carry the opposite charge. Combining them to ZWIX-CSPs seems to be a good choice and given broad application in separation ionizable chiral analytes[51].

To find out which ionizable groups are more effective, CSPs with different ionizable groups were tested. In doing so the cation exchange (CX) groups were varied where different carboxylic- and sulfonic acids were used. It turned out that with sulfonate-type CX-sites bounded to the C_9 position of the cinchona alkaloid high enantioselective recognition for chiral zwitterionic analytes was dissovered[50].

2. Results and Discussion

2.1. Synthesis of novel - Zwitterionic Ion Exchange Chiral Stationary Phases (ZWIX-CSPs)

An important focus of the diploma thesis was the synthesis of new zwitterionic chiral stationary phases. The idea is based on combining a chiral anion exchange part with a chiral cation exchange part to one zwitterionic ion exchanger. **CSP 1**, **CSP 2** and **CSP 3** were synthesized for the HPLC-evaluation.

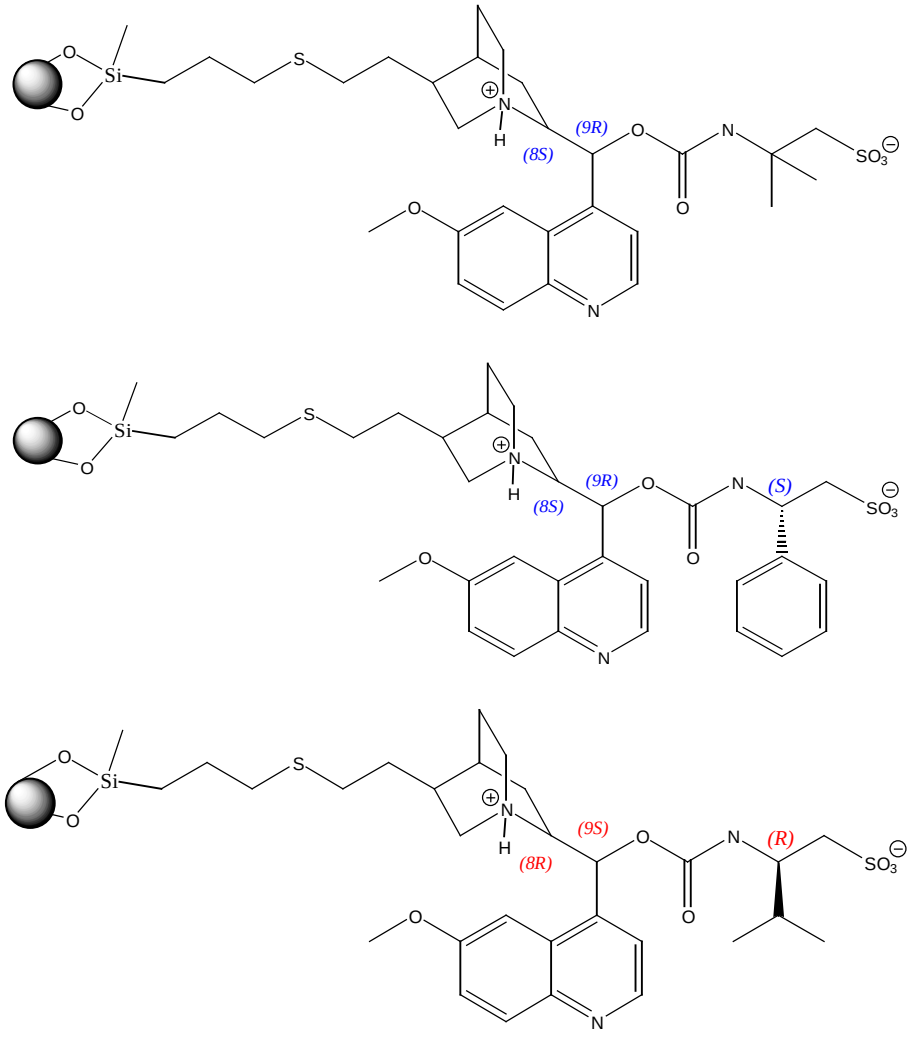


Figure 2.1.: *synthesized CSPs used for the measurements*

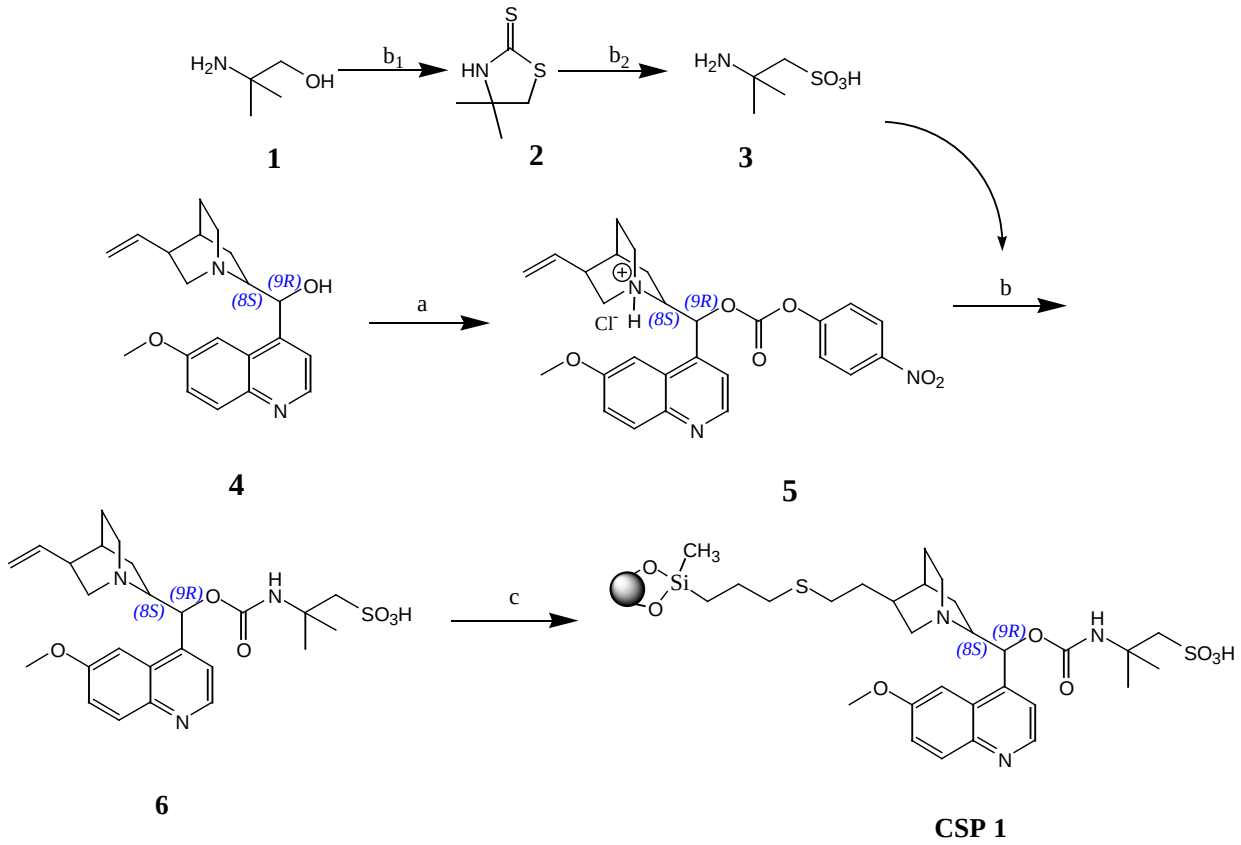
Table 2.1 shows the short names, full names and the selector coverage of the used CSPs.

CSP Number	Short name	Full name	dimension	coverage µmol [SO]g-1 [CSP]
CSP 1	dimethyl-Tau-QN	dimethyl-taurine-quinine	250 x 3 mm ID, 3 µm	255
CSP 2	2-(S)-phenyl-Tau-QN	2-(S)-phenyl-taurine-quinine	250 x 3 mm ID, 3 µm	186
CSP 3	2-(R)-isopropyl-Tau-QD	2-(R)-isopropyl-taurine-quinidine	250 x 3 mm ID, 3 µm	274
CSP 4	2-(S)-isopropyl -Tau-QN	2-(S)-isopropyl-taurine-quinine	250 x 3 mm ID, 3 µm	203
CSP 5	2-(S)-isobutyl-Tau-QN	2-(S)-isobutyl-taurine-quinine	250 x 3 mm ID, 3 µm	265
CSP 6	ACHSA-Tau-QN	2-cyclohexyl-taurine-quinine	250 x 3 mm ID, 3 µm	232
CSP 7	Tau-QN	taurine-quinine	250 x 3 mm ID, 3 µm	190
CSP 8	2-(R)-phenyl-Tau-QN	2-(R)-phenyl-taurine-quinine	150 x 4 mm ID, 5 µm	260

Table 2.1.: *names and selector coverage of all evaluated CSPs*

The selector coverage is in the range from 186 to 275 µmol g^{-1}. In former experiments by our working group it could be shown that good enantioseparation was achieved with selector coverage over 200 µmol g^{-1} [52].

2.1.1. Synthesis of dimethyl-Tau-QN (CSP 1)

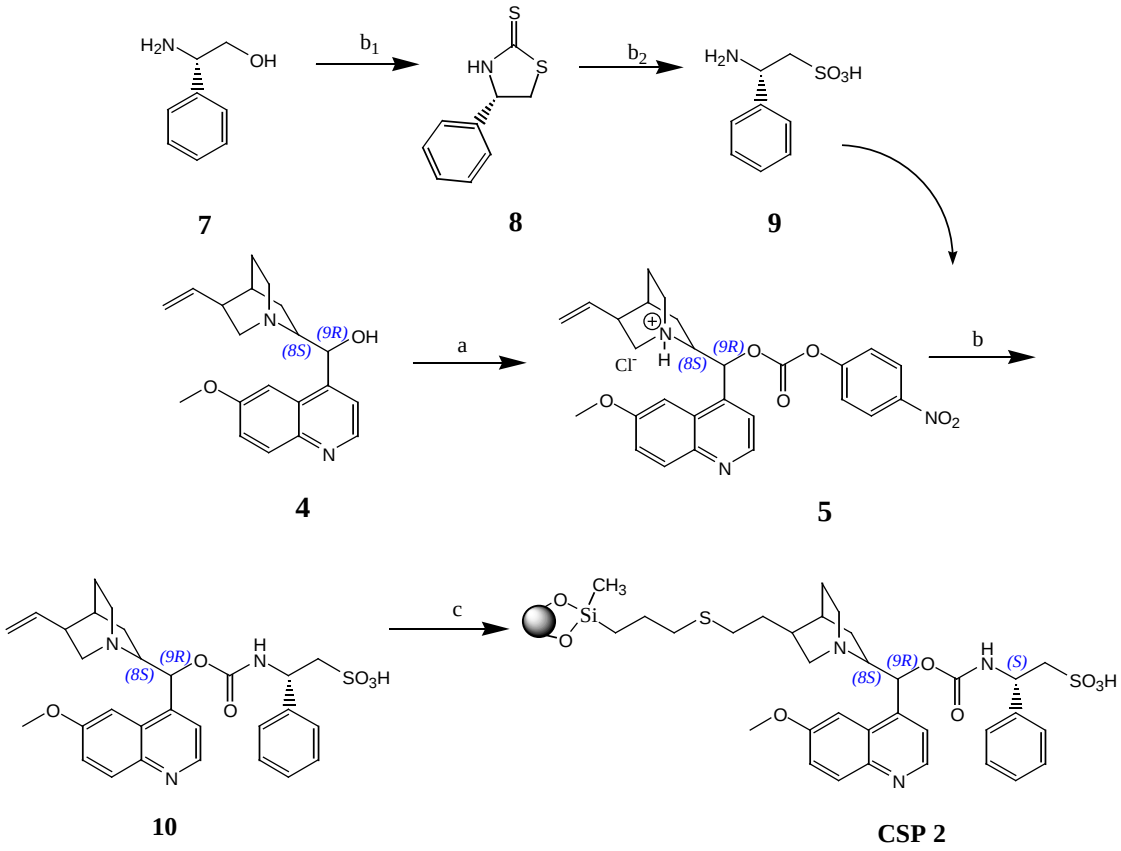


Scheme 1: *Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b_1) CS_2, KOH, 100 °C, 16 h, (b_2) HCOOH, H_2O_2, 0°C, 16 h, (b) 1)* ***3****, BSA, CH_2Cl_2, reflux, 48 h, 2)* ***5****, CH_2Cl_2, r.t., 24 h, (c) AIBN, MeOH, reflux, 6 h.*

Scheme 1: quinine was converted with 4-nitrophenyl chloroformate to **5**. The activated ester of **5** was stored under N_2-atmosphere because it is prone to hydrolysis. Under alkaline conditions the amino secondary alcohol (2-amino-2,2-dimethyl-1-propanole) reacted with CS_2 and formed the thiazolidine-thione. The free amino group reacted first with carbon disulfide to generate N-acyl amino alcohol hydrogen sulfate that underwent an intramolecular sulfur S_{N2}-reaction[53]. **2** was worked up with an extraction using DCM and the raw material was purified by flash chromatography (to separate the co-product 4,4-dimethyl-1,3-oxazolidine-2-thione). H_2O_2 and formic acid formed peroxy formic acid which oxidized **2** to the aminosulfonic acid **3**[54]. In more detail and according to the proposed reaction mechanism thiazolidine-2-thiones are oxidized to

an anthiazolidine-2-thione-1,1-dioxides cyclic sulfur compound. Then by an acid-catalyzed hydrolysis a 2-aminoalkanesulfinic acid was generated by removal of S=C=O. After that the 2-amino-alkanesulfinic acid oxidized to the aminosulfonic acid with peroxy formic acid. Because of the exothermic reaction an ice bath was necessary to cool the reaction. Recrystallization in MeOH yielded pure **3**. For the zwitterionic selector **6** the activated ester of quinine reacts with the silylated aminosulfonic acid. For solubility in the aprotic DCM, the aminosulfonic acid **3** had to be silylated **3**. By a nucleophilic addition of the silylated aminosulfonic acid to the activated ester of quinine the zwitterionic selector was formed. To achieve a high yield of the selector it was important that the silylation of the aminosulfonic acid was completed. For removing 4-Nitrophenol and remaining quinine and methoxycarbonate of quinine from the selector **6** a flash chromatography was applied. The last step c of the synthesis pathway was very delicate. The selector was covalently immobilized on the silica gel by a radical addition reaction on to thiol-modified silica via the vinyl-double bond of the QN-selector. Former experiments by the working group showed that the selector coverage on the endcapped 3-mercaptopropylmethyl modified silica gel was high when using as little solvent as possible[53].

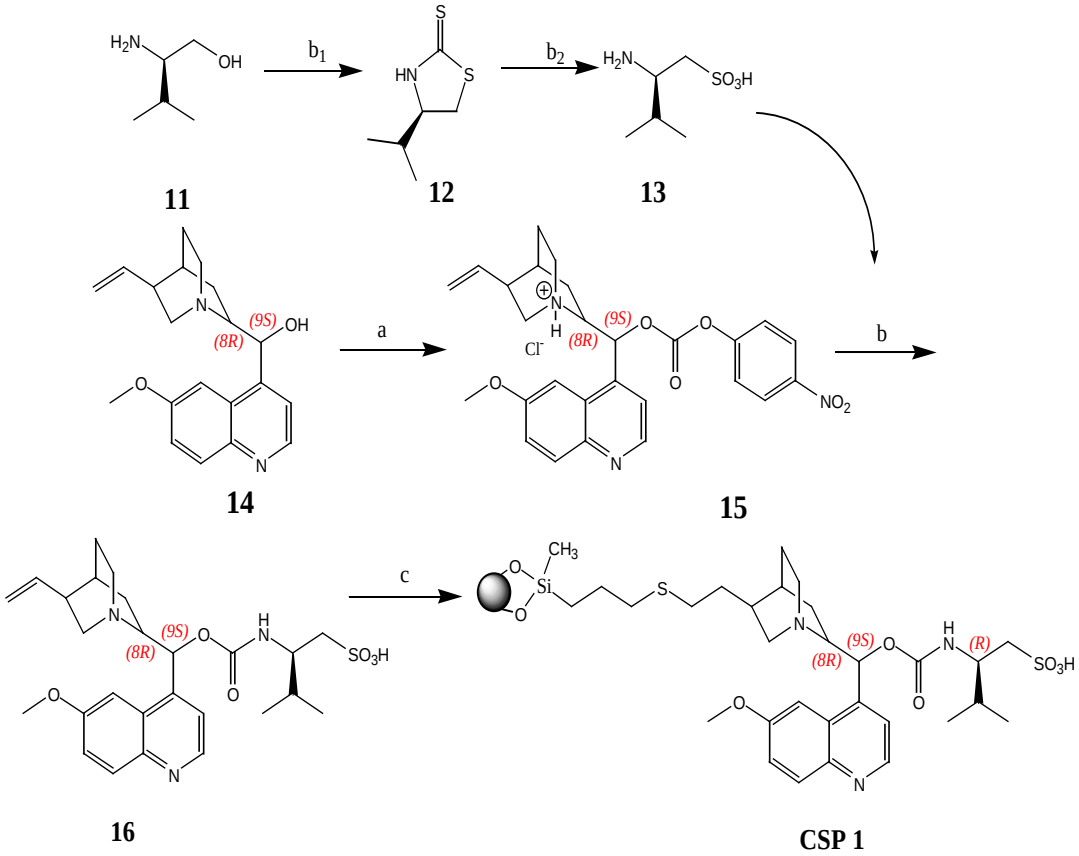
2.1.2. Synthesis of 2-(S)-phenyl-Tau-QN (CSP 2)



Scheme 2: *Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b_1) CS_2, KOH, 100 °C, 16 h, (b_2) HCOOH, H_2O_2, 0°C, 16 h, (b) 1)* ***9****, BSA, CH_2Cl_2, reflux, 48 h, 2)* ***5****, CH_2Cl_2, r.t., 24 h, (c) AIBN, MeOH, reflux, 6 h.*

Scheme 2: The procedure was the same like in **2.1.1**. The sole difference was that flash chromatographic purification was not necessary because the co-product of **8** (4-(S)-4-phenyl-1,3-oxazolidine-2-thione) existed in an insignificant small amount. Only the final immobilization step **c** was repeated because with the amount of AIBN reported in the literature, a selector loading of only 85 µmol g^{-1} could be achieved. Therefore the ten-times higher amount of AIBN was used in the second immobilization trial. The SO coverage was higher with 186 µmol g^{-1}. The reasons why the coverage was low in the first trial could be that during the radical addition reaction some oxygen got into the apparatus through the connection-part of the mechanical stirrer, or for that kind of SO more AIBN was needed for proper immobilization. Further investigations were not carried out due to time issues.

2.1.3. Synthesis of (R)-isopropyl-Tau-QD (CSP 3)



Scheme 3: *Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b_1) CS_2, KOH, 100 °C, 16 h, (b_2) CH_3COOH, H_2O_2, 0°C, 16 h, (b) 1)* ***13****, BSA, CH_2Cl_2, reflux, 48 h, 2)* ***15****, CH_2Cl_2, r.t., 24 h, (c) AIBN, MeOH, reflux, 6 h.*

Scheme 3: For synthesizing **CSP3** the same synthetic steps as in the **2.1.1** were applied. Sole difference was that quinidine was used as starting material. The co-product ((4R)-4-(propan-2-yl)-1,3-oxazolidine-2-thione) of **12** was removed by flash chromatography. Instead of using peroxy formic acid, peroxy acetic acid (reaction by H_2O_2 and acetic acid) was used to form the aminosulfonic acid. The immobilization was repeated because with the amount of AIBN reported in the literature, a selector loading of only 79 µmol g^{-1} could be achieved. Therefore a ten-times amount of AIBN was used like for in synthesizing **CSP 2**. For the repetition of the immobilization a new silica gel batch was used and the selector coverage was 274 µmol g^{-1}.

3. HPLC-Evaluation of ZWIX-CSPs

An evaluation using a set of 75 amino acids was carried out. The chromatographic parameters which are discussed during the evaluation are the retention factor k_i, the selectivity coefficient α_{ij}, the resolution R_S and the plate number N_i. All CSPs were evaluated separately and were compared later with each other.

3.1 Further used CSPs for Evaluation

In our working group further CSPs were synthesized. In **figure 3.1 CSP 4** to **CSP 8** are pictured. All these CSPs are quinine-based.

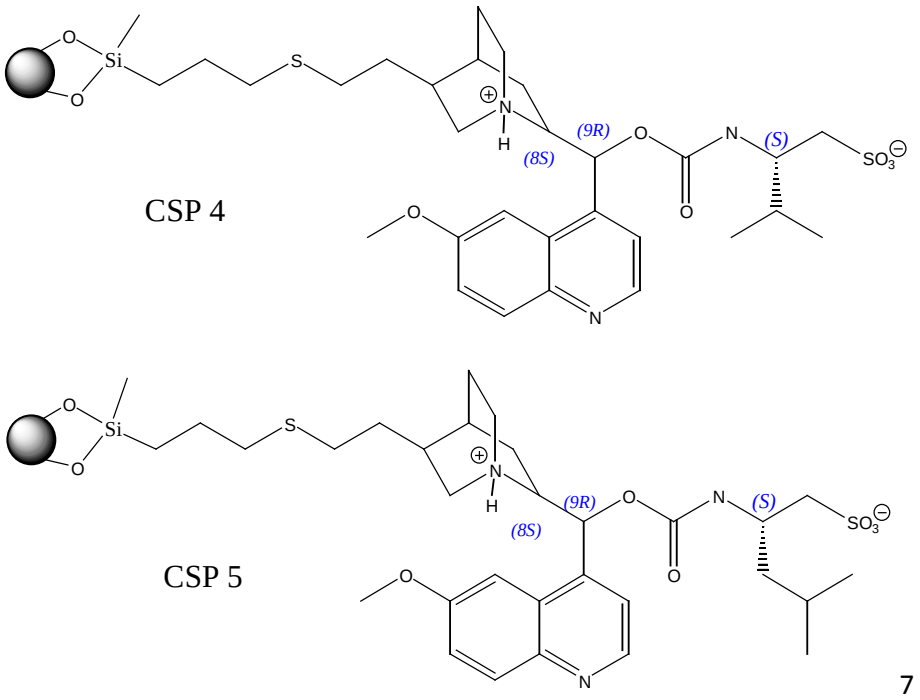
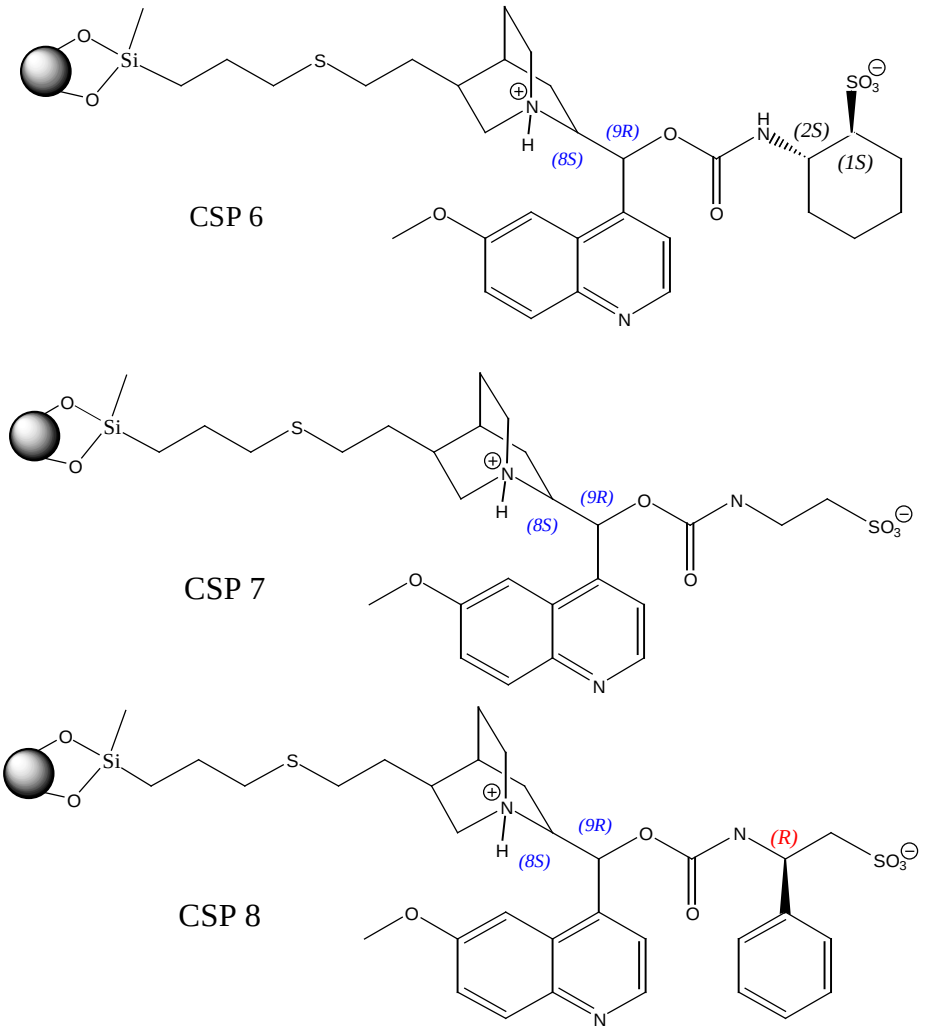


Figure 3.1.: *further CSPs, which were used for the evaluation.*



Continue Figure 3.1.: *further CSPs, which were used for the evaluation.*

3.2. Mobile Phase

For the evaluation of the CSPs a polar organic mobile phase was used. A polar organic mobile phase consists of a polar organic solvent with basic and acidic additives, which behave like co- and counter-ions in ion exchange chromatography. The ion strength depends on the concentration of the co-iones and counter ions, on the pH-value and on the ion-charge in the mobile phase. Former investigations were done to ascertain the best mobile phase conditions for separation of basic, acidic or zwitterionic analytes.

The most applicable mobile phase for the use of chiral zwitterionic stationary phases turned out to be a polar organic solvent consisting of MeOH and ACN, for which MeOH is the bulk phase. Moreover the ratio of acidic to basic additives in the mobile phase was tested from 4:1 to 0.5:1 in former studies[51]. Best performance of the ZWIX-mode by means of short analysis time, high efficiency and enantioselectivity with MeOH or low volume of ACN in MeOH.

The studies demonstrated that the best acid-to-base-ratio regarding separation performance was 2:1. The type of acid and basic additive was tested. It turned out that the type of basic additive had no effect on the retention, but the type of acidic additives had a strong influence on the retention. Studies were done with acid additives like TFA, FA and HOAc. It was found out that enantioselectivity was best with HOAc. When using FA the resolution was the best when using protic MeOH and aprotic ACN as mobile phase. Using only MeOH in the mobile phase the hydrogen bonds are suppressed and π-π-interactions play a greater role. With adding a high amount of ACN the π-π-interactions are reduced and hydrogen bonding is favored. It is of interest how this change of interactions effects on the elution order of enantiomers.

With the help of these former evaluations a mobile phase consisting of methanol as bulk phase and 50 mM FA and 25 MM DEA as acidic and basic additives was a compromise of high separation performance and proper retention times.

In this work the composition of the bulk phase was systematically changed from 100 % MeOH to 50 % MeOH/ACN. With using 75 % ACN in the mobile phase limited solubility of the analytes in the mobile phase occurred and therefore no meaningful results were achieved. Because of the upcoming difficulty of measuring, basic amino acids were measured with acidic additive of 100 mM instead of 50 mM FA in 100 % MeOH (acid/base ratio of 4/1) only during the characterization of **CSP 2** and **CSP 3**.

3.3. Analytes

For the evaluation of the CSPs a broad analyte set was chosen, comprising 75 racemic amino acids and aminosulfonic acids (including cysteic acid and homocysteic acid). Furthermore, 52 single enantiomers were used for determination of the elution order. In three cases when (L)-enantiomers were not available (D)-enantiomers were applied.

The analyte set contained proteinogenic, halogenic derivatives of phenylalanine (including Baclofen), hydroxy AAS, β-AAS, α-alkylated (mostly methylated) AAS, N-methylated AAS, cyclic AAS, "Nor"-AAS and other AAS which could not be classified in the groups listed before like kynurenine or 1-naphtylalanine.

The amino acids were split in aromatic (= UV-active) and aliphatic (= non-UV-active) groups and measured by two different instruments, where one instrument had an UV/VIS-Detector and the other a charged areasol detector (CAD). All amino acids were commercially available or were kind gifts of other working groups. (2R)-2-amino-3-sulfopropanoic acid was synthesized in house. α-aminosulfonic acids like 1-amino-3-methylbutane-1-sulfonic acid and 2-amino-methyl-propane-1-sulfonic acid were removed from the analyte set because they were not stable for several weaks. Besides, 1,3-thiazolidine-4-carboxylic acid was removed from the analytes set. The used racemate and (S)-enantiomer of pyrrolidin-2-ylmethansulfonic acid was impure (impurity was seen after the eluted analyte). The whole set of analytes is shown in (**Figure 3.2 – 3.11**).

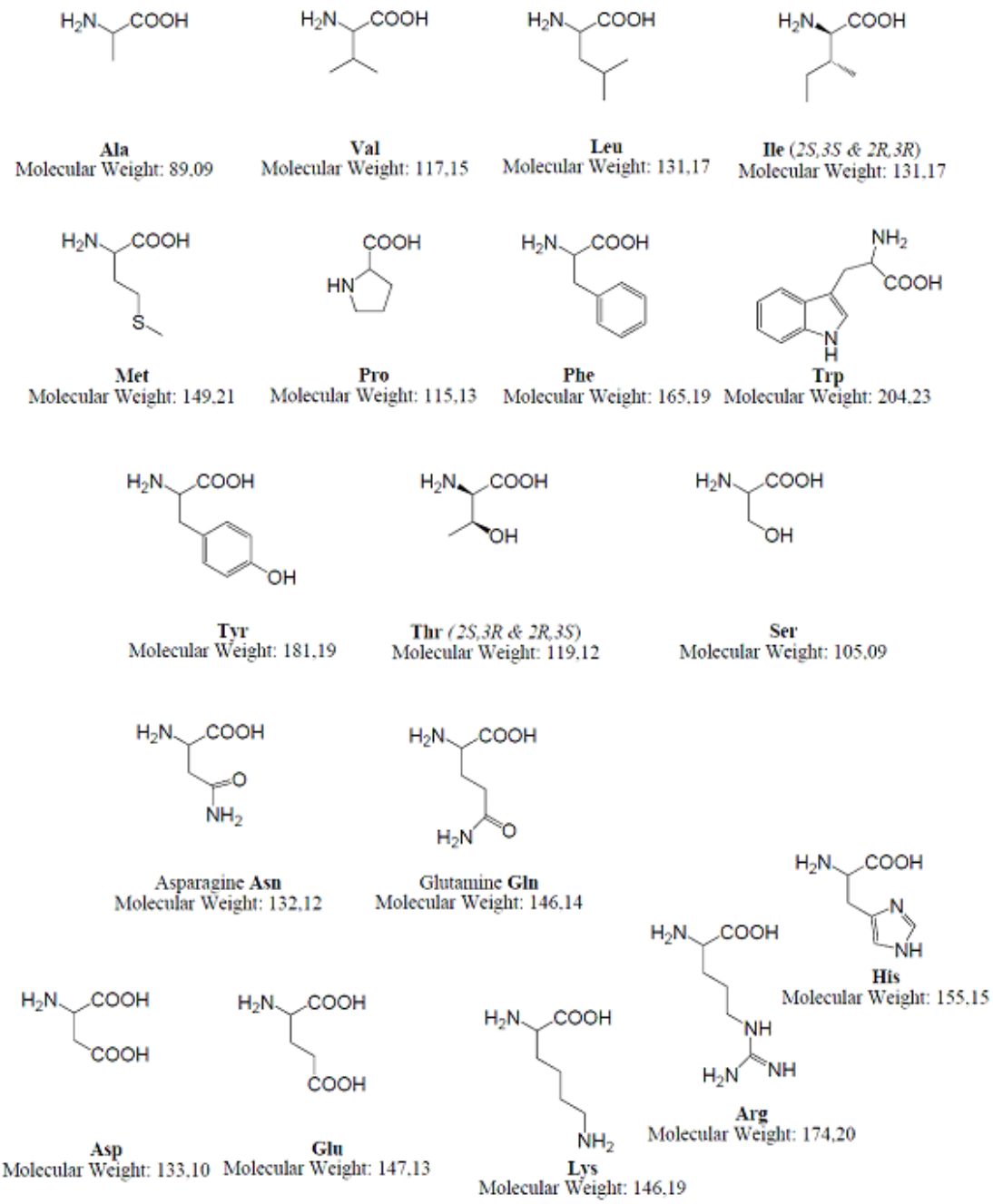


Figure 3.2.: *Proteinogenic AAS (exclusive glycine and cysteine), for all proteinogenic AAS the (L)-enantiomer is also measured*

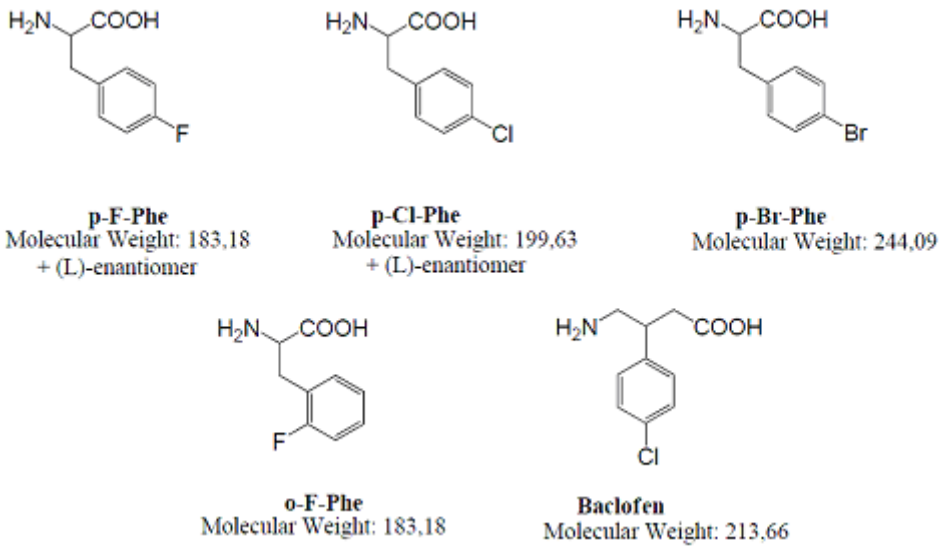


Figure 3.3.: *Halogen derivatives of phenylalanine (including baclofen)*

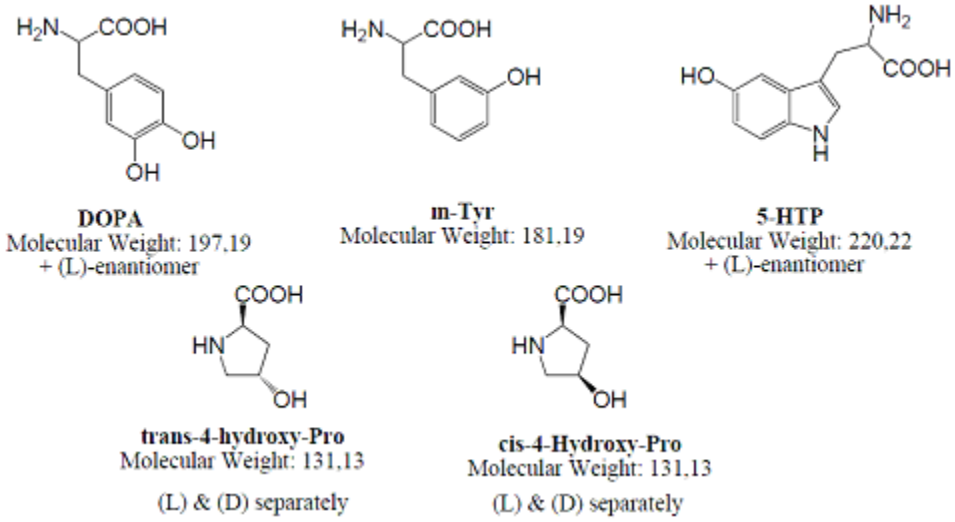


Figure 3.4.: *Hydroxy derivatised AAS*

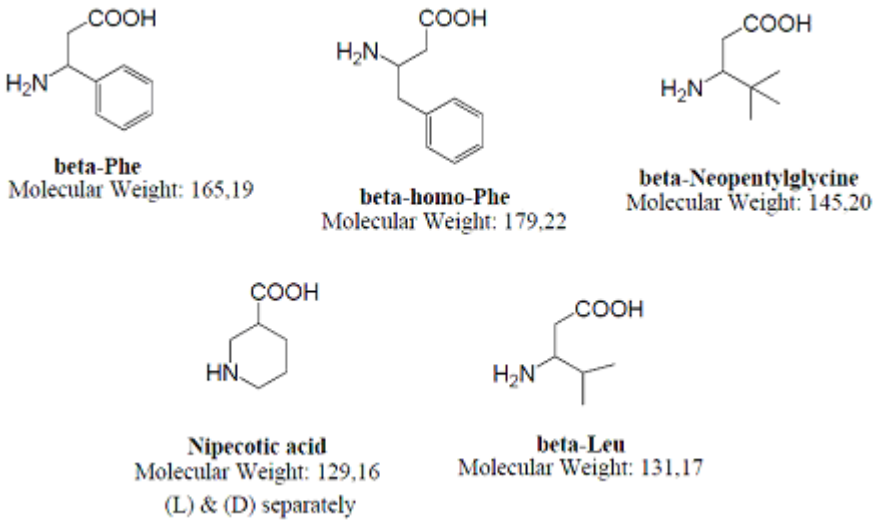


Figure 3.5.: *β-AAS*

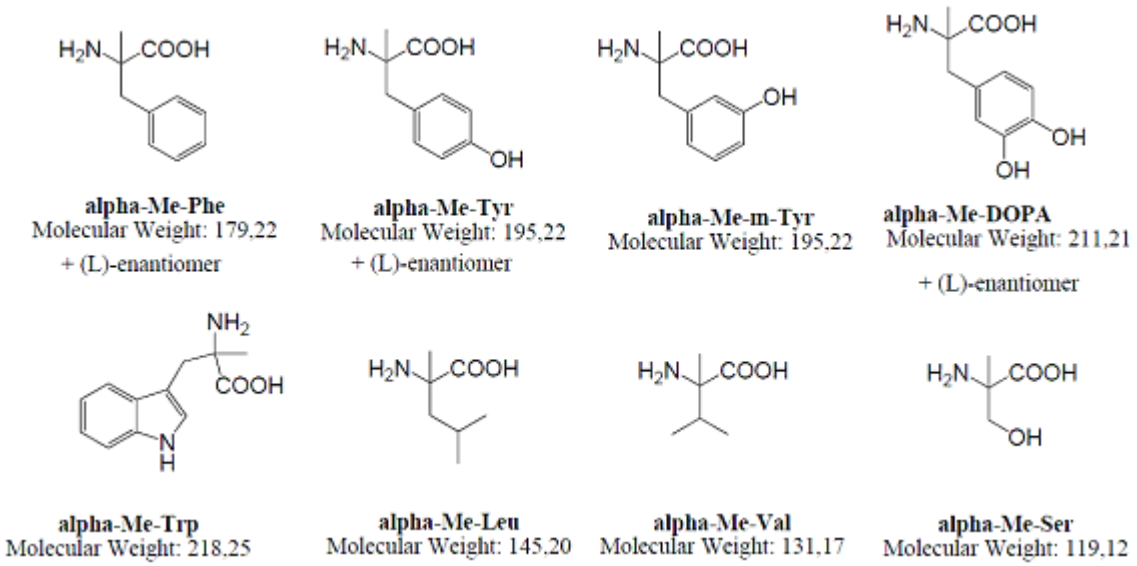


Figure 3.6.: *α-methylated AAS*

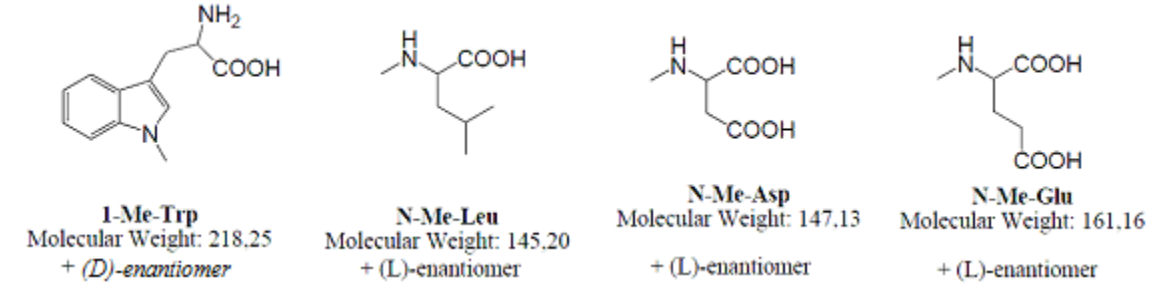
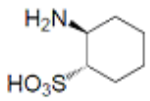
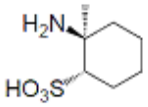


Figure 3.7.: *N-methylated AAS*

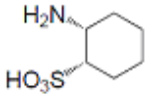
8) aminosulfonic acids



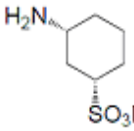
trans-2-ACHSA
(trans-racemate)
Molecular Weight: 179,24



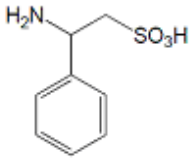
trans-2-Me-2-ACHSA
(trans-racemate)
Molecular Weight: 193,26



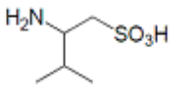
cis-2-ACHSA
(>99% cis-racemate)
Molecular Weight: 179,24



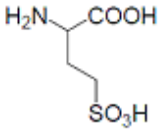
cis-3-ACHSA
(>95% cis-racemate)
Molecular Weight: 179,24



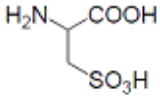
2-phenyl-Tau
Molecular Weight: 201,24



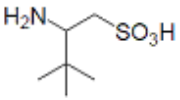
2-isopropyl-Tau
Molecular Weight: 167,23



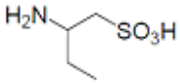
Homocysteic acid
Molecular Weight: 183,18
+ (L)-enantiomer



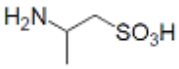
Cysteic acid
Molecular Weight: 169,16
+ (L)-enantiomer
(monohydrate)



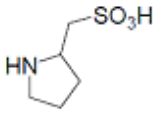
2-tert-Bu-Tau
Molecular Weight: 181,25
+ (S)-enantiomer



2-Et-Tau
Molecular Weight: 153,20
+ (S)-enantiomer

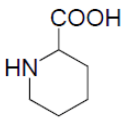


2-Me-Tau
Molecular Weight: 139,17
+ (S)-enantiomer

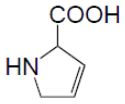


Pro-Me-SO3H
Molecular Weight: 165,21
+ (S)-enantiomer

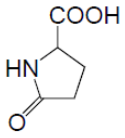
Figure 3.8.: *Aminosulfonic acids*



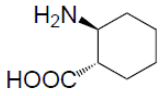
Pipecolic acid (Homoproline)
Molecular Weight: 129,16
+ (L)-enantiomer



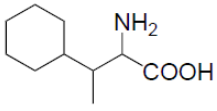
3,4-Dehydroproline
Molecular Weight: 113,11



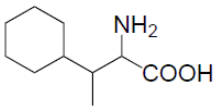
5-oxo-Proline
Molecular Weight: 129,11



trans-2-aminocyclohexanecarboxylic acid
Molecular Weight: 143,18

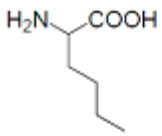


erythro-beta-methyl-cyclohexylalanine
Molecular Weight: 185,26

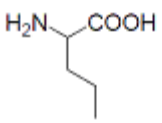


threo-beta-methyl-cyclohexylalanine
Molecular Weight: 185,26

Figure 3.9.: *cyclic AAS*

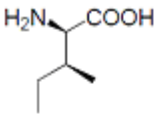


Nor-Leu
Molecular Weight: 131,17
+ (L)-enantiomer

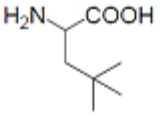


Nor-Val
Molecular Weight: 117,15
+ (L)-enantiomer

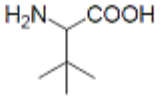
Figure 3.10.: *"Nor"-AAS*



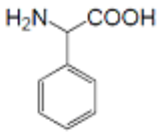
***allo*-Ile** (*2S,3R & 2R,3S*)
Molecular Weight: 131,17
+ (L)-enantiomer



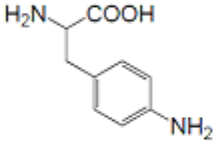
Neopentylglycine
Molecular Weight: 145,20
+ (L)-enantiomer



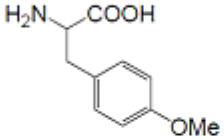
tBu-Glycine
Molecular Weight: 131,17
+ (L)-enantiomer



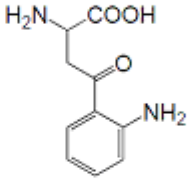
Phenylglycine
Molecular Weight: 151,16
+ *(D)-enantiomer*



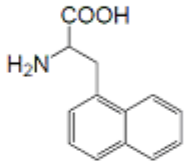
p-Amino-Phe
Molecular Weight: 180,20



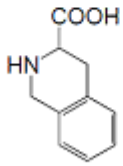
OMe-Tyr
Molecular Weight: 195,22
+ (L)-enantiomer



Kynurenine
Molecular Weight: 208,21
+ (L)-enantiomer

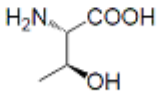


1-Naphtylalanine
Molecular Weight: 215,25
+ *(D)-enantiomer*



Tic
Molecular Weight: 177,20

+ (S)-enantiomer



allo-Thr (*2S,3S & 2R,3R*)
Molecular Weight: 119,12

+ (L)-enantiomer

Figure 3.11.: *remaining AAS (not classified)*

3.4. Materials and Methods for Evaluation

The chromatographic measurements were carried out either on a 1260 series HPLC system from Agilent Technologies (Waldbronn, Germany) consisting of an autosampler, a binary pump with four channels and a variable wavelength detector (VWD) for all aromatic amino acids and derivatives thereof. These analytes were detected by the VWD at a wavelength of 254 nm. The other instrument used a 1200 series HPLC system from Agilent Technologies (Waldbronn, Germany) consisting of an autosampler, a binary pump with four channels and a corona charged aerosol detector (CAD) from ESA Bioscience, Inc. (Chelmford, USA) for all aliphatic amino acids and derivatives thereof. After using the columns they were washed (7 column volumens) and stored in MeOH.

All measured chromatograms were edited with Chemstation B.04.03 chromatographic data software from Agilent Technologies and all other needed data were evaluated by Microsoft excel.

Elution was performed in isocratic mode with a flow rate of 0.4 mL/min at (25.0 $\pm$ 0.1°C). The void time of all columns was determined with 50 µl to 100 µl acetone in MeOH (whole volume 1 ml) The injection volume (including determination of the void volume) was 10 µl.

The analytes were dissolved either in MeOH or in a methanol-water solution. Aminosulfonic acids were dissolved in pure water. The analyte concentrations were 1.0 $\pm$ 0.1 mg/ml. For determination of the elution order of the enantiomers in a racemic mixture, a single enantiomer (in almost all samples the (L)-enantiomer) was measured, from which the absolute configuration was already known.

3.5. Comparison of ZWIX-CSPs

The evaluation of all eight zwitterionic CSPs was arranged by a set of aromatic, aliphatic amino acids and aminosulfonic acids. The results are discussed in this chapter. To characterize the performance of each CSP the common chromatographic parameters (k_i, α, R_S and N) of each CSP were compared. Differences in these parameters determine the separation performance of the CSPs.

CSP 1 (dimethyl-Tau-QN) and **CSP 7** (Tau-QN) are of zwitterionic character but carry no chiral center on the cation exchange part.

CSP 5 (2-(S)-isobutyl-Tau-QN) has been packed badly, therefore the comparison of the plate number N of the columns is not meaningful.

CSP 4 (2-(S)-isopropyl-Tau-QN) and **CSP 6** (ACHSA-Tau-QN) were compared to each other. **CSP 6** is a "Benchmark CSP" with the best results for separation of amino acids in former studies[50, 51].

Moreover, **CSP 1** (dimethyl-Tau-QN) and **CSP 7** (Tau-QN) were compared to determine if a chiral center at the SCX-part or just a steric hindering group is crucial for pronounced enantioselectivity towards zwitterionic analytes.

CSP 2 (2-(S)-phenyl-Tau-QN) and **CSP 8** (2-(R)-phenyl-Tau-QN) were compared to determine if elution order switches when changing the configuration of the chiral center at the SCX-part.

CSP 3 (2-(R)-isopropyl-Tau-QD) and **CSP 4** (2-(S)-isopropyl-Tau-QN) were compared to each other to discuss the elution order of the enantiomers when switching from QN to QD based selectors.

The influence of the SCX-part side chains particular CSPs (**CSP 2**, **4**, **5** and **6**) were compared to each other.

Finally to mention, the measurement of the basic and acidic amino acids was difficult and did not give good results. The used mobile phase-conditions are not the best for separating such amino acids.

Retention Factor k_i

The retention factor k_i is calculated from the void time t_0 of the column and of the retention time t_i of the analyte.

$$k_i = \frac{t_0 - t_i}{t_0} \qquad [3.1]$$

The retention factor k_i specifies the binding strength between the chiral selector attached on the silica gel and the selectant (analytes). When the retentions factor k_i becomes larger, the stronger the interaction between selector and analyte will be.

Selectivity coefficient/ separation factor α

The separation factor α describes the selectivity and gives information about the capability of a chromatographic system. Calculated by dividing of the retention factors of the second eluted enantiomer (k_2) through the first (k_1) eluted enantiomer gives α.

$$\alpha_{1,2} = \frac{k_2}{k_1} \qquad [3.2]$$

Different retention factors lead to α–values over 1.00. An α-value of 1.00 means that there is no (enantio)separation of the two enantiomers. High α-values allow baseline separation at the baseline and show good application for preparative separations.

Resolution R_S

The resolution is one of the significant parameters and shows the quality of a separation.

The resolution R_S depends on the retention factor k_i, on the selectivity coefficient α and of the plate number N. Baseline separation occurs when R_S-value is over 1.50. Values between 1.00 and 1.50 mean partial separation. High resolution values are necessary for preparative chromatography applications.

Theoretical plate number N

The plate number N provides information about column performance. Furthermore, N provides information about the packing quality of a column and the selector coverage on the stationary phase. Low N-values are in many cases sign of peak tailing.

The peak shape can be described by the asymmetry factor A_S = a/b. The asymmetric factor is defined as the distance b (distance from the peak center line to the back slope) divided by the distance a (distance from the peak center line to the front slope). These distances are made at 10 % of the maximum peak height. When the value of the asymmetric factor is near 1, the peak is almost symmetric, when the value is over 1 the peak is tailing, when the value is under 1 peak fronting occurs.

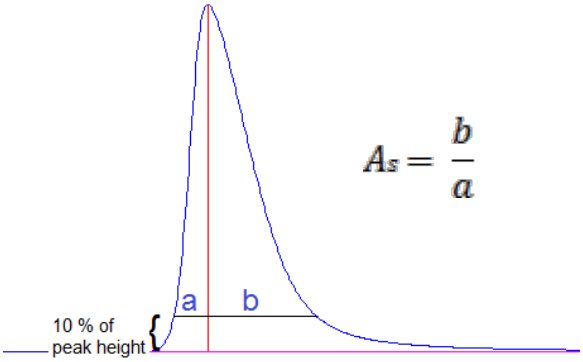


Figure 3.1.: *Asymmetric factor*

Retention studies

As it is seen in **table 3.1** all zwitterionic stationary phases have different selector coverage. The difference in the selector loading from the lowest to the highest coverage accounts for 88 µmol g^{-1}. Former investigation in the working group showed that the height of selector loading influence on the amount of the interactions between the selector and the selectand and therefore a increasing of the retention time[52].

In **Table 3.1** the average of the retention factor k_1 of aromatic and aliphatic amino acids and aminosulfonic acids of all CSPs were also compared to each other. 100 % MeOH with 50 mM FA and 25 mM DEA were used as mobile phase.

k_1-values							
	Dimethyl-Tau-QN CSP 1	2-(S)-phenyl-Tau-QN CSP 2	2-(R)-Iso-P-Tau-QD CSP 3	2-(S)-Iso-P-Tau-QN CSP 4	2-(S)-Iso-B-Tau-QN CSP 5	ACHSA-Tau-QN CSP 6	Tau-QN CSP 7
Selector loading [µmol g^{-1}]	255	186	274	203	265	232	190
aliphatc AAS	0.81	0.78	1.23	1.64	2.02	2.26	0.77
aromatic AAS	0.76	0.83	1.21	1.66	1.50	1.72	1.18
aminosulfonic acids	0.68	1.08	1.52	2.06	2.56	2.10	0.61
all analytes	0.77	0.85	1.27	1.71	1.93	2.06	0.88

Table 3.1.: *average k_1-values of certain zwitterionic analyte groups*

When considering the k_1-values, it can be interpreted that the average retention in **CSP 1** is the lowest, following by the retention factors of **CSP 2** and **CSP 7**. **CSP 6** shows stronger retention towards almost all analytes.

All CSPs differ in the SCX-part of the selector. It is of interest how the different SCX-part of each CSP affects the interaction between analyte and selector and also the retention of the analytes. Investigation on different CSPs and different mobile phases was done by our working group[51] before. It was discovered that retention of zwitterionic analytes increased with increasing ACN in the mobile phase. On the other hand the presence of a higher ACN amount leads to decrease of enantioselectivity-values and resolution-values in many cases. Besides, using a higher amount of aprotic ACN a degradation of peak efficiency could be observed and thus, baseline separation could be more difficult to achieve.

Retention studies with all three mobile phases (pure MeOH, MeOH with 25 % ACN and MeOH with 50 % ACN) were realized with **CSP 6** (whole analyte set), **CSP 1**, **CSP 5** (both aromatic analytes only) and with **CSP 4** (aliphatic analytes only). For the majority of these analytes it was observed that α-values and R_S-values were increasing. The opposite behavior was also observed at certain analytes, like α-methyl-DOPA (as displayed in the **figure 3.1** and **3.2**).

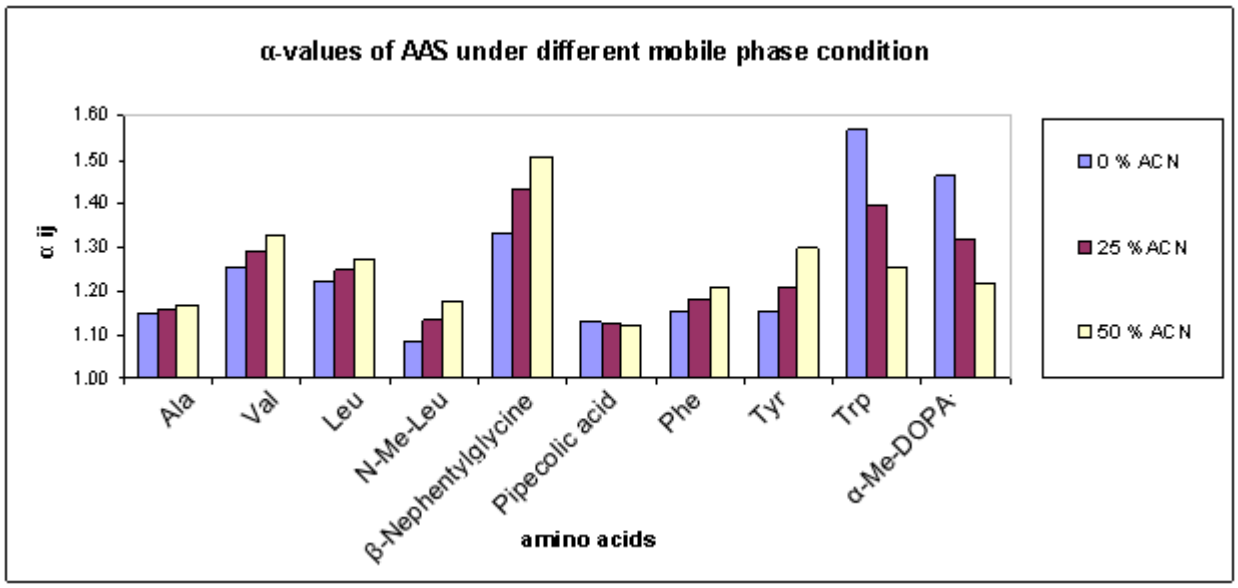


Figure 3.1.: *trend of α-values of certain zwitterionic analytes on* ***CSP 6***

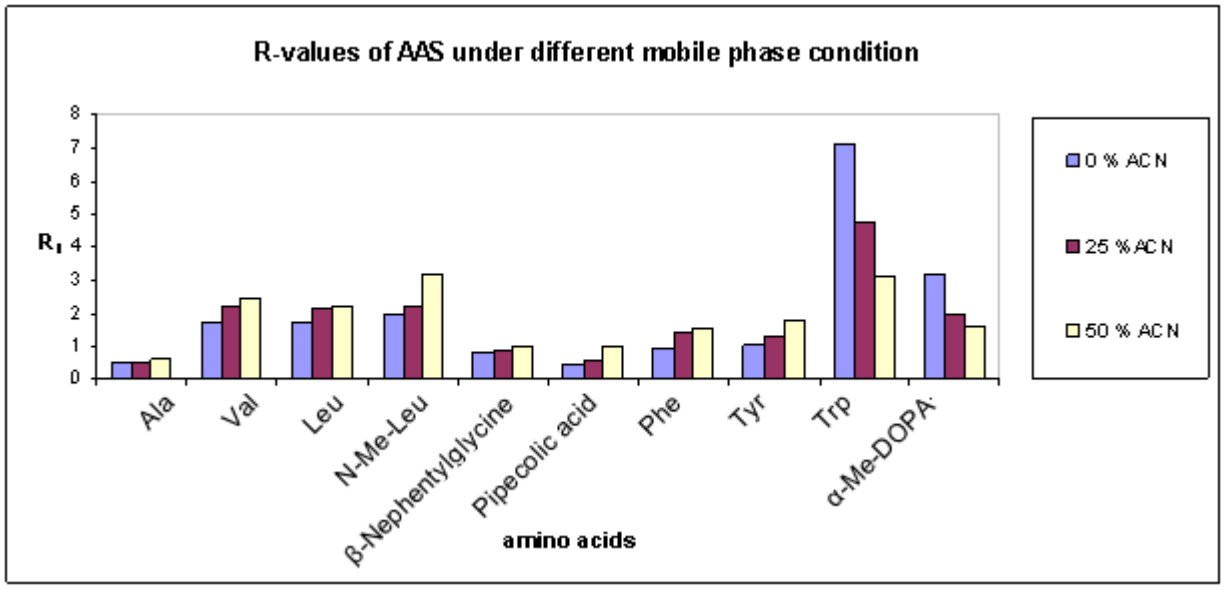


Figure 3.2.: *trend of resolution-values of certain zwitterionic analytes on* ***CSP 6***

It was clearly visible that the retention factor k_1 increased with higher amount of ACN in the mobile phase. The k_1-values increased up to 97 % in average (comparing data of all analytes measured on **CSP 1** to **CSP 7**) when using 50 % ACN in mobile phase compared to 100 % MeOH. In **figure 3.3** and **figure 3.4** changes of k_1-values and α-values are shown.

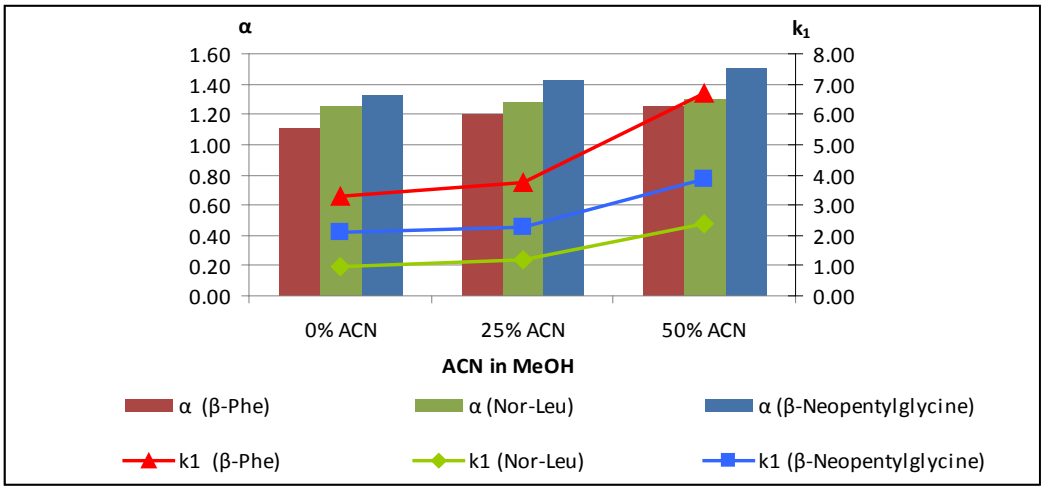


Figure 3.3. *α-values and k_1-values of β-phenylalanine, Nor-leucine and β-neopentylglycine with pure MeOH, 25 % and 50 % ACN in MeOH on* ***CSP 6***

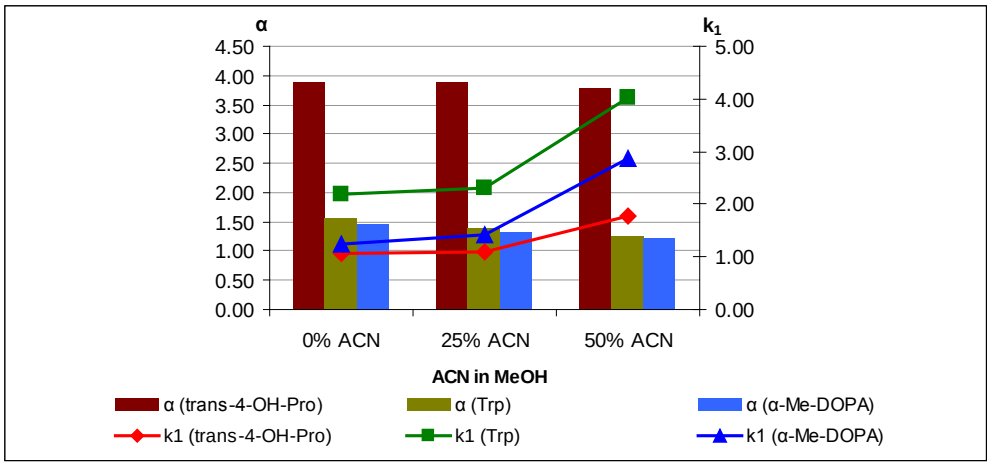


Figure 3.4. *α-values and k_1-values of trans-4-hydroxyproline, tryptophan and α-methyl-DOPA with in MeOH, 25 % and 50 % ACN in MeOH on* ***CSP 6***

Using these CSPs it was observed that the separation factor α and the resolution R_S increased with higher amount of ACN depending on the analytes. For the majority of the analytes the values of enantioselectivity and resolution increased with higher ACN-amount, as it was pictured in **figure 3.3**. The separation factor decreased with higher ACN-content as pictured in **figure 3.4**. The retention factors of all analytes were increasing with higher ACN-amount. The α-values increased with increasing the ACN-amount in the mobile phase with the exception that

the α-values of tryptophan and tryptophan derivatives and of many α-methylated amino acids decreased.

In **figure 3.5** and **3.6** changes of the parameters R_S and N_2 were pictured, where in **figure 3.5** an increase of R_S with higher ACN amount and in **figure 3.6** a decrease of R_S with higher ACN amount was seen. The values of the plate number N_2 of the second eluted enantiomer seemed to become smaller at higher amount of aprotic solution.

The resulting peaks are not symmetric. A tailing under every mobile phase condition could be observed, where in 50/50 MeOH/ACN the peak-tailing was the greatest.

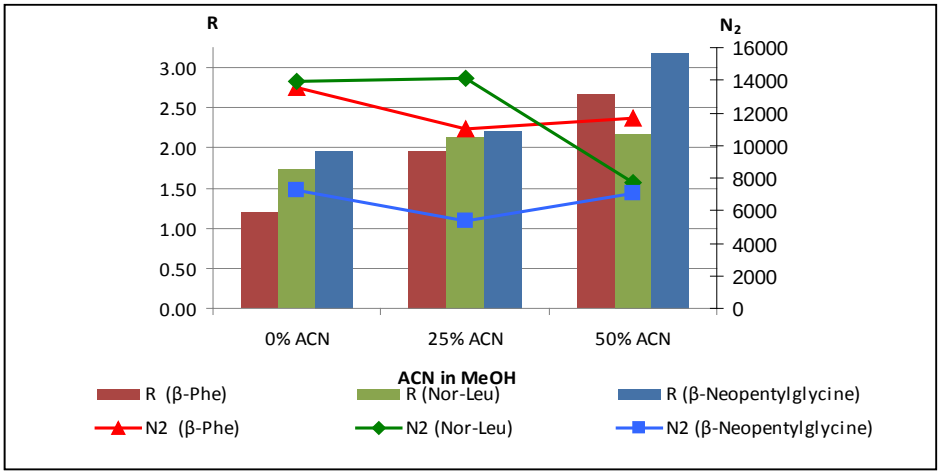


Figure 3.5. *resolution-values and N_2-values of β-phenylalanine, Nor-leucine and β-Neopentylglycine in pure MeOH, 25 % and 50 % ACN in MeOH on* ***CSP 6***

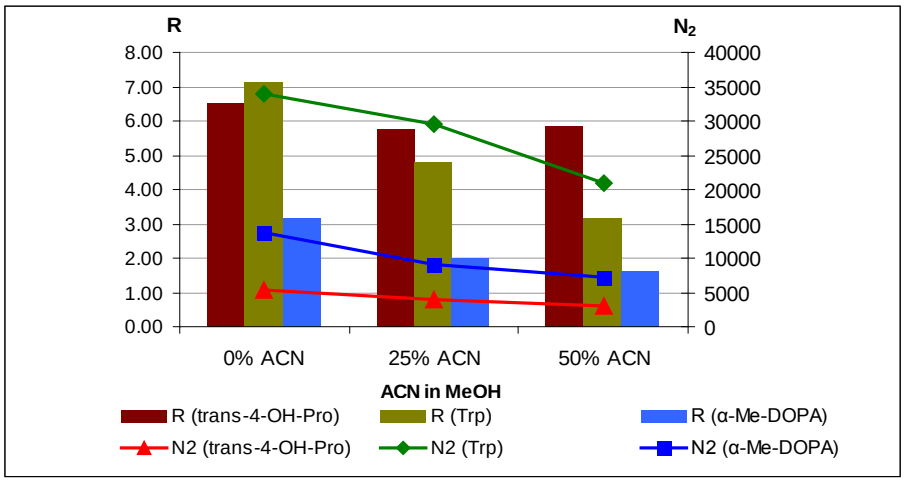


Figure 3.6. *resolution-values and N_2-values of trans-4-hydroxyproline, tryptophan and* α-methyl-DOPA *and pure MeOH, 25 % and 50 % ACN in MeOH on* ***CSP 6***

Figure 3.7 depicts three chromatograms of tert-leucine under three different mobile phase conditions. The two enantiomeric peaks of tert-leucine were better separated and higher retained with higher ACN amount in the mobile phase. In contrary, the peaks show stronger tailing. The peaks became smaller and broader. By calculating the asymmetry factor it was confirmed that the tailing became stronger with higher ACN-amount in the mobile phase.

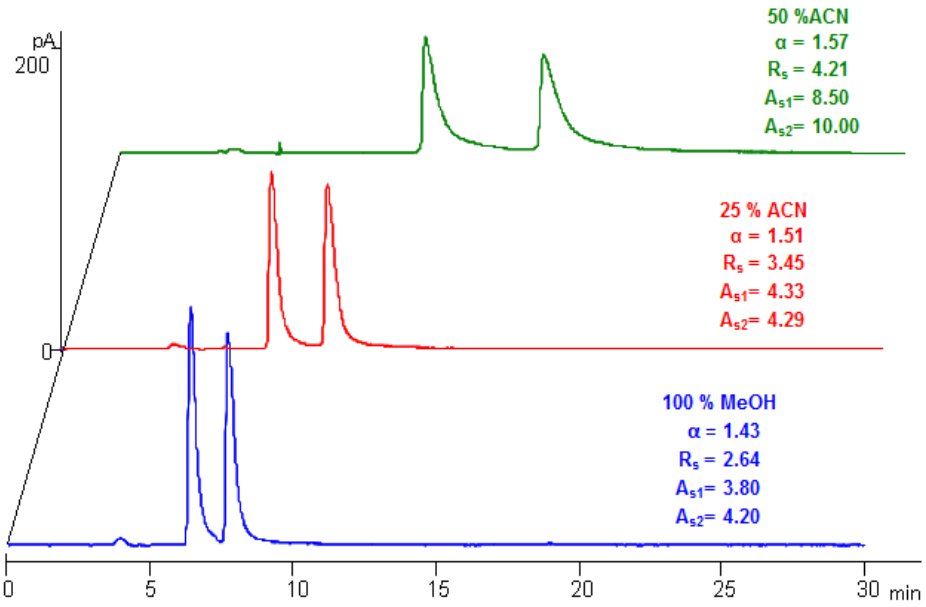


Figure 3.7. *HPLC enantiomer separations of tert-leucine on ACHSA-Tau-QN (CSP 6) with 100 % MeOH, 25 % ACN and 50 % ACN in the mobile phase (t_0=3.40 min)*

Figure 3.8 shows separation of 5-HTP. The selectivity and resolution decreased with increasing the ACN amount. At 50 % ACN in the mobile phase there was no baseline separation. The asymmetric factor became smaller whicht indicated that tailing was smaller in mobile phase with higher amount of aprotic solvent.

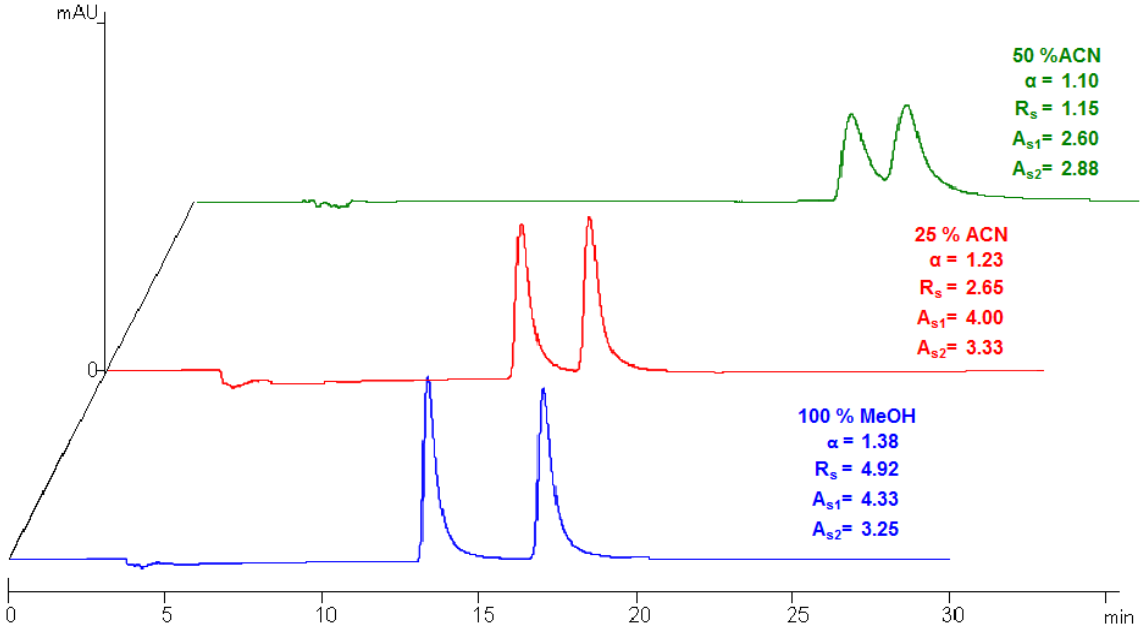


Figure 3.8. *HPLC enantiomer separations of 5-HTP on ACHSA-Tau-QN (CSP 6) with 100 % MeOH, 25 % ACN and 50 % ACN in the mobile phase (t_0=3.40 min)*

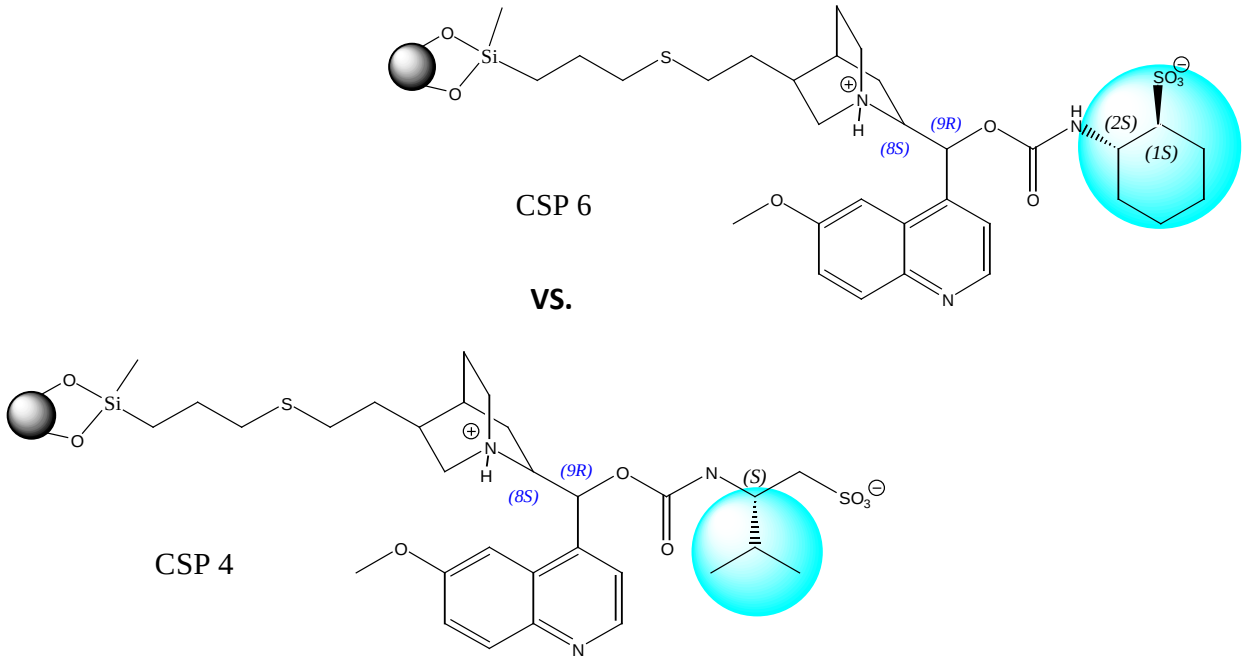
After evaluating the first CSPs with all three mobile phase conditions (100 % MeOH, 25 % ACN and 50 % ACN) it was seen that for determining changes in selectivity and resolution only two mobile phases (100 % MeOH and 50 % ACN) were necessary and the mobile phase with 25 % ACN could be omitted.

Comparing all data of the analytes with 100 % MeOH and 50 % ACN in MeOH, the strongest decrease of enantioselectivity was seen on **CSP 3** (47 % of the analytes), the less on **CSP 6** (25 % of the analytes). For aromatic analytes the greatest decrease of separation factor α was on **CSP 7** (79 % of the analytes). When comparing all α-values and R_S-values of all zwitterionic analytes used on all CSPs with all mobile phases, there were 60 % of all α-values and 58 % of all R_S-values increased with increasing ACN-amount. At 10 % of all analytes the α-values increased where R_S-

values decreased because of worse peak shapes. At 8 % of all analytes the R_S-values increased where the α-value decreased.

To sum up, resolution-values were increasing on average with higher ACN-amount. For many analytes the separation factor α on diverse CSPs was increasing with higher ACN content as well. Nevertheless, the opposite behavior occurred on all CSPs for certain analytes like tryptophan and tryptophan derivatives. Using **CSP 3** had the lowest decrease in resolution while **CSP 6** had the most.

3.6. ACHSA-Tau-QN (CSP 6) vs. 2-(S)-isopropyl-Tau-QN (CSP 4)



Previous studies revealed that **CSP 6** and **CSP 4** showed best results in enantioselectivity and resolution. Both CSPs are of the quinine-type. The selectors of these two CSPs differ only in their side chain of the SCX-moiety. **CSP 6** consists of a cyclohexane moiety with absolute configurations (1S) and (2S) at the aminosulfonic acid part. **CSP 4** has an isopropyl group with a (S)-configuration. The cyclohexyane moiety is a larger and more spatially demanding group than the isopropyl moiety and is also more rigid.

β-AAS

In former research of the working group, it was confirmed that β-amino acid enantiomers were strongly retained and partly better enantioseparated than α-amino acids[55]. Comparison of enantioselectivity values between **CSP 6** and **4** revealed higher α-values for **CSP 4**. Of all β-AAS nipecotic acid had the best values. With 50 % ACN in the mobile phase all β-AAS were baseline separated on both CSPs.

	CSP 6 100 % MeOH			CSP 4 100 % MeOH			CSP 6 50 % ACN			CSP 4 50 % ACN		
β-AAS	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**
Nipe	R-S	**1.99**	**4.08**	R-S	**2.23**	**5.69**	R-S	**2.40**	**5.74**	R-S	**2.89**	**10.03**
β-Leu	n.d.	**1.06**	**0.64**	n.d.	**1.06**	**0.56**	n.d.	**1.23**	**2.91**	n.d.	**1.23**	**3.57**
β-Neopentylgly	n.d.	**1.33**	**2.00**	n.d.	**1.35**	**2.59**	n.d.	**1.50**	**3.19**	n.d.	**1.53**	**4.39**
β-Phe	n.d.	**1.11**	**1.20**	n.d.	**1.15**	**1.95**	n.d.	**1.26**	**2.67**	n.d.	**1.31**	**5.79**
β-homo-Phe	n.d.	**1.03**	**0.52**	n.d.	**1.00**	**0.00**	n.d.	**1.10**	**1.92**	n.d.	**1.11**	**1.84**
Average values		**1.30**	**1.69**		**1.36**	**2.16**		**1.50**	**3.29**		**1.62**	**5.12**

Table 3.2.: *β-AAS measured on CSP 4 and 6*

α-methylated AAS

Several zwitterionic CSP were able to enantioseparate α-methylate amino acids in former studies[49].

CSP 4 and **6** separated only a part of α-methylated AAS enantiomers. All used α-methylated AAS could be partially separated on **CSP 4** using 100 % MeOH, but only three analytes - all are aromatic - were baseline separated. The best α-value and R_S-value in all two mobile phases had α-methylated tryptophan. The elution order of α-methylated tyrosine changed on both CSPs when using higher ACN amount in the mobile phase. In pure MeOH the aromatic α-methylated amino acids were better separated on both CSPs.

	CSP 6 100 % MeOH			CSP 4 100 % MeOH			CSP 6 50 % ACN			CSP 4 50 % ACN		
α -methylated AAS	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**
α-Me-Leu	n.d.	**1.00**	**0.00**	n.d.	**1.08**	**0.49**	n.d.	**1.12**	**1.00**	n.d.	**1.18**	**1.32**
α-Me-Val	n.d.	**1.00**	**0.00**	n.d.	**1.02**	**0.16**	n.d.	**1.00**	**0.00**	n.d.	**1.00**	**0.00**
α-Me-Ser	n.d.	**1.13**	**0.79**	n.d.	**1.11**	**0.59**	n.d.	**1.11**	**0.85**	n.d.	**1.08**	**0.54**
α-Me-Phe	n.d.	**1.13**	**0.49**	D-L	**1.18**	**0.59**	n.d.	**1.00**	**0.00**	n.d.	**1.00**	**0.00**
α-Me-Tyr	D-L	**1.17**	**0.88**	D-L	**1.14**	**0.67**	L-D	**1.07**	**0.53**	L-D	**1.07**	**0.54**
α-Me-m Tyr	n.d.	**1.42**	**2.70**	n.d.	**1.42**	**2.54**	n.d.	**1.29**	**2.03**	n.d.	**1.31**	**2.22**
α-Me-DOPA	D-L	**1.46**	**3.18**	D-L	**1.52**	**2.52**	D-L	**1.22**	**1.62**	D-L	**1.29**	**1.68**
A-Me-Trp	n.d.	**3.40**	**20.61**	n.d.	**3.56**	**20.51**	n.d.	**2.14**	**13.28**	n.d.	**2.07**	**13.31**
Average values		**1.46**	**3.58**		**1.50**	**3.51**		**1.24**	**2.41**		**1.25**	**2.45**

Table 3.3.: *α –methylated AAS measured on CSP 4 and 6*

Aminosulfonic AAS

On **CSP 6** the aminosulfonic acids were stronger retained. Cysteic acid and homocysteic acid had larger retention factors than the other analytes and also showed a strong peak tailing. On both CSPs these two aminosulfonic acids could not be enantioseparated. The analytes 2-tert-butyltaurine, 2-ethyltaurine, 2-isopropyltaurine and 2-phenyltaurine were baseline separated on both CSPs with both mobile phase conditions. The elution order did not change with higher ACN amount.

	CSP 6 100 % MeOH			CSP 4 100 % MeOH			CSP 6 50 % ACN			CSP 4 50 % ACN		
aminosulfonic acids	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**
Homocysteic acid	n.d.	**1.00**	**0.00**	n.d.	**1.00**	**0.00**	n.d.	**1.00**	**0.00**	n.d.	**1.11**	**0.60**
Cysteic acid	n.d.	**1.00**	**0.00**	L-D	**1.11**	**0.60**	n.d.	**1.00**	**0.00**	n.d.	**1.13**	**0.49**
trans-2-ACHSA	n.d.	**1.05**	**0.48**	n.d.	**1.09**	**0.60**	n.d.	**1.08**	**0.55**	n.d.	**1.05**	**0.39**
cis-2-ACHSA	n.d.	**1.37**	**1.85**	n.d.	**1.26**	**1.18**	n.d.	**1.37**	**1.47**	n.d.	**1.18**	**0.68**
cis-3-ACHSA	n.d.	**1.11**	**0.66**	n.d.	**1.16**	**0.87**	n.d.	**1.03**	**0.28**	n.d.	**1.04**	**0.29**
trans-2-Me-2-ACHSA	n.d.	**1.10**	**1.33**	n.d.	**1.49**	**5.31**	n.d.	**1.00**	**0.00**	n.d.	**1.19**	**3.83**
2-tert-Bu-Tau	R-S	**2.46**	**5.77**	R-S	**2.79**	**6.47**	R-S	**2.52**	**4.28**	R-S	**2.62**	**4.97**
2-Et-Tau	R-S	**1.39**	**2.33**	R-S	**1.52**	**2.48**	R-S	**1.43**	**1.96**	R-S	**1.54**	**2.23**
2-Me-Tau	R-S	**1.08**	**0.56**	R-S	**1.16**	**0.83**	R-S	**1.08**	**0.51**	R-S	**1.17**	**0.69**
Pro-Me-SO_3H	R-S	**1.21**	**1.04**	R-S	**1.34**	**1.35**	///	**2.10**	**7.19**	R-S	**1.22**	**0.73**
2-isopropyl-Tau	D-L	**1.47**	**4.41**	D-L	**1.63**	**2.99**	D-L	**1.53**	**3.99**	D-L	**1.67**	**2.58**
2-phenyl-Tau	D-L	**1.21**	**2.57**	D-L	**1.37**	**2.75**	D-L	**1.16**	**1.70**	D-L	**1.31**	**1.82**
Average values		**1.29**	**1.75**		**1.41**	**2.12**		**1.36**	**1.83**		**1.35**	**1.61**

Table 3.4.: *aminosulfonic acids measured on CSP 4 and 6*

Aromatic AAS

Almost all aromatic amino acids could be enantioseparated. A majority of the analytes was baseline separated where the most were baseline separated with a mobile phase of 50 % A CN on **CSP 4**. A change of elution order was only seen for α-methylated tyrosine when measuring with higher ACN amount in the mobile phase. On average the α-values and the R_S-values decreased when using 50/50 MeOH/ACN mobile phase.

	CSP 6 100 % MeOH			CSP 4 100 % MeOH			CSP 6 50 % ACN			CSP 4 50 % ACN		
aromatic amino acids	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**
Phe	L-D	**1.15**	**0.96**	L-D	**1.16**	**1.06**	L-D	**1.21**	**1.56**	L-D	**1.19**	**1.17**
p-F-Phe	L-D	**1.13**	**1.31**	L-D	**1.11**	**1.10**	L-D	**1.19**	**2.03**	L-D	**1.18**	**2.13**
p-Cl-Phe	L-D	**1.14**	**1.47**	L-D	**1.12**	**1.48**	L-D	**1.19**	**1.29**	L-D	**1.20**	**2.49**
p-Br-Phe	n.d.	**1.14**	**1.80**	n.d.	**1.13**	**1.62**	n.d.	**1.18**	**2.12**	n.d.	**1.21**	**2.66**
o-F-Phe	n.d.	**1.16**	**1.81**	n.d.	**1.16**	**1.14**	n.d.	**1.21**	**2.80**	n.d.	**1.21**	**1.63**
β-Phe	n.d.	**1.11**	**1.20**	n.d.	**1.15**	**1.95**	n.d.	**1.26**	**2.67**	n.d.	**1.31**	**5.79**
β-homo-Phe	n.d.	**1.03**	**0.52**	n.d.	**1.00**	**0.00**	n.d.	**1.10**	**1.92**	n.d.	**1.11**	**1.84**
α-Me-Phe	n.d.	**1.13**	**0.49**	D-L	**1.18**	**0.59**	n.d.	**1.00**	**0.00**	n.d.	**1.00**	**0.00**
p-Amino-Phe	n.d.	**1.16**	**0.79**	n.d.	**1.14**	**0.77**	n.d.	**1.27**	**1.15**	n.d.	**1.23**	**1.14**
DOPA	L-D	**1.07**	**0.59**	n.d.	**1.00**	**0.00**	L-D	**1.16**	**0.83**	L-D	**1.07**	**0.37**
α-Me-DOPA	D-L	**1.46**	**3.18**	D-L	**1.52**	**2.52**	D-L	**1.22**	**1.62**	D-L	**1.29**	**1.68**
Tyr	L-D	**1.15**	**1.06**	L-D	**1.13**	**0.86**	L-D	**1.30**	**1.78**	L-D	**1.24**	**1.61**
m-Tyr	n.d.	**1.09**	**0.73**	n.d.	**1.06**	**0.46**	n.d.	**1.13**	**0.96**	n.d.	**1.08**	**0.59**
α-Me-Tyr	D-L	**1.17**	**0.88**	D-L	**1.14**	**0.67**	L-D	**1.07**	**0.53**	L-D	**1.07**	**0.54**
α-Me-m Tyr	n.d.	**1.42**	**2.70**	n.d.	**1.42**	**2.54**	n.d.	**1.29**	**2.03**	n.d.	**1.31**	**2.22**
OMe-Tyr	L-D	**1.14**	**1.20**	L-D	**1.15**	**1.36**	L-D	**1.17**	**1.21**	L-D	**1.19**	**1.72**
Trp	D-L	**1.57**	**7.11**	D-L	**1.61**	**7.62**	D-L	**1.25**	**3.14**	D-L	**1.26**	**3.21**
α-Me-Trp	n.d.	**3.40**	**20.61**	n.d.	**3.56**	**20.51**	n.d.	**2.14**	**13.28**	n.d.	**2.07**	**13.31**
1-Me-Trp	D-L	**1.33**	**4.49**	D-L	**1.36**	**4.98**	D-L	**1.10**	**1.29**	D-L	**1.12**	**1.64**
5-HTP	D-L	**1.38**	**4.92**	D-L	**1.43**	**5.23**	D-L	**1.10**	**1.15**	D-L	**1.13**	**1.32**
Baclofen	n.d.	**1.25**	**3.35**	n.d.	**1.28**	**5.43**	n.d.	**1.32**	**3.99**	n.d.	**1.34**	**6.99**
Phenylglycine	L-D	**1.06**	**0.77**	L-D	**1.04**	**0.54**	L-D	**1.09**	**1.26**	n.d.	**1.00**	**0.00**
Kynurenine	L-D	**1.24**	**2.63**	L-D	**1.10**	**1.31**	L-D	**1.23**	**1.93**	L-D	**1.12**	**1.61**
1-Naphtylalanine	L-D	**1.14**	**1.50**	L-D	**1.12**	**1.68**	L-D	**1.16**	**1.48**	L-D	**1.14**	**1.74**
Tic	S-R	**1.33**	**2.30**	S-R	**1.32**	**2.20**	S-R	**1.29**	**1.94**	S-R	**1.27**	**2.08**
Average values		**1.29**	**2.73**		**1.30**	**2.70**		**1.22**	**2.16**		**1.21**	**2.38**

Table 3.5.: *aromatic amino acids measured on CSP 4 and 6*

Proteinogenic AAS

Independent of using one of the two CSP in addition with one of the mobile phase conditions the (L)-enantiomers eluted first expect from the two acidic amino acids aspartic acid and glutamic acid. Proline-enantiomers were best separated. With 100 % MeOH using **CSP 4** serine

could be partial separated. On **CSP 6** the majority of these amino acids could be baseline separated.

	CSP 6 100 % MeOH			CSP 4 100 % MeOH			CSP 6 50 % ACN			CSP 4 50 % ACN		
proteinogenic AAS	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**	**EO**	**α**	**R_S**
Ala	L-D	**1.15**	**0.50**	L-D	**1.13**	**0.48**	L-D	**1.17**	**0.61**	L-D	**1.11**	**0.60**
Val	L-D	**1.26**	**1.74**	L-D	**1.22**	**1.51**	L-D	**1.33**	**2.42**	L-D	**1.22**	**1.94**
Leu	L-D	**1.22**	**1.72**	L-D	**1.22**	**1.63**	L-D	**1.27**	**2.21**	L-D	**1.19**	**2.03**
Ser		**1.00**	**0.00**	L-D	**1.09**	**0.56**		**1.00**	**0.00**		**1.00**	**0.00**
Thr	L-D	**1.23**	**1.33**	L-D	**1.25**	**1.31**	L-D	**1.30**	**2.02**	L-D	**1.30**	**1.46**
Phe	L-D	**1.15**	**0.96**	L-D	**1.16**	**1.06**	L-D	**1.21**	**1.56**	L-D	**1.19**	**1.17**
Tyr	L-D	**1.15**	**1.06**	L-D	**1.13**	**0.86**	L-D	**1.30**	**1.78**	L-D	**1.24**	**1.61**
Trp	D-L	**1.57**	**7.11**	D-L	**1.61**	**7.62**	D-L	**1.25**	**3.14**	D-L	**1.26**	**3.21**
Ile	L-D	**1.26**	**2.01**	L-D	**1.24**	**2.02**	L-D	**1.33**	**2.44**	L-D	**1.24**	**2.58**
Met	L-D	**1.14**	**1.19**	L-D	**1.10**	**0.96**	L-D	**1.17**	**1.20**	L-D	**1.11**	**1.04**
Lys	L-D	**1.16**	**1.04**	L-D	**1.18**	**2.27**		n.d.		L-D	**1.00**	**0.00**
Pro	L-D	**1.57**	**4.01**	L-D	**1.56**	**4.30**	L-D	**1.86**	**5.85**	L-D	**1.94**	**5.96**
Asn		**1.00**	**0.00**	L-D	**1.20**	**1.00**	L-D	**1.33**	**1.56**		**1.00**	**0.00**
Gln	L-D	**1.16**	**0.69**		**1.00**	**0.00**	L-D	**1.27**	**0.93**	n.d.	**1.26**	**1.38**
Asp		**1.00**	**0.00**	D-L	**1.05**	**0.33**		**1.00**	**0.00**		**1.00**	**0.00**
Glu		**1.00**	**0.00**	D-L	**1.10**	**0.59**	D-L	**1.15**	**1.02**	n.d.	**1.15**	**0.66**
Arg	n.d.	**1.07**	**0.50**	n.d.	**2.37**	**1.38**		n.d.			n.d.	
His		**1.00**	**0.00**	L-D	**1.40**	**1.59**		n.d.		n.d.	**1.58**	**0.87**
Average values		**1.15**	**0.98**		**1.27**	**1.33**		**1.27**	**1.69**		**1.22**	**1.32**

Table 3.6.: *proteinogenic acids measured on CSP 4 and 6*

CSP 6 achieved better results of average α-values and R_S-values. Besides, the retention-value was greater for almost all analytes when using **CSP 6.** For phenylalanine and all phenylalanine derivatives the retention factors were nearly ($\pm$ 0.05) the same.

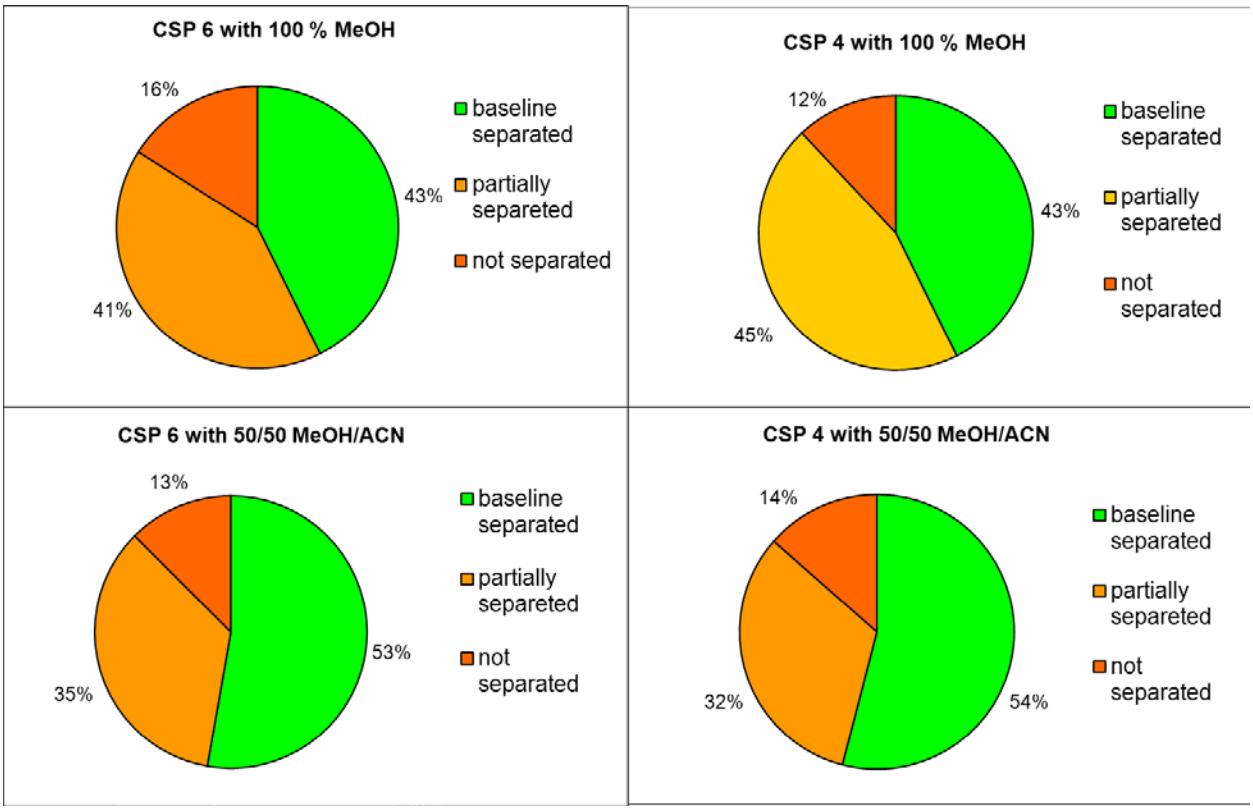
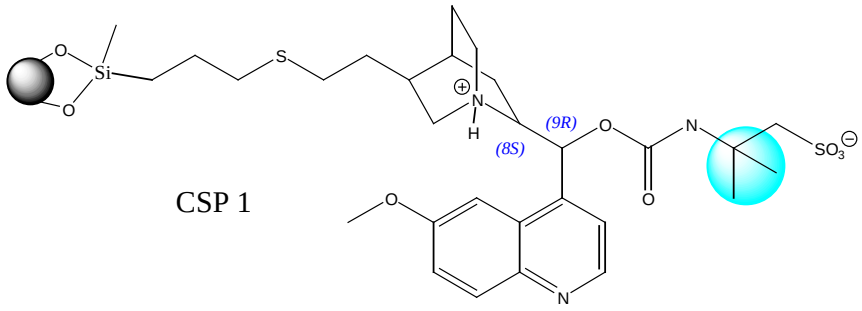


Figure 3.9.: *ratio of baseline separation, partial separated and not separated analytes*

To sum up, in many cases the results with 2-(S)-isopropyl-Tau-QN are the same with ACHSA-Tau-QN. When using 100 % MeOH the same set of analytes could be baseline separated, with higher aprotic amount 2-(S)-isopropyl-Tau-QN could baseline separate two analytes more.

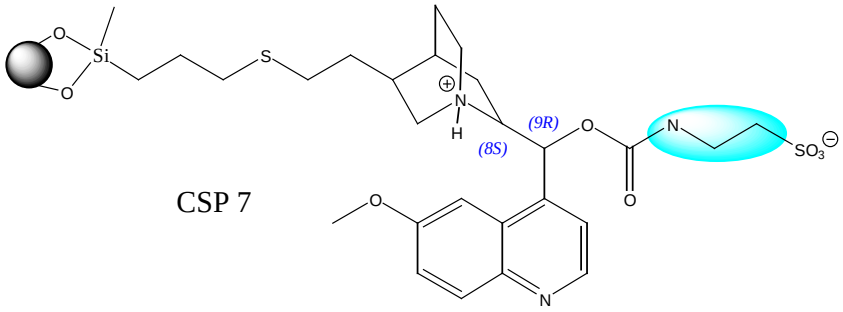
CSP 4 seems to be a competitive CSP to **CSP 6** regarding to in enantioseparation of amino acids.

3.7. Tau-QN (CSP 7) vs. Dimethyl-Tau-QN (CSP 1)



CSP 1

VS.



CSP 7

CSP 1 and **CSP 7** are similar. They both are according to their structure based on quinine. The sole difference is in the SCX-part of the selectors. Where Tau-QN (**CSP 7**) has no side chain, **CSP 1** has a dimethyl-group. Compared to all other zwitterionic CSPs, **CSP 1** and **CSP 7** do not carry chiral information on the cation-exchange site.

Is a chiral center on the SCX-part or just a steric hindering group for amino acid-enantioseparation necessary or are stereogenic centers in the QN sufficient?

It was compared how many analytes were enantioseparated on each CSP. It was distinguished between not separated, partly separated ($0.1 < R_S \leq 1.5$) and baseline separated ($R_S > 1.5$)

There were a small number of analytes which are baseline separated. **CSP 1** did not separate the majority of analytes at any mobile phase condition. When using **CSP 7** with 50 % ACN in the mobile phase the majority of the analytes (53 %) are at least partial separated, where 20 % of the analytes are baseline separated.

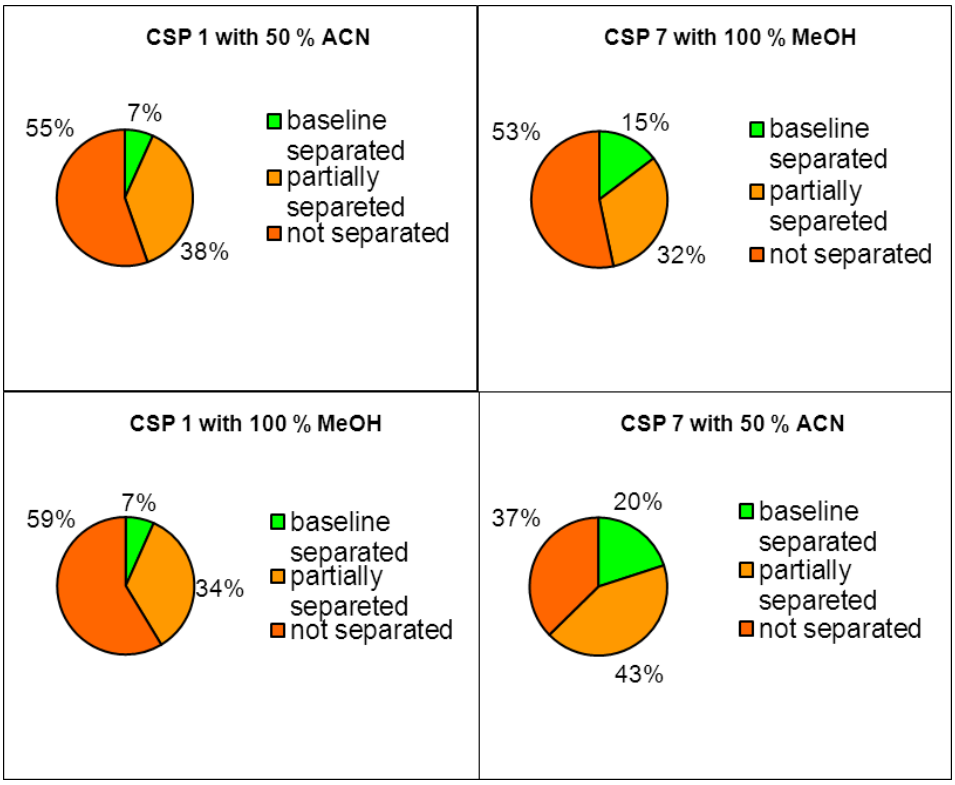


Figure 3.10.: *ratio of baseline separation, partial separated and not separated analytes*

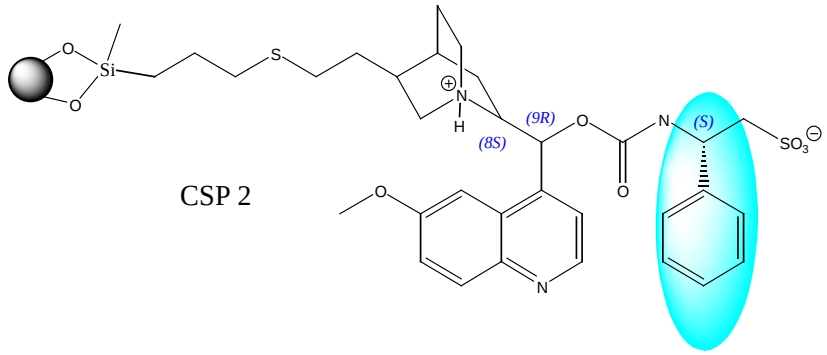
According to the data (appendix) all tryptophan and tryptophan derivatives were baseline separated on **CSP 1** and **7** on both mobile phases.

The dimethyl-group does not strongly affect the retention. The retention factors between the two CSPs were almost the same when using 100 % MeOH. When using 50 % ACN in the mobile phase the analytes' retention factors were larger on **CSP 1**.

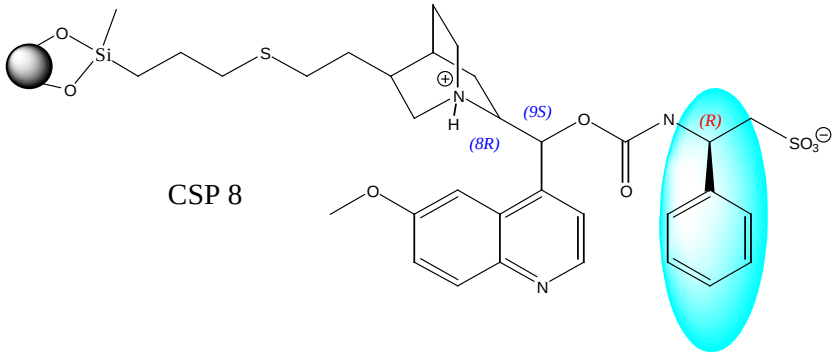
To summarize, using both CSPs with 100 % MeOH more than 50 % of the analytes could not be enantioseparated. Under the same mobile phase condition **CSP 7** could baseline separate 8 % more analytes than **CSP 1** with a steric hindering group on the SCX-part. When changing the mobile phase to more aprotic character the amount of separated analytes kept the same. In contrary, on **CSP 7** the number of separated analytes increased.

The results showed that a stereogenic center on the SCX-part is necessary for enantioseparation and that a sole steric hindering group is not sufficient for braod enantioselectivity towards amino acids.

3.8.: 2-(S)-phenyl-Tau-QN (CSP 2) vs. 2-(R)-phenyl-Tau-QN (CSP 8)



VS.



CSP 2 and **CSP 8** are both based on QN and have the same phenyl-group on position 2 of the SCX-part, only the chiral center is of opposite absolute configuration. The phenyl-group in **CSP 2** has the (S)-configuration and in **CSP 8** the group is in (R)-configuration.

The difference in selector coverage between these two CSPs makes 74 µmol g^{-1}.For the reason that the column-dimensions of **CSP 2** and **8** are different, only the selectivity values can be compared with each other to give valuable information (**table 3.1**). On **CSP 8** only a selected number of amino acids from the set were measured (almost just the analytes where the single enantiomer was available and thus the elution order could be determined).

Both CSPs inhibit a stereogenic center at the SCX-moiety. How does the chiral information on the SCX-part affect the enantioseparation and does a change of configuration in the SCX-part lead to a change of elution order or does the change only depend from QN/QD in general?

	CSP 2 100 % MeOH		CSP 8 100 % MeOH	
Analyt	**EO**	**α**	**EO**	**α**
Ala		**1.10**		**1.00**
Val	**L-D**	**1.15**		**1.00**
Leu	**L-D**	**1.15**		**1.00**
Thr	**L-D**	**1.18**		**1.00**
Ile	**L-D**	**1.16**		**1.00**
Met	**L-D**	**1.06**		**1.00**
Lys	**L-D**	**1.12**	**L-D**	**1.15**
Pro	**L-D**	**1.32**	**L-D**	**1.07**
Asn		**1.15**		**1.08**
Asp	**D-L**	**1.04**	**L-D**	**1.05**
Arg		**1.06**	**L-D**	**1.10**
trans-4-OH-Pro	**L-D**	**2.84**	**L-D**	**1.53**
cis-4-OH-Pro		**1.12**	**D-L**	**1.33**
Nor-Leu	**L-D**	**1.18**		**1.00**
Nor-Val	**L-D**	**1.17**		**1.00**
Nipe	**R-S**	**1.67**	**S-R**	**1.09**
Pipe	**L-D**	**1.11**		**1.00**
allo-Ile	**L-D**	**1.17**		**1.00**
Neopentylgly	**L-D**	**1.29**		**1.00**
tert-Leu	**L-D**	**1.26**		**1.00**
2-tBu-Tau	**R-S**	**2.22**		**1.00**
2-Et-Tau	**R-S**	**1.55**		**1.00**
2-Me-Tau	**R-S**	**1.19**		**1.00**
Pro-Me-sulfonic acid	**S-R**	**1.87**		**1.00**
2-isopropyl-Tau	**D-L**	**1.57**		**1.00**
2-phenyl-Tau	**D-L**	**1.34**	**D-L**	**1.20**

Table 3.7.: *elution order and resolution-values using100 % MeOH*

With 2-(R)-phenyl-Tau-QN (**CSP 2**) a great amount of aliphatic amino acids could be separated, whereas on 2-(S)-phenlyl-Tau-QN (**CSP 8**) in many cases no separation occurred (**table 3.7**). **CSP 8** showed similar separation profile than Tau-QN (**CSP 1**). For certain analytes (aspartic acid and nipecotic acid) a change in elution order was observed.

It seems that the (R)-configuration of the side chain in the SCX-part does not favorer enantioseparation of zwitterionjic analytes compared to the (S)-configuration.

The measurement of the aliphatic analytes was only done with 100 % MeOH in the mobile phase. In contrast, the aromatic analytes could be separated on **CSP 8**. In **table 3.8** and **3.9** the data of the aromatic analytes are shown.

	CSP 2		**CSP 8**	
	100 % MeOH		100 % MeOH	
Analyt	**EO**	**α**	**EO**	**α**
Phe		**1.00**		**1.00**
Trp	**D-L**	**1.81**	**D-L**	**2.14**
5-HTP	**D-L**	**1.88**	**D-L**	**1.86**
α-Me-Tyr	**D-L**	**1.20**	**D-L**	**1.23**
α-Me-DOPA	**D-L**	**1.50**	**D-L**	**1.34**
1-Me-Trp	**D-L**	**1.42**	**D-L**	**1.26**
Kynurenine	**L-D**	**1.06**		**1.00**
Tic	**S-R**	**1.21**	**S-R**	**1.13**
Average values				

Table 3.8.: *elution order and α-values using 100 % MeOH*

	CSP 2		CSP 8	
	50 /50 MeOH/ACN		50 /50 MeOH/ACN	
Analyt	EO	α	EO	α
Phe	L-D	1.05		1.00
Trp	D-L	1.45	D-L	1.92
5-HTP	D-L	1.49	D-L	1.58
α-Me-Tyr		1.00	D-L	1.08
α-Me-DOPA	D-L	1.24	D-L	1.12
1-Me-Trp	D-L	1.20	D-L	1.14
Kynurenine		1.00		1.00
Tic	S-R	1.19	S-R	1.12
Average values				

Table 3.9.: *elution order and resolution-values using 50 /50 MeOH/ACN*

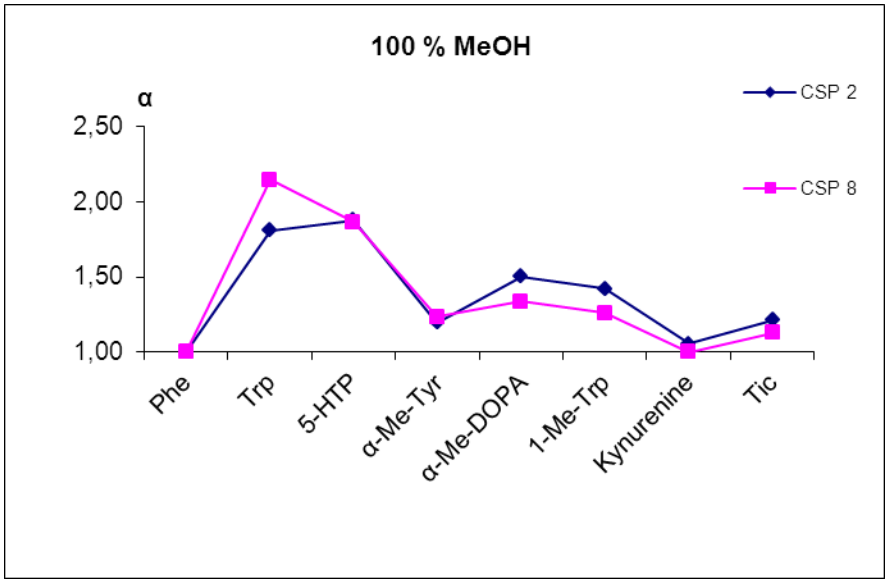


Figure 3.11.: *α-values of aromatic analytes with 100 % MeOH*

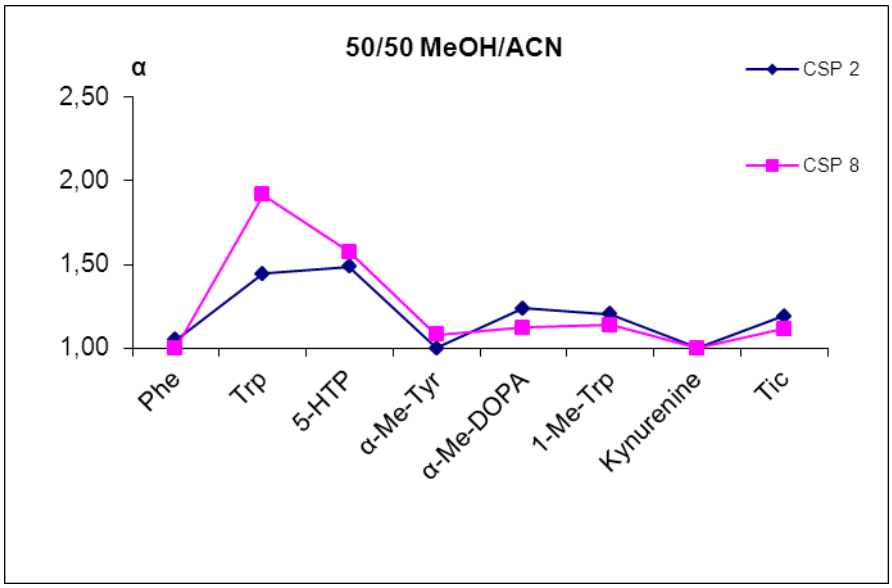


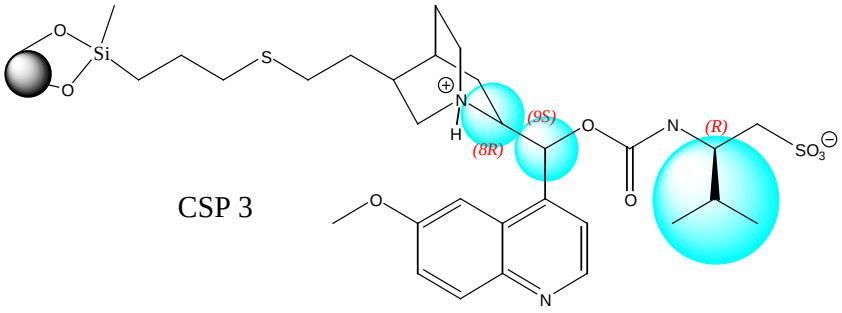
Figure 3.12.: *α-values of aromatic analytes with 100 % MeOH*

The α-values of the majority of the aromatic analytes were smaller on 2-(S)-Phenyl-Tau-QN (**CSP 8**). Only tryptophan and 5-HTP clearly showed better separation on **CSP 8**.
When using the aromatic analytes as well no change of elution order was seen.

A chiral information at the SCX-part of a selector compared to selectors without a stereogenic centre at the SCX-part supports enantioseparation. The 2-(S)-configuration of the phenyl-moiety on the SCX-part combined with the QN on the WAX-part of the selector leads to enantioseparation in many cases. On the other hand, the 2-(R)-configuration of the phenyl-moiety does not seem to support the separation of enantiomers. It seems that this configuration combined with the QN-moiety is badly suited for enantioseparation.
Besides, with changing only the configuration in the SCX-part no effective change of elution orders occurred. Only for two aliphatic amino acids this phenomenon could be observed.

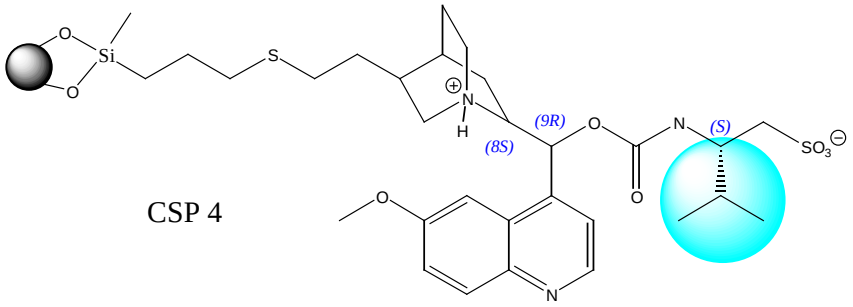
Thus, the change of configuration only in the SCX-part only is not sufficient for the change of elution order of the zwitterionic analytes.

3.9. 2-(R)-isopropyl-Tau-QD (CSP 3) vs. 2-(S)-isopropyl-Tau-QN (CSP 4)



CSP 3

VS.



CSP 4

It is well-established by former studies[44] that quinine and quinidine-based selectors are behaving as pseudo-enantiomers to each other and showing similar enantioselectivites for specific analytes but with a switching of the elution order of the enantiomers.

2-(S)-isopropyl-Tau-QN (**CSP 4**) is the pseudoenantiomeric contra-part of 2-(R)-isopropyl-Tau-QD (**CSP 3**). The comparison of these two CSP is important for the evaluation. It is of interest if which part of the selector is responsible for the change of the elution order.

CSP 3 is QD-based with the chiral SCX-side chain in (R)-configuration and **CSP 4** has a QN-moiety with the chiral SCX-side chain in the (S)-configuration.

It is interesting if that phenomenon of switching elution orders of enantiomers occurs when comparing these two zwitterionic CSPs.

In the following **tables 3.10 – 3.14** the chromatographic parameter are shown.

β-AAS

β-AAS were baseline separated, when using 100 % MeOH. Using 50 % aprotic ACN in the mobile phase all β-AAS were baseline separated where nipecotic acid gave exceptionally high R_S-values. Selectivity- and resolution-values increased with increasing amount of ACN.

	CSP 3 100 % MeOH			CSP 4 100 % MeOH			CSP 3 50 % ACN			CSP 4 50 % ACN		
β-AAS	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**
Nipe	1.23	**2.52**	**9.02**	2.22	**2.23**	**5.69**	2.72	**2.99**	**11.80**	3.82	**2.89**	**10.03**
β-Leu	2.06	**1.11**	**1.35**	2.40	**1.06**	**0.56**	5.05	**1.33**	**4.66**	5.13	**1.23**	**3.57**
β-Neopentylgly	1.88	**1.36**	**3.40**	2.01	**1.35**	**2.59**	4.29	**1.59**	**6.18**	3.98	**1.53**	**4.39**
β-Phe	0.82	**1.26**	**2.12**	1.17	**1.15**	**1.95**	5.89	**1.56**	**4.32**	7.09	**1.31**	**5.79**
β-homo-Phe	2.36	**1.00**	**0.00**	3.22	**1.00**	**0.00**	7.05	**1.18**	**3.20**	7.77	**1.11**	**1.84**
Average values		**1.45**	**3.18**		**1.36**	**2.16**		**1.73**	**6.03**		**1.62**	**5.12**

Table 3.10.: *β-AAS measured on CSP 3 and 4*

α-methylated AAS

With higher aprotic content in mobile phase the selectivity-values decreased. On both CSPs α-methylated tryptophan was separated best. On average, aliphatic α-methylated AAS had smaller α-values than the aromatic ones. It is to mention that α-methyl-meta-tyrosine could be better separated than α-methyl-tyrosine.

	CSP 3 100 % MeOH			CSP 4 100 % MeOH			CSP 3 50 % ACN			CSP 4 50 % ACN		
α-methylated AAS	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**
α-Me-Leu	0.31	**1.00**	**0.00**	0.44	**1.08**	**0.49**	1.32	**1.09**	**0.53**	1.35	**1.18**	**1.32**
α-Me-Val	0.40	**1.00**	**0.00**	0.55	**1.02**	**0.16**	2.53	**1.12**	**0.90**	1.65	**1.00**	**0.00**
α-Me-Ser	0.68	**1.25**	**1.30**	0.94	**1.11**	**0.59**	1.59	**1.00**	**0.00**	2.61	**1.08**	**0.54**
α-Me-Phe	3.18	**1.08**	**0.64**	4.16	**1.18**	**0.59**	3.18	**1.06**	**0.68**	4.16	**1.00**	**0.00**
α-Me-Tyr	0.66	**1.12**	**0.46**	1.05	**1.14**	**0.67**	0.66	**1.00**	**0.00**	1.05	**1.07**	**0.54**
α-Me-m Tyr	0.53	**1.63**	**5.57**	0.82	**1.42**	**2.54**	0.53	**1.50**	**7.39**	0.82	**1.31**	**2.22**
α-Me-Trp	0.56	**3.04**	**11.08**	0.83	**3.56**	**20.51**	0.56	**1.91**	**6.96**	0.83	**2.07**	**13.31**
α-Me-DOPA	0.66	**2.11**	**3.09**	1.98	**1.52**	**2.52**	1.45	**1.85**	**5.10**	1.98	**1.29**	**1.68**
Average values		**1.53**	**2.77**		**1.50**	**3.51**		**1.32**	**2.70**		**1.25**	**2.45**

Table 3.11.: *α-methylated AAS measured on CSP 3 and 4*

Aminosulfonic AAS

When separating aminosulfonic acids it was observed that with **CSP 4** better resolution values could be achieved, also when using 50 % aprotic ACN in the mobile phase. Nearly none aminosulfonic acid could be separated with 50/50 mobile phase condition on **CSP 3**.

	CSP 3 100 % MeOH			CSP 4 100 % MeOH			CSP 3 50 % ACN			CSP 4 50 % ACN		
aminosulfonic acids	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**	**k_1**	**α**	**R_S**
Homocysteic acid	1.76	**1.18**	**1.21**	2.69	**1.00**	**0.00**	2.09	**1.26**	**1.19**	2.96	**1.11**	**0.60**
Cysteic acid	2.20	**1.00**	**0.00**	2.19	**1.11**	**0.60**	2.59	**1.00**	**0.00**	2.28	**1.13**	**0.49**
trans-2-ACHSA	2.02	**1.00**	**0.00**	2.71	**1.09**	**0.60**	2.52	**1.00**	**0.00**	3.46	**1.05**	**0.39**
cis-2-ACHSA	1.11	**1.22**	**0.56**	1.37	**1.26**	**1.18**	1.60	**1.00**	**0.00**	1.87	**1.18**	**0.68**
cis-3-ACHSA	1.02	**1.00**	**0.00**	2.36	**1.16**	**0.87**	2.42	**1.00**	**0.00**	4.86	**1.04**	**0.29**
trans-2-Me-2-ACHSA	1.19	**1.32**	**2.01**	1.44	**1.49**	**5.31**	2.00	**1.00**	**0.00**	1.82	**1.19**	**3.83**
2-tert-Bu-Tau	1.06	**2.27**	**2.93**	1.18	**2.79**	**6.47**	1.41	**2.04**	**1.69**	1.52	**2.62**	**4.97**
2-Et-Tau	1.69	**1.43**	**1.25**	2.27	**1.52**	**2.48**	1.97	**1.65**	**1.16**	2.85	**1.54**	**2.23**
2-Me-Tau	1.95	**1.00**	**0.00**	2.81	**1.16**	**0.83**	2.69	**1.00**	**0.00**	3.65	**1.17**	**0.69**
Pro-Me-SO_3H	1.31	**1.12**	**0.82**	1.86	**1.34**	**1.35**	1.50	**1.00**	**0.00**	2.03	**1.22**	**0.73**
2-isopropyl-Tau	1.45	**1.56**	**1.60**	1.91	**1.63**	**2.99**	1.86	**1.55**	**0.79**	2.36	**1.67**	**2.58**
2-phenyl-Tau	1.44	**1.28**	**1.22**	1.91	**1.37**	**2.75**	2.57	**1.00**	**0.00**	2.57	**1.31**	**1.82**
Average values		**1.31**	**1.68**		**1.34**	**1.69**		**1.31**	**2.09**		**1.35**	**1.94**

Table 3.12.: *aminosulfonic acids measured on CSP 3 and 4*

Aromatic AAS

Almost all aromatic analytes could be enantioseparated on both CSPs using them under each mobile phase condition. When using 50 % ACN in the mobile phase 72 % of the aromatic analytes were baseline separated in both CSPs and when using 100 % MeOH as mobile phase 80 % of the aromatic analytes were baseline separated on **CSP 3** and only 44 % on **CSP 4**. The majority of baseline separation (80 %) was found when using **CSP 3** in 100 % MeOH. Tryptophan and tryptophan derivatives displayed greater selectivity values on **CSP 4**.

	CSP 3 100 % MeOH			CSP 4 100 % MeOH			CSP 3 50 % ACN			CSP 4 50 % ACN		
aromatic amino acids	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
Phe	0.80	**1.34**	**3.49**	1.03	**1.16**	**1.06**	2.21	**1.34**	**4.32**	2.50	**1.19**	**1.17**
p-F-Phe	0.84	**1.33**	**1.89**	1.10	**1.11**	**1.10**	2.38	**1.39**	**2.38**	2.60	**1.18**	**2.13**
p-Cl-Phe	1.05	**1.39**	**3.33**	1.34	**1.12**	**1.48**	2.94	**1.46**	**3.62**	3.14	**1.20**	**2.49**
p-Br-Phe	1.16	**1.42**	**4.39**	1.48	**1.13**	**1.62**	3.22	**1.48**	**5.14**	3.40	**1.21**	**2.66**
o-F-Phe	0.78	**1.35**	**3.57**	1.00	**1.16**	**1.14**	2.18	**1.37**	**5.12**	2.29	**1.21**	**1.63**
β-Phe	0.82	**1.26**	**2.12**	1.17	**1.15**	**1.95**	2.02	**1.56**	**4.32**	2.49	**1.31**	**5.79**
β-homo-Phe	2.36	**1.00**	**0.00**	3.22	**1.00**	**0.00**	5.89	**1.18**	**3.20**	7.09	**1.11**	**1.84**
α-Me-Phe	3.18	**1.08**	**0.64**	4.16	**1.18**	**0.59**	7.05	**1.06**	**0.68**	7.77	**1.00**	**0.00**
p-Amino-Phe	0.49	**1.31**	**3.35**	0.66	**1.14**	**0.77**	1.44	**1.31**	**4.47**	1.68	**1.23**	**1.14**
DOPA	1.36	**1.00**	**0.00**	1.71	**1.00**	**0.00**	3.68	**1.00**	**0.00**	4.30	**1.07**	**0.37**
α-Me-DOPA	0.66	**2.11**	**3.09**	1.05	**1.52**	**2.52**	1.84	**1.85**	**5.10**	2.62	**1.29**	**1.68**
Tyr	0.86	**1.28**	**2.12**	1.20	**1.13**	**0.86**	2.42	**1.30**	**2.32**	2.89	**1.24**	**1.61**
m Tyr	1.01	**1.17**	**0.71**	1.34	**1.06**	**0.46**	2.92	**1.14**	**1.32**	3.43	**1.08**	**0.59**
α-Me-Tyr	0.53	**1.12**	**0.46**	0.82	**1.14**	**0.67**	1.61	**1.00**	**0.00**	2.17	**1.07**	**0.54**
α-Me-m Tyr	0.56	**1.63**	**5.57**	0.83	**1.42**	**2.54**	1.55	**1.50**	**7.39**	1.98	**1.31**	**2.22**
OMe-Tyr	0.92	**1.39**	**3.89**	1.21	**1.15**	**1.36**	2.38	**1.40**	**4.99**	2.74	**1.19**	**1.72**
Trp	1.45	**1.26**	**2.92**	1.98	**1.61**	**7.62**	3.27	**1.05**	**0.59**	3.93	**1.26**	**3.21**
α-Me-Trp	0.83	**3.04**	**11.08**	1.35	**3.56**	**20.51**	1.94	**1.91**	**6.96**	2.94	**2.07**	**13.31**
1-Me-Trp	1.69	**1.14**	**1.75**	1.94	**1.36**	**4.98**	3.42	**1.06**	**0.55**	3.68	**1.12**	**1.64**
5-HTP	1.78	**1.14**	**1.52**	2.24	**1.43**	**5.23**	3.66	**1.00**	**0.00**	4.48	**1.13**	**1.32**
Baclofen	2.72	**1.32**	**2.28**	4.63	**1.28**	**5.43**	8.96	**1.42**	**3.88**	11.92	**1.34**	**6.99**
Phenylglycine	0.87	**1.22**	**1.57**	1.31	**1.04**	**0.54**	2.70	**1.11**	**1.39**	3.20	**1.00**	**0.00**
Kynurenine	1.39	**1.26**	**3.34**	1.98	**1.10**	**1.31**	3.13	**1.28**	**4.13**	3.79	**1.12**	**1.61**
1-Naphtylalanine	1.20	**1.53**	**5.31**	1.60	**1.12**	**1.68**	3.01	**1.56**	**4.65**	3.44	**1.14**	**1.74**
Tic	0.91	**1.34**	**4.07**	1.11	**1.32**	**2.20**	1.94	**1.32**	**4.92**	2.17	**1.27**	**2.08**
Average values		**1.38**	**2.90**		**1.30**	**2.70**		**1.32**	**3.26**		**1.21**	**2.38**

Table 3.13.: *aromatic acids measured on CSP 3 and 4*

Proteinogenic AAS

When using more aprotic content in the mobile phase the average values of selectivity and resolution were increasing on **CSP 3** and the values were decreasing on **CSP 4**.

The retention-factors were higher on **CSP 4** for every analyte with both mobile phases. In many cases retention clearly increased in both mobile phases.

	CSP 3 100 % MeOH			CSP 4 100 % MeOH			CSP 3 50 % ACN			CSP 4 50 % ACN		
proteogenic amino acids	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
Ala	0.70	**1.22**	**1.55**	0.89	**1.13**	**0.48**	2.28	**1.18**	**1.79**	2.38	**1.11**	**0.60**
Val	0.60	**1.35**	**2.13**	0.74	**1.22**	**1.51**	2.06	**1.27**	**2.39**	2.12	**1.22**	**1.94**
Leu	0.74	**1.34**	**3.12**	0.86	**1.22**	**1.63**	2.41	**1.31**	**3.96**	2.35	**1.19**	**2.03**
Ser	1.05	**1.00**	**0.00**	1.26	**1.09**	**0.56**	3.42	**1.00**	**0.00**	3.80	**1.00**	**0.00**
Thr	0.77	**1.26**	**0.80**	1.03	**1.25**	**1.31**	2.37	**1.29**	**1.20**	2.67	**1.30**	**1.46**
Ile	0.65	**1.33**	**1.92**	0.80	**1.24**	**2.02**	2.27	**1.24**	**2.34**	2.25	**1.24**	**2.58**
Phe	0.80	**1.34**	**3.49**	1.03	**1.16**	**1.06**	2.21	**1.34**	**4.32**	2.50	**1.19**	**1.17**
Tyr	0.86	**1.28**	**2.12**	1.20	**1.13**	**0.86**	2.42	**1.30**	**2.32**	2.89	**1.24**	**1.61**
Trp	1.45	**1.26**	**2.92**	1.98	**1.61**	**7.62**	3.27	**1.05**	**0.59**	3.93	**1.26**	**3.21**
Met	0.99	**1.24**	**1.55**	1.24	**1.10**	**0.96**	3.02	**1.27**	**1.88**	3.01	**1.11**	**1.04**
Lys	3.77	**1.23**	**1.98**	10.05	**1.18**	**2.27**		n.d		13.48	**1.00**	**0.00**
Pro	0.94	**1.80**	**6.72**	1.07	**1.56**	**4.30**	1.43	**2.34**	**10.15**	1.44	**1.94**	**5.96**
Asn	1.20	**1.15**	**1.27**	1.54	**1.20**	**1.00**	2.74	**1.24**	**2.41**	7.57	**1.00**	**0.00**
Gln	0.86	**1.26**	**0.69**	1.24	**1.00**	**0.00**	2.22	**1.25**	**0.78**	2.61	**1.26**	**1.38**
Asp	2.53	**1.00**	**0.00**	3.20	**1.05**	**0.33**	6.56	**1.00**	**0.00**	7.44	**1.00**	**0.00**
Glu	1.38	**1.00**	**0.00**	1.79	**1.10**	**0.59**	4.20	**1.00**	**0.00**	4.67	**1.15**	**0.66**
Arg	4.68	**1.00**	**0.00**	2.64	**2.37**	**1.38**		n.d			n.d	
His	1.61	**1.37**	**2.24**	4.45	**1.40**	**1.59**		n.d		9.71	**1.58**	**0.87**
Average values		**1.24**	**1.60**		**1.27**	**1.33**		**1.28**	**2.24**		**1.22**	**1.32**

Table 3.14.: *proteinogenic amino acids measured on CSP 3 and 4*

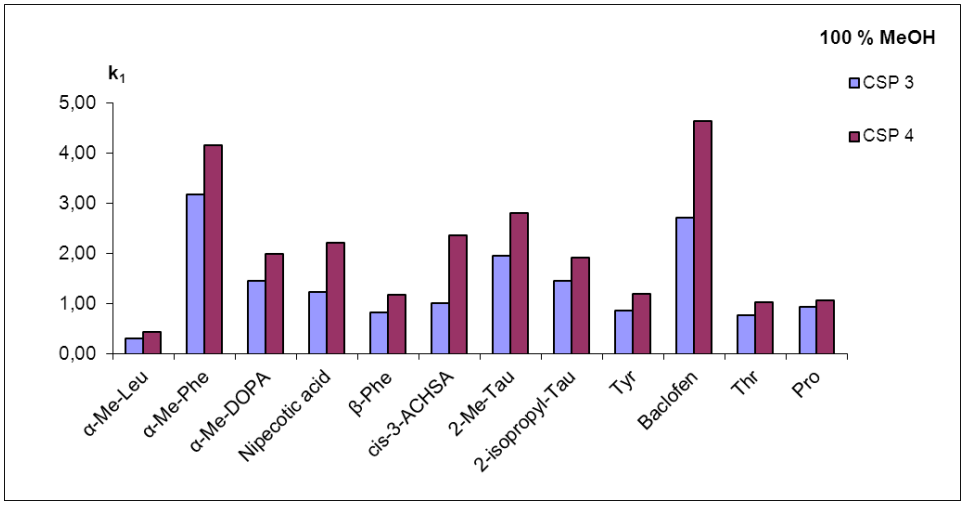


Figure 3.13.: *retention-factors shown on CSP 3 and CSP 4 for certain analytes*

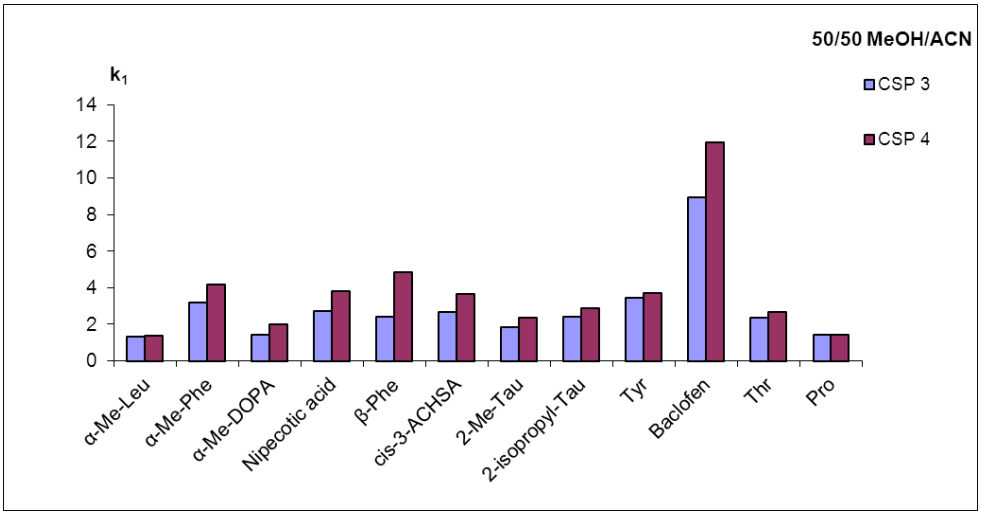


Figure 3.14.: *retention-factors shown on CSP 3 and CSP 4 for certain analytes*

The k_1-values are higher on **CSP 4**. Maybe the isopropyl moiety in (S)-configuration assisted in the interactions between the amino acid analytes and the SO or quinidine is the reason.

The resolution-values of the analytes differ enormously between the two CSPs, but the "separation power" is similar and thus, the analytes can be at least baseline separated on both CSPs. The configuration of the side chain and of the cinchona alkaloid seemed to play a big role in that case.

Change of elution order

According to Lindner and Lämmerhofer[43] the elution order of N-protected amino acids changes when changing from the tert-butyl-QN-AX selector to the tert-butyl-QD-AX selector. Does this phenomenon cause with chiral amino acids at chiral zwitterionic selectors?

In **table 3.15** the elution orders of chosen analytes were listed. Listed L-D means that the L-enantiomer elutes before the D-enantiomer.

	100 % MeOH		50 % ACN	
	CSP 3	CSP 4	CSP 3	CSP 4
analytes	**EO**	**EO**	**EO**	**EO**
α-Me-Phe	L-D	D-L	///	///
α-Me-Tyr	///	D-L	///	**L-D**
α-Me-DOPA	L-D	D-L	L-D	D-L
Nipecotic acid	S-R	R-S	S-R	R-S
Homocysteic acid	D-L	///	D-L	///
Cysteic acid	///	L-D	///	///
2-tert-Bu-Tau	S-R	R-S	S-R	R-S
2-Et-Tau	S-R	R-S	S-R	R-S
2-Me-Tau	///	R-S	///	R-S
Pro-Me-SO_3H	S-R	R-S	///	R-S
2-isopropyl-Tau	L-D	D-L	L-D	D-L
2-phenyl-Tau	L-D	D-L	n. d.	D-L
Phe	D-L	L-D	D-L	L-D
p-F-Phe	D-L	L-D	D-L	L-D
p-Cl-Phe	D-L	L-D	D-L	L-D
DOPA	///	///	///	L-D
Tyr	D-L	L-D	D-L	L-D
OMe-Tyr	D-L	L-D	D-L	L-D
Trp	L-D	D-L	L-D	D-L
1-Me-Trp	L-D	D-L	**D-L**	D-L
5-HTP	L-D	D-L	///	D-L
Phenylglycine	D-L	L-D	D-L	///
Kynurenine	D-L	L-D	D-L	L-D
1-Naphtylalanine	D-L	L-D	D-L	L-D
Tic	R-S	S-R	R-S	S-R
Ala	n.d.	L-D	///	L-D
Val	D-L	L-D	D-L	L-D
Leu	D-L	L-D	D-L	L-D
Ser	///	L-D	///	///
Thr	///	L-D	D-L	L-D
Ile	///	L-D	D-L	L-D
Met	D-L	L-D	D-L	L-D
Lys	///	L-D	///	///
Pro	D-L	L-D	D-L	L-D
Asn	D-L	L-D	D-L	///
Gln	D-L	///	D-L	///

Table 3.15.: *elution order when using CSP 3 and 4 (n.d.: not determined, /// no value)*

Asp	///	D-L	///	///
Glu	///	D-L	///	///
Glu	///	D-L	///	///
His	///	L-D	///	///
allo-Ile	D-L	L-D	D-L	L-D
Neopentylglycine	D-L	L-D	D-L	L-D
tert-Leu	D-L	L-D	D-L	L-D
Pipecoloc acid	D-L	L-D	D-L	L-D
N-Me-Leu	L-D	D-L	L-D	D-L
trans-4-OH-Pro	**L-D**	**L-D**	**L-D**	**L-D**
cis-4-OH-Pro	///	**D-L**	**D-L**	///
Nor-Leu	D-L	L-D	D-L	L-D
Nor-Val	D-L	L-D	D-L	L-D

Continue table 3.15.: *elution order when using CSP 3 and 4 (n.d.: not determined, /// no separation)*

By testing the whole analyte set it was confirmed that the "pseudo"-enantiomeric behavior of QN and QD was decisive resulting in switching elution orders of enantiomers. Therefore one can state that the quinine and quinidine moiety were crucial for recognition of the chiral analytes.

Figure 3.14 and **3.15** depict enantioseparation of proline on **CSP 3** and **CSP 4**. The elution order changed when using the pseudo-enantiomeric CSP but with changing the mobile phase elution order was preserved.

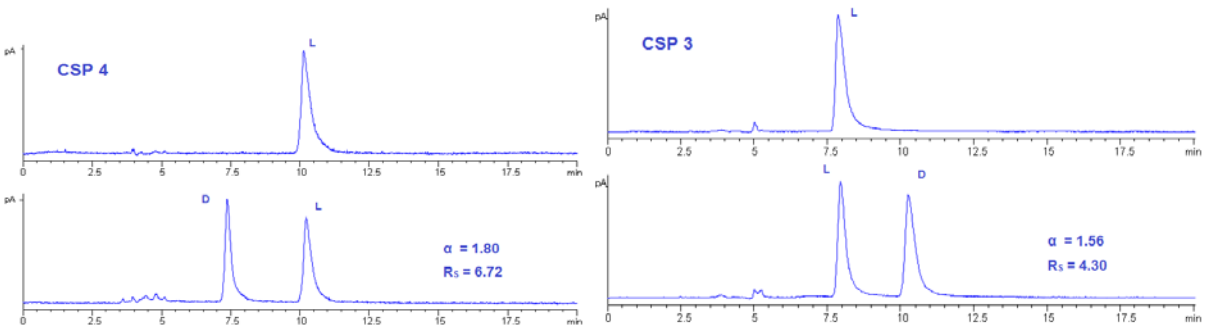


Figure 3.15.: right: *Proline on CSP 3, 100 % MeOH;* ***left:*** *Proline on CSP 4, 100 % MeOH*

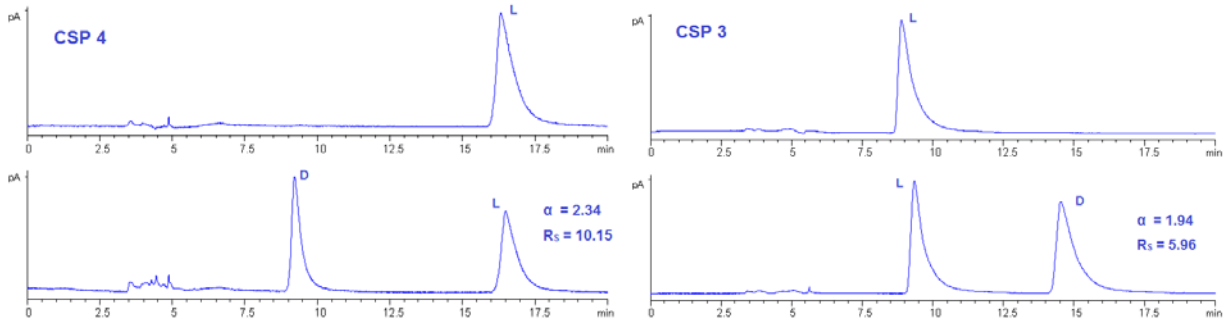


Figure 3.16.: right: *Proline on CSP 3, 50/50 MeOH/ACN;* ***left:*** *Proline on CSP 4, 50/50 MeOH/ACN*

Figure 3.16 shows 5-HTP enantiomers that changed the elution order when changing the CSPs. In this case the use of higher aprotic solvent amount led to a much better resolution-value.

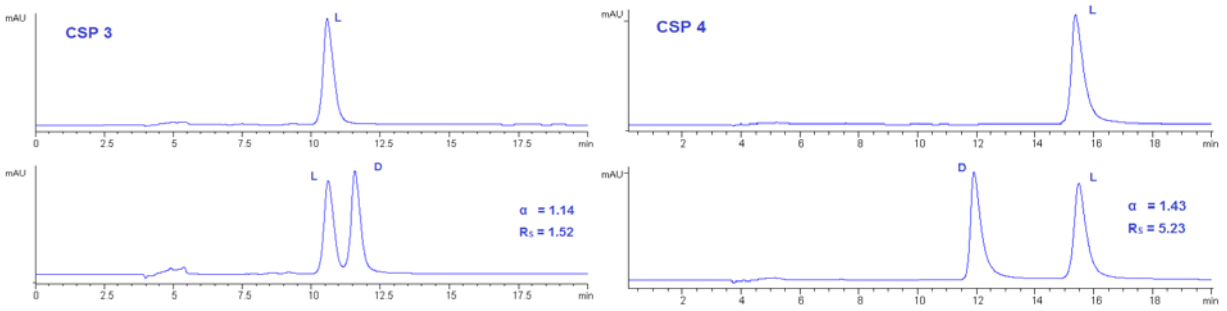


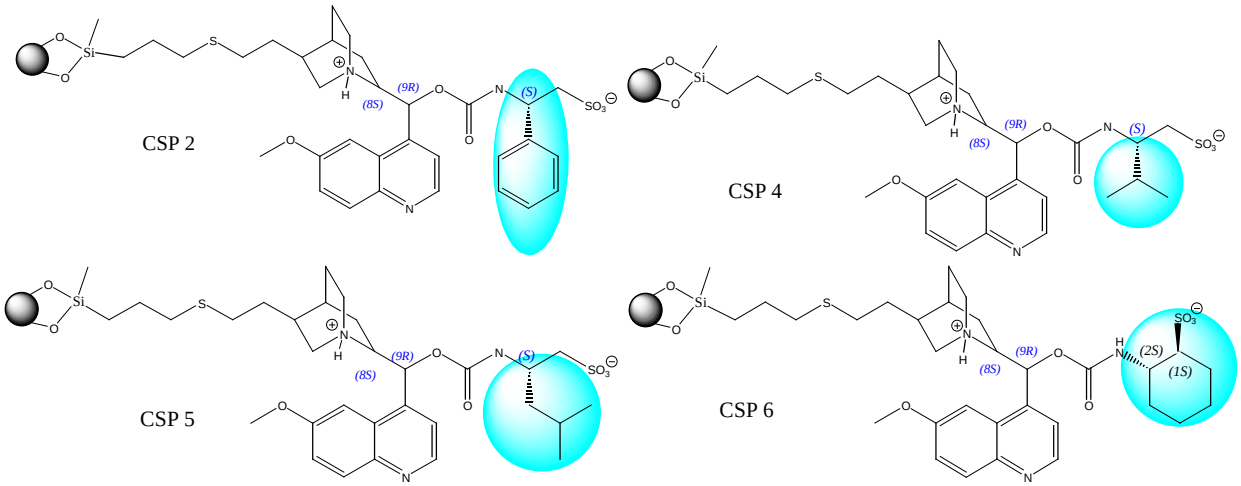
Figure 3.17.: right: *5-HTP on CSP 3, 100 % MeOH;* ***left:*** *5-HTP on CSP 4, 100 % MeOH*

In some cases the elution order did not change when switching from **CSP 3** to **CSP 4**. These exceptions were trans-4-hydroxy-proline where no change in elution order could be observed and cis-4-hydroxy-proline (also see **table 3.15**).

A reversal of elution order for certain analytes also occurred when the mobile phase was changed. With changing the mobile phase the elution order of α-methyl-tyrosine-enantiomers switched when using **CSP 4** and of 1-methyl-tryptophan-enantiomers when using **CSP 3**.

To sum up, when changing from (R)-isopropyl-Tau-QD to (S)-isopropyl-Tau-QN a change of elution order for almost all analytes could be observed. When the mobile phase on the same CSP was changed the elution order did not change for all but one case. With changing not only the configuration in the SCX-part but also the configuration in the WAX-part (from QD to QN) of the selector, it was shown that the phenomenon of switching elution orders depends on the configuration in the WAX-part of the selector. QN and QD seem to be the driving force for chiral recognition and therefore elution orders.

3.10. Comparison: side chain of CSP 2, 4, 5 and 6



By testing different ZWIX-CSPs the question was posed which chiral side chain gave the best selectivity. To answer this question four CSPs were chosen to be compared to each other. The side chains have steric groups of different size, structure and they are all in (S)-configuration.

The k_1-values of different analytes were compared to each other.

CSP 2 has an aromatic ring, where additional π-π-interactions to the quinoline moiety of the ZWIX-SO can be found. **CSP 5** has a similar, but more sterically demanding side chain than **CSP 4**.

To see which CSPs interact strongly with the analytes the k_1-values were compared. **CSP 2** had the lowest retention and **CSP 6** the highest retention. When comparing k_1-values from aromatic analytes on **CSP 4** and **5** greater k_1-values were observed on **CSP 4**. The same results can be seen when comparing the k_1-values of phenylalanine derivatives of **CSP 4** and **6**.

Hoping that the aromatic ring of **CSP 2** would interact with the aromatic analytes and support retention, this was not the case. The phenyl ring was maybe too sterically demanding to allow better interaction between the analyte and the selector.

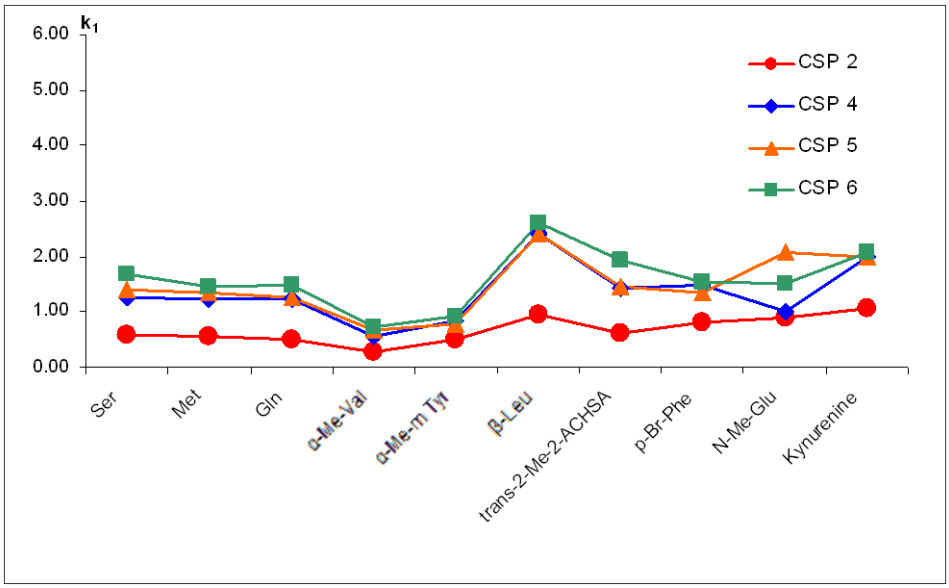


Figure 3.18.: *retention-factors of certain analytes using 100 % MeOH*

Figure 3.18 depicts that on **CSP 2** the retention-factor of each analyte was smaller than the retention factors of analytes measured on the other CSPs with a non-aromatic side chain.

When measuring with MeOH/ACN (50/50) the β-AAS showed greater retention on **CSP 4** compared with the retention-values of **CSP 5** and **CSP 6**. This also counted for the aromatic analytes. According to the k_1-values the isopropyl-moiety of **CSP 4** supported stronger retention to amino sulfonic acids than the isobutyl-moiety of **CSP 5**. Again the k_1-values were similar when using **CSP 4**, **5** and **6** with higher amount of aprotic solvent in the mobile phase.

In both mobile phase conditions the k_1-values measured on ACHSA-Tau-QN (**CSP 6**) were the highest where as phenyl-Tau-QN (**CSP 2**) exhibited the lowest retention.

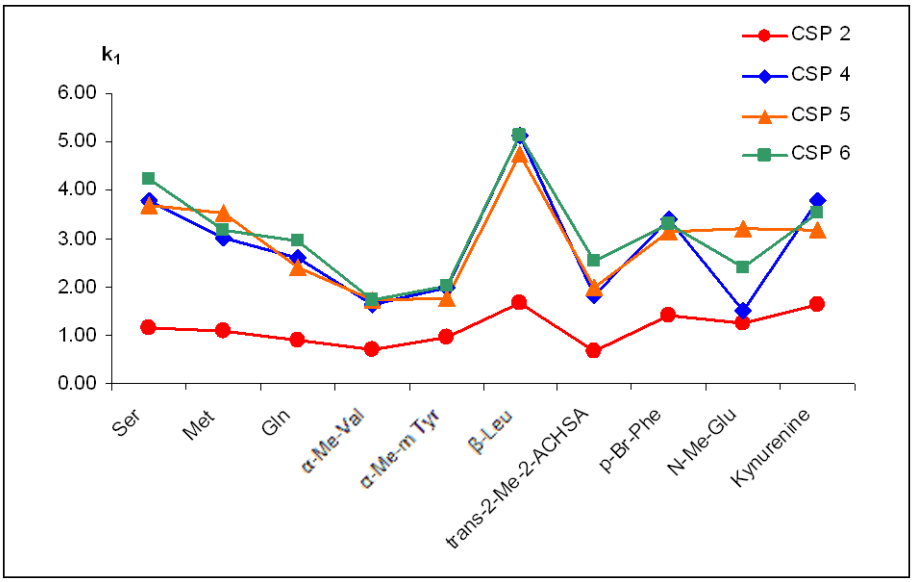


Figure 3.19.: *retention-factors of certain analytes using 50 /50 MeOH/ACN*

Considering histogram **3.20** the majority of the analytes had selectivity-factors between 1.10 and 1.49. 2-(S)-isoprop-Tau-QN (**CSP 4**) showed the biggest number of high selectivity-values. On phenyl-Tau-QN (**CSP 2**) compared with the other CSPs had the greatest number of non-separated enantiomers.

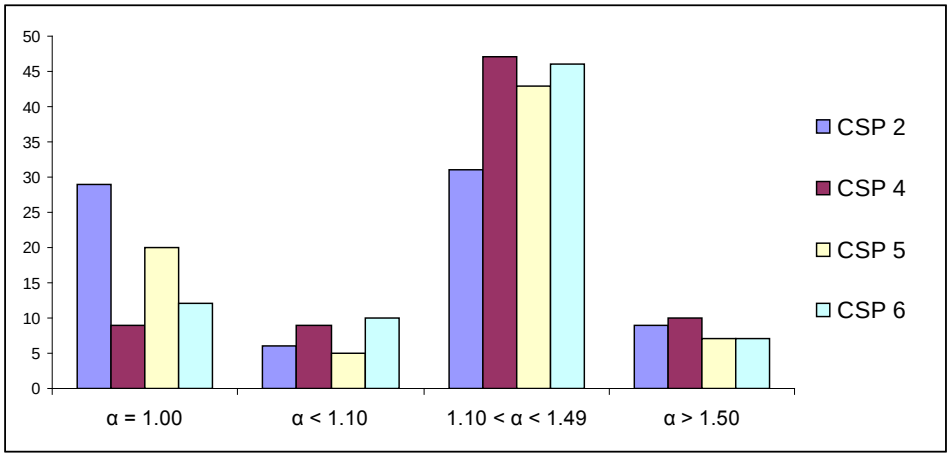


Figure 3.20.: *number of analytes which have different α-values*

When comparing one analyte on four different CSPs it was seen that there were some differences in the peak shape. Crucial for the peak shape is the asymmetry factor A_S.

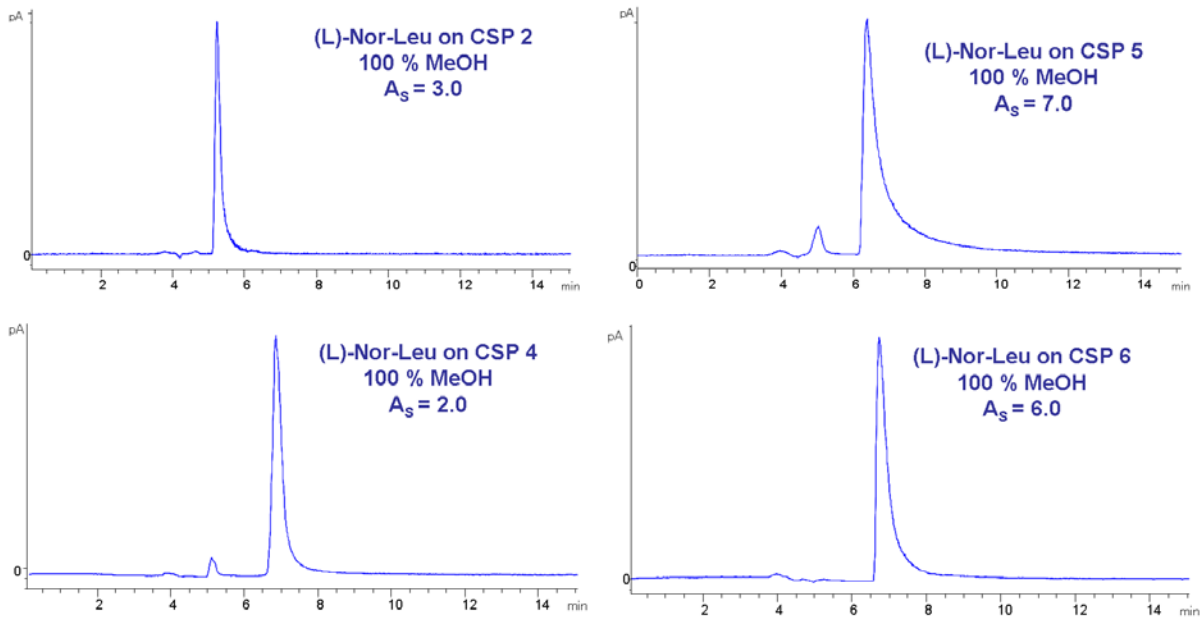


Figure 3.21.: *peak tailing of (L)-nor-leucine using different CSPs*

The A_S-values in the **figure 3.21** show that peak tailing occurs in different dimension on every four CSPs. 2-(S)-isopropyl-Tau-QN (**CSP 4**) has the lowest A_S-value and the smallest peak tailing. The strongest peak tailing occurs when using 2-(S)-isobutyl-Tau-QN (**CSP 5**), where it is not sure if the strong tailing comes from the kind of the SCX-part or from the bad packing of the column.

Comparing all four CSPs 2-(S)-isopropyl-Tau-QN (**CSP 4**) gave the best values, followed by the ACHSA-Tau-QN (**CSP 6**).

CSP 2 gave worse results like the other CSPs (most amino acids could not be enantioseparated) but the peak shape was even better than the peak shape on **CSP 6**. **CSP 4** showed the best separation-values compared to the other and also the fewest amount of non-separated amino acids. Furthermore, the peak shapes on **CSP 4** seems to be the best.

The results revealed that the isopropyl-moiety (**CSP 4**) is most beneficial for overall enantioselectivity of the zwitterionic analytes.

4. Experimental Part

4.1. Materials and Methods

Mass spectrometry measurements were performed on a PE Sciex API quadrupol mass spectrometer with an electrospray ionization source with 1100 Series CL/MSD Trap ion-trap MS-system. The mass spectrometer was hyphenated with a HPLC (Agilent 1100 Serien) of Agilent technologies. The measurements were done in positive ionization mode.

LC-MS measurements were carried out on a reversed phase column (RP-C_{18} Phenomenex Gemini, 150 x 3 mm ID, 3 µm) and a mobile Phase consisting of methanol (with 0.1 % formic acid) and water (with 0.1% formic acid). It was done in isocratic with 50/50, 55/45 and 60/40 MeOH/water, respectively.

Elemental–Analysis (CHNS) of the immobilized selector were performed on a CHNS-O Element analyzer (Carlo Erba, now Thermo Scientic, Germany) and the selector coverage of the silica was calculated from mass per cent of nitrogen.

^{1}H-NMR and ^{13}C-NMR were recorded at room temperature with a Bruker DRX400 and DRX600 spectrometer. The spectra were recorded in CD_3OD, $CDCl_3$ and D_2O and the solvent signals were used as reference signals. The raw data were processed with SpinWorks software (Version 3).

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} from Merck (Darmstadt, Germany). Column chromatography was performed on silica gel 60 (0.040-0.063 mm, Merck).

MeOH (gradient grade quality, Merck), DCM (VWR), PE (Baker), EA (Fluka), toluene (VWR), DEE (Merck) and DCM (Fluka), distilled over CaH_2, were used as solvents during the synthesis.

For the synthesis of the chiral stationary phases the following substances were used: quinine and quinidine (both Buchler, Germany), CS_2 (Fluka), KOH (Riedel-de Haen), H_2O_2 (30 %, Merck), HCOOH (98%, Fluka), CH_3COOH (98 %, Fluka*),* 4-Nitrophenyl chloroformate (>97%, Aldrich), 2-amino-2-methyl-1-propanol (> 97 %, Fluka), (2S)-2-amino-2-phenylethaneol and (2R)-2-amino-3-methylbutaneol (both Aaron chemistry Gmbh, Germany).

The silica gel (3 µm, 120 Å, Daiso, Japan) was modified and endcapped in house. All CSPs were prepared by immobilization of the chiral selector onto 3-mercaptopropyl-modified and endcapped silica gel via radical addition with AIBN (Merck). The selector loading were

calculated on the nitrogen content (w-% N). Analysis of the filtrate by ESI-MS confirmed selector integrity during immobilization conditions. Each CSP was packed by Bischoff Chromatography (Leonberg, Germany) into stainless steel columns (each 250 x 3 mm I.D.). All chemical reactions were carried out under nitrogen atmosphere.

(2R)-2-amino-3-sulfopropanoic acid (D-Cysteic acid)

Into a 50 ml flask 2.5 ml (77 mmol, 31 eq.) H_2O_2 (30 %), were added portionwise to 12.5 ml (331 mmol, 133 eq.) of formic acid (98%). The solution was stirred in an ice bath (0°C) for one hour. Peroxyformic acid was formed. After adding 0.313 g (2.5 mmol) of D-Cysteine the mixture was stirred over night at 0-5 °C and was covered with aluminium foil. The next day the solution was evaporated under reduced pressure (around 10^{-2} atm). Then ethanol was added for recrystallisation. The yield was 0.18 g (43 %) of white crystals.

^{1}H-NMR [$CDCl_3$]: δ = 4.08 (s, 1H), 2.99 (s, 1 H), 2.94 (s, 1 H). ^{13}C-NMR [$CDCl_3$]: δ = 127.0 (CH), 50.4 (CH_2), 49.5 (CH_2).

4.2. Synthesis of CSP 1, CSP 2 and CSP 3

2, Dimethylthiazolidine-2-thione

In a 250 ml flask 1.72 g (19 mmol) of 2-amino-2-methyl-1-propanole was put and dissolved in 100 ml aqueous solution KOH (1 M) under stirring. After adding 6.2 ml (100 mmol) CS_2 it was refluxed using an oil bath at 100 °C (solution turned red). It was stirred overnight at 100 °C (the solution turned green). After extracting with dichloromethane (3 x 50 ml) it was dried over Na_2SO_4. A co-product (4,4-dimethyl-1,3-oxazolidine-2-thione) was also formed. The co-product was separated from the product by flash chromatography (PE : EA 3:1). The yield of **2** as white crystals was 0.98 g (35 %).

^{1}H-NMR [$CDCl_3$]: δ = 8.02 (s, 1H), 3.34 (s, 2H), 1.51 (s, 6H).

Because of less amount of **2** all the reaction was repeated:

For synthesizing **2** 2.71 g of 2-amino-2-methyl-1-propanole (30 mmol), 9.1 ml of CS_2 (150 mmol) and 150 ml KOH (1M) were used. The yield of **2** as white crystrals was 2.02 g (50 %).

^{1}H-NMR [$CDCl_3$]: δ = *not seen* (br, s, 1H, NH_3), 3.33 (s, 2H, CH_2S), 1.50 (s, 6H, $2CH_3$). ^{13}C-NMR [$CDCl_3$]: δ = 199.2 (C=S), 68.0 (C_4), 46.2 (CH_2), 27.3 (CH_3).

3, 2-amino-2-methylpropane-1-sulfonic acid

Into a 50 ml flask 5.8 ml H_2O_2 (30 %) was added portionwise to 28.9 ml of formic acid (98%). The solution was stirred in an ice bath (0°C) for one hour. Peroxy formic acid was formed. After adding 0.85 g of dimethylthiazolidine-2-thione (5.8 mmol) the mixture was stirred over night at 0-5°C and was covered with aluinium foil. The next day the solution was evaporated in a pear shape flask under reduced pressure (around 10^{-2} atm). Then ethanol was added. After recrystallisation in methanol into the fridge was done overnight it was washed with acetonitrile. The yield was 0.40 g (45%) of white crystals.

^{1}H-NMR [$CDCl_3$]:δ = 3.19 (s, 2H); 1.45 (s, 6H): ^{13}C-NMR [$CDCl_3$]: δ = 58.1 (CH_2) , 52.8 (C_4) , 25.7 (CH_3).

Because of less amount of **3** all the reaction was repeated:

For **3** were 2.02 g of **2** (13.7 mmol), 13.7 ml of H_2O_2 (30 %) and 68.5 ml of HCOOH (98%) used. The yield of **3** (white crystals) was 0.61 g (29%).

^{1}H-NMR [D_2O]: δ = 3.22 (s, 2H, CH_2S); 1.19 (s, 6H, $2CH_3$)**.** ^{13}C-NMR [D_2O]: δ = 57.9 (CH_2), 52.8 (C_4), 25.5 (CH_3).

5, 9-O-(4-nitrophenyloxycarbonyl)-quinine hydrochloride

In a three-necked flask 8.02 g of quinine (**4**, 24.66 mmol) were dissolved in 550 ml of toluene followed by azeotropic distillation (removal of 1/3 of the solvent) using a Dean-Stark-apparatus. After cooling the mixture to room temperature (r. t.) under N_2-atmosphere 5.06 g of 4-nitrophenyl chloroformate (25 mmol) were added. The mixture was stirred at r. t. under

N_2-atmosphere overnight. After filtration a pale yellow precipitation were yielded quantitatively. The solid was washed with n-heptane (2 x 30 ml), dried under vacuum and stored under N_2-atmosphere to avoid hydrolysis.

6, dimethyl-taurine-quinine-selector

After building the apparatus 80 ml of dichlormethane were distilled over a drying agent (CaH_2). After adding 0.40 g of 2-amino-2methylpropane-1-sulfonic acid (2.6 mmol) into the three-necked flask, the apparatus was put under a N_2-stream and charged with the dry solvent under stirring. Under stirring 1.94 ml of BSA (7.8 mmol) was added via syringe. The mixture was refluxed (oil bath temperature 100 °C). Therefore the mixture not become clear overnight 1.43 ml of BSA (5.8 mmol) was added. The mixture became clear and was cooled to r. t. under N_2-atmosphere.

Then 1.37 g of **1** (2.6 mmol) was added portionswise and stirred overnight under N_2-atmosphere. The reaction was quenched with 2 ml MeOH (gradient grade quality) for one hour. Purification (4-Nitrophenol and quinine byproducts were separated) by flash column chromatography (DCM : MeOH 10 : 1 – 3 : 1) yielding 0.27 g (21 %) of **6** as white crystals.

Because of less amount of **6** all the reaction was repeated:

For the synthesis of **6** were used 0.61 g of **3** (4.0 mmol), 2.10 g of **5** (4.0 mmol, 1 eq.), 5.0 ml of BSA (20.0 mmol, 5 eq.) and 80 ml of dried DCM. The yield of **6** (white crystals) was 0.51 g (25 %).

^{1}H-NMR [d_4-MeOD]: δ =8.714 (d, 1H), 8.00 (d, 1H), 7.62 (d, 1H), 7.50 (dd, 1H), 7.45 (s, 1H) 6.66 (s, 1H), 5.79 (m,1H), 5.11 (d, 1H), 5.03 (d, 1H), 4.04 (s, 3H), 3.81 (s, 1H), 3.65 (s, 1H), 3.40 (d, 1 H), 2.94 (d, 1H), 2.71 (s, 1H) 2.22-2.14 (m, 2H), 1.88 (m, 1H), 1.72 (m, 1H), 1.54 (s, 2 H), 1.48 (s, 3H), 1,42 (s, 3H). ^{13}C-NMR [d_4-MeOD]: δ = 160.8 (C_{ar}), 155.3 (C=O), 148.74 (C_{ar}H), 145.5 (C_{ar}), 143.6 (C_{ar}), 138.9 (CH=), 132.3 (C_{ar}H), 128.0 (CH_2=), 127.4 (C_{ar}), 124.3 (C_{ar}H), 120.5 (C_{ar}H), 102.7 (C_{ar}H), 71.1 (CH), 60.6 (CH), 59.4 (CH_2SO_3H), 57.2 (OCH_3), 45.6 (CH_2), 38.3 (CH_2), 38.3 (CH), 28.9 (CH_3), 28.4 (C_4), 28.3 (CH_3), 25.0 (CH_2), 20.7 (CH_2).

(ESI, positive): 504.2 $[M + H]^+$.

CSP 1, immobilization of the chiral selector 6

After 2.4 g of (endcapped mercaptopropylsilane modified silica (3 µm) was weighed into the flask, 540 mg of the selector **6** was added to the mixture and to remove the oxygen a gentle N_2-stream was bubbled through for ten minutes. Then 60 mg of AIBN (0.4 mmol) were added. The reaction mixture was refluxed under a N_2-stream for seven hours. After that, it was cooled to r. t. , the modified silica gel was collected by filtration, resuspended in 4.8 ml (2ml per gram silica) of MeOH (gradient grade quality), washed with MeOH (3 x 2 ml) and with DCM (2 x 2 ml). The filtrates were saved for selector recovery. At last the silica gel was transferred to a drying cabinet and dried under vacuum at 60 °C overnight. Elemental analysis: 1.071 % N results in SO loading of 255 µmol g^{-1}.

8, (4S)-4-phenyl-1,3-thiazolidine-2-thione

In a 250 ml flask 4.11 g of (2S)-2-amino-2-phenylethanol (**7,** 30 mmol) was dissolved in 150 ml aqueous solution KOH (1 M) under stirring. After adding 9.1 ml of CS_2 (100 mmol), it was refluxed at 100 °C (using an oil bath) overnight. The solution turned light yellow. Then it was extracted with DCM (3 x 70 ml), dried over Na_2SO_4. A co-product ((4S)-4-phenyl-1,3-oxazolidine-2-thione) was also formed, but in less amount (controlled by TLC with PE : EA 3:1 and RP-HPLC with water : MeOH 50 : 50, 0.1 % FA; confirmed that there was very low amount of the co-product). Thus, a separation by flash chromatography was not necessary. The product was evaporated and dried under vacuum. The yield of **8** as white crystals was 2.80 g (48 %).

^{1}H NMR [$CDCl_3$]: δ = *not seen* (br, s, 1H), 7.42 (m, 5H), 5.34 (dd, 1H), 3.88 (dd, 1H), 3.50 (dd, 1H). ^{13}C-NMR [$CDCl_3$]: δ = 129.8 ($C_{ar}H$) , 129.7($C_{ar}H$), 126.6 ($C_{ar}H$), 51.3 (CH) , 42.0 (CH_2).

9, (2S)-2-amino-2-phenylethanesulfonic acid

Into a 250 ml flask 12.7 ml of H_2O_2 (35 %) (179 mmol, 71 eq.) was added portionwise to 63.6 ml of formic acid (98%) (766 mmol, 307 eq.). The solution was stirred in an ice bath (0°C) for one hour. Peroxyformic acid was formed. After adding 2.48 of **8** (12.7 mmol) the mixture was stirred overnight at 0-5°C with a cooling-aggregate. After that, the solution was evaporated DEE and

EtOH were added and the flask was put into the fridge for recrystallisation overnight. At last it was washed in diethyl ether and put in drying cabinet overnight. The Yield of **9** as white crystals was 0.89 g (35 %).

^{1}H-NMR [D_2O]: δ = 7.43 (m, 5H, ArH), 4.70 (m, 1H, CH), 3.54 (dd, 1H in CH_2), 3.47 (dd, 1H in CH_2). ^{13}C-NMR [D_2O]: δ = 130.2 ($C_{ar}H$), 129.8 ($C_{ar}H$), 127.5 ($C_{ar}H$), 53.6 (CH_2), 52.4 (CH).

10, 2-(S)-phenyl-taurine-quinine-selector

80 ml of dichlormethane was distilled and collected over a drying agent (CaH_2). After adding 0.82 g of **9** (4.1 mmol) into the three-necked flask, the apparatus was put under a N_2-stream and charged with the dry solvent under stirring. Under stirring 5.04 ml of BSA (12.4 mmol, 5 eq.) was added via syringe. The mixture was refluxed (oil bath temperature 100 °C) under N_2-atmosphere). After that, the mixture was cooled to r. t. 2.14 g of **5** (4.1 mmol, 1 eq.) was added portionswise and stirred overnight under N_2-atmosphere. The reaction was quenched with 2 ml MeOH (gradient grade quality) for one hour. Purification (4-Nitrophenol and quinine byproducts were separated) by flash column chromatography (DCM : MeOH 9 : 1 – 6 : 1 – 3 : 1) yielded 1.12 g (50 %) of **10** as crème-brown crystals.

^{1}H-NMR [CD_3OD]: δ =8.88 (d, 1H), 8.12 (d, 1H), 7.97 (d, 1H), 7.75 (dd, 1H), 7.68 (d, 1H), 7.32 (m, 3H), 7.30 (m, 3H), 7.03 (s, 1H), 5.25 (m, 1H), 5.17 (d, 1H), 4.75 (m, 1H), 4.07 (s, 3H), 3.87 - 3.72 (m, 2 H), 3.68 (s, 1H), 3.49 (m, 1H), 3.23 (d, 1H), 3.13 (d, 1H), 2.30 - 2.17 (m, 2H), 2.03 (m, 2H), 1.82 (m, 1H). ^{13}C-NMR [CD_3OD]: δ = 160.3 (C_{ar}), 155.6 (C=O), 148.3 ($C_{ar}H$), 145.0 (C_{ar}), 143.5 (C_{ar}), 139.5 (CH=), 131.2 ($C_{ar}H$), 131.0 ($C_{ar}H$), 130.2 ($C_{ar}H$), 128.8 ($C_{ar}H$), 125.6 (C_{ar}), 120.9 ($C_{ar}H$), 119.6 ($C_{ar}H$), 118.1 (CH=), 102.2 ($C_{ar}H$), 71.4 (CH), 60.2 (CH), 58.1 (OCH_3), 55.3 (CH_2), 54.3 (CH), 46.4 (CH_2), 39.6 (CH_2), 38.93 (CH), 35.0 (CH_2), 28.8 (CH), 25.5 (CH_2), 21.7 (CH_2). MS (ESI, positive): 552.4 $[M+H]^+$.

CSP 2, immobilization of the chiral selector 10

After 2.4 g of endcapped mercaptopropylsilane modified silica (3 µm) was weight into the flask, 560 mg of the selector **10** was added to the mixture and to remove the oxygen a gentle N_2-stream was bubbled through for ten minutes. Then 60 mg of AIBN (0.4 mmol) were added. The reaction mixture refluxed (oil bath temperature 75-77 °C) under a N_2-stream for seven hours. After that, it was cooled to r. t. , the modified silica gel was collected by filtration, resuspended in 4.8 ml (2ml per gram silica) of MeOH (gradient grade quality), washed with MeOH (3 x 2 ml) and with DCM (2 x 2 ml). The filtrates were saved for selector recovery.

At last the silica gel was transferred to a drying cabinet and dried under vacuum at 60 °C over-night. Subjected to elmental analysis: 0.357 % N 85 µmol Selector pro gram silica (19 %) was immobilized. Because the immobilization was very bad, it was repeated with another approach: For repetition of the immobilization 2.3 g of the endcapped mercaptopropylsilane modified silica (3 µm, immobilized before with 85 µmol), 250 mg of the selector **10**, 300 mg of AIBN and 38 ml of MeOH (gradient grade quality). Subjected to the elemental analysis: 0.781 % N results in SO loading of 186 µmol g^{-1}.

12, 4-(R)-4-(propan-2-yl)-1,3-thiazolidine-2-thione

In a 250 ml flask 3.09 g of (2R)-2-amino-3-methylbutan-1-ol (**11**, 30 mmol)) was put and dissolved in 150 ml aqueous solution KOH (1 M) under stirring. After adding 9.1 ml CS_2 (150 mmol, 5 eq.) it was refluxed at 100 °C (using an oil bath) overnight. The solution turned orange-red. It was stirred overnight (the solution turned dark red). After extracting with DCM (3 x 50 ml) it was dried over Na_2SO_4. A co-product ((4R)-4-(propan-2-yl)-1,3-oxazolidine-2-thione) was also formed. The co-product was separated from the product by flash chromatography (PE : EA 3:1) yielding 3.53 g (73 %) of **12** (white crystals).

^{1}H-NMR [$CDCl_3$]: δ = 4.10 (dd, 1H), 3.58 (dd, 1H),3.38 (dd, 1H), 2.00 (dqq, 1H), 1.08 (d, 3H), 1.04 (d, 3H). ^{13}C-NMR [$CDCl_3$]: δ = 67.7 (CH), 36.7 (CH_2), 32.6 (CH), 19.3 (CH_3), 18.8 (CH_3).

Because the immobilization was very bad, the whole preparation of the selector **16** and therefore **12** was repeated with another approach:

For **12** two approaches were made. For the first were used 3.43 g of **11** (33 mmol, 1 eq.), 9.1 ml of CS_2 (150 mmol, 4.5 eq.) and 150 ml of KOH (1M).

For the second experiment were used 2.51 g of **11** (24 mmol, 1 eq.), 7.4 ml of CS_2 (122 mmol, 5 eq.) and 150 ml KOH (1M). The solids of the first and of the second approaches were united. The co-product was separated from the product by flash chromatography (PE : EA 3 : 1). The product was evaporated and dried under vacuum.

The whole yield of **12** as white crystals by flash chromatography was 3.04 g (75 %).

^{1}H-NMR [$CDCl_3$]: δ = 8.04 (br s, 1H), 3.99 (dd, 1H), 3.42 (dd, 1H), 3.28 (dd, 1H), 1.91 (dqq, 1H), 0.98 (d, 3H), 0.96 (d, 3H).^{13}C-NMR [$CDCl_3$]: δ = 201.5 (C=S), 70.4 (CH), 36.4 (CH), 32.45 (CH_2), 19.2 (CH_3), 18.6 (CH_3).

13, (2R)-2-amino-3-methylbutane-1-sulfonic acid

Into a 150 ml flask 15 ml of H_2O_2 (35 %) (447 mmol, 35 eq.) were added portionwise to 75 ml of formic acid (98%) (1.66 mol, 130 eq.) . The solution was stirred in an ice bath (0°C) for one hour. Peroxyformic acid was formed. After adding 2.41 g of **12** (15 mmol) to the mixture, it was stirred overnight at 0-5°C and covered with aluminium foil. The next day the solution was put in a pear shaped flask and evaporated under reduced pressure. A light yellow viscous substance was formed. Then diethyl ether and ethanol were added and the flask put into the fridge. No precipitation was formed, so it was evaporated under reduced pressure again, this time methanol was added and the flask put into the fridge for recrystallisation. At least it was washed in diethyl ether and put in drying cabinet overnight. The yield of **13** as white crystals was 0.79 g (32 %).

^{1}H-NMR [D_2O]: δ= 3.51 (dd, 1H), 3.30 (dd, 1H), 3.09 (dd, 1H), 2.05 (m, 1H), 0.99 (d, 3H), 0.98 (d, 3H). ^{13}C-NMR [D_2O]: δ = 54.2 (CH), 49.7 (CH_2) , 30.6 (CH), 17.6 (CH_3), 17.0 (CH_3).

Because the immobilization was very bad, the whole preparation of the selector **16** and therefore **13** was repeated with another approach:

For **13** were used 3.06 g of **12** (19 mmol), 19ml of H_2O_2 (35 %) (586 mmol, 31 eq.) and 95 ml of H_3CCOOH (98 %) (2.52 mol, 133 eq.). The yield of **13** as white crystals was 0.94 g (30 %).

^{1}H-NMR [D_2O]: δ = 3.49 (d, 1H), 3.22 (dd, 1H), 3.10 (dd, 1H), 2.06 (m, 1H), 0.98 (d, 3H), 0.96 (d, 3H). ^{13}C-NMR [D_2O]: δ = 54.2 (CH), 49.8 (CH_2), 30.6 (CH), 17.7 (CH_3), 17.0 (CH_3).

15, 9-O-(4-nitrophenyloxycarbonyl)-quinidine hydrochloride

In a three-necked flask 8.02 g of quinine (24.66 mmol) were dissolved in 300 ml of toluene followed by azeotropic distillation (removal of 1/3 of the solvent) using a Dean-Stark-apparatus. After cooling the mixture to room temperature (r. t.) under N_2-atmosphere 5.04 g of 4-nitrophenyl chloroformate (25 mmol, 1.01 eq.) were added. The mixture was stirred at r. t. under N_2-atmosphere overnight. After filtration a pale yellow precipitation was separated. The solid was washed with n-heptane (2 x 30 ml), dried under vacuum and stored under N_2-atmosphere to avoid hydrolysis. The yield of **15** as white solid was 12.42 g (96 %).

16, (2R)-isopropyl-taurine-QD-selector

After building the apparatus 80 ml of DCM was distilled and collected over a drying agent (CaH_2). After adding 0.67 g of **13** (4.0 mmol) into the three-necked flask, the apparatus was put under a N_2-stream and charged with the dry solvent under stirring. With a syringe 4.94 ml of BSA (12 mmol, 5 eq.) was added under stirring. The mixture was refluxed (oil bath temperature 100 °C) under N_2-atmosphere. After the mixture was cooled to r. t.

Then 2.10 g of **15** (4.0 mmol, 1 eq.) was added portionswise and stirred overnight under N_2-atmosphere. The reaction was quenched with 2 ml MeOH (gradient grade quality) for one hour. Purification (4-Nitrophenol and remaining active ester were separated) by flash column chromatography (DCM : MeOH 9 : 1 – 6 : 1 – 2 : 1) yielded 1.05 g (50 %) of **16** as crème-brown crystals.

^{1}H-NMR [CD_3OD]: δ = 8.69 (d, 1H), 7.95 (d, 1H), 7.62 (d, 1H), 7.54 (d, 1H), 7.42 (d, 1H), 7.09 (s, 1H), 6.26 (h, 1H), 5.27 (d, 1H), 3.99 (s, 3H), 3.95 (m, 1H), 3.71 (m, 1H), 3.61 (m, 1H), 3.21-3.14 (m, 2H), 3.01(m, 2H), 2.95(m, 2H), 2.64(dd, 1H), 2.41 (m, 1H), 1.98 (s, 1H), 1.82 (m, 3H), 1.46 (m, 1H), 0.88 (d, 2H), 0.85 (d, 2H). ^{13}C-NMR [CD_3OD]: δ =160.3 (C_{ar}), 156.9 (C=O), 147.9 ($C_{ar}H$), 145.0 (C_{ar}), 144.3 (Car), 139.4 (CH=), 131.5 ($C_{ar}H$), 127.7 (Car), 124.2 ($C_{ar}H$), 119.6 ($C_{ar}H$), 117.6 (CH_2=), 102.3 ($C_{ar}H$), 72.0 (CH), 60.1 (CH), 57.1 (CH_2SO_3H), 55.6 (OCH_3), 55.2 (CH), 54.5 (CH2), 50.8 ($2CH_2$), 50.4 (CH2), 39.2 (CH), 33.5 (CH), 29.2 (CH_3), 24.6 (CH2), 21.2 (CH2), 19.7 (CH_3), 18.3 (CH_3). (ESI, positive): 518.4 $[M+H]^+$.

Because the immobilization was very bad, the preparation of the selector **16** was repeated with another approach:

For **16** were used 1.02 g of **13** (61 mmol), 3.21 g of **15** (61 mmol, 1 eq.), 7.55 ml BSA (305 mmol, 5 eq.) and 100 ml of absoluted DCM. The yield of **16** (crème-brown crystals) was 2.36 g (75 %). ^{1}H-NMR [CD_3OD]: δ = 8.70 (d, 1H), 7.95 (d, 1H), 7.62 (d, 1H), 7.53 (s, 1H), 7.43 (d, 1H), 7.07 (s, 1H), 6.25 (m, 1H), 5.26 (d, 1H), 4.00 (s, 3H), 3.94 (m, 1H), 3.70 (m, 1H), 3.59 (t, 1H), 3.20-3.13 (m, 2H), 3.01 (m, 2H), 2.93 (m,1H), 2.63 (dd, 1H), 2.39 (m, 1H), 1.97 (s, 1H), 1.81 (m, 3H), 1.46 (m, 1H), 0.88 (d, 2H), 0.85 (d, 2H). ^{13}C-NMR [CD_3OD]: δ =160.3 (C_{ar}), 157.0 (C=O), 147.9 ($C_{ar}H$), 145.0 (C_{ar}), 144.4 (C_{ar}), 139.3 (CH=), 131.5 ($C_{ar}H$), 127.7 (C_{ar}), 124.2 ($C_{ar}H$), 119.6 ($C_{ar}H$), 117.5 (CH_2=), 102.3 ($C_{ar}H$), 72.1 (CH), 60.1 (CH), 57.1 (CH_2SO_3H), 55.6 (OCH_3), 55.2 (CH), 54.5 (CH_2), 50.9 ($2CH_2$), 50.3 (CH_2), 39.3 (CH), 33.5 (CH), 29.2 (CH_3), 24.7 (CH_2), 21.2 (CH_2), 19.7 (CH_3), 18.3 (CH_3). (ESI, positive): 504.2 $[M + H]^+$.

CSP 3, Immobilization of chiral selector 16

After 2.4 g of (endapped mercaptopropylsilane modified silica (3 µm) was weighed into the flask, 600 mg of the selector **16** were added (solved in 15 ml MeOH) to the mixture and to remove the oxygen a gentle N_2-stream was bubbled through for ten minutes. Then 67 mg of AIBN (0.4 mmol) was added (solved in 5 ml MeOH). The reaction mixture refluxed (oil bath temperature at 75-77 °C) under a N_2-stream for seven hours. After it was cooled to r. t. , the modified silica gel was collected by filtration, resuspended in 4.8 ml (2ml per gram silica) of MeOH (gradient grade quality), washed with MeOH (3 x 2 ml) and with DCM (2 x 2 ml). The filtrates were saved for selector recovery. At least the silica gel was transferred to a drying cabinet and dried under vacuum at 60 °C overnight. Subjected to elmental analysis: 0.333 % N in SO loading of 79 µmol g^{-1} (18 %) was immobilized.

Because of the immobilization was very bad, the immobilization of the selector **16** was repeated with another approach:

For **CSP 3** were used 2.5 g of endcapped mercaptopropylsilane modified silica (3 µm), 582 mg of the selector **16**, 600 mg of AIBN (37 mmol) and 30 ml of MeOH (HPLC grade quality).

Elemental analysis: 1.152 % N results in SO loading of 274 µmol/g.

List of Abbreviations

///	no value, no data
AAS	free amino acids
ACHSA	aminocyclohexanesulfonic acid
AIBN	Azo-bis-isobutyronitril
AX	anion exchanger
BSA	O,N-Bistrimethylsilylacetamid
CAD	charged aerosol detector
CD	cylodextrine
CDA	chiral derivatizing additives
CMPA	chiral mobile phase agent
CLEC	chiral ligand exchange chromatography
CS_2	carbon disulfide
CSP	chiral stationary phase
CX	cation exchanger
DCM	dichloromethane
DEA	diethylamine
DEE	diethyl ether
EA	ethyl acetate
e. g.	exampla gratia, for example
ESI	electrospray ionisation
EtOH	ethanol
FA	formic acid
FDA	American Food and Drug administration
GC	gas chromatography
H_2O_2	hydrogen peroxide
HCOOH	formic acid
H_3CCOOH	acetic acid

HPLC	high performance liquid chromatography
IMCI	intramolecular counterion
KOH	potassium hydroxide
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
MeOH	methanol
MS	mass spectrometry
n. d.	not determined
NMR	nuclear magnetic resonance
NP	normal phase mode
PE	petrol ether
PO	polar organic mode
RP	reversed phase mode
QN	quinine
QD	quinidine
SCX	strong cation exchanger
r. t.	room temperature
w-%	mass percent
WAX	weak anion exchanger
WAX-CSP	weak anion exchanger - chiral stationary phase
ZWIX	zwitterionic ion exchange

Abbreviations of analytes

1-Me-Trp	1-methyl-tryptophan
1-Naphtylalanine	1-naphtylalanine
2-Et-Tau	2-amino-butyl-1-sulfonic acid
2-Me-Tau	2-amino-propyl-1-sulfonic acid
2-tert-Bu-Tau	2-amino-3,3-dimethylbutane-1-SA
3,4-Hydroxyproline	3,4-hydroxyproline
5-HTP	5-hydroxy-tryptophan
5-oxo-Proline	5-oxo-proline
Ala	alanine
allo-Ile	allo-isoleucine
allo-Thr	allo-threonine
α-Me-DOPA	α-methyl-dihydroxyphenylalanine
α-Me-Leu	α-methyl-leucine
α-Me-Phe	α-methyl-phenylalanine
α-Me-Ser	α-methyl-serine
α-Me-Trp	α-methyl-tryptophan
α-Me-m Tyr	α-methyl-meta-tyrosine
α-Me-Tyr	α-methyl-tyrosine
α-Me-Val	α-methyl-valine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Baclofen	baclofen
β-homo-Phe	β-homo-phenylalanine
β-Leu	β-leucine
β-Neopentylglycine	β-neopentylglycine
β-Phe	β-phenylalanine
cis-2-ACHSA	cis-2-amino-cyclohexyanecarboxylic acid
cis-3-ACHSA	cis-3-amino-cyclohexyanecarboxylic acid
cis-4-OH-Pro	cis-4-hydroxy-proline
Cysteic acid	cysteic acid
DOPA	3,4-dihydroxyphenylalanine
erythro-beta	erythro-β-methyl-cycloalanine
Gln	glutamine
Glu	glutamic acid
His	histidine
Homocysteic acid	homocysteic acid
Ile	iso-leucine

isopropyl-Tau	2-amino-3-methylbutane-1-sulfonic acid
Kynurenine	kynurenine
Leu	leucine
Lys	lysine
m-Tyr	meta-tyrosine
Met	methionine
N-Me-Asp	N-methyl-aparaginsäure
N-Me-Glu	N-methyl-glutamine acid
N-Me-Leu	N-methyl-leucine
Neopentylglycine	neopentylglycine
Nipe	nipecotic acid
Nor-Leu	nor-leucine
Nor-Val	nor-valine
oF-Phe	ortho-fluoro-phenylalanine
OMe-Tyr	O-methyl-tyrosine
p-Amino-Phe	para-amino-phenylalanine
p-Br-Phe	para-bromo-phenylalanine
pCl-Phe	para-chloro-phenylalanine
p-F-Phe	para-fluoro-phenylalanine
Phe	phenylalanine
phenyl-Tau	2-amino-2-phenylethanesulfonic acid
Phenylglycine	phenylglycine
Pipe	pipecolic acid
Pro	proline
Pro-Me-SO_3H	pyrrolidine-2-methanesulfonic acid
Ser	serine
Tic	1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid
tert-Leu	tert-leucine
Thr	threonine
threo-beta	threo-β-methyl-cycloalanine
trans-2-ACHSA	trans-2-amino-cyclohexyanecarboxylic acid
trans-2-amino	trans-2-aminocyclohexanecarboxylic acid
trans-4-OH-Pro	trans-4-hydroxy-proline
trans-2-Me-2-ACHSA	trans-2-methyl-amino-cyclohexyanecarboxylic acid
Trp	tryptophan
Tyr	tyrosine
Val	valine

Conclusion

The focus of the diploma thesis was the synthesis and chromatographic evaluation of novel zwitterionic CSPs. Different aminosulfonic acid functionalities were attached to cinchona alkaloid scaffold to synthesize chiral zwitterionic CSPs. By evaluation with HPLC it was characterized which CSP was promising for enantioseparation of zwitterionic analytes. A set of 75 different amino acids, amino acid derivatives and aminosulfonic acids was applied for evaluation.

In detail, dimethyl-Tau (**CSP 1**) was compared to Tau-QN (**CSP 7**) to prove if chiral information in the SCX-part is necessary for a successful enantioseparation.

CSP 2 having a side chain with (S)-configuration at the SCX-part was compared with **CSP 8** exhibiting opposite configuration at the SCX-moiety to investigate how a different configuration in the SCX-part influences enantioseparation and elution order of enantiomers.

The QD-based **CSP 3** was compared to the "pseudo-enantiomeric" QN-based **CSP 4** to observe if a change in elution orders occurs.

Moreover, four QN-based CSPs with different side chains in the SCX-part were compared to each other to determine which chiral side chain gives the best results in enantioseparation. Additionally, variations of the bulk mobile phase were carried out by changing the type and amount of the polar organic solvents. Its influence on chromatographic parameters as there are retention, enantioselectivity and resolution was discussed.

Resolution-values and separation factors were increasing on average with higher ACN-amount in the mobile phase.

In doing so, it was seen that enantioseparation was best with **CSP 4** and **CSP 6** where **CSP 4** affords good results for almost all amino acids. CSPs without any chiral information like **CSP 1** and **CSP 7** were not suitable for enantioselective separations of the zwitterionic test compounds.

With changing only the configuration in the SCX-part of the selector no effective change in elution order occurred.

When changing the cinchona alkaloid scaffold from quinine to quinidine and the configuration in the SCX-part from (S) to (R) a change in elution order for all but one analyte could be observed. Therefore, quinine and quinidine seem to be the driving force for chiral recognition in ZWIX-selectors.

To summarize, synthesis of novel zwitterionic CSP were carried out successfully. By chromatographic evaluation new findings on enantioselective recognition were achieved.
The synthesis path of these zwitterionic selectors and their application in enantioseparation of amino acids seems to be promising for future investigations.

Zusammenfassung

In dieser Arbeit wurden die Synthese und die Evaluierung von neuen zwitterionischen chiralen stationären Phasen (CSPs) diskutiert. Diese zwitterionischen Selektoren (SO) bestehen aus einem Chinin- oder Chinidin-basierenden schwachen Anionenaustauscher (WAX), an dem durch chemische Modifikation ein starker Kationenaustauscher (SCX) in Form einer Sulfonsäure angebracht wurde. Durch Immobilisierung an ein modifiziertes Kieselgel wurde somit eine Zwitterionenaustauscherphase (ZWIX-CSP) hergestellt.

Es wurden drei verschiedene ZWIX-CSPs synthetisiert und zusammen mit fünf weiteren ZWIX-CSPs chromatographisch verglichen. Mittels HPLC-Evaluierung sollte bestimmt werden welcher Selektor die besten Ergebnisse bezüglich Enantiomerentrennung für zwitterionische Analyten liefert. Dafür wurde ein Set von 75 Aminosäuren und Aminosulfonsäuren verwendet.

Es wurde festgestellt, dass eine chirale Seitenkette an der Kationenaustauscherseite nötig ist um Enantioselektivität für einen Großteil der Analyte zu erzielen.

Im Weiteren wurde der Einfluss der Konfiguration im SCX-Teil als auch der Einfluss von QN und QD im WAX-Teil auf das Potential für Enantiomerentrennung und auf die Elutionsreihenfolge hin untersucht. Dabei wurde beobachtet, dass eine alleinige Änderung der Konfiguration im SCX-Teil für die Umkehr der Elutionreihenfolge nicht ausreichend ist, sondern bei ZWIX-CSPs dieses Phänomen noch immer durch QN und QD im Selektor gesteuert wird.

Durch Evaluierung aller acht CSPs konnte herausgefunden werden, dass im Durschnitt die Auflösung und die Selektivität gute Werte aufweisen und sich bei einigen Analyten mit zunehmendem aprotischen Anteil in der mobilen Phase verbessern.

Zusammenfassend kann festgehalten werden, dass diese zwitterionischen Phasen erfolgreich synthetisiert wurden. Durch die Evaluierung von diversen ZWIX-Phasen konnten viele Erkenntnisse in Bezug auf Enantiomerentrennung und Elutionsreihenfolge von verschieden Aminosäuren gewonnen werden. Diese Phasen zeigten bei einzelnen zwitterionischen Aminosäuren eine gute Trennung. Durch Modifikation der Aminosulfonsäure-Gruppe bei der Synthese lassen sich weitere neuartige ZWIX-Phasen herstellen. Das Forschungsgebiet ist noch nicht abgeschlossen und es befinden sich weitere neue ZWIX-CSPs in Entwicklung.

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Appendix

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		3.68	5.80	5.80	0.58	0.58	**1.00**	**0.00**	615	615	2460	2460
Trp	D-L	3.68	7.44	10.00	1.02	1.72	**1.68**	**5.79**	4109	7405	16436	29620
Tyr		3.68	6.19	6.19	0.68	0.68	**1.00**	**0.00**	757	757	3028	3028
p-F-Phe		3.68	5.69	5.69	0.55	0.55	**1.00**	**0.00**	5163	5163	20652	20652
pCl-Phe		3.68	6.12	6.12	0.66	0.66	**1.00**	**0.00**	4722	4722	18888	18888
p-Br-Phe		3.68	6.38	6.38	0.74	0.74	**1.00**	**0.00**	4520	4520	18080	18080
oF-Phe		3.68	5.70	5.70	0.55	0.55	**1.00**	**0.00**	512	512	2048	2048
Baclofen		3.68	6.75	6.75	0.83	0.83	**1.00**	**0.00**	836	836	3344	3344
DOPA		3.68	6.85	6.85	0.86	0.86	**1.00**	**0.00**	864	864	3456	3456
m Tyr		3.68	6.29	6.29	0.71	0.71	**1.00**	**0.00**	713	713	2852	2852
5-HTP	D-L	3.68	7.87	10.70	1.14	1.91	**1.67**	**5.29**	3772	5979	15088	23916
β-Phe		3.68	6.81	7.31	0.85	0.99	**1.16**	**0.78**	2101	1874	8404	7496
β-homo-Phe		3.68	7.15	7.52	0.95	1.05	**1.11**	**1.17**	9799	7897	39196	31588
α-Me-Phe		3.68	5.42	5.42	0.47	0.47	**1.00**	**0.00**	284	284	1136	1136
α-Me-Tyr		3.68	6.21	6.21	0.69	0.69	**1.00**	**0.00**	520	4464	2080	17856
α-Me-m Tyr		3.68	5.76	6.69	0.57	0.82	**1.45**	**1.49**	727	4656	2908	18624
α-Me-DOPA	D-L	3.68	6.30	7.54	0.71	1.05	**1.47**	**1.03**	308	975	1232	3900
α-Me-Trp		3.68	6.97	14.60	0.89	2.97	**3.32**	**16.79**	6197	11118	24788	44472
1- Me-Trp	D-L	3.68	7.27	9.87	0.98	1.68	**1.72**	**6.11**	5157	7827	20628	31308
Phenylglycine	D-L	3.68	6.07	6.42	0.65	0.75	**1.15**	**1.22**	8430	7202	33720	28808
p-Amino-Phe		3.68	6.17	6.17	0.68	0.68	**1.00**	**0.00**	711	711	2844	2844
OMe-Tyr		3.68	6.18	6.18	0.68	0.68	**1.00**	**0.00**	1647	1647	6588	6588
Kynurenine		3.68	7.75	7.75	1.11	1.11	**1.00**	**0.00**	2573	2573	10292	10292
1-Naphtylalanine	L-D	3.68	6.95	6.95	0.89	0.89	**1.00**	**0.00**	8229	2616	32916	10464
Tic	S-R	3.68	6.05	6.64	0.64	0.81	**1.25**	**0.63**	325	2410	1300	9640
AVERAGE VALUES							**1.24**	**1.61**				

Table i: *aromatic amino acids on dimethyl-Tau-QN (CSP 1), 100 % MeOH, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		3.68	5.99	5.99	0.63	0.63	**1.00**	**0.00**	5774	5774	23096	23096
Trp	D-L	3.68	7.57	9.42	1.06	1.56	**1.47**	**3.55**	3313	5399	13252	21596
Tyr		3.68	6.50	6.50	0.76	0.76	**1.00**	**0.00**	1075	1075	4300	4300
p-F-Phe		3.68	6.04	6.04	0.64	0.64	**1.00**	**0.00**	4306	4306	17224	17224
pCl-Phe		3.68	6.50	6.50	0.77	0.77	**1.00**	**0.00**	3126	3126	12504	12504
p-Br-Phe		3.68	6.79	6.79	0.85	0.85	**1.00**	**0.00**	2910	2910	11640	11640
oF-Phe		3.68	6.04	6.04	0.64	0.64	**1.00**	**0.00**	499	499	1996	1996
Baclofen		3.68	6.94	6.94	0.89	0.89	**1.00**	**0.00**	885	885	3540	3540
DOPA		3.68	7.15	7.15	0.94	0.94	**1.00**	**0.00**	421	421	1684	1684
m Tyr		3.68	6.58	6.58	0.79	0.79	**1.00**	**0.00**	1274	1274	5096	5096
5-HTP	D-L	3.68	7.73	9.56	1.10	1.60	**1.45**	**2.92**	2489	3635	9956	14540
β-Phe		3.68	7.10	7.68	0.93	1.09	**1.17**	**0.67**	1147	1255	4588	5020
β-homo-Phe		3.68	n.d.									
α-Me-Phe		3.68	5.87	5.87	0.59	0.59	**1.00**	**0.00**	508	508	2032	2032
α-Me-Tyr	D-L	3.68	6.14	6.43	0.67	0.75	**1.12**	**0.48**	1144	2729	4576	10916
α-Me-m Tyr		3.68	6.10	6.82	0.66	0.85	**1.30**	**1.10**	1204	1962	4816	7848
α-Me-DOPA	D-L	3.68	7.47	7.47	1.03	1.03	**1.00**	**0.00**	865	193	3460	772
α-Me-Trp		3.68	7.23	12.55	0.97	2.41	**2.49**	**12.11**	5581	10568	22324	42272
1- Me-Trp	D-L	3.68	7.28	8.98	0.98	1.44	**1.47**	**3.40**	3318	5394	13272	21576
Phenylglycine	D-L	3.68	6.32	6.76	0.72	0.84	**1.17**	**1.41**	7535	6386	30140	25544
p-Amino-Phe		3.68	6.32	6.32	0.72	0.72	**1.00**	**0.00**	970	970	3880	3880
OMe-Tyr		3.68	6.38	6.38	0.73	0.73	**1.00**	**0.00**	1322	1322	5288	5288
Kynurenine		3.68	7.83	7.83	1.13	1.13	**1.00**	**0.00**	1737	1737	6948	6948
1-Naphtylalanine		3.68	7.26	7.26	0.97	0.97	**1.00**	**0.00**	668	668	2672	2672
Tic	S-R	3.68	6.14	6.85	0.67	0.86	**1.29**	**0.70**	702	638	2808	2552
AVERAGE VALUES							**1.16**	**1.10**				

Table ii: *aromatic amino acids on dimethyl-Tau-QN (CSP 1), 75/25 MeOH/ACN, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		3.68	7.50	7.50	1.04	1.04	**1.00**	**0.00**	5071	5071	20284	20284
Trp	D-L	3.68	9.90	11.98	1.69	2.26	**1.34**	**2.31**	1989	2734	7956	10936
Tyr		3.68	8.67	8.67	1.36	1.36	**1.00**	**0.00**	516	516	2064	2064
p-F-Phe		3.68	7.61	7.61	1.07	1.07	**1.00**	**0.00**	2174	2174	8696	8696
pCl-Phe		3.68	8.40	8.40	1.28	1.28	**1.00**	**0.00**	1447	1447	5788	5788
p-Br-Phe		3.68	8.88	8.88	1.41	1.41	**1.00**	**0.00**	1060	1060	4240	4240
oF-Phe		3.68	7.40	7.89	1.01	1.14	**1.13**	**0.56**	1653	975	6612	3900
Baclofen		3.68	9.31	9.31	1.53	1.53	**1.00**	**0.00**	1953	3656	7812	14624
DOPA		3.68	10.35	10.35	1.81	1.81	**1.00**	**0.00**	13	13	52	52
m Tyr		3.68	8.52	8.52	1.32	1.32	**1.00**	**0.00**	1399	1399	5596	5596
5-HTP	D-L	3.68	10.13	12.24	1.75	2.33	**1.33**	**1.80**	1320	1604	5280	6416
β-Phe		3.68	9.37	10.26	1.55	1.79	**1.16**	**0.81**	1557	1072	6228	4288
β-homo-Phe		3.68	9.54	9.90	1.59	1.69	**1.06**	**0.63**	6516	3671	26064	14684
α-Me-Phe	D-L	3.68	7.21	7.48	0.96	1.03	**1.08**	**0.50**	3231	2883	12924	11532
α-Me-Tyr		3.68	8.10	8.10	1.20	1.20	**1.00**	**0.00**	683	683	2732	2732
α-Me-m Tyr		3.68	7.75	8.74	1.10	1.37	**1.24**	**1.38**	2314	1984	9256	7936
α-Me-DOPA		3.68	10.33	10.33	1.81	1.81	**1.00**	**0.00**	29	29	116	116
α-Me-Trp		3.68	9.40	15.70	1.55	3.27	**2.10**	**10.43**	5196	8395	20784	33580
1- Me-Trp	D-L	3.68	9.18	10.97	1.50	1.98	**1.32**	**1.79**	1330	2025	5320	8100
Phenylglycine	L-D	3.68	7.97	8.71	1.17	1.37	**1.17**	**1.27**	3531	3163	14124	12652
p-Amino-Phe		3.68	7.94	7.94	1.16	1.16	**1.00**	**0.00**	684	684	2736	2736
OMe-Tyr		3.68	7.90	7.90	1.15	1.15	**1.00**	**0.00**	1333	1333	5332	5332
Kynurenine		3.68	10.02	10.02	1.72	1.72	**1.00**	**0.00**	12245	12245	48980	48980
1-Naphtylalanine		3.68	8.91	9.36	1.42	1.54	**1.09**	**0.45**	3267	702	13068	2808
Tic	S-R	3.68	7.60	8.56	1.06	1.33	**1.25**	**0.90**	1119	784	4476	3136
AVERAGE VALUES							**1.13**	**0.91**				

Table iii: *aromatic amino acids on dimethyl-Tau-QN (CSP 1), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3,68	5,32	5,32	0,45	0,45	**1,00**	**0,00**	346	346	1384	1384
Val		3,68	5,17	5,17	0,40	0,40	**1,00**	**0,00**	1887	1887	7548	7548
Leu	L-D	3,68	5,24	5,44	0,42	0,48	**1,13**	**0,61**	4992	3388	19968	13552
Ser		3,68	5,99	5,99	0,63	0,63	**1,00**	**0,00**	735	735	2940	2940
Thr		3,68	5,74	5,74	0,56	0,56	**1,00**	**0,00**	700	700	2800	2800
Ile		3,68	5,04	5,04	0,37	0,37	**1,00**	**0,00**	333	333	1332	1332
Met		3,68	5,88	5,88	0,60	0,60	**1,00**	**0,00**	4074	4074	16296	16296
Lys		3,68	13,58	13,58	2,69	2,69	**1,00**	**0,00**	38	38	152	152
Pro		3,68	5,94	5,94	0,61	0,61	**1,00**	**0,00**	1657	1657	6628	6628
Asn		3,68	6,34	6,34	0,72	0,72	**1,00**	**0,00**	892	892	3568	3568
Gln		3,68	5,84	5,84	0,59	0,59	**1,00**	**0,00**	409	409	1636	1636
Asp	L-D	3,68	9,99	10,28	1,71	1,79	**1,05**	**0,41**	12242	1491	48968	5964
Glu		3,68	7,32	7,32	0,99	0,99	**1,00**	**0,00**	1887	1887	7548	7548
Arg		3,68	13,83	13,83	2,76	2,76	**1,00**	**0,00**	111	111	444	444
His		3,68	18,01	18,01	3,89	3,89	**1,00**	**0,00**	7	7	28	28
trans-4-OH-Pro	L-D	3,68	5,51	7,31	0,50	0,99	**1,99**	**1,84**	363	1340	1452	5360
cis-4-OH-Pro		3,68	7,37	7,83	1,00	1,13	**1,13**	**0,60**	1151	2132	4604	8528
Nor-Leu	L-D	3,68	5,21	5,34	0,42	0,45	**1,09**	**0,50**	10113	4613	40452	18452
Nor-Val	L-D	3,68	5,22	5,32	0,42	0,44	**1,06**	**0,43**	14325	6133	57300	24532
Nipecotic acid		3,68	7,06	7,06	0,92	0,92	**1,00**	**0,00**	1384	1384	5536	5536
β-Leu		3,68	5,80	6,09	0,58	0,65	**1,14**	**0,49**	1060	2715	4240	10860
α-Me-Leu		3,68	4,88	4,88	0,33	0,33	**1,00**	**0,00**	1099	1099	4396	4396
α-Me-Val		3,68	5,07	5,13	0,38	0,39	**1,05**	**0,26**	5213	9725	20852	38900
α-Me-Ser		3,68	5,87	5,87	0,60	0,60	**1,00**	**0,00**	1010	1010	4040	4040
N-Me-Leu	L-D	3,68	4,93	5,01	0,34	0,36	**1,06**	**0,37**	8616	8374	34464	33496
trans-2-ACHSA		3,68	5,86	6,18	0,59	0,68	**1,14**	**0,37**	396	2201	1584	8804
cis-2-ACHSA		3,68	5,21	5,26	0,41	0,43	**1,03**	**0,08**	867	1570	3468	6280
cis-3-ACHSA		3,68	5,87	5,87	0,59	0,59	**1,00**	**0,00**	524	524	2096	2096
β-Neopentylglycine		3,68	5,55	5,55	0,51	0,51	**1,00**	**0,00**	495	495	1980	1980
Homocysteic acid		3,68	8,61	8,61	1,34	1,34	**1,00**	**0,00**	1620	1620	6480	6480
Cysteic acid	L-D	3,68	7,88	8,39	1,14	1,28	**1,12**	**0,83**	3465	2369	13860	9476
Pipecoloc acid	L-D	3,68	5,39	5,72	0,47	0,55	**1,19**	**0,97**	4975	3920	19900	15680
3,4-Hydroxyproline		3,68	5,55	5,86	0,51	0,59	**1,17**	**0,63**	2339	1904	9356	7616
5-oxo-Proline		3,68	4,97	5,36	0,35	0,46	**1,30**	**0,58**	357	5854	1428	23416
allo-Ile		3,68	5,04	5,04	0,37	0,37	**1,00**	**0,00**	541	541	2164	2164
Neopentylglycine		3,68	5,25	5,45	0,43	0,48	**1,12**	**0,61**	5445	3669	21780	14676
tert-Leu		3,68	4,99	4,99	0,36	0,36	**1,00**	**0,00**	450	450	1800	1800
allo-Thr		3,68	5,24	5,24	0,42	0,42	**1,00**	**0,00**	360	360	1440	1440
N-Me-Asp		3,68	10,01	10,01	1,72	1,72	**1,00**	**0,00**	1880	1880	7520	7520
N-Me-Glu		3,68	6,77	6,77	0,84	0,84	**1,00**	**0,00**	517	517	2068	2068
trans-2-Me-2-ACHSA		3,68	5,52	5,95	0,50	0,62	**1,24**	**1,44**	5993	5733	23972	22932
erythro-beta		3,68	5,66	6,04	0,54	0,64	**1,19**	**0,51**	540	2176	2160	8704
threo-beta		3,68	5,70	5,70	0,55	0,55	**1,00**	**0,00**	2240	2240	8960	8960
trans-2-amino		3,68	6,91	7,62	0,88	1,07	**1,22**	**0,54**	256	1222	1024	4888
2-tert-Bu-Tau		3,68	4,97	4,97	0,35	0,35	**1,00**	**0,00**	179	179	716	716
2-Et-Tau	S-R	3,68	5,47	5,98	0,49	0,63	**1,29**	**0,52**	167	8122	668	32488
2-Me-Tau		3,68	5,61	5,61	0,52	0,52	**1,00**	**0,00**	156	156	624	624
Pro-Me-SO_3H	S-R	3,68	8,75	9,37	1,38	1,55	**1,12**	**0,57**	583	2440	2332	9760
2-isopropyl-Tau		3,68	5,28	5,28	0,43	0,43	**1,00**	**0,00**	157	157	628	628
2-phenyl-Tau		3,68	5,28	5,28	0,43	0,43	**1,00**	**0,00**	158	158	632	632
AVERAGE VALUES							**1,08**	**0,26**				

Table iv: *aliphatic amino acids on dimethyl-Tau-QN (CSP 1), 100 % MeOH, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3.68	7.41	7.41	1.01	1.01	**1.00**	**0.00**	365	365	1460	1460
Val		3.68	7.04	7.04	0.91	0.91	**1.00**	**0.00**	1114	1114	4456	4456
Leu	L-D	3.68	7.06	7.43	0.92	1.02	**1.11**	**0.55**	2542	1352	10168	5408
Ser		3.68	8.77	8.77	1.38	1.38	**1.00**	**0.00**	208	208	832	832
Thr		3.68	7.84	7.84	1.13	1.13	**1.00**	**0.00**	396	396	1584	1584
Ile		3.68	6.81	6.81	0.85	0.85	**1.00**	**0.00**	778	778	3112	3112
Met		3.68	7.97	7.97	1.16	1.16	**1.00**	**0.00**	955	955	3820	3820
Lys		3.68	27.68	27.68	6.52	6.52	**1.00**	**0.00**	21	21	84	84
Pro		3.68	7.00	7.00	0.90	0.90	**1.00**	**0.00**	844	844	3376	3376
Asn		3.68	8.70	8.70	1.36	1.36	**1.00**	**0.00**	111	111	444	444
Gln		3.68	7.47	7.47	1.03	1.03	**1.00**	**0.00**	161	161	644	644
Asp		3.68	15.40	15.40	3.18	3.18	**1.00**	**0.00**	153	153	612	612
Glu		3.68	11.57	11.57	2.14	2.14	**1.00**	**0.00**	309	309	1236	1236
Arg		3.68	42.71	42.71	10.61	10.61	**1.00**	**0.00**	33	33	132	132
His		3.68	n.d.									
trans-4-OH-Pro	L-D	3.68	6.94	8.93	0.89	1.43	**1.61**	**1.11**	410	267	1640	1068
cis-4-OH-Pro		3.68	9.04	9.04	1.46	1.46	**1.00**	**0.00**	207	207	828	828
Nor-Leu	L-D	3.68	7.14	7.33	0.94	0.99	**1.06**	**0.32**	6333	1176	25332	4704
Nor-Val	L-D	3.68	7.17	7.34	0.95	1.00	**1.05**	**0.33**	11050	1454	44200	5816
Nipecotic acid		3.68	9.41	9.41	1.56	1.56	**1.00**	**0.00**	376	376	1504	1504
β-Leu		3.68	7.47	8.08	1.03	1.20	**1.16**	**1.28**	4529	4018	18116	16072
α-Me-Leu		3.68	6.65	6.65	0.81	0.81	**1.00**	**0.00**	1856	1856	7424	7424
α-Me-Val		3.68	7.17	7.17	0.95	0.95	**1.00**	**0.00**	897	897	3588	3588
α-Me-Ser		3.68	8.36	8.36	1.27	1.27	**1.00**	**0.00**	496	496	1984	1984
N-Me-Leu	L-D	3.68	5.95	6.11	0.62	0.66	**1.07**	**0.41**	4566	3415	18264	13660
trans-2-ACHSA		3.68	6.23	6.23	0.69	0.69	**1.00**	**0.00**	230	230	920	920
cis-2-ACHSA		3.68	4.96	5.39	0.35	0.46	**1.33**	**0.37**	341	316	1364	1264
cis-3-ACHSA		3.68	6.58	6.58	0.79	0.79	**1.00**	**0.00**	10495	9896	41980	39584
β-Neopentylglycine		3.68	7.50	7.94	1.04	1.16	**1.12**	**0.40**	483	1440	1932	5760
Homocysteic acid		3.68	8.24	8.24	1.24	1.24	**1.00**	**0.00**	1184	1184	4736	4736
Cysteic acid	L-D	3.68	7.17	7.83	0.95	1.13	**1.19**	**0.59**	783	714	3132	2856
Pipecoloc acid	L-D	3.68	6.92	7.58	0.88	1.06	**1.20**	**0.99**	2029	1805	8116	7220
3,4-Hydroxyproline		3.68	6.25	6.61	0.70	0.80	**1.14**	**0.45**	2134	592	8536	2368
5-oxo-Proline		3.68	4.99	4.99	0.36	0.36	**1.00**	**0.00**	203	203	812	812
allo-Ile		3.68	6.79	6.79	0.84	0.84	**1.00**	**0.00**	1215	1215	4860	4860
Neopentylglycine	L-D	3.68	7.18	7.45	0.95	1.03	**1.08**	**0.41**	5776	919	23104	3676
tert-Leu	L-D	3.68	7.18	7.48	0.95	1.03	**1.09**	**0.32**	1032	873	4128	3492
allo-Thr		3.68	7.24	7.24	0.97	0.97	**1.00**	**0.00**	322	322	1288	1288
N-Me-Asp		3.68	12.26	12.26	2.33	2.33	**1.00**	**0.00**	390	390	1560	1560
N-Me-Glu		3.68	8.59	8.59	1.33	1.33	**1.00**	**0.00**	352	352	1408	1408
trans-2-Me-2-ACHSA		3.68	5.62	6.48	0.53	0.76	**1.44**	**2.57**	6171	4593	24684	18372
erythro-beta		3.68	8.28	8.81	1.25	1.39	**1.12**	**0.51**	1449	854	5796	3416
threo-beta		3.68	7.78	7.78	1.11	1.11	**1.00**	**0.00**	1123	1123	4492	4492
trans-2-amino		3.68	10.17	11.76	1.76	2.19	**1.25**	**1.01**	957	669	3828	2676
2-tert-Bu-Tau		3.68	4.96	4.96	0.35	0.35	**1.00**	**0.00**	151	151	604	604
2-Et-Tau	R-S	3.68	5.18	5.63	0.41	0.53	**1.30**	**0.47**	1271	281	5084	1124
2-Me-Tau	R-S	3.68	5.42	5.86	0.47	0.59	**1.25**	**0.46**	1396	301	5584	1204
Pro-Me-SO_3H	S-R	3.68	8.61	9.00	1.34	1.45	**1.08**	**0.34**	452	2872	1808	11488
2-isopropyl-Tau		3.68	4.96	5.35	0.35	0.45	**1.30**	**0.44**	669	494	2676	1976
2-phenyl-Tau		3.68	5.18	5.66	0.41	0.54	**1.33**	**0.50**	366	706	1464	2824
AVERAGE VALUES							1.09	0.28				

Table v: *aliphatic amino acids on dimethyl-Tau-QN (CSP 1), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		3.68	5.90	5.90	0.60	0.60	**1.00**	**0.00**	5237	5237	20948	20948
Trp	D-L	3.68	7.32	10.27	0.99	1.79	**1.81**	**7.19**	5988	8754	23952	35016
Tyr		3.68	6.22	6.22	0.69	0.69	**1.00**	**0.00**	1494	1494	5976	5976
p-F-Phe		3.68	5.89	5.89	0.60	0.60	**1.00**	**0.00**	4739	4739	18956	18956
pCl-Phe		3.68	6.38	6.38	0.73	0.73	**1.00**	**0.00**	4266	4266	17064	17064
p-Br-Phe		3.68	6.66	6.66	0.81	0.81	**1.00**	**0.00**	5503	5503	22012	22012
oF-Phe		3.68	5.81	5.81	0.58	0.58	**1.00**	**0.00**	692	692	2768	2768
Baclofen		3.68	8.89	9.84	1.42	1.67	**1.18**	**1.23**	2288	2392	9152	9568
DOPA		3.68	6.79	6.79	0.85	0.85	**1.00**	**0.00**	3085	3085	12340	12340
m Tyr		3.68	6.36	6.36	0.73	0.73	**1.00**	**0.00**	1250	1250	5000	5000
5-HTP	D-L	3.68	7.32	10.52	0.99	1.86	**1.88**	**7.98**	6645	9115	26580	36460
β-Phe		3.68	8.74	9.51	1.38	1.58	**1.15**	**2.03**	10412	8753	41648	35012
β-homo-Phe		3.68	9.99	9.99	1.72	1.72	**1.00**	**0.00**	4396	4396	17584	17584
α-Me-Phe		3.68	5.32	5.32	0.44	0.44	**1.00**	**0.00**	375	375	1500	1500
α-Me-Tyr	D-L	3.68	5.52	5.88	0.50	0.60	**1.20**	**0.56**	862	2018	3448	8072
α-Me-m Tyr		3.68	5.55	6.43	0.51	0.75	**1.47**	**4.01**	11749	12197	46996	48788
α-Me-DOPA	D-L	3.68	5.92	7.04	0.61	0.91	**1.50**	**2.87**	3801	5049	15204	20196
α-Me-Trp		3.68	6.70	14.99	0.82	3.07	**3.74**	**17.13**	6257	9143	25028	36572
1- Me-Trp	D-L	3.68	7.50	9.12	1.04	1.48	**1.42**	**4.57**	8093	9496	32372	37984
Phenylglycine		3.68	6.25	6.25	0.70	0.70	**1.00**	**0.00**	7946	7946	31784	31784
p-Amino-Phe		3.68	6.17	6.17	0.68	0.68	**1.00**	**0.00**	868	868	3472	3472
OMe-Tyr		3.68	6.32	6.32	0.72	0.72	**1.00**	**0.00**	1237	1237	4948	4948
Kynurenine	L-D	3.68	7.63	7.85	1.07	1.13	**1.06**	**0.52**	8880	3439	35520	13756
1-Naphtylalanine		3.68	7.05	7.05	0.92	0.92	**1.00**	**0.00**	3719	3719	14876	14876
Tic	S-R	3.68	6.18	6.72	0.68	0.83	**1.21**	**0.91**	1121	3634	4484	14536
AVERAGE VALUES							**1.26**	**1.96**				

Table vi: *aromatic amino acids on 2-(S)-phenyl-Tau-QN (CSP 2), 100 % MeOH, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	7.60	7.80	1.06	1.12	**1.05**	**0.55**	16099	4073	64396	16292
Trp	D-L	3.68	9.72	12.42	1.64	2.37	**1.45**	**3.64**	3195	3950	12780	15800
Tyr		3.68	8.39	8.39	1.28	1.28	**1.00**	**0.00**	364	364	1456	1456
p-F-Phe		3.68	7.72	7.72	1.10	1.10	**1.00**	**0.00**	1496	1496	5984	5984
pCl-Phe		3.68	8.62	8.62	1.34	1.34	**1.00**	**0.00**	1175	1175	4700	4700
p-Br-Phe		3.68	8.89	9.16	1.41	1.49	**1.05**	**0.56**	8144	3744	32576	14976
oF-Phe		3.68	7.43	7.43	1.02	1.02	**1.00**	**0.00**	599	599	2396	2396
Baclofen		3.68	14.35	16.75	2.90	3.55	**1.23**	**2.49**	4063	4215	16252	16860
DOPA		3.68	9.49	9.49	1.58	1.58	**1.00**	**0.00**	1062	1062	4248	4248
m Tyr		3.68	8.62	8.62	1.34	1.34	**1.00**	**0.00**	853	853	3412	3412
5-HTP	D-L	3.68	9.41	12.20	1.56	2.32	**1.49**	**4.47**	4137	5387	16548	21548
β-Phe		3.68	12.60	15.21	2.42	3.13	**1.29**	**5.05**	12286	11089	49144	44356
β-homo-Phe		3.68	13.65	14.83	2.71	3.03	**1.12**	**2.26**	12656	11160	50624	44640
α-Me-Phe		3.68	6.80	6.80	0.85	0.85	**1.00**	**0.00**	969	969	3876	3876
α-Me-Tyr		3.68	7.71	7.71	1.09	1.09	**1.00**	**0.00**	1072	1072	4288	4288
α-Me-m Tyr		3.68	7.19	8.25	0.95	1.24	**1.30**	**3.42**	10395	9526	41580	38104
α-Me-DOPA	D-L	3.68	8.08	9.13	1.20	1.48	**1.24**	**0.95**	1211	824	4844	3296
α-Me-Trp		3.68	8.85	15.96	1.41	3.34	**2.37**	**10.57**	3702	7037	14808	28148
1- Me-Trp	D-L	3.68	9.13	10.24	1.48	1.78	**1.20**	**1.96**	4423	4890	17692	19560
Phenylglycine		3.68	8.15	8.15	1.21	1.21	**1.00**	**0.00**	5547	5547	22188	22188
p-Amino-Phe		3.68	7.69	7.69	1.09	1.09	**1.00**	**0.00**	483	483	1932	1932
OMe-Tyr		3.68	7.90	7.90	1.15	1.15	**1.00**	**0.00**	697	697	2788	2788
Kynurenine		3.68	9.70	9.70	1.63	1.63	**1.00**	**0.00**	961	961	3844	3844
1-Naphtylalanine		3.68	9.12	9.12	1.48	1.48	**1.00**	**0.00**	2106	2106	8424	8424
Tic	S-R	3.68	7.44	8.16	1.02	1.22	**1.19**	**0.64**	775	776	3100	3104
AVERAGE VALUES							**1.16**	**1.46**				

Table vii: *aromatic amino acids on 2-(S)-phenyl-Tau-QN (CSP 2), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3.83	5.33	5.49	0.39	0.43	**1.10**	**0.59**	8056	5884	32224	23536
Val	L-D	3.83	5.16	5.35	0.35	0.40	**1.15**	**0.79**	8060	6503	32240	26012
Leu	L-D	3.83	5.31	5.54	0.39	0.45	**1.15**	**0.73**	5549	4252	22196	17008
Ser		3.83	6.13	6.13	0.60	0.60	**1.00**	**0.00**	387	387	1548	1548
Thr	L-D	3.83	5.69	6.02	0.49	0.57	**1.18**	**1.30**	9482	7997	37928	31988
Ile	L-D	3.83	5.21	5.43	0.36	0.42	**1.16**	**0.83**	7719	5651	30876	22604
Met	L-D	3.83	5.96	6.08	0.56	0.59	**1.06**	**0.42**	10928	4725	43712	18900
Lys	L-D	3.83	11.64	12.58	2.04	2.29	**1.12**	**0.54**	956	662	3824	2648
Pro	L-D	3.83	5.92	6.60	0.55	0.72	**1.32**	**2.14**	6503	6038	26012	24152
Asn		3.83	6.30	6.68	0.65	0.75	**1.15**	**0.44**	634	1317	2536	5268
Gln		3.83	5.79	5.79	0.51	0.51	**1.00**	**0.00**	2532	2532	10128	10128
Asp	D-L	3.83	12.26	12.57	2.20	2.28	**1.04**	**0.31**	10145	1102	40580	4408
Glu		3.83	8.49	8.49	1.22	1.22	**1.00**	**0.00**	1208	1208	4832	4832
Arg		3.83	14.55	15.22	2.80	2.98	**1.06**	**0.30**	1267	424	5068	1696
His		3.83	10.32	10.32	1.70	1.70	**1.00**	**0.00**	23	23	92	92
trans-4-OH-Pro	L-D	3.83	5.78	9.38	0.51	1.45	**2.84**	**2.62**	330	649	1320	2596
cis-4-OH-Pro		3.83	9.38	10.03	1.45	1.62	**1.12**	**0.51**	446	2878	1784	11512
Nor-Leu	L-D	3.83	5.23	5.49	0.37	0.43	**1.18**	**0.87**	5960	5073	23840	20292
Nor-Val	L-D	3.83	5.20	5.43	0.36	0.42	**1.17**	**0.60**	3748	2582	14992	10328
Nipecotic acid	R-S	3.83	7.50	9.96	0.96	1.60	**1.67**	**3.76**	2545	3164	10180	12656
β-Leu		3.83	7.51	7.82	0.96	1.04	**1.09**	**0.84**	7915	5805	31660	23220
α-Me-Leu		3.83	4.69	4.69	0.22	0.22	**1.00**	**0.00**	4102	4102	16408	16408
α-Me-Val		3.83	4.90	4.90	0.28	0.28	**1.00**	**0.00**	1360	1360	5440	5440
α-Me-Ser		3.83	5.63	5.63	0.47	0.47	**1.00**	**0.00**	779	779	3116	3116
N-Me-Leu		3.83	4.92	4.92	0.29	0.29	**1.00**	**0.00**	999	999	3996	3996
trans-2-ACHSA		3.83	7.47	7.92	0.95	1.07	**1.12**	**0.50**	805	1849	3220	7396
cis-2-ACHSA		3.83	6.06	6.06	0.58	0.58	**1.00**	**0.00**	331	331	1324	1324
cis-3-ACHSA		3.83	7.24	7.24	0.89	0.89	**1.00**	**0.00**	725	725	2900	2900
β-Neopentylglycine		3.83	7.01	8.03	0.83	1.10	**1.32**	**2.32**	4454	4918	17816	19672
Homocysteic acid		3.83	12.28	12.28	2.21	2.21	**1.00**	**0.00**	1706	1706	6824	6824
Cysteic acid		3.83	11.19	11.19	1.92	1.92	**1.00**	**0.00**	1729	1729	6916	6916
Pipecoloc acid	L-D	3.83	5.46	5.64	0.43	0.47	**1.11**	**0.56**	5940	4142	23760	16568
3,4-Hydroxyproline		3.83	5.74	6.67	0.50	0.74	**1.49**	**2.58**	4829	4640	19316	18560
5-oxo-Proline		3.83	5.27	5.45	0.38	0.42	**1.12**	**0.72**	8193	6847	32772	27388
allo-Ile	L-D	3.83	5.19	5.43	0.36	0.42	**1.17**	**0.85**	6828	5327	27312	21308
Neopentylglycine	L-D	3.83	5.52	6.02	0.44	0.57	**1.29**	**1.79**	7179	6705	28716	26820
tert-Leu	L-D	3.83	5.10	5.43	0.33	0.42	**1.26**	**1.34**	7687	6804	30748	27216
allo-Thr		3.83	5.53	5.53	0.44	0.44	**1.00**	**0.00**	234	234	936	936
N-Me-Asp	L-D	3.83	11.74	12.05	2.07	2.15	**1.04**	**0.35**	6266	1619	25064	6476
N-Me-Glu		3.83	7.23	7.23	0.89	0.89	**1.00**	**0.00**	3950	3950	15800	15800
trans-2-Me-2-ACHSA		3.83	6.17	7.02	0.61	0.83	**1.36**	**2.85**	8192	7641	32768	30564
erythro-beta		3.83	5.85	6.28	0.53	0.64	**1.21**	**1.43**	1020	4774	4080	19096
threo-beta		3.83	5.68	5.97	0.48	0.56	**1.15**	**0.49**	1751	1475	7004	5900
trans-2-amino		3.83	9.10	9.10	1.38	1.38	**1.00**	**0.00**	508	508	2032	2032
2-tert-Bu-Tau	R-S	3.83	5.78	8.17	0.51	1.13	**2.22**	**2.17**	365	1102	1460	4408
2-Et-Tau	R-S	3.83	6.66	8.22	0.74	1.15	**1.55**	**1.45**	754	773	3016	3092
2-Me-Tau	R-S	3.83	7.12	7.74	0.86	1.02	**1.19**	**0.53**	577	711	2308	2844
Pro-Me-SO_3H	S-R	3.83	12.52	20.09	2.27	4.25	**1.87**	**4.10**	946	1532	3784	6128
2-isopropyl-Tau	D-L	3.83	6.38	7.83	0.67	1.04	**1.57**	**1.40**	608	904	2432	3616
2-phenyl-Tau	D-L	3.83	6.61	7.56	0.73	0.97	**1.34**	**1.11**	916	1331	3664	5324
AVERAGE VALUES							1.22	0.88				

Table viii: *aliphatic amino acids on 2-(S)-phenyl-Tau-QN (CSP 2), 100 % MeOH, 50mM FA & 25mM DEA*

	Analyt	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
D,L	Ala	L-D	3.83	7.18	7.43	0.88	0.94	**1.07**	**0.50**	7227	2032	28908	8128
D,L	Val	L-D	3.83	6.94	7.45	0.81	0.95	**1.16**	**1.04**	4003	3067	16012	12268
D,L	Leu	L-D	3.83	7.15	7.60	0.87	0.98	**1.13**	**0.89**	3922	3028	15688	12112
D,L	Ser	D-L	3.83	8.22	8.71	1.15	1.28	**1.11**	**0.46**	1089	925	4356	3700
D,L	Thr	L-D	3.83	7.95	8.56	1.08	1.24	**1.15**	**0.59**	1480	755	5920	3020
D,L	Ile	L-D	3.83	6.97	7.52	0.82	0.96	**1.17**	**1.13**	4383	3121	17532	12484
D,L	Met	L-D	3.83	8.05	8.31	1.10	1.17	**1.06**	**0.43**	5935	1820	23740	7280
D,L	Lys		3.83	n.d.									
D,L	Pro	L-D	3.83	6.44	7.74	0.68	1.02	**1.50**	**3.09**	4713	4518	18852	18072
D,L	Asn		3.83	8.05	8.52	1.10	1.22	**1.11**	**0.29**	617	296	2468	1184
D,L	Gln	L-D	3.83	7.32	8.18	0.91	1.14	**1.25**	**0.65**	651	470	2604	1880
D,L	Asp		3.83	18.20	18.20	3.76	3.76	**1.00**	**0.00**	475	475	1900	1900
D,L	Glu		3.83	13.53	13.53	2.53	2.53	**1.00**	**0.00**	745	745	2980	2980
D,L	Arg		3.83	41.14	44.63	9.75	10.66	**1.09**	**0.27**	911	80	3644	320
D,L	His		3.83	n.d.									
DL	trans-4-OH-Pro	L-D	3.83	6.42	11.44	0.68	1.99	**2.94**	**2.57**	388	312	1552	1248
DL	cis-4-OH-Pro		3.83	11.49	11.49	2.00	2.00	**1.00**	**0.00**	265	265	1060	1060
DL	Nor-Leu	L-D	3.83	6.95	7.55	0.82	0.97	**1.19**	**1.13**	3333	2706	13332	10824
DL	Nor-Val	L-D	3.83	6.98	7.45	0.82	0.95	**1.15**	**0.85**	3175	2397	12700	9588
SR	Nipecotic acid	R-S	3.83	9.28	14.01	1.43	2.66	**1.87**	**4.97**	2193	2573	8772	10292
DL	β-Leu		3.83	10.20	11.69	1.66	2.05	**1.23**	**2.60**	6404	5387	25616	21548
DL	α-Me-Leu		3.83	6.12	6.31	0.60	0.65	**1.08**	**0.54**	7196	3725	28784	14900
DL	α-Me-Val		3.83	6.49	6.49	0.69	0.69	**1.00**	**0.00**	2519	2519	10076	10076
DL	α-Me-Ser		3.83	7.72	8.21	1.02	1.15	**1.13**	**0.58**	1678	1255	6712	5020
DL	N-Me-Leu	D-L	3.83	5.61	5.78	0.47	0.51	**1.10**	**0.44**	3278	3229	13112	12916
	trans-2-ACHSA		3.83	7.45	7.45	0.95	0.95	**1.00**	**0.00**	413	1773	1652	7092
	cis-2-ACHSA		3.83	6.18	6.18	0.62	0.62	**1.00**	**0.00**	377	377	1508	1508
	cis-3-ACHSA		3.83	8.62	8.62	1.25	1.25	**1.00**	**0.00**	365	3320	1460	13280
DL	β-Neopentylglycine		3.83	9.14	11.54	1.39	2.02	**1.45**	**3.55**	3689	3783	14756	15132
DL	Homocysteic acid		3.83	11.23	11.23	1.93	1.93	**1.00**	**0.00**	1392	1392	5568	5568
DL	Cysteic acid		3.83	9.78	9.78	1.56	1.56	**1.00**	**0.00**	1033	1033	4132	4132
DL	Pipecoloc acid	L-D	3.83	6.66	6.94	0.74	0.81	**1.10**	**0.63**	4262	3104	17048	12416
DL	3,4-Hydroxyproline		3.83	6.07	7.56	0.59	0.98	**1.67**	**2.26**	1749	1686	6996	6744
DL	5-oxo-Proline		3.83	5.18	5.33	0.35	0.39	**1.11**	**0.62**	8514	6837	34056	27348
DL	allo-Ile	L-D	3.83	7.01	7.54	0.83	0.97	**1.17**	**0.99**	3599	2455	14396	9820
DL	Neopentylglycine	L-D	3.83	7.40	8.37	0.93	1.19	**1.27**	**1.78**	3776	3023	15104	12092
DL	tert-Leu	L-D	3.83	7.01	7.97	0.83	1.08	**1.30**	**2.13**	4888	4138	19552	16552
DL	allo-Thr		3.83	7.31	7.31	0.91	0.91	**1.00**	**0.00**	386	386	1544	1544
DL	N-Me-Asp	L-D	3.83	12.88	13.74	2.36	2.59	**1.10**	**0.55**	2174	737	8696	2948
DL	N-Me-Glu		3.83	8.67	8.67	1.26	1.26	**1.00**	**0.00**	2215	2215	8860	8860
RS	trans-2-Me-2-ACHSA		3.83	6.41	7.16	0.67	0.87	**1.29**	**2.42**	7793	7560	31172	30240
RS	erythro-beta		3.83	8.30	9.28	1.17	1.43	**1.22**	**1.24**	2655	1578	10620	6312
RS	threo-beta		3.83	7.73	8.34	1.02	1.18	**1.16**	**0.81**	2355	1491	9420	5964
RS	trans-2-amino		3.83	13.67	15.68	2.57	3.09	**1.20**	**0.77**	504	521	2016	2084
RS	2-tert-Bu-Tau	S-R	3.83	5.30	8.12	0.38	1.12	**2.92**	**2.06**	283	486	1132	1944
RS	2-Et-Tau	S-R	3.83	6.59	8.40	0.72	1.19	**1.66**	**1.21**	404	398	1616	1592
RS	2-Me-Tau	S-R	3.83	7.30	7.86	0.91	1.05	**1.16**	**0.36**	358	420	1432	1680
RS	Pro-Me-SO_3H	R-S	3.83	11.63	19.08	2.04	3.98	**1.96**	**3.06**	583	671	2332	2684
RS	2-isopropyl-Tau	D-L	3.83	6.15	8.06	0.61	1.11	**1.82**	**1.34**	357	436	1428	1744
RS	2-phenyl-Tau	D-L	3.83	6.79	7.76	0.77	1.03	**1.33**	**0.80**	486	681	1944	2724
	AVERAGE VALUES							**1.28**	**1.03**				

Table ix: *aliphatic amino acids on 2-(S)-phenyl-Tau-QN (CSP 2), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	D-L	3.82	6.87	7.91	0.80	1.07	**1.34**	**3.49**	10460	9422	41840	37688
Trp	L-D	3.82	9.34	10.80	1.45	1.83	**1.26**	**2.92**	5451	7598	21804	30392
Tyr	D-L	3.82	7.10	8.02	0.86	1.10	**1.28**	**2.12**	4631	4992	18524	19968
p-F-Phe	D-L	3.82	7.03	8.10	0.84	1.12	**1.33**	**1.89**	2542	3183	10168	12732
pCl-Phe	D-L	3.82	7.82	9.39	1.05	1.46	**1.39**	**3.33**	5011	5563	20044	22252
p-Br-Phe		3.82	8.25	10.10	1.16	1.64	**1.42**	**4.39**	7845	7487	31380	29948
oF-Phe		3.82	6.80	7.84	0.78	1.05	**1.35**	**3.57**	10687	9583	42748	38332
Baclofen		3.82	14.20	17.56	2.72	3.60	**1.32**	**2.28**	1825	1882	7300	7528
DOPA		3.82	9.00	9.00	1.36	1.36	**1.00**	**0.00**	1296	1296	5184	5184
m Tyr		3.82	7.68	8.34	1.01	1.18	**1.17**	**0.71**	977	1480	3908	5920
5-HTP	L-D	3.82	10.61	11.58	1.78	2.03	**1.14**	**1.52**	4078	5727	16312	22908
β-Phe		3.82	12.84	15.22	2.36	2.99	**1.26**	**2.12**	2777	2322	11108	9288
β-homo-Phe		3.82	15.96	15.96	3.18	3.18	**1.00**	**0.00**	4365	4365	17460	17460
α-Me-Phe	L-D	3.82	5.69	5.85	0.49	0.53	**1.08**	**0.64**	8944	8523	35776	34092
α-Me-Tyr		3.82	5.85	6.10	0.53	0.60	**1.12**	**0.46**	974	5361	3896	21444
α-Me-m Tyr		3.82	5.94	7.28	0.56	0.91	**1.63**	**5.57**	11759	12543	47036	50172
α-Me-DOPA	L-D	3.82	6.32	9.10	0.66	1.38	**2.11**	**3.09**	610	2245	2440	8980
α-Me-Trp		3.82	6.98	13.41	0.83	2.51	**3.04**	**11.08**	2031	9823	8124	39292
1- Me-Trp	L-D	3.82	10.26	11.14	1.69	1.92	**1.14**	**1.75**	6601	8322	26404	33288
Phenylglycine	D-L	3.82	7.14	7.89	0.87	1.07	**1.22**	**1.57**	3559	4648	14236	18592
p-Amino-Phe		3.82	6.97	7.95	0.82	1.08	**1.31**	**3.35**	10572	10129	42288	40516
OMe-Tyr	D-L	3.82	7.34	8.70	0.92	1.28	**1.39**	**3.89**	8107	8776	32428	35104
Kynurenine	D-L	3.82	9.11	10.51	1.39	1.75	**1.26**	**3.34**	9152	8648	36608	34592
1-Naphtylalanine	D-L	3.82	8.39	10.82	1.20	1.83	**1.53**	**5.31**	6602	7384	26408	29536
Tic	R-S	3.82	7.31	8.49	0.91	1.22	**1.34**	**4.07**	12590	11224	50360	44896
AVERAGE VALUES							1.38	2.90				

Table x: *aromatic amino acids on 2-(R)-isopropyl-Tau-QD (CSP 3), 100 % MeOH, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	D-L	3.82	12.24	15.06	2.21	2.94	**1.34**	**4.32**	7825	6425	31300	25700
Trp	L-D	3.82	16.30	16.98	3.27	3.45	**1.05**	**0.59**	4108	2824	16432	11296
Tyr	D-L	3.82	13.06	15.79	2.42	3.14	**1.30**	**2.32**	2443	2580	9772	10320
p-F-Phe	D-L	3.82	12.89	16.45	2.38	3.31	**1.39**	**2.38**	1522	1570	6088	6280
pCl-Phe	D-L	3.82	15.05	20.18	2.94	4.29	**1.46**	**3.62**	2300	2620	9200	10480
p-Br-Phe		3.82	16.10	21.96	3.22	4.75	**1.48**	**5.14**	4220	4649	16880	18596
oF-Phe		3.82	12.14	15.25	2.18	2.99	**1.37**	**5.12**	9595	7172	38380	28688
Baclofen		3.82	38.02	52.33	8.96	12.71	**1.42**	**3.88**	2120	2644	8480	10576
DOPA		3.82	17.88	17.88	3.68	3.68	**1.00**	**0.00**	609	609	2436	2436
m Tyr		3.82	14.96	16.49	2.92	3.32	**1.14**	**1.32**	3154	2797	12616	11188
5-HTP		3.82	17.80	17.80	3.66	3.66	**1.00**	**0.00**	1218	1218	4872	4872
β-Phe		3.82	26.30	38.96	5.89	9.20	**1.56**	**4.32**	1891	2068	7564	8272
β-homo-Phe		3.82	30.72	35.62	7.05	8.33	**1.18**	**3.20**	8238	7012	32952	28048
α-Me-Phe		3.82	9.32	9.64	1.44	1.52	**1.06**	**0.68**	7113	5830	28452	23320
α-Me-Tyr		3.82	9.98	9.98	1.61	1.61	**1.00**	**0.00**	1082	1082	4328	4328
α-Me-m Tyr		3.82	9.74	12.68	1.55	2.32	**1.50**	**7.39**	12580	12932	50320	51728
α-Me-DOPA	L-D	3.82	10.85	16.85	1.84	3.41	**1.85**	**5.10**	1494	3040	5976	12160
α-Me-Trp		3.82	11.24	18.02	1.94	3.72	**1.91**	**6.96**	2321	5061	9284	20244
1- Me-Trp	D-L	3.82	16.88	17.61	3.42	3.61	**1.06**	**0.55**	3746	2024	14984	8096
Phenylglycine	D-L	3.82	14.13	15.22	2.70	2.99	**1.11**	**1.39**	5302	6018	21208	24072
p-Amino-Phe		3.82	11.53	13.95	2.02	2.65	**1.31**	**4.47**	9274	8627	37096	34508
OMe-Tyr	D-L	3.82	12.89	16.54	2.38	3.33	**1.40**	**4.99**	6243	6684	24972	26736
Kynurenine	D-L	3.82	15.76	19.15	3.13	4.02	**1.28**	**4.13**	7604	6984	30416	27936
1-Naphtylalanine	D-L	3.82	15.30	21.74	3.01	4.69	**1.56**	**4.65**	2763	2945	11052	11780
Tic	R-S	3.82	11.24	13.59	1.94	2.56	**1.32**	**4.92**	11876	10140	47504	40560
AVERAGE VALUES							1.32	3.26				

Table xi: *aromatic amino acids on 2-(R)-isopropyl-Tau-QD (CSP 3), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3.80	6.46	7.03	0.70	0.85	**1.22**	**1.55**	5771	5024	23084	20096
Val	D-L	3.80	6.07	6.85	0.60	0.80	**1.35**	**2.13**	5316	4560	21264	18240
Leu	D-L	3.80	6.61	7.57	0.74	0.99	**1.34**	**3.12**	8702	8166	34808	32664
Ser		3.80	7.78	7.78	1.05	1.05	**1.00**	**0.00**	931	931	3724	3724
Thr		3.80	6.72	7.47	0.77	0.97	**1.26**	**0.80**	947	921	3788	3684
Ile		3.80	6.26	7.07	0.65	0.86	**1.33**	**1.92**	5133	3253	20532	13012
Met	D-L	3.80	7.57	8.48	0.99	1.23	**1.24**	**1.55**	3165	2774	12660	11096
Lys		3.80	18.12	21.47	3.77	4.65	**1.23**	**1.98**	2204	2209	8816	8836
Pro	D-L	3.80	7.38	10.23	0.94	1.69	**1.80**	**6.72**	6942	6819	27768	27276
Asn	D-L	3.80	8.36	9.04	1.20	1.38	**1.15**	**1.27**	4471	4179	17884	16716
Gln	D-L	3.80	7.06	7.91	0.86	1.08	**1.26**	**0.69**	577	599	2308	2396
Asp		3.80	13.43	13.43	2.53	2.53	**1.00**	**0.00**	756	756	3024	3024
Glu		3.80	9.03	9.03	1.38	1.38	**1.00**	**0.00**	625	625	2500	2500
Arg		3.80	21.58	21.58	4.68	4.68	**1.00**	**0.00**	721	721	2884	2884
His		3.80	9.92	12.21	1.61	2.21	**1.37**	**2.24**	1857	1905	7428	7620
trans-4-OH-Pro	L-D	3.80	8.50	14.92	1.24	2.93	**2.37**	**2.72**	302	467	1208	1868
cis-4-OH-Pro		3.80	14.94	14.94	2.93	2.93	**1.00**	**0.00**	292	292	1168	1168
Nor-Leu	D-L	3.80	6.23	7.16	0.64	0.88	**1.38**	**2.27**	4248	4346	16992	17384
Nor-Val	D-L	3.80	6.20	7.01	0.63	0.84	**1.33**	**1.76**	3412	3324	13648	13296
Nipecotic acid	S-R	3.80	8.48	15.58	1.23	3.10	**2.52**	**9.02**	4682	3352	18728	13408
β-Leu		3.80	11.62	12.49	2.06	2.29	**1.11**	**1.35**	6103	5180	24412	20720
α-Me-Leu		3.80	4.98	4.98	0.31	0.31	**1.00**	**0.00**	777	777	3108	3108
α-Me-Val		3.80	5.33	5.33	0.40	0.40	**1.00**	**0.00**	2082	2082	8328	8328
α-Me-Ser		3.80	6.40	7.06	0.68	0.86	**1.25**	**1.30**	2745	2956	10980	11824
N-Me-Leu	L-D	3.80	5.05	5.29	0.33	0.39	**1.19**	**0.55**	2236	2284	8944	9136
trans-2-ACHSA		3.80	11.48	11.48	2.02	2.02	**1.00**	**0.00**	544	544	2176	2176
cis-2-ACHSA		3.80	8.02	8.93	1.11	1.35	**1.22**	**0.56**	369	490	1476	1960
cis-3-ACHSA		3.80	7.67	7.67	1.02	1.02	**1.00**	**0.00**	102	102	408	408
β-Neopentylglycine		3.80	10.96	13.51	1.88	2.55	**1.36**	**3.40**	4071	4403	16284	17612
Homocysteic acid	D-L	3.80	10.48	11.68	1.76	2.07	**1.18**	**1.21**	2278	1810	9112	7240
Cysteic acid		3.80	12.16	12.16	2.20	2.20	**1.00**	**0.00**	1306	1306	5224	5224
Pipecoloc acid	D-L	3.80	6.28	7.05	0.65	0.86	**1.31**	**1.88**	4398	4128	17592	16512
3,4-Hydroxyproline		3.80	7.25	9.75	0.91	1.57	**1.72**	**5.38**	5354	5397	21416	21588
5-oxo-Proline		3.80	4.51	4.63	0.19	0.22	**1.18**	**0.56**	7090	6678	28360	26712
allo-Ile	D-L	3.80	6.22	7.10	0.64	0.87	**1.36**	**2.08**	4236	3769	16944	15076
Neopentylglycine	D-L	3.80	7.11	8.58	0.87	1.26	**1.44**	**3.85**	6457	7143	25828	28572
tert-Leu	D-L	3.80	6.16	7.50	0.62	0.97	**1.57**	**4.11**	6864	7183	27456	28732
allo-Thr	L-D	3.80	6.32	6.87	0.66	0.81	**1.22**	**0.86**	1669	1669	6676	6676
N-Me-Asp	D-L	3.80	10.59	11.63	1.79	2.06	**1.15**	**1.16**	3186	2052	12744	8208
N-Me-Glu	L-D	3.80	6.35	6.59	0.67	0.73	**1.09**	**0.58**	4254	3932	17016	15728
trans-2-Me-2-ACHSA		3.80	8.32	9.75	1.19	1.57	**1.32**	**2.01**	2298	2298	9192	9192
erythro-beta		3.80	7.51	9.31	0.98	1.45	**1.49**	**5.13**	9290	9040	37160	36160
threo-beta		3.80	7.71	8.86	1.03	1.33	**1.30**	**1.53**	1686	2229	6744	8916
trans-2-amino		3.80	14.39	15.28	2.79	3.02	**1.08**	**0.83**	3372	2838	13488	11352
2-tert-Bu-Tau	S-R	3.80	7.84	12.98	1.06	2.41	**2.27**	**2.93**	358	795	1432	3180
2-Et-Tau	S-R	3.80	10.23	12.99	1.69	2.42	**1.43**	**1.25**	439	455	1756	1820
2-Me-Tau		3.80	11.22	11.22	1.95	1.95	**1.00**	**0.00**	313	313	1252	1252
Pro-Me-SO_3H	S-R	3.80	8.78	9.40	1.31	1.47	**1.12**	**0.82**	2304	2330	9216	9320
2-isopropyl-Tau	L-D	3.80	9.33	12.40	1.45	2.26	**1.56**	**1.60**	482	538	1928	2152
2-phenyl-Tau	L-D	3.80	9.27	10.79	1.44	1.84	**1.28**	**1.22**	1071	1008	4284	4032
AVERAGE VALUES							1.31	1.72				

Table xii: *aliphatic amino acids on 2-(R)-isopropyl-Tau-QD (CSP 3), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3,80	12,46	14,05	2,28	2,70	**1,18**	**1,79**	3879	3308	15516	13232
Val	D-L	3,80	11,64	13,78	2,06	2,63	**1,27**	**2,39**	3387	3134	13548	12536
Leu	D-L	3,80	12,96	15,80	2,41	3,16	**1,31**	**3,96**	6770	6238	27080	24952
Ser		3,80	16,78	16,78	3,42	3,42	**1,00**	**0,00**	999	999	3996	3996
Thr	D-L	3,80	12,82	15,47	2,37	3,07	**1,29**	**1,20**	659	653	2636	2612
Ile	D-L	3,80	12,44	14,48	2,27	2,81	**1,24**	**2,34**	3934	3724	15736	14896
Met	D-L	3,80	15,26	18,38	3,02	3,84	**1,27**	**1,88**	1718	1619	6872	6476
Lys		3,80	n.d.									
Pro	D-L	3,80	9,22	16,49	1,43	3,34	**2,34**	**10,15**	4955	5298	19820	21192
Asn	D-L	3,80	14,20	16,75	2,74	3,41	**1,24**	**2,41**	3585	3329	14340	13316
Gln	D-L	3,80	12,22	14,37	2,22	2,78	**1,25**	**0,78**	398	366	1592	1464
Asp		3,80	28,71	28,71	6,56	6,56	**1,00**	**0,00**	274	274	1096	1096
Glu		3,80	19,78	19,78	4,20	4,20	**1,00**	**0,00**	392	392	1568	1568
Arg		3,80	n.d.									
His		3,80	n.d.									
trans-4-OH-Pro	L-D	3,80	16,02	20,07	3,22	4,28	**1,33**	**0,83**	196	244	784	976
cis-4-OH-Pro	D-L	3,80	20,02	23,88	4,27	5,28	**1,24**	**0,71**	212	325	848	1300
Nor-Leu	D-L	3,80	12,20	14,88	2,21	2,91	**1,32**	**2,94**	3299	3818	13196	15272
Nor-Val	D-L	3,80	12,12	14,48	2,19	2,81	**1,28**	**2,13**	2198	2400	8792	9600
Nipecotic acid	S-R	3,80	14,13	34,64	2,72	8,11	**2,99**	**11,80**	3250	3125	13000	12500
β-Leu		3,80	22,98	29,35	5,05	6,72	**1,33**	**4,66**	5969	5785	23876	23140
α-Me-Leu		3,80	8,81	9,27	1,32	1,44	**1,09**	**0,53**	1664	1715	6656	6860
α-Me-Val		3,80	13,41	14,55	2,53	2,83	**1,12**	**0,90**	1985	1910	7940	7640
α-Me-Ser		3,80	9,86	9,86	1,59	1,59	**1,00**	**0,00**	1605	1605	6420	6420
N-Me-Leu	L-D	3,80	6,76	7,56	0,78	0,99	**1,27**	**1,04**	1401	1379	5604	5516
trans-2-ACHSA		3,80	13,38	13,38	2,52	2,52	**1,00**	**0,00**	234	234	936	936
cis-2-ACHSA		3,80	9,88	9,88	1,60	1,60	**1,00**	**0,00**	108	108	432	432
cis-3-ACHSA		3,80	13,01	13,01	2,42	2,42	**1,00**	**0,00**	67	67	268	268
β-Neopentylglycine		3,80	20,08	29,73	4,29	6,82	**1,59**	**6,18**	3491	4587	13964	18348
Homocysteic acid	D-L	3,80	11,75	13,78	2,09	2,63	**1,26**	**1,19**	980	844	3920	3376
Cysteic acid		3,80	13,66	13,66	2,59	2,59	**1,00**	**0,00**	574	574	2296	2296
Pipecoloc acid	D-L	3,80	9,86	12,06	1,59	2,17	**1,36**	**2,68**	2867	2834	11468	11336
3,4-Hydroxyproline		3,80	8,74	14,36	1,30	2,78	**2,14**	**7,56**	3622	4018	14488	16072
5-oxo-Proline		3,80	4,64	4,77	0,22	0,26	**1,16**	**0,66**	9619	8351	38476	33404
allo-Ile	D-L	3,80	12,40	14,42	2,26	2,79	**1,24**	**2,09**	2946	3198	11784	12792
Neopentylglycine	D-L	3,80	14,08	17,32	2,71	3,56	**1,31**	**3,83**	5045	6028	20180	24112
tert-Leu	D-L	3,80	12,22	16,66	2,22	3,39	**1,53**	**5,80**	5394	5953	21576	23812
allo-Thr	L-D	3,80	12,74	13,93	2,35	2,67	**1,13**	**0,70**	1051	916	4204	3664
N-Me-Asp	D-L	3,80	14,81	18,03	2,90	3,74	**1,29**	**1,90**	1649	1401	6596	5604
N-Me-Glu	L-D	3,80	8,40	8,99	1,21	1,37	**1,13**	**0,82**	2582	2092	10328	8368
trans-2-Me-2-ACHSA		3,80	11,38	11,38	2,00	2,00	**1,00**	**0,00**	768	768	3072	3072
erythro-beta		3,80	16,77	22,35	3,41	4,88	**1,43**	**6,22**	7553	7687	30212	30748
threo-beta		3,80	17,05	18,78	3,49	3,94	**1,13**	**0,88**	1351	1285	5404	5140
trans-2-amino		3,80	31,18	40,48	7,21	9,65	**1,34**	**1,70**	637	738	2548	2952
2-tert-Bu-Tau	S-R	3,80	9,18	14,79	1,41	2,89	**2,04**	**1,69**	135	288	540	1152
2-Et-Tau	S-R	3,80	11,30	16,15	1,97	3,25	**1,65**	**1,16**	138	204	552	816
2-Me-Tau		3,80	14,01	14,01	2,69	2,69	**1,00**	**0,00**	145	145	580	580
Pro-Me-SO_3H		3,80	9,48	9,48	1,50	1,50	**1,00**	**0,00**	1121	1121	4484	4484
2-isopropyl-Tau	L-D	3,80	10,86	14,74	1,86	2,88	**1,55**	**0,79**	120	103	480	412
2-phenyl-Tau		3,80	13,58	13,58	2,57	2,57	**1,00**	**0,00**	333	333	1332	1332
AVERAGE VALUES							**1,31**	**2,09**				

Table xiii: *aliphatic amino acids on 2-(R)-isopropyl-Tau-QD (CSP 3), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	7.47	8.07	1.03	1.20	**1.16**	**1.06**	3917	2449	15668	9796
Trp	D-L	3.68	10.97	15.45	1.98	3.20	**1.61**	**7.62**	7282	8832	29128	35328
Tyr	L-D	3.68	8.08	8.66	1.20	1.36	**1.13**	**0.86**	2625	2243	10500	8972
p-F-Phe	L-D	3.68	7.71	8.16	1.10	1.22	**1.11**	**1.10**	7073	5177	28292	20708
pCl-Phe	L-D	3.68	8.60	9.21	1.34	1.51	**1.12**	**1.48**	8790	6460	35160	25840
p-Br-Phe		3.68	9.12	9.80	1.48	1.67	**1.13**	**1.62**	9535	6787	38140	27148
oF-Phe		3.68	7.37	7.97	1.00	1.17	**1.16**	**1.14**	3366	3421	13464	13684
Baclofen		3.68	20.71	25.48	4.63	5.93	**1.28**	**5.43**	11389	10868	45556	43472
DOPA		3.68	9.98	9.98	1.71	1.71	**1.00**	**0.00**	1509	1509	6036	6036
m Tyr		3.68	8.60	8.88	1.34	1.41	**1.06**	**0.46**	5337	2398	21348	9592
5-HTP	D-L	3.68	11.92	15.49	2.24	3.21	**1.43**	**5.23**	5829	7104	23316	28416
β-Phe		3.68	15.52	17.34	3.22	3.72	**1.15**	**1.95**	5540	4514	22160	18056
β-homo-Phe		3.68	18.98	18.98	4.16	4.16	**1.00**	**0.00**	5867	5867	23468	23468
α-Me-Phe	D-L	3.68	6.10	6.55	0.66	0.78	**1.18**	**0.59**	436	5826	1744	23304
α-Me-Tyr	D-L	3.68	6.70	7.13	0.82	0.94	**1.14**	**0.67**	1815	1959	7260	7836
α-Me-m Tyr		3.68	6.72	8.00	0.83	1.18	**1.42**	**2.54**	3093	3669	12372	14676
α-Me-DOPA	D-L	3.68	7.53	9.55	1.05	1.60	**1.52**	**2.52**	1562	2097	6248	8388
α-Me-Trp		3.68	8.64	21.36	1.35	4.81	**3.56**	**20.51**	8468	9805	33872	39220
1- Me-Trp	D-L	3.68	10.82	13.40	1.94	2.64	**1.36**	**4.98**	7835	9752	31340	39008
Phenylglycine	L-D	3.68	8.49	8.70	1.31	1.37	**1.04**	**0.54**	9816	6759	39264	27036
p-Amino-Phe		3.68	7.99	8.62	1.17	1.34	**1.14**	**0.77**	2211	1362	8844	5448
OMe-Tyr	L-D	3.68	8.14	8.80	1.21	1.39	**1.15**	**1.36**	4965	4756	19860	19024
Kynurenine	L-D	3.68	10.96	11.72	1.98	2.19	**1.10**	**1.31**	6938	5408	27752	21632
1-Naphtylalanine	L-D	3.68	9.57	10.30	1.60	1.80	**1.12**	**1.68**	10357	7271	41428	29084
Tic	S-R	3.68	7.75	9.06	1.11	1.46	**1.32**	**2.20**	2609	3928	10436	15712
AVERAGE VALUES							**1.30**	**2.70**				

Table xiv: *aromatic amino acids on 2-(S)-isopropyl-Tau-QN (CSP 4), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	12.88	14.62	2.50	2.97	**1.19**	**1.17**	1679	1163	6716	4652
Trp	D-L	3.68	18.15	21.88	3.93	4.94	**1.26**	**3.21**	4171	5341	16684	21364
Tyr	L-D	3.68	14.30	16.87	2.89	3.58	**1.24**	**1.61**	1567	1514	6268	6056
p-F-Phe	L-D	3.68	13.24	14.95	2.60	3.06	**1.18**	**2.13**	5683	4494	22732	17976
pCl-Phe	L-D	3.68	15.23	17.54	3.14	3.77	**1.20**	**2.49**	5441	4621	21764	18484
p-Br-Phe		3.68	16.20	18.81	3.40	4.11	**1.21**	**2.66**	5915	4488	23660	17952
oF-Phe		3.68	12.09	13.88	2.29	2.77	**1.21**	**1.63**	2469	2090	9876	8360
Baclofen		3.68	47.54	62.39	11.92	15.95	**1.34**	**6.99**	10856	10641	43424	42564
DOPA	L-D	3.68	19.50	20.55	4.30	4.58	**1.07**	**0.37**	2375	421	9500	1684
m Tyr		3.68	16.29	17.31	3.43	3.70	**1.08**	**0.59**	2076	1202	8304	4808
5-HTP	D-L	3.68	20.18	22.25	4.48	5.05	**1.13**	**1.32**	3014	2848	12056	11392
β-Phe		3.68	29.78	37.92	7.09	9.30	**1.31**	**5.79**	9498	9153	37992	36612
β-homo-Phe		3.68	32.28	35.47	7.77	8.64	**1.11**	**1.84**	7507	5830	30028	23320
α-Me-Phe		3.68	9.87	9.87	1.68	1.68	**1.00**	**0.00**	1774	1774	7096	7096
α-Me-Tyr	L-D	3.68	11.66	12.20	2.17	2.31	**1.07**	**0.54**	1400	4607	5600	18428
α-Me-m Tyr		3.68	10.98	13.24	1.98	2.60	**1.31**	**2.22**	2276	2265	9104	9060
α-Me-DOPA	D-L	3.68	13.31	16.09	2.62	3.37	**1.29**	**1.68**	971	1631	3884	6524
α-Me-Trp		3.68	14.50	26.10	2.94	6.09	**2.07**	**13.31**	7068	9863	28272	39452
1- Me-Trp	D-L	3.68	17.23	18.82	3.68	4.11	**1.12**	**1.64**	5474	5676	21896	22704
Phenylglycine		3.68	15.46	15.46	3.20	3.20	**1.00**	**0.00**	1774	1774	7096	7096
p-Amino-Phe		3.68	12.85	14.97	2.49	3.07	**1.23**	**1.14**	1746	576	6984	2304
OMe-Tyr	L-D	3.68	13.76	15.70	2.74	3.26	**1.19**	**1.72**	2799	2711	11196	10844
Kynurenine	L-D	3.68	17.65	19.37	3.79	4.26	**1.12**	**1.61**	5345	4307	21380	17228
1-Naphtylalanine	L-D	3.68	16.33	18.15	3.44	3.93	**1.14**	**1.74**	5783	3437	23132	13748
Tic	S-R	3.68	11.65	13.83	2.17	2.76	**1.27**	**2.08**	2286	2428	9144	9712
AVERAGE VALUES							**1.21**	**2.38**				

Table xv: *aromatic amino acids on 2-(S)-isopropyl-Tau-QN (CSP 4), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.84	7.24	7.68	0.89	1.00	**1.13**	**0.48**	565	2167	2260	8668
Val	L-D	3.84	6.68	7.32	0.74	0.91	**1.22**	**1.51**	4794	4087	19176	16348
Leu	L-D	3.84	7.15	7.86	0.86	1.05	**1.22**	**1.63**	5019	4438	20076	17752
Ser	L-D	3.84	8.67	9.11	1.26	1.37	**1.09**	**0.56**	2865	1529	11460	6116
Thr	L-D	3.84	7.81	8.81	1.03	1.30	**1.25**	**1.31**	1944	1811	7776	7244
Ile	L-D	3.84	6.89	7.64	0.80	0.99	**1.24**	**2.02**	6187	6233	24748	24932
Met	L-D	3.84	8.59	9.07	1.24	1.36	**1.10**	**0.96**	6034	4464	24136	17856
Lys	L-D	3.84	42.43	49.43	10.05	11.88	**1.18**	**2.27**	1343	1343	5372	5372
Pro	L-D	3.84	7.95	10.27	1.07	1.68	**1.56**	**4.30**	4630	4549	18520	18196
Asn	L-D	3.84	9.76	10.94	1.54	1.85	**1.20**	**1.00**	1351	1153	5404	4612
Gln		3.84	8.58	8.58	1.24	1.24	**1.00**	**0.00**	2315	2315	9260	9260
Asp	D-L	3.84	16.14	16.69	3.20	3.35	**1.05**	**0.33**	5130	751	20520	3004
Glu	D-L	3.84	10.72	11.38	1.79	1.97	**1.10**	**0.59**	1032	2646	4128	10584
Arg		3.84	13.97	27.89	2.64	6.27	**2.37**	**1.38**	38	100	152	400
His	L-D	3.84	20.90	27.65	4.45	6.20	**1.40**	**1.59**	733	422	2932	1688
trans-4-OH-Pro	L-D	3.84	7.50	18.01	0.95	3.69	**3.88**	**6.00**	650	650	2600	2600
cis-4-OH-Pro	D-L	3.84	17.88	20.69	3.66	4.39	**1.20**	**1.09**	984	965	3936	3860
Nor-Leu	L-D	3.84	6.90	7.70	0.80	1.01	**1.26**	**1.93**	5088	4922	20352	19688
Nor-Val	L-D	3.84	6.96	7.61	0.81	0.98	**1.21**	**1.61**	5600	4808	22400	19232
Nipecotic acid	R-S	3.84	12.36	22.80	2.22	4.94	**2.23**	**5.69**	1126	1726	4504	6904
β-Leu		3.84	13.05	13.55	2.40	2.53	**1.06**	**0.56**	3991	3034	15964	12136
α-Me-Leu		3.84	5.52	5.65	0.44	0.47	**1.08**	**0.49**	7257	7252	29028	29008
α-Me-Val		3.84	5.95	5.99	0.55	0.56	**1.02**	**0.16**	15041	7801	60164	31204
α-Me-Ser		3.84	7.44	7.83	0.94	1.04	**1.11**	**0.59**	2310	2091	9240	8364
N-Me-Leu	L-D	3.84	5.97	5.98	0.55	0.56	**1.01**	**0.85**	5278	4226	21112	16904
trans-2-ACHSA		3.84	14.23	15.12	2.71	2.94	**1.09**	**0.60**	1464	1622	5856	6488
cis-2-ACHSA		3.84	9.11	10.46	1.37	1.72	**1.26**	**1.18**	1295	1089	5180	4356
cis-3-ACHSA		3.84	12.91	14.39	2.36	2.75	**1.16**	**0.87**	1079	963	4316	3852
β-Neopentylglycine		3.84	11.54	14.26	2.01	2.71	**1.35**	**2.59**	2169	2666	8676	10664
Homocysteic acid		3.84	14.16	14.16	2.69	2.69	**1.00**	**0.00**	918	918	3672	3672
Cysteic acid	L-D	3.84	12.24	13.14	2.19	2.42	**1.11**	**0.60**	1810	818	7240	3272
Pipecoloc acid	L-D	3.84	6.86	7.41	0.79	0.93	**1.18**	**1.23**	4238	3720	16952	14880
3,4-Hydroxyproline		3.84	7.49	10.37	0.95	1.70	**1.79**	**4.55**	3098	3237	12392	12948
5-oxo-Proline		3.84	4.56	4.56	0.19	0.19	**1.00**	**0.00**	418	418	1672	1672
allo-Ile	L-D	3.84	6.77	7.50	0.76	0.95	**1.25**	**1.45**	3494	2952	13976	11808
Neopentylglycine	L-D	3.84	7.60	9.01	0.98	1.35	**1.38**	**3.04**	5229	4999	20916	19996
tert-Leu	L-D	3.84	6.65	7.69	0.73	1.00	**1.37**	**2.30**	4192	3921	16768	15684
allo-Thr		3.84	7.09	7.09	0.85	0.85	**1.00**	**0.00**	767	2798	3068	11192
N-Me-Asp		3.84	12.97	12.97	2.38	2.38	**1.00**	**0.00**	1342	1342	5368	5368
N-Me-Glu		3.84	7.66	7.66	1.00	1.00	**1.00**	**0.00**	320	320	1280	1280
trans-2-Me-2-ACHSA		3.84	9.35	12.06	1.44	2.14	**1.49**	**5.31**	6811	7251	27244	29004
erythro-beta		3.84	8.10	9.44	1.11	1.46	**1.31**	**2.84**	5818	5337	23272	21348
threo-beta		3.84	7.68	8.78	1.00	1.29	**1.29**	**2.25**	4894	4257	19576	17028
trans-2-amino		3.84	16.73	16.73	3.36	3.36	**1.00**	**0.00**	1988	1988	7952	7952
2-tert-Bu-Tau	R-S	3.84	8.38	16.53	1.18	3.31	**2.79**	**6.47**	1347	1707	5388	6828
2-Et-Tau	R-S	3.84	12.54	17.10	2.27	3.46	**1.52**	**2.48**	983	1094	3932	4376
2-Me-Tau	R-S	3.84	14.63	16.39	2.81	3.27	**1.16**	**0.83**	891	848	3564	3392
Pro-Me-SO_3H	R-S	3.84	10.99	13.40	1.86	2.49	**1.34**	**1.35**	755	752	3020	3008
2-isopropyl-Tau	D-L	3.84	11.16	15.80	1.91	3.12	**1.63**	**2.99**	1101	1295	4404	5180
2-phenyl-Tau	D-L	3.84	11.19	13.89	1.91	2.62	**1.37**	**2.75**	2327	2886	9308	11544
AVERAGE VALUES							**1.34**	**1.69**				

Table xvi: *aliphatic amino acids on 2-(S)-isopropyl-Tau-QN (CSP 4), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.84	8.46	9.04	1.21	1.36	**1.12**	**0.48**	460	1874	1840	7496
Val	L-D	3.84	7.94	8.85	1.07	1.30	**1.22**	**1.68**	4263	3538	17052	14152
Leu	L-D	3.84	8.45	9.35	1.20	1.44	**1.20**	**1.65**	4560	3955	18240	15820
Ser	L-D	3.84	10.46	11.11	1.72	1.89	**1.10**	**0.58**	1682	1378	6728	5512
Thr	L-D	3.84	9.18	10.75	1.39	1.80	**1.29**	**1.54**	1614	1458	6456	5832
Ile	L-D	3.84	8.22	9.27	1.14	1.41	**1.24**	**2.17**	5486	5004	21944	20016
Met	L-D	3.84	10.09	10.70	1.63	1.79	**1.10**	**1.01**	5527	4137	22108	16548
Lys		3.84	12.74	12.74	2.32	2.32	**1.00**	**0.00**	23	23	92	92
Pro	L-D	3.84	7.70	10.82	1.01	1.82	**1.81**	**5.60**	4346	4511	17384	18044
Asn	L-D	3.84	10.57	12.41	1.75	2.23	**1.27**	**1.20**	987	839	3948	3356
Gln		3.84	9.43	10.62	1.46	1.77	**1.21**	**0.82**	420	1621	1680	6484
Asp		3.84	n.d.									
Glu		3.84	12.63	13.41	2.29	2.49	**1.09**	**0.48**	710	1658	2840	6632
Arg		3.84	n.d.									
His		3.84	24.83	24.83	5.47	5.47	**1.00**	**0.00**	251	251	1004	1004
trans-4-OH-Pro	L-D	3.84	7.56	18.77	0.97	3.89	**4.02**	**5.36**	550	676	2200	2704
cis-4-OH-Pro		3.84	18.68	19.97	3.87	4.20	**1.09**	**0.53**	555	2264	2220	9056
Nor-Leu	L-D	3.84	8.08	9.17	1.11	1.39	**1.26**	**2.01**	4176	3990	16704	15960
Nor-Val		3.84	8.16	9.08	1.13	1.37	**1.21**	**1.76**	4683	4045	18732	16180
Nipecotic acid	D-L	3.84	12.97	26.72	2.38	5.96	**2.51**	**6.80**	1108	1859	4432	7436
β-Leu		3.84	14.88	16.68	2.88	3.34	**1.16**	**1.61**	3607	2965	14428	11860
α-Me-Leu		3.84	6.30	6.69	0.64	0.74	**1.16**	**0.97**	4617	3772	18468	15088
α-Me-Val		3.84	6.95	6.95	0.81	0.81	**1.00**	**0.00**	3061	3061	12244	12244
α-Me-Ser		3.84	8.88	9.33	1.31	1.43	**1.09**	**0.54**	2681	1545	10724	6180
N-Me-Leu	D-L	3.84	5.98	6.49	0.56	0.69	**1.24**	**1.29**	4426	3735	17704	14940
trans-2-ACHSA		3.84	13.68	13.68	2.56	2.56	**1.00**	**0.00**	753	753	3012	3012
cis-2-ACHSA		3.84	9.04	10.24	1.36	1.67	**1.23**	**0.93**	994	819	3976	3276
cis-3-ACHSA		3.84	14.14	14.14	2.68	2.68	**1.00**	**0.00**	270	270	1080	1080
β-Neopentylglycine		3.84	12.80	16.88	2.34	3.40	**1.45**	**3.40**	2205	2684	8820	10736
Homocysteic acid		3.84	12.66	12.66	2.30	2.30	**1.00**	**0.00**	652	652	2608	2608
Cysteic acid	D-L	3.84	10.26	11.08	1.67	1.89	**1.13**	**0.66**	1025	1356	4100	5424
Pipecoloc acid		3.84	7.40	8.02	0.93	1.09	**1.17**	**1.18**	3614	3315	14456	13260
3,4-Hydroxyproline		3.84	7.12	10.63	0.85	1.77	**5.08**	**2.34**	2296	2928	9184	11712
5-oxo-Proline		3.84	4.24	4.24	0.10	0.10	**1.00**	**0.00**	385	385	1540	1540
allo-Ile	L-D	3.84	7.97	8.98	1.08	1.34	**1.25**	**1.47**	2577	2334	10308	9336
Neopentylglycine	L-D	3.84	8.86	10.64	1.31	1.77	**1.36**	**3.09**	4449	4665	17796	18660
tert-Leu	L-D	3.84	7.88	9.54	1.05	1.49	**1.41**	**2.67**	3099	3218	12396	12872
allo-Thr	L-D	3.84	8.54	8.54	1.22	1.22	**1.00**	**0.00**	687	2104	2748	8416
N-Me-Asp		3.84	13.00	13.00	2.39	2.39	**1.00**	**0.00**	230	230	920	920
N-Me-Glu		3.84	7.69	7.69	1.00	1.00	**1.00**	**0.00**	292	292	1168	1168
trans-2-Me-2-ACHSA		3.84	9.12	11.46	1.38	1.99	**1.44**	**4.71**	6478	7253	25912	29012
erythro-beta		3.84	9.75	11.81	1.54	2.08	**1.35**	**3.28**	4860	4635	19440	18540
threo-beta		3.84	9.11	10.46	1.37	1.73	**1.26**	**2.14**	4034	3711	16136	14844
trans-2-amino		3.84	19.54	21.34	4.09	4.56	**1.11**	**1.17**	2900	2795	11600	11180
2-tert-Bu-Tau	R-S	3.84	8.02	15.52	1.09	3.04	**2.79**	**5.03**	745	1191	2980	4764
2-Et-Tau	R-S	3.84	11.83	16.30	2.08	3.25	**1.56**	**2.21**	762	779	3048	3116
2-Me-Tau	R-S	3.84	13.91	15.67	2.62	3.08	**1.17**	**0.78**	695	686	2780	2744
Pro-Me-SO_3H	R-S	3.84	9.85	11.63	1.57	2.03	**1.30**	**1.03**	639	601	2556	2404
2-isopropyl-Tau	D-L	3.84	10.49	15.04	1.73	2.92	**1.68**	**2.64**	797	948	3188	3792
2-phenyl-Tau	D-L	3.84	10.86	13.28	1.83	2.46	**1.35**	**2.06**	1507	1860	6028	7440
AVERAGE VALUES							**1.41**	**1.64**				

Table xvii: *aliphatic amino acids on 2-(S)-isopropyl-Tau-QN (CSP 4), 75/25 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.84	12.98	14.01	2.38	2.65	**1.11**	**0.60**	695	1477	2780	5908
Val	L-D	3.84	11.99	13.79	2.12	2.59	**1.22**	**1.94**	3592	2769	14368	11076
Leu	L-D	3.84	12.86	14.60	2.35	2.80	**1.19**	**2.03**	4697	3678	18788	14712
Ser		3.84	18.41	18.41	3.80	3.80	**1.00**	**0.00**	269	269	1076	1076
Thr	L-D	3.84	14.09	17.22	2.67	3.49	**1.30**	**1.46**	895	838	3580	3352
Ile	L-D	3.84	12.48	14.55	2.25	2.79	**1.24**	**2.58**	5875	3762	23500	15048
Met	L-D	3.84	15.38	16.62	3.01	3.33	**1.11**	**1.04**	3963	2293	15852	9172
Lys		3.84	55.58	55.58	13.48	13.48	**1.00**	**0.00**	1111	1111	4444	4444
Pro	L-D	3.84	9.35	14.55	1.44	2.79	**1.94**	**5.96**	2905	3084	11620	12336
Asn		3.84	32.88	32.88	7.57	7.57	**1.00**	**0.00**	239	239	956	956
Gln		3.84	13.87	16.49	2.61	3.30	**1.26**	**1.38**	967	1091	3868	4364
Asp		3.84	32.39	32.39	7.44	7.44	**1.00**	**0.00**	156	156	624	624
Glu		3.84	21.77	24.49	4.67	5.38	**1.15**	**0.66**	262	1128	1048	4512
Arg		3.84	n.d.									
His		3.84	41.11	62.60	9.71	15.31	**1.58**	**0.87**	33	143	132	572
trans-4-OH-Pro	L-D	3.84	9.89	26.66	1.58	5.95	**3.77**	**6.74**	1163	784	4652	3136
cis-4-OH-Pro		3.84	26.45	26.45	5.89	5.89	**1.00**	**0.00**	664	664	2656	2656
Nor-Leu	L-D	3.84	12.24	14.33	2.19	2.73	**1.25**	**2.06**	2830	2693	11320	10772
Nor-Val	L-D	3.84	12.30	14.03	2.21	2.66	**1.20**	**1.85**	3651	2867	14604	11468
Nipecotic acid	R-S	3.84	18.49	46.24	3.82	11.05	**2.89**	**10.03**	1651	2466	6604	9864
β-Leu		3.84	23.54	28.04	5.13	6.31	**1.23**	**3.57**	6904	6531	27616	26124
α-Me-Leu		3.84	9.04	9.97	1.35	1.60	**1.18**	**1.32**	3034	2769	12136	11076
α-Me-Val		3.84	10.16	10.16	1.65	1.65	**1.00**	**0.00**	2213	2213	8852	8852
α-Me-Ser		3.84	13.85	14.68	2.61	2.83	**1.08**	**0.54**	905	2272	3620	9088
N-Me-Leu	D-L	3.84	7.49	8.51	0.95	1.22	**1.28**	**1.86**	3800	3196	15200	12784
trans-2-ACHSA		3.84	17.13	17.78	3.46	3.63	**1.05**	**0.39**	825	6680	3300	26720
cis-2-ACHSA		3.84	11.00	12.32	1.87	2.21	**1.18**	**0.68**	598	546	2392	2184
cis-3-ACHSA		3.84	22.49	23.23	4.86	5.05	**1.04**	**0.29**	626	4391	2504	17564
β-Neopentylglycine		3.84	19.11	27.18	3.98	6.08	**1.53**	**4.39**	2202	2841	8808	11364
Homocysteic acid		3.84	15.21	16.42	2.96	3.28	**1.11**	**0.60**	483	2766	1932	11064
Cysteic acid		3.84	12.58	13.72	2.28	2.57	**1.13**	**0.49**	365	744	1460	2976
Pipecoloc acid	L-D	3.84	10.08	11.15	1.63	1.91	**1.17**	**1.29**	2888	2460	11552	9840
3,4-Hydroxyproline		3.84	8.60	14.18	1.24	2.69	**2.17**	**5.82**	1951	2505	7804	10020
5-oxo-Proline		3.84	4.73	4.73	0.23	0.23	**1.00**	**0.00**	1236	8240	4944	32960
allo-Ile	L-D	3.84	12.33	14.20	2.21	2.70	**1.22**	**2.17**	4083	3582	16332	14328
Neopentylglycine	L-D	3.84	13.33	16.40	2.47	3.27	**1.32**	**2.82**	3159	2900	12636	11600
tert-Leu	L-D	3.84	12.05	15.57	2.14	3.06	**1.43**	**3.94**	3828	3834	15312	15336
allo-Thr		3.84	14.29	14.29	2.72	2.72	**1.00**	**0.00**	602	1348	2408	5392
N-Me-Asp		3.84	17.52	19.21	3.56	4.00	**1.12**	**0.48**	365	498	1460	1992
N-Me-Glu	D-L	3.84	9.58	10.40	1.50	1.71	**1.14**	**0.57**	732	830	2928	3320
trans-2-Me-2-ACHSA		3.84	10.84	12.20	1.82	2.18	**1.19**	**3.83**	7032	8320	28128	33280
erythro-beta		3.84	15.57	19.80	3.06	4.16	**1.36**	**3.57**	3730	3465	14920	13860
threo-beta		3.84	14.19	16.50	2.70	3.30	**1.22**	**2.03**	3099	2805	12396	11220
trans-2-amino		3.84	33.02	38.18	7.60	8.95	**1.18**	**2.31**	3917	4171	15668	16684
2-tert-Bu-Tau	R-S	3.84	9.68	19.13	1.52	3.99	**2.62**	**4.97**	733	1047	2932	4188
2-Et-Tau	R-S	3.84	14.78	20.74	2.85	4.40	**1.54**	**2.23**	618	793	2472	3172
2-Me-Tau		3.84	17.83	20.19	3.65	4.26	**1.17**	**0.69**	276	1017	1104	4068
Pro-Me-SO_3H	R-S	3.84	11.63	13.31	2.03	2.47	**1.22**	**0.73**	527	422	2108	1688
2-isopropyl-Tau	D-L	3.84	12.90	18.98	2.36	3.94	**1.67**	**2.58**	606	848	2424	3392
2-phenyl-Tau	D-L	3.84	13.71	16.82	2.57	3.38	**1.31**	**1.82**	1154	1399	4616	5596
AVERAGE VALUES							**1.35**	**1.94**				

Table xviii: *aliphatic amino acids on 2-(S)-isopropyl-Tau-QN (CSP 4), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	7.04	7.73	0.91	1.10	**1.21**	**1.16**	5148	1502	20592	6008
Trp	D-L	3.68	10.26	13.14	1.79	2.57	**1.44**	**2.98**	1795	2983	7180	11932
Tyr	L-D	3.68	7.76	8.38	1.11	1.28	**1.15**	**0.61**	1485	723	5940	2892
p-F-Phe	L-D	3.68	7.27	7.75	0.98	1.11	**1.13**	**0.72**	2149	1951	8596	7804
pCl-Phe	L-D	3.68	8.10	8.72	1.20	1.37	**1.14**	**0.71**	1498	1474	5992	5896
p-Br-Phe		3.68	8.62	9.31	1.34	1.53	**1.14**	**0.65**	1317	1022	5268	4088
oF-Phe		3.68	7.03	7.61	0.91	1.07	**1.17**	**0.68**	1354	1083	5416	4332
Baclofen		3.68	16.47	20.65	3.48	4.62	**1.33**	**3.28**	3112	3676	12448	14704
DOPA		3.68	10.46	10.46	1.84	1.84	**1.00**	**0.00**	36	36	144	144
m Tyr		3.68	8.36	8.36	1.27	1.27	**1.00**	**0.00**	6357	1640	25428	6560
5-HTP	D-L	3.68	11.24	14.09	2.06	2.83	**1.38**	**2.51**	1559	2498	6236	9992
β-Phe		3.68	14.11	14.96	2.84	3.07	**1.08**	**0.80**	3792	2531	15168	10124
β-homo-Phe		3.68	15.88	16.56	3.32	3.50	**1.06**	**0.71**	5690	3925	22760	15700
α-Me-Phe		3.68	6.03	6.26	0.64	0.70	**1.10**	**0.53**	2598	4546	10392	18184
α-Me-Tyr	D-L	3.68	6.45	6.82	0.75	0.85	**1.13**	**0.65**	1939	2427	7756	9708
α-Me-m Tyr		3.68	6.51	7.67	0.77	1.09	**1.41**	**2.42**	3004	3968	12016	15872
α-Me-DOPA		3.68	9.54	9.54	1.59	1.59	**1.00**	**0.00**	49	49	196	196
α-Me-Trp		3.68	8.14	17.33	1.21	3.71	**3.06**	**14.58**	5221	7371	20884	29484
1- Me-Trp	D-L	3.68	10.22	12.05	1.78	2.28	**1.28**	**1.60**	1135	2046	4540	8184
Phenylglycine	L-D	3.68	7.52	8.07	1.05	1.19	**1.14**	**1.10**	4039	3732	16156	14928
p-Amino-Phe		3.68	7.61	8.23	1.07	1.24	**1.16**	**0.58**	1326	623	5304	2492
OMe-Tyr	L-D	3.68	7.80	8.46	1.12	1.30	**1.16**	**0.57**	708	907	2832	3628
Kynurenine		3.68	10.96	10.96	1.98	1.98	**1.00**	**0.00**	373	373	1492	1492
1-Naphtylalanine	L-D	3.68	8.98	9.68	1.44	1.63	**1.13**	**0.58**	1435	726	5740	2904
Tic	S-R	3.68	7.39	8.79	1.01	1.39	**1.38**	**1.44**	890	1369	3560	5476
AVERAGE VALUES							1.25	1.55				

Table xix: *aromatic amino acids on 2-(S)-isobutyl-Tau-QN (CSP 5), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3,68	8,08	9,11	1,19	1,48	**1,24**	**1,26**	4396	988	17584	3952
Trp	D-L	3,68	11,47	13,42	2,12	2,65	**1,25**	**1,33**	820	1650	3280	6600
Tyr	L-D	3,68	9,03	10,01	1,45	1,72	**1,18**	**0,58**	889	335	3556	1340
p-F-Phe	L-D	3,68	8,51	9,22	1,31	1,50	**1,15**	**0,69**	1238	1127	4952	4508
pCl-Phe	L-D	3,68	9,60	10,50	1,61	1,85	**1,15**	**0,65**	949	772	3796	3088
p-Br-Phe		3,68	10,31	11,25	1,80	2,06	**1,14**	**0,55**	859	499	3436	1996
oF-Phe		3,68	8,14	8,98	1,21	1,44	**1,19**	**0,67**	887	695	3548	2780
Baclofen		3,68	17,65	23,09	3,79	5,27	**1,39**	**3,10**	1882	2428	7528	9712
DOPA		3,68	n.d.									
m Tyr		3,68	9,40	9,75	1,56	1,65	**1,06**	**0,37**	5561	817	22244	3268
5-HTP	D-L	3,68	11,30	13,69	2,07	2,72	**1,31**	**1,11**	771	1389	3084	5556
β-Phe		3,68	16,05	18,63	3,36	4,06	**1,21**	**1,69**	2192	1958	8768	7832
β-homo-Phe		3,68	n.d.									
α-Me-Phe		3,68	6,89	6,89	0,87	0,87	**1,00**	**0,00**	2409	2409	9636	9636
α-Me-Tyr		3,68	7,49	7,49	1,03	1,03	**1,00**	**0,00**	1862	1862	7448	7448
α-Me-m Tyr		3,68	7,25	8,40	0,97	1,28	**1,32**	**1,90**	2553	2790	10212	11160
α-Me-DOPA		3,68	n.d.									
α-Me-Trp		3,68	8,97	16,03	1,44	3,36	**2,34**	**10,71**	4318	6929	17272	27716
1- Me-Trp	D-L	3,68	11,18	12,17	2,04	2,31	**1,13**	**0,69**	1029	1109	4116	4436
Phenylglycine		3,68	9,39	9,39	1,55	1,55	**1,00**	**0,00**	385	385	1540	1540
p-Amino-Phe		3,68	8,45	9,40	1,30	1,55	**1,20**	**0,57**	776	313	3104	1252
OMe-Tyr	L-D	3,68	8,83	9,72	1,40	1,64	**1,17**	**0,89**	1228	1515	4912	6060
Kynurenine	L-D	3,68	11,63	12,01	2,16	2,26	**1,05**	**0,19**	1523	279	6092	1116
1-Naphtylalanine	L-D	3,68	10,46	11,18	1,84	2,04	**1,11**	**0,41**	1522	332	6088	1328
Tic	S-R	3,68	8,13	9,66	1,21	1,62	**1,35**	**1,24**	698	973	2792	3892
AVERAGE VALUES							1,22	1,30				

Table xx: *aromatic amino acids on 2-(S)-isobutyl-Tau-QN (CSP 5), 75/25 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3,68	11,37	13,11	2,09	2,56	**1,23**	**0,88**	1118	413	4472	1652
Trp	D-L	3,68	16,75	18,69	3,55	4,08	**1,15**	**0,75**	550	1046	2200	4184
Tyr	L-D	3,68	13,73	15,79	2,73	3,29	**1,21**	**0,58**	402	188	1608	752
p-F-Phe	L-D	3,68	12,22	13,72	2,32	2,73	**1,18**	**0,72**	499	748	1996	2992
pCl-Phe	L-D	3,68	14,18	16,10	2,85	3,38	**1,18**	**0,73**	382	754	1528	3016
p-Br-Phe		3,68	15,29	17,42	3,16	3,73	**1,18**	**0,73**	372	682	1488	2728
oF-Phe		3,68	11,41	13,02	2,10	2,54	**1,21**	**0,67**	446	389	1784	1556
Baclofen		3,68	30,71	41,76	7,35	10,35	**1,41**	**3,84**	2188	2879	8752	11516
DOPA	L-D	3,68	14,22	19,59	2,86	4,32	**1,51**	**1,03**	177	164	708	656
m Tyr		3,68	14,70	14,70	2,99	2,99	**1,00**	**0,00**	3190	460	12760	1840
5-HTP	D-L	3,68	17,72	19,30	3,82	4,25	**1,11**	**0,59**	1046	599	4184	2396
β-Phe		3,68	25,05	30,97	5,81	7,42	**1,28**	**1,80**	1188	1141	4752	4564
β-homo-Phe		3,68	26,52	28,41	6,21	6,72	**1,08**	**1,03**	4570	2890	18280	11560
α-Me-Phe		3,68	9,31	9,31	1,53	1,53	**1,00**	**0,00**	1388	1388	5552	5552
α-Me-Tyr		3,68	10,81	10,81	1,94	1,94	**1,00**	**0,00**	736	736	2944	2944
α-Me-m Tyr		3,68	10,16	11,85	1,76	2,22	**1,26**	**1,29**	1170	1101	4680	4404
α-Me-DOPA		3,68	16,11	16,11	3,38	3,38	**1,00**	**0,00**	77	77	308	308
α-Me-Trp		3,68	12,72	21,30	2,46	4,79	**1,95**	**7,98**	2959	4946	11836	19784
1- Me-Trp		3,68	16,61	16,61	3,51	3,51	**1,00**	**0,00**	481	481	1924	1924
Phenylglycine		3,68	12,64	13,77	2,43	2,74	**1,13**	**0,74**	1010	1373	4040	5492
p-Amino-Phe		3,68	13,74	13,74	2,73	2,73	**1,00**	**0,00**	29	29	116	116
OMe-Tyr	L-D	3,68	12,48	14,17	2,39	2,85	**1,19**	**0,77**	614	579	2456	2316
Kynurenine	L-D	3,68	15,37	16,89	3,18	3,59	**1,13**	**0,54**	664	429	2656	1716
1-Naphtylalanine	L-D	3,68	15,53	16,69	3,22	3,54	**1,10**	**0,36**	945	226	3780	904
Tic	S-R	3,68	11,01	13,34	1,99	2,63	**1,32**	**1,06**	313	790	1252	3160
AVERAGE VALUES							**1,19**	**1,04**				

Table xxi: *aromatic amino acids on 2-(S)-isobutyl-Tau-QN (CSP 5), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.39	6.71	6.71	0.98	0.98	**1.00**	**0.00**	216	216	864	864
Val	L-D	3.39	6.27	6.97	0.85	1.06	**1.25**	**1.16**	1957	1833	7828	7332
Leu	L-D	3.39	6.70	7.46	0.98	1.20	**1.23**	**1.10**	1599	1775	6396	7100
Ser		3.39	8.14	8.14	1.40	1.40	**1.00**	**0.00**	361	361	1444	1444
Thr	L-D	3.39	7.28	8.11	1.15	1.39	**1.21**	**0.54**	832	256	3328	1024
Ile	L-D	3.39	6.38	7.22	0.88	1.13	**1.28**	**1.34**	2061	1754	8244	7016
Met	L-D	3.39	7.92	8.25	1.34	1.44	**1.07**	**0.34**	3245	554	12980	2216
Lys	D-L	3.39	21.78	24.03	5.43	6.10	**1.12**	**0.34**	662	95	2648	380
Pro	L-D	3.39	7.24	9.26	1.14	1.73	**1.52**	**2.25**	1222	1478	4888	5912
Asn		3.39	8.81	9.48	1.60	1.80	**1.12**	**0.37**	1467	197	5868	788
Gln		3.39	7.67	7.67	1.26	1.26	**1.00**	**0.00**	183	183	732	732
Asp		3.39	28.83	28.83	7.52	7.52	**1.00**	**0.00**	153	153	612	612
Glu	D-L	3.39	13.79	15.05	3.07	3.45	**1.12**	**0.58**	891	597	3564	2388
Arg		3.39	25.17	25.17	6.44	6.44	**1.00**	**0.00**	93	93	372	372
His		3.39	23.83	23.83	6.04	6.04	**1.00**	**0.00**	4271	4271	17084	17084
trans-4-OH-Pro	L-D	3.39	6.93	15.50	1.05	3.58	**3.42**	**3.48**	136	598	544	2392
cis-4-OH-Pro		3.39	15.43	15.43	3.56	3.56	**1.00**	**0.00**	642	642	2568	2568
Nor-Leu	L-D	3.39	6.42	7.19	0.90	1.12	**1.25**	**1.06**	1334	1469	5336	5876
Nor-Val	L-D	3.39	6.47	7.11	0.91	1.10	**1.21**	**0.89**	1450	1489	5800	5956
Nipecotic acid	R-S	3.39	10.22	17.35	2.02	4.13	**2.04**	**3.24**	508	725	2032	2900
β-Leu		3.39	11.56	11.87	2.42	2.51	**1.04**	**0.47**	7683	3714	30732	14856
α-Me-Leu		3.39	5.28	5.28	0.56	0.56	**1.00**	**0.00**	710	710	2840	2840
α-Me-Val		3.39	5.66	5.66	0.67	0.67	**1.00**	**0.00**	982	982	3928	3928
α-Me-Ser		3.39	7.12	7.12	1.10	1.10	**1.00**	**0.00**	360	360	1440	1440
N-Me-Leu		3.39	5.53	5.74	0.63	0.69	**1.10**	**0.48**	3034	2411	12136	9644
trans-2-ACHSA		3.39	11.68	11.68	2.45	2.45	**1.00**	**0.00**	719	719	2876	2876
cis-2-ACHSA		3.39	7.76	9.42	1.29	1.78	**1.38**	**1.68**	1404	1092	5616	4368
cis-3-ACHSA		3.39	10.52	11.33	2.11	2.35	**1.11**	**0.62**	1130	1078	4520	4312
β-Neopentylglycine		3.39	10.26	12.55	2.03	2.71	**1.33**	**1.86**	1205	1551	4820	6204
Homocysteic acid		3.39	24.57	24.57	6.26	6.26	**1.00**	**0.00**	995	995	3980	3980
Cysteic acid		3.39	23.51	23.51	5.94	5.94	**1.00**	**0.00**	711	711	2844	2844
Pipecoloc acid	L-D	3.39	6.45	7.02	0.91	1.07	**1.19**	**0.89**	1881	1672	7524	6688
3,4-Hydroxyproline		3.39	7.09	9.73	1.09	1.88	**1.72**	**1.68**	337	592	1348	2368
5-oxo-Proline		3.39	6.22	6.50	0.84	0.92	**1.10**	**0.68**	4401	3510	17604	14040
allo-Ile	L-D	3.39	6.41	7.26	0.89	1.14	**1.28**	**1.28**	1617	1750	6468	7000
Neopentylglycine	L-D	3.39	7.02	8.41	1.07	1.48	**1.38**	**1.71**	1298	1567	5192	6268
tert-Leu	L-D	3.39	6.14	7.24	0.81	1.14	**1.40**	**1.92**	2079	2259	8316	9036
allo-Thr		3.39	6.74	6.74	0.99	0.99	**1.00**	**0.00**	478	478	1912	1912
N-Me-Asp		3.39	25.11	25.11	6.42	6.42	**1.00**	**0.00**	111	111	444	444
N-Me-Glu		3.39	10.43	10.43	2.08	2.08	**1.00**	**0.00**	1147	1147	4588	4588
trans-2-Me-2-ACHSA		3.39	8.32	10.41	1.46	2.08	**1.42**	**4.36**	5999	6152	23996	24608
erythro-beta		3.39	7.38	8.95	1.18	1.64	**1.39**	**1.51**	1131	1560	4524	6240
threo-beta		3.39	7.25	8.36	1.14	1.47	**1.29**	**1.37**	1408	1499	5632	5996
trans-2-amino		3.39	14.34	14.34	3.24	3.24	**1.00**	**0.00**	1001	1001	4004	4004
2-tert-Bu-Tau	R-S	3.39	7.62	14.44	1.25	3.27	**2.61**	**5.71**	1078	1568	4312	6272
2-Et-Tau	R-S	3.39	10.45	14.01	2.09	3.14	**1.50**	**2.23**	946	941	3784	3764
2-Me-Tau	R-S	3.39	11.65	13.02	2.44	2.85	**1.16**	**0.74**	772	690	3088	2760
Pro-Me-SO_3H	R-S	3.39	9.12	11.11	1.69	2.28	**1.35**	**1.36**	792	745	3168	2980
2-isopropyl-Tau	D-L	3.39	9.56	13.24	1.82	2.91	**1.60**	**2.62**	985	1115	3940	4460
2-phenyl-Tau	D-L	3.39	9.67	11.34	1.86	2.35	**1.27**	**1.85**	1849	2468	7396	9872
AVERAGE VALUES							**1.27**	**1.03**				

Table xxii: *aliphatic amino acids on 2-(S)-isobutyl-Tau-QN (CSP 5), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3.39	12.76	12.76	2.77	2.77	**1.00**	**0.00**	153	153	612	612
Val		3.39	10.96	13.02	2.24	2.85	**1.27**	**0.85**	296	528	1184	2112
Leu	L-D	3.39	11.96	13.79	2.53	3.07	**1.21**	**0.64**	269	399	1076	1596
Ser		3.39	15.83	15.83	3.68	3.68	**1.00**	**0.00**	82	82	328	328
Thr	L-D	3.39	13.73	15.82	3.05	3.67	**1.20**	**0.42**	311	85	1244	340
Ile	L-D	3.39	11.48	13.74	2.39	3.06	**1.28**	**0.76**	204	400	816	1600
Met		3.39	15.34	15.34	3.53	3.53	**1.00**	**0.00**	105	105	420	420
Lys		3.39	n.d.									
Pro	L-D	3.39	8.60	12.85	1.54	2.80	**1.81**	**3.31**	856	1372	3424	5488
Asn		3.39	16.60	16.60	3.90	3.90	**1.00**	**0.00**	44	44	176	176
Gln		3.39	11.50	11.50	2.40	2.40	**1.00**	**0.00**	85	85	340	340
Asp		3.39	22.34	22.34	5.60	5.60	**1.00**	**0.00**	30	30	120	120
Glu	D-L	3.39	19.20	32.77	4.67	8.68	**1.86**	**0.51**	445	238	1780	952
Arg		3.39	n.d.									
His		3.39	n.d.									
trans-4-OH-Pro	L-D	3.39	9.38	21.84	1.77	5.45	**3.08**	**3.09**	131	333	524	1332
cis-4-OH-Pro		3.39	21.77	21.77	5.43	5.43	**1.00**	**0.00**	138	138	552	552
Nor-Leu	L-D	3.39	11.27	13.20	2.33	2.90	**1.25**	**0.68**	212	411	848	1644
Nor-Val	L-D	3.39	11.37	12.94	2.36	2.82	**1.20**	**0.59**	318	356	1272	1424
Nipecotic acid		3.39	31.20	33.88	8.22	9.01	**1.10**	**0.67**	643	1509	2572	6036
β-Leu		3.39	19.51	23.05	4.76	5.81	**1.22**	**2.82**	4864	4414	19456	17656
α-Me-Leu		3.39	8.27	9.03	1.44	1.67	**1.16**	**0.84**	1699	1292	6796	5168
α-Me-Val		3.39	9.28	9.28	1.74	1.74	**1.00**	**0.00**	1190	1190	4760	4760
α-Me-Ser		3.39	12.68	12.68	2.75	2.75	**1.00**	**0.00**	207	207	828	828
N-Me-Leu	D-L	3.39	7.26	7.95	1.15	1.35	**1.18**	**0.95**	1853	1730	7412	6920
trans-2-ACHSA		3.39	13.70	13.70	3.05	3.05	**1.00**	**0.00**	592	592	2368	2368
cis-2-ACHSA		3.39	9.09	11.01	1.69	2.25	**1.34**	**1.10**	555	515	2220	2060
cis-3-ACHSA		3.39	15.46	18.63	3.57	4.50	**1.26**	**1.21**	684	672	2736	2688
β-Neopentylglycine		3.39	16.55	22.93	3.89	5.77	**1.49**	**2.69**	945	1253	3780	5012
Homocysteic acid		3.39	26.63	26.63	6.87	6.87	**1.00**	**0.00**	169	169	676	676
Cysteic acid		3.39	22.05	22.05	5.51	5.51	**1.00**	**0.00**	374	374	1496	1496
Pipecoloc acid	L-D	3.39	9.24	10.33	1.73	2.05	**1.19**	**1.07**	1637	1363	6548	5452
3,4-Hydroxyproline		3.39	8.78	13.35	1.59	2.94	**1.85**	**1.14**	68	202	272	808
5-oxo-Proline		3.39	6.31	6.57	0.86	0.94	**1.09**	**0.60**	3780	3366	15120	13464
allo-Ile	L-D	3.39	12.03	14.03	2.55	3.14	**1.23**	**0.57**	202	234	808	936
Neopentylglycine	L-D	3.39	12.38	15.31	2.66	3.52	**1.33**	**0.82**	149	390	596	1560
tert-Leu	L-D	3.39	11.23	15.16	2.32	3.48	**1.50**	**2.19**	847	884	3388	3536
allo-Thr		3.39	12.60	12.60	2.72	2.72	**1.00**	**0.00**	143	143	572	572
N-Me-Asp		3.39	36.60	36.60	9.81	9.81	**1.00**	**0.00**	48	48	192	192
N-Me-Glu		3.39	14.27	14.27	3.21	3.21	**1.00**	**0.00**	904	904	3616	3616
trans-2-Me-2-ACHSA		3.39	10.09	12.30	1.98	2.63	**1.33**	**4.00**	6149	7004	24596	28016
erythro-beta		3.39	13.87	19.55	3.10	4.77	**1.54**	**1.71**	365	441	1460	1764
threo-beta		3.39	13.49	16.22	2.99	3.79	**1.27**	**0.75**	210	333	840	1332
trans-2-amino		3.39	27.67	31.94	7.17	8.44	**1.18**	**1.21**	1297	1024	5188	4096
2-tert-Bu-Tau	R-S	3.39	8.63	16.52	1.55	3.88	**2.51**	**4.09**	412	942	1648	3768
2-Et-Tau	R-S	3.39	12.13	17.05	2.58	4.04	**1.56**	**2.04**	476	692	1904	2768
2-Me-Tau	R-S	3.39	14.09	16.40	3.16	3.85	**1.22**	**0.75**	551	524	2204	2096
Pro-Me-SO_3H	R-S	3.39	9.72	11.14	1.87	2.29	**1.22**	**0.66**	308	471	1232	1884
2-isopropyl-Tau	D-L	3.39	10.99	15.98	2.25	3.72	**1.66**	**2.22**	406	766	1624	3064
2-phenyl-Tau	D-L	3.39	11.93	13.99	2.52	3.13	**1.24**	**1.36**	1134	1202	4536	4808
AVERAGE VALUES							**1.29**	**0.99**				

Table xxiii: *aliphatic amino acids on 2-(S)-isobutyl-Tau-QN (CSP 5), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	7.48	8.06	1.03	1.19	**1.15**	**0.96**	2076	3496	8304	13984
Trp	D-L	3.68	11.68	16.25	2.18	3.42	**1.57**	**7.11**	6549	8508	26196	34032
Tyr	L-D	3.68	8.47	9.21	1.30	1.50	**1.15**	**1.06**	2917	2353	11668	9412
p-F-Phe	L-D	3.68	7.71	8.24	1.10	1.24	**1.13**	**1.31**	6994	5673	27976	22692
pCl-Phe	L-D	3.68	8.70	9.38	1.36	1.55	**1.14**	**1.47**	6572	5738	26288	22952
p-Br-Phe		3.68	9.32	10.10	1.53	1.74	**1.14**	**1.80**	9042	7494	36168	29976
oF-Phe		3.68	7.45	8.05	1.03	1.19	**1.16**	**1.81**	10059	8071	40236	32284
Baclofen		3.68	18.42	22.13	4.01	5.01	**1.25**	**3.35**	5263	5467	21052	21868
DOPA	L-D	3.68	10.70	11.20	1.91	2.04	**1.07**	**0.59**	4583	1844	18332	7376
m Tyr		3.68	9.06	9.54	1.46	1.59	**1.09**	**0.73**	3928	2695	15712	10780
5-HTP	D-L	3.68	13.37	17.05	2.63	3.63	**1.38**	**4.92**	5791	7469	23164	29876
β-Phe		3.68	15.74	17.04	3.28	3.63	**1.11**	**1.20**	4036	3377	16144	13508
β-homo-Phe		3.68	19.13	19.55	4.20	4.31	**1.03**	**0.52**	19261	5765	77044	23060
α-Me-Phe		3.68	6.32	6.65	0.72	0.81	**1.13**	**0.49**	650	5025	2600	20100
α-Me-Tyr	D-L	3.68	7.01	7.58	0.90	1.06	**1.17**	**0.88**	1576	2748	6304	10992
α-Me-m Tyr		3.68	7.10	8.55	0.93	1.32	**1.42**	**2.70**	2631	4314	10524	17256
α-Me-DOPA	D-L	3.68	8.30	10.44	1.26	1.84	**1.46**	**3.18**	2726	3459	10904	13836
α-Me-Trp		3.68	9.10	22.10	1.47	5.00	**3.40**	**20.61**	8066	10723	32264	42892
1- Me-Trp	D-L	3.68	11.02	13.44	2.00	2.65	**1.33**	**4.49**	7186	9372	28744	37488
Phenylglycine	L-D	3.68	8.55	8.85	1.32	1.40	**1.06**	**0.77**	9728	7243	38912	28972
p-Amino-Phe		3.68	8.28	9.01	1.25	1.45	**1.16**	**0.79**	2033	1074	8132	4296
OMe-Tyr	L-D	3.68	8.28	8.95	1.25	1.43	**1.14**	**1.20**	4067	3634	16268	14536
Kynurenine	L-D	3.68	11.31	13.12	2.07	2.57	**1.24**	**2.63**	5235	4877	20940	19508
1-Naphtylalanine	L-D	3.68	9.85	10.70	1.68	1.91	**1.14**	**1.50**	6024	4751	24096	19004
Tic	S-R	3.68	7.85	9.24	1.13	1.51	**1.33**	**2.30**	3083	3373	12332	13492
AVERAGE VALUES							1.29	2.73				

Table xxiv: *aromatic amino acids on ACHSA-Tau-QN (CSP 6), 100 % MeOH, 50mM FA & 25mM DEA*

Analyt	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3.68	8.21	9.02	1.23	1.45	**1.18**	**1.39**	4178	2912	16712	11648
Trp	D-L	3.68	12.14	15.48	2.30	3.21	**1.39**	**4.80**	5269	7392	21076	29568
Tyr	L-D	3.68	9.21	10.37	1.50	1.82	**1.21**	**1.32**	2207	1845	8828	7380
p-F-Phe	L-D	3.68	8.49	9.24	1.31	1.51	**1.16**	**1.55**	5686	4970	22744	19880
pCl-Phe	L-D	3.68	9.64	10.59	1.62	1.88	**1.16**	**1.64**	5083	4744	20332	18976
p-Br-Phe		3.68	10.30	11.35	1.80	2.08	**1.16**	**2.06**	7792	6735	31168	26940
oF-Phe		3.68	7.96	8.69	1.16	1.36	**1.17**	**1.47**	5611	3724	22444	14896
Baclofen		3.68	21.36	26.41	4.81	6.18	**1.29**	**3.30**	3706	4091	14824	16364
DOPA	L-D	3.68	11.50	12.35	2.12	2.35	**1.11**	**0.58**	2296	632	9184	2528
m Tyr		3.68	9.93	10.61	1.70	1.88	**1.11**	**0.76**	2702	1719	10808	6876
5-HTP	D-L	3.68	13.34	15.54	2.63	3.22	**1.23**	**2.65**	4350	5442	17400	21768
β-Phe		3.68	17.50	20.30	3.75	4.52	**1.20**	**1.96**	2842	2764	11368	11056
β-homo-Phe		3.68	n.d.									
α-Me-Phe		3.68	6.73	6.88	0.83	0.87	**1.05**	**0.25**	1914	2304	7656	9216
α-Me-Tyr		3.68	7.91	7.91	1.15	1.15	**1.00**	**0.00**	1131	1131	4524	4524
α-Me-m Tyr		3.68	7.66	9.01	1.08	1.45	**1.34**	**1.93**	1511	3573	6044	14292
α-Me-DOPA	D-L	3.68	8.95	10.63	1.43	1.89	**1.32**	**1.99**	2040	2274	8160	9096
α-Me-Trp		3.68	9.71	19.60	1.64	4.33	**2.64**	**16.26**	6574	11376	26296	45504
1- Me-Trp	D-L	3.68	11.18	12.63	2.04	2.43	**1.19**	**2.44**	5663	7418	22652	29672
Phenylglycine	L-D	3.68	9.20	9.63	1.50	1.62	**1.08**	**1.04**	9247	7740	36988	30960
p-Amino-Phe		3.68	8.54	9.56	1.32	1.60	**1.21**	**0.95**	1727	818	6908	3272
OMe-Tyr	L-D	3.68	8.87	9.69	1.41	1.63	**1.16**	**1.21**	3075	2947	12300	11788
Kynurenine	L-D	3.68	11.59	13.48	2.15	2.66	**1.24**	**2.04**	2755	3166	11020	12664
1-Naphtylalanine	L-D	3.68	10.50	11.44	1.85	2.11	**1.14**	**1.37**	4729	3710	18916	14840
Tic	S-R	3.68	8.11	9.45	1.20	1.57	**1.30**	**1.91**	2442	2587	9768	10348
AVERAGE VALUES							1.25	2.29				

Table xxv *aromatic amino acids on ACHSA-Tau-QN (CSP 6), 75/25 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	L-D	3,68	11,98	13,71	2,25	2,73	**1,21**	**1,56**	2507	1901	10028	7604
Trp	D-L	3,68	18,48	22,24	4,02	5,04	**1,25**	**3,14**	4030	5237	16120	20948
Tyr	L-D	3,68	14,30	17,46	2,89	3,75	**1,30**	**1,78**	1274	1247	5096	4988
p-F-Phe	L-D	3,68	12,43	14,08	2,38	2,83	**1,19**	**2,03**	4846	3846	19384	15384
pCl-Phe	L-D	3,68	14,60	16,65	2,97	3,52	**1,19**	**1,29**	3675	3271	14700	13084
p-Br-Phe		3,68	15,79	18,02	3,29	3,90	**1,18**	**2,12**	4384	3967	17536	15868
oF-Phe		3,68	11,34	12,98	2,08	2,53	**1,21**	**2,80**	9121	5612	36484	22448
Baclofen		3,68	38,82	49,99	9,55	12,58	**1,32**	**3,99**	3751	4283	15004	17132
DOPA	L-D	3,68	18,77	21,14	4,10	4,74	**1,16**	**0,83**	894	708	3576	2832
m Tyr		3,68	15,59	17,13	3,24	3,66	**1,13**	**0,96**	2028	1423	8112	5692
5-HTP	D-L	3,68	20,87	22,63	4,67	5,15	**1,10**	**1,15**	3357	3134	13428	12536
β-Phe		3,68	28,39	34,72	6,71	8,44	**1,26**	**2,67**	2777	2906	11108	11624
β-homo-Phe		3,68	31,46	34,25	7,55	8,31	**1,10**	**1,92**	9538	7164	38152	28656
α-Me-Phe		3,68	9,50	9,50	1,58	1,58	**1,00**	**0,00**	2499	2499	9996	9996
α-Me-Tyr	L-D	3,68	11,98	12,55	2,25	2,41	**1,07**	**0,53**	1094	4926	4376	19704
α-Me-m Tyr		3,68	11,17	13,34	2,04	2,62	**1,29**	**2,03**	2068	2161	8272	8644
α-Me-DOPA	D-L	3,68	14,20	16,46	2,86	3,47	**1,22**	**1,62**	2073	1817	8292	7268
α-Me-Trp		3,68	14,66	27,16	2,98	6,38	**2,14**	**13,28**	5599	9809	22396	39236
1- Me-Trp	D-L	3,68	15,93	17,14	3,33	3,66	**1,10**	**1,29**	4738	5204	18952	20816
Phenylglycine	L-D	3,68	13,58	14,46	2,69	2,93	**1,09**	**1,26**	7018	5921	28072	23684
p-Amino-Phe		3,68	12,07	14,36	2,28	2,90	**1,27**	**1,15**	1270	475	5080	1900
OMe-Tyr	L-D	3,68	13,01	14,59	2,54	2,96	**1,17**	**1,21**	2077	1589	8308	6356
Kynurenine	L-D	3,68	16,71	19,76	3,54	4,37	**1,23**	**1,93**	2083	2201	8332	8804
1-Naphtylalanine	L-D	3,68	15,29	17,11	3,16	3,65	**1,16**	**1,48**	3149	2505	12596	10020
Tic	S-R	3,68	10,90	12,97	1,96	2,52	**1,29**	**1,94**	2036	1985	8144	7940
AVERAGE VALUES							1,22	2,16				

Table xxvi: *aromatic amino acids on ACHSA-Tau-QN (CSP 6), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.40	6.98	7.52	1.05	1.21	**1.15**	**0.50**	608	836	2432	3344
Val	L-D	3.40	6.55	7.35	0.93	1.16	**1.26**	**1.74**	3802	3521	15208	14084
Leu	L-D	3.40	6.96	7.75	1.05	1.28	**1.22**	**1.72**	4105	4026	16420	16104
Ser		3.40	9.12	9.12	1.68	1.68	**1.00**	**0.00**	705	705	2820	2820
Thr	L-D	3.40	7.91	8.94	1.33	1.63	**1.23**	**1.33**	1956	1833	7824	7332
Ile	L-D	3.40	6.73	7.60	0.98	1.24	**1.26**	**2.01**	4638	4208	18552	16832
Met	L-D	3.40	8.39	9.07	1.47	1.67	**1.14**	**1.19**	4179	3469	16716	13876
Lys	L-D	3.40	31.16	35.62	8.16	9.48	**1.16**	**1.04**	806	1140	3224	4560
Pro	L-D	3.40	7.32	9.55	1.15	1.81	**1.57**	**4.01**	3487	3845	13948	15380
Asn		3.40	10.76	10.76	2.17	2.17	**1.00**	**0.00**	724	724	2896	2896
Gln	L-D	3.40	8.48	9.29	1.50	1.73	**1.16**	**0.69**	470	2276	1880	9104
Asp		3.40	23.81	23.81	6.00	6.00	**1.00**	**0.00**	523	523	2092	2092
Glu		3.40	12.49	12.49	2.67	2.67	**1.00**	**0.00**	1323	1323	5292	5292
Arg		3.40	43.64	46.41	11.84	12.65	**1.07**	**0.50**	150	79	600	316
His		3.40	17.17	17.17	4.05	4.05	**1.00**	**0.00**	28	28	112	112
trans-4-OH-Pro	L-D	3.40	7.03	17.53	1.07	4.16	**3.90**	**6.50**	465	1326	1860	5304
cis-4-OH-Pro		3.40	17.54	17.54	4.16	4.16	**1.00**	**0.00**	632	632	2528	2528
Nor-Leu	L-D	3.40	6.76	7.62	0.99	1.24	**1.26**	**1.74**	3283	3480	13132	13920
Nor-Val	L-D	3.40	6.78	7.54	1.00	1.22	**1.22**	**1.62**	3904	3602	15616	14408
Nipecotic acid	R-S	3.40	10.86	18.24	2.19	4.37	**1.99**	**4.08**	822	1212	3288	4848
β-Leu		3.40	12.29	12.78	2.62	2.76	**1.06**	**0.64**	5887	3337	23548	13348
α-Me-Leu		3.40	5.53	5.53	0.63	0.63	**1.00**	**0.00**	2487	2487	9948	9948
α-Me-Val		3.40	5.88	5.88	0.73	0.73	**1.00**	**0.00**	2191	2191	8764	8764
α-Me-Ser		3.40	7.50	8.02	1.21	1.36	**1.13**	**0.79**	2575	2012	10300	8048
N-Me-Leu	D-L	3.40	5.65	5.84	0.66	0.72	**1.09**	**0.48**	4537	2579	18148	10316
trans-2-ACHSA		3.40	14.53	15.13	3.27	3.45	**1.05**	**0.48**	2844	1831	11376	7324
cis-2-ACHSA		3.40	8.92	10.96	1.62	2.22	**1.37**	**1.85**	1351	1266	5404	5064
cis-3-ACHSA		3.40	12.51	13.55	2.68	2.99	**1.11**	**0.66**	1429	899	5716	3596
β-Neopentylglycine		3.40	10.52	12.89	2.10	2.79	**1.33**	**2.00**	1330	1813	5320	7252
Homocysteic acid		3.40	22.27	22.27	5.55	5.55	**1.00**	**0.00**	969	969	3876	3876
Cysteic acid		3.40	20.28	20.28	4.96	4.96	**1.00**	**0.00**	804	804	3216	3216
Pipecoloc acid	L-D	3.40	6.52	6.92	0.92	1.03	**1.13**	**0.82**	3307	2863	13228	11452
3,4-Hydroxyproline		3.40	7.14	10.24	1.10	2.01	**1.83**	**4.29**	2006	2576	8024	10304
5-oxo-Proline		3.40	5.22	5.38	0.53	0.58	**1.09**	**0.45**	3294	3097	13176	12388
allo-Ile	L-D	3.40	6.70	7.57	0.97	1.23	**1.26**	**1.64**	2979	2789	11916	11156
Neopentylglycine	L-D	3.40	7.41	8.92	1.18	1.62	**1.38**	**2.96**	4024	4176	16096	16704
tert-Leu	L-D	3.40	6.46	7.76	0.90	1.28	**1.43**	**2.64**	3148	3448	12592	13792
allo-Thr	D-L	3.40	7.27	7.56	1.14	1.22	**1.07**	**0.36**	1621	1169	6484	4676
N-Me-Asp	L-D	3.40	15.43	16.51	3.54	3.86	**1.09**	**0.54**	1475	753	5900	3012
N-Me-Glu		3.40	8.50	8.50	1.50	1.50	**1.00**	**0.00**	901	901	3604	3604
trans-2-Me-2-ACHSA		3.40	10.01	10.69	1.94	2.14	**1.10**	**1.33**	6646	6315	26584	25260
erythro-beta		3.40	7.76	8.94	1.28	1.63	**1.27**	**1.93**	2789	3150	11156	12600
threo-beta		3.40	7.39	8.32	1.17	1.45	**1.23**	**1.63**	3129	3053	12516	12212
trans-2-amino		3.40	15.99	15.99	3.70	3.70	**1.00**	**0.00**	1656	1656	6624	6624
2-tert-Bu-Tau	R-S	3.40	7.80	14.23	1.29	3.18	**2.46**	**5.77**	1262	1789	5048	7156
2-Et-Tau	R-S	3.40	12.57	16.11	2.70	3.74	**1.39**	**2.33**	1339	1513	5356	6052
2-Me-Tau	R-S	3.40	14.70	15.56	3.32	3.58	**1.08**	**0.56**	960	2840	3840	11360
Pro-Me-SO_3H	S-R	3.40	10.23	11.66	2.01	2.43	**1.21**	**1.04**	1043	1002	4172	4008
2-ispropyl-Tau	D-L	3.40	10.36	13.63	2.05	3.01	**1.47**	**4.41**	3496	4897	13984	19588
2-phenyl-Tau	D-L	3.40	11.27	12.92	2.31	2.80	**1.21**	**2.57**	5003	6429	20012	25716
AVERAGE VALUES							**1.27**	**1.42**				

Table xxvii: *aliphatic amino acids on ACHSA-Tau-QN (CSP 6), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	*k1*	*k2*	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3.40	7.85	8.55	1.31	1.51	**1.16**	**0.53**	829	473	3316	1892
Val	L-D	3.40	7.40	8.56	1.18	1.52	**1.29**	**2.24**	4009	3653	16036	14612
Leu	L-D	3.40	7.79	8.87	1.29	1.61	**1.25**	**2.11**	4441	4023	17764	16092
Ser		3.40	10.73	10.73	2.16	2.16	**1.00**	**0.00**	504	504	2016	2016
Thr	L-D	3.40	8.98	10.49	1.64	2.09	**1.27**	**1.61**	1699	1741	6796	6964
Ile	L-D	3.40	7.68	8.97	1.26	1.64	**1.30**	**2.56**	4744	4082	18976	16328
Met	L-D	3.40	9.35	10.43	1.75	2.07	**1.18**	**1.39**	4079	3352	16316	13408
Lys		3.40	43.34	43.34	11.75	11.75	**1.00**	**0.00**	1281	1281	5124	5124
Pro	L-D	3.40	7.20	10.03	1.12	1.95	**1.75**	**5.32**	3796	4548	15184	18192
Asn	L-D	3.40	10.07	11.81	1.96	2.47	**1.26**	**1.30**	1173	990	4692	3960
Gln	L-D	3.40	8.99	10.20	1.65	2.00	**1.22**	**0.72**	274	1195	1096	4780
Asp		3.40										
Glu	D-L	3.40	13.90	14.96	3.09	3.40	**1.10**	**0.70**	1925	1180	7700	4720
Arg		3.40	32.53	32.53	8.57	8.57	**1.00**	**0.00**	116	116	464	464
His	L-D	3.40	20.02	26.58	4.89	6.82	**1.39**	**0.82**	95	95	380	380
trans-4-OH-Pro	L-D	3.40	7.10	17.82	1.09	4.24	**3.90**	**5.75**	372	1002	1488	4008
cis-4-OH-Pro	D-L	3.40	17.50	17.50	4.15	4.15	**1.00**	**0.00**	699	699	2796	2796
Nor-Leu	L-D	3.40	7.55	8.72	1.22	1.56	**1.28**	**2.14**	3506	3522	14024	14088
Nor-Val	L-D	3.40	7.52	8.65	1.21	1.54	**1.27**	**2.07**	4352	3824	17408	15296
Nipecotic acid	R-S	3.40	10.81	19.27	2.18	4.67	**2.14**	**3.62**	489	803	1956	3212
β-Leu		3.40	13.22	14.73	2.89	3.33	**1.15**	**1.89**	5579	4430	22316	17720
α-Me-Leu		3.40	6.06	6.29	0.78	0.85	**1.09**	**0.55**	4415	2960	17660	11840
α-Me-Val		3.40	6.55	6.55	0.93	0.93	**1.00**	**0.00**	2962	2962	11848	11848
α-Me-Ser		3.40	8.59	9.19	1.53	1.70	**1.12**	**0.76**	2353	1780	9412	7120
N-Me-Leu	D-L	3.40	5.78	6.10	0.70	0.79	**1.13**	**0.57**	1845	1803	7380	7212
trans-2-ACHSA		3.40	12.76	12.76	2.75	2.75	**1.00**	**0.00**	444	444	1776	1776
cis-2-ACHSA		3.40	8.21	10.03	1.42	1.95	**1.38**	**1.35**	643	836	2572	3344
cis-3-ACHSA		3.40	12.53	13.02	2.69	2.83	**1.05**	**0.38**	908	3413	3632	13652
β-Neopentylglycine		3.40	11.14	14.48	2.28	3.26	**1.43**	**2.21**	970	1333	3880	5332
Homocysteic acid		3.40	18.08	18.08	4.32	4.32	**1.00**	**0.00**	957	957	3828	3828
Cysteic acid	D-L	3.40	10.41	15.38	2.06	3.52	**1.71**	**2.04**	247	775	988	3100
Pipecoloc acid	L-D	3.40	6.82	7.26	1.01	1.13	**1.13**	**0.87**	3411	3014	13644	12056
3,4-Hydroxyproline		3.40	6.78	10.28	0.99	2.02	**2.03**	**5.13**	2079	2888	8316	11552
5-oxo-Proline		3.40	4.89	5.04	0.44	0.48	**1.10**	**0.34**	1103	4218	4412	16872
allo-Ile	L-D	3.40	7.54	8.78	1.22	1.58	**1.30**	**2.02**	2833	2841	11332	11364
Neopentylglycine	L-D	3.40	8.19	10.11	1.41	1.97	**1.40**	**3.28**	3807	4030	15228	16120
tert-Leu	L-D	3.40	7.28	9.24	1.14	1.72	**1.51**	**3.45**	3085	3680	12340	14720
allo-Thr		3.40	8.50	8.50	1.50	1.50	**1.00**	**0.00**	381	381	1524	1524
N-Me-Asp	L-D	3.40	14.69	16.30	3.32	3.79	**1.14**	**0.88**	1385	968	5540	3872
N-Me-Glu		3.40	8.82	8.82	1.59	1.59	**1.00**	**0.00**	2642	2642	10568	10568
trans-2-Me-2-ACHSA		3.40	9.32	9.79	1.74	1.88	**1.08**	**1.08**	8017	7625	32068	30500
erythro-beta		3.40	9.02	10.96	1.65	2.22	**1.34**	**2.97**	3680	3831	14720	15324
threo-beta		3.40	8.48	9.74	1.49	1.87	**1.25**	**1.89**	2960	2968	11840	11872
trans-2-amino		3.40	20.01	21.34	4.88	5.28	**1.08**	**0.67**	1919	1594	7676	6376
2-tert-Bu-Tau	R-S	3.40	7.12	12.73	1.10	2.74	**2.50**	**4.18**	535	1242	2140	4968
2-Et-Tau	R-S	3.40	11.28	14.61	2.32	3.30	**1.42**	**2.04**	957	1055	3828	4220
2-Me-Tau	R-S	3.40	13.33	14.08	2.92	3.14	**1.08**	**0.51**	901	2297	3604	9188
Pro-Me-SO_3H	S-R	3.40	20.72	38.82	5.10	10.42	**2.04**	**7.05**	1523	2674	6092	10696
AVERAGE VALUES							1.35	1.68				

Table xxviii: *aliphatic amino acids on ACHSA-Tau-QN (CSP 6), 75/25 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]
Ala	L-D	3,40	11,76	13,16	2,46	2,87	**1,17**	**0,61**	503	457	2012
Val	L-D	3,40	11,10	13,61	2,27	3,00	**1,33**	**2,42**	2557	2093	10228
Leu	L-D	3,40	11,69	13,93	2,44	3,10	**1,27**	**2,21**	2838	2360	11352
Ser		3,40	17,80	17,80	4,24	4,24	**1,00**	**0,00**	470	470	1880
Thr	L-D	3,40	14,09	17,26	3,14	4,08	**1,30**	**2,02**	1649	1571	6596
Ile	L-D	3,40	11,39	14,04	2,35	3,13	**1,33**	**2,44**	2174	2229	8696
Met	L-D	3,40	14,20	16,08	3,18	3,73	**1,17**	**1,20**	1746	1321	6984
Lys		3,40									0
Pro	L-D	3,40	8,68	13,24	1,55	2,90	**1,86**	**5,85**	2720	3525	10880
Asn	L-D	3,40	14,89	18,71	3,38	4,50	**1,33**	**1,56**	830	705	3320
Gln	L-D	3,40	13,40	16,06	2,94	3,72	**1,27**	**0,93**	251	768	1004
Asp		3,40	5,71	5,71	0,68	0,68	**1,00**	**0,00**	10164	10164	40656
Glu	D-L	3,40	25,71	29,14	6,56	7,57	**1,15**	**1,02**	1207	950	4828
Arg		3,40									0
His		3,40									0
trans-4-OH-Pro	L-D	3,40	9,43	26,18	1,77	6,70	**3,78**	**5,86**	378	773	1512
cis-4-OH-Pro	L-D	3,40	25,03	26,03	6,36	6,66	**1,05**	**0,36**	1753	1013	7012
Nor-Leu	L-D	3,40	11,43	13,88	2,36	3,08	**1,31**	**2,17**	2066	1941	8264
Nor-Val	L-D	3,40	11,27	13,40	2,31	2,94	**1,27**	**2,10**	2552	2238	10208
Nipecotic acid	R-S	3,40	15,92	33,44	3,68	8,84	**2,40**	**5,74**	668	1361	2672
β-Leu		3,40	20,87	24,85	5,14	6,31	**1,23**	**2,91**	5045	4071	20180
α-Me-Leu		3,40	8,49	9,10	1,50	1,68	**1,12**	**1,00**	3654	2993	14616
α-Me-Val		3,40	9,28	9,28	1,73	1,73	**1,00**	**0,00**	2447	2447	9788
α-Me-Ser		3,40	13,24	14,29	2,89	3,20	**1,11**	**0,85**	2421	1697	9684
N-Me-Leu	D-L	3,40	7,09	7,74	1,08	1,28	**1,18**	**0,99**	2462	1766	9848
trans-2-ACHSA		3,40	16,04	17,00	3,72	4,00	**1,08**	**0,55**	686	2380	2744
cis-2-ACHSA		3,40	10,00	12,47	1,94	2,67	**1,37**	**1,47**	752	691	3008
cis-3-ACHSA		3,40	19,58	20,13	4,76	4,92	**1,03**	**0,28**	1022	3343	4088
β-Neopentylglycine		3,40	16,55	23,18	3,87	5,82	**1,50**	**3,19**	1164	1762	4656
Homocysteic acid		3,40	21,99	21,99	5,47	5,47	**1,00**	**0,00**	736	736	2944
Cysteic acid		3,40	18,65	18,65	4,49	4,49	**1,00**	**0,00**	886	886	3544
Pipecoloc acid	L-D	3,40	8,99	9,66	1,64	1,84	**1,12**	**0,98**	3231	2701	12924
3,4-Hydroxyproline		3,40	8,09	13,37	1,38	2,93	**2,13**	**4,77**	1197	1760	4788
5-oxo-Proline		3,40	5,31	5,42	0,56	0,59	**1,06**	**0,30**	9355	1724	37420
allo-Ile	L-D	3,40	11,10	13,60	2,26	3,00	**1,32**	**2,20**	1896	1906	7584
Neopentylglycine	L-D	3,40	12,09	15,56	2,56	3,58	**1,40**	**3,12**	2380	2552	9520
tert-Leu	L-D	3,40	10,67	14,79	2,14	3,35	**1,57**	**4,21**	2699	2727	10796
allo-Thr		3,40	13,29	13,29	2,91	2,91	**1,00**	**0,00**	395	395	1580
N-Me-Asp	L-D	3,40	19,18	22,02	4,64	5,48	**1,18**	**0,99**	821	821	3284
N-Me-Glu		3,40	11,54	11,54	2,39	2,39	**1,00**	**0,00**	763	763	3052
trans-2-Me-2-ACHSA		3,40	12,01	12,01	2,53	2,53	**1,00**	**0,00**	3306	3306	13224
erythro-beta		3,40	14,18	18,35	3,17	4,40	**1,39**	**2,72**	1815	1805	7260
threo-beta		3,40	12,72	15,13	2,74	3,45	**1,26**	**1,97**	2066	2067	8264
trans-2-amino		3,40	31,83	36,24	8,36	9,66	**1,16**	**2,41**	5612	5517	22448
2-tert-Bu-Tau	R-S	3,40	8,37	15,96	1,46	3,69	**2,52**	**4,28**	607	857	2428
2-Et-Tau	R-S	3,40	14,21	18,90	3,18	4,56	**1,43**	**1,96**	707	815	2828
2-Me-Tau	R-S	3,40	17,35	18,40	4,10	4,41	**1,08**	**0,51**	701	2497	2804
Pro-Me-SO_3H	R-S	3,40	26,36	51,66	6,75	14,19	**2,10**	**7,19**	1306	2532	5224
2-isopropyl-Tau	D-L	3,40	12,03	16,59	2,54	3,88	**1,53**	**3,99**	2168	2837	8672
2-phenyl-Tau	D-L	3,40	14,33	16,09	3,21	3,73	**1,16**	**1,70**	3204	3673	12816
AVERAGE VALUES							**1,36**	**1,94**			

Table xxix: *aliphatic amino acids on ACHSA-Tau-QN (CSP 6), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe	D-L	3.75	5.77	5.88	0.54	0.57	**1.06**	**0.53**	13303	10873	53212	43492
Trp	D-L	3.75	7.40	9.52	0.98	1.54	**1.58**	**6.31**	8605	11829	34420	47316
Tyr		3.75	6.25	6.25	0.67	0.67	**1.00**	**0.00**	1743	1743	6972	6972
p-F-Phe		3.75	5.72	5.72	0.53	0.53	**1.00**	**0.00**	4131	4131	16524	16524
pCl-Phe	D-L	3.75	5.90	6.14	0.57	0.64	**1.11**	**0.47**	1350	3703	5400	14812
p-Br-Phe		3.75	6.25	6.34	0.67	0.69	**1.04**	**0.40**	21502	9282	86008	37128
oF-Phe		3.75	5.83	5.83	0.56	0.56	**1.00**	**0.00**	7027	7027	28108	28108
Baclofen		3.75	7.96	8.33	1.13	1.22	**1.09**	**1.08**	10253	8840	41012	35360
DOPA	D-L	3.75	6.76	7.18	0.80	0.92	**1.14**	**0.91**	3459	3675	13836	14700
m Tyr		3.75	6.18	6.48	0.65	0.73	**1.12**	**1.15**	9972	8956	39888	35824
5-HTP	D-L	3.75	8.10	10.62	1.16	1.83	**1.58**	**5.84**	6153	8860	24612	35440
β-Phe		3.75	7.36	7.36	0.96	0.96	**1.00**	**0.00**	2813	2813	11252	11252
β-homo-Phe		3.75	7.85	7.85	1.10	1.10	**1.00**	**0.00**	9313	9313	37252	37252
α-Me-Phe	D-L	3.75	5.39	5.89	0.44	0.57	**1.31**	**2.59**	13954	13383	55816	53532
α-Me-Tyr	D-L	3.75	5.74	6.45	0.53	0.72	**1.36**	**1.74**	1944	7344	7776	29376
α-Me-m Tyr		3.75	5.91	6.96	0.58	0.86	**1.48**	**4.64**	13070	12900	52280	51600
α-Me-DOPA	D-L	3.75	6.43	7.86	0.71	1.10	**1.53**	**4.31**	6253	8632	25012	34528
α-Me-Trp		3.75	7.14	12.68	0.90	2.39	**2.64**	**13.43**	6282	11941	25128	47764
1- Me-Trp	D-L	3.75	7.28	8.46	0.94	1.26	**1.33**	**3.41**	6641	10260	26564	41040
Phenylglycine		3.75	6.03	6.03	0.61	0.61	**1.00**	**0.00**	9884	9884	39536	39536
p-Amino-Phe		3.75	6.29	6.29	0.68	0.68	**1.00**	**0.00**	600	600	2400	2400
OMe-Tyr	L-D	3.75	6.12	6.29	0.63	0.68	**1.07**	**0.64**	10299	7599	41196	30396
Kynurenine		3.75	7.56	7.74	1.02	1.06	**1.05**	**0.57**	13499	7560	53996	30240
1-Naphtylalanine		3.75	6.92	6.92	0.85	0.85	**1.00**	**0.00**	1755	1755	7020	7020
Tic	S-R	3.75	5.97	6.22	0.59	0.66	**1.11**	**1.13**	13129	11090	52516	44360
AVERAGE VALUES							**1.22**	**1.97**				

Table xxx: *aromatic amino acids on Tau-QN (CSP 7), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		3.75	7.05	7.05	0.88	0.88	**1.00**	**0.00**	6001	6001	24004	24004
Trp	D-L	3.75	9.04	11.01	1.41	1.94	**1.37**	**4.22**	6869	7895	27476	31580
Tyr		3.75	8.11	8.11	1.17	1.17	**1.00**	**0.00**	3391	3391	13564	13564
p-F-Phe		3.75	7.01	7.01	0.87	0.87	**1.00**	**0.00**	5663	5663	22652	22652
pCl-Phe		3.75	7.65	7.65	1.04	1.04	**1.00**	**0.00**	6598	6598	26392	26392
p-Br-Phe		3.75	8.02	8.02	1.14	1.14	**1.00**	**0.00**	7234	7234	28936	28936
oF-Phe		3.75	7.16	7.16	0.91	0.91	**1.00**	**0.00**	8682	8682	34728	34728
Baclofen		3.75	10.11	10.80	1.70	1.88	**1.11**	**1.62**	10559	9042	42236	36168
DOPA		3.75	9.10	9.10	1.43	1.43	**1.00**	**0.00**	888	888	3552	3552
m Tyr		3.75	8.09	8.36	1.16	1.23	**1.06**	**0.62**	7611	4346	30444	17384
5-HTP	D-L	3.75	9.75	11.94	1.60	2.19	**1.37**	**3.11**	3628	3945	14512	15780
β-Phe		3.75	9.10	9.69	1.43	1.59	**1.11**	**1.17**	6916	4694	27664	18776
β-homo-Phe		3.75	9.71	9.86	1.59	1.63	**1.03**	**0.45**	21690	8590	86760	34360
α-Me-Phe	D-L	3.75	6.74	7.30	0.80	0.95	**1.19**	**2.21**	12747	12044	50988	48176
α-Me-Tyr	L-D	3.75	7.78	8.54	1.08	1.28	**1.19**	**2.33**	9324	10869	37296	43476
α-Me-m Tyr		3.75	7.57	8.75	1.02	1.34	**1.31**	**4.03**	12519	12479	50076	49916
α-Me-DOPA	D-L	3.75	8.59	10.00	1.29	1.67	**1.29**	**2.02**	2921	2807	11684	11228
α-Me-Trp		3.75	8.85	13.99	1.36	2.73	**2.01**	**11.06**	8316	10703	33264	42812
1- Me-Trp	D-L	3.75	8.63	9.25	1.30	1.47	**1.13**	**2.12**	7146	7192	28584	28768
Phenylglycine		3.75	7.44	7.44	0.99	0.99	**1.00**	**0.00**	7032	7032	28128	28128
p-Amino-Phe		3.75	7.62	7.62	1.03	1.03	**1.00**	**0.00**	5887	5887	23548	23548
OMe-Tyr		3.75	7.41	7.41	0.98	0.98	**1.00**	**0.00**	4947	4947	19788	19788
Kynurenine		3.75	8.82	8.82	1.35	1.35	**1.00**	**0.00**	3684	3684	14736	14736
1-Naphtylalanine		3.75	8.28	8.28	1.21	1.21	**1.00**	**0.00**	6261	6261	25044	25044
Tic	S-R	3.75	6.89	7.24	0.84	0.93	**1.11**	**1.34**	12782	10366	51128	41464
AVERAGE VALUES							**1.13**	**1.45**				

Table xxxi: *aromatic amino acids on Tau-QN (CSP 7), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		3.63	4.88	4.88	0.34	0.34	**1.00**	**0.00**	4988	4988	19952	19952
Val		3.63	5.14	5.14	0.42	0.42	**1.00**	**0.00**	4225	4225	16900	16900
Leu	L-D	3.63	5.21	5.29	0.43	0.46	**1.05**	**0.46**	17154	12624	68616	50496
Ser		3.63	5.86	5.86	0.61	0.61	**1.00**	**0.00**	2442	2442	9768	9768
Thr		3.63	5.64	5.64	0.55	0.55	**1.00**	**0.00**	1311	1311	5244	5244
Ile		3.63	5.19	5.19	0.43	0.43	**1.00**	**0.00**	7429	7429	29716	29716
Met		3.63	5.76	5.76	0.59	0.59	**1.00**	**0.00**	3638	3638	14552	14552
Lys		3.63	14.82	16.44	3.08	3.53	**1.14**	**1.52**	3791	3284	15164	13136
Pro	L-D	3.63	5.58	5.87	0.54	0.62	**1.15**	**1.31**	11032	9983	44128	39932
Asn		3.63	6.23	6.23	0.72	0.72	**1.00**	**0.00**	5611	5611	22444	22444
Gln		3.63	5.74	5.74	0.58	0.58	**1.00**	**0.00**	603	603	2412	2412
Asp		3.63	8.88	8.88	1.45	1.45	**1.00**	**0.00**	832	832	3328	3328
Glu		3.63	6.89	6.89	0.90	0.90	**1.00**	**0.00**	807	807	3228	3228
Arg		3.63	17.58	17.94	3.84	3.94	**1.03**	**0.29**	11903	1430	47612	5720
His		3.63	8.25	8.65	1.27	1.38	**1.09**	**0.44**	2651	868	10604	3472
trans-4-OH-Pro	L-D	3.63	5.70	7.21	0.57	0.99	**1.73**	**1.46**	399	966	1596	3864
cis-4-OH-Pro		3.63	7.24	7.24	0.99	0.99	**1.00**	**0.00**	445	445	1780	1780
Nor-Leu		3.63	5.24	5.24	0.44	0.44	**1.00**	**0.00**	5135	5135	20540	20540
Nor-Val		3.63	5.21	5.21	0.44	0.44	**1.00**	**0.00**	6219	6219	24876	24876
Nipecotic acid	R-S	3.63	7.28	7.91	1.00	1.18	**1.17**	**1.82**	8349	7023	33396	28092
β-Leu		3.63	6.39	6.49	0.76	0.79	**1.04**	**0.45**	19518	9062	78072	36248
α-Me-Leu		3.63	4.74	4.90	0.30	0.35	**1.15**	**0.50**	5731	2439	22924	9756
α-Me-Val		3.63	5.16	5.16	0.42	0.42	**1.00**	**0.00**	13363	13363	53452	53452
α-Me-Ser		3.63	5.65	5.84	0.56	0.61	**1.09**	**0.79**	10518	7861	42072	31444
N-Me-Leu		3.63	4.90	4.90	0.35	0.35	**1.00**	**0.00**	1232	1232	4928	4928
trans-2-ACHSA		3.63	5.86	5.86	0.61	0.61	**1.00**	**0.00**	474	474	1896	1896
cis-2-ACHSA		3.63	5.36	5.36	0.48	0.48	**1.00**	**0.00**	1084	1084	4336	4336
cis-3-ACHSA		3.63	6.44	6.44	0.77	0.77	**1.00**	**0.00**	706	706	2824	2824
β-Neopentylglycine		3.63	6.04	6.31	0.66	0.74	**1.12**	**0.84**	5917	5521	23668	22084
Homocysteic acid		3.63	4.73	7.83	0.30	1.16	**3.82**	**0.49**	962	2081	3848	8324
Cysteic acid		3.63	6.91	6.91	0.90	0.90	**1.00**	**0.00**	685	685	2740	2740
Pipecoloc acid	L-D	3.63	5.36	5.48	0.48	0.51	**1.07**	**0.62**	20352	10426	81408	41704
3,4-Hydroxyproline		3.63	5.52	5.88	0.52	0.62	**1.19**	**1.41**	8395	7628	33580	30512
5-oxo-Proline		3.63	4.70	4.83	0.29	0.33	**1.12**	**0.74**	12582	10808	50328	43232
allo-Ile		3.63	5.19	5.19	0.43	0.43	**1.00**	**0.00**	7011	7011	28044	28044
Neopentylglycine	L-D	3.63	5.29	5.45	0.46	0.50	**1.09**	**0.66**	10199	7042	40796	28168
tert-Leu		3.63	5.13	5.13	0.41	0.41	**1.00**	**0.00**	9658	9658	38632	38632
allo-Thr		3.63	5.29	5.29	0.46	0.46	**1.00**	**0.00**	3771	3771	15084	15084
N-Me-Asp		3.63	8.82	8.82	1.43	1.43	**1.00**	**0.00**	2693	2693	10772	10772
N-Me-Glu		3.63	4.39	4.39	0.21	0.21	**1.00**	**0.00**	6208	6208	24832	24832
trans-2-Me-2-ACHSA		3.63	5.28	5.38	0.46	0.48	**1.06**	**0.54**	23259	9259	93036	37036
erythro-beta		3.63	5.71	5.71	0.57	0.57	**1.00**	**0.00**	9843	9843	39372	39372
threo-beta		3.63	5.57	5.57	0.54	0.54	**1.00**	**0.00**	1310	1310	5240	5240
trans-2-amino		3.63	7.61	7.61	1.09	1.09	**1.00**	**0.00**	1471	1471	5884	5884
2-tert-Bu-Tau		3.63	5.86	5.86	0.61	0.61	**1.00**	**0.00**	98	98	392	392
2-Et-Tau		3.63	5.61	5.61	0.55	0.55	**1.00**	**0.00**	479	479	1916	1916
2-Me-Tau		3.63	5.78	5.78	0.59	0.59	**1.00**	**0.00**	257	257	1028	1028
Pro-Me-SO_3H	S-R	3.63	7.30	9.03	1.01	1.49	**1.47**	**1.73**	1035	1082	4140	4328
2-ispropyl-Tau		3.63	5.56	5.56	0.53	0.53	**1.00**	**0.00**	358	358	1432	1432
2-phenyl-Tau		3.63	5.55	5.55	0.53	0.53	**1.00**	**0.00**	1269	1269	5076	5076
AVERAGE VALUES							**1.11**	**0.32**				

Table xxxii: *aliphatic amino acids on Tau-QN (CSP 7), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala	L-D	3,63	6,73	6,86	0,85	0,89	**1,04**	**0,49**	15429	7101	61716	28404
Val	L-D	3,63	6,53	6,65	0,80	0,83	**1,04**	**0,47**	15305	6872	61220	27488
Leu	L-D	3,63	6,63	6,84	0,83	0,88	**1,07**	**0,65**	7059	6530	28236	26120
Ser		3,63	7,67	7,67	1,11	1,11	**1,00**	**0,00**	2862	2862	11448	11448
Thr	L-D	3,63	7,15	7,35	0,97	1,02	**1,06**	**0,49**	10339	3081	41356	12324
Ile	L-D	3,63	6,65	6,80	0,83	0,87	**1,05**	**0,51**	11885	6765	47540	27060
Met	L-D	3,63	7,31	7,48	1,01	1,06	**1,05**	**0,49**	15856	4379	63424	17516
Lys		3,63	n.d.									
Pro	L-D	3,63	6,12	6,60	0,68	0,82	**1,19**	**1,85**	10089	9283	40356	37132
Asn	L-D	3,63	7,57	7,69	1,08	1,12	**1,03**	**0,34**	25231	3636	100924	14544
Gln	L-D	3,63	6,85	7,34	0,89	1,02	**1,15**	**0,63**	1788	1053	7152	4212
Asp		3,63	12,20	12,20	2,36	2,36	**1,00**	**0,00**	1643	1643	6572	6572
Glu	D-L	3,63	9,03	9,50	1,49	1,62	**1,09**	**0,55**	3247	1258	12988	5032
Arg	L-D	3,63	41,87	43,27	10,53	10,92	**1,04**	**0,34**	7516	745	30064	2980
His		3,63	10,31	10,82	1,84	1,98	**1,08**	**0,19**	2077	99	8308	396
trans-4-OH-Pro	L-D	3,63	6,23	8,37	0,71	1,30	**1,83**	**2,03**	903	689	3612	2756
cis-4-OH-Pro		3,63	8,15	8,15	1,24	1,24	**1,00**	**0,00**	1963	1963	7852	7852
Nor-Leu	L-D	3,63	6,57	6,75	0,81	0,86	**1,06**	**0,58**	8861	6030	35444	24120
Nor-Val	L-D	3,63	6,60	6,74	0,82	0,86	**1,05**	**0,50**	13769	6043	55076	24172
Nipecotic acid	R-S	3,63	8,65	9,78	1,38	1,69	**1,22**	**2,45**	7079	5884	28316	23536
β-Leu		3,63	7,86	8,31	1,17	1,29	**1,11**	**1,27**	9321	7748	37284	30992
α-Me-Leu		3,63	6,03	6,25	0,66	0,72	**1,09**	**0,86**	10826	8804	43304	35216
α-Me-Val		3,63	6,33	6,52	0,74	0,80	**1,07**	**0,65**	9024	6565	36096	26260
α-Me-Ser		3,63	7,33	7,60	1,02	1,09	**1,07**	**0,73**	8317	5739	33268	22956
N-Me-Leu		3,63	5,62	5,62	0,55	0,55	**1,00**	**0,00**	5047	5047	20188	20188
trans-2-ACHSA		3,63	5,59	5,59	0,54	0,54	**1,00**	**0,00**	556	556	2224	2224
cis-2-ACHSA		3,63	5,26	5,26	0,45	0,45	**1,00**	**0,00**	703	703	2812	2812
cis-3-ACHSA		3,63	6,81	7,29	0,88	1,01	**1,15**	**0,57**	974	1339	3896	5356
β-Neopentylglycine		3,63	7,21	7,80	0,99	1,15	**1,16**	**1,64**	7360	6664	29440	26656
Homocysteic acid	D-L	3,63	7,14	7,47	0,97	1,06	**1,09**	**0,48**	956	1987	3824	7948
Cysteic acid		3,63	6,38	6,38	0,76	0,76	**1,00**	**0,00**	507	507	2028	2028
Pipecoloc acid	L-D	3,63	6,44	6,62	0,77	0,82	**1,06**	**0,64**	9417	8319	37668	33276
3,4-Hydroxyproline		3,63	5,75	6,29	0,58	0,73	**1,25**	**1,70**	6281	5476	25124	21904
5-oxo-Proline		3,63	4,58	4,73	0,26	0,30	**1,16**	**0,94**	14316	13128	57264	52512
allo-Ile	L-D	3,63	6,58	6,74	0,81	0,86	**1,05**	**0,52**	10852	5575	43408	22300
Neopentylglycine	L-D	3,63	6,58	6,92	0,81	0,90	**1,12**	**1,05**	7532	6359	30128	25436
tert-Leu	L-D	3,63	6,64	6,86	0,83	0,89	**1,07**	**0,76**	9602	7761	38408	31044
allo-Thr		3,63	6,71	6,71	0,85	0,85	**1,00**	**0,00**	4927	4927	19708	19708
N-Me-Asp		3,63	9,82	9,82	1,71	1,71	**1,00**	**0,00**	801	801	3204	3204
N-Me-Glu		3,63	6,16	6,16	0,70	0,70	**1,00**	**0,00**	1526	1526	6104	6104
trans-2-Me-2-ACHSA		3,63	5,31	5,31	0,46	0,46	**1,00**	**0,00**	7133	7133	28532	28532
erythro-beta		3,63	7,45	7,70	1,05	1,12	**1,06**	**0,62**	7107	5116	28428	20464
threo-beta		3,63	6,97	7,16	0,92	0,97	**1,06**	**0,46**	7051	3263	28204	13052
trans-2-amino		3,63	10,11	10,54	1,78	1,90	**1,07**	**0,51**	2902	2129	11608	8516
2-tert-Bu-Tau	R-S	3,63	4,95	5,60	0,36	0,54	**1,49**	**0,54**	175	611	700	2444
2-Et-Tau		3,63	5,53	5,53	0,52	0,52	**1,00**	**0,00**	605	605	2420	2420
2-Me-Tau		3,63	5,54	5,54	0,52	0,52	**1,00**	**0,00**	714	714	2856	2856
Pro-Me-SO_3H	S-R	3,63	6,57	8,24	0,81	1,27	**1,57**	**1,64**	940	768	3760	3072
2-isopropyl-Tau		3,63	5,26	5,26	0,45	0,45	**1,00**	**0,00**	392	392	1568	1568
2-phenyl-Tau		3,63	5,32	5,32	0,47	0,47	**1,00**	**0,00**	6261	3058	25044	12232
AVERAGE VALUES							**1,10**	**0,57**				

Table xxxiii: *aliphatic amino acids on Tau-QN (CSP 7), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Ala		1,27	2,49	2,49	0,96	0,96	**1,00**	**0,00**	1096	1096	7307	7307
Val		1,27	2,28	2,28	0,80	0,80	**1,00**	**0,00**	1745	1745	11633	11633
Leu		1,27	2,31	2,31	0,82	0,82	**1,00**	**0,00**	1246	1246	8307	8307
Thr		1,27	2,60	2,60	1,05	1,05	**1,00**	**0,00**	2053	2053	13687	13687
Ile		1,27	2,30	2,30	0,81	0,81	**1,00**	**0,00**	1728	1728	11520	11520
Met		1,27	2,77	2,77	1,18	1,18	**1,00**	**0,00**	1453	1453	9687	9687
Lys	L-D	1,27	7,74	8,71	5,09	5,86	**1,15**	**0,95**	n.d.			
Pro	L-D	1,27	2,85	2,96	1,24	1,33	**1,07**	**0,53**	n.d.			
Asn		1,27	2,95	3,09	1,32	1,43	**1,08**	**0,58**	n.d.			
Asp	L-D	1,27	5,47	5,67	3,31	3,46	**1,05**	**0,36**	n.d.			
Arg	L-D	1,27	12,54	13,69	8,87	9,78	**1,10**	**0,55**	n.d.			
trans-4-OH-Pro	L-D	1,27	2,73	3,50	1,15	1,76	**1,53**	**2,00**	912	1244	6080	8293
cis-4-OH-Pro	D-L	1,27	3,51	4,24	1,76	2,34	**1,33**	**1,75**	1230	1524	8200	10160
Nor-Leu		1,27	2,32	2,32	0,83	0,83	**1,00**	**0,00**	1072	1072	7147	7147
Nor-Val		1,27	2,33	2,33	0,83	0,83	**1,00**	**0,00**	1168	1168	7787	7787
Nipecolic acid	S-R	1,27	3,96	4,21	2,12	2,31	**1,09**	**0,60**	n.d.			
Pipecolic acid		1,27	2,67	2,67	1,10	1,10	**1,00**	**0,00**	1286	1286	8573	8573
allo-Ile		1,27	2,29	2,29	0,80	0,80	**1,00**	**0,00**	1716	1716	11440	11440
Neopentylglycine		1,27	2,32	2,32	0,83	0,83	**1,00**	**0,00**	717	717	4780	4780
tert-Leu		1,27	2,27	2,27	0,79	0,79	**1,00**	**0,00**	1562	1562	10413	10413
2-tBu-Tau		1,27	2,28	2,28	0,80	0,80	**1,00**	**0,00**	205	205	1367	1367
2-Et-Tau		1,27	2,48	2,48	0,95	0,95	**1,00**	**0,00**	440	440	2933	2933
2-Me-Tau		1,27	2,55	2,55	1,01	1,01	**1,00**	**0,00**	432	432	2880	2880
2-isopropyl-Tau		1,27	2,37	2,37	0,87	0,87	**1,00**	**0,00**	251	251	1673	1673
2-phenyl-Tau	D-L	1,27	2,53	2,78	0,99	1,19	**1,20**	**0,80**	n.d.			
AVERAGE VALUES							1,06					

Table xxiv: *aliphatic amino acids on 2-(R)-phenyl-Tau-QN (CSP 8), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		1.51	2.76	2.76	0.83	0.83	**1.00**	**0.00**	2625	2625	17500	17500
Trp	D-L	1.51	3.88	6.59	1.57	3.36	**2.14**	**6.01**	2524	1983	16827	13220
5-HTP	D-L	1.51	4.41	6.91	1.92	3.58	**1.86**	**4.99**	2132	2004	14213	13360
α-Me-Tyr	D-L	1.51	2.79	3.09	0.85	1.05	**1.23**	**1.37**	n.d.			
α-Me-DOPA	D-L	1.51	3.39	4.02	1.25	1.66	**1.34**	**1.93**	2164	2030	14427	13533
1-Me-Trp	D-L	1.51	3.60	4.14	1.38	1.74	**1.26**	**1.83**	2859	2586	19060	17240
Kynurenine		1.51	3.86	3.86	1.56	1.56	**1.00**	**0.00**	2589	2589	17260	17260
Tic	S-R	1.51	2.98	3.17	0.97	1.10	**1.13**	**0.68**	n.d.			
AVERAGE VALUES							1.37					

Table xxxv: *aromatic amino acids on 2-(R)-phenyl-Tau-QN (CSP 8), 100 % MeOH, 50mM FA & 25mM DEA*

analytes	EO	t0	t1	t2	k1	k2	alpha	Res.	N1	N2	N1[m-1]	N2[m-1]
Phe		1.51	3.76	3.76	1.49	1.49	**1.00**	**0.00**	3059	3059	20393	20393
Trp	D-L	1.51	5.46	9.08	2.62	5.01	**1.92**	**5.63**	2080	2025	13867	13500
5-HTP	D-L	1.51	6.21	8.92	3.11	4.91	**1.58**	**3.92**	1793	2022	11953	13480
α-Me-Tyr	D-L	1.51	4.55	4.79	2.01	2.17	**1.08**	**0.59**	n.d.			
α-Me-DOPA	D-L	1.51	5.41	5.88	2.58	2.89	**1.12**	**0.82**	n.d.			
1-Me-Trp	D-L	1.51	4.59	5.02	2.04	2.32	**1.14**	**1.04**	n.d.			
Kynurenine		1.51	4.73	4.73	2.13	2.13	**1.00**	**0.00**	2719	2719	18127	18127
Tic	S-R	1.51	3.85	4.12	1.55	1.73	**1.12**	**0.65**	n.d.			
AVERAGE VALUES							1.24					

Table xxxvi: *aromatic amino acids on 2-(R)-phenyl-Tau-QN (CSP 8), 50/50 MeOH/ACN, 50mM FA & 25mM DEA*