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Review

Chiral liquid chromatography contribution to the determination of the absolute configuration of enantiomers

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Abstract

The review covers examples in which chiral HPLC, as a source of pure enantiomers, has been combined with classical methods (X-ray, vibrational circular dichroism (VCD), enzymatic resolutions, nuclear magnetic resonance (NMR) techniques, optical rotation, circular dichroism (CD)) for the on- or off-line determination of absolute configuration of enantiomers. Furthermore, it is outlined that chiral HPLC, which associates enantioseparation process and classical purification process, opens new perspectives in the classical determination of absolute configuration by chemical correlation or chemical interconversion methods. The review also contains a discussion about the various approaches to predict the absolute configuration from the retention behavior of the enantiomers on chiral stationary phases (CSPs). Some examples illustrate the advantages and limitations of molecular modeling methods and the use of chiral recognition models. The assumptions underlying some of these methods are critically analyzed and some possible emerging new strategies are outlined.

Keywords: Reviews; Chiral stationary phases, LC; Enantiomer separation; Circular dichroism; Chemical correlation

Contents

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1. Introduction

This review will focus on methods utilizing chiral HPLC in the determination of the absolute configuration of enantiomers. It highlights and summarizes some key findings presented in recent publications and discoveries of original approaches.

As this review is also written by practitioners, some practical examples are described and to some extent many personal and original viewpoints enlarge the perspective of this paper.

The past decade has seen a maturation of chiral HPLC with an increasing number in the availability of chiral stationary phases (CSPs) [1–5], as well as an improvement in the capacities of this approach. Today, there are more and more examples where chiral HPLC can compete with chemical processes in providing more economically favorable processes. Chiral HPLC offers singular advantages since it provides generally short development time and often yields 100% enantiomeric excess (e.e.) of the required enantiomer. As a result, chemists who have already own in-house capability and resources to deal with absolute configuration, should consider the advantageous combination of these technologies with chiral HPLC.

One of the most remarkable areas of application of chiral HPLC is its exploitation for the determination of the absolute structures of naturally occurring molecules. Biological compounds often contain several stereocentres and in these situations, classical approaches may involve several complex steps. The task to establish the absolute configuration can be quick and effective using chiral HPLC since it can be done in one step from the comparison of the retention time of the unknown synthetic or natural sample with those of reference enantiomers whose absolute configuration is well known.

The different specific techniques coupled to chiral HPLC and ways to their efficient use will be the focused interest of the first part of this review. We will then survey the progress of the pioneering chemical correlation methods made to date in the context of its combination with chiral HPLC. At last, several aspects of the challenge aiming at predicting absolute configuration from the retention data obtained by chiral HPLC will be featured. More attention and discussion will be paid to the chiral recognition models which can be the way for new insights into both determination of absolute configuration and the enantiorecognition mechanisms. In our opinion, these efforts should be devoted first to commercially available CSPs.

2. Preparative chiral HPLC combined with physicochemical methods for the determination of the absolute configuration

2.1. Methodology

An assumption underlying the separation of enantiomers by chiral HPLC is that it usually gives higher e.e. than other methods. Although widely employed and documented in the literature, the strategies involving the combination of chiral HPLC and physicochemical methods do not emphasize enough the advantages of such an approach. As an introduction, we have summarized the different steps in Fig. 1. First, chiral chromatography provides pure enantiomers from a synthetic racemate or an enantiomerically enriched sample.

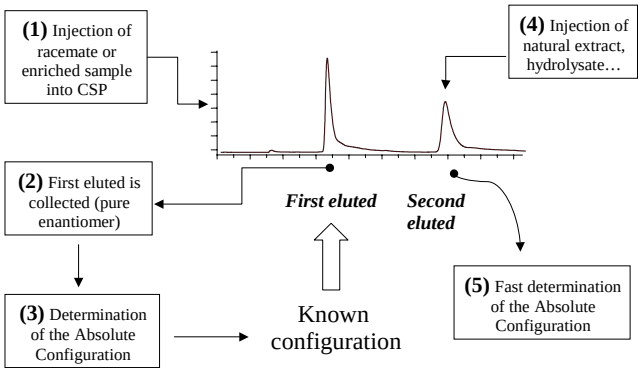

Fig. 1. Sequence of tasks involved in establishing the absolute configuration and highlighting the role of chiral HPLC.

Next, the absolute configuration of the isolated enantiomers, first eluted or second eluted is then determined using techniques already validated for pure enantiomers of whatever origin. In some cases, the techniques for determining absolute configurations may be applied either “on-line” or in a “stop-flow” manner, in that case the collection of optically pure sample is not required. Then, having in hand pure enantiomers of known configuration, the order of elution is easily determined on the initial CSP or any other CSPs. At last, the knowledge of the absolute configuration of the eluted enantiomers on a given CSP allows the determination of the absolute configuration of the same optically active compounds available in minute amounts from natural product extracts, metabolites, hydrolysates, screening results of asymmetric synthesis or enzymatic resolutions. This goal is achieved by comparison of the retention time with authentic sample (co-injection is preferred) and the method can be improved using chirality detector such as polarimeter or circular dichroism (CD).

Among the techniques validated for the determination of absolute configuration, X-ray determination, vibrational circular dichroism (VCD), nuclear magnetic resonance (NMR) or CD have been thoroughly applied. Some of them have already been or will be in future coupled to chiral HPLC. In the following sections, we have selected some examples to illustrate these applications.

2.2. X-ray determination

The absolute configuration can be determined by X-ray analysis of a crystalline derivative formed between one enantiomer isolated by chiral HPLC and an optically pure compound of known configuration. The absolute configuration

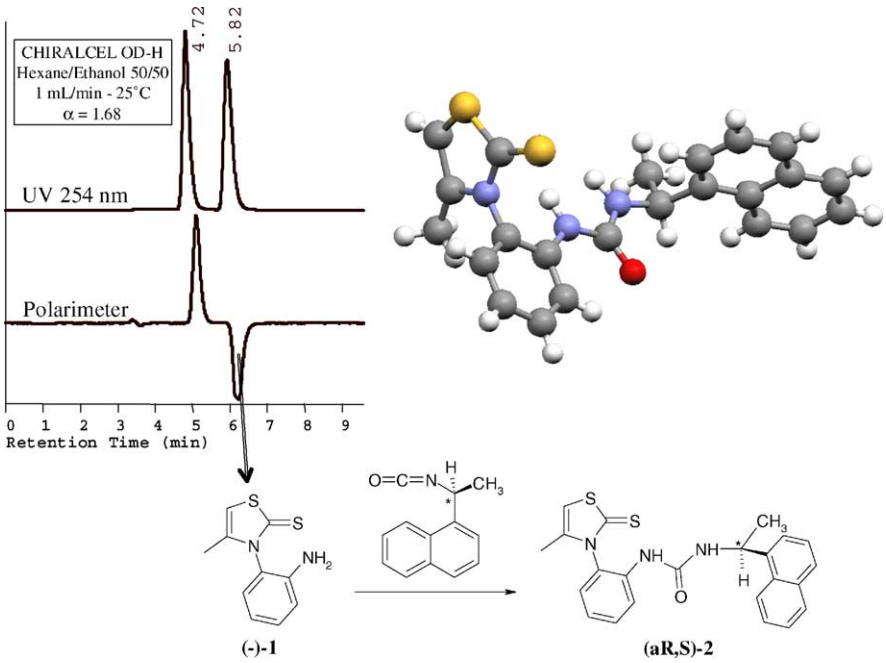

Fig. 2. An illustrative example of the determination of absolute configuration combining chiral chromatography, derivatization experiment and X-ray crystallography.

of the enantiomer is thus obtained relatively to the known configuration of the derivatizing agent. This method is interesting since classical X-ray analysis is involved. In some cases, the derivatization affords by purpose or incidentally crystalline compound from liquid or oily enantiomers for which the direct X-ray method would have not been applicable. The enantiomer under study shall present at least an anchoring bond to render the derivatization possible and sometimes the two enantiomers should be tested with the same derivatizing agent in order to obtain a crystalline diastereomer suitable for X-ray analysis. Fig. 2 is an example from our own laboratory to illustrate this. It reports the determination of the absolute configuration of enantiomers of *N*-2-aminophenyl-thiazolinthione derivative. The last eluted enantiomer (the (−) form in hexane/EtOH 50:50) was collected and reacted with an optically pure isocyanate to give crystalline urea diastereomer. X-ray afforded the absolute configuration (−)-(aR)-(M) of the atropisomer relatively to the known configuration of isocyanate framework.

As another example, the absolute configuration of indole-3-succinic acid enantiomers which were obtained by semi-preparative chromatography on Cyclobond I 2000 RSP, was determined by X-ray crystallography on cinchonidine indole succinate salt [6].

Direct X-ray determination using anomalous X-ray scattering developed by Bijvoet et al. for molecules having at least one relatively heavy atom (sulfur or larger) [7,8] or using multiple scattering X-ray experiments for light atom molecules [9–13], afforded hundreds of absolute configurations. An interesting example of the conjunction between chiral HPLC and absolute configuration was reported by Bringmann et al. [10] in the study of the atropisomeric lactone whose structure is displayed in Fig. 3. This lactone crystallizes as a conglomerate of the M and the P forms and thus, as a precondition for the CD and X-ray absolute configuration determination, a reliable HPLC analysis on a CSP was required so that one can detect immediately on the chiral column if the chosen crystal corresponds to the first or second eluted enantiomer. On that column (Chiralcel OF) it was found that the P form is eluted first.

2.3. Vibrational circular dichroism

VCD is the extension of CD from electronic to vibrational transitions in molecules. VCD offers a new and powerful approach to the determination of absolute configuration in chiral molecules (for reviews see [14–16]). The basic principle is the comparison of measured VCD spectra with simulated VCD spectra issued from quantum mechanical ab initio methods without adjustable parameters or with density functional theory (DFT) calculations. The calculation can be

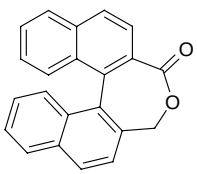

Fig. 3. Chemical structure of one atropisomeric lactone that forms a conglomerate of the two enantiomers (mechanical mixture of M and P crystals). Identification of the crystals by chiral HPLC was a prerequisite for the determination of the absolute configuration [9].

limited to molecular fragment containing the chiral center with some caution regarding the conformational states.

The increase in power and speed of computer as well as recent advances in computation algorithm and in performance of DFT's functionals will extend the range of molecules for which VCD determinations of absolute configuration can be carried out [17,18]. Furthermore, dedicated VCD instruments are now commercially available from Bomem/BioTools, Jasco and Bruker [19].

Within the passed 2 years, chiral HPLC and determination of absolute configuration by VCD spectroscopy have been associated for 1-(2-methylnaphthyl)sulfoxide [20], an oxathiane [21], a cruciferous phytoalexin related metabolites [22] and a naphthyl-2-piperidyl methanol derivative [23].

We presume that the combination of VCD spectroscopy and semi-preparative chiral HPLC will become a familiar technique for the determination of the absolute configuration of organic enantiomers. One limitation is the scan time (1 h) and required concentration (ca. 0.1 M) which prevents on-line detection for analytical experiment. We may expect that these issues will improve in the future.

2.4. Enzymatic resolutions

Enantiomeric ratios obtained in enzymatic resolutions can be used as a method for the prediction of absolute configuration. The usefulness of this approach was demonstrated in the following example. The absolute configuration of the first and second eluted enantiomers of α-hydroxyglycine derivatives on amylose tris-(3,5-dimethylphenylcarbamate) (Chiralpak AD-R) was determined from the retention time of the unreacted enantiomer remaining after the peptidylamidoglycolate lyase enzymatic conversion. On the basis of the specificity of the enzyme reaction, the absolute configuration of the unreacted enantiomer could be assigned as (*R*) [24].

2.5. NMR techniques

Various NMR techniques are also available for the determination of the absolute configuration. They involve the formation of stable diastereomers by covalent bonding or labile diastereomeric complexes in the case of chiral solvating agent [25,26]. They rely on the differential anisotropy of the substituents in diastereomeric forms such as differential analysis between the (*S*)- and (*R*)-Mosher's esters in the case of an alcohol [27–34] or other ester derivatives [35–37]. The absolute configuration of a secondary alcohol can be deduced from the ^{1}H NMR spectra of a single methoxyphenylacetic ester derivative (MPA, either the (*R*) or the (*S*) form) recorded at two different temperatures [38].

The Mosher method was extended by using a diamagnetic lanthanide complex [39] or combined with lipase- and subtilisin-catalyzed kinetic resolutions of secondary alcohols [40]. The study of about twenty alcohols of known configuration and diverse structural features was used to validate a method for the determination of the absolute configuration using in situ complexation of α-methoxyphenylacetic acid esters with barium(II). The proposed procedure is simple, fast, and inexpensive because it requires a very small amount of sample, only one derivatization, and the recording of only two ^{1}H NMR spectra at room temperature [41].

The recent advances in the development of chiral derivatizing and solvating agents [42] which facilitate the determination of enantiomeric excess and absolute configuration have been recently reviewed [43]. Moreover, a practical guide for the assignment of the absolute configuration of alcohols, amines and carboxylic acids by NMR with auxiliary reagent was presented. This guide includes the information required for the judicious selection of the most suitable auxiliary reagent: MPA, α-methoxy-α-trifluoromethylphenylacetic acid (MTPA), *tert*-Butoxycarbonyl (Boc)-phenylglycine (BPG), (9-anthryl)methoxyacetic acid (9-AMA) and ethyl 2-hydroxy-2-(9-anthryl) acetate (9-AHA), derivatization procedures and NMR conditions (solvent and temperature) for each substrate, as well as a critical account on the reliability, scope and limitations of these applications [44,45].

NMR was also used to determine the absolute configuration of chiral sulfoxides [46], amines [47–49], alkoxybenzyl-cyclopentanol derivatives [50]. Chiral silylating reagents have been used recently to derivatize chiral allylic alcohols, which do not withstand ester-based methodologies [51]. Microscale derivatization conditions have been defined.

Recently, coupled achiral HPLC–NMR emerged as a very sensitive method for the determination of absolute configuration. Derivatized mixtures of enantiomers (including racemates) of chiral secondary alcohols with a pure enantiomer of the auxiliary reagent, 9-anthrylmethoxyacetic acid [(*R*)- or (*S*)-9-AMA)] allowed the determination of the e.e. and the assignment of the absolute configuration in a single operation and with just a few sample micrograms [52–54]. The determination of the absolute configuration of 6-alkylated α-pyrones from *Ravensara crassifolia* by LC–NMR was described recently. The conventional analysis of the purified Mosher ester derivatives by ^{1}H NMR was replaced by a rapid and sensitive method in which the α-pyrones were analyzed under isocratic reversed-phase LC–NMR conditions prior to and after derivatisation reactions [55,56]. These coupled techniques are even more interesting if the derivatization step is performed in a precolumn. In the case of chiral HPLC, on-line derivatization should be performed in post column fashion and coupled to the NMR.

Achiral HPLC has also been used for determining the absolute configurations of chiral compounds via the relative retention time of diastereomeric adduct obtained from *N*-succinimidyl α-methoxyphenylacetate (SMPA) and α-chiral amine adducts on silica. This original method is based on the hypothesis of a "priority number" [57]. This number relies on the relative size and the hydrogen bond donor ability of the substituents used in conjunction with the Newman projection examination of the two diastereomeric

forms. The diastereomer, which presents both groups of the same rank on the same side of the amide bond, is predicted to be eluted last. This method can be used for mixture of enantiomers of the amine with one enantiomer of the SMPA as well as for the determination of the absolute configuration of a single enantiomer of the amine through reaction with two enantiomers of SMPA. Despite the collection of hypothesis, the concept certainly deserves more attention than it has been given in the literature (only six citations within 10 years).

2.6. Optical rotation

The field of optical rotation is currently becoming a powerful structural tool due to the advances in quantum mechanics and the availability of faster desktop computer. The field has been reviewed recently by Polavarapu [58]. Two levels of theory for calculation of optical rotation, DFT and non-correlated Hartree–Fock (HF) theory, have been recently evaluated for trioxabicyclooctane derivatives by Stephens et al. [59]. The authors concluded that "predictions of the optical rotations of chiral organic molecules with quantum mechanical calculations of accuracy sufficient to permit their application to the determination of absolute configuration are now practicable". Nevertheless, the choice of the level of theory used is of critical importance. Indeed, quick HF calculations with small basis set (such as 6-31G*) are claimed to provide absolute configuration with very little effort, but the predicted specific rotation may be largely different from experimental ones. Inclusion of electronic correlation effects, as well as an appropriate extended basis set which includes diffuse function (e.g. DFT/aug-cc-pVDZ), seems to improve the agreement with optical rotation experimental values.

In the future, one may imagine coupling chiral HPLC and polarimetric detection with these calculations. As the polarimetric detection [60–62] provides the sign of the rotation in the elution solvent at very low concentration, it is not difficult to foresee the interest of such a method enabling to correlate the sign of the rotation with the absolute configuration. Even if one limitation may be the dependence of the sign of rotation with the elution solvent [63], we expect that polarimetric detection directly coupled with calculations will develop in the future.

2.7. Circular dichroism

2.7.1. Application methods of circular dichroism

Liquid chromatography has been successfully applied for the determination of absolute configuration of naturally occurring optically pure samples using achiral LC–CD coupling. In addition, chromatographic methods for resolution of enantiomers and CD of the resolved enantiomers have also been combined in a very efficient way for studying the absolute configuration of organic compounds. Several studies, which were not possible or too time-consuming before the event of this combination, have been performed on a large variety of enantiomers. These two coupled techniques are characterized by their high sensitivity and consequently one of the main interest of combining chiral HPLC and CD is the low amount of compound needed for the absolute configuration determination. Furthermore the possibility to purify the analyte during the chromatographic process is highly profitable. Basically two methods have been used: the CD spectra can be acquired "on-line" or "off-line". In the former case, the CD spectra is performed in the elution solvent of the chromatography. In the latter case the elution solvent may be evaporated, then the CD can be obtained in any suitable solvent. An advantage of the "off-line" method compared to the "on-line" method is the possibility to modify the structure of the separated enantiomer with a derivatizing agent useful for the CD approach. Coupling chiral HPLC and CD has been also largely applied for the study of the dynamic processes involving racemization.

Examples of "on-line" and "off-line" applications of CD are discussed in the two following sections.

2.7.2. Off-line examples of circular dichroism

Several examples involving low-pressure chiral chromatography on microcrystalline cellulose triacetate (or other chiral supports) and CD have been provided by the Lund's school [64–72]. The uncertainties, if any, to determine the absolute configuration are thus independent of the chromatographic method, and rest on the CD treatment [73] which can be performed at different levels of sophistication. These various approaches are beyond the scope of this review [74,75].

An interesting aspect of CD is its exploitation for the validation of chromatographic results. A correlation was established between the observed CD spectra and absolute configuration of a series of 3-arylphthalide, then these results were employed to validate the retention order observed on a Pirkle CSP containing the (*S*)-*N*-(3,5-dinitrobenzoyl)leucine as chiral selector [76].

Other interesting examples of chiral HPLC–CD combination include the works of Yang et al. [77], Bringmann et al. [10,78], Bekker et al. [79], Fleischhauer et al. [80], Caccamese et al. [81] and Pescitelli et al. [82].

More recently, CD was used for the determination of the absolute configuration of hydroxylated metabolites formed during the in vitro biotransformation of thalidomide enantiomers [83]. The CD spectra of individual incubation extracts were recorded in the "stopped flow" method [84,85] during achiral chromatography and compared with the calculated CD spectra obtained by various quantum chemical methods. The racemic reference compounds were separated on a Chiralpak AD column and their CD recorded from 200 to 350 nm as required for the comparison with the calculated spectra.

A series of enantiomers of [60]fullerene bisadducts possessing an inherent chiral addition pattern has been recently studied on Chiralcel OD and Chiralpak AD columns under

various eluting conditions [86]. The orders of elution were monitored by on-line CD detection and the absolute configurations of the collected enantiomers were determined by off-line CD spectra. This paper deserves to be mentioned since authors commenting the relationship between order of elution and absolute configuration concluded: " it seems to be difficult to estimate the absolute configuration only from the elution order of enantiomers under any elution conditions. The elution order is apparently dependent on the nature of addends and stationary phases rather than the absolute configuration itself, in contrast with CD spectra".

2.7.3. On-line examples of circular dichroism

A series of aryl–alkyl–carbinols have been resolved on a Pirkle ionic column and the eluted peaks were monitored by on-line CD detection. Application of the sector rule at 270 nm allowed the determination of the order of elution: the (*S*) enantiomer was the less retained enantiomer. This order fits the one determined on the base of recognition mechanism. For binaphthol derivative, the order of elution was determined applying the exciton model at 220 nm. In the case of the dibenzoate of *trans*-1,2-cyclohexanol separated on Chiralpak OT(+), the "dibenzoate chirality rule" was applied for absorption at 235 nm. The (*S*,*S*) absolute configuration was assigned to the less retained enantiomer. This seminal work showed that the absolute configuration of chiral eluates can be determined, on-line, by examination of the sign of the CD at a suitable wavelength. The choice of the wavelength is a key issue in this approach [87]. Finally, a further example illustrating the efficiency of chiral HPLC coupled to CD detection was the successful determination of the absolute configuration of synthetic pterocarpans [88].

3. Analytical chiral HPLC combined with chemical correlation methods for the determination of the absolute configuration

3.1. Overview

The chemical correlation method, a time-honored way to establish absolute configuration of enantiomer requires in its classical form rather large quantities of product and very clean purification methods. The principle of the chemical correlation method is whether to chemically transform the enantiomer of unknown configuration into one enantiomer of known configuration, or to start the synthesis from an enantiomer of known configuration to obtain the enantiomer of unknown configuration. Sometimes, depending on the reaction difficulties, the two approaches may be combined and in that case the comparison will be performed on a common intermediate. It is clear that the chemical transformations must not affect the chiral center or must transform it in a well-known stereochemical manner. In the classical method the sensor is the sign of the optical rotation.

The chemical correlation method has been strongly revitalized by chiral HPLC for two main reasons:

(i) Partial racemization is easily detected and quantified thanks to the enantioselection by the chiral support.
(ii) By-products are easily detected and discarded thanks to the separative properties of the support.

The chromatographic technique brings further advantage in allowing the use of far less products.

Additionally, chiroptical on-line detectors have further extended the application field and the user's comfort, however we will see that one has to take some precautions when using these detectors.

At this stage, an important remark should be made: in the classical chemical correlation method, the sequence of chemical reactions is performed on an optically pure or enriched material. Here, the optical rotation will be generally used for comparison and there is in principle no need to run the sequence of reactions on the racemate. However, in the case of coupling with the chiral chromatographic method, we do recommend to have at each step the corresponding racemate readily available either from syntheses or commercial sources.

Furthermore, this concept is fundamental for the sample on which the comparison is performed because the availability of both enantiomers is essential to validate the chromatographic chiral separation. Indeed, this is a method to check the clean separation of the enantiomers at each step or to decide at which step the comparison will be the most efficient.

Chiral HPLC may provide the affiliation between the source of enantiomers of known configuration and the same enantiomers resulting either from degradation of complex molecules or from new asymmetric processes. This is of course an obvious application, which is closely linked to the chemical correlation method. It is worth highlighting again here that chiral HPLC has an advantage over the classical chiroptical methods: its process combines both enantiorecognition and separation. Additionally, chiroptical methods require purification steps to prevent contamination by chiral intermediates or catalysts.

3.2. Case studies

Most of the studies follow this straightforward strategy. First, one has to find a chiral stationary phase and operating conditions which cleanly separate the racemate. Then, once the chiral HPLC separation is achieved, the order of elution can be easily determined or confirmed by injection of pure or enantiomerically enriched sample whose absolute configuration is well established. Finally, after this preparing work, the unknown isomer can be analyzed and directly determined from the comparison of its retention time with the reference enantiomer ones.

Several case studies illustrate well this concept. For example, the absolute configuration of 3-*N*,*N*-dimethylamino-

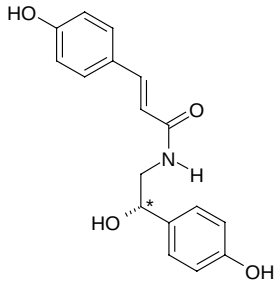

Fig. 4. Chemical structure and absolute configuration (*S*) of natural *N*-*p*-coumaroyl-octopamine found in potato tuber tissue as determined by chiral chromatography using a Chiral Ru-1 stationary phase.

3-phenylpropionic acid in taxuspines derivatives was determined by the comparison of the retention data with authentic samples. Retention times of standard 3-(*S*)- and 3-(*R*)-*N*,*N*-dimethylamino-3-phenylpropanoic acids were 14.7 and 15.9 min, respectively on a Sumichiral OA-5000 column (2.0 mM $CuSO_4$ in 98:2 H_2O/CH_3OH, flow rate 1 ml/min) and that of the hydrolysate was found to be 15.9 min [89].

Other examples are worth mentioning. In order to identify the absolute configuration of *N*-*p*-coumaroyl-octopamine found in potato tuber tissue, the synthesis was performed from *p*-coumaric acid and optically enriched octopamine. The separation was performed on Ceramospher Chiral Ru-1 (available from Shiseido) in pure methanol and the order of elution was determined. By comparing the chromatographic behavior of synthetic (enriched) and naturally occurring samples, the configuration of the natural product was found to be (*S*) [90] (Fig. 4).

A domain in which chiral HPLC contributes as an invaluable tool is the determination of the absolute configuration of amino-acid residues in complex molecules. For example, lyngbyabellin B (Fig. 5), an analogue of a potent microfilament-disrupter, was ozonized (−78 °C in dichloromethane) and subsequently subjected to acid hydrolysis (6 M HCl at 110 °C, 12 h). The hydrolyzate

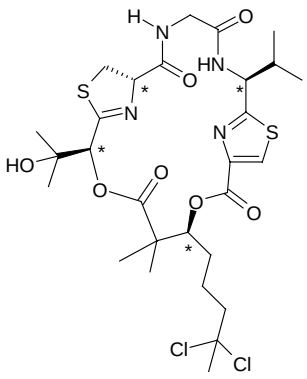

Fig. 5. Absolute stereochemistry of lyngbyabellin B. Hydrolysis of this complex molecule has been accomplished to obtain the corresponding amino acid residues and thus full absolute configuration was easily accessible by chiral HPLC.

was analyzed on a ligand exchange column containing *N*,*S*-dioctyl-(D)-penicillamine coated on octadecylsilanized silica (Chirex 3126 column or Sumichiral OA-5000 for the Japanese version, eluted with a copper sulfate solution). L-Valine and a D/L ratio of 3:1 for cysteic acid were identified by comparison with authentic samples. It is clear that a partial racemization occurred for cysteic acid under the very drastic condition of hydrolysis. However, the absolute configuration of the thiazolidine framework could be deduced from these results [91]. Obviously, this straightforward possibility to detect partial racemization is important in many fields and gives to chiral HPLC a decisive advantage compared to other methods.

In some cases, the absolute configuration of a multistereogenic center compound is determined from a degradation in several pieces. One excellent work on providing a method to address such a situation is the configurational assignment of antifungal cyclododecapeptide, lobocyclamide B which was established by a combination of chiral HPLC and Marfey's methods. In this example, L-valine and D-glutamine (as D-glutamate) amino-acid residues were again identified by chiral HPLC on a D-penicillamine-based column [92].

This method has also been used in several instances for the configurational assignment of natural peptides [93–95]. It is important to note that, for such peptide studies, commercially available chiral columns based on ligand exchange are perfectly suitable. Indeed, most 2-amino acids and 2-hydroxy acids have been evaluated on ligand-exchange CSPs and elution order of their enantiomers are today well known. We have already mentioned above some applications of a ligand-exchange chiral column based on D-penicillamine. Another notable example involves a ligand exchange chiral column composed of (L)-proline bonded to silica gel (Chiralpak WH) which was successfully used for the identification of (*R*)-2-hydroxyisovaleric acid from clavariopsin A hydrolyzate [96]. For anyone interested in learning more about this important area of research, see the recent review by Davankov [97].

Alternatively, the use of chiral crown-ethers was also proved successful as powerful enantioselective agents for the determination of the absolute configuration of amino-acids. Crownpak CR(+) (available from Daicel) is the most commonly used column based on a chiral crown-ether specifically designed for chiral analysis of primary amino groups. An example of an original application of Crownpak CR(+) is the determination of the absolute configuration of amino-acids found in the hydrolysate of some antifungal cyclodepsipeptides [98] or valinoctins A and B [99]. In view of its straightforward use, chiral HPLC may advantageously replace for such purposes the chromatographic achiral method, which consists in the derivatization of the amino acid into its *o*-phthaldialdehyde and (L) or (D)-cysteine derivative [100–103].

Hydrolyzates containing underivatized amino acids may be advantageously analyzed on macrocyclic antibiotic chiral

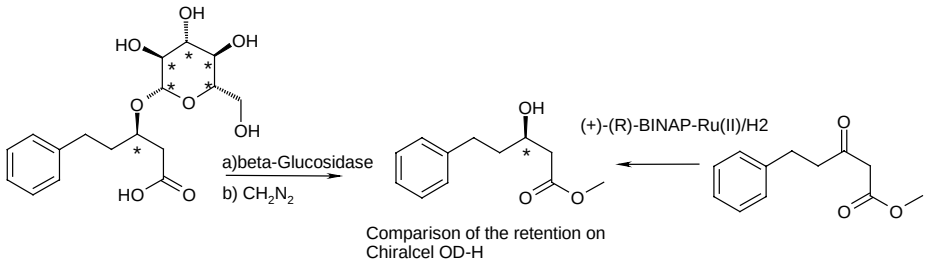

Fig. 6. Absolute configuration of methyl 3-hydroxy-5-phenylpentanoate contained in *Polygonum salicifolium* was elucidated using the known enantioselectivity sense of the asymmetric reduction of methyl 3-oxo-5-phenylpentanoate.

columns such as Chirobiotic T or Chirobiotic R from Astec. The separations and the orders of elution are perfectly established on these CSPs for natural and unnatural amino acids (vide infra).

Degradation method is not limited to large structures containing amino-acids or α-hydroxy-acid constituents. Triterpenoid saponins, dianchinenosides E and F, G and H are diastereomers, which differ by the stereochemistry of the 1,2-propanediol residues esterified to the C-23 of their aglycones. The propane-diols were obtained by $LiAlH_4$ reduction. They were converted into a primary tosylate and then subjected to co-HPLC comparison with authentic *R*-(−)- and *S*-(+)-propane-1,2-diol tosylates on a chiral column (Chiralcel OC). The propanediol fragment in dianchinenoside E and G have a (*R*) configuration, the (*S*) configuration is found in dianchinenoside F and H [104].

Asymmetric synthesis of β-amino alcohols of moderate enantiomeric purity were obtained from aldehyde enamine, hydroboration and oxidation processes. The absolute configuration of the preferred enantiomer was established by chiral HPLC comparison of the retention with authentic β-amino alcohols prepared from chiral epoxides of known absolute configuration [105].

The absolute configuration of methyl 3-hydroxy-5-phenylpentanoate (Fig. 6) obtained by enantioselective reduction of the corresponding 3-oxo derivative was inferred from the known mechanism of reduction by (+)-(*R*) or (-)-(*S*)-BINAP-Ru(II)/H_2. Comparison of the chiral HPLC data (Chiralcel OD-H column) of the prepared methyl 3-hydroxy-5-phenylpentanoate and those of the natural one found in the constituent of *Polygonum salicifolium* provided the absolute configuration of the natural derivative [106].

The degradation method and the obtention of optically pure reference compounds may be quite complex. An illustration of such a difficulty is the investigation of the absolute configuration of the pseudodistomin C [107]. In this work, the authors were able to disclose the absolute configuration of this natural piperidine alkaloid using a chiral HPLC analysis on a Chiralpak AD column. HPLC retention time of a tetraacetate sample directly derived from the natural compound (shown in Fig. 7) was compared with those of two synthetic D- and L-tetraacetates of known configuration and found to have the same retention as the D enantiomer. These synthetic references D- and L-tetraacetate were prepared in 16 steps from the oxazolidine homoallyl alcohol itself derived respectively from D-serine or L-serine. The "natural" tetraacetate was obtained by ozonolysis, reduction and peracetylation.

Next example is from our laboratory work and concerns chiral separation of 1,2-diaminocyclohexane derivatives [108]. Starting from the commercially available technical mixture of 1,2-diaminocyclohexane (*RR*, *SS* and meso forms in very similar amount), the diimine derivatives were prepared by reaction with salicaldehyde (Fig. 8). Injection on Chiralcel OD-H or Sumichiral OA-2500 chiral columns afforded three-well resolved peaks. Since the three peaks are of similar surface, on-line polarimetric detection was used to identify the meso form which happened to be eluted second on Chiralcel OD-H and third on Sumichiral OA-2500. The first eluted enantiomer presents the same sign of rotation on both CSPs, and since the eluent is the same, it is the same stereoisomer which is eluted first. Injection of authentic sample formed from (*R*,*R*)-diaminocyclohexane shows that the (*R*,*R*) form corresponds to the minus isomer which is eluted in second. In summary on the Chiralcel OD-H column the first peak corresponds to the (*S*,*S*) configuration, the second one to the meso arrange-

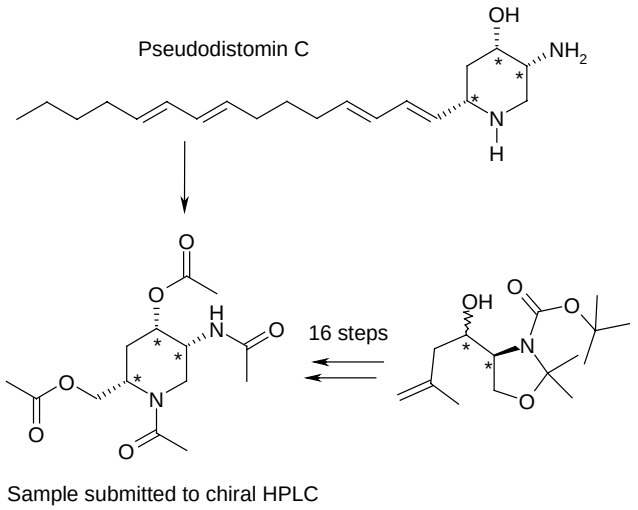

Fig. 7. Absolute configuration of the pseudodistomin C was determined via its corresponding tetraacetate derivative and two synthetic D- and L-tetraacetate of known configuration obtained in 16 steps.

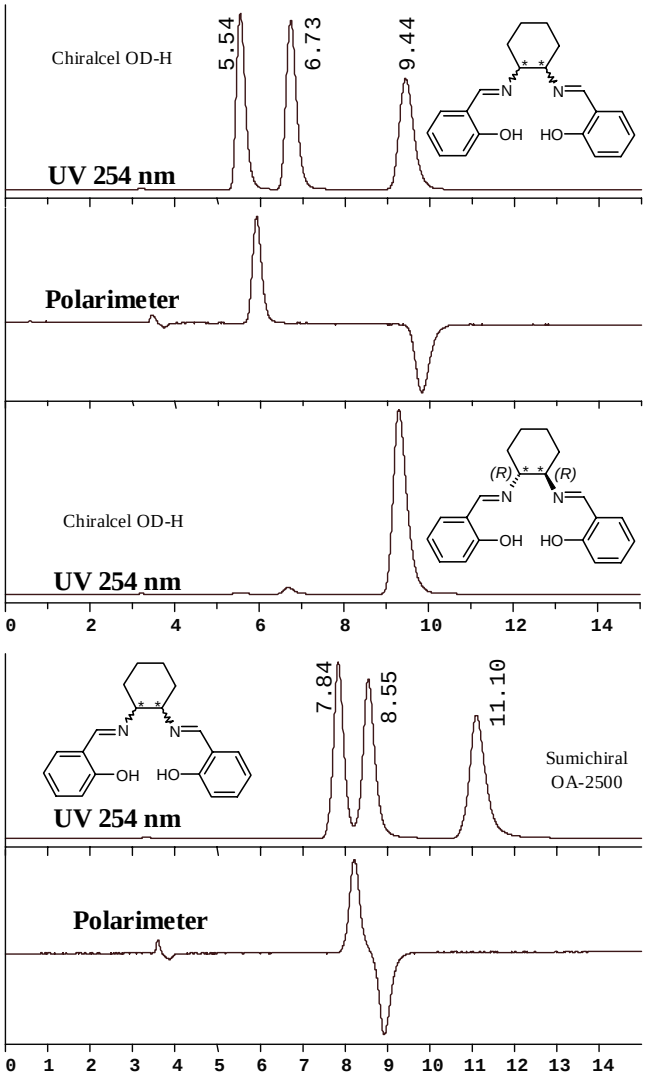

Fig. 8. A Chiral HPLC process for the determination of the absolute configuration of 1,2-diaminocyclohexane (author's unpublished work). Sample was derivatized into diimine derivatives and subjected to chiral analysis on two different CSPs (Chiralcel OD and Sumichiral OA-2500) using the same eluent. The elution sequence of (*RR*), (*SS*) and meso stereoisomers was well established and corroborated by the independent injection of the authentic (*RR*) standard and direct match of elution times and sign of rotation provided by the on-line polarimetric detection. This method has been found attractive for any 1,2-diaminocyclohexane derivative.

ment and the third one to the (*R*,*R*) configuration. The order of elution is thus established on both chiral phases. The absolute configuration of any 1,2-diaminocyclohexane derivative arising from an enantioselective process will be immediately determined provided it can be chemically transformed into the reference diimine. This holds for instance for any of the resolution process of technical diaminocyclohexane.

As a result, Chiral HPLC combined with chemical correlation method represents a very powerful tool in the determination of the absolute configuration of natural products as well metabolites which are available in minute quantities. In these cases the chemistry on natural samples shall be at minimal.

3.3. Case studies involving a chemical transformation

In other strategies, enantiomers of unknown configuration are resolved from rac-synthetic material by semi-preparative chiral HPLC. They are chemically transformed into enantiomers of known configuration. In a classical way, the comparison with authentic samples can be based on any of the chiroptical properties such as optical rotation, CD or VCD. However, as stated previously, these methods require purified samples and significant amount of compounds. This is why chiral chromatography may be advantageously chosen to perform the comparison with authentic samples. A prerequisite is that the enantiomers of the reference sample can be conveniently separated on a chiral column, and this study shall come first.

We have chosen to illustrate this methodology with an example dealing with the stereochemistry assignment of fruit-piercing noctuid pheromone. The resolution of model insect pheromonal cis-monoepoxy racemates derived from (*Z*,*Z*)-6,9-dienes was achieved on a Chiralpak AD column for both 6,7- and 9,10-epoxides with a C17–C23 straight chain. In order to determine the absolute configuration, the isolated enantiomers were totally hydrogenated and their retention on Chiralcel OJ-R CSP compared to the retention of enantiomers of known configuration independently prepared by a Sharpless epoxidation reaction. Utilizing these results, it was established that an abdominal tip extract of one fruit piercing moth included (9*S*,10*R*)-(*Z*)-9,10-epoxy-6-henicosene as a main sex pheromone component [109]. A similar methodology was used for mulberry looper sex pheromone [110].

These examples are instructive since:

(i) Two different chiral columns were used: one for the semi-preparative work and another for the analytical determination.
(ii) The reference enantiomers of known configuration were obtained by asymmetric catalysis with 80–85% e.e. Chiral chromatography avoided further enantiomeric purification.
(iii) Minute amount of the natural pheromone sample was used. It is worth noting that these experiments were performed without the use of a chirality detector.

In an other example, the determination of the absolute configuration of the optically active 3-methoxy-4-hydroxyphenylglycol (MHPG) purified from human urine was established by a combination of chemical correlation and chiral HPLC. (D)-MHPG was obtained by chemical transformation of (D)-norepinephrine. Comparison of the retention time obtained on a Chiral Ru-1 HPLC column for synthetic rac-MHPG, synthetic (D)-MHPG and optically active MHPG isolated from human urine respectively, allowed a straightforward determination of absolute configuration of the latter product [111]. The authors drew the attention on

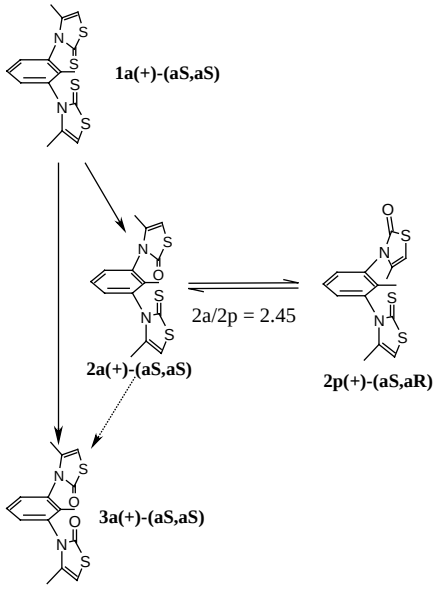

Fig. 9. Interconversions through stereospecific reactions (arrows stereospecific reaction, dotted arrows possible stereospecific reactions); interconversions through rotation equilibrium around C–N pivot bond (double arrows).

the sensitivity of retention times to various conditions (in the present case, the amount of water in the eluent and the sample), it is a very general remark and we will recommend co-injection of the products as a final checking in case of small difference in retention times (see for instance the section on inversion of elution order).

We recently reported [112] a combination of chiral HPLC with polarimetric detection in the determination of absolute configuration by chemical interconversion method. The method used the affiliation between signs of rotation of polarized light during chemical interconversion which occurred with conservation of the absolute configuration and also during known rotation around a single pivot bond. First, suitable chiral column and conditions for the simultaneous separation (one injection) of the enantiomers of all the inter-converting racemates were found. This separation was monitored by UV and polarimetric detections. Then, the (+) enantiomer of the trans-bis-thiazoline thione **1a** (Fig. 9) was transformed at room temperature into thiazoline-thione thiazolin-one derivative **2a** and bis-thiazolin-one **3a**. All of them were found to correspond to the (+) enantiomer. The absence of racemization during the inter-conversion process is easily checked by comparing the chromatogram of the resulting mixture with the chromatogram obtained for the racemates. On heating, single rotation around the pivot bond of the thiazolinone heterocycle in *trans*-**2a** afforded the (+) enantiomer of the *cis*-**2p** derivative whereas **3a** racemized. The absolute configuration of the starting (+)-**1a** enantiomer was determined by X-ray and thus the absolute configuration of the three related enantiomers was derived as indicated in the figure. The use of chiral HPLC in tandem with a chirality detector gave a decisive advantage since such correlation can be performed on a mixture of a very limited quantity of compounds. The chemical transformations leading to **3a** from **1a** via **2a** are not complete, some of them being to some extent reversible and thus a mixture of **1a**, **2a** and **3a** can be analyzed without tedious purification steps. Another particularly interesting affiliation of the sign of rotation has also been used in the study of DDT compounds [113,114].

Caution: When a method relies on the sign of rotation of the polarized light monitored on line by a polarimetric detector, it is important to recall that the observed signal is the sign of the enantiomer in the eluting solvent [63]. Francotte [115] and our group [63] have shown that one should be careful when using polarimetric detection since an apparent inversion of elution may be advocated whereas the change of the sign is simply due to the solvent-inversion of the sign for the same enantiomer. It is clear that the use of the chemical correlation method monitored by polarimetric detection requires a determination of the sign of rotation of the enantiomer of known configuration in the solvent of elution. Furthermore, some commercially available polarimeters are monitoring a full range of wavelengths at the same time, which may lead to confusing results in the case of anomalous ORD curve [63,116]. Thus, it is not sufficient to perform the measurement at a single wavelength; ORD curves are preferred.

Obviously, chiral HPLC combining the advantages of a separation technique and an enantioselective process had a decisive impact on the determination of absolute configuration by providing chemically and optically pure enantiomers in a relatively fast manner.

4. Determination of the absolute configuration from the retention data obtained on CSPs

In the previous sections, liquid chromatography on chiral support was mainly used to provide optically pure enantiomers for which the absolute configuration was determined using various methods. Chiral HPLC proved an inestimable tool in this respect, since analytical methods can be easily scaled up to semi-preparative separation if necessary. The main issue was to find a column and conditions suitable to achieve a good separation whatever the mechanism of chiral recognition.

If one goes back to the fundamental aspect of the separation of enantiomers on chiral stationary phases by HPLC, it is well known that chiral separation between two enantiomers will occur when the diastereomeric labile complexes formed with the chiral support differ in stability. The enantiomer which gives the complex of higher stability will be eluted in second. In fact, the picture of a single complex for each enantiomer is obviously an oversimplification. Chiral separation will occur when the summation of the energies of the various complexes between the support and one enantiomer will differ from the summation of the energies corresponding to the complexes between the same support and the

opposite enantiomer. The energies and the spatial arrangement of these complexes are depending on the solvent which is used for the elution. Thus, if one can estimate which enantiomer interacts more strongly with the chiral support in the conditions of analysis, the absolute configuration can be determined from the simple consideration of the elution order. This is a fundamental principle which holds for all hundreds of CSPs (more than 1300) which have been described in the literature and whatever their mode of chiral recognition.

The result from this introduction is that the retention of the two enantiomers shall be available and examination of the two set of complexes must be considered. *Chiral chromatography will never be able to assign the absolute configuration of a single enantiomer without information about the retention of the second one*. Therefore, it will not be possible to determine the absolute configuration of a single optically pure product coming, for instance, from an extraction process of natural source or from highly enantioselective synthetic process without comparison with the analytical data of the corresponding racemic sample.

This very optimistic introduction must be tempered by considering the very small difference in Gibbs free energies corresponding to a successful baseline separation. An easily observable separation corresponding to $\alpha = 1.05$ entails a difference in energy of 0.12 kJ/mol. This is in sharp contrast with the difference in diastereomeric transition state energies encountered in asymmetric catalysis in which a 98% e.e. corresponds to a 12 kJ/mol difference.

The retention factors k_1 and k_2 reflect the interaction of the first and second eluted enantiomer with the chiral support under the chromatographic conditions (solvent, temperature, flow, achiral support if any). These retention factors are related to the Gibbs free energy. $\ln\alpha$ is related to the difference in Gibbs free energies:

$$\Delta G_1^\circ = -RT\ln k_1, \qquad \Delta G_2^\circ = -RT\ln k_2,$$
$$\ln\alpha = \ln\left(\frac{k_2}{k_1}\right) = \frac{\Delta G_2^\circ - \Delta G_1^\circ}{RT}$$

In the complexes between a support and one enantiomer, any classical interactions operating between molecular objets may develop and contribute to the adsorption energy. Among them, dipole–dipole interaction, hydrogen bonding, charge-transfer (π–π) interaction, van der Waals forces and CH–π interactions are often involved. All these interactions are distance dependent. Some of them are operating at short distances and they vanish quite rapidly as soon as the interacting groups are distant. Some others are still active in the long distance range. For instance, electrostatic attraction may be used for a long-range recognition between positively and negatively charged part of the analyte and the support respectively. These remarks gave rise to the concept of "point of interaction" or "interaction site" between the selector and the selectand. For instance, an hydrogen bond accepting site on the support may interact with one hydrogen bond donating site on the enantiomer. In an amide group, the N–H hydrogen is prone to develop an hydrogen bond with an accepting group such as the oxygen of a ketone or amide.

As soon as one approaches each enantiomer with the chiral support, a pair series of diastereomeric complexes can be formed with different energy contents. The resulting energies in the complexes must be different whatever the number of interacting groups since the geometric relationships between all the constitutive atoms of the complexes will be different. These changes in geometric relationships are not limited to the intermolecular part but they may also come from an intramolecular contribution, since conformational changes of the chiral selector and/or the bonded enantiomer may occur in the complexes. The difference in energy may be insufficient to give rise to an observable separation in the case of too weak discriminating interactions, or if different complexes with opposite sense of stereoselectivity interfere and thus may potentially lower the global enantiorecognition.

As we already indicated, more than 1300 different CSPs have been described in the literature and new ones will continue to appear [117]. Several attempts to sort these CSPs into four or five classes have been proposed in the literature [118] and are still in use [119]. These classifications are generally established according to the nature of the interactions offered by the host selector (CSP) to the guest selectand (enantiomers) with a strong emphasis on its capability to engender or not inclusion interactions. In our opinion, this conception might be somewhat artificial and to some extent confusing since the same type of interactions will be operating in very different chemical classes of support.

We prefer a classification of the CSPs into two categories [120–122]: molecular CSPs ('brush type') and polymeric CSPs. This classification embraces the concept of "independent" chiral selectors in opposition to "cooperative" chiral selectors. In the first category, the selector can be a very simple chiral molecule such as (*R*) or (*S*)-1-(1-naphthyl)ethylamine or a much more complex one as vancomycin or teicoplanin antibiotics but always presents a well-defined and delineated molecular structure. Usually linked to a silica inert support, this well-defined structure will operate on its own and independently from the next anchored chiral selector which will duplicate the same molecular recognition behavior. In these CSPs, a single active site or several sites can be identified and molecular modeling can be used to scan the different conformational states of the selector. A molecular selector which is attached to a polymeric structure in such a way that it will not be in close proximity of the next molecular selector gives also rise to a molecular CSP. These selectors will also operate independently from each other. This is the case for instance of the poly-Whelk-O1 [123–125] in which the chiral selector is the same as in the classical Whelk-O1 column, but is attached to a polymeric siloxane backbone.

In the second class, the CSP is composed of synthetic or naturally occurring chiral polymers, which have been functionalized. Synthetic polymers will be for instance

a poly(meth)acrylamide obtained by polymerization of methacryloyl-substituted optically pure amines. Naturally occurring chiral polymer examples are polysaccharides such as cellulose and amylose which led to the well known Chiralcel and Chiralpak series from Daicel. A large number of chiral centers are in a close proximity and thus the three-dimensional structure of the polymer will play a decisive role in the multiplicity and geometry of the chiral sites. The three-dimensional structures are strongly dependent on the mode of coating on the support [126], the type of support, the swelling or surface modifications caused by the solvent of elution [127], the temperature (phase transition) or the memory effect of the previous uses of the selector. Furthermore, if theses polymeric structures are bonded to silica, they will exhibit different chromatographic behaviours and results depending on the mode of bonding [128]. These difficulties arising from the three-dimensional structure modifications and the covering of the polymer may also be illustrated by the inversion of elution order for some derivatives when using dried or wetted hexane alone or in mixture with alcoholic modifier on Chiralcel OD [129,130].

Some very minor modification of analysis conditions may also affect the chiral separation. Nakamura et al. [131] reported that the separation of an α-methylketone on Chiralcel OD column using hexane as eluent is lost if the sample is prepared in pure hexane without addition of 10% diethyl ether. We have all sometimes experienced such dramatic incidence of the solvent used during sample preparation on the Chiralcel or Chiralpak phases and this behavior is far out of reach to be rationalized in terms of chiral recognition mechanisms. Similar difficulties are revealed in studying the effect of solvent of elution on the three-dimensional structures of the polymeric phases. This can be well illustrated by the inversion of elution order observed when the modifier or the solvent of elution is changed [132–134]. Inversions of elution order occurring during chiral separations have been recently reviewed by Okamoto [135,136] and we recently drew the attention on the true or apparent inversion of elution using on line polarimetric detection [63].

As a result, the variability of the three-dimensional structures and the resulting occurrence of several interdependent chiral sites in these polymers may be at the origin of their remarkable range of successful applications in terms of molecular diversity but on the other hand we feel it will be a serious limitation for the prediction of the order of elution with a high probability of success and in an efficient way.

Nevertheless, in a very interesting study, Yashima et al. used a combination of NMR studies of soluble cellulose phenylcarbamate derivatives together with molecular modeling to propose a convincing chiral discrimination rationale for some of these polymeric CSPs and a few selectands [122]. This cornerstone paper and other molecular modeling from the same group [137–140] have been largely discussed in a review by Lipkowitz [119]. Remarkable results were obtained in the comparison of calculated interactions with the experimental data showing that the calculation is a possible tool for the prediction of the absolute configuration. Unfortunately these are heavy calculations and the number of examples is limited. In fact a simple model working for a large series of derivatives is not yet available. NMR is particularly useful to foresee the interacting points between selectors and selectands but the transfer of the results from solution or solid state NMR to actual polymeric phases might prove difficult generally because of the limitations already mentioned in the previous examples. This transfer is much easier for molecular CSPs, and as it has been already pointed out, NMR studies have been widely used to determine the absolute configuration of the more interacting enantiomer (vide supra).

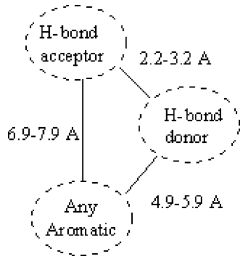

Fig. 10. Illustration of the "enantiophore concept" applied to Chiralcel OD. This model is built from the extraction of the most frequent common structural features found in a data set of three-dimensional molecules resolved on Chiralcel OD column.

We recently proposed the concept of "enantiophore" for these polymeric phases. This concept is somewhat derived from the topological pharmacophore concept. In our case, the goal is to extract the most frequent common structural features which may be relevant for enantioselectivity. We have called such invariants enantiophores. For Chiralcel OD the enantiophore model (Fig. 10) was inferred from a large set of three-dimensional structures taken from a three-dimensional version of CHIRBASE database. Through a three-dimensional flexible structure search it was found that 94% of the structures having a conformer fitting the enantiophore requirement were separated. Since most of the data used in this study were unfortunately available without the relevant absolute configurations associated to the first and second enantiomer respectively, the proposed enantiophore is not chiral. An aromatic group (π), a hydrogen bond donating center (HBD) and a hydrogen bond accepting center (HBA) separated with some distance flexibility are situated at the three corners of a triangle which cannot be used for the prediction of the elution order, since it is achiral. However, one may intuitively anticipate that a refinement of this enantiophore model, taking into account the order of elution when available on a large set of diverse structures fitting the model, will indicate if the enantiophore triangle is adopted (satisfied) in the sense (π) → HBD → HBA or (π) → HBA → HBD for the more retained enantiomer. Thus, if we can orient the corners of the triangle, the order of elution in terms of absolute configuration may possibly be available in a rather simple manner with a good

probability. This approach may prove useful but at present we believe it will be always associated with a probability without a claim for total confidence. One limitation of this approach comes from the possible occurrence of remote effect of the groups which are situated far away from the chiral center of the enantiomer and which may interact in an unpredictable manner with the three-dimensional backbone of the selector. These remote interactions are far less common in the molecular selectors based on simple molecular framework provided that they are attached to the inert support by an adequately designed linker.

In the present state of the art the chiral recognition models aiming at the prediction of the elution order are much more developed for molecular CSPs. The chiral selectors are seemingly simpler to model and in several cases they have been designed through a rational approach in order to provide the different interaction points.

However, as a general rule, prediction of the elution order through sophisticated or simple modeling will be difficult in the following cases:

(i) High conformational flexibility in the selectand and (or) the selector.
(ii) Presence of competitive sites on the selector and (or) the selectand.

Flexibility and competing interaction sites are not favorable for an easy guess of the structures and relative energies of the different diastereomeric complexes.

Let us now take as a case example, the well known 3,5-dinitrobenzoyl-phenylalanine (DNBPG) covalently bonded to aminopropyl silica (Fig. 11) pioneered by Pirkle's group, and the series of CSPs based on other amino acids such as leucine, tyrosine, alanine, valine with the same linker and same derivatization. In these selectors, several well defined interaction points are offered to the chiral ligand: two dipole amides, two hydrogen bond donating and accepting sites generated by the NH and C=O of the amide groups respectively and a π accepting group.

These CSPs are rather flexible, a property which has been advocated in molecular CSPs to offer much more possibilities to accommodate a larger range of selectands and they offer two amide groups in competition for hydrogen bonding or dipole interaction with the selectand. Under these circumstances, chiral recognition models are generally proposed to account for the results of elution order but are hardly predictive. As an illustrative example, the chiral separation and order of elution of a naphthylamide derivative on DNBPG was explained by the interaction model reported in Fig. 12 and it was stated in the text that " detailed discussion would require knowledge of the conformational preferences of both CSP and solutes" [141].

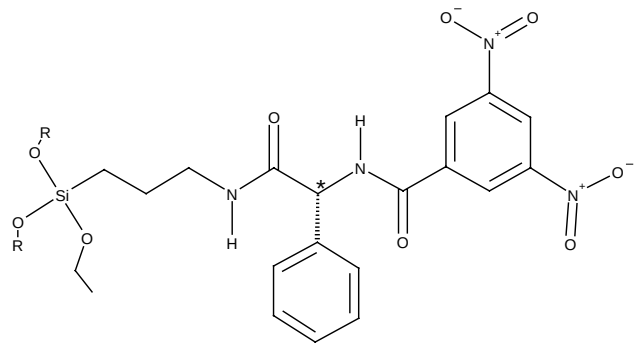

Fig. 11. The so-called (D)-Pirkle DNBPG covalent CSP. This molecular CSP, based on 3,5-dinitrobenzoyl-(D)-phenylalanine, possesses well identified interaction sites via the amide moieties (H-bond donor and acceptor sites) and the aromatic rings.

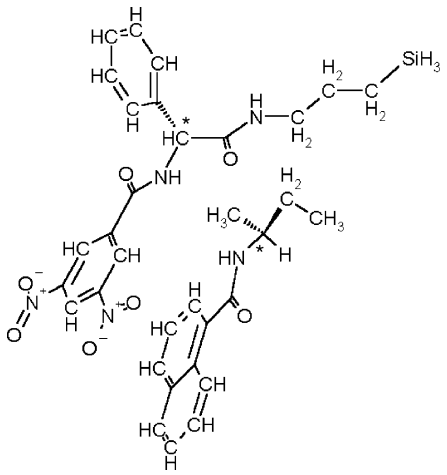

Fig. 12. A chiral recognition model supposed to explain the sequence of the elution order of *N*-(1-naphthoyl)-2-butylamine on the Pirkle DNBPG covalent CSP.

Such an occurrence of several competing sites on the selector is also well exemplified for other brush-type CSPs. This is especially the case of most of the CSPs available from Sumika and referenced as Sumichiral trade name. As exemplified in Fig. 13, the Sumichiral family exhibits different types and combinations of functional groups and stereogenic features.

At this point, it is particularly interesting to compare two chiral selectors: the one found in the Sumichiral OA-2500 and the one used in the design of the Whelk-O1 (see Fig. 14). These two CSPs are both able to separate some free carboxylic acids [142,143], a possibility which is rarely encountered in the brush-type CSPs. They both present a naphthyl π donating group and a dinitrobenzoyl π accepting group which are separated by the same number of bonds, and amide functions. All these elements are recognized as potential points of interaction. However, Sumichiral OA-2500 is much more flexible than the Whelk-O1 and presents two competing amide sites. The flexibility in OA-2500 is detrimental to the perpendicular arrangement of the two π accepting and donating groups which is observed in Whelk-O1. In addition, the occurrence of the two amides in competition renders even more difficult the claim of an effective similitude of the two selectors towards an enantiomeric solute.

In Whelk-O1, conformational rigidity reinforced by the pseudo-axial position of the single amide group and the

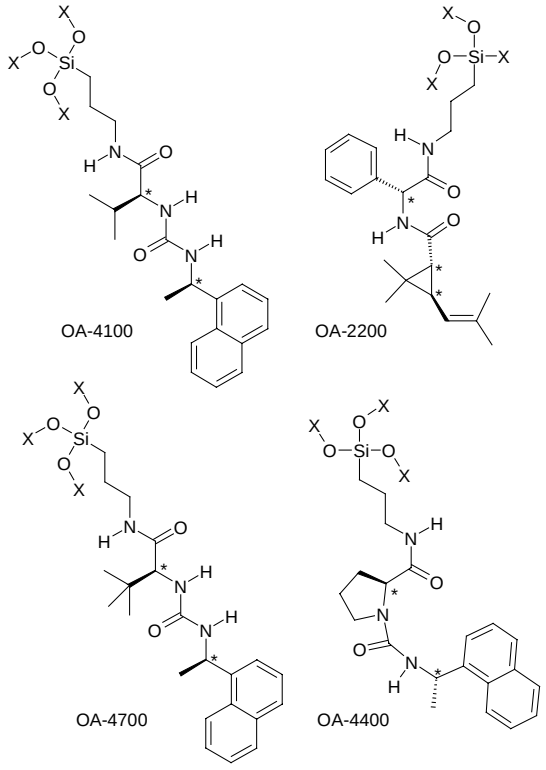

Fig. 13. A selection of commonly-used chiral selectors in the design of Sumichiral CSPs. These CSPs belong to the molecular CSP category (brush-type).

absence of competing sites is a considerable advantage for a priori proposal of a chiral recognition mechanism for various enantiomeric compounds. We have reported in Fig. 15 a cartoon representation of the two enantiomeric versions of the Whelk-O1 CSPs. In this cartoon, the pointing hydrogen of the amide group **a**, the π accepting group **c** and the π donating group **b** are schematized. The right side of the cartoon corresponds to the (3*R*,4*S*) Whelk-O1 and the left side corresponds to the (3*S*,4*R*) which is the opposite enantiomer version of the CSP. The hydrogen **a** is situated into the plane of drawing, the π donating group **b** is situated up to the plane and the π accepting group **c** down to the plane. This arrangement mimics the minimized conformational state of the selector.

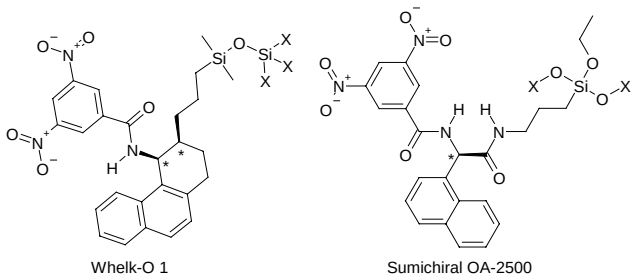

Fig. 14. Whelk-O1 and Sumichiral OA-2500 have similar structural features. Even so, Whelk-O1 has demonstrated exceptional degree of enantioselectivity for a very broad range of enantiomeric solutes.

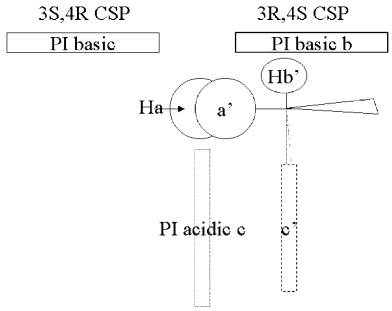

Fig. 15. A general model representing the chiral recognition mechanism of the Whelk-O1 for various enantiomers. *CSP features*: **a**, hydrogen of the amide group; **b**, π donating group; **c**, π accepting group. *Interacting enantiomer features*: **a**′, hydrogen bond accepting group; **c**′, π donating group; **b**′, a hydrogen able to form a CH–π interaction.

For enantiomeric solutes presenting a rigid conformation, the enantiomer having at the chiral center a π donating group **c**′ interacting with the π accepting group **c** (face to face interaction) [144], a hydrogen bond accepting group **a**′ involved in an hydrogen bond with **a** and a hydrogen **b**′ forming CH–π interaction with **b**, will be the more retained on the (3*R*,4*S*) CSP and the less retained on the (3*S*,4*R*) CSP. In this figure, the enantiomer interacting favorably with the (3*R*,4*S*) Whelk-O1 column has been incorporated.

This model holds for aryl lactones [145] and their benzoanalogues [146], 2-aryltetrahydrofuran [145], 2-arylcyclanones [145], 5-phenylthiazolidine-2,4-dione [147], *N*-Boc-2-phenylpyrrolidine [148], 2-aryloxiranes [149], arylbicyclic ether [80,144], arylhydantoins [150].

Rigid conformations are encountered in aryl allenic acids and their ester or amide derivatives. These compounds are nicely separated on Whelk-O1 [151] and the model already described above in Fig. 15 can be applied if one reduces the axial chirality to a chirality center. Conformational effects in amides and bulky esters reduced the enantioselectivity but did not change the order of elution.

Highly rigid conformations are also found in *N*-aryl-heterocyclic atropisomers such as *N*-arylthiazoline-2-thione or *N*-arylthiazolinones derivative (Fig. 16).

In these compounds the *N*-aryl group and the heterocycles are situated in two orthogonal planes. The R substituent on the aryl ring will be situated outside of the cleft in the more retained enantiomer according to Fig. 16. The heteroatom X is forming a H bond with the NH of the selector, face-to-face (π–π) and face-to-edge (CH–π) interactions [152] are quite satisfied.

Double substitution in o, o′ positions reduced the separation. A large variety of these atropisomers have been separated in the literature with an order of elution consistent with the model [153]. However, we have recently detected some exceptions in the case of some 1,3-bis-thiazoline(thi)one toluene derivatives of known absolute configurations [112].

For enantiomers having higher flexibility, i.e. which are able to present several conformational states, the models are more difficult to apply in a simple manner. We can say that

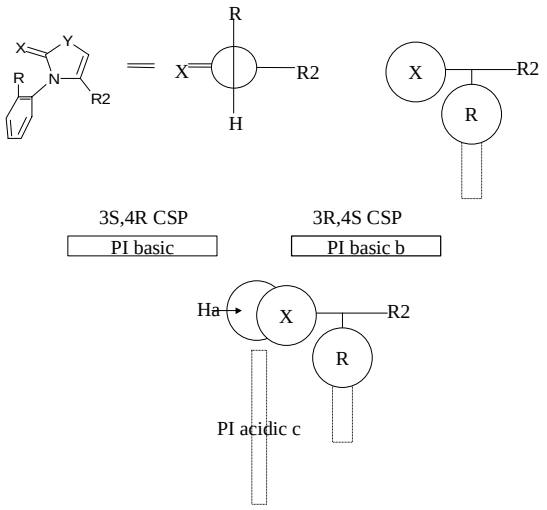

Fig. 16. A general model representing the chiral recognition mechanisms of *N*-aryl-heterocyclic atropisomers on the Whelk-O1 CSP. Upper part, construction of a schematic representation of axially chiral N-aryl-heterocyclic atropisomers (X = heteroatom O or S) showing that the axial chirality may be transposed to a chirality center, bottom fitting to the CSP model of Fig. 15. **a**, **b** and **c** as in Fig. 15.

in these cases, simple consideration on the conformational states may lead to wrong results and the confidence level in the prediction will decrease with increasing flexibility. However, the model described in the previous cartoon of Fig. 15 was shown to operate for α-aryl-α-hydroxymethane phosphonates [154], free naproxen [155], imine derivative of amino acid ester [156], amides of 2-aryloxypropionic acid [157]. Some exceptions were reported [158], for 2-arylpropionic acid amides [157,159] and pivaloyl or acetyl phenethyl amide [160,161].

In the case of flexible molecules, the conformation of the enantiomers will be optimized by molecular modeling calculation. The preferentially populated conformations are approached by computer to the rigid selector in order to estimate which enantiomer will develop interactions with the three interacting sites of the Whelk-O1 column. One assumption is that both selector and enantiomer do not undergo conformation changes in the diastereomeric complexes. Recently, Wolf et al. [162] used PM3 calculations for several aryl primary amines derivatized with Boc or Z group. The experimental differences in Gibbs energies were between 0.07 and 2.2 kJ/mol, however for the seven studied compounds, the order of elution was correctly predicted without using minimization of the energy in the diastereomeric complexes. More complex calculations including located or Monte-Carlo approaches offer a better description of the difference in energy of the two series of diastereomeric complexes [163].

A decade ago, several CSPs based on macrocyclic antibiotic were introduced by Armstrong's group [164,165] and their uses have been recently reviewed [166]. Vancomycin, teicoplanin and ristocetin were covalently bonded to silica and marketed by Astec under the trade-names Chirobiotic V, Chirobiotic T and Chirobiotic R, respectively. They can be operated with a polar organic mobile phase (POM) or with a reversed phase mode (RPM). A large number of compounds have been successfully separated on these selectors. Chirobiotic T and Chirobiotic R were found particularly suitable for the chiral analysis of underivatized amino acids and dipeptides. A "point of interaction" is clearly identified: teicoplanin and ristocetin have in common a primary amino group which under protonation offers an anchoring group for the ionized carboxylate group of the amino acids. It has been observed that the order of elution of naturally occurring amino acids on Chirobiotic T is L before D with no exception and the D-terminated dipeptides are more retained than the L-terminated ones [167]. For unnatural α-amino acids, the elution sequence is also L enantiomer before D enantiomer and for β-methyl α-amino acids having two stereogenic centers, the enantiomers eluting second have the R configuration at the carbon atom adjacent to the carboxyl group [168,169]. The same observations hold for Chirobiotic R however some exceptions have been observed for a couple of dipeptides and dansyl-amino acids [170,171]. Some dependence of the elution order with the elution mode have been observed in the case of Chirobiotic R [172]. Chirobiotic T can be used to determine the absolute configuration of α-amino acids with a high confidence level if one applies the aforementioned observations. However, we believe that further efforts should be devoted to provide a simple predictive model demonstrating the other points of interaction.

The enantioseparation of amino acids and hydroxyl acid by chiral ligand-exchange chromatography (CLEC) has been recently reviewed. [97,173–175]. It involves the formation of well defined square planar Cu(II) complexes between a chiral selector (generally *N*,*N*-substituted amino acid) and the selectand. These well defined complexes are appropriate for the prediction of the more retained enantiomer for bifunctional or trifunctional ligands. The selectors have been bonded to different supports such as polystyrene type resins or silica. In another approach *N*-alkyl-(L)-hydroxyproline has been coated on achiral RP silica, the alkyl group being the anchoring group. Chiral recognition model shown in Fig. 17 is reliable for the prediction of the configuration of the more retained enantiomer on that CSP. The D-enantiomer

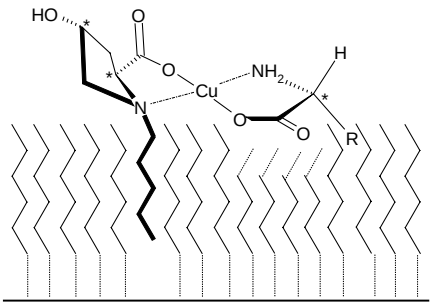

Fig. 17. A general model representing the chiral recognition mechanism of R amino acid by CLEC on *N*-alkylhydroxyproline coated on RP silica.

of the selectand with a R lipophilic group will be the more retained due to the stabilizing interaction developed with the alkyl chains of the RP silica.

In conclusion, the confidence in the absolute configuration of enantiomers determined from their elution order on a CSP and based on simple model of recognition decreases as soon as the selector and the enantiomer may adopt several low lying conformations which can be further modified by solvent effect. In case of flexibility, sophisticated calculations taking into account the solvent shall be undertaken. The cost-effectiveness and confidence level of these calculations shall be compared to other methods of determination of absolute configuration.

5. Conclusion

We have reviewed the importance of chiral HPLC in the investigation of absolute configuration. Methods through which chiral HPLC can be coupled have been described. It has been seen that there are some excellent works on providing original methods and strategies for exploring the stereochemistry of molecules. An important consideration is that chiral HPLC can be introduced in different ways and at different stages of the process. We have seen for instance that chiral HPLC can be extremely useful for the minute amount preparation of highly enantiomerically pure enantiomers. In many cases, the availability of stereochemically pure enantiomers is a crucial step. On the other hand, chiral HPLC can provide outstanding achievements in the chemical correlation procedures. It has also the advantage to overcome one of the main problems of complex asymmetric synthesis, since it allows to detect easily any possible racemizing conditions that are detrimental to a reliable assignment of absolute configuration. Finally it is worth recalling that chiral HPLC does not only afford the separation. It also proceeds through chiral molecular interactions and thus gives access to structural information about each individual enantiomers.

Another area of developing interest is the prediction of absolute configuration based solely on the chromatographic experiments and modeling study. This concept has also been a key theme of this review. There are still today a number of reasons, such a prediction remains very difficult to obtain. More extensive studies providing reliable results about the elution order may greatly contribute to improve the models. This should be an important part of the research efforts in the future.

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