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State-of-the-art and recent developments of immobilized polysaccharide-based chiral stationary phases for enantioseparations by high-performance liquid chromatography (2013-2017)

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Abstract

Polysaccharide-based chiral stationary phases have been recognized as one of the most powerful ones for high performance liquid chromatography (HPLC) separations of chiral compounds in analytical and also in preparative scale.

Immobilized polysaccharide-based chiral stationary phases constitute a remarkable achievement due to their stable nature on working with standard or common solvents and also with those prohibited for using with coated phases.

This review is mainly focused on the *i.* applications of these chiral stationary phases in numerous fields of HPLC separations; *ii.* comparative aspects between immobilized *vs.* coated polysaccharide-derived phases, and *iii.* revision of several theoretical studies such as enantiorecognition mechanism, mobile phase composition and column temperature effects.

Keywords: Chiral compounds; Chiral recognition mechanisms; Enantioseparations; High-performance liquid chromatography; Immobilized *vs.* Coated; Polysaccharide-based chiral stationary phases.

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1   Introduction

Stereospecific recognition of chiral molecules and enantiomer discrimination are fundamental phenomena in life sciences. The chiral nature of living systems has evident implications on biologically active compounds interacting with them, biological systems are sensitive to stereochemistry and different responses can be observed when comparing the activities of an enantiomeric pair. Therefore stereochemistry has impact in chemical fields that are dealing with bioactive xenobiotic compounds, such as drugs, agrochemicals, food additives, fragrances, pollutants, etc. [1–3]. To deal stereoselectivity issues in such research, enantioselective liquid chromatography emerged as the method of choice, for both analytical and preparative purposes [4,5]. The direct approach using, a chiral stationary phase (CSP mode) is nowadays the method of choice for its relative simplicity. This mode employs chiral adsorbents, in most cases spherical porous silica supports functionalized with a chiral selector that is covalently linked or physically adsorbed.

Among a large number of CSPs developed the polysaccharides and its derivatives are highly used as chiral selectors for HPLC. It is because of their high chiral recognition ability. Polysaccharides are polymers with glucose units linked via β(1→4) (cellulose) or α(1→4) (amylose) linkages. Native amylose and cellulose are easily converted to a variety of derivatives with higher enantiorecognition ability [6]. Nowadays, amylose and cellulose derivatives synthetized by different workers are tribenzoate, *tris*-(4-methylbenzoate), triphenylcarbamate, *tris*-(3,5-dimethylphenylcarbamate), *tris*-[(R)-1-phenylethylcarbamate], *tris*-[(S)-1-methylphenylcarbamate], *tris*-(3,5-dichlorophenylcarbamate), etc. These derivatives were firstly coated by simple physically adsorption of chiral selectors on macroporous silica gel supports [7,8]. Therefore, they can be used only with a limited range of solvents as mobile phases. Then only low-polarity solvents, such as mixtures of alkanes (n-pentane, n-hexane, n-heptane, etc.) and alcohols (methanol, ethanol, 2-propanol, etc.), or sometimes, acetonitrile can be used for chiral separations under normal phase mode.

Unfortunately, commonly used organic solvents, prohibited with these CSPs may be useful to resolve some racemates, are used as sample diluents and are also required for the determination of the chiral recognition mechanisms using NMR and other spectroscopic techniques [6,9]. Besides, some stereospecific reactions are possible only in these solvents and, hence, its progress monitoring is not possible using coated CSPs. Therefore, versatility in the solvent selection is highly desired.

These coated CSPs drawbacks can be solved by preparing immobilized phases [10,11]. Presently, many columns, having polysaccharides immobilized on silica gel, are commercially available **(Table 1)**. Several immobilization methods to covalently fix the polysaccharide derivatives on silica gel were carried out [10–16], they include: linkage with (1) diisocyanates, (2) at the reactive terminal, (3) radical polymerization of vinyl groups, (4) photo irradiation, (5) click reaction and (6) polycondensation of the alkoxysilyl groups [14,17–31]. The high chiral recognition ability is usually due to the regular helical structure of them. Therefore, it is well known that on the coated-type CSPs, particularly those derived from cellulose benzoate derivatives, the coating conditions on silica gel significantly influence their chiral recognition ability [7,8,12,14].

Supercritical fluid chromatography (SFC) extend the use of the same polysaccharide CSPs as those for HPLC, providing a complementary choice of the GC and HPLC methods. **Fig. 1** shows the distribution of enantiomeric excess (ee) determination methods, CSPs for chiral HPLC and type of polysaccharide-based CSPs reported in the Angewandte Chemie International Edition in 2012. In that year, the journal published about 2,100 communication papers and at least 199 of them reported ee determinations. As well as the methods in **Fig. 1**, HPLC and SFC analyses were predominantly peformed with the cellulose-based CSPs, Chiralcel OD and OJ, and the amylose-based CSPs, Chiralpak AD and AS. The chemical name of these CSPs are shown in **Table 1**. Some columns include several brand names, such as CellCoat (Kromasil), RegisCell (Regis), Eurocel 01 (Knauer), Lux Cellulose-1 (Phenomenex), Sepapak-1 (Sepaserve) and Chiral Cellulose-C (YMC), Astec (Sigma-Aldrich), Reprosil Chiral (Dr. Maisch GmbH), besides Chiralcel and

Chiralpak (Daicel), respectively. Chiralpak IA and Chiralpak IB, were first time commercialized in 2004. The highly successful Chiralpak IC CSP was introduced in 2008 [32], and by 2016, the alphabetic sequence had expanded to "IG".

The present review highlights recent developments on immobilized polysaccharide-based chiral stationary phases for liquid phase separation techniques. Emphasizing in HPLC applications dating between 2013 and 2017. Topics such as chiral recognition ability of coated *vs.* immobilized CSPs, chiral recognition mechanisms, influence of column temperature and mobile phase composition are extensively discussed. Finally, this review tries to give current weak points or shortcomings that could be overcome with future developments in order to wider the applications of these promising CSPs.

2   Comparison between coated-type and immobilized-type CSPs

Chiral recognition ability of the immobilized-type CSPs can be different from the coated ones, and often is slightly lower. This is because the formation of a regular structure of the polysaccharide chains becomes difficult by the incorporation of different side groups necessary for the immobilization.

Okamoto et al. described in 1987 the first preparation of polysaccharide-based CSPs with 3,5-dichloro and 3,5-dimethylphenylcarbamate bonded covalently to γ-aminopropylsilica gelmatrix [24]. The resulting CSPs were tested with (n-hexane/2-propanol) in order to compare the results with coated CSPs. The stability of the 3,5-dichlorophenylaminocarbonyl-derived CSP was higher than the correspondingly coated CSP. However, a certain decrease in the enantioselectivity values was observed. A method modification involving a regioselective fixation was published for the same research group in 1994 [23]. The chromatographic behavior of the obtained CSPs, was compared to the coated CSPs and again a reduction in the enantioselectivity was observed. The results obtained for analogous selectors fixed by different positions were also compared. Interesting results were obtained when a small amount of chloroform was

used in the mobile phase. Some changes in the chiral discrimination ability of the chiral selectors were observed. Certain analytes, that were not resolved when the classical mobile phases were used, could be separated using mobile phases containing 5% of chloroform. This effect was attributed to a change in the conformation of the chiral selector in this solvent.

Andersson et al. compared the recognition abilities of the immobilized-type CSPs, Chiralpak IA and Chiralpak IB, with the corresponding coated-type, Chiralpak AD and Chiralcel OD, respectively [33], for 48 racemic compounds, and confirmed that coated-type CSPs often exhibit better recognitions. Coated CSPs, however, are not capable of resolving certain drugs and pharmaceuticals, especially in reaction mixtures when "prohibited solvents" are essential.

Zhang et al. [34] compared the chiral separation of bupivacaine under identical chromatographic conditions acetonitrile/diethylamine (100:0.1, v/v) on Chiralpak AD (coated) and Chiralpak IA (immobilized), better resolution was reported on the latter column. Chen et al. [35], compared the chiral recognition of immobilized CSPs and reported that chemically bonded CSPs greatly extend the choice of solvents and better resolution of several test enantiomers was observed on the immobilized CSPs with the addition of tetrahydrofuran and chloroform to the mobile phase. Aboul-Enein et al. [36,37] compared the chiral recognition capabilities of the Chiralpak IA column with those of Chiralpak AD for a variety of racemates and noted a complementary working nature of these two columns. Moreover, the reversal of the enantiomer elution order between coated and covalently immobilized versions of cellulose- and amylose-based (3,5-dimethylphenylcarbamates) has been reported [16]. Although the chemical composition of the stationary phase in the immobilized and coated columns is similar, the chiral recognition in both columns is not the same. Indeed, the immobilization of the chiral selector on silica did affect its chiral recognition ability showing different (often lower) resolving ability than the coated in the same experimental conditions. This is probably due to the change in the polymer configuration and/or supramolecular structure due to the

immobilization on the support, different conformations or rigidity of coated and covalently immobilized polysaccharide derivatives can be the reason of such a discrepancy in their chiral recognition.

Kalíková et al. [38] compared two commercially available polysaccharide-based CSPs containing amylose tris-(3,5-dimethylphenylcarbamate) chiral selector, coated and immobilized on silica support, under different separation conditions. The immobilized CSP, Chiralpak IA, and coated CSP, Chiralpak AD-RH, had comparable enantiorecognition potential for acidic analytes in the RP-HPLC mode. Chiralpak AD-RH seemed to be a better choice for the separation of basic analytes, despite Chiralpak IA exhibited higher retention for most of these analytes.

Adhikari et al. separated aliphatic amines including amino alcohols by NP-HPLC using six covalently bonded and four coated CSPs based on amylose and cellulose. Chiralpak IE showed the best enantiomer separation for most analytes among the covalently bonded CSPs, while Chiralpak AD-H and Amylose-1 generally showed higher enantiomer separation among the coated type CSPs [39]. Cirilli et al. compared a coated Chiralpak AD column with the new commercial immobilized-type amylose tris-(3-chloro-5-methylphenylcarbamate) Chiralpak IG for the determination of ricobendazole. Using the IG CSP under polar organic mode (100% MeOH or 100% EtOH), retention factors were about two-fold higher and the enantioseparation was lower than that obtained with the Chiralpak AD CSP even though the α-values remained fairly high (α>2), pure methanol gave the best enantioselectivity conditions [40].

Ferretti et al. [41] developed a simple HPLC method for separating triclabendazole sulfoxide (TCBZ-SO) enantiomers. First, the authors employed commercial columns with broad chiral discrimination ability, such as Chiralpak IA, IB and IC then no resolution or modest enantioselectivity values were obtained. Surprisingly, when they used a Chiralpak AS-H (amylose tris-[(S)-α-methylphenylcarbamate] coated and also the Chiralpak IF complete resolution for TCBZ-SO was achieved. At 25 ºC, with n-hexane/ethanol/trifluoroacetic acid (70:30:0.1, v/v/v) as mobile phase, Chiralpak AS-H gave an enantioselectivity value (α=1.87) higher than Chiralpak IF (α=1.26). However, since the immobilization of

the chiral selector, it was possible to explore new selectivity profiles using mobile phases containing ethyl acetate, dichloromethane, tetrahydrofuran and acetone [42,43]. With the "non-standard" elution mixture n-hexane/ethanol/ethyl acetate/trifluoroacetic acid (70:1:30:0.1, v/v/v/v), the TCBZ-SO enantiomers were well resolved with enantioseparation and resolution factor values of 1.45 and 5.17, respectively [41]. Gallinella et al. reported the analysis of oxaliplatin (platinum(II) anticancer drug) carried out with a Chiralpak IC column under optimized chromatographic conditions (column temperature= 40 ºC, flow rate= 1.0 mL min^{-1}, mobile phase= acetonitrile/water (100:5, v/v)). Oxaliplatin enantiomers were better resolved in a shorter analysis time (8 min) than required in the application of the method established by regulatory agencies (21 min) using Chiralcel OC column. The developed method demonstrates the applicability of hydrophilic interaction liquid chromatography (HILIC) mode for the enantioseparation in a better, simpler and faster way respect to the method reported in the current Pharmacopoeias. And also has the potential to provide reliable and effective LC–MS analyses of pharmaceutical formulation containing oxaliplatin [44].

3   Applications

Applications of immobilized polysaccharides CSPs (Chiralpak columns) are summarized in the **Tables 2-4**. It is well known that a single CSP cannot be universally used for chiral resolution of all racemic compounds. **Tables 2-4** provides CSPs trade names and their most relevant applications published between 2013-2017. Some interesting applications are discussed here. Various pharmaceutical, biological and veterinary compounds **(Table 2),** pesticides **(Table 3)** and organic, polyaromatic, natural and synthetic compounds **(Table 4)** have been resolved on immobilized polysaccharides CSPs at both analytical and semi/preparative scales [40,41,45–51]. In all these cases, the authors used reversed or normal phase modes. Other solvents, for example, chloroform and 2-propanol mixture with a Chiralpak IA, were used for

analytical and semipreparative separation of chiral 9,9-Spirobifluorenes derivatives obtaining R_s>>1.5 and enantioselectivity factor about 4.3 [47].

Many papers are aimed to the determination of herbicides [103,104,108,110], insecticides [48,100,97,107,105], fungicides [102,109,106,101,99] or antiviral drugs [98] from many different chemical families, including aryloxyphenoxy propionic acids, pyridines, oxadiazines, phenylpyrazoles, acylalanines, pyrethroids, triazoles, α-aminophosphonates, sulfoxides, etc. have been determinated in environmental or food samples (soil, natural waters, fruits, vegetables, etc.). Pharmaceutical compounds recognized as potential anti-carcinogen agents [44,45,56,92,64], α- and β- adrenergic receptor blockers [51,92,59,65–68,60], nonsteroidal anti-inflammatory drugs [38], antihistamines [92,74], proton pump inhibitors [50,94,73,79,81,88,96,76], retroviral drugs [55,84], antihyperglycemic drugs [83], antihelminthic agents [40,57], anti-hypertensive drugs [41,53,85], L-type calcium channel [72,89], broad-spectrum antimicrobials [95,70], nootropic drugs [90,91], anticoagulant drugs [69], etc. have also been determined, especially in commercial products or biological samples (pharmaceutical formulations, human urine, human or animal blood, human plasma, animal serum, etc.). Another heterogeneous group of analytes, including connectors for building metal-organic frameworks [116,117,124], intermediates for the synthesis of pharmaceuticals [111], natural and synthetic compounds [39,113,121,120], biological compounds [82,112,58,71,78,86,61–63,77,75], natural alkaloids [121], biomolecules [115,54], organometallic compounds [119] and chiral metabolites [103,108,100,97,102,59,63,52] has been enantioseparated with these immobilized polysaccharides CSPs (see **Table 2-4**). **Fig. 2** shows the number of publications reported in the last five years for chiral separations obtained with all available Chiralpak columns under HPLC and also SCF methods. The noticeable difference in the number of HPLC and SCF papers published for Chiralpak IF and IG must be undoubtedly due that these columns are the newest in the series, and they have incipient use in HPLC methodologies. It is expected that SFC applications increase over time.

4   Theoretical studies using polysaccharide CSPs

4.1. Chiral recognition mechanisms

Selector-selectand complexes are thought to be primarily mediated via hydrogen bonds as well as π-π interactions and van der Waals forces [125–127]. Especially the carbamate linkage allows some flexibility of the aromatic rings for maximizing π-π interactions and van der Waals forces upon binding with the solute. The mobile phase composition may modulate the recognition process. Thus, reversal of the enantiomer elution order depending on the composition of the mobile phase has been observed [16]. Furthermore, based on a systematic study for the enantioseparation of polyhalogenated 4,4'-bipyridines on polysaccharide-based chiral stationary phases including structure-separation relationships as well as computational evaluation of the geometries and electron distribution of selectors and selectands. Peluso and coworkers pointed out a contribution of halogen bonding interaction for analyte stereorecognition by cellulose tris-(3,5-dimethylphenylcarbamate) [116,117]. Studies on the elucidation of the chiral recognition mechanism of polysaccharide chiral selectors have been performed by various techniques including chromatography, NMR spectroscopy, vibrational circular dichroism spectroscopy or molecular modeling as summarized earlier in [1,125–128], while applications to chiral separations as well as studies on the separation ability of the selectors can be found in [12–14,16,124,129,130].

Hydrogen bonding and π-π interactions were the major factors for the HPLC separation of the four stereoisomers of 5-bromo-3-ethyl-3-(4-nitrophenyl)piperidine-2,6-dione on amylose *tris*-(3,5-dimethylphenylcarbamate) as derived from molecular docking [56]. Based on modeling results and stereo-interactions of all the stereoisomers (SS, RR, RS, SR) at supramolecular level with the CSP constituted by attractive electrostatic forces among amide groups and two carboxylic groups of stereoisomers and amino, amide, hydroxyl and oxide groups of the chiral selector, π–π interactions and hydrogen bonding, the authors could explain the elution order for the stereoisomers.

The importance of π-π interactions in the chiral recognition process was also discussed for the separation of the enantiomers of a pyrrolo[2, 3- c] pyridine drug by SFC [131]. While both enantiomers formed hydrogen bonds with the selector amylose *tris*-(3,5-dimethylphenylcarbamate), only the stronger retained (R)-enantiomer established π-π interactions between the pyrrolo[2, 3-c] pyridine moiety and 3,5-dimethylphenylcarbamate substituents. Docking studies revealed that the complex with the strongly retained (S)-enantiomer was stabilized by two π-π interactions of both phenyl rings with 4-methylbenzoate substituents of two separate glucose units. The thioamide group formed a hydrogen bond with two oxygen atoms of a glucose residue. In contrast, the weaker retained (R)-enantiomer displayed only one π-π interaction between the phenyl ring in position 3 and a 4-methylbenzoate substituent as well as one hydrogen bond between the thioamide group and a glucose residue. Further enantioseparation studies supported by molecular modeling include the enantioseparations of pheniramine, oxybutynin, cetirizine and brinzolamide by amylose *tris*-(3,5-dimethylphenylcarbamate) [132] and also the optical isomers of atracurium besylate on cellulose *tris*-(3,5-dimethylphenylcarbamate) [133].

Differences in the formation of hydrogen bonds between the enantiomers of propranolol and cellulose *tris*-(3,5-dimethylphenylcarbamate) derived from molecular modeling were used to explain the elution order observed in HPLC [66]. The less retained (S)-enantiomer formed a hydrogen bond between the hydroxy group of the selectand and the carbamate carbonyl group of the selector while the stronger retained (R)-enantiomer formed hydrogen bonds with the carbohydrate backbone due to deeper insertion into the chiral cavities. π-π Interactions were not considered in this study although they appear very likely based on the structures of selector and selectand.

Quantum chemical calculations of a set of chiral pyrrolidin-2-one derivatives were used to predict structure-enantioselective retention relationships for amylose *tris*-(3,5-dimethylphenylcarbamate) as chiral stationary phase in the normal phase mode [134]. Although direct solute-selector interactions were not modeled, the polar surface area of the solutes as well as a significant role of charge-transfer interactions between the

stationary phase and the analyte enantiomers were derived from the calculations, which allowed the prediction of the enantioseparation of related structures.

Several works deal with the effect of the mobile phase composition and the analyte chemical structure on the enantioseparation of various racemates. For example, the effect of acidic and basic additives to 2-propanol and acetonitrile based mobile phases on the enantiomer elution order of chiral β-blockers and other basic drugs on commercial polysaccharide chiral columns has been reported [135,136]. The influence of the 2,2'-alkyl chains of atropisomeric 1,1'-bibenzimidazole derivatives on an amylose *tris*-(3,5-dimethylphenylcarbamate) stationary phase was studied and it was noted that the chain length affected binding strength (accounted for analysis time) as well as enantiomer elution order [122]. The effect of halogen substitution in a large variety of structurally diverse analytes has also been summarized recently [124]. Ramisetti et al. [95] studied the mechanism of separation between a Chiralpak IE column and R-Besinfloxacin. A large number of interactions between selectand enantiomers and chiral selector can be envisaged including dipole–dipole, hydrogen bonding and π–π interactions depending on the experimental conditions. The probable mechanism for chiral recognition is predominantly ionic, making it either an essential acid or an essential base to be added as mobile phase additive. In this work, a good selectivity was achieved by the addition of both acidic trifluoroacetic acid, and basic diethylamine.

4.2. Effect of mobile phase composition

Polysaccharide-type CSPs are most frequently used with organic eluents, however, enantiomer resolution by aqueous eluent has largely been used [43,137] too. The choice of RP-mode was often related to analyte scarce retentivity, low sample solubility of polar species and lack of enantioselectivity in organic eluents. In the last decades, an additional reason to prefer RP methods has been their suitability for LC–MS applications [43]. RP chromatography is often complementary to normal mode.

If sample components are soluble in aqueous medium and an HPLC separation on polysaccharide-derived column will be tested, there are two possibilities:

(a) Developing a method in polar organic mode.

(b) Developing a method in reversed-phase conditions.

For an efficient screening in the polar organic mode on immobilized CSPs, the choice of the solvents often includes: 100% acetonitrile (ACN), 100% methanol (MeOH) and/or 100% ethanol (EtOH) (or their mixtures).

This preliminary screening allows to identify potential enantiorecognition with these columns. If this strategy is not successful it would be necessary switching to the RP-mode. The first choice of modifier to be combined with water is ACN. MeOH can also be useful and, THF seems to be less versatile.

The presence of water has often an unpredictable impact on the enantiorecognition ability. Water promotes a strong solvation effect on the polymeric chiral selector and a substantial weakening of several types of interactions, such as the hydrogen bonding, involved in the chiral recognition mechanism. Despite of the exhaustive number of studies on the chiral discrimination ability, there is a lacking of information concerning the retention behavior of polysaccharide-type CSPs under aqueous-organic conditions. Okamoto and Kaida [138] and Kummer and Werner [139] have pointed out that only at high water concentrations the adsorption mechanism on polysaccharide derivatives is controlled by hydrophobic interactions. At low water concentrations an alternative retention behavior typical of the hydrophilic interaction liquid chromatography (HILIC) could be observed. Chankvetadze et al. have related the HILIC behavior to a competition between water and analyte for hydrogen binding sites of the polymeric selector [16,140].

Materazzo et al. [57] separated albendazole and fenbendazole sulfoxides (ABZ-SO and FBZ-SO, respectively) using Chiralpak ID and Chiralpak IF colums with mixtures of acetonitrile/water (100:180 and 100:80, v/v, respectively) as mobile phases (**Fig. 3a**). Good enantioresolution of FBZ-SO was performed using analogue CSP/mobile phase combinations (**Fig. 3b**). The authors hypothesized that the HILIC and

RPLC retention behaviors of the immobilized-type CSPs depend on strong binding sites located outside the chemical portions involved in the immobilization of the selector.

Mobile phase additives are routinely used with the main purpose of improving peak shape and efficiency of basic/acidic compounds by minimizing peak tailing and/or broadening. The additives are usually used at very low proportion (0.05–0.5%, v/v) and include mainly diethylamine (DEA), triethylamine (TEA), isopropylamine (iPA), 2-aminoethanol (AE), ethylenediamine (EDA) or trifluoroacetic acid (TFA), acetic acid (AA) and formic acid (FA). Among the basic additives, DEA is the most commonly used and has proved to be effective for screening and method development of many basic compounds [40,50,51,67,68,60,74,73,55,84,95,111,58,78,62,75,122,87,93,80] (see **Tables 2-4**). As a general rule, basic additives are employed for enantiomer separation of basic compounds, while acidic additives are essential for acidic ones. Basic additives in the mobile phase for elution of acidic compounds generally lead to no elution [129,141] or very long retention times with peaks in broad hump accompanied by total collapse of separation. The usefulness of acidic additives, nevertheless, can sometimes be extended beyond the category of acidic compounds. Several studies carried out on certain coated polysaccharide-based CSPs showed significant effects of acidic additive on basic compounds [141–144]. This phenomenon was also observed with the immobilized polysaccharide-based columns, most notably on CHIRALPAK IB [129]. For molecules with a certain amphoteric nature, combinations of TEA and TFA can also be used in order to improve peak shapes (ion-pairing strategy) [43,73,95].

Several studies carried out on certain polysaccharide-based CSPs of immobilized type showed significant effects of acidic additive (or acidic with basic mixture additive) mainly on basic compounds [41,103,104,108,101,73,53,95,90,91,58,86,63,115,52] (see **Tables 2-4**).

For β-blockers enantiomer resolution on Chiralpak IB, both DEA (basic) and TFA (acidic) were added to a n-hexane/ethanol (80:20, v/v) mobile phase. A change as low as 0.1% for the mobile phase additive produced a notable shift on enantioselectivity [129].

4.3. Effect of temperature on retention and enantioselectivity

Several studies using different CSPs [40,53,145–150] demonstrated that the column temperature should be optimized in enantioselective HPLC separations.

Generally, solute transfer from the mobile to the stationary phase is an exothermic process, and consequently Tthe retention factor k often decreases with increasing temperature.

The retention mechanism is widely explained as a mixed mechanism involving solute–solvent, solute–sorbent, and solvent–sorbent interactions. The detailed thermodynamic study of these complex retention behaviors requires van't Hoff analysis over a sufficiently wide range of modifier concentrations and the development of a thermodynamic retention model. The retention behavior of solutes can be described reasonably well using a combination of thermodynamic analysis and a retention model, as was previously implemented for solutes with distinct functional groups on Chiralpak IA [151] and Chiralpak IE [150] sorbents.

Cirilli et al. [40] studied the temperature impact on retention, enantioselectivity and resolution of ricobendazole. The authors used a Chiralpak IG column and its temperature was changed in the 25–45 ºC range at intervals of 5 ºC, using methanol as mobile phase. In all cases, retention decreased with increasing temperature. Furthermore, the ln k terms were linearly related ($r^2 > 0.990$) to the inverse of the temperature. In contrast, the influence of temperature on enantioselectivity was modest and the ln α values did not follow a linear trend as the temperature increased. It is interesting to note that although the enantioselectivity changed slightly with temperature, the maximum resolution value 10.90 was at 45 ºC. The calculated $\Delta(\Delta H^o)$ and $\Delta(\Delta S^o)$ values were positive for IG CSPs (0.43 kcal mol^{-1} and 3.00 kcal mol^{-1}) indicated that the chiral resolution of RBZ on the IG was entropically driven (i.e. $|\Delta(\Delta H^o)| < |T\Delta(\Delta S^o)|$).

Recently, Ferretti et al. studied the temperature effect on the enantioresolution parameters of the halogenated sulfanyl-benzimidazole derivative triclabendazole using a Chiralpak IF column. The ln k terms were linearly related to the inverse of the absolute temperature (r^2 > 0.9850). Retention increased with temperature only for the IF CSP/n-hexane/EtOH/ethyl acetate/trifluoroacetic acid (70:1:30:0.1, v/v/v/v) condition. In most cases, the enantioselectivity and resolution remained almost unchanged or decreased when the column temperature increased from 25 to 40 °C [41].

On the other hand, Cirilli's group developed a direct HPLC method for the separation of Bicalutamide (BCT) enantiomers by using a Chiralpak IA column. Enantioselective conditions were achieved using standard normal phase mixtures of n-hexane-alcohol (ethanol or 2-propanol) and a "non-standard" mobile phase containing ethyl acetate. For the first eluent n-hexane/ethanol (70:30, v/v) the effect of column temperature on enantioselectivity and retention was unidirectional. The van't Hoff plots followed a classical linear pattern and the retention factors of both BCT enantiomers as well as the enantioselectivity decreased as the column temperature increased. The BCT van't Hoff plots are markedly different when n-hexane/ethyl acetate/ethanol (100:30:5, v/v/v) is used as eluent. The ln α vs. $1/T$ plot was non-linear and the resolution factor reached a maximum value at 40 °C (R_s= 11.08). It is worthwhile noting that this resolution value, is slightly higher than that obtained with standard NP elution mode (R_s= 10.67) (see **Fig. 4**) [45].

Ferretti et al. [50] studied column temperature influence on the chromatographic behavior of lansoprazole (LAN) using a Chiralpak IC and ethanol/water (50:50, v/v) as mobile phase. The retention and enantioselectivity factors were recorded at 25, 30, 35 and 40 °C. The resolution factor incremented from 2.63 to 3.05 within the temperature range, mainly due to an improvement in column efficiency. Besides, at 40 °C an appreciable reduction of the analysis time (from about 12 to 8 min) was observed. And an impurity (IMP-A) partially resolved was also studied in the same temperature range. The authors focused attention towards the isolation of enantiopure samples of both chiral compounds and their stereochemical assignment. The NP-HPLC resolutions were carried out with the same column. Mixtures of n-

hexane/ethanol/diethylamine (60:40:0.1 and 40:60:0.1, v/v/v, respectively) were selected as the best mobile phases to provide enantioseparation of LAN and IMP-A, respectively. The results demonstrate that the resolution reached an optimum value when the column temperature was set at 35 ℃.

5   Future Perspectives

5.1. Liquid chromatography coupled to mass spectrometry

Liquid chromatography–mass spectrometry (LC–MS) applications have grown significantly since 1980, primarily due to the introduction of the atmospheric pressure ionization (API) technique for the LC–MS interfaces, which overcomes the robustness and sensitivity issues of the earlier LC–MS interfacing ones. Enantiomeric LC–MS separation techniques combine the resolving power of HPLC and the sensitivity and specificity of mass spectrometric detection [152].

In recent literature (2013-2017), we have not found polar or normal-phase mode applications of total chiral LC–MS separations using immobilized polysaccharide-based CSPs. However, polar organic and NP-LC-MS applications have been found in not so recent literature [153]. The mobile phase incompatibility issue with normal-phase mode was overcome by using a make-up liquid. However, this post-column addition reduces enantioresolution; therefore, further work should be done for the optimization of chromatographic and mass spectrometric ionization conditions.

All chiral applications were found in the LC reversed-phase mode using tandem mass spectrometry (LC-MS/MS), which is attributed to its compatibility with API-based MS ionization techniques. Pharmaceutical [81,96,76,89,70], biomedical [61,54] and agrochemical [103,104,107,102,101] applications were the dominant separation fields, and to a lesser extent are other industries such as fragrances and food, and also environmental studies.

The promise of wider applications based on the polar organic mode of chiral LC–MS using different immobilized CSPs has not yet been realized. Although it possesses the advantages of compatibility with API sources and high-resolution efficiency in a short analysis time. The primary reason may be that scientists are less familiar with this hybrid mode of LC–MS techniques and because of the infrequent use of high-throughput sample preparations using pure organic-based liquid handling techniques.

5.2. Miniaturization of chiral separations

Downscaling chromatographic techniques from conventional to micro or nano-HPLC is considered a "green analysis approach". Instrumental miniaturization results in smaller solvent consumption and reduced waste production compared to the full-size laboratory instruments [154]. Several studies [155–157] show the feasibilty of miniaturization from conventional HPLC to nano-LC, capillary-LC (CLC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) for chiral separations with polysaccharide based-CSPs. An early study attempted the simulatenous enantioseparation of thalidomide and its hydroxylated metabolites using three different polysaccharide-type CSPs under conventional HPLC, CLC, and CE [155]. Baseline separation of the six peaks was achieved using conventional HPLC; however, for CLC and CE, this was not possible. Hence, column length had to be increased to provide the same baseline separation displayed by the conventional column. These results show that the performance of conventional size HLPC columns is not yet comparable to the miniaturized counterpart, mainly due to the lower CS content in them. Although several applications have been reported [158,159], further work is necessary in this field, e.g., new CSPs development, availability of commercial capillary columns and less expensive instrumentation. Finally, the use of chip technology seems to be very promising. Here dead volumes are minimized providing, in addition to short analysis time, high efficiency and resolution.

5.3. Chemometric analysis

In enantiomeric purity determinations, the enantiomer of interest has to be usually quantitatively analyzed at levels below 1% (minority peak) of the main enantiomer. Higher accuracy in detection and quantitation of the minor signal close to the major peak can be obtained as larger the enantioresolution is. These large enantioresolution factors are not so easily achieved in chiral chromatography at a reasonably analysis time [160], and it is very often observed a partial overlapping between both enantiomer profiles, causing a loss in the quantitation accuracy. Chemometric analysis of multivariate chromatographic data for quantitative purposes is becoming popular, particularly for the study of complex samples [161–163]. The expressions 'chromametrics' [164] and 'chroMAThography' [165] have been coined to describe the combination of chromatography and chemometrics/mathematics. Recently, it has been demonstrated how chiral liquid chromatography combined with multivariate techniques, specifically unfolded-partial least-squares regression (U-PLS), provides a powerful analytical methodology to face unresolved profiles [166–168]. Chemometric methods coupled to chromatographic-spectroscopy data can be a powerful analytical strategy in the following circumstances: (1) to reduce analysis time by using stronger mobile phases to elute earlier all the interesting peaks and (2) to circumvent the usual lower enantioresolution factors achieved in reversed-phase chiral systems as compared to the larger normal phase enantioselectivities and, last but not least, to reduce analysis cost.

6 Concluding remarks

It is well known the extremely broad applicability showed by polysaccharide type CSPs in HPLC chiral analysis. One of the most remarkable recent advancement for this family constitutes the immobilization of

polysaccharide derivatives of cellulose and amylose onto a chromatographic support, allowing them to extend the choice of solvents used as mobile phases, even to "non-standard" solvents which are prohibited for the coated ones. Then immobilized CSPs have great versatility in method development, can be used to monitor certain stereospecific reactions carried out only in prohibited solvents, and enable preparative scale chromatography. Although they have many, it is important to know that the applications of immobilized CSPs are not yet fully explored and studies are still in progress.

Moreover, these immobilized CSPs admit column downscaling and then the miniaturized techniques developed with them contribute to the current trends towards green chemistry. Still effort must be put in instrumental improvements and technological advances that allow coupling MS detection and extend applications of these CSPs used under normal or polar organic modes, respectively. Finally, the development of combined chemometric methods with spectroscopic-chromatographic data as a powerful analytical strategy for the analysis of strongly overlapped enantiomeric peak profiles is not yet exploited.

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Figure Captions

Figure 1. Distribution of enantiomeric excess determinations reported in Angew. Chem. Int. Ed. in 2012. The numbers in parentheses are the amount of papers published in each category. **(A)** for different separation techniques **(B)** for different types of CSPs used for HPLC and SFC. **(C)** for different chemical nature of polysaccharide-based CSPs. OD, AD, OJ and AS are coated-type and IA, IB, IC, ID and IE are immobilized-type CSPs. (Reproduced with permission of Elsevier from Ref. [14] © 2014 Elsevier).

Figure 2. Number of publications reported in the last five years for chiral separations obtained with Chiralpak columns by Scopus database under (a) HPLC, and (b) SCF methods.

Figure 3. HPLC chromatograms illustrating the enantioseparation obtained on the Chiralpak ID (left) and Chiralpak IF (right) columns for **(a)** albendazole sulfoxide (ABZ-SO) and **(b)** fenbendazole sulfoxide (FBZ-SO). Mobile phase composition corresponding to polar organic (POLC), hydrophilic interactions (HILIC) and reversed phase (RPLC) liquid chromatography modes, are indicated in the Figure. UV detection at 254 nm; flow rate: 1 mL min^{-1}; column temperature: 25 ºC. (Reproduced with permission of Elsevier from Ref. [57] © 2014 Elsevier)**.**

Figure 4. HPLC chromatograms obtained at different temperatures under NP and RP liquid chromatography modes for lansoprazole (LAN) and an impurity (IMP-A). Left side (NP mode): Chiralpak IC column (250 mm x 4.6 mm I. D.). Mobile phase composition: n-hexane/ethanol/diethylamine (60:40:0.1, v/v/v) for LAN and n-hexane/ethanol/diethylamine (40:60:0.1, v/v/v) for IMP-A. Right side (RP mode): Chiralpak IC-3 column (100 mm x 4.6 mm). Mobile phase composition: ethanol/water (50:50, v/v). UV detection at 280 nm; flow rate, 1.0 mL/min; column temperature, from top to bottom: 40, 35, 30, and 25 ºC. (Reproduced with permission of Wiley and Sons from Ref. [50] © 2016 John Wiley & Sons).

Table 1: Name of various polysaccharide-based commercial columns.

Trade name	Chemical name	Trade name	Chemical name
Cellulose based CSPs		**Amylose based CSPs**	
Chiralcel OB[a] (c) Chiralcel OB-H[a] (c) Eurocel 02[b] (c)	Tribenzoate	Chiralpak AD[a] (c) Chiralpak AD-R[a] (c) Chiralpak AD-RH[a] (c) Lux Amylose-1[c] (c) CHIRAL ART Amylose-C[f] (c) Reprosil Chiral-AM[i] (c) AmyCoat[d] (c) RegisPack[e] (c)	*tris*-(3,5-dimethylphenylcarbamate)
Chiralcel OJ[a] (c) Chiralcel OJ-R[a] (c) Lux Cellulose-3[c] (c) Eurocel 03[b] (c) CHIRAL ART Cellulose-SJ[f] (c)	*tris*-(4-methylbenzoate)	Chiralpak IA[a] *(i)* Lux i-Amylose-1[c] *(i)* CHIRAL ART Amylose-SA[f] *(i)*	
		Chiralpak AR[a] (c)	*tris*-[(R)-1-phenylethylcarbamate]
Chiralcel CMB[a] (c)	*tris*-(3-methylbenzoate)	Chiralpak AS[a] (c) Chiralpak AS-H[a] (c) Chiralpak AS-RH[a] (c)	*tris*-[(S)-α-methylphenylcarbamate
Chiralcel OC[a] (c) Eurocel 04[b] (c)	Triphenylcarbamate	Chiralpak AY[a] (c) Chiralpak AY-H[a] (c) Chiralpak AY-RH[a] (c) Lux Amylose-2[c] (c) Sepapak-3[g] (c) RegisPack CLA-1[e] (c)	*tris*-(5-chloro-2-methylphenylcarbamate)
Chiralcel OD[a] (c) Chiralcel OD-H[a] (c) Chiralcel OD-R[a] (c) Chiralcel OD-RH[a] (c) Lux Cellulose-1[c] (c) Eurocel 01[b] (c) CHIRAL ART Cellulose-C[f] (c) Sepapak-1[g] (c) Astec Cellulose DMP[h] (c) Reprosil Chiral-OM[i] (c) CelluCoat[d] (c) RegisCell[e] (c)	*tris*-(3,5-dimethylphenylcarbamate)		
Chiralpak IB[a] *(i)* CHIRAL ART Cellulose-SB[f] *(i)*		Chiralpak AZ[a] (c) Chiralpak AZ-H[a] (c) Chiralpak AZ-RH[a] (c)	*tris*-(3-chloro-4-methylphenylcarbamate)
Chiralcel OF[a] (c)	*tris*-(4-chlorophenylcarbamate)	Chiralpak IF[a] *(i)*	
Chiralcel OG[a] (c)	*tris*-(4-methylphenylcarbamate)	Chiralpak ID[a] *(i)*	*tris*-(3-chlorophenylcarbamate)
Chiralcel OA[a] (c)	triacetate on silica gel	Chiralpak IE[a] *(i)*	*tris*-(3,5-dichlorophenylcarbamate)

Chiralcel CTA[a] (c)	triacetate microcrystalline	Chiralpak IG[a] *(i)*	*tris*-(3-chloro-5-methylphenylcarbamate)
Chiralcel OK[a] (c)	Tricinnamate		
Chiralcel OX[a] (c) Chiralcel OX-H[a] (c) Chiralcel OX-RH[a] (c) Lux Cellulose-4[c] (c) Sepapak-4[g] (c)	*tris*-(4-chloro-3-methylphenylcarbamate)		
Chiralcel OZ[a] (c) Chiralcel OZ-H[a] (c) Chiralcel OZ-RH[a] (c) Lux Cellulose-2[c] (c) Sepapak-2[g] (c)	*tris*-(3-chloro-4-methylphenylcarbamate)		
Sepapak-5[g] (c) Chiralpak IC[a] *(i)* Lux i-Cellulose-5[c] *(i)* CHIRAL ART Cellulose-SC[f] *(i)*	*tris*-(3,5-dichlorophenylcarbamate)		

[a] Supplier: Daicel Chemical Industries; [b] Knauer; [c] Phenomenex; [d] Kromasil; [e] Regis Technologies Inc.; [f] YMC Europe GMBH; [g] Sepaserve; [h] Sigma-Aldrich; [i] Dr. Maisch GmbH. *(i)*: Immobilized polysaccharide-based CSPs, (c): Coated polysaccharide-based CSPs.

Table 2: Chiral resolution of different pharmaceutical, biological and veterinary compounds on immobilized polysaccharide-based.

Compound (Sample)	Chromatographic conditions	Chiralpak column	Chromatographic parameters	Ref.
BMT-094817 (Human plasma)	0.1% HFor/ACN:IPA (50:50) (55:45), 1mL/min, 50°C, LC-MS/MS	**IA-3** 150×4.6mm, 3μm (A)	R_s=1.90, α=1.50	[52]
6-substituted carbamoyl benzimidazoles (Standard stock solution)	(a)MTBE/MeOH/HFor, (b)MTBE/EtOH/HFor, (c)MTBE/PrOH/HFor, (d)MTBE/IPA/HFor, (e)MTBE/BuOH/HFor, (f)MTBE/t-BuOH/HFor, alcohol (1.37M) in MTBE with 0.1% HFor, 1mL/min, 25°C, 271 and 286nm	**IA** and **IC** 250×4.6mm, 5μm (A)	**IA** (a)R_s≤3.26, α≤1.24; (b)R_s≤3.40, α≤1.56; (c)R_s≤2.38, α≤1.20; (d)R_s=0.00, α=1.00; (e)R_s≤2.38, α≤1.47; (f)R_s≤1.95, α≤1.18 **IC** (a)R_s≤7.08, α≤2.25; (b)R_s≤7.90, α≤2.42; (c)R_s≤6.42, α≤2.41; (d)R_s≤6.89, α≤2.63; (e)R_s≤6.65, α≤2.72; (f)R_s≤5.03, α≤2.59	[53]
Phosphatidylcholine hydroperoxide with 13S-hydroperoxy-9Z,11E-octadecadienoic acid (Soy lecithin)	MeOH/NH$_4$Ac 5mM (80:20), 1.0mL/min, 40°C, LC-MS/MS	**IA**, **IB**, **IC**, **ID**, **IE** and **IF** 250×4.6mm, 5μm (A)	**IA** R_s=1.9, **IB** R_s=2.2, **IC** R_s=0.0, **ID** R_s=3.6, **IE** R_s=nd, **IF** R_s=0.0	[54]
Bicalutamide and impurities (Standard stock solution)	(a)n-Hex/EtOH (70:30), (b)n-Hex/IPA (65:35), (c)n-Hex/EA/EtOH (100:30:5), 1mL/min, 25 °C, 280nm	**IA** 250×4.6mm, 5μm (A) **IA** 250×10mm, 5μm (S)	(a)R_s=3.12-13.79, α=1.57-3.56 (b)R_s=3.00-9.07, α=1.74-2.71 (c)R_s=1.83-9.96, α=1.28-2.15	[45]
Nadolol (Standard stock solution)	(a)IPA/ACN with 0.1% DEA (10:90), (b)EtOH/n-Hex/DEA (20:80:0.1), (c)EtOH/n-Hep/DEA (20:80:0.1), 1mL/min, 23°C	**IA** 250×4.6mm, 5μm (A), home-packed with 20 μm (P)	(a)$R_{s2,1}$=3.95, $R_{s3,2}$=2.89, $R_{s4,3}$=9.18 (b)$R_{s2,1}$=1.72, $R_{s3,2}$=2.91, $R_{s4,3}$=4.03 (c)$R_{s2,1}$=2,74, $R_{s3,2}$=2.88, $R_{s4,3}$=5,19	[51]
Darunavir (Dried blood spot)	n-Hex/EtOH/DEA (75:25:0.1), 1mL/min, 20°C, 266nm	**IA** 250×4.6mm, 5μm (A)	R_s>1.5	[55]
5-bromo-3-ethyl-3-(4-nitrophenyl)-piperidine-2,6-dione (Standard stock	100%ACN, 1mL/min, 27 °C, 254nm	**IA** 250×4.6mm, 5μm (A)	R_{s1}=3.88, α_1=1.22 R_{s2}=7.04, α_2=1.69 R_{s3}=8.22, α_3=1.49	[56]

solution)				
Profens (Standard stock solution)	(a)HFor (pH=2.10)/ACN (60:40), (b) HFor (pH=2.10)/ACN (70:30), 1mL/min, 25°C, 220-264nm	**IA** 250×4.6mm, 5μm (A)	(a)$R_s \leq$4.75, $\alpha \leq$1.22 (b)$R_s \leq$4.36, $\alpha \leq$1.23	[38]
Albendazole and fenbendazole sulfoxides (ABZ-SO and FBZ-SO) (Synthetic compounds)	(a)100%ACN, (b)100%MeOH, (c) EtOH/w (100/5), (d) ACN/w (100:10), (e) ACN/w (100:20), (f) ACN/w (100:5), 1.0mL/min, 25 °C, 254nm	**IA-3**, **ID-3**, **IE-3** and **IF-3** 100×4.6mm, 3μm (A)	**IF-3** (a)α_{ABZ-SO}=3.89, α_{FBZ-SO}=4.04 **ID-3** (a)α_{ABZ-SO}=3.56, (b)α_{FBZ-SO}=3.29 **IE-3** (c)R_s=5.06, α_{FBZ-SO}=1.99 **IA-3** (c)R_s=3.65, α_{ABZ-SO}=2.16 **ID-3** (d)R_s=6.95, α_{ABZ-SO}=3.34 **IF-3** (e)R_s=5.74, α_{ABZ-SO}=3.35 **ID-3** (f)R_s=6.28, α_{FBZ-SO}=3.35 **IF-3** (d)R_s=6.83, α_{FBZ-SO}=4.77	[57]
Steroisomers and geometrical isomers of pitavastatin (Drugs)	*n*-Hep/BuOH/MeOH/HFor/DEA (94:3.5:2.5:0.2:0.1), 1.0mL/min, 35°C, 250nm	**IA** 250×4.6mm, 5μm (A)	R_s~1.5	[58]
Naftopidil and o-desmethyl metabolites (Rat feces)	MeOH/ACN/acetate buffer (pH=5.3, 5mM) (3:33:22), 0.5mL/min, 25°C λ_{ex}=290nm and λ_{em}=340nm	**IA** 250×4.6mm, 5μm (A)	R_s~1.5	[59]
Carvedilol (Human urine)	MeOH/EtOH/w/DEA (64:15:12:0.3), 1.5mL/min, 35°C, 240nm	**IA-3** 100×4.6mm, 3μm (A)	R_s=9.27, α=2.78	[60]
OTX015 (Mice plasma)	0.2% ammonia/ACN (20:80), 1.2mL/min, 40°C, LC-MS/MS	**IA** 250×4.6mm, 5μm (A)	R_s=4.86	[61]
OTX015 (Bulk drug)	MeOH/DEA (100:0.1), 1mL/min, 35°C, 254nm	**IA** 250×4.6mm, 5μm (A)	R_s=3.74, α=1.76	[62]
Pidotimod (Standard stock solution)	MTBE/ACN/TFA (35:65:0.2), 1.0mL/min, 25°C, λ=210nm	**IA** 250×4.6mm, 5μm (A)	R_s=3.8, α=1.57	[63]

Purine Derivatives (Standard stock solution)	n-Hex/IPA (90:10), 1.0mL/min, 35°C, 254nm	**IB** 250×4.6mm, 5μm (A)	R_s=0.0-1.99, α=1.03-1.09	[64]
Bevantolol (Standard stock solution)	ACN/buffer KH_2PO_4 20mM, pH=4.5 (25:75), 0.8 mL/min, 25°C, 259nm	**IB** 250×4.6mm, 5μm (A)	R_s=3.90, α=1.26	[65]
Propranolol (Rat Serum)	n-Hex/EtOH/TEA (95:5:0.4), 0.6mL/min, λ_{ex}=290nm and λ_{em}=375nm	**IB** 250×4.6mm (A)	R_s=3.37, α=1.24	[66]
β-receptor blockers (Standard stock solution)	(a)n-Hex/IPA/DEA (70:30:0.5), (b)n-Hex/EtOH/DEA (70:30:0.5), (c)n-Hex/PrOH/DEA (70:30:0.5), 1.0mL/min, 30°C, 275nm	**IB** 150× 4.6mm (A)	(a)R_s≤3.80, α≤5.51 (b)R_s≤4.54, α≤3.78 (c)R_s=0.74-5.95, α≤5.55	[67]
β-receptor blockers (Bulk drug)	n-Hex/EtOH/DEA (70:30:0.5), 0.8mL/min, 20°C, 295, 274, 224 and 223nm	**IB** 250× 4.6mm, 5μm (A)	R_s=3.07-12.2, α=1.2-2.4	[68]
Acenocoumarol (Tablets formulations)	n-Hex/EtOH (70:30), 0.5mL/min, 25°C, 383nm	**IB** 250× 4.6mm, 10μm (A)	R_{s1}=0.81, α_1=1.051 R_{s2}=1.38, α_2=1.109 R_{s3}=1.08, α_3=1.100 R_{s4}=1.1, α_4=1.131	[69]
Miconazole (Rat plasma)	ACN/buffer NH_4HCO_3 5mM (80:20), 0.6 mL/min, 20°C, LC-MS/MS	**IC** 250×4.6mm, 5μm (A)	R_s>1.5	[70]
α-Hydroxyallylphosphonates and Phosphonoallylic Carbonate Derivatives (Synthetic compounds)	n-Hex/IPA (90:10), 1.0mL/min, 25°C, 210nm	**IC-3** 100×4.6mm, 3μm (A)	R_s≤5.9, α=1.04-1.63	[71]
Dihydropyridines (Standard stock solution)	(a)n-Hex/IPA (85:15), (b)n-Hex/IPA (92:8), (c)n-Hex/IPA/EtOH (97:2:1), 1.0mL/min, 25°C, 230nm	**IC** 250×4.6mm, 5μm (A)	(a)R_s=5.65-5.80, α=1.47-1.48 (b)R_s=1.76-1.92, α=1.15-1.18 (c)R_s=1.47-1.84, α=1.14-1.15	[72]

Proton pump inhibitors (Standard stock solution)	(a)*n*-Hex/EtOH/DEA (50:50:0.1), (b)*n*-Hex/EtOH/DEA/TFA (50:50:0.1:0.1), 1.0mL/min, 30 °C, 280 and 300nm	IC 250×4.6mm, 5µm (A)	(a)R_s=4.91-10.15, α=1.51-1.99 (b)R_s=5.0-10.2, α=1.46-1.98	[73]
Lansoprazole and impurities (Standard stock solution)	(a)*n*-Hex/EtOH/DEA (60:40:0.1), (b)EtOH/w (50/50),1.0mL/min, 25°C, 280nm	IC 250×4.6mm, 5µm (A) IC-3 250 ×4.6mm, 3µm (A) IC 250×10mm, 5µm (S)	IC (a)R_s=5.36-5.67, α=1.79-2.00 IC-3 (b)R_s=1.18-2.63, α=1.26-1.50	[50]
Antihistamines (Standard stock solution)	(a)*n*-Hex/IPA/DEA (95:5:0.1), (b)*n*-Hex/EtOH/DEA (95:5:0.1), 0.8mL/min, 25°C, 227 and 262nm	IC 250×4.6mm, 5µm (A)	(a)R_s≤5.75, α=1.05-1.36 (b)R_s=0.63-2.82, α=1.04-1.15	[74]
Ezetimibe optical isomers (Bulk drug)	*n*-Hex/IPA/DEA (90:10:0.1), 1.0mL/min, 25°C, 256nm	IC 250× 4.6mm, 5µm (A)	$R_{s1,2}$=3.02, $\alpha_{1,2}$=1.37 $R_{s2,3}$=2.15, $\alpha_{2,3}$=1.25 $R_{s3,4}$=2.20, $\alpha_{3,4}$=1.23	[75]
Proton pump inhibitors (Wastewater and river water)	ACN/ 5mM NH$_4$Ac (60:40), 0.6mL/min, 15°C, LC-MS/MS	IC 250× 4.6mm, 5µm (A)	R_s>1.5	[76]
10-hydroxycamptothecin (Synthetic compounds)	*n*-Hex/EtOH (50:50), 1.0mL/min, 40°C, 270nm	IC 250× 4.6mm, 5µm (A)	R_s=3.2	[77]
3-(S)-Quinuclidinol (Synthetic compounds)	*n*-Hex/EtOH/IPA/DEA (80:8:12:0.4), 0.8mL/min, 15°C, 230nm	IC 250× 4.6mm, 5µm (A)	R_s=11.4, α=2.18	[78]
Rabeprazole and impurities (Pharmaceutical formulations)	Gradient programme: (A)phosphate buffer at pH=7.0, (B)ACN, t(min)/B (v/v): 0.0/35, 20.0/70, 25.0/35, 30.0/35, 35°C, 1.0mL/min, 282nm	IC 250×4.6mm, 5µm (A)	R_s=15.35 (Imp-B), 9.45 (S-Rab), 3.95 (R-Rab), 13.89 (Imp-A)	[79]
Xeljanz (Active pharmaceutical ingredients and tablets)	*n*-Hex/EtOH/DEA (65:35:0.1), 0.9mL/min, 40°C, 289nm	IC 250×4.6mm, 10µm (A)	R_s=2.07	[80]
Lansoprazole (Human plasma)	10mM NH$_4$Ac with 0.05% HAc/ACN (50:50), 0.6mL/min, 30°C, LC-MS/MS	IC 150×4.6mm, 5µm (A)	R_s~1.5	[81]

Carboxilate ester of evacetrapib (Standard stock solution)	n-Hex/MTBE/IPA (90:9:1), 1.0mL/min, 40°C, 260nm	**IC-3** 250 ×4.6mm, 3µm (A)	R_s=1.96 (between the trans-R-enantiomers and the penultimate)	[82]
Oxaliplatin (Standard stock solution)	(a)ACN/w (100:5), 40°C, (b)MEOH/w (100:5), (c) EtOH, 1.0mL/min, 25°C, 210nm	**IC-3** 100×4.6mm, 3µm (A)	(a)R_s=5.79, α=2.03, (b)α=1.81 (c)R_s=1.81, α=2.04	[44]
Pioglitazone (Rat plasma)	n-Hex/IPA (70:30), 1.0mL/min, 35°C, 225nm	**IC** 250×4.6mm, 5µm (A)	R_s=3.43	[83]
Darunavir (Standard stock solution)	(a)n-Hex/IPA/DEA (65:35:0.1), (b)n-Hex/EtOH (65:35:0.1), (c)n-Hex/1-PrOH (65:35:0.1), 1.2mL/min, 25°C, 266nm	**IC** 250 ×4.6mm (A)	(a)R_s=6.96, α=2.20 (b)R_s=3.48, α=1.50 (c)R_s=5.13, α=1.98	[84]
Indapamide (Rat whole blood)	n-Hex/IPA (70:30), 0.8mL/min, 25°C, 240nm	**IC** 250×4.6mm, 5µm (A)	R_s<2	[85]
Nipecotic acid (Synthetic compounds)	20mM HFor and 20mM N,N-diisopropyleethylamine in ACN, 1.0mL/min, 40°C, 490nm	**ID-3** 250 ×4.6mm, 3µm (A)	R_s=5.44	[86]
Declatasvir and its enantiomers (Standard stock solution)	Gradient programme: (A) MeOH/DEA (100/0.1), (B) ACN/DEA (100/0.1), t(min)/B (v/v): 0.0/10,10.0/80, 15.0/80, 15.1/10, 25.0/10, 40°C, 1.0mL/min, 315nm	**ID-3** 250×4.6mm, 3µm (A)	$R_{s1,2}$=4.52, $\alpha_{1,2}$=1.27 $R_{s2,3}$=2.56, $\alpha_{2,3}$=1.11 $R_{s3,4}$=6.21, $\alpha_{3,4}$=1.34	[87]
Omeprazole and impurities (Standard stock solution)	ACN/w (50:50), 1.0 mL/min, 40°C, 280nm	**ID-3** 100×4.6mm, 3µm (A)	R_s>1.5 α=3.11; α=2.56; α=1.10	[88]
Efonidipine (Human plasma)	ACN/w (60:40), 0.4mL/min, 25°C, LC-MS/MS	**ID** 250×4.6mm, 5µm (A)	R_s=3.02, α=1.13	[89]
Oxiracetam (Beagle dog and rat plasma)	n-Hex/EtOH/TFA (78:22:0.1), 1.0mL/min, 35°C, 214nm	**ID** 250 ×4.6mm, 5µm (A)	R_s>>1.5	[90,91]
Several pharmaceutical compounds (Standard stock solution)	n-Hex/IPA (90:10), 1.0mL/min, 25°C, 219 and 270nm	**ID** 250×4.6mm, 5µm (A) **ID-3** 150×3mm, 3µm (A)	**ID** R_s=1.33-7.71, α=1.24-4.69 **ID-3** R_s=2.25-3.85, α=2.70-3.34	[92]

Triazole drugs and diastereomeric mixture (Standard stock solution)	n-Hex/IPA/EtOH/DEA (60:35:5:0.1), 1.0mL/min, 25°C, 230nm	ID 250 ×4.6mm, 5µm (A)	$R_{s1,2}$=-, α_1=7.67 $R_{s2,3}$=2.12, α_2=8.65 $R_{s3,4}$=6.27, α_3=12.03 $R_{s3,4}$=8.16, α_4=18.25	[93]
Proton pump inhibitors (Standard stock solution)	(a)100%MeOH, (b)100%EtOH, (c)100%THF, (d)100%ACN, 1.0mL/min, 25°C, 280nm	**ID-3** 100 ×4.6mm, 3µm (A) **IE-3** 100 ×4.6mm, 3µm (A)	**ID-3** (a)$\alpha\leq$2.94, (b)$\alpha\leq$1.59, (c)α=1.00, (d)α=1.18-3.15 **IE-3** (a)$\alpha\leq$1.24, (b)$\alpha\leq$1.40, (c)α=1.00, (d) α=1.18-1.62	[94]
Besifloxacin (Bulk drug and formulations)	DCM/IPA/TFA/DEA (90:10:0.3:0.5), 1.5mL/min, 15°C	**IE-3** 250×4.6mm, 3µm (A)	R_s=2.07, α=2.14	[95]
Pantoprazole (Human plasma)	10mM NH$_4$AcACN with 0.1% HAc/ACN (28:72), 0.5mL/min, 30°C, LC-MS/MS	**IE** 150×4.6mm, 3µm (A)	R_s>1.5	[96]
Triclabendazole (Synthetic compound)	(a)n-Hex/EtOH/EA/TFA (70:1:30:0.1), (b)n-Hex/IPA/TFA (70:30:0.1), (c)n-Hex/EtOH/TFA (70:30:0.1), (d) ACN/w (100:100), 1.0mL/min, 25°C, 294nm	**IF-3** 250×4.6mm, 3µm (A) and (S)	(a)R_s=5.17, α=1.45 (b)R_s=1.54, α=1.20 (c)R_s=2.67, α=1.26 (d)R_s=1.99	[41]
Ricobendazole (Synthetic compound)	n-Hex/IPA/EtOH/DEA (60:35:5:0.1), 1.0mL/min, 25°C, 230nm	**IG-3** 250×4.6mm, 3µm (A) **IG** 250×10mm (S)	(a)R_s=10.90; α=2.35, (b)R_s=14.25; α=2.41	[40]

(A)=analytical, (S)=semipreparative, (P)=preparative, MeOH=methanol, EtOH=ethanol, IPA=2-propanol, PrOH=1-propanol, BuOH=1-butanol, t-BuOH=tert-butanol, ACN=acetonitrile, THF=tetrahydrofuran, w=water, DEA=diethylamine, TEA=triethylamine, HFor=Formic acid, TFA=Trifluoroacetic acid, n-Hex=n-hexane, n-Hep=n-heptane, EA=ethyl acetate, DCM=dichloromethane, MTBE=methyl tert-butyl ether, nd=no detected.

Table 3: Chiral resolution of different pesticides on immobilized polysaccharide-based CSPs.

Compound (Sample)	Chromatographic conditions	Chiralpak column	Chromatographic parameters	Ref.
Pyriproxyfen and metabolites (Standard stock solution)	(a)n-Hex/EtOH (95:5), (b)ACN/water (50:50), 0.3-0.8mL/min, 15°C, 220, 230 and 270nm	**IA**, **IB** and **IC** 250×4.6mm, 5µm (A)	**IA** (a)R_s=2.39-5.64, α=1.11-1.45 **IB** (b)R_s=3.31, α=1.08	[97]
Dufulin (α-aminophosphonate) (Soil)	n-Hex/EtOH (90:10), 1.0mL/min, 25°C	**IA** 250×4.6mm, 5µm (A)	R_s>1.5	[98]
Indoxacarb (Natural waters)	n-Hex/IPA (70:30), 1.0mL/min, 30 °C, 310nm	**IA** 250×4.6mm, 5µm (A) **IA** 250× 10mm, 5µm (S)	R_s>1.5	[48]
Vinclozolin (Soil)	(a)n-Hex/IPA (98:2), (b)n-Hex/IPA (95:5), (c)n-Hex/EtOH (98:2), (d) n-Hex/BuOH (95:5), 0.8mL/min, 20°C, 220nm	**IB** 250×4.6mm, 5µm (A)	(a)R_s=2.90, α=1.15 (b)R_s=2.51, α=1.14 (c)R_s=2.67, α=1.16 (d)R_s=2.56, α=1.13	[99]
Phenylpyrazole pesticides and metabolites (Standard stock solution)	(a) n-Hex/IPA (95:5), (b) n-Hex/EtOH (95:5), (c) PE/IPA (98:2), (d) PE/EtOH (95:5), 1.0mL/min, 30°C, 230nm	**IB** 250×4.6mm, 5µm (A)	(a)R_s=1.62-2.30, α=1.25-1.26 (b)R_s=1.67-3.40, α=1.18-1.38 (c)R_s=1.56-16.82, α=1.22-6.71 (d)R_s=1.67-9.40, α=1.22-2.61	[100]
Benalaxyl and benalaxyl acid (Soil)	ACN/w/HFor (90:10:0.1), 0.5mL/min, 20°C, LC-MS/MS	**IC** 250×4.6mm (A)	R_s~1.5	[101]
Furalaxyl (Residues in *Tenebrio molitor larvae* and wheat bran)	ACN/w (80:20), 0.4mL/min, 20°C, LC-MS/MS	**IC** (A)	R_s~1.5	[102]
Fenoxaprop-ethyl and its metabolites (Soil with earthworms)	MeOH/w/HFor (75:25:0.1), 0.5 mL/min, 20°C, LC-MS/MS	**IC** 250×4.6mm (A)	R_s~1.5	[103]
Fluazifop (flu) and fluazifop-	(a)MeOH/ 0.1% aqueous HFor (80:20),	**IC** 250×4.6mm, 5µm (A)	(a)R_s=1.57, α=1.24 (flu-butyl)	[104]

butyl (flu-butyl) (Water and soil)	(b)MeOH/ 0.1% aqueous HFor (68:32), 0.5mL/min, 25°C, LC-MS/MS		(b)R_s=1.87, α=1.18 (flu)	
Fenpropathrin (Soil)	(a)MeOH/w (1000:0 to 70:30), (b)ACN/w (65:35 to 50:50), 0.8mL/min, 230nm	**IC** 250×4.6mm, 5μm (A)	(a)R_s=-, α=1.00 (b)R_s=0.34-0.64, α=1.03-1.03	[105]
Tanikolide and intermediates (Standard stock solution)	(a)n-Hex/IPA (90:10), (b)n-Hex/EtOH (95:5), 0.8mL/min, 25°C, 206 and 230nm	**IC** 250×4.6mm, 5μm (A)	(a)R_s=1.47-8.24, α=1.21-1.62 (b)R_s=1.03-2.54, α=1.09-1.13	[106]
Indoxacarb (Green tea)	ACN/w (60:40), 0.8mL/min, 30°C, LC-MS/MS	**IC** 250×4.6mm, 5μm (A)	R_s~1.5	[107]
Diclofop-methyl and diclofop (Loach liver microsomes in vitro)	n-Hex/IPA/TFA (96:4:0.1), 1.0mL/min, 20°C, 260nm	**IC** 250×4.6mm (A)	R_s>1.5	[108]
Triazole fungicides (Groundwater and river water)	(a)PE/EtOH (95:5), (b)n-Hex/IPA (95:5), 1.0mL/min, 20°C, 220 and 230nm	**IC** 250×4.6mm (A)	(a)R_s=1.36-9.14, α=1.10-1.51 (b)R_s=1.38-8.42, α=1.12-1.46	[109]
Napropamide (Tomato, cucumber, rape, cabbage, and soil)	n-Hex/IPA (85:15), 1.0mL/min, 35°C, 225nm	**IC** 250×4.6mm (A)	R_s=11.75, α=1.11	[110]

(A)=analytical, (S)=semipreparative, MeOH=methanol, EtOH=ethanol, IPA=2-propanol, BuOH=1-butanol, ACN=acetonitrile, w=water, HFor=Formic acid, TFA=Trifluoroacetic acid, n-Hex=n-hexane, PE=petroleum ether.

Table 4: Chiral resolution of organic, polyaromatic, natural and synthetic compounds on immobilized polysaccharide-based CSPs.

Compound	Chromatographic conditions	Chiralpak column	Chromatographic parameters	Ref.
Chiral Sulfoxides (Standard stock solution)	(a)n-Hep/IPA/DEA (90:10:0.1), (b)n-Hep/EtOH/DEA (90:10:0.1) 1mL/min, 20°C, 254 and 230nm	**IA** 250×4.6mm, 5µm (A)	(a)R_s≤1.88, $α$≤1.14 (b)R_s≤5.91, $α$≤1.49	[111]
Underivatized chiral primary amines (Standard stock solution)	(a)ACN/IPA/BA (97:3:0.1), (b)n-Hep/EtOH/BA (90:10:0.1), 2.0mL/min, 25°C, 280nm	**IA**, **IB**, **IC**, **ID**, **IF** 250×4.6mm, 5µm (A)	**IA** (a) $α$=1.54-4.33; (b)$α$=1.44-2.64 **IB** (a)$α$=1.83; (b)$α$=1.59 **IC** (a)$α$=1.47; (b)$α$=1.17-3.26 **ID** (a)$α$=1.76-2.81; (b)$α$=1.23-2.53 **IF** (a)$α$=2.58-3.11; (b)$α$=1.38	[112]
Cationic hetero[6]helicenes (Synthetic compounds)	(a)MeOH/30mM KPF$_6$ (90:10), (b)ACN/ 30mM NH$_4$Ac (90:10), 1mL/min, 25°C, 420, 370 and 450nm	**IA** 250×4.6mm, 5µm (A) **ID** ~~2×25 cm~~ 250×20mm; 5 µm ~~(P)~~ (S)	(a)$α$=1.11-1.14, (b)$α$=1.08	[46]
β-aminoketones (Synthetic compounds)	(a)n-Hex/EtOH (95:5), (b)IPA, 0.2-0.5mL/min, 25°C, 250, 260, 275 and 301nm	**IA** and **IB** 150×4.6mm, 5µm (A)	**IA** (a)R_s≤1.89, $α$≤1.11 **IB** (a)R_s=0.92-1.67, $α$=1.06-1.07 (b)R_s≤0.62, $α$≤1.11	[113]
Chiral 9,9 Spirobifluorenes derivatives (Synthetic compounds)	(a)CHCl$_3$/IPA (95:5), 300nm (b)CHCl$_3$/IPA (90:10), 325nm, 0.5mL/min	**IA** 250×4.6mm, 5µm (A) **IA** 250×10mm, 5µm (S)	R_s>>1.5, $α$=4.3	[47]
3,5-disubstituted hydantoins (Synthetic compounds)	(a)n-Hex/EtOH (80:20), (b)n-Hex/PrOH (80:20), (c)n-Hex/IPA (80:20), (d)n-Hex/BuOH (80:20), (e)n-Hex/t-BuOH (80:20), 0.5mL/min, 25°C, 220nm	**IA** 250×4.6mm, 5µm (A)	(a)$α$=1.33-2.21, (b)$α$=1.13-2.97 (c)$α$≤1.71, (d)$α$=1.62-2.16 (e)$α$=1.08-1.55	[114]
N-protected amino acids (Standard stock solution)	n-Hex/EtOH/TFA (75:25:0.5), 1.0 mL/min, 30°C, 310nm	**IA** 250×4.6mm (A)	R_s=1.16-1.76, $α$=1.10-1.22	[115]

| Compound	Conditions	Column	Results	Ref
Polyhalogenated 4,4'-bipyridines (Synthetic compounds)	(a)n-Hex/IPA (90:10), (b)MeOH, (c)MeOH/IPA (50:50), (d)EtOH, (e)ACN, (f)n-Hep/THF (95:5), (g)n-Hep/THF/DCM (90:5:5), (h)n-Hex/IPA/MeOH (90:5:5), 0.4-0.8mL/min, 22°C, 220, 254, 280 and 360nm	**IA** and **IC** 250×4.6mm, 5µm (A)	**IC** (a)$R_s \leq 3.4$, $\alpha \leq 1.34$; (b)R_s=1.0-4.2, α=1.12-1.51; (c)R_s=1.8-4.6, α=1.27-1.71; (d)R_s=2.5-4.5, α=1.51-1.70; (e)R_s=3.7-5.9, α=1.54-1.97; (f)R_s=0.7-4.9, α=1.06-1.59; (g)R_s=0.6-3.6, α=1.04-1.53 **IA** (h)R_s=0.7-4.2, α=1.12-1.47	[116]
Aliphatic amines as nitrobenzoxadiazole derivatives (Synthetic compounds)	n-Hex/IPA (95:5), 1.0mL/min, 25°C, λ_{ex}=470nm and λ_{em}=530nm	**IA, IB, IC, ID, IE** and **IF** 250×4.6mm, 5µm (A)	**IA** $R_s \leq 6.06$, $\alpha \leq 1.44$, **IB** $R_s \leq 1.0$, $\alpha \leq 1.04$, **IC** $R_s \leq 8.73$, $\alpha \leq 1.63$, **ID** R_s=1.31-4.30, α=1.11-1.40, **IE** $R_s \leq 10.89$, $\alpha \leq 4.44$, **IF** R_s=1.03-1.29, α=1.22-2.61	[39]
Atropisomeric 3,3',5,5'-tetrasubstituted-4,4'-bipyridines (Synthetic compounds)	(a)n-Hex/IPA (90:10), (b)EtOH, (c)EtOH/IPA (80:20), 0.4-0.8mL/min, 22°C, 220, 254, 280 and 360nm	**IA** and **IC** 250×4.6mm, 5µm (A)	**IA** (a)$R_s \leq 10.0$, $\alpha \leq 2.59$, (b)$R_s \leq 3.6$, α=1.06-1.65, (c)R_s=0.6-5.4, α=1.07-1.60 **IC** (a)$R_s \leq 30.6$, $\alpha \leq 8.33$, (b)$R_s \leq 7.4$, $\alpha \leq 2.08$	[117]
Troger´s Base derivatives (Synthetic compounds)	(a)n-Hex/DCM (75:25), 1.5mL/min, (b)n-Hex/DCM (80:20), 1.5mL/min	**IA** 250×4.6mm, 5µm (A) **IA** 250×10mm, 5µm (S)	(a)R_s=5.58, α=1.55 (*rac*-9) (b)R_s=5.65, α=1.30 (*rac*-12)	[118]
Polyarsenic derivatives (Synthetic compounds)	DCM/n-Hex (2:8), 1.0mL/min, λ=254nm	**IA** 250×4.6mm, 5µm (A)	$R_s \gg 1.5$	[119]
Racemic 4,12-difunctionalized [2,2]paracyclophanes (Synthetic compounds)	n-Hex/EtOH (75:25), 1.0mL/min	**IC** 250×4.6mm, 5µm (A) **IC** 250×10mm, 5µm (S)	$R_s \gg 1.5$	[49]
γ-substituted butanolide (Synthetic compounds)	(a)n-Hex/IPA (70:30), (b)n-Hex/EtOH (70:30), 0.8mL/min, 25°C, 254nm	**IC** 250×4.6mm, 5µm (A)	(a)R_s=2.11-4.77, α=1.10-1.21 (a)R_s=0.86-2.75, α=1.03-1.11	[120]

Homoharringtonine and Intermediates (Synthetic compounds)	(a)n-Hex/EtOH (90:10), (b)n-Hex/IPA (90:10),0.8mL/min, 25°C, 220nm	**IC** 250×4.6mm, 5µm (A)	(a)R_s≤13.71, α≤1.73 (b)R_s≤13.05, α≤1.81	[121]
Tris-(3-indolyl)-phosphane oxides (Standard stock solution)	(a)DCM/IPA/DEA (100:15:0.1), -15°C, (b)DCM/IPA/DEA (100:10:0.1), -10°C, (c)DCM/IPA/DEA (100:1:0.1), 20°C, (d)ACN/w/DEA (100:100:0.1), -5°C, 1.0mL/min, 280nm	(I) **IC** 250×4.6mm (A) (II) **IC** 150×4.6 mm (A)	(I) (a)R_s=2.70, α=1.67 (I) (b)R_s=2.42, α=1.63 (I) (c)R_s=2.65, α=1.33 (II) (d)R_s~1.5	[122]
Farnesyl phenolic compounds (Extract of Genoderma sinense)	n-Hex/IPA (65:35)	**IE** (A)	R_s~1.5	[123]

(A)=analytical, (S)=semipreparative, ~~(P)=preparative~~, MeOH=methanol, EtOH=ethanol, IPA=2-propanol, BuOH=1-butanol, t-BuOH=*tert*-butanol, ACN=acetonitrile, THF=tetrahydrofuran, w=water, DEA=diethylamine, BA=butyl amine, TFA=Trifluoroacetic acid, n-Hex=n-hexane, n-Hep=n-heptane, DCM=dichloromethane.

Highlights

- Polysaccharide (amylose and cellulose) based CSPs are widely reviewed.

- Fundamental aspects of their enantiorecognition ability in HPLC are discussed.

- Comparison between coated and immobilized CSPs are done.

- Advantages and applications of immobilized ones are described.
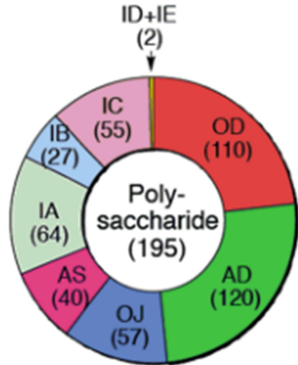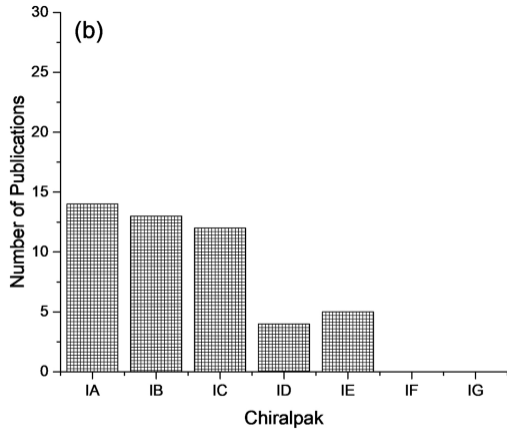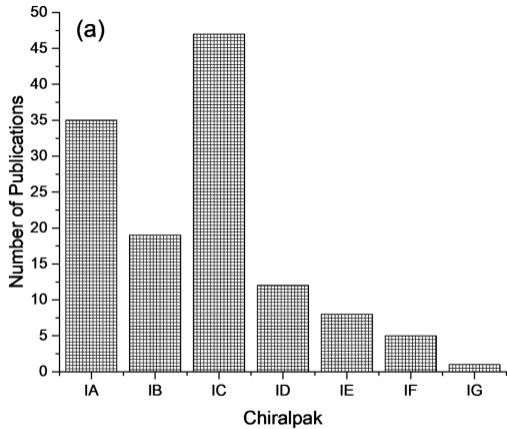
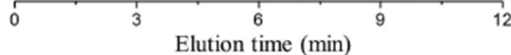- Perspective and future trends are summarized.
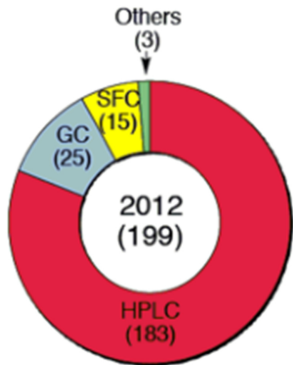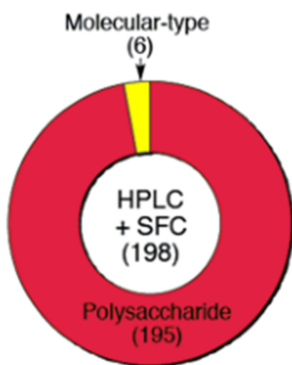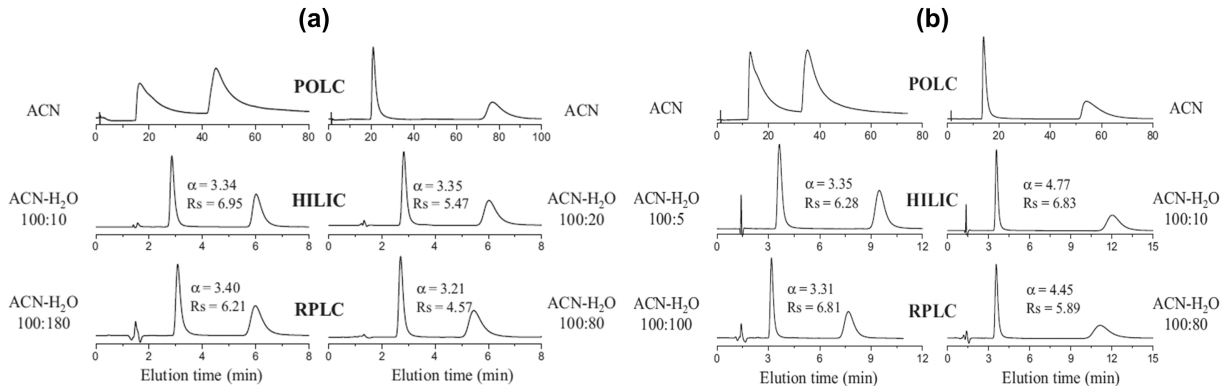
Figure 1

Figure 2

Figure 3

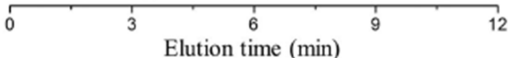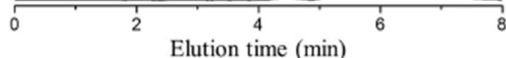**NP MODE**

LAN

$k_1 = 0.90$
$\alpha = 1.81$ Rs = 5.57

$k_1 = 0.96$
$\alpha = 1.80$ Rs = 5.84

$k_1 = 1.02$
$\alpha = 1.79$ Rs = 5.64

$k_1 = 1.08$
$\alpha = 1.79$ Rs = 5.67

Elution time (min)

RP MODE

$k_1 = 2.68$
$\alpha = 1.49$ Rs = 3.05

$k_1 = 3.05$
$\alpha = 1.49$ Rs = 2.97

$k_1 = 3.46$
$\alpha = 1.50$ Rs = 2.80

$k_1 = 3.99$
$\alpha = 1.50$ Rs = 2.63

Elution time (min)
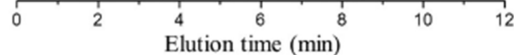
IMP-A

$k_1 = 1.07$
$\alpha = 1.92$ Rs = 5.46

$k_1 = 1.14$
$\alpha = 1.94$ Rs = 5.71

$k_1 = 1.19$
$\alpha = 1.97$ Rs = 5.59

$k_1 = 1.21$
$\alpha = 2.00$ Rs = 5.36

Elution time (min)

$k_1 = 2.31$
$\alpha = 1.26$ Rs = 1.26

$k_1 = 2.12$
$\alpha = 1.26$ Rs = 1.27

$k_1 = 2.41$
$\alpha = 1.27$ Rs = 1.21

$k_1 = 2.84$
$\alpha = 1.26$ Rs = 1.18

Elution time (min)

Figure 4