Focusing Review

# Development of Practical Chiral Stationary Phases for Chromatography and Their Applications

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**Abstract**

Chromatography is a very useful method for the direct separation of enantiomers. However, about 30–40 years ago, commercially available chiral stationary phases were very limited. We have developed many novel chiral stationary phases for gas and liquid chromatography, and found these phases were effective practically to the separation and analysis of various chiral compounds.

*Keywords: chiral stationary phase, gas chromatography, liquid chromatography enantiomer separation*

## 1. Introduction

It was well known chromatography was one of the most useful methods for the direct resolution of enantiomers. Many workers challenged in this field, but all efforts remained fruitless for many years. The breakthrough was accomplished about 30–40 years ago by the unambiguous separation with some chiral stationary phases by Gil−Av et al. [1] in gas chromatography or by Davankov et al. [2] in liquid chromatography.

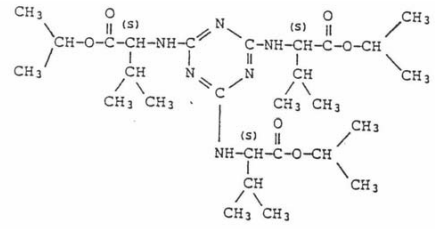
However, in those days, efficient chiral stationary phases were very few and not yet commercially available. Therefore, the conversion of enantiomers to their diastereomers with suitable chiral derivatization reagents was usually used for the separation and analysis of chiral compounds. For example, in the case of the separation of eight isomers of allethrin, which contains one chiral centre in the alcohol moiety (allethrolone) and two chiral centers in the acid moiety (chrysanthemic acid), two components obtained by the hydrolysis were converted to their diastereomers and separated to two and four isomers by gas chromatography with conventional achiral stationary phases. Such process was very complicated and the practical effective chiral stationary phases for the direct separation were desired earnestly.

We have prepared many novel chiral stationary phases and examined their enantioselectivities. Fortunately, some of them showed good enantioselectivity, and we could use them effectively for the separation and analysis of various chiral compounds.

## 2. Gas Chromatography

Since the pioneering work of Gil−Av and coworkers [1], many publications have appeared dealing with the enantiomer separation by gas chromatography (GC) using chiral stationary phases comprised of amino acids or amines. In these phases hydrogen bonding association and charge transfer interaction should play the main role for the chiral recognition. We have prepared many similar chiral stationary phases and found these novel phases were effective for the separation of various classes of organic chiral compounds [3−13].

It was noticed the thermal property and enantioselectivity of stationary phases were improved by the bond of chiral selectors with the *s*−triazine ring. A typical example is CSP−1[3] and the enantiomer separation of some amino acids on this phase is shown in Figure 1.



CSP−1

We have achieved the direct enantiomer separation of racemic *cis* and *trans* chrysanthemic acid with a *s*−triazine derivative of tripeptide ester (CSP−2) [12] as shown in Figure 2.

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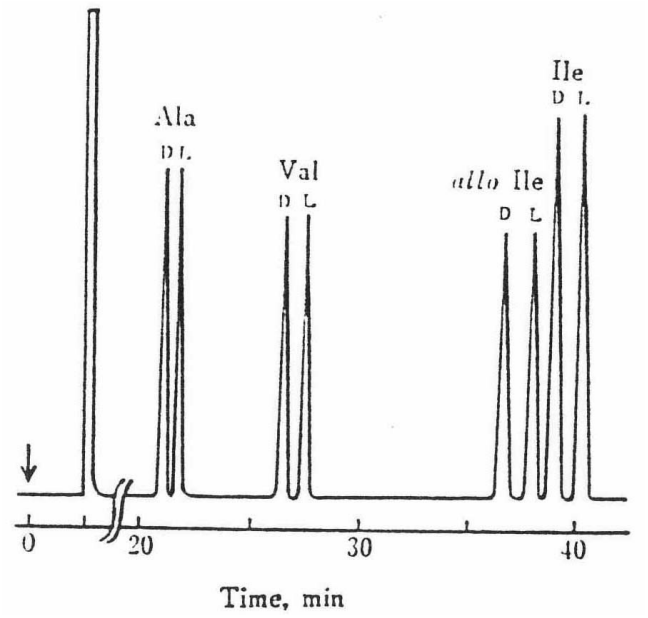
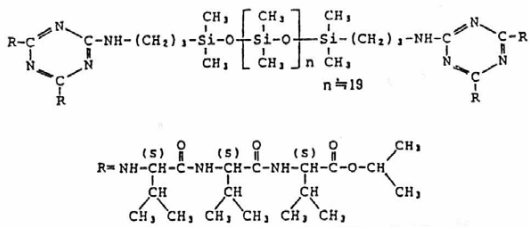



**Figure 1.** Enantiomer separation of N–TFA isopropyl esters of DL –alanine, DL–valine, DL–alloisoleucine and DL–isoleucine by GC (Ref. [3] ).
Column, glass capillary (60 m×0.25 mmI.D.) coated with CSP–1, Carrier gas, helium (0.8 ml/min), Temperature, 100℃, Detection, FID



CSP–2

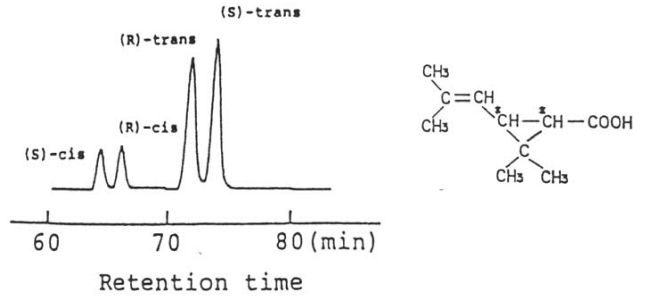
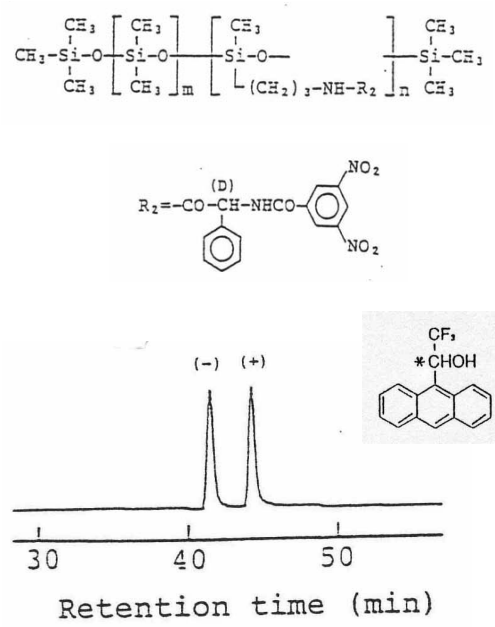



**Figure 2.** Enantiomer separation of *cis* and *trans* chrysanthemic acid by GC (Ref. [12] )
Column, fused silica capillary (25 m×0.25 mmI.D.) coated with CSP–2, Carrier gas, helium (0.8 ml/min), Temperature, 120℃, Detection, FID

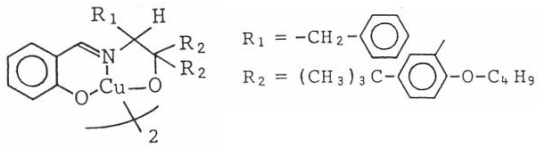
It is interesting that the chiral selector in CSP–3 [13] is the effective chiral selector used in high performance liquid chromatography (HPLC) [23]. The excellent resolution of racemic 2, 2, 2–trifluoro–1– (9–anthryl) ethanol was achieved with CSP–3 by GC as shown in Figure 3, and this result showed the interaction for the chiral recognition in GC and HPLC was common.




CSP–3

**Figure 3.** Enantiomer separation of racemic 2, 2, 2–trifluoro–1–(9 –anthryl) ethanol by GC (Ref [13].)
Column, fused silica capillary (25 m×0.25 mm.I.D) coated with CSP–3, Carrier gas, helium (0.8 ml/min), Temperature, 180'C, Detection, FID

The first gas chromatographic resolution of olefins on an optically active rhodium complex of the camphor derivative was accomplished by Schurig [14]. This result suggested the complexation GC should applicable to various conceivable ligand metal



CSP–4

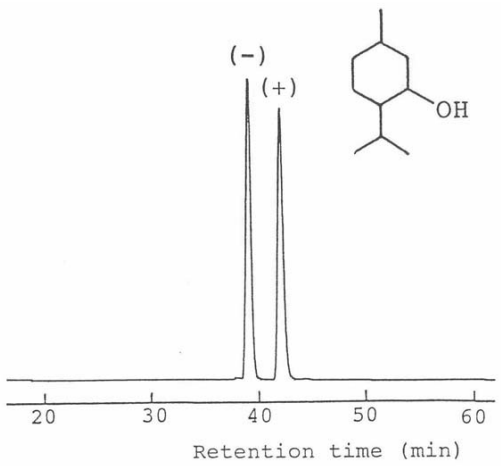



**Figure 4.** Enantiomer separation of racemic menthol by GC (Ref [13] )
Column, fused silica capillary (25 m×0.25 mmI.D) coated with CSP–4 (10% in amino silicone oil), Carrier gas, helium, Temperature, 70℃, Detection, FID

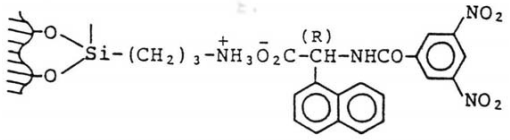
complexation. We have achieved the direct separation of some α–hydroxy acid ester and aminoalcohol enantiomers with the copper (II) complex of Schiff's base of a chiral aminoalcohol (CSP–4) [15, 16. 17, 13]. Although the peak shape of chromatogram was rather asymmetric at first [15], we could obtain excellent chromatograms in the enantiomer separation of aliphatic alcohols on this phase by the aid of amino–silicone oil as shown in Figure 4 [13].

### 3. Liquid Chromatography

It is generally considered that the chiral recognition mechanism is essentially common in both GC and HPLC. Hydrogen bonding association, π–π interaction, dipole–dipole interaction and ligand metal complexation etc. may play important role.

We have prepared various chiral stationary phases comprised of amino acids, amines, and carboxylic acids used in GC as chiral selectors and found some of them were effective for the enantiomer separation by HPLC [18–22].

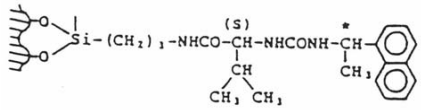
It was noticed CSP–5 containing (R)–1–naphthylglycine as the chiral selector showed excellent enantioselectivity [22]. This phase is a modification of the well known phase containing (R)–phenylglycine as the chiral selector [23]. The superior value of the separation factor obtained on CSP–5 suggested the naphthyl group attached to an asymmetric carbon atom could play the effective role in the chiral recognition.



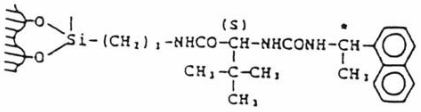

CSP–5

It was already reported an ureid formed by the condensation of phosgene with L–valine isopropyl ester gave good chiral recognition in GC by Feibush et al. [24], but such phase has never been used in HPLC. We have prepared novel urea derivatives of L–valine or (R)–1–(α–naphthyl) ethylamine as chiral stationary phases for HPLC and found the excellent separation was achieved with these phases. [25, 26] These results showed that the urea group bonded to the asymmetric carbon atom was responsible as the active site for the diastereomeric hydrogen bonding association in the chiral recognition.

As we indicated previously the second chiral constituent in amide stationary phase was effective to the chiral recognition in HPLC as well as in GC [9, 19], we prepared urea derivative stationary phases CSP–6 and CSP–7 derived from L–valine and (S)– or (R)–1–(α–naphthyl) ethylamine, which contain two asymmetric carbon atoms attached to two nitrogen atoms of the urea group. [27]. As expected enantioselectivities of these two phases were clearly improved, and it was interesting the elution orders in the separation of some racemic compounds on these phases suggested




CSP–6
CSP–7




CSP–8
CSP–9

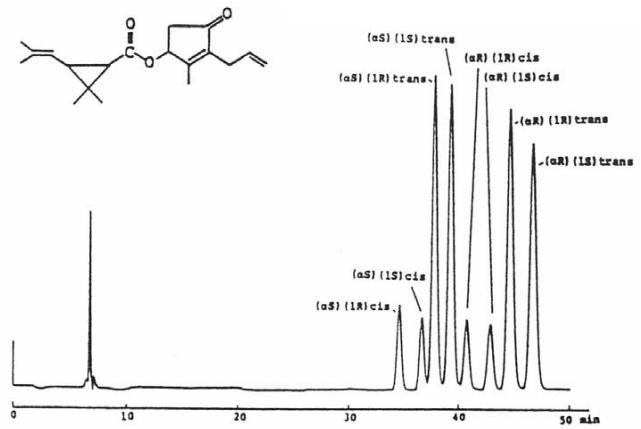
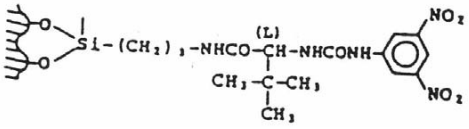



**Figure 5.** Separation of eight isomer mixture of allethrin by HPLC (Ref [32] )
Column, 25 cm×4 mmI. D. Stationary phase, CSP–8, Mobile phase, hexane–1, 2–dichloroethane–ethanol (500/30/0.15), Flow rate, 1.0 ml/min at room temperature, Detection, UV.

each of two chiral centers could contribute to the chiral recognition respectively. [28]

We have also prepared several similar urea derivatives containing another amino acid moieties such as (R)–phenylglycine, (S)–*tert*–leucine (CSP–8, CSP–9), (S)–proline and (S)–indoline–2–carboxylic acid instead of (S)–valine in CSP–6 and CSP–7 [29, 30]. These phases showed very characteristic enantioselectivities and a wide range of chiral compounds were resolved. It was noticed the chemical structure of amino acid moiety controlled their enantioselectivities sensitively. The beautiful separation of the eight isomer mixture of allethrin, which was only partially resolved with the Pirkle type chiral phase [31], was accomplished with CSP–8 as shown in Figure 5 [32]

It was also noticed the direct enantiomer separation of various carboxylic acids was achieved on 3, 5–dinitrophenylurea derivatives derived from D–phenylglycine, L–valine, and L–*tert*–leucine (CSP–10) using aqueous mobile phases [33]. As an example, the enantiomer separation of racemic *cis* and *trance* chrysanthemic acid on CSP–10 is shown in Figure 6.




CSP–10

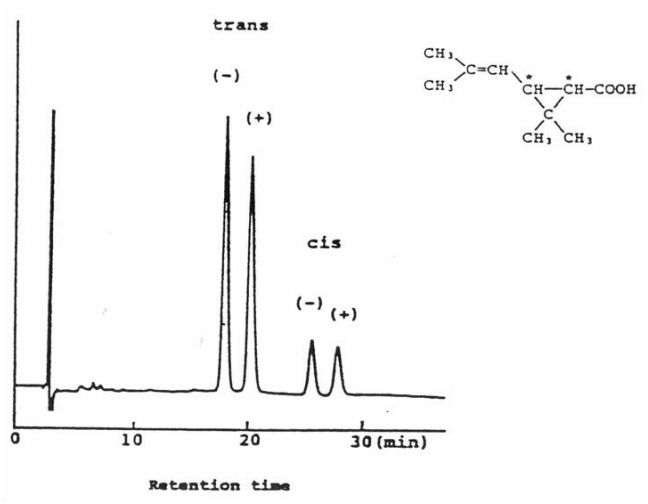



**Figure 6.** Enantiomer separation of racemic *cis* and *trans* crysanthemic acid by HPLC (Ref [33] )
Column, 250×4.6 mm I.D, Stationary phase, CSP−10, Mobile phase, 0.1 M ammonium acetate in water−tetrahydrofuran (60/40), Flow rate, 1.0 ml/min at room temperature, Detection; UV.

Davankov et al. [34] demonstrated a novel chiral phase system which is composed of a reversed phase packings coated with an appropriate resolving agent and a hydro−organic eluent containing the complexing metal ion is very advantageous in chiral ligand exchange HPLC.

We have found CSP−4 which was effective for the complexation gas chromatography was very promising as a coating agent on reverse phase material in HPLC [35, 36]. The direct enantiomer separation of aminoalcohols, amino acids and amines was achieved on this phase using hydro−organic eluents with copper (II) ion. An example of chromatogram is shown in Figure 7.

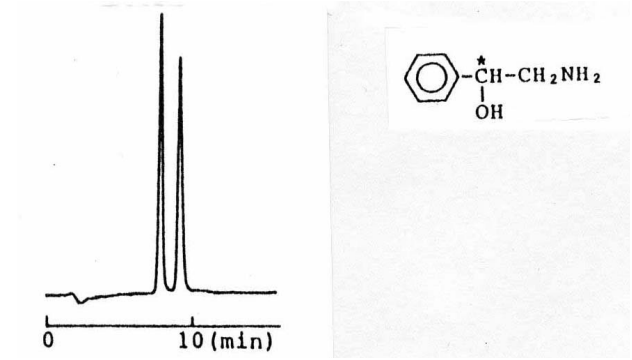
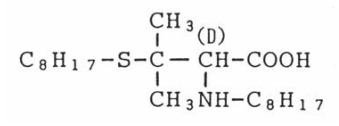
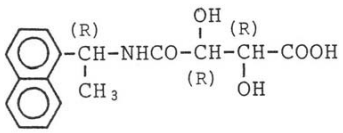



**Figure 7.** Enantiomer separation of racemic 2−amino−1−phenylethanol by HPLC (Ref [35])
Column, ODS (150×4.6 mm I.D), Stationary phase, CSP−4, Mobile phase 1 mM copper (II) sulphate in water, Flow rate, 1.0 ml/min at room temperature, Detection, UV

We have prepared another novel chiral ligand−exchange coating agents (CSP−11 and CSP−12) [37, 38]. Direct enantiomer




separation of many amino acids, amino alcohols and hydroxyl acids was accomplished using octadecylsilanized silica coated with these agents and water or hydro−organic eluents containing copper (II) ion as mobile phases. It was noticed that enantiomers of carboxylic acids and amines were also resolved on copper (II)−complexes of CSP−11 and CSP−12. [39] These results suggest some polar groups in these enantiomers may play a cooperative role in forming of the metal ligand complexes.

We have achieved the adequate separation of racemic amino acids and amines in the form of their derivatives with CSP−12 ionically bonded to the amino−silica gel using organic mobile phases [40]. This result showed CSP−12 could act as a bifunctional chiral selector, and similar derivatives of (1 R, 3 R)−tartaric acid could also act as bifunctional chiral selectors.

## 4. Conclusion

We have developed many novel chiral stationary phases which mainly belonging to the hydrogen−bonding, π−π interaction and ligand metal complexation type for GC and HPLC, and shown these phases were very efficient for the direct resolution of a wide range of enantiomers. Some of these phases are now commercially available and they are very effective for the routine analysis of various chiral compounds.

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