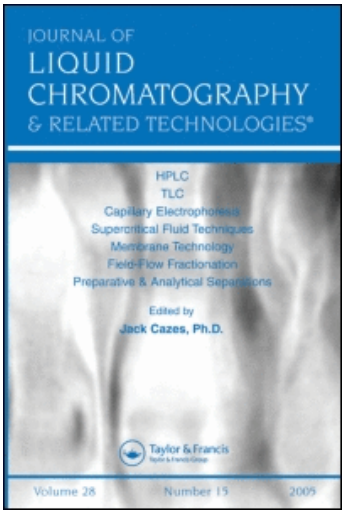

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Chiral Separations: A Comparison of Hplc and TLC

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CHIRAL SEPARATIONS: A COMPARISON OF HPLC AND TLC

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ABSTRACT

Comparisons are made for separations attained in normal-phase HPLC and TLC involving N-carbobenzyloxy-glycyl-L-proline and (+/-)-10-camphorsulfonic acid as chiral mobile phase additives/chiral counter ions (CMA). Possible reasons for differences in stereoselectivity of β-cyclodextrin CMA and chiral stationary phases (CSP) are discussed. In addition, differences in solubility and proposed separation mechanisms for native cyclodextrins (CD) versus derivatized CD are discussed. Inherent differences in high performance liquid chromatographic (HPLC) and thin-layer chromatographic (TLC) methods are outlined. The pros and cons of using HPLC and TLC are considered.

INTRODUCTION

The significance of stereoisomerism in relation to biological activity has been recognized since 1848 when Pasteur reported the first separation of enantiomers from a racemic mixture of tartaric salts (1). The resolution of enantiomers (optical isomers) has

traditionally presented a greater challenge than the development of stereospecific syntheses (2). The development of rapid and efficient chiral separation methods involving the use of chiral counter ions/chiral mobile phase additives (CMA) in thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) and chiral stationary phases (CSP) in TLC, HPLC and gas chromatography (GC) has altered this situation. With the resultant boom in chiral separations, manufacturers in the drug and food and beverage industries have the means to enhance their ability to tailor the preparation of products which contain optically active compounds. Synthetic organic chemists now have many efficient, accurate methods to check their reaction progress and purity (% composition of each enantiomer, quality control, etc.) Recently, the Food and Drug Administration (FDA) has issued guidelines on the submission of New Drug Applications (NDAs) which addresses the question of stereochemistry in the manufacturing of drug substances (3). The Food, Drug and Comestic (FD & C) Act requires a complete description of the "methods used in the manufacture of the drug" which contains testing to demonstrate its identity, strength, quality and purity. Consequently, the decision of whether to market the racemate in preference to a pure enantiomer must now be justified by the submission of relevant information.

In this paper, chiral separations in HPLC and TLC are discussed. First, the advantages of each chromatographic method are evaluated. Then, the inherent differences in HPLC and TLC are noted. Specific examples of normal-phase (NP) and reversed-phase (RP) chiral separations are given and compared for both TLC and HPLC. For example, the use of N-carbobenzyloxy-glycyl-L-proline (ZGP) and 10-

camphorsulfonic acid (CSA) as CMA in NP-TLC is evaluated and comparisons are made between the separations achieved in HPLC versus TLC. Results are also presented for separations attained using maltosyl β-CD as a CMA in RP-TLC. The use of toluoyl β-CD as a CSP in NP-HPLC is discussed. Possible chiral separation mechanisms are considered for the use of derivatized CD in reversed-phase and normal-phase media.

Comparison of TLC and HPLC

TLC has achieved popularity due to its simplicity, low cost and wide versatility and applicability. In his review, Stahl states that TLC is the easiest and most economical chromatographic technique for fast separation and visual evaluation (4). The main advantage of TLC over HPLC has been the ability to screen a large number of compounds concurrently. One problem that has been noted is the elongation of spot size and/or irregular spot size for polar compounds using this method. The introduction of high performance TLC has helped in regulating this problem. The spot diameter is controlled by molecular diffusion in these high performance plates (5). In some cases, TLC could be used to determine what mobile phase to use in HPLC if the same CSP or CMA is utilized.

Several important differences exist between HPLC and TLC. The major advantage that HPLC has over TLC is better reproducibility and greater sensitivity. In HPLC, the column is equilibrated with the mobile phase before use whereas the stationary phase is initially dry in TLC. This has resulted in several important differences. The quantity of mobile phase in the bed varies according to location on

the TLC plate so the phase ratio is variable during development. Second, heat is given off when a solvent wets a dry bed and this results in a small temperature differential between the solvent front and the rest of the bed. Third, in TLC, the mobile phase may change composition during development of a binary or more complex solvent due to differential adsorption. In addition, the velocity of the solvent front is inversely proportional to the distance that the solvent front has traveled so the flow rate is not constant with time or distance. An advantage of HPLC is that the analyst can choose between gradient or isocratic elution. It is also possible to change the flow rate in HPLC to optimize separations. Precise control of pH and ionic strength during the separation process and ease of temperature control are additional advantages offered by high performance LC. Despite these differences, it is sometimes possible to attain similiar results with both chromatographic methods.

CMA have been the mainstay in TLC whereas in HPLC the preferred method is CSP. The amount of chiral agent in the mobile phase may change with TLC due to preferential adsorption of the chiral selector as the solvent front migrates along the chromatographic bed. In contrast, in HPLC, the column is pre-equilibrated with the CMA so the concentration of the CMA remains constant during development. The following equation can be used to interrelate HPLC and TLC if the concentration of the CMA remains constant during development of the TLC plate or if a CSP with an isocratic mobile phase is being used.

$$R_f = 1/(1 + k')$$

where: R_f = retention factor in TLC

k' = capacity factor in HPLC

Theoretically, it is possible to choose the mode which best suits the needs of the analyst. The main obstacle to this idea has been the limited commercial availability of CSP for TLC. There has been a greater number of commercially available CSP for HPLC. However, these columns can be expensive and may damage easily. A viable alternative to CSP is the use of achiral stationary phases with the appropriate CMA. Some disadvantages of using CMA are: (1) the method of detection must be compatible with the CMA used. For example, if a compound which absorbs ultraviolet light is used as a CMA, one can use an ultraviolet detector in HPLC and adjust the baseline accordingly but in TLC it would not be possible to detect the samples using an ultraviolet detector. (2) the isolation of a pure chiral analyte may be difficult. Advantages of using CMA are: (1) it is easy to change the chiral selector being used; (2) the lifetime of conventional achiral columns is typically longer than chiral columns.

Chiral Separations by TLC

There have been relatively few reports of CSP in TLC and only for a limited number of compounds. Yuasa announced the partial separation of DL-tryptophan on a crystalline cellulose-coated plate (6). Wainer, et al. announced the separation of racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol on a chiral dinitrobenzoyl phenylglycine bonded phase (7). Brunner et al. announced the use of a naphthylethyl urea TLC-CSP for the separation of enantiomeric

amides (8). Alak and Armstrong reported the separation of several racemic amino acids and ferrocene derivatives on a β-cyclodextrin bonded phase (9). Weinstein (10), Grinberg and Weinstein (11), and Gunther, et al. (12) reported the separation of several racemic dansyl amino acids on reversed-phase (RP) plates impregnated with copper (II) complexes of chiral alkyl α-amino acid derivatives. At this time, the only CSP which are commercially available for TLC are the ternary complex-ligand exchange plates. Some of the problems in developing chiral TLC plates were discussed by Wilson (13).

There have been even fewer reports on using CMA in TLC analysis than CSP. Enantiomers of aromatic amino alcohols have been separated using CSA and ZGP as chiral counter ions in NP-TLC (14). The formation of diastereomeric pairs of ions with the chiral ion interaction agent is the basis for the resolution of optical isomers. Enantiomers have identical chemical and physical properties in an isotropic environment but diastereomers may display dissimiliar distribution properties in isotropic environments; this difference makes it possible to separate diastereomeric ion-pairs using non-chiral stationary phases.

Cyclodextrins (CD) have proven useful as chiral stationary phases (CSP) and as chiral mobile phase additives/chiral counter ions (CMA) but their low solubility in hydro-organic solvents has limited their applicability as CMA in the reversed-phase (RP) mode. It is possible to increase the solubility of β-CD by using saturated urea solutions or by synthetically modifying the native molecule. Chiral separations have been achieved in TLC using β-CD as a CMA in saturated urea solutions (15). Derivatized forms of β-CD have also proven useful as CMA in TLC (16,17). The use of maltosyl β-CD as a

CMA was evaluated in this paper and comparisons are made between the native and the derivatized CD used as CMA in RP-TLC. The chiral separation mechanism involved in the reversed-phase mode is thought to be due to the formation of CD inclusion complexes. Each enantiomer complexes the CD cavity differently, so disparate retention times are noted for the optical isomers.

Chiral Separations by HPLC

In 1981, Pirkle introduced the first commercially available HPLC-CSP (18). The number of commercially available HPLC-CSP has risen to over thirty and has continued to grow annually (19). There have been several reviews of HPLC-CSP which were designed to help determine which CSP to use for a particular problem (20-24). It should be noted that research has concentrated on HPLC while the progress in TLC has advanced much more slowly. Many enantiomeric compounds can be resolved on conventional achiral LC columns by using suitable chiral additives to the mobile phase both in reversed-phase (25) and normal-phase (26-28) modes. The selectivities of these CMA sometimes differ from those of available CSP.

Comparison of HPLC and TLC Results

Chiral Selectors as NP-CMA Results which are attained with TLC-CMA are similiar to those which can be obtained using the same HPLC-CMA. Several aromatic amino alcohols have been resolved using a mobile phase containing ZGP or CSA by NP-HPLC (26-28) and

by NP-TLC(14). The chiral separation mechanism for each chromatographic method (HPLC and TLC) in this case is the formation of diastereomeric ion-pairs.

Separations achieved for aromatic amino alcohols in HPLC involved the (+) antipode of 10-camphorsulfonate whereas the same work performed in TLC used the (-) antipode. It was possible to obtain better stereoselectivity in HPLC with the ZGP than with the CSA for the aminoalcohols. In TLC, ZGP and CSA demonstrated unique abilities in separating enantiomeric aminoalcohols. Of the eight enantiomers resolved in this TLC study; two compounds could only be separated using ZGP, two compounds could only be resolved using CSA and four of the compounds analyzed could be separated using either CMA. Of the enantiomers which could be resolved with either CMA, a higher stereoselectivity was obtained in four out of five of these compounds (Table I) when ZGP was the CMA. There were also differences in the effects which resulted from increased concentrations of CMA in the mobile phase. It was difficult to dissolve CSA in the main solvent (dry dichloromethane). Solubility problems were encountered, in TLC, if the concentration of CSA was above 10mM so it was not possible to study the effects of increased concentrations of this CMA. It was possible to vary the concentration of ZGP in the TLC study from 6.5-16.8 mM without any significant change in the retention factor (R_f) or the separation factor (α) (Table II). The capacity factors (k') for the enantiomers usually decrease with an increase in the concentration of the counter ion. This is probably due to increased competition between the counter ion and the diastereomeric ion-pair for adsorption sites on the stationary phase.

Table I. Compounds Separated Using ZGP or CSA as Chiral Mobile Phase Additives

Compounds	R_{f1}	R_{f2}	α	Conditions
Phenylpropanol-amine	0.06	0.26	4.3	6.9 mM ZGP + 5 mM TEA[a]
Octopamine	0.15	0.33	2.2	7.9 mM CSA[a]
Pindolol	0.07	0.12	1.7	6.7 mM ZGP + 5 mM TEA[b]
Norphenylephrine	0.05	0.26	5.2	5.9 mM ZGP + 5 mM TEA[a]
	0.03	0.14	4.7	6.8 mM CSA[c]
Propranolol	0.08	0.20	2.5	6.7 mM ZGP + 5 mM TEA[c]
	0.05	0.20	4.0	9.3 mM CSA[c]
Isoproterenol	0.14	0.38	2.7	5.9 mM ZGP + 5 mM TEA[a]
	0.12	0.30	2.5	6.8 mM CSA[a]
Metoprolol	0.11	0.17	1.5	10.7 mM CSA[b]
Timolol	0.26	0.51	2.0	5.8 mM ZGP[a]
	0.39	0.55	1.4	13.9 mM CSA[b]

[a]HP Silica gel plates with 75/25 (v/v) methylene chloride/methanol
[b]DIOL plates with 95/5 (v/v) methylene chloride/2-propanol
[c]HP Silica gel plates with 90/10 (v/v) methylene chloride/methanol

NOTE: All separations achieved with ZGP contained 5 mM TEA.

Source: Duncan et. al (14)

Table II. Effect of Varying Concentration of ZGP on Enantioselectivity for Propranolol in TLC Study

Concentration of ZGP (mM)	R_{f1} range	R_{f2} range	α (average)
6.5	0.00-0.14	0.14-0.33	1.7
6.8	0.00-0.13	0.13-0.36	1.9
13.6	0.01-0.11	0.11-0.30	1.8
16.8	0.05-0.12	0.12-0.27	1.6

*Experimental conditions: The mobile phase consisted of 90/10, methylene chloride/methanol. The stationary phase was HP-KF silica gel. The method of detection was a Schimadzu dual wavelength TLC scanner (CS-910).

There were several mobile phase dissimilarities which were noted in this comparison between HPLC and TLC when ZGP and CSA are used as CMA. The addition of triethylamine (TEA) to the mobile phase in HPLC decreased retention times but did not effect stereoselectivity. The use of TEA in the mobile phase in TLC gave different results with different compounds (Table III) and no general trend was noted. Acetonitrile, tetrahydrofuran, 1-pentanol and ethyl acetate could be used as polar modifiers in HPLC but 1-pentanol was the only modifier which did not contribute to peak asymmetry. Solute movement in TLC was restricted to mobile phases which contained an alcohol as co-solvent. The R_f values in TLC increased with an increase in the amount of alcohol in the mobile phase. This is as expected because the solutes travel farther on the plate if

Table III. The Effect on Enantioselectivity when TEA is added to Mobile Phases Containing ZGP in TLC Studies

Compound	with TEA			without TEA		
	R_{f1}	R_{f2}	α	R_{f1}	R_{f2}	α
Propranolol	0.08	0.20	2.5	0.10	0.30	3.0
Isoproterenol	0.14	0.38	2.7	0.04	0.10	2.5
Phenylpropranol-amine	0.06	0.26	4.3	0.08	0.32	4.0
Timolol	0.26	0.51	2.0	0.20	0.40	2.0

*Experimental conditions: The mobile phase consisted of 75/25, methylene chloride/methanol. The stationary phase was HP-KF silica gel. A Schimadzu dual wavelength TLC scanner(CS-910) was used for detection.

interactions with the mobile phase are enhanced. Similiarly, an increase in the alcohol content resulted in decreased retention times in HPLC The α values changed, in TLC, if the amount of alcohol in the mobile phase was varied. The water content of the mobile phase of HPLC and TLC (and the stationary phase in TLC) has an influence on the retention and stereoselectivity for these studies performed using ZGP and CSA as CMA. It is not possible to attain separations in HPLC or TLC if there is much water present. This is presumably due in part to competition for the chiral counter ion by the water. In addition, the formation of the neutral diastereomeric association complex is promoted by a nonpolar medium.

In HPLC, the preferred stationary phase was the DIOL. In TLC, the largest number of compounds were separated with the high performance silica gel plates although it was necessary to use the DIOL plates to resolve some of the analytes. The only compound which could be separated on either type of TLC plate was timolol.

Chiral selectors as RP-CMA Recent investigations into the use of CD as chiral separation agents involve the utilization of different types of cycodextrin derivatives as CMA. Derivatization of some of the CD hydroxyls results in enhanced aqueous solubility relative to the native CD (16). Some of the compounds which can be separated using maltosyl β-CD as a CMA in RP-TLC are shown in Table IV. The stereoselectivity of native β-CD is different from that of derivatized β-CD although there are certain similiarities. Similiar mobile phases are used and some compounds such as dansyl amino acids can be separated using either type of CD. Studies conducted with derivatized CD in TLC indicated that the degree of substitution is an important factor. A delicate balance must be attained so that the degree of derivatization is adequate to increase the selectivity and solubility of the CMA but not large enough to obstruct the formation of an inclusion complex or to unnecessarily lengthen development times. A large number of substituents about the mouth of the CD cavity may interfere with the formation of an inclusion complex, which is thought to be important for chiral recognition. The aqueous solubility of hydroxypropyl β-CD appears to increase as the degree of substitution increases. Increases in solubility allow larger concentrations of the CMA which may lead to enhanced

Table IV. Compounds Separated Using Maltosyl β-Cyclodextrin as CMA

Enantiomers	Rf1	Rf2	α	Reversed Phase Plate Type
(1) DL-Alanine-β-naphthylamide	0.71	0.66	1.08	ethyl
(2) Dansyl DL-leucine	0.22	0.16	1.38	octadecyl
(3) Dansyl DL-valine	0.40	0.31	1.29	diphenyl
(4) DL-Methionine-β-naphthylamide	0.39	0.34	1.15	ethyl
(5) N'-(2-Naphthylmethyl)nornicotine	0.50	0.30	1.67	ethyl
Diastereomers				
(1) Cinchonidine/Cinchonine	0.23	0.12	1.92	ethyl
(2) Quinidine/Quinine	0.09	0.03	3.00	ethyl*

*Analysis using diphenyl plate produced the same value for selectivity (α).
The mobile phase consists of: 30/70, acetonitrile/water which contains 0.4M maltosyl-β-CD and 0.6 M NaCl.

Source: Duncan and Armstrong (17)

enantioselectivity. Unfortunately, maltosyl derivatized β-CD having different degrees of substitution were unavailable. The development times were longer for the derivatized CD than for the native CD (in saturated urea solution) because a greater concentration of CMA was used and the viscosity of the solution increases as the concentration of the CMA increases.

Chiral Selectors as CSP Results obtained with TLC-CSP are comparable to those obtained using the same HPLC-CSP. β-CD has been used successively as a bonded TLC (9) phase but attempts to coat a TLC plate with β-CD were unsuccessful (13). The problem with coating the plates was due to the low solubility of β-CD in organic solvents.

Most of the prior work done with CD has been in the reversed-phase mode but recent developments with derivatized CD columns utilized the normal-phase (NP) mode (28). The first successful CD-based, normal phase separation of enantiomers was accomplished using derivatized CD (Table V). The derivatized CD used in NP-HPLC contained a greater number of interaction sites than the derivatized CD used in RP-TLC. For NP work involving CD, the hydrophobic cavity is presumably filled with the nonpolar solvent and the probability of the formation of an inclusion complex is much lower than in reversed-phase. The new derivatized CD phases are not exhaustively derivatized and therefore have residual chirally selective hydrogen bonding sites as well as additional hydrogen bonding sites and aromatic groups for π-π interactions. In addition, the solvents used in normal phase work do not compete with the

Table V. Normal Phase LC Separation Data for Toluoyl Derivatized β-CD

Compound	k'1	α	Mobile phase
1. (R,R)-(S,S)-(±)-N,N'-bis-(α-methylbenzyl)-sulfamide	2.1	1.08	90:10, hex:ipa
2. (R,S)-α-methoxyphenyl-acetic acid	2.6	1.27	99.5:0.5, EtOH:HoAC
3. (±) glutethimide	3.1	1.08	90:10, hex:ipa
4. (R,S)-2,2'-binaphthyldiyl-17-thiacrown 5	5.2	1.08	95:5, hex:ipa
5. N-(3,5-Dinitrobenzoyl)-DL-leucine	2.4	1.06	99:1, EtOH:HOAc
6. N-(3,5-Dinitrobenzoyl)-DL-phenylglycine	17.3	1.08	99:1, EtOH:HOAc
7. N-(3,5-Dinitro-2-pyridyl)-DL-tryptophan	10.1	1.04	99:1, EtOH:HOAc
8. N-(3,5-Dinitro-2-pyridyl)-DL-phenylalanine	17.5	1.01	99.5:0.5 EtOH:HOAc

Abbreviations:
Hexane - hex, Isopropranol - ipa, EtOH - ethanol, and HOAc - glacial acetic acid
Source: Armstrong et. al (29)

analyte for the residual hydrogen bonding sites on the derivatized CD. Derivatization of analytes by the addition of a dinitrobenzoyl group expands the range of compounds which can be separated by providing additional sites for interaction with the chiral agent. It is possible to attain different selectivities with the derivatized CD in comparison to the native CD although some compounds can be separated using either chiral agent. At this time, research is being conducted on the use of these new derivatized CD-CSP in reversed-phase HPLC.

COMPARISON: CD-CMA AND CD-CSP

The majority of reports of CD-CMA are done in TLC whereas most applications of CD-CSP are for work in HPLC or GC. Although many compounds separate well with both methods, it is interesting to note that sometimes there is a poor correlation between the enantiomeric separations obtained with CD-CMA versus CD-CSP. Reasons for this discrepancy may be: (1) β-CD is linked to the silica gel via an 8-10 atom spacer in order to form the CSP. These spacer arms may provide additional interaction sites for the complexed analyte and/or these arms may restrict motion of the CD; (2) if the CD is in the mobile phase, it is available for multiple complexation. The most notable difference that has been observed between the use of CD as CMA and CSP in reversed-phase media is the reversal of elution order of enantiomers. For example, with DL amino acid derivatives, the D enantiomer elutes ahead of the L enantiomer when the chiral agent is in the mobile phase but a reversal of the retention

behavior is noted if the chiral agent is bonded to the stationary phase, exclusively (15).

CONCLUSIONS

Empirical results denote the complementary nature of HPLC and TLC chiral separations. The selectivities may differ slightly between the two chromatographic methods but it is feasible to use TLC to screen large numbers of compounds for HPLC analysis. HPLC provides greater sensitivity and better reproducibility while TLC offers low cost and wide versatility. Rapid growth in chiral separations technology has provided a variety of viable techniques. The equipment available for the arsenal of a chiral separations analyst continues to grow annually.

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