REVIEW

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Progress in the separation of enantiomers of chiral drugs by TLC without their prior derivatization

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1. Introduction

A pharmacist, who is an expert on the molecules of bioactive substances and on the activities of these substances in living systems, more and more often encounters stereochemical problems. Among these are the different stereoselective activities of drugs depending on the elements of asymmetry in the structures of drug molecules [1]. Some drug molecules may be chiral and can exist in isomeric forms representing non-superimposable mirror images called enantiomers. Analysis of the enantiomeric purity of drugs is very important during production and storage of drugs already in use. The same holds for different steps of investigations on the synthesis and isolation from natural sources of new drug candidates, and on their fate in the body with respect to the stereoselectivity of their metabolism and steric differentiation of pharmacokinetic parameters of enantiomers. In the analysis of enantiomers, chromatographic methods [1, 2] have become very popular, mainly because of their relative simplicity and fast results compared with some tedious and time consuming methods, including fractional crystallization [1].

Although TLC is one of the long known analytic techniques, enantioselective TLC, like enantioselective GC [2] is a relatively new domain of pharmaceutical analysis and is still in the development stage. An increasing number of enantiomeric drugs have been separated by TLC [3]. Compared to HPLC [2, 4], which is the most widely used direct method for enantioseparation [2, 5] and CE [6, 7], the number of enantiomer separations performed by TLC is small but the potential for increased use has been demonstrated [3].

This review continues previous publications [8, 9, 11, 12] and summarizes contributions on the separation of enantiomers of chiral drugs by TLC without their prior derivatization published since l990. Advantages and disadvantages of enantioselective TLC are discussed elsewhere [9−12].

Two approaches are used for the TLC separation of enantiomers. One involves the use of chiral stationary phases and will be discussed in chapter 2 and the relevant references are summarized in Table 1. The other entails the use of a chiral component in the mobile phase, see chapter 3 and Table 2. In some cases enantiomers are separated even in achiral systems [13], see chapter 4.

2. Drug enantiomer separation by TLC in systems with chiral stationary phases

Similarly to HPLC [2, 4], the chromatographic systems with a chiral stationary phase (CSP) were used for the separation of enantiomers of drugs by TLC without their prior derivatization more often than systems with a chiral component in the mobile phase. Layers with a chiral selector were often prepared by impregnation of available non chiral layers [14]. Methods of impregnation of thin layer material with a variety of reagents and the role of impregnating reagents in resolving compounds on these layers were discussed earlies [14].

2.1. Polymers

2.1.1. Cellulose, cellulose derivatives and other polysaccharides

Cellulose was probably the first chiral selector used in enantioselective TLC [15, 16], but is still under investigation [17−21].

The effect of various cations and anions in the aqueous eluent, the effect of changing the polarity of the eluent by addition of methanol and the effect of the kind of cellulose used as the stationary phase were studied on the TLC separation of D- and L-tryptophan enantiomers [17]. Though commercially available (COM) Merck Art 5577 DC Plastikfolien and Art 5787 HPTLC Fertigplatten lead to the separation of enantiomers, COM cellulose layers Macherey Nagel MN Polygram Cel 300 are not useful for that purpose. In another study [18], a much better separation of tryptophan enantiomers was achieved on microcrystalline cellulose than on "native" cellulose. On microcrystalline cellulose, thin layers Merck Art 5577 developed with $CuSO_4$ solution, the chiral discrimination for tryptophan [17, 19] and oxitriptan [19] was decreased due to the presence of $CuSO_4$. A TLC system comprising cellulose-aqueous solution of α-cyclodextrin as a chiral component in the mobile phase was also investigated for the separation of tryptophan enantiomers [20]. The chiral effects were found essentially additive for cellulose and cyclodextrin. The best separation of enantiomers was obtained with a system involving microcrystalline cellulose thin layers (Merck Art 5577), 1 M NaCl and 40 mM α-cyclodextrin solution as eluent at a temperature of 7 °C [21]. After visualization by iodine vapor, it was possible to detect one enantiomer in the presence of the other down to the relative concentration of 1%. The mechanism of chiral recognition on cellulose is not yet completely

clarified even though a significant role is attributed to the cellulose structure and the hydroxyl groups [11].

Cellulose triacetate (CTA) is a well known sorbent too, but it is still studied and used in enantioselective TLC [22–27]. The chiral recognition of CTA depends strongly on its structure and the type of eluent. It enhances with increased crystallinity of the polysaccharide [11]. Typically, it is used in the reversed phase chromatography mode [22–26]. Home-made TLC layers of CTA allow better separation of enantiomers then commercially available ones [22]. Home-made microcrystalline CTA plates with sodium carboxymethylcellulose as binder and with ethanol–water mixture 80 + 20 (v/v) used as a mobile phase made the separation of enantiomers of aminoglutethimide possible (detection with p-dimethylaminobenzaldehyde) [22]. Better resolution was achieved on home-made microcrystalline CTA plates with silica gel 60 GF_{254} as binder and an ethanol-water mixture 50 + 50 as the mobile phase. This chromatographic system with variation of the mobile phase to 40 + 60 allowed also the separation of enantiomers of flurbiprofen, (detection UV light) [22]. On the same thin layer enantiomers of carprofen were separated with a 2-propanol-water mixture 60 + 40 [23] and with methanol-water 80 + 20 mixture the enantiomers of naringenin and taxifolin was resolved (detection by UV illumination at 254 and 364 nm and densitometric at 260 nm) [24]. With an ethanol-water 70 + 30 eluent pinocembrin enantiomers were separated and with the same modified mobile phase some other racemic flavanones could be resolved [25]. Another study [27] compares the separation of enantiomers of chlormezanone, chloroquine and methaqualone on column and thin-layer plate of microcrystalline CTA with ethanol 95% and its mixtures with water at different pH and temperature. Chiral plates were found to be more sensitive to eluent composition and pH than chiral columns [27].

Cellulose triphenylcarbamate derivatives have been used as new CSP's in TLC for the resolution of the enantiomers of the β-blockers bupranolol and propranolol [28]. For preparation of TLC plates each of eight examined cellulose derivatives were mixed with microcrystalline cellulose. While the best resolution of bupranolol enantiomers was obtained on cellulose tris-(2,3-dimethy1phenyl carbamate) CSP, the optimum resolution of propranolol enantiomers was achieved on cellulose tris-(3,5-dimethylphenyl carbamate), with the mobile phase hexane-2-propano1 80 : 20 v/v in both cases. Despite some problems which included difficult preparation of stable thin layers on glass and difficult detection due to the intense UV absorption of the CSP's these results demonstrated the potential of cellulose triphenylcarbamates as CSP's for the direct and rapid (within 30 min) resolution of racemates by TLC.

Further, the polysaccharide galactomannan (guaran) was examined as CSP in TLC. The chiral selectivity of galactomannan was further enhanced by a ligand-exchange reaction of a polymer with a tetracoordinated borate group [29]. The paper reports the effective separation of several amino acid racemates on silica gel impregnated with sodium tetraborate gelled guaran. The amino acids cystine, isoleucine, leucine and proline were examined with the mobile phase 2-propanol-water 7 : 3, alanine and valine were treated with a phenol-water 4 : 1 mixture and aspartic acid, DOPA, glutamic acid, phenylalanine, serine, threonine, tryptophan and tyrosine were separated by a butanol-acetic acid-water 3 : 1 : 1 mixture. The detection of amino acids was achieved with ninhydrin [29].

Amino derivatives of polysaccharides used in enantioselective TLC included chitin, chitosan and their derivatives [30–32]. By TLC with chitin impregnated with Cu(II) ions as a stationary phase and methanol or ethanol as eluents, the racemates of alanine, leucine, threonine end valine were resolved into spot pairs [30]. Racemates of alanine, leucine, methionine, serine, threonine and valine were resolved on chitin-Cu(II) layers using a methanol-water 1 + 1 mixture as a binary mobile phase. The retention of the amino acids increased with increasing concentrations of acetonitrile in a ternary mobile phase and the separation of enantiomers deteriorated [31]. Enantiomers of alanine, leucine, methionine, serine, threonine and valine were resolved with the greatest differences in their R_f values on a chitin-Cu(II) layer with methanol-water-acetonitrile 1 : 1 : 0,1 v/v mobile phase; the spots of amino acids were visualized with ninhydrin. Analogous investigations on a chitosan-Cu(II) bed were carried out, but did not result in successful separation [32]. The possibilities of enantiomer separation on chitin-Cu(II) layers seem to be uncertain if we consider, that even the non enantiomeric glycine gives rise to two separated spots of different colors [30, 31] in the same way as do racemic amino acids [11].

2.1.2. Molecularly imprinted polymers

Molecular imprinting is a technique for preparing specific recognition sites in synthetic polymers. During the imprinting process, the imprint molecule binds reversibly to the functional monomer and should be stripped from the rigid polymer. After polymerization the imprint molecule is extracted from the polymer matrix producing recognition sites within the polymer network with an affinity for the original print molecule or molecules structurally related. A combination of molecular imprinting and TLC [33] led to a new group of CSP's for enantioselective TLC of drugs without their prior derivatization [34–36]. Molecularly imprinted polymers (MIP's) for TLC were prepared using quinine as imprint molecule and two different monomers, methacrylic acid and itaconic acid [34]. For preparing of TLC plates on glass microscope slides MIP and anhydrous $CaSO_4$ were gradually mixed with water and a small amount of ethanol. The system consisting of the polymer prepared from methacrylic acid as CSP and acetonitrile as a mobile phase allowed the separation of ephedrine racemate into the enantiomers, the system with the polymer prepared from itaconic acid and with acetonitrile or methanol as a mobile phase allowed the enantiomeric resolution of the norephedrine racemate. Here, UV light of 366 nm was used for detection [34]. Further, polymers of the same two monomeric acids were prepared and investigated using (−)-pseudoephedrine and (−)-norephedrine [35]. The enantiomers of ephedrine, epinephrine, norephedrine and pseudoephedrine were separated using a mobile phase consisting of either acetonitrile or methanol or with these solvents with 1% acetic acid (methacrylic acid polymers) or 1–l0% acetic acid (itaconic acid polymers). The spots were detected with ninhydrin reagent. Only small differences in the selectivity of MIP's based on methacrylic acid or itaconic acid prepared using (−)-norephedrine were observed. The best resolution was achieved for the enantioseparation of norephedrine on plates based on MIP of (−)-norephedrine using itaconic acid as a monomer with methanol as a mobile phase [35]. In a study [36] of MIP's imprinted with (+)-ephedrine, (+)-norephedrine and (+)-pseudoephedrine

Table 1: Enantioselective separation on CSP

Drug	Chiral selector	Ref.
Alanine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[48]
Aminoglutethimide	CTA	[22]
Arginine	(1*R*,3*R*,5*R*)-2-Azabicyclo[3,3,0]octan-3-carboxylic acid	[51]
Aspartam	Hydroxyproline derivative-Cu(II), COM	[43]
Aspartic acid	Borated guaran	[29]
Atenolol	L-Arginine or L-lysine	[40]
Atropine	L-Aspartic acid	[37]
Bupranolol	Cellulose triphenylcarbamate derivatives	[28]
Carprofen	CTA	[23]
Colchicine	L-Aspartic acid	[37]
Cystine	Borated guaran	[29]
DOPA	Borated guaran	[29]
	L-N^{τ}-n-Decylhistidine-Cu(II)	[49]
Ephedrine	MIP of quinine	[34]
	MIP of (−)-norephedrine or (−)-pseudoephedrine	[35]
	MIP of (+)-ephedrine or (+)-pseudoephedrine	[36]
Epinephrine	MIP of (−)-norephedrine or (−)-pseudoephedrine	[35]
	MIP of (+)-pseudoephedrine	[36]
Flurbiprofen	CTA	[22]
	(−)-Brucine	[54]
Glutamic acid	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[48]
Histidine	(1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid-Cu(II)	[50]
	(1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid	[51]
Ibuprofen	L-Arginine	[38]
	(−)-Brucine	[54]
Isoleucine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[47]
Isoprenaline	MIP of (+)-norephedrine	[36]
Lactic acid	Hydroxyproline derivative-Cu(II), COM	[47]
Leucine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[47]
Lysine	(1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid	[51]
Methionine	Hydroxyproline derivative-Cu(II), COM	[46, 47]
α-Methyltyrosine	L-N^{τ}-n-Decylhistidine-Cu(II)	[49]
Metoprolol	L-Arginine or L-lysine	[40]
Naringenin	CTA	[24]
Norephedrine	MIP of quinine	[34]
	MIP of (−)-norephedrine or (−)-pseudoephedrine	[35]
	MIP of (+)-ephedrine or (+)-pseudoephedrine	[36]
Oxitriptan	Cellulose, COM	[19]
Oxprenolol	MIP of (+)-ephedrine or (+)-pseudoephedrine or (+)-norephedrine	[36]
Phenylalanine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[46, 48]
	L-N^{τ}-n-Decylhistidine-Cu(II)	[49]
Pindolol	MIP of (+)-ephedrine or (+)-norephedrine or (+)-pseudoephedrine	[36]
Pinocembrin	CTA	[25]
Proline	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[48]
Propranolol	Cellulose triphenylcarbamate derivatives	[28]
	MIP of (+)-ephedrine or (+)-norephedrine or (+)-pseudoephedrine	[36]
	L-Arginine or L-lysine	[40]
Pseudoephedrine	MIP of (−)-norephedrine or (−)-pseudoephedrine	[35]
	MIP of (+)-ephedrine or (+)-norephedrine or (+)-pseudoephedrine	[36]
Salbutamol	MIP of (+)-ephedrine or (+)-norephedrine	[36]
Serine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[47]
	(1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid-Cu(II)	[50]
Taxifolin	CTA	[24]
Threonine	Borated guaran	[29]
Thyroxine	Hydroxyproline derivative-Cu(II),COM	[45]
α-Tocopherol	Hydroxyproline derivative-Cu(II), COM	[44]
Tryptophan	Cellulose, COM	[17–20]
	Cellulose, COM (+ α-CD)	[20, 21]
	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[46, 48]
	L-N^{τ}-n-Decylhistidine-Cu(II)	[49]
	(1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid-Cu(II)	[50]
Tyrosine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[48]
	L-N^{τ}-n-Decylhistidine-Cu(II)	[49]
Valine	Borated guaran	[29]
	Hydroxyproline derivative-Cu(II), COM	[46, 47]
	(1*R*,3*R*,5*R*)-2-Azabicyclo[3,3,0]octan-3-carboxylic acid	[51]

were prepared using methycrylic acid as a monomer. Prepared MIP's were processed and coated on a glass support as thin layer. Enantiomers of ephedrine, epinephrine, isoproterenol, norephedrine, pseudoephedrine and salbutamol were separated with 5–10% acetic acid in acetonitrile (ninhydrin or acidified $KMnO_4$ detection reagent), racemates of oxprenolol, pindolol and propranolol were separated with 5–7% acetic acid in dichloromethane as a mobile phase (anisaldehyde detection reagent) [36].

2.2. Amino acids and their derivatives

Enantiomers of amino acids were used in several cases for the impregnation of TLC layers based on silica gel G [37, 38, 40]. Enantioselective retention of corresponding analyte enantiomers was achieved in this case by intermediate formation of diastereomeric salts. Resolution of racemic colchicine and hyoscyamine (atropine) on thin layers impregnated with L-aspartic acid as the chiral selector is reported by Bhusnan and Ali [37]; chromatograms were developed at 0 °C with chloroform-acetic acid-n-butanol-water 6 : 4 : 3 : 1 v/v, the spots were visualized in an iodine chamber. An ionic interaction between anions of optically pure aspartic acid and the large cations of components of racemic mixtures of alkaloides is considered to produce diastereomers leading to enantiomeric separation [37]. The resolution of ibuprofen racemate into its enantiomers was achieved on silica gel plates impregnated with L-arginine. Chromatograms were developed at 32 °C using acetonitrile-methanol-water 5 : 1 : 1 v/v as the solvent system; the spots were detected in an iodine chamber [38]. The method was successful in resolving as little as 0.1 μg of the enantiomeric mixture which is a much lower quantity than many of the reported HPLC detection limits [39]. The resolution of atenolol, metoprolol and propranolol racemates was achieved on silica gel plates impregnated with L-arginine or L-lysine using acetonitrile-methanol 16 : 2–14 : 6 as eluent and detection with iodine [40].

More often, amino acids and their derivatives were used as CSP's in the presence of Cu(II) ions [l0, 41, 43–50]. In this cases the enantioseparation is based on a chiral ligand exchange and the different stability of the intermediately formed diastereomeric copper complexes of the chiral selector and enantiomer to be separated. Two commercially available layers have been on the market, both are based on hydroxyproline derivative-Cu(II) complex as a chiral selector. For differences between both layers see [42]. On Chiralplate® (Macherey-Nagel), two pairs of enantiomers of aspartame with acetonitrile-methanol-water 200 : 50 : 50 v/v as the mobile phase were separated and detected with ninhydrin [43]. Further α-tocopherol racemate was resolved with an eluent consisting of 2-propanol-water-methanol 8.5 + 1.0 + 0.5 v/v (detection under UV light at 254 nm) [44]. Thyroxine enantiomers were separated with an acetonitrile-methanol-water 60 : l5 : l5 mixture and detected under UV light at 254 nm [45]. The relation between capacity factors and the mobile phase composition (mixtures of phosphate buffer pH 7 with acetonitrile, methanol or dioxane) was examined in a TLC study of the enantiomers of selected amino acids including methionine, phenylalanine, tryptophan, and valine [46]. It has been shown that the chromatographic behavior of ternary mixed-ligand complexes of amino acids can be described by an equation used in reversed phase chromatography.

On Chir® (Merck) HPTLC plates the selected acids including lactic acid, iso-leucine, leucine, methionine, serine, valine have been separated into their enantiomers using a mobile phase consisting of acetonitrile-water 3 + 2 v/v for hydroxy acids and acetonitrile-methanol-water 4 + 1 + 1 v/v for amino acids. A new optical topological index was proposed which allows the distinction between isomers of D and L configuration [47]. In a paper of the same author [48] the amino acids alanine, glutamic acid, phenylalanine, proline, tyrosine and tryptophan were separated into their enantiomers with the same mobile phase and a new valence optical topological index and valence optical Gutman index were proposed. A new chiral ligand exchange selector L-N^{τ}-n-decylhistidine was synthesized [49]. It was suitable for the modification of an RP-C_{18} stationary phase (Merck RP-18 WF_{254} S HPTLC plate) with Cu(II)-acetate and has separation power for racemic aromatic amino acids as DOPA, α-methyltyrosine and phenylalanine, tyrosine and tryptophan with water-tetrahydrofuran-methanol-acetonitrile 52.9 : 33.9 : 7.3 : 5.9 mixture as a mobile phase [49]. The enantiomeric resolution of amino acids histidine, serine and tryptophan was achieved on silica gel plates impregnated with (1*R*,3*R*,5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid, which is a nonproteinogenic α-amino acid, and $CuSO_4$ [50]. Resolution was successful in different combinations of acetonitrile-methanol-water in the range from 3 : 1 : 1 to 8 : 1 : 1. The same chiral selector was used for the impregnation of the silica gel as an ion-pair forming reagent without a Cu(II)-compound and at pH 8 [51]. Arginine, histidine, leucine, lysine and valine racemates were resolved with acetonitrile-methanol-water mixtures as mobile phases based on an ion-pair separation mechanism [51]. The spots of amino acids were detected with ninhydrin [46–5l]. Since the publication of the ionic and covalent modification of amino propyl bonded HPTLC plates with *N*-(3,5-dinitrobenzoyl)(*R*)(−)-α-phenylglycine and *N*-(3,5-dinitrobenzoyl)-L-leucine and their use for the separation of enantiomers of drugs like atenolol, metoprolol, propranolol, hexobarbital, lorazepam, oxazepam in the year 1989 [52], CSP's of this type and their applications in the enantioselective TLC of drugs without their prior derivatization were not developed until now.

2.3. Other compounds

In analogy to a study of the separation of enantiomers of amino acids [53] (−)-brucine was used as a chiral selector in two-dimensional enantioseparation of some 2-arylpropionic acids as flurbiprofen and ibuprofen. Silica gel G was impregnated with (−)-brucine at a pH between 6 and 7, the mobile phase for the first dimension was acetonitrile-methanol 16 : 3 and for the second dimension acetonitrile-methanol-water l6 : 3 : 0.4 v/v; iodine vapor was used for detection [54]. It was believed that interactions between the chiral selector and the racemates provided in situ formation of diastereomers on the impregnated plate, and consequently, resolution. The method was successful in resolving as little as 0.1 μg of both (±)-flurbiprofen and (±)-ibuprofen [54].

3. Systems with a chiral component in the mobile phase

In contradiction to HPLC [2, 4], CSP's based on cyclodextrins (CD's) and their derivatives were not used in direct enantioselective TLC. These compounds were used as the chiral component in the mobile phase and their applications were severally extended [25, 55–57]. A mobile phase consisting of a mixture of 0.15 M β-CD in 32%

Table 2: Enantioselective separation in CMP comprising systems

Drug	Chiral selector	Ref.
Alprenolol	ZGP	[69]
Arginine	[2]-O-[(*R*)-2-Hydroxypropyl]-β-CD	[55]
Atenolol	D-10-Camphorsulfonic acid	[67]
Bendroflume-thiazide	Vancomycin	[71]
Citrulline	[2]-O-[(*R*)-2-Hydroxypropy]-β-CD	[55]
Coumachlor	Vancomycin	[71]
DOPA	α-CD or β-CD	[57]
Epinephrine	β-CD	[57]
Fluoxetine	Hydroxypropyl-β-CD	[56]
Glutamine	[2]-O-[(*R*)-2-Hydroxypropyl]-β-CD	[55]
Histidine	[2]-O-[(*R*)-2-Hydroxypropyl]-β-CD	[55]
Hydrastine	BSA	[62]
Indoprofen	Vancomycin	[71]
Isoprenaline	ZGP or (1*R*)-(−)-ammonium-10-camphor-sulfonate	[66]
Lysine	[2]-O-[(*R*)-2-Hydroxypropyl]-β-CD	[55]
Methotrexate	BSA	[64]
Metoprolol	(1*R*)-(−)-Ammonium-10-camphorsulfonate	[66]
	ZGP	[69]
Norfenefrine	ZGP or (1*R*)-(−)-ammonium-10-camphor-sulfonate	[66]
Octopamine	(1*R*)-(−)-Ammonium-10-camphorsulfonate	[66]
Pindolol	ZGP	[66]
	D-10-Camphorsulfonic acid	[67]
Propafenone	D-10-Camphorsulfonic acid	[67]
Propranolol	(1*R*)-(−)-Ammonium-10-camphorsulfonate or ZGP	[66]
	D-10-Camphorsulfonic acid	[67]
	ZGP	[69]
Thyronine	α-CD or β-CD	[57]
Timolol	ZGP or (1*R*)-(−)-ammonium-10-camphor-sulfonate	[66]
Tryptophan	α-CD (+ cellulose, COM)	[20, 2l]
	β-CD	[57]
Tyrosine	α-CD or β-CD	[57]
Valine	[2]-O-[(*R*)-2-Hydroxypropyl]-β-CD	[55]
Warfarin	BSA	[64]

aqueous urea solution and 2% NaCl-acetonitrile 80 + 20 v/v was used for the enantioselective separation of racemic flavanones, like naringenin and pinocembrin on Sil C18−50/UV_{254} plates [25]. Arginine, citrulline, glutamine, histidine, lysine and valine racemates were separated on silica gel Kieselgel 60F_{254} Plastikfolien which were developed with water containing 6.5 mM 2-O-[(*R*)-2-hydroxypropyl]-β-CD-acetonitrile 2.5 : 1 or 2 : 1.5 v/v; spots were detected with salicylaldehyde [55]. Formation of inclusion-complexes between CD's and fluoxetine and its metabolite norfluoxetine have been investigated [56]. Hydroxypropyl-β-CD as a mobile phase additive allows the successful separation of the enantiomers of these solutes on silica gel 60F_{254} plates impregnated with 2.5% paraffin oil. The mobile phase was methanol-aqueous buffer pH 4.5 with 2.5 mM of the chiral additive 55 + 45 or 60 + 40 at pH 6, spots were located under UV light 254 nm [56]. Some racemic amino acids and aromatic amino alcohols (DOPA, epinephrine, thyronine, tyrosine) were resolved using cellulose thin layer with the mobile phase methanol-β-CD saturated solution of urea-formic acid 7 : 2 : 1 (pH 4.5) or methanol-0.2 M α-CD solution of urea-formic acid 7 : 2 : 1 at pH 4.5 [57]. Without CD separation did not occur, underlining that CD play an important role in these enantiomeric separations [57]. For another application of α-CD see chapter 2.1.1. and refs. [20, 21].

Attention was paid to study chromatographic systems with bovine serum albumin (BSA) as mobile phase additive [25, 58−65]. Only in two instances, [62] and [64], these systems were used successfully for the enantioselective separation of drugs without their prior derivatization. Hydrastine enantiomers were separated on Sil C_{18}-50/UV_{254} plates with 0.05 M $NaHCO_3$/Na_2CO_3 eluent containing 10% 2-propanol and 5% BSA [62]. Methotrexate racemate was resolved on RP-18W/UV_{254} plates with 0.5 M acetic acid eluent containing 2% 2-propanol and 6−8% BSA. The warfarin racemate was resolved with 0.5 M sodium acetate containing 2% 2-propanol and more then 5% BSA. In both cases the spots were detected by UV light. The role of BSA in the enantioseparation of the different racemates was discussed [64].

In the direct enantioselective TLC of drugs as components of the mobile phase ammonium-10-camphorsulfonate [66−68] enantiomers were used as chiral counter ions. Several aromatic amino alcohol racemates including metoprolol, timolol and isoprenaline, norfenefrine, octopamine and propranolol were resolved on diol HPTLC and high performance silica gel plates using a mobile phase containing (1*R*)-(−)-ammonium-10-camphorsulfonate. Mobile phases were mixtures of dichloromethane-2-propanol 95 + 5 v/v and dichloromethane-methanol 75 + 25 or 90 + 10 v/v containing 6.8−l3.9 mM of a chiral selector; the detection was achieved by a UV 254 nm lamp [66]. Four aromatic amino alcohol racemates as atenolol, pindolol, propafenone, and propranolol were resolved on general-purpose silica gel GF_{254} plates with dichloromethane-methanol 70 + 30−50 + 50 v/v and 6.8 mM of ammonium-D-l0-camphorsulfonate as a chiral mobile phase aditive at 5 °C [67]. The aromatic alcohol amino drugs Bataroc and Labarol enantiomers were separated under similar conditions at 2−4 °C [68].

Another compound used in enantioselective TLC as chiral ion-pairing agent is *N*-benzoxycarbonylglycyl-L-proline (ZGP) [66, 69]. Racemates of isoprenaline, norfenefrine, phenylpropanolamine, propranolol and timolol were resolved on high performance silica gel plates with dichloromethane-methanol 75 + 25 or 90 + 10 v/v containing 5.8−6.9 mM ZGP and 5 mM of triethylamine. The racemate of pindolol was resolved on HPTLC diol plates with dichloromethane-2-propanol 95 + 5 v/v containing 6.7 mM ZGP and 5 mM triethylamine [66]. Enantiomers of alprenolol, metoprolol and propranolol were separated on diol HPTLC plates with dichloromethane containing 5 mM ZGP. Saturation of the chromatographic system with water has a beneficial effect both for reproducibility and chromatographic performance [69]. Comparison of enantioselective TLC and HPLC are made for separations attained in normal-phase HPLC and TLC involving ZGP and 10-camphorsulfonic acid as chiral counter ions [70]. Bendroflumethiazide, coumachlor, indoprofen and warfarin racemates were resolved on chemically bounded Diphenyl-F reversed-phase plates with vancomycin as a chiral selector. The mobile phase was 0.6 M NaCl aqueous solution containing vancomycin-acetonitrile 60 + 40−100 + 20, in the case of bendroflumethiazide 85 + 5 + triethylammonium acetate buffer of pH 4.1, spots were visualized under an UV lamp [71].

4. Achiral systems

D- and L-lactic acid enantiomers were separated on COM precoated silica gel plates impregnated with an aqueous solution of Cu(II)-acetate. The mobile phase was dioxane-

water 9 + l, the two enantiomers appear as blue spots [72]. Though the complex formation with Cu(II) has been held responsible for the resolution of enantiomers no efforts to estimate the difference in stabilities of complexes corresponding to D-, and L-isomers have been made [13, 72].

5. Conclusion

Enantioselective TLC of drugs without their prior derivatization is still a developing field of pharmaceutical analysis. Similarly to the recent trends in enantioselective HPLC, the increase in the palette of commercially available thin layers with CSP's can be expected in the future. The development of home-made CSP's based on impregnation of achiral stationary phases by chiral selectors and CSP's based on molecularly imprinted polymers will certainly continue. Among the chiral additives in the mobile phase for the separation of ionic drugs, particularly chiral counter ions are promising.

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