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Review

Chiral separation of pharmaceuticals possessing a carboxy moiety

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Abstract

The separation of carboxylic enantiomers in the pharmaceutical field using high-performance liquid chromatographic and capillary electrophoretic techniques is reviewed. The techniques used for chiral separation include diastereomer derivatization, a chiral mobile phase, a chiral stationary phase (high-performance liquid chromatography) and chiral additives (capillary electrophoresis). Practical and conventional separation systems for pharmaceutical applications, such as pharmacokinetics, optical purity testing and stability studies, are described. A comprehensive collection of applications to carboxylic drugs and other carboxylic compounds of pharmaceutical interest is listed in the tables. The characteristics of each enantioseparation method are also discussed briefly.

Keywords: Reviews; Enantiomer separation; Carboxylic acids

Contents

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1. Introduction

Recent reports have shown that the stereochemical composition of pharmaceutical carboxylic acids may be rapidly and accurately determined by chiral separation using high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE). Chromatographic techniques such as thin-layer chromatography (TLC), gas chromatography (GC), supercritical fluid chromatography (SFC), HPLC [1,2] and CE [3,4], frequently have been used for chiral separation. The development of these methods is shown in Fig. 1. In the past two decades, the development of these methods for enantioselective separation has attracted great interest, since it became evident that the biological activity of compounds of pharmaceutical interest is mostly restricted to one of the enantiomers (e.g. vitamins, anti-inflammatory drugs and anti-bacterial drugs). There can be qualitative and quantitative differences in the pharmacological activity of the enantiomers. Some types of enantiomers are biologically active compounds and are called 'eutomers' and the inactive ones are called 'distomers'. The distomers can exhibit unexpected adverse reactions, antagonistic activities or toxic effects. Even if these adverse reactions are not drastic, the distomer has to be metabolized and represents an unnecessary burden for the organism. Therefore, it is necessary to develop analytical methods for the chiral separation to control optical purity and to gain an understanding of the clinical, pharmacological and pharmacodynamic modes of actions, etc.

In the early stages of chromatographic chiral resolution, some attempts were made using GC. However, the compounds that this technique could be applied to were limited and they often needed derivatization steps to volatilize them. A few TLC attempts were performed using ligand-exchange or inclusion complex mechanisms. In the 1980s, chiral separation using HPLC made rapid progress in the pharmaceutical field. The advantages of HPLC are (1) high reproducibility, (2) high resolution using ODS columns, (3) broad detection spectrum of UV, (4) the variety of mobile phases that can be used, etc. The HPLC method is still considered as a standard procedure. Currently, CE has been found to be an alternative to chromatographic techniques and several chiral separation principles that have been applied successfully in HPLC have been transferred to CE. The advantages of CE are that it requires small amounts of chiral selector and solvents. This permits the use of expensive reagents and makes it easy to change the selector and the electrolyte when screening to determine optimal conditions.

Compared with compounds possessing an amino moiety, applications for the chiral separation of carboxylic compounds are limited, even if the above

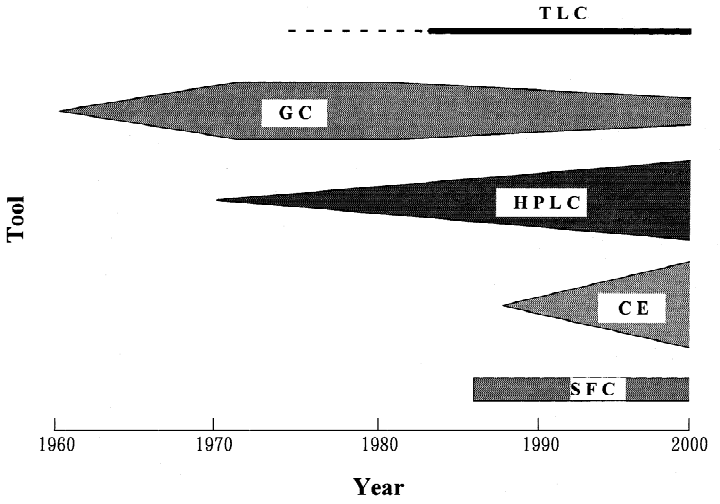

Fig. 1. Method development for chromatographic and electrophoretic chiral separations.

Table 1
Derivatization to diastereomers for chiral separations using HPLC

Drug	Chiral reagent (condensing agent)	Reacting conditions	Phase	Detection	Reference
Indoprofen	L-Leucinamide (ethyl chloroformate)	r.t. 3 min	RP	UV	[15]
Ketoprofen	L-Leucinamide (ethyl chloroformate)	r.t. 2 min	RP	UV	[19]
Lipoic acid	D-Phenylalanine (OPA)	r.t. 10 min	RP	FL	[17]
Naproxen	Anthryl ethylamine (WSC)	40°C 1.5 h	NP	FL	[11]
	DANE (HOBT+WSC)	4°C 45 min	NP	FL	[18]
NSAIDs	DAPEA (DPDS+TPP)	r.t. 2.5 h	RP	FL	[6]
	APMB (DPDS+TPP)	r.t. 2 h	NP	FL	[7]
	DBD-APy, NBD-APy ABD-APy (DPDS+TPP)	r.t. 2 h	RP	FL	[8]
	DBD-APy, NBD-Apy ABD-Apy (DPDS+TPP)	r.t. 2 h	RP	CL	[9]
	APMB (DPDS+TPP)	r.t. 2 h	NP RP	FL	[10]
	NEA (DMAP-carbodiimide)	r.t. 1.5 h	NP	UV	[14]
	L-1-Aminoethyl-4-dimethyl-aminonaphthalene (E-DMAP-carbodiimide)	r.t. 3 h	NP	FL	[16]
Ofloxacin Ketoprofen	Leucinamide (DPP+TEA)	r.t. 10 min	RP	UV	[12]
Ofloxacin	Leucinamide (DPP+TEA)	r.t.	RP	UV	[13]
Phenyl carboxylic acid	L-NSPC (DCC)	r.t. 60 min	NP	UV	[20]
Temafloxacin (methyl ester)	L-NSPC (TEA)	r.t. 10 min	NP	UV	[108]

Table 2
Detection methods for chiral mobile phase chromatography

Method	Detection type	Application
Ultraviolet absorption (UV)	Direct detection	[27,29,31–34,23]
	Indirect detection	Negative peak detection [21,22,35]
	Postcolumn derivatization	Fe(III) reaction, 430 nm [25,26,28,30,36]
Fluorescence (FL)	native FL	Ofloxacin [23,24]
	Postcolumn reaction	Amino acids
Electrochemical detection (ECD)	Direct detection	(High sensitivity, low generality) DOPA [37]

chromatographic procedures are used. This review will mainly be devoted to recent developments and applications from the early 1980s.

2. Indirect separation by derivatization to diastereomers

The use of DNP- or DNB-derivatization for chiral separation on HPLC was reported by Pirkle et al. [5]. Pirkle's method is excellent since it can be applied to many compounds over a wide area. In this section, the formation of diastereomeric derivatives using chiral derivatization reagents will be described. The diastereomeric pairs formed can be separated using achiral chromatography. In general, diastereomeric derivatization of the carboxylic group is more complicated and requires an additional reaction step than needed for the amino group. A suitable derivatization reagent has to be of high optical purity. Also, no racemization can take place during the derivatization process. However, the diastereomeric derivatizing method is one of the most effective procedures for small molecules, and for aliphatic and nonchromophore compounds.

Goto et al. [11] designed L-1-aminoethyl-4-dimethyl-aminonaphthalene as a highly sensitive, chiral derivatization reagent for carboxylic compounds. The diastereomers were efficiently resolved by normal-phase chromatography. With fluorescence detection, the detection limit was 0.1 ng. Björkman [15] reported an HPLC assay for the enantiomers of indoprofen. The method involved the extraction of unchanged drug, conversion to a mixed anhydride with ethylchloroformate and derivatization with leucinamide. The derivatizing reaction was rapid and the diastereomers formed were efficiently resolved by reversed-phase chromatography.

Toyo'oka et al. [9] reported the peroxyoxalate chemiluminescence detection of naproxen enantiomers after diastereomeric derivatization. The detection limits (signal-to-noise ratio of 2) of 4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-amino-pyrrolidin-1-yl)-2,1,3-benzoxadiazole naproxen (DBD-APy-Nap), 4-(aminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole naproxen (ABD-APy-Nap) and 4-nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole naproxen (NBD-APy-Nap) after separation by HPLC were 0.49, 1.9 and 15 fmol, respectively.

A specific plasma assay for the enantiomers of α-lipoic acid was achieved using chemical reduction to the dithiol enantiomers, and their precolumn chiral derivatization with *o*-phthalaldehyde in the presence of D-phenylalanine [17]. The two diastereomeric derivatives were separated by reversed-phase HPLC with fluorescence detection. The working range of the assay is between 15 ng/ml (lower limit of quantitation) and 1000 ng/ml for either enantiomer. Other applications of the chiral derivatization method are listed in Table 1.

3. Chiral mobile-phase HPLC method

Different approaches have been used to separate enantiomers in LC. Chiral stationary phases have considerable practical advantages but the use of chiral selectors in the mobile phase in combination with achiral adsorbents often gives rise to a more versatile system. This section presents approaches for the separation of enantiomeric carboxylic compounds using chiral mobile phase methods, such as the ligand-exchange method, the ion pair method and others.

3.1. Ligand-exchange (LE) methods

The wide success achieved in the separation of underivatized amino acid enantiomers by HPLC are based on diastereomeric ternary complex (ligand–metal complex) formation in a mobile phase. However, applications of the LE method were limited except for amino acids and their derivatives.

In the case of the chiral separation of compounds possessing a carboxylic moiety, the LE mobile phase method might be a powerful tool if the detection system is improved. The mobile phase containing ligand–metal complex has strong UV absorption at wavelengths under 230 nm, so fluorescence (FL) or electrochemical detection (ECD) have been effective. The detection methods used in LE mobile phase systems are listed in Table 2. Some attempts using chiral mobile-phase methods for HPLC of carboxylic compounds are shown in Table 3.

Horikawa et al. [26] reported the separation of

Table 3
Chiral mobile phase methods for HPLC of carboxylic compounds

Compound	Chiral additive	Stationary phase	Separation mode	Detection	Phase	Reference
DOPA	Aspartame L-Asp–L-Phe–O–CH_3 + Metal ion	ODS	LE	UV	RP	[31] [32]
	N,*N*,*N*′,*N*′-tetra methyl-(*R*)-propanediamine-1,2 + metal ion	ODS	LE	UV	RP	[34]
	L-Phenylalanine + metal ion	ODS	LE	ECD	RP	[37]
9-(3,4-Dihydroxy-butyl)guanine	L-Phenylalanine + metal ion	ODS	LE	UV	RP	[29]
Hydroxy acids	*N*,*N*-Dialkyl–L-AA + metal ion	OS	LE	Postcolumn reaction [Fe(III)]	RP	[25]
	AA + metal ion	ODS	LE	Postcolumn reaction [Fe(III)]	RP	[26]
	N^2-Alkyl-(*S*)-AA	Dynamically coated ODS	LE	Postcolumn reaction [Fe(III)]	RP	[28]
	L-Phenylalanine + metal ion	ODS	LE	Postcolumn reaction [Fe(III)]	RP	[30]
	L-Phenylalaninamide + metal ion	ODS	LE	Postcolumn reaction [Fe(III)]	RP	[36]
Methyl DOPA	L-Proline + metal ion	ODS	LE	UV	RP	[27]
NSAIDs	Quinine	Porous graphite carbon	Counter ion	Indirect UV	NP	[35]
Phenyl carboxylic acid	HSA	ODS	Affinity	Indirect UV	RP	[21]
	Quinine	Diol	Counter ion	Indirect UV	NP	[22]
Pyridone carboxylic acid	Amino acid + metal ion	ODS	LE	UV FL	RP	[23] [24]
4-Thiazolidine carboxylic acid	(2′*S*, 4*R*, 2′*RS*)-*N*-(2′-hydroxy dodecyl)-4-hydroxyproline + metal ion	ODS	LE	UV	RP	[33]
Thyroid hormones	L-Proline + metal ion	ODS	LE	UV	RP	[27]

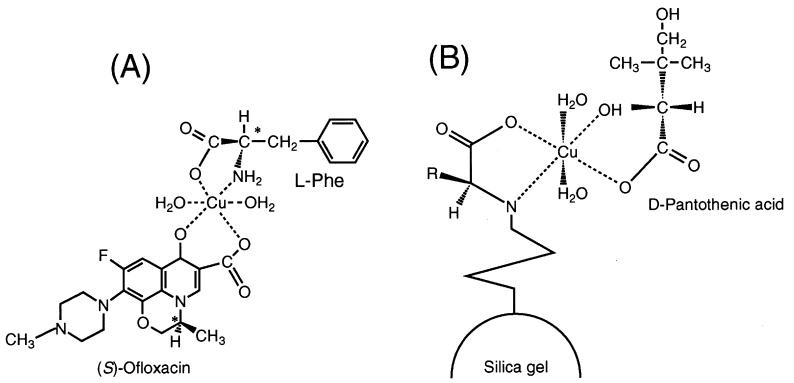

Fig. 2. Proposed structures of ternary complexes. (A) (*S*)-Ofloxacin–L-phenylalanine in the chiral mobile phase and (B) pantothenic acid on the ligand exchange CSP with copper ion (from Refs. [23,48]).

enantiomers of underivatized α-hydroxy acids, including tartaric and glyceric acids, on a reversed-phase column using Cu(II) complexes of underivatized L-amino acids as eluents; the problem of detecting the hydroxy acids in the column effluent containing Cu(II)–amino acid complexes was solved by employing a postcolumn color reaction of the hydroxy acids with iron(III).

Gilon et al. [31] have reported the enantioselective resolution of DOPA using aspartame, which is a dipeptide methyl ester, and a metal complex as the chiral additives. In later years, Aso et al. [37] reported an optical purity test method for L-DOPA preparations using a chiral mobile phase containing an L-phenylalanine–copper(II) complex with ECD. The detection limits obtained with an UV detector and an electrochemical detector were compared, and it was found that the electrochemical detector could detect significantly lower levels of D-DOPA in the presence of L-DOPA than the UV detector.

LE mobile phase methods are simple and can be used with conventional HPLC columns, but their applications have been limited to the separation of amino acids, hydroxy carboxylic acids and their derivative compounds (Table 3). Arai et al. [23] studied the applicability of the LE method to pyridone carboxylic compounds using a chiral mobile phase containing an amino acid–copper(II)

Table 4
Characterization of CSPs

CSP type	Selector	Mobile phase (main mode)	Note
Ligand exchange amino acid derivatives		Reversed-phase	Applied compounds: amino acids hydroxy carboxylic acids quinolone carboxylic acids
Affinity	Proteins (BSA, HSA, OVM, AGP, etc.)	Reversed-phase	Broad separation spectrum
Brush	Amino acid naphthyl ethylamine dinitrobenzoyl-derivative	Normal phase	In many cases, DNP- or DNB-derivatization is needed
Polysaccharide helical polymer	Cellulose amylose derivatives, etc.	Normal phase	Commercially available (ex. Chiralcel series, etc.)
Inclusion	Cyclodextrin	Reversed-phase	Reference to CD application

Table 5
Chiral stationary phase (CSP) methods for HPLC of carboxylic compounds

Compound	CSP type	Condition	Reference
Abscisic acid (plant hormone)	Cellulose derivative polymer	Hexane–2-propanol–TFA (80:20:1, v/v/v)	[41]
3-Amino-ε-capro-lactam	LE	1 m*M* Copper(II) sulfate in water	[42]
Aromatic carboxylic acids	Inclusion complex (β-CD)	0.1 *M* PB (pH 4.2)–acetonitrile (65:35, v/v)	[43]
Citronellic acid aromatic carboxylic acids	Ergoline skeletons (as chiral selector)	0.05 *M* Acetate buffer–methanol	[44]
Clidanac NSAIDs	Brush type (urea derivative)	Ammonium acetate in methanol	[45]
Hydroxy acids	Urea polymer (+L-leucinamide)	0.01 *M* Acetate buffer (pH 5.0)–methanol (80:20, v/v)	[61]
	ODS coated with *N*,*N*-dioctyl-L-alanine (LE)	2 m*M* $CuSO_4$ in aqueous acetonitrile (10%)	[62]
Ketoprofen Ibuprofen Proglumide	Protein affinity (OVM)	20 m*M* PB (pH 5.5–6.0)	[52]
Ketoprofen	Chitin-coated aminopropyl silica	Hexane–2-propanol (90:10, v/v)	[55]
	polysaccharide derivative	Hexane–2-propanol–TFA (90:10:0.1, v/v/v)	[109]
	polysaccharide derivative	Heptane–2-propanol–TFA (98:2:0.5, v/v/v)	[56]
Mandelic acid phenylacetate-related compounds	Polysaccharide derivative	Hexane–2-propanol–TFA (80:20:1, v/v/v)	[57]
NSAIDs	Protein affinity (BSA)	PB (pH 5.9–7.8)	[58]
	Protein affinity (BSA)	100 m*M* PB (pH 7.4)–1-propanol (95:5, v/v)	[49]
	Protein affinity (α1-AGP)	PB (pH 7.0) containing 0.5% 2-propanol+DMOA	[50]
	Protein affinity (BSA)	20 m*M* PB (pH 8.0)+acetonitrile	[51]
	Cellulose or amylose derivative polymer	Hexane–2-propanol–TFA (80:20:1, v/v/v) or (95:5:1, v/v/v)	[53]
Naproxen	Protein affinity (α-chymotrypsin)	PB (pH 5.3)	[54]
Ofloxacin	Protein affinity (BSA)	0.2 *M* PB (pH 8.0) containing 3% 2-propanol	[63]
Pantolactone	Brush type (urea derivative)	*n*-Hexane–1,2-dichloroethane–ethanol (50:10:1, v/v/v)	[46]
Pantothenic acid	LE	2 m*M* Copper(II) sulfate in water–acetonitrile (95:5, v/v)	[47]
Pantoic acid pantothenic acid	LE	2 m*M* $CuSO_4$ in aqueous acetonitrile (10%)	[48]
Tropic acid mandelic acid baclofen	Inclusion complex (β-CD)	0.1 *M* PB (pH 4.2 or 6.5) –acetonitrile (65:35, v/v)	[59] [60]
Temafloxacin (acetylated)	Protein affinity (OVM)	0.02 *M* PB (pH 7.0)–acetonitrile (92:8, v/v)	[108]

Table 6
Enantiomer separation of carboxylic drugs by CE using different selectors

Drug	Selector	BGE	Reference
Baclofen	β-CD (20 mM) 50 mM STDC	30 mM PB, 10 mM boric acid, (pH 7)	[66]
	18C6TCA (10 mM)	20 mM Tris-phosphate (pH 2.06)	[67]
Carprofen	β-CD (coated Chirasil-Dex) CEC	20 mM PB (pH 7)	[68]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomycin (2 mM)	0.1 M PB (pH 7)	[70]
	Teicoplanin (2 mM)	0.1 M PB (pH 6)	[71,72]
	TM-β-CD (10 mM)	75 mH formate (pH 4)	[73]
	β-CD-NH_2 (20 mM)+ TM-β-CD (10 mM)	34 mM PB (pH 2.3)	[74]
Carnitine	derivatized with FLEC	50 mM PB (pH 2.6) 20 mM TBAB	[75]
Cicloprofen	β-CD (coated Chirasil-Dex) CEC	20 mM PB (pH 7)	[68]
	SBE-β-CD (2 mM)	20 mM PB (pH 2.5)	[76]
	HP-β-CD (120 mM)	0.1 M PB (pH 2.5)	[76]
	HP-β-CD (60 or 120 mM)	50 mM borate (pH 9.5) 5% 1-propanol, 50 mM STC	[77]
Fenoprofen	β-CD (15 mM)	0.2 M MES (pH 4.5), 0.2% HEC	[78]
	HP-β-CD (20 mM)	0.2 M MES (pH 4.41), 0.2% HEC	[79]
	β-CD (15 mM)	0.6 M MES (pH 4.65), 0.2% HEC	[80]
	TM-β-CD (30 mM)	0.1 M MES (pH 5)	[81]
	Dextrin (10%)	0.1 M PB (pH 6 or 7)	[82]
	Vancomysin (2 mM)	0.1 M PB (pH 6)	[70,71]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Teicoplanin (2 mM)	0.1 M PB (pH 6)	[71,72]
	MeNH-β-CD (5 mM)	50 mM PB, 50 mM acetic acid	[83]
	β-CD (10 mM)	0.2 M MES (pH 4.6)	[84]
Flurbiprofen	TM-β-CD (30 mM)	0.1 MES (pH 5)	[81]
	β-CD (coated Chirasil-Dex)	CEC: 20 mM PB (pH 7)	[68]
	Avidin (0.025 mM)	50 mM PB (pH 6) 10% ethanol	[85]
	Maltooligosaccharides (2.5–10%)	10 mM PB (pH 7)	[86]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomycin (2 mM)	0.1 M PB (pH 7) SDS	[87]
	Dextrin (6%)	20 mM PB (pH 2.5)	[88]
	β-CD-NH_2 (20 mM) +TM-β-CD (10 mM)	34 mM PB (pH 2.3)	[74]
Folinic acid	Avidin (0.025 mM)	50 mM PB (pH 6) 10% 10% ethanol	[85]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomycin (2 mM)	0.1 M PB (pH 7)	[87]
Ibuprofen	β-CD (15 mM)	200 mM MES (pH 4.5) 0.2% HEC	[89]
	TM-β-CD (30 mM)	100 mM MES (pH 5)	[81]
	β-CD or γ-CD (coated Chirasil-Dex)	20 mM PB (pH 7)	[68]
	Avidin (0.025 mM)	50 mM PB (pH 6) 10% ethanol	[85]
	BSA (1 mg/ml)	10 mM PB (pH 7.12) 5% Dextran	[90]
	Maltooligosaccharides (2.5–10%)	10 mM PB (pH 7)	[86]
	Vancomycin (2 mM)	0.1 M PB (pH 7)	[87]
	Dextrin (5%)	20 mM PB (pH 2.5)	[88]
	β-CD (15 mM)	0.2 M MES (pH 4.6)	[91]
Indoprofen	TM-β-CD (30 mM)	0.1 M MES (pH 5)	[81]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomycin (2 mM)	0.1 M PB (pH 7) SDS	[87]
	Teicoplanin (2 mM)	0.1 M PB (pH 6)	[71,72]
	TM-β-CD (10 mM)	35 mM formate (pH 4)	[73]

Table 6 (Continued)

Drug	Selector	BGE	Reference
Isolysergic acid	γ-CD (30 mM)	0.1 M PB (pH 2.5)	[92]
Ketoprofen	TM-β-CD (30 mM)	0.1 M MES (pH 5)	[81]
	Avidin (0.025 mM)	50 mM PB (pH 6) 10% ethanol	[85]
	Dextrin (10%)	0.1 M PB (pH 6 or 7)	[82]
	Dextrin 10 (15 mM)	20 mM TAPS-6.5 mM Tris (pH 7.7), 4% ethanol	[93]
	Maltooligosaccharides (2.5–10%)	10 mM PB (pH 7)	[86]
	Vancomycin (2 mM)	0.1 M PB (pH 6) SDS	[87]
	Teicoplanin (2 mM)	0.1 M PB (pH 6)	[71,72]
	β-CD-NH_2 (20 mM)+ TM-β-CD (10 mM)	34 mM pB (pH 2.3)	[74]
Kynunerine	HSA (1 mg/ml)	10 mM borate (pH 9.5)	[94]
N-(2-Mercaptopro-pionic)-acid	derivatized with OPA-L-Phe or L-Tyr	MEKC	[95]
Naproxen	HP-β-CD (10 mM)	0.2 M MES-TBA (pH 5) 0.4% polymeric additive	[96]
	HP-β-CD (5 mM)	0.2 M MES (pH 4.86) 0.2 % HEC	[79]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomycin (2 mM)	0.1 M PB (pH 7)	[87]
	Dextrin (6%)	20 mM PB (pH 2.5)	[88]
	HP-β-CD (10 mM)	25 mM acetate (pH 4.6)	[91]
	β-CD-NH_2 (20 mM) +TM-β-CD (10 mM)	34 mM PB (pH 2.3)	[74]
NSAIDs	Cu-vancomycin complex (4 mM)	0.1 M PB (pH 5) formamide (80:20)	[97]
	tri-OMe-β-CD	20 mM PB-20 mM triethanolamine (pH 5.0)	[98]
Ofloxacin	BSA (0.4%)	0.1 M PB (pH 8)	[99]
Ofloxacin and analogues	γ-CD (20 mM)+ 10 mM Zn (II) Sulfate	10 mM acetate (pH 6.5)	[100]
	5 mM vancomycin	0.1 mM PB (pH 3–5)	[102]
Phenoxy acid herbicides (Silvex etc.)	Octylmaltopyranoside (60 mM)	200 mM PB (pH 6.5)	[101]
Pranoprofen	TM-β-CD (10 mM)	35 mM formate (pH 4)	[81]
Suprofen	TM-β-CD (30 mM)	0.1 M MES (pH 5)	[81]
	Ristocetin (2 mM)	0.1 M PB (pH 6)	[69]
	Vancomysin (2 mM)	0.1 M PB (pH 7)	[87]
	Teicoplanin (2 mM)	0.1 M PB (pH 6)	[71,72]
	TM-β-CD (10 mM)	75 mM formate (pH 4)	[73]
	β-CD-NH_2 (20 mM)+ TM-β-CD (10 mM)	34 mM PB (pH 2.3)	[74]
Tiaprofenic acid	MeNH-β-CD (5 mM) or $(MeNH)_7$-β-CD	50 mM phosphoric acid, 50 mM acetic acid 50 mM boric acid and NaOH (pH 7)	[83]

complex, and UV or fluorescence detection. The proposed structure of the ternary complex of ofloxacin, which is one of pyridone carboxylic: antibacterial reagent, and amino acid with copper (II) complex are shown in Fig. 2.

In recent years, Galaverna et al. [36] introduced an

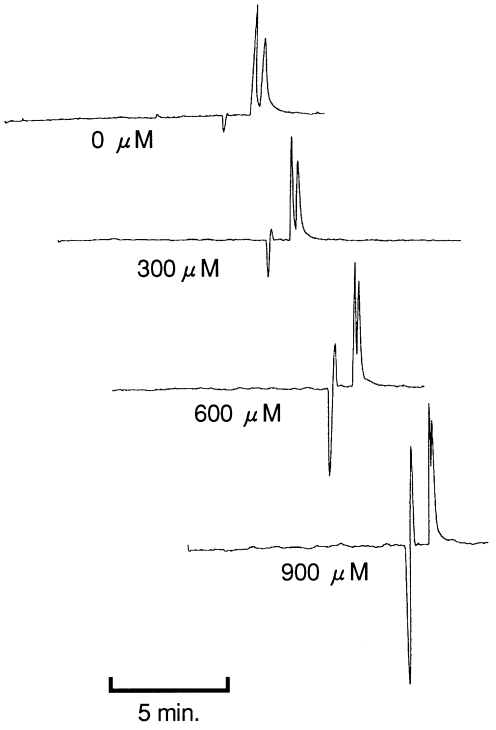

Fig. 3. Influence of the coadditive ketoprofen on the electropherograms of ofloxacin enantiomer by capillary affinity zone electrophoresis using BSA. BSA concentration, 60 μ*M*; applied voltage, 20 kV. The carrier buffer solution (20 m*M* phosphate buffer, pH 7.5) contained 3% methanol in order to dissolve the ketoprofen. Detection was at a wavelength of 300 nm using an uncoated capillary with an effective length of 50 cm and a temperature of 23°C. Sample (0.2 mg/ml) were electrically injected at 15 kV for 3 s (from Ref. [103]).

alternative approach to the LE method using copper(II) complexes of N^2-alkyl-(*S*)-amino acid amides as the chiral selector for dynamically coated chiral stationary phases in RP-HPLC. The methods could be used to obtain the direct enantiomeric separation of unmodified hydroxy acids by elution with aqueous or mixed aqueous–organic solutions containing copper(II) sulfate or acetate. The dynamically coated chiral stationary phase maintained its separation ability for about three months. The column could easily be restored by recovering the selector with methanol and repeating the loading procedure.

Linder and Hirschböck [38] have suggested some requirements for applying the LE mobile phase method, which are listed above:

1. nature of the ligand atoms (N, O, S, P)
2. functionality of the ligand atom (e.g. N in amine, amide or nitrile)
3. size of the chelate rings to be performed (four, five, six or seven-membered rings)
4. number of chelate rings that can be accomplished by the ligand molecules (e.g. bi- or tridentate ligand)
5. steric effect determined by the size and position of the optically active substituents on the chelating ligand molecules
6. nature of the central metal ion to be chelated, its oxidation state, as well as its coordination number (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{3+}, etc.)
7. temperature, solvents in which the chelation process should take place, pH and the buffer system (summarized as mobile phase conditions).

3.2. Ion-pair method

A chiral counter-ion added to the mobile phase could be used to separate enantiomers of acids and amines. The basis for resolution is the formation of diastereomeric and ionic complexes with different stabilities or distribution properties between the mobile and the stationary phases. Cinchona alkaloids, such as quinine and quinidine, are effective counter-ions for the separation of enantiomers of carboxylic and sulfonic acids with hydrogen-bonding functions on conventional achiral stationary phases. The chiral counter-ions are generally used with organic mobile phases of low polarity to promote a high degree of ion-pair formation. However, in some cases, chiral ions have been used as additives in reversed-phase chromatography. Optically active zwitterions could be used for stereoselective separation.

Pettersson and Schell [22] reported the chiral separation of phenyl carboxylic acids using quinine as a chiral ion-pairing reagent. In this system, the UV background of mobile phase was strong, but a negative peak of solutes was observed. The detection method is called ‘indirect detection’.

Karlsson and Pettersson [35] reported the use of porous graphitic carbon as an adsorbing phase for the direct separation of enantiomeric acids using a chiral counter-ion to separate the enantiomeric acids. The

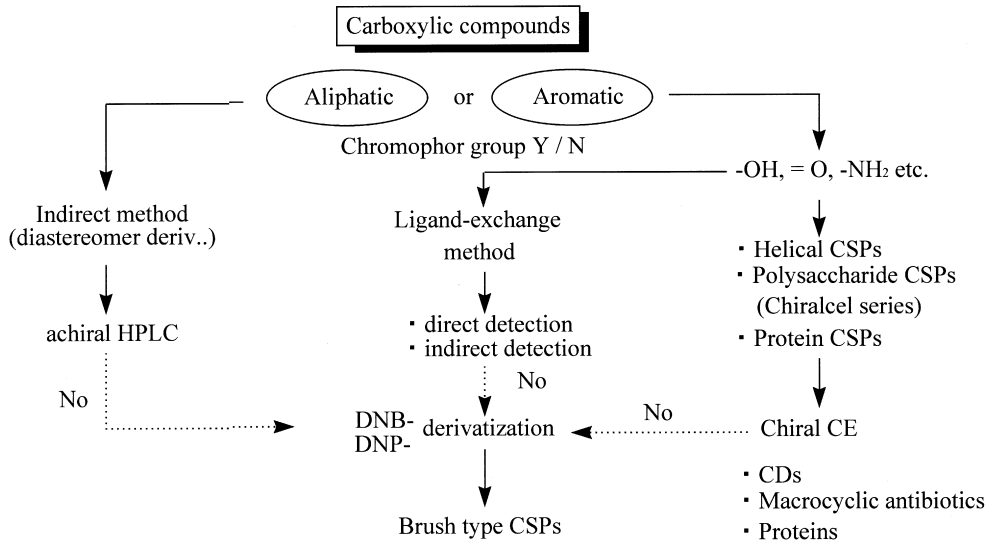

Fig. 4. Selection of the separation procedure.

strong UV-absorbing quinine improved the detection of solutes with low UV-absorbing properties by indirect detection.

3.3. Other chiral mobile phase methods

Some alternative chiral mobile phase methods have been used in an attempt to separate enantiomers of carboxylic compounds using HPLC. For example, am inclusion complex using cyclodextrin (CD), affinity interaction using protein and host–guest interaction with crown ethers etc. However, they have only been used successfully in the field of carboxylic drugs, and attempts are being made to transfer to a chiral CE method.

4. Chiral stationary phase HPLC method

The first commercially available HPLC chiral stationary phase (HPLC–CSP) was introduced by Pirkle in 1981 [39]. The large number of chiral phases presents analysts with several different possibilities for the development of an assay. Wainer [40] classified the HPLC–CSPs into five basic types in terms of how the solute–CSP complexes are formed. They are as follows and are also summarized in Table 4.

Type I: The solute–CSP complexes are formed by attractive interactions, such as hydrogen bonding, π–π interactions, dipole stacking, and so on. These are the CSPs developed by Pirkle and those that operate in a similar fashion.

Type II: The primary mechanism for the formation of the solute–CSP complex is through attractive interactions but where inclusion complexes also play an important role.

Type III: The primary mechanism for the formation of the solute–CSP complex is the inclusion of the solute in a chiral cavity of the CSP. These are the cellulose triacetate–CSPs, the CD–CSPs (CD–CSP) and the phenylmethacrylate polymers, etc.

Type IV: The solute is part of a diastereomeric metal complex. These are chiral ligand exchange CSPs.

Type V: The CSP is based on an immobilized protein and the solute–CSP complexes are based on combinations of hydrophobic and polar interactions. These are the CSPs based upon bovine serum albumin (BSA–CSP) and α-1-acid glycoprotein (AGP–CSP).

Now, CSPs for the efficient chiral separation of carboxylic drugs, such as ligand-exchange CSPs (type IV), polysaccharide derivative CSPs (type III), brush type CSPs (type I) and others will be described. Their applications are listed in Table 5.

4.1. Ligand-exchange CSPs

Efficient tools for the chiral separation of carboxylic compounds are LE–CSPs and LE–mobile phase methods. Almost all of the LE–CSPs have conjugated amino acids or amino acid derivatives on a silica gel surface as the chiral selector. Some enantiomers of aliphatic compounds lacking a chromophore could be analyzed using the LE–CSP method. The analytes form a chelate complex in the mobile phase containing metal ion and can be detected at around 254 nm. Chiral separations of pantothenic acid and pantoic acid are achieved using an *N*,*N*-dioctyl–L-alanine conjugated ODS column with a $CuSO_4$ aqueous solution as the mobile phase [48,62].

Also, the optical resolution of 2-hydroxy acids was achieved using CSP. The optical resolution of mandelic acid derivatives and of C_2 to C_5 2-hydroxy acids was performed without derivatization. The column could resolve amino acids and 2-hydroxy acids. For their selective detection, a postcolumn method was employed in which the specific color reaction of 2-hydroxy acids with iron(III) was utilized. For the analysis of foodstuffs in which amino acids and 2-hydroxy acids often coexist, the present method was found to be effective in distinguishing between these enantiomers.

Ôi et al. [42,47] designed new LE–CSPs. The CSPs were conjugated amino acids on aminopropyl silica gel. 3-Amino-ε-caprolactam and pantothenic acid enantiomers were separated using these LE–CSPs. The proposed structure of a ternary complex on LE–CSP is shown in Fig. 2B. The ternary complex of one enantiomer may assume a *trans* conformation around a planar face formed by chelate bonding and that of another isomer a *cis* conformation. The enantioselectivity is dependent on several factors, but is mostly ascribed to differences in the relative stabilities of the ternary complex of chiral ligand, metal ion and applied enantiomer.

4.2. Polysaccharide derivative CSPs

Polysaccharide derivative CSPs are widely used in the field of chiral separation. In particular, the Chiralcel series of CSPs are the most efficient CSPs in practical terms and do not require derivatization. Okamoto et al. [41,53,57] have designed different kinds of polysaccharide helical polymers as Chiralcel series CSPs containing a good deal of application data. In this section, some applications regarding chiral separation of carboxylic compounds using polysaccharide helical type CSPs will be described.

About a decade ago, Okamoto et al. [53,57] examined different kinds of cellulose or amylose Tris(phenylcarbamate) derivatives as CSP packing materials. Various mono- and dicarboxylic acids enantiomers were resolved on the CSPs by using normal phases. They reported the direct optical resolution of abscisic acid, an important plant hormone, on cellulose Tris(3,5-dimethylphenylcarbamate) [41]. In addition, Okamoto et al. reported the direct optical resolution of anti-inflammatory drugs by HPLC using Tris 3,5-dimethylphenylcarbamate's of cellulose and amylose as CSPs (see Table 5). The CSPs are commercially available as the Chiralcel series.

Oliveros et al. [56] prepared benzoates of cellulose and linked them to allyl silica gel by means of a radical reaction. The chiral recognition ability of the resulting materials when used as HPLC chiral stationary phases was evaluated using heptane–2-propanol and heptane–chloroform mixtures as mobile phases.

4.3. Brush types CSPs

In many cases, the use of brush type CSPs requires DNP- or DNB-derivatization to initiate π–π interaction between analytes and CSPs. In this section, direct resolution using brush type CSPs will be reviewed. Ôi et al. [45,46] developed new brush type CSPs containing urea derivatives. Chiral separations of clidanac, nonsteroidal anti-inflammatory drugs (NSAIDs) and pantolactone were achieved using these CSPs without derivatization in a normal phase.

Recently, some new brush type CSPs were designed by Sinibaldi et al. [44] using an ergoline

skeleton conjugated CSP. This CSP was applied to the chiral separation of citronellic acid and aromatic carboxylic acids.

Cass et al. [55] investigated the use of bis(aryl carbamate) derivatives of chitin coated onto microporous organosilane-modified silica. The chiral resolution of ketoprofen was achieved on this CSP using hexane–2-propanol (90:10, v/v) as the mobile phase.

Several years ago, a chiral urea polymer was prepared as a CSP packing material [61], but it may not be classified as brush type CSP. It was obtained by copolymerization of urea with formaldehyde in the presence of an optically active amino acid derivative. Its application to the resolution of some organic acid racemates with reversed-phase chromatography was demonstrated.

4.4. Other types of CSP (affinity, inclusion chromatography)

Some attempts using protein affinity CSPs and CD inclusion complex CSPs are reviewed. Chiral selectors of protein affinity CSPs are bovine serum albumin (BSA), human serum albumin (HSA), α1-acid glycoprotein (α1-AGP), ovomucoid (OVM), etc. Some anti-inflammatory agents have been separated into enantiomers on all types of protein phases [50–52]. Negatively charged analytes, such as carboxylic compounds, become significantly more retained when the pH is decreased on all of the protein columns.

The charged analyte makes the application of ion-pairing mobile-phase additives interesting. Miwa et al. [52], using ovomucoid columns, found that cationic (tetrabutylammonium) as well as anionic (octylsulfonate) additives constantly decreased all k' values, although this phenomenon was most drastic with anionic additives. In no case was the change associated with any significantly increased α-value. By contrast, Hermansson and Eriksson [50] reported that using an AGP–CSP the cationic modifier *N*,*N*-dimethyloctylamine (DMOA) in the cases NSAIDs causes increased α values, mainly as a result of increased k_2' values. The very large effect on naproxen, where 5 m*M* DMOA in the mobile phase (phosphate buffer, pH 7.0, 0.5% 2-propanol) led to a remarkable increase in α, from 1.05 to 4.5. Whether the effects resulted from the use of different protein phases, types of cationic modifiers or some other factors is unclear.

Feitsma et al. [59,60] investigated the enantiomeric separation of some aromatic carboxylic acids by HPLC using a covalently bonded β-CD stationary phase. They succeeded in resolving racemic cyclohexylphenylacetic acid and racemic cyclohexylphenylglycolic acid, the latter being the acid moiety of the anticholinergic drug oxyphenonium bromide.

5. Chiral separation using CE

Among the analytical techniques currently used, CE is a powerful tool for chromatographic or electrophoretic resolution. Enantiomeric separations by CE have been widely studied and the results have been reported by several authors [64,65]. This section provides an overview of chiral separations in the field of carboxylic pharmaceuticals using CE.

Enantiomer separations of carboxylic drugs by CE using different selectors are listed in Table 6.

5.1. Inclusion complexation CE

CDs are oligosaccharides that are made up of several D(+)-glucopyranose units. Despite the fact that CDs with six to twelve units have been separated, only those with six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units are in frequent use. The hydroxy groups in positions 2, 3 and 6 are available for derivatization. The various derivatives have been synthesized, and it has been shown that the enantioselectivity can vary drastically among them.

The most frequently used derivative for carboxylic compounds is heptakis (2,3,6-Tris-*O*-methyl) cyclodextrin (TM-CD). NSAID enantiomers have been separated using TM-CD [73,74,81,98]. TM-CD and β-CD (a frequently used CD) are classified as neutral cyclodextrins and possess the same sized truncated cone with a hydrophobic cavity and a hydrophilic surface. The molecular size of NSAIDs may be suitable for the hydrophobic cavity of the CD.

Schurig et al. [106] demonstrated the concept of

unified enantioselective chromatography through the chiral separation of hexobarbital by GC, HPLC, SFC and CE using the same capillary coated with Chirasil-Dex. Several NSAID enantiomers were resolved on Chirasil-Dex using capillary electrochromatography [68,104,105].

Recently, the combination of ligand-exchange and inclusion interactions was investigated as a new mode for chiral separations. Horimai et al. [100] mixed γ-CD with a solution of Zn(II)–D-phenylalanine and used this dual selector system to successfully separate pyridone carboxylic antibacterial reagents. In the near future, the CE method with CDs may have the broadest application in the field of chiral separation.

5.2. Chiral affinity CE

Several proteins, such as serum albumins, AGP and OVM, have been used for chiral separation. After their successful use as chiral selectors in HPLC, proteins also found their way into CE. Most of the separations were done with the proteins as additives in the background electrolyte (BGE), according to the principle of affinity electrokinetic chromatography. Chiral separations of flurbiprofen, folinic acid, ibuprofen and ketoprofen were achieved using avidin as a chiral selector [90].

Enantiomers of ibuprofen were separated using dextran as a run buffer additive in addition to BSA by means of affinity capillary electrophoresis. By adding different amounts of dextran to the run buffer, the net velocity of BSA could be adjusted to a desired rate. Qualitative information pertaining to the binding interaction of the samples with BSA could be obtained by this method. Chiral CE using serum albumin was also found to be useful for studies of enantioselective drug–protein binding and displacement interactions [103,107]. Typical electropherograms obtained using serum albumin as a chiral selector in the presence of competitive drug at various concentrations are shown in Fig. 3.

These electropherograms shows that, if competitive displacement occurs on the same binding site of albumin, the enantioselectivity decreases. Thus, it is possible that exploring the selectivity of proteins will become a significant application of CE.

5.3. Use of macrocyclic antibiotics

Armstrong et al. [69,70,87] introduced macrocyclic antibiotics as a new type of chiral selector that shows enantioselectivity for a broad spectrum of compounds.

Macrocyclic glycopeptide antibiotics have an aglycone portion consisting of fused macrocyclic rings that form a ‘basket’ shape and have several pendent sugar moieties. They are positively charged under the conditions applied and have been used in the chiral separation of a broad spectrum of anionic solutes. About 350 compounds have been resolved successfully with glycopeptide antibiotics, including NSAIDs, hydroxy acids, quinolone carboxylic acid and N-blocked amino acids [69,70,87,102].

Macrocyclic glycopeptide antibiotics are suitable as chiral selectors for carboxylic compounds. The most versatile selector among the macrocyclic glycopeptide antibiotics was found to be ristocetin A. About 120 compounds, including N-blocked amino acids, NSAIDs and hydroxy acids were resolved into their enantiomers [69].

Disadvantages of using macrocyclic antibiotics as chiral selectors in CE are the UV absorption up to 250 nm and its limited stability. Recently, aminoglucoside antibiotics were also found to be applicable as chiral selectors. It is expected that this promising technique will find broad application in the future.

6. Selection of the chiral separation procedure for carboxylic compounds

The first recommended method for the chiral separation of carboxylic compounds is HPLC using polysaccharide derivative CSPs. At present, the analytical accuracy of HPLC is still better than that of CE. In many cases, finding a suitable procedure is still based on trial and error, both in HPLC and CE. However, procedure selection depends on the purpose it is to fulfill, such as in pharmacokinetic studies, quality testing, stability studies and molecular interaction studies, etc. If the compound possesses chelating ability, then ligand exchange is an attractive method. If the purpose is a pharmacokinetic study, then the derivatization method

will be useful. The most versatile selectors may be CDs, followed by macrocyclic antibiotics and proteins in the case of the CE method. Finally, if a suitable chiral procedure is not found, the brush type (Pirkle type) CSP method after DNP- or DNB-derivatization will be a reliable approach.

Some suggestions for choosing a chiral separation method for carboxylic compounds are shown in Fig. 4.

7. Conclusions

Chromatography and electrophoresis have proven to be efficient techniques for the chiral separation of carboxylic drugs. Diastereomer derivatization by HPLC is a classical method, and it is reliable for predicting the separation on an achiral stationary phase and it is easy to introduce a strong chromophore. The HPLC–CSP method is standard and many CSPs are now commercially available. In many cases, HPLC and CE are complementary with respect to enantioselectivity. In recent times, separation principles are being transferred from HPLC to CE. CE is preferred over HPLC because of its flexibility regarding choice of chiral selector and conditions and the ease of operation. Recent advances in the combination of analytical system will help to extend the spectrum of applications of chiral HPLC and CE.

8. Abbreviations

AA	amino acid
ABD-APy	4-(aminosulfonyl)-7-(3-amino-pyrrolidin-1-yl)-2,1,3-benzoxadiazole
AGP	α_1-acid glycoprotein
APMB	(−)-2-[4-(1-amino-ethyl)phenyl]benzoxazole
BGE	Background electrolyte
BSA	bovine serum albumin
CD	cyclodextrin
CL	chemiluminescence
CSP	chiral stationary phase
18C6TCA	(+)-18-crown-6-tetracarboxylic acid
DANE	1-(4-dimethylamino-1-naphthyl)ethylamine
DAPEA	1-(4-dansylaminophenyl)ethylamine
DBD-APy	4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-amino-pyrrolidin-1-yl)-2,1,3-benzoxadiazole
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DMAP	dimethylaminopropyl
DMOA	dimethyloctylamine
DNB	dinitrobenzoyl
DNP	dinitrophenyl
DPDS	2,2′-dipyridyl disulfide
DPP	diphenyl phosphine
E-DMAP-carbodiimide:	1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride
ECD	electrochemical detection
FL	fluorescence
FLEC	(−)-[1-(9-fluorenyl)-ethyl-]chloroformate
HEC	hydroxyethylcellulose
HOBT	1-hydroxybenzotriazole
HP-β-CD	Hydroxypropyl-β-cyclodextrin
HSA	human serum albumin
LE	ligand exchange
L-NSPC	*S*-(−)-*N*-1-(2-naphthyl sulfonyl)-2-pyrrolidine carbonylchloride
MEKC	micellar electrokinetic chromatography
MES	2-(*N*-morpholino)ethanesulfonic acid
MeNH-β-CD	6-methylamino–β-cyclodextrin
$(MeNH)_7$-β-CD	heptamethylamino–β-cyclodextrin
NBD-APy	4-nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole
NEA	napthylethyl amine
NP	normal phase
NSAID	nonsteroidal anti-inflammatory drug
ODS	octadecylsilanized silica gel
OPA	*o*-phthalaldehyde
OS	octylsilanized silica gel
OVM	ovomucoid
PB	phosphate buffer
RP	reversed-phase

r.t.	room temperature
STDC	sodium taurodeoxycholate
TEA	triethylamine
TFA	trifluoroacetic acid
TM-β-CD	heptakis(2,3,6-tri-*O*-methyl)–β-CD
TPP	triphenylphosphine
Tris	Tris(hydroxymethyl)aminomethane
WSC	water-soluble carbodiimide
β-CD-NH_2	mono(6-amino-6-deoxy)-β-cyclodextrin

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