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Review on Enantiomeric Separation of Drugs by HPLC

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Abstract

Optical Activity, Or Chirality, Is an Important Characteristic of Many Chemical Compounds. Various Industries Use Organic Compounds with Stereo Genic Centers, Which Can Affect Their Properties. Investigating The Attributes of Each Drug Enantiomer Is Crucial, As Only One Typically Exhibits Activity. Enantioselective Chromatography Plays a Valuable Role in Producing Pure Compounds and Developing Chiral Drugs. Advancements In Chiral Methods Within the Pharmaceutical Industry, Traditionally Reliant on Liquid Chromatography, Should Be Assessed.

Key words: Chiral stationary phase, Chiral additives, Derivatization, Enantioselectivity, Enantiomer, Diastereomeric adducts, Resolution, Tailing factor, Separation factor, Resolution factor, HPLC, Chiral, and Achiral

Introduction

Methods for the Resolution of Racemic Compounds

Chiral separation techniques in the synthesis of pharmaceutical intermediates include crystallization, kinetic resolutions, membrane-based separations, and chromatographic methods for separating enantiomers.

Indirect Method

The indirect method of enantiomeric separation uses optically active reagents to derivatize enantiomers, followed by separation as diastereomeric derivatives. Different reaction rates make this process complex, resulting in varying proportions of Dia stereoisomers. Chiral derivatization and diastereomeric formation in HPLC have been documented for drug enantiomers and xenobiotic compounds. Various chiral derivatizing agents like ß-D glucopyranosyl isothiocyanate and acetylmandeloylchloride have been used for enantiomer separation. New agents like (S)-N-(4-nitrophenoxycarbonyl) phenyl alanine methoxy ethyl ester have also been used for amino acid separation. Bevantolol can be directly separated on a Chiralcel OD column or indirectly using chiral derivatizing agents.

Direct Method

Separating enantiomers in chiral liquid chromatography can be done through direct methods. This involves chiral recognition by the stationary phase or through a diastereomeric complex created by a chiral component in the mobile phase. Various methods exist based on sorbent type.

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Use of an Intrisically Chiral, Polymeric Stationary Phase of
Either Natural Origin or Synthetic Polymers

i. Polysaccharide Derivatives
ii. Proteins
iii. Chiral Synthetic Polymers

Bonded Synthetic Chiral Selectors

i. Crown Ethers
ii. Metal Complexes
iii. Chiral Stationary Phase Based on Charge Transfer Complexation
iv. Other Types of Selection

CSP Based on Urea and Amide Derivatives
Macrocyclic Antibiotics

Technique Based on Addition of Chiral Constituents To Mobile Phase

Modern high-performance liquid chromatography (HPLC) sorbents are effective for achieving high column efficiency. Cyclodextrins and their derivatives are potent chiral selectors that can be applied to various organic compounds. Researchers have investigated the retention behaviour of different compounds using cyclodextrins as chiral additives in HPLC. Various enantiomers, such as procaine hydrochloride, naproxen, nomifensine, and epinephrine, were successfully separated using different cyclodextrins as mobile phase additives. Sulphated ß-cyclodextrin and other cyclodextrins were used as chiral selectors in RP-HPLC to resolve enantiomers. Teicoplanin and cellulose protein were also used as chiral selectors in HPLC and capillary electrophoresis. Different methods and techniques were developed for enantioselective separations of various compounds, including voriconazole, terbutaline, pheniramine, and ibuprofen. Levofloxacin HCl was separated using a chiral mobile phase additive in HPLC. Teicoplanin served as a chiral selector for amino acids, peptides, and cyclic amides and amines in HPLC. Overall, these studies highlight the effectiveness of using modern HPLC techniques with chiral selectors for enantioseparations.

HPLC separation: chiral drugs, β-cyclodextrins.

The use of chiral selectors in conjunction with achiral columns offers advantages such as flexibility, a wide range of additives, and cost savings compared to using chiral stationary phases. Cyclodextrins (CDs), specifically beta-cyclodextrin, are commonly used as chiral mobile phase additives in liquid chromatography. The separation mechanism involves the formation of inclusion complexes, where the solute is included in the CD cavity. Factors that influence the enantioseparation process include the stability of the CD complexes, adsorption on the stationary phase, and adsorption of free solute molecules on the CD layer. The size of the CD cavity is critical in forming stable inclusion complexes. Different interactions between enantiomers and CDs, such as dipole-dipole, hydrogen bonding, and hydrophobic interactions, play a role in chiral separation. Poor solubility of CDs in aqueous organic solvents can be improved by the addition of urea or through chemical modification of the CD hydroxyl groups. The use of chemically modified CDs can enhance chromatographic separations by changing the inclusive complex strength. In a study, propylsilane, hexylcaine, octyl silane, octadecylsilane, and a nonporous octadecylsilane column were evaluated with native and derivatized beta-CDs in the mobile phase. Chiral drugs such as chlorthalidone, terbutaline, and oxazepam were investigated. The nonporous column showed benefits such as lower organic modifier usage and faster retention times.

Experimental

Materials

β-Cyclodextrin, hydroxypropyl β-Cyclodextrin, methyl β-Cyclodextrin, sulphated β-Cyclodextrins were obtained from TCI and American Maize Company. Various drugs were purchased from Sigma Chemical Co. Chlorthalidone and fenoprofen standards were from USP. HPLC-grade solvents were purchased from various suppliers.

Instrumentation

Chromatography was conducted using a Beckman HPLC system with a Beckman solvent delivery module, Rheodyne injector, and Waters Millipore spectrophotometer. Chromatograms were recorded using a Spectra-Physics integrator. HPLC columns from various manufacturers were purchased.

Chromatographic Conditions

Separations were done at 23°C with UV detection set at specific maxima for each analyte: 240nm for oxazepam, lorazepam, and temazepam, 265nm for

ketoprofen, fenoprofen, and ibuprofen, 275nm for terbutaline and chlorthalidone, and 250nm for trimipramine and trimeprazine. Mobile phases contained 5-20mM concentrations of CDs in acetonitrile and aqueous trifluoroacetic acid (pH 4.0). Flow rates varied for different columns. Stock solutions of 1µg/mL were prepared in respective mobile phases.

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EXPERIMENTAL

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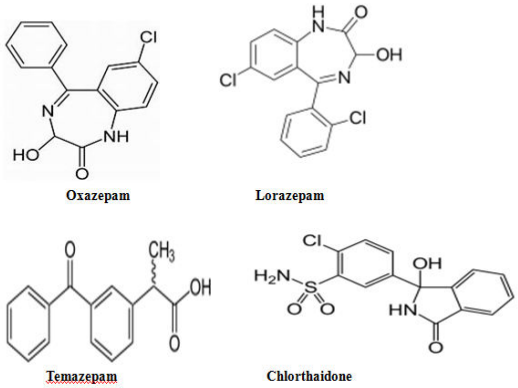

Fig 1: Chemical Structures of Analytes Studied

Results and Discussion

Preliminary results in the lab showed that only chlorthalidone and terbutaline enantiomers were resolved on specific columns with β-CD or HP-β-CD added to the mobile phase. Chlorthalidone required a specific mobile phase condition, while terbutaline enantiomers didn't. The use of a 50mM ammonium acetate buffer improved peak sharpness for both chlorthalidone and terbutaline enantiomers on some columns. However, none of the analysed compounds, including chlorthalidone and terbutaline, could be separated on other columns with CDs added to the mobile phase. The newly introduced nonporous reversed phase octadecylsilane column showed success in separating chlorthalidone enantiomers, as well as other compounds. Mobile phase compositions and retention factors were investigated, and most enantiomeric pairs had a resolution greater than 1. The nonporous column required a low concentration of organic modifier and provided short retention times for analysis.

Conclusion

Chiral compounds can be easily studied through chromatographic techniques to determine their absolute configuration, even in small quantities. Chiral chromatography is a mature field but continues to evolve to meet the demands for enantiomer determination, especially in the pharmaceutical industry. New chiral selectors are leading to a re-evaluation of established principles.

Table 1

Resolution (Rs) of Lorazepam, Temazepam, Oxazepam, and Chlorthalidone Enantiomers on Nonporous Octadecylsilane Column

Analyte	Mobile Phase Composition (v/v)[a]		Retention Factors		
	A	B	k_1	k_2	Rs
Lorazepam	98	2	6.04	7.00	1.05
	96	4	6.60	7.56	1.05
	94	6	6.25	7.37	1.02
	92	8	6.54	7.33	1.00
	90	10	6.46	7.13	0.90
Temazepam	98	2	8.58	10.85	1.34
	96	4	8.25	10.10	1.25
	94	6	7.23	8.54	1.14
	92	8	7.42	8.50	1.10
	90	10	7.37	8.22	1.00
Oxazepam	98	2	3.79	5.60	1.97
	96	4	4.66	6.51	1.90
	94	6	4.19	6.00	1.74
	92	8	4.60	6.16	1.74
	90	10	4.11	5.50	1.65
Chlorthalidone	98	2	4.27	5.79	1.78
	96	4	--[b]	--	--
	94	6	--	--	--
	92	8	--	--	--
	90	10	--	--	--

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