CHAPTER 1

INTRODUCTION
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Hypertension is considered multifactorial in origin and is caused by a breakdown of the control mechanisms, which regulate cardiac output, blood volume, sodium balance and systemic vascular resistance. It is observed that blood pressure increases with age, and thus the prevalence of hypertension increases from about 25% in the middle-aged population, and to round 50% in those aged over 75, with risk factor for coronary heart diseases, cerebrovascular diseases, chronic renal failure and congestive heart failure. It is therefore, rapidly becoming a foremost problem in developing countries and is considered as the principal cause of stroke that leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death. Along with this, it is also a major contributor to cardiac failure, renal insufficiency and dissecting aneurysm of the aorta; hence, antihypertensive drugs have been chosen for the present study [1]. As far as treatment is concerned, only a few patients having hypertension, which includes adrenal disease, renal artery stenosis, unilateral renal parenchymal disease etc., are amenable to surgical correction. Rest of the patients requires treatment with antihypertensive drugs and, because this must continue indefinitely, its acceptability in terms of dosage frequency and side effects must be considered majorly. The choice of treatment should be tailored to the individual since side effects are rarely obtrusive and impotence in particular. In addition, these drugs do not have unfavourable effects on glucose or lipid metabolism and, but may protect against development of arterial disease in later life.
In recent years, most of the drugs available are in the combination of low doses of two different drugs with an additive effect, which may have fewer side effects than the higher doses of one agent. Antihypertensive drugs such as atenolol, metoprolol propranolol, hydrochlorothiazide and nifedipine are the group of the drugs that are widely used in the treatment of edema, hypertension, hypoparathyroidism, hyperthyroidism, angina pectoris, vascular disease, glaucoma, acute myocardial arrhythmias etc. [2], either alone or in combination. Hence, in order to ensure quality and stability of the final product, the analyst must be able to separate these mixtures into individual components prior to quantitation analysis. These drugs are selected, since, these drugs are preferred as first line therapy for hypertension; including efficacy, side effect profile and cost. In addition, they are generally well tolerated, relatively inexpensive and widely given alone and in combinations [3]. \( \beta \)-blockers have a useful role in patients with exaggerated tachycardia or an anxiety component to their hypertension, while for many patients calcium antagonists are the best tolerated and most effective drugs. Thus, if a single agent provides inadequate blood pressure control, a second can be added-ideally as a combined preparation for ease of administration and better compliance, while persistent hypertension requires the addition of a third agent and, in the most severe cases, four or more different drugs may be necessary.

Also, drugs when administered, in formulation, are not in pure form but contain several excipients like binders, fillers, disintegrants, diluents, colours, flavours etc. In addition, they may contain impurities in the final product, which generally are confined to those arising from incomplete and side reactions, or due to environmental conditions
(i.e. oxidation, reduction, hydrolysis etc.). This has necessitated the chemist for determining the assay of drug since drug impurities induce toxicity, which may prove to be fatal. In addition, the comparison of the relative efficacy of the different dosage forms of the same drug entity requires the analysis of the active ingredient in biological matrices, such as blood, urine and tissues, which is necessary to study bioavailability or bioequivalence study. Hence, quality assurance and control have become very important parameters for the complete assay of drug within the limits of accuracy and precision. In addition, the impurity check of the drug substance is currently the most important analysis, thereby giving essentially the fingerprint of the chemical synthesis and of the stability studies. Literature cites several conventional methods like volumetry, gravimetry etc., but today they are being fastly replaced by instrumental techniques viz. spectrophotometry, GC, CE, HPLC etc., which allows even traces of impurities to be separated and detected and finds numerous applications in the field of science like medicine, clinical chemistry, pharmaceutical, agricultural, forensic, metallurgy, oceanography etc.

As drug purity is equated with drug safety, enormous effort is expended by the pharmaceutical industries to minimize and control the impurity levels in its products. This effort begins at the initial stages of pharmaceutical development and continues into quality control of the final products. Impurity assays by liquid chromatography are developed and validated to support the manufacturing process, for stability study of the formulations and for release assays of each lot of bulk drugs. Since impurity levels are often very low in most pharmaceutical bulk products, the choice of the method is very
important. The method must be accurate, sensitive, selective, reproducible and convenient.

Taking this into view, the literature for the separation of antihypertensive drugs viz. atenolol, propranolol, metoprolol, hydrochlorothiazide and nifedipine of the recent five years have been overviewed. Separation of drugs via chromatographic methods is further discussed by two methods since some of these drugs exist in their racemic forms.

I ACHIRAL METHODS

II CHIRAL METHODS

Separation of the drugs via achiral methods

In chromatography, High Performance Liquid Chromatography (HPLC) has expanded to include ion chromatography, affinity, immunoaffinity, and chiral chromatography in addition to the more common modes of adsorption, ion-exchange, normal phase, reversed phase and size exclusion chromatography. An abundant number of packing materials have been developed of which some are highly specialized for specific applications and some have multipurpose use. However, use of reversed phase packings helped to give high resolution separations that could not be readily accomplished by ion-exchange, adsorption, or normal phase chromatography. Initially silica was the only solid support but other supports gradually began to evolve. Organic
resins have comeback after declining its popularity in 1970s and early 1980s. These supports have advantages, as do polymer-coated silicas, especially carbons also have great potential as solid supports. They are chemically unreactive, can be used over a wide pH range, are highly reproducible and stereoselective, can be modified by adsorption of high molecular weight materials, and can be used for size exclusion.

Different methods have been reported in the literature for the separation of antihypertensive drugs. Most of these drugs have been separated in combination with other antihypertensive drugs, while some with other class of drugs in RP-mode using variety of columns under different conditions with polar mobile phases. As far as drugs are concerned, most of the drugs are either polar or semipolar in nature and hence reversed phase chromatography is more preferred compared to normal phase in pharmaceutical industries. The pharmaceutical industry is facing the challenge of ensuring quality and purity in drug production. As pressure from regulator increases, detecting trace impurities in drugs becomes vital. In such cases, separation has been carried out by different reversed phase columns and polar mobile phases using various detectors viz. UV detector, Fluorescence detector, Refractive index detector, Electrochemical detector etc. In the present work, a review of literature of last five years have only been taken into consideration.

A modified HPLC method with fluorimetric detection has been developed to determine the pharmacokinetics and comparative bioavailability of atenolol tablets manufactured by two companies [4]. Chiap et al. have separated atenolol from human plasma on C₈ column using UV detector [5], while the same group of the authors have
also reported the automated liquid chromatographic determination of atenolol in plasma using dialysis and trace enrichment on a cation exchange precolumn [6]. Quantification of atenolol in human plasma has been carried out by HPLC [7] and Capillary Zone Electrophoresis method [8] having linear range 25-800 ng/mL and 30-585 ng/mL, respectively. Mislanova and Hutta have studied influence of various biological matrices like plasma, blood microdialysate etc., on chromatographic performance for the determination of β-blockers viz. atenolol, pindolol and propranolol using alkyl-diol silica precolumn [9]. A general method has been developed for the detection of β-blockers viz. alprenolol, atenolol, betaxolol, bupranolol, butofilolol, nadolol, oxprenolol, penbutolol and timolol in human urine by GC-MS-MS-EI with an average sensitivity limit of 20 ng/mL [10]. Kriikku and co-workers have carried out analysis of β-blockers viz. oxprenolol, atenolol, timolol, propranolol, metoprolol and acebutolol in human urine by combination of isotachophoresis and zone electrophoresis with poly (methyl methacrylate) chip system [11]. Also, optimization of the separation of β-blockers like atenolol, sotalol, betaxolol and metoprolol have been carried out by Servais et al. by ion pair capillary electrophoresis in non-aqueous media using univariate and multivariate approaches [12]. Simultaneous determination of β-blockers viz. (atenolol, sotalol, diacetolol, carteolol, nadolol, pindolol, acebutolol, metoprolol, celiprolol, oxprenolol, labetalol, propranolol, tertatolol and betaxolol); (alprenolol, atenolol, metoprolol, nadolol, pindolol, propranolol, sotalol and timolol); and (acebutalol, alprenolol, atenolol, betaxolol, bisoprolol, varvedilol, celiprolol, esmolol, labetalol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol and timolol) have been carried out be HPLC
methods using UV and fluorescence [13]; photodiode-array UV [14]; and mass spectrometry [15] detectors, respectively. Atenolol in combination with different calcium channel blockers viz. nifedipine [16], amlodipine [17, 18] and amlodipine, nifedipine, nimodipine and nitrendipine [19] have been separated by RP mode. Abdel-Hamid has separated β-blockers viz. atenolol, propranolol, pindolol and acebutalol along with certain antiepileptic drugs using LC-MS method, which proved to be faster, more sensitive and specific [20]. Also, atenolol alone [21, 22] and in combination with β-blockers [23-26] and chlorthalidone [27] have been separated using variety of mobile phases. Bhatia et al. have separated atenolol in combination with antihypertensive drugs like hydrochlorothiazide and amiloride on cyanide bonded column using acetonitrile-phosphate buffer as mobile phase [28]. In a similar way, Hermansson et al. have separated atenolol, propranolol and ibuprofen with three different columns viz. Zorbax-CN column, Hypersil Elite C_{18} column and CT-Sil C_{18} column, respectively using acetonitrile and phosphate buffer in different proportions [29]. An HPTLC method has been reported for atenolol with calcium channel blocker amlodipine [30, 31] and nitrendipine [32] using different mobile phases. Similarly, Dul’tseva et al. have reported separation of atenolol but by TLC and GLC method in which, GLC was found to be suitable for the chemical toxicology analysis [33].

Propranolol has been separated on different columns like 15 μm Shimpack CLC-ODS column [34], 5 μm Inertsil ODS 80 A column [35], 5 μm Bondapack C_{18} column [36] and column containing polystyrene seed particles with ~1 μm diameter [37] and in each case detection has been carried out by fluorescence detector. Further, propranolol has
been analysed using various methods like fluorescence optosensor [38], continuous flow method based on chemiluminescence [39], phosphorescence [40], and direct chromatographic detection of α-napthoxylactic acid in urine [41]. Panchagnula and co-workers have carried out RP-LC method with UV detection for simultaneous quantitation of indinavir and propranolol from ex-vivo rat intestinal permeability studies [42] with limit of detection 40 and 30 ng/mL, respectively. Further, propranolol and metoprolol have been detected by AAS and spectrophotometric method [43], and rapid electrochemical detection in pharmaceutical preparations using stabilized lipid film [44]. Braza et al. have developed two liquid chromatographic methods with fluorimetric detection to quantify atenolol and propranolol in human plasma [45], while Pulgarin and co-workers have used first derivative non-linear variable-angle synchronous fluorescence spectrometry for atenolol, propranolol, dipyridamole and amiloride to improve the selectivity of fluorescence measurements without loss of sensitivity [46]. El-Saharty has reported an efficient RP-HPLC method for the determination of furosemide and propranolol with linear range 0.1-200 and 5-200 µg/mL, respectively [47]. Du et al. have separated propranolol, caffeine and phenytoin sodium in Kapuben tablets on Inertsil ODS column with linear range 3-24, 20-160 and 35-280 µg/mL [48]. A size exclusion chromatographic method has been reported for propranolol using Amberlite LA₂ as anion exchangers and methyl isobutylketone and water as mobile phase and its detection has been done by NMR spectroscopy [49]. Jonczyk and Nowakowska have separated propranolol along with hydrochlorothiazide and triamterene by HPLC and spectrophotometric methods. It was concluded that the results from both the methods
results from both the methods were comparable and could be successfully applied to
assay in their pharmaceutical preparation [50]. Yamini et al. have studied solubilities of
phenazopyridine, propranolol and methimazole in supercritical carbon dioxide and results
shows good self-consistency of the data obtained using semi empirical model [51].

Alpdogan and Sungar have separated metoprolol on tL-bondapak C18 column after
forming Cu (II) - dithiocarbamate complex by precolumn derivatization of secondary
amino group of metoprolol with CS2 and CuCl2 in presence of ammonia [52]. Metoprolol
and &-hydroxy metoprolol from urine and plasma samples have been separated on
Novapak C18 column and C4/E column, respectively in RP mode in acidic pH and
fluorimetry detectors have made detection [53, 54]. Metoprolol and alprenolol have been
separated on two different columns viz. 5Am Hypercarb column and Lichrosorb column
in which results have indicated that the separation was more robust using Lichrosorb
column than the another one [55]. Metoprolol along with other &-blockers viz.
bisoprolol, carazolol, propranolol and alprenolol have been separated on Lichrocart RP
column using acetonitrile and 0.02 M sodium dihydrogen phosphate (1:3) as mobile
phase [56]. Braza and co-workers have developed two HPLC methods for the individual
determination of bisoprolol and metoprolol in human plasma with fluorimetric detection
having linear range 6.25-200 ng/mL for both [57]. Metoprolol tartarate along with
allopurinol, procaterol hydrochloride, dipyridamole, timepidium bromide and
mequitazine in pharmaceutical preparation have been determined by HPLC method and
this method was found to be rapid and was suitable for QC-analysis of commercial
samples [58].
Hydrochlorothiazide has been separated on Hypersil C_{18} column and Nucleosil C_{18} column with acetonitrile - 0.1 % acetic acid as mobile phase in different proportion having detection limit of 1 ppm and 0.2 ppb, respectively [59, 60]. Razak has carried out electrochemical study for the determination of hydrochlorothiazide in urine and tablets [61]. Hydrochlorothiazide in presence of various angiotensin converting enzyme inhibitor viz. benazepril [62], ramipril [63, 64], captopril [65], cilazapril [66], enalapril [67, 68], fosinopril [69], quinapril [70], and angiotensin receptor antagonist viz. losartan [71-73], valsartan [74], and diuretic viz amiloride [75, 76] have been separated by RP-HPLC method. Manna et al. have done simultaneous determination of benazepril hydrochloride, fosinopril sodium, ramipril and hydrochlorothiazide in pharmaceutical formulations by liquid chromatographic ion pairing method [77]. Further, Zecevic and co-workers have separated hydrochlorothiazide in combination with methyldopa and amiloride in Alatan tablets [78]. While Ferraro et al. have carried out chemometric determination of amiloride hydrochloride, atenolol, timolol maleate and hydrochlorothiazide in synthetic mixtures of pharmaceutical formulations [79]. HPLC and spectrophotometric method have been reported for the determination and separation of hydrochlorothiazide and other drugs like benazepril [80, 81], lisinopril [82], captopril [83], amiloride [84, 85], losartan [86, 87], valsartan [88, 89] and fosinopril [90]. Further, HPLC and TLC method for hydrochlorothiazide alone [91] and in presence of benazepril [92, 93] have been reported. El-Gindy et al. have reported spectrophotometric and HPTLC determination of hydrochlorothiazide in combination with lisinopril and losartan [94].
Nifedipine has been separated on different RP column viz. Lichrocart RP -18 [95], Pecosphere C_18 [96], Hypersil RP C_18 [97] and Novapak C_{18} column [98] with different mobile phase but best linear range was obtained on Hypersil C_{18} column which was 2-200 ng/mL with diazepam as internal standard. Determination of nifedipine from human plasma by solid-phase extraction have been carried out by RP-HPLC having linear range 5-400 ng/mL [99], 2-200 ng/mL [100], and 10-200 ng/mL [101] using different mobile phase with limit of quantification to be 5 ng/mL, 2 ng/mL, and 3 ng/mL, respectively. Castro et al. have developed first derivative spectrophotometric and liquid chromatographic methods for determination of nifedipine in oil/water/oil multiple microemulsions during stability studies [102]. Nifedipine in combination with rifampicin is separated on radiapak C_{18} column at pH 4 using UV detector [103]. GC, spectrophotometry and HPLC method have been used for determining nifedipine with mefruside [104] and acebutolol hydrochloride [105] using UV detector for HPLC and flame ionization detector in GC. Results further have revealed that, all these methods show good linearity, precision and reproducibility. Nifedipine in combination with other antihypertensive drugs viz. diltiazem, dimethyl diltiazem, deacetyl diltiazem, verapamil, norverapamil, nitrendipine and dehydronitrendipine have been separated on 4-\text{m} novapak C_{18} column using imipramine as internal standard [106]. Thus, the achiral methods reported in the literature for the separation of chosen drugs viz. atenolol, metoprolol, propranolol, hydrochlorothiazide and nifedipine have been discussed as above. Separation of chiral drugs viz. atenolol, metoprolol, and propranolol via chiral chromatographic methods is discussed in detail as follows.
Separation of drugs via chiral methods

The majority of the new drugs approved and most often prescribed, have at least one asymmetric centre, and 75-90% are marketed as racemates. Since, most often only one of the enantiomer is responsible for a compound’s activity and in exceptional case, both the isomers may be active and in some cases, the correct ratio of enantiomers is necessary for maximum response. Also, it is well known that stereochemistry of these compounds have dramatic effects on their properties, especially in a biological environment and due to this, the enantiomers will differ in absorption, distribution, protein binding and affinity to the receptor [107]. Thus, it is required to study the properties of individual drug enantiomers. Considering this, it is appropriate to assess the progress of chiral methods in pharmaceutical industries that have traditionally been carried out by liquid chromatography.

Methods for the resolution of chiral drugs via indirect and direct methods are described below.

The ideal way to obtain pure drug enantiomers would be enantioselective synthesis. This is, however, not always easy and usually complicated as well as expensive. Therefore, the separation of racemic mixtures of intermediate or final products is often required. The classical methods for the chiral separation that has been used for the resolution of pharmaceutical intermediates includes crystallization, kinetic resolutions, reaction resolution combinations, membrane based separations etc.
The most practically useful technique for the separation of chiral analyte includes "Separation Methods" based on indirect and direct chromatographic separation.

(A) INDIRECT METHOD

Indirect method includes derivatization of enantiomers via highly pure optically active reagent, followed by separating them as diastereomeric derivatives, which differ in their chemical and physical behaviours and therefore can be separated on achiral stationary phase. However, derivatization represents an additional step that can involve undesirable side reactions, formation of decomposed products and racemization. Furthermore, the chiral derivatization reagent has to be of high enantiomeric purity and the presence of derivatizable groups in the analyte is a prerequisite. Nevertheless, this approach circumvents the need for expensive columns with chiral stationary phase and is more flexible.

The most frequently used chiral derivatization reagents are 1-(9-fluorenyl) ethylchloroformate and o-phthal dialdehyde in combination with chiral thiols [111]. Kleidernigg et al. [112] used (O, O'-R, R)- diacylated tartaric acid anhydrides for the derivatization of 2-blockers. Same group of authors have introduced a new chiral derivatization reagent, (1R, 2R) - or (1S, 2S)-N- [(2-isothiocyanato) cyclohexyl]-3, 5 dinitrobenzoylamide (DDITC) for the derivatization of primary and secondary amines and amino alcohols [113]. 1-(6-Methoxy-2-naphthyl)-ethyl isothiocyanate (NAP-IT) and 2-(6-methoxy-2-naphthyl)-1-propylchloroformate (NAP-C) were used by Buschges et al.
workers have developed RP-HPLC method for atenolol enantiomers in rat hepatic microsome after chiral derivatization with 2,3,4,6-tetra-O-acetyl- 2-D-glycopyranosyl isothiocynate having detection limit 0.055 µg/mL [126]. While Zhou and co-workers have derivatized propranolol enantiomers in transgenic Chinese hamster CHL using 2,3,4,6-tetra-O-acetyl- 2-D-glycopyranosyl isothiocynate as derivatizing agent [127]. Kim et al. have derivatized certain β-blockers (acebutolol, arotinolol, betaxolol, bisoprolol, celiprolol, metoprolol and pindolol) with (-) menthyl chloroformate and have resolved their diastereomeric derivatives on achiral column [128]. Chiral purity test of metoprolol enantiomers have been studied by Kim and co-workers by direct method using Chiralcel OD column and by indirect method via derivatization with (-) menthyl chloroformate and then separating on Inertsil C8 column in RP-mode [129]. Direct enantioselective analysis of metoprolol in plasma was carried out using Chiralpak AD and Chiralcel OD columns while indirect analysis was done by using S-(-) menthyl chloroformate as diastereomeric derivatives [130]. Further, various other chiral derivatizing agents like S(-)-1-(1-naphthyl)ethyamine, R(+)-K-methylbenzylamine [131- 133], (S)-1-phenylethylamine [134] and amino acid based CDA like (S) - N- (4-nitrophenoxycarbonyl ) phenyl alanine methoxy ethyl ester [135], L- phenylalanine methyl ester[136] and 1-fluoro-2,4-dinitrophenyl-5-L-alanine amine [137] have been used for the separation of different group of drugs.
for the derivatization of adrenoreceptor antagonists and antiarythmic drugs [114].

Bruckner and Wachsmann have synthesized N-[4-[(S)-1-carbamoyl-2-methylpropylamino]-6-chloro-[1,3,5]triazin-2-yl] -L-phenylalanine starting from cyanuric chloride and used this reagent for the indirect enantioseparation of amino acids [115].

Toyo'oka has given an excellent overview of fluorescent chiral derivatization reagents [111]. A new chiral fluorescent tagging reagent, (1R, 2R)-N-[(2-isothiocyanato)cyclohexyl]-6-methoxy-4-quinolinylamide was prepared by Kleidernigg and Lindner and applied to amino acids and amines [116]. Al-Kindy et al. have synthesized a series of new fluorescent chiral benzoxadiazole-amino acid derivatives for amines [117]. Yasaka et al. have reported the preparation of (S)-(→)-1-methyl-2- (6,7-dimethoxy-2,3-naphthyalimido)ethyl trifluoromethansulfonate as chiral fluorescent derivatization reagent for carboxylic acids [118]. More recently, Inoune et al. [119] have introduced 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride as a chiral fluorescent labeling reagent for prolyl dipeptides. Indirect resolution of 2-blockers viz. propranolol, oxprenolol, pindolol, metoprolol and atenolol have been carried out by RP capillary electro chromatographic method using (+)-1-(9-fluorenyl)ethyl chloroformate as a derivatizing agent and then separating on octadecysilanized silica gel based stationary phase [120].

Derivatives of isocyanates including (1R, 2R)-1,3-diacetoxy-1- (4-nitrophenyl)-2-propyl isothiocyanate, R-(+)-1-phenylethylisocyanate, S-(−)-K:methylbenzyl isocyanate and S (+)-napthyl ethyl isocyanate have been used for the enantioseparation of series of 9-blockers, racemic amino alcohols and terbutaline enantiomers [121-125]. Li and his co-
(B) DIRECT METHOD

Separation of enantiomers through direct method includes two fundamentally different cases, depending upon whether enantiodifferentiation takes place through a chiral recognition effect by the stationary phase or by a chiral constituent of the mobile phase forming a diastereomeric complex in situ during the chromatographic process.

From an experimental point of view, a clear distinction can be made between the uses of chiral column (i.e., a column containing a chiral stationary phase) together with an achiral mobile phase, and the use of an achiral column together with a chiral mobile phase. In the later case, however, the actual mode of chiral separation will depend on the relative affinity of the chiral constituent for the stationary phase and the analyte, respectively. In one extreme case, the chiral constituent may become strongly adsorbed on the stationary phase, thereby converting it into a CSP, and the separation process would then be regarded as a chiral recognition by the CSP thus generated. In the opposite case, the chiral constituent has a much lower affinity for the stationary phase than for the analyte. This means that diastereomeric complexes are generated in the mobile phase and the separation takes place as normal LC separation of diastereomers. Thus, direct approach, which makes use of columns with chiral stationary phases, is more convenient and applicable for the separations on preparative scale, but requires a collection of expensive columns to solve a variety of problems. In case of mobile phase additive, though it represents a simple and flexible alternative, is not always applicable, since, the
mobile phase containing the chiral selector cannot be reused and hence the technique cannot be applied with expensive reagents.

Chiral sorbents for LC in case of chiral columns may further be classified with respect to their general structure types. Some are based on synthetic or natural polymers and are totally and intrinsically chiral. Other consists of chiral selector of low molecular weight, which is bound to a hard, incompressible matrix, usually silica. There are also sorbents consisting of polymers anchored to silica in order to give improved column performance, but the difficulty is that, each new class of enantiomeric compound would appear to require a different stationary phase.

A rough classification of various modes of chiral LC is given below, which is mainly based on the general nature of chromatographic sorbent, without regard to the type of retention process involved, which is often quite complex and difficult to evaluate in detail.

**Natural (polysaccharides and protein derivatives) and Synthetic intrinsically chiral polymeric stationary phases**

**POLYSACCHARIDE DERIVATIVES**

The linear polysaccharide cellulose represents the most common organic compound of all with chemical constitution of a linear poly -β-D-1,4-glucoside. Gubitz and co-workers have observed that the native cellulose showed only weak chiral
recognition ability [138]. Considering this, Hesse and Hagel have discovered that on acetylation of cellulose which yielded microcrystalline cellulose triacetate (CTA-I) produces a tertiary structure upon swelling and forms chiral cavities which are able to include stereoselectivity with aromatic residues and gave better resolution compared to that of natural cellulose [139]. Thus, with the advent of time, number of papers and review articles has been reported for the separation of drugs using polysaccharide phase. Most of them have been excluded and only relevant references have been quoted over here.

The chromatographic resolution of enantiomers of numerous pharmaceutical intermediates and final products with the chiral discrimination mechanism regarding polysaccharide phases have been discussed by Miller and co-workers [140] and Aboul-Enein [141]. Several acidic racemic drugs [142] and 2-blockers [143] have been separated on cellulose derivatives. Similarly, various other chiral drugs like metipranolol and desacetylmetipranolol [144], and propranolol analogues [145] have been separated via a Chiralcel OD column using different mobile phases. Ferdam and Modier have separated enantiomers and diastereomers of propranolol derivatives on cellulose tris (3,5 dimethyl phenyl carbamate) [146] while Rumiantsev and Ivanova have determined propranolol content by achiral NP-HPLC and their enantiomeric ratio by chiral HPLC using silica bonded cellulose tris(3,5 dimethyl phenyl carbamates) [147]. Santoro and co-workers have described the separation and quantitative determination of atenolol isomers using Chiralcel OD column and hexane-ethanol-diethylamine as mobile phase [148]. Singh et al. have developed a simple, rapid, precise and accurate method for separating the enantiomers of atenolol and metoprolol in tablet preparation on a Chiralcel OD
column with hexane-ethanol-diethylamine-acetic acid as mobile phase in different proportions [149]. In a similar way, Svensson et al. have separated metoprolol analogues along with several racemic amino alcohols on a Chiralcel OD column using chemometrics [150]. Direct stereoselective separation of four stereoisomers of α-hydroxymetoprolol in human plasma and urine has been carried out on Chiralpak AD column [151]; and Chiralcel OD-R and Chiralcel OD-H column [152]. Kim et al. have determined metoprolol enantiomers in human urine by coupled achiral-chiral column, quantifying metoprolol and internal standard first on silica column and then separating enantiomers on Chiralcel OD CSP [153] with detection limit 25ng/mL for each enantiomers. β-adrenergic blocking drugs viz. propranolol and atenolol were resolved by HPLC using Chiralcel OD-H and Chiralpak AD as CSP with separation factor in the range 1.34-4.55 and resolution 1.50-10.65 [154]. Yang et al. have derivatized propranolol, atenolol and metoprolol with a fluorogenic reagent 4-(N-chloroformylmethyl-N-methyl)amino-7-N,N-dimethylaminosulfonyl-2,1,3-benoxadiazole (DBD-COCl). Further, propranolol has been separated on Chiralcel OD-R and the other two (i.e. atenolol and metoprolol) on Chiralcel OJ-R column [155]. Schmid et al. have done comparative study of chiral resolution of several β-blockers on cellulose tris (3, 5-dimethyl phenyl carbamate) phase's viz. (Chiralcel OD NP-column, Chiralcel OD-RH RP column and Chiralcel OD-RH RP narrow bore column). In this condition, sixteen β-blockers were resolved under NP conditions and eleven under RP conditions [156]. Ali and Aboul-Enein have carried out chiral resolution of some clinical used drugs namely β-blockers (metoprolol, teratolol, tolamolol, nebivolol), antifungal
agent (miconazole), antihypertensive agent (cromakalin) and anti-inflammatory agent (etodolac) on cellulose tris (3,5-dichlorophenylcarbamate) CSP [157].

![Chemical structure of cellulose](image)

*Fig. 1 Cellulose, linear poly [1 → 4-β-D-glucose]*

The other widespread polysaccharide which is built from (+) D-glucose units is starch. Substitution of cellulose by amylase was found to result in different enantioselectivity [158]. Contrary to cellulose, left-handed 4/1 helical structure is postulated for amylase [159]. Zhou et al. have synthesized amylopectin -tris (phenyl carbamate) as a CSP and separated racemic biphenyl diesters of acidic drugs [160]. Greiser et al. have described direct, preparative enantioselective chromatography of propranolol hydrochloride and thioridazine hydrochloride [161]. Comparative enantioseparation of certain drugs on four different polysaccharide type CSPs viz. Chiralcel OJ, Chiralpak AD, Chiralcel OD and Cellulose tris (3,5- dichlorophenyl carbamates) have been done with polar organic mobile phases, however the study
confirms that the Cellulose tris (3,5-dichlorophenyl carbamates) has high potency for the enantioseparation over other three CSPs [162].

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\text{Fig. 2 Amylopectin, 6-branched poly [1} \rightarrow 4\alpha-D-glucose]\]

The new crystalline carbohydrates, so-called dextrins form inclusion complexes with various compounds of the correct size due to its relatively non-polar central cavity and the stability of the complex is largely dependent on the hydrophobic and steric character of the guest [163]. Cyclodextrins have been used in HPLC both in the form of chiral mobile phase additives as well as CSPs. Bressolle et al. have given an overview of the use of cyclodextrins in HPLC and CE [164]. Radulovic and co-workers have investigated inclusion complex formation between metoprolol tartrate and \(\beta\)-cyclodextrins, which further indicated the potential applications of precolumn derivatization for enantiomeric separation of \(\beta\)-blockers [165]. In a similar way, Park and co-workers have studied inclusion complexes of metoprolol and carboxymethyl \(\beta\)-
cyclodextrin. Binding studies performed using HPLC, UV-spectrophotometry, and CE has revealed that a complex with 1:1 stoichiometry was predominant [166].

Duval and co-workers have discussed the use of mono-2-pentenyl β- cyclodextrin and mono-6-pentenyl β- cyclodextrin based chiral stationary phase in HPLC and SFC for enantioseparation of aminogluthethimide and thalidomide and revealed that hetero atom present in spacer arm was essential for the chiral recognition mechanism [167]. Schurig et al. have developed a new chiral polymer column, Chirasil-Dex which found to have a unified enantioselective chromatographic approach utilizing common methods of GLC, SFC, LC (open tubular and packed ) and CEC for chiral separation [168]. Propranolol analogues have been separated on sulphated β-cyclodextrin bonded stationary phase [169]. Similarly, an approach has been made to use an analytical column packed with novel perphenylcarbamate β-cyclodextrin bonded CSP to separate propranolol enantiomers using triethylammonium acetate buffer and methanol mixture as mobile phase [170]. A chemically bonded β-cyclodextrin chiral stationary phase for HPLC has been prepared by Tazerouti et al. and using this chiral stationary phase, various racemic analytes such as aminoalcohol, adrenergic β-blockers, benzodiazepine anxiolytics like arylopropionic acid antiinflammatory agents and herbicides like aryloxy propionic acids and esters have been separated [171]. A comparative study has been carried out by Ng et al. between commercially available cyclodextrin based column (CYCLOBOND I 2000 SN) and newly synthesized novel perfunctionalized cyclodextrin CSP immobilized onto aminized silica gel by a single ureido chemical bond. Studies have revealed that ureido bonded CSP column showed unique properties especially on enantioseparation of β-
adrenergic blockers and amine containing racemic compounds that proved to be complementary to CYCLOBOND column that can separate acidic compounds more effectively [172].

Fig. 3 β-cyclodextrin
PROTEINS

Proteins are biomolecules that are directly responsible for cellular structure and functions. The ability of proteins to bind drugs stereoselectively has been utilized for the chromatographic separation of drug enantiomers. Proteins consist of chiral amino acid building blocks, which form three-dimensional structure, whereby hydrophobic, electrostatic interactions and hydrogen bonds are assumed to be the possible interactions responsible for chiral recognition. During the developments of enantiomeric separations in HPLC, a variety of protein chiral selectors like bovine serum albumin, α₁-acid glycoprotein, conalbumin and cellulose typically immobilized to silica-based carriers have been used as stationary phase, though they may also be used as mobile phase components [173]. Haginaka has given comprehensive review reports on the developments of protein based CSPs and their application [174]. Peyrin et al. have used Human Serum Albumin column to investigate measurement selectivity data using a new chiral recognition model [175]. With the same column, Haque et al. have also obtained the baseline resolution of several drug enantiomers [176]. Martinez-Pla and co-workers have developed fast enantiomeric separation of propranolol by affinity capillary electrophoresis using human serum albumin as chiral selector to control the application of propranolol in quality control of pharmaceuticals [177]. α-Adreno receptor antagonists have been separated on Human Serum Albumin and α₁-Acid Glycoprotein (AGP) using HPLC methods while, the same have also been separated by Capillary Electrophoresis using heptakis (2,6-di-o-methyl)-β-cyclodextrin (DMCO) 1-hydroxy propyl -β-
cycloextrin (HPCD) & β-Cycloextrin as chiral selector [178]. A different study has been carried out by Goetmar et al. with respect to adsorption isotherms of enantiomers of β-blockers namely metoprolol, alprenolol and propranolol on cellobiohydrolase-1 immobilized on silica gel in the concentration range between 0.25 μM and 1.7 mM at pH 5.0, 5.5 and 6.0 [145], while, Fornstedt and co-workers have separated atenolol in microdialysis and plasma samples by Chiral CBH column with Lichrospher precolumn [180].

Another human plasma protein called α₁-acid glycoprotein (AGP) or ovomucoid present in a concentration of 55-140 mg per 100 mL of plasma has been claimed as the main cationic binding protein in the humans [181]. Five basic hydrophobic drugs alprenolol, propranolol, promethazine, chlorphenaramine and disopyramide have been separated on α₁. AGP column with diamine sparteine as cationic mobile phase modifiers [182], similarly Berthault et al. have separated several β-blockers by chiral AGP column [183].

**CHIRAL SYNTHETIC POLYMERS**

Synthetic chiral polymers can be produced either by the use of chiral reagent or by a chiral catalyst. In the first case, a chiral derivatization of a suitable monomer is performed and the product is then polymerized to form a polymer network having chiral substituents. In the second case, the monomer is polymerized under the influence of a chiral catalyst, which will produce an optically active polymer, since the stereoregulatory
influence of the catalyst will yield an isotactic polymeric structure of a certain preferred helicity. Thus, CSP prepared in this manner are relatively economic and excellent for the optical resolution of racemates. Nakano has given a comprehensive review on the synthesis and application of chiral synthetic polymeric CSPs [183]. Krause et al. studied different separation modes i.e. normal phase and reverse phase nano HPLC and CEC with and without pressure assistance in fused silica capillaries packed with silica gel which was modified by covalent attachment of poly-N-acryloyl -l-phenylalanine ethylester (chiraspher®) or by coating with cellulose tris (3,5-dimethylphenylcarbamate) [185]. Two affinity adsorbents for the vancomycin group antibiotics have been prepared by immobilizing D- alanine and D, L-alanine, respectively, onto crosslinked polyacrylamide resin through Mannich reaction by Yuan et al. and they further have studied adsorption of N- dimethylvancomycin on these adsorbents [186].

Fig. 4 Synthetic polymers
Molecular imprinting technique includes construction of polymer network cavities by synthetic means to fit only one of two enantiomers. The principle rests on an imitation of an enzyme's binding site, which can usually be regarded as a chiral cavity or cleft in the protein, often highly specific with respect to binding of substrate enantiomers because of the precise steric requirements for multiple bond attachment. Since the experimental technique can be compared with making a plaster cast from an original template, it is also called "Molecular Imprinting". Hence, the molecules of a particular compound act as templates around which a rigid polymeric network is cast. Specialized review articles have been reported in the literature regarding molecular imprinted polymers [187-189]. Amino acid derivatives and peptides have been separated by antibody mimicking polymers as CSP and chiral separation has showed higher load capacity, increased selectivity with better resolving capability [190]. Similarly, Liao and co-workers have prepared polymeric molecule transporters using molecular imprinting techniques with D-tryptophan, D-phenylalanine and D-histidine as the template and further revealed that molecular "receptors" prepared using molecular imprinting techniques could potentially be used for the separation of enantiomers through serial enantioselective transports [191]. Owens and co-workers have reviewed the potential of analytical techniques based on molecular imprinting from view point of bio and pharmaceutical analysis and focussed on how these benefits have been used for the improvement in the quality of analytical procedures [192]. In this regard, Aboul-Enein and Al-Duraibi have developed direct isocratic method for enatioseparation of propranolol using newly developed chirose C1 CSP as chiral polymer [193]. Haginaka and Sakai have prepared uniformed size MIP for
(S)-propranolol by a multistep swelling and thermal polymerization method using methacrylic acid and ethylene glycol dimethacrylate as a host functional monomer and cross linker, respectively. These MIP have proved to have some specific recognition for (S)-propranolol and moderate recognition for some structurally related β-adrenergic antagonists but no recognition for other basic, acidic or neutral compounds [194]. Martin et al. have investigated three propranolol derived MIPs as selective sorbents for the solid phase extraction of β-blockers. Results have revealed that aqueous based elution solvents gave little or no selectivity for propranolol analogues compared to elution using toluene based solvents, which showed significant selective extraction [195]. Further, same group of authors have prepared propranolol derived MIP against the drug tamoxifen, which surprisingly showed considerable selectivity towards tamoxifen, and was indeed much more selective than the MIP prepared using tamoxifen as the imprint molecule [196]. In addition, Suedee et al. have prepared several MIPs using the enantiomers of either β-blockers drugs R-(+)-propranolol, R-(+) or S-(-) atenolol, or the NSAID, S-(+)-naproxen and S-(+)-ibuprofen as print molecules. They have coated these polymers on glass supports and have resolved racemates of propranolol and ketoprofen [197]. Thus, chiral phases based on MIPs have high antibody like selectivity, however, suffers from relatively low efficiency and the restricted range of applicability, since one special phase shows chiral recognition only for the same molecule used as template or very closely related compounds.
**Bonded Synthetic Chiral Selectors**

The CSPs described under bonded synthetic chiral selectors are all characterized by their well-defined molecular structures bonded to some solid support, usually silica.

**CROWN ETHERS**

Crown ethers are macrocyclic polyethers, which are known to form host-guest complexes with alkali- and alkaline earth-metal ions as well as primary ammonium cations. Cram et al. have been the first to synthesize optically active crown ethers for optical resolution purposes [198] and this principle was then transferred to an LC separation technique by the use of the chiral crown ether in the mobile phase or covalently bound to a silica support [199]. They prepared crown ether phases based on polystyrene or silica for classical LC and demonstrated the applicability of these phases to the chiral separations. Moreover, substituents of the crown ether are perpendicular to the plane of the macrocyclic ring, forming a chiral barrier, which divides the space available for the substituents at the chiral centre of the analyte into two domains. Thus, two different diastereomeric inclusion complexes are formed.

Several selected amino acid enantiomers have been separated using Crownpak R- (+) column and enantiomeric impurities as low as 0.001% (10ppm) was determined by this method [200]. Machida et al. have used (+)18-Crown-6-tetracarboxylic acid on 3-
amino propyl silanized silica gel to separate thirteen DL-amino acids out of eighteen and seven racemic amino alcohols [201]. A CSP derived from (+) - (18-crown-6)-2,3,11-12-tetra carboxylic acid has been used for direct separation of an antibacterial agent, fluoroquinolines, however the resolution depends not only on the contents, but also on the type of acidic and organic modifiers in mobile phase and on column temperature [202]. Nishiaok and co-workers have developed novel CSPs covalently bonded with chiral pseudo crown ether containing phenyl groups as chiral barrier that has high ability of discriminating enantiomers in both RP and NP mode for the separation of wide range of chiral amines, amino alcohols and amino acids especially for the hydrophobic amines [203].

![Chiral crown ether](image)

*Fig. 5 Chiral crown ether*
**METAL COMPLEXES (CHIRAL LIGAND EXCHANGE)**

Chiral ligand exchange chromatography has been extensively studied by Davankov and co-workers, the pioneers, in resolving α-amino acids [204, 205]. Here, the chiral recognition on chiral stationary phases is based on the formation of ternary mixed metal complexes between the selector and the analyte ligand.

<table>
<thead>
<tr>
<th>Mobile Phase (m)</th>
<th>A&lt;sub&gt;m&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary Phase (s)</td>
<td>A&lt;sub&gt;s&lt;/sub&gt; + MS&lt;sub&gt;s&lt;/sub&gt; ↔ AMS&lt;sub&gt;s&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

A : Analyte  
M : Metal  
S : Selector

The ability of transition metals to participate in complex formation has been exploited earlier for the purpose of enantiomeric separation. Since, diastereomeric complexes are formed from amino acid ligands of opposite configuration, any difference in stability of these complexes will of course result in different chromatographic mobilities of the amino acid enantiomers. The original phases prepared by Davankov for classical column chromatography were based on polystyrene-divinylbenzene polymers containing amino acid residues complexed with metal ions. These phases showed remarkable enantioselectivity for amino acids.
Kurganov has extensively reported chiral chromatographic separations based on ligand exchange chromatography [206]. Wachsmann and Bruckner have reported the synthesis of a new chiral ligand exchange chromatography phase by binding L-proline or L-lysine to aminopropylsilanized silica through a triazine spacer. While the first phase was found to be suitable to resolve underivatized amino acids and N-(2,4-dinitrophenyl) amino acids, the second phase resolved dansylamino acids [207]. Wan et al. have synthesized chiral selectors derived from L-proline and L-phenylalanine by alkylation and arylation. The selectors containing C7, C9, C12-chains or methoxybenzyl, naphthylmethyl or anthrylmethyl groups were adsorbed onto the surface of porous graphitic carbon. Using this, authors have resolved thirty-six racemic amino acids [208]. Ma et al. have separated amino acid enantiomers using polyvinyl alcohol with L-proline pendant as CSP in ligand exchange chromatography [209].

CSP BASED ON CHARGE TRANSFER COMPLEXATION

The chiral selectors have been characterized by the operation of an aromatic π-π bonding interaction as an essential element of the retention process. This technique that includes interactions between so called π-acceptor and π-donor molecules has been first introduced by Pirkle. A selector of this type is designed to have a cleft, consisting of π-acidic and π-basic aromatic parts directed almost perpendicular to each other, to which one enantiomer is preferentially bound. The enantioselectivity binding has been shown by NMR studies to be due to a simultaneous face-to-face and face-to-edge π-π interaction.
These phases (commercially available as Whelk-O™, Regis Co.) typically operate under normal phase conditions with hexane containing a retention modifier like propan-2-ol. Chilmonczyk et al. have enantioseparated several clinically used racemic drugs on N- (3,5-dinitrobenzoyl) -3-amino-4-phenylazetidin-2-one bound to silica known as pirkle-1J CSP and they have explained chiral recognition mechanism involved in it by using molecular modeling semi-empirical AM1 method [211].

Vinkovic and co-workers have investigated mechanism of chiral recognition in the enantioseparation of 2-aryloxypropionic acids on new brush type CSPs comprising N-3, 5, 6-trichloro-2, 4-dicyanophenyl -L-α-amino acids bound to γ-aminopropyl silica [212], while Lin et al. have used CSP based on α-amino acid and pyrrolidine disubstituted cyanuric chloride for the separation of amino acids [213]. Petersen et al. have investigated eighteen β-blockers using Pirkle type α-Burke CSP, of which fifteen have been successfully separated by NP-HPLC within an acceptable analysis time [214]. Eaginger et al. have separated (R)- and (S)- propranolol in human plasma have been separated on (R, R) dinitrobenzoyl diamino cyclohexane CSP using dichloromethane-methanol as mobile phase [215]. Cleveland has studied separation of chiral drugs of certain classes (like antihypertensive, antiarrhythmics, antianginals, diuretics, adrenergic drugs, anti inflammatory and analgesic compounds, topical anesthetics, antihistamines and antimalarial) on Pirkle concept CSP [216]. Machida et al. have synthesized a tartaric diamide phase bearing a p-chlorophenyl residue and applied it to the resolution of 1, 2-diols, 2, 2'-dihydroxy-1, 1'-binaphthyl and some β-blockers [217].
Enantiomers of N-aryloxazolinones, N-arylthiazolinones & their thia analogs [218]; as well as aryl propionic acid, non steriodal antiinflammatory drug, aryl epoxides, sulfoxides, alcohols, amides and esters [219] on Whelk-01 column have been studied and the results revealed that the separation has been achieved in spite of an element of uncertainty due to the presence of 2π-basic interaction sites. A number of racemic thiazide diuretics and their analogues have been resolved on two diastereomeric chiral stationary phases prepared from (S)- or (R)- α [1-(6,7- dimethyl) -napthyl] - 10-dodecenyl-amine and (S)-2- phenyl propanoic acid by Hyun and Pirkle and studies showed former CSP to be better than latter one [220].

![Selector based on π-π interaction](image-url)

*Fig. 6 Selector based on π-π interaction*
OTHER TYPES OF SELECTION

Though the bonded selectors are reasonably well understood in terms of their chiral recognition behaviour, a number of other selectors have been developed which probably act by more complicated and less well-understood mechanisms.

CSP BASED ON UREA AND AMIDE DERIVATIVES

Very promising CSPs based on amide or urea derivatives have recently been prepared and studied. The amides have only one H-bonding acceptor/donor group, and can form only a single bond with a neighboring selector-molecule. The free rotation along the single bond, and the alternative positions on the L-peptide molecule, where such a bond can be formed, prevented establishing a predominant selector/selectand and associate that would lead to chiral selectivity. To enhance chiral selectivity of a complex formed through a single bond, the bonding site of the selector was placed between two asymmetric centers, as can be seen in the structure of the "L-urea" phase. The C₂-symmetry around the bonding site should maximize the number of the different associates and maximize the contribution of the complex(es) where the asymmetric carbons of the selector and selectand are brought to close proximity.

Norephedrine enantiomers have been separated on urea derivative as chiral stationary phase using hexane, dichloroethane and isopropanol, and studies have been done by
altering the organic modifier by substituting ethanol, acetonitrile and tetrahydrofuran for isopropanol to decrease the retention time [221]. Similarly, Zou and Yun have separated racemic propranolol using amide derived CSP using hexane-1, 2-dichloroethane-ethanol as mobile phase system in composition (71-26-3) [191] while, Zhang et al. have directly separated atenolol on amide derived CSP via NP-HPLC using n-hexane - 1,2-dichloroethane - methanol as mobile phase. Further, chiral resolution of atenolol with propranolol and celiprolol has also been discussed [222]. Zou and Yun have resolved celiprolol enantiomers by using NP-mode with urea derivative as CSP [223]. In contrast, formoterol, labetalol, nadolol, indenolol and U-54494A have been resolved by urea type CSP column of (S)-indoline-2-carboxylic acid and R-(1) (α-napthyl) ethyl amine by Aboul-Enein and Al-Duraibi [225].

\[
\begin{align*}
R' & \quad C \quad O \\
R & \quad \quad C \quad N \quad H \\
R' & \quad \quad N \quad C \quad R
\end{align*}
\]

\[
R = -\text{CH}(\text{CH}_3)_2 \\
R = -\text{CO}_2\text{CH}(\text{CH}_3)_2
\]

*Fig. 7 Derivatized urea phase*
MACROCYCLIC ANTIBIOTICS

One of the latest and perhaps the most advanced classes of chiral selectors includes the macrocyclic antibiotics, which may either be immobilized or covalently bonded to a solid support and also be used as a chiral additive. These chiral selectors are of a relatively complex structure and the mechanism behind their chiral discrimination ability is virtually unknown, although it has been suggested that they act by a combination of hydrogen bonding, π-π complexation, dipole stacking, inclusion and steric interactions [226]. Vancomycin contains 18 stereogenic centres, whereas the others used, rifamycin B, thiostrepton and teicoplanin, contain 9, 17 and 23, respectively. These selectors are immobilized via a two to three carbon spacer to the silica surface and columns packed with these sorbents are now commercially available (such as Chirobiotic™, Astec Corp.) and have been used for the resolution of large number of different racemates. Vancomycin, teicoplanin and rifamycin are the major antibiotics that have been used in both HPLC and CE to separate wide variety of enantiomers [227]. Rojkovocova et al. have summarized properties of the macrocyclic antibiotics viz. vancomycin, teicoplanin and ristocetin A which have been used as stationary phases in HPLC for the separation of various enantiomeric drugs and their derivatives [228]. Lamprecht et al. carried out an enantioselective analysis of (R)- and (S)-atenolol in urine samples by HPLC column switching setup using Lichrospher ADS restricted access column and Chirobiotic T column and effluent were detected by fluorescence detector [229]. Mistry and co-workers have developed sensitive and stereoselective HPLC assay
for the determination of enantiomers of metoprolol and diastereoisomers of α-hydroxymetoprolol using chirobiotic T bonded phase column with linear range 0.5-100 ng/mL and 1-100 ng/mL, respectively [230]. Mislanova et al. have reported direct HPLC determination of (R)- and (S)- propranolol in rat microdialysate using on-line two column switching procedures viz. RP-18 ADS precolumn coupled with ovomucoid analytical column and RP-8 ADS precolumn with a teicoplanin analytical column giving limit of detection 10 ng/mL and 15 ng/mL for (R)- and (S)- propranolol, respectively [231]. A number of compounds including amino acid and their related compounds, compounds with a ring containing stereogenic centre, compounds bearing aromatic structures near their stereogenic centres and alcohols have been tested for enantioseparation on these two CSPs [232].

Vancomycin

Rifamycin

*Fig. 8 Antibiotics*
Technique based on addition of chiral constituents to mobile phase

Modern RP-HPLC sorbents are excellent materials for the achievement of high column efficiency. However, the principle of using mobile phase additives in RP-LC in order to regulate the retention behaviour of an analyte is widely used today. By the use of an optically active counter-ion, diastereomeric pairs have been separated on an achiral ordinary RP-column. Many of the principles had been already described, which are based on covalently bound chiral phases, which can also be applied to the technique of adding the chiral selector to the mobile phase.

Cyclodextrins and their derivatives are currently the most powerful chiral selectors, which are applicable to wide range of organic compounds. The enantiomeric separation of metoprolol and its metabolites in human urine was carried out by Capillary Electrophoresis using carboxymethyl-β-cyclodextrin as chiral selector [233]. Shuang and Choi have described retention behaviour of procaine hydrochloride on an Alltima octadecyl silica C\textsubscript{18} column with a mobile phase containing negatively charged carboxymethyl β-cyclodextrin as a chiral additive by RP-HPLC and the results show that proposed method can be successfully applied to real sample analysis [234]. Mc Murtrey \textit{et al.} have resolved enantiomers of dihydroxyphenylalanine and selected salsolinol derivatives using sulfated β-cyclodextrin as chiral selector with conventional RP-ODS column, however sulfated β-cyclodextrin gave very effective resolution of salsolinol derivatives with little less effective resolution for dihydroxyphenylalanine [235]. Li and co-workers have performed enantiomeric resolution of epenephrine, isoproterenol and
ephedrine by RP-mode using β-cyclodextrin, DM-β-cyclodextrin and TM-β-cyclodextrin as mobile phase additive and results showed that chromatographic systems with a dynamically generated stationary phase with methylated β-cyclodextrin proved to be a versatile tool for enantiomeric separation [236].

Dolezalova and Tkaczykova have reviewed direct HPLC and CE separation for enantiomers of interest by using teicoplanin and sulfobutylether-β-cyclodextrin as chiral selectors. They have also described ligand exchange liquid chromatography with N,N-dimethyl-L-phenylalanine[237]. In a similar way, aryloxyphenoxypropanoic acid and an antitumor agent have been separated by using teicoplanin as the chiral selector in HPLC and hydroxypropyl cyclodextrins in CE method with the result showing that CE assay is much less precise and accurate than HPLC [238].

Gotmar et al. have studied the influence of the solute hydrophobicity on the enantioselective adsorption of β-blockers on cellulose protein, which has been used as chiral selector, and results have showed the hydrophobicity of propranolol is greater than alprenolol and metoprolol [239]. Fornstedt and co-workers have studied the complex dependence of the selectivity factor on pH of mobile phase for propranolol enantiomers in which cellulose protein was used as chiral selector [240]. β-lactoglobulin has been used as CSP in HPLC while as chiral additive in CE in separation of several chiral acidic, basic and uncharged drugs [241]. Teicoplanin, a macrocyclic antibiotic has also been used as a CSP and as chiral selector in HPLC for amino acid, peptides. α-OH carboxylic acid and neutral analytes including cyclic amides and amines [242].

41
An alternative technique in ligand exchange chromatography is the use of chiral metal complexes as additives to the mobile phase in combination with achiral stationary phases. In this case, a monodendate (MS) or bidendate selector metal complex (SMS) is present in the mobile phase and forms a mixed selector-analyte complex (AMS). Partition between the mobile \((m)\) and the stationary phase \((s)\) takes place according to the following equilibria:

\[
\begin{align*}
\text{Mobile Phase} & : A_m + MS_m \rightleftharpoons AMS_m \\
\text{Stationary Phase} & : A_s + MS_s \rightleftharpoons AMS_s
\end{align*}
\]

A : Analyte  
M : Metal  
S : Selector

Dolezalova and Tlaczykova have determined D-DOPA in levodopa by chiral ligand exchange chromatography with an ordinary \(C_{18}\) column and a chiral mobile phase containing N, N-dimethyl L-phenyl alanine and Cu (II) acetate by HPLC on a teicoplanin column. However, HPLC methods have been proved much more sensitive than polarimetric methods [243]. Using the same approach, Bazylak and Aboul-Enein have
separated stereoisomers of labetalol [244], carbuterol [245] and clenbuterol enantiomers [246] by RP-HPLC on conventional Octadecyl silica packed column and double helical chelate (-) (M) (λ, Δ) - 4, 4' - (S) -methyl - (2R) - (propylethane - diyldiimino) bis (pent -3 - en-2-onato) nickel (II) as chiral mobile phase additive. In addition, Jiang et al. have determined R (+) isomer and its related substances in levofloxacin HCl on C\textsubscript{18} column with 0.008 M L-phenylalanine and methanol as a mobile phase [247].
AIM AND SCOPE

The review of the literature work reveals that impurity assays in pharmaceutical industries by liquid chromatography are developed and validated to support the manufacturing process, for stability study of the formulations and for release assays of each lot of bulk drugs. Since, impurity levels are often very low in most pharmaceutical bulk products, the choice of the method is very important. The method must be accurate, sensitive, selective, reproducible and convenient. The development of such reliable analytical methods enables quantitative assessments required to ensure that the product, which reaches to the public, is fit for its purpose or not. For this, liquid chromatography is the premier method for stability and impurity assays of pharmaceuticals, which allows traces of impurities to be separated and detected.

Apart from this, in recent years, the impact of chirality in the design, development and utilization of drugs has gained widespread recognition. As a result, there has been a dramatically increasing demand for chiral separations. When an analyst faces the task to develop a separation method for a given problem, it is important that there exists sufficient background knowledge on the potentials of the techniques available in relation to the analyte(s) of interest, so that a rational choice can be made towards the best possible solution. For the separation of non-chiral analytes, extensive theoretical knowledge is available. Yet, despite various efforts, when it comes to the resolution of chiral analytes, knowledge about the basic principles is still fragmentary and most of the phase systems available are non-predictable with regard to their enantioselectivity and
retention properties for a given chiral problem. Keeping this in view, there emerged a need to synthesize a sorbent that can separate an active ingredient from the given drug. The present work involves the synthesis of polymeric sorbent and using that resolution of antihypertensive drugs has been carried out. Further, a SFC method has also been described for bioequivalence study of atenolol in human plasma.
PRESENT INVESTIGATION

The present work describes the synthesis of amide based stationary phases, their characterization and applications.

A novel method for preparing stationary phase based on amberlite XAD-4 resin is presented. Functionalized polymers were obtained by reactions which involves Friedel-Crafts acetylation, oxidation and chloroformoylation of the resin. CSP viz. \( m(+)[\alpha\text{-methyl benzyl carboxamide}] \) XAD-4 was synthesized by reacting \( R(+)\text{-1-phenylethylamine} \) with chloroformoyl XAD-4 under weakly alkaline conditions. An amino acid based stationary phase was synthesized by reacting methyl ester L- histidine dihydrochloride with chloroformoyl XAD-4 to give \( m- [2\text{- carbamoyl-3-(4- imidazolyl)} \text{methyl propanoate}] \) XAD-4. These synthesized compounds were characterized by mp, elemental analysis, FT-IR spectra and SEM analysis.

The newly synthesized \( m(+)[\alpha\text{-methyl benzyl carboxamide}] \) XAD-4 chiral resin was used for the resolution of \( \beta\)-blockers viz. atenolol, metoprolol and propranolol by column chromatography. Physico-chemical properties of the resin viz. moisture content, swelling, density, void volume were determined for the synthesized resin. Hydrogen bonding and \( \pi - \pi \) interactions are supposed to be the major analyte – chiral stationary phase interactions. The resin was highly selective for the separation of the selected \( \beta\)-blockers with high resolution.
Separation and estimation of antihypertensive drugs viz. propranolol, hydrochlorothiazide, atenolol and nifedipine has been carried out on \( m -[2\text{-carbamoyl-3-(4-imidazolyl)} \text{methyl} \text{propanoate}] \) XAD-4 stationary phase. Antihypertensive drugs were absorbed in methanol media and separated by eluting them with the mixture of acetonitrile : sodium acetate – acetic acid buffer (3:7, v/v) (pH = 4.1) solution. The proposed method was further applied to the analysis of tablets containing these drugs.

A new and novel separation technique is developed for atenolol using Supercritical Fluid Chromatography, which is cost effective, viable and speedy technique with usage of less toxic solvents and hence, is an ecofriendly method. The method was applied to determine atenolol in blood plasma for its bioequivalence study. Thus, the developed method is accurate, sensitive, selective, reproducible and convenient.
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