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CHIRAL AGP FOR THE RESOLUTION OF ENANTIOMERS - AN OVERVIEW

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ABSTRACT

Chiral seperation also called chiral resolution,is a procedure used to seperate the two isomers of a racemic compound. Many Chiral stationary phases(CSP'S) have been manufactured for the seperation of enantiomers.Among them Chiral-AGP is the protein based chiral column . α_1-acid glycoprotein (AGP) is a very stable protein, which tolerates pure organic solvents,high temperatures and high and low pH. The CHIRAL-AGP is a reversed phase column giving many possibilities to affect both the retention and the enantioselectivity. The solutes are retained by three types of forces Ionic binding (charged solutes), Hydrophobic interaction and Hydrogen bonding. Based on the product type, particle size, internal diameter and column length many columns have been manufactured by Chiral Technologies. Chiral AGP has a broad range of applicability and it has been used for the separation of many basic drugs, environmental pollutents,1,4 benzodiazepines. It is also suitable with MS detection of many drugs.

Keywords: Chiral stationary phases, α_1-acid glycoprotein (AGP) and enantioselectivity.

INTRODUCTION

Chilarity is now an integral part of drug research and development and the regulatory process. The Food and Drug Administration (FDA, U.S.A.), and regulatory authorities in Europe, China, and Japan have provided guidelines indicating that preferably only the active enantiomer of a chiral drug should be brought to market. Although they have the same chemical structure, most isomers of chiral drugs exhibit marked differences in biological activities such a pharmacology, toxicology, pharmacokinetics, metabolism etc. In the last 20 years technical innovations have made the synthesis of single enantiomers more feasible, the regulatory authorities have gradually made it a requirement that when drugs at development stage made up of racemic mixture, the producer must access the efficacy of single enantiomers and choose giving reasons which of the two to market. So, in the future they will have to be a gradual reduction in the marketing of new drugs made up of racemic mixtures.

In general many methods have been developed for the resolution of chiral drugs by using analytical instruments like high performance liquid chromatography(HPLC), gas chromatography(GC), supercritical fluid chromatography(SFC),Capillaryelectrophoresis(CE), LC/MS, Gel electrophoresis etc. Chiral molecules can be resolved directly by Chiral stationary phases(CSP'S) or indirectly by using chiral dervetizing reagents represented in Figure 1. Among all the developed methods, direct method of resolution by using Chiral stationary phases(CSP'S) was the best one. Many Chiral stationary phases were been developed and there in the market. But among all of them, protein based Chiral stationary phases (CSP'S) were gained lot of commercial importance because of their stability in variety of organic modifiers, direct reversed phase resolution of Chiral molecules, availability in both analytical and semi-preparative sizes.

The first choice when developing methods on proteins-CSP'S is CHIRAL-AGP (α_1-acid glyco protein). AGP (α_1-acid glycoprotein) also termed as orosomucoid (ORM) is a very stable, which tolerates pure organic solvents, high temperature and high and low pH.

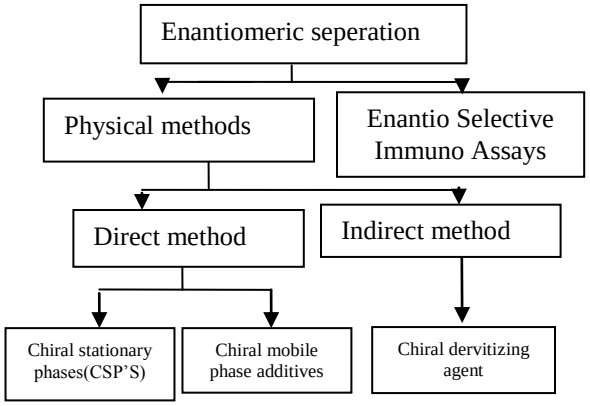

Figure 1: Methods for resolution of enantiomers.

AGP is the chiral selector in the CHIRAL-AGP column. CHIRAL-AGP is the second generation chiral separation column based on the use of α1-acid glycoprotein (AGP) as the chiral selector. Through a patented process α_1-AGP has been immobilized on porous, spherical silica particles (5µm). The surface chemistry of the silica proves a stable chiral separation material with extremely broad applicability. Racemic amines, acids and nonprotolytic compounds can be resolved directly, without derivatization. The column enables resolution of a very large number of chiral compounds from different compound classes. This is due to the unique nature of the chiral stationary phase, and the fact that enantioselectivity can be induced by choosing a proper mobile phase composition. Phosphate buffers with addition of organic modifiers are used as mobile phase. (1- and 2-propanol and acetonitrile are the most frequently used modifiers). Enantioselectivity and retention can be regulated by changing the mobile phase composition; i.e, the pH, the concentration or the nature of the organic modifier[1]. The column temperature also affects these parameters. according to USP nomenclature of columns, the CHIRAL-AGP is named as L-4.

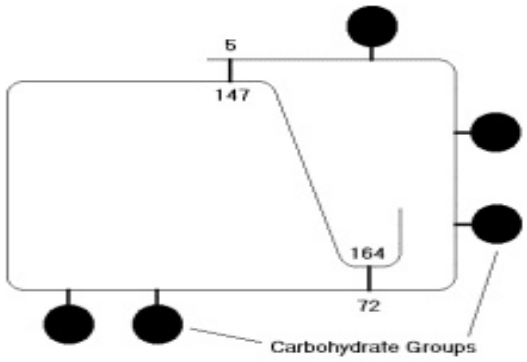

Figure.2: Structure of α1-acid glycoprotein (AGP)

PRINCIPAL OF SEPARATION USING CHIRAL-AGP

The CHIRAL-AGP is a reversed phase column giving many possibilities to affect both the retention and the enantioselectivity. Schematic drawing of α_1-acid glycoprotein (AGP) represented in Figure -2.The unique property of AGP column is, the chiral bonding properties of the stationary phase can be changed dynamically. Enantioselectivity can be induced and improved by simple changes of the mobile phase composition. The solutes are retained by three types of forces.

a. Ionic binding(charged solutes)
b. Hydrophobic interaction
c. Hydrogen bonding

The relative contribution of the different forces to the retention of the solutes, depend of the nature of the analyte. Analytes containing charged groups, hydrogen bonding groups and hydrophobic parts can be retained by interaction with corresponding groups on the chiral selector. The separation can be affected by pH, buffer concentration, type of buffer, organic modifier concentration, type of organic modifier.

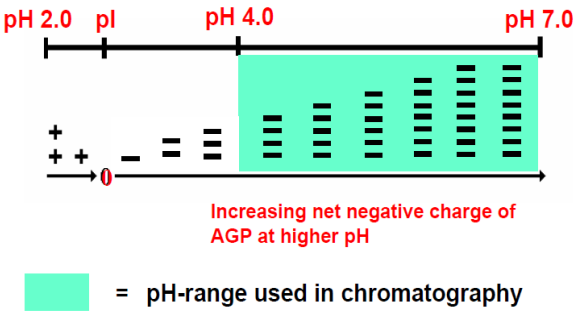

Figure 3: Net charge of AGP at different pH.

The most important tool in the method development is the mobile phase pH, which affects the ionization of both solutes and the protein stationary phase.AGP has a low isoelectric point (pI) of 2.7. The characteristics of AGP were represented in Table -1. This means at pH 2.7 the column has a net zero charge. From pH 2.7 to 7, the net negative charge on the AGP molecule increases, providing increased retention of positively –charged analytes, like amines. The net charge of AGP at different pH is represented in Figure-3.

TYPES OF CHIRAL-AGP COLUMNS

Based on the product type, particle size, internal diameter and column length many columns have been manufactured and they are listed in Table -2[2]. The

protein stationary phases (PSPs) were originally developed and manufactured by ChromTech Ltd, U.K. Chiral Technologies Europe acquired ChromTech in 2009 and they were one of the manufacturer of these widely-recognized PSPs.

APPLICATION OF CHIRAL–AGP

At pharmaceutical companies, hospitals, universities and chemical industry CHIRAL-AGP is used for the analysis of enantiomeric purity and for bioanalysis. A growing application area, due to the exceptional applicability of the phase, is isolation of pure enantiomers on semi preparative columns. Many works have been reported on Chiral-AGP and all those are discussed here in detail.

A sensitive, enantioselective, coupled column high performance liquid chromatographic assay has been done for determination of amlodipine enantiomers in human plasma[3]. Chiral chromatography is performed on an alpha 1-acid glycoprotein column (i.e. Chiral-AGP) and the eluted enantiomers are trapped and compressed on two short columns before final achiral chromatography on a narrow bore column (i.e. Zorbax SB-Ph) using electrochemical detection.

Felodipine enantiomers have been resolved on Chiral-AGP column by retention model using micellar mobile phases[2]. The model assumes the presence of two stereoselective sites and each enantiomer was found to interact with different sites. Addition of a chiral aliphatic alcohol, (+)-(S)-2-octanol, preferentially interacted with the binding site for (−)-(S)-felodipine. The retention of the solutes was effectively controlled by adding small quantities ($<1.63 \times 10^{-3} M$) of the nonionic detergent Tween 20 to the mobile phase. The separation factor ($\alpha = 1.74$) was unaffected by the detergent concentration in the presence of 1.0 m *Mn*-octylamine.

The resolution of twenty-five 3-chiral and 5-chiral 1,4-benzodiazepines and related compounds was studied on a Chiral-AGP column[5]. The majority of the benzodiazepines were separated with high separation factors and high resolution. The enantioselectivity was influenced by the nature and the concentration of the organic modifier in the mobile phase, as well as by the pH. Relationship between the structure and enantioselective retention is hydrophobic and hydrogen-bonding interactions.

A method has been developed on four different *N*-substituted amino acid derivatives inorder to find out the effect of Tertiary Alcohol Additives on Enantioselectivity of the Chiral-AGP Column[6]. The group of solvents typically used as mobile phase additives includes methanol, ethanol, 1-propanol, 2-propanol, and acetonitrile. The mobile phase consisted of a pH 7 phosphate buffer with the addition of organic solvent to control retention.
A method has been developed for chiral resolution of four hydrophobic amines i.e alprenolol, oxprenolol, trimipramine and propronolol on Chiral-AGP by using a three factor central composite face design (CCF)[7]. The variables (factors) pH and the concentration of the micellar agent Tween 20 and heptanoic acid.

The retentions and enantiomeric resolutions of remoxipride, propranolol, and trimipramine were studied using a CHIRAL-AGP column with micellar mobile phases and aliphatic, anionic additives[8]. From the study it was found that , presence of the aliphatic acid was essential in order to increase the enantiomeric selectivity.

Chiral-AGP was used for the separation of basic compounds[9]. It has been used for the separation of many basic drugs like verapamil, epibatidine, leukotriene D_4 antagonist ,vamicamide, barbiturates, idrapil, thalidomide, methadone. Few environmental pollutants like 2-(2,4-Dichloro phenoxy) propionic acid, Aryloxypropionate have been separated using Chiral-AGP[7].

The second generation H_1 histamine receptor agonist,cetrizine was studied in rat plasma using Chiral-AGP column[10].the enantiomers were detected at a wavelength 230nm and covered the concentration range of 2.5-200 µg/ml in plasma. The method did not detected any pharmacokinetic difference between the enantiomers.Chiral AGP also suitable for LC/MS resolution of enantiomers[11]. The drugs resolved were listed in the table 3. The enantiomeric resolution of almost 157 acidic,basic and neutral drugs have been done using Chiral-AGP[12].

CONCLUSION

CHIRAL-AGP is the second generation chiral separation column based on the use of α1-acid glycol protein (AGP) as a Chiral selector. It has a broadest applicability of all the chiral columns available. It separated amines, acids and non-proteolytes. Chiral AGP retains the solutes by ionic bonding ,hydrophobic bonding and hydrogen bonding. In Chiral AGP the enantioselectivity and the retention can be regulated by pH of the buffer, nature and concentration of the buffer and also nature and concentration of the organic/inorganic modifier.

Chiral AGP is also compatable for LC/MS methods in the resolution of enantiomers.

Hence Chiral AGP is considered as one of the most stable, and first choice among all the protein based stationary phases.

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Table -1 : Characteristics of α1-acid glycoprotein (AGP).

Peptide chain	183 aa
Carbohydrate content	4%
Molecular weight	40000
Isoelectric point (pI)	2.7

Table 2: Different dimensions of CHIRALPAK®AGP manufactured by Diacel

Column length(mm)	Internal diameter(mm)	Product type	Particle size (µm)
50	4	Analytical	5
50	3	Analytical	5
50	2	Analytical	5
100	4	Analytical	5
100	3	Analytical	5
100	2	Analytical	5
100	10	Semi-preparative	5
150	4	Analytical	5
150	3	Analytical	5
150	2	Analytical	5
150	10	Semi-preparative	5

Table 3: Different drugs resolved on LC/MS by using Chiral AGP

Drug name	Mobile phase	Retention time of enantiomers
Desmethylsibutramine	5% acetonitrile in 10Mm ammonium acetate buffer Ph 4.1	1.05,1.66
Etodolac	15% acetonitrile in 10Mm ammonium acetate buffer	0.78,1.66
Pindolol	7% acetonitrile in 10Mm ammonium acetate buffer	5.21,6.53
Cyamemazine	4% acetonitrile in 10Mm ammonium acetate buffer Ph 4.0	4.57,7.46
proglumide	9% acetonitrile in 10Mm ammonium acetate buffer	0.88,1.77

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