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Ph.D. Dissertation

Enantiodiscrimination Studies on Chiral Amines and Acids Using Polysaccharide-derived Chiral Stationary Phases

Graduate School of Chosun University

College of Pharmacy

Suraj Adhikari

Enantiodiscrimination Studies on Chiral Amines and Acids Using Polysaccharide-derived Chiral Stationary Phases

다당류로부터 유도된 키랄 고정상을 이용한 키랄아민과 카르복실산의 광학이성질체의 구별연구

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Graduate School of Chosun University

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Suraj Adhikari

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Advisor: Prof. Wonjae Lee, Ph.D.

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College of Pharmacy

Suraj Adhikari

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Chairman : Prof. Hong, Joon Hee (Chosun University)

Member : Prof. Hwang, Seung Rim (Chosun University)

Member : Prof. Kang, Jong Seong (Chungnam Natl. University)

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Abstract (Korean)

다당류로부터 유도된 키랄 고정상을 이용한 키랄아민과 카르복실산의 광학이성질체의 구별연구

수라즈 아드히카리

지도교수: 이원재.

조선대학교 대학원 약학과

키랄성 물질의 광학분리는 제약산업의 주요 관심 분야이다. 우리 생체 시스템은 고도의 광학이성질체로 이루어져 있기에, 키랄 의약품의 두 거울상 이성질체는 이들 수용체나 효소에 따라 입체화학적으로 다르게 반응하기 때문에 서로 다른 생리학적 특징을 나타낼 수 있다. 따라서, 하나의 거울상 이성질체는 생물학적 활성 및 질병 치료효과가 있는 반면, 또 다른 형태의 거울상 이성질체는 질병 치료효과는 없으면서 부작용을 야기시킬 수 있다. 그리하여, 키랄 제약산업에서 광학활성물질 분리분석법과 함께, 광학적으로 순수한 광학이성질체를 제조하기 위한 요구가 증가하고 있다. 따라서, 하나의 형태의 순수한 광학이성질체 화합물을 분리분석 또는 제조하기 위해서는 효율적이고 검증된 키랄분석방법의 지속적인 개발이 요구된다. 다양한 키랄 고정상을 사용하는 키랄 고성능 액체 크로마토그래피는 키랄 약물의 거울상 이성질체의 분석에 가장 효과적이고 널리 사용되는 방법 중 하나이다. 키랄 아민과 키랄 카르복실 산은 키랄 의약품 및 키랄 약물 후보물질의 핵심 중간체로 알려져 있기에 키랄 의

약품 합성과정에서 매우 중요하다. 본 논문들의 주요 내용은 키랄 아민 및 카르복실산의 거울상 이성질체 분리뿐 만 아니라 이들의 광학순도 측정 및 다당류 유래 키랄 고정상을 사용하는, 편리하고, 효과적인 순상 키랄 액체 크로마토그래피 분석법 개발에 대한 연구내용이다. 본 연구에 사용된 키랄 아민과 키랄 카르복실 산은 낮은 자외선 흡수를 나타내기 때문에, 여러 잠재적인 방향족 유도체 시약으로 유도체화를 수행하였는데 이들의 방향족 구조를 통해 높은 검출 감도가 가능하면서도 효과적인 광학분리를 위한 상호작용 부위를 제공하고자 하였다. 보다 심층적인 연구를 위해, 유도체화된 분석물질과 키랄 고정상 사이의 키랄 인식 메커니즘으로 안정화된 에너지와 용출 순서를 확인하기 위해 컴퓨터-보조 분자 모델링 연구를 수행하였다. 이론적인 컴퓨터 모델링 연구를 통해 얻은 결합 에너지의 차이는 액체 크로마토그래피 실험에서 광학분리된 분리결과 및 용출 순서와 일치하였다.

제 2장에서는, 아미노알코올을 포함한 키랄 지방족 아민 화합물을 순상 액체 크로마토그래피에 의한 거울상 이성질체의 광학분리 하기위해 형광성질을 띠는 유도체 시약인 4-chloro-7-nitro-2,1,3-benzoxadiazole 을 사용하여 유도체화하였다. 자외선 및 형광 검출 을 동시에 수행하면서 여러 다당류에서 유도된 키랄 고정상을 사용하였다. 키랄 선택자가 공유결합 된 6 개의 키랄 고정상가운데, Chiralpak IE는 대부분의 분석물에 대해 가장 좋은 광학분리 및 분리인자를 나타냈다. 일반적으로 아밀로오스 유도체의 키랄 선택자가 코팅 된 Chiralpak AD-H 및 Lux Amylose-1 키랄 고정상은 셀룰로오스 유도체의 키랄 선택자가 코팅 된 Chiralcel OD-H 및 Lux Cellulose-1 키랄 고정상보다 NBD 유도체화 키랄 아민 화합물이 우수한 광학분리를 나타내었다. 시판중

인 (R) 및 (S)-류신올 화합물 내의 광학불순물은 두 분석 물질에서 모두 0.06% 였다. 검증 실험을 통해, 이 개발된 분석법은 자외선 및 형광의 동시 검출 하에서 NBD 유도체로서 아미노 알코올을 포함하는 키랄 지방족 아민의 분리에 대해 신뢰할 수 있는 키랄분석법임을 입증하였다.

제 3장에서는 여러 다당류 유래 키랄 고정상에서 2-hydroxynaphthaldimine유도체로서의 키랄성 아민을 광학분리하는 키랄 크로마토그래피 방법을 서술하였다. 이 연구에서는 2-hydroxynapthaldehyde를 처음으로 유도체화 시약으로 도입하여 검출 민감도를 높이고 키랄 광학분리를 위한 적절하게 반응하는 부위를 제공하였다. 실험에서 사용된 키랄 고정상 중에서, 셀룰로오스 유도된 키랄 고정상은 아밀로오스 유도된 키랄 고정상 보다 우수한 거울상 이성질체 분리를 보였다. 특히, 셀룰로오스계 키랄 선택자가 공유결합된 Chiralpak IC는 최상의 광학 분리 및 분해인자를 나타내었다. 유도체화 시약으로 2-hydroxynaphthaldehyde를 사용하여 개발된 분석방법을 사용하여 (R)- 및 (S)-류신올의 거울상 이성질체 광학순도를 측정하였는데, 조사된 이들 분석물의 광학불순물은 0.06-1.20% 였다. 이 분석방법은 ICH 지침에 따라 검증실험되어, 순상 액체 크로마토그래피 에서 자외선 검출하에 2-hydroxynaphthaldimine 유도체로서 키랄 지방족 아민의 거울상 이성질체 광학분리 분석법은 본 연구에 적합하며 효과적으로 적용가능하다는 것이 입증되었다.

제 4 장에서는, 다양한 키랄 카르복실 산을 미량 검출이 용이하도록 1-naphthylamides 유도체화한 후 이들을 대상으로 한 광학분리를 아밀로오스 및 셀룰로

오스 유도체에 기초한 10 개의 액체 크로마토그래피 키랄 고정상 를 사용하여 수행하였다. 대부분의 분석물의 광학분리 실험에서 기준분리와 분리계수 결과가 양호했다. 일반적으로, 코팅형태 컬럼에서 관찰된 거울상 이성질체 광학선택성은 공유결합형태 컬럼에서 관찰된 결과보다 더 좋은 것으로 나타났다. 특히, 2-aryloxypropionic acid 분석물질의 유도체를 제외하고는, 1-naphthylamide 유도체로서의 키랄 카르복실 산의 광학분리에서 다른 키랄 고정상 에 비해 코팅형 Lux Cellulose-1 컬럼의 성능이 우수하였다. 1-나프틸 그룹의 높은 자외선 흡광도 성질과 검증된 분석법 결과를 바탕으로, 다당류 유래 키랄 고정상 를 사용하여 본 연구에서 개발된 1-naphthylamide 유도체로서 다양한 키랄 카르복실 산의 거울상 이성질체 광학분리는 매우 유용하고 실제적인 것으로 보아진다.

제 5 장에서는, 3 개의 방향족 알데히드 유도체 화제를 사용하여 아밀로오스와 셀룰로오스 유래 키랄 고정상 에 대해 키랄 아민의 순상 액체 크로마토그래피 거울상 이성질체 분리를 수행 하였다. 3 가지 나프틸 알데히드 유도체 시약의 일반적인 액체 크로마토그래피 에 대한 광학분리분석을 수행하였고 ~~UV~~ 자외선 검출 하에서 아밀로오스와 셀룰로오스 기반 키랄 고정상 에서의 실험결과를 비교하였다. 키랄 아민의 거울상 이성질체 아민에 대한 유도체 시약 종류 및 키랄 고정상 유형의 구조적 형태에 따른 결과를 비교하였다. 본 연구에서 셀룰로오스를 기초로 한 키랄 고정상의 2-hydroxynaphthaldimine 유도체의 광학분리를 제외하고는, 아밀로오스에서 유도된 키랄 고정상 에서 얻은 거울상 이성질체 광학분리는 셀룰로오스 유래 키랄 고정상 결과보다 더 컸다. 3 종류의 나프틸 유도체화 시약을 사용하여 가장 구조적으로 유연한

2-naphthaldimine 유도체는 구조적으로 유연한 아밀로오스에 기초한 키랄 고정상 에서 가장 큰 광학분리를 나타내었으며 그 반대도 마찬가지였다. 본 실험의 키랄 물질과 키랄 고정상을 대상으로 분자 모델링 연구를 수행하고자 AutoDock 및 PyMOL 소프트웨어를 사용하였다. 분자 모델링 결과에서, 아밀로오스 tris(3,5-dimethylphenylcarbamate)의 키랄 선택기를 갖는 아밀로오스 유도된 키랄 고정상 에서 2-naphthaldimine 및 2-hydroxynaphthaldimine 유도체를 포함하는 키랄 인지 메커니즘을 밝혀냈다. 수소 결합, π-π 및 소수성 상호 작용이 키랄 광학분리와 관련된 주요 힘이었다는 것이 관찰되었다. 얻어진 이론적인 데이터는 실험으로 수행된 키랄 액체 크로마토그래피 분석의 실험 결과와 잘 일치한다.

6 장에서 유도체시약으로 fluorene-2-carboxaldehyde를 사용하여 자외선 검출 하에서 순상 액체 크로마토그래피 를 이용하여 아미노 알코올을 포함한 키랄 지방족 아민의 거울상 이성질체를 처음으로 광학분리하였다. 아밀로오스 또는 셀룰로오스 계 키랄 선택자가 공유결합된 6개 키랄 고정상 및 코팅된 4 개 다당류 유래 키랄 고정상 중에서, 실험한 분석물질에 대해서 Chiralpak IE 및 Chiralpak IF 컬럼상에서는 부분적으로 또는 기준분리로 광학분리분석되었다. 특히, 셀룰로오스 유래의 키랄 고정상는 특히 아미노 알코올 분석물에 대해 가장 우수한 광학분리를 나타내었다. 류시놀의 경우, 셀룰로오스 tris(3,5-dimethylphenylcarbamate)로 동일한 키랄 선택자가 사용된 코팅되거나 공유결합된 키랄 고정상 모두에서 가장 우수한 거울상 이성질체 분리 및 분리인자를 나타내었다. 방향족기를 함유한 α-메틸 벤질아민의 거울상 이성질체 광학분리에서도 효과적이었으며 우수한 거울상 이성질체 분리 및 분해능을 보였다.

개발된 분석법을 검증하기 위해 일중 및 일간 정확성 및 정밀성 실험이 수행됨으로, 아미노 알코올을 비롯한 키랄성 아민을 fluorene-2-carboxaldimine 유도체로 광학분리하는데 매우 정확하고 선택적임을 입증하였다.

Abstract (English)

Enantiodiscrimination Studies on Chiral Amines and Acids Using Polysaccharide-derived Chiral Stationary Phases

Suraj Adhikari

Advisor: Prof. Wonjae Lee, Ph.D.

College of Pharmacy

Graduate School of Chosun University

Chiral separation has become a major concern in the pharmaceutical industry. In highly enantioselective biological environment of our living system, the two enantiomers of a chiral pharmaceutical will react differently with their complementary receptor or enzyme and produce diverse biological activities. In fact, one enantiomer may be biologically active and produce desired activity, while the other may create various side effects and problems in our body. In this context, there is an increasing demand for the analysis of chiral compounds and the preparation of optically active and pure stereomers or enantiomers for biologically important pharmaceuticals or drugs. Accordingly, for the enantiomerically pure pharmaceuticals, the constant development of efficient and validated analytical methods for enantiomeric separation and analysis of racemic chiral pharmaceuticals is required. Chiral high performance liquid chromatography (HPLC) using various chiral stationary phases (CSPs) is one of the most effective and widely used method for the resolution and analysis of the enantiomers of a chiral drug. Chiral amines and acids are of particular interest in pharmaceuticals as they were found as key intermediates in drugs and drug candidates.

This dissertation is designated to develop simple, convenient, validated and readily available normal phase chiral HPLC methods for the enantiomer separation of chiral amines and acids as well as the determination of enantiomeric purity and their absolute configuration using polysaccharide-derived CSPs. Since the chiral amines and acids used in the study show low ultraviolet (UV) absorption, achiral derivatization using several potent derivatizing agents was performed to provide good interaction sites for enantiodiscrimination as higher detection sensitivity for analytes. Computer-aided molecular modeling study was performed to ascertain the mechanism of chiral recognition and the elution order between the investigated analytes and the employed CSP. The differences in the binding energies corresponded to the separation and the elution order of investigated analytes in the HPLC experiments.

In Chapter 2, several chiral aliphatic amines including amino alcohols were derivatized using a fluorogenic achiral derivatizing agent, 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) for enantiomeric resolution by normal phase HPLC on six covalently bonded and four coated type polysaccharide-derived CSPs under simultaneous UV and fluorescence detection (FLD). Out of six covalently bonded CSPs, Chiralpak IE showed the best enantiomer separation and resolution for most of the analytes. Amylose-derived coated type CSPs, Chiralpak AD-H and Lux Amylose-1 generally exhibited better enantiomer separation of NBD derivatized chiral amines than cellulose-derived Chiralcel OD-H and Lux Cellulose-1 CSPs. The impurity of commercially available (R)- and (S)-leucinol was found to be 0.06% for both analytes. After the validation experiments, this developed method proved to be precise and enantioselective for the separation of chiral aliphatic amines including amino alcohols as NBD derivatives under simultaneous UV and FL detection.

In Chapter 3, a chromatographic method for enantiomer separation of chiral amines as 2-hydroxynaphthaldimine derivatives on several polysaccharide-derived CSPs was developed. 2-Hydroxynapthaldehyde was introduced as a potent derivatizing agent for the first time in this study to enhance detection sensitivity and also to provide suitable interaction sites for chiral separation. Amongst the examined CSPs, in general, the cellulose-derived CSPs showed better enantiomer separation than the amylose-derived CSPs. In particular, the covalently bonded type Chiralpak IC with cellulose-based chiral selector showed the best enantioseparation and resolution. The developed analytical method using 2-hydroxynaphthaldehyde as derivatizing agent was employed to determine the enantiomeric purity of commercially available (R)- and (S)-leucinol and the impurities of the investigated analytes from two distributors were found to be 0.06-1.20%. This analytical method was validated in accordance with ICH guidelines and it proved to be sensitive, precise and applicable for the enantiomer separation of chiral aliphatic amines as 2-hydroxynaphthaldimine derivatives under UV detection on normal phase HPLC.

In Chapter 4, the liquid chromatographic enantiomer separation of various chiral acids as 1-naphthylamides was performed using ten CSPs derived from amylose and cellulose tris phenylcarbamates. Most of the analytes under consideration were base-line separated with good separation and resolution factors. In general, the enantioselectivities observed on coated type columns were better than those on covalently bonded type columns. Especially, the performance of coated type Lux Cellulose-1 column was superior for the enantiomer separation and resolution of chiral acids as 1-naphthylamide derivatives to those of the other CSPs, except for 2-aryloxypropionic acid derivatives. Owing to the strong UV absorbance of the 1-naphthyl group and method validation results, the analytical method developed in this study could be valuable and sensitive for the enantiomer separation of various chiral acids as 1-naphthylamide derivatives using

polysaccharide-derived CSPs.

In Chapter 5, normal phase liquid chromatographic enantiomer separation of chiral amines was performed on amylose and cellulose-derived CSPs using three aromatic aldehyde derivatizing agents. Enantiomeric separation ability of three aromatic aldehyde derivatizing agents on normal HPLC was studied and compared between amylose and cellulose-based CSPs under UV detection. The impact of the structural makeup of both derivatizing agents and CSP types on enantiodiscrimination of chiral amines was also elucidated. The enantioselectivities and resolutions obtained on amylose-derived CSPs were greater than on cellulose-derived CSPs, except for 2-hydroxynaphthaldimine derivatives on cellulose-derived CSPs. The most conformationally flexible 2-naphthaldimine derivatives using three kinds of derivatizing agents afforded the greatest enantioseparation on conformationally flexible amylose-derived CSPs and vice-versa. Molecular modeling studies were performed and compared using AutoDock and PyMOL software to elucidate the chiral recognition mechanism involving 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives on amylose derived CSP having the chiral selector of amylose tris(3,5-dimethylphenylcarbamate). It was observed that hydrogen bonds, π-π and hydrophobic interactions were the major forces involved during chiral separation. The theoretical data obtained were in good accordance with the chromatographic results from the developed chiral HPLC method.

In Chapter 6, fluorene-2-carboxaldehyde was used as an achiral derivatizing agent for the first time to separate the enantiomers of chiral aliphatic amines including amino alcohols by normal phase HPLC under UV detection. Among six covalently bonded and four coated type polysaccharide-derived CSPs of amylose or cellulose-based chiral selectors, all the analytes were either partially or base-line separated on Chiralpak IE and Chiralpak IF columns. In particular,

cellulose-derived CSPs showed the best enantiomer separation especially for amino alcohol analytes as fluorene-2-carboxaldimine derivatives. 2-amino-4-methyl-1-pentanol analyte (leucinol) showed the best enantiomer separation and resolution on both coated type and covalently bonded CSPs of the same chiral selector as cellulose tris(3,5-dimethylphenylcarbamate). Among the chiral aliphatic amines, enantiomer separation of α-methylbenzylamine containing aromatic group was effective and showed the better enantiomer separation and resolution. Intra- and interday accuracy and precision experiments were done to evaluate the presently developed analytical method and proved to be quite precise and selective for the separation of chiral amines including amino alcohols as fluorene-2-carboxaldimine derivatives.

Index Terms: Chiral amine, Chiral acid, Chiral high performance liquid chromatography, Enantiomer separation, Polysaccharide-derived chiral stationary phase, Molecular docking

Abbreviation

ADT	AutoDock Tools
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CS	Chiral Selector
CSP	Chiral Stationary Phase
FL	Fluorescence
FMOC	Fluorenylmethoxycarbonyl
FMOC-Cl	Fluorenylmethoxycarbonyl chloride
FDA	Food and Drug Administration
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
LGA	Lamarckian Genetic Algorithm
LOD	Limit of Detection
LOQ	Limit of Quantification
NBD	Nitrobenzoxadiazole
NBD-Cl	4-chloro-7-nitro-2,1,3-benzoxadiazole
PHT	Phthaloyl
RSD	Relative Standard Deviation
SFC	Supercritical Fluid Chromatography

TA	Tartaric Acid
TLC	Thin-Layer Chromatography
UV	Ultraviolet

CHAPTER 1

General Introduction

"I call any geometrical figure, or group of points, chiral, and say it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself".

Kelvin, 1904

1.1 Chirality-a critical factor

Our daily life encounters with the phenomenon of chirality such as shaking of hands, left foot does not fit in the right shoe, and tightening of a screw always in the same direction which goes unnoticed. The term, chiral, derived from the Greek word 'chier' which means 'hand'. Chirality or handedness is the geometric property of a molecule that occurs in two asymmetric forms and non-superimposible with its mirror image regardless of the same atomic numbers or bond orders [1]. This phenomenon is due to the differences in the three-dimensional orientation of different substituents attached to a single central atom generating left and right-hand versions of the same molecule. The classical example of chirality is human left and right hand as they are mirror images of each other but not superimposible. On molecular level, generally chirality is caused by sp3-hybridized tetrahedral carbon atom having four different substituents attached to it [2].

There is always a debate about whether chirality and underlying stereochemistry essential for the emergence and evolution of chemically based life. It is no doubt that stereochemistry provides valuable information compared with their non-stereoisomeric analogs. The most important fundamental aspect shown by living organism is homochirality or the signature of life [3]. The monomeric unit of DNA and RNA, deoxyribose and ribose sugar, which contain and transfer genetic information, are all right-handed (D-form). Proteins that are essential for the structure and chemical transformations in the living cells are constructed from α-amino acids having same

configuration of L-form. If there is no uniform chirality found in the monomeric units which build central molecules of life, our current life forms could not exist. Due to the homochirality shown by biomolecules, chirality is a decisive factor in chemistry of life.

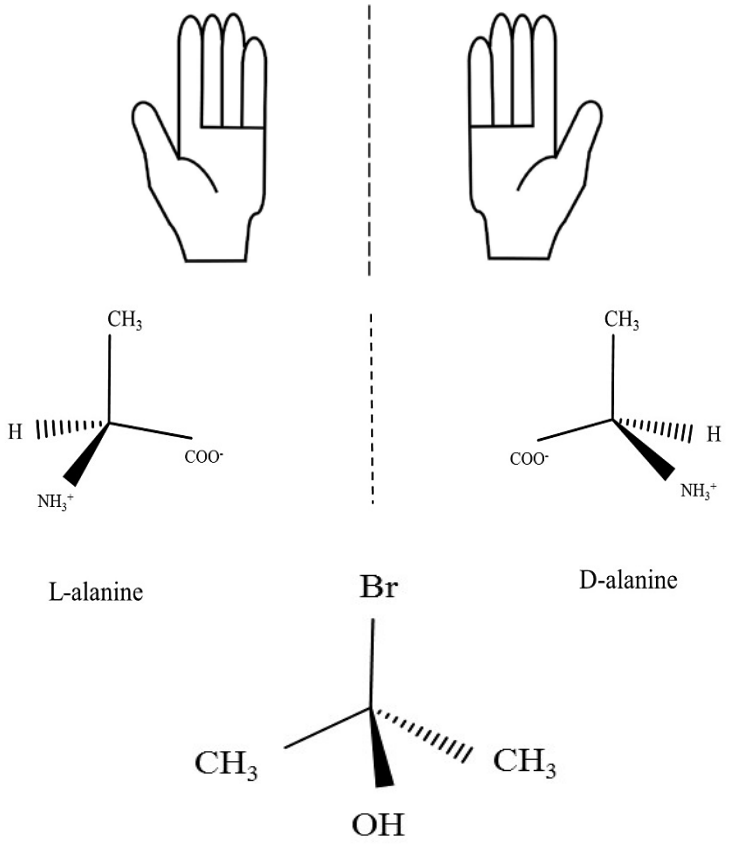

Figure 1.1: (Top) The mirror image of a left hand is not superposable and rather is a right hand, (Middle) Chirality phenomenon shown by α-amino acid alanine as the mirror images are non-superimposable, (Bottom) A chiral carbon (stereogenic center) with four different substituents

Chirality is a ubiquitous and important property of living nature as many important molecules of life such as amino acids and sugars which form the central molecules of life (proteins and nucleic

acids) are chiral in nature [4,5]. Molecular recognition occurs by the specific non-covalent interaction between the biomolecules which include enzyme-substrate, receptor-ligand, DNA-protein, sugar-lectin and antigen-antibody interactions and is sensitive to stereochemistry [6]. Therefore, it is not unusual to get different biological responses to the stereoisomers or enantiomers of a chiral pharmaceutical. There are countless biological pathways of the life cycle which are strictly enantioselective and interaction of racemic chiral pharmaceuticals with an asymmetric biological molecule such as a receptor or enzyme results in different pharmacological activity. Thus, chirality is a fundamental characteristic of nature and pervades the living words. We have been under its constant influence throughout evolution as a result of asymmetrical nature of the environment [4]. The ability to discriminate between chiral molecules is prerequisite for almost all research involving chirality and very important in biochemical development also [5,7].

1.2 Chiral Molecule

Mislow stated that “a chiral molecule is an object if, and only if, it is not superposable on its mirror image; otherwise it is achiral” [8]. The two mirror images are commonly known as enantiomers [9]. The biological activity of chiral molecules strictly relies upon their stereoselectivity as living system comprises of a highly chiral environment which shows different biological response to enantiomers of chiral drugs.

In general, a chiral molecule is a molecule having at least one asymmetric carbon. However, sulfur, phosphorus and nitrogen can sometimes also form chiral molecules like some drugs as omeprazole, cyclophosphamide and methaqualone. The chirality elements which are responsible for molecular asymmetry due to the spatial arrangement of atoms are as follows: chiral centre, chiral axis, chiral plane, atropisomerism, and molecular strain.

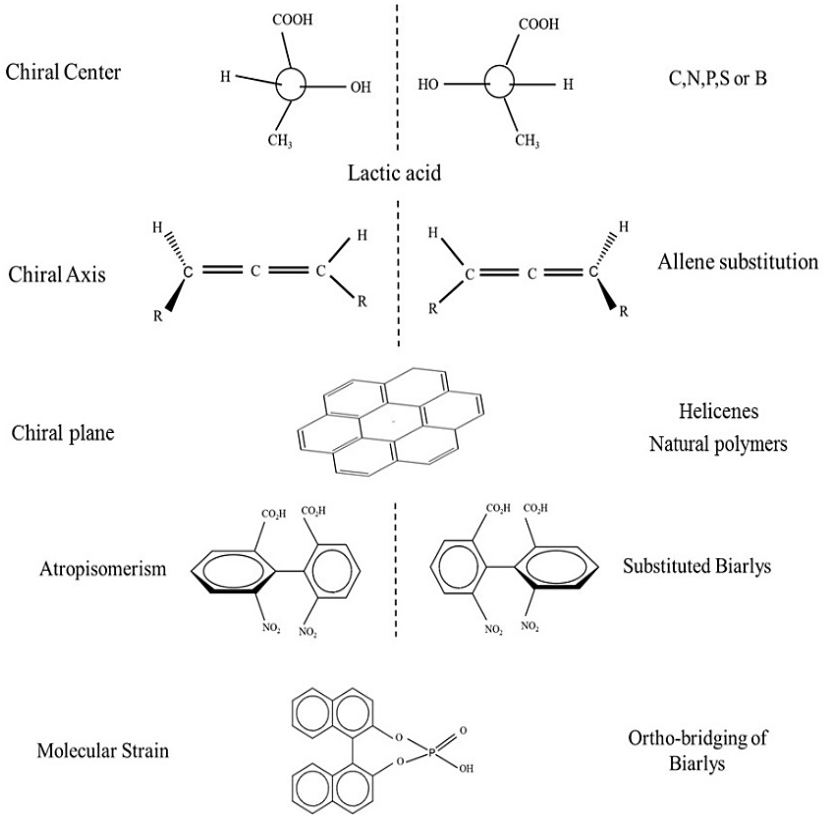

Figure 1.2: Chirality elements responsible for molecular asymmetry (Source: Chiral Guide)

The simplest way to identify chiral molecule is to look for the asymmetric carbon atom (stereogenic center) first. If no asymmetry is observed, then, the next step is to look at the plane of symmetry of the whole molecules and other atoms such as sulfur and nitrogen which can also confer chirality. If there is one stereogenic center or asymmetric carbon found, a pair of enantiomer results. The 2^n rule applies for the molecule having two or more stereogenic centers [10].

1.3 Stereochemistry of Chiral Molecules

Isomers are the molecules having same molecular formula but different structural arrangement [11]. The result of isomerism yields various compounds which possess different properties from each other. There are three types of isomerism as constitutional, configurational, and conformational [11,12]. Among them, conformational isomerism represents the spatial relationship of every atom in a molecule.

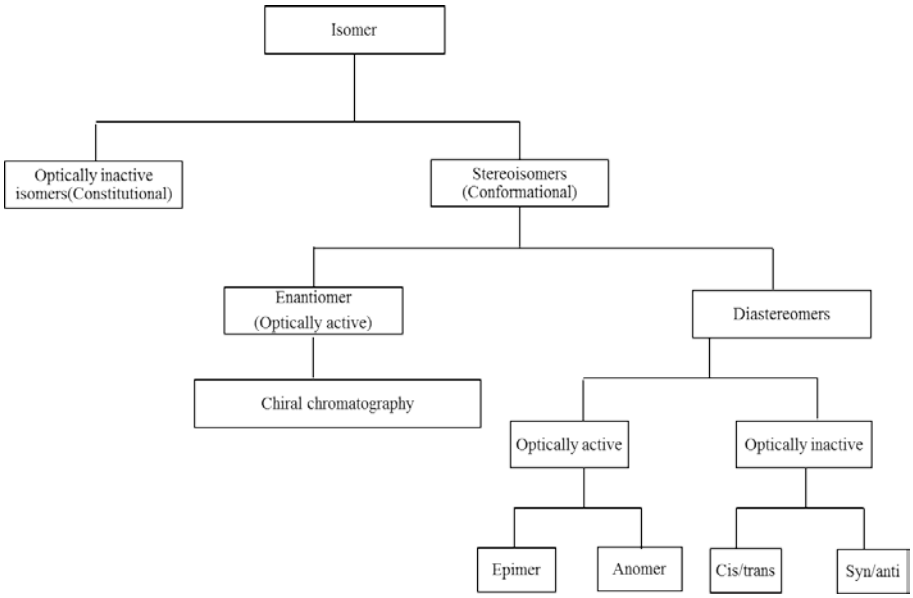

Figure 1.3: The field of stereochemistry [11]

Stereochemistry also known as 3D chemistry is the study of the differences in the spatial arrangements of atoms in molecule. Molecules that are isomeric but have a different spatial arrangement are called stereoisomers [10]. Symmetry classifies stereoisomers as enantiomers or diastereomers [13]. A chiral molecule having one stereocenter (a carbon atom or another atom

bearing four different atoms or groups of atoms) has two stereoisomers which are non-superimposable with their mirror image called enantiomers or optical isomers. They have the same chemical formula, the same physical and chemical properties [13,14]. In contrast, molecule with two or more chiral centers can exist as diastereoisomers. They are stereoisomers that are not mirror images of each other and have different relative configuration [14]. This study is focused on the separation of enantiomers as they show different pharmacological activities when entered in the chiral environment of living body.

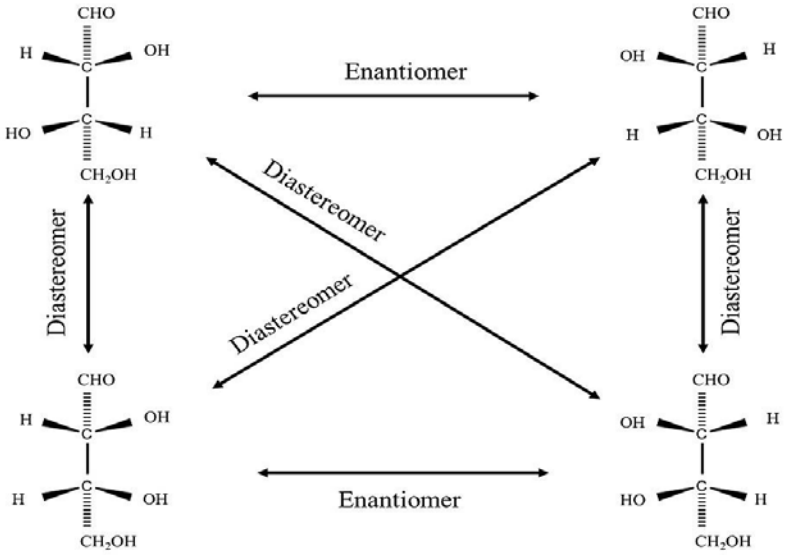

Figure 1.4: Basic understanding of enantiomer and diastereomer using 2,3,4-Trihydroxybutanal [10]

1.4 Pasteur's Contribution

Molecular stereochemistry was discovered by a French chemist and biologist Louis Pasteur (1848) based on his work on the resolution of tartaric acid into its enantiomeric form [15]. The historic chain of events emerged gradually in the middle of nineteenth century after the observation of a property called as optical activity [16]. In 1811, French physicist Francois Arago was the first to notice optical rotation after observing the rotation of the plane of linearly polarized light passing through quartz crystals. Afterwards, in 1815, French physicist and astronomer Jean-Baptiste Biot noted that there are certain natural organic compounds which rotate plane polarized light in the non-crystalline state suggesting that this optical activity was actually a molecular property. Furthermore, in 1838, he discovered tartaric acid (TA) from grape juice after fermentation to rotate plane polarized light in a clockwise direction but he made the opposite observation for racemic acid. Ten years later, Pasteur worked with tartaric acid where he observed that crystallization of the sodium ammonium salt of this acid produce mirror-image crystal which he isolated by hand and rotates the plane polarized light in opposite direction. Thus, he concluded that these two crystalline forms were dissymmetric, non-superimposable mirror images and their equimolar solutions showed equal but opposite optical activity.

In 1848, for the first time, optical activity in mirror-image pairs of crystals was connected to the optical activity of their corresponding solutions by Pasteur. The observation thus made, marked the advent of our understanding of optical activity in nature and its association with mirror symmetry at the molecular level. Based on these observations, Pasteur also concluded that optical activity is actually based on dissymmetry, not only at the crystalline level, but also at the molecular level. Pasteur work led the way for Van"t Hoff and Le Bel and in 1874; they postulated the tetrahedral nature of carbon (a molecule having a tetrahedral carbon with four different substituents attached

and may exist as a pair of isomers) which provide the platform for the modern stereochemistry. However, Pasteur used French term dissymetrie (dissymmetry) to this observed phenomenon of handedness. The term chirality that we call today was coined by Lord Kelvin in 1894 [17]. Still, Pasteur's resolution of (-)-TA is considered as the most elegant experiments in the history of chemistry.

1.5 Enantiomer Separation and Pharmaceutical Industries

As the critical molecules of our living system are entirely chiral, the discrimination of chiral compounds into its enantiomers is of great interest. Chiral separation and analysis of chiral drugs are vital in pharmaceutical industry in order to discard the unwanted isomer from the preparation and to find an optimal therapeutic effect for the patients [18]. Enantiomers administered alone may have different action within the body than a marketed racemate. Enantiomer may differ in pharmacodynamics properties (interactions with enzymes and receptors, pharmacological and toxicological activities), pharmacokinetic properties (absorption, distribution, metabolic conversion, biotransformation, and excretion of the drug) as well as in clinical pharmacology properties (therapeutic index and response, bioavailability, adverse drug reactions, drug-drug interactions) [19]. One isomer may be more active isomer for the desired therapeutic activities while the other may be inactive or even active for different action, in worst cases, contributing to side-effects, displaying toxicity, or acting as antagonist [20]. The (-) enantiomers of opiates are potent narcotic analgesics whereas their (+) enantiomers are useful anti-tussive agents (propoxyphene, dextromethorphan). The (S)-enantiomers of α-arylpropionic acids including (S)-ibuprofen are responsible for the anti-inflammatory effect and useful in treatment of rheumatoid arthritis. On the other hand, (R)-ibuprofen is inactive.

The interest for the single enantiomeric chiral drugs significantly increased after the tragic experience encountered by using a drug called thalidomide. This racemic drug was marketed in 1960 as a sedative drug but later on it was found that therapeutical activity was shown exclusively by R-(+)-enantiomer. After the birth of several hundred malformed infants, it was discovered that the S-(-)-enantiomer of this racemic drug was teratogenic [21]. The U.S. Food and Drug Administration (FDA), in 1992, issued a guideline that for chiral drugs; pharmaceutical companies must study each enantiomer separately for their pharmacological effects before marketing. As a result, chiral separation and using of a single enantiomeric chiral drug is a prime interest in pharmaceuticals over the past 30 years.

The advantages of using single enantiomeric drug are as follows:

- Enhanced biological activity and reduction of dosage form to half.
- There will be fewer or no side effects after discarding the unwanted enantiomer. The unwanted enantiomer is generally a waste and there will be reduction in the generation of waste.
- Drug variability of the patients is reduced and a greater confidence in setting a single dose regimen.
- The other enantiomer can also be used for the different indications as well as interaction with other common is also reduced.
- A new commercial opportunity of ‘Racemic Switching’ of chiral drugs. The introduction of new single enantiomeric chiral drug from existing commercialized racemic pharmaceuticals.

Moreover, enantiomeric separation has also found in much application in other fields such as in agrochemicals, pheromones, food additives, flavors and fragrances [22-24]. They also represent the chemicals with high economic and scientific impact and there is an increasing demand for optically pure compounds. For example, the (R)-enantiomer of the compound limonene has orange odor, while the (S)-enantiomer contains lemon odor [22].

1.6 Chiral Chromatography

Chromatography is a separation tool for separating components from complex mixtures and is either preparative or analytical. Basically, it consists of a mobile and stationary phase where the desired analytes are separated from a mixture based on differential affinities of the analytes towards the mobile and stationary phases [25].

Chiral chromatography commonly known as enantioselective chromatography involves in the separation of stereoisomers. The desired enantiomeric species are separated due to the enantioselective interaction with the chiral selector(s) of a chromatographic system. For this, either the mobile phase or the stationary phase must be made chiral for differing affinities between the analytes [26]. The high demand for the accurate enantiomer analysis (separation and purity determination) especially in pharmaceutical drug research as well as food chemistry, agrotechnology, forensic sciences and classical organic chemistry leads for the rapid development of sensitive and reliable chiral chromatographic methods.

The common chromatographic techniques used for separating the racemates are listed as follows:

- High-Performance Liquid Chromatography (HPLC)
- Gas Chromatography (GC)

- Thin-Layer Chromatography (TLC)
- Capillary Electrophoresis (CE)
- Capillary Electrochromatography (CEC)
- Supercritical Fluid Chromatography (SFC)

Among them, liquid chromatographic enantiomer separation particularly using HPLC with different types of chiral stationary phases is considered to be the most powerful methods and highly applicable technology available for obtaining pure enantiomers as well as determination of enantiomeric composition [27]. HPLC can separate enantiomers that are non-volatile, polar or organic and is considered to be more versatile than chiral GC.

1.6.1 Chiral HPLC

Separation, preparation and analysis of chiral isomers can be performed using chiral HPLC. Chiral HPLC is recognized as a one of the best, fast and highly selective method which can be successfully applied for the research and development of chiral drugs [28]. It has become a method of choice in many field of science dealing with chiral compounds [29].

Chiral HPLC components

A typical HPLC system consists of the following components

- Mobile Phase
- Solvent Reservoir
- Column
- Pump
- Injector

- Detector
- Data Acquisition and Control System

Enantiomer separation by chiral HPLC can be performed either directly using chiral stationary phases (CSPs) or chiral additives in the mobile phase, or it can also be done indirectly by the derivatization of the analytes. In indirect method, the derivatization of analytes requires lot of time and formation of unwanted products or racemization may occurs. Furthermore, optically pure chiral derivatizing agents may not be available. Direct methods of chiral separation are based on the interaction of analytes with chiral selectors (CSs) either incorporated into the stationary phase or chemically bonded to or immobilized onto the surface of a solid support or simply dissolved in the liquid stationary phase. The direct separation by chiral stationary phases has advantages not only to determine the optical purity but also obtaining the optical isomers in large scale [29]. Recent commercially developed powerful CSPs with excellent chiral discrimination abilities, wide applicability, and high loading capacity for both in analytical and preparative separation has been attracting analysts for enantiomeric separation using direct HPLC method [29]. This current study is about the direct enantiomeric separation of chiral amines and acids by normal phase HPLC using chiral stationary phases.

1.6.2 Mechanism of Chiral Separation

Fischer's lock and key principle (1894) states that the drug molecules refer to the keys which fit into the locks in the body for a particular biological response. The lock in the living systems is chiral which generally exist in one enantiomeric form. Therefore, only one enantiomer of the key molecule (drug) should be fit for eliciting a response.

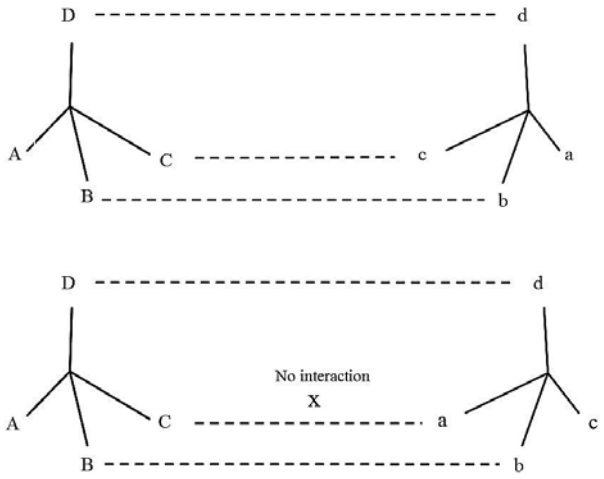

Figure 1.5: Three-point rule for chiral mechanisms [30]

For enantiorecognition mechanisms, Dalgliesh (1952) postulated ‘three-point rule’ from his observation on paper chromatographic studies of certain aromatic amino acids [4,30]. It states that three points of interactions between the chiral molecules and CS are necessary to achieve the chiral recognition and discrimination. Additionally, at least one of the three interactions must be stereoselective in both chiral selector and the enantiomers to form diastereomeric complexes and thereby enabling chiral separation.

There are number of chiral recognition models being purposed for the resolution of the enantiomers which are mostly based on the three point interaction rule by Dalgliesh. For example, in the Brush type or Pirkle columns, the chiral selectors are capable of ionic or covalent bonding. The complex formation that occurs between the chiral molecule and CSP involves a combination of interaction forces as hydrogen bonding, π-π interactions, dipole/dipole interactions and steric effects [31]. In this type of CSPs also, the chiral selector (CS) interacts with one enantiomer at

three active sites, while for the other at only two active sites forming two transient complexes. These transient complexes formed are of different stabilities and thus the chiral selector recognize the two enantiomers and discriminate them.

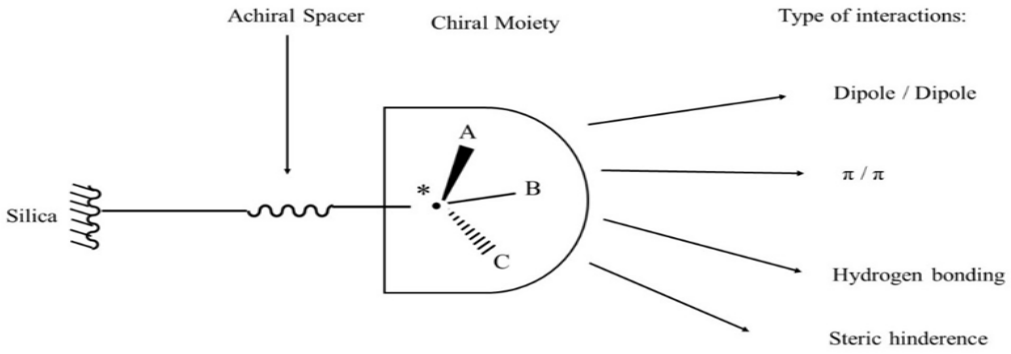

Figure 1.6: Pirkle interaction force for chiral recognition and discrimination [Source: Chiral Guide, (31)]

1.6.3 Chiral Stationary Phases

The purpose of chiral stationary phases or chiral columns is to show the different affinities towards the two enantiomers of a chiral drug so that a difference is created between their retention times which are large enough to discriminate enantiomers [32]. In fact, there is no such universal chiral stationary phase that has the ability to separate all classes of racemic compounds. Selecting a good chiral stationary phase is always challenging part of chiral chromatography. While choosing chiral stationary phases, analysts must consider some important factors such as solubility profile of analytes in mobile phase, structural makeup of the analyte, cost while using column, mobile phase, analysis time, or method robustness [33].

The common CSPs that are being used for enantiomer separation of different class of chiral compounds are as follows:

- Polysaccharide Based
- Brush or Pirkle Type
- Crown Ethers Type
- Protein Based
- Cyclodextrin Type
- Ligand Exchange Type
- Macrocyclic Antibiotic Type

1.6.3.1 Polysaccharide Derived Chiral Stationary Phases

Polysaccharides are naturally occurring optically active biopolymers comprising several units of monosaccharide attached with each other through a glycosidic bond which incorporate various levels of chiral information in their perfectly defined structures [34]. There are several types of polysaccharides such as amylose, cellulose, galactosamine, chitosan, chitin, xylan, dextran etc. and some of them have been investigated as possible chiral selectors for chiral HPLC [35]. Among these, cellulose and amylose are the most functional polysaccharides because of their higher enantioselective abilities, sensitivities and reproducibilities for chiral resolution as well as availability in pure form make them widely applicable chiral selector for the analytical and preparative scale separation of enantiomers. They are made from D-glucose unit linked by either 1,4 α or β glycosidic bonds. These polymers contains large number of chiral active sites because of the presence of several stereogenic centers of the glucopyranose units and thus exhibit a high probability of chiral site interaction with the analytes. Conformational chirality shown by amylose polymer due to the flexible helical makeup of the polymer backbone is also responsible for the

superior chiral recognition [36]. The mechanisms evolved on the enantiodiscrimination of the enantiomers are based on the spatial effects of cavities built by the polysaccharide, steric effects, π-π interactions, hydrogen bonding and dipole-dipole [37,38].

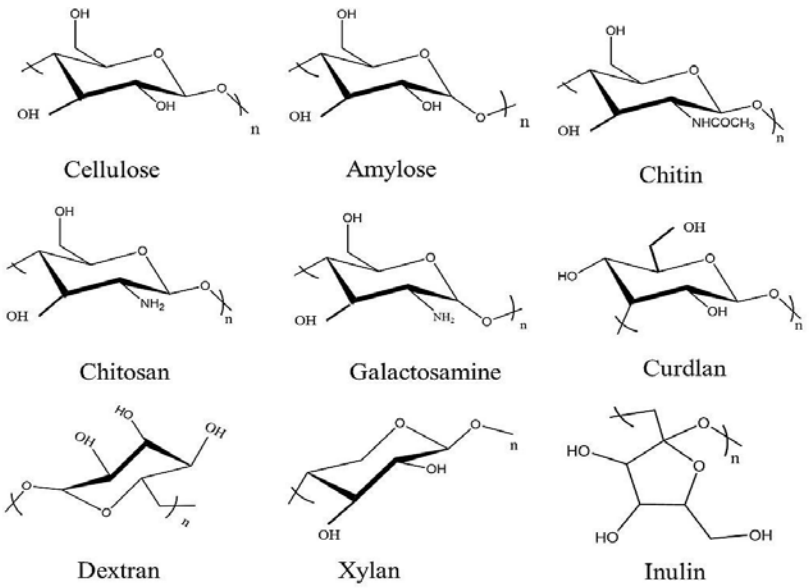

Figure 1.7: Several polysaccharides used as chiral selectors for chiral stationary phases

Phenylcarbamates and ester derivatives of amylose and cellulose are the most successful, effective and applicable CSPs for chiral separation, and commercialized mostly under Chirpak, Chiralcel or Phenomenox. Especially, the developed tris-phenylcarbamates of amylose and cellulose as chiral selectors showed the best enantiorecognition performance and are the most widely used CSPs [39]. These amylose and cellulose-derived polysaccharide CSPs can be either coated or immobilized on chromatographic support mainly amino propyl silica [40].

Coated type polysaccharide Chiral Stationary Phases

In the early 1980s, Japanese researcher came up with the great idea of derivatizing polysaccharides to escalate their chiral recognition abilities and then coating them on a silica gel support. After that, these coated CSPs have become routine tools for preparative and analytical enantiomer separations in academic and industrial settings. There are different types of coated chiral columns such as Chiralcel OD, Chiralcel OD-H, Chiralpak AD-H, Lux Amylose-1 and Lux Cellulose-1 having tris phenylcarbamates of amylose and cellulose. These types of CSPs are only compatible to limited number of solvents due to the solubility of chiral selectors which narrow their applicability. Solvents such as dichloromethane, chloroform, ethyl acetate, tetrahydrofuran, dioxane, toluene and acetone are incompatible with polysaccharide-derived coated CSPs as they induce swelling and/or dissolution of physically adsorbed chiral selectors [34].

Covalently bonded polysaccharide Chiral Stationary Phases

A new series of immobilized polysaccharide-derived CSPs has become commercially available which are prepared by chemically linking the chiral selector on silica gel matrix [41,42]. Because of their immobilized nature, they show broad application scope and high preparative potential with enhanced robustness, practically vast solvent compatibility with long withstand property of columns for extended range of applications. Covalently fixing of chiral selector on silica gel matrix is done by the three processes: fixation by means of bio-functional reagent, fixation by means of polysaccharide derivatized by a polymeric group and fixation of mixed derivatives of polysaccharide [41]. At first, they are Chiralpak IA, Chiralpak IB and Chiralpak IC and most recently, Chiralpak ID, Chiralpak IE and Chiralpak IF are also commercially available [40].

Table 1.1: Commercially available and commonly used polysaccharide-derived chiral columns [Source: Diacel Company (Tokyo, Japan) and Phenomenox (Torrance, CA, USA)]

Chiral columns derived from polysaccharide derivatives			
Type	Chiral selector	Chiral HPLC column	Supplier
Coated type	Cellulose ester derivatives	Chiralcel OA, OB, OB-H, OJ, OJ-H, OJ-RH, OK	Diacel
	Cellulose carbamates	Chiralcel OC, OD, OD-H, OD-R, OD-RH, OF, OG, OX, OX-R, OZ, OZ-R	Diacel
		Lux Cellulose-1, 2, 3, 4	Phenomenex
	Amylose carbamates	Chiralpak AD, AD-H, AD-RH, AS, AS-H, AS-RH, AY, AZ Chiralcel AD-R, AS-R, AY-R	Diacel
		Lux Amylose-1 and 2	Phenomenex
Covalently bonded type	Cellulose carbamates	Chiralpak IB, IC	Diacel
	Amylose carbamates	Chiralpak IA, ID, IE, IF	Diacel

1.7 Contributions of Dissertation

This present dissertation aims at contributing to the field of separation science. Since the two enantiomers in a chiral drug exhibit different biological activities, the development of convenient and reliable analytical methods for enantiomeric separation are vital in the field of chirotechnology. Chiral amines, acids and their derivatives are important chiral analogs that are widely employed in the pharmaceutical, agrochemical and chemical industry as chiral building blocks of powerful pharmacophores for the synthesis of many pharmaceuticals and biologically active molecules. In this regard, the enantiomer separation of chiral amines and acids is a need to obtain the desired optically pure (R)- or (S)-enantiomer for the development of pure chiral pharmaceuticals. In this dissertation, I mainly focus on the enantiomeric separation and resolution of chiral aliphatic amines and acids using polysaccharide-derived chiral stationary phases under normal phase HPLC. Convenient and validated analytical methods for enantiomeric separation of chiral amines and acids were developed using direct chiral HPLC. The direct separation by chiral stationary phases has advantages not only to determine the optical purity but also obtaining the optical isomers in large scale. Achiral derivatization was also done using various achiral derivatizing agents to provide good interaction sites for enantiodiscrimination as well as to increase the detection sensitivity for analytes. Several validated analytical methods are presented in this study for the better understanding of enantiomer separation as well as chiral discrimination mechanism of the mentioned chiral compounds. Furthermore, computer simulations were exploited for the exact chiral recognition that occurs during the separation of analytes.

1.8 Organization of Dissertation

This dissertation consists of total six chapters. It begins with a general overview about chirality, the importance of chiral separation and the need for producing single enantiomeric drugs as well as the methods for separating the desired enantiomers. The following five chapters that contain enantiodiscrimination studies of chiral amines and acids are the finished research projects during my tenure of Ph.D. study. All these projects were designed by my advisor Prof. Dr. Wonjae Lee and me. I am the primary researcher and author of these finished research manuscripts.

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CHAPTER 2

Validated Chiral HPLC Method for Enantiomeric Separation of Chiral Aliphatic Amines or Amino Alcohols as Nitrobenzoxadiazole Derivatives under Simultaneous Ultraviolet and Fluorescence Detection

Abstract

In this study, several chiral aliphatic amines including amino alcohols were derivatized using a fluorogenic agent, 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) for enantiomeric resolution by normal phase HPLC. Among the six covalently bonded and four coated type polysaccharide-derived chiral stationary phases (CSPs), Chiralpak IE (covalently bonded CSP) showed a superior enantiomer separation for most analytes. The other CSPs also showed good enantioselectivity except for Chiralpak IB. On the other hand, Chiralpak AD-H and Lux Amylose-1 as coated type CSPs generally exhibited better enantiomer separation of NBD derivatized chiral amines. The developed analytical technique was also applied to determine the optical purity of commercially available (R)- and (S)-leucinol and impurity was found to be 0.06% in both enantiomers. The developed method was thoroughly validated and proved to be an accurate, precise as well as enantioselective for separation of chiral aliphatic amines as NBD derivatives under the simultaneous ultraviolet (UV) and fluorescence (FL) detection.

Key words: Chiral amine, Chiral selector, Chiral stationary phase, Enantiomer separation, Nitrobenzoxadiazole derivative

2.1 Introduction

55% of the drugs that are currently marketed are chiral compounds and 21% among them are being in use as racemates having the equimolar mixtures of two enantiomers [1,2]. These two enantiomers in a chiral drug possess the same chemical structure but exhibit distinct differences in their biological activities. Thus, in pharmaceutical industry, enantiomer separation and evaluation of chiral drugs is a vital task to find those unwanted isomers and discard them for an optimal therapeutic control in patients [1,3]. Chiral amines are present in biologically active natural products, and are essential in synthetic drugs or act as building blocks of powerful pharmacophores for defining new pharmaceutical drugs [4]. So, their enantiomer separation as well as preparative process is of great interest during drug development process in pharmaceutical industry [3,5]. High performance liquid chromatography (HPLC) using the chiral stationary phases (CSPs) is most widely used and is more predictable chromatographic method for the separation of enantiomers [3,5-7]. Polysaccharide (cellulose and amylose) derivatives are widely used as chiral selectors for CSPs in liquid chromatography and exhibit the unique chiral recognition for the broad range of chiral compounds [7-11]. In our group, several research have been performed for the enantiomeric separation of chiral amines, amino acids as well as their esters as 9-anthraldimine, benzophenone imine, fluorenylmethoxycarbonyl (FMOC) or phthaloyl (PHT) derivatives under normal HPLC conditions using several polysaccharide-derived CSPs [12-16]. 9-anthryl (or benzophenone) moiety of amines as well as the N-protected FMOC (or PHT) group of amino acids served as good interaction sites for enantiodiscrimination by the polysaccharide-derived CSPS. In addition, the achiral derivatization group of these analytes has significant advantages in increasing the detection sensitivity.

The 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) group has previously been applied as a

derivatizing agent for enantiomer separation of amino acids and analysis was done by Imai and Zaitsu research groups [17-19]. In these reports, the determination of amino acids as NBD derivatives were performed in wine and biological samples using reversed-phase HPLC under fluorescence detection. Pirkle type CSPs, such as Chiralpak QN-AX, Chiralpak QD-AX and Sumochiral OA 2500 series were used for reversed chiral HPLC. However, the enantiomer separation of chiral aliphatic amines as NBD derivatives has not been reported on polysaccharide-derived CSPs by normal HPLC. Based on our previous studies involving 9-anthryl and benzophenone imine moieties for chiral amines or amino acids [14-16], it is expected that the aromatic NBD group will interact well with the CSP for chiral recognition and significantly enhanced the detection sensitivity of chiral aliphatic amines. Therefore, we describe in this study, a convenient and validated chromatographic enantiomer separation method of chiral aliphatic amines including amino alcohols as NBD derivatives on polysaccharide-derived CSPs under simultaneous UV and FL detection by normal phase HPLC.

2.2 Experimental Section

2.2.1 Reagents and Sample Preparation

Chiral aliphatic amines employed in the study along with 4-chloro-7-nitro-2,1,3-benzooxadiazole (NBD-Cl) and sodium bicarbonate ($NaHCO_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grades of hexane, 2-propanol and ethanol for the mobile phase were obtained from Burdick & Jackson (Morristown, NJ, USA). The NBD derivatives were prepared by stirring chiral aliphatic amines and NBD chloride (2 equivalents) with surfeit sodium bicarbonate (10 equivalents) in ethanol for 6h at room temperature, according to the modified procedure for preparation of 9-anthraldimine derivatives [16].

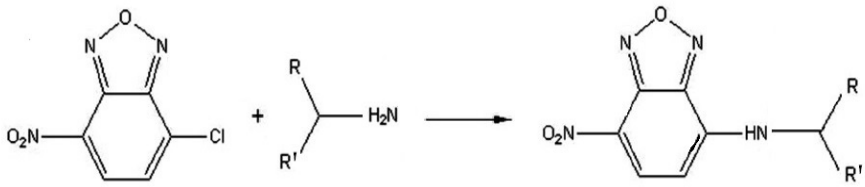

Figure 2.1: Preparation of chiral amines as NBD derivatives

2.2.2 Instrumentation and Experimental Conditions

An Agilent 1100 HPLC system (Palo, Alto, CA, USA) with micro-vacuum degasser, a G1310A isocratic pump, an automatic sample injector, a thermostatic column compartment and a HP1046A programmed fluorescence detector was used for the chromatographic analysis. The six covalently bonded Chiralpak IA [amylose tris(3,5-dimethylphenylcarbamate)], Chiralpak IB [cellulose tris(3,5-dimethylphenylcarbamate)], Chiralpak IC [cellulose tris(3,5-dichlorophenylcarbamate)], Chiralpak ID [amylose tris(3-chlorophenylcarbamate)], Chiralpak IE [amylose tris(3,5-dichlorophenylcarbamate)], and Chiralpak IF [amylose tris(3-chloro-4-methylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) columns were obtained from Daicel Company (Tokyo, Japan). The four coated type Chiralcel OD-H [cellulose tris(3,5-dimethylphenylcarbamate)] and Chiralpak AD-H [amylose tris(3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) columns were purchased from Daicel Company (Tokyo, Japan); Lux Cellulose-1 [cellulose tris(3,5-dimethylphenylcarbamate)] and Lux Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) columns were procured from Phenomenex (Torrance, CA, USA). Separation of enantiomer using chiral HPLC was performed at room temperature with a flow rate of 1 mL/min. The isocratic mobile phase employed for the whole separation procedure was 10-30% 2-propanol/hexane (v/v). A simultaneous detection of UV 337 nm and FL (excitation 470 nm and

emission 530 nm) were employed for enantiomeric separation.

2.2.3 Method Validation

The developed liquid chromatographic separation method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [20]. Validation procedure was performed using Chiralpak IC column with mobile phase of 30% 2-propanol/hexane (v/v). To measure limit of detection (LOD) and limit of quantification (LOQ) determination, samples from stock solution were taken and diluted to obtain a 3:1 and 10:1 signal to noise ratios [20]. The accuracy as well as precision experiments for both intra- and interday evaluation were performed at three enantiomeric purity of S-leucinol (98.4, 96.4 and 94.4%) as NBD derivatives. Spiking of (R)-enantiomer was carried out with (S)-enantiomer to obtain the required purity.

2.3 Results and Discussion

The enantiomeric separation of chiral aliphatic amines as NBD derivatives on polysaccharide-derived CSPs were performed using normal HPLC under simultaneous UV and FL detection. Several polysaccharide-derived amylose and cellulose triscarbamates or esters chiral selectors were used for the covalently bonded and coated type CSPs [7,10]. The covalently bonded type CSPs are Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak ID, Chiralpak IE and Chiralpak IF, while the coated type CSPs are Chiralcel OD-H, Chiralpak AD-H, Lux Cellulose-1 and Lux Amylose-1. Tables 2.1 and 2.2 show the enantiomer separation of several chiral amines and amino alcohols as NBD derivatives on covalently bonded and coated type polysaccharide-derived CSPs, respectively. The degree of enantioselectivity depends upon structural differences of the polysaccharide-derived

chiral selector (amylose or cellulose derivative), nature of analyte (amine or amino alcohol) and type of column (coated or covalently bonded) [7,10]. Among the six covalent chiral columns listed in Table 2.1, in general, Chiralpak IE showed the best enantiomeric separation and resolution, while Chiralpak IB showed the worst. The other four CSPs also displayed pretty good base-line enantiomer separation. The degree of enantioselectivity of the six covalently bonded chiral columns was as follows: Chiralpak IE > Chiralpak IC > Chiralpak IA > Chiralpak ID > Chiralpak IF >> Chiralpak IB. In case of coated type CSPs shown in Table 2.2, in general, the performance of Chiralpak AD-H and Lux Amylose-1 with the chiral selector of amylose tris(3,5-dimethylphenylcarbamate) was superior to that of Chiralcel OD-H and Lux Cellulose-1 with the same cellulose tris(3,5-dimethylphenylcarbamate) chiral selector.

Interestingly, it was observed that the enantiomer separation of the aliphatic amines (entry 1-3) was selectively effective on Chiralcel OD-H and Lux Cellulose-1, while that of amino alcohols (entries 4-9) on Chiralpak AD-H or Lux Amylose-1. The order of enantioselectivity in case of the coated CSPs was Lux Amylose-1 ~ Chiralpak AD-H > Lux Cellulose-1 >> Chiralcel OD-H. As the performance of amylose-derived CSPs (Chiralpak AD-H and Lux Amylose-1) was generally better than that of cellulose-derived CSPs (Chiralcel OD-H and Lux Cellulose-1) in Table 2.2, the same trend was also observed in Table 2.1 where Chiralpak IE with amylose tris(3,5-dichlorophenylcarbamate) showed much superior enantioselectivity than Chiralpak IC with cellulose tris(3,5-dichlorophenylcarbamate). Figure 2.2 shows typical chromatograms of 1-amino-2-propanol as NBD derivative on Chiralpak ID and Lux Amylose-1 under simultaneous UV and FL detection.

Accordingly from Table 2.1 and Table 2.2, the comparison between coated and covalent type columns with same chiral sorbents can be made. The performances in enantiomer separation of three columns, i.e. Chiralpak IA, Chiralpak AD-H, and Lux Amylose-1 with amylose tris(3,5-dimethylphenylcarbamate) as the chiral selector were examined. All analytes were generally resolved in both types of columns, but the coated type Chiralpak AD-H and Lux Amylose-1 showed greater selectivity with better separation and resolution factors than the covalent type Chiralpak IA. Similarly, for the same cellulose tris(3,5-dimethylphenylcarbamate) derived CSPs (Chiralpak IB, Chiralcel OD-H, and Lux Cellulose-1), enantiomeric separation on the coated CSPs, Lux Cellulose-1 and Chiralcel OD-H, was superior than that on the covalently bonded Chiralpak IB, and was in the order: Lux Cellulose-1 > Chiralcel OD-H >> Chiralpak IB. The low enantioselectivity on the immobilized CSPs is due to the lack of ordered arrangement of chiral selectors bonded to the silica of the CSPs [10,21]. Despite the fact that the enantioselectivity on the covalently bonded CSPs was generally lower than on the coated type CSPs with same chiral selector, the former may afford significant advantages in use of much wider range of solvents because of their higher column stability [10-13,21].

Table 2.1: Separation of the enantiomers of chiral amines as NBD derivatives on covalently bonded CSPs

Entry	Analytes	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.14	2.31[a]	2.02	1.04	3.90[a]	0.89	1.10	13.60	2.53
2	1,2-Dimethylpropylamine	1.00	3.00[a]	-	1.00	4.80[a]	-	1.10	14.90	2.43
3	1-Methylheptylamine	1.09	2.35[a]	1.20	1.04	3.85[a]	0.64	1.03	12.80	0.89
4	2-Amino-1-butanol	1.28	5.19	3.19	1.00	5.67	-	1.50	11.30	8.73
5	2-Amino-1 hexanol	1.42	3.00	4.75	1.00	3.79	-	1.53	9.83	8.49
6	2-Amino-4-methyl-1-pentanol	1.33	2.83	2.80	1.00	3.72	-	1.63	9.10	6.66
7	2-Amino-1-pentanol	1.44	3.09	6.06	1.00	4.44	-	1.57	10.40	8.24
8	1-Amino-2-propanol	1.14	5.55	2.05	1.00	7.60	-	1.00	13.74	-
9	2-Amino-1-propanol	1.36	6.20	3.01	1.00	6.96	-	1.42	13.65	5.05
Entry	Analytes	Chiralpak ID			Chiralpak IE			Chiralpak IF		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.13	7.69[a]	1.99	1.10	13.08[b]	1.19	1.13	2.65	1.26
2	1,2-Dimethylpropylamine	1.16	10.27[a]	2.73	1.44	12.48[b]	4.26	1.07	3.40	1.03
3	1-Methylheptylamine	1.11	9.00[a]	1.89	1.24	19.19[b]	2.63	1.13	3.18	1.37
4	2-Amino-1-butanol	1.22	3.47	1.59	3.89	4.24[b]	10.60	1.29	4.58	2.06
5	2-Amino-1 hexanol	1.19	3.00	1.31	3.87	4.11[b]	8.79	1.21	3.43	1.86
6	2-Amino-4-methyl-1-pentanol	1.26	2.71	2.11	4.18	2.37[b]	9.94	1.29	3.30	1.47
7	2-Amino-1-pentanol	1.21	2.98	2.13	4.44	3.67[b]	10.89	1.24	3.71	2.28
8	1-Amino-2-propanol	1.40	5.45	4.30	1.00	9.72[b]	-	1.18	6.16	1.77
9	2-Amino-1-propanol	1.28	3.96	3.57	3.32	4.75[b]	9.71	1.24	5.52	1.87

Mobile phase: 20% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 337 nm; fluorescence, 470 nm excitation, 530 nm emission, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]10% 2-propanol/hexane (v/v), [b]30% 2-propanol/hexane (v/v)

Table 2.2: Separation of the enantiomers of chiral amines as NBD derivatives on coated type CSPs

Entry	Analytes	Chiralcel OD-H			Lux Cellulose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	2.33	4.54	4.11	2.12	6.28	5.68
2	1,2-Dimethylpropylamine	1.52	5.93	2.91	1.26	6.78	2.04
3	1-Methylheptylamine	2.01	7.71	4.59	1.53	9.16	3.67
4	2-Amino-1-butanol	1.00	8.08	-	1.11	8.92	1.30
5	2-Amino-1 hexanol	1.13	5.86	0.84	1.10	7.79	0.94
6	2-Amino-4-methyl-1-pentanol	1.00	5.45	-	1.05	6.76	0.37
7	2-Amino-1-pentanol	1.00	6.23	-	1.00	7.96	-
8	1-Amino-2-propanol	1.00	10.13	-	1.12	11.32	1.56
9	2-Amino-1-propanol	1.14	8.25	0.89	1.19	10.43	2.31
Entry	Analytes	Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.20	1.93[a]	2.18	1.17	2.11[a]	2.13
2	1,2-Dimethylpropylamine	1.00	2.86[a]	-	1.00	3.05[a]	-
3	1-Methylheptylamine	1.11	1.85[a]	1.02	1.13	1.99[a]	1.55
4	2-Amino-1-butanol	1.51	2.95	5.35	1.48	3.29	5.58
5	2-Amino-1 hexanol	1.65	2.73	4.75	1.63	3.02	5.17
6	2-Amino-4-methyl-1-pentanol	1.69	2.54	5.02	1.58	2.96	5.26
7	2-Amino-1-pentanol	1.72	2.76	6.58	1.68	3.09	7.45
8	1-Amino-2-propanol	1.14	4.56	1.75	1.18	4.94	2.74
9	2-Amino-1-propanol	1.70	3.04	6.59	1.67	3.42	8.60

Mobile phase: 20% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 337 nm; fluorescence, 470 nm excitation, 530 nm emission, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]10% 2-propanol/hexane (v/v)

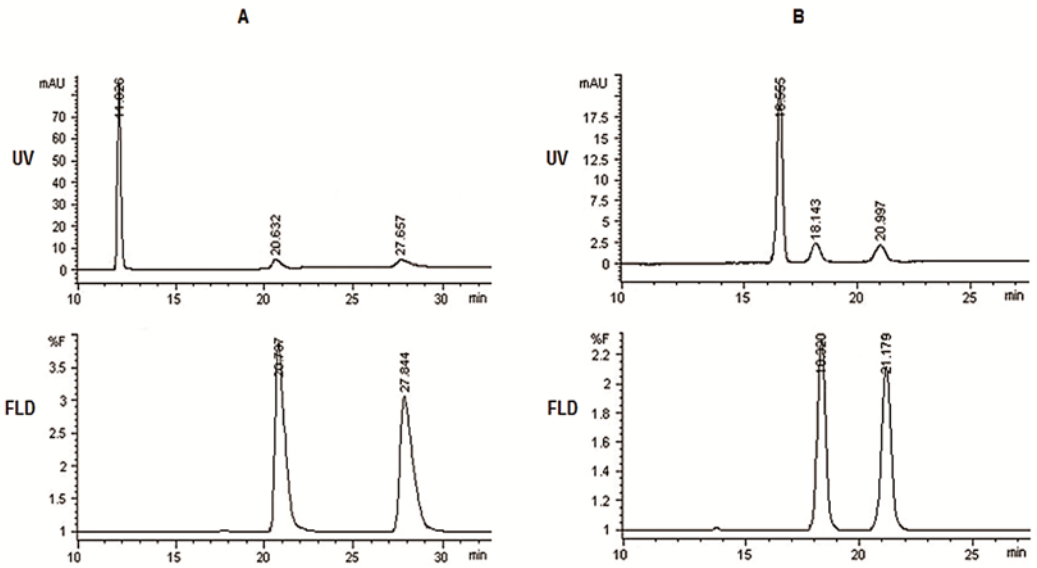

Figure 2.2: Representative chromatograms for enantiomer separation of 1-amino-2-propanol as NBD derivatives on (A) Chiralpak ID and (B) Lux Amylose-1 under simultaneous UV and FLD. Mobile phase: 20% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 337 nm; fluorescence, 470 nm excitation, 530 nm emission

To evaluate the developed chiral analytical method, intra- and interday experiments for accuracy and precision as well as LOD and LOQ determination were performed using (R)- and (S)-leucinol as NBD derivatives on Chiralpak IC. The accuracy and precision of the analytical method were estimated at three (S)-leucinols (enantiomeric purities of 98.4, 96.4 and 94.4%) as NBD derivatives under FL detection and are shown in Table 2.3. The accuracy for intra- and interday assay for the developed chiral method was determined to be 100.97-102.96% and 101.10-103.09%, respectively, while the precision for intra- and interday assay in terms of relative standard deviation (RSD) was found to be 0.30-1.22% and 0.78-1.40%, respectively. Owing to accuracy and precision results, the developed analytical method proved to be suitable and sensitive for enantiomer separation of chiral amines as NBD derivatives. LOD is the lowest concentration of analyte that produces an analytical

signal which is distinguishable from analytical noise at S/N of 3:1[20]. LOQ represents the concentration of analytes in a sample that can be quantitatively determined with an acceptable precision at S/N ratio of 10:1. The amount of (R)-leucinol as NBD derivative for LOD determination was found to be 7.5 ng and 0.5 ng under UV and FL detection, respectively. Also, the amount of (R)-leucinol for LOQ determination was 19.80 ng and 1.30 ng under UV and FL detection. The analytical method precision for both LOD and LOQ determination was less than 1% RSD. The sensitivity of FL detection to UV detection was 15:1 for the investigated chiral amine.

Enantiomeric purities of commercially available (R)- and (S)-2-amino-4-methyl-1-pentanol (entry 6, leucinol, Sigma-Aldrich) were measured, according to this developed analytical method. Figure 2.3 shows chromatograms of (R)- and (S)-leucinol as NBD derivatives on Chiralpak IC, eluted using 30% 2-propanol/hexane (v/v) under simultaneous UV and FL detection. The enantiomeric impurities were found to be 0.06% for both analytes, as shown in Table 2.4. An interesting fact was observed as the unreacted NBD-Cl peak as a starting material appeared at about 11 minutes under UV detection at 337 nm (Figure 2.3). However, it was not found under FL detection, because the used fluorogenic reagent, NBD-Cl is fluorescence inactive prior to derivatization. It results in affording simple analytical chromatograms under fluorescence detection, which is an additional advantage of this analytical method along with high sensitivity by fluorescence detection. The analyte of 2-amino-4-methyl-1-pentanol (leucinol) showed consistent second eluted isomer as (R)-leucinol on all the resolved CSPs except for Chiralpak IA and Chiralpak IC. As shown in Figure 2.3, (S)-leucinol was the more retained enantiomer on Chiralpak IC.

Table 2.3: Intra- and interday accuracy and precision of the analytical method validated using of three (S)-leucinols as NBD derivatives

Enantiomeric purity of (S)-leucinol (%)	Intraday (n=6)		Interday (n=6)	
	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
98.4	102.96	0.30	103.09	1.41
96.4	102.20	0.72	101.97	0.83
94.4	100.97	1.22	101.10	0.78

See experimental section for chromatographic conditions

Table 2.4: Determination of enantiomeric purity of commercially available (R)- and (S)-leucinol as NBD derivatives on Chiralpak IC

Entry	Analyte	R:S ratio[a]
1	(R)-leucinol	99.94 : 0.06
2	(S)-leucinol	0.06 : 99.94

[a]Average value of four replicates. See experimental section for chromatographic conditions

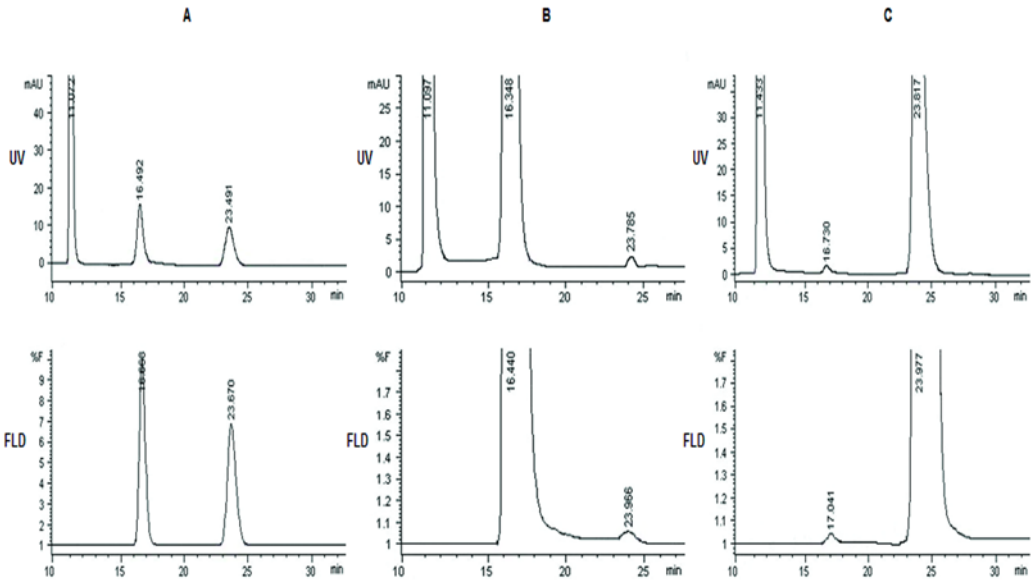

Figure 2.3: Representative chromatograms for enantiomeric separation of (A) rac-leucinol, (B) (R)-leucinol (R:S=99.94:0.06) and (C) (S)-leucinol (R:S=0.06:99.94) as NBD derivatives on Chiralpak IC under simultaneous UV and FLD. Mobile phase: 30% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 337 nm; fluorescence, 470 nm excitation, 530 nm emission

2.4 Conclusion

A derivatized chiral HPLC method was developed and validated for the enantiomeric separation of chiral aliphatic amines as NBD derivatives using normal phase HPLC under simultaneous UV and FL detection. Covalently bonded and coated type CSPs based on different amylose and cellulose derivatives were used and their performances were compared on the basis of mentioned chiral selectors. Chiralpak IE showed the best enantiomer separation for most analytes among the bonded CSPs, while Chiralpak AD-H and Lux Amylose-1 generally showed higher enantiomer separation among the coated type CSPs. The developed analytical method was applied to determine the enantiomeric purity of commercially available (R)- and (S)-leucinol and it proved to be sensitive, selective and suitable for the enantiomeric separation of chiral aliphatic amines as NBD derivatives.

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CHAPTER 3

2-Hydroxynaphthaldehyde as a Derivatizing Agent for Enantiomeric Separation of Chiral Aliphatic Amines using Polysaccharide-derived Chiral Stationary Phases under Normal HPLC

Abstract

The chromatographic enantiomer separation of chiral amines as 2-hydroxynaphthaldimine derivatives was performed on several coated and covalently bonded polysaccharide-derived chiral stationary phases (CSPs). A new potent derivatizing agent of 2-hydroxynapthaldehyde for enantiomer separation of chiral aliphatic amines was introduced not only to enhance detection sensitivity but also to provide suitable interaction sites for chiral separation. Amongst the examined CSPs, in general, the cellulose-derived CSPs showed better enantiomer separation than the amylose-derived CSPs. In particular, the covalently bonded type Chiralpak IC with cellulose-based chiral selector showed the best enantioseparation and resolution. The developed analytical method using 2-hydroxynaphthaldehyde as derivatizing agent was subjected to determine the enantiomeric purity of commercially available (R)- and (S)-leucinol where the impurities of the investigated analytes from two suppliers were found to be 0.06-1.20%. This analytical method was validated in accordance with ICH guidelines and it proved to be sensitive and applicable for the enantiomer separation of chiral aliphatic amines as 2-hydroxynaphthaldimine derivatives under ultraviolet (UV) detection.

Keywords: Chiral amine, Chiral stationary phase, Enantiomer separation, 2-Hydroxynaphthaldimine derivative

3.1 Introduction

Many pharmaceutical preparations which are being sold as racemic mixtures contain active ingredients having more than one stereoisomer or enantiomer [1]. The utmost concern is the recognition of these different enantiomers as they do not necessarily have to be identical, or even having desirable biological activity [2,3]. In fact, if a chiral pharmaceutical is administered as a single enantiomer, the verification of chiral purity in the term of their toxicological and pharmacological effects must be established or studied [3]. Thus, the difference in behavior of enantiomeric drug demands more selectivity and specificity of individual isomers in racemic drugs as well as optically active compounds in the term of analytical methods for the separation and analysis of enantiomers to ensure proper development of commercial pharmaceutical products [4-6].

Since a surplus of present commercialized drugs contains chiral amino groups, enantiomer analysis of chiral aliphatic amines has been an area of present research [7]. Various analytical methods have been reported and applied for the enantiomer separation of chiral compounds [8-10]. Among them, high performance liquid chromatography (HPLC) using the chiral stationary phases (CSPs) is most widely used and is more predictable method for the separation of enantiomers [4-6,10,11]. The resolution of enantiomers by direct HPLC using chiral stationary phases has been a subject of intensive investigation and an intriguing field. Polysaccharide (cellulose or amylose) derivatives exhibit the unique chiral recognition for the broad range of chiral compounds and widely used as CSPs by chiral HPLC [5,12,13].

The aliphatic chiral amine used in this study show low UV absorption and therefore a precise and accurate analytical method to enhance the detection and chiral discrimination is desirable. Derivatization of analytes with an achiral reagent is a better choice to achieve greater separability

or higher detection sensitivity. For this, proper derivatizing agents are required to provide as good interaction sites for enantiodiscrimination as well as to increase the detection sensitivity for analytes [14]. In our previous study, several derivatizing agents such as 9-anthrylaldehyde, benzophenone imine, phthaloyl anhydride and fluorenylmethoxycarbonyl chloride (FMOC-Cl) were used for enantiomer separation of chiral amines or amino acids [15-19]. In other groups, 2-hydroxynaphthaldehyde has been used as derivatizing agent to determine tranexamic acid in pharmaceutical preparation and blood, glutamine in cerebrospinal fluid as well as some other amino acids [20-22]. However, there is no attempt made to exploit 2-hydroxynaphthaldehyde as a potent achiral derivatizing agent for enantiomeric separation of chiral aliphatic amines. Therefore, in this study, we focus the establishment of 2-hydroxynapthaldehyde as a potent derivatizing agent for the first time to separate the enantiomer of chiral aliphatic amines using polysaccharide-derived CSPs. We also expected that the aromatic 2-hydroxynaphthyl moiety of the derivatized analytes might interact with the chiral selector of CSP for enantiomer separation as well as the enhanced detection of chiral aliphatic amines [15-18]. Herein, we describe a convenient and validated enantiomeric separation method of chiral aliphatic amines including amino alcohols as 2-hydroxynaphthaldimine derivatives on polysaccharide-derived CSPs under ultraviolet detection by normal HPLC.

3.2 Materials and Methods

3.2.1 HPLC and Chiral Columns

An Agilent 1100 HPLC system (Palo Alto, CA, USA) was used for the liquid chromatographic analysis which consists of a vacuum degasser with G1310A isocratic pump and an automatic sample injector with a thermostatic column compartment. The covalently bonded type derived from

polysaccharides Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak ID, Chiralpak IE and Chiralpak IF (250mm × 4.6mm, I.D., 5 µm) were purchased from Daicel Company (Tokyo, Japan). The coated type Chiralcel OD-H and Chiralpak AD-H (250mm × 4.6mm, I.D., 5 µm) were obtained from Daicel Company (Tokyo, Japan) whereas Lux Cellulose-1 and Lux Amylose-1 (250mm × 4.6mm, I.D., 5µm) were procured from Phenomenex (Torrance, CA, USA) respectively.

3.2.2 Chemicals and Experimental Conditions

HPLC-grade 2-propanol, ethanol, and hexane for the mobile phase were obtained from Burdick & Jackson (Morristown, NJ, USA). Chiral aliphatic amines including amino alcohols, 2-hydroxynaphthaldehyde and magnesium sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Haverhill, MA, USA). The commercially available (R)- and (S)-leucinols for the determination of enantiomeric purity and specific configuration were procured from Sigma-Aldrich (Supplier 1) (St. Louis, MO, USA) or Alfa Aesar (Supplier 2) (Haverhill, MA, USA). Chiral separation and resolution using HPLC was performed at room temperature with a flow rate of 1 mL/min using 3% 2-propanol/hexane (v/v) for aliphatic amines or 10% 2-propanol/hexane (v/v) for amino alcohols as the mobile phase under UV 254nm detection.

3.2.3 Sample Preparation by Derivatization

The 2-hydroxynaphthaldimine derivatives were prepared by stirring chiral primary amines and 2-hydroxynapthaldehyde (1-2 equivalents) with excess magnesium sulphate (7 equivalents) in ethanol at room temperature for 6 h, according to the modified procedure for preparation of 9-anthraldimine derivatives [18]. Mild reaction conditions were preferred in order to avoid the racemization of the derivatized samples. The obtained solution was filtered to remove excess

magnesium sulphate and was further diluted in 2-propanol for the direct injection in normal HPLC.

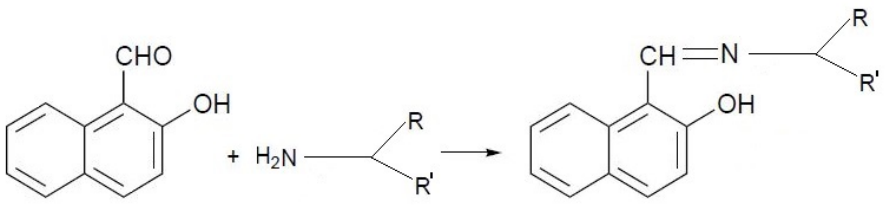

Figure 3.1: Preparation of chiral aliphatic amines as 2-hydroxynaphthaldimine derivatives

3.2.4 Method Validation

Validation experiments were done in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [23]. Limit of detection (LOD), limit of quantification (LOQ), linearity, intra- and interday accuracy as well as precision were measured to assess the analytical method. The validation of the developed method was carried out using a Lux Cellulose-1 column with a mobile phase of 10% 2-propanol/hexane (v/v). A signal-to-noise ratios of 3:1 and 10:1 were used to achieve LOD and LOQ, respectively, by injecting a series of prepared dilute solutions of (R)- and (S)-leucinol. Detector response linearity was assessed by preparing five sample solutions of (R)- and (S)-leucinol covering from LOQ to 150%. The calibration curve was obtained by plotting peak response of (R)- and (S)-leucinol versus its concentration using least square method. Intra- and interday accuracy and precision were evaluated at three enantiomeric purity level (98.4, 96.4 and 94.4%) of (R)- and S-leucinol as 2-hydroxynaphthaldimine derivatives.

3.3 Results and Discussion

The enantiomeric separation of chiral amines as 2-hydroxynaphthaldimine derivatives on covalently bonded and coated type polysaccharide-derived CSPs was performed using normal phase HPLC. The employed polysaccharide-derived CSPs comprise of derivatized cellulose and amylose tris carbamates or esters (chiral selectors) coated or chemically linked on silica gel support. Also, the backbone of chiral selectors (amylose-derived) is helical in structure consisting of a groove that is chiral in nature which significantly favors the binding of one enantiomer over the other and ultimately results in separation [12]. Table 3.1 and 3.2 show the enantiomer separation data of chiral aliphatic amines (entries 1-3) and amino alcohols (entries 4-9) on six covalently bonded and four coated type CSPs with n-hexane in 2-propanol as mobile phase. Most analytes under consideration were base-line separated with good resolution. In general, Chiralpak IC with cellulose tris(3,5-dimethylphenylcarbamate) showed the best enantiomer separation except two analytes (entries 8 and 9). Additionally, the enantioselectivities of chiral aliphatic amines (entries 1-3) on Chiralpak IC and Chiralpak ID were superior to those on the other covalently bonded CSPs. Like the covalently bonded CSPs in Table 3.1, coated type CSPs in Table 3.2 (Chiralcel OD-H or Lux Cellulose-1) having cellulose tris(3,5-dimethylphenylcarbamate) as the chiral selector showed enhanced enantiomer separation than CSPs (Chiralpak AD-H or Lux Amylose-1) with amylose tris(3,5-dimethylphenylcarbamate). Especially, the enantiodiscrimination of 2-amino-4-methyl-1-pentanol (entry 6, leucinol) was best on cellulose-derived CSPs (Chiralcel OD-H and Lux Cellulose-1). The differences observed between the cellulose- and amylose-based CSPs cannot be generalized based on the polysaccharide backbone only but it should be mentioned that overall results under this study depicted that the cellulose-based CSPs showed higher separation and resolution than amylose-based CSPs. Previously in our group, 9-anthraldehyde was used as a derivatizing agent for the enantiomeric separation of chiral amines on polysaccharide-derived CSPs.

Compared to the 9-anthraldimine derivatives, the 2-hydroxynaphthaldimine derivatives of this present study exhibited much greater enantioselectivities and sensitivities for the same covalently bonded and coated type CSPs [18]. Presumably, the 2-hydroxynaphthaldimine moiety of chiral amines for enantiomer resolution might function as a better interaction site than the corresponding 9-anthraldimine group of the bulky 9-anthryl structure with the same chiral selectors.

Table 3.1: Enantioseparation of chiral amines as 2-hydroxynaphthaldimine derivatives on covalently bonded CSPs

Entry	Analytes	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.46	2.23	5.99	1.61	1.80	4.98	1.97	9.25	12.74
2	1,2-Dimethylpropylamine	1.50	3.27	6.83	1.94	2.16	7.77	1.78	14.51	11.35
3	1-Methylheptylamine	1.08	2.85	1.28	1.05	2.78	0.56	1.64	12.33	8.42
4	2-Amino-1-butanol	1.38	2.86	3.83	1.51	4.46	2.95	1.86	8.45	9.97
5	2-Amino-1 hexanol	1.33	2.45	3.43	1.59	2.62	3.80	1.96	6.93	10.04
6	2-Amino-4-methyl-1-pentanol	1.14	2.10	1.45(R)[a]	2.09	1.75	5.62(S)[a]	1.90	5.78	9.74(S)[a]
7	2-Amino-1-pentanol	1.35	2.64	3.64	1.66	3.37	4.03	1.95	7.50	10.18
8	1-Amino-2-propanol	2.07	5.14	7.50	1.23	6.79	1.36	1.03	10.80	0.51
9	2-Amino-1-propanol	1.59	3.22	5.37	1.18	4.88	1.14	1.03	6.73	0.33
Entry	Analytes	Chiralpak ID			Chiralpak IE			Chiralpak IF		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	2.27	4.66	9.83	1.10	7.54	1.23	1.39	3.91	5.97
2	1,2-Dimethylpropylamine	2.54	6.31	14.00	1.09	8.54	1.08	1.55	5.11	7.49
3	1-Methylheptylamine	1.59	6.91	3.89	1.08	7.00	1.04	1.20	5.14	3.16
4	2-Amino-1-butanol	1.47	6.53	6.89	1.19	8.35	2.26	1.28	5.01	3.65
5	2-Amino-1 hexanol	1.55	5.66	6.30	1.18	7.10	1.52	1.33	4.08	3.57
6	2-Amino-4-methyl-1-pentanol	1.23	4.21	3.13(R)[a]	1.21	6.11	2.06(R)[a]	1.23	3.25	2.95(R)[a]
7	2-Amino-1-pentanol	1.47	6.20	6.21	1.19	7.77	1.67	1.29	4.28	3.52
8	1-Amino-2-propanol	1.00	14.49	-	1.02	14.33	0.24	1.32	3.48	3.79
9	2-Amino-1-propanol	1.77	4.23	9.88	1.16	3.89	1.44	1.31	2.41	3.16

Mobile phase: 3% 2-propanol/hexane (v/v) (entries 1-3, chiral aliphatic amines) and 10% 2-propanol/hexane (v/v) (entries 4-9, amino alcohols), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]the absolute configuration of the second eluted enantiomer

Table 3.2: Enantioseparation of chiral amines as 2-hydroxynaphthaldimine derivatives on coated type CSPs

Entry	Analytes	Chiralcel OD-H			Lux Cellulose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.84	3.94	7.28	1.88	4.74	8.47
2	1,2-Dimethylpropylamine	2.35	4.74	8.93	2.50	5.20	12.76
3	1-Methylheptylamine	1.15	6.50	1.69	1.30	8.10	2.57
4	2-Amino-1-butanol	1.39	2.96	4.03	2.50	4.93	9.21
5	2-Amino-1 hexanol	1.36	2.67	3.77	2.29	4.41	8.61
6	2-Amino-4-methyl-1-pentanol	4.46	2.04	12.94(S)[a]	4.04	2.76	15.66(S)[a]
7	2-Amino-1-pentanol	1.36	2.78	3.55	2.30	4.69	7.87
8	1-Amino-2-propanol	2.00	2.43	5.35	2.05	7.81	8.82
9	2-Amino-1-propanol	1.72	2.02	3.44	1.63	2.42	4.61
Entry	Analytes	Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.25	2.84	3.37	1.33	3.09	5.31
2	1,2-Dimethylpropylamine	1.29	4.54	4.56	1.38	4.58	6.13
3	1-Methylheptylamine	1.05	3.44	0.62	1.04	3.78	0.62
4	2-Amino-1-butanol	1.39	2.99	4.05	1.39	3.19	4.89
5	2-Amino-1 hexanol	1.36	2.66	3.73	1.38	2.91	4.67
6	2-Amino-4-methyl-1-pentanol	1.15	2.27	1.70(R)[a]	1.15	2.81	1.95(R)[a]
7	2-Amino-1-pentanol	1.37	2.80	3.69	1.40	3.12	5.53
8	1-Amino-2-propanol	1.38	1.79	2.37	1.62	1.90	5.65
9	2-Amino-1-propanol	1.52	1.16	3.47	1.58	2.81	1.95

Mobile phase: 3% 2-propanol/hexane (v/v) (entries 1-3, chiral aliphatic amines) and 10% 2-propanol/hexane (v/v) (entries 4-9, amino alcohols), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]the absolute configuration of the second eluted enantiomer

Also, comparison between covalently bonded (Chiralpak IB) in Table 3.1 and coated type (Chiralcel OD-H and Lux Cellulose-1) CSPs in Table 3.2, which contains the same chiral selectors of cellulose tris(3,5-dimethylphenylcarbamate) was made. All the analytes were discriminated with good separation and resolution factors in both type of CSPs, but in general the coated type CSPs showed greater enantioselectivity than the covalently bonded CSPs. The lowered enantioselectivities on the bonded CSP is due to the lack of ordered arrangement of the chiral selector bonded to the slilca matrix [24,25]. Consistent trends were observed in case of the elution

order in both coated and covalently bonded CSPs having the same chiral selector. The (S)-enantiomer of 2-amino-4-methyl-1-pentanol (entry 6, leucinol) was selectively retained on Chiralpak IB (covalently bonded type), Chiralcel OD-H, and Lux Cellulose-1(coated type) having same cellulose tris(3,5-dimethylphenylcarbamate) as chiral selector. Likewise, the (R)-enantiomer was preferentially retained on same chiral selector columns of amylose tris(3,5-dimethylphenylcarbamate) and shown in Table 3.1 and 3.2. It is considered that the different elution orders were attributed to the different backbones of the amylose- or cellulose-derived chiral selectors [26,27]. It is also worth noting that the chemical linkage or covalently bonding between the chiral selectors on silica gel offers a much wider range of solvents for separation and eventually higher column stability [19,25]. Figure 3.2 shows typical chromatograms for the enantiomeric resolution of the chiral aliphatic amines on the covalently bonded and coated type CSPs.

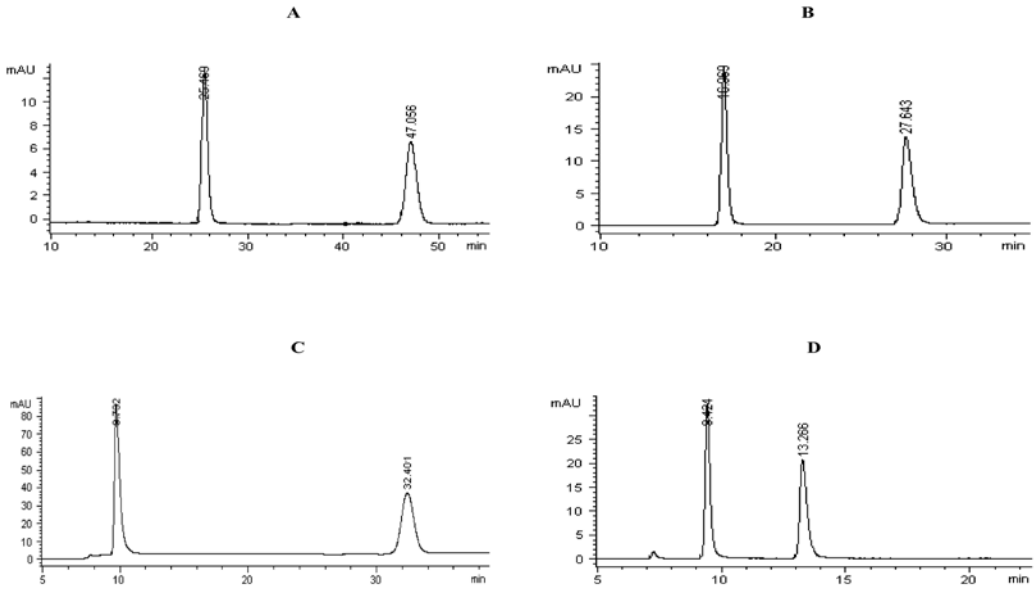

Figure 3.2: Typical chromatograms of the enantiomeric separation of chiral amines as 2-hydroxynaphthaldimine derivatives on covalently bonded and coated type CSPs; (A) 2-amino-1-hexanol on Chiralpak IC, (B) 2-amino-1-propanol on Chiralpak ID, (C) 2-amino-4-methyl-1-pentanol on Chiralcel OD-H and (D) 1-amino-2-propanol on Lux Amylose-1. Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm

The LOD and LOQ concentration determined from signal-to-noise-ratio of 3:1 and 10:1 of the developed analytical method for (R)-leucinol were found to be 1.36 and 4.14 µg/mL and for (S) leucinol 1.49 and 4.75 µg/mL, respectively. Therefore, the developed method is believed to have adequate sensitivity for the detection and estimation of (R)- and (S)-leucinol. In linearity test, the correlation coefficient obtained was 0.999 for (R)-leucinol with equation of calibration curve Y=1.9614x - 0.1639 over the loading range of 4.14 (LOQ) to 24.75 µg/mL, and for (S)-leucinol correlation of 0.999 with the equation of calibration curve Y=1.6101x - 0.2442 over the loading range of 4.75 (LOQ) to 28.50 µg/mL; the data obtained reveal that an excellent correlation existed between the peak area and concentration of (R) and (S)-leucinol. Intra- and interday accuracy and precision were carried out using (R)- and (S)-leucinol as 2-hydroxynaphthaldimine derivatives on Lux Cellulose-1 with a mobile phase of 10% 2-propanol/hexane (v/v). Table 3.3 shows chromatographic data from three (R)- and (S)-leucinol samples with enantiomeric purities of 98.4, 96.4 and 94.4% for the determination of the accuracy and precision of the developed analytical method. The intra- and interday accuracy for (R)-leucinol were 101.05-103.05% and 101.03-103.04%, respectively, while the intra- and interday precision were found to be 0.67-0.86% and 0.62-0.82%, respectively. Similarly, intra- and interday accuracy of the developed analytical method in the case of (S)-leucinol were 101.07-103.06% and 101.08-103.07%, respectively, while the intra- and interday precision were found to be 0.20-0.55% and 0.25-0.45%, respectively.

Table 3.3: Intra- and interday accuracy and precision of the analytical method validated using of three (R)- and (S)-leucinols as 2-hydroxynaphthaldimine derivatives on Lux Cellulose-1

Enantiomeric purity of (R)- and (S)-leucinol (%)	Intraday (n=9)		Interday (n=9)		Intraday (n=9)		Interday (n=9)	
	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
98.4	103.05	0.67	103.04	0.45	103.06	0.55	103.07	0.45
96.4	102.12	0.75	102.08	0.24	102.03	0.36	102.01	0.24
94.4	101.05	0.86	101.03	0.25	101.07	0.20	101.08	0.25

See experimental section for chromatographic conditions

The developed analytical method using 2-hydroxynaphthaldehyde as a potent derivatizing agent was applied to the determination of enantiomeric purity also as a test of the applicability of the method. The enantiomeric purities of commercially available (R)- and (S)-2-amino-4-methyl-1-pentanol (entry 6 of Tables 3.1 and 3.2, leucinol) were determined on Lux Cellulose-1, and the percentage of impurities was found to be 0.06% for both analytes of Supplier 1. In case of Supplier 2, the enantiomeric impurities of both (R)- and (S)-leucinols were found to be 1.20 and 0.15%, respectively. Figure 3.3 shows the chromatograms for the enantiomeric purity determination of racemic, (R)- and (S)-leucinol (Supplier 2) as 2-hydroxynaphthaldimine derivatives on Lux Cellulose-1.

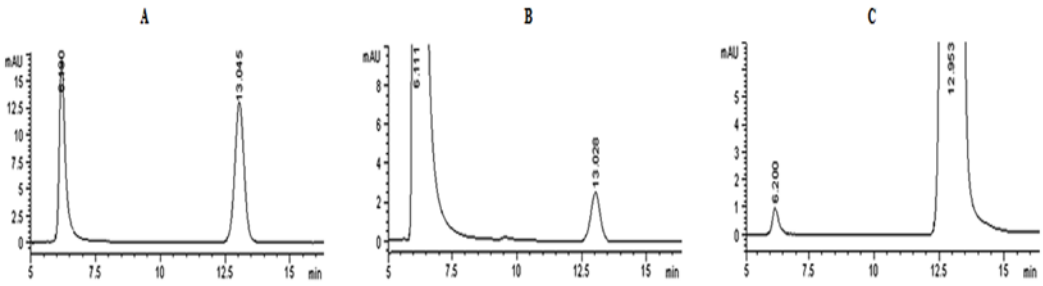

Figure 3.3: Chromatograms for enantiomeric purity check of (A) rac-leucinol, (B) (R:S=98.80:1.20) and (C) (S)-leucinol (R:S=0.15:99.85) as 2-hydroxynaphthaldimine derivatives on Lux Cellulose-1. Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm

3.4 Conclusion

This is the first reported analytical method establishing 2-hydroxynapthaldehyde as a potent derivatizing agent for enantiomeric separation of chiral aliphatic amines including amino alcohol using normal HPLC. The enantioselectivity was affected by in the structural makeup of the used chiral selector (polysaccharide amylose or cellulose derivative), the nature of the investigated analyte (amine or amino alcohol), and the type of CSP (covalently bonded or coated-type). Among the investigated CSPs, in general, cellulose-derived chiral selector columns, such as Chiralpak IC (covalently bonded) and Lux Cellulose-1 (coated type) showed the superior enantioselectivity and resolution. This described analytical method proved to be suitable and selective for the separation of chiral amines as 2-hydroxynaphthaldimine derivatives after the validation experiment and is expected to be applied for the determination of enantiomeric purities which is the prime interest in pharmaceutical industry for developing and marketing enantiomerically pure chiral drugs.

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78:473-479

CHAPTER 4

Enantiomeric Discrimination and Resolution of 1-Naphthylamides of Several Chiral Acids and Normal Phase HPLC

Abstract

The liquid chromatographic enantiomer separation of various chiral acids was performed using several chiral stationary phases (CSPs). The CSPs employed in this study were six covalently bonded and four coated type CSPs derived from amylose and cellulose derivatives as chiral selectors. The degree of enantioseparation is affected by the structure of chiral acids and the CSPs used. The performance of the coated type Lux Cellulose-1 was superior to those of the other CSPs, except for 2-aryloxypropionic acid derivatives. Owing to the strong ultraviolet (UV) absorbance of the 1-naphthyl group, the convenient and validated chromatographic method developed in this present study could be useful for the enantiomer separation of various chiral acids as 1-naphthylamide derivatives using polysaccharide-derived CSPs.

Keywords: Chiral acid, Chiral stationary phase, Enantiomer separation, 1-Naphthylamide derivative

4.1 Introduction

In pharmaceuticals, chiral acids have been widely used as chiral drugs and important building blocks and their enantiomeric separation for the development of pure chiral drugs as well as their analogs has been of great interest [1]. Two enantiomers show same physical and chemical properties and accordingly they are differentiated only under chiral environments. Therefore, in living systems which are strictly enantioselective, two enantiomers shows different chemical properties and biological properties. Consequently, only one enantiomer of chiral drugs may show desired biological activities, while the other enantiomer exhibits undesired or toxic effects [1,2]. In the field of chirotechnology, several analytical methods have been developed to discriminate the enantiomer of various chiral acids [1,3]. Among them, liquid chromatographic enantiomer separation using chiral stationary phases (CSPs) is known to be one the most convenient, accurate and versatile methods [3-5]. Polysaccharides-derived CSPs are very selective and powerful in the resolution of wide range of racemates and are widely used as CSPs [5,6]. For the better chiral separation and recognition, the understanding of interaction between enantiomer and chiral selector is very important as both mechanisms go together [6]. Especially, Brush or Pirkle type CSPs having chiral selectors made up small molecules have an advantage in understanding of the chiral interaction between enantiomer and chiral selector [3,5]. Previously, in our research group, direct enantiomeric separation and resolution of non-steroidal anti-inflammatory drugs, N-protected amino acids, pyrethroic acids and 2-aryloxypropionic acids was performed using Pirkle type or polysaccharide type CSPs [7-11]. Moreover, several indirect enantiomer separations and resolutions of chiral analytes have been reported also by our research group [12-17]. These derivatization processes used in this study is expected to enhance significantly the detection sensitivity as well as enantioselectivity. Especially, for 2-aryloxypropionic acids separation, 1- and 2-napthylamide derivatives have been utilized on two Pirkle type CSPs [13]. In addition, several

enantiomer resolutions of chiral amines, amino alcohols and amino acid esters as α- and β-naphthoyl, 9-anthraldimine and benzophenone imine derivatives have been reported using polysaccharide-derived or Pirkle type CSPs [12,13,15,16,17].

The previously employed aromatic groups such as the naphthoyl groups or 9-anthryl moiety not only provide a good interaction site for enantiomer separation, but also play an vital role in enhancing the detection of analytes. Obviously, there is no doubt that the use of these derivatizing groups in enantiomer separation could enhance the chiral discrimination [12,13,15,17]. The hydrazide derivatives of 2-aryloxypropionic acids have been used to form the complex between amino moiety with crown ether type CSPs for the appropriate interaction site during chiral recognition and resolution [14]. As other typical application example, enantiomer separation of 2-methylbutyric acid as 1-naphthyl derivative was done very recently to determine the absolute configuration of a natural product of marinopyrone D [18]. In this study, therefore, we perform achiral derivatization of several chiral acids using aromatic 1-napthylamine with an aim to develop a convenient chromatographic method for separating the enantiomers of various chiral acids as 1-naphthylamides using normal HPLC on polysaccharide-derived CSPs.

4.2 Materials and Methods

4.2.1 Liquid Chromatography

Two high performance liquid chromatography (HPLC) systems were used in the all chromatographic analysis. These were the Waters Breeze HPLC system, which consisted of a Waters 1525 binary HPLC pump, 2487 dual absorbance detector and 717 plus auto sampler, and the other was an HP series 1100 with a G1310A Isopump and an automatic sample injector. Covalently

bonded type CSPs of Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak ID, Chiralpak IE, and Chiralpak IF as well as coated type CSPs of Chiralpak AD-H and Chiralpak OD-H (250 mm × 4.6 mm, I.D., 5 μm) were purchased from Daicel Chemical Company (Tokyo, Japan) (Daicel chiral columns, 2013). The other two coated type CSPs of Lux Amylose-1 and Lux Cellulose-1 (250 mm × 4.6 mm, I.D., 5 μm) were purchased from Phenomenex (Torrance, CA, USA). High performance liquid chromatography was performed at room temperature and a flow rate of 1 mL/min; 5-30% 2-propanol/hexane (v/v) were used as mobile phases. The entire chromatographic analyses were performed under UV 215 nm detection.

4.2.2 Chemicals

HPLC grades of solvents were obtained from Burdick & Jackson (Morristown, NJ, USA). The racemic and (S)-analyte of ibuprofen (Sigma-Aldrich, St. Louis, MO, USA) and 2-methylbutyric acid (Santa Cruz Biotechnology, Dallas, TX, USA) as well as 2-aryloxypropionic acids and 1-naphthylamine (Sigma-Aldrich, St. Louis, MO, USA) were commercially available. N-t-butoxycarbonyl (BOC) and phthaloyl (PHT) protected amino acids have been used in previous studies [8, 10]. The 1-naphthylamide derivatives of several chiral acids were prepared according to the conventional method [19]. The resulting solution was filtered to remove the insoluble solids, diluted with 2-propanol, and then directly injected into the HPLC system.

4.2.3 Method Validation

Several validation experiments were performed in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [20]. The limit of detection (LOD) and limit of quantification (LOQ) of (S)-ibuprofen enantiomer

was achieved by injecting the prepared dilute solutions to obtain a 3:1 and 10:1 signal to noise ratios using 20% 2-propanol/hexane under UV 215 nm. Linear leastsquare regression analysis was used for detector response linearity and was performed by preparing the series of sample solutions of (S)-ibuprofen enantiomer from 6.0 ng (LOQ) to 15.0 ng in diluent. To obtain intra- and interday accuracy and precision, racemic ibuprofen was spiked with (S)-ibuprofen.

4.3 Results and Discussion

The chromatographic enantiomer separation of various chiral acids as 1-naphthylamides was performed using polysaccharide-derived six covalently bonded and four coated type CSPs, respectively. The degree of enantioselectivity relies on the structure of the amylose or cellulose-derived chiral selector, nature of analyte (such as protected amino acids or 2-aryloxypropionic acid) and column type (covalently bonded or coated) [4]. Table 4.1 shows the chromatographic result of the enantiomer separation of the 1-naphthylamides of various chiral acids using six covalently bonded type CSPs. In general, all the chiral amine analytes were resolved on Chiralpak IA, Chiralpak ID and Chiralpak IF columns with good separation and resolution factors. In particular, Chiralpak IA showed the significantly higher enantiodiscrimination for analytes 1-4 than the other covalently bonded CSPs, while Chiralpak IE showed the lowest enantioseparation for most analytes. The order of enantioselectivity for the covalently bonded CSPs was: Chiralpak IA > Chiralpak ID > Chiralpak IF > Chiralpak IB > Chiralpak IC > Chiralpak IE.

Table 4.2 shows the results of the four coated type CSPs of Chiralpak AD-H, Lux Amylose-1, Chiralcel OD-H and Lux Cellulose-1. All CSPs showed significantly greater enantioseparation for the entries 1-4 analytes as 1-naphthylamide derivatives. On the other hand, Lux Cellulose-1 showed the best enantioseparation and resolution for all other analytes, except for 2-aryloxypropionic acid

derivatives (entries 7, 8). Chiralpak AD-H and Lux Amylose-1 showed high enantioseparation for most analytes except for N-PHT phenylalanine and/or (3-chlorophenoxy) propionic acid as 1-naphthylamide derivatives. The performance of Chiralpak AD-H was comparable to that of Lux Amylose-1 with the same amylose tris(3,5-dimethylphenylcarbamate) in Table 4.2. Almost all the analytes resolved showed consistent trend of elution orders on both coated and covalently bonded type CSPs as shown in Tables 4.1 and 4.2.

Among all the CSPs in Tables 4.1 and 4.2, the coated type cellulose-derived Lux Cellulose-1 showed the best enantiomer separation, except for 2-aryloxypropionic acid derivatives (entries 7, 8). In general, the enantioselectivities observed on the coated type columns in Table 4.2 were better than those on the covalently bonded type columns in Table 4.1, except for 2-aryloxypropionic acid derivatives. For example, it was observed that the coated type CSP Chiralpak AD-H and Lux Amylose-1 in Table 4.2 showed better results than that of the covalently bonded type CSP Chiralpak IA in Table 4.1, which contain the same amylose tris(3,5-dimethylphenylcarbamate) as the chiral selector. Similarly, the coated type CSP Chiralcel OD-H and Lux Cellulose-1 in Table 4.2 exhibited better result than the covalently bonded type CSP Chiralpak IB in Table 4.1, which have the same cellulose tris(3,5-dimethylphenylcarbamate) as the chiral selector. Low enantioselectivity observed in covalently bonded CSPs might be the lack of ordered arrangement of the chiral selector bonded to the silica matrix [9,11]. Although the enantiomer separation was generally lower than that the coated type CSP with the same chiral selector, it is noteworthy that the covalently bonded CSP provides an advantage of solvent versatility because of the higher column stability of the immobilized chiral selector [10,11].

Table 4.1: Enantiomer separation of various chiral acids as 1-naphthylamides on covalently bonded CSPs

Entry	Analyte	Chiralpak IA				Chiralpak IB				Chiralpak IC			
		α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.
1	Ibuprofen	2.28	0.90[a]	8.77	R	1.76	1.75[a]	7.11	R	1.44	2.13[a]	5.39	R
2	2-Methylbutyric acid	1.24	2.70	2.72	R	1.22	5.77	2.46	R	1.20	4.36	3.47	R
3	N-BOC leucine	2.24	1.25	7.60	R	1.00	0.63	-	-	1.00	1.12	-	-
4	N-BOC phenylalanine	1.77	2.34	6.63	R	1.60	1.25	4.03	R	1.34	2.24	2.96	S
5	N-PHT leucine	1.05	3.01[a]	0.52	S	1.11	6.81[a]	1.17	S	1.19	7.61[a]	2.68	S
6	N-PHT phenylalanine	1.06	5.14[a]	0.76	S	1.10	5.30[a]	1.08	S	1.00	18.15[a]	-	-
7	(3-Chlorophenoxy)propionic acid	1.08	2.71[b]	1.15	-	1.17	2.33	2.10	-	1.73	3.16	9.13	-
8	(2,4-Dichlorophenoxy)propionic acid	1.01	3.36[b]	0.33	-	1.32	3.01[b]	3.26	-	1.11	2.36[b]	1.76	-
Entry	Analyte	Chiralpak ID				Chiralpak IE				Chiralpak IF			
		α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.
1	Ibuprofen	1.47	1.41[a]	5.38	R	1.18	2.29[a]	1.78	R	1.30	1.52[a]	2.92	R
2	2-Methylbutyric acid	1.07	2.76	1.29	R	1.00	4.31	-	-	1.04	3.91	0.82	R
3	N-BOC leucine	1.50	2.18	4.30	R	1.39	2.47	1.58	R	1.82	1.40	6.01	R
4	N-BOC phenylalanine	1.49	4.18	5.95	S	1.00	5.36	-	-	1.09	2.65	1.04	R
5	N-PHT leucine	1.06	7.75[a]	1.53	S	1.00	0.98[a]	-	-	1.14	6.01[a]	1.96	R
6	N-PHT phenylalanine	1.32	7.83[c]	3.87	S	1.24	1.76[a]	2.17	S	1.17	9.57[a]	3.68	S
7	(3-Chlorophenoxy)propionic acid	1.05	2.33	0.90	-	1.12	3.12	1.26	-	1.06	2.08	0.75	-
8	(2,4-Dichlorophenoxy)propionic acid	1.09	1.93[b]	1.24	-	1.17	2.11[b]	1.53	-	1.09	1.85[b]	1.07	-

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 215 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, Conf.: the absolute configuration of the second eluted enantiomer, [a]20% 2-propanol/hexane (v/v), [b]5% 2-propanol/hexane (v/v), [c]30% 2-propanol/hexane (v/v)

Table 4.2: Enantiomer separation of various chiral acids as 1-naphthylamides on coated type CSPs

Entry	Analyte	Chiralpak AD-H				Lux Amylose-1			
		α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.
1	Ibuprofen	2.68	0.93	7.75	R	2.81	0.85	8.80	R
2	2-Methylbutyric acid	1.28	5.35[a]	4.77	R	1.30	5.09[a]	5.69	R
3	N-BOC leucine	2.50	1.39[b]	9.73	R	2.37	1.40[b]	7.71	R
4	N-BOC phenylalanine	1.97	2.43[b]	7.40	R	1.70	2.53[b]	7.14	R
5	N-PHT leucine	1.06	2.65	0.78	S	1.07	3.16	1.11	S
6	N-PHT phenylalanine	1.00	6.07	-	-	1.00	7.00	-	-
7	(3-Chlorophenoxy)propionic acid	1.10	3.62[a]	1.64	-	1.00	3.72[a]	-	-
8	(2,4-Dichlorophenoxy)propionic acid	1.06	4.63[a]	1.07	-	1.35	3.71[a]	3.47	-
Entry	Analyte	Chiralcel OD-H				Lux Cellulose-1			
		α	k'_1	R_s	Conf.	α	k'_1	R_s	Conf.
1	Ibuprofen	2.37	3.55	9.55	R	2.32	4.53	11.58	R
2	2-Methylbutyric acid	1.28	4.52	2.97	R	1.29	6.58	3.55	R
3	N-BOC leucine	7.78	1.25[b]	14.45	R	8.51	1.51[b]	23.01	R
4	N-BOC phenylalanine	1.88	2.86[b]	5.43	R	1.90	3.49[b]	7.42	R
5	N-PHT leucine	1.38	6.14	3.31	S	1.40	6.70	4.47	S
6	N-PHT phenylalanine	1.23	12.72	1.74	S	1.31	14.94	3.77	S
7	(3-Chlorophenoxy)propionic acid	1.00	7.03[b]	-	-	1.00	12.18[b]	-	-
8	(2,4-Dichlorophenoxy)propionic acid	1.00	8.36[b]	-	-	1.00	11.03[b]	-	-

Mobile phase: 20% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 215 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, Conf.: the absolute configuration of the second eluted enantiomer, [a]5% 2-propanol/hexane (v/v), [b]10% 2-propanol/hexane (v/v)

Interestingly, it was observed that the chiral acid analytes (entries 1-4 of ibuprofen, 2-methylbutyric acid and BOC amino acids) were much better resolved than the other analytes (entries 5-8 of PHT amino acids and 2-aryloxypropionic acids) in Tables 4.1 and 4.2. Undoubtedly, 1-naphthyl moiety of the derivatized analytes (entries 1-4) might serve as a good interaction site with the chiral selector of the CSP [13]. However, 1-naphthyl moiety or the aromatic 2-aryloxy (or phthaloyl) group of the analytes (entries 5-8) could competitively interact with the chiral selectors of the CSP for chiral recognition, resulting in the poor enantioseparation of entries 5-8 analytes. Two commercially available (S)-ibuprofen and (S)-2-methylbutyric acid as 1-naphthylamide derivatives were applied to check enantiomeric purity, according to the presently developed

analytical method. Table 4.3 shows that the enantiomeric impurity obtained using Chiralpak IC for (S)-ibuprofen and Chiralpak IA for (S)-2-methylbutyric acid and found to be 0.36% and 0.86%, respectively. Figure 4.1 shows the representative chromatograms of two analytes as 1-naphthylamides for the determination of enantiomeric purity. The results obtained from the method validation study using (S)-ibuprofen as 1-naphtylamide derivative on Chiralpak IC are shown in Table 4.4. The intra- and interday accuracy was found to be 100.90-102.94 and 100.92-100.97%, respectively, while intra- and inter-day precision calculated in terms of relative standard deviation was 0.46-2.19 and 0.33-2.23%, respectively. In the linearity test, the correlation coefficient was 0.9998 for (S)-ibuprofen as 1-naphtylamide derivative over a loading amount range of 6.0-15.0 ng, as shown in Figure 4.2. These results indicated that there is a strong correlation existed between the peak area and concentration of (S)-ibuprofen. The amount of (S)-ibuprofen as 1-naphtylamide derivative for LOD and LOQ determination was found to be 2.0 and 6.0 ng under UV 215 nm detection, respectively.

Table 4.3: Determination of enantiomeric purity of commercially available (S)-ibuprofen and (S)-2-methylbutyric acid as 1-naphthylamide derivatives

Analyte	R : S ratio	Company
(S)-ibuprofen	0.36 : 99.64	Sigma-Aldrich
(S)-2-methylbutyric acid	0.86 : 99.14	Santa Cruz Biotechnology

Flow rate: 1mL/min, UV 215 nm detection, Mobile phase: 5% 2-propanol/hexane (v/v)

Table 4.4: Intra- and interday accuracy and precision of the analytical method validated using of (S)-ibuprofen as 1-napthylamide derivative at three purity levels

Enantiomeric purity of (S)-ibuprofen (%)	Intraday (n=6)		Interday (n=6)	
	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
98.6	102.94	0.79	102.97	0.77
97.6	102.23	0.46	102.17	0.33
96.6	100.90	2.19	100.92	2.23

Flow rate: 1mL/min, UV 215 nm detection, Mobile phase: 20% 2-propanol/hexane (v/v)

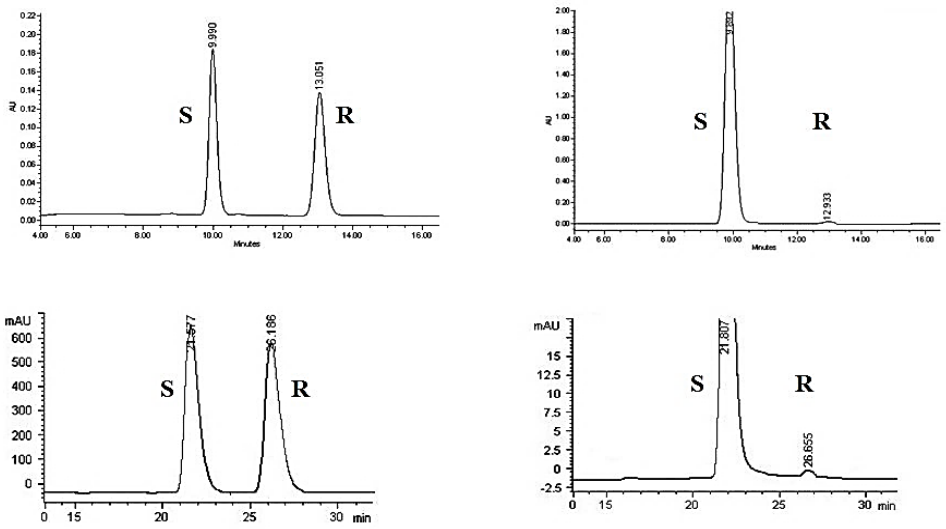

Figure 4.1: Chromatograms of enantiomer resolution of 1-naphthylamides of racemic ibuprofen (top left) and commercially available (S)-ibuprofen (top right) using Chiralpak IC with 1-naphthylamides of racemic 2-methylbutyric acid (bottom left) and commercially available (S)-2-methylbutyric acid (bottom right) using Chiralpak IA. See chromatographic conditions in Table 4.4

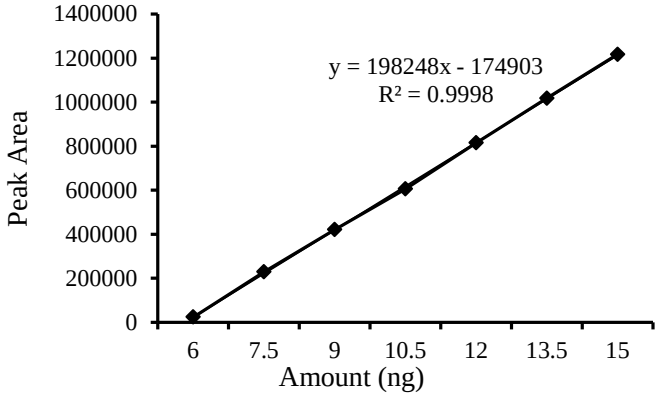

Figure 4.2: Linearity graph

4.4 Conclusion

For the normal HPLC analysis, the liquid chromatographic enantiomer separation method of various chiral acids as 1-naphthylamide derivatives was developed in this study using several polysaccharide-derived CSPs. Among all the CSPs investigated, the coated type Lux Cellulose-1 showed the greatest enantiomer separation of the chiral acids as 1-naphthylamide derivatives, except for 2-aryloxypropionic acid derivatives. The developed analytical method after validation experiments proved to be accurate, reliable and sensitive. Owing to the strong UV detection sensitivity and enhanced enantioselectivity afforded by the 1-naphthyl group of this study, this convenient liquid chromatographic enantiomer separation method is expected to be very useful for the resolution and determination of enantiomeric purity of various chiral acids as 1-naphthylamide derivatives.

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CHAPTER 5

Enantiomer Separation and Molecular Modeling Study of Chiral Amines as Several Naphthaldimine Derivatives on Amylose and Cellulose-derived Chiral Stationary Phases

Abstract

Enantiomer separation of chiral amines using three aromatic aldehyde derivatizing agents on normal high performance liquid chromatography (HPLC) was studied and compared between amylose and cellulose-based chiral stationary phases (CSPs) under ultraviolet (UV) detection. In particular, amylose-based CSPs showed the enhanced enantioselectivity and resolution, except for 2-hydroxynaphthaldimine derivatives on cellulose-derived CSPs. The most conformationally rigid 2-hydroxynaphthaldimine derivatives using three kinds of derivatizing agents afforded the greatest enantioseparation on conformationally rigid cellulose-derived CSPs and vice versa. Chiral recognition mechanism as well as the elution order of the investigated analytes were determined by docking simulation studies. The enantiomeric discrimination of analytes was controlled by hydrogen, π-π, hydrophobic, steric, etc interactions. The described method is fast, reproducible, precise and selective, which can be used successfully for evaluating the enantiomers of the reported chiral amines.

Keywords: Aromatic aldehyde derivatizing agent, Chiral amine, Enantiomer separation, Docking simulation, Normal phase mode

5.1 Introduction

Stereoselectivity is often a characteristic feature of enzymatic reaction, messenger-receptor interaction and metabolic process [1]. In search for safer and more effective drugs, recent trend in pharmaceuticals research and development is mostly inclined towards the generation of enantiomerically pure compounds [1]. Optically active amines form a large group of compounds of both biochemical and pharmaceutical interest [2,3]. So, the efforts are made to develop a convenient and precise chiral analytical techniques to separate and quantify their enantiomeric composition [3,4]. A lot of progress has been made in analyzing these substances due to the rapid development of liquid chromatography and new derivatization procedures which enhance separation, detection, as well as resolution. An investigation using normal liquid chromatographic (HPLC) resolution with polysaccharide-derived chiral stationary phases (CSPs) of chiral amines is of considerable importance because it can provide a suitable and convenient method for determining enantiomeric purity, absolute configuration as well as preparatively resolving large quantity of these interesting molecules [5-7]. Polysaccharide (cellulose and amylose-derived) CSPs exhibits the excellent chiral recognition for the broad range of chiral compounds and are widely used as CSPs for liquid chromatographic method [7-11]. In this present study, three different naphthaldehydes are used as derivatizing agents to enhance the enantiomeric separation and ultraviolet (UV) detection of the investigated chiral amines as the corresponding naphthaldimine derivatives. It is expected that these additional naphthaldimine moieties will function to provide better interaction sites with chiral selectors of CSPs for enantiodiscrimination [10]. Also, three chiral amines (α-methylbenzylamine, 2-amino-4-methyl-1-pentanol and 2-amino-1-propanol) for this study were selected for their different structural features as they contain either aromatic group or aliphatic hydroxyamine. Thus, the aim of this study is facilitate and develop a stereoselective HPLC-UV method with amylose and cellulose tris carbamates as CSPs to exploit and understand

different enantiodiscrimination mechanism in terms of structural differences of CSPs, chiral amine analytes and naphthaldehyde derivatizing agents using docking simulations.

5.2 Experimental Section

5.2.1 Chemicals

The derivatizing agents, 1-naphthaldehyde, 2-naphthaldehyde, 2-hydroxynaphthaldehyde as well as racemic, (R)- and (S)-α-methylbenzylamine, (R)- and (S)-2-amino-4-methyl-1-pentanol, (R)- and (S)-2-amino-1-propanol and magnesium sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Haverhill, MA, USA). HPLC-grade 2-propanol, ethanol, and hexane for the mobile phase were obtained from Burdick & Jackson (Morristown, NJ, USA). All the solvents used were degassed by an ultrasonic bath (Branson, MI, USA).

5.2.2 Derivatization for Sample Preparation

The derivatized analytes for the normal HPLC were prepared by stirring α-methylbenzylamine or 2-amino-4-methyl-1-pentanol or 2-amino-1-propanol and the corresponding three aromatic naphthaldehyde derivatizing agent (1-2 equivalents) with excess magnesium sulfate (7 equivalents) in 2-propanol at room temperature for 6 h, according to a procedure for the preparation of 9-anthraldimine derivatives [11]. The resulted mixture was then filtered to remove excess magnesium sulfate and diluted to the required concentration for separation.

5.2.3 Apparatus and HPLC Methods

For HPLC, an Agilent (Palo Alto, CA, USA) 1100 system incorporating a vacuum degasser with a G1310A isocratic pump and an automatic sample injector with a thermostatic column

compartment was used. Covalently bonded and coated type chiral columns derived from either amylose or cellulose tris carbamates were used for the analysis. Covalently bonded chiral columns as Chiralpak IA [amylose tris(3,5-dimethylphenylcarbamate)], Chiralpak IB [cellulose tris(3,5-dimethylphenylcarbamate)], and Chiralpak IC [cellulose tris(3,5-dichlorophenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) were purchased from Daicel Company (Tokyo, Japan). The coated type Chiralcel OD-H [cellulose tris(3,5-dimethylphenylcarbamate)] and Chiralpak AD-H [amylose tris(3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) columns were obtained from Daicel Company (Tokyo, Japan); Lux Cellulose-1 [cellulose tris(3,5-dimethylphenylcarbamate)] and Lux Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5μm) columns were procured from Phenomenex (Torrance, CA, USA). All separations were carried out at room temperature with a flow rate of 1 mL/min under UV 254 nm detection. The mobile phases were prepared in volume ratio and are indicated in Tables 5.1-5.6.

5.2.4 Calculation

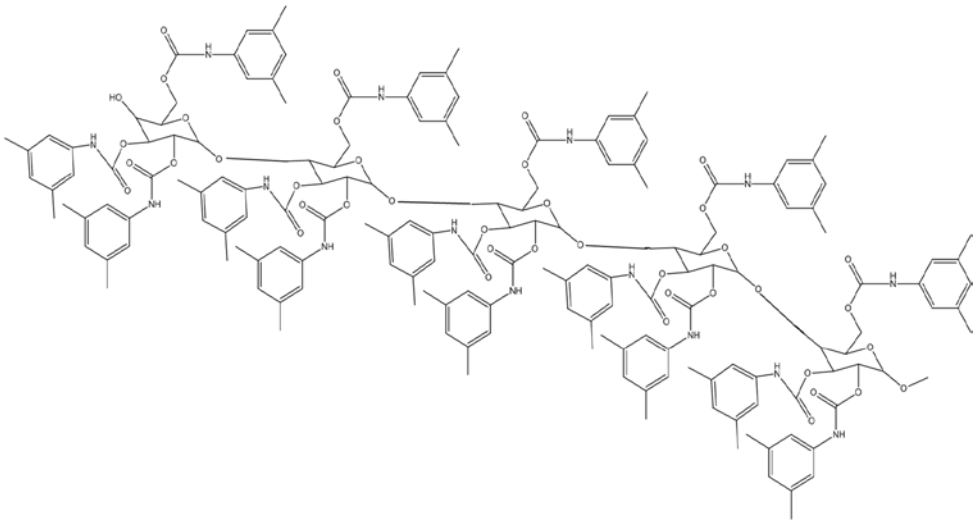
The retention factor (k′) was calculated from (t_R-t_0) where t_R and t_0 are the retention times of the analyte and the void volume obtained, respectively. The separation factor (α) was calculated from k'_2/k'_1 where k'_1 and k'_2 are the retention factors of the first and second isomers eluted. The resolution (R_S) of the investigated analytes was calculate from the equation: $2(t_2-t_1)/(W_1+W_2)$, t_1 and t_2, the retention times of the first and second eluted isomers; W_1 and W_2, the baseline peak width of the first and second eluted isomers.

5.2.5 Molecular Docking Simulations

Simulation studies were carried out on a computer having configurations of Intel® Pentium®

Gold CPU (3.10 GHz) with Windows 10 education operating system. The ChemBioDraw Ultra (12.0 version) was used to the draw the structures of the ligands and the receptor. The structures were then cleaned and optimized to 3D and saved in PDB format for the docking simulations. The ligands used in this study were 2-naphthaldimine derivatives of (R)- and (S)-leucinol as well as 2-hydroxynaphthaldimine derivatives of (R)- and (S)-leucinol. The receptor used was amylose-derived CSP having amylose tris(3,5-dimethylphenylcarbamate) as chiral selector and shown in Figure 5.1 (2D and 3D structure). AutoDock 4.2.6 (Scripps Research Institute, La Jolla, CA, USA) and PyMOL (2.2 version) software was used for docking [12]. AutoDock Tools (ADT) 1.5.6. was employed for processing amylose and ligand structures prior to docking [13]. During receptor preparation, Kollman and Gasteiger charges were computed. During ligand preparation, Gasteiger charges and rotatable bonds were assigned. Ligands were treated as flexible while the amylose structure was kept rigid in the docking protocol. AutoGrid program was employed to generate 3D affinity grid fields. A grid of size 70 × 70 × 70 Å with 0.375 Å spacing was used for docking. A total of 100 docking runs, 25×10^5 energy evaluations and 27,000 iterations were carried out using Lamarckian genetic algorithm method (LGA) [14]. Default settings were used for all other parameters. Amylose-ligand interactions and scoring functions were utilized for choosing the docked poses. Additionally, LigPlot+ v.2.1 software was also applied for the assessment of the hydrophobic interactions.

A



B

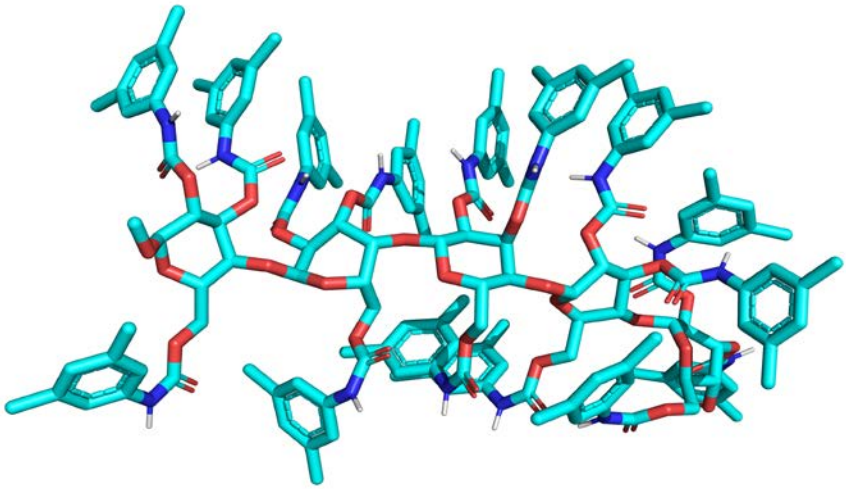


Figure 5.1: (A) 2D and (B) 3D optimized structures of receptor, amylose tris(3,5-dimethylphenyl carbamate)

5.3 Results and Discussion

Enantiomer separation results of α-methylbenzylamine, 2-amino-4-methyl-1-pentanol (leucinol) and 2-amino-1-propanol (alaninol) as three chiral amines using three naphthaldehyde derivatizing agents on amylose and cellulose-based polysaccharide CSPs under normal phase mode are summarized in Tables 5.1-5.6. For enantiomer separation of three chiral amines as three kinds of naphthaldimine derivatives, attempts were made to comprehensively compare the enantiomer separation and resolution behaviors of immobilized versus coated polysaccharide type CSPs, and amylose versus cellulose-derived chiral selectors. The enantiomeric separation results obtained from α-methylbenzylamine (Tables 5.1 and 5.2), 2-amino-4-methyl-1-pentanol (Tables 5.3 and 5.4 leucinol) and 2-amino-1-propanol (Tables 5.5 and 5.6, alaninol) as three corresponding naphthaldimine derivatives was more or less comparable with each other among the CSPs used.

As shown in Table 5.1-5.6, the enantiomers of chiral amines as naphthaldimine derivatives were resolved with baseline separation on several CSPs of amylose and cellulose-derivatives. The enantioselectivities of three chiral amines as three naphthaldimine derivatives on amylose-derived CSPs (Chiralpak IA, Chiralpak AD-H and Lux Amylose-1) were superior to those on cellulose-derived CSPs (Chiralpak IB, Chiralpak IC, Chiralcel OD-H and Lux Celluose-1), except for 2-hydroxynaphthaldimine derivatives on cellulose-derived CSPs. The structural characteristics of CSP as well as the structure of derivatizing agent have an effect on the enantiodiscrimination between analyte and CSP [9,15,16]. In this study, the impact of different substituents in the same backbone structure of naphthyl derivatizing agents on enantiomeric resolution was fully elucidated. Also, the enantioselectivities observed on coated amylose-derived CSPs (Chiralpak AD-H and Lux Amylose-1) in Tables 5.2, 5.4 and 5.6 were generally greater than those on covalently bonded amylose-derived CSP (Chiralpak IA) in Tables 5.1, 5.3 and 5.5. In particular, coated amylose-

derived CSPs (Chiralpak AD-H and Lux Amylose-1) using 2-naphthaldehyde as a derivatizing agent provided greater enantioselectivity and resolution than any other CSP. For an example, the highest enantiomer separations of 2-amino-1-propanol as 2-naphthaldimine derivative were achieved by Lux Amylose-1 and Chiralpak AD-H (α=2.03, 2.09; Rs=7.42, 7.45, Table 5.6). Figure 5.2 shows the typical chromatograms for enantiomeric separation of 2-amino-1-propanol and α-methylbenzylamine as two naphthaldimine derivatives on Chiralpak IA and Lux Amylose-1.

Table 5.1: Enantiomeric separation of α-methylbenzylamine using three naphthaldehyde derivatizing agents on covalently bonded CSPs

S.N.	Derivatizing agent	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1-Naphthaldehyde	1.27	0.23	1.29(R)[a]	1.00	0.24	-	1.00	0.22	-
2	2-Naphthaldehyde	1.37	0.51	2.58(R)	1.09	0.30	0.41(S)	1.00	0.31	-
3	2-Hydroxynaphthaldehyde	1.46	3.63	7.14(R)	1.62	1.96	4.83(R)	1.25	7.12	4.08(R)

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.2: Enantiomeric separation of α-methylbenzylamine using three naphthaldehyde derivatizing agents on coated type CSPs

S.N	Derivatizing agent	Chiralcel OD-H			Lux Cellulose-1			Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1-Naphthaldehyde	1.07	0.50	0.44(R)[a]	1.07	0.54	0.57(R)	1.26	0.29	1.15(R)	1.35	0.25	1.09(R)
2	2-Naphthaldehyde	1.09	0.65	0.81(S)	1.14	0.70	1.18(S)	1.52	0.73	4.18(R)	1.45	0.72	4.20(R)
3	2-Hydroxynaphthaldehyde	1.92	3.62	7.14(R)	1.88	4.09	8.41(R)	1.28	4.96	4.27(R)	1.34	4.84	6.03(R)

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.3: Enantiomeric separation of 2-amino-4-methyl-1-pentanol using three naphthaldehyde derivatizing agents on covalently bonded CSPs

S.N.	Derivatizing agents	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'1	Rs	α	k'1	Rs	α	k'1	Rs
1	1-Naphthaldehyde	1.14	0.81	0.99(R)[a]	1.71	0.63	3.68(S)	1.26	0.75	1.75(S)
2	2-Naphthaldehyde	1.78	0.94	5.55(R)	1.09	0.69	0.□3(S)	1.29	0.94	2.33(S)
3	2-Hydroxynaphthaldehyde	1.14	1.93	1.70(R)	2.19	1.43	6.37(S)	1.88	5.29	9.61(S)

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.4: Enantiomeric separation of 2-amino-4-methyl-1-pentanol using three naphthaldehyde derivatizing agents on coated bonded CSPs

S.N.	Derivatizing agents	Chiralcel OD-H			Lux Cellulose-1			Chiralpak AD-H			Lux Amylose-1		
		α	k'1	Rs	α	k'1	Rs	α	k'1	Rs	α	k'1	Rs
1	1-Naphthaldehyde	2.88	1.36	6.20(S)[a]	2.96	1.73(S)	5.13	1.11	0.97(R)	0.67	1.11	1.02(R)	1.03
2	2-Naphthaldehyde	1.16	1.60	1.58(S)	1.11	2.14(S)	1.03	1.82	1.08(R)	5.11	1.86	1.17(R)	7.08
3	2-Hydroxynaphthaldehyde	4.46	2.04	12.94(S)	4.00	2.78(S)	15.57	1.15	2.09(R)	1.72	1.15	2.46(R)	2.25

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.5: Enantiomeric separation of 2-amino-1-propanol using three naphthaldehyde derivatizing agents on covalently bonded CSPs

S.N.	Derivatizing agent	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1-Naphthaldehyde	1.40	1.07	3.01(R)[a]	1.16	1.30	1.13(S)	1.14	1.42	1.30(S)
2	2-Naphthaldehyde	1.77	1.38	5.62(R)	1.00	1.58	-	1.21	1.86	2.15(S)
3	2-Hydroxynaphthaldehyde	1.59	3.22	5.37(R)	1.18	4.88	1.14(S)	1.03	6.73	0.33(R)

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.6: Enantiomeric separation of 2-amino-1-propanol using three naphthaldehyde derivatizing agents on coated type CSPs

S.N.	Derivatizing agent	Chiralcel OD-H			Lux Cellulose-1			Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1-Naphthaldehyde	1.43	2.16	3.61(S)[a]	1.39	2.61	3.41(S)	1.62	1.15	3.19(R)	1.58	1.14	3.99(R)
2	2-Naphthaldehyde	1.00	2.46	-	1.00	2.97	-	2.09	1.47	7.45(R)	2.03	1.58	7.42(R)
3	2-Hydroxynaphthaldehyde	1.72	2.02	3.44(S)	1.63	2.42	4.61(S)	1.52	1.16	3.47(R)	1.58	2.81	1.95(R)

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor; [a]the absolute configuration of the second eluted enantiomer

Table 5.7: Determination of enantiomeric purity of commercially available (R)- and (S)-α-methylbenzylamine as 2-hydroxynaphthaldimine derivatives on Chiralcel OD-H

Entry	Analyte	R:S ratio[a]
1	(R)-α-methylbenzylamine	99.37 : 0.63
2	(S)-α-methylbenzylamine	0.55 : 99.45

[a]Average value of four replicates. See Table 5.2 for chromatographic conditions

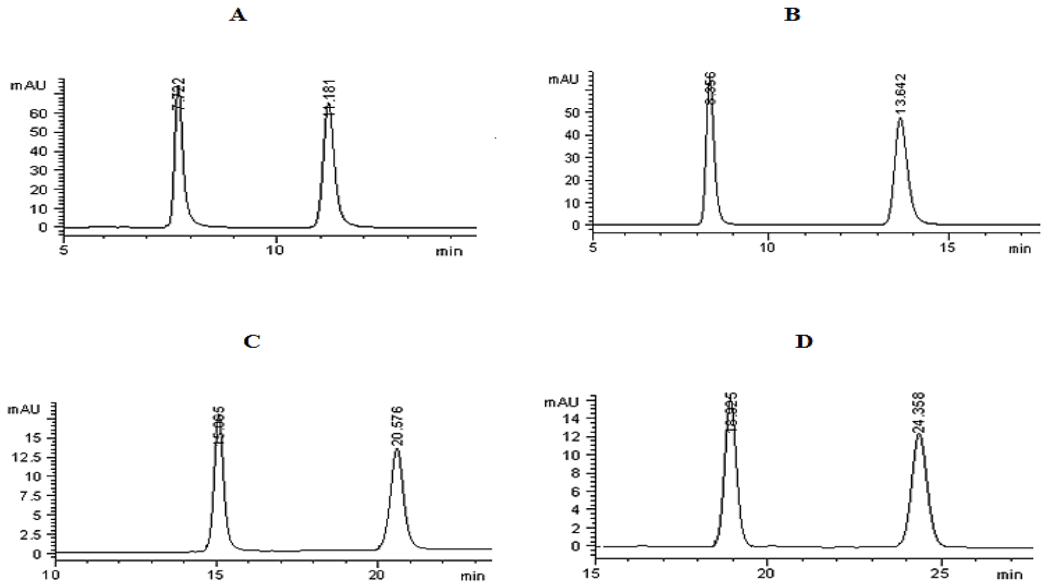


Figure 5.2: Typical chromatograms for the enantiomeric separation of (A) 2-naphthaldimine derivatives of 2-amino-1-propanol on Chiralpak IA, and (B) on Lux Amylose-1, (C) 2-hydroxynaphthaldimine derivatives of α-methylbenzylamine on Chiralpak IA, and (D) on Lux Amylose-1. Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm

Compared to the amylose-based CSPs of the helical structure, the cellulose-derived CSPs with a straight chain type polymer are conformationally rigid [17,18]. In terms of conformational rigidity among three naphthaldimine derivatives of chiral amines, 2-hydroxynaphthaldimine derivatives with an intramolecular hydrogen bonding interaction by 2-hydroxy moiety are the most conformationally rigid analytes. On the contrary, 2-naphthaldimine derivatives are the most conformationally flexible analytes because in case of 1-naphthaldimine derivatives, their peri-hydrogens function as the barrier for their free rotation. It is worth noted from the result that 2-hydroxynaphthaldimine derivatives of the most conformationally rigid analyte (entry 3) among three derivatized analytes afforded generally the greatest enantioseparation on conformationally

rigid cellulose-derived CSPs (Chiralpak IB, Chiralpak IC and Chiralcel OD-H). As mentioned before, it is interesting that 2-naphthaldimine derivatives of the most conformationally flexible analyte (entry 2) afforded the highest enantioseparation on conformationally flexible amylose-derived CSPs (Chiralpak IA, Chiralpak AD-H, Lux Amylose-1).

Consequently, two matched patterns between analyte kinds of derivatizing agents and CSP kinds provided the highest enantioselectivities on two different type CSPs based on cellulose or amylose-derivatives in this study; (a) conformationally rigid 2-hydroxynaphthaldimine derivatives (entry 3) on conformationally rigid cellulose-derived CSPs, especially in Tables 5.3 and 5.4, (b) conformationally flexible 2-naphthaldimine derivatives (entry 2) on conformationally flexible amylose-derived CSPs, especially in Tables 5.5 and 5.6. It is also interesting that the retention factors were generally highest in the case 2-hydroxynapthaldehyde derivatives (entry 3) using the same mobile phase conditions. Especially, the greater enantioselectivities of its derivatives were shown on conformationally rigid cellulose type CSPs than on conformationally flexible amylose type CSPs. It implies that there is a strong interaction between conformationally rigid 2-hydroxynapthaldehyde derivatives and the conformationally rigid chiral selectors of cellulose-derived CSPs and suggests that the transient diastereomeric complex fits strongly each other under thermodynamically favorable environments [18-20] The results that the greatest enantioselectivities of conformationally flexible 2-naphthaldimine derivatives (entry 2) were obtained on conformationally flexible amylose type CSPs was likely related to three-dimensional structures of the amylose helical structure with chiral cavities. The identical elution orders between all three naphthaldimine derivatized analytes on amylose-derived CSPs were seen, as (R)-enantiomers being selectively eluted. However, the consistency in elution orders was not always observed on cellulose-derived CSPs. It indicates that there might be a similar predominant chiral recognition

mechanism on amylose-derived CSPs during enantiodiscrimination process, not as like on cellulose-derived CSPs. The present developed analytical method was applied to determine the enantiomeric purity of (R)- and (S)-α-methylbenzylamine (Alfa Aesar company) as 2-hydroxynaphthaldimine derivatives on Chiralcel OD-H (entry 3 of Table 5.2). The enantiomeric impurities of (R)- and (S)-α-methylbenzylamine reagents were found to be 0.63 and 0.55%, respectively, and shown in Table 5.7. Figure 5.3 shows the chromatograms for the enantiomeric separation of racemic, (R)- and (S)-α-methylbenzylamine as 2-hydroxynaphthaldimine derivatives on Chiralcel OD-H.

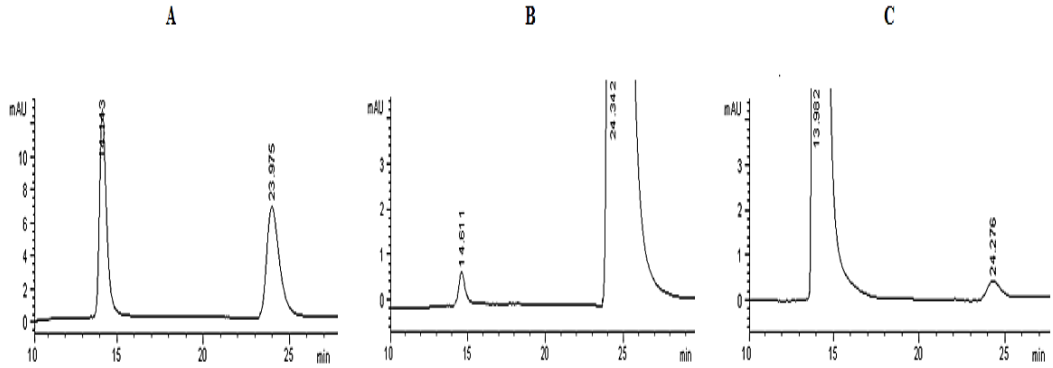


Figure 5.3: Chromatograms for the determination of enantiomeric purity of (A) racemic α-methylbenzylamine, (B) (R)-α-methylbenzylamine (R:S=99.37:0.63) and (C) (S)-α-methylbenzylamine (R:S=0.55:99.45) as 2-hydroxynaphthaldimine derivatives on Chiralcel OD-H. Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm

Amylose-derived chiral column having amylose tris(3,5-dimethylphenylcarbamate) as chiral selector was used for the molecular docking simulations. In amylose tris(3,5-dimethylphenylcarbamate) chiral selector columns, the phenylcarbamate residues are oriented in such a way that chiral helical grooves are formed near the carbohydrate backbone which

incorporate various levels of chiral information in their perfectly defined structures [21]. Enhanced chiral discrimination of a chiral molecule occurs if the ligand (chiral molecule) better fit into the chiral grooves of the selector [21,22]. The modeling study is considered as one of the important tools for exploiting the chiral recognition mechanism involved between the enantiomers and the chiral selector during the chiral separation process [21]. In this study also, the investigated four enantiomers as 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives were fixed well enantioselectively in the chiral helical grooves of amylose for the better enantioseparation and resolution of chiral amines. The fitting of the enantiomers were stabilized by diverse interactions that occur during the chiral separation process [22,23]. Table 5.8 shows the molecular modeling data of the ligands [(R)- and (S)-leucinol as 2-naphthaldimine derivatives and 2-hydroxynaphthaldimine derivatives] with the receptor amylose tris(3,5-dimethylphenylcarbamate). The binding affinities of (R)- and (S)-leucinol as 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives were 6.39 and 5.85, and 5.74 and 5.62 (kcal/mole). The difference between (R)- and (S)-leucinol as 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives in the enantiorecognition process gave rise to the significant differences in binding energy and then lead to the chiral discrimination of the enantiomers under given experimental conditions. The major forces involved in the interaction were hydrogen bondings, π-π and hydrophobic interactions. It was also noted that three hydrogen bonds were formed between each ligand and helical amylose as chiral selector in all four investigated enantiomers. These hydrogen bonds were formed among the oxygen and hydrogen atoms of OH group of (R)- or (S)-leucinol or hydrogen atom present in the naphthalene ring and different oxygen and hydrogen atoms of the chiral selector. In addition, the total atoms involved during weaker hydrophobic interaction are clearly shown in the Table 5.8. The 3D docking model of four enantiomers of (R)- and (S)-leucinol as 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives with amylose tris(3,5-dimethylphenylcarbamate) as chiral

selector is shown in the Figure 5.4. It is clear from the docking pose that all enantiomers interacted with tris(3,5-dimethylphenylcarbamate) amylose chiral selector in a different fashion and fitted well stereospecifically in the chiral helical grooves of amylose. Besides, hydrogen bondings, π-π, and hydrophobic interactions, the other forces observed such as van der Waal's forces and steric effects also contributed significantly in the chiral recognition of the stereomers of the reported molecule [22-24].

The elution order of the four enantiomers can be predicted and explained using the molecular docking simulations results [22,23]. As shown in experimental Table 5.9, the secondly eluted enantiomer in helical amylose tris(3,5-dimethylphenylcarbamate) chiral selector columns was (R)-enantiomer in both 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives. Therefore, the arrangement of elution of the compounds was in order of (S)-leucinol > (R)-leucinol. The values of the binding affinities of compounds as 2-naphthaldimine and 2-hydroxynapthaldimine derivatives were in the order of S-leucinol (-5.85) < R-leucinol (-6.39) and S-leucinol (-5.62) < R-leucinol (-5.74). Higher binding energy resulted to the stronger retention of the enantiomer in the chiral selector and thus resulting into late elution [23]. Thus, the predicted order of elution of analytes by molecular docking simulation was also (S)-leucinol > (R)-leucinol in both cases which clearly supported the order of elution of the enantiomers as shown in chromatographic experimental data Table 5.9.

Compared to the difference in binding energy (ΔΔE) between enantiomers (R)- and (S)-leucinol as 2-napthaldimine and 2-hydroxynapthaldimine derivatives, and chiral selector, it was found that small differences in the binding energies can lead to the enhanced separation and resolution of the enantiomers [24]. The binding energy difference (ΔΔE) between (R)- and (S)-leucinol as 2-

nahpthaldimine and 2-hydroxynapthaldimine derivatives were 0.54 and 0.12 kcal/mole, respectively. These results were also in the good agreement with the experimental data as 2-naphthaldimine derivatives (α=1.86, Rs=7.08, Table 5.9) showed better chiral separation and resolution than 2-hydroxynaphthaldime derivatives (α=1.15, Rs=2.25, Table 5.9).

Table 5.8: Molecular modeling data of (R)-leucinol and (S)-leucinol as 2-naphthaldimine and 2-hydroxynaphthaldimine derivatives on amylose chiral selector

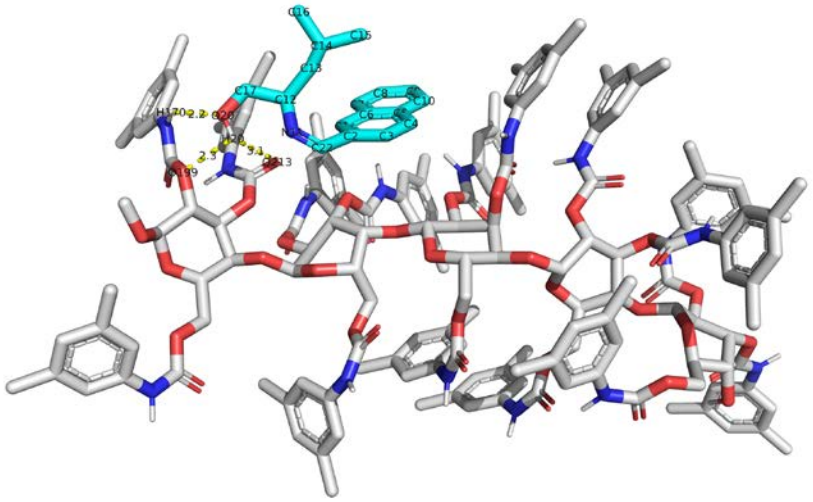
R/S enantiomer	Binding energy ΔE (kcal/mol)	Number of hydrogen bonds	Difference in binding energy ΔΔE (kcal/mol)	Atoms involved in H-bonding (distance in Å)	Atoms involved in hydrophobic interactions	Number of π–π interactions
(R)-leucinol as 2-naphthaldimine derivative	-6.39	3	-0.54	Amylose:H170: :O20 of OH group of (R)-leucinol (2.2) Amylose:O199: :H20 of OH group of (R)-leucinol (2.3) Amylose:O213: :H20 of OH group of R-leucinol (3.0)	Amylose: :C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, N11, C12, C16, C17, O20	2
(S)-leucinol as 2-naphthaldimine derivatives	-5.85	3		Amylose:H170: :O20 of OH groupof (S)-leucinol (2.2) Amylose:O199: :H20 of OH group of (S)-leucinol (2.3) Amylose:O213: :H20 of OH group of (S)-leucinol (3.1)	Amylose: :C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, N11, C12, C16, C17, O20	2
(R)-leucinol as 2-hydroxynaphthaldimine derivative	-5.74	3	-0.12	Amylose:H131: :O17 of OH group of (R)-leucinol (2.4) Amylose:O167: :H17 of OH group of (R)-leucinol (1.8) Amylose:H141: :O17 of OH group of (R)-leucinol (2.2)	Amylose: :C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, N11, C12, C13, C14, C15, C16, O17, C21, O22	1
(S)-leucinol as 2-hydroxynapthaldimine derivative	-5.62	3		Amylose:O96: :H10 of OH group of (S)-leucinol (2.2) Amylose:H81: :O14 of OH group of (S)-leucinol (2.1) Amylose:O213: :H19 of OH group attached to naphthalene ring (2.2)	Amylose: :C1, C2, C3, C4, C5, C6, C7, C8, C10, N11, C12, O14, C15, C21, O22	3

Table 5.9: Chromatographic data of 2-amino-4-methyl-1-pentanol (leucinol) as 2 naphthaldimine and 2-hydroxynaphthaldimine derivatives on amylose tris(3,5-dimethylphenylcarbamate) chiral selector columns

Amylose-derived chiral columns	Racemic leucinol as 2-naphthaldimine derivatives		Racemic leucinol as 2-hydroxynaphthaldimine derivatives		Secondly eluted enantiomer
	α	R_s	α	R_s	
Chiralpak IA	1.78	5.55	1.14	1.70	R
Chiralpak AD-H	1.82	5.11	1.15	1.72	R
Lux Cellulose-1	1.86	7.08	1.15	2.25	R

Mobile phase: 10% 2-propanol/hexane (v/v), Flow rate: 1 mL/min, Detection: UV 254 nm, α: Separation factor, Rs: Resolution factor

A



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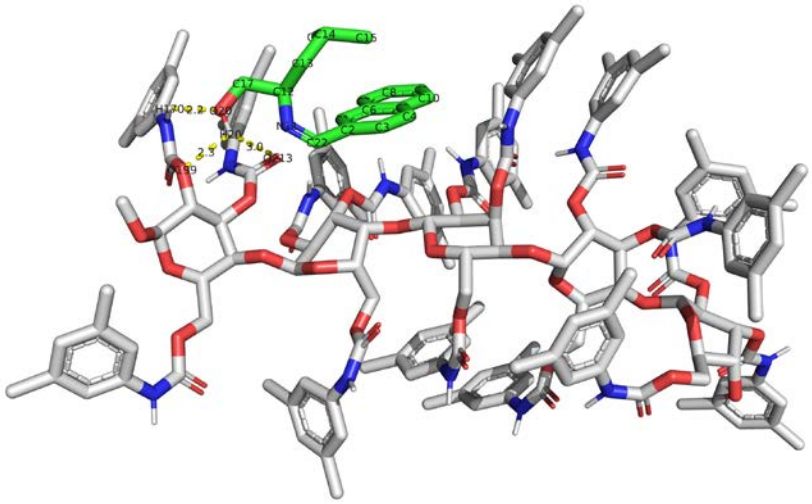

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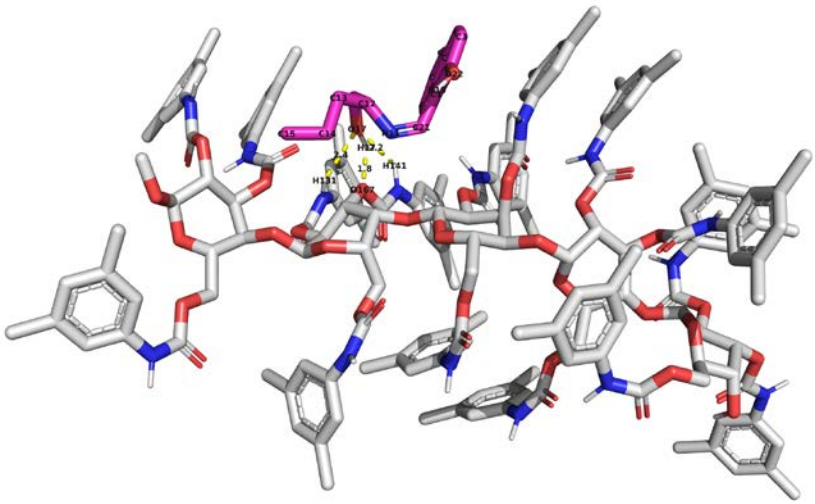

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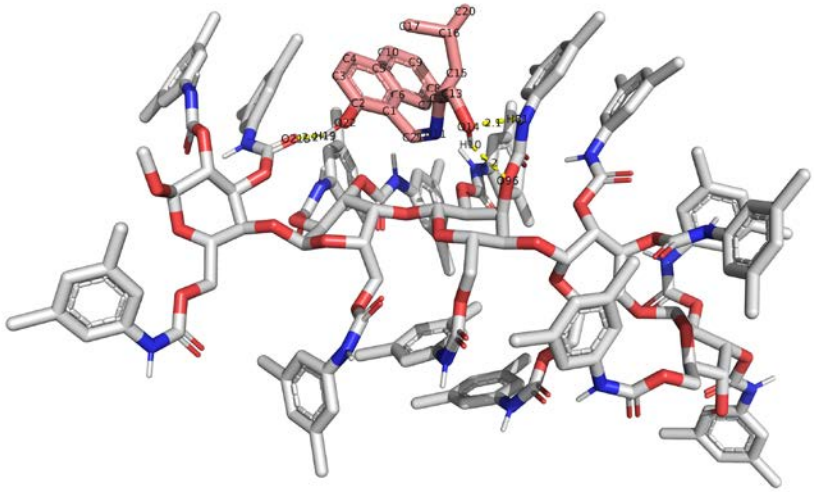

Figure 5.4: 3D docking pose of enantionmers of (A) (R)-leucinol and (B) (S)-leucinol as 2-naphthaldimine derivatives, and (C) (R)-leucinol and (D) (S)-leucinol as 2-hydroxynaphthaldimine derivatives with tris(3,5-dimethylphenylcarbamate) amylose chiral selector

5.4 Conclusion

For the search of better enantiomeric separation and resolution of chiral amines, their enantiodiscrimination using three naphthaldehyde derivatizing agents was performed and compared using several polysaccharide-derived CSPs. The structure of naphthaldimine derivatizing agents and CSP type (amylose or cellulose derivatives of chiral selectors and covalently bonded or coated-type) used in this study have a significant impact on enantiomeric discrimination of analytes. Overall, the performance in terms of enantiomer resolving ability was better with amylose-based CSPs, except for 2-hydroxynaphthaldimine derivatives on cellulose-derived CSPs. The modeling studies confirmed mechanism of chiral recognition and elution orders which support the experimental results obtained. This HPLC-UV analytical method seems quite suitable, selective and useful for the analysts as well as pharmaceuticals in understanding the use of derivatizing agents to enhance separation of chiral amine isomers.

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CHAPTER 6

High-Performance Liquid Chromatography Separation of Chiral Amines Enantiomers Using Polysaccharide-derived Chiral Stationary Phases

Abstract

A new, convenient, isocratic and derivatized analytical method for the separation of chiral aliphatic amine including amino alcohols was developed. Fluorene-2-carboxaldehyde was used as an achiral derivatizing agent for the first time to separate the enantiomers of chiral aliphatic amines including amino alcohols by normal phase HPLC under ultraviolet (UV) detection. The enantiomer separation of chiral aliphatic amines including amino alcohols as fluorene-2-carboxaldimine derivatives was performed on six covalently bonded and four coated type polysaccharide-derived chiral stationary phases (CSPs) of either amylose or cellulose-based chiral selectors and the results were compared. All the analytes were either partially or base-line separated on Chiralpak IE and Chiralpak IF columns. Cellulose-derived CSPs showed the best enantiomer separation especially for amino alcohol analytes. Also, the coated type CSPs, Chiralcel OD-H and Lux Cellulose-1, showed the better enantiomer selection and resolution than the covalently bonded Chiralpak IB. Among the chiral aliphatic amines, enantiomer separation of α-methylbenzylamine containing aromatic group was selectively effective and showed the fairly good enantiomer separation and resolution.

Key words: Chiral amine, Chiral selector, Chiral stationary phase, Enantiomer separation, Fluorene-2-carboxaldimine derivatives

6.1 Introduction

The chiral selective biological pathways of our living system interact with racemic chiral pharmaceuticals differently and metabolize each enantiomer by a separate pathway [1-3]. Therefore, it is not surprising to get different biological responses to the enantiomers of a chiral pharmaceutical [2]. One enantiomer may exhibit the desired biological activity while the other may show undesired or toxic activity [3-5]. Accurate and simple analytical methods which can discriminate specific enantiomers have been of great interest in pharmaceuticals for the development of enantiomerically pure chiral drugs [4-7]. Chiral aliphatic amines are of particular interest in pharmaceuticals as they were found as key intermediates in drugs and drug candidates [8-12]. Simple and efficient strategies are highly desirable for the preparation of chiral aliphatic amines building blocks in synthesizing a number of chiral pharmaceutical products [9]. Besides preparation, the enantiomeric separation of chiral amines is of prime importance as the availability of enantiopure compound is necessary for the development of single enantiomeric drug with desired therapeutic activity [4-6,11-13]. Tremendous efforts have been made in the field of chirotechnology to develop effective analytical techniques for enantiomer separation and resolution of chiral compounds [13]. Among them, the resolution of a racemic chiral compound into its individual enantiomers by liquid chromatography (HPLC) on various chiral stationary phases (CSPs) has been the most convenient and versatile methods for the exploration and development of chiral drugs [6,13,14]. In particular, the direct separation of enantiomers by CSPs has been recognized to be the most advantageous means for determining the optical purity of enantiomers and also for obtaining optical isomers on a large scale [13,14]. Polysaccharides, such as cellulose and amylose, are naturally occurring biopolymers which exhibit the unique chiral recognition for the broad range of chiral compounds due to the incorporation of various levels of chiral information in their perfectly defined structures [15,16]. The esters and carbamates of polysaccharides either

coated or covalently bonded on silica gel have been extensively explored and are widely used as CSPs for liquid chromatographic method [16]. To achieve better enantiomer separability as well as higher detection sensitivity, derivatization with an achiral reagent before the enantioseparation is the best choice [17]. In this study, we developed a convenient and precise analytical method to separate the enantiomers of chiral aliphatic amines including amino alcohols using fluorene-2-carboxaldehyde as a derivatizing agent on ten polysaccharide-derived chiral columns under normal phase HPLC. In our previous study also, 4-chloro-7-nitro-1,2,3-benzoxadiazole and 2-hydroxynapthaldehyde were used as derivatizing agent to provide good interaction sites as well as to increase the detection sensitivity for chiral discrimination of aliphatic amines [17,18]. Fluorene-2-carboxaldehyde has never been exploited as achiral derivatizing agent for enantiomeric separation and resolution of chiral amines by normal phase HPLC under ultraviolet (UV) detection. Comparison of the chromatographic results between the same chiral selectors but with the different chiral columns of either coated or covalently bonded were also made in this study.

6.2 Experimental Section

Liquid chromatographic analysis was performed at room temperature using an Agilent HPLC system of HP series 1100 (Palo, Alto, CA, USA) which consists of a micro-vacuum degasser, a G1310A isocratic pump, an automatic sample injector, and an HP1046A programmed fluorescence detector. Chiral aliphatic amines including amino alcohols with fluorene-2-carboxaldehyde (derivatizing agent) and magnesium sulfate used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Haverhill, MA, USA). HPLC-grade 2-propanol, ethanol, and hexane for the mobile were the product of Burdick & Jackson (Morristown, NJ, USA). The fluorene-2-carboxaldimine derivatives were prepared according to the conventional method [19]. The resulting solution was filtered to remove the insoluble magnesium sulfate, diluted with 2-

propanol, and then injected into the HPLC system. Covalently bonded type CSPs of Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak ID, Chiralpak IE, and Chiralpak IF as well as coated type CSPs of Chiralpak AD-H and Chiralpak OD-H (250 mm × 4.6 mm, I.D., 5 μm) were obtained from Daicel Chemical Company (Tokyo, Japan). The other two coated type CSPs of Lux Amylose-1 and Lux Cellulose-1 (250 mm × 4.6 mm, I.D., 5 μm) were purchased from Phenomenex (Torrance, CA, USA). Chiral separation and resolution using normal HPLC was carried out under UV 310 nm detection with a flow rate of 1 mL/min and 3 or 10% 2-propanol/hexane (v/v) as mobile phase.

6.3 Results and Discussion

Enantiomer separation and resolution of chiral aliphatic amines including amino alcohols was performed on six covalently bonded and four coated type CSPs derived from amylose or cellulose phenylcarbamates under normal phase HPLC. The conventional mobile phase system (2-propanol/hexane) was used for the entire enantiomeric separation process. The performance of each CSP for the investigated chiral amines as fluorene-2-carboxaldimine derivatives is summarized in Table 6.1 and Table 6.2. The nature of the mobile phase, the employed CSP, and the used analyte type have significant impact on chromatographic parameters of enantioseparation and retention factor [15,20]. Most of the analytes under the consideration were discriminated with good enantioseparation and resolution factors. In Table 6.1, out of the six covalently bonded CSPs, Chiralpak IE and Chiralpak IF showed the best enantiomer separation as all the analytes were either partially or base-line separated. The other CSPs also exhibited good separation and resolution of the investigated analytes except for chiral aliphatic amines (entries 1-4). In particular, the enantioselectivities of amino alcohols (entries 5 and 6) were superior to those of chiral aliphatic amines (entries 1-4). Especially, the analyte, 2-amino-4-methyl-1-pentanol (entry 6, leucinol) showed the best separation and resolution (α=3.15, k'_1=0.71, Rs=7.00) on covalently bonded CSP

(Chiralpak IB) having chiral selector of cellulose tris(3,5-dimethylphenylcarbamate). Interestingly, among the four analytes of chiral aliphatic amines (entries 1-4), α-methylbenzylamine showed the fairly good enantiomer separation and resolution than the other three analytes. The presence aromatic group in case of α-methylbenzylamine might serve as a good interaction site for enhanced chiral discrimination as fluorene-2-carboxaldimine derivatives.

Table 6.2 shows the enantiomer separation data of chiral aliphatic amines (entries 1-4) and amino alcohols (entries 5 and 6) as fluorene-2-carboxaldimine derivatives on four coated type CSPs. Similar in trend but superior performance in enantioseparation of analytes was observed on coated type CSPs. The enantioseparation of amino alcohols (entries 5 and 6) was selectively effective than chiral aliphatic amines (entries 1-4) on coated type CSPs also. Especially, the enantioseparation was best on cellulose-derived CSPs (Chiralcel OD-H and Lux Cellulose-1). The highest enantiodiscrimination (α=5.55, k'_1=1.07, Rs=14.06) was shown by 2-amino-4-methyl-1-pentanol analyte (entry 6, leucinol, Table 6.2) on Lux Cellulose-1 having chiral selector of cellulose tris(3,5-dimethylphenylcarbamate).

Table 6.1: Enantiomeric separation of chiral amines as fluorene-2-carboxaldimine derivatives on covalently bonded CSPs

Entry	Analytes	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.10	0.27	0.40	1.00	0.22		1.48	0.36	2.13
2	1,2-Dimethylpropylamine	1.00	0.30		1.00	0.23		1.14	0.36	0.60
3	1-Methylheptylamine	1.00	0.29		1.00	0.19		1.23	0.37	1.01
4	α-Methylbenzylamine	1.12	0.67	0.95(R)[a]	1.15	0.33	0.71(S)	1.00	0.38	-
5	2-Amino-1-propanol	1.29	1.68	2.11(R)	1.66	1.68	2.67(S)	1.07	2.84	0.70(S)
6	2-Amino-4-methyl-1-pentanol	1.29	1.17	1.98(R)	3.15	0.71	7.00(S)	1.10	1.63	0.76(S)
Entry	Analytes	Chiralpak ID			Chiralpak IE			Chiralpak IF		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.13	0.25	0.55	1.27	0.46	1.34	1.11	0.38	0.59
2	1,2-Dimethylpropylamine	1.07	0.25	0.40	1.16	0.40	0.68	1.07	0.35	0.55
3	1-Methylheptylamine	1.00	0.27		1.20	0.46	0.79	1.10	0.38	0.43
4	α-Methylbenzylamine	1.13	0.42	0.98(R)	1.20	0.59	1.41(R)	1.12	0.47	0.97(R)
5	2-Amino-1-propanol	1.23	2.54	3.65(R)	1.06	3.32	0.78(R)	1.32	2.34	3.50(R)
6	2-Amino-4-methyl-1-pentanol	1.32	1.13	2.43(R)	1.07	2.07	0.59(R)	1.40	1.45	3.26(R)

Mobile phase: 3% 2-propanol/hexane (v/v) (entries 1-4, chiral aliphatic amines) and 10% 2-propanol/hexane (v/v) (entries 5 and 6, amino alcohols), Flow rate: 1 mL/min, Detection: UV 310 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]the absolute configuration of the second eluted enantiomer

Table 6.2: Enantiomeric separation of chiral amines as fluorene-2-carboxaldimine derivatives on coated type CSPs

Entry	Analytes	Chiralcel OD-H			Lux Cellulose-1		
		A	k'_1	R_s	α	k'_1	R_s
1	1,3-Dimethylbutylamine	1.00	0.30		1.00	0.28	
2	1,2-Dimethylpropylamine	1.00	0.33		1.00	0.31	
3	1-Methylheptylamine	1.00	0.26		1.00	0.26	
4	α-Methylbenzylamine	1.06	0.70	0.50(S)[a]	1.11	0.72	1.14(S)
5	2-Amino-1-propanol	2.59	1.53	9.32(S)	2.60	1.76	10.70(S)
6	2-Amino-4-methyl-1-pentanol	4.84	0.90	10.47(S)	5.55	1.07	14.06(S)
Entry	Analytes	Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	A	k'_1	R_s
1	1,3-Dimethylbutylamine	1.10	0.33	0.49	1.13	0.29	0.55
2	1,2-Dimethylpropylamine	1.00	0.36		1.00	0.33	
3	1-Methylheptylamine	1.00	0.33		1.00	0.30	
4	α-Methylbenzylamine	1.13	1.04	1.35(R)	1.13	1.02	1.59(R)
5	2-Amino-1-propanol	1.40	1.81	3.45(R)	1.36	2.09	2.23(R)
6	2-Amino-4-methyl-1-pentanol	1.34	1.38	1.92(R)	1.63	1.43	2.54(R)

Mobile phase: 3% 2-propanol/hexane (v/v) (entries 1-4, chiral aliphatic amines) and 10% 2-propanol/hexane (v/v) (entries 5 and 6, amino alcohols), Flow rate: 1 mL/min, Detection: UV 310 nm, k'_1: Retention factor of the first eluted enantiomer, α: Separation factor, Rs: Resolution factor, [a]the absolute configuration of the second eluted enantiomer

Likewise, the performances of three columns, i.e., Chiralpak IA (covalently bonded) and Chiralpak AD-H, and Lux Amylose-1 (coated type) with same chiral selector of amylose tris(3,5-dimethylphenylcarbamate) were examined and compared from Table 6.1 and Table 6.2. In general, most of the analytes were resolved in both kinds of CSPs, but the coated type columns Chiralpak AD-H and Lux Amylose-1 showed better enantioselectivity and separation of chiral amines as fluorene-2-carboxaldimine derivatives than the covalently type Chiralpak IA (Table 6.1). Similarly, the covalently bonded (Chiralpak IB) and coated type (Chiralcel OD-H and Lux Cellulose-1), which all contains the chiral selector as cellulose tris(3,5-dimethylphenylcarbamate) were also compared. Similar trend as coated type CSPs showed greater enantiomer separation than covalently

bonded CSP. The low enantioselectivities on the immobilized CSPs might be the lack of ordered arrangement of chiral selectors during the bonding with the silica of the CSPs [20]. In this study, in general, the performance of cellulose-based CSPs was best among all the columns used. The typical comparative chromatograms of enantiomer resolution of racemic 2-amino-1-propanol and 2-amino-4-methyl-1-pentanol as fluorene-2-carboxaldimine derivatives on Chiralpak IB and Lux Cellulose-1 are shown in Figure 6.1.

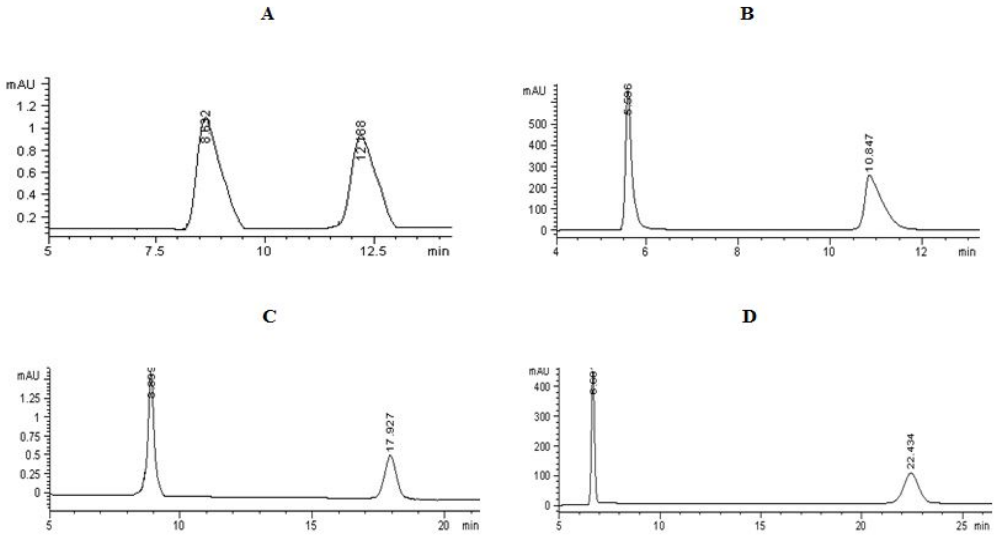

Figure 6.1: Chromatograms of the enantiomeric separation of chiral amines as fluorene-2-carboxaldimine on cellulose-derived CSPs; (A) 2-amino-1-propanol, (B) 2-amino-4-methyl-1-pentanol (leucinol) on Chiralpak IB, (C) 2-amino-1-propanol and (D) 2-amino-4-methyl-1-pentanol (leucinol) on Lux Cellulose-1. Mobile phase: 10% 2-propanol/hexane (v/v), Flow Rate:1 mL/min, Detection: 310 nm

Consistent chiral selector-dependent elution order were observed for the CSPs with the identical chiral selectors of either amylose or cellulose tris(3,5-dimethylphenylcarbamate). The (R)-

enantiomer was selectively retained in case of amylose-derived CSPs, whereas (S)-enantiomer was selectively retained on cellulose-derived CSPs. The conformational differences in the structure of amylose and cellulose-derived CSPs lead to the different chiral recognition mechanism for enantiomer separation and ultimately different elution order [16,21].

Intra- and interday accuracy and precision of the developed analytical method were evaluated using (R)- and (S)-leucinol (entry 6, leucinol) as fluorene-2-carboxaldimine derivatives on Lux Cellulose-1 with 10% 2-propanol/hexane (v/v) as a mobile phase [22]. Table 6.3 shows the data for the determination of the accuracy and precision of the analytical method using three (R)- and (S)-leucinol samples of enantiomeric purities: 98.4, 96.4, and 94.4%. The accuracy assay of intra- and interday for (R)-leucinol was determined to be 100.60-102.94% and 100.93-102.81%, respectively, while the precision for intra- and interday assay was found to be 0.35-1.02% and 0.34-0.87%, respectively. On the other hand, intra- and interday accuracy assay of (S)-leucinol were 101.07-103.03% and 101.12-103.05%, respectively, while the intra- and interday precision of the developed normal phase HPLC method were found to be 0.46-0.80% and 0.38-0.69%, respectively. Based on these obtained chromatographic results, this developed analytical method demonstrated to be a suitable method for enantiomer separation of chiral amines as fluorene-2-carboxaldimine derivatives.

Table 6.3: Intra- and interday accuracy and precision of the developed analytical method on Lux Cellulose-1

Enantiomeric purity of (R)- and (S)-leucinol (%)	Intra-day (n=9)		Inter-day (n=9)		Intra-day (n=9)		Inter-day (n=9)	
	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
98.4	102.94	0.35	102.81	0.47	103.03	0.58	103.05	0.49
96.4	101.75	0.66	101.87	0.34	102.01	0.46	101.92	0.38
94.4	100.60	1.02	100.93	0.87	101.07	0.80	101.12	0.69

See experimental section for chromatographic conditions

6.4 Conclusion

The derivatizing agent, fluorene-2-carboxaldehyde, is very effective for the separation of several chiral amines and amino alcohols using polysaccharide-derived CSPs under UV 310 nm detection. Among the investigated six covalently bonded and four coated CSPs, the enantioselectivities of amino alcohols were superior to those of chiral aliphatic amines. Especially, cellulose derived CSPs showed the best separation of amino alcohols than amylose-derived CSPs. Coated type CSPs showed enhanced enantioseparation and resolution of investigated analytes when compared with the same chiral selectors as either amylose or cellulose tris(3,5-dimethylphenylcarbamate). It is expected that the present developed analytical method can be successfully applied for enantiomer separation of chiral amines as fluorene-2-carboxaldimine derivatives under normal phase HPLC.

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Publications

1. **A convenient and validated enantiomer separation of chiral aliphatic amines as nitrobenzoxadiazole derivatives on polysaccharide-derived chiral stationary phases under simultaneous ultraviolet and fluorescence detection**

   Adhikari S, Kang JS, Lee W

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2. **Liquid chromatographic enantiomer separation of 1-naphthylamides of chiral acids using several amylose- and cellulose-derived chiral stationary phases**

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3. **Chiral separation using chiral crown ethers as chiral selectors in chirotechnology: a review**

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4. **Liquid chromatographic separation of the enantiomers of N-fluorenylmethoxycarbonyl α-amino acids on a covalently bonded type polysaccharide-derived chiral stationary phase under fluorescence detection**

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5. **Liquid chromatographic enantiomeric separation of chiral aliphatic amines using 2-hydroxynaphthaldehyde as a derivatizing agent on polysaccharide-derived chiral stationary phases**

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6. **Determination of chemical and enantiomeric purity of α-amino acids and their methyl esters as N-fluorenylmethoxycarbonyl derivatives using amylose-derived chiral stationary phases**

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7. **Enantiodiscrimination of chiral amines as naphthaldimine derivatives on polysaccharide-derived chiral stationary phases by normal phase HPLC**

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