Journal of Food and Drug Analysis
1993, 1(4) : 335-339

藥物食品分析
第一卷　第四期

A Simple Methodology for Preparing Synthetic Multiple-Interaction Chiral Stationary Phase Column for Chiral Drug Analysis

GERALD K. SHIU

Generic Drugs Research Laboratories, Food and Drug Administration
200 C Street, S.W. Washington, D.C. 20204, U.S.A.

ABSTRACT

A simple methodology for preparing synthetic multiple-interaction (SMI) chiral stationary phase (CSP) column is described in this report. The column was synthesized by an *in situ* derivatizing procedure and can be easily prepared with a standard analytical HPLC set-up. The column is a no frills, low cost one, and can be tailored to various packing particle sizes and column lengths. The efficiency of the column is high; over 5,000 plates per 10 cm. Applications on resolving some racemic drugs in plasma and formulation are illustrated.

Key words : Chiral stationary phase, Enantiomers resolution, Column preparation.

INTRODUCTION

There are two major HPLC methods for the resolution of drug enantiomers, namely, indirect chiral chromatography methods based on the formation of diastereomeric derivatives, and direct enantiomeric separation using enantioselective chiral stationary phases (CSPs). Enantiomeric contamination from reagents can be a potential limiting factor for successful chiral analysis by the diastereomeric derivative method [1]. On the other hand, the majority of the chiral molecules can be easily resolved on CSP columns without derivatization. Even if modification of the molecule is needed, an achiral derivatizing agent can be utilized, and hence eliminating the analysis error from enantiomeric contamination[2].

Recently, many synthetic multiple-interaction (SMI) CSPs have become commercially available. These CSPs provide a wide range of selectivity and versatility for enantiomeric separations. Unfortunately, high cost of these commercial CSP columns make them less affordable for routine applications. In this report, a no frills, easy to prepare SMI-CSP is described for our readers. This CSP column is rapidly prepared by a very simple and convenient *in-situ* technique. It can be customized to specific column lengths and particle sizes for particular analytical applications. Applications of this CSP column for drug products will also be demonstrated.

MATERIALS AND METHODS

I . *Reagents and Materials*

R-(−)-1-(1-naphthyl)ethyl isocyanate (R-NEI C), 2-ethoxy-1-ethoxycarbony1-1,2-dihydroquioline (EEDQ), para-nitrobenzylamine HC1 (PNBA HCl), racemic ibuprofen, and ibuprofen isomers were commercially available from Aldrich Chemical Co. (Milwaukee, WI). All other reagents and solvents used were reagent or HPLC grade.

II. *Chromatographic system and conditions*

A Waters HPLC system consisting of a model 715 Ultra WISP sample processor, M600E system controller, M991 photodiode array detector and M5200 printer plotter equipped with data analysis software was used in this study. The chromatographic separation of ibuprofen by the prepared CSP column was carried out with a mobile phase consisting of heptane : isopropanol (95 : 5) at a flow rate of 1.5 ml/min and the eluent was monitored at 235 nm.

III. *Standard solutions*

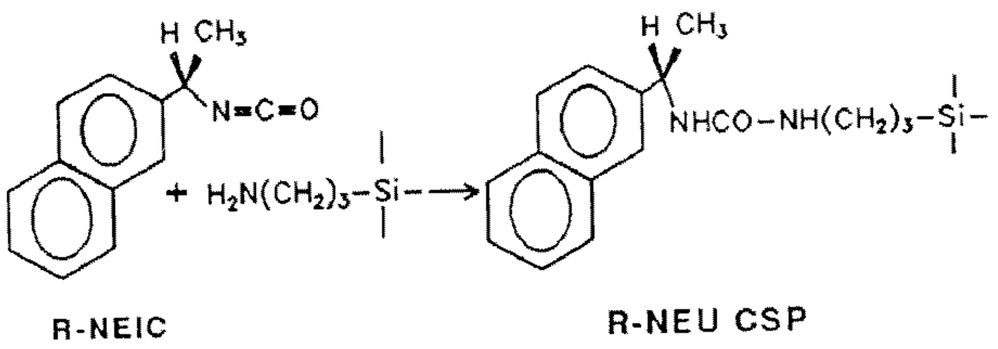
A 2% (W/V) R-NEIC solution was prepared in methylene chloride. EEDQ solution was prepared by dissolving 24 mg of EEDQ in 10 ml of ethylene chloride. PNBA solution was prepared by extracting PNBA with ethylene chloride from PNBA HCl solution. Briefly, 50 mg of PNBA HCl was dissolved in 50 ml of 0.2N NaOH, and then extracted with 50 ml of ethylene chloride. After centrifugation, the organic solvent was separated and dried with sodium sulfate. A 0.02% (W/V) solution of tridecanoic acid was prepared in ethylene chloride as an internal standard for the analysis of ibuprofen enantiomers.

IV. CSP column preparation

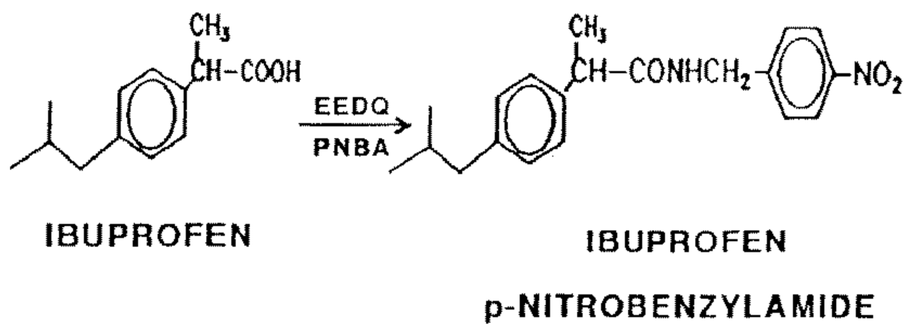
The perparation of this CSP column can be applied to all commercially available amino columns of any column length and particle size. In our study, a 3 micron aminopropylsilanized silica column of 100 mm × 4.6 mm I.D. (Regis Chemical Co., Morton Grove, IL) was installed in the HPLC system. The detector was disconnected during the column preparation. About 100 ml of 2% R-NEIC solution was delivered to the amino column at 2 ml/min at room temperature (Scheme 1). The eluent was discarded for the first ten minutes and then recycled with the remaining R-NEIC solution for 2 hours. The column was then washed with about 200 ml of methylene chloride at a flow of 2 ml/min. After reconnecting the detector, the column was washed with an additional 100 ml of methylene chloride and then switched to heptane : isopropanol (80 : 20) solution until a level baseline was observed. Usually this could be achieved in a couple of hours.

V. *Analysis of ibuprofen enantiomers*

Although many chiral molecules can be re-



Scheme 1



Scheme 2

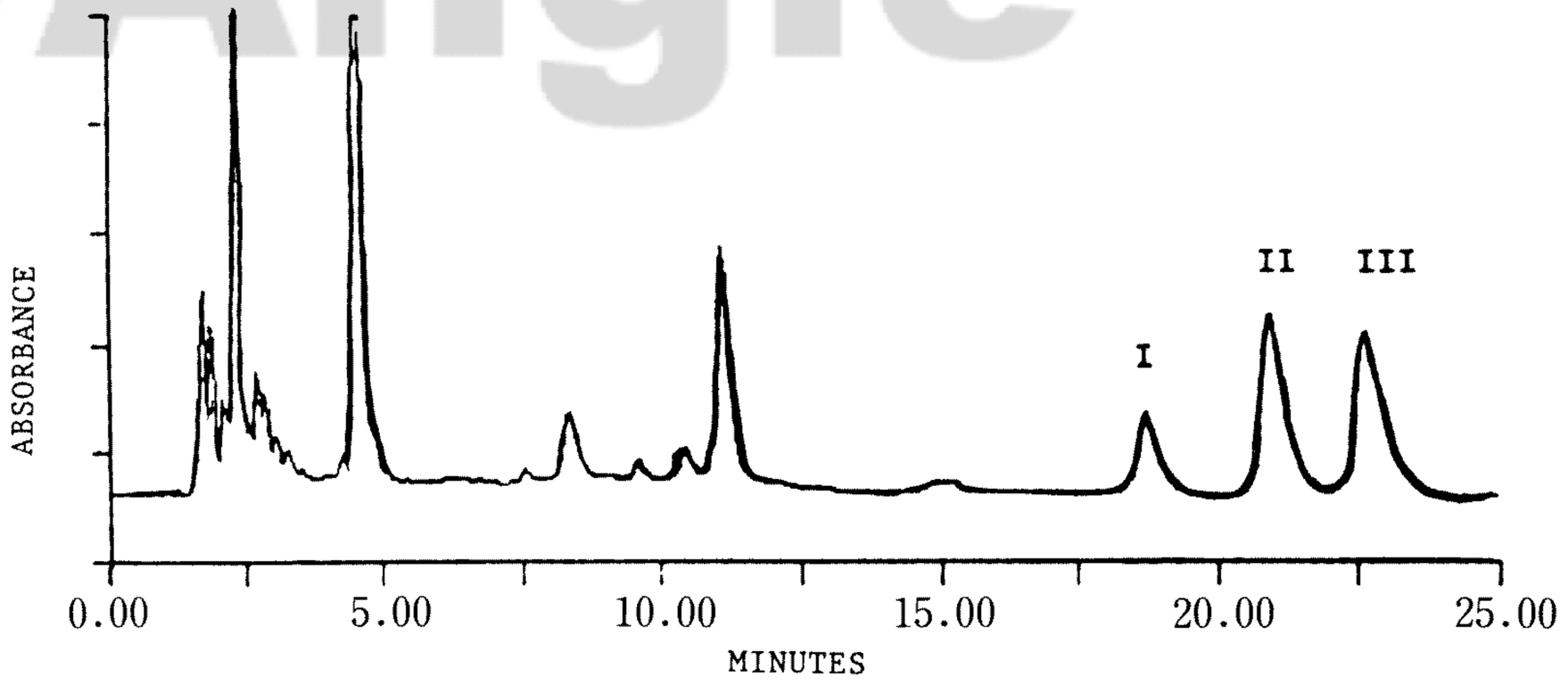


Figure 1. A typical chromatogram of the enantiomeric resolution of S-ibuprofen (II) and R-ibuprofen (III) as their para-nitrobenzylamides in plasma. The internal standard is shown as peak I.

solved on CSP column directly, a modification of the molecule to enhance the pi-pi aromatic interaction with the CSP is often desirable. Such a modification, usually with an achiral derivatizing agent that does not introduce any enantiomeric contamination, also improve sensitivity to a certain degree.

In the analysis of ibuprofen plasma samples , 0.1 ml of 0.02% tridecanoic acid (I.S.) and 0.2 ml of diluted H_2SO_4 were added to 0.5 ml of plasma. The mixture was extracted with 3 ml of isooctane : isopropanol (95 : 5). The organic solvent was dried, then refluxed for 10 minutes in 1 ml of EEDQ and 5 ml of PNBA solution (Scheme 2). After cooling to room temperature, 10 ml of ethylene chloride was added, and then washed with 10 ml of 0.2 N NaOH, 1 N HCl and water respectively. The organic solvent was dried with sodium sulfate and evaporated to dryness. The residue was reconstituted with mobile phase and aliquot injected into the above described CSP column.

RESULTS AND DISCUSSION

Currently more than one hundred SMI-CSPs that involve pi-pi interaction and hydrogen bonding or dipole interaction can be found in the literature. The utilization of chiral naphthylethylurea silica gel for racemic amine and amino acid separation was first described by Oi *et al* [3] . The simple one step *in situ* column preparation technique described above, however, was developed in our FDA laboratories. This technique applies a synthetic method for urea formation and is similar to the method described by Pirkle *et al.* [4] In preparation, the chiral naphthylethyl isocyanate (either R– or S–) is reacted to the primary amino group of the stationary packing instantaneously to form a stable covalent urea linkage. No side reactions were observed. In fact , the preparation method is so simple that only a standard analytical HPLC set-up is needed for the column preparation. Suitability of this NEU-CSP preparation for biological sample has also been demonstrated by analysis of phenylpropanolamine. [5]

The described method generated a very efficient column for ibuprofen enantiomer separation. The number of theoretical plates, N, was more than 5,000 counts per 10 cm column length. The capacity factor (k') and the separation factor (α) were about 15 and 1.09 respectively, and independent of the column length. A baseline resolution (Rs=1.5) for R(–)–and S(+)–ibuprofen was achieved by a single 10 cm co-

lumn but could be considerably improved by two 10 cm columns in series (Rs=2) for biological samples. A typical chromatogram for R(−)− and S(+)−ibuprofen in plasma as their para−nitrobenzyl amides is shown in figure 1.

Successful resolution for enantiomers of amphetamine, methamphetamine and tryptophan were also achieved by utilizing this CSP column [6,7]. In the case of amphetamine and methamphetamine, samples were derivatized at room temperature in less than one minute with (3,5-dinitrophenyl)isocyanate. The enantiomeric 3,5-dinitrobenzoyl amide derivatives were then resolved on the NEU-CSP column[6]. The method was extremely sensitive and a trace isomeric contamination of less than 0.1% of dextro-methamphetamine (street drug "speed") in a nasal decongestant containing legal OTC drug levo-methamphetamine could be determined.

CONCLUSION

The NEU-CSP column described here is a very simple and easy to prepare column that can resolve a variety of chiral drugs. Though the column preparation and its applications have been investigated and used extensively in our laboratories for enantiomeric resolution of a variety of drugs, the column preparation has not been fully publicized and utilized by the others. Recently, this type of column has become available commercially with a high cost. It was the author's intention in this report to disseminate this simple know-how to all interested readers to take the advantage of this low cost alternative. With a little bit of imagination and a spirit of exploration, further applications to other chiral drugs will no doubt be achieved.

REFERENCES

1. Ahn, H.Y., Wu, S.T., Shiu, G.K., Doyle, T. D., Brunner, C.A. and Trafton W.F. 1991. Potential racemization during enantiomeric analysis of ibuprofen. Pharm. Research. 8 : 31.
2. Brunner, C.A., Ahn, H.Y., Shiu, G.K. and Doyle T.D. 1992. Concerning the optimization of HPLC chiral resolutions. 106th AOAC Annual meeting, Cincinnati, OH., U. S.A.
3. Oi, N., Kithara, T., Doi, T. and Yamamoto S. 1983. Urea derivatives of chiral amino acids and amines as novel stationary phases for enantiomer separation by high performance liquid chromatography. Bunseki Kagaku (Jap. Anal.) 32 : 345-346.
4. Pirkle, W.H., Mahler, G. and Hyun M.H. 1986. Separation of the enantiomers of 3,5-dinitrophenyl carbamates and 3,5-dinitrophenyl ureas. J. Liq. Chromatogr. 9 : 443-453.
5. Doyle, T.D., Brunner, C.A. and Vick J.A. 1991. Enantiomeric analysis of phenylpropanolamine in plasma via resolution of dinitrophenylurea derivatives on a HPLC chiral stationary phase. Biomed. Chromatogr. 5 : 43-46.
6. Doyle, T.D., and Brunner C.A. 1989. Application of chiral HPLC determination of trace amounts of dextro- in levo-methamphetamine. 103rd Association of Official Analytical Chemists (AOAC) Annual meeting, St. Louis, MO., U.S.A.
7. Doyle, T.D. 1993. Development and validation of chiral methodology. Symposium on Analytical Aspects for New Drug Applications, Pittsburgh Conference, Atlanta, GA., U.S.A.

Correspondence to : Gerald K. Shiu

Accepted for Publication: September. 15. 1993

Journal of Food and Drug Analysis 1993. 1(4)

以光學活性靜相管柱分析光學活性藥物

GERALD K. SHIU(蕭　參)

Generic Drugs Research Laboratories, Food and Drug Administration
200 C Street, S.W. Washington, D.C. 20204, U.S.A.

摘　要

本報告係描述一種簡單且容易製備的合成多重交互作用光學活性管柱，管柱固定相以衍生步驟合成，且易裝置於一般標準型的分析用高效液相層析儀。此管柱是一簡單，價低廉，各種大小的填充粒子與管柱長度皆適合，管柱的效率高，每10公分多於5000板，本文並以實例說明此管柱於分析生物體液及藥劑中消旋性藥物之應用。