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Short communication

Separation of naproxen enantiomers by supercritical/subcritical fluid chromatography

Yiwen Yang *, Baogen Su, Quanhong Yan, Qilong Ren

Institute of Pharmaceutical Engineering, Department of Chemical and Biochemical Engineering, Zhejiang University, 38 Zheda Road, Hangzhou 310027, China

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Abstract

An isocratic supercritical/subcritical fluid chromatography method for the separation of naproxen enantiomers on the Kromasil CHI-TBB column was investigated. The mobile phase was composed of supercritical CO_2 with 2-propanol as modifier. The experimental conditions were temperature 293 K–323 K, pressure 9.4 MPa–21.3 MPa, and 2-propanol concentration 6%–15% (by mass), respectively. The enthalpic contribution to the overall enantiomer transfer energy was more important than the entropic contribution in the temperature range examined. The preferred operation conditions were 293 K, 9.4 MPa, and the concentration of 2-propanol in the mobile phase 11% (by mass).

Keywords: Naproxen; Chiral; Enantiomer separation; Supercritical fluid chromatography

1. Introduction

Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid) is a non-steroidal anti-inflammatory drug with analgesic and anti-pyretic properties. It has one chiral center which gives rise to two optical isomers and their pharmacological activity resides in *S*-(+)-enantiomer, while the *R*-(−)-enantiomer causes some unwanted side effects. Therefore, it is marketed as a single enantiomer [1].

Several methods have been proposed to separate the naproxen enantiomers. Yuan et al. [2] resolved *S*-(+)-naproxen from the racemate by inclusion crystallization using *N*-octyl-D-(−)-glucamine as the chiral host. Koul et al. [3] resolved it by enzymatic kinetic method from the racemate methyl ester using *Trichosporon* sp. in kg level. Pirkle and Welch [4] separated the enantiomers of underivatized naproxen by chiral stationary phase (CSP) HPLC. Healy et al. [5] separated the naproxen enantiomers on an ODS column with methyl-β-cyclodextrin as a mobile phase additive using conventional and nano-LC. Lei and Tan [6] prepared a new chiral stationary phase by molecular imprinting, and separated naproxen enantiomers by affinity chromatography. Lelièvre and Gareil [7] studied the separations of non-steroidal anti-inflammatory drugs of the family of arylpropionics acids including naproxen by capillary electrophoresis in different pH buffers containing cyclodextrins.

Supercritical fluid chromatography has proven to be widely applicable for the separation of enantiomers on chiral stationary phases (CSPs) [8]. Due to the unique properties of supercritical/subcritical fluids, high efficiency and resolution and good selectivity can be obtained in short analysis times. It may be used to separate enantiomers both analytically and preparatively. Medvedovici et al. [9] evaluated the separation of different types of racemates including naproxen on the polysaccharide CSPs (Chiralcel OD and Chiralpak AD), the macrocyclic antibiotic CSPs (Chirobiotic V and Chirobiotic T), and the brush type CSPs (Chirex 3022 and Chirex 3005) by subcritical fluid chromatography.

The aim of this work is to explore the feasibility of enantiomer separation of naproxen by supercritical/subcritical fluid chromatography on the Kromasil CHI-TBB column. An isocratic mobile phase was used which was composed of supercritical CO_2 with 2-propanol as modifier. The effects of

* Corresponding author. Tel.: +86 571 85967039; fax: +86 571 87952773.
E-mail address: ceywyang@zju.edu.cn (Y. Yang).

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temperature, pressure, and the concentration of modifier on the capacity factor, selectivity, and resolution were discussed.

2. Experimental

2.1. Instrumentation

The experimental apparatus was modified from the solubility determination apparatus in supercritical CO_2 as reported previously [10]. The carbon dioxide was delivered from a cylinder to the cooling coil, pressurized by a syringe pump with a constant flow mode, mixed with 2-propanol, and then flew through the preheater, the six way valve, and the column, all of which were immersed in a water thermostat. A packed Kromasil CHI-TBB column (250 mm × 4.6 mm i.d., 5 μm, Eka Chemicals AB, Bohus, Sweden) was selected as the chiral stationary phase, which was made by bonding a polymer of O,O′-bis (4-*tert*-butylbenzoyl)-*N*,N′-diallyl-L-tartar diamide chiral monomer and hydrosilane to silica. The sample solution was prepared in 2-propanol at 1.006 mg/ml, and introduced into the column via a high-pressure six way valve fitted with a 20-μl sample loop. The peaks were detected at 254 nm using a UV–vis detector (UV Linear UVIS 200) and the signals from the detector were processed by a chromatograph work station and a computer. The pressure was controlled by a manually adjustable back-pressure regulator. The gas exiting from the restrictor was expanded to atmospheric pressure and its volumetric flow-rate was 710 ml/min at room temperature.

2.2. Chemicals

Carbon dioxide with 99.99% purity was obtained from Mingxing Gas Co. Ltd. (Hangzhou, China). 2-Propanol (chromatography grade) was from Tianjin Shield Company (Tianjin, China). Racemic naproxen with a purity of more than 98.5% was donated from Tianxin Pharmachem Co. Ltd. (Tiantai Country, Zhejiang Province, China). *S*-(+)-naproxen standard (free acid, USP grade) was purchased from Sigma (Shanghai, China).

3. Results and discussion

3.1. Repeatability

Typical chromatogram is shown in Fig. 1. The *S*-(+)-naproxen was eluted earlier than *R*-(−)-naproxen. It can be seen that the chiral separation of racemic naproxen on Kromasil CHI-TBB column was very well.

The repeatable experiments were done at 17.8 MPa, 293 K, and the concentration of 2-propanol in the mobile phase 11% (by mass). The relative standard deviations (R.S.D.) ($n = 4$) of capacity factors of *S*-naproxen and *R*-naproxen, selectivity, and resolution were 1.60%, 1.86%, 0.31%, and 0.34%, respectively, which showed a good repeatability.

3.2. Effect of temperature and pressure on capacity factor

The capacity factors of *S*-naproxen and *R*-naproxen are plotted against pressure as shown in Figs. 2 and 3, respectively, with the concentration of 2-propanol in the mobile phase being 11% (by mass). The capacity factor decreases with pressure at constant temperature. It is because that as the pressure increases at constant temperature, the density of CO_2 increases too, which enhances the solubility of naproxen in CO_2. The capacity factor increases with temperature at constant pressure.

3.3. Effect of temperature and pressure on resolution

In Fig. 4, the resolution of naproxen is plotted against the pressure with the concentration of 2-propanol in the mobile

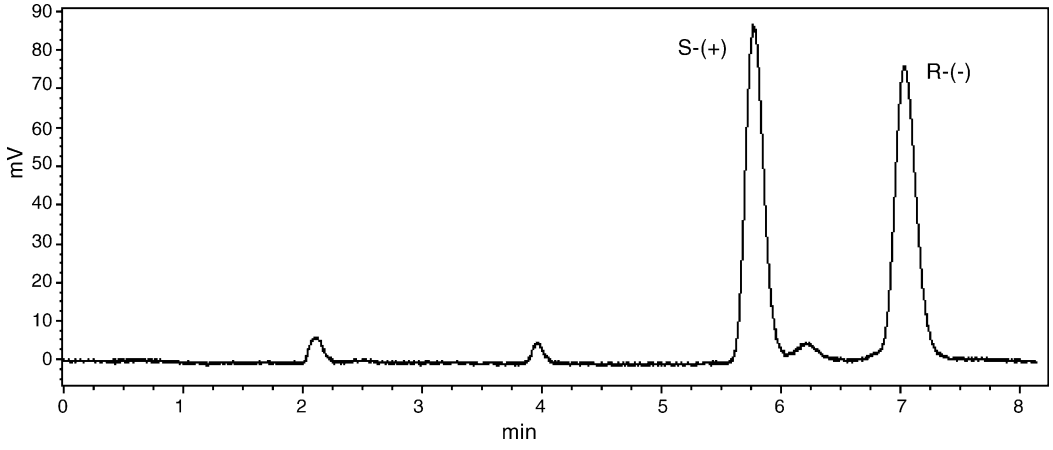

Fig. 1. Typical chromatogram of racemic naproxen on Kromasil CHI-TBB column. (Operation condition: 293 K, 17.8 MPa, the concentration of 2-propanol in the mobile phase 11% (by mass.))

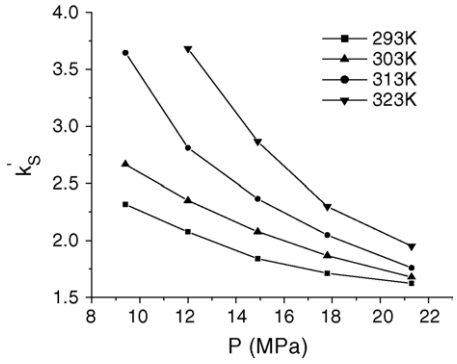

Fig. 2. Plot of k' of *S*-naproxen vs. pressure (the concentration of 2-propanol in the mobile phase 11% (by mass)).

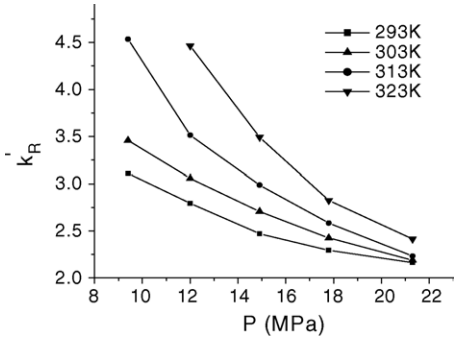

Fig. 3. Plot of k' of *R*-naproxen vs. pressure (the concentration of 2-propanol in the mobile phase 11% (by mass)).

phase being 11% (by mass). The resolution decreases with pressure at constant temperature. It decreases with temperature at constant pressure as well. From Sections 3.2 and 3.3, it may be concluded that the preferred operation temperature and pressure in the range examined were 293 K and 9.4 MPa, respectively.

3.4. Effect of temperature on selectivity

As described by Stringham and Blackwell [11], and Smith et al. [12], the selectivity in SFC may be related to temperature

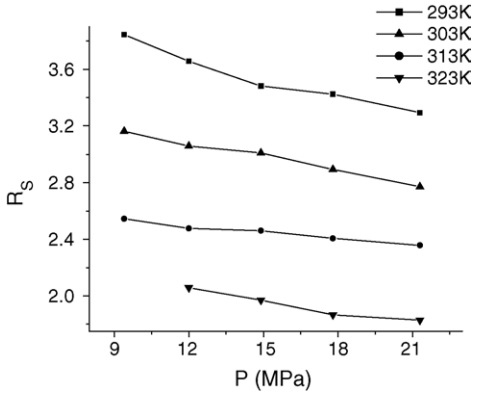

Fig. 4. Plot of R_S vs. pressure (the concentration of 2-propanol in the mobile phase 11% (by mass)).

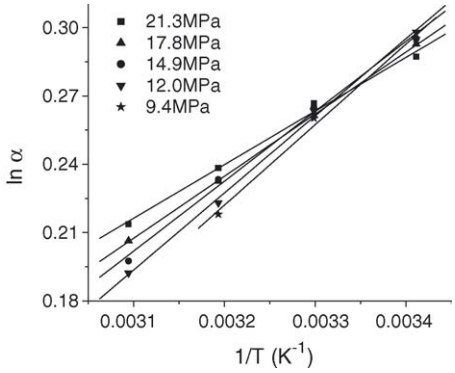

Fig. 5. Plot of $\ln\alpha$ vs. $1/T$ (the concentration of 2-propanol in the mobile phase 11% (by mass)).

as:

$$\ln\,\alpha = -\frac{\Delta_{R,S}\,\Delta H^0}{RT} + \frac{\Delta_{R,S}\,\Delta S^0}{R} \quad (1)$$

where α is selectivity (calculated as k'_R/k'_S), $\Delta_{R,S}\,\Delta H^0$ and $\Delta_{R,S}\,\Delta S^0$ are the differences in the enthalpy and entropy terms for the enantiomers, R is the ideal gas constant, T is absolute temperature. Selectivity is determined by an enthalpic contribution that decreases with temperature and an entropic contribution that should be independent of temperature. Potting $\ln\alpha$ of naproxen enantiomers at constant pressure versus $1/T$ (see Fig. 5) gives straight lines and their linear correlation coefficients are not less than 0.99. It is obvious that the selectivity increases as temperature decreases. The differences in the enthalpy and entropy at different pressure are calculated from the slopes and *y*-intercepts of the Van't Hoff plots shown in Fig. 5, respectively, which are summarized in Table 1.

As shown in Table 1, $|\Delta_{R,S}\,\Delta H^0| > |T\,\Delta_{R,S}\,\Delta S^0|$ keeps in the experimental range (293 K–323 K). Thus, the separation of naproxen enantiomers by supercritical (or subcritical) fluid chromatography on the Kromasil CHI-TBB chiral stationary phase is "enthalpically driven" in the range examined.

3.5. Effect of 2-propanol concentration in the mobile phase

Experiments were carried out under preferred conditions of pressure 9.4 MPa, temperature 293 K. The results are shown in Table 2. The capacity factors, selectivity, and resolution of enantiomers decreased with the concentration of

Table 1
Thermodynamic parameters for naproxen enantiomers

P (MPa)	$\Delta_{R,S}\,\Delta H^0$ (J/mol)	$\Delta_{R,S}\,\Delta S^0$ (J/mol K)	$T\,\Delta_{R,S}\,\Delta S^0$ at 323 K (J/mol)
9.4	−2940	−7.56	−2442
12.0	−2810	−7.11	−2297
14.9	−2550	−6.23	−2012
17.8	−2270	−5.30	−1712
21.3	−1960	−4.27	−1379

Table 2
Effect of 2-propanol concentration on capacity factors and resolution

2-Propanol concentration (%, by mass)	k'_R	k'_S	α	R_S
5.7	7.08	4.91	1.44	5.76
11	3.11	2.32	1.34	3.85
15	1.57	1.19	1.32	2.57

2-propanol in the mobile phase. Reducing the concentration of 2-propanol in the mobile phase increased the resolution of enantiomers. However, the capacity factors also increased, the enantiomers eluted later, so that the sample throughput was lower. The higher was the concentration of 2-propanol in the mobile phase, the more was the consumption of modifier solvent. When the capacity factors were small, the effects of 2-propanol concentration on capacity factors were less. Therefore, the suitable capacity factors were between 2 and 5. As a result, the preferred concentration of 2-propanol in the mobile phase was 11% (by mass) in the range examined.

Acknowledgements

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