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Short communication

Separation of enantiomers of ibuprofen on chiral stationary phases by packed column supercritical fluid chromatography

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Abstract

A packed column supercritical fluid chromatography (SFC) method for the separation of ibuprofen enantiomers on a chiral stationary phase and CO_2 with modifier as mobile phase has been developed at an analytical scale. Among 11 different stationary phases the Kromasil CHI-TBB phase showed by far the best separation properties. The influence of different modifiers, injection solvents, temperature, and pressure, and density of the fluid, respectively, on the separation behavior has been studied. It was found that the separation behavior strongly depends on the type of modifier and the modifier content. Temperature and pressure are of less influence.

Keywords: Chiral stationary phases, SFC; Enantiomer separation; Supercritical fluid chromatography; Ibuprofen

1. Introduction

Ibuprofen [2-(4-isobutylphenyl) propionic acid], is a non-steroidal anti-inflammatory drug (NSAID) belonging to the group of propionic acid derivatives. Nowadays ibuprofen is sold as a racemate. Though the pharmacological activity resides in the *S*(+)-enantiomer, the *R*(−)-enantiomer causes some unwanted side effects.

Today preparative chromatography of enantiomers is mostly carried out by liquid chromatography (LC). The use of supercritical (or subcritical) fluid chromatography (SFC) may result in higher production rates as the resolution per time in general is better in SFC. Other advantages of preparative SFC over preparative HPLC include substantial waste reduction, facilitated product recovery and feasibility of solvent recycling.

Several direct and indirect liquid chromatographic analytical methods involving a variety of chiral and achiral phases for resolution of ibuprofen enantiomers have been reviewed [1]. A packed polysiloxane-based chiral stationary phase was used for the separation of different enantiomers in HPLC and SFC [2]. A good separation factor between the ibuprofen isomers was found in SFC with isopropanol–acetic acid in carbon dioxide as mobile phase and it was even better in HPLC with ethanol–acetic acid in hexane. A comparison of LC and SFC for the separation of ibuprofen and other enantiomers was done for a cyclodextrin stationary phase [3] and for a pirkle-type stationary phase (Whelk-O 1) [4]. For ibuprofen determination by SFC with carbon dioxide–isopropanol–trifluoroacetic acid or carbon dioxide–methanol, respectively, as mobile phase higher

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resolutions but lower separation factors than those by LC were found for both packed columns. The application of Chiralcel OJ phases for the analytical separation of ibuprofen and other frequently used drugs was evaluated [5]. In the SFC mode with a modifier of acetonitrile–trifluoroacetic acid–triethylamine in carbon dioxide no separation occurred. With methanol–trifluoroacetic acid–triethylamine in carbon dioxide separation was possible and it was even better than in HPLC with isopropanol–acetic acid in hexane. The enantioselective separation of ibuprofen by packed column SFC on a Chiralpak AD column with methanol in carbon dioxide as mobile phase has been investigated [6,7]. Anyway, separation factors and conditions reported in the literature are not satisfactory for preparative SFC separations.

The aim of this project was to develop an SFC method with a binary mobile phase (without an additive) for the separation of ibuprofen enantiomers at an analytical scale which is suitable for an upscaling to preparative chromatography like simulated moving bed (SMB) SFC [8].

2. Experimental

2.1. Analytical SFC system

A commercial SFC G1205A system (Hewlett-Packard, Little Falls, USA) with an SFC pump, a modifier pump and a HP 1050 high-pressure diode array detector was used for analytical chromatography. The SFC system was equipped with a HP 7673 autosampler connected to a Rheodyne valve with a fixed 5-μl volume external loop. This system, operated in the downstream mode, allowed independent pressure and flow control. The columns were kept at a constant temperature in the instrument's column oven. Data were collected and processed on HP ChemStation.

The SFC method can be optimized by variation of the stationary phase, the mobile phase, the modifier (type and concentration), the temperature and the pressure, respectively, density of the fluid. For our investigations we used 11 different analytical chiral columns as stationary phases. The mobile phase consisted of a variety of modifiers entrained in carbon dioxide at 0–20% (v/v) and column outlet pressure ranged from 100 to 190 bar. Column temperature varied from 30 to 50°C. Modifiers included ethanol, isopropanol and ethyl acetate. The total flow of the mobile phase was kept constant at 2 ml/min (pump setting).

2.2. Materials

Ibuprofen USP 23 (99.3%) was kindly donated from Omya Peralta (Hamburg, Germany). For method development solutions of about 1% ibuprofen either in isopropanol or in *n*-hexane have been prepared. Carbon dioxide with a purity of more than 99.995% was obtained from Linde (Hamburg, Germany). Isopropanol, ethanol, benzene, toluene, diisopropylether, and trichlorethane of analytical grade and ethyl acetate and *n*-hexane of SupraSolv grade were all purchased from Merck (Darmstadt, Germany). Butylbenzene (~99%), 1,3,5-tri-*tert*.-butylbenzene (≥97%), and *S*-ibuprofen (≥99%) were obtained from Fluka (Deisenhofen, Germany). Analytical (25×0.46 cm) chiral columns Chiralcel OB-H, OJ, and OD, Chiralpak AD and AS were from Daicel/Chiral Technologies (Illkirch, France), Chirobiotik T and V were from Astec/btr (Wilmington, USA), Kromasil CHI-DMB and CHI-TBB were kindly donated from Eka Chemicals (Bohus, Sweden), Whelk-O 1 and L-ChiraSpher NT from Merck.

3. Results and discussion

3.1. Influence of stationary phase and mobile phase composition

Different chiral stationary phases were tested with regard to their suitability to separate the two ibuprofen enantiomers. The experiments were done at a constant temperature of 40°C and a column outlet pressure of 160 bar. The content of modifier isopropanol was varied. The samples were dissolved in isopropanol. The wavelength for UV detection was 220 nm.

In Fig. 1 and Fig. 2 the separation factor α and, respectively, the peak resolution R_s between the two ibuprofen enantiomers are shown as functions of the isopropanol content in the mobile phase. The higher

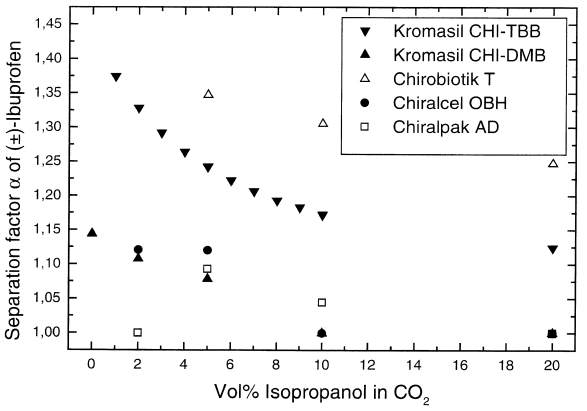

Fig. 1. Separation factor α of (±)-ibuprofen as a function of isopropanol content in CO_2 for different chiral columns at 40°C and 160 bar.

the modifier concentration the lower is the separation factor and the peak resolution, as expected. The highest separation factor (α=1.37) was reached on the Kromasil CHI-TBB phase where the peak resolution R_s was the highest (R_s=4.4) also. A maximum number of theoretical plates of $N_{2\ max}$=7900 was obtained on the Kromasil CHI-TBB phase with 3% (v/v) isopropanol in CO_2.

Although also the Chirobiotik T phase showed a good separation factor (α=1.35), the enantiomers eluted late and broad there, so that the peak resolution and the number of theoretical plates ($N_{2\ max}$= 1360) are not nearly as good as they are on the Kromasil CHI-TBB phase. Separation of the ibuprofen enantiomers could also be seen on the Kromasil CHI-DMB, Chiralcel OBH and Chiralpak AD phases. The determined separation factor and resolution on Chiralpak AD with modifier isopropanol are lower as reported in the literature with methanol modifier [6,7]. No separation occurred on Chiralcel OD, Chiralcel OJ, Chiralpak AS, Whelk-O 1, Chiraspher NT, and Chirobiotik V.

Because of the good results on the Kromasil CHI-TBB phase all further investigations were fixed on this phase.

3.2. Method optimization on Kromasil CHI-TBB

3.2.1. Influence of different solvents as modifier

Different modifiers, isopropanol, ethanol and ethyl acetate were used. The experiments were done on the Kromasil CHI-TBB phase at a constant temperature of 40°C and a column outlet pressure of 160 bar. The percentages of modifier were varied. The samples were dissolved in *n*-hexane. Detection was done at 220 nm with ethanol modifier and 254 nm with isopropanol and ethyl acetate.

In Fig. 3 the separation factor α between the two ibuprofen enantiomers is shown as a function of the retention factor of the second eluting enantiomer K_2 for different modifier solvents (5, 10, and 20%, v/v, each). As seen before, the higher the modifier concentration (for all modifiers) the lower is the separation factor (and the peak resolution). With

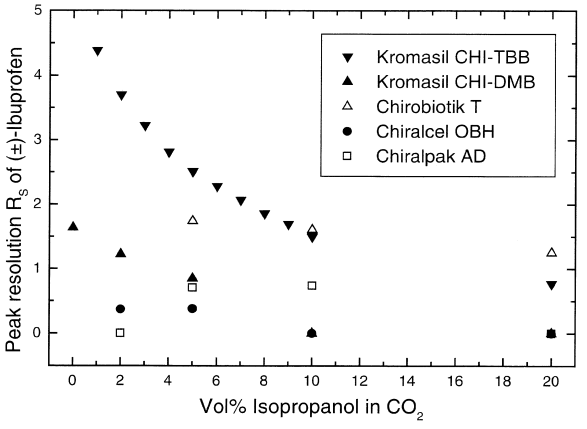

Fig. 2. Peak resolution R_s of (±)-ibuprofen as a function of isopropanol content in CO_2 for different chiral columns at 40°C and 160 bar.

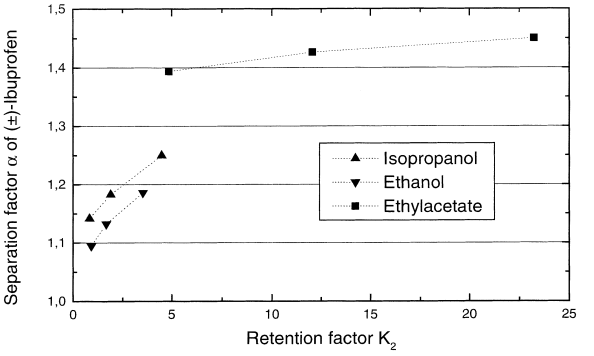

Fig. 3. Separation factor α of (±)-ibuprofen on a Kromasil CHI-TBB analytical column as a function of retention factor K_2 for different modifier solvents (5, 10, and 20%, v/v, each) at 40°C and 160 bar.

isopropanol as modifier better separation factors as with ethanol have been obtained. The highest separation factor was reached with ethyl acetate as modifier where the peak resolution was the highest, too. But with ethyl acetate the enantiomers eluted very late, so that the resolution per time is lower than with isopropanol. This leads to ineffective analysis times.

3.2.2. Influence of temperature and pressure

First, experiments were done at 160 bar with 6 and 8% (v/v) isopropanol in the mobile phase. The temperature was varied between 30 and 50°C. It was found that with increasing temperature the separation factor is substantially, but the peak resolution only marginally, decreasing.

Finally, a pressure variation between 100 and 250 bar was done at two temperatures and with two isopropanol contents in the mobile phase. It was found that while the separation factor is marginally increasing with increasing pressure the peak resolution is marginally decreasing.

4. Conclusions

An SFC method for the separation of ibuprofen enantiomers on a Kromasil CHI-TBB stationary phase and CO_2 with isopropanol as mobile phase has been developed at an analytical scale. Suitable chromatographic conditions for the separation were found with 4 to 7% (v/v) isopropanol in CO_2 as mobile phase. The up-scaling for the preparative separation by SMB-SFC have been published elsewhere [9].

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