Separation of Flurbiprofen Enantiomers on a Chiral Stationary Phase by Supercritical Fluid Chromatography

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Abstract

A Sub- and Supercritical Fluid Chromatography System was developed for separation of chiral isomers of flurbiprofen, a non-Steroidal Anti-Inflammatory Drug (NSAID). S-isomer is used for various pain treatments such as Rheumatoid Arthritis and Osteoarthritis while R-isomer is used in cancer research. The column utilized for this method was a Pirkle brush-type Whelk-O 1 analytical column packed with 10μm particles. Carbon dioxide is used as a mobile phase with isopropanol and sec-butanol employed as a modifier. The temperature was varied to provide sub and supercritical conditions for the alcohol – carbon dioxide mixture. The effect of temperature on the separation factor, peak resolution and retention time is studied. The adsorption isotherms are used to abstract isotherm information for scale up and synthesis of operating policies for fractionation through simulated moving bed. Isotherms were obtained at the optimum conditions for separation. Elution techniques such as Elution by a Chromatographic Point and peak maxima as well as frontal analysis technique were used in order to determine isotherms for single component adsorption as well as isotherms for competitive adsorption at finite concentrations of the solutes.

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1. Introduction

Preparative, chiral supercritical fluid chromatography (SFC) is drawing extensive attention for separation of enantiomers due to the green characteristics of supercritical fluids. Supercritical fluid chromatography is most often used with carbon dioxide as a mobile phase and an organic modifier such as some type of an organic solvent (alcohol) [1]. In addition to some significant advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations, it is basically preferred to HPLC due to significantly less generation of hazardous waste. Main advantages of supercritical fluids for preparatory chromatography include solvent waste reduction as well as facilitated product recovery, lower solvent cost and the possibility for solvent recycling [2].

The chiral drug being studied in this body of work is Flurbiprofen, a non-steroidal anti-inflammatory drug (NSAID), which as of late is being studied for its chiral-related properties. There are several reasons to separate the two isomers (R and S). One is that pure S-Flurbiprofen, used fro treatment of arthritis would allow for a smaller dosage and possibly a reduction in the severity or occurrence of side effects. Another is that more recently R-Flurbiprofen has been studied for potential uses in as cancer treatment drug.

Chromatographic techniques and technologies are currently in wide spread use for analytical and preparative purposes in industry [3]. In many cases, the only method to obtain pure components is chromatography; this is especially true for isomers and enantiomers. Chiral separations are relatively new capabilities that are based on the elution chromatography model, but special stationary phases have to be utilized to achieve separation. The most often produced stationary phases are made of polysaccharides or proteins, but these are much more delicate than the standard C_{18} and C_8 columns and tend to have a much shorter operational life expectancy [4]. The reason for this delicate nature is that chiral stationary phases are not covalently bonded to the silica support like the C_{18} and C_8 columns.

Several different modifiers were used in the experiments to find what organic or combination of organics produced the best results. The modifiers that were used in the experiments were around 10 volume percent in carbon dioxide. The specific organics used as modifiers were isopropanol, and sec-butanol.

The objective of the research is to find best modifier(s) and the optimal conditions for scale-up to preparative supercritical fluid chromatography applications. For this purpose, elution techniques such as Elution by a Chromatographic Point and peak maxima as well as frontal analysis technique were used in order to determine isotherms for single component adsorption as well as isotherms for competitive adsorption at finite concentrations of the solutes.

2. Experimental

2.1. Experimental Set-up

The SFC system used is shown on Figure 1. The apparatus consisted of a 500mL ISCO syringe pump used for the delivery of carbon dioxide. A Waters 600E HPLC pump was used for delivery of the various modifiers. A Hewlett Packard 1050 UV/vies detector, fitted with a 400bar flow cell, was used for detection. Sample delivery was accomplished via a Perkins-Elmer ISS-900 auto sampler equipped with a Rheodyne valve which has a pressure rating up to 350bar. A circulating bath was a 15 gallon aquarium and a circulating heater was used to heat the water bath. The outlet line of the syringe pump was connected to a pre-heating coil of ¼ in O.D. and 30 feet in total length through the top part of the column heater to assure that the carbon dioxide was also at

the desired temperature before sample introduction. The Regis (R, R) Whelk-O 1 chiral chromatographic column used in this work was packed with 10μm particles. The 10μm particles were used because the larger packing is more durable and better encapsulated than the 5μm particles. The Whelk-O 1 column is pressure rated to 7000 psi, and is stable up to 60°C. An automated backpressure regulator made by Thar Designs, BPR-A-200B, was used to keep the pressure at desired levels. A recirculating chiller, Lauda E100 econoline RE120, supplied the coolant to the ISCO syringe pump cooling-jacket. A BNC-1120 connector block along with a 6240E data acquisition card was purchased from National Instruments for use with the Labview 6.1 data acquisition software. The data were analyzed by employing the Microcal Origin 6.0 graphical software.

2.2. Materials and Reagents

Carbon dioxide (99.8% purity) was purchased from Air Gas. HPLC grade Isopropanol (99.9% purity), Sec-butanol (99.5% purity), were supplied by Sigma Aldrich. Flurbiprofen Mix, R-isomer and S-isomer were all supplied by Sigma Aldrich. A 25cm × 4.6mm internal diameter column packed with (R, R) Whelk-O 1 material, 10μm particle size was purchased from Regis Technologies.

2.3. Procedure

The re-circulating chiller was adjusted to supply a temperature of –4.0°C to the cooling jacket of the syringe pump to prevent the vaporization of carbon dioxide. The low temperature allowed for fast reloading of the syringe pump with carbon dioxide. The column heater was adjusted to the desired temperature. The syringe pump was set to the desired flow rate and the HPLC pump's set point was set usually to deliver 10% v/v of modifier into the mobile phase of carbon dioxide. The automated backpressure regulator was set to the desired pressure. The UV/visible detector was set for detection at 220nm. The mobile phase flow rate was 1 ml/min. The auto sampler was programmed to inject a 10μL sample. The sample concentrations for the work contained here were all 1.0 mg/mL of racemic Flurbiprofen for separation experiments.

3. Method for isotherm determination

Adsorption isotherms of the S and R enantiomers were determined using elution chromatography. The retention volume in an elution peak is proportional to the derivative of adsorption. The isotherms were calculated from single peaks, the height of the peak being proportional to the concentration of the enantiomer in the fluid phase [5].

4. Results and discussion

4.1. Effect of temperature, pressure and modifier on separation factor and peak resolution

Several different modifiers were used in the experiments to find what organic or combination of organics produced the best results. The modifiers that were used in the experiments were around 10 volume percent in carbon dioxide. The specific organics used as modifiers were isopropanol, sec-butanol and combinations of equal amounts by volume of isopropanol and sec-butanol. Separation factors were evaluated in order to find out which modifier worked best. Highest separation factors were obtained with isopropanol.

Separation with isopropanol was tested at 30^{o}C 35^{o}C and 40^{o}C. Higher temperatures produced lower separation factors, a trend that was expected and observed. The effect of pressure was not uniform for different modifiers. Different modifiers showed different trends. When isopropanol was used as a modifier, higher pressures produced slightly higher separation factors.

Peak resolution is another indication of the degree of separation and peak resolutions were evaluated in order to determine the best modifier and optimum conditions for ibuprofen separation. Highest peak resolution was obtained with 10% *v/v* isopropanol modifier (Figure 1).

5. Conclusions

A method was developed to separate the enantiomers of flurbiprofen on a Whelk-O 1 stationary phase and using various modifiers in Supercritical Fluid Chromatography. Thus far isopropanol (total *v/v* 10%) produced the best peak resolution of 1.1018 at 30°C and 100bar. The work expands previous work to different modifiers and their mixes.

6. Acknowledgements

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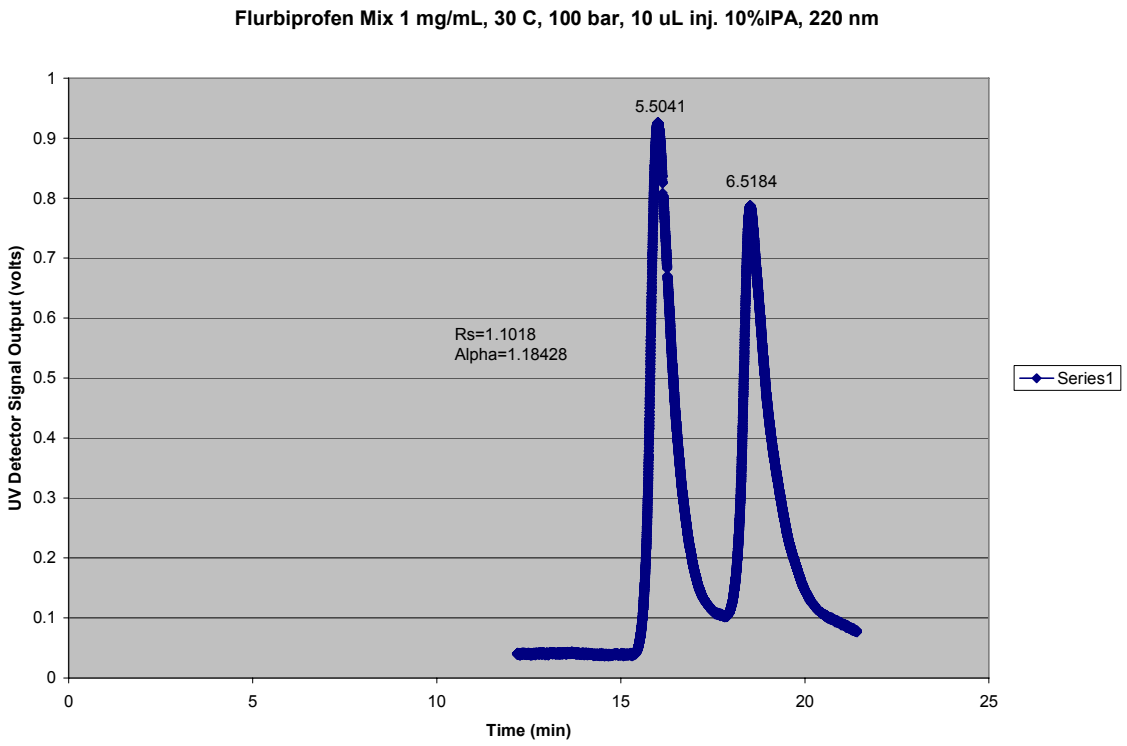

Figure 1: Flurbiprofen Separation 1mg/mL Sample at 30°C

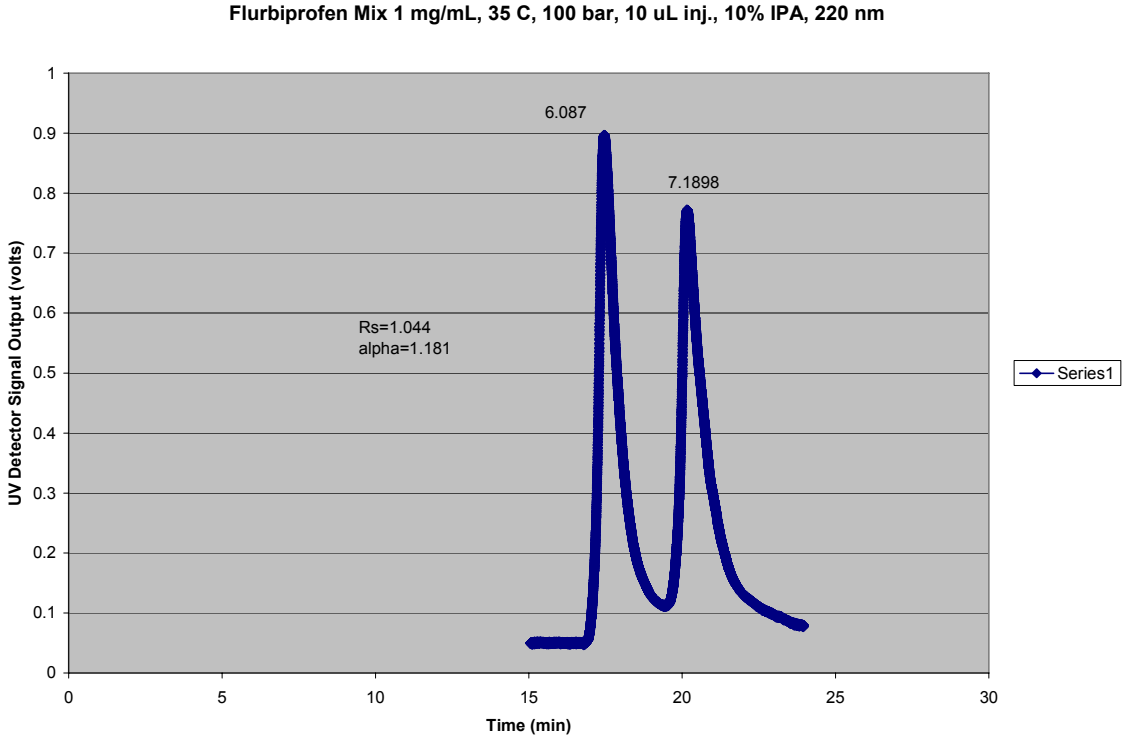

Figure 2: Flurbiprofen Separation 1mg/mL Sample at 35°C

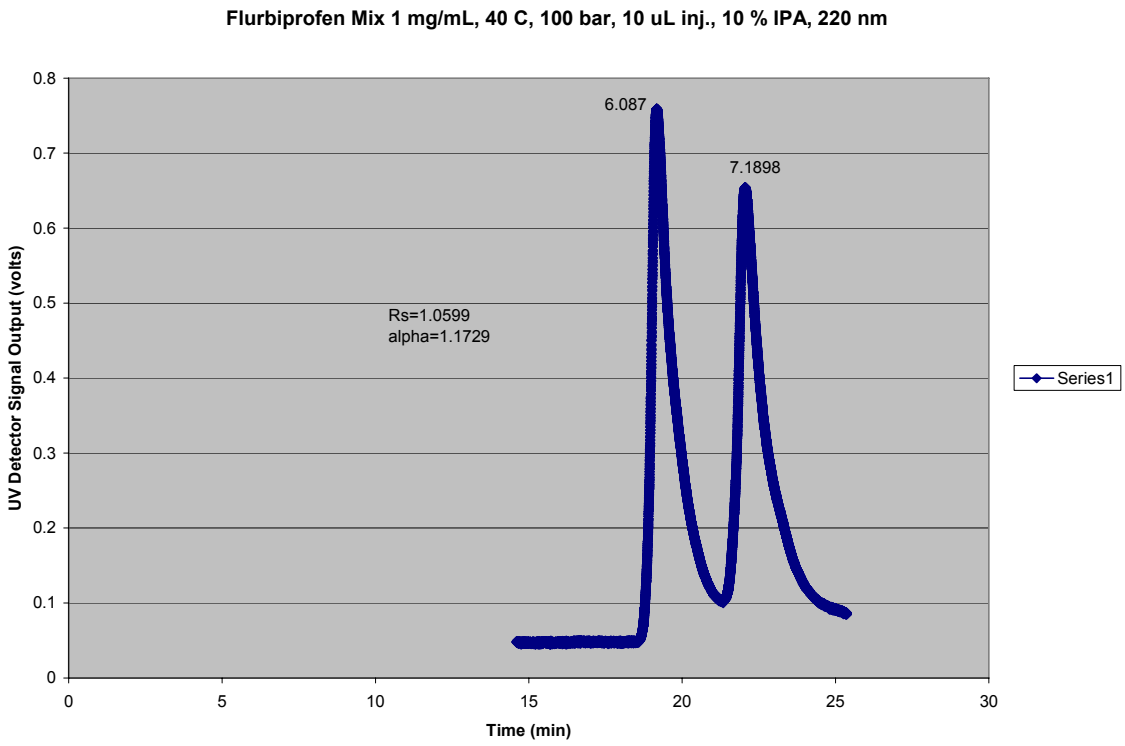

Figure3: Flurbiprofen Separation 1mg/mL Sample at 40^{o}C