SEPARATION OF FLURBIPROFEN AND IBUPROFEN ENANTIOMERS ON A CHIRAL STATIONARY PHASE USING SUPERCRITICAL FLUIDS

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A Supercritical Chromatography System is developed for separation of chiral isomers. The systems studied include ibuprofen as well as 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 several different organics employed as a modifier. The effect of pressure, temperature, flow rate and modifier content on the separation factor, peak resolution and retention time is analyzed. Elution chromatography is used to abstract isotherm information for scale up and synthesis of operating policies for fractionation through simulated moving bed.

INTRODUCTION

Supercritical fluid chromatography (SFC) is most often used with carbon dioxide as a mobile phase and an organic modifier such as some type of organic alcohol (1). It has some significant advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations and significantly less generation of hazardous waste. Some of the main advantages for preparatory chromatography include solvent waste reduction, facilitated product recovery, lower solvent cost and the possibility for solvent recycling (2).

Two chiral drug systems were studied in this body of work. The first is Ibuprofen, a nonsterodial anti-inflammatory drug which as of late is being studied for its chiral-related properties. There are several reasons to separate the two isomers (R and S). The first is that the S-isomer works more than twice as fast as the racemic mixture. This allows for therapeutic applications that require more speed than would be impossible with the mixture. The other reason is that the effective dosage of the drug is decreased. The second system is 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.

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.

The effects of pressure on separation factors and peak resolutions were found at 100bar, 150bar, 200bar, and sometimes at 250bar. The effects of temperature on separation factors and peak resolutions were found at 35°C, 40°C and 50°C. Some experiments were done to find the effect of flow rate on peak resolutions and separation factors.

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, ethanol, methanol, ethyl acetate, acetone, and combinations of equal amounts by volume of isopropanol and ethanol, isopropanol and methanol, ethanol and methanol, ethyl acetate and isopropanol, as well as a 9.4% and 0.6% combination of hexane and isopropanol.

The objective of the research is to find optimal conditions for scale-up to preparative supercritical fluid chromatography applications.

EXPERIMENTAL SFC SET-UP

The SFC apparatus consisted of a 100mL 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/vis detector, fitted with a 400bar flow cell, was used for detection. Sample delivery was accomplished via a Perkin-Elmer ISS-900 auto sampler equipped with a Rheodyne valve with a pressure rating up to 350bar. A circulating bath column heater was designed and built. The outlet line of the syringe pump was run down and through the back 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 was analyzed by employing the Microcal Origin 6.0 graphical software.

RESULTS AND DISCUSION

Effect of temperature and pressure using isopropanol as a modifier

Higher flow rates and higher temperatures produced lower separation factors, a trend that was expected and displayed in Figure 1. Higher pressures produced slightly higher separation factors and slightly decreasing peak resolution numbers.

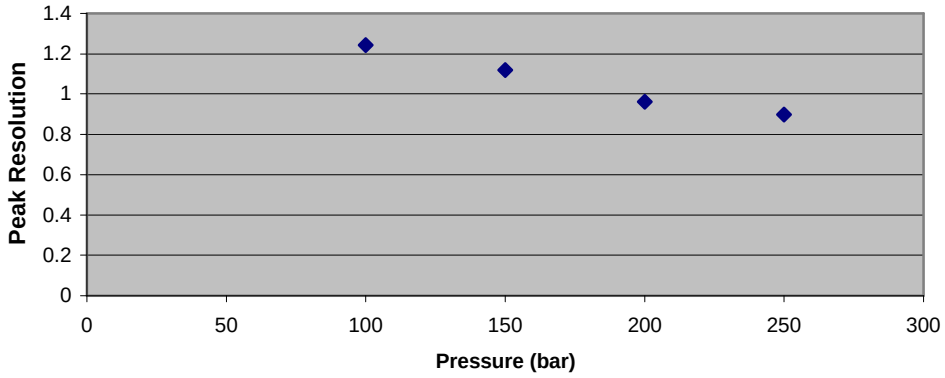

Figure 1. Effect of Pressure on Peak Resolution at 35^{o}C

Effect of various solvents as modifier

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, ethanol, methanol, ethyl acetate, acetone, and combinations of equal amounts by volume of isopropanol and ethanol, isopropanol and methanol, ethanol and methanol, ethyl acetate and isopropanol, as well as a 9.4% and 0.6% combination of hexane and isopropanol. The ones that worked best were isopropanol and ethanol. Combining the two in a 50:50 mixture and 10% *v/v* gave the best results. Good results were also achieved using a combination of methanol and ethanol 50:50, and isopropanol and methanol 50:50. Figure 2 depicts the various data points obtained from the before mentioned results. Ethyl Acetate was used by itself at 10% *v/v* and no separation was observed. Methanol worked and the retention times were noticeably reduced, but the separation and the resolution were drastically worse. A 50:50 combination of methanol and isopropanol was used which combined the lower retention times of methanol and the better separations of isopropanol. In comparison, the separation and resolution was more desirable than just isopropanol. Since a mobile phase of 94% hexane and 6% isopropanol had worked so well for separating ibuprofen in HPLC, that mixture was combined with SFC. Carbon dioxide was combined with a 9.4% hexane and 0.6% isopropanol (total *v/v* 10%) mixture and used as a modifier. The hexane modifier did not work well at all at supercritical conditions so no comparison of results in various publications and in this work was possible with isopropanol and

combinations of isopropanol and ethanol. This figure includes results from sub-critical experiments.

The effect of pressure and modifier is summarized in Table 1.

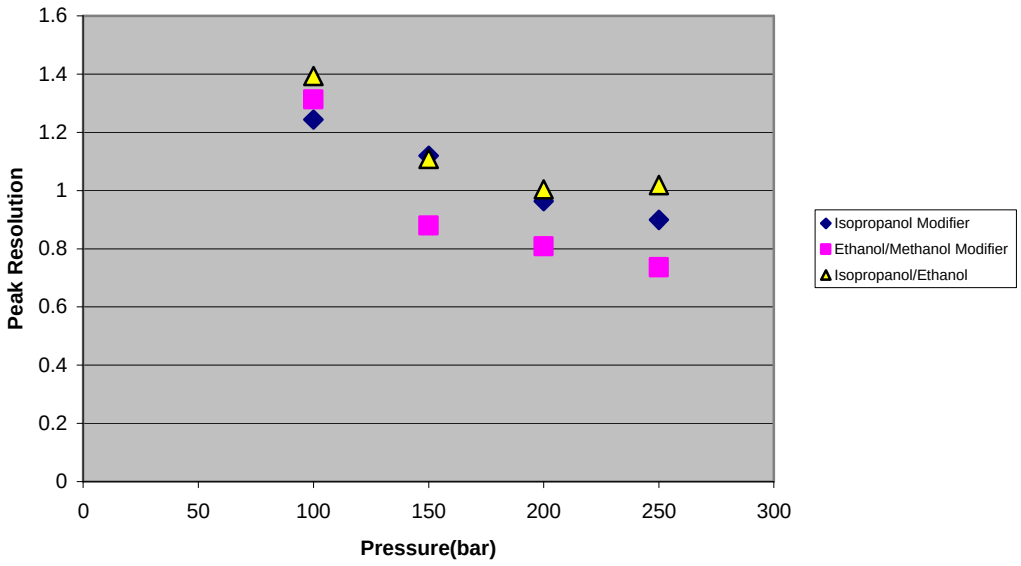

Figure 2. Effect of Pressure on Peak Resolution, Various Modifiers

Effect of Modifier Content v/v % on Peak Resolution and Separation Factor in SFC

The results mirrored reported research with other columns: as the *v/v%* of modifier is lowered the greater the separation [5]. The peak resolution was also better as the modifier percentage was decreased. However, as the modifier percent was decreased the retention time of the sample increased greatly. In this work, more time was spent on shorter analysis times because rather long retention times are undesirable if the conditions are to be transferred to a preparatory chromatography process.

Some of the key results such as theoretical plate numbers with the 10% Isopropanol as the modifier is given on Table 2.

Other Chiral Systems and Path to Scale Up

The second system studied is 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 separation factors and Peak resolutions were satisfactory but not high as ibuprofen case. Furthermore, Elution chromatography is used to abstract isotherm information for scale up and synthesis of operating policies for fractionation through simulated moving bed.

Table 1. Peak Resolutions and Separation Factors at 35°C

Separation Factor			
Modifier Comparison @ 35°C vs. Pressure (bar)			
P (bar)	IPA	IPA/Ethanol	Ethanol/Methanol
100	1.0953	1.1919	1.1012
150	1.0878	1.2232	1.2130
200	1.0803	1.2437	1.2441
250	1.0769	1.2947	1.2011
Peak Resolution			
Modifier Comparison @ 35 °C vs. Pressure (bar)			
P (bar)	IPA	Ethanol/methanol	IPA/ ethanol
100	1.2435	1.3126	1.3929
150	1.1196	0.8798	1.1087
200	0.9637	0.8078	1.0034
250	0.8986	0.7362	1.0186

Table 2. Theoretical Plate Numbers for Isopropanol Experiments

10% IPA Theoretical Plate Numbers				
P	35°C		50°C	
(bar)	N	H	n	H
100	2988.295	0.008366	2720.357	0.00919
150	3046.473	0.008206	2670.208	0.009363
200	2708.176	0.009231	2311.156	0.010817
250	2806.794	0.008907	3087.301	0.008098

CONCLUSIONS

A method was developed to separate the enantiomers of ibuprofen and flurbiprofen on a Whelk-O 1 stationary phase and using various modifiers in Supercritical Fluid Chromatography. Several modifiers were used for analysis. One combination of 5% isopropanol and 5% ethanol modifier (total *v/v* 10%) achieved the best peak resolution of 1.39 at 35°C. Isopropanol came close at 1.24 at 35°C and 100bar. 5% ethanol and 5% methanol produced a peak resolution of 1.31 at 35°C and 100bar. When the 9.4% hexane and 0.6% isopropanol modifier were used in the supercritical region it produced results that were not very good and not as good as just using isopropanol.

The results were found to be similar to previous experiments done in 1994 by Blum, Lynam and Nicolas, except that the columns that are produced today have advanced in several areas (2). The improvements of the column led to improved results for the separation.

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