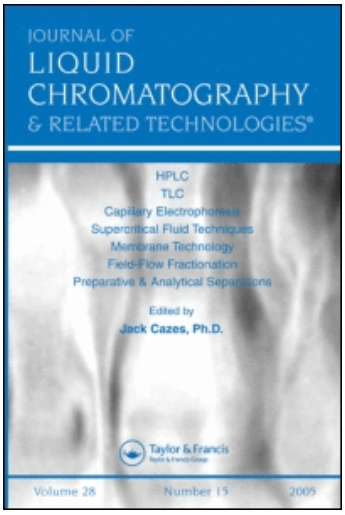

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APPLICATION OF PACKED COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY FOR SEPARATION OF BROMOSULFONE FROM PROCESS RELATED IMPURITIES

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ABSTRACT

Sub- or supercritical fluid chromatography (SFC) is a viable alternative method for separations that are not optimal under reversed phase conditions. 2-Bromo-4′-(methylsulfonyl) acetophenone (bromosulfone) was found to undergo on-column degradation under reversed phase conditions. An SFC separation was developed wherein bromosulfone could be resolved from seven process-related impurities within a run time of five minutes. The effect of column type, modifier type, temperature, and pressure were investigated during the method development.

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INTRODUCTION

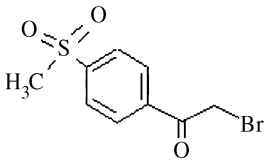
Within the past two decades sub- or supercritical fluid chromatography (SFC) has established itself as a viable separation technique. Much of its growth can be attributed to instrumentation innovations, which facilitated the use of modifiers in packed column mode. SFC can be considered as a form of normal phase chromatography as it uses a stationary phase whose polarity is greater than that of the mobile phase. Carbon dioxide is the most commonly utilized mobile phase. However, the use of pure carbon dioxide is limited due to its low solvent strength. Carbon dioxide has a solvent strength similar to that of pentane or hexane (1,2). Pure carbon dioxide is best utilized for less polar solutes such as PAH's (3) and fossil fuels (4–6). For separations in the pharmaceutical industry, most drug candidates are too polar for the use of SFC with pure carbon dioxide as the eluent. The polarity of most drugs requires the use of a polar modifier in addition to carbon dioxide as the mobile phase (7–11).

For an optimal separation, the polarity of the solute should lie between that of the stationary phase and the mobile phase. If there is a large difference in polarity between the two phases and the polarity of the solute is closer to that of the stationary phase then the solute is strongly retained. This case applies for the use of pure carbon dioxide for the elution of polar drugs. To reduce the strong retention, a polar modifier such as methanol, isopropanol, or acetonitrile is added to the mobile phase. The modifier increases the polarity of the mobile phase. Additionally, the modifier also occupies active sites of the stationary phase, which also serves to reduce retention (12,13). The relationship between concentration of modifier and retention is non-linear and is a function of the non-linear relationship between concentration and solvent strength. However, the relationship between retention and solvent strength is, for the most part, linear (14,15). Small additions of modifier can result in large increases in solvent strength (16). For example, the addition of 9.5% methanol increases the solvent strength to more than halfway to the solvent strength of pure methanol (2).

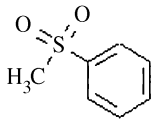
Even with the use of modifier and modifier gradients, SFC does not possess the ability to tune mobile phase polarity over as wide a range as when utilizing reversed phase chromatography with gradient elution. It is this limitation, which most renders SFC incapable of surpassing reversed phase chromatography usage in the pharmaceutical industry. However, SFC does have its niche for applications where reversed phase chromatography cannot be utilized or optimized, such as for cases where the solute is too polar or is incompatible with an aqueous environment. Normal phase chromatography in these cases has been the traditional alternative.

In actuality, SFC is normal phase chromatography with some advantages over the conventional form. The low viscosity and high diffusivity of supercritical fluids leads to higher column efficiencies relative to conventional HPLC (17). Thus, SFC can give more rapid separations than HPLC, or for a given analysis

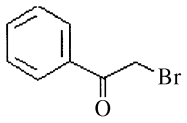
time give a better separation. The use of relatively high percentages of modifier has little effect on the efficiency (18,19). One can also use a strong polar modifier, such as methanol at high percentages which is impossible when using hexane in normal phase HPLC. Additionally, one has the ability to run modifier gradients, which are not conventionally utilized in normal phase chromatography because of the long



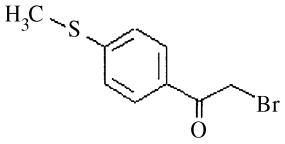
(1)
Bromosulfone



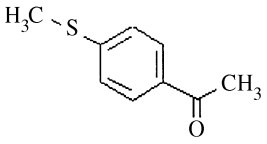
(2)
Phenylmethylsulfone



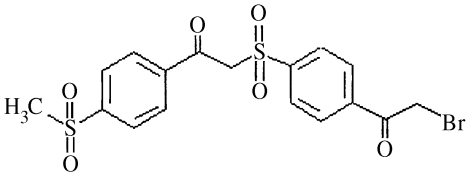
(3)
2-Bromoacetophenone



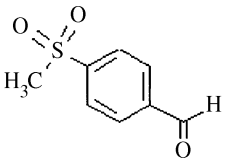
(4)
Bromosulfide



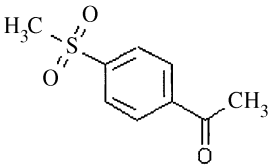
(5)
Ketosulfide



(6)
Bromosulfone Dimer



(7)
4-Methylsulfonylbenzaldehyde



(8)
Ketosulfone

Figure 1. The structures of bromosulfone and its process related compounds.

re-equilibration times required (20). These advantages have led to extensive utilization of SFC for enantiomeric resolutions (21–25). SFC can also be applied in cases where the solute of interest is unstable under aqueous conditions.

This paper describes a separation of 2-bromo-4′-(methylsulfonyl)acetophenone (bromosulfone), which is unstable in water, from seven process related compounds using SFC (Figure 1). The nature of the separation and the dominating mechanisms are investigated, as well as the effect of varying such parameters as type of modifier and temperature.

EXPERIMENTAL

Instrumentation

SFC experiments were performed with a Berger SFC Instrument (Newark, DE, USA) equipped with dual pumps, a diode array UV detector, oven, and an autosampler. The instrument was controlled by the HP Chemstation software. LC was performed with an Agilent 1100 series LC, and LC-MS was performed with an Agilent LC/MSD (Palo Alto, CA). The UV detection wavelength was set at λ 240 nm.

Three types of columns were investigated in the SFC study, Zorbax silica (250 × 4.6 mm; 5 μm particles), Zorbax CN (250 × 4.6 mm; 5 μm particles), and Zorbax phenyl (250 × 4.6 mm; 5 μm particles) [Agilent, Palo Alto, CA]. A YMC ODS-AQ (Milford, MA) was used for the reversed phase LC portion of the investigation (250 × 4.6 mm, 5 μm particles).

Chemicals and Reagents

Carbon dioxide used in this research study was SFC grade obtained from Air Products, Inc. (NJ, USA). Four types of modifiers were investigated, acetonitrile, 80/20 methylene chloride/acetonitrile, methanol, and 2-propanol. Methylene chloride and 2-propanol were obtained from Aldrich Chemical Company, Inc. (WI, USA). Methanol was obtained from EM Science (Gibbstown, NJ) and acetonitrile from Fisher Scientific (Fairlawn, NJ). Phosphate buffer (10 mM adjusted to pH 3.5 with phosphoric acid) was used for the aqueous portion of the LC mobile phase and HPLC grade acetonitrile for the organic modifier. Potassium phosphate was obtained from Fisher Scientific and phosphoric acid from EM Science. For the LC-MS studies, a mobile phase of 0.1% formic acid and acetonitrile was utilized. Formic acid was obtained from J. T. Baker (Phillipsburg, NJ).

Bromosulfone and the seven potential impurities (Figure 1) were obtained from Department of Process Research and Development, Merck & Co., Inc. (Rahway, NJ, USA) and Aldrich Chemical Company, Inc. (WI, USA). The samples were dissolved and diluted to the desired concentration with acetonitrile.

RESULTS AND DISCUSSION

Stability of Bromosulfone Under Reversed Phase Conditions

Initial steps toward development of a method were performed under reversed phase conditions. Under ambient conditions significant fronting was observed for bromosulfone, which could be reduced but not eliminated by reducing the column temperature to 5°C (Figure 2). This fronting was attributed to some form of on-column degradation, especially considering its temperature dependence. Under reversed phase conditions, optimal resolution of all eight components could be affected in 30 minutes provided the column temperature was kept at 5°C or less.

Possible modes of degradation for bromosulfone include nucleophilic substitution of the bromo group with a hydroxy group to form an alcohol or addition to the ketone group to form a gem diol (Figure 3). The latter type of reaction tends to be fast and reversible (26). Elucidation into the type of degradation involved was undertaken using LC-MS. A sample of bromosulfone dissolved in 0.1% formic acid was left to sit for 1 hour then analyzed. In addition to the fronting, two early eluting peaks with m/z corresponding to the alcohol ($m/z = 215$) and the gem diol ($m/z = 296$) were observed. Additionally, analysis

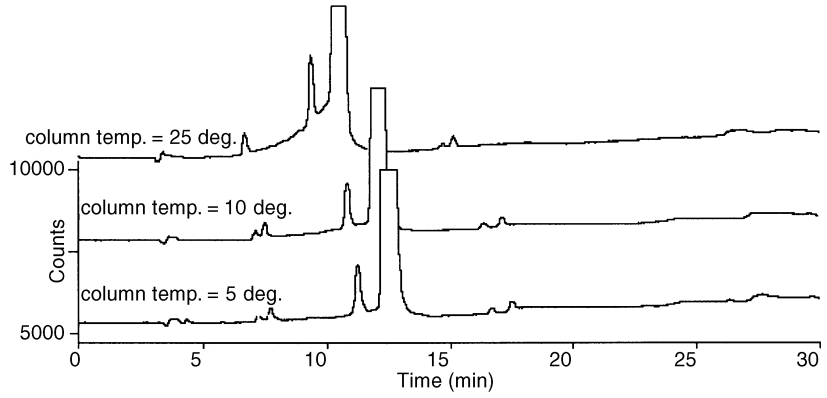
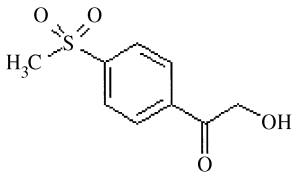
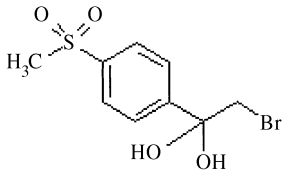


Figure 2. Reversed phase HPLC of bromosulfone under different column temperatures.



Alcohol



Diol

Figure 3. Degradation products of bromosulfone.

of the fronting region of the bromosulfone peak also indicated the presence of m/z corresponding to both the alcohol and gem diol. Evidently, the bromosulfone degrades to both species with the alcohol being the dominant product.

Development and Optimization of SFC Method for Bromosulfone

In cases where utilization of reversed phase chromatography is not optimal, normal phase chromatography is considered as a viable alternative. SFC was considered, as the retention mechanism would be the same as conventional normal phase chromatography. Additionally, there would be the advantages of higher efficiency and potential use of higher flow rates without compromising a large loss of efficiency.

The initial mobile phase chosen was carbon dioxide modified with an alcohol. Methanol and isopropanol were the alcohols used. With these modifiers, the solutes were un-retained or weakly retained, even at low alcohol concentrations on all three types of columns, cyano, phenyl, and silica. In fact, with the cyano and phenyl column, retention was weak even in the presence of pure carbon dioxide. Structural analysis of the eight solutes indicates the absence of strong hydrogen bond donors. The modes of interactions between solute and stationary phase would likely be through dipolar interactions or through hydrogen

bonding with the hydrogen bond acceptors of the solutes. However, the alcohols themselves are very polar with strong hydrogen bond accepting and donating properties. The competition that they provide for interactions with the stationary phase thus renders the solutes to be very weakly retained. Additionally, peak fronting was observed for the bromosulfone with column temperature at 55°C or higher indicating degradation through reaction with the alcohol.

The second mobile phase chosen for method development was carbon dioxide modified with acetonitrile. Acetonitrile is a non-basic localizing solvent with a high enough polarity to allow for flexible tuning of the mobile phase to elute all components. However, it does not provide for the strong hydrogen bonding interactions observed with the alcohols. With the cyano and phenyl columns, the compounds were weakly retained and co-eluted. Changes of pressure and temperature did not improve the selectivities of the co-eluting peaks. On the silica column, which provides for stronger interaction with the solute, retention and resolution were observed. In addition, no degradation of bromosulfone was observed when acetonitrile was used as the modifier, even at high column temperature.

To optimize the method, the concentration of acetonitrile was varied from 11% (v/v) to 17% (v/v) at an outlet pressure of 200 bar and column temperature at 40°C with total flow rate of 3.0 mL/min. The resolution between bromosulfone and 4-methylsulphonylbenzaldehyde began to diminish at 17% (v/v) acetonitrile. A modifier concentration of 15% (v/v) acetonitrile was chosen as the final modifier concentration for this study to sustain a balance between selectivity coefficients α and retention times of the compounds.

The range of outlet pressures studied was 100–250 bars with 15% (v/v) acetonitrile as modifier, 3.0 mL/min total flow rate and 40°C column temperature. The capacity factors of all solutes decreased as the outlet pressure was increased. The effect of pressure at constant column temperature on retention times under these conditions is relatively simple. An increase of pressure leads to an increase of density, thus, increasing the solvation capability of the mobile phase leading to a decrease in retention of the compounds (27,28). The optimal SFC separation for this work was obtained at 200 bars outlet pressure.

The effect of column temperature was investigated. The temperature was varied from 30°C to 60°C with 15% (v/v) acetonitrile, 3.0 mL/min total flow rate, and 200 bars of outlet pressure. For phenylmethylsulfone, 2-bromoacetophenone, bromosulfide, and ketosulfide, which were the early eluted compounds, temperature variation had negligible effect on their capacity factors (data not shown). For 4-methylsulfonylbenzaldehyde, bromosulfone, and ketosulfone, which were the later eluted compounds, an increase in column temperature led to an increase in capacity factors of these compounds (Figure 4).

The effect of temperature on SFC retention is very complex. The temperature not only affects the density of the mobile phase, but also the solubility

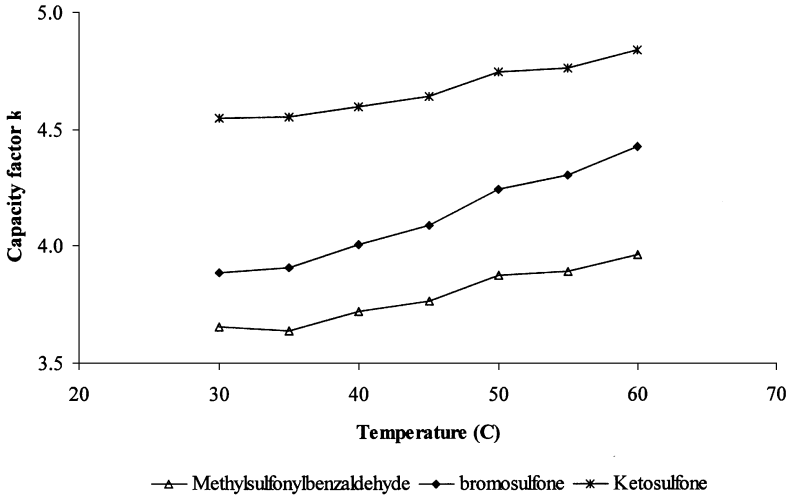


Figure 4. Effect of temperature on the capacity factors.

parameters of both the solute and the supercritical fluid. Retention in SFC can be described as a combination of conventional normal phase retention characteristics and solubility (29–33). Retention is, thus, governed by the heat of adsorption of the solute on the stationary phase and the heat of solution for the solute in the mobile phase (34,35). It is, thus, not uncommon to see plots of log capacity factor versus reciprocal temperature demonstrating a maxima with a positive slope at high temperature and a negative slope at lower temperatures (36,37). In these cases, at low temperature ranges, the capacity factor will increase with increasing temperature as solvation in the mobile phase dominates (38). A general rule which appears to apply to most cases, is that the variation in SFC retention due to temperature approximately follows the variations in mobile phase density when the concentration of modifier is lower than 15% (v/v) at low temperatures (28). In this work, an increase in temperature caused a decrease in mobile phase density, which led to an increase in capacity factors of the compounds. The optimum temperature was chosen at 40°C to sustain a balance between selectivity and run time.

The optimum SFC conditions were found to be at a pressure of 200 bars, 40°C column temperature, 15% (v/v) acetonitrile, and 3.0 mL/min total flow rate, using a silica column. The weaker hydrogen bond donating and accepting ability and high polarity coefficient of acetonitrile made it the ideal organic modifier. Moreover, acetonitrile as the modifier avoided the possible degradation of bromosulfone during the analysis. Bromosulfone and its seven potential impurities could be separated in 5 minutes with acceptable selectivity under these conditions. A chromatograph of the eight compounds (0.3 mg/mL) obtained under these

conditions is shown in Figure 5. The method was found to be linear for all eight compounds within the concentration range of 0.5 μg/mL to 1000 μg/mL ($R^2 = 0.999$) when using a 5 μL injection. The limit of detection, based on a signal to noise ratio of 3:1, was estimated at 0.5 μg/mL for all the seven potential impurities of bromosulfone. The limit of quantitation was found to be 1.0 μg/mL,

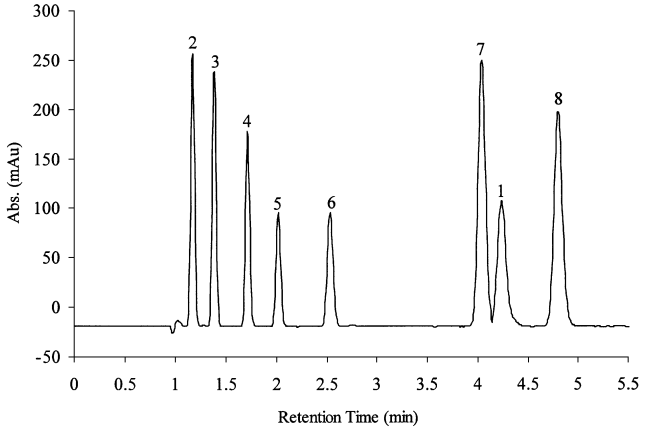


Figure 5. SFC separation of bromosulfone and its seven process related compounds using a Zorbax Silica 250 × 4.6 mm, 5 μm column. Conditions: 15% modifier (acetonitrile) in carbon dioxide at 40°C and 200 bar outlet pressure with 3.0 mL/min total flow rate.

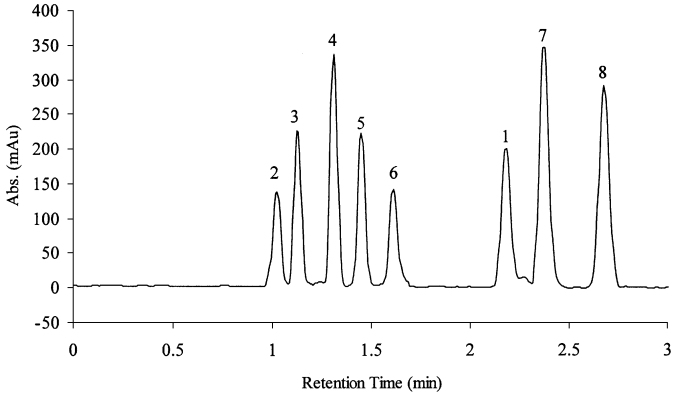


Figure 6. SFC separation of bromosulfone and its seven process related compounds with 50% modifier (80/20 methylene chloride/acetonitrile) in carbon dioxide. Other conditions are the same as those in Figure 5.

based on the satisfaction of relative standard deviation (RSD) of injection precision less than 15% with a signal to noise ratio of 10:1, for all the impurities.

As in conventional normal phase chromatography, one can tune selectivity by variation of the type of modifier (non-localizing, localizing, basic etc). One can also use ternary mixtures with combinations of classes of modifiers. A binary mixture of organic modifier, which contained 80% (v/v) of methylene chloride and 20% (v/v) of acetonitrile, was also investigated in this work. The non-localized methylene chloride alone could not elute bromosulfone out from the column within 30 minutes. Combined with highly localized acetonitrile, the organic modifier mixture (80/20 CH_2Cl_2/CH_3CN) eluted and separated all eight compounds within three minutes at 50% (v/v) modifier concentration (Figure 6). Although a faster separation can be achieved with 80/20 CH_2Cl_2/CH_3CN compared to with acetonitrile alone, it is not recommended in this work because of

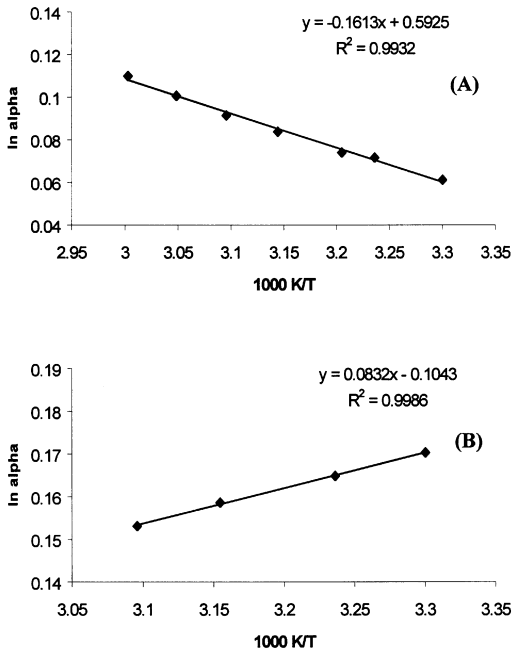


Figure 7. Effect of temperature on selectivity between bromosulfone and 4-methyl-sulfonylbenzaldehyde. (A) 15% acetonitrile in carbon dioxide; (B) 50% methylene chloride/acetonitrile (80/20) in carbon dioxide. Other conditions: 200 bar outlet pressure; total flow rate at 3.0 mL/min; 30–60°C temperature; 5 microliter injection; and UV detection at 240 nm.

the much higher modifier concentration required. Such high concentrations of methylene chloride are not recommended for use in the SFC by the manufacturers.

It was also noted that compared to using acetonitrile alone as the modifier, using this binary organic modifier switched the elution order between bromosulfone and 4-methylsulfonylbenzaldehyde (Figure 7). This switch in elution order indicates that the properties of methylene chloride do, in fact, affect the interaction between these two solutes and the stationary phase in a different way than using pure acetonitrile.

CONCLUSION

The packed column SFC method developed in this work offers a fast separation (≤ 5 min) with good selectivities between bromosulfone and its seven potential impurities. Moreover, it eliminates the risk of bromosulfone degradation during the analysis, which was a major concern for reverse phase HPLC method development. The method is sensitive enough to detect the impurities at 0.5 μg/mL level.

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