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Purification method development for chiral separation in supercritical fluid chromatography with the solubilities in supercritical fluid chromatographic mobile phases

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Abstract

A comprehensive approach was applied to develop a chiral purification method for an analyte that was found to be unusually difficult to scale-up in supercritical fluid chromatography (SFC). This was performed by studying major factors such as the solubility of an analyte in SFC mobile phases, impurity profiles, and cycle time. For this case study, the solubility in SFC mobile phase was measured by a packed column technique, coupled with a novel trapping mechanism to enhance measurement precision in SFC conditions. The solubility studies in SFC mobile phases suggested a couple of possible SFC mobile phases, in which the analyte would potentially be most soluble. The SFC methods were developed to purify a sample containing 15% of an impurity, after considering impurity profiles and cycle times of several potential methods in addition to SFC mobile phase solubility. An equal volume mixture of acetonitrile and ethanol was chosen for the final purification method, since this mixture demonstrated the relatively high SFC solubility among all solvent combinations with enhanced resolution between the analyte and the impurity as well as the shortest run time. The solubility of the compound was also determined in various organic solvents using a high throughput solubility screening system to better understand relative change of solubility from neat solution to SFC mobile phases.

Keywords: SFC; Preparative SFC; SFC solubility; SFC purification method development; Organic solubility

1. Introduction

Preparation of an optically pure compound is very important in the compound synthesis to drug testing cycle in the pharmaceutical industry [1,2]. Enantiomers have been shown in many cases to demonstrate different biological activities in living systems [3]. Therefore, it is essential to have a single enantiomer (10 mg to 10 g) in order to test activity and toxicity in the pharmaceutical discovery stage [4]. Thus, the process to get a pure enantiomer is a critical step.

Since the first commercial instrument became available in the 1990s [5], supercritical fluid chromatography (SFC) has become an increasingly important purification tool to obtain enantiomerically pure compounds, especially in the early drug discovery stage due to its high speed and separation efficiency compared to LC [6–13]. In addition, the reduction of solvent to be removed from the purified sample, as well as reduced solvent waste after chromatographic separation, makes SFC extremely attractive to discovery-purification laboratories in the pharmaceutical industry [14,15].

Chromatographic throughput is defined as the amount of purified chemical produced per unit time and unit weight of stationary phase [16]. Throughput in preparative chromatography is dependent on separation factors, resolutions, and solubilities of compounds in mobile phases. Maximum loadability with the shortest separation time is an important factor to consider in purification method development. Others have already worked to maximize column loadability by dissolving samples in a strong mobile phase and subsequently diluting with a weak mobile phase at the head of the column [17]. Berger and Fogel-

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man discovered that by injecting a sample in organic mobile phases before mixing with the high-pressure CO_2 modifier they could significantly shorten the time of separation process [18].

Handling a poorly soluble compound is non-trivial in both analytical and preparative chromatography [19,20]. A compound with poor solubility is more problematic in preparative purification than analytical chromatography because the typical ratio of sample to both mobile and stationary phases is much higher in preparative chromatography. Reduced solubility in the SFC mobile phase relative to the injection solvent may cause sample precipitation in the chromatographic system.

Measuring solubility in supercritical fluidic conditions has been one of the major research topics of the supercritical fluid extraction field [21–23]. Solubility has been determined by using a phase monitor, observing formation of a single phase or by directly connecting a saturated chamber to a detection device such as UV or flame ionization [24–27]. Chromatographic retention times have also used to measure solubilities in a liquid or supercritical fluid mobile phase [28,29]. A simple and classic way of measuring solubility of an analyte in an SFC mobile phase with an organic modifier is to pack the analyte into a chromatographic column [30]. The analyte can then be eluted after the appropriate equilibration time and quantified by SFC with UV detection. This method has limited application when studying equilibrium solubility due to the complication of kinetics.

In this paper, a modified technique to study solubility in SFC mobile phase incorporating a novel trapping set-up to improve measurement precision is presented. With this technique, an SFC chiral purification method for a poorly soluble sample was developed based on chromatographic data coupled with newly obtained SFC solubility data. An initial attempt to scale-up resulted in failure, because the solubility of the analyte in SFC was not fully considered. Three major considerations in a successful scale-up in SFC are the impurity profile, the cycle time, and SFC solubility. Traditionally, measuring SFC solubility had been overlooked, because it had been difficult and time consuming. By incorporating a newly developed system for measuring SFC solubility, this task has been greatly facilitated. Now, considering the impurity profile, the cycle time and SFC solubility, a successful purification method for a poorly soluble analyte is presented.

2. Experimental section

2.1. Materials

SFC-grade carbon dioxide was obtained from BOC Gases (Murray Hill, NJ, USA). Methanol (MeOH), ethanol (EtOH), 2-propanol (IPA), 1-propanol (PA) and acetonitrile (MeCN) were HPLC-grade from Mallinckrodt Baker (Muskegon, MI, USA). Ethylene glycol dimethyl ether (DME), diethylamine (DEA) and trifluoroacetic acid (TFA) were obtained from Sigma–Aldrich (Milwaukee, WI).

2.2. Racemate

The racemate employed in this study was a proprietary pharmaceutical intermediate prepared at Amgen Inc. (Thousand Oaks, CA). This compound contains secondary and tertiary amines. The chiral center is located at the five-member ring. One alkyl chain is connected to the chiral center. Two different batches of the racemate were used in the study. One was 15 g batch of purity greater than 98%. This batch of the racemate was initially utilized for the solubility measurement. The other 50 g batch of the racemate of purity close to 85% with a major impurity of 15% was purified.

2.3. Analytical SFC instrumentation

The SFC instrument was a Berger SFC unit with an FCM1200 flow control module, a dual pump control module, a TCM2100 thermal column module (temperature can be controlled from 7 to 150 °C), a column selection valve capable of switching between six columns and a solvent control valve for up to six modifiers to be selected. The aforementioned equipment was from Mettler-Toledo Autochem (Newark, DE, USA). The SFC was equipped with an Agilent 1100 photodiode array detector with a high-pressure flow cell (Agilent Technologies, Palo Alto, CA, USA). The autosampler unit was a CTC LC Mini PAL (Leap Technologies, Carrboro, NC, USA). A Waters ZQ bench top single quadrapole mass spectrometer (Waters, Milford, MA, USA) with an atmospheric pressure chemical ionization (APCI) source was coupled to the analytical SFC system. The software in the analyses was Berger MassWare™ v. 4.01 and MassLynx™ v. 4.0 SP1.

2.4. Preparative SFC instrumentation

The preparative SFC system was a Berger Multigram™ II. The components were the Separator Control Module (SCM)-2500, Electronics Control Module (ECM)-2500, CO_2 Solvent Delivery Module, Modifier Solvent Delivery Module, Direct Expansion Probe Chiller, UV Variable Wavelength Detector, Cavro XL3000 Modular Digital Pump (injector), ventilated collection cabinet, and a waste containment vessel. The aforementioned equipment was from Mettler-Toledo Autochem (Newark, DE, USA). The software in the purification was Berger SFC ProNTo™ v. 1.5.305.15.

2.5. Chiral packed columns

The analytical chiral packed columns were Chiralpak AD-H and AS-H, as well as Chiralcel OD-H and OJ-H. All columns were purchased from Chiral Technologies (Exton, PA, USA). Dimensions of the columns were 150 mm × 4.6 mm ID with 5 μm particle size for analytical method development. For preparative SFC, the chiral packed column was Chiralpak AD-H (250 mm × 21 mm, 5 μm) from Chiral Technologies (Exton, PA, USA).

2.6. Determination of solubility in supercritical fluid mobile phases by analytical SFC instrumentation

Berger's analytical SFC instrumentation was modified to measure solubility in supercritical fluids. A stainless steel column (30 mm × 21 mm ID) was dry packed with approximately 7 g of 50 μm ODS-AQ silica gel mixed with approximately 60–100 mg of the finely ground analyte. Each end of the stainless tube was capped with a 0.5 μm frit to prevent leakage of silica material into the SFC detector. The packed column with the analyte was serially connected to an analytical SFC column (Cyano column, 5 μm particle size, 150 mm × 4.6 mm ID, Berger Instrument, Inc.) to further prevent any solid particle flow to the outlet pressure regulator. A schematic diagram for SFC solubility measurement set-up, consisting of two six-port switching valves equipped with a multiposition microelectric valve actuator and a manual controller (Valco Instruments Co. Inc., Houston, TX), is shown in Fig. 1. Five discrete steps were employed to measure SFC solubility. The packed column (solubility chamber) was initially pressurized under the appropriate SFC conditions and filled with the SFC mobile phase. The chamber was then isolated from the inlet pump. After isolation at 35 °C for at least 15 min, the chamber was reconnected to the pump for approximately 2–5 s allowing the saturated solution from the chamber to be trapped in a sample loop (typically 5 or 10 μL). This was followed by the isolation of the sample loop from the flowing system to remove excess sample solution in all plumbing lines except the sample loop. After 3–5 min of isolation, the sample loop was reconnected to inject the sample to the SFC system for quantification. The mobile phase typically consisted of 50% organic modifier and 50% carbon dioxide with a total flow rate of 4.0 mL/min. The concentration of the solution was determined at 270 nm.

2.7. Determination of solubility in organic modifiers using the Symyx Solubility System

The Symyx Solubility System in this study was manufactured by Symyx Technologies, Inc. (Santa Clara, CA). The system consisted of a solid dispensing robot, a liquid handling robot and an Agilent 1100 HPLC [31,32]. The organic modifiers chosen for solubility screening were MeOH, EtOH, IPA, MeCN and DME containing 0.2% DEA. To study dissolution by co-solvent effect, binary mixtures (alcohol and MeCN, alcohol and DME, and MeCN and DME) were also screened at 50/50 (v/v). The upper limit of solubility measurement was set to 100 mg/mL.

3. Results and discussion

3.1. Analytical SFC chiral separations in various solvent mixtures

The four chiral technology columns (AD-H, AS-H, OD-H and OJ-H), were screened by SFC with MeOH (0.2% DEA) as an organic modifier. Partial separation was obtained with the OJ-H column. Both OD-H and AS-H columns did not give any hint of separation under these conditions. The best separation was achieved with the AD-H column.

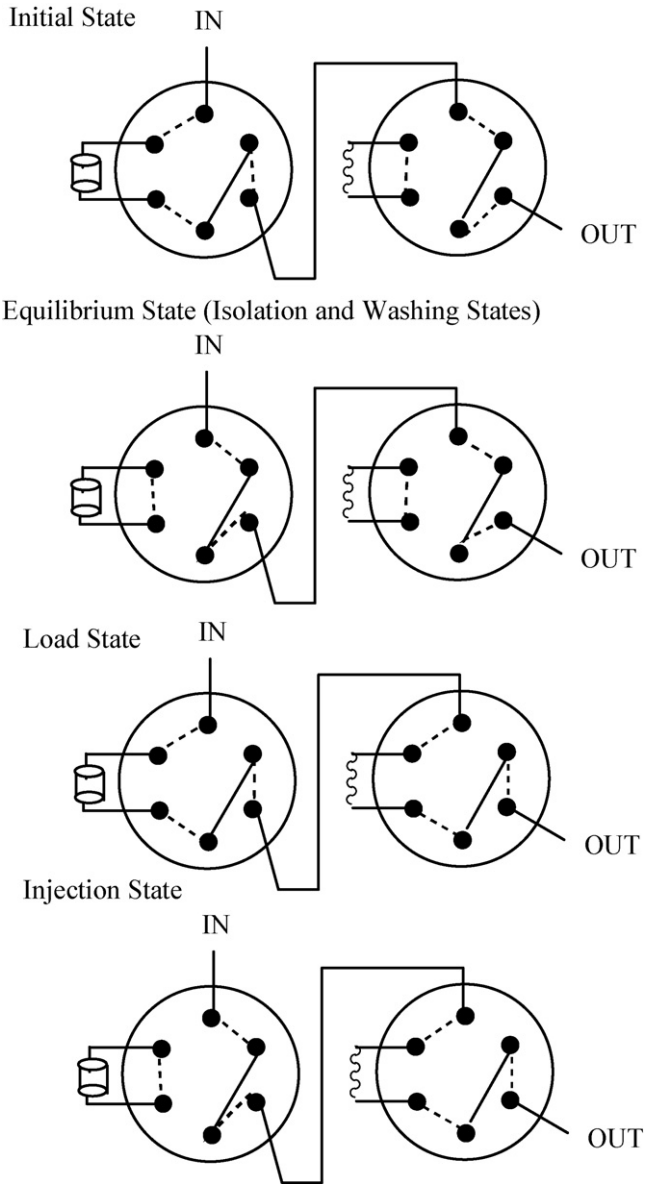

Fig. 1. Schematic diagram for SFC solubility measurements. IN and OUT ports were connected to the pump and UV detector, respectively.

The enantiomeric separation on the AD-H column was further studied with the three most common SFC organic modifiers (MeOH, EtOH and IPA). The mobile phase with IPA yielded the best separation followed by EtOH and MeOH as shown in Fig. 2.

SFC chiral separation was then further evaluated with the AD-H column using several mixtures of organic solvents in the mobile phase. Chromatographic characteristics such as capacity factors, separation factors and resolutions (with various solvent mixtures, together with the six pure solvents) are listed in Table 1.

Good resolutions were achieved using protic solvents such as MeOH, EtOH, IPA and PA. Among them, IPA showed the best resolution (Rs = 9.05). Poor resolutions were achieved with

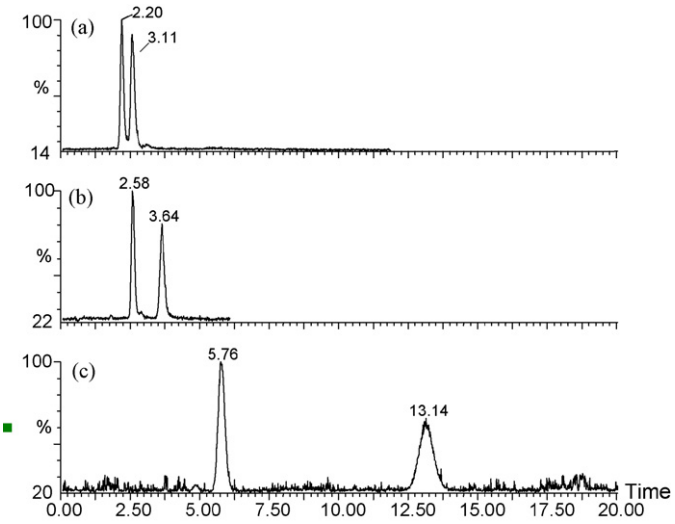

Fig. 2. TIC chromatograms of the compound in the mobile phases of 65% carbon dioxide and 35% of the three most common organic modifiers in SFC such as (a) methanol, (b) ethanol and (c) isopropanol on AD-H. All organic modifiers contained 0.2% diethylamine as a base additive. The flow rate was 4.0 mL/min, and the oven temperature was set to 35 °C. Outlet pressure was set to 100 bar. The sample concentration was approximately 1.0 mg/mL and the injection volume was 10 μL.

MeCN (Rs = 0.88) and DME (Rs = 0.32), suggesting that mixing these aprotic solvents with other protic solvents in the SFC mobile phases decreased separations as listed in Table 1. When an equal volume of MeOH was mixed with either MeCN or DME, resolution was too poor to be useful for scale-up purification.

The equal volume mixture of DME or MeCN with protic solvents (EtOH and IPA) resulted in decreased separation factors and resolutions with shorter run times, compared to those obtained with the corresponding single protic solvents. Equal volume mixtures of protic solvents with either MeCN or DME (except the combinations of EtOH/DME) resulted in successful separations with separation factors (>1.3) and resolutions (>2.4) with reasonably short separation times ($k_2 < 2.8$). These mixtures were further tested with a sample from the second batch containing a 15% impurity. The SFC chromatograms are shown in Fig. 3. From the several conditions tested, MeCN/EtOH (50/50, v/v) and EtOH alone allowed baseline separation of the impurity from the two enantiomers, suggesting these two methods could be used for further scale-up purification methods.

Table 1
Effect of organic modifier compositions on the SFC chiral separations

Composition (v/v)	k_1	k_2	α	Rs
MeOH	2.49	3.94	1.58	1.13
MeOH/DME, 50/50	1.71	1.87	1.09	0.10
MeOH/MeCN, 50/50	1.86	2.14	1.15	1.02
EtOH	3.10	4.78	1.54	3.76
EtOH/DME, 50/50	1.79	2.30	1.28	1.95
EtOH/MeCN, 50/50	1.81	2.44	1.35	2.45
IPA	8.14	19.86	2.44	9.05
IPA/DME, 50/50	1.56	2.35	1.51	2.69
IPA/MeCN, 50/50	1.71	2.78	1.62	3.39
PA	2.86	4.81	1.68	4.43
PA/DME, 50/50	1.19	1.62	1.36	1.44
PA/MeCN, 50/50	1.38	2.06	1.49	2.45
MeCN	4.78	5.79	1.21	0.88
DME	2.40	2.78	1.16	0.32

Mobile phases consisted of 65% carbon dioxide and 35% of the organic modifiers with 0.2% DEA as a base additive. The AD-H column (4.6 mm × 15 cm, 5 μm) was used. The flow rate was 4.0 mL/min and the oven temperature was set to 35 °C. The sample concentration was approximately 1.0 mg/mL and the injection volume was 10 μL. The back pressure regulator was set to 100 bar. Chromatograms were drawn from the average UV absorption signal between 210 and 320 nm.

Temperature and pressure are two variables that can potentially affect separation. These effects were studied using an equal volume mixture of IPA and DME as an organic modifier as shown in Fig. 4. Changing the outlet pressure had a minimal impact on separation in the range of pressure employed in the current study (100–200 bar). With higher outlet pressure, the resolution decreased slightly from 2.69 (at 100 bar) to 2.24 (at 200 bar). By increasing oven temperature, the resolution increased from 2.69 (at 35 °C) to 3.21 (at 50 °C). The enhanced resolution with temperature was most likely due to the higher mass transfer. Overall, both temperature and pressure showed little effect on an analytical scale and therefore were not further explored for scale-up.

3.2. Solubility measurement in organic solvents and initial SFC scale-up attempt

As with any newly obtained solid sample, the first step is dissolving the sample in an organic solvent. The solubility of the compound in neat MeOH, EtOH, IPA, MeCN and DME was determined using a Symyx solubility screening system. Solubility data in the various organic solvents are given in Table 2. Generally, the solubility of the compound was higher in aprotic solvents than in any protic solvent studied. The greatest solubility in a single solvent was obtained with DME (>100 mg/mL), while IPA showed the lowest solubility (3 mg/mL). Single solvents such as MeOH, EtOH, IPA, or MeCN demonstrated lower solubility (≤12 mg/mL). All solvents in the current study showed enhanced solubility upon mixing with DME. In every case of mixing an equal volume of a solvent with DME, solubility was greater than or equal to 50 mg/mL. Solubility in the 50% DME mixtures with protic solvents was consistently greater than those in the equal percentage mixtures of MeCN with protic solvents.

It was notable that solubility was greatly enhanced in the binary solvents systems containing both MeCN and a protic solvent such as in EtOH/MeCN (v/v 50/50, 38 mg/mL) versus either EtOH (8 mg/mL) or MeCN (12 mg/mL), respectively. The co-solvent effect on the enhanced solubilities in the mixture of other protic solvents (i.e., MeOH, EtOH and IPA with MeCN) was also observed. Among all conditions explored, using DME as co-solvent enhanced solubility the most.

With organic solubility data available, initial scale-up purification without consideration of the SFC solubility was attempted using IPA/DME. This mixture was chosen since it has the highest SFC separation factor ($\alpha = 1.51$) among the three DME co-solvent mixtures studied, even though it had a slightly lower

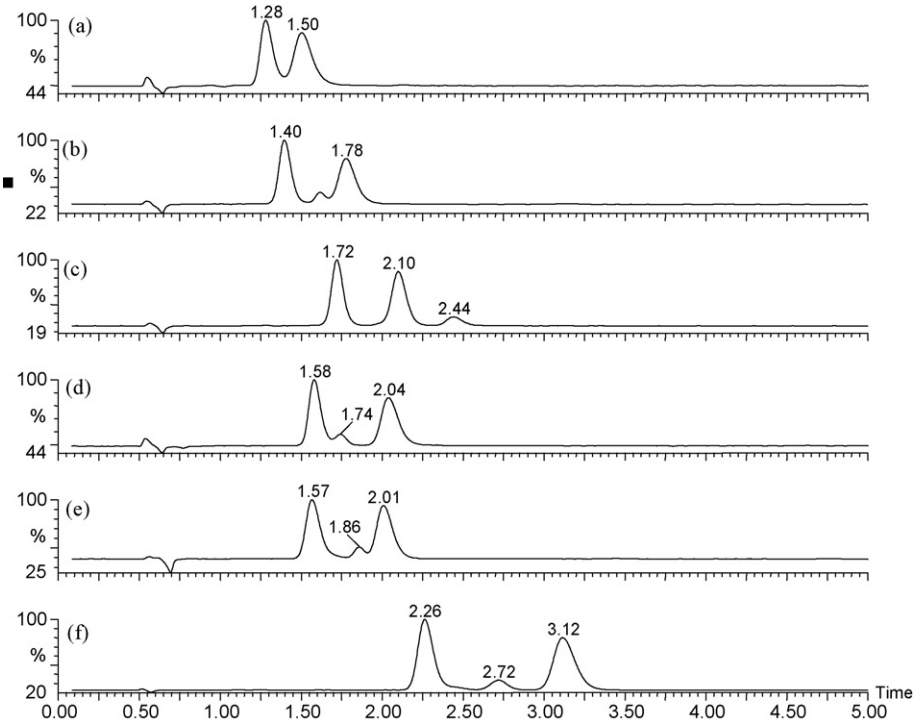

Fig. 3. TIC chromatogram of the separation of the racemic compound (two major peaks) and an impurity (the minor peak) in the mobile phases of 65% carbon dioxide and 35% of the equal volume mixtures of (a) PA/DME, (b) PA/MeCN, (c) MeCN/EtOH, (d) IPA/DME, (e) IPA/MeCN and (f) EtOH alone. The flow rate was 4.0 mL/min, and the oven temperature was set to 35 °C. Outlet pressure was set to 100 bar. The sample concentration was approximately 1.0 mg/mL and the injection volume was 10 μL.

organic solubility. While DME was used in the process of the study, it is less ideal in preparative chromatography due to the potential formation of peroxide, and special care is necessary.

It was obvious from the first injection of 50 mg of sample that no resolution could be achieved together with long tailing peaks indicating sample precipitation in the system, as shown in Fig. 5. Sample precipitation was attributed to the poor solubility of the compound in the SFC mobile phase. After the second injection of 25 mg, the system became over-pressurized, thus preventing further exploration.

3.3. Solubility measurement in supercritical chromatographic mobile phase

After the initial failed scale-up attempt utilizing organic solubility data, solubility of the analyte in SFC was measured.

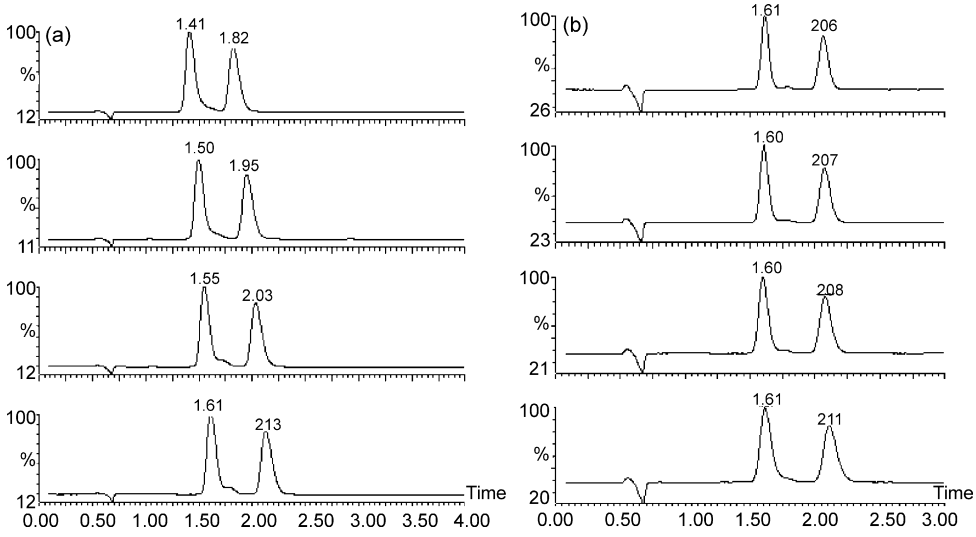

Fig. 4. The effects of pressure and temperature on separation in the SFC mobile phase consisting of 50% carbon dioxide and 50% equal volume mixture of IPA/DME with 0.2% DEA as a base modifier. (a) From the top, 200, 160, 130 and 100 bar, (b) from the top, 50, 45, 40 and 35 °C. The flow rate was 4.0 mL/min. The sample concentration was approximately 1.0 mg/mL and the injection volume was 10 μL.

Table 2
Solubility of the analyte in various organic solvents at 27 °C

Composition (v/v)	Solubility (mg/mL, $n = 1$)
MeOH	10
EtOH	8
IPA	3
MeCN	12
DME	≥100
MeOH/DME, 50/50	59
EtOH/DME, 50/50	68
IPA/DME, 50/50	54
MeOH/MeCN, 50/50	37
EtOH/MeCN, 50/50	38
IPA/MeCN, 50/50	34
MeCN/DME, 50/50	60

The measurement error was typically less than 5% relative standard deviation.

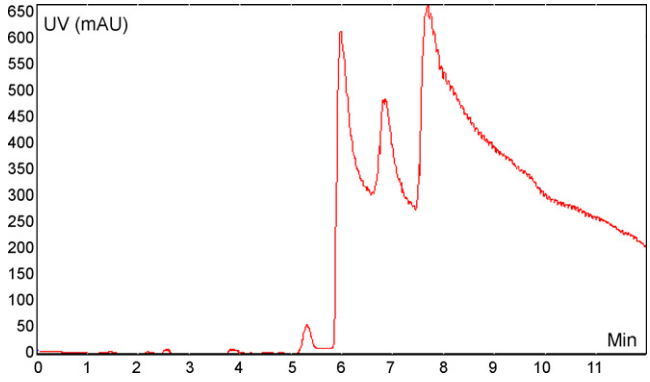

Fig. 5. Preparative SFC chromatogram showing a single 50 mg injection of sample. Flow rate was 55 mL/min, and the mobile phase consisted of 65% carbon dioxide and 35% IPA/DME with 0.2% DEA. The oven was set to 40 °C. The back pressure regulator was set to 100 bar. Sample concentration was 50 mg/mL in DME. The injection volume was 1.0 mL.

This was done by serially connecting a chromatographic column packed with ODS silica (mixed with finely ground analyte) with two six-port switching valves for isolation and sample loop as described in Section 2. An example chromatogram demonstrating the elution of the sample component from the solubility measurement set-up is shown in Fig. 6 for the mobile phase consisting of carbon dioxide with 50% IPA as an organic modifier. The three repetitive peaks at 13, 17 and 23 min (marked with arrows in the figure), each preceded by a saturated peak, correspond to the actual sample peaks from a sample loop. The preceding saturated signals correspond to the elution of excess sample during the sample loop filling step as described in Section 2. The small relative standard deviation (0.4%) of the three

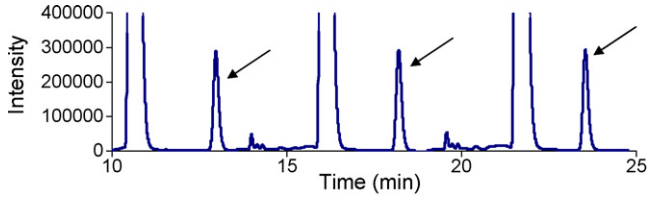

Fig. 6. Example chromatogram demonstrating the elution of the analyte for the SFC solubility measurement. The mobile phase consisted of 50% carbon dioxide and 50% IPA modified with 0.2% DEA as a base additive.

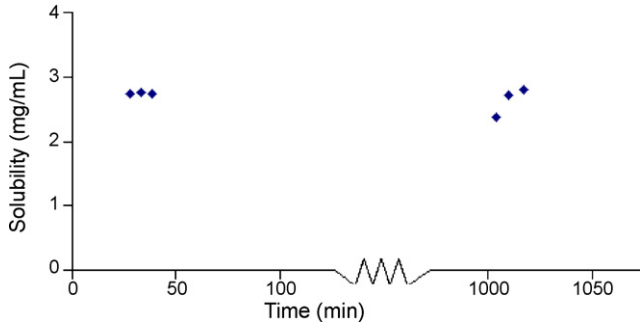

Fig. 7. Solubility of the analyte in SFC mobile phase consisting of 50% carbon dioxide and 50% of IPA as an organic modifier with 0.2% DEA. The oven temperature was maintained at 35 °C and the chamber was set to 100 bar.

repetitive samplings suggests precise solubility measurements. To study a proper equilibration time, samplings were carried out at 15, 30, 50 min and overnight (approximately 17 h) after isolation from the flowing system; the resultant solubilities are shown in Fig. 7. There were no significant variations in solubilities of the sample over the studied time periods. The solubility chamber appeared to reach equilibrium after greater than 15 min of isolation, as demonstrated in Fig. 7. Further solubility measurements were then measured at least 15 min to ensure system equilibrium.

The SFC solubilities of the compounds in various SFC mobile phases are listed in Table 3. Many phenomena were revealed with the solubility measurements in the SFC mobile phase. There is an obvious contrast in solubility trends between the protic and aprotic organic modifiers. Most surprisingly, the solubility of the compound in DME was significantly reduced in the corresponding SFC mobile phase. The solubility in neat DME is greater than 100 mg/mL, but it is only 4.3 mg/mL under the SFC conditions. This suggests that the interaction between DME and the analyte was greatly disturbed by the addition of carbon dioxide to the system. It has been shown by NMR that amines can form a carbamic acid under supercritical fluid conditions [33,34]. The dramatic decrease in solubility of the analyte in SFC conditions with DME might be attributed to the formation of a carbamic acid, which is expected to have a poor solubility in aprotic solvent.

Table 3
Solubility of the analyte in various supercritical fluid chromatographic conditions at 35 °C

Solvent	Solubility in SFC (mg/ml)	
	Mean (n)	%R.S.D.
IPA	2.8 (3)	0.4
IPA:DME (50/50, v/v)	4.0 (3)	2.7
MeCN	5.1 (2)	3.8
DME	4.3 (3)	2.6
EtOH	14.2 (3)	1.0
EtOH:MeCN (50/50, v/v)	6.5 (3)	7.1
MeOH	73.1 (3)	1.8

SFC conditions: 50% carbon dioxide and 50% of the organic modifiers with 0.2% DEA as a base additive.

Similar reduction in SFC solubility was observed for the case of an equal volume mixture of IPA and DME, perhaps due to solubility reduction by DME. An approximately 2.5-fold reduction in SFC solubility was observed for MeCN. A nearly six-fold decrease in SFC solubility was observed for the equal volume mixture of EtOH and MeCN, suggesting that the organic solubility was not necessary a predictor of SFC solubility.

More than a seven-fold increase in SFC solubility with MeOH compared to neat MeOH is notable. The SFC solubility of the analyte with EtOH was approximately two-fold higher than the organic solubility. Interestingly, solubilities of the analyte with IPA were almost equal in both the organic and SFC conditions.

The purpose of the solubility measurement in SFC conditions was to determine the mobile phase composition with a good solubility to maximize sample loading. MeOH appeared to have the highest solubility in SFC conditions, but the poor separation prevented further exploration. Considering solubility data (Table 3) and the analytical SFC separation (Fig. 3), either EtOH or an equal volume mixture of EtOH/MeCN was one of the best potential conditions for further SFC scale-up purification method development.

3.4. A high throughput SFC purification method

Upon consideration of impurity profiles and solubility data obtained in the SFC mobile phases, either EtOH/MeCN or EtOH as an organic modifier was selected for the potential SFC purification method. EtOH appeared to be a good candidate for the scale-up experiment if one considers only solubility data and the analytical scale separation. However, in this mobile phase, the impurity elutes between the two desired enantiomers, consequently preventing this method from being useful for purification, since high organic content (50%) was necessary to shorten the purification time, resulting in overlap of the impurity with the early eluting enantiomer. A lower organic content such as 35%, together with a potentially higher flow rate, could be an alternative way to achieve high throughput purification for the analyte studied.

A short cycle time requirement and impurity profile suggested that EtOH/MeCN was the best of all organic modifier conditions. Final purification was performed by injecting 25 mg of sample on an AD-H column (2.1 cm × 25 cm, 5 μm particle size) at a flow rate of 55 mL/min. The mobile phase consisted of 50% carbon dioxide and a 50% equal volume mixture of MeCN and EtOH, with 0.2% DEA as a base additive. A high percentage

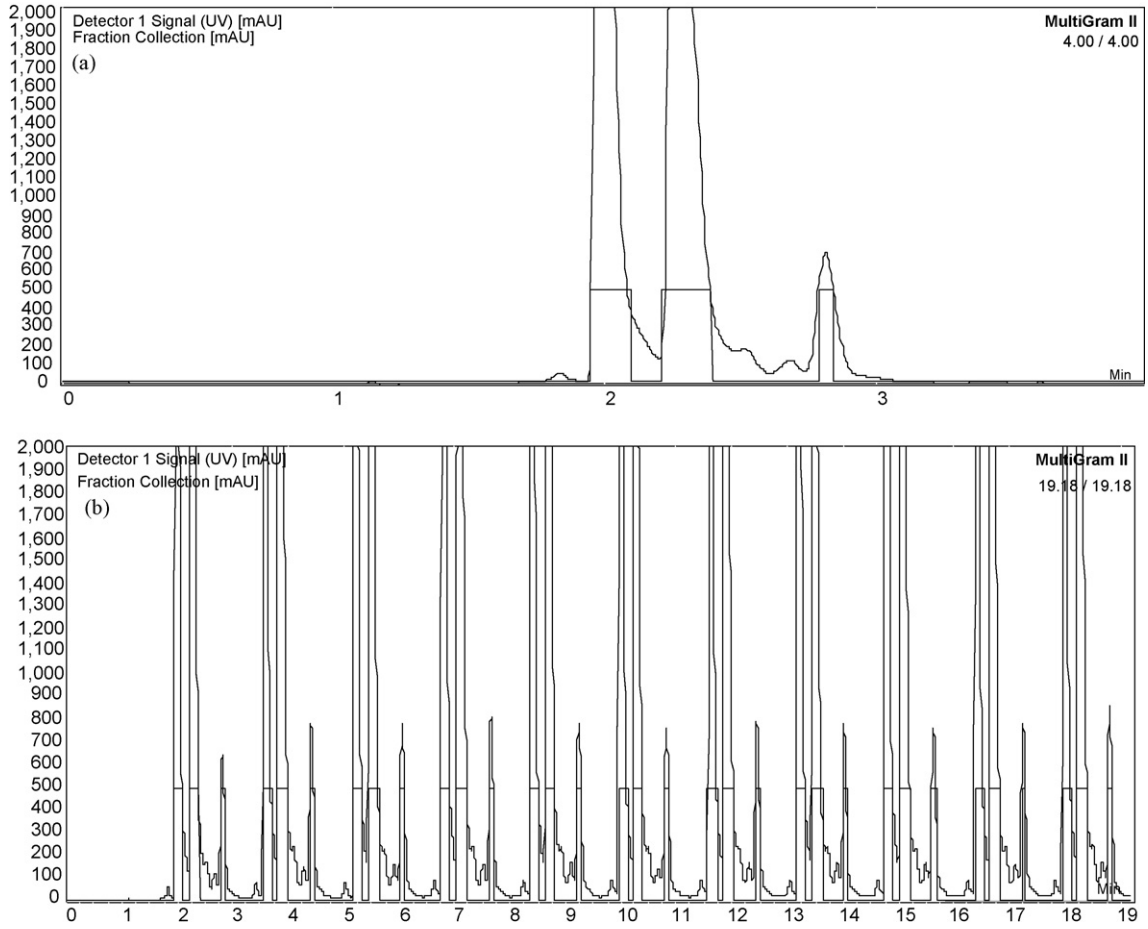

Fig. 8. Preparative SFC chromatograms showing a single injection (a) and stacked injections with a 1.6 min cycle time (b). Flow rate was 55 mL/min. The mobile phase consisted of 50% carbon dioxide and 50% EtOH/MeCN (50/50, v/v) with 0.2% DEA as a base modifier. Injection amount was 25 mg and the oven was set to 40 °C. The back pressure regulator was set to 100 bar.

of organic modifier (i.e. 50% organic modifier for preparative versus 35% for analytical) was utilized to shorten cycle time to 1.6 min. Outlet pressure was set at 100 bar and the oven temperature was kept at 40 °C. The sample was dissolved either in DME or an equal volume mixture of EtOH/MeCN. Representative chromatograms, together with stacking injections, are shown in Fig. 8. The final purification throughput was approximately 0.8 g/h. The enantiomeric excesses of both enantiomers were greater than 98%.

4. Conclusions

In this study, precise measurement of the solubilities in SFC mobile phases was demonstrated by the use of a packed column and two six port switching valves equipped with a sample loop. As a case study, a comprehensive approach was conducted to develop a high throughput SFC chiral purification method for a poorly soluble analyte that was difficult to scale-up. This was done by using an optimized organic mobile phase modifier selected on the basis of solubility measurement in SFC mobile phases in conjunction with chromatographic data.

Knowledge obtained from this new SFC solubility measuring device made it possible to handle a challenging purification more efficiently. The SFC solubility data for the analyte in the current study revealed a probable reason for the initial failed attempts and facilitated the development of a better method. This resulted in reduced turn around time and prevented system downtime.

Solubility data of the analyte in organic solvents was helpful to find the best organic solvent to dissolve the analyte; however, SFC solubility measurements indicated no obvious correlation with organic solubilities. Among all observations, the seven-fold enhanced solubility in MeOH modified SFC mobile phase and more than twenty-fold decrease in DME modified SFC mobile phase compared to neat forms were the most notable.

Overall, the current SFC solubility set-up may not be practical for routine work, but proved to be more efficient for a sample with poor solubilities in SFC mobile phases. Work is on going to study solubility of large number of commercially available organic molecules in SFC conditions.

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