Rapid Isolation of a Minor API Impurity Using SFC, HPLC/MS, and SFC/MS

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Introduction

The process of isolating impurities and degradents from batch lots of drug substance is challenging and an intensive use of chromatography. Small amounts of any unknown component having a relative abundance in the batch of greater than 0.1% must be isolated for structure elucidation. If synthesis of the compound is intractable, chromatography is often employed to isolate larger amounts for use as reference standard or for *in vivo* testing.

The high efficiency of SFC for isocratic isolation and purification work is well known, but development of an SFC method specific for a targeted minor component in a complex mixture is difficult. Almost universally, stability indicating methods identifying minor API components are reversed-phase HPLC gradients, and SFC is a normal phase chromatography that is run isocratically to access the efficiency of injection stacking.

To resolve this difficulty, we have developed a co-configured HPLC/MS and SFC/MS system that uses the same mass spectrometer as a detector for either chromatography. Using this system we can identify the mass associated with the desired component in the RP-HPLC trace and develop methods for isolating that component by preparative SFC. HPLC/MS is then used to confirm the success of the process.

Experimental

HPLC / MS System

LC:	Alliance HT 2795
Column:	XBridge Phenyl, 4.6 x 150 mm, 5µ
UV Detector:	2996 PDA
MS:	Waters ZQ Mass Spectrometer, ESI+
Software:	MassLynx

Analytical SFC / MS System

SFC:	Thar Investigator
Columns:	(S,S) Whelk0-1 , 4.6 x 150 mm, 5µ RegisPack, 4.6 x 100mm, 5µ Kromasil
UV Detector:	2996 PDA
MS:	Waters ZQ Mass Spectrometer, ESI+
Software:	MassLynx

Preparative SFC System

SFC:	Thar Prep 80
Columns:	(S,S) Whelk0-1 , 2 x 25 cm, 5µ RegisPack, 2 x 25 cm, 5µ Kromasil
UV Detector:	Gilson 151
Software:	Thar SuperChrom™

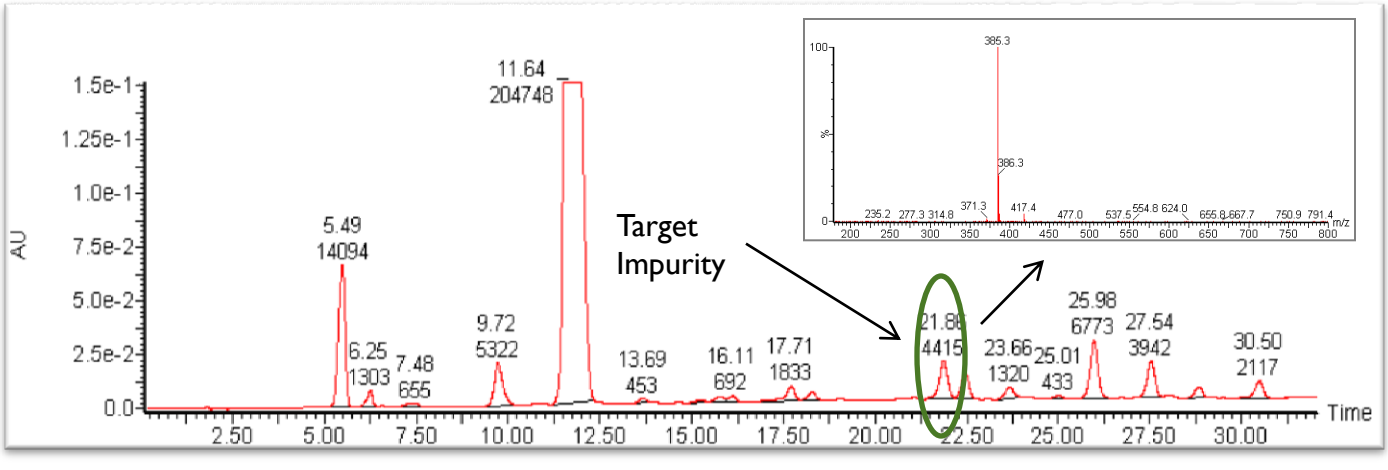

Figure 1 : HPLC (UV_{295}) of crude material following forced degradation. The peak of interest has m/z 385 (main peak is m/z 371).

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Results and Discussion

Figure 1 shows the HPLC/UV chromatogram produced using a standard method (Table 1) from a 3.5 gram lot of a developmental drug subjected to acid and heat stress in a rapid forced degradation experiment. Several peaks were isolated for further characterization, but for the purposes of this discussion we will focus on the desired peak at 21.8 minutes. The forced degradation enhanced this peak to 2.1% of the main peak area based on the preferred UV wavelength of 295 nm.

HPLC/MS using the method in Table 1 was used to determine that this peak had a nominal MH+ mass of 385 Da.

Column:	Waters XBridge Phenyl 4.6 x 150 mm 5 µm
Mobile Phase A:	10mM Ammonium Formate in water to pH 4.0
Mobile Phase B:	10mM Ammonium Formate in Methanol: Water (90:10) to pH 4.0
Flow Rate	1 ml/min
Gradient:	10 – 40% B over 30 minutes, 40 -100% B over 5 minutes, hold at 100% B for 4 minutes, return to initial conditions
Run Time:	50 minutes
Detection:	UV Diode Array 220 – 400 nm

Table 1: Standard RP-HPLC Method

Isolation of this peak by repeated preparative reverse-phase gradient runs was deemed impractical due to the volume of buffer and time required using a 50 minute method. SFC is of interest for such separations due to several advantages:

•Significantly faster chromatographic run time
•Lowered solvent consumption
•Low volume fraction collection with highly volatile solvents
•Rapid processing of bulk material using injection stacking

SFC/MS Method Development

Multiple chiral and achiral columns were screened in an effort to find an appropriate separation. The objective in preparative SFC is an isocratic method that disperses the desired peak from most others BUT STILL elutes all peaks in a reasonable time frame. Injections are then stacked so that the desired peak is not overlapped with any other peak when repeated injections are on column.

An isocratic method (Table 2) was developed to achieve the separation shown in Figure 4. The method uses a chiral stationary phase; however, achiral stationary phases had unique selectivity as well – the choice to use a chiral selector was results-based. MS (Figure 2) identified the target eluting at 1.45. The MS spectral purity indicates the presence of a co-eluting peak at m/z 372. Even with the co-eluting impurity, this method was chosen for scale up because of the resolution of the target from the main peak. In preparative separations of a minor peak, we gain a great advantage if the target elutes before the main peak – it can be collected at higher on-column loads without interference from the overloaded main peak.

Development of a method would not be possible without the use of the MS data. However, once the peak is identified its UV absorbance is used to set up collection times in the preparative process.

Column:	(S,S) Whelk0-1 from Regis Technologies (Morton Grove, IL) 4.6 x 150 mm, 5 µm
Co-Solvent:	Methanol with 0.1% Isopropylamine
Isocratic Method	35% Co-Solvent
Flow Rate	4 ml/min
Pressure:	100 Bar
Run Time:	9 minutes
Detection:	UV: 230 nm MS: ES+ 180 – 800 at ~1200 amu /sec

Table 2: Analytical SFC/ MS Method

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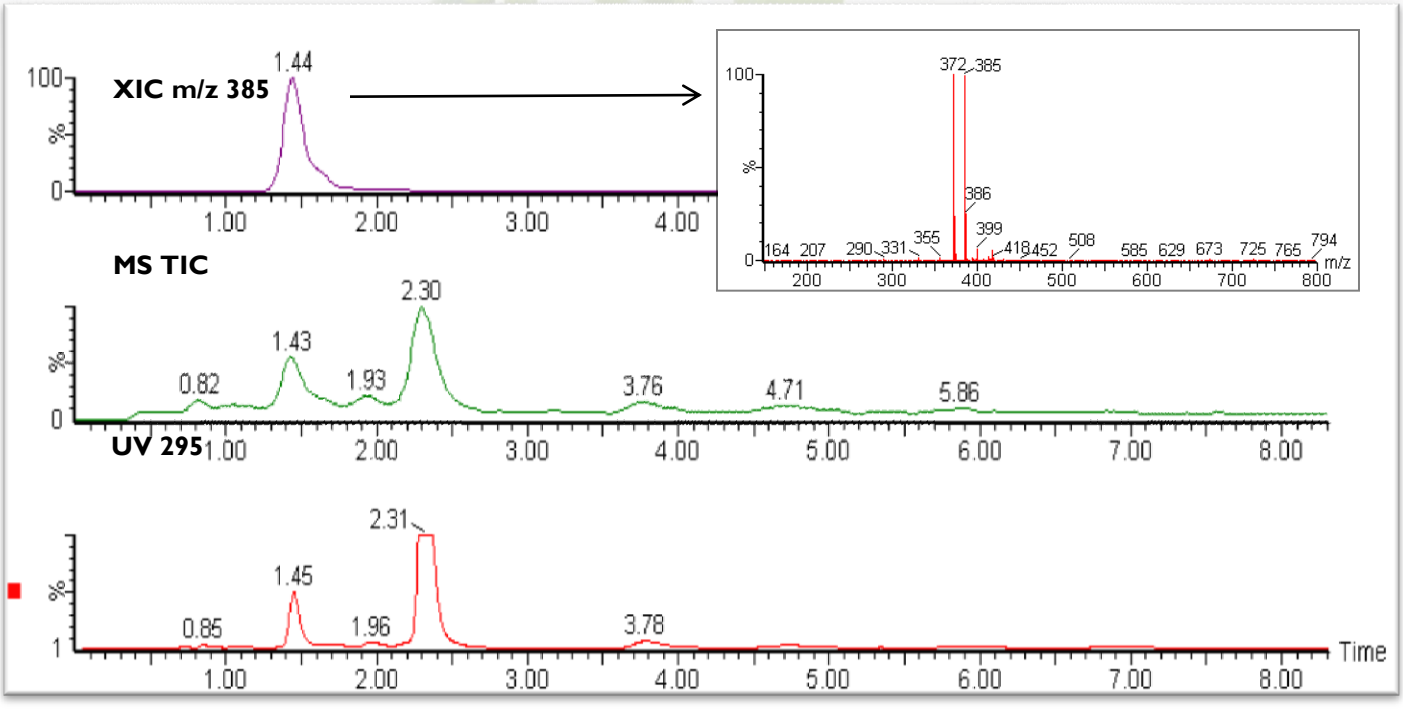

Figure 2: Isocratic (see Table 2) SFC / UV / MS of crude sample

Preparative Scale SFC

A total of 3.5 grams of crude material was available for purification. The goal was to obtain about 20 - 50 milligrams of material for structural characterization. The scaled up preparative SFC outlined in Table 3. A representative single injection chromatogram is shown in Figure 3.

The method allowed 200 mg of crude to be injected every 8 minutes. This corresponds to a production rate of 1.5 g / hour, and the processing was complete in 2.3 hours.

Column:	(S,S) Whelk0-1 from Regis Technologies (Morton Grove, IL) 3 x 25 cm, 5 µm
Co-Solvent:	Methanol with 0.1% Isopropylamine
Isocratic Method	35% Co-Solvent
Flow Rate	80 g/min
Pressure:	100 Bar
Sample Load	200 mg milligram / injection
Stack Time:	8 minutes (1.5 g/ hour)
Detection:	UV: 230 nm

Table 3: Preparative SFC Method for isolation of 1.45 min peak

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Preparative Scale SFC

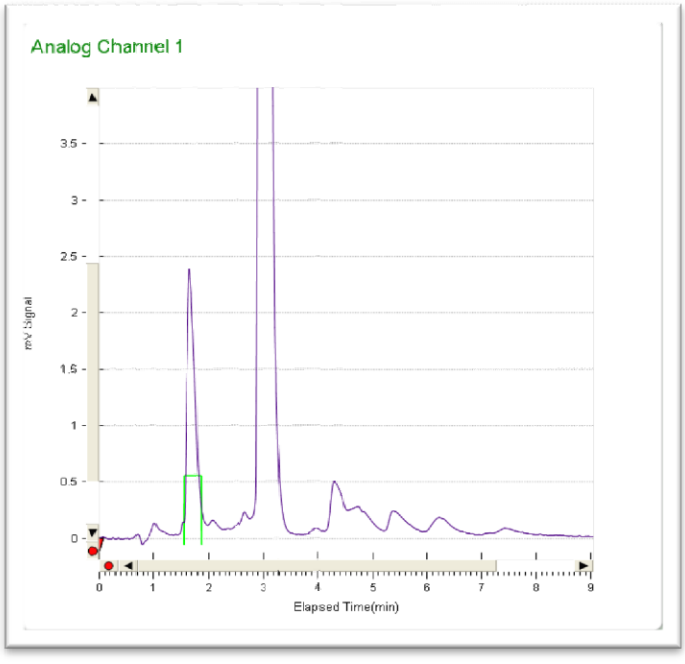

Figure 3: Preparative SFC fractionation of 1.45 min peak

The target peak was 26 seconds wide, and the flow rate of 80 g/min combined CO2/MeOH (65:35) resulted in collection of 12 mL methanol solution of the target peak. The total collected fraction volume was about 225 mL. This concentrated fraction was rapidly dried by rotary evaporation, a benefit of SFC that helps limit decomposition.

After drying approximately 250 mg of solid material remained from the collected fraction. This "primary fraction" was analyzed using the standard HPLC method (Table 1) which assessed the target at 21.8 minutes as roughly 30% pure (Figure 4). This was not unexpected, as the SFC/MS data in Figure 2 indicated at least one other ionizable compound co-eluting with the target. The fraction, however, was substantially cleaner than the crude batch, and further purification proved straightforward.

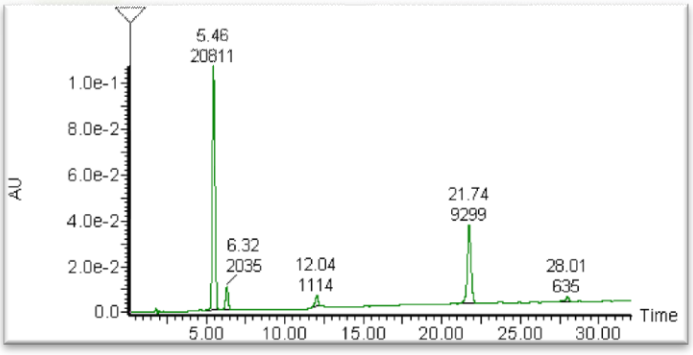

Figure 4: HPLC (UV 295nm) of primary fraction at 1.45 min

Development of Second SFC Method

The primary fraction was screened against a variety of stationary phase / mobile phase combinations, just as was done with the original material. An analytical method using a different stationary phase was selected and scaled to the preparative method outlined in Table 4. SFC/MS was used to identify the 1st eluting major peak as the target of interest. The preparative SFC chromatogram is shown in Figure 5.

Column:	RegisPack from Regis Technologies (Morton Grove, IL) 3 x 25 cm, 5 µm
Co-Solvent:	Isopropanol with 0.1% Isopropylamine
Isocratic Method	35% Co-Solvent
Flow Rate	80 g/min
Pressure:	100 Bar
Sample Load	40 mg milligram / injection
Stack Time:	8 minutes
Detection:	UV: 230 nm

Table 4: Preparative SFC Method of fraction from crude

Second SFC Purification

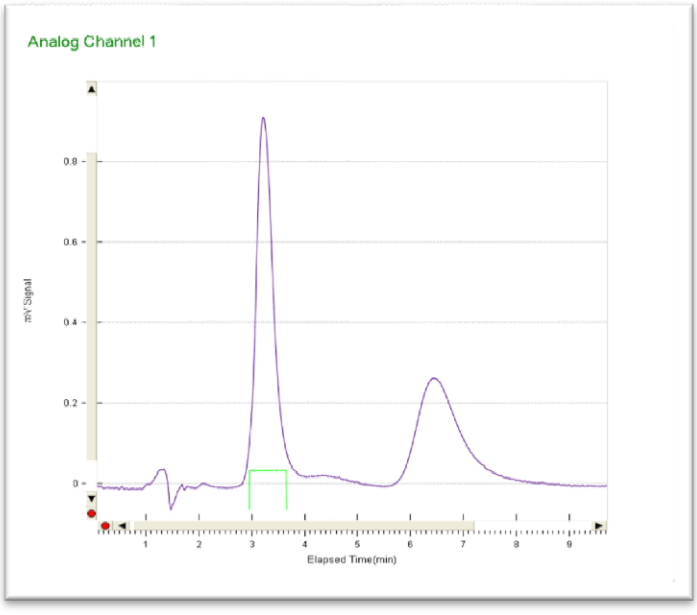

Figure 5: Second preparative SFC fractionation, selecting the peak at 3.3 min

In this case only three injections were necessary to process the 250 mg of "primary fraction" to yield 38 mg of the target compound. This was assessed at 95% purity using the standard HPLC method (Table 1), and was used for further structure elucidation work by NMR.

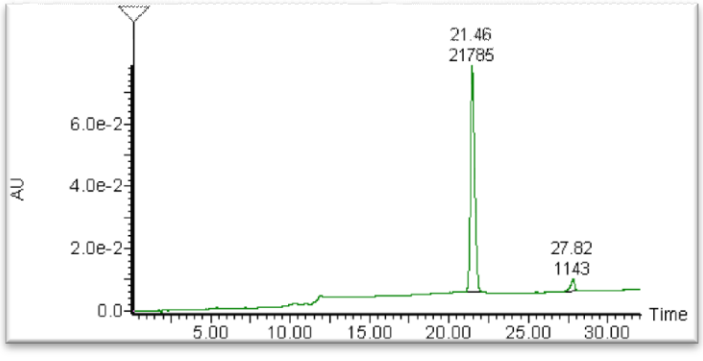

Figure 96 Final HPLC assessment (UV 295nm) of target.

Conclusions

SFC is a viable and valuable technology for efficient separation of minor components from complex mixtures. However, to do this process efficiently one must have a way to definitively identify the desired minor peak(s). We have found that complex mixtures are most frequently assayed using RP-HPLC, and that a system that couples MS detection to not only the HPLC analysis but also to analytical SFC is a key tool that makes this work possible.

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