

## Mass-Directed Fraction Collection for Preparative SFC

### Collection of a 0.2% impurity using a Jasco Prep SFC coupled with the expression Compact Mass Spectrometer

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#### Introduction

Supercritical fluid chromatography (SFC) is a rapidly growing technique. Initially used almost exclusively only for thermally labile and chiral compounds, it is now accepted as a versatile analytical and purification technique applied across a broad range of compounds. It is higher-throughput than regular HPLC and Prep-LC and offers the 'green' advantages of much lower solvent consumption, consumable and disposal costs. SFC is compatible with a wide range of detection methods including UV, electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometry.

#### Experimental

|                         |  |
|-------------------------|--|
| Mass spectrometer:      | <u>expression</u> CMS  |
| SFC:                    | Jasco Prep SFC System  |
| Total flow:             | 70 ml/min  |
| BPR:                    | 120 bar  |
| Column:                 | Waters Viridis Silica<br>2-Ethylpyridine OBD Prep<br>Column, 100Å, 5 µm,<br>19 mm x 250 mm   |
| Co-solvent:             | Methanol   |
| Gradient:               | 5-25% in 7 min.  |
| Splitter configuration: | 100 cm (2 x 50 cm) PEEKSil<br>- 0.025 mm ID x 1/16" OD<br>coupled with ZDV union   |
| Makeup pump flow:       | 8 mL/min MeOH (constant<br>flow for 30% modifier and<br>below)   |
| Interface:              | Jasco ChromNav set up to read two<br>analog outputs from <u>expression</u><br>CMS mapped to the total ion<br>chromatogram (TIC) and one<br>selected extracted ion<br>chromatogram (XIC)mg/mL |
| Sample:                 | Flavone 0.02 mg/mL (0.2%),<br>Carbamazepine 10 mg/mL (100%)<br>Dissolved in methanol   |

#### Objective

To determine the purity of collection of a small (0.2%) impurity in a concentrated sample matrix using the extracted ion chromatogram (XIC) for mass directed fraction collection.

#### Methods

- 1. Pre-Purification Analytical:** A small aliquot is injected to determine whether the compound target mass is present and to measure the UV purity of the sample prior to purification. The combination of target mass being found and the sample's purity determine whether a sample moves to the preparative stage and which preparative narrow method, based on target retention time, will be automatically selected for the purification process.
- 2. Purification:** 20 injections of 0.7 mL were made and the target mass extracted using fraction collection based on the method identified in Pre-purification. Mass directed triggering using XIC for Flavone *m/z* 223.1.
- 3. Post-Purification Analytical:** A small aliquot is injected from each tube of collected fraction material and the final sample purity measured.

## Instrument Set up



JASCO Preparative SFC system with fraction collection and Advion expression CMS



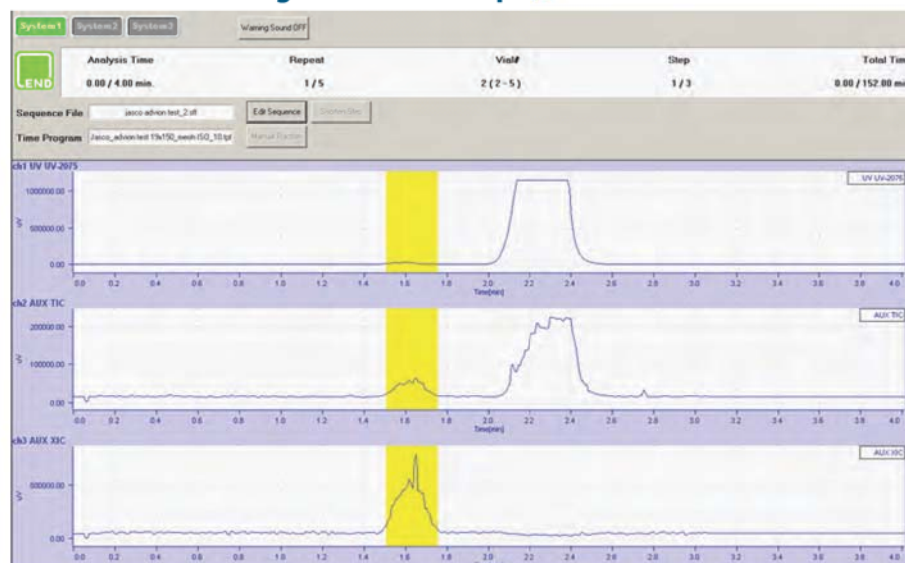
ZDV splitting tees post-UV detection, 1<sup>st</sup> tee post VWD splits in the solvent make-up for the fraction collector and travels to 2<sup>nd</sup> tee that splits flow to the CMS via 100 cm x 0.025 mm PEEKsil.



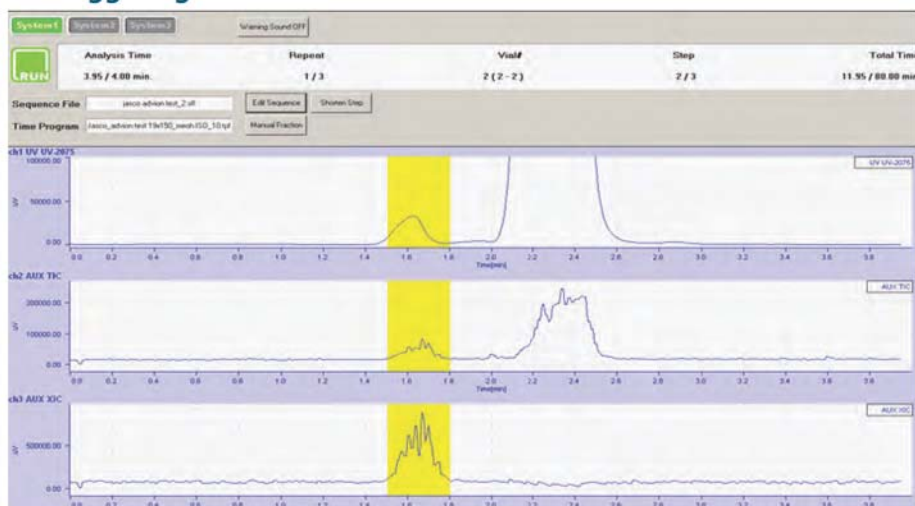
ZDV splitting tee pre-CMS splits in the LC/MS make-up solvent for ionization and sample stream from 2<sup>nd</sup> ZDV tee PEEKsil line.

## Results

### 0.2% Flavone in 10mg/mL Carbamazepine



### Zoom chromatogram of 0.2% Flavone in 10mg/mL Carbamazepine. MS Triggering with XIC



## Conclusions

- The CMS can be simply interfaced to both analytical scale and prep scale SFC using a simple passive split before the UV detector
- All fractions collected of each analyte were tested analytically and showed that each was 99.9% pure or greater
- Limit determination at 0.2% Flavone in the presence of Carbamazepine - Using the XIC signal to trigger the fraction collector, a 0.2 wt % peak of Flavone was collected at 100% purity