Application with Mass Directed Preparative SFC Utilizing the JASCO Preparative SFC platform and Advion expression Compact Mass Spectrometer

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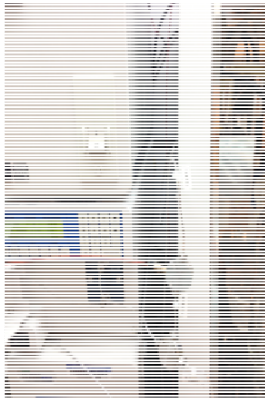
ABSTRACT
In this study we evaluated the performance of a Preparative SFC-MS system combining the JASCO Preparative SFC with the Advion expression CMS (Compact Mass Spectrometer) utilizing a simplified passive splitter for mass-directed fraction collection. The initial test explored a wide range of flow rates both isocratic and gradient to test performance and reliability of the passive splitter, coinciding timing of MS and UV signal and the clearance of the source with highly concentrated samples. The system was further evaluated testing the recovery of main peaks as well as small low level impurities using either TIC or XIC fraction triggering. Purity of the collected peaks was also determined.

OBJECTIVES
Test #1: Three component STD, gradient elution, using TIC mass-directed collection as trigger.

Test #2: Three component STD, gradient elution, using UV-directed collection with comparison versus mass directed collection.

Test #3 – 0.2% impurity collection to determine LOQ limitations using XIC mass-directed collection as trigger.

INSTRUMENTATION SET-UP



ZDV splitting Tee's post UV detection, 1st Tee post VWD splits in the solvent make-up for the fraction collector and travels to 2nd Tee that splits flow to the CMS via 100 cm x 0.1 mm PEEKSil.

JASCO Preparative SFC system with fraction collection and Advion expression

ZDV splitting Tee pre CMS, splits in the LCMS make-up solvent for ionization and sample stream from 2nd ZDV Tee PEEKSil line.

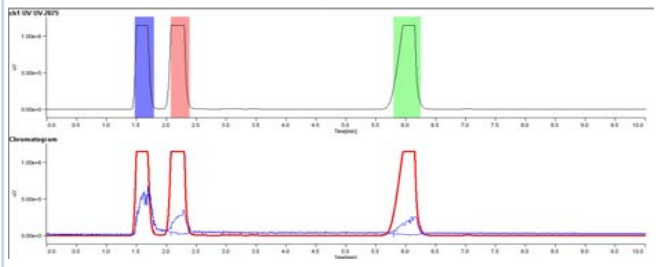
MATERIALS & METHODS
STD 1 for TIC and UV trigger collection
Peak A = Flavone, 477.8 mg/50 ml (9.56 mg/ml)
Peak B = Carbamazepine, 478.2 mg/50 ml (9.56 mg/ml)
Peak D = Sulfamethazine, 473.9 mg/50 ml (9.48 mg/ml)
Total concentration: 1429.9 mg/50 mL MeOH (28.60 mg/mL), 700 µL injections

STD 2 for XIC trigger collection
Peak A = Flavone, 20.4 mg/25 ml -> 610 µL / 25 mL (~0.019 mg/ml)
Peak B = Carbamazepine, 248.7 mg/25 ml (9.95 mg/ml)
Total concentration: 269.1 mg/25 mL MeOH (9.97 mg/mL), 700 µL injections

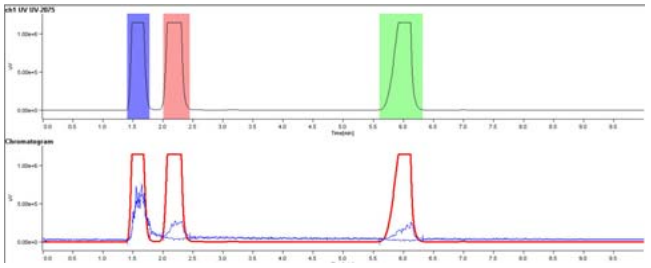
Chromatographic Conditions:
- Column: Viridis Silica 2-Ethylpyridine OBD Prep Column, 100Å, 5 µm, 19 mm X 250 mm Part number, 186004946
- Co-solvent: 100% Methanol
- Gradient: 5-25% in 7 min (5-25% in 7 min, hold 25% for 30 sec, 25-5% in 10 sec, hold 5% for 2.5 min)
- Total Flow: 70 ml/min
- Outlet Pressure: 120 bar
- Splitter configuration: 100 cm (2 x 50 cm) using PEEKSil - 0.1 mm ID x 1/16" OD, part number 0624300, coupled with ZDV union.
- Fraction collector makeup pump flow: 8 mL/min MeOH (constant flow for 30% modifier and below)
- Advion CMS makeup pump flow: 1 mL/min 90:10 v/v Methanol : Water with 0.1% Formic Acid (to aid with ionization and band broadening)

RESULTS
- JASCO Preperative SFC/ Advion CMS system was first evaluated by collecting three analyte fractions by both UV and MS-TIC triggering. Chromatorgraphy was optimized for the peak shape of all three analytes by implementing gradient elution and also a make-up pump for the CMS to reduce band broading upon gas expansion from the "system leak" created from the Tee split for the CSM eluent.
- Timing delay between UV and MS signals was determined to be 3.6 seconds.
- All fractions collected of each analyte were tested analytically and showed that each was 99.9% pure or greater.
- Difference between the recoveries of that were triggered by UV verses the fractions triggered by MS-TIC were less than 2% for the flavone and sulfamethazine peak and 6% for the carbamazepine.

Test #1 - TIC triggered Fraction collection (5 injections)



Test #2 - UV triggered Fraction collection (5 injections)



JASCO/ ADVION SFC PREP recovery

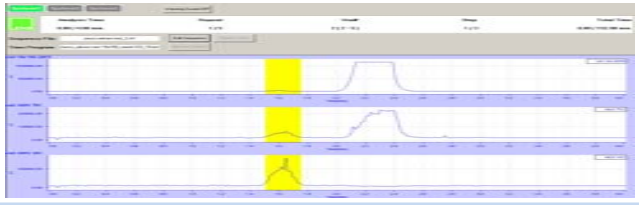
UV trigger	Theoretical amount injected on Column	Sample Recovered	Recovery	
Flavone	33.446	31.7 mg	**94.8 %**	
Carbamazepine	33.474	33.5 mg	**100.1 %**	
Sulfamethazine	33.173	31.3 mg	**94.4 %**	
TIC trigger	**Theoretical amount injected on Column**	**Sample Recovered**	**Recovery**	**Difference Vs. UV triggering**
Flavone	46.824	44.4 mg*	**94.8 %**	**0.0%**
Carbamazepine	33.474	31.4 mg	**93.8 %**	**6.3%**
Sulfamethazine	33.173	31.8 mg	**95.9 %**	**-1.6%**

* Two additional fractions of Flavone were collected

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.

Test #3 - 0.2% Flavone in 10 mg/ml Carbamazepine. MS triggering with XIC



CONCLUSIONS
Mass-directed fraction collection with a simplified passive splitter on the Preparative JASCO SFC-Advion MS system proved very successful. The MS accepted high concentrations of sample material and peak shapes and rear peak edges did not appear to be extended much longer than the UV trace. Low, Middle and High isocratic flow rates all showed comparable MS source clearance leading to minimal tailing beyond the UV. Gradients further minimized peak tailing as expected on the UV and this was also seen on the TIC and XIC. Peak purities for all main band purifications were ≥99.9% pure and minor impurity collections showed equivalent success yielding 100% pure fractions. Acceptable recoveries for all collected fractions were 94% or better.