MASS DIRECTED FRACTION COLLECTION BY REVERSE PHASE FLASH CHROMATOGRAPHY COUPLED TO THE EXPRESSION® CMS

Instrumentation

Mass Spec: expression® CMS
Purification: puriFlash® XS 530
Coupling: Advion Interchim Scientific® MS Splitter

Authors

Advion Interchim Scientific®

In this application note, we compare the use of a flash chromatography system with a UV detector alone versus the same system paired with a mass spectrometer. The data highlights the impact on workflow efficiency and underscores the importance of selecting the appropriate splitter.

Introduction

The following application material will compare the use of a flash chromatography system with a UV detector alone versus the same system paired with a mass spectrometer. The data highlights the impact on workflow efficiency and underscores the importance of selecting the appropriate splitter.

Figure 1: The puriFlash® XS 530with UV detector

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Example #1: Purification from UV signal only

The Advion Interchim Scientific® puriFlash XS 530 with a UV detector only (figure 1) is used to monitor compounds eluting.

UV detection is based on the absorption of ultraviolet light by compounds containing chromophore groups. These groups (such as conjugated double bonds or aromatic groups) absorb light at specific wavelengths.

Method

EXPERIMENTAL SETUP (Figure 2)

- **Flash Chromatography System:** puriFlash® XS 530 with UV detector (200-400nm)
- **Column:** puriFlash® Column 15µm C18-HQ F0080 34 mL/min
- **Sample:** Mixture of 1 mL (5 compounds 20mg/ml each)

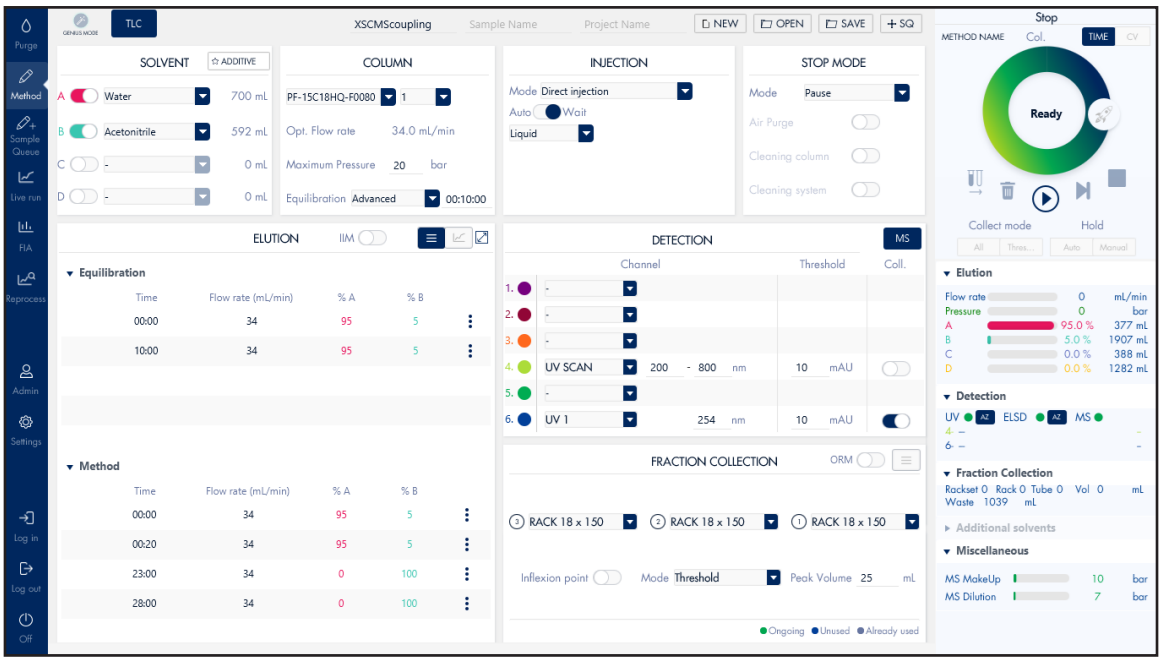

Figure 2: The puriFlash® InterSoft® X software, show here, controls the full workflow for UV detection and fraction collection

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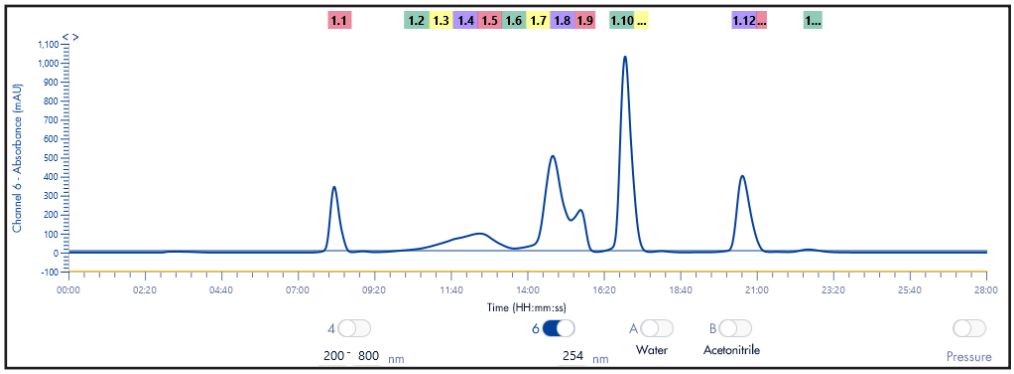
Results

UV signal 254nm for collection (Figure 3):



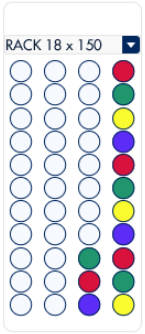


Figure 3:

Left: UV 254nm detection & collection

Right: Collection rack corresponding to the purification from UV signal

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The compounds are collected in 15 tubes of 25ml. Each collected fraction will be analyzed by LC/MS (liquid chromatography coupled with mass spectrometry) in another lab. LC/MS analysis after purification by flash chromatography is a key step in verifying the purity and identity of isolated compounds but it requires time for sample preparation and analysis.

Example #2: Purification from MS signal

We are going to perform the same separation with the Advion Interchim Scientific® expression® CMS coupled to the Flash Chromatography XS 530 system (Figure 4) for mass directed fraction collection. Typically, a UV detector is used to monitor compounds eluting. A limitation of the UV detector is that it relies on compounds having a chromophore in the chemical structure, hence an analyte of interest may not be detectable if it lacks a suitable UV chromophore or coelutes with another compound.

In addition, solvent systems can interfere with and hinder UV absorbance. The CMS provides the ultimate sensitivity, unambiguously identifying residual starting materials, desired product ions, side reactants and impurities by their molecular weight despite co-eluting compounds and solvent peaks.

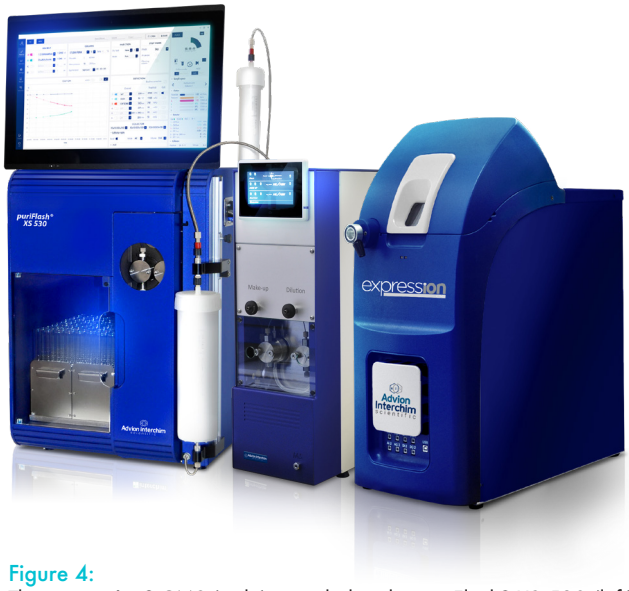

Figure 4:
The expression® CMS (right), coupled to the puriFlash® XS 530 (left) seamlessly via the MS Splitter (center)

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FIA Injection

In order to identify by mass our compounds of interest in our mix to purify, we are going to make a FIA (Flow injection analysis) by diluting a part of our mixture and introducing with a syringe into the expression® CMS thanks to the front injection valve of the system. With the integrated puriFlash® InterSoft® X software, the ion source parameters for the mass spectrometer are available to program within the same interface. Both ESI and APCI, in both positive or negative ion mode are available. (fig 5.1 & 5.2)

5.1

Figure 5.1: Identification of our first compounds of interest *m/z* 222

5.2

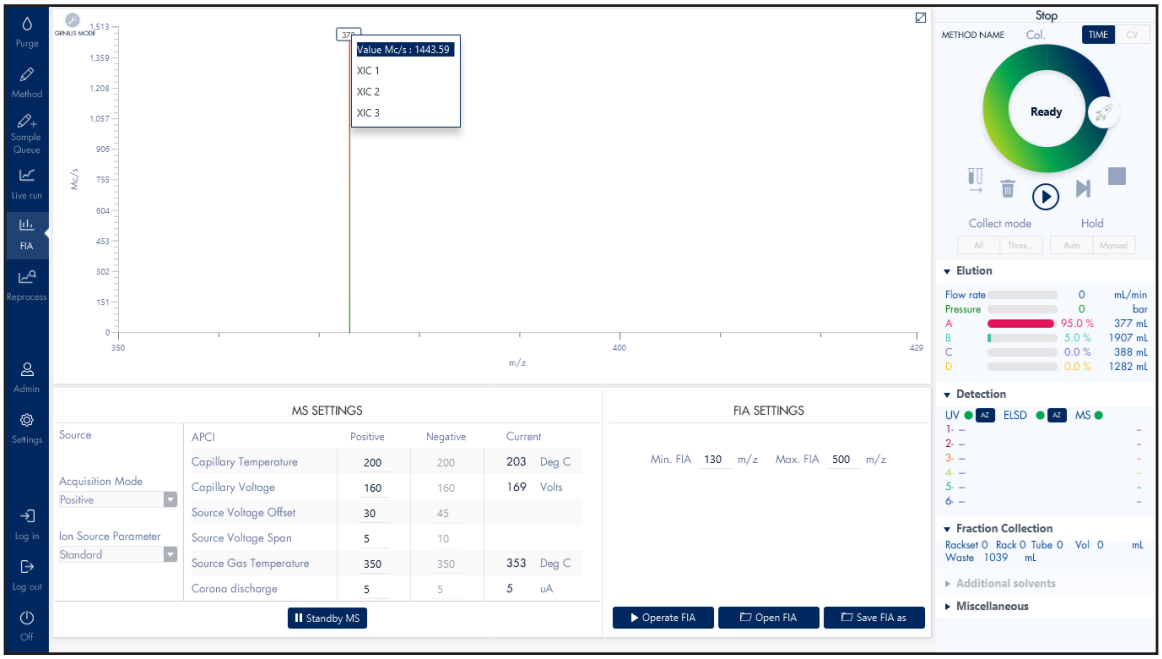

Figure 5.2: Identification of our second compounds of interest *m/z* 372

The *m/z* of interest are added directly to the purification method by clicking on the label in order to collect from these *m/z* signals.

Method

EXPERIMENTAL SETUP (Figure 6)

- **Flash Chromatography System:** puriFlash® XS 530 with UV detector (200-400nm)
- **Mass Spectrometer:** expression® CMS
- **puriFlash MS splitter:** make up pump 0.1ml/min, dilution pump 0.5ml/min, valve 1.5s
- **Solvent:** Acetonitrile+0.1% formic acid
- **Column:** puriFlash® Column 15µm C18-HQ F0080 34 mL/min
- **Sample:** Mixture of 1 mL (5 compounds 20mg/ml each)

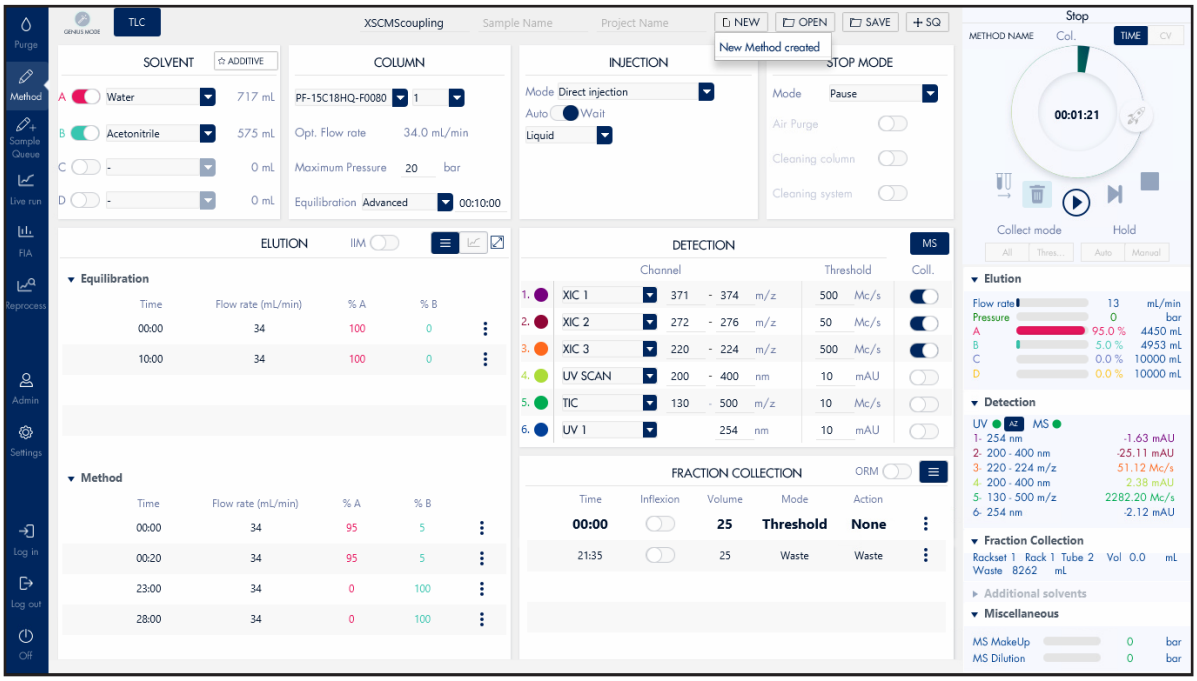

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Figure 6:
The puriFlash® InterSoft® X software, show here, controls the full workflow for the mass-directed fraction collection, including the operation and data management from the mass spectrometer.

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Figure 7:
APCI source & MS splitter parameters:

The ion source and MS splitter parameters are available to program directly in the method pannel.

Results

UV signal 254nm for visualization & XIC of 2 compounds for collections (Figures 8.1 & 8.2):

8.1

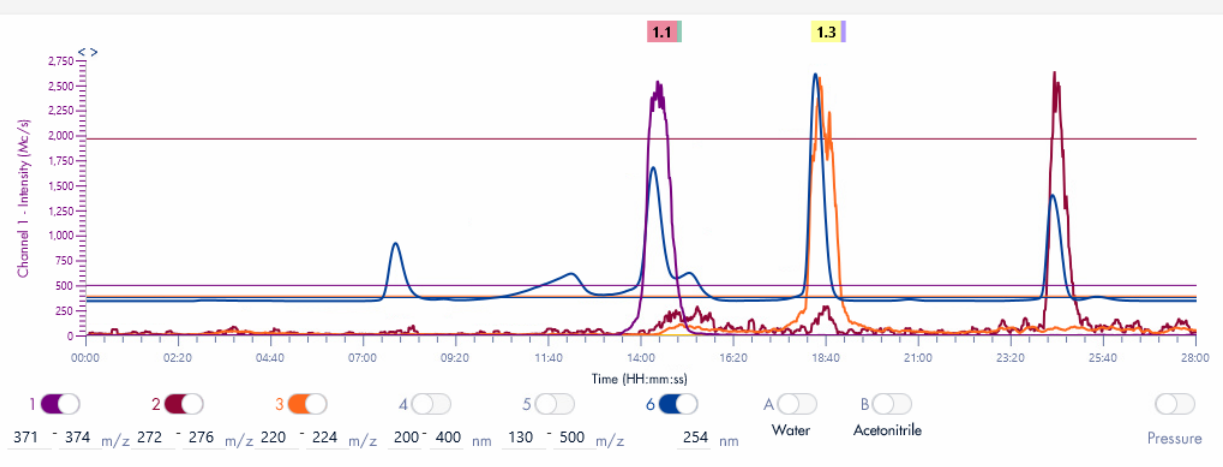

Figure 8.1: UV 254nm, XIC 371-374*m/z*, XIC 272-275*m/z*, XIC 220-224*m/z* signals

8.2

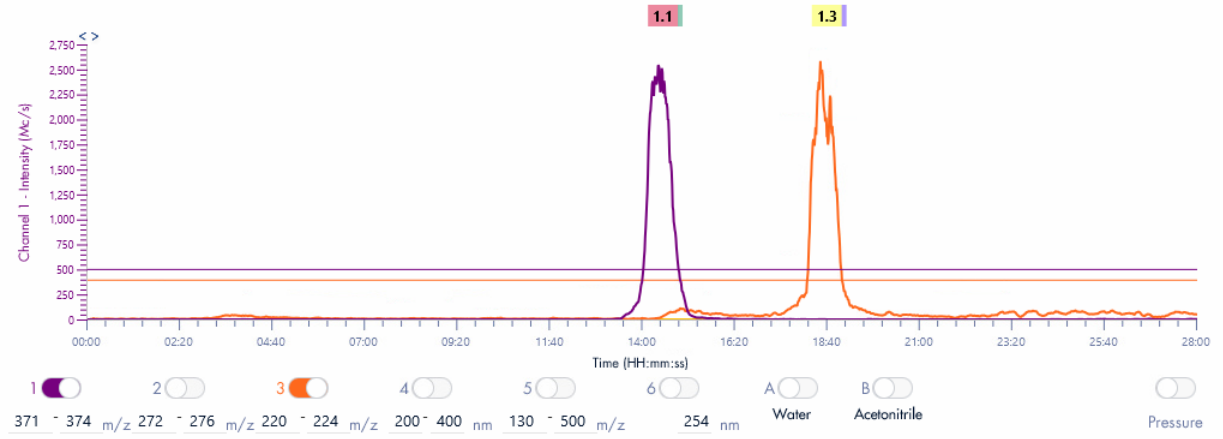

Figure 8.2: XIC 371-374*m/z*, XIC 220-224*m/z* signals

The synchronization between UV signal and mass signals is perfect, and the compounds are collected in only 4 tubes of 25 ml (Figure 9).

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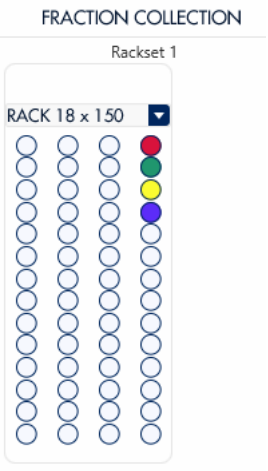
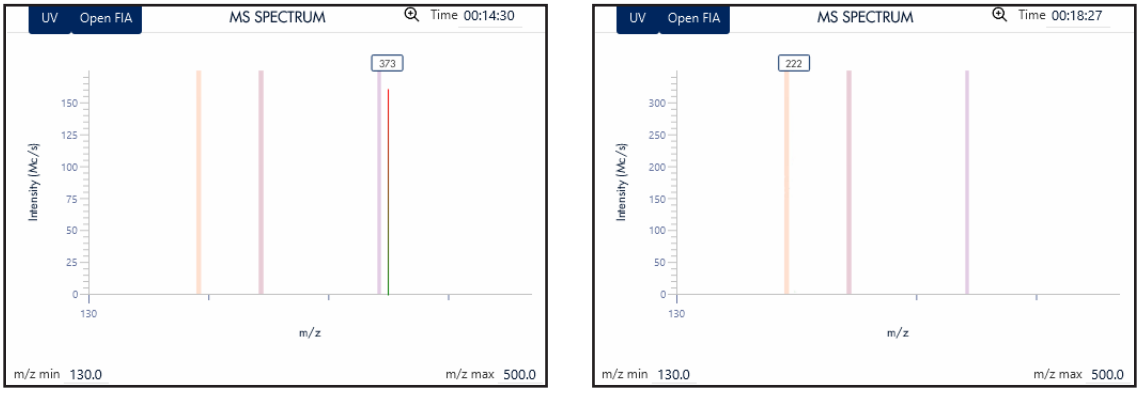


Figure 9: Collection rack corresponding to the purification from mass signals

No need to make other analysis, the visualization of the mass spectrum for each peak is available directly on ISX software (Figure 10).

Mass directed fraction collection greatly speeds up the chemist's workflow with confidence in the content and purity of the collected fractions by eliminating off-line TLCs, workup, and core lab LC/MS assays.



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3. Importance of the splitter

MS splitter vs Standard dynamic splitter (Figure 11): The same purification has been performed with a standard dynamic splitter valve (the most common on the market). The backpressure generated by this splitter was higher to 20 bars (max pressure that can withstand the column) so the system detected an overpressure that can't be solved even in decreasing the flowrate.

Figure 11: MS splitter includes make up & dilution pumps vs standard dynamic splitter valve without make up pump

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Due to this back pressure generated it becomes difficult or impossible to work with a flash column with a flowrate higher than 25ml/min.

Thanks to the innovative technology of the MS splitter, it doesn't generate any back pressure, even if the flow is high. You can work with all kinds of column dimensions and all of the puriFlash® 5 systems.

The post-dilution integrated into this interface allows the injection of samples without concentration limitations and without the risk of detector saturation.

Conclusion

The high flow rate and highly concentrated eluate from the flash chromatography system is continuously sampled using the MS splitter, diluted, and introduced to the CMS using a MS compatible solvent. The mass-to-charge ratio (*m/z*) of each compound are detected and correlated to the UV peaks. The two target products are collected based on a common fragment ion. The affordability and capability of the Advion Interchim Scientific® expression® CMS and puriFlash® systems makes the instruments of choice for organic and medicinal synthetic chemists who desire definitive identification of their compounds while optimizing chromatographic separations.