Application Note

Agilent OpenLab

Purify with Ease Using Mass-Based Fraction Collection with InfinityLab Pro iQ and OpenLab CDS

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Abstract

Fraction collection is a powerful tool in liquid chromatography for purifying synthesis products or isolating low-abundance components. When guided by detector signals, especially mass spectrometry, collection can be highly selective and efficient. The Agilent 1290 Infinity II Preparative LC/MSD System, InfinityLab Pro iQ Plus single quadrupole mass spectrometer (MS), combined with Agilent OpenLab CDS software, brings mass-based fraction collection into routine workflows with ease. This application note demonstrates the process applied to an elderberry extract targeting flavone glycosides.

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Introduction

Fraction collection in liquid chromatography (LC) is a well-established technique for isolating specific components from complex mixtures. Whether purifying synthetic products, enriching low-abundance impurities, or isolating bioactive compounds from natural sources, fraction collection enables targeted downstream analysis and processing. Traditionally, collection has been guided by ultraviolet (UV) detectors that are straightforward to use but have limited sensitivity and compound selectivity.

Mass-based fraction collection (MBFC) significantly enhances selectivity and sensitivity by using mass spectrometry (MS) to trigger collection based on molecular weight of the targets. This approach provides unmatched specificity, allowing users to isolate only the compounds of interest even in the presence of closely eluting or spectrally similar species. However, MBFC can introduce complexity, particularly when configuring methods to detect the correct ion species and interpreting results involving multiple targets.

This application note demonstrates how the InfinityLab Pro iQ Plus single quadrupole mass detector, added to a 1290 Infinity II Preparative LC system controlled by OpenLab CDS software, enables mass-based fraction collection of flavone glycosides from an elderberry extract. The system simplifies method setup and data review by automatically creating adducts, summary signals and reports while preserving the advantages of mass triggered fraction collection.

Experimental

Samples and solvents

An ethanolic natural product extract of Sambucus nigra (black elderberry) from Deutsche Homöopathie-Union was purchased at a local pharmacy. The samples were filtered through 15 mm 0.2 µm nylon syringe filters (part number 5190-5088) and refrigerated after opening.

The InfinityLab Acetonitrile (ACN) gradient grade for LC (part number 5191-5100) and trifluoro acetic acid (VWR, Bruchsal, Germany) were used as mobile phase B. InfinityLab ACN for LC/MS (part number 5191-5101) and LC/MS grade formic acid (part number G2453-85060) were used for the preparation of the MS makeup solvent. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Software and instrumentation

The experiments were conducted on a 1290 Infinity II Preparative LC/MSD system connected to Agilent OpenLab CDS version 2.8 update 08 with Feature Pack 2 (FP02) consisting of the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/ Fraction Collector (G7158B)
- Agilent 1260 Infinity III Diode Array Detector WR (G7115A) with 0.3 mm preparative flow cell (option #084)
- Agilent 1260 Infinity III Isocratic Pump (G7110B)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A) with delay coils for 4–8 mL/min (option #211)
- Agilent Pro iQ Plus LC/MS (G6170A)

Gradient and method parameters used throughout are summarized in Table 1 and Table 2.

Table 1. Chromatography method parameters 1290 Infinity II Preparative LC/MSD system.

Parameter	Value		
Column	Agilent Prep 100Å C18, 10 x 50 mm, 5 µm (part number 446905-802)		
Mobile Phase A	0.1% Trifluoro acetic acid in water		
Mobile Phase B	0.1% Trifluoro acetic acid in acetonitrile		
Gradient	Time [min]	%B	mL/min
	Start conditions	5	0
	0	5	7
	10	25	
	11	98	
	13	98	
	14	5	
Makeup Solvent	70/30 Water/ACN, 0.1% formic acid		
Makeup Fow	1.5 mL/min		
Flow Splitter	Split ratio 1500:1, dilution factor 1:300		
Injection Volume	700 µL		
UV Detector	270 nm, 4 nm bandwidth, reference 360 nm		

Table 2. Agilent Pro iQ Plus and fraction collection settings.

Parameter	Value
Ion Source	ESI
Polarity	Positive and negative
Drying Gas Temperature	300 °C
Gas Flow	13 L/min
Nebulizer Pressure	35 psi
Capillary Voltage	4,000 V
	Scan Settings Positive and Negative
Scan Range (m/z)	100–1000
Scan Time (ms)	143
Fragmentor Voltage	75 V
Gain	1
	Target Compound Settings (SIM per adduct)
Adducts	[M+H]+ , [M+Na]+, [M−H]−
Fragmentor Voltage	75 V
Gain	1
	Fraction Collection Settings
Detection Mode	Threshold
Threshold	10,000 cps
Peak Duration	No limit

Results and discussion

Sambucus nigra (black elderberry) extract is known to contain various flavonoids and glycosylated species. From a literature search and online databases, a list of flavone glycosides fractionation targets and sum formulas was compiled (Table 3). The fraction collection method was provided with the sum formulas of the targets, and the software automatically created a list of signals for the selected adducts to consider ([M+H]+ , [M+Na]+, [M−H]−). A more detailed overview of acquisition method settings, including target compound definition, trigger threshold setting, and sequence-based purification is provided in another publication[1].

In addition to the positive and negative scan, this amounted to a total of 15 target ions that were monitored during the separation. The new Pro iQ Plus with OpenLab CDS version 2.8 FP2 allows users to enter an unlimited number of target compounds. There is no absolute limit on the number of adducts that can be monitored however, dwell time and desired data acquisition rate determine a practical limit.

Table 3. Target compounds for mass-based fractionation of the elderberry extract.

Target Compound	Sum Formula	Monoisotopic Mass (Da)
Rutin (quercetin 3-rutinoside)	C27H30O16	610.2
Isoquercetin (quercetin 3-glucoside)	C21H20O12	464.1
Kaempferol 3-rutinoside	C27H30O15	594.2
Isorhamnetin 3-rutinoside	C28H32O16	624.2
Isorhamnetin 3-glucoside	C22H22O12	478.1

Figure 1 shows the resulting UV chromatogram of injecting the neat elderberry sample using the described method. Both the OpenLab CDS data acquisition and analysis applications automatically highlight the fractions on all available signals when a target was found and collected. The fraction result layout in the data analysis application shows tooltips, a summary table, and a visual representation of the fraction collector to help in identifying the location in the instrument. In this specific sample, all five target compounds were found by their respective adducts and five fractions were collected between retention time of 7 to 9 minutes.

For the elderberry sample the first fraction collected from 7.3 to 7.6 min of retention time coincided with the most abundant peak in the UV chromatogram and was labeled as a fraction triggered by both the rutin and isoquercetin target compounds. To provide an overview of the target compounds and their possibly many adducts the data analysis application presents total ion chromatogram (TIC) signals of all selected ion monitoring (SIM) traces for all observed adducts (TIC SIM). Figure 2 shows the TIC signals for all positive (Fig 2A) and negative adducts (Fig 2B) monitored during the run. Additionally, for every target compound, a TIC SIM signal is computed for both positive and negative adducts of the compound, a TIC SIM signal is computed for both positive and negative adducts of the respective compound (Fig 2C and 2D).

Figure 1. Fraction layout in OpenLab CDS data analysis. A) Chromatograms are highlighted with fraction collection regions. B) The fraction results table presents a tabular summary of the collected fractions and the triggering targets. C) The sample containers component highlights the sample vial used and the location of the collected fractions.

As shown in Figure 2D the main intensity for the peak at 7.5 min retention time were the adducts of the target compound rutin (610.2 Da). However, the isoquercetin target (464.1 Da) showed two peaks in the summed SIM signal (Figure 2 C) that was also present at 7.5min. A mass spectrum was extracted from the full scan positive data at 7.5 min as shown in Figure 3. It was found that the presence of a second peak for isoquercetin was likely a result of in-source fragmentation of rutin leading to a loss of a rhamnose sugar unit (−146 Da). This is a known fragmentation pathway for this compound[2], and a similar pattern was observed for the targets isorhamnetin-rutinoside and the respective glucoside. Because this fragmentation is an effect of the electrospray ionization it is of no consequence for the collected fractions.

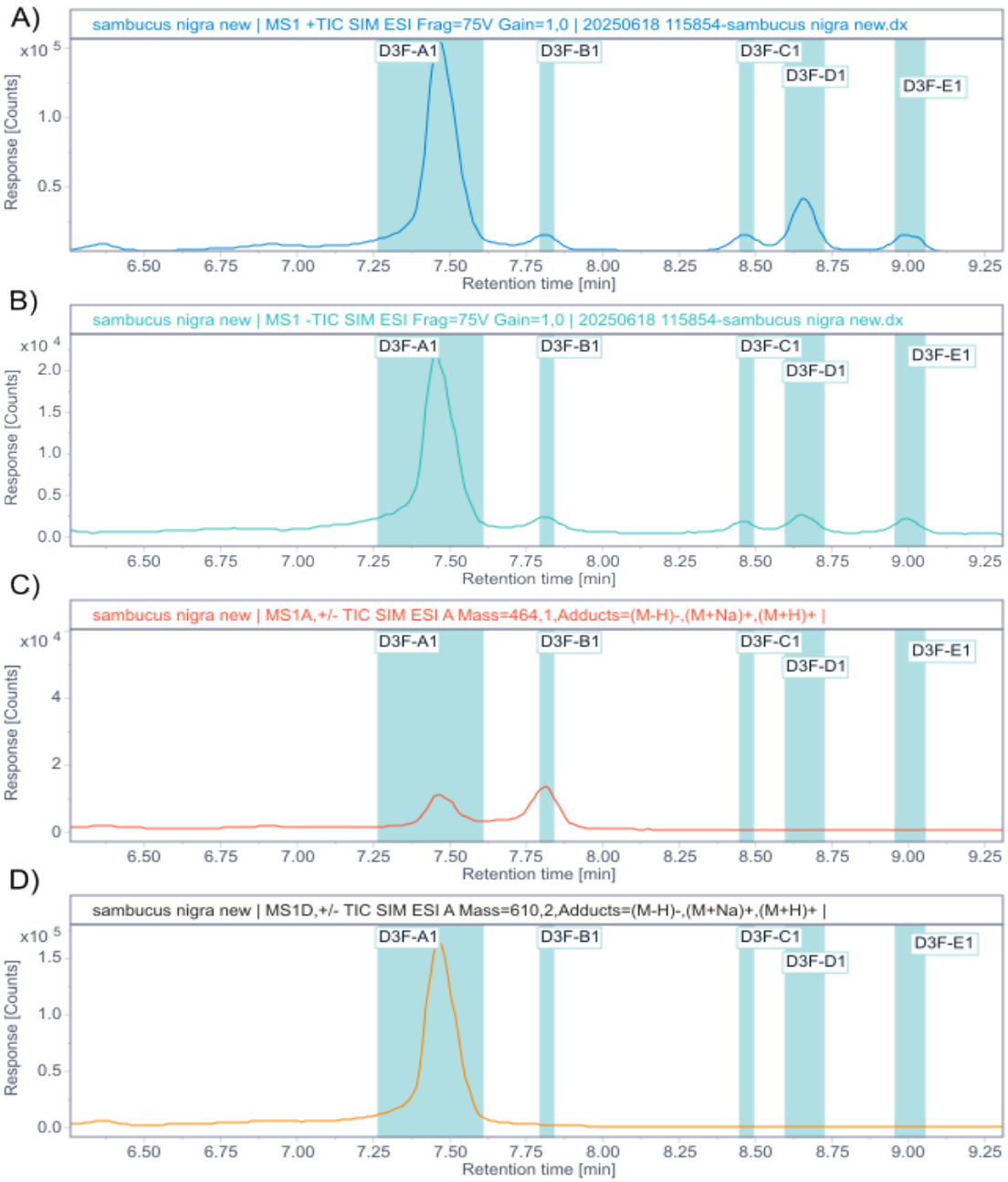

Figure 2. A) TIC SIM for all positive adducts. B) TIC SIM for negative adducts. C) TIC SIM for all positive and negative adducts of target isoquercetin D) TIC SIM for all adducts of rutin.

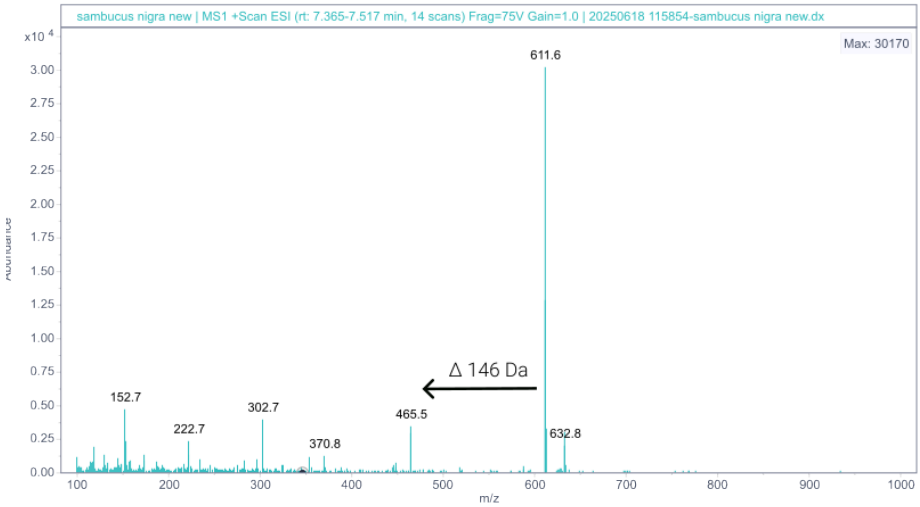

Figure 3. Mass spectrum of the region around the rutin main peak showing rutin main peak [M+H]+ and peak after loss of a rhamnose.

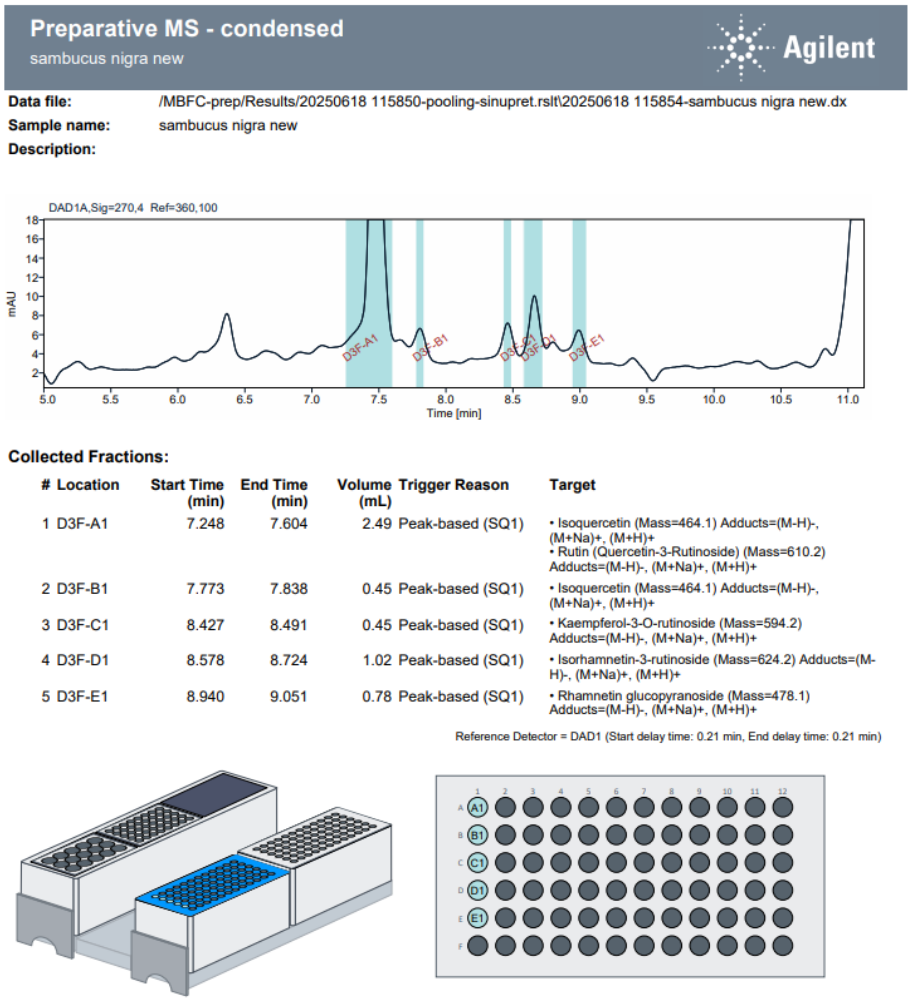

Figure 4. Customizable fraction collection report showing a close up of the UV signal highlighted with the fraction collection regions, fraction results table with all collected fractions, and rendering of the fraction collection vessel.

To conclude the analysis and fractionation of the sample, a condensed report was generated for the fractionation results. The report was customized from the existing template to show only key information alongside the UV chromatogram (Fig 4). For routine execution, the report template can be attached to a processing method to be automatically generated.

Conclusion

This application note illustrates how MBFC with InfinityLab Pro iQ Plus and OpenLab CDS simplifies compound isolation even in complex mixtures targeting multiple compounds. With automated adduct handling, customizable reporting, and no hard limits on monitored targets, the system is designed for versatility and ease of use. As demonstrated with the natural product extract, multiple targets with multiple adducts each can be monitored and reviewed with ease. This makes the solution ideal for a wide range of fractionation needs whether working with synthetic mixtures, impurities, or natural extracts, users benefit from streamlined workflows and high-confidence results.

References

1. Improve the Productivity of Purification Workflows. Agilent Technologies technical overview, publication number 5994-8532EN, **2025**.
2. Study of the Glycosylated Secondary Metabolites in Tea (Camellia sinensis L.) Using UHPLC/Q-TOF/MS, Agilent Technologies application note, publication number 5991-8066EN, **2017**.

To learn more about OpenLab CDS, visit:
www.agilent.com/chem/openlabcds

To learn more about InfinityLab Pro iQ Plus, visit:
www.agilent.com/lcms/pro-iq-series

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