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Mass-Based Fraction Collection (MBFC) for Scalable Purification in Pharma and Biopharma Applications

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Introduction

Preparative HPLC is widely applied for the separation and isolation of individual compounds from complex mixtures, including natural product extracts, synthetic intermediates, and process reaction mixtures. The increasing complexity of samples often results in numerous byproducts, making the selective isolation of target analytes more challenging. Mass detectors are frequently employed to enhance the selectivity of fraction collection.

The Agilent InfinityLab Pro iQ Series Mass Detectors[1] integrate with Agilent preparative HPLC systems to enable mass-based fraction collection with high selectivity and sensitivity. Target compounds can be defined by chemical formula, allowing precise selection of adducts and charge states, as well as the application of compound-specific trigger thresholds. In addition, single or multiple masses can be excluded from collection using advanced trigger combinations. To further increase flexibility in purification workflows, override parameters for target formulas and thresholds can be assigned on a sequence line basis. This feature, among others, is described in-depth in a technical overview[2].

This poster demonstrates the advantages of the InfinityLab Pro iQ Series Mass Detector in diverse preparative HPLC applications. Mass-based detection with fraction collection boosts selectivity and sensitivity, allowing precise isolation of target compounds. Formula-based targeting, mass exclusion, and customizable triggers offer robust, flexible solutions for diverse purification workflows.

Figure 1. 1290 Infinity II Preparative LC/MSD System.

Experimental

Instrument configuration

All experiments were conducted on an Agilent 1290 Infinity II Preparative LC/MSD system comprising the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/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 Preparative Column Compartment (G7163B)
- 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 InfinityLab Pro iQ Plus LC/MS (G6170A)

Column

Agilent Prep 100Å C18, 10 × 50 mm, 5 µm, preparative LC column (part number 446905-802)

Software

Agilent OpenLab CDS, version 2.8 FR2 or later

Solvents/chemicals

InfinityLab Acetonitrile (ACN) Gradient Grade for LC (part number 5191-5100*) was used as mobile phase B.
InfinityLab ACN for LC/MS (part number 5191-5101*) and LC/MS grade formic acid (part number 5191-4549) was 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).

* Only available in select countries.

Results and Discussion

Defining Target Compounds and Adducts

Mass spectrometric detectors differ from universal detectors such as refractive index detectors because signal intensity depends on molecular structure and ionization efficiency. Two compounds at the same concentration may produce very different responses: some form stable adducts (protonated, sodium, or potassium), while others undergo in-source fragmentation and lose functional groups. These behaviors are strongly influenced by the sample matrix, buffer composition, and solvent system.

Such variability makes it difficult to predict which ion species will appear, complicating both data interpretation and fraction collection. OpenLab CDS addresses this variability by allowing users to define a compound's molecular formula or exact mass and specify expected adducts (see Figure 2). The software calculates mass-to-charge ratios, generates SIM traces, and consolidates them into a single trigger. This ensures fraction collection occurs reliably—without the need to select only the most abundant adduct.

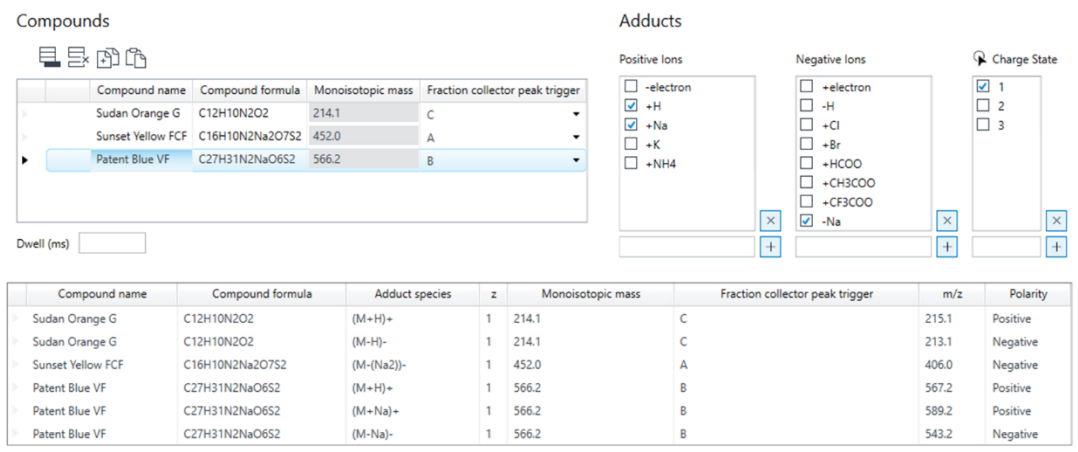

Figure 2. Target compounds windows of the Agilent Pro iQ acquisition method in Agilent OpenLab CDS.

Multiple Mass-based Triggers in OpenLab CDS

In this example, three dyes formed different adducts, resulting in signal intensities that varied by up to a factor of 1,000 (see Figure 3). By setting up three independent mass-based triggers with tailored thresholds, fraction collection was optimized for each dye without capturing baseline noise.

Thanks to this multi-trigger approach, all compounds were collected cleanly into separate fractions—something that would not have been possible with a single trigger setting. The method supports up to four individual trigger thresholds, enabling fine-tuned control for complex separations.

This strategy is particularly valuable when working with mixtures containing diverse compound classes (e.g., natural products) or when isolating a synthesis product alongside unreacted starting materials, ensuring selective and efficient fraction collection.

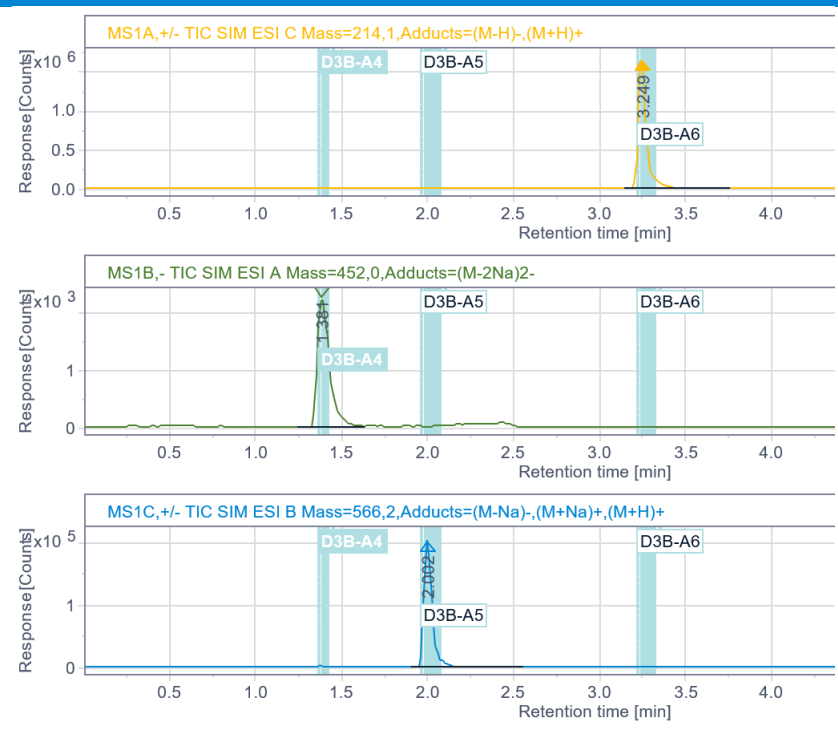

Figure 3. Summed SIM traces of three dyes collected with three different trigger settings.

Monitoring Multiple Charge States in Mass Detection

Large biomolecules such as peptides or oligonucleotides often form multiply charged ions, making it difficult to predict which charge state will dominate the mass spectrum.

The Pro iQ method in OpenLab CDS addresses this challenge by allowing multiple charge states to be defined for a single target. Users simply enter the molecular formula or monoisotopic mass, and the software automatically calculates the corresponding *m/z* values. These are used to generate SIM signals, which are then combined into one unified trigger for fraction collection. In this example, angiotensin I was monitored across charge states from +1 to +5 (see Figure 4). OpenLab CDS generated all necessary *m/z* traces, applied a trigger threshold of 10,000 cps, and enabled precise fraction collection under standard LC conditions.

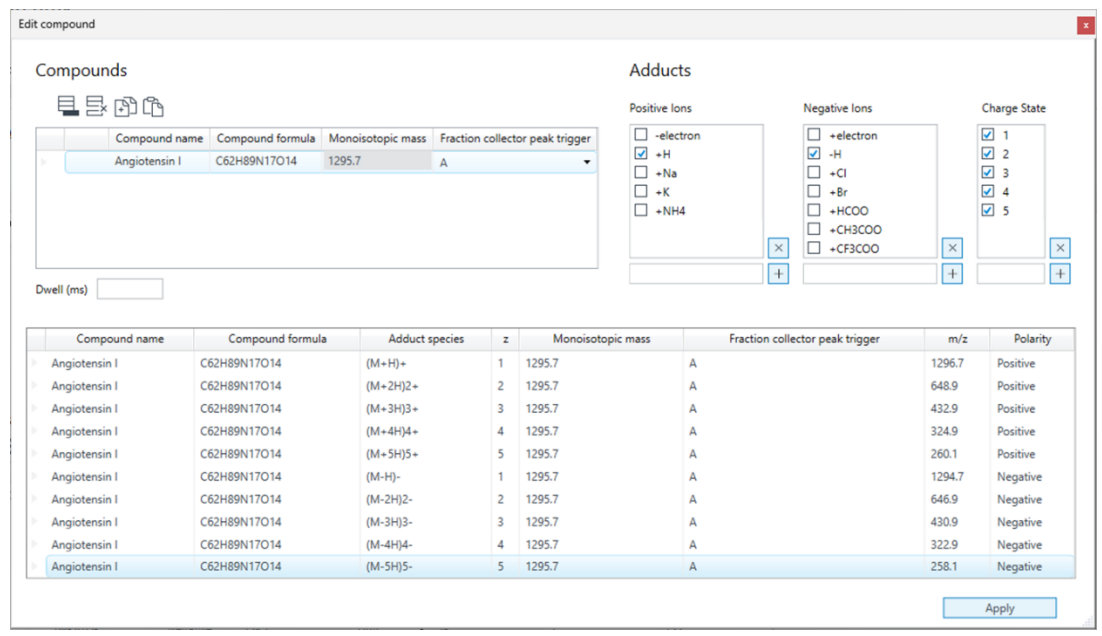

Figure 4. Target compounds window of the Agilent Pro iQ method in Agilent OpenLab CDS showing a peptide as target with +H/−H adducts and charge states of one through five. Note the automatic calculation of the m/z traces based on the monoisotopic mass and charges.

Results and Discussion

This approach ensures reliable purification of multiply charged species—without the need to choose a single ion in advance. To illustrate this feature, a crude sample of angiotensin I was separated and purified using the mass detector settings depicted in Figure 4

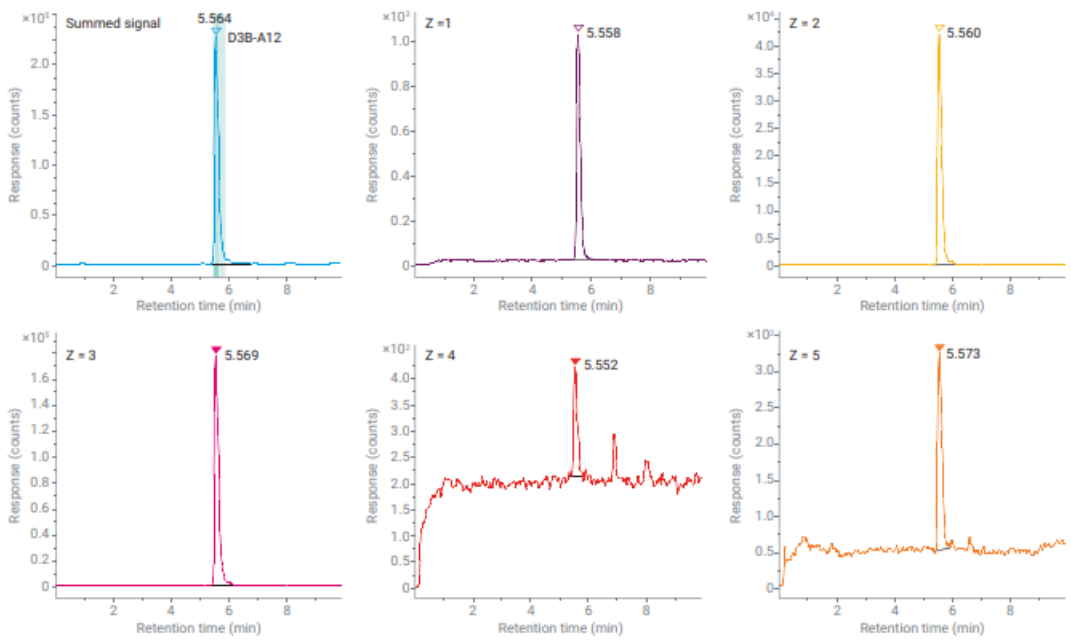

Figure 5. Summed and single SIM traces of angiotensin I monitored as protonated species with different charges (Z = 1 to 5). Note the different Y-axis scales

The different scales of the y-axes show clearly in this fractionation of angiotensin, Z = 2 and 3 were the main contributors to the summed signal that triggered fraction collection by a threshold of 10,000 cps. These results underline the gain in confidence of successful fraction collection when the new software features are used with the Pro iQ Mass Detector.

Excluding Unwanted Masses From Collection

Some samples contain unwanted impurities that cannot be completely separated from target peaks using chromatography. In these cases, the targets can be singled out using a mass detector. To avoid an impurity peak tailing into the target, OpenLab CDS can exclude the impurity target mass (by Boolean NOT logic) and pause fraction collection when an impurity is above a set threshold.

Figure 5 shows the UV and SIM traces for a target and exclusion mass. The threshold for the target caffeine is exceeded after 0.85 minutes, but no collection happens because the exclusion mass is also above its threshold. Only after the exclusion mass signal has fallen below the threshold will the collection start at 0.94 minutes. Re-analyzing the fraction for purity showed an increase from 93% to > 99% when the impurity was excluded[2].

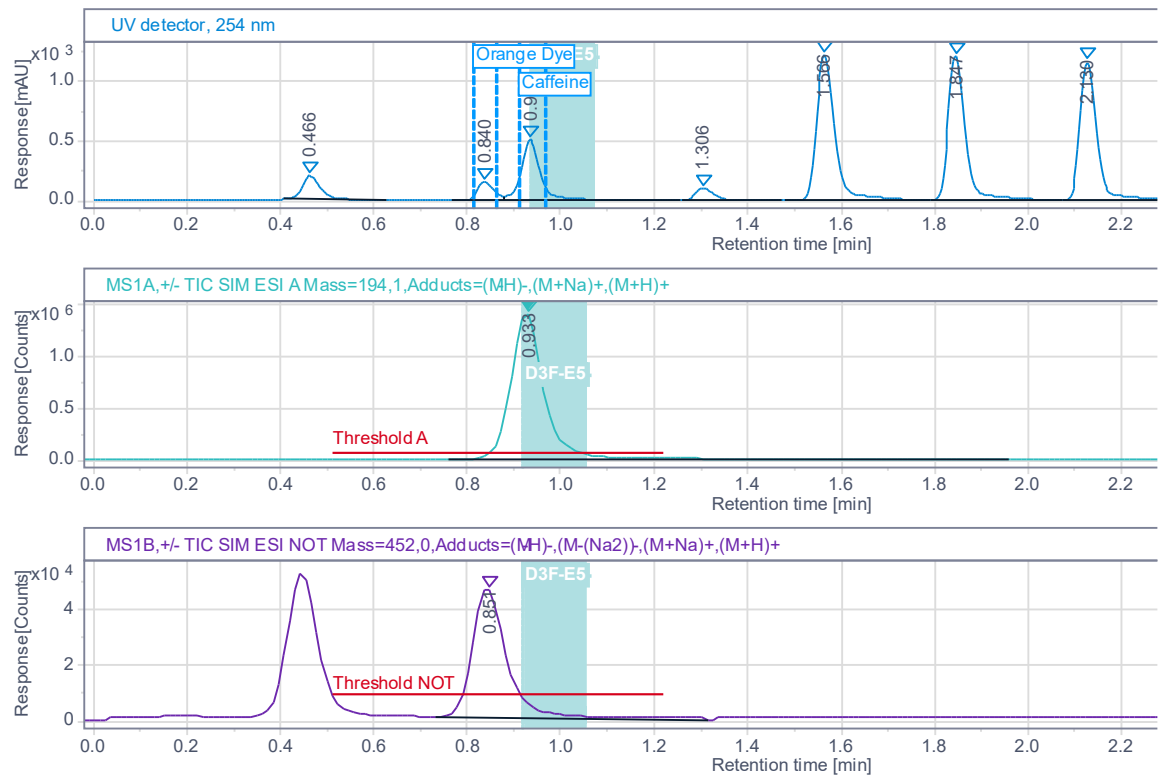

Figure 6. Using the exclusion trigger (Boolean NOT logic) to avoid contamination of the caffeine fraction (turquoise) by the impurity (purple trace). The collection is delayed until the NOT signal is below the threshold.

Conclusions

- Mass-based fraction collection in Agilent OpenLab CDS is tailored for purification workflows.
- By defining target compounds with formulas, adducts, and charge states, setup becomes easier, and errors are reduced.
- Four mass-based triggers, including a NOT logic to exclude impurities, deliver higher purity outcomes.
- Flexible override options for target mass, trigger threshold, and more enable fine-tuning at the sample level.
- Combined with the Agilent InfinityLab Pro iQ Series Mass Detector, these features significantly boost purification productivity.

References

1.Harness the Power of Pro. *Agilent Technologies brochure*, publication number 5994-8330EN, **2025**.

2.Improve the Productivity of Purification Workflows. *Agilent Technologies application note*, publication number 5994-8532EN, **2025**

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