Advantages of Mass-based Fraction Collection

Application Note

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Abstract

The Agilent 1200 Series purification system[1], in combination with the Agilent 6100 Single Quadrupole LC/MS[2], is an optimal, highly automated system for time, peak and mass-based fraction collection.
In this Application Note the advantages of mass based fraction collection relative to time and peak based fraction collection are briefly described.

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Introduction

In modern drug discovery large numbers of highly pure compounds are required to assure reliable results in primary activity screening. The only purification technique that can be automated, is fast and leads to excellent sample purities, is preparative HPLC. Modern, automated purification systems offer various ways to collect fractions. The fraction collector can usually be triggered manually by a user; or fractions can be collected based on retention time windows or on signals from UV or other detectors, or on a trigger mass using a single quadrupole MSD. In this Application Note, the advantages of mass-based fraction collection are briefly explained.

Results and discussion

Manual fraction collection

Manual fraction collection allows the user to collect only the fractions that should specifically be triggered. Since this method requires direct human interference, it is the least automated way of collecting fractions, and is therefore not often used.

Time-based fraction collection

Fraction collection based on retention time windows is usually only used to pre-fractionate complex samples, for example, natural product extracts[3]. This technique is not often used in drug discovery, since a single peak can be cut into two fractions, and the overall number of collected fractions is very high (figure 1).

Peak-based fraction collection

Peak-based fraction collection using a UV detector signal[4], for example, is more targeted and produces fewer fractions than time-based fraction collection. However, several fractions are usually collected per sample, and each fraction must be analyzed on an analytical system with an MSD, to identify the fraction containing the compound of interest. The advantage of peak-based fraction collection using a UV signal is the high purity of the resulting fractions[5] (figure 2).

Mass-based fraction collection

Mass-based fraction collection is only possible if the mass of the target compound is known to the user. Depending on the mobile phase used, different adducts, for example, $[M+H]^+$ or $[M+Na]^+$ lead to a trigger mass on which the MSD triggers the fraction collector. Because of the high specificity of the MS, very often only one

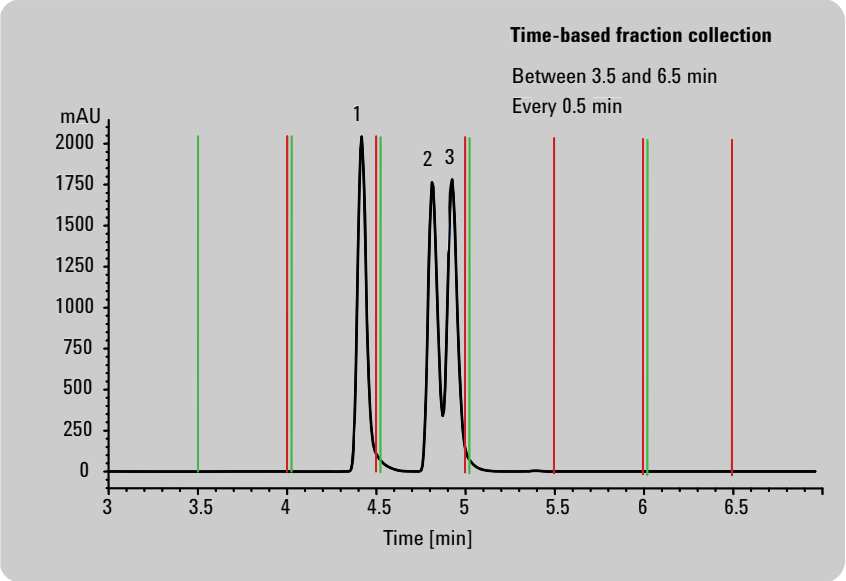

Figure 1
Result of time-based fraction collection, retention time windows of 0.5 min between 3.5 and 6 minutes. Six fractions were collected. Peaks 2 and 3 were collected in the same fraction.

fraction per sample is collected. However, the presence of isomers or other compounds, which coincidently have the same mass, can lead to a higher number of fractions[6]. Even if the system configuration is optimized[7], the purity of the collected fraction is usually slightly lower than peak-based fraction collection (figure 3).

Conclusion

The advantages and disadvantages of mass-based fraction collection can be summarized as follows:

Advantages:

- Small number of fractions per sample, often only one fraction per sample.
- Only target compound collected (or compounds with the same mass).
- No need to analyze fractions to determine which one contains the compound of interest.

Disadvantages:

- Target mass must be known
- Purity of fractions slightly lower than for UV based collection.

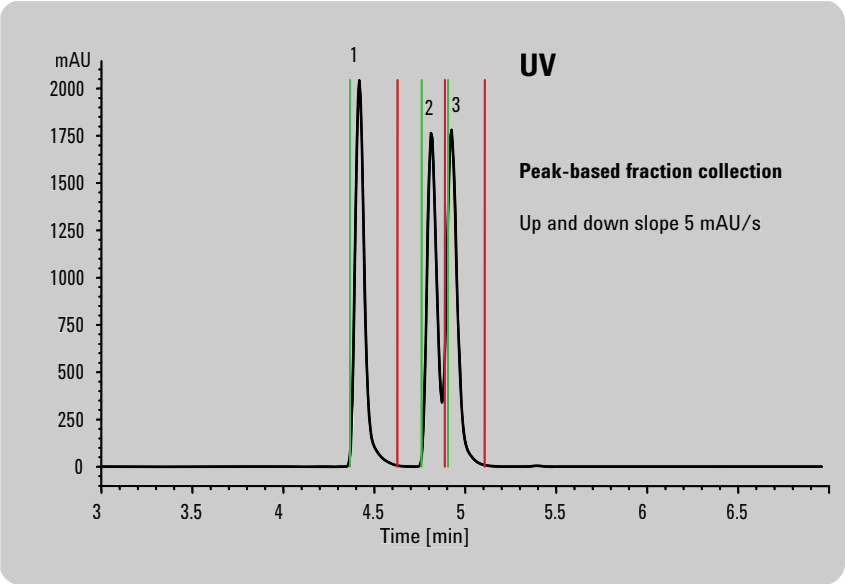

Figure 2
Result of peak-based fraction collection, up and down slope of 5 mAU/s. Three fractions were collected, purity of compound 2: 98 %.

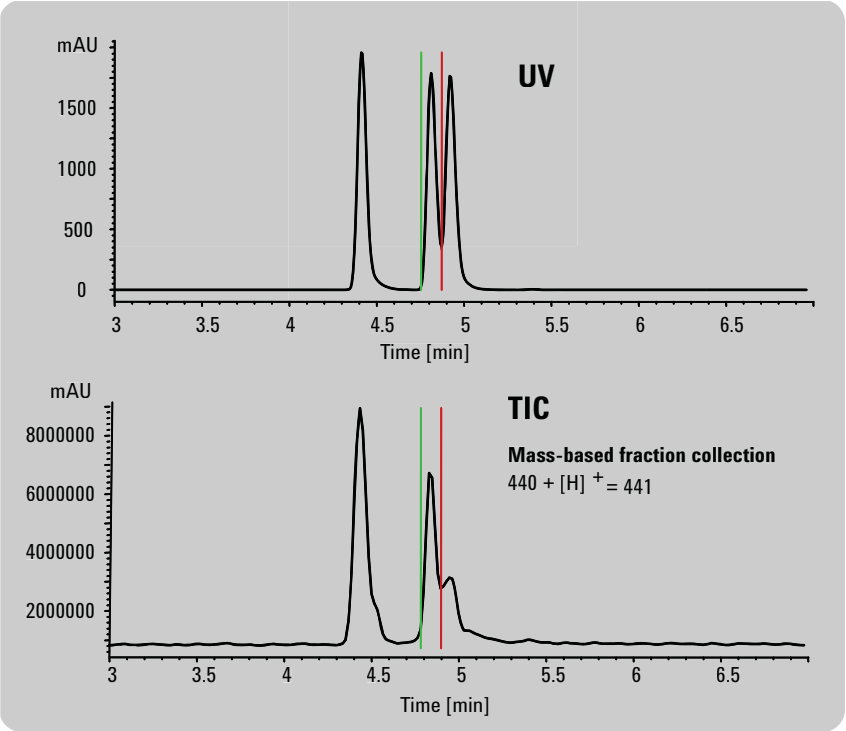

Figure 3
Result of mass based fraction collection, target mass 440, adduct [H]+ equals trigger mass of 441. One fraction was collected, purity of compound 2: 93 %.

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Published December 1, 2006
Publication Number 5989-5937EN

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