Mass Detection for Chromatographers: How New Technology Is Changing the Way We Think About Mass Spectrometry and Its Use In Routine Workflows

by Dr Stephanie N. Harden, Waters Corporation, Baden-Dättwil, Switzerland
stephanie_harden@waters.com

Under ever-increasing demands to improve efficiency and reduce costs, the implementation of rigid processes, standardisation and high throughput workflows have made a significant difference to the way quality control (QC) laboratories operate. The perceived, and sometimes real, challenges associated with the introduction of relatively complex analytical technology, like mass spectrometry, into such an environment have meant that the benefits of such a change have been realised only by a limited few. With recent advances in detector technology making mass information more accessible to chromatographers, however, innumerable applications for this kind of technology are emerging.

In this article we discuss the feasibility of introducing mass detection into routine QC workflows and describe the application of the technology to the analysis of small molecules and biomolecules.

Addressing the Unmet Analytical Need

Historically, UV detection has been favoured in many laboratories for its ease of use, robustness, and reliability. However, some of the inherent challenges include analytes that do not have a response in a UV channel, coelutions, and unknowns, any of which can require an orthogonal approach such as mass detection. When used in tandem with UV detection, mass detection undoubtedly does offer benefits, particularly in the routine QC environment, but implementation of the technology has traditionally been viewed as challenging. Instrumentation is typically highly sophisticated, relatively expensive and requires trained operators and regular maintenance to maximise uptime. Moreover, where processes and practices have been in place for many years within an organisation, and the results are acceptable, then there is little impetus to change.

So why change? In fact, there are various reasons, relating for example to the need to address specific analytical challenges where optical detection is non-responsive, or where there is a need for greater selectivity than optical detection can provide. There may be a need for greater confidence in results, which can be provided by the use of an orthogonal detection technique, or a requirement to adhere to increasingly low limits of detection for specific classes of components. At the same time, significant and continued developments in technology, such as improvements in ease of use, lower running costs and lower power consumption, are now providing better return on investment in comparison to earlier generation mass spectrometers.

Figure 1. Empower 3 CDS software provides a simple view of mass and UV chromatograms and spectra, enabling fast peak identification and determination of peak homogeneity.

Considerations for Introducing Mass Spectrometry into the QC Environment

In many quality control laboratories, the standard platform for chromatographic separations is HPLC, however, the use of UHPLC and UPLC is becoming more widespread. Rapid, high resolution separations are enabling better asset utilisation and increased throughput without jeopardising the quality of separations. However, with chromatographic peak

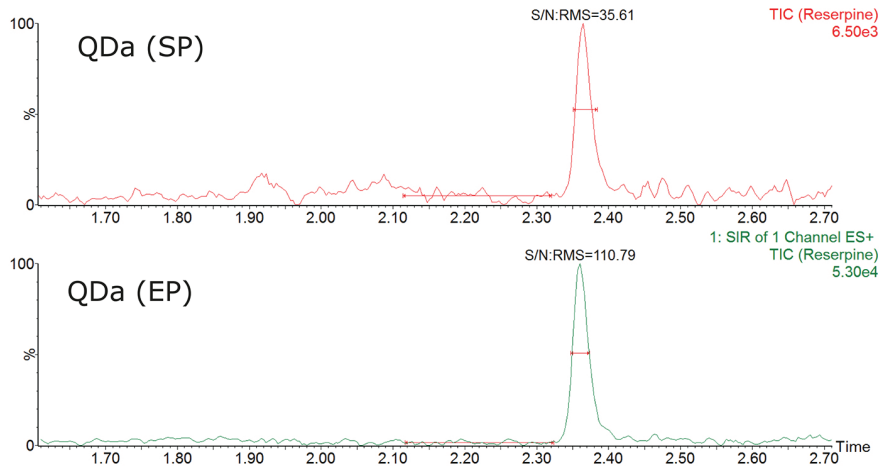

Figure 2. Sensitivity comparison for 1pg Reserpine on column, measured on the SP and EP versions in combination with an ACQUITY H-Class system. A 5µL injection of 200 fg/µL Reserpine gave 1pg on column, with a flow rate of 0.4 mL/min. The ACQUITY QDa was operated in SIR mode. Chromatographic peak width was approximately 1.4 seconds (FWHM).

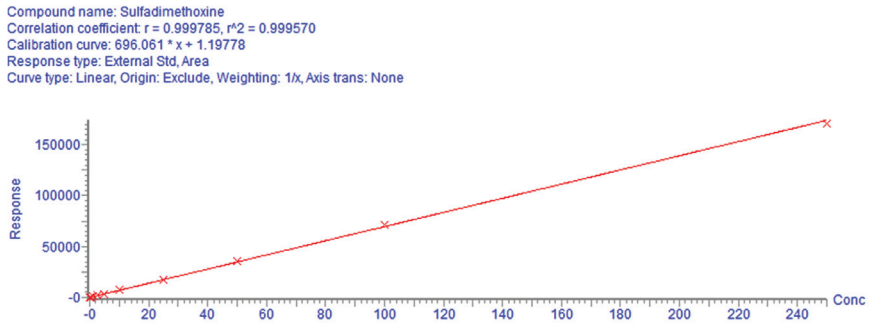

Figure 3. ACQUITY QDa Detector Performance linearity over 4 orders of magnitude (0.025 pg/µL to 250 pg/uL). Calibration points were run in duplicate with all individual replicates within ±10% of the nominal concentration (±15% at LOQ).

widths in the range of 1-3 seconds, older generation quadrupole mass spectrometers lack the appropriate scan rate to maintain the 15-20 data points across the chromatographic peak, which are required for accurate and reliable quantification. Therefore, for mass data to be considered appropriate for use with rapid chromatography in the productivity-driven QC laboratory, data acquisition rate must be sufficiently high to accommodate narrow peak widths.

For QC labs to consider adopting mass detection, attention also needs to be given to ease and degree of maintenance, up-time and reliability, running costs, availability of bench space, as well as to the ease with which accurate and repeatable results are obtained. Transferability of technology is also important, whether to an adjacent laboratory, for use by another operator, or when implemented at facilities in other geographies: a fundamental requirement is to ensure that consistent results can be obtained.

Finally, it is good practice to avoid phosphate buffers or high salts (e.g. sodium and potassium salts) in combination with mass detection as even trace levels of these interfere with the ionisation process and can cause blockages. Where existing QC methods use non-volatile buffers, and mass information is required, an increasingly popular solution is to configure a 2D chromatographic system in combination with a mass detector [1]. In this way, a selected peak from a USP chromatographic method can be directly transferred to the mass detector without the need for fraction collection for redevelopment of the USP method. Configuring the system for at-column dilution would further ensure the quality and repeatability of the mass spectral data collected from the transferred peak.

A New Generation of Mass Detectors for the Chromatographer

Recently, we have seen the introduction of a new wave of more compact mass detectors [2] developed to bring the benefits of mass analysis to chromatographers using analytical or purification LC and SFC systems. One example, the ACQUITY QDa Detector has been specifically designed to lower the barrier for adoption of MS technology into a variety of disciplines and across all phases in product development and QC. This new generation of detectors has the performance characteristics that rival conventional instruments, but a footprint similar to a photodiode array (PDA) detector. The ACQUITY QDa system, in particular, is designed to be integrated within a chromatographic stack and for the QC laboratory, where space is often a limiting factor, this can be a major consideration.

Significant attention has been paid to reducing the complexity of instrument operation, including the design of the electrospray ion source to ensure that even novice users can generate reproducible data of high quality. The ion source has a fixed, optimised geometry, and fixed, optimised gas flows. The design enables a novice user to obtain a good result for a wide variety of components of different chemical nature, without having to change the tuning parameters.

A unique feature of the ACQUITY QDa Detector is the automated, invisible calibration, which runs upon start-up. The calibration runs provide the user with confidence that the data is accurate and precise, with a full, automated calibration and resolution check completing in approximately 20 minutes. The standardisation of the tuning parameters together with the pre-programmed calibration checks facilitate the integration of the system into standard operating procedures and ensure consistency between different operators and different laboratories.

The pre-optimised positioning of the instrument's electrospray capillary also provides a good balance between sensitivity and long-term robustness, thereby minimising the requirement for routine cleaning of parts. Where the emphasis is on throughput and up-time, operators should

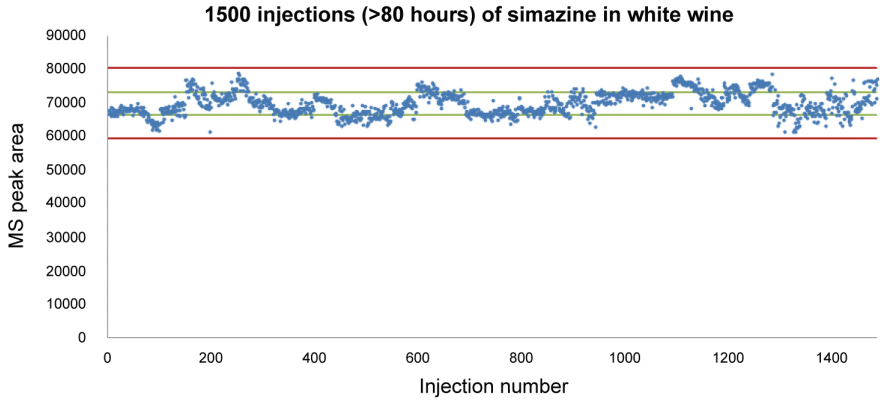

Figure 4. Peak area for 1500 injections of simazine spiked into white wine (red lines indicate 15%, green lines 5% from the mean).

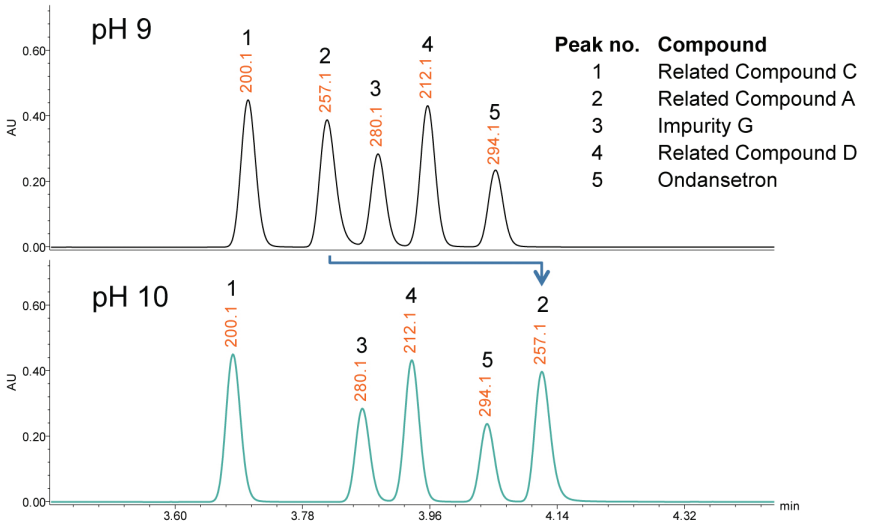

Figure 5. Effect of mobile phase pH on the separation of ondansetron (peak 5) and related impurities, analysed with the ACQUITY UPLC H-Class with PDA and QDa detectors, a CORTECS C_{18+} column and Empower 3 FR2 software. In this example a change of 1 pH unit resulted in a shift of 0.4 minutes for peak 2. The nominal mass of each component is populated at the top of the chromatographic peak, facilitating peak tracking.

not be burdened with time-consuming instrument maintenance, and with this in mind, the sample aperture on the ACQUITY QDa is now designed as a consumable item.

Data Review

Reviewing results from qualitative or quantitative studies that utilise a mass detector should also not be an obstacle to realising the benefits of mass information. Within the most recent release of Empower 3 chromatography data system (CDS) software, data review of LC/UV projects is made using a single window for reviewing mass and UV data simultaneously (Figure 1). The lower part of the window displays both the UV and mass data, including the UV chromatogram, a total ion chromatogram (TIC) and an extracted ion chromatogram (XIC) for the separation. The XIC is an overlay of multiple components extracted from the TIC. This ability to view extracted ions related to specific components allows the ACQUITY QDa to be used as a highly selective detector, displaying only the masses or components of interest. In the UV chromatogram, integrated peaks are automatically labelled with the corresponding mass of the component, which is important for fast, simple data review.

The top part of the window in Figure 1 displays the UV and MS spectra for specific components. This combination of both UV and MS information can be a significant advantage in making a rapid, sound assessment with regard to spectral purity of components. This graphical user interface (GUI) streamlines the review of both UV and MS data and serves to facilitate the fast adoption of the technology.

Sensitivity

In order to address different analytical needs, two variants of the mass detector have been developed, a Standard Performance (SP) version and an Enhanced Performance (EP) version. To demonstrate the high level of sensitivity achievable on the EP version, and to make a comparison with the SP version, a standard Reserpine sensitivity test was performed on both (Figure 2). The SP version requires only a small vacuum pump, which is fitted to the back of the module, so where this version meets the specific needs of the laboratory in terms of detection limits, further reductions are made in terms of required laboratory space and noise compared with earlier generation mass spectrometers.

Linearity

Where the ACQUITY QDa is used in tandem with an optical UV detector, UV data usually remains the primary quantitative data source. MS data is used either to confirm the UV quantification data or to supplement the overall dynamic range (where the MS is more sensitive and the concentrations are below the LOQ of the UV detector). In cases where the objective is to quantify very low abundance components relative to very high abundance components in the same analytical run, e.g. in the case of a pharmaceutical impurity in the presence of an abundant API, the mass detector offers a wider linear dynamic range. Figure 3 illustrates the linearity of response over 4 orders of magnitude achievable with the ACQUITY QDa.

Robustness

To highlight the high level of robustness of the system, facilitated in part by the new sample cone design, a long-term study of repeated injections has been performed. In Figure 4, a solution of white wine has been spiked with Simazine and diluted to the equivalent of the maximum residual level (MRL) of 10pg/µL. Wine is a particularly challenging matrix due to the high sugar content that can rapidly coat electrode surfaces and result in charging effects. Despite this, the mass detector proved robust, acquiring data for greater than 80 hours and over 1500 injections. No single acquisition was outside 15% of the mean value and the overall relative standard deviation (RSD) was 4.8%, with no obvious drift associated with the detection technology over this period.

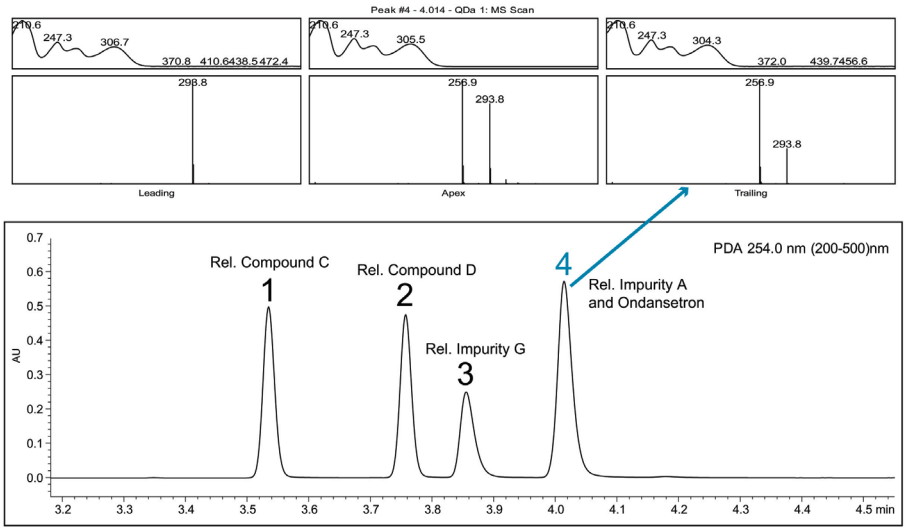

Figure 6. Peak homogeneity determination for an API and related impurities.

Power Consumption

Organisations are now paying increasingly more attention to energy consumption and efficiency. Due to the compact nature of this system through miniaturisation of many of its components, the ACQUITY QDa boasts an 85% reduction in power consumption over previous generation single quadrupole systems, resulting in significant savings in electricity usage and laboratory cooling costs. The push-button design of the system and non-prohibitive pump down time enable users to switch the ACQUITY QDa off when not in use. Furthermore, maintenance costs are lower as the system has fewer serviceable parts than previous generation systems.

Streamlining Analytical Workflows and Enabling Faster Decisions

For the analytical chemist in late pharmaceutical development and QC, chromatographic method development, method transfer and method validation can take up a considerable amount of time. However, systematic workflows [3] with enabling software tools [4] can serve to reduce some of the manual tasks involved and release analysts to carry out other activities, thereby improving productivity.

Whether optimising methods or troubleshooting out-of-specification results, the addition of a mass detector to a chromatographic system with UV detection provides useful orthogonal data, not only giving greater assurance that all of the components in a sample are detected and resolved, but also facilitating chromatographic peak tracking. Figure 5 shows the effect of small changes in mobile phase pH during the optimisation of a method for ondansetron and related impurities [3]. Even a change of 1 pH unit changes the retentivity and selectivity for this class of compounds. However, since each chromatographic peak is labelled with the mass (m/z) of the component, the shifting peaks can immediately be identified. In this example prior knowledge of the pK_as would allow the separation scientist to ensure that the mobile phase pH was prepared away from the pK_as of the compounds under investigation, however for many separations where multiple pK_as exist it may require some scouting of mobile phase compositions to find an optimum pH. The generation of mass information alongside the UV data helps to significantly speed up decision-making during the sometimes highly complex method development process and eliminates the need to check analyte retention times using individual standards.

The complimentary nature of UV and mass information is also clear when confirming peak homogeneity. The LC/UV chromatogram in Figure 6 shows the separation of a small molecule active pharmaceutical ingredient (API) and related impurities. The UV peak purity plots for the leading edge, apex and trailing edge of the peak for component number 4 are indicative of a spectrally pure peak. However, when the mass spectra across the chromatographic peak are examined, the changes in distribution of the ions present show that at least two components are co-eluting.

Strengthening Process Control and Quality Assurance in Biotherapeutic Production

In addition to the analysis of small molecules, the ACQUITY QDa can be used as an orthogonal detection technique for the analysis of biotherapeutics, such as peptides and released glycans. Mobile phases most often used in routine peptide experiments are amenable with mass analysis and importantly, the detection range of the system (30 to 1250 m/z) permits the analysis of multiply charged peptides [5].

A very recent development in labelling reagents for the analysis of released N-glycans has made analysis by mass detection even more interesting for these components [6]. Analysis using conventional labelling techniques based on 2-aminobenzamide (2-AB) results in a mixture of released glycans that are readily detected by fluorescence, but are challenging to detect using an electrospray ionisation mass analyser due to ion suppression effects. The development of the new reagent, RapiFluor-MS, for the labelling of released N-glycans has resulted in at least a 2-fold improvement by fluorescence detection and more notably, up to 100-fold signal increase by MS. Additionally, the RapiFluor-MS label results in the generation of ions with higher charge states (typically doubly-charged ions), compared with the ions resulting from glycans labelled with 2-AB (typically singly-charged ions).

With the increase in MS signal for these labelled components and the bias towards the generation of doubly-charged ions afforded by this approach, the addition of a compact mass detector to a chromatography system for glycan profiling is now a very attractive solution for improving monitoring capabilities in the production environment with minimal cost and effort. Furthermore, this new labelling technology reduces sample preparation time from a day to less than one hour, offering the opportunity to completely transform glycan analysis workflows [6].

Conclusions

As advances in technology continue to be made in the field of mass spectrometry, an open minded attitude will help organisations to fully realise the benefits that the next generation of mass detectors can bring in terms of improved productivity and quality. Indeed, with mass information now considered as easy to generate as PDA

data and with the barriers to adoption considerably lower than in former times, there is now an opportunity to significantly reduce the costs associated with poor quality, challenging detection limits, out-of-specification results, lengthy delays in decision making, etc. Moving forward, greater confidence in results, higher quality data, faster, more informed decisions and more efficient workflows will be key drivers for success.

References

1. Thomas E. Wheat, Daniel S. Root, Aparna Chavali, Patricia McConville (2014) Using Mass Spectral Detection with USP Methods. Poster presented at HPLC, New Orleans, Louisiana, 11-15 May 2015. Source: www.waters.com, library number: PSTR134799558

2. Daniel Kenny, John Pugh, Dave Gordon, Richard Moulds, Kate Whyatt, Marcus Dawber, Ian Trivett, Howard Read (2014) Design and Performance of a Highly Compact Single Quadrupole Mass Spectrometer. Poster presented at ASMS, 15-19 June 2014. Source: www.waters.com, library number PSTR134802287.

3. Paula Hong and Patricia McConville (2015) White paper: A Complete Solution to Perform a Systematic Screening Protocol for LC Method Development. Source: www.waters.com, library number LITR134836562.

4. Margaret Maziarz, Mark Wrona, and Sean M. McCarthy (2014) Increasing Efficiency of Method Validation for Metoclopramide HCl and Related Substances with Empower 3 MVM Software. Source: www.waters.com, library number APNT134808554.

5. Robert E. Birdsall, Eoin Cosgrave, Sean M. McCarthy, Joe Fredette, Scott Berger and Weibin Chen (2015) Adding Cost Effective Mass Detection as an Orthogonal Technique for Improved Productivity and Confidence in the Analysis of Protein Biotherapeutics. Poster presented at WCBP Washington DC, 27-29 January 2015. Source: www.waters.com, library number PSTR134829560.

6. Eoin FJ Cosgrave, Matthew A Lauber, Stephan M Koza, and Sean M McCarthy (2015) Routine Monitoring of N-Glycans using a Novel Labeling Reagent with Fluorescence and Mass Detection. Poster presented at WCBP Washington DC, 27-29 January 2015. Source: www.waters.com, library number PSTR134829579.