SEAMLESS INTEGRATION OF MASS DETECTION INTO THE UV CHROMATOGRAPHIC WORKFLOW

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Historically UV detection has been favored 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. While mass detection offers a number of benefits when used in tandem with UV detection, incorporating it into an existing UV workflow can be time-consuming and laborious. Advances in both instrumentation and software now make mass detection a technique that is much more accessible to chromatographers. In this white paper, we describe the features of the ACQUITY® QDa® Detector and Empower® 3 Software that have been purposefully designed to enable analysts to readily incorporate mass detection within a UV chromatographic workflow.

INTRODUCTION

A liquid chromatography (LC) system that includes an ultraviolet (UV) detector serves as the chromatographer's traditional method development tool. Incorporating mass detection capabilities enhances the analytical range and efficacy of such systems. Specifically, it provides the chromatographer with additional analytical capabilities, including the detection of analytes that give no UV response; identifying non-homogeneous or coeluting peaks; providing information to help identify a novel or unknown sample component; and tracking peaks.

In the past, setting up and verifying the performance of quadrupole instruments before acquiring mass data required significant expertise. This process includes multiple steps such as calibration and tuning the detector for a specific analyte and chromatographic conditions. Consequently, for analysts new to mass spectrometry, such a process can present a significant challenge.

In recent years, instrument developers have invested heavily in improving the reliability and ease of use of mass spectrometry hardware, and many of these improvements have lead to increased system stability and more robust equipment. Automation of the hardware and acquisition software has also been an area of focus. These technological advances have given rise to mass detectors that are as intuitive to operate as UV detectors, and capable of generating high-quality mass-spectral data.[1] This white paper will describe integrating one such mass detector, the ACQUITY QDa Detector, with chromatography data software (CDS), Empower 3 Software, into the UV chromatographic workflow.

Automated startup

Mass detection provides many benefits to UV liquid chromatographic separations, from providing an orthogonal mode of detection to streamlining method development. Yet mass spectrometers typically require a start-up procedure that is manual and can be time-consuming. For many instruments, part of that procedure involves operating a vacuum pump (or pumps) and establishing a flow of gas for many hours prior to operation of the instrument. In addition, before data acquisition can occur, calibration and resolution checks of the instrument should be performed, requiring the analyst to manually prepare samples and ensure enough calibrant for the process to complete. These procedures can require a significant amount of the analyst's time.

With the ACQUITY QDa Detector, however, startup is fully automated. Operating the system from a cold start requires simply turning it on. The instrument's automated startup tests include a source pressure test, critical read-back checks, and calibration and resolution verification – all occurring within minutes of startup. The calibration and resolution verification tests use an internal calibrant, which eliminates the need for separate sample preparation. If a calibration check is required, the analyst can select it from a drop-down menu. Once run, the calibration check automatically generates a report that evaluates the results and applies the new data as an updated calibration (Figure 1).

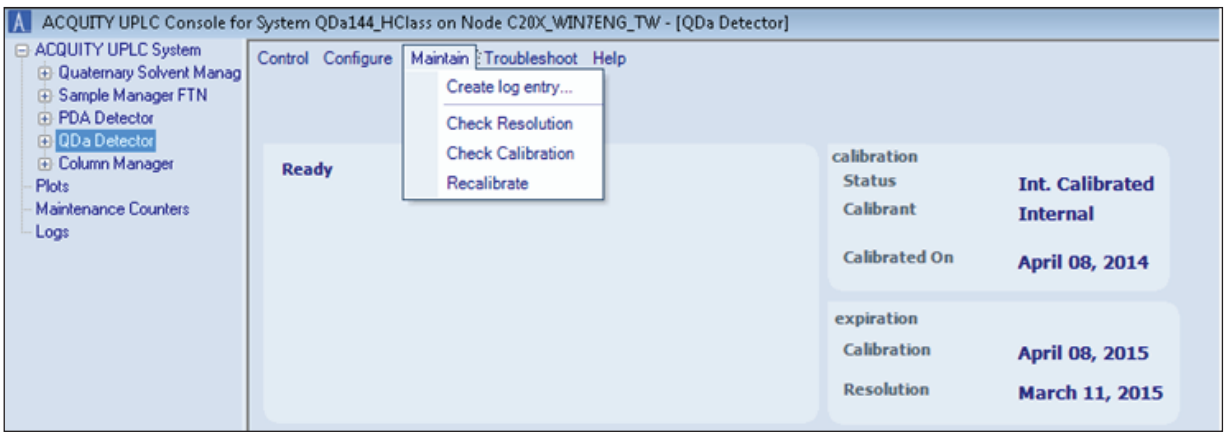


Figure 1. An internal calibrant in the mass detector enables automated calibration and resolution. This automation eliminates the need for manual sample preparation and provides added confidence to an analyst who is new to mass spectrometry.

Empower 3 Software simplifies adding mass detection to a chromatography project. For example, analysts wishing to collect mass detection create the appropriate system in the software, and select the appropriate detection techniques in the project. Like photodiode-array (PDA) detection, mass detection is included in the software as a project option and is incorporated into the instrument method (Figure 2).

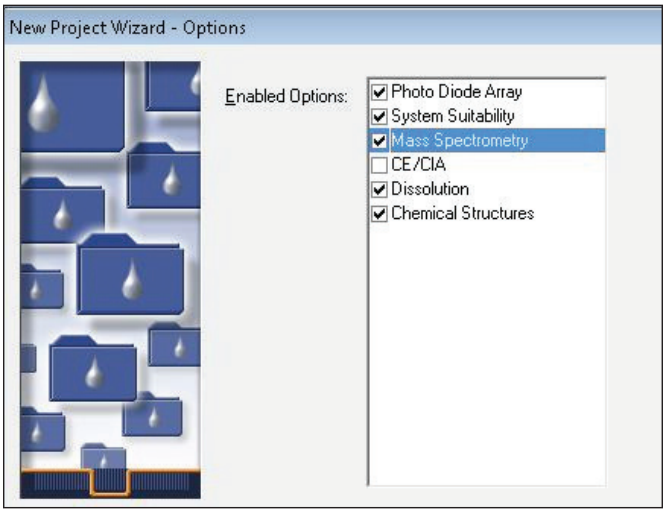


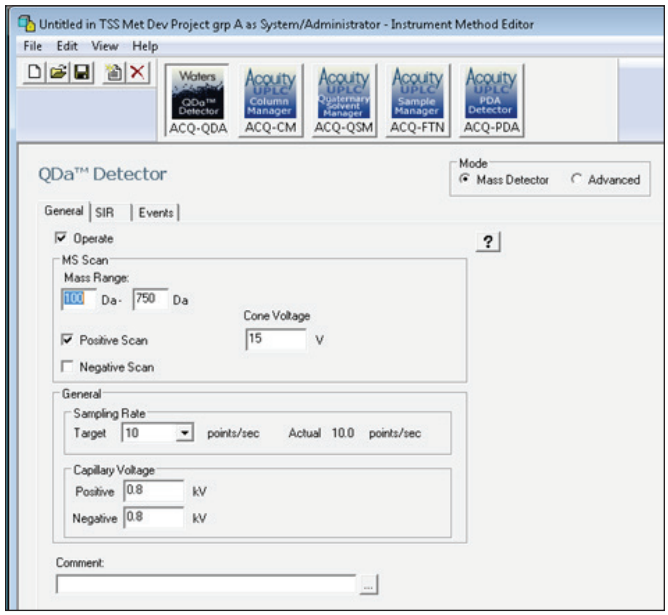
Figure 2. Empower 3 Software makes adding mass detection to any chromatographic project a straightforward operation. The switch-on capability in the CDS allows the chromatographer to select necessary workflows, processing capabilities, and detectors during project setup. These features can also be incorporated into existing project.

Acquiring mass spectral data with minimal adjustments

For MS quantification studies, optimizing the various MS detection parameters is often necessary. This process, which can include adjusting parameter settings that affect ionization, can prove time-consuming and challenging. If the parameters are not chosen carefully, the response of the analytes can be negatively affected.

With the advancements in mass detection hardware and software, the ACQUITY QDa Detector requires adjustment of far fewer parameters. Defining an instrument method requires selecting a function (full-scan or selected ion recording (SIR)) and a mass range or value (Figure 3). The default settings provide quality data for a range of compounds. If adjustment is necessary, a few key parameter settings, each relating to a specific chromatographic property, are adjustable. For example, the probe temperature relates mostly to flow rate and mobile phase composition. The capillary voltage relates mostly to flow rate. The cone voltage is truly sample-specific, and the data rate is defined by chromatographic-peak width and the "range" of the MS data to be acquired.

Full Scan



Selected Ion Recording (SIR)

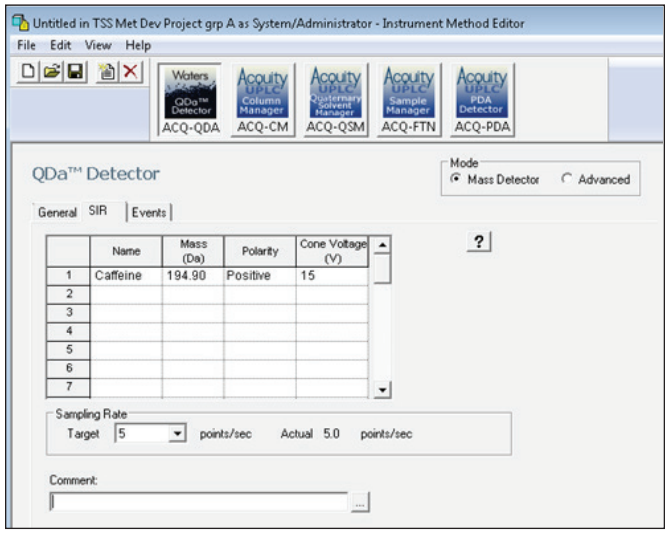


Figure 3. Defining a mass detector instrument method requires minimal input. The default parameters enable the analyst new to mass spectrometry to acquire quality data for a wide range of compounds.

Figure 4 shows the separation of flavonoids analyzed in both UV and full-scan, positive-electrospray ionization mode. The mass spectral data confirming peak identity were collected using the default detector settings.

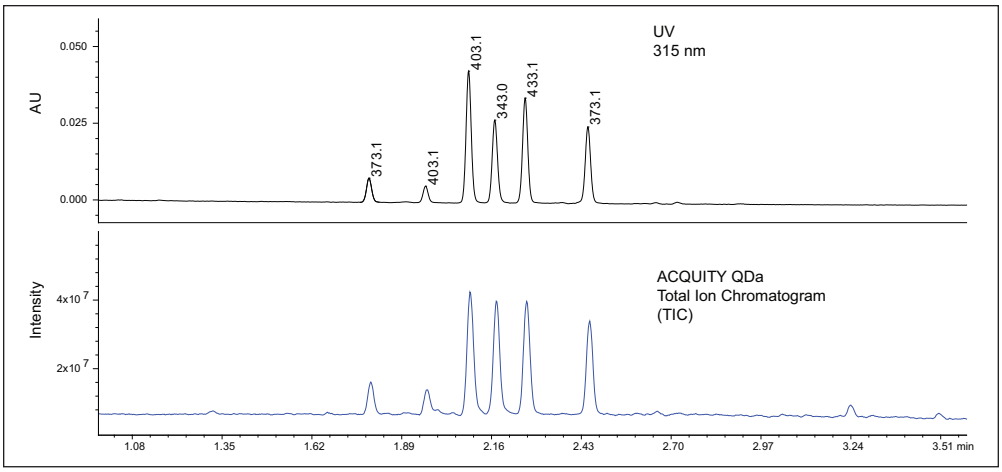


Figure 4. The default mass detector parameters provide reliable mass spectra, which can provide information to help identify a novel or unknown sample component, and to perform peak tracking. For example, in the analysis of orange extract by UV and mass detection, mass data allows for confirmation of the presence of flavonoids.

Comprehensive mass and UV spectral data review

Historically, integrating mass-spectral data into the workflow of a chromatographer performing UV analysis has proven challenging. Both sets of data can contain individual chromatographic and spectral windows. Processing and reviewing the results of studies is, therefore, often cumbrous, requiring evaluation of UV and mass-spectral data individually. By providing a single window for reviewing mass and UV information simultaneously (Figure 5), Empower 3 Software simplifies data evaluation.

The chromatographic display appearing in the lower portion of the window includes both the UV chromatogram and mass data – a total-ion chromatogram (TIC) and in its bottom trace an extracted-ion chromatograms (XIC) for the separation. Automatically generated, the XIC is an overlay of the extracted-ion chromatograms corresponding to the apex base peak extracted from the TIC for each integrated UV peak (Figure 8).

The spectral information for each peak appears in the upper portion of the screen. It shows the UV and mass spectra. To help identify possible coelutions, these spectra can be displayed as three separate views: 1) the base peak at apex (Figure 5); 2) a combined view that shows the apex and (average) combined spectra; and 3) a purity view, which displays the leading, apex, and trailing segments of a peak (figure 8). This combined view is assembled automatically according to specifications in the processing method.

Figure 5. Empower 3 Software provides a view of mass and UV chromatograms and spectra. The UV chromatogram can be labeled with most intense ion at the peak apex. This view provides the tools for streamlined peak tracking and identification of coeluting peaks.

Summarizing results into a report

Many chromatography data systems, including Empower 3, make use of a relational database to store, catalog, and retrieve data. The relational database provides data traceability required for compliance-ready management of MS data, and more efficient result reporting, as compared with flat-file chromatography and mass spectrometry data systems. These databases facilitate the use of configurable report templates. Once positioned in a report, objects such as tables, plots, chromatograms, spectra, etc. automatically receive data from the relational database during report generation.

A configurable report template receiving information directly from the database renders many types of data. Using a base template, the report displays text-based information (titles and tables) as well as chromatograms and spectra. Figure 6 demonstrates that simultaneously displayed UV and mass-based chromatograms can appear in the same configurable report using Empower 3 Software.

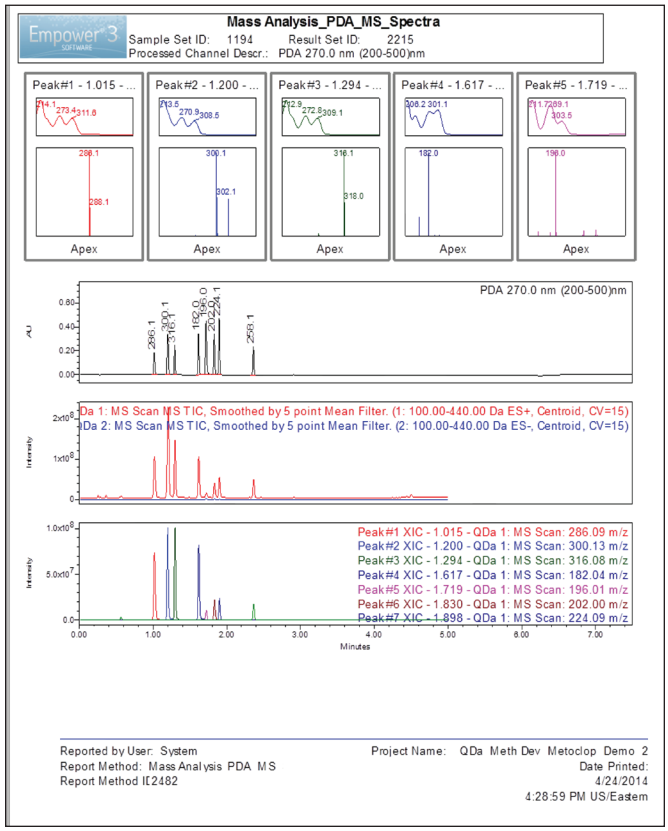


Figure 6. A relational database CDS displays text, chromatograms and spectra in configurable report. The report displays UV and MS chromatographic and spectral results in a single plot.

Quantitative studies may require thorough documentation, particularly while adhering to compliance regulations. Chromatography data software based on a relational database simplifies quantitative reporting because all necessary information can appear in the report template. Figure 7 illustrates this feature within Empower 3 Software, with a quantitative measurement of leucine enkephalin.

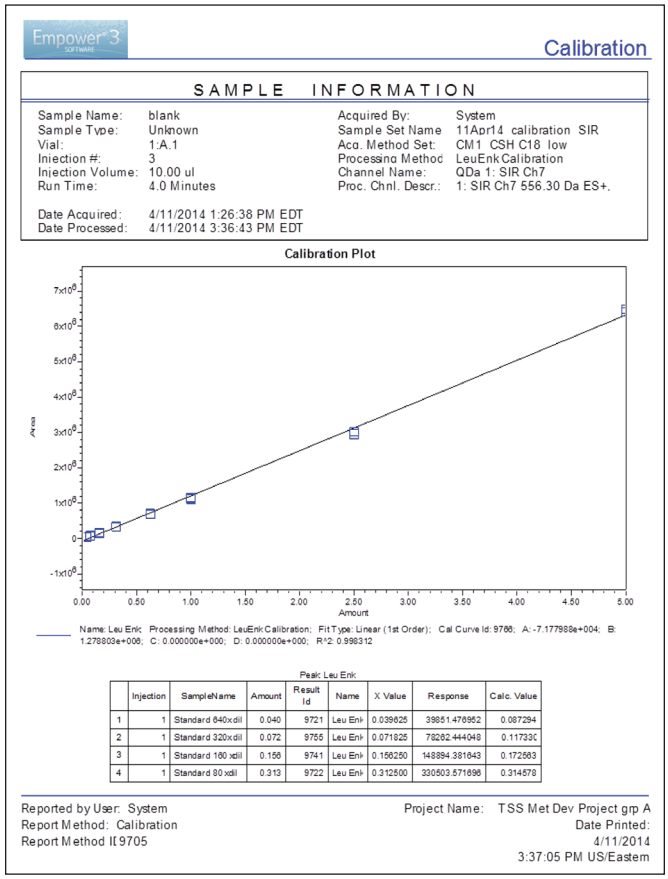


Figure 7. The CDS system's relational database allows all the related information for the calibration report for leucine enkephalin to be displayed in a single report.

Mass detector aids method development

Chromatographic method development can be labor-intensive, requiring a significant investment in analysts' time. Adding mass spectrometry to method development studies offers the opportunity to simplify and accelerate the method development process.

Analytical chemists have known a single detection technique provides insufficient information for missed peaks and coelutions. For those compounds that do not absorb UV, an orthogonal detection technique is required. To address some of these challenges, multiple detectors can be used for analysis of

a single sample, dependent on a different physical or chemical property of the molecule. Mass detection represents one such orthogonal detection mechanism since it requires that a gas-phase ion be generated.

Figure 8 illustrates the advantage of orthogonal MS detection over UV-only detection. The LC/UV chromatogram shows the presence of four peaks in a chromatographic separation. To ensure peak purity, the UV and MS spectra of each peak can be examined. Analysis of the last peak shows no differences in the UV spectra across the peak, the expected pattern for a homogeneous peak. Yet when the mass spectra across the peak are similarly examined, the changes in distribution of the masses at *m/z* 256.9 and *m/z* 293.8 indicate the elution of at least two distinct analytes in that time range, which demonstrates that five, not four, chromatographically eluting analytes exist in this separation.

Mass spectral data can help track the individual sample components as their relative elution times and order change during the process of separation development.[2] Figure 9 illustrates selectivity as a function of mobile phase pH during method development. LC/UV alone would require confirming the analyte retention times by comparing the analyte with authentic standards or, alternatively, by examining the UV spectrum for each analyte. The former task is time-consuming, and the latter can be difficult for closely related species. Using mass detection, however, the selectivity change is instantly noted by tracking the mass-to-charge ratio of the individual analytes. Moreover, this information can be displayed as the peak label in both UV and mass chromatograms.

In addition, when using a CDS that is designed to process mass spectral data, such as Empower 3 FR3, added tools can assist in analysis of mass spectral data. For example, the MS Peak Tracking function can be used to monitor analytes in a series of chromatograms. This may be of particular usefulness when performing method development where each analyte's retention time may vary under different method conditions such as mobile phase pH, shown in Figure 10. Using the predominate mass-to-charge ratio of each peak, the CDS can automatically track analytes for all results. The added ability to summarize the results by user-defined text such as assigned mass, provides the ability to summarize the results in an easy to read table for evaluation or reporting of the results.[3]

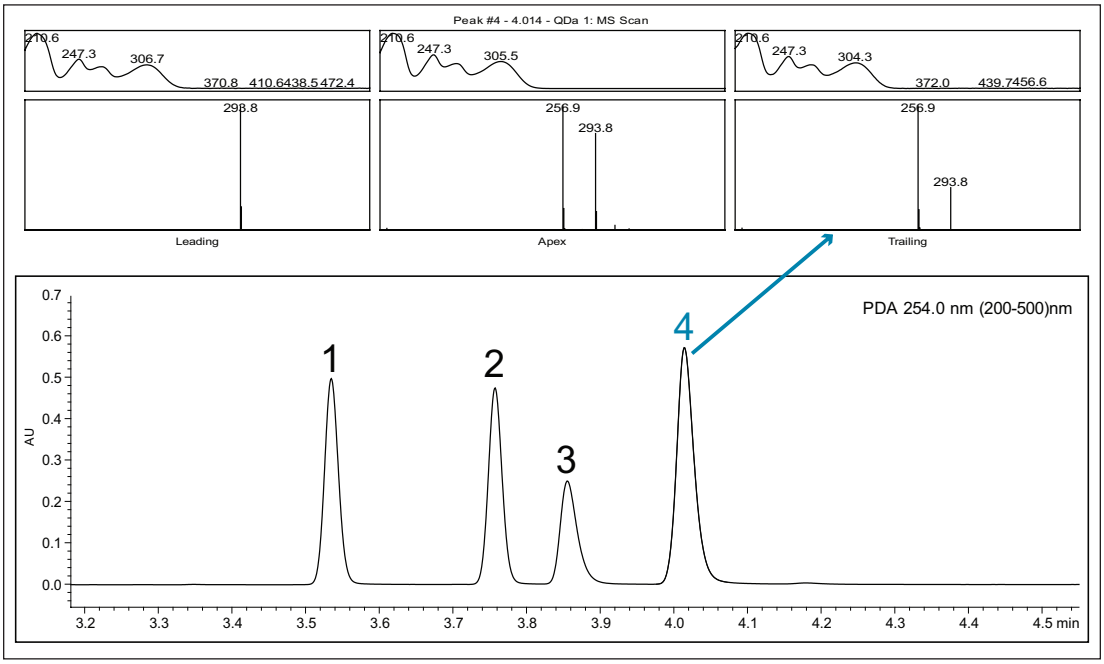


Figure 8. For the analysis of an API and related impurities, UV detection alone indicates the presence of four analytes by the number of chromatographically separated peaks. The addition of mass detection suggests the presence of two coeluting analytes present in peak 4. Analysis of the leading, apex, and trailing segments of the peak demonstrate varying ion ratios of the two major ions, thereby confirming the coelution.

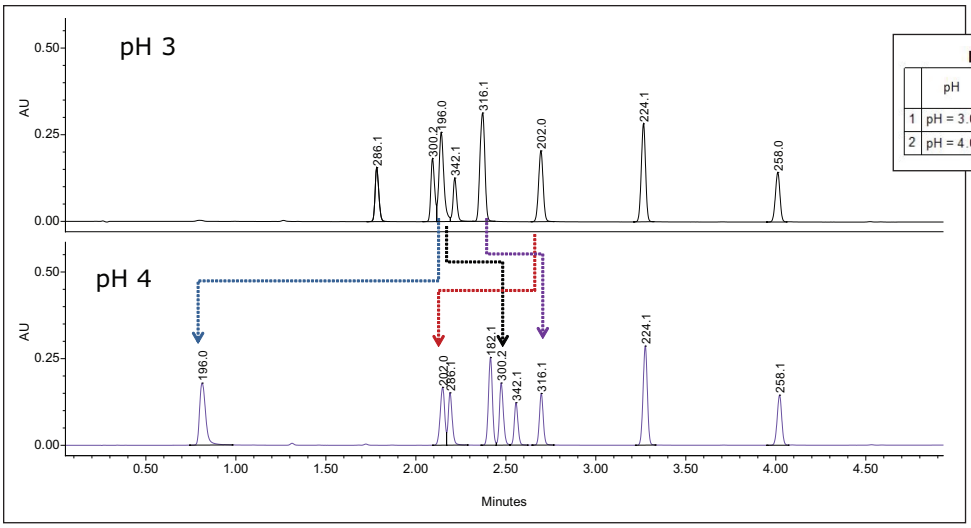


Figure 9. Mass detection provides a straightforward approach to tracking selectivity changes. For example, an API and related impurities was analyzed under different mobile phase pH conditions. Peak tracking, using the most intense ion at the peak apex, enables identification of selectivity differences without injecting standards individually for each set of chromatographic conditions.

Retention Time Summarized by Assigned Mass

	pH	182 (min)	196 (min)	202 (min)	224 (min)	258 (min)	286 (min)	300 (min)	316 (min)	342 (min)
1	pH = 3.0		2.141	2.695	3.265	4.011	1.783	2.092	2.371	2.216
2	pH = 4.0	2.415	0.813	2.149	3.275	4.021	2.190	2.474	2.697	2.556

Figure 10. Empower 3 report table for MS Peak Tracking with varying mobile phase pH. This report table was tailored to summarize and track retention times of the chromatograms in Figure 9, using a defined text string (Assigned Mass).

CONCLUSIONS

Analytes that lack a UV-detectable chromophore require a means of orthogonal detection such as mass detection. Until recently, incorporating mass detection as part of the UV chromatographic workflow has required MS method development and instrument expertise. Also, reviewing chromatographic and spectral data for both detectors has traditionally meant viewing each set of data separately, making data evaluation burdensome. Utilizing an intuitive, automated mass detector, such as the ACQUITY QDa Detector, controlled by a compliant-ready data system like Empower 3 Software provides these benefits:

- Simple on/off capability of the mass detector, reducing startup time and enabling data acquisition within minutes.
- Using an "intelligent" CDS system, mass detection can be integrated as part of the workflow by selecting the appropriate instrument modules and detection capability from among the project properties.
- Automated startup and instrument checks provide greater confidence in results.
- Reviewing both chromatographic and spectral results from UV and mass detectors in a single window enables efficient, peak-by-peak data review.
- The ability of mass detection to identify coeluting peaks provides additional information.

With the addition of an automated mass detector that has been designed to be a synergistic element of a chromatographic system, analytical chemists performing UV chromatographic separations now have the ability to significantly increase their method development capabilities without the complexity of a traditional mass spectrometer.

References

1. Hong P, McConville PR. *Combining Mass and UV Spectral Data with Empower 3 Software to Streamline Peak Tracking and Coelution Detection*. Application Note 720004846EN, Waters Corporation, 2014.
2. Maziarz M, McCarthy SM, and Wrona M. *Improving Effectiveness in Method Development by Using a Systematic Screening Protocol for a USP Method (Metoclopramide HCl and Related Substances).* Application Note 720005026EN, Waters Corporation, 2014.
3. Maziarz, M. and Wrona M. *Streamline Method Development with Empower 3 MS Peak Tracking*. Technology Brief 720005721EN, Waters Corporation, 2016.

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