Integration of MS and UV Data for Peak Tracking in HPLC Method Development

by Richard Verseput, President S-Matrix Corporation
1594 Myrtle Avenue Eureka, CA 95501 USA Phone: 707-441-0405 E-mail: richard.verseput@smatrix.com

Joseph A. Turpin, Director, Chromatography Products and Services, S-Matrix Corporation
Purdue Technology Center, 5225 Exploration Drive, Suite S-2357, Indianapolis, IN 46241 USA, Phone: 317-514-3458, E-mail: joseph.turpin@smatrix.com

High Performance Liquid Chromatography (HPLC) is the most widely used analytical technique in support of pharmaceutical drug development. Advances in instrumentation design, column technology (UHPLC), software, and automation have led to reductions in method development cycle time, as well as the "greening" of the technique with the accompanying reduction in solvent usage and waste. These advancements, along with the ability to connect and obtain data from multiple detectors, will likely maintain its position as the lead analytical platform for the foreseeable future.

The introduction of Quality by Design (QbD) and Lifecycle Management concepts into pharmaceutical method development promise to improve the quality of methods throughout the method lifecycle. Quantitative characterization of a robust analytical method design space, a central element of the QbD methodology, has been consistently demonstrated to improve method performance and repeatability, thereby reducing downstream failures [1, 2, 3]. This analytical method design space has recently been referred to in Analytical QbD circles as the method operable design region (MODR) [4]. Software such as Fusion QbD (S-Matrix Corporation, Eureka, CA USA) promotes this approach with the ability to generate statistically defensible multi-factor designed experiments, automatically transcribe these experiments into ready-to-run sequences and instrument control methods within the Chromatography Data Software (CDS), and directly import all experiment results from the CDS for automated modeling and visualization.

To fully realize the benefits of analytical QbD supporting technologies must also be able to address the separation of all potential impurities and degradation products in complex samples. Samples containing molecules lacking chromophores, or ones for which UV absorbance changes with

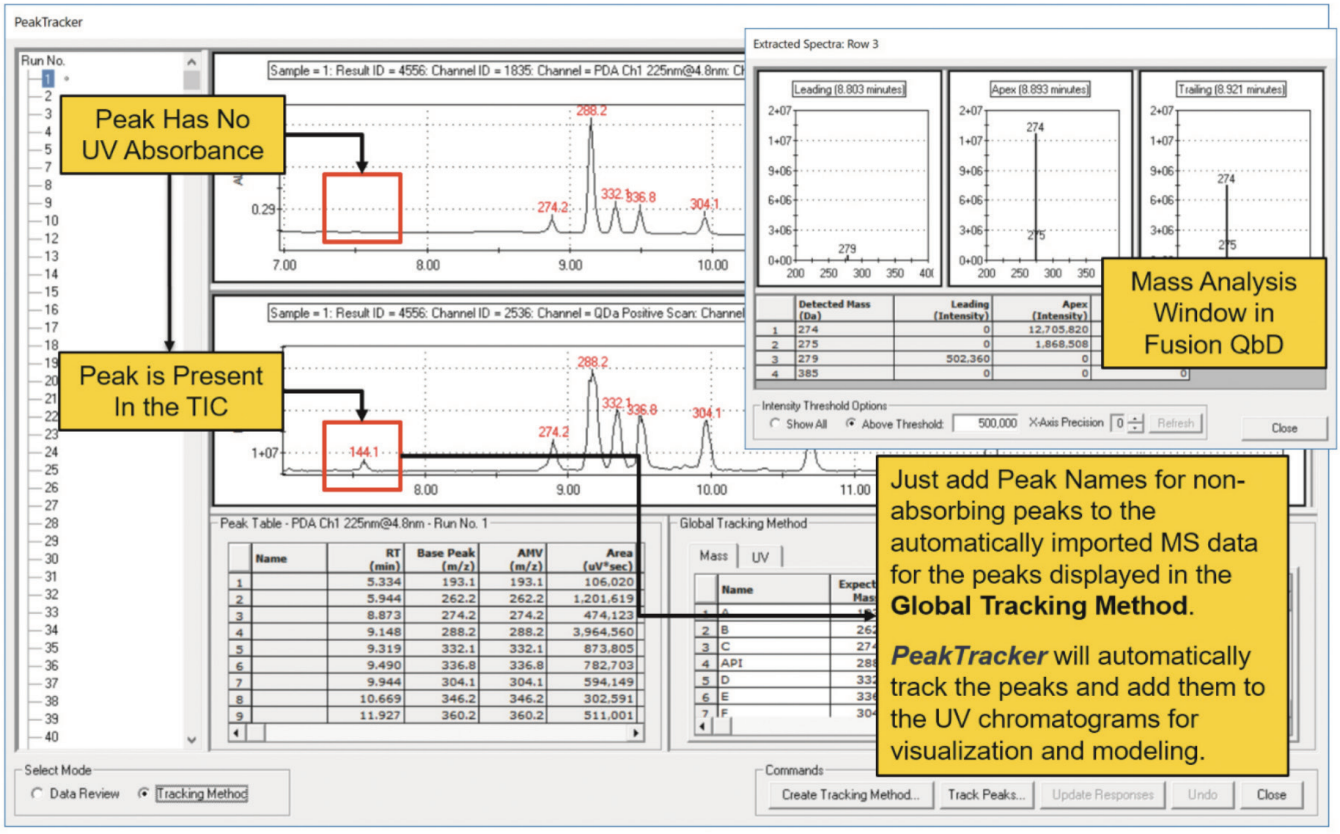

Figure 1 – Non-absorbing Peak

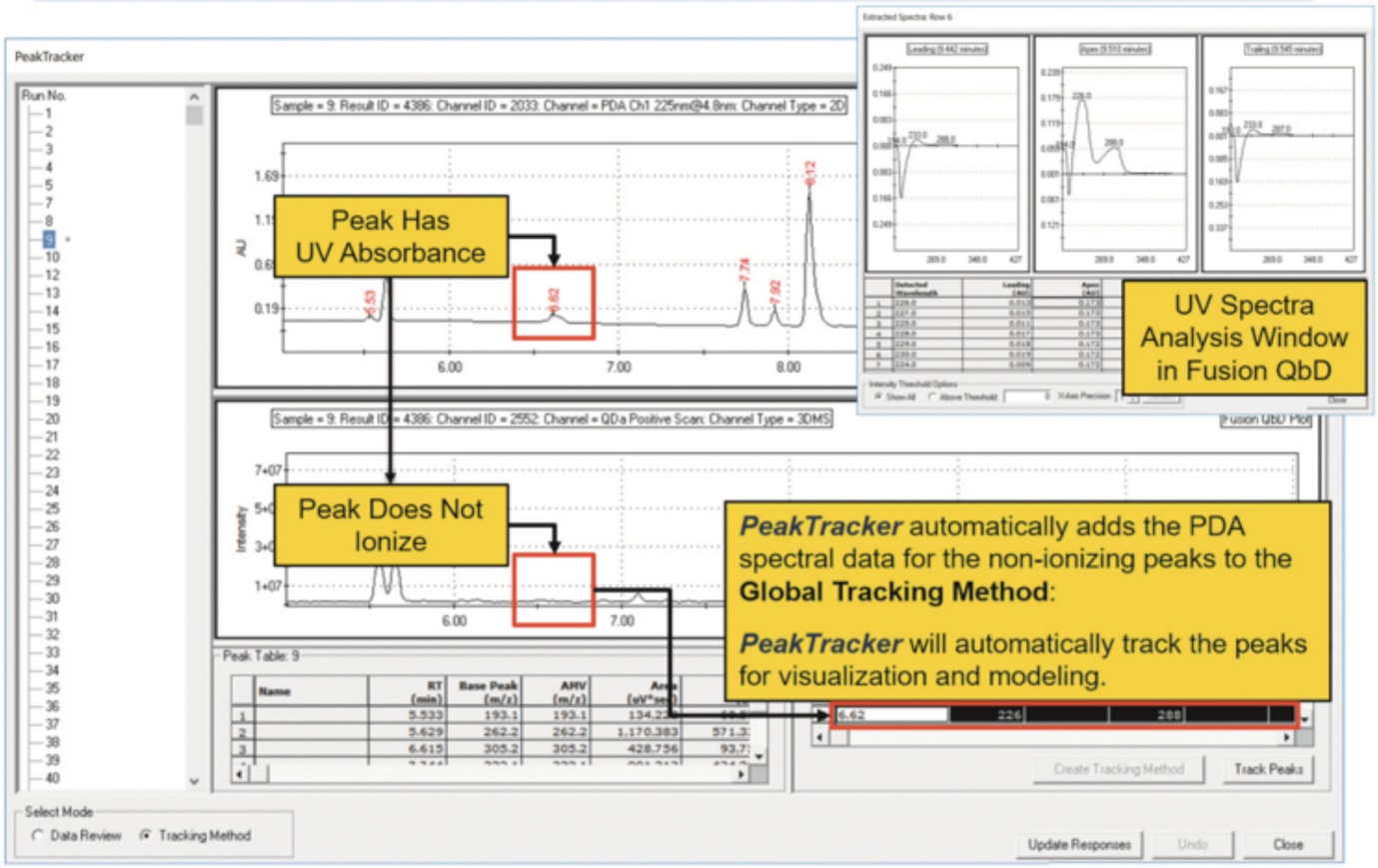

Figure 2 – Non-ionizing Peak

pH, complicate method development. Utilizing MS detection to identify and track these problematic sample compounds would therefore greatly improve method development effectiveness, resulting in increased performance of, and confidence in, the final method. Though there are a number of MS instruments available to assist with identification of products and impurities, it has long been the desire of the chromatographer to combine the confirmatory power of the MS with familiar chromatography data systems and UV detection typically used in method development. The recent introduction of the Acquity QDa, a miniaturized mass spectrometric detector, coupled with the Empower Chromatography Data Software (CDS) by Waters Corporation (Milford, MA.) has made it both simple and economical to integrate MS data into method development.

To support QbD method development, screening and optimization experiments are conducted that focus primarily on the factors most likely to affect separation – for example factors such as column chemistry, solvent composition, pH, column temperature, and gradient slope in reversed phase chromatography. Statistically designed experiments provide an extremely efficient sampling of a multi-factor experimental region relative to a brute force all-possible-combinations approach, and also provide much more knowledge-based data than the incremental trial-and-error approach. Designed experiments have the added benefit of enabling the user to study important parameters in combination so that their interactive influences on method performance can be characterized and visualized.

The most valuable and useful knowledge obtainable from method development experimental results is the exact nature of the combined factor effects on the retention and peak shape of each sample molecule as the factor level setting combinations are varied across the experimental region. Extracting this knowledge normally requires the ability to unambiguously locate and track the shape and retention changes of each sample peak across the experiment data set – in other words, peak tracking. Tracking each peak in each experiment chromatogram is a normally a manual and challenging effort, even with the facilitated tools available in chromatography data software. Peak tracking becomes even more complex when two or more peaks co-elute or change elution order between experiment runs.

The limitations of peak tracking using Photodiode Array (PDA) spectral data are well known [5]. Manually tracking peaks without the benefit of confirmatory mass data requires spiking experiments, or carefully controlled sample mixtures that enable the determination of simple migrations or co-elution to be observed via area or peak height responses. Utilizing mass data from an integrated mass spectrometer greatly facilitates peak identification, but to date has required additional manual manipulation of increased amounts of data.

To alleviate this problem, S-Matrix has developed PeakTracker™ – a powerful new UV/MS peak tracking technology to automate, optimize, and simplify the use of PDA and MS data in LC and LC/MS method development. Fully integrated into S-Matrix's Fusion QbD software for LC and LC/MS method development, PeakTracker uses 3D PDA spectral data augmented with standard UV peak results data to automatically identify each peak in each experiment chromatogram. PeakTracker also automatically utilizes 3D mass spectral data for experiments run on LC systems configured with the Waters Acquity QDa Mass Detector (QDa). Complex separation and tracking challenges PeakTracker can automatically address include:

- Auto-deconvolution of partially and completely co-eluted peaks.

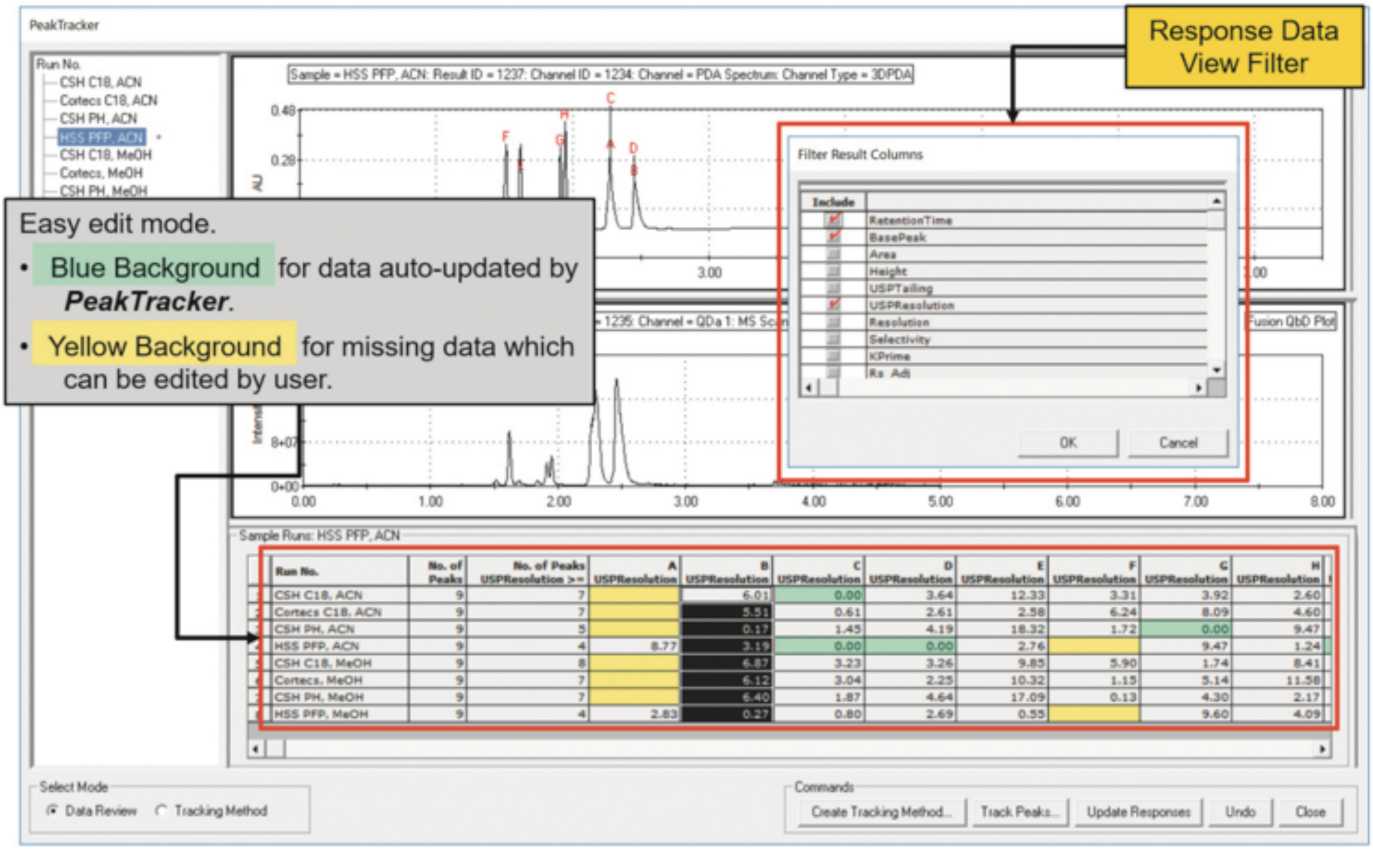

Figure 3 – Simple Data Review Display

When two peaks co-elute, one peak of the co-eluted peak pair will be "hidden" in the UV chromatogram. Standard UV results data such as Retention Time and Resolution will be missing for this peak for all experiment runs in which the peaks co-elute. This negatively impacts prediction models derived from the method development experiment results. Using PDA and MS spectral data to automatically locate "hidden" peaks and fill in missing results data can dramatically improve the quality of prediction models.

- Two or more peaks with identical mass data. There are many circumstances in which two peaks will have the same parent mass value, and therefore the same mass-to-charge ratio (m/z). A solution in these cases would be to use a mass spectrometer capable of fragmenting all ionizable compounds coupled with a spectral library for identification. However, this capability is unavailable in many labs charged with developing LC methods. Utilizing an economical mass detector, and coupling it with automated diagnostics utilizing UV spectral data and standard peak results data provides a unique solution to this problem.
- Non-absorbing and non-ionizing compounds.
  In most cases it is desirable to have a mass spectrometer compatible LC method which resolves all sample compounds. This goal is complicated when the sample contains compounds which either do not absorb, as shown in Figure 1 (no UV data), or do not ionize, as shown in Figure 2 (no MS data). These cases require coupling the PDA and MS spectral data into the automated peak tracking protocol. This enables creating a merged chromatogram which contains all of the peaks and the associated results data needed for data modeling.

PeakTracker also incorporates the additional features and functions needed to complete the tracking workflow. These include (1) a paired graphical and numerical display of the MS and PDA spectra data for each peak, as shown in Figures 1 and 2, respectively, along with facilitation tools for manual manipulations to tracking results, and (2) a stacked display of the the UV chromatogram and Total Ion Chromatogram (TIC) for each experiment run for simple visual comparisons and tracking confirmation. In addition, as shown in Figure 3, PeakTracker displays a user-filterable table of the UV peak results with highlight colors to easily identify tracking updates to peak data – for example, updates to missing data for co-eluted peaks and added data for non-absorbing peaks merged into the UV chromatogram. Once tracking is complete, PeakTracker automatically maps compound names to all of UV results data computed by the CDS for all identified peaks in the experiment chromatograms for automated modeling and visualization. Automated peak tracking which fully utilizes PDA and MS data within a chromatography data framework greatly simplifies the integration of MS data into the method development workflow. Further, the ability to incorporate non-absorbing peaks into UV experiment chromatograms directly supports the development of MS compatible HPLC methods, which can be of great benefit to both production and quality control.

References

[1] R. Verseput, J. Turpin, Chromatography Today, August / September 2015, 64

[2] A. Schmidt, C. Wess, Journal of Liquid Chromatography & Related Technologies, 37 (2014) 18

[3] B. Debrus, D. Guillarme, S. Rudaz, Journal of Pharmaceutical and Biomedical Analysis, 84 (2013) 215– 223.

[4] J. Kaufman, D. Mans, FDA-DPA, Presentation to the CASSS CMC Strategy Forum Europe 2015

[5] J. Strasters, H. Billiet, L. Galan, B. Vandeginste, Journal of Chromatography A, Volume 499, 19 January 1990, Pages 499-522