ASMS 2011

Fast and Sensitive Target Compound Quantification and Confirmation with a New Triple Quadrupole Acquisition Function

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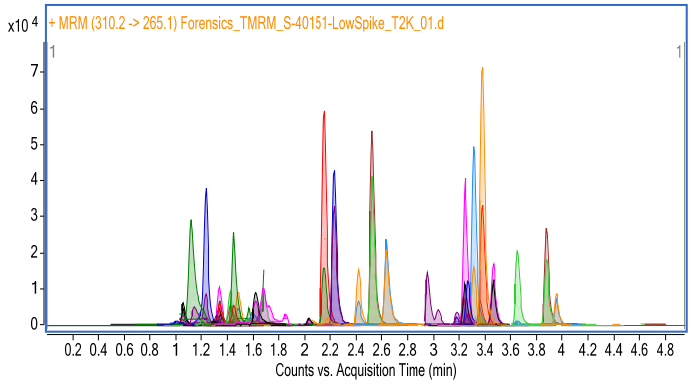
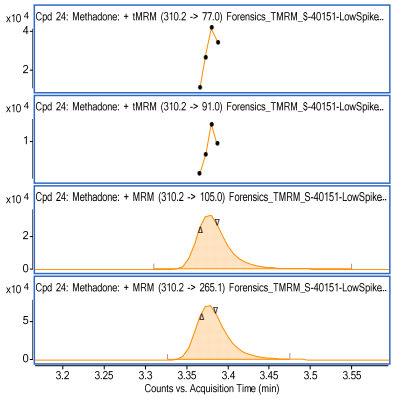
WP 209
Agilent Technologies
Santa Clara, CA
June 2011

Introduction

Two primary ion transitions (a 'target' and 'qualifier') are often used in multiple reaction monitoring (MRM) to confirm the presence of target analytes in samples based upon their retention time and qualifier/target ratio. Additional MRMs (i.e. secondary ion transitions) could be used to provide more complete confirmation of compound identity, however, the amount of time available for MRMs in complex, multi-analyte sample analysis often limits their use. This time restriction can be largely overcome by using a data dependent acquisition approach to dynamically monitor the primary ion transitions within specific RT windows[1,] and 'trigger' the secondary ion transitions only when the primary transitions of the target compound are detected above a set threshold. We refer to this new QQQ acquisition mode as triggered-DMRM.

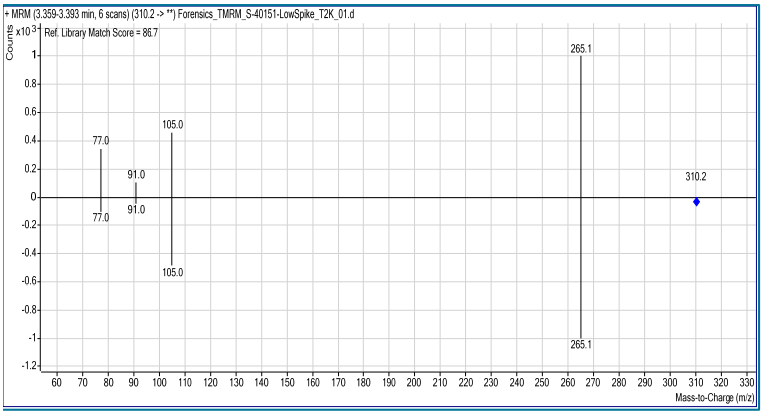




Methadone secondary ion transitions (upper left EICs) are triggered when the primary ion transitions (lower left EICs) are detected above a specific threshold. The start (Δ) and end ( ) of triggering is indicated on the two primary EIC traces.

In this example, 4 data points ('repeats') are acquired for the secondaries, while the primaries (target and qualifier ions), are consistently monitored and detected within their Delta RT window.

A key advantage of triggered-DMRM acquisition is that the combined MS data (primaries + secondaries) results in MS spectra which can be used to create (and subsequently to search), an MS reference library of target compounds.



Experimental

Analytical grade individual standards and mixtures were obtained from the following suppliers:

Agilent Technologies, Inc. (Little Falls, DE):

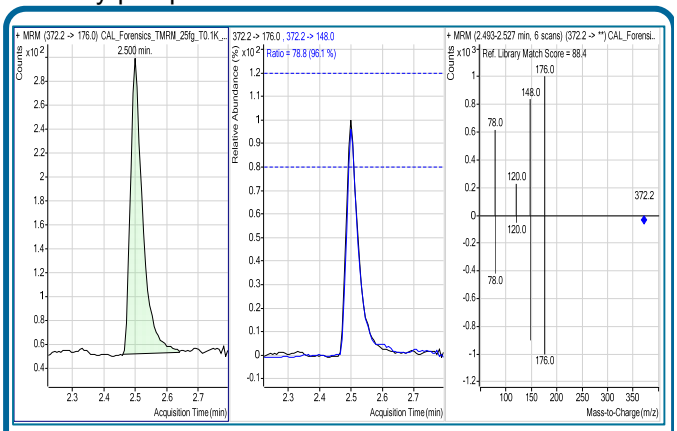
• LC/MS Toxicology Test Mixture, 5190-0470, 28 compounds

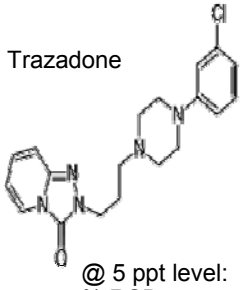
Cerilliant Corporation (Round Rock, TX):
• 1.0 mg/ml standard solutions of benzoylecgonine, *m*-OH-benzoylecgonine, flunitrazepam, norflunitrazepam, and 7-aminoflunitrazepam in methanol.

Synthetic biological matrices (control and spiked urine and serum reference samples) were obtained from Medichem Diagnostica (Steinenbronn, Germany):
• Base-Line U, REF 40201 Lyophilized Urine Control
• Drug U-Confirmation REF 40572, 28 analytes
• Base-Line S, REF 40151 Lyophilized Serum Control
• BTMF Series S-Plus, REF 41653 2/10-B, 15 analytes

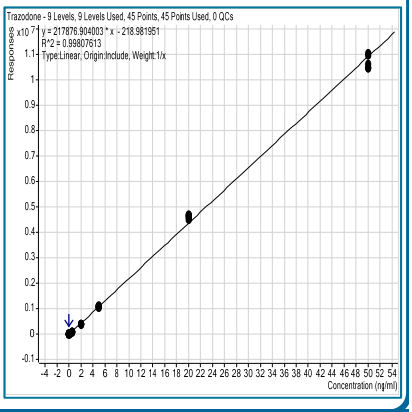
Samples were cleaned up by offline SPE utilizing Bond Elute Plexa PCX 30 mg tubes (12108301). This is a mixed-mode polymeric sorbent which effectively extracts basic drugs (cation exchange) and retains acidic and neutral drugs on its hydrophobic portion. The SPE method protocol is listed in reference [2].

UHPLC/QQQ acquisition methods were developed utilizing the MassHunter Forensics and Toxicology Dynamic MRM Database (G1734-64000). Data was acquired in triggered-Dynamic MRM mode on a 6490 triple quadrupole mass spectrometer with thermal gradient focusing technology and differentially-pumped dual ion funnels.





@ 5 ppt level:
% RSD = 7.7



Results and Discussion

The specific MRM transitions (precursor and product ion selections, collision energies, etc.) were optimized for each analyte using an automated software acquisition tool (Optimizer) or from QTOF analysis at six collision energies.

Triggered-DMRM data acquisition differs from targeted QTOF MS/MS acquisition in that for each MRM transition associated with a particular analyte, the collision energy is optimized to yield the highest signal response. Moreover, the raw data can be acquired at a high duty cycle ensuring low level detection of multiple analytes in complex samples with fast chromatography. The averaged composite spectra generated are also more unique when compared to spectra obtained from fixed collision energy product ion scans.

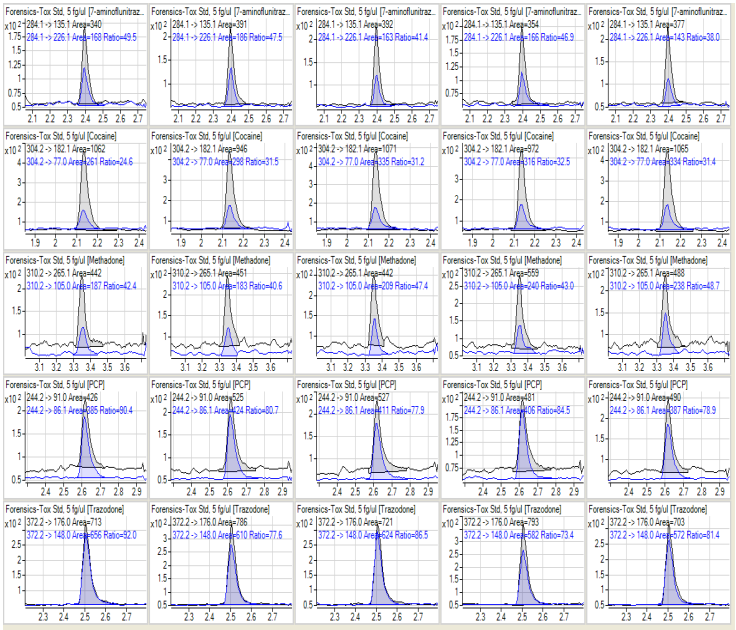
Summary of the acquisition method development workflow:

(1.) First, analyze a multi-analyte, medium-level calibration standard in static MRM mode using a fixed 2 ms dwell time.

(2.) Automatically update the method to a dynamic-MRM method with RTs and Delta RT detection windows. Add additional MRM transitions ('secondaries').

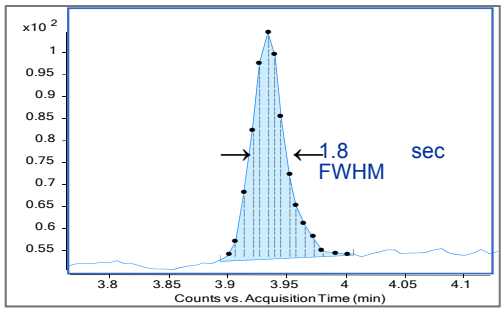
(3.) Select/enable the triggered-DMRM checkbox and enter number of repeats. (These are the '*triggered*' data points). Reanalyze the same standard, then create an MS Reference Library from the triggered-DMRM data file in Quant DA.

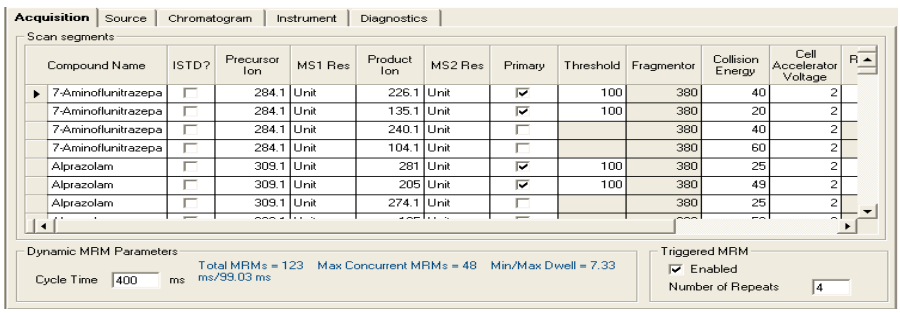
Several triggered-dynamic MRM data sets (e.g. pesticides, forensics, photo-initiators, and peptides) were analyzed to compare target/qualifier ratios among the various MRM scan types. The triggered-dynamic MRM and dynamic-MRM quantitative results were similar for multi-analyte data sets of 200+ ion transitions. Static MRM data was more variable, particularly at low detection levels due to shorter, fixed dwell times, and therefore, lower S/N.



1290 Infinity LC	Parameters and Run Conditions
Column	ZORBAX Eclipse Plus-C18, RRHD, 2.1x100 mm, 1.8 µm, 80Å
TCC	60 ^{0}C
Mobile Phase	A = H_2O w/5 mM NH_4HCO_2 + 0.01% formic B = 95:5 ACN/H_2O w/5 mM NH_4HCO_2 + 0.01% formic
Flow Rate	0.5 ml/min
Injection Volume	5 µl
Needle Wash	5 sec (75:25 MeOH/H_2O w/0.1% formic)
Gradient	Initial: 10% B 0.5 min: 15% B 3.0 min: 50% B 4.0 min: 98% B (hold 2 min)

6490A QQQ	Source / Acquisition Conditions
Source/Ion Polarity	Agilent Jet Stream - ESI / Positive ion mode
Nebulizer Pressure	30 psig N2
Sheath Gas Temp Sheath Gas Flow	375 ^{0}C 12 L/min N2
Drying Gas Temp Drying Gas Flow	200 ^{0}C 12 L/min N2
Capillary Voltage Nozzle Voltage	3500 V 500 V
Acquisition Mode Quad Resolution	Targeted-DMRM Unit resolution (0.7 Da FWHM) for Q1 & Q2

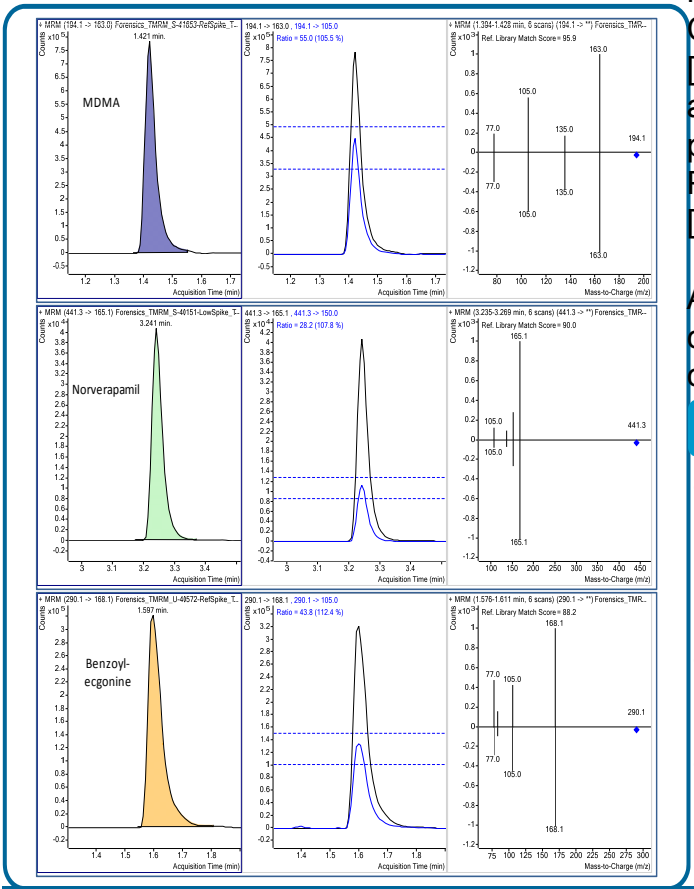




Results and Discussion

Creating an MS reference library within Quantitative Data Analysis (Version B.05.00) is completely automated for the user. A Library Editor tool is also provided for modifying library entries or adding additional compound information.

The 'goodness of fit', or match score for spectral matches depends on several factors. The MS reference library is created with "pure spectra" i.e. MS spectra originating from pure compounds with no background ions. Each ion in the averaged composite spectrum has an inherent relative height to that of the base peak. When a sample spectrum is compared with one in the reference library, a normalized dot product of the two spectra (i.e. ions and relative height) is calculated ('dot product'-based match factor [3.]). Also the number of ions entered into the reference spectrum is a factor which contributes to the match score. Less ions to compare decreases the match factor weighting. Matrix effects such as ion suppression or background ion contamination can result in lower match scores. The analyte peak maximization (all related ions typically display a similar chromatographic profile when overlayed), provides confidence that all ions are associated with a specific compound. As part of the data acquisition method, it is very important for the user to specify an acquisition duty cycle consistent with obtaining a sufficient number of data points across chromatographic peaks. This helps to ensures good quantitation and predictable ion profiles.



During triggered dynamic-MRM acquisition, the ability to trigger several times in succession (number of repeats) results in an averaged composite MS spectrum which can be compared with the reference library spectrum to obtain a library match score. Also the ability to reset the trigger ('rearm') allows detection of closely eluting diastereomers which often have similar mass spectra. Some future SW enhancements could include a criteria for triggering only if the ratio of the primary ion abundances (qualifier/target) is within a user-defined percentage range, for example 20%. Firmware algorithms to trigger several times near the apex of the analyte peak would provide the best MS spectra and S/N since ion abundances are at their highest level and more constant. These improvements would allow optimal data acquisition rates, result in fewer false positives, and better match scores when detecting multiple analytes in complex matrices.

References

[1.] G.T. Overney, H.E. Bunting, et al. 18th International Mass Spectrometry Conference (IMSC 2009, Bremen, Germany), PPM: 292 Dynamic Multiple Reaction Monitoring (DMRM) - Key Concepts & Capabilities.

[2.] W. Hudson, A. Junker-Buchheit, Fractionation of acidic, neutral and basic drugs from plasma with polymeric SPE cation exchange, Bond Elute Plexa PCX (Agilent application note SI-01013).

[3.] S.E. Stein, JASMS, Vol. 10, 8, (August 1999), 770-781,
An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data.

Conclusions

- Low PPT level detection and confirmation of toxicological compounds utilizing off-line SPE UHPLC/MSMS triggered-DMRM acquisition and reference library match scoring
- User-friendly workflows, and method development tools
- Automatically-generated MS Reference Library