Screening To Quantitation: DDA Of Extractables To Determine Markers Of Interest For Quantitation Using Tof MRM With A Multi-Reflecting Time-of-Flight Mass Spectrometer

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Introduction

Due to the concern about the safety of components from plastic, it is crucial to screen for potential extractables and leachables (E&L) from pharmaceutical packaging and medical devices.[1]

Regulations and standards are in place to ensure safety limits are met. Analytical instrumentation needs to be highly sensitive to detect low level chemical species to meet expected screening thresholds.[2]

A data dependent acquisition (DDA) mode can be used to determine markers of interest in a complex mixture providing highly specific MS/MS spectra. The DDA generated product ions can then be utilized for targeted quantitation of leachables with time-of-flight (TOF) multiple reaction monitoring (TOF MRM).

Here we report a DDA approach, to determine markers qualitatively. Curated fragment ions were then packaged for a targeted leachable quantitative analysis which was subsequently undertaken with a highly sensitive Tof MRM mode.

Figure 1. Waters Xevo™MRT Mass Spectrometer and ACQUITY™ Premier System

Experimental

A compound mix (SST) containing typical extractables was injected. Data were acquired using a DDA method to determine fragment ions. Survey data were collected with a 10/20 Hz scan rate over the range of 50-1200 *m/z*. MS/MS utilized a scan rate of 20/50 Hz with the top number of 10 ions chosen for MS/MS. The switch criteria from survey to MS/MS used an intensity threshold exceeding 20000 counts per second (cps), whilst the switch back was below 10000 cps or a 3 s timeout. Fragmentation utilized a collision energy ramp from 10-75 V over the full *m/z* range.

A Tof MRM method was optimized using the isolated DDA fragments and then used to quantify the SST mix spiked into the solution from a nasal spray device.

LC CONDITIONS: ACQUITY Premier System	
Column	ACQUITY CORTECS™ C18, 90 Å (1.6 µm, 2.1 x 100 mm)
Mobile Phase A / B	Water + 1 mM ammonium acetate + 0.1% formic acid / Methanol
Flow Rate	0.3 mL/min
Column Temp.	50 ºC
Injection volume	1 µL
Gradient run time	15 minutes

MS CONDITIONS: Xevo MRT MS	
Acquisition mass range	*m/z* 50-1200
Source/Desolvation temp	120 ºC / 550 ºC
Desolvation/Cone gas flow	800 / 50 L/hr
Column Temp.	ESI+ 2.5 kV,
Collision energy	Low: 6 eV High: 20-60 eV

Data Management
The waters_connect™ Software Platform was used for data acquisition and the UNIFI™ Application and MS Quan application out of the waters_connect™ Software Platform were used for data processing.

Results

To test the DDA method the standard was injected using a data independent acquisition (DIA) mode[3] reported previously[4] and using DDA acquisitions with and without an include list. All compounds in the SST mix were identified across all modes. The mass accuracy RMS across all DDA injections and analytes was ≤1 ppm.

The DDA MS/MS spectra were compared to DIA MS/MS spectra, highlighting highly specific spectra due to the reduction in fragment spectral peaks that were not attributed to the precursor. For example, for Cyasorb 2908, the number of peaks in the MS/MS spectra at retention time 9.08 minutes, was reduced by 33% in the DDA spectra (Figure 2). For the standards spiked into matrix the number of peaks was reduced by 48% in the DDA spectra of Cyasorb 2908 compared to DIA (Figure 2).

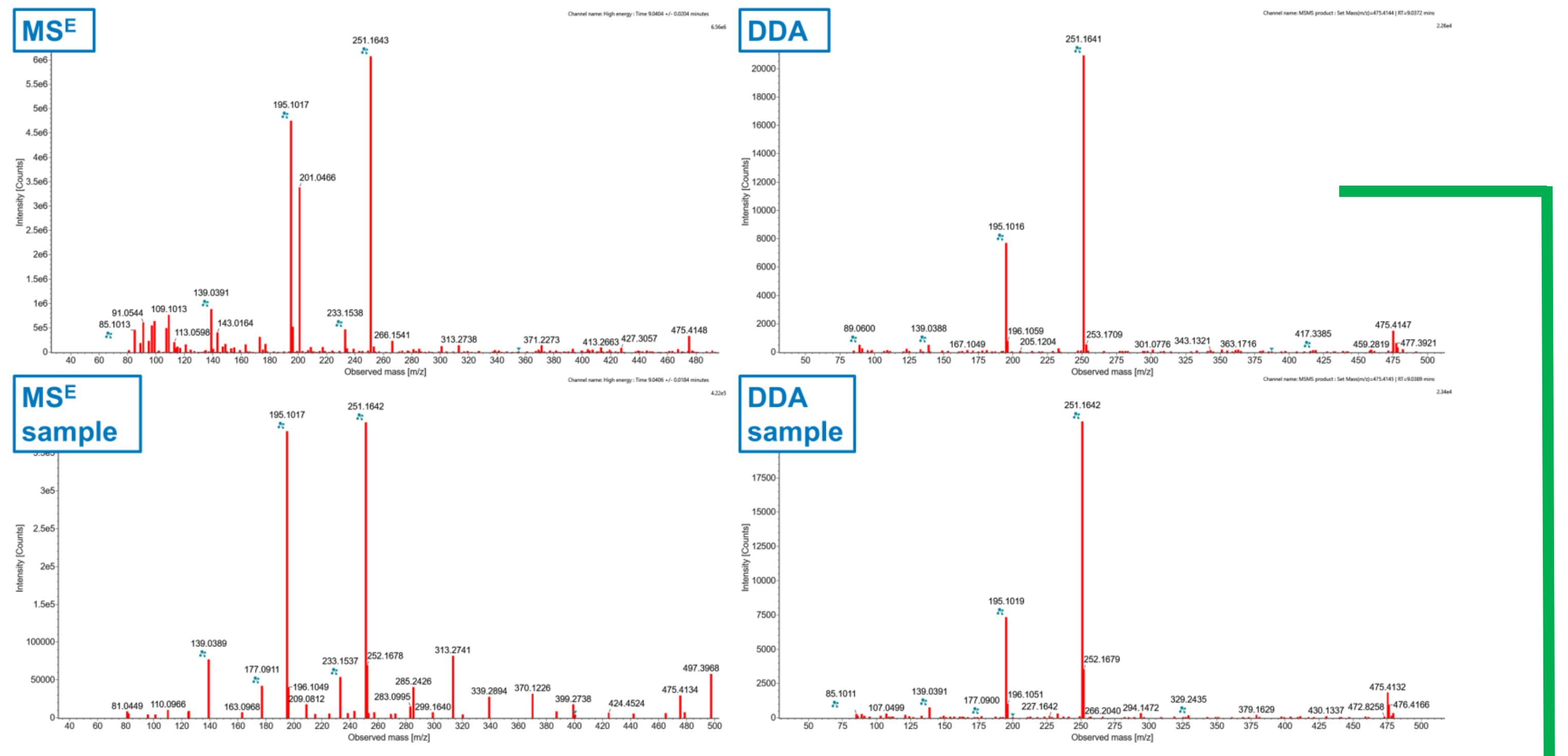


Figure 2. Cyasorb 2908 MS/MS spectra compared for DIA vs DDA (top) and DIA vs DDA of sample (bottom).

Compound specific peaks from the DDA processed spectra were selected for Tof MRM transitions and were optimized for quantitation (Figure 3). Tof MRM utilizes an Enhanced Duty Cycle (EDC) mode, where target ions are trapped and released with timing synchronization with the pusher. Ion utilization over a specific *m/z* range approaches 100%, increasing the sensitivity of the assay.[5]

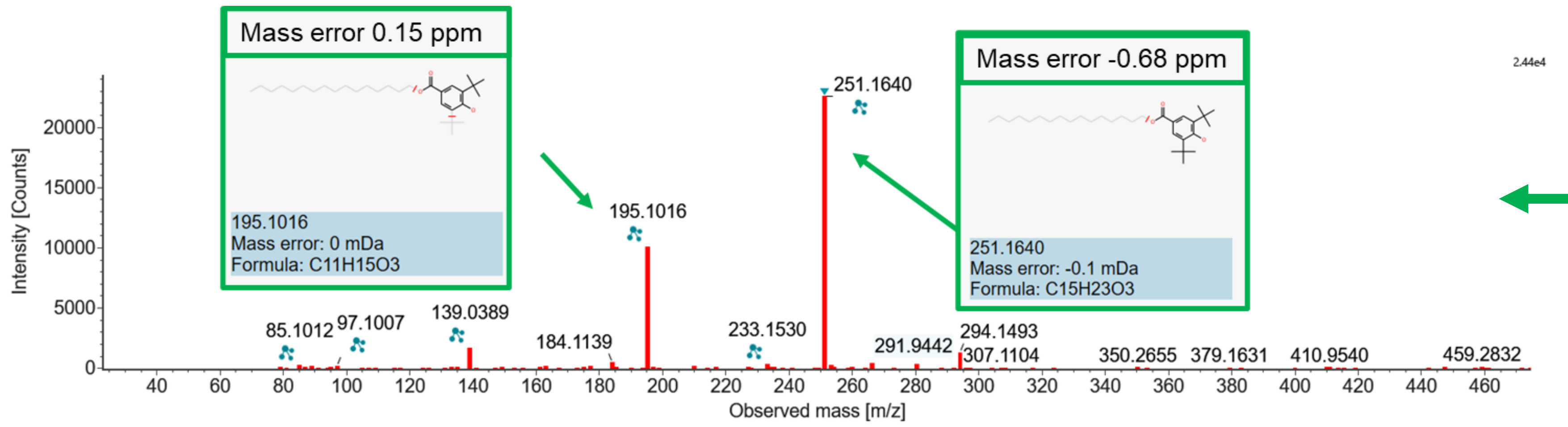
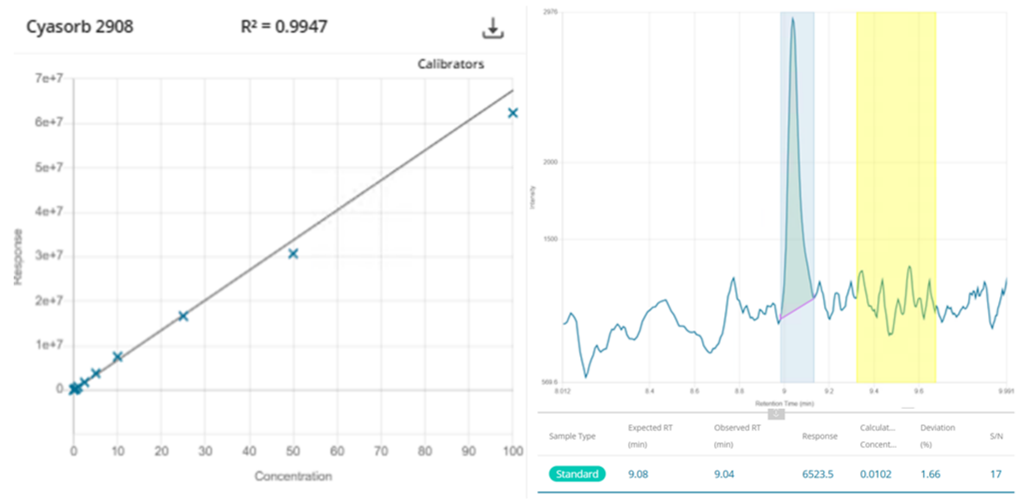


Figure 3. Cyasorb 2908 MS/MS spectral fragments selected for Tof MRM.

Calibration curves were created and acquired alongside the spiked nasal solution. The Cyasorb 2908 calibration curve was linear from 0.01 ng/mL (S/N 17) to 100 ng/mL (R2 0.9947) (Figure 4). The concentration of the spiked compound in the sample was calculated to be 9.39 ng/mL (actual concentration 10 ng/mL) (Table 1).



Sample Type	Expected RT (min)	Observed RT (min)	Response	Calculat... Concent...	Deviation (%)	S/N
Standard	9.08	9.04	6523.5	0.0102	1.66	17

Figure 4. Cyasorb 2908 calibration curve, and the 0.01 ng/mL calibrator.

Compound Name	Injection Name	Response	Calculated Concentration (ng/mL)	Expected Concentration (ng/mL)	% Deviation	Signal to Noise
Cyasorb 2908	MeOH		Not Detected		No level	
Cyasorb 2908	C1	6524	0.0102	0.01	1.66	23
Cyasorb 2908	C2	30563	0.0458	0.05	-8.37	44
Cyasorb 2908	C3	169817	0.252	0.25	0.93	361
Cyasorb 2908	C4	320404	0.476	0.5	-4.88	413
Cyasorb 2908	C5	691305	1.03	1	2.56	1572
Cyasorb 2908	C6	1746722	2.59	2.5	3.63	2414
Cyasorb 2908	C7	3738160	5.54	5	10.88	1687
Cyasorb 2908	C8	7514851	11.1	10	11.44	2926
Cyasorb 2908	C9	16627641	24.7	25	-1.37	321
Cyasorb 2908	C10	30690825	45.5	50	-8.98	3294
Cyasorb 2908	C11	62370842	92.5	100	-7.51	4793
Cyasorb 2908	IPA Blank		Not Detected			
Cyasorb 2908	IPA spiked	6892748	10.2	10		2097
Cyasorb 2908	IPA spiked	7084012	10.5	10		5721
Cyasorb 2908	IPA spiked	7137459	10.6	10		1684
Cyasorb 2908	Solution_spiked	6229393	9.24	10		1734
Cyasorb 2908	Solution_spiked	6416805	9.52	10		2515
Cyasorb 2908	Solution_spiked	6345176	9.41	10		2143
Cyasorb 2908	IPA Blank		Not Detected			

Table 1. Calculated concentration of Cyasorb 2908 in the spiked sample.

Conclusions

- **DDA to Tof MRM workflow in extractables screening to determine markers of interest for quantification with a multi-reflecting TOF platform**
- **DDA, compared to DIA, significantly reduces the number of peaks not attributed to the precursor resulting in highly specific MS/MS spectral data.**
- **Tof MRM with EDC allows for highly sensitive targeted quantitation on a high-resolution mass spectrometer.**

References

1. USP-NF/PF, <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. https://doi.usp.org/USPNF/USPNF_M7127_03_01.html
2. ISO 10993-18:2020 Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process, https://www.iso.org/standard/64750.html
3. Stevens D., Cabovska B., Bailey A. Detection and Identification of Extractable Compounds from Polymers. Waters Application Note 720004211. January 2012.
4. Sanig R., Kirk J., Gethings L., Lock R. Increased Identification Confidence for Extractables Screening Using the Xevo™ MRT Mass Spectrometer. Waters Application Note 720008970. August 2025.
5. Daly M., Gethings L., Hughes C., Lock R., Syed N. Tof MRM for the Quantification of Peptide Biomarkers in Human Glioblastoma with the Xevo™ MRT Mass Spectrometer. Waters Application Note 720008972, October 2025.

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