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Application Note

Integrated Single-injection Quan/Qual Characterization of Extractables and Leachables Using a Benchtop Multi-Reflecting Time-of-Flight Mass Spectrometer

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Abstract

When screening for extractables and leachables (E&L) from pharmaceutical packaging and medical devices, it is critical to identify all extractables found at levels above the analytical evaluation threshold for toxicological assessment and to quantify low levels of leachable compounds. An extractables screening workflow was reported previously using a benchtop multi-reflecting time-of-flight mass spectrometer (MRT MS) for consistent low- to sub-ppm mass accuracy, allowing for increased extractables identification confidence. Once extractables are identified, a leachables experiment needs to be undertaken, this can be undertaken on the same platform with a highly sensitive time-of-flight multiple reaction monitoring (Tof MRM) analysis. On the same instrument, an advanced mixed mode acquisition can be used, combining quan/qual analysis in the same injection.

Here, a combined analysis workflow is demonstrated using the benchtop MRT MS. Tof MRM for targeted quantitation was acquired simultaneously with data-independent acquisition (DIA) for confident extractables screening. This dual acquisition approach enables quantitation of known leachables while also supporting

concurrent screening and identification of potential new extractables.

Benefits

- Streamlined E&L workflow with combined quantitation and characterization in one injection. Eliminates the need for separate Quan/Qual analyses, improving overall operational efficiency.
- High sensitivity quantitation using scheduled Time-of-flight multiple reaction monitoring (Tof MRM). Tof MRM with enhanced duty cycle improves sensitivity whilst retaining the advantages of high-resolution mass spectrometry (HRMS).
- Comprehensive qualitative coverage through concurrent DIA. Retrospective data interrogation of extractables compounds for long term risk monitoring.

Introduction

Medical devices, pharmaceutical packaging, and manufacturing components, contain different chemicals, including polymers, polymer additives such as antioxidants, slip agents, colorants, and other compounds. These chemicals, their impurities, and degradation products can migrate out of the materials resulting in potentially unsafe substances. Due to this, there are regulations, standards, and guidance in place to ensure that safety limits for the consumer are met.[1-3]

Worst-case studies are undertaken to find extractables at levels above the analytical evaluation threshold, and these must be identified and reported for toxicological assessment.[4] Extractables then deemed to be leachables under normal use need to be routinely quantified. Analytical instrumentation needs to be highly sensitive to detect low level chemical species to meet expected screening thresholds.

E&L assessments are moving from one off experiments to lifecycle management which can be seen with the release of draft ICH Q3E guidelines from 2025. This takes into account changes that happen in the process of the products, for example, changes to drug product formulation or changes in suppliers and manufacturing process and helps to ensure that risks are constantly managed.[5] With this in mind, being able to retrospectively interrogate data is critical to long term risk monitoring. The Xevo™ MRT P10 Mass Spectrometer (Figure 1) utilizes an advanced mixed mode acquisition to combine quantitative and qualitative data within one injection.

Here, a combined analysis approach on one benchtop MRT MS is reported. A Tof MRM mode for targeted

quantitation was acquired simultaneously with a DIA mode for confident extractables screening. This dual acquisition schema supports quantitation of known leachables and concurrent monitoring of potential new extractables through screening and identification of unknowns.

Figure 1. Waters Xevo MRT P10 Mass Spectrometer and ACQUITY™ Premier System.

Experimental

Sample Description

A commercial nasal spray was purchased, and the neat solution was removed for analysis. The nasal container closure system was extracted and previously acquired and reported.[6] The nasal spray solution was diluted 1 in 10 with isopropanol and spiked with the Waters E&L system suitability test mix (SST) mix and injected in triplicate on the instrument.

Method Conditions

Eluate was analyzed using an advanced mixed mode acquisition incorporating, in parallel, targeted and

untargeted acquisitions (DIA scans (MS^E) with scheduled targeted Tof MRM within a single injection). In addition, a nasal spray solution was spiked with the E&L SST mix to investigate the results in a complex matrix.

LC Conditions

LC system:	ACQUITY Premier System
Column(s):	ACQUITY CORTECS™ C_{18} Column, 90 Å, (1.6 µm, 2.1 x 100 mm)
Column temperature:	50 °C
Injection volume:	1 µL
Flow rate:	0.3 mL/min
Mobile phase A:	Water + 1 mM ammonium acetate + 0.1% formic acid
Mobile phase B:	Methanol
Gradient:	Mobile phase B was held at 2% for 0.5 minutes before being ramped to 98% over 5.5 minutes then held for 7 minutes. It was then reduced to 2% for 2 minutes.

MS Conditions

MS system:	Xevo MRT P10 Mass Spectrometer
Ionization mode:	ESI+/ESI-
Acquisition range:	*m/z* 50-1200

Source temperature:	120 °C
Desolvation temperature:	550 °C
Desolvation gas flow:	800 L/hr
Cone gas flow:	50 L/hr
Capillary voltage:	2.5 kV
Collision energy:	Low energy: 6 eV High energy ramp: 20-40 eV
Cone voltage:	40 V

Data Management

The waters_connect™ Software Platform was used for data acquisition and the UNIFI™ Application and MS Quan Application were used for data processing.

Results and Discussion

MS^E is a data independent approach where alternating low and high collision energy is applied, enabling the acquisition of both precursor and fragment ions throughout the entire chromatographic run.[7] This acquisition mode is effective for extractables screening workflows where the accurate mass of precursor and fragment ions with low- to sub- ppm mass accuracy is critical to make confident screening identifications.

To ensure that targeted leachables quantitation can be undertaken on the same instrument platform, increased sensitivity is needed to meet low E&L screening thresholds. The Xevo MRT P10 Mass Spectrometer uses Tof MRM which 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.[8,9]

These two modes were run concurrently in the same injection providing both the sensitive, targeted data analysis and the high mass accuracy precursor and fragment ion data for every peak in the chromatogram. The E&L SST mix was run as a calibration curve and spiked into the solution from a nasal spray device and each injection acquired under mixed mode conditions. Figure 2 shows the MRM transitions compared to the extracted ion chromatogram (XIC) of Ethanox™ 1330 from MS^E mode, acquired in the same injection.

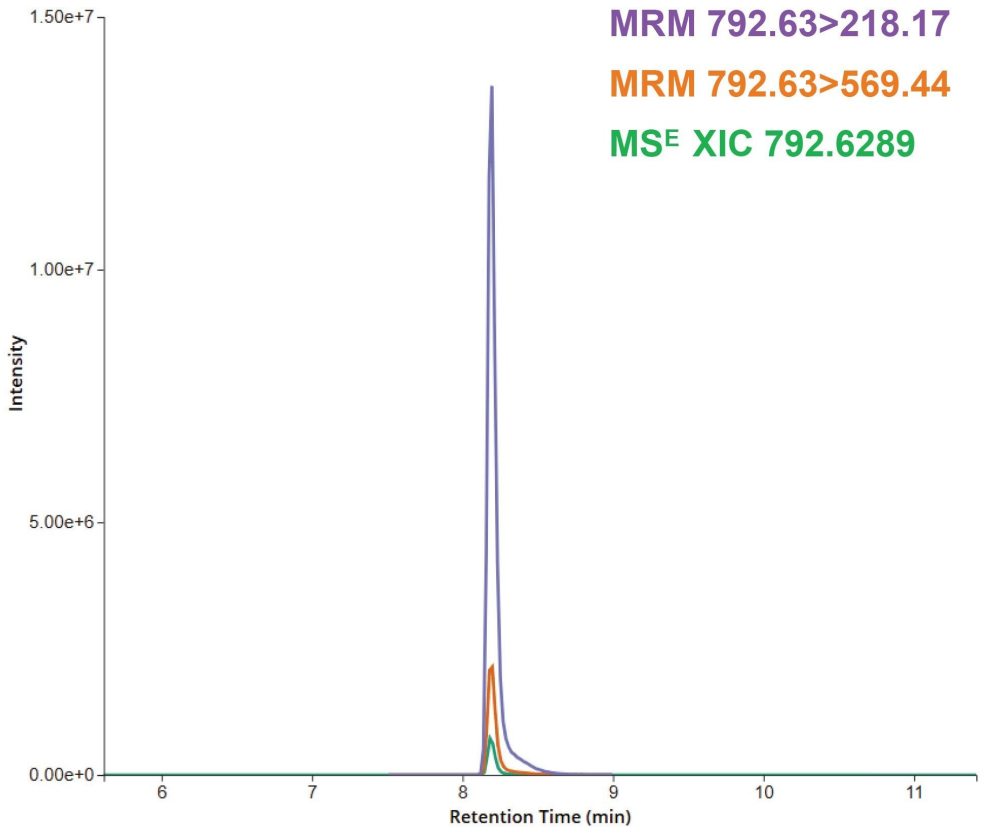

Figure 2. Overlaid chromatograms of MS^E and two MRM transitions acquired in the same injection for Ethanox 1330.

Optimized Tof MRM transitions were acquired to quantify E&L compounds at trace levels. The propylparaben calibration curve, in negative ionization mode, was linear from 0.01 ng/mL (signal to noise (S/N) 15) to 100 ng/mL (R^2 0.99). An average of the concentrations of the replicate spiked compound in the sample was calculated to be 10.04 ng/mL (actual concentration 10 ng/mL) (Figure 3 and Table 1).

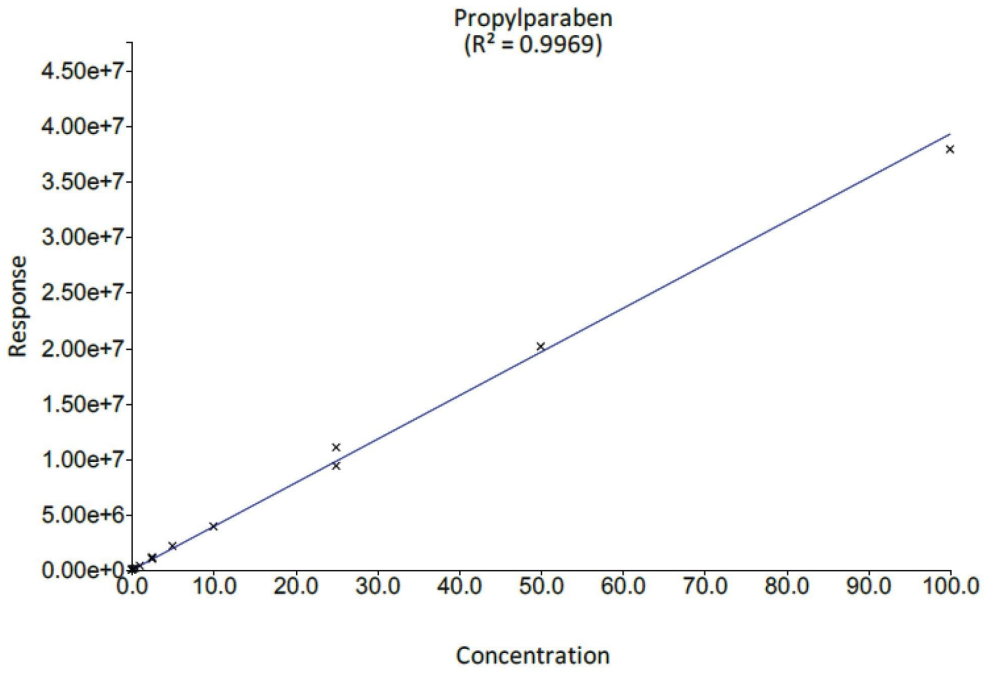

Figure 3. Propylparaben calibration curve.

Compound Name	Injection Name	Response	Calculated Concentration (ng/mL)	Expected Concentration (ng/mL)	% Deviation	Signal to Noise
Propylparaben	C1	7353.9	0.011	0.01	9.87	15
Propylparaben	C2	22830.1	0.05	0.05	0.66	23
Propylparaben	C3	39139	0.09	0.1	-8.21	45
Propylparaben	C4	94486	0.23	0.25	-7	79
Propylparaben	C5	168754	0.42	0.5	-15.74	132
Propylparaben	C6	353783	0.89	1	-10.83	474
Propylparaben	C7	984017	2.49	2.5	-0.25	176
Propylparaben	C8	2140121	5.43	5	8.66	190
Propylparaben	C9	3896294	9.90	10	-1.03	206
Propylparaben	C10	11037388	28.10	25	12.2	204
Propylparaben	C11	9368267	23.80	25	-4.77	191
Propylparaben	C12	20134730	51.20	50	2.36	204
Propylparaben	C13	37927199	96.40	100	-3.59	212
Propylparaben	IPA spiked	3999580	10.20			215
Propylparaben	IPA spiked	3773181	9.58			205
Propylparaben	IPA spiked	3659393	9.30			204
Propylparaben	Solution_spiked	3550718	9.02			209
Propylparaben	Solution_spiked	3781450	9.61			201
Propylparaben	Solution_spiked	4452750	11.30			222

Table 1. Calculated concentration of propylparaben in the spiked sample.

The MS^E data, acquired during the same experiment, were screened against the Waters E&L library for identification. The spiked standards were confidently identified in addition to other library matches, including erucamide (Figure 4). Data generated in this study provided mass accuracies with an RMS of low- to sub-ppm. This significantly narrows the window of possible compounds and vastly increases the confidence in the identifications returned.

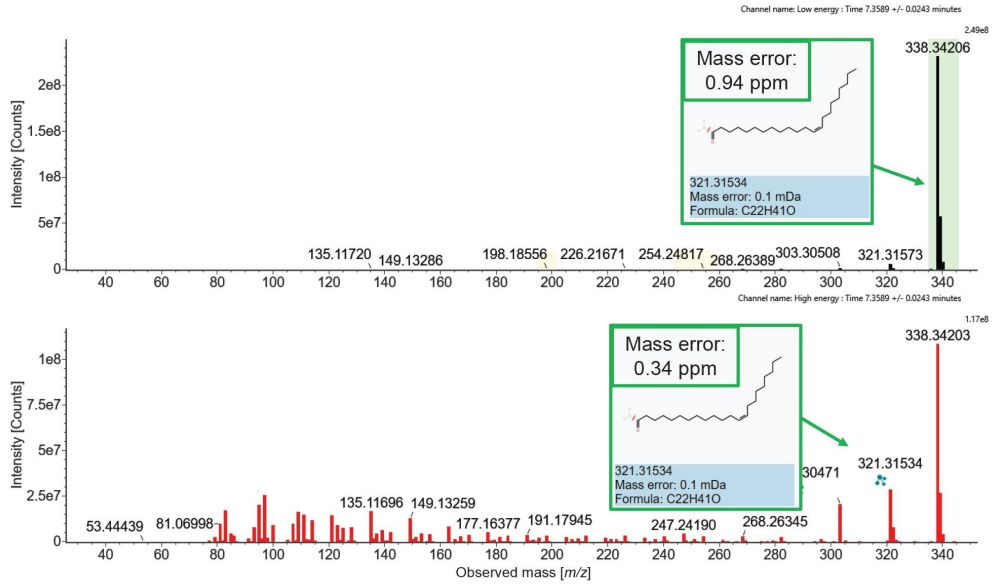

Figure 4. Putative identification of protonated m/z 338.3417 (mass error 0.94 ppm) as erucamide from MS^E, with a corresponding mass fragment, m/z 321.3152 (mass error 0.34 ppm).

Conclusion

As E&L screening moves to a lifecycle risk management approach, technology improvements can help to streamline this process. Advanced mixed mode acquisition of quan/qual data in one injection on the Xevo MRT P10 Mass Spectrometer enables retrospective interrogation of data so that an analyst is able to continuously risk assess their product.

Tof MRM with EDC acquisition mode allows for highly sensitive targeted quantitation of leachables with HRMS. Concurrent MS^E mode acquisition facilitates retrospective data interrogation with high mass accuracy precursor and fragment ion data for confident identifications.

The Xevo MRT P10 Mass Spectrometer is an effective platform for both characterization of extractables with high mass accuracy data for confident identifications and also, for targeted leachables analysis with the highly sensitive Tof MRM mode.

References

1. USP-NF/PF, <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. ⟨1664⟩ Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems < https://doi.usp.org/USPNF/USPNF_M7127_03_01.html>

2. USP-NF/PF, <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems. ⟨1663⟩ Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems <https://doi.usp.org/USPNF/USPNF_M7126_03_01.html>

3. Norwood D., Paskiet D., Ruberto M., Feinberg T., Schroeder A., Poochikian G., Wang Q., Deng T., Degrazio F., Munos M., Nagao L. *Pharmaceutical Research*. 25. 727–39, 2008.

4. ISO 10993-18:2020 Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process, ISO 10993-18:2020 <https://www.iso.org/standard/64750.html>

5. ICH Q3E Guideline for extractables and leachables, August 2025. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-ich-q3e-guideline-extractables-leachables_en.pdf <https://www.ema.europa.eu/en/documents/scientific-guideline/draft-ich-q3e-guideline-extractables-leachables_en.pdf>

6. 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.

7. Stevens D., Cabovska B., Bailey A. Detection and Identification of Extractable Compounds from Polymers. Waters Application Note 720004211. January 2012.

8. 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.

9. Tomcyzk, N., Wallace, A., Richardson, K., Grzyb, A., Wildgoose, J. Targeted High Resolution Quantification with Tof-MRM and HD-MRM. Waters Application Brief, 720004728 <https://www.waters.com/nextgen/gb/en/library/application-notes/2013/targeted-high-resolution-quantification-with-tof-mrm-and-hd-mrm.html> . June 2013.

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