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Applikationsbericht

Natural Products Solution on the Xevo™ G3 QTof Mass Spectrometer

Lisa Reid, Mark Towers, Lee A. Gethings, Jayne Kirk

Waters Corporation

Abstract

The rapid, accurate screening of natural products utilizing the Waters™ Xevo G3 QTof Mass Spectrometer demonstrates a powerful, robust, and flexible platform for natural product analysis. With a dynamic range of up to five orders of magnitude, a ~30,000 FWHM mass resolution and a high mass accuracy: typically within 2 ppm for small molecule applications, this compact benchtop QTof Mass Spectrometer is ideally suited as a routine platform for natural products screening and authenticity testing. Offering a fully flexible solution, data can be acquired using a diverse range of acquisition methods ***e.g.*** MS, MS^E, MRM, DDA, or SONAR, and comes with the option of two instrument control software platforms: waters_connect™ or MassLynx™ depending upon laboratory compliance requirements.

Benefits

- Robust, reproducible system with high mass accuracy, excellent mass resolution, and a wide linear dynamic range for natural product active compounds
- A versatile system offering a choice from multiple acquisition methods depending upon assay objectives
- Comprehensive screening libraries available affording rapid data processing and reporting
- Option of instrument control software depending upon laboratory compliance requirements

Introduction

Within the EU and USA, any natural products supplied, claiming therapeutic properties, must adhere to strict regulations. These allow only products assessed by authorities such as the Medicine and Healthcare Products Regulatory Agency (MHRA) or Food and Drug Agency (FDA) to be sold. These agencies demand and enforce that a rigorous quality control process be in place to which manufacturers must adhere, these prove that the products have been manufactured to strict standards and ensure they contain a consistent and clearly marked dose of any active ingredients. The regulation means manufacturers and contract testing organizations must unequivocally demonstrate the provenance, purity, and quantity of the natural materials contained within their products.

Should manufacturers and/or authorities wish to quality control the levels of the active components found within natural products then liquid chromatography-mass spectrometry (LC-MS) is a reliable, well-established platform for their measurement. These systems are able to separate the various isomeric species (***e.g.***, catechin and epicatechin) and further specificity to differentiate confidently the components of interest from other high abundance and complex matrix analytes such as flavonoid species ***e.g.*** kaempferol quercetin, or polyphenol species such as theogallin. To ensure speed and efficiency of testing it is beneficial if the method demonstrates a wide linearity range for these highly variable natural compounds.

For thousands of years green tea has been prepared by rapid steaming and drying freshly harvested leaves of the plant *Camellia sinensis.*[1] Green tea contains a range of phenolic compounds, specifically the flavon-3-ols known as catechins.[2] Catechins are a class of molecules well documented as possessing anti-inflammatory- and antioxidant properties and are thought to contribute to the health benefits associated with drinking the product. The antioxidant levels in commercially available green tea can, however, vary significantly based upon - but not limited to - conditions of growth, harvest timing, processing, storage, and final preparation.[1] There is also potential for adverse health effects notably hepatic toxicity associated with green tea consumption, in particular: excessive catechin exposure and the possibility for contamination with pyrrolizidine alkaloids (PA).[2]

The method outlined in this document identifies and quantifies known constituents of green tea, utilizing chromatographic retention time, accurate mass, and fragmentation pattern (both theoretical and analytically derived). With a focus on the active components (catechins) the selectivity of the method and the advantages of a flexible platform offering multiple different acquisition modes is demonstrated.

Experimental

Sample Description

Green Tea Matrix (p/n: 186006962 <https://www.waters.com/nextgen/global/shop/standards--reagents/186006962-green-tea-extract-mix.html> , Waters Wilmslow, UK), was diluted in 25:75 v/v methanol:water to a concentration of 8.25 mg/mL, vortexed, sonicated for 30 minutes and centrifuged at 10,300 g for 10 minutes at 4˚C to remove particulates.

The catechin standard curve was produced using a catechins 100 µg/mL standard purchased from Sigma (Dorset, UK) diluted in 25:75 v/v methanol:water.

LC Conditions

LC system:	ACQUITY™ I-Class Premier FTN
Column(s):	ACQUITY Premier UPLC™ HSS T3 (100 mm x 2.1 mm, 1.8 µm)
Column temperature:	40 °C
Injection volume:	1 µL
Flow rate:	0.6 mL/min
Mobile phase A:	Water 0.1% formic acid
Mobile phase B:	Acetonitrile 0.1% formic acid
Gradient green tea:	99% A hold 0.5 minutes, 99%–65% A 0.5–16 minutes, 1% A hold 16–18 minutes, re-equilibrate initial conditions 18–20 minutes.

MS Conditions

MS system:	Xevo G3 QTof
Capillary voltage (kV):	1.5 in Positive ion mode
Sampling cone:	20 V
Source offset:	80 V
Source temperature:	120 °C
Desolvation temperature:	600 °C
Cone gas flow:	150 L/hr
Desolvation flow:	800 L/hr
StepWave mode:	Soft Transmission[3]
Detector auto gain:	On
Analyzer mode:	Sensitivity
Data format:	MS^E continuum/MRM/DDA/SONAR
Acquisition range:	50–1200 Da
SONAR acquisition\range:	250–500 Da (10 Da window)
Scanning speed:	10 Hz (5 Hz for SONAR)
Fragmentation CE:	25–45 V

Lockspray flow:	10 µL/min
Lockspray settings:	Average 3 scans every 60 seconds
IDC:	Off

Results and Discussion

This assay was performed on a commercially available green tea matrix and a catechins standard mixture, these were prepared by a simple dilution of liquid standards or reconstitution of lyophilized matrix, followed by centrifugation to remove particulates. No clean-up of the green tea matrix was performed.

Using an ACQUITY Premier UPLC chromatography System and a Xevo G3 QTof Mass Spectrometer a 1 µL injection of an 8.25 mg/mL green tea matrix standard and a catechin standard were analyzed in positive ionization mode with MS^E, MRM, DDA, and SONAR acquisition modes. Utilizing the new StepWave™ design, data was acquired with soft ionization parameters, ensuring pre-quad precursor fragmentation was minimal and providing optimal transmission of analytes.[3]

The data displayed in Figure 1 demonstrates the selected component response for a single injection of catechin standards (compound displayed is epicatechin-3-gallate), the visual includes a table providing all analytical data for the selected identified compound, an XIC of the chromatographic peak for a selected sample and the selected component, and both the high and low energy spectra for the selected analyte. Stacked plots allow both spectra (precursor and fragment ions) to be visually matched, the high energy spectrum visual includes an indication of positively identified fragments, these can be expanded to show proposed corresponding chemical structure (as displayed).

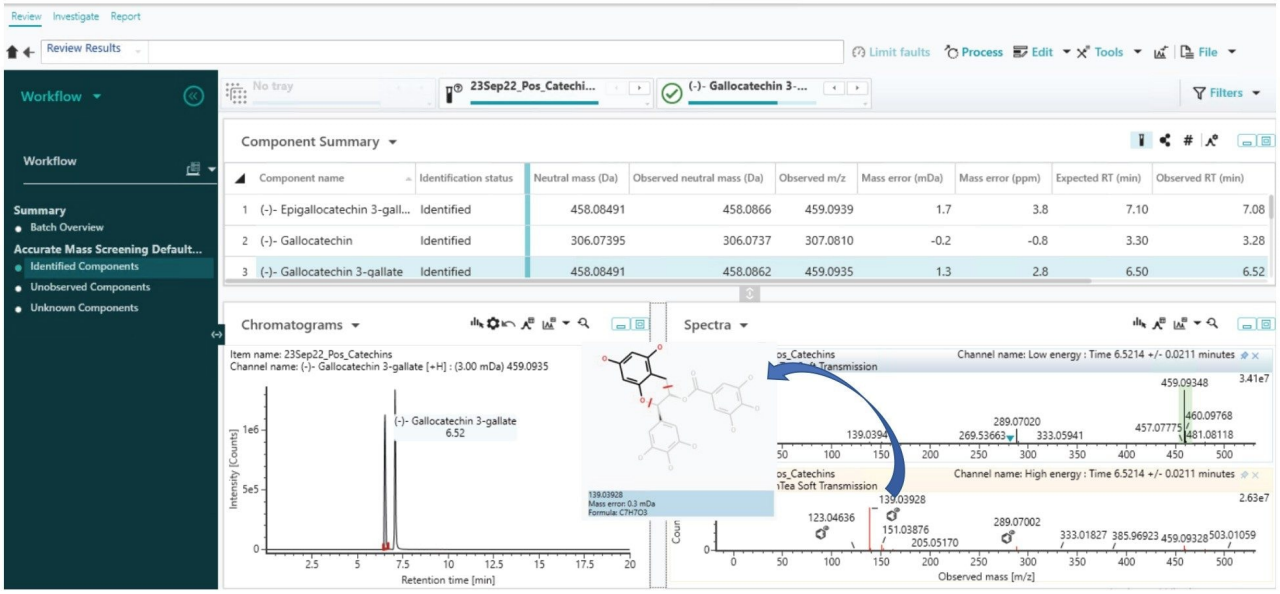


Figure 1. waters_connect/UNIFI™ display showing table of identifications in one selected injection with a summary for each compound, an XIC for a selected compound (in this case Gallocatechin-3-gallate), with a low and high energy spectra for the selected compound. The high energy spectra indicates identified fragments which can be expanded to see fragment mass information and structure as shown.

The Xevo G3 QTof Mass Spectrometer offers the user the ability to choose the instrument control software between MassLynx and waters_connect allowing users to tailor their experience to their laboratory environment. The waters_connect informatics platform allows users to utilize the UNIFI application for data processing, within which these data were processed against a downloadable *Camelia Sinensis* library. This library was used to aid compound identification based upon chromatographic retention time (for a given method), mass accuracy, and presence of fragment ions compared to a theoretical fragmentation pattern. When an MS^E screening acquisition has been performed and processed within the UNIFI application it is possible to view "unidentified" components as well as those contained within the library. Unidentified compounds can be filtered into or out of the processing session, depending upon whether the focus is on confirmation of known constituents or for comprehensive screening.

The data generated from an injection of green tea, when processed through the *Camelia Sinensis* filtered UNIFI library containing 27 natural green tea compounds, gave 24 matched identifications (eight confirmed with standards, 16 putative with mass and theoretical fragmentation matching only) for positive ion polarity and 23

matched identifications for negative ion polarity (seven confirmed with standards, 16 putative with mass and theoretical fragmentation matching only).

The MS^E, a data independent mode of analysis (DIA) allows all ions within the analysis mass range to pass through the quadrupole for detection in the low energy channel and will fragment all ions within the mass range and display all fragment data within the high energy channel. The high energy spectra will display all ions from co-eluting species as all ions are transmitted though the quadrupole. This is the least restrictive acquisition method for generating precursor and product ion information and provides information rich data sets.

The Tof-MRM functionality is a DIA acquisition on the Xevo G3 QTof Mass Spectrometer, it follows the same principle as an MRM experiment on a tandem quadruple Mass Spectrometer and is ideal for obtaining quantitative information. The experiment is targeted, with precursor ions chosen by the user selected in the quadrupole and fragmented in the collision cell. Both the low energy precursor information and high energy/product ion information will be available as channels in the dataset. A high degree of sensitivity can be obtained in this mode and is therefore well suited to experiments where targeted quantitative information is required.

The SONAR functionality, a DIA mode of acquisition utilizes scanning quadrupole technology, by selecting the mass range of interest ***e.g.*** 200–500 Da the quadrupole filters ions within user determined mass windows ***e.g.*** 2 Da, these are then fragmented within the collision cell and detected at any one time. This enables each compound with a mass difference greater than the set window to be fragmented individually and data stored within the high energy function. This mode of acquisition provides clean spectra without the loss of compound data from either manually setting analytes of interest (MRM) or only selecting those ions with the highest signal (DDA). Due to the nature of a scanning quadrupole this data will be of lower intensity than that produced by the alternative acquisition modes. It is recommended to use a scanning quadrupole mass range of ≤300 Da width, to improve sensitivity of the acquisition, compound masses which fall outside of this range will not be acquired.

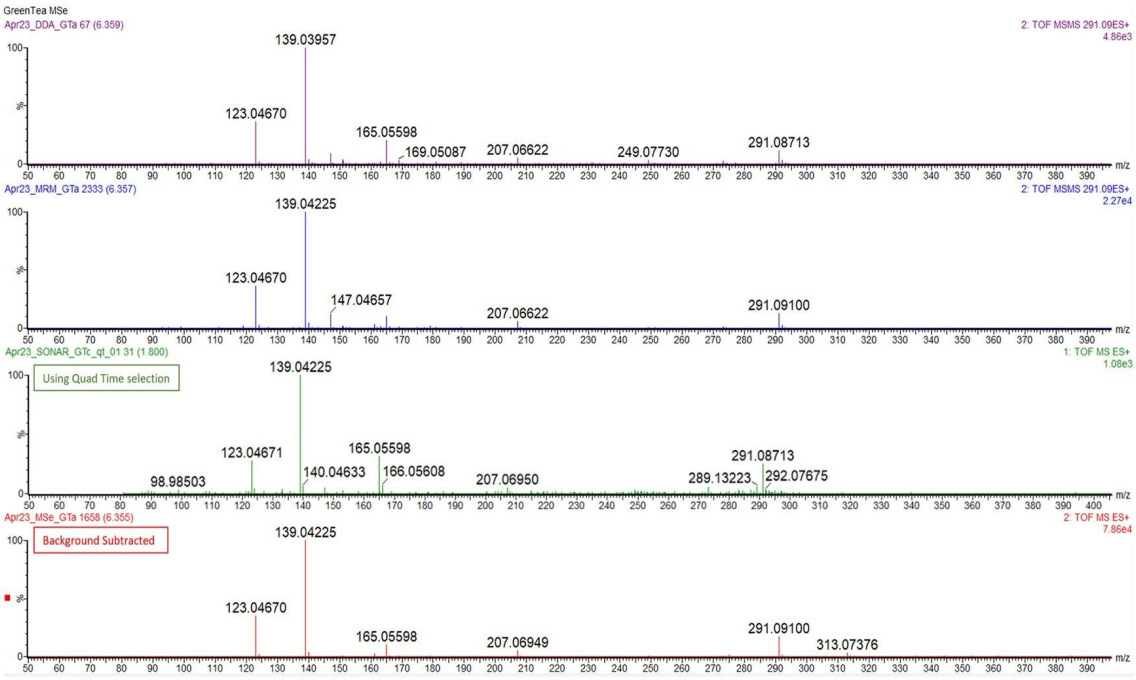
For more information please see: Waters White Paper 720006033 < https://www.waters.com/waters/library.htm?locale=en_US&cid=134908444&lid=134944704> .

The Data Dependent Acquisition (DDA) mode isolates and fragments precursor ions based on intensity, this can be using an include list or charge state (typically used for biomolecules) or can be based upon ion intensity. Once isolated by the quadrupole, ions are then fragmented within the collision cell and product ions analyzed. As the quadrupole is used to select and transmit ions of interest an MS/MS product ion spectrum is obtained, aiding in precursor ion assignment and identification. Setting for DDA examples can be optimized to ensure the

appropriate number of precursor ions are selected within a complex matrix, while minimizing switching on matrix or background ions.

When a green tea sample was analyzed using all the acquisition modes detailed above. The targeted/semi-targeted: MRM, DDA, and SONAR acquisition modes provide enhanced spectral clarity and increases confidence in product ion identification. Figure 2 shows a comparison of high energy spectra for epicatechin over the four analysis modes. In positive ionization mode, Figure 3 the analyte of interest predominantly fragments into *m/z* 123.1 (formula $C_7H_7O_2$), *m/z* 139.0 (formula $C_7H_7O_3$), *m/z* 147.0 (formula $C_9H_7O_2$), *m/z* 165.0 (formula $C_9H_9O_3$). All fragment ions were observed in all analysis modes. In the MS^E acquisition mode, however, there are a number of additional ions generated from the fragmentation of co-eluting species. These are reduced with the background subtract functionality available as a tune paramater option during acquisition. In acquisition modes where the quadruploe is isolating precursor fragment ions, interference from co-eluting peaks is reduced or eliminated.

The MS^E acquisition mode is ideal mode for screening of unknown compounds. The SONAR Tof MRM and DDA analyses reduce the complexity of the high energy spectra removing ions produced from either the background or co-eluting (alternative *m/z*) species making these modes better suited to more targeted/semi-targeted applications.



High energy spectra showing the MSE, MRM, DDA and SONAR analysis of Epicatechin Precursor m/z 291.1 Da. Spectra A (red) shows background subtracted MSE high energy spectra for Epicatechin, spectra B (green) shows quad time selected SONAR high energy spectra for Epicatechin, spectra C (blue) shows the MRM high energy spectra for Epicatechin and spectra D (purple) shows the DDA high energy spectra for Epicatechin.

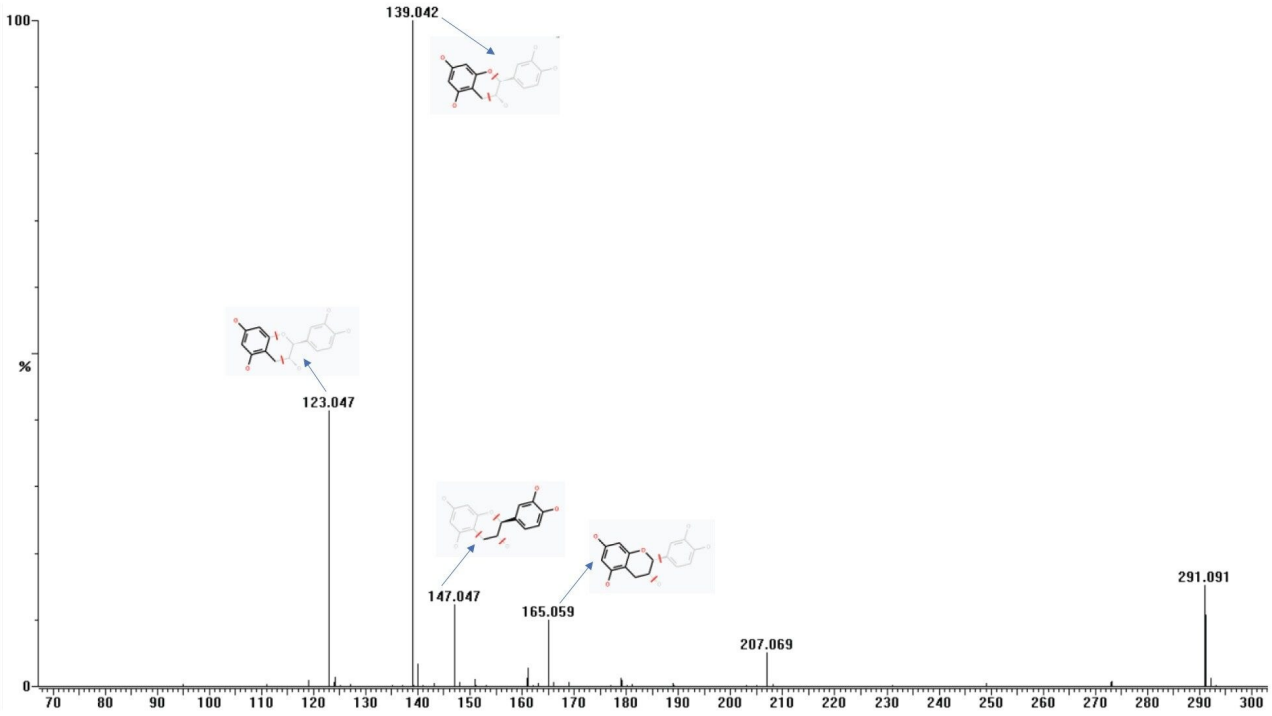


Figure 3. Expected high energy spectra (MRM) showing the fragmentation pattern of Epicatechin Precursor m/z 291.1 Da.

Conclusion

The Xevo G3 QTof platform offers a powerful solution for natural product screening applications.

The range of acquisition modes provides flexibility to the analyst depending on the questions being asked. Whether it be a targeted experiment of known compounds or comprehensive screening of a complex sample there is a suitable experiment type provided by this platform.

Choice of software platform allows for a tailored experience based upon laboratory environment, application, or user requirements. The new StepWave design enables transmission optimization based on fragility of compounds of interest, ensuring improved sensitivity for target analytes. Along with excellent mass accuracy,

sensitivity, and a wide dynamic range the Xevo G3 QTof Mass Spectrometer is equipped with all the necessary tools for effective natural product analyses.

References

1. Pinto, G; Illiano, A; Carpentieri, A; Spinelli, M; Melchiorre, C; Fontanarosa, C; Di Serio, M; Amoresano, A. Quantification of Polyphenols and Metals in Chinese Tea Infusions by Mass Spectrometry, *Foods* 9(6):835; June 2020.
2. Younes, M; Aggett, P; *et al*. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Scientific Opinion on the Safety of Green Tea Catechins, *EFSA Journal* 16(4); April 2018.
3. Lisa Reid, David Pickles. Improved Transmission of Labile Species on the Xevo™ G3 QTof Mass Spectrometer with the StepWave™ XS, Waters Application Note 720007794, November 2022.

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UNIFI Scientific Information System <https://www.waters.com/waters/nav.htm?cid=134801648>

waters_connect <https://www.waters.com/nextgen/global/products/informatics-and-software/waters_connect.html>

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