Waters™

Note d'application

Green Tea Screening Using the ACQUITY RDa Detector

Lisa Reid, Lauren Mullin, Robert S. Plumb, Giorgis Isaac, Emmanuelle Claude, Jayne Kirk

Waters Corporation

Abstract

A method for rapid, accurate profiling of green tea extract utilizing the Waters ACQUITY RDa Detector, a high-resolution LC-MS time-of-flight (ToF) mass analyzer. The instrument exhibits a dynamic range of up to 3 orders of magnitude and a high mass accuracy consistently within ±5 ppm, demonstrated with catechin compounds. This compact benchtop time-of-flight mass spectrometer is ideally suited for natural products screening and authenticity testing.

Combining the ACQUITY RDa Detector with the waters_connect Software platform and UNIFI application allows data acquisition and processing to be performed in tandem. The incorporation of easily tailored workflows and library searching simplifies and speeds up analysis and processing times – producing clearly displayed results, easily converted to a report format to enable rapid decision making.

Benefits

- Compound identification in green tea matrix using accurate mass and chromatography specific retention time matching
- Comprehensive screening library affording rapid data processing in both positive and negative ion polarity
- Advanced data visualization, processing, and reporting tools
- Robust, reproducible system with high mass accuracy and a suitable dynamic range for catechins

Introduction

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 is a widely consumed beverage and driving this consumption are the perceived health benefits found from certain components, such as the flavonoids, as well as recreational intake for other compounds found within the leaves, such as caffeine (a methylxanthine stimulant and adenosine receptor antagonist).

Green tea contains a range of phenolic compounds, specifically the flavon-3-ols (known as catechins).[1] Catechins, a class of molecules well documented as possessing anti-inflammatory and antioxidant properties, are present in green tea. They are thought to contribute to the health benefits associated with drinking the product, one example being cancer prevention.[1,2] The antioxidant levels in commercially available green tea, however, can vary significantly based upon but not limited to conditions of growth,

harvest timing, processing, storage, and final preparation.[3] There is also potential for adverse health effects, notably hepatic toxicity, associated with green tea consumption, specifically excessive catechin exposure and the possibility for contamination with pyrrolizidine alkaloids (PA).[3]

Liquid chromatography-mass spectrometry (LC-MS) is a well-established, reliable platform for the measurement of components found within green tea products. These systems are able to separate the various isomeric species (e.g., catechin and epicatechin) and further specificity to confidently differentiate 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.

The method outlined below identifies known constituents of green tea utilizing chromatographic retention time, accurate mass, and fragmentation pattern (based upon both theoretical and analytically derived). With particular focus on the active components (catechins), the high selectivity and the wide dynamic range of the system have been proven. The ACQUITY RDa Detector, being a time-of-flight mass analyzer, has an advantage over traditional, selected ion monitoring mode quantification assays in that the data is acquired in "full scan" mode (meaning that no pre-selection of ions occurs prior to detection). These conditions make the instrument ideal for purity and contamination assessment of raw materials prior to manufacturing. Coupled to waters_connect using the UNIFI app, the data acquisition and processing is contained within a single software platform ensuring the entire workflow is rapid and streamlined.

Experimental

Sample Description

Green tea matrix (p/n: 186006962, Waters Wilmslow, UK), was diluted in 25:75 v/v methanol:water to a concentration of 2.5 mg/mL, vortexed, sonicated for 30 minutes and centrifuged at 13,000 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. The catechin standard mix contains a certified multi-component mix of caffeine and seven of the common green tea catechins: epigallocatechin 3-gallate, catechin, epicatechin, epicatechin 3-gallate, gallocatechin, gallocatechin 3-gallate, and catechin 3-gallate.

LC Conditions

LC system:	ACQUITY UPLC I-Class FTN
Column(s):	ACQUITY UPLC HSS T3 (100 mm x 2.1 mm, 1.8 µm)
Column temp.:	40 °C
Injection volume:	10 µL
Flow rate:	0.6 mL/min
Mobile phase A:	Water 0.1% formic acid
Mobile phase B:	Acetonitrile 0.1% formic acid
Gradient:	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:	ACQUITY RDa Detector
Ionization mode:	Both negative and positive ion modes
Acquisition range:	50–2000
Scanning speed:	10 Hz
Capillary voltage:	0.8 kV Neg, 1.5 kV Pos
Cone voltage:	30 V Neg, 40 V Pos

Fragmentation cone voltage:	60–120 V
IDC:	On
Software:	waters_connect with UNIFI v1.9.12

The ACQUITY RDa Detector automatically performs a system setup as part of its SmartMS technology. This means all calibration, tuning, and lockmass optimization are performed by the instrument prior to analysis and checked between each injection thus removing the requirement to perform these tasks manually. This requires the following solutions:

Lockmass solution:	ACQUITY RDa Lockmass Kit (Waters p/n: 186009298)
Calibration solution:	ACQUITY RDa Calibration and Wash Kit (Waters pn: 186009183)
Wash solution:	ACQUITY RDa Calibration and Wash Kit (Waters pn: 186009183)

Results and Discussion

The LC-MS analysis was performed on a commercially available green tea matrix and catechin standard mixture by a simple dilution of liquid standards or reconstitution of lyophilized matrix, followed by centrifugation to remove particulates. No cleanup of the green tea matrix was performed.

Using an ACQUITY UPLC I-Class inlet and an ACQUITY RDa Detector a 10 µL injection of a green tea matrix standard mix (8 mg/mL) was analyzed in both positive and negative ion acquisition modes. Utilizing UNIFI Software, component matching was performed against a *Camellia sinensis* scientific library. This library can be 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. The LC-MS data generated was searched against a green tea filtered UNIFI library that contains 27

compounds commonly identified within green tea. Positive ion mode data gave 23 matched identifications (7 confirmed with standards, 16 putative mass accuracy matching and theoretical fragmentation only) catechin -3-O-gallate did not appear in the green tea sample at a high enough concentration for identification, however, was successfully identified within the standard mix. Negative ion mode data gave 24 matched identifications (7 confirmed with standards, 17 putative matching mass accuracy and theoretical fragmentation only). Figure 1 shows a typical review pane from UNIFI clearly indicating the compound identified, an XIC of the chromatographic peak, low and high cone voltage mass spectra, and a navigable table of other identified compounds within the sample. In the channel 2 (fragmentation cone voltage) mass spectrum precursor and fragment ions can be seen, and retention time matched with the low energy mass spectrum. The UNIFI processing informatic platform is also able to tabulate “unidentified” components. 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.

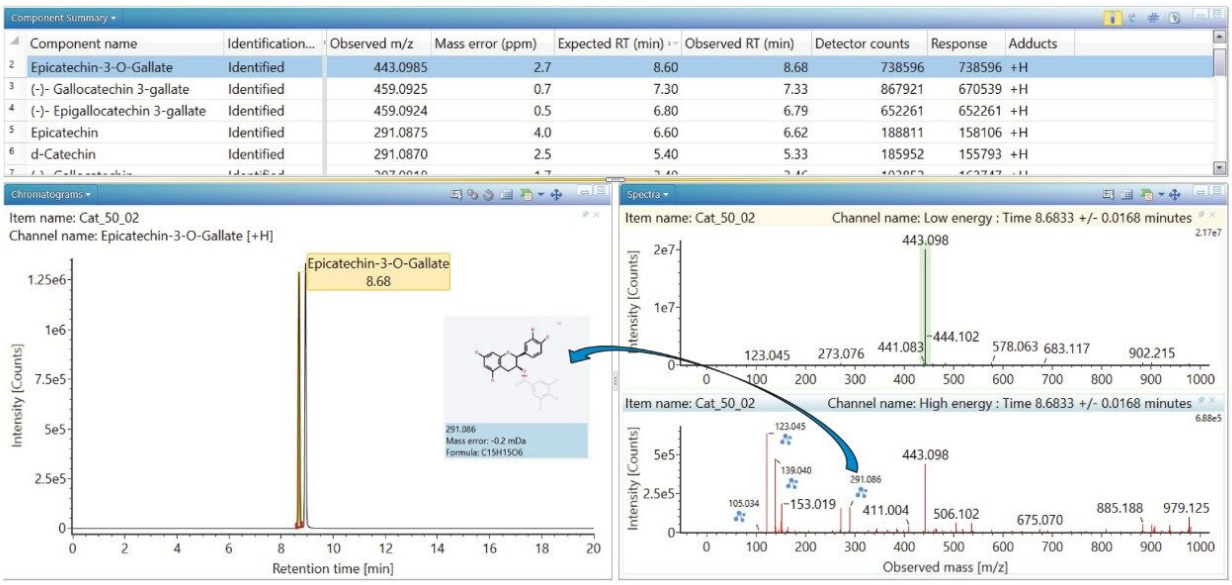

Figure 1. UNIFI display showing table of identifications in a catechin standard injection with a summary for each compound, an XIC for the identified epigallocatechin-3-gallate, and a low and high cone voltage mass spectra for the epigallocatechin-3-gallate. The high energy spectra indicates identified fragments which can be expanded to see fragment mass information and structure as shown.

In addition to library searching, the system dynamic range was tested to ensure data confidence at typical analysis levels. For this assessment, a dilution series was prepared using commercially available catechin standards with the range: 0.01 µg/mL to 25 µg/mL, injecting 10 µL of each concentration level resulted in a

range of 0.1 ng to 250 ng material on column. This analysis was performed in positive ion mode and demonstrated between 2 and 3 orders of magnitude for these compounds on the ACQUITY RDa Detector. At 1 ng on column all 8 catechin compounds had a signal to noise ratio of greater than 400, and repeat injection reproducibility %RSD of less than 4.7%. The ULOQ for all catechin standard mix was 100 ng on column. At 250 ng on column the chromatography showed signs of overloading with peak broadening, however repeat injection reproducibility was still acceptable with a response %RSD of less than 6.7% for all compounds.

To assess the within sample dynamic range capabilities of the system, a commercially available green tea matrix was analyzed at three pre-particulate concentrations equivalent to: 0.8 mg/mL (10x dilution), 8.3 mg/mL (typical analysis level), and 82.5 mg/mL (10x concentrated) of total green tea components. The analysis demonstrates the system suitability for endogenous catechin detection at varying sample strengths and potential varying compound concentration levels within different samples. At a typical analytical concentration of 8 mg/mL (total green tea component mix), all compounds were within the established dynamic range, i.e less than 10 µg/mL (or 100 ng on column) of each individual catechin. At 10x the typical concentration (83 mg/mL), all compounds (except epigallocatechin-3-gallate since this compound exceeded the range when injected at this high a concentration) were within the established dynamic range of the system. At 0.8 mg/mL a 10x dilution from the typical concentration 5 out of 8 catechin compounds were within the established dynamic range. The three compounds below the established dynamic range were catechin, catechin-3-gallate, and gallocatechin-3-gallate, peaks could be seen for these compounds with signal to noise greater than three but their response was less than that seen for 1 ng of standard on column.

Figure 2 demonstrates the selected component response over the whole analysis (compound displayed is epigallocatechin-3-gallate). As before, the visual includes a table giving all analytical data for the selected identified compound, an XIC of the chromatographic peak for a selected sample and the selected component, and a bar chart clearly showing response for the compound in each injection over the analytical session. This gives an immediate visual comparison for the entire analysis so that patterns, anomalies, or batch effects can be rapidly assessed.

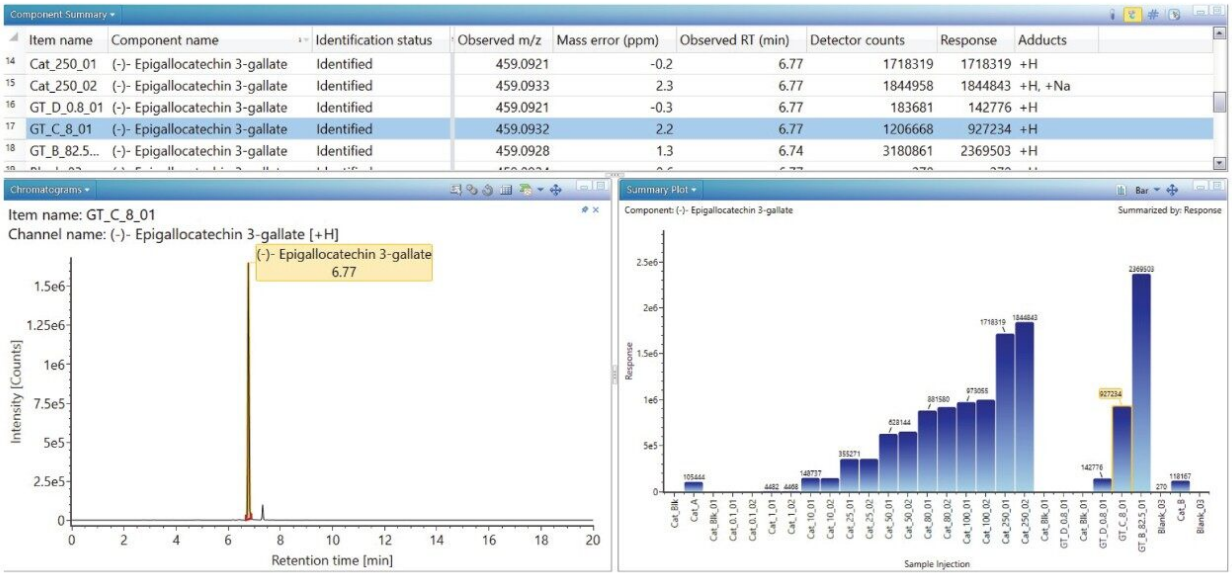

Figure 2. UNIFI display showing table of injections with a summary of the selectred compound for each injection, the XIC for selected compound, and a summary plot providing a visual response for the selected compound over every injection in the analyses for the epigallocatechin-3-gallate.

The analysis of the standard dilution series and matrix injections totaled >12 hours continuous acquisition. Over the entire analysis the chromatographic retention time for each of the 8 catechin standards varied by a maximum of 0.05 minutes (3 seconds) with a maximum RSD of 0.3% seen on the earliest eluting peak (gallocatechin).

The mass accuracy for each of the standards was assessed and the maximum ppm deviation was ±4.9, the average error for all compounds and injections was ±0.9 ppm over the entire analysis. The signal intensity and peak area injection to injection has been assessed using duplicate injections of each sample demonstrating that within the established dynamic range of the instrument, the maximum variance in peak response observed between duplicate injections was 7.6% with an average variance of 2.2%. The signal intensity and peak area robustness for the entire analytical session was assessed using an injection of catechin standards at the beginning and end of the analytical session. Across all catechin standards investigated a 3.6% (average) change was observed for signal count and a 3.6% (average) difference in calculated response was seen between injection 1 and injection 35 (>12 hours of analysis time).

Conclusion

The ACQUITY RDa Detector offers a robust and effective tool for natural product screening applications including but not limited to green tea analysis. With excellent signal reproducibility, mass accuracy, and a wide dynamic range, this LC-MS system is perfectly placed for any natural products laboratory. Coupled to the UNIFI application: library searching, processing, and report generation are simple and rapid. All acquisition and processing are contained within the one software platform eliminating the need to export results compromising data integrity and allowing for complete compliance readiness upon audit trail activation.

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. Forester, S; Lamert, J. Antioxidant Effects of Green Tea, *Mol Nutr Food Res*. 2011 Jun; 55(6): 844–854.
3. 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.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

BioAccord LC-MS System for Biopharmaceuticals <https://www.waters.com/waters/nav.htm?cid=135005818>

UNIFI Scientific Information System <https://www.waters.com/134801648>

Natural Products Application Solution with UNIFI <https://www.waters.com/134777097>

720007226, April 2021