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Application Note

Ginseng Compound Screening using the ACQUITY RDa Detector

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Abstract

The rapid, accurate screening of ginseng utilizing the Waters ACQUITY RDa Detector, a high-resolution LC-MS time-of-flight (ToF) mass analyzer. With a dynamic range of up to 3 orders of magnitude and a high mass accuracy consistently within ±5 ppm for both product and fragment ions, this compact benchtop time-of-flight mass spectrometer is ideally suited as a workhorse 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, produces clearly displayed results which are easily converted to a report format.

Benefits

- Comprehensive screening library affording rapid data processing in both positive and negative polarity
- Advanced data visualization, processing, and reporting tools
- Compound identification in ginseng matrix using accurate mass and fragment ion matching
- Robust, reproducible system with high mass accuracy a dynamic range of up to 3 orders of magnitude for ginsenosides

Introduction

For millennia the roots of ginseng have been prepared and employed for various therapeutic purposes, attributed to the active compounds ginsenosides. Today, many species of ginseng can be found within commercially available natural therapeutic products for example: Asian ginseng *(Panax ginseng),* Japanese ginseng (*Panax japanoicus),* American ginseng (*Panax quinquefolium),* and Sanchi Notoginseng *(Panax notoginseng).* The various species are believed to have different and sometimes opposing therapeutic properties, potentially due to the ginsenoside profile which has been seen to differ greatly depending upon Panax variety.[1]

The saponins known as ginsenosides are comprised of two main groups: the protopanaxadiol (PPD) group includes Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3; and the protopanaxatriol (PPT) includes Rg1, Re, Rf, Rg2, and Rh1.[2] It is thought that American ginseng is richer in certain ginsenosides found within the protopanaxadiol (PPD) group such as Rb1 and Rd, while Korean ginseng is thought to be richer in the protopanaxatriol (PPT)

group with certain ginsenosides such as Rf not being seen present within the American ginseng. In addition to the differences in the ginsenoside profile between the species, the phytochemical profile may also be impacted by factors such as: the age of the root, time of harvest, storage conditions, or preparation processes – with particular note that some ginsenosides exist naturally in a malonyl form (such as Rg1 and Rd) being thermally unstable they may be converted into their corresponding ginsenosides upon steaming.[3]

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 organisations must unequivocally demonstrate the provenance, purity and quantity of the natural materials contained within their products.

The method outlined in this document identifies known constituents of ginseng based upon chromatographic retention time, accurate mass and fragmentation pattern (based upon both theoretical and analytically derived). With a focus on the active components (ginsenosides) selectivity and dynamic range has 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 all data is acquired in “full scan” mode, meaning that no pre-selection of ions occurs prior to detection. Making this instrument ideal for purity and contamination assessment of raw materials prior to manufacturing. Coupled to the UNIFI Software package library searching, processing, and report generation are simple, rapid, and contained within the one platform eliminating the need to export results or data and compromise data integrity.

Experimental

Sample Description

Ginseng USP extract (p/n: Y0001029 Sigma Dorset, UK), was diluted in 70:30 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 ginsengoside standard curve was produced using a ginsenosides standard (p/n: G-015-1ML Sigma Dorset, UK), diluted in 70:30 methanol:water.

LC Conditions

LC system:	ACQUITY UPLC I-Class FTN
Column(s):	ACQUITY UPLC HSS T3 (100 mm x 2.1 mm, 1.8 µm) (p/n: 186003539)
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:	95%–70% A 0–3 minutes, hold 70% A 3–7 minutes, 70%–55% A 7–12 minutes, 55%–5% A 12–18 minutes, hold 1% A 18–20 minutes, re-equilabrate to initial conditions 20–22 minutes

MS Conditions

MS system:	ACQUITY RDa Detector
Ionization mode:	Both positive and negative
Acquisition range:	50–2000
Scanning speed:	10 Hz
Capillary voltage:	0.7kV Neg, 1.5kV Pos
Cone voltage:	30V Neg, 40V Pos

Fragmentation cone voltage:	120–170V
IDC:	On
Software:	waters_connect with UNIFI 1.9.12

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

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

Results and Discussion

This assay was performed on a commercially available ginseng matrix and ginsenosides 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 ginseng matrix was performed.

Using an ACQUITY UPLC I-Class System chromatography system and an ACQUITY RDa Detector a 10 µL injection of a 2.5 mg/mL ginseng matrix standard was analyzed in both positive and negative ion acquisition modes. Utilizing the UNIFI Informatics platform, component matching was performed against a Panax 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. The data generated from this analysis, when processed through a ginsenoside

filtered UNIFI library containing 22 ginsenoside compounds, gave 18 matched identifications (8 confirmed with standards, 10 putative with mass and theoretical fragmentation matching only) for negative polarity, and 17 matched identifications for positive polarity (8 confirmed with standards, 9 putative with mass and theoretical fragmentation matching only).

Figure 1 shows a typical review pane from UNIFI clearly indicating the compound identified (in this case Rg1), an XIC of the chromatographic peak, low and high energy spectra and a navigable table of other identified compounds within the sample. The high energy spectrum precursor and fragment ions can be visually matched with the low energy spectrum displayed above the high energy spectrum. A feature of the high energy spectrum visual includes an indication of positively identified fragments, these can be expanded to show proposed corresponding chemical structure (as displayed).

The UNIFI processing 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.

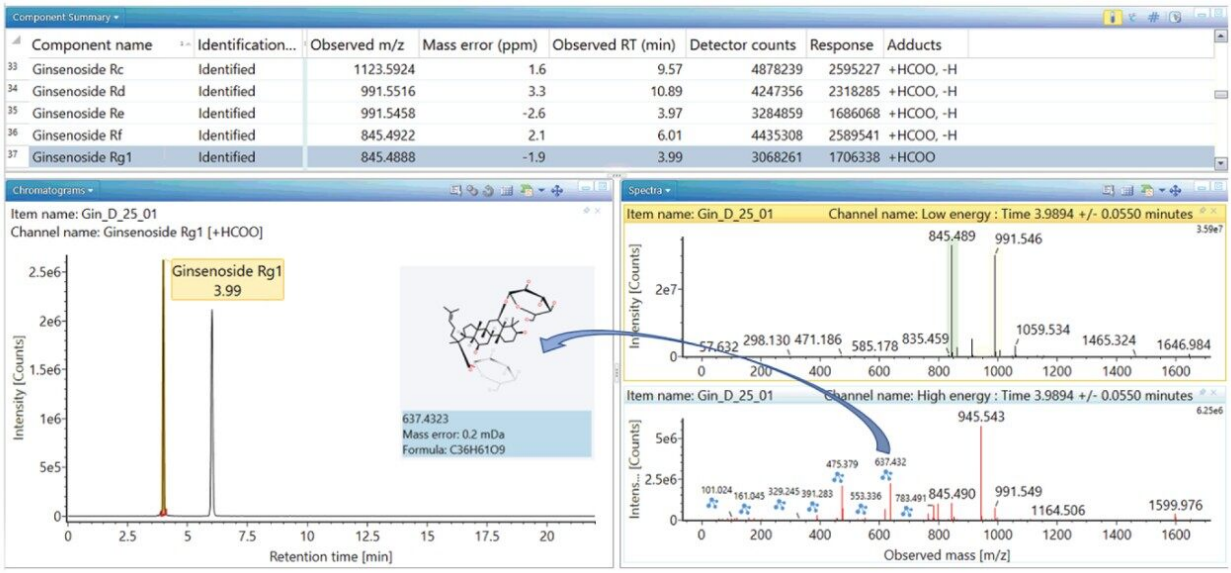

Figure 1. UNIFI display showing table of identifications in one selected injection with a summary for each compound, an XIC for a selected compound (in this case ginsenoside Rg1), and a low and high energy spectra for the selected compound. The high energy spectra indicates identified fragments which can be expanded to see fragment mass information and structure as shown.

In addition to library searching, the system applicability dynamic range was tested to ensure data confidence

at typical analysis levels. For this assessment, a dilution series was prepared using commercially available ginsenoside standards with the range: 0.001 µg/mL to 50 µg/mL, injecting 10 µL of each point resulted in a range of 10 pg (0.01 ng) to 500 ng material on column. This analysis was performed in negative 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 ginsenoside compounds had a signal to noise ratio of greater than 400, and repeat injection reproducibility of less than 1.4% RSD. Injecting 0.01 ng on column peaks could be seen with signal to noise ratio (S/N) of greater than 5, and injecting 0.1 ng on column peaks could be seen with signal to noise ratio of greater than 70, however the response repeat injection reproducibility for both these concentrations was >10% RSD and therefore deemed too variable for reliable statistical analysis. The upper limit of system performance for all ginsenoside compounds within the standard mix was determined to be 250 ng on column. At 500 ng on column the chromatography showed signs of overloading with peak broadening, however repeat injection reproducibility was still good with a response %RSD of less than 4.3% for all compounds.

In addition to the commercially available standard mix, a ginseng test matrix was analysed at five concentrations, pre-particulate concentration equivalent to 0.25 mg/mL, 2.5 mg/mL, 6.3 mg/mL, 12.5 mg/mL, and 25 mg/mL (2.5 µg to 250 µg material on column), to demonstrate the system suitability for endogenous ginsenoside detection at varying concentrations. At a typical analytical concentration of 2.5 mg/mL, all compounds were within the established dynamic range, i.e less than 25 µg/mL (or 250 ng of individual ginsenoside compound on column). The details of which ginsenoside compounds were observed within the established dynamic range of the system at each ginseng concentration are given in Table 1. In the higher concentrations of matrix some ginsenoside signals exceed the established dynamic range however the response %RSD between duplicate injections remained excellent.

Ginsenoside identity	Concentration of Ginseng matrix (mg/mL)				
	0.25	2.5	6.3	12.5	25
Ginsenoside Rb1			0.1 % RSD		
Ginsenoside Rb2				1.2 % RSD	
Ginsenoside Rc				1.1 % RSD	
Ginsenoside Rd					1.1 % RSD
Ginsenoside Re					1.0 % RSD
Ginsenoside Rf					0.7 % RSD
Ginsenoside Rg1			1.1 % RSD		
Ginsenoside Rg2					0.4 % RSD

Table 1. Demonstrating the compounds which fall within the dynamic range of the instrument at varying ginseng concentrations. Green box indicates within the established dynamic range, blue indicates outside of established dynamic range. The numbers within the boxes indicate the variance (calculated as percentage deviation of response) between duplicate injections.

A green box, in Table 1, indicates within the established dynamic range (based upon response value) and a blue box indicates outside of the established dynamic range, the number with the red box indicates the response %RSD for the duplicate injections for the compound at the concentration level.

The data displayed in Figure 2 demonstrates the selected component response over the whole analysis (compound displayed is ginsenoside Rg2), 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.

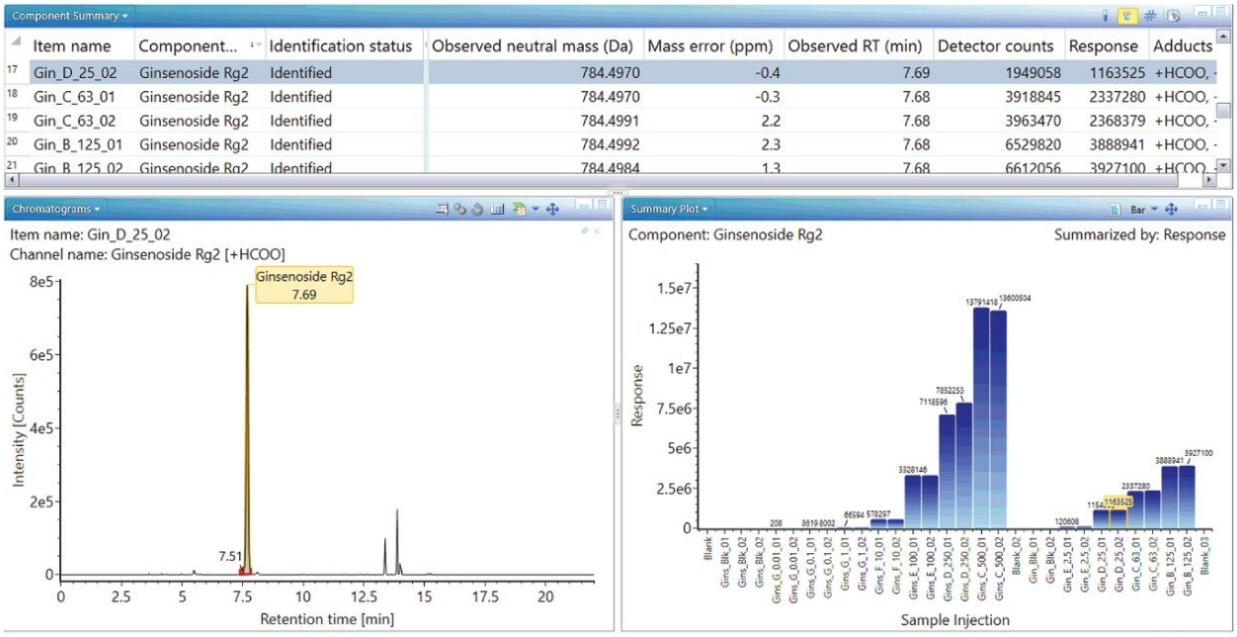

Figure 2. UNIFI display showing table of injections with a summary for each sample, the XIC for a selected compound, and a summary plot providing a visual of response for the selected compound (ginsenoside Rg2 in this case) over every injection in the analyses.

Analyzing standard dilution series and matrix injections as one continuous analysis exceeded 24 hours of non-stop analysis time which the system comfortably achieved. The chromatographic retention time for each of the 8 ginsenoside standards varied by a maximum of 0.05 minutes (3 seconds) with a maximum RSD of 0.2% over the entire analysis.

The mass accuracy for each of the standards over the entire analysis was assessed and deviated by a maximum of ±4.7 ppm for and demonstrated an average ppm error of ±1.3 over the whole 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 seen between duplicate injections is 4.6% for response with an average variance of 1.5%, including both the pure standard and ginseng matrix injections. The signal intensity and peak area robustness for the entire analytical session was assessed using an injection of 100 ng (on column) ginsenoside standards at the beginning and end of the analysis, across all ginsenoside compounds only a 7.8% (average) change was observed for signal count and only a 8.0% (average) difference in calculated response was seen between injection 1 and injection 80 (>27 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 Ginseng analysis.

With excellent signal reproducibility, great mass accuracy and a wide dynamic range, this LC-MS System is perfectly placed as a compact workhorse for any natural products laboratory. Coupled to waters_connect using the UNIFI the data acquisition and processing is contained within a single software platform, ensuring the entire workflow is rapid, streamlined, and allows for complete compliance with audit trail activation.

References

1. Sengupta, S, Toh, SA, Sellers, LA, Skepper, JN, Koolwijk, P, Leung, HW, Yeung, HW, Wong, RN, Sasisekharan, R, Fan, TP. Modulating Angiogenesis: The Yin and the Yang in Ginseng. Circulation. 2004, 110, 1219–1225. doi: 10.1161/01.CIR.0000140676.88412.CF. Epub 2004 Aug 30. PMID: 15337705.
2. Zhu, J, Liang, Y, Yue, S, Fan, G, Zhang H, Zhang M. Combination of Panaxadiol and Panaxatriol Type Saponins and Ophioponins From Shenmai Formula Attenuates Lipopolysaccharide-induced Inflammatory Injury in Cardiac Microvascular Endothelial Cells by Blocking NF-kappa B Pathway, Journal of Cardiovascular Pharmacology. 2017, 69(3), 140–146. doi: 10.1097/FJC.0000000000000450
3. Wang, C; Ni, M; Sun, S; Li, X; He, H; Mehendale, S; Yuan, C. Detection of Adulteration of Notoginseng Root Extract with Other *Panax* Species by Quantitative HPLC Coupled with PCA. *J. Agric. Food Chem.* 2009, *57*, 2363–2367.

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