CHARACTERIZATION OF GLUCOSINOLATES IN *ARABIS SAGITTATA* EXTRACTS USING A MULTI-REFLECTING Q-TOF MASS SPECTROMETER

Emma Marsden-Edwards[1], Isabel Riba[1], Martin Palmer[1], Vera Wewer[2], Sabine Metzger[2]
1. Waters, Wilmslow, UK. 2. University of Cologne, Cologne, Germany.

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INTRODUCTION

Plants produce a large variety of chemical compounds that are believed to protect them from herbivore or pathogen attack. One such group of compounds are glucosinolates (GSLs), which are a diverse group of sulfur-rich plant secondary metabolites that have been associated with defence response against herbivore or pathogenic attack (Figure 1).

Over 100 GSLs have been identified, with many more uncharacterized. The diversity of GSLs is attributed to different amino acid precursors and further multiple modifications of the side chain during their biosynthesis. (Figure 2). The high variability of GSLs poses a complex analytical challenge, which is compounded by the presence of a wide chemical diversity of primary and secondary metabolites, present in differing concentrations in plant extracts.

Here we demonstrate the benefit of using a high resolving power (>300,000 FWHM) multi-reflecting Q-Tof to detect GSLs and confidently identify them based on accurate mass and their naturally occurring fine isotope structure.

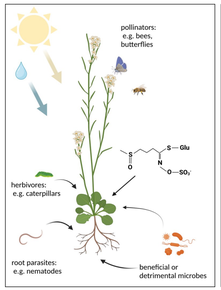

Figure 1: Plant secondary metabolite production is influenced by abiotic factors like light and water availability and biotic stress by herbivores and parasites. (Figure created with biorender).

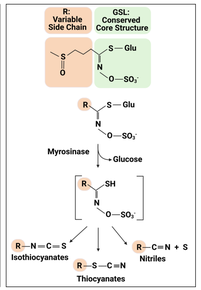

Figure 2: Molecular structure of glucosinolates and degradation pathway by myrosinase. The variable side chain (R, here shown for glucoraphanin) is derived from amino acids and classified as aliphatic, indolic or aromatic.

Acknowledgement:
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METHODS

A mixture of 16 glucosinolate standards and *Arabis sagittata* extracts were analysed by UHPLC/MS using a charged surface hybrid UPLC column, coupled to a SELECT SERIES™ MRT[1] multi-reflecting Q-Tof.

SAMPLE PREPARATION

Arabis sagittata extracts were prepared in a two-step protocol by grinding leaves in liquid nitrogen with subsequent extractions of 80% and 50% methanol (including 5 µM sinigrin as IS), with immediate inactivation of the enzyme myrosinase at 80 °C.

LC/MS CONDITIONS

A Waters ACQUITY™ Premier UPLC™ system was fitted with an XSelect™ Premier CSH C18 3.5 µm 2.1 x 150 mm column at 25 °C, MPA: H_2O + 0.1% formic acid, MPB: MeOH + 0.1% formic acid with a gradient from 2% MPB held for two minutes ramping to 100% MPB over 10 minutes, followed by a wash and equilibration step. The LC was coupled to an electrospray source operating in negative ion mode, with an electrospray voltage of 1 kV and a cone voltage 30 V.

MS METHOD

Data for both the GSLs standards and *Arabis sagittata* extracts were acquired in MRT mode (single pass of the multi-reflecting Tof, resolving power >200,000 FWHM) as a data independent acquisition, MS^E, at 10 Hz with a CE ramp of 20 to 50 V applied to the high energy data, the GSLs standards were also acquired in REM mode (two passes of the multi-reflecting Tof[2], resolving power >300,000 FWHM) by flow injection. Data were lockmass corrected with leucine enkephalin (*m/z* 554.26202) to provide ppb mass accuracy.

DATA PROCESSING

Data were processed using waters_connect™.

RESULTS AND DISCUSSION

The chromatographic base peak intensity plot for the identified GSLs standards is shown in **Figure 3**, all 16 GSLs were identified from a search of a custom library. Components were considered to be identified where their retention time was within 0.1 minutes of the library entry and the mass accuracy was less than 2 ppm on the precursors and 2 mDa for their *in-silico* derived fragment ions.

Two chromatographic peaks of differing relative abundances were observed for three components (glucobrassicin, glucocapparin and neoglucobrassicin) demonstrating the complexity of GSLs characterization owing to the isomeric variability obtained from their complex structures, this is further compounded by the common fragmentation pathways observed for GSLs, **Figure 4,** with isomers typically not yielding unique fragment ions.

The chromatographic base peak intensity plot for the GSLs identified in the *Arabis sagittata* sample is shown in **Figure 5**, for this extract 15 GSLs were putatively identified from the library. The observed mass accuracy for the identified GSLs in the extract are summarized in **Table 1**, the overall mass accuracy was **588 ppb RMS**. Six of the identified components in the extract were observed at the same retention time as the standards, whereas two components were observed at a different retention time (glucocapparin and 8-(Methylthio)-3-oxooctyl GSL), which further demonstrates the challenges of GSLs identification.

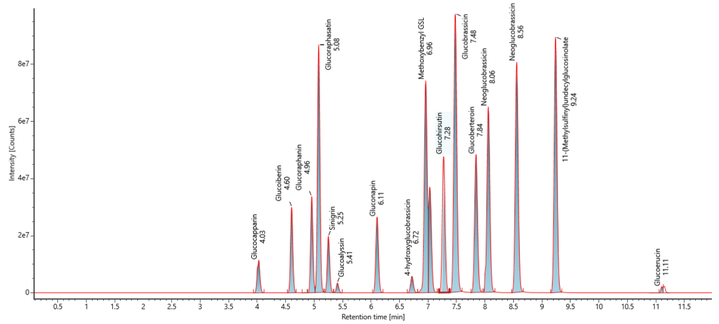

Figure 3: Chromatographic base peak intensity plot for the identified GSLs standards.

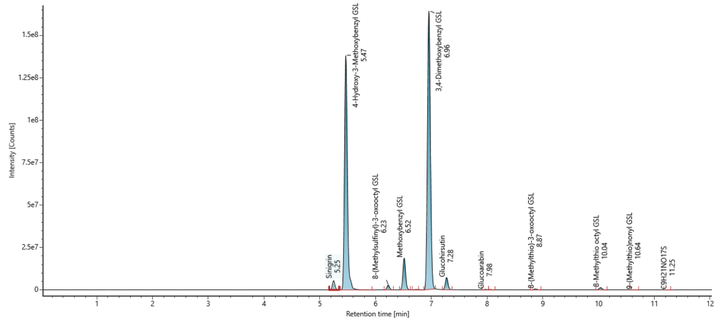

Figure 5: Chromatographic base peak intensity plot for the GSLs identified in the Arabis sagittata sample.

Table 1: Observed mass accuracy for the GSLs putatively identified in the *Arabis sagittata* sample.

Component name	Formula	Observed *m/z*	Mass error / ppb	Observed t_R / min
Sinigrin	$C_{10}H_{17}NO_9S_2$	358.02745	704	5.25
4-Hydroxy-3-Methoxybenzyl GSL	$C_{15}H_{21}NO_{11}S_2$	454.04862	641	5.47
Glucocapparin	$C_8H_{15}NO_9S_2$	332.01178	699	5.47
8-(Methylsulfinyl)-3-oxooctyl GSL	$C_{16}H_{29}NO_{11}S_3$	506.08287	-247	6.23
Methoxybenzyl GSL	$C_{15}H_{21}NO_{10}S_2$	438.05304	-855	6.52
4-hydroxyglucobrassicin	$C_{16}H_{20}N_2O_{10}S_2$	463.04864	-35	6.71
3,4-Dimethoxybenzyl GSL	$C_{16}H_{23}NO_{11}S_2$	468.06423	552	6.96
Glucocapparin	$C_8H_{15}NO_9S_2$	332.01189	1031	6.96
Glucohirsutin	$C_{16}H_{31}NO_{10}S_3$	492.10338	-723	7.28
Glucoarabin	$C_{17}H_{33}NO_{10}S_3$	506.11956	344	7.98
Neoglucobrassicin	$C_{17}H_{22}N_2O_{10}S_2$	477.06447	328	8.06
8-(Methylthio)-3-oxooctyl GSL	$C_{16}H_{29}NO_{10}S_3$	490.08800	-171	8.87
8-Methylthio octyl GSL	$C_{16}H_{31}NO_9S_3$	476.10884	39	10.04
9-(Methylthio)nonyl GSL	$C_{17}H_{33}NO_9S_3$	490.12468	440	10.64

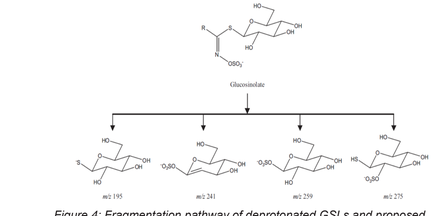

Figure 4: Fragmentation pathway of deprotonated GSLs and proposed group specific MS/MS product ions at m/z 195, 241 and 259 and 275[3].

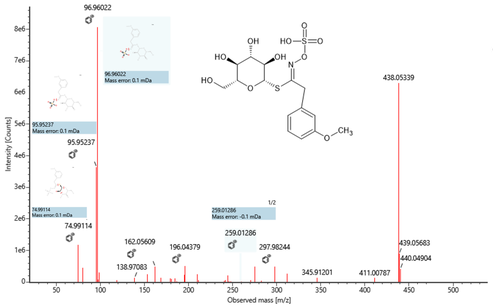

Figure 6: Fragment ion spectrum of methoxy benzyl GSL identified in the Arabis sagittata sample.

Table 2: Observed mass accuracy for the fragment ions of methoxy benzyl GSL.

Formula	Observed *m/z*	Mass error / mDa	Mass error / ppm
$C_6H_{11}O_8S_2$	274.9901	-0.011	-0.04
$C_6H_{11}O_9S$	259.0129	-0.073	-0.28
$C_6H_8NO_5S$	242.0129	0.053	0.22
$C_6H_9O_5S$	241.0026	0.229	0.95
$C_6H_{11}O_5S$	195.0333	0.022	0.11
$C_4H_7O_4$	119.0351	0.112	0.94
HSO_4	96.96022	0.119	1.23
SO_4	95.95237	0.096	1.00
SO_3	79.95748	0.122	1.52
C_2H_3OS	74.99114	0.133	1.78

A representative high energy fragment ion spectrum from the *Arabis sagittata* extract is shown in **Figure 6**, with *in-silico* generated structures for methoxy benzyl GSL, the observed mass accuracy for the fragment ions are summarised in **Table 2**, with sub-ppm mass accuracy observed for fragment ions > 100 *m/z* and 1-2 ppm for ions < 100 *m/z*. Excellent mass accuracy is maintained for both the precursors and fragment ions, which increases confidence in the identification.

The GSLs standard mix was infused and data acquired in REM mode, where ions undergo a second pass of the Tof analyzer resulting in an increase in resolving power to >300,000 FWHM[2].

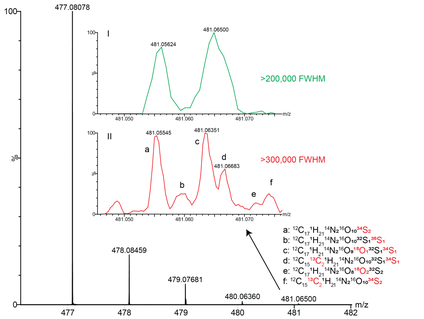

Figure 7: Deprotonated molecular ion of Methoxyglucobrassicin ($C_{17}H_{22}N_2O_{10}S_2$); Inset I: partially resolved fine isotope structure acquired in MRT mode at >200,000 FWHM; Inset II: resolved fine isotope structure revealed in REM mode at >300,000 FWHM.

These data were then compared to that acquired in MRT mode, an example for methoxyglucobrassicin is shown in **Figure 7** , where for the A+4 isotope (*m/z* 481.07) a partially resolved fine isotope structure is observed for the MRT mode data (Inset I, green trace), whereas the REM mode (two passes of the Tof analyzer where resolving power is >300,000 FWHM), data (Inset II, red trace) shows greater detail and clarity of the fine isotope structure, which further increases confidence in empirical formulae designation. This is especially the case for GSLs, where the number of sulfur, nitrogen and oxygen atoms can vary having the ability to acquire data with resolved fine isotope structure will significantly aid empirical formula attribution.

CONCLUSION

- **A range of Glucosinolate (GSLs) standards have been chromatographically separated and matched to a custom library.**
- **GSLs were successfully identified in an extract of *Arabis sagittata.***
- **Excellent mass accuracy was observed for the identified GSLs in the *Arabis sagittata* with an overall RMS error of 588 ppb.**
- **High mass accuracy was also observed for the fragment ions of GSLs, with sub-ppm accuracy observed for ions > 100 *m/z*.**
- **Resolved fine isotope structure has been demonstrated for methoxyglucobrassicin aiding determination of empirical formula.**
- **The combination of high resolving power and excellent mass accuracy on both precursor and fragment ions means that the SELECT SERIES MRT is ideally suited to the characterisation of complex small molecules such as glucosinolates.**

References
1. Cooper-Shepherd et al, *J. Am. Soc. Mass Spectrom.* 2023, 34, 2, 264–272 (https://doi.org/10.1021/jasms.2c00281)
2. Johnson et al, in review.
3. Lee et al, *Rapid Commun. Mass Spectrom.* 2008; 22: 2825–2834 (https://doi.org/10.1002/rcm.3669

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