THE SEARCH FOR THE "REAL THING", *HOODIA GORDONII* CONTENT IN COMMERCIALLY AVAILABLE DIET SUPPLEMENTS BY ACQUITY UPLC MS/MS

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INTRODUCTION

Hoodia gordonii, family Asclepiadaceae, is a succulent found throughout the semi-arid environments of southern Africa (Figure 1). While traditionally used for a variety of purposes by local peoples its utilization in the Kalahari region as an appetite suppressant by hunters has made it a sought-after ingredient in the diet supplement industry. However, due to its slow growth and limited distribution, the export of *Hoodia* is now strictly controlled.

The appetite suppressant activity of Hoodia has been linked to a steroidal glycoside within the plant, known as P57 (Figure 2). This chemical has been demonstrated to affect neurons in the brain associated with food intake[3]. To date, it is the only chemical constituent extracted from *Hoodia* with any anorectic activity.

Effective quality control and screening of *Hoodia* plant material and extracts must focus on the detection and determination of P57 content. Previous High Performance Liquid Chromatography methods have been effective in resolving this compound from interfering substances, but the long runs and lower-sensitivity of UV and single quadropole mass spectrometry detection used can limit the quality of the results.

The Waters® ACQUITY UPLC® has a demonstrated record of increasing throughput while maintaining or exceeding the resolving power of an HPLC assay. The Waters TQD can bring to bear high selectivity and sensitivity. Coupling these two technologies can effectively address the need for quick, selective and sensitive quality control and screening.

This poster summarizes the transfer of a previously successful HPLC method to ACQUITY UPLC MS/MS using Waters software tools and the application of this method to the screening and quantification of P57 content in a series of nutritional supplements.

Figure 1. The succulent Hoodia gordonii, Asclepiadaceae.

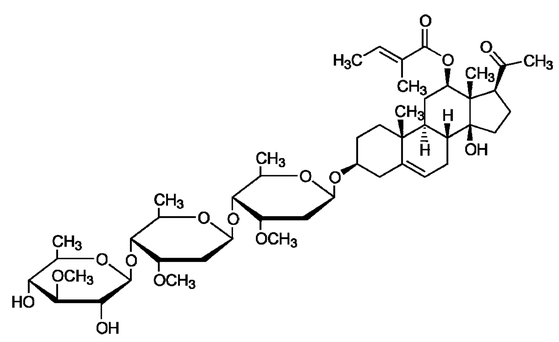

Figure 2. The Oxypregnane glycoside P57.

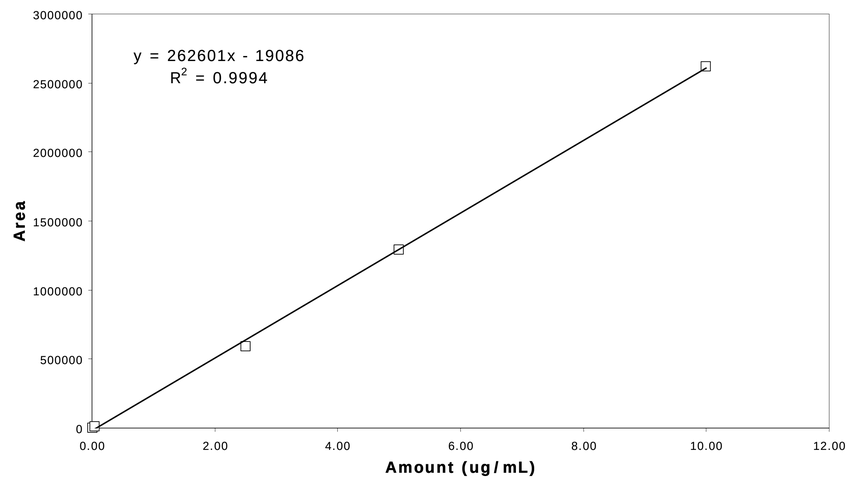

Figure 6. P57 Calibration curve 761.4 > 97.1.

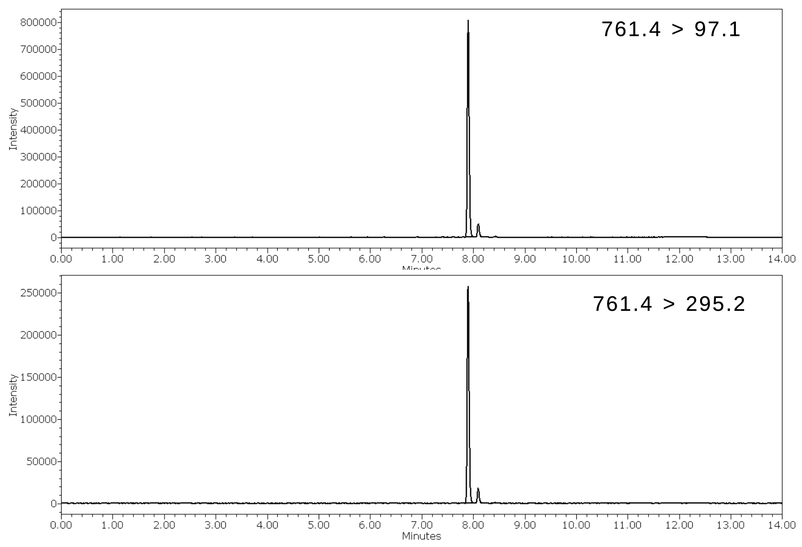

Figure 4. MRM chromatograms of P57 using transistions provided by Intellistart.

Product (dosage form)	µg P57 per gram
Product 1 (capsule)	Not found
Product 2 (capsule)	Not found
Product 3 (capsule)	0.025
Product 4 (caplet)	1.50
Product 5 (caplet)	13.5
Product 6 (geltab)	0.012
Product 7 (caplet)	19.4
Product 8 (capsule)	0.025

TABLE 1. Results of the UPLC MS/MS analysis of Hoodia-containing supplements.

METHOD

Method Transfer

The original HPLC method of analysis used in this transfer was from the work of Avula et al.[2] and was selected as a preferred candidate for transfer due to its documented resolution of P57 related glycosides. Column selection was made using the Waters Column Selectivity chart. ACQUITY UPLC instrumental parameters were smoothly generated from the original HPLC method by the ACQUITY UPLC Columns Calculator (Figure 3).

Transferred UPLC Method Parameters

UPLC System	Waters ACQUITY UPLC System
Column	ACQUITY UPLC HSS C18 2.1 x 100 mm, 1.8µm
Mobile Phase A	0.1% Formic acid in Water
Mobile Phase B	0.1% Formic acid in Acetonitrile
Flow Rate	0.60 mL/min.
Gradient	15%-90%B over 12 minutes
Injection Volume	1.7 µL
Sample Temperature	15°C
Column Temperature	35°C
Detection	Waters TQD
Data	Empower™ 2 CDS

MS Conditions

MS system	Waters ACQUITY TQD
Ionization mode	ES+
Acquisition mode	Multiple Reaction Monitoring MRM 1 766.4 > 97.1 MRM 2 766.4 > 295.2
Capillary Voltage	2.3 kV
Cone Voltage	50 V
Desolvation Gas	600 L/h
Cone Gas	50 L/h
Source Temperature	150°C
Desolvation Temperature	350°C
Collision Gas	Argon, 0.1 mL/min.

Extraction

Portions of each sample (about 1.0 g) were weighed from either capsule contents or tablets crushed to fine powder in a mortar and pestle, then transferred to 15 mL polypropylene centrifuge tubes. Geltab contents were expressed into the tubes following piercing of the capsule. Optima grade methanol was then added (5 mL) and the tube vortexed for 30 seconds. Samples were sonicated for 20 minutes then centrifuged for 30 minutes at 3000 rpm. Aliquots of the supernatants were passed through a 0.2 µm filter into 2 mL HPLC vials and analyzed.

Figure 3. Main window of Column Selectivity Chart and the result window of the ACQUITY Columns Calculator showing the gradient table of the transferred method.

RESULTS AND DISCUSSION

The transferred UPLC method effectively resolved P57 from its interferences and yielded an analysis with a single injection run time of 14 minutes (85 minutes less than the original HPLC assay). No attempt was made to further optimize the instrumental method generated by the ACQUITY UPLC Columns Calculator. This will be done in future work.

Initial MS scans indicated the presence of Na and 2Na adducts of the parent molecule, as well as a 761.4 fragment ion (indicative of the loss of a glucosyl residue and –OH). This was also observed in the literature[1]. Adduct formation was quite intractable. As in the literature, the 761.4 m/z fragment was used as the base ion for this analysis. The P57 standard material available for this study was 88% pure and contained possible isomeric forms

The Waters Intellistart™ software of the TQD automatically tuned the instrument to the 761.44 ion and generated transitions to be used in the MRM experiments for this analysis. The transitions selected by the software are shown in example chromatograms below (Figure 4). The primary transition (761.4 > 97.1) was used for peak confirmation and quantification and the secondary transition (761.4 > 295.2) was used for peak confirmation purposes only. A Product Ion Scan was also performed to confirm these transition ions (Figure 5).

A positive result for the presence of P57 in the nutritional supplements tested was confirmed by the following criteria:

1— All monitored ions were present

2— A peak was present with the retention time of 7.888 ± 0.003 minutes (~0.05%RSD).

3— Ratios of the primary and secondary transitions fell within the range observed in standard and spiked samples.

The amount of P57 in samples with peaks meeting the above criteria was calculated from a 5 point linear calibration curve (r^2 = 0.9994) (Figure 6). At this writing, a formal LOQ had not been established. Standard concentrations ranged from 0.01 to 10 µg/mL. Of the 8 samples extracted for analysis, 6 contained observable levels of P57 (Table 1). Amounts varied widely. Two capsule products contained no observable P57 and two other capsule products had only trace levels. The single geltab tested also contained only trace quantities of P57. The caplet products analyzed all contained easily measurable amounts of P57 with one product at 1.5 µg/g and the other two at 13.5 and 19.4 µg/g, respectively.

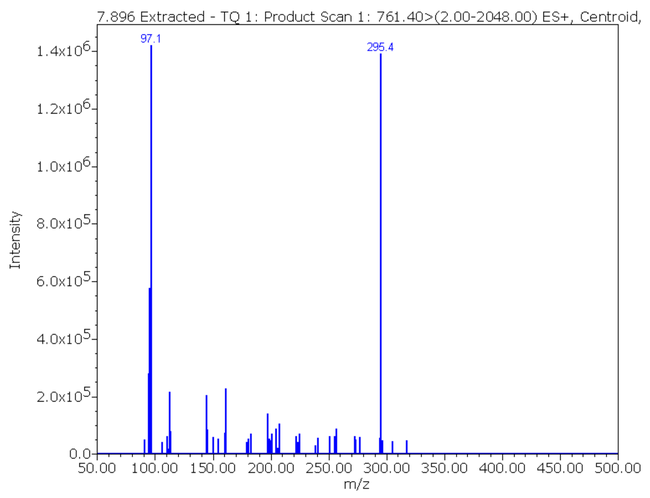

Figure 5. Product Ion Scan for P57 from Hoodia gordonii.

CONCLUSION

- **The original HPLC run time was reduced to 14 minutes from the original 100 minutes upon transfer to the ACQUITY UPLC® using Waters software tools: a tremendous increase in throughput. Further optimization is possible and will increase throughput even more.**
- **Intellistart™ performed all tuning and method development for the assay. A Product Ion Scan was performed and confirmed the transitions generated from Intellistart.**
- **P57 determination in the nutritional supplements was accomplished using a combination of excellent retention time precision, the presence of the 2 MRM transition ions provided by Intellistart, and stable ion ratios between the primary and secondary MRM transitions.**
- **P57 content between products varied widely. Two of the products analyzed contained no observable P57.**

Figure 7. Waters ACQUITY UPLC/TQD system.

REFERENCES

1. Avula, B, Wang, Y., Pawar R, Shukla, Y. and Khan, I (2006) J. AOAC Int. 89:606—611.
2. Avula, B, Wang, Y., Pawar R, Shukla, Y. and Khan, I (2007) J. AOAC Int. 90:1526 - 1531.
3. MacLean, D.B, and Lou, L. (2004) Brain Res. 1020: 1-11

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