METABOLOMICS APPROACH FOR THE AUTHENTICATION OF VARIOUS BOTANICALS AND DIETARY SUPPLEMENTS USING UPLC/QTOF-MS

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HIGHLIGHTS

- **Authentication and chemical identification of different botanicals *Hoodia, Terminalia* and chamomile using novel analytical and informatics tools.**
- **A simple step by step application of high resolution LC-MS coupled with Progenesis QI and multivariate statistical analysis is described.**
- **The significantly changing major hoodigoside marker compounds that differentiate between *H. gordonii* and commercial products were identified.**

INTRODUCTION

Hoodia gordonii is traditionally used by San tribe in South Africa for its appetite suppressant properties which is a rich source of pregnane glycosides (1). Adulteration of products claiming to contain *H. gordonii* is possible by other species or even other genera and developing techniques for rapid product analysis becomes crucial for product validity and safety. More than 150 products are currently marketed as tablets, capsules, liquid tinctures, liquid gels, powders, juice, snack bars, protein shakes, lollipops, patch, chewing gum, tea, *Hoodia* cappuccino, and beauty products.

Multivariate statistical analysis was used as a tool to extract relevant chemical information from the mass spectrometry data to easily differentiate the differences between *Terminalia* species, chamomile varieties, and authentication of *Hoodia* products. The UPLC-QToF-MS based chemical fingerprinting with multivariate statistical analysis (MVA) was able to correctly distinguish botanicals and their commercial products. This work can be used as a basis to assure the quality of botanicals and commercial products. This poster focuses on *H. gordonii* and authentication of the commercial dietary supplements

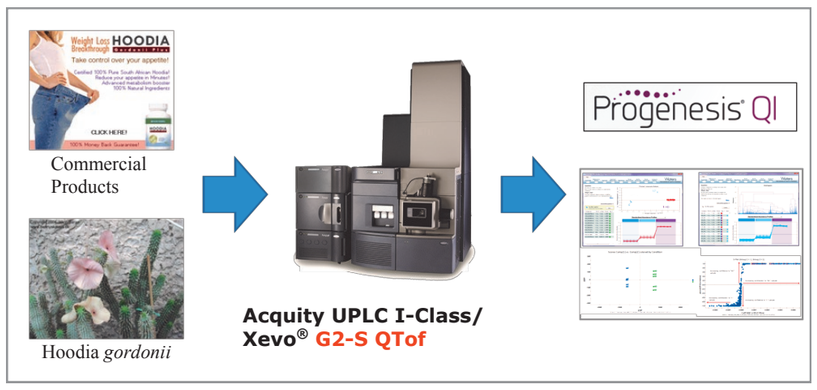


Figure 1. *The metabolomics workflow used for the different botanicals (Hoodia gordonii, Terminalia and chamomile) and commercial dietary supplements. The reference various botanical samples and corresponding commercial dietary supplements were extracted and analyzed using an Acquity UPLC-I Class with Xevo G2-XS QTOF for LC/MS data acquisition. The data was then imported into Progenesis QI (Nonlinear Dynamics, Newcastle, UK) for multivariate analysis and identification of significant chemical markers.*

METHODS

SAMPLE EXTRACTION

- 500 mg of dry Hoodia gordonii plant samples or an adequate amount of capsule content were weighted and sonicated in 2 mL of methanol for 30 min at 40 °C.
- The solution was centrifuged for 15 min at 950 g and the supernatant was transferred to a 10 mL tube.
- The procedure was repeated four times and the supernatants were combined.
- The final volume was adjusted to 10 mL with methanol and passed through 0.45 µm PTFE membrane. The first 1.0 mL was discarded and the remaining volume was collected in an LC sample vial.
- QC sample was created by mixing 10 µL of each sample.

INSTRUMENTAL CONDITIONS

LC CONDITIONS:

LC system: ACQUITY UPLC I-Class with FTN Sample Manager
Column: ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 µm, 40°C

Sample temp: 15°C

Mobile Phase: Gradient elution, A: Water (0.1% formic acid) & B: acetonitrile (0.1% formic acid)

Time	Flow	%A	%B	Curve
0	0.600	80	20	Initial
0.5	0.600	80	20	6
25	0.600	5	95	6
30	0.600	1	99	1
32	0.600	98	20	1

MS CONDITIONS:

MS system: Xevo G2-XS QTof MS
Acquisition range: 100-1800 Da (0.1s scan rate)
Acquisition mode: MS^E, ESI^- and ESI^+ in resolution mode
Capillary voltage: 3.0 kV (ESI^+)/2.5 kV (ESI^-)
Cone voltage: 30 V, Collision energy (eV): low CE: 6/High CE: 15-45eV
Source temp: 120°C, Desolvation temp: 550°C

QC was checked to ensure system suitability throughout the data acquisition.

DATA ANALYSIS:

Data analysis performed using:
Progenesis QI
www.nonlinear.com

RESULTS AND DISCUSSION

Sample Information

	Product ID	Description	Sample Type
1	3476	Pure Health	Product
2	3515	Hoodia Max +	Product
3	3525	Product -8 Hoodia Supreme	Product
4	3622	Product -9 Hoodia 911	Product
5	3700	Hoodia Hoodia	Product
6	5001	Dex L10	Product
7	H-57	HG3	Product
8	HG-4	Product -3 Desert Burn	Product
9	2799	*Hoodia gordonii*	*gordonii*
10	2821	*Hoodia gordonii*	*gordonii*
11	2926	*Hoodia gordonii*	*gordonii*
12	2927	*Hoodia gordonii*	*gordonii*
13	3126	*Hoodia gordonii*	*gordonii*
14	3229	*Hoodia gordonii*	*gordonii*
15	3560	*Hoodia gordonii*	*gordonii*
16	2925	*H. gordonii*	*gordonii*

Table 1. shows the 8 *H. gordonii* samples and 8 commercially available dietary supplements claiming to contain *H. gordonii*.

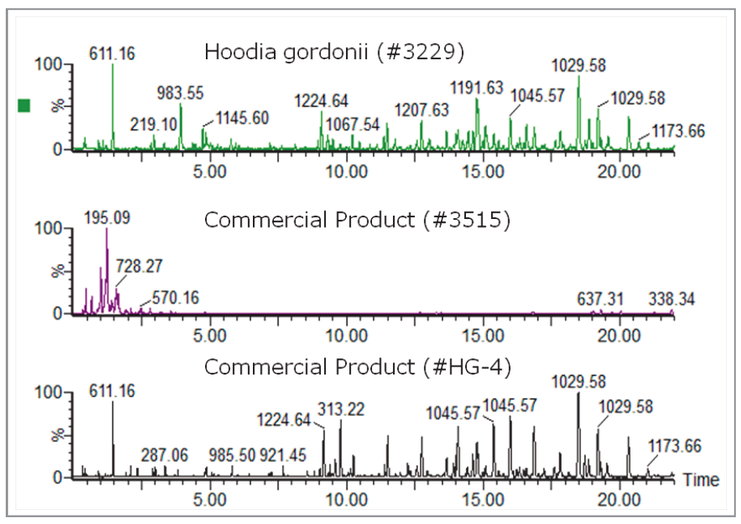
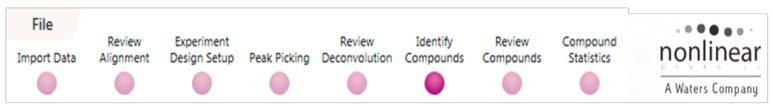


Figure 2. Representative chromatograms of *H. gordonii* and commercial dietary supplement extracts analyzed by UPLC-QToF-MS in positive ion ESI mode.

Data Analysis Using Progenesis QI



Intuitive Step by Step Workflow

The Progenesis QI software adopts an intuitive step by step workflow to perform comparative high resolution UPLC-MS metabolomics data analysis. The key to data processing and analysis is the ability of the software to distinguish biological variation and metabolic changes from analytical interferences. It is crucial that each sample is randomized and injected a minimum of three times to ensure that the data analysis is statistically valid. For this study the botanicals and commercial products were randomized and injected three times with a set of QC pooled sample runs.

Figure 3. Shows the total peak picked of 10,982 exact mass precursor ion and retention time pair used for chemical finger printing. Progenesis QI allows for the differential analysis of high resolution UPLC-MS metabolomics data across a large sample set. This strategy can be used for the identification and quantitation of potential chemical markers.

Multivariate Statistical Analysis (MVA)

The data from Progenesis QI analysis was exported to EZinfo for detailed multivariate statistical analysis such as principal component analysis (PCA, Figure 4), orthogonal partial least squares – discriminant analysis (OPLS-DA) and S-plot (Figure 5). Using these MVA tools, regardless the complexity of the sample, it allows an easy determination of the features that change between the different botanical samples for further identification and targeted analysis. MVA was performed for the acquired metabolomic data to identify whether the metabolite profiles of the different botanical samples could be used to differentiate and possibly identify the chemical markers that significantly contribute to the difference between the Hoodia gordonii and commercial products.

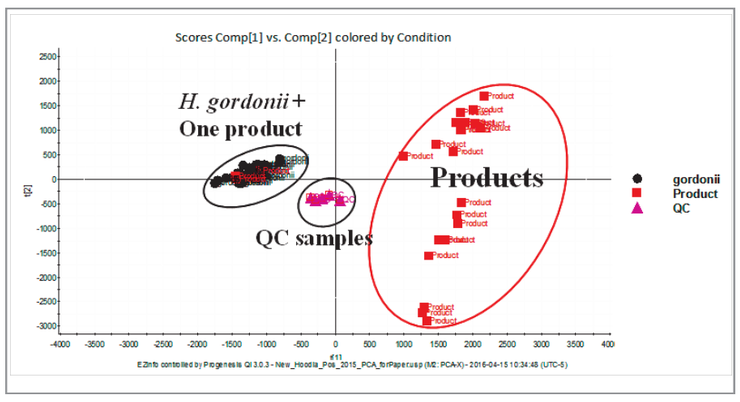


Figure 4. The PCA Scores Plot of the entire Hoodia *gordonii* and commercial products in positive ion mode. All 8 reference *H. gordonii* samples analyzed were grouped more closely compared to the dietary supplements. Only the **HG-4** commercial dietary supplement was clustered with reference *H.* gordonii.

As the ultimate goal was to identify key chemical markers that are statistically significant to *H. gordonii*, the 8 *H. gordonii samples* were set as group 1, and all other commercial dietary supplements except for one product (HG-4 which was clustered with *H. gordonii* samples) as group 2. The resulted S-plot is shown in Fig. 6.

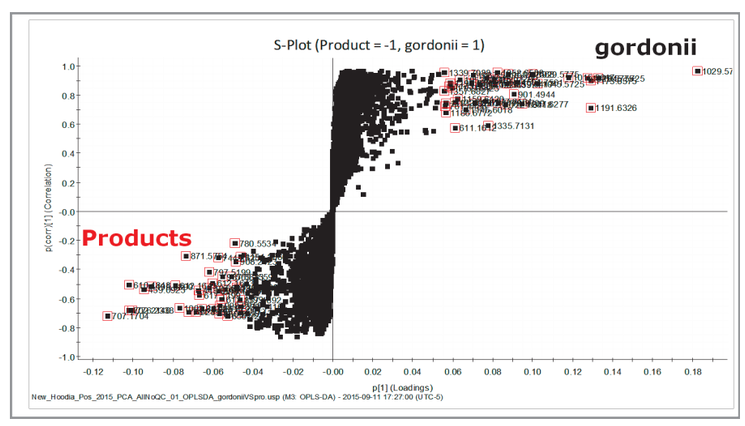


Figure 5. S-Plot of Hoodia *gordonii* and commercial dietary supplement samples. The significant chemical markers (highlighted in red in the top right) obtained from the S-Plot for *H. gordonii* were imported back into Progenesis QI for automatic compound identification.

Compound Identification of Significant Chemical Markers

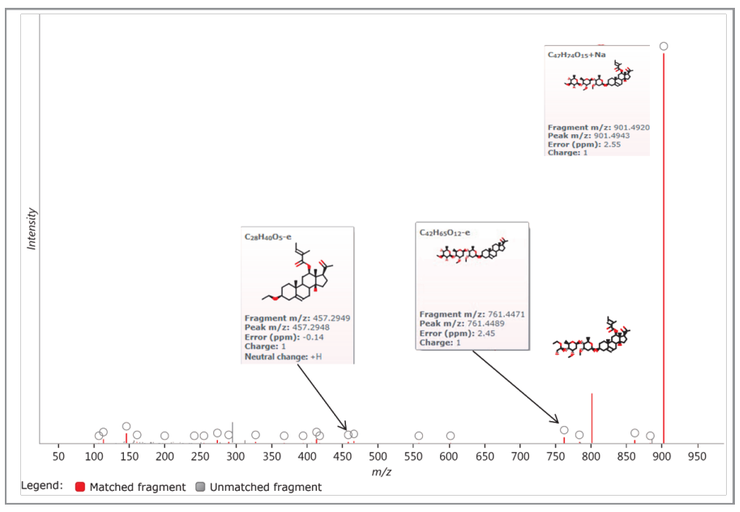


Figure 6. Fragmentation trace for compound 14.07_901.4944 in the positive ion mode with the identification of compound P57 a key marker for Hoodia *gordonii* using Chemspider search in Progenesis QI. The experimental spectra which are matched against the in silico fragment ions are highlighted in red. The circle on the top of the red spectra shows the structure, measured m/z, theoretical m/z and corresponding mass error in ppm.

CONCLUSIONS

- **Large scale LC-MS metabolomics data analysis is complex and very time consuming. A simple step by step application of high resolution LC-MS coupled with Progenesis QI and multivariate statistical analysis is described for the authentication of various botanicals and commercial dietary supplements.**
- **All 8 reference *H. gordonii* samples analyzed were grouped more closely compared to the commercial dietary supplements. Only the HG-4 commercial dietary supplement was authenticated as *H. gordonii*.**
- **The significantly changing major hoodigoside marker compounds that differentiate between *H. gordonii* and commercial products were identified.**
- **Identification based on precursor exact mass, isotopic distribution and fragmentation pattern provides confidence in compound identification.**

REFERENCES

1. F.R. van Heerden, M.R. Horak, V.J. Maharaj, R. Vleggaar, J.V. Senabe, P.J. Gunning, An appetite suppressant from Hoodia species. Phytochemistry 68 (20) (2007) 2545–2553.

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