Profiling Hoodia Extracts by HPLC with Charged Aerosol Detection, Electrochemical Array Detection, and Principal Component Analysis

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Overview

Purpose: To combine fast and sensitive HPLC methods with chemometric analysis to profile botanical extracts from the botanical supplement *Hoodia gordonii*.

Methods: Hoodia extracts were analyzed by gradient reversed-phase high performance liquid chromatography with either a charged aerosol detector or photodiode array detector in series with an electrochemical array detector (the spectro-electro array). Chromatographic data were interrogated by chemometric software to identify patterns related to differences in plant species, origin or processing.

Results: Unsupervised chemometric evaluation of the information rich data obtained from HPLC with charged aerosol detection and electrochemical array detection correctly identified differences in sample origin and processing.

Introduction

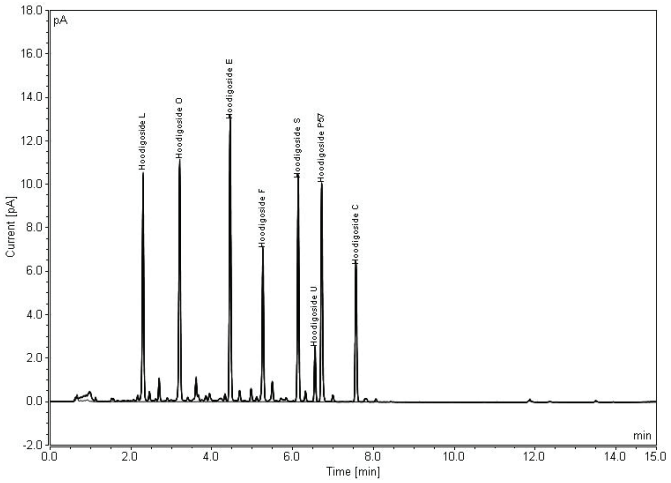
Hoodigosides are oxypregnane steroidal glycosides abundant in *Hoodia gordonii* and related plants native to the deserts of southwestern Africa. These plants, used traditionally to ease hunger during long hunting expeditions, enjoy wide use today in dietary supplements purported to aid in appetite suppression and weight loss.

Presented are two approaches to profile Hoodia related products. In the first approach, Hoodia extracts were analyzed by HPLC with charged aerosol detection. Eight hoodigosides isolated from dried plant material are separated within 15 min on a solid core C8 analytical column that delivers superb resolution with low backpressure. With low-nanogram sensitivity, the charged aerosol detector responds uniformly to all non-volatile species including the target analytes, degradation products and impurities that may not possess a chromophore.

In the second approach, Hoodia extracts were analyzed by HPLC with an online electrochemical array detector that responds to redox active compounds such as polyphenols. Each of the sixteen sensors in the electrochemical array detects analytes at a unique potential to yield picogram on-column sensitivity for diverse compounds that vary widely in redox properties. The chromatograms resulting from this voltammetric approach often clearly resolve co-eluting compounds.

These two techniques provide complementary data that are used to quantify known compounds. The complex patterns of known and unknown compounds revealed by the detectors were also interrogated by pattern recognition software to support inferences on product quality, authenticity, adulteration, or origin. Limits of detection, linear and dynamic range, precision, and pattern recognition results are compared for the two approaches.

FIGURE 1. HPLC with charged aerosol detection identifies hoodigosides in a standard with precision and sensitivity; overlay of seven injections, 10 mg/L



Methods

HPLC with Charged Aerosol Detection

HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with a: - DAD–3000RS Diode Array Detector and a - Corona™ ultra RS™ Charged Aerosol Detector: Nebulizer Temperature: 25 °C Power function: 1.00 Data collection rate: 20 Hz
Data Analysis:	Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.1 SR1
Column:	Thermo Scientific™ Accucore™ C8, 4.6 × 150 mm; 2.6 µm
Temp.:	40 °C
Injection Vol.:	10 µL
Flow Rate:	2.0 mL/min
Mobile Phase A:	Deionized water
Mobile Phase B:	Acetonitrile, Fisher Scientific™ Optima™ LCMS
Gradient:	40%B to 47%B in 2.5 min; to 95%B in 15 min; hold 10 min.

HPLC with Spectro-Electro Array Detection

HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RS system with a - DAD–3000RS Diode Array Detector - CoulArray™ Electrochemical Array Detector: 16-channel array from 0 to +900 mV versus Pd in +60 mV steps
Data Analysis:	Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System 6.8 and CoulArray 3.0 software.
Column:	Thermo Scientific™ Acclaim™ C18, 3 × 150 mm; 3 µm
Temperature:	35 °C
Flow Rate:	0.65 mL/min
Injection Vol.:	10 µL
Mobile Phase A:	20 mM Monobasic sodium phosphate, 3% acetonitrile, 0.2% tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM Monobasic sodium phosphate, 50% acetonitrile, 10% tetrahydrofuran, pH 3.45
Mobile Phase C:	Methanol, Optima LCMS

Gradient:

Time (min)	Flow (mL/min)	%A	%B	%C	Curve
0	0.65	92	5	3	5
2	0.65	92	5	3	7 (concave)
30	0.65	0	97	3	7 (concave)
45	0.65	0	97	3	5
45	0.65	92	5	3	5
50	0.65	92	5	3	5

Sample Preparation

50 mg powder from a commercially available capsule was placed into a 2 mL centrifuge tube. One 1 mL methanol was added and the mixture vortexed for 10 min. The supernatant was transferred to a 5 mL volumetric flask. Extraction was repeated two times with fresh methanol and the extracts combined. After bringing to volume with methanol and mixing, an aliquot was centrifuged in a 1 mL centrifuge tube at 13,000 rpm for 10 min and the supernatant transferred to a glass HPLC autosampler vial.

Data Analysis

Principal components analysis (PCA) and cluster analysis (HCA) were used to investigate pattern differences in chromatograms represented as detector response versus retention time. Data were aligned by using the Pattern Recognition Wizard in CoulArray for Windows 3.0 software and imported into Pirouette® Comprehensive Chemometrics modeling software version 4.5 for PCA and HCA.

Results

Chromatography

Method 1: HPLC-Charged aerosol detection

A mixture of eight hoodigosides eluted with baseline resolution within 10 min from the Accucore C8 column under an aqueous/solvent gradient ranging from 40–95% acetonitrile over 15 min (Figure 1). After separation was achieved, the column was washed for an additional 10 min with 95% acetonitrile to ensure complete elution of hydrophobic compounds that might be present in sample extracts. Good precision and ample response can be seen in this overlay of seven injections of a 10 mg/L standard and diluent blank.

The chromatograms obtained from sample extracts are very complex, with many peaks eluting before, during and after the retention time window of the hoodigoside standards. Figure 2 shows example chromatograms obtained from extracts of dried *Hoodia gordonii* mixed parts. In most cases, the individual hoodigosides were resolved sufficiently to permit their accurate identification and quantification in the sample mixtures. Some of the extracts included peaks that coeluted with the standard hoodigosides. Note that in many of the extracts there were several hydrophobic compounds that eluted between 15 and 25 min that are not well detected at 220 nm, but were well detected by the charged aerosol detector. Calibration curves were obtained from standard injections ranging from 0.10 to 100 mg/L (not shown). The calibration curves spanned four orders of magnitude, had a quadratic fit to the data and yielded a correlation coefficient of > 0.999 for each standard hoodigoside.

FIGURE 2. HPLC-CAD chromatograms of methanolic extracts from *Hoodia gordonii*, *Hoodia gordonii* (from Namibia), and *Opuntia leptocaulis*

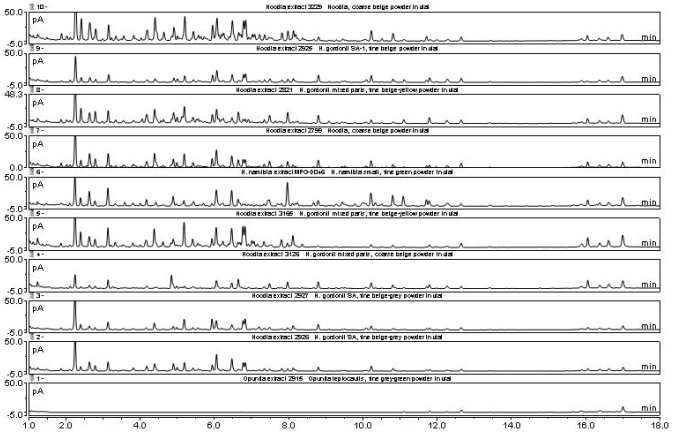


FIGURE 3. PCA score plot of CAD data, only hoodigoside response were used for PCA analysis

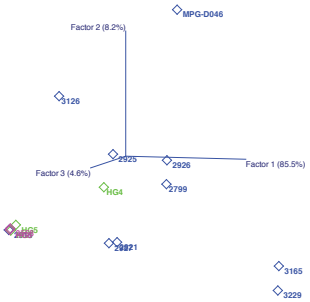


Figure 3 shows the PCA score plot of the hoodigosides data acquired by charged aerosol detection. Capsule samples HG1 and HG5, tablet samples HG-6 and 3558, and sample vial 2915 containing *Opuntia leptocaulis* form a distinct cluster. The primary difference between these clusters is identified as hoodigoside content. (A description of the pattern recognition analysis is provided below.)

Method 2: HPLC-Spectro-Electro Array.

Chromatographic results:

The Spectro-Electro Array makes use of both spectrophotometric and electrochemical data. While UV detection provides identification and quantification of the major components in a sample, EC array detection is incredibly sensitive and is capable of measuring compounds missed by UV. Furthermore, the EC array can voltammatrically resolve compounds that co-elute chromatographically, and the redox behavior of a compound reacting across the array provides qualitative information that can be used for analyte identification/authentication. The CoulArray is particularly suitable for generating chemical fingerprints of supplements containing endogenous electroactive compounds that are essential to flavor, color and stability. The HPLC method used with the Spectro-Electro Array was optimized to resolve the earlier-eluting more polar components in the samples. Illustrative examples of HPLC-ECD Array chromatograms obtained for a few of the samples analyzed are shown in Figure 3–5. As can be seen, these extracts contain many species that are electrochemically active.

FIGURE 3: HPLC-ECD Array chromatogram of *Hoodia gordonii* mixed parts extract #3165

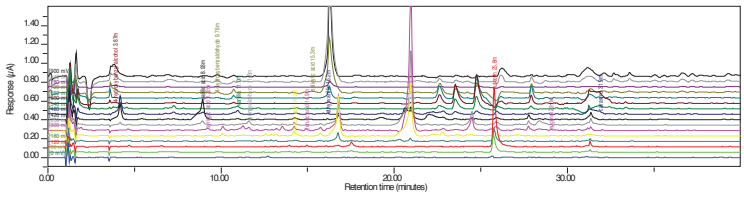


FIGURE 4: HPLC-ECD Array chromatogram of Hoodia commercial product capsule extract HG4

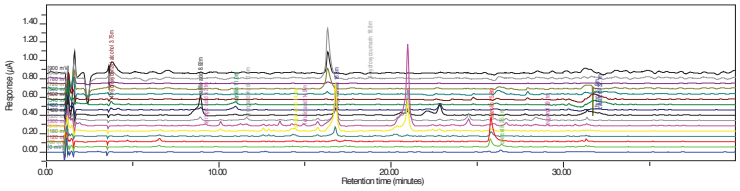
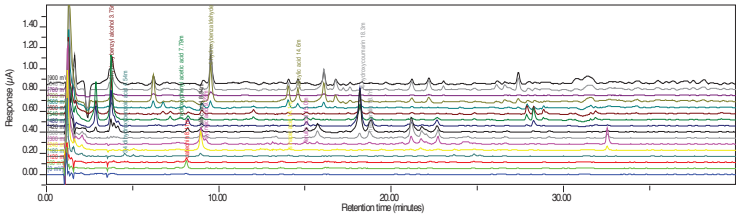


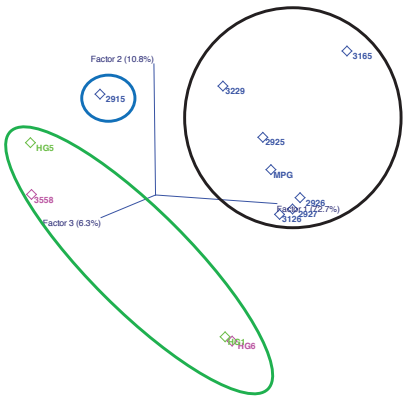
FIGURE 5: HPLC-ECD Array chromatogram of Hoodia commercial product tablet extract HG6



Pattern Recognition Analysis

Chromatographic data from either the charged aerosol detector or the Spectro-Electro array detector were pre-processed to align peak retention times by using the pattern recognition wizard in CoulArray 3.0 software and then imported into Pirouette software for PCA and HCA. PCA is a mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. It is mostly used as a tool in exploratory data analysis and for making predictive models. The greatest variance by any projection of the data is found in the first coordinate (Factor 1), the second greatest variance is found on the second coordinate (Factor 2), and so on. Thus samples with different patterns in their chromatographic or electrochemical profile are distinguished from each other based on their relative position in three- dimensional space. The relationships among these data were used to investigate authenticity and differences in processing, e.g., dried plant parts, tablet, or capsule.

FIGURE 6. PCA score plot for ECD array data from channels 1–16.



A score plot from the HPLC-Charged aerosol detection data is shown in Figure 3 and discussed in that section. A score plot from PCA analysis of the ECD array (CoulArray) data is shown in Figure 6. Samples 2821, 2799 and HG4 were excluded as outliers before PCA analysis because of problems with the chromatography. PCA readily identifies three major groups among the remaining samples. One group contains only sample 2915, from the non-hoodia species *Opuntia leptocaulis*. Another group corresponds to the samples processed into capsules or tablets. The third set, within the black circle, corresponds to authentic *Hoodia gordonii* dried plant material. In each case, the results from the unsupervised chemometric analysis correspond to plainly observable characteristics of the samples, allowing us to verify the accuracy of the approach. The real power of this combined chromatographic/chemometric approach is that it can be implemented in a more or less automated way to quickly screen unknown samples.

Conclusion

- Gradient HPLC with charged aerosol or electrochemical array detection are simple approaches that generate information-rich chemical profile data. Both detectors can reveal analytes overlooked by UV detection alone.

- Chemical profile data can be imported into pattern recognition software and with principal components analysis used to readily identify product authenticity.

- The combined chromatographic/chemometric approach was verified by applying it to known test samples, but can be readily applied to quickly screen unknown samples.

References

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