Application Update: 184

Mogroside V Determination by HPLC with Charged Aerosol and UV Detections

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Key Words

- Glycosides
- HILIC
- Natural Sweetners
- Sugar Substitutes

Introduction

Luo han kuo fruit (*Siraitia grosvenori* Swingle) has long been used in traditional Asian medicine. Recently cucurbitane-type and other triterpene glycosides have been isolated from the fruit and investigated for numerous potential health benefits such as antioxidant activity, anticancer effects, and antihyperglycemic effects.[1] Many of these compounds are intensely sweet and therefore have also been investigated as sugar substitutes and flavor enhancers. Extracts of luo han kuo fruit used as sweeteners were acknowledged as Generally Recognized as Safe (GRAS) based on a GRAS submission to the U.S. FDA in January of 2010.[2]

Typical reversed-phase high-performance liquid chromatography (HPLC) methods to determine these glycosides are challenging due to the lack of a strong, specific chromophore in the compound. Other detection methods, such as charged aerosol detection, can be used to improve triterpene glycoside quantification. In this work, mogroside V (Figure 1) is determined in a luo han kuo beverage by both charged aerosol and UV detections. This triterpene glycoside is separated on the Thermo Scientific Acclaim Trinity P1 column using 81/19 acetonitrile/ammonium formate buffer at pH = 3.0. The developed method uses hydrophilic interaction liquid chromatography (HILIC) conditions suitable for the trimode column, allowing separation of multiple terpene glycosides. The same method has also been used to separate steviol glycosides.[3] The volatile mobile phase makes charged aerosol detection possible, which adds further flexibility to the method for detection of such glycosides.

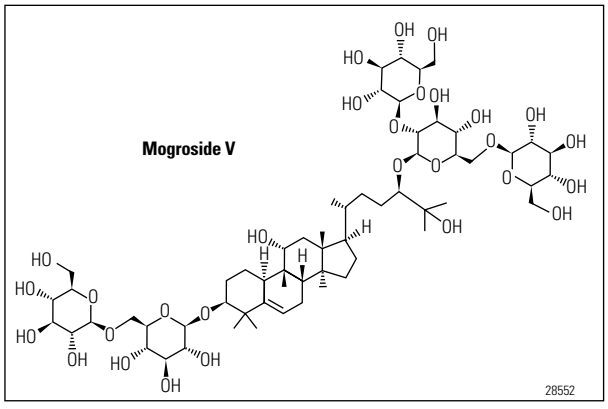

Figure 1: Chemical structure of mogroside V.

Equipment

Thermo Scientific Dionex UltiMate Rapid Separation LC (RSLC) system including:

- SRD-3600 Integrated Solvent and Degasser (P/N 5035.9230)
- HPG-3400RS Binary Pump with Solvent Selector Valves (P/N 5040.0046)
- WPS-3000TRS Analytical Autosampler (P/N 5840.0020)
- TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
- DAD-3000RS Diode Array Detector (P/N 5082.0020)

Thermo Scientific Dionex Corona *ultra* Charged Aerosol Detector (P/N 70-9298)

Polypropylene injection vials with caps and septa, 300 µL (Thermo Scientific Dionex P/N 055428)

Nalgene™ Filter Unit, 0.2 µm nylon membrane, 1 L capacity (Thermo Scientific Nalgene P/N 164-0020)

Reagents and Standards

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better

pH buffer, 4.00 (VWR P/N BDH4018-500ML)

pH buffer, 2.00 (VWR P/N BDH5010-500ML)

Stevia Standards Kit, (ChromaDex P/N KIT-00019566-005) containing:

- Rebaudioside A
- Stevioside
- Rebaudioside B
- Rebaudioside C
- Dulcoside A
- Steviolbioside
- Rebaudioside D

Mogroside V standard (ChromaDex P/N ASB-00013881)

Formic Acid (Sigma-Aldrich P/N 06440)

Ammonium Formate (Sigma-Aldrich P/N 51691)

Acetonitrile (Honeywell P/N 015-4)

Sample

Brand A: Luo han kuo beverage, supplied as a pair of water-soluble cubes (15.2 g)

Conditions

Column:	Acclaim™ Trinity™ P1 (3 µm), 2.1 × 100 mm (Thermo Scientific Dionex P/N 071389) Acclaim Trinity P1 (3 µm), 2.1 × 10 mm guard column (P/N 071391) with guard holder (P/N 069580)
Mobile Phase:	81/19 acetonitrile/10 mM ammonium formate, pH = 3.0
Flow Rate:	0.3 mL/min
Inj. Volume:	5 µL
Temperature:	20 °C
Detection:	Diode Array UV-vis detector, 210 nm Charged aerosol detector, nebulizer temperature, 10 °C
System Backpressure:	~1500 psi
Noise:	~0.15 mAU (UV) ~0.07 pA (charged aerosol detection)
Run Time:	10–30 min, depending on sample matrix

Preparation of Solutions and Reagents

Mobile Phase Preparation

Transfer 0.63 g of ammonium formate to a 1 L bottle and add 1000 g (1000 mL) of DI water. Adjust the pH of the resulting 10 mM ammonium formate solution to 3.00 ± 0.05 by adding 1700 µL of formic acid. Using a precleaned (with DI water) 0.2 µm nylon filter unit, filter the stock buffer to remove any insoluble particles that may be present.

Transfer 192.5 mL (192.5 g) 10 mM ammonium formate solution to a 1 L volumetric glass flask and fill to the mark. The resulting solution will be approximately 644 g acetonitrile. Mix well. After converting the mass of acetonitrile to a volume, based on density, this method prepares a solution of 81/19 (v/v) acetonitrile/ammonium formate. Mixing aqueous ammonium formate and acetonitrile is endothermic and the solution will cool, resulting in a substantial reduction in volume. This volume change may cause variability in the actual mobile phase composition. These changes in the mobile phase composition will change analyte retention times, and for this reason, gravimetric preparation of the mobile phase will provide the most consistent retention times between mobile phase preparations. Allow the solution to return to ambient temperature before use.

Standards and Sample Solutions

Standards

Prepare a 2.0 mg/mL stock standard of mogroside V by adding 1.4 mg to 700 µL of mobile phase. Then use this stock standard to prepare standards of 0.06 mg/mL to 0.5 mg/mL of mogroside A by appropriate dilution in mobile phase. Steviol glycoside standards were added to standard solutions, in addition to mogroside V. For further details on these standards, see Application Note 293.[3]

Samples

Dissolve cubes in 100 mL of DI water. Further dilute a 100 µL sample aliquot by a factor of 20 in acetonitrile. Samples that show precipitates should be filtered through a 0.2 µm polyethersulfone (PES) membrane syringe filter.

Precautions

Take care to consistently prepare the mobile phase. Changes in the ionic strength, pH, or organic content of the mobile phase can lead to shifts in analyte retention times. If chromatographic resolution decreases without a change in overall peak shape, reprepare the ammonium formate buffer, paying close attention to the amount of ammonium formate and the final pH. Increasing the amount of acetonitrile by up to 5% in the mobile phase will increase retention times, which may improve the resolution for complex samples; however, the late-eluting peak sensitivity will decrease due to peak broadening from dispersion during the isocratic elution.

Metal contamination of the column will reduce both column efficiency and capacity. If reduced retention times and poor peak shape are observed, remove the Corona™ *ultra*™ Charged Aerosol Detector from the flow path and follow the column wash procedure in Section 4 of the Thermo Fisher Scientific, Inc. (formerly Dionex Corp.) Acclaim Trinity P1 column manual.[4] Be sure to thoroughly equilibrate the column with the ammonium formate mobile phase before reconnecting the Corona *ultra* detector.

For this work, a column temperature of 20 °C was chosen to maximize resolution between the steviol glycoside dulcoside A and components within stevia extracts.[3] For mogroside V, column temperatures between 20–30 °C may be used. The use of a temperature-controlled column compartment is highly recommended to ensure consistent retention times.

Results and Discussion

Separation

Figure 2 shows the separation of a mixed standard containing steviol glycosides and mogroside V within 10 min. In foods there is the potential presence of multiple sweeteners. In this example, steviol glycosides are well resolved from mogroside V, with the later eluting well after the steviol glycosides.

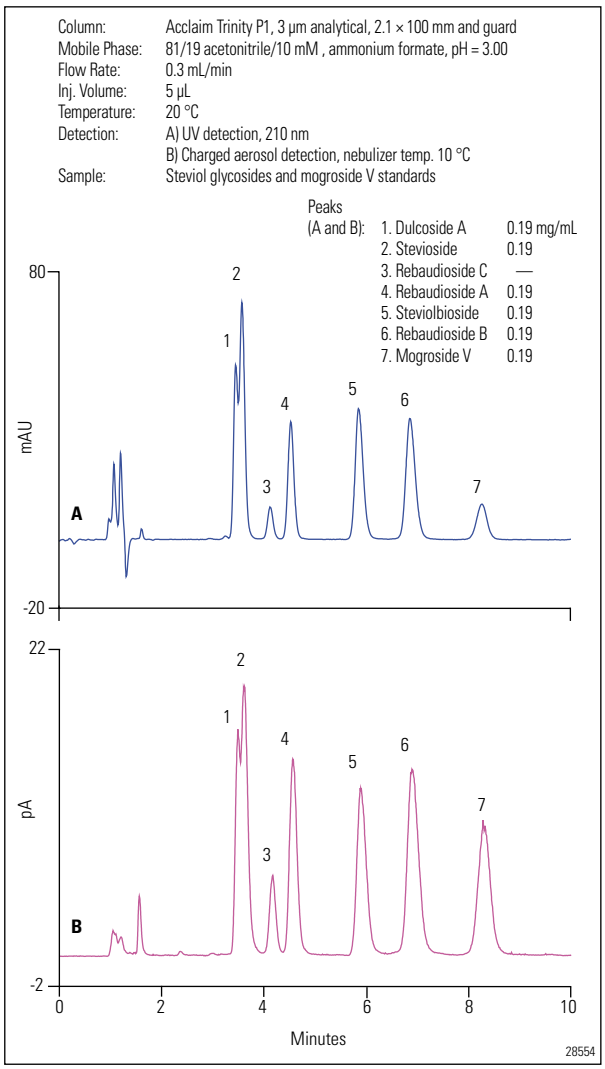

Figure 2: Separation of steviol glycosides and mogroside V standards on the Acclaim Trinity P1 column.

Quantification Assay Linearity and LOD

Table 1 shows the correlation between peak area and concentration for mogroside V determined using UV and charged aerosol detections. As shown, the coefficients of determination are 0.9995 and 0.9991 for mogroside V, by UV (210 nm) and charged aerosol detection, respectively. Calibration curves using charged aerosol detection are inherently nonlinear and were fit with quadratic curves. This nonlinearity is the result of physical interactions that contribute to the detection technique. To fit the calibration curves for charged aerosol detection, use the quadratic fitting option within the Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software. Coefficient of determination values reported within Chromeleon CDS software are from linear fits of converted data.

The limit of detection (LOD), determined as 3× the signal-to-noise (S/N) ratio, for mogroside V was 1.4 µg/mL for charged aerosol detection and 7.0 µg/mL for UV detection. This is a fivefold difference in sensitivity between the two detection methods. A similar improvement in detection was determined when evaluating the Limit of Quantification (LOQ) by injecting standards that resulted in a signal which was 10× the S/N ratio.

Sample Analysis

Sucrose, a component of many beverages, including the one analyzed here, can potentially interfere with mogroside V determination. With the proposed method, sucrose elutes early and mogroside V is well retained. Mogroside V can be detected by both UV (210 nm) or charged aerosol detection with equivalent results in this sample (Figure 3.) However, for samples that contain natural products such as fruit extracts, sodium may be present. Under these conditions, sodium elutes at 20.4 min and the run time must be extended to avoid coelution of the sodium with analyte peaks in subsequent injections.

Table 1: Mogroside V calibration (0.007–0.28 mg/mL), LOD, LOQ, and method precision.

Detector	Coeff. of Deter.	Calibration Model	LOD (µg/mL)	LOQ (µg/mL)	RT (min)	RT (RSD)	Peak Area	Peak Area (RSD)
UV: 210 nm	0.9995	Linear	7.0	22.0	8.03	0.16	0.926 mAU*min	1.67
Charged Aerosol Detection	0.9991	Quadratic	1.4	4.6	8.07	0.07	1.72 pA*min	0.75

Precision values calculated for seven injections of a 70 µg/mL standard.

An expansion of the chromatogram shown in Figure 3 is shown in Figure 4. Charged aerosol detection is more sensitive to mogroside V than UV detection. In addition to the luo han kuo beverage, a mixed glycoside standard detected using charged aerosol detection is shown for retention time reference. Mogroside V is easily identified.

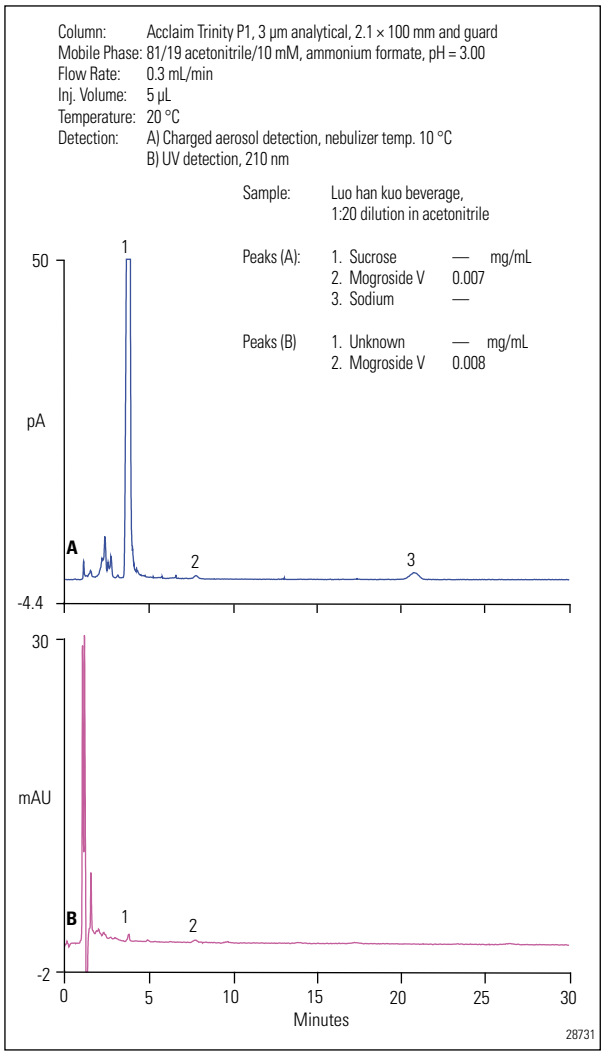

Figure 3: Separation of mogroside V in a luo han kuo beverage as detected by A) charged aerosol detection and B) UV, 210 nm. Note the good separation between sucrose and mogroside V in chromatogram A.

Precision and Accuracy

The chromatographic precision for determination of mogroside V is listed in Table 1. Retention time and peak area precisions are also listed in Table 1. Retention time precision (n = 7), as RSD, was very good at <0.2. Peak area precision (RSD) was <2.0. Mogroside V (0.050 mg/mL) was spiked into table-top sweeteners to evaluate separation from other components as well as recoveries from the sample matrix. Recoveries ranged from 89–105% with charged aerosol detection and 88–103% by UV detection, demonstrating method accuracy.

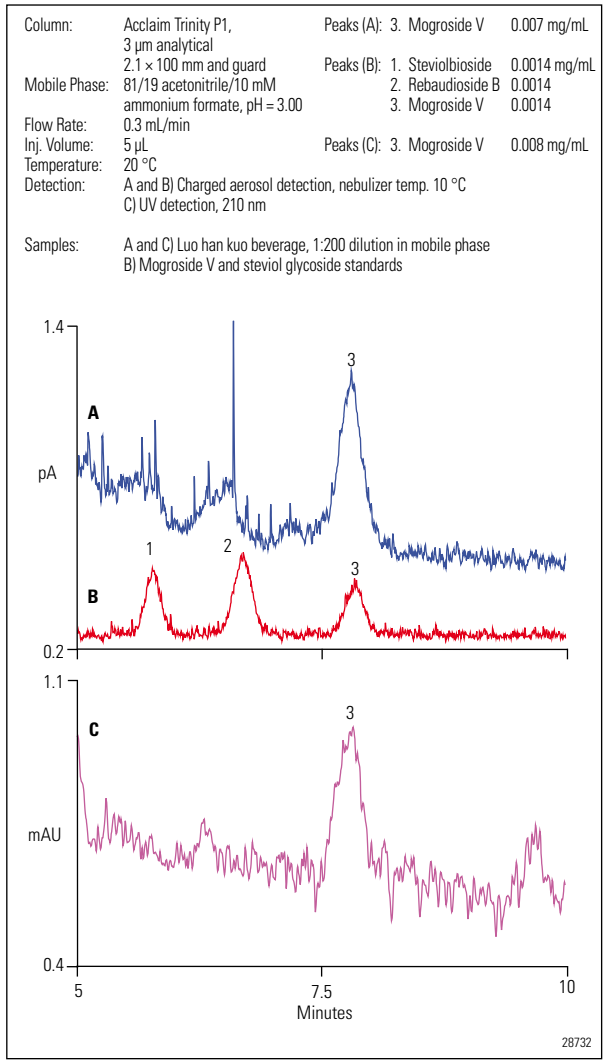

Figure 4: Expanded view of the separation of mogroside V detected by charged aerosol detection (A) and UV, 210 nm (C). Chromatogram B shows the separation of a mixed terpene glycoside standard for comparison.

Conclusion

Here the separation of mogroside V is shown both in a mixture of standards and in a commercially available beverage. The glycoside is separated on the Acclaim Trinity P1 column using 81/19 acetonitrile/ammonium formate buffer at pH = 3.0. This method uses HILIC conditions suitable for the trimode column, allowing separation of multiple terpene glycosides, and has also been used to separate steviol glycosides. The volatile mobile phase makes charged aerosol detection possible, which adds further method flexibility and improved detection sensitivity.

Suppliers

VWR, 1310 Goshen Parkway, West Chester, PA 19380 U.S.A., Tel: 800-932-5000. www.vwr.com

Fisher Scientific, One Liberty Lane, Hampton, NH 03842 U.S.A., Tel: 800-766-7000. www.fishersci.com

Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178 U.S.A., Tel: 800-325-3010. www.sigma-aldrich.com

ChromaDex, 10005 Muirlands Blvd, Suite G, First Floor, Irvine, CA 92618U.S.A., Tel: 949-419-0288. www.chromadex.com

References

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2. U.S. FDA, Agency Response Letter to GRAS Notice No. GRN 000301, Jan 15, 2010.
3. Hurum, D.; Rohrer, J. Application Note 293: Steviol Glycoside Determination by HPLC with Charged Aerosol and UV Detections Using the Acclaim Trinity P1 Column, LPN2976, 2011. Thermo Scientific: Documents, Applications.www.dionex.com/.
4. Thermo Fisher Scientific, Product Manual for Acclaim Trinity P1 Columns. Document No. 065306-02. 2010, Sunnyvale, CA.

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