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File: ■ Monk Fruit (*Siraitia grosvenorii*, Cucurbitaceae)
■ Sweeteners
■ HPLC-MS

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RE: HPLC-ESI-MS/MS Method Simultaneously Quantifies Eight Mogrosides of Monk Fruit

Luo Z, Shi H, Zhang K, Qin X, Guo Y, Ma X. Liquid chromatography with tandem mass spectrometry method for the simultaneous determination of multiple sweet mogrosides in the fruits of *Siraitia grosvenorii* and its marketed sweeteners. *J Sep Sci*. November 2016;39(21):4124-4135.

Monk fruit (MF) (luo han guo; *Siraitia grosvenorii*, Cucurbitaceae) has been used for thousands of years as both a natural sweetener and traditional medicine. Recent interest in MF as a natural sweetener and its generally recognized as safe (GRAS) status has resulted in new MF sweeteners in the US market. The active ingredients in MF that act as natural sweeteners are cucurbitane-type triterpenoids known as "mogrosides." The eight major mogrosides found in MF are mogroside III (MIII), mogroside IVa (MIVA), mogroside IV, mogroside V (MV), mogroside VI (MVI), iso-mogroside V, 11-oxomogroside-V, and siamenoside I (SI). Mogroside type and quantity in MF will differ depending on ripeness. Ripe MF contains mostly MV, the most plentiful sweet-tasting mogroside, whereas unripe MF contains mostly mogroside IIE and MIII, which are, respectively, bitter and tasteless.[1-3] Ripe MF is difficult to distinguish by appearance only, and quality can differ considerably among cultivation sites.[1-3] This study discusses the development and validation of a high-performance liquid chromatography with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) method for simultaneous quantification of the eight primary mogrosides of MF from samples of harvested fruits and marketed sweeteners.

A mixed standard solution of all eight mogrosides was analyzed via HPLC at varying concentrations to generate calibration curves (peak area versus concentration) for each mogroside. Mogroside standards were purchased from Chengdu Must Bio-Technology (Sichuan, China). A total of 10 batches of MF (MF1 through MF10) were obtained. Five batches were supplied by the Guangxi Branch Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medicinal Science, Peking Union Medical College, and five were purchased from Guangxi Province, China. Three sweeteners were purchased from Xi'an Jinheng Chemical (Shanxi, China). Ultrasound-assisted solid-liquid extraction of mogrosides in methanol/water (80/20, per volume) was found to give optimal results

while being convenient, cost effective, and highly reproducible. Samples of dried MF were prepared by homogenization, mixing with methanol/water (80/20, per volume), sonication, and filtering. Sweetener samples were also mixed with methanol/water (80/20, per volume), sonicated, and filtered. The HPLC system used was an Agilent Technologies 1260 Series LC system (Agilent; Santa Clara, California). After preliminary experiments, Agilent Poroshell 120 SB C_{18} columns were chosen for chromatographic separation of the eight mogrosides for producing good results with a short analytical time. Acetonitrile and methanol mobile phases were tested at different flow rates (0.2-0.5 mL min^{-1}), and acetonitrile/water eluent (both containing 0.1% formic acid) with a 0.25 mL min^{-1} flow rate showed the best separation and symmetric peak shapes. Gradient elution was used for HPLC analysis to avoid long retention times and bad peak shapes noted on some mogrosides with isocratic runs. Satisfactory separation of the eight mogrosides was achieved in 10 minutes. "Good linearity ($r^2 \geq 0.9984$) was achieved within the investigated ranges for all of the analytes," the authors observe. Retention times, precursor and product ions, and ion ratios were compared to identify the mogrosides. Optimum mass spectrometric behaviors were analyzed by introducing each standard compound individually into the mass spectrometer to optimize the ESI source parameters. The negative ionization mode showed higher sensitivity for determining quantity; therefore, the $[M-H]^-$ ion was measured for each compound. Multiple reaction monitoring (MRM) scanning was employed for quantification and optimized for maximum $[M-H]^-$ and fragment/product ions generated for each mogroside.

Intra- and inter-day variability was calculated as a precision check, based on analysis of three mogroside standards, at low, intermediate, and high concentrations compared to sample MF3. Intra- and inter-day relative standard deviations (RSDs) for all were less than 3.73% and 3.91%, respectively, indicating precision of quantification. The authors summarize recovery results—"The average recoveries of all of the compounds ranged from 91.22 to 106.58%, and the RSDs were less than 3.79%, which demonstrated that the method was accurate for simultaneous quantitative evaluation of the eight mogrosides in [MF]." Stability was evaluated by analyzing the prepared solution of MF3, kept at room temperature, every six hours for 24 hours, and it was found to be stable (RSD values at peak area < 3.01%). Good repeatability was determined after six independently prepared solutions of MF3 were analyzed, with RSD less than 3.42%. Matrix effects were calculated for each compound, as endogenous substances present in a complex sample such as MF can lead to ion suppression or signal enhancement. It was determined that any matrix effects were minor and would not interfere with accurate analysis.

The quantity of each mogroside and total mogrosides varied significantly in the analyzed MF batches (8.83 to 18.46 mg/g total mogrosides). MV, present at 5.77-11.75 mg/g, made up 49.29-66.96% of total mogrosides in the MF samples. SI, which like MV tastes sweet, was the second most abundant mogroside. MIII and MIVA were detected only in MF3 and MF8, indicating these batches may not have been fully ripe. MVI was not detected in any sample. MV was also the main component in the tested sweeteners, accounting for 75% of total mogrosides. A small amount of MIVA was present in one sweetener. No MIII or MVI was present. The total contents of the sweeteners varied greatly, with different amounts of MF extract added and blended with other less intensely sweet substances, so the mogroside contents were, as expected, lower than those of MF samples.

The developed HPLC-ESI-MS/MS method was checked for precision, repeatability, accuracy, stability, and sensitivity, culminating in mogroside quantifications of MF and sweetener samples analyzed. In line with current industry trends to better establish the identity of botanical ingredients, a test to identify *and* quantify the mogrosides of MF before commercial use would be a valuable tool for market standardization.

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References

[1]Lu F, Li D, Fu C, et al. Studies on chemical fingerprints of *Siraitia grosvenorii* fruits (Luo Han Guo) by HPLC. *J Nat Med*. January 2012;66(1):70-76.
[2]Zhang H, Yang H, Zhang M, et al. Identification of flavonol and triterpene glycosides in Luo-Han-Guo extract using ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry. *J Food Compost Anal*. March 2012;25(2):142-148.
[3]Li D, Ikeda T, Huang Y, et al. Seasonal variation of mogrosides in Lo Han Kuo (*Siraitia grosvenori*) [*sic*] fruits. *J Nat Med*. July 2007;61(3):307-312.