ACS OMEGA

Open Access



http://pubs.acs.org/journal/acsodf

Article

# Quality Evaluation of *Tripterygium* Glycoside Tablets Based on Quantitative Band-Selective 2D $^{1}H-^{13}C$ HSQC and $^{1}H$ NMR Fingerprinting

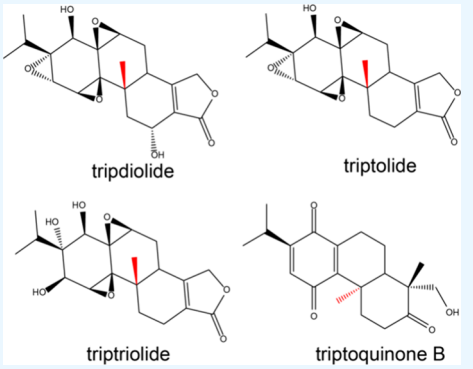
Youwen Chen, Yadan Wang, Yiwen Xu, Shuangcheng Ma,* Huiying Yang,* Yuanyan Liu,* and Xianfu Wu*

Cite This: *ACS Omega* 2024, 9, 27321−27328

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Tripterygium glycoside tablets (TGTs) are preparations extracted and purified from *Tripterygium wilfordii* Hook. F and are extensively utilized in the treatment of autoimmune diseases, such as rheumatoid arthritis (RA). However, variations in production processes among manufacturers can lead to challenges in quality control and clinical utilization of TGTs. A band-selective 2D $^{1}H-^{13}C$ HSQC quantification method was applied for the determination of 13 active ingredients in TGTs. This method was validated following the guidelines of USP-NF 2022. The results demonstrated that the quantitative method exhibited excellent signal resolution, as well as sufficient accuracy, sensitivity, and stability. In addition, the $^{1}H$ NMR spectra of TGTs from three manufacturers underwent analysis using principal component analysis and orthogonal partial least-squares discriminant analysis. The results revealed significant differences among the TGTs from the three manufacturers, with manufacturer 2 and manufacturer 3 demonstrating superior product consistency compared to manufacturer 1. A quality evaluation system for TGTs was developed based on band-selective 2D $^{1}H-^{13}C$ HSQC and $^{1}H$ NMR, encompassing both quality markers and fingerprinting. This system offers reliable approaches and insights for enhancing the quality control of natural products.




## 1. INTRODUCTION

*Tripterygium wilfordii* Hook. F (TwHF) has long been used as a traditional herbal medicine to treat various autoimmune and inflammatory diseases in China.[1] Recent pharmacological and phytochemical studies have shown that TwHF has significant anti-inflammatory and immunosuppressive activity studies, and diterpenes, triterpenes, and sesquiterpene alkaloids are the main active components in TwHF.[2−5] *Tripterygium* Glycoside Tablets (TGTs) is a preparation that is specifically enriched with diterpenes, triterpenes, and sesquiterpene alkaloids from the root of TwHF and has become the first-line therapy for RA in China.[6−8] Triptolide and wilforlide A are defined as quality markers in the current quality standards set by the National Medical Products Administration of China, in which the content of triptolide should be less than 10 $\mu$g/tablet and the content of wilforlide A should be more than 10 $\mu$g/tablet.[9] The TGTs contain hundreds of compounds, with terpenoids, triterpenes, and alkaloids comprising the majority. In addition to triptolide and wilforlide A, compounds such as tripdiolide, triptoquinone B, wilforgine, wilforine, celastrol, demethylzeylasteral have also been reported to exhibit significant anti-inflammatory activity.[1] Therefore, it is necessary to quantitate these active ingredients in the TGTs. Due to the structural similarity among these compounds (Figures 1−3), it is challenging to separate and quantify them using conventional chromatographic methods. Hence, it is imperative to establish a method with a high resolution for the quantitative analysis of multiple active ingredients in TGTs. Furthermore, TGTs taken by RA patients may come from multiple manufacturers. As an herbal extract, the chemical compositions of TGTs from different manufacturers vary, likely attributed to their different production processes. Even different batches of TGTs from the same manufacturer may also have poor product consistency.[7] Maintaining product consistency is crucial for the clinical efficacy of the medications. Therefore, it is necessary to establish a rapid and reliable method for evaluating the consistency of the TGTs from various manufacturers.

NMR can provide structural information such as chemical shifts, *J*-coupling coefficients, and number of peaks for compound identification, and also enables accurate quantification by peak area.[10,11] $^{1}H$ NMR can rapidly characterize the

Received: February 26, 2024
Revised: June 1, 2024
Accepted: June 4, 2024
Published: June 13, 2024

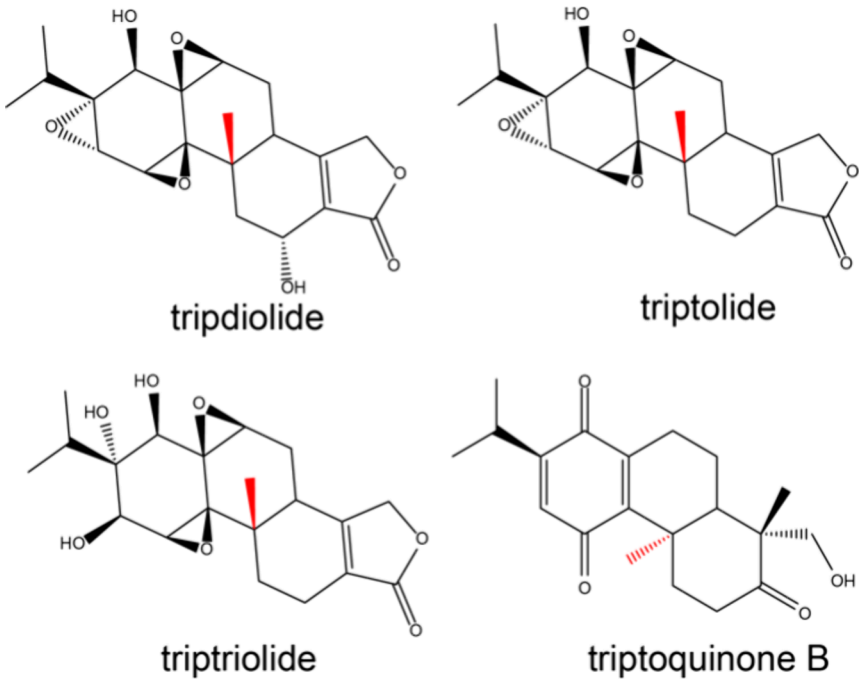



**Figure 1.** Structures of four diterpenes. The quantitative signals are marked in red.

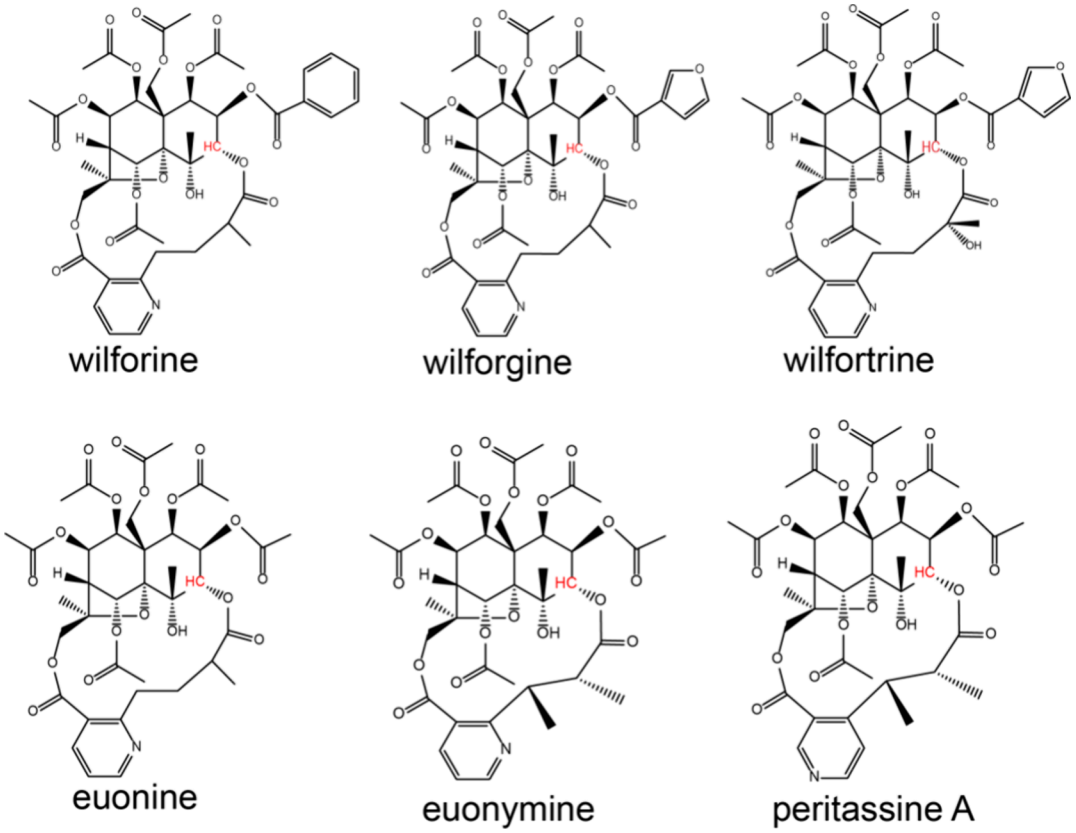



**Figure 2.** Structures of six alkaloids. The quantitative signals are marked in red.

protons of all compounds in complex mixtures, and the spectra are as characteristic as fingerprints.[12] Based on multivariate statistical analysis, $^{1}$H NMR fingerprinting plays a great role in the classification of herbal plants by species, geographic origin, and seasonal differences, as well as in the identification of authenticity, production, and batches of herbal preparations.[13−17] Quantitative NMR is highly reproducible and versatile.[18,19] However, for complex natural products, $^{1}$H NMR spectra sometimes show severe peak overlap, which makes it difficult to achieve signal separation and accurate integration. 2D $^{1}$H−$^{13}$C HSQC obtains high resolution while maintaining acceptable sensitivity by extending the signal to the $^{13}$C



https://doi.org/10.1021/acsomega.4c01878
ACS Omega 2024, 9, 27321−27328

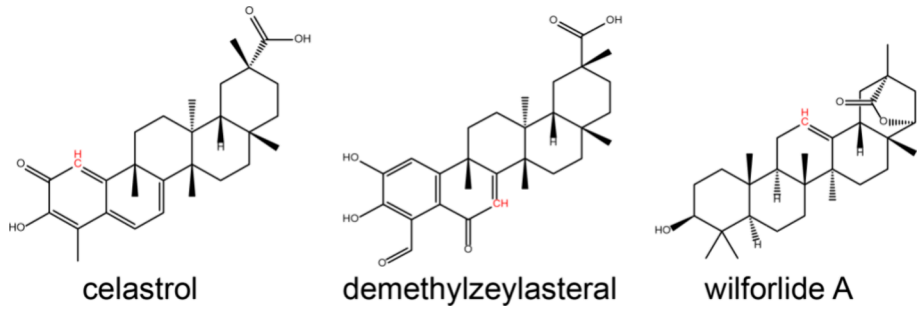



**Figure 3.** Structures of three triterpenes. The quantitative signals are marked in red.

dimension.[19] Therefore, 2D $^{1}H-^{13}C$ HSQC experiments are increasingly used for the quantitative analysis of complex mixtures, involving a range of natural products, body fluids, and food products.[20−25] Band-selective 2D $^{1}H-^{13}C$ HSQC is a 2D NMR spectrum with high resolution due to the excitation of a specified region resulting in a much higher sampling density of that region.[26,27] Even for botanical extracts containing structural analogs, band-selective 2D $^{1}H-^{13}C$ HSQC can significantly separate the signals of multiple analogs. For example, Xiao et al. completed the identification and quantification of 12 lignans in *Sambucus williamsii* Hance by band-selective 2D $^{1}H-^{13}C$ HSQC.[27]

In recent years, multicomponent quantification by LC-MS and bioactivity determination have gradually become the primary strategies for quality evaluation of TGTs,[6,28] but NMR is still not mentioned even as a stable and rapid method. In this study, we determined 13 active ingredients in TGTs from 3 manufacturers by band-selective 2D $^{1}H-^{13}C$ HSQC spectra. The linearity, accuracy, precision, and quantitation limit of the method were validated by USP-NF 2022. In addition, we acquired $^{1}H$ NMR spectra of 65 batches of TGTs from 3 manufacturers. Combined with PCA and OPLS-DA, consistency evaluation and classification of TGTs from three manufacturers were achieved using $^{1}H$ NMR spectra. This work combines quantitative band-selective 2D $^{1}H-^{13}C$ HSQC, $^{1}H$ NMR, and multivariate statistical analysis to comprehensively evaluate TGTs from 3 manufacturers in terms of both quality markers and product consistency.

## 2. RESULTS AND DISCUSSION

**2.1. Band-Selective 2D $^{1}H-^{13}C$ HSQC Spectra of TGTs.** The regular 2D $^{1}H-^{13}C$ HSQC spectra of TGTs exhibit severe signal overlap (Figure 4). To minimize signal interference between terpenoids, triterpenes, and alkaloids, C−H pairs with similar chemical environments and distinguishable from the other classes were selected as quantitative signals for each class of compounds (Figures 1−3). Band-selective 2D $^{1}H-^{13}C$ HSQC spectra with high resolution can be obtained by exciting the region in which the quantitative signals of terpenoids, triterpenes, and alkaloids are located. It provided excellent signal separation and thus contributed to signal identification. Based on spectra of standards (Figures S1−S13), 13 compounds were identified from band-selective 2D $^{1}H-^{13}C$ HSQC spectra of TGTs (Figure 4), respectively. The content of the two compounds in the TGTs was estimated by an external standard curve.

**2.2. Method Validation.** *2.2.1. Lineraty and Range.* To verify the linearity, a series of standard solutions of 13 compounds were prepared, respectively. The standard curves were constructed by plotting the peak volumes of quantitative signals versus the concentrations (Figures S14−S26). The regression equations and coefficients of determination ($R^2$) for these compounds are given in Table 1. All standard curves exhibited good linearity with $R^2$ greater than 0.99. Based on the equations, the contents of 13 compounds in TGTs from three manufacturers were calculated (Table 2).

*2.2.2. Accuracy.* The accuracy was evaluated by spiking experiments. Since we were unable to obtain a blank preparation without the 13 compounds, we assessed the recovery by adding different levels of standards to the samples to be tested. The samples prepared by manufacturer 2's TGTs were spiked with different levels (high, medium, and low levels) of reference standards. Quantification of 13 compounds in synthetic samples was performed according to an established method. Comparing the results obtained with the theoretical values, the recoveries of 13 compounds ranged from 95.25% to 104.36% (Table 3).

*2.2.3. Precision.* Six samples were prepared in parallel to assess the repeatability. The mean RSD values of 13 compounds ranged from 0.09 to 2.50% (Table 3). In addition, three different analysts performed the analytical procedure twice at different times to assess the intermediate precision, and the RSD values of 13 compounds ranged from 1.98 to 3.15%. The precision validation criteria for compounded pharmaceutical finished products in USP-NF 2022 state that the RSD should not be more than 2.0% (repeatability) or 3.0% (intermediate precision). Considering the complex composition of TGTs, the precision of this quantitative method basically meets the pharmacopoeial requirements.

*2.2.4. Quantitation Limit.* The quantitation limit was estimated by calculating the standard deviation of the integrated noise from six regions on band-selective 2D $^{1}H-^{13}C$ HSQC spectra and then dividing by the slope of the calibration line and multiplying by 10. The quantitation limit of 13 compounds ranged from 0.04 to 0.46 mM.

*2.2.5. Robustness.* The results of quantitative 2D $^{1}H-^{13}C$ HSQC are affected by parameters such as the relaxation time and temperature. With other parameters fixed, D1 was changed to 2.5 s, and the probe temperature was replaced by 303 K. The data obtained were compared with the original data (*t* test) and no significant differences were found. This indicates that the method has a certain degree of robustness.

**2.3. Consistency Evaluation and Classification of TGTs Based on $^{1}H$ NMR.** To assess the consistency of TGTs from different manufacturers, PCA was performed on the data sets of $^{1}H$ NMR spectra from 38 batches of TGTs (D1-D38, Figures S27−S29). Figure 5A shows the score plot for PCA, a five-component model explaining 100% of the variance. The contribution of principal component 1 (PC1) and principal component 2 (PC2) to the total variance was


https://doi.org/10.1021/acsomega.4c01878
ACS Omega 2024, 9, 27321−27328

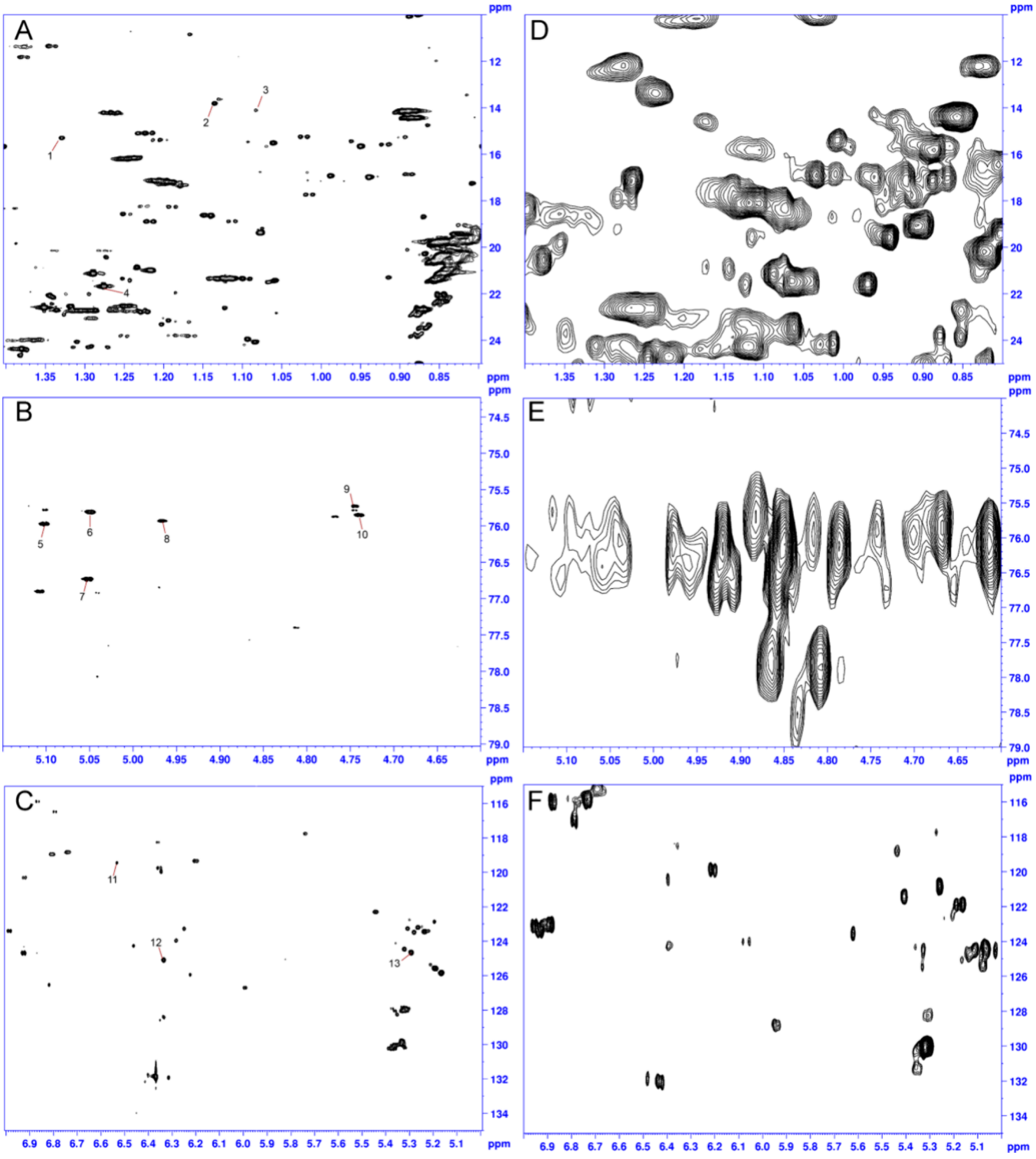



**Figure 4.** 2D $^{1}H-^{13}C$ HSQC spectra of TGTs. (A, B, C) Band-selective 2D $^{1}H-^{13}C$ HSQC spectra acquired based on the quantitative signals of diterpenes, alkaloids, and triterpenes, respectively. (D, E,F) Zoomed-out corresponding regions of the regular 2D $^{1}H-^{13}C$ HSQC spectra.

66.7 and 13.1%, respectively. The TGTs from the 3 manufacturers were well separated on the PCA score plot, with samples from each manufacturer largely within the 95% confidence interval. It indicates that TGTs from different manufacturers have significant differences and TGTs from the same manufacturer have a high degree of similarity and consistency. In terms of dispersion, the points of manufacturers 2 and 3 were significantly more concentrated than those of manufacturer 1, indicating that the drug quality of manufacturers 2 and 3 was more stable.

In addition, an OPLS-DA classification model was developed for TGTs from 3 manufacturers based on the $^{1}H$ NMR spectra from 38 batches of TGTs (Figure 5B). The data were divided into 7 folds for cross-validation, $R^2$ is 0.97, and $Q^2$ is 0.96 when only component 1 and component 2 are considered, indicating that the OPLS-DA model for TGTs



https://doi.org/10.1021/acsomega.4c01878
ACS Omega 2024, 9, 27321−27328

Table 1. Regression Equation of 13 Compounds

| no. | compound | regression equation | $R^2$ |
|---|---|---|---|
| 1 | tripdiolide | $y = 8.16 \times 10^{10}x + 1.02 \times 10^{8}$ | 0.9999 |
| 2 | triptolide | $y = 8.06 \times 10^{10}x + 2.55 \times 10^{8}$ | 0.9999 |
| 3 | triptriolide | $y = 8.14 \times 10^{10}x + 1.62 \times 10^{8}$ | 0.9997 |
| 4 | triptoquinone B | $y = 8.13 \times 10^{10}x + 4.41 \times 10^{8}$ | 0.9998 |
| 5 | wilforine | $y = 3.98 \times 10^{10}x + 1.86 \times 10^{9}$ | 0.9985 |
| 6 | wilforgine | $y = 3.95 \times 10^{10}x + 2.28 \times 10^{9}$ | 0.9983 |
| 7 | wilfortrine | $y = 3.97 \times 10^{10}x + 1.59 \times 10^{9}$ | 0.9984 |
| 8 | euonine | $y = 3.97 \times 10^{10}x + 1.63 \times 10^{8}$ | 0.9989 |
| 9 | euonymine | $y = 3.79 \times 10^{10}x + 6.57 \times 10^{9}$ | 0.9978 |
| 10 | peritassine A | $y = 3.98 \times 10^{10}x - 2.02 \times 10^{9}$ | 0.9996 |
| 11 | celastrol | $y = 4.61 \times 10^{10}x + 3.32 \times 10^{8}$ | 0.9998 |
| 12 | demethylzeylasteral | $y = 4.60 \times 10^{10}x + 1.31 \times 10^{8}$ | 0.9999 |
| 13 | wilforlide A | $y = 4.42 \times 10^{10}x + 8.13 \times 10^{8}$ | 0.9996 |

from the 3 manufacturers has good prediction.[29] Additionally, we collected $^1$H NMR of another 27 batches of TGTs and used these spectral data sets as the prediction set to validate the OPLS-DA model.[30,31] By importing the prediction set ($x1-x27$) into the OPLS-DA model and setting these samples to no class, the $Y_{Pred}$ value, the probability of the prediction set being classified into the specified class, can then be read from the "Classification List" module of SIMCA 14.1. A sample is considered to belong to a specified class when the $Y_{Pred}$ value is greater than 0.65, and when the $Y_{Pred}$ value is less than 0.3, the sample is considered not to belong to that class. The Classification List of the prediction set showed the prediction results for samples $x1-x27$ (Table S1), which is consistent with the actual classification. It meant that the sensitivity, specificity, and accuracy of the developed model were 100%.[30]

## 3. CONCLUSIONS

In this study, a quantitative band-selective 2D $^1$H−$^{13}$C HSQC method was applied for the determination of 13 active ingredients in TGTs from 3 manufacturers. The established quantitative method was validated according to USP-NF 2022, and the results showed that the method was sufficiently accurate, precise, and sensitive. The results showed the great potential of band-selective 2D $^1$H−$^{13}$C HSQC for the quantification of multiple components of herbs. In addition, $^1$H NMR of 65 batches of TGTs from the three manufacturers were collected, and the consistency of TGTS from each manufacturer was evaluated and a classification model of TGTs was developed based on PCA and OPLS-DA. The $^1$H NMR fingerprinting of TGTs from the same manufacturer all showed a high degree of similarity, while products from manufacturers 2 and 3 had better consistency than those from manufacturer 1. Compared with the original method for quality evaluation of TGTs, the established system achieves quality control of TGTs in two dimensions: quality markers and overall chemical profile, which is of significance for the quality evaluation of natural products and their related preparations.

Table 3. Validation Results of 13 Compounds in TGTs

| compound | recovery | repeatability | intermediate precision | LOQ (mM) |
|---|---|---|---|---|
| tripdiolide | 103.21% | 1.67% | 2.31% | 0.05 |
| triptolide | 103.10% | 0.09% | 2.15% | 0.04 |
| triptriolide | 104.36% | 2.50% | 3.15% | 0.04 |
| triptoquinone B | 98.97% | 1.23% | 1.98% | 0.06 |
| wilforine | 97.25% | 1.64% | 2.42% | 0.44 |
| wilforgine | 96.59% | 1.65% | 2.15% | 0.41 |
| wilfortrine | 98.56% | 1.78% | 3.02% | 0.46 |
| euonine | 99.02% | 1.48% | 2.81% | 0.35 |
| euonymine | 97.62% | 2.27% | 2.65% | 0.36 |
| peritassine A | 95.25% | 1.93% | 2.37% | 0.41 |
| celastrol | 97.18% | 2.18% | 3.24% | 0.08 |
| demethylzeylasteral | 101.76% | 1.14% | 2.05% | 0.07 |
| wilforlide A | 99.05% | 1.16% | 2.28% | 0.11 |

## 4. MATERIALS AND METHODS

**4.1. Sample Preparation.** A randomly selected batch of TGT from each manufacturer was ground separately into a powder. The powder equal to 20 tablets was dispersed in 30 mL of methanol and extracted by ultrasonication for 30 min. For the determination of diterpenes and alkaloid compounds in TGTs, the methanol extract was evaporated and redissolved with a small amount of ethyl acetate and then purified by Sep-Pak Alumina N cartridges (Waters, Part No. WAT054630), using 30 mL of ethyl acetate as eluent. The eluate was evaporated and redissolved with an appropriate amount of $CDCl_3$ to fix the final volume of 1 mL. For the determination of triterpenes in TGTs, the methanol extract was evaporated and redissolved with a small amount of ethyl acetate and then purified by Sep-Pak Silica cartridges (Waters, Part NO. WAT036920). The eluate was evaporated and redissolved

Table 2. Contents of 13 Compounds in TGTs from Three Manufacturers

| | content ($\mu$g/tablet) | | |
|---|---|---|---|
| compound | manufacturer 1 ($n$ = 6) | manufacturer 2 ($n$ = 6) | manufacturer 3 ($n$ = 6) |
| tripdiolide | 1.51 ± 0.05 | 7.36 ± 0.03 | 3.84 ± 0.05 |
| triptolide | 3.80 ± 0.06 | 7.28 ± 0.04 | 4.95 ± 0.03 |
| triptriolide | 1.70 ± 0.06 | 3.73 ± 0.05 | 1.51 ± 0.04 |
| triptoquinone B | 12.97 ± 0.12 | 43.61 ± 0.56 | 35.33 ± 0.52 |
| wilforine | 80.17 ± 1.23 | 50.04 ± 0.87 | 65.06 ± 1.07 |
| wilforgine | 125.85 ± 1.52 | 72.39 ± 0.95 | 95.36 ± 2.31 |
| wilfortrine | 128.20 ± 2.21 | 61.25 ± 1.05 | 44.42 ± 0.85 |
| euonine | 41.10 ± 0.77 | 26.84 ± 0.39 | 46.93 ± 0.52 |
| euonymine | 50.96 ± 1.20 | 17.80 ± 0.26 | 38.69 ± 1.17 |
| peritassine A | 57.94 ± 1.07 | 34.94 ± 0.86 | 64.05 ± 0.96 |
| celastrol | 2.37 ± 0.07 | 8.23 ± 0.19 | 61.23 ± 0.79 |
| demethylzeylasteral | 4.09 ± 0.08 | 44.15 ± 0.36 | 37.19 ± 0.24 |
| wilforlide A | 44.89 ± 0.54 | 51.28 ± 0.40 | 25.36 ± 0.38 |



https://doi.org/10.1021/acsomega.4c01878
ACS Omega 2024, 9, 27321−27328

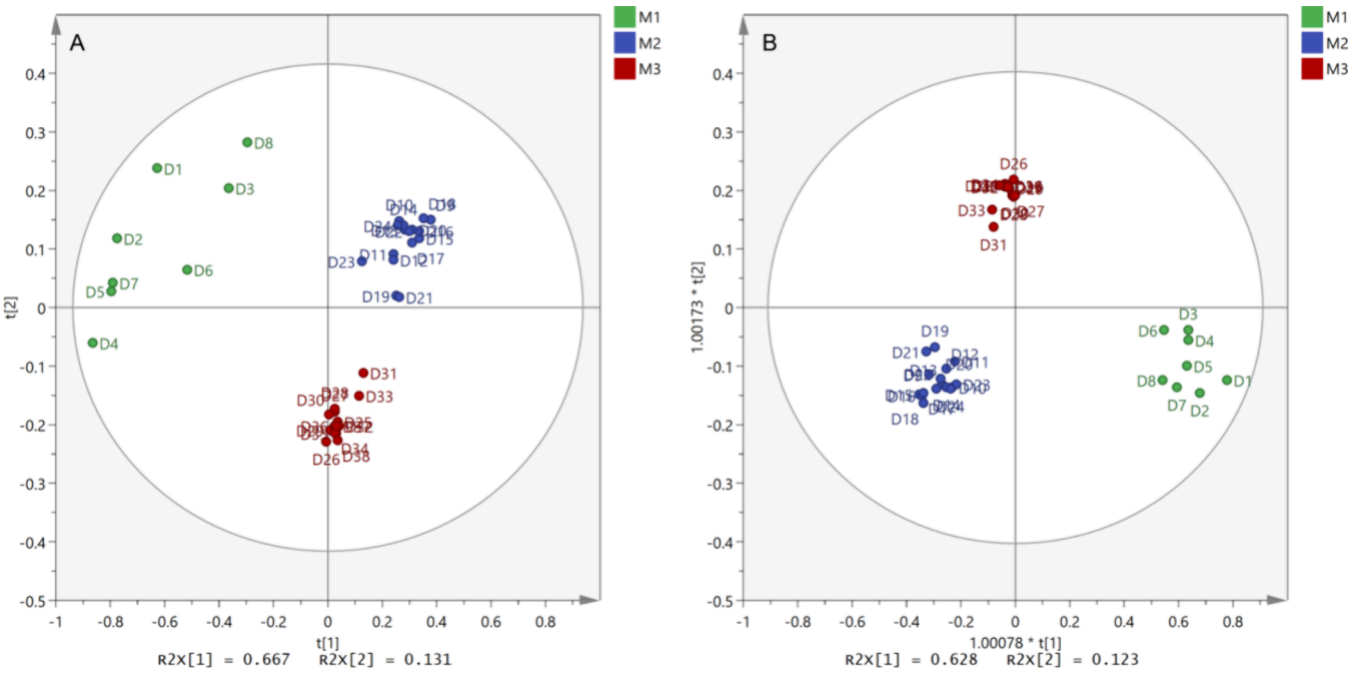



**Figure 5.** Score plot of the PCA model (A) and OPLS-DA model (B). Each point in the plot represents the $^{1}$H NMR spectrum of an individual sample, and samples from different manufacturers are marked with different colors.

with an appropriate amount of $CDCl_3$ to fix the final volume of 1 mL. All samples to be tested were prepared six times in parallel. The samples with a concentration of 20 tablets·mL$^{-1}$ were used separately for band-selective 2D $^{1}$H−$^{13}$C HSQC experiments.

On the other hand, 65 batches of TGT powder (equivalent to five tablets) from three manufacturers were individually weighed and extracted by ultrasonication with 20 mL of methanol for 30 min. The extract was filtered, evaporated, and redissolved with 1 mL of $CDCl_3$. Finally, 500 $\mu$L of the solutions were taken for $^{1}$H NMR experiments. A schematic diagram of the sampling method is provided in the supplementary data (Figure S30).

**4.2. Band-Selective 2D $^{1}$H−$^{13}$C HSQC Experiments and Data Processing.** All spectra were recorded on a Bruker Ascend 600 nuclear magnetic resonance spectrometer (Bruker Corp., Switzerland). The $T_1$ values of the quantitative signals of the 13 compounds were determined by the standard experiment "PROTONT1″ and the $T_1$ values ranged from 0.71 to 1.20 s. The band-selective 2D $^{1}$H−$^{13}$C HSQC experiments were performed with the Bruker shsqcetgpsisp2.2 pulse program, which uses a band-selective shaped $^{13}$C refocusing pulse to avoid spectral folding. For diterpenes, the excitation region covered 0.8−1.4 ppm in F2 ($^{1}$H channel) and 10−25 ppm in F1 ($^{13}$C channel); the region of alkaloids was ranged of 4.5−5.3 ppm in F2 and 74.2−79 ppm in F1; the region of triterpenes was ranged of 5−7 ppm in F2 and 115−135 ppm in F1. Other acquisition parameters for both experiments were consistent as follows: temperature at 298 K; 16 dummy scans; 32 scans; a 6 s relaxation delay; a 101 receiver gain; 1024 points collected in F2 and 256 increments in F1. Zero filling was applied to 4 K in F2 and 1 K in F1. A $^{1}$H 90° hard pulse was automatically calibrated before spectra acquisition. All the spectra data were processed using the Topspin 4.1.3 software for manual phase correction and automatic baseline correction. The automatic peak detection routine ("peak picking") in the 2D mode was employed in the spectral region of interest, with the following parameters: mi 0.01; maxi 1; ppdiag 1; ppresol 5; ppiptyp parabolic; psign all. The peaks were integrated using the automatic peak integration of the topspin software and setting the threshold to 0.001.

**4.3. $^{1}$H NMR Fingerprinting and Multivariate Statistical Analysis.** $^{1}$H NMR spectra of the samples were recorded by using a standard Bruker zg30 pulse sequence. A total of 63,536 data points were collected with a spectral width of 20 ppm, a relaxation delay of 1 s, 128 repetitions, and a receiver gain of 16. Phase and baseline corrections were performed manually for all spectra by Topspin 4.1.3 software. To compare the $^{1}$H NMR fingerprinting of TGTs from three manufacturers, the $^{1}$H NMR spectra were divided into buckets of 0.04 ppm on the Bruker AssureNMR 2.2 software. The solvent peak (7.26−7.30 ppm) was removed from the data set. The remaining data were imported into SIMCA 14.1 (Umetrics, Sweden) for Pareto scaling before PCA and OPLS-DA.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01878.

$^{1}$H NMR and 2D $^{1}$H−$^{13}$C HSQC spectra of 13 compounds; $^{1}$H NMR spectra of TGTs from three manufacturers; classification list of prediction set; and schematic diagram of the sampling method (DOCX)

## ■ AUTHOR INFORMATION

### Corresponding Authors

**Shuangcheng Ma** − *National Institutes for Food and Drug Control, Beijing 102629, P.R. China;* orcid.org/0000-0003-0466-857X; Email: masc@nifdc.org.cn



https://doi.org/10.1021/acsomega.4c01878
*ACS Omega* 2024, 9, 27321−27328

**Huiying Yang** − *National Institutes for Food and Drug Control, Beijing 102629, P.R. China*; Email: yanghuiying@nifdc.org.cn

**Yuanyan Liu** − *School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, P.R. China*; orcid.org/0000-0003-3840-5292; Email: yyliu_1980@163.com

**Xianfu Wu** − *National Institutes for Food and Drug Control, Beijing 102629, P.R. China*; orcid.org/0000-0001-7675-0543; Email: wuxf99@163.com

### Authors

**Youwen Chen** − *School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, P.R. China; National Institutes for Food and Drug Control, Beijing 102629, P.R. China*

**Yadan Wang** − *National Institutes for Food and Drug Control, Beijing 102629, P.R. China*

**Yiwen Xu** − *National Institutes for Food and Drug Control, Beijing 102629, P.R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c01878

### Author Contributions

Y.C.: conceptualization, investigation, experiment, writing—review and editing. Y.W.: formal analysis, material collection, experiment, writing—review and editing. Y.X.: supervision, investigation, software. Y.L.: design, formal analysis, project administration. X.W.: methodology, literature research, experimental validation. S.M.: material collection, supervision, investigation.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors kindly acknowledge the financial support from National Institutes for Food and Drug Control of China.

## ■ REFERENCES

(1) Zhang, Y.; Mao, X.; Li, W.; Chen, W.; Wang, X.; Ma, Z.; Lin, N. Tripterygium wilfordii: An inspiring resource for rheumatoid arthritis treatment. *Med. Res. Rev.* **2021**, *41* (3), 1337−1374.

(2) Lv, H.; Jiang, L.; Zhu, M.; Li, Y.; Luo, M.; Jiang, P.; Tong, S.; Zhang, H.; Yan, J. The genus Tripterygium: A phytochemistry and pharmacological review. *Fitoterapia* **2019**, *137*, No. 104190.

(3) He, L.; Liang, Z.; Zhao, F.; Peng, L.; Chen, Z. Modulation of IL-37 expression by triptolide and triptonide in THP-1 cells. *Cell Mol. Immunol.* **2015**, *12* (4), 515−518.

(4) Yang, J. H.; Luo, S. D.; Wang, Y. S.; Zhao, J. F.; Zhang, H. B.; Li, L. Triterpenes from Tripterygium wilfordii Hook. *J. Asian Nat. Prod. Res.* **2006**, *8* (5), 425−429.

(5) Gao, C.; Huang, X.; Bai, M.; Wu, J.; Li, J.; Liu, Q.; Li, L.; Song, S. Anti-inflammatory sesquiterpene pyridine alkaloids from Tripterygium wilfordii. *Fitoterapia* **2015**, *105*, 49−54.

(6) Gao, X.; Du, X.; An, L.; Wang, Y.; Wang, L.; Wu, Z.; Huang, C.; He, X. Wilforine, the Q-marker and PK-maker of Tripterygium glycosides tablet: Based on preparation quantitative analysis and PK-PD study. *Phytomedicine* **2019**, *54*, 357−364.

(7) Wang, Y.; Dai, Z.; Yan, J.; Wu, X.; Ma, S. Comprehensive Evaluation of the Quality of Tripterygium Glycosides Tablets Based on Multi-Component Quantification Combined with an In Vitro Biological Assay. *Molecules* **2022**, *27* (16), 5102.

(8) Su, M. X.; Zhou, W. D.; Lan, J.; Di, B.; Hang, T. J. Rapid and sensitive analysis of multiple bioactive constituents in tripterygium glycosides tablets using liquid chromatography coupled with time-of-flight mass spectrometry. *J. Sep. Sci.* **2015**, *38* (5), 804−812.

(9) National Medical Products Administration. *National Pharmaceutical Standards: WS3-B-3350−98−2011 [S]*; China, 2011.

(10) Convertini, R.; Patz, C.; Kumar, K.; May, B.; Andlauer, W.; Schweiggert, R. (1)H NMR spectrometry for methanol quantification in apple wines and ciders as optimized by comparison to SIDA-HS-GC-MS. *Food. Chem.* **2022**, *387*, No. 132912.

(11) Ghiviriga, I. Nuclear Magnetic Resonance Method for Measuring Water Solubility of Organic Compounds. *Anal. Chem.* **2023**, *95* (5), 2706−2712.

(12) Ellis, D.; Brewster, V.; Dunn, W.; Allwood, J.; Golovanov, A.; Goodacre, R. Fingerprinting food: current technologies for the detection of food adulteration and contamination. *Chem. Soc. Rev.* **2012**, *41* (17), 5706−5727.

(13) Freitas, J. V. B.; Alves Filho, E. G.; Silva, L. M. A.; Zocolo, G. J.; de Brito, E. S.; Gramosa, N. V. Chemometric analysis of NMR and GC datasets for chemotype characterization of essential oils from different species of Ocimum. *Talanta* **2018**, *180*, 329−336.

(14) Brahmi, F.; Nguyen, A. T.; Nacoulma, A. P.; Sheridan, H.; Wang, J.; Guendouze, N.; Madani, K.; Duez, P. Discrimination of Mentha species grown in different geographical areas of Algeria using (1)H-NMR-based metabolomics. *J. Pharm. Biomed. Anal.* **2020**, *189*, No. 113430.

(15) Hu, B.; Cao, Y.; Zhu, J.; Xu, W.; Wu, W. Analysis of metabolites in chardonnay dry white wine with various inactive yeasts by (1)H NMR spectroscopy combined with pattern recognition analysis. *AMB Express* **2019**, *9* (1), 140.

(16) Pariyani, R.; Ismail, I. S.; Ahmad Azam, A.; Abas, F.; Shaari, K. Identification of the compositional changes in Orthosiphon stamineus leaves triggered by different drying techniques using (1) H NMR metabolomics. *J. Sci. Food. Agric.* **2017**, *97* (12), 4169−4179.

(17) Zhao, H.; Xu, J.; Ghebrezadik, H.; Hylands, P. J. Metabolomic quality control of commercial Asian ginseng, and cultivated and wild American ginseng using (1)H NMR and multi-step PCA. *J. Pharm. Biomed. Anal* **2015**, *114*, 113−120.

(18) Bourafai-Aziez, A.; Jacob, D.; Charpentier, G.; Cassin, E.; Rousselot, G.; Moing, A.; Deborde, C. Development, Validation, and Use of (1)H-NMR Spectroscopy for Evaluating the Quality of Acerola-Based Food Supplements and Quantifying Ascorbic Acid. *Molecules* **2022**, *27* (17), 5614.

(19) Lhoste, C.; Lorandel, B.; Praud, C.; Marchand, A.; Mishra, R.; Dey, A.; Bernard, A.; Dumez, J. N.; Giraudeau, P. Ultrafast 2D NMR for the analysis of complex mixtures. *Prog. Nucl. Magn. Reson. Spectrosc.* **2022**, *130−131*, 1−46.

(20) Lewis, I.; Schommer, S.; Hodis, B.; Robb, K.; Tonelli, M.; Westler, W.; Sussman, M.; Markley, J. Method for determining molar concentrations of metabolites in complex solutions from two-dimensional 1H-13C NMR spectra. *Anal. chem.* **2007**, *79* (24), 9385−9390.

(21) Boerkamp, V. J. P.; Merkx, D. W. H.; Wang, J.; Vincken, J. P.; Hennebelle, M.; van Duynhoven, J. P. M. Quantitative assessment of epoxide formation in oil and mayonnaise by (1)H-(13)C HSQC NMR spectroscopy. *Food. Chem.* **2022**, *390*, No. 133145.

(22) Talebi Amiri, M.; Bertella, S.; Questell-Santiago, Y. M.; Luterbacher, J. S. Establishing lignin structure-upgradeability relationships using quantitative (1)H-(13)C heteronuclear single quantum coherence nuclear magnetic resonance (HSQC-NMR) spectroscopy. *Chem. Sci.* **2019**, *10* (35), 8135−8142.

(23) (23) Hu, K.; Westler, W. M.; Markley, J. L. Simultaneous quantification and identification of individual chemicals in metabolite mixtures by two-dimensional extrapolated time-zero (1)H-(13)C HSQC (HSQC(0)). *J. Am. Chem. Soc.* **2011**, *133* (6), 1662−1665.

(24) Rai, R.; Sinha, N. Fast and accurate quantitative metabolic profiling of body fluids by nonlinear sampling of 1H−13C two-dimensional nuclear magnetic resonance spectroscopy. *Anal. chem.* **2012**, *84* (22), 10005−10011.

(25) Heikkinen, S.; Toikka, M.; Karhunen, P.; Kilpeläinen, I. Quantitative 2D HSQC (Q-HSQC) via suppression of J-dependence

of polarization transfer in NMR spectroscopy: application to wood lignin. *J. Am. Chem. Soc.* **2003**, *125* (14), 4362−4367.
(26) Girreser, U.; Ugolini, T.; Cicek, S. S. Quality control of Aloe vera (Aloe barbadensis) and Aloe ferox using band-selective quantitative heteronuclear single quantum correlation spectroscopy (bs-qHSQC). *Talanta* **2019**, *205*, 120109.
(27) Xiao, H. H.; Lv, J.; Mok, D.; Yao, X. S.; Wong, M. S.; Cooper, R. NMR Applications for Botanical Mixtures: The Use of HSQC Data to Determine Lignan Content in Sambucus williamsii. *J. Nat. Prod.* **2019**, *82* (7), 1733−1740.
(28) Qu, L.; Xiao, Y.; Jia, Z.; Wang, Z.; Wang, C.; Hu, T.; Wu, C.; Zhang, J. Comprehensive two-dimensional liquid chromatography coupled with quadrupole time-of-flight mass spectrometry for chemical constituents analysis of tripterygium glycosides tablets. *J. Chromatogr. A* **2015**, *1400*, 65−73.
(29) Kang, C.; Zhang, Y.; Zhang, M.; Qi, J.; Zhao, W.; Gu, J.; Guo, W.; Li, Y. J. F. c. Screening of specific quantitative peptides of beef by LC-MS/MS coupled with OPLS-DA. *Food. Chem.* **2022**, *387*, No. 132932.
(30) Kharbach, M.; Viaene, J.; Yu, H.; Kamal, R.; Marmouzi, I.; Bouklouze, A.; Heyden, Y. J. J. o. c. A. Secondary-metabolites fingerprinting of Argania spinosa kernels using liquid chromatography-mass spectrometry and chemometrics, for metabolite identification and quantification as well as for geographic classification. *J. Chromatogr. A* **2022**, *1670*, No. 462972.
(31) Khan, S.; Rai, A.; Singh, A.; Singh, S.; Dubey, B.; Lal, R.; Negi, A.; Birse, N.; Trivedi, P.; Elliott, C.; Ch, R. Rapid metabolic fingerprinting with the aid of chemometric models to identify authenticity of natural medicines: Turmeric, Ocimum,and Withania somnifera study. *J. Pharm. Anal.* **2023**, *13* (9), 1041−1057.


https://doi.org/10.1021/acsomega.4c01878
*ACS Omega* 2024, 9, 27321−27328