Interbatch quality control of the extract from *Artemisia frigida* Willd. by spectrum–effect relationship between HPLC fingerprints and the total antioxidant capacity

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**ABSTRACT**

Similarity analysis of complicated chromatographic profiles is a potential protocol for the authentication and quality control of traditional Chinese medicine to guarantee prospective biological activity. In this work, a high-performance liquid chromatography (HPLC) method was established to identify and control the inter-assay quality of extract from *Artemisia frigida* Willd. Meanwhile, a partial least squares regression (PLSR) was applied to construct a spectrum–effect relationship between the HPLC fingerprints and the total antioxidant capacity of the extract from *Artemisia frigida* Willd. The similarity correlation coefficient of 10 batches of extract was ≥0.948 could guarantee the 10 batches of showed the inter-assay stability of antioxidant capacity of extract from *Artemisia frigida* Willd. Moreover, PLSR analysis showed that four efficacy-related components were closely related to antioxidant effect. Therefore, this study provided a flexible and efficient method for the quality and the stable biological activity assessment of *Artemisia frigida* Willd.

**ARTICLE HISTORY**

Received 15 September 2021
Revised 4 February 2022
Accepted 7 March 2022

**KEYWORDS**

*Artemisia frigida* Willd.; Extract; High performance liquid chromatography; Total antioxidant capacity; Spectrum–effect relationship

**Introduction**

Traditional Chinese Medicine (TCM) has been increasingly employed as complementary and alternative medicine to treat or prevent disease.\(^{[1,2]}\) However, the chemical composition of TCM with rich medicinal potential is extremely complex. Therefore, in today’s world, the quality control of TCM has always been a problem to be overcome. Over the past 20 years, the HPLC fingerprint has been advocated by the World Health Organization (WHO) as an effective approach for the quality control and identification of medicinal herbs,\(^{[3,4]}\) which can reflect its chemical composition on the whole. Hence, the HPLC fingerprinting technique has gained international recognition as a powerful, user-friendly, and practical method for ensuring the quality of the raw materials and products of TCM.\(^{[5,6]}\)

*Artemisia frigida* Willd. (*Artemisia frigida*) is a plant belonging to the genus *Artemisia* of the Compositae family and is mainly distributed in Inner Mongolia. Based on research literature, researches on *Artemisia frigida* have focused on chemical composition\(^{[7]}\) pharmacological effect,\(^{[8–10]}\) such as anti-tumor effect,\(^{[11]}\) anti-inflammatory effect,\(^{[12]}\) antibacterial and antioxidant effect,\(^{[13]}\)
and feeding value.\textsuperscript{[10,12–14]} As is known to all, the intra-assay quality stability of samples affects the intra-assay bioactivity stability of samples. Therefore, it is essential to study the quality control method of the intra-assay extracts from \textit{Artemisia frigida}.

In this work, firstly, we used HPLC to establish the chromatographic fingerprints method for the extract from \textit{Artemisia frigida}. Secondly, to further clarify the intra-assay bioactivity stability of samples, the total antioxidant capability was detected based on the FRAP method. The spectrum–effect relationship between HPLC fingerprints and the antioxidant effect of the extract from \textit{Artemisia frigida} was established using a PLSR method. This aims of this work was to build a spectrum–effect combination method for quality control ensuring the bioactivity stability of inter-batch of the extract from \textit{Artemisia frigida}, which provide a foundation for the further study of functional components and the application of the extract from \textit{Artemisia frigida}.

\section*{Materials and methods}

\subsection*{Plant material}

The sample of the aerial parts of \textit{Artemisia frigida} was collected from Hailar, Inner Mongolia, China in September, 2019. A voucher specimen was deposited at the Feed Research Institute (Specimen number: AFW20190901).

\subsection*{Reagents}

Analytical grade phosphoric acid, acetic acid, formic acid, and trifluoroacetic acid for preparation of mobile phase were purchased from the Agela Technologies (Tianjin, China). Chromatographic grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Analytical grade methanol was purchased from Beijing Chemical Works (Beijing, China). Utilizing the Millipore Milli-Q water purification system (Billericia, MA, USA) to obtain high-purity water.

\subsection*{Sample preparation}

Naturally dry aboveground parts of \textit{Artemisia frigida} were pulverized with a micro-powder grinder and handed via a 60 mesh analytical sieve. 20 g sieved sample was extracted by 800 mL distilled water, and then extracted by ultrasonic extraction at 50°C and 500 W for 30 min. This mixture was centrifuged at 5000 rpm for 15 min, discarding precipitation, this collected supernatant was concentrated at 40 ± 2°C, concentrated to about 5 mL and stored at the −80°C refrigerator, lyophilized into powder and stored at −20°C. A 100 mg/mL of the extract from \textit{Artemisia frigida} solution was prepared with 1 mL ultrapure water and filtered through a 0.45 μm membrane filter. All 10 batches of extract from \textit{Artemisia frigida} were prepared by the above methods.

\subsection*{Instrumental conditions}

HPLC analyses were performed by Shimadzu HPLC system composed of an LC-20 AD pump, an autosampler (SIL-20A), a system controller (CBM-20A), a column temperature chamber (CT0-20A) and a UV–vis diode array detector (SPD-M20A 230 V), and an Kromasil C18 column (250 mm × 4.6 mm; 5 μm), Agilent ZORBAX SB-C18 column (150 mm × 4.6 mm; 5 μm), Agilent Eclipse XDB-C18 column (100 mm × 2.1 mm; 3.5 μm) were used.
**Method validation**

Precision of the method was tested by evaluating six consecutive injections of the same sample solution. The stability of the sample was proved by assessing the data of the same sample at 0, 2, 4, 6, 12, 24 h. Repeatability testing was performed using six sample solutions from the same batch. The relative standard deviations (RSDs) of reproducibility, precision, and intermediate precision were computed according to the relative peak area (RPA) and relative peak time (RPT) of each characteristic peak, and peak 9 was selected as the reference peak.

**Antioxidant activity**

The ferric reducing antioxidant power (FRAP) assay was performed according to method of Benzie & Strain. The experiment is founded on reducing complex Fe$^{3+}$-TPTZ to the ferrous form by antioxidants at low pH.$^{[15]}$ This change is observed by detecting the absorption at 585–605 nm. Briefly, a volume of 180 µL working liquid was incubated with 5 µL of the same concentration extract for 3–5 min at 37°C. Absorbance value was measured at 593 nm. Ferrous sulfate standards (0.15 mM to 1.5 mM) were prepared. The total antioxidant capacity of the samples was calculated from the standard curve and expressed as mmol of Fe$^{2+}$ equivalents per g of the extract from *Artemisia frigida.*$^{[16]}$

**Partial least squares regression (PLSR)**

The peak areas (X) of the common peaks of the fingerprint profiles of the extract from *Artemisia frigida* and the total antioxidant capacity (Y) were analyzed by PLSR using SIMCA-P software (Version 14.0), and the standardized regression coefficients and the projection of importance (VIP) values of the variables were calculated.

**Data analysis**

All related coefficient for complete chromatographic profiles was computed applying the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). The data of the total antioxidant capacity of 10 batches of extract from *Artemisia frigida* were shown as means ± SD of three independent experiments. Statistical comparison using variance analysis with Tukey post hoc, and $P < .05$ was considered statistically significant.

**Result and discussion**

**Optimization of HPLC conditions**

Through full-wavelength scanning, 230 nm was determined to be the ideal detection wavelength with more characteristic peaks and abundant Chromatographic information (Figure 1a). Then, to obtain a chromatogram with better separation of adjoining peaks inside a quick time, the column, mobile phase, modifier, flow rate, and column temperature on the separation effect of HPLC were researched. The elution and separation effects of the four different columns (the Kromasil C18 column (250 mm × 4.6 mm; 5 µm), Agilent ZORBAX SB-C18 column (150 mm × 4.6 mm; 5 µm), Agilent Eclipse XDB-C18 column (100 mm × 2.1 mm; 3.5 µm) were compared, respectively, under the same chromatographic conditions. In this study, the Kromasil C18 column (250 mm × 4.6 mm; 5 µm) was found to be the best for separating samples (Figure 1b). As shown as in Figure 1c, acetonitrile can be used as a mobile phase to obtain more chromatographic peaks and better separation performance than methanol as a mobile phase. Therefore, the acetonitrile-water system was added as the chromatographic conditions. Another important factor is that acid can be counted as a modifier to better separation.$^{[17]}$ Therefore, four common acids were analyzed: 0.1% formic acid, 0.1% trifluoroacetic...
acid, 0.1% acetic acid, and 0.1% phosphoric acid, the result is shown in Figure 1d, compared with the other three acids, 0.1% trifluorooxycetic acid has a more significant improvement in peak shape and resolution. Furthermore, three-column temperatures (25°C, 30°C, and 35°C) were researched to improve the analysis efficiency. The results showed that keeping the column temperature at 25°C could improve the separation and chromatographic information (Figure 1e). Different flow rates (0.8 and 1.0 mL/min) were also researched, as shown in Figure 1f, the final results showed that a suitable separation and shorter analysis time could be acquired when the mobile phase at a flow rate of 1 mL/min. In this experiment, this loading volume was 10 μL. Gradient conditions of Artemisia frigida were installed as follows: 0–0.01 min: solvent B (acetonitrile) kept at 90%; 0.01–70 min: acetonitrile followed a linear change, from 5 to 80%; 75–90 min: solvent B was set at 90%.

**Method validation**

HPLC fingerprint analysis is not a quantitative method. The assessed parameters and validation aspects vary from the common assaying technique. This technology has been known as an agile and efficient method that is able to assess the quality control of TCM.

All samples were analyzed under optimum chromatographic conditions. The average chromatogram of the samples was defined as the standardized characteristic fingerprint of the extract from Artemisia frigida. In this work, the 17 chromatographic peaks were used as the
characteristic peaks to identify and evaluate the quality of the extract from *Artemisia frigida* (Figure 2). Because there is no particular component to make the external standard method, among all of the characteristic peaks, peak 9 displays a high and stable content, which was selected as the internal reference peak to compute the relative retention time (RRT) and relative peak area (RPA). The calculating formulas of RRT and RPA were $RRT = \frac{RT_{peak}}{RT_{peak\_9}}$ and $RPA = \frac{PA_{peak}}{PA_{peak\_9}}$, respectively. To assess the stability of the samples in 24 h, the same sample was kept at room temperature and was successively injected at 0, 2, 4, 6, 12, and 24 h under suitable condition. This result showed that the RSD of RRT and RPA of 17 common peaks were <0.33 and <3.85%, respectively (Table 1). The precision of the HPLC fingerprint method was evaluated by successive analysis of six replicated tests of the same sample in one day under the optimum conditions. The results showed that the RSD of RRT and RPA of 17 common peaks were <0.25 and <2.81%, respectively (Table 1). The repeatability test was performed by injecting independently processed six batches of extract from *Artemisia frigida* solution. The results showed that the RSD of RRT and RPA of 17 common peaks were <0.30 and <4.47%.

Table 1. The data of precision, repeatability, and stability of the method.

| No. | RRT (%) | RSD (%) | RPA (%) | RRT (%) | RSD (%) | RPA (%) | RRT (%) | RSD (%) | RPA (%) | RSD (%) |
|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1   | 0.15    | 0.14    | 0.12    | 0.65    | 0.15    | 0.19    | 0.31    | 0.66    | 0.15    | 0.14    | 1.38    |
| 2   | 0.55    | 0.13    | 0.33    | 0.98    | 0.54    | 0.28    | 0.46    | 3.55    | 0.54    | 0.33    | 1.13    |
| 3   | 0.63    | 0.22    | 0.07    | 0.71    | 0.62    | 0.26    | 0.16    | 3.05    | 0.62    | 0.28    | 0.88    |
| 4   | 0.68    | 0.23    | 0.07    | 0.99    | 0.67    | 0.20    | 0.24    | 1.38    | 0.67    | 0.25    | 0.88    |
| 5   | 0.72    | 0.24    | 0.06    | 2.81    | 0.72    | 0.18    | 0.28    | 0.68    | 0.72    | 0.24    | 0.79    |
| 6   | 0.83    | 0.20    | 0.10    | 1.51    | 0.82    | 0.16    | 0.47    | 0.92    | 0.82    | 0.22    | 0.10    |
| 7   | 0.88    | 0.18    | 0.09    | 1.88    | 0.88    | 0.15    | 0.47    | 1.54    | 0.88    | 0.22    | 0.10    |
| 8   | 0.95    | 0.17    | 0.11    | 1.10    | 0.95    | 0.14    | 0.38    | 2.11    | 0.95    | 0.21    | 0.12    |
| 9   | 1.00    | 0.17    | 1.00    | 0.42    | 1.00    | 0.12    | 1.00    | 4.47    | 1.00    | 0.20    | 1.00    |
| 10  | 1.05    | 0.25    | 0.17    | 1.32    | 1.04    | 0.12    | 0.25    | 3.25    | 1.04    | 0.19    | 0.14    |
| 11  | 1.06    | 0.17    | 0.05    | 2.63    | 1.06    | 0.12    | 0.17    | 3.93    | 1.06    | 0.18    | 0.05    |
| 12  | 1.33    | 0.13    | 0.11    | 1.43    | 1.33    | 0.12    | 0.28    | 1.34    | 1.33    | 0.17    | 0.12    |
| 13  | 1.47    | 0.08    | 0.04    | 0.34    | 1.47    | 0.28    | 0.15    | 1.66    | 1.47    | 0.11    | 0.04    |
| 14  | 1.48    | 0.08    | 0.06    | 0.72    | 1.48    | 0.29    | 0.16    | 1.41    | 1.48    | 0.10    | 0.06    |
| 15  | 1.49    | 0.08    | 0.11    | 2.74    | 1.49    | 0.30    | 0.25    | 2.21    | 1.50    | 0.10    | 0.11    |
| 16  | 1.52    | 0.08    | 0.10    | 1.08    | 1.52    | 0.06    | 0.22    | 1.91    | 1.52    | 0.10    | 0.10    |
| 17  | 1.63    | 0.07    | 0.08    | 0.67    | 1.63    | 0.05    | 0.15    | 1.22    | 1.63    | 0.10    | 0.08    |

Figure 2. A characteristics fingerprint of extracts from *Artemisia frigida* Wild. peaks 1–17 were used as common peaks.
respectively (Table 1). As can be seen from the above result, the RSDs of RRTs and RPAs of stability, repeatability, and precision were within 0.05–0.33% and 0.34–4.47%, respectively. This method is stable for the analysis of extract from *Artemisia frigida*.

**Quality control analysis of artemisia frigida**

Similarity evaluation has been widely recognized to control the quality of TCM effectively. In order to assure the consistency of each batch, the chromatographic fingerprint similarity of 10 batches of extract from *Artemisia frigida* (labeled as S1–S10) were analyzed by the Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2004 Version A). The fingerprint data were imported into this software, and S1 sample was used as the reference spectrum. The similarity values between the fingerprints of 10 batches of extract from *Artemisia frigida* were summarized (Table 2), and the simulative mean chromatogram was derived (Figure 3) from the fingerprints of each sample.

![Figure 3](image_url)

**Figure 3.** The overlay HPLC fingerprints of 10 batches samples of *Artemisia frigida* Willd. (1–10) by a similarity evaluation system that analyses sample similarity against a generated reference chromatogram (R).

### Table 2. The similarities and the total antioxidant capacity of 10 batches of the *Artemisia frigida* Willd.

| Sample No. | Similarities | Total antioxidant capacity (mmol/g) |
|------------|--------------|-------------------------------------|
| Control    | 0.784 ± 0.023|
| 1          | 0.988        | 1.294 ± 0.005                       |
| 2          | 0.985        | 1.212 ± 0.003                       |
| 3          | 0.988        | 1.278 ± 0.009                       |
| 4          | 0.948        | 1.186 ± 0.004                       |
| 5          | 0.988        | 1.277 ± 0.005                       |
| 6          | 0.985        | 1.285 ± 0.002                       |
| 7          | 0.965        | 1.312 ± 0.006                       |
| 8          | 0.992        | 1.284 ± 0.002                       |
| 9          | 0.986        | 1.295 ± 0.007                       |
| 10         | 0.962        | 1.289 ± 0.004                       |
**Total antioxidant capacity**

In order to assess whether 10 batches of extract from *Artemisia frigida* have ferric reducing antioxidant power (FRAP), the total antioxidant capacity (300 μg/mL of extract) of 10 batches of extract from *Artemisia frigida* was measured. The results of the total antioxidant capacity of 10 batches of extract from *Artemisia frigida* are extremely steady without significant difference, as shown as in Table 2, revealing that good quality control can support the steadiness of antioxidant effect.

**Partial least squares regression (PLSR)**

As demonstrated in Figure 4, the peaks 2, 3, 4, 8, 10, 11, 12, and 16 of the extract from *Artemisia frigida* were positively correlated with the antioxidant level, while the remaining 10 peaks were negatively correlated. As shown as in Figure 5, the results showed that the VIP values of peak 10, 9, 1, and 2 were >1, indicating that the corresponding components of extract from *Artemisia frigida* performed an essential position in the antioxidant effect.

**Conclusion**

In this study, a method of HPLC fingerprint was established to reflect the types and number of chemical components of the extracts from *Artemisia frigida*. From the similarity of 10 batches of samples (≥0.948), implying that the peak-to-peak distribution pattern of the fingerprint is stable and repeatable, which indicates *Artemisia frigida* from the same region has a high chemical composition similarity. Moreover, the antioxidant effect of the extract from *Artemisia frigida* suggests that it may contain antioxidant components, and the relationship between the bioactive properties and HPLC fingerprint was analyzed by a PLSR method. Based on the above analytical techniques, the results

![Figure 4](image-url)
showed that peaks 1, 2, 9, 10 were the main antioxidant components of extract from *Artemisia frigida*, and these four components should be focused on in our follow-up study. In sum, a method for quality control of extract from *Artemisia frigida* by spectrum–effect combination is of great significance for further ensuring the safety and efficacy of inter-assay extract from *Artemisia frigida*.

**Acknowledgments**

This work was supported by the National Key Research and Development Program of China (Grant No. 2016YFE0113300), the Natural Science Foundation of Tianjin City (20JCQNJC00730), the Fundamental Research Funds for Central Non-profit Scientific Institution (Grant No. 1610382018003) and the Open Project Program of Key Laboratory of Feed Biotechnology, Ministry of Agriculture and Rural Affairs (Grant No. KLFB-IFR-202005). The authors declare no conflict of interest.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This work was supported by the Fundamental Research Funds for Central Non-profit Scientific Institution [Grant No. 1610382018003]; Open Project Program of Key Laboratory of Feed Biotechnology, Ministry of Agriculture and Rural Affairs [Grant No. KLFB-IFR-202005]; Shandong Provincial Youth Innovation Technology Support Program [2019]Ke117]National Key Research and Development Program of China [Grant No. 2016YFE0113300]; Tianjin Natural Science Foundation [20JCQNJC00730].
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