Range of Molecular Weight of Different Effective Components in the Aqueous Extract of *Angelica-Radix Hedysari* Ultrafiltration Content by HPLC-MS

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Summary: The molecular weight range of effective components in the aqueous extract of *Angelica-Radix Hedysari* (1:5) was analyzed, providing a foundation for understanding its chemical composition and pharmacodynamics. Ultrafiltration technology was used to prepare different forms of the extract, including water extract powder, ultrafiltration powder, ultrafiltration saponin component powder, and ultrafiltration polysaccharide component powder. High-performance liquid chromatography-mass spectrometric (HPLC-MS) were used to analyze these extracts. Comparison between the components of *Angelica-Radix Hedysari* using HPLC and HPLC-MS showed that the ultrafiltration products contained fewer components than the original extract powder. However, the content of some components in the ultrafiltration products increased. The components with molecular weight greater than 10 kDa were removed during ultrafiltration, resulting in fewer components in the ultrafiltration products compared to *Angelica Hongqi* extract powder.

Keywords: Aqueous extract of *Angelica-Radix Hedysari*, Water extraction ultrafiltration, HPLC-MS, HPLC, Saponins, Polysaccharide

Introduction

Angelica sinensis, the dried root of the plant Diels, family Umbelliferae, is a common Chinese medicine widely used in clinical practice. It contains various active components such as volatile oil, ferulic acid, and polysaccharides, which are known for their effects in invigorating and promoting blood circulation, among other benefits [1]. *Angelica-Radix Hedysari* ultrafiltration is derived from the decoction of *Angelica sinensis* as part of the traditional Chinese medicine (TCM) formulation known as "differentiation on endogenous". This formulation combines *Radix Hedysari* and *Angelica sinensis* in a ratio of 5:1, providing the effects of supplementing Qi, generating blood, and warming while removing heat. *Radix Hedysari* is reputed for its abilities to support righteousness, expel evil, supplement Qi, and strengthen the foundation. Its active components include saponins, organic acids, flavonoids, red sandalwood, and polysaccharides [2-4]. The combination is mainly used to treat conditions such as blood deficiency and fever [5].

The use of ultrafiltration membrane separation technology in TCM allows for the convenient, efficient and pollution-free separation and purification of medicinal components [6-8]. This technique employs selective permeation membranes and external pressure to selectively allow the passage of certain components, achieving separation and purification.

In this study, we focused on refining the aqueous extract of *Angelica-Radix Hedysari* using ultrafiltration technique. This process aimed to isolate different components and analyze them by HPLC and HPLC-MS. The rationale behind this study lies in the need to better understand the chemical composition and pharmacodynamics of *Angelica-Radix Hedysari*, as it holds significant potential in traditional medicine for treating various ailments. By identifying and quantifying the molecular weight range of the functional groups of its effective components, we aim to provide a scientific basis for its medicinal use, thus enhancing its application in modern medical practice.

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Experimental

Instruments

High performance liquid chromatograph (Tsushima, Japan); Ultra high performance liquid chromatography-mass spectrometry (Waterworld, xev0g2qtof/acquityuplc); Negative pressure vacuum freeze dryer (Japan heto company, powerdryl3000); Ultrafiltration device (Gansu Academy of membrane science and Technology).

Materials

The standard of *Angelica-Radix Hedysari* is provided by Gansu Institute of drug control (China); Formic acid and acetonitrile are chromatographic grade and purchased from Merck company (USA); Ceramic membrane and PNA hollow fiber ultrafiltration membrane are provided by Gansu Academy of membrane science and technology (China); *N*-butanol and absolute ethanol were purchased from Nanjing Chemical Reagent Company (Jiangsu, China).

Preparation of different components of water extract

Firstly, 500 g *Angelica* Decoction Pieces and 2500 g *Hedysari* decoction pieces were mixed, soaked and decocted, and after that, the liquid medicine was poured out. Next, the remaining angelica and Hedysarum residue were supplemented with water and subject to decoction, and the decocting solution was gathered. Subsequently, two decoctions were mixed, filtered with sterile absorbent cottons, heated, concentrated to paste, and then frozen and dried into powder under negative pressure and vacuum to obtain the original solution powder of water extract. Later, 10% *Anglica-Radix Hedysari* decoction was taken out, cooled to room temperature, subject to ultrafiltration through ceramic membrane microfiltration and PNA hollow fiber ultrafiltration membrane, then heated and concentrated to paste. The paste was then frozen and dried into powder under negative pressure and vacuum to obtain water-extracted ultrafiltration powder. After that, the water was added to extract 200 g sample of ultrafiltration powder. Briefly, the 600 mL distilled water was supplemented, and the sample was stirred to completely dissolve and extracted with *N*-butanol for 3-4 times, 600 mL each time; the *N*-butanol phase was saponins, and the organic solvent was distilled to obtain saponin dry powder. Afterwards, the remaining aqueous phase in the extraction process of saponin components in the water extraction ultrafiltration product was collected and added with anhydrous ethanol for alcohol precipitation for 4 times based on the water-soluble alcohol precipitation method. Then, the sediment was gathered and subject to freeze-drying under negative pressure and vacuum to obtain polysaccharide dry powder. The different components of the aqueous extract of *Angelica-Radix Hedysari* (1:5) were shown in Fig. 1 (A-D).

High-performance liquid chromatography (HPLC) analysis

The samples of aqueous extract, ultrafiltration, saponin and polysaccharide of *Angelica* and *Hedysarum* standard were dissolved with 50% methanol and microwave assisted dissolution. Gradient elution: methanol: 0.1% phosphoric acid aqueous solution, 0-20 min, 3:97; 20-40 min 20:80; 40-50 min 60:40; 0-60 min 90:10. Shimadzu lc-20ad HPLC (Tsushima, Japan), detector spd-m20a, chromatographic conditions: detection wavelength 275 nm, inertsil Ods-spg8 chromatographic column (5 μm, 4.6*150 mm). The sample injection amount of the original solution of the aqueous extract of *Angelica-Radix Hedysari* (1:5) formula is 20 μL, and the injection amount of other samples is 10 μL.

HPLC-mass spectrometric (MS) Analysis

Take 30 mg ultrapure water of each sample to constant volume to 25 mL, filter with 0.2 μm membrane, and inject 2 μL. Chromatographic conditions: water HSS T3 column, column temperature 30 ℃, sample room temperature 10 ℃, scanning range ultraviolet detector (PDA), wavelength range 190-400 nm, flow rate: 0.2 $mL \cdot min^{-1}$, mobile phase A is water + 1% formic acid solution; Mobile phase B is acetonitrile solution. Gradient elution: (0~5 min, 100% a → 44% A; 5~10 min, 44% a → 95% A). Optimization of saponin gradient: (0~5 min, 100% A → 50% A). MS conditions: the electrospray ion source (ESI source), positive ion mode, and electrospray voltage: 3.0 kV; Taper hole voltage: 40 V. Detection range of mass number: 300~1500 m/z.

Results and Discussion

Analysis of different components of Angelica-Radix Hedysari (1:5) by HPLC

The results (Fig. 2) showed that the HPLC fingerprint of the *Angelica* standard and the *Radix Hedysari* Standard overlapped with the HPLC fingerprint of different components of the aqueous extract of *Angelica-Radix Hedysari* (1:5). However, some peaks were not fully covered, suggesting the presence of new substances formed during the decoction and water extraction process.

Fig. 1: Different components of aqueous extract of *Anglica-Radix Hedysari* (1:5). (A) Original solution powder of aqueous extract; (B) Ultrafiltration powder; (C) Saponin dry powder; (D) Polysaccharide dry powder.

Analysis of different components in aqueous extracts by HPLC-MS

The MS fingerprint (3.12 min) of the aqueous extract of *Angelica-Radix Hedysari* (1:5) identified 12 major mass spectrum fragments with molecular weights of 211.1628, 227.1370, 249.1677, 289.1049, 351.1839, 362.2479, 365.1637, 447.2931, 475.3256, 610.3775, 615.3314, and 616.3358 (Fig. 3A). Additional MS fingerprint at various retention times identified other significant fragments, suggesting a complex mixture of components in the aqueous extract (Fig. 3B-F).

The HPLC fingerprint of the original aqueous extract of *Angelica-Radix Hedysari* (1:5) displayed nine significant peaks, which were 0.33 min, 0.39 min, 0.49 min, 1.05 min, 1.66 min, 2.01 min, 5.02 min, 5.15 min, and 7.79 min. The HPLC fingerprint of the ultrafiltration product showed seven significant peaks, which were 0.31 min, 0.39 min, 0.48 min, 1.05 min, 1.66 min, 5.14 min, and 7.79 min respectively (Fig. 4). The results indicated that ultrafiltration removed some components while retaining others.

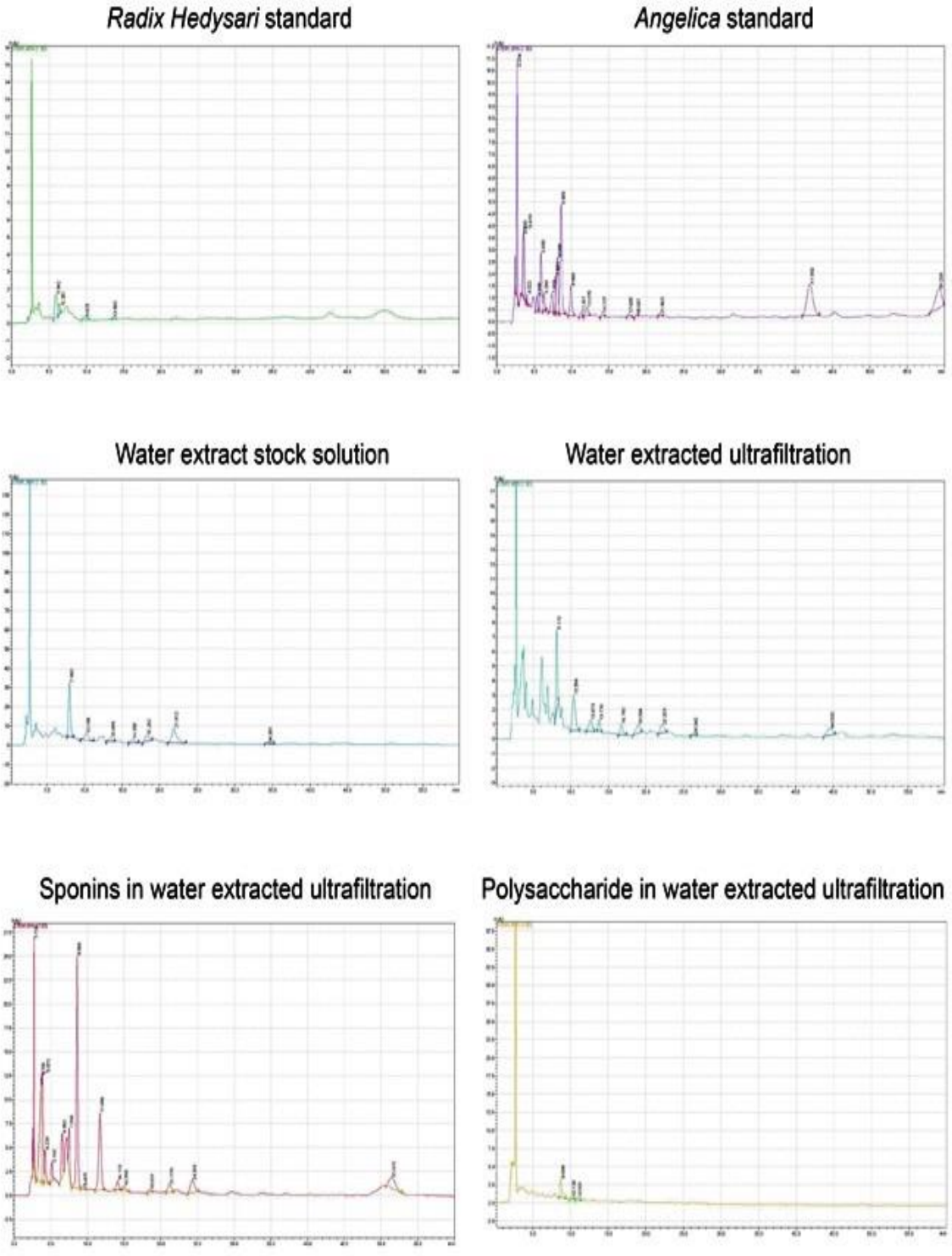

Fig. 2: The fingerprint of each sample was detected by HPLC.

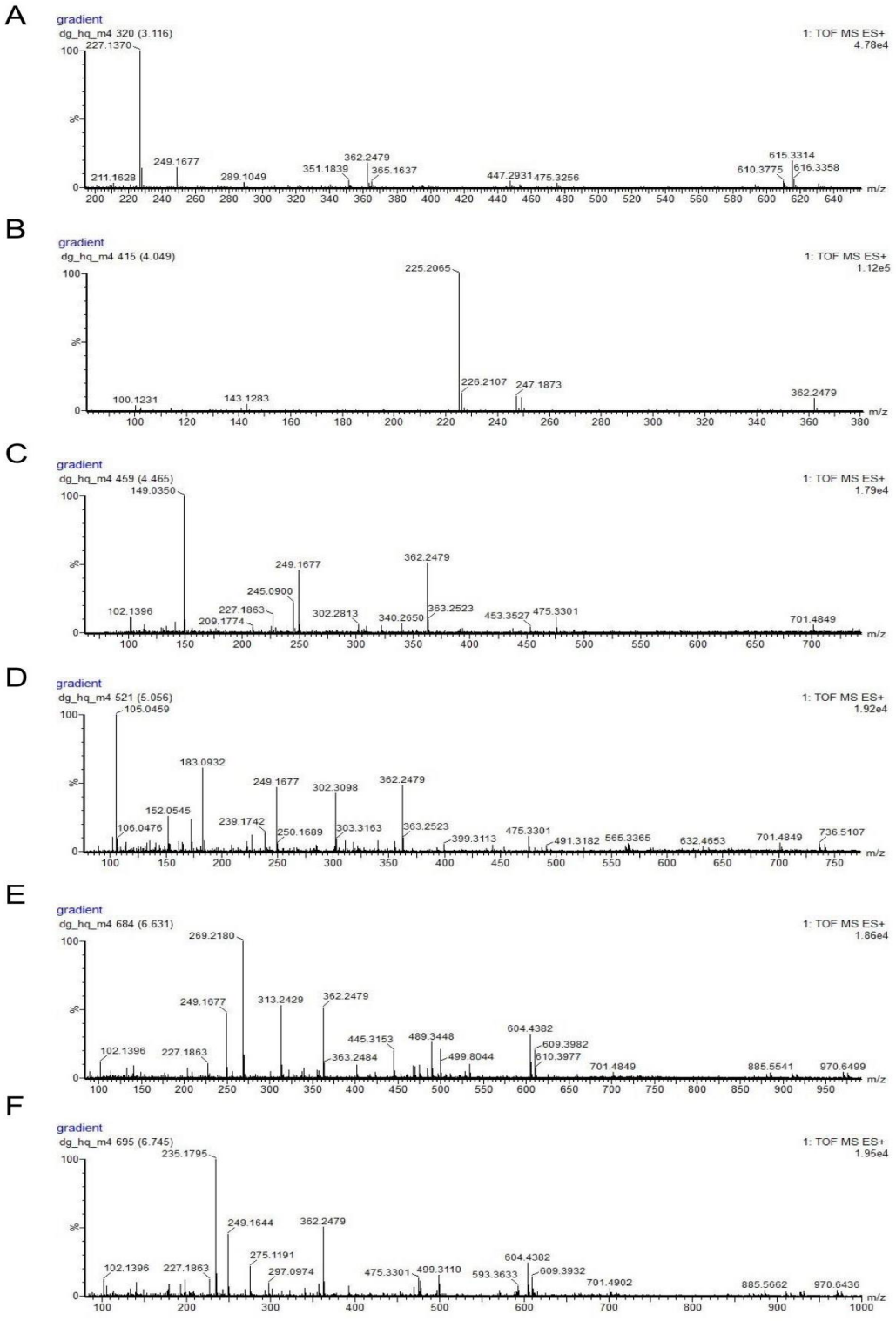

Fig.3: MS fingerprint of original solution components of aqueous extract of *Angelica -Radix Hedysari* (1:5). (A) 3.12 min; (B) 4.05 min; (C) 4.47 min; (D) 5.06 min; (E) 6.63 min; (F) 6.75 min.

HPLC fingerprint analysis of original liquid components and ultrafiltration products

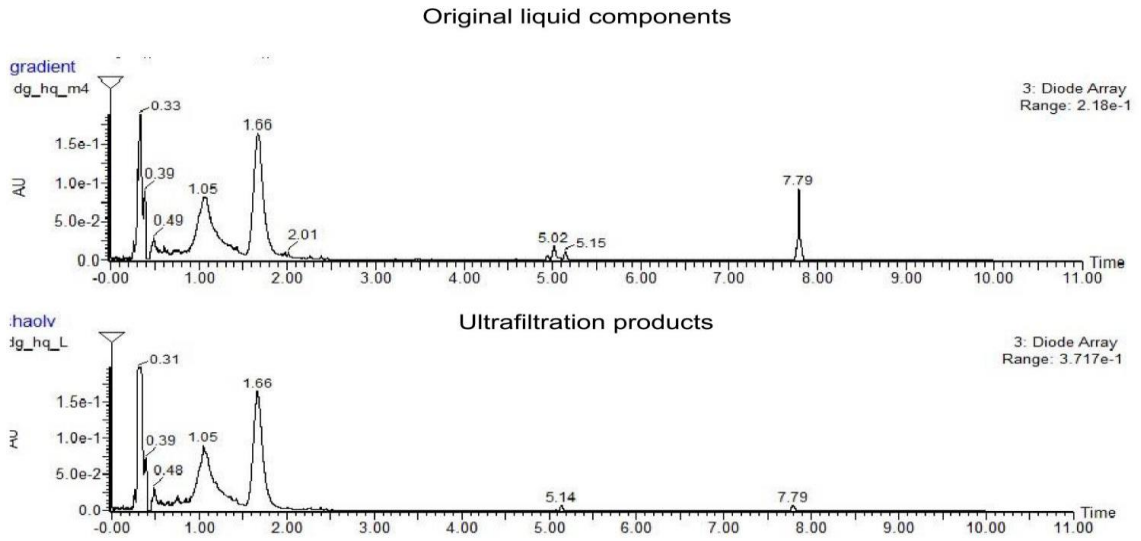

Fig. 4: HPLC fingerprint of original liquid components and ultrafiltration products of aqueous extract of *Angelica-Radix Hedysari* (1:5).

Total ion flow diagram fingerprint analysis of original liquid components and ultrafiltration products

The total ion flow diagram fingerprint of the original liquid components in the aqueous extract of *Angelica-Radix Hedysari* (1:5) showed multiple peaks at different retention times, while the ultrafiltration product showed exhibited fewer peaks, demonstrating the selective removal of certain components during ultrafiltration (Fig. 5).

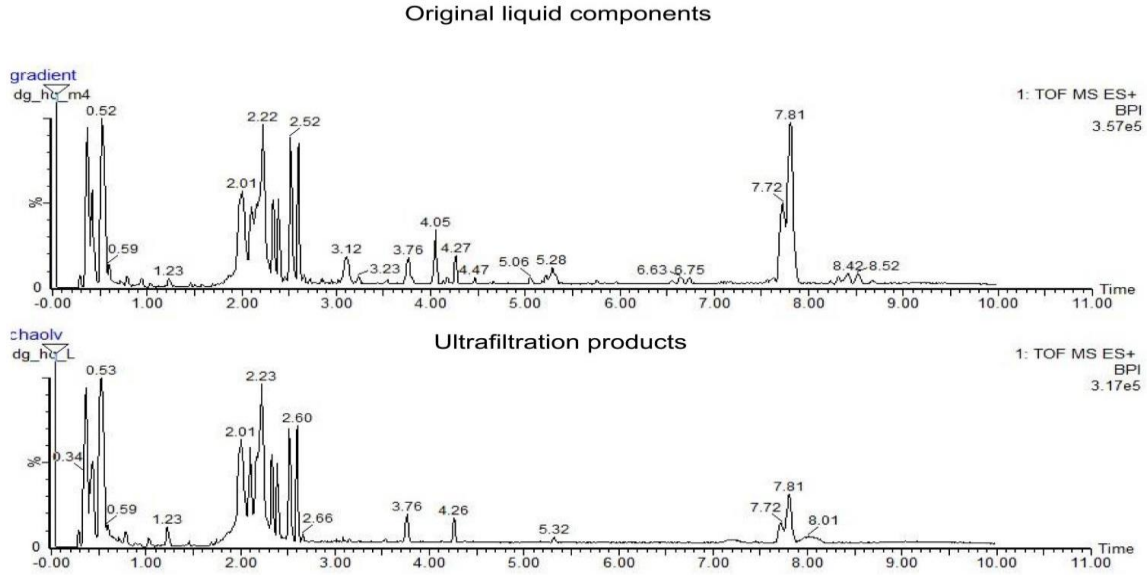

Fig. 5: Total ion flow diagram fingerprint of original liquid components and ultrafiltration products of aqueous extract of *Angelica-Radix Hedysari* (1:5).

MS fingerprint analysis of original liquid components and ultrafiltration products

The MS fingerprint of the aqueous extract of *Angelica-Radix Hedysari* (1:5) identified 15 major fragments, while the ultrafiltration product showed 14 major fragments with some variations in molecular weights, indicating changes in composition due to ultrafiltration (Fig. 6).

Analysis of saponin components in the water extraction ultrafiltration of Angelica-Radix Hedysari (1:5).

The MS analysis of saponin components in the water extract ultrafiltration product of *Angelica-Radix Hedysari* (1:5) identified 14 mass spectrum fragments with significant molecular weights (Fig. 7A). The UPLC of saponin components in the water extraction ultrafiltration of *Angelica-Radix Hedysari* (1:5) revealed 12 peaks with various retention times, indicating the presence of concentrated saponin components (Fig. 7B). The total ion flow diagram exhibited 18 significant peaks, suggesting the successful isolation and concentration of saponins through ultrafiltration (Fig. 7C).

Further detailed MS analysis at key retention times highlighted major fragments. For example, at 2.71 min fragments with m/z values such as 322.2606, 340.2688, and 362.2517 were identified (Fig. 8A). Similarly, at 3.09 min, six significant fragments included m/z values of 407.2333, 435.3388, 453.3483, 454.3499, 475.3301, and 476.3333 (Fig. 8B). Additionally, at 3.26 min, five significant fragments were revealed (Fig. 8C), and 11 significant fragments were identified at 3.72 min (Fig. 8D). The identification of these specific m/z values provides a detailed understanding of the molecular composition of the saponin components in the ultrafiltration product.

The above findings highlighted the complex nature of the saponin components present in the water extract ultrafiltration of Angelica-Radix Hedysari. The identification of multiple significant mass spectrum fragments and peak times underscores the efficacy of ultrafiltration in isolating and concentrating specific bioactive components. By focusing on key m/z values and their corresponding retention times, we can predict the presence of specific bioactive components in the extract, providing a clearer understanding of its potential therapeutic applications.

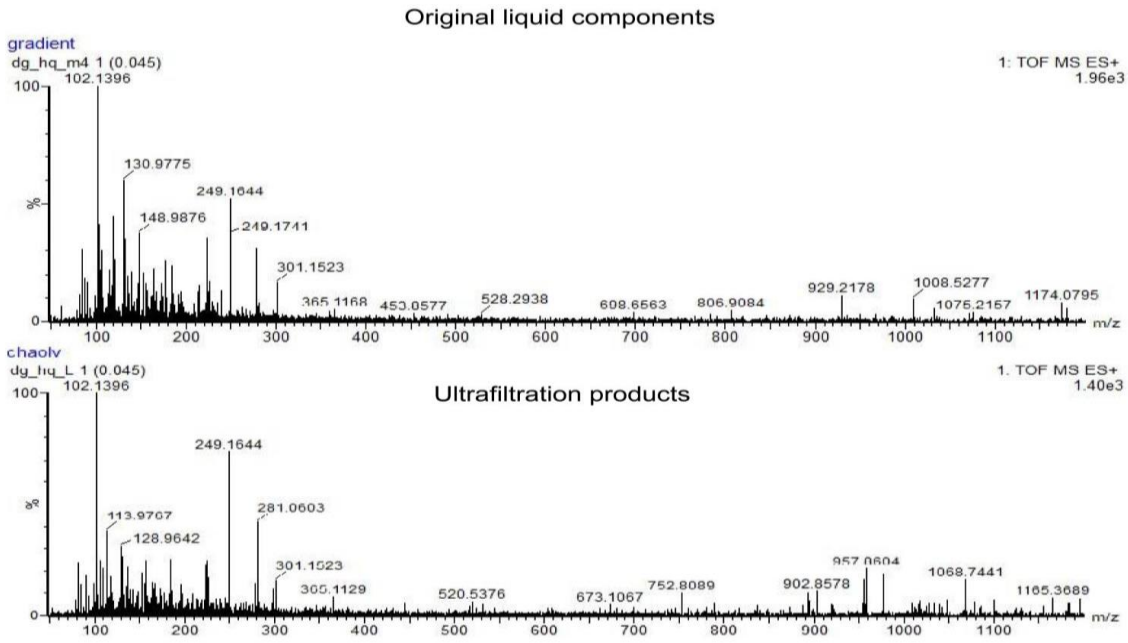

Fig. 6: Mass spectrum fingerprint of original liquid components and ultrafiltration products of aqueous extract of *Angelica-Radix Hedysari* (1:5).

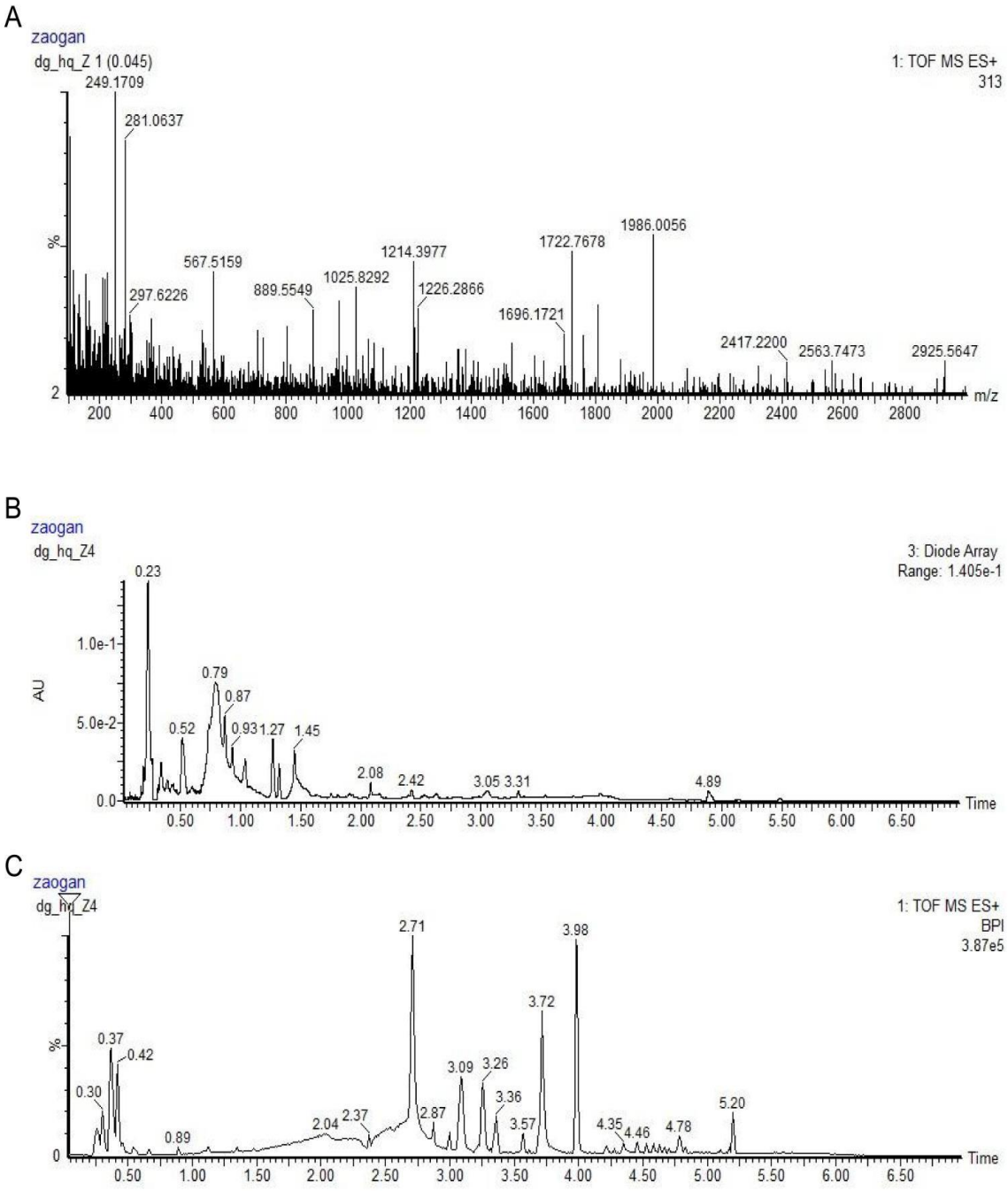

Fig. 7: Analysis of saponin components in water extract ultrafiltration of *Angelica-Radix Hedysari* (1:5). (A) MS; (B) UPLC; (C) Total ion flow diagram.

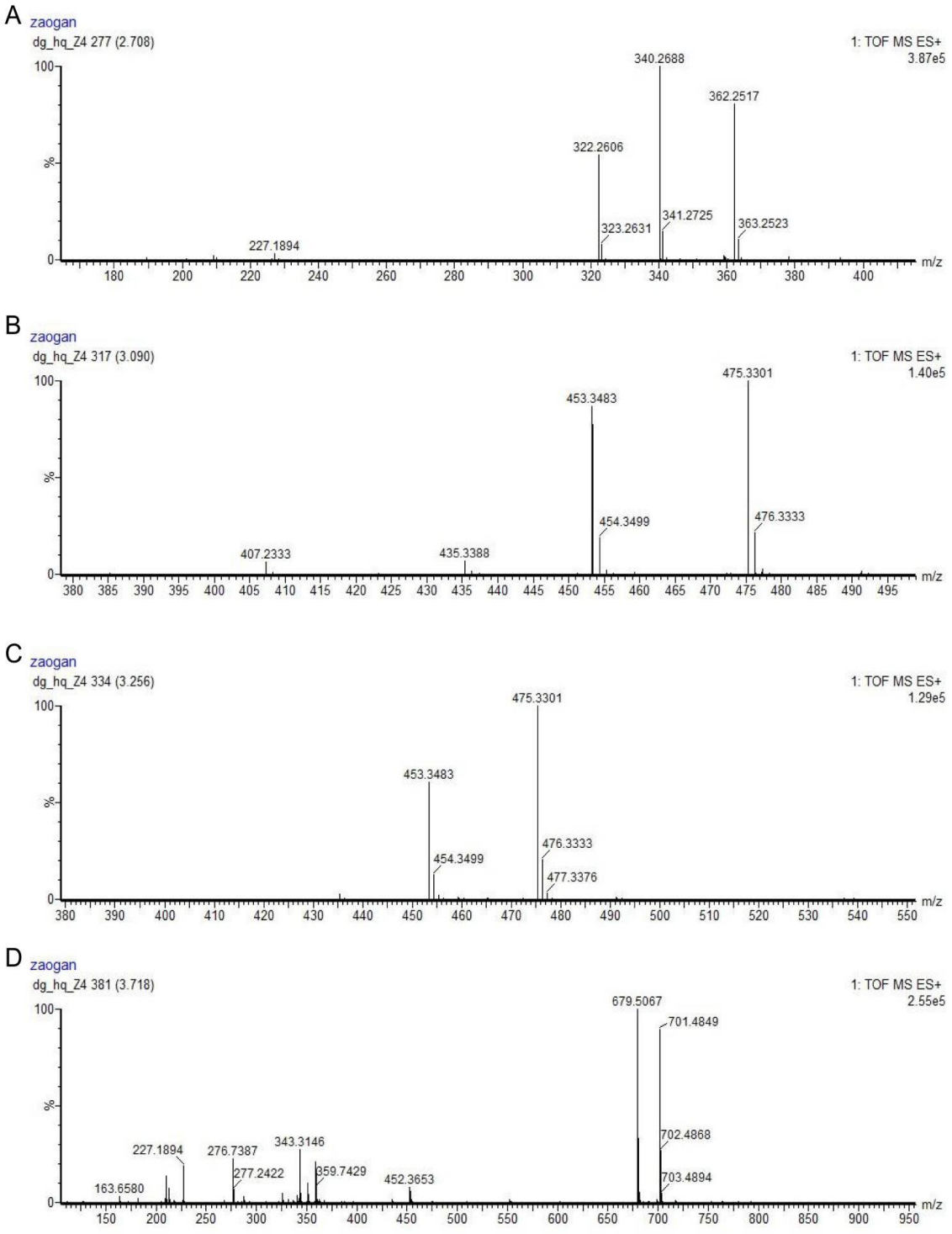

Fig. 8: MS of the main peaks in the total ion flow diagram of saponin components in the water extraction ultrafiltration of *Angelica-Radix Hedysari* (1:5). (A) 2.71 min; (B) 3.09 min; (C) 3.26 min; (D) 3.72 min.

TCM compound often uses compound prescriptions rather than single- drug preparations to achieve therapeutic efficacy. Understanding the material basis of these compounds is crucial for exploring their pharmacological mechanism [9]. The *Angelica-Radix Hedysari* ultrafiltration membrane extract, as a pure TCM compound, has demonstrated regulatory effects on immune function, anti-tumor properties, and benefits in energy metabolism with minimal side effects on human body and high safety [10-12].

HPLC is a vital tool in medical research for separating and analyzing medicinal components [13-15], served as a means of quality control [16-18]. In this study, HPLC was used to identify the fingerprints of crude total saponins in *Angelica-Radix Hedysari* extract powder and ultrafiltration products. It was found that in the HPLC fingerprint, the chromatographic peaks of extract powder at 7.992 min, 10.185 min, 13.496 min, 16.355 min, 18.252 min and 21.872 min were similar to those of ultrafiltration at 8.112 min, 10.394 min, 13.776 min, 16.750 min, 18.984 min, and 22.203 min. The extract powder peaked in 34.381 min, but the ultrafiltration did not peak here, suggesting that ultrafiltration effectively removed or reduced certain components. The peak of ultrafiltration products appeared at 12.673 min, 26.342 min, and 44.552 min, but the peak of extract powder did not appear, suggesting that ultrafiltration significantly increased certain components. For the same amount of sample solution, the injection amount of *Angelica-Radix Hedysari* extract powder is 20 μL and that of ultrafiltration is 10 μL, indicating that ultrafiltration improves the content of components in *Angelica-Radix Hedysari* extract powder.

LC-MS technology, combining the super separation ability of HPLC with the high sensitivity and specificity of mass spectrometry, provided a detailed analysis of the different components in the aqueous extract of *Angelica-Radix Hedysari*. The comparison between HPLC and LC-MS results revealed significant differences in component omposition and concentration, suggesting that ultrafiltration enhances the medicinal efficacy of the extract by modifying its chemical profile. Specifically, the comparison of UPLC between extract powder and ultrafiltration showed that the peak values of ultrafiltration decreased or disappeared significantly at 2.01 min, 5.02 min, and 7.79 min. Additionally, the comparison of total ion flow diagram between extract powder and ultrafiltration showed that the peaks of ultrafiltration decreased or disappeared at 3.12 min, 4.05 min, 4.47 min, 5.06 min, 6.63 min, 6.75 min, 7.72 min, 7.81 min, 8.01 min, 8.42 min, and 8.52 min. The comparison between original liquid and ultrafiltration showed that the overall molecular weight distribution were similar, whereas ultrafiltration product had a significant increase in specific mass spectrum fragments (e.g., m/z values 113.9707, 128.9642, and 1165.3689). This suggested that ultrafiltration enhances the concentration of specific bioactive components, potentially improving the pharmacological effects of the extract.

However, there are inherent limitations. The complexity of TCM components presents significant challenges in achieving comprehensive qualitative analysis. The MS fragments generated during analysis require further comparison with reference standards, including retention time, UV spectrum, and mass spectrum to fully realize qualitative analysis. Due to the complexity of TCM components, relying solely on reference substances to characterize all chemical constituents is impractical. As a result, this study did not conduct exhaustive analysis of all specific compound components within the water-extracted ultrafiltration (0-10 kDa) and saponin components. Future research should incorporate additional analytical techniques and a broader range of reference standards to achieve a more comprehensive characterization of the chemical components in TCM extracts.

Conclusions

This study successfully identified key saponin components in the water extraction ultrafiltration product of *Angelica-Radix Hedysari* (1:5) using HPLC-MS and UPLC. The ultrafiltration process significantly altered the composition of the extract, enhancing the concentration of specific components and potentially improving its pharmacological effects. These findings provide a scientific basis for the use of *Angelica-Radix Hedysari* in traditional medicine and support its potential applications in modern therapeutic practices.

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