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ORIGINAL ARTICLE

Comparative Study of Ultra-High-Performance Supercritical Fluid Chromatography and Ultra-High-Performance Liquid Chromatography to Simultaneous Determination of Ten Components in *Radix hedysari*

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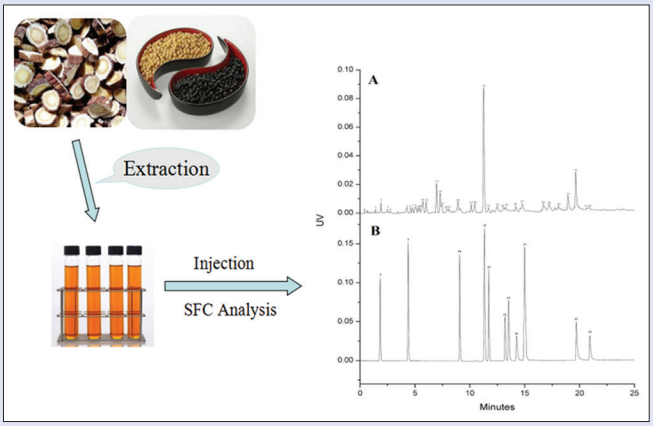
ABSTRACT

Background: In China, dried root of Hedysarum polybotrys Hand-Mazz, known as Hong Qi (*Radix hedysari* [RH]), is one of the important traditional Chinese medicines (TCMs). The long analysis time was a major limitation when high-throughput techniques are required for research or quality control purposes. **Materials and Methods:** It is necessary to develop a high-throughput method for the determination of multiple components of TCMs by investigating the separation characteristics of the target with ultra-high-performance liquid chromatography (UHPLC) and ultra-high-performance supercritical fluid chromatography (UHPSFC). **Objective:** This study intends to establish a high-throughput method for simultaneous determination of ten compounds in RH by UHPSFC and to develop a standardized method to comprehensively evaluate the quality of RH samples. In addition, this study was based on UHPLC and UHPSFC systems to understand and compare the effects of different separation parameters on ten target compounds in RH. A high-throughput and reliable solid-phase extraction method for simultaneous detection of ten target compounds in RH was established and validated. **Results:** The optimal analysis method was achieved using HSS C_{18} SB column, gradient elution, flow rate of 1.5 mL/min, diode array detection at 270 nm, and back pressure at 12.41 Mpa. The separation method was validated sufficiently by examining the precision, recoveries, linearity, range, limit of detection, and limit of quantification and was successfully applied to quantify the 25 RH samples. In addition, the comparative study also demonstrated the complementary nature of the two separation modes. **Conclusion:** This is the first study that ten compounds in RH were separated and analyzed simultaneously with UHPSFC-diode array detector. In addition, under the separation conditions of UHPSFC and UHPLC, the comparison of chromatographic parameters shows that a combination of these two separation techniques efficiently separates the ten target compounds in RH. The results obtained in this study are useful for future TCM quality control and high-throughput analysis.

Key words: Comparative study, high-throughput analysis, *Radix hedysari*, ultra-high-performance supercritical fluid chromatography

SUMMARY

- A rapid ultra-high-performance supercritical fluid chromatography (UHPSFC) method with high resolution and reproducibility was developed and validated to quantify ten active compounds in *Radix hedysari* (RH)
- These active compounds have been described for the first time in RH
- The effect of UHPSFC parameters on the separation of active compounds was studied in detail
- Although the method presented in this study was designed for RH, it can be used for the determination of other compounds in different traditional Chinese medicines
- In addition, different separation parameters were compared using UHPSFC and UHPLC. These studies also demonstrated the complementary nature of the two separation modes.



Abbreviations used: ACN: Acetonitrile; BPR: Back pressure regulator; DAD: Diode array detector; RA: *Radix astragali*; RH: *Radix hedysari*; SF: Supercritical fluid; TCM: Traditional Chinese medicine; UHPSFC: Ultra-high-performance supercritical fluid chromatography.

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INTRODUCTION

In China, Hong Qi (*Radix hedysari* [RH]), dried root of Hedysarum polybotrys Hand-Mazz, is one of the most important traditional Chinese medicines (TCMs). In Hong Kong, as well as some countries in Southeast Asia, RH and Huang Qi (*Radix astragali*, RA) are considered crude drugs and RH is considered to be of better quality than RA.[1] In addition, the compounds in RH, which include the organic acid, flavonoids, saponins, coumarins, polyphenols, and terpenoids,

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give it a number of pharmacological activities.[2-4] Vanillic acid and ferulic acid have many biological properties, including antioxidant and anticancer activities.[5] Genistein, calycosin-7-O-β-D-glucoside, 3-hydroxy-9,10-dimethoxypterocarpane, liquiritigenin, formononetin, and formononetin-7-O-β-D-glucoside, having antioxidant, antiviral, and antithrombogenic properties, are commonly considered an important class of compounds in RHs.[1,6] In recent years, various methods have been developed to analyze and quantify the compounds in TCMs, including thin-layer chromatography and ultraviolet (UV)-Vis methods, which have been applied for the determination of polyphenols in some medicinal plants.[7,8] The use of HPLC-MS/MS to analyze flavonoids has also been reported many times.[3,6,9] Moreover, flavonoids have been analyzed by GC or GC-MS.[10,11] HPLC has been successfully and widely used in the analysis of compounds in TCMs.[3,12-14] During the last decade, there has been remarkable progress in the column materials and instruments used for LC, especially with the emergence of ultra-high-performance liquid chromatography (UHPLC) in 2004. UHPLC combines a packed columns of sub-2 μm particles and an instrumental system that can withstand pressures up to 15,000 psi (103.42 Mpa). UHPLC methods offer significantly reduced analysis time compared to those of traditional LC or HPLC.[15] The long analysis time is a major limitation when high-throughput techniques are needed for research or quality control purposes. Highly efficient separation can be achieved by increasing the temperature of the mobile phase (more than 60°C) in LC in conjunction with sub-2 μm particle-containing columns. However, this strategy may be harmful to silicon-based stationary phases and thermally unstable compounds, especially above 100°C. As we know, SFC can improve chromatographic efficiency and resolution.[16,17] Supercritical fluids are an inexpensive mobile phase that has liquid-like densities, low viscosity, and high gas-like diffusivity. Moreover, supercritical fluid chromatography (SFC) is a fast and reliable equipment for separation and quantification that consumes a small amount of solvent. The SFC application in TCM analysis dated back to the 1990s, when the SFC was used as an extension of gas chromatography. Currently, the SFC combined with different detectors showed good analytical applicability for a variety of compounds in different TCMs, from nonpolar compounds such as terpenes,[18] carotenoids,[19] and fatty-acids[20] to more polar compounds such as flavonoids[21,22] and alkaloids.[23,24] However, as far as we know, rarely, reports have been published on the simultaneous analysis and determination of ten compounds in RHs from different cultivated regions, especially using ultra-high-performance supercritical fluid chromatography (UHPSFC). Thus, the aim of this study was to use in ultra-high-performance supercritical fluid chromatography (UHPSFC) coupled to diode array detector (DAD) to establish a high-throughput detection and standardized method for the comprehensive assessment of RH sample quality. Ten target compounds were (1) vanillic acid, (2) calycosin, (3) ferulic acid, (4) coumarin, (5) formononetin, (6) 3-hydroxy-9,10-dimethoxypterocarpane, (7) formononetin-7-O-β-D-glucoside, (8) calycosin-7-O-β-D-glucoside, (9) genistein, and (10) liquiritigenin. Moreover, the separation conditions of cosolvent, stationary phase, back pressure, flow rate, additives, and column temperature were investigated. Ten target compounds were simultaneously isolated and determined by UHPSFC-DAD within 20 min. The method was then applied to the detection of ten analytes in actual samples of RH. This is the first study on the simultaneous isolation and determination of ten compounds using UHPSFC-DAD. In addition, another purpose of this study was to test the effects of different stationary phase and temperature on the separation of the ten target compounds by UHPLC and UHPSFC techniques.

MATERIALS AND METHODS

Chemicals, reagents, and samples

Standards of vanillic acid, 3-hydroxy-9,10-dimethoxypterocarpane, calycosin, formononetin, liquiritigenin, ferulic acid, formononetin-7-O-β-D-glucoside, genistein, calycosin-7-O-β-D-glucoside, and

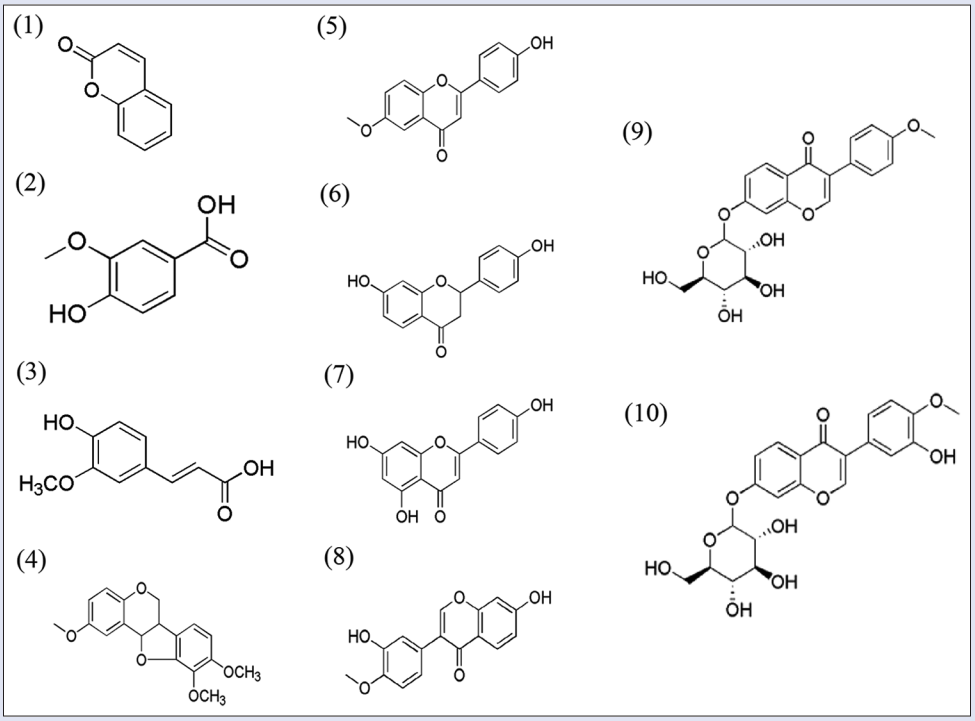

Figure 1: Chemical structure of ten target compounds. (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

coumarin [Figure 1] were obtained from the Dr. Ehrenstorfer GmbH (Augsburg, Germany). Formic acid was obtained from Sigma (St. Louis, USA). Food-grade carbon dioxide (purity >99.93%) was stored in 50 kg cylinders and purchased from Hui Neng (Lan Zhou, China). Other solvents and chemicals were of analytical HPLC grade.

Twenty-five RH samples were purchased from local pharmacy shops in Gansu, China. All the samples were identified by Professor Zhi-gang Ma (Department of Pharmacognosy, Lanzhou University, Lanzhou, China). All RH samples were kept away from light, moisture, and stored at room temperature before analysis.

Standard preparation and calibration

Samples of the ten standard reference compounds, namely, vanillic acid, 3-hydroxy-9,10-dimethoxypterocarpane, genistein, formononetin, calycosin, formononetin-7-O-β-D-glucoside, liquiritigenin, coumarin, ferulic acid, and calycosin-7-O-β-D-glucoside, were weighed precisely and dissolved in methanol to prepare 400 μg/mL standard stock solutions. The standard working solution was made by diluting the standard stock solutions in cosolvent to six different levels, 0.20, 0.40, 4.00, 40.00, 100.00, and 200.00 μg/mL, and these were stored in brown glass flasks at 4°C.

Sample preparation

Each sample of RH was passed through a 60-mesh screen. 2.0 g homogeneous RH powder was weighed, and 40 mL 75% (v/v) ethanol was used as the extraction solvent. Then, it was extracted in an ultrasonic water bath for 20 min at room temperature and centrifuged at 12,000 r/min for 5 min. The upper solution was evaporated to a nearly dry state with a rotary evaporator at 50°C. The extract was redissolved in 2.0 mL of methanol and diluted to 10.0 mL with deionized water. Subsequently, before loading samples, Oasis HLB cartridge (200 mg, 6 mL) was activated with 5 mL methanol and water, respectively. After loading samples, washed SPE cartridge with 5.0 mL deionized water and then eluted with 3.0 mL methanol. The flow rate for all the SPE steps was approximately 1.0 mL/min. Finally, the elution solution was concentrated to a nearly dry state under the action of N_2 stream, and then redissolved with 1.0 mL methanol, and through a 0.22 μm membranes (Agilent, USA) for UHPSFC and/or UHPLC analysis.

Ultra-high-performance supercritical fluid chromatography analysis

This study was analyzed using the ultra-performance convergence chromatography (UPC^2) system (Waters, USA) equipped with a binary solvent delivery pump, DAD, a back pressure regulator, and a column oven and controlled by Empower 3 software. HSS C_{18} SB (150 mm × 2.1 mm, 1.7 μm) column was used. Gradient elution (eluent A: CO_2; eluent B: 0.1% formic acid in methanol, v/v) was used in this study. The elution procedure was as follows: 99.0% A (initial), 99%–93% A (0–10.0 min), and 93%–80% A (10–20 min). Finally, 99% A was used to rebalance the system and column for 2 min. The back pressure is set at 12.41 MPa. The column temperature was 70°C and the sample temperature was 20°C. Sample injection volume was 1.0 μL and the flow rate was 1.5 mL/min. Detection wave was 280 nm.

Ultra-high-performance liquid chromatography analysis

At the same time, the ultra-high-performance chromatography (UHPLC) system (Waters, USA) was also used in the study, which was equipped with a binary solvent delivery pump, DAD, and controlled by Empower 2 software. In this study, HSS C_{18} SB was used as a chromatographic column. Sample injection volume was 1.0 μL, the column temperature was 30°C, and the sample temperature was 20°C. Detection wave was 280 nm. The elution gradient (eluent A: methanol containing 0.1% formic acid; eluent B: water containing 0.1% formic acid, v/v) was as follows: 15.0% A (initial), 15%–25% A (0–5.0 min), 25%–40% A (5–40 min), 40%–75% A (40–80 min), and 75%–90% A (80–100 min). Finally, 15% A was used to rebalance the system and column for 3 min. Flow rate was set at 0.3 mL/min.

RESULTS AND DISCUSSION

Selection of the stationary phase

The complexity of RH samples can make UHPSFC separation quite challenging. In order to select the most suitable UHPSFC separation column for target compounds, BEH 2-EP, BEH, HSS C_{18} SB, and CSH fluoro-phenyl were studied in this study. These stationary phases can be divided into three groups. BEH and BEH 2-EP constitute the first group. These stationary phases can form different polar interactions with solutes, such as dipole-induced dipole and dipole–dipole interactions.[21] It is necessary to note that BEH 2-EP was particularly suitable for UHPSFC analysis of alkaline compounds, which were mainly reflected in providing excellent peak shape for alkaline compounds. The second group was the CSH fluoro-phenyl stationary phase (charged surface hybrid bonded to fluoro-phenyl). It can be formed with additional kinds of retention interactions such as aromaticity (π-π) interaction, dipole–dipole interaction, H-bonding interactions, and hydrophobic interaction. The last group was the HSS C_{18} SB stationary phase (a normal inorganic silica phase bonded to the C_{18} group), which forms a charge transfer and dispersion interactions.[25] Standards of ten analytes were used as target compounds, and the separation conditions were described in "UHPSFC analysis." In this study, all the compounds (except for calycosin-7-O-β-D-glucoside [10]) were eluted under the described conditions and the calycosin (8) peak in the first group columns showed substantial tailing [Figure 2a and b]. Mainly because the BEH and BEH 2-EP are suitable for separating polar compounds,[25,26] the retention of polar compounds on the first group columns was stronger. In addition, elution orders (E.o) of the nine compounds from the BEH column were different from that of the BEH 2-EP column. This might be because the solid phase contained 2-ethylpyridine groups, which changed the selectivity of the chromatographic column. Therefore, BEH and BEH 2-EP phases were not suitable for the separation of the target compounds in UHPSFC. Moreover, serious tailing was observed for calycosin (8) and calycosin-7-O-β-D-glucoside (10) of CSH fluoro-phenyl phase [Figure 2c]. The ten target compounds were baseline separated. Among the three groups of chromatographic columns tested, the HSS C_{18} SB provided the best separation of the ten standards [Figure 2d]. All target compounds were baseline separated within 20 min. Therefore, even if the peak shape and baseline were not ideal under these conditions, HSS C_{18} SB column was selected for further optimization.

Selection of the cosolvent

Carbon dioxide (CO_2) as a supercritical fluid is too nonpolar for the solvent power of polar compounds such as organic acids and glycosides.[27,28] The spiked of polar cosolvent (including acetonitrile, isopropanol, ethanol, and methanol) was necessary to prompt elution and improve separation efficiency.[29] Because of different polarities and properties of the four cosolvents, the optimization of the cosolvents for the UHPSFC separation was studied using these four common organic solvents. Under the same chromatographic conditions (Section UHPSFC analysis), extremely strong retentions of calycosin (8), formononetin-7-O-β-D-glucoside (9), and calycosin-7-O-β-D-glucoside (10) were observed when acetonitrile and isopropanol were used as the cosolvents [Figure 3a and b]. With ethanol as the cosolvent [Figure 3c], only calycosin-7-O-β-D-glucoside (10) has a

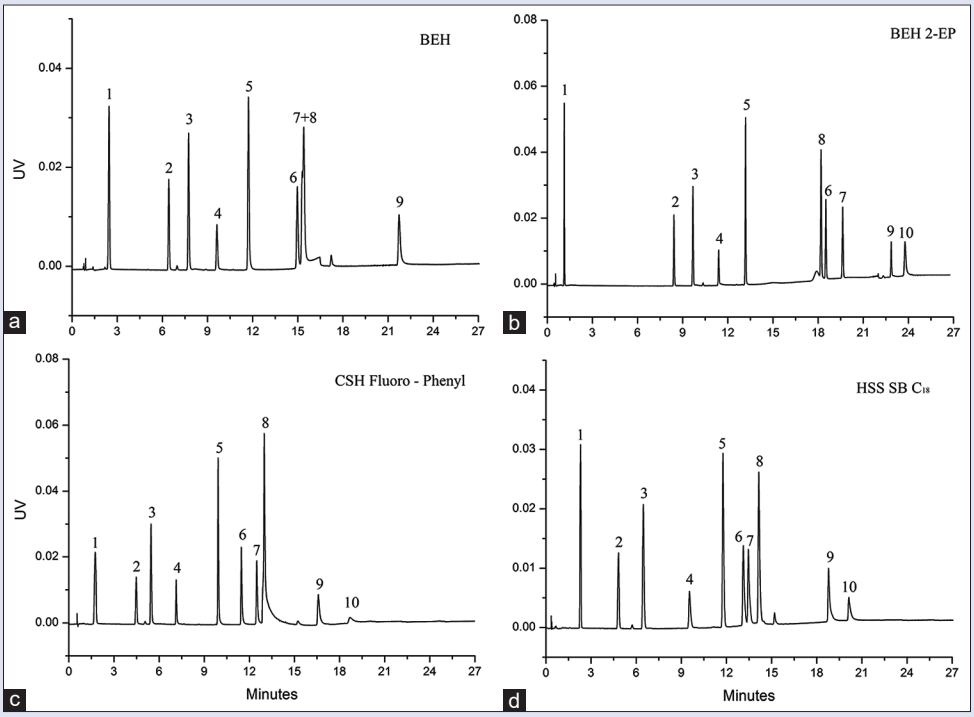

Figure 2: The separation of ten target compounds on four different stationary phases: (a) Acquity UPC² BEH; (b) Acquity UPC² BEH 2-EP; (c) Acquity UPC² CSH fluoro-phenyl; (d) Acquity UPC² HSS C_{18} SB using gradient elution with 1%–20% of methanol in 20 min at 1.5 mL/min. The temperature was set at 70°C and back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

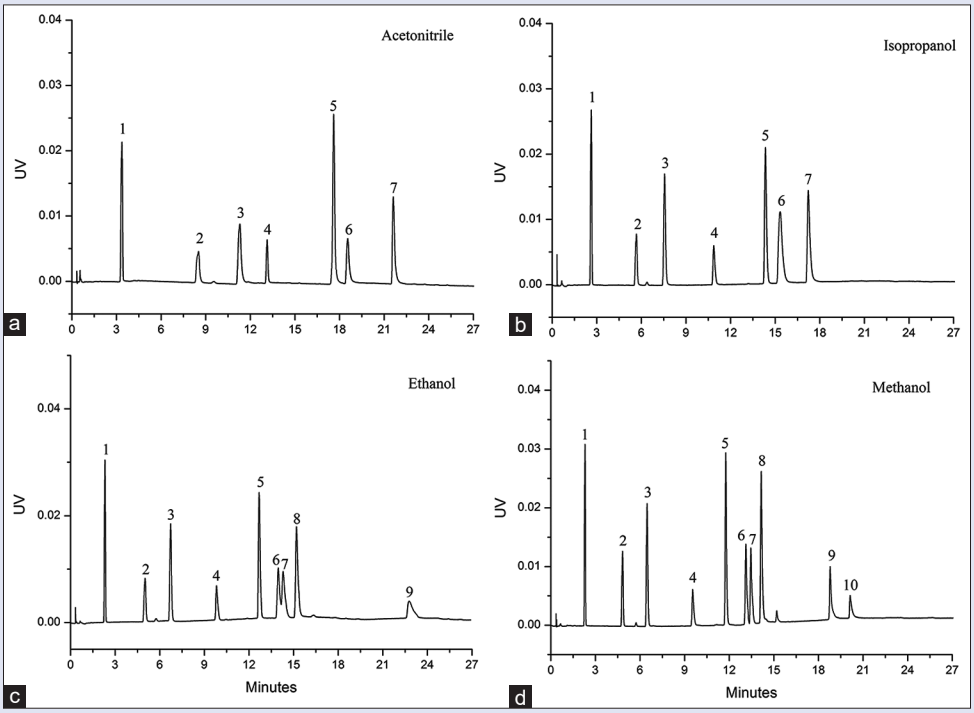

Figure 3: (a) Acetonitrile; (b) isopropanol; (c) ethanol; and (d) methanol on the separation of ten target compounds; the HSS C_{18} SB column using gradient elution with 1%–20% of methanol in 20 min at 1.5 mL/min. The temperature was set at 70°C and back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

strong retention (no elution). When methanol was used as the cosolvent [Figure 3d], the resolution and tR of formononetin-7-O-β-D-glucoside (9), calycosin-7-O-β-D-glucoside (10), and calycosin (8) were significantly improved. Therefore, methanol is the best choice for cosolvent in this study. Moreover, this study also found that alcohol (isopropanol, ethanol, and methanol) had higher solvation ability than acetonitrile, which had very weak solvation ability to stationary phase and target compounds. This phenomenon is similar to the results reported by Nováková *et al.*[29]

Selection of the back pressure

The back pressure can affect supercritical fluid density. Density is important for the mobile phase solvation power and consequently for the retention and selectivity. Therefore, the effect of back pressure between 1600 psi (11.03 Mpa) and 2200 psi (15.16 Mpa) was also tested to optimize the separation of the analytes. As shown in Figure 4, as back pressure increased, the retention time (tR) of coumarin (1), vanillic acid (2), ferulic acid (3), 3-hydroxy-9,10-dimethoxypterocarpane (4), formononetin (5), liquiritigenin (6), genistein (7), and calycosin (8) decreases. This was consistent with the reports by Gibitz Eisath *et al.*[27] and Nováková *et al.*,[29] which found that effect of back pressure on retention time is quit straightforward; at fixed temperature, as the pressure increases, the density of the mobile phase changes, resulting in reduced tR of the target compounds. However, in the range of tested back pressure, the effect of back pressure on the tR of formononetin-7-O-β-D-glucoside (9) and calycosin-7-O-β-D-glucoside (10) was quite limited. The reason may be the relatively high proportion of cosolvents in the mobile phase. In conclusion, when gradient elution was used, the earliest eluted compounds were significantly affected by the change in back pressure because of the lower proportion of cosolvent in the mobile phase at the beginning of the elution gradient. On the other hand, due to the higher proportion of mobile phase cosolvent at the end of elution gradient, compounds at the latest elution were less affected by the change of back pressure.

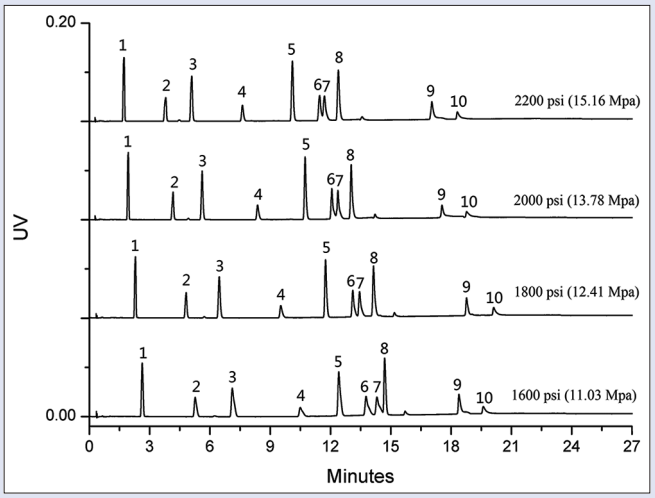

Figure 4: The back pressure range 11.03 Mpa to 15.16 Mpa on the separation of ten target compounds; the HSS C_{18} SB column using gradient elution with 1%–20% of methanol (containing 0.1% formic acid, v/v) in 20 min at 1.5 mL/min. The temperature was set to 70°C. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

Selection of the temperature

The temperature change causes the solute vapor pressure and the supercritical fluid density change. Moreover, the stationary phase may be unstable at higher temperatures. Therefore, it is important to determine the appropriate temperature to ensure the stability of the stationary phase and optimized the separation. In this study, temperature effects were tested in the 30°C–80°C range. As shown in Figure 5, increasing the temperature from 30°C to 80°C increased the resolution of liquiritigenin (6) and genistein (7) and decreases the tR of all target compounds. In addition, when the column temperature was 70°C, all ten target compounds were baseline separated, but when the column temperature was 80°C, the peaks of liquiritigenin (6), genistein (7), calycosin (8), and calycosin-7-O-β-D-glucoside (10) trailed and the peak shape of formononetin-7-O-β-D-glucoside (9) was poor. Therefore, the best column temperature was found to be 70°C because this temperature not only provided suitable tR of all target compounds but also maintained satisfactory peak shapes. Moreover, Nováková *et al.*[29] and Lou *et al.*[30] recently reported that temperature and pressure affected not only the density and vapor pressure of supercritical fluids but also the strength of the interactions between the solute, the mobile phase, and stationary phase. Thus, pressure and temperature can be used to fine-tune separation method, although they cannot be accurately predicted.

Selection of the flow rate

To further decrease the analysis time, flow rates (1.0 mL/min, 1.5 mL/min, and 2.0 mL/min) were investigated in the optimization process. Slower flow rates cause peak broadening and increased tR of the target compounds. In this study, changes in the flow rate did not improve the peak shapes. As shown in Figure 6, when the flow rate was higher than 1.5 mL/min, liquiritigenin (6) and genistein (7) were not baseline separated. Considering the pressure of the system, the resolution and sensitivity of the target compound, 1.5 mL/min was used as the best flow rate.

Comparison of ultra-high-performance supercritical fluid chromatography and ultra-high-performance liquid chromatography methods

Comparison of the stationary phases

In this study, it is also quit necessary to compare the selectivity of UHPSFC and UHPSFC according to different stationary phases. To get as close as possible to the separation conditions of UHPSFC and UHPLC, methanol was used as the cosolvent and/or the mobile phase in this study. The tested stationary phases were the same in both methods. It is important to note that BEH column was a polar stationary phase, which is inappropriate for reversed-phase elution conditions. BEH 2-EP column was not normally used in RP-LC; some mixed-mode retention may be possible on this column. These reasons lead to the low retention and bad peak shapes of all target compounds in BEH and BEH 2-EP (data not shown). Only HSS C_{18} SB and CSH fluoro-phenyl were normally employed in RPLC mode and appear to be suitable to achieve this separation. Therefore, the retention and selectivity of the two stationary phases can be directly compared. In this study, two different stationary phases were evaluated and compared using UHPLC and UHPSFC techniques and the results are shown in Figure 7. Moreover, the difference between UHPLC and UHPSFC was evaluated by comparing the tR, E.os, capacity factor (k'), and peak resolution (Rs) of the target compounds under different columns. The UHPLC separation conditions are described in "Section UHPLC analysis." First, under the selected gradient conditions, all ten target

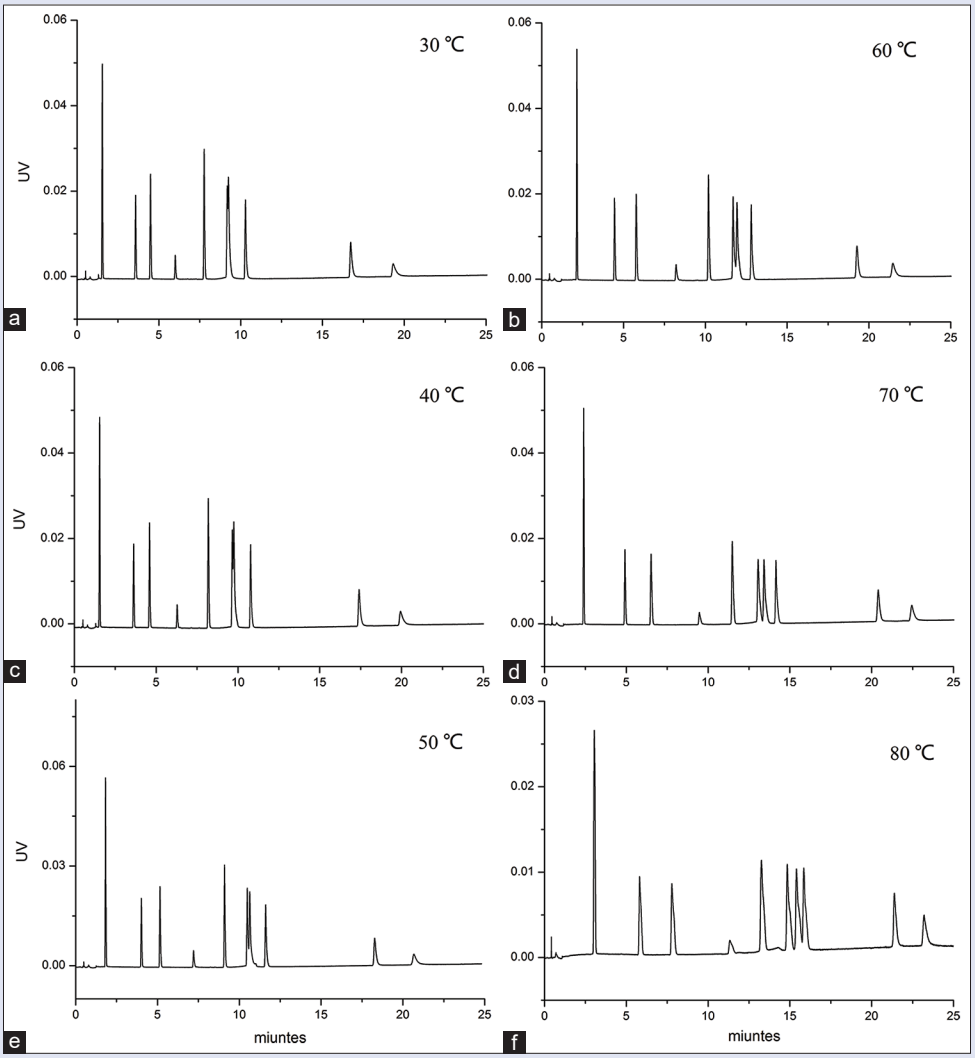

Figure 5: (a) 30°C; (b) 40°C; (c) 50°C; (d) 60°C; (e) 70°C, and (f) 80°C on the separation of ten target compounds; the HSS C_{18} SB column using gradient elution with 1%–20% of methanol in 20 min at 1.5 mL/min. The back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

compounds in the mixture were partially separated [Rs >0.86; Table 1] on the HSS C_{18} SB column [Figure 7a] within 70 min and completely separated [Rs >2.11; Table 1] on the CSH fluoro-phenyl column [Figure 7b]. By comparing the chromatograms obtained from UHPLC and UHPSFC [Figures 2 and 7], a shorter analysis time was observed for the latter. For instance, the analytical time was 70 min in UHPLC, but only 20 min in UHPSFC. On all measured stationary phases, k' values of all target compounds were <4.95. In addition, HSS C_{18} SB and CSH fluoro-phenyl columns demonstrated very different separation patterns. Under the conditions of UHPLC, the target compound peak resolution of CSH fluoro-phenyl columns was better than HSS C_{18} SB, but the opposite trend was observed under the conditions of UHPSFC. Finally, in the comparative study of different stationary phases under UHPLC and UHPSFC conditions, it can be concluded that the HSS C_{18} SB and CSH fluoro-phenyl more effectively separate the target compounds. These studies have also shown the complementarity of the two separation systems, and UHPSFC can serve as a powerful separation technique for adjusting selectivity when UHPLC was unable to separate some mixtures, and vice versa.

Comparison of the effect of temperature

In UHPSFC and UHPLC, the retention strength and elution strength of the analyte are affected by the density of the mobile phase, which was related to its composition, temperature, and pressure.[30] In addition, in UHPSFC and UHPLC, the effect of temperature on the retention strength of compounds was multifactorial, which is very complicated to explain. Therefore, in this study, HSS C_{18} SB column and gradient elution was selected. Under UHPSFC conditions, the temperature changed from 30°C to 70°C [Figure 5], and under UHPLC conditions, the temperature changed from 30°C to 60°C [Figure 8]. The effect of temperature on compound separation was evaluated using the Rs, E.o, k', and tR. Table 2 shows the parameters of Rs, E.o, k', and tR for the analysis of target compounds by both UHPSFC and UHPLC. As summarized in Table 2, the k' for the target compounds was in the range of 1.96–54.95 for UHPSFC and 3.07–32.08 for UHPLC. In addition, in combination with the E.o and parameter k' of the target compounds, it can be concluded that the parameter k' of the target compound varies with changes in temperature. Between UHPSFC and UHPLC, the change in temperature and the change of k' is opposite. This phenomenon may be due to the distribution of target

compounds between the mobile phase (or cosolvent) and the different columns (stationary phase) under the different conditions of UHPSFC and UHPLC. The parameter Rs for the analysis of target compounds was in the range of 0.37–15.07 for UHPSFC and 0.82–13.47 for UHPLC (except for the coeluted peak). It is noteworthy that under the conditions of UHPSFC, the Rs of the target compounds increases with an increase in temperature. Conversely, the Rs of the target compound decreases with increasing temperatures under the conditions of UHPLC. In addition, the E.o of coumarin (1) and calycosin-7-O-β-D-glucoside (10) changed between 60°C and 30°C, which may be due to the different distribution ratios between the stationary and mobile phase of different compounds.

Method validation

In this study, several method validation parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), correlation determination (R^2), accuracy, and recovery (trueness) were studied.

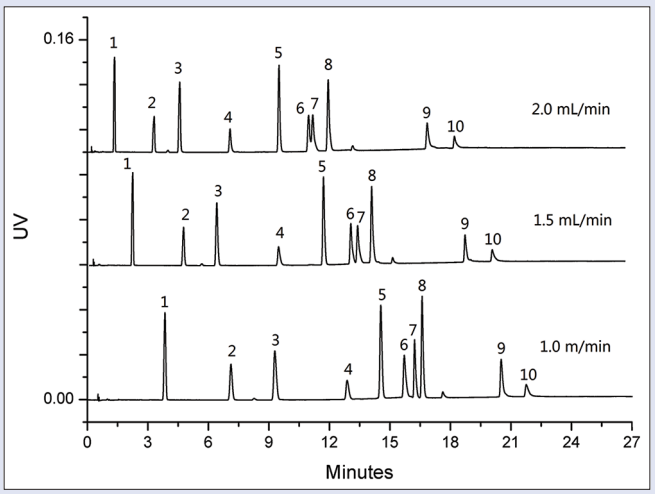

Figure 6: The influence of a flow rate on the separation of ten target compounds. The change in the flow rate of the mobile phase; 1.0 mL/min, 1.5 mL/min; and 2.0 mL/min on HSS C_{18} SB column. The analyses were performed using methanol (containing 0.1% formic acid, v/v) as a cosolvent, the temperature was set at 70°C and back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

Calibration curves were prepared from the stock solutions, which were diluted with cosolvent to generate working solutions with concentrations ranging from 0.2 to 400 μg/mL. Average concentrations are presented in Table 3 (each concentration was injected 3 times). Regression analyses determined the correlation coefficient (r^2) values ranged from 0.9989 to 0.9998. Under the optimized separation conditions, the LOD and LOQ were tested at S/N ratios of 3 and 10, respectively. LODs ranged from 0.2 μg/mL to 0.4 μg/mL and the LOQs ranged from 0.5 μg/mL to 1.0 μg/mL. The results showed that UHPSFC-DAD was sensitive to the determination of the target compound.

Intra- and interday variations were used to determine the precision of the developed assay ($n = 6$, variations were expressed in RSD). The RSDs of the analytes were <4.1% for interday precision and <3.5% for intraday precision [Table 4]. The extract of RH-1 was analyzed by replicate injection after 0, 1, 2, 4, 6, 8, 12, 18, 24, and 48 h to determine the stability of the sample. Table 4 shows the stability of the ten target compounds. The RSDs were <3.3%.

The trueness of the method was investigated by measuring the recovery, which was assessed by adding three different concentrations (low, medium, and high) of standard compounds to RH-1, for which the concentrations of the analytes were known. The sample was extracted and analyzed under optimized method. Each standard was tested at each concentration five times. Detailed procedure for the preparation of the spiked samples and average recoveries for compounds 1–10 are given in Table 5. The recoveries were between 96.58% and 104.50%, and the RSDs were <4.6%. The peak purity of the target compounds were calculated by Empower 3 software, and separation method selectivity was evaluated.

Analysis of ten compounds

The established UHPSFC-DAD method was successfully used to the investigation of ten analytes in the RH obtained from different growing regions. The identities of the compounds in the chromatograms were confirmed based on their tR and UV spectra. The contents of the ten analytes in the 25 samples are summarized in Table 6. A representative chromatography of the mixture of standards and standard samples (RH-1) under the selected conditions is shown in Figure 9. The concentrations of the ten compounds in sample RH-1 decreased in the order formononetin > formononetin-7-O-β-D-glucoside > 3-hydroxy-9,10-dimethoxypterocarpane > vanillic acid > calycosin-7-O-β-D-glucoside > liquiritigenin > calycosin > genistein > coumarin > ferulic acid, and this order is similar to what has been reported previously.[1,3] Moreover, samples purchased from Minxian county in Gansu province (mean content of 308.60 μg/mL) contained higher

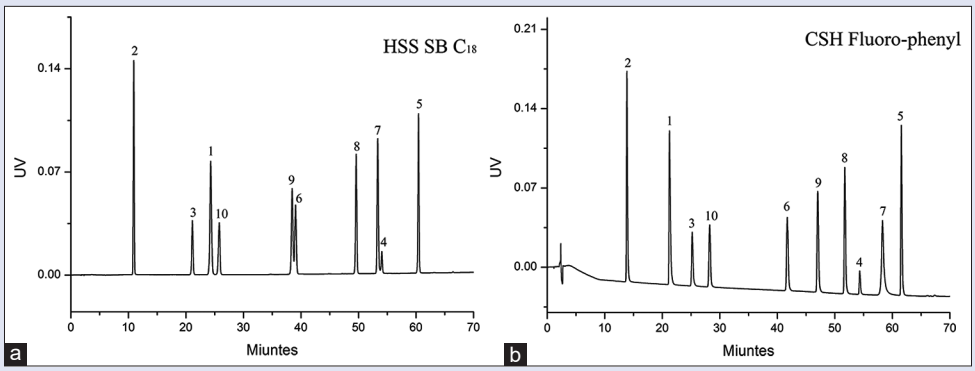

Figure 7: Selectivity of different UHPLC sub-2 μm particles stationary phases. (a) HSS C_{18} SB; (b) CSH fluoro-phenyl; using gradient elution with 15-90% of methanol in 70 min at 0.3 mL/min. The temperature was set at 30°C. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

Table 1: The result of resolution, retention factor, retention time, and elution order for different stationary phases

Stationary phase	Separation type	Chromatographic parameter										
HSS C_{18} SB	UHPSFC	E.o	1[a]	2	3	4	5	6	7	8	9	10
		tR	2.22	4.64	6.27	9.36	11.47	12.80	13.18	13.64	17.97	19.43
		k	4.95	11.48	15.85	24.15	29.82	33.41	34.43	35.67	47.31	51.22
		Rs	NA[b]	7.11	3.83	6.67	4.65	3.34	1.01	1.23	9.48	2.75
	UHPLC	E.o	2	3	1	10	9	6	8	7	4	5
		tR	10.93	21.12	24.31	25.79	38.47	39.07	49.60	53.35	54.05	60.44
		k	5.11	10.81	12.60	13.43	20.51	20.85	26.74	28.84	29.23	32.80
		Rs	NA	12.61	2.84	1.19	13.47	0.86	12.90	4.32	1.02	8.57
CSH fluoro-phenyl	UHPSFC	E.o	1	2	3	4	5	6	7	8	9	10
		tR	1.76	4.48	5.46	7.13	9.92	11.46	12.50	13.00	16.58	19.11
		k	2.22	7.20	9.00	12.06	17.16	19.99	21.89	22.80	29.36	9.88
		Rs	NA	8.25	2.67	4.54	7.03	3.54	2.38	1.11	7.10	6.88
	UHPLC	E.o	2	1	3	10	6	9	8	4	7	5
		tR	13.86	21.26	25.20	28.24	41.72	47.03	51.74	54.33	58.29	61.57
		k	5.74	9.34	11.26	12.74	19.29	21.88	24.16	25.43	27.35	28.95
		Rs	NA	6.54	3.30	2.67	11.60	4.41	4.07	2.62	2.69	2.11
BEH 2-EP	UHPSFC	E.o	1	2	3	4	5	8	6	7	9	NA
		tR	1.13	8.42	9.68	11.39	13.17	18.20	18.52	19.64	23.79	
		k	1.51	17.63	20.41	24.20	28.14	39.25	39.96	42.44	51.63	
		Rs	NA	28.36	4.06	4.80	5.03	17.09	1.28	3.87	9.69	
	UHPLC	E.o						NA				
		tR										
		k										
		Rs										
BEH	UHPSFC	E.o	1	2	3	4	5	6	7	8	9	NA
		tR	2.45	6.43	7.17	9.63	11.72	14.97	15.29	15.40	21.74	
		k	2.12	7.74	8.83	11.23	13.89	18.02	18.43	18.56	26.62	
		Rs	NA	13.41	3.79	9.83	8.37	8.37	1.40	0.35	13.26	
	UHPLC	E.o						NA				
		tR										
		k										
		Rs										

[a]The compound numbers are the same as Figure 2d. [b]NA: Not available, UHPSFC: Ultra-high-performance supercritical fluid chromatography; UHPLC: Ultra-high-performance liquid chromatography

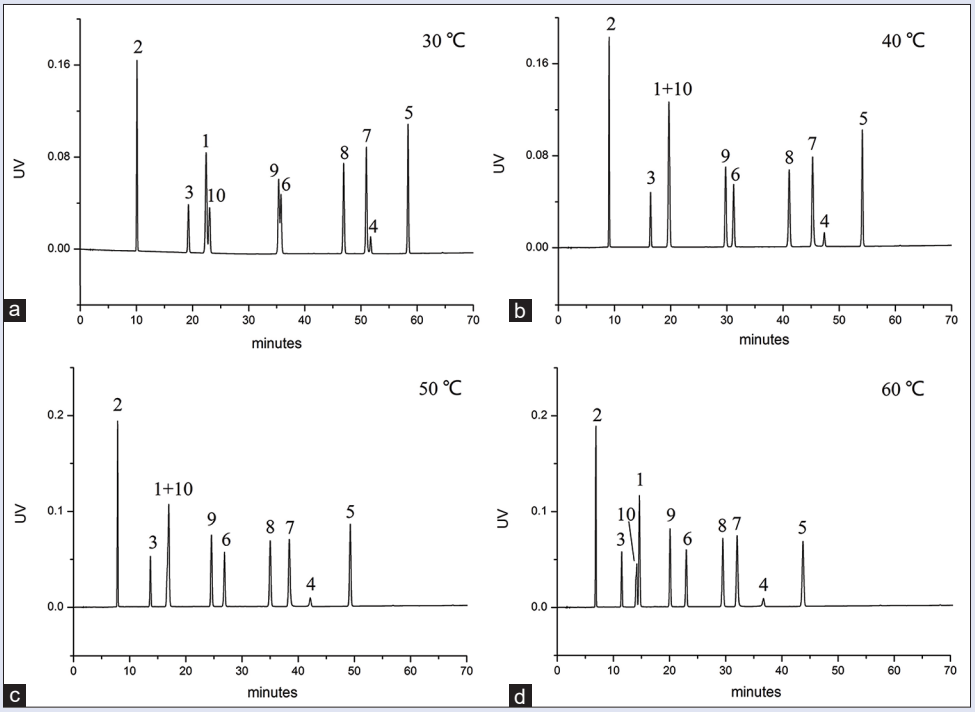

Figure 8: Selectivity of different UHPLC temperatures: (a) 30°C; (b) 40°C; (c) 50°C; and (d) 60°C on the separation of ten target compounds; the separation of target compounds on a HSS C_{18} SB column using gradient elution with 15%–90% of methanol in 70 min at 0.3 mL/min. The back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4) 3-hydroxy-9,10-dimethoxypterocarpane; (5) formononetin; (6) liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

Table 2: The result of resolution, retention factor, retention time, and elution order for different temperature

Temperatures	Separation type	Chromatographic parameter										
30°C	UHPSFC	E.o	1[a]	2	3	4	5	6	7	8	9	10
		tR	1.54	3.57	4.49	6.00	7.77	9.17	9.26	10.29	16.73	19.32
		k	1.96	5.86	7.61	10.52	13.91	16.61	16.77	18.74	31.10	36.08
		Rs	NA[b]	9.30	3.23	5.17	5.57	5.89	0.37	2.96	14.31	4.93
	UHPLC	E.o	2	3	1	10	9	6	8	7	4	5
		tR	10.93	21.12	24.31	25.79	38.47	39.07	49.60	53.35	54.05	60.44
		k	5.11	10.81	12.60	13.43	20.51	20.85	26.74	28.84	29.23	32.80
		Rs	NA	12.61	2.84	1.19	13.47	0.86	12.90	4.32	1.02	8.57
40°C	UHPSFC	E.o	1	2	3	4	5	6	7	8	9	10
		tR	1.54	3.62	4.60	6.29	8.19	9.66	9.75	10.77	17.40	19.94
		k	1.95	5.93	7.79	11.02	14.66	17.47	17.64	19.60	32.27	37.12
		Rs	NA	9.70	3.43	6.30	6.53	5.91	0.37	2.92	15.07	4.82
	UHPLC	E.o	2	3	1+10		9	6	8	7	4	5
		tR	9.03	16.42	19.69		29.78	31.21	41.10	45.27	47.34	54.12
		k	4.08	8.23	10.07		15.75	16.55	22.11	24.46	25.63	29.44
		Rs	NA	10.86	3.68		9.66	1.28	8.68	3.66	2.07	7.69
50°C	UHPSFC	E.o	1	2	3	4	5	6	7	8	9	10
		tR	1.82	4.02	5.15	7.20	9.10	10.48	10.64	11.61	18.28	20.67
		k	2.68	7.13	9.43	13.58	17.41	20.22	20.53	22.50	36.00	40.84
		Rs	NA	9.95	3.68	6.52	5.92	5.78	0.75	2.85	14.28	4.34
	UHPLC	E.o	2	3	1+10		9	6	8	7	4	5
		tR	7.96	13.69	16.96		24.55	26.88	35.02	38.42	42.16	49.27
		k	3.56	6.85	8.72		13.07	14.41	19.07	21.02	23.16	27.23
		Rs	NA	9.49	3.56		7.28	2.52	8.28	3.05	3.52	7.32
60°C	UHPSFC	E.o	1	2	3	4	5	6	7	8	9	10
		tR	2.15	4.45	5.77	8.21	10.19	11.70	11.94	12.81	19.27	21.46
		k	3.57	8.47	11.28	16.47	20.68	23.89	24.39	26.25	39.99	44.66
		Rs	NA	8.30	4.31	7.65	5.45	4.94	0.91	2.36	13.96	4.15
	UHPLC	E.o	2	3	10	1	9	6	8	7	4	5
		tR	6.89	11.49	14.17	14.63	20.09	22.98	29.48	32.02	36.71	43.75
		k	3.07	5.78	7.37	7.64	10.87	12.57	16.41	17.91	20.69	24.84
		Rs	NA	8.82	4.48	0.82	7.73	3.33	6.86	2.34	4.54	6.90
70°C	UHPSFC	E.o	1	2	4	3	5	6	7	8	9	10
		tR	2.40	4.92	6.52	9.47	11.48	13.06	13.42	14.15	20.40	22.45
		k	4.29	9.85	13.38	19.90	24.34	27.83	28.62	30.23	44.03	48.56
		Rs	NA	9.98	5.01	8.42	5.04	4.19	1.04	1.74	12.43	3.73
80°C		E.o	1	2	4	3	5	6	7	8	9	10
		tR	3.04	5.81	7.79	11.34	13.26	14.48	15.40	15.85	21.39	23.20
		k	6.31	12.98	17.73	26.25	30.87	33.81	36.02	37.10	50.41	54.79
		Rs	NA	7.37	4.28	7.15	3.50	2.17	1.86	1.05	12.06	3.34

[a]The compounds numbers are the same as Figure 2d. [b]NA. NA: Not available

Table 3: Linearity, limit of detections, and limit of quantifications in the determination of target compounds

Analytes	tR (min)	Regressive equation	Linear range (μg/mL)	r^2	LOD (μg/mL)	LOQ (μg/mL)
1[a]	2.31	y=3715.6x + 4095.2	0.50-200.00	0.9998	0.20	0.50
2	4.73	y=1574.5x − 2666.6	0.50-200.00	0.9995	0.20	0.50
3	6.35	y=1435.9x − 301.55	0.50-200.00	0.9991	0.20	0.50
4	9.41	y=1574.7x + 2952.3	0.50-200.00	0.9993	0.20	0.50
5	11.59	y=5468.3x + 35169	0.50-200.00	0.9995	0.20	0.50
6	12.91	y=4133.7x − 946.81	0.50-200.00	0.9994	0.20	0.50
7	13.29	y=5845.4x + 3131.6	0.50-200.00	0.9995	0.20	0.50
8	13.89	y=4731.8x − 642.12	0.50-200.00	0.9997	0.20	0.50
9	18.13	y=2467.3x + 15144	1.00-200.00	0.9994	0.40	1.00
10	19.39	y=2578.4x + 10134	1.00-200.00	0.9989	0.40	1.00

[a]The compound numbers are the same as Figure 2d. LOD: Limit of detection, LOQ: Limit of quantification

concentrations of the compounds than did the samples obtained from other growing regions. Second, not all the samples contained ferulic acid. In addition, of the 25 RH samples, the samples from Minxian country (Gansu province) had significantly higher contents of formononetin (206.04 μg/mL) than those from other origins. Wild RH (RH-21 and RH-22) had lower contents of formononetin (48.54 μg/mL and 41.41 μg/mL, respectively). However, wild RH samples had higher contents of vanillic acid and calycosin-7-O-β-glucoside. This results show that analyses conducted using UHPSFC are consistent with those reported previously[4] and suggested that the differences between the ratios of the contents of calycosin-7-O-β-glucoside/vanillic acid and formononetin/formononetin-7-O-β-glucoside could be used as to distinguish the geographic origins of RH samples. Finally, this study showed that UHPSFC can be applied to the detection of compounds in TCMs.

Table 4: Stability, interday, and intraday precision of the investigated compounds

Analytes	Concentration (mg/kg)	Intra-day (*n*=6)		Inter-day (*n*=6)		Stability RSD (%)
		Found (mg/kg)	RSD (%)	Found (mg/kg)	RSD (%)	
1	5.0	4.8[a]	0.9	4.9	1.2	0.9
	50.0	49.9	0.9	49.8	1.0	
	100.0	99.8	1.1	100.8	1.4	
2	5.0	4.9	0.7	4.8	1.1	1.3
	50.0	49.8	0.9	49.9	1.3	
	100.0	99.8	1.1	99.7	1.3	
3	2.5	2.4	1.2	2.5	1.4	1.2
	25.0	25.0	1.6	24.9	2.1	
	50.0	49.9	1.2	49.7	1.3	
4	10.0	9.9	1.1	9.9	1.2	1.4
	100.0	99.6	1.5	99.8	2.0	
	200.0	198.8	1.3	199.4	1.4	
5	5.0	4.9	0.9	5.1	1.2	0.9
	50.0	49.6	1.0	49.9	1.5	
	100.0	101.1	1.1	100.0	1.5	
7	2.5	2.4	1.1	2.5	1.2	0.9
	25.0	24.9	1.4	24.6	2.1	
	50.0	49.9	1.2	49.8	1.3	
8	2.5	2.4	1.2	2.4	1.3	1.1
	25.0	24.8	1.3	24.5	1.8	
	50.0	49.6	1.6	49.9	1.3	
9	5.0	5.0	1.9	4.9	3.1	3.3
	50.0	49.7	1.7	50.1	2.9	
	100.0	99.6	2.3	100.0	3.5	
10	5.0	4.9	2.3	5.01	2.5	2.8
	50.0	49.8	3.2	50.0	3.8	
	100.0	100.5	3.5	101.6	4.1	

[a]The compound numbers are the same as Figure 2d. RSD: Relative standard deviation

Table 5: Recoveries for the assay of ten target compounds in *Radix hedysari* - 1

Analytes	Original (μg/mL)	Spiked (μg/mL)	Found (μg/mL)[b]	Recovery (%)[c]	RSD (%, *n*=5)
1[a]	1.81	0.5	2.38	103.15	1.6
		5.0	6.86	100.70	1.4
		25.0	26.77	99.84	1.4
2	6.97	0.5	7.37	98.71	1.5
		5.0	12.18	101.75	1.2
		25.0	31.82	99.54	1.3
3	NQ	0.5	0.50	100.80	1.8
		5.0	5.08	101.60	1.9
		25.0	24.94	99.76	1.8
4	9.69	0.5	10.33	101.34	2.0
		5.0	15.13	103.01	2.5
		25.0	34.73	100.11	2.6
5	104.5	25	130.82	101.02	2.5
		100	203.95	99.73	2.1
		200	300.21	98.59	1.9
6	6.73	0.5	7.21	99.75	2.6
		5.0	11.55	98.47	3.0
		25.0	30.64	96.58	3.2
7	1.04	0.5	1.49	96.71	1.8
		5.0	5.95	98.45	1.6
		25.0	25.43	97.67	1.1
8	3.72	0.5	4.25	100.80	1.8
		5.0	8.86	101.60	1.9
		25.0	28.65	99.76	1.8
9	19.85	0.5	20.26	99.56	3.9
		5.0	25.97	104.50	4.1
		25.0	44.31	98.79	4.1
10	2.48	0.5	2.96	99.35	4.3
		5.0	7.56	101.04	4.6
		25.0	28.12	102.32	3.9

[a]The analyte numbers are the same as Figure 2d. [b]The data were present as average of three determinations, [c]Recovery (%) = 100 × (amount founds - original amount)/amount spiked. NQ: Not quantify

Table 6: Contents of investigated compounds in *Radix hedysari* (*n*=3, unit: μg/mL)

Number	1[a]	2	3	4	5	6	7	8	9	10
RH-1	1.81±0.05[b]	6.97±0.13	NQ	9.69±0.19	104.5±3.25	6.73±0.02	1.04±0.02	3.72±0.12	19.85±1.12	2.48±0.25
RH-2	1.15±0.02	6.07±0.12	ND	4.41±0.12	184.17±4.37	0.69±0.01	1.27±0.02	5.57±0.11	83.56±2.56	5.99±0.44
RH-3	2.47±0.08	6.92±0.18	ND	11.25±0.20	100.55±2.24	5.34±0.03	3.85±0.03	4.07±0.08	69.53±2.13	8.35±0.35
RH-4	1.92±0.05	4.91±0.14	NQ	3.87±0.10	73.65±1.58	2.35±0.02	5.75±0.03	4.86±0.13	28.71±1.25	5.96±0.15
RH-5	2.03±0.04	8.03±0.09	NQ	2.97±0.09	67.91±2.14	3.57±0.02	3.08±0.05	5.08±0.12	36.21±2.04	3.21±0.21
RH-6	4.05±0.17	10.29±0.19	NQ	8.42±0.18	119.03±3.12	3.61±0.01	2.11±0.03	5.55±0.09	67.08±2.87	12.19±1.29
RH-7	3.26±0.09	8.37±0.15	0.79±0.02	5.27±0.11	95.02±2.25	2.84±0.03	1.03±0.01	3.24±0.10	75.78±2.01	11.61±1.08
RH-8	3.26±0.08	9.22±0.14	NQ	8.86±0.14	103.39±3.01	8.70±0.03	20.19±0.11	5.44±0.15	53.24±1.68	7.53±0.86
RH-9	2.29±0.05	8.23±0.16	ND	6.43±0.14	97.99±2.23	4.96±0.02	16.51±0.19	2.15±0.06	58.74±1.67	7.25±0.95
RH-10	2.16±0.03	5.16±0.09	0.97±0.01	5.62±0.12	89.02±2.01	6.54±0.02	4.08±0.03	2.72±0.08	38.45±1.14	5.37±0.54
RH-11	2.20±0.04	3.88±0.15	1.02±0.02	13.71±0.21	101.42±3.34	6.12±0.03	3.06±0.03	3.51±0.09	42.24±1.54	8.23±0.47
RH-12	1.13±0.07	4.54±0.09	NQ	8.10±0.18	93.62±2.21	5.91±0.02	4.41±0.02	2.69±0.11	28.01±1.05	6.93±0.27
RH-13	1.95±0.05	4.90±0.12	NQ	9.66±0.15	72.22±1.17	7.85±0.03	8.29±0.03	4.93±0.20	28.99±0.99	3.73±0.09
RH-14	2.52±0.12	4.50±0.10	NQ	6.78±0.09	76.36±1.79	2.64±0.01	1.08±0.01	3.38±0.21	24.76±1.34	4.97±0.17
RH-15	2.37±0.09	12.78±0.18	0.72±0.02	13.63±0.19	106.91±3.54	3.61±0.03	4.08±0.04	4.51±0.14	48.87±2.07	5.41±0.16
RH-16	2.67±0.02	7.44±0.09	0.63±0.01	8.31±0.15	206.04±4.25	2.80±0.03	3.06±0.09	3.36±0.08	68.57±2.16	6.35±0.20
RH-17	1.16±0.03	2.98±0.11	ND	10.76±0.18	99.41±2.68	8.76±0.03	1.04±0.01	2.27±0.05	61.43±2.09	7.67±0.12
RH-18	1.27±0.02	4.77±0.14	ND	3.68±0.09	82.61±3.07	3.61±0.02	4.41±0.16	3.29±0.11	33.03±1.57	9.51±0.14
RH-19	3.83±0.05	8.15±0.08	NQ	9.92±0.11	113.35±2.39	2.84±0.01	4.08±0.15	4.78±0.12	67.23±2.01	9.23±0.14
RH-20	1.80±0.03	8.52±0.13	ND	11.44±0.12	61.44±2.12	8.70±0.02	3.06±0.06	2.04±0.08	57.41±1.87	5.41±0.09
RH-21	2.35±0.05	11.55±0.15	ND	18.54±0.18	48.54±1.58	2.64±0.01	2.29±0.02	5.89±0.21	23.56±1.05	14.26±0.10
RH-22	2.64±0.03	14.45±0.26	NQ	21.41±1.01	41.41±2.01	3.61±0.01	1.08±0.02	7.25±0.23	28.12±0.89	13.67±0.03
RH-23	1.12±0.03	4.57±0.19	ND	14.79±0.11	74.79±2.09	2.81±0.02	2.08±0.01	5.17±0.19	18.57±0.91	2.39±0.02
RH-24	2.80±0.04	7.25±0.19	ND	11.25±0.09	71.25±1.99	8.74±0.03	3.06±0.03	3.56±0.14	31.66±1.06	3.57±0.06
RH-25	1.65±0.03	5.25±0.16	NQ	8.98±0.03	84.98±0.23	3.61±0.02	1.04±0.01	8.05±0.18	24.8±1.11	2.69±0.03

[a]The analyte numbers are the same as Figure 2d, [b]The data were present as average of triplicates. NQ: Not quantify; ND: Not detected

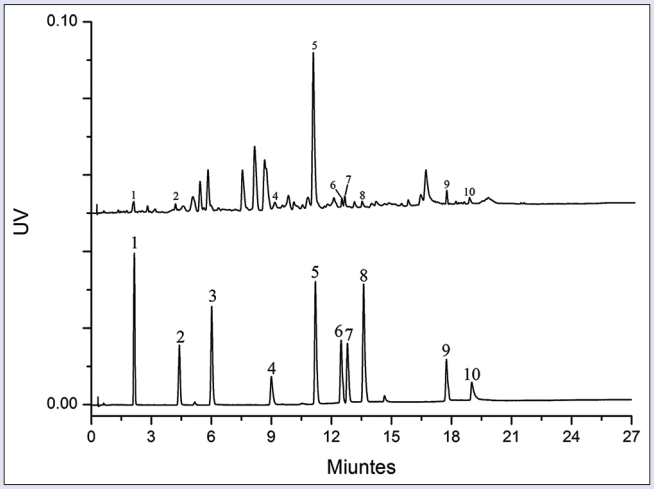

Figure 9: Ultra-high-performance supercritical fluid chromatography chromatographic of sample (RH-1) and mixture standards. The separation of ten target compounds on a HSS C_{18} SB column using gradient elution with 1%–20% of methanol in 20 min at 1.5 mL/min. The column temperature was set at 70°C and back pressure to 12.41 Mpa. Compounds: (1) Coumarin; (2) vanillic acid; (3) ferulic acid; (4)3-hydroxy-9,10-dimethoxypterocarpane;(5)formononetin;(6)liquiritigenin; (7) genistein; (8) calycosin; (9) formononetin-7-O-β-D-glucoside; and (10) calycosin-7-O-β-D-glucoside

CONCLUSION

A fast UHPSFC method with high resolution, sensitivity, and reproducibility was developed and validated to quantify ten compounds in RH. These compounds have been described for the first time in RH. In the optimized UHPSFC method, methanol and CO_2 were used as mobile phases and gradient elution was performed, and these target compounds were successfully separated on HSS C_{18} SB column. The optimized separation method can accurately determine and quantify all ten compounds within 20 min. The method utilized SPE as the pretreatment. Under the optimal conditions, the target compounds were quantitatively recovered from the samples. Moreover, consumption of cosolvent was greatly reduced in the analysis, indicating that UHPSFC was a greener and more environmentally friendly separation system than traditional separation system. Although the method presented in this study was designed for RH, it can be applied to the determination of compounds in other TCMs. Obviously, sample pretreatment and/or chromatographic conditions may require specific modifications for different TCMs. The purpose of this study was to compare the separation mechanisms of ten target compounds under different separation systems. The results show that changes in selectivity are more apparent under various UHPLC conditions than under UHPSFC conditions. These results can be explained by the properties of the stationary phase bonds themselves and the effects of the interactions between the target and different mobile phases and different stationary phases in UHPLC and UHPSFC systems. In addition, analysis time of the UHPSFC was faster than that of the UHPLC, which affords high-throughput analysis of target compounds in complex TCMs. Finally, under the separation conditions of UHPSFC and UHPLC, the comparison of chromatographic parameters (Rs, k', and E.o) shows that a combination of these two separation techniques efficiently separate the ten target compounds in RH. The results obtained in this study will be useful in quality control and high-throughput analysis of TCMs in future.

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Conflicts of interest

There are no conflicts of interest.

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