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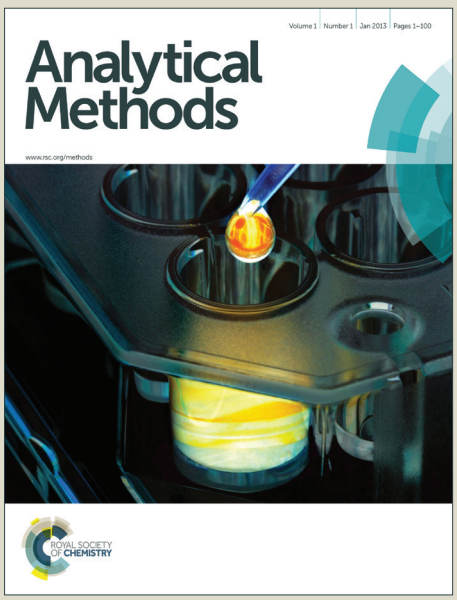

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Development and validation of an ultra-high-performance liquid chromatography for the determination of sennoside A and sennoside B in laxatives based on optimal chromatographic parameters

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Abstract Sennoside A and sennoside B are the major bioactive components in laxative herbs like rhubarb, senna, etc. The previously reported techniques for quantitative analysis of sennoside A and sennoside B have the disadvantages of defective peak purity and stability. This study investigates the influence of analytical parameters on efficiency of separation of sennoside A and sennoside B in rhubarb by ultra-high-performance liquid chromatography (UHPLC). The chromatographic parameters of column temperature and flow rate have a non-linear relationship with theoretical plate number and symmetry factor; the optimal column temperature was 30 °C and the optimal flow rate was 0.20 mL/min. A new UHPLC analytical method was developed based on these optimal parameters. The chromatographic peak purity of sennoside A and sennoside B in rhubarb was satisfactory. This UHPLC-based analytical method was successfully applied for quantitative determination of sennosides A and sennoside B in two sources of rhubarbs, *Cassia angustifolia* Vahl and Paidu Yangyan Capsule. There are remarkable content differences between sennoside A (more than 19 times) and sennoside B (more than 18 times) in the two species of rhubarb tested. In order to make the analytical method widely applicable, the analysis parameters of UHPLC have been converted into those of HPLC. In conclusion, the devised technique is suitable for quality control of laxative herbs and diet drugs containing sennoside A and sennoside B.

1. Introduction

Rhubarb (Da Huang in Chinese) is an herb of worldwide fame. It has historically been used in clinical therapy under traditional Chinese medical theories owing to its multiple pharmacological effects including laxative, hemostatic, and antibacterial effects [1–3]. Rhubarb is officially listed in the Chinese, European and Japanese Pharmacopoeia [4–6], from three important plant sources: the roots of *Rheum tanguticum* Maxim.ex Balf, *Rheum. palmatum* L., and *Rheum. officinale* Baill. Rhubarb is nowadays used in the clinical treatment of chronic renal failure, acute pancreatitis, icteric hepatitis, and cancer, under the guidance of modern medical theories [7–12].

Despite extensive phytochemical investigations and pharmacological studies on rhubarb, a full quality control of rhubarb has been limited. Sennoside A and sennoside B is a pair of bianthrone-derived isomers (Fig. 1). Several important pharmacological effects of sennosides include relaxing the bowels, bacteriostasis [13, 14], insulin sensitization, and protecting nerve cells [15, 16]. They generally exist as major bioactive components in traditional laxatives [17], such as rhubarb, senna, and the diet drugs (Fig. 2), indicating that the content determination of sennoside A and sennoside B is crucial to the safety and efficacy of the laxative herbs and diet drugs.

Quantitative determination of sennoside A and sennoside B in rhubarb and diet drugs have always been difficult because of the lack of a simple analytical method in the Chinese and European Pharmacopoeia [4, 5]. In fact, only the Japanese pharmacopoeia provides a technique for the quantitative determination of sennoside A in rhubarb, but without satisfactory chromatographic resolution [6]. The literatures previously reported on the analysis of sennoside A and sennoside B in related drugs without good resolution and shorter analysis time [18–24]. The prevalent disadvantage of quality control of laxative herbs and diet pills is the lack of a simple and widely applicable quantitative analytical method for sennoside A and sennoside B.

In this study, we aim to resolve the problems of low peak purity and low resolution of the existing chromatographic analytical methods for sennoside A and sennoside B, to this end, the influence of analytical parameters on the chromatographic separation efficiency were systematically investigated, and the optimized parameters were obtained. A simple ultra-high-performance liquid chromatography (UHPLC) analytical method was applied successfully to simultaneous quantification of sennoside A and sennoside B in *Rheum tanguticum* Maxim.ex Balf, *Rheum. palmatum* L., *Cassia angustifolia* Vahl and Paidu Yangyan capsule.

2. Experimental

2.1 Materials and reagents

Nine batches of rhubarb raw materials (samples Rh01–Rh09) were collected from the Sichuan and Gansu provinces, China. All the samples were identified as *Rheum tanguticum* Maxim.ex Balf. (samples Rh01–Rh05), *Rheum palmatum* L. (samples Rh06–Rh09). Two batches of *Cassia angustifolia* Vahl were purchased from 302 Military Hospital of the Chinese People's Liberation Army (samples Ca01–Ca02), two batches of Paidu Yangyan Capsule were purchased from the Beijing Jia Shi-tang drugstore chain (samples Ph01–Ph02, drug batch number: 140757).

Sennoside A and sennoside B supplied by National Institutes for Food and Drug Control were used as chemical standard references for quantitative analysis; the batch numbers of sennoside A and sennoside B are 110824-201301 and 110825-201301, respectively. The sennoside A and sennoside B have purity more than 99.00%, as validated by HPLC. Methanol, acetonitrile, and phosphoric acid [85% (w/w) in H_2O] for quantitative analysis were of HPLC grade (Thermo Fisher Scientific, USA). High-purity water was obtained from Millipore Milli Q water purification system (Millipore, Synergy, USA). All other chemical reagents were purchased from Sigma-Aldrich, China.

2.2 UHPLC instrumentation and conditions

UHPLC was performed by a Waters Acquity ultra-high-performance liquid chromatograph system equipped with a binary solvent delivery pump (Waters, USA), an auto sample manager, and a photoelectric diode array detector (PDA). Data collection and integration were performed by the Empower 2 software. The chromatographic separation was performed using a Waters Acquity BEH C18 column (2.1 mm × 100 mm, 1.7μm). The mobile phase, consisting of a mixture of acetonitrile (A) and 0.1% (v/v) aqueous phosphoric acid (B), had a flow rate of 0.20 mL/min. The binary gradient elution protocol was as follows: 0.00–5.00 min with 8–12% A, 5.01–10.00 min with 12–13% A, 10.01–15.00 min with 13–15% A, 15.01–20.00 min with 15–17% A, 20.01–25.00 min with 17–21% A, 25.01–30.00 min with 21–60% A. The detector wavelength was set at 340 nm. The injection volume was 2.0 μL and the column temperature was maintained at 30 °C.

2.3 Standard solution preparation

The standard reference substances sennoside A and sennoside B were accurately weighed into a 50-mL brown volumetric glass flask, dissolved in 50 mL 0.1% (w/w) $NaHCO_3$ aqueous solution containing 40% methanol to make a stock solution of 46.00–74.00 μg/mL, which is stable for 1 month when stored at 4 °C. Different concentrations of working solutions for UHPLC analysis were diluted from this stock solution.

2.4 Sample solution preparation

Each of nine rhubarb samples (0.2000–0.2100g) was accurately weighed into a 50-mL brown volumetric glass flask with 25 mL 0.1% (w/w) $NaHCO_3$ aqueous solution and was then extracted by ultrasonic treatment for 40 min (500 W, 40 KHz). After ultrasonic processing, the lost weight was made up using 0.1% (w/w) $NaHCO_3$ aqueous solution. Each sample solution was filtered through a 0.22-μm microfiltration membrane (Agilent Technologies, USA) before UHPLC injection.

2.5 Optimization of UHPLC parameters

Given that isomers sennoside A and sennoside B were difficult to separate from other stronger polar compounds, two mobile phase systems, including acetonitrile–water and methanol–water with different gradients, were compared. Considering the resolution and the smooth baseline, the acetonitrile–water system was preferred. Using a photoelectric diode array detector, we performed a full wavelength scan in the ultraviolet region, from 200 to 440 nm, for each sennoside standard solution. Sennoside B showed strong absorption peaks at 268 nm and 358 nm, while sennoside A showed strong absorption at 269 nm and 340 nm. 340 nm was preferred. In order to achieve robust chromatographic separation, important analytical parameters, including retention time, resolution, theoretical plate number, and symmetry factor, were optimized by altering the column temperature and flow rate.

2.6 Validation of the UHPLC analytical method

The UHPLC method was rigorously validated in terms of sensitivity, accuracy stability, and linearity according to the Chinese Pharmacopoeia of "Traditional Chinese Medicine Quality analysis Methods Validation Guidelines" and the International Conference on Harmonization Guidelines (ICH Q2B, validation of analytical procedures, methodology)[25, 26].

2.6.1 Sensitivity of the analytical method

The limit of detection (LOD) and the limit of quantification (LOQ) for sennoside A and sennoside B were analyzed to evaluate the sensitivity of the developed UHPLC method. The LOD and LOQ for sennoside A and sennoside B were estimated by injecting a series of diluted solutions with known concentrations, which were detected at a signal/noise (S/N) ratio of about 3: 1 and 10: 1, respectively.

2.6.2 Linear regression equation and calibration curve of the analytical method

The linear regression equation (y = ax+b) and calibration curve were designed to investigate the correlation between the peak area (y) and the injection concentration (x, μg/ml) of each sennoside. Six injections were performed to obtain absorption plots for concentrations ranging from 0.46 to 74.00 μg/mL. Solutions for the linearity test were prepared by diluting the mixed standard stock solutions to the desired level. A linearity study was also carried out at the LOQ level.

2.6.3 Precision and stability of the analytical method

Intra- and interday variations were used to evaluate the precision of the developed UHPLC method. The relative standard deviation (RSD) was used to evaluate the variation range of the results. Intra- and interday repeatability was determined by six replicate analyses on sample Rh03, within one and two consecutive days, respectively. The stability of the analyzed components in the sample was tested by analyzing sample Rh03; the peak areas of the analyzed components at 0, 2, 4, 8, 24, and 48 h were recorded, and the variations in the content were expressed as RSD.

2.6.4 Accuracy of the analytical method

The accuracy of the quantitative analysis method was evaluated on the basis of the matrix spike recovery for each standard substance. A certain amount of sennoside A and sennoside B standards was added to sample Rh03 (0.1000–0.1100g); then, the mixtures were extracted as described in the sample solution preparation, and analyzed using the developed UHPLC method. The recovery of each added standard substance was calculated by the following formula: recovery% = $[(C_{mea} - C_{sam})/(C_{add})]\times 100$, where C_{mea} is the measured amount of the mixture of sample Rh03; C_{sam} and C_{add} represent the mean value of the detected sennoside A and sennoside B in sample Rh03 and added standard substances, respectively.

2.6.5 Robustness of the analytical method

To verify the robustness of the method, the final experimental conditions were altered and the resolution was examined. The flow rate, percentage of organic modifier, and column temperature were varied by (±) 3.0%, (±) 3.0%, and (±) 3 °C, respectively.

3 Results and discussion

3.1 Development of the UHPLC method

In this study, the target components were identified by comparing their retention times and UV spectra with those of the standard solution. The peak purity of the target components in these samples was verified by a photodiode array (PDA) detector. Peak purity tests showed that the chromatographic peak purity of each sennoside was not attributable to more than one component, indicating successful chromatographic separation. The results of the peak purity tests are shown in Fig. 4.

The resolution, symmetry factor, and theoretical plate number of sennoside B, which was relatively more difficult to separate from stronger polar compounds, showed a remarkable decrease when the flow rate was greater than 0.20 mL/min, although the retention time dramatically decreased. Considering the retention time,

symmetry factor, and system backpressure, a flow rate of 0.20 mL/min was preferred. The resolution, symmetry factor, and theoretical plate number obtained for sennoside A and sennoside B showed a slight improvement with an increase in column temperature from 22 °C to 30 °C but decreased above 30 °C. All the retention times decreased as the column temperature increased. The results showed that existed an optimal parameter as a turning point which column temperature was 30 °C and flow rate was 0.20 mL/min. Hence, considering the resolution, retention time and symmetry factor, a column temperature of 30 °C was preferred. At this column temperature and a flow rate of 0.20 mL/min, satisfactory chromatographic separation was achieved under a system backpressure of 12000 psi. The detailed results are shown in Fig. 3.

3.2 Validation of the UHPLC method

Linearity, sensitivity, precision, stability, and accuracy analyses were carried out to validate the developed UHPLC method. Linear regression equations of the two isomers were obtained at six concentration levels in triplicate, as summarized in Table 1. The linearity was expressed in terms of the correlation coefficient (R^2). The results showed excellent correlation between the peak areas and the concentration of UHPLC injection, with R^2 at 0.9997–0.9998. The LOD and LOQ of the two isomers are summarized in Table 1. The RSDs for inter- and intraday variation repeatability are summarized in Table 2. The overall inter- and intraday variations were not more than 1.77% and 1.04%, respectively. The variations in the concentrations of the analyzed components in sample Rh03 were 0.53%–1.76%, indicating that the analyzed components in the sample solutions were stable for at least 48 h. The calculated recoveries of the two isomers are summarized in Table 3. The recoveries of the investigated components ranged from 99.62% to 100.39%, and the RSDs were less than ±5.0%, thus indicating the good reliability and accuracy of the method. The test results showed satisfactory chromatographic resolutions under the conditions where the analytical parameters were varied.

3.3 Sample analysis

The developed UHPLC method was applied to the simultaneous quantitative of sennoside A and sennoside B in nine batches of rhubarb samples. The typical UHPLC-UV chromatograms of the standard reference solution, sample Rh03, and sample Rh08 are shown in Fig. 5. The amounts of individual sennoside in nine batches of samples were calculated, as shown in Table 4. More specifically, the individual amount of sennoside A ranged 0.52 mg/g in sample Rh09 to 10.42 mg/g in sample Rh03, i.e., the latter was more than 19 times the former. The amount of sennoside B ranged from 0.27 mg/g in sample Rh09 to 5.34 mg/g in sample Rh03; the latter was more than 18 times the former. The test results indicated remarkable content discrepancy between *Rheum tanguticum* Maxim. ex Balf. (samples Rh01–Rh05) and *Rheum palmatum* L. (samples Rh06–Rh09). Therefore, determination of the sennoside A and sennoside B content is crucial for the quality control of rhubarb.

Considering sennoside A and sennoside B as the major bioactive components in *Cassia angustifolia* Vahl and Paidu YangYan capsule, the developed analytical method was successfully applied to the two laxatives; the results indicated that this method is robust and has the potential for widespread practicability. The individual amount of sennoside B ranged from 0.17 mg/g in sample Ph02 to 0.26 mg/g in sample Ph01, indicating that the latter was present in more than 52.94% concentration as compared to the former. The amount of sennoside A ranged from 0.25 mg/g in sample Ph02 to 0.33 mg/g in sample Ph01, indicating that the latter was present in more than 32.00% concentration compared to the former. Detailed results are listed in Table 4, and the typical UHPLC-UV chromatograms are shown in Fig. 5.

3.4 Transformation from UHPLC to HPLC

Most of the drug inspection institutions only use high-performance-liquid chromatographs (HPLC); hence, the analysis parameters of UHPLC have been converted into those of HPLC. Using the Waters Acquity Column Calculator software 1.45 (Waters Corporation, USA), the binary gradient elution procedures of UHPLC transferred to those for HPLC, as follows: 0.00–10.00 min with 8–12% A, 10.01–

22.00 min with 12–13% A, 22.01–35.00 min with 13–15% A, 35.01–50.00 min with 15–17% A, 50.01–60.00 min with 17–21% A, and 60.01–70.00 min with 21–60% A. The Agilent 1260 HPLC instrument was equipped with a ZORBAX Eclipse plus C18 column (4.6×250 mm, 5μm), and the detector wavelength was set at 340 nm. The injection volume was 10.0μL, while the column temperature was maintained at 30 °C. The results indicated that the new chromatographic conditions can be well applied to HPLC. The typical HPLC-UV chromatograms are shown in Fig. 6.

3.5 Application

Although the total contents of the five anthraquinones in rhubarb were quantitatively determined for quality control in the Chinese Pharmacopoeia (15mg/g) [4], the contents of two isomers, sennoside A and sennoside B, in different rhubarb species varied remarkably, which influenced the quality. Therefore, controlling the concentrations of individual bioactive components (sennoside A and sennoside B) is recommended rather than modifying the total content of the five anthraquinones. Compared with the analytical method used for rhubarb in the literatures previously reported [22-24], the advantages of the proposed method including simple analysis conditions, excellent chromatographic resolution and shorter analysis time. Compared with the analytical method used for *Cassia angustifolia* Vahl in the Chinese pharmacopoeia [27], the present method is simple because organic reagent (tetrahydrofuran, THF) is avoided and mild conditions are sufficient as no ion pair reagent (tetraheptylammonium bromide) or acetate buffer solution is used. There is no quality criterion mentioned in the current Chinese Pharmacopoeia for Paidu YangYan Capsule, but the total sennoside A and sennoside B content should be no less than 0.40 mg/g.

4 Conclusions

Quality control of the *Rheum tanguticum* Maxim.ex Balf, *Rheum. palmatum* L., *Cassia angustifolia* Vahl and Paidu Yangyan capsule is crucial to ensure the safety and efficacy of widely used laxative herbs and diet drugs. This study will be beneficial for

the standardization of sennoside A and sennoside B in laxative herbs and related pharmaceutical preparations, and the developed UHPLC method will be a promising tool for improving the quality control of these laxative herbs and diet drugs.

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Conflict of interests

The authors declared no conflict of interests.

References

1 W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin, Springer, Berlin, Heidelberg, 1992, 855-875.

2 WHO Monographs on Selected Medicinal Plants, World Health Organization, Geneva, 1999, 231-240.

3 K.C. Huang. The Pharmacology of Chinese Herbs, CRC Press, Boca Raton, FL, 1993, 185-186.

4 Pharmacopoeia of PR China, The State Pharmacopoeia Commission of PR China, Chemical Industry Press, Beijing, 2010, 22-23.

5 European Pharmacopoeia, 7th ed., European Directorate for the Quality of Medicines, Council of Europe, Strasbourg, 2011, 2363-2364.

6 Japanese Pharmacopoeia, 16th ed., Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, Tokyo, 2011, 1723-1724.

7 Sanada H, Nihon Jinzo Gakkai Shi, 1996, 38, 379-387.

8 B. Wan, H.Y. Fu, J.T. Yin, F. Xu, Scand J Gastroenterol, 2014, 49, 1375-1384.

9 Y.L. Zhao, J.B. Wang, G.D. Zhou, L.M. Shan, X.H. Xiao, Basic Clin Pharmacol Toxicol, 2009, 104, 463-469.

10 H.M. Yu, Y.F. Liu, Y.F. Cheng, L, M. Hou, Lung Cancer, 2008, 59, 219-226.

11 Q.Huang, G.D. Lu, H.M. Shen, C.M. Chung, Choon Nam Ong, Medicinal Research Reviews, 2007, 27, 609-630.

12 W.J. Wu, N. Hu, Q.W. Zhang, P. Li, R. Yan, Y.T. Wang. Chemico-Biological Interactions, 2014, 219, 18-27.

13 SHARMA R A, BHARDWAJ R, SHARMA P, etal. Antimicrobial activity of sennosides from Cassia pumila Lamk [J]. J Med Plants Res, 2012, 6(19): 3591-3595.

14 ROUIBI A, SAIDI F, BOUTOUMI H. Extraction and characterization of some active

principles of the Senna (Cassia acutifolia) of the arid region of Tamanrasset and their antimicrobial effects [J]. Bullet Univ Agri, 2007, 63 (64): 400-404.

15 CHOI S B, KO B S, PARK S K, etal. Insulin sensitizing and α-glucoamylase inhibitory action of sennosides, rheins and rhaponticin in Rhei Rhizoma [J]. Life Sci, 2006, 78 (9): 934-942.

16 LEE H H, LEE Y H, YANG L L, etal. Effects of natural anthraquinones on neuronal survival in rat brain neuron [J]. J Cerebr Blood F Met, 2005, 25 (1):449.

17 Kon R, Ikarashi N, Nagoya C, Takayama T, Kusunoki Y, Ishii M, Ueda H, Ochiai W, Machida Y, Sugita K, Sugiyama K, J Ethnopharmacol, 2014, 152, 190-200.

18 Yamasaki K, Kawaguchi M, Tagami T, Sawabe Y, Takatori S.Takatori S, J Nat Med, 2010, 64, 126-132.

19 Satoh K, Seto T, Miyatake N, Hamano T, Shioda H, Onishi K, Yakugaku Zasshi, 1999, 119, 88-92.

20 Morinaga O, Uto T, Sakamoto S, Tanaka H, Shoyama Y, Fitoterapia, 2009, 80, 28-31.

21 Morinaga O, Uto T, Sakamoto S, Putalun W, Lhieochaiphant S, Tanaka H, Shoyama Y, Phytochem Anal, 2009, 20, 154-158.

22 Gao Xiao-yan, Lu jian-qiu, HPLC-DAD simultaneous determination of 7 anthraquinone derivatives in Rhubarb. Chin J Pharm Anal, 2010, 30, 1636-1641.

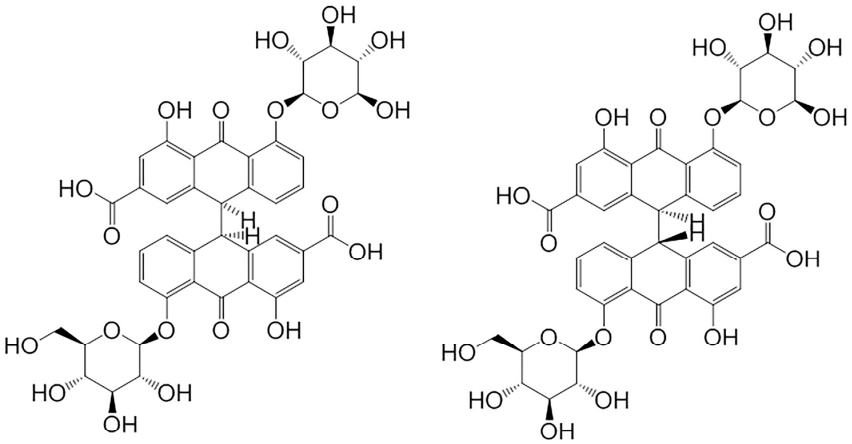
23 Cheng Xiao-li, Wei Sheng-li, Liu Chun-sheng, etal. RP-HPLC-DAD Method for Simultaneous Determination of Nine Active Constituents in Rhubarb. Chinese Journal of Experimental Traditional Medical Formulae, 2013, 19, 99-102.

24 Lin CC, Wu CI, Lin TC, Sheu SJ. Determination of 19 rhubarb constituents by high performance liquid chromatography-ultraviolet-mass spectrometry. J Sep Sci, 2006, 29, 2584-2593.

25 Pharmacopoeia of PR China, The State Pharmacopoeia Commission of PR China, Chemical Industry Press, Beijing, 2010, 130-131.

26 ICH Topic Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH /281/95). Step 4. Consensus Guideline, the European Agency for the Evaluation of Medicinal Products, 6 Nov 1996.

27 Pharmacopoeia of PR China, The State Pharmacopoeia Commission of PR China, Chemical Industry Press, Beijing, 2010, 326-327.



sennoside A sennoside B

Figure. 1. The chemical structures of sennoside A and sennoside B

Figure. 2. Typical laxative herbs and diet drugs: (A) *Rheum.tanguticum* Maxim.ex Balf. (B) *Cassia angustifolia* Vahl. (C) Paidu Yangyan capsule

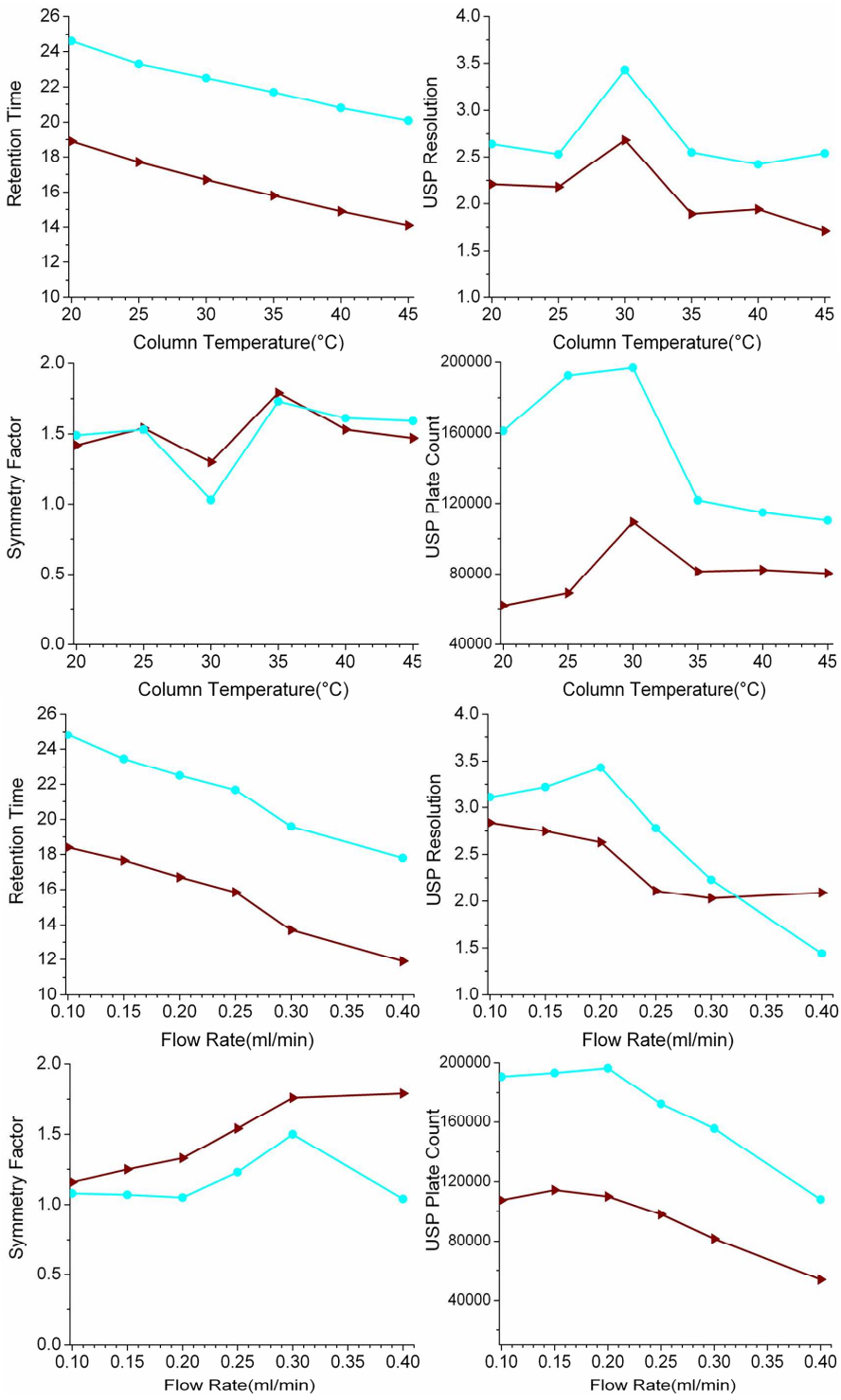


Figure. 3. Influence of column temperature and flow rate on UHPLC chromatographic parameters of sennoside A (—●—) and sennoside B (—►—)

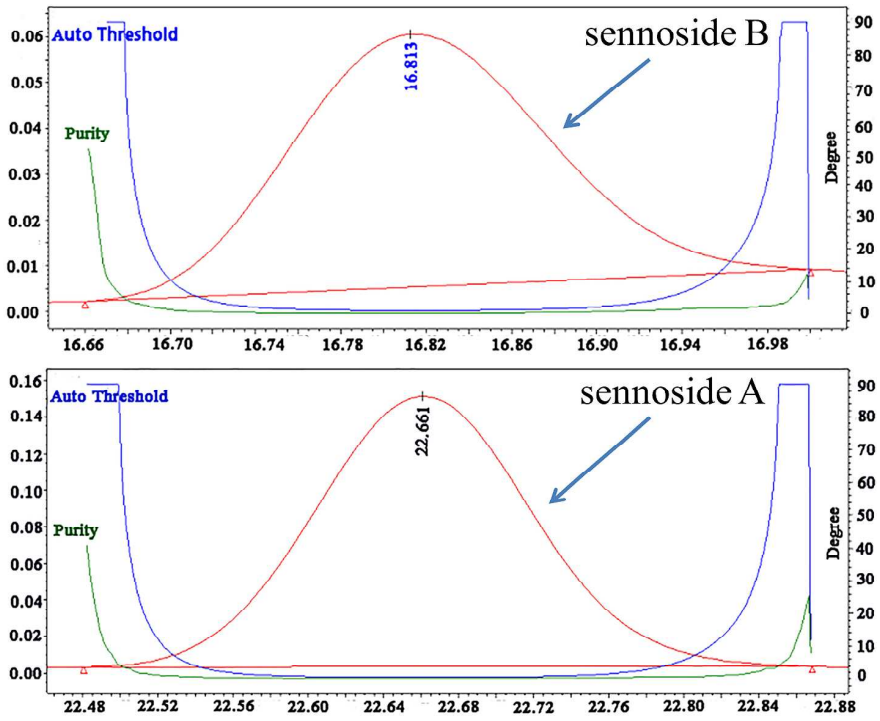


Figure. 4 Chromatographic peak purity of sennoside B and sennoside A

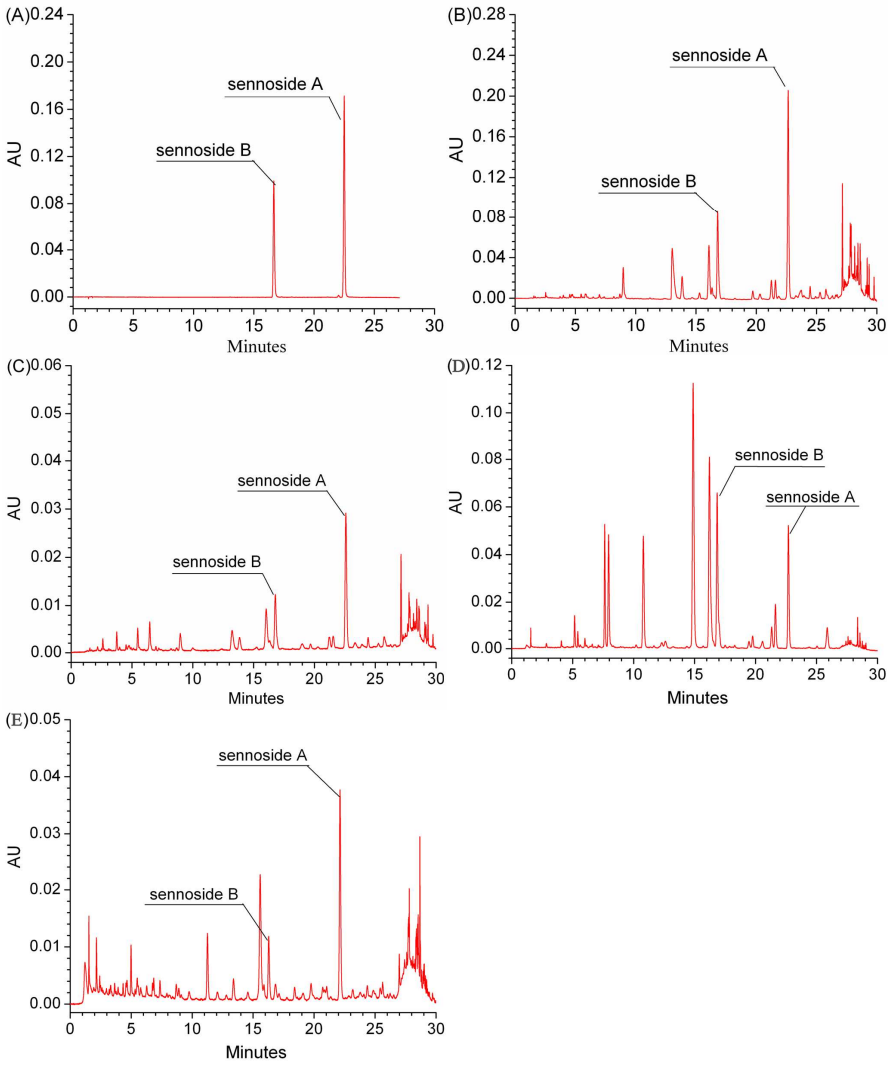


Figure. 5 Typical UHPLC-UV chromatograms of (A) Standard substance solution, (B) Sample Rh08: *Rheum palmatum* L, (C) Sample Rh03: *Rheum tanguticum* Maxim.ex Balf, (D) Sample Ca01: *Cassia angustifolia* Vahl, (E) Sample Ph01: Paidu Yangyan capsule

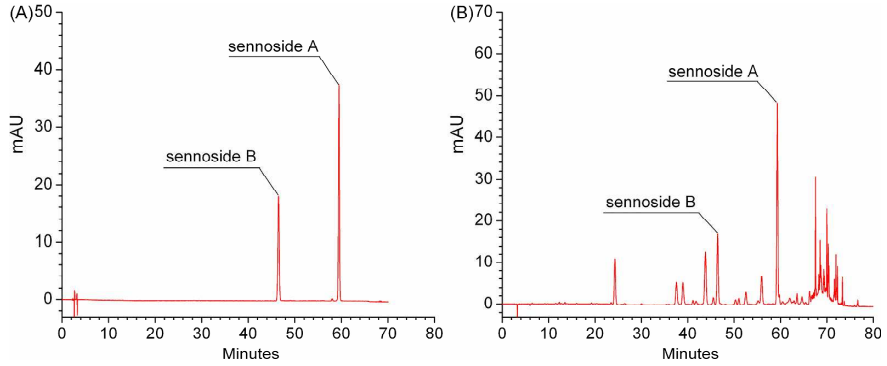


Figure. 6 Typical HPLC-UV chromatograms of (A) Standard substance solution (B) Sample Rh03: *Rheum tanguticum* Maxim.ex Balf.

Table 1

Linearity and sensitivity of the UHPLC analysis

Components	RT(min)	linear regression equation	R^2	Linear range (μg/mL)	LOD [b] (μg/mL)	LOQ [c] (μg/mL)
sennoside B	16.813	$y=4.1\times10^6x-2142.46$ [a]	0.9998	0.46-46.00	0.05	0.20
sennoside A	22.661	$y=4.1\times10^6x-3079.67$	0.9997	0.74-74.00	0.07	0.24

[a] y is peak area, x is analyte concentration (μg/mL).

[b] LOD: Limit of detection (S/N=3.0).

[c] LOQ: Limit of quantification (S/N=10.0)

Table 2

Precision test of the UHPLC analysis

Components	intra-day precision (mean ± SD, n=6)		inter-day precision (mean ± SD, n=6)	
	Content (mg/g)	RSD [a] (%)	Content (mg/g)	RSD (%)
sennoside B	5.34±0.03	1.04	5.33±0.04	1.77
sennoside A	10.42±0.05	0.93	10.40±0.05	1.32

[a] RSD(%)=100×SD/mean

Table 3

Recovery of the UHPLC analysis (mean ± SD, n=6)

Components	Amount detected in sample Rh03 (C_{sam}, mg)	added amount (C_{add}, mg)	Amount detected in the mixture of sample Rh03 and added component (C_{det}, mg)	Recovery (%)
sennoside B	2.65± 0.02	2.62± 0.06	5.26 ± 0.03	99.62 ± 1.14
sennoside A	5.17± 0.01	5.11± 0.07	10.30 ± 0.05	100.39 ± 0.98

Table 4

Analyzing the results of different samples (mg/g, mean ± SD, n = 3)

sample number	sampling site	sennoside B (mg/g)	sennoside A (mg/g)	Total content (mg/g)
Rheum.tanguticum Maxim.ex Balf.				
Rh 01	Sichuan, Zoige county	4.67± 0.09	9.96± 0.04	14.63± 0.13
Rh 02	Sichuan, Zoige county	4.06± 0.06	9.32± 0.05	13.38± 0.11
Rh 03	Sichuan, Zoige county	5.34± 0.04	10.42± 0.03	15.76± 0.07
Rh 04	Sichuan, Zoige county	4.86± 0.05	10.26± 0.04	15.12± 0.09
Rh 05	Sichuan, Taopin county	5.01± 0.03	9.96± 0.05	14.97± 0.08
Rheum.palmatum L.				
Rh 06	Gansu, Qingyang city	--[a]	--	--
Rh 07	Gansu, Tianshui city	--	--	--
Rh 08	Gansu, Li county	0.27± 0.04	0.61± 0.03	0.88± 0.07
Rh 09	Gansu, Zuanglang county	0.35± 0.02	0.52± 0.01	0.87± 0.03
Cassia angustifolia Vahl.				
Ca 01	Yunnan, Zhaotong county	5.32± 0.05	6.01± 0.02	11.33± 0.07
Ca 02	Yunnan, Zhaotong county	5.18± 0.04	6.14± 0.05	11.32± 0.09
Diet drugs (Paidu Yangyan capsule)				
Ph 01	Beijing city	0.26± 0.07	0.33± 0.04	0.59± 0.11
Ph 02	Beijing city	0.17± 0.06	0.25± 0.03	0.42± 0.09

--[a] ＜LOQ: Limit of quantification was not reached.

The influence of analytical parameters on the chromatographic separation efficiency were systematically investigated, and the optimized parameters were obtained. A simple UHPLC method was applied to simultaneous quantification of sennoside A and sennoside B in laxatives.