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Article

Simultaneous Quantification of Three Chemical Types Bioactive Compounds in Radix Isatidis and Its Relevant Pharmaceutical Dosage Forms by HPLC-DAD

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A simple and rapid high performance liquid chromatography coupled with diode array detector (HPLC-DAD) method was developed for simultaneous quantification of three chemical types of bioactive compounds including an alkaloid (epigoitrin), six nucleosides (uracil, cytidine, hypoxanthine, uridine, guanosine and adenosine), and four phenylpropanoids (syringin, clemastanin B, indigoticoside A and isolariciresinol) in Radix Isatidis and its relevant pharmaceutical dosage forms. The developed method was successfully applied to analyze 32 batches of samples with good linearity (R^2, 0.9966-0.9999), precisions (relative standard deviation (RSD), 0.24-4.61%), repeatability (RSD, 0.39-4.93%), stability (RSD, 1.18-4.29%) and recovery (range, 95.4-104.6%) of the eleven compounds. The limits of detection (LOD) and limits of quantification (LOQ) of the method ranged 0.0383-0.1744 and 0.1550-0.5467 μg mL^{-1}, respectively. The results indicated that the newly established HPLC-DAD method was sensitive, precise, accurate and reproducible. It could be applied to the quality control of Radix Isatidis and its relevant pharmaceutical dosage forms.

Keywords: Radix Isatidis, HPLC-DAD, alkaloid, phenylpropanoid, nucleosides

Introduction

The well-known traditional Chinese medicine Radix Isatidis (common name: Banlangen) is the dried root of the plant *Isatis indigotica* Fort. It belongs to the Cruciferae family and widely distributed in the north and central of China.[1,2] Radix Isatidis are one of the most frequently used traditional Chinese medicine for treatment of influenza, bacterial infection, fever and epidemic hepatitis.[3] Because of the unique pharmacological activities, Radix Isatidis has been developed into various dosage forms such as granules, tablets, capsules and altapharmas.

Chemical studies showed that Radix Isatidis contains various compounds such as alkaloids, phenylpropanoids, nucleosides and organic acids.[4] Epigoitrin as an alkaloid was used as a marker compound of Radix Isatidis in the 2015 edition of the Chinese Pharmacopoeia.[1] Uracil, cytidine, hypoxanthine, uridine, guanosine and adenosine are the main nucleosides in Radix Isatidis.[5] The latest studies showed that nucleosides were important bioactive compounds related to anti-inflammatory, immunoregulation, antitumor and antivirus activities.[6-9] Clemastanin B, indigoticoside A, isolariciresinol and syringin are the major phenylpropanoids isolated from Radix Isatidis.[10-12] In our previous studies,[13] we found that clemastanin B and syringin had strong inhibitory effects on influenza FM1. Clemastanin B was found to inhibit different subtypes of human (H1N1, including swine-origin H1N1; H3N2 and influenza B) and avian influenza viruses (H6N2, H7N3, H9N2) at different magnitudes of activity.[14] From the latest studies, indigoticoside A could inhibit herpes simplex virus (HSV) and influenza A virus.[15]

Qualitative and quantitative analysis of major bioactive compounds are very important for the quality control of Radix Isatidis. It is well known that traditional Chinese medicine (TCM) exerts its activities through multiple components at multiple targets, and the determination of multiple components has also been well accepted for the quality control of TCM.[16] Many analytical methods including high performance liquid chromatography (HPLC),[17,18] capillary zone electrophoresis (CZE)[19] and ultra-high performance liquid chromatography coupled

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with triple quadrupole mass have been developed for the determination of one of the three chemical types bioactive compounds (alkaloids, nucleosides and phenylpropanoids) in Radix Isatidis or its pharmaceutical dosage forms.[5,20] However, to the best of our knowledge, no method has been developed for simultaneous analysis of alkaloids, phenylpropanoids, nucleosides in Radix Isatidis or its relevant pharmaceutical dosage forms.

In order to control the quality of the herbs and its pharmaceutical dosage forms from different regions or manufacturers, it is very necessary to develop a simple and effective HPLC method for simultaneously quantifying these three different chemical types of bioactive compounds.

In our research, a simple and reliable HPLC coupled with diode array detector (DAD) method has been developed for simultaneous determination of these eleven main bioactive compounds (Figure 1) in Radix Isatidis and its pharmaceutical dosage forms. To our knowledge, this is the first report on the simultaneous quantification of alkaloid, nucleosides and phenylpropanoids in Radix Isatidis and its pharmaceutical dosage forms. We expected that this HPLC-DAD method would be applied to monitor these bioactive compounds for the quality control of Radix Isatidis and its pharmaceutical dosage forms.

Experimental

Materials and chemicals

Raw Radix Isatidis samples were purchased from a medical market in Tongling City of Anhui Province (China). The botanical origins were identified by Professor Jianwei Chen, Nanjing University of Chinese Medicine. The voucher specimens were deposited at the Herbarium in Nanjing University of Chinese Medicine (Nanjing, China). Twenty batches of pharmaceutical dosage forms of Radix Isatidis produced by different manufacturers were purchased from local drug stores (Table 1). Multi-herb Radix Isatidis granule (S28, S29) was composed of Radix Isatidis and Folium Isatidis according to the proportion of 2:3. Radix Isatidis tea (S30) only contained Radix Isatidis. The pharmaceutical dosage forms of Radix Isatidis were prepared according to the methods of the Pharmacopoeia of the People's Republic of China.[21]

Uracil, cytidine, hypoxanthine, uridine, guanosine, epigoitrin and adenosine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Syringin, clemastanin B, indigoticoside A and isolariciresinol were separated and purified in our laboratory. The purity of all compounds was above 98% which was determined by HPLC-DAD evaporative light-scattering detector (ELSD). The structures (Figure 1) were elucidated by their ultraviolet (UV), infrared (IR), mass spectrometry (MS), ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and 2D NMR data.[10-12] Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA). The solvents and chemicals used were of analytical grade or HPLC grade.

Preparation of standard solutions

The standard stocks were accurately weighed and dissolved in water/methanol (20:80, v/v). Stock standard solutions containing uracil (1.64 mg mL^{-1}), cytidine (1.56 mg mL^{-1}), hypoxanthine (1.57 mg mL^{-1}), uridine (1.32 mg mL^{-1}), guanosine (1.39 mg mL^{-1}), epigoitrin (1.44 mg mL^{-1}), adenosine (1.25 mg mL^{-1}), syringin (1.15 mg mL^{-1}), clemastanin B (1.34 mg mL^{-1}), indigoticoside A (1.36 mg mL^{-1}) and isolariciresinol (1.25 mg mL^{-1}). A series of standard working solutions for the calibration curves were obtained by serial dilution of the stock solutions. All stock solutions were stable for 1 month stored in a refrigerator at 4 °C.

Sample preparation

The Radix Isatidis and its relevant pharmaceutical dosage forms were powdered to a homogeneous size, sieved through a No. 20 mesh, and further dried at 60 °C until a constant weight was obtained. The extraction was performed under the optimized conditions: the dried powder of samples (1.0 g) were accurately weighed and placed into 50 mL conical flasks, and then mixed with 20 mL 80% methanol, weighed accurately, ultrasonic extraction for 1 h at 40 °C (40 kHz, 500 W) (Kunshan Ultrasound Instrument Company, China). Finally, the extracted solution was adjusted to the original weight by adding 80% methanol. After centrifugation at 12,000 × g for 10 min, the supernatant was collected. The supernatant was filtrated by 0.45 μm microfiltration membrane prior to injection into HPLC system.

HPLC analysis

All chromatographic measurements were performed on a Waters 2695 liquid chromatography system (Waters, USA), equipped with a vacuum degasser, a quaternary low-pressure mixing pump, an autosampler, a thermostated column compartment and a Waters 2998 photodiode array detector. The chromatography column was a Waters Symmetry C18 column (250 × 4.6 mm, 5 μm). Gradient elution with (A) water and (B) methanol was 0-12 min,

Table 1. Summary of the tested samples of Radix Isatidis and its relevant pharmaceutical dosage forms

No.	Raw herbs and its dosage forms	Regions collected or manufacturer	Batch No.
S1	Radix Isatidis	Xinxiang, Hennan Province	120224
S2	Radix Isatidis	Chifeng, Neimenggu Province	120307
S3	Radix Isatidis	Heixi, Gansu Province	120318
S4	Radix Isatidis	Gansu Province	120216
S5	Radix Isatidis	Shanxi, Province	120208
S6	Radix Isatidis	Daqing, Heilongjiang Province	120324
S7	Radix Isatidis	Anhui Provinve	120217
S8	Radix Isatidis	Anhui Provinve	120207
S9	Radix Isatidis	Fengning, Henbei Province	120223
S10	Radix Isatidis	Mianyang, Sichuan Province	120302
S11	Radix Isatidis	Tongling, Anhui Province	111209
S12	Radix Isatidis	Nantong, Jiangsu Province	120615
S13	Radix Isatidis buccal tablet	XinChunDu Bio-pharm Co., Ltd.	130501
S14	Radix Isatidis buccal tablet	XinChunDu Bio-pharm Co., Ltd.	121201
S15	Radix Isatidis granule	Guangxi Medicinal Botanical Garden pharmaceutical factory	130407
S16	Radix Isatidis granule	Kunming Shenghuo Pharmaceutical Group Co., Ltd.	20130417
S17	Radix Isatidis granule	Nanning Weiwei Pharmacy Co., Ltd.	130430
S18	Radix Isatidis granule	Jiilin Jinbao Pharmaceutical Co., Ltd.	130801
S19	Radix Isatidis granule	Xinxiang Zuojinming Pharmaceutical Co., Ltd.	20130730
S20	Radix Isatidis granule	Jiangxi Xincheng Pharmaceutical Co., Ltd.	20130404
S21	Radix Isatidis granule	Guangxi Qianzhen Pharmaceutical Co., Ltd.	130417
S22	Radix Isatidis granule	Guangxi Tiantianle Pharmaceutical Co., Ltd.	121203
S23	Radix Isatidis granule	Hubei Wudang Jinding Pharmaceutical Co., Ltd.	130601
S24	Radix Isatidis granule	Shandong Kongfu Pharmaceutical Co., Ltd.	20130413
S25	Radix Isatidis granule	Shandong Sanjiu Pharmaceutical Co., Ltd.	130471
S26	Radix Isatidis granule	Jiangxi Yaodu Zhangshu Pharmaceutical Co., Ltd.	120717
S27	Radix Isatidis granule	Yunnanbaiyao Group Co., Ltd.	201012
S28	multi-herb Radix Isatidis granule	Hebei Wansui Pharmaceutical Co., Ltd.	130508
S29	multi-herb Radix Isatidis granule	Nanjing Tongrentang Pharmaceutical Co., Ltd.	130313
S30	Radix Isatidis tea	Guangdong Heping Pharmaceutical Co., Ltd.	130101
S31	Radix Isatidis altapharma	TASLY Pharmaceutical Group Co., Ltd.	20130301
S32	Radix Isatidis capsule	Hebei Longhai Pharmaceutical Co., Ltd.	130401

3-10% B; 12-17 min, 10-20% B; 17-25 min, 20% B; 25-35 min, 20-30% B; 35-55 min, 30-40% B. The flow rate was set at 1.0 mL min^{-1} and the column temperature was 30 °C. The injection volume was 10 μL. The UV detection wavelengths were set at 254 and 280 nm. The identification of the investigated compounds was confirmed by comparison of their retention times and UV spectra with those standards under the same conditions.

Validation of the developed method

Calibration curves, limits of detection (LOD) and quantification (LOQ)

Linear regression analysis for each of the eleven compounds was performed by the external standard method. Each calibration curve was analyzed with seven different concentrations using the same HPLC condition as described above in triplicate. The calibration curves were calculated based on linear regression analysis of the integrated peak areas (y) *versus* concentrations (x, μg mL^{-1}). The regression equation was calculated in the form of $y = ax + b$. The limits of detection (LOD) and quantification (LOQ) for each analyte under present chromatographic conditions were determined at the signal-to-noise ratio (S/N) for each compound of about 3 and 10, respectively.

Precision, repeatability and stability

The intra- and inter-day precisions were investigated by determining a known concentration working standard solution in six replicates within one day and three consecutive days, respectively. To confirm the repeatability, six different working solutions prepared from the same

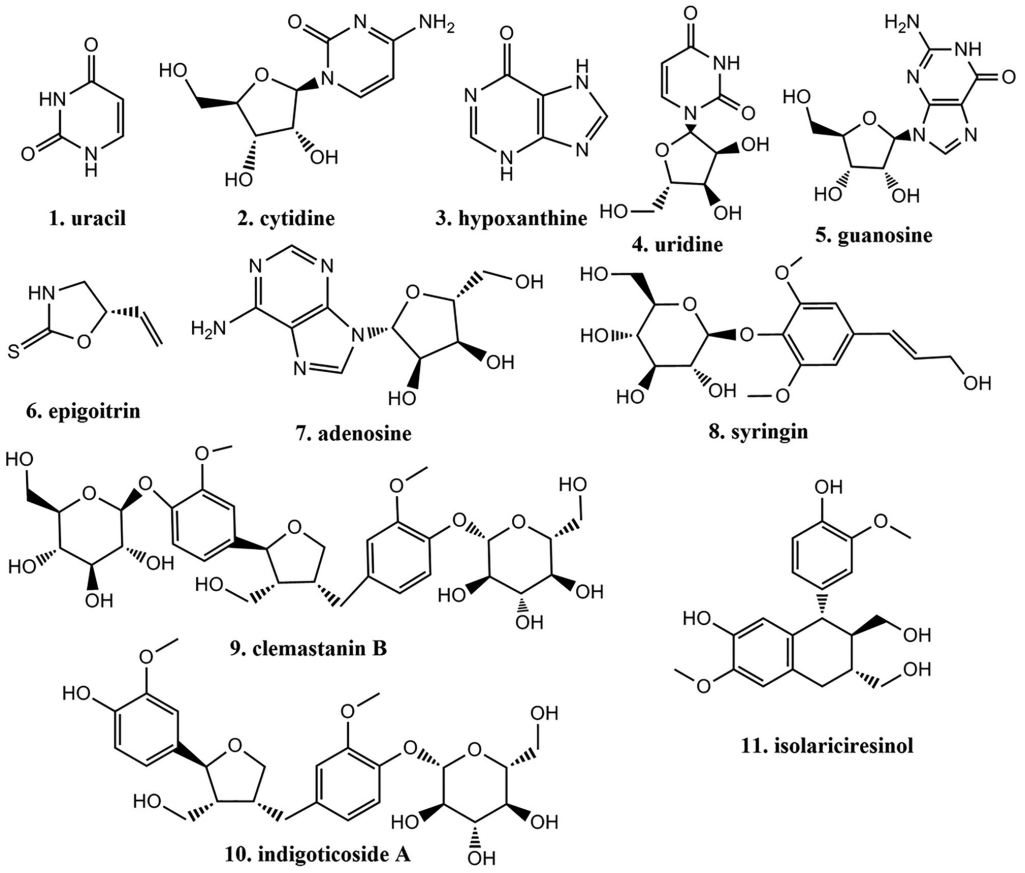

Figure 1. The chemical structures of the eleven analytes in Radix Isatidis.

sample were analyzed. For stability test, the same sample solution was analyzed at different time points (0, 2, 4, 6, 8, 12, 24 and 48 h). The relative standard deviation (RSD) was taken as a measure of precision, repeatability and stability.

Recovery

The recovery was used to evaluate accuracy of the method. Recovery was performed by adding the certain concentration standard stock solutions at low, medium and high levels (80, 100 and 120%) into Radix Isatidis samples (S2, Neimenggu Province; 0.5 g), The mixture was extracted and analyzed by the method mentioned above. Three replicates were performed for the test. The quantity of each analyte was subsequently obtained from the corresponding calibration curve. The recovery was calculated as follow:

$$\text{Recovery (\%)} = \frac{\text{(amount found – original amount)}}{\text{amount spiked}} \times 100 \quad (1)$$

Results and Discussion

Optimization of the extraction conditions

In order to obtain satisfactory extraction efficiency, variables involved in the procedure such as extraction method, extraction solvent, extraction solvent volume, extraction time, extraction temperature were optimized. There was no significant difference between ultrasonic extraction and refluxing extraction, but ultrasonic extraction was more convenient and was applied in the experiments. It was found that 80% methanol was the most efficient extraction solvent among the tested different concentrations of methanol (0, 10, 30, 50, 80 and 100%) (Figure 2A). Different solvent volumes were examined, and an extraction efficiency of 20 mL solvent volume was equivalent to that of 30 and 40 mL (Figure 2B). In addition, the efficiencies of ultrasonic extraction were measured in different extraction times (10, 30, 45, 60, 90, and 120 min). The results demonstrated that the extraction efficiency at 60 min had no significant difference with that at 90 min, or even at 120 min (Figure 2C). In our study, extraction was carried out at six different temperatures (20, 30, 40, 50, 60 and 70 °C). The extraction yield of the eleven compounds increased with the increase of extraction temperature from 20 to 40 °C. Then, the yield began to decrease as the temperature increased from 40 to 70 °C (Figure 2D). Therefore, the optimal sample extraction conditions were prepared by ultrasonic extraction with 20 mL of 80% methanol for 60 min at 40 °C.

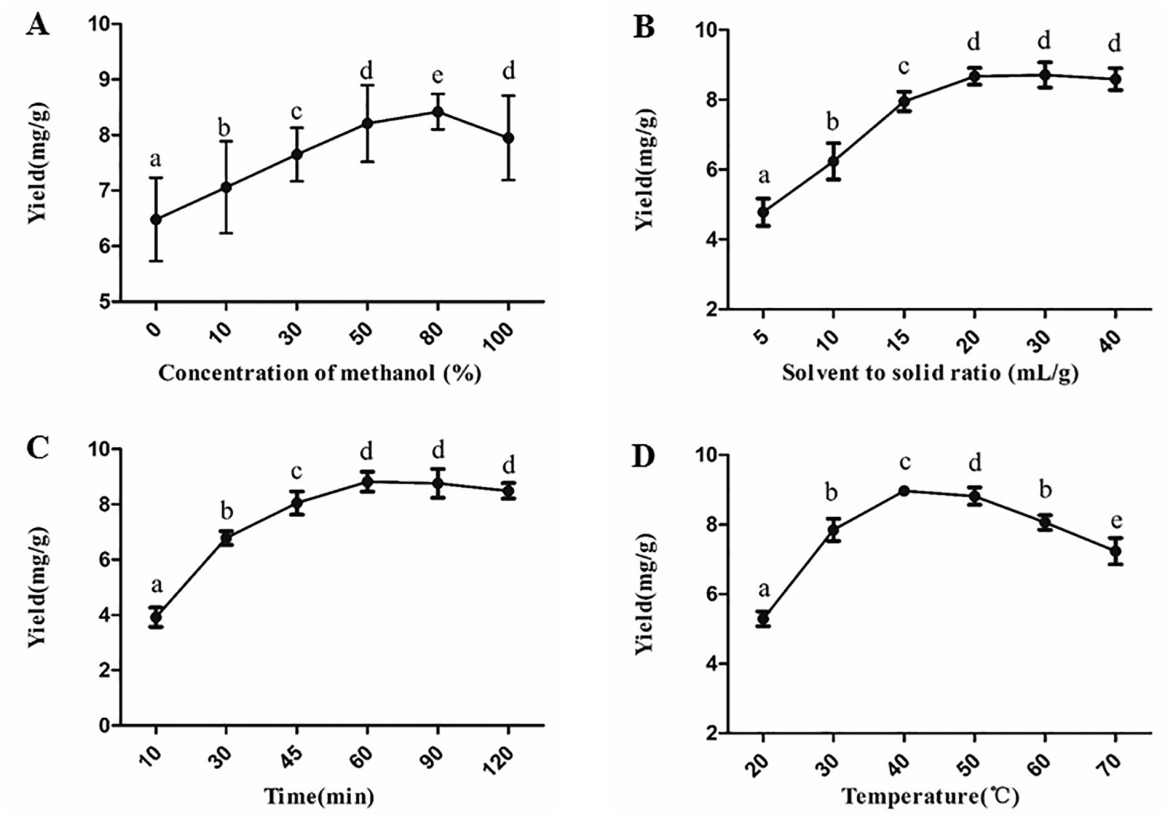

Figure 2. Effects of different extraction conditions on the total yield of eleven compounds. (A) extraction solvent concentration; (B) extraction ratio of liquid/solid; (C) extraction time; (D) extraction temperature. The data with different lowercase letters are significantly different ($p < 0.05$).

Optimization of HPLC conditions

To confirm the detection wavelengths, wavelengths from 200 to 400 nm were scanned for the eleven investigated compounds. On the basis of ultraviolet (UV) absorption of the eleven compounds, the alkaloid and nucleosides showed better UV absorption at 254 nm, but the four phenylpropanoids was at 280 nm. Therefore, the detection wavelength was set at 254 nm for uracil, cytidine, hypoxanthine, uridine, guanosine, epigoitrin and adenosine, and at 280 nm for syringin, clemastanin B, indigoticoside A and isolariciresinol. Considering polarity of the eleven compounds, the gradient elution was used to achieve a better separation. In our previous research, it was found that the nucleosides were difficult to obtain good separation in high proportion of organic solvents because of their high and similar polarity.[5] So the proportion of organic phase slowly increased from 3 to 10% in 12 min. As shown in Figure 3, the nucleosides were completely separated with good peak symmetry.

Different mobile phases (methanol-water, acetonitrile-water and methanol-acid aqueous solution) were tested and compared in order to obtain good resolution of all analytes. It was shown that the optimum separation was achieved by methanol-water. The addition of acid in mobile phase was found to have no significant improvement on the chromatographic behavior. Instead, the addition of acid could cause damage to chromatographic columns. Finally, the methanol-water system was chosen as the mobile phases of our experiments. Three different brands of chromatographic columns were investigated for separation, such as Hanbon Hedera C18 (250 × 4.6 mm, 5 μm), Waters Symmetry C18 (250 × 4.6 mm, 5 μm) and Agilent Lichrospher C18 (250 × 4.6 mm, 5 μm). As a result, the Waters Symmetry C18 column was selected because it provided the best resolution and peak shape. The representative chromatograms of the eleven standard analytes and samples were shown in Figure 3. The eleven standard compounds were well separated within 50 min and other compounds in the samples did not interfere with the analysis of the eleven analytes.

Validation of the developed method

Calibration curves, limits of detection and quantification

As shown in Table 2, a good linearity ($R^2 > 0.9966$) was observed in calibration curves over the concentration ranges investigated. LODs of eleven analytes ranged from 0.0383 to 0.1744 μg mL^{-1} and LOQs varied from 0.1550 to 0.5467 μg mL^{-1}.

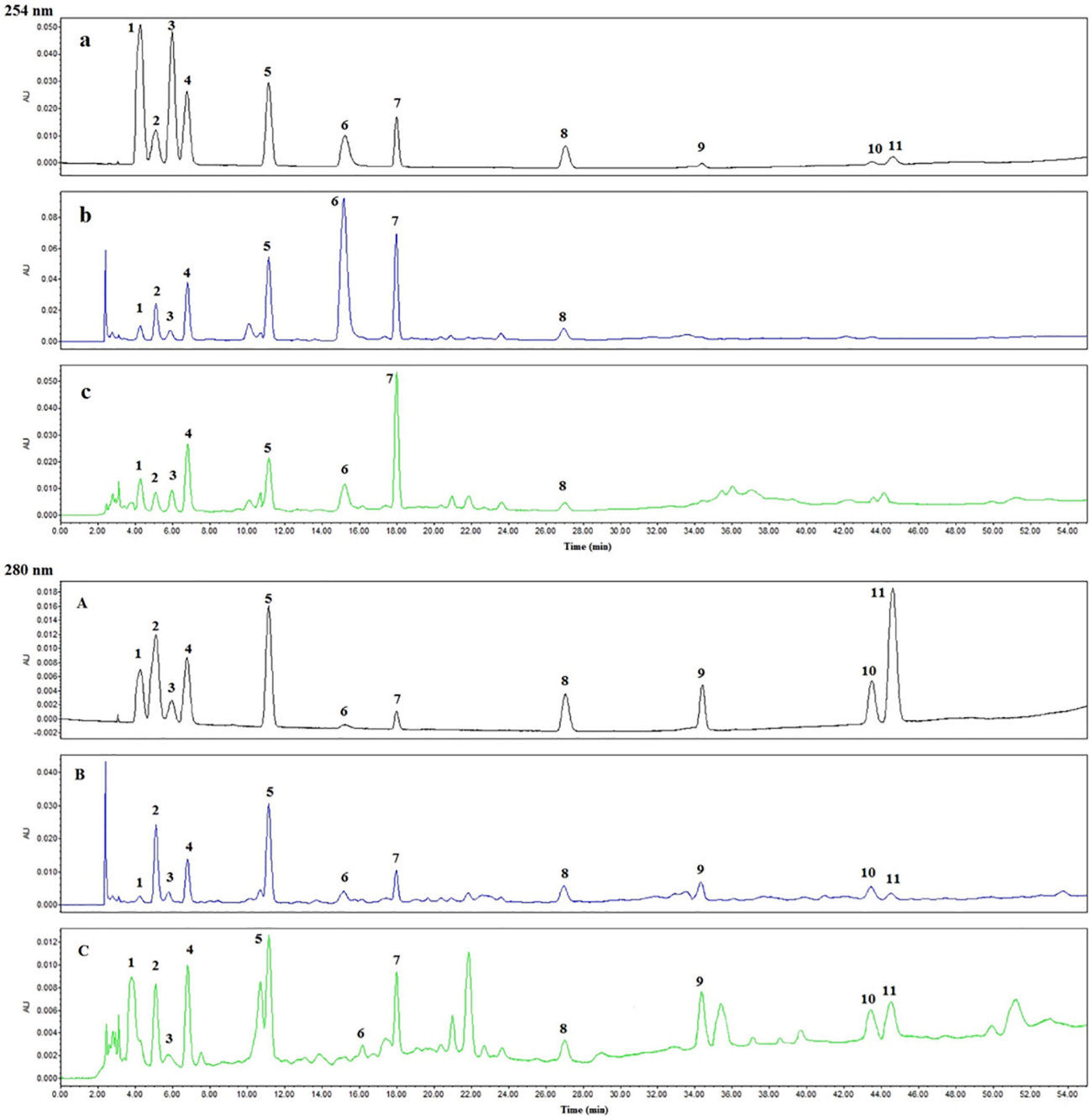

Figure 3. HPLC chromatograms of mixed standards (a, A); Radix Isatidis sample (b, B) and Radix Isatidis granule sample (c, C). (a, b, c) HPLC chromatograms monitored at 254 nm; (A, B, C) HPLC chromatograms monitored at 280 nm. (**1**) Uracil; (**2**) cytidine; (**3**) hypoxanthine; (**4**) uridine; (**5**) guanosine; (**6**) epigoitrin; (**7**) adenosine; (**8**) syringin; (**9**) clemastanin B; (**10**) indigoticoside A and (**11**) isolariciresinol.

Precision, repeatability, stability and accuracy

The results of precision, repeatability and stability were listed in Table 3. The intra- or inter-day precisions calculated as RSD were within the range of 0.27-3.92% or 2.15-4.61% for the eleven analytes. Six independent samples of Radix Isatidis (S2) were analyzed in parallel by the established method for the evaluation of repeatability. The RSD values of eleven compounds were within the range from 0.39 to 4.93%, which revealed good repeatability of the method. Besides, the results of the stability study demonstrated that the sample was very stable over two days with RSDs less than 4.29% for the eleven analytes.

As shown in Table 4, the mean recovery rates of all the components varied from 95.4 to 104.6%, and the RSDs were less than 4.6%, which indicated that the developed method manifested good accuracy for determination of all the constituents.

Table 2. Regression equations, LODs and LOQs for eleven markers

Analyte	RT / min	Regression equation	R^2	Linear range / (μg mL^{-1})	LOD / (μg mL^{-1})	LOQ / (μg mL^{-1})
1	4.29	y = 45687x – 72716	0.9966	1.3-328.0	0.1367	0.5467
2	5.19	y = 13275x – 16858	0.9987	0.8-195.0	0.0520	0.1560
3	6.07	y = 36983x – 31723	0.9999	0.6-157.0	0.1744	0.5233
4	6.91	y = 26782x – 30428	0.9994	1.1-264.0	0.0413	0.1650
5	11.31	y = 25200x – 23861	0.9999	1.1-278.0	0.0397	0.1390
6	15.53	y = 13158x – 28076	0.9998	1.4-360.0	0.1601	0.4802
7	18.08	y = 12315x – 10992	0.9998	0.8-208.3	0.0521	0.2083
8	27.35	y = 10661x – 11908	0.9991	0.8-191.7	0.0383	0.1150
9	34.76	y = 5477.7x – 6110	0.9999	1.1-268.0	0.0419	0.1675
10	43.91	y = 6404.4x – 10273	0.9972	1.1-272.0	0.0907	0.2267
11	45.01	y = 20957x – 27180	0.9999	1.0-250.0	0.1389	0.4167

RT: retention time; LOD: limit of detection; LOQ: limit of quantification.

Table 3. Precision, repeatability and stability of eleven markers

Analyte	Precision (RSD / %)		Repeatability RSD / % (n = 6)	Stability (48 h) (n = 8, RSD / %)
	Intra-day (n = 6)	Inter-day (n = 6)		
1	2.54	3.59	1.92	1.18
2	0.52	3.99	1.53	4.12
3	0.36	4.26	2.75	4.09
4	0.56	4.37	2.25	2.40
5	0.24	4.28	1.21	4.29
6	3.92	4.61	4.93	4.11
7	0.27	4.43	0.39	3.59
8	0.43	3.80	4.55	3.78
9	1.85	2.15	2.01	2.00
10	1.20	4.36	3.01	3.45
11	2.01	3.31	3.61	4.02

Application

The optimized HPLC-DAD method was applied to simultaneously determine the eleven major bioactive compounds in Radix Isatidis and its pharmaceutical dosage forms from different provinces or pharmaceutical factories. The mean contents of uracil, cytidine, hypoxanthine, uridine, guanosine, epigoitrin, adenosine, syringin, clemastanin B, indigoticoside A and isolariciresinol in Radix Isatidis and its relevant pharmaceutical dosage forms from three parallel determinations were revealed in Table 5. As shown in Table 5, there were significant differences in the content of the target analytes in different Radix Isatidis samples, which could be due to environmental or climate factors such as altitude, humidity, light conditions and temperature and so on. Among the eleven compounds, the mean content of epigoitrin was the highest (1286.28 μg g^{-1}). Because of the high content, epigoitrin was selected as the unique marker of Radix Isatidis in the 2015 edition of the Chinese Pharmacopoeia.[21]

Of all the raw herbs analyzed, the total content of the eleven compounds in Radix Isatidis of Neimenggu Province (S2) was the highest (8953.70 μg g^{-1}), followed by Hennan Province (S1), Gansu Province (S3) and Shanxi Province (S5). The total content of these bioactive compounds in the pharmaceutical dosage forms of Radix Isatidis was lower than the raw Radix Isatidis samples. The low concentration of bioactive compounds in these pharmaceutical dosage forms may be due to the inefficient extraction process, poor herb quality, decomposition of compounds during the extraction procedure, or the excessive dilution in the preparation of the final product.[22] In addition, it suggested that the variations of the contents of eleven compounds were apparent in the pharmaceutical dosage forms of Radix Isatidis from different manufacturers. The variation of the contents was mainly derived from the different quality of the raw material and the place of production.[23]

Conclusions

The major problems faced by the herbal industry are the inconsistency and adulteration due to several reasons. It is necessary to develop quality control methods to authenticate the sample and its dosages form. This research describes a simple and rapid high performance liquid chromatography coupled with diode array detector (HPLC-DAD) method for simultaneous quantification of three chemical types of bioactive components in Radix Isatidis and its relevant pharmaceutical dosage forms. The proposed HPLC method has been applied successfully to simultaneous

Table 4. Recoveries of eleven markers (n = 3)

Analyte	Original / mg	Spiked / mg	Found / mg	Mean recovery / %	RSD / %
Uracil	0.037	0.026	0.064	104.5	4.1
		0.033	0.069	99.9	2.2
		0.039	0.076	99.3	2.8
Cytidine	0.323	0.250	0.579	102.7	0.8
		0.312	0.636	100.2	1.4
		0.374	0.702	101.2	1.7
Hypoxanthine	0.023	0.019	0.042	97.1	3.7
		0.024	0.048	104.6	2.1
		0.028	0.050	95.4	4.1
Uridine	0.260	0.211	0.473	100.9	1.4
		0.264	0.521	98.7	0.6
		0.317	0.583	101.9	3.0
Guanosine	0.451	0.334	0.777	97.6	1.4
		0.417	0.865	99.4	2.8
		0.500	0.949	99.5	0.8
Epigoitrin	2.245	1.872	4.068	97.4	3.7
		2.304	4.476	96.8	1.6
		2.736	4.951	98.9	1.6
Adenosine	0.658	0.525	1.192	101.7	1.7
		0.650	1.312	100.7	1.2
		0.775	1.437	100.5	1.2
Syringin	0.199	0.161	0.356	97.5	2.0
		0.196	0.390	97.5	3.9
		0.230	0.425	98.1	2.2
Clemastanin B	0.049	0.039	0.087	97.5	4.4
		0.048	0.099	104.5	4.0
		0.058	0.104	95.8	4.4
Indigoticoside A	0.198	0.163	0.358	98.1	4.6
		0.204	0.395	96.6	2.7
		0.245	0.446	101.5	2.3
Isolariciresino	0.034	0.028	0.061	98.6	4.3
		0.034	0.069	104.0	2.5
		0.040	0.074	99.4	2.1

determination of different structural multi-components (an alkaloid, six nucleosides and four phenylpropanoids) in twelve batches raw Radix Isatidis from different provinces in China and twenty batches pharmaceutical dosage forms of Radix Isatidis from different manufacturers. Additionally, the method was validated for acceptable levels of linearity, precision, repeatability, stability and accuracy. Our research might serve as a sound foundation for further study and better quality control of Radix Isatidis and its pharmaceutical dosage forms.

Acknowledgments

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Table 5. Contents (μg g^{-1}) of the eleven analytes in Radix Isatidis and its relevant pharmaceutical dosage forms

Sample	1[b]	2	3	4	5	6	7	8	9	10	11	Total
S1[a]	68.13	419.51	42.07	388.19	636.98	5363.19	849.72	98.89	102.65	393.15	65.83	8428.31
S2	73.16	645.92	46.74	520.67	901.83	4490.08	1315.87	398.20	97.55	395.90	67.80	8953.70
S3	64.79	438.05	34.31	349.59	562.60	4854.48	1062.00	78.57	438.53	288.81	53.15	8224.88
S4	46.74	93.96	62.48	118.46	192.47	1430.73	597.37	57.86	282.36	113.20	22.15	3017.78
S5	58.07	398.93	37.78	376.19	510.98	4800.49	1157.47	[c]ND	217.78	132.43	28.43	7718.56
S6	60.98	354.52	33.23	294.10	480.67	5343.65	191.86	277.83	49.82	240.58	43.49	7370.73
S7	64.46	237.20	30.62	28.46	416.21	1106.36	799.93	[c]ND	221.74	88.57	21.69	3015.23
S8	57.17	233.78	17.85	315.27	505.93	760.17	1170.01	56.24	128.46	128.74	27.19	3400.82
S9	72.14	265.17	46.69	271.43	390.79	4461.38	95.52	201.61	546.04	223.05	39.51	6613.33
S10	87.49	721.70	75.64	508.94	789.90	2538.86	55.48	63.94	358.48	124.36	30.13	5354.92
S11	86.86	430.65	74.15	388.17	545.57	1098.97	802.59	28.22	371.39	119.09	27.59	3973.24
S12	52.55	289.32	30.49	289.84	481.43	1509.40	1113.22	62.03	126.49	103.36	[c]ND	4058.13
S13	130.3	906.67	76.46	301.68	112.21	960.70	922.45	109.90	156.32	51.23	[c]ND	3727.93
S14	129.61	628.53	17.16	592.11	508.48	380.83	1054.57	114.28	203.21	83.11	[c]ND	3711.89
S15	19.58	27.59	14.24	35.79	27.92	33.98	42.94	13.72	15.65	50.66	23.40	305.47
S16	17.67	31.35	13.75	53.71	50.41	40.36	133.42	78.93	[c]ND	113.59	12.97	546.17
S17	20.22	28.81	15.02	53.20	44.79	27.66	74.77	78.21	[c]ND	47.36	17.62	407.66
S18	37.99	107.96	71.48	100.88	98.33	118.45	464.20	44.36	[c]ND	[c]ND	[c]ND	1043.65
S19	19.68	31.52	12.43	50.93	31.23	39.04	66.49	14.17	15.62	39.58	[c]ND	320.69
S20	17.80	29.02	12.60	45.47	39.07	47.04	98.29	14.59	[c]ND	[c]ND	[c]ND	303.87
S21	44.96	130.22	47.86	212.21	155.01	116.39	[c]ND	155.52	[c]ND	39.75	[c]ND	901.92
S22	22.32	54.58	11.01	92.41	56.79	51.63	175.06	17.81	[c]ND	22.11	[c]ND	503.72
S23	20.79	55.92	28.15	75.75	81.11	99.44	148.05	24.75	[c]ND	24.82	18.45	577.22
S24	22.74	39.33	32.46	84.66	71.37	51.35	135.07	45.91	[c]ND	[c]ND	16.22	499.11
S25	21.67	34.89	10.48	51.12	29.39	50.63	108.40	22.00	22.79	25.50	15.88	392.75
S26	20.42	31.89	11.61	56.81	33.41	37.33	97.87	18.88	52.49	41.46	17.87	420.04
S27	34.14	86.54	12.47	171.14	101.37	212.61	31.95	63.27	164.60	51.04	27.87	957.01
S28	[c]ND	95.65	30.41	186.61	91.88	60.24	176.95	28.40	28.67	58.77	[c]ND	757.59
S29	26.06	47.62	25.34	26.41	102.21	106.09	18.75	184.35	109.89	50.10	[c]ND	696.82
S30	[c]ND	[c]ND	21.91	14.32	28.54	32.60	97.77	18.04	[c]ND	36.67	15.14	264.98
S31	[c]ND	[c]ND	34.50	405.06	416.89	906.26	1514.89	110.08	211.06	150.81	33.85	3783.40
S32	[c]ND	[c]ND	93.07	381.79	77.30	30.64	337.50	32.80	411.52	[c]ND	21.94	1386.56
mean	43.70	215.53	35.14	213.79	267.91	1286.28	480.98	83.78	188.40	115.64	29.46	2960.60

[a]The samples' numbers are the same as in Table 1; [b]the compounds numbers are the same as in Figure 1; [c]ND: not detected.

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