Identification and Comparative Determination of Rhodionin in Traditional Tibetan Medicinal Plants of Fourteen *Rhodiola* Species by High-Performance Liquid Chromatography-Photodiode Array Detection and Electrospray Ionization-Mass Spectrometry

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Using the HPLC/PDA/ESI/MS method, a comparative analysis of rhodionin (RH) was undertaken in order to conduct a qualitative and quantitative study in 38 batches of fourteen species of *Rhodiola* for quality control purposes. Alongside of this RH analysis, a simultaneous determination of salidroside (SA), tyrosol (TY), and gallic acid (GA) was carried out. *Rhodiola* plants are a popularly used ethnodrug from the Qinghai-Tibetan plateau of China. The identity of RH was unambiguously determined based on the quasimolecular ions in negative ESI-MS mode. This method was validated in respect to sensitivity, linearity, precision, repeatability and recovery using optimized chromatographic conditions. The linear calibration curve was acquired with $R^2>0.999$, and the limit of detection (S/N=3) was estimated to be 43.75 μg/g. The relative standard deviations (RSDs) of the intra- and inter-day precisions were 0.75% and 0.50%, respectively. The repeatability was evaluated by a replicated analysis of samples with the RSD value found within 0.67%. The recovery rates varied within the range of 98.79—100.08% with RSD less than 1.10%. In the present study, the content of RH was quantified within 0.4192—4.7260 mg/g for 16 batches of *R. crenulata*. It was also found in eight other species plants. The results demonstrated that RH is a useful characteristic standard compound for quality evaluation and chemical differentiation among species of *Rhodiola*. The study also indicated that the analytical procedure is precise, reproducible and a potential tool for both quality assessment and species identification.

Key words *Rhodiola*; rhodionin; ethnodrug; *Rhodiola crenulata*; quality evaluation

The genus *Rhodiola* L. (Crassulaceae) comprises about 96 species found in the alpine regions of Asia and Europe. China contains a total of 73 species, 2 subspecies and 7 varieties.[1] *Rhodiola* plants are mainly distributed in southwest and northwest China, with most species located in Tibet and in Sichuan province. In China, *Rhodiola* species have been used as an important adaptogen, hemostatic, and tonic in traditional Tibetan medicines (TTM) for thousands of years.[2] Most notably, the roots and rhizomes of *R. crenulata* (Radix et Rhizoma Rhodiolae Crenulatae; RC) have the best quality and have been accepted by the Pharmacopoeia of China (2005).[3] In recent years, many pharmacological activities and different efficacies have been reported, such as: anti-anoxia,[4] antifatigue,[5] anti-aging,[6] antioxidant,[7] enhancement in learning and memory,[8] anti-tumor,[9] and anti-radiation.[10]

Flavonoids, phenylpropanoids, and organic acids—the major effective constituents in *Rhodiola*—are generally regarded as the index for quality assessment. Salidroside (SA), tyrosol (TY), and gallic acid (GA) (Figs. 1a, b, c) are commonly used as markers to control the quality.[3,11,12] However, both SA and TY are not characteristic compounds in *Rhodiola*, since these also exist in other genus.[13] It is also well-known that GA widely exists in many medicinal plants. In addition, the identification of the closely related species of *Rhodiola* is often difficult due to their generally similar morphology. As a result, attempts to distinguish between other genus and *Rhodiola* plants with RC using the above mentioned chemical markers have met with little success. To solve this problem, a characteristic compound for evaluating the quality and chemical differentiation of RC is desirable.

Rhodionin (Herbacetin 7-*O*-α-L-rhamnopyranoside, RH) (Fig. 1d) is a characteristic flavonoid compound found only in *Rhodiola* plants that has not been considered in previous quality control efforts. A recent study revealed that RH possesses anti-thrombotic activities. RH and SA may play a role in the antitumor effect, but IC_{50} of RH was distinctly lower than SA.[14] Also, RH is recognized to be involved in learning and memory.[8] Therefore, it appears worthwhile to quantitatively determine the concentration of RH in herbs of *Rhodiola*.

However, very little work on the contents of RH is on record.[15] Even where studies exist, they only focus on five species of *Rhodiola* plants. The other thirteen species, with the exception of *R. kirilowii*, are ignored. In order to accurately evaluate quality and efficiency, it is therefore necessary to focus on developing a new characteristic and effective chemical marker in herbs. In this paper, a HPLC-PDA-MS

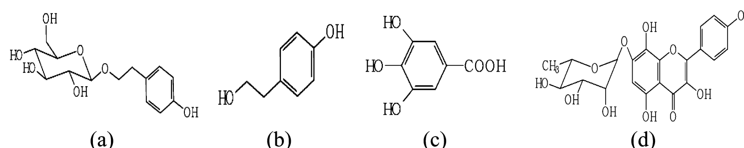

Fig. 1. Chemical Structures of (a) Salidroside (SA), (b) Tyrosol (TY), (c) Gallic Acid (GA), and (d) Rhodionin (RH)

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method with a comprehensive validation protocol was developed to determine the RH contents in fourteen species of *Rhodiola*.

Experimental

Materials and Reagents Samples were collected during flowering and fruiting time in the western Sichuan province and Tibet. Specifications of the samples evaluated in the present study are shown in Table 1. The species were identified by Prof. Hao Zhang (West China School of Pharmacy, Sichuan University, Chengdu, P. R. China). Voucher specimens were deposited in the Herbarium of Pharmacognosy, West China School of Pharmacy, Sichuan University (WCU).

Three reference compounds of SA, TY, and GA were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol (Merck, Darmstadt, Germany) was used as the mobile phase for HPLC. Analytical grade solvents including methanol, acetic acid and ethyl acetate were purchased from Merck Company Inc. for the extraction of herbs and column chromatography. Ultra-pure water was prepared by a Milli-Q50 water purification system (Millipore, Bedford, MA, U.S.A.). A semipreparative column (Alltima C_{18}, 10.0 mm×250 mm, 5 μm) was used in a semipreparative HPLC isolation.

HPLC System and Conditions Quantitative analyses were performed on a Shimadzu LC-10ATvp system, equipped with a LC-10ATvp binary pump, SPD-M10Avp photodiode array detector (PDA), CTO-10Asvp column oven, SCL-10Avp system controller and CLASS-VP workstation (Shimadzu Corp., Kyoto, Japan). An ultrasonic cleaner was used for extraction.

The chromatographic separation for RH was performed on a Shim-pack VP-ODS analytical column (5 μm, 4.6 mm×150 mm) with a guard column (C_{18}, 5 μm, 4.6 mm×7.5 mm) used. An isocratic elution (water : methanol=55 : 45) system was employed. The flow rate was 1 ml/min and the column temperature was set at 35 °C. The UV detector was set at the maximum absorption wavelength, *i.e.*, 332 nm, of RH. For chromatographic analyses of SA, TY, and GA, a Diamonsil C_{18} column (5 μm, 4.6 mm×250 mm) was used. The mobile phase consisted of methanol (A) and 1% acetic acid in water (B) using a gradient program of 5—12% A in 0—10 min and 12—25% A in 10—40 min. The detection wavelength was set at 278 nm for acquiring chromatograms. The other chromatographic conditions were identical to the one used for RH analysis.

HPLC-MS System and Conditions An Agilent-1100 API 3000 LC-MS system with electrospray ionization source (Aglient Corporation, MA, U.S.A.) was used for mass spectrometric determination. The chromatographic conditions for HPLC-MS analysis were identical to the one used for HPLC-PDA analysis. The ESI-MS spectrum conditions were optimized in negative-ion mode with the following parameters: nebulizer gas flow 4 l/min, curtain gas flow 7 l/min, collision gas flow 5 l/min, gas temperature 550 °C, scan range 100—600 *m*/*z*, declustering potential −55 V, focusing potential −400 V, entrance potential −10 V, collision energy −23 V, collision cell exit potential −10 V, and ionspray voltage −4.5 kV.

Isolation of RH from RC The standard compound RH was isolated as follows: RC powder (100 g) was extracted three times with methanol under

Table 1. Contents of Four Constituents in Roots and Rhizomes of Fourteen Species of *Rhodiola*

Sample code	Locality	Elevation (m)	Date of collection	Contents of four constituents (mg/g)[a]			
				Rhodionin	Salidroside	Tyrosol	Gallic acid
RC01	Danba, Sichuan, China	4550	August, 2006	3.8408±0.0626	4.6647±0.0324	2.5365±0.0217	1.3518±0.0160
RC02	Danba, Sichuan, China	4300	July, 2006	4.7260±0.0643	2.0242±0.0176	10.6496±0.0685	1.8376±0.0289
RC03	Hongyuan, Sichuan, China	4100	September, 2006	0.9997±0.0129	2.0168±0.0106	1.8995±0.0102	2.8468±0.0254
RC04	Heishui, Sichuan, China	4300	August, 2007	0.4192±0.0074	1.1682±0.0089	8.6600±0.0591	1.1406±0.0093
RC05	Baoxing, Sichuan, China	4100	July, 2007	0.9109±0.0140	2.7837±0.0214	8.7453±0.0683	0.8536±0.0043
RC06	Xiaojin, Sichuan, China	4200	July, 2006	1.0311±0.0092	2.6199±0.0203	9.2015±0.0323	1.1794±0.0176
RC07	Xiaojin, Sichuan, China	4500	August, 2006	1.4411±0.0304	4.3381±0.0421	8.2599±0.0301	0.9305±0.0061
RC08	Songpan, Sichuan, China	4300	September, 2006	1.1930±0.0195	3.9822±0.0305	2.0528±0.0328	3.3281±0.0490
RC09	Linzhi, Tibet, China	4500	September, 2006	2.4195±0.0735	3.1322±0.0287	8.7014±0.0574	1.5260±0.0188
RC10	Hailuogou, Sichuan, China	4400	July, 2006	2.5502±0.0409	1.8157±0.0096	7.9613±0.0538	2.0710±0.0276
RC11	Wenchuan, Sichuan, China	4500	June, 2006	2.1259±0.0503	2.6663±0.0254	6.9383±0.0405	0.7163±0.0043
RC12	Wenchuan, Sichuan, China	4400	August, 2006	2.3498±0.0658	3.3180±0.0327	8.2876±0.0630	1.0462±0.0075
RC13	Kangding, Sichuan, China	4200	July, 2006	1.0707±0.0073	3.1085±0.0283	10.3701±0.0656	0.8264±0.0079
RC14	Pali, Tibet, China	4300	September, 2006	0.5703±0.0093	10.2992±0.0565	2.9009±0.0233	1.4049±0.0161
RC15	Jiulong, Sichuan, China	4400	August, 2007	0.7428±0.0044	2.4712±0.0214	6.8246±0.0495	0.9677±0.0063
RC16	Jiulong, Sichuan, China	4500	August, 2007	0.7464±0.0154	3.5365±0.0397	3.1779±0.0397	6.8842±0.0597
RFA01	Wenchuan, Sichuan, China	4400	June, 2006	0.2987±0.0018	—	—	0.5281±0.0031
RFA02	Jiulong, Sichuan, China	4300	August, 2007	0.6237±0.0038	0.0852±0.0019	0.3796±0.0020	0.5734±0.0040
RFA03	Baoxing, Sichuan, China	4100	July, 2007	0.4807±0.0094	—	—	0.2401±0.0013
RK01	Wenchuan, Sichuan, China	3300	July, 2006	—	0.1438±0.0059	0.5838±0.0036	0.7540±0.0072
RK02	Wenchuan, Sichuan, China	3500	July, 2006	—	4.1099±0.0485	2.3644±0.0244	1.0195±0.0057
RK03	Baoxing, Sichuan, China	3400	July, 2007	—	1.9220±0.0112	1.3935±0.0177	1.5021±0.0170
RB	Wenchuan, Sichuan, China	4400	July, 2006	0.2134±0.0058	2.5801±0.0317	0.9359±0.0082	0.3951±0.0021
RQ01	Kangding, Sichuan, China	4000	July, 2006	—	5.7866±0.0696	0.9289±0.0071	0.5048±0.0022
RQ02	Wenchuan, Sichuan, China	4400	June, 2006	—	2.4625±0.0234	0.4351±0.0024	0.9181±0.0059
RY01	Kangding, Sichuan, China	3250	July, 2006	1.2096±0.0220	1.2370±0.0088	0.9916±0.0056	0.5920±0.0028
RY02	Xiaojin, Sichuan, China	3100	August, 2006	1.5219±0.0375	0.9159±0.0054	1.2976±0.0171	0.8645±0.0066
RH01	Wenchuan, Sichuan, China	2780	July, 2006	1.1800±0.0345	1.2293±0.0078	0.3333±0.0018	0.5631±0.0049
RH02	Kangding, Sichuan, China	3050	July, 2006	5.7621±0.0921	0.5400±0.0045	0.2443±0.0018	0.0775±0.0011
RE01	Wenchuan, Sichuan, China	3800	July, 2006	—	—	—	0.2614±0.0015
RE02	Hailuogou, Sichuan, China	3400	July, 2006	0.0426±0.0018	—	—	0.2040±0.0012
RD01	Wenchuan, Sichuan, China	3500	July, 2006	—	—	—	0.4261±0.0053
RD02	Baoxing, Sichuan, China	3600	July, 2007	—	—	—	0.3909±0.0035
RW	Wenchuan, Sichuan, China	2410	July, 2006	1.6283±0.0260	0.6226±0.0064	0.4203±0.0021	0.6693±0.0049
RP	Kangding, Sichuan, China	3500	July, 2006	—	0.1573±0.0017	1.8176±0.0190	0.2440±0.0018
RS	Wenchuan, Sichuan, China	4400	June, 2006	—	1.6275±0.0181	0.7815±0.0051	0.2718±0.0010
RA	Hongyuan, Sichuan, China	4100	September, 2006	0.2506±0.0056	—	0.3178±0.0022	0.2931±0.0024
RFO	Muli, Sichuan, China	3500	September, 2006	0.3605±0.0043	2.0602±0.0229	0.6952±0.0037	0.6281±0.0041

RC01—RC16: *R. crenulata*, RFA01—RFA03: *R. fastigata*, RK01—RK03: *R. kirilowii*, RB: *R. brevipetiolata*, RQ01—RQ02: *R. quadrifida*, RY01—RY02: *R. yunnanensis*, RH01—RH02: *R. henryi*, RE01—RE02: *R. eurycarpa*, RD01—RD02: *R. discolor*, RW: *R. wolongensis*; RP: *R. purpureoviridis*, RS: *R. scabrida*, RA: *R. alsia*, RFO: *R. forrestii*. *a*) The value is mean±S.D. (*n*=3). —: absence.

sonication (each 300 ml) for 30 min. The extract was then suspended in water and partitioned with ethyl acetate. Next, the EtOAc-soluble fraction was subjected to polyamide solid-phase extraction and semipreparative HPLC for separation and purification. The polyamide (60—80 mesh, LJ, Jingsu, China) columns (12 g) were prepared. Each column added the EtOAc-soluble fraction, pre-diluted in 40 ml of ultra-pure water, and was loaded. Sequential elution was done with 300 ml ultra-pure water, and 400 ml 95% methanol. The 95% methanol fraction was concentrated to a minimum volume and 1 ml of the concentrate was diluted with 5 ml ultra-pure water prior to loading. The mobile phase for semipreparative HPLC consisted of water and methanol (60 : 40, v/v) at a flow rate of 5 ml/min. The detection wavelength was maintained at 332 nm. Identity and purity of the RH were confirmed by melting point, chromatographic (TLC, HPLC) and spectroscopic (NMR, LC-MS) methods in reference to literature values.[16] The structure of rhodionin (RH) is shown in Fig. 1d. The yield and purity of the isolated RH was found to be 1.32 mg/g and 97.43% by HPLC-UV, respectively.

Standard Solution Preparation Accurately weighed 17.5 mg (RH), 25.5 mg (SA), 16 mg (TY), and 2.15 mg (GA) were introduced into a 50 ml volumetric flask and made up to the volume with methanol as stock standard solution. Aliquots of 0.1, 0.25, 0.5, 0.75, 1, 1.5, and 2 ml stock standard solutions were transferred into 10 ml volumetric flasks and made up to the volume with methanol as working calibration solutions. An aliquot of 10 μl of solution for each calibration was injected for HPLC analysis.

Sample Solution Preparation A 0.3 g (RH) or 0.1 g (SA, TY, and GA) pulverized sample powder was accurately weighed and then extracted with 8 ml of methanol by sonicating at room temperature for 30 min. The extractions were repeated three times. After centrifugation at 3000 rpm for 5 min, the supernatants were combined and diluted to 25 ml with extraction solvent. The 2 ml solutions were filtered through a syringe filter (0.45 μm). An aliquot of 10 μl was subjected to HPLC- PDA and HPLC-ESI-MS analysis.

Results and Discussion

Optimization of the Isolation Process for RH Sonication was chosen as the extraction method in the present study. The extract was then partitioned with ethyl acetate and subjected to polyamide solid-phase extraction to remove the major extraneous compounds for reducing the load capacity during subsequent semipreparative HPLC purification. Ultra-pure water was used to elute unbound compounds. Observation of TLC showed that the amount of RH was more abundant while the non-flavonoids impurities were minimized using polyamide column chromatography.

Optimization on the Preparation of Sample Solution For the extraction of RH, SA, TY, and GA, sonication was chosen as the extraction method for its confirmed efficacy and ease of handling. Prior to sample analysis, the optimum extraction conditions were determined. Different extract solvent compositions (methanol, water, 50% methanol and 80% methanol), procedures (sonication and refluxing) and times (10, 20, 30, 45 min) were further compared. It was concluded that the most efficient method involved a repeated sonication of the plant materials with methanol.

Identification of RH in *Rhodiola* Species by HPLC-MS Apart from comparing retention time (t_R), RH was further identified by HPLC-ESI-MS analysis. Since the RH-MS spectra were only acquired in negative-ion modes, the negative-ion mode was adopted. In the MS spectra, the pseudo-molecular ion $[M-H]^-$ ($m/z=447$) was consistently observed. Cleavage of the glucose unit, such as [M−H−rhamnose]$^-$ ($m/z=285$) as the base peak, and $[M-C_6H_{11}O_4]^-$ ($m/z=301$), can be easily observed for peak RH in HPLC chromatograms in the standard solution and samples of nine species (Figs. 2a, b, c, e, g, h, i, k, n, o). Their fragmentation patterns were well matched with the chemical structures and the reports in the literature.[17]

Linearity and Limit of Detection (LOD) Linearity was determined using seven standard solutions of different concentrations. Calibration curves were constructed by the value of the area of peak (Y) and the concentration of standard solutions (X mg/l). The linearity calibration curve factors are listed in Table 2. For the four quantified constituents, a good linearity with $R^2>0.999$ was achieved. These regression equations were used for quantifying four constituents in all sample solutions.

The limit of detections (LODs) of RH, SA, TY, and GA in samples was determined based on visual evaluation with a signal-to-noise ratio of about 3 : 1. The LODs were estimated to be 0.525 mg/l, 1.152 mg/l, 0.766 mg/l, and 9.936 μg/l, which were equivalent to 43.75 μg/g, 288.00 μg/g, 191.50 μg/g, and 2.48 μg/g in solid samples. Moreover, the quantitation limits were determined based on a signal-to-noise ratio of about 10 : 1 for five replicated analyses of spiked matrix blank. The quantitation limits of RH, SA, TY, and GA were found to be 0.875 mg/l, 1.728 mg/l, 1.149 mg/l, and 29.808 μg/l, equivalent to 72.92 μg/g, 432.00 μg/g, 287.25 μg/g, and 7.45 μg/g in solid samples.

Method Precision and Repeatability The intra- and inter-day precisions were determined by analyzing five consecutive injections of the standard solutions during a single day and three a day on three consecutive days, respectively. To confirm the repeatability, five different working solutions prepared from the same sample were analyzed. The RSDs of RH, SA, TY, and GA were 0.75%, 0.76%, 0.25%, 1.09% for intra-day assays and 0.50%, 1.32%, 0.90%, 1.29% for inter-day assays. The RSDs of the content of RH, SA, TY, and GA in sample replicates were estimated to be 0.67%, 0.79%, 1.11% and 0.82% ($n=5$). The sample stability test precisions were determined with one sample during 2 d. The RSDs of RH, SA, TY, and GA were 1.13%, 0.72%, 1.93% and 1.39%, respectively. These results indicated that the samples remained stable during this period.

Recovery The recovery rate was determined using spiked samples with different concentration levels of 80%, 100%, and 120% of RH, SA, TY, and GA in the samples, respectively. The recovery rates are listed in Table 3.

Sample Analysis The contents of RH, SA, TY, and GA

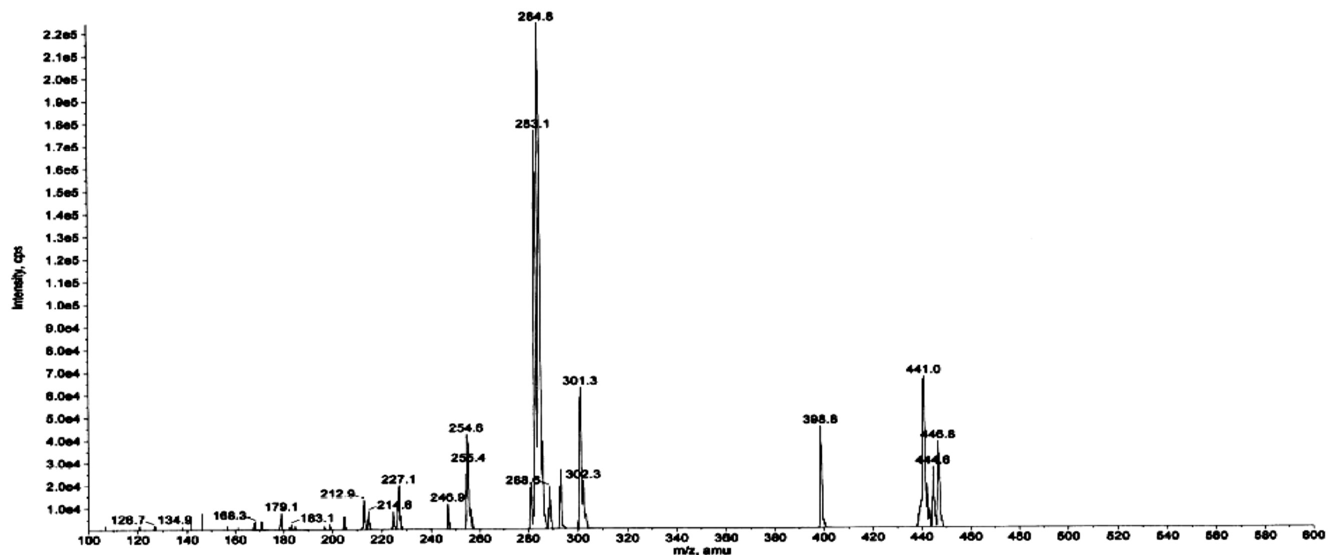
Table 2. Linearity Calibration Curve Factors of Four Constituents

Compound	Slope (A)	Intercept (B)	R^2	Concentration (mg/l)
Rhodionin	−1249.941	+10398.687	0.9997	3.50—70.0
Salidroside	2547.542	+4324.546	0.9996	5.10—102.0
Tyrosol	1867.515	+5486.821	0.9996	3.20—64.0
Gallic acid	8903.842	+56975.071	0.9997	0.43—8.6

Table 3. Recovery of Four Constituents

Spike level (%)	Recovery of four constituents (%)[a]			
	Rhodionin	Salidroside	Tyrosol	Gallic acid
80	100.08±1.10	99.26±0.94	99.38±0.29	101.05±0.87
100	98.79±0.64	98.89±0.77	99.11±0.96	98.92±0.84
120	98.92±0.61	98.66±0.36	98.57±0.60	98.89±0.99
Mean	99.26±0.94	98.87±0.42	99.02±0.64	99.62±1.31

a) The value is mean±RSD ($n=3$).



(a)

RH

(b)

RH

(c)

(d)

RH

(e)

(f)

RH

(g)

Fig. 2

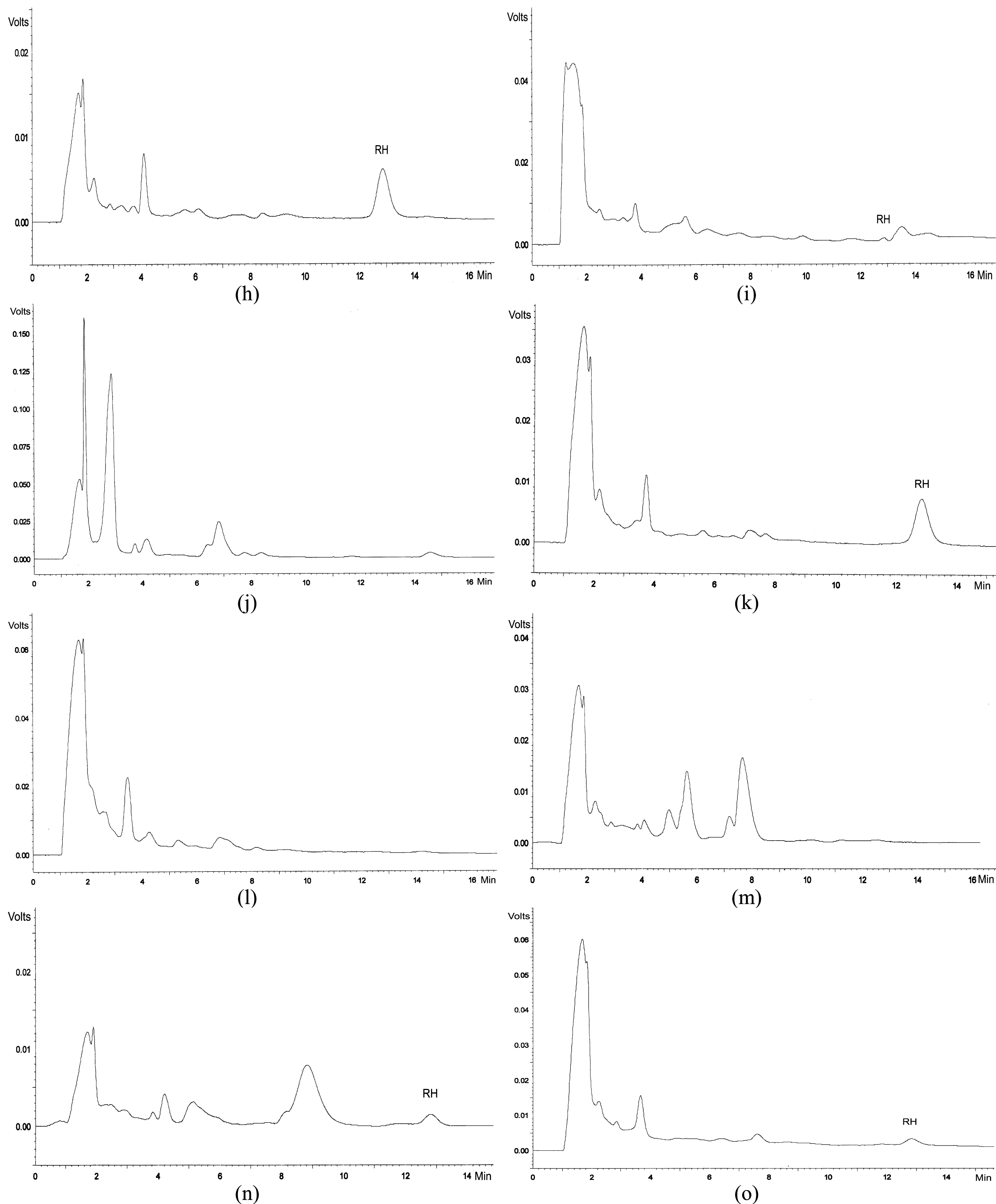


Fig. 2. On-Line LC-ESI Mass Spectrum of (a) Rhodionin (RH) in Negative Ionization Mode, and HPLC Chromatogram of Rhodionin (RH) in Roots and Rhizomes of (b) *R. crenulata*, (c) *R. fastigata*, (d) *R. kirilowii*, (e) *R. brevipetiolata*, (f) *R. quadrifida*, (g) *R. yunnanensis*, (h) *R. henryi*, (i) *R. eurycarpa*, (j) *R. discolor*, (k) *R. wolongensis*, (l) *R. purpureoviridis*, (m) *R. scabrida*, (n) *R. alsia*, and (o) *R. forrestii*.

in 38 batches of fourteen species of *Rhodiola* are presented in Table 1. The contents of RH, SA, TY, and GA in RC were within the ranges of 0.4192—4.7260 mg/g, 1.1682—10.2992 mg/g, 1.8995—10.6496 mg/g, and 0.7163—6.8842 mg/g, respectively. These results showed that the content deviation is quite different in various batches of RC. This could probably be attributed to the variation of habitat, climate, circumstances and soil conditions. Therefore, it is necessary and critical to establish a quality control method to ensure the batch-to-batch uniformity of the herbal medicine and

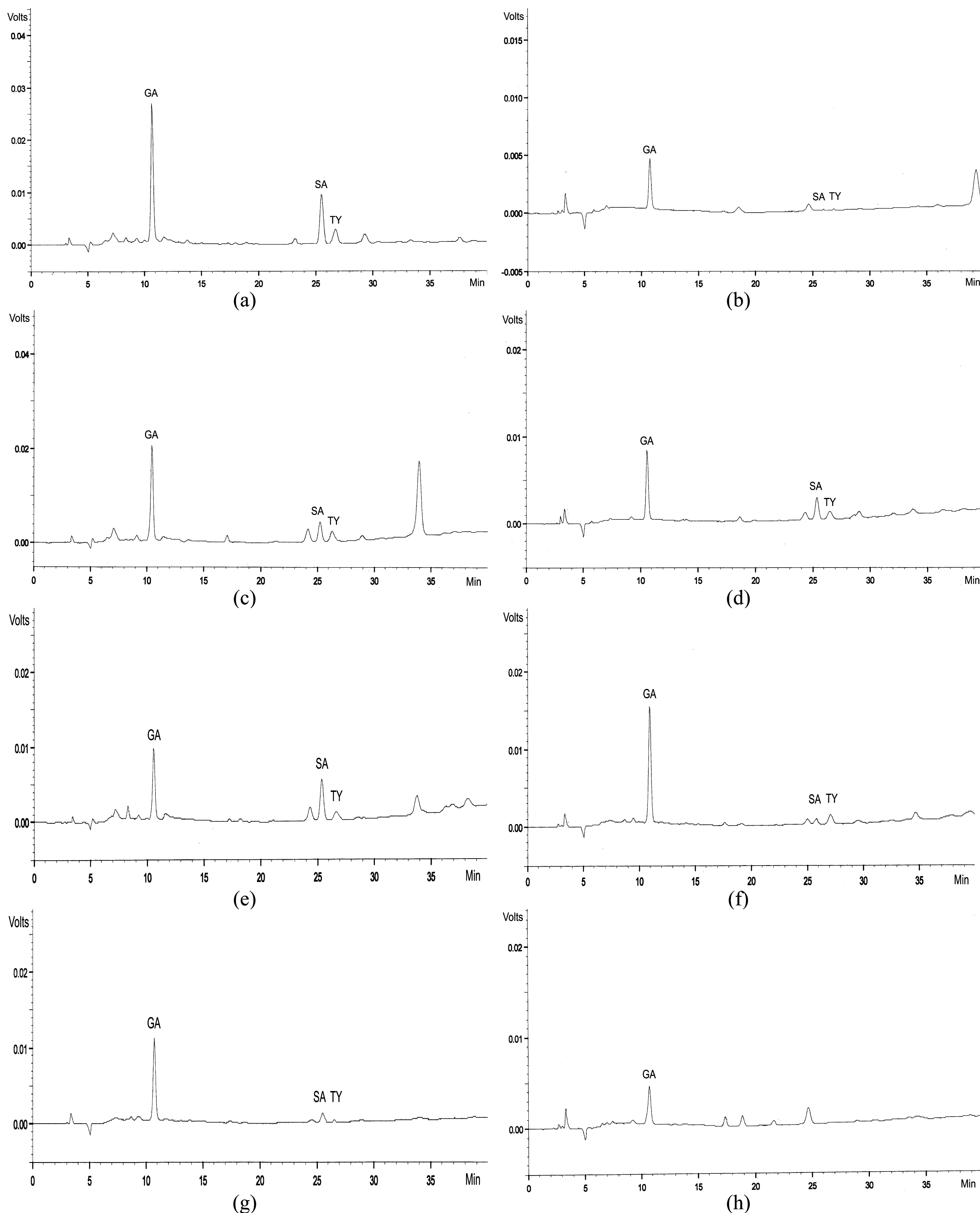


Fig. 3

preparations.

Nine species contain RH, although this is highest in *R. crenulata*, *R. yunnanensis*, *R. henryi*, *R. fastigata*, and *R. wolongensis*. RH is absent in *R. discolor*, *R. purpureoviridis*, and *R. scabrida* which have not recorded in the medicinal properties (Figs. 2b—o). SA has been found in the eleven species. It is higher in *R. crenulata*, *R. quadrifida*, *R. kirilowii*, and *R. brevipetiolata* than in the other seven species. TY existed in twelve species, with *R. crenulata*, *R. kirilowii*, *R. yunnanensis*, and *R. purpureoviridis* containing the highest levels. As can be seen, GA widely existed in each species, while the corresponding contents of *R. crenulata* and *R. kirilowii* are higher than those of other species (Figs. 3a—n).

The results indicated that the four quantified constituents' contents of RC are higher than those of other species. During an herbal drug market survey, it was observed that RC has the best quality and is commonly in use as a TTM named "Suo-Luo-Ma-Bao", derived mainly from *R. crenulata*, *R. fastigata*, and *R. yunnanensis*.[2] These three herbs which contain higher effective chemical marker RH are commonly being sold under the same name. However, the contents of SA and TY are very low or absent in *R. fastigata*. Although RH is not found in *R. kirilowii* and *R. quadrifida*, both species have some distinct differences in clinical application compared with RC.[18,19] In fact, *R. kirilowii* is known as another TTM named "Ga-Du-Er."[2] We conclude that for the sake of safety, efficacy and quality control for RC, RH is useful as a characteristic compound for chemical differentiation and quality evaluation.

Conclusion

In this study, the qualitative and quantitative analysis of RH in fourteen species plants of *Rhodiola* was undertaken using HPLC/PDA/ESI/MS. At the same time, a simultaneous

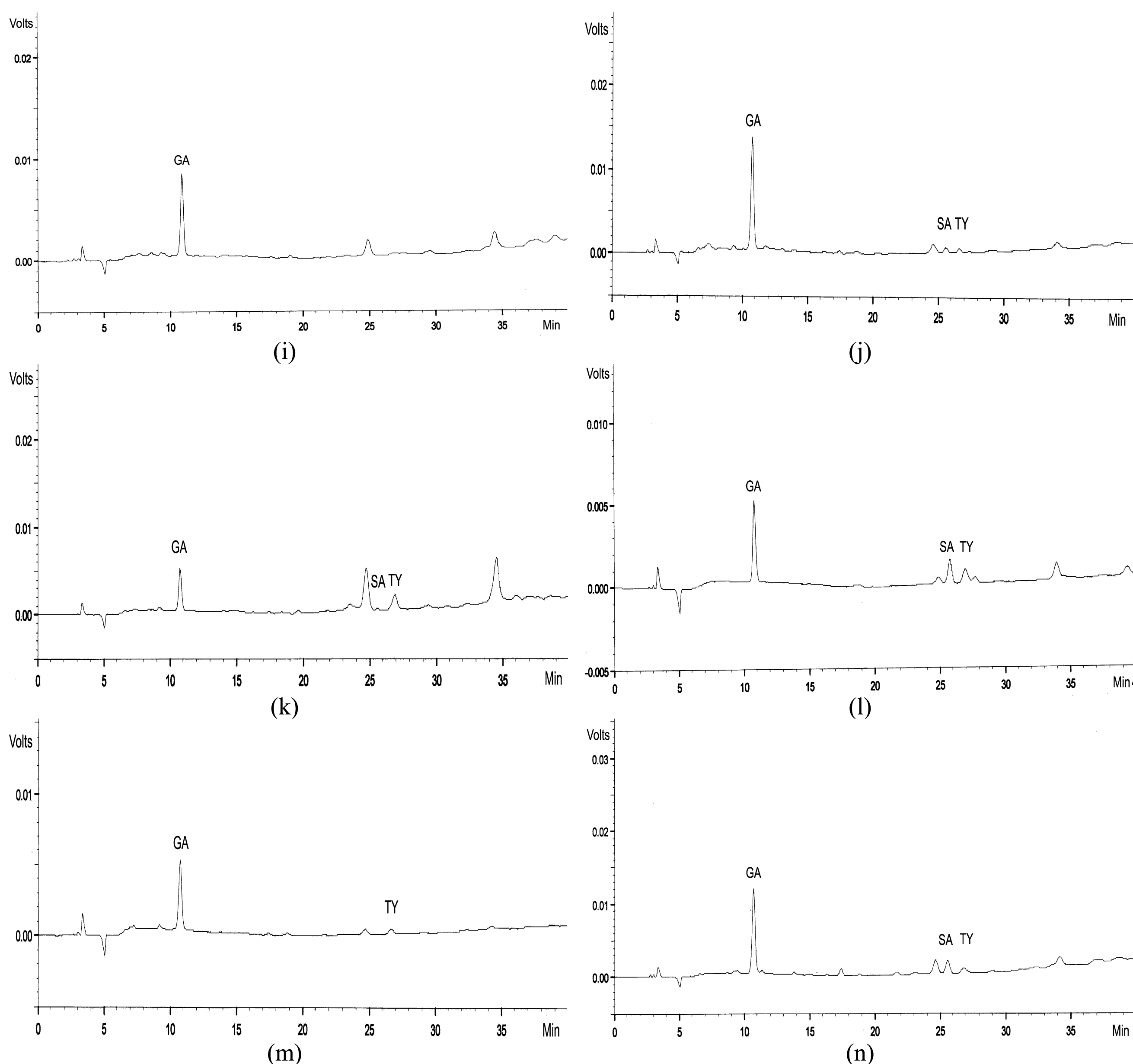


Fig. 3. HPLC Chromatogram of Salidroside (SA), Tyrosol (TY), and Gallic Acid (GA) in Roots and Rhizomes of (a) *R. crenulata*, (b) *R. fastigata*, (c) *R. kirilowii*, (d) *R. brevipetiolata*, (e) *R. quadrifida*, (f) *R. yunnanensis*, (g) *R. henryi*, (h) *R. eurycarpa*, (i) *R. discolor*, (j) *R. wolongensis*, (k) *R. purpureoviridis*, (l) *R. scabrida*, (m) *R. alsia*, and (n) *R. forrestii*.

determination of SA, TY, and GA by HPLC/PDA was carried out. The method has been successfully applied to analyze the marker compound RH in 38 batches of fourteen species from different habitats. We conclude that RH is useful as a characteristic standard compound for quality evaluation as well as for chemical differentiation between closely related *Rhodiola* medicinal plants.

Compared with the currently used quality control procedures, this method permits a much more reasonable and efficient manner to ensure the efficacy, safety, and batch-to-batch uniformity for RC. The proposed method is rapid and reproducible and could be readily utilized as a new quality control technique for RC and RC-derived herbal products. In addition, it is potentially useful as a tool in the accurate evaluation of different sources of *Rhodiola*.

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