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Simultaneous quantification of polyherbal formulations containing *Rhodiola rosea* L. and *Eleutherococcus senticosus* Maxim. using rapid resolution liquid chromatography (RRLC)

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ABSTRACT

An RRLC method capable of simultaneous identification and rapid quantification of six biologically active compounds (salidroside, tyrosol, rosarin, rosavin, rosin, rosiridin) in *Rhodiola rosea* L. and two active compounds (eleutheroside B and eleutheroside E) in *Eleutherococcus senticosus* Maxim. was developed. The chromatographic analyses were performed on a reversed phase Phenomenex C18 (2)-HST column at 40 °C with a neutral mobile phase (purified water and acetonitrile) gradient system at a flow rate of 1.0 ml/min and UV detection at 205 and 220 nm simultaneously. Baseline separation of eight active compounds was achieved within 8 min. This developed method provides good linearity ($R > 0.9997$), precision (RSD < 1.99%) and recovery of the bioactive compounds. The RRLC method developed is capable of controlling the quality of *R. rosea* and *E. senticosus* raw herbs, commercial extracts, as well as polyherbal formulations containing *R. rosea* and *E. senticosus* as ingredients. This RRLC method is accurate and sensitive; in addition, it greatly increases sample analysis throughput with reduced analysis time, which is suitable for routine quality control analysis.

1. Introduction

Adaptogens are natural bio-regulators that increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors; example of these are *Eleutherococcus senticosus* Maxim. and *Rhodiola rosea* L. [1]. A number of studies have demonstrated the efficacy of these adaptogens in stress-induced disorders of central nervous, cardiovascular and immune systems, and they have been used as adjuvants to other medicines to enhance curative effects in conditions such as, chronic pneumonia, chronic tuberculosis, vascular dystonia, cancer (reduction of metastasis), and in reducing the debilitating effects of radiotherapy and chemotherapy [2].

R. rosea has been categorized as an adaptogen by Russian researchers due to its observed ability to increase resistance to a variety of chemical, biological, and physical stressors [3,4]. The pharmacological effects of *R. rosea* have been studied extensively, including the CNS stimulating, neuro-, cardio- and hepato-protective effects, life-span increasing, MAO-A inhibitory, immunotropic, antiviral, anti-inflammatory and antibacterial activity [5]. *E. senticosus* grows in abundance in northeastern China, particularly in the Heilongjiang province, as well as Inner Mongolia, North Korea, and Siberia [6]. It is adaptogenic and an effective, anti-aging medicinal herb [7]. In addition, *E. senticosus* regulates endocrine secretions, adrenal cortex, and blood sugar levels [7].

Phytochemical investigations show that there are three important classes of bioactive constituents in *R. rosea*: phenylethanoids (salidroside, *p*-tyrosol), phenylpropanoid glycosides (rosarin, rosavin, rosin), and monoterpene (rosiridin) that are responsible for the bioactivity of *R. rosea* [8]. Characteristic feature of *R. rosea* is the presence of phenylpropanoids rosavin, which was not detected in 21 other genus *Rhodiola* species morphologically similar to *R. rosea* [9,10]. On the market, the term rosavins refers to three phenylpropanoid glycosides: rosavin, rosin and rosarin [11,12]. In present days, commercial *R. rosea* extracts are standardized for the contents of both salidroside and rosavins. Besides this, rosiridin is contained in *R. rosea* in an amount of about 3% and should also be considered as a marker compound [13].

Isolation and structural studies on several diterpenoids [14], triterpenoid saponins [15], and phenolic components [16] from

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Eleutherococcus species have been reported previously. Among these, the lignan compounds, eleutheroside B (syringin) and eleutheroside E ((−) syringaresinol-di-O-13-Dglucoside) have been identified as the key bioactive compounds for *E. senticosus*.

Literature survey reveals that a variety of methods have been reported to analyze the active constituents in *R. rosea* using analytical methods including capillary zone electrophoresis [17–19], liquid chromatography (LC) coupled with UV [8,12,20,21] or MS [21–23]. All of the listed methods suffer from longer analysis time of over 30 min and subsequently much solvent consumption. For *E. senticosus* analysis, several methods have been reported to analyze the bioactive active compounds, such as thin-layer chromatography-densitometry [24], HPLC [25]. An HPLC analysis method for *E. senticosus* was provided in USP [26] using H_2O and acetonitrile as mobile phase; however, this method takes longer than 30 min to complete. With the objective of reducing analysis time and maintaining good efficiency, there has been substantial focus on high-speed chromatographic separations.

RRLC is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. A study conducted by Guillarme et al. has shown that rapid resolution technology is capable of obtaining very high resolution in both isocratic and gradient modes for a wide range of compounds from molecular weight 160–1000 g mol^{-1} [27]. As efficiency and speed of analysis has become of a great importance in many application of liquid chromatography, especially in the field of pharmaceutical analysis, where it is important to increase throughput and reduce analysis costs, RRLC could play a significant role in the future of liquid chromatography. Therefore, an effective and reliable method, which is capable of analyzing the major bioactive compounds in *R. rosea* and *E. senticosus* to ensure its quality and efficacy, is necessary for qualitative and quantitative analysis of polyherbal formulations.

In today's natural health products market, polyherbal formulations that contain two or more herbal ingredients are very popular because of the joint contributions of multi-components. In this study, we have established an RRLC method to examine the contents of the active compounds, i.e. salidroside, tyrosol, rosarin, rosavin, rosin, rosiridin, eleutheroside B and eleutheroside E simultaneously in *R. rosea* and *E. senticosus*. A back pressure of 420 bar was observed during analyses and hence an RRLC system, which is capable of running up to 600 bar is necessary. This method is intended not only to evaluate the quality of *R. rosea* and *E. senticosus* raw materials, but also be efficient in the quality control of commercial polyherbal formulated products.

2. Materials and methods

2.1. Chemicals and materials

The reference standards of salidroside (purity: 98.3%), tyrosol (purity: 98.1%), rosarin (purity: 98.6%), rosavin (purity: 99.2%), and rosin (purity: 98.1%) were purchased from ChromaDex (Irvine, USA); eleutheroside B (purity: 99.6%), eleutheroside E (purity: 99.1%) and rosiridin were purchased from Biopurify (Chengdu, China), where rosiridin reference standard was used for identification purpose only. Four samples of dried *R. rosea* roots (H1, H2, H3 and H4) were provided by Alberta Agriculture Canada and a supplier in Shanxi province, China; one fresh *R. rosea* root sample (H5) was provided by a Canadian supplier. Twelve commercial *Rhodiola* extracts samples (P1-Lot1, P2-Lot1, P2-Lot2, P3-Lot1, P3-Lot2, P4-Lot1, P5-Lot1, P5-Lot2, P5-Lot3, P5-Lot4, P5-Lot5, P5-Lot6) and 5 commercial *E. senticosus* powder extracts samples (E1, E2, E3, E4 and E5) were collected from 8 suppliers in China. Seven other *Rhodiola* commercial extracts samples (P6-Lot1, P6-Lot2, P6-Lot3, P7-Lot1, P7-Lot2, P8-Lot1 and P8-Lot2) were provided by three Canadian suppliers. Six commercial polyherbal formulated capsules (RE1, RE2, RE3, RE4, RE5 and RE6) containing *R. rosea* and *E. senticosus* ingredients were purchased from a local drug store.

CH_3CN and methanol (MeOH) was of HPLC grade (Fisher Scientific, Ottawa, Canada). Deionized H_2O was purified by a Nanopure Ultrapure water system (Barnstead, USA) for all solutions and dilutions.

2.2. Preparation of standard solutions and samples

The stock reference standard solution of salidroside, tyrosol, rosarin, rosavin, and rosin were prepared at concentration of 1.0 mg/ml in pure methanol (MeOH), stock solution of eleutheroside B and eleutheroside E were prepared at 0.1 mg/ml in pure MeOH. Reference standard rosiridin was used for identification purpose only. The mixture of the reference compounds stock solution was also prepared. All the reference standard solutions were stored at 4 °C in a refrigerator. The reference standards in solution were stable at least for 24 h at room temperature.

Dried *R. rosea* roots, commercial *R. rosea* extracts, *E. senticosus* extracts and commercial polyherbal formulated capsules were kept in the desiccator. Fresh *R. rosea* roots were stored in a freezer at −10 °C and dried in an oven at 50 °C for 24 h before analysis. About 5 g of dried *R. rosea* roots were ground into fine powder and 0.1 g were accurately weighed and transferred into a 10 ml volumetric flask, ultrasonic extracted at 37 °C with 75% MeOH aq. solution for 15 min and then made up to volume. For *Rhodiola* and *E. senticosus* extracts, the same procedures were followed except using pure MeOH as extraction solvent for efficiency. Prior to RRLC analysis all samples were filtered through a 0.20 μm membrane filter. Every sample was injected in triplicates and the relative standard deviation (RSD) was calculated for all the samples. For the commercial polyherbal formulated capsules, the contents of 20 capsules were transferred to a flask and 0.10 g of capsule powder was accurately weighed into a 10 ml volumetric flask and ultrasonic extracted with pure MeOH as stated above.

2.3. Chromatography conditions

Analysis was carried out using an Agilent series 1200 RRLC instrument (Agilent, CA, US) equipped with a binary pump, a micro vacuum degasser, a multi-wavelength (MW) detector, an autosampler, and a thermostated column. Optimum resolution and peak shape were obtained on a Luna C18-HST (High Speed Technology) column (2.5 μm, 100 mm × 3.0 mm) from Phenomenex (Torrance, CA, U.S.A.). The mobile phase consisted of purified water (H_2O) (A) and acetonitrile (CH_3CN) (B). At a flow rate of 1.0 ml/min, the linear gradient was as follows: 0–6 min, 6–17% B; 6–7 min, 17–19.7% B; 7–9 min, 19.7–19.7% B, 9–10 min, 19.7–100% B. The detection wavelength varies as follows: 0–3.5 min, 205 nm; 3.5–6.5 min, 220 nm; 6.5–8 min, 205 nm. The column temperature was 40 °C and the injection volume was 1 μl. Each run was followed by a 2 min post run and an equilibration period for 5 min. The injection volume was 1 μl for all analysis. Data acquisition and processing were performed by ChemStation revision B.01.01 software.

3. Results and discussion

3.1. Method development

Basic chromatographic conditions, such as, mobile phase composition, elution gradients, column temperature and UV detec-

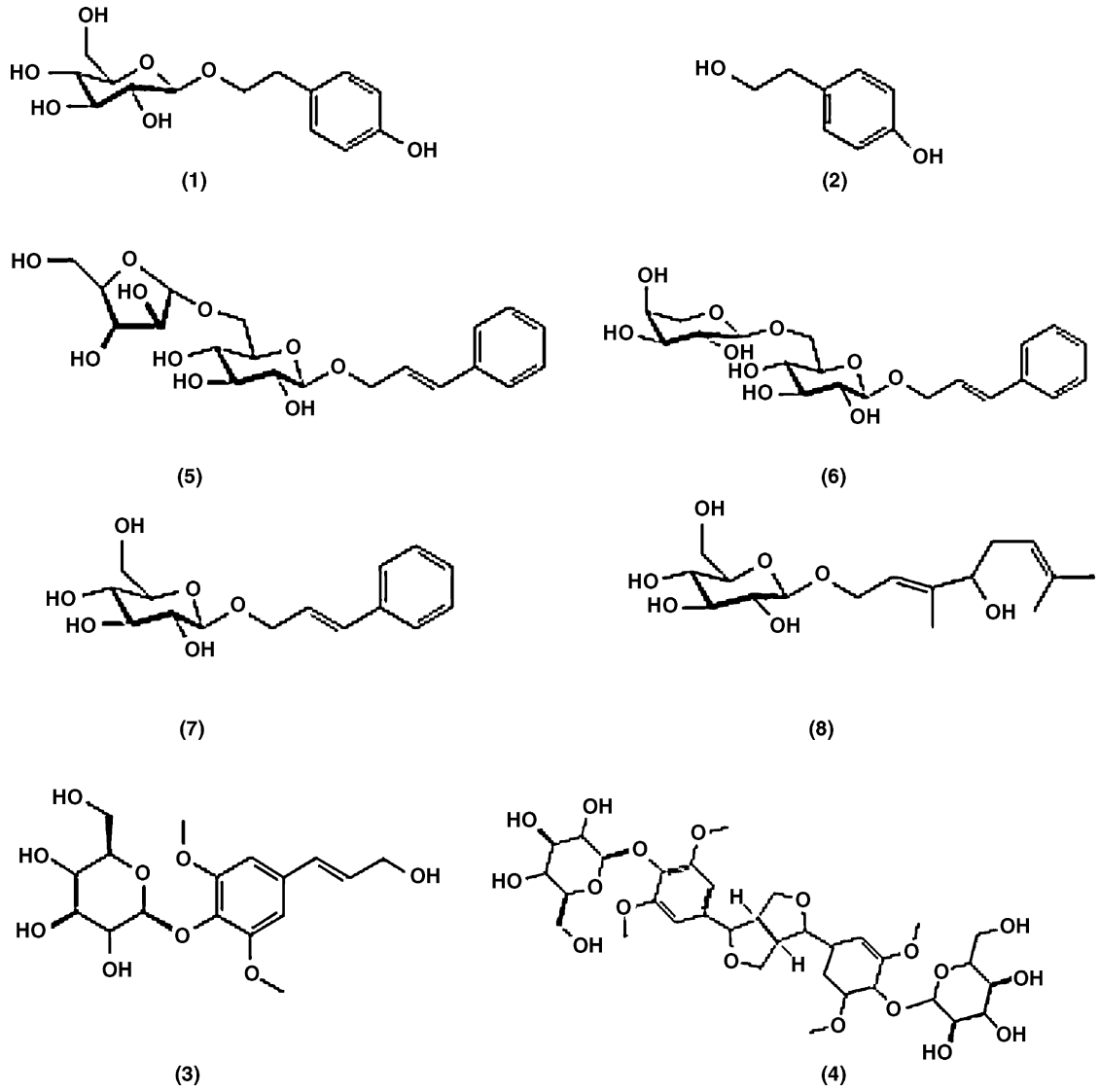

Fig. 1. Chemical structures of six bioactive compounds in *Rhodiola rosea* roots: (**1**) salidroside, (**2**) tyrosol, (**5**) rosarin, (**6**) rosavin, (**7**) rosin, (**8**) rosiridin and two bioactive compounds in *Eleutherococcus senticosus* (**3**) eleutheroside B, (**4**) eleutheroside E.

tion wavelength were optimized while developing the RRLC method. In conventional HPLC, it is a common practice to add ionization suppressants, such as phosphoric acid (H_3PO_4) and formic acid (HCO_2H) to mobile phases to improve chromatographic separations. In this study, various mobile phases including H_2O–methanol (MeOH), 0.05% (v/v) H_3PO_4 solution–MeOH, H_2O–acetonitrile (CH_3CN), 0.1% HCO_2H solution–CH_3CN, and 0.05% H_3PO_4 solution–CH_3CN, were evaluated and compared. CH_3CN was chosen over MeOH for its ability to reduce backpressure and achieve better separation. Ionization suppressants did not improve the peak shape and resolution of the six active compounds, hence H_2O–CH_3CN was selected as the mobile phase.

Selecting a proper detection wavelength is vital to ensure the active compounds are detected precisely. The active compounds **1**, **2**, **5**, **6**, **7** and **8** in *R. rosea* had characteristic absorption at 205 nm; active compounds **3** and **4** in *E. senticosus* had maximum absorption at 220 nm. For the quality control of commercial polyherbal formulated capsules, detection wavelength was selected as 205 nm (0–3.5 min), 220 nm (3.5–6.5 min), and 205 nm (6.5–8 min) to simultaneously analyze all bioactive compounds in polyherbal formulations containing *R. rosea* and *E. senticosus* (Fig. 1).

3.2. Optimization of extraction conditions

Prior to sample analysis, the optimal extraction conditions of the biologically active compounds in *R. rosea* roots were examined. Previous work by Ma et al. [8] showed that refluxing and ultrasonication using MeOH produced comparable extraction yields of rosavins. In this study, ultrasonication was selected for its simplicity and shorter extraction time. Variables in the extraction procedures, such as solvent strength and extraction time were optimized on an authentic *R. rosea* root sample (H3). As shown in Fig. 2, the extraction yield of rosavins increased as the concentration of MeOH in H_2O (v/v) increased from 20 to 75%; further increases of MeOH concentration to 100% had a negative effect on rosavins extraction yield. Fig. 2 shows that both 15 and 20 min of ultrasonication resulted in comparable results for rosavins content, indicating 15 min of ultrasonication was sufficient for *R. rosea* root extraction. The highest extraction effectiveness of *R. rosea* roots was achieved with 75% MeOH aq. solution with 15 min of ultrasonication at 37 °C.

Table 1
Calibration curves, limit of detection (LOD), limit of quantification (LOQ) ($n = 3$) and recovery data ($n = 3$) for seven reference compounds: salidroside, tyrosol, rosarin, rosavin, rosin, eleutheroside B and eleutheroside E in *Rhodiola rosea* and *Eleutherococcus senticosus*.

Compounds	Salidroside	Tyrosol	Rosarin	Rosavin	Rosin	Eleutheroside B	Eleutheroside E
Regression equation	$y = 351.24X - 0.97$	$y = 864.60X - 0.61$	$y = 725.16X + 0.87$	$y = 1081.57X + 1.95$	$y = 953.07X + 1.10$	$y = 1400.21X + 1.10$	$y = 953.07X + 1.10$
R	0.9997	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
LOD (μg/ml) ($n = 3$)	0.36	0.85	0.52	0.69	0.65	0.19	0.25
RSD%	0.14	0.36	0.24	0.09	0.23	0.13	0.19
LOQ (μg/ml) ($n = 3$)	0.86	1.78	1.14	1.59	1.62	0.55	0.71
RSD%	0.19	0.42	0.31	0.14	0.30	0.15	0.19
Recovery % ($n = 3$)	99.6	100.3	98.9	100.9	99.5	100.5	99.7
RSD%	1.12	1.15	1.23	1.01	1.42	1.11	0.98

3.3. Method validation

3.3.1. Calibration, sensitivity, linearity, and accuracy

The quantitative method was assessed by linearity, sensitivity, and accuracy. Calibration curves were prepared by plotting the peak area of marker compounds against the corresponding concentrations. The regression lines are linear in the concentration range studied and the corresponding coefficients of correlation are shown in Table 1. Peaks were assigned by spiking the samples with standard compounds, and comparing of the UV spectra and retention time. Good linear relationships ($R = 0.9997$ for salidroside, and 0.9999 for tyrosol, rosarin, rosavin, rosin, eleutheroside B and E) are demonstrated over a range of 50–800 μg/mL. The mean regression equations for the seven marker compounds are listed in Table 1. Fig. 5A shows the separation of mixed marker compounds (**1**) salidroside, (**2**) tyrosol, (**3**) eleutheroside B, (**4**) eleutheroside E, (**5**) rosarin, (**6**) rosavin, (**7**) rosin and (**8**) rosiridin.

Limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined on the basis of response and slope of each regression equation at a signal to noise ratio (S/N) of 3 and 10, respectively. The LOD for the seven marker compounds ranged from 0.19 to 0.85 μg/ml ($n = 3$, RSD ranged from 0.09 to 0.24%); the corresponding LOQ ranged from 0.55 to 1.78 μg/ml ($n = 3$, RSD ranged from 0.14 to 0.42%).

The accuracy of the analytical method was evaluated using the recovery test. The recovery tests of this method were studied by spiking a known quantity of the references to 0.1 g of the *R. rosea* samples and *E. senticosus* samples. The fortified samples were then extracted and quantified as described before. Each sample was analyzed in triplicate. The recovery values were obtained by comparing the results from samples and fortified samples. The mean recoveries are from 98.9 to 100.9% with RSD less than 1.42% for seven reference compounds.

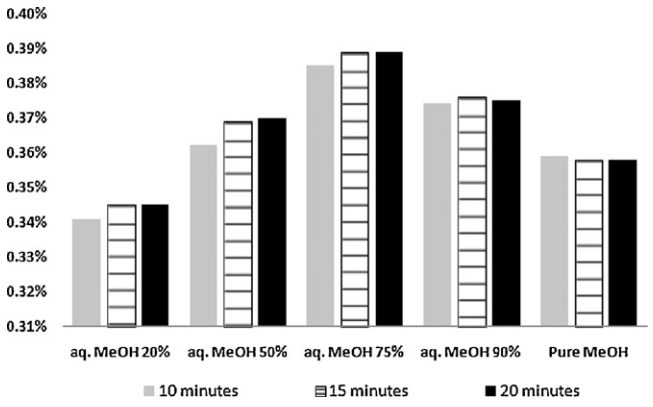

Fig. 2. Rosavins yields from *R. rosea* roots sample H3 using various extraction conditions at 37 °C. *x*-Axis consists of five blocks representing the extraction solvent strength varying from 20% MeOH aq. solution to pure MeOH; *y*-axis shows the tested rosavins contents using different extraction conditions. Each block has three bars representing different ultrasonication times varying from 10 to 20 min.

3.3.2. Precision, tailing factors, and resolution

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by testing a commercial polyherbal formulated capsules RE-1 at the same condition during the same day. The intermediate precision was studied by analyzing and comparing the assays on each of the 3 successive days. As shown in Table 2, the intra-day and inter-day RSD of retention time ranged from 0.04 to 0.92% and 0.18–1.98%, respectively. The intra-day and inter-day RSD of peak area ranged from 0.34 to 1.91% and 0.71–1.99%, respectively. The results show that the variance for both retention time and peak area for triplicate injections of the same sample analyzed on 3 successive days tended to be higher than the variance for a single day. The tailing factor of 7 reference compounds ranged from 1.01 to 1.07; the resolution ranged from 1.57 to 3.66.

3.4. Sample analysis

Four samples of dried and one sample of fresh *Rhodiola* roots, as well as 18 batches of commercial *R. rosea* roots extracts and 5 batches of commercial *E. senticosus* extracts were tested. Six batches of commercial polyherbal formulated capsules were also tested for the contents of seven active compounds. All investigated compounds were identified by comparing the retention times of the peaks with those of the reference compounds eluted in parallel under the same conditions and spiking the sample with stock standard solutions of the reference compounds further confirmed the identities of the peaks.

3.4.1. R. rosea roots and extract testing

Five samples of *Rhodiola* roots and 18 batches of *Rhodiola* commercial extract have been analyzed by the described method. Fig. 3A–C show the characteristic RRLC chromatogram of authentic *R. rosea* dried roots (H3), fresh roots (H5), and commercial extract (P5-Lot3), respectively. The optimum extraction and RRLC analysis method were applied to 18 samples of commercial *R. rosea* roots extracts collected from 8 suppliers to calculate and compare the contents of the 5 marker compounds.

Table 3 shows that 6 out of 18 (33.3%) commercial *Rhodiola* extracts namely, P1-Lot1, P3-Lot1, P6-Lot1, P6-Lot2, P8-Lot1, and P8-Lot2 are not *R. rosea* species as claimed by suppliers since the marker compounds of *R. rosea* species: rosarin, rosavin, and rosin were not detected in these samples. Five out of 18 (27.8%) commercial *R. rosea* extracts namely, P3-Lot2, P4-Lot1, P5-Lot2, P7-Lot1, and P7-Lot2 did not meet the claimed bioactive compounds' contents standards. Specifically, in these five samples, two samples (P4-Lot1and P7-Lot2) contained lower salidroside content than the claimed one; three samples (P3-Lot2, P5-Lot2, and P7-Lot1) contain lower rosavin or rosavins content.

3.4.2. E. senticosus extract testing

Table 4 summarizes the contents of eleutheroside B and eleutheroside E in five samples of commercial *E. senticosus* extracts.

Table 2
Precision, tailing factor and resolution ($n = 3$).

		Retention time (min)	Peak area	Tailing factor	Resolution[a]
Salidroside					
Intra-day	Mean	2.37	41.58	1.07	1.57
	RSD (%)	0.29%	0.34%	0.14%	0.71%
Inter-day	Mean	2.39	52.36	1.07	1.57
	RSD (%)	1.54%	1.06%	0.15%	0.84%
Tyrosol					
Intra-day	Mean	2.88	15.23	1.02	1.84
	RSD (%)	0.92%	1.56%	0.46%	0.65%
Inter-day	Mean	2.89	15.54	1.02	1.84
	RSD (%)	1.13%	1.89%	041%	0.93%
Rosarin					
Intra-day	Mean	6.79	26.41	1.01	2.41
	RSD (%)	0.48%	0.96%	0.05%	1.12%
Inter-day	Mean	6.82	26.87	1.02	2.40
	RSD (%)	0.95%	1.14%	0.14%	1.27%
Rosavin					
Intra-day	Mean	7.01	54.46	1.03	2.41
	RSD (%)	0.27%	0.65%	0.84%	1.12%
Inter-day	Mean	7.04	54.11	1.03	2.40
	RSD (%)	1.98%	0.71%	1.21%	1.27%
Rosin					
Intra-day	Mean	7.37	17.12	1.05	1.97
	RSD (%)	0.04%	0.81%	0.86%	0.93%
Inter-day	Mean	7.37	17.93	1.05	1.98
	RSD (%)	0.63%	1.25%	1.78%	1.24%
Eleutheroside B					
Intra-day	Mean	3.38	37.16	1.03	1.78
	RSD (%)	0.07%	1.91%	0.47%	1.01%
Inter-day	Mean	3.39	37.62	1.03	1.78
	RSD (%)	0.18%	1.99%	0.65%	1.39%
Eleutheroside E					
Intra-day	Mean	6.45	38.62	1.04	3.66
	RSD (%)	0.14%	1.42%	0.81%	0.87%
Inter-day	Mean	6.44	38.45	1.04	3.67
	RSD (%)	0.27%	1.96%	1.31%	1.29%

[a] Resolution is calculated using the most adjacent peak.

Table 3
The contents of 6 marker compounds: salidroside, tyrosol, rosarin, rosavin, rosin, rosavins in 18 claimed *R. rosea* roots extracts collected from 8 suppliers ($n = 3$).

Sample	Claim (mg/g)	Contents (mg/g)					
		Salidroside	Tyrosol	Rosarin	Rosavin	Rosin	Rosavins
P1-Lot1	Salidroside: 0	10.1 ± 0.01	ND[a]	ND	ND	ND	ND
P2-Lot1	Rosavins: 30	40.9 ± 0.02	4.65 ± 0.01	7.12 ± 0.02	19.64 ± 0.01	5.75 ± 0.01	32.51 ± 0.04
P3-Lot1	Salidroside: 30	30.18 ± 0.01	5.14 ± 0.02	ND	ND	ND	ND
P3-Lot2	Rosavins: 30	36.5 ± 0.02	2.49 ± 0.01	6.84 ± 0.01	15.7 ± 0.01	5.67 ± 0.01	28.21 ± 0.03
P4-Lot1	Salidroside: 10	2.97 ± 0.01	0.52 ± 0.005	1.28 ± 0.01	2.48 ± 0.01	0.68 ± 0.006	4.44 ± 0.026
P5-Lot1	Salidroside: 30	42.9 ± 0.02	2.27 ± 0.01	2.72 ± 0.01	5.32 ± 0.007	1.47 ± 0.005	9.51 ± 0.022
P5-Lot2	Rosavin: 30	36.55 ± 0.02	2.64 ± 0.01	9.39 ± 0.03	26.2 ± 0.02	6.32 ± 0.01	41.91 ± 0.06
P5-Lot3	Salidroside: 0; Rosavins: 30	22.35 ± 0.02	1.81 ± 0.01	6.39 ± 0.01	20.68 ± 0.01	4.41 ± 0.01	31.48 ± 0.03
P5-Lot4	Rosavins: 30	21.56 ± 0.01	1.84 ± 0.01	6.86 ± 0.01	20.15 ± 0.02	4.68 ± 0.01	31.69 ± 0.04
P5-Lot5	Salidroside:100	132 ± 0.04	9.35 ± 0.02	2.33 ± 0.01	3.22 ± 0.006	1.86 ± 0.004	7.41 ± 0.02
P5-Lot6	Salidroside: 30	36 ± 0.02	2.42 ± 0.01	9.32 ± 0.01	27.12 ± 0.03	6.31 ± 0.01	42.75 ± 0.05
P6-Lot1	Salidroside: 30	31.59 ± 0.01	2.83 ± 0.01	ND	ND	ND	ND
P6-Lot2	Salidroside: 20	32.3 ± 0.01	2.83 ± 0.01	ND	ND	ND	ND
P6-Lot3	Salidroside: 10	17.4 ± 0.01	1.39 ± 0.01	3.52 ± 0.01	6.02 ± 0.01	2.94 ± 0.006	12.48 ± 0.026
P7-Lot1	Rosavin: 30	27.73 ± 0.01	4.16 ± 0.01	10.86 ± 0.01	26.1 ± 0.02	6.59 ± 0.01	43.55 ± 0.04
P7-Lot2	Salidroside: 30	15.1 ± 0.01	2.22 ± 0.01	0.09 ± 0.001	0.333 ± 0.002	0.08 ± 0.001	0.5 ± 0.004
P8-Lot1	Salidroside: 10	16.3 ± 0.01	2.12 ± 0.01	ND	ND	ND	ND
P8-Lot2	Salidroside: 30	46.36 ± 0.02	4.71 ± 0.01	ND	ND	ND	ND

[a] Not detected.

Table 4
The contents of two marker compounds: eleutheroside B and eleutheroside E in five commercial *E. senticosus* extracts ($n = 3$).

Sample	Claim (mg/g)	Eleutheroside B (mg/g)	Eleutheroside E (mg/g)	Total eeutherosides (mg/g)
E1	Total eleutherosides ≥ 8	2.2 ± 0.01	6.2 ± 0.01	8.4 ± 0.02
E2	Total eleutherosides ≥ 8	2.4 ± 0.01	6.8 ± 0.01	9.2 ± 0.02
E3	Total eleutherosides ≥ 8	3.1 ± 0.02	8.7 ± 0.02	11.8 ± 0.04
E4	Total eleutherosides ≥ 8	2.5 ± 0.01	6.9 ± 0.01	9.4 ± 0.02
E5	Total eleutherosides ≥ 8	2.1 ± 0.01	5.9 ± 0.01	8.0 ± 0.02

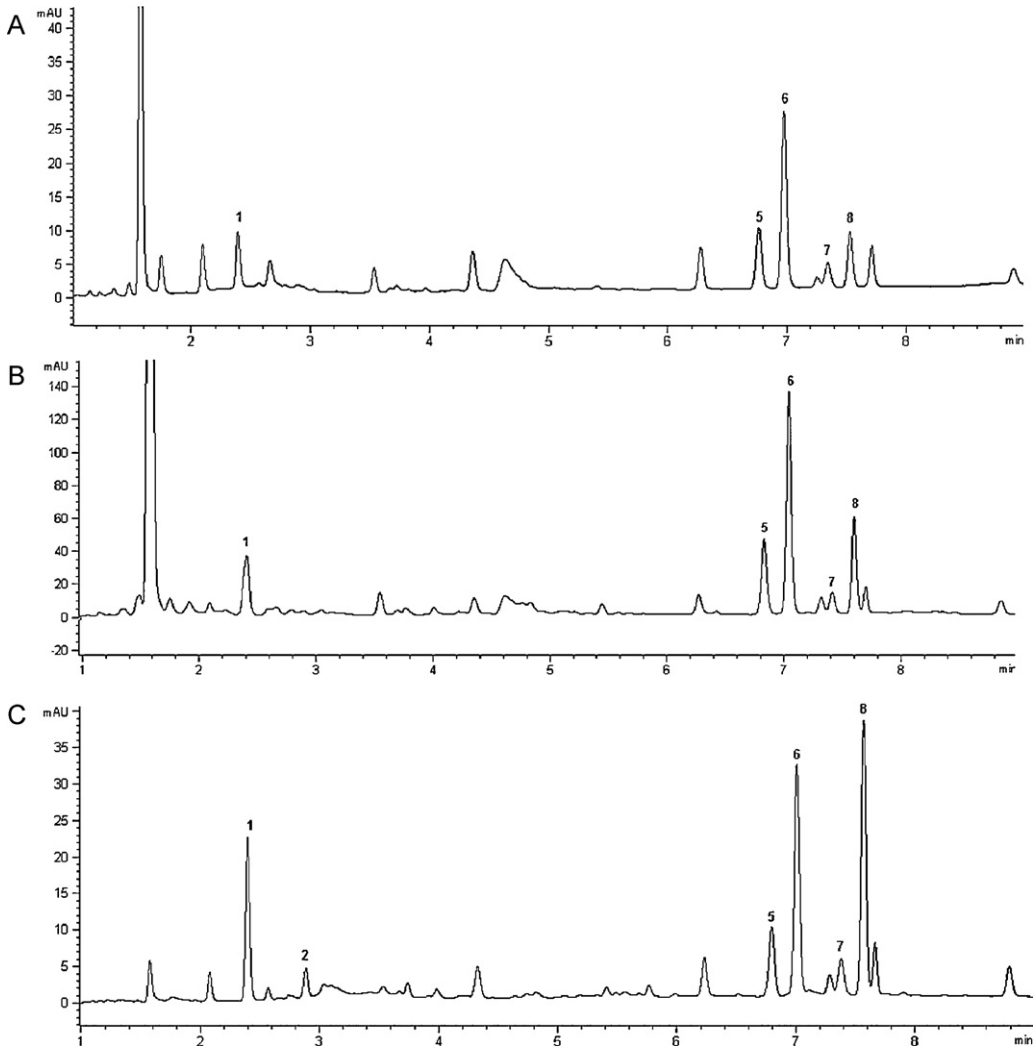

Fig. 3. RRLC chromatograms of (A) dried *R. rosea* roots (H3), (B) fresh *R. rosea* roots (H5), (C) commercial *R. rosea* extract (P5-Lot3).

The five samples claim not less than 8 mg/g of total eleutherosides content and the results are satisfactory. A typical RRLC chromatogram of *E. senticosus* extract (E1) is shown in Fig. 4.

3.4.3. Results of commercial polyherbal formulated products testing

The RRLC method developed was successfully applied to simultaneous determination of the eight marker compounds in six samples of commercial polyherbal formulated capsules. Fig. 5 shows the RRLC profile of (A) mixed marker compounds reference standards and (B) commercial polyherbal formulated capsule RE-2 (Table 5).

Although the commercial polyherbal formulated capsules RE-1, RE-2, RE-03, RE-4, RE-5 and RE-6 all contain *R. rosea* and *E. senticosus* ingredients, they show considerable variations in the contents of rosavins and total eleutherosides. The contents of rosavins and total eleutherosides B and E detected in RE-1, RE-5 and RE-6 were lower than claimed contents; in other words, three out of six samples failed the quality tests. This might be caused by variations in the manufacturing process. The developed RRLC method in this

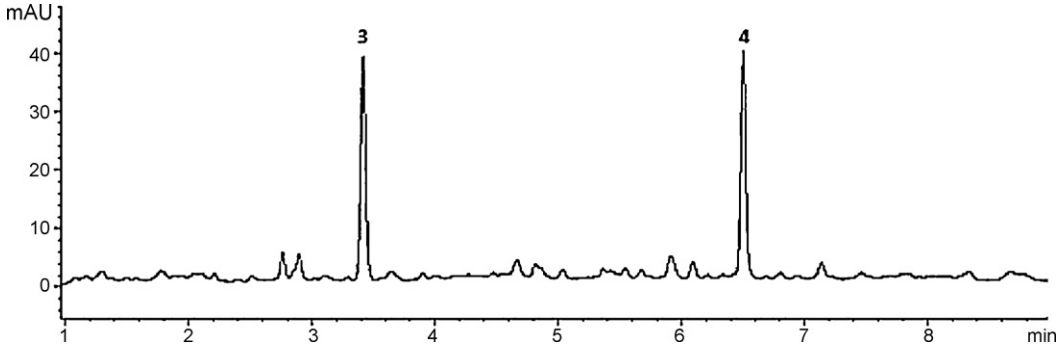

Fig. 4. RRLC chromatogram of a commercial *E. senticosus* extract (E1).

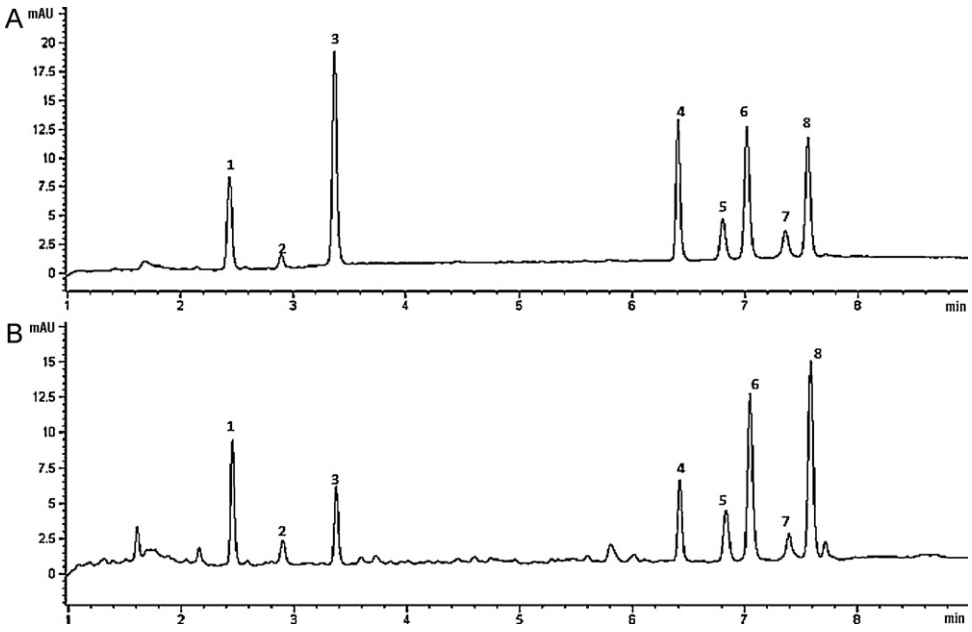

Fig. 5. RRLC chromatograms of (A) marker compounds reference standard mixture, (B) commercial formulated product capsule (RE-2).

Table 5
The marker compounds contents in six samples of commercial formulated capsules ($n = 3$).

Sample	Claims (mg/capsule)	Rosavins (mg/capsule)	Total eleutherosides (mg/capsule)
RE-1	Rosavins: 1.3 Total eleutherosides: 0.6	1.12 ± 0.01	0.58 ± 0.005
RE-2	Rosavins: 3.0Total eleutherosides: 0.44	3.15 ± 0.01	0.46 ± 0.005
RE-3	Rosavins: 0.3 Total eleutherosides: 0.8	0.32 ± 0.006	0.82 ± 0.008
RE-4	Rosavins: 0.9Total eleutherosides: 0.08	0.85 ± 0.008	0.075 ± 0.001
RE-6	Rosavins: 2.1 Total eleutherosides: 0.6	1.71 ± 0.01	0.45 ± 0.004

study could be used for fast simultaneous determination of all eight marker compounds in commercial polyherbal formulated products containing *R. rosea* and *E. senticosus*.

4. Conclusion

R. rosea and *E. senticosus* are two of the most extensively studied adaptogens in both pharmacological and clinical studies. In the present study, we have successfully developed an RRLC method to control the quality of commercial formulated products which contain *R. rosea* and *E. senticosus* components. This RRLC method is capable of simultaneously analyzing six biological compounds, i.e. salidroside, tyrosol, rosarin, rosavin, rosin, rosiridin in *R. rosea* and two biological compounds, i.e. eleutheroside B and eleutheroside E in *E. senticosus* with high resolution in a single run within 8 min. Additionally, this method was fully validated with respect to precision, repeatability and accuracy.

This method allows strict quality control not only for raw material *R. rosea* and *E. senticosus* extracts, but also for commercial polyherbal formulated products. The analyses revealed that the quality issue of commercial *R. rosea* extracts was a big concern because the characteristic bioactive compounds, i.e. rosarin, rosavin and rosin of *R. rosea* were not detected in 33.3% of the commercial *R. rosea* extract samples tested. Furthermore, there was also a big variation between the actual rosavins content and the amount claimed by suppliers. No deviation was observed in the five batches of commercial *E. senticosus* extracts quantitatively. This method was further applied to analyze six samples of commercial polyherbal formulated capsules collected on the market that contain *R. rosea* and *E. senticosus* ingredients.

Our results also showed that this readily available, rapid and reliable method is suitable for routine analysis and effective quality control of raw materials and finished products. The main advantage of RRLC is particularly a significant reduction of analysis time, which meant also reduction in solvent consumption. Our experiments showed four times analysis shortening, while solvent consumption decreased four times. According to GMP regulations, routine tests should be performed by quality control (QC) laboratory on every batch of raw materials and finished products. From this point of view, RRLC is more convenient for complex analytical determination of pharmaceutical preparations because analysis duration, solvent consumption and consequently analysis cost is a very important aspect in many QC laboratories. Therefore, RRLC is an attractive alternative to conventional HPLC technique in routine QC analysis, especially in situations where high sample throughput and fast analytical speed are needed.

Health Canada, FDA and many other Natural Health Product Regulatory Affairs departments are becoming more strict with the identification of herbs and botanical products; therefore in natural health product industry, it is crucial to establish a rapid analytical method not only to analyze a single herb, but also to evaluate and control the quality of commercial polyherbal natural health products [28].

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