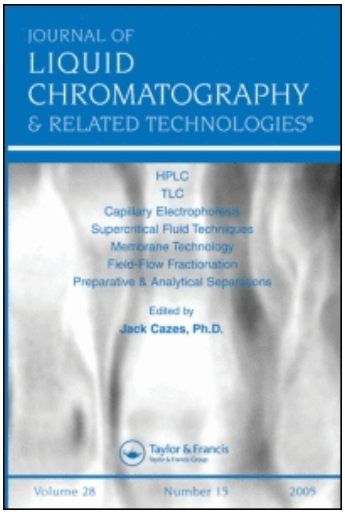

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Sensitive Determination of Saponins in *Radix et Rhizoma Notoginseng* by Charged Aerosol Detector Coupled with HPLC

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Abstract: With continuous development in analytical instruments over recent years, high performance liquid chromatography (HPLC) with various detectors has become very important tools for routine analysis and quality control of TCMs and botanical medicines. As a new "mass" detector, charged aerosol detector (CAD) provides an alternative detection method, which ultraviolet (UV) is unable to achieve for components owning almost no chromophore in its chemical structures with the lower sensitivity. With the purpose of evaluating the applicability of CAD for the analysis of TCMs, an HPLC-CAD method for simultaneous determination of saponins in *Radix et Rhizoma Notoginseng* ("Sanqi" in chinese) was established in this contribution, which was successfully applied for quantitation of seven saponins, notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rg_2, Rh_1, and Rd, in thirty batches of Sanqi samples. Meanwhile, the LODs and LOQs of UV, ELSD and CAD were compared and the results showed that

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the CAD method exhibited a lower LOD ($0.01 \sim 0.15\,\mu g$) and LOQ ($0.04 \sim 0.41\,\mu g$) than UV and ELSD. Furthermore, the CAD exhibited a steadier baseline in gradient elution compared with UV at 203 nm. Also, the HPLC-CAD method presents many apparent advantages of high sensitivity, steady baseline in gradient elution, easy operating, and it was expected to be a sensitive and universal method for analysis of TCMs containing weak UV absorption compounds. It was demonstrated that the content of seven saponins in *Radix et Rhizoma Notoginseng* did not significantly correlate with the original work nor with the conventional quality standard.

Keywords: Charged aerosol detector, evaporative light scattering detector, HPLC, *Panax notoginseng*, *Radix et Rhizoma Notoginseng*, saponins

INTRODUCTION

With the development of analytical instruments, HPLC has become a principal tool for the analysis of TCMs and botanical medicines. The UV detector was the main detector for HPLC analysis because of its steady performance and high sensitivity for those compounds with chromophores in the chemical structure. However, the analysis of terpenes, saponins, saccharides, and some alkaloids is difficult to perform by HPLC-UV for there are no chromophores in the chemical structure and the signal generated by UV is weak. So, analysis of those components in TCMs and botanical medicines has become a difficult problem in pharmaceutical analysis.

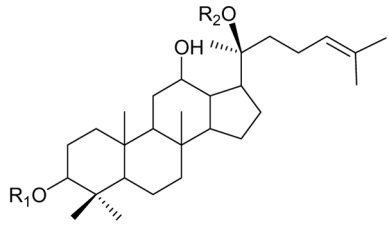
To make the analysis more accurate and precise, there continues to be a strong requirement for improvements in sensitivity, selectivity, and other performance characteristics, which to a large extent is strongly dependent on the performance of the detection. To solve the problem of the detection of compounds which has end absorption even without UV absorption, a variety of detection techniques coupled to HPLC instruments have been exploited in the quality analysis of TCMs, such as refractive index (RI), low wavelength UV, mass spectrometry (MS), and evaporative light scattering detector (ELSD). In contrast to specific detectors, the UV detector is sensitive for compounds with chromophores. However, the sensitivity is not accurate enough for those compounds with end absorption (200–210 nm) or even no chromophores in the chemical structure. Moreover, it may lead to baseline drift when gradient elution is employed. Mass spectrometry is considered as a universal detection method, but low repeatability of quantitative analysis with LC-MS, besides the high price of the instruments, limited its use for routine analysis. Refractive index (RI) detection is widely used as a universal technique, but it suffers from limited sensitivity, and it cannot be used in gradient elution analysis. ELSD is considered as a universal detector and

it can provide identification and determination of non-UV absorption compounds and presents higher sensitivity than a UV detector at 200–210 nm. However, the day-to-day reproducibility obtained with ELSD is not very good, which leads to the need for regular recalibration, especially for the determination of those with a low content of TCMs.[1] Therefore, researching and developing a new detector, which the response magnitude of analytes is independent from chemical structure or characteristics of the analytes itself, has been a key problem and a concerned topic for analysts.

Fortunately, a new "mass" detector based upon the innovative technology of charged aerosol detection has been developed by Dixson and Peterson (2002),[2] which can be used to determine compounds with weak UV absorption and low content at the same time. In the CAD, aerosol particles are charged with an ionized gas. After the removal of high mobility particles, the aerosol particles are then electrically measured. Similar to ELSD, CAD provides a universal detection for semi-volatile or nonvolatile analytes and the response is independent of analyte structural properties with or without a strong chromophore.[3] Recently, it has been used as a powerful tool for determination of oligosaccharides,[4] lipids,[5] enantiomeric ratios,[6] and pharmaceutical cleaning validation.[7] Currently, there are no reports on the examining and application of CAD on the analysis of TCMs.

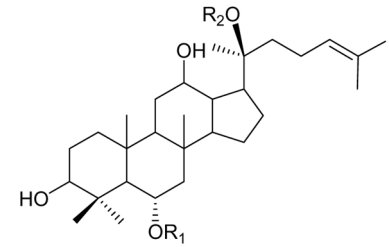
Saponins are one kind of the main effective ingredients in TCMs and botanical medicines, such as *Radix Ginseng*, *Radix Panacis Quinquefolii*, *Radix et Rhizoma Notoginseng*, and *Radix Platycodi*. Therefore, they have been considered as important chemical markers for the quality control of TCMs. However, it is difficult to determine these kinds of compounds by a UV detector, for they mostly subject to no chromophores in the chemical structure and the ultraviolet absorption is weak or only has end absorption. Till now, detection of saponins still has been a difficult issue in the analysis of TCMs.

Radix et Rhizoma Notoginseng (the root and rhizoma of *Panax notoginseng* (Burk.) F. H. Chen) also called "Sanqi" or "Sanchi", is one of the most famous traditional Chinese medicines used for promoting blood circulation. Current pharmacological studies revealed that it possessed important activities of antihypertensive, antithrombotic, antiatherosclerotic, and neuroprotective actions.[8,9] The chemical investigations certified that its main active constituents were saponins, which belongs to the type of 20(S)-protopanaxadiol (Diol) and 20(S)-protopanaxatriol (Triol),[10] see Figure 1. Therefore, these saponins were generally considered as chemical markers for the quality control of *Radix et Rhizoma Notoginseng*. Several methods have been developed for the analysis of the saponins, such as capillary supercritical fluid chromatography,[11] thin layer chromatography,[12–14] gas chromatography,[15,16] high performance liquid chromatography,[17–21] etc. Among them, HPLC coupled with different detectors was the most common used method for



Compd.	R_1	R_2
4	Glc(2-1)Glc	Glc(2-1)Glc
7	Glc(2-1)Glc	Glc

20(S)-Protopanaxadiols



Compd.	R_1	R_2
1	Glc(2-1)Xyl	Glc
2	Glc	Glc
3	Glc(2-1)Rha	Glc
5	Glc(2-1)Rha	H
6	Glc	H

20(S)-Protopanaxatriols

Figure 1. Chemical structures of seven saponins from *Radix et Rhizoma Notoginseng*.

determination of the saponins. However, previous investigations about their determination[22–25] were only focused on the separation of the saponins, such as the simultaneous determination of 11 saponins in *Radix et Rhizoma Notoginseng* by HPLC-ELSD which was performed by Jian-Bo Wan, et al. in 2005,[26] and it was recently reported that a more rapid method of ultra performance liquid chromatography coupled with photo diode array detection (UPLC-PDA) was performed within 12 min.[10]

In this contribution, with the aim of examining the feasibility and performance of CAD on the analysis of TCMs, a HPLC-CAD method has been established and it was successfully applied to the analysis of seven saponins (notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rg_2, Rh_1, and Rd) in thirty batches of samples of *Radix et Rhizoma Notoginseng*. Meanwhile, the LODs and LOQs of UV, ELSD, and CAD were

compared. Taken together, we found that there were some desirable characteristics and features in the analysis of CAD on the identification and determination of saponins. The result provides a reference for the application of CAD as a universal detector on the analysis of TCMs and botanical medicines.

EXPERIMENTAL

Chemicals and Materials

Thirty batches of Sanqi samples were collected from different places in China (Table 7) identified by Professor Pengfei Tu. The voucher specimens were deposited at the Modern Research Center for Traditional Chinese Medicine, Peking University. Analytical grade methanol was purchased from Beijing Chemical Factory (Beijing, China). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). Deionized water was prepared by using a Millipore Milli Q-Plus system (Millipore, Bedford, MA). Standards of notoginsenoside R_1(**1**), ginsenosides Rg_1(**2**), Re (**3**), and Rb_1(**4**), were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and ginsenoside Rd (**7**) was kindly provided by Dr. Xiu-Ming Cui (Wenshan Prefecture Sanqi Research Institute, Wenshan City, Yunnan Province, China). Rg_2(**5**) and Rh_1(**6**) were purchased from Zhongxi Company (Beijing, China). The purity of these reference compounds were determined to be more than 98% by normalization of the peak areas detected by HPLC analysis.

Sample Preparation

Approximately 1.0 g of the finely powered and sieved (80 mesh, 180 ± 7.6 μm) *Radix et Rhizoma Notoginseng* was refluxed with 25 mL methanol for 1.5 h. The solution was filtered through a 0.22 μm nylon filter membrane (Agilent Technologies) before the injection into the HPLC system.

HPLC Analysis

The analysis was carried out on an Agilent chromatography system (Agilent 1100 series, including a vacuum degasser, quaternary pump, autosampler, thermostated column compartment, and a UV detector and coupled with CAD (Corona^{TM}, ESA Inc., Chelmsford, MA). An Agilent 1100 HPLC instrument equipped with a Sedex 75 ELSD was used in this experiment. A Zorbax Eclipse XDB column (250 mm × 4.6 mm,

5 μm ID) from Agilent Technologies was used for separations. The mobile phase consisted of water (A) and acetonitrile (B) using the following gradient program: 0–40 min, 19% B; 40–45 min, 19–31% B; 45–75 min, 31% B; 75–80 min, 31–48% B and then equilibrated with 19% B for 10 minutes. The column temperature was kept constant at 30°C. The flow rate was set at 1.0 mL/min and the injection volume was fixed at 10 μL. The UV wavelength was monitored at 203 nm. ELSD was set to a probe temperature of 45°C, a gain of 8, and the nebulizer gas nitrogen (N_2) of 2.5 bar. The N_2 pressure of the CAD was adjusted to 35 psi and the response range was set to 100 pA.

Calibration Curves, LOD, and LOQ

The standard solutions containing from 0.025 mg/mL to 5.000 mg/mL, corresponding to test ranges of seven saponins, were prepared in parallel, 10 μL of each sample was injected into the HPLC-CAD system, and calibration curves were constructed and their linear ranges determined. Since CAD response was nonlinear[3,27] and it can be represented by $y = ax^b$, where 'y' refer to peak area, while 'x' to analyte amount, and 'b' to the exponential response factor, respectively. The slope was determined from a log-log plot of response versus concentration for these seven compounds. Therefore, calibration of CAD was constructed by a double logarithmic plot.[1,27,28] The linearity was evaluated by linear regression analysis calculated by the least square regression method. LODs and LOQs of three detectors, including CAD, ELSD, and UV, 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. All calibration curves, LOD, LOQ, of the seven saponins by three different detectors were shown in Table 3.

Precision, Repeatability, and Accuracy

The precision of the HPLC-CAD method was determined by intra- and inter-day variations. Samples containing 1.0 g of the pulverized *Radix et Rhizoma Notoginseng* were weighed, extracted, and analyzed as described in Sample Preparation Section. The intra-day variability was performed in triplicate on the same sample extracted on a single day, while the inter-day variability was carried out in triplicate in another independent sample extracted on three different days. Variations were expressed by the relative standard deviations (RSD). The recovery test was used to evaluate the accuracy of this method. Accurate amounts of seven saponins were added to 1.0 g of *Radix et Rhizoma Notoginseng*, and then extracted and analyzed. The average recoveries were determined by the

equation: Recovery (%) = (Observed amount − Original amount)/Spiked amount × 100%, and RSD (%) = (SD/mean) × 100%.

RESULTS AND DISCUSSION

Comparison of CAD, ELSD, and UV

UV spectroscopic modes of detection are often used as the workhorse in the pharmaceutical laboratory since most pharmaceutical compounds possess a chromophore. They are inexpensive, easy to use, and typically applicable. However, there are several cases, primarily when the analytes do not have a very strong chromophore, where alternative modes of detection are necessary. In 1994, the first ELSD was used for analysis in China. This type of detector can detect all solutes that are less volatile than the mobile phase. More recently, a charged aerosol detector, with a similar principle of ELSD and more sensitive than ELSD[27] was introduced. Moreover, the response factor towards different analytes is reasonably uniform at a fix chromatographic condition such as a given composition of the mobile phase for ELSD and CAD. But it was reported that they changed as the organic content of the mobile phase changes. The differences were also investigated; the CAD behaved as a mass sensitive detector whose response did not change when the effluent from the column was diluted with the compensation stream from the second pump for the mobile compensation.[29]

As QC of TCMs is becoming a hot topic worldwide, identification and determination of saponins by various approaches have been reported.[30] In this experiment, a new method was established by CAD coupling HPLC to analyze seven saponins in *Radix et Rhizoma Notoginseng*. Additionally, LODs and LOQs of three detectors (CAD, ELSD, and UV) were examined by injecting various amounts of standard compounds **1–7** (Table 3). After this, the linear ranges of CAD and ELSD for compounds **1–7** were investigated. Sample solutions with the same concentration were injected and then chromatograms were achieved from CAD, ELSD, and UV, respectively (Figure 4). By comparison of the peak area of seven compounds, it can be found that response of CAD is apparently higher than the other two, except **4**, which exhibited a fairly sensitive response on ELSD. Mean values of peak area were calculated as 7.66 times of CAD to ELSD while 16.76 times of CAD to UV. So, a conclusion can easily be drawn that response of most saponins in *Radix et Rhizoma Notoginseng* by CAD presents the highest one in the determination of saponins (Figure 5). This result is in accordance with the previous reports on CAD.[3,4,7,28,31,32]

In order to comprehensively evaluate the performance of three detectors on saponins in *Radix et Rhizoma Notoginseng*, the sensitivity

which is the main performance index of a detector was investigated. Then, analysis by HPLC coupled with UV, ELSD, and CAD were performed, respectively, under the fixed chromatographic conditions (as described in HPLC section) with the same injection of notoginsenoside R_1, ginsenosides Rg_1, and Rb_1. Sensitivity of the detector was calculated as the variation value of the peak area divided by the change of the quantity of sample injection (Table 6). It evidently showed that the mean sensitivity of CAD was 2.07 times that of ELSD.

According to the use of CAD coupled to the HPLC system, there are still some aspects which are not so satisfied for identification and quantification. For example, there are only two controllable parameters (Gas pressure and response range) on CAD which actually limit the optimization room for detection. In addition, both ELSD and CAD are operated on a similar principle involving evaporation of the mobile phase, so their flow rate cannot be set as high as UV for it cannot ensure a certain extent volatilization of mobile phase transported into detectors.

Although CAD is not as perfect as we considered, it is still the best selection for the analysis of saponins and their analogs for its remarkable sensitivity, easy to operate, and its outstanding performance on reproducibility. In the present work, HPLC coupled with CAD was applied to determine seven major saponins (**1–7**) in 30 batches of *Radix et Rhizoma Notoginseng* samples collected from different places in China. The results showed that HPLC-CAD provided benefits in terms of increasing dynamic range and sensitivity compared to other two detectors (Tables 1–3).

Extraction Method Development

It is widely recognized that sample preparation is the bottleneck of most analytical procedures. In order to obtain quantitative extraction, the

Table 1. Regression data for the seven saponins by HPLC-CAD

Compd.	Calibration curve (n = 7)	R^2	Linear range (μg)
N-R_1	$y = 1.005x + 3.449$	0.9996	0.50–17.5
G-Rg_1	$y = 0.951x + 3.496$	0.9994	0.06–11.3
G-Re	$y = 0.989x + 3.330$	0.9980	0.10–17.5
G-Rb_1	$y = 0.773x + 3.980$	0.9950	0.10–16.0
G-Rg_2	$y = 0.830x + 3.291$	0.9978	0.10–18.4
G-Rh_1	$y = 0.934x + 3.189$	0.9982	0.10–12.0
G-Rd	$y = 0.860x + 3.715$	0.9980	1.13–20.0

N-: notoginsenoside
G-: ginsenoside

Table 2. Linearity of calibration curve for seven saponins by HPLC-ELSD

Compd.	Calibration curve (n = 7)	R^2	Linear range (μg)
N-R_1	y = 1.648x + 2.626	0.9994	0.20–7.05
G-Rg_1	y = 1.569x + 2.805	0.9979	0.15–8.20
G-Re	y = 1.312x + 1.592	0.9972	0.13–5.25
G-Rb_1	y = 1.618x + 2.692	0.9916	0.10–6.90
G-Rg_2	y = 0.932x + 1.968	0.9949	1.00–18.0
G-Rh_1	y = 0.605x + 0.443	0.9961	0.50–20.0
G-Rd	y = 1.536x + 0.361	0.9948	3.00–11.0

different extraction methods, including reflux, soxhlet, and ultrasonic extraction, and several variables involved in the procedure, such as solvent and extraction time, were optimized. The water, methanol, and 95% aqueous ethanol, were used for optimization of extraction solvent. The results showed that the efficiency of refluxing extraction was the highest among the three different extraction methods (Figure 2), and methanol was the proper solvent for extraction of saponins from *Radix et Rhizoma Notoginseng* (Figure 3). Hence, refluxing extraction was chosen as a preferred method. The extraction time for the highest amount of saponins was determined at 1.5 h.

Optimization of HPLC Conditions

HPLC coupled with three different detectors were explored in this research. The selection was guided by obtaining chromatograms with better resolution of adjacent peaks. The HPLC conditions developed in this study offered full peak-to-baseline resolution of the seven saponins present in *Radix et Rhizoma Notoginseng* in one single run. The wavelength

Table 3. LOD, LOQ for seven saponins of UV, ELSD, and CAD

	UV		ELSD		CAD	
Compd.	LOD (μg)	LOQ (μg)	LOD (μg)	LOQ (μg)	LOD (μg)	LOQ (μg)
N-R_1	0.03	0.13	0.04	0.13	0.05	0.16
G-Rg_1	0.04	0.28	0.08	0.16	0.03	0.13
G-Re	0.02	0.63	0.19	0.75	0.15	0.41
G-Rb_1	0.05	0.30	0.05	0.17	0.01	0.05
G-Rg_2	0.12	0.45	0.15	0.82	0.02	0.05
G-Rh_1	0.13	0.45	0.18	0.48	0.12	0.04
G-Rd	1.83	4.60	0.08	0.30	0.02	0.15

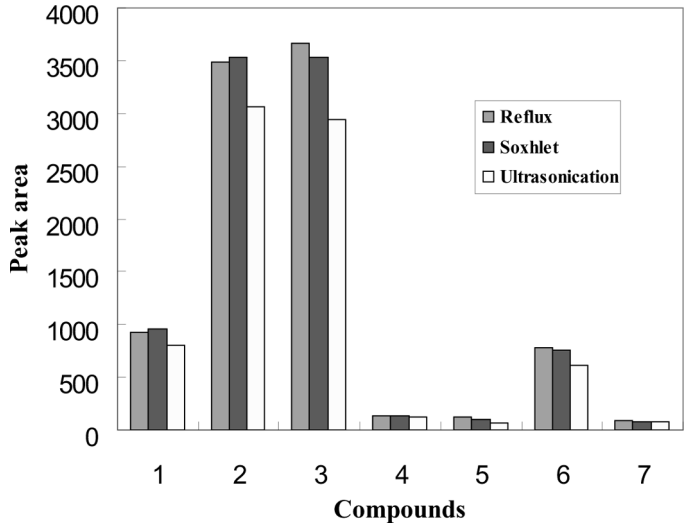


Figure 2. Extraction efficiency of saponins in *Radix et Rhizoma Notoginseng* with different sample preparation methods.

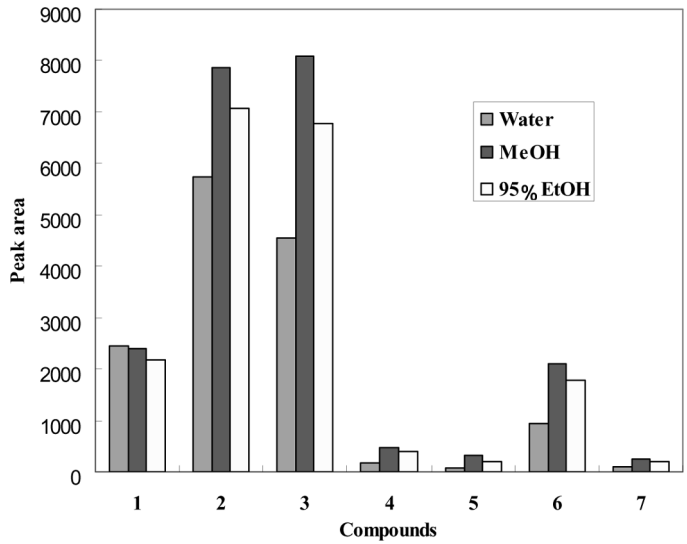


Figure 3. Extraction efficiency of saponins in *Radix et Rhizoma Notoginseng* with different solvent systems.

Table 4. Intra- and Inter-day variations of the HPLC method for determination of compounds **1–7**

	Intra-day precision (n = 3)		Inter-day precision (n = 3)	
Compd.	Content (μg/g)	RSD (%)	Content (μg/g)	RSD (%)
N-R_1	11582±179	1.55	9634±166	1.72
G-Rg_1	32681±274	0.84	27939±296	1.06
G-Re	10229±160	1.56	11278±219	1.94
G-Rb_1	17503±205	1.17	14689±267	1.82
G-Rg_2	4022±133	3.32	6421±215	3.45
G-Rh_1	5956±154	2.58	7848±223	2.84
G-Rd	7324±124	1.70	7340±130	1.78

RSD (%) = (SD/mean) × 100.

Table 5. Recovery of the HPLC method of the seven saponins

Compd.	Original (μg)	Spiked (μg)	Observed (μg)	Recovery (%)	RSD (%)	Mean (%)	SD
N-R_1	963.4	960.0	1907.1	98.3			
	963.4	770.0	1738.0	100.6	2.0	100.4	1.96
	963.4	1150.0	2138.7	102.2			
G-Rg_1	2793.9	2790.0	5491.8	96.7			
	2793.9	2230.0	5019.4	99.8	2.3	99.2	2.26
	2793.9	3350.0	6180.7	101.1			
G-Re	1127.8	1120.0	2265.8	101.6			
	1127.8	900.0	2025.1	99.7	1.4	100.1	1.39
	1127.8	1350.0	2463.0	98.9			
G-Rb_1	1468.9	1460.0	2934.7	100.4			
	1468.9	1170.0	2676.3	103.2	2.7	100.5	2.70
	1468.9	1760.0	3190.2	97.8			
G-Rg_2	624.1	620.0	1239.8	99.3			
	624.1	500.0	1096.1	94.4	2.6	96.6	2.50
	624.1	750.0	1344.1	96			
G-Rh_1	784.8	780.0	1543.0	97.2			
	784.8	620.0	1383.1	96.5	1.4	97.6	1.35
	784.8	940.0	1716.4	99.1			
G-Rd	730.4	730.0	1450.1	98.6			
	730.4	580.0	1310.9	100.1	1.8	100.3	1.81
	730.4	870.0	1619.5	102.2			

Recovery (%) = (observed amount − original amount)/spiked amount × 100, and RSD (%) = (SD/mean) × 100.

Table 6. Comparison of sensitivity of ELSD and CAD by QI, PA, ratio of S_c and S_e parameters

Compd.	CAD			ELSD			Ratio
	QI*	PA*	S_c*	QI	PA	S_e*	S_c/S_e
N-R_1	1.00	2990.60	—	2.80	2313.30	—	—
	2.00	5629.00	2638.40	3.60	3470.10	1446.00	1.82
	4.00	11325.00	2848.00	4.40	4784.80	1643.38	1.73
	5.00	14352.00	3027.00	5.20	6516.00	2164.00	1.40
	6.00	16792.00	2440.00	6.00	8664.20	2685.25	0.91
	7.50	19954.50	2108.33	7.00	10558.90	1894.70	1.11
G-Rg_1	0.70	2174.40	—	2.80	3615.65	—	—
	3.50	10727.40	3054.64	3.60	5105.05	1861.75	1.64
	7.00	20254.00	2721.89	4.40	6288.80	1479.69	1.84
	10.50	29484.20	2637.20	5.20	7650.25	1701.81	1.55
	14.00	38301.60	2519.26	6.00	9709.00	2573.44	0.98
	17.50	46131.90	2237.23	8.00	17341.20	3816.10	0.59
G-Rb_1	5.00	33449.60	—	1.60	928.00	—	—
	7.50	45316.60	4746.80	2.00	1170.00	605.00	7.85
	10.00	56400.60	4433.60	3.00	3157.40	1987.40	2.23
	12.50	66266.80	3946.48	4.00	6598.90	3441.50	1.15
	15.00	74895.30	3451.40	6.00	9850.10	1625.60	2.12
	17.50	91006.40	6444.44	7.00	11436.00	1585.90	4.06
Mean							2.07

QI: quantity of injecting sample: PA: peak area: S_c: sensitivity of CAD; S_e: sensitivity of ELSD.

of the UV detector was set at 203 nm. As to ELSD, under the fixed chromatographic conditions, the temperature of the drift tube and the flow rate of nebulizer gas (pressure) were evaluated by injections of ginsenoside Rg_1 (**2**) at different detector temperatures from 40°C to 60°C and the pressure from 2.0 to 3.5 bar. The drift tube temperature of 45°C and gas pressure of 2.5 bar were selected for detection of the analytes by comparing peak area values. These optimized parameters allow a complete solvent evaporation with negligible baseline noise. As the two controllable parameters of CAD, the N_2 pressure was set at 35 psi, according to the recommended value by the manufacturer, and the response range was set at 100 pA after a wide assay from 100 to 500 pA.

Method Validation

The linearity, regression, and precision of seven saponins were performed using the developed HPLC-CAD method. The high correlation

Table 7. Contents of saponins in different *Radix et Rhizoma Notoginseng* samples

No.	Quantitya (heads)[a]	Collected place	Content (%, w/w)[b]							
			N-R_1*	G-Rg_1	G-Re	G-Rb_1	G-Rg_2	G-Rh_1	G-Rd	T.S.
PN-1	80	Qiubei, Yunnan	0.09	2.05	0.15	2.24	0.23	0.18	0.14	5.04
PN-2	40	Qiubei, Yunnan	0.13	2.21	0.14	2.37	0.08	0.16	0.09	5.19
PN-3	80	Maguan, Yunnan	0.06	2.13	0.16	2.20	0.21	0.13	0.14	4.99
PN-4	80	Wenshan, Yunnan	0.06	2.10	0.04	3.02	0.05	0.01	0.04	5.28
PN-5	40	Maguan, Yunnan	0.05	2.09	0.06	2.22	0.18	0.15	0.10	4.85
PN-6	60	Qiubei, Yunnan	0.03	2.11	0.11	2.23	0.26	0.15	0.08	4.99
PN-7	120	Qiubei, Yunnan	0.09	2.21	0.08	2.35	0.06	0.14	0.06	5.03
PN-8	40	Qiubei, Yunnan	0.05	2.14	0.01	2.39	0.18	0.09	0.04	4.86
PN-9	80	Masupo, Yunnan	0.05	2.18	0.08	2.80	0.01	0.10	0.04	5.22
PN-10	120	Masupo, Yunnan	0.11	2.24	0.13	3.06	0.08	0.16	0.09	5.88
PN-11	40	Wenshan, Yunnan	0.06	2.06	0.20	3.30	0.30	0.17	0.14	6.19
PN-12	60	Qiubei, Yunnan	0.04	2.07	0.05	3.05	0.13	0.07	0.11	5.51
PN-13	60	Masupo, Yunnan	0.01	2.10	0.10	2.92	0.28	0.12	0.08	5.63
PN-14	40	Masupo, Yunnan	0.01	2.16	0.07	3.01	0.21	0.08	0.04	5.54
PN-15	x-1	Beijing, Market	0.03	2.12	0.09	2.20	0.21	0.12	0.11	4.87
PN-16	x-2	Beijing, Market	0.07	2.09	0.10	2.02	0.24	0.21	0.12	4.83
PN-17	x-3	Jingxi-3, Guangxi	0.13	2.27	0.19	2.18	0.13	0.20	0.16	5.3
PN-18	x-4	Jingxi-2, Guangxi	0.09	2.19	0.07	2.30	0.01	0.06	0.05	4.82
PN-19	x-5	Jingxi-1, Guangxi	0.08	2.07	0.15	3.04	0.29	0.16	0.14	5.89
PN-20	80	Xilin county, Baise	0.16	2.29	0.14	3.19	0.02	0.04	0.13	5.94
PN-21	80	Longlin village, Tianlin county, Baise	0.16	2.29	0.13	2.79	0.02	0.03	0.09	5.52
PN-22	80	Baile village, Tianlin county, Baise	0.15	2.29	0.14	3.20	0.05	0.04	0.11	5.97

PN-23	80	Xintan village, Jingxi county, baise	0.13	2.28	0.15	3.12	0.04	0.03	0.11	5.85
PN-24	80	Longlin county, Baise	0.19	2.30	0.15	2.80	0.03	0.06	0.15	5.73
PN-25	80	Baiyunshan village, Debao county, Baise	0.15	2.29	0.15	3.19	0.03	0.02	0.09	5.93
PN-26	80	Baiyunshan village, Debao county, Baise	0.16	2.30	0.15	3.22	0.05	0.05	0.12	6.03
PN-27	80	Napo village, Tianyang county, Baise	0.17	2.29	0.18	2.21	0.05	0.02	0.13	5.02
PN-28	80	Longsang village, Tianyang county, Baise	0.17	2.29	0.16	2.23	0.04	0.03	0.13	5.02
PN-29	80	Silin village, Tiandong county, Baise	0.16	2.29	0.17	2.51	0.05	0.03	0.14	5.31
PN-30	80	Pingguo county, Baise	0.15	2.26	0.16	3.19	0.10	0.12	0.12	6.08
Mean content			0.10	2.19	0.12	2.69	0.12	0.10	0.10	5.32

[a]Quantity (heads): the head numbers of Sanqi per 500 g.

[b]Content: mean value of samples ($n = 2$).

"x": means those medicinal substances which were purchased from different drugstores or from different markets and the specification of them were not so clear in detail.

T.S.: means total content of the seven saponins in *Radix et Rhizoma Notoginseng*.

coefficient ($R^2 > 0.995$) values indicated good correlations between investigated compounds concentrations and their peak areas within the test ranges (Table 2). Both intra-day and inter-day repeatability (R.S.D.) of 7 peaks area detected for the investigated components were less than 4.0% (Table 4). Besides, validation studies shown in Table 5 suggested the analytical method we developed had good accuracy with the overall recovery of 94.4–103.2% for the analytes concerned. Therefore, the HPLC-CAD method was precise, accurate, and sensitive for simultaneous quantitative evaluation of seven major saponins in *Radix et Rhizoma Notoginseng*.

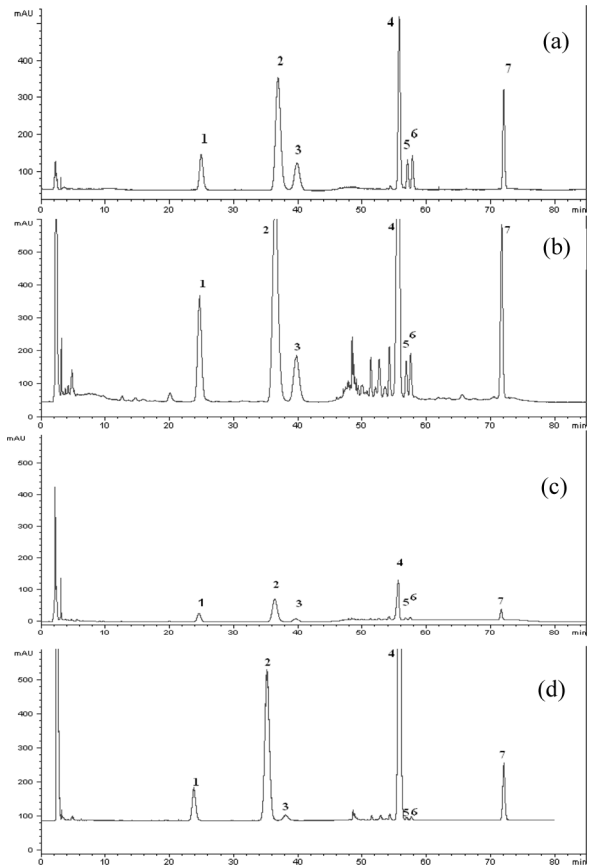


Figure 4. Typical HPLC chromatograms of mixed standards with CAD (**A**) and methanol extracts of *Radix et Rhizoma Notoginseng* with the detectors of CAD (**B**), UV (**C**), and ELSD (D). Compd.1–7: Notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rg_2, Rh_1, and Rd, respectively.

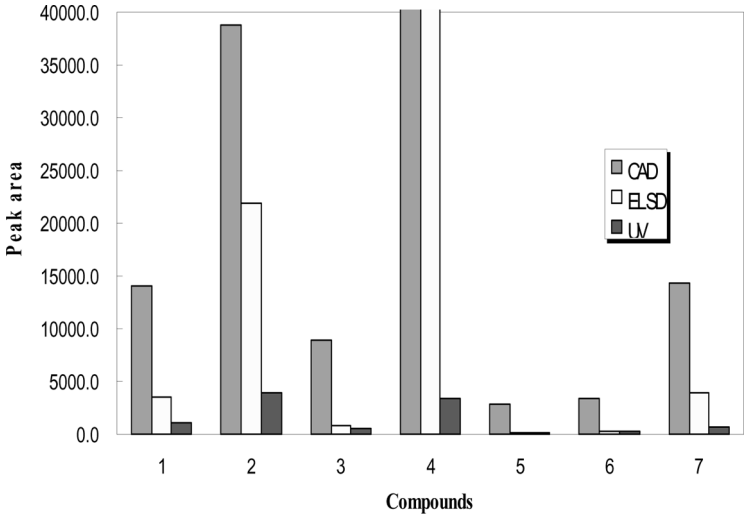


Figure 5. CAD, UV, and ELSD response of seven investigated saponins in *Radix et Rhizoma Notoginseng*.

Identification and Quantitation of Investigated Compounds

The identification of investigated compounds was carried out by comparison of their retention times on chromatograms generated from different detectors with those standards in the same condition, and by spiking the samples with stock standard solutions. Using the optimized HPLC condition coupled with CAD, the contents of seven target compounds in *Radix et Rhizoma Notoginseng* from different habitats were determined (Table 7). Their representative chromatograms are shown in Figure 4. Among the thirty investigated samples, the mean content of compounds **1–7** are 0.10%, 2.19%, 0.12%, 2.69%, 0.12%, 0.10%, and 0.10%, respectively. The results showed that ginsenosides Rg_1 (**2**) and Rb_1 (**4**) are the two major constituents in *Radix et Rhizoma Notoginseng* while the content of others were relatively lower.

According to traditional Chinese custom, Sanqi was divided into different quantity types by its weight and color, such as 40 heads (numbers of Sanqi per 500 g), 60 heads, 80 heads, etc. As common sense, it was considered that the smaller of the heads number (the bigger in size), the better the quality is. Content comparison of total saponins among different heads of the investigated samples was shown with the single factor analysis of variance. The statistical analysis was performed by SPSS 11.5 for windows (SPSS Inc., Chicago, IL, USA) and the output showed that the value of F was 4.956 while P was 0.181. Upon the results of the seven

saponins examined, it can be concluded that there was no close linkage between the content of saponins and the head quantities of Sanqi, and investigation of different samples showed that there is no obvious difference on the content of saponins, which were collected from different places or drugstores.

CONCLUSIONS

This is the first report on the determination of seven major saponins in *Radix et Rhizoma Notoginseng*, namely notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, Rg_2, Rh_1, and Rd by a HPLC-CAD method. It is also the first application of CAD coupled with HPLC for the qualification of TCMs. A detailed comparison of this method with HPLC-UV and HPLC-ELSD in terms of several parameters proved that this newly developed HPLC-CAD method owned the superiority of good reproducibility and excellent sensitivity for saponins determination. This newly established method was successively applied to determine seven major saponins in 30 batches of *Radix et Rhizoma Notoginseng* from different places in China. As a relative universal and powerful analysis method, the HPLC-CAD is expected to be applied for the determination of saponins and those compounds with weak or even no UV absorption in other TCMs or herbal medicines.

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