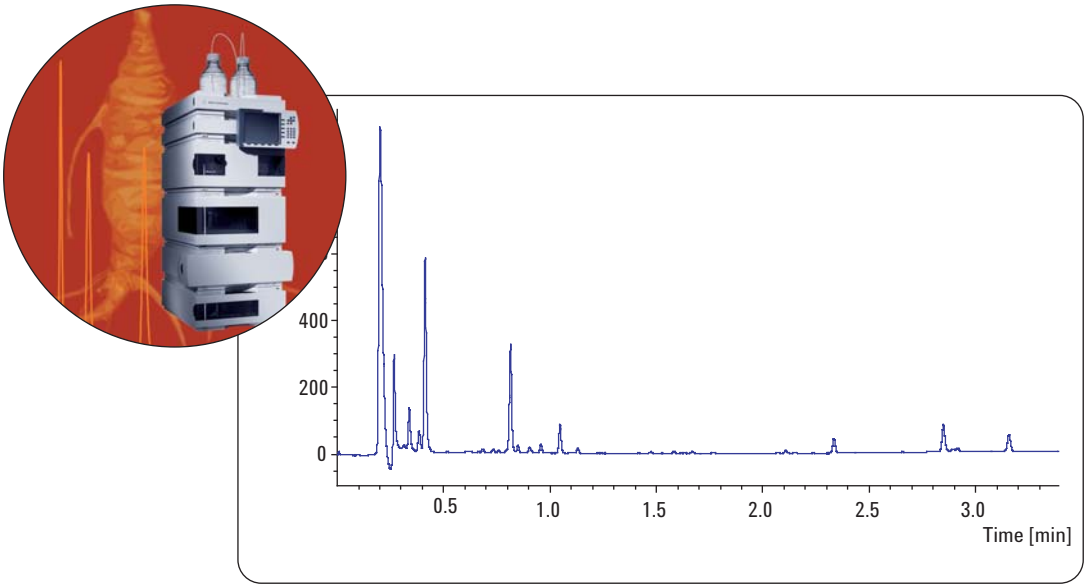
Fast, high-resolution analysis of notoginseng by rapid resolution liquid chromatography

Application Note

Zhi-xiu Xu



Abstract

This Application Note describes:

- The development of rapid resolution liquid chromatography (RRLC) method for the analysis of notoginseng.
- The results of method transfer from conventional HPLC to RRLC.
- The use of the RRLC method to shorten run times while maintaining good resolution of complex components, thereby increasing sample throughput and lowering costs.
- The chromatograms obtained with optimized methods for the different parts of the notoginseng plant, which show different peak profiles and different concentrations of certain saponins.

Agilent Equipment:
1200 Series Rapid Resolution LC system ZORBAX XDB C18 RRHT column

Application Area:
Traditional Chinese Medicine

Agilent Technologies

Introduction

Traditional Chinese Medicines (TCMs) have a long history of use in China and their therapeutic effects are well known in China and other Asia countries such as Korea and Japan. In Western countries the use of TCMs as food supplements or nutriments is becoming more and more popular. More than 11,000 kinds of TCMs have been used over time. Research and quality control of TCMs rely heavily on instrumental separations and the performance of these separations.

In this study a rapid resolution liquid chromatography (RRLC) method for the analysis of notoginseng was developed. The conventional HPLC analysis method was transferred easily to RRLC using Agilent's method translator. Different extraction methods were used to produce different samples from different parts of the notoginseng plant. These samples were separated with optimized methods and the resulting chromatograms showed different peak profiles. This Application Note also shows the quantitative results. Using the faster and better methods developed in this study, quality control departments will be able to reduce analysis times and increase sample throughput. Using these methods also reduced the cost of solvent as well as improved the overall analysis process.

Notoginseng, which is also known as in Chinese as Sanqi or Tianqi, is an important and highly valued traditional medicine in China. It has been cultivated for about 400 years and more than 85 % of the notoginseng production originates from the Yunnan Province, China. Notoginseng is known for its
efficiency in promoting blood circulation, removing blood stasis, inducing blood clotting, relieving swelling and alleviating pain. Current pharmacological studies revealed that Panax notoginseng possesses anticarcinogenic and hepatoprotective properties, as well as protective effects on cardiovascular and cerebrovascular systems[1]. Compared with the well known Panax ginseng and Panax quinquefolium (American Ginseng), the profile of the saponins in Panax notoginseng is similar because they belong to the same genus. Notoginseng is used mostly in south China in different traditional Chinese formulations. The famous traditional Chinese formulations Yun Nan Bai Yao and Pien Tze Huang also contain Panax notoginseng.

The complex matrixes of TCMs always present major challenges for quality control and research. The 2005 edition of the China Pharmacopeia lists Materia Medica Sanqi and Chinese patent medicines such as Sanqi Shangyao Pian as containing notoginseng saponin R1, which must be analyzed in these formulations. The typical run time for the analysis of notoginseng saponin is longer than 60 minutes. With more and more research on TCMs, scientists realized that control of only one or two components is not enough to determine the quality of a TCM and that it is necessary to develop other quality control methods that separate more components. Separating complex samples such as TCMs requires longer analysis times than synthetic drugs. When sample throughput is also an issue, having similar or better performance with shorter analysis times would be an ideal solution for this situation. With RRLC it is possible to develop methods with shorter run times and with better performance. RRLC methods also give users the benefits of time and solvent savings. For manufacturing quality control, using RRLC means batches of products can be released faster compared with using conventional HPLC methods

Experimental

Equipment

For development of the RRLC method an Agilent 1200 Series Rapid Resolution LC system with the following modules was used:

Agilent 1200 Series RRLC system consisting of:

- Agilent 1200 Series binary pump SL with vacuum degasser
- Agilent 1200 Series high-performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series diode array detector SL with micro flow cell (2 µL volume, 3 mm path length)
- Agilent ChemStation B.02.01 SR1 for data acquisition and evaluation
- Agilent ZORBAX XDB C18 RRHT column, 4.6 x 50 mm, 1.8 µm particle size

For comparison with conventional HPLC a standard configuration of an Agilent 1100 Series LC with the following modules was used:

- Agilent 1100 Series binary pump with degasser
- Agilent 1100 Series autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode array detector
- Agilent ZORBAX SB C18 column, 4.6 x 50 mm, 5 µm particle size

Samples and sample preparation

- Notoginseng saponin R1, ginsenoside Rg1 and ginsenoside Rb1 standards were purchased from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP), China. Gensenoside Re and ginsenoside Rc standards were purchased from Sigma-Aldrich, USA.
- Notoginseng caudexes and leaf extracts (NCLE) were kindly provided by a customer's laboratory. The caudexes and leaves were extracted with 60% ethanol, the solvent was evaporated to dryness and the residue dissolved in n-butanol, the solvent was then evaporated to dryness again and the residual yellow powder was dissolved in methanol and used for injection.
- Notoginseng root extracts (NRE) were kindly provided by a customer's laboratory. The raw components were extracted with water, filtered through a marcoporous membrane and the filtrate evaporated to dryness. The residual white powder was dissolved in methanol and used for injection.
- Notoginseng was purchased from a local drug store. The brand was Tong Ren Tang. The raw material was extracted with water/methanol (30/70, v/v), treated ultrasonically for 30 minutes, filtered through a 0.22 µm membrane and the clear filtrate was used for injection.
- Water, acetonitrile and methanol were purchased from Fisher, USA.

Method translation

The conventional HPLC method using the column packed with 5 µm particles needed to be transferred to an RRLC method using a column packed with 1.8 µm particles. Agilent's method translator (available on CD for Agilent 1200 Series Rapid Resolution LC system, publication number 5989-5130EN) was used for this purpose and made the process easy and quick (figure 1). Depending on individual requirements it might be necessary to make further modifications to the transferred method based on the different factors listed by the method translator. For details of how to translate manually a conventional HPLC method to an RRLC method, further Application Notes are available from Agilent[2,3,4].

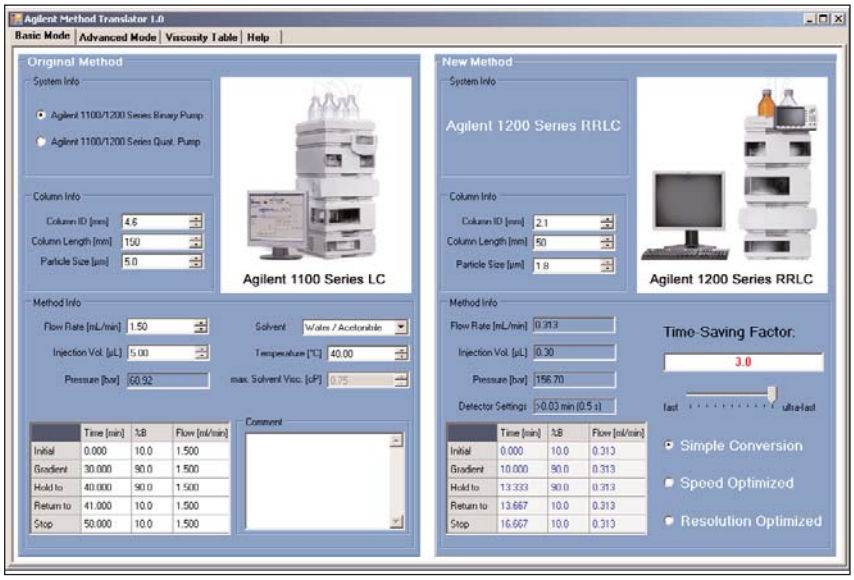


Figure 1
Agilent's method translator for transfer of standard HPLC methods to RRLC technology.

Results and discussion

Chromatograms

According to results of pharmacological studies, different parts of the notoginseng plant have different therapeutic effects as a result of different components being present. Specific extraction methods were used with the objective of obtaining more effective components. The notoginseng samples were analyzed with conventional HPLC methods and RRLC methods to reveal different peak profiles. Figure 2 shows the chromatogram of notoginseng caudexes and leaf extracts (NCLE) analyzed using the conventional HPLC method. Typically, this type of separation requires more than 60 minutes[5]. As described in the experimental section, the RRLC method used for analyzing the notoginseng samples was developed based on the conventional HPLC method. The same NCLE sample was analyzed with the conventional HPLC method and the RRLC method 1. The chromatograms are shown in figures 2 and 3. The peaks in figure 3 were narrower than those in figure 2. This means that the peak capacity increased for the com-

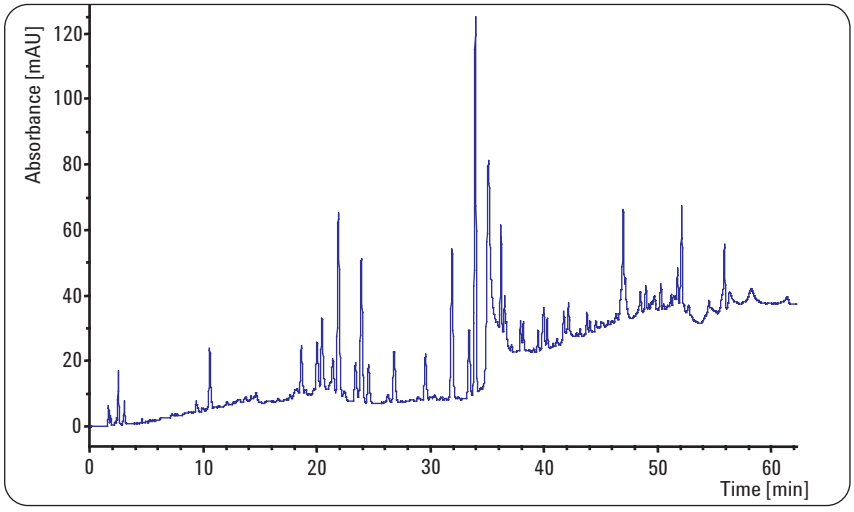


Figure 2
Chromatogram of NCLE analyzed using a conventional HPLC method on an Agilent 1100 Series LC system. See table 1 for chromatographic conditions.

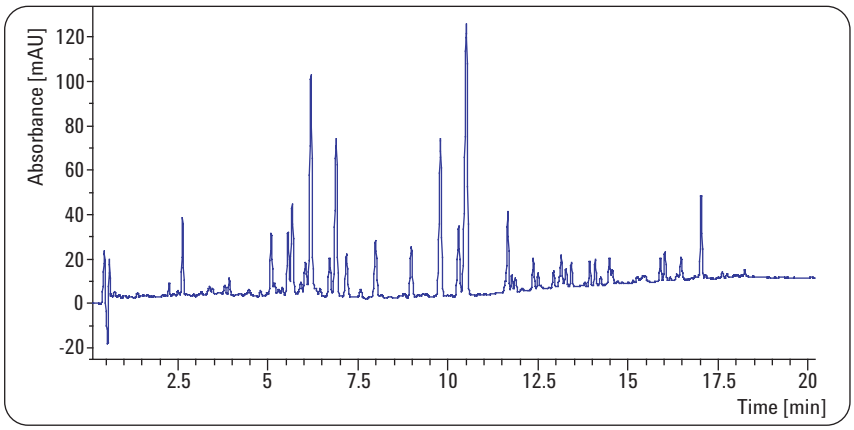


Figure 3
Chromatogram of NCLE, analyzed using RRLC method 1 on an Agilent 1200 Series RRLC system. See table 1 for chromatographic conditions.

Chromatographic conditions for traditional method with Agilent 1100 Series LC:		**Chromatographic conditions for RRLC method 1:**		**Chromatographic conditions for RRLC method 2:**		**Chromatographic conditions for RRLC method 3:**	
Column:	Agilent ZORBAX SB-C18, 4.6 x 250 mm, 5 µm particle size	Column:	Agilent ZORBAX XDB C18, 4.6 x 50 mm, 1.8 µm particle size	Column:	Agilent ZORBAX XDB C18, 4.6 x 50 mm, 1.8 µm particle size	Column:	Agilent ZORBAX XDB C18, 4.6 x 50 mm, 1.8 µm particle size
Mobile phase:	A: Water; B: ACN	Mobile phase:	A: Water; B: ACN	Mobile phase:	A: Water; B: ACN	Mobile phase:	A: Water; B: ACN
Gradient:	0 min, 20 %B; 10 min, 30 %B; 20 min, 35 %B; 30 min 40 %B; 40 min, 60 %B; 50 min, 100 %B	Gradient:	0 min, 20 %B; 3 min, 30 %B; 7 min, 35 %B; 10 min 40 %B; 13 min, 60 %B; 17 min, 100 %B	Gradient:	0 min, 20 %B; 1.5 min, 30 %B; 5 min, 35 %B; 7 min 40 %B; 10 min, 60 %B; 12 min, 100 %B	Gradient:	0 min, 30 %B; 2.5 min, 60 %B; 5 min, 100 %B
Flow rate:	1.0 mL/min	Flow rate:	1.0 mL/min	Flow rate:	1.5 mL/min	Flow rate:	2.5 mL/min
Injection volume:	5 µL	Injection volume:	5 µL	Injection volume:	5 µL	Injection volume:	5 µL
Diode array detection:	203 nm ±8 nm, Ref. 360 nm ±100 nm	Diode array detection:	203 nm ±8 nm, Ref. 360 nm ±100 nm	Diode array detection:	203 nm ±8 nm, Ref. 360 nm ±100 nm	Diode array detection:	203 nm ±8 nm, Ref. 360 nm ±100 nm

Table 1
Chromatographic conditions.

plex samples. The resolution or separation performance of the RRLC analysis was better than conventional HPLC. At the same time the run time was reduced dramatically from 60 to 20 minutes. Another factor that should be considered is the selectivity of the columns for conventional HPLC and RRLC, which should be kept the same. If the new RRLC column has totally different selectivity properties than the conventional HPLC column, transferring the method makes no sense. In this study peak profiles and elution sequence were the same so that it was not necessary to identify the peaks again in the new chromatogram. Further, the UV spectra from the diode array detector helped to do further confirmation.

Figure 4 shows the chromatogram of the NCLE sample analyzed by RRLC method 2. Increasing the flow rate to 1.5 mL/min reduced the run time. At the same time it was possible to maintain the same or comparable performance as the results shown in figure 3. For more complex samples, shorter run times mean less peak capacity. Hence users should choose a balance between peak capacity and run time.

As mentioned in the experimental section, three kinds of samples were obtained from different origins. Figure 5 shows the chromatogram of the notoginseng root extraction (NRE) sample analyzed with the RRLC method 1. This sample was extracted from the notoginseng root using a different procedure. The peak profiles show that NRE and NCLE contain different kinds of saponins and other components.

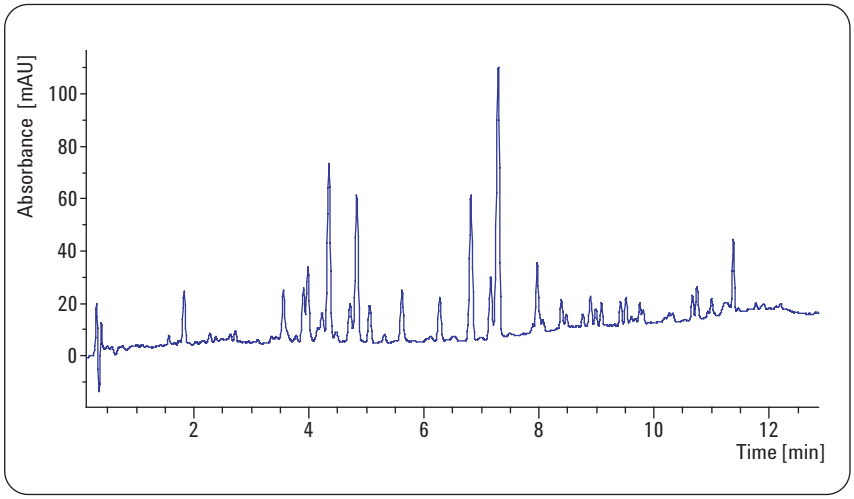


Figure 4
Chromatogram of NCLE, analyzed using RRLC method 2 on an Agilent 1200 Series RRLC system. See table 1 for chromatographic conditions.

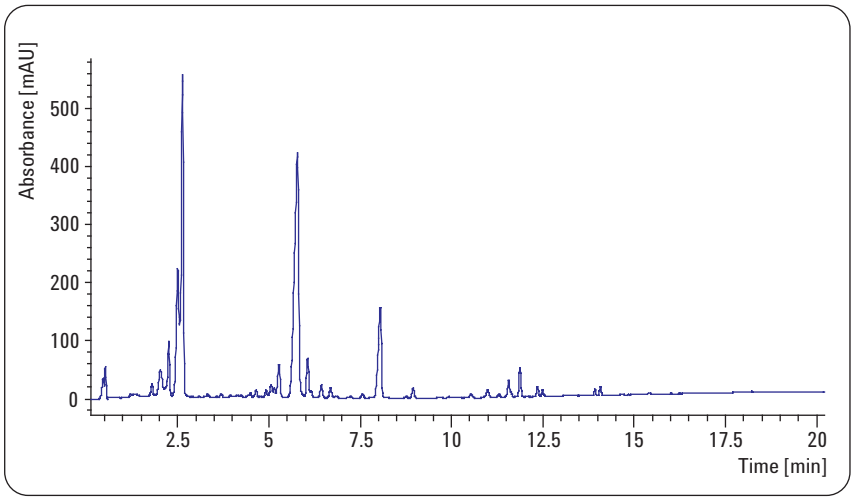


Figure 5
Chromatogram of NRE, analyzed using RRLC method 1 on an Agilent 1200 Series RRLC system. See table 1 for chromatographic conditions.

To test if the developed RRLC method could be used with other notoginseng samples, we purchased notoginseng from a local TCM store. The brand was Tong Ren Tang and the notoginseng had already been ground to powder at TCM store. The chromatograms of the notoginseng analyses are shown in figures 6 and 7. RRLC method 1 was used to obtain the chromatogram in figure 6 and RRLC method 3 was used in figure 7. Compared with the conventional HPLC method, which needs more than 60 minutes, the chromatogram in figure 7 demonstrates that the analysis can be done in 4 minutes.

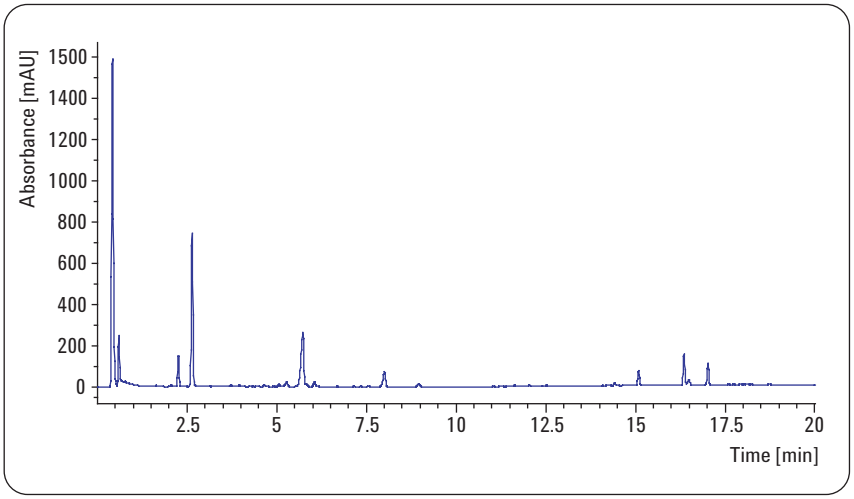


Figure 6
Chromatogram of notoginseng from Tong Ren Tang, analyzed using RRLC method 1 on an Agilent 1200 Series RRLC system. See table 1 for chromatographic conditions.

Notoginseng caudexes and leaf extracts are more complex than the other samples analyzed and RRLC method 1 or 2 should be used to obtain analysis times while maintaining resolution. For less complex samples such as Tong Ren Tang notoginseng extractions, the faster method can be used to complete the analysis in a shorter time as shown in figure 7.

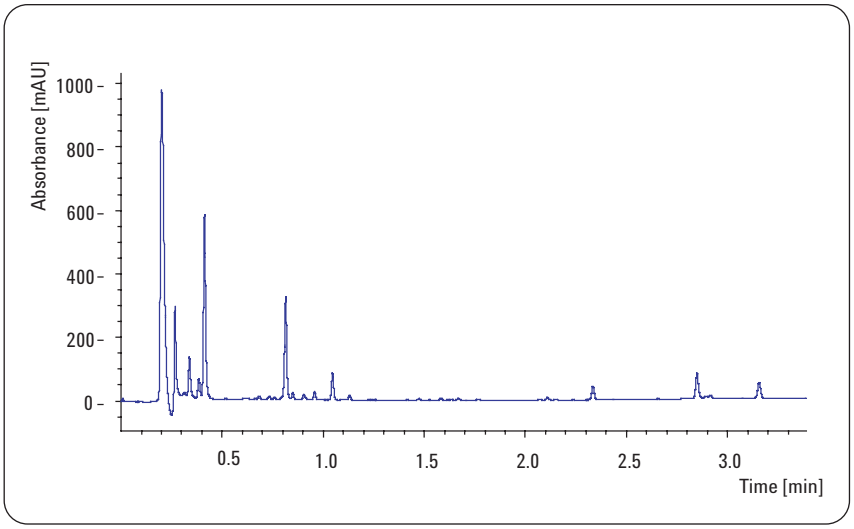


Figure 7
Chromatogram of notoginseng from Tong Ten Tang, analyzed using RRLC method 3 on an Agilent 1200 Series RRLC system. See table 1 for chromatographic conditions.

Quantitative results

The standards listed in the experimental section were analyzed using RRLC method 2. Using different levels of concentrations of the standards, a correlation curve was created and used to determine the concentrations of the three samples. Table 2 shows the quantitative results. NCLE contained many kinds of saponins but did not contain Rc or Re. NRE contained fewer kinds of saponins but did contain the five standards analyzed in this study. The Tong Ren Tang notoginseng extraction did not contain many kinds of saponins and did not contain Rc or Re. The concentrations of R1, Rb1 and Rg1 were higher than in NCLE and NRE. The concentration of notoginseng R1 ranged from 0.029 to 0.89 µg/µL across all samples. The quantitative method developed in this study can be deployed easily in the quality control of notoginseng with different extraction methods.

Standard	Formula Y: peak area x: amount (0.1 µg/µL)	Correlation	Concentration in NCLE (µg/µL)	Concentration in NRE (µg/µL)	Concentration in Tong Ren Tang notoginseng powder (µg/µL)
R1	Y = 54.03850x + 0.395722	0.99995	0.029	0.48	0.89
Rb1	Y = 71.64751x – 0.159297	1.0000	0.18	4.64	2.136
Re	Y = 93.85331x + 3.83488	0.99982	N/A	1.28	N/A
Rg1	Y = 151.66579x + 0.501417	1.0000	0.07	1.52	1.54
Rc	Y = 11.12002x + 0.128832	0.99980	N/A	0.025	N/A

Table 2
Quantitative results of the saponin standards.

Conclusion

Traditional Chinese Medicines are complex natural products and their analysis by conventional HPLC requires a high performance system and long run times. The RRLC methods developed in this study demonstrated how to shorten run times while maintaining good resolution of complex components. The chromatograms show the different peak profiles corresponding to the different notoginseng extraction samples. Taking the requirements of the application into consideration, RRLC with a faster flow rate can be deployed to achieve a faster analysis method. The quantitative methods developed in this study can be implemented easily as a quality control method for notoginseng products. Using RRLC methods maintain or give even better performance while at the same time reducing the run time dramatically, thereby increasing sample throughput and saving costs.

References

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