Applications of Ultra-Performance Liquid Chromatography to Traditional Chinese Medicines

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Abstract

Ultra-performance liquid chromatography (UPLC), as a development of the chromatographic technique, was used in the quality control of Panax ginseng C.A. Mey. In the study, the UPLC methods were transferred from conventional high-performance liquid chromatography (HPLC) methods according to chromatographic equations. The results demonstrated that UPLC analysis methods could be transferred from HPLC without loss of efficiency. Compared with conventional HPLC, UPLC made a surprising 10-fold increase in speed and 20-fold decrease in solvent consumption. The study indicated UPLC as a suitable alternative to HPLC and can be reliably applied to quality control of traditional Chinese medicines.

Introduction

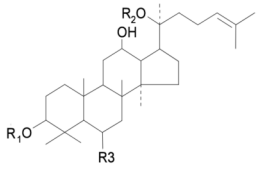
Traditional Chinese medicines and UPLC

In the field of chromatographic analysis, the identification and quantification of components of traditional Chinese medicines (TCM) is one of the main challenges for high-performance liquid chromatography (HPLC). TCM, singularly or in combinations, contain a myriad of compounds in complex matrices in which no single active constituents is responsible for the overall efficacy (1). It is required to determine several ingredients synchronously to quality control the TCM. In the Chinese Pharmacopeia (Ch.P), the analysis of ginsenosides of *Panax ginseng* C.A. Mey. is one of the most tedious chromatographic tasks (2). The multi-step gradient elution is used to quantify the contents of ginsenoside Rg1, Re, and Rb1. It is well-known that the ginsenosides are triterpene saponins derived from the tetracyclic dammarane skeleton and are divided into two main groups: protopanaxadiols, such as Rb1, Rc, and Rd, as well as protopanaxatriols such as Rg1 and Re (Figure 1). Because of the complexity of the compounds, the whole gradient time is more than 110 min, including 100 min for separation elution and 10 min for re-equilibration. Customarily, *Panax ginseng* is used as a main ingredient in many formulae. The combination of two or more herbals and considering its complexities, the analysis of *Panax ginseng* in Ch.P is just the simplest thing.

Recently, ultra-performance liquid chromatography (UPLC), as a development of the chromatographic technique, shows advantages in fast analysis without reducing separation efficiency (3–10). The adjustments of the UPLC instrument have been introduced in previous references (5), all which mention the advancements of speed, resolution, and sensitivity. However, few studies to date have compared the methods of UPLC and HPLC in the analysis of TCM (9,10). The main aim of this work was to make comparison of UPLC and HPLC analyses in evaluating ginsenosides of *Panax ginseng*, an important TCM.

Method transfer process

The main developments of UPLC are the column packed with bridged ethylsiloxane/silica hybrid (BEH) materials, and the particle size is 1.7 µm. The BEH can resist the maximum back-pressure up to 15,000 psi, which is a breakthrough compared to conventional HPLC. Fortunately, it does not change the chemistry of stationary phases for its identical ligands, such as octadecylsilyl, in BEH with conventional column packed materials. It is the base of method transfer process. The injection



Saponins	R1	R2	R3
Ginsenoside Re	-H	-Glc	-O-Glc2-Rha
Ginsenoside Rg1	-H	-Glc	-O-Glc
Ginsenoside Rb1	-Glc2- Glc	-Glc6- Glc	-H
Ginsenoside Rb2	-Glc2- Glc	-Glc6- Ara(p)	-H
Ginsenoside Rc	-Glc2- Glc	-Glc6- Ara(f)	-H
Ginsenoside Rd	-Glc2- Glc	-Glc	-H

Figure 1. Structures of the main saponins in Panax ginseng and Panax quinquefolium. Glucose, glc; Rhamnose, Rha; Arabinose in pyranose form, Ara(p); Arabinose in furanose form, Ara(f).

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volume and the flow rate, two main parameters in the transfer process, have to be modified according to the column dimensions (11,12).

The target injected volume (V_T) can be calculated according to the following equation:

$$V_T = V_O \times \frac{d^2_T}{d^2_O} \times \frac{L_T}{L_O} \quad \text{Eq. 1}$$

where O and T refer to the original and target methods, respectively, and where d and L are the diameter and length of the columns, respectively.

In order to get identical analysis efficiencies and resolutions, the flow rate must be adjusted in proportion to the square ratio of the column diameters for constant linear velocity. Therefore, the target flow rate (F_T) can be calculated according to the following equation:

$$F_T = F_O \times \frac{d^2_T}{d^2_O} \quad \text{Eq. 2}$$

The multi-step gradient elution can be decomposed as a combination of multi-segment, and each segment duration should be scaled in proportion to the column volume and the reciprocal of the flow rate. For the slope segment, the initial and final gradient composition must be constant, and each step gradient time can be calculated as:

$$t_{n \times T} = t_{n \times O} \frac{F_O}{F_T} \times \frac{V_{col \times T}}{V_{col \times O}} \quad \text{Eq. 3}$$

where $t_{n\cdot O}$ and $t_{n\cdot T}$ are the No.n segment duration of the original and target gradient elution, respectively. $V_{col\cdot T}$ and $V_{col\cdot O}$ are the volumes of the original and target columns, respectively. The previous equations have been used in the study for scaling the primitive parameters of UPLC according to HPLC.

Table I. Gradient Mobile Phases of UPLC and HPLC

Mobile phase	Acetonitrile (%)	18	18	29	29	40	19	19
	Water (%)	82	82	71	71	60	81	81
Elution Time (min)	HPLC (min)*	0	35	55	70	100	101	110
	UPLC (min)†	0	8.8	13.8	17.5	25	25.1	27
	UPLC (min)‡	0	3.7	5.8	7.4	10.5	10.6	11.5

* Flow rate = 1.0 mL/min. † Flow rate = 0.21 mL/min. ‡ Flow rate = 0.50 mL/min.

Table II. Comparisons of System Suitability Data (SST) of HPLC and UPLC

Methods	HPLC			UPLC					
Flow ratio (mL/min)	1.00			0.21			0.50		
Standards	Rg_1	Re	Rb_1	Rg_1	Re	Rb_1	Rg_1	Re	Rb_1
Resolutions	–	3.2	1.8	–	2.1	2.3	–	1.8	1.7
Retention factors	33.7	37.7	76.3	36.6	39.5	82.1	17.4	18.9	40.3
Symmetry factors	0.99	0.95	0.86	1.03	0.96	0.91	1.03	1.02	0.95
Selectivity factors	–	1.12	1.02	–	1.08	1.02	–	1.09	1.02
LOD (ng)	3.3	3.6	4.5	1.0	1.0	1.5	1.0	1.0	1.5
LOQ (ng)	11	12	15	3.3	3.3	4.5	3.3	3.3	4.5
Contents (mg/g)	1.69	1.62	3.00	1.65	1.61	2.97	1.65	1.61	2.97
n_{peaks}		64			77			56	

Peak capacity

Peak capacity, a useful measure of comparative separating power of different analytical systems, is defined as the number of peaks which can be resolved in a certain time interval (7,12). In the gradient LC, peak capacity is a function of column efficiency, gradient time, flow rate, and analyte characteristics. Equation 4 is often used for the estimation of peak capacity (n_{peaks}) (7,13).

$$n_{peaks} = 1 + \frac{t_g}{\overline{W}} \quad \text{Eq. 4}$$

where t_g and w are the overall gradient time and the average value of the peak width at the baseline value, respectively. In practice, the representative peaks are often chosen to provide an estimate of overall peak capacity.

Experimental

Instrumentation

An Acquity UPLC System (Waters, Milford, MA) was used, including a binary solvent manager with a maximum delivery flow rate of 2 mL/min, a sample manager with a 10 µL loop, a 2996 TUV detector, and a column oven. The following columns were connected in this study: Acquity BEH C_{18} (50 mm × 2.1 mm, 1.7 µm). The data acquisition rate was 20 Hz for UPLC experiments. Another conventional Waters HPLC system was used for the comparison, which included a 2695 system and a 2996 PDA detector. The column was a reversed-phase column Hypersil ODS-2 (200 mm × 4.6 mm, 5 µm, Thermo, Boston, MA). The data acquisition rate was 1 Hz for HPLC experiments. All the previous data acquisition, data handling, and instrument control were performed by Empower Software. An ultrasonic cleaner (T660/H, Elma, Germany) was used for extraction.

Materials

Panax ginseng and standards of ginsenoside Rg_1, Re, and Rb_1 were obtained from National Identification Center for Pharmaceutical and Bio-Product (NICPBP, Beijing, China). The HPLC-grade acetonitrile was obtained from Sigma Aldrich (Bornem, Belgium). Reverse osmosis water (18MΩ, Simplicity 185, Millipore, Molsheim, France) was used for all the solutions and dilutions.

Sample preparation

A 1-g powder of dried materials was refluxed with 50 mL trichloromethane for 3 h. This extraction solution was filtrated through an analytic filter. The residue was evaporated to dryness at a water bath and dissolved in 50 mL water-saturated *n*-butanol with a supersonic process for 30 min. The solution was filtrated with an analytic filter, and 25 mL of filtrated solution was evaporated to dryness at a water bath. The dry extract was dissolved in 5 mL methanol, and suspended particles were then filtrated through a 0.22-µm membrane filter.

HPLC procedures

According to Ch.P (2), the separations for *Panax ginseng* were performed by multi-step gradient elution shown in Table I. The flow rate was 1.0 mL/min. The detection wavelength and the column temperature were set at 203 nm and 25°C, respectively. The injecting volume was 10 µL.

UPLC procedures

The UPLC separation mobile phase for *Panax ginseng* (Table I) were transferred directly from HPLC linear gradient elution with the previously mentioned equations except the flow rate and the injection volume. According to Equation 2, the transferred flow rate was 0.21 mL/min. In order to develop a faster analysis method, the flow rate was optimized and finally adapted to 0.5 mL/min. The detection wavelength and column temperature were the same as those of HPLC, respectively. The transferred inject volume was 0.5 µL. It was optimized and finally adapted to 2 µL.

Results and Discussion

Regression curve, recovery, and sensitivity

The linearity of the detector response was investigated by injecting different volumes of the ginsenosides in the HPLC and UPLC systems, respectively. The responses were linear in the determined ranges in this study (the linear ranges not shown). The relative coefficients were more than 0.9996 for ginsenoside Rg_1, Re, and Rb_1 in HPLC and UPLC, respectively. Recoveries for the three ginsenosides were determined by standard addition method in HPLC and UPLC, respectively. The recoveries were within the range of 97.5–102.5%, and the relative standard deviation (RSD) value was better than 2.5%, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of ginsenoside Rg_1, Re, and Rb_1 in both HPLC and UPLC systems had been estimated as three and ten times the signal-to-noise (S/N), respectively (Table II). The results demonstrated that both methods had good accuracy and precision. But the UPLC method obtained a lower LOD and LOQ than that from HPLC, which could be explained by the UPLC instrument's solvent delivery module and detector cell dispersion. It was the reason that the HPLC and UPLC chromatogram differs slightly from each other (Figure 1).

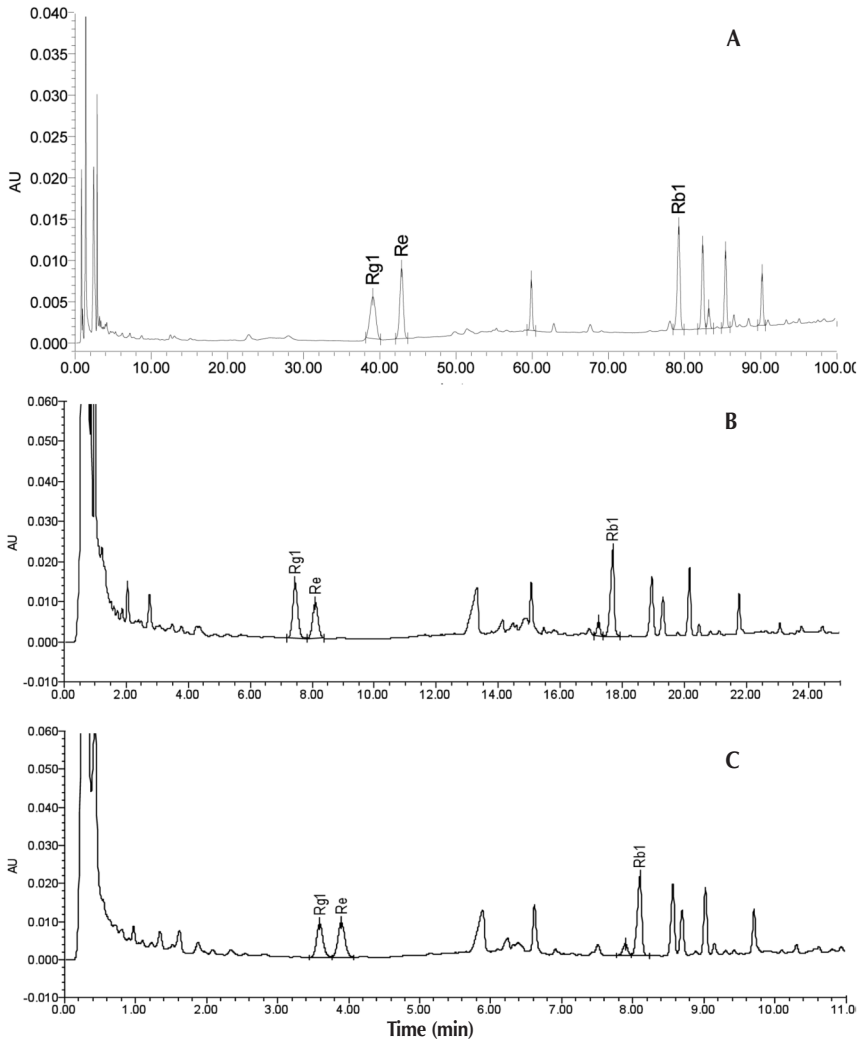

Figure 2. The (A) HPLC and (B and C) UPLC analyses of Panax ginseng. Conditions A: Hypersil ODS-2 column (200 mm × 4.6 mm, 5 µm); the mobile phases shown in Table I; flow rate 1.0 mL/min; detection 203 nm; temperature 25°C; injection 10 µL; acquisition rate 1 Hz. Conditions B: Acquity BEH C_{18} column (50 mm × 2.1 mm, 1.7 µm); the mobile phases shown in Table I; flow rate 0.21 mL/min; detection 203 nm; temperature 25°C; injection 2 µL; acquisition rate 20 Hz. Conditions C: flow rate 0.5 mL/min (other conditions same as conditions B).

Quantification of ginsenosides contents

The three ginsenosides in *Panax ginseng* were determined by HPLC and UPLC, respectively. The results were summarized in Table II. In this study, no obvious difference was shown in the yield of individual ginsenoside in *Panax ginseng*.

Comparisons of analysis time and efficiency

The HPLC method in Ch.P had a 110-min analysis including a 100-min separation and 10-minute re-equilibration for the analysis of *Panax ginseng* (Figure 2). The peak capacity was 64 (Table II). In the UPLC system, the initial flow rate of 0.21 mL/min had a separation time of about 25 min, and the peak capacity was 77 (Table II). Although the analysis time shortened about four times, the separation power increased about two-tenths. The optimal flow rate obviously decreased the separation time, making 10.5 min enough and shortening the rate about 10 times. The peak capacity was 56 (Table II). Comparing to the HPLC, the optimal method had not decreased the separation power. In both methods, all the analytes had an acceptable resolution and efficiency.

Optimization of the flow rate

In the study, the Equation 2 used for the flow rate transferring was different from

that reported in the previous paper (12). The reported equation considered the particle size, but in practice, it could not bring a required separation results such as unacceptable resolution of Rb_1. If the transferring equation took no account of the particle size, the separation results would be satisfactory. In the study, the transferred flow rate was 0.21 mL/min according to Equation 2. For a faster separation, the flow rate can be optimized according to Equation 3. It was increased from 0.21 mL/min to 0.50 mL/min. The optimization of the flow rate made the separation time shorten accordingly ~ 2.4 times with acceptable peak resolutions of the determined components. The results investigated that the Equation 2 is a good choice in the flow rate transferring from HPLC to UPLC.

Conclusion

The application of UPLC has been investigated in the analysis of ginsenosides of *Panax ginseng*. The results indicated that the UPLC method could be developed rapidly from HPLC according to some equations. In comparison to HPLC, the UPLC has obvious advantages in the TCM analysis. The main advantage is that the significant reduction of analysis time, which means reduction in solvent consumption but keeping the equivalent separation power. The SST data show that peak asymmetry, resolution, selectivity, and retention values were not markedly different. Only the LOD and LOQ had obviously decreased, which meant higher sensitivity. Because of the difference of the HPLC and UPLC, chromatograms differ slightly from each other.

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