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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 669–678
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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A rapid HPLC–ESI-MS/MS for qualitative and quantitative analysis of saponins in "XUESETONG" injection

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Received 2 August 2005; received in revised form 4 November 2005; accepted 4 November 2005
Available online 15 December 2005

Abstract

'XUESETONG' injection, one of the most widely used proprietary medicines in traditional Chinese medicine, consists of total saponins made from *Panax notoginseng*, which is a highly valued and important Chinese medicinal herb. It is used to treat cardiovascular diseases. In order to control the quality of XUESETONG injection, a rapid HPLC–ESI-MS/MS method was developed for qualitative and quantitative determination of the saponins. The analyses were performed on SB-C18 column using gradient elution in 25 min. Full scan and time programmed selected reaction monitoring (SRM) were used for qualitative and quantitative analysis of saponins, respectively. Twenty-seven saponins were identified and nine of them including notoginsenoside R1, ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf and Rg1 were quantified. Ten XUESETONG injections were analyzed and compared. The results showed that there is a great variation among different samples. In conclusion, the developed method is rapid, accurate and sensitive for qualitative and quantitative analysis of saponins in XUESETONG injection. Moreover, it also can be used for the quality control of *P. notoginseng* raw material and its preparations.

Keywords: XUESETONG injection; *Panax notoginseng*; Total saponins; HPLC–ESI-MS/MS; Quality control; Notoginsenoside; Ginsenoside

1. Introduction

'XUESETONG' injection consists of total saponins made from *Panax notoginseng* (commonly known as Tianqi or Sanqi), which is a highly valued and important Chinese medicinal herb produced mainly in Yunnan Province of China, is one of the most widely used proprietary medicines in traditional Chinese medicine. It is used to treat cardiovascular diseases. The dammarane-type saponins, which include ginsenosides and notoginsenosides, contribute to pharmacological activity [1–4]. In general, the curative effect of traditional Chinese medicine is an integrative result of a number of bioactive compounds. Therefore, analysis of saponins is helpful to control the quality of XUESETONG injection. Actually, several methods, including HPTLC [5–7], HPLC–UV [8–10], HPLC–ELSD [11], have been used for analysis of saponins in *P. notoginseng*. However, these methods suffer from low resolution and reproducibility [5–7] or low sensitivity and long analytical time [8–11]. Up to date, qualitative identification and quantitative determination of saponins in *P. notoginseng* were not performed by HPLC–MS, though the technique has been applied to identify *P. notoginseng* from *Panax ginseng* and *Panax quinquefolius* [12] and the saponins in *P. ginseng* [13–16]. On the other hand, the analytical time of most previous studies was more than 60 min [12,15,16]. In our current study, a rapid HPLC–ESI-MS/MS method was first attempted to analyze saponins in XUESETONG injection. Using this method, 27 saponins were identified and nine saponins were quantitatively determined in 25 min.

2. Experimental

2.1. Chemicals, standards and samples

Acetonitrile for liquid chromatography was purchased from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Riedel-de Haën (Seelze, Germany). Chemicals not mentioned here were from standard sources. Water was prepared using a Millipore Milli Q-Plus system (Millipore, Bedford, MA).

The standards of ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf and Rg1 were purchased from ChromaDex Company (Santa,

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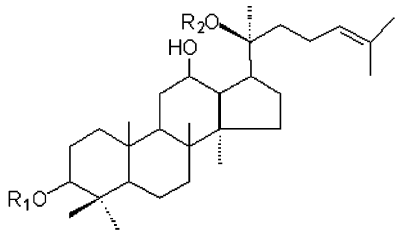
doi:10.1016/j.jpba.2005.11.003

Ana, USA). Notoginsenoside R1 was kindly supplied by Kunming Institute of Botany, Scientific Academy of China (Kunming, China). The standard saponins were first dissolved in initial mobile phase (8 mM ammonium acetate aqueous solution/acetonitrile; 8/2; v/v) at ~0.5 mg/ml as a stock solution. 1 ml of the stock solution was transferred to a 2 ml volumetric flask, which was brought up to its volume with the mobile phase. A series dilution was performed so as to obtain the desired concentration. Filtered through a 0.45 μm membrane (Econofilter RC, Agilent Technologies), a certain volume of standard solution was injected for LC–MS analysis.

Ten samples of XUESETONG injection (25–50 mg/ml) were collected in the market. They were manufactured by four pharmaceutical companies in China, named A, B, C and D, respectively. The samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China. A certain volume of the injection, according to its nominal content of total saponins, was transferred to a 50 ml volumetric flask which was brought up to its volume with the mobile phase so as to obtain the concentration of total saponins at about 0.5 mg/ml. Filtered through a 0.45 μm membrane, 10 μl of the solutions were injected for LC–MS analysis. Spiked injection was produced by mixing test sample solution and the reference compounds at the ratio of 1:1.

2.2. HPLC–UV–MS analysis

Analysis were performed on an Agilent 1100 Series LC/MSD Trap system (Agilent Technologies, Palo Alto, CA), equipped with a vacuum degasser, a quaternary pump, an autosampler, a column compartment, a DAD detector and an ion-trap mass spectrometer with electrospray ionization interface, connected to an Agilent LC/MSD Trap Software. A ZORBAX SB-C18 column (2.1 × 150 mm i.d., 5 μm) and a ZORBAX ODS C18 guard column (4.6 × 12.5 mm i.d., 5 μm) were used. Solvents that constituted the mobile phase were (A) 8 mM aqueous ammonium acetate and (B) acetonitrile. The elution conditions applied were: 0–3 min, linear gradient 20–25% B; 3–20 min, linear gra-

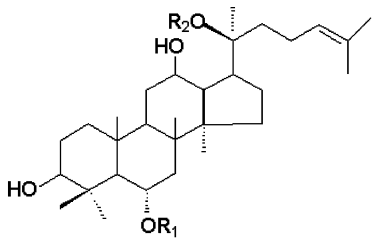


R_1=R_2=H 20(S)-Protopanaxadiol

20(S)-Protopanaxadiol

Saponin	R_1	R_2	M.W.
Ginsenoside F2	glc	glc	784
Ginsenoside Ra1	glc(2-1)glc	glc(6-1)arap(4-1)Xyl	1210
Ginsenoside Ra2	glc(2-1)glc	glc(6-1)araf(4-1)Xyl	1210
Ginsenoside Ra3	glc(2-1)glc	glc(6-1)glc(3-1)Xyl	1240
Ginsenoside Rb1	glc(2-1)glc	glc(6-1)glc	1108
Ginsenoside Rb2	glc(2-1)glc	glc(6-1)arap	1078
Ginsenoside Rb3	glc(2-1)glc	glc(6-1)xyl	1078
Ginsenoside Rc	glc(2-1)glc	glc(6-1)araf	1078
Ginsenoside Rd	glc(2-1)glc	glc	946
Ginsenoside Rd (iso.)	glc(2-1)glc	glc(20-R)	946
Ginsenoside Rg3	glc(2-1)glc	H	784
Gypenosid IX	glc	glc(6-1)xyl	916
Gypenosid XII	glc	glc(6-1)glc	946
Notoginsenoside Fa	glc(2-1) glc(2-1)xyl	glc(6-1)glc	1240
Notoginsenoside Fc	glc(2-1) glc(2-1)xyl	glc(6-1)xyl	1210
Notoginsenoside Fe	glc	glc(6-1)araf	916
Notoginsenoside R4	glc	glc(6-1) glc(6-1)xyl	1078

Fig. 1. Structure of main saponins in *P. notoginseng*.



R_1=R_2=H 20(S)-Protopanaxatriol

20(S)-Protopanaxatriol

Saponin	R_1	R_2	M.W.
Ginsenoside F1	H	glc	638
Ginsenoside Re	glc(2-1)rha	glc	946
Ginsenoside Rf	glc(2-1)glc	H	800
Ginsenoside Rg1	glc	glc	800
Ginsenoside Rg2	glc(2-1)rha	H	784
Ginsenoside Rh1	glc	H	638
Notoginsenoside R1	glc(2-1)xyl	glc	932
Notoginsenoside R2	glc(2-1)xyl	H	770

Note: Glc, β-D-glucose; Rha, α-L-rhamnose; Arap, α-L-arabinose(pyranose); Araf, α-L-arabinose(furanose); Xyl= β-D-xylose

Fig. 1. (*Continued*).

dient 25–50% B; 20–30 min, linear gradient 50–80% B; and finally, reconditioning steps of the column was 20% B isocratic for 15 min. The flow-rate was 0.4 ml/min and the system operated at 25 °C. Peaks were detected at 203 nm of UV detection and negative ion mode of MS and MS/MS detection. ESI-MS conditions were as follows: drying gas N_2, 7 l/min, temperature 325 °C, pressure of Nebulizer 25 psi, source voltage 3.5 kV. ESI-MS/MS conditions: isolation width 4, fragment amplification 1.5. Scan range of both ESI-MS and ESI-MS/MS was 200–1400 u.

3. Results and discussion

Nine reference compounds ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1 and notoginsenoside R1 (Fig. 1) were analyzed in order to optimize the MS conditions and calibrate the quantitative determination. The trials showed that the negative ion mode was more sensitive than the positive ion mode. The HPLC–ESI-MS chromatogram exhibited good agreement with the HPLC–UV chromatogram (Fig. 2). A good chromatographic separation of saponins in XUESETONG injection was achieved on reversed-phase using a linear gradient of 8 mM aqueous ammonium acetate and acetonitrile.

3.1. Qualitative analysis

Fig. 3A–K showed the 27 peaks detected by using selective ion monitoring (SIM) in XUESETONG injections. They were tentatively identified by careful studies of the MS and MS/MS spectra and by comparison with literature data [15–21]. The identification of peaks as ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg3 and notoginsenoside R1, respectively; was also confirmed by spiked injection of the reference compounds.

Example of peaks of *m*/*z* 946 was ginsenoside Re, ginsenoside Rd, gypenoside XII and ginsenoside Rd isomer (Fig. 3G). The retention times were 8.1 min (P14), 13.4 min (P15), 16.1 min (P16) and 16.8 min (P17), respectively. The MS/MS spectra of

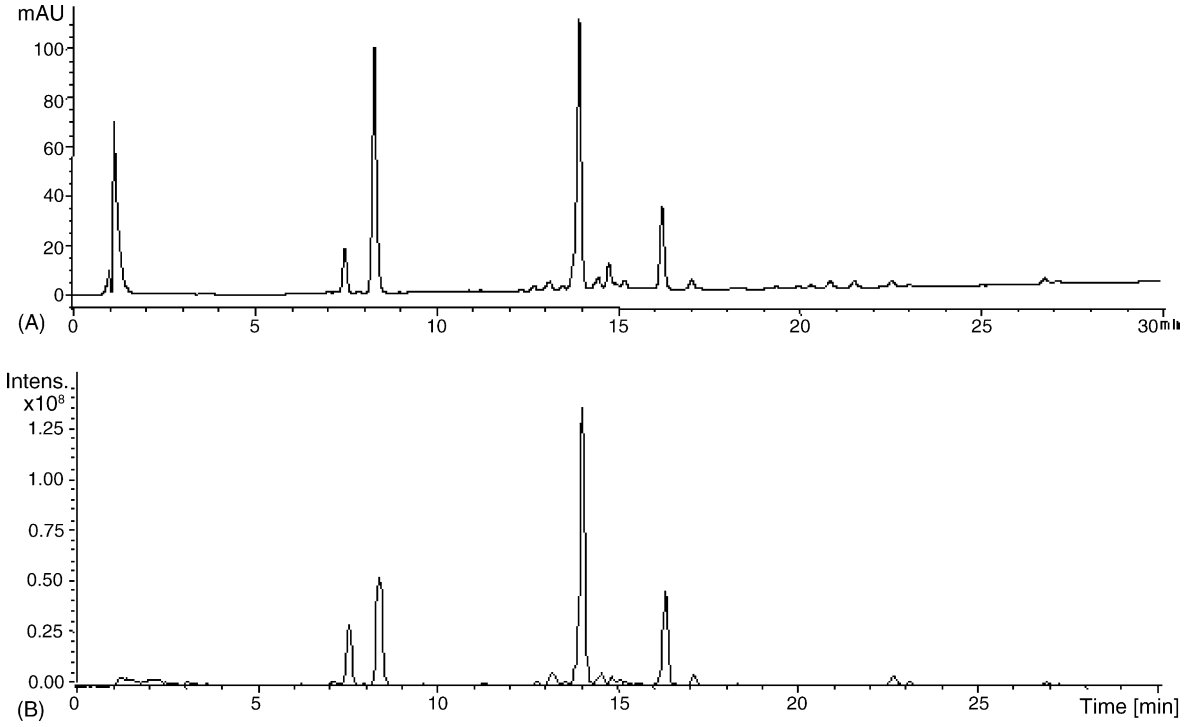


Fig. 2. HPLC chromatograms of XUESETONG injection detected by (A) UV and (B) MS detectors.

ginsenosides exhibited a fragmentation pattern corresponding to the loss of the glycosidic units (Table 1, Fig. 4). In addition, an [aglycone-H] ion at *m*/*z* 475 corresponding to the (20S)-protopanaxatriol aglycon moiety was visible for peak P14. Peaks P15, P16 and P17 showed an [aglycone-H] ion at *m*/*z* 459 corresponding to the (20S)-protopanaxadiol aglycon moiety. Peaks P14 and P16 were identified as ginsenosides Re and Rd, confirmed by spiking with standards. Apropos of the peak appear at 13.4 min, the MS data had *m*/*z* 783 $[M–H\text{-}Glc]^-$, *m*/*z* 621 $[M–H\text{-}2Glc]^-$ and *m*/*z* 459 $[M–H\text{-}3Glc]^-$. The fragment of *m*/*z* 459 was derived from sapogenin produced by the saponin release all linked glucose, which was in accordance with the fragmen-

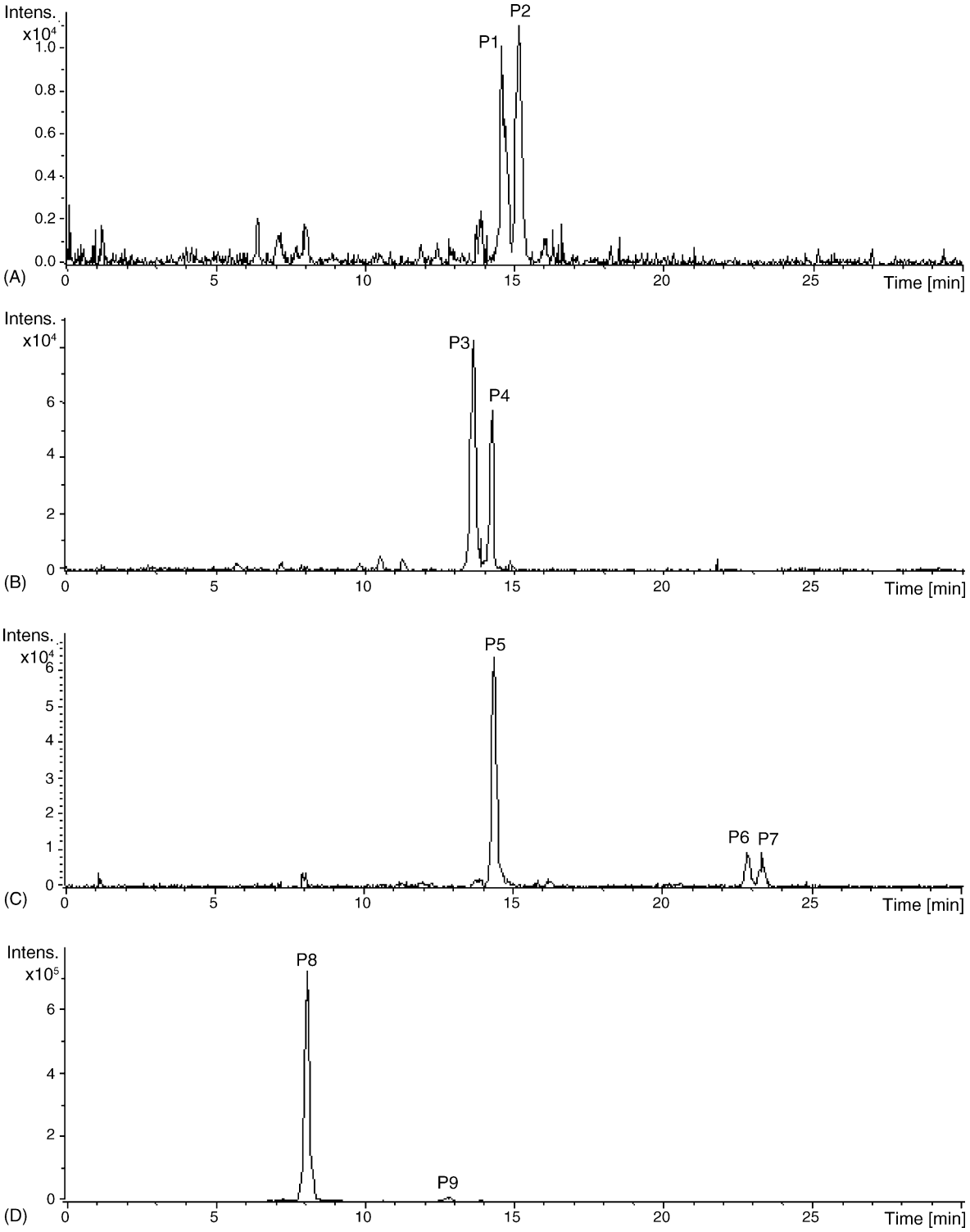


Fig. 3. Selected ion chromatogram of XUESETONG injection at *m*/*z* (A) 638, (B) 770, (C784), (D) 800, (E) 916, (F) 932, (G) 946, (H) 1078, (I) 1108, (J) 1210, (K) 1240.

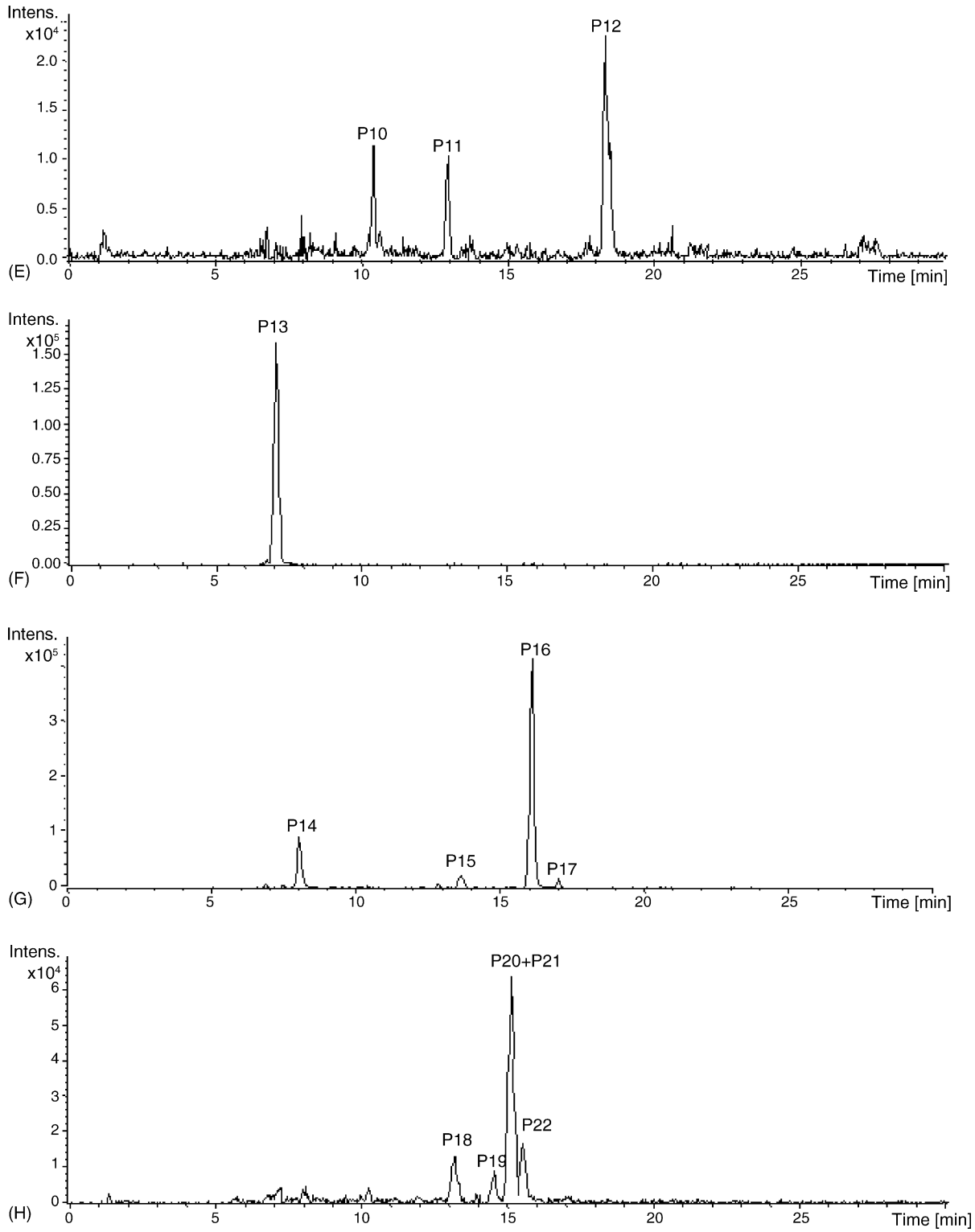


Fig. 3. (*Continued*).

tation pathway of gypenoside XII and ginsenoside Rd isomer. According to the references [12,15], the peak at 16.8 min was identified as the isomer of ginsenoside Rd, which product ion were similar to those of ginsenoside Rd. Therefore, Peak P15 was identified as gypenoside XII.

Using mentioned method above, 27 compounds, including four unknown saponins, as well as ginsenoside F1, Fc (or Ra1/Ra2,), Ra3, Rb1, Rb2, Rb3, Rc, Rd, Rd isomer, Re, Rf, Rg1, Rg2, Rg3, Rh1, notoginsenoside F2, Fa, Fe, R1, R2, R4, gypenoside IX and gypenoside XII, were identified (Table 1).

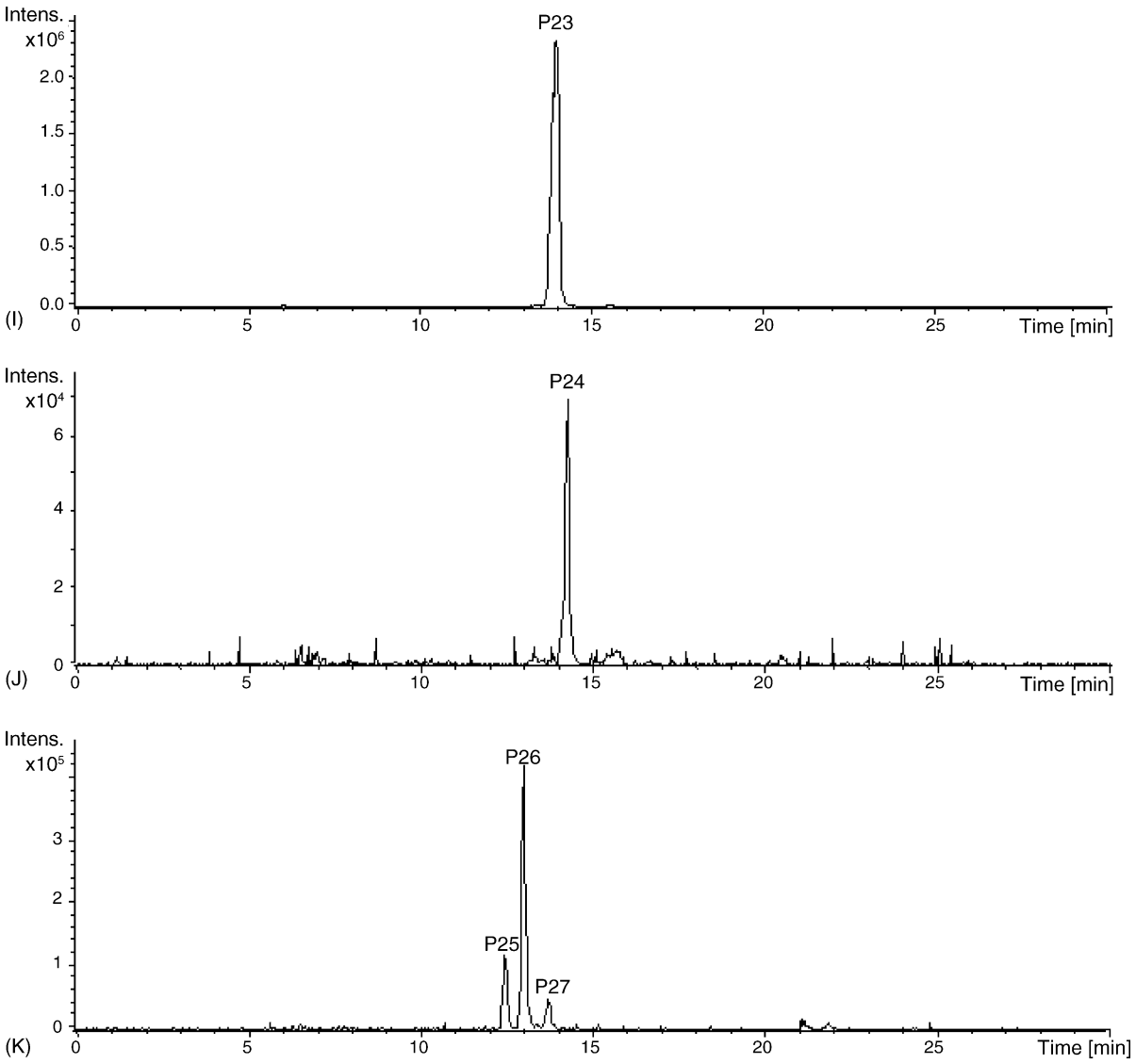


Fig. 3. (*Continued*).

3.2. Quantitative analysis

The number of ions chosen has an effect on the sensitivity of quantitative analysis. The advantage of SIM is achieved through spending more time monitoring the ions of interest—the more ions being monitored, then the less time will be spent on each of them and the lower the increase in sensitivity of SIM over full scanning. When SIM is being carried out in conjunction with chromatography, a further consideration is that an adequate number of cycles of measurement must be made to define the shape and intensity of the chromatographic response exactly, or otherwise inaccurate and imprecise measurements will be made (Fig. 5). In order to increase sensitivity and specificity of quantification, time programmed selective reaction monitoring (SRM) of ginsenoside Rb1 (1108 → 946), Rb2 (1078 → 946), Rc (1078 → 946), Rd (946 → 784), Re (946 → 800), Rf (800 → 637), Rg1 (800 → 637) and notoginsenoside R1 (932 → 800) was performed using five segments (Fig. 6).

3.2.1. Validation of the method

In order to determine the linearity of notoginsenoside R1, ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, and Rg1, a series of concentration for standards solution were injected and analyzed by LC–ESI-MS/MS under time programmed SRM. This was done by injecting 10 μl of different concentrations on column using the chromatographic conditions described above. All measurements were done in triplicate and data were processed by LC/MSD Trap Software (Version 4.2). The linearity ranges for the investigated compounds were reported in Table 2.

The short term (12 h) repeatability as well as the long term (72 h) repeatability of nine saponins for quantitative determination was calculated for ten runs. The peak area of selected

Table 1
HPLC–ESI-MS and HPLC–ESI-MS/MS ions of saponins in XUESETONG injection (*m/z* with relative abundance (%) in parentheses)

No.	Peak	Identification	RT (min)	MS $[M–H]^-$	MS/MS *m/z* (%)
1	P13	Notoginsenoside R1	7.1	932	799(100) $[M–H\text{-}Xyl]^-$; 769(32) $[M–H\text{-}Glc]^-$; 637(10) $[M–H\text{-}Xyl\text{-}Glc]^-$; 475(1)Agl
2	P8	Ginsenoside Rg1	7.9	800	637(100) $[M–H\text{-}Glc]^-$; 475(10)Agl
3	P14	Ginsenoside Re	8.1	946	799(45) $[M–H\text{-}Rha]^-$; 783(100) $[M–H\text{-}Glc]^-$; 765(20) $[M–H\text{-}Glc\text{-}H20]^-$; 637(63) $[M–H\text{-}Glc\text{-}Rha]^-$; 619(21) $[M–H\text{-}Glc\text{-}Rha\text{-}H20]^-$; 475(20)Agl
4	P10	Unknown	10.4	916	783(100) $[M–H\text{-}(Ara/Xyl)]^-$; 637(42) $[M–H\text{-}(Ara/Xyl)\text{-}Rha\text{-}Glc]^-$; 475(3)Agl
5	P25	Ginsenoside Ra3/notoginsenoside Fa/unknown	12.3	1240	1107(100) $[M–H\text{-}Xyl]^-$; 1077(50) $[M–H\text{-}Glc]^-$; 945(15) $[M–H\text{-}Xyl\text{-}Glc]^-$; 783(8) $[M–H\text{-}Xyl\text{-}2Glu]^-$; 621(2) $[M–H\text{-}Xyl\text{-}3Glc]^-$
6	P18	Notoginsenoside R4/unknown	12.8	1078	945(100) $[M–H\text{-}(Ara/Xyl)]^-$; 915(29) $[M–H\text{-}Glc]^-$; 783(48) $[M–H\text{-}(Ara/Xyl)\text{-}Glc]^-$; 621(4) $[M–H\text{-}(Ara/Xyl)\text{-}2Glc]^-$; 459(1)Agl
7	P9	Ginsenoside Rf	12.9	800	637(100) $[M–H\text{-}Glc]^-$; 475(45)Agl
8	P26	Ginsenoside Ra3/notoginsenoside Fa/unknown	13.0	1240	1107(100) $[M–H\text{-}Xyl]^-$; 1077(1) $[M–H\text{-}Glc]^-$; 945(1) $[M–H\text{-}Xyl\text{-}Glc]^-$; 783(1) $[M–H\text{-}Xyl\text{-}2Glc]$
9	P11	Notoginsenoside Fe/gypenoside IX	13.1	916	783(100) $[M–H\text{-}Araf/Xy]^-$; 621(5) $[M–H\text{-}Araf/Xyl\text{-}Glc]^-$;459(1)Agl
10	P15	Gypenoside XII	13.4	946	783(100) $[M–H\text{-}Glc]^-$; 621(10) $[M–H\text{-}2Glc]^-$; 459(3)Agl
11	P23	Ginsenoside Rb1	13.5	1108	945(100) $[M–H\text{-}Glc]^-$; 783(36) $[M–H\text{-}2Glc]^-$; 621(7) $[M–H\text{-}3Glc]^-$; 459(1)Agl
12	P3	Notoginsenoside R2/unknown	13.6	770	637(100) $[M–H\text{-}Xyl]^-$; 475(5)Agl
13	P27	Ginsenoside Ra3/notoginsenoside Fa/unknown	13.7	1240	1107(100) $[M–H\text{-}Xyl]^-$; 1077(7) $[M–H\text{-}Glc]^-$; 945(3) $[M–H\text{-}Xyl\text{-}Glc]^-$; 783(1) $[M–H\text{-}Xyl\text{-}2Glc]^-$
14	P19	Ginsenoside Rc	14.1	1078	945(100) $[M–H\text{-}Araf]^-$; 915(12) $[M–H\text{-}Glc]^-$; 783(35) $[M–H\text{-}Araf\text{-}Glc]^-$; 621(4) $[M–H\text{-}Araf\text{-}2Glc]^-$; 459(1)Agl
15	P24	Ginsenoside Fc/Ra1/Ra2	14.2	1210	1077(100) $[M–H\text{-}Xyl]^-$; 1047(5) $[M–H\text{-}Glc]^-$; 945(10) $[M–H\text{-}2Xyl/(Xyl\text{-}Ara)]^-$; 783(6) $[M–H\text{-}2Xyl\text{-}Glc/(Xyl\text{-}Ara\text{-}Glc)]^-$
16	P4	Notoginsenoside R2/unknown	14.3	770	637(100) $[M–H\text{-}Xyl]^-$; 475(5)Agl
17	P5	Ginsenoside Rg2	14.7	784	637(100) $[M–H\text{-}Rha]^-$; 621(11) $[M–H\text{-}Glc]^-$; 475(23)Agl
18	P1	Ginsenoside F1/Rh1	14.8	638	475(100) $[M–H\text{-}Glc]^-$
19	P20	Ginsenoside Rb2	14.9	1078	945(100) $[M–H\text{-}Arap]^-$; 915(27) $[M–H\text{-}Glc]^-$; 783(49) $[M–H\text{-}Arap\text{-}Glc]^-$; 621(4) $[M–H\text{-}Arap\text{-}2Glc]^-$; 459(1)Agl
20	P21	Ginsenoside Rb3	15.0	1078	945(100) $[M–H\text{-}Xyl]^-$; 915(29) $[M–H\text{-}Glc]^-$; 783(48) $[M–H\text{-}Xyl\text{-}Glc]^-$; 621(4) $[M–H\text{-}Xyl\text{-}2Glc]^-$; 459(1)Agl
21	P2	Ginsenoside F1/Rh1	15.2	638	475(100) $[M–H\text{-}Glc]^-$
22	P22	Notoginsenoside R4/unknown	15.3	1078	945(100) $[M–H\text{-}(Ara\text{ or }Xyl)]^-$; 915(29) $[M–H\text{-}Glc]^-$; 783(48) $[M–H\text{-}(Ara/Xyl)\text{-}Glc]^-$; 621(4) $[M–H\text{-}(Ara/Xyl)\text{-}2Glc]^-$; 459(1)Agl
23	P16	Ginsenoside Rd	16.1	946	783(100) $[M–H\text{-}Glc]^-$; 621(10) $[M–H\text{-}2Glc]^-$; 459(3)Agl
24	P17	Ginsenoside Rd (iso)	16.8	946	783(100) $[M–H\text{-}Glc]^-$; 621(10) $[M–H\text{-}2Glc]^-$; 459(3)Agl
25	P12	Notoginsenoside Fe/gypenoside IX	18.3	916	783(100) $[M–H\text{-}Araf/Xyl]^-$; 621(5) $[M–H\text{-}Araf/Xyl\text{-}Glc]^-$; 459(1)Agl
26	P6	Notoginsenoside F2	22.7	784	621(100) $[M–H\text{-}Glc]^-$; 459(8) $[M–H\text{-}2Glc]^-$
27	P7	Ginsenoside Rg3	23.2	784	621(100) $[M–H\text{-}Glc]^-$; 459(10) $[M–H\text{-}2Glc]^-$

ions was relatively stable. The R.S.D. of short and long term repeatability for tested compounds were 0.50–4.81% and 0.52–5.58%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were defined as the signal-to-noise ratio of 3 and 10, respectively. The LOD (LOQ) values were shown in Table 2. The results showed that there was good sensitivity for tested compounds in our calibrated system.

In order to determine the accuracy, a known amount of nine investigated compounds was added into the XUESETONG injection. The samples were subjected to LC–ESI-MS/MS. The recovery of the tested compounds was within the range of 95.5–100.0%, with R.S.D. for nine analytes was between 2.2 and 5.0%, where $n = 3$ (Table 2).

3.2.2. Application to quantitative determination of XUESETONG injection

Ten different batches of XUESETONG injection from four pharmaceutical manufacturers were tested. Time programmed SRM chromatograms of nine standard saponins and XUESETONG injection was shown in Fig. 6. Based on the chromatogram, we see that ginsenoside Rg1 and Re, as well as ginsenoside Rb2 and Rb3 cannot be separated, respectively. However, the advantage of the mass spectrometer is that mass

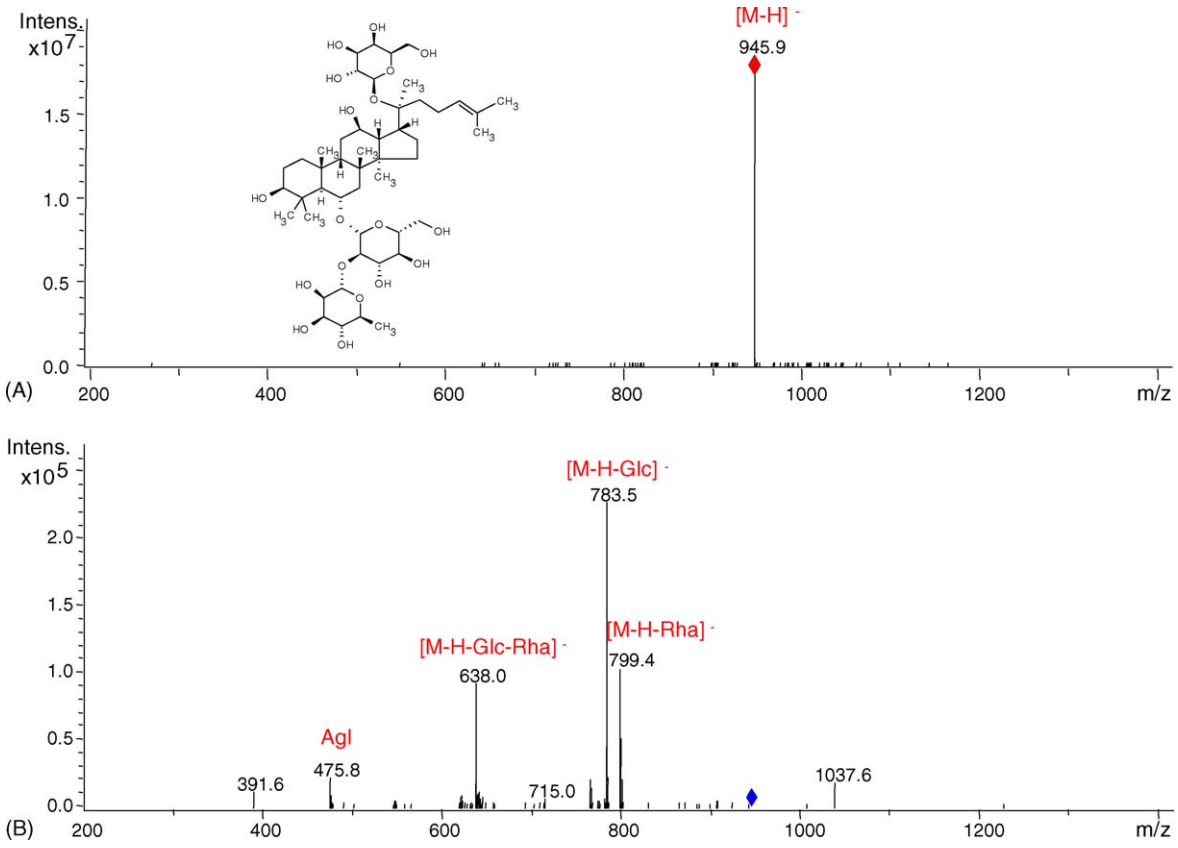


Fig. 4. HPLC–ESI–MS and HPLC–ESI–MS/MS of peak P14 (ginsenoside Re).

can be used as a discriminating feature and this may allow quantitative measurements to be made on unresolved components. Therefore, individual quantitative determination of ginsenoside Rg1 and Re was available. But total content of ginsenoside Rb2 and Rb3 was determined only because their precursors and ion products were same, and ginsenoside Rb2 was used as calibrating standard. By using the calibration curve of each investigated compound, the amount of analytes in XUESETONG injection was determined within the linear range. Then the calculation was as follows:

$$\text{Percentage of analyte in XUESETONG injection} = 100 \times \frac{\text{Amount of analyte}}{\text{Nominal amount of total saponins in the injection}}$$

$$\text{Percentage of analyte in nine investigated saponins} = 100 \times \frac{\text{Amount of analyte}}{\text{Total amount of nine investigated saponins in the injection}}$$

Table 2
Linear regression data and validation of developed method for investigated compounds in XUESETONG injection

Standard	Target ion (SRM)	Regression equation ($n = 7$)	R^2	Linear range (μg/μl)	Recovery (%)		LOD (pg/μl)	LOQ (pg/μl)
					Mean	R.S.D.		
Ginsenoside Rb1	1108 → 946	$y = 1312.08x + 668040.72$	0.9950	0.0200–1.0900	98.3	2.9	65	809
Rb2	1078 → 946	$y = 2787.43x + 144301.00$	0.9981	0.0026–0.1300	97.7	3.4	104	332
Rc	1078 → 946	$y = 5929.04x + 9404.40$	0.9996	0.0002–0.0100	99.6	4.8	100	350
Rd	946 → 784	$y = 3304.03x + 744030.43$	0.9970	0.0100–0.5000	98.5	4.7	3	10
Re	946 → 800	$y = 708.82x + 791501.81$	0.9973	0.0488–0.2928	95.5	2.2	122	1220
Rf	800 → 637	$y = 3479.70x + 26254.14$	0.9979	0.0128–0.064	100.0	4.2	53	107
Rg1	800 → 637	$y = 220.22x + 1591821.43$	0.9985	0.4350–1.0880	98.8	3.7	4	11
Notoginsenoside R1	932 → 800	$y = 882.85x + 173385.58$	0.9948	0.0104–0.3120	98.3	5.0	104	208

r^2, squares of correlation coefficients for the standard curves; percentage of relative standard deviation (R.S.D.) for three replicates; LOD, limit of detection; LOQ, limit of quantification.

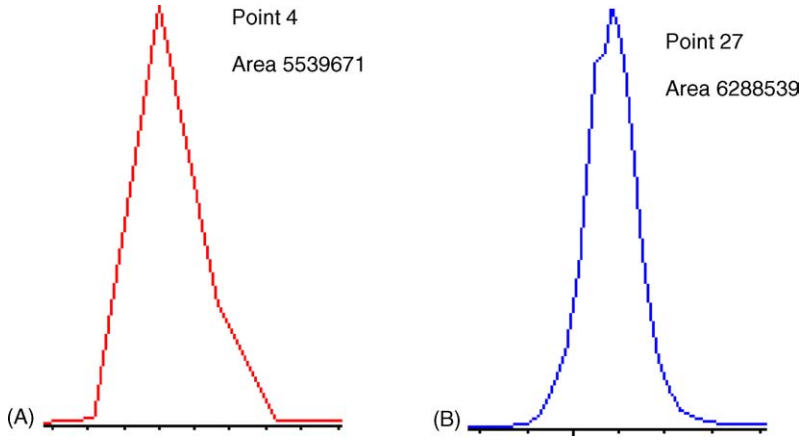


Fig. 5. Effect of the number of data points defining the signal on peak shape.

Table 3 shows the summary results. There is a great variation for the contents of investigated saponins in different XUESETONG injection, even though the samples came from the same pharmaceutical factory. Hierarchical cluster analysis was performed based on nine peaks characteristics of investigated saponins in the injection. A method named as average linkage between groups was applied, and Squared Euclidean distance was selected as measurement. In this way, the groups are represented by their mean values for each variable. Fig. 7 shows the results on the tested 10 samples of XUESETONG injection, which are divided into two main clusters. It suggests that there is difference in the quality of XUESETONG injection. Actually, the saponins for XUESETONG injection have two sources, root or rhizomes of *P. notoginseng*. It may be why the quality of XUESETONG injection in different manufacturers or different batches is obviously different.

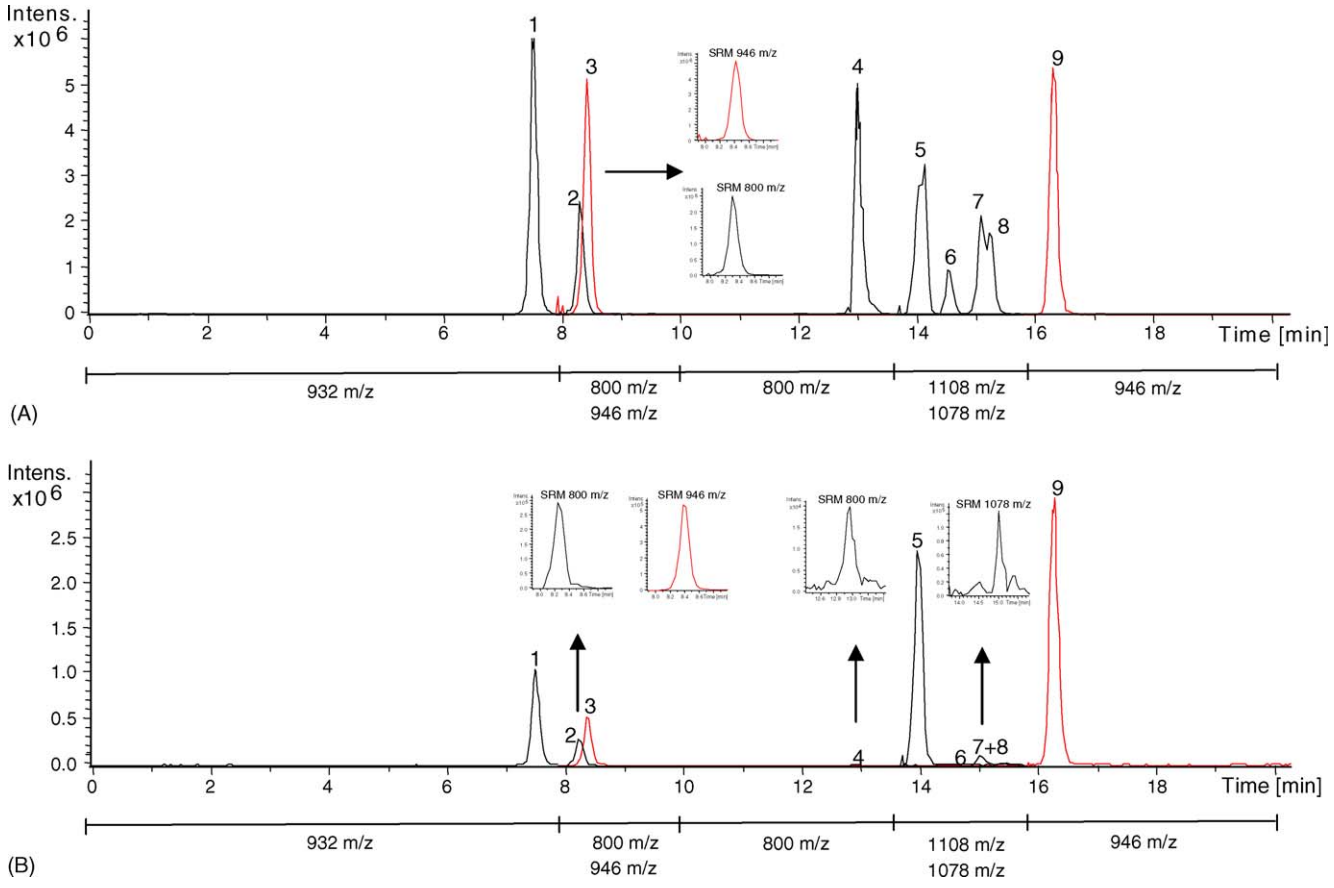


Fig. 6. Time programmed selected reaction monitoring chromatograms of (A) standard saponins and (B) XUESETONG injection. Notoginsenoside R1 (1), ginsenoside Rg1 (2), Re (3), Rf (4), Rb1 (5), Rc (6), Rb2 (7), Rb3 (8), and Rd (9).

Table 3
The percentage of individual saponin in nominal amount of total saponins and in total of nine investigated saponins (in parenthesis) from different XUESETONG injection

Samples	Notoginsenoside	Ginsenoside						
	R1	Rg1	Re	Rf	Rb1	Rc	Rb2 + Rb3	Rd
S1	10.50[a] (10.39)	37.92 (37.51)	4.55 (4.50)	0.04 (0.04)	37.92 (37.51)	0.03 (0.03)	0.52[b] (0.51)	9.60 (9.50)
S2	10.43 (10.37)	39.00 (38.78)	4.39 (4.37)	0.05 (0.05)	37.49 (37.28)	0.03 (0.03)	0.56 (0.56)	8.61 (8.56)
S3	9.89 (9.67)	33.63 (32.88)	4.89 (4.78)	0.06 (0.06)	41.58 (40.65)	0.03 (0.03)	0.68 (0.66)	11.52 (11.26)
S4	8.12 (8.66)	23.04 (24.57)	3.40 (3.63)	0.10 (0.11)	44.87 (47.85)	0.03 (0.03)	0.77 (0.82)	13.44 (14.33)
S5	10.38 (10.65)	21.30 (21.86)	1.99 (2.04)	0.21 (0.22)	46.00 (47.20)	0.04 (0.04)	0.93 (0.95)	16.62 (17.05)
S6	9.12 (9.60)	26.13 (27.51)	2.14 (2.25)	0.22 (0.23)	42.76 (45.02)	0.04 (0.04)	0.83 (0.87)	13.74 (14.47)
S7	6.63 (7.00)	29.65 (31.33)	3.48 (3.68)	0.10 (0.11)	40.24 (42.51)	0.03 (0.03)	0.73 (0.77)	13.78 (14.56)
S8	10.25 (10.14)	22.61 (22.36)	3.52 (3.48)	0.08 (0.08)	50.37 (49.82)	0.03 (0.03)	0.80 (0.79)	13.43 (13.28)
S9	7.62 (7.79)	28.03 (28.67)	1.99 (2.04)	0.19 (0.19)	45.49 (46.53)	0.03 (0.03)	0.82 (0.84)	13.60 (13.91)
S10	6.87 (7.13)	22.82 (23.67)	3.01 (3.12)	0.26 (0.27)	48.36 (50.17)	0.03 (0.03)	0.77 (0.80)	14.27 (14.80)

[a] Calibrated as ginsenoside Rb2.
[b] The data was presented as average of three replicates (R.S.D.s <5%).

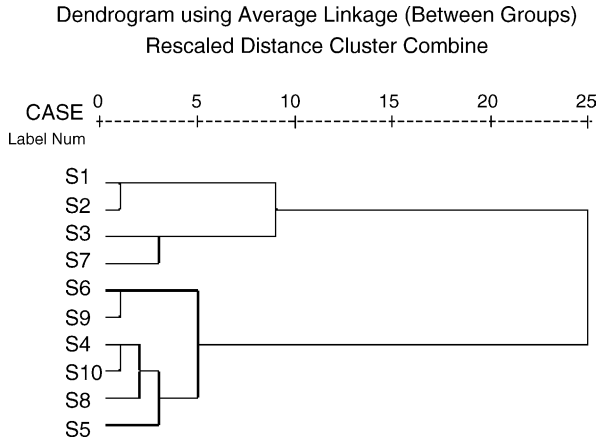


Fig. 7. Dendrogram resulting from average linkage between groups hierarchical cluster analysis. The hierarchical clustering was done by SPSS software. A method named as average linkage between groups was applied, and Squared Euclidean distance was selected as measurement. Dendrogram resulting from the contents of nine investigated saponins including notoginsenoside R1, ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf and Rg1. S1, S2 and S3: Pharmaceutical Factory A; S4, S5 and S6: Pharmaceutical Factory B; S7 and S8: Pharmaceutical Factory C; S9 and S10: Pharmaceutical Factory D.

4. Conclusion

The developed HPLC–ESI-MS/MS method is rapid and sensitive. It can be used for qualitative and quantitative determination of gensinosides and notoginsenosides, which is helpful to improve the quality control of *P. notoginseng* and its pharmaceutical preparations such as XUESETONG injection.

Acknowledgements

We are grateful to Sandy Lao from our institute for her expert technical assistance. The research was supported grants from University of Macau (RG029/04-05S/C86 to S.P. Li).

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