Korean J. Ginseng Sci.
Vol. 19, No. 2, 134~137(1995)

Analysis of Ginsenosides by Thermospray LC/MS

Man Ki Park, Jeong Hill Park*, Gwi Seo Hwang[1], Mi-Young Lee and In-Jeong Park
College of Pharmacy, Seoul National University, Seoul 151-742, Korea
[1]*College of Oriental Medicine, Kyongwon University, Kyungki 461-701, Korea*
(Received July 15, 1995)

Abstract□Ginseng saponins were analyzed by thermospray (TSP) LC/MS method using ODS column and with acetonitrile/ammonium acetate solution. Optimal condition for TSP LC/MS was found as follows; capillary temperature: 330℃, repeller voltage: 200 V, and concentration of ammonium acetate: 0.05 M. Panaxadiol and panaxatriol type saponins showed characteristic fragment ions. The calibration curve of ginseng saponin showed good linearity with a correlation coefficient of 0.99. Detection limits using selected ion monitoring (SIM) technique were improved by 10~200 times compared to conventional HPLC/UV detection method.

Key words□*Panax ginseng*, analysis of ginsenosides, thermospray LC/MS, selected ion monitoring (SIM).

Introduction

Ginseng is a well-known herbal medicine in the Orient. Reported activities of ginseng include sedative,[1] antifatigue,[2] hyperglycemic,[3] and anti-tumor[4] activities. Ginsenosides are known to be the major constituent of ginseng.

Many reports described analytical methods for the ginsenosides which include colorimetry,[5] TLC,[6] GC,[7,8] HPLC,[9~11] ion chromatography (IC)[12] and radioimmunoassay.[13,14] Among these techniques, HPLC is the most widely accepted method since it provides quantitative analytical data of individual ginsenosides. However, UV detection at short wavelength limits the detection sensitivity.

Mass spectrometer is an universal detector which can detect almost all organic compounds with good sensitivity and selectivity.[15] Furthermore, it provides valuable information on the structure of the compound. In this respect, mass spectrometer is one of the most ideal detector for the chromatography. The problem in using mass spectrometer as a liquid chromatographic detector arises from the nature of the mass spectrometer and liquid chromatography. Mass spectrometer principally works in high vacuum and deals with gas-state analyte, while liquid chromatography works with liquid mobile phase and analyte molecule in solution. Recently, many interfaces have been developed to connect LC and mass spectrometer.

Thermospray (TSP) LC/MS interface was first introduced by Vestal, and successfully applied to many compounds.[15] Normal HPLC flow rate of 1~2 ml/min can be directly introduced to mass spectrometer through this interface. In the TSP interface, column effluent containing volatile electrolyte passes through the hot stainless steel capillary, where minute charged droplets are formed. The droplets lose the solvent in the ionization chamber, and gas-state ionized analyte is introduced into the mass analyzer. Reverse phase HPLC which uses high percentage of water as mobile phase is easily applied in TSP ionization method. Since TSP ionization is a mild ionization process, labile compound also can be analyzed.

In this paper, we examined the analysis of ginsenosides by TSP LC/MS.

Materials and Methods

1. Reagents and chemicals

*To whom correspondence should be addressed.

Five kinds of ginsenoside standards were generous gift from Korean Tobacco and Ginseng Research Institute and 5 kinds of ginsenosides were isolated in our laboratory. Six-year old white ginseng was purchased from the local botanical market in Seoul. Distilled deionized water was prepared with Barnstead ultrapure water system (USA). Acetonitrile was of HPLC grade and other chemicals were of reagent grade.

2. Chromatography

The mass spectrometer was VG Trio-2 quadrapole mass spectrometer (VG Masslab, Manchester, UK) with VG thermospray ion source and VG thermospray probe. Young-In 910 pump (Young-In Sci. Co., Korea) with Rheodyne Model 7125 injector was used for HPLC. Hitachi L-4200 UV/Vis detector (Hitachi, Japan) was used to monitor ginseng saponins. Zorbax ODS column (8 cm×4.6 mm, 5 μm, USA) was used for the separation of ginsenosides.

3. Optimization of the condition of TSP LC/MS

The temperature of TSP capillary, repeller voltage, and concentration of the electrolyte affect the detection sensitivity and fragmentation in TSP interface.[15] To optimize these parameters the peak intensity of ginsenoside Rg1 was observed under various conditions. The repeller voltage was varied from 100 to 300 V, and capillary temperature from 280 to 340℃. The effect of the concentration of the electrolyte (ammonium acetate) in the mobile phase was also examined.

4. TSP LC/MS spectra of ginsenosides

TSP LC/MS spectra of panaxadiol, panaxatriol, and 10 kinds of ginsenosides were obtained by flow injection at the optimal condition of each saponin.

5. Detection limit and dynamic linear range

To determine the dynamic linear range, the standard solution of each ginsenoside was injected at its optimal condition and calibration curve was obtained. The detection limit was determined with the S/N ratio of 3. To compare the result with that of HPLC/UV detection method, ginsenoside standard solution was injected with mobile phase of 25% acetonitrile and was detected at UV 207 nm.

Results and Discussion

1. Optimization of TSP LC/MS condition

Capillary temperature (OC) and repeller voltage (SEL) were varied to observe their effect on the signal of ginsenosides. In the case of ginsenoside Rg_1, the optimal condition was found at 280℃ (OC) and 200 V (SEL) (Fig. 1, 2). The peak intensity showed the maximum when the concentration of ammonium acetate in mobile phase was 0.05 M (Fig. 3).

2. TSP LC/MS spectra of ginseng saponins

The TSP LC/MS spectral data of ginseng saponins which were obtained by flow injection analysis are summarized in Table 1. The peaks at m/z 443,

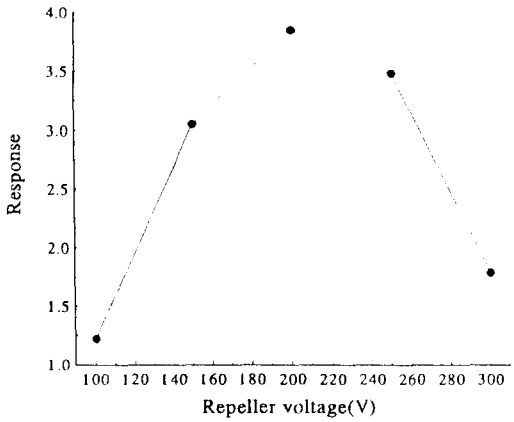

Fig. 1. The effect of repeller voltage on the total ion current of ginsenoside Rg_1 (Zorbax ODS column, 25% CH_3CN in 0.05 M NH_4Ac, 1 ml/min).

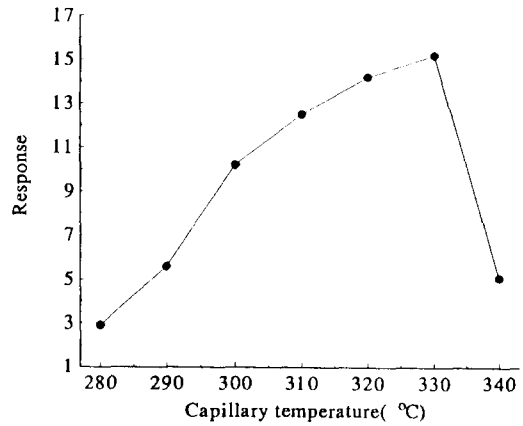

Fig. 2. The effect of capillary temperature on the total ion current of ginsenoside Rg_1 (conditions are as in Fig. 1).

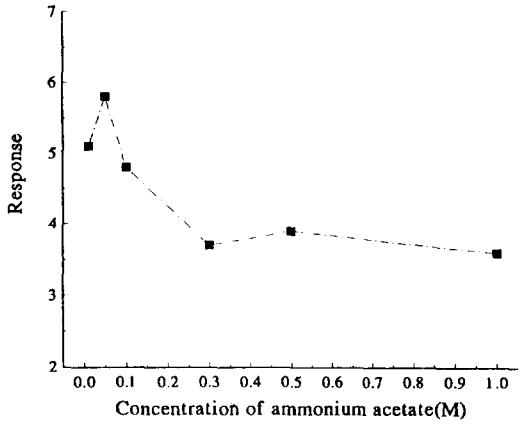

Fig. 3. The effect of the concentration of the electrolyte on the peak intensity of sodium adduct ion of ginsenoside Rf (flow injection, 20% CH_3CN in ammonium acetate aqueous solution, flow rate : 1 ml/min).

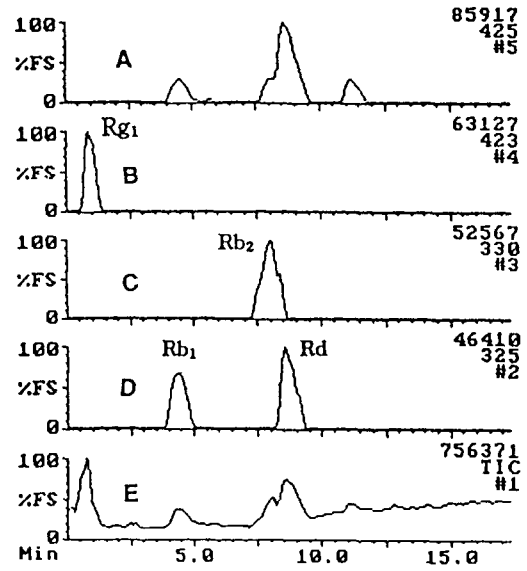

Fig. 4. TSP LC/MS chromatogram of some ginsenosides, A~D : m/z 425, 423, 330 and 325, E : total ion current (Zorbax ODS column, gradient elution : 30%→50% CH_3CN in 0.05 M ammonium acetate, 1 ml/min).

425, 407 appeared in the spectra of panaxadiol type saponins, which arised from the extraction of several molecules of water from protopanaxadiol. Panaxatriol type saponins showed peaks at m/z 459, 441, 423, 405 which also arised from the extraction of several molecule of water from their aglycon, protopanaxatriol. The panaxatriol and panaxadiol type saponins could be distinguished by these peaks. The peak at m/z 360 in the spectrum of ginsenoside Rb1 was arised from glucose-glucose fragment, and m/z 330 peak in the spectrum of ginsenoside Rb2 was from glucose-arabinose.

3. Determination of ginsenosides by SIM

Fig. 4 shows TSP LC/MS chromatogram of ginsenosides on ODS column. Though a small drift was observed in total ion chromatogram (TIC, Fig. 4E) due to the change of eluent composition by gradient

Table 1. LC/TSP-MS spectral peaks of ginseng saponins

Ginsenoside	M.W.	Formula	m/z
Rb_1	1108	$C_{54}H_{92}O_{23}$	343, 361, 365, 407, 784, 806, 1130, 1131
Rb_2	1078	$C_{53}H_{90}O_{22}$	313, 331, 407, 784, 1101
Rc	1078	$C_{53}H_{90}O_{22}$	331, 360, 407, 426, 785, 1078, 1101
Rd	946	$C_{48}H_{82}O_{18}$	424, 785, 969
Rg_3	784	$C_{48}H_{82}O_{18}$	342, 407, 425, 750, 783, 784, 808
PD	460	$C_{30}H_{54}O_3$	127, 407, 425, 443, 461
Re	946	$C_{48}H_{82}O_{18}$	345, 406, 424, 442, 459, 604, 639, 784, 946, 969
Rf	800	$C_{42}H_{72}O_{14}$	361, 406, 424, 441, 765, 800, 823
Rg_1	800	$C_{42}H_{72}O_{14}$	405, 423, 441, 603, 638, 823, 859
Rg_2	784	$C_{42}H_{72}O_{13}$	406, 424, 442, 749, 784, 808
Rh_1	638	$C_{36}H_{62}O_9$	406, 424, 442, 603, 621, 639, 661
PT	476	$C_{30}H_{54}O_4$	127, 405, 423, 441, 459, 477

PD : panaxadiol, PT : panaxatriol.

Table 2. Detection limits of ginseng saponins in TSP LC/MS

Ginseno-side	Detection limit (ng)		mz*
	SIM	Scan	
Rb_1	4.9	1000	360
Rb_2	11.4	1200	312 330 342 407 425
Rc	21.8	4000	312 342 425
Rd	31.7	1500	342 407 425
Re	10.9	1800	405 423 441
Rg_1	1.4	500	405 423 441
Rg_2	7.4	400	423
Rg_3	32.8	8200	342 407 425
Rh_1	8.9	400	423

*Selected m/z for SIM (selected ion monitoring)

elution, the peaks of each saponin in the selected ion monitoring (SIM) chromatogram (A~D) did not show any drift. At m/z 425 (Fig. 4A), only panaxadiol-type saponins appeared and panaxatriol-type saponins appeared at m/z 423 (Fig. 4B). The peaks of ginsenoside Rb_2 and Rd were clearly separated at their characteristic peaks of m/z 330 and 325 (C and D), while they were overlapped in TIC (E).

4. Detection limit and dynamic linear range

The detection limit was determined in both scan mode and SIM mode. Intense peaks were monitored up to five for each saponins. The detection limits were lowered by two orders compared to scan mode or UV detection method (Table 2). The detection limit of ginsenoside Rg1 was 400ng in UV detection, while it was 1.4 ng in TSP LC/MS with SIM mode. The calibration curve of ginsenoside Re showed the correlation coefficient of 0.99 in the range of 0.25~20 μg in SIM mode.

요 약

인삼사포닌을 열분무(thermospray) LC/MS에 의하여 ODS 컬럼과 아세토니트릴/암모늄아세테이트 이동상을 이용하여 검출하는 방법을 검토하였다. 열분무 모세관의 온도는 330℃, repeller voltage는 200 V, 암모늄아세테이트의 농도는 0.05 M이 적당하였다. 파낙사다이올계와 트리올계 사포닌은 서로 다른 분열피크를 나타내어 검출 피크를 바꿈으로써 이 두 계열의 사포닌을 분류할 수 있었다. 검량선은 상관계수 0.99의 양호한 직선성을 나타내었고 선택이온 검출법(selected ion monitoring)을 이용한 분석의 경우 일반적인 HPLC/UV 검출법보다 10~200배 검출한계가 낮아졌다.

References

1. Kim, H. S., Oh, K. W., Lee, M. K., Back, D. Y., Rheu, H. M. and Seong Y. H. : *Proceedings of International Symposium on Korean Ginseng*, Seoul, Korea, p. 36 (1990).
2. Chang, Y. S. and Chang, I. M. : *Proceedings of International Symposium on Korean Ginseng*, Seoul, Korea, p. 142 (1990).
3. Wang, B. X., Yang, M., Jin, Y. L., Cui, X. Y. and Wang, Y. : *Proceedings of International Symposium on Korean Ginseng*, Seoul, Korea, p. 196 (1990)
4. Yamamoto, H., Katano, M. and Matsunaga, H. : *Proceedings of International Symposium on Korean Ginseng*, Seoul, Korea, p. 102 (1990).
5. Woo, L. K., Han, H. H., Baik, D. W. and Park, D. S. : *J. Pharm. Soc. Kor.*, **17**, 129 (1973).
6. Sanada, S., Shibata, S. and Shoji, J. : *Yakugaku Zasshi*, **98**, 1048 (1978).
7. Bombardelli, E., Bonat, A., Gabetta, B. and Martinelli, E. M. : *J. Chromatogr.*, **196**, 121 (1980).
8. Park, M. K., Park, J. H., Kang, J. S., Lee, M. Y., Park, Y. I., Yu, S. J. and Han, B. H. : *Kor. J. Ginseng Sci.*, **17**(1), 35 (1993).
9. Ko, S. R., Choi, K. J., Kim S. C. and Kim, M. W. : *Kor. J. Pharmacogn.*, **20**, 170 (1989).
10. Soldati, F. and Sticher, O. : *Planta Medica*, **38**, 348 (1980).
11. Park, M. K., Kim, B. K., Park, J. H., Shin, Y. G. and Cho, K. H. : *J. Liq. Chromatogr.*, 18, 2077 (1995).
12. Park, M. K., Park, J. H., Lee, M. Y., Kim, S. J. and Park, I. J. : *J. Liq. Chromatogr.*, **17**, 1171 (1994).
13. Han, B. H. and Han, Y. N. : *Yakhak Hoeji*, **25**, 43 (1981).
14. Sankawa, U., Sung, C. K., Han, B. H., Akiyama, T. and Kawashima, K. : *Chem. Pharm. Bull.*, **30**, 1907 (1982).
15. Park, J. H. : *Pharmacon*, **21**, 30 (1991).