Development of Sensitive LC/MS/MS Methods for Simultaneous Qualitative and Quantitative Analysis of Glycosides in TCMs

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Feng Zhong, Tom Biesenthal, Carmai Seto and Takeo Sakuma
AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

ABSTRACT

The adduct ion species of ginsenosides were studied and their relative intensities were found to be correlated with source temperature. LC/MS/MS methods were developed to quantify ginsenosides based on both sodiated species in positive ion mode and deprontonated species in negative ion mode. Calibration curves were generated and LLOQs were determined for each ginsenoside in both positive and negative modes. Lower LLOQs,10 fg/uL, were achieved in positive mode using sodiated species. Linear Dynamic ranges of ginsenosides are typically between 3 and 5 orders of magnitude without isotopically labeled internal standards. Absolute sensitivities and signal-to-noise were also compared with conventional methods.

INTRODUCTION

Traditional Chinese Medicines(TCMs) are receiving significant attention because of their pharmacological benefits to human health. Ginseng, ginkgo, along with other herbal medicine has been used to treat various illnesses for centuries. Many bioactive ingredients in TCMs contain sugar moieties, such as ginsenosides in ginseng, ginkgolides and bilobalide in ginkgo, flavonoid glycosides in many plants.

These glycosides are non-volatile and polar in nature. They tend to form multiple ion species in ESI-MS analysis, such as protonated $[M + H]^+$ and sodiated $[M + Na]^+$ in positive mode, and deprotonated $[M - H]^-$ and formic acid adduct $[M +FA - H]^-$ in negative ion mode, if formic acid is present in mobile phase. In general, $[M + H]^+$ of ginsenosides are much more labile than their sodiated species $[M + Na]^+$. $[M + H]^+$ ions are not often observed or only existed in the form of loss of water, $[M – H_2O + H]^+$. However, the $[M + H]^+$ species produce richer fragments useful for structure elucidation than sodiated species. The sodiated species produce only glycosidic bond cleavage with limited structure information. In negative mode, $[M - H]^-$ and $[M +FA - H]^-$ also provide useful structure information.

As researchers are trying to characterize the major bioactive ingredients of each TCM and to understand their pharmacokinetic profiles, development of sensitive analytical methods for these type of compounds are essential to support pharmacokinetic studies. We demonstrates a few sensitivity LC/MS/MS methods that can be easily adapted to any qualitative and quantitative analysis of ginsenosides, ginkgolides and flavonoide glycosides compounds.

MATERIALS AND METHODS

Sample Preparation:
HPLC grade ginsenosides M, Rc, Rd, Re, Rf and Rg2 were dissolved in methanol and diluted in water. Other glycosides, hesperidin, rutin, digitoxin, bilobalide, ginkolide A and B were also prepared in the same way.

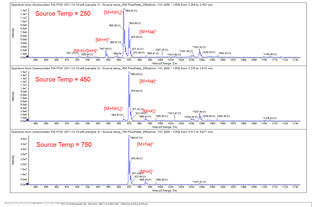
HPLC Conditions:
An Agilent 1200 LC system with a GL Sciences Inertsil ODS-SP, 2.1x 75 mm, 3µm column with a gradient of eluent A water/acetonitrile (95/5) + 0.1% formic acid + 2mM ammonium acetate and eluent B water/acetonitrile (5/95) + 0.1% formic acid + 2mM ammonium acetate was used at a flow rate of 500µL/min. The injection volume was set to 5 µL.

MS/MS Conditions:
An AB SCIEX QTRAP® 6500 LC/MS/MS system with IonDrive™ Turbo V source and Electrospray Ionization (ESI) probe was used. MRM methods for the 6 ginsenosides were developed using 2 MRM transitions per compound for both positive and negative ion mode. Every sample was injected three times in positive and negative polarity.

RESULTS

Ginsenoside Adducts

Ammonium acetate and formic acid were purposely added in to mobile phase, not only to achieve better LC separation, but also to promote the ammoinum adducts in positive mode and formic acid adduct in negative ion mode. In positive mode, $[M+H]^+$, $[M+NH_4]^+$, and $[M+Na]^+$ of ginsenosides are present, and their relative intensities of adduct ions correlate with source temperature shown in figure 1. At the low source temperature, ammonium adduct and protonated ions dominated the spectra in positive mode. As the source temperature went up, metal adduct ions not only survived with the heat, but also intensified. The CID fragmentation pattern of ammonium adduct is similar to $[M+H]^+$, rich and useful structure elucidation.



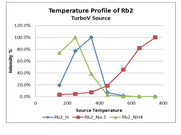


Figure 1. Full scan spectra of ginsenoside Rb1 obtained at the source temperature settings of 250, 450, and 750 ºC in positive mode (left) and source temperature profile for each ion specie of Rb2.

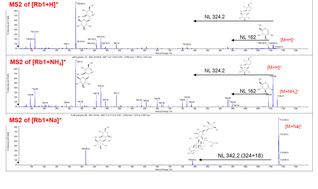
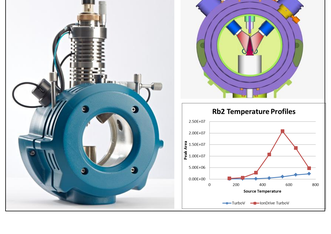


Figure 2. CID spectra of $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$. Ammonium adduct CID fragmentation pattern is similar to $[M+H]^+$, rich and useful for structure elucidation. One way to improve compound characterization is to use ammonium adduct MS2 spectra by adding ammonium in mobile phase and using low source to promote ammonium adducts. The sodiated species produce only glycosidic bond cleavage with limited structure information.

IonDrive™ Turbo V source



The IonDrive™ Turbo V source was designed for providing more heat and better desolvation and ionizations, especially at high flow rates. It features increased heater diameter (11 mm ID vs. 4 mm ID on Turbo V™ ion source) for improved ionization, larger "sweet spot", less performance variability and improved design over Turbo V™.

The IonDrive™ Turbo V source works really well with heat-loving compounds like ginsenosdies. The temperature profile of [Bb2 + Na]+ obtained on the IonDrive™ Turbo V source show a significant response gain over Turbo V™ source.

A back-to-back comparison between IonDrive™ Turbo V source and conventional Turbo V™ source was performed on 6 ginsenoside mixture. S/N gains, similar to signal gains are compound dependent. Most gain was obtained on high mass ginsenosides, like Rb1 and Rc.

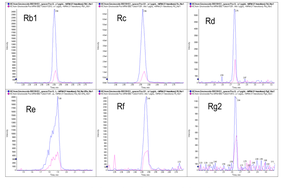


Figure 3. XICs of the six ginsenosides from IonDrive™ Turbo V source (blue) and conventional Turbo V™ source (purple).

			Averaged Area (counts)		Averaged Signal-to-Noise		Response Gain	S/N Gain
Peak Name	Q1	Q3	BHS1	TurboV1	BHS1	TurboV1		
Rb1_Na-1	1131.6	789.6	4.37E+04	6.21E+03	9.30E+02	1.30E+02	7.0	7.2
Rb1_Na-2	1131.6	365.2	1.04E+05	1.45E+04	2.15E+03	3.10E+02	7.1	6.9
Rc_Na-1	1101.6	789.6	1.11E+05	1.80E+04	2.35E+03	3.77E+02	6.2	6.2
Rc_Na-2	1101.6	335.2	1.00E+05	1.55E+04	2.11E+03	3.22E+02	6.5	6.5
Rd_Na-1	969.6	789.6	1.11E+05	1.84E+04	2.25E+03	3.91E+02	6.0	5.7
Rd_Na-2	969.6	365.2	1.29E+04	2.33E+03	2.60E+02	4.76E+01	5.5	5.5
Re_Na-1	969.6	789.6	1.07E+05	2.81E+04	8.41E+02	2.28E+02	3.8	3.7
Re_Na-2	969.6	349.2	1.30E+04	3.91E+03	1.06E+02	3.26E+01	3.3	3.3
Rf_Na-1	823.5	481.5	4.29E+03	1.52E+03	7.88E+01	2.56E+01	2.8	3.1
Rf_Na-2	823.5	365.2	1.62E+05	4.61E+04	3.20E+03	9.30E+02	3.5	3.4
Rg2_Na-2	807.5	481.5	3.32E+03	2.01E+03	4.55E+01	3.68E+01	1.7	1.2
Rg2_Na-3	807.5	349.2	1.14E+04	7.72E+03	2.15E+02	1.53E+02	1.5	1.4

Table 1. IonDrive™ Turbo V source signal and S/N gains over conventional Turbo V™ source

Quantitation of Ginsenosides

Quantitation was easily performed in both positive and negative mode since the same LC condition was used for both modes. 4 – 5 orders of linear dynamic range with 10-100 fg/uL LLOQ were achieved for all 6 ginsenosides tested. Better linear range can be achieved if deuterated IS was available. Lower LLOQs and wider linear range were achieved on positive mode except for Rf and Rg2, where better LLOQ and linear range were obtained in negative mode. The unusual behavior can be due to the readily loss of water in Rf and Rg2 in positive mode.

	Positive Mode			Negative Mode		
Ginsenoside	LLOQ (fg/uL)	Linear Range (fg/uL)	orders of magnitudes	LLOQ (fg/uL)	Linear Range (fg/uL)	orders of magnitudes
Rb1	10	10-100,0000	4	100	100-10,000	2
Rc	10	10-100,0000	4	100	100-100,000	3
Rd	10	10-1,000,000	5	1000	1000-100,000	2
Re	10	10-100,000	4	1000	1000-1,000,000	3
Rf	10	10-10,000	3	100	100-1,000,000	4
Rg2	1000	N/A	N/A	100	100-1,000,000	4

Table 2. Quantitation results for ginsenosides in both positive and negative mode.

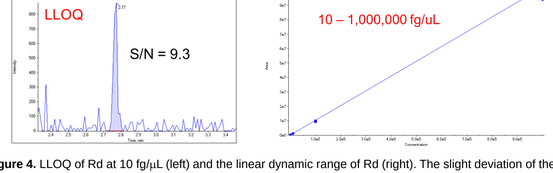


Figure 4. LLOQ of Rd at 10 fg/µL (left) and the linear dynamic range of Rd (right). The slight deviation of the highest concentration was due to the source saturation since no deuterated internal standard was used.

Other Glycosides

Other glycosides from TCMs, like hesperidin, rutin, digitoxin, bilobalide, ginkolide A and B, were also explored. Rutin and hesperidin exhibited similar behavior like ginsenosides, the others did not. Protonated species dominated the spectra at low source temperature, while sodiated species at high source temperature.

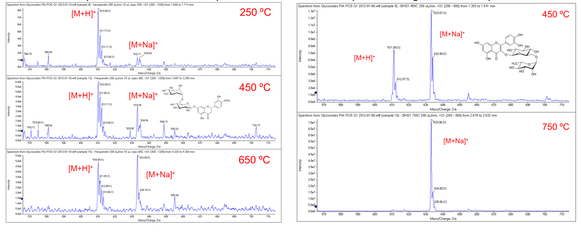


Figure 5. Q1 scan of hersperidin (left) and rutin (right) at different source temperatures. The sodiated ions were promoted at higher source temperature. The protonated ions were not.

CONCLUSIONS

The relative intensities of protonated and adduct ions of ginsenosides correlate with ion source temperature. Ammonium adducts and protonated ions prefer relatively low source temperature, while metal adduct ions are in favor of high source temperature.

The new IonDrive™ Turbo V source, which provides more heat and better desolvation, benefits the sodiated adduct ions. Significant sensitivity gain of ginsenosides over conventional Turbo V™ source was observed on all tested ginsenosides. The overall sensitivity gain is between two times to seven times, with the most gain on high mass ginsenosides, like Rb1 and Rc.

LC/MS/MS methods were developed to quantify ginsenosides based on both sodiated species in positive ion mode and deprontonated species in negative ion mode. Lower LLOQs, 10 fg/uL , were achieved in positive mode using sodiated species than negative mode. Linear Dynamic ranges of ginsenosides are typically between 3 and 5 orders of magnitude without deuterated internal standards.

TRADEMARKS/LICENSING