Identification of Ginsenosides Using the SCIEX X500R QTOF System

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Background

Ginseng is one of the most valued herbs. It has properties of nourishing vital strength, tranquilizing the mind, promoting secretions, and supplementing deficiencies. Modern medical research shows that ginseng is effective in preventing cancer, countering aging, acting as an antiarrhythmic agent, and has hypoglycemic, hypolipidemic, and immune-stimulating properties. Its main active components are ginsenosides.

Ginsenosides are triterpenoid chemical compounds; based on the glycosyl structure, they can be divided into tetracyclic triterpenes of the dammarane type and pentacyclic triterpenes of the oleanane type. The dammarane type can be divided into ginseng diols and ginseng triols. Because ginsenosides have many components, different species and sources yield differences in composition [1], so a full identification of the ginsenoside composition and accurate analysis of its structure currently requires extensive literature and document research. At the same time, the analytical result obtained by different technologies can be quite difficult to verify with data, which can complicate quality evaluation and material basis.

The SCIEX X500R QTOF high resolution mass spectrometer requires a single injection to collect high quality MS and MS/MS data. Combined with an expansive high resolution MS/MS database of Traditional Chinese Medicine (TCM) active ingredients, SCIEX OS software automatically determines the theoretical molecular weight and isotope pattern distribution and simultaneously matches it with the MS/MS database. Comprehensive scoring allows intuitive, rapid, and accurate ginsenoside component identification.

The SCIEX high resolution database of Chinese medicine is based on “Chinese pharmacopeia” Part 1, TCM active ingredients. It includes almost a thousand compounds such as saponins, flavonoids, flavonoid glycosides, triterpenes, phenylethyl glycosides, and organic acids.

This document describes the workflow for analysis of ginsenosides in Chinese medicine using the SCIEX OS ultra-efficient data processing software and the high resolution Chinese medicine MS/MS database on the SCIEX X500R QTOF high resolution mass spectrometer system.

The software has a simple user interface, and the workflow is intuiative.

Experimental Process

1. Using TOF-IDA- mode (Top 10 MS/MS per cycle), inject a sample and simultaneously obtain primary precursor ions and secondary daughter ion information. This allows confidence in the compound identification and allows all data to be acquired ina single shot alleviating time involved in a two-injection workflow.
2. Search known ginsenoside components; according to the accurate mass, isotope distribution, and Chinese medicine matching data, identify the compounds.
3. Use Ginsenoside Rg2 as an internal standard to verify accuracy of match results.
4. Use the accurate mass, characteristic fragment ions, and relative retention times to enhance identification of ginsenoside isoforms.
5. A total of 51 commonly observed ginsenoside components have already been identified.

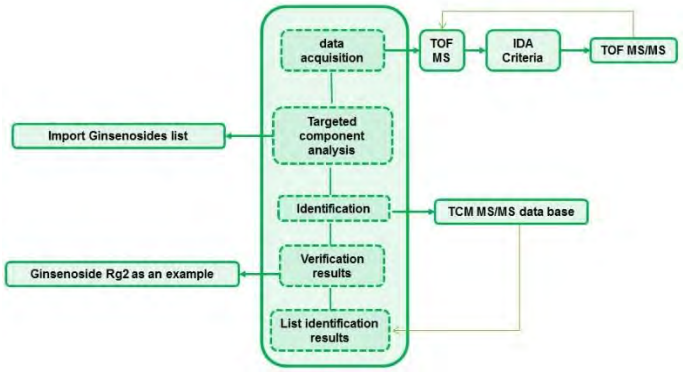


Figure 1 Workflow for using the SCIEX OS X500R QTOF high resolution mass spectrometer and the Chinese medicine MS/MS database to identify ginsenoside components

Sample Preparation

1. Accurately measure 5.0g ginseng powder into a 50mL centrifugation tube.
2. Add 25mL 90% methanol water, agitate 5 min.
3. Immerse in an ice bath overnight.
4. Ultrasonicate 30 min, at 4 deg. C, then centrifuge at 10000r/min for 12 min.

5. Remove the supernatant and pass through a 0.22μm filter.

Liquid Chromatography (LC) Conditions

Chromatographic Column: Phenomenex Kinetex C18, 2.1*100mm, 2.6μm;

Mobile phase: Gradient elution is used

Negative ions: A is H_2O (containing 0.05% formic acid); B is acetonitrile;

Flow rate: 0.25mL/mL

Column temperature: 40℃

Injection volume: 3μL

Table 1. Elution conditions

Time (min)	A%	B%
0	90	10
0.5	90	10
5.0	50	50
35.0	10	90
40.0	0	100
40.1	90	10
45.0	90	10

Mass Spectrometry Conditions

Scanning method: TOF-IDA-10 MS/MS qualitative;

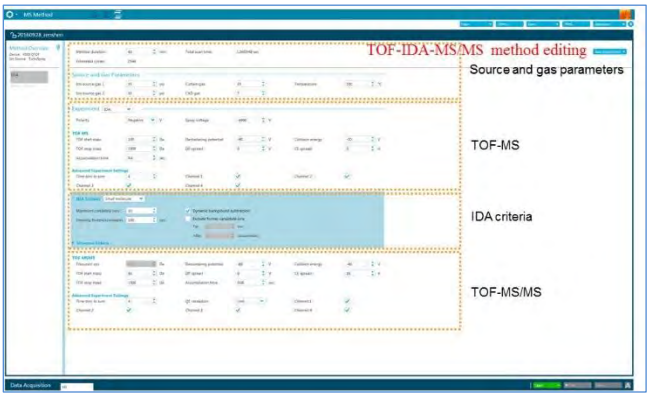
ESI ion source parameters:

Air curtain gas CUR: 35psi;

IS voltage: -4500V: Source temperature: 550℃

Cone voltage: -80V;

Atomizing gas GAS1: 55psi; Auxiliary gas GAS2: 55psi



Application of SCIEX OS Software for Ginsenoside Analysis

SCIEX OS Software platform provides simultaneous mass spectrometer control, method editing, data analysis, and result reporting.

1. Data acquisition

Data acquisition is performed on extracted samples according to conditions described. The Explorer data processing options can be used to open the acquired high-resolution data, and perform any data QC as in Fig. 2.

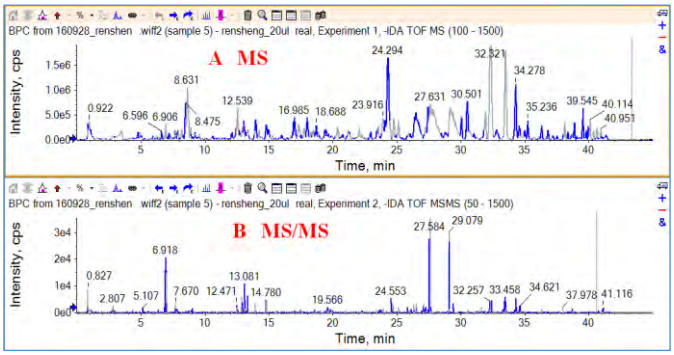


Figure 2. Acquired high-resolution TOF MS-IDA-TOF MS/MS data. Fig. 2A shows a full TOF MS scan, and Fig. 2B is an IDA TOF MS/MS spectrogram of a sample.

2. Editing of data processing methods

Using targeted component analysis, one can enter or copy known ginsenosides to the component options, including their name and molecular composition, as shown in Fig. 3.

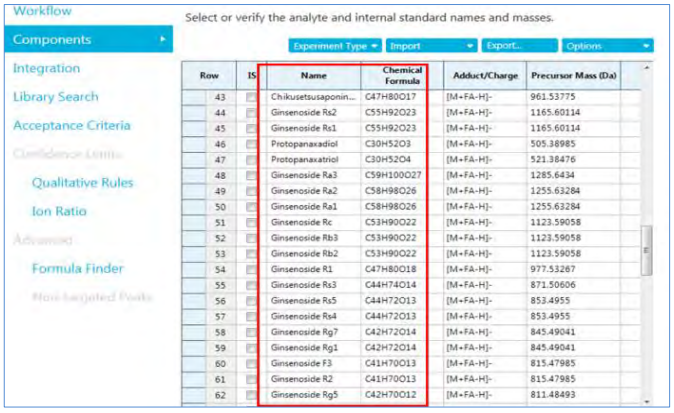


Figure 3. Input chemical compound list

At the same time, select the database to search (this study uses the TCM MS/MS Library) and configure the confidence levels, as shown in Fig. 4.

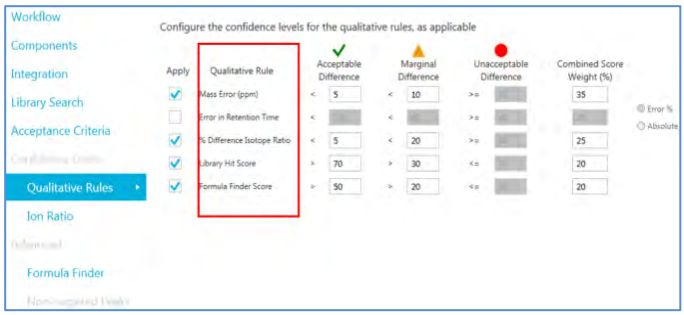


Figure 4. Setting SCIEX OS Software confidence levels

Confidence intervals are primarily used in compound identification and verification, including theoretical mass numbers, isotope distribution, retention times (can be omitted if unavailable), and MS/MS spectrum matching in the database. Each score is calculated based on the configuration, and an overall score is determined based on the weight of the four parameters.

3. Data processing and Results Viewing

Use built-in processing methods to processing data. Select “process,” and the software will list results based on 4 established confidence intervals. Use the “signal indicator” to easily obtain the results, including MS/MS matched results.Identification of Ginsenoside Rg2 is given as an example; results are shown in Fig.5.

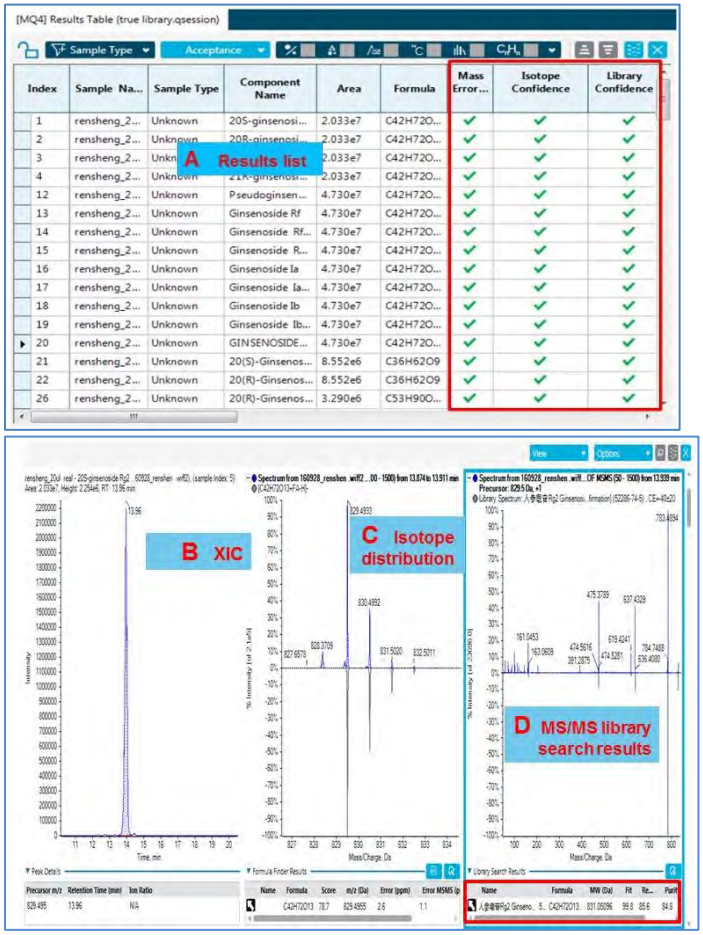


Figure 5. Screening results display

Fig. A is the display of the data processing results; B is the display of an extracted ion chromatogram. Retention time is useful for compound verification. Fig. C shows the mass spectrum and isotope distributions; the upper portion is the actual measured value, the lower, gray part shows the theoretical MS pattern, and the two match well. Fig. D is the mirror image display for the MS/MS database match results: the upper, blue portion is the MS/MS spectrum acquired for ginsenoside, and the lower, gray portion shows the MS/MS spectrum from the database. Results are compared clearly. The lower right corner shows database search results, and with an overall score above 90, matching results are excellent.

Verification of identification results

For ginsenoside in the negative ion mode, the excimer ion peaks are mainly present at [M-H]¯ and [M+HCOOH-H]; the structure of the saponin component is described by its secondary signature fragment loss of HCOOH and daughter ions of sugars, e.g.: -46 -162 (Loss HCOOH& Glu); -46-146 (Loss HCOOH& Rhamnose) or -46-132 (Loss HCOOH& arabinose), and fragment 161 forms readily. For ginsenoside Rg2 secondary fragment structure analysis, see Fig. 6.

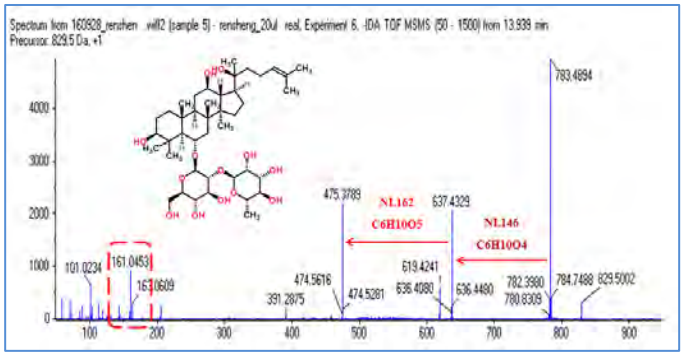


Figure 6. Secondary structural analysis spectrogram for ginsenoside Rg2

Using MS/MS information, a neutral loss (NL) loss of 162 and 146, and the signature fragment for dammarane triol at m/z 475, this structure can be described as dammarane triol +glucose+rhamnose, which is similar to ginsenoside Rg2 and matches search results.

Ginsenosides are made of saponins and sugars such as glucose, rhamnose, and arabinose that may be linked at various positions on the saponin. At the same time, since saponins and sugars have different structures, they may form stereoisomers. In database searches, isomers link to secondary signature fragment ion information, retention time, and relevant literature [2]. Verification of known ginsenosides is necessary, and secondary fragment information is critical for structural identification of Chinese medicine components

Table 2. List of identified ginsenosides

Index	Compound name	Molecular formula	[M+HCOOH-H]-	ss Error (ppm)	Retention time (min)	MS/MS
1	20R-ginsenoside Rg2	C42H72O13	829.4944	2.30	13.93	m/z783, 637, 475,391,161
2	20S-ginsenoside Rg2	C42H72O13	829.4944	1.78	14.01	m/z783, 637, 475,391,161
3	20S-ginsenoside F2	C42H72O13	829.4944	1.50	21.05	m/z783, 621, 459,161
4	20R-ginsenoside F2	C42H72O13	829.4944	1.10	20.10	m/z783, 621, 459,161
5	20S-ginsenoside Rg3	C42H72O13	829.4944	-0.80	26.09	m/z783, 621,459,375,161
6	20R-ginsenoside Rg3	C42H72O13	829.4944	-0.80	26.54	m/z783, 621,459,375,161
7	Ginsenoside Rg4	C42H70O12	811.4838	-1.20	7.79	m/z765,619,457,161
8	Ginsenoside F4	C42H70O12	811.4838	-1.70	12.22	m/z765,619,457,161
9	Ginsenoside Ic	C42H70O12	811.4838	-0.90	28.58	m/z765,619,457,161
10	Ginsenoside Rk1	C42H70O12	811.4838	-1.20	29.04	m/z765,603,441,161
11	Ginsenoside Rk1 (isomer)	C42H70O12	811.4838	1.40	41.33	m/z765,603,441,161
12	Pseudoginsenoside F11	C42H72O14	845.4893	-1.60	12.52	m/z799,637,475,161
13	Ginsenoside Rf	C42H72O14	845.4893	-1.90	8.63	m/z799,637,475,161
14	Ginsenoside Rf (isomer)	C42H72O14	845.4893	-1.60	8.35	m/z799,637,475,161
15	Ginsenoside Rg7	C42H72O14	845.4893	-2.00	7.64	m/z799,637,475,161
16	Ginsenoside Rg1(Ginsenoside A2)	C42H72O14	845.4893	-1.60	7.40	m/z799,637,475,161
17	Ginsenoside F1	C36H62O9	683.4365	0.90	15.85	m/z637,475,391,161
18	20(S)-Ginsenoside Rh1	C36H62O9	683.4365	0.70	14.23	m/z637,475,391,161
19	20(R)-Ginsenoside Rh1	C36H62O9	683.4365	0.90	14.10	m/z637,475,391,161
20	Ginsenoside Re	C48H82O18	991.5472	-0.84	8.47	m/z945, 799,637,475
21	Ginsenoside Rd	C48H82O18	991.5472	-0.70	21.22	m/z945,783,621,459,375,161
22	Ginsenoside Rd (isomer)	C48H82O18	991.5472	-0.70	22.83	m/z945,783,621,459,161
23	pseudo-Ginsenoside RT2	C41H70O14	831.4737	-1.50	7.41	m/z785,653,491,391
24	Ginsenoside Rb2	C53H90O22	1123.5895	-1.90	18.45	m/z1077,945,783,621,459
25	20(S)-Ginsenoside Rc	C53H90O22	1123.5895	-2.10	19.44	m/z1077,945,783,621,459
26	20(R)-Ginsenoside Rc	C53H90O22	1123.5895	-1.90	19.76	m/z1077,945,783,621,459
27	Ginsenoside Rb1	C54H92O23	1153.6001	-1.00	17.99	m/z1107, 945,783,621,459
28	20(S)-Ginsenoside-Rh2	C36H62O8	667.4416	-0.50	29.52	m/z621,459,375
29	20(R)-Ginsenoside-Rh2	C36H62O8	667.4416	-0.50	30.50	m/z621,459,375
30	Ginsenoside Rd+Acetylation	C50H84O19	1033.5578	-1.40	20.26	m/z987, 945, 928, 783, 621,459
31	Ginsenoside Re+Acetylation	C50H84O19	1033.5578	-1.52	20.74	m/z987, 945, 928, 783, 621,459
32	Pseudoginsenoside RT5	C36H62O10	699.4314	-1.10	7.75	m/z699,653,491,329,161
33	Ginsenoside Ra1	C58H98O26	1255.6317	-0.63	17.23	m/z1209,1077,945,783,621,459
34	Ginsenoside Ra2	C58H98O26	1255.6317	-0.70	18.40	m/z1209,1077,945,783,621,459
35	Chikusetsusaponin III	C47H80O17	961.5367	-1.00	23.65	m/z915,783,621,459,375
36	Ginsenoside Rs2	C55H92O23	1165.6001	1.60	20.17	m/z1119,1077,1059,945,783,621,459
37	Ginsenoside Rs2 (isomer)	C55H92O23	1165.6001	1.84	19.57	m/z1119,1077,1059,945,783,621,459
38	Ginsenoside Rs1	C55H92O23	1165.6001	1.00	18.67	m/z1119,1077,1059,945,783,621,459
39	Ginsenoside Rs1 (isomer)	C55H92O23	1165.6001	1.00	17.73	m/z1119,1077,1059,945,783,621,459
40	Ginsenoside R1	C47H80O18	977.5316	-1.20	7.87	m/z 931, 799, 637, 475, 161
41	Ginsenoside F3	C41H70O13	815.4788	-1.40	13.04	m/z 161, 391, 475, 637, 769
42	Ginsenoside F3 (isomer)	C41H70O13	815.4788	-1.44	11.22	m/z 161, 391, 475, 637, 769
43	Pseudo-ginsenoside RT1	C47H74O18	971.4846	0.40	9.64	m/z 161, 763
44	Ginsenoside Rs3	C44H74O14	871.5050	0.40	25.25	m/z 161, 459, 621,783
45	Ginsenoside R2	C41H70O13	815.4788	-1.40	10.52	m/z 161, 391, 475, 637, 769
46	Ginsenoside Ra3	C59H100O27	1285.6423	-2.09	15.97	m/z 1239, 1077, 945, 783, 621
47	Ginsenoside Ra3 (isomer)	C59H100O27	1285.6423	-2.10	17.52	m/z 1239, 1077, 945, 783, 621
48	Ginsenoside Rb3	C53H90O22	1123.5895	-1.00	18.45	m/z 1077, m/z 1123
49	Ginsenoside Rb3 (isomer)	C53H90O22	1123.5895	-1.13	19.44	m/z 1077, m/z 1123
50	Ginsenoside Rk3	C36H60O8	665.4259	-1.30	20.68	m/z 161, 619
51	Protopanaxatriol	C30H52O4	521.3837	-1.00	21.26	m/z521,475,391
52	Protopanaxatriol(isomer)	C30H52O4	521.3837	-1.03	22.05	m/z521,475,391
53	Ginsenoside Ro	C48H76O19	1001.4952	2.40	25.07	m/z 955,793,631,455

Conclusions

This study used the high-resolution SCIEX X500R QTOF System for identification of ginsenoside components. It uses SCIEX OS software along with the TCM MS/MS database for rapid, accurate identification of 53 ginsenoside components, showing strong resolving power and illustrating the benefits of the high-resolution SCIEX database in traditional Chinese medicine analysis. The high-resolution MS/MS TCM database contains almost a thousand TCM active ingredient MS/MS spectra; automatic data extraction can be used for matching and greatly decreases the identification time for Chinese medicines. It also allows for simple and accurate component identification.

The SCIEX X500R QTOF high-resolution system is the right tool when identifying Chinese medicine components. The IDA workflow can be used to ensure the integrity of the acquisition, and TOF MS and MS/MS data can be obtained for all components. The X500R's front end has all the advantages of a triple quadrupole mass spectrometer, greatly improving its quantification capabilities, sensitivity, stability, and linear range.

SCIEX OS system software integrates instrument control, method editing, data acquisition, and reporting. It can perform simultaneous qualitative and quantitative analysis, wirelessly connect to other software, and simplify analytic workflow.

References

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