A Mass Spectrometry Applications Guide to

Elevate Your Natural Products Analysis

SCIEX

The Power of Precision

Contents

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X500 QTOF System Applied to Natural Products Testing

Contents

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Contents

Research on the Different Composition of Smilax Glabra from Different Regions by High Resolution Mass Spectrometry Using the SCIEX X500R QTOF System

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Introduction

China has a long tradition of using herbs of diverse types as medicine. *Smilax Glabra* is one of the most common Chinese herbal medicines. *Smilax Glabra* has many components, including volatile ingredients, sterols, fatty acids, phenols and flavonoids. *Smilax Glabra* is an antitumor, anti-arteriosclerosis drug that is used for treatment of coronary disease. It is also a treatment for angina pectoris. *Smilax Glabra* grows mainly in the Yangtze River area and the southern provinces. *Smilax Glabra* from different places varies in efficacy and quality due to differences in environmental conditions and climate. Differentiating between *Smilax Glabra* from different regions is important to ensure *Smilax Glabra*'s therapeutic efficacy.

The SCIEX X500R QTOF System is a revolutionary high-resolution mass spectrometry system. As the first platform developed especially for high throughput laboratory testing, it is stable, reliable, and powerful. The fully updated hardware design offers rapid scanning in combination with powerful information-dependent acquisition (IDA); using a single injection, TOF-MS accurate mass numbers and TOF-MS/MS secondary fragmentation spectra can be obtained. SCIEX's unique MarkerView™ Software can be used to perform statistical analysis on differences in *Smilax Glabra* from different regions. It can automatically correlate TOF-MS and TOF-MS/MS secondary fragmentation spectra. Chinese medicine ingredients are complex, and currently identification primarily relies on mass number, fragmentation ions, plus online and photo research. These methods waste time and effort, and they produce results of poor accuracy. SCIEX has a high-resolution MS/MS database with almost 900 Chinese medicine ingredients. Combined with primary exact mass numbers and isotope distribution, one can quickly and accurately make identifications.

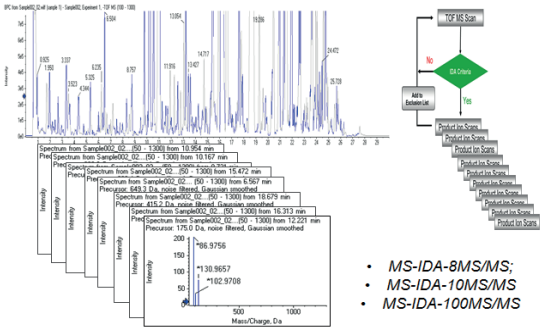
This application note describes using the SCIEX X500R QTOF System including updated SCIEX OS Software for mass spectrographic control and data acquisition and analysis. With a single sample injection, one can analyze differences with MarkerView Software and the high-resolution MS/MS Chinese medicine database to perform Chinese medicine component differential analysis.

Experimental Process

1. Three varieties of *Smilax Glabra* from four different regions - Guangdong Jiangmen, Guangdong Heyuan, Guangxi Jiazhou and Hunan - were collected, dried and crushed.
2. Using the X500R's high-resolution mass spectrum TOF-MS-IDA-12 MS/MS mode with a single sample injection, TOF-MS and TOF-MS/MS mass spectra were obtained.
3. MarkerView Software cluster analysis revealed a statistically significant difference between components and markers.
4. Using primary accurate mass numbers, secondary fragments, and the high-resolution MS/MS Chinese medicine database for comparison, database searches and structural verification were performed for the different components.

X500R QTOF System High Resolution Mass Spectrometry Screening Workflow

Contents



- *MS-IDA-8MS/MS;*
- *MS-IDA-10MS/MS*
- *MS-IDA-100MS/MS*

Preprocessing Method

Using 4 groups of Smilax Glabra from different areas, weigh about 1.0 g of Smilax Glabra powder, take 3 equal samples from each group, add 90%formic acid-aqueous solution 10 mL, vortex 10 min, then ultrasonicate at room temperature for 30 min, remove supernatant and centrifuge at 13000 rpm for 10 min, extract supernatant and use as sample.

Chromatographic Conditions

Chromatographic Column: Phenomenex Kinetex C18, 100 x 4.6 mm, 2.6 µm;
Mobile phase: A: 0.01% Formic acid Water/B: Acetonitrile

Flow rate: 0.4 mL/min;

Column temperature: 40ºC;

Injection volume: 10 µL

Table 1. Elution conditions

Time (min)	A%	B%
0.0	95	5
5.0	55	45
15.0	20	80
20.0	5	95
25.0	5	95
25.1	95	5
30.0	95	5

Mass Spectrometry Method

Scanning method: TOF-MS -IDA-12MS/MS;

Ion source: ESI source double spray technology

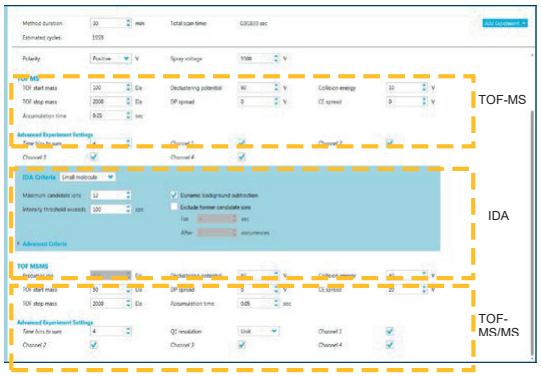
Scanning range: m/z 50-2000

CDS automatic calibration

Mass Spectrum Parameter Establishment

ESI ion source parameters:

Air curtain gas CUR: 35psi; Collision Gas CAD: 7
IS voltage: 5500V/-4500V; Source temp: 550ºC
Atomizing gas GAS1: 55psi
Auxiliary gas GAS2: 55psi
DP voltage: ± 60 V
Collision energy: 40 ±20V
Dynamic Background Subtraction



MarkerView™ Data Processing

MarkerView's advanced processing algorithm finds the peaks, and data alignment compensates for small variations in the retention time and mass number. The differences between samples can be normalized. Before the data is processed, known background ion interference or uncommon ions in the sample can be canceled out.

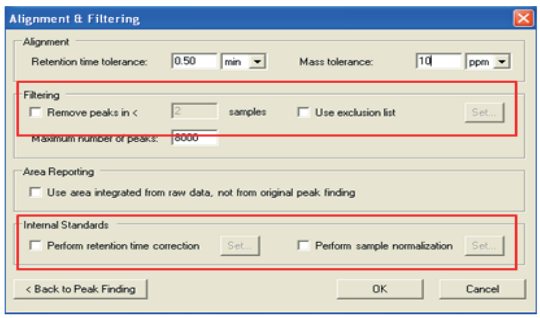


Figure 1 Advanced processing algorithm finds characteristic peaks by MarkerView™ Software

Contents

All samples underwent supervised PCA analysis and received a PCA score and a loading chart: The loading chart reflects the variables of intergroup differences; a high load value generally indicates a significant effect on separation.

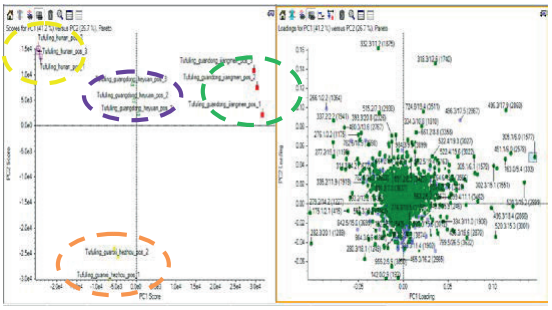


Figure 2 The PCA Score and loading diagram of Glabrous Greenbrier Rhizome from different regions

The PCA Score Plot and loading chart show *Smilax Glabra* samples from 4 different areas are well differentiated along the PC1 dimension, showing large differences between the 4 groups. Taking m/z 305.0649, RT 5.97 min as an example, it is clear that this component's load value is high, and the content of *Smilax Glabra* in samples from Guangdong Jiangmen is higher than those from Guangdong Heyuan, Guangxi Jiazhou, or Hunan.

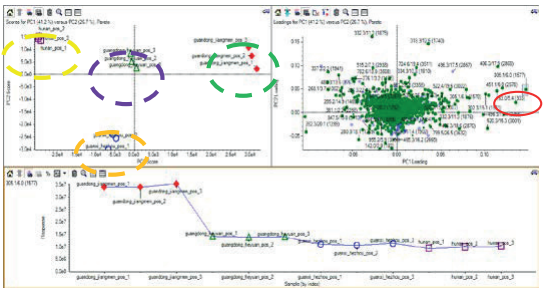


Fig. 3 The content change of m/z 305.0654, RT5.98min in different groups

The MarkerView Software intuitively shows differences between samples in terms of primary accurate mass numbers, isotope ratios, and TOF-MS/MS secondary spectrograms; it is convenient and accurate.

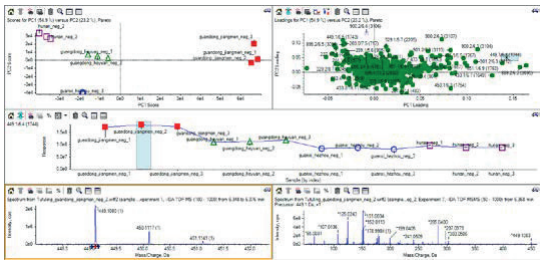


Figure 4 MarkerView software automatically associated with the raw data

Chinese Medicine Database Identification of Chemical Compounds

The SCIEX high resolution Chinese medicine database is based on the "Chinese pharmacopeia" Chinese medicine ingredients, including almost 900 compounds such as saponins, flavonoids, flavonoid glycosides, triterpenes, phenylethyl glycosides and organic acids. The SCIEX OS Software's MS/MS database allows entry of component lists. Peak extraction and database matching use SCIEX OS Software's unique confidence parameter configuration and confidence intervals to identify and verify compounds. Using primary accurate mass numbers, retention times, isotope ratios, database scoring, and molecular formula scoring, an overall score is automatically calculated and listed. A "signal indicator" evaluates results simply, clearly, and intuitively.

Figure 5 The confirmed result of m/z 305.0654, RT 5.98 min

Fig. 5A is the extracted ion flow chart for a compound; its retention time can be verified. Fig. 5B displays the mea primary mass number and isotope ratio. A mirror image

Contents

comparison is performed with the chemical compound's theoretical values from the database. Fig. 5C shows mirror image comparisons of secondary spectra, namely between MS/MS spectra and database spectra.

Summary of Identification Results

Using MarkerView™ data analysis and MS/MS secondary database matching result verification, *Smilax Glabra* differences from different areas were verified:

Mass	Adduct	Formula	Compound	Origins
449.1085	M-H	C21H22O11	Astilbin	
527.374	M-H	C33H52O5	Pachymic acid	Surface layer,Sclerotium
453.3376	M-H	C30H46O3	Betulonicacid	
525.3588	M-H	C33H50O5	Dehydropachymic acid	Surface layer,Sclerotium
511.3432	M-H	C32H48O5	Acetyl-11-keto-β-boswellic acid	
433.1134	M-H	C21H22O10	Engeletin	
283.0609	M-H	C16H12O5	Calycosin	
525.3581	M-H	C28H48O6.HCOOH	Epibrassinolide +HCOOH	
471.3483	M-H	C30H48O4	Echinocystic acid	
305.0649	M+H	C15H12O7	Dihydroquercetin	
453.3376	M-H	C30H46O3	Dehydrotrametenolic acid	Surface layer,Sclerotium
525.3588	M-H	C33H50O5	3-epi-dehydropachymic acid	Sclerotium
Mass	**Adduct**	**Formula**	**Compound**	**Origins**
285.0748	M+H	C16H12O5	Biochanin A	
433.113	M-H	C21H22O10	Isoengeletin	
511.3432	M-H	C32H48O5	Poricoic acid AM	Surface layer
449.1085	M-H	C21H22O11	Smilsibin	
525.3588	M-H	C33H50O5	Poricoic acid AE	
455.3535	M-H	C30H48O3	Trametenolic Acid	Surface layer
527.374	M-H	C33H52O5	3-O-Acetyltumulosic acid	
449.1085	M-H	C21H22O11	Neosmilsibin	
453.3376	M-H	C30H46O3	3beta-Hydroxylanosta-7,9(11),24-trien-21-oic acid	Sclerotium
511.3432	M-H	C32H48O5	3-O-acetyl-16α-hydroxydehydrotrametenolic acid	Sclerotium
453.3376	M-H	C30H46O3	Pinicolic acid A	Surface layer,sclerotium
455.3531	M-H	C30H48O3	Ursolic Acid	
511.3432	M-H	C32H48O5	3β-hydroxy-16α-acetoxy-lanosta-7,9(11),24-trien-21-oic acid	Sclerotium

MarkerView Software T-test Data Processing

MarkerView Software can also use T-test data analysis to find different components. The MarkerView Software volcano plot shows log fold change versus p-value plots. The smaller the p-value, the more significant the differences between the log values of m/z at either end of the X axis.

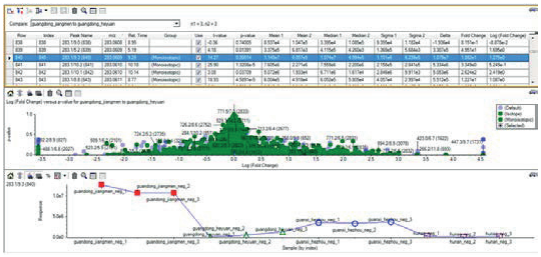


Figure 6 Mapping of log ratio change and p-value

Figure 7 The confirmed result of m/z 283.0609, RT 9.28 min

The SCIEX secondary high-resolution MS/MS database helps to quickly identify and verify compounds.

MarkerView Software Welch T-test Function

MarkerView1.3 Welch T-test has a group alignment function to enhance result accuracy. New box plots appear automatically and identify parallelism among samples within the group.

Figure 8 Box and whiskers plots figure out the parallelism between the test samples

ChemSpider Database Search and Rapid Verification

SCIEX OS Software automatically correlates with the ChemSpider database, which enhances the database capability to rapidly verify chemical compound names. The Fragment Pane fragment analysis function helps rapidly verify chemical compound secondary fragment analysis.

Contents

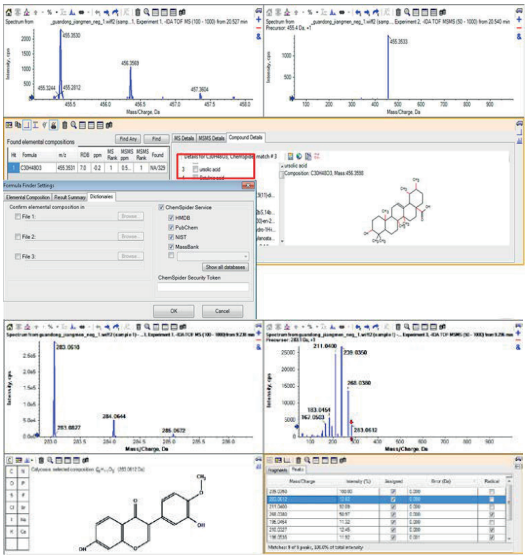


Figure 9 ChemSpider database can help identifying compounds rapidly

Conclusions

The SCIEX X500R high-resolution system's rapid scan speed and dynamic background subtraction (DBS) function can help to complete sample preprocessing and efficiently remove background interference. A single needle injection can yield both primary and secondary spectral information, suitable for high-throughput analysis of differences in traditional Chinese medicine. SCIEX OS Software allows for rapid data acquisition, analysis, and database searching; one-touch processing is simple, fast, and convenient.

MarkerView analytic software uses PCA and T-test for analysis, which reveal differences in components quickly and intuitively. It automatically correlates raw data to provide TOF-MS and TOF-MS/MS spectra that bolster analysis. The newly added Welch T-test function offers clearer and more practical results.

This study applied the SCIEX X500R high-resolution liquid chromatography system to analyze differences in TCM components. The powerful SCIEX OS Software and analytic MarkerView Software are well integrated; primary accurate mass number, secondary comprehensive fragment analysis, and isotope ratios can quickly verify compounds. The secondary high-resolution database links to the online ChemSpider database for automated matching and rapid verification of differences, yielding accurate and reliable results. The efficient integration of Chinese medicine identification solutions from SCIEX offers comprehensive analysis.

References

1. Chemical Constituents and Pharmacological Properties of Poria cocos.

2. Mycology, cultivation, traditional uses, phytochemistry and pharmacology of Wolfiporia cocos (Schwein) Ryvarden et Gilb.: A review.

3. Comparative study of lanostane-type triterpene acids in different parts of Poria cocos (Schw.) Wolf by UHPLC-Fourier transform MS and UHPLC-triple quadruple MS.

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Contents

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 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, and the powerful information dependent acquisition (IDA) function creates high resolution TOF-MS and TOF-MS/MS spectrograms. Combined with an expansive high resolution MS/MS database of Chinese medicine active ingredients, the software automatically determines the theoretical molecular weight and isotope 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 of the SCIEX X500R QTOF high resolution mass spectrometer system.

The software has a simple user interface, and the workflow is clear. Data results are high quality and efficient.

Experimental Process

1. Using TOF-IDA-10 MS/MS mode, inject a sample and simultaneously obtain primary precursor ions and secondary daughter ion information. This saves time and increases work efficiency.
2. Input known ginsenoside components; according to the accurate mass number, isotope distribution, and Chinese medicine matching data, identify the compounds and
3. Use Ginsenoside Rg2 as a standard to verify accuracy of match results.
4. Use the accurate molecular weight, characteristic secondary fragment ions, and relative retention times to enhance identification of ginsenoside isoforms.
5. 51 commonly observed ginsenoside components have already been identified.

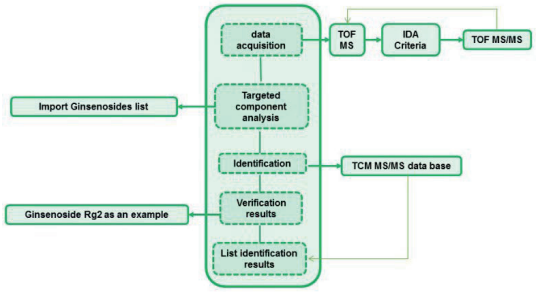


Figure 1 Workflow for using the SCIEX OS X500R QTOF high resolution mass spectrometer and the Chinese medicine MS/MS database to identify ginsenoside components

Preprocessing Method

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 0.2.

Contents

Liquid phase 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 method

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

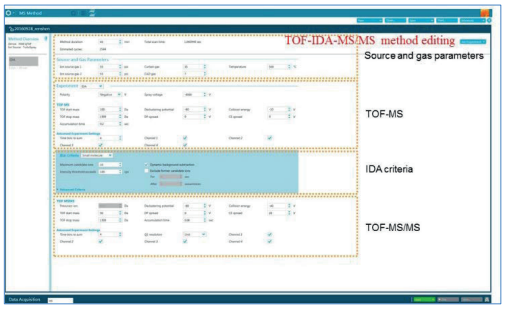
ESI ion source parameters:

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 the Identification of Ginsenosides

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

1. Data acquisition

Data acquisition is performed on processed samples according to edited liquid phase and mass spectrometry methods. The Explorer data processing options can be used to open the acquired high-resolution data, as in Fig. 2.

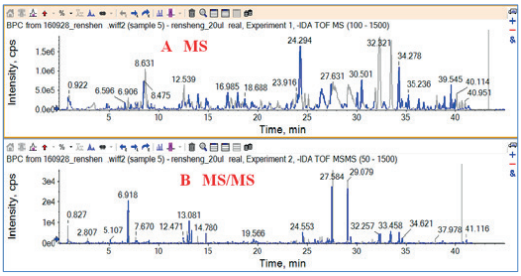


Figure 2. Acquired high-resolution TOF MS-IDA-TOF MS/MS data. Fig. 2A shows a full TOF MS scan, and Fig. 2B is an IDA TOF MS/MS spectrogram of a sample.

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.

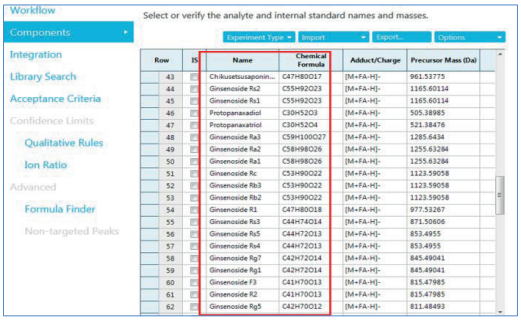


Figure 3. Input chemical compound list

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.

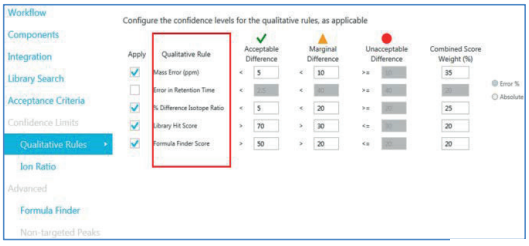


Figure 4. Setting SCIEX OS Software confidence levels

Contents

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, viewing processing results

Using built-in processing methods to open the processing method needed, select “process,” and the software will list results based on 4 established confidence intervals. Use the “signal indicator” to easily obtain the results, including secondary spectrographic match results and results at a glance. Identification of Ginsenoside Rg2 is given as an example; results are shown in Fig.5.

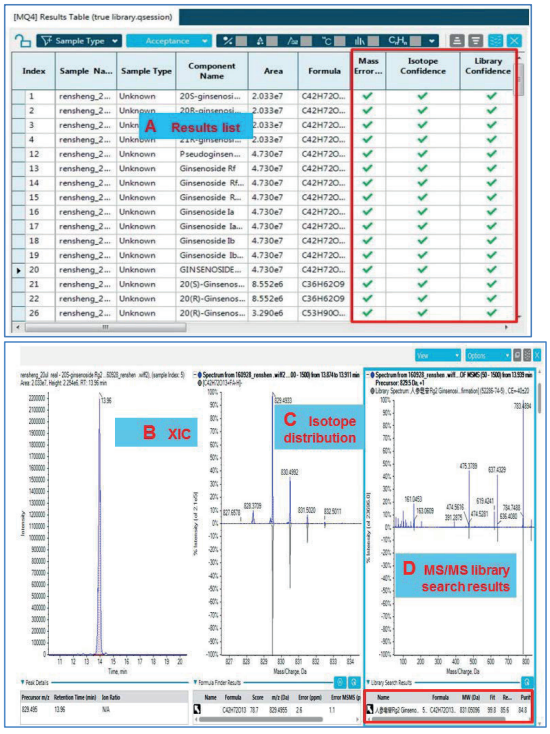


Figure 5. Screening results display

Fig. A is the display of the data processing results; B is the display of an extracted ion flow chart. Retention time is useful for compound verification. Fig. C shows the display for measured mass numbers and isotope distributions; the upper portion is the actual measured value, the lower, gray part shows the theoretical value, 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 good.

Verification of identification results

For ginsenoside in ESI negative ion mode, in the primary mass spectrum, 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.

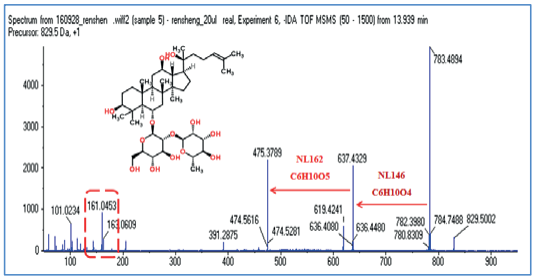


Figure 6. Secondary structural analysis spectrogram for ginsenoside Rg2

Using MS/MS information, NL loss 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

Contents

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

Contents

Conclusions

This study used the high-resolution SCIEX X500R QTOF System for identification of ginsenoside components. It uses SCIEX OSsoftware 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 Chinese medicine identification. 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 updated SCIEX X500R QTOF high-resolution system is very sensitive and discriminating 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. X500R's front end has all the advantages of a triple quadrupole mass spectrometer, greatly improving its quantification capabilities, sensitivity, stability, and linear range.

The fully updated 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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2. Zhang Xiaoxu, Wang Hongping, Yang Yang, et al. Rapid identification of saponins in sunflower ginseng by ultra performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry [J]. China Medical Journal, 2015, 12(9), 130-136.

Document number: RUO-MKT-02-6090-A

Contents

Chemical Components Identification of Cistanche Deserticola Using the X500R QTOF System

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Introduction

Cistanche Deserticola was first recorded in the "*Shen Nong Materia Medica*"; it is also known as "*Dayun*," "*Rousong Rong*," and "*Zong rong*." As traditional herbal medicine, it has the properties of nourishing the kidney yang, improving bloodflow, acting as a laxative, immune stimulation, and other effects[1]. In 1983, the Japanese scholar H. Kobayashi and others began to study the chemical composition of *Cistanche Deserticola*[2], and since then it has become a popular topic in Chinese medicine research that has generated great interest both domestically and abroad over the last 30 years. *Cistanche Deserticola* belongs to the class of plants containing phenolic glycosides, iridoids and their glycosides, and lignans and their glycosides.

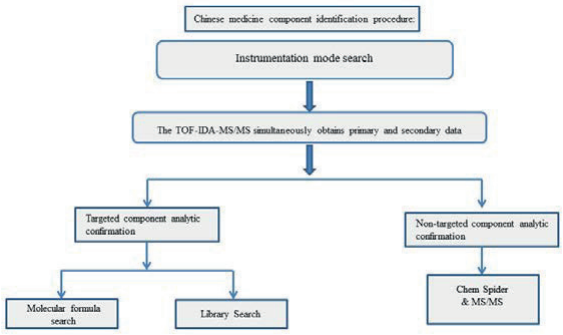
Quadrupole time-of-flight (QTOF) mass spectrometry is a sensitive and specific tool for identification of Chinese medicine components that has gradually become indispensable to research. This technology has overcome traditional technical challenges with retrospective analyses of single injections that permit extraction of important data and the most comprehensive acquisition of sample information. Using exact mass and high resolution TOF-MS and TOF-MS/MS data allows for simultaneous, highly specific targeted and non-targeted qualitative analysis. However, the complexity of instrument operation and software use have vastly limited the spread and development of this technology. Here we introduce a new QTOF system that uses a revolutionary N-type geometry-based TOF path, intuitive software, and accurate molecular weight techniques that are easier to use in Chinese medicine component identification.

The benefits of this method are as follows:

1. The X500R uses the durable, industry-approved Turbo V™ ion source and air curtain gas interface design, which has a strong anti-contamination feature;
2. SCIEX OS Software has integrated acquisition, processing, and reporting functions on a single platform; the interface is intuitive, easy to master, and has one-touch auto-adjust correction to ensure that analysts with any degree of expertise can obtain high quality, reliable data;
3. Using SCIEX OS Software to process data to identify Chinese medicine components is simple and permits rapid extraction of useful information, thus improving efficacy;
4. It derives more accurate and reliable identification results from Chinese medicine libraries containing MS/MS spectra;
5. High-resolution MS/MS Chinese medicine databases are based on the "Chinese pharmacopeia" Part 1 TCM active ingredients; including component references in the pharmacopeia and active ingredients in the herbs, there are nearly 900 compounds.

Experimental Process

1. Using TOF-MS-IDA MS/MS mode, inject a sample and simultaneously obtain primary precursor ions and corresponding secondary spectra;
2. Using SCIEX OS Software targeted screening, confirmation of target compounds, and secondary spectra along with screening of Chinese medicine standards and matching methods can increase accuracy and work productivity.
3. SCIEX OS Software's non-targeted identification workflow uses library searches and complete unknown searches in ChemSpider to verify results, ensuring more components are identified with a simpler workflow.



Samples and Preprocessing Method

Sample source:

Purchased from Shanghai pharmacies in sliced form

Preprocessing method:

1. Slices were crushed to form powder;
2. 0.9mg was weighed and immersed in 3mL methanol for 40 min;

Contents

3. The sample from step 2) was ultrasonicated 1 h;
4. Centrifugation and removal of the supernatant to use as a sample were performed.

Liquid Phase Conditions

Chromatographic Column: XSelect HSS T3, 2.1*150mm, 3.5μm;

Mobile phase: Gradient elution was used

Mobile phase: A is 0.1% formic acid water-2mM NH4FA
B is 95% acetonitrile-5% water-2mM NH4FA

Flow rate: 0.5mL/min

Column temperature: 40ºC

Amount inserted: 5μL

Mass Spectrometry Method

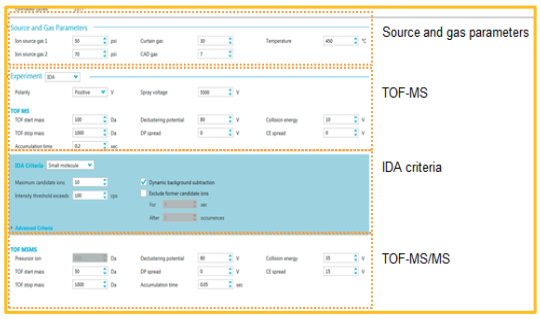
Scanning method: TOF MS-IDA-15 MS/MS qualitative screening

ESI ion source parameters:

Air curtain gas CUR: 35psi; IS voltage: 5500V/-4500V

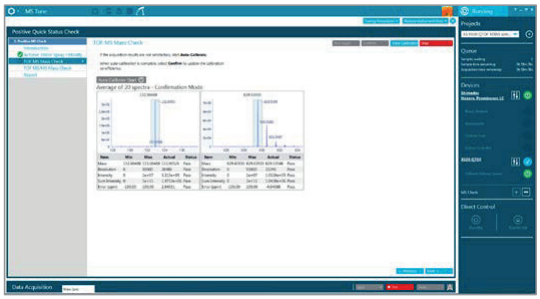
Source temperature: 600ºC

Atomizing gas GAS1: 55psi; Auxiliary gas GAS2: 60psi



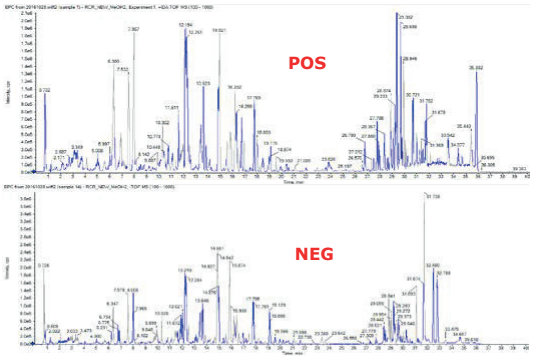
Instrumentation mode search:

One-touch (select MS Check on the lower right), fully automated TOF-MS and TOF-MS/MS correction mode ensures that analysts of any expertise level can obtain accurate, reliable, reproducible data.



Ion Data Plots

Positive and negative ion mode BPC's:

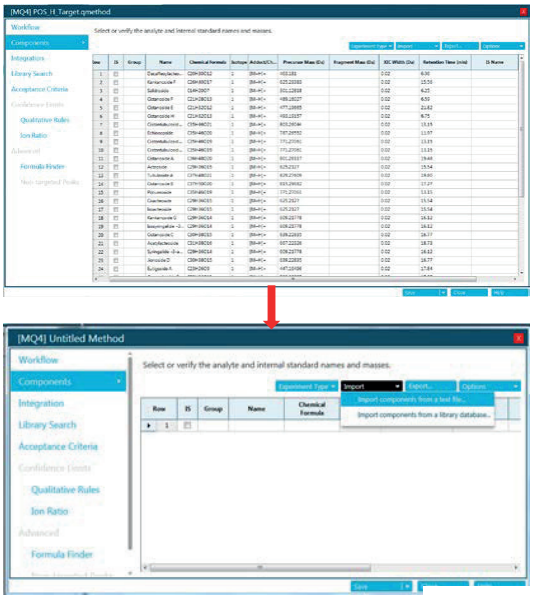


Simple Chinese Medicine Component Identification Procedure

1. Targeted component identification workflow

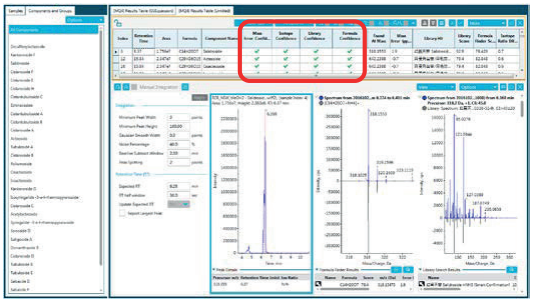
1.1: Molecular formula search

Only the chemical compound name and molecular formula are required; these can be input directly or imported using Excel's copy and paste function to create a processing method.



Contents

Once a results table is created, quantitative and qualitative results can be viewed in the same window. A red/green indicator system is used to indicate mass accuracy, retention time, isotope type, and confidence in identification by database matching.



SCIEX OS software lets users filter results and display only those compounds meeting acceptance criteria and falling within confidence intervals defined by the user. It can quickly find targeted results in large databases.

The TCM database of MS/MS spectra allows for secondary matching and yields more reliable results (grey color in the database indicates MS/MS data).

The literature contains names and molecular formulas of phenolic glycoside active ingredients. Using the above process for the identification of target components, it was determined that the sample contains 39 types of phenolic glycosides:

Component Name	Formula	Found At Mass (NH4)	RT (min)	Mass Error (ppm)
Veroascose glycoside	C20H30O12	480.208	6.17	0.951
Kankanoside F	C26H40O17	642.26	6.16	-0.749
Salidroside	C14H20O7	318.155	6.38	0.241
Cistanoside F	C21H28O13	506.187	6.74	0.898
Cistanoside E	C21H32O12	494.223	8.04	0.449
Cistanoside H	C21H32O13	510.219	7.24	0.997
Cistanche tubulosa glycoside C	C35H46O21	820.287	10.3	-0.413
Echinacea glycoside	C35H46O20	804.291	12.21	-0.733
Cistanche tubulosa glycoside A	C35H46O19	788.296	13.62	-0.982
Cistanche tubulosa glycoside B	C35H46O19	788.296	13.62	-0.898
Cistanoside A	C36H48O20	818.307	13.84	-0.55
Calamus glycosides	C29H36O15	642.239	14.83	-0.6
Tubuloside A	C37H48O21	846.301	14.94	-0.965
Isoacteoside	C29H36O15	642.239	14.83	-0.6
Kankanoside G	C29H36O14	626.244	16.42	-0.871
Isosyringalide -3-a-l-rhamnopyranoside	C29H36O14	626.244	16.42	-0.871
Cistanoside C	C30H38O15	656.255	17.11	0.739
Acetylfuran glycoside	C31H38O16	684.249	17.77	-0.723
Syringalide -3-a-l-rhamnopyranoside	C29H36O14	626.244	16.42	-0.871
Jionoside D	C30H38O15	656.255	17.11	0.739
Phenylethyl glycoside B	C29H36O13	610.249	18.22	-0.789
Tubuloside B	C31H38O16	684.249	17.77	-0.723
Tubuloside E	C31H38O15	668.254	19.55	-0.638
Salsaside D/F	C31H38O15	668.254	19.55	-0.638
Salsaside E	C32H40O16	698.267	19.93	0.997
Cistansinenside A	C32H40O16	698.267	14.83	-0.6
Cistanoside G	C20H30O11	464.213	14.83	-0.6
2-acetylacteoside	C31H38O16	684.249	16.42	-0.871
Cistanche tubulosa glycoside B2	C35H46O19	788.295	16.42	-0.871
Lipedoside A1 Isosyringalide 3-rhamnoside	C29H36O14	626.244	17.11	0.739
campneoside I	C30H38O16	672.249	17.77	-0.723
campneoside II	C29H36O16	658.234	16.42	-0.871
crenatoside	C29H34O15	640.224	18.22	-0.789
Tubuloside C	C43H54O24	972.334	17.77	-0.723
Tubuloside D	C43H54O23	956.339	19.55	-0.638
Cistanoside I	C21H28O12	490.193	19.55	-0.638
Cistantubulose A1	C27H38O18	668.24	6.19	0.585
Cistantubulose A2	C27H38O17	652.246	7.63	0.894
Kankanoside H1/H2	C37H48O20	830.307	16.3	

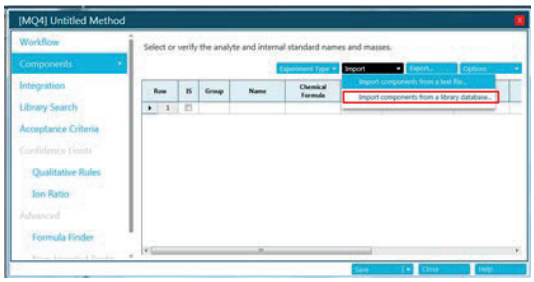
Contents

Besides the phenolic glycoside active components, *Cistanche* also contains a large number of compounds such as iridoids, glycosides and lignans. The identification results are as follows:

The iridoids, glycosides and lignans identification list:

Component Name	Formula	Found At Mass (NH4)	RT (min)	MassError (ppm)
mussaenoside acid/8-epiloganic acid	C16H24O10	394.17	3.16	-0.934
Gluroside	C15H24O8	350.181	7.97	-0.509
Kankanoside A/O/P	C16H26O8	364.196	10.42	-0.402
Leonuride/Kankanoside L	C15H24O9	366.176	5.02	-0.014
8-epideoxyloganic acid	C16H24O9	378.176	9.21	0.306
6-deoxycatalpol	C15H22O9	364.16	6.3	-0.764
catalpol	C15H22O10	380.156	3.27	0.985
bartsioside/antirrhide	C15H22O8	348.165	7.54	-0.175
Kankanoside B / phelypaeside	C15H24O10	382.171	2.68	-0.368
adoxoside acid	C17H26O10	408.187	4.29	0.995
Kankanoside D	C15H26O7	336.202	11.22	0.891
Kankanoside N	C16H28O8	366.212	11.95	-0.986
(+)-pinoresinol-O-β-D-glucopyranoside	C26H32O11	538.228	15.52	-0.786
(+)-syringaresinol-O-β-D-glucopyranoside	C28H36O13	598.249	16.2	-0.952
liriodendrin	C34H46O18	760.302	13.01	-0.64
syringin	C17H24O9	390.176	8.14	0.896

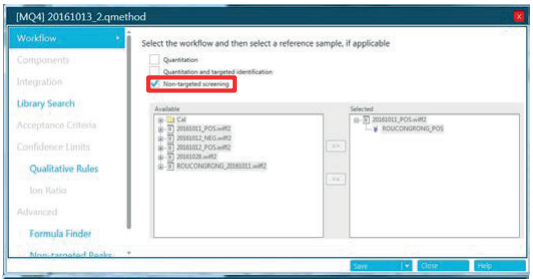
1.2: Database Search:

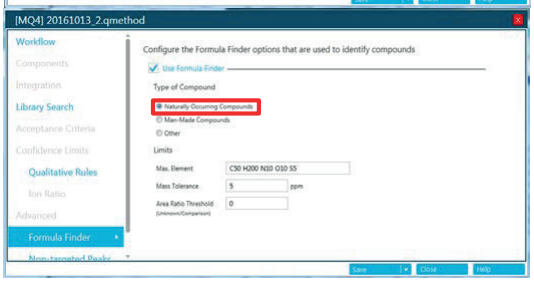


53 compounds were obtained from matching identification in the TCM database: 43 positive ions, 22 negative ions, and 12 repeats, listed below. Besides active phenolic glycosides, iridoids, glycosides and lignans, Cistanche also contains mannitol, leucine, and geniposidic acid. See appendix for list.

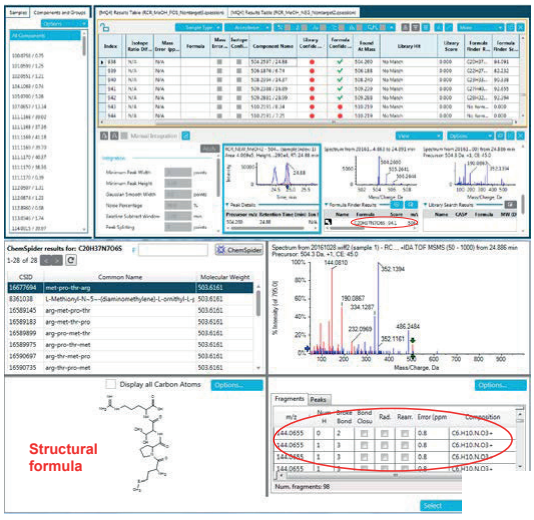
2. Non-targeted component analysis

Non-targeted component identification can be performed with the built-in ChemSpider search function to determine the classification and type of unknowns. For non-targeted component identification, simply choose the "non-targeted" mode, and the molecular formula search will develop a processing method. The workflow is as follows:





For complete unknowns, molecular formula search results are shown in a peak browser window in the lower part of the TOF-MS mass spectrum; with ChemSpider database search, results are listed by priority and the structural information obtained in ChemSpider is automatically compared with the MS/MS spectrum obtained, providing secondary feedback for rapid identification.



Contents

Summary

1. Rapid high-resolution data acquisition; a single injection yielded high-resolution TOF MS and MS/MS data, with 39 identifiable phenolic glycoside active components and 16 iridoids, lignans and glycosides;
2. A TCM database of secondary spectra provides additional matching information, and the software automatically provides a database match score. Using the score, one can easily, quickly, and accurately identify Chinese medicine components;
3. The device is simple and has one-touch auto-adjust correction to ensure that analysts with any degree of expertise can obtain high quality, reliable data;
4. The new SCIEX OS software version integrates data acquisition, processing (quantitative and qualitative), display, reporting, and database management. It solves the difficulties that many users face with an intuitive and easy-to-use interface;
5. Both the targeted and non-targeted screening workflows are simple, and the built-in method guide helps users accurately and rapidly create methods.

References

6. ISBN 978-7-5067-7337-9, PRC pharmacopeia [S]. Beijing: National pharmacopeia commission, 2015
7. Lei, Song Zhihong, Tu Pengfei; Research advances in the chemical composition of plants of the genus Cistanche, “Chinese Herbal Medicine,” Volume 34, No. 5, May 2003, 473-476.

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Contents

Analyzing Different Compositions of Polygala from Different Regions Using the X500R QTOF System

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Introduction

Authentic herbs come from specific locations that are traditionally known for these high-quality products. Authentic herbs have become synonymous with traditional Chinese medicine and form a comprehensive material standard for evaluating the quality of Chinese herbal medicines. Authentic herbs thus play a unique and important role in authentication and quality control of Chinese herbal preparations. Authenticity of Chinese medicine has become an important guarantee of high herbal quality.

Polygala is one of the main Chinese herbal medicines, one of 85 traditional Chinese herbal medicine exports, and one of 42 species of level 3 protected wild products in China[1]. The 2010 "Chinese Pharmacopoeia" divides Polygala herbs into those derived from the plant leaves of Polygalaceae and those made from dried Polygala leaves and roots. They have the properties of sedation, promoting heart and kidney circulation, acting as an expectorant, and decreasing swelling. They are used to treat insomnia, excessive dreaming, forgetfulness, and fear caused by poor heart and kidney circulation[2]. The commercial Polygala industry depends on the Polygala supply, which is found in an area bounded by the desert to the south and the Yangtze River to the north. It is grown mainly in Shanxi, Shaanxi, Henan, and Hebei, under the traditional notion of "Shanxi - large quantity, Shaanxi - high quality"[3].

Currently, the identification and analysis of Chinese herbal medicine components is quite challenging. These components underlie the pharmacodynamic efficacy of Chinese medicinal products. Herein lies the key to modernizing Chinese medicine. How to quickly identify the active ingredient and its structure, as well as how to identify the differences between the active ingredients of authentic and inauthentic herbs, are urgent problems that must be solved.

This study used the SCIEX high resolution X500R QTOF mass spectrometer for data acquisition and used the accompanying MarkerView™ analytic software to statistically analyze differences between components. This study involved Chinese medicine (e.g., Polygala) that includes components from different regions. This method makes component identification more effective, faster and a better reflection of the integrity and unique nature of the sample tested. In turn, it highlights the differences between Polygala components from different sources and provides a new framework for quality evaluation of Chinese herbal medicines.

The high resolution X500R's hardware design, including N-type ion path technology, time of flight tube design, and a stable and durable Turbo V™ ion source, ensures that under routine testing conditions, sample identification is more stable, higher quality, and more reliable for the long term. The X500R's high-sensitivity, high-resolution analysis and accurate mass-to-charge ratio analysis, combined with the intelligent TOF-MS-IDA-MS/MS acquisition mode, truly achieve the goal of collection of high-quality, accurate primary and secondary mass spectrometry data by single injection, and quickly provide the most accurate qualitative screening results.

SCIEX X500R QTOF mass spectrometry system with ExionLC™ liquid chromatography system and SCIEX OS workstation

Study Design

1. Samples of Polygala herbs from different sources were obtained and assigned to groups, each containing 6 samples.
2. TOF-IDA-MS/MS mode was used for data acquisition; one injection allowed simultaneous collection of various components' primary ions and secondary daughter ions.
3. The MarkerView™ Software was used to analyze differences in components and identify statistically significant differences between groups for use as markers.
4. After entering mass spectrometry data on primary ions and secondary daughter ions into SCIEX OS Software, the components were matched with the SCIEX high resolution MS/MS Chinese medicine database or the ChemSpider online database; differences in components were identified.

Contents

Study Design Workflow

1. Acquire high-resolution primary TOF-MS and secondary TOF-MS/MS spectra

2. MarkerView Software, Perform statistical analysis of data, to find variant ions

3. Structural identification software Structural identification and secondary analysis of variant ions

Materials and Methods

This study collected Polygala herbs from 4 regions: Chengcheng, Shaanxi; Shangluo, Shaanxi; Shanxi; and Hebei. After the samples were dried, they were cut into small pieces and dried in the oven at 40 degrees C for 18 h. After removal, they were crushed and filtered through a 20 mesh sieve, placed in the dryer, and then used.

Sample Preparation

Carefully weigh out about 1.0 g of Polygala powder of consistent weight, add 50 mL of 70% methanol aqueous solution, ultrasonicate 30 min., centrifuge for 10 min at 13000 rpm, and take the supernatant for injection.

Chromatographic Conditions

Chromatographic Column: Phenomenex Kinetex F5, 100*3.0 Mobile phase: A is ultrapure water/B is acetonitrile;

Gradient elution was performed as shown below:

Time (min)	A%	B%
0.0	95	5
5.0	90	10
15.0	85	15
20.0	80	20
25.0	75	25
30.0	70	30
35.0	65	35
40.0	10	90
45.0	10	90
45.1	90	5
50.0	90	5

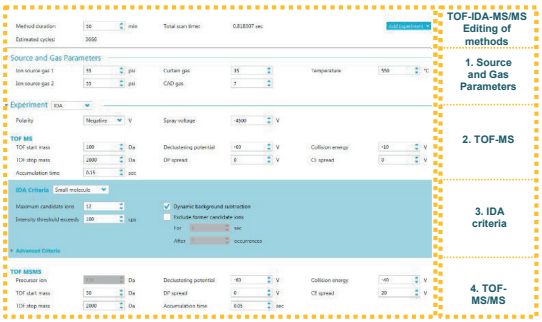
Flow rate: 0.4 mL/min ;

Column temperature: 40℃ ;

Amount inserted: 5 μL

Mass Spectrometry Method

Scanning method: TOF-IDA MS/MS qualitative screening;
Ion source: ESI source
Mass spectrum parameters are established in 4 steps:



Chromatogram

Typical ion base peak chromatograms (BPC) for four groups of polygala samples from different sources; see fig. 1 below:

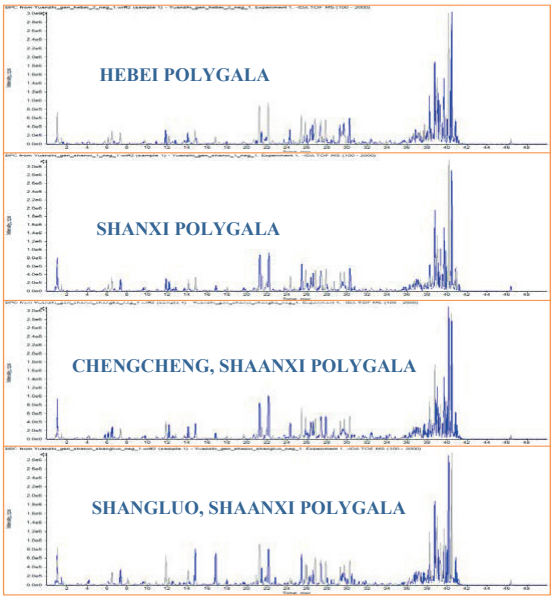


Figure 1. Typical BPC for four polygala samples from different sources

Chromatographic peak retention reproducibility was very good among the four Polygala samples from different sources. Many baseline analysis separation peaks were obtained on th showing good chromatographic separation.

Contents

MarkerView™ Data Processing

The MarkerView™ Software was used for preliminary data extraction of chromatographic peaks. Identification and integration were performed on chromatographic peaks with a retention time of 0 - 50 min; the three-dimensional data was transformed into a two-dimensional data matrix, including variables (*m/z*_RT), number observed (24 samples), and the integral area. This study found 994 variables (*m/z*_RT).

PCA-DA processing and Library Database Search

All samples underwent supervised PCA analysis, and their Score and Loading chart is as shown in Fig.2:

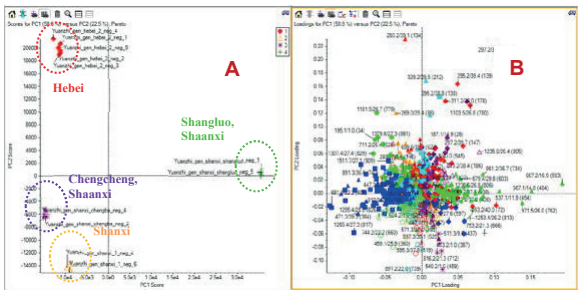


Figure 2 A) Polygala samples from different sources, PCA Score Plot; B) Polygala samples from different sources, PCA Loading Plot;

Fig. 2 Score Plot shows Polygala samples from the 4 different areas are well separated, meaning that there are large differences between groups.

Using Polygala products sourced from different areas, take m/z 667.2 (RT=16.9 min) as an example. For *m/z* 667.2 in the figure below, showing content differences in samples from different areas, the line plot shows that Chengluo, Shaanxi Polygala has a Tenuifoliside B2 content that is approximately 5 times that of the 3 other areas, as in Fig. 3:

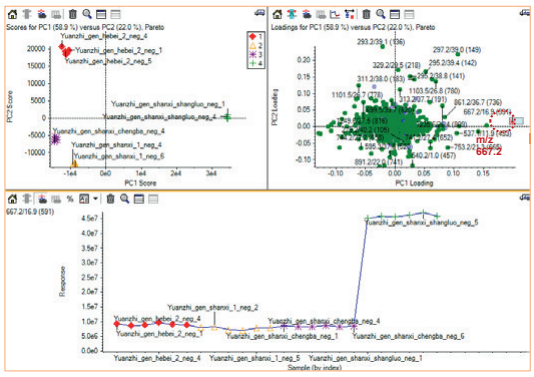


Figure 3 Polygala Marker: *m/z* (667.2), (RT=16.9 min)

Polygala characteristic marker *m/z* 667.2, retention time 16.9 min, SCIEX OS identification of the marker is: Tenuifoliside B2, $C_{30}H_{36}O_{17}$, *m/z* (MS)= 667.1875, *m/z* (MS/MS) = 461.1288, 367.1035, 239.0557, 205.0498, 190.0265. Using Library search, identification results in
Fig. 4-1 shows that secondary fragment matching is good, with the main fragment structural analysis shown in Fig. 4-2:

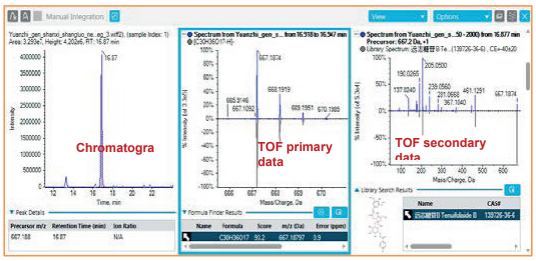


Figure 4-1. Polygala Marker m/z 667.2 via SCIEX OS structural attribution results

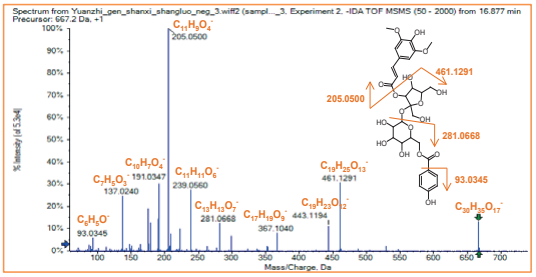


Figure 4-2. Polygala Marker m/z 667.2 secondary fragment attribution and main fragment structural analysis

T-test data processing

All samples underwent T-test data processing; results are in Fig. 5. Fig. A is the volcano plot, expressed as log fold change vs. p-value; as the X axis is approached, more ions are located at both ends of the X axis, indicating a greater difference between them. Fig. B is a line plot, and Fig. C is a box plot, showing the content relationships between the samples.

Contents

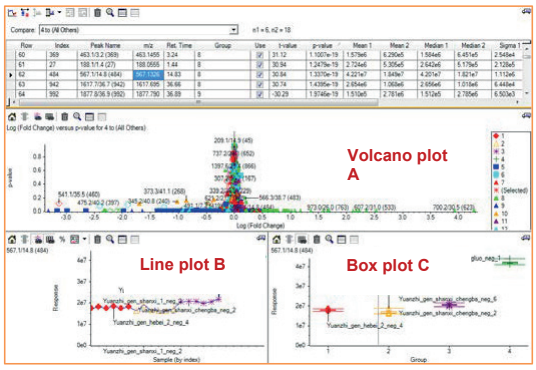


Figure 5 Log (Fold Change) versus p-values data processing

T-experimentally ($p < 0.005$) differentiated ion scans appear in line plot B and box plot C. Compound *m/z* 567.1 (RT 14.8 min) is significantly different in the Shangluo, Shaanxi Polygala, so it is used as a marker. Its structure is identified with SCIEX OS software's ChemSpider online structural identification for markers. Results are in Fig. 6:

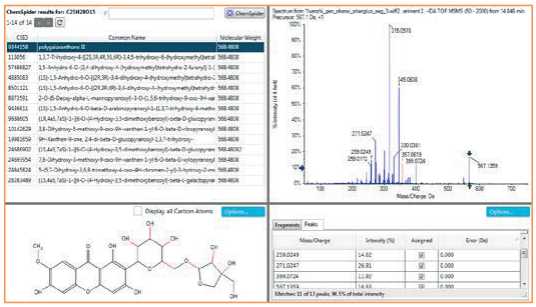


Figure 6-1. Polygala Marker: m/z (567.1), (RT 14.8 min)

Marker identified as: Polygalaxanthone III, $C_{25}H_{28}O_{15}$, *m/z* (MS)= 567.1359, *m/z* (MS/MS) = 345.0608, 315.0510, 399.0724, 271.0247; its online secondary fragment matching is good.

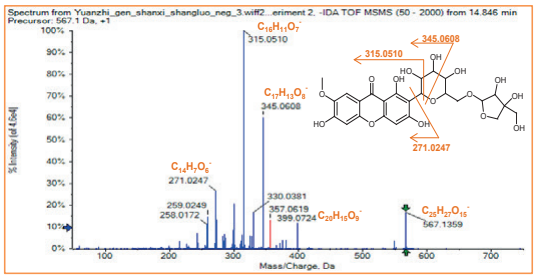


Figure 6-2. Polygala Marker m/z 567.1 secondary fragment attribution and main fragment structure analysis

SCIEX OS compound structural identification process

Using the SCIEX OS Formula Finder function, based on this ion's primary mass spectrum exact mass number and isotope ratio, the likely molecular formula was identified. At the same time, mass spectrometry fragmentation patterns and the ion's secondary mass spectrum mass number verified the molecular formula.

Using Polygala products sourced from different areas, take m/z 1379.4083 (RT 30.22 min) as an example. With the Formula Finder function, based on an exact mass number and isotope distribution, the molecular formula was determined to be $C_{62}H_{76}O_{35}$. Its TOF MS mass deviation was -0.8 ppm, and 17 TOF MS/MS fragments' mean mass deviation was 0.9 ppm. Results are shown in Fig. 7:

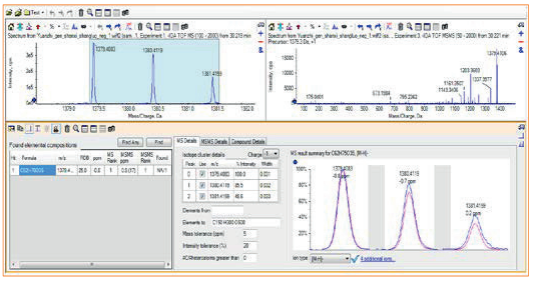


Figure 7. Polygala Marker m/z (1379.4083) molecular formula calculated with Formula Finder

The MS/MS fragmentation molecular formula is shown with green dots; see Fig. 8 for the fragmentation molecular formula, which is consistent with the mass spectrum fragmentation pattern.

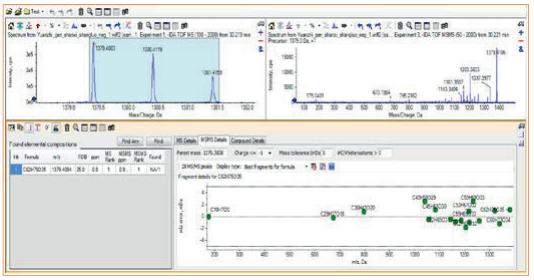


Figure 8. Polygala Marker m/z (1379.4083) secondary mass spectrum element component fitting

Contents

ChemSpider online structural identification, an additional verification of the structure, identified this marker as: Tenuifoliose A2, $C_{62}H_{76}O_{35}$, *m/z* (MS)= 1379.4083, *m/z* (MS/MS) = 1203.3603,1337.3977, 1161.3507, 1143.3406, 795.2362, 175.0401. Results are shown in Fig. 9.

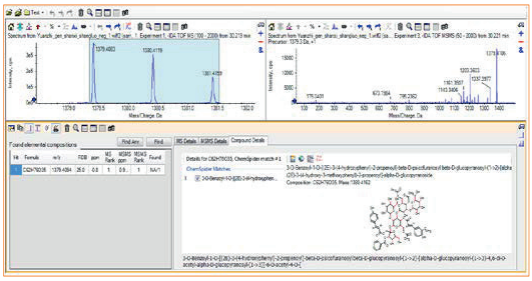


Figure 9-1. Polygala Marker m/z (1379.4083)

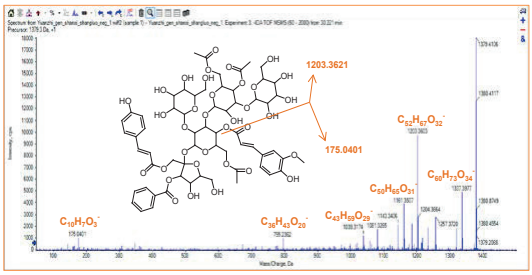


Figure 9-2. Polygala Marker m/z (1379.4083) secondary fragment attribution and main fragment structure analysis

Data Processing Results

Loading results and T-experimental results are combined to find the differentiated ions and the exact mass number and retention time of the potential biomarker are shown in Table 1.

This study identified 11 signature markers in Polyagala from different areas; of these, No. 1-2 are sucrose esters, No. 3–8 are oligosaccharides, No. 9-10 are triterpenoid saponins, No. 11 is xanthone. A summary is shown in Table 1-1. Table 1-2 shows the content differences in the 11 signature markers.

Table 1-1: Summary of signature ions of 4 Polygala samples from different sources

No.	m/z (MS)	RT (min)	Ion	Formula	Mass error (ppm)	Tentative identification	Major fragment ion (MS/MS)
1	667.1874	16.9	[M-H]⁻	C30H36O17	-0.8	Tenuifoliside B2	461.1291, 367.1040, 239.0560, 205.0500, 190.0265, 137.0240
2	753.2243	17.24	[M-H]⁻	C34H42O19	-0.7	3, 6'-Disinapoyl sucros	547.1647, 529.1542, 367.1024, 223.0602, 205.0507, 190.0263
3	1223.3681	24.7	[M-H]⁻	C55H68O31	0.8	Tenuifoliose S	1077.3292, 1059.3173, 955.2944, 931.2927, 753.2247
4	1233.3725	34.8	[M-H]⁻	C53H70O33	-0.1	Tenuifoliose V	1057.3263, 1015.3101, 839.2826, 465.2248
5	1265.3776	27.3	[M-H]⁻	C57H70O32	-0.1	Tenuifoliose K	1119.3386, 1101.3335, 1077.3305, 997.3054, 145.0299
6	1379.4083	30.22	[M-H]⁻	C62H76O35	-0.8	Tenuifoliose A2	1203.3603,1337.3977, 1161.3507, 1143.3406, 795.2362, 175.0401
7	1495.4586	25.79	[M-H]⁻	C67H84O38	0.6	Tenuifoliose L	1349.3947, 1307.3890, 1203.3589, 1161.3475, 1143.3385, 795.2381
8	1525.4692	25.84	[M-H]⁻	C68H86O39	0.5	Tenuifoliose F	1379.4050, 1337.4001, 1203.3599, 1185.3515, 1161.3516, 795.2363
9	1571.6874	36.87	[M-H]⁻	C75H112O35	-0.5	Onjisaponin B	1566.3539, 1542.6812, 1347.6365, 567.1939, 425.3044
10	1469.6571	37.06	[M-H]⁻	C71H106O32	-0.8	Onjisaponin Z	1439.6490, 1245.6042, 455.3163, 405.1402, 237.0766
11	567.1359	14.85	[M-H]⁻	C25H28O15	-0.7	Polygalaxanthone III	399.0724, 345.0608, 315.0510, 271.0247

No.	Compound	Response			
		Chengcheng of Shaanxi	Shangluo of Shaanxi	Shanxi	Hebei
1	Tenuifoliside B2	1	5	1	1
2	3, 6'-Disinapoyl sucrose	1	5	1	1
3	Tenuifoliose S	4	1	4	4
4	Tenuifoliose V	4	1	4	4
5	Tenuifoliose K	10	1	10	10
6	Tenuifoliose A2	1	6	1	1
7	Tenuifoliose L	4	1	4	4
8	Tenuifoliose F	8	1	8	8
9	Onjisaponin B	1	4	1	1
10	Onjisaponin Z	10	1	10	10
11	Polygalaxanthone III	1	3	1	1

Note: Numbers within the table show only the fold-relationship of the same compound (e.g., Shangluo, Shaanxi Tenuifoliside B2 content is 5 times that of Chengcheng, Shaanxi; Shanxi; and Hebei).

Experimental Conclusion

TheX500R high-resolution LC system was used to analyze 24 Polygala samples, and grouped analysis found 11 signature markers responsible for sample variation. These included 2 sucrose esters, 6 oligosaccharides, 2 triterpenoid saponins, and 1 xanthone.

Analysis of the differences among the 11 markers revealed that the content of Shangluo, Shaanxi Polygala is clearly different from that of other locations. Main component analysis software MarkerView™ helped to differentiate between Polygala samples, for example using the 11 signatures that differentiate Shangluo, Shaanxi Polygala from that of the 4 other locations.

Contents

Screening and Identification of Natural Products in Citrus Oil

Non-Target Analysis using SWATH® Acquisition, Compound Identification Using the NIST Library

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[1] SCIEX, Framingham, USA, [2] SCIEX, Redwood City, USA

Overview

Analysis of natural products in citrus oils by non-targeted acquisition techniques is described using the SCIEX X500R QTOF system and MS/MS library matching against the NIST mass spectral library for compound identification. The polymethoxylated flavonoids of the non-volatile fraction of the citrus oils are of interest in describing the screening workflow. An important feature of the method was that the same acquisition run was used to monitor both the natural products and screen for pesticide suspects. Several hundred natural products were identified using the non-target peak finding processing. Using a targeted screening list, 14 compounds were detected in the 3 citrus oils (Valencia orange, lemon and grapefruit).

Introduction

Citrus oils are widely used in the food and cosmetic industries for their flavour and fragrance properties. In addition, citrus oils are increasing employed as therapeutic agents due to their apparent antibacterial, antifungal and anticarcinogenic activities.[1] The non-volatile fraction of citrus oils contains coumarins, furocoumarins, and polymethoxylated flavonoids (PMFs). Specifically, the PMFs have shown anti-inflammatory and anti-carcinogenic effects.[2] Therefore, identification of these natural products in citrus oils is important to assess therapeutic content. In addition, it is important to screen for pesticide compounds to ensure safety.

Non-target acquisition (NTA) techniques are beneficial for chemical screening since they allow for wide compound coverage without sacrificing scan time. QTOF instruments, such as the X500R, are ideal for NTA because they collect high resolution accurate mass data on both the precursors and their fragments at cycle times which are satisfactory for HPLC and UPLC peak widths. SWATH® Acquisition is a NTA technique that is advantageous over traditional information dependent acquisition (IDA) techniques. SWATH Acquisition collects MS/MS spectra on all precursor compounds, whereas IDA is limited because only the most intense precursors trigger MS/MS. Therefore, SWATH Acquisition will typically result in greater unknown compound identifications.

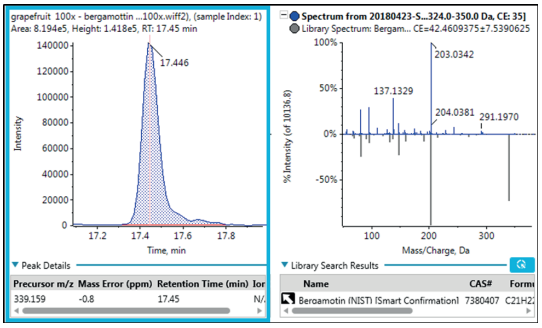

Figure 1. Identification of Bergamottin in Grapefruit Peel Oil Extract. Left panel shows precursor XIC (extraction width = 20 mDa), right panel shows experimental MS/MS pattern (top) and NIST MS/MS library match for bergamottin (bottom).

Accurate identification of unknown compounds is greatly strengthened by matching experimental MS/MS fragmentation patterns with published library databases. The NIST MS/MS database contains fragmentation spectra for ~14,000 compounds and has recently been converted to a SCIEX LibraryView™ format for easy searching. For example, Figure 1 shows the NIST library match for bergamottin in grapefruit peel oil.

Key Workflow Benefits

- Flexibility of QTOF workflow and data acquisition means endogenous natural products and trace pesticide residues can be analyzed in the same sample injection
- The addition of the NIST MS/MS library significantly improves the ability for unknown compound identification.
- SWATH Acquisition ensures that MS/MS information was collected on all precursors, resulting in MS/MS information available for peak ID

Contents

Experimental

Sample Preparation: Three cold-pressed citrus oils (Valencia orange, lemon and red grapefruit) were received from a commercial producer. For analysis, the oils were diluted 100-fold in acetonitrile and thoroughly vortexed.

Chromatography: The SCIEX ExionLC™ AD LC system was utilized and chromatographic separation was achieved under gradient conditions using the Phenomenex® Luna Omega Polar C18 column (100 Å, 2.1 x 100 mm, 1.7 µm particle size). The mobile phases were water (0.1% formic acid, 5mM ammonium formate) and acetonitrile (0.1% formic acid) with a flow rate of 200 µL/min. The column oven was maintained at 40°C and the injection volume was 1 µL.

Table 1. LC Gradient Program. Using a flow rate of 200 µL/min, injection volume = 1 µL.

Step	Time (min)	A (%)	B (%)
0	0.0	95	5
1	20.0	5	95
2	25.0	5	95
3	25.1	95	5
End	32.0		

Mass Spectrometry: The SCIEX X500R QTOF system with Turbo V™ source using the electrospray ionization (ESI) probe war run in positive ion mode. Source and gas conditions are presented in Table 2. Data were collected using SWATH Acquisition with variable window widths. The SWATH Acquisition windows were chosen to minimize the precursor Q1 density during the pesticde screening since the natural product molecular weights were unknown at time of analysis (Table 3). Using variable SWATH Acquisition windows help reduce the complexity of MS/MS fragmenention spectrum thus improving library matching and improvng unknown identification. TOF MS (scan range: 100-1500 Da) parameters were DP = 80 V, CE = 10 V and accumulation time = 0.10 sec. The TOF MS/MS (scan range 50-1000 Da) parameters were DP = 80 V, CE = 35 V, CES = +/-15 V and accumulation time = 0.05 sec. The collision energy spread (CES) ensures that a comphrensive MS/MS pattern is collected.

Table 2: Source, Gas and Temperature Conditions.

Parameter	Value
Curtain Gas (CUR)	30 psi
Collision Gas (CAD)	12
IonSpray Voltage (IS)	*5500 V*
Temperature (TEM)	*550°C*
Nebulizer Gas (GS1)	*50 psi*
Heater Gas (GS2)	*50 psi*

Data Processing: All data processing was performed within the SCIEX OS software 1.5. Data were initially processed using the "non-target peaks" module in Analytics. This module uses the peak finding algorithm to extract features from the TOF MS TIC and then assigns the applicable MS/MS fragmentation spectrum to that precursor. The peak detection sensitivity was set to the middle level since the natural products were expected to be in high concentration. To aid in the identification of extracted features, experimental MS/MS fragmentation spectra were searched against the National Institute of Technology (NIST) MS/MS Spectral Library. This NIST database is beneficial in this workflow because it contains a large number of natural products. Data review efficiency was optimized by utilizing the qualitative rule "traffic lights" which filtered out features which did not have corresponding NIST library hits.

In addition, a list of 15 PMFs was used for targeted screening (Table 4). In the processing method, the user-defined chemical formulae and adduct/charge were used to calculate the exact mass (extracted mass width = 20 mDa). Compounds were positively identified if the mass error was <5 ppm, experimental isotope ratio was within 20% of the theoretical value, and MS/MS library matching score for fit was >70.

Contents

Table 3: Variable SWATH Acquisition Windows.

Start	End	Width
100	175	75
174	200	26
199	225	26
224	250	26
249	275	26
274	300	26
299	325	26
324	350	26
349	375	26
374	400	26
399	425	26
424	450	26
449	475	26
474	500	26
488	525	26
524	1000	476

Results

Pesticides

A components list of 274 pesticides was built and retention times were determined from the authentic standards. Overall, 19 unique pesticides were detected in the 3 citrus oil samples (10 pesticides per sample). Analytes were considered positively detected if they met the following criteria: mass error <5 ppm, isotope pattern error <20%, retention time error <2.5% and library fit score >70.

An example detection for azoxystrobin in lemon peel oil is shown in Figure 2. The XIC shows that the retention time difference was 0.2%, precursor mass error was -0.7 ppm, isotope ratio difference was 6.1% and the library fit score was 100.

These results demonstrate that both the natural products and pesticides can be analyzed in the same acquisition method, highlighting the flexibility of the X500R QTOF system.

Table 4: Components List for Targets PMF Screening.

Parameter	Formula	Adduct	Precursor Mass (Da)
5,7-Dimethoxycoumarin	*C11H10O4*	*[M+H]+*	*207.0652*
Bergapten	*C12H8O4*	*[M+H]+*	*217.0495*
Osthol	*C15H16O3*	*[M+H]+*	*245.1172*
Meranzin	*C15H16O4*	*[M+H]+*	*261.1121*
Isomeranzin	*C15H16O4*	*[M+H]+*	*261.1121*
Naringenin	*C15H12O5*	*[M+H]+*	*273.0758*
Aurapten	*C19H22O3*	*[M+H]+*	*299.1642*
Bergamottin I	*C21H22O4*	*[M+H]+*	*339.1591*
Bergamottin II	*C21H22O4*	*[M+H]+*	*339.1591*
Tetra-O-Methylscutellarein	*C19H18O6*	*[M+H]+*	*343.1176*
Epoxybergamottin	*C21H22O5*	*[M+H]+*	*355.154*
Tangeretin	*C20H20O7*	*[M+H]+*	*373.1282*
Sinensetin	*C20H20O7*	*[M+H]+*	*373.1282*
Nobiletin	*C21H22O8*	*[M+H]+*	*403.1387*
3,5,6,7,8,3',4'-Heptamethoxyflavone	*C22H24O9*	*[M+H]+*	*433.1493*

Natural Products: Non-Target Peak Processing

The non-target peak finding algorithm extracted ~1500 features between the 3 citrus peel samples. However, not all features yielded good chromatographic peaks and strong MS/MS spectra. Manually reviewing each chromatogram would be tedious and time consuming. Therefore, the qualitative rule “traffic lights” was used to quickly filter the data. Since no prior knowledge of the compounds were known, the results table was filtered to only show positive library matches (i.e. library fit score >70).

After filtering the data to display only positive library hits, the data was sorted by either area count, or the area ratio of comparison (ratio of sample area count to blank area count) to focus on the dominant natural products.

After filtering and sorting the data, many natural flavonoids (e.g. 5,6,7,3',4'-pentamethoxyflavone, Figure 3), PMFs (e.g. osthol) and pesticides (e.g. pyraclostrobin) were identified through MS/MS spectral matching with the NIST library. The diversity of identified compound classes demonstrates extensive coverage of the NIST mass spectral library as well as the broad application of the chromatography and SWATH Acquisition method.

Contents

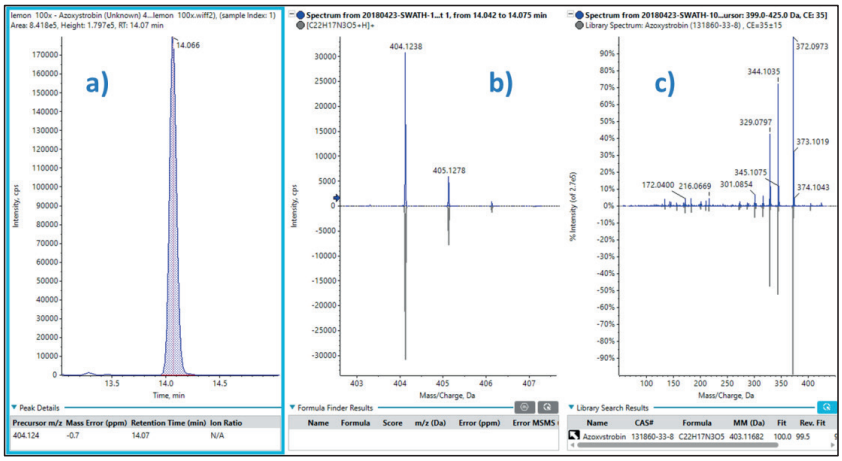

Figure 2. Peak Review Pane for the Detection of Azoxystrobin in Lemon Peel Oil. Panels show: TOF MS XIC (a), MS spectrum (b) and MS/MS spectrum (c) showing comparison between experimental (top) and library match (bottom).

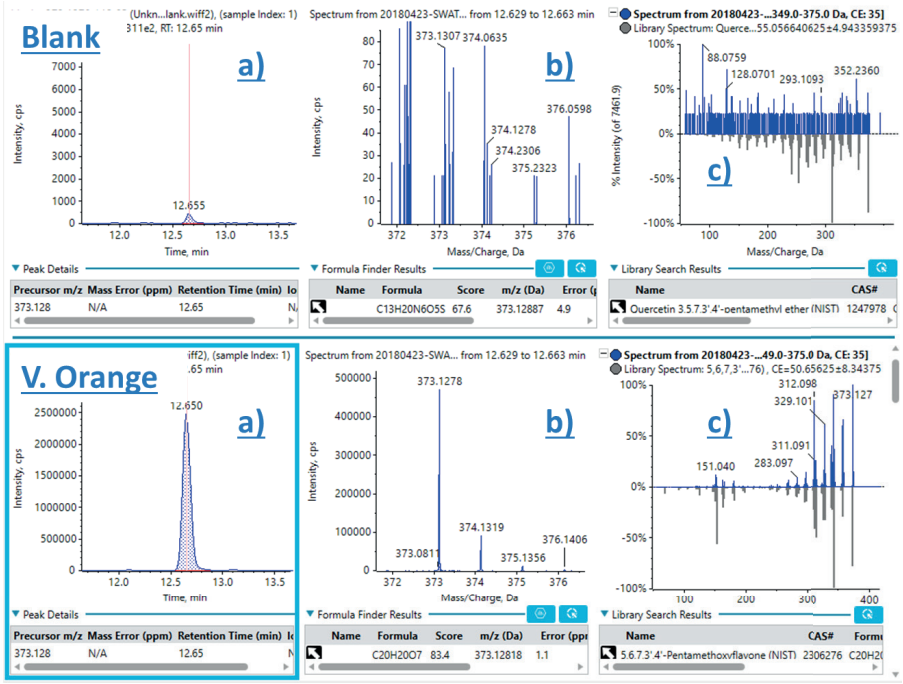

Figure 3. Peak Review Pane. Easy visualization of m/z 373.1278 (RT = 12.65 min) showing blank (top) and Valencia Orange Peel oil (bottom) show the a) TOF MS XIC b) MS spectrum and c) MS/MS spectrum with NIST library match for 5,6,7,3',4'-pentamethoxyflavone.

Contents

Natural Products: Targeted Screening

The non-target workflow demonstrates the ability of the X500R with SWATH Acquisition and MS/MS library matching to broadly identify natural products such as the PMFs. Therefore, the sample data was reprocessed with a targeted components list to focus on 15 specific PMFs of interest. For the targeted screening workflow, the precursor XIC mass is calculated from the chemical formula and adduct/charge state. MS/MS library matching was used to confirm the compound identity. Since retention time was unknown, the "retention time mode" in the components table was set to "find 5 peaks" (Figure 6). This feature extracts the 5 peaks, at each XIC mass, that possess "acceptable" mass errors (i.e. <5 ppm) – the retention time was confirmed by comparing to the MS/MS library match.

All 15 targeted PMFs were detected in at least 1 citrus oil sample through "acceptable" mass error (<5 ppm), isotope ratio difference and positive MS/MS library hit (Figure 1, Table 5). Grapefruit had the most individual PMFs detected with all compounds observed with the exception of epoxybegamottin.

It was not possible to distinguish between meranzin & isomeranzin, as well as between tangeretin & sinensetin since these pairs are structural isomers (i.e. identical accurate masses) and have similar MS/MS fragmentation spectra. For example, tangeretin and sinesetin differ by the relative position of 1 methoxy group. Therefore, authentic standards will be needed to confirm these compounds by their retention times.

Two peaks were present in the XIC for bergamottin and both peaks showed positive MS/MS library matches for bergamottin. Thus, it is possible that these are also structural isomers and additional experiments will be needed to elucidate the definitive structure. Further, 3,5,6,7,8,3',4'-hexamethoxyflavone did not have a positive MS/MS library match but did have acceptable mass error and isotope ratio difference for all samples.

Table 5: Individual PMFs Detected in Citrus Oil.

Compound	RT (min)	Valencia Orange	Lemon	Grapefruit
5,7-Dimethoxycoumarin	*11.7*		✔	✔
Bergapten	*11.9*			✔
Osthol	*14.9*	✔		✔
Meranzin	*12.0, 12.2*	✔	✔	✔
Isomeranzin	*12.0, 12.2*	✔	✔	✔
Naringenin	*10.6*			✔
Aurapten	*17.7*			✔
Bergamottin I	*17.5*		✔	✔
Bergamottin II	*18.3*		✔	✔
Tetra-O-Methylscutellarein	*13.4*	✔	✔	✔
Epoxybergamottin	*15.5*	✔	✔	
Tangeretin	*13.9, 12.6*	✔	✔	✔
Sinensetin	*13.9, 12.6*	✔	✔	✔
Nobiletin	*13.3*	✔		✔
3,5,6,7,8,3',4'-Heptamethoxyflavone	*13.6*	✔*	✔*	✔*

Contents

Conclusions

A comprehensive suite of pesticides and natural products were identified in citrus oils through non-target SWATH Acquisition with MS/MS fragmentation spectral matching against the NIST library. Non-target data processing demonstrated the potential to detect hundreds of natural products whereas a targeted screening list was used to focus on 15 individual PMFs.

A unique feature of this workflow is that both chemical classes – pesticides and natural products – were analyzed in the same injection, thus greatly simplifying the analysis.

References

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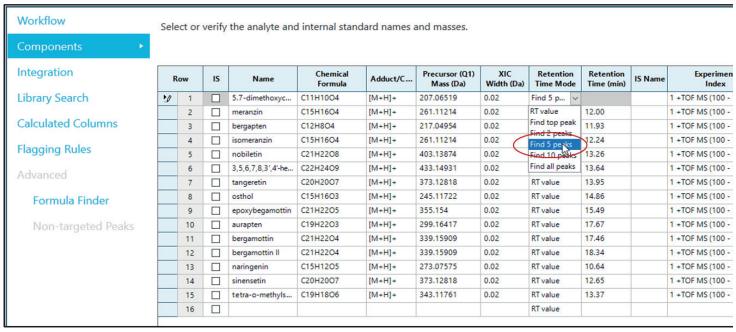

Figure 6. SCIEX OS Analytics Module Showing the Retention Time Mode Feature of "Find 5 Peaks".

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Contents

A Mass Spectrometry Applications Guide to
Elevate Your Natural Products Analysis

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Contents

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