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.

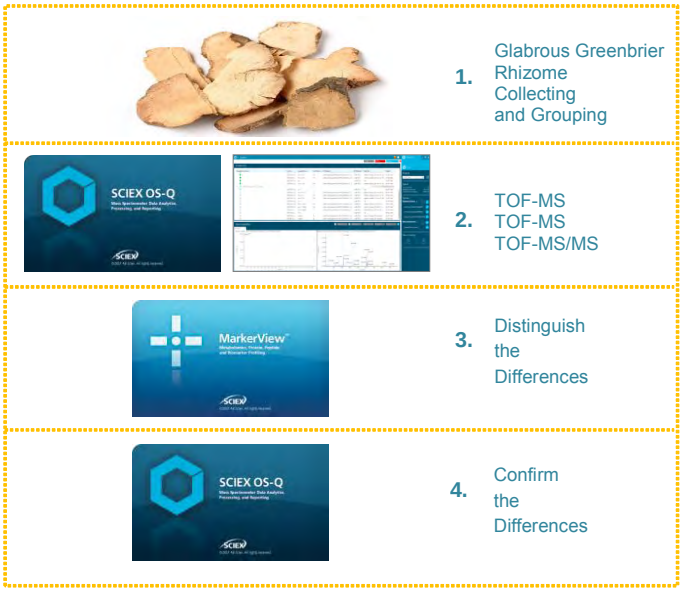
The SCIEX X500R QTOF System is a revolutionary high-resolution mass spectrometry system. As the first platform developed especially for high throughput laboratory testing, it is stable, reliable, and powerful. The fully updated hardware design offers rapid scanning in combination with powerful information-dependent acquisition (IDA); using a single injection, TOF-MS accurate mass 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 accurate mass (MS1), fragment ions (MS2), online and literature 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 the exact mass and isotope distribution, one can quickly and accurately make identifications.

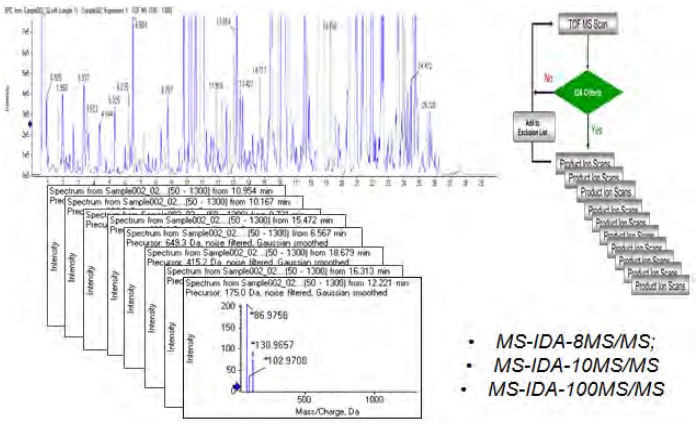
This application note describes using the SCIEX X500R QTOF System including updated SCIEX OS Software for mass spectrographic control and data acquisition and analysis. With a single sample injection, one can analyze differences with MarkerView Software and the high-resolution MS/MS Chinese medicine 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 TOF-MS-IDA- mode (Top 12 MS/MS per cycle), MS/MS mass spectra were obtained in a single sample injection.
3. MarkerView Software data analysis revealed a statistically significant difference between sample groups interrogated.
4. Using the MS, MS/MS, and the high-resolution MS/MS Chinese medicine database for comparison, database searches and structural verification were performed for the different samples.

X500R QTOF System High Resolution Mass Spectrometry Screening Workflow





- *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 (Top12 MS/MS);

Ion source: ESI source double spray technology

Scanning range: m/z 50-2000

CDS automatic calibration

Mass Spectrum Parameters

ESI ion source parameters:

Air curtain gas CUR: 35psi; Collision Gas CAD: 7

IS voltage: 5500V/-4500V; Source temp: 550ºC

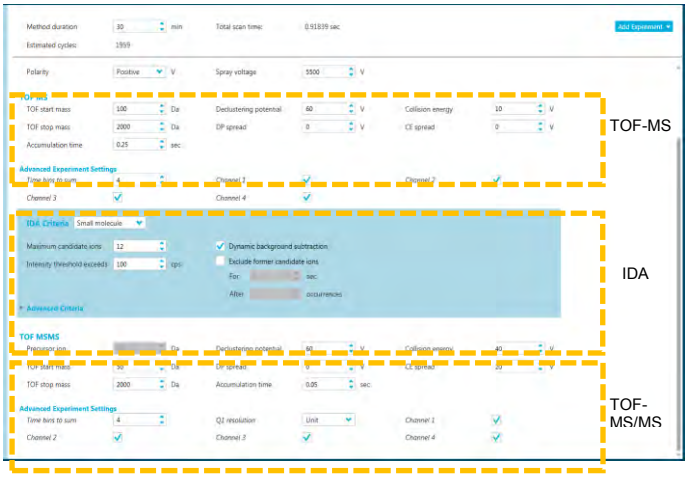
Atomizing gas GAS1: 55psi

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 accurate mass. The differences between samples can be normalized. Before the data is processed, known background ion interference or uncommon ions in the sample can be removed.

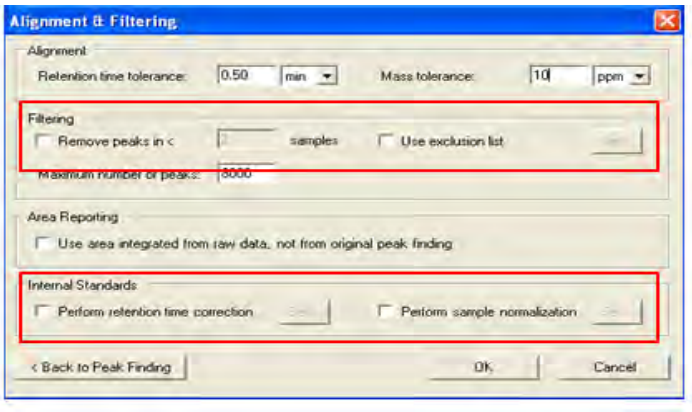


Figure 1 Advanced processing algorithm finds characteristic peaks by MarkerView Software

All samples underwent supervised principal component analysis (PCA).The scores plot highlights any differences/trends amongst the sample groups. The loadings plot reflects the variables responsible for the separation.

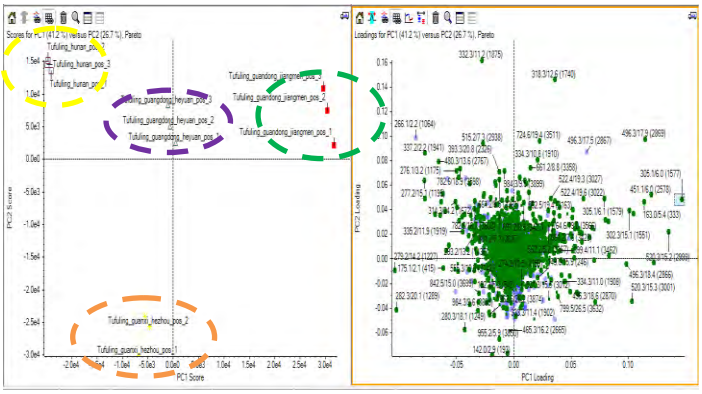


Figure 2 The PCA Score and loading diagram of Glabrous Greenbrier Rhizome from different regions

The PCA Score Plot and loading chart show *Smilax Glabra* samples from 4 different areas are well differentiated along the PC1 dimension, showing the largest variation between the 4 groups. Taking m/z 305.0649 at retention time 5.97 min as an example, it is clear that the the impact of this variable on the loadingss high, and the concentration of *Smilax Glabra* in samples from Guangdong Jiangmen is higher than those from Guangdong Heyuan, Guangxi Jiazhou, or Hunan.

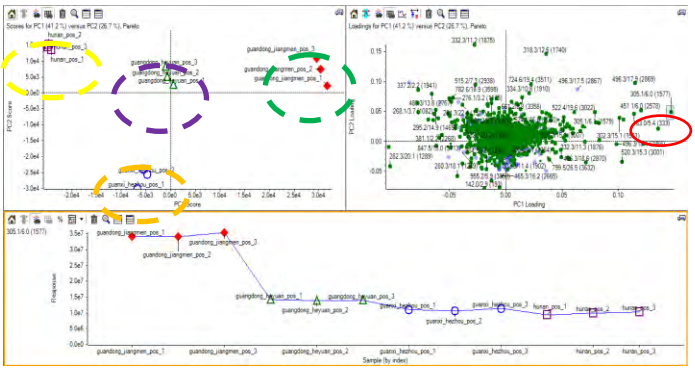


Fig. 3 The content change of m/z 305.0654, RT5.98min in different groups

MarkerView Software intuitively shows differences between samples in terms of accurate mass, isotope ratios, and MS/MS; needed for confirmation.

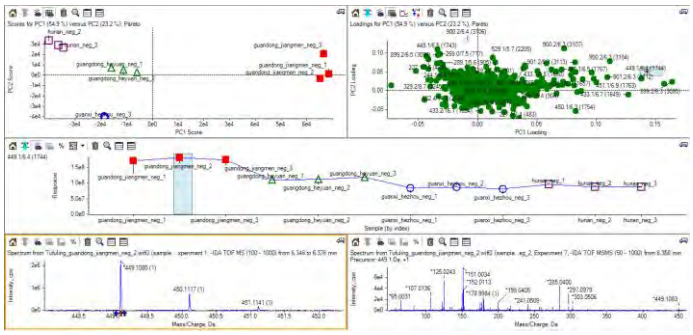


Figure 4 MarkerView software automatically associated with the raw data

Chinese Medicine Database Searching

The SCIEX high resolution Chinese medicine database is based on the “Chinese pharmacopeia” Traditional Chinese Medicine (TCM) ingredients, including almost 900 compounds such as saponins, flavonoids, flavonoid glycosides, triterpenes, phenylethyl glycosides and organic acids. Peak extraction and database matching in SCIEX OS Software’s allows a user to identify and verify compounds in their samples. Using accurate mass, 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 measured MS spectrum and isotope ratio. A mirror image comparison is performed with the chemical compound’s theoretical values from the database. Fig. 5C shows mirror image comparisons of the MS/MS spectra, namely between acquired and database hit.

Summary of Identified Results

Using MarkerView data analysis and MS/MS database matching results were verified, *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
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	Smitilbin	
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	Neosmitilbin	
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 Statistics

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

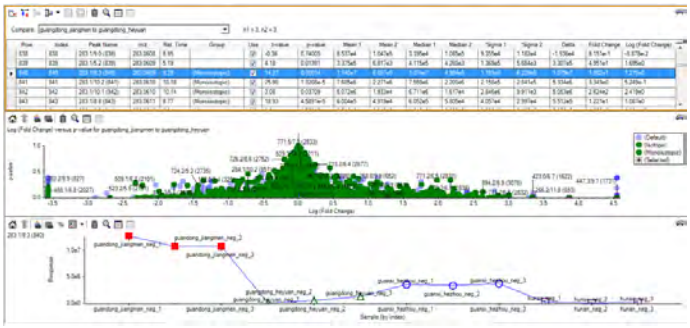


Figure 6 Mapping of log ratio change and p-value

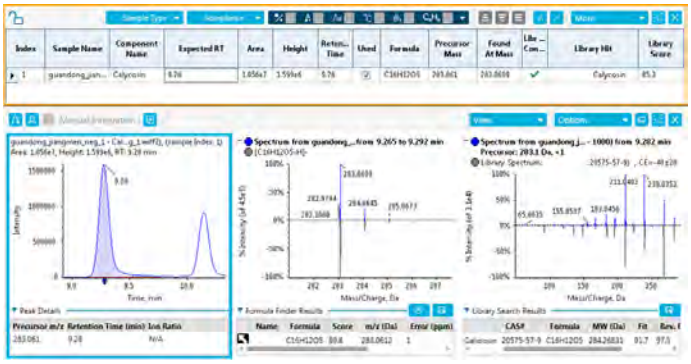


Figure 7 The confirmed result of m/z 283.0609, RT 9.28 min

SCIEX MS/MS database helps to quickly identify and verify compounds with confidence.

A 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 compounds. The Fragment Pane analysis function helps rapidly verify chemical compounds by theoretically fragmenting a structure and matching the MS/MS to the actually experimental data collected..

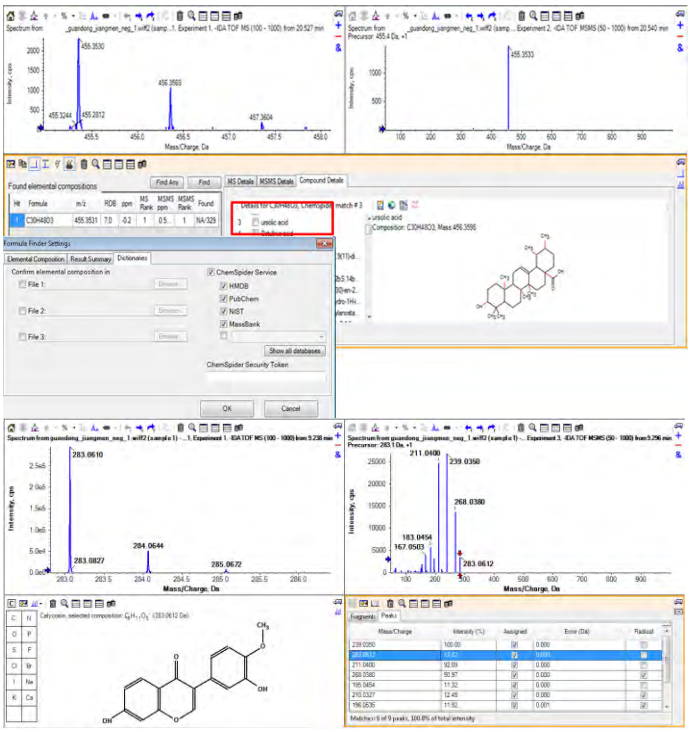


Figure 9 ChemSpider database can help identifying compounds rapidly

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 injection workflow allows the collection of high quality MS and MS/MS 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 data acquisition, processing and reviewing is simple, fast, and convenient.

MarkerView data analysis software applies multivariate (PCA) and univariate (t-test) analyses to find trends/differences between sample groups quickly and intuitively.

This study applied the SCIEX X500R high-resolution system to analyze differences between TCM samples. The powerful SCIEX OS Software and MarkerView Software are well integrated; accurate mass, fragment ions data, and isotope ratios can quickly verify compounds. The high-resolution MS/MS 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.

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