

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 new N-type geometry-based TOF path, intuitive software, and accurate molecular weight techniques that are easier to use in Chinese medicine component identification.

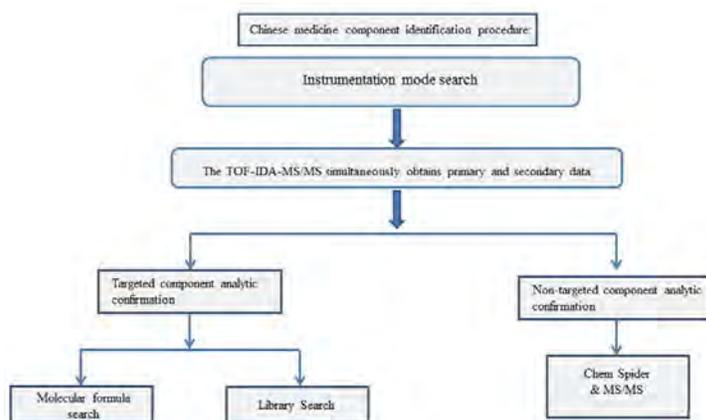
The benefits of this method are as follows:

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;

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

Liquid Chromatography (LC) 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: 40°C

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: 600°C

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

Source and gas parameters

TOF-MS

IDA criteria

TOF-MS/MS

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.

MS Tune

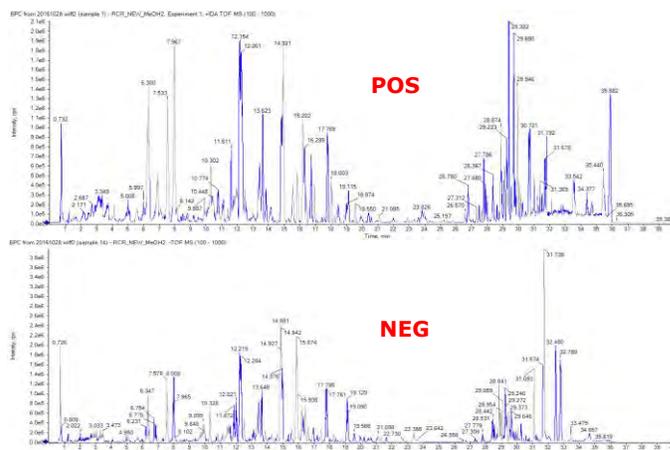
Finalize QMS Status Check

TOF MS/MS Check

Average of 20 spectra - Confirmation Mode

Scan	Retention	Mass	Abundance	Isotope
1	11.611	116.073	100	116.073
2	11.611	117.073	100	117.073
3	11.611	118.073	100	118.073
4	11.611	119.073	100	119.073
5	11.611	120.073	100	120.073
6	11.611	121.073	100	121.073
7	11.611	122.073	100	122.073
8	11.611	123.073	100	123.073
9	11.611	124.073	100	124.073
10	11.611	125.073	100	125.073
11	11.611	126.073	100	126.073
12	11.611	127.073	100	127.073
13	11.611	128.073	100	128.073
14	11.611	129.073	100	129.073
15	11.611	130.073	100	130.073
16	11.611	131.073	100	131.073
17	11.611	132.073	100	132.073
18	11.611	133.073	100	133.073
19	11.611	134.073	100	134.073
20	11.611	135.073	100	135.073
21	11.611	136.073	100	136.073
22	11.611	137.073	100	137.073
23	11.611	138.073	100	138.073
24	11.611	139.073	100	139.073
25	11.611	140.073	100	140.073
26	11.611	141.073	100	141.073
27	11.611	142.073	100	142.073
28	11.611	143.073	100	143.073
29	11.611	144.073	100	144.073
30	11.611	145.073	100	145.073
31	11.611	146.073	100	146.073
32	11.611	147.073	100	147.073
33	11.611	148.073	100	148.073
34	11.611	149.073	100	149.073
35	11.611	150.073	100	150.073
36	11.611	151.073	100	151.073
37	11.611	152.073	100	152.073
38	11.611	153.073	100	153.073
39	11.611	154.073	100	154.073
40	11.611	155.073	100	155.073
41	11.611	156.073	100	156.073
42	11.611	157.073	100	157.073
43	11.611	158.073	100	158.073
44	11.611	159.073	100	159.073
45	11.611	160.073	100	160.073
46	11.611	161.073	100	161.073
47	11.611	162.073	100	162.073
48	11.611	163.073	100	163.073
49	11.611	164.073	100	164.073
50	11.611	165.073	100	165.073
51	11.611	166.073	100	166.073
52	11.611	167.073	100	167.073
53	11.611	168.073	100	168.073
54	11.611	169.073	100	169.073
55	11.611	170.073	100	170.073
56	11.611	171.073	100	171.073
57	11.611	172.073	100	172.073
58	11.611	173.073	100	173.073
59	11.611	174.073	100	174.073
60	11.611	175.073	100	175.073
61	11.611	176.073	100	176.073
62	11.611	177.073	100	177.073
63	11.611	178.073	100	178.073
64	11.611	179.073	100	179.073
65	11.611	180.073	100	180.073
66	11.611	181.073	100	181.073
67	11.611	182.073	100	182.073
68	11.611	183.073	100	183.073
69	11.611	184.073	100	184.073
70	11.611	185.073	100	185.073
71	11.611	186.073	100	186.073
72	11.611	187.073	100	187.073
73	11.611	188.073	100	188.073
74	11.611	189.073	100	189.073
75	11.611	190.073	100	190.073
76	11.611	191.073	100	191.073
77	11.611	192.073	100	192.073
78	11.611	193.073	100	193.073
79	11.611	194.073	100	194.073
80	11.611	195.073	100	195.073
81	11.611	196.073	100	196.073
82	11.611	197.073	100	197.073
83	11.611	198.073	100	198.073
84	11.611	199.073	100	199.073
85	11.611	200.073	100	200.073
86	11.611	201.073	100	201.073
87	11.611	202.073	100	202.073
88	11.611	203.073	100	203.073
89	11.611	204.073	100	204.073
90	11.611	205.073	100	205.073
91	11.611	206.073	100	206.073
92	11.611	207.073	100	207.073
93	11.611	208.073	100	208.073
94	11.611	209.073	100	209.073
95	11.611	210.073	100	210.073
96	11.611	211.073	100	211.073
97	11.611	212.073	100	212.073
98	11.611	213.073	100	213.073
99	11.611	214.073	100	214.073
100	11.611	215.073	100	215.073

Positive and negative ion mode BPC's:



Compound Identification Procedure

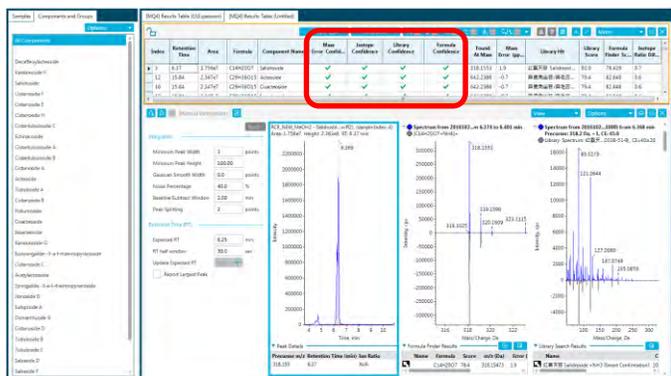
1. Targeted component identification workflow

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.

Row	IS	Group	Name	Chemical Formula	Isotope	Adduct (CH)	Precursor Mass (Da)	Fragment Mass (Da)	MS Width (Da)	Retention Time (min)	IS Name
1			Dimethylacetone	C ₅ H ₁₀ O	1	[M+H] ⁺	88.1031	88.1031	0.02	1.09	
2			Karabioside F	C ₂₀ H ₃₀ O ₁₂	1	[M+H] ⁺	625.2389	625.2389	0.02	15.54	
3			Sakuranoside	C ₁₄ H ₂₀ O ⁺	1	[M+H] ⁺	203.1283	203.1283	0.02	6.25	
4			Ginsenoside F	C ₃₂ H ₅₄ O ₁₁	1	[M+H] ⁺	609.3657	609.3657	0.02	6.59	
5			Ginsenoside E	C ₃₂ H ₅₄ O ₁₂	1	[M+H] ⁺	477.3865	477.3865	0.02	21.82	
6			Ginsenoside H	C ₃₂ H ₅₄ O ₁₃	1	[M+H] ⁺	493.3937	493.3937	0.02	6.75	
7			Ginsenoside G	C ₃₂ H ₅₄ O ₁₄	1	[M+H] ⁺	603.4044	603.4044	0.02	11.15	
8			Echinacoside	C ₁₈ H ₂₈ O ₁₀	1	[M+H] ⁺	381.2052	381.2052	0.02	11.97	
9			Cristobalucoside	C ₃₀ H ₄₈ O ₁₀	1	[M+H] ⁺	471.2951	471.2951	0.02	13.15	
10			Cristobalucoside	C ₃₀ H ₄₈ O ₁₁	1	[M+H] ⁺	487.3023	487.3023	0.02	13.15	
11			Ginsenoside A	C ₃₀ H ₄₈ O ₁₀	1	[M+H] ⁺	401.2817	401.2817	0.02	19.46	
12			Achraside	C ₂₀ H ₃₀ O ₁₁	1	[M+H] ⁺	403.2327	403.2327	0.02	15.54	
13			Tachycinol A	C ₁₈ H ₂₈ O ₁₁	1	[M+H] ⁺	403.2389	403.2389	0.02	19.65	
14			Ginsenoside B	C ₃₂ H ₅₄ O ₁₀	1	[M+H] ⁺	611.2982	611.2982	0.02	17.27	
15			Polygonoside	C ₃₀ H ₄₈ O ₁₀	1	[M+H] ⁺	371.2194	371.2194	0.02	13.15	
16			Cristobalucoside	C ₃₀ H ₄₈ O ₁₁	1	[M+H] ⁺	403.2327	403.2327	0.02	17.54	
17			Isachneoside	C ₂₀ H ₃₀ O ₁₁	1	[M+H] ⁺	403.2327	403.2327	0.02	15.54	
18			Karabioside G	C ₂₀ H ₃₀ O ₁₄	1	[M+H] ⁺	609.2178	609.2178	0.02	16.12	
19			Isopolygonoside B	C ₃₀ H ₄₈ O ₁₄	1	[M+H] ⁺	609.2178	609.2178	0.02	16.12	
20			Cristobalucoside C	C ₃₀ H ₄₈ O ₁₅	1	[M+H] ⁺	639.2835	639.2835	0.02	16.77	
21			Achyroside	C ₂₀ H ₃₀ O ₁₄	1	[M+H] ⁺	467.2328	467.2328	0.02	16.73	
22			Synepigalbin A	C ₃₀ H ₄₈ O ₁₄	1	[M+H] ⁺	609.2178	609.2178	0.02	16.12	
23			Isopolygonoside D	C ₃₀ H ₄₈ O ₁₅	1	[M+H] ⁺	639.2835	639.2835	0.02	16.77	
24			Echinacoside A	C ₁₈ H ₂₈ O ₁₀	1	[M+H] ⁺	447.1698	447.1698	0.02	17.84	

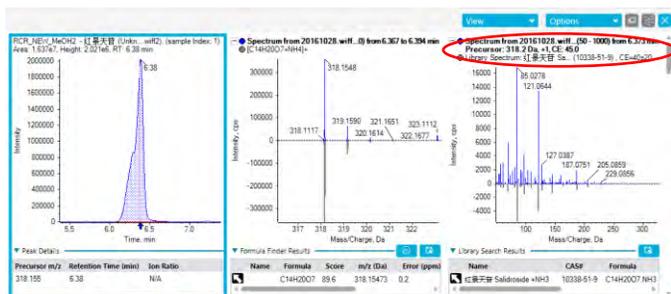
Once a results table is created, quantitative and qualitative results can be viewed in the same window. A red/green indicator system is used to indicate mass accuracy, retention time, isotope type, and confidence in identification by database matching.



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

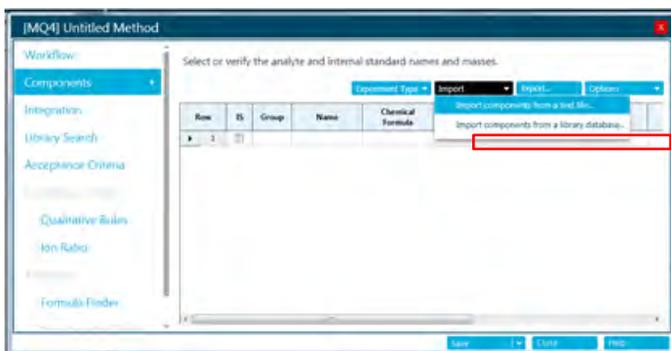
Kankanoside F	C26H40O17	642.26	6.16	-0.749
Salidroside	C14H20O7	318.155	6.38	0.241
Cistanoside F	C21H28O13	506.187	6.74	0.898
Cistanoside E	C21H32O12	494.223	8.04	0.449
Cistanoside H	C21H32O13	510.219	7.24	0.997
Cistanche tubulosa glycoside C	C35H46O21	820.287	10.3	-0.413
Echinacea glycoside	C35H46O20	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
Acetylfuluran 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
Lipodeside 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
Cistan tubulose A1	C27H38O18	668.24	6.19	0.585
Cistan tubulose A2	C27H38O17	652.246	7.63	0.894
Kankanoside H1/H2	C37H48O20	830.307	16.3	-0.977

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
Glucoside	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
(+)-pinosresinol-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

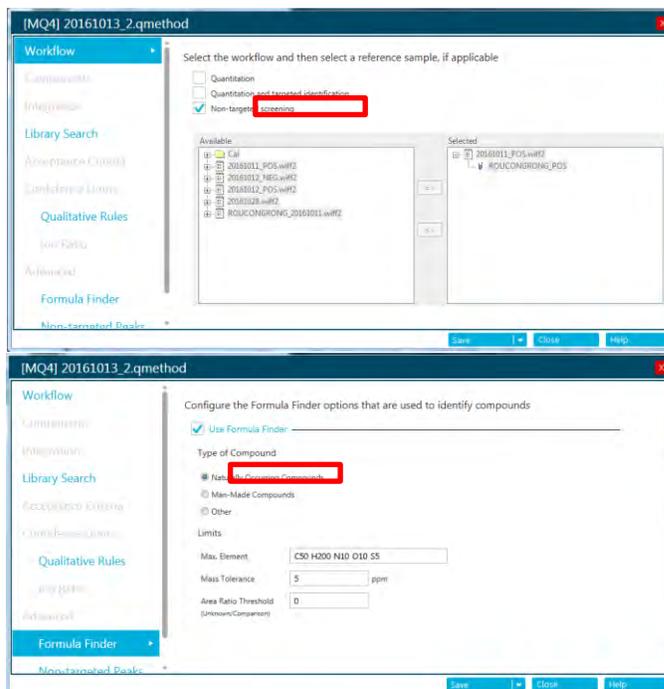
1.2: Database Search:



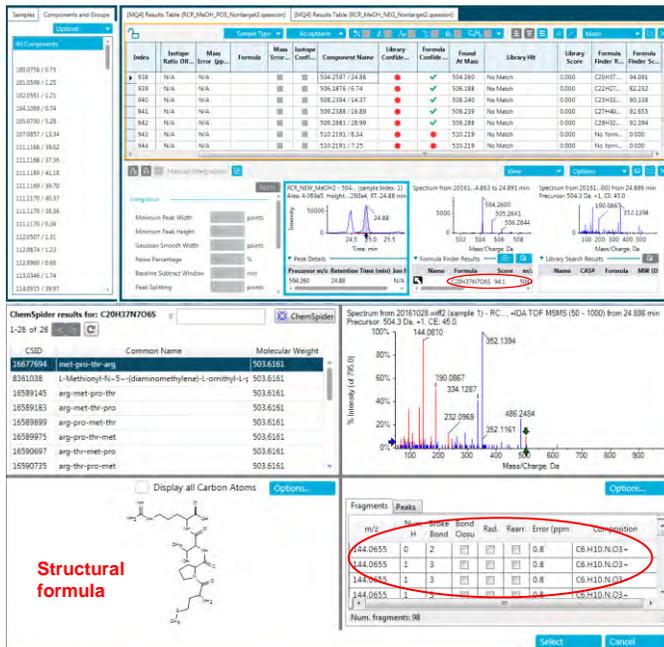
53 compounds were obtained from matching identification in the TCM database: 43 positive ions, 22 negative ions, and 12 repeats, listed below. Besides active phenolic glycosides, iridoids, glycosides and lignans, Cistanche also contains mannitol, leucine, and geniposidic acid. See appendix for list.

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.



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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