Chemical Components Identification of Cistanche Deserticola Using the X500R QTOF System

Li Yuejie, Cheng Haiyan, Liu Ting, Li Lijun, Jin Wenhai
SCIEX, Asia Pacific Application Support Center (Shanghai), China

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.

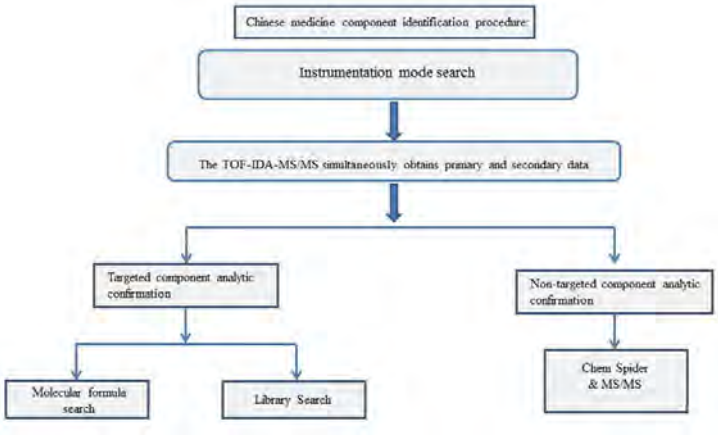
Quadrupole time-of-flight (QTOF) mass spectrometry is a sensitive and specific tool for identification of Chinese medicine components that has gradually become indispensable to research. This technology has overcome traditional technical challenges with retrospective analyses of single injections that 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;

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 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 NH_4FA
B is 95% acetonitrile-5% water-2mM NH_4FA

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:

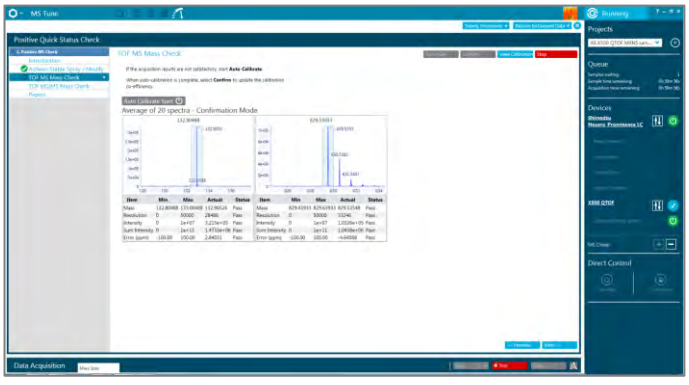
Air curtain gas CUR: 35psi; IS voltage: 5500V/-4500V

Source temperature: 600ºC

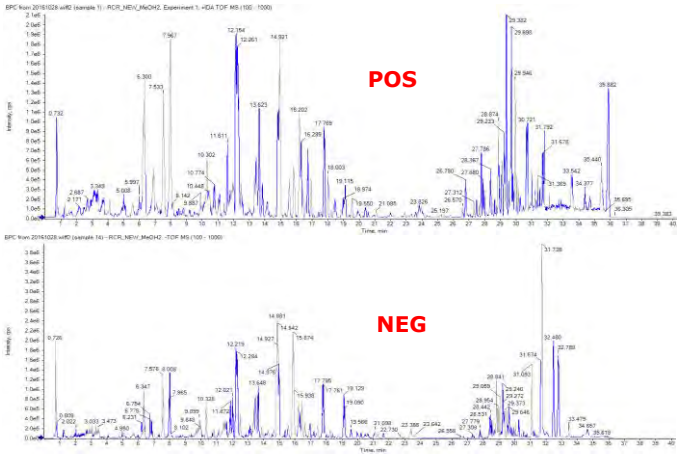
Atomizing gas GAS1: 55psi; Auxiliary gas GAS2: 60psi

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.



Positive and negative ion mode BPC's:

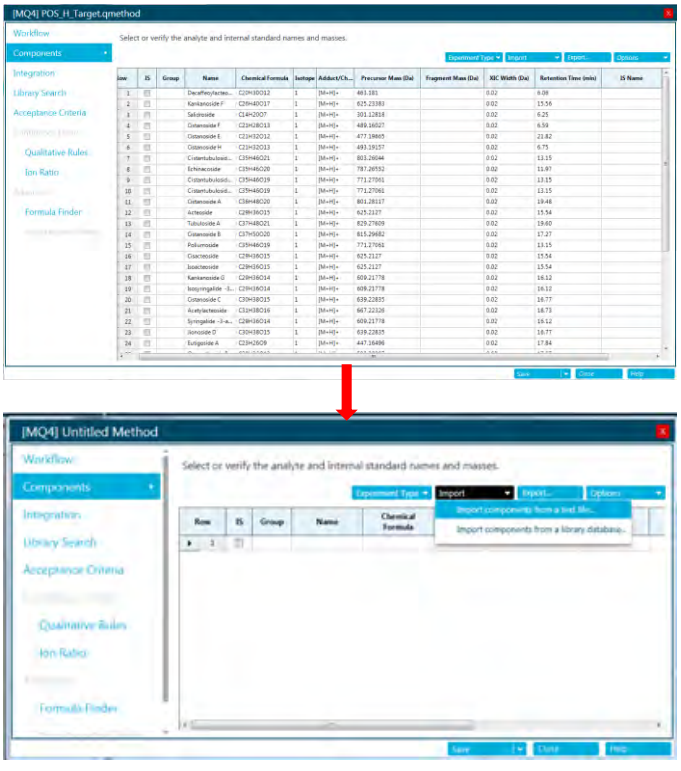


Compound Identification Procedure

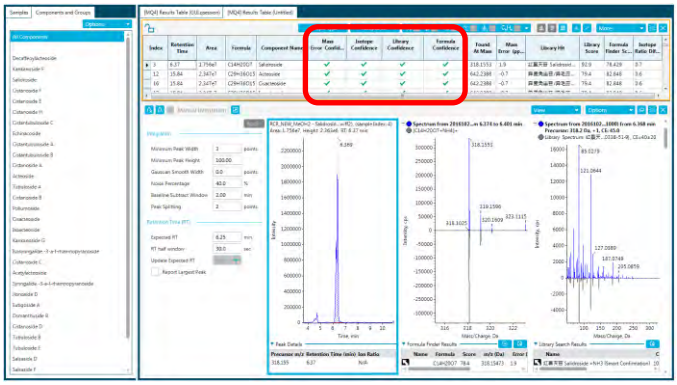
1. Targeted component identification workflow

1.1: Molecular formula search

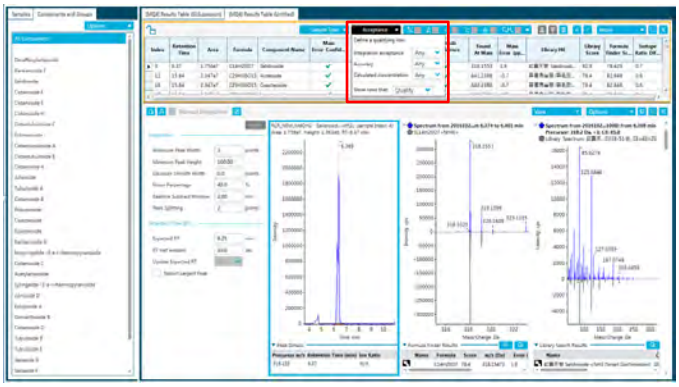
Only the chemical compound name and molecular formula are required; these can be input directly or imported using Excel's copy and paste function to create a processing method.



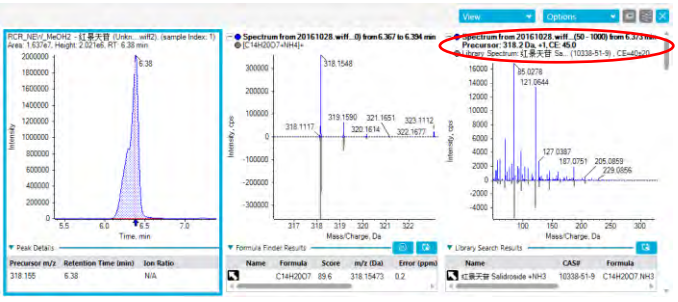
Once a results table is created, quantitative and qualitative results can be viewed in the same window. A red/green indicator system is used to indicate mass accuracy, retention time, isotope type, and confidence in identification by database matching.



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:

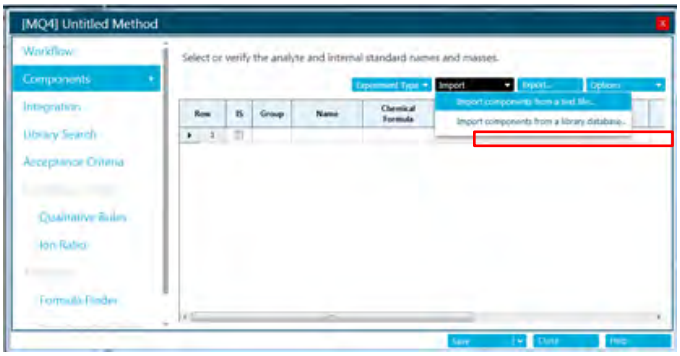
Component Name	Formula	Found At Mass (NH4)	RT (min)	Mass Error (ppm)
Veroascose glycoside	C20H30O12	480.208	6.17	0.951
Kankanoside F	C26H40O17	642.26	6.16	-0.749
Salidroside	C14H20O7	318.155	6.38	0.241
Cistanoside F	C21H28O13	506.187	6.74	0.898
Cistanoside E	C21H32O12	494.223	8.04	0.449
Cistanoside H	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	-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
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

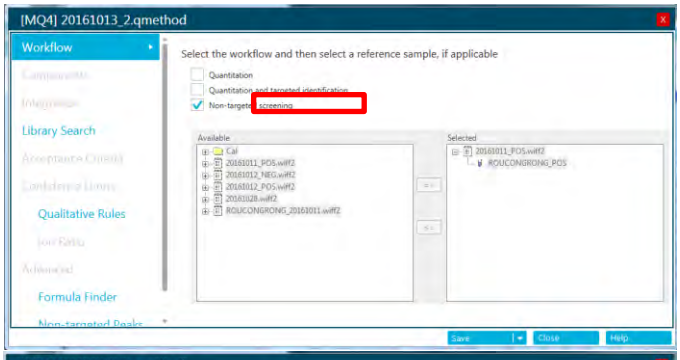
1.2: Database Search:

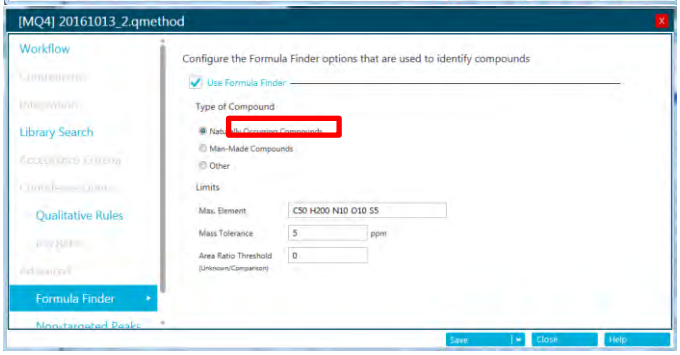


53 compounds were obtained from matching identification in the TCM database: 43 positive ions, 22 negative ions, and 12 repeats, listed below. Besides active phenolic glycosides, iridoids, glycosides and lignans, Cistanche also contains mannitol, leucine, and geniposidic acid. See appendix for list.

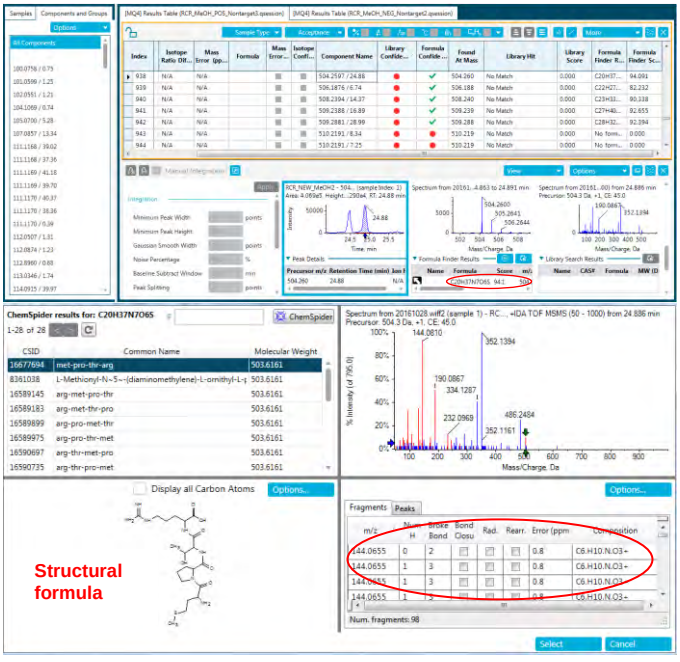
2. Non-targeted component analysis

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.



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.

Document number: RUO-MKT-02-6091-A

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices