A Novel UHPLC Orbitrap HRMS Approach for Monitoring Sulfur Fumigation Abuse in Drying Process of Traditional Chinese Medicine

Poster Note 64417

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Overview

Purpose: To demonstrate the application of fine isotope pattern filter with ultra high resolution mass spectrometry data for sulfur fumigation abuse monitoring in Traditional Chinese Medicine.

Methods: Sulfur fumigation processed herb powder was analyzed by Thermo Scientific^{TM} Orbitrap Velos Pro^{TM} high resolution mass spectrometer. MS data processing was performed on Thermo Scientific^{TM} Compound Discoverer^{TM} software.

Results: The fine isotope pattern screening method was successfully applied in study of sulfur-fumigated Pueraria species with twelve major sulfur derivatives, eleven of which were tentatively identified according to their HRMS^n data which is not known to occur naturally in these samples. The factors which may effect the accuracy of screening result were also evaluated.

Introduction

Traditional Chinese medicine has been used for disease treatment for thousands of years and still plays a very important role in current medical system in China and worldwide. Recently, due to the benefits of reducing the drying duration, preventing against insects , maintaining better appearance and even keeping moist to gain more weigh, sulfur fumigation has been commonly employed for fast drying medicinal materials during post-harvest handling instead of traditional natural drying process. However, determination of SO_2 involves complicated reactions to release and capture free SO_2 and was not sensitive enough. Benefit from the ultra high resolving power of Orbitrap Velos Pro mass spectrometer up to 100,000 FWHM, a novel fine isotopic pattern screening method was developed according to the separation of ^{34}S with $^{13}C2$ and ^{18}O isotopic pattern with mass shift of only 0.00981 amu.

Methods

Sample Preparation

Powdered samples (40 mesh, 0.2 g of Yege (YG, root of Pueraria lobata （Willd.）Ohwi), 0.1 g of Gegenqinlian tablets and Yufengningxin tablets, 0.8 g of Fenge (FG, root of Pueraria thomsonii Benth.) were extracted with 30 mL 30% ethanol for 30 minutes of ultrasonic extraction, filtered.

Liquid Chromatography:

Chromatographic separation was performed using an Thermo Scientific^{TM} UltiMate 3000 Binary RSLC system, equipped with a binary solvent delivery system, an autosampler, and a diode-array detection (DAD). Zorbax Eclipse Plus C18 column (100 × 2.1 mm, 1.8 *μ*m) was used for separation. The column temperature was set at 35 ˚C. The flow rate was 0.5 mL/min. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), gradient elution was processed as table 1.

Table 1. Gradient elution program

时间(min)	A	B
0	91	9
9.5	91	9
25.5	83.5	16.5
33	77	23
44	46	54
45	0	100
50	0	100
50.1	91	9
60	91	9

Mass Spectrometry

Samples were analyzed by an Orbitrap Velos Pro mass spectrometer (Fig.1) using HESI-Ⅱ probe in negative ion mode.

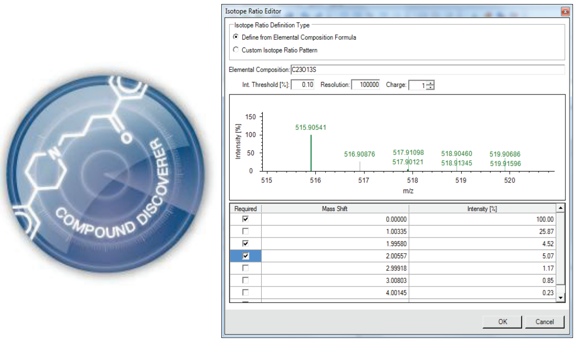
Ion source parameters: heat temperature, 300 ˚C; capillary temperature, 350 ˚C; spray voltage, 3.2 kV; sheath gas flow, 45 arb units ; auxiliary gas flow, 5 arb units.

The resolution was set to 100,000 FWHM for full scan screening and 30,000 FWHM for Data dependent MS^n (DDA). Scan range was from 150 to 1400 amu, the isolation window for DDA acquisition was set as 2 amu, collision energy 40%,

Fig.1 Orbitrap Velos Pro mass spectrometer

Data Analysis

Data was processed by Thermo Scientific^{TM} Compound Discoverer^{TM} software for sulfur derivatives related peaks detection, paeoniflorin sulfonate was selected as reference molecule with the formula of $C_{23}H_{28}O_{13}S$ (Fig.2),multiple nodes were constructed in a work flow to optimize the factors which may impact the precision of screening result.



Isotope Ratio Definition Type
● Define from Elemental Composition Formula
○ Custom Isotope Ratio Pattern

Elemental Composition: C23O13S
Int. Threshold [%]: 0.10 Resolution: 100000 Charge: 1

Required	Mass Shift	Intensity [%]
☑	0.00000	100.00
☐	1.00335	25.87
☑	1.99580	4.52
☑	2.00557	5.07
☐	2.99918	1.17
☐	3.00803	0.85
☐	4.00145	0.23

Fig.2 Compound Discoverer software and interface of isotope pattern screening

Results

The separation of isotope pattern at ultra high resolution

Isotopic pattern filter was used mainly in study of Cl- and Br-containing compounds due to their distinct isotope patterns, but it has seldomly been used for screening of S-containing compounds, because the ^{34}S isotopic ion will be drastically affected by the contribution of $^{13}C_2$ and ^{18}O, which might leads to many false positives if the resolving power is not high enough. As the resolution capabilities have been increased along with the development of MS technique, mass spectrometers can now be able to separate the extremely close isobars and resolve the fine isotopic pattern of the small molecules. Take $C_{25}H_{24}O_{15}S$ for example, Figure 2 demonstrates the evolution of the A_2 as the resolution arrange from 30K to 100K. From a broad singlet to absolute two peaks, the improved resolution definitely enhances the recognition ability of the algorithm, which makes it possible to extract ^{34}S information directly.

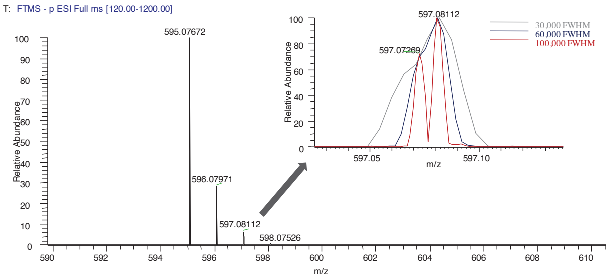
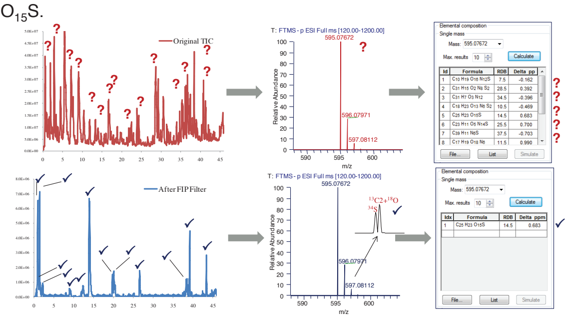


Fig.3 Evolution of isotope pattern A_2 at different resolutions.

Sulfur derivatives search

There is no effective method for sulfur derivatives screening with conventional identification work flow, it will take lots of time to find them out from complex ms signal manually and may lead to confusing results: numerous formulas will be generated even the mass accuracy is better than 1ppm. Base on the ultra high resolution ms data provided by Orbitrap technology, Fine Isotope Pattern (FIP) filter can now be used to screen sulfur atom containing mass signal with specific tolerance (typically 5ppm mass tolerance for more accurate result and 60% intensity tolerance for more comprehensive result). significant sulfur-related peaks can be detected significantly after processed by FIP filter. The number of sulfur atoms can even be counted through the ratio of fine isotopes, then most of false positive formula could be excluded, leading to a more accurate result. Take 595.07672 *m/z* for example, ratio of ^{34}S is 4.6% and ratio of $^{13}C2+^{18}O$ is 6.2% leading to the only element composition: $C_{25}H_{23}O_{15}S$.



Elemental composition
Single mass
Mass: 595.07672
Max. results 10

Id	Formula	RDB	Delta pp
1	$C_{10}H_{19}O_{16}N_{12}S$	7.5	-0.162
2	$C_{31}H_{15}O_2N_8S_2$	28.5	0.392
3	$C_{31}H_7O_3N_{12}$	34.5	-0.396
4	$C_{18}H_{23}O_{13}N_6S_2$	10.5	-0.469
5	$C_{25}H_{23}O_{15}S$	14.5	0.683
6	$C_{23}H_{11}O_5N_{14}S$	25.5	0.700
7	$C_{39}H_{11}N_8S$	37.5	-0.703
8	$C_{17}H_{19}O_{18}N_6$	11.5	0.990

Elemental composition
Single mass
Mass: 595.07672
Max. results 10

Idx	Formula	RDB	Delta ppm
1	$C_{25}H_{23}O_{15}S$	14.5	0.683

Fig.4 Conventional identification work flow and FIP filter work flow

Structural characterization of the sulfur derivatives

$C_{25}H_{23}O_{15}S$ shows core skeleton of daidzin according to the HRMS^n spectra (Figure4), the peak at *m/z* 253.0505 indicate existing of O-glycosyl. Also it contains a succinyl group, which makes it prone to lose CO_2. The obtained ion at m/z 551.0867 then produced two different ions at m/z 253.0505 ($C_{15}H_9O_4$) and 297.0283 ($C_9H_{13}O_9S$), respectively. The former is glycone and the latter is glycosyl group. Obviously, the sulfonyl is attached at the glycosyl part. Therefore, $C_{25}H_{23}O_{15}S$ is characterized as O-sulfonyl-succinyl-daidzin.

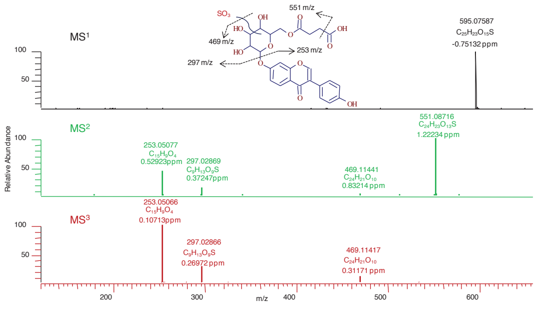


Fig.5 HRMS^n spectra of $C_{25}H_{23}O_{15}S$

The fine isotope screening was successfully applied in study of sulfur-fumigated Pueraria species with twelve major sulfur derivatives, eleven of them were tentatively identified according to their HRMS^n data in these samples (Figure5), by monitoring the eleven not naturally occurring components, the sulfur-fumigating situation of Gegen and Fenge products were evaluated.

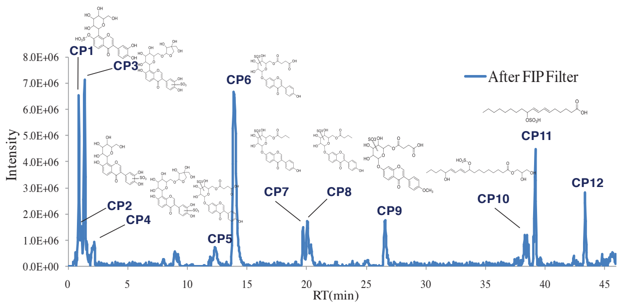


Fig.6 Proposed structures of detected sulfur derivatives

Impact factors

Resolving power has a big impact for mass accuracy for the final result both in MS data acquisition and screening setting. Both of them are evaluated to demonstrate the importance of resolution and mass accuracy.

The common resolution of 30K shows in Fig.6 A gave lots of false positives while a moderate resolution of 60K can reduced many false positives but still could not detect the sulfur-related peaks exactly(Fig.6 B). Due to the completely separation of A_2 ^{34}S and A_2 $^{13}C_2+^{18}O$ peaks, the resolution of 100k showed a really perfect filter results(Fig.6 C) which means the ^{34}S FIP filter could not be effective under the resolution of 60K.

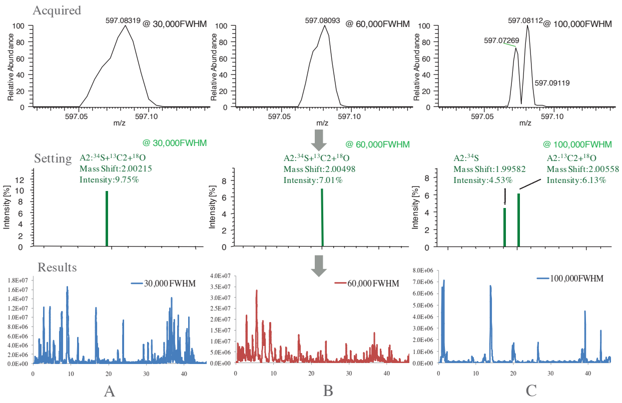


Fig.7 Comparison of FIP filter results at different mass resolution

As settings arrange from 15 to 5 ppm, the narrowed mass tolerance windows remarkably reduce the false positive peaks, which means an accurate screening result needs mass deviation less than 5 ppm. Otherwise, the sulfur-related peaks will be buried in interference signal.

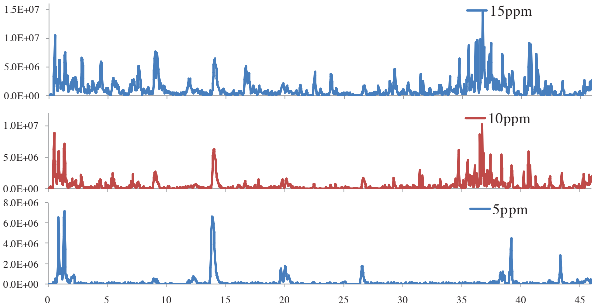


Figure. 8. Comparison of FIP filter results at different mass tolerance

Conclusion

As the resolution capabilities have been increased along with the development of MS technique, mass spectrometers can now be able to resolve the fine isotope pattern, therefore, a novel fine isotopic pattern screening method was developed according to the separation of ^{34}S with ^{13}C2 and ^{18}O isotopic pattern which was successfully applied in study of sulfur-fumigated Pueraria species. The comparison result also shows that ultra high resolution, high mass accuracy and novel FIP algorithm are three major factors for this method.

Furthermore, the novel FIP method is also a potent tool for not only S-containing compounds but also other specific element such as ^{15}N or ^{18}O has a wide range of application:

a) Discovery of specific element containing natural products from TCM

b) Drug metabolites screening in complex biological matrix

c) Definitive elemental composition determination in complex de novo applications

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