Identification of the Metabolites of Tetrachloronamide by QTRAP® 4500 Complex Mass Spectrometry System

Identification of the metabolites of SYP-9080 by QTRAP 4500 Mass Spectrometry System

Si Dandan, Liu Ting, Jin Wenhai
SCIEX, Pacific Applications Support Center (Beijing). China

Introduction

The bisamide insecticides developed in the 2000's are very effective against pests that attack rice and other crops and are also safe for non-target organisms. These types of chemical compounds are ryanodine receptor agonists that show no cross-resistance to current insecticides that work by other mechanisms. There are currently 3 commercial varieties of these compounds: flubendiamide entered the market in 2006, chlorantraniliprole in 2007, and cyantraniliprole in 2013. Shenyang Chemical Industry Research Co., Ltd. has led the market with chlorantraniliprole compounds. They have modified its structure with its benzene ring substituents and pyrazole substituents, and in 2008 they developed the highly effective pesticide tetrachloronamide (SYP-9080). It entered the market in 2014 and has been patented in China and the U.S. Mammalian toxicity is low and it is useful against Lepidoptera including Spodoptera exigua, Plutella xylostella, Mythimna separata, and Chilo suppressalis.[1]

This study used the SCIEX QTRAP 4500 quadrupole-linear ion trap complex mass spectrometry system with the specialized metabolite research software LightSight® Software to identify tetrachloronamide metabolites in rat urine and feces samples.

The QTRAP 4500 mass spectrometry system has the full advantages of triple quadrupole mass spectrometry: High sensitivity, durability, reproducibility, and anti-contamination functions; rapid switching between quadrupole and linear ion trap mode for a set IDA workflow. Triple quadrupole and linear ion trap Survey Scans combined with triggered enhanced ion scanning (EPI) and intelligent DBS functions ensure that high-quality secondary spectra of low-concentration chemical compounds can be obtained. This makes for easier, faster identification of chemical compounds at various concentrations within samples. MRM-IDA-EPI scanning mode, mainly used in research, is a highly sensitive method for qualification and quantitation of metabolites.

Combined with the LightSight Software, several IDA acquisition methods can be automatically set and combined with parent drug information for convenient and fast metabolite searching and identification. The experimental workflow is as follows:

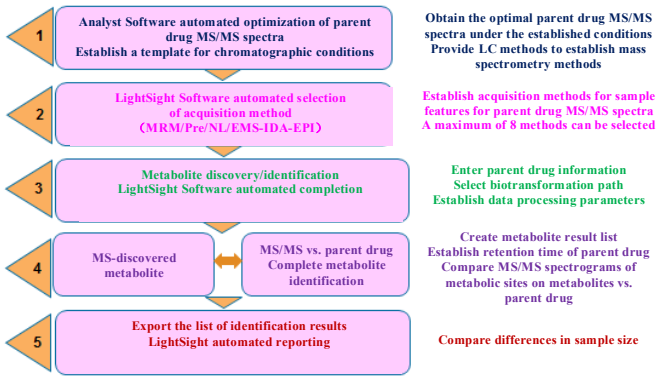


Figure 1: QTRAP 4500 integrates with LightSight Software's metabolite identification workflow

Sample information

1. Urine sample

Sample and blank: 4mL urine was filtered through a 0.45um filter; the sample was adsorbed to the SPE column (prior to adding sample, the column was rinsed with 3mL of acetonitrile and 3mL of water); after the sample was adsorbed, the column was eluted with 3mL of ultrapure water, then 3mL of acetonitrile, and the acetonitrile eluent fraction was collected. The solvent was evaporated until dry, and 250uL acetonitrile/water (50: 50) mixed solvent solution was added and vortexed. Then the mixture was centrifuged and the supernatant was extracted.

2. Feces sample

Sample and blank: An appropriate amount of feces was taken; 10 times the sample size in acetonitrile was mixed in. It was centrifuged and 3mL of supernatant was removed. The solvent was evaporated until dry; 250uL acetonitrile/water (50: 50) mixed solvent solution was added, vortexed, and centrifuged. Then the supernatant was removed.

Liquid phase conditions

Chromatographic Column: Kinetex C18 100 X 3.0 mm, 2.6 μm Phenomenex Analytical Column,

The mobile phase: Phase A: Water + 5mM Ammonium formate + 0.05 % formic acid;

Phase B: 95% acetonitrile + 5mM Ammonium formate + 0.05 % formic acid;

Flow rate: 0.4mL / min;

Elution method: Gradient elution 15 min

Column temperature: 40°C;

Volume injected: 5uL

Mass spectrometry method

Scanning method: MRM-IDA-EPI targeted search for predicted metabolites;

Pre/NL-IDA-EPI semi-targeted search for special metabolites;

EMS-IDA-EPI non-targeted search for high-concentration metabolites;

Dynamic background subtraction: DBS On

ESI ion source parameters:

Curtain Gas: 20psi; Collision Gas: High;

IS: 5500V/-4500V; TEM: 575°C;

GAS1: 60psi; GAS2: 60psi

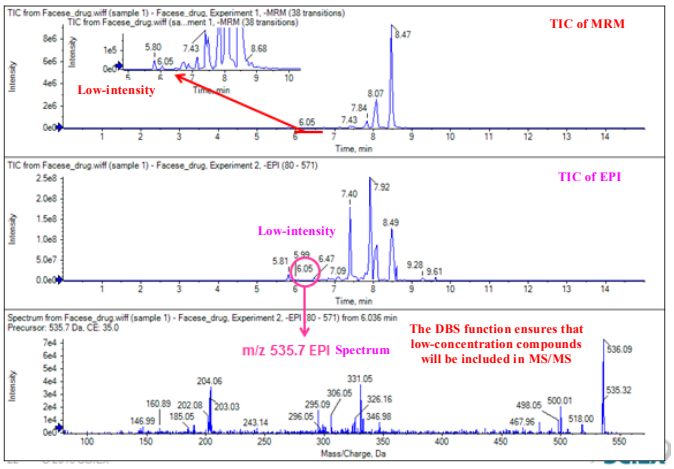


Figure 2: DBS ensures that MS/MS spectra will include low concentration metabolites

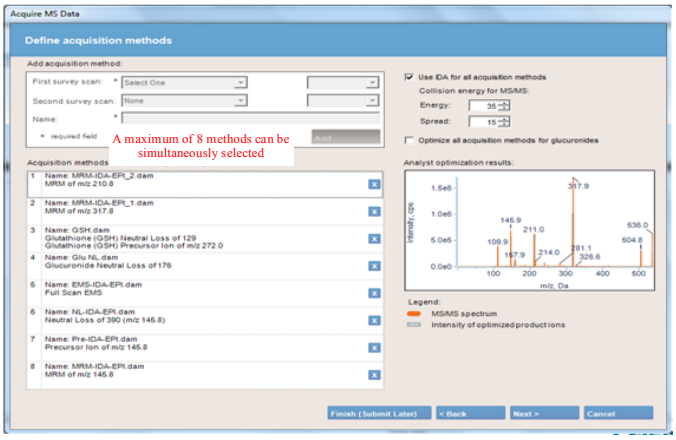


Figure 3. LightSight Software automatically sets several IDA acquisition methods

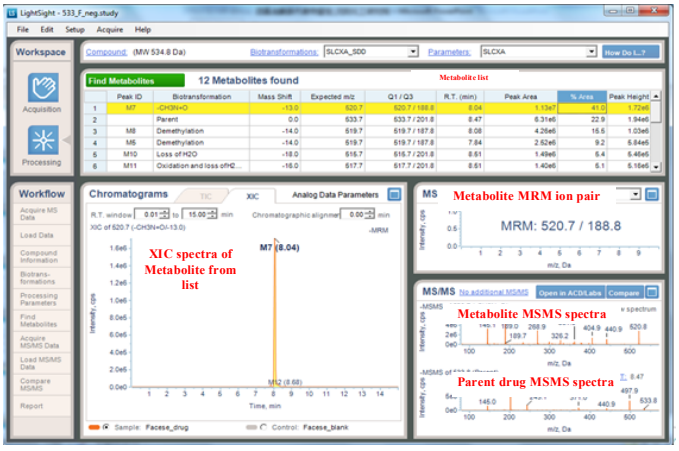


Figure 4: LightSight Software simply and rapidly completes data processing

LightSight Software

LightSight® Software is specialized, complete metabolite identification software that integrates with QTRAP series mass spectrometers. The software includes metabolite conversion pathways from reputable domestic and foreign literature and a list of almost 100 in vivo/in vitro biotransformations. As shown in Figure 3, the software can establish several simultaneous IDA acquisition methods (maximum 8) based on information related to parent drugs. The methods complement each other to provide the most comprehensive metabolite spectral information. The software can also use sample-blank comparison. It can establish data processing parameters and biotransformations either based on or independent of the acquisition method. Data processing is easy, fast and complete; see Fig. 4 for the LightSight Software data processing interface. It contains an intuitive list of metabolites, XIC spectra, parent drug and metabolite MS/MS Spectra, and other comparative information and can integrate with third party software ACD for complete structural analysis, metabolic site identification, and proposals of possible structures.

Metabolite identification results

Table 1: Metabolites identified in urine sample - results list

Index	Met ID	Biotransformation	Mass Shift	m/z	Q1 / Q3	R.T. (min)	Peak Area	Peak Height
1	Parent	Parent	0	535.8	535.8 / 145.8	8.46	1.51E+06	4.11E+05
2	M12	Loss of 300.8	-300.8	235	235.0 / 235.0	3.30	4.49E+07	5.92E+06
3	M13	Loss of 330.8	-330.8	205	205.0 / 205.0	4.09	5.18E+06	7.26E+05
4	M14	Loss of 199.9	-199.9	335.9	335.9 / 145.9	5.62	4.78E+05	1.27E+05
5	M15	Loss of 316.8	-316.8	219	219.0 / 219.0	5.66	1.48E+07	3.93E+06
6	M16	Loss of 302.8	-302.8	233	233.0 / 233.0	6.16	1.70E+06	2.14E+05
7	M17	Oxidation and loss of H2O and CH2	-16	517.7	517.7 / 201.8	6.48	4.84E+05	1.77E+05
8	M18	Loss of 345.8	-345.8	190	190.0 / 190.0	6.87	4.46E+06	1.42E+06
9	M19	Loss of 140.8	-140.8	395	395.0 / 395.0	7.33	1.24E+06	1.65E+05
10	M20	Demethylation + Oxidation	2	535.7	535.7 / 187.8	7.42	2.33E+06	7.10E+05
11	M21	Oxidation	16	551.8	551.8 / 145.8	7.43	4.24E+04	7.10E+03
12	M4	-CH3N	-29	506.8	506.8 / 145.8	7.82	1.25E+06	2.35E+05
13	M6	Loss of H2O and CH2	-32	503.8	503.8 / 145.8	7.96	6.89E+05	2.20E+05
14	M8	-CH3N+O	-13	520.7	520.7 / 188.8	8.07	2.49E+07	6.93E+06
15	M9	Demethylation	-14	519.7	519.7 / 187.8	8.11	1.19E+05	4.65E+04
16	M10	Loss of H2O	-18	517.8	517.8 / 145.8	8.50	6.12E+05	1.54E+05
17	M11	Oxidation and loss of H2O and CH2	-16	517.7	517.7 / 201.8	8.50	3.68E+06	1.16E+06

Note: Pink signifies positive ion mode detection, and blue signifies negative ion mode detection.

Table 2: Metabolites identified in fecal sample - results list

Index	Met ID	Biotransformation	Mass Shift	m/z	Q1 / Q3	R.T. (min)	Peak Area	Peak Height
1	Parent	Parent	0	535.8	535.8 / 145.8	8.49	3.62E+06	9.60E+05
2	M1	Oxidation and loss of H2O	-2	531.7	531.7 / 199.8	6.67	2.22E+04	7.29E+03
3	M2	Demethylation + Oxidation	2	535.7	535.7 / 201.8	6.05	7.65E+04	9.07E+03
4	M3	Oxidation	16	549.7	549.7 / 201.8	7.53	3.54E+04	9.93E+03
5	M4	-CH3N	-29	506.8	506.8 / 145.8	7.83	2.60E+07	9.52E+06
6	M5	Oxidation	16	551.8	551.8 / 145.8	7.83	1.58E+05	2.05E+04
7	M6	Loss of H2O and CH2	-32	503.8	503.8 / 145.8	7.92	1.67E+05	4.27E+04
8	M7	Oxidation and loss of H2O	-2	531.7	531.7 / 199.8	7.98	9.53E+04	3.11E+04
9	M8	-CH3N+O	-13	520.7	520.7 / 188.8	8.04	1.13E+07	1.72E+06
10	M9	Demethylation	-14	519.7	519.7 / 187.8	8.08	4.26E+06	1.03E+06
11	M10	Loss of H2O	-18	515.7	515.7 / 201.8	8.50	1.49E+06	5.46E+05
12	M11	Oxidation and loss of H2O and CH2	-16	517.7	517.7 / 201.8	8.51	1.40E+06	5.16E+05

QTRAP 4500 integrates with LightSight Software. Data acquisition and analysis of urine and feces samples require the LC-MS method above. Identification results appear as in Lists 1 and 2, showing 17 tetrachloronamide-related metabolites in the urine and 12 in the fecal sample. There are more metabolites in the urine sample than the fecal sample, and the types and quantities vary greatly. All identified metabolites are Class I; no Class II metabolites were discovered.

Metabolites identified in the samples showed: In the urine sample, metabolites produced by the breakdown of the parent drug were detected, and a small quantity of oxidation, demethylation and dehydration metabolites were also identified. In the fecal sample, more oxidation, dehydration, demethylation, dehydromethylation and deaminomethylation metabolites were found, and the quantities were higher than in the urine sample. Analysis of parent drug secondary spectra and comparison to metabolite secondary spectra completes the structural analysis and identifies the potential metabolic pathways and sites.

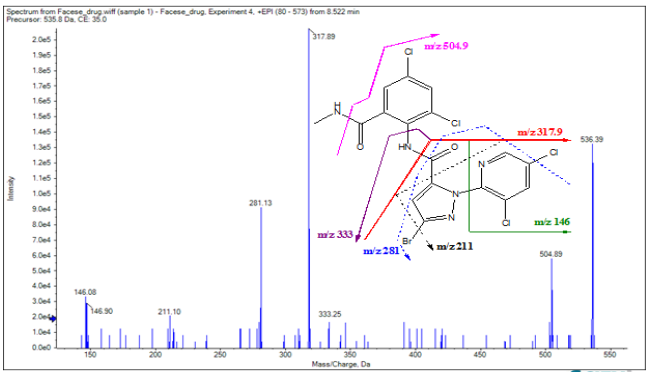


Figure 5: Principal tetrachloronamide fragments in positive ion mode and structural analysis diagram

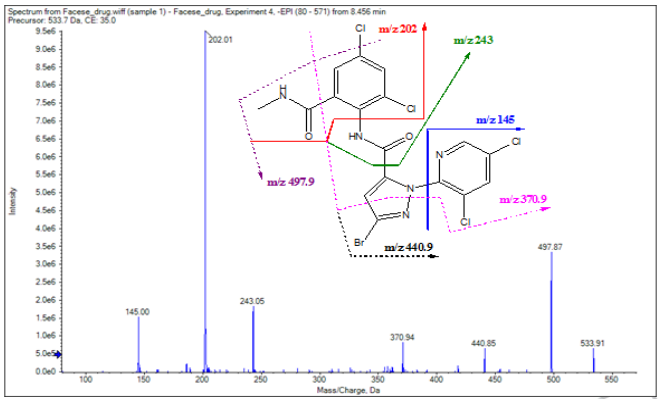


Figure 6: Principal tetrachloronamide fragments in negative ion mode and structural analysis diagram

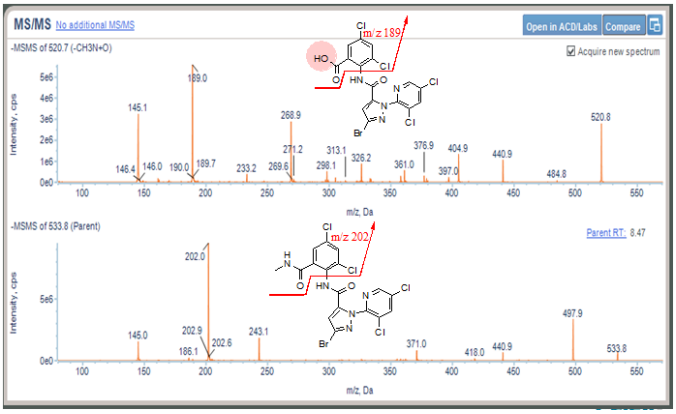


Figure 7： Metabolite structural analysis display and identification of metabolic sites

As shown in Figs. 5 and 6: Principal parent drug tetrachloronamide fragments in positive ion mode include: 146, 211, 281, 318, 333, 505; fragments in negative ion mode: 145, 202, 243, 371, 441, 498; structure attribution of secondary fragments was performed and the structural analysis is as shown. Fig. 7 shows a comparison between secondary spectra of metabolites vs. parent drug, main fragment ion differences, structural analysis attribution, and metabolic pathways and sites. The proposed metabolite structures appear in the appendix; consult it for detailed structural diagrams.

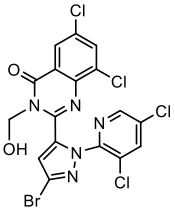
Summary

1. SCIEX QTRAP® 4500 triple quadrupole-linear ion trap complex mass spectrometry has rapid pole-trap switching speed, plus intelligent dynamic background subtraction (DBS) functions; it uses IDA workflow with several pre-scanning mode integration methods to perform tetrachloronamide metabolite identification tasks.
2. LightSight® Software can automatically set several methods based on parent drug information; the software includes almost 100 in vivo and *in vitro* metabolic pathways. By setting parameters and comparing samples to blanks, it processes metabolite data. It can connect to third-party software ACD for structural analysis, determination of metabolic pathways and sites, and proposal of potential structures. All these functions make metabolite identification easy - from method selection to data processing.
3. Application of this method to analyze urine and feces samples identified a total of 21 metabolites. Types and quantities of metabolites in each sample were different. All were Class I, none were Class II. This method is an easy and fast way to detect and identify different metabolites of varying concentrations; thus, it is a recommended workflow.

References:

1. Li Bin, Yang Huibin, Wang Junfeng, Song Yuquan, Synthesis and Insecticidal Activity of Si Lv Chong Xian'an. Modern Argochemicals (2014) Vol.13; No.3

Appendix: Structures of metabolites identified in urine and fecal samples



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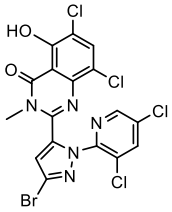

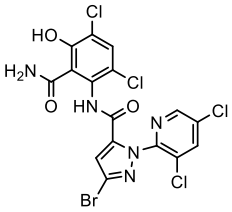

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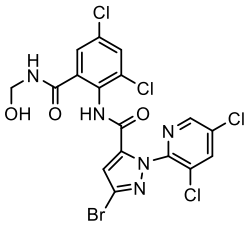

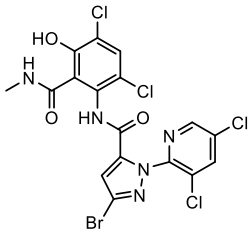

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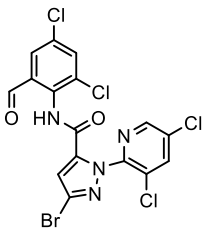

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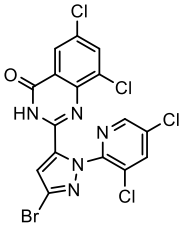

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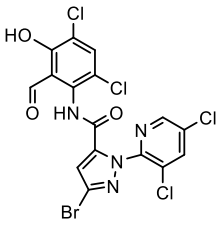

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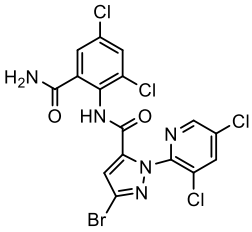

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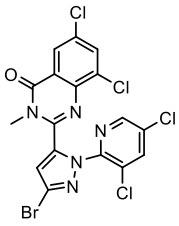

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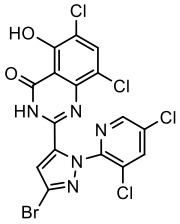

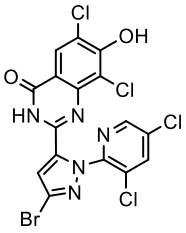

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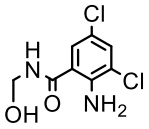

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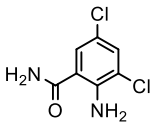

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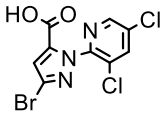

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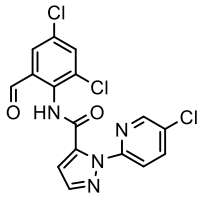

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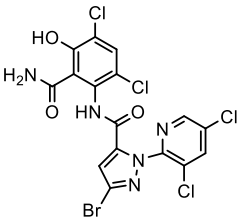

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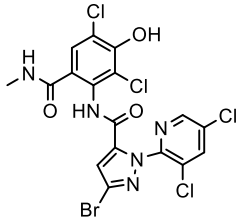

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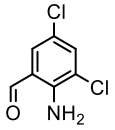

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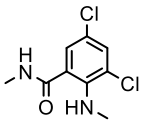

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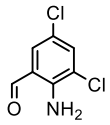

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