X500R QTOF

A COMPENDIUM OF RELATED RESEARCH APPLICATIONS

SCIEX

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X500R QTOF FOOD TESTING

Pesticide analysis in food

Elevate your food testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pesticides in food extracts using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software..

Sample Prep

Step 1
- 10g homogenized sample (hydrate with water if needed).

Step 2
- Add 10mL acetonitrile and internal standard

Step 3
- Extract with vigorous shaking for 1 minute

Step 4
- Add QuEChERS salt mix (Phenomenex roQ KS0-8909), shake vigorously for 1 minute.

Step 5
- Centrifuge for 10 minutes at 9000 rpm.

Step 6
- Transfer 1mL supernatent to dSPE tube (Phenomenex roQ KS0-8916,8913,8914, or 8915 depending on sample type.

Step 7
- Shake vigorously for 30 seconds

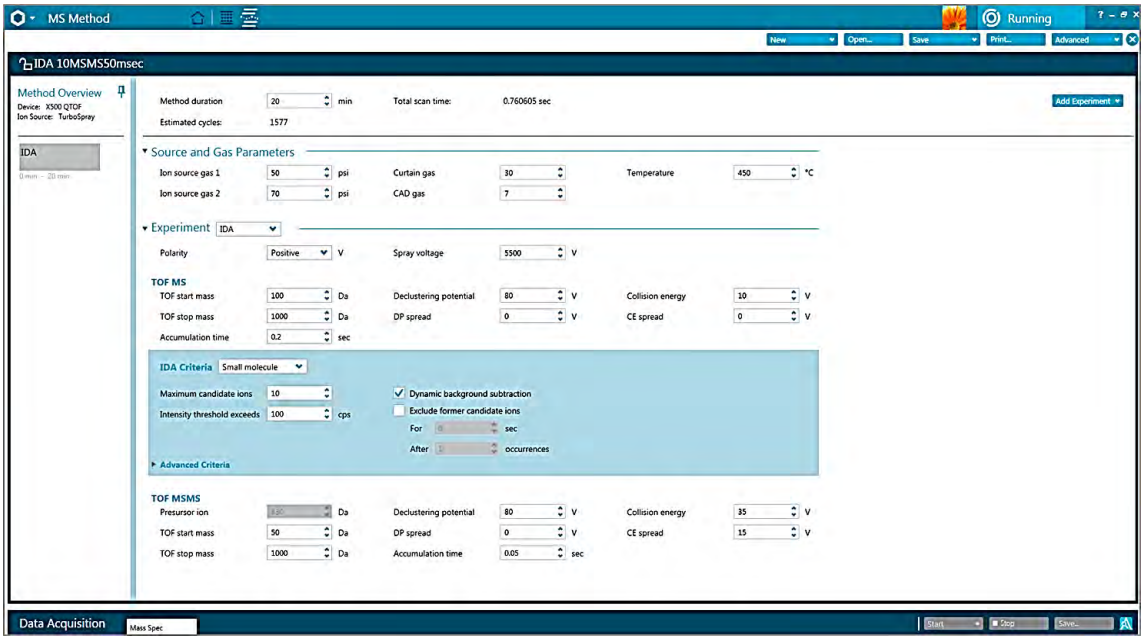
Step 8
- Dilute 100µL of extract to 1mL in water for MS analysis

Suggested sample preparation conditions based on the QuEChERS method (*QuEChERS European standard method 15662).*

LC Method

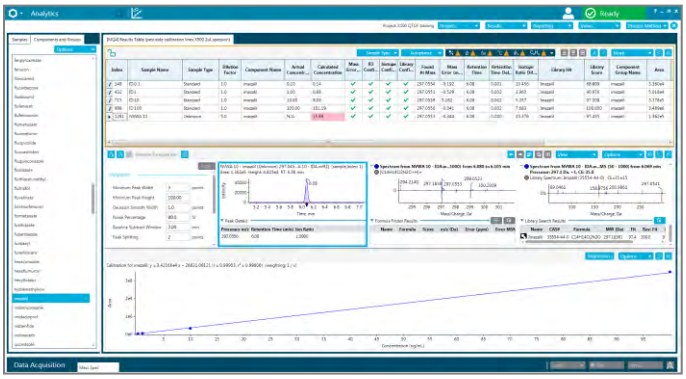
Column	Phenomenex Kinetex Biphenyl, 50 x 2.1 mm, 2.6 um	
Mobile Phase A	*5 mM ammonium formate in water*	
Mobile Phase B	*5 mM ammonium formate in methanol*	
Flow rate	*0.5 mL/min*	
Column temperature	*40 ℃*	
Injection volume	*2 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*10*
	0.5	*10*
	2.00	*30*
	9.0	*60*
	11.0	*80*
	12.0	*95*
	15.0	*95*
	16.0	*10*
	20.0	*10*

MS Method



Suggested IDA (Information Dependent Acquisition) conditions for routine food contaminant testing as displayed in SCIEX OS.

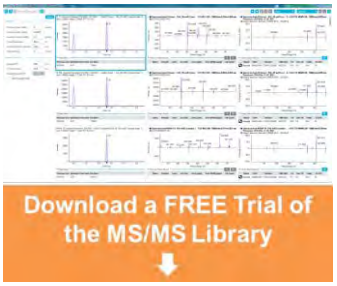
Data Processing



Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of pesticide compounds including molecular formula and accurate mass.



Download a free trial of the pesticide high resolution MS/MS library, containing 557 compounds.

[Learn more at sciex.com/X-Pesticides](sciex.com/X-Pesticides).

Document number: RUO-MKT-07-2861-A

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Antibiotic analysis in food

Elevate your food testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to detect, quantify, and confirm antibiotic vet drugs in tissue extracts using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software..

Sample Prep

Step 1
- 1g Tissue homogenized with 10mL extraction solvent (1:5) Water Acetonitrile

Step 2
- Vortex, shake vigorously for 5 minutes, centrifuge (5000rpm, 5 minutes)

Step 3
- Decant supernatent to 15mL tube & add 500mg C18 sorbent.

Step 4
- Vortex, shake 30 sec, centrifuge (5000rpm, 1 minute).

Step 5
- Collect 5mL aliquot of extract and reduce in volume to <1mL.

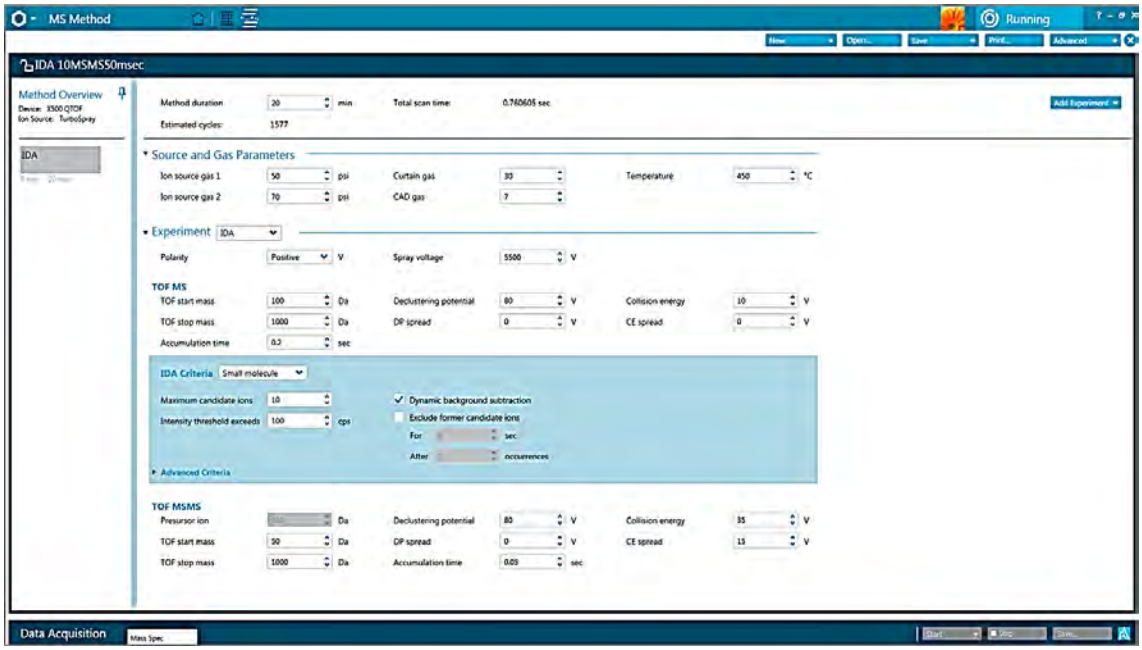
Step 6
- Bring to final volume of 1mL w/water and filter for analysis

Sample prep protocol adopted from:
Mastovska & Lightfield, J. Chrom. A., 2008, 1202, 118-123

LC Method

Column	Phenomenex Gemini 3µm C18 110Å column, 50 x 2.0mm	
Mobile Phase A	*0.1% formic acid in water*	
Mobile Phase B	*0.1% formic acid in methanol*	
Flow rate	*0.5 mL/min*	
Column temperature	*40℃*	
Injection volume	*10 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*2*
	0.3	*2*
	7.27	*80*
	7.37	*99*
	10.9	*99*
	11	*2*
	15	*2*

MS Method



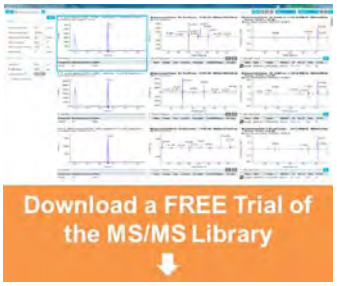
Suggested IDA (Information Dependent Acquisition) conditions for routine food contaminant testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of antibiotic compounds including molecular formula and accurate mass.



Download a free trial of the antibiotic high resolution MS/MS library, containing 244 compounds.

Learn more at sciex.com/X-Antibiotics.

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Mycotoxin analysis in food

Elevate your food testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to detect, quantify, and confirm mycotoxins in food extracts using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1
- Add 4mL extraction solvent (79/20/1. V/V/V) Acetonitrile/Water/Acetic Acid per 1g sample.

Step 2
- Extract on a rotary shaker for 90 minutes

Step 3
- Centrifuge.

Step 4
- Dilute 350µL of extract with 350µL 20/79/1 (v/v/v) Acetonitrile/Water/Acetice Acid.

Step 5
- Filter into vial for analysis.

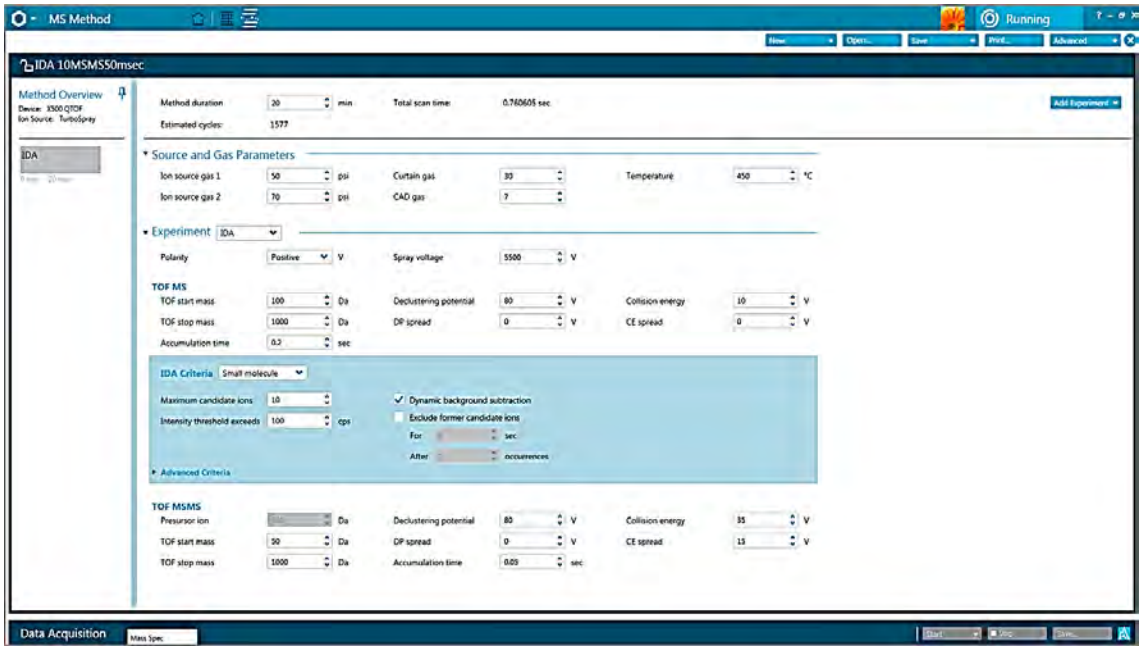
Sample prep protocol based : Sulyok M, Krska R, Schumacher R (2010) Food Chem 119:408-416

SCIEX OS can deliver faster method set-up

LC Method

Column	Phenomenex Gemini C18, 150 x 4.6 mm, 5 µm	
Mobile Phase A	*5 mM ammonium acetate + 1% acetic acid in water*	
Mobile Phase B	*5 mM ammonium acetate + 1% acetic acid in methanol*	
Flow rate	*1.0 mL/min*	
Column temperature	*25℃*	
Injection volume	*5 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*0*
	2	*0*
	14	*100*
	18	*10*
	18.1	*0*
	20.5	*0*

MS Method



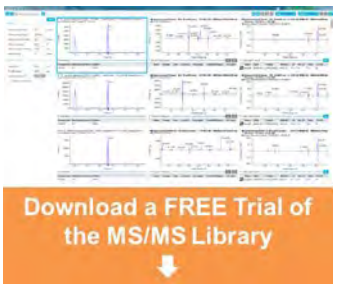
Suggested IDA (Information Dependent Acquisition) conditions for routine food contaminant testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of mycotoxin compounds include molecular formula and accurate mass.



Download a free trial of the mycotoxin high resolution MS/MS library, containing 288 compounds.

Learn more at sciex.com/X-Mycotoxins.

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Document number: RUO-MKT-07-2860-A

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Pesticide and Potency Testing for the Cannabis Industry

Elevate your confidence in cannabis testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pesticides, mycotoxins, cannabinoids and terpenes in plant edible samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1 • Weight 0.1g sample

Step 2 • Sonicate in 9mL methanol for 5 minutes

Step 3 • Bring to 10mL final volume

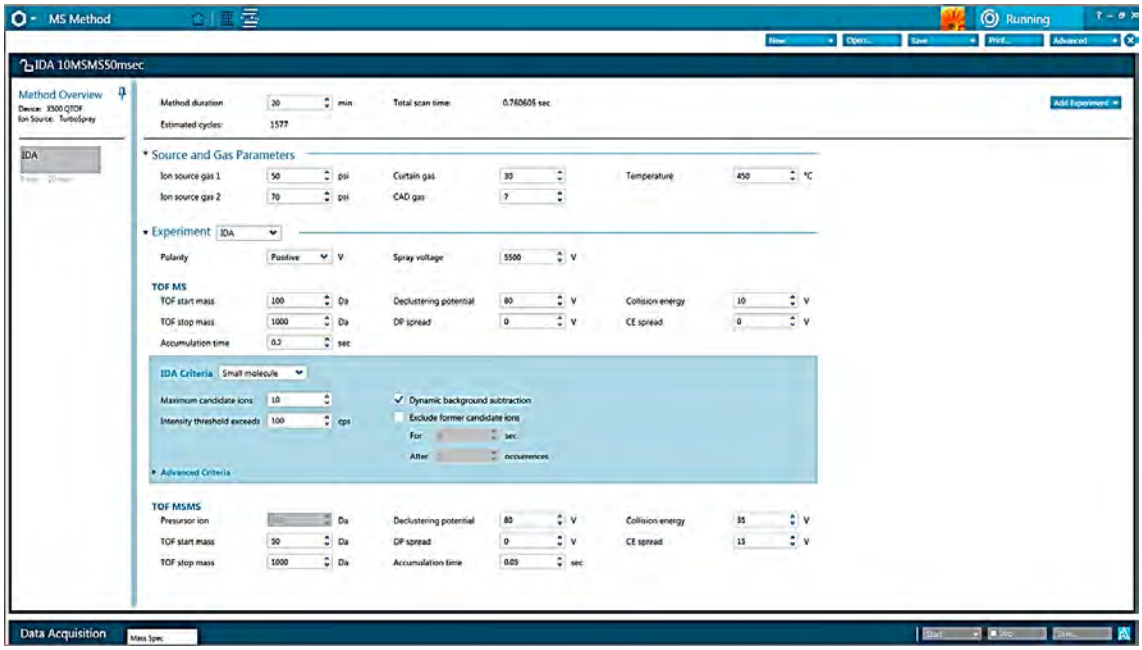
Step 4 • Centrifuge and draw off 1mL for analysis.

SCIEX OS can deliver faster method set-up

LC Method

Column	Restek Raptor ARC-18 50 x 2.1mm, 2.7µm	
Mobile Phase A	*0.1% formic acid, 5mM ammonium formate in water*	
Mobile Phase B	*0.1% formic acid, 5mM ammonium formate in acetonitrile*	
Flow rate	*0.4 mL/min*	
Column temperature	*40 ℃*	
Injection volume	*5 µL*	
Gradient profile	**Time (min)**	**% B**
	0	*30*
	0.5	*30*
	4	*95*
	5	*95*
	5.1	*30*

MS Method



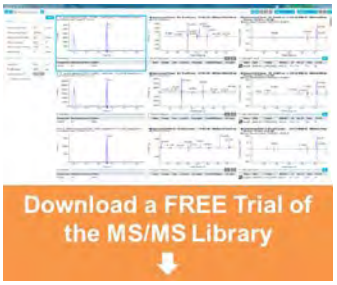
Suggested IDA (Information Dependent Acquisition) conditions for routine food contaminant testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of applicable compounds including molecular formula and accurate mass.



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Document number: RUO-MKT-07-2859

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Perfect Balance to Elevate your Lab's Performance

Using the X500R QTOF System and SCIEX OS Software to Identify and Quantify Food Residues

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[1]SCIEX Concord, Ontario (Canada), [2]SCIEX Framingham, Massachusetts (USA), [3]SCIEX Darmstadt (Germany)

Overview

Here we present results using a new method to identify and quantify pesticide residues in food using the SCIEX X500R QTOF system. Samples were extracted using a QuEChERS method and analyzed by LC-HR-MS/MS. Limits of quantitation of 10 µg/kg were achieved for every compound after 10x dilution of the extract to minimize possible matrix effects.

Target compounds were automatically identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching using SCIEX OS software. In the same data processing step, compounds were quantified and unknown samples were flagged when a user-defined reporting level was exceeded.

Introduction

Recent advancements in LC-MS/MS technology, including hybrid systems like quadrupole-quadrupole Time-of-Flight (QTOF), now provide the ability to perform targeted and non-targeted screening in food samples on a routine basis.[3]

The SCIEX X500R QTOF system is a robust, high performance high resolution MS/MS system designed for routine use providing:

- Sensitivity to easily detect compounds at maximum residue levels
- Resolving power to remove interference from complex food matrices
- Linearity to quantify over up to 3 orders of magnitude
- Mass accuracy to identify compounds following regulatory guidelines
- Confident identification using MS/MS spectra and ion ratios
- Industry leading robustness of Turbo V™ source and Curtain Gas™ interface

Full scan chromatograms are very rich in information and easily contain thousands of ions from any residue present in the sample, including the food matrix itself. Powerful software is needed to explore the high resolution MS/MS spectra generated to get answers and results from these complex data.

The SCIEX OS software is a single platform for MS control, data processing, and reporting and provides:

- Simple software workflows that deliver reliable results
- Simultaneous identification and quantitation
- Quick data review and reporting utilizing customizable flagging and filtering of results

Experimental

Standards

A standard mix of 200 pesticides was used to prepare serial dilutions for quantitative analysis.

Sample preparation

EU proficiency test samples and food samples from a local supermarket were extracted using a QuEChERS procedure following guideline EN 15662/2007. Sample extracts were diluted 10x to minimize possible matrix effects.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AC system with a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and methanol with 5 mM ammonium formate buffer at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile). The injection volume was 5 µL.

Table 1. Gradient conditions used for the separation of pesticides

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	0.5	90	10
2	2.0	70	30
3	9.0	40	60
4	11.0	20	80
5	12.0	5	95
6	15.0	5	95
7	16.0	90	10
8	20.0	90	10

MS/MS Detection

The SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI) was used.

Mass calibration was achieved using the integrated calibrant delivery system (CDS) with the TwinSprayer probe (dual ESI needle).

High resolution data were acquired using an IDA method consisting of a TOF-MS survey (100-1000 Da for 100 msec) and up to 20 dependent MS/MS scans (50-1000 Da for 35 msec). MS/MS fragmentation was achieved using CE of 35 V with a collision energy spread (CES) of ±15 V.

Dynamic background subtraction (DBS) was activated for best MS/MS coverage, and no inclusion list was used to also allow retrospective unknown identification without the need for a second injection to acquire MS/MS data.

Data Acquisition and Processing

All data were acquired and processed using SCIEX OS software version 1.0, which showcases a thoughtfully designed user interface that is fast to learn and delivers improved lab productivity.

Results and Discussion

X500R Performance Characteristics

Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm are often sufficient to separate the analytes of interest from interfering matrices and, thus, are identified as the set requirements for compound identification in various guidelines.[1, 2]

The X500R QTOF system utilizes N-optics design to maximize resolution while maintaining benchtop design and a minimized footprint. Its resolving power increases with mass range providing ~30000 to 40000 for the typical molecular weight range of pesticides.

The 4 mm orifice leading into the TOF accelerator delivers resolution without compromise in sensitivity. The sensitivity of the X500R QTOF system is comparable to a SCIEX QTRAP® 5500 system operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 µg/kg levels (Figure 1).

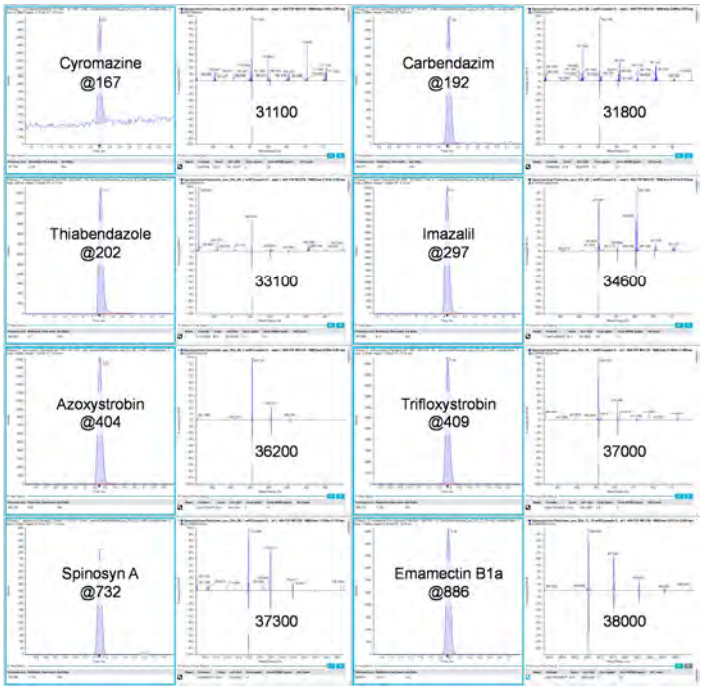

Figure 1. Sensitivity and resolution of different pesticides, left: XIC of the molecular ion of each compound ± 5 mDa at 1 ng/mL (Emamectin at 10 ng/mL), right: TOF-MS spectrum of molecular ion with achieved resolution value (average of seven X500R QTOF systems)

The X500R QTOF system achieves stable mass accuracy of less than 2 ppm by using a heated TOF configuration, with 6 heater drones throughout the TOF path to maintain mass accuracy and robustness. In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant delivery path for reliable auto-calibration. The CDS setup maintains mass accuracy over long periods of time by automatically calibrating in batch mode (it is recommended to infuse a calibrant standard every hour or two).

Furthermore, the X500R QTOF's mass accuracy is supplemented by legendary dynamic transmission control and dynamic background calibration, introduced in 2010 with the TripleTOF® system and optimized over time.

Figure 2 shows an example of mass accuracy for a selected pesticide detected over a wide concentration range. Paclobutrazol was quantified from 0.1 to 1,000 ng/mL with good linearity (r^2 = 0.9993). Excellent mass accuracy was achieved (-0.2 to 0.91 ppm) at all levels, even at the highest concentration of 10,000 ng/mL which was above the upper limit of quantitation for this analyte.

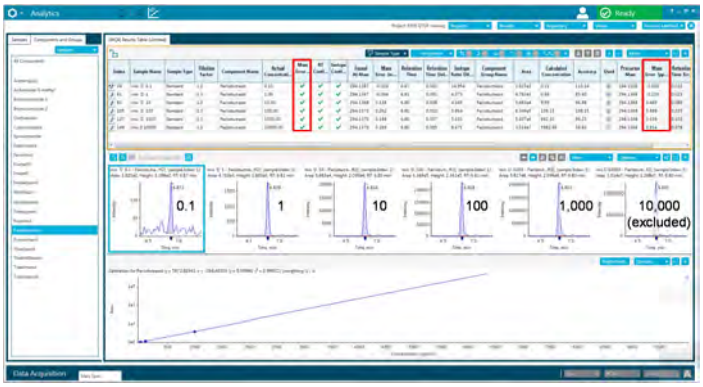

Figure 2. Detection of Paclobutrazol from 0.1 to 10,000 ng/mL with good linearity (0.1 to 1,000 ng/mL) and mass errors of < 1 ppm even at the highest concentration above the upper limit of quantitation

Despite the high selectivity of high resolution MS detection, there is a risk of false positive findings due to interfering isomers and matrix signals. As a result food testing guidelines require the detection of the "molecular ion" and "at least one fragment ion", and for "a higher degree of confidence in identification, further evidence may be gained from additional mass spectrometric information. For example, evaluation of full scan spectra, isotope pattern, adduct ions, additional accurate mass fragment ions… (in MS/MS)".[2]

The example shown in Figure 3 highlights the need of fragment ion detection to confidently differentiate between isomers.

The pesticides Prometon and Terbumeton have identical molecular formulae ($C_{10}H_{19}N_5O$) and as a result the identical molecular ion and isotope pattern. The retention time difference of less than 0.1 min, due to highly similar structures, is not sufficient to differentiate both pesticides.

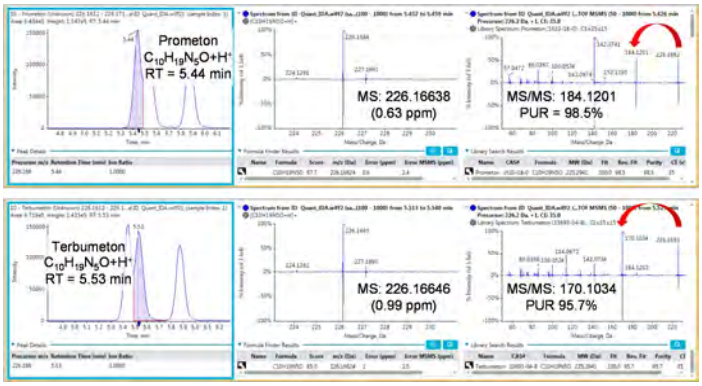

Figure 3. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

However, the two compounds have unique and characteristic fragment ions, $C_7H_{14}N_5O^+$ and $C_6H_{12}N_5O^+$, respectively, which can be used for identification. Molecular and fragment ions have been measured with good mass accuracy of < 5 ppm and less < 1 mDa, respectively.

Processing Workflow for Targeted Identification and Quantitation in SCIEX OS Software

Extracted Ion Chromatograms (XIC) of all target analytes are generated based on user input (chemical formula and expected retention time). MS and MS/MS information is automatically evaluated if an XIC signal is detected and compounds are identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching. Qualitative rules are defined in the processing method and can be used for results review and filtering (Figures 4a and b).

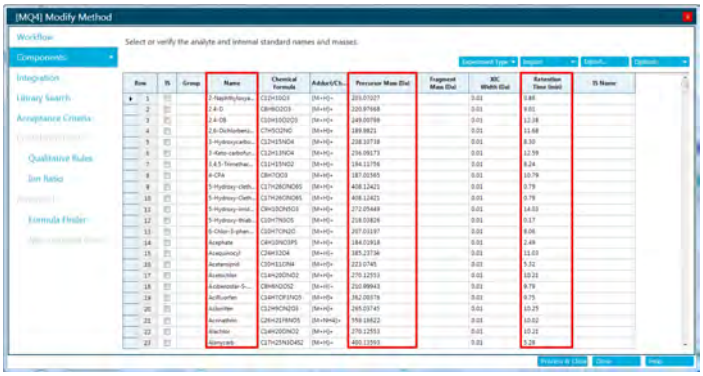

Figure 4a. Method editor in SCIEX OS software, user input for target compounds including chemical formula to calculate precursor ion mass and expected retention time

Figure 4b. Method editor in SCIEX OS software, user input for qualitative rules (traffic lights) to enable easy results review and filtering

In the same data processing step standard calibration lines are generated to automatically calculate concentrations in unknown samples (Figure 2).

Results of EU Proficiency Test Samples

Two samples of an EU proficiency test for pesticides and fruits and vegetables were extracted and analyzed for pesticides. Results are listed in Table 2. Retention time errors were less than 0.1 min and mass errors were between -1.20 and 1.17 ppm and were well below the required 5 ppm (SANTE/11945/2015).

Concentrations were assigned for pesticides present in the SCIEX iD*Quant*™ standards kit for pesticide analysis.

Table 2. Pesticides identified and quantified in two EU proficiency test (EUPT) samples based on matching retention time (RT), accurate mass and isotope pattern and MS/MS library searching

Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
EUPT 1					
Acetamiprid	0.00	0.09	2.2	100.0	449
Acrinathrin	0.00	0.61	1.0	98.9	-
Buprofezin	0.01	0.32	1.1	100.0	204
Chlorpyrifos	0.00	-0.78	3.3	95.2	-
Cypermethrin	0.01	-0.27	4.9	99.2	-
Cyprodinil	0.01	-0.17	1.1	100.0	374
Diazinon	0.00	-0.20	1.7	100.0	-
Difenoconazole	0.00	0.22	1.8	100.0	1092
Fenamiphos	0.00	-1.74	1.3	99.9	-
Fenamiphos-sulfone	0.00	-0.26	1.7	100.0	-
Fenamiphos-sulfoxide	0.00	-0.94	1.3	97.1	-
Fenhexamid	0.02	0.16	0.6	100.0	871
Fludioxonil (-)	0.01	-0.69	0.8	99.6	236
lambda-Cyhalothrin	0.00	0.42	2.4	99.0	-
Methoxyfenozide	0.02	0.63	12.2	100.0	94.0
Pirimicarb	0.02	-0.37	0.3	100.0	478
Pyridaben	0.01	0.41	3.1	100.0	1063
Spinosyn A	0.01	-0.24	3.3	100.0	366
Spinosyn D	0.01	1.17	13.3	N/A	57.4
Tetraconazole	0.01	-0.36	9.3	100.0	111

Table 2. cont. (sample 2)

Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
EUPT 2					
Atrazine	0.00	0.12	7.3	100.0	
Cadusafos	0.00	-1.20	2.3	99.2	
Carbetamide	0.02	-1.02	16.3	100.0	
Demeton-S-methyl-sulfone	0.00	0.21	0.4	99.7	
Ethoprophos	0.00	-0.47	1.7	98.7	
Fenpropidin	0.00	-0.34	2.2	100.0	
Fipronil (-)	0.00	0.20	7.3	100.0	
Flubendiamide (-)	0.00	0.11	8.9	0.0	
Fluometuron	0.01	-0.03	0.9	99.9	
Fuberidazole	0.02	-0.56	1.3	99.7	
Furathiocarb	0.01	-0.31	2.3	100.0	
Metosulam	0.00	-0.42	1.7	100.0	
Prosulfocarb	0.00	-0.54	1.2	100.0	
Secbumeton	0.00	0.06	1.6	100.0	
Spiromesifen	0.01	-0.84	5.9	99.0	

(-): identified in negative polarity

Figures 5a and 5b show screenshots of the result table used for pesticide identification in proficiency test samples.

Figure 5a. Pesticides identified in proficiency test sample 1 in positive polarity based on matching retention time, accurate mass, isotope pattern and MS/MS library searching (note: Fludioxonil was identified in negative polarity)

Figure 5b. Pesticides identified in proficiency test sample 2 in positive polarity based on matching retention time, accurate mass, isotope pattern and MS/MS library searching (note: Fipronil and Flubendiamide were identified in negative polarity)

No false positive results were reported. MS/MS data and mass spectral library searching were crucial to differentiate and correctly identify structural isomers. Library searching results were reported as FIT and in all cases were above 90%.

The pesticide Flubendiamide was not present in our MS/MS libraries. Here the built-in 'Fragments Tool' of SCIEX OS was used to compare the structure of the suspected compound with the high resolution MS/MS spectrum. All measured fragment ions matched the theoretical fragmentation pathway, resulting in a tentative identification of Flubendiamide.

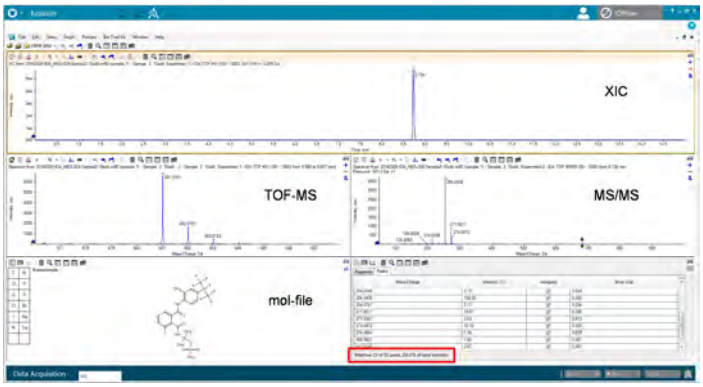

Figure 6. Tentative identification of Flubendiamide based on a comparison of the HR-MS/MS spectrum with the theoretical fragmentation pathway

Results of Store-bought Samples

Fruit and vegetable samples obtained from a local supermarket were extracted and tested for pesticide residues. Results above 10 µg/kg are listed in Table 3.

Table 3. Pesticides identified and quantified in store-bought fruit and vegetable samples based on matching retention time (RT), accurate mass and isotope pattern and MS/MS library searching

Sample / Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
Banana					
Buprofezin	0.01	0.32	3.5	100.0	341
Imazalil	0.02	0.79	15.1	91.5	565
Thiabendazole	0.01	-1.51	13.9	97.6	444
Blueberry			n.d.		
Carrot			n.d.		
Grapes					
Boscalid	0.01	-0.80	8.8	97.2	115
Buprofezin	0.01	0.22	7.3	99.6	17.3
Cyprodinil	0.01	-0.87	3.3	94.8	412
Imidacloprid	0.01	-0.58	14.6	96.1	82.5
Pyraclostrobin	0.00	-1.31	4.8	100.0	46.7
Lemon					
Imazalil	0.02	0.74	7.3	94.7	1080
Pyrimethanil	0.01	-0.77	1.0	99.2	164
Pyriproxyfen	0.01	0.43	11.4	95.3	31.6
Organic banana					
Spinosyn D	0.00	2.33	19.8	100.0	12.6
Organic strawberry					
Spinosyn A	0.01	0.55	9.1	100.0	13.9
Spinosyn D	0.01	1.63	6.0	99.4	33.3
Spinach			n.d.		
Strawberry					
Acetamiprid	0.08	-0.35	6.5	98.7	19.2
Boscalid	0.00	-0.49	4.9	99.3	161
Myclobutanil	0.00	-0.31	13.9	100.0	85.0
Pyraclostrobin	0.00	1.33	16.3	99.0	40.5
Pyrimethanil	0.00	0.32	4.7	97.3	391
Tomato *(n.d.)*			n.d.		

n.d.: no pesticide detected

Summary

A new method to identify and quantify pesticide residues in food samples was developed using the SCIEX X500R QTOF system. Qualitative and quantitative data processing was performed in SCIEX OS software.

The method was successfully applied to EU proficiency test samples and store-bought fruit and vegetable samples. Samples were extracted using a QuEChERS procedure and analyzed using LC-HR-MS/MS. Limits of quantitation of 10 µg/kg were achieved for all compounds after 10x dilution the extracts to minimize possible matrix effects.

Pesticides were automatically identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching using SCIEX OS software. In the same data processing step compounds were quantified and unknown samples were flagged when a user-defined reporting level was exceeded.

Acknowledgement

The authors thank Amadeo Fernandez-Alba (EURL) Almeria, Spain for providing EUPT samples.

References

1 EU Commission Decision ‘concerning the performance of analytical methods and the interpretation of results’ #2002/657/EC

2 EU Commission Guidance Document: ‘on analytical quality control and method validation procedures for pesticides residues analysis in food and feed’ #SANTE/11945/2015

3 André Schreiber et al.: ‘Using the X500R QTOF System and SCIEX OS Software to Quickly Identify Unknowns in Food Samples’ Application Note SCIEX (2016) # RUO-MKT-02-3761-A

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Perfect Balance to Elevate your Lab's Performance

Using the X500R QTOF System and SCIEX OS Software to Quickly Identify Unknowns in Food Samples

André Schreiber[1], Yuji Aita[2], and Jianru Stahl-Zeng[3]
[1]SCIEX Concord, Ontario (Canada), [2]SCIEX Tokyo (Japan), [3]SCIEX Darmstadt (Germany)

Overview

Here we present results using a new method to identify unexpected chemical residues and contaminants in food using the SCIEX X500R QTOF system. Samples were extracted using a QuEChERS method and analyzed by LC-HR-MS/MS.

Unknown compounds were automatically identified by using a non-target peak finding algorithm followed by sample-control-comparison to separate matrix and sample specific signals from true contaminations. TOF-MS and MS/MS data for ions of interest were automatically processed using formula finding and searched against mass spectral libraries and online databases, such as ChemSpider, for identification. The SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unexpected chemicals in food.

Introduction

Hybrid LC-MS/MS systems like quadrupole-quadrupole Time-of-Flight (QTOF) provide the ability to perform targeted and non-targeted screening in food samples on a routine basis.

The SCIEX X500R QTOF system is a robust, high performance high resolution MS/MS system designed for routine use providing:

- Sensitivity to easily detect compounds at relevant concentrations
- Resolving power to remove interference from complex food matrices
- Linearity over up to 3 orders of magnitude to identify compounds at different concentration levels
- Mass accuracy to identify compounds following regulatory guidelines
- Confident identification using MS/MS spectra and ion ratios
- Industry leading robustness of Turbo V™ source and Curtain Gas™ interface

Full scan chromatograms are very rich in information and easily contain thousands of ions from any chemical present in the sample, including the food matrix itself. Powerful software is needed to explore the high resolution MS/MS spectra generated to get answers and results from these complex data.

The SCIEX OS software is a single platform for MS control, data processing and reporting, and provides:

- Simple software workflows that deliver reliable results
- Automated identification of unknowns
- Quick data review and reporting utilizing customizable flagging and filtering of results

Experimental

Sample preparation

Food samples from a local supermarket were extracted using a QuEChERS procedure following guideline EN 15662/2007. Sample extracts were diluted 10x to minimize possible matrix effects.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AC system with a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and methanol with 5 mM ammonium formate buffer at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile).

The injection volume was 5 µL.

Table 1. Gradient conditions used for unknown screening

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	0.5	90	10
2	2.0	70	30
3	9.0	40	60
4	11.0	20	80
5	12.0	5	95
6	15.0	5	95
7	16.0	90	10
8	20.0	90	10

MS/MS Detection

The SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI) was used.

Mass calibration was achieved using the integrated calibrant delivery system (CDS) with the TwinSprayer probe (dual ESI needle).

High resolution data were acquired using an IDA method consisting of a TOF-MS survey (100-1000 Da for 100 msec) and up to 20 dependent MS/MS scans (50-1000 Da for 35 msec). MS/MS fragmentation was achieved using CE of 35 V with a collision energy spread (CES) of ±15 V.

Dynamic background subtraction (DBS) was activated for best MS/MS coverage, and no inclusion list was used to also allow retrospective unknown identification without the need for a second injection to acquire MS/MS data.

Data Acquisition and Processing

All data were acquired and processed using SCIEX OS software version 1.0, which showcases a thoughtfully designed user interface that is fast to learn and delivers improved lab productivity.

Results and Discussion

X500R Performance Characteristics

Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm are often sufficient to separate the analytes of interest from interfering matrices and, thus, are identified as the set requirements for compound identification in various guidelines.[1, 2]

The X500R QTOF system utilizes N-optics design to maximize resolution while maintaining benchtop design and a minimized footprint (Figure 1). Its resolving power increases with mass range providing ~30000 to 40000 for the typical molecular weight range of pesticides.[3]

The 4 mm orifice leading into the TOF accelerator delivers resolution without compromise in sensitivity. The sensitivity of the X500R QTOF system is comparable to a SCIEX QTRAP® 5500 system operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 µg/kg levels.[3]

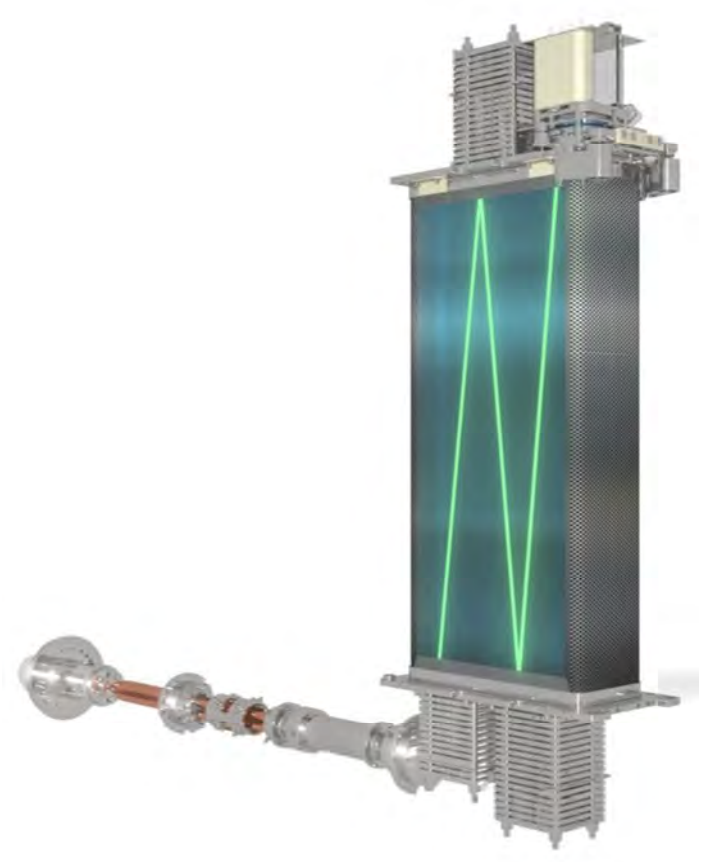

Figure 1. N-optics design of the X500R QTOF system to maximize resolution while maintaining benchtop design and a minimized footprint, 6 heater drones are integrated into the TOF path to maintain mass accuracy and robustness

The X500R QTOF system achieves stable mass accuracy of less than 2 ppm by using a heated TOF configuration, with 6 heater drones throughout the TOF path to maintain mass accuracy and robustness. In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant

delivery path for reliable auto-calibration. The CDS setup maintains mass accuracy over long periods of time by automatically calibrating in batch mode (it is recommended to infuse a calibrant standard every hour or two).

Furthermore, the X500R QTOF's mass accuracy is supplemented by legendary dynamic transmission control and dynamic background calibration, introduced in 2010 with the TripleTOF® system and optimized over time.

While accurate mass measurement of the molecular ion is important for empirical formula finding, this is not the only information available. Combining all available accurate mass MS and MS/MS information is crucial to minimize the list of potential formulae. Figures 2, 3 and Table 2 illustrate that the number of formulae can be reduced from over 200 to a single match by not only using the accurate mass of the molecular ion but also including the isotope pattern and MS/MS matching in the formula-finding algorithm.

Using the combined scoring of MS and MS/MS matches, SCIEX OS lists the most likely chemical formula at the top of results table. Also, SCIEX OS downloads a ChemSpider hit count for each calculated formula which further assists in identifying the correct result (Figure 2).

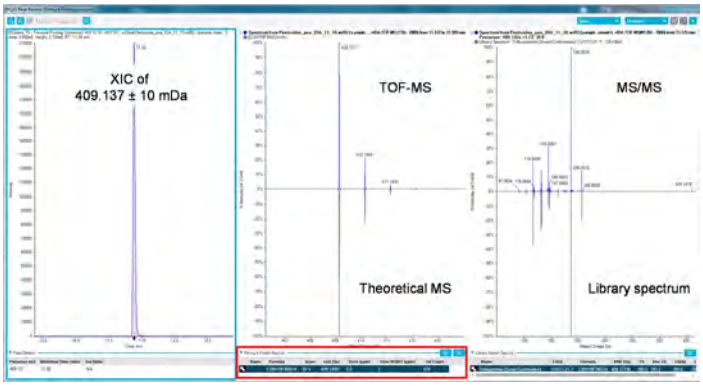

Figure 2. TOF-MS and MS/MS spectra used for empirical formula finding, results are ranked by a combined score using MS and MS/MS information, and when combined with the ChemSpider hit count, can be used to quickly find the correct match

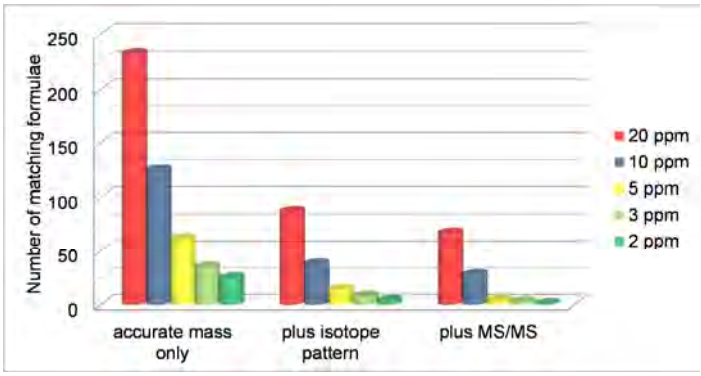

Figure 3. Number of matching molecular formulae depending on the information and mass accuracy used for empirical formula finding (elements allowed $C_{49}H_{75}Br_3Cl_5F_3I_3N_{10}O_{10}PS_3$)

Table 2. Ranking of matching formulae using MS and MS/MS information collected for Trifloxystrobin, the MS rank combines mass accuracy and isotope pattern matching and the MS/MS rank combines mass accuracy and number of ions (n)

Hit	Formula	MS Rank	ppm	MS/MS Rank	ppm (n=11)
1	$C_{20}H_{19}F_3N_2O_4$	2	0.3	2	2.0
2	$C_{21}H_{15}F_3N_6$	9	-2.9	4	3.0
3	$C_{18}H_{16}N_8O_4$	4	0.9	6	4.8
4	$C_{15}H_{17}FN_8O_5$	11	-1.9	5	4.8
5	$C_{16}H_{13}FN_{12}O$	7	-5.2	10	9.0
6	$C_{14}H_{20}F_3N_6O_3P$	22	2.8	1	2.0
7	$C_{16}H_{21}N_6O_5P$	7	-3.1	11	9.4
8	$C_{23}H_{18}F_2N_2O_3$	9	3.1	14	9.4
9	$C_{21}H_{23}F_2O_4P$	1	-0.9	24	22.1
10	$C_{19}H_{21}FN_2O_7$	16	-8.4	12	9.4

In addition to more efficient formula finding, MS/MS spectra are also needed for structural elucidation. Without MS/MS spectra it is impossible to conclude a correct structure from a molecular formula alone.

The example shown in Figure 4 highlights the need of fragment ion detection to confidently differentiate between isomers Prometon and Terbumeton.

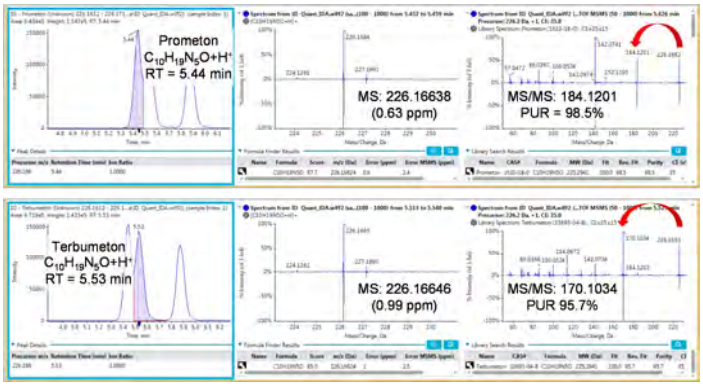

Figure 4. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

Processing Workflow for Unknown Identification in SCIEX OS Software

Extracted Ion Chromatograms (XIC) are generated using a non-target peak finding algorithm. No masses or retention times are provided to find chromatographic features. Sample-control-comparison is used to separate matrix and sample-specific signals from true contaminations.

High resolution TOF-MS and MS/MS data of ions of interest are automatically processed using:

- MS/MS library searching to identify compounds already present in existing libraries
- Empirical formula finding based on TOF-MS and MS/MS
- ChemSpider searching
- Comparison of structures retrieved from ChemSpider against the acquired HR-MS/MS spectra

The method editor in SCIEX OS software to setup parameters and criteria for unknown identification is shown in Figure 5a-c.

Figure 5a. Method editor in SCIEX OS software for unknown identification, selection of sample and control-sample for non-target peak finding

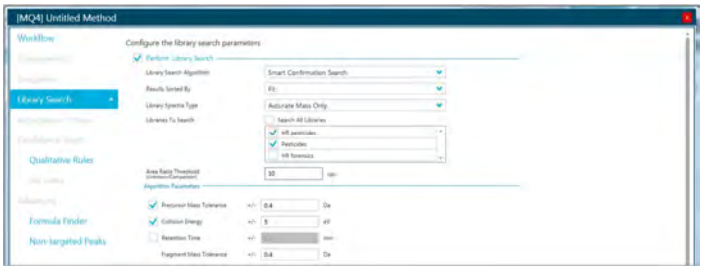

Figure 5b. Method editor in SCIEX OS software for unknown identification, configuration of library search parameters

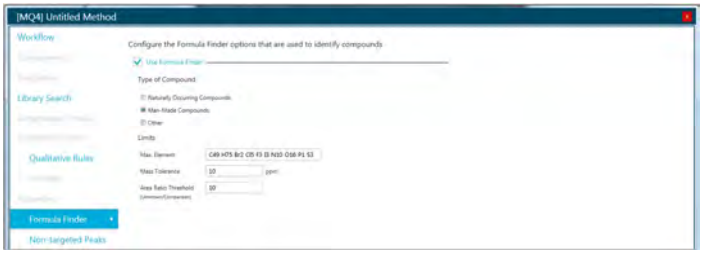

Figure 5b. Method editor in SCIEX OS software for unknown identification, configuration of formula finding options

SCIEX offers true HR-MS/MS spectral libraries for over 2500 compounds, including pesticides, veterinary drugs, toxins, fluorochemicals, pharmaceuticals, and illicit drugs.

Results of Unknown Identification

Two samples of bell pepper, including an organic pepper, were extracted and analyzed using the developed LC-HR-MS/MS method in positive and negative polarity. Both samples were processed using the described non-target workflow.

A total of 2358 (positive polarity) and 1563 (negative polarity) chromatographic features were identified using the non-target peak finding algorithm. Less than 50 features were found to be characteristic for the contaminated bell pepper after sample-control-comparison using an area ratio of 10.

Results can be sorted and filtered for easy data review after performing sample-control-comparison. Library searching and formula finding results and scores are listed in the result table. More details and a visual display of XIC, TOF-MS and MS/MS for both samples can be found in peak review (Figure 6).

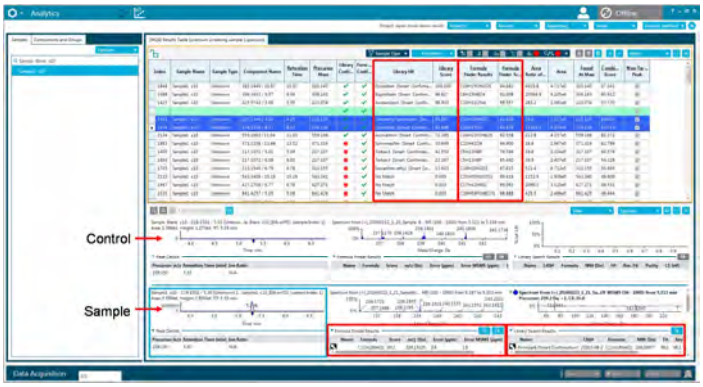

Figure 6. Results display after non-target screening, library searching and formula finding results are displayed in the table (top) and chromatograms and spectra with result details can be reviewed (bottom)

Formula finding results are displayed below the TOF-MS spectrum in the peak review window. Results are automatically ranked by mass accuracy (MS and MS/MS) and the matching of the isotope pattern. In addition the ChemSpider hit count is listed to quickly identify the correct match. The formulae can be searched against ChemSpider. Structural information from ChemSpider will be automatically compared against the acquired MS/MS spectrum to provide feedback for a quick identification.

Examples of tentatively identified pesticides in the bell pepper sample are shown in Figures 7, 8 and 9.

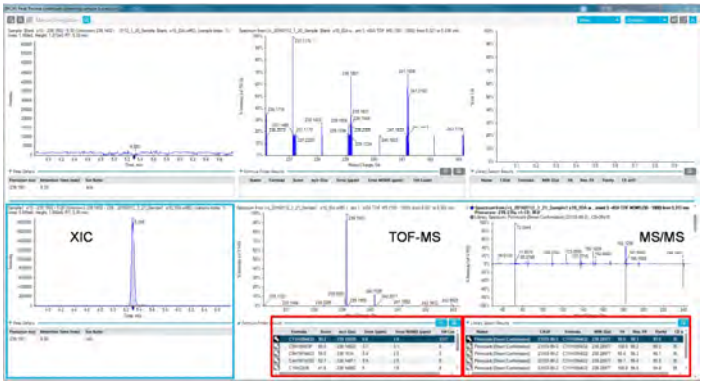

Figure 7a. Review of XIC of m/z 239.150 at RT 5.3 min and spectra with a found formula of $C_{11}H_{18}N_4O_2$

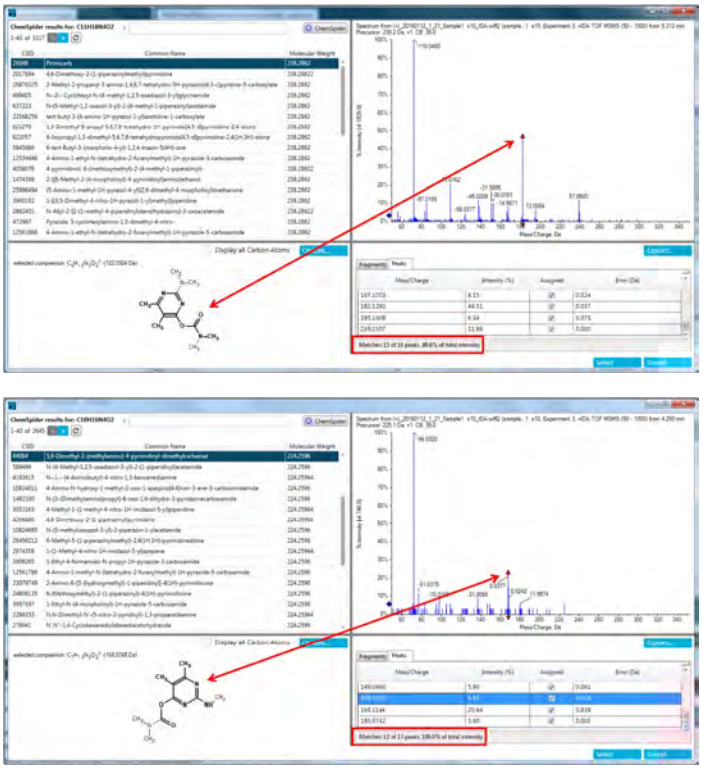

Figure 7b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Pirimicarb (top) and also of its metabolite Desmethyl-pirimicarb (bottom), both compounds were confirmed by MS/MS library searching

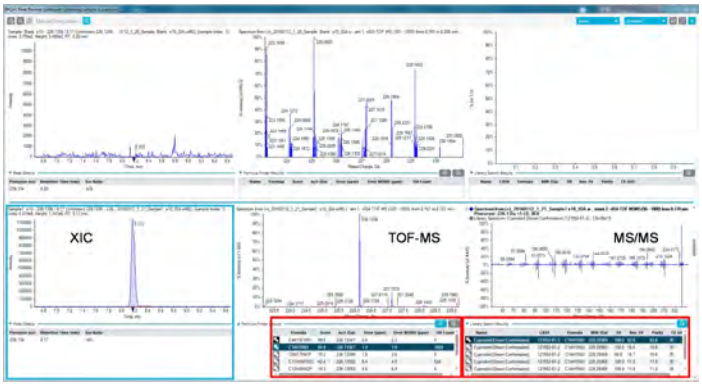

Figure 8a. Review of XIC of m/z 226.134 at RT 8.2 min and spectra with a found formula of $C_{14}H_{15}N_3$, although ranked second based on mass accuracy the high ChemSpider hit count revealed the correct match

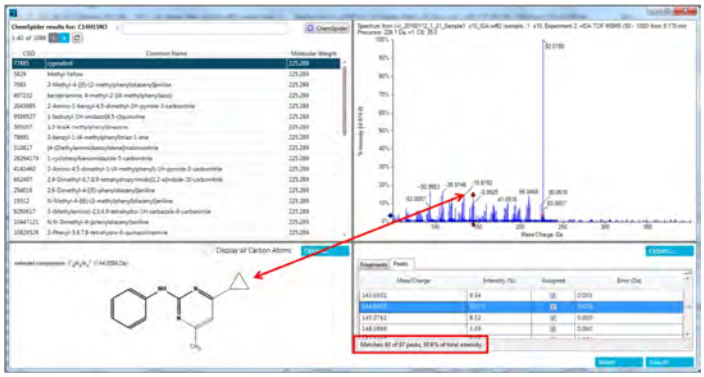

Figure 8b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Cyprodinil, this compound was confirmed by MS/MS library searching

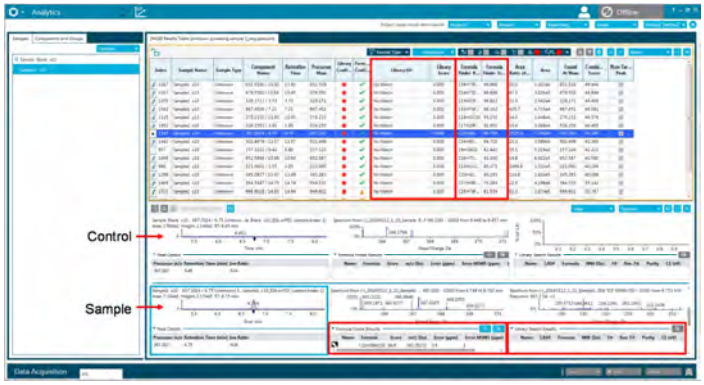

Figure 9a. Results display after non-target screening of the negative polarity data, review of XIC of m/z 367.203 at RT 6.7 min and spectra with a found formula of $C_{22}H_{28}N_2O_3$

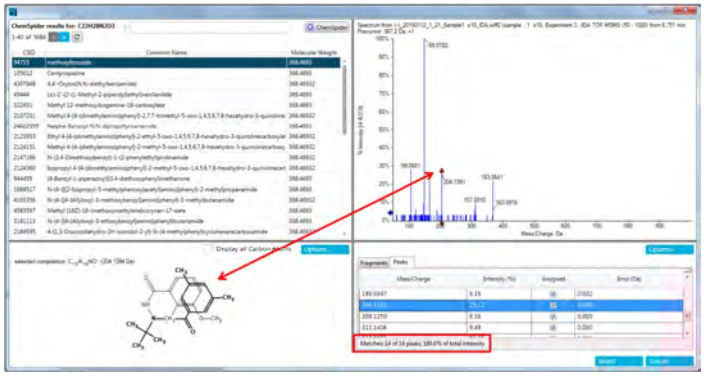

Figure 9b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Methoxyfenozide

Summary

A new method to identify unexpected chemical residues and contaminants in food samples was developed using the SCIEX X500R QTOF system. Store-bought food samples were extracted using a QuEChERS procedure and analyzed by LC-HR-MS/MS.

Data processing was performed in SCIEX OS software. The processing workflow consists of peak finding using a non-target algorithm (no masses or retention times were provided to find chromatographic features). Automatic sample-control-comparison was used to separate matrix and sample specific signals from true contaminations. In a final step, tools such as empirical formula finding, MS/MS library searching and online database searching was used for identification.

The method was successfully applied to tentatively identify pesticide residues in vegetable samples.

References

1 EU Commission Decision ‘concerning the performance of analytical methods and the interpretation of results’ #2002/657/EC

2 EU Commission Guidance Document: ‘on analytical quality control and method validation procedures for pesticides residues analysis in food and feed’ #SANTE/11945/2015

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X500R QTOF WATER TESTING

Environmental Method

Pesticide analysis in water

Elevate your environmental testing with the X500R QTOF System

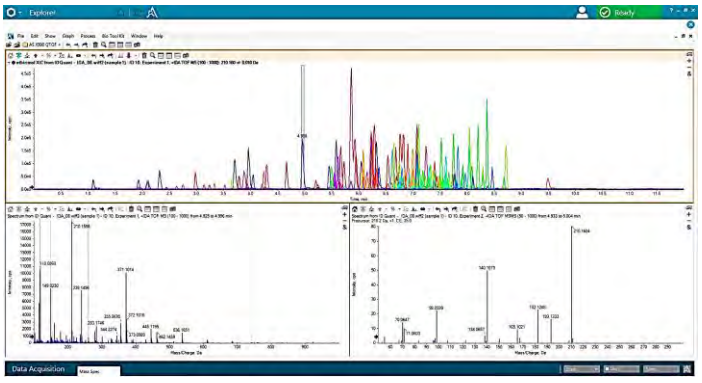
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pesticides in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1

- Direct aqueous injection

SCIEX OS can deliver faster method set-up

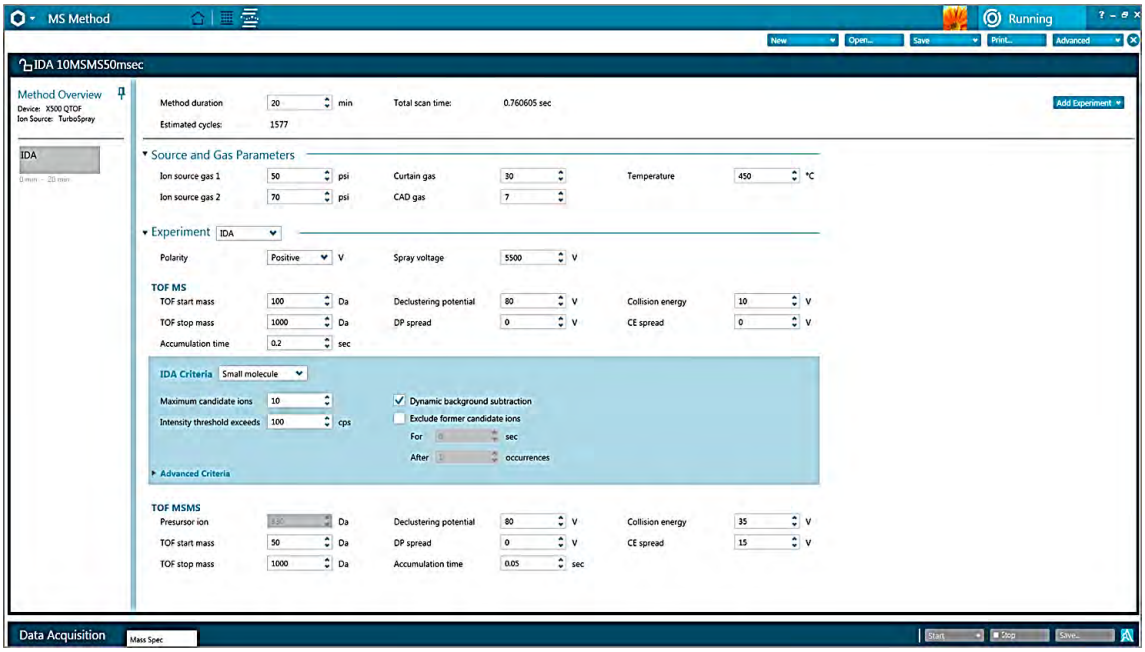


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Phenomenex Kinetex Biphenyl, 100 x 2.1 mm, 2.6 um	
Mobile Phase A	*5 mM ammonium formate in water*	
Mobile Phase B	*5 mM ammonium formate in methanol*	
Flow rate	*0.5 mL/min*	
Column temperature	*30 ℃*	
Injection volume	*100 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*0*
	10	*90*
	13	*90*
	13.1	*10*
	15	*10*

MS Method



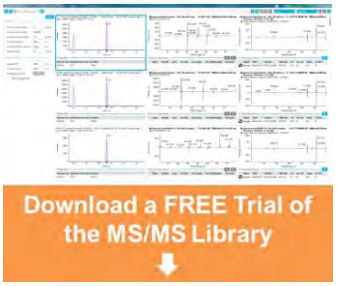
Suggested IDA (Information Dependent Acquisition) conditions for routine environmental testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of pesticides compounds including molecular formula and accurate mass.



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PPCP analysis in water

Elevate your environmental testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pharmaceuticals and personal care products in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

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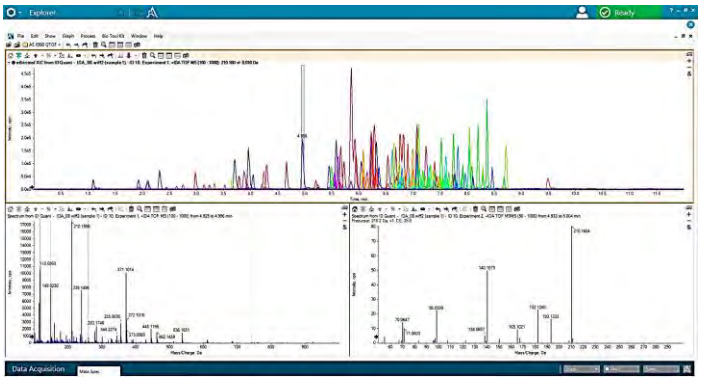
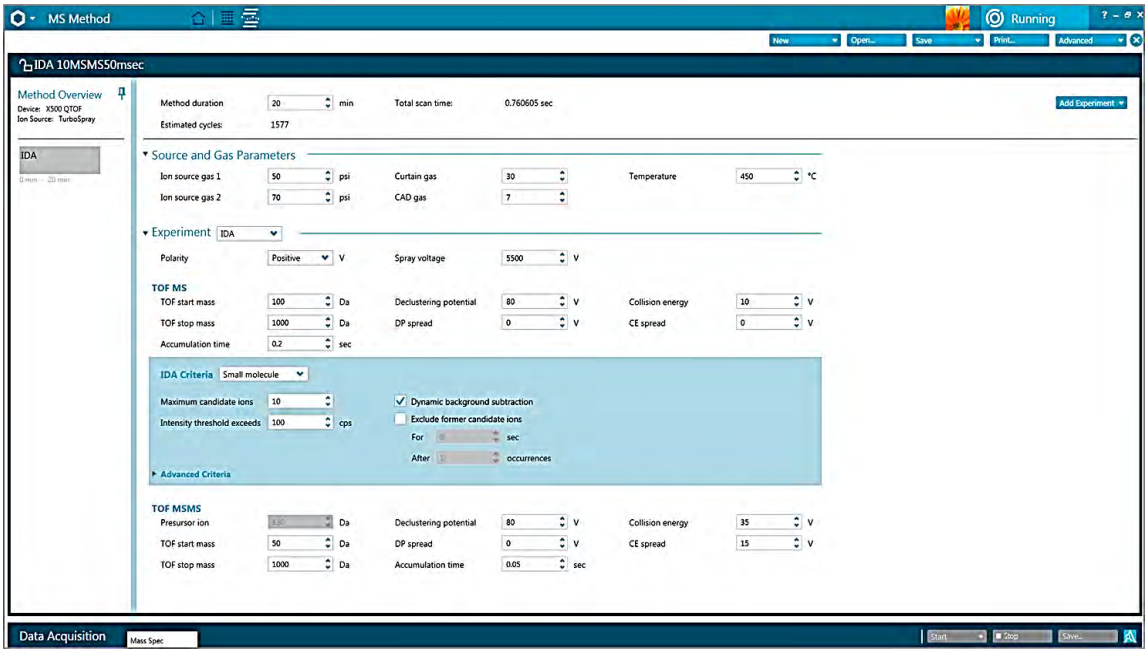


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Phenomenex Kinetex Biphenyl, 100 x 2.1 mm, 2.6 µm column	
Mobile Phase A	*0.1% formic acid in water*	
Mobile Phase B	*0.1% formic acid in methanol*	
Flow rate	*0.6 mL/min*	
Column temperature	*30 ℃*	
Injection volume	*100 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*2*
	1	*2*
	7	*65*
	7.1	*100*
	9	*100*
	9.1	*2*
	12	*2*

MS Method

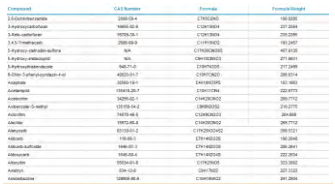


Suggested IDA (Information Dependent Acquisition) conditions for routine environmental testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries



Download the XIC List

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Illicit drugs analysis in water

Elevate your environmental testing with the X500R QTOF System

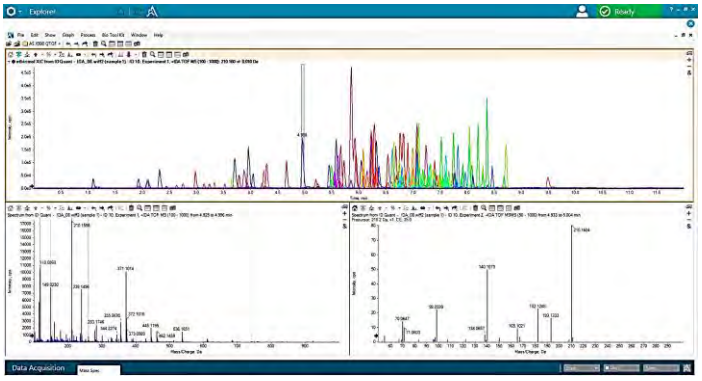
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm illicit drugs in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1
- Samples acidified to pH 2 using HCL and stored in the dark at 4 C until required

Step 2
- Transfer 1mL of sample into an analytical vial

SCIEX OS can deliver faster method set-up

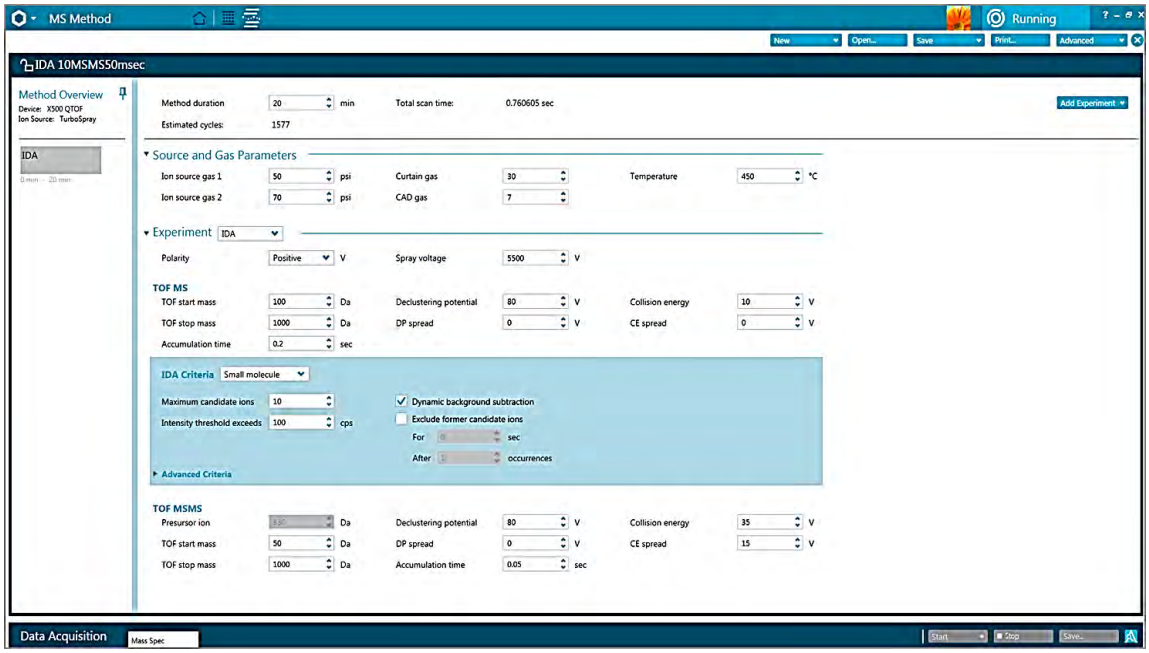


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Phenomenex Kinetex C18, 100 x 4.6 mm, 5 um column	
Mobile Phase A	*0.1% formic acid in water + 2mM ammonium formate*	
Mobile Phase B	*Acetonitrile*	
Flow rate	*0.9 mL/min*	
Column temperature	*30 ℃*	
Injection volume	*100 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*2*
	1	*2*
	7	*65*
	7.1	*100*
	9	*100*
	9.1	*2*
	12	*2*

MS Method

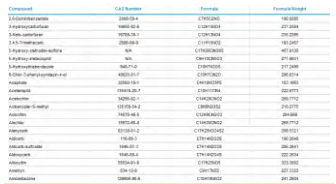


Suggested IDA (Information Dependent Acquisition) conditions for routine environmental testing as displayed in SCIEX OS.

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries



Download the XIC List

Download a free XIC compound list detailing a full list of illicit drug compounds including molecular formula and accurate mass.

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X500R QTOF FORENSIC ANALYSIS

Forensic Identification and Quantification Workflows Delivered on a Revolutionary Designed QTOF and SCIEX OS Software

Igniting your routine forensic testing with the new SCIEX X500R QTOF System

Xiang He[1] and Adrian Taylor,[2]

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Overview

Quadrupole Time-of-Flight (QTOF) mass spectrometry is becoming the desired technology for sensitive and selective screening workflows in a forensic toxicological setting. The technology overcomes many challenges faced when using traditional techniques and more significantly captures all information about the sample in one injection to allow for retrospectively mining the data. Using the accurate mass and mass resolution information from both TOF-MS and TOF-MS/MS acquired data allows for simultaneous highly specific targeted quantitation and non-targeted screening. Here we describe a new benchtop QTOF system with revolutionary N geometry TOF designed flight path and new, intuitive software for easy adoption of accurate mass technology to forensic testing. We demonstrate that the new hardware and software combined allow a high level of confidence for compound identification and quantification from urine samples in one seamless workflow.

Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of compounds and metabolites. Triple quadrupole based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode have become the preferred method to routinely deliver highly selective and sensitive quantitative results, but are limited to targeted screening only.

With an increasing demand for retrospective and non-targeted analyses of forensic toxicological samples, high resolution, accurate mass and full scan mass analyzers are gaining popularity. The adoption of the technology has been restricted by more complicated to use and more expensive instrumentation compared to their nominal mass counterparts. Here we introduce a revolutionary new Quadrupole Time-of-Flight (QTOF) mass spectrometer that contains advances in engineering design to bring the high performance TOF-MS and TOF-MS/MS capabilities into a compact benchtop platform.

Figure 1: The SCIEX ExionLC™ AC HPLC system (left), the SCIEX X500R QTOF System (middle) and SCIEX OS Software (right).

The SCIEX X500R QTOF mass spectrometer is part of a complete workflow from the fully integrated SCIEX ExionLC™ Systems to the freshly designed SCIEX OS software; a new user interface for simultaneous identification and quantification workflows (Figure 1.)

SCIEX X500R QTOF System

The new benchtop SCIEX X500R QTOF System with revolutionary N geometry TOF designed flight path has been engineered for simplicity, service accessibility and minimized footprint. N TOF geometry, versus V geometry, gives the same effective flight path length for ions and therefore resolution, but in a smaller overall foot print. This has been accomplished with an extra mirror in the TOF chamber without a loss in transmission (Figure 2). To maintain stable mass accuracy the system uses a simple heated TOF vacuum chamber design. This consists of 6 discreet heater drones maintaining a constant TOF chamber temperature, insulating against ambient temperature changes (Figure 2).

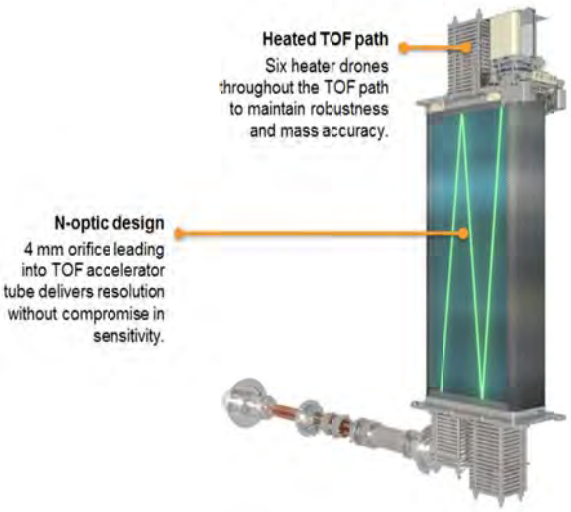

Figure 2. SCIEX X500R QTOF System and Technology Advances

The system has been designed to maximize robustness and uptime

- Integrated Calibrant Delivery System and Turbo V™ Source with TwinSpray probe (Figure 3), allows seamless mass accuracy auto-calibrations during long runs.
- Service Accessibility
  - Easy QJet® and Turbo pump access for fast and efficient maintenance, increasing system uptime
  - Segmented TOF vacuum chamber – allows easy access to detector while protecting sensitive accelerator.

Figure 3. Integrated Calibrant Delivery System and Turbo V™ Source with TwinSpray probe

Figure 4 shows the mass accuracy stability of the SCIEX X500R QTOF System when analyzing multiple urine samples, spiked with various concentrations of analytes, without auto-calibration, over a ten hour period. The majority of compounds are shown to be within a 1 ppm mass accuracy over this time period.

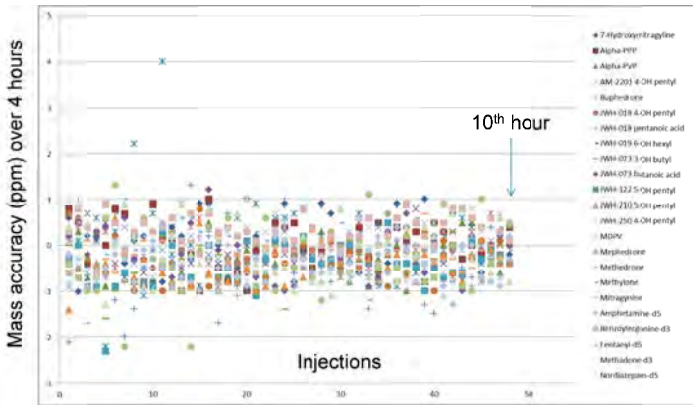

Figure 4. Mass Accuracy Stability of the SCIEX X500R QTOF System in the Analysis of Urine Samples

Figure 5 shows the resolution for both TOF-MS and TOF-MS/MS masses sampled over a seven day time period on a SCIEX X500R QTOF System.

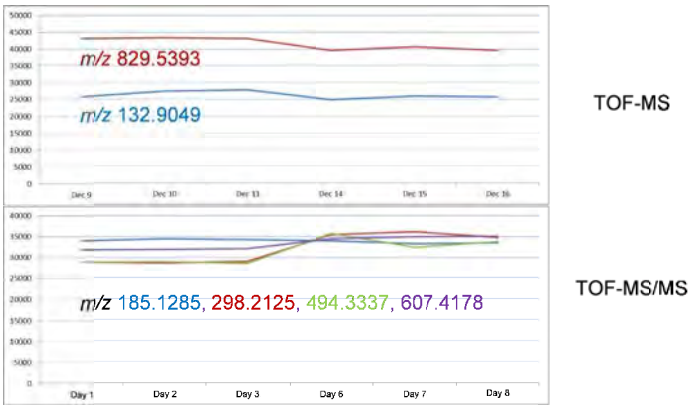

Figure 5. Resolution of the SCIEX X500R QTOF System Over a Week's Period for Selected *m/z*; both TOF-MS and TOF-MS/MS

Figure 6 shows a representative linear dynamic range of the SCIEX X500R QTOF System showing 4 orders for the Asenapine compound.

SCIEX OS Software

SCIEX OS Software is a single software platform for LC and MS control, data processing as well as reporting.

Figure 6. Linearity of the SCIEX X500R QTOF System shown for Asenapine (0.5 ng/mL to 1000 ng/mL)

The SCIEX OS software is intuitive and logical, segregated into Acquisition, Processing and Management work spaces (Figure 7). In the Acquisition work space there are separate method editors for the LC and MS parameters as well as batch creation and queue panes. The Processing allows for simultaneous identification and quantification. The Management workspace allows the adjustment of hardware, software and user settings.

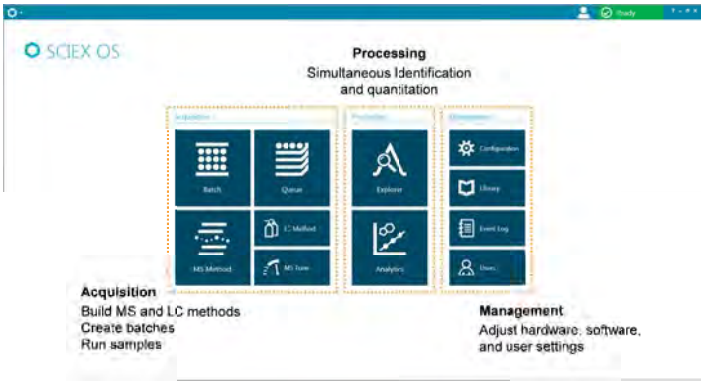

Figure 7. Home Page of SCIEX OS Software. Single Software Platform for LC/MS Control, Data Processing and Reporting.

Acquisition

The SCIEX OS software has a simplified step by step acquisition method setup with only relevant parameters being visible. Figure 8 shows the setup for an Information Dependent Acquisition method for the analysis of small molecules and the intuitive steps that are taken to input the MS parameter values.

For a quick instrument status check, the Manual Tune guides the user through the steps to perform a quick review of the system performance, perform an auto-calibration and report out the test result prior to running a batch (Figure 9).

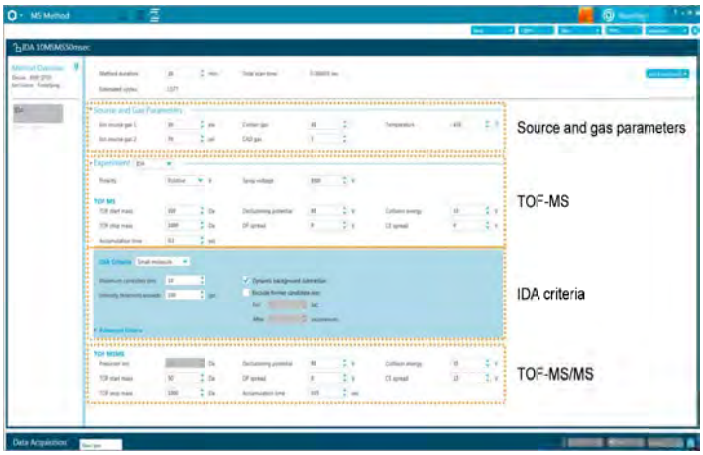

Figure 8. SCIEX OS Software MS Acquisition Method

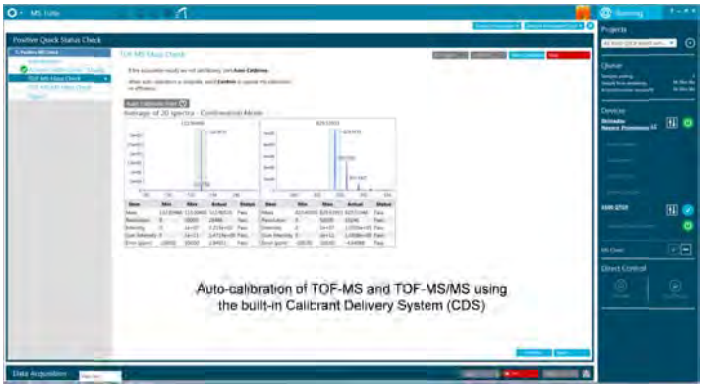

Figure 9. SCIEX OS Software MS Tune Allows for Quick Instrument Status Check via Simple Step by Step Instructions

Building a batch is assisted by the smart grid design allowing copy/paste, fill down, auto increment and import/export. Figure 10 shows the batch editor and the link to the auto-calibration setup.

Once the batch has been submitted to the queue the Auto-Cal samples are inserted as shown in the Queue Manager in Figure 11. The SCIEX OS software allows for detailed instrument status including monitoring and recording of LC pressure traces as well as direct control of the individual components of the system (Figure 11).

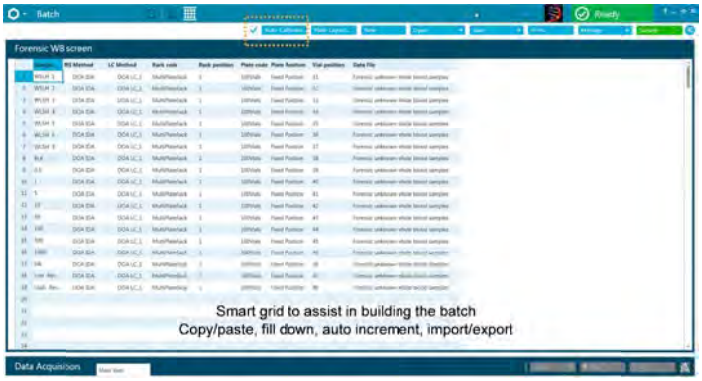

Figure 10. SCIEX OS Software Batch Editor and Setup for Auto-Calibration

Figure 11. SCIEX 0S Software Queue Manager with Inserted Auto-Cal Samples and Detailed Instrument Status Panel

Processing- Analytics

Once a results table is generated, quantitative and qualitative results can be reviewed in the same panel (Figure 12). A Traffic light system indicates the confidence of the identification based on accurate mass, retention time, isotopic pattern and library matching. Compounds calculated to be above the cutoff concentration in unknown samples are flagged. In the same work space the peak integration, spectra and calibration lines can be displayed.

Figure 12. SCIEX OS Software Allows the Simultaneous Review of Qualitative and Quantitative Results

The SCIEX OS Software allows the user to filter the results to only show compounds that pass acceptance criteria and are detected with user defined confidence (Figure 13)

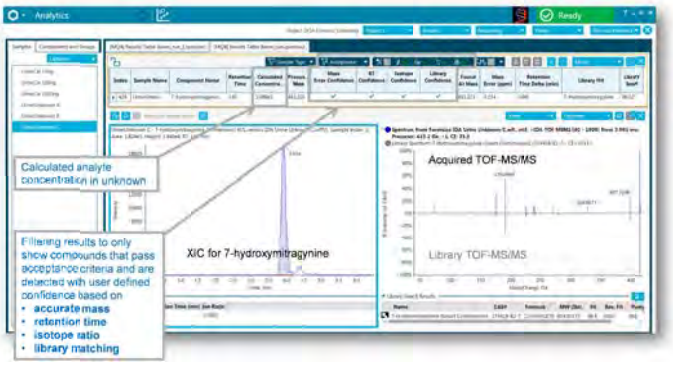

Figure 13. SCIEX OS Software Filtering Criteria

Finally results can be reported out using the *Create Report* functionality (Figure 14)

Figure 14. SCIEX OS Software Report Generation

Acquisition Workflows on the SCIEX X500R QTOF System with SCIEX OS Software

Information Dependent Acquisition

Information Dependent Acquisition (IDA) is a non-targeted data acquisition (Figure 15). It allows for TOF-MS quantification and provides high confidence in screening with MS/MS information that uses high selectivity through unit Q1 resolution. IDA-MS/MS provides the most interference-free fragmentation information.

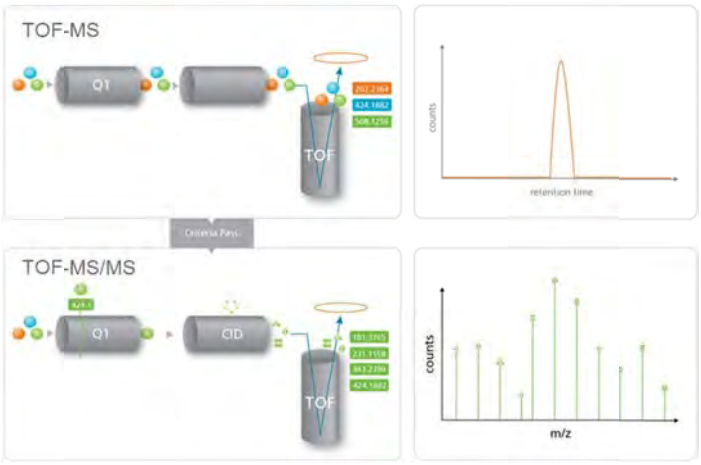

Figure 15. Information Dependent Acquisition

When creating an IDA acquisition, MS and MS/MS settings are all contained in a single User Interface. Figure 16 shows the parameters used in the IDA experiments described in this technical note. In this example, one TOF-MS survey scan and up to 16 dependent TOF-MS/MS scans are triggered from the survey scan, in each data cycle.

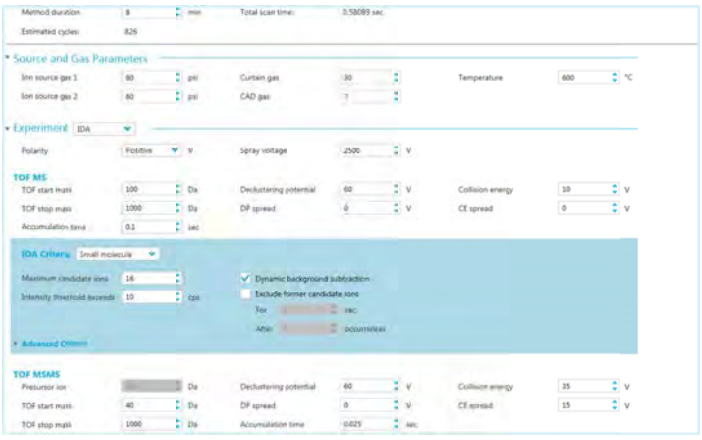

Figure 16. SCIEX OS Software Information Dependent Acquisition Method Editor

Due to the high scanning speed (up to 100 Hz for single collision energy) on SCIEX X500R QTOF systems, almost all potential compound targets in the samples can be confirmed with confident MS/MS library matching.

IDA-MS/MS is a non-targeted data acquisition method and the user needs to define the maximum number of candidates in each data cycle. More intense ions take higher priority within any data cycle, so for less abundant species especially in complex sample matrices, the associated MS/MS information might be missed. Therefore, an unbiased MS/MS data acquisition approach that collects MS/MS information for everything at all times (MS/MS^{All}) will solve this potential concern.

SWATH® Acquisition

SWATH® acquisition (Figure 17) is non-targeted and provides MS/MS information for everything in the sample, all the time. Each scan cycle in SWATH® Acquisition starts with a TOF-MS experiment. The acquisition approach therefore allows for screening and quantification from both TOF-MS and TOF-MS/MS acquired data.

Most of the existing MS/MS^{All} techniques collect MS and MS/MS information for all ions in an alternating fashion, i.e. MS scan of all precursor ions, followed by MS/MS scan of the fragments of all precursor ions. Without precursor ion selection, such approaches suffer from insufficient sensitivity, selectivity and narrower linear range compared to IDA-MS/MS.

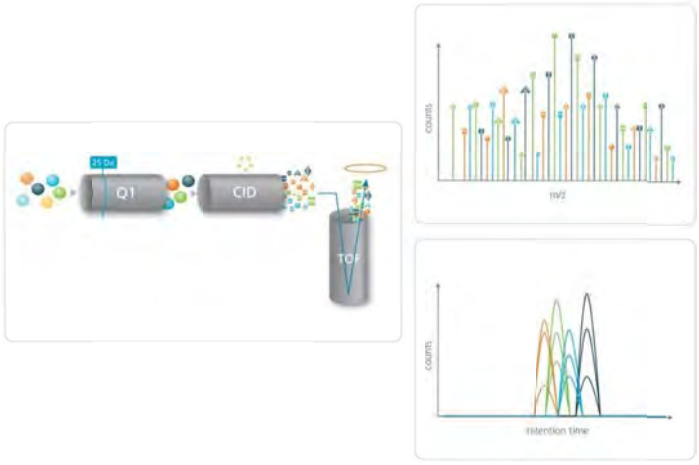

Figure 17. SWATH® Acquisition

SWATH® acquisition uses either a fixed or a variable Q1 isolation window, as part of a TOF-MS/MS experiment, which is stepped across the mass range of interest. Figure 18 shows the SWATH® acquisition method editor in the SCIEX OS Software, with the example of 16 looped TOF-MS/MS experiments, each with a different (variable) Q1 isolation window, that are required to cover the mass range of interest (120 to 500 *m/z*).

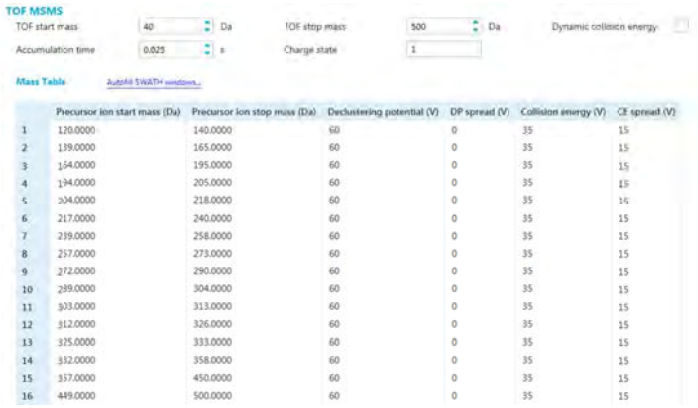

Figure 18. SCIEX OS Software SWATH® Acquisition Method Editor

By varying the Q1 isolation window for each TOF-MS/MS experiment we are able to separate compounds with similar mass into different SWATH® Acquisition windows so that we minimize the amount of convolution (multiple precursor ions generating common fragment ions at the same time) in each TOF-MS/MS experiment (Figure 19).

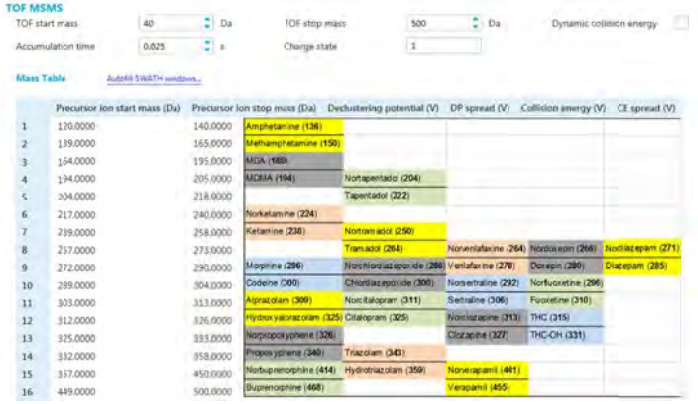

Figure 19. Constructing Variable SWATH® Acquisition Window Sizes for each Looped TOF-MS/MS Experiment to Minimize Convolution in the SCIEX OS Software

MRM^HR

MRM^{HR} (High Resolution Multiple Reaction Monitoring) is a targeted data acquisition for quantification purposes and can be unscheduled or scheduled. Compound dependent parameters can be optimized for each MRM^{HR}.

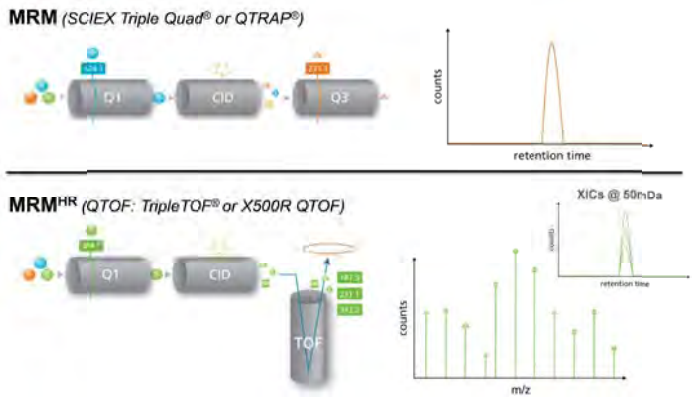

Figure 20. Comparison of MRM^{HR} with traditional (unit resolution) MRM

To help transition familiarity of MRM performed on a triple quadrupole to MRM^{HR} performed on the SCIEX X500R QTOF system, the SCIEX OS Software has a unique way of building the MRM^{HR} method to have the look and feel of performing traditional MRM experiments by allowing the input of the precursor ion mass (MRM Q1 equivalent mass) and accurate fragment mass (MRM Q3 equivalent nominal mass) (Figure 21). These transitions can easily be imported from the SCIEX high resolution 1700 compound MS/MS forensic spectral library to include up to 5 transitions per compound.

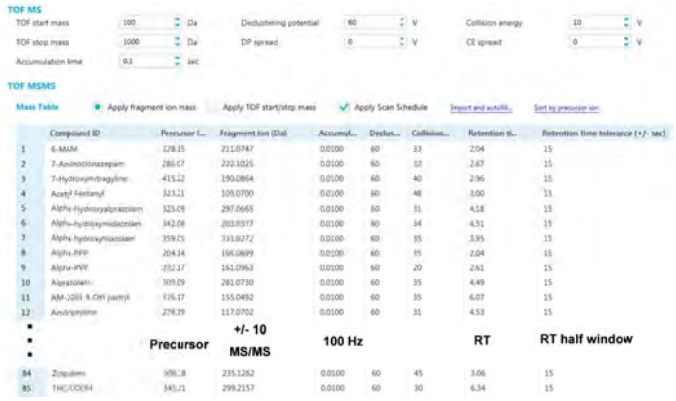

Figure 21. SCIEX OS Software *Scheduled* MRM^{HR} Method Editor, Fragment Ion Mass ± 10 *m/z*

The quantification method is then generated automatically from the acquisition method (Figure 22).

Figure 22. Automatically generating the SCIEX OS Software MRM^{HR} Quantification Method from the SCIEX OS Software MRM^{HR} Acquisition Method

Alternatively, if the fragment masses are not known at the time of the acquisition method creation, then the traditional MRM^{HR} setup is still achievable by inputting the TOF start and stop masses (Figure 23).

Figure 23. *Scheduled* MRM^{HR} Method Editor, MS/MS Full Scan

Materials and Methods

Compound list and spiking solutions

Table 1 lists the 93 compounds plus internal standards. All were procured from Cerilliant Corporation (Round Rock, TX). Two spiking solutions in methanol were prepared: one for analytes (**SA**) and the other for internal standards (**SIS**). Concentrations of all the analytes in the spiking solution **SA** are listed in Table 1.

Compounds in black font are in the regular panel (72 analytes) and the ones in blue font are the additional 21 analytes in the extended panel (93 analytes). Internal standards are shown in grey background.

Calibrator preparation

Blank human urine was spiked with solution **SA** to prepare calibrators. Four levels of calibrators were prepared. Actual concentrations varied for each compound, however the concentration ratio between these calibrators was always (in descending order): 20:6:2:1. For instance, the four different concentrations (in descending order) for fentanyl in the calibrators were: 20, 6, 2 and 1 ng/mL, while those of gabapentin were: 1000, 300, 100 and 50 ng/mL.

Sample preparation

1. 100 µL urine sample was mixed with 25 µL IMCS Rapid Hydrolysis Buffer, 20 µL IMCSzyme and 10 µL **SIS**. Both IMCS Rapid Hydrolysis Buffer and IMCSzyme were acquired from IMCS (Columbia, SC). Hydrolysis time was typically between 30 and 60 min at 55°C.
2. After hydrolysis was complete, 0.2 mL methanol and 0.625 mL water were added to the mixture.
3. The mixture was then centrifuged at 21,000 *g* for 10 min.
4. The supernatant was transferred to a glass vial with insert for analysis by LC-MS/MS.

Liquid Chromatography

Liquid Chromatography analysis was performed on the SCIEX ExionLC™ AC HPLC system at 30°C. Separation was achieved using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6 µm, 00B-4495-E0), with a Phenomenex SecurityGuard ULTRA UHPLC Phenyl (AJ0-8774) and ULTRA holder (AJ0-9000). Mobile phase A (MPA) was ammonium formate in water. Mobile phase B (MPB) was formic acid in methanol. The LC flow rate was 0.5 mL/min and the LC run-times investigated were 8.0 and 2.0 minutes. Injection volume was 10 µL.

Table 1: List of analytes and internal standards, and their concentrations in spiking solution (for preparation of calibrators)

Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)
6-MAM	*1000*	*Gabapentin*	*10000*	*Naloxone*	*5000*	*Pentobarbital*	*10000*
7-Aminoclonazepam	*5000*	*Hydrocodone*	*5000*	*Naltrexone*	*5000*	*Secobarbital*	*10000*
7-Hydroxymitragynine	*1000*	*Hydromorphone*	*5000*	*N-desmethyltapentadol*	*5000*	*THC-COOH*	*2000*
Acetyl Fentanyl	*200*	*Imipramine*	*5000*	*Norbuprenorphine*	*2000*	*6-MAM-d3*	
Alpha-Hydroxyalprazolam	*5000*	*JWH 122 5-OH pentyl*	*1000*	*Norcodeine*	*5000*	*Amphetamine-d5*	
Alpha-Hydroxymidazolam	*5000*	*JWH 19 6-OH hexyl*	*1000*	*Nordiazepam*	*5000*	*Benzoylecgonine-d3*	
Alpha-Hydroxytriazolam	*5000*	*JWH 210 5-OH-pentyl*	*1000*	*Norfentanyl*	*200*	*Buprenorphine-d4*	
Alpha-PPP	*1000*	*JWH-018 4-OH pentyl*	*1000*	*Norhydrocodone*	*5000*	*Carisoprodol-d7*	
Alpha-PVP	*1000*	*JWH-018 pentanoic acid*	*1000*	*Normeperidine*	*5000*	*Codeine-d6*	
Alprazolam	*5000*	*JWH-073 3-OH butyl*	*1000*	*Noroxycodone*	*5000*	*Fentanyl-d5*	
AM-2201 4-OH pentyl	*1000*	*JWH-073-butanoic acid*	*1000*	*Norpropoxyphene*	*10000*	*Hydrocodone-d6*	
Amitriptyline	*5000*	*JWH-250-N-4-OH pentyl*	*1000*	*Nortriptyline*	*5000*	*Hydromorphone-d6*	
Amphetamine	*10000*	*JWH-073-butanoic acid*	*1000*	*O-Desmethyltramadol*	*5000*	*JWH 018 4-OH pentyl-d5*	
Benzoylecgonine	*5000*	*JWH-250-N-4-OH pentyl*	*1000*	*Oxazepam*	*5000*	*JWH 019 6-OH hexyl-d5*	
Buphedrone	*1000*	*Lorazepam*	*5000*	*Oxycodone*	*5000*	*MDPV-d8*	
Buprenorphine	*2000*	*MDA*	*10000*	*Oxymorphone*	*5000*	*Meperidine-d4*	
Carisoprodol	*10000*	*MDEA*	*10000*	*PCP*	*2500*	*Mephedrone-d3*	
Clomipramine	*5000*	*MDMA*	*10000*	*Pregabalin*	*10000*	*Meprobamate-d7*	
Codeine	*5000*	*MDPV*	*1000*	*Propoxyphene*	*10000*	*Methadone-d3*	
Cotinine	*5000*	*Meperidine*	*5000*	*Protriptyline*	*5000*	*Methamphetamine-d5*	
Cyclobenzaprine	*5000*	*Mephedrone*	*1000*	*RCS4-4-OH-pentyl*	*1000*	*Methylone-d3*	
Desalkylflurazepam	*5000*	*Meprobamate*	*10000*	*Ritalinic Acid*	*5000*	*Mitragynine-d3*	
Desipramine	*5000*	*Methadone*	*10000*	*Sufentanil*	*200*	*Morphine-d6*	
Desmethyldoxepin	*5000*	*Methamphetamine*	*10000*	*Tapentadol*	*5000*	*Nordiazepam-d5*	
Dextromethorphan	*5000*	*Methedrone*	*1000*	*Temazepam*	*5000*	*Nortriptyline-d3*	
Diazepam	*5000*	*Methylone*	*1000*	*Tramadol*	*5000*	*Oxycodone-d6*	
Dihydrocodeine	*5000*	*Methylphenidate*	*5000*	*Zolpidem*	*5000*	*Oxymorphone-d3*	
Doxepin	*5000*	*Midazolam*	*5000*	*Amobarbital/pentobarbital*	*10000*	*THC-COOH-d3*	
EDDP	*10000*	*Mitragynine*	*1000*	*Butabarbital*	*10000*	*Butalbital-d5*	
Fentanyl	*200*	*Morphine*	*5000*	*Butalbital*	*10000*	*Secobarbital-d5*	

Grey background: IS

SCIEX OS Software Processing

Identification and Quantification Results

Defining the retention time and accurate precursor and fragment mass for each analyte is performed first (Figure 24) followed by setting up the library searching parameters.

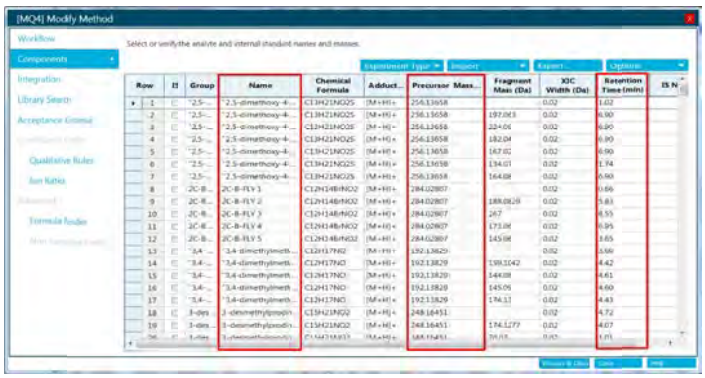

Figure 24. Defining the Retention Time, Accurate mass of Precursor and Fragment Ions

Defining the qualifying components includes setting accuracy tolerance levels for calibrants and controls as well as flagging integration discrepancies. Qualifying definitions also includes defining the identification criteria and setting the confidence levels at which mass error, error in retention time, isotope pattern and library matching scores are deemed an acceptable difference, marginal difference or unacceptable difference (Figure 25).

Figure 25. Defining the Identification and Quantification Qualifying Components in the SCIEX OS Software

Results and Discussion

As part of evaluating the new SCIEX X500R QTOF to perform simultaneous identification and quantification of compounds from forensically related samples routinely, we investigated two LC gradients. We evaluated each methods capabilities to elute all analytes throughout the entire gradient as evenly as possible in order to maximize triggering IDA MS/MS for all components, reduce the MRM^{HR} concurrency for quality of data (*Scheduled* MRM^{HR}), resolve isobaric species and alleviate ion suppression caused by co-elution of excessive number of analytes. Figure 26 shows the Extracted Ion Chromatograms (XICs) for the 8.0 minute run and Figure 27 show the XICs for the 2.0 minute run.

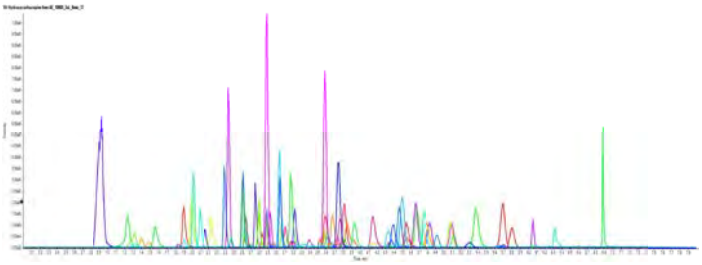

Figure 26. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 8.0 Minute LC Runtime

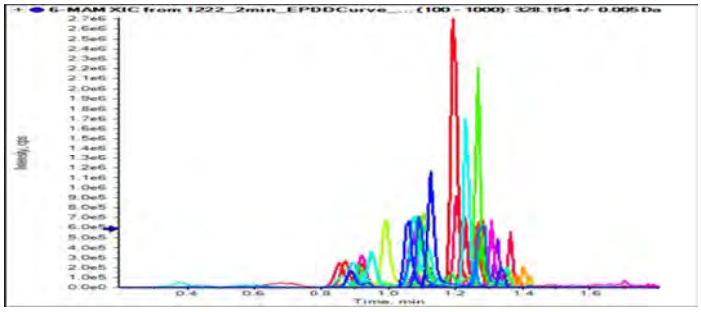

Figure 27. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 2.0 Minute LC Runtime

Information Dependent Acquisition

With the ability to provide the most interference free fragmentation information for library searching in a non-targeted acquisition, the IDA workflow provides the highest confidence screening using MS/MS information. Figure 28 shows the multiple screening criteria that are used for identification purposes in the SCIEX OS Software's easy to understand user interface.

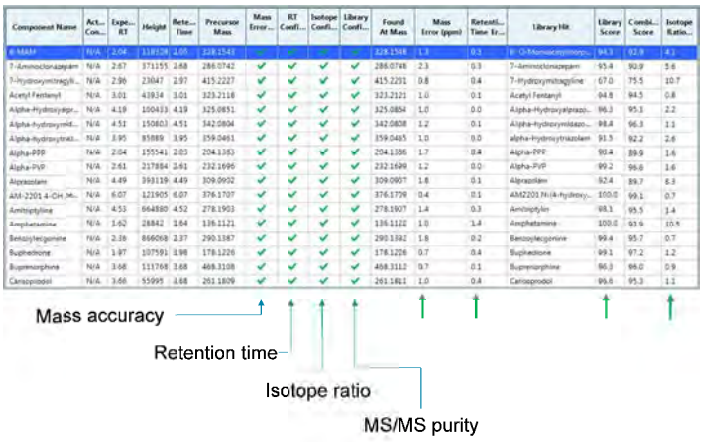

Figure 28 Screening and Identification Results from an IDA Experiment

The importance of acquiring quality MS/MS data for identification purposes, and not to solely rely on the accurate mass of the precursor ion, is demonstrated in Figures 29, 30 and 31. Each figure demonstrates how, by acquiring MS/MS data, we can distinguish between structural isobaric compounds. In each example shown, isobaric compounds are barely chromatographically separated and so the presence of either or both the compounds cannot be identified by either accurate mass of the precursor ions or confidently by retention time if there is any drift in retention of the compounds. The highest confidence is gained through library MS/MS comparisons.

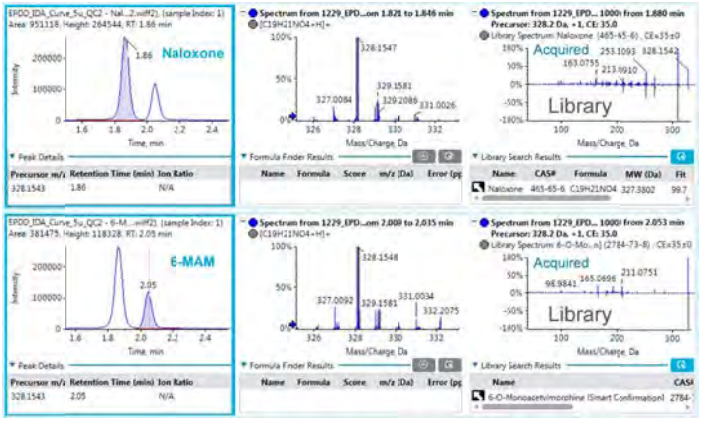

Figure 29. High Confidence Identification of Naloxone and 6-MAM Isobaric Compounds Gained through Library MS/MS Comparisons

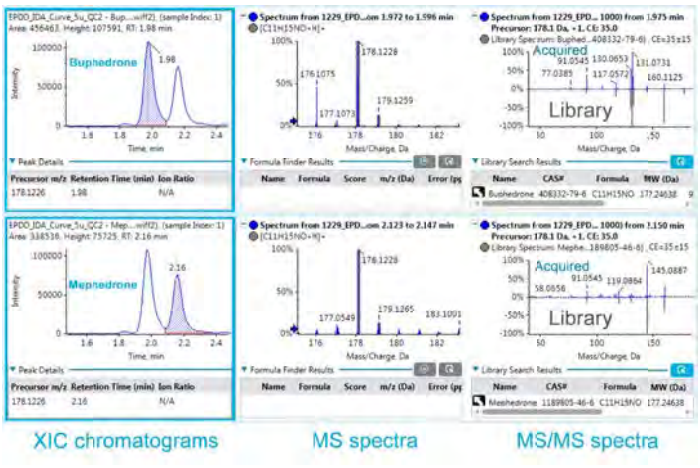

Figure 30. High Confidence Identification of Buprenorphine and Mephedrone Isobaric Compounds Gained through Library MS/MS Comparisons

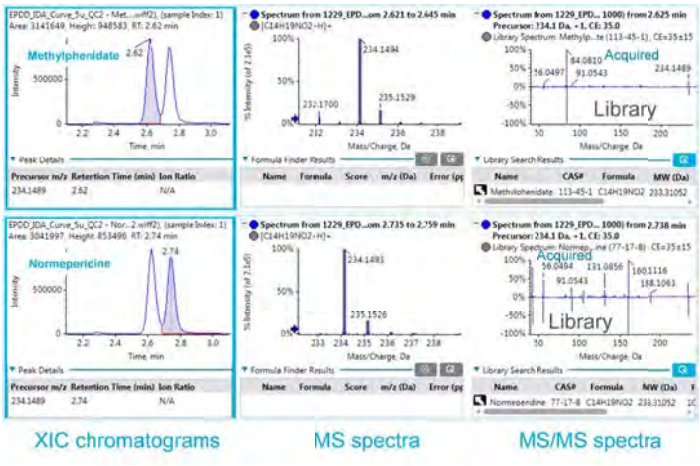

Figure 31. High Confidence Identification of Methylphenidate and Normeperidine Isobaric Compounds Gained through Library MS/MS Comparisons

Figures 32 and 33 show selected compound examples of XICs from the TOF-MS information acquired as part of the IDA workflow. This information can be used for quantification purposes.

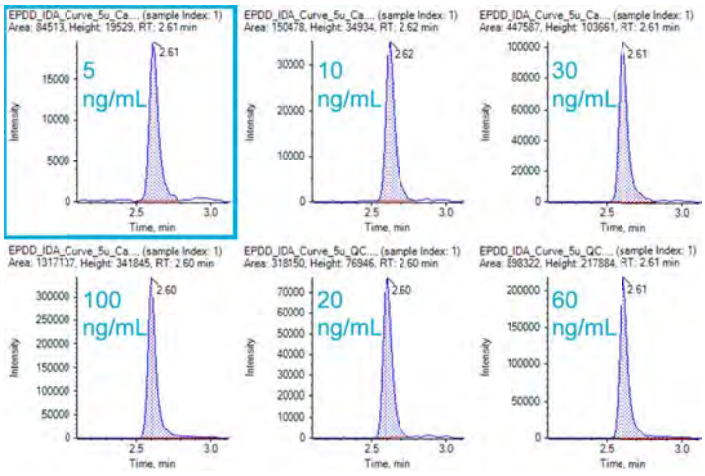

Figure 32. XICs of α-PVP in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

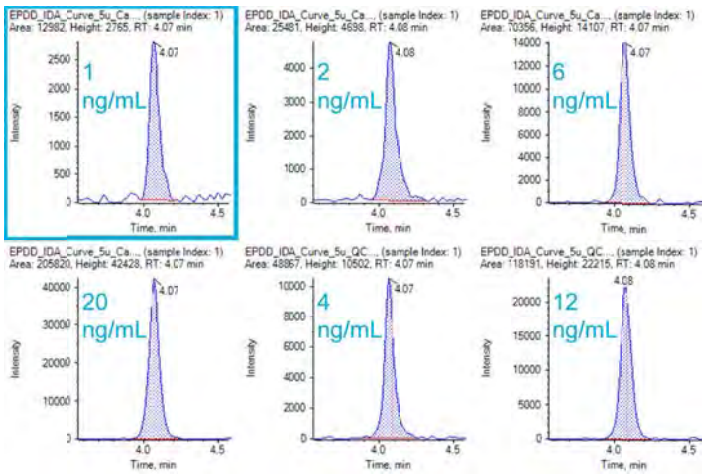

Figure 33. XICs of Sufentanil in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

Figure 34 shows representative calibration curves obtained from the IDA experiment.

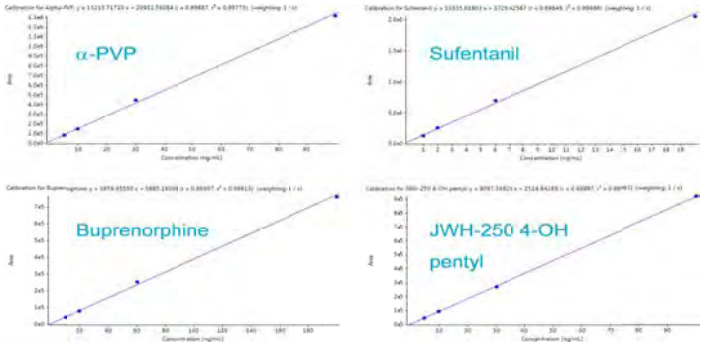

Figure 34. Representative Calibration Curves for Selected Compounds Showing that the TOF-MS information can be used for Quantification in an IDA Workflow

SWATH® Acquisition Results

SWATH® Acquired data can be processed in a similar way to processing IDA data for screening purposes. Again this uses multiple criteria for confidence in identification; most importantly using MS/MS library matching. Figure 35 shows a result of this from the 8.0 minute LC run which resulted in a high true positive rate of 98%.

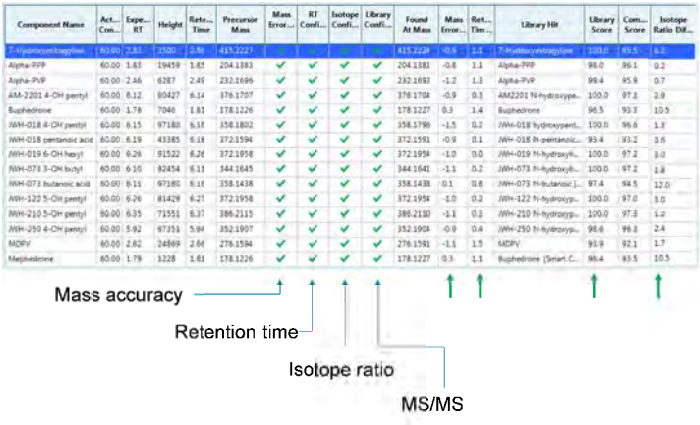

Figure 35. Processed SWATH® Acquired Data using Multiple Identification Criteria; including MS/MS Library Matching

With traditional IDA-MS/MS, quantitation can only be performed from TOF-MS mode and not from the *in situ* sporadic TOF-MS/MS data points. In contrast, due to the continual and looped MS/MS scan function, quantification from fragment ions is achievable from SWATH® acquisition. Better selectivity from the fragment ion information (Figure 36) relative to parent ion information, allows more sensitive detection in MS/MS mode of lower concentration species in complex matrices.

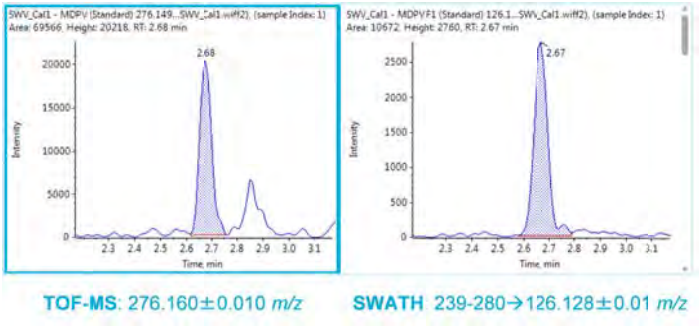

Figure 36. Gains in Selectivity with the Ability to Extract Out a Specific Fragment Ion From Variable Window SWATH® Acquired Data Compared to Extracted Accurate Mass of the Precursor Ion

Figure 37 shows identification and quantification results for a synthetic drug obtained from SWATH® Acquisition using the 8.0 min LC run time. This compound was not in the original targeted list but retrospective interrogation of the data from this unknown

sample allowed for its identification without having to re-inject the sample again.

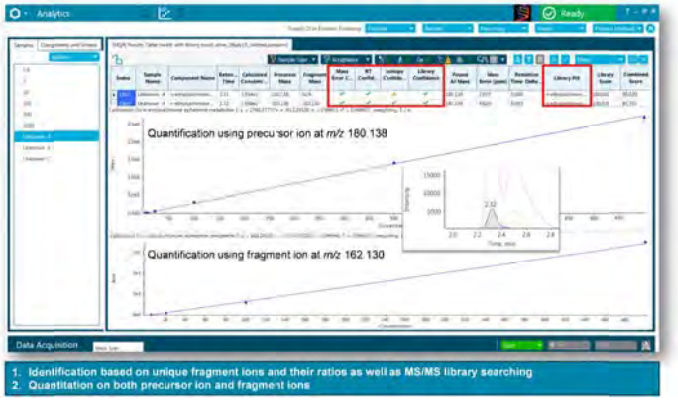

Figure 37. Identification and Quantification Results for n-Ethylcathinone Ephedrine Metabolite Compound Anlaysed by SWATH® Acquisition

The n-ethylcathinone ephedrine metabolite compound was identified based on unique fragment ions and their ratios as well as a library searching match (Figure 38). In a SWATH® acquisition experiment, not only can confirmation of the presence of compounds be made through MS/MS library matching and ion ratio calculations but because of the ability to extract out many unique fragment ions from the SWATH® acquired MS/MS data we can also determine the concentration based on quantification of either or both the precursor and fragment ions depending on which has less interferences.

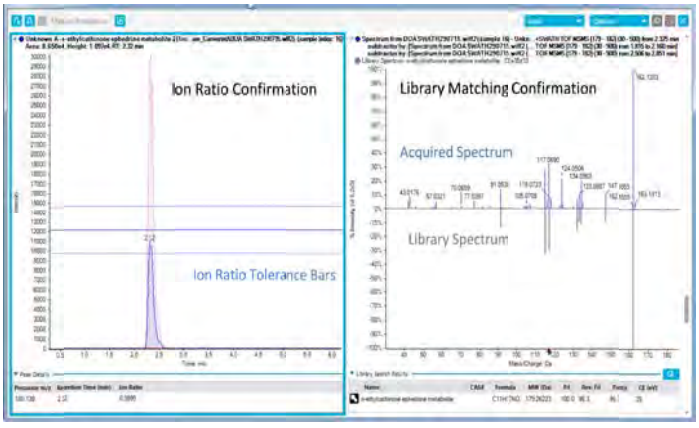

Figure 38. Extraction of Unique Fragment Ions From SWATH® Acquisition and Using Both Ion Ratio and Library Matching to Confirm Presence of n-Ethylcathinone Ephedrine metabolite in an Unknown Urine Sample

When investigating using a 2.0 minute LC run time as part of the SWATH® acquisition, we were able to accomplish good quantification results. Figure 39 shows representative calibration curves obtained from the ultra-fast screening experiment.

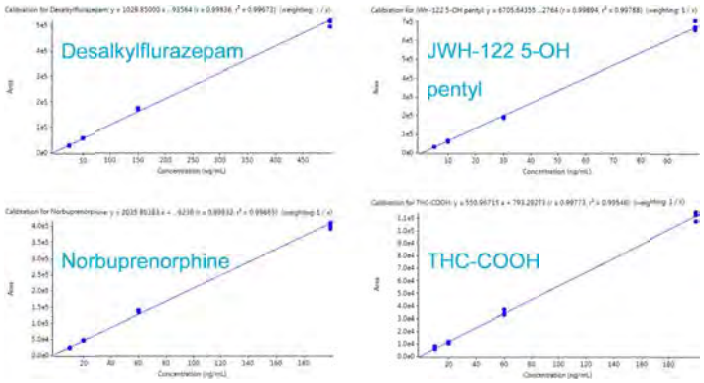

Figure 39. Representative Calibration Curves Generated from the SWATH® Acquisition using a 2.0 minute LC Runtime (n=3)

Sensitivity examples are shown in Figures 40, 41 and 42 for selected compounds.

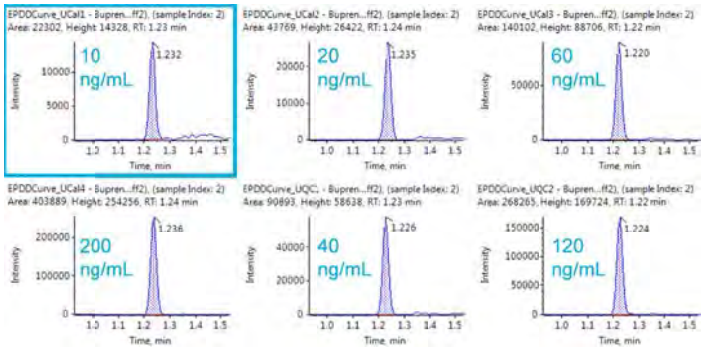

Figure 40. XICs of Buprenorphine at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

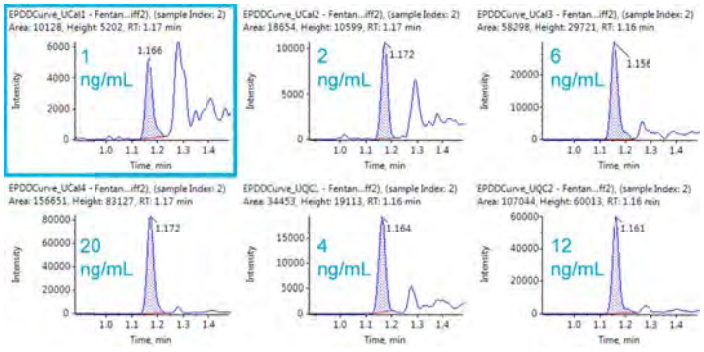

Figure 41. XICs of Fentanyl at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

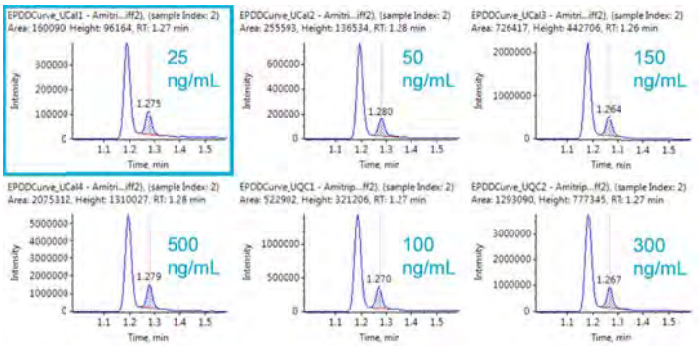

Figure 42. XICs of Amitriptyline at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

In the SWATH® Acquisition, MS/MS information is always available and so we can confirm the presence of the compound through MS/MS library matching (Figures 43 and 44) at the same time as determining how much of the compound is present.

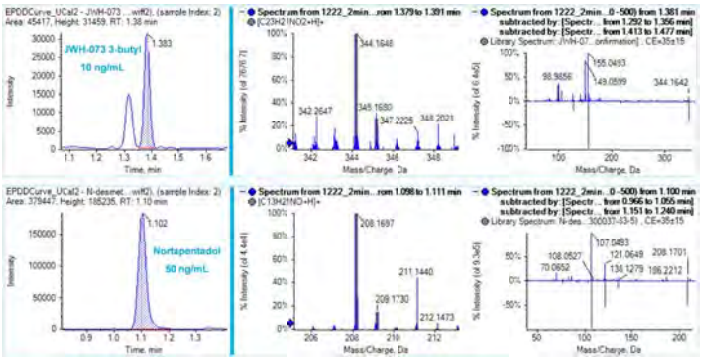

Figure 43. Confident Identification of JWH-073 3-Butyl and Nortapentadol from SWATH® Acquisition Through Library Searching

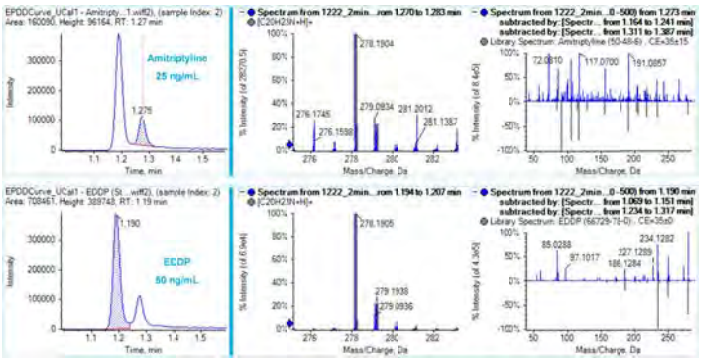

Figure 44. Confident Identification of Amitriptyline and EDDP from SWATH® Acquisition Through Library Searching; Showing LC Separation Between Isomers was Still Achievable with this Fast Method

At the cutoff concentrations, library matching worked well with 80% of compounds yielding greater than 70% hit score (Figure 45).

Sample Name	Component Na...	Actual Concentration	Height	Retention Time	Precursor Mass	Mass Error ...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error...	Retention Time...	Library Hit	Library Score	Combi... Score	Isotope Ratio Dif...
EPDDCurve_UCal2	Alpha-Hydroxyal...	50.00	39322	1.31	325.0851	✔	✔	✔	✔	325.0851	0.0	0.00	Alpha-hydroxya...	100.0	95.1	16.6
EPDDCurve_UCal2	Alpha-hydroxym...	50.00	54742	1.32	342.0804	✔	✔	✔	✔	342.0804	0.0	0.00	Alpha-hydroxy...	100.0	99.3	1.8
EPDDCurve_UCal2	Buprenorphine	20.00	26422	1.24	468.3108	✔	✔	✔	✔	468.3107	-0.3	0.01	Buprenorphine	100.0	97.9	2.9
EPDDCurve_UCal2	Desalkylflurazep...	50.00	39330	1.33	289.0538	✔	✔	✔	✔	289.0539	0.2	0.01	Desalkylflurazep...	100.0	98.2	1.4
EPDDCurve_UCal2	EDDP	100.00	76837	1.20	278.1903	✔	✔	✔	✔	278.1906	0.8	0.00	EDDP	100.0	96.1	1.3
EPDDCurve_UCal2	Fentanyl	2.00	10599	1.17	337.2274	✔	✔	✔	✔	337.2276	0.5	0.01	Fentanyl	100.0	97.3	0.7
EPDDCurve_UCal2	Hydrocodone	50.00	61526	0.92	300.1594	✔	✔	✔	✔	300.1597	0.9	0.01	Hydrocodone	100.0	94.9	8.3
EPDDCurve_UCal2	JWH-018 4-OH...	10.00	29593	1.39	358.1802	✔	✔	✔	✔	358.1801	-0.1	0.01	JWH-018 hydro...	100.0	98.0	4.6
EPDDCurve_UCal2	JWH-018 6-OH h...	10.00	44558	1.40	372.1958	✔	✔	✔	✔	372.1959	0.3	0.01	JWH-019 N-hyd...	100.0	98.7	4.4
EPDDCurve_UCal2	JWH-073 3-OH...	10.00	31459	1.38	344.1645	✔	✔	✔	✔	344.1648	0.9	0.01	JWH-073 N-hyd...	100.0	94.3	5.1
EPDDCurve_UCal2	JWH-122 5-OH...	10.00	44558	1.40	372.1958	✔	✔	✔	✔	372.1958	0.3	0.01	JWH-019 N-hyd...	100.0	96.7	4.4
EPDDCurve_UCal2	Methamphetami...	100.00	91875	0.92	150.1277	✔	✔	✔	✔	150.1277	-0.4	0.00	Methamphetam...	100.0	98.0	0.6
EPDDCurve_UCal2	Methylphenidate	50.00	195247	1.07	234.1489	✔	✔	✔	✔	234.1490	0.8	0.00	Methylphenidate	100.0	98.4	0.7
EPDDCurve_UCal2	Norbuprenorphi...	20.00	27123	1.17	414.2639	✔	✔	✔	✔	414.2641	0.5	0.00	Norbuprenorphi...	100.0	97.5	2.3
EPDDCurve_UCal2	Norclazepam	50.00	44694	1.35	271.0633	✔	✔	✔	✔	271.0635	0.8	0.00	Norclazepam	100.0	95.5	3.7
EPDDCurve_UCal2	Noroxycodone	50.00	23623	0.90	302.1387	✔	✔	✔	✔	302.1389	-0.2	0.01	Noroxycodone	100.0	97.9	4.3
EPDDCurve_UCal2	Tapentadol	50.00	203039	1.09	222.1852	✔	✔	✔	✔	222.1854	0.7	0.00	Tapentadol	100.0	95.6	1.1
EPDDCurve_UCal2	Temazepam	50.00	58735	1.34	301.0738	✔	✔	✔	✔	301.0738	-0.2	0.01	Temazepam	100.0	98.1	1.2
EPDDCurve_UCal2	Tramadol	50.00	169458	1.06	264.1958	✔	✔	✔	✔	264.1961	1.0	0.00	Tramadol	100.0	95.9	0.6
EPDDCurve_UCal2	Zolpidem	50.00	277586	1.13	308.1757	✔	✔	✔	✔	308.1760	0.8	0.00	Zolpidem	100.0	98.1	1.9
EPDDCurve_UCal2	7-Aminoclonaze...	50.00	78113	1.12	286.0742	✔	✔	✔	✔	286.0744	0.9	0.01	7-Aminoclonaze...	99.8	95.0	3.2
EPDDCurve_UCal2	Alprazolam	50.00	123684	1.32	309.0902	✔	✔	✔	✔	309.0904	0.8	0.00	Alprazolam	99.8	95.7	4.0
EPDDCurve_UCal2	Diazepam	50.00	110405	1.36	285.0789	✔	✔	✔	✔	285.0791	0.7	0.01	Diazepam	99.7	95.5	3.4
EPDDCurve_UCal2	Nalfenone	50.00	56359	0.91	342.1700	✔	✔	✔	✔	342.1698	-0.4	0.00	Nalfenone	99.7	97.3	3.0
EPDDCurve_UCal2	Oxymorphone	50.00	6763	0.41	302.1387	✔	✔	✔	✔	302.1389	0.8	0.02	Oxymorphone	99.7	94.2	3.5
EPDDCurve_UCal2	Amphetamine	100.00	3665	0.88	136.1121	✔	✔	✔	✔	136.1120	-0.3	0.00	Amphetamine	99.6	97.1	4.1

Figure 45. Library Searching and Identification of Compounds in the 2.0 Minute Method at Cutoff Concentration Levels

MRM^HR

MRM^{HR} is a purely targeted data MS/MS acquisition and can be unscheduled or scheduled. The only non-targeted and therefore retrospective capability is through the TOF-MS experiment which is performed at the beginning of every scan. The power of the workflow however, is its selectivity capabilities through the accurate mass of unique fragment ions for quantification purposes. This is demonstrated in Figure 46 where MRM^{HR} is compared to the MRM analysis, extracted at nominal mass, and the extraction of the accurate mass of the precursor ion from a TOF-MS experiment. The compound is not able to be distinguished from the high background and interferences of the nominal mass experiment and not even by the extraction of the accurate mass of the precursor ion from the full scan TOF-MS experiment. It is not until we extract out two unique accurate mass fragment ions from the MRM^{HR} experiment that we achieve the selectivity required to detect this compound by removal of the background and interferences and increase the S/N; improving the quantification capabilities. Another example of this selectivity gain over the accurate mass of the precursor ion is demonstrated in Figure 47 where a visible improvement in S/N is gained for the analysis of buprenorphine by the MRM^{HR} approach.

Figure 46. Increased Selectivity with MRM^{HR}; Avoiding False Negatives. Example given is a Feed Sample Tested Positive for NP Semicarbazide

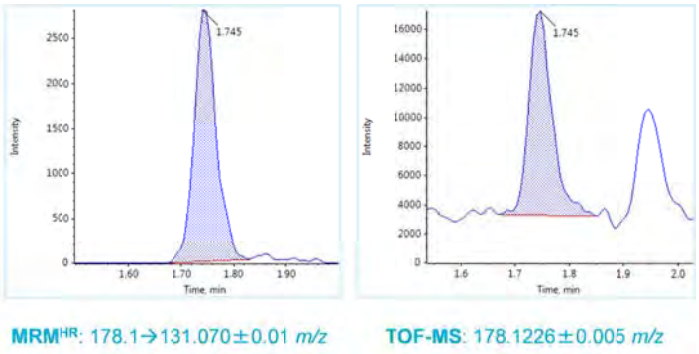

Figure 39. Scheduled MRM^{HR} Selectivity Compared to TOF-MS; Buprenorphine (5ng/mL in urine, 10 fold dilution, 10 µL injection)

Quantification performance of the MRM^{HR} is demonstrated in Figure 40 for the 8.0 minute LC-MS/MS method.

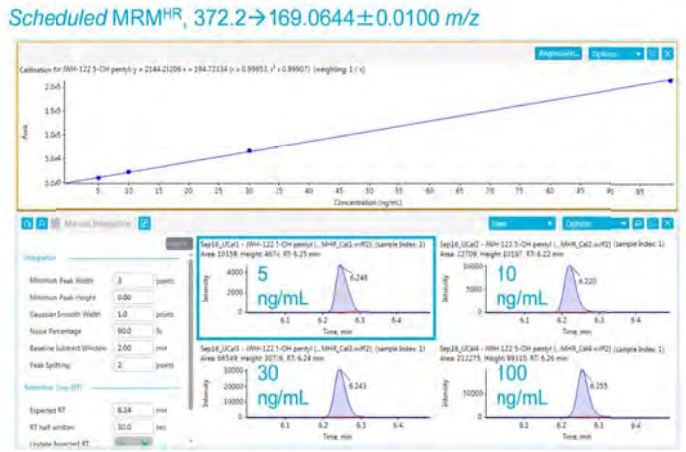

Figure 40. MRM^{HR} Quantification Results for JWH-122 5-OH Pentyl in urine (Urine was diluted 10-fold, 10 µL injection)

Negative Mode Performance

Figures 41 and 42 show a couple of examples of negative mode performance of the SCIEX X500R QTOF System.

Figure 41. Negative Mode Performance of SCIEX X500R QTOF System for Analysis of Amo/pentobarbital

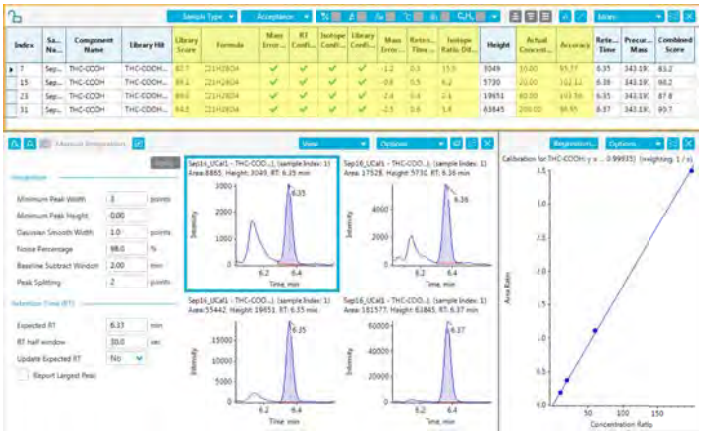

Figure 42. Negative Mode Performance of SCIEX X500R QTOF System for Analysis of THC-COOH

Conclusion

The arrival of the next generation QTOF, with the launch of the SCIEX X500R QTOF System and SCIEX OS Software, brings the powerful performance capabilities of the high resolution accurate mass technology to the routine identification and quantification forensic workflows.

- Hardware
  - SCIEX ExionLC™ Systems
    - Fully controlled by SCIEX OS software
    - Improved software integration for better stability
  - SCIEX X500R QTOF System
    - N-geometry design (same effective flight path length for ions and therefore resolution than V-geometry, but in a smaller overall footprint)
    - Heated TOF path for mass accuracy stability

        - Minimized footprint, engineered for simplicity and service accessibility
- Software
    - SCIEX OS Software
        - Intuitive and logical single software platform for LC control, MS control, data processing and reporting.
        - New user interface
        - Simultaneous identification and quantitation

We have described the screening and quantification workflows of the SCIEX X500R QTOF System. Each workflow is straightforward to setup in the newly designed SCIEX OS Software and depending on the end users requirements we have demonstrated in this technical note the strengths of each workflow. Each provides TOF-MS and TOF-MS/MS analysis, both data being crucial in confidently identifying and quantifying forensic compounds.

- TOF-MS
- TOF-MS/MS
    - IDA
        - Non-targeted data acquisition
        - MS quantitation
        - Highest confidence screening with MS/MS information
    - MRM^{HR}
        - Targeted data acquisition for quantitation purpose
        - Can be performed unscheduled or scheduled
    - SWATH® Acquisition (with variable windows)
        - Non-targeted data acquisition
        - MS/MS for everything all the time
        - Screening and quantitation (MS/MS)
            - Library Searching and Ion Ratio

We evaluated different LC runtime methods. The longer method aided eluting all analytes throughout the entire gradient as evenly as possible in order to maximize triggering IDA MS/MS for all components and reduce the MRM^{HR} concurrency for quality of data (*Scheduled* MRM^{HR}). The library searching worked well for the SWATH® Acquisition in the 2.0 minute LC runtime with MS/MS information always being available with this MS/MS^{ALL} approach.

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SCIEX

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Forensic drug screening analysis

Elevate your forensic testing with the X500R QTOF System

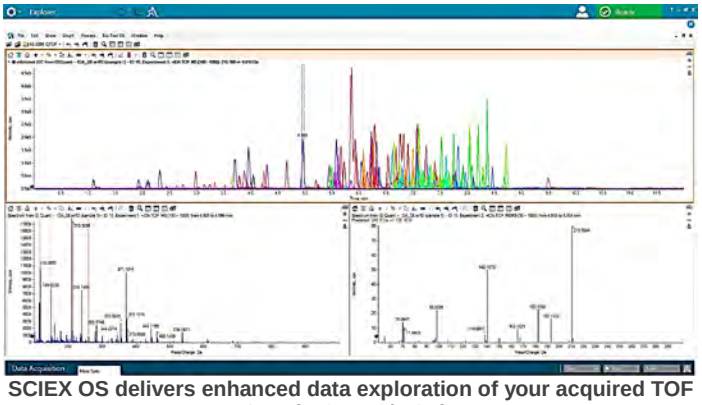
Method details and access to HR-MS/MS libraries to screen for forensic drugs in urine samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1

- Dilute urine samples 5 times with the following dilutent: 90% Mobile Phase A (5mM ammonium formate in water): 10% Mobile Phase B (Methanol with 0.05% formic acid)

SCIEX OS can deliver faster method set-up

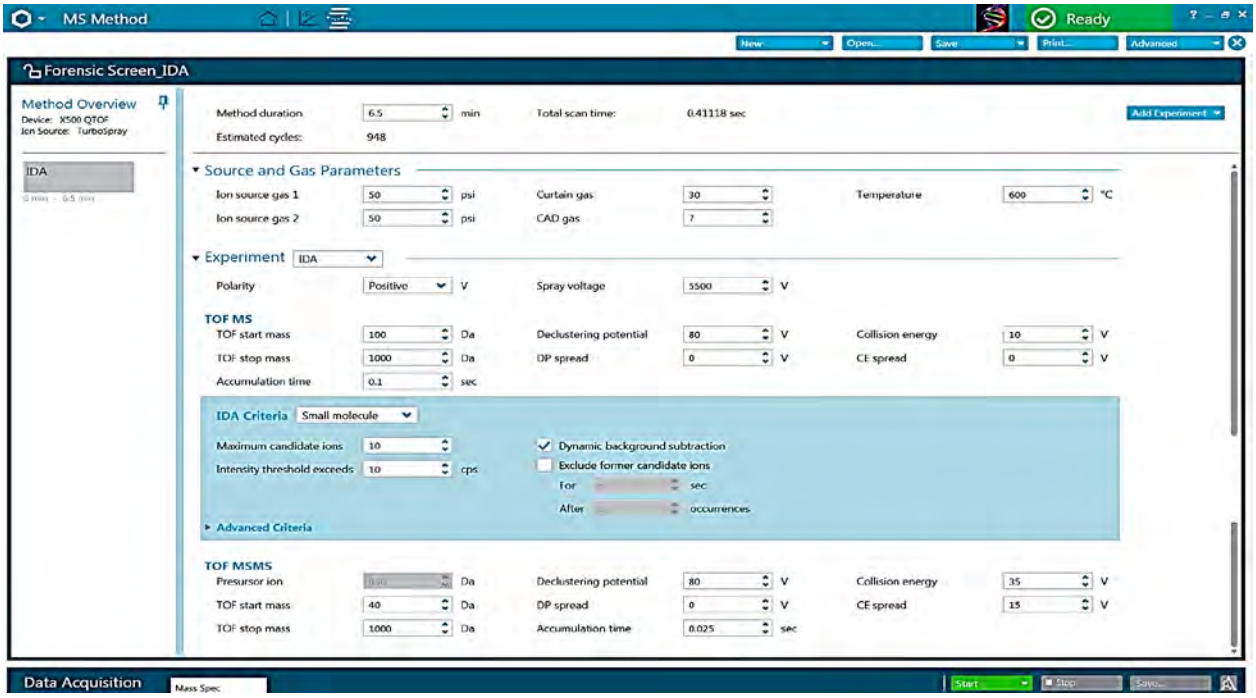


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Phenomenex Kinetex Biphenyl, 100 x 3.0 mm, 2.6 um column	
Mobile Phase A	*0.1% formic acid in water*	
Mobile Phase B	*0.1% formic acid in methanol*	
Flow rate	*0.6 mL/min*	
Column temperature	*30 ℃*	
Injection volume	*10 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*2*
	1	*2*
	7	*65*
	7.1	*100*
	9	*100*
	9.1	*2*
	12	*2*

MS Method



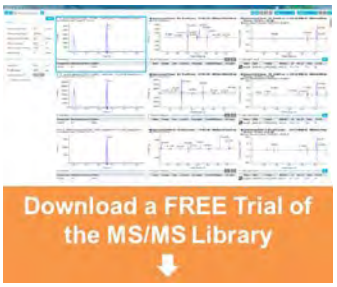
Suggested IDA (Information Dependent Acquisition) conditions for routine forensic drug screening as displayed in SCIEX OS

Data Processing

Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries

Download a free XIC compound list detailing a full list of forensic drug compounds including molecular formula and accurate mass.



Download a free trial of the forensic high resolution MS/MS library, containing 1703 compounds.

Learn more at sciex.com/X-Forensics.

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Forensic Method

Forensic drug screening analysis

Elevate your forensic testing with the X500R QTOF System

Method details and access to HR-MS/MS libraries to screen for forensic drugs in blood extracts using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1
- Add 0.2ml of blood into centrifuge tube

Step 2
- Add 0.8mL of cold acetonitrile dropwise while vortexing

Step 3
- Centrifuge for 10 minutes at 5000rpm

Step 4
- Transfer supernatant to clean glass vials and dry down.

Step 5
- Reconstitute in 200µL of 20% methanol in water.

Step 6
- Vortex for 1 minute and centrifuge for 10 minutes at 10,000xg

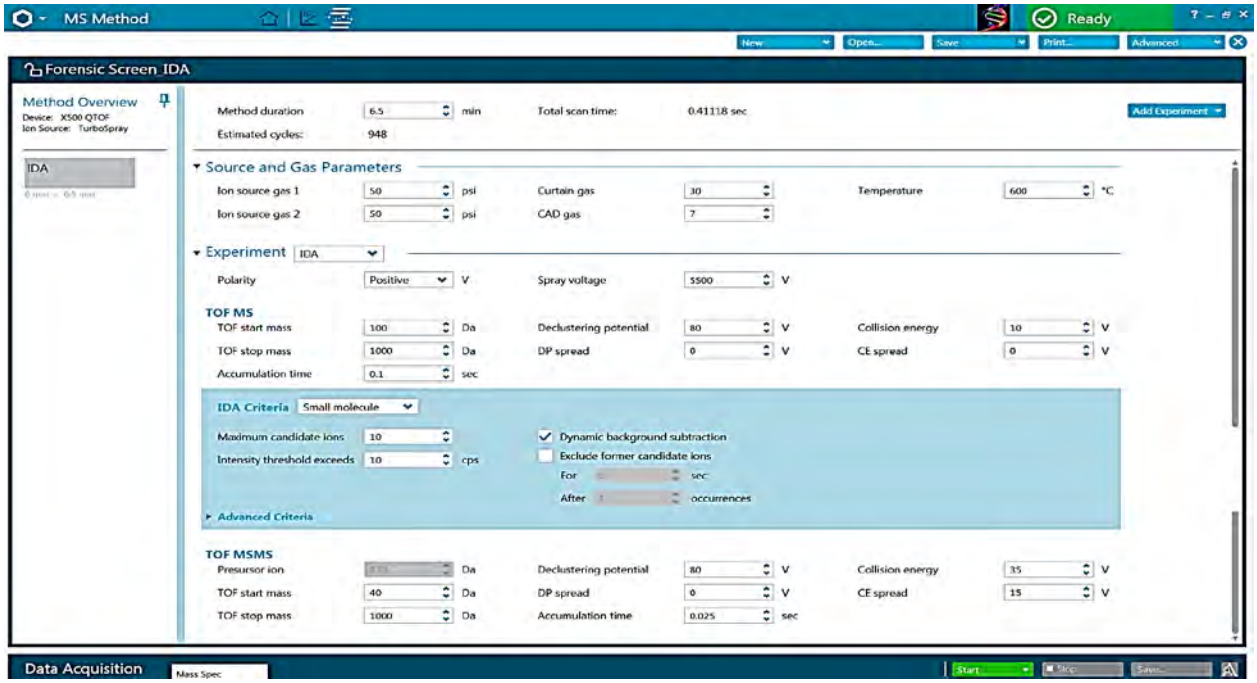
Step 7
- Transfer supernatant to clean glass HPLC vials

SCIEX OS can deliver faster method set-up

LC Method

Column	Phenomenex Kinetex Biphenyl, 100 x 3.0 mm, 2.6 um column	
Mobile Phase A	*0.1% formic acid in water*	
Mobile Phase B	*0.1% formic acid in methanol*	
Flow rate	*0.6 mL/min*	
Column temperature	*30℃*	
Injection volume	*10 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*2*
	1	*2*
	7	*65*
	7.1	*100*
	9	*100*
	9.1	*2*
	12	*2*

MS Method



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Data Processing

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X500R High Resolution Libraries

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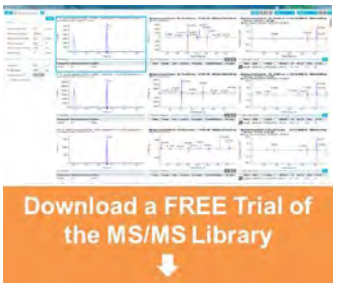

Download a free trial of the forensic high resolution MS/MS library, containing 1703 compounds.

Learn more at sciex.com/X-Forensics.

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