X500R QTOF

COMPENDIUM OF RELATED RESEARCH APPLICATIONS
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X500R QTOF System with SWATH® Acquisition for Pesticide Residue Screening in Fruits and Vegetables

Xiaojing Sun, Haiyan Cheng, Lijun Li, Wenhai Jin
SCIEX, Asia Pacific Application Support Center (Shanghai), China

Introduction

It is widely accepted that modern Chinese agriculture has a long history of excessive chemical fertilizer, pesticide, and herbicide use, and this has not only resulted in reduced nutrient content in food, but also in a variety of chemical residues that have harmed human health. It has been confirmed that pesticide residues may interfere with the body's endocrine effects on the immune system and hematopoietic system, and can even cause in-vivo fetal visceral hypoplasia or deformity in pregnant women. Weighed against the benefits of consuming more fruits and vegetables, people have recently become more heavily concerned about the widespread existence of pesticide residues and the excessive damage they can cause to the human body.

With the continued development and popularization of liquid chromatography / mass spectrometry, more and more pesticide residue detection technologies are being developed based on the LC-MS/MS system. The most commonly used pesticide residue screening method includes the MRM-IDA-EPI system, which is based on QTRAP® system and high resolution TOF-IDA-MSMS technology. While the SWATH technology is based on high-resolution systems, it also combines the advantages of IDA and MRM by dividing the mass range of the parent ion into multiple mass windows and allowing all ions in each window to collide with each other and fragment, resulting in fragmentation information for all ions in the entire mass range. SWATH® technology's measurement of second-order fragmentation differs from the IDA, in which only the selected ions are triggered, ensuring the continuity of all ion debris and achieving SWATH's second degree of quantification. By customizing the unique variable window settings, the size of the mass window is automatically adjusted according to the quantity of ions, ensuring the collection of high-quality data.

The SCIEX High Resolution Mass Spectrometry X500R QTOF system provides high resolution, high accuracy, high sensitivity and high linearity range scan speeds, making SCIEX SWATH technology not only popular for protein macromolecules but also for small molecule pesticide residue screening. The X500R QTOF system uses newly designed SCIEX OS software to achieve an all-in-one whole process analysis with instrument control, data acquisition and data processing. The software has the built-in SWATH method of setup and powerful automatic deconvolution capabilities. This simple and convenient design meets food safety field use requirements.

Experimental considerations:

1. Collect and process samples of fruits and vegetables, and measure the actual SWATH data
2. Prepare Standard Curve, Test 190 Pesticide Standard SWATH data
3. Screening of Pesticide Residues in Vegetables and Fruits
4. The pesticide residue was quantified at two levels

Sample treatment:

1 • Weigh10g of mashed sample into a clean tube

2 • Add 10mL of Acetonitrile with 1% acetic acid, votex for 1min

3 • Add 1.5g of NaAC, 6g of MgSO4, votex, then Centrifuge for 5 mins

4 • Precipitate 8mL of supernatant with Agela clean package

5 • Centrifuge for 5 mins, transfer supernatant for analysis

The QuEChERS method was used to pretreat received samples: 1 leek, 2 cauliflower, 3 bean, 4 jujube (after washing), 5 jujube (not cleaned), 6 pear.

Chromatographic Methods

Chromatography column: Phenomenex Kinetex C18, 100*2.1 mm, 2.6μm

Mobile phase: A: Contains 5mM ammonium acetate in water; B: Contains 5mM ammonium acetate in methanol gradient elution

Flow rate: 0.4mL/min

Column temperature: 40℃

Input volume: 10μL

Time (min)	B%
0	*3*
1	*3*
2	*45*
19	*95*
22	*95*
22.1	*5*
25	*5*

Mass Spectrometry Method

Scanning method: SWATH® Acquisition methods

Ion source: ESI+source CDS automatic calibration

Table 1: Ion source parameters

IS Voltage: 5500V	*Air curtain gas CUR: 35psi*
Atomizing gas GS1: 55 psi	*Auxiliary gas GS2: 55 psi*
Source Temperature TEM: 550°C	*Collision Gas CAD: 7*
Collision energy CE ± CES: 35 ±15V	*Air curtain gas CUR: 35psi*

Data acquisition and SWATH setup process

IDA (Information Dependent Acquisition) uses TOF/MS Survey Scan to pre-scan. When a peak ion is successfully triggered and detected, the scan mode is switched to Q1 and the parent ion is selected to acquire a high sensitivity MS/MS secondary spectrum of the target ion. SWATH distributes all the ions into successive windows, and all the ions in each window are transferred to the collision chamber and broken into second-order MS/MS debris and then traced back to the parent ion through the software's powerful de-convolution function. Thus all of the second-order fragments of all abundant ions can be obtained through this technique, which ensures that the secondary information of the low-content target is included, allowing the trace residue screening to become more complete and accurate.

Unique intelligent variable windows, according to the distribution of ions in the sample, set narrow windows in the high density distribution areas and set up wider windows in the regions with fewer ions to ensure high-quality secondary mass spectra are collected for all ions.

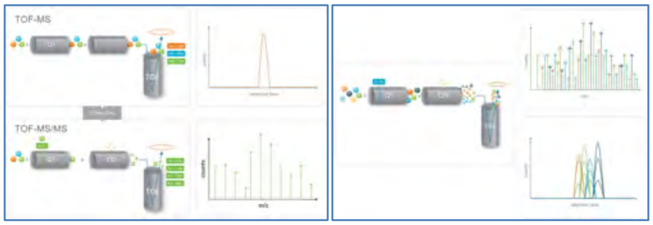


Figure 1 Left IDA schema; Right SWATH schema

Polarity: Positive
Spray voltage: 5500 V

TOF start mass: 100 Da
Declustering potential: 80 V
Collision energy: 10 V
TOF stop mass: 630 Da
DP spread: 0 V
CE spread: 0 V
Accumulation time: 0.2 s

Time bins to sum: 4
Channel 1 ✓
Channel 2 ✓
Channel 3 ✓
Channel 4 ✓

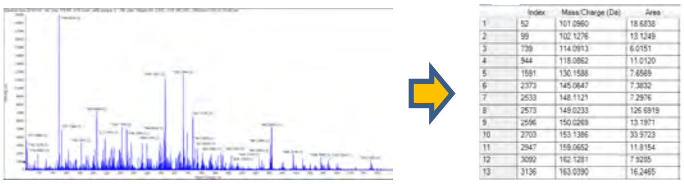
TOF start mass: 50 Da
TOF stop mass: 630 Da
Dynamic collision energy ☐
Accumulation time: 0.05 s
Charge state: 1

	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)	Time bins to sum
1	99.5000	159.5000	80	0	35	15	4
2	158.5000	203.0000	80	0	35	15	4
3	202.0000	229.3000	80	0	35	15	4
4	228.3000	252.2000	80	0	35	15	4

Figure 2 SWATH method settings

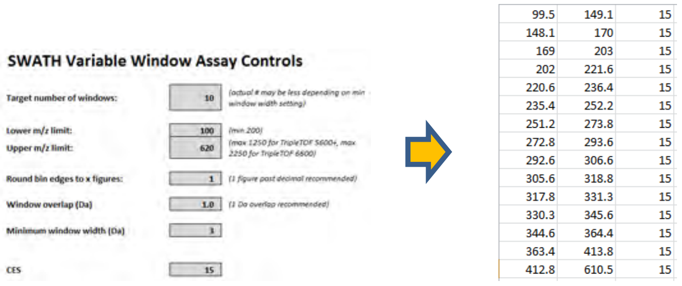
In the SCIEX OS Software, choose "Experiment" and then pick the SWATH Acquisition mode. The software then automatically lists the required parameters for the SWATH mode. Mass Table is for the Q1 window.

The Variable Window Calculator can be based on TOF/MS's parent ion to automatically calculate the SWATH smart variable window. The mode can be established by copying and pasting to the Mass Table, which is a method that is simple, rapid, and easy to use.



	Index	Mass/Charge (Da)	Area
1	52	101.0960	18.6838
2	99	102.1276	13.1249
3	739	114.0913	6.0151
4	944	118.0862	11.0120
5	1581	130.1588	7.6569
6	2373	145.0647	7.3832
7	2533	148.1121	7.2976
8	2573	149.0233	126.6919
9	2596	150.0269	13.1971
10	2703	153.1386	33.9723
11	2947	159.0652	11.8154
12	3092	162.1281	7.9285
13	3136	163.0390	16.2465

Figure 3 TOF/MS's parent ion



SWATH Variable Window Assay Controls

Target number of windows: 10 (actual # may be less depending on min window width setting)

Lower m/z limit: 100 (min 200)

Upper m/z limit: 620 (max 1250 for TripleTOF 5600+, max 2250 for TripleTOF 6600)

Round bin edges to x figures: 1 (1 figure past decimal recommended)

Window overlap (Da) 1.0 (1 Da overlap recommended)

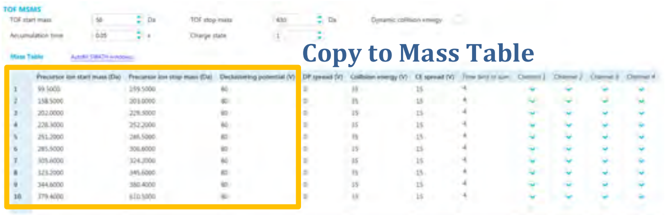
Minimum window width (Da) 1

CES 15

99.5	149.1	15
148.1	170	15
169	203	15
202	221.6	15
220.6	236.4	15
235.4	252.2	15
251.2	273.8	15
272.8	293.6	15
292.6	306.6	15
305.6	318.8	15
317.8	331.3	15
330.3	345.6	15
344.6	364.4	15
363.4	413.8	15
412.8	610.5	15

Q1 Window

Figure 4 Variable Window Calculator



Copy to Mass Table

Establish SWATH® Acquisition method and initial test of 190 varieties of standard pesticide products.

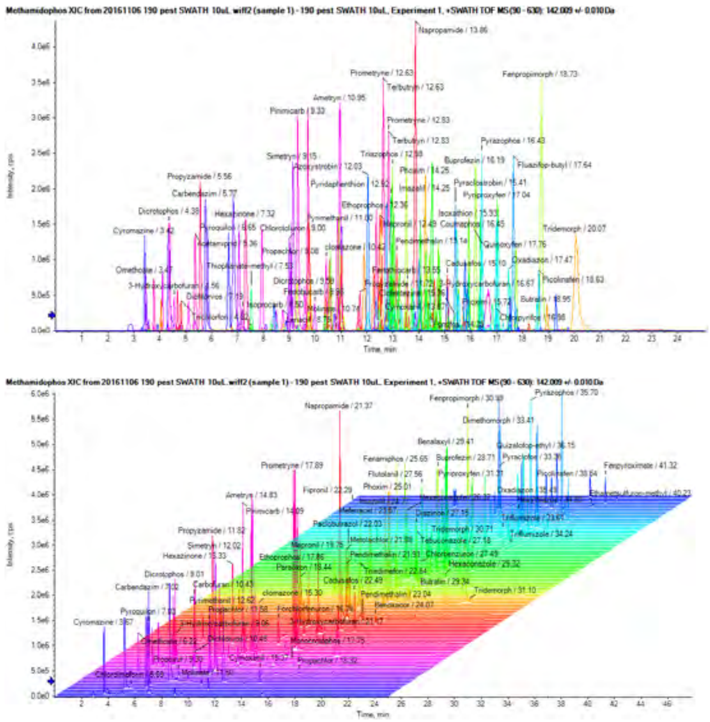


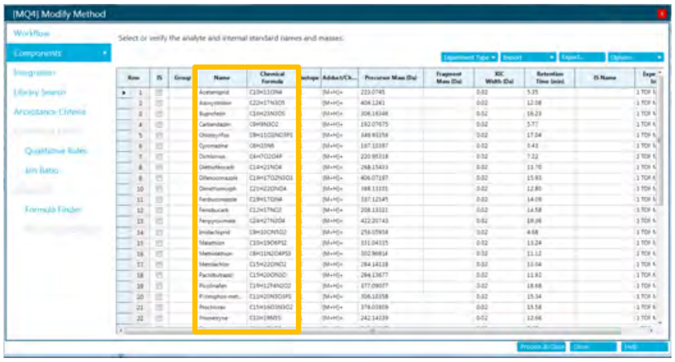
Figure 5 Chromatogram of 190 varieties of pesticide residue standard products collected by SWATH.

Data Analysis

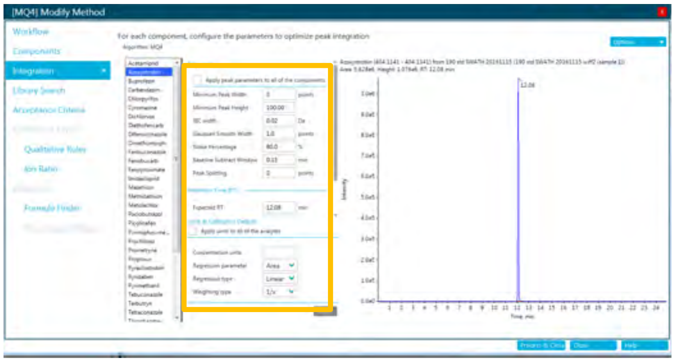
1. Qualitative screening

Test SWATH data of 1 (leek), 2(cauliflower), 3 (kidney beans), 4 (jujube, washed), 5 (winter jujube, unwashed), and 6 (pear). Use X500R SCIEX OS Software to perform data analysis by passing four confidence conditions: mass accuracy, retention time, isotope distribution and secondary library matching to screen pesticide residues in the 6 samples.

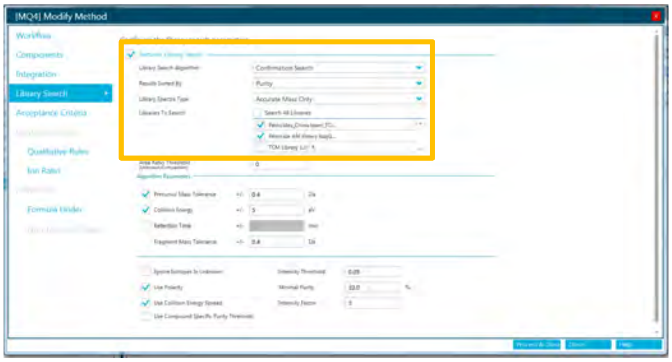
1. Select the standard product data to establish screening methods; import the screening list



2. Set the quantitative integration parameters



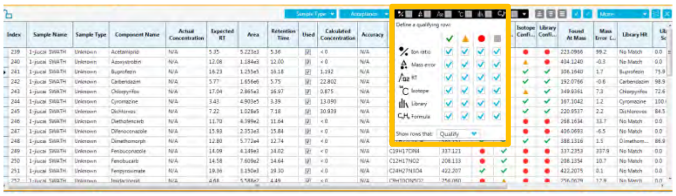
3. Set the library search criteria



4. Set the screening confidence conditions

5. One time import of all standard product and samples' SWATH® data to perform screening

6. Filter results through the Mass error, RT, Isotope, Library



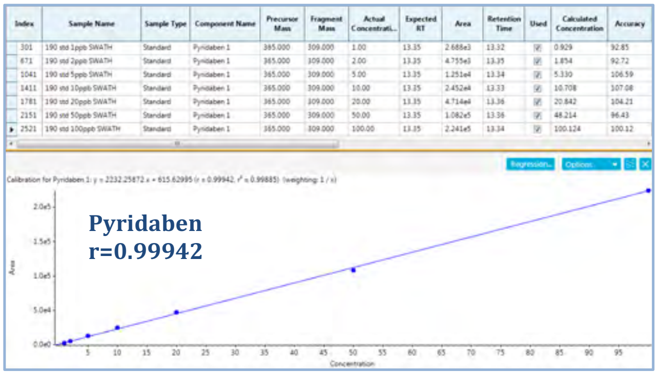
7. Obtain he results of screening for each sample

Chromatogram isotope pattern MS/MS FIT

2. SWATH second degree quantification

Using TOF/MS's first degree quantitative data in complex matrix samples has disadvantages such as high baseline noise and a narrow linear range, etc. The SCIEX OS Software in the X500R QTOF system can be used to directly copy and paste the ion pairs of compounds when a quantitative method is established, obtaining the results of the second quantification by using the MRM^{HR} method to process SWATH data.

Preparation of 190 kinds of pesticide on the standard curve1ng/mL~100ng/mL established the second degree quantitative SWATH® method to obtain second degree quantitative linear relationships, see Fig. 6.



Index	Sample Name	Sample Type	Component Name	Precursor Mass	Fragment Mass	Actual Concentrati...	Expected RT	Area	Retention Time	Used	Calculated Concentration	Accuracy
301	190 std 1ppb SWATH	Standard	Pyridaben 1	365.000	309.000	1.00	13.35	2.688e3	13.32	☑	0.929	92.85
671	190 std 2ppb SWATH	Standard	Pyridaben 1	365.000	309.000	2.00	13.35	4.755e3	13.35	☑	1.854	92.72
1041	190 std 5ppb SWATH	Standard	Pyridaben 1	365.000	309.000	5.00	13.35	1.251e4	13.34	☑	5.330	106.59
1411	190 std 10ppb SWATH	Standard	Pyridaben 1	365.000	309.000	10.00	13.35	2.452e4	13.33	☑	10.708	107.08
1781	190 std 20ppb SWATH	Standard	Pyridaben 1	365.000	309.000	20.00	13.35	4.714e4	13.36	☑	20.842	104.21
2151	190 std 50ppb SWATH	Standard	Pyridaben 1	365.000	309.000	50.00	13.35	1.082e5	13.36	☑	48.214	96.43
2521	190 std 100ppb SWATH	Standard	Pyridaben 1	365.000	309.000	100.00	13.35	2.241e5	13.34	☑	100.124	100.12

Calibration for Pyridaben 1: y = 2232.25872 x + 615.62995 (r = 0.99942, r^2 = 0.99885) (weighting: 1 / x)

Figure 6 Quantitative linearity using pyridaben as an example

For use of the high sensitivity and high selectivity SWATH second degree quantitative method to quantify the pesticide residues contained in leek, cauliflower, kidney bean, winter jujube (washed), winter jujube (unwashed), and pear, please see the table below for the pesticide residues contained in the above samples.

Detected pesticides (unit: ng / mL)

	Leek	Cauliflower	Kidney Bean	Winter Jujube (washed)	Winter Jujube (unwashed)	Pear
Carbendazim	*8.7*		*1*		*1.4*	
Insecticide	*4.5*					
Methylpyrimidine	*3.5*					
Prometryn	*33*					
Pyrimethanil	*23*		*270*			
Thiophanate-methyl	*2.1*					
Imidacloprid		*1.2*			*580*	*3.4*
Propoxur		*50*	*13.7*			
Tebuconazole		*1.1*	*4.5*			*3.4*
Acetamiprid			*3.7*		*5.1*	*86*
Kresoxim			*8.2*	*15*	*22*	
Streptozotocin			*35*	*18*	*74*	
Buprofezin					*160*	
Fenpyroximate					*61*	
Paclobutrazol					*140*	
Triadimefon					*23*	

Summary

The experiment used the SCIEX X500R QTOF system's SWATH technology to screen pesticide residues in six varieties of vegetables and fruits, among which leeks, kidney beans and jujube contained 6 or more types of pesticide residue. In particular, in the jujube, the imidacloprid content reached 580 ppb, far exceeding the limits of pesticide residue standards; buprofezin and paclobutrazol content also exceeded100ppb. Through the analysis of the washed jujube samples we found that although the pesticide residue is extremely high, it is fortunately possible to be washed off with detergent. Even so, the washed jujube still contains more than 10ppb of kresoxim-methyl and pyraclostrobin.

This experiment established the SWATH® screening and quantitative methods for residues of the190 most commonly used types of pesticides for the Ministry of Agriculture risk assessment. SWATH technology obtained the primary and secondary data of all pesticide residues by entering the samples only a single time. The highly sensitive secondary spectra were still able to identify each compound in the spectral library and obtained the secondary spectra even when the pesticide content in the sample was very low, using four confidence conditions: mass accuracy, isotope distribution, retention time, and secondary library matching to corroborate.

Any ion's continuous chromatographic peak data in the SWATH secondary spectrum can be used as the basis for quantitative data analysis. When the sample in the first class chromatographic peak has matrix interference, SWATH second degree quantification can effectively reduce the background noise and eliminate interfering ions so that the quantitative results are more accurate and reliable. 190 kinds of standard curve pesticide residues were profiled, and qualitative and quantitative analysis of the 6 samples was performed.

Setting up the SWATH method is very easy. One can directly establish methods by going to SCIEX OS software's built-in SWATH options, and through the Variable Windows, the settings allow the user to intelligently partition the ions' Q1 mass window, to ensure that each ion can receive high quality SWATH data, in order to meet the qualitative and quantitative needs.

References

1. *André Schreiber, SCIEX, Concord, Ontario (Canada),* X500R and SWATH for pesticide screening RUO-MKT-11-4711-A
2. *SCIEX*, European Union Reference Laboratory (EURL-FV) Almeria, Spain and the EMEA team, Analysis of Regulated Pesticides in Baby Food Using SCIEX X500R QTOF

Food and Environmental

Use of X500R QTOF for Monitoring Unexpected Additives in Nutritional Supplements

Zhao Xianglong, Cheng Haiyan, Li Lijun, Jin Wenhai
SCIEX, Pacific Applications Support Center (Guangzhou), China

Introduction

Nutritional supplements can supplement necessary nutrients and are believed to support recovery from illness. Generally, these products promote a particular effect or claimed function[1]; thus, in typical use, people often incorrectly believe they have a definite treatment efficacy. They are often linked to the alleviation of certain illnesses. In order to maximize these functions, manufacturers may add related drugs in order to increase their efficacy without including them as a listed ingredient. According to reports and discoveries from actual monitoring cases, unexpected additives to nutritional supplements are generally selected because they relate to the health product effects or address the additive side effects or functions; the additive usually takes the form of one or more drug additives, drug derivatives, etc.[5] Because these additives are generally high-dose, drug interactions can be unclear. Thus, a great potential hazard exists for human health [2-4]; the China Food and Drug Administration (CFDA) “Health product potential illegal additives list” clearly stipulates monitoring processes for additives in 6 different types of nutritional supplements: those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. The purpose is to protect consumers’ health.

SCIEX’s X500R QTOF high resolution mass spectrometry system can be used for rapid monitoring of additives in nutritional supplements; after sample injection, a first order mass accuracy number and second order fragmentation spectrum are simultaneously obtained. Currently, over 50 additives can quickly be qualitatively confirmed in this way. Matrix interference in complex matrices can be overcome for specific screening of additives; preprocessing is even simpler and more convenient. The new SCIEX OS software fully integrates instrument control, data collection, data handling, and other processes. The workflow is more intuitive and smarter; this method provides an efficient means for rapid, high-throughput monitoring of nutritional supplements for additives.

Experimental Process

1. Collect samples of 6 types of nutritional supplements currently on the market - those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. Perform simple preprocessing.
2. Use TOF MS-IDA MS/MS mode for data collection; after sample injection, obtain first order ion and second order ion fragmentation spectrograms.
3. The mass accuracy number, isotope distribution, retention time, and standard library alignment are used for positive verification of samples and checking the accuracy of sample monitoring results.
4. Monitoring reports systematically summarize sample screening results; the report content can be tailored to specific requirements.

X500R high-resolution mass spectrometry screening workflow:

1. Both TOF-MS-IDA-MS/MS And TOF-MS/MS data gathered in the same injection

2. SCIEX OS is the integral software used to perform this analysis

3. Screening results and report generation

Preprocessing Method

1. Use tablets ground into a powder, granules from inside capsules, or liquid samples; weigh accurately a 1.0gsample, and place in a 10mLcentrifuge tube;
2. Add 5mL acetonitrile and agitate 2 min;
3. Vortex 2 min;
4. Centrifuge at 4℃ at 10000 Rpm for 15min;
5. Dissolve the supernatant 1-fold;
6. Pass through a 0.22μm filter and directly inject sample;

Liquid Phase Conditions

Chromatographic Column: Phenomenex Kinetex C18, 2.1*100mm, 2.6μm;

Elution gradient

Time (min)	A%	B%
0	*95*	*5*
5.0	*55*	*45*
15.0	*20*	*80*
20.0	*5*	*95*
25.0	*5*	*95*
25.1	*95*	*5*
30	*95*	*5*

Positive ion mode: A: 0.1% Formic acid Water; B: 0.1% Formic acid Acetonitrile;

Negative ion mode: A: Water; B: Acetonitrile;

Flow rate: 0.3mL/min;

Column temperature: 40℃;

Amount inserted: 10 μL;

Mass Spectrometry Method

Scanning method: TOF MS-IDA MS/MS

Ion source: ESI source

Scanning range: m/z 50-2000

CUR gas: 30 PSI

Collision gas CAD: 7

IS voltage: 5500V/-4500V

Source temperature: 600℃

Atomizing gas GAS1: 55 PSI

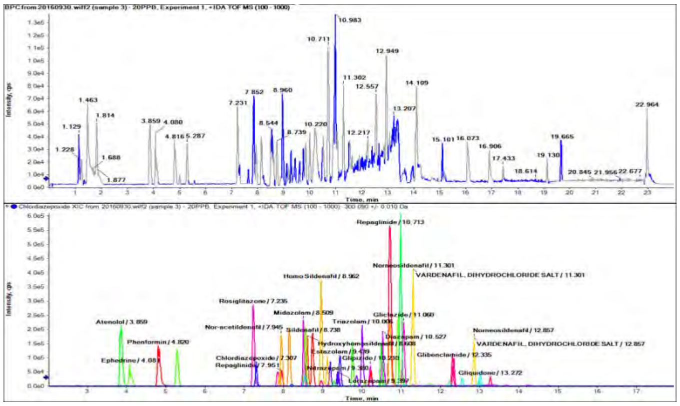
Auxiliary gas GAS2: 70 PSI

DP voltage: ± 60V

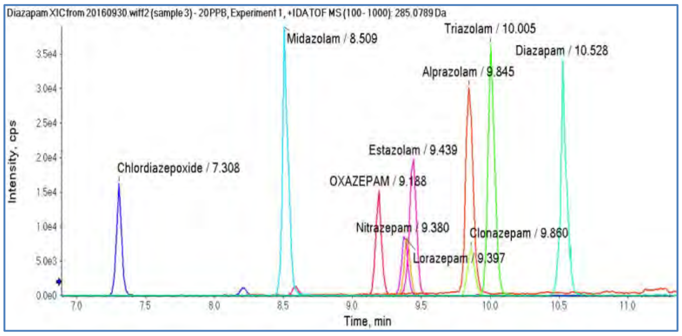
Collision energy: 35 ± 15V

Unexpected Additive Screening Method

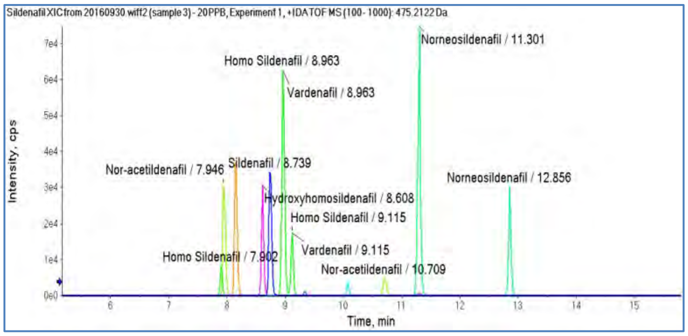
Injection of a single sample simultaneously monitors for over 50 unexpected additives:



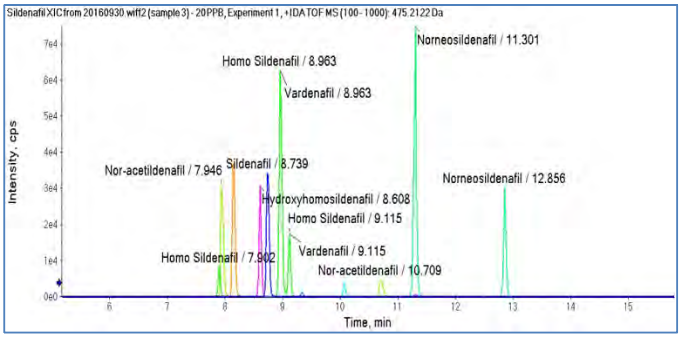
1. 10 sedative-hypnotic mixtures (20ppb), ion extraction flow diagram (XIC) appears below:



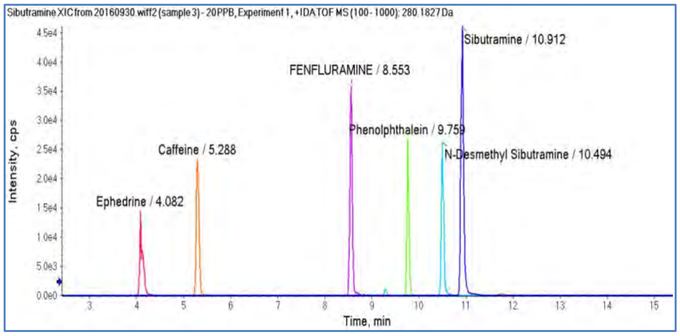
2. 7 blood glucose-lowering drugs (concentration 20ppb); ion extraction flow diagram (XIC) appears below:



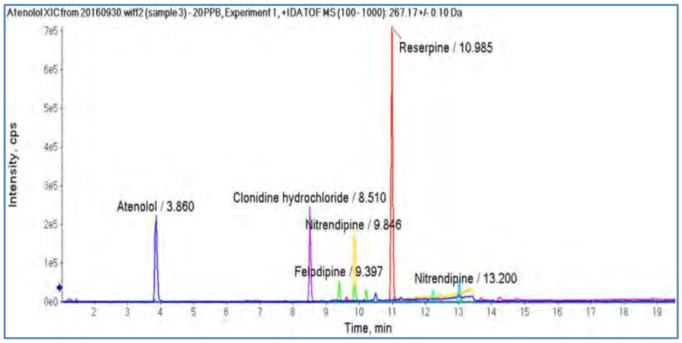
3. 8 impotence drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



4. 6 weight loss drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;

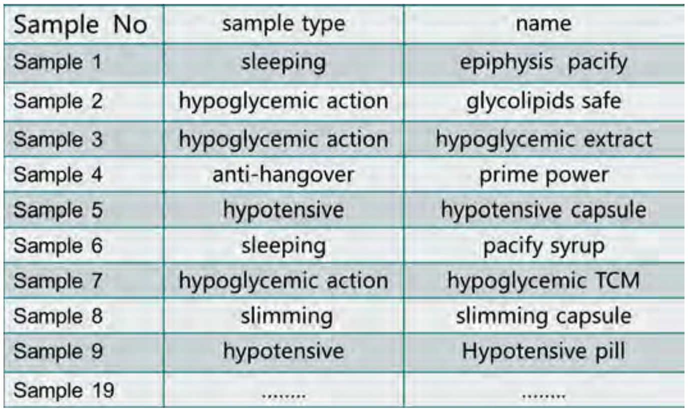


5. 5 blood pressure-lowering drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



Sample Information

Following the CFDA's "Health product potential illegal additives list" 6 different nutritional supplements were randomly selected, including those for weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening. Samples came from 19 different brands;



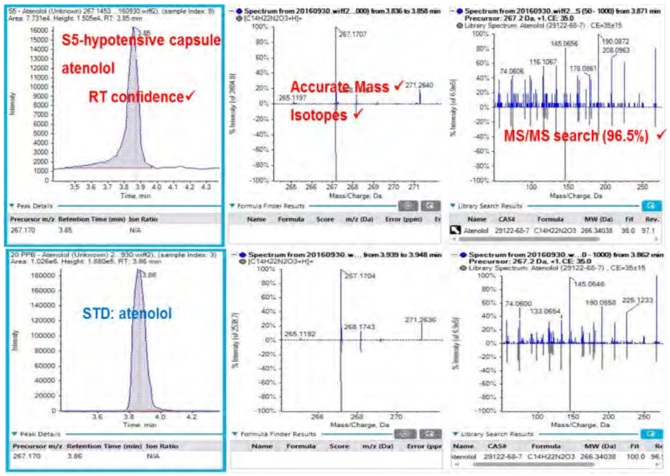
Sample No	sample type	name
Sample 1	sleeping	epiphysis pacify
Sample 2	hypoglycemic action	glycolipids safe
Sample 3	hypoglycemic action	hypoglycemic extract
Sample 4	anti-hangover	prime power
Sample 5	hypotensive	hypotensive capsule
Sample 6	sleeping	pacify syrup
Sample 7	hypoglycemic action	hypoglycemic TCM
Sample 8	slimming	slimming capsule
Sample 9	hypotensive	Hypotensive pill
Sample 19	……..	……..

Experimental Results

Blood Pressure-Lowering Drugs

1. Sample no. 5 - atenolol positive

Sample no. 5 is a blood pressure-lowering capsule; it claims to have a rapid effect and prolonged use can control blood pressure.



Precursor m/z	Retention Time (min)	Ion Ratio
267.170	3.85	N/A

Name	CAS#	Formula	MW (Da)	Fit	Rev.
Atenolol	29122-68-7	C14H22N2O3	266.34038	98.0	97.1

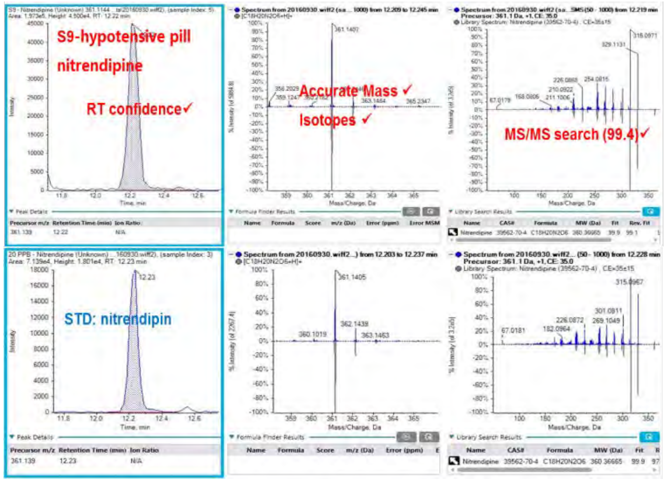
Precursor m/z	Retention Time (min)	Ion Ratio
267.170	3.86	N/A

Name	CAS#	Formula	MW (Da)	Fit	Re
Atenolol	29122-68-7	C14H22N2O3	266.34038	100.0	96.

Screening with the X500R QTOF system showed Sample no. 5 contains large amounts of the additive atenolol. Prolonged use of high-dose atenolol can lead to serious side effects including decreased vision, breathing difficulties, weakness, depression, unexplained rash and ankle swelling and other symptoms.

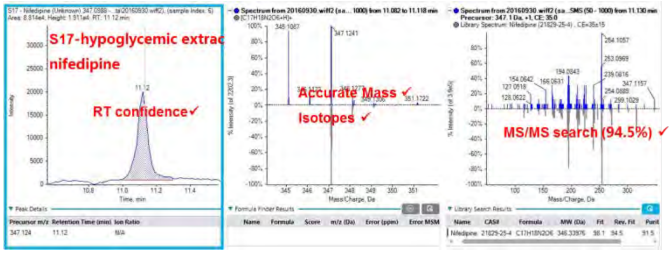
2. Sample no. 9 - nitrendipine positive

Sample no. 9 is from a brand of blood pressure-lowering tablet; screening shows a definite quantity of nitrendipine. The product claims to contain pure and natural extracts with no side effects, but prolonged oral nitrendipine can cause diseases like allergic hepatitis, rash, and even exfoliative dermatitis.



3. Sample no. 17 - nifedipine positive

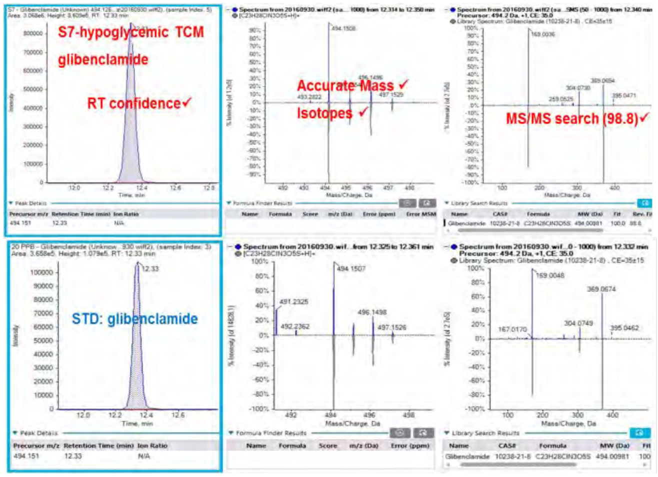
Sample no. 17 is from a brand of blood pressure-lowering Chinese medicine; screening shows a nifedipine additive. It claims to lower blood pressure with Chinese medicine, falsely advertising an anti-hypertensive effect.

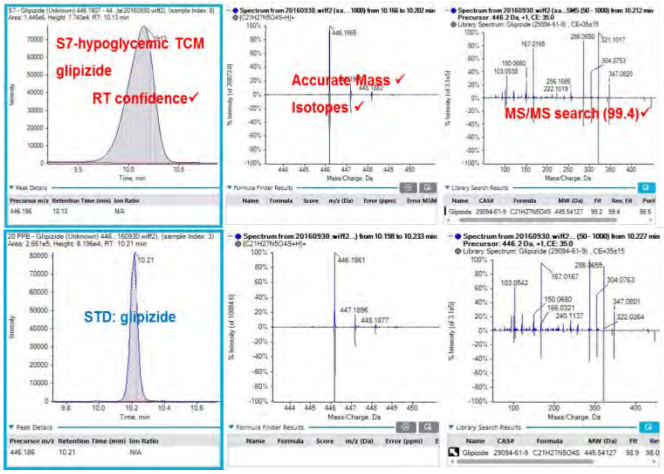


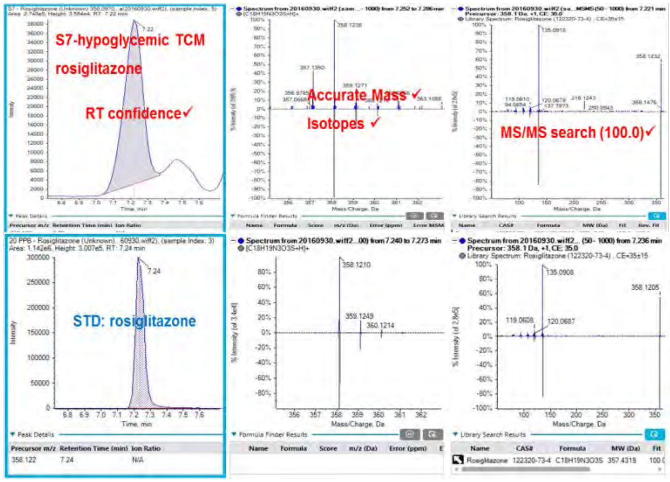
Glucose-Lowering Drugs

1. Sample no. 7 - glibenclamide, glipizide, rosiglitazone positive

Sample no. 7 is a brand of glucose- and lipid-lowering capsule; test results show sample no. 7 contains the 3 glucose-lowering drugs glibenclamide, glipizide, and rosiglitazone as additives. Improper use of sulfonylureas such as glibenclamide and glipizide can cause hypoglycemia; patients can rarely develop rash, erythema multiforme, edema, and liver and kidney damage. Thiazolidinediones like rosiglitazone can cause slight hypersensitivity and mild headache when used incorrectly or at improper doses.

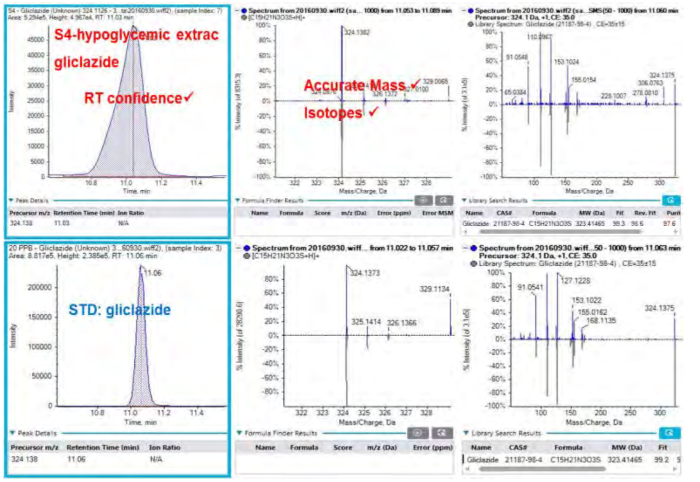






2. Sample no. 4 - Gliclazide positive

Sample no. 4 is a brand of plant extract; it is mainly used to stabilize blood sugar. Screening results show an addition of glicazide, which produces a definitive glucose-lowering effect.

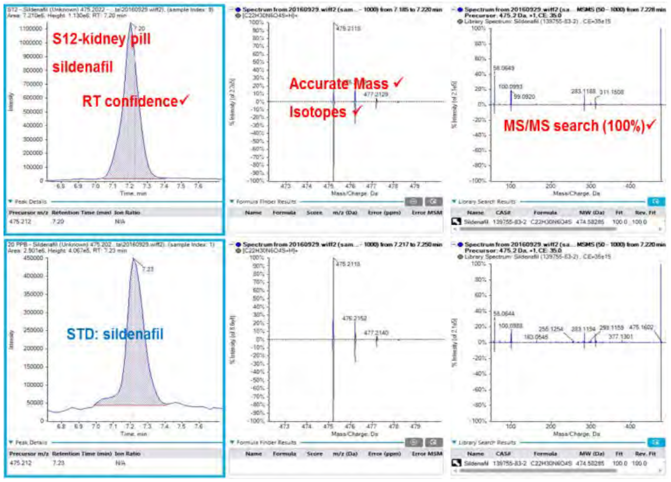


Glucose-lowering drugs are low-cost; they are common "functional components" added to nutritional supplements. These chemical drugs are often used to treat diabetes, as they have a clear hypoglycemic effect. However, their side effects are also quite evident; prolonged use can lead to hypoglycemia and kidney damage, even leading to death.

Anti-Fatigue/Impotence

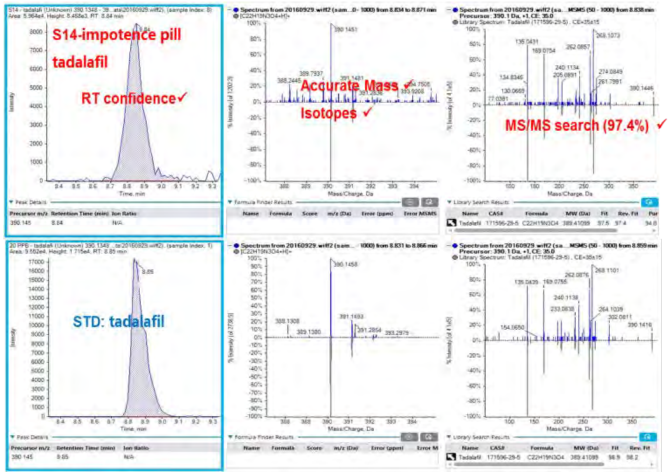
1. Sample no. 12 - sildenafil positive

Sample no. 12 is a kidney health product for the elderly; its description states it is pure Chinese medicine and contains several flavors of medicine. Screening shows an addition of large quantities of sildenafil in order to achieve its claimed kidney effects.



2. Sample no. 14 - tadalafil positive

Sample no. 14 is a brand of impotence health product. Impotence products are the most frequently found to contain additives. In order to accelerate the speed of onset, additives are generally used in large quantities; screening results showed sample no. 14 contained tadalafil.



When not used under the guidance of a specialized physician, prolonged use of nutritional supplements containing "impotence" additives can severely harm the body. Side effects can include dizziness, fainting, and even hearing loss.

Screening results appear in the table:

1. The problem of additives in nutritional supplements is widespread; additives appear in many samples;

2. Blood sugar- and pressure-reducing products contain many different additives; they generally take the form of multiple drugs, and use of Chinese medicine is especially serious.

3. Anti-fatigue and impotence health care products generally contain large amounts of additives;

Sample No	sample name	positvie results
Sample 1	epiphysis pacify	--------
Sample 2	glycolipids safe	--------
Sample 3	hypoglycemic action	--------
Sample 4	hypoglycemic extrac	gliclazide
Sample 5	hypotensive capsule	atenolol
Sample 6	pacify syrup	--------
Sample 7	hypoglycemic TCM	glipizide、rosiglitazone、glibenclamide
Sample 8	slimming capsule	--------
Sample 9	hypotensive pill	nitrendipine
Sample 12	kidney pill	sildenafil
Sample 14	impotence pill	tadalafil
Sample 17	hypoglycemic extrac	nifedipine

Summary

This study randomly selected 19 nutritional supplements commonly found on the market; these covered 7 glucose- and blood pressure-lowering products, 5 anti-fatigue, anti-impotence products, 4 sleep aids, and 3 weight loss products. Screening results showed that blood pressure-lowering and glucose-lowering products most commonly contained additives, especially those products advertised to use Chinese medicine extracts to lower blood sugar. Representative samples of blood pressure-lowering capsules showed a high rate of positive results. The main additives were atenolol, nitrendipine, nifedipine, glibenclamide, glipizide, rosiglitazone, gliclazide and other inexpensive and readily available glucose- and blood pressure-lowering drugs, impotence, anti-fatigue/immune system-enhancing additives were generally sildenafil or tadalafil. Additives take the form of one or many drugs; some additives are present in amounts several times therapeutic doses. Thus, they can be quite hazardous to consumer health.

The SCIEX X500R QTOF high resolution mass spectrometry system was used for rapid monitoring of 50 different additives in 6 types of nutritional supplements. Its high sensitivity detected small concentrations of additives, its rapid scanning and effective overcoming of complex matrix interference ensure that after sample injection, a first order mass accuracy number (TOF-MS) and second order fragmentation spectrum (TOF-MS/MS) are simultaneously obtained. Combined with the high-quality additive library, accurate qualitative screening for additives in complex matrices can be performed.

Health product additive screening methods using the X500R QTOF system are reliable, simple, and rapid. The system provides an efficient approach to additive screening of nutritional supplements, and it ensures health and safety product quality; it is critical in the fight against the use of potentially harmful additives.

References

[1] State Bureau of Technical Supervision. GB 16740-1997 General Standards for Health Food (Functions) [S]. Beijing: China Standards Press, 1997.

[2] Yin Shi'an, Wang Yin. Discussion of Health Food Safety [J]. Chinese Journal of Preventive Medicine, 2006, 40(2): 141-143.

[3] Wang Lanming. Analysis and Thoughts on China's Food Safety Management Work [J]. Food Science, 2004, 25(7): 187-192.

[4] Huang Xianglu, Wang Jingwen, Cao Jin. Monitoring and analysis of illegal additives in blood sugar-lowering health foods [J]. Food Science, 2014, 35(4): 149-151.

[5] Virginia M. Wheatley, John Spink. Defining the Public Health Threat of Dietary Supplement Fraud, Comprehensive Reviews in Food Science and Food Safety, 2013, 10.1111/1541-4337.12033.

Food and Environmental

Use of X500R QTOF for Monitoring of Additives in Cosmetics

Yang, Cheng Haiyan, Li Lijun, Jin Wenhai
SCIEX, Pacific Applications Support Center (Beijing), China

Introduction

With the gradual increase in people's living standards, cosmetics have become a necessity for daily life. As they are used at every stage of life from infancy into old age, their functions have gradually evolved from the traditional areas of cleanliness and skin care to the domains of beautification and beauty enhancement[1]. Some businesses add undocumented ingredients to cosmetic products to enhance their function and attract consumers. The use of these additives can potentially cause many adverse health effects in people. Issues with the safe use of cosmetics have attracted the attention of domestic and international research teams. The European Union Cosmetics Regulation EC 1223/2009[2] and China's "Sanitary Regulations for Cosmetics" [3] both clearly prohibit the use of hormones, antibiotics, and disinfectants in cosmetics. Because the cosmetics market is wide-ranging, and prohibited additives are quite diverse, we have established a highly selective, high-throughput method for rapid screening and quantification of prohibited drugs in cosmetics. It allows relevant teams to significantly improve their monitoring capabilities and consumer protection.

The benefits of this method are as follows:

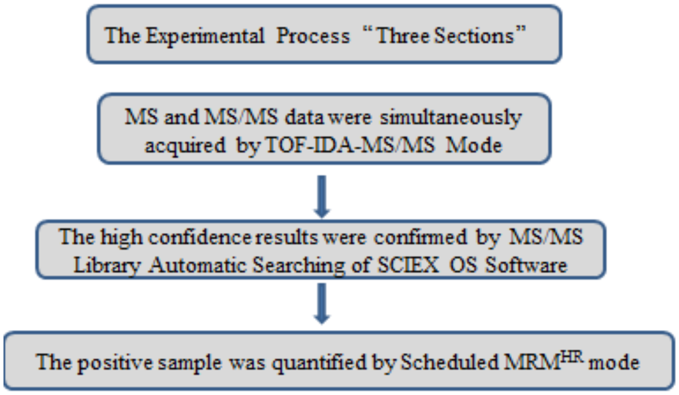
1. In this method, sample preparation is simple and efficient; for 35 samples, it takes only 2 hours from preprocessing to the start of testing. The previous obstacle of tedious procedures such as the need for SPE purification during preprocessing has been resolved;
2. The scope of compounds that may be monitored is wide; there are 240 total illegal additives commonly found in cosmetics, including glucocorticoids, disinfectants, tetracyclines, and hormones.
3. One sample injection can be tested for 200 compounds in a testing time of 24 min., which is fast and efficient.
4. Performing simultaneous identification and quantification in the truest sense, the X500R system has a spectral library of prohibited cosmetics additives and four stages (first-order error, retention time, isotope abundance matching, spectral library matching) for identification purposes. It is simple and fast and ensures accurate, reliable screening results. Concerning quantification, the X500R system MRM^{HR} ion matching quantification is comparable to quadrupole MRM.
5. This method establishes retention time stage, screening sequences, and spectral libraries for 240 compounds; all can be used directly, saving method development time and costs and acting as a powerful cosmetics monitoring tool.

Experimental Process

Data collection: Using TOF-IDA MS/MS mode, inject a sample and simultaneously obtain primary precursor ions and secondary product ions; work time can be saved and efficiency increased via standard library matching of two sub-ions.

Positive sample determination: Using library search, with positive match and reverse match, determine the overall degree of match. Efficiently eliminate false positive results, making qualitative results more accurate and reliable.

Positive sample accurate quantification: Because of the high selectivity and high throughput of the X500R *Scheduled* MRM^{HR}, its scanning mode allows accurate quantification of prohibited additives found in positive samples.



Automated monitoring report generation: Include sample screening library search results and quantitative results; report formats can be tailored to your needs.

Preprocessing method:

1. Emulsions, solid samples, liquid samples: Weigh accurately 0.5g or 0.5mL into a 15mL centrifuge tube;
2. Add 10 mL of 85% aqueous methanol and agitate vigorously for 2 min;
3. Vortex 2 min;
4. Centrifuge at 4℃ at 10000 Rpm for 10 min;
5. Dissolve the supernatant in ultrapure water 1-fold;
6. Pass 1 mL through a 0.22μm filter and insert for testing;

Liquid phase conditions:

Chromatographic Column: Phenomenex Kinetex C18, 2.1*100mm, 1.7μm;

Mobile phase: Gradient elution was used

Positive ions: A is 0.1% formic acid; B is acetonitrile;

Negative ions: A is ultrapure water; B is acetonitrile;

Flow rate: 0.3 mL/min;

Column temperature: 40 ℃;

Amount injected: 10 μL;

Mass spectrometry method:

Scanning method: TOF-IDA MS/MS qualitative screening;

Scanning method: *Scheduled* MRM^{HR} positive sample accurate quantification;

ESI ion source parameters:

Air curtain gas CUR: 30 psi;

Collision gas CAD: 7 psi;

IS Voltage: 5500V/-4500 V;

Source temperature: 600 ℃;

Atomizing gas GAS 1: 55psi;

Auxiliary gas GAS 2: 70psi;

Compound information:

Table 1 240 prohibited additives - information

Glucocorticoid	Name	Molecular Formula	CAS
1	Triamcinolone	$C_{21}H_{27}FO_6$	124-94-7
2	Prednisolone	$C_{21}H_{28}O_5$	50-24-8
......42 species	......	......	......

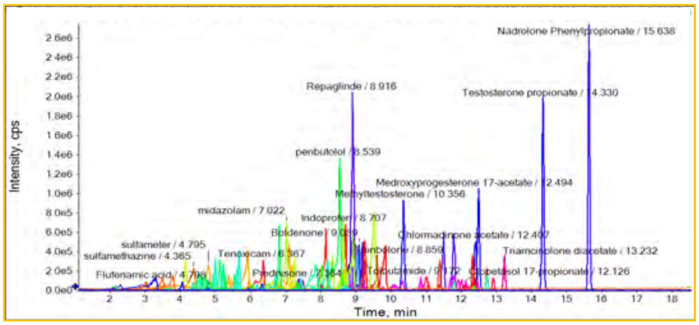
Sulfonamides and Xacin	Name	Molecular Formula	CAS
1	Sulfadiazine (SDZ)	$C_{10}H_{10}N_4O_2S$	68-35-9
2	Lomefloxacin	$C_{17}H_{19}F_2N_3O_3$	98079-51-7
......181 species	......	......	......

Tetracycline	Name	Molecular Formula	CAS
1	Tetracycline	$C_{22}H_{24}N_2O_8$	60-54-8
2	Oxytetracycline	$C_{22}H_{24}N_2O_9$	79-57-2
......9 species	......	......	......

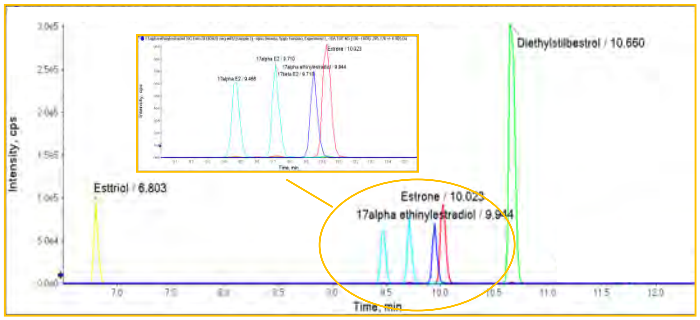
Sexhormone	Name	Molecular Formula	CAS
1	Estradiol	$C_{18}H_{24}O_2$	50-28-2
2	Estriol	$C_{18}H_{24}O_3$	50-27-1
......8 species	......	......	......

Chromatogram:

Positive ion mode: For 200 prohibited cosmetics additives (5 ng/mL), including sulfonamides, quinolones, glucocorticoids, androgens, product ion chromatograms from precursor ions are below:



Negative ion mode: 8 estrogens (5 ng/mL) product ion chromatograms from precursor ions appear below:



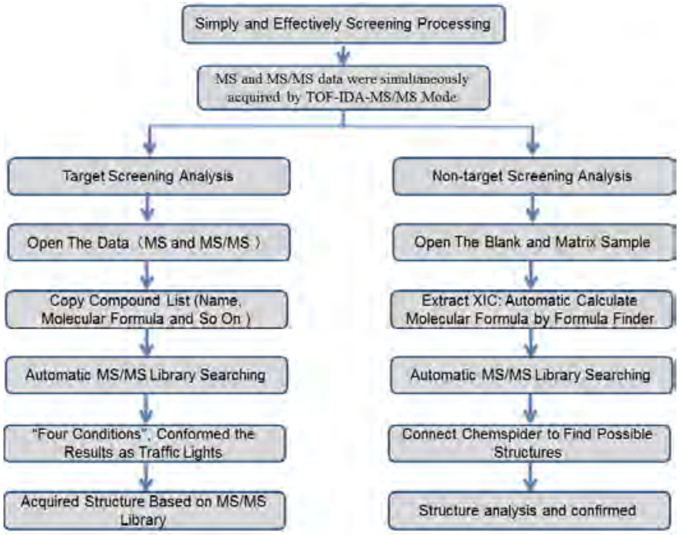
Sample Collection

Following the definition of "cosmetics" in the national "Sanitary Regulations for Cosmetics," 35 cosmetic products of different types, brands, and functions were collected; the products principally came from colleagues, Taobao, small companies, market stalls, free samples, hotels, etc.

Facial Mask	mulsion、Humectant、cream
Hei Li Tou Bai Dong	Ji Yuan Heng Run
Zai Sheng Pian	Kang Zhou Yang Yan Bao Shi
Mei Yan Xiu Hu Yin Xing	Qing Shuang Mei Bai
Ji Ling Duo Xiao Chan Si	Bao Shi Shen Ceng
Dong Li Bu Shui	Bao Shi Water
…… and so on	……and so on

Screening Flowchart

Simple, efficient screening flowchart: Includes analysis of target unknowns and complete unknowns.

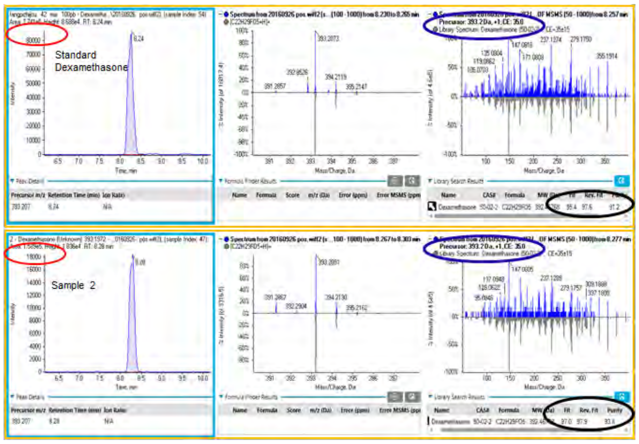


Screening Results

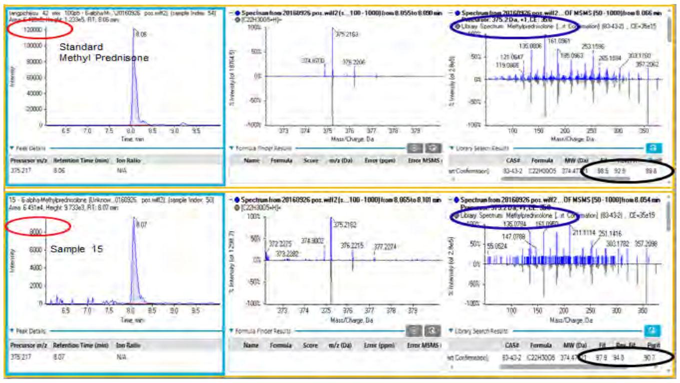
1. Glucocorticoids:

Mask sample No. 2 contains the glucocorticoid dexamethasone, Mask sample No. 15 contains the glucocorticoid methylprednisolone, and Mask sample No. 17 contains the glucocorticoid prednisone. Chromatograms are as follows:

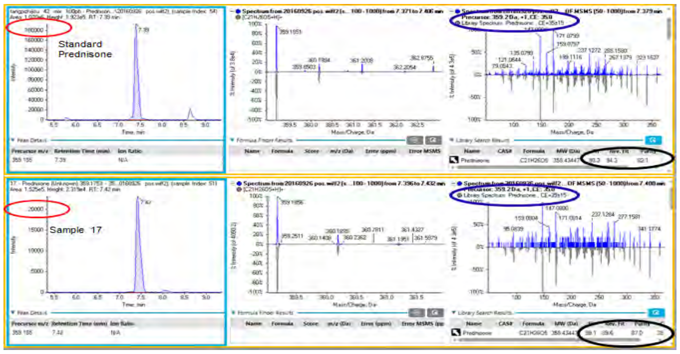
Mask sample no. 2 chromatogram:



Mask sample no. 15 chromatogram:



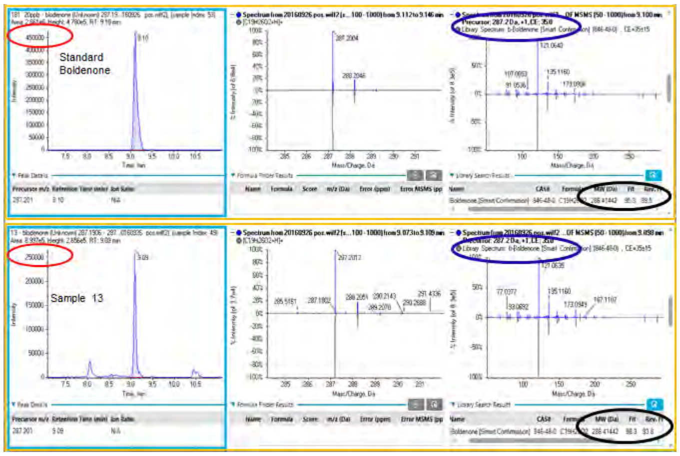
Mask sample no. 17 chromatogram:



Hazard: Studies show that glucocorticoids can influence glucose, lipids, protein biosynthesis and metabolism. Clinically, glucocorticoids act as anti-infectious drugs and can inhibit fibroblast proliferation; they decrease serotonin formation and have a definitive skin-whitening effect. However, long-term use of cosmetics containing these may lead to skin thinning, capillary expansion, and hair follicular atrophy; once use is discontinued, skin may redden, itch, become flushed, and develop papules, desquamation, and other time-dependent dermatitis. Excessive use might also cause an increase in blood sugar, hypertension, osteoporosis, decreased immune function, obesity, and other hazards. Thus, China's "Sanitary Regulations for Cosmetics" and the EC cosmetics regulations state clearly that glucocorticoids are prohibited from cosmetics.

2. Sex hormones:

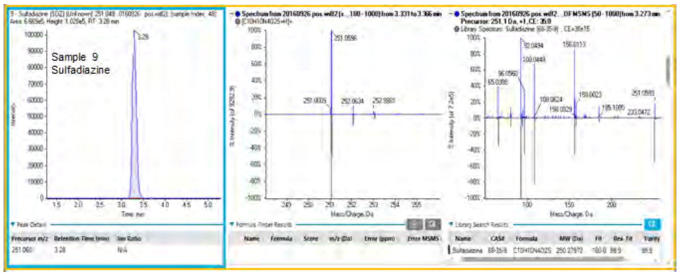
No. 13 Emulsion sample containing the androgen progesterone. The chromatogram is as follows:



Hazards: Progesterone is a hormone used in the treatment of infertility and menopause. It's topical application is believed to promote skin elasticity, but it's long term adverse effects have halted it's use. Chinese cosmetics standards, cosmetics sanitary regulations, and EC cosmetics regulations clearly state that androgens are prohibited from cosmetics. These regulations serve to prevent consumers from unknowingly using cosmetics with prohibited androgen additives, a health safety hazard.

3. Sulfonamide antibiotics:

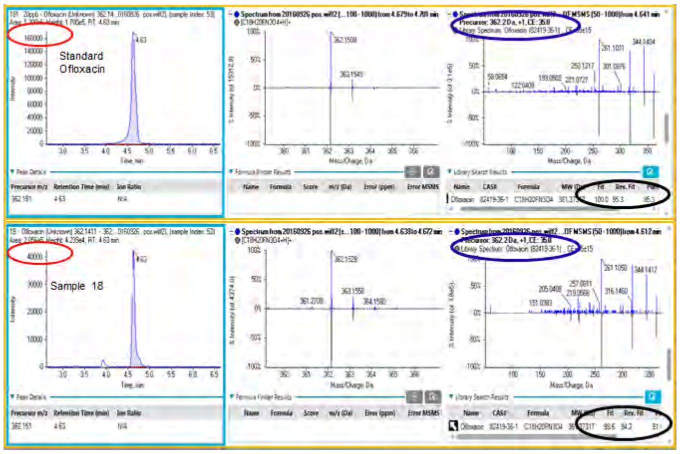
Sample No. 9- Moisturizing lotion containing sulfonamidopyrimidine Sample No. 9- Moisturizing lotion chromatogram:



Hazard: Sulfonamide drugs are mainly used to prevent and treat bacterial infections. They have a wide antimicrobial spectrum, are chemically stable, convenient to use, easy to manufacture, etc. Their bactericidal activity is elaborated through inhibition of bacterial growth and proliferation by disruption of folic acid metabolism. Clinical research shows that sulfonamides have a definitive anti-acne and anti-pimple function. However, because sulfonamides have a relatively long in vivo acting time and half-life, long-term use can cause accumulation in the human body, producing human health hazards and leading to drug-resistant pathogens. Based on the Chinese "Sanitary Standard for Cosmetics," "Sanitary Regulation for Cosmetics," EU Cosmetics Regulation (Council Directive 76/768/EEC), and Japan's Pharmaceutical Affairs Law, sulfonamides are prohibited from use as components in cosmetics.

4. Quinolone antibiotics:

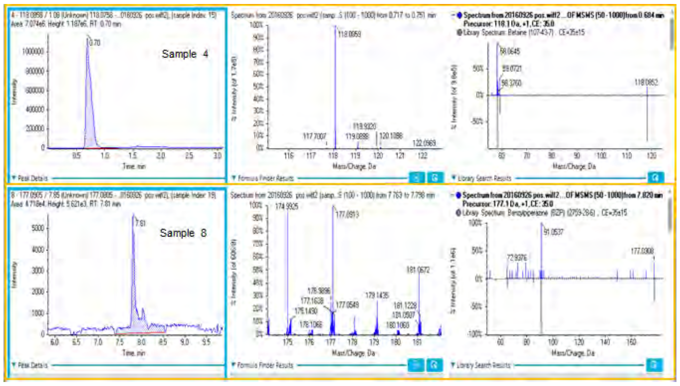
Sample No. 18 - Cleansing emulsion containing ofloxacin. The chromatogram follows:



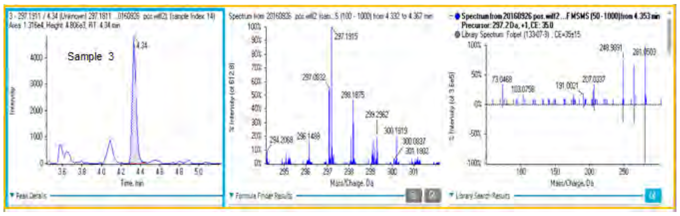
Hazard: Ofloxacin is a quinolone drug. This drug is quite effective as an antibiotic, and it is especially active against gram-negative bacteria and other antibiotic-resistant bacteria. It has a wide spectrum of activity, is well absorbed, easy to take, and low-cost. It is used for treating a wide variety of clinical infections. However, when this drug is added to cosmetics and distributed over the body surface, particularly on the face, lips, and other sensitive skin and blood vessel-rich mucosal surfaces, it may undergo rapid absorption through capillaries or mucus membranes and gradually damage the normal mucosa of the skin surface, causing adverse reactions like rash and rapid-type hypersensitivity.

5. Other components: Not all cosmetics contain hazardous ingredients, of course. There are also nutritive components.

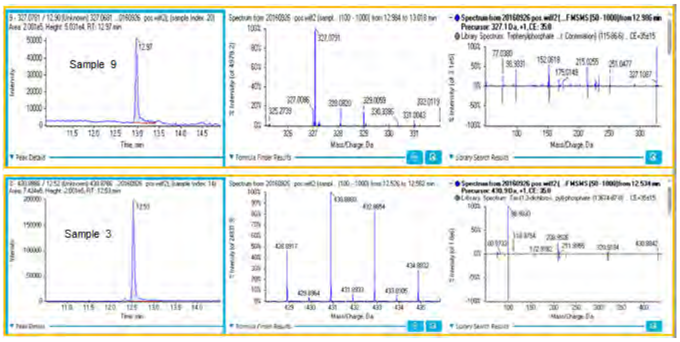
a. For example, Sample No. 4 moisturizer contains betaine, which is a water-soluble disinfectant. It is not harmful to skin and is commonly found in shaving cream. Sample No. 8 is a high-end brand of Hydrosol containing benzyl piperazine pharmaceutical intermediates, which improve anti-allergy function and enhance the effectiveness of the immune system; the chromatogram is below:



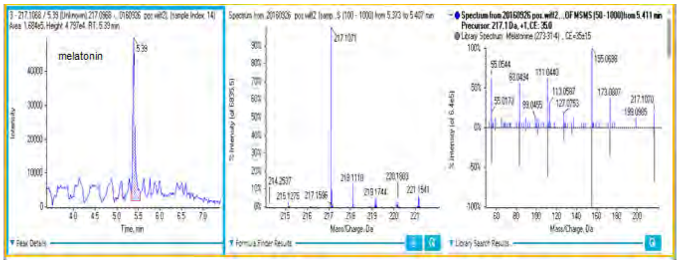
b. For example, sample no. 3 contains folpet, which is a disinfectant with a broad spectrum of protection. Its human and animal toxicities are low, but it is a human mucus membrane irritant and is toxic to fish. It stimulates plant growth and development. It is relatively insoluble in water at room temperature but is soluble in alkali or at high temperatures.



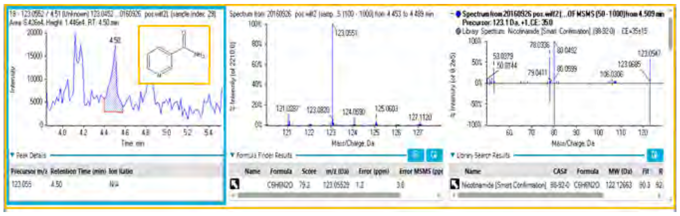
c. Most cosmetics contain triphenyl phosphate compounds; these are flame retardants and stabilizers, according to the literature: These cosmetics are nonvolatile and make skin translucent, soft, resilient, and very light-stable. In addition, they are not readily absorbed by the skin and are not prohibited under the cosmetics health regulations;



d. In addition, most cosmetics contain melatonin, which has a whitening effect; the literature reports that melatonin can regulate natural sleep/wake cycles, counter aging, improve immune function, prevent cancer, etc. Toxicity in humans has not been observed;



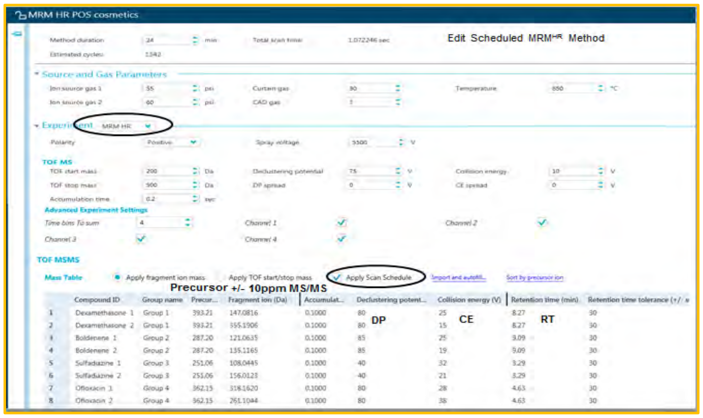
e. Many cosmetics contain vitamin B3, nicotinamide. This is a known nutrient in cosmetics.



This component has 3 main functions: first, it can accelerate metabolism, promote the sloughing of melanin-containing keratinocytes, thus improving whitening. Second, it can act on previously produced melanin, decreasing its outward cell migration towards the surface. Third, it can promote epidermal protein synthesis, improving skin texture and moisture, and countering aging. Nicotinamide has some drawbacks; the most significant is that the impurity nicotinic acid in it can be stimulating to the skin. Some consumers may experience allergic reactions like skin reddening after using products with high nicotinamide content. This is widely seen as an unacceptable problem with nicotinamide. Thus, nicotinamide use in cosmetics is still under investigation in terms of impurity control; formulation design and process also have an influence on this.

Quantitative Aspects

Quantitative method establishment: Use quadrupole ion pair mode:



Method duration: 24 min
Total scan time: 1.072246 sec
Estimated cycles: 1342

Ion source gas 1: 55 psi
Curtain gas: 30
Temperature: 550 °C
Ion source gas 2: 60 psi
CAD gas: 7

Experiment: MRM HR
Polarity: Positive
Spray voltage: 5500 V

TOF start mass: 200 Da
Declustering potential: 75 V
Collision energy: 10 V
TOF stop mass: 500 Da
DP spread: 0 V
CE spread: 0 V
Accumulation time: 0.2 sec

Time bins To sum: 4
Channel 1 ☑
Channel 2 ☑
Channel 3 ☑
Channel 4 ☑

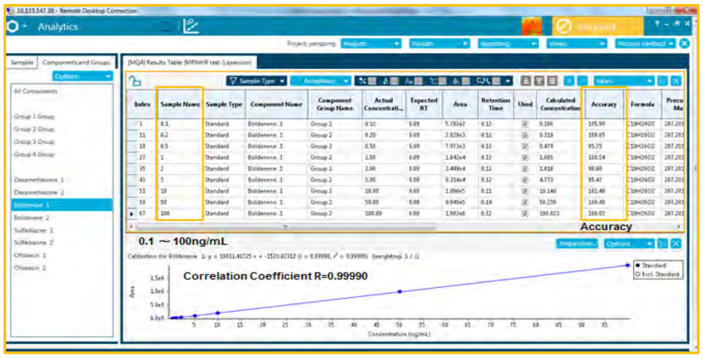
Mass Table ● Apply fragment ion mass ○ Apply TOF start/stop mass ☑ Apply Scan Schedule

	Compound ID	Group name	Precur...	Fragment ion (Da)	Accumulat...	Declustering potent...	Collision energy (V)	Retention time (min)	Retention time tolerance (+/- s
1	Dexamethasone 1	Group 1	393.21	147.0816	0.1000	80	25	8.27	30
2	Dexamethasone 2	Group 1	393.21	355.1906	0.1000	80	15	8.27	30
3	Boldenene 1	Group 2	287.20	121.0635	0.1000	85	25	9.09	30
4	Boldenene 2	Group 2	287.20	135.1165	0.1000	85	19	9.09	30
5	Sulfadiazine 1	Group 3	251.06	108.0445	0.1000	40	32	3.29	30
6	Sulfadiazine 2	Group 3	251.06	156.0123	0.1000	40	21	3.29	30
7	Ofloxacin 1	Group 4	362.15	318.1620	0.1000	80	28	4.63	30
8	Ofloxacin 2	Group 4	362.15	261.1044	0.1000	80	38	4.63	30

Quantitative results:

Use *Scheduled* MRM^{HR} scanning mode for accurate quantification; the standard curve is as follows:

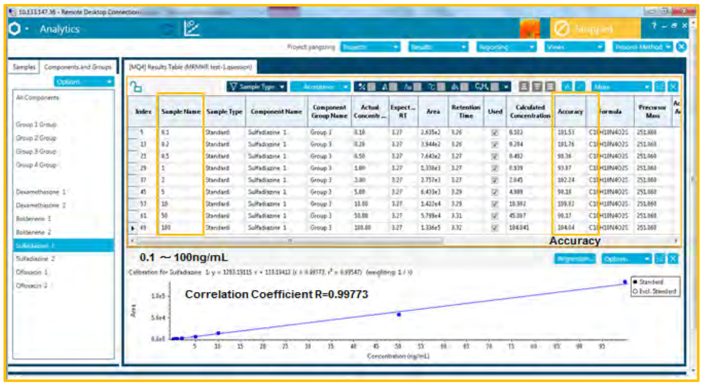
Androgen progesterone



Index	Sample Name	Sample Type	Component Name	Component Group Name	Actual Concentrati...	Expected RT	Area	Retention Time	Used	Calculated Concentration	Accuracy	Formula
1	0.1	Standard	Boldenene 1	Group 2	0.10	9.09	5.792e2	9.12	☑	0.106	105.90	C19H26O2
11	0.2	Standard	Boldenene 1	Group 2	0.20	9.09	2.028e3	9.11	☑	0.219	109.65	C19H26O2
19	0.5	Standard	Boldenene 1	Group 2	0.50	9.09	7.973e3	9.13	☑	0.479	95.75	C19H26O2
27	1	Standard	Boldenene 1	Group 2	1.00	9.09	1.842e4	9.13	☑	1.005	100.54	C19H26O2
35	2	Standard	Boldenene 1	Group 2	2.00	9.09	3.449e4	9.12	☑	1.816	90.80	C19H26O2
43	5	Standard	Boldenene 1	Group 2	5.00	9.09	9.314e4	9.12	☑	4.773	95.47	C19H26O2
51	10	Standard	Boldenene 1	Group 2	10.00	9.09	1.996e5	9.11	☑	10.140	101.40	C19H26O2
59	50	Standard	Boldenene 1	Group 2	50.00	9.09	9.948e5	9.14	☑	50.238	100.48	C19H26O2
67	100	Standard	Boldenene 1	Group 2	100.00	9.09	1.982e6	9.12	☑	100.023	100.02	C19H26O2

Calibration for Boldenene 1: $y = 19831.41325 x + -1520.92312$ ($r = 0.99990$, $r^2 = 0.99980$) (weighting: 1 / x)

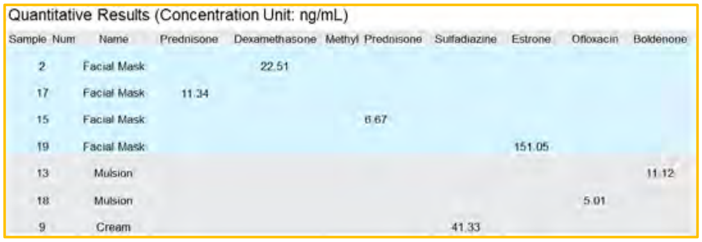
Sulfadiazine



Index	Sample Name	Sample Type	Component Name	Component Group Name	Actual Concentr...	Expect... RT	Area	Retention Time	Used	Calculated Concentration	Accuracy	Formula	Precursor Mass
5	0.1	Standard	Sulfadiazine 1	Group 3	0.10	3.27	2.635e2	3.26	☑	0.102	101.53	C10H10N4O2S	251.060
13	0.2	Standard	Sulfadiazine 1	Group 3	0.20	3.27	3.944e2	3.26	☑	0.204	101.76	C10H10N4O2S	251.060
21	0.5	Standard	Sulfadiazine 1	Group 3	0.50	3.27	7.643e2	3.27	☑	0.492	98.36	C10H10N4O2S	251.060
29	1	Standard	Sulfadiazine 1	Group 3	1.00	3.27	1.338e3	3.27	☑	0.939	93.87	C10H10N4O2S	251.060
37	2	Standard	Sulfadiazine 1	Group 3	2.00	3.27	2.757e3	3.27	☑	2.045	102.24	C10H10N4O2S	251.060
45	5	Standard	Sulfadiazine 1	Group 3	5.00	3.27	6.403e3	3.29	☑	4.989	99.78	C10H10N4O2S	251.060
53	10	Standard	Sulfadiazine 1	Group 3	10.00	3.27	1.422e4	3.29	☑	10.992	109.92	C10H10N4O2S	251.060
61	50	Standard	Sulfadiazine 1	Group 3	50.00	3.27	5.799e4	3.31	☑	45.087	90.17	C10H10N4O2S	251.060
69	100	Standard	Sulfadiazine 1	Group 3	100.00	3.27	1.336e5	3.32	☑	104.041	104.04	C10H10N4O2S	251.060

Calibration for Sulfadiazine 1: $y = 1283.19115 x + 133.19412$ ($r = 0.99773$, $r^2 = 0.99547$) (weighting: 1 / x)

Quantitative results:



Quantitative Results (Concentration Unit: ng/mL)

Sample Num	Name	Prednisone	Dexamethasone	Methyl Prednisone	Sulfadiazine	Estrone	Ofloxacin	Boldenone
2	Facial Mask		22.51					
17	Facial Mask	11.34						
15	Facial Mask			6.67				
19	Facial Mask					151.05		
13	Mulsion							11.12
18	Mulsion						5.01	
9	Cream				41.33			

The quantitative results table shows:

1. According to requirements of the “Sanitary Regulations for Cosmetics”: All compounds in the table are illegal additives;

2. Glucocorticoids are widespread throughout masks;

3. Many hormones, sulfonamides, quinolones, and other disinfectants are found in emulsions and creams;

4. The major brand names are not excluded; they too may add prohibited substances;

Summary

This study collected 35 common cosmetics, including 10 different brands of mask and 25 types of emulsions, moisturizers, and creams. Experimental results show many glucocorticoids are found in masks, and other cleaning emulsions and moisturizers contain hormones and antibiotics. In addition, several cosmetics were found to contain nutritive components: for example, vitamin B3, melatonin, etc.; a high-end brand of hydrosol contains benzyl piperazine pharmaceutical intermediates. These promote immune function.

This article uses the novel X500R high resolution mass spectrometer to establish screening and quantification techniques for prohibited additives in cosmetics via liquid chromatography tandem mass spectrometry. Because X500R has a highly sensitive and strong anti-matrix interference capability, sample preprocessing is simplified, increasing work efficiency. This method is widely applicable; it includes 240 illegal substances commonly found in cosmetics. It has a dedicated spectral cosmetics library; in combination with rapid X500R scanning, it can simultaneously collect the most abundant and highest quality primary and secondary spectrograms from complex matrices. This ensures screening results are accurate and reliable. Because X500R sensitivity to prohibited additives in cosmetics is on the nanogram level and below, the X500R system’s MRM^{HR} quantitative analysis capability is comparable to that of quadrupole MRM.

This method is fully compliant with customer requirements; application of a high resolution mass spectrometer to screen for and monitor prohibited additives in cosmetics is a significant advance.

References

1. Journal of Chromatography A, Volume 1461, 26 August 2016, Pages 78 – 83;

2. EU cosmetics regulation (Regulation EC 1223/2009);

3. PRC “Sanitary Regulation for Cosmetics” (2015);

Document number: RUO-MKT-02-5389-A

Food and Environmental

Rapid and Sensitive Analysis of Antibiotics in Children's Urine Using the X500R QTOF System

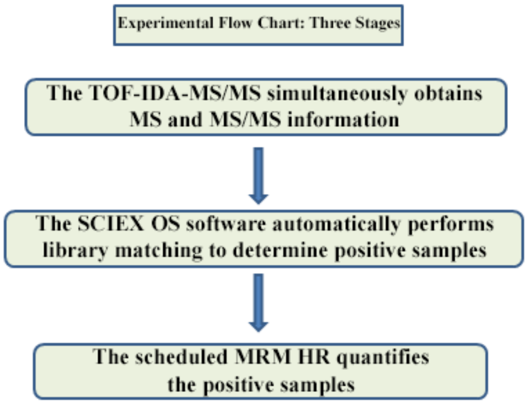
Binjie Liu, Haiyan Chen, Lei Yi, Wan Wang, Lijun Li, Wenhai Jin
SCIEX, Asia & Pacific area Application Support Center(Beijing), China

Introduction

Antibiotics are a class of secondary metabolites produced by microorganisms (including bacteria, fungi, and actinomycetes) which restrain the growth and survival of other microorganisms, as well as analogous compounds that are chemically synthesized or semi-synthesized. In 1928, Alexander Fleming discovered penicillin and officially began using it in clinical settings in 1941, marking the arrival of antibiotic era. In recent decades, antibiotics have evolved by leaps and bounds, with more than 200 varieties on the market. The annual use of antibiotics is alarming and amounts to tens of tons, including not only human antibiotics, but also large amounts used in animal breeding. The abuse of antibiotics has caused serious pollution to food and the environment. It is widely known that many antibiotics can cause liver and kidney damage, allergies, local irritation, and other side effects to the human body. Because the physiological functions of childrens' organs are still immature, direct and indirect antibiotic exposure may cause negative effects on the development of their bodies. Aside from clinical and prescribed medication, children also are more likely to ingest antibiotics from meat, eggs, milk, other food, and water pollution. Therefore, the monitoring of level of antibiotics in biological samples has become a major research area.

In this study, we use the SCIEX X500R **Quadrupole-Time of Flight Mass Spectrometer** to build screening and quantitative methods. Urine samples containing more than 200 types of antibiotics from hundreds of children were screened, and the positive samples were quantitatively analyzed.

The X500R **Quadrupole-Time of Flight Mass Spectrometer system** features the world's fastest sampling rate, the intelligent TOF MS-ID / MS / MS sampling mode, and a stable and durable ion source, making it ideal for the analysis of a large number of complex matrix samples (Figure 1). This system truly achieves the goal of instant, comprehensive collection of high-quality primary and secondary mass spectrometry data by needle injection. It allows the target compound to pass the screening of "Four Critical Points": precursor mass accuracy, compound isotope pattern, retention time, and high-precision mass accuracy, providing the most accurate qualitative screening in a timely manner. SCIEX has a professional spectral library with hundreds of antibiotics, which delivers added confirmatory proof of the analyte detected.



In addition, the excellent sensitivity of the SCIEX X500R and unique *Scheduled* MRM^{HR} function is comparable to the quantitative function of triple quadrupole (see Figure 2). The experiment procedures are as follows:

Sample Collection

A total of 114 urine samples were collected. 55 boys and 59 girls from Beijing participated, 12 of whom were 2-5 years old and 110 of were 8-11 years old. Samples were from from morning urine. Samples were immediately stored in a -80 refrigerator after collection.

Pretreatment Method

1. Sample hydrolysis

Urine hydrolysis: 1000 µL juvenile urine, +200 µL ammonium acetate buffer solution (pH 5.0) +15 µL glucuronidase. Allow the mixture to undergo hydrolysis reaction at 37°C overnight. Add 100 µL of McIlvaine-Na2 EDTA buffer to mixture.

2. SPE extraction

1) **Activate:** Water-activate the Cleanert MAS-MIX SPE cartridge (60 mg / 3 mL) using 1 mL of methanol and 1 mL of water.

2) **Sample loading:** Load 1300 µL of urine hydrolysate sample.

3) **Leaching:** Leach with 1 mL of 5% aqueous methanol

4) **Elution:** 2 mL (ammonia: methanol: water) = 5: 85: 10

5) **Dissolution after nitrogen blowdown:** 35 nitrogen blow to dry, 20% methanol dissolved in water to 200 µL, mix with sample.

Aqueous phase conditions

Chromatographic column: Phenomenex Analytical Column, Kinetex 2.6 µm F5 100 Å, 100 X 3.0 mm

Mobile phase Phase A consists of 0.1% formic acid; Phase B consists of 0.1% acetonitrile

Flow rate: 0.4 mL / min

Column temperature: 40ºC

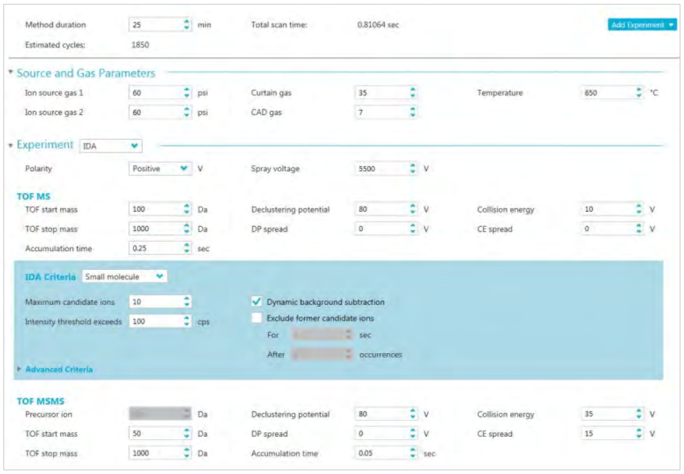
Mass Spectrometry Method

Scanning method: TOF-IDA-MS/MS qualitative screening

Scanning method: *Scheduled* MRM^{HR} positive sample accurate quantification

ESI ion source parameters:

Air curtain air CUR: 30 PSI;
Collision gas CAD: 7
IS voltage: 5500V / -4500V;
Source temperature: 600;
Atomizing gas GAS1: 60 PSI;
Auxiliary gas GAS2: 60 PSI



Method duration 25 min
Total scan time: 0.81064 sec
Estimated cycles: 1850

Source and Gas Parameters
Ion source gas 1 60 psi
Curtain gas 35
Temperature 650 °C
Ion source gas 2 60 psi
CAD gas 7

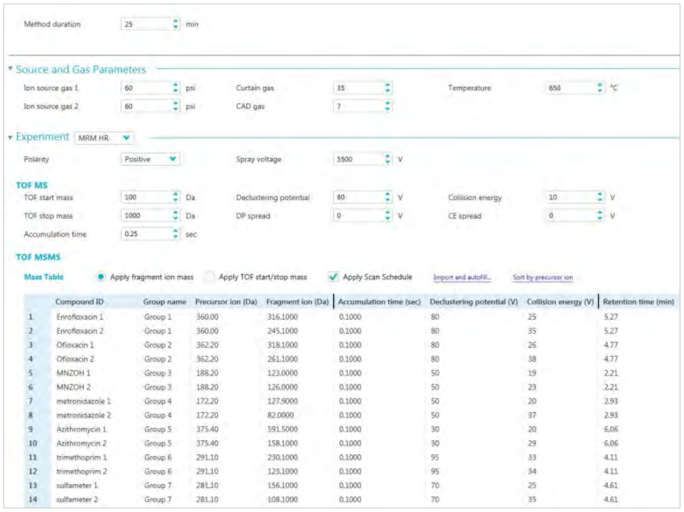
Experiment IDA
Polarity Positive V
Spray voltage 5500 V

TOF MS
TOF start mass 100 Da
Declustering potential 80 V
Collision energy 10 V
TOF stop mass 1000 Da
DP spread 0 V
CE spread 0 V
Accumulation time 0.25 sec

IDA Criteria Small molecule
Maximum candidate ions 10
Intensity threshold exceeds 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences
Advanced Criteria

TOF MSMS
Precursor ion Da
Declustering potential 80 V
Collision energy 35 V
TOF start mass 50 Da
DP spread 0 V
CE spread 15 V
TOF stop mass 1000 Da
Accumulation time 0.05 sec

Figure 1 Screening method TOF MS-IDA-MS / MS method editing interface



Method duration 25 min

Source and Gas Parameters
Ion source gas 1 60 psi
Curtain gas 35
Temperature 650 °C
Ion source gas 2 60 psi
CAD gas 7

Experiment MRM HR
Polarity Positive
Spray voltage 5500 V

TOF MS
TOF start mass 100 Da
Declustering potential 80 V
Collision energy 10 V
TOF stop mass 1000 Da
DP spread 0 V
CE spread 0 V
Accumulation time 0.25 sec

TOF MSMS
Mass Table ● Apply fragment ion mass ○ Apply TOF start/stop mass ☑ Apply Scan Schedule

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumulation time (sec)	Declustering potential (V)	Collision energy (V)	Retention time (min)
1	Enrofloxacin 1	Group 1	360.00	316.1000	0.1000	80	25	5.27
2	Enrofloxacin 2	Group 1	360.00	245.1000	0.1000	80	35	5.27
3	Ofloxacin 1	Group 2	362.20	318.1000	0.1000	80	26	4.77
4	Ofloxacin 2	Group 2	362.20	261.1000	0.1000	80	38	4.77
5	MNZOH 1	Group 3	188.20	123.0000	0.1000	50	19	2.21
6	MNZOH 2	Group 3	188.20	126.0000	0.1000	50	23	2.21
7	metronidazole 1	Group 4	172.20	127.9000	0.1000	50	20	2.93
8	metronidazole 2	Group 4	172.20	82.0000	0.1000	50	37	2.93
9	Azithromycin 1	Group 5	375.40	591.5000	0.1000	30	20	6.06
10	Azithromycin 2	Group 5	375.40	158.1000	0.1000	30	29	6.06
11	trimethoprim 1	Group 6	291.10	230.1000	0.1000	95	33	4.11
12	trimethoprim 2	Group 6	291.10	123.1000	0.1000	95	34	4.11
13	sulfameter 1	Group 7	281.10	156.1000	0.1000	70	25	4.61
14	sulfameter 2	Group 7	281.10	108.1000	0.1000	70	35	4.61

Figure 2 MRM^{HR} quantitative editing interface

Screening of target antibiotics

A total of more than 200 varieties of antibiotics in eight categories were screened in this study including: 8 varieites of penicillins, 38 cephalosporins, 18 macrolide compounds, 10 lincomycin compounds, 18 Tetracycline compounds, 39 quinolones, 41 imidazolium compounds, and 50 sulfonamides and sulfonamide compounds.

Screening test results

The extracted ion chromatogram for more than 200 antibiotic compounds under the above LC-MS conditions (see Fig. 3).

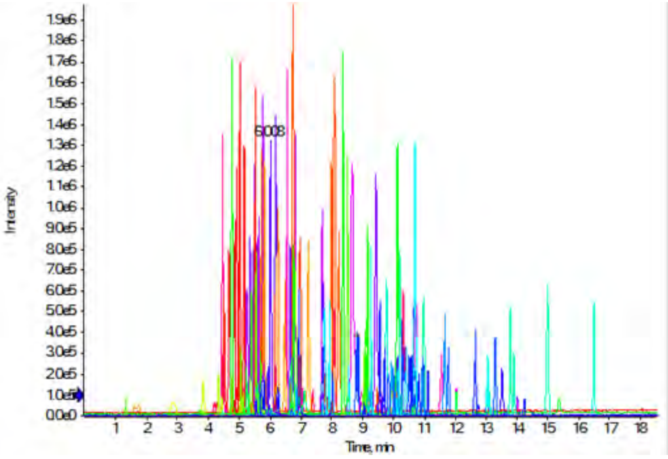


Figure 3 The extracted ion chromatogram for more than 200 antibiotic compounds

SCIEX OS software's integrated interface is highly automated, easy to use and self-explanatory. The software automatically screens target compounds on "Four Critical Points": compound mass error, isotope distribution, retention time and secondary fragments spectral configuration to ensure the accuracy of the results.

As shown in Fig. 4, according to the results shown by the software, azithromycin was detected in several samples. The green check mark on mass error, isotopic distribution, retention time and secondary fragments spectral configuration means they matched well. The mass deviation of azithromycin in several samples was less than 1 ppm, which indicated the instrument had mass accuracy and stability. The match scores between measured second-order spectra and the database were all over 90 points, which indicated that the instrument retained excellent secondary fragmentation performance even with complex matrix samples.

According to the experimental results, eight compounds in four categories were detected in 104 samples (see Table 1). This includes enrofloxacin and ofloxacin in the quinolone category, which were detected, respectively, in 1 and 13 of the samples, accounting for 0.88% and 11.40% of the total sample size, respectively. Three examples of the sulfonamides category, namely Sulfamonomethoxine, trimethoprim, sulfamethoxazole pyrimidine were found in 5, 11, and 4 of the samples, accounting for 4.39%, 9.65% and 3.51% of the total sample size, respectively. The macrocyclic lactone azithromycin was detected in 11 samples, accounting for 9.65% of the total sample. Metronidazole and metronidazole of imidazole were detected in 12 and 13 samples respectively, accounting for 10.53% and 11.40% of the total sample size respectively.

The detection rate is shown in Fig 6. A total of 39 samples were found to be positive for antibiotics, accounting for 34.2% of the total samples. More than one kind of antibiotic (as many as three) were found in some samples. In some samples, hydroxylmetronidazole was detected. This may be caused by the internal biotransformation of metronidazole.

Table 1 Compounds tested in urine samples and urine sample numbers

Compound Name	Molecular formula	Child urine sample number (#)
Enrofloxacin	*C19H22FN3O3*	*90*
Ofloxacin	*C18H20FN3O4*	*37、38、56、58、63、64 、65 、66 、68、 69、 85 、90、 92*
Hydroxymetronidazole	*C6H9N3O4*	*3 、4、7、13、16、18、19、31、63、80、81、95、112*
metronidazole	*C6H9N3O3*	*3 、4、7、13、16、18、19、31、 63、 80、 95、112*
Sulfamonomethoxine	*C11H12N4O3S*	*19、63、66、68、69*
Trimethoprim (TMP)	*C14H18N4O3*	*55、63、66、68、69、72、80、86、87、90、91*
Sulfameter (SMD)	*C11H12N4O3S*	*63、66、68、69*
Azithromycin	*C38H72N2O12*	*7、8、21、23、41 、47、 50、 57、67、72 、74*

Food and Environmental

Profiling and Identification of Hop-Derived Bitter Compounds in Beer Using LC-HR-MS/MS

André Schreiber
[1]SCIEX Concord, Ontario (Canada)

Overview

Here we present a method to study the profile of hop-derived bitter compounds using LC coupled to high resolution mass spectrometry.

Diluted beer samples were injected directly into the SCIEX X500R QTOF system. Data were processed using both a targeted list of accurate masses of molecular ions of known hop-derived compounds in SCIEX OS software and a non-targeted analysis by performing statistical data processing (Principal Components Analysis, PCA) in MarkerView™ software.

Introduction

Beer is the world's most widely consumed beverage (after tea and water) and probably the oldest alcoholic beverage.[1] Beer has attracted consumers over centuries due to its refreshing character, attractive aroma, and typical bitter taste.

The production of beer is called brewing, which involves the fermentation of starches, mainly derived from cereal grains (most commonly malted barley, although wheat, corn, and rice are widely used). Most beer is flavored with hops, which add bitterness and act as a natural preservative, though other flavorings such as herbs or fruit may occasionally be included.[2]

Aroma-active volatiles as well as nonvolatile bitter compounds of beers have been thoroughly investigated in recent decades.

The typical hop-derived beer bitterness is caused by adding cones, pellets, or extracts of hop during wort boiling. A number of isomerization processes have been identified to be of major importance for bitter taste development in the final beer product. The so-called isoxanthohumol (Figure 1), identified as a bitter compound in beer, was found to be generated from the hop-derived xanthohumol, during wort boiling. Moreover, *trans*- and *cis*-iso-α-acids have been identified as the major bitter contributors in beer and were demonstrated to be generated upon a rearrangement reaction of their hop-derived precursors, the α-acids. Following the α-acids, the second major constituents of hop are the β-acids (Figure 1), but there are almost no data available on the direct contribution of these compounds to beer bitterness or on their role in the generation of bitter-tasting conversion products during wort boiling.[3, 4]

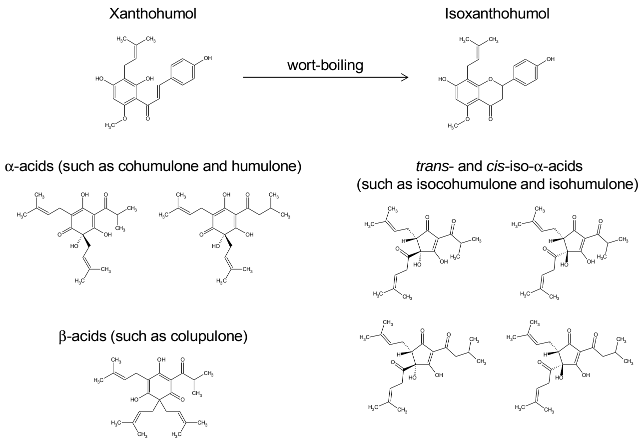


Figure 1. Hop-derived bitter compounds and their isomerization processes during the wort boiling

Here an LC-HR-MS/MS based method is presented using the SCIEX X500R QTOF system to identify hop-derived bitter compounds in beer based on accurate mass MS and MS/MS data after information dependent acquisition (IDA) followed by targeted and non-targeted data processing in SCIEX OS and MarkerView™ software.

Experimental

Beer Samples

- Store-bought samples from the Liquor Control Board of Ontario (LCBO)
- Degassed and diluted 2x with water
- Injection of 5 µL

LC Separation

- ExionLC™ AD system
- Phenomenex Luna Omega 1.6 µm Polar C18 (50 x 2.1 mm)
- Gradient of water/methanol + 5 mM ammonium formate at a flow of 0.5 mL/min (Table 1)

Table 1. Gradient conditions used for the identification hop-derived bitter compounds

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	1.0	90	10
2	6.0	10	90
3	7.0	10	90
4	7.1	90	10
8	10.0	90	10

MS/MS Detection

The SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI) was used in positive polarity. Ion source temperature was set to 450ºC and IS voltage was set to 5500 V.

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 200 msec) and up to 10 dependent MS/MS scans (50-1000 Da for 50 msec). Declustering Potential (DP) was set to 80 V and MS/MS fragmentation was achieved using a Collision Energy (CE) of 35 V with a Collision Energy Spread (CES) of ±15 V.

Dynamic background subtraction (DBS) was activated to achieve the most complete MS/MS coverage. No inclusion list was used which allowed non-target 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. In addition, MarkerView™ software version 1.3 was used for statistical processing using Principal Components Analysis (PCA).

Results and Discussion

X500R Performance Characteristics and Data Acquisition Workflows

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 resolution for the typical molecular weight range of ingredients and potential contaminants in beer (Figure 2).

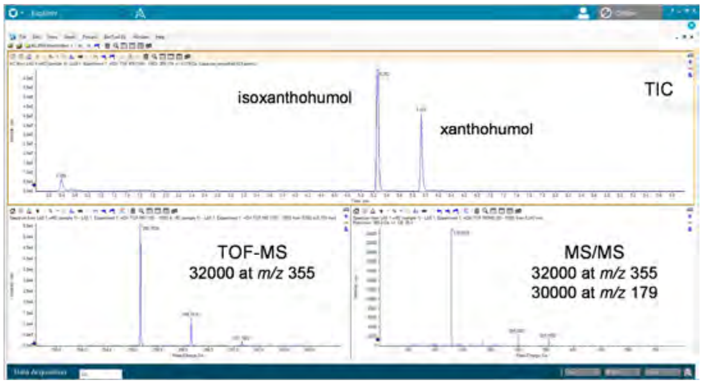


Figure 2. Resolution in TOF-MS and MS/MS mode achieved for isoxanthohumol

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 and by using the dynamic background calibration software algorithm. 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.

In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant delivery path for reliable auto-calibration (Figure 3), maintaining mass accuracy over long periods of time by automatically calibrating in batch mode.

Figure 3. TwinSprayer ESI probe showing the independent inlet for LC and calibrant

The accurate measurement of a molecular ion is insufficient for compound identification. Single stage MS information can only be used for empirical formula finding, and accurate mass MS/MS data are absolutely necessary to identify chemical structures based on the molecular fingerprint saved into the pattern of fragment ions.

Using IDA, simultaneous acquisition of TOF-MS and MS/MS data in a single data file for each sample was possible. Up to 10 MS/MS spectra were automatically collected for each chromatographic data point (Figure 4).

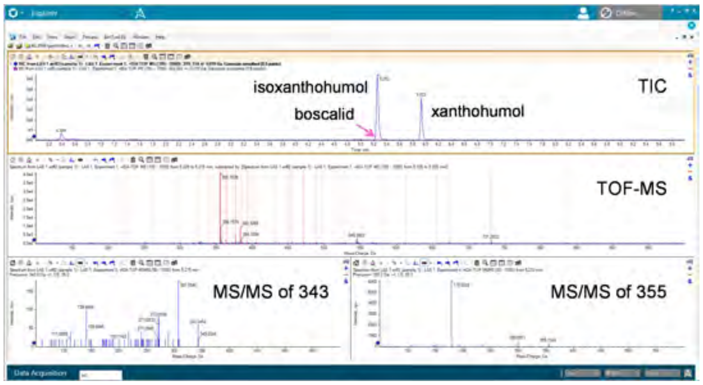
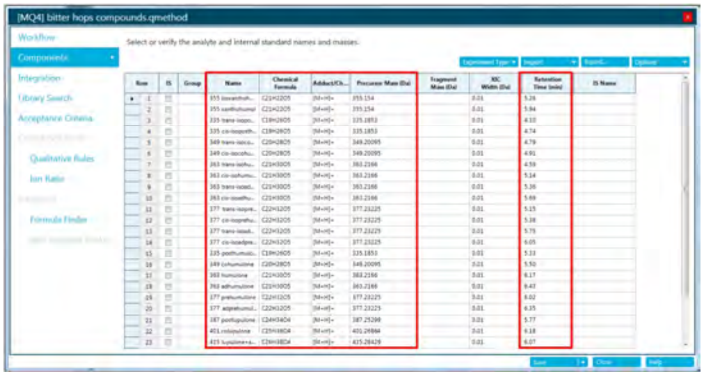


Figure 4. Simultaneous acquisition of TOF-MS and MS/MS spectra using IDA, the example shows spectra of isoxanthohumol (355) and the pesticide boscalid (343)

Processing Workflow for Target Identification in SCIEX OS Software

A targeted processing method was built to profile for 26 known hop-derived bitter compounds (Figure 5). Retention times were established by comparing the chromatographic profiles of beer samples to results published in literature.[3]

During targeted processing, Extracted Ion Chromatograms (XIC) of all 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.



Select or verify the analyte and internal standard names and masses.

Figure 5. Targeted processing method to profile known hop-derived bitter compounds, XIC will be generated based on provided chemical formula and retention time)

During this project a small HR-MS/MS library was generated to assist future identification by also utilizing the MS/MS fragmentation (Figure 6).

Figure 6. Updating HR-MS/MS libraries in SCIEX OS software to assist future compound identification

Results of Analyzing 40 Beer samples

The amounts of xanthohumol (X) and isoxanthohumol (IX) in a beer can provide information about how hops were used during the beer making process. Figure 7 shows the intensity for IX and X in 3 different beers. The observed X/IX ratio of the pilsner and Weissbier is in line with published data.[3]

Figure 7. Intensity of isoxanthohumol (IX) and xanthohumol (X) and the X/IX ratio in different beers (left to right German pilsner, German Weissbier of the same brewery and dry-hoped IPA produced in Ontario, Canada)

The concentrations of IX, X and X/IX from the beer samples are summarized in Figure 8. This data reflectins different styles of beer making. It is obvious that the high X/IX ratio in Pale Ales and India Pale Ale (IPA) is caused by the late addition of hops during the boiling and fermentation process.[4]

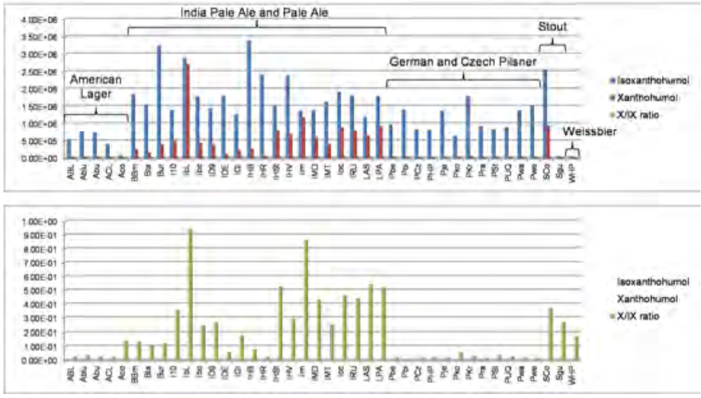


Figure 8. Intensity of isoxanthohumol (IX) and xanthohumol (X), top, and the X/IX ratio, bottom, for 40 different beers including different styles. The data reflect the differences of beers produced in different countries

Isoxanthohumol (IX), isocohumulone (ICH), and isohumulone (IH) were the 3 most abundant hop-derived bitter compounds detected in beer. ICH and IH and other iso-α-acids were present in their *trans*- and *cis*- form (Figure 9). The intensity ratio of *trans*/*cis* acids varied between 0.02 to 0.15 (American Lagers < European Pilsners < American Pal Ales and IPA).

Figure 9. Intensity of *cis*- and *trans*-isocohumulone, isohumulone, cohumulone, and humulone in a Czech pilsner, the *trans*/*cis* ratio was 0.06

The measure of bitterness in beer is the IBU (International Bittering Units). A plot of the sum of the peak areas of IX, ICH and IH against the IBU for different beers is shown in Figure 10.

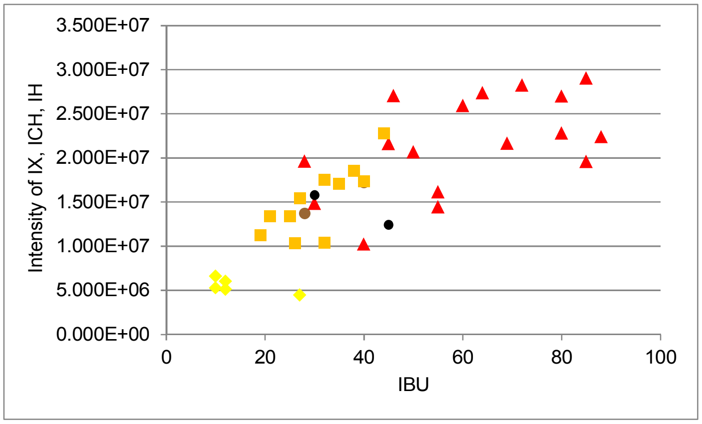


Figure 10. Correlation of intensity of IX, ICH and IH against the published IBU for different beer styles (yellow: American lagers, orange: European pilsners, red: Pale Ales and India Pale Ales, brown: Bock, and black: Stout)

Beer Profiling using Statistical Data Analysis

Statistical data analysis, such as Principal Components Analysis (PCA), can be used to profile and compare different beverage samples. Figure 11 shows the PCA scores plot for 40 different beers. Beers of similar style group together. The location of a sample in the plot indicates a specific flavor or color (i.e. lighter beer vs. a more hoppy/bitter beer). The PCA loadings plot assists in finding characteristic markers (*m/z*-RT). Once these markers are found, the corresponding chemical can be identified using formula finding based on accurate mass MS and MS/MS followed by ChemSpider searching and MS/MS elucidation.

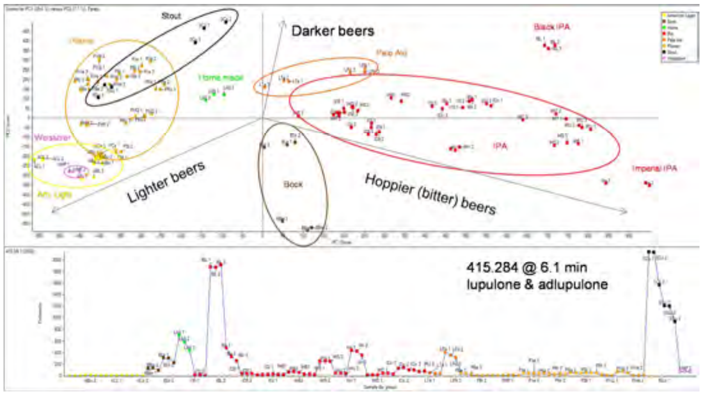


Figure 11. PCA scores plot used to profile and map different beer styles, the loading plot (not shown here) assisted to find characteristic *m/z*-RT

The β-acids lupulone and adlupulone were found in higher concentrations in darker beers, such as stout and black IPA, as the profile plot (bottom trace) in Figure 11 shows. Both compounds were identified using the described automated software tools in SCIEX OS software (Figure 12).

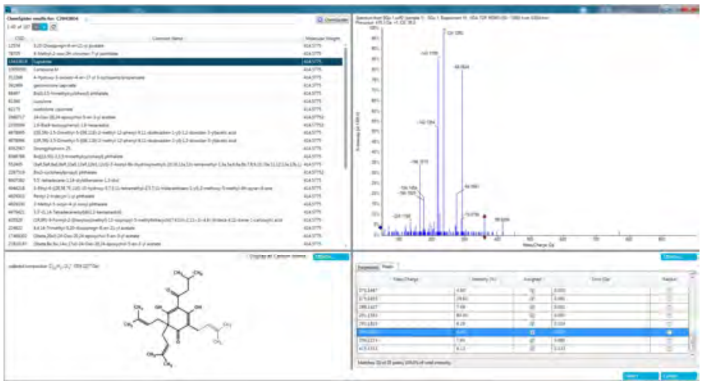


Figure 12. Identification of lupulone and adlupulone in an Irish stout based on formula finding followed by ChemSpider searching and MS/MS elucidation

Summary

The SCIEX X500R QTOF system was used to analyze 40 different beer samples in positive polarity ESI using information dependent acquisition of MS and MS/MS spectra. Samples were processed using a targeted approach to profile 26 known hop-derived bitter compounds in SCIEX OS software. Samples were also processed using PCA in MarkerView™ software.

This study shows that LC-HR-MS/MS is a valuable tool to study flavor and aroma profile in food and beverage samples.

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Publication number: RUO-MKT-02-4481-A

LC-MS/MS Analysis of Emerging Food Contaminants

Identification of Artificial Colors and Dyes in Food Samples using LC-HR-MS/MS

André Schreiber[1] and Paul Winkler[2]
[1]SCIEX Concord, Ontario (Canada) and [2]SCIEX Redwood City, California (USA)

Overview

Here we present a novel LC-HR-MS/MS method that was used to identify artificial colors and dyes in food samples.

High resolution MS and MS/MS data were collected using a SCIEX X500R QTOF system in negative Electrospray Ionization (ESI). Non-target peak finding, sample-control-comparison followed by identification based on empirical formula finding and ChemSpider database searching was performed in SCIEX OS software. In addition, statistical data processing was done in MarkerView™ software.

Introduction

Artificial colors and dyes are used in food to make it visually more appealing and “flavorful” since people associate certain colors with certain flavors.

However, some dyes are banned because they are toxic and carcinogenic. Other dyes are approved for use in foods and regulated by Codex Alimentarius, the US-FDA, EFSA etc. Nature derived color additives (pigments derived from vegetables, minerals or animals) are exempt from certification.[1-3]

Recent research shows a link between the presence of artificial colors in food and behavioral problems of children.[4-5] These findings have resulted in public concern about the use of artificial dyes.

Analytical methods used to test for the presence of banned colors and dyes in food include TLC-UV/VIS, LC-UV/VIS, and LC-MS. Such methods have limited selectivity and sensitivity and are therefore only used for target analysis. Recent advancements in LC-HR-MS technology provide the ability to perform targeted and non-targeted screening in food samples on a routine basis. The exact mass and MS/MS data provided by these instruments contain enough information to confidently identify known food ingredients and contaminants and also to identify unknown chemicals that may also be present in the sample.

Artificial colors and dyes in food samples were identified using the SCIEX X500R QTOF system. MS detection was performed using information dependent acquisition (IDA) to simultaneously collect accurate mass MS and MS/MS information.

Compounds were automatically identified. SCIEX OS was used to automatically process the data using a non-target peak finding algorithm and sample-control-comparison to locate unique peaks in the sample. MarkerView™ software and statistical data processing was used to separate matrix and sample specific signals from true contaminants. TOF-MS and MS/MS data of ions of interest were automatically processed using empirical formula finding and searched against online databases, such as ChemSpider, for identification. The SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unknown chemicals in food.

Experimental

Samples

Store-bought “Icing Colors” were diluted 10,000x using a sugar solution prepared by dissolving 10 g of sugar in 10 mL water (LC grade) to mimic the icing sugar matrix typically used for baking.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AD system with a Phenomenex Luna Omega 1.6 µm Polar C18 (50 x 2.1 mm) 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 identification of food dyes

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	1.0	90	10
2	6.0	10	90
3	7.0	10	90
4	7.1	90	10
8	10.0	90	10

MS/MS Detection

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

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 scan (100-1000 Da for 200 msec) and up to 10 dependent MS/MS scans (50-1000 Da for 50 msec). Declustering Potential (DP) was set to -80 V and MS/MS fragmentation was achieved using a Collision Energy (CE) of CE of -35 V with a collision energy spread of ±15 V.

Dynamic background subtraction (DBS) was activated for best MS/MS coverage. No inclusion list was used, which allowed non-target 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. In addition, MarkerView™ software version 1.3 was used for statistical processing using Principal Components Analysis (PCA).

Results and Discussion

X500R Performance Characteristics and Data Acquisition Workflows

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

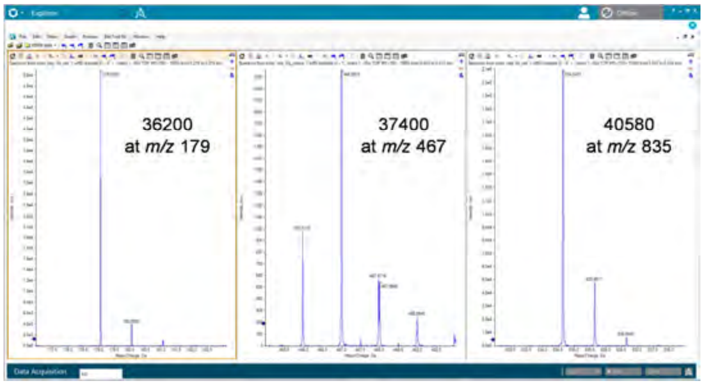

Figure 1. Resolution of different chemicals detected in negative polarity ESI in diluted dye samples

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 and by using the dynamic background calibration software algorithm. 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.

In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant delivery path for reliable auto-calibration (Figure 2), maintaining mass accuracy over long periods of time by automatically calibrating in batch mode.

Figure 2. TwinSprayer ESI probe showing the independent inlet for LC and calibrant

The accurate mass measurement of a molecular ion is insufficient for compound identification. Single stage MS information can only be used for empirical formula finding. Because many different chemicals have identical molecular formulas accurate mass MS/MS data are absolutely necessary to identify chemical structures based on the molecular fingerprint observed in the MS/MS spectrum.

Using IDA, simultaneous acquisition of TOF-MS and MS/MS into a single data file for each sample was possible. Up to 10 MS/MS spectra were automatically collected for each chromatographic data point (Figure 3).

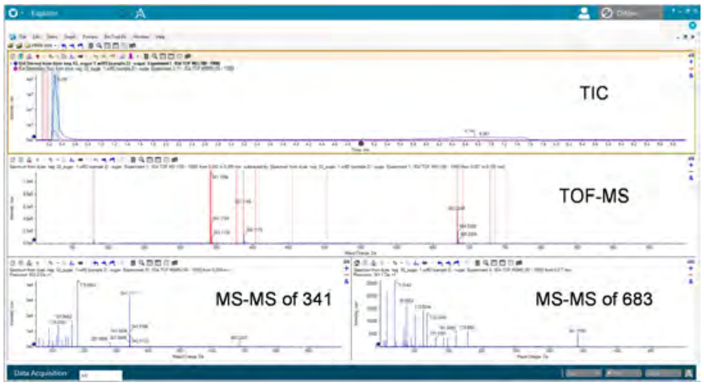

Figure 3. Simultaneous acquisition of TOF-MS and MS/MS using IDA, the example shows spectra of sucrose and sucrose dimer of the matrix

Processing Workflow for Non-Target Identification in SCIEX OS Software

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

Figure 4 shows Total Ion Chromatograms (TIC) of samples analyzed. It can be seen that the TIC are dominated by matrix components (sugars) eluting at ~0.3 min. The main dyes in the red and blue sample can be found, but minor components and ingredients in the yellow and brown sample are not visible.

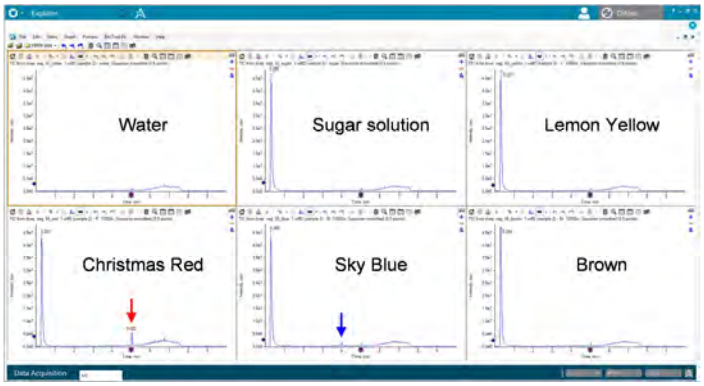

Figure 4. TIC of a blank (water), matrix (sugar solution) and for dyes samples, peak finding without software tools is very complicated or even impossible

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

- Simple software workflows that deliver reliable results
- Automated feature detection based on non-target peak finding followed by sample-control-comparison
- Automated compound identification using empirical formula finding followed by library and online database searching
- Quick data review and reporting utilizing customizable flagging and filtering of results

The workflow to setup non-target data processing is illustrated in Figures 5a and b.

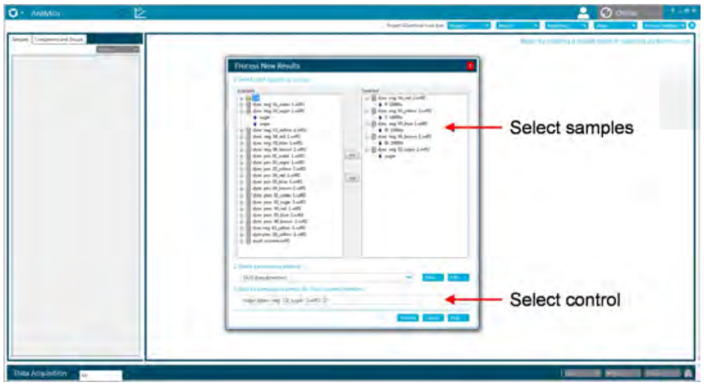

Figure 5a. Selecting unknown sample(s) and control sample for non-target data processing and sample-control comparison in SCIEX OS software

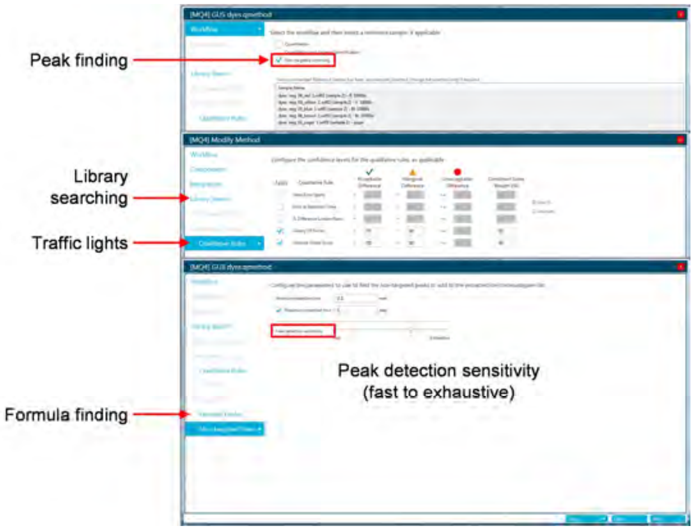

Figure 5b. Setup of non-target peak finding criteria and identification tools, including MS/MS library searching and empirical formula finding, criteria for traffic lights are set for later data review and filtering

Data Review during Non-Target Identification in SCIEX OS Software

After non-target peak finding and sample-control-comparison the results are displayed (Figure 6). The results table can be sorted and filter using the traffic lights. The Peak review will automatically provide XIC, TOF-MS and MS/MS data for both the sample and control sample.

Figure 6. Results display after non-target peak finding and sample-control comparison, results were filtered by formula finding score (>70%) and sorted by intensity

Zooming into the TOF-MS spectrum provides details of formula finding, including mass error in TOF-MS and MS/MS and the number of structures found in ChemSpider for each possible formula (Figure 7).

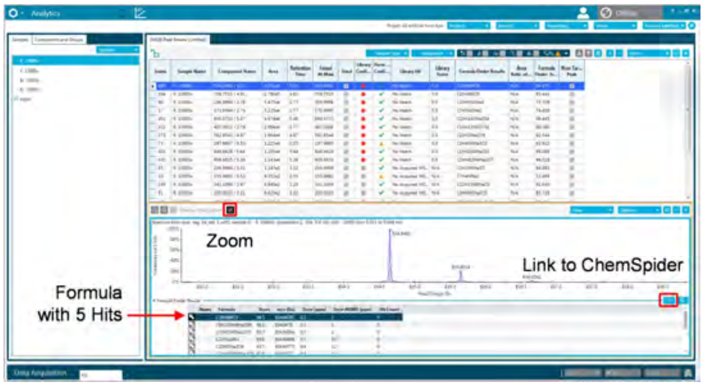

Figure 7. Zoom into TOF-MS to display detailed results of formula finding, the most likely formula has a mass error of 0.3 ppm in TOF-MS an average mass error of 1 ppm of all fragments in MS/MS and 5 matching structures in ChemSpider

From the TOF-MS display the formula can be linked to ChemSpider. The ChemSpider display will list all matching structures, automatically sorted by number of references. The selected structure is automatically fragmented in-silico and compared against the accurate mass MS/MS spectrum.

Using this workflow the main ingredient in the red food coloring was quickly identified as Erythrosine (Figure 8).

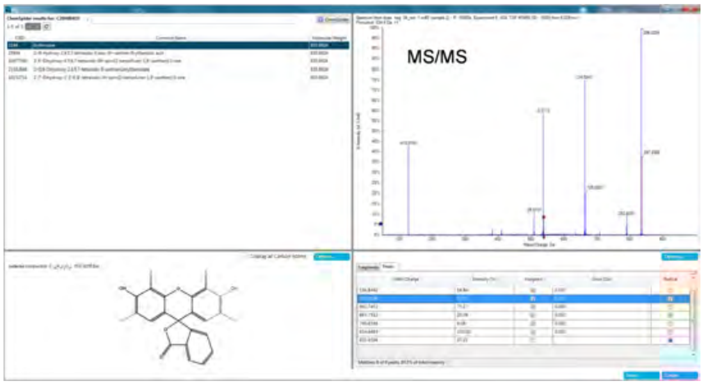

Figure 8. ChemSpider search results and in-silico fragmentation assisting to quickly identify Erythrosine in red food coloring

Results of Analyzing Food Coloring

Identified artificial dyes and by-products are summarized in Table 2. Figures 9, 10 and 11 show further examples of identification based in ChemSpider searching and MS/MS elucidation.

Table 2. Artificial dyes and by-products identified in samples

Sample	*m/z* - RT	Area %*	Formula	Formula Finder Score (%)	Mass error (ppm)	MS/MS error (ppm)	Identification
Red	834.6480 / 5.02	88.5	$C_{20}H_8I_4O_5$	94.5	0.3	1.0	**Erythrosine**
	708.7515 / 4.91	6.1	$C_{20}H_9I_3O_5$	93.4	0.5	0.9	Erythrosine-I
	890.6733 / 5.47	1.0	$C_{23}H_{12}I_4O_6$	92.0	0.8	0.8	Erythrosine+C_3H_4O
	407.0012 / 2.76	0.6	$C_{16}H_{12}N_2O_7S_2$	96.2	0.1	1.0	**Sunset Yellow**
	582.8543 / 4.87	0.4	$C_{20}H_{10}I_2O_5$	92.1	0.1	2.1	Erythrosine-I2
Yellow	197.9867 / 0.53		$C_7H_4NO_4S^-$	88.9	0.7	2.0	in-source fragment
	466.9974 / 0.53	90.3	$C_{16}H_{12}N_4O_9S_2$	92.0	0.2	2.0	**Tartrazine**
	224.0134 / 0.53		$C_8H_6N_3O_3S^-$	93.6	0.7	0.5	in-source fragment
	501.9503 / 0.56	9.7	contains 2 Cl^-				not identified
Blue	747.1508 / 4.01	90.1	$C_{37}H_{36}N_2O_9S_3$	86.9	0.3	3.3	**Brilliant Blue**
	577.1473 / 4.49	5.4	$C_{30}H_{30}N_2O_6S_2$	92.5	0.2	1.7	Brilliant Blue - $C_7H_6O_3S$ (by-product)
	184.9909 / 0.52	4.5	$C_7H_6O_4S$	78.1	2.1	2.3	3-Formylbenzene-sulfonic acid (by-product)
Brown	407.0012 / 2.76	33.9	$C_{16}H_{12}N_2O_7S_2$	93.3	0.2	1.6	**Sunset Yellow**
	451.0277 / 3.31	24.8	$C_{18}H_{16}N_2O_8S_2$	86.6	0.5	3.0	**Allura Red**
	834.6480 / 5.02	19.1	$C_{20}H_8I_4O_5$	92.8	0.1	1.9	**Erythrosine**
	501.9503 / 0.56	9.3	contains 2 Cl^-				not identified

* Area % includes monoisotopic peak, isotopes, adducts (i.e. Na+), multiply charged ions and in-source-fragments

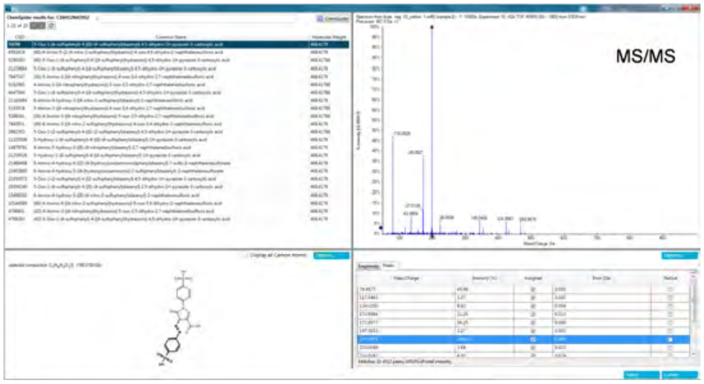

Figure 9. ChemSpider search results and in-silico fragmentation assisting to quickly identify Tartrazine in yellow food coloring

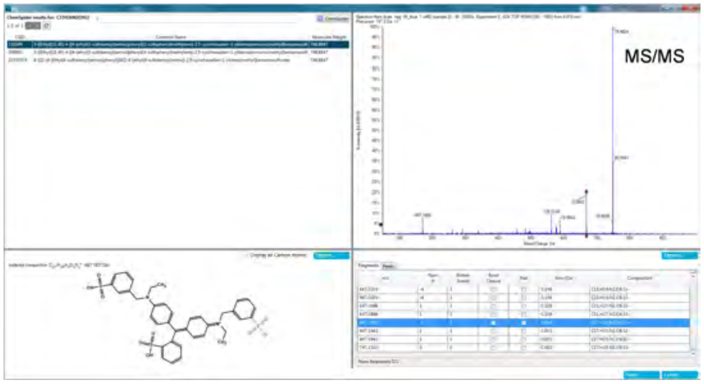

Figure 10. ChemSpider search results and in-silico fragmentation assisting to quickly identify Brilliant Blue in blue food coloring

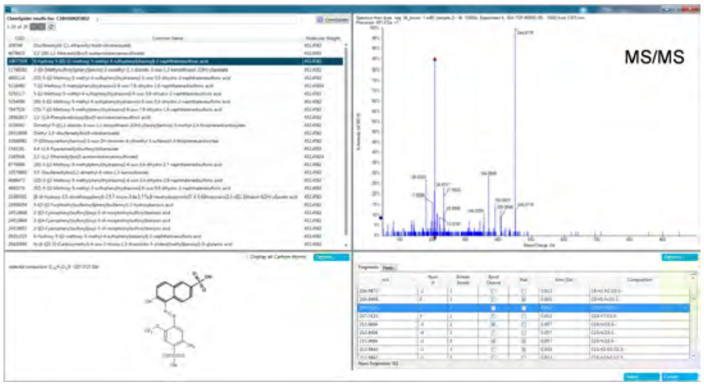

Figure 11. ChemSpider search results and in-silico fragmentation assisting in the quick identification of Allura Red in the brown food coloring

Statistical Data Analysis to Identify Unknowns

Statistical data analysis is an alternative to simple sample-control-comparison. Tools, such as Principal Components Analysis (PCA), can be used to identify characteristic markers in complex samples and at lower levels. Figure 12 shows an example of PCA performed in MarkerView™ software to find ingredients in food dyes.

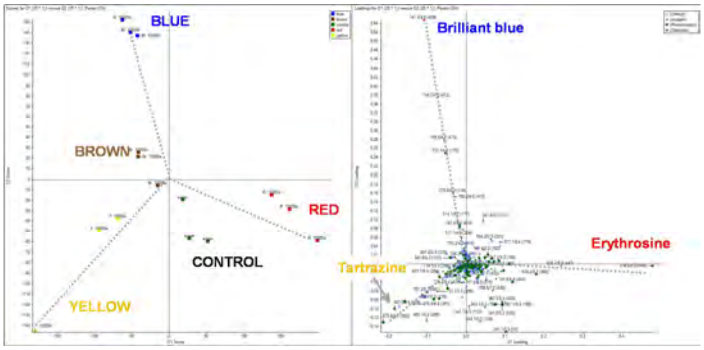

Figure 12. PCA as an alternative to sample-control comparison quickly finds differences between samples (score plot shown left) and helps to identify characteristic *m/z*-RT using the above described tools

Summary

A new non-target LC-HR-MS/MS based approach to quickly identify artificial colors in food samples was developed using the SCIEX X500R QTOF system.

Negative polarity ESI TOF-MS and MS/MS data acquired using information dependent acquisition were processed in SCIEX OS and MarkerView™ software. Characteristic *m/z*-RT were further processed using empirical formula finding and ChemSpider searching. The major compounds in food coloring were quickly identified using automated and intuitive software workflows in SCIEX OS.

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Publication number: RUO-MKT-02-4482-A

Perfect Balance to Elevate your Lab's Performance

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

André Schreiber[1], April Quinn-Paquet[2], and Jianru Stahl-Zeng[3]
[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).

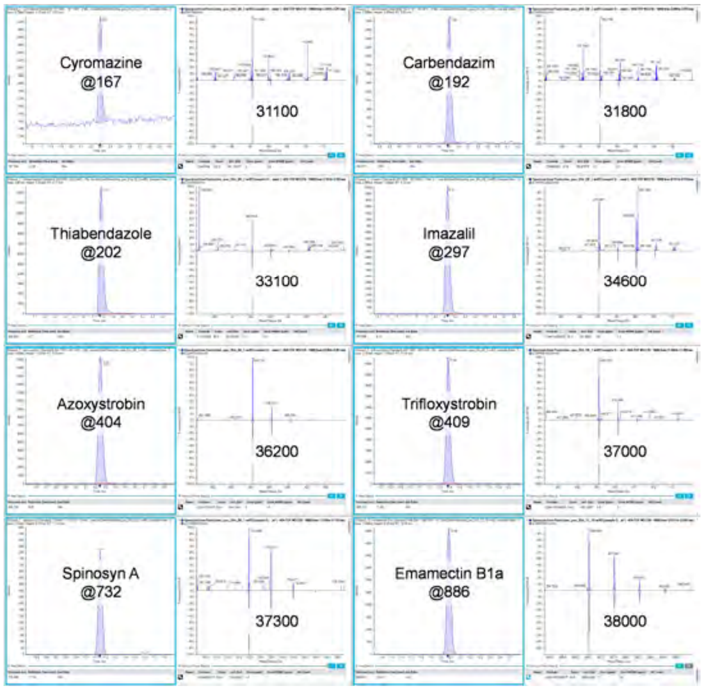


Figure 1. Sensitivity and resolution of different pesticides, left: XIC of the molecular ion of each compound ± 5 mDa at 1 ng/mL (Emamectin at 10 ng/mL), right: TOF-MS spectrum of molecular ion with achieved resolution value (average of seven X500R QTOF systems)

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.

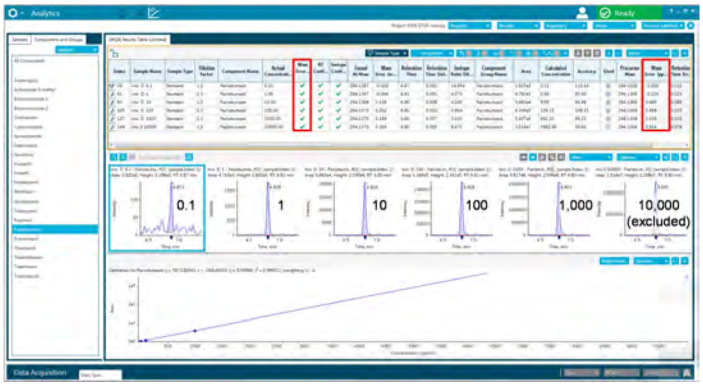


Figure 2. Detection of Paclobutrazol from 0.1 to 10,000 ng/mL with good linearity (0.1 to 1,000 ng/mL) and mass errors of < 1 ppm even at the highest concentration above the upper limit of quantitation

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.

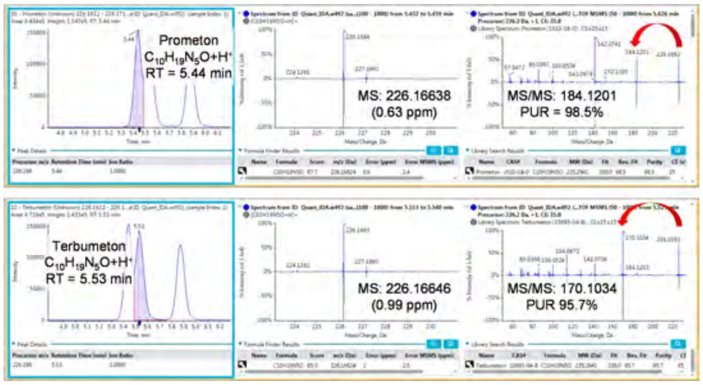


Figure 3. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

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).

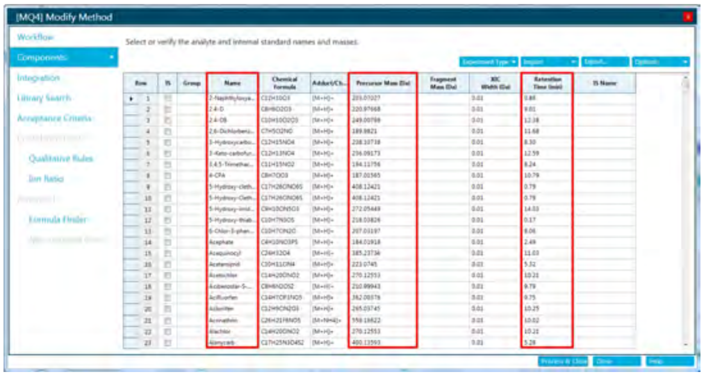


Figure 4a. Method editor in SCIEX OS software, user input for target compounds including chemical formula to calculate precursor ion mass and expected retention time

Configure the confidence levels for the qualitative rules, as applicable

Apply	Qualitative Rule	Acceptable Difference	Marginal Difference	Unacceptable Difference	Combined Score Weight (%)
☑	Mass Error (ppm)	5	10		30
☑	Error in Retention Time	0.1	0.2		30
☑	% Difference Isotope Ratio	20	40		20
☑	Library Hit Score	70	50		20
☐	Formula Finder Score				

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.

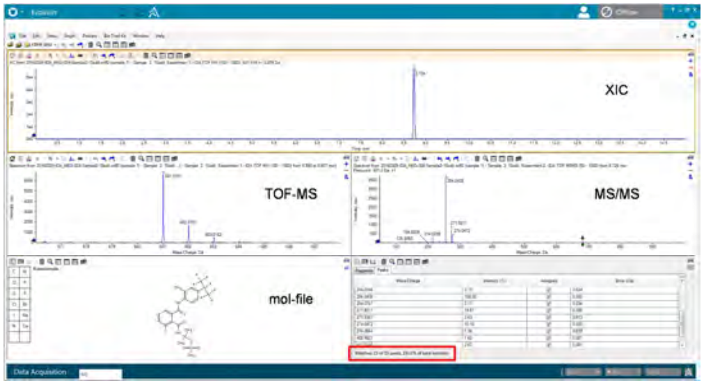


Figure 6. Tentative identification of Flubendiamide based on a comparison of the HR-MS/MS spectrum with the theoretical fragmentation pathway

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

Publication number: RUO-MKT-02-3760-A

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]

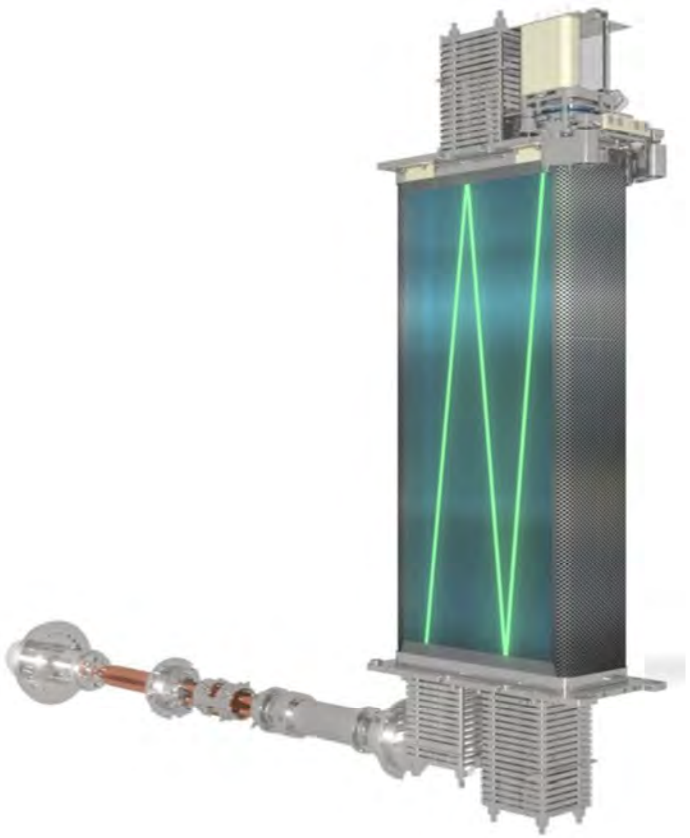


Figure 1. N-optics design of the X500R QTOF system to maximize resolution while maintaining benchtop design and a minimized footprint, 6 heater drones are integrated into the TOF path to maintain mass accuracy and robustness

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).

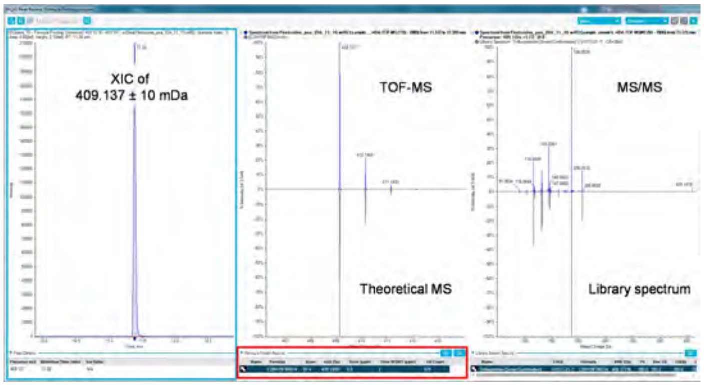


Figure 2. TOF-MS and MS/MS spectra used for empirical formula finding, results are ranked by a combined score using MS and MS/MS information, and when combined with the ChemSpider hit count, can be used to quickly find the correct match

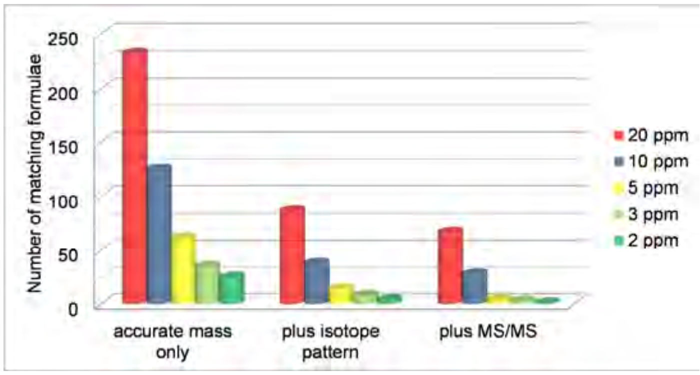


Figure 3. Number of matching molecular formulae depending on the information and mass accuracy used for empirical formula finding (elements allowed $C_{49}H_{75}Br_3Cl_5F_3I_3N_{10}O_{10}PS_3$)

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.

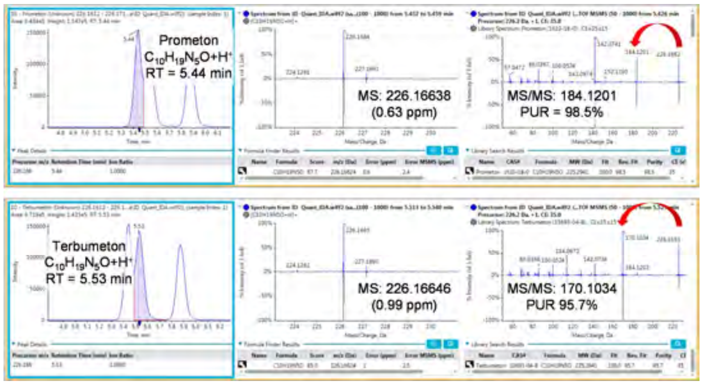


Figure 4. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

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.

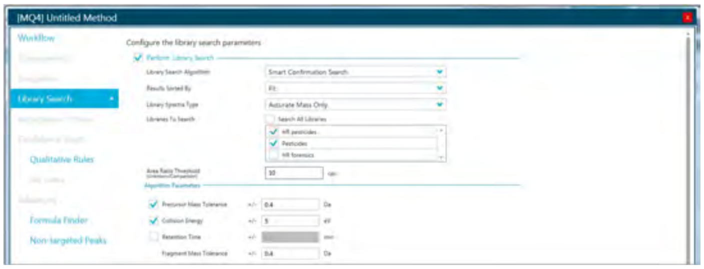
Select the workflow and then select a reference sample, if applicable

☐ Quantitation
☐ Quantitation and targeted identification
☑ Non-targeted screening

The recommended Reference Sample has been automatically selected. Change the selection only if required.

Sample Name
2 Data EURL 008.wiff2 (sample 1) - org banana
2 Data EURL 014.wiff2 (sample 1) - banana

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



Configure the library search parameters

☑ Perform Library Search

Library Search Algorithm: Smart Confirmation Search
Results Sorted By: Fit
Library Spectra Type: Accurate Mass Only
Libraries To Search: ☐ Search All Libraries
☑ HR pesticides
☑ Pesticides
☐ HR forensics
Area Ratio Threshold (Unknown/Comparison): 10

Algorithm Parameters

☑ Precursor Mass Tolerance +/- 0.4 Da
☑ Collision Energy +/- 5 eV
☐ Retention Time +/- min
Fragment Mass Tolerance +/- 0.4 Da

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

Configure the Formula Finder options that are used to identify compounds

☑ Use Formula Finder

Type of Compound
○ Naturally Occurring Compounds
● Man-Made Compounds
○ Other

Limits
Max. Element: C49 H75 Br2 Cl5 F3 I3 N10 O16 P1 S3
Mass Tolerance: 10 ppm
Area Ratio Threshold (Unknown/Comparison): 10

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).

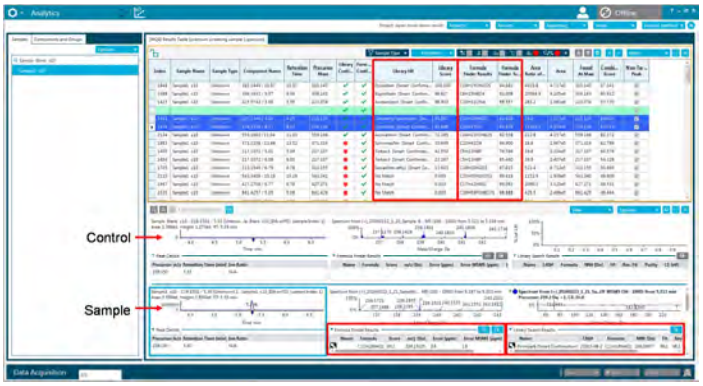


Figure 6. Results display after non-target screening, library searching and formula finding results are displayed in the table (top) and chromatograms and spectra with result details can be reviewed (bottom)

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.

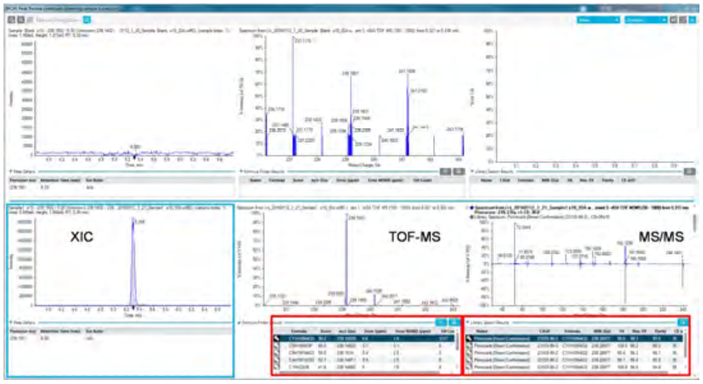


Figure 7a. Review of XIC of m/z 239.150 at RT 5.3 min and spectra with a found formula of $C_{11}H_{18}N_4O_2$

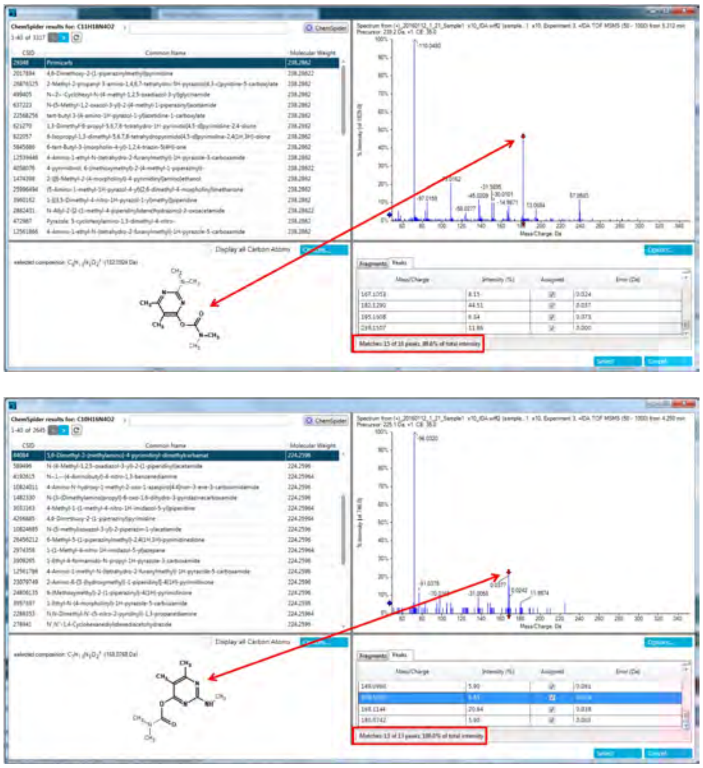


Figure 7b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Pirimicarb (top) and also of its metabolite Desmethyl-pirimicarb (bottom), both compounds were confirmed by MS/MS library searching

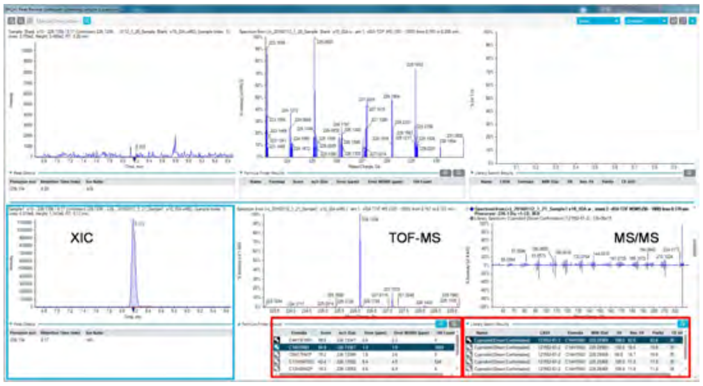


Figure 8a. Review of XIC of m/z 226.134 at RT 8.2 min and spectra with a found formula of $C_{14}H_{15}N_3$, although ranked second based on mass accuracy the high ChemSpider hit count revealed the correct match

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

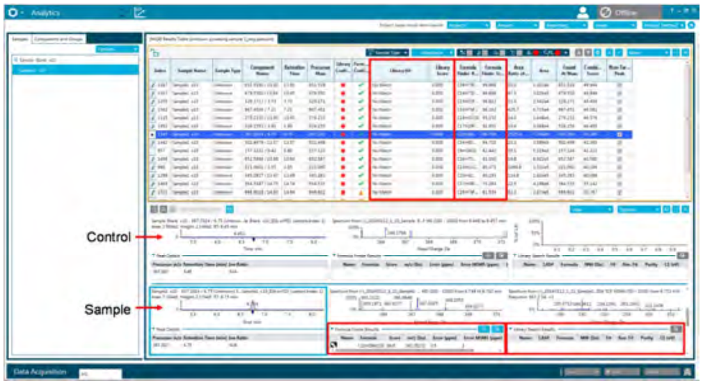


Figure 9a. Results display after non-target screening of the negative polarity data, review of XIC of m/z 367.203 at RT 6.7 min and spectra with a found formula of $C_{22}H_{28}N_2O_3$

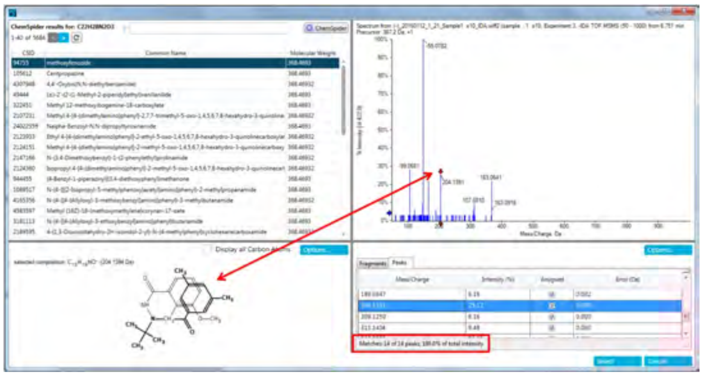


Figure 9b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Methoxyfenozide

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

[3] André Schreiber et al.: ‘Using the X500R QTOF System and SCIEX OS Software to Identify and Quantify Food Residues’ Application Note SCIEX (2016) # RUO-MKT-02-3760-A

Publication number: RUO-MKT-02-3761-A

Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
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Food Method

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

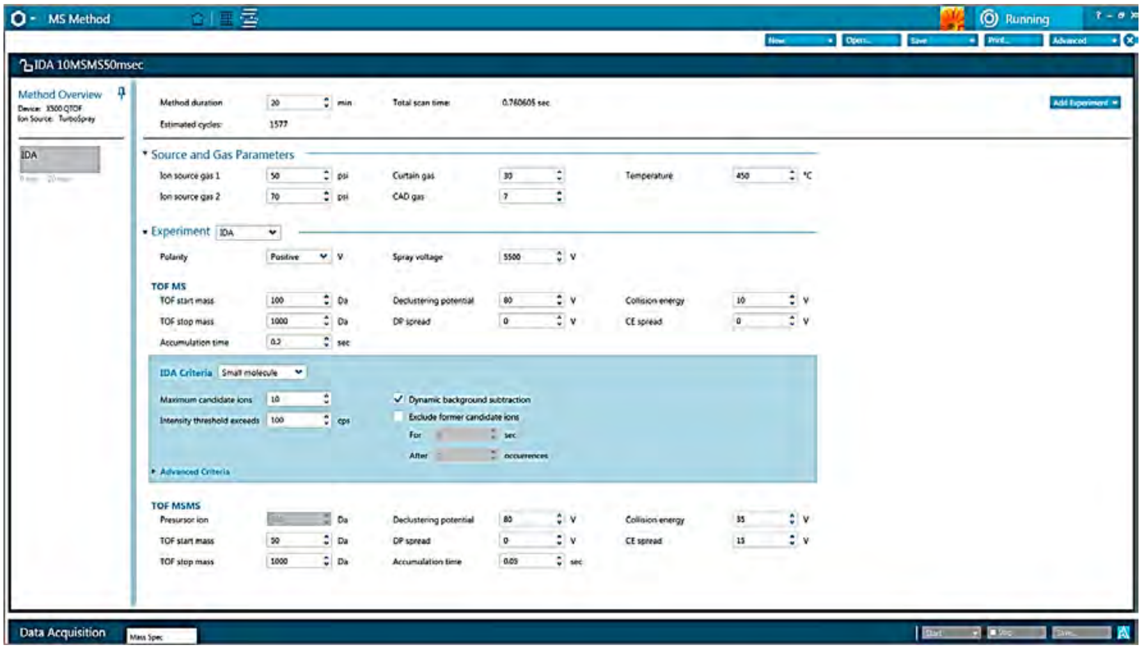
Step 4
- Centrifuge and draw off 1mL for analysis.

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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA

Method duration: 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters
Ion source gas 1: 50 psi
Ion source gas 2: 70 psi
Curtain gas: 30
CAD gas: 7
Temperature: 450 °C

Experiment: IDA
Polarity: Positive V
Spray voltage: 5500 V

TOF MS
TOF start mass: 100 Da
TOF stop mass: 1000 Da
Accumulation time: 0.2 sec
Declustering potential: 80 V
DP spread: 0 V
Collision energy: 10 V
CE spread: 0 V

IDA Criteria: Small molecule
Maximum candidate ions: 10
Intensity threshold exceeds: 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For: sec
After: occurrences
Advanced Criteria

TOF MSMS
Presursor ion: Da
TOF start mass: 50 Da
TOF stop mass: 1000 Da
Declustering potential: 80 V
DP spread: 0 V
Accumulation time: 0.05 sec
Collision energy: 35 V
CE spread: 15 V

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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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

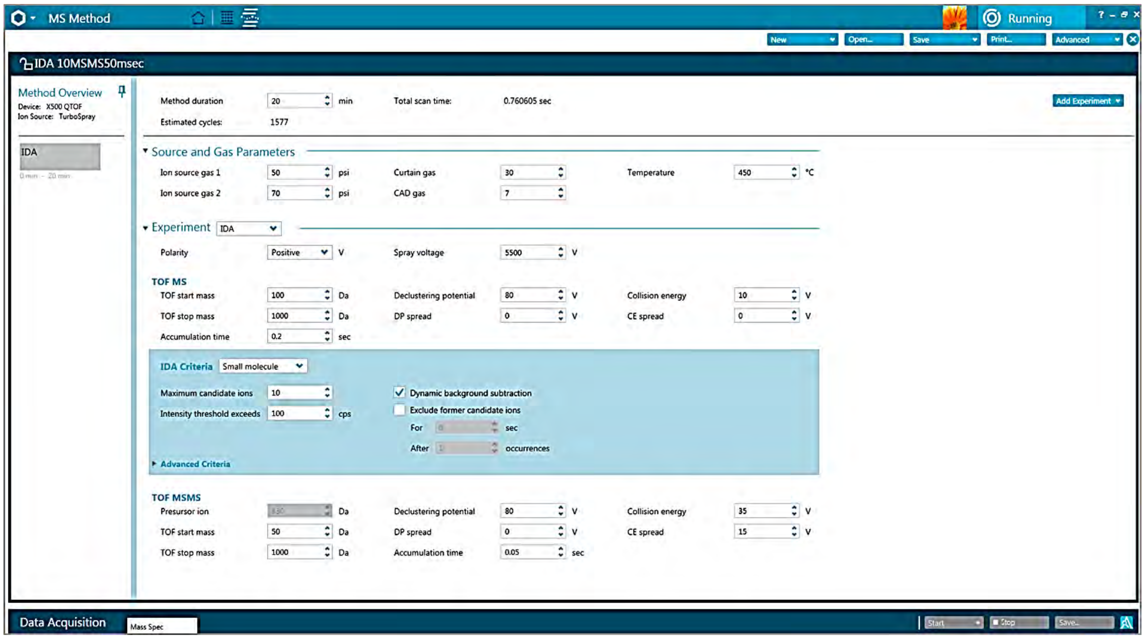
Step 1	• 10g homogenized sample (hydrate with water if needed).
Step 2	• Add 10mL acetonitrile and internal standard
Step 3	• Extract with vigorous shaking for 1 minute
Step 4	• Add QuEChERS salt mix (Phenomenex roQ KS0-8909), shake vigorously for 1 minute.
Step 5	• Centrifuge for 10 minutes at 9000 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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA
0 min – 20 min

Method duration 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters
Ion source gas 1 50 psi
Ion source gas 2 70 psi
Curtain gas 30
CAD gas 7
Temperature 450 °C

Experiment IDA
Polarity Positive V
Spray voltage 5500 V

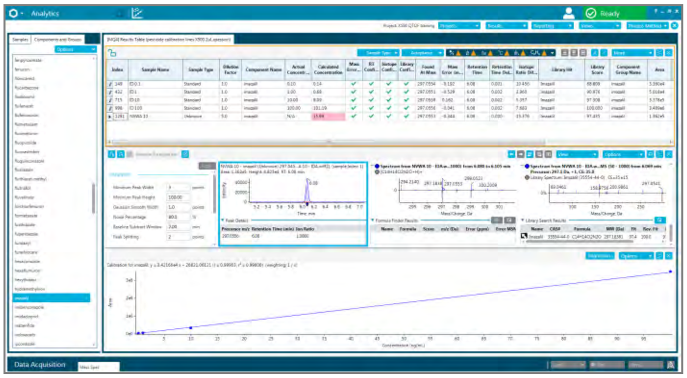
TOF MS
TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.2 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule
Maximum candidate ions 10
Intensity threshold exceeds 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For 0 sec
After 1 occurrences
Advanced Criteria

TOF MSMS
Presursor ion 330 Da
TOF start mass 50 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.05 sec
Collision energy 35 V
CE spread 15 V

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

Data Processing



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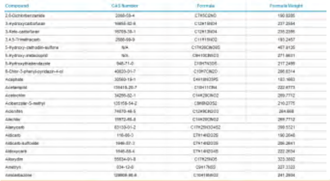
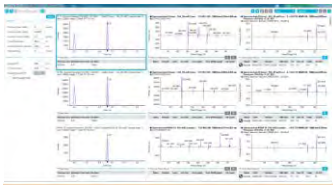
X500R High Resolution Libraries



Download the XIC List

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 MS/MS Library

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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

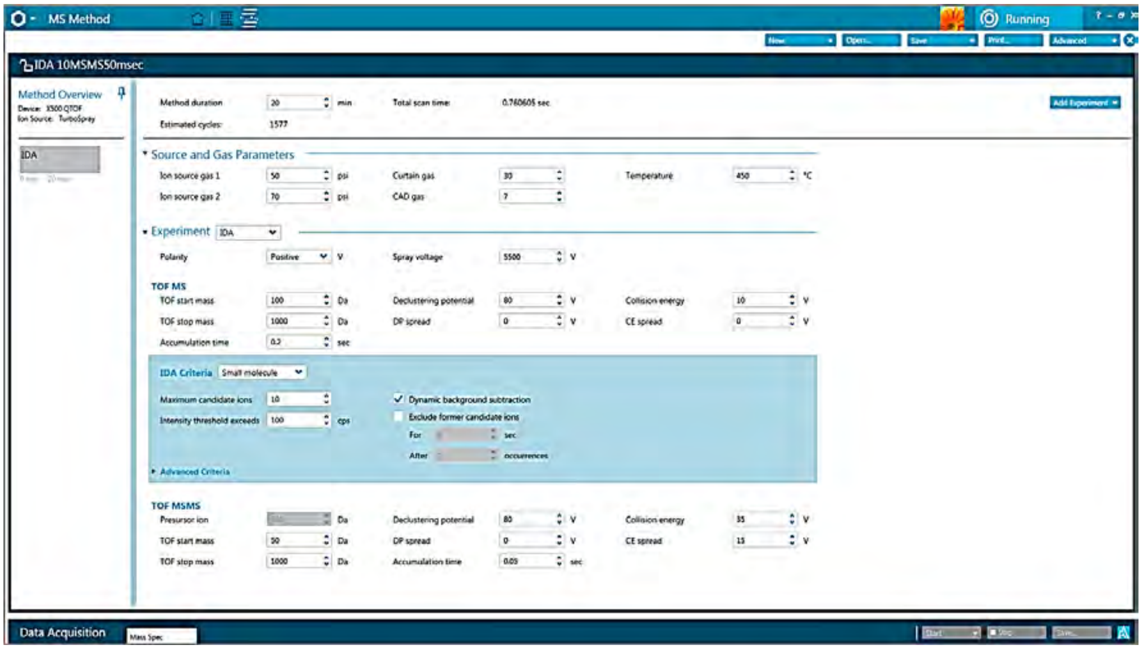
Step 1	• 1g Tissue homogenized with 10mL extraction solvent (1:5) Water Acetonitrile
Step 2	• Vortex, shake vigorously for 5 minutes, centrifuge (5000rpm, 5 minutes)
Step 3	• Decant supernatent to 15mL tube & add 500mg C18 sorbent.
Step 4	• Vortex, shake 30 sec, centrifuge (5000rpm, 1 minute).
Step 5	• 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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA

Method duration 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters
Ion source gas 1 50 psi
Curtain gas 30
Temperature 450 °C
Ion source gas 2 70 psi
CAD gas 7

Experiment IDA
Polarity Positive V
Spray voltage 5500 V

TOF MS
TOF start mass 100 Da
Declustering potential 80 V
Collision energy 10 V
TOF stop mass 1000 Da
DP spread 0 V
CE spread 0 V
Accumulation time 0.2 sec

IDA Criteria Small molecule
Maximum candidate ions 10
Intensity threshold exceeds 100 cps
✓ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences
Advanced Criteria

TOF MSMS
Presursor ion Da
Declustering potential 80 V
Collision energy 35 V
TOF start mass 50 Da
DP spread 0 V
CE spread 15 V
TOF stop mass 1000 Da
Accumulation time 0.05 sec

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.

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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.

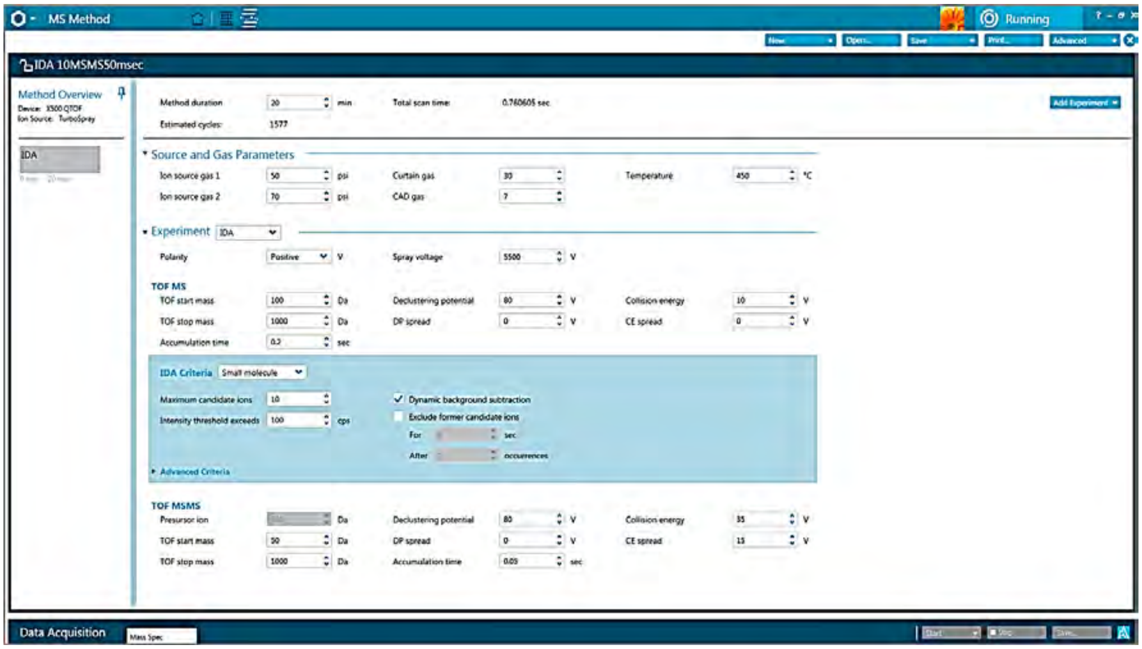
Sample prep protocol based : Sulyok M, Krska R, Schumacher R (2010) Food Chem 119:408-416

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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA

Method duration: 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters

Ion source gas 1: 50 psi
Ion source gas 2: 70 psi
Curtain gas: 30
CAD gas: 7
Temperature: 450 °C

Experiment: IDA

Polarity: Positive V
Spray voltage: 5500 V

TOF MS

TOF start mass: 100 Da
TOF stop mass: 1000 Da
Accumulation time: 0.2 sec
Declustering potential: 80 V
DP spread: 0 V
Collision energy: 10 V
CE spread: 0 V

IDA Criteria: Small molecule

Maximum candidate ions: 10
Intensity threshold exceeds: 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences

Advanced Criteria

TOF MSMS

Presursor ion Da
TOF start mass: 50 Da
TOF stop mass: 1000 Da
Declustering potential: 80 V
DP spread: 0 V
Accumulation time: 0.05 sec
Collision energy: 35 V
CE spread: 15 V

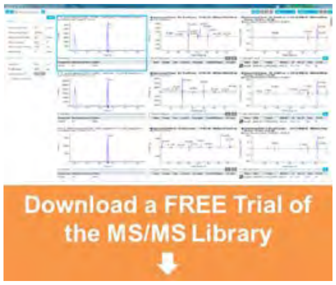
Suggested IDA (Information Dependent Acquisition) conditions for routine food contaminant testing as displayed in SCIEX OS.

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.

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X500R QTOF WATER TESTING

Drinking water analysis using Large Volume Injection

Elevate your environmental testing with the X500R QTOF System

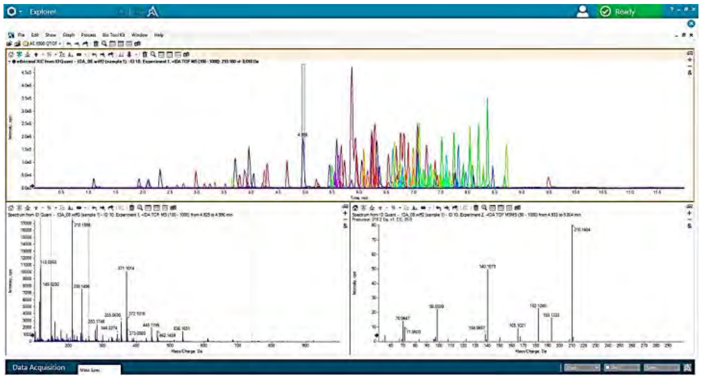
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm 460 compounds (pesticides, herbicides pharmaceuticals and personal care products) in drinking water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1

- Direct aqueous injection

SCIEX OS can deliver faster method set-up

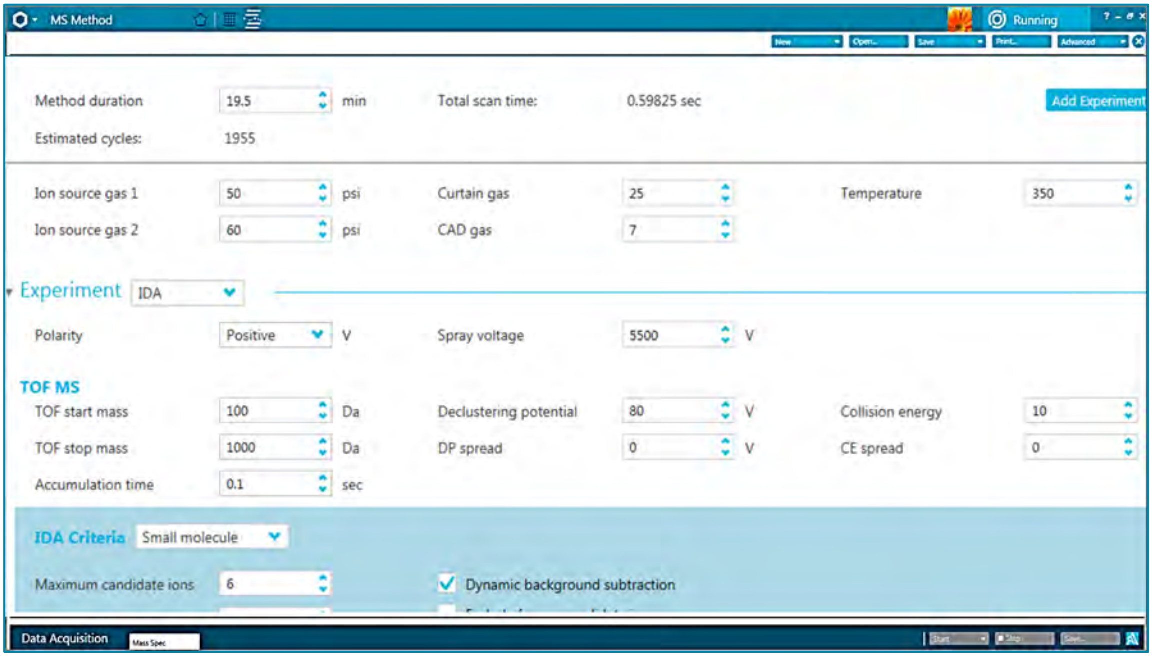


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Acquity HSS T3, 1.8µm 2.1 x 100mm	
Mobile Phase A	*5mM ammonium acetate in water*	
Mobile Phase B	*5mM ammonium acetate in methanol*	
Flow rate	*0.4 mL/min*	
Column temperature	*30 ℃*	
Injection volume	*100 uL*	
Gradient profile	**Time (min)**	**% B**
	0	*20*
	13	*80*
	15	*80*
	15.1	*20*
	17	*20*

MS Method



Method duration 19.5 min
Total scan time: 0.59825 sec
Estimated cycles: 1955

Ion source gas 1 50 psi
Ion source gas 2 60 psi
Curtain gas 25
CAD gas 7
Temperature 350

Experiment IDA

Polarity Positive V
Spray voltage 5500 V

TOF MS

TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.1 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10
CE spread 0

IDA Criteria Small molecule

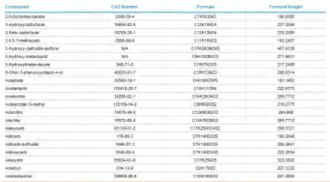
Maximum candidate ions 6
☑ Dynamic background subtraction

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

Data Processing

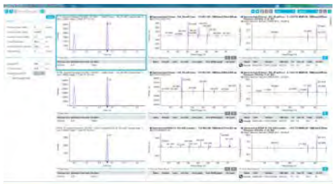
Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries



Download the XIC List

Download your free XIC compound lists detailing Pesticides and PPCP compounds including molecular formula and accurate mass.



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Pesticide analysis in water

Elevate your environmental testing with the X500R QTOF System

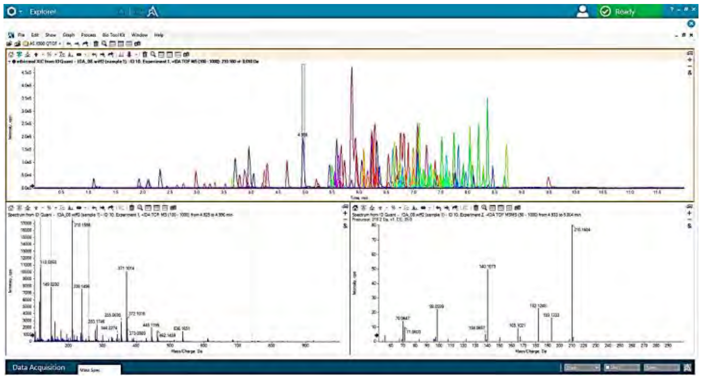
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pesticides in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1

- Direct aqueous injection

SCIEX OS can deliver faster method set-up

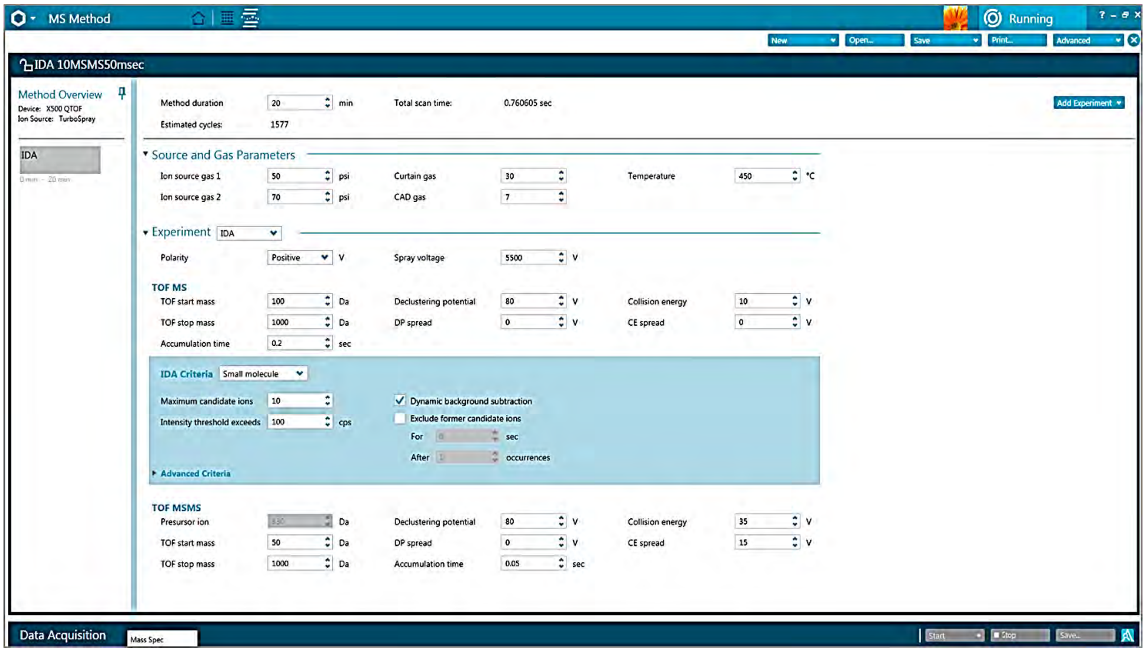


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA
0 min - 20 min

Method duration 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters
Ion source gas 1 50 psi
Ion source gas 2 70 psi
Curtain gas 30
CAD gas 7
Temperature 450 °C

Experiment IDA
Polarity Positive V
Spray voltage 5500 V

TOF MS
TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.2 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule
Maximum candidate ions 10
Intensity threshold exceeds 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For 0 sec
After 1 occurrences
Advanced Criteria

TOF MSMS
Presursor ion 830 Da
TOF start mass 50 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.05 sec
Collision energy 35 V
CE spread 15 V

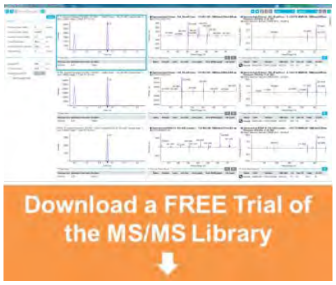
Suggested IDA (Information Dependent Acquisition) conditions for routine environmental testing as displayed in SCIEX OS.

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.



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

Learn more at sciex.com/X-Pesticides

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PPCP analysis in water

Elevate your environmental testing with the X500R QTOF System

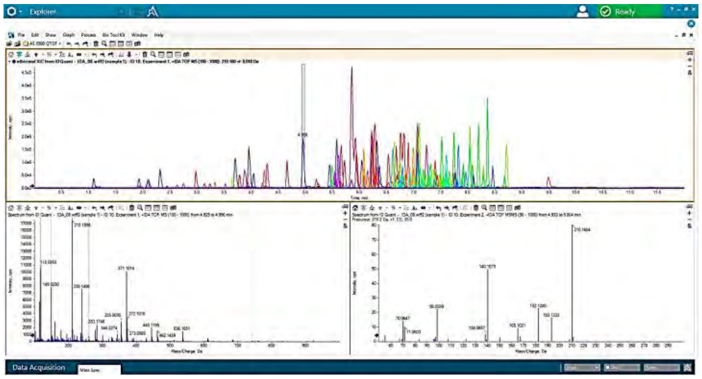
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm pharmaceuticals and personal care products in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

Sample Prep

Step 1

- Direct aqueous injection

SCIEX OS can deliver faster method set-up

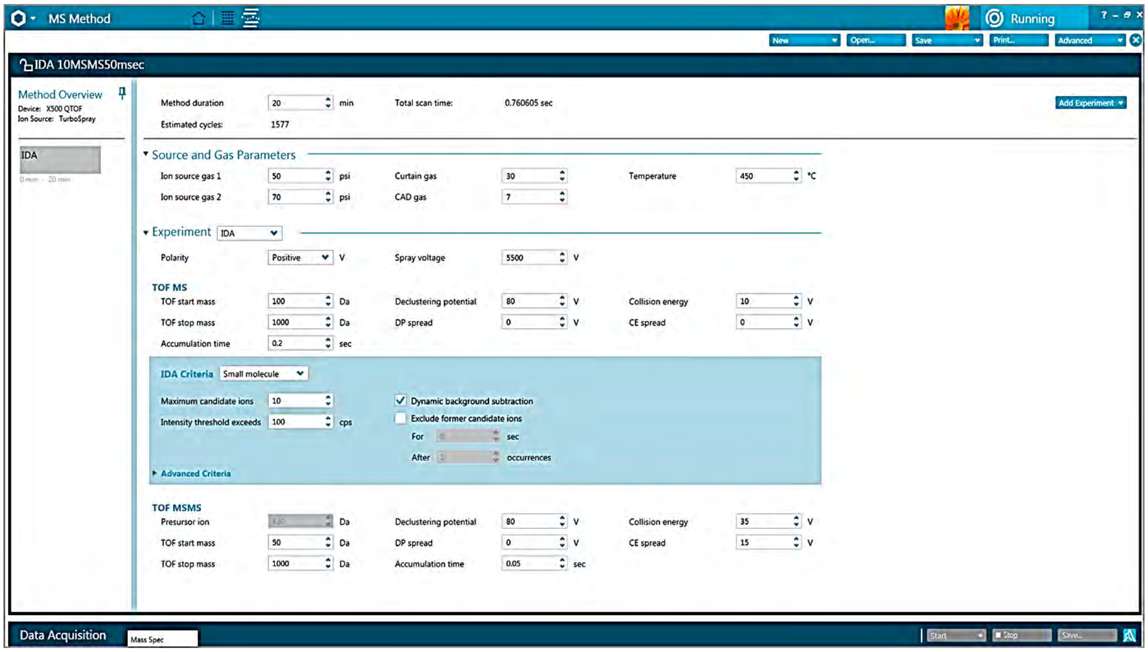


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

LC Method

Column	Phenomenex Kinetex Biphenyl, 100 x 2.1 mm, 2.6 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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA
0 min – 20 min

Method duration 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters
Ion source gas 1 50 psi
Ion source gas 2 70 psi
Curtain gas 30
CAD gas 7
Temperature 450 °C

Experiment IDA
Polarity Positive V
Spray voltage 5500 V

TOF MS
TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.2 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule
Maximum candidate ions 10
Intensity threshold exceeds 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For 0 sec
After 1 occurrences
Advanced Criteria

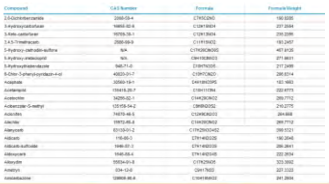
TOF MSMS
Presursor ion 850 Da
TOF start mass 50 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.05 sec
Collision energy 35 V
CE spread 15 V

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

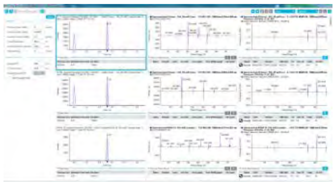
Data Processing

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

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Illicit drugs analysis in water

Elevate your environmental testing with the X500R QTOF System

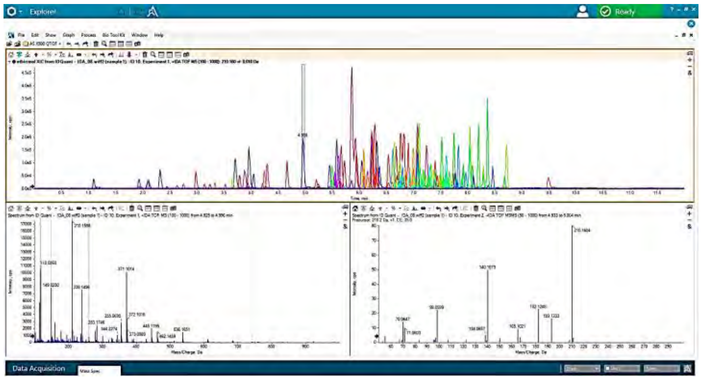
Method details and access to HR-MS/MS libraries to detect, quantify, and confirm illicit drugs in water samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

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

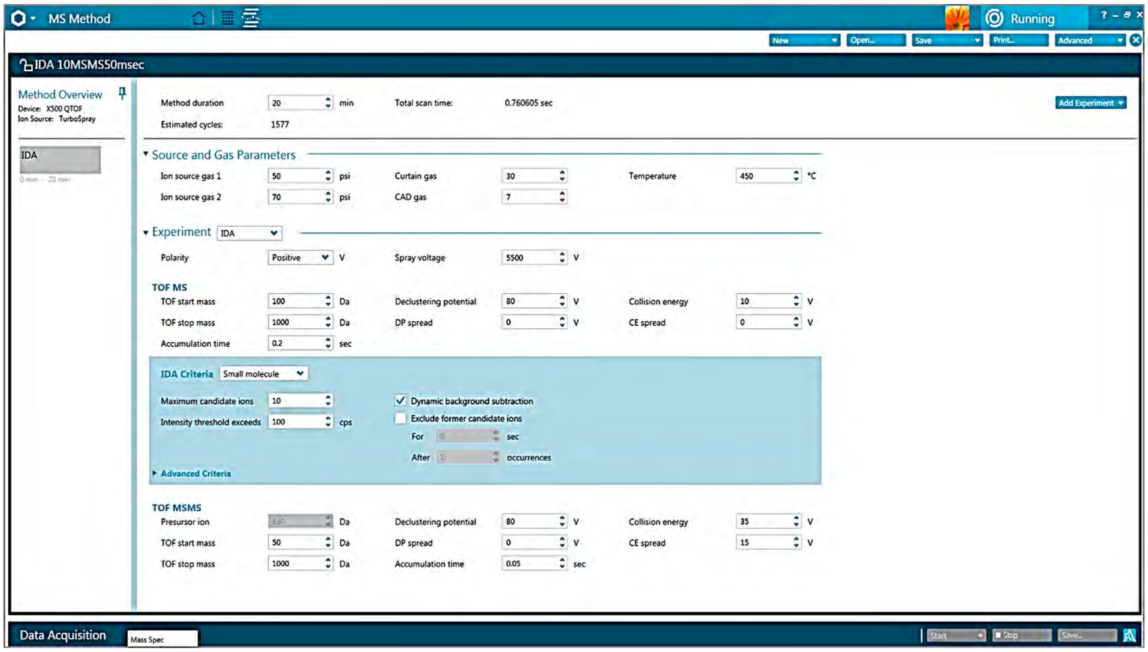


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

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



IDA 10MSMS50msec

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA
0 min - 20 min

Method duration 20 min
Total scan time: 0.760605 sec
Estimated cycles: 1577

Source and Gas Parameters

Ion source gas 1 50 psi
Ion source gas 2 70 psi
Curtain gas 30
CAD gas 7
Temperature 450 °C

Experiment IDA

Polarity Positive V
Spray voltage 5500 V

TOF MS

TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.2 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule

Maximum candidate ions 10
Intensity threshold exceeds 100 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences

Advanced Criteria

TOF MSMS

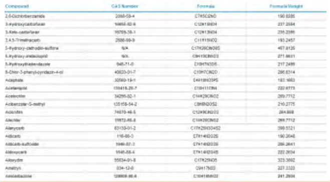
Presursor ion Da
TOF start mass 50 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.05 sec
Collision energy 35 V
CE spread 15 V

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

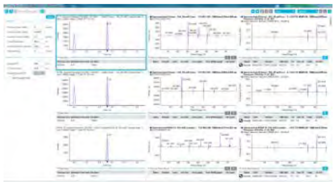
Data Processing

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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X500R QTOF FORENSIC ANALYSIS

Forensic

Using MS/MS^{All} with SWATH® Acquisition for Forensic Designer Drug Analysis with SCIEX X500R QTOF System and SCIEX OS Software

Adrian M. Taylor[1], Xiaohong Chen[2], Xiang He[2], Michael Jarvis[1] and Alexandre Wang[2]
[1]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8 Canada; [2]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA

Overview

In this technical note, we investigated the use of MS/MS^{All} with SWATH® acquisition for screening applications in a forensic toxicological setting with particular focus on bath salts. We demonstrate that SWATH® acquisition is a viable tool for identification and quantification in biological samples. A SWATH® (variable window) data acquisition was used that enabled sensitive quantitation of lower concentration species in complex matrices utilizing the more selective MS/MS information as well as using both ion ratio and MS/MS library searching for confident identification purposes.

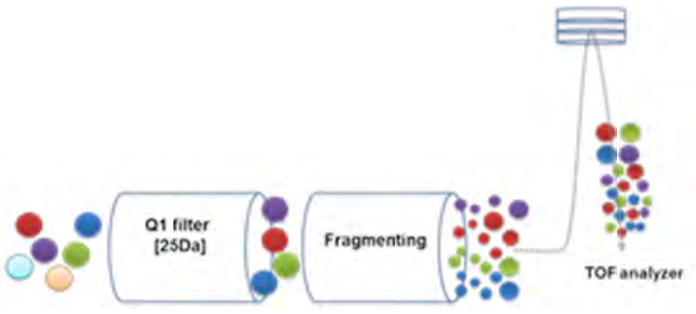


Figure 1: MS/MS^{All} with SWATH® Acquisition

Introduction

Bath Salts refer to a group of drugs containing one or more synthetic chemicals related to cathinone, one of the psychoactive principles naturally found in khat (*Catha edulis* Forsk). Some of the early synthetic cathinones such as mephedrone were first synthesised in the 1920s. However they became popular only in recent years when underground chemists began to use them in designer drugs. Since then, hundreds of other designer drugs or “legal highs” have been reported.

Chemically cathinones are similar to amphetamine and behave as central nervous system stimulants. New synthetic cathinones are constantly emerging, and their widespread availability makes it difficult for regulatory agencies to stay abreast of this major public health threat.

Many techniques have been used for bath salts screening including immunoassay, gas chromatography mass spectrometry (GC-MS), and liquid chromatography tandem mass spectrometry (LC-MS/MS). Limitations in current technologies include insufficient selectivity and sensitivity, cross-reactivity, difficulty in adding new compounds and lack of retrospective analytical capability. Single stage high resolution accurate mass platforms (e.g. Time-of-flight or TOF) solved some of these challenges but have not been able to provide clean and characteristic MS/MS information for high-confidence structural confirmation. Quadrupole TOF (QTOF) mass spectrometers combine quadrupole with TOF analyzer and enable selection of precursor ions within narrow *m/z* window (< 1 a.m.u.) before fragmentation in Q2 and separation of the fragment ions in the TOF, thereby provide high-quality MS/MS information for data analysis.

For screening applications, users often have no prior knowledge about the number and the identities of the drugs in the samples, but need to report possibly all correct identifications (true positives) and not to report erroneous compounds (false positives) or miss correct compounds (false negatives). Constantly emerging synthetic cathinones pose additional challenge since new drugs cannot be detected by existing, targeted analytical methods. Therefore, the collected screening data should contain the necessary information for confident identification of any drug in the sample, dictating a non-targeted data acquisition approach for both MS and MS/MS levels.

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. 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 2.)

Figure 2: The SCIEX ExionLC™ AC HPLC system (left), the SCIEX X500R QTOF System (middle) and SCIEX OS Software (right).

One of the acquisition methods that can be efficiently set up on the new X500R is MS/MS^{All} with SWATH$^{®}$ acquisition. This MS acquisition method allows recording MS/MS information of everything at all times during the LC gradient. In every data cycle, the instrument acquires TOF-MS information; then it sequentially acquires MS/MS information of all precursor ions across a specified mass range in pre-divided mass windows. SWATH$^{®}$ acquisition significantly improves the MS/MS data quality over traditional MS/MS^{All} techniques by allowing sequentially programed MS/MS experiments, therefore more selective MS/MS data collection (Figure 3).

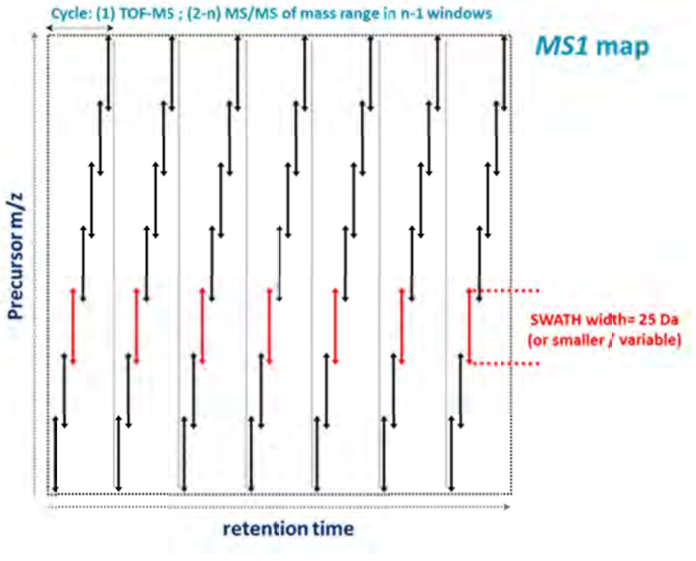


Figure 3: Principle of SWATH™ Acquisition, Demonstrated Using Fixed Window Size of 25 Da.

In this technical note, we investigated the use of SWATH$^{®}$ acquisition for identification and quantification of bath salts. We evaluated improvement on detection sensitivity with MS/MS information for quantitation purposes as well as identification based on unique fragment ions and their ratios and MS/MS library searching.

Experimental

Sample Preparation

Internal Standards. 6-APB-d5, Buphedrone Ephedrine Metabolite –d3 and 4-Fluoro-methamphetamine-d5 were used as internal standards. They were mixed and diluted in methanol at concentration of 1000 ng/mL as IS spiking solution.

Dilute and Shoot. Calibration curve with a mix of known bath salts standards were prepared in human drug free urine. Then 10 µL IS spiking solution was added to 100µL of urine samples which include both the calibration standards and unknown forensic samples. The mix was then diluted 5-fold with 90:10 (v:v), 0.1% formic acid in water : 0.1% formic acid in methanol followed by ultra-centrifugation. Injection volume was 10 µL.

Liquid Chromatography

HPLC separation was performed at 30 °C on a reversed-phase HPLC column (50 × 2.1 mm). Mobile phases used were water and methanol with appropriate additives. The LC flow rate was 0.5 mL/min and the LC runtime was 6.5 minutes.

MS and MS/MS Conditions

MS and MS/MS data were collected using SWATH$^{®}$ acquisition on the new benchtop SCIEX X500R QTOF System with SCIEX OS software, each SWATH$^{®}$ acquisition scan beginning with a TOF-MS experiment. Table 1 lists the data acquisition methods and source conditions. Variable SWATH$^{®}$ acquisition window size was applied to accommodate the application of three internal standards, ensuring that the labeled internal standards were not in the same SWATH$^{®}$ acquisition window as the none-labeled analytes.

Table 1: Data Acquisition Parameters Used for Analyzing Urine Samples

	SWATH$^{®}$ acquisition; variable
TOF-MS	100 to 700 *m/z*, 0.1 sec
Precursors of MS/MS	114 to 500 *m/z*, in 29 windows (variable size)
MS/MS	30 to 500 *m/z* x 29
Collision energy ramp	20 to 50 V
Total cycle time	**0.973 sec**

Table 2: List of Drug targets For Urinary Analysis

Drugs analyzed in positive mode

2,5-Dimethoxy-4-n-propylthiophenethylamine	*Flephedrone*
2C-B-FLY	*Vigabatrin*
3,4-Dimethylmethcathinone	*Vilazodone*
3-Desmethylprodine	*MDPBP*
4-Ethylmethcathinone	*MDPV*
4-Fluoroamphetamine	*Methedrone*
4-Fluoromethamphetamine	*Methiopropamine*
4-Fluorotropacocaine	*Methoxetamine*
4-Methylephedrine	*Methylhexanamine*
4-Methylethcathinone	*Naloxone-N-Oxide*
4-Methyl-N-ethyl-norephedrine	*Naphyrone*
Cocaine-N-oxide	*Zopiclone-N-oxide*
5-apb	*n-Ethylcathinone*
6-apb	*n-Ethylcathinone ephedrine metabolite*
5-Iodo-2-aminoindane	*Pentedrone*
Codeine-6beta glucuronide	*Salvinorin B*
Alpha-PVP	*Tiagabine*
Alpha-Pyrrolidinopropiophenone	Butylone
n-Desmethylmirtazapine	Desomorphine
Bromo-Dragonfly	Desoxypipradrol
Buphedrone ephedrine metabolite	Etizolam

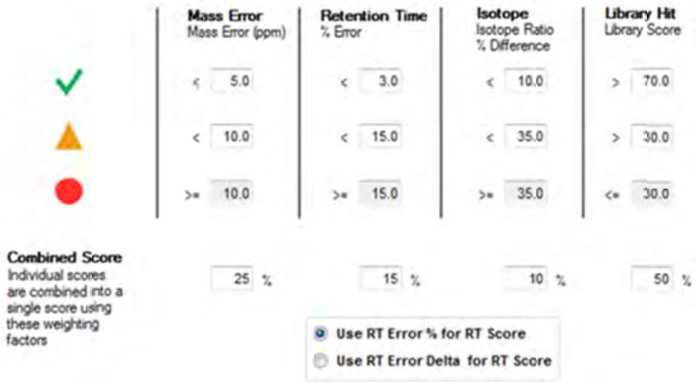
List of Target Compounds

Data was acquired in a non-targeted fashion but analyzed in a targeted way. A calibration mix was prepared that contained over 50 forensically relevant drugs; thus a targeted list consisting of 56 drugs was constructed for post-acquisition data processing containing the bath salt (detailed in Table 2) and designer drug related compounds.

Data Analysis: Confidence Settings and Screening Criteria

Data was processed in SCIEX OS software version 1.0. Reporting was performed also in SCIEX OS software with customized report templates.

Figure 4 is an example of the confidence setting used for screening. Four main confidence criteria were used for positive identification determination, which were mass error (M), RT error (R), isotope ratio difference (I), and library score (L). Subsequently, a combined score (C) was computed based on these four confidence categories (MRIL) with custom weightings. Finally, when there was no comparison sample (blank sample or sample spiked with drugs at reference level), the absolute peak intensity was used as an additional criteria to help reduce false positive rate.



	Mass Error Mass Error (ppm)	Retention Time % Error	Isotope Isotope Ratio % Difference	Library Hit Library Score
✓	< 5.0	< 3.0	< 10.0	> 70.0
▲	< 10.0	< 15.0	< 35.0	> 30.0
●	>= 10.0	>= 15.0	>= 35.0	<= 30.0
Combined Score Individual scores are combined into a single score using these weighting factors	25 %	15 %	10 %	50 %

● Use RT Error % for RT Score

○ Use RT Error Delta for RT Score

Figure 4. Confidence Settings in SCIEX OS Software

Results and Discussion

Figure 5 shows a representative quantitative linear dynamic range of the SCIEX X500R QTOF System showing 4 orders for the Etizolam compound (0.5 to 1000 ng/mL in urine) in TOF-MS mode. The figure also shows that even at high concentrations the mass error is still below 1.5 ppm. An example from the table in Figure 6 is for the standard at 2000 ng/mL in which the mass error is -0.6 ppm.

Figure 5. Linearity of the SCIEX X500R QTOF System Shown for Etizolam (0.5 ng/mL to 1000 ng/mL)

MS/MS^{All} with SWATH$^{®}$ Acquisition

Accurate mass TOF MS full scan provides the advantage of a generic methodology. It is a non-targeted method that allows later data re-interrogation to serach for unanticipated drugs. This is particularly important in the scenario of designer drugs where new drugs emerge on a monthly basis. But full scan TOF MS approach, in a lot of cases, is not selective enough when analyzing biological samples where matrix interference is common. SWATH® acquisition is a non-targeted method providing selective MS/MS detection of every single analyte in addition to the full scan TOF MS data.

SWATH® acquisition allows MS/MS to be acquired all the time and it is therfore possible to use that information to confidently distinguish the presence or absence of structural isomers that fragment to produce unique fragmentation pathways and therefore unique fragment ions.

Although the screening data in the technical note was acquired in a non-targeted fashion, it was anayzed in a targeted way, which means a compound list was pre-assembled to perform the targeted analysis. This list contained the chemical formulas (for extracted ion mass calculation of the precursor molecular ion), mass extraction window, retention times and retention time window. The list also contained the accurate mass of several unique fragment ions for each compound.

Figure 6 shows an example where two structural isomers are barely chromatographically separated and relying on retention time alone may not accurately identify the presence of the correct isomer. Accurate mass of the precursor molecular ion will also not be able to distinguish between the two isomers. SWATH® acquisition however gurantees the collection of MS/MS data which through library searching against the SCIEX High Resolution MS/MS Forensic Spectral Library (Version 2.0) has allowed the confident identification of the 3,4 Dimethylmethcathinone and 4-Ethylmethcathinone (both having an accurate mass of 192.138 with a chemical formula of $C_{12}H_{17}NO+H^+$).

Further to library searching of MS/MS data, acquiring MS/MS all the time, allows the extraction of unique fragment masses which provide a clean extracted ion chromatogram trace for quantifcation purposes; while the TOF-MS equivalent trace of the extracted precursor ion, will in many cases show interference. Figure 7 shows the same example of 3,4 Dimethylmethcathinone and 4-Ethylmethcathinone structural isomers used previously, where the interference in TOF MS trace (pink) is eliminated in the MS/MS traces of the unique fragment ions (blue) *m/z* 159.10425 and 145.0886 for Dimethylmethcathinone and 4-Ethylmethcathinone respectively. Top pane A is the example of 3,4 Dimethylmethcathinone (RT=4.41 min), and the bottom pane (B) refers to 4-Ethylmethcathinone (RT= 4.61min).

Another example of the gain in specificity of choosing a unique fragment ion of a compound over reliance on the accurate mass of the precursor molecular ion is demonstrated in Figure 8. SWATH® acquisition collects all fragment ion data across the whole LC peak and therfore allows the ability to choose the correct ion that is free of interference and high background for more specific detection and quantification. Figure 8 shows an example of n-ethylcathinone ephedrine metabolite; a structural isomer to 4-methylephedrine and buphedrone ephedrine metabolite. The compounds are barely chromatographically separated and extraction of the precursor ion produces high background and interfering peaks. N-ethylcathinone ephedrine metabolite has a unique fragment ion at *m\z* 117 that can be extracted out to remove interfering ions and reduction in the background. The fragment ion at *m/z* 132.080 wouldn't be chosen for quantification purposes as it doesn't provide enough specificity.

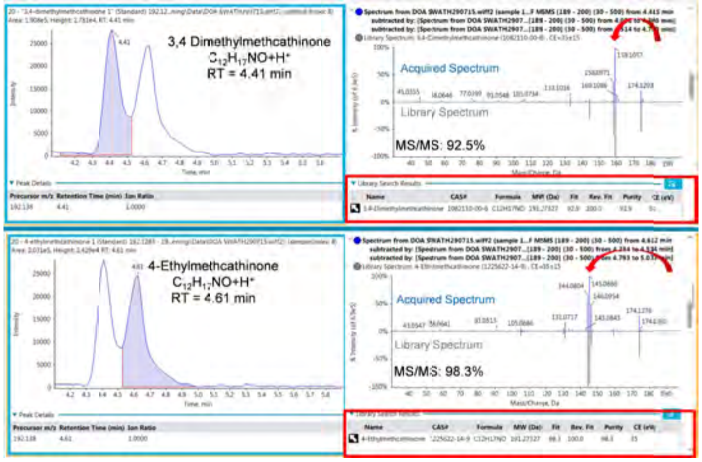


Figure 6. Confident Identification of Structural Isomers using MS/MS Data Collected from a SWATH® Acquisition Experiment

A third example of the specificity advantage gained by the ability to extract fragment ion information from SWATH® acquired data is shown in Figure 9. SWATH® acquisition is a truly non-targeted method providing selective MS/MS detection of every single analyte in addition to the full scan TOF MS data. In the situation when LC is not separating the structural isomer interferences, extraction of unique fragments will provide a clean extracted ion chromatogram, while the TOF MS trace will show interference. Figure 9 shows two examples where the interference in TOF MS trace (green) is eliminated in the MS/MS trace (blue). Top pane A is the example of Cocaine-N-Oxide (RT=6.99), and the bottom pane B refers to the internal standard 6-apb-d5.

The specificity of a fragment ion is not only important in the correct identifcation of a compound but also in accurate and precise quantification. Methoxetamine is given as an example in Figure 10, where the extraction of the unique fragment ion at *m/z* 121.06 (blue trace) has allowed the removal of an interfering peak that is seen in the precursor ion extracted ion chromatogram for *m/z* 248.1645 (pink trace).

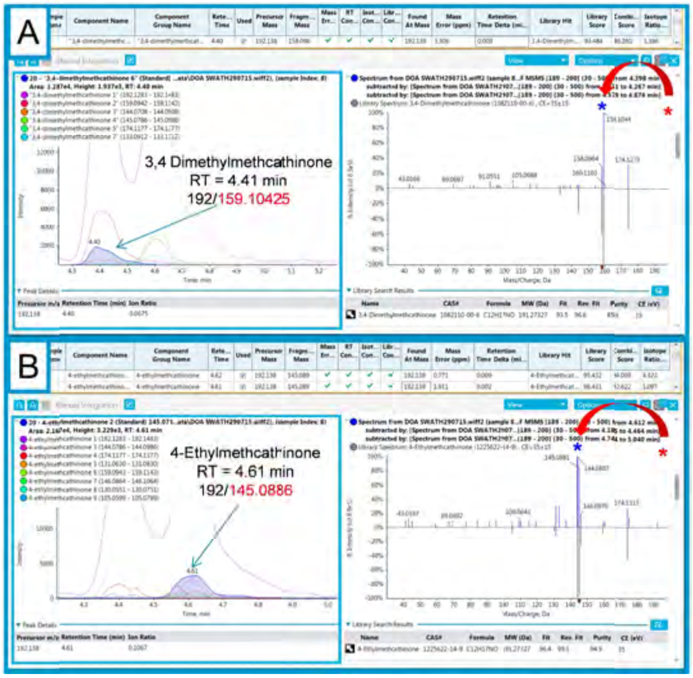


Figure 7. Confident Identification of Structural Isomers using SWATH® Acquisition based on Extraction of Unique Fragment Ions. **TOF MS scan pink trace vs. MS/MS blue trace. (A) 3,4 Dimethylmethcathinone; RT=4.41 min, MS/MS extracted using fragment ion at *m/z* 159.10425. (B) 4-Ethylmethcathinone, RT=4.61min. MS/MS extracted using fragment ion at *m/z* 145.0886.**

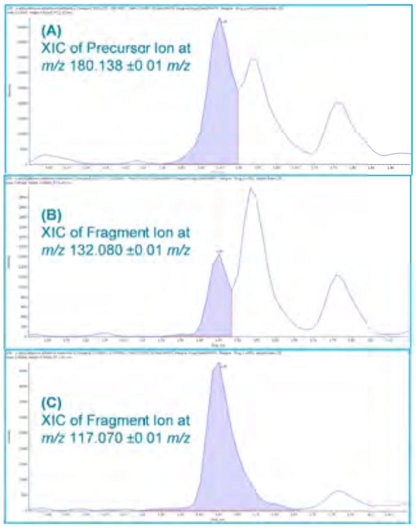


Figure 8. Ability to Extract Compound Unique Fragment Ions from SWATH® Acquired Data (A) XIC of precursor ion for n-ethylcathinone ephedrine metabolite at m/z 180.138; a structural isomer to 4-methylephedrine and buphedrone ephedrine metabolite. (B) XIC of fragment ion at m/z 132.080 (C) XIC of -ethylcathinone ephedrine metabolite unique fragment ion at m/z 117.070.

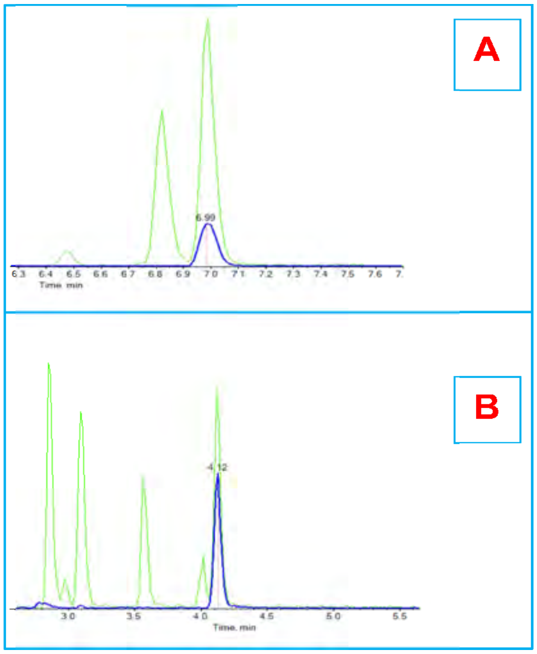


Figure 9. TOF MS Scan (Green Trace) vs. SWATH® Acquired TOF-MS/MS (Blue Trace). (A) Cocaine-N-Oxide, RT=6.99 min, MS/MS extracted using fragment 182.1166. (B)Internal standard, 6-apb-d5, RT=4.12min. MS/MS extracted using fragment 133.0619.

This clean up allows for more efficient integration and reliable quantification with high precision.

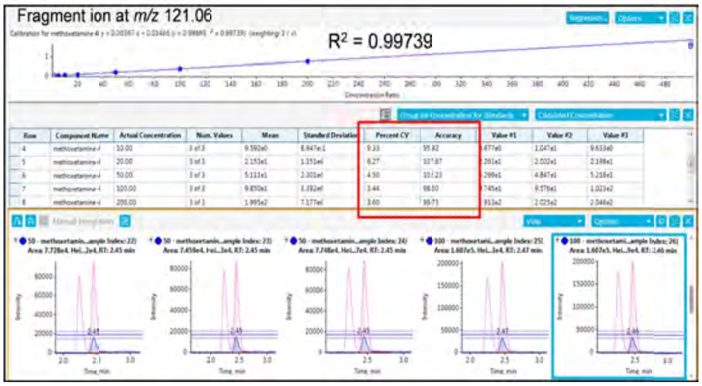


Figure 10. Quantification Statistics, Calibration line (ng/mL) and Extracted Ion Chromatograms (Qualifier, Quantifier ions overlayed) for Methoxetamine Compound from SWATH® Acquired Data.

As already suggested by Figure 10, SCIEX OS software allows for quantification and ion ratio/library searching confirmation to be performed simultaneously and for the results of this processing to be displayed together (Figure 11). Figure 11 shows the result table for a SWATH® acqusiition sample set containing calibrators and spiked unknown urine samples. Results can be displayed and mined via sample or by a specific compound/ compound group. The results table has customisable column displays, one selection being the *Calculated Concentration*. Samples that have compounds above a cutoff concentration can be flagged, as shown by the red highlighted cell for Unknown A, in the *Calculated Concentration* results table column (Figure 11).

Figure 11.Simultaneous Identification and Quantification in SCIEX OS Software. **Reviewing qualitative and quantitative results from SWATH® Acquired Data in the same software window.**

As well as determining the amount of compound identified that is present in the sample, the SCIEX OS software also indicates the confidence in the compound identification through the traffic light columns using accurate mass, retention time, iostope pattern and library searching as the criteira.

For a selected compound, the viewer can review the peak integration of the extracted ion chromatogram as well as the TOF-MS and TOF-MS/MS spectra and also the calibration line. As this data was acquired through SWATH® acquisition, post acqusition data processing can also include the extraction of the precursor ion and mutiple accurate mass fragment ions and therefore ion ratio determinations. The extracted ion chromatogram for both the qualifier and quantifier ions can be overlayed with the ion ratio lines displayed.

Figure 12 shows that the results table can be filtered in various ways dependent on sample type and acceptance criteria (based on integration accetance, accuracy and calculated concentation). In the example shown, the software has been set up to only show unknown samples that show a positive finding with a compound above a specified cutoff level of 10 ng/mL.

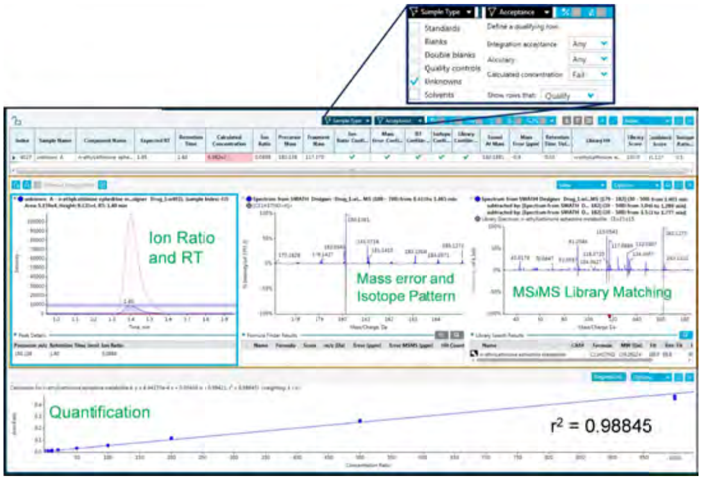


Figure 12. Unknown Sample Analysis. Calculated Concentration (Quant) with Ion Ratio and Library Searching from SWATH® Acquired Data. **Filtering the results table to only show the positive findings.**

Figure 13 shows an example of a calibration series for 4-methyl-N-ethyl-norephedrine displaying the quantification results as well as the ion ratio confirmation with both the qualifier and quantifier extracted ion chromatograms for each of the calibrators. Library searching confirmation was performed at the same time as ion ratio confirmation and generation of a calibration curve.

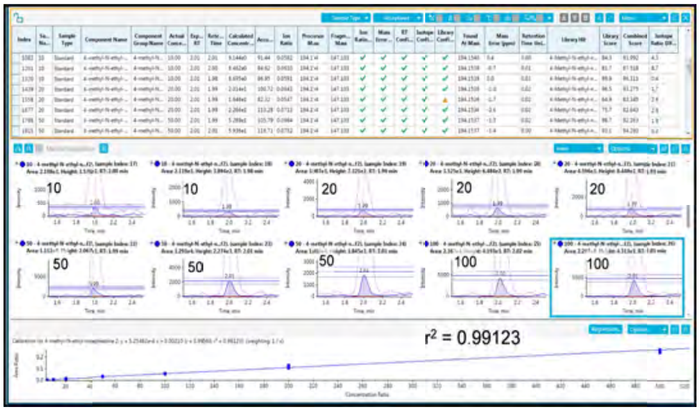


Figure 13. Quantification Results with Ion Ratio/Library Searching Confirmation from SWATH® Acquired Data. **Example using calibrators for 4-methly-N-ethyl-norephedrine.**

Figure 14 shows that acquiring data via SWATH® acquisition allows multiple ions in which to qant from, retrospectively chosing a different one if a unique interference is found in a one off sample. Figure 14 is also another example showing that even at high concentration, good mass accuracy is obtained.

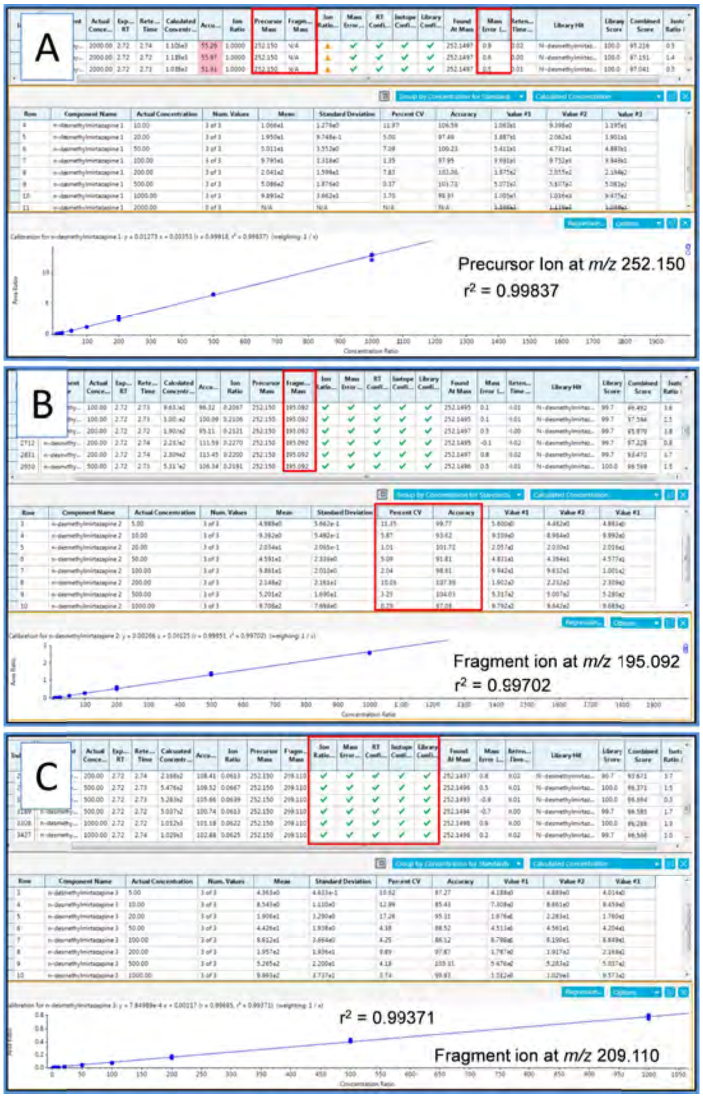
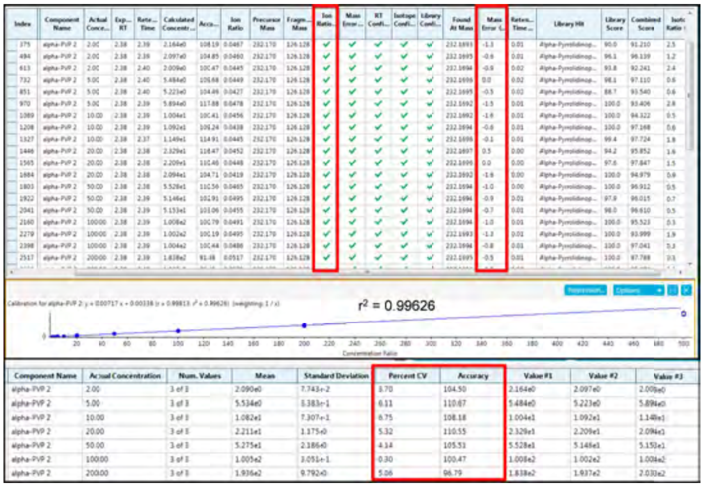


Figure 14. For the Same Compound, Multiple Ions can be Chosen in which to Quantify From After using SWATH® Acquisition. **Results are given for the precursor ion at *m/z* 252.150 [A] and two fragment ions, at m/z 195.092 [B] and 209.110 [C] for N-Desmethylmitrapazine.**

As an example of the comprehensive information that can be extracted from a SWATH® acqusition sample set, Figures 15,16 and 17 show the qunatifiaction performance (precision and accuracy), ion ratio and library searching results for a calibrator set for the alpha-PVP compound.



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
alpha-PVP 2	2.00	3 of 3	2.090e0	7.743e-2	3.70	104.50	2.164e0	2.097e0	2.009e0
alpha-PVP 2	5.00	3 of 3	5.534e0	3.383e-1	6.11	110.67	5.484e0	5.223e0	5.894e0
alpha-PVP 2	10.00	3 of 3	1.082e1	7.307e-1	6.75	108.18	1.004e1	1.092e1	1.149e1
alpha-PVP 2	20.00	3 of 3	2.211e1	1.175e0	5.32	110.55	2.329e1	2.209e1	2.094e1
alpha-PVP 2	50.00	3 of 3	5.275e1	2.186e0	4.14	105.51	5.528e1	5.148e1	5.153e1
alpha-PVP 2	100.00	3 of 3	1.005e2	3.051e-1	0.30	100.47	1.008e2	1.002e2	1.004e2
alpha-PVP 2	200.00	3 of 3	1.936e2	9.792e0	5.06	96.79	1.838e2	1.937e2	2.030e2

Figure 15. Quantification (Preciision and Accuracy), Ion Ratio and Library Matching for an Alpha-PVP Calibration Set from SWATH® Acquired Data.

Figure 16. Extracted Ion Chromatograms for Alpha-PVP (ng/mL) Fragment Ion from SWATH® Acquired Data

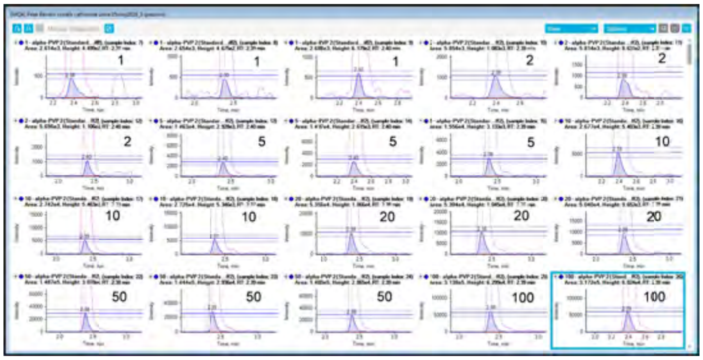


Figure 17. Alpha-PVP; Ion Ratio Chromatograms for Calibrators with Tolerance Lines from SWATH® Acquired Data

Unknown Sample Identification and Quantitation:

Unknown forensic samples were prepared and analyzed the same way as the standards by dilute and shoot. Data from thirteen unknown samples were acquired using SWATH® acquisition and processed in a targeted fashion, mining the data for the targeted list of bath salt compounds. The fact that quantitation can be done with MS informaiton (TOF-MS full scan) or MS/MS information from SWATH® acquisition is demonstrated here using an unknown forensic sample donated by a forensic lab.

In unknown sample 6, two peaks are present at RT=2.9 min and RT=3.1 min, (shown in Figure 18, peak A and B), when extracting out the precursor ion for buphedrone ephedrine metabolite (BEM, formula: $C_{11}H_{17}NO$) at *m/z* of 179.1301. From the TOF MS scan (dark blue trace) and RT, both peaks can potenticially indicate the presence of buphedrone ephedrine metabolite. Upon closer analysis of the MS/MS extraction, peak A showed overlay of buphedrone ephedrine metabolite fragment ions at m/z 133.09, 162.13 and 91.05. However peak B doesn't have these fragment peaks. Even though BEM has a retention time of 3.1 minutes, the MS/MS data for this peak suggests otherwise. Further analysis on the MS/MS spectra confirms the chromatographic inaccuracy.

In Figure 18, pane C showed acquired MS/MS spectrum (blue) at RT=2.9 min matches well with the grey library spectrum of BEM. On the other hand, pane D acquired spectrum at RT=3.1 doesn't show similiarity to BEM library spectrum, nor does it match up to the library spectrum of Mexiletine (shown in grey in D). Thus we conclude that sample 6 is identified to be positive with BEM. Retention time can be influenced by many factors, incluing column life time, sample matrix (e.g. pH value). In this particular case, even though the standards were prepared with real human urine, it is from a different individual compared to unknown sample 6. It is not surprising that we see slight difference in the retention time.

For BEM, calibration line generation using the precursor ion from the TOF-MS experiment as well as all three fragment ions from the SWATH® acquisition experiment demonstrated good linearity from 1-1000 ng/mL with r values over 0.99. Unknown sample 6 is calculated to contain 108 ng/mL BEM. Figure 19, left, shows the integrated peak of BEM (extracted using fragment ion at *m/z* 162.13). The right pane in Figure 19 shows the linearity of all 4 calibration lines.

Conclusion

In this technical note, we described the use of MS/MS^{All} with SWATH® acquisition for forensic drug screening with the QTOF X500R system and SCIEX OS software. We demonstrated that SWATH® acquisition is a viable tool for screening of bath salts in human urine.

Non-targeted SWATH® acquisition affords retrospective data analysis which is critically important for the fast emerging new designer drugs.

It was shown in this study that, due to the continual and looped MS/MS scan function and better selectivity with the fragment ion information, SWATH® acquisition enabled quantification in MS/MS mode of low concentration species in complex matrix and also allowed for ion ratio confirmation determinations in combination with library searching for confident identifications. We successfully identified and quantified buphedrone ephedrine metabolite in an unknown sample which contained 108 ng/mL BEM.

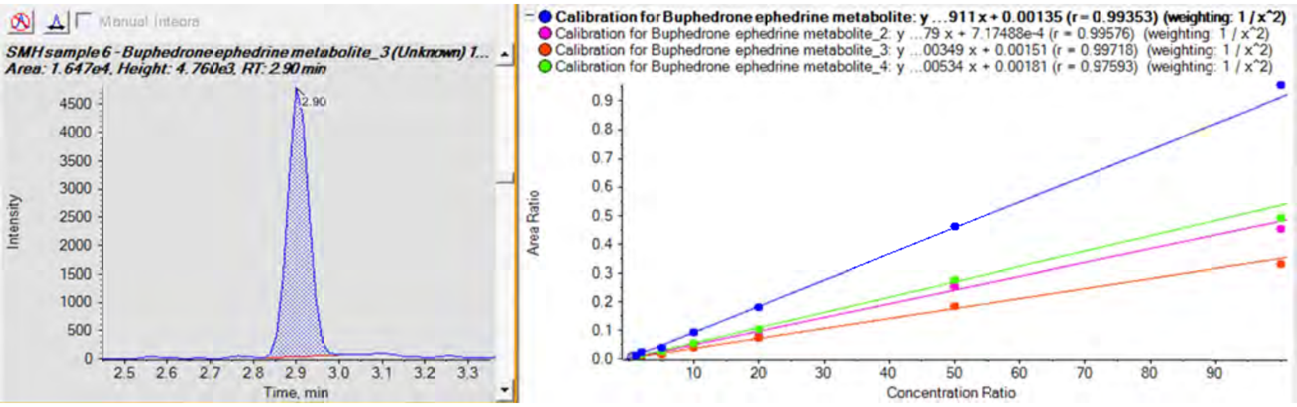


Figure 19. Left, Integrated Extracted Ion Chromatogram for BEM Fragment Ion at *m/z* 162.13 from SWATH® Acquired Data for Unknown Sample 6. Right, Representative Calibration Curves for the BEM Precursor Ion at *m/z* 179.1301 from the TOF MS Experiment and Three Fragment Ions from the SWATH® Acquisition Experiment.

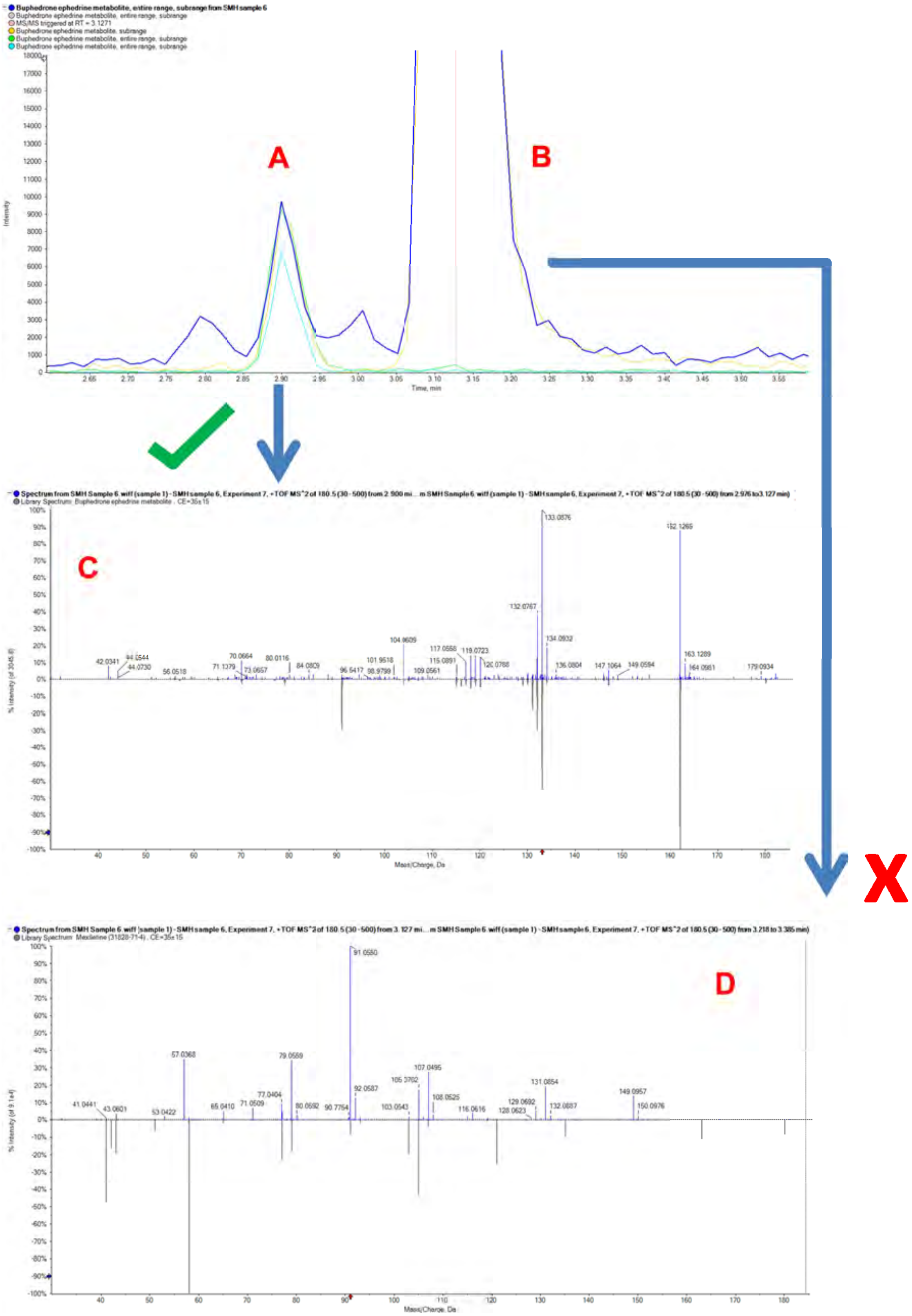


Figure 18. Extracted Ion Chromatograms and MS/MS Data for Unknown Sample Six. **Top pane: overlaid extracted ion chromatograms of precursor ion (*m/z* 179.1301; TOF-MS) and fragment ions (at m/z 133.09, 162.13 and 91.05; SWATH® acquisition) for buphedrone ephedrine metabolite from unknown sample #6, containing peaks A (RT=2.9 min) and B (RT=3.1 min). (C) Library matching of buphedrone ephedrine metabolite (peak A), acquired MS/MS spectrum (blue) and library spectrum (grey). (D) Library matching of unknown peak B, acquired MS/MS spectrum (blue) and library spectrum (grey).**

Document number: RUO-MKT-02-4542-A

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Forensic

SCIEX

Identification and Quantification of Forensic Compounds using MRM^{HR} on the SCIEX X500R QTOF System with SCIEX OS Software

Adrian M. Taylor[1], Xiang He[2] and Alexandre Wang[2]
[1]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8 Canada; [2]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA

Overview

In this technical note, we investigated the use of MRM^{HR} (Figure 1), on the SCIEX X500R QTOF, for targeted quantification applications in a forensic toxicological setting. We demonstrate that MRM^{HR} is a viable tool for identification and quantification in biological samples. MRM^{HR} data acquisition enabled sensitive quantitation of low concentration species in complex matrices utilizing the selective high resolution, accurate mass MS/MS information as well as using ion ratio for identification purposes.

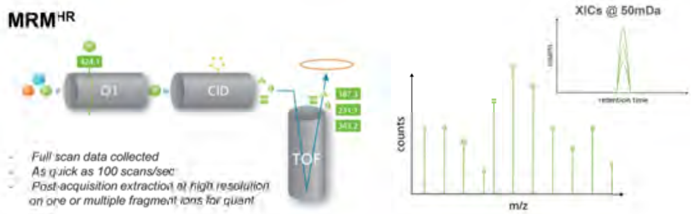


Figure 1. High Resolution MRM (MRM^{HR})

Introduction

MRM^{HR} (High Resolution Multiple Reaction Monitoring) is a targeted data acquisition method for quantification purposes using a high resolution accurate mass QTOF instrument. Here we demonstrate the technique on 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. 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 2).

MRM^{HR} uses the same selectivity principle of the unique fragment ion concept that is gained in an MRM experiment performed on a triple quadrupole. The difference is that in a regular MRM experiment selectivity is gained by nominal mass Q3 selection of a unique fragment ion, but in MRM^{HR} selectivity is gained by post acquisition extraction of the accurate mass of the unique fragment ion from a full scan TOF-MS/MS product ion experiment (Figure 1). In contrast to the nominal mass workflow, now we have the capability of extracting out the high resolution accurate mass of one or multiple fragment ions for quantification purposes and retrospectively chosing a different one if a unique interference is found in a one off sample. This is achieved from one single product ion experiment of the compounds molecular ion, as a opposed to multiple MRM experiments for each unique fragment ion performed on the triple quadrupole workflow.

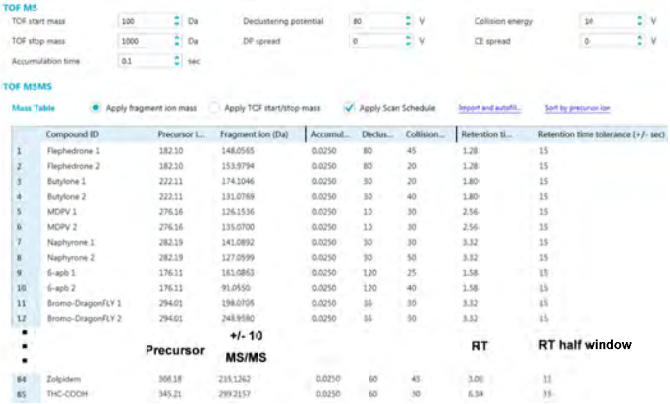
Figure 2: The SCIEX ExionLC™ AC HPLC system (left), the SCIEX X500R QTOF System (middle) and SCIEX OS Software (right).

The MRM^{HR} experimental design consists of a TOF-MS experiment, in order to capture the accurate mass of the precursor ion, followed by a series of looped TOF-MS/MS experiments of different precursor ions corresponding to the target list of compounds that are being analyzed.

MRM^{HR} Workflow on the SCIEX X500R QTOF System

To help transition from the familiarity of MRM performed on a triple quadrupole to MRM^{HR} performed on the SCIEX X500R QTOF system, the SCIEX OS Software has been designed with an option that allows a unique way of building the MRM^{HR}

method. It has the look and feel of performing traditional MRM experiments by allowing the input of the accurate mass of the precursor ion (MRM Q1 equivalent mass) and accurate fragment mass (MRM Q3 equivalent nominal mass) in the acquisition method (Figure 3). These transitions can easily be imported from the SCIEX high resolution 1700 compound MS/MS forensic spectral library (Forensic HR-MS/MS Spectral Library version 2.0) to include up to 5 transitions per compound.



TOF MS

TOF start mass: 100 Da
TOF stop mass: 1000 Da
Accumulation time: 0.1 sec
Declustering potential: 80 V
DP spread: 0 V
Collision energy: 10 V
CE spread: 0 V

TOF MSMS

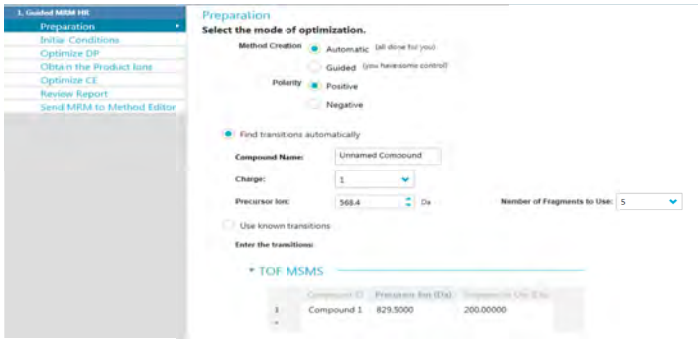
Mass Table: ● Apply fragment ion mass ○ Apply TOF start/stop mass ✓ Apply Scan Schedule

	Compound ID	Precursor i...	Fragment ion (Da)	Accumul...	Declus...	Collision...	Retention ti...	Retention time tolerance (+/- sec)
1	Flephedrone 1	182.10	148.0565	0.0250	80	45	1.28	15
2	Flephedrone 2	182.10	153.9794	0.0250	80	20	1.28	15
3	Butylone 1	222.11	174.1046	0.0250	30	20	1.80	15
4	Butylone 2	222.11	131.0769	0.0250	30	40	1.80	15
5	MDPV 1	276.16	126.1536	0.0250	10	30	2.56	15
6	MDPV 2	276.16	135.0700	0.0250	10	30	2.56	15
7	Naphyrone 1	282.19	141.0892	0.0250	30	30	3.32	15
8	Naphyrone 2	282.19	127.0599	0.0250	30	50	3.32	15
9	6-apb 1	176.11	161.0863	0.0250	120	25	1.58	15
10	6-apb 2	176.11	91.0550	0.0250	120	40	1.58	15
11	Bromo-DragonFLY 1	294.01	198.0705	0.0250	35	30	3.32	15
12	Bromo-DragonFLY 2	294.01	248.9580	0.0250	35	30	3.32	15
▪ ▪ ▪								
84	Zolpidem	308.18	235.1262	0.0250	60	45	3.00	11
85	THC-COOH	345.21	299.2157	0.0250	60	30	6.34	15

Figure 3. SCIEX OS Software *Scheduled* MRM^{HR} Method Editor, Applying Fragment Ion Mass ± 10 *m/z*

Guided MRM^{HR} Mode

Alternatively the optimum intense fragment ions can be determined together with optimization of the compound dependent parameters, for each MRM^{HR} transition, using *Guided MRM^{HR}* mode in the SCIEX OS software. This mode guides the user through a series of steps in order to determine the optimum MRM^{HR} transitions (Figure 4) which at the end automatically generates an acquisition method containing all optimized transitions.



Select the mode of optimization.

Method Creation: ● Automatic (all done for you) ○ Guided (you have some control)
Polarity: ● Positive ○ Negative

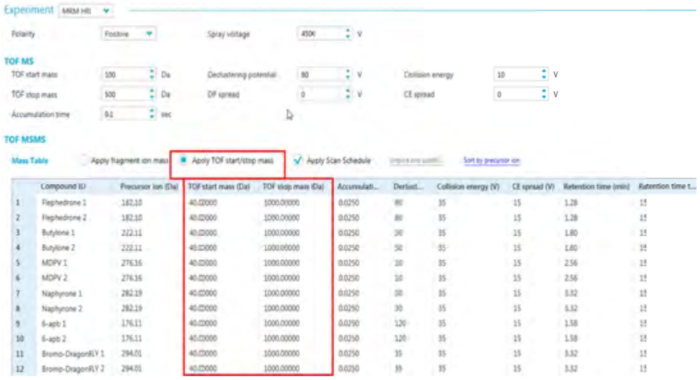
● Find transitions automatically
Compound Name: Unnamed Compound
Charge: 1
Precursor Ion: 568.4 Da
Number of Fragments to Use: 5

☐ Use known transitions
Enter the transitions:

	Compound ID	Precursor Ion (Da)	[illegible]
1	Compound 1	829.5000	200.00000

Figure 4. *Guided MRM^{HR}* mode in the SCIEX OS Software: MRM^{HR} Transition Optimization

If the accurate mass of the fragment ions are not known or compound optimization is not performed then a start/stop mass for each looped TOF-MS/MS experiment of different precursor masses can be set up in the traditional MRM^{HR} procedure (Figure 5). This allows for post-acquisition data processing extraction of one or multiple unique accurate mass fragment ions.



Experiment: MRM HR
Polarity: Positive
Spray voltage: 4500 V

TOF MS

TOF start mass: 100 Da
TOF stop mass: 500 Da
Accumulation time: 0.1 sec
Declustering potential: 80 V
DP spread: 0 V
Collision energy: 10 V
CE spread: 0 V

TOF MSMS

Mass Table: ○ Apply fragment ion mass ● Apply TOF start/stop mass ✓ Apply Scan Schedule

	Compound ID	Precursor ion (Da)	TOF start mass (Da)	TOF stop mass (Da)	Accumulati...	Declust...	Collision energy (V)	CE spread (V)	Retention time (min)	Retention time t...
1	Flephedrone 1	182.10	40.02000	1000.00000	0.0250	80	35	15	1.28	15
2	Flephedrone 2	182.10	40.02000	1000.00000	0.0250	80	35	15	1.28	15
3	Butylone 1	222.11	40.02000	1000.00000	0.0250	30	35	15	1.80	15
4	Butylone 2	222.11	40.02000	1000.00000	0.0250	50	35	15	1.80	15
5	MDPV 1	276.16	40.02000	1000.00000	0.0250	10	35	15	2.56	15
6	MDPV 2	276.16	40.02000	1000.00000	0.0250	10	35	15	2.56	15
7	Naphyrone 1	282.19	40.02000	1000.00000	0.0250	30	35	15	3.32	15
8	Naphyrone 2	282.19	40.02000	1000.00000	0.0250	30	35	15	3.32	15
9	6-apb 1	176.11	40.02000	1000.00000	0.0250	120	35	15	1.58	15
10	6-apb 2	176.11	40.02000	1000.00000	0.0250	120	35	15	1.58	15
11	Bromo-DragonFLY 1	294.01	40.02000	1000.00000	0.0250	35	35	15	3.32	15
12	Bromo-DragonFLY 2	294.01	40.02000	1000.00000	0.0250	35	35	15	3.32	15

Figure 5 SCIEX OS Software *Scheduled* MRM^{HR} Method Editor, Applying TOF Start/Stop Mass

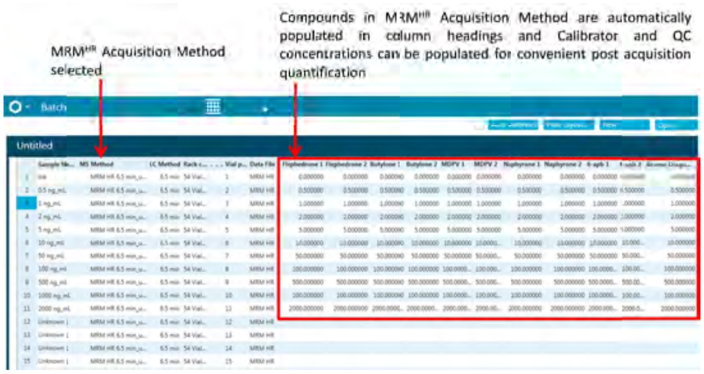
Scheduled MRM^{HR} Algorithm

In order to reduce the number of concurrent MRM^{HR} experiments at any given time and allow for more looped experiments (i.e. monitoring of more compounds) in a scan cycle, but also maintain the same cycle time and accumulation times, MRM^{HR} can be scheduled. *Scheduled* MRM^{HR} algorithm adjusts detection windows automatically depending on the retention time of the compound. Each MRM^{HR} experiment is monitored only across its expected elution time. Figures 3 and 5 show that each TOF-MS/MS experiment (MRM^{HR} transition), for each precursor ion, can have an associated retention time allotted. Each precursor ion's TOF-MS/MS experiment will only be monitored at that retention time with, in these examples shown, a retention time tolerance of ±15 seconds.

MRM^{HR} Batch and Post- Acquisition Processing Method Building

For convenience during batch building, once an MRM^{HR} acquisition method is selected in the MS Method column, the compounds in the MRM^{HR} acquisition method are automatically listed in the headings of automatically created compound columns. This allows the concentrations for the analytes in the calibrators and QCs to then be populated for post-acquisition calibration line generation (Figure 6).

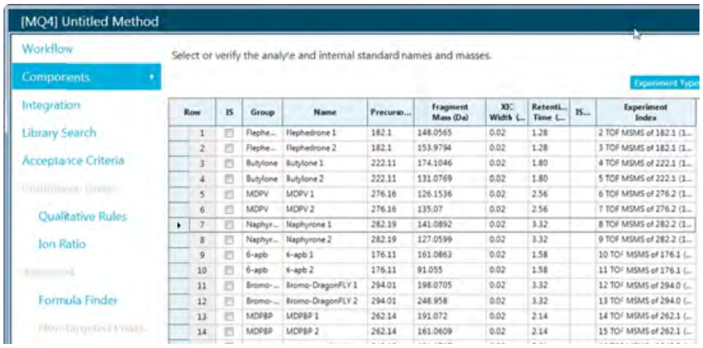
During creating a post-acquisition quantification method, once a reference sample is selected, the components table is automatically populated, from the acquisition method, with the MRM^{HR} transitions for each compound (Figure 7), allowing for efficient integration of the extracted fragment ions.



MRM^{HR} Acquisition Method selected

Compounds in MRM^{HR} Acquisition Method are automatically populated in column headings and Calibrator and QC concentrations can be populated for convenient post acquisition quantification

Figure 6. Batch Editor; Convenient MRM^{HR} Compound Quantification Setup



[MQ4] Untitled Method

Select or verify the analyte and internal standard names and masses.

Row	IS	Group	Name	Precurso...	Fragment Mass (Da)	XIC Width (...	Retenti... Time (...	IS...	Experiment Index
1	☐	Flephe...	Flephedrone 1	182.1	148.0565	0.02	1.28		2 TOF MSMS of 182.1 (1...
2	☐	Flephe...	Flephedrone 2	182.1	153.9794	0.02	1.28		3 TOF MSMS of 182.1 (1...
3	☐	Butylone	Butylone 1	222.11	174.1046	0.02	1.80		4 TOF MSMS of 222.1 (1...
4	☐	Butylone	Butylone 2	222.11	131.0769	0.02	1.80		5 TOF MSMS of 222.1 (1...
5	☐	MDPV	MDPV 1	276.16	126.1536	0.02	2.56		6 TOF MSMS of 276.2 (1...
6	☐	MDPV	MDPV 2	276.16	135.07	0.02	2.56		7 TOF MSMS of 276.2 (1...
7	☐	Naphyr...	Naphyrone 1	282.19	141.0892	0.02	3.32		8 TOF MSMS of 282.2 (1...
8	☐	Naphyr...	Naphyrone 2	282.19	127.0599	0.02	3.32		9 TOF MSMS of 282.2 (1...
9	☐	6-apb	6-apb 1	176.11	161.0863	0.02	1.58		10 TOF MSMS of 176.1 (...
10	☐	6-apb	6-apb 2	176.11	91.055	0.02	1.58		11 TOF MSMS of 176.1 (...
11	☐	Bromo-...	Bromo-DragonFLY 1	294.01	198.0705	0.02	3.32		12 TOF MSMS of 294.0 (...
12	☐	Bromo-...	Bromo-DragonFLY 2	294.01	248.958	0.02	3.32		13 TOF MSMS of 294.0 (...
13	☐	MDPBP	MDPBP 1	262.14	191.072	0.02	2.14		14 TOF MSMS of 262.1 (...
14	☐	MDPBP	MDPBP 2	262.14	161.0609	0.02	2.14		15 TOF MSMS of 262.1 (...

Figure 7. Automatic Populating of MRM^{HR} transitions into the Components Table of the Quantification Processing Method

Experimental

Sample Preparation

<u>*Internal Standards*</u>. 6-APB-d5, Buphedrone Ephedrine Metabolite –d3 and 4-Fluoro-methamphetamine-d5 were used as internal standards (Cerilliant, Round Rock, Texas). They were mixed and diluted in methanol at concentration of 1000 ng/mL as IS spiking solution.

Dilute and Shoot. Calibration curve with a mix of known compound standards (Cerilliant, Round Rock, Texas) were prepared in human drug free urine. Then 10 µL IS spiking solution was added to 100µL of urine samples which include both the calibration standards and unknown forensic samples. The mix was then diluted 5-fold with 90:10 (v:v), 0.1% formic acid in water : 0.1% formic acid in methanol followed by ultra-centrifugation. Injection volume was 10 µL.

Liquid Chromatography

HPLC separation was performed at 30 °C on a reversed-phase HPLC column (50 × 2.1 mm). Mobile phases used were water and methanol with appropriate additives. The LC flow rate was 0.5 mL/min and the LC runtime was 6.5 minutes.

Table 1: List of Drug targets For Urinary Analysis

Drugs analyzed in positive mode

2,5-dimethoxy-4-n-propylthiophenethylamine	*Flephedrone*
2C-B-FLY	*Lacosamide*
3,4-dimethylmethcathinone	*Lurasidone*
3-desmethylprodine	*MDPBP*
4-ethylmethcathinone	*MDPV*
4-fluoroamphetamine	*Methedrone*
4-fluoromethamphetamine	*Methiopropamine*
4-fluorotropacocaine	*Methoxetamine*
4-methylephedrine	*Methylhexanamine*
4-methylethcathinone	*Naloxone-N-Oxide*
4-methyl-N-ethyl-norephedrine	*Naphyrone*
4-methylnitrosamino-1-3-pyridyl-1-butanol	*n-desmethylcyclobenzaprine*
5-apb	*n-desmethylmirtazapine*
6-apb	*n-ethylcathinone*
5-Iodo-2-aminoindane	*n-ethylcathinone ephedrine metabolite*
7-hydroxymitragynine	*N-nitrosoanabasine*
Alpha-PVP	*N-nitrosonornicotine*
Alpha-Pyrrolidinopropiophenone	*NNK*
Asenapine	*Norcotinine*
Bromo-Dragonfly	*Pentedrone*
Buphedrone ephedrine metabolite	*Ractopamine*
Butylone	*Retigabine*
Carbamazepine-10,11-epoxide	*Salvinorin B*
Cocaine-N-oxide	*Tiagabine*
Codeine-6beta glucuronide	*Trans-3'-Hydroxycotinine*
Desomorphine	*Vigabatrin*
Desoxypipradrol	*Vilazodone*
Etizolam	*Zopiclone-N-oxide*

MS and MS/MS Conditions

MS and MS/MS data were collected using MRM^{HR} acquisition on the new benchtop SCIEX X500R QTOF System with SCIEX OS software, each acquisition scan beginning with a TOF-MS experiment. *Guided MRM^{HR}* mode in the SCIEX OS software was used to aid in determining the optimal MRM^{HR} transitions for each target compound and to optimize the compound dependent parameters for each MRM^{HR} transition. The *Guided MRM^{HR}* was set up to automatically create MRM^{HR} acquisition methods containing two looped TOF-MS/MS experiments (2 transitions) per compound corresponding to the two most selective and intense fragment ions for each compound.

List of Target Compounds

Data was acquired in a targeted fashion and processed in a targeted way. A calibration mix was prepared that contained over 50 forensically related drugs; thus a targeted list consisting of 56 drugs (Table 1) was automatically constructed in the processing method for post-acquisition data processing from the MRM^{HR} acquisition method for 56 compounds

Results and Discussion

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 8 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 9 where a visible improvement in S/N is gained for the analysis of buprenorphine by the MRM^{HR} approach.

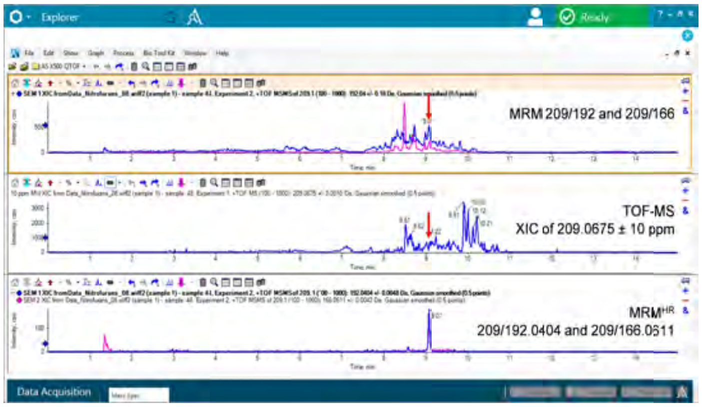
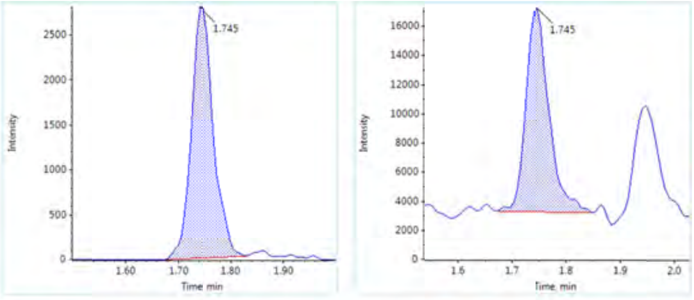


Figure 8. Increased Selectivity with MRM^{HR}; Avoiding False Negatives. Example given is a Feed Sample Tested Positive for NP Semicarbazide



MRM^{HR}: 178.1→131.070±0.01 *m/z* **TOF-MS**: 178.1226±0.005 *m/z*

Figure 9. *Scheduled* MRM^{HR} Selectivity Compared to TOF-MS; Buprenorphine (5ng/mL in urine, 10 fold dilution, 10 µL injection)

As this data was acquired through MRM^{HR} acquisition, post acqusition data processing can include the extraction of the precursor ion and multiple accurate mass fragment ions and therefore ion ratio determinations. The extracted ion chromatogram for both the qualifier and quantifier ions can be overlayed with the ion ratio lines displayed. Figure 10 shows an MRM^{HR} calibration series, using Naphyrone as an example, and displaying the quantification results as well as the ion ratio confirmation with the overlayed qualifier and quantifier extracted ion chromatograms for each of the calibrators. Further examples using Butylone, Bromo-DragonFLY, MDPBP, 2,5-Dimethoxy-4-n-Propylthiophenethylamine, 4-Fluoroamphetamine, 2C-B-FLY, 4-Fluoromethamphetamine and 5-IODO-2-aminoindane are provided in Figures 11, 12, 13, 14,15, 16, 17 and 18 respectively.

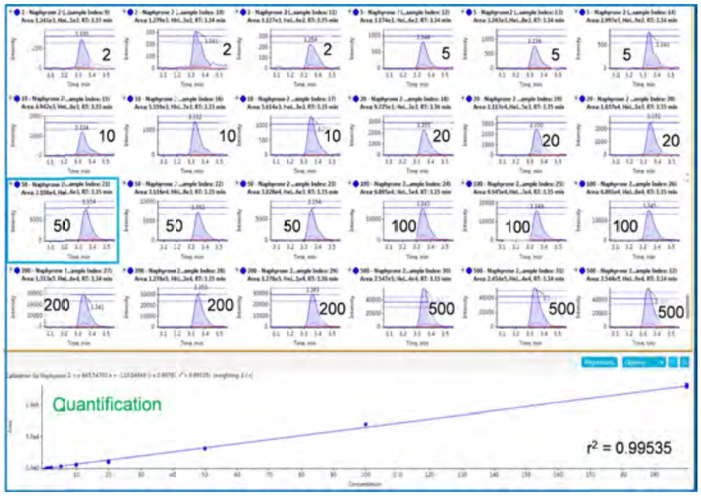


Figure 10. MRM^{HR} Ion Ratios and Quantification; Naphyrone (ng/mL)

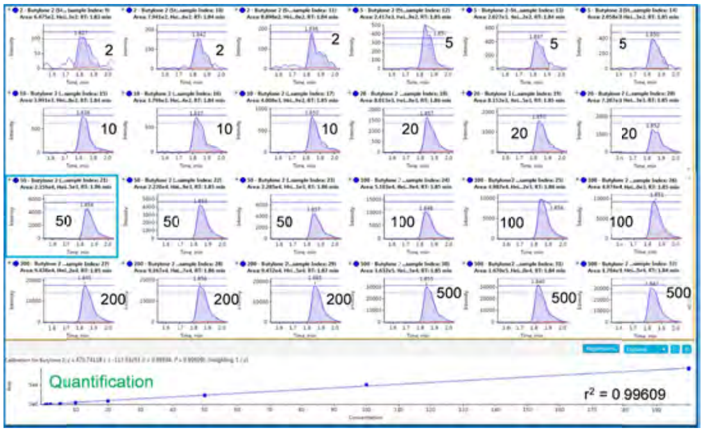


Figure 11. MRM^{HR} Ion Ratios and Quantification; Butylone (ng/mL)

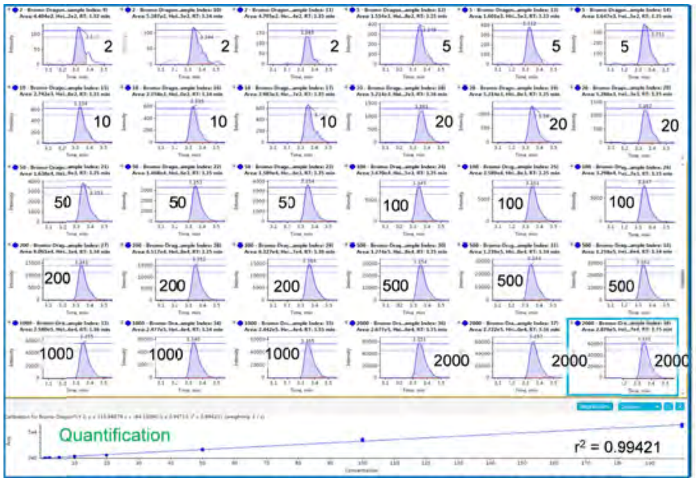


Figure 12. MRM^{HR} Ion Ratios and Quantification; Bromo-Dragon FLY (ng/mL)

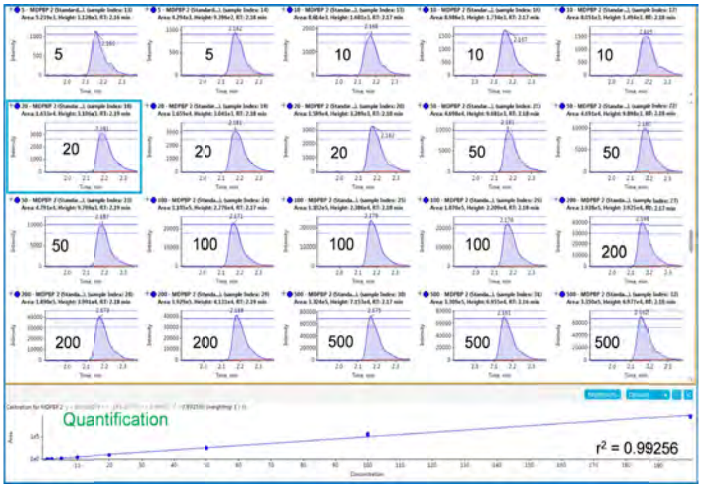


Figure 13. MRM^{HR} Ion Ratios and Quantification; MDPBP (ng/mL)

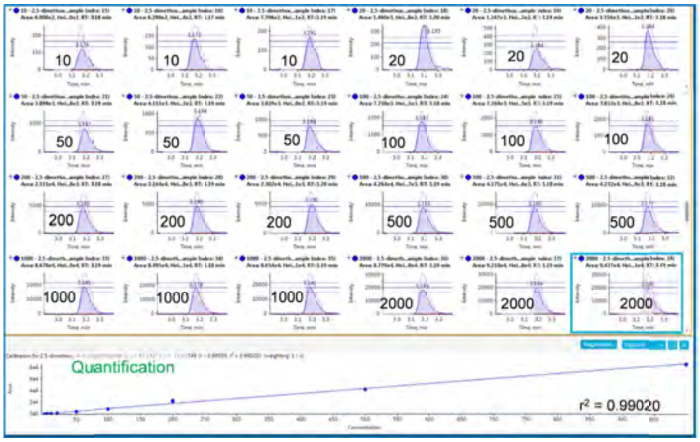


Figure 14. MRM^{HR} Ion Ratios and Quantification; 2,5-Dimethoxy-4-n-Propylthiophenethylamine (ng/mL)

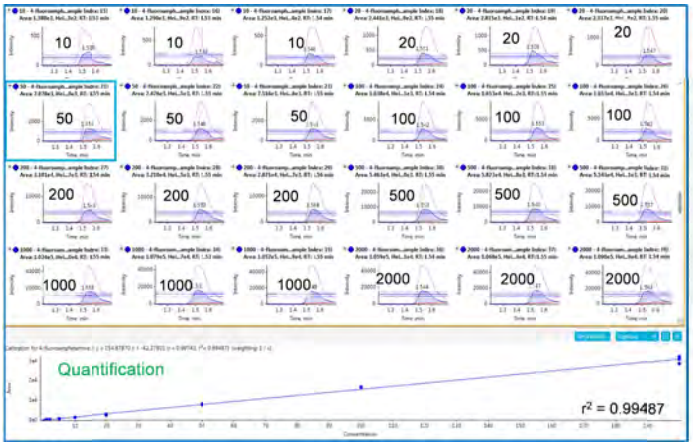


Figure 15. MRM^{HR} Ion Ratios and Quantification; 4-Fluoroamphetamine (ng/mL)

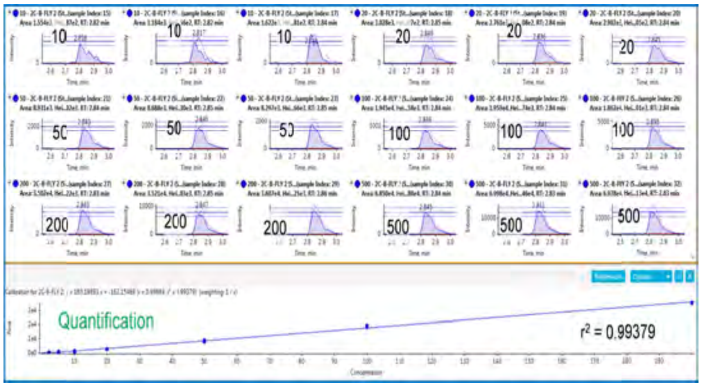


Figure 16. MRM^{HR} Ion Ratios and Quantification; 2C-B-FLY (ng/mL)

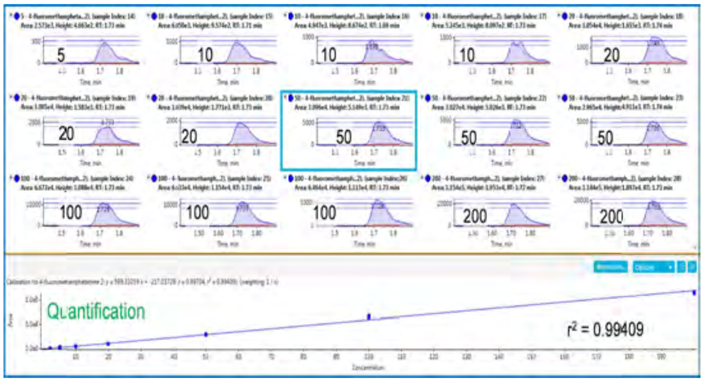


Figure 17. MRM^{HR} Ion Ratios and Quantification; 4-Fluoromethamphetamine (ng/mL)

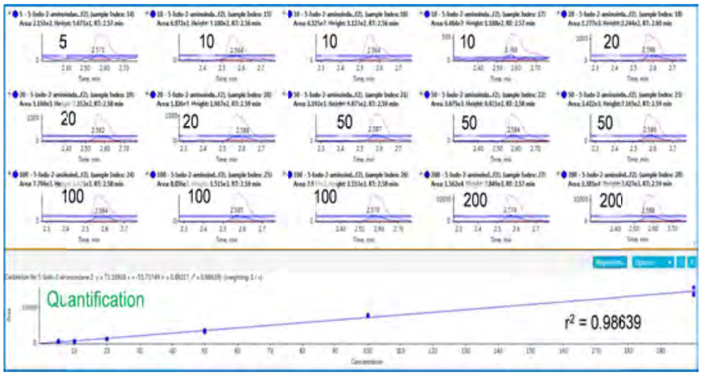
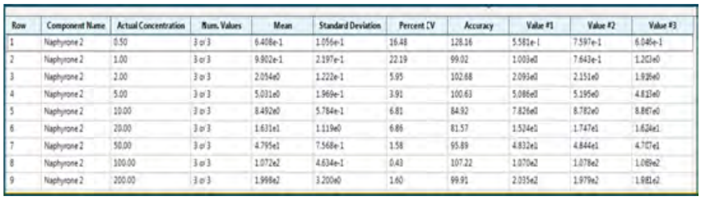


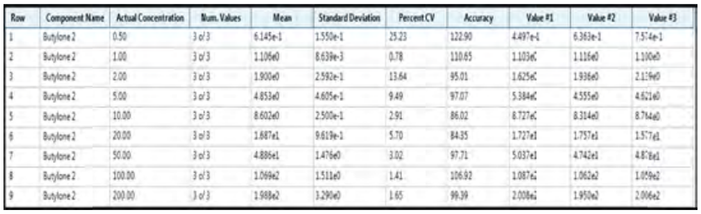
Figure 18. MRM^{HR} Ion Ratios and Quantification; 5-IODO-2-aminoindane (ng/mL)

Figures 19 to 24 show representative examples of quantification performance of the MRM^{HR} approach.



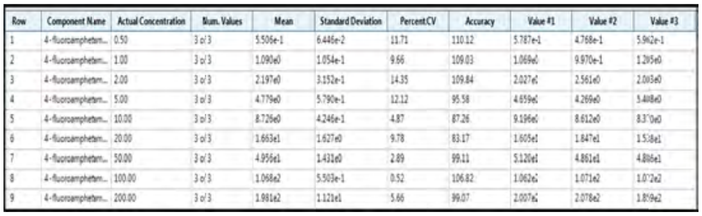
Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Naphyrone 2	0.50	3 of 3	6.408e-1	1.056e-1	16.48	128.16	5.581e-1	7.597e-1	6.046e-1
2	Naphyrone 2	1.00	3 of 3	9.902e-1	2.197e-1	22.19	99.02	1.003e0	7.643e-1	1.203e0
3	Naphyrone 2	2.00	3 of 3	2.054e0	1.222e-1	5.95	102.68	2.093e0	2.151e0	1.918e0
4	Naphyrone 2	5.00	3 of 3	5.031e0	1.969e-1	3.91	100.63	5.086e0	5.195e0	4.813e0
5	Naphyrone 2	10.00	3 of 3	8.492e0	5.784e-1	6.81	84.92	7.826e0	8.782e0	8.867e0
6	Naphyrone 2	20.00	3 of 3	1.631e1	1.119e0	6.86	81.57	1.524e1	1.747e1	1.624e1
7	Naphyrone 2	50.00	3 of 3	4.795e1	7.568e-1	1.58	95.89	4.832e1	4.844e1	4.707e1
8	Naphyrone 2	100.00	3 of 3	1.072e2	4.634e-1	0.43	107.22	1.070e2	1.078e2	1.069e2
9	Naphyrone 2	200.00	3 of 3	1.998e2	3.200e0	1.60	99.91	2.035e2	1.979e2	1.981e2

Figure 19. Example of MRM^{HR} Quantification Performance – Statistics (n=3); Naphyrone



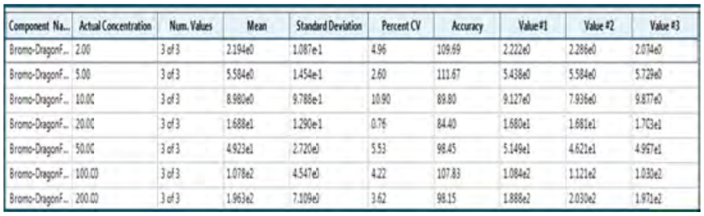
Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Butylone 2	0.50	3 of 3	6.145e-1	1.550e-1	25.23	122.90	4.497e-1	6.363e-1	7.574e-1
2	Butylone 2	1.00	3 of 3	1.106e0	8.639e-3	0.78	110.65	1.103e0	1.116e0	1.100e0
3	Butylone 2	2.00	3 of 3	1.900e0	2.592e-1	13.64	95.01	1.625e0	1.936e0	2.139e0
4	Butylone 2	5.00	3 of 3	4.853e0	4.605e-1	9.49	97.07	5.384e0	4.555e0	4.621e0
5	Butylone 2	10.00	3 of 3	8.602e0	2.500e-1	2.91	86.02	8.727e0	8.314e0	8.764e0
6	Butylone 2	20.00	3 of 3	1.687e1	9.619e-1	5.70	84.35	1.727e1	1.757e1	1.577e1
7	Butylone 2	50.00	3 of 3	4.886e1	1.476e0	3.02	97.71	5.037e1	4.742e1	4.878e1
8	Butylone 2	100.00	3 of 3	1.069e2	1.511e0	1.41	106.92	1.087e2	1.062e2	1.059e2
9	Butylone 2	200.00	3 of 3	1.988e2	3.290e0	1.65	99.39	2.008e2	1.950e2	2.006e2

Figur 20. Example of MRM^{HR} Quantification Performance – Statistics (n=3); Butylone



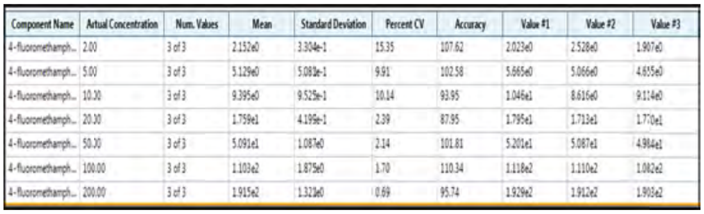
Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	4-fluoroamphetam...	0.50	3 of 3	5.506e-1	6.446e-2	11.71	110.12	5.787e-1	4.768e-1	5.962e-1
2	4-fluoroamphetam...	1.00	3 of 3	1.090e0	1.054e-1	9.66	109.03	1.069e0	9.970e-1	1.205e0
3	4-fluoroamphetam...	2.00	3 of 3	2.197e0	3.152e-1	14.35	109.84	2.027e0	2.561e0	2.003e0
4	4-fluoroamphetam...	5.00	3 of 3	4.779e0	5.790e-1	12.12	95.58	4.659e0	4.269e0	5.408e0
5	4-fluoroamphetam...	10.00	3 of 3	8.726e0	4.246e-1	4.87	87.26	9.196e0	8.612e0	8.370e0
6	4-fluoroamphetam...	20.00	3 of 3	1.663e1	1.627e0	9.78	83.17	1.605e1	1.847e1	1.538e1
7	4-fluoroamphetam...	50.00	3 of 3	4.956e1	1.431e0	2.89	99.11	5.120e1	4.861e1	4.886e1
8	4-fluoroamphetam...	100.00	3 of 3	1.068e2	5.503e-1	0.52	106.82	1.062e2	1.071e2	1.072e2
9	4-fluoroamphetam...	200.00	3 of 3	1.981e2	1.121e1	5.66	99.07	2.007e2	2.078e2	1.859e2

Figure 21. Example of MRM^{HR} Quantification Performance – Statistics (n=3); 4-Fluroamphetamine



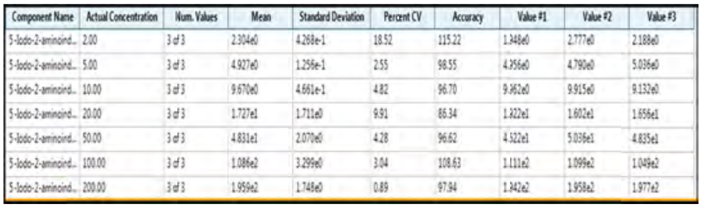
Component Na...	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
Bromo-DragonF...	2.00	3 of 3	2.194e0	1.087e-1	4.96	109.69	2.222e0	2.286e0	2.074e0
Bromo-DragonF...	5.00	3 of 3	5.584e0	1.454e-1	2.60	111.67	5.438e0	5.584e0	5.729e0
Bromo-DragonF...	10.00	3 of 3	8.980e0	9.788e-1	10.90	89.80	9.127e0	7.936e0	9.877e0
Bromo-DragonF...	20.00	3 of 3	1.688e1	1.290e-1	0.76	84.40	1.680e1	1.681e1	1.703e1
Bromo-DragonF...	50.00	3 of 3	4.923e1	2.720e0	5.53	98.45	5.149e1	4.621e1	4.997e1
Bromo-DragonF...	100.00	3 of 3	1.078e2	4.547e0	4.22	107.83	1.084e2	1.121e2	1.030e2
Bromo-DragonF...	200.00	3 of 3	1.963e2	7.109e0	3.62	98.15	1.888e2	2.030e2	1.971e2

Figure 22. Example of MRM^{HR} Quantification Performance – Statistics (n=3); Bromo-DragonFly



Component Name	Artual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
4-fluoromethamph...	2.00	3 of 3	2.152e0	3.304e-1	15.35	107.62	2.023e0	2.528e0	1.907e0
4-fluoromethamph...	5.00	3 of 3	5.129e0	5.081e-1	9.91	102.58	5.665e0	5.066e0	4.655e0
4-fluoromethamph...	10.00	3 of 3	9.395e0	9.525e-1	10.14	93.95	1.046e1	8.616e0	9.114e0
4-fluoromethamph...	20.00	3 of 3	1.759e1	4.199e-1	2.39	87.95	1.795e1	1.713e1	1.770e1
4-fluoromethamph...	50.00	3 of 3	5.091e1	1.087e0	2.14	101.81	5.201e1	5.087e1	4.984e1
4-fluoromethamph...	100.00	3 of 3	1.103e2	1.875e0	1.70	110.34	1.118e2	1.110e2	1.082e2
4-fluoromethamph...	200.00	3 of 3	1.915e2	1.321e0	0.69	95.74	1.929e2	1.912e2	1.903e2

Figure 23. Example of MRM^{HR} Quantification Performance – Statistics (n=3); 4-Fluromethamphetamine



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
5-Iodo-2-aminoind...	2.00	3 of 3	2.304e0	4.268e-1	18.52	115.22	1.348e0	2.777e0	2.188e0
5-Iodo-2-aminoind...	5.00	3 of 3	4.927e0	1.256e-1	2.55	98.55	4.356e0	4.790e0	5.036e0
5-Iodo-2-aminoind...	10.00	3 of 3	9.670e0	4.661e-1	4.82	96.70	9.362e0	9.915e0	9.132e0
5-Iodo-2-aminoind...	20.00	3 of 3	1.727e1	1.711e0	9.91	86.34	1.322e1	1.602e1	1.656e1
5-Iodo-2-aminoind...	50.00	3 of 3	4.831e1	2.070e0	4.28	96.62	4.522e1	5.036e1	4.835e1
5-Iodo-2-aminoind...	100.00	3 of 3	1.086e2	3.299e0	3.04	108.63	1.111e2	1.099e2	1.049e2
5-Iodo-2-aminoind...	200.00	3 of 3	1.959e2	1.748e0	0.89	97.94	1.342e2	1.958e2	1.977e2

Figure 24. Example of Quantification Performance – Statistics (n=3); 5-Iodo-2-aminoindane

Conclusion

The newly designed QTOF instrument coupled with the intuitive SCIEX OS software has made MRM^{HR} acquisition straightforward to setup and run. The MRM^{HR} approach provides TOF-MS and TOF-MS/MS analysis, both types of data being crucial in confidently identifying and quantifying forensic compounds. The MRM^{HR} experiment can be performed unscheduled and scheduled. *Scheduled* MRM^{HR} algorithm adjusts detection windows automatically depending on the retention time of the compound. Each MRM^{HR} experiment is monitored only across its expected elution time and therefore reducing the number of concurrent MRM^{HR} experiments at any given time and allow for more looped experiments (i.e. monitoring of more compounds) in a scan cycle.

MRM^{HR} is a targeted data acquisition enabling sensitive quantitation of low concentration species in complex matrices utilizing the selective high resolution, accurate mass MS/MS information as well as using ion ratio for identification purposes.

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]

[1]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA; [2]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8 Canada.

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).

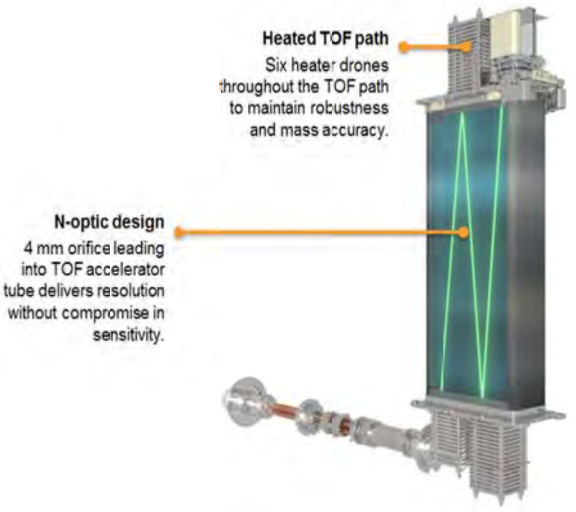


Figure 2. SCIEX X500R QTOF System and Technology Advances

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.

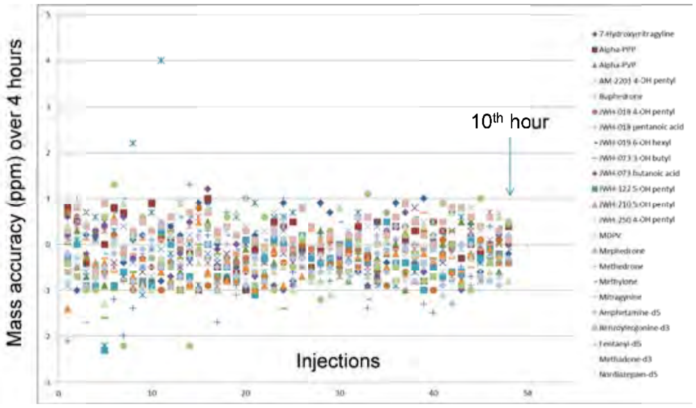


Figure 4. Mass Accuracy Stability of the SCIEX X500R QTOF System in the Analysis of Urine Samples

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.

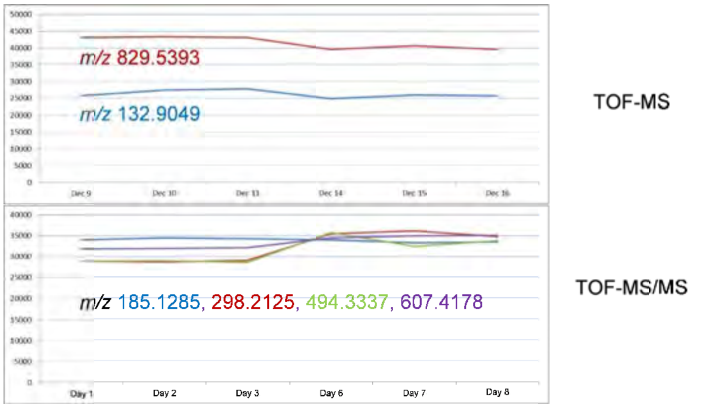


Figure 5. Resolution of the SCIEX X500R QTOF System Over a Week's Period for Selected *m/z*; both TOF-MS and TOF-MS/MS

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.

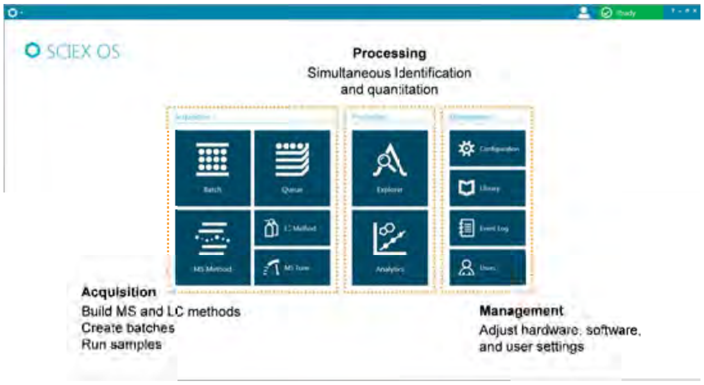


Figure 7. Home Page of SCIEX OS Software. Single Software Platform for LC/MS Control, Data Processing and Reporting.

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).

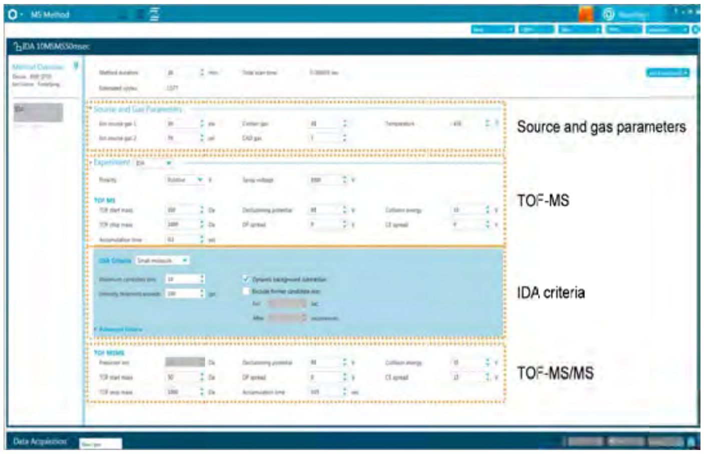


Figure 8. SCIEX OS Software MS Acquisition Method

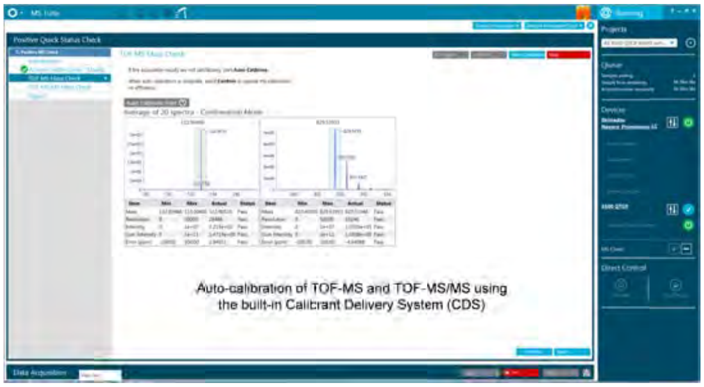


Figure 9. SCIEX OS Software MS Tune Allows for Quick Instrument Status Check via Simple Step by Step Instructions

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).

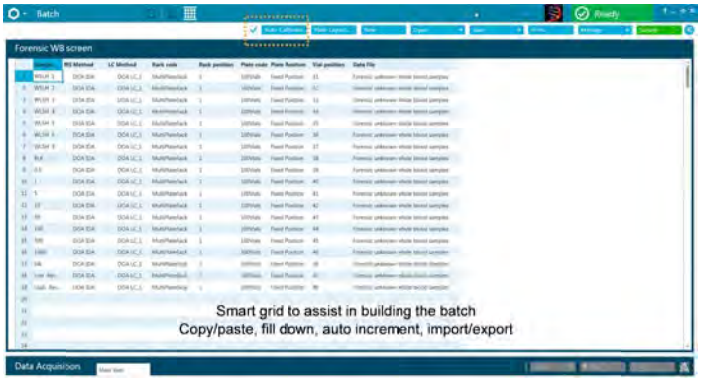


Figure 10. SCIEX OS Software Batch Editor and Setup for Auto-Calibration

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)

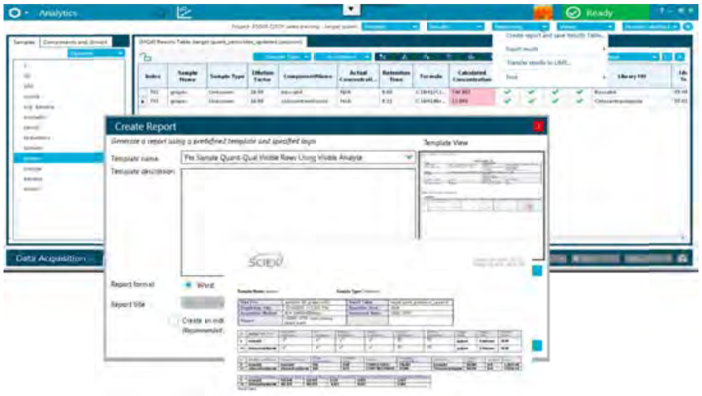


Figure 14. SCIEX OS Software Report Generation

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.

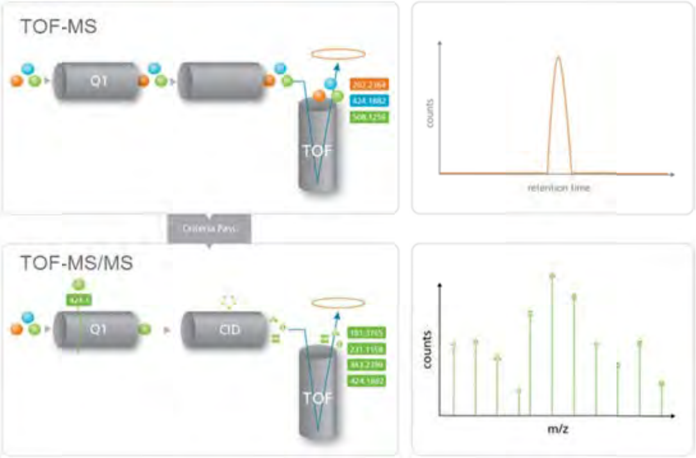
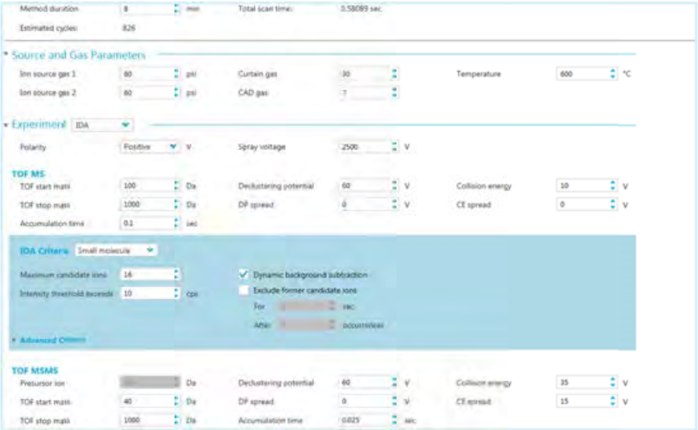


Figure 15. Information Dependent Acquisition

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.



Method duration: 8 min
Total scan time: 0.58089 sec
Estimated cycles: 826

Source and Gas Parameters
Ion source gas 1: 60 psi
Curtain gas: 30
Temperature: 600 °C
Ion source gas 2: 60 psi
CAD gas: 7

Experiment: IDA
Polarity: Positive
Spray voltage: 2500 V

TOF MS
TOF start mass: 100 Da
Declustering potential: 60 V
Collision energy: 10 V
TOF stop mass: 1000 Da
DP spread: 0 V
CE spread: 0 V
Accumulation time: 0.1 sec

IDA Criteria: Small molecule
Maximum candidate ions: 16
☑ Dynamic background subtraction
Intensity threshold exceeds: 10 cps
☐ Exclude former candidate ions
For: sec
After: occurrences
Advanced Criteria

TOF MSMS
Precursor ion: Da
Declustering potential: 60 V
Collision energy: 35 V
TOF start mass: 40 Da
DP spread: 0 V
CE spread: 15 V
TOF stop mass: 1000 Da
Accumulation time: 0.025 sec

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.

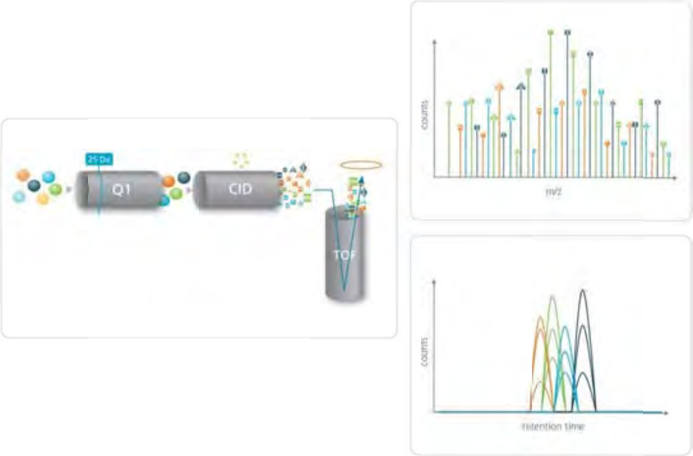
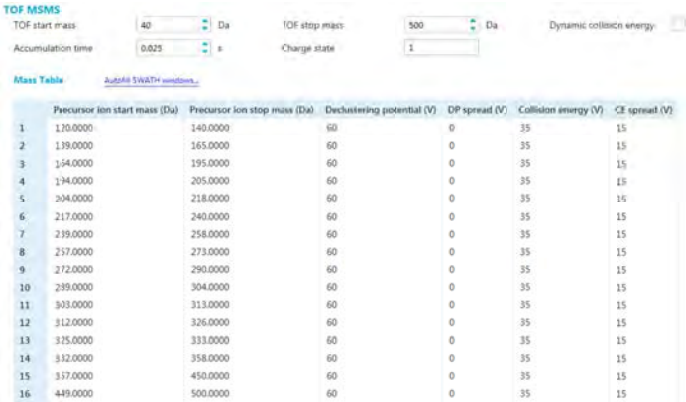


Figure 17. SWATH® Acquisition

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*).



TOF MSMS

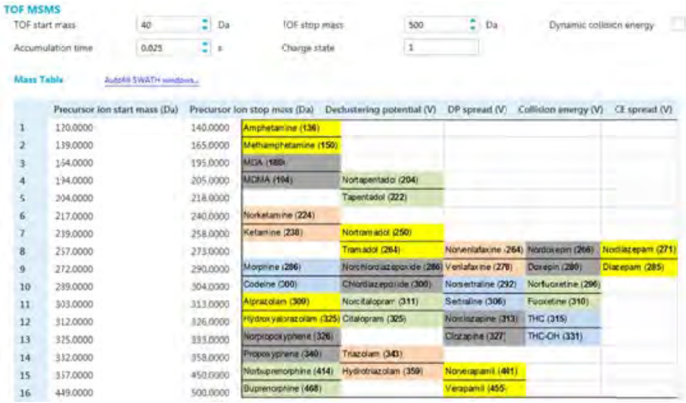
TOF start mass 40 Da
TOF stop mass 500 Da
Dynamic collision energy ☐
Accumulation time 0.025 s
Charge state 1

Mass Table AutoFill SWATH windows...

	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)
1	120.0000	140.0000	60	0	35	15
2	139.0000	165.0000	60	0	35	15
3	164.0000	195.0000	60	0	35	15
4	194.0000	205.0000	60	0	35	15
5	204.0000	218.0000	60	0	35	15
6	217.0000	240.0000	60	0	35	15
7	239.0000	258.0000	60	0	35	15
8	257.0000	273.0000	60	0	35	15
9	272.0000	290.0000	60	0	35	15
10	289.0000	304.0000	60	0	35	15
11	303.0000	313.0000	60	0	35	15
12	312.0000	326.0000	60	0	35	15
13	325.0000	333.0000	60	0	35	15
14	332.0000	358.0000	60	0	35	15
15	357.0000	450.0000	60	0	35	15
16	449.0000	500.0000	60	0	35	15

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).



TOF MSMS

TOF start mass 40 Da
TOF stop mass 500 Da
Dynamic collision energy ☐
Accumulation time 0.025 s
Charge state 1

Mass Table AutoFill SWATH windows...

	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)	
1	120.0000	140.0000	Amphetamine (136)				
2	139.0000	165.0000	Methamphetamine (150)				
3	164.0000	195.0000	MDA (180)				
4	194.0000	205.0000	MDMA (194)	Nortapentadol (204)			
5	204.0000	218.0000		Tapentadol (222)			
6	217.0000	240.0000	Norketamine (224)				
7	239.0000	258.0000	Ketamine (238)	Nortramadol (250)			
8	257.0000	273.0000		Tramadol (264)	Norvenlafaxine (264)	Nordoxepin (266)	Nordiazepam (271)
9	272.0000	290.0000	Morphine (286)	Norchlordiazepoxide (286)	Venlafaxine (278)	Doxepin (280)	Diazepam (285)
10	289.0000	304.0000	Codeine (300)	Chlordiazepoxide (300)	Norsertraline (292)	Norfluoxetine (296)	
11	303.0000	313.0000	Alprazolam (309)	Norcitalopram (311)	Sertraline (306)	Fluoxetine (310)	
12	312.0000	326.0000	Hydroxyalprazolam (325)	Citalopram (325)	Norclozapine (313)	THC (315)	
13	325.0000	333.0000	Norpropoxyphene (326)		Clozapine (327)	THC-OH (331)	
14	332.0000	358.0000	Propoxyphene (340)	Triazolam (343)			
15	357.0000	450.0000	Norbuprenorphine (414)	Hydroxytriazolam (359)	Norverapamil (441)		
16	449.0000	500.0000	Buprenorphine (468)		Verapamil (455)		

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.

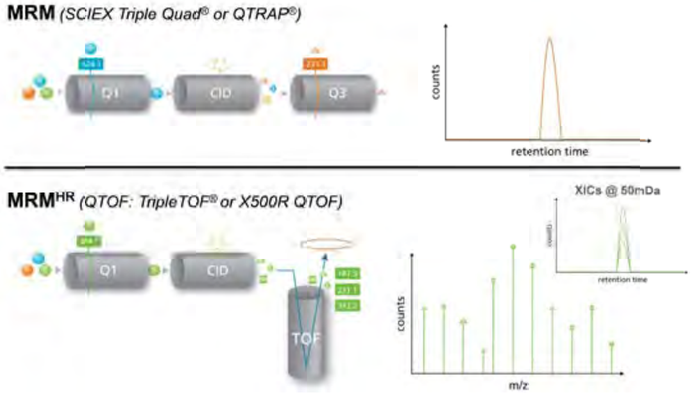
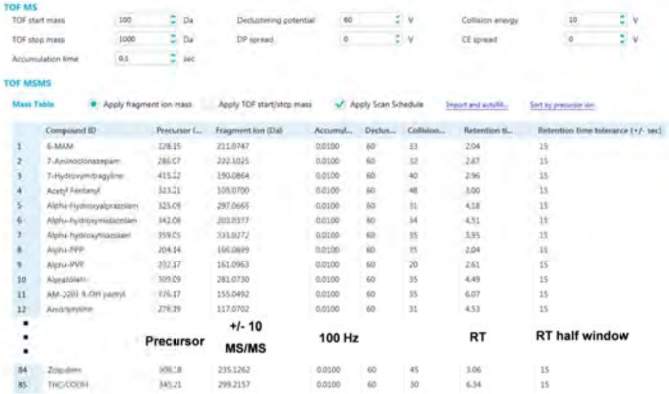


Figure 20. Comparison of MRM^HR with traditional (unit resolution) MRM

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.



TOF MS

TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.1 sec
Declustering potential 60 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

TOF MSMS

Mass Table ● Apply fragment ion mass ☐ Apply TOF start/stop mass ✓ Apply Scan Schedule Import and autofill... Sort by precursor ion

	Compound ID	Precursor I...	Fragment Ion (Da)	Accumul...	Declus...	Collision...	Retention ti...	Retention time tolerance (+/- sec)
1	6-MAM	328.15	211.0747	0.0100	60	33	2.04	15
2	7-Aminoclonazepam	286.07	222.1025	0.0100	60	32	2.67	15
3	7-Hydroxymitragyline	415.22	190.0864	0.0100	60	40	2.96	15
4	Acetyl Fentanyl	323.21	105.0700	0.0100	60	48	3.00	15
5	Alpha-Hydroxyalprazolam	325.08	297.0665	0.0100	60	31	4.18	15
6	Alpha-hydroxymidazolam	342.08	203.0377	0.0100	60	34	4.51	15
7	Alpha-hydroxytriazolam	359.05	331.0272	0.0100	60	35	3.95	15
8	Alpha-PPP	204.14	105.0699	0.0100	60	15	2.04	15
9	Alpha-PVP	232.17	161.0963	0.0100	60	20	2.61	15
10	Alprazolam	309.09	281.0730	0.0100	60	35	4.49	15
11	AM-2201 N-OH pentyl	376.17	155.0492	0.0100	60	35	6.07	15
12	Amitriptyline	278.19	117.0702	0.0100	60	31	4.53	15
⋮		Precursor	+/- 10 MS/MS	100 Hz			RT	RT half window
84	Zolpidem	308.18	235.1262	0.0100	60	45	3.06	15
85	THC-COOH	345.21	299.2157	0.0100	60	30	6.34	15

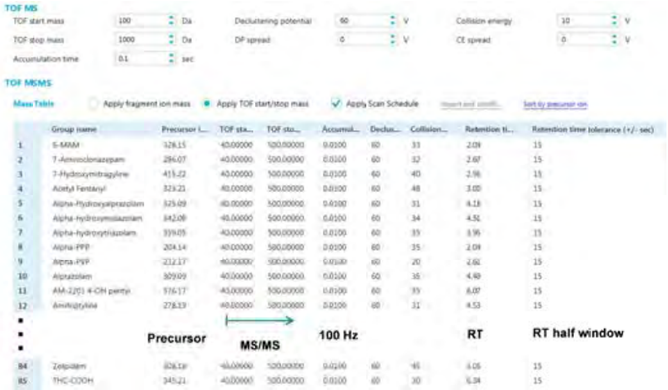
Figure 21. SCIEX OS Software *Scheduled* MRM^HR Method Editor, Fragment Ion Mass ± 10 *m/z*

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

Row	IS	Group	Name	Precursor Ma...	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	IS...	Experiment Index
1	☐	6-MAM	6-MAM	328.154	211.0747	0.02	2.04		2 TOF MSMS of 328.2 (40 - 500)
2	☐	7-Aminoclonazepam	7-Aminoclonazepam	286.074	222.1025	0.02	2.67		3 TOF MSMS of 286.1 (40 - 500)
3	☐	7-Hydroxymitragyline	7-Hydroxymitragyline	415.223	190.0864	0.02	2.96		4 TOF MSMS of 415.2 (40 - 500)
4	☐	Acetyl Fentanyl	Acetyl Fentanyl	323.212	105.07	0.02	3.00		5 TOF MSMS of 323.2 (40 - 500)
5	☐	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	325.085	297.0665	0.02	4.19		6 TOF MSMS of 325.1 (40 - 500)
6	☐	Alpha-hydroxymidazolam	Alpha-hydroxymidazolam	342.08	203.0177	0.02	4.51		7 TOF MSMS of 342.1 (40 - 500)
7	☐	Alpha-hydroxytriazolam	Alpha-hydroxytriazolam	359.046	331.0272	0.02	3.94		8 TOF MSMS of 359.0 (40 - 500)
8	☐	Alpha-PPP	Alpha-PPP	204.138	105.0699	0.02	2.04		9 TOF MSMS of 204.1 (40 - 500)
9	☐	Alpha-PVP	Alpha-PVP	232.17	161.0963	0.02	2.61		10 TOF MSMS of 232.2 (40 - 500)
10	☐	Alprazolam	Alprazolam	309.09	281.073	0.02	4.49		11 TOF MSMS of 309.1 (40 - 500)
11	☐	AM-2201 4-OH pentyl	AM-2201 4-OH pentyl	376.171	155.0492	0.02	6.07		12 TOF MSMS of 376.2 (40 - 500)
12	☐	Amitriptyline	Amitriptyline	278.19	117.0701	0.02	4.53		13 TOF MSMS of 278.2 (40 - 500)
13	☐	Amphetamine	Amphetamine	136.112	91.0553	0.02	1.63		14 TOF MSMS of 136.1 (40 - 500)
14	☐	Benzoylecgonine	Benzoylecgonine	290.139	168.1021	0.02	2.56		15 TOF MSMS of 290.1 (40 - 500)
15	☐	Buphedrone	Buphedrone	178.123	131.07	0.02	1.98		16 TOF MSMS of 178.1 (40 - 500)
16	☐	Buprenorphine	Buprenorphine	468.311	414.2636	0.02	3.67		17 TOF MSMS of 468.3 (40 - 500)
17	☐	Carisoprodol	Carisoprodol	261.181	55.0565	0.02	3.66		18 TOF MSMS of 261.2 (40 - 500)
18	☐	Clomipramine	Clomipramine	315.182	86.0959	0.02	5.36		19 TOF MSMS of 315.2 (40 - 500)
19	☐	Codeine	Codeine	300.159	215.1109	0.02	1.87		20 TOF MSMS of 300.2 (40 - 500)
20	☐	Cotinine	Cotinine	177.102	80.0496	0.02	1.83		21 TOF MSMS of 177.1 (40 - 500)
21	☐	Cyclobenzaprine	Cyclobenzaprine	276.175	215.0878	0.02	4.28		22 TOF MSMS of 276.2 (40 - 500)
22	☐	Desalkylflurazepam	Desalkylflurazepam	289.054	140.0264	0.02	4.42		23 TOF MSMS of 289.1 (40 - 500)
23	☐	Desipramine	Desipramine	267.180	72.0823	0.02	4.26		24 TOF MSMS of 267.2 (40 - 500)
24	☐	Desmethyldoxepin	Desmethyldoxepin	266.154	107.0491	0.02	3.60		25 TOF MSMS of 266.2 (40 - 500)
25	☐	Dextromethorphan	Dextromethorphan	272.201	215.1458	0.02	3.84		26 TOF MSMS of 272.2 (40 - 500)
26	☐	Diazepam	Diazepam	285.079	154.0424	0.02	5.43		27 TOF MSMS of 285.1 (40 - 500)

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).



TOF MS

TOF start mass: 100 Da
TOF stop mass: 1000 Da
Accumulation time: 0.1 sec
Declustering potential: 60 V
DP spread: 0 V
Collision energy: 10 V
CE spread: 0 V

TOF MSMS

Mass Table ○ Apply fragment ion mass ● Apply TOF start/stop mass ☑ Apply Scan Schedule

	Group name	Precursor I...	TOF sta...	TOF sto...	Accumul...	Declus...	Collision...	Retention ti...	Retention time tolerance (+/- sec)
1	6-MAM	328.15	40.00000	500.00000	0.0100	60	33	2.09	15
2	7-Aminoclonazepam	286.07	40.00000	500.00000	0.0100	60	32	2.67	15
3	7-Hydroxymitragyline	415.22	40.00000	500.00000	0.0100	60	40	2.96	15
4	Acetyl Fentanyl	323.21	40.00000	500.00000	0.0100	60	48	3.00	15
5	Alpha-Hydroxyalprazolam	325.09	40.00000	500.00000	0.0100	60	31	4.18	15
6	Alpha-hydroxymidazolam	342.08	40.00000	500.00000	0.0100	60	34	4.51	15
7	Alpha-hydroxytriazolam	359.05	40.00000	500.00000	0.0100	60	35	3.96	15
8	Alpha-PPP	204.14	40.00000	500.00000	0.0100	60	35	2.09	15
9	Alpha-PVP	232.17	40.00000	500.00000	0.0100	60	20	2.61	15
10	Alprazolam	309.09	40.00000	500.00000	0.0100	60	36	4.49	15
11	AM-2201 4-OH pentyl	376.17	40.00000	500.00000	0.0100	60	35	6.07	15
12	Amitriptyline	278.19	40.00000	500.00000	0.0100	60	31	4.53	15
▪									
▪									
▪									
84	Zolpidem	308.19	40.00000	500.00000	0.0100	60	45	3.05	15
85	THC-COOH	345.21	40.00000	500.00000	0.0100	60	30	6.34	15

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)

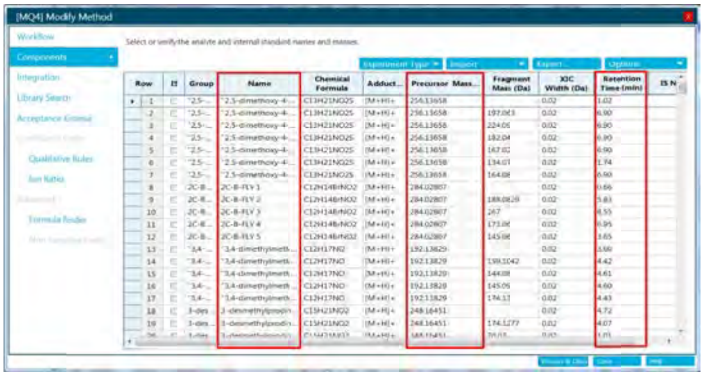
Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)
6-MAM	*1000*	*Gabapentin*	*10000*	*Naloxone*	*5000*	*Pentobarbital*	*10000*
7-Aminoclonazepam	*5000*	*Hydrocodone*	*5000*	*Naltrexone*	*5000*	*Secobarbital*	*10000*
7-Hydroxymitragynine	*1000*	*Hydromorphone*	*5000*	*N-desmethyltapentadol*	*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.

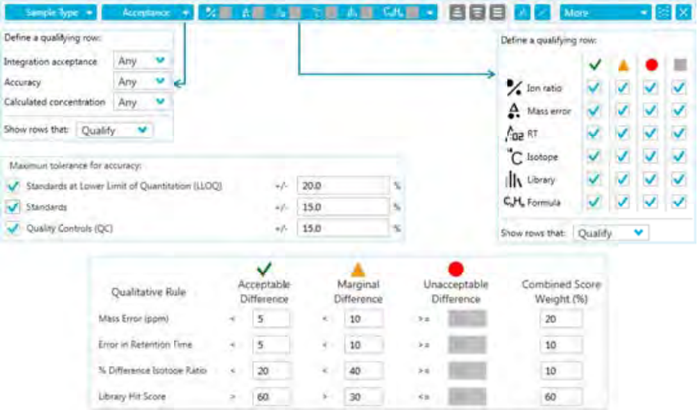


Select or verify the analyte and internal standard names and masses.

Row	IS	Group	Name	Chemical Formula	Adduct...	Precursor Mass...	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	IS N
1	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658		0.02	1.02	
2	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	197.063	0.02	6.90	
3	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	224.06	0.02	6.90	
4	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	182.04	0.02	6.90	
5	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	167.02	0.02	6.90	
6	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	134.07	0.02	1.74	
7	☐	2,5-...	2,5-dimethoxy-4-...	C13H21NO2S	[M+H]+	256.13658	164.08	0.02	6.90	
8	☐	2C-B...	2C-B-FLY 1	C12H14BrNO2	[M+H]+	284.02807		0.02	0.66	
9	☐	2C-B...	2C-B-FLY 2	C12H14BrNO2	[M+H]+	284.02807	188.0828	0.02	5.83	
10	☐	2C-B...	2C-B-FLY 3	C12H14BrNO2	[M+H]+	284.02807	267	0.02	4.55	
11	☐	2C-B...	2C-B-FLY 4	C12H14BrNO2	[M+H]+	284.02807	173.06	0.02	0.95	
12	☐	2C-B...	2C-B-FLY 5	C12H14BrNO2	[M+H]+	284.02807	145.06	0.02	3.65	
13	☐	3,4-...	3,4-dimethylmeth...	C12H17NO	[M+H]+	192.13829		0.02	3.66	
14	☐	3,4-...	3,4-dimethylmeth...	C12H17NO	[M+H]+	192.13829	159.1042	0.02	4.42	
15	☐	3,4-...	3,4-dimethylmeth...	C12H17NO	[M+H]+	192.13829	144.08	0.02	4.61	
16	☐	3,4-...	3,4-dimethylmeth...	C12H17NO	[M+H]+	192.13829	145.05	0.02	4.60	
17	☐	3,4-...	3,4-dimethylmeth...	C12H17NO	[M+H]+	192.13829	174.13	0.02	4.43	
18	☐	3-des...	3-desmethylprodin...	C15H21NO2	[M+H]+	248.16451		0.02	4.72	
19	☐	3-des...	3-desmethylprodin...	C15H21NO2	[M+H]+	248.16451	174.1277	0.02	4.07	

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).



Define a qualifying row:
Integration acceptance: Any
Accuracy: Any
Calculated concentration: Any
Show rows that: Qualify

Maximum tolerance for accuracy:
☑ Standards at Lower Limit of Quantitation (LLOQ) +/- 20.0 %
☑ Standards +/- 15.0 %
☑ Quality Controls (QC) +/- 15.0 %

Define a qualifying row:
Show rows that: Qualify

Qualitative Rule		✓ Acceptable Difference		▲ Marginal Difference		● Unacceptable Difference	Combined Score Weight (%)
Mass Error (ppm)	<	5	<	10	>=		20
Error in Retention Time	<	5	<	10	>=		10
% Difference Isotope Ratio	<	20	<	40	>=		10
Library Hit Score	>	60	>	30	<=		60

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.

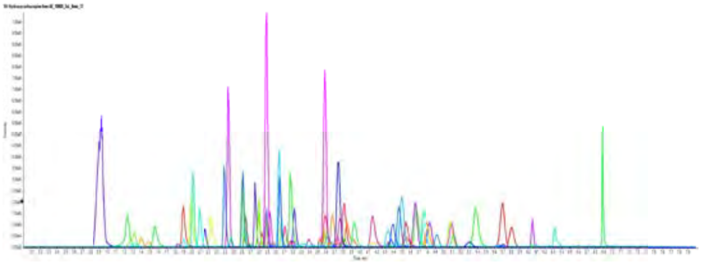


Figure 26. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 8.0 Minute LC Runtime

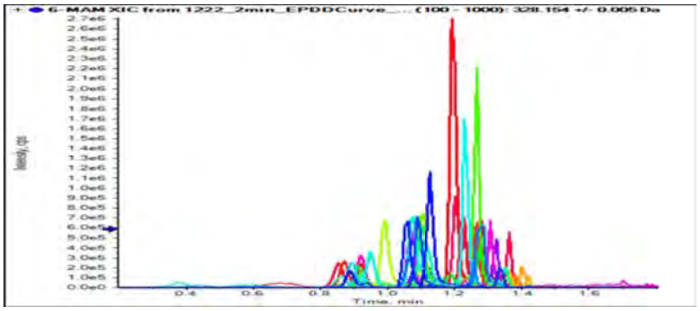
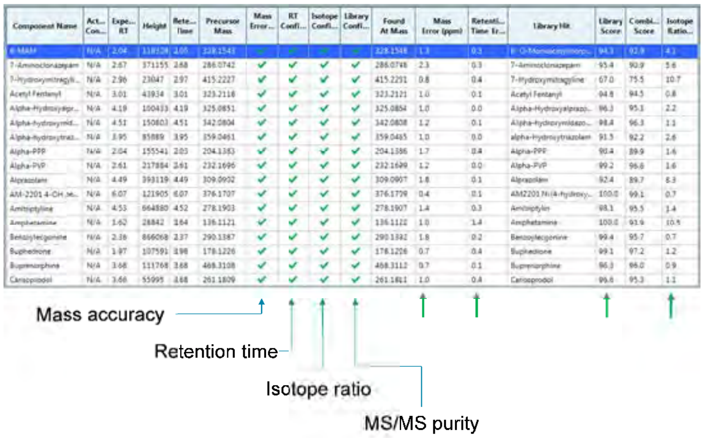


Figure 27. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 2.0 Minute LC Runtime

Information Dependent Acquisition

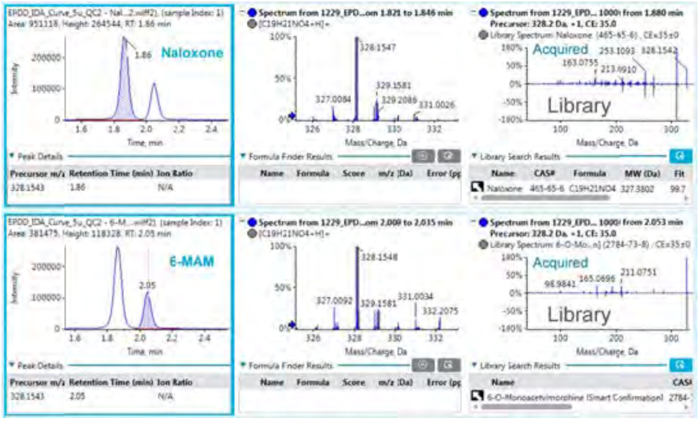
With the ability to provide the most interference free fragmentation information for library searching in a non-targeted acquisition, the IDA workflow provides the highest confidence screening using MS/MS information. Figure 28 shows the multiple screening criteria that are used for identification purposes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



Component Name	Act... Con...	Expe... RT	Height	Rete... Time	Precursor Mass	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error (ppm)	Retenti... Time Er...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
6-MAM	N/A	2.04	118328	2.05	328.1543	✓	✓	✓	✓	328.1548	1.3	0.3	6-O-Monoacetylmorph...	94.3	92.9	4.1
7-Aminoclonazepam	N/A	2.67	371155	2.68	286.0742	✓	✓	✓	✓	286.0748	2.3	0.3	7-Aminoclonazepam	95.4	90.9	5.6
7-Hydroxymitragyli...	N/A	2.96	23047	2.97	415.2227	✓	✓	✓	✓	415.2231	0.8	0.4	7-Hydroxymitragyline	67.0	75.5	10.7
Acetyl Fentanyl	N/A	3.01	43934	3.01	323.2118	✓	✓	✓	✓	323.2121	1.0	0.1	Acetyl Fentanyl	94.8	94.5	0.8
Alpha-Hydroxyalpr...	N/A	4.19	100433	4.19	325.0851	✓	✓	✓	✓	325.0854	1.0	0.0	Alpha-Hydroxyalprazo...	96.3	95.1	2.2
Alpha-hydroxymid...	N/A	4.51	150803	4.51	342.0804	✓	✓	✓	✓	342.0808	1.2	0.1	Alpha-hydroxymidazo...	98.4	96.3	1.1
Alpha-hydroxytriaz...	N/A	3.95	85889	3.95	359.0461	✓	✓	✓	✓	359.0465	1.0	0.0	alpha-Hydroxytriazolam	91.5	92.2	2.6
Alpha-PPP	N/A	2.04	155541	2.03	204.1383	✓	✓	✓	✓	204.1386	1.7	0.4	Alpha-PPP	90.4	89.9	1.6
Alpha-PVP	N/A	2.61	217884	2.61	232.1696	✓	✓	✓	✓	232.1699	1.2	0.0	Alpha-PVP	99.2	96.6	1.6
Alprazolam	N/A	4.49	393119	4.49	309.0902	✓	✓	✓	✓	309.0907	1.8	0.1	Alprazolam	92.4	89.7	6.3
AM-2201 4-OH pe...	N/A	6.07	121905	6.07	376.1707	✓	✓	✓	✓	376.1709	0.4	0.1	AM2201 N-(4-hydroxy...	100.0	99.1	0.7
Amitriptyline	N/A	4.53	664880	4.52	278.1903	✓	✓	✓	✓	278.1907	1.4	0.3	Amitriptylin	98.1	95.5	1.4
Amphetamine	N/A	1.62	28842	1.64	136.1121	✓	✓	✓	✓	136.1122	1.0	1.4	Amphetamine	100.0	93.9	10.5
Benzoylecgonine	N/A	2.38	866068	2.37	290.1387	✓	✓	✓	✓	290.1392	1.8	0.2	Benzoylecgonine	99.4	95.7	0.7
Buphedrone	N/A	1.97	107591	1.98	178.1226	✓	✓	✓	✓	178.1226	0.7	0.4	Buphedrone	99.1	97.2	1.2
Buprenorphine	N/A	3.68	111768	3.68	468.3108	✓	✓	✓	✓	468.3112	0.7	0.1	Buprenorphine	96.3	96.0	0.9
Carisoprodol	N/A	3.66	55995	3.68	261.1809	✓	✓	✓	✓	261.1811	1.0	0.4	Carisoprodol	99.6	95.3	1.1

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.



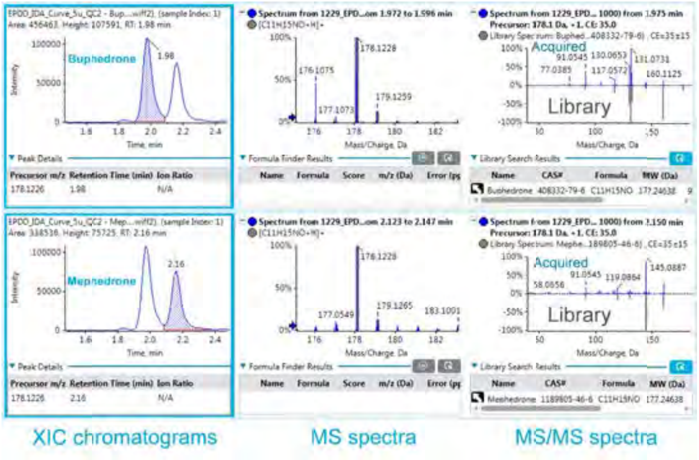
Precursor m/z: 328.1543, Retention Time (min): 1.86, Ion Ratio: N/A

Naloxone 465-65-6 C19H21NO4 327.3802 99.7

Precursor m/z: 328.1543, Retention Time (min): 2.05, Ion Ratio: N/A

6-O-Monoacetylmorphine (Smart Confirmation) 2784-

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



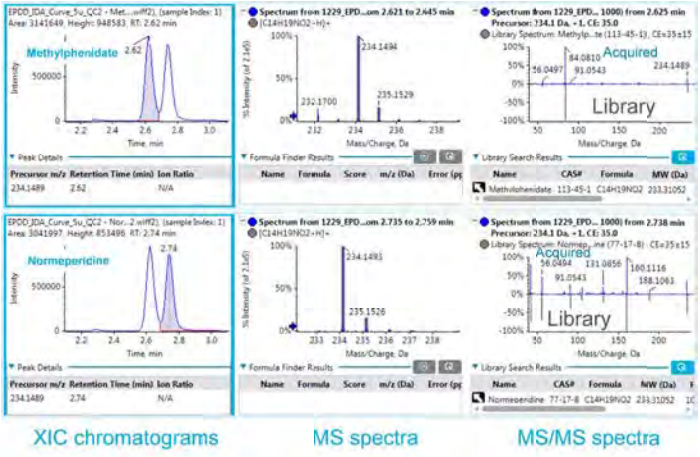
Precursor m/z: 178.1226, Retention Time (min): 1.98, Ion Ratio: N/A

Buphedrone 408332-79-6 C11H15NO 177.24638

Precursor m/z: 178.1226, Retention Time (min): 2.16, Ion Ratio: N/A

Mephedrone 1189805-46-6 C11H15NO 177.24638

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



Precursor m/z: 234.1489, Retention Time (min): 2.62, Ion Ratio: N/A

Methylphenidate 113-45-1 C14H19NO2 233.31052

Precursor m/z: 234.1489, Retention Time (min): 2.74, Ion Ratio: N/A

Normeperidine 77-17-8 C14H19NO2 233.31052

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.

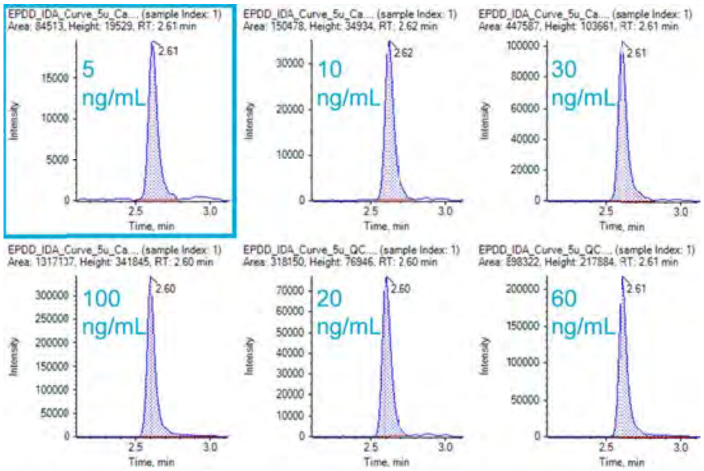


Figure 32. XICs of α-PVP in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

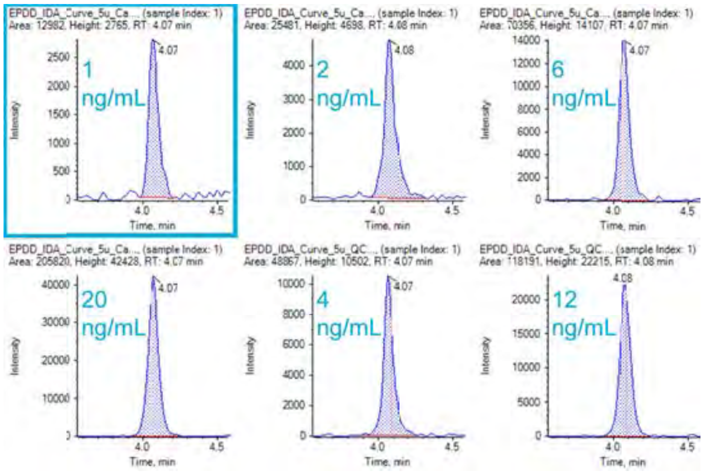


Figure 33. XICs of Sufentanil in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

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

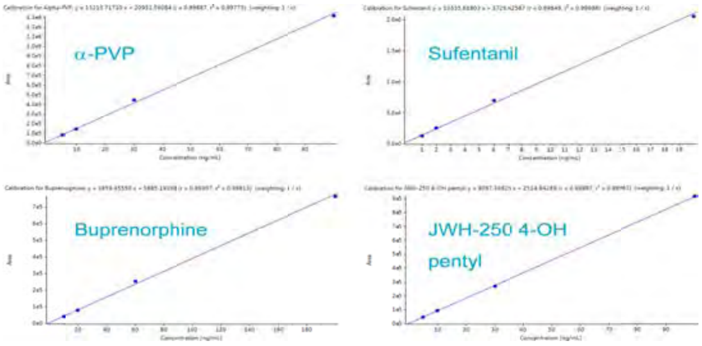
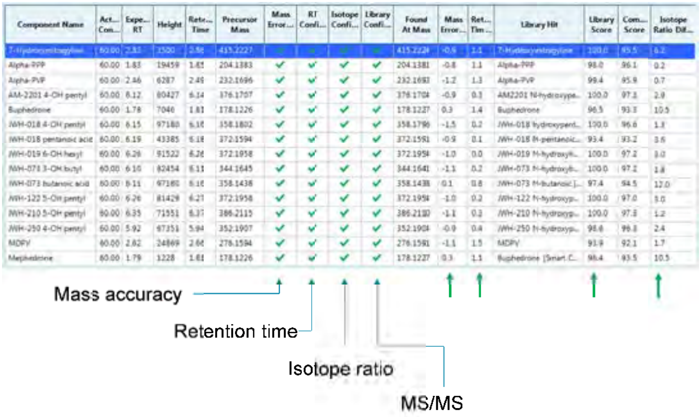


Figure 34. Representative Calibration Curves for Selected Compounds Showing that the TOF-MS information can be used for Quantification in an IDA Workflow

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%.



Component Name	Act... Con...	Expe... RT	Height	Rete... Time	Precursor Mass	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error...	Ret... Tim...	Library Hit	Library Score	Com... Score	Isotope Ratio Dif...
7-Hydroxymitragynine	60.00	2.33	1500	2.36	415.2227	✓	✓	✓	✓	415.2224	-0.6	1.1	7-Hydroxymitragynine	100.0	85.5	6.2
Alpha-PPP	60.00	1.83	19459	1.85	204.1383	✓	✓	✓	✓	204.1381	-0.8	1.1	Alpha-PPP	98.0	96.1	0.2
Alpha-PVP	60.00	2.46	6287	2.49	232.1696	✓	✓	✓	✓	232.1693	-1.2	1.3	Alpha-PVP	99.4	95.9	0.7
AM-2201 4-OH pentyl	60.00	6.12	80427	6.14	376.1707	✓	✓	✓	✓	376.1704	-0.9	0.3	AM2201 N-hydroxype...	100.0	97.3	2.8
Buphedrone	60.00	1.78	7046	1.81	178.1226	✓	✓	✓	✓	178.1227	0.3	1.4	Buphedrone	96.5	93.3	10.5
JWH-018 4-OH pentyl	60.00	6.15	97180	6.16	358.1802	✓	✓	✓	✓	358.1796	-1.5	0.2	JWH-018 hydroxypent...	100.0	96.6	1.3
JWH-018 pentanoic acid	60.00	6.19	43385	6.18	372.1594	✓	✓	✓	✓	372.1591	-0.9	0.1	JWH-018 N-pentanoic...	93.4	93.2	3.6
JWH-019 6-OH hexyl	60.00	6.26	91522	6.26	372.1958	✓	✓	✓	✓	372.1954	-1.0	0.0	JWH-019 N-hydroxyh...	100.0	97.2	3.0
JWH-073 3-OH butyl	60.00	6.10	82454	6.11	344.1645	✓	✓	✓	✓	344.1641	-1.1	0.2	JWH-073 N-hydroxyb...	100.0	97.2	1.8
JWH-073 butanoic acid	60.00	6.11	97160	6.16	358.1438	✓	✓	✓	✓	358.1438	0.1	0.8	JWH-073 N-butanoic]...	97.4	94.5	12.0
JWH-122 5-OH pentyl	60.00	6.26	81429	6.27	372.1958	✓	✓	✓	✓	372.1954	-1.0	0.2	JWH-122 N-hydroxyp...	100.0	97.0	3.0
JWH-210 5-OH pentyl	60.00	6.35	71551	6.37	386.2115	✓	✓	✓	✓	386.2110	-1.1	0.3	JWH-210 N-hydroxyp...	100.0	97.3	1.2
JWH-250 4-OH pentyl	60.00	5.92	67351	5.94	352.1907	✓	✓	✓	✓	352.1904	-0.9	0.4	JWH-250 N-hydroxyp...	98.6	96.3	2.4
MDPV	60.00	2.82	24869	2.66	276.1594	✓	✓	✓	✓	276.1591	-1.1	1.5	MDPV	93.9	92.1	1.7
Mephedrone	60.00	1.79	1228	1.81	178.1226	✓	✓	✓	✓	178.1227	0.3	1.1	Buphedrone [Smart C...	96.4	93.5	10.5

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.

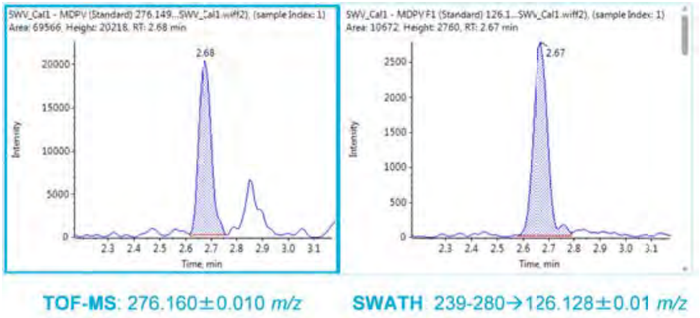


Figure 36. Gains in Selectivity with the Ability to Extract Out a Specific Fragment Ion From Variable Window SWATH® Acquired Data Compared to Extracted Accurate Mass of the Precursor Ion

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.

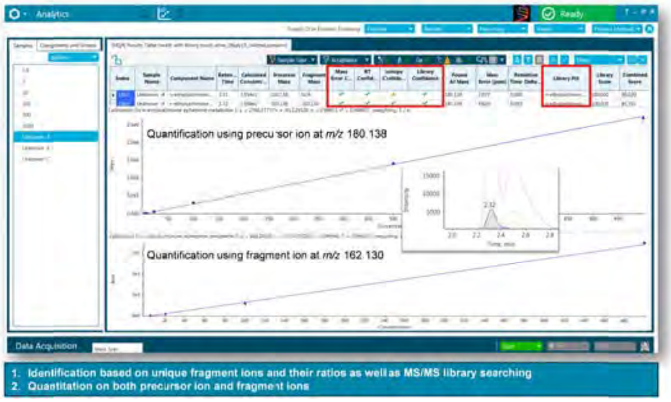


Figure 37. Identification and Quantification Results for n-Ethylcathinone Ephedrine Metabolite Compound Anlaysed by SWATH® Acquisition

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.

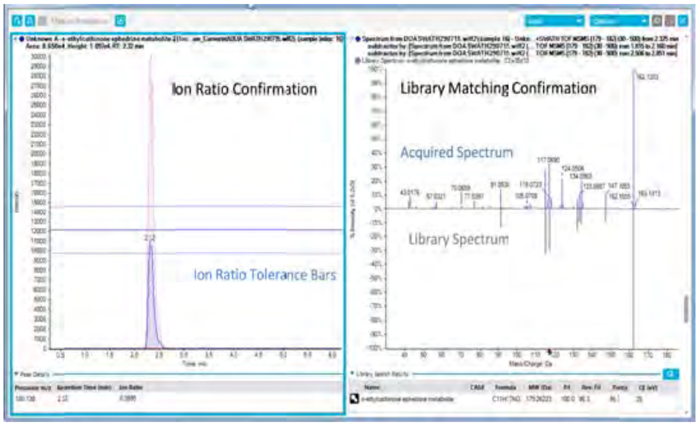


Figure 38. Extraction of Unique Fragment Ions From SWATH® Acquisition and Using Both Ion Ratio and Library Matching to Confirm Presence of n-Ethylcathinone Ephedrine metabolite in an Unknown Urine Sample

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.

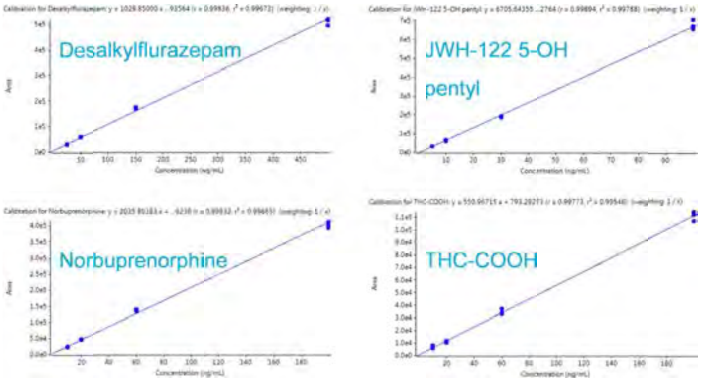


Figure 39. Representative Calibration Curves Generated from the SWATH® Acquisition using a 2.0 minute LC Runtime (n=3)

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

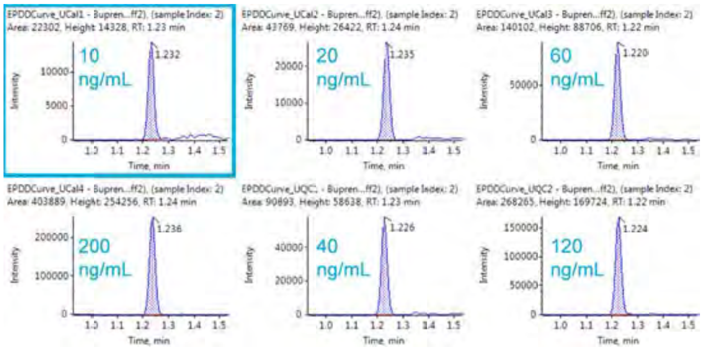


Figure 40. XICs of Buprenorphine at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

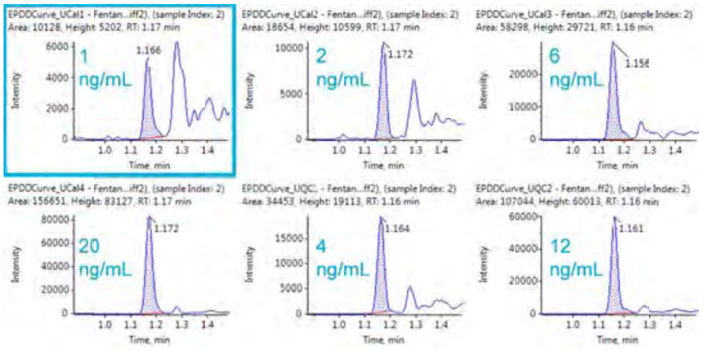


Figure 41. XICs of Fentanyl at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

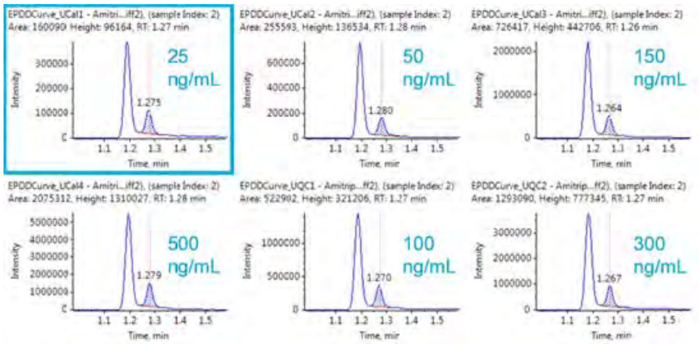


Figure 42. XICs of Amitriptyline at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

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.

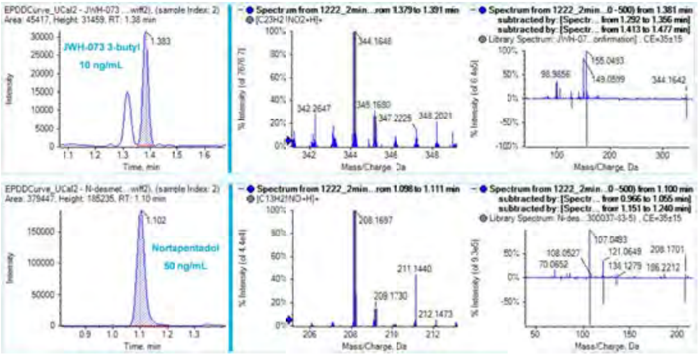


Figure 43. Confident Identification of JWH-073 3-Butyl and Nortapentadol from SWATH® Acquisition Through Library Searching

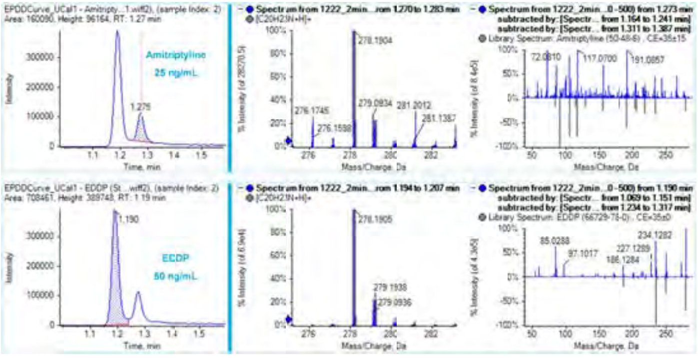


Figure 44. Confident Identification of Amitriptyline and EDDP from SWATH® Acquisition Through Library Searching; Showing LC Separation Between Isomers was Still Achievable with this Fast Method

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 Conce...	Height	Rete... Time	Precursor Mass	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error...	Ret... Tim...	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	15.6
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	97.8	2.9
EPDDCurve_UCal2	Desalkylflurazep...	50.00	39350	1.33	289.0538	✓	✓	✓	✓	289.0539	0.2	0.01	Desalkylflurazep...	100.0	98.2	1.4
EPDDCurve_UCal2	EDDP	100.00	768375	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	3.3
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-019 6-OH h...	10.00	44558	1.40	372.1958	✓	✓	✓	✓	372.1959	0.3	0.01	JWH-019 N-hyd...	100.0	96.7	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	✓	✓	✓	✓	372.1959	0.3	0.01	JWH-019 N-hyd...	100.0	96.7	4.4
EPDDCurve_UCal2	Methamphetami...	100.00	33875	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	Nordiazepam	50.00	44694	1.35	271.0633	✓	✓	✓	✓	271.0635	0.8	0.00	Nordiazepam	100.0	95.5	3.7
EPDDCurve_UCal2	Noroxycodone	50.00	23623	0.90	302.1387	✓	✓	✓	✓	302.1386	-0.2	0.01	Noroxycodone	100.0	97.9	4.3
EPDDCurve_UCal2	Tapentadol	50.00	203639	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	96.1	1.9
EPDDCurve_UCal2	7-Aminoclonaze...	50.00	76113	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	Naltrexone	50.00	56359	0.91	342.1700	✓	✓	✓	✓	342.1698	-0.4	0.00	Naltrexone	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	1.5
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.

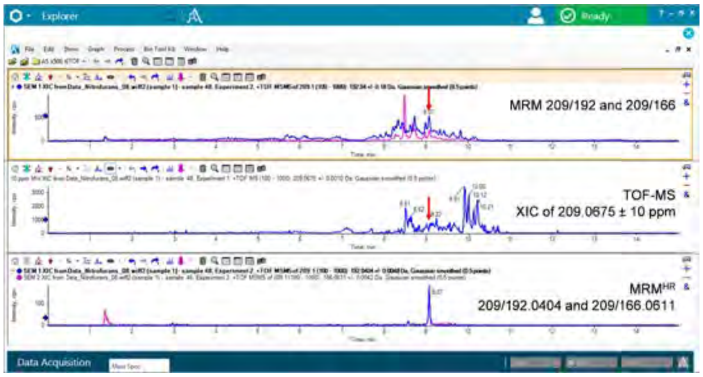
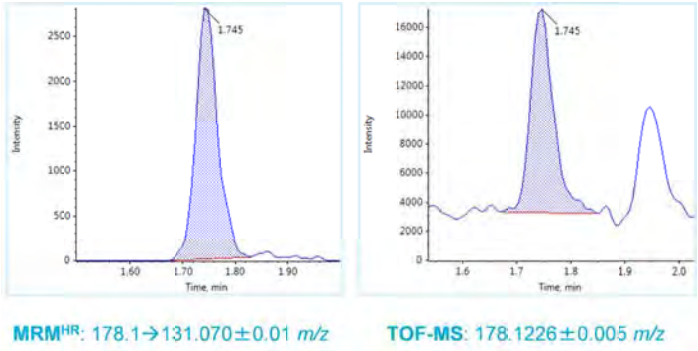


Figure 46. Increased Selectivity with MRM^{HR}; Avoiding False Negatives. Example given is a Feed Sample Tested Positive for NP Semicarbazide



MRM^{HR}: 178.1→131.070±0.01 *m/z*

TOF-MS: 178.1226±0.005 *m/z*

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.

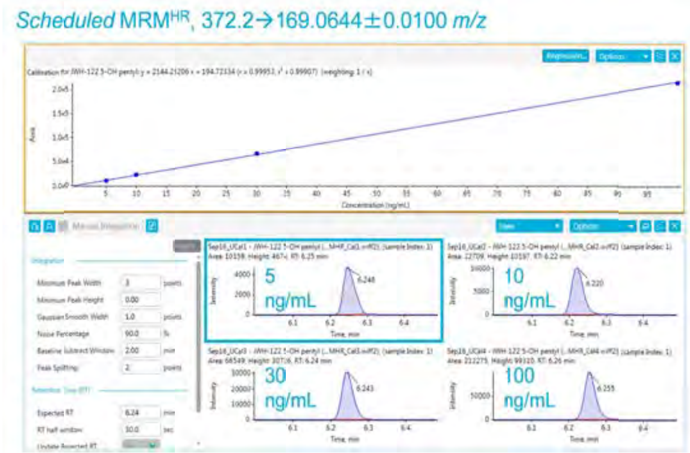


Figure 40. MRM^{HR} Quantification Results for JWH-122 5-OH Pentyl in urine (Urine was diluted 10-fold, 10 µL injection)

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.

Document number: RUO-MKT-02-3786-A

Anti-Doping Analysis

Identify the drug cheats with the X500R QTOF System!

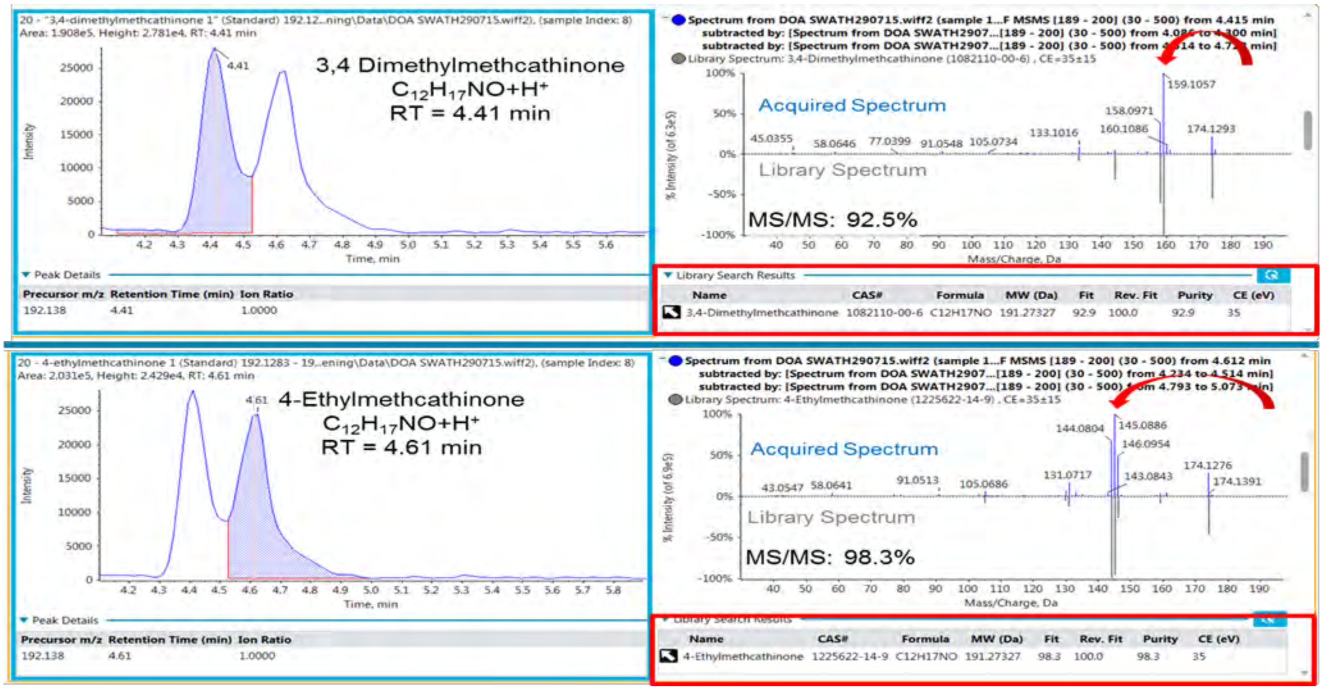
The new SCIEX X500R QTOF System and SCIEX OS are bringing the power of accurate mass technology to the routine environment; your latest sport doping tool to help you win the fight against doping and to catch the cheaters!

With this new technology there is nowhere for the cheaters to hide! A perfectly balanced system designed with optimal resolution and sensitivity.

All information from the sample is captured for easy retrospective analysis - Using the unique mode of MS/MSALL with SWATH® Acquisition both MS and MS/MS data is captured on everything in the sample

No more need to store samples; reanalyze the data not the sample

Figure 1



20 - "3,4-dimethylmethcathinone 1" (Standard) 192.12...ning\Data\DOA SWATH290715.wiff2), (sample Index: 8)
Area: 1.908e5, Height: 2.781e4, RT: 4.41 min

3,4 Dimethylmethcathinone
$C_{12}H_{17}NO+H^+$
RT = 4.41 min

Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
192.138	4.41	1.0000

Spectrum from DOA SWATH290715.wiff2 (sample 1...F MSMS [189 - 200] (30 - 500) from 4.415 min
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.086 to 4.300 min]
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.514 to 4.728 min]
Library Spectrum: 3,4-Dimethylmethcathinone (1082110-00-6) , CE=35±15

Acquired Spectrum

Library Spectrum

MS/MS: 92.5%

Library Search Results

Name	CAS#	Formula	MW (Da)	Fit	Rev. Fit	Purity	CE (eV)
3,4-Dimethylmethcathinone	1082110-00-6	C12H17NO	191.27327	92.9	100.0	92.9	35

20 - 4-ethylmethcathinone 1 (Standard) 192.1283 - 19...ening\Data\DOA SWATH290715.wiff2), (sample Index: 8)
Area: 2.031e5, Height: 2.429e4, RT: 4.61 min

4-Ethylmethcathinone
$C_{12}H_{17}NO+H^+$
RT = 4.61 min

Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
192.138	4.61	1.0000

Spectrum from DOA SWATH290715.wiff2 (sample 1...F MSMS [189 - 200] (30 - 500) from 4.612 min
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.234 to 4.514 min]
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.793 to 5.073 min]
Library Spectrum: 4-Ethylmethcathinone (1225622-14-9) , CE=35±15

Acquired Spectrum

Library Spectrum

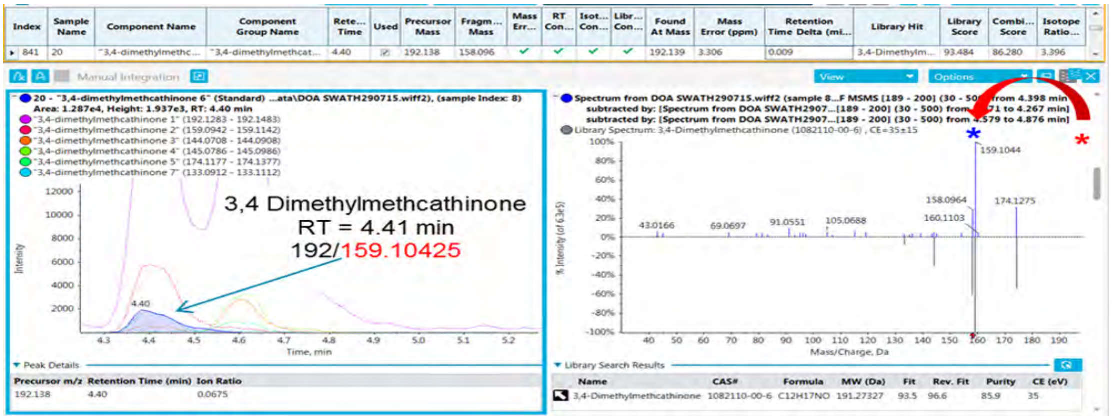
MS/MS: 98.3%

Library Search Results

Name	CAS#	Formula	MW (Da)	Fit	Rev. Fit	Purity	CE (eV)
4-Ethylmethcathinone	1225622-14-9	C12H17NO	191.27327	98.3	100.0	98.3	35

Using our unique SWATH® Acquisition Workflow you can confidently identify the isomers of your analyte based upon their unique fragment ions and ratios. Due to the high degree of accuracy in the identification of doping substances the X500R is the solution to your Anti-Doping testing.

Figure 2



Index	Sample Name	Component Name	Component Group Name	Rete... Time	Used	Precursor Mass	Fragm... Mass	Mass Err...	RT Con...	Isot... Con...	Libr... Con...	Found At Mass	Mass Error (ppm)	Retention Time Delta (mi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
841	20	"3,4-dimethylmethc...	"3,4-dimethylmethcat...	4.40	☑	192.138	158.096	✓	✓	✓	✓	192.139	3.306	0.009	3,4-Dimethylm...	93.484	86.280	3.396

20 - "3,4-dimethylmethcathinone 6" (Standard) ...ata\DOA SWATH290715.wiff2), (sample Index: 8)
Area: 1.287e4, Height: 1.937e3, RT: 4.40 min
"3,4-dimethylmethcathinone 1" (192.1283 - 192.1483)
"3,4-dimethylmethcathinone 2" (159.0942 - 159.1142)
"3,4-dimethylmethcathinone 3" (144.0708 - 144.0908)
"3,4-dimethylmethcathinone 4" (145.0786 - 145.0986)
"3,4-dimethylmethcathinone 5" (174.1177 - 174.1377)
"3,4-dimethylmethcathinone 7" (133.0912 - 133.1112)

Spectrum from DOA SWATH290715.wiff2 (sample 8...F MSMS [189 - 200] (30 - 50 from 4.398 min
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 71 to 4.267 min]
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.579 to 4.876 min]
Library Spectrum: 3,4-Dimethylmethcathinone (1082110-00-6) , CE=35±15

Peak Details

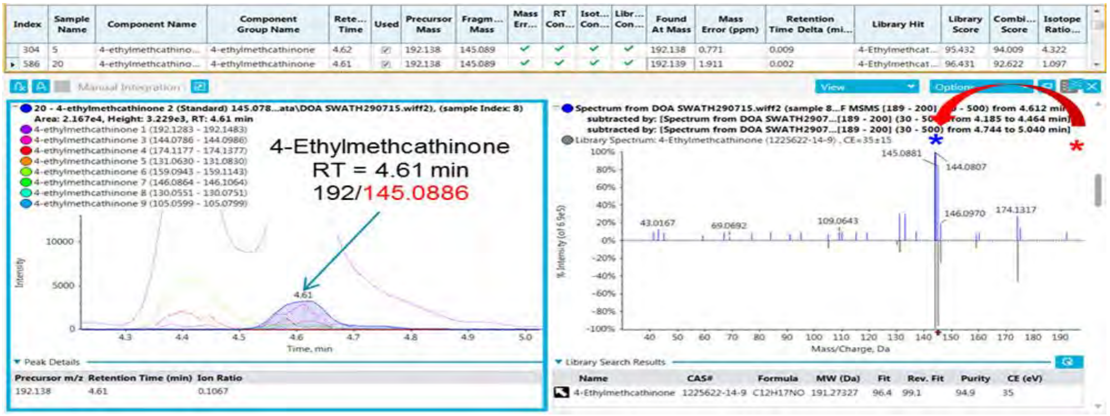
Precursor m/z	Retention Time (min)	Ion Ratio
192.138	4.40	0.0675

Library Search Results

Name	CAS#	Formula	MW (Da)	Fit	Rev. Fit	Purity	CE (eV)
3,4-Dimethylmethcathinone	1082110-00-6	C12H17NO	191.27327	93.5	96.6	85.9	35

The X500R SWATH® Workflow can confidently detect the presence of doping substances and their isomers at low concentration levels. The two images (figures 1 and 2) illustrate that even at a low concentration the spectrum information matches that of our >1700 compound High Resolution Library.

Figure 3



Index	Sample Name	Component Name	Component Group Name	Rete... Time	Used	Precursor Mass	Fragm... Mass	Mass Err...	RT Con...	Isot... Con...	Libr... Con...	Found At Mass	Mass Error (ppm)	Retention Time Delta (mi...	Library Hit	Library Score	Combi... Score	Isotope Ratio...
304	5	4-ethylmethcathino...	4-ethylmethcathinone	4.62	☑	192.138	145.089	✓	✓	✓	✓	192.138	0.771	0.009	4-Ethylmethcat...	95.432	94.009	4.322
586	20	4-ethylmethcathino...	4-ethylmethcathinone	4.61	☑	192.138	145.089	✓	✓	✓	✓	192.139	1.911	0.002	4-Ethylmethcat...	96.431	92.622	1.097

20 - 4-ethylmethcathinone 2 (Standard) 145.078...ata\DOA SWATH290715.wiff2), (sample Index: 8)
Area: 2.167e4, Height: 3.229e3, RT: 4.61 min
4-ethylmethcathinone 1 (192.1283 - 192.1483)
4-ethylmethcathinone 3 (144.0786 - 144.0986)
4-ethylmethcathinone 4 (174.1177 - 174.1377)
4-ethylmethcathinone 5 (131.0630 - 131.0830)
4-ethylmethcathinone 6 (159.0943 - 159.1143)
4-ethylmethcathinone 7 (146.0864 - 146.1064)
4-ethylmethcathinone 8 (130.0551 - 130.0751)
4-ethylmethcathinone 9 (105.0599 - 105.0799)

Spectrum from DOA SWATH290715.wiff2 (sample 8...F MSMS [189 - 200] - 500) from 4.612 mi
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 50 rom 4.185 to 4.464 min]
subtracted by: [Spectrum from DOA SWATH2907...[189 - 200] (30 - 500) from 4.744 to 5.040 min]
Library Spectrum: 4-Ethylmethcathinone (1225622-14-9) , CE=35±15

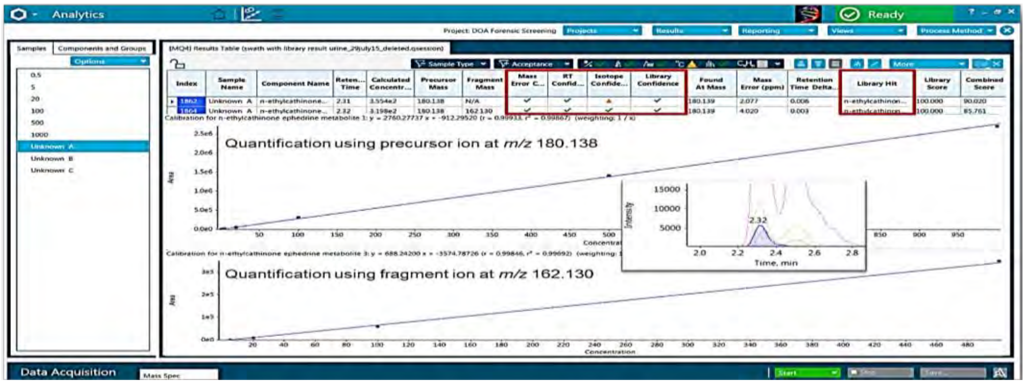
Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
192.138	4.61	0.1067

Library Search Results

Name	CAS#	Formula	MW (Da)	Fit	Rev. Fit	Purity	CE (eV)
4-Ethylmethcathinone	1225622-14-9	C12H17NO	191.27327	96.4	99.1	94.9	35

Figure 4



[MQ4] Results Table (swath with library result urine_29july15_deleted.qsession)

Index	Sample Name	Component Name	Reten... Time	Calculated Concentr...	Precursor Mass	Fragment Mass	Mass Error C...	RT Confid...	Isotope Confide...	Library Confidence	Found At Mass	Mass Error (ppm)	Retention Time Delta...	Library Hit	Library Score	Combined Score
1862	Unknown A	n-ethylcathinone...	2.31	3.554e2	180.138	N/A	✓	✓	▲	✓	180.139	2.077	0.006	n-ethylcathinon...	100.000	90.020
1864	Unknown A	n-ethylcathinone...	2.32	3.198e2	180.138	162.130	✓	✓	✓	✓	180.139	4.020	0.003	n-ethylcathinon...	100.000	85.761

Calibration for n-ethylcathinone ephedrine metabolite 1: y = 2760.27737 x + -912.29520 (r = 0.99933, r² = 0.99867) (weighting: 1 / x)

Calibration for n-ethylcathinone ephedrine metabolite 3: y = 688.24200 x + -3574.78726 (r = 0.99846, r² = 0.99692) (weighting: 1

Using the X500R with SWATH® acquisition and SCIEX OS the following is achieved:-

1) Identification based on unique fragment ions and their ratios as well as MS/MS library searching

2) Quantitation on both precursor ion and fragment ions

Learn more at sciex.com/X500R

Document number: RUO-MKT-07-3041-A

For Research Use Only. Not For Use In Diagnostic Procedures.

Forensic Method

Forensic drug screening analysis

Elevate your forensic testing with the X500R QTOF System

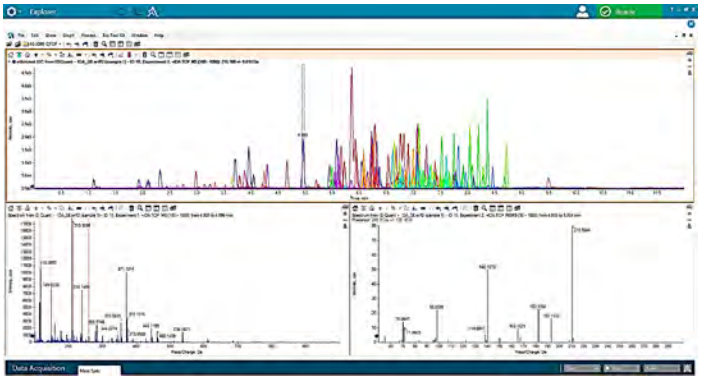
Method details and access to HR-MS/MS libraries to screen for forensic drugs in urine samples using HPLC coupled with the X500R QTOF system, powered by SCIEX OS Software.

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

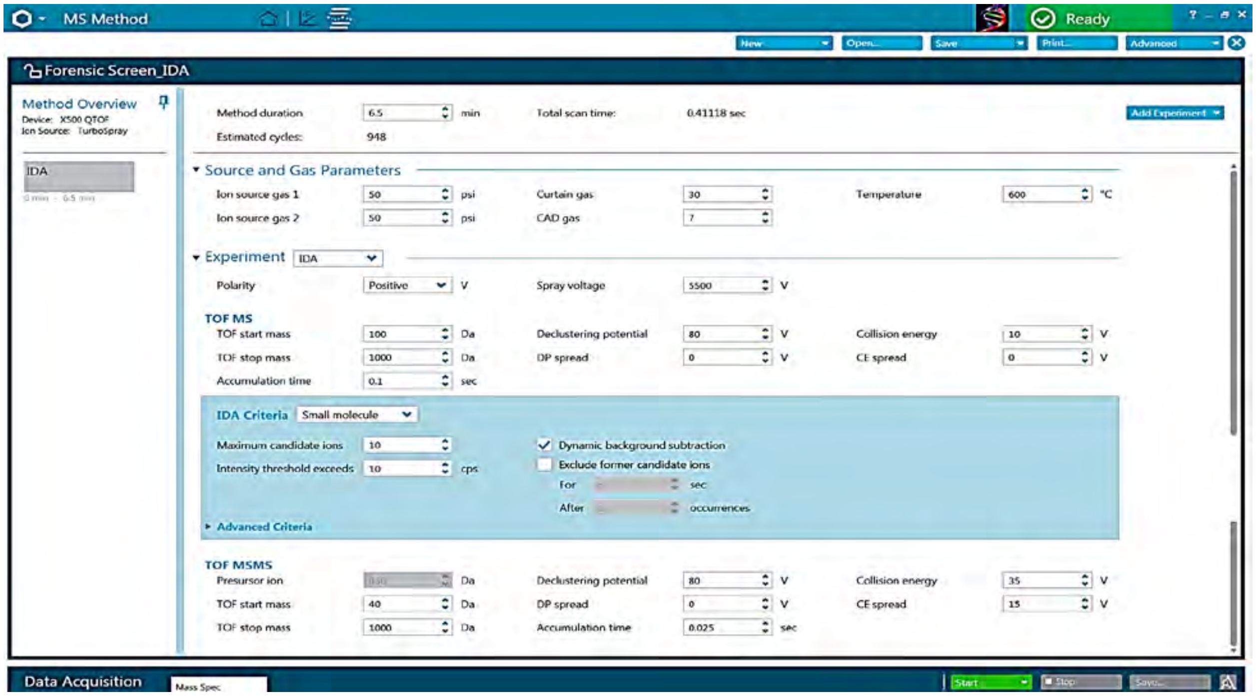


SCIEX OS delivers enhanced data exploration of your acquired TOF MS and TOF MS/MS data

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



Forensic Screen_IDA

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA
0 min - 6.5 min

Method duration 6.5 min
Total scan time: 0.41118 sec
Estimated cycles: 948

Source and Gas Parameters

Ion source gas 1 50 psi
Ion source gas 2 50 psi
Curtain gas 30
CAD gas 7
Temperature 600 °C

Experiment IDA

Polarity Positive V
Spray voltage 5500 V

TOF MS

TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.1 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule

Maximum candidate ions 10
Intensity threshold exceeds 10 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences

Advanced Criteria

TOF MSMS

Presursor ion 0.00 Da
TOF start mass 40 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.025 sec
Collision energy 35 V
CE spread 15 V

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.](https://sciex.com/X-Forensics)

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Document number: RO-MKT-07-2866-A

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

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

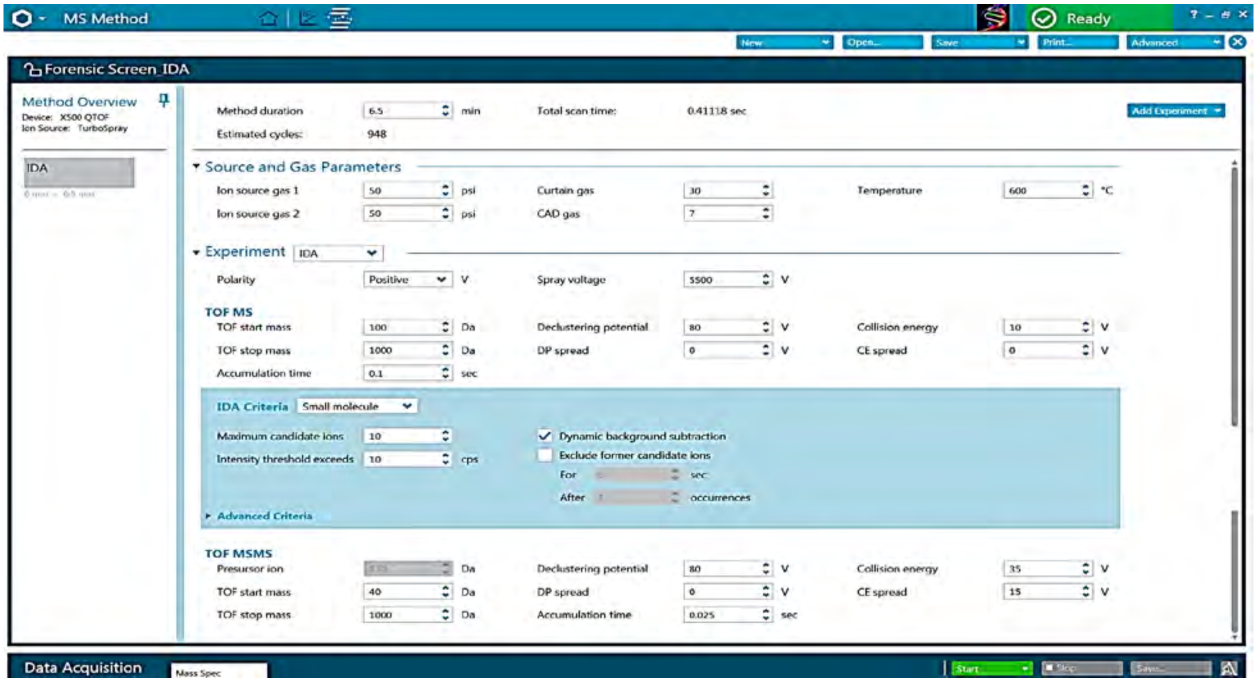
Step 7
- Transfer supernatant to clean glass HPLC vials

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*

SCIEX OS can deliver faster method set-up

MS Method



Forensic Screen_IDA

Method Overview
Device: X500 QTOF
Ion Source: TurboSpray

IDA

Method duration 6.5 min
Total scan time: 0.41118 sec
Estimated cycles: 948

Source and Gas Parameters

Ion source gas 1 50 psi
Ion source gas 2 50 psi
Curtain gas 30
CAD gas 7
Temperature 600 °C

Experiment IDA

Polarity Positive V
Spray voltage 5500 V

TOF MS

TOF start mass 100 Da
TOF stop mass 1000 Da
Accumulation time 0.1 sec
Declustering potential 80 V
DP spread 0 V
Collision energy 10 V
CE spread 0 V

IDA Criteria Small molecule

Maximum candidate ions 10
Intensity threshold exceeds 10 cps
☑ Dynamic background subtraction
☐ Exclude former candidate ions
For sec
After occurrences
Advanced Criteria

TOF MSMS

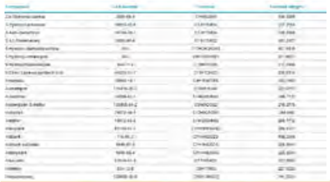
Presursor ion Da
TOF start mass 40 Da
TOF stop mass 1000 Da
Declustering potential 80 V
DP spread 0 V
Accumulation time 0.025 sec
Collision energy 35 V
CE spread 15 V

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

Data Processing

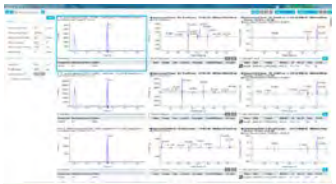
Review your results with utmost efficiency using SCIEX OS for simultaneous quantitation and MS/MS library confirmation.

X500R High Resolution Libraries



Download the XIC List

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 MS/MS Library

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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Document number: RUO-MKT-07-2865-A

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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RUO-MKT-03-4305-B

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