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第二期 --- 生物監測和毒理學

(Biomonitoring and toxicology)

SCIEX

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目錄

應用文章

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Automated Sample Preparation for the Monitoring of Ethanol Metabolites in Urine by LC-MS/MS

Beckman Coulter Biomek 4000 Workstation and SCIEX Triple Quad™ 4500 LC-MS/MS system

Lekh Sharma[1], Michael Kowalski[2], Michael Jarvis[3], Amy Gibson[2] and Navaline Quach[2]
[1]Vision Laboratories, LLC, Chattanooga, TN, USA; [2]Beckman Coulter Life Sciences, Indianapolis, IN, USA; [3]SCIEX, Concord, ON, Canada

Abstract

Ethylglucuronide (EtG) and Ethylsulphate (EtS) are stable Phase II metabolites of ethanol, which can be detected in urine until several days after alcoholic beverage consumption. In this application note, we describe an LC-MS/MS method for the analysis of EtG and EtS in urine employing a simple, automated sample preparation methodology using a liquid handling-based automation system. Automation of the sample preparation for analysis by LC-MS/MS has the potential to increase throughput, reduce active bench time, and minimize the opportunity for human error. Our method enabled the quantification of EtG and EtS over a large concentration range, spanning from 0.5X to 10X the cut-off value, with accuracies ranging from 97-104%, and CV less than 4% taking into account both sample preparation and LC-MS/MS analysis.

Introduction

LC-MS/MS is a powerful technology that is increasingly used by forensic laboratories for the monitoring of drugs in urine samples. The time and effort to process these samples can be significant and the required resources increase with sample number. In addition, the likelihood of errors increases with sample throughput. These challenges can be minimized by automating the sample preparation prior to analysis by LC-MS/MS. In addition to minimizing active sample processing time, automation can also increase the consistency of results by reducing user-to-user sample processing variability and the opportunity for errors.

In this study the Biomek 4000 Workstation (Figure 1) was used to automate the sample processing for the forensic screening of two metabolites of ethanol, EtG and EtS, in human urine on the SCIEX Triple Quad™ 4500 LC/MS/MS system. The cut-off values were 500 ng/mL and 100 ng/mL, for EtG and EtS, respectively. Standard curves were generated across five concentration levels (0.5X, 1X, 3X, 5X, 10X cut-off value) with excellent accuracy, precision, and curve linearity.

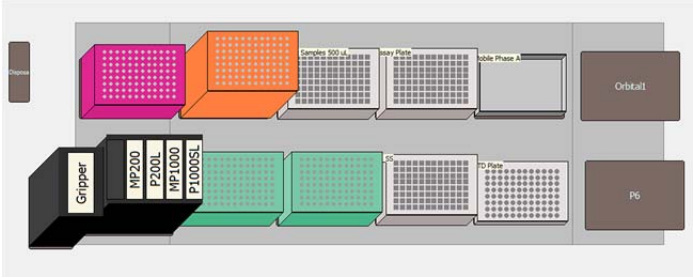
Materials and Methods

Automated Sample Preparation

Table 1 describes the steps in the automated sample preparation protocol for the analysis of EtG and EtS. Briefly, pre-cleared urine controls and samples were diluted with mobile phase A in a 96-well plate, a deuterated ethyl glucoronide internal standard was added to each well, and the samples were thoroughly mixed using an orbital shaker. The 96-well plate was centrifuged offline to clear any precipitate and 200 µL of the supernatant were

Figure 1. Beckman Coulter Biomek 4000 Workstation (left) and SCIEX Triple Quad™ 4500 LC-MS/MS system (right).

Figure 2. Software representation of the Biomek 4000 deck illustrating the tips, plates, and reservoirs utilized for the automated alcohol analysis method.



transferred to a new plate for analysis by LC-MS/MS.

This sample preparation was automated on a Biomek 4000 Workstation utilizing single and 8-channel pipetting tools. To initiate sample processing, a user simply places tips, plates, and reservoirs on the deck as directed in the software (Figure 2) and then highlights the wells containing samples and controls. Reagents were added to full plate columns using the multichannel tools while partial columns (if any) were added using the single channel tool.

Upon completion of the method, a text file containing information for each sample well (i.e. original sample barcodes) is generated in a format that allows direct import into the Analyst software for LC-MS/MS analysis. This ensures that sample data is propagated throughout the workflow.

Analyses by LC-MS/MS

HPLC separation was performed using a Shimadzu Prominence LC-20XR system and mass spectrometric detection was performed using the SCIEX Triple Quad™ 4500 LC-MS/MS system (Figure 1), equipped with Turbo V™ ionization source (Temperature = 600°C; Gas1 = 60; Gas2 = 50; Curtain Gas = 25). The temperature of the autosampler was set at 15°C.

The prepared samples were injected onto the system, and the chromatographic separation was achieved using a Phenomenex Synergi 2.5 µm Hydro-RP 100A column (50 x 3.0 mm), at 40°C. The separation employed a binary gradient of mobile phases A (HPLC-grade water with 0.1% formic acid) and B (methanol with 0.1% formic acid). The LC-MS/MS data acquisition was done using the Analyst 1.6.2 software. MultiQuant™ 3.0.1 software was used for data processing, and reporting. Two MRM transitions were used to monitor each analyte, and a single MRM transition was used to monitor the internal standard EtG-D5 (see Table 2). The Scheduled MRM™ algorithm was employed, to maximize the acquisition dwell time for each analyte and thereby improve data quality.

The LC-MS/MS analysis was performed in negative ionization mode (Ion Spray voltage = -4500). 10 µl of sample were injected and run at a flow rate of 0.7 mL/min for a 5 min run time with the following time profile for mobile phase B: 0-1.5 minute hold at 0% B; 1.5-2.5 minute ramp from 0-80% B; 2.5-3.0 minute hold at 80% B; 3.0-4.0 minute decrease to 0% B, 4.0-5.0 minute hold (re-equilibrate) at 0% B (Figure 3). Each analyte was monitored

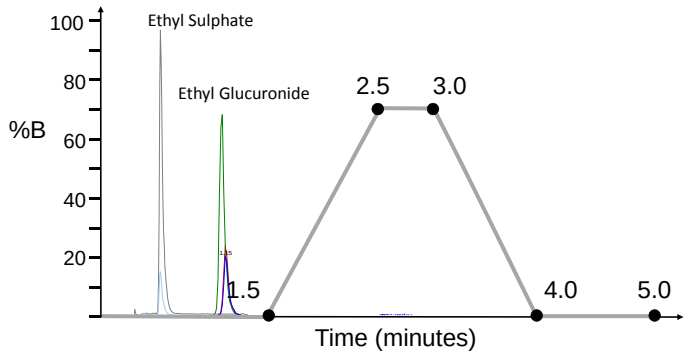
Table 1. Automated sample preparation protocol for LC-MS/MS analysis of alcohol in human urine samples

Step 1	Barcoded urine samples are added to a deepwell plate (“Samples 500 µL”) and precleared by centrifugation at 4500 rpm in an Allegra X-30R (Beckman Coulter).	Manual
Step 2	100 µL of samples, calibrators, and QC controls are transferred to a deepwell plate (“Assay”).	Automated
Step 3	850 µL of Mobile Phase A are added to each well.	Automated
Step 4	50 µL of internal standard (deuterated ethyl glucuronide, EtG-D5) are added to each well and the plate is shaken to mix.	Automated
Step 5	Samples are centrifuged at 4500 rpm in an Allegra X-30R, for 15 minutes.	Offline
Step 6	200 µL of supernatant are transferred to a flat-bottomed plate (“STD”) for analysis.	Automated
Step 7	Analysis by LC-MS/MS system.	

Table 2. MS/MS Conditions for the analysis of EtG and EtS.

	Q1	Q3	CE (V)
Ethyl Glucuronide 1	220.9	85.1	*-20*
Ethyl Glucuronide 2	220.9	*74.9*	*-18*
Ethyl Sulphate 1	*124.8*	*97*	*-18*
Ethyl Sulphate 2	*124.8*	*79.7*	*-40*
Ethyl Glucuronide-D5	*225.9*	*85.1*	*-24*

Figure 3. HPLC gradient (% Mobile Phase B) for the analysis of EtG and EtS on the SCIEX Triple Quad™ 4500 LC-MS/MS system, using negative electrospray ionization (ESI), with a run-time of 5 minutes. Overlaid is a representative chromatogram displaying the analytes at their respective cut-off concentration levels.



during a 60-second detection window centered on the expected Retention Time.

Results

A full plate of 96 samples (including calibrators and controls) can be processed in under one hour, including the offline centrifugation. The reproducibility of the automated sample preparation protocol was assessed by preparing and analyzing replicates (n=6) of each calibration standard for EtG and EtS. A total of 5 concentration levels were prepared and analyzed, across a concentration range from 0.5x – 10x the cut-off levels. Calibration curves are shown in Figure 4, and display excellent linearity, accuracy and precision across the entire concentration range. Representative chromatograms at the cut-off level (500 ng/mL EtG, and 100 ng/mL EtS) are displayed in Figure 5, demonstrating the excellent sensitivity of this method. Table 3 and 4 summarize the accuracy and coefficient of variation (CV) across the five concentration levels. The accuracies ranged from 97% to 104%. The CVs were less than 4% at all concentration levels.

Figure 4. Calibration curves plotting Area Ratio (Y-axis) vs. Concentration Ratio (X-axis) for (a) EtG, and (b) EtS.

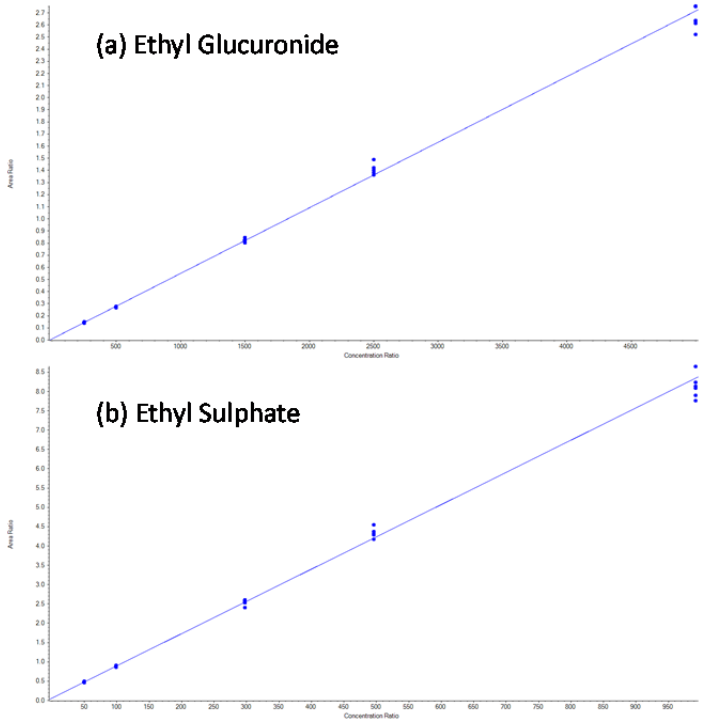
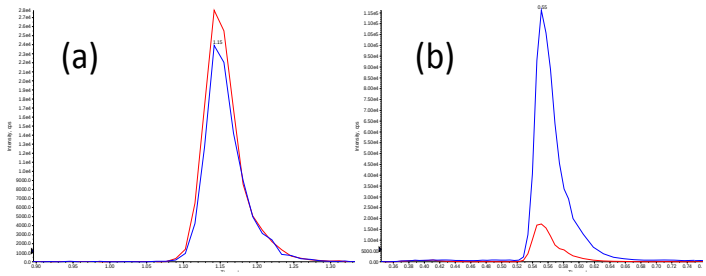


Figure 5. Representative chromatograms showing two MRMs per analyte at the cut-off level for (a) EtG (500 ng/mL), and (b) EtS (100 ng/mL), demonstrating the excellent sensitivity of this analysis.



Conclusion

This study describes a successful application of an automated sample preparation protocol for analysis of metabolites of ethanol, EtG and EtS, in urine samples by LC-MS/MS. The Beckman Coulter Biomek 4000 Workstation was used to prepare the calibration curves and urine samples prior to end-point analysis. The SCIEX Triple Quad™ 4500 LC-MS/MS system was used for the identification and quantification of EtG and EtS in the processed samples. In summary, this automated method offers a simple, rapid, accurate and reproducible solution for the quantitation of ethanol metabolites that is suitable for implementation in routine testing laboratories.

Table 3. Statistical summary (n=6) for the quantitation of EtG by LC-MS/MS, using automated sample preparation on the Biomek 4000 workstation.

Fold Cutoff Value	Mean Conc. (ng/mL)	Accuracy (%)	CV (%)
0.5	252.3	100.9	3.8
1	487.9	97.6	2.4
3	1507.8	100.5	1.8
5	2582.8	103.3	3.2
10	4883.9	97.7	3.4

Table 4. Statistical summary (n=6) for the quantitation of EtS by LC-MS/MS, using automated sample preparation on the Biomek 4000 workstation.

Fold Cutoff Value	Mean Conc. (ng/mL)	Accuracy (%)	CV (%)
0.5	49.7	100.3	3.6
1	98.1	99	2.3
3	297.0	99.8	2.8
5	513.3	103.5	2.9
10	966.1	97.4	3.8

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SCIEX

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

International Sales
For our office locations please call the division headquarters or refer to our website at
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SCIEX QTRAP®系统对血液中有毒生物碱的定性与定量分析

Identification and Quantitation of Poisonous Alkaloids in Blood by SCIEX QTRAP®

张景然，刘冰洁，李立军，郭立海
Zhang Jingran, Liu Bingjie, Li Lijun, Guo Lihai
SCIEX　亚太应用支持中心，上海
SCIEX China, Shanghai

Key words: QTRAP®; 4500; Poisonous Alkaloids; MRM-IDA-EPI;

引言

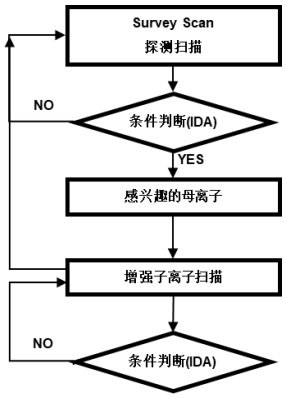
生物碱是存在于自然界（主要为植物）中的一类含氮碱性有机化合物，大多数生物碱具有杂环状结构。很多生物碱是中药的有效成分，其在具有生物活性的同时也具有一定的毒性，过量摄入过量会产生副作用。生物碱是引发食物中毒的主要原因之一，有些生物碱还会被犯罪分子利用进行违法犯罪类活动。

由于有毒生物碱的成分复杂多样、含量低、毒性大、生物检材基质复杂等原因，为公安司法的鉴定工作带来难题。因此，本实验使用QTRAP®质谱独有的复合功能和扫描方式建立了血液中31种有毒生物碱的快速筛查、鉴定和定量的方法。

SCIEX QTRAP®质谱系统将行业标准的三重四极杆质谱技术与专利的线性加速离子阱技术相结合。不仅具有三重四级杆质谱的所有功能，离子阱技术更能提供超快的扫描速度，以及具有提高两个数量级以上的二级碎片灵敏度。QTRAP®质谱并不仅是两种质谱技术简单的叠加，其能够瞬时从三重四极杆模式切换到线性加速离子阱模式，故可智能化的将这两类质谱的扫描方式相结合，实现一针进样同时获得不同扫描模式下的数据。本实验使用QTRAP®质谱系统独有的MRM-IDA-EPI扫描模式（见图1），实现一针进样同时获得定性与定量数据。

实验方法特点

1. 检测方法简单快速：单针分析时间8 min即可完成31种有毒生物碱的定量与定性分析。31种生物碱的提取离子流色谱图见图2。



步骤1，探测扫描（可有多种方式， 如MRM、中性丢失(NL)、母离子(Precursor)扫描等）；

步骤2，系统自动判断：探测扫描采集到离子信号是否符合IDA的各项限定条件

步骤3，当步骤2的条件满足时，系统自动快速切换（<1ms）为线性离子阱模式，进行增强子离子扫描（EPI），获得高质量MS^2谱图；

图1. 一针进样同时获得定性（EPI）与定量（MRM）数据。

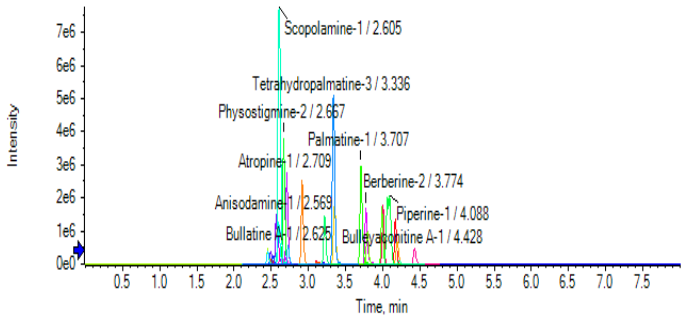


图2. 31种有毒生物碱MRM提取离子流色谱图。

2. 强大的定性功能：QTRAP®质谱系统独有的EPI模式，即增强型子离子扫描模式，可得到灵敏度更高（与三重四极杆相比可提高两个数量级以上），且不同能量碎裂的全质量范围的二级碎片谱。软件通过与标准品谱库的自动比对，使定性结果更准确。

3. 强大的定量功能：QTRAP®质谱系统具有与三重四极杆质谱完全一致的定量性能。提供pg级的灵敏度，以及出色的仪器重现性和稳定性，保证定量准确。
4. 目标物定性与定量一针完成：QTRAP®质谱系统独有的MRM-IDA-EPI复合扫描模式一针进样同时获得MRM定量数据以及高灵敏度的二级碎片全谱数据（EPI）。实现同时进行目标化合物的定性和定量分析。
5. 高质量的二级谱图为化合物的定性提供依据，结合SCIEX OS软件可以自动将化合物二级碎片谱图与数据库进行匹配，快速完成目标化合物的筛查和定性。

仪器设备

液质系统： SCIEX ExionLC™液相 + QTRAP® 4500质谱系统

实验方法

前处理方法：

参照《血液中45 种有毒生物碱成分的液相色谱-串联质谱检验方法》（SF/Z JD0107015-2015）

液相方法：

色谱柱：Phenomenex Kinetex F5 (100 × 3.0 mm, 2.6 µm)

流速：0.4 mL/min；

柱温：40 ℃；

进样量：5 µL；

洗脱方式：梯度洗脱；

质谱方法：

扫描方式：MRM-IDA-EPI;

离子源：ESI+源

IS电压: 3000 V（+）

源温度 TEM: 550 ℃

气帘气 CUR: 25 psi　　碰撞气 CAD: 7 psi

雾化气 GS1: 45 psi　　辅助气 GS2: 55 psi

EPI碰撞能量CE：35 ± 15 V；

结果与讨论

定性结果

SCIEX OS 软件的集成界面，具有高自动化、简单易用，一目了然的特点。软件自动根据化合物的保留时间和二级碎片谱图进行目标化合物筛查和定性。

如图3，根据软件结果显示，在某样本中检测出阿托品，其保留时间和二级碎片谱图均为绿色对勾，说明匹配良好。实测二级谱图与数据库匹配Purity 得分为96.6 分，体现QTRAP®质谱的EPI 的独有优势，即便在复杂基质中的低浓度目标物仍能得到高质量的二级谱图，保证定性结果的准确性。

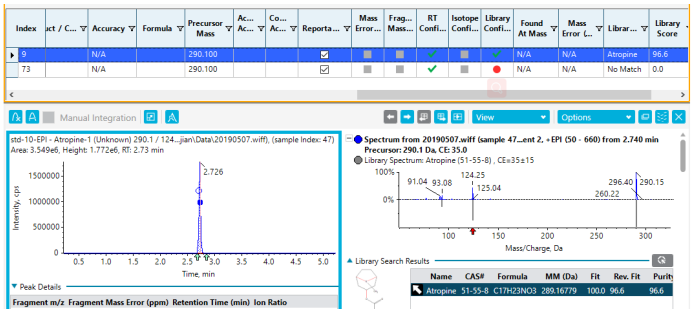


图3. 样品中检测出的阿托品色谱图及其二级谱图与数据库匹配结果。

定量结果

标准曲线及检出限

将空白血液样品按照“前处理方法”提取净化后，得到空白基质，使用空白基质配制线性范围为0.1-100 ng/mL的基质标准溶液。实验结果表明（见表1），各目标化合物的线性关系良好，相关系数均大于0.995，检测结果满足血液中有毒生物碱的检测要求。

使用空白基质溶液配制6份浓度为1 ng/mL的质控样品，通过计算6份样品的RSD考察方法的重现性，数据结果表明（见表2）方法的重现性良好，满足检测要求。

表1. 31种有毒生物碱的线性方程及定量限。

编号	化合物名称	定量限 ng/mL	线性范围 ng/mL	线性方程	相关系数
1	石山碱甲	0.04	0.1-100	y = 11858.20084 x + 686.74202	r=0.99872
2	喜树碱	0.1	0.1-100	y = 7904.43557 x + 200.34331	r=0.99727
3	氧化苦参碱	0.1	0.1-100	y = 6.10628e4 x + 864.44780	r=0.99813
4	消旋山莨菪碱	0.02	0.1-100	y = 2.81196e5 x + 999.11354	r=0.99754
5	阿托品	0.01	0.1-100	y = 3.11947e5 x + 868.88403	r=0.99625
6	盐酸小檗碱	0.05	0.1-100	y = 3.25780e5 x + 3596.32447	r=0.99807
7	马钱子碱	0.1	0.1-100	y = 25233.25228 x + 835.26718	r=0.99722
8	雪山一支蒿甲素	0.05	0.1-100	y = 7.16212e4 x + 881.18653	r=0.99718
9	草乌甲素	0.05	0.1-100	y = 1.06230e5 x + 2535.19434	r=0.99943
10	天然辣椒素	0.05	0.1-100	y = 1.92689e5 x + 1078.78189	r=0.99782
11	秋水仙碱	0.1	0.1-100	y = 21495.35124 x + 1605.94851	r=0.99685
12	二氢辣椒素	0.02	0.1-100	y = 1.60055e5 x + 901.72743	r=0.99677
13	二羟丙茶碱	0.1	0.1-100	y = 3.83407e4 x + 2823.32456	r=0.99791
14	粉防己碱	0.1	0.1-100	y = 16124.19051 x + -2287.64120	r=0.99681
15	高三尖杉酯碱	0.02	0.1-100	y = 3.70479e5 x + 591.91404	r=0.99813
16	次乌头碱	0.02	0.1-100	y = 1.53941e5 x + 5763.40045	r=0.99817
17	药根碱	0.02	0.1-100	y = 3.03593e5 x + 1760.69140	r=0.99808
18	新乌头碱	0.02	0.1-100	y = 2.03892e5 x + 6005.47920	r=0.99705
19	合成辣椒素	0.02	0.1-100	y = 4.31062e5 x + 5758.71996	r=0.99809
20	盐酸巴马汀	0.02	0.1-100	y = 3.88577e5 x + 2178.03855	r=0.99682
21	毒扁豆碱	0.01	0.1-100	y = 6.07215e5 x + 1001.25454	r=0.99838
22	胡椒碱	0.05	0.1-100	y = 4.45212e5 x + 5216.17661	r=0.99628
23	原阿片碱	0.02	0.1-100	y = 1.12835e5 x + 1131.57049	r=0.99571
24	利血平	0.1	0.1-100	y = 21186.88628 x + -700.11755	r=0.99949
25	东莨菪碱	0.002	0.1-100	y = 5.73171e5 x + 6609.02175	r=0.99539
26	青藤碱	0.01	0.1-100	y = 1.75872e5 x + 608.62341	r=0.99714
27	槐定碱	0.1	0.1-100	y = 5.18948e4 x + 1985.17326	r=0.99780
28	士的宁	0.1	0.1-100	y = 7.47103e4 x + 1172.95841	r=0.99848
29	延胡索乙素	0.01	0.1-100	y = 5.30766e5 x + 1658.33279	r=0.99649
30	可可碱	0.1	0.1-100	y = 6830.25059 x + 1320.11258	r=0.99549
31	茶碱	0.1	0.1-100	y = 4.93122e4 x + 8033.83516	r=0.99575

表2. 31种有毒生物碱的重现性数据。

编号	化合物名称	实际浓度 ng/mL	平行样数量	平均计算浓度 ng/mL	RSD %
1	石山碱甲	1.00	6 of 6	0.93	2.94
2	喜树碱	1.00	6 of 6	1.02	3.18
3	氧化苦参碱	1.00	6 of 6	0.94	1.39
4	消旋山莨菪碱	1.00	6 of 6	1.02	4.51
5	阿托品	1.00	6 of 6	0.99	4.24
6	盐酸小檗碱	1.00	6 of 6	0.98	2.01
7	马钱子碱	1.00	6 of 6	0.88	3.06
8	雪山一支蒿甲素	1.00	6 of 6	0.99	2.82
9	草乌甲素	1.00	6 of 6	0.91	5.35
10	天然辣椒素	1.00	6 of 6	1.01	4.10
11	秋水仙碱	1.00	6 of 6	0.95	4.02
12	二氢辣椒素	1.00	6 of 6	0.98	1.52
13	二羟丙茶碱	1.00	6 of 6	1.00	4.22
14	粉防己碱	1.00	6 of 6	0.84	2.10
15	高三尖杉酯碱	1.00	6 of 6	1.02	2.42
16	次乌头碱	1.00	6 of 6	0.98	4.39
17	药根碱	1.00	6 of 6	0.99	3.40
18	新乌头碱	1.00	6 of 6	1.04	2.83
19	合成辣椒素	1.00	6 of 6	0.99	5.65
20	盐酸巴马汀	1.00	6 of 6	1.00	2.69
21	毒扁豆碱	1.00	6 of 6	0.97	2.59
22	胡椒碱	1.00	6 of 6	1.00	2.17
23	原阿片碱	1.00	6 of 6	1.02	2.45
24	利血平	1.00	6 of 6	0.92	3.49
25	东莨菪碱	1.00	6 of 6	0.96	1.63
26	青藤碱	1.00	6 of 6	0.97	1.04
27	槐定碱	1.00	6 of 6	0.88	4.99
28	士的宁	1.00	6 of 6	0.96	3.91
29	延胡索乙素	1.00	6 of 6	1.01	3.28
30	可可碱	1.00	6 of 6	1.02	3.41
31	茶碱	1.00	6 of 6	1.05	3.92

总结

1. 本文应用SCIEX QTRAP® 4500系统独有的MRM-IDA-EPI模式，建立了血液中31种有毒生物碱的定量和定性筛查方法。一针进样同时得到准确高质量的MRM数据和EPI数据，使得定量和定性筛查可以一针完成，省时省力。
2. SCIEX 专利技术的Turbo V™ 离子源，业内最高的离子源温度设计和专利技术的主动排空功能带来高离子化效率和极强的抗污染能力。在日常大批量样本检测过程中仍保证的稳定的高灵敏度和重现性。
3. QTRAP®质谱独有的EPI扫描模式，利用碰撞池的多能量碎裂功能以及离子阱质量分析器的富集功能，可得到更全质量范围的二级碎片谱，且具有与三重四极杆质谱子离子扫描模式相比大于两个数量级以上的灵敏度。即便对复杂基质中的低浓度目标化合物仍能得到高质量的二级碎片谱，通过与标准谱库的匹配使定性结果更准确。
4. SCIEX OS 软件自动化高，操作简单，可在同一界面完成定性和定量分析，界面直观，省时省力。软件可以自动根据化合物的保留时间和二级碎片谱图进行目标化合物筛查和定性。
5. 高质量的二级谱图为化合物的定性提供依据，在没有标准品的情况下，也可以快速完成目标化合物的筛查和定性。
6. 该方法能快速准确的对血液中31种有毒生物碱进行定性和定量分析，化合物灵敏度远高于标准要求。该方法和实验思路同样适用于SCIEX 其它型号QTRAP®产品。为公安法医领域提供方法参考。

SCIEX中国公司

北京分公司
地址：北京市朝阳区酒仙桥中路24号院 1号楼5层
电话：010-58081388
传真：010-58081390

上海公司及亚太区应用支持中心
地址：上海市长宁区福泉北路518号 1座502室
电话：021-24197200
传真：021-24197333

广州分公司
地址：广州市天河区珠江西路15号 珠江城1907室
电话：020-85100200
传真：020-38760835

全国免费垂询电话：800 820 3488，400 821 3897　网址：www.sciex.com.cn　微博：@SCIEX

QTRAP® 5500对常见14种抗凝血类杀鼠药的定性定量分析

Qualitative and Quantitative Analysis of 14 Common Anticoagulant Rodenticides by QTRAP® 5500

吴海军，赵祥龙，李立军，郭立海

Wu haijun，Zhao Xianglong，Li Lijun，Guo Lihai

SCIEX亚太应用支持中心 广州

SCIEX China, Guangzhou

Key words: QTRAP® 5500,Anticoagulant rodenticides

引言

抗凝血杀鼠药是目前广泛使用的一类慢性杀鼠药，由4-羟基香豆素或茚二酮母体结构衍生而来，具有高效、广谱、毒性相对较低、不易引起二次中毒、具有特效解毒药等特点，在生产、生活中被人们广泛应用。但有关鼠药误食、自杀、投毒等案件常有发生，为配合公安破案，及中毒病人的临床诊断和治疗，需对中毒人血样及接触性器材进行快速定性和定量检测。目前常用分光光度法、薄层色谱法、高效液相色谱法、液相色谱质谱联用法。其中液相色谱质谱联用法结合了色谱分离和质谱结构确认技术的优势，具有灵敏度高、稳定性好。结合公安破案分析样本的多样性，复杂性，基质干扰严重而常常出现假阴或假阳的特点，本文采用QTRAP®质谱系统，一针进样同时获得常见14种抗凝血类杀鼠药的定性的二级全谱，以及MRM定量的色谱峰。

抗凝血杀鼠药检测的难点和挑战：

- 公安破案分析样本具有多样性，复杂性。如尿液、血样、组织等生物性样本基质干扰特别严重，常会出现假阳、假阴的问题，需要花大量时间从前处理到方法优化来确认结果可靠性。
- 常用分光光度法、薄层色谱法、高效液相色谱法等方法分析种类单一，分析时间长，准确定性定量能力差。

本文实验方案技术特点和优势：

- Analyst® 1.7软件中MRM-IDA-EPI扫描模式采集，分析时间短、灵敏度高，6 min完成一次检测，同时获得每一种鼠药的EPI定性的二级全谱，及MRM定量色谱峰面积（见图1），最终快速排除假阴、假阳的定性判断，准确的定量分析；
- 浓度范围为0.1-20 μg/L，具有良好的线性相关性，r均大于0.99，此实验方法灵敏度高；
- SCIEX OS-Q分析软件可以同时自动批量处理EPI定性二级谱图匹配及MRM定量的数据，快速高通量完成大量样本的定性定量工作。

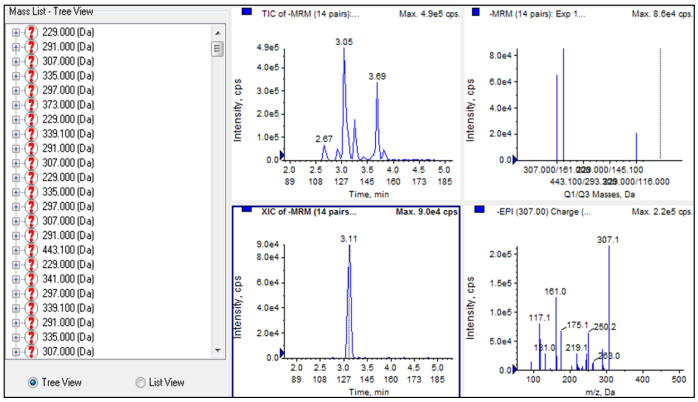


图1. 浓度为0.5 ng/mL的14种抗凝血杀鼠药MRM的TIC、XIC图及EPI的二级全谱。

实验方法

1. 液相方法：

流动相A: 5 mmol/L甲酸铵水溶液

流动相B: 5 mmol/L甲酸铵甲醇溶液

色谱柱： ACQUITY UPLC BEH C18 1.7 μm 2.1 × 100 mm

流速：0.25 mL/min；柱温：40 ℃；

洗脱程序：梯度洗脱；

2. 质谱方法：

QTRAP® 5500质谱的MRM-IDA-EPI采集参数如表1、表2：

表1. 负离子模式：ESI-，EPI：50-550 Da。

Parameter	Setting
Curtain Gas (CUR)	30
IonSpray Voltage（IS）	-4500
Temperature (TEM)	550
Ion Source Gas (GS1)	55
Ion Source Gas (GS2)	55
Collision Energy（CE）	-35
Collision Energy Spread（CES）	-35 ± 15

表2. 14种抗凝血类杀鼠药的质谱参数。

名称	英文	母离子	碎片离子	去簇电压	碰撞电压
杀鼠灵	Warfarin	307	161*	-110	-27
			250.1	-110	-31
杀鼠醚	Coumatetralyl	291	141.1*	-120	-36
			106.1	-120	-35
溴鼠灵	Brodifacoum	521	135*	-100	-48
			187	-100	-49
溴敌隆	Bromadiolone	525.1	250*	-120	-48
			273	-120	-49
鼠得克	Difenacoum	443.1	293.3*	-100	-45
			134.9	-100	-43
氟鼠灵	Flocoumafen	541.1	382.2*	-85	-37
			161.1	-85	-45
氯鼠酮	Chlorphacinon	373	200.9*	-70	-30
			145.1	-70	-31
敌鼠	diphacinone	339.1	167.1*	-90	-33
			145	-90	-31
鼠完	pinone	229	145.1*	-100	-30
			116	-100	-45
噻鼠灵	Difethialone	537	151*	-60	-48
			203	-60	-50
克鼠灵	coumafuryl	297	161*	-60	-27
			240	-60	-27
氯灭鼠灵	coumachlor	341	284*	-60	-31
			161	-60	-31
杀鼠酮	Valone	229	116*	-100	-45
			172	-100	-30
敌鼠灵	Melitonin	335	161*	-50	-25
			117	-50	-57

*表示定量离子对

3. 样品前处理

参照SF/Z JD0107018-2018前处理方法，对生物性样本及器材采用乙酸乙酯反复提取，氮吹、定容，上机。

结果与讨论

1. 抗凝血类杀鼠药的定性判断

QTRAP® 5500的质谱系统，采用MRM-IDA-EPI扫描方式，一针进样获得每种鼠药的EPI定性数据及MRM的定量数据。使用SCIEX OS-Q软件自动批量的对鼠药进行定性分析，鼠药的EPI定性通过自动与标准品建立的Library谱库进行全范围碎片的比对，最终得出定性的结果，如图2中氟鼠灵二级全谱匹配，结果Fit，Rev.Fit，Purity都在90以上，确认该物质就是氟鼠灵。所以，QTRAP®中EPI功能的定性判断，减少工作者需要花大量时间从前处理到方法优化来确认结果可靠性，避免假阴假阳的误判，同时保证结果的时效性。

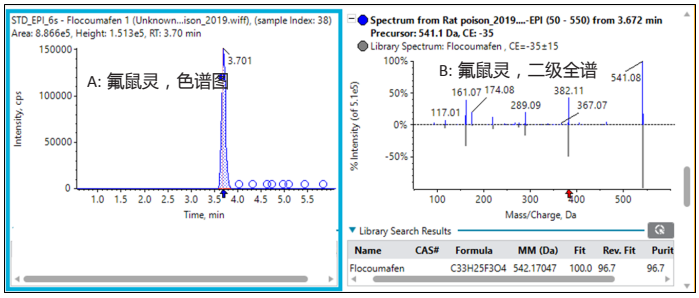


图2. SCIEX OS-Q软件批量同时处理EPI二级全谱的定性图（B）及MRM的定量色谱峰（A）。

2. 线性范围

14种抗凝血类杀鼠药在浓度为0.1-20 μg/L范围线性良好（r>0.99），保证不同浓度水平样品的准确定量（见图3）。

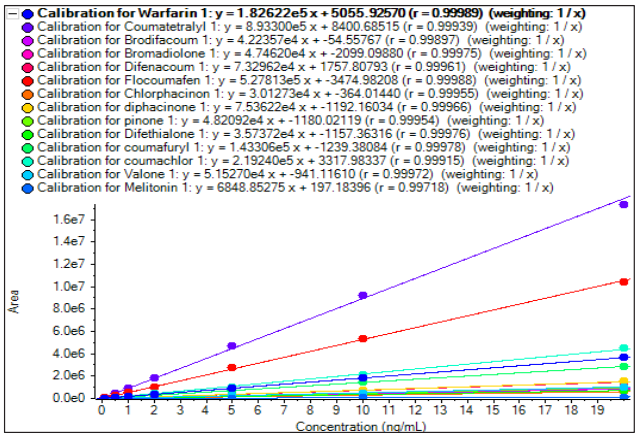


图3. 14种抗凝血类杀鼠药的工作曲线。

3. 定量限

浓度为0.1-0.2 μg/的 14种抗凝血类杀鼠药化合物的色谱图（见图4），QTRAP 5500质谱高灵敏度的特性，保证了抗凝血类杀鼠药在复杂的基质样本测定的准确性。

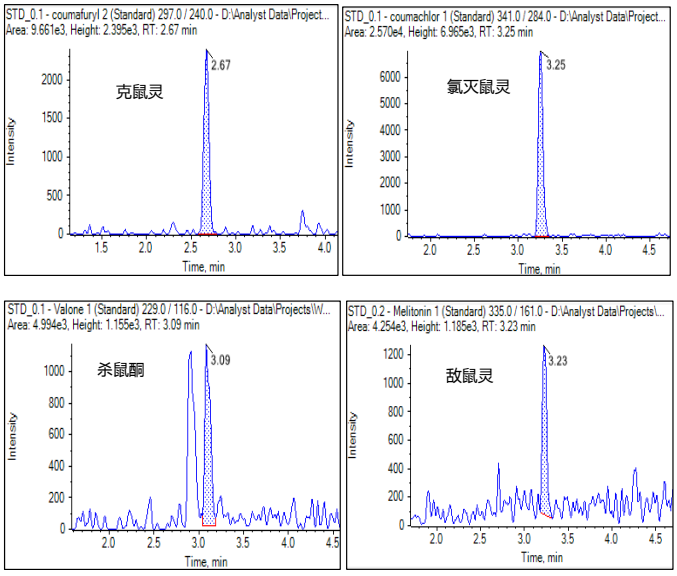


图4. 浓度为0.1-0.2 ng/mL的14种抗凝血类杀鼠药的色谱图。

总结：

- 本实验基于SCIEX QTRAP® 5500质谱系统，建立了QTRAP®对常见的14种抗凝血类杀鼠药的定性定量分析；结果表明本实验方法分析速度快，定性分析准确，检测灵敏度高、定量准确。
- 在Analyst® 1.7软件，MRM-IDA-EPI的扫描模式下，6 min完成一次检测，一针进样同时采集EPI定性的二级全谱，及MRM定量的色谱峰。减小工作者需要花大量时间从前处理到方法优化来确认结果可靠性，避免假阴假阳的误判。同时，获取的MRM数据保证定量的准确。因此，QTRAP®质谱系统完成复杂基质样本的精准的定性定量分析，保证结果的时效性。
- SCIEX OS-Q分析软件可以同时自动批量处理EPI定性二级谱图匹配及MRM定量的数据。快速高通量完成大量样本的定性定量工作。

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SCIEX中国公司

北京分公司
地址：北京市朝阳区酒仙桥中路24号院1号楼5层
电话：010-58081388
传真：010-58081390

上海公司及亚太区应用支持中心
地址：上海市长宁区福泉北路518号1座502室
电话：021-24197200
传真：021-24197333

广州分公司
地址：广州市天河区珠江西路15号珠江城1907室
电话：020-85100200
传真：020-38760835

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An accurate and sensitive method for the quantification of 33 pyrrolizidine and 21 tropane alkaloids in plant-based food matrices

Using the SCIEX QTRAP® 6500+ LC-MS/MS System

[1]Jianru Stahl-Zeng, [2]Jack Steed, [1]Jasmin Meltretter
[1]SCIEX, Germany; [2]SCIEX, UK

Recent incidents of poisoning from pyrrolizidine and tropane alkaloids, present in cereals distributed in food aid packages, has heightened awareness regarding the lack of regulations around these naturally occurring toxins. Several scientific opinions have been put forward by EFSA[2-6] and maximum residues limits for Europe[7] are under consideration. The issue is of such importance that a joint FAO/WHO expert meeting has been held and a special safety evaluation has been produced.[8]

In anticipation of coming legislation, a sensitive and robust analytical method will be required to meet the increased need for routine testing of plant-based food and commodities. Here, a method has been developed for the detection and quantification of 54 alkaloids in plant-based food matrices using the SCIEX QTRAP 6500+ LC-MS/MS System.[1]

Methods

Chromatography: Chromatographic separation was achieved using an ExionLC™ System with a Phenomenex Luna Omega C18 column with a 1.6 µm particle size. Various mobile phases were tried as detailed by Dzuman et al.[1] A final method with a run-time of 22 minutes was developed to give the most efficient resolution with respect to analysis time.

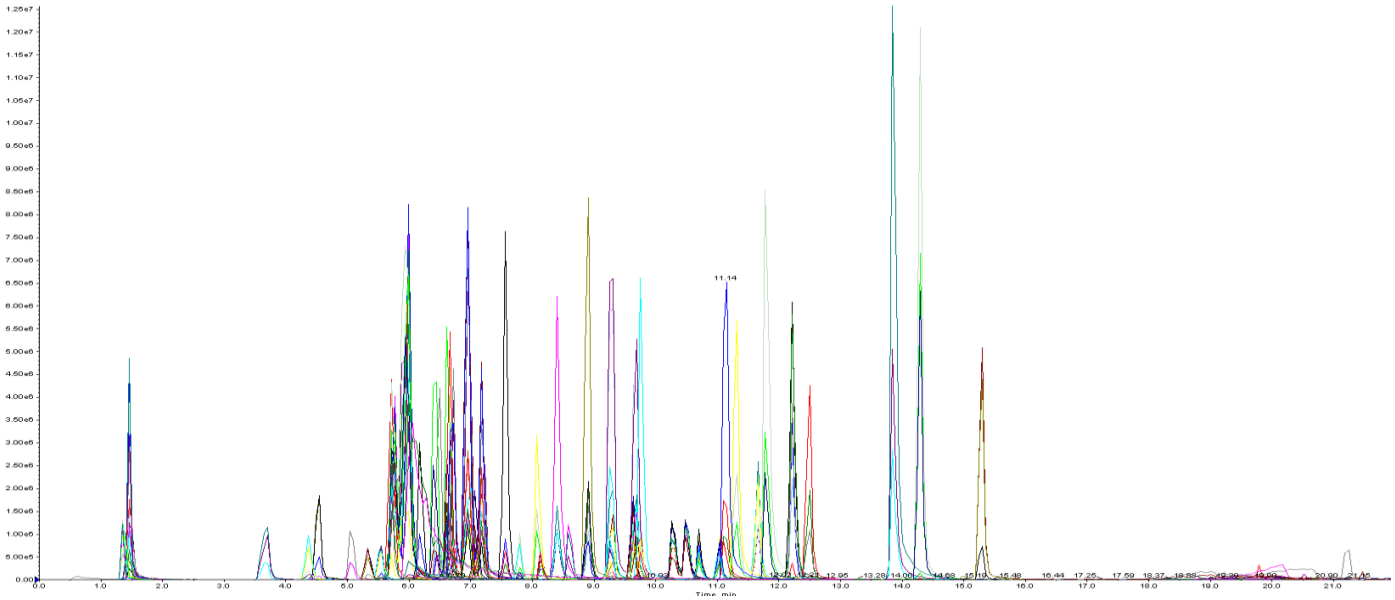

Figure 1. Chromatographic separation of the 54 alkaloids. The XIC overlays shown here for a 10 ng/mL standard solution highlight the distribution of elution that was possible in the method. This helps to achieve accurate quantification of all 54 alkaloid analytes within a 22-minute run time.

Mass spectrometry: MS analysis was performed on the QTRAP 6500+ System using the IonDrive™ Turbo V Ion Source in electrospray ionization mode. All 54 analytes were optimized to determine compound dependent parameters: entrance potential (EP), declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP). The instrument was operated in positive ion mode with source parameters optimized according to the mobile phase selected in each experiment. See reference 1 for full method details.

Separation of isomers

With this method, baseline chromatographic separation was achieved for 49 of the 54 alkaloids (Figure 1). However, even utilizing UHPLC and a slow gradient to maximize separation, reverse phase chromatography is unable to separate five of the compounds due to containing two groups of isomers. This lack of separation has previously been noted in several other publications [9-11], so the upcoming EFSA regulation is expected to allow for certain sums of the most difficult-to-separate isomers to be reported. However, if separation of these compounds is deemed necessary, options such as HILIC chromatography[1] or the use of SelexION® Technology, which can be added to the QTRAP 6500+ System, can aid in isomeric separation and has been covered in references 1 (HILIC) and 12 (SelexION Technology).

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Acknowledgments

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Appendix

Table 1: List of compounds which have been analyzed and verified using this method, along with group information.

Compound name	Group	Compound name	Group	Compound name	Group
6-Beta-hydroxytropinone	*Tropane alkaloids*	*Echimidine*	*Pyrrolizidine alkaloids*	*Echimidine N-oxide*	*Pyrrolizidine alkaloid N-oxides*
Anisodamine		*Echinatine*		*Echinatine N-oxide*	
Anisodine		*Erucifoline*		*Erucifoline N-oxide*	
Apoatropine		*Europine*		*Europine N-oxide*	
Aposcopolamine		*Heliotrine*		*Heliotrine N-oxide*	
Atropine		*Indicine*		*Indicine N-oxide*	
Convolamine		*Intermedine*		*Intermedine N-oxide*	
Convolidine		*Jacobine*		*Jacobine N-oxide*	
Convolvine		*Lasiocarpine*		*Lasiocarpine N-oxide*	
Fillalbine		*Lycopsamine*		*Lycopsamine N-oxide*	
Homatropine		*Monocrotaline*		*Monocrotaline N-oxide*	
Hydroxymethyl-atropine		*Retronecine*		*Retrorsine N-oxide*	
Littorin		*Retrorsine*		*Senecionine N-oxide*	
Noratropine		*Senecionine*		*Seneciphylline N-oxide*	
Nortropinone		*Seneciphylline*		*Senecivernine N-oxide*	
Norscopolamine		*Senecivernine*			
Phenylacetoxytropane		*Senkirkine*			
Pseudotropine		*Trichodesmine*			
Scopolamine					
Tropine					
Tropinone					

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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Biomonitoring of glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), in human urine

Using the QTRAP 6500+ system

I. Beser[1], E. Carbonell[1], P. Ruiz[1], C. Coscollá[1], J. Lliberia[2] and A. Morla[2]
[1]Public Health Laboratory of Valencia, Spain; [2]SCIEX, France

Glyphosate (N-(phosphonomethyl)-glycine, Gly) is an herbicide that was introduced to the market in 1974 and has become the most widely used agricultural herbicide in the world in the last decade. It has been related to several types of cancer[1] and is classified as "probably carcinogenic to humans" (Group 2A) by the International Agency for Research on Cancer (IARC, 2017).[2] Conversely, the European Food Safety Authority (EFSA) claimed that Gly is "unlikely to pose a carcinogenic hazard to humans," establishing an acceptable daily intake (ADI) value of 0.5 mg/kg of bodyweight (BW) per day (mg/kg BW/day) (EFSA, 2015). Another potential source of toxicity is aminomethylphosphonic acid (AMPA). AMPA is a metabolite of Gly and has a similar toxicological profile to Gly. The structural similarities of the compounds involved are shown in Figure 1.

AMPA can be detected in urine primarily due to its consumption in food and water and, to a lesser extent, due to human metabolism of Gly.[3] AMPA accumulates in soil and therefore can be found in the environment.[4] For biomonitoring studies, the parent compound in urine is an appropriate biomarker for Gly exposure. The presence of the Gly metabolite, AMPA, in urine is a biomarker of direct exposure to AMPA and the small fraction of Gly that is bio-transformed to AMPA. In this study, the levels of Gly and AMPA present in the urine of Spanish breastfeeding mothers[5] was assessed.

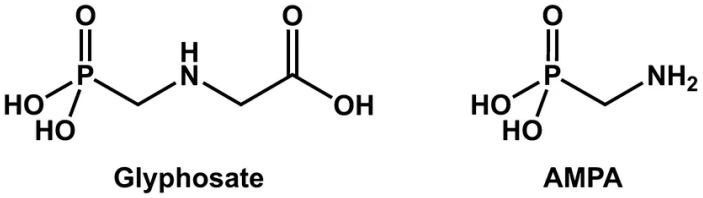

Figure 1: Glyphosate and its metabolite, AMPA.

Key features of the QTRAP 6500+ system

- Simple solid phase extraction to isolate target analytes from human urine
- Eight-minute runtime to analyze the target analytes
- QTRAP 6500+ system for high sensitivity assays in bioanalysis
- IonDrive Turbo V ion source for improved ionization efficiency at high flows and more robustness
- Up to 6 orders of magnitude linear dynamic range
- Detection limits in the low-ng/mL range from human plasma

Methods

Sample preparation: Urine samples (5 mL) were extracted by solid phase extraction (SPE) with SAX cartridges, a process which briefly consisted of these tasks:

- Condition cartridges with methanol and water
- Load samples onto wet cartridges
- Wash with methanol and dry under vacuum
- Elute with strong acid solution
- Evaporate eluates to dryness
- Reconstitute in water with 0.005% formic acid and transfer to vials for injection

Liquid chromatography: Chromatographic separation was accomplished with a Metrosep A Supp 5 (100 mm × 4.0 mm, 5 µm) column equipped with a Metrosep A Supp 5 Guard/4.0 column, at a flow rate of 0.7 mL/min. Mobile phase A was 0.02% formic acid in water and mobile phase B was 100mM ammonium bicarbonate. A 200 µL sample of extract was injected into the UHPLC system.

Mass spectrometry: MS/MS detection was performed using the QTRAP 6500+ system equipped with the IonDrive Turbo V ion source and operated with the electrospray ionization (ESI) interface in negative ionization mode. Multiple reaction monitoring (MRM) mode and QTRAP MS3 mode were employed. Dwell and cycle times were optimized according to the scan modes used. Mass spectrometry parameters are shown in Table 1.

Table 1. Mass spectrometry parameters for each compound

Compound	Q1 Ion	Q3 Ion	DP	CE	CXP
Glyphosate (MRM)	167.9	62.9[a]	-30	-26	-29
Glyphosate (MRM³)	167.9	*150→63.0*[b]	-30	-15	*0.05*[c]
Glyphosate-(13C,15N)	170.0	*63.0*	-30	-22	-29
AMPA	109.9	*62.8*	-35	-26	-29
AMPA-(13C,15N,D2)	*114.0*	*63.0*	-35	-26	-29

[a]Quantitative ion ; [b]Confirming ion ; [c]Excitation energy

Data processing: Data were acquired using Analyst software and processed using SCIEX OS software.

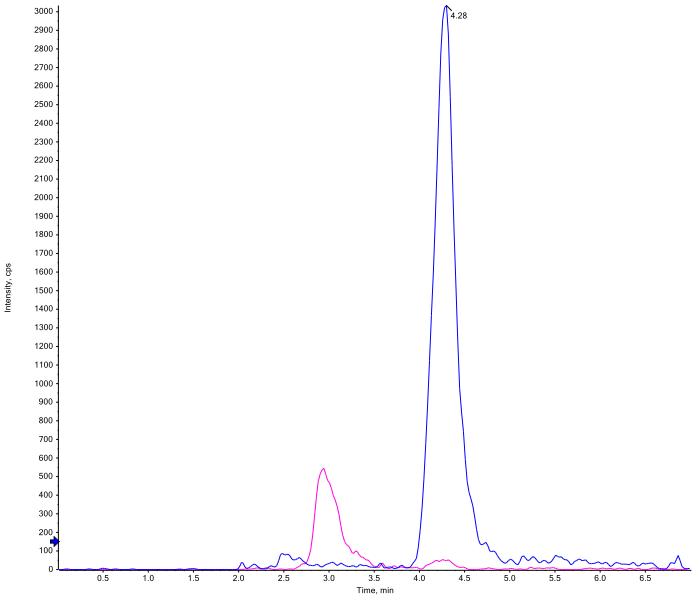

Figure 2: Examples of chromatograms obtained for a quality control sample. The concentration of the measured analytes was found to be 1 µg/L for glyphosate (blue) and AMPA (pink).

Analytical performance

Chromatographic conditions were optimized and good retention of the target analytes was demonstrated (Figure 2). Several identification criteria were implemented for Gly and AMPA. First, the maximum retention time deviation between each analyte and its internal isotope-labeled standards (ILIS) in each sample had to be ±0.1 min. Second, for Gly, at least 2 ion transitions were required for quantification and confirmation purposes, respectively. For the ILIS, only the quantitative ion was required and only 1 transition was achieved for AMPA. Third, the signal-to-noise ratio of the peaks had to be ≥3. Fourth, the analyte peaks of both products' ions had to overlap in the chromatograms. Last, the ion ratio had to be within ±30% of the ion ratio range, which is the average for standards of the same sequence.

We determined the creatinine concentration in urine using Jaffé's reaction method to adjust the Gly and AMPA urinary concentrations. Performance criteria for the proposed method were generated considering SANTE 12682/2019[6] and the Bioanalytical Method Validation Guidance for Industry.[7] The average recovery ranged from 84% to 113% for Gly and from 95% to 117% for AMPA. For both analytes, the RSD was lower than 13%, indicating good precision. The lower limit of quantification (LLOQ) was 0.1 µg/L for both analytes (Table 2).

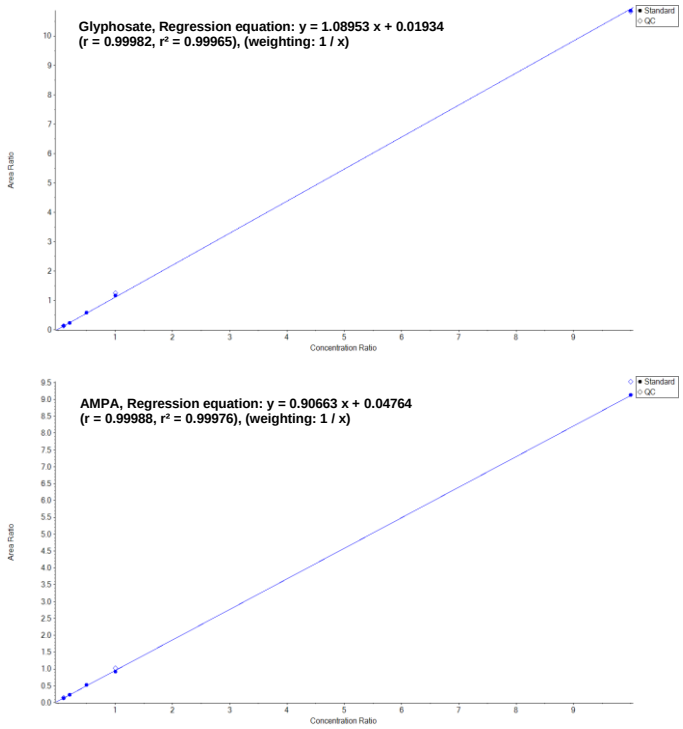

Figure 3. Calibration curve examples for glyphosate and AMPA.

For quantification purposes, a 6-point, matrix-matched calibration curve and ILIS were employed. The linear dynamic range (LDR) was established from 0.1 to 10 µg/L and the accuracy of all calibrators was in the range of ±20% (Figure 3). A series of quality control samples was also run and prepared by spiking blank urine with standard solutions to levels of 0.1, 1 and 10 µg/L. Likewise, a reagent blank (water) and a blank sample were included in each batch to check for process contamination. The method successfully fulfilled the requirements of the German External Quality Assessment Scheme (G-EQUAS) intercomparison program, round 66, in 2020.

Table 2. Performance parameters for the proposed analytical method (n=3).

Compound	R^2	LLOQ (µg/L)	Spiked level (µg/L)	Precision, RSD (%)	Recovery (%)
Glyphosate	0.996-0.999	0.1	0.1 1 10	13 4 1	84-109 105-113 97-99
AMPA	0.997-0.999	0.1	0.1 1 10	8 12 4	95-111 100-117 96-98

LLOQ: Lower limit of quantification; RSD: Relative standard deviation.

Conclusions

This was the first work to assess the levels of Gly and AMPA in urine from and risk of Spanish breastfeeding mothers. In this study, the pesticide Gly and its main metabolite AMPA were analyzed in urine samples of Spanish lactating mothers with good sensitivity and linearity. Ruiz P *et al*. report that glyphosate and AMPA had detection frequencies of 54% and 60%, with means of 0.12 µg/L and 0.14 µg/L, respectively.[5] Their studied population had similar levels of both analytes to those reported in other parts of the world. They detected Gly and AMPA in the urine of different groups within their population, including vulnerable groups, similar to the breastfeeding mothers included in the present study. Given the prevalence of these compounds and their potential effects on the human body, it would be important to include these two compounds in the scope of analyzed substances in human biomonitoring programs.

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Rapid LC-MS/MS method for monitoring bio-relevant levels of per- and polyfluoroalkyl substances (PFAS) in serum

Using Scheduled MRM™ Algorithm on the SCIEX QTRAP® 6500+ LC-MS/MS System

Pierre Negri[1] and Scott Krepich[1]
[1]*SCIEX, Redwood City, CA, USA*

Per- and polyfluoroalkyl substances (PFAS) are pervasive compounds used in a variety of industrial applications and found in a wide range of consumer products such as cookware, stain repellant, flame-retardant and coatings.[1] PFAS are considered environmental factors due to their persistent and bioaccumulating nature. A recent biomonitoring study conducted by the National Health and Nutrition Examination Survey (NHANES) on a nationally representative sample of the U.S. population found that more than 98% of the people tested had multiple congeners of PFAS present in their bodies.[2]

Bioaccumulation of PFAS in the human body resulting from environmental exposure is a growing public health concern. Recent studies have linked PFAS exposure to adverse health outcomes including childhood health complications, reduction in kidney functions, thyroid disease, hormone suppression, decreased fertility, increased cholesterol levels and diabetes, among others.[3,4,5,6] Given the prevalence and ubiquitous nature of PFAS in the environment and every-day consumer products (including our drinking water supply), there is a critical need to develop quantitative tools capable of accurately and precisely detecting low-levels of PFAS in biological fluids to inform the extent of their bioaccumulation and overall impact on the human body. Close biomonitoring of PFAS levels will help evaluate their toxicity and further understand the health consequences associated with their bioaccumulation over time in exposed human populations.

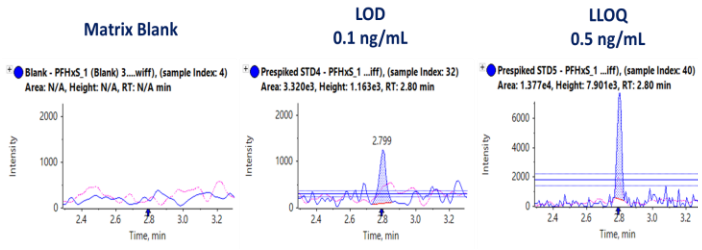

Figure 1: Accurate and sensitive detection of PFAS extracted from serum samples. Representative extracted ion chromatograms (XICs) of the qualifier ion for PFHxS, one of the most persistent PFAS measured at the highest levels in exposed human population. From left to right: in matrix blank, at 0.1 ng/mL (LOD) and 0.5 ng/mL (LLOQ).

In this technical note, a quantitaitive workflow for the analysis of 22 PFAS in serum was developed using the SCIEX QTRAP 6500+ System. This targeted screening workflow provides a fast analytical method capable of accurately quantifying sub-nanogram per mL levels of PFAS in the human body.

Key features of LC-MS/MS method for accurate quantification of PFAS in serum

- Simple sample preparation procedure enables fast and efficient extraction of PFAS from serum samples
- Modifications to the LC system components and consumables were made to reduce the risk of system-related PFAs interferences and minimize the impact of background contamination
- Rapid (6.5 minutes) and high-throughput acquisition method using the Scheduled MRM Algorithm Pro in SCIEX OS Software enables accurate quantification of 22 PFAS at bio-relevant concentrations in serum
- Method demonstrated excellent reproducibility and linearity for PFAS concentrations ranging from 0.5 to 100 ng/mL, with R^2 values greater than 0.99, while maintaining accuracy and precision across the calibration range
- Presented workflow provides a fast and easily implemented solution to accurately measure bioaccumulation levels of PFAS in serum
- Method is adaptable for high-throughut biomonitoring studies aimed at determining the potential toxic effects of PFAS bioaccumulation associated with human exposure

Experimental details

Standards and internal standards: A total of 22 PFAS and 15 mass-labeled internal standards were purchased from Wellington Laboratories (Guelph, ON, Canada) and prepared using Baker's HPLC-grade methanol. A list of all the PFAS included in this panel is summarized in Table 1.

Calibrator preparation: A 1 µg/mL stock standard solution mixture containing the 22 PFAS was prepared by diluting the stock standard solutions with methanol. The resulting 1 µg/mL stock standard solution mixture was used to spike 50 µL of serum in order to prepare a series of 9 calibrator solutions covering concentations ranging from 0.01 to 100 ng/mL. A 1 µg/mL stock internal standard solution mixture containing the mass-labeled internal standards was prepared similarly by diluting the stock standard solutions with methanol. This solution mixture was used to prepared a 5 ng/mL mass-labeled internal standard solution in 0.1 M formic acid. High density polyethylene or polypropylene consumables (Eppendorf tubes, pipette tips, HPLC vials, etc) were used to minimize PFAS contamination from external sources.

Sample preparation: PFAS were extracted from 50 µL serum samples by using a protein precipitation procedure summarized in Figure 2.

Liquid chromatography: UHPLC separation was performed on a Phenomenex Gemini® C18 column (50 x 2 mm, 3 µm, 00B-4439-B0) at 25ºC on a SCIEX ExionLC™ AC System. A Phenomenex Luna C18(2) column (30 x 2 mm, 5 µm, 00A-4252-Y0) was installed between the pump mixing chamber and the analytical column used for separation. This additional column served as a delay column to isolate PFAS contamination leaching from the LC system components and minimize the risk of system-related PFAS interfering with real signals from the sample during the analytical run.

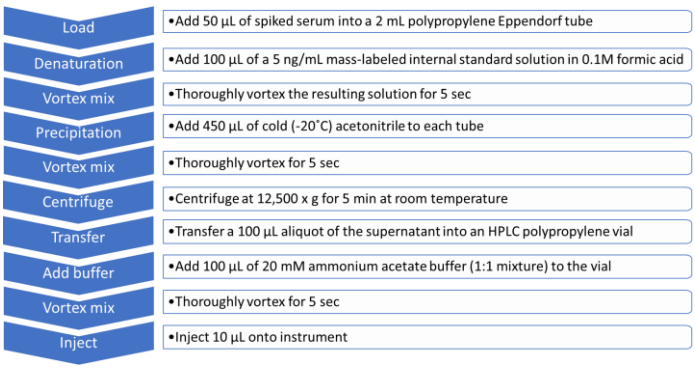

Figure 2. Protein precipitation procedure for serum samples. A 10-step protein precipitation procedure was used for extracting the PFAS from serum samples for MS analysis.

In addition, the fluoroethylene and Teflon tubing on the ExionLC AC System pumps and degasser was replaced with PEEK tubing to minimized the impact of PFAS background contamination and leaching. The FEB and PTFE tubings from the rinse solvent lines, the needle seal, the sample holding loop, pump seal, pump lining and degasser unit were also replaced with PEEK tubing to avoid system-related interferences. Additionally, the PTFE frits and rotor seals were replaced with stainless-steel parts. Mobile phases used for analytical separation were ammonium acetate in water (mobile phase A) and formic acid in methanol (mobile phase B) prepared from HPLC and LC-MS/MS grade solvents. The LC flow rate was 0.6 mL/min and the total run time was 6.5 min. The injection volume was 10 µL. Each extracted sample was injected in triplicate over the course of two consecutive days.

Mass spectrometry: Data were collected using a SCIEX QTRAP 6500+ System with a IonDrive™Turbo V Ion Source, operated with electrospray ionization (ESI) in negative mode. The ion source parameters were optimized for the LC conditions using the compound optimization (FIA) feature in Analyst® Software. A single acquisition method consisting of 57 MRM transitions (42 for the PFAS and 15 for the mass-labeled internal standards) was created in Analyst Software. The Scheduled MRM Algorithm was used to optimize data sampling across each peak and maximize the dwell times used, ensuring reliable integration, quantification and confirmation for each of the PFAS in the panel.

Data analysis: Data processing was performed using SCIEX OS Software. Detection and integration of the peaks from the background was achieved using the MQ4 algorithm. Quantitative analysis was performed in the Analytics module of the software where calibration curves, concentration calculations, assay precision and accuracy statistics were automatically generated.

LC system optimization and use of appropriate laboratory consumables leads to reduction in PFAS contamination

PFAS are pervasive and persistent compounds that have the potential to accumulate and contaminate the LC systems used in analytical testing. As a result, measures were implemented to reduce the risk of outside, ambient and system-related PFAS contamination. The most critical measure was the inclusion of a delay column between the autosampler and LC pumps to trap ambient and system-related PFAS and ensure they will be retained away from the sample signals.

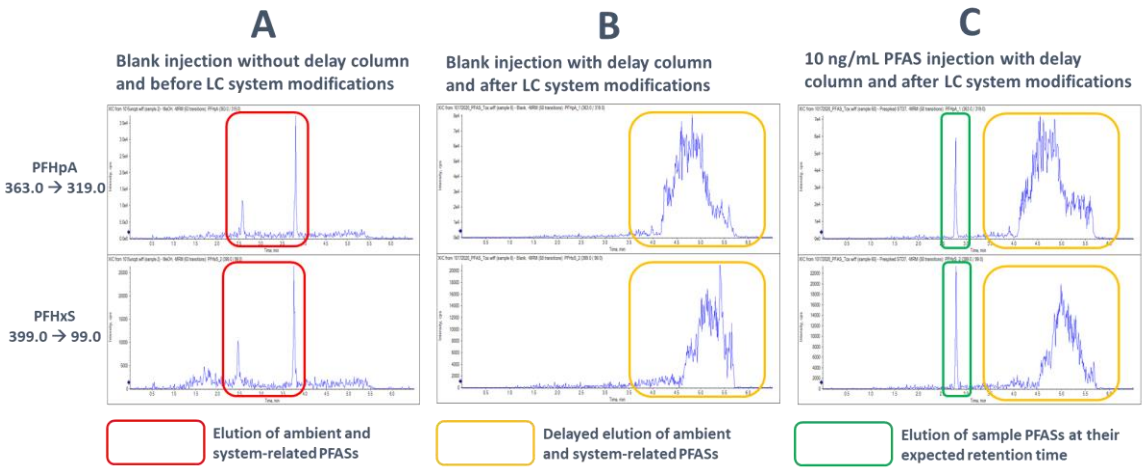

Figure 3. Benefits of using a delay column for PFAS analysis. XICs for PFHpA (top row) and PFHxS (bottom) showing (A) background and system-related contamination signals resulting from a blank sample injection before the system modifications were made showing, (B) broader and delayed contaminant peaks caused by the system-related PFAS that were held up following the addition of the delay column, and (C) sharp peaks resulting from the PFAS in the samples followed by the delayed and broad contamination peaks caused by the delayed column. The modifications made to the LC components significantly reduced the impact of system-related PFAS interferences and enabled accurate quantification of PFAS in serum samples.

Figure 3 summarizes the chromatographic response following the addition of the delay column and shows the extracted ion chromatograms (XICs) for PFHpA (top row) and PFHxS (bottom row) before and after the hardware modifications were made on the LC system.

Figure 3A shows the background signal resulting from a blank sample injection before the system modifications were made. Sharp PFAS peaks resulting from the ambient LC system contamination were observed throughout the chromatograms, including at the retention times at which the sample peaks were expected.

Figure 3B shows the background signal of the same blank sample injection after the system modifications were made (including the addition of the delay column). This configuration eliminated the PFAS interference peaks at the expected analyte retention times and produced a much broader and delayed contaminant peak caused by the system-related PFAS that were held up by the delay column.

Figure 3C shows the XICs resulting from a 10 ng/mL injection of a sample containing all the PFAS in the panel with the system modification. The XICs show a sharp peak resulting from the PFAS in the samples followed by the same broad and delayed contamination peak shown in Figure 3B. The addition of the delay column and the modifications made to the LC system components together minimized the impact of system-related PFAS contamination and ensured the analytical integrity of this quantitative workflow.

Minimizing system-related interference results in accurate measurements of bio-relevant PFAS levels from serum samples

Chromatographic separation of PFAS, including the two compounds that contain both branched and linear isotopes (PFOS and PFHxS), is critical to ensure reliable and accurate quantification. Figure 4 shows the chromatographic profile on an injection of the neat, 10 ng/mL standard solution containing the 22 PFAS. The choice of column, gradient and optimized mobile phase composition resulted in the baseline separation that was needed to correctly distinguish all isomers. As seen in Figure 4, the delayed contamination peaks caused by the delayed column did not interfere with the PFAS sample peaks.

Blank serum samples were spiked with the standard mixture containing the 22 PFAS at concentrations ranging from 0.01 to 100 ng/mL. These standard solutions were extracted using the protein precipitation procedure and injected in triplicate. Data were processed in the Analytics module in SCIEX OS Software 2.0 using the MQ4 Algorithm.

Calibration curves were generated for each of the PFAS in the panel and plotted across 9 calibration levels ranging from 0.1 to 100 ng/mL to evaluate the linearity of the method for the serum-spiked control samples. Figure 5 shows the resulting calibration curves for the native linear perfluoroalkylsulfonates (A) and the native linear perfluoroalkyl carboxylic acids (B). These calibration curves demonstrated excellent linearity covering 3 orders of magnitude with R^2 greater than 0.99 for all of the PFAS in the panel with the exepction of PFODA. The system modifications implemented in this method were critical in attaining linearity achieved in this workflow.

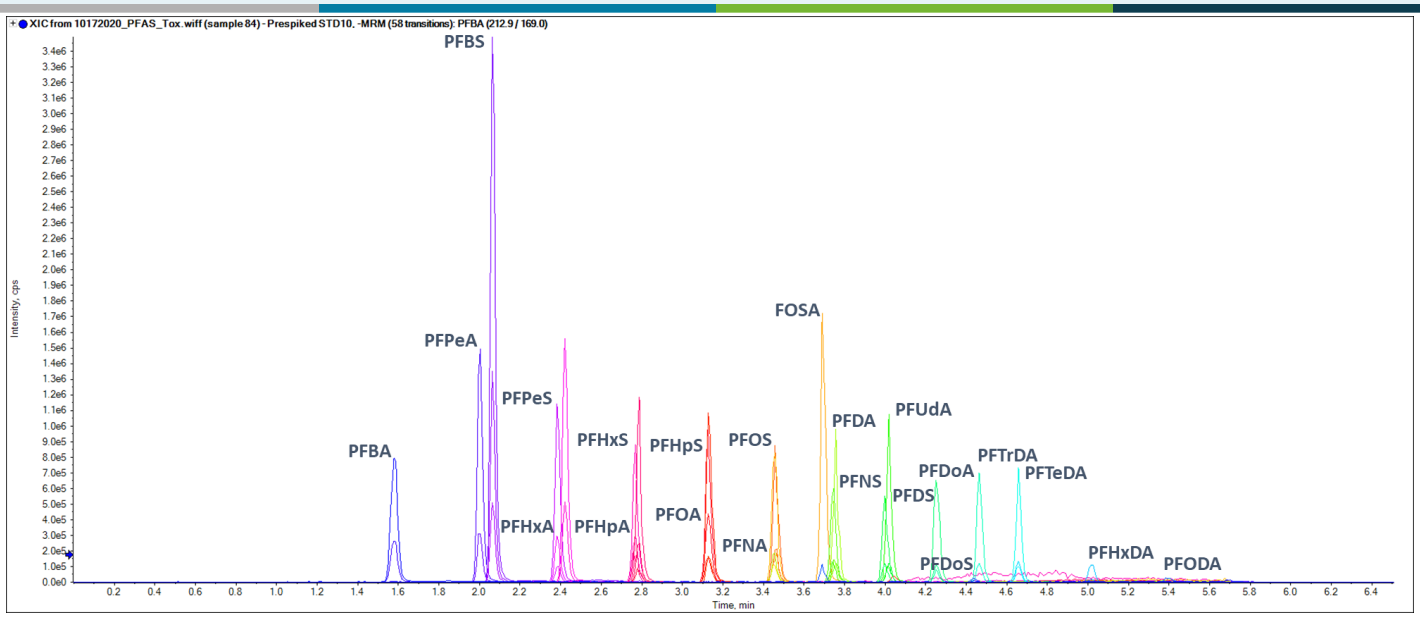

Figure 4. Chromatographic profile of the 22 PFAS monitored in this study. Extracted ion chromatograms (XICs) resulting from the optimized data acquisition method using a 10 ng/mL neat standard mixture. The combination of the optimized mobile phase composition and column choice enabled baseline separation of the PFAS from the injected sample, including branched and linear isotopes such as PFOS and PFHXs.

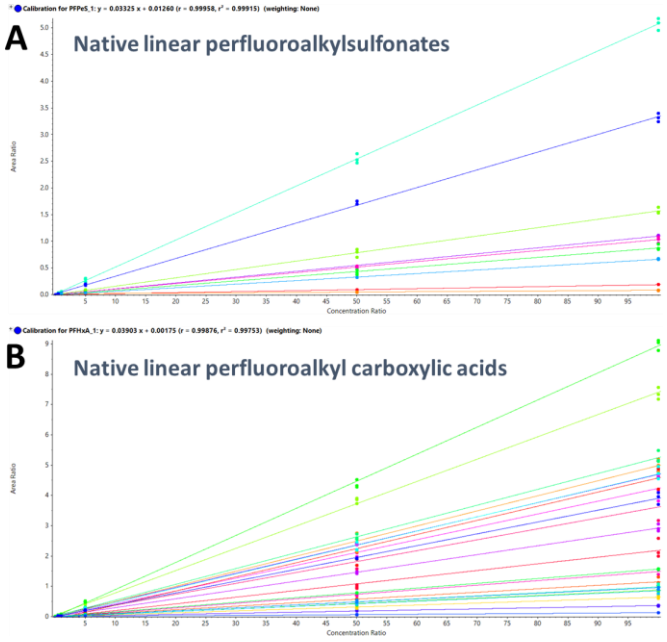

Figure 5. Excellent linearity for the 22 PFAS. Regression curves resulting from the calibration series from 0.5 to 100 ng/mL for A) native linear perfluoroalkylsulfonates and B) native linear perfluoroalkyl carboxylic acid extracted from serum samples. R^2 values greater than 0.99 were observed for all of the PFAS in the panel.

Detection method enables reproducible and accurate quantification of PFAS in serum

The ability to accurately detect low levels of PFAS extracted from serum samples is critical to inform the extent of their accumulation in the human body. The series of calibrators was injected to evaluate the ability to quantify PFAS across a wide range of concentrations. Figure 6 shows representative extracted ion chromatograms (XIC) for the two MRM transitions monitored for PFHxS and PFOA, two PFAS commonly measured in the exposed population. Five levels of calibrators were used to determine the ion ratio criteria for the quantifier and qualifier ions of PFHxS and PFOA. The series of XIC traces for the two compounds showed a high level of consistency and precision, as evidenced by the acceptance criteria (20% or less) of all the ion ratios across the calibration series ranging from 0.5 (LLOQ) to 100 ng/mL. However, the majority of the PFAS showed detectable signal below the LLOQ. Figure 1 shows the XICs for PFHxS for the matrix blank (left), at 0.1 ng/mL (middle) and 0.5 ng/mL (right). The signal at 0.1 ng/mL is the limit of detection (LOD) for PFHxS as it is well above the blank signal. Similar peaks were observed below the LLOQ for the PFAS in the panel. In addition, the assay showed great reproducibility over the course of the two consecutive days the data were acquired (inter-day peak area variations of 5% or less were observed for the 22 PFAS across the calibration range).

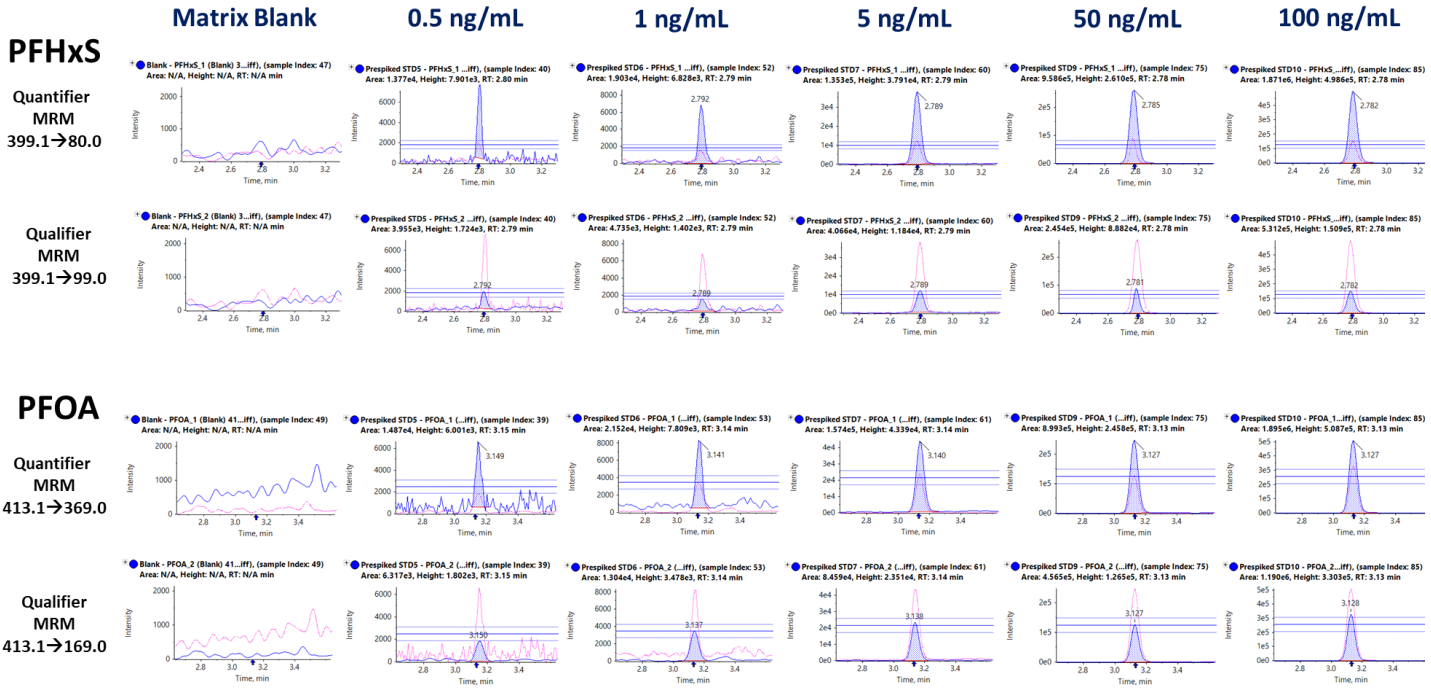

Figure 6. Accurate quantification of bioaccumulating PFAS in serum. Extracted ion chromatograms (XICs) for A) PFHxS and B) PFOA, two of the most persistent and common PFAS measured in the exposed human population. The XICs display overlays of both the quantifier and qualifier ions from 0.5 to 100 ng/mL as well as the tolerance in the form of the ion ratio line overlay that help in visualizing the confidence levels for the ion ratios. All ion ratios passed the ion ratio acceptance criteria (20% or less) across the calibration range (0.5 to 100 ng/mL).

The lower limit of quantification (LLOQ) for each of the PFAS included in the panel was determined based on the lowest concentration at which the integrated peak area of the analyte was quantifiable, with calculated concentration accuracy between 80 and 120%, precision (%bias) below 20%, ion ratio acceptance criteria of less than 20% and maintaining a linear calibration curve with an R^2 value of at least 0.99.

Table 1 summarizes the quantitative performance of the workflow and includes the calibration range, linear correlation coefficient (R^2 value), LLOQ, as well as the accuracy and precision values at the LLOQ for each of the 22 PFAS monitored in this workflow. PFBS and PFODA are the only two PFAS in the panel that proved challenging to quantify accurately due to their ubiquitous presence in the analytical system. This was evidenced by their detection in blank and solvent injections and as a result, their pervasiveness impacted their detection limits in this workflow. PFBS was quantified accurately from 5 to 100 ng/mL however PFODA could not be quantified.

The assay as a whole demonstrated excellent reproducibility, linearity, precision and accuracy for all the other PFAS in the panel. This method achieved the required levels of robustness and qualitative performance necessary to accurately measure sub ng/mL levels of PFAS from serum samples.

Conclusions

A robust and sensitive workflow for the detection of PFAS in serum samples using the SCIEX QTRAP 6500+ System was successfully developed. The addition of a delay column and the modifications made to the LC system components reduced the risk of system-related PFA interferences. The combination of a simple sample preparation procedure with a fast LC separation enabled accurate and sensitive detection of 22 PFAS down to sub ng/mL levels. The assay showed excellent reproducibility, precision, accuracy and linearity, with an LLOQ of 0.5 ng/mL, LOD of 0.1 ng/mL and an R^2 of greater than 0.99 for the vast majority of the PFAS in the panel with the exception of PFBS and PFODA. The excellent precision and accuracy observed at the LLOQ is highlighted in Table 1.

Overall, the developed method provides a robust and accurate method for bio-monitoring of low-levels of PFAS in biological fluids. Therefore, the presented workflow is readily adaptable for high-throughput toxicology investigations aimed at determining the extent of PFAS bio-accumulation and its broader impact on human health.

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Table 1. Statistical results for the 22 PFAS monitored in this workflow. The table includes calibration range, linear correlation coefficient (R^2 Value), and LLOQ, as well as the accuracy and precision at the LLOQ for each of the PFAS MRM transitions monitored.

Compound	Calibration Range (ng/mL)	Linear Correlation (R^2)	LLOQ (ng/mL)	Accuracy at LLOQ (%)	Precision at LLOQ (%)
PFBS 1	*0.5-100*	*0.99876*	*0.5*	*101.70*	*10.16*
PFPeA 1	*0.1-100*	*0.99896*	*0.1*	*99.41*	*12.21*
PFBS 1	*5-100*	*0.99647*	*5*	*99.55*	*5.51*
PFBS 2	*5-100*	*0.99689*	*5*	*101.50*	*3.68*
PFHxA 1	*0.5-100*	*0.99753*	*0.5*	*109.93*	*19.61*
PFHxA 2	*0.5-100*	*0.99791*	*0.5*	*102.34*	*11.19*
PFPeS 1	*0.5-100*	*0.99793*	*0.5*	*90.54*	*8.97*
PFPeS 2	*0.5-100*	*0.99846*	*0.5*	*106.62*	*6.88*
PFHpA 1	*0.5-100*	*0.99331*	*0.5*	*114.43*	*6.60*
PFHpA 2	*0.5-100*	*0.99685*	*0.5*	*111.71*	*6.76*
PFHxS 1	*0.5-100*	*0.99780*	*0.5*	*84.15*	*5.96*
PFHxS 2	*0.5-100*	*0.99741*	*0.5*	*80.98*	*1.33*
PFOA 1	*0.5-200*	*0.99885*	*0.5*	*96.41*	*10.31*
PFOA 2	*0.5-200*	*0.99570*	*0.5*	*104.74*	*3.12*
PFHpS 1	*0.5-100*	*0.99359*	*0.5*	*116.49*	*1.59*
PFHpS 2	*0.5-100*	*0.99386*	*0.5*	*115.21*	*3.58*
PFNA 1	*0.5-100*	*0.99151*	*0.5*	*110.17*	*0.88*
PFNA 2	*0.5-100*	*0.99237*	*0.5*	*99.73*	*9.95*
FOSA 1	*0.5-100*	*0.99702*	*0.5*	*89.92*	*1.35*
PFOS 1	*0.5-100*	*0.99294*	*0.5*	*104.67*	*16.54*
PFOS 2	*0.5-100*	*0.99932*	*0.5*	*108.33*	*14.24*
PFDA 1	*0.5-100*	*0.99557*	*0.5*	*100.17*	*7.09*
PFDA 2	*0.5-100*	*0.99002*	*0.5*	*91.69*	*2.00*
PFNS 1	*0.5-100*	*0.99214*	*0.5*	*101.09*	*11.33*
PFNS 2	*0.5-100*	*0.99721*	*0.5*	*99.06*	*15.41*
PFUdA 1	*0.5-100*	*0.99835*	*0.5*	*94.08*	*9.71*
PFUdA 2	*0.5-100*	*0.99524*	*0.5*	*107.37*	*3.33*
PFDS 1	*0.5-100*	*0.99477*	*0.5*	*94.23*	*1.89*
PFDS 2	*0.5-100*	*0.99651*	*0.5*	*111.42*	*0.23*
PFDoA 1	*0.5-100*	*0.99634*	*0.5*	*107.00*	*3.59*
PFDoA 2	*0.5-100*	*0.99950*	*0.5*	*99.49*	*16.56*
PFTrDA 1	*0.5-100*	*0.99732*	*0.5*	*93.24*	*3.47*
PFTrDA 1	*0.5-100*	*0.99495*	*0.5*	*102.89*	*16.07*

Table 1. Statistical results for the 22 PFAS monitored in this workflow. Continued.

Compound	Calibration Range (ng/mL)	Linear Correlation (R^2)	LLOQ (ng/mL)	Accuracy at LLOQ (%)	Precision at LLOQ (%)
PFTeDA 1	*0.5-100*	*0.99761*	*0.5*	*96.96*	*19.47*
PFTeDA 2	*1-100*	*0.99443*	*1*	*90.17*	*11.51*
PFHxDA 1	*0.5-100*	*0.99465*	*0.5*	*111.05*	*2.38*
PFHxDA 2	*10-100*	*0.99481*	*1*	*95.32*	*19.95*
PFODA 1	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
PFODA 2	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
PFDoS 1	*1-100*	*0.99263*	*1*	*96.55*	*7.40*
PFDoS 2	*5-100*	*0.99680*	*5*	*100.08*	*16.05*

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Fast screening method for 86 forensic analytes in human urine

Using QTRAP® LC-MS/MS System

When a urine specimen is acquired for clinical analysis it is put through a presumptive, or screening, test prior to confirmation analysis. Traditionally enzyme linked immunosorbent assay (ELISA) has been used for screening to identify the presence of a compound class in a given sample. However, multiple ELISA kits are often required for a comprehensive panel increasing the cost of analysis. The difficulty of producing new antibodies for each assay also makes it a challenge for immunoassays to rapidly adapt to include new analytes.

While the ability of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to simultaneously analyze across multiple compound classes has made it the gold standard for confirmation analysis, it offers many benefits to screening as well. This screening analysis utilizes multiple reaction monitoring (MRM) scans as well as an Enhanced MS (EMS) scan.

For each MRM scan, a specified precursor ion is selected in Q1, which is fragmented to produce a unique product ion that is monitored in Q3; therefore, yielding sensitive and specific data for the analytes of interest. When the *Scheduled* MRM™ Algorithm is then used optimal cycle and dwell times can be established to produce high quality data in a short overall run time.

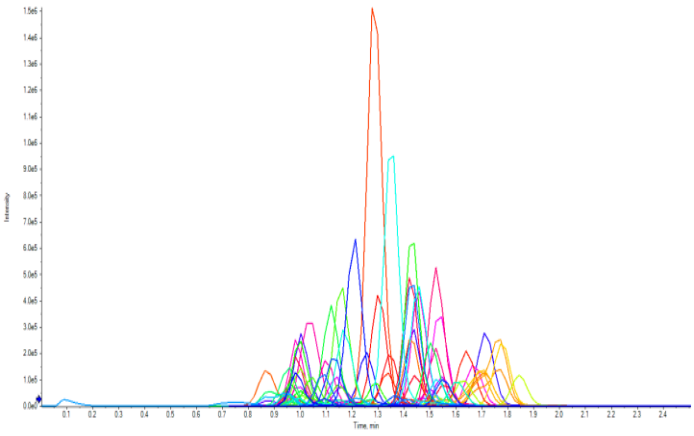

Figure 1: Elution profile of all the 86 compounds in screening panel.

For this project, a rapid screening method of 86 compounds in human urine was developed using a SCIEX QTRAP System to collect both MRM and EMS data within a single injection. With it's simple to perform sample preparation and fast acquisition time (2.5 min), this method is optimal for forensic laboratory screening.

Key features of QTRAP System technology and Scheduled MRM™ Algorithm

- Rapid, robust approach allows direct detection of 86 forensic analytes in human urine with simple sample preparation and fast chromatography
- Targeted MRM workflow allows sensitive detection with high selectivity, reduced matrix effects and higher confidence in results
- Additional QTRAP System allows for enhanced scan functions that further improve confidence in results, enabling spectral identification and library searching.
- Scheduled MRM™ Algorithm enables the use of fast chromatography without loss of data integrity, allowing for faster throughput and turnaround times.

Methods

Compound list and spiking solutions: Table 1 lists the 86 compounds plus 6 internal standards in the panel. Two spiking solutions in methanol were prepared: one for analytes (SA) and the other for internal standards (SIS).

Calibrator and Control preparation: Blank human urine was spiked with solution SA to prepare calibrators. A single calibrator and three quality controls (QCs) were prepared. Actual concentrations varied for each compound; however, the calibrator was prepared at the cutoff (CO) and three QCs at 50% CO, 3xCO, and 10xCO.

Sample preparation: 100 µL urine sample was mixed with 25 µL IMCS Rapid Hydrolysis Buffer, 20 µL IMCSzyme and 10 µL SIS. Hydrolysis time was typically between 30 and 60 min at 55°C.After hydrolysis was complete, 0.8 mL of diluent was added to the mixture. The mixture was then centrifuged at 21,000 g for 15 min. The supernatant was transferred to a glass vial with insert for analysis by LC-MS/MS.

Liquid Chromatography: Chromatographic analysis was performed on a SCIEX HPLC system. Separation was achieved using a Phenomenex Luna C18 column. Mobile phase A (MPA) was ammonium formate in water. Mobile phase B (MPB) was formic acid in methanol. The LC flow rate was varied throughout the run from 0.2 mL/min to 1 mL/min to allow for necessary equilibration of the column in the 2.5 min LC run-time. The injection volume used was 5 µL.

Mass Spectrometry: The system was run using ESI positive mode. Individual voltages were optimized for each individual component. Scheduled MRM Algorithm was optimized to ensure best data quality. Enhanced MS Scan, where used, was run over a 100-600Da window.

Table 1: List of compounds and internal standards analyzed.

Compounds	Compounds	Compounds	Compounds	Internal Standards
6-MAM	*Desipramine*	*MDA*	*Norfentanyl*	*Amphetamine-d5*
7-Aminoclonazepam	*Desmethyldoxepin*	*MDEA*	*Norhydrocodone*	*Fentanyl-d5*
7-Hydroxymitragynine	*Dextromethorphan*	*MDMA*	*Normeperidine*	*JWH 018 4-OH pentyl-d5*
Acetyl Fentanyl	*Diazepam*	*MDPV*	*Noroxycodone*	*Methadone-d3*
Alpha-Hydroxyalprazolam	*Dihydrocodeine*	*Meperidine*	*Norpropoxyphene*	*Methamphetamine-d5*
Alpha-Hydroxymidazolam	*Doxepin*	*Mephedrone*	*Nortriptyline*	*Morphine-d6*
Alpha-Hydroxytriazolam	*EDDP*	*Meprobamate*	*O-Desmethyltramadol*	
Alpha-PPP	*Fentanyl*	*Methadone*	*Oxazepam*	
Alpha-PVP	*Gabapentin*	*Methamphetamine*	*Oxycodone*	
Alprazolam	*Hydrocodone*	*Methedrone*	*Oxymorphone*	
AM-2201 4-OH pentyl	*Hydromorphone*	*Methylone*	*PCP*	
Amitriptyline	*Imipramine*	*Methylphenidate*	*Pregabalin*	
Amphetamine	*JWH-018 4-OH pentyl*	*Midazolam*	*Propoxyphene*	
Benzoylecgonine	*JWH-018 pentanoic acid*	*Mitragynine*	*Protriptyline*	
Buphedrone	*JWH-019 6-OH hexyl*	*Morphine*	*RCS4-4-OH-pentyl*	
Buprenorphine	*JWH-073 3-OH butyl*	*Naloxone*	*Ritalinic Acid*	
Carisoprodol	*JWH-073 butanoic acid*	*Naltrexone*	*Sufentanil*	
Clomipramine	*JWH-081 5-OH pentyl*	*N-desmethyltapentadol*	*Tapentadol*	
Codeine	*JWH-122 5-OH pentyl*	*Norbuprenorphine*	*Temazepam*	
Cotinine	*JWH-210 5-OH pentyl*	*Norcodeine*	*Tramadol*	
Cyclobenzaprine	*JWH-250 4-OH pentyl*	*Nordiazepam*	*Zolpidem*	
Desalkylflurazepam	*Lorazepam*			

Sample and calibrator preparation

IMCSzyme was selected as the hydrolysis enzyme for this study. Using this enzyme, the hydrolysis of glucuronide-conjugated analytes was completed in a shorter time frame compared to other traditional beta-glucuronidase enzymes. Furthermore, less interferences were observed when using IMCSzyme, which proved beneficial for LC column life and MS maintenance.

Human urine was established as the necessary matrix for the calibrator and all controls in this method. Due to the rapid nature of the gradient, interferences present in human urine more closely match that of unknown samples. The method was tested using both human urine and purchased synthetic urine; however, spiked samples in synthetic urine often fell outside of the expected range when compared against data points collected using human urine.

QTRAP System data types used

Scheduled MRM Algorithm: An LC gradient was designed to ensure that the retention times of the various compounds were evenly distributed throughout the gradient to reduce the MRM concurrency. This enabled maximum sampling of every LC peak, while maintaining optimal dwell times for each MRM transition. The Scheduled MRM Algorithm ensured optimal data quality even during regions of the chromatography where the MRM concurrency was very high. A minimum of 5 data points was achieved across every peak, providing the necessary peak definition for a screening method. A typical chromatogram is shown in Figure 1.

Enhanced MS Scan: Enhanced MS Scan was run over a 100-600Da window and was collected to assist in the identification of an unknown peak. Figure 2 shows the total ion chromatogram (TIC) of EMS of the entire 2.5-min run, a 100 Da window extracted ion chromatogram (XIC) around 240 with EMS, and the EMS mass spectrum at 1.15-min. In order to further identify the analyte of interested it would be necessary to run a secondary experiment in which an Enhanced Product Ion (EPI) spectrum is collected for comparison with a library.

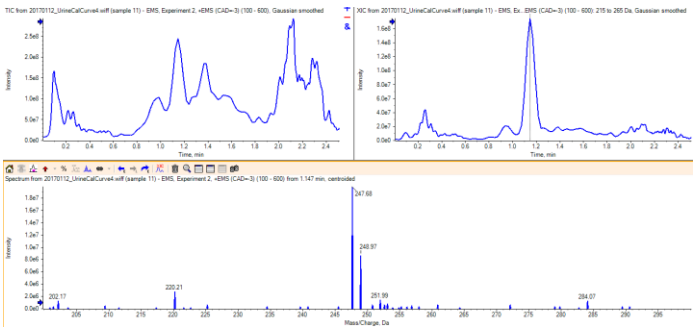

Figure 2: Enhanced MS data. (Clockwise from top left) Total ion chromatogram (TIC) of EMS of the entire 2.5-min run, 100-Da window extracted ion chromatogram (XIC) around 240 Da with EMS, EMS mass spectrum at 1.15-min.

Analytical sensitivity

A single-point calibration was used for all 86 analytes, which yielded accurate results for low, medium, and high QCs. Figure 3 shows a typical calibration curves for alpha-PVP with excellent linearity fitted through zero. The XICs of the quality controls for alpha-PVP are shown over three replicate sets of injections (Figure 3, bottom).

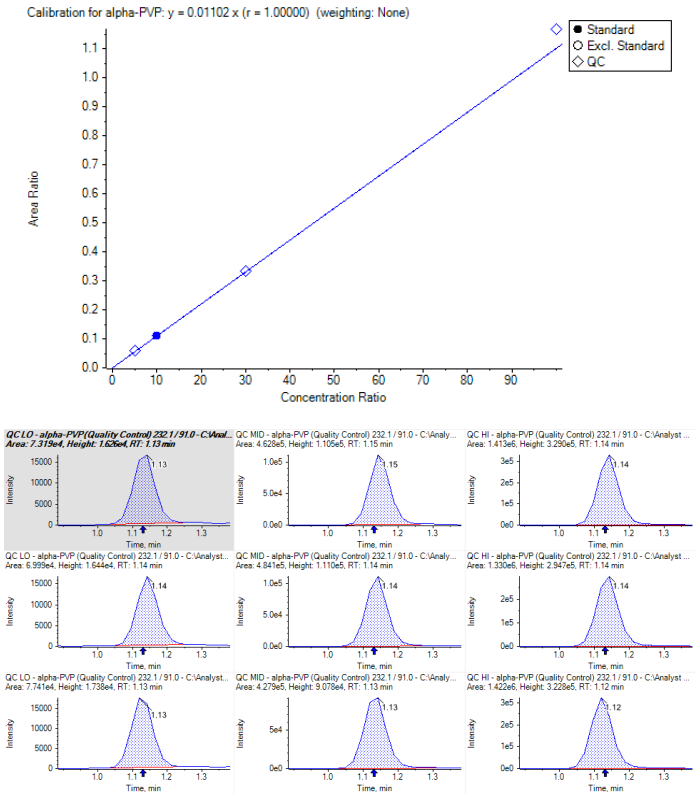

Figure 3: Quantitative data for alpha-PVP. (Top) Typical calibration curve of alpha-PVP plotted with quality controls. (Bottom) Extracted ion chromatograms (XICs) of alpha-PVP (n=3, quality controls. (A) 5 ng/mL; (B) 30 ng/mL; (C) 100 ng/mL.

Conclusions

This method provides a fast screening technique for 86 forensic analytes making use of both the Scheduled MRM Algorithm and Enhanced MS scan in a single injection. The SCIEX QTRAP System technology produces high quality data for all analytes, while utilizing samples prepared by rapid enzyme hydrolysis and a simple dilute and shoot pretreatment.

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LC-MS/MS法测定人血浆中有机磷酸酯类化合物

Determination of OPEs in human plasma with LC-MS/MS system

刘丹，黄超，李国庆

Liu Dan, Huang Chao, Li Guoqing

SCIEX应用支持中心，中国

SCIEX, China

前言

有机磷阻燃剂（Organophosphateflameretardants，OPFRs）是一类被广泛添加到塑料制品、建筑材料、电子产品、家具和纺织用品等中作为阻燃剂、增塑剂和防沫剂的化合物。近年来，随着传统溴代阻燃剂的逐步禁用，有机磷阻燃剂因其资源丰富、阻燃效果显著，正成为其主要替代品，在全球范围内得到快速发展，应用量逐年递增。

本研究所指OPFRs主要为有机磷酸酯（Organophosphates，OPEs）。人体可通过皮肤、呼吸道和经口等途经吸入或摄入OPEs。大量的研究证实了OPEs有潜在的神经毒性，会造成生殖紊乱、内分泌干扰甚至致癌作用，被列为持久/高迁移性有机化合物，引起了人们对其潜在危害的担忧。

就OPEs的人群暴露而言，目前国内外采用的生物标志物分为两种。第一种是胆碱酯酶，但此类生物标志物并非特异性标志物，可以被其他有机磷类化合物，如有机磷农药等抑制。第二种是有机磷酸酯母体和其代谢产物，此类标志物性质稳定易于测定，结构如图1。

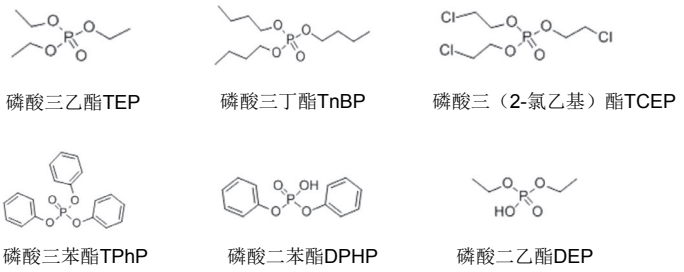

图 1.有机磷酸酯阻燃剂及其代谢物的结构图

因此，本研究建立了一种高灵敏度、简便快捷的有机磷酸酯类阻燃剂及其代谢产物的检测方法，用于测定血浆中有机磷酸酯暴露情况。

1 实验部分

1.1 血浆样品前处理

样品采集：使用EDTA抗凝真空管采集患者静脉血。尽快（2 h内）离心分离血浆。

样品处理：取400 µL血浆样品于2 mL EP管中，加入混合内标，加入甲酸，涡旋混匀之后，加入有机试剂进行液液萃取，震荡混匀5 min，离心5 min(10000 rpm)。取全部上层有机相转移至另外新的EP管中，置于真空冷冻干燥机中挥干。用30%甲醇水复溶，涡旋混匀后进样分析。

1.2 色谱条件

色谱柱为Kinetex F5；柱温设定为40℃；进样量为5 µL。洗脱梯度表见表1。

表1. 洗脱梯度

时间（min）	流速(mL/min)	A(%)	B(%)
0.5	0.3	80	20
1.0	0.3	30	70
3.0	0.3	10	90
4.5	0.3	0	100
4.6	0.3	80	20
5.5	0.3	80	20

1.3 质谱条件

采用电喷雾离子源（Electrospray Ionization, ESI）和多反应监测（Multiple Reaction Monitoring, MRM）模式进行质谱扫描。离子源参数：加热气（GS1）和辅助加热气（GS2）分别为40 psi和40 psi，脱溶剂气温度为 600℃；气帘气（ Curtain Gas, CUR ）为 30 psi，碰撞气（Collision Gas, CAD）为9 psi；喷雾针（Ionspray, IS）电压为+5500/-4500 V。为了获取较好的稳定性和灵敏度，各化合物监测离子对的去簇电压（Declustering Potential, DP）和碰撞电压（Collision Energy, CE），目标物定量离子对、定性离子对以及内标物监测离子对等参数均经过系统优化，离子对信息见表2。

表2. 待测组分和内标物质的质谱参数

化合物	Q1	Q3	ID	DP	CE
磷酸三乙酯	183	99.1	TEP-1	58	27
	183	155	TEP-2	56	13
磷酸三（2-氯乙基）酯	285.1	63.1	TCEP-1	72	41
	285.1	99	TCEP-2	72	31
磷酸三苯酯	327.1	77.1	TPHP-1	118	60
	327.1	152.1	TPHP-2	120	55
磷酸三丁酯	267.1	99.1	TNBP-1	67	27
	267.1	155	TNBP-2	66	15
磷酸二苯酯	249	78.9	DPHP-1	-97	-60
	249	92.9	DPHP-2	-81	-47
磷酸二乙酯	153.1	63.1	DEP-1	-53	-86
	153.1	124.8	DEP-2	-53	-15

2 结果与讨论

2.1 回归方程及线性

在本实验条件下，几种有机磷酸酯阻燃剂及其代谢物在0.05~20 ng/mL（DPHP: 0.01~4 ng/mL）范围内线性良好，r值大于0.995如表3。

表3. 有机磷酸酯及其代谢物的线性回归方程、相关系数和定量下限

目标化合物	线性回归	相关系数	LLOQ（ng/mL）
TEP	Y=0.5323χ+0.3683	r=0.995	0.05
TCEP	Y=0.9961χ-0.1314	r=0.998	0.05
TPHP	Y=0.9539χ-0.1091	r=0.997	0.05
TnBP	Y=0.3053χ+0.0380	r=0.996	0.05
DPHP	Y=1.2417e5χ+4129.38	r=0.995	0.01
DEP	Y=4.3797e4χ+1942.04	r=0.995	0.05

2.2 色谱图

有机磷酸酯阻燃剂及其代谢物的色谱图如图2，峰形和灵敏度良好。

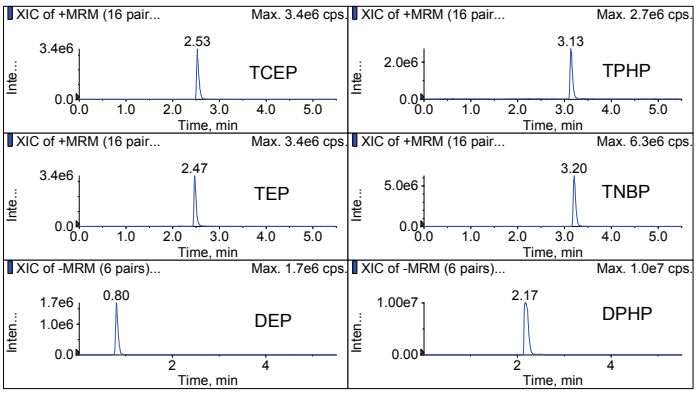

图 2.有机磷酸酯及其代谢物的色谱图

2.3 性能验证参数

采用低、高两个浓度水平的质控品，每个浓度平行测定3次，得到日内精密度RSD在1.56%~13.6%范围内。低、高两个浓度水平质控品，每个浓度平行测定3次，连续测定3天，得到日间精密度RSD在3.88%~13.51%之间。（见表4）。

表4. 有机磷酸酯及其代谢物的批内和批间精密度（%）

目标化合物	日内精密度RSD%		日间精密度RSD%	
	低浓度	高浓度	低浓度	高浓度
TEP	3.97	6.79	9.97	13.51
TCEP	4.2	1.56	4.31	3.88
TPHP	2.45	3.72	4.62	4.64
TnBP	4.13	2.82	10.19	8.60
DPHP	13.6	6.12	8.65	7.5
DEP	7.87	3.81	11.00	5.83

总结

本方法基于SCIEX 质谱平台，建立了可对有机磷阻燃剂进行定性定量检测的液相色谱串联质谱方法。所建方法能够很好的排除内源性干扰，且灵敏度满足要求，方法稳定性好准确性高，能满足实际检测需求。适用于快速高效的对人血浆当中有机磷酸三酯和有机磷酸二酯化合物进行生物检测。

仅限专业展会等使用、仅向专业人士提供的内部资料

SCIEX

SCIEX中国

北京分公司
北京市朝阳区酒仙桥中路24号院
1号楼5层
电话：010-5808-1388
传真：010-5808-1390

全国咨询电话：800-820-3488, 400-821-3897

上海公司及中国区应用支持中心
上海市长宁区福泉北路518号
1座502室
电话：021-2419-7200
传真：021-2419-7333

官网：sciex.com.cn

广州分公司
广州市天河区珠江西路15号
珠江城1907室
电话：020-8510-0200
传真：020-3876-0835

官方微信：SCIEX-China

PFAS identification in serum and plasma using data-independent acquisition and MS/MS library matching

SWATH acquisition using the SCIEX X500 series QTOF system and the SCIEX Fluorochemical HR-MS/MS library 2.0

Craig M. Butt[1], Casey Burrows[1], Frederick G. Strathmann[2] and Joseph W. Homan[2]
[1]SCIEX, USA, [2]NMS Labs, PA, USA

Per- and poly-fluorinated alkyl substances (PFAS) are used extensively in consumer and industrial products.[1] Although they have been highly beneficial to society, it is now widely recognized that health effects such as immunosuppression and serum lipid alteration are linked to continued exposure and accumulation of PFAS in the body.[2]

Evidence for organic fluorine compounds in human serum was first discovered over 50 years ago,[3] however the LC-MS/MS techniques necessary to identify them were not widely available until the early 2000s. Targeted LC-MS/MS strategies for PFAS detection provide high confidence and accuracy, but only detect a limited subset of compounds. In contrast, non-targeted strategies using high resolution accurate mass instruments provide greater coverage and can be used to identify unknown compounds. For example, MS/MS spectra can be matched to library databases for identification.

In this technical note, the SCIEX X500 series QTOF system was used to analyze PFAS in human serum and plasma. An unbiased and non-targeted workflow with automatic library matching allowed for wide analyte coverage. Additionally, a streamlined sample preparation strategy provided greater laboratory efficiency, while maintaining sample integrity.

Key features of SWATH acquisition for PFAS identification

- High resolution accurate mass SWATH acquisition data provide multiple levels of evidence for identification and compound confirmation (i.e. mass error, isotope pattern, MS/MS library matching)
- High resolution PFAS MS/MS library covers negatively charged, positively charged and zwitterionic compound classes and allows users to add custom compounds
- SCIEX OS allows the user to add MS/MS spectra from authentic standards or sample data for those compounds not yet in the library such as novel PFAS compounds

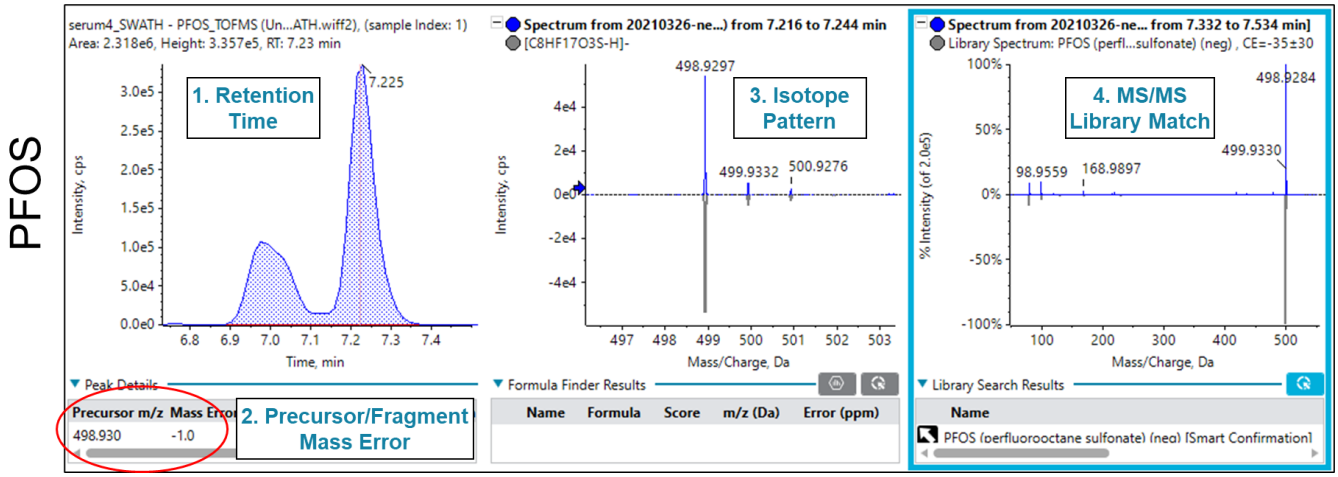

Figure 1. Multiple features for compound identification. High resolution accurate mass LC-MS/MS data from the X500 series QTOF system using SWATH acquisition provides multiple types of data for identification and confirmation of compounds. Data are shown for perfluorooctane sulfonate (PFOS), including (1) retention time, (2) precursor ion and fragment ion mass error, (3) isotope pattern and (4) MS/MS library match.

Methods

Sample preparation: Five pooled samples were prepared from discarded serum and/or plasma specimens. Samples (0.5 mL) were denatured using 2 mL of acetonitrile with 1% formic acid and then centrifuged. The supernatant was passed through a Captiva EMR-lipid cartridge (300 mg), the eluent evaporated under a nitrogen stream and the sample reconstituted in 200 µL of 1:1 water: methanol (1% formic acid).

Analytical standards: The EPA 533 PFAS analyte list was used to build the initial suspect screening list. Analytical standards were purchased from Wellington Laboratories (Guelph, ON, Canada) and analyzed to confirm retention times.

Chromatography: Chromatography was performed using an ExionLC system. Analytes were separated using a Phenomenex Gemini C18 column (110 Å, 100 x 3 mm, 3 µm particle size) using gradient conditions and a flow rate of 0.5 mL/min. A delay column was used to separate PFAS compounds originating from the LC. The mobile phases were water (A) and methanol (B), both with 10 mM ammonium acetate. The column oven was 40˚C and the injection volume was 10 µL. Initial conditions were 20% B, held for 1 min and then increased to 99% B over 3.5 min.

Mass spectrometry: Analysis was performed on the X500 series QTOF system with the Turbo V ion source using electrospray ionization (ESI) in negative ion mode. Data were collected using SWATH acquisition mode with a TOF MS experiment followed by 16 TOF MS/MS experiments using Q1 windows spanning 100 to 1000 Da. The TOF MS and each MS/MS experiment used an accumulation time of 50 ms, for a total cycle time of 0.85 s. Table 1 lists the variable Q1 windows used for SWATH acquisition and other related parameters. SWATH acquisition windows were carefully chosen to avoid overlap with co-eluting analytes, for example perfluorononanoic acid (PFNA) and perfluorooctane sulfonate (PFOS). The TOF MS scan ranged from 100 to 1000 Da using DP=-50 V and CE=-5 V. The source and gas conditions were: GS1= 60, GS2= 60, CUR= 35, CAD= 10, TEM= 500˚C, ISV = -4500 V.

Data processing: Data acquisition and data processing were performed using Analytics within the SCIEX OS software. Analytics links the precursor extracted ion chromatogram (XIC) with the appropriate MS/MS fragmentation pattern. Library matching was automatically performed using the SCIEX Fluorochemical HR-MS/MS library 2.0, which contains 252 PFAS compounds. These compounds cover negative, positive and zwitterionic compound classes, and include both legacy and novel PFAS compounds, such as those originating from AFFF (aqueous film forming foam) and AFFF-impacted water.

Table 1. SWATH acquisition windows and parameters.

Item	Start mass (Da)	Stop mass (Da)	Range (Da)	DP (V)	CE (V)
1	*100*	*175*	*75*	*50*	*35 +/- 30*
2	*174*	*225*	*51*	*50*	*35 +/- 30*
3	*224*	*275*	*51*	*50*	*35 +/- 30*
4	*274*	*320*	*46*	*50*	*35 +/- 30*
5	*319*	*345*	*26*	*50*	*35 +/- 30*
6	*344*	*375*	*31*	*50*	*35 +/- 30*
7	*374*	*420*	*46*	*50*	*35 +/- 30*
8	*419*	*445*	*26*	*50*	*35 +/- 30*
9	*444*	*475*	*31*	*50*	*35 +/- 30*
10	*474*	*520*	*46*	*50*	*35 +/- 30*
11	*519*	*545*	*26*	*50*	*35 +/- 30*
12	*544*	*594*	*50*	*50*	*35 +/- 30*
13	*593*	*643*	*50*	*50*	*35 +/- 30*
14	*642*	*795*	*153*	*50*	*35 +/- 30*
15	*794*	*845*	*51*	*50*	*35 +/- 30*
16	*844*	*1000*	*156*	*50*	*35 +/- 30*

Reducing bias through study design

There are approximately 5000 PFAS compounds used in global commerce. For this reason, most people in the world have been exposed to and have measurable concentrations of PFAS compounds in their blood.[2,4] It has been shown that approximately 95% of the population have PFAS in their blood, and in particular PFOS (perfluorooctane sulfonic acid) and PFOA (perfluorooctanoic acid).

Serum is a complex matrix that contains many compounds that can interfere with PFAS detection and identification.[5] As a result, serum samples often undergo preparation specifically intended to reduce these interferences. These preparation strategies, however, can bias both the overall composition of the final extract, as well as the concentrations of individual PFAS compounds. For this study, a simplified sample preparation, consisting of only denaturation, centrifugation and highly selective lipid/matrix removal, was used to reduce bias induced by sample clean-up and handling.

It is now recognized that there are many PFAS "unknowns" that are not detected using traditional targeted analyses. In fact, the proportion of undetected PFAS unknowns is increasing in recent years potentially due to the introduction of replacement compounds.[6] Thus, non-targeted strategies using high resolution accurate mass instruments are typically now used for the confidence they confer in the identification of PFAS compounds. Because of the high number of PFAS compounds and overlap with matrix interferences (which are potentially even more profound with the simplified sample preparation used here), the addition of high resolution accurate mass analysis is necessary to distinguish between and correctly identify the many PFAS and confounding matrix compounds present in samples.[5]

SWATH acquisition is a type of data independent acquisition (DIA), that uses an unbiased approach for the acquisition of MS/MS data from complex samples. In contrast to the more traditional information dependent acquisition (IDA), which relies on a set of preconfigured criteria for precursor selection and subsequent MS/MS acquisition, SWATH acquisition captures MS/MS spectra on all detectable compounds. SWATH acquisition uses a wide Q1 isolation window to step across the mass range to sequentially acquire MS/MS data for every precursor ion. Once the data have been acquired, they are stored as a digital archive of the sample which can also be used for retrospective analysis, if necessary. Data processing algorithms then deconvolute the fragment data to assign precursor ions to product ions.

In this study, 16 variable width SWATH acquisition windows were used to step across the entire precursor mass range to fragment all PFAS precursors (Table 1). Variable width windows were used to reduce the total number of precursors in certain densely populated regions and thereby reduce the complexity of the MS/MS spectra and increase specificity. Once the data were collected, the entire data file was subjected to suspect screening to determine whether specific compounds were present in the samples. In addition, MS/MS library matching was performed for compound confirmation.

Results

Figure 1 shows the SWATH acquisition data for perfluorooctane sulfonate (PFOS) identified in the serum sample. Multiple lines of evidence support the identification of PFOS including:

- the retention time ✓
- the accurate precursor mass with low mass error ✓
- the isotope pattern observed for the precursor mass ✓
- the library match to the MS/MS fragment data ✓

Figures 2, 3 and 4 show similar data for perfluorobutanoic acid (PFBA), PFNA and perfluoroheptane sulfonic acid (PFHpS), which were also identified in the serum sample. The multiple lines of evidence outlined above confirm the presence of these compounds in the serum sample.

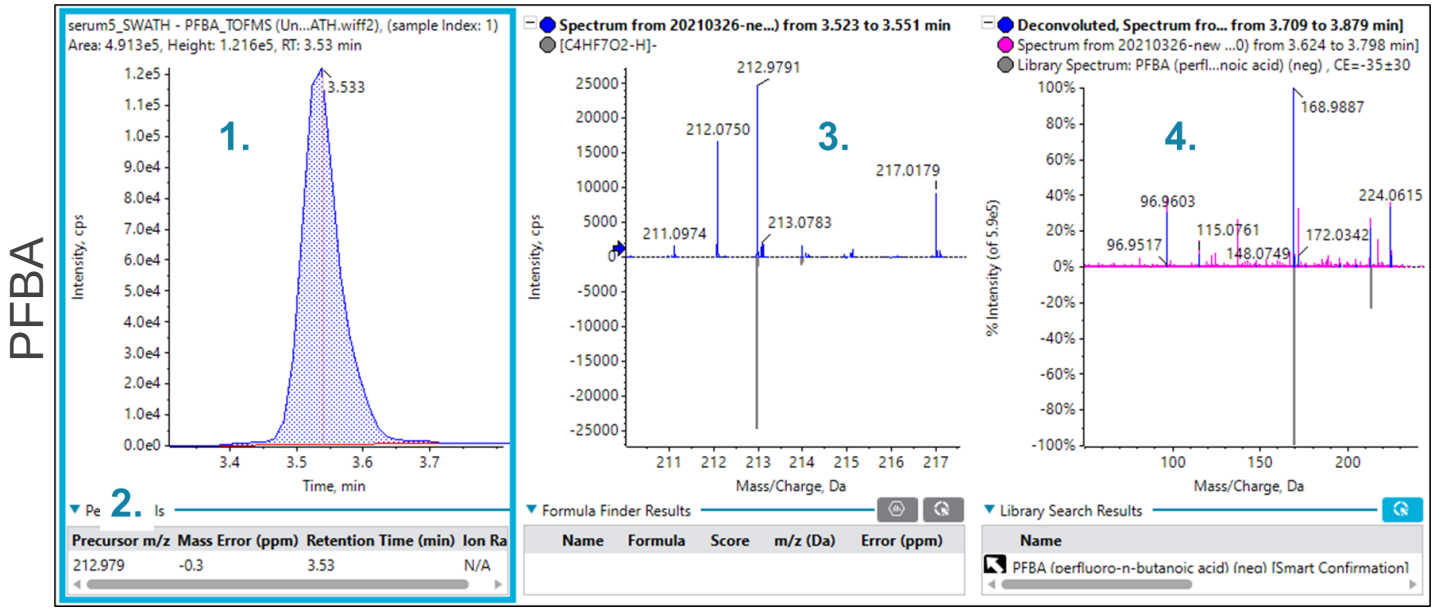

Figure 2. Perfluorobutanoic acid (PFBA) found in serum. SWATH acquisition analysis shows multiple levels of confirmation for PFBA, including 1) retention time, 2) precursor mass with m/z 212.979 and very low mass error (-0.3 ppm), 3) precursor isotope pattern in the center, and 4) MS/MS spectrum and library match on the top right and bottom right, respectively.

PFNA

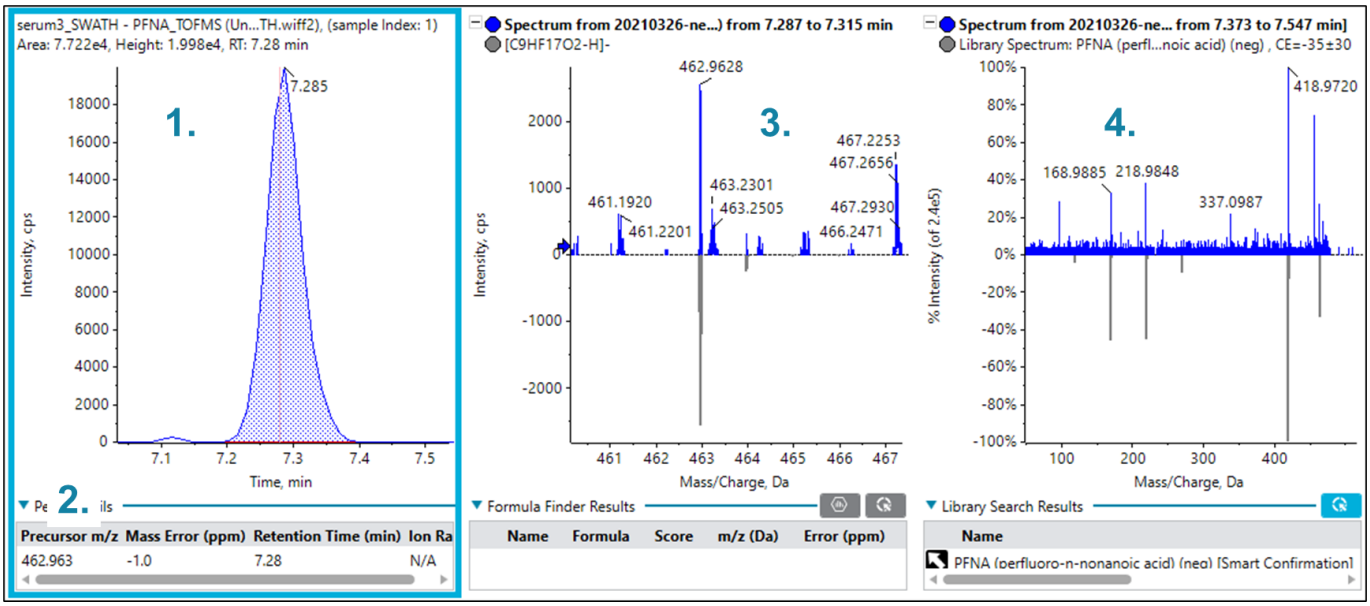

Figure 3. Perfluorononanoic acid (PFNA) found in serum. SWATH acquisition analysis shows multiple levels of confirmation for including 1)retention time, 2) precursor mass with m/z 462.963 and low mass error (-1.0 ppm), 3) precursor isotope pattern in the center, and 4) MS/MS spectrum and library match on the top right and bottom right, respectively.

PFHpS

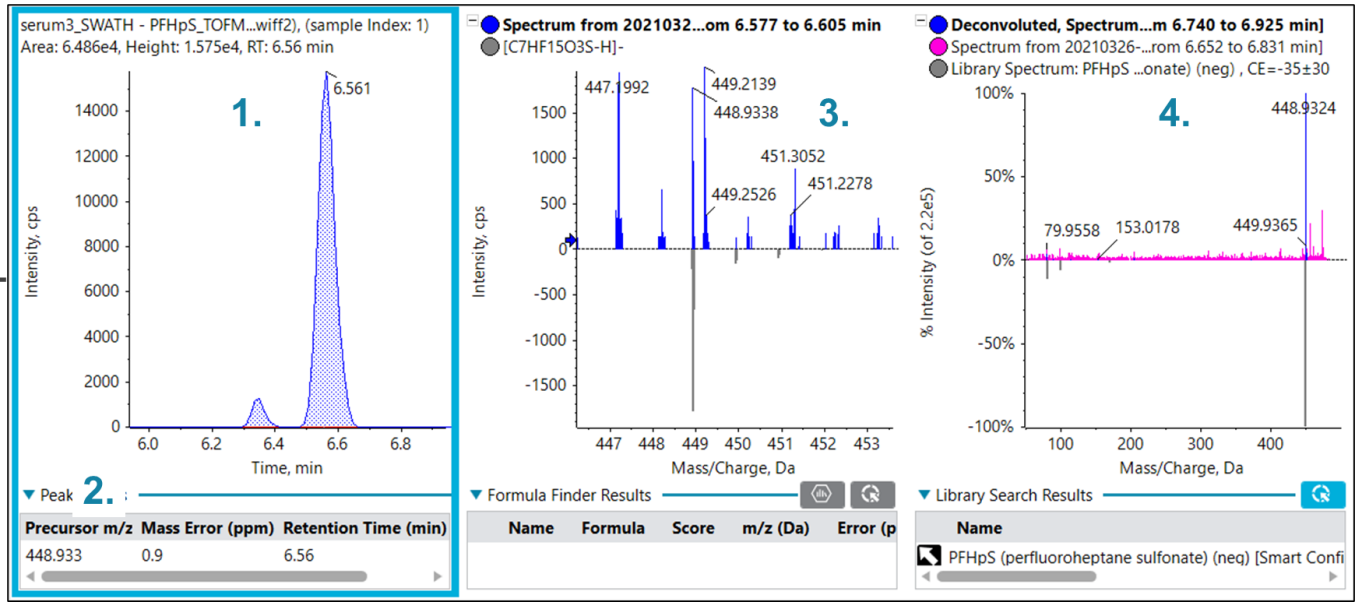

Figure 4. Perfluoroheptane sulfonic acid (PFHpS) found in serum. SWATH acquisition analysis shows multiple levels of confirmation for PFHpS, including 1) retention time, 2) the precursor mass with m/z 448.933 and low mass error (-0.9 ppm), 3) precursor isotope pattern, 4) MS/MS spectrum and library match on the top right and bottom right, respectively.

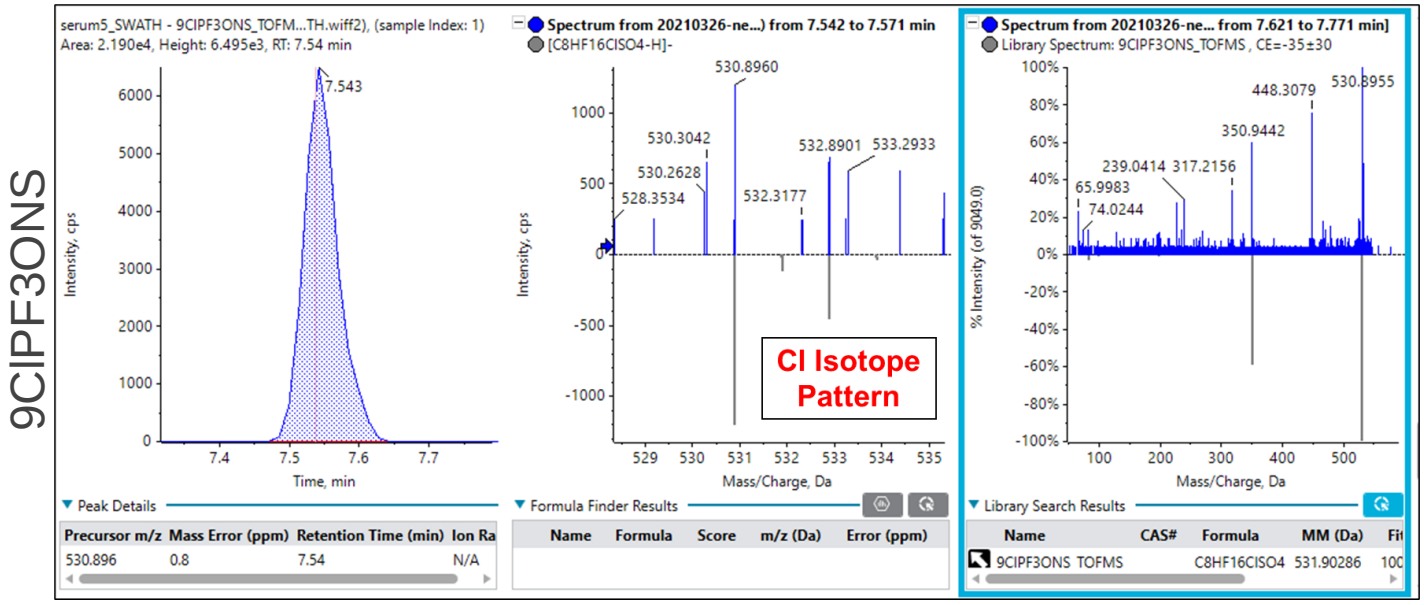

Figure 5. 9ClPF3ONS found in serum. An unknown compound was found in the sample. Careful examination of the TOF MS isotope pattern indicated the presence of a chlorine atom (M+2 isotope ~1/3 intensity of the molecular ion) and the precursor mass matched the PFOS replacement compound 9ClPF3ONS. The data generated by running an authentic standard for 9ClPF3ONS were added to the fluorochemical library. Re-processing the serum data and comparing them against the library now identifies the unknown compound as 9ClPF3ONS.

Figure 5 shows an interesting situation in which a compound was not initially identified – despite being included on the suspect screening list – since the fluorochemical library did not have a MS/MS spectrum for this chemical. Careful examination of the TOF MS isotope pattern indicated that the compound likely contained a chlorine atom. Note that because of the wider Q1 isolation windows, the full isotope pattern was preserved in the MS/MS data, enabling the detection of the distinctive isotope pattern for Cl. Further, the precursor mass matched that of the PFOS replacement compound, 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9ClPF3ONS) with a low mass error.

An authentic standard of 9ClPF3ONS was obtained and run using SWATH acquisition to confirm the identity of the unknown PFAS compound. The MS/MS spectrum generated for 9ClPF3ONS was added to the fluorochemical library. Re-processing the serum sample data and comparing it against the updated fluorochemical library now showed a positive identification of the unknown as 9ClPF3ONS. Notably, this positive identification of 9ClPF3ONS was accomplished using previously collected data, as SWATH acquisition creates a digital archive of the sample. This retrospective analysis could prove valuable in the future as more novel PFAS compounds are reported.

Conclusions

With the high number of known PFAS compounds in existence today and the growing number of new and unknown PFAS, a non-targeted LC-MS/MS method provides the most comprehensive strategy for PFAS identification. SWATH acquisition is a non-targeted data *independent* acquisition (DIA) workflow and therefore provides an unbiased approach for capturing MS/MS data for all detectable PFAS when utilizing the appropriate polarity and ionization technique. SWATH acquisition provides high resolution accurate mass data on precursors and product ions, isotope information, retention time and MS/MS library matching to help identify and confirm compounds from highly complex matrices. Moreover, the crude serum sample preparation used here minimizes losses from extensive sample handling, preserving the integrity of the sample and allowing data capture on as many known and unknown PFAS compounds as possible.

The ability of SCIEX OS software to allow the user to rapidly add MS/MS fragmentation spectra into new or existing libraries results in enhanced flexibility for compound identification during non-target analysis. Coupled with the in-depth retrospective data processing possible with SWATH acquisition, this workflow will allow users to easily adapt as new PFAS compounds are discovered.

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Headquarters
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SCIEX China, Beijing

关键词： X500R；致毒农药；SWATH

Key words: X500R; Toxic pesticides; SWATH

引言

众所周知，为防止疾病传播和农作物损失，大量农药被农业社区用于控制虫害，杂草，及其它植物疾病。我国农药的使用量和中毒比例都位居世界前茅。其中有机磷类、氨基甲酸酯类和拟除虫菊酯类农药毒性强，危害大。人如果接触、吸入或服入农药，会出现头晕、头痛、全身乏力、多汗、恶心、呕吐、腹痛、腹泻、胸闷、呼吸困难等症状，严重时甚至会造成死亡。这些农药也常常出现在公安司法的投毒案件中。为了更好的帮助案情判定，本实验应用四极杆-飞行时间质谱X500R建立了全血样本中有机磷、氨基甲酸酯类、除虫菊酯类等在日常案件中常见的中毒农药的筛查和定量方法。

四极杆-飞行时间（QTOF）质谱作为一种高质量精度质谱，目前已大量应用于农药，兽药残留的筛查工作。SCIEX X500R QTOF系统能够在高扫描速度下同时保持高分辨率、高质量准确度、高灵敏度和宽线性范围。其独创的SWATH®（Sequential Windowed Acquisition of all Theoretical mass spectra）技术，能够将母离子分成不同的采集窗口，并将各窗口母离子一起碎裂，从而得到连续的全质量轴二级谱图。SCIEX OS专利的去卷积功能，准确为每个每个碎片与母离子匹配，从而得到最全面的准确的二级碎片全谱信息。此外，X500R QTOF 利用采集到的二级碎片信息，可实现媲美三重四极杆的MRM定量功能，从而一针快速获得全面的和高质量的定性和定量结果。

本实验采用X500R QTOF高分辨质谱，应用SWATH®智能分段扫描方式建立了全血样本中110种常见中毒农药的一针筛查定性和定量方法。

实验特点：

1. X500 系列质谱独有的Turbo V™离子源和气帘气接口设计，具有很强的抗污染能力，从而保证复杂基质样品的重现性。
2. 使用SWATH®扫描方式可一针得到样品中所有离子的一级和二级全谱信息。因此数据可溯源，非常适合公安法医系统毒物检测。
3. SCIEX OS 软件可在同一界面同时进行筛查定性和定量分析，无需软件或者界面的切换，大大提高工作效率。
4. 定性分析：应用SWATH®数据，通过一级质量精度、同位素分布、二级碎片信息、保留时间四个维度，更准确的进行筛查和定性。
5. 定量分析：对于SWATH®数据，可随意选择灵敏度高的任意碎片或者母离子进行定量分析，OS软件还可自动给出定性离子和定量离子的离子丰度比（与三重四极杆质谱相同）。通过自定义运算规则，可自动对符合规则的样本做标识，阳性结果一目了然，便于快速出报告。
6. 一针进样20分钟内完成110种致毒农药的筛查分析，所有化合物在0.5~100 ng/mL范围内线性良好。提取回收率均可达到70%~120%。

样品处理：

1. 准确吸取200 µL血液样品至2 mL离心管中。
2. 加入600 µL乙腈，旋涡混合1 min。
3. 12000转离心10 min。
4. 吸取上清液，直接上样分析。

色谱方法：

色谱柱：Phenomenex Kinetex F5, 100 × 2.1 mm, 2.6 µm；

流动相： A：含2 mM甲酸铵-水
B：含2 mM甲酸铵-甲醇
梯度洗脱

流速：0.4 mL/min；

柱温：40℃；

进样量：5 µL

Time [min]	Flow [mL/min]	B.Conc [%]
0.50	0.4000	15.0
2.50	0.4000	50.0
13.00	0.4000	80.0
14.00	0.4000	98.0
17.00	0.4000	98.0
17.10	0.4000	15.0
20.00	0.4000	15.0

质谱方法：

扫描方式：SWATH®采集模式

离子源：ESI+

CDS自动校正

离子源参数：

IS电压: 5500 V　　气帘气CUR: 35 psi

雾化气GS1: 55 psi　　辅助气GS2: 50 psi

源温度TEM: 550 ℃　　碰撞气CAD: 7

去簇电压 DP: 60 V　　碰撞能量CE ± CES: 35 ± 15 V

可变SWATH设置流程

智能可变窗口SWATH®（Sequential Windowed Acquisition of all Theoretical mass spectra）可根据样本中一级离子的分布，在分布密集的离子区域设置较窄的采集窗口，反之，设置较宽的采集窗口，从而保证在所有离子均可获得高质量的准确的二级碎片全谱（见图1）。

通过扫描一级质谱图得到样品的离子分布信息，Variable Window Calculator 可变窗口计算器可根据离子分布信息自动计算合理的SWATH®扫描窗口，从而建立可变窗口的SWATH®采集方法。

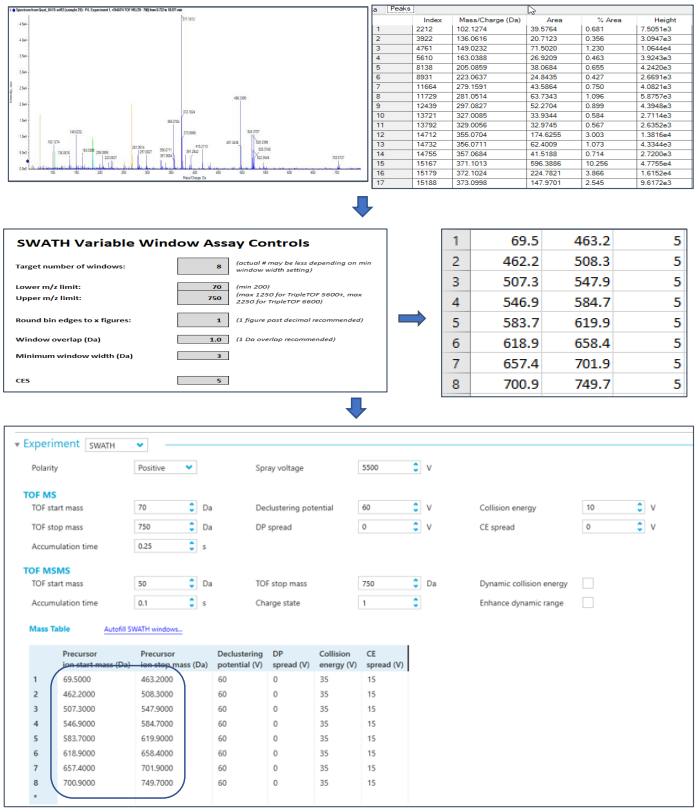


图1. 智能可变SWATH扫描窗口设置流程。

实验结果

1. 一针进样完成了110种致毒农药的一级和二级谱图采集，110种致毒农药的提取离子流图（图2）SWATH®一针进样，获得高质量的样品一级和二级全谱信息，通过一级质量精度、保留时间、同位素分布和二级库匹配四大置信条件进行化合物筛查和鉴定。

针对目标化合物的筛查，SCIEX OS可通过将已知化合物的名称和分子式导入软件，设置相关的离子加合方式进行筛查。相关筛查结果，其阳性结果可以以红绿灯方式（图3）显示，结果一目了然。

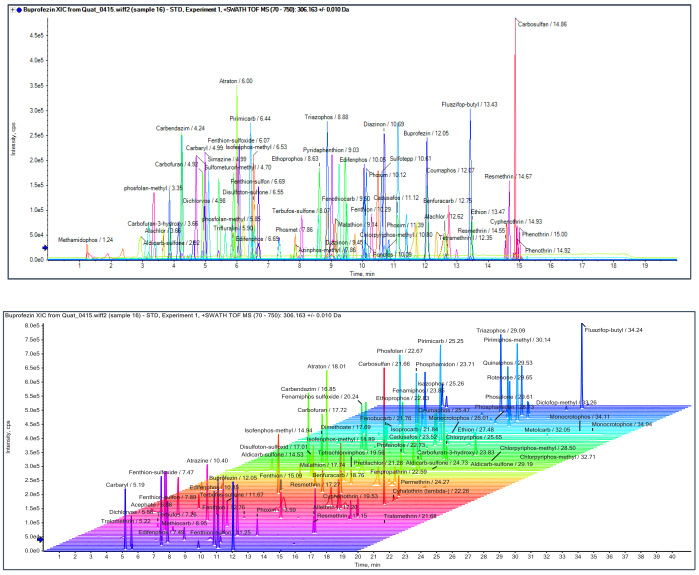


图2. 110种致毒农药的提取离子流图（20 ng/mL）。

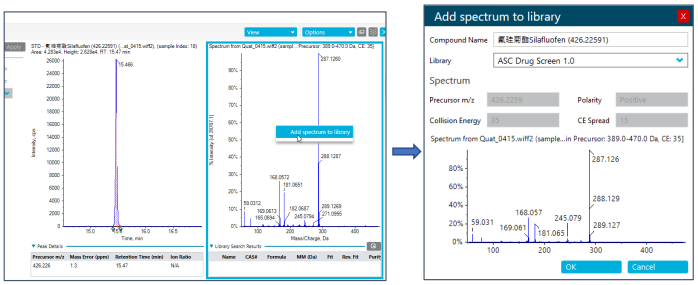
84 of 113 rows | Filters: 10 | Qualify for Rul

Index	Sample Name	Component Name	Expected RT	Mass Error (...	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...
2311	P4	亚胺硫磷Phosm...	7.88	1.0	✓	✓	✓	✓
2314	P4	胺菊酯ITetramet...	12.35	0.8	✓	✓	✓	✓
2268	P4	倍硫磷亚砜Fent...	6.08	0.3	✓	✓	✓	✓
2318	P4	乙拌磷砜Disulfo...	6.56	0.9	✓	✓	✓	●
2269	P4	甲硫威Methioca...	7.35	-0.3	✓	✓	✓	✓
2358	P4	硫环磷Phosfola...	4.27	0.1	✓	✓	✓	✓
2276	P4	西玛津Simazine...	4.99	-0.5	✓	✓	✓	✓
2274	P4	哒嗪硫磷Pyrida...	9.05	0.8	✓	✓	✓	✓
2264	P4	敌敌畏Dichlorv...	4.99	0.0	✓	✓	✓	▲
2355	P4	甲拌磷砜Phorat...	6.50	0.9	✓	✓	✓	✓
2333	P4	蝇毒磷Coumap...	12.07	1.2	✓	✓	✓	✓
2280	P4	特丁硫磷碎片2...	12.48	0.3	✓	✓	✓	●
2326	P4	莠去通Atraton (...	6.01	-0.4	✓	✓	✓	●
2338	P4	苯线磷Fenamip...	9.46	0.3	✓	✓	✓	✓
2265	P4	敌瘟磷Edifenph...	10.07	0.7	✓	✓	✓	✓
2316	P4	三唑酮Triadimef...	8.90	0.6	✓	✓	✓	✓

图3. 红绿灯式筛查结果显示。

2. SCIEX OS支持自建二级谱库，一键即可将获得的毒物二级谱图信息加入相关数据库（图4）。

1）将化合物添加到指定数据库



2）设置数据库搜索

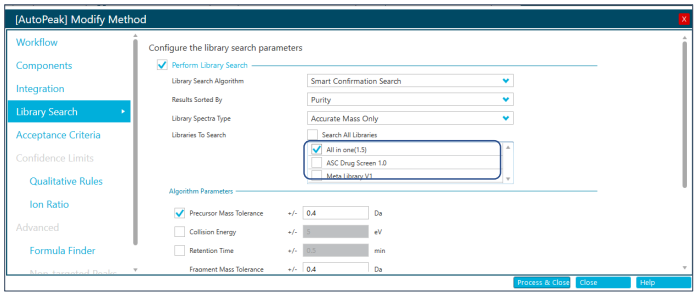


图4. 利用SCIEX OS软件快速自建谱库。

3. 良好的仪器和方法稳定性

在血液样品中添加浓度为5 ng/mL的标品，连续进样6针，所得定量RSD均在2.5%以下（图5），说明该方法具有良好的重现性。图5为甲萘威、克百威、苯线磷、乐果、敌敌畏等部分化合物的稳定性示例。

Index	Sample Name	Sample T...	Component...	Compound...	Actual Concentr...	Expected RT	Area	Component Group Name	Retent... Time	Retenti... Time D...	U...	Calcula Concent
1	5 ng/mL	Standard	甲萘威Carbaryl...	Quantifiers	5.00	4.99	2.177e5		4.99	0.00	☑	degener
2	5 ng/mL	Standard	克百威Carbofur...	Quantifiers	5.00	4.93	1.451e5		4.92	0.01	☑	degener
3	5 ng/mL	Standard	苯线磷Fenamip...	Quantifiers	5.00	9.46	2.779e5		9.45	0.01	☑	degener
4	5 ng/mL	Standard	乐果Dimethoate...	Quantifiers	5.00	3.89	5.625e4		3.88	0.01	☑	degener
5	5 ng/mL	Standard	敌敌畏Dichlorv...	Quantifiers	5.00	4.99	1.138e5		4.98	0.01	☑	degener
6	5 ng/mL	Standard	特丁硫磷Terbuf...	Quantifiers	5.00	3.86	1.195e4		3.86	0.00	☑	degener
7	5 ng/mL	Standard	残杀威Propoxur...	Quantifiers	5.00	4.91	1.597e4		4.90	0.01	☑	degener

Group by Concentration for Standards | Area

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Value #1	Value #
1	甲萘威Carbaryl (202.08626)	5.00	6 of 6	2.203e5	2.805e3	1.27	2.177e5	2.228e5
2	克百威Carbofuran (222.11247)	5.00	6 of 6	1.433e5	1.990e3	1.39	1.451e5	1.414e5
3	苯线磷Fenamiphos (304.11308)	5.00	6 of 6	2.722e5	6.267e3	2.30	2.779e5	2.665e5
4	乐果Dimethoate (230.0069)	5.00	6 of 6	5.736e4	1.215e3	2.12	5.625e4	5.847e4
5	敌敌畏Dichlorvos (220.95318)	5.00	6 of 6	1.114e5	2.656e3	2.38	1.138e5	1.090e5
6	特丁硫磷Terbufos (289.05141)	5.00	6 of 6	1.216e4	2.243e2	1.84	1.195e4	1.236e4
7	残杀威Propoxur (210.11247)	5.00	6 of 6	1.624e4	3.007e2	1.85	1.597e4	1.652e4
8	氟氯苯菊酯Cyfluthrin(NH4) (451.09...	5.00	6 of 6	7.957e3	1.529e2	1.92	7.818e3	8.097e3

图5. 基质添加样品的重现性（5 ng/mL）。

X500R Q-TOF具有非常好的质量稳定性，连续进样24小时，各化合物的一级质量偏差均小于1 ppm（图6），另以杀线威源内裂解碎片，甲氰菊酯，杀螨菊酯为例，其质荷比分别为90.055，350.175，564.122，在低中高三个质荷比其质量偏差分别为-0.3 ppm，-0.1 ppm和1.0 ppm，均在1 ppm以内（图7），表明X500R在不同质量范围均拥有良好的质量稳定性，从而最大程度的保证数据结果的可靠性。

Index	Sample N...	Component Name	Formula	Precursor Mass	Found At Mass	Mass Error ...	Mass Error (...	Retent... Time	Sample T...
5809	P4	噻嗪酮Buprofezin (306.16346)	C16H23N...	306.163	306.1635	✓	0.2	12.06	Quality Cont...
5813	P4	二嗪磷Diazinon (305.10833)	C12H21N...	305.108	305.1084	✓	0.2	10.70	Quality Cont...
5816	P4	敌瘟磷Edifenphos (311.03239)	C14H15O...	311.032	311.0326	✓	0.7	10.06	Quality Cont...
5822	P4	倍硫磷亚砜Fenthion-sulfoxide (295.022...	C10H15O...	295.022	295.0223	✓	0.3	6.08	Quality Cont...
5823	P4	甲硫威Methiocarb (Mercaptodimethur) (...	C11H15N...	226.090	226.0896	✓	-0.3	7.35	Quality Cont...
5836	P4	吡噻硫磷Pyridaphenthion (341.07194)	C14H17N...	341.072	341.0722	✓	0.8	9.03	Quality Cont...
5837	P4	卡呋萄酮Resmethrin (339.19547)	C22H26O3	339.195	339.1955	✓	0.2	14.68	Quality Cont...
5840	P4	西玛津Simazine (202.0854)	C7H12ClN5	202.085	202.0853	✓	-0.5	4.99	Quality Cont...
5841	P4	甲脲噻隆Sulfometuron-methyl (365.091...	C15H16N...	365.091	365.0918	✓	1.0	4.69	Quality Cont...
5865	P4	烯丙菊酯Allethrin I (303.19547)	C19H26O3	303.195	303.1955	✓	0.2	13.00	Quality Cont...
5870	P4	烯丙菊酯Allethrin II (303.19547)	C19H26O3	303.195	303.1955	✓	0.2	13.00	Quality Cont...
5873	P4	倍硫磷Fenthion (279.0273)	C10H15O...	279.027	279.0275	✓	0.6	10.30	Quality Cont...
5882	P4	乙草胺Acetochlor (270.12553)	C14H20Cl...	270.126	270.1256	✓	0.3	9.00	Quality Cont...
5884	P4	丙硫克百威Benfuracarb (411.19482)	C20H30N...	411.195	411.1951	✓	0.8	12.76	Quality Cont...
5886	P4	甲草胺Alachlor (270.12553)	C14H20Cl...	270.126	270.1256	✓	0.3	9.00	Quality Cont...
5919	P4	甲基异柳磷碎片231Isofenphos-methyl F...	C8H7O4PS	230.988	230.9876	✓	0.4	10.50	Quality Cont...
5920	P4	甲基异柳磷碎片273Isofenphos-methyl F...	C11H13O...	273.034	273.0346	✓	0.5	10.50	Quality Cont...
5921	P4	马拉硫磷Malathion (331.04334)	C10H19O...	331.043	331.0437	✓	1.0	9.15	Quality Cont...

图6. 基质添加样品的质量准确度。

Index	Sample Na...	Component Name	Formula	Precursor Mass	Found At Mass	Mass Err...	Mass Error (...	Retent... Time
62	sample1	杀螨菊酯Acrinathrin (564.12161)	C26H21F6NO5	564.122	564.1222	✓	1.0	15.24
63	sample1	倍硫磷-砜Fenthion-sulfon (311.01713)	C10H15O5PS2	311.017	311.0174	✓	0.8	6.69
65	sample1	甲硫威Methiocarb (Mercaptodimethur) (226.08963)	C11H15NO2S	226.090	226.0897	✓	0.3	7.35
66	sample1	杀线威（草肟威）碎片90Oxamyl Fragm 90 (90.0...	C3H7NO2	90.055	90.0549	✓	-0.3	3.08
68	sample1	西玛津Simazine (202.0854)	C7H12ClN5	202.085	202.0852	✓	-1.0	4.99
70	sample1	特丁磷亚砜Terbufos-sulfoxid (305.04632)	C9H21O3PS3	305.046	305.0465	✓	0.6	7.82
71	sample1	特丁磷亚砜碎片187Terbufos-sulfoxid Fragm 187 (...	C4H11O2PS2	187.001	187.0010	✓	-0.2	7.81
75	sample1	阿特拉津Atrazine (216.10105)	C8H14ClN5	216.101	216.1010	✓	-0.1	6.01
76	sample1	甲基硫环磷phosfolan-methyl (227.99125)	C5H10NO3PS2	227.991	227.9914	✓	0.6	3.35
77	sample1	丙硫克百威碎片190Benfuracarb Fragm 190 (190.0...	C8H15NO2S	190.090	190.0895	✓	-0.4	12.75
78	sample1	甲草胺碎片238Alachlor Fragm 238 (238.09932)	C13H16ClNO	238.099	238.0992	✓	-0.4	12.62
79	sample1	丁草胺碎片238Butachlor Fragm 238 (238.09932)	C13H16ClNO	238.099	238.0992	✓	-0.4	12.62
81	sample1	甲氰菊酯Fenpropathrin (350.17507)	C22H23NO3	350.175	350.1750	✓	-0.1	14.59
83	sample1	甲基异柳磷碎片231Isofenphos-methyl Fragm 231...	C8H7O4PS	230.988	230.9876	✓	0.1	10.50
84	sample1	甲基异柳磷碎片273Isofenphos-methyl Fragm 273...	C11H13O4PS	273.034	273.0347	✓	0.7	10.50
86	sample1	氧乐果碎片183Omethoate Fragm 183 (182.98754)	C4H7O4PS	182.988	182.9876	✓	0.5	2.37
88	sample1	腈菊酯碎片286Tetramethrin I Fragm 286 (286.180...	C18H23NO2	286.180	286.1804	✓	0.9	12.35
89	sample1	三唑酮Triadimefon (294.10038)	C14H16ClN3O2	294.100	294.1005	✓	0.5	8.90
90	sample1	砜拌磷Disulfoton-sulfoxid (291.03067)	C8H19O3PS3	291.031	291.0309	✓	0.8	6.21

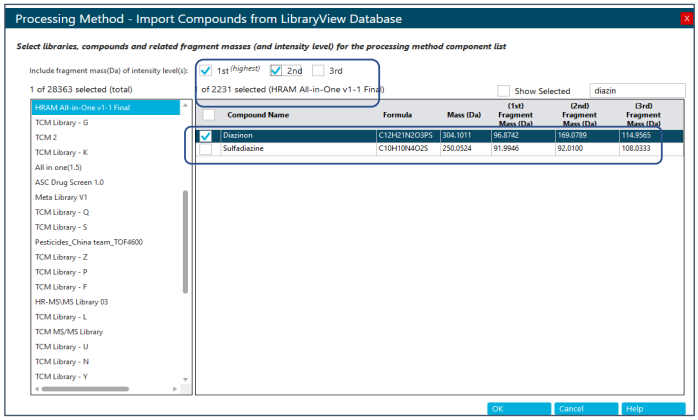
图7. X500R在全质量轴的质量准确度。

4. 定量处理SWATH®数据

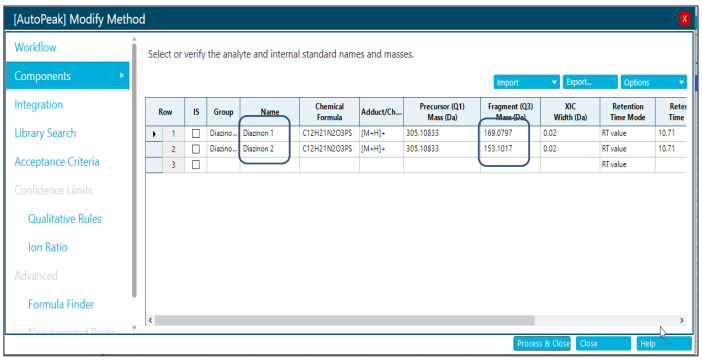
使用OS 软件对SWATH®数据进行定量处理，可任意选择母离子及合适的碎片离子作为定量离子和定性离子，可以得到相关碎片信息的Ion Ratio比率，并且具有线性范围宽，背景噪音低等优点。

其使用的离子对有两种方式可以导入，一是通过EXCEL将相关碎片离子拷贝进入离子列表，二是通过LibraryView软件将标准谱库中碎片信息导入离子列表（图8）。

1）从LibraryView数据谱库选择相关离子对导入，一般可按强度导入



2）设置定量及定性离子



3）设置离子比率置信空间

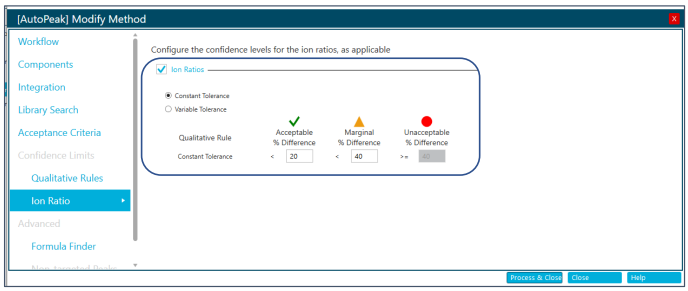


图8. 通过LibraryView导入定量及定性离子对。

以二嗪磷为例，以碎片169.08作为定量离子进行定量分析，结果显示其在0.2~100 ng/mL之间线性良好（r2>0.99）,且在整个线性范围内准确度都在80-120 %之间。以碎片153.102作为定性离子，计算得到标品离子丰度比在0.75左右，样本的离子丰度为0.7575，符合国标关于《定性确证时相对离子丰度的最大允许偏差》当相对离子丰度>20%时，离子丰度比在±20%之内的规定（图9）。

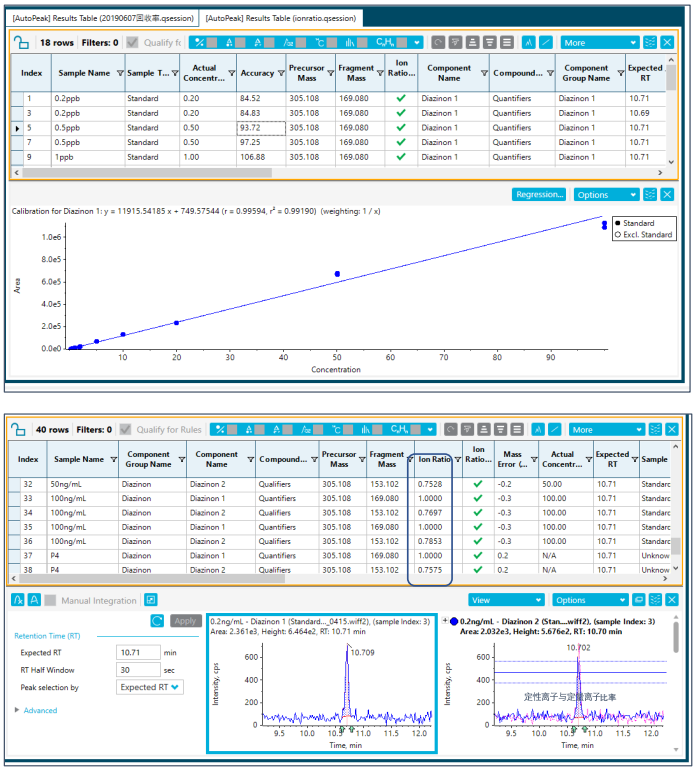


图9. 定量线性及Ion Ratio（以二嗪磷为例）。

5. 对110种致毒农药进行基质添加回收率实验，大部分样品在添加上机浓度为0.5, 1, 10 ng/mL时回收率均可达到70%~120%，可直接用于致毒农药的筛查。

总结

本次实验通过SCIEX X500R QTOF系统的SWATH®技术，对110种致毒农药进行筛查。

1. 使用X500R 的SWATH®方法对基质样本中致毒农药进行筛查分析，结合其智能窗口分配功能，使每个化合物均可采集到质量可靠的二级图谱，且软件可以一键将新化合物加入谱图数据库，方便后期未知物筛查。

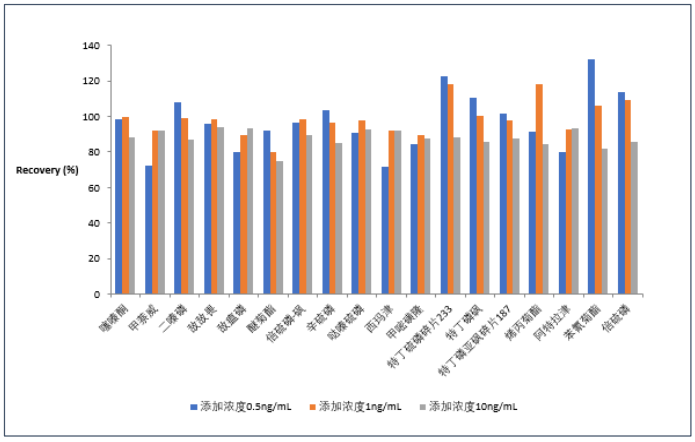


图10. 部分致毒农药的不同浓度添加回收率结果。

2. 一针进样即可得到复杂基质样品中待测物的高质量一级与二级质谱数据，且质量精度均小于1 ppm，配合同位素分布、保留时间、二级质谱图四个维度可快速准确地提供筛查结果。

3. 结合Turbo V™离子源的X500R Q-TOF质谱，具有优秀的抗干扰能力，血液样品连续进样，其RSD<2.5 %。由于离子源的抗干扰能力较强，所以可以采用相对简单的前处理方法，从而节约时间，提高通量。

4. 使用乙腈直接沉淀蛋白的方式处理样品，80%以上样品回收率在70~120%之间。

5. 使用二级碎片定量能够有效的降低背景噪音，排除干扰离子，使定量的结果更加准确可靠。大部分致毒农药的均在0.5~100 ng/mL之间有良好的线性，r^2>0.99，可用于实际样品定量分析。

参考文献

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SCIEX中国公司

北京分公司
地址：北京市朝阳区酒仙桥中路24号院1号楼5层
电话：010-58081388
传真：010-58081390

上海公司及亚太区应用支持中心
地址：上海市长宁区福泉北路518号1座502室
电话：021-24197200
传真：021-24197333

广州分公司
地址：广州市天河区珠江西路15号珠江城1907室
电话：020-85100200
传真：020-38760835

全国免费垂询电话：800 820 3488，400 821 3897　网址：www.sciex.com.cn　微博：@SCIEX

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台灣塞爾克斯應用生技有限公司 | 11492 台北市內湖區瑞光路631號4F
SCIEXNow Toll Free : 0800-818-978 或 service.taiwan@sciex.com