# Rapid and Sensitive Analysis of a 93-Compound Forensic Panel in Urine using the QTRAP®/Triple Quad™ 4500 LC-MS/MS System

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## Overview

In this technical note, we describe a rapid and sensitive analysis of a comprehensive panel of forensic compounds in human urine using the ExionLC™ AC HPLC system and the QTRAP®/Triple Quad™ 4500 LC-MS/MS system (Figure 1). This forensic panel contains 93 compounds, and a total of 212 MRM transitions (including internal standards) are monitored. Some compounds ionize preferentially in positive mode, while others ionize preferentially in negative mode; therefore this method takes advantage of rapid polarity switching. The total LC runtime is 6.5 minutes, which can be further accelerated for a smaller panel of compounds. Sample preparation is based on enzymatic hydrolysis and a simple “dilute and shoot” methodology.

## Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for simultaneous quantitation of multiple compounds in forensic samples. Multiple Reaction Monitoring (MRM) detection is the gold standard for quantitation purposes because of its speed, specificity and sensitivity. All these attributes are critical for quantitative analysis of a comprehensive forensic compound panel.

There are challenges associated with the monitoring of large numbers of compounds in a single MRM method. Ideally, cycle times should remain as small as possible, to ensure that a large number of data points are acquired across each LC peak. However, as the number of analytes in a panel increases, the cycle time increases accordingly. In order to maintain the desired cycle time, one may choose to decrease the dwell times for individual MRM transitions, however this will inevitably decrease data quality. Therefore, we have employed the *Scheduled* MRM™ algorithm to intelligently monitor MRMs only during the appropriate retention time windows, thus decreasing the number of concurrent MRMs monitored at any point in time, allowing both the cycle time and dwell time to remain optimal. The *Scheduled* MRM™ algorithm has been re-optimized in the Analyst 1.6.3 software to provide superior data quality at high levels of MRM multiplexing (Figure 2).

In this study we present a rapid, robust and sensitive analysis of a comprehensive forensic panel consisting of 93 compounds in human urine using the QTRAP®/Triple Quad™ 4500 LC-MS/MS system. Owing to the inclusion of several barbiturates in the panel which ionize preferentially in negative mode, a polarity switching method has been implemented. Due to a high number of MRM transitions (212 MRMs in total, including the internal standards) and a short LC runtime (6.5 min), a newly optimized *Scheduled* MRM™ algorithm is used.

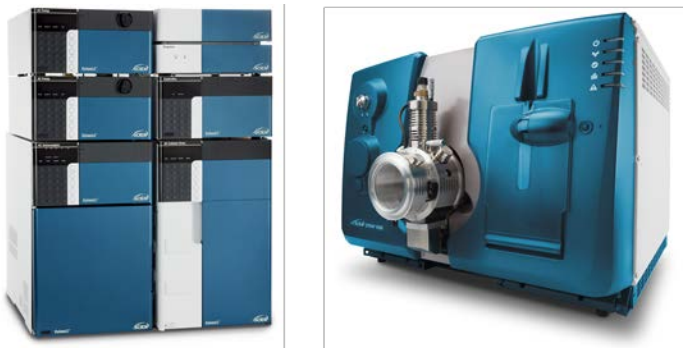

**Figure 1: The SCIEX ExionLC™ AC HPLC system (left) and the SCIEX QTRAP® 4500 LC-MS/MS System (right).**

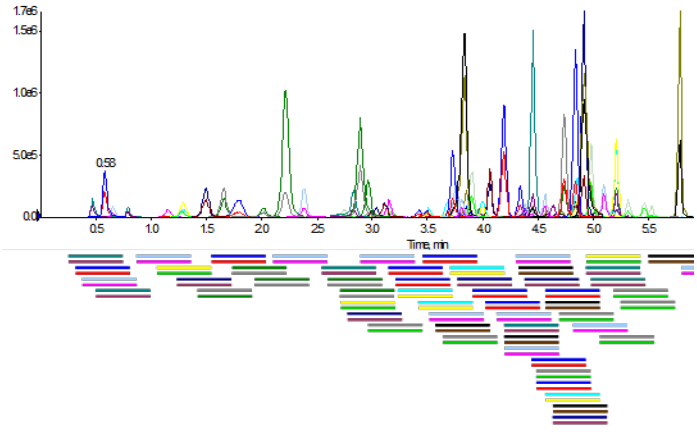


**Figure 2: The *Scheduled* MRM™ algorithm allows the detection of many more MRMs using fast LC while maintaining optimal cycle time and dwell times.**

## Materials and Methods

### *Compound list and spiking solutions*

Table 1 lists the 93 compounds plus internal standards in the extended panel. All were procured from Cerilliant Corporation (Round Rock, TX). Two spiking solutions in methanol were prepared: one for analytes (**SA**) and the other for internal standards (**SIS**). Concentrations of all the analytes in the spiking solution **SA** are listed in Table 1. Compounds in black font are in the regular panel (72 analytes) and the ones in blue font are the additional 21 analytes in the extended panel (93 analytes). Internal standards are shown in grey background.

### *Calibrator preparation*

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

### *Sample preparation*

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

### *Liquid Chromatography*

Liquid Chromatography analysis was performed on the SCIEX ExionLC™ AC HPLC system at 30°C. Separation was achieved using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 4.6 mm, 2.6 µm, 00B-4495-E0), with a Phenomenex SecurityGuard ULTRA UHPLC Phenyl (AJ0-8774) and ULTRA holder (AJ0-9000). Mobile phase A (MPA) was ammonium formate in water. Mobile phase B (MPB) was formic acid in methanol. The LC flow rate was 1 mL/min and the LC run-time was 6.5 min. Injection volume was 5 µL.

For the autosampler, the needle rinse solution was methanol:acetonitrile:isopropanol (1:1:3, v/v/v). The Rinse sequence was as follows:

- Rinsing volume: 1 mL
- Needle stroke: 54 mm
- Rinsing speed: 35 µL/sec
- Sampling speed: 15 µL/sec
- Rinse dip time: 5 sec
- Rinse mode: before and after aspiration.

### *MS and MS/MS condition*

- Curtain gas (CUR): 30
- Collision gas (CAD): Medium
- IonSpray Voltage (IS): 2500 V (positive) and -4500 V (negative)
- Temperature (TEM): 650°C
- Ion Source Gas 1 (GS1): 60
- Ion Source Gas 2 (GS2): 50

The Declustering Potential (DP), Collision Energy (CE), and Collision Cell Exit Potential (CXP) voltages were optimized for each individual component, as described in Table 2 below.

### *General scan timings for Scheduled MRM™ algorithm and MRM table*

- Target Scan Time (positive) = 0.2 sec
- Target Scan Time (negative) = 0.05 sec
- MRM detection window = 20 sec

## Results and Discussion

### *Sample 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.

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

| Compounds | (ng/mL) | Compounds | (ng/mL) | Compounds | (ng/mL) | Compounds | (ng/mL) |
|---|---|---|---|---|---|---|---|
| 6-MAM | 1000 | Gabapentin | 10000 | Naltrexone | 5000 | Secobarbital | 10000 |
| 7-Aminoclonazepam | 5000 | Hydrocodone | 5000 | N-desmethyltapentadol | 5000 | THC-COOH | 2000 |
| 7-Hydroxymitragynine | 1000 | Hydromorphone | 5000 | Norbuprenorphine | 2000 | | |
| Acetyl Fentanyl | 200 | Imipramine | 5000 | Norcodeine | 5000 | 6-MAM-d3 | |
| Alpha-Hydroxyalprazolam | 5000 | JWH-018 4-OH pentyl | 1000 | Nordiazepam | 5000 | Amphetamine-d5 | |
| Alpha-Hydroxymidazolam | 5000 | JWH-018 pentanoic acid | 1000 | Norfentanyl | 200 | Benzoylecgonine-d3 | |
| Alpha-Hydroxytriazolam | 5000 | JWH-019 6-OH hexyl | 1000 | Norhydrocodone | 5000 | Buprenorphine-d4 | |
| Alpha-PPP | 1000 | JWH-073 3-OH butyl | 1000 | Normeperidine | 5000 | Carisoprodol-d7 | |
| Alpha-PVP | 1000 | JWH-073 butanoic acid | 1000 | Noroxycodone | 5000 | Codeine-d6 | |
| Alprazolam | 5000 | JWH-081 5-OH pentyl | 1000 | Norpropoxyphene | 10000 | Fentanyl-d5 | |
| AM-2201 4-OH pentyl | 1000 | JWH-122 5-OH pentyl | 1000 | Nortriptyline | 5000 | Hydrocodone-d6 | |
| Amitriptyline | 5000 | JWH-210 5-OH pentyl | 1000 | O-Desmethyltramadol | 5000 | Hydromorphone-d6 | |
| Amphetamine | 10000 | JWH-250 4-OH pentyl | 1000 | Oxazepam | 5000 | JWH 018 4-OH pentyl-d5 | |
| Benzoylecgonine | 5000 | Lorazepam | 5000 | Oxycodone | 5000 | JWH 019 6-OH hexyl-d5 | |
| Buphedrone | 1000 | MDA | 10000 | Oxymorphone | 5000 | MDPV-d8 | |
| Buprenorphine | 2000 | MDEA | 10000 | PCP | 2500 | Meperidine-d4 | |
| Carisoprodol | 10000 | MDMA | 10000 | Pregabalin | 10000 | Mephedrone-d3 | |
| Clomipramine | 5000 | MDPV | 1000 | Propoxyphene | 10000 | Meprobamate-d7 | |
| Codeine | 5000 | Meperidine | 5000 | Protriptyline | 5000 | Methadone-d3 | |
| Cotinine | 5000 | Mephedrone | 1000 | RCS4-4-OH-pentyl | 1000 | Methamphetamine-d5 | |
| Cyclobenzaprine | 5000 | Meprobamate | 10000 | Ritalinic Acid | 5000 | Methylone-d3 | |
| Desalkylflurazepam | 5000 | Methadone | 10000 | Sufentanil | 200 | Mitragynine-d3 | |
| Desipramine | 5000 | Methamphetamine | 10000 | Tapentadol | 5000 | Morphine-d6 | |
| Desmethyldoxepin | 5000 | Methedrone | 1000 | Temazepam | 5000 | Nordiazepam-d5 | |
| Dextromethorphan | 5000 | Methylone | 1000 | Tramadol | 5000 | Nortriptyline-d3 | |
| Diazepam | 5000 | Methylphenidate | 5000 | Zolpidem | 5000 | Oxycodone-d6 | |
| Dihydrocodeine | 5000 | Midazolam | 5000 | Amobarbital/pentobarbital | 10000 | Oxymorphone-d3 | |
| Doxepin | 5000 | Mitragynine | 1000 | Butabarbital | 10000 | THC-COOH-d3 | |
| EDDP | 10000 | Morphine | 5000 | Butalbital | 10000 | Butalbital-d5 | |
| Fentanyl | 200 | Naloxone | 5000 | Phenobarbital | 10000 | Secobarbital-d5 | |

Blue font: extended panel. Grey background: IS.

**Table 2-1: MRM Table (part 1)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *6-MAM-d3 IS* | *331.1* | *165* | *1.79* | *100* | *10* | *49* | *8* |
| *Amphetamine-d5 IS* | *141.1* | *93* | *1.7* | *30* | *10* | *21* | *8* |
| *Benzoylecgonine-d3 IS* | *293.1* | *171.2* | *2.17* | *80* | *10* | *25* | *12* |
| *Buprenorphine-d4 IS* | *472.3* | *400.2* | *2.77* | *120* | *10* | *52* | *18* |
| *Carisoprodol-d7 IS* | *267.1* | *180* | *2.99* | *35* | *10* | *11* | *12* |
| *Codeine-d6 IS* | *306.2* | *152.2* | *1.6* | *100* | *10* | *83* | *10* |
| *Fentanyl-d5 IS* | *342.3* | *105.1* | *2.59* | *90* | *10* | *50* | *8* |
| *Hydrocodone-d6 IS* | *306.2* | *202.1* | *1.85* | *100* | *10* | *39* | *14* |
| *Hydromorphone-d6 IS* | *292.1* | *185.1* | *1.2* | *100* | *10* | *39* | *12* |
| *JWH 018 4-OH pentyl-d5 IS* | *363.1* | *155.1* | *4.44* | *100* | *10* | *35* | *14* |
| *JWH 019 6-OH hexyl-d5 IS* | *377.2* | *155.1* | *4.42* | *90* | *10* | *27* | *12* |
| *MDPV-d8 IS* | *284.1* | *134.1* | *2.28* | *70* | *10* | *27* | *15* |
| *Meperidine-d4 IS* | *252.2* | *224.1* | *2.26* | *86* | *10* | *29* | *16* |
| *Mephedrone-d3 IS* | *181.1* | *148.1* | *1.98* | *46* | *10* | *26* | *6* |
| *Meprobamate-d7 IS* | *226.1* | *165.1* | *2.33* | *26* | *10* | *11* | *8* |
| *Methadone-d3 IS* | *313.2* | *105.1* | *3.29* | *60* | *10* | *35* | *14* |
| *Methamphetamine-d5 IS* | *155.2* | *92* | *1.84* | *45* | *10* | *25* | *6* |
| *Methylone-d3 IS* | *211.1* | *163.1* | *1.7* | *60* | *10* | *25* | *12* |
| *Mitragynine-d3 IS* | *402.2* | *174.1* | *2.81* | *71* | *10* | *40* | *12* |
| *Morphine-d6 IS* | *292.1* | *152* | *1.01* | *90* | *10* | *75* | *12* |
| *Nordiazepam-d5 IS* | *276.1* | *140* | *4* | *95* | *10* | *40* | *10* |
| *Nortriptyline-d3 IS* | *267.1* | *233.1* | *3.45* | *56* | *10* | *20* | *14* |
| *Oxycodone-d6 IS* | *322.1* | *247.1* | *1.79* | *75* | *10* | *40* | *8* |
| *Oxymorphone-d3 IS* | *305.1* | *230.1* | *1.1* | *90* | *10* | *40* | *8* |
| *PCP-d5 IS* | *249.3* | *96.1* | *2.5* | *56* | *10* | *41* | *8* |
| *6-MAM 1* | *328.1* | *165* | *1.8* | *100* | *10* | *48* | *6* |
| *6-MAM 2* | *328.1* | *211.1* | *1.8* | *100* | *10* | *34* | *8* |
| *7-Aminoclonazepam 1* | *286.1* | *121.1* | *2.27* | *80* | *10* | *37* | *13* |
| *7-Aminoclonazepam 2* | *286.1* | *250.1* | *2.27* | *80* | *10* | *29* | *13* |
| *7-Hydroxymitragynine 1* | *415.2* | *190.1* | *2.4* | *85* | *10* | *50* | *8* |
| *7-Hydroxymitragynine 2* | *415.2* | *226.1* | *2.4* | *85* | *10* | *30* | *10* |
| *Acetyl fentanyl 1* | *323.1* | *188.1* | *2.38* | *90* | *10* | *31* | *10* |
| *Acetyl fentanyl 2* | *323.1* | *105.1* | *2.38* | *90* | *10* | *50* | *8* |
| *Alpha-Hydroxyalprazolam 1* | *325.1* | *297.1* | *3.5* | *60* | *10* | *40* | *15* |
| *Alpha-Hydroxyalprazolam 2* | *327.1* | *299.1* | *3.5* | *60* | *10* | *35* | *15* |
| *Alpha-Hydroxymidazolam 1* | *342.1* | *203.1* | *3.66* | *60* | *10* | *36* | *10* |

**Table 2-2: MRM Table (part 2)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *Alpha-Hydroxymidazolam 2* | *342.1* | *168.1* | *3.66* | *60* | *10* | *53* | *11* |
| *Alpha-Hydroxytriazolam 1* | *359.1* | *331.1* | *3.24* | *90* | *10* | *37* | *12* |
| *Alpha-Hydroxytriazolam 2* | *359.1* | *239.1* | *3.24* | *90* | *10* | *60* | *12* |
| *alpha-PPP 1* | *204.1* | *105* | *1.86* | *80* | *10* | *30* | *9* |
| *alpha-PPP 2* | *204.1* | *133* | *1.86* | *80* | *10* | *26* | *14* |
| *alpha-PVP 1* | *232.1* | *91* | *2.21* | *80* | *10* | *30* | *10* |
| *alpha-PVP 2* | *232.1* | *126.1* | *2.21* | *80* | *10* | *32* | *12* |
| *Alprazolam 1* | *309.1* | *281.1* | *3.72* | *80* | *10* | *35* | *10* |
| *Alprazolam 2* | *309.1* | *205.1* | *3.72* | *80* | *10* | *50* | *10* |
| *AM 2201 4-OH pentyl 1* | *376.1* | *155.1* | *4.42* | *65* | *10* | *31* | *10* |
| *AM 2201 4-OH pentyl 2* | *376.1* | *127.1* | *4.42* | *65* | *10* | *71* | *8* |
| *Amitriptyline 1* | *278.1* | *233.1* | *3.38* | *70* | *10* | *23* | *12* |
| *Amitriptyline 2* | *278.1* | *91* | *3.38* | *70* | *10* | *27* | *7* |
| *Amphetamine 1* | *136* | *119* | *1.71* | *40* | *10* | *12* | *10* |
| *Amphetamine 2* | *136* | *91* | *1.71* | *40* | *10* | *40* | *8* |
| *Benzoylecgonine 1* | *290.1* | *168* | *2.17* | *80* | *10* | *37* | *12* |
| *Benzoylecgonine 2* | *290.1* | *105* | *2.17* | *80* | *10* | *40* | *8* |
| *Buphedrone 1* | *178.1* | *131.1* | *1.87* | *50* | *10* | *28* | *11* |
| *Buphedrone 2* | *178.1* | *132.1* | *1.87* | *50* | *10* | *22* | *12* |
| *Buprenorphine 1* | *468.3* | *55* | *2.8* | *120* | *10* | *85* | *8* |
| *Buprenorphine 2* | *468.3* | *414.2* | *2.8* | *120* | *10* | *46* | *18* |
| *Carisoprodol 1* | *261.1* | *176* | *2.97* | *35* | *10* | *11* | *12* |
| *Carisoprodol 2* | *261.1* | *97* | *2.97* | *35* | *10* | *24* | *7* |
| *Clomipramine 1* | *315.1* | *86.1* | *3.95* | *26* | *10* | *23* | *12* |
| *Clomipramine 2* | *315.1* | *58.1* | *3.95* | *26* | *10* | *67* | *16* |
| *Codeine 1* | *300.1* | *215.1* | *1.62* | *100* | *10* | *35* | *12* |
| *Codeine 2* | *300.1* | *165* | *1.62* | *100* | *10* | *50* | *12* |
| *Cotinine 1* | *177* | *80* | *1.78* | *70* | *10* | *28* | *8* |
| *Cotinine 2* | *177* | *98* | *1.78* | *70* | *10* | *25* | *9* |
| *Cyclobenzaprine 1* | *276.1* | *215.1* | *3.2* | *80* | *10* | *58* | *12* |
| *Cyclobenzaprine 2* | *276.1* | *216.1* | *3.2* | *80* | *10* | *34* | *12* |
| *Desalkylflurazepam 1* | *289.1* | *140* | *3.61* | *90* | *10* | *40* | *10* |
| *Desalkylflurazepam 2* | *289.1* | *226* | *3.61* | *90* | *10* | *38* | *16* |
| *Desipramine 1* | *267.1* | *72* | *3.28* | *70* | *10* | *50* | *8* |
| *Desipramine 2* | *267.1* | *193* | *3.28* | *70* | *10* | *50* | *12* |
| *Desmethyldoxepin 1* | *266.1* | *107* | *2.82* | *70* | *10* | *29* | *8* |

**Table 2-3: MRM Table (part 3)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *Desmethyldoxepin 2* | *266.1* | *235.1* | *2.82* | *70* | *10* | *21* | *10* |
| *Dextromethorphan 1* | *272.1* | *171.1* | *2.69* | *70* | *10* | *50* | *12* |
| *Dextromethorphan 2* | *272.1* | *215.1* | *2.69* | *70* | *10* | *30* | *14* |
| *Diazepam 1* | *285.1* | *193* | *4.27* | *80* | *10* | *40* | *10* |
| *Diazepam 2* | *285.1* | *154* | *4.27* | *80* | *10* | *35* | *8* |
| *Dihydrocodeine 1* | *302.1* | *199.1* | *1.57* | *95* | *10* | *44* | *14* |
| *Dihydrocodeine 2* | *302.1* | *201.1* | *1.57* | *95* | *10* | *40* | *14* |
| *Doxepin 1* | *280.1* | *107* | *2.81* | *70* | *10* | *45* | *10* |
| *Doxepin 2* | *280.1* | *115* | *2.81* | *70* | *10* | *60* | *8* |
| *EDDP 1* | *278.1* | *234.1* | *2.71* | *80* | *10* | *50* | *12* |
| *EDDP 2* | *278.1* | *186* | *2.71* | *80* | *10* | *46* | *10* |
| *Fentanyl 1* | *337.1* | *188.1* | *2.6* | *90* | *10* | *31* | *10* |
| *Fentanyl 2* | *337.1* | *105.1* | *2.6* | *90* | *10* | *50* | *8* |
| *Gabapentin 1* | *172.1* | *137* | *1.32* | *50* | *10* | *20* | *10* |
| *Gabapentin 2* | *172.1* | *95* | *1.32* | *50* | *10* | *30* | *8* |
| *Hydrocodone 1* | *300.1* | *199* | *1.87* | *100* | *10* | *50* | *12* |
| *Hydrocodone 2* | *300.1* | *128* | *1.87* | *100* | *10* | *70* | *10* |
| *Hydromorphone 1* | *286.1* | *185* | *1.21* | *100* | *10* | *39* | *10* |
| *Hydromorphone 2* | *286.1* | *157* | *1.21* | *100* | *10* | *51* | *8* |
| *Imipramine 1* | *281.1* | *86* | *3.28* | *80* | *10* | *25* | *6* |
| *Imipramine 2* | *281.1* | *58* | *3.28* | *80* | *10* | *51* | *6* |
| *JWH 018 4-OH pentyl 1* | *358.1* | *155.1* | *4.45* | *100* | *10* | *37* | *14* |
| *JWH 018 4-OH pentyl 2* | *358.1* | *127.1* | *4.45* | *100* | *10* | *63* | *10* |
| *JWH 018 Pentanoic acid 1* | *372.1* | *155.1* | *4.48* | *90* | *10* | *33* | *14* |
| *JWH 018 Pentanoic acid 2* | *372.1* | *127.1* | *4.48* | *90* | *10* | *71* | *10* |
| *JWH 019 6-OH hexyl 1* | *372.2* | *155.1* | *4.57* | *90* | *10* | *27* | *12* |
| *JWH 019 6-OH hexyl 2* | *372.2* | *127.1* | *4.57* | *90* | *10* | *69* | *10* |
| *JWH 073 3-OH butyl 1* | *344.1* | *155.1* | *4.39* | *100* | *10* | *33* | *14* |
| *JWH 073 3-OH butyl 2* | *344.1* | *127.1* | *4.39* | *100* | *10* | *70* | *8* |
| *JWH 073 Butanoic acid 1* | *358.2* | *155.1* | *4.38* | *90* | *10* | *33* | *10* |
| *JWH 073 Butanoic acid 2* | *358.2* | *127.1* | *4.38* | *90* | *10* | *65* | *10* |
| *JWH 081 5-OH-pentyl 1* | *388.1* | *185.1* | *4.4* | *100* | *10* | *31* | *18* |
| *JWH 081 5-OH-pentyl 2* | *388.1* | *114.1* | *4.4* | *100* | *10* | *100* | *12* |
| *JWH 122 5-OH pentyl 1* | *372.1* | *169.1* | *4.59* | *90* | *10* | *32* | *14* |
| *JWH 122 5-OH pentyl 2* | *372.1* | *141.1* | *4.59* | *90* | *10* | *40* | *12* |
| *JWH 210 5-OH-pentyl 1* | *386.1* | *183.1* | *4.71* | *100* | *10* | *45* | *18* |

**Table 2-4: MRM Table (part 4)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *JWH 210 5-OH-pentyl 2* | *386.1* | *155.1* | *4.71* | *100* | *10* | *41* | *6* |
| *JWH 250-4-OH pentyl 1* | *352.1* | *121.1* | *4.25* | *90* | *10* | *35* | *12* |
| *JWH 250-4-OH pentyl 2* | *352.1* | *91* | *4.25* | *90* | *10* | *65* | *8* |
| *Lorazepam 1* | *321.1* | *275.1* | *3.4* | *80* | *10* | *40* | *10* |
| *Lorazepam 2* | *321.1* | *229.1* | *3.4* | *80* | *10* | *40* | *10* |
| *MDA 1* | *180.1* | *105* | *1.87* | *40* | *10* | *30* | *8* |
| *MDA 2* | *180.1* | *133.1* | *1.87* | *40* | *10* | *25* | *10* |
| *MDEA 1* | *208.1* | *163.1* | *2.08* | *100* | *10* | *20* | *12* |
| *MDEA 2* | *208.1* | *105.1* | *2.08* | *100* | *10* | *35* | *8* |
| *MDMA 1* | *194* | *163* | *1.97* | *100* | *10* | *20* | *10* |
| *MDMA 2* | *194* | *135* | *1.97* | *100* | *10* | *26* | *10* |
| *MDPV 1* | *276.1* | *175.1* | *2.29* | *70* | *10* | *30* | *8* |
| *MDPV 2* | *276.1* | *205.1* | *2.29* | *70* | *10* | *25* | *10* |
| *Meperidine 1* | *248.1* | *220.1* | *2.26* | *86* | *10* | *28* | *10* |
| *Meperidine 2* | *248.1* | *174* | *2.26* | *86* | *10* | *26* | *10* |
| *Mephedrone 1* | *178.1* | *145.1* | *2.02* | *46* | *10* | *26* | *6* |
| *Mephedrone 2* | *178.1* | *144.1* | *2.02* | *46* | *10* | *38* | *10* |
| *Meprobamate 1* | *219* | *158* | *2.33* | *26* | *10* | *11* | *8* |
| *Meprobamate 2* | *219* | *97.2* | *2.33* | *26* | *10* | *19* | *6* |
| *Methadone 1* | *310.1* | *265* | *3.3* | *80* | *10* | *25* | *18* |
| *Methadone 2* | *310.1* | *105.1* | *3.3* | *80* | *10* | *33* | *14* |
| *Methamphetamine 1* | *150* | *119* | *1.85* | *60* | *10* | *15* | *8* |
| *Methamphetamine 2* | *150* | *91* | *1.85* | *60* | *10* | *45* | *6* |
| *Methedrone 1* | *194.1* | *161.1* | *1.88* | *50* | *10* | *26* | *6* |
| *Methedrone 2* | *194.1* | *146.1* | *1.88* | *50* | *10* | *36* | *6* |
| *Methylone 1* | *208.1* | *160.1* | *1.7* | *60* | *10* | *25* | *12* |
| *Methylone 2* | *208.1* | *132.1* | *1.7* | *60* | *10* | *32* | *14* |
| *Methylphenidate 1* | *234.1* | *84.1* | *2.21* | *31* | *10* | *60* | *8* |
| *Methylphenidate 2* | *234.1* | *56* | *2.21* | *31* | *10* | *65* | *8* |
| *Midazolam 1* | *326.1* | *291.1* | *3.66* | *101* | *10* | *45* | *22* |
| *Midazolam 2* | *326.1* | *249.1* | *3.66* | *101* | *10* | *49* | *18* |
| *Mitragynine 1* | *399.2* | *174.1* | *2.79* | *71* | *10* | *59* | *12* |
| *Mitragynine 2* | *399.2* | *159.1* | *2.79* | *71* | *10* | *37* | *12* |
| *Morphine 1* | *286* | *152* | *1.02* | *90* | *10* | *75* | *12* |
| *Morphine 2* | *286* | *165* | *1.02* | *90* | *10* | *50* | *12* |
| *Naloxone 1* | *328.1* | *212.1* | *1.6* | *90* | *10* | *49* | *14* |

**Table 2-5: MRM Table (part 5)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *Naloxone 2* | *328.1* | *253.1* | *1.6* | *90* | *10* | *35* | *18* |
| *Naltrexone 1* | *342.1* | *267.2* | *1.75* | *86* | *10* | *39* | *18* |
| *Naltrexone 2* | *342.1* | *282.1* | *1.75* | *86* | *10* | *37* | *20* |
| *N-desmethyl-Tapentadol 1* | *208.1* | *107* | *2.18* | *120* | *10* | *30* | *10* |
| *N-desmethyl-Tapentadol 2* | *208.1* | *121* | *2.18* | *120* | *10* | *25* | *10* |
| *Norbuprenorphine 1* | *414.3* | *55* | *2.43* | *141* | *10* | *90* | *12* |
| *Norbuprenorphine 2* | *414.3* | *83* | *2.43* | *141* | *10* | *70* | *12* |
| *Norcodeine 1* | *286.1* | *152* | *1.53* | *80* | *10* | *75* | *10* |
| *Norcodeine 2* | *286.1* | *165* | *1.53* | *80* | *10* | *58* | *10* |
| *Nordiazepam 1* | *271.1* | *140.1* | *4.03* | *71* | *10* | *37* | *10* |
| *Nordiazepam 2* | *271.1* | *165.1* | *4.03* | *71* | *10* | *37* | *14* |
| *Norfentanyl 1* | *233.1* | *84.1* | *2.12* | *50* | *10* | *23* | *8* |
| *Norfentanyl 2* | *233.1* | *150.1* | *2.12* | *50* | *10* | *23* | *12* |
| *Norhydrocodone 1* | *286.1* | *199* | *1.83* | *70* | *10* | *39* | *11* |
| *Norhydrocodone 2* | *286.1* | *241.1* | *1.83* | *70* | *10* | *32* | *11* |
| *Normeperidine 1* | *234.1* | *160* | *2.27* | *45* | *10* | *30* | *14* |
| *Normeperidine 2* | *234.1* | *42* | *2.27* | *45* | *10* | *45* | *6* |
| *Noroxycodone 1* | *302.1* | *187.1* | *1.77* | *40* | *10* | *33* | *10* |
| *Noroxycodone 2* | *302.1* | *227.1* | *1.77* | *40* | *10* | *40* | *11* |
| *Norpropoxyphene 1* | *308.1* | *100* | *2.85* | *50* | *10* | *18* | *10* |
| *Norpropoxyphene 2* | *308.1* | *44* | *2.85* | *50* | *10* | *50* | *6* |
| *Nortriptyline 1* | *264.1* | *233.1* | *3.39* | *56* | *10* | *17* | *4* |
| *Nortriptyline 2* | *264.1* | *117.1* | *3.39* | *56* | *10* | *25* | *4* |
| *O-Desmethyltramadol 1* | *250.1* | *58.1* | *1.8* | *55* | *10* | *97* | *19* |
| *O-Desmethyltramadol 2* | *250.1* | *42* | *1.8* | *55* | *10* | *97* | *4* |
| *Oxazepam 1* | *287.1* | *241.1* | *3.55* | *76* | *10* | *31* | *18* |
| *Oxazepam 2* | *287.1* | *269.2* | *3.55* | *76* | *10* | *24* | *22* |
| *Oxycodone 1* | *316.1* | *241.1* | *1.79* | *75* | *10* | *38* | *8* |
| *Oxycodone 2* | *316.1* | *256.1* | *1.79* | *75* | *10* | *33* | *8* |
| *Oxymorphone 1* | *302* | *227.1* | *1.1* | *86* | *10* | *39* | *18* |
| *Oxymorphone 2* | *302* | *198.1* | *1.1* | *86* | *10* | *57* | *14* |
| *PCP 1* | *244.1* | *159.1* | *2.5* | *56* | *10* | *19* | *10* |
| *PCP 2* | *244.1* | *91.1* | *2.5* | *56* | *10* | *41* | *8* |
| *Pregabalin 1* | *160.1* | *55.1* | *1.27* | *58* | *10* | *31* | *8* |
| *Pregabalin 2* | *160.1* | *97.1* | *1.27* | *58* | *10* | *19* | *8* |
| *Propoxyphene 1* | *340.1* | *266.1* | *3.05* | *46* | *10* | *20* | *10* |

**Table 2-6: MRM Table (part 6)**

| Analyte | Q1 | Q3 | RT (min) | DP | EP | CE | CXP |
|---|---|---|---|---|---|---|---|
| *Propoxyphene 2* | *340.1* | *91* | *3.05* | *46* | *10* | *60* | *8* |
| *Protriptyline 1* | *264.1* | *155* | *3.2* | *76* | *10* | *29* | *10* |
| *Protriptyline 2* | *264.1* | *191* | *3.2* | *76* | *10* | *43* | *16* |
| *RCS4-4-OH-pentyl 1* | *338.2* | *135* | *4.16* | *90* | *10* | *40* | *8* |
| *RCS4-4-OH-pentyl 2* | *338.2* | *77* | *4.16* | *90* | *10* | *75* | *8* |
| *Ritalinic Acid 1* | *220.1* | *84* | *2.07* | *50* | *10* | *60* | *8* |
| *Ritalinic Acid 2* | *220.1* | *56* | *2.07* | *50* | *10* | *62* | *8* |
| *Sufentanil 1* | *387.1* | *238* | *3.01* | *46* | *10* | *27* | *16* |
| *Sufentanil 2* | *387.1* | *111.1* | *3.01* | *46* | *10* | *49* | *10* |
| *Tapentadol 1* | *222.1* | *107* | *2.19* | *100* | *10* | *40* | *8* |
| *Tapentadol 2* | *222.1* | *121* | *2.19* | *100* | *10* | *26* | *8* |
| *Temazepam 1* | *301.1* | *255.1* | *3.9* | *70* | *10* | *50* | *8* |
| *Temazepam 2* | *301.1* | *177.1* | *3.9* | *70* | *10* | *50* | *10* |
| *Tramadol 1* | *264.1* | *58.1* | *2.17* | *66* | *10* | *103* | *8* |
| *Tramadol 2* | *264.1* | *42.2* | *2.17* | *66* | *10* | *103* | *6* |
| *Zolpidem 1* | *308.1* | *235.1* | *2.49* | *85* | *10* | *60* | *12* |
| *Zolpidem 2* | *308.1* | *219* | *2.49* | *40* | *10* | *77* | *6* |
| *THC-COOH-d3 IS* | *346.1* | *302.1* | *4.73* | *-110* | *-10* | *-28* | *-11* |
| *Butalbital-d5 IS* | *228.1* | *42* | *2.74* | *-65* | *-10* | *-40* | *-7* |
| *Secobarbital-d5 IS* | *242.1* | *42* | *3.06* | *-90* | *-10* | *-40* | *-7* |
| *Amobarbital/Pentobarbital 1* | *225.1* | *42* | *2.86* | *-60* | *-10* | *-40* | *-7* |
| *Amobarbital/Pentobarbital 2* | *225.1* | *182* | *2.86* | *-60* | *-10* | *-18* | *-10* |
| *Butabarbital 1* | *211.1* | *42* | *2.53* | *-60* | *-10* | *-40* | *-7* |
| *Butabarbital 2* | *211.1* | *168* | *2.53* | *-60* | *-10* | *-18* | *-9* |
| *Butalbital 1* | *223.1* | *42* | *2.6* | *-65* | *-10* | *-40* | *-7* |
| *Butalbital 2* | *223.1* | *180* | *2.6* | *-65* | *-10* | *-16* | *-10* |
| *Phenobarbital 1* | *231.1* | *42.1* | *2.42* | *-70* | *-10* | *-40* | *-7* |
| *Phenobarbital 2* | *231.1* | *188* | *2.42* | *-70* | *-10* | *-14* | *-10* |
| *Secobarbital 1* | *237.1* | *42.1* | *3.06* | *-90* | *-10* | *-40* | *-7* |
| *Secobarbital 2* | *237.1* | *194.1* | *3.06* | *-77* | *-10* | *-17* | *-10* |
| *THC-COOH 1* | *343.1* | *299.1* | *4.73* | *-110* | *-10* | *-28* | *-11* |
| *THC-COOH 2* | *343.1* | *245.1* | *4.73* | *-110* | *-10* | *-36* | *-9* |

Blue font: negative mode.

## *LC performance*

A Phenomenex Kinetex Phenyl-Hexyl column (50 × 4.6 mm, 2.6 μm) was used for LC separation. A guard column was used for LC column protection. Using a rapid 6.5-minute LC gradient, we achieved separation of various isobaric compounds in the panel. In addition, the size and dimension of this column yielded less than 3000 psi column pressure at 1 mL/min flow rate throughout the gradient while still maintaining the narrow LC peak width. Under these conditions, at 20% organic content in the injected solution, we did not notice any peak front-tailing for those early eluters including gabapentin, morphine and oxymorphone. Figure 3 shows the elution profile of all the compounds, in which the top trace shows the extracted ion chromatograms (XICs) for the barbiturates and THC-COOH (negative mode) and the bottom trace the XICs for the remaining compounds in positive mode. Figure 4 shows the LC separation of several isobaric compounds in the panel.

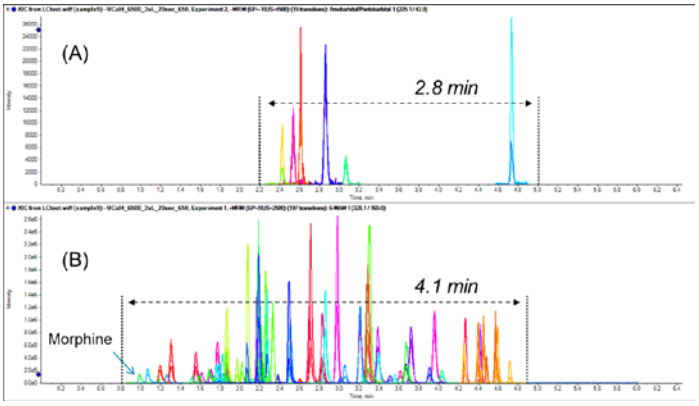


**Figure 3: Elution profile of all the 93 compounds in the panel. (A) Compounds in negative mode. (B) Compounds in positive mode.**

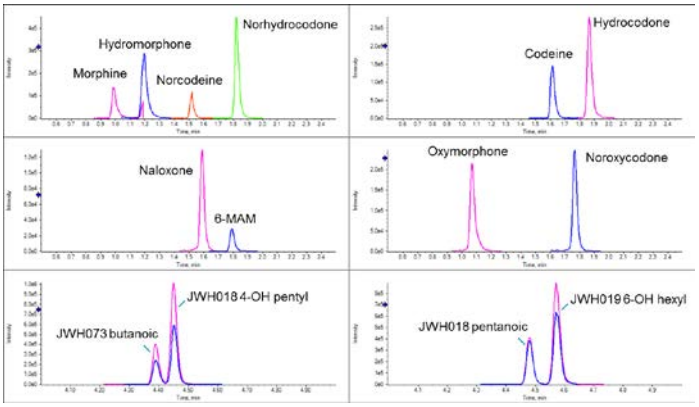


**Figure 4: LC separation of isobaric compounds.**

## *Scheduled* MRM™

We designed the LC gradient 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 re-optimized *Scheduled* MRM™ algorithm in Analyst® 1.6.3 ensured optimal data quality even during regions of the chromatogram when the MRM concurrency was very high. Most MRM transitions had 15 or more data points across the LC peaks. A minimum of 10 data points was achieved across every peak (Figure 5).

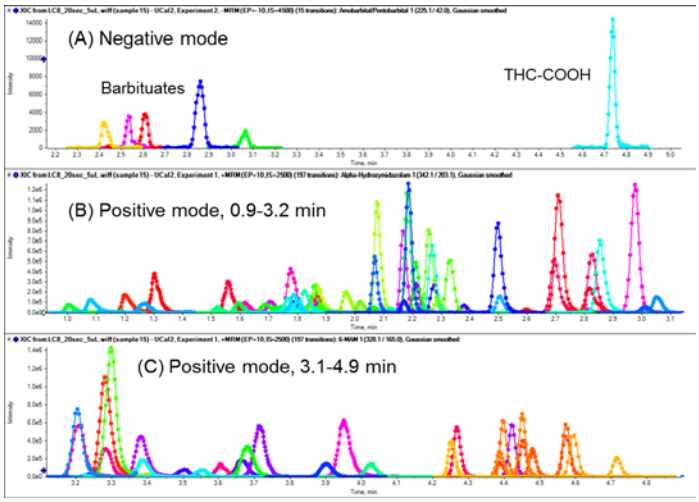


**Figure 5: Representative chromatogram, highlighting the number of data points across each LC peaks. (A) Negative mode. (B) and (C) Positive mode.**

## *Analytical sensitivity*

The processed urine sample had a final dilution factor of 10. With only 5 μL injection volume (equivalent of 0.5 μL unprocessed urine), all compounds were detected with ease. For a few analytes such as fentanyl/norfentanyl/acetyl fentanyl/sufentanil, buprenorphine/norbuprenorphine, 6-MAM and THC-COOH, the lowest calibrator concentration was between 1 and 10 ng/mL. For instance, for acetyl fentanyl, the concentration at the lowest calibrator level was 1 ng/mL, which is equivalent to a mere 0.5 pg on-column. Figure 6 shows XICs of the quantifier MRM of acetyl fentanyl (323.1 → 188.0 m/z). Three replicate set of injections were shown (row 1 to 3). Figure 7 shows XICs for THC-COOH in negative mode. As can be observed from the data shown, in both cases, the analyte MRM signal was strong at the lowest calibrator level, suggesting the possibility of reaching even lower LOQ. Figure 8 shows typical calibration curves for the following analytes: codeine, nortriptyline, temazepam and THC-COOH. Excellent linearity and reproducibility was observed across the entire concentration ranges assessed in this effort.

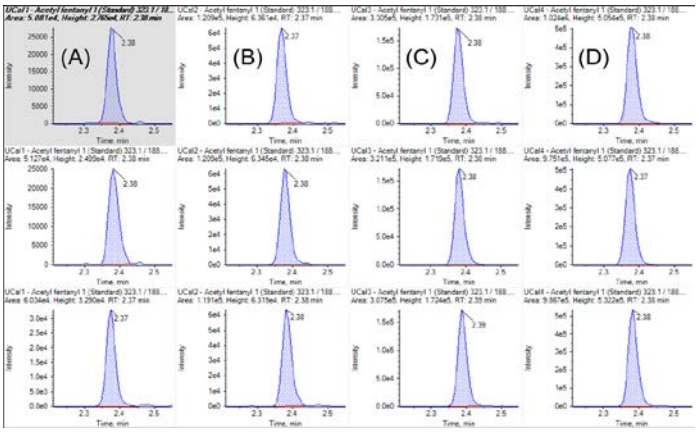


**Figure 6: Extracted ion chromatograms (XICs) of the quantifier MRM of acetyl fentanyl (n=3, 323.1 → 188.0 m/z). (A) 1 ng/mL; (B) 2 ng/mL; (C) 6 ng/mL; (D) 20 ng/mL.**

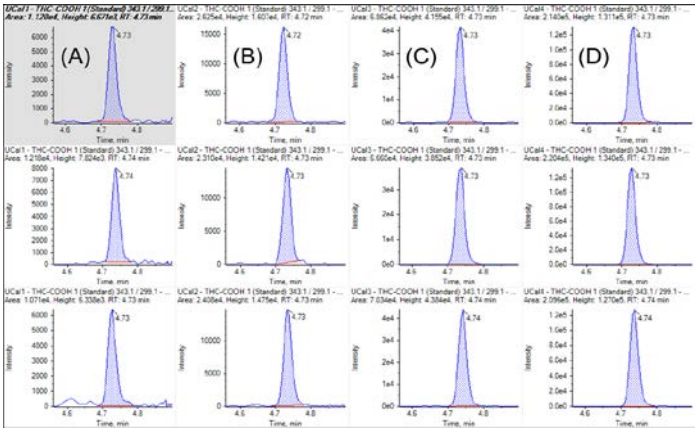


**Figure 7: Extracted ion chromatograms (XICs) of the quantifier MRM for THC-COOH (n=3, 343.1 → 299.1 m/z). (A) 10 ng/mL; (B) 20 ng/mL; (C) 60 ng/mL; (D) 200 ng/mL.**

### *Polarity Switching and Sample Throughput*

It was essential to employ rapid polarity switching in order to accommodate 93 compounds (more than 200 MRMs) within a single, rapid data acquisition method. Compared to two separate injections (one for each polarity), this rapid polarity switching method offers a significant improvement in sample throughput. In the current extended panel consisting of 93 compounds, the LC runtime is 6.5 minutes. With a smaller panel (e.g. 72 compounds), we can easily reduce the LC runtime to 5.5 minutes (Figure 9).

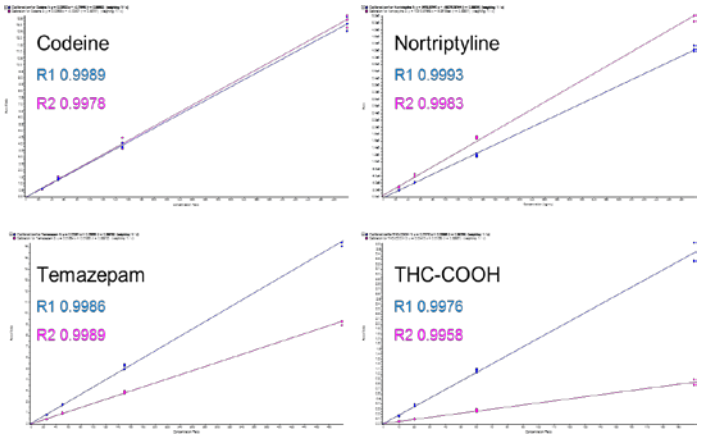


**Figure 8: Typical calibration curves for selected compounds.**

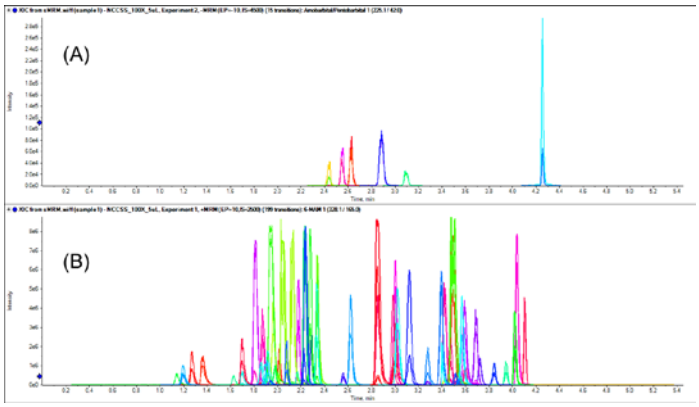


**Figure 9: Elution profile of a smaller panel (72 compounds). (A) Compounds in negative mode. (B) Compounds in positive mode.**

## Conclusion

A rapid and sensitive method for the LC-MS/MS analysis of a 93-compound forensic panel in human urine was developed using the SCIEX ExionLC™ AC HPLC system and the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system. The method takes advantage of the re-optimized *Scheduled* MRM™ algorithm, and the fast polarity switching capability of the 4500 series, to deliver high throughput and high-quality data. This method utilized a dilute-and-shoot sample preparation procedure. Excellent linearity and precision were observed for all the compounds across the relevant calibration range.

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