Rapid DUID screening of 17 drugs of abuse in plasma and saliva

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System with an ExionLC™ AD System

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The offense of driving under the influence of drugs, or DUID, typically relates to driving while being intoxicated on mind-aterning substances such as prescribed or illegal drugs. This serious crime can result in serious DUI charges and carries significant financial, legal, and social penalties. These substances are known to act on the central nervous system and impair the driver's alertness, concentration and judgment as well as their overall ability to operate a vehicle safely. Over the years, drug-impaired driving has become a prevalent cause of motor vehicle fatalities worldwide. As a result, detecting the presence of these drugs in the impaired driver's system is paramount to law enforcement. They require comprehensive drug screening approaches to confirm the presence of these substances and support evidence to prosecute the DUID charge.

Traditionally, DUID screens are either performed by immunoassay or GC-MS. However, immunoassays are known to suffer from cross-reactivity, have poor sensitivity and are prone to a high rate of false positives. On the other hand, GC-MS requires lengthy sample preparation and derivatization which considerably slows down the analytical process. As a result, there is a critical need to develop rapid, robust and comprehensive drug screening methods for positive identification and accurate quantification of these substances from biological specimens.

In this technical note, a simple and fast sample preparation procedure was used in combination with the SCIEX Triple Quad 3500 LC-MS/MS System for picogram to sub-nanogram per mL detection of 17 drugs in plasma and saliva samples. This targeted screening method is shown to provide a fast and sensitive quantitative solution for high-throughput detection of these substances typically screened in DUID cases.

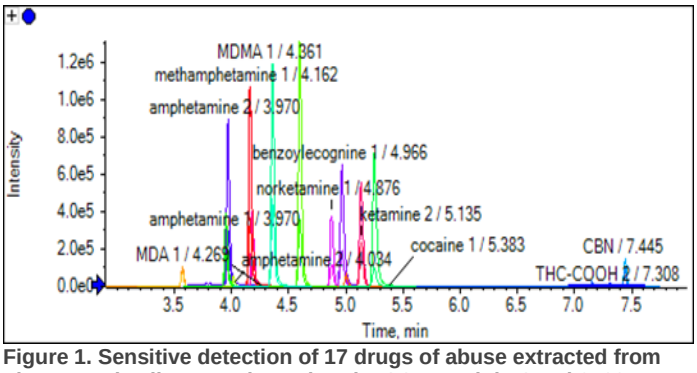

Figure 1. Sensitive detection of 17 drugs of abuse extracted from plasma and saliva samples using the SCIEX Triple Quad 3500 System. Extracted Ion Chromatogram (XIC) showing baseline separation of the 17 drugs of abuse screened in this DUID panel.

Key advantages for sensitive detection of 17 drugs of abuse in plasma and saliva samples

- Fast and simple sample preparation procedure enabled efficient extraction of drugs from plasma and saliva samples
- Rapid (10 minutes) and high-throughput MRM acquisition method provides accurate and sensitive quantitation of the 17 drugs in the panel, enabling pg to sub-ng/mL detection limits in plasma and saliva samples
- Fast polarity switching capabilities on the SCIEX Triple Quad 3500 System enabled detection of ions in positive and negative mode in a single run, negating the need for a two injection workflow traditionally performed
- Workflow demonstrated excellent linearity for concentrations ranging from 0.1 to 50 ng/mL with R values greater than 0.995
- Excellent precision and accuracy as demonstrated by RSD values less than 5% for multiple quality control samples acquired at three different concentration levels
- Analyte extraction recoveries were demonstrated to be between 70 and 120% for the majority of the drugs in the DUID panel
- Method enabled identification and quantification of drugs present in real plasma and saliva case samples, demonstrating the robustness of the developed workflow in complex biological matrices
- Method can be easily implemented by law enforcement authorities as a fast and robust screening method to test drivers suspected of DUID

Methods

Sample preparation: A total of 17 drugs commonly screened for in DUID cases were selected for this panel. The full list of the drugs included in this DUID panel is summarized in Table 1. Drugs were extracted from plasma and saliva samples by using a protein precipitation procedure. In short, 300 µL of plasma or saliva was added to a centrifuge tube to which 900 µL of acetonitrile was added and vigorously vortexed for 30 seconds. The samples were then centrifuged for 10 min at 12,000 rpm. 250 µL of the supernatant was transferred out to a glass tube and completely dried for 20 minutes at 60˚C under a gentle nitrogen flow. The residues were reconstituted with 100 µL of an acetonitrile/water (20:80, v/v) solution. The protein precipitation procedure is summarized in Figure 2.

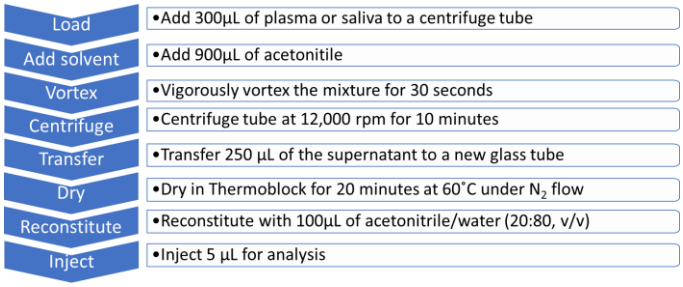

Figure 2. Protein precipitation procedure for plasma and saliva samples. An 8-step protein precipitation procedure was used for extracting the 17 drugs from plasma and saliva samples for MS analysis.

Table 1. List of the 17 drugs along with their molecular formula included in the DUID panel.

Compound Name	Molecular Formula
6-acetylmorphine	$C_{19}H_{21}NO_4$
Morphine	$C_{17}H_{19}NO_3$
Cocaine	$C_{17}H_{21}NO_4$
Benzoylecognine	$C_{17}H_{21}NO_4$
Cannabinol	$C_{21}H_{26}O_2$
11-nor-9-carboxy-THC	$C_{21}H_{28}O_4$
Methamphetamine	$C_{10}H_{15}N$
Amphetamine	$C_9H_{13}N$
MDMA	$C_{11}H_{15}NO_2$
MDA	$C_{10}H_{13}NO_2$
Ketamine	$C_{13}H_{16}ClNO$
Methcathinone	$C_{10}H_{13}NO$
Heroin	$C_{21}H_{23}NO_5$
3,4-Methylene dihydro-N-ethyl-amphetamine	$C_{12}H_{17}NO_2$
Norketamine	$C_{12}H_{14}ClNO$
Cannabidiol	$C_{21}H_{30}O_2$
Tetrahydrocannabinol	$C_{21}H_{30}O_2$

Liquid chromatography: UHPLC separation was performed on a Phenomenex Kinetex Biphenyl (100 × 3 mm, 2.6µm, 00D-4622-Y0) held at 40°C on a SCIEX ExionLC AC System. Mobile phases used consisted of ammonium formate, methanol, and appropriate additives. The flow rate was 0.4 mL/min. The injection volume was 5 µL and the total LC runtime was 10 minutes.

Mass spectrometry: MS and MS/MS data were collected using the Scheduled MRM™ Algorithm on the SCIEX Triple Quad 3500 LC-MS/MS System. The 17 drugs were detected in positive and negative electrospray ionization (ESI) modes using the polarity switching capabilities of the instrument. Two MRM transitions per compound were used to allow confirmation and quantification of the drugs. Samples were injected in triplicate.

Data analysis: Data was acquired in Analyst® Software 1.7 and processed in MultiQuant™ Software 3.0.3.

Scheduled MRM Algorithm ensures sensitive detection and accurate quantitation of drugs of abuse extracted from plasma and saliva

Control plasma and saliva samples spiked with the mixture of the 17 drugs of abuse were prepared at various concentrations ranging from 0.1 to 50 ng/mL. These standard mixtures were extracted using the aforementioned procedure and injected in triplicate to build a data processing method.

Figure 1 shows the chromatographic profile of the 17 drugs of abuse in the DUID panel in a control plasma sample at final drug concentration of 5 ng/mL. The 10-minute long gradient in combination with the column selection and the choice of mobile phase composition resulted in baseline separation of the 17 drugs, as seen in the extracted ion chromatogram (XIC) traces shown in Figure 1.

The linearity of the method was assessed for both the plasma-spiked and the saliva-spiked control samples. Calibration curves were generated for each of the drugs in the DUID panel and plotted across seven calibration levels ranging from 0.1 to 50 ng/mL. Figure 3 shows the resulting calibrations curves for the plasma-spiked (A) and the saliva-spiked (B) control samples. The regression curves showed excellent correlation with R values greater than 0.995 for all 17 drugs in the DUID panel, regardless of the matrix from which they were extracted. The results demonstrate the robustness of the extraction method across a wide variety of drug chemistries and the wide applicability of the protein precipitation procedure for both plasma and saliva samples.

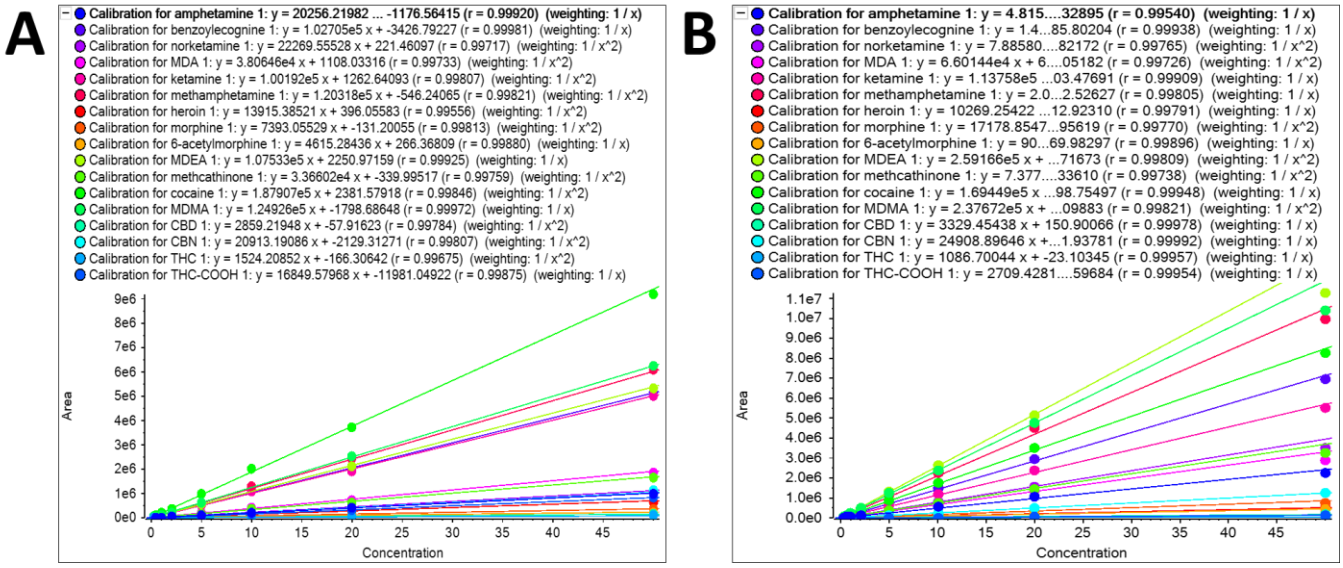

Figure 3: Excellent linearity for the 17 drugs in the DUID panel. Calibration curves resulting from the calibration series for the 17 drugs from 0.1 to 50 ng/mL extracted from (A) plasma and (B) saliva samples. R values greater than 0.995 were observed for all 17 drugs, regardless of the matrix from which they were extracted.

Achieving high sensitivity is essential to any toxicology workflow that requires accurate quantification of low levels of drugs in complex biological matrix. The sensitivity of the workflow was investigated by determining the lower limit of quantitation (LLOQ) for the 17 drugs of abuse in both plasma and saliva matrix. These values were determined based on the lowest concentration at which the integrated peak area of the analyte was quantifiable. Table 2 shows the LLOQ for each of the drugs extracted from plasma and saliva samples. The LLOQ values range from 0.0025 to 0.25 ng/mL for all the drugs in the DUID panel, demonstrating the sensitivity of the workflow on the SCIEX Triple Quad 3500 System.

Developing toxicology workflows that are both robust and reproducible is key to attaining reliable quantification of drugs in biological specimens. The reproducibility of the assay was assessed by spiking the control plasma and saliva samples at three concentration levels (1, 5 and 50 ng/mL). Three replicate injections were performed to determine the precision of measurement (expressed as percent variation coefficient, CV%). Table 3 shows the average reproducibility values at each of the three calibrator levels for each of the drugs of abuse in both plasma and saliva matrix. Overall, the assay showed great reproducibility at all three calibration levels with %CV values between 0.47 and 6.15% for the drugs in plasma and 0.6 and 5.74% for the drugs in saliva, respectively.

Table 2. LLOQ values for the 17 drugs of abuse included in the DUID panel in both plasma and saliva matrix.

Compound Name	LLOQ in plasma (ng/mL)	LLOQ in saliva (ng/mL)
6-acetylmorphine	*0.01*	*0.04*
Morphine	*0.01*	*0.05*
Cocaine	*0.0025*	*0.0025*
Benzoylecognine	*0.01*	*0.01*
Cannabinol	*0.25*	*0.25*
11-nor-9-carboxy-THC	*2*	*1*
Methamphetamine	*0.05*	*0.1*
Amphetamine	*0.1*	*0.25*
MDMA	*0.005*	*0.01*
MDA	*0.1*	*0.2*
Ketamine	*0.01*	*0.01*
Methcathinone	*0.1*	*0.2*
Heroin	*0.02*	*0.02*
3,4-Methylene dihydro-N-ethyl-amphetamine	*0.005*	*0.01*
Norketamine	*0.01*	*0.03*
Cannabidiol	*0.05*	*0.25*
Tetrahydrocannabinol	*0.025*	*0.05*

Table 3. Average results showing the precision of measurement (expressed as percent variation coefficient, % CV, n=3) for each of the 17 drugs of abuse at the three concentration levels (1, 5 and 50 ng/mL) extracted from plasma and saliva matrix.

Compound	% CV in Plasma Matrix			% CV in Saliva Matrix		
	1 ng/mL	5 ng/mL	50 ng/mL	1 ng/mL	5 ng/mL	50 ng/mL
6-acetylmorphine	5.02	2.9	0.48	1.05	2.16	0.64
Morphine	3.63	2.21	0.51	0.65	0.82	1.74
Cocaine	4.11	3	2.7	1.12	1.24	2.88
Benzoylecognine	3.17	1.57	1.4	1.87	2.98	2.26
Cannabinol	2.13	1.43	6.15	2.64	2.86	3.53
11-nor-9-carboxy-THC	3.11	1.37	1.38	1.17	0.6	2.96
Methamphetamine	1.01	2.22	1.42	3.91	3.18	1.53
Amphetamine	3.21	1.11	2.3	3.38	4.14	0.68
MDMA	3.67	1.35	1.64	2.34	1.26	0.75
MDA	1.24	1.96	1.35	1.73	5.74	2.13
Ketamine	2.89	2.77	1.42	2.65	3.16	1.92
Methcathinone	3.06	1.76	2.54	2.88	2.79	4.43
Heroin	1.45	2.42	1.71	1.84	1.96	1.74
3,4-Methylene dihydro-N-ethyl-amphetamine	4.5	0.47	1.02	1.5	2.21	1.87
Norketamine	1.86	1.55	0.87	2.79	1.55	3.12
Cannabidiol	3.12	3.23	1.66	4.35	3.41	2.38
Tetrahydrocannabinol	1.83	0.88	4.57	4.46	2.37	1.62

Fast and efficient sample preparation procedure leads to high recovery of drugs in plasma and saliva samples

Fast and reliable sample extraction procedures are critical to accurately measure the concentration of drugs from complex biological matrix. The recovery of the drugs included in this panel was calculated to assess the efficiency of the protein precipitation procedure for plasma and saliva samples. The recovery values were calculated for each of the three concentration levels (1, 10 and 50 ng/mL) by expressing ratio of the peak areas of each analyte spiked before and after the extraction procedure as a percentage. Table 4 lists the recoveries of each drug at the three concentration levels for plasma and saliva samples. The protein precipitation procedure used in this experiment demonstrated recoveries between 70.57 and 118.8% for the drugs in plasma and 55.54 and 114.97% for the drugs in saliva, respectively. The recovery values for all the drugs and drug metabolites in this panel enabled reliable and reproducible quantitation in both matrices.

Evaluating method in case samples

Developing a robust workflow is key to its full implementation to routine testing in the forensic laboratory. The robustness of the workflow was investigated by analyzing a plasma case sample from a driver suspected of operating a motor vehicle under the influence of drugs. The sample was subject to the protein precipitation procedure and screened using the developed method. Amphetamine and methamphetamine were detected in the case sample at concentrations of 21.42 and 155.73 ng/mL, respectively. Figure 4 shows the extracted ion chromatograms of each of the two drugs were detected in the driver's sample. The results from the analysis demonstrated the robustness of the DUID screening workflow and showed that the combined sample preparation procedure with Scheduled MRM Algorithm on the SCIEX Triple Quad 3500 System enabled accurate identification and sensitive detection of drugs in real case samples.

Table 4. Average (n=3) recovery values for each of the 17 drugs of abuse at the three concentration levels (1, 5 and 50 ng/mL) extracted from plasma and saliva matrix.

Compound	Recovery Values in Plasma Matrix			Recovery Values in Saliva Matrix		
	1 ng/mL	5 ng/mL	50 ng/mL	1 ng/mL	5 ng/mL	50 ng/mL
6-acetylmorphine	*91.63*	*111.30*	*114.97*	*86.94*	*99.23*	*82.31*
Morphine	*111.20*	*102.30*	*100.64*	*75.18*	*82.67*	*76.24*
Cocaine	*101.48*	*108.14*	*94.38*	*91.85*	*97.60*	*85.09*
Benzoylecognine	*100.34*	*93.70*	*102.83*	*92.65*	*97.03*	*86.97*
Cannabinol	*92.31*	*85.83*	*102.25*	*71.65*	*55.54*	*57.23*
11-nor-9-carboxy-THC	*101.62*	*89.04*	*76.65*	*-*	*73.70*	*85.60*
Methamphetamine	*80.90*	*70.88*	*81.26*	*93.39*	*97.67*	*85.32*
Amphetamine	*84.39*	*81.38*	*87.23*	*95.41*	*97.48*	*80.58*
MDMA	*99.02*	*87.38*	*96.05*	*98.15*	*101.27*	*82.84*
MDA	*106.69*	*84.77*	*92.78*	*101.04*	*100.69*	*83.91*
Ketamine	*98.41*	*88.26*	*95.31*	*91.60*	*93.89*	*85.78*
Methcathinone	*84.72*	*70.57*	*73.83*	*85.26*	*86.33*	*79.50*
Heroin	*107.58*	*85.58*	*93.54*	*93.32*	*94.37*	*88.46*
3,4-Methylene dihydro-N-ethyl-amphetamine	*95.99*	*91.26*	*92.93*	*107.48*	*107.76*	*87.98*
Norketamine	*94.54*	*90.54*	*95.50*	*108.60*	*99.62*	*87.41*
Cannabidiol	*98.13*	*85.54*	*97.30*	*76.80*	*71.10*	*65.07*
Tetrahydrocannabinol	*87.39*	*82.29*	*95.63*	*74.71*	*61.44*	*63.30*

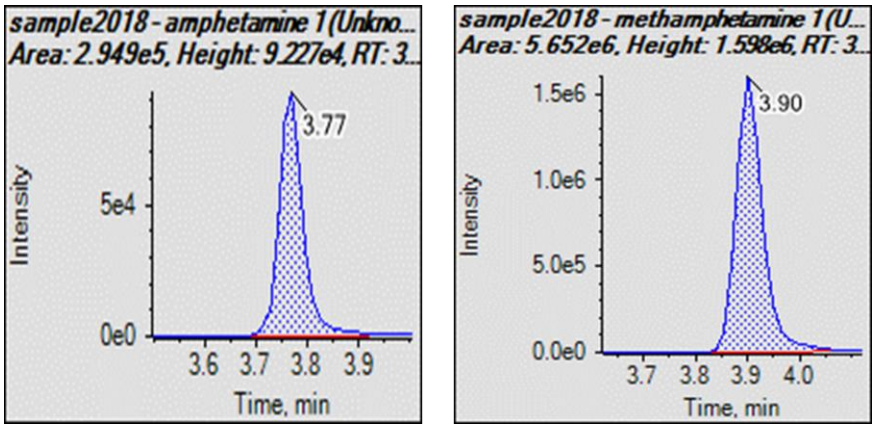

Figure 4. Positive identification of two drugs of abuse in a plasma case sample.
Extracted Ion Chromatogram (XIC) traces showing positive identification of amphetamine and methamphetamine at concentrations of 21.42 and 155.73 ng/mL, respectively.

Conclusions

The combination of fast and robust protein precipitation procedure with optimized chromatography on the SCIEX Triple Quad 3500 System allowed efficient and sensitive detection of 17 drugs of abuse typically screened in DUID case. The broad applicability of the sample preparation procedure enabled extraction of these drugs from plasma and saliva matrix at pg to low ng/mL levels and with high recoveries.

- An 8-step extraction procedure using a protein precipitation efficiently extracted drugs from plasma and saliva samples
- Scheduled MRM Algorithm enabled analysis of 17 different drugs in a single 10 minute method
- Excellent linearity across seven calibration levels ranging from 0.1 to 50 ng/mL was achieved, with R values greater than 0.995 for all the drugs in the DUID panel
- Excellent reproducibility of measurement was achieved at all three calibration (1, 5 and 50 ng/mL) levels with %CV values between 0.47 and 6.15% for the drugs in plasma and 0.6 and 5.74% for the drugs in saliva, respectively
- Extraction method demonstrated recoveries between 70.57 and 118.8% for the drugs in plasma and 55.54 and 114.97% for the drugs in saliva, respectively
- Developed workflow enabled confident identification and accurate quantification of drugs in a real case plasma sample, demonstrating the applicability of the method for screening DUID sample
- This robust method provides the necessary evidence for toxicologists, law enforcement agencies and other traffic safety personel to successfully investigate and prosecute DUID cases
- Overall, the efficiency of the extraction method combined with the analytical performance of the SCIEX Triple Quad 3500 System is providing a robust method for screening DUID case samples, making it easily adaptable into a forensic toxicology laboratory

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