Modernizing the forensic lab with LC-MS/MS technology

Innovative SCIEX analytical tools for the rapid identification of drugs of abuse in forensic samples

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The abuse of opioids and other classes of drugs continues to be a serious public health problem around the world. Investigators report a sevenfold increase in the number of drivers killed in car crashes while under the influence of drugs (i.e., Forensic DUID cases).[1] Unlike alcohol, there is no reliable forensic test for driving while impaired by other drugs, limiting the ability of law enforcement efforts to save lives.

Forensic laboratories use GC-MS as the golden standard analysis tool technique of choice for drug analysis. Recently, the United States Department of Transportation (USDOT) has expanded the list of regulated forensic compounds for motorists abuse to include four "semi-synthetic" opioids: hydrocodone, hydromorphone, oxycodone and oxymorphone.[2] Typical forensic analyses of these analytes with GC-MS require extensive sample preparation and derivatization, which limits the throughput of the toxicological examination.

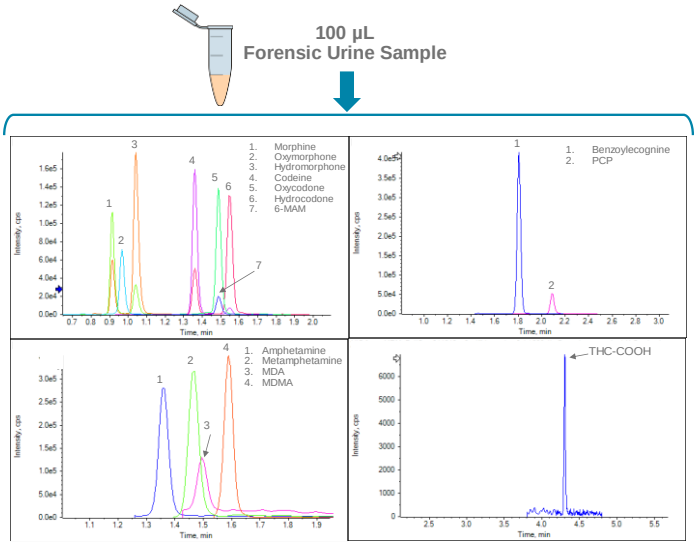

Figure 1. Rapidly analyze all USDOT regulated forensic compounds from a single injection. Extracted Ion Chromatograms showing the comprehensive analysis of forensic compounds mixed in a urine sample. A rapid polarity switching method is used to include THC-COOH using negative electrospray ionization, as well as other compounds (e.g., Oxycodone) that ionize well in positive electrospray mode.

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) offers the speed, selectivity and sensitivity required to reliably identify different classes of illicit compounds in a forensic sample within a single workflow. In this study, a rapid method for the analysis of the updated USDOT forensic compounds in urine samples using SCIEX Triple Quad™ LC-MS/MS Systems is described.

Features of SCIEX Triple Quad™ LC-MS/MS Systems for forensic drug analysis

- Industry leading Turbo V™ Source efficiently ionizes compounds, delivering highly efficient desolvation for stable and sensitive performance while analyzing complex biological matrices. (e.g., urine/blood)
- Scheduled MRM™ Pro Algorithm intelligently uses information of retention times to automatically optimize MRM dwell time of each transition and total cycle time of the experiment resulting in best data quality
- Curved LINAC® Collision Cell design improves data quality to ensure fewer peaks are missed and optimal sensitivity is achieved for all drugs of abuse
- SCIEX OS Software data analysis component allows fast data processing, with less manual intervention and streamlined reporting tools, enabling forensic laboratories to release results more efficiently

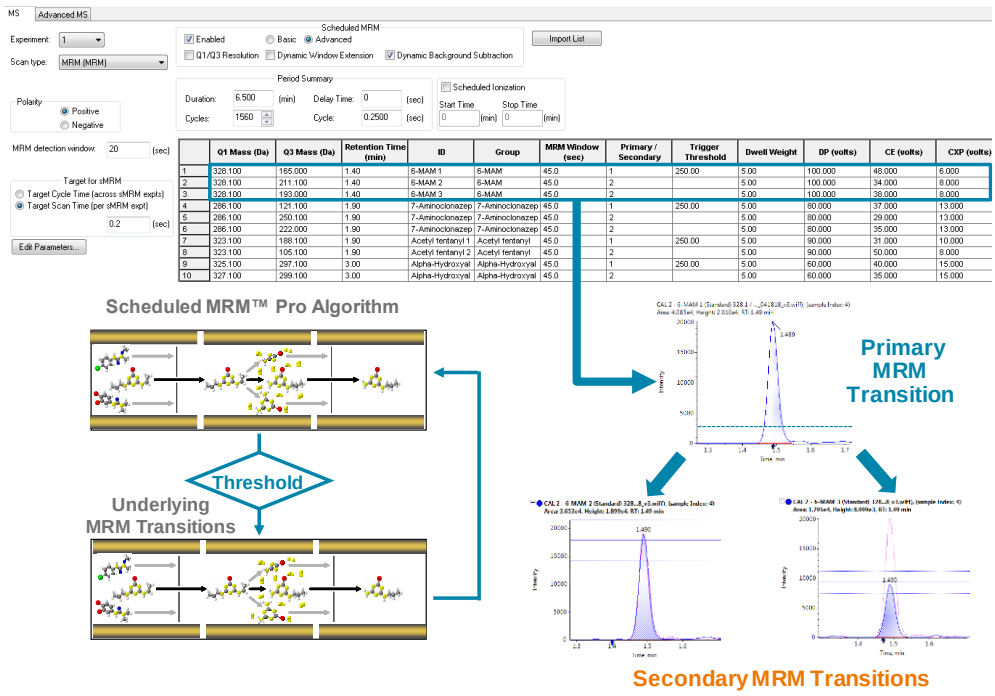

Figure 2. The Scheduled MRM Pro Algorithm aids in the acquisition of multiple of compounds based on a list of Multiple Reaction Monitoring (MRM) transitions, retention times, and compound IDs. The algorithm intelligently uses information of retention times to automatically optimize MRM dwell time of each transition resulting in best data quality. Three MRM transitions were monitored for each analyte (e.g.6-Acetylmorphine) Two secondary MRM transitions were triggered to calculate ion ratios of quantifier and qualifier ions for compound identification.

Methods

Sample preparation: Control urine matrix samples were spiked with reference standards (Cerilliant, Round Rock, Texas) of the compounds listed on Table 1. Samples were enzymatically hydrolyzed, and centrifuged. Supernatant was diluted 10-fold with 90:10 (v:v), water: 0.1% formic acid in methanol.

LC conditions: HPLC separation was performed on Phenomenex Kinetex Phenyl-hexyl (50 × 4.6 mm, 2.6 µm, 00B-4495-E0) on the ExionLC™ AC System. Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate in water and methanol with formic acid, respectively. The LC flowrate was 1 mL/min, and the total LC runtime was 6.5 min. Injection Volume varied on the different instruments evaluated:

- 20 µL on the SCIEX Triple Quad 3500 LC-MS/MS System
- 5 µL on the SCIEX Triple Quad 4500 LC-MS/MS System

MS and MS/MS Conditions: 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.

Data acquisition: Data was acquired with Analyst® software 1.7, the *Scheduled* MRM Pro algorithm was used with a target cycle time of 0.2 sec and compound dependent detection windows and thresholds. One primary MRM and two secondary MRM transitions were monitored per analyte (Figure 2).

Data review and processing*:* Targeted data processing was performed using SCIEX OS Software data analysis component. Positive analyte identification was determined by establishing the following variable tolerance ion ratio criteria:

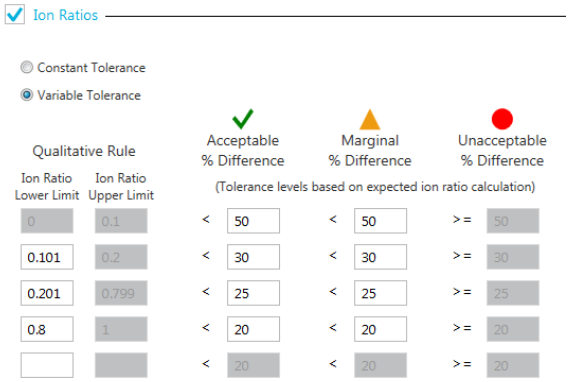

Figure 3. Ion ratio scores. Scores are calculated automatically and visualized using traffic lights in the results review pane.

Intelligent forensic compound identification using the Scheduled MRM Pro Algorithm

Figure 4 shows the identification of morphine spiked at 50 ng/mL in urine matrix using the SCIEX Triple Quad 4500 LC-MS/MS System. The Scheduled MRM Pro algorithm in Analyst software 1.7 enabled the automatic triggering of secondary MRM transitions (286→165 and 286→152) after the detecting morphine's primary MRM transition (286→201). This feature further optimizes the MRM scheduling resulting in an increase of data quality and confidence in forensic analyte detection at low ng/mL concentration levels.

Identification of the updated USDOT forensic compounds was further evaluated using *Scheduled* MRM Pro algorithm meeting SWGTOX analytical criteria (analytes at the lowest concentrations that are capable of reproducibly providing symmetrical peaks and the minimum mass spectral identification ratios, while maintaining a bias of ±20% and % RSDs of <20%).[3]

Table 2 summarizes the average ion ratios and %RSDs obtained for all the secondary MRM transitions of the USDOT forensic compounds. Table 3 details the compound identification criteria results based on retention time error and ion ratio % bias of all secondary MRM transitions. All results were automatically calculated using the SCIEX OS Software data analytics component.

Table 1. List of USDOT forensic regulated forensic compounds and analytical cutoff concentrations (ng/mL).

Compounds	Forensic Screen Cutoff Concentration	Forensic Confirmation Cutoff Concentration
Marijuana metabolite (THC-COOH)	*50 ng/ml*	*15 ng/ml*
Cocaine Metabolite (Benzoylecgonine)	*150 ng/mL*	*100 ng/mL*
Phencyclidine (PCP)	*25 ng/mL*	*25 ng/mL*
Amphetamine	*500 ng/mL*	*250 ng/mL*
Methamphetamine	*500 ng/mL*	*250 ng/mL*
MDMA/MDA	*500 ng/mL*	*250 ng/mL*
Codeine/Morphine	*2000 ng/mL*	*2000 ng/mL*
6-Acetylmorphine	*10 ng/mL*	*10 ng/mL*
Hydrocodone	*300 ng/mL*	*100 ng/mL*
Hydromorphone	*300 ng/mL*	*100 ng/mL*
Oxymorphone	*100 ng/mL*	*100 ng/mL*
Oxycodone	*100 ng/mL*	*100 ng/mL*

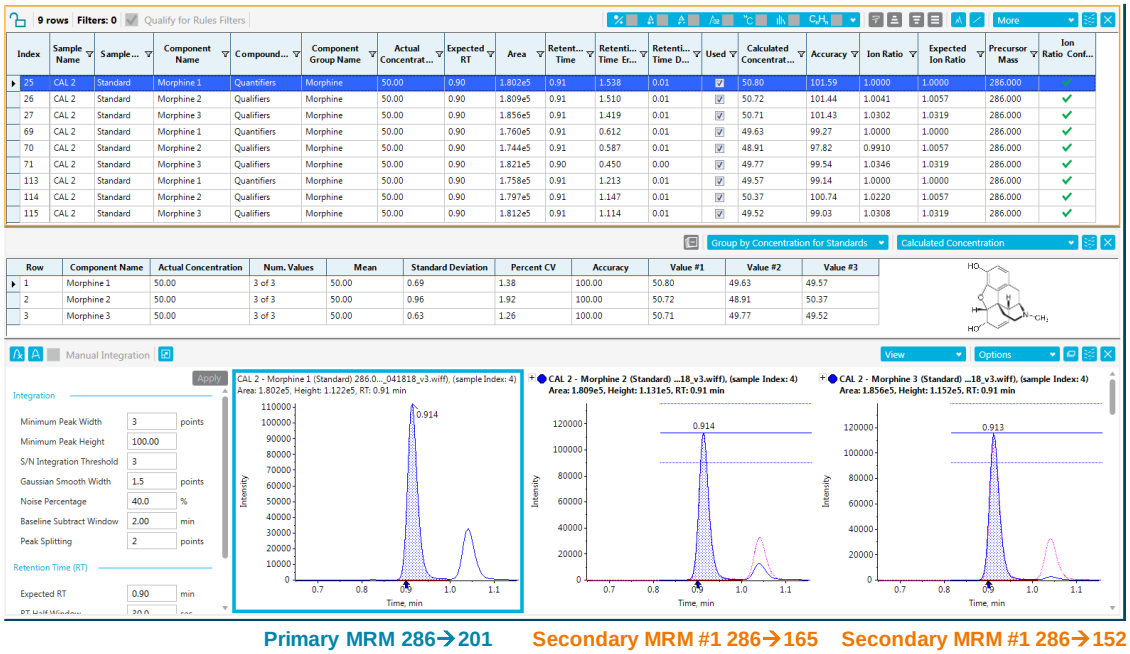

Figure 4. Quickly retrieve analyte specific information present in a forensic sample. View of all compounds present in a forensic urine sample using SCIEX OS Software data analytics component. (Top) List of all quantitative results obtained for morphine spiked at 50ng/mL in urine matrix (Bottom) XICs obtained for morphine showcasing all 3 MRM Transitions overlaying qualifier and quantifier ions with ion ratio lines.

Table 2. List of forensic compounds analyzed in a single method using the Scheduled MRM Pro Algorithm with average ion ratios and reproducibility (%RSD).

Forensic Analyte	Urine Matrix Spiked Concentration	Average Ion Ratio (n=3) Secondary MRM # 1	%RSD	Average Ion Ratio (n=3) Secondary MRM # 2	%RSD
THC-COOH	*10 ng/mL*	*0.2488*	*9.69%*	*0.1253*	*10.65%*
Benzoylecgonine	*50 ng/mL*	*0.7474*	*0.38%*	*0.5232*	*1.21%*
Phencyclidine (PCP)	*5 ng/mL*	*0.8633*	*2.47%*	*1.0109*	*3.94%*
Amphetamine	*50 ng/mL*	*0.4870*	*2.58%*	*0.1944*	*5.93%*
Methamphetamine	*50 ng/mL*	*2.6868*	*0.73%*	*0.6090*	*8.53%*
MDMA	*50 ng/mL*	*0.4454*	*11.23%*	*0.2612*	*7.98%*
MDA	*50 ng/mL*	*0.6547*	*9.85%*	*0.3211*	*10.33%*
Morphine	*50 ng/mL*	*1.0057*	*1.55%*	*1.0319*	*0.23%*
Hydromorphone	*25 ng/mL*	*0.5464*	*2.75%*	*0.1734*	*4.90%*
Codeine	*50 ng/mL*	*1.0035*	*10.80%*	*0.8295*	*12.23%*
Hydrocodone	*25 ng/mL*	*0.6098*	*9.10%*	*0.7030*	*4.92%*
6-Acetylmorphine	*5 ng/mL*	*0.8899*	*1.46%*	*0.4311*	*5.48%*
Oxymorphone	*25 ng/mL*	*0.6111*	*3.25%*	*2.4937*	*4.07%*
Oxycodone	*25 ng/mL*	*0.8407*	*4.00%*	*2.7161*	*6.65%*

It is important to highlight that the Phenomenex Kinetex Phenyl-Hexyl column enabled the rapid separation of various isobaric compounds targeted in this method. Under the conditions used, peak front-tailing for early eluting compounds like morphine, codeine and oxymorphone was not noticed. Figure 1 shows the LC separation of all forensic compounds targeted in this method.

Streamlining forensic analysis using SCIEX Triple Quad LC-MS/MS Systems

Typical forensic toxicology analysis methods using GC/MS are throughput limited (i.e., >15 min per sample) due to analyte volatility, derivatization techniques and other physicochemical properties. SCIEX Triple Quad LC-MS/MS systems offer the advantage of speed and sensitivity that streamlines the reliable identification of drugs of abuse present in forensic samples.

Figure 5 shows representative XICs of the quantifier MRMs of notable USDOT forensic compounds measured (THC-COOH and 6-Acetylmorphine) using an entry level LC/MS platform like the SCIEX Triple Quad 3500 LC-MS/MS system. Three replicate set of injections were performed, obtaining excellent reproducibility with RSD values less than 10% for all compounds. Complete quantitative analysis performance for multiple classes

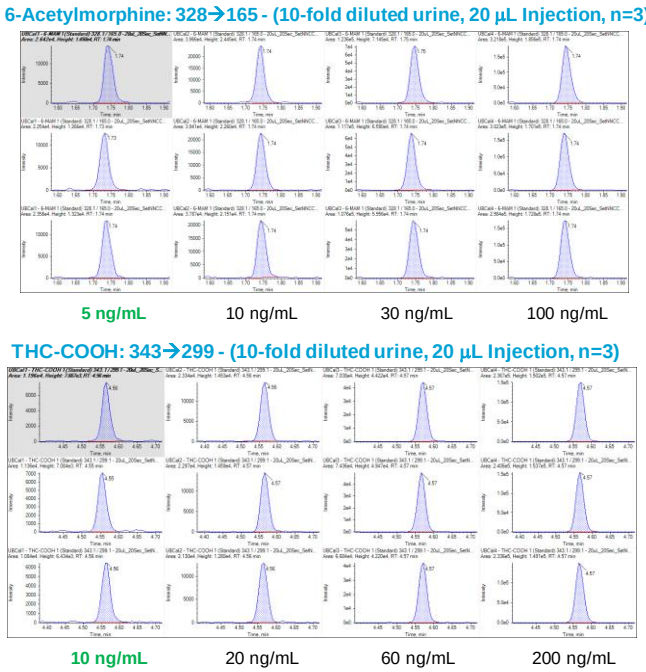

Figure 5. SCIEX Triple Quad Systems offer selectivity and speed to reproducibly analyze USDOT forensic compounds. XICs of 6-Acetylmorphine (Top) and THC-COOH (Bottom) obtained at different concentrations in urine matrix.

Table 3. Forensic compound identification criteria results obtained in a single method using the Scheduled MRM Pro Algorithm.

Forensic Analyte	Expected RT (min)	Actual RT (min)	RT Error (+/-min)	Expected Ion Ratio Secondary MRM #1	Ion Ratio Secondary MRM #1	% Bias	Expected Ion Ratio Secondary MRM #2	Ion Ratio Secondary MRM #2	% Bias
THC-COOH	*4.3*	*4.3*	*0.00*	*0.2488*	*0.2682*	*7.80%*	*0.1253*	*0.1405*	*12.16%*
Benzoylecgonine	*1.8*	*1.81*	*0.01*	*0.7474*	*0.7443*	*0.41%*	*0.5232*	*0.5193*	*0.75%*
Phencyclidine (PCP)	*2.1*	*2.09*	*-0.01*	*0.8633*	*0.8634*	*0.02%*	*1.0109*	*1.0263*	*1.52%*
Amphetamine	*1.4*	*1.41*	*0.01*	*0.4870*	*0.4747*	*2.53%*	*0.1944*	*0.182*	*6.36%*
Methamphetamine	*1.5*	*1.51*	*0.01*	*2.6868*	*2.7086*	*0.81%*	*0.6090*	*0.6135*	*0.74%*
MDMA	*1.64*	*1.65*	*0.01*	*0.4454*	*0.3963*	*11.03%*	*0.2612*	*0.2851*	*9.15%*
MDA	*1.2*	*1.21*	*0.01*	*0.6547*	*0.6399*	*2.26%*	*0.3211*	*0.3029*	*5.67%*
Morphine	*0.9*	*0.91*	*0.01*	*1.0057*	*1.0041*	*0.16%*	*1.0319*	*1.0302*	*0.16%*
Hydromorphone	*1.03*	*1.04*	*0.01*	*0.5464*	*0.5634*	*3.10%*	*0.1734*	*0.164*	*5.40%*
Codeine	*1.35*	*1.35*	*0.00*	*1.0035*	*0.992*	*1.14%*	*0.8295*	*0.7863*	*5.21%*
Hydrocodone	*1.53*	*1.54*	*0.01*	*0.6098*	*0.6016*	*1.35%*	*0.7030*	*0.7054*	*0.34%*
6-Acetylmorphine	*1.49*	*1.49*	*0.00*	*0.8899*	*0.8943*	*0.49%*	*0.4311*	*0.4394*	*1.93%*
Oxymorphone	*0.96*	*0.97*	*0.01*	*0.6111*	*0.6238*	*2.08%*	*2.4937*	*2.5372*	*1.74%*
Oxycodone	*1.5*	*1.49*	*-0.01*	*0.8407*	*0.8347*	*0.72%*	*2.7161*	*2.5333*	*6.73%*

of forensic compounds has been previously demonstrated using both SCIEX Triple Quad 3500 and 4500 LC-MS/MS Systems.[4,5]

The data analysis component of SCIEX OS Software, is designed to provide a centralized results grid for streamlined review and efficient sample report processing. Retention time errors, analyte concentration and ion ratios score are calculated automatically and visualized using “traffic lights” Compounds identified with high confidence are indicated using green check symbols.

Figure 6 shows a customized report generated by SCIEX OS Software, after the analysis of an unknown forensic urine sample using the the Scheduled MRM Pro algorithm previously described. Oxycodone was confidently identified based in the sample, and a sample report was efficiently generated to include all MRM transitions peak areas, retention times, ion ratio confidence scores and accuracy acceptance results for further laboratory processing.

Conclusions

A rapid and analysis method for the analysis of the updated USDOT forensic compounds in urine samples using SCIEX Triple Quad LC-MS/MS Systems was successfully developed.

- The Scheduled MRM Pro algorithm in Analyst software 1.7 aids in the acquisition of multiple of compounds by automatically triggering secondary MRM transitions, resulting in an increase of data quality and confidence in forensic analyte detection at low ng/mL concentration levels.
- SCIEX Triple Quad LC-MS/MS Systems offer the advantage of speed and sensitivity as opposed to traditional GC-MS approaches, to streamline the identification of multiple classes of USDOT forensic compounds (e.g., THC-COOH) at different concentrations in a single analytical workflow.
- The data analysis component of SCIEX OS Software, provided a simplified interface for streamlined data review based upon a “traffic light” display and simplified sample report generation to maximize the forensic laboratory operational efficiency.

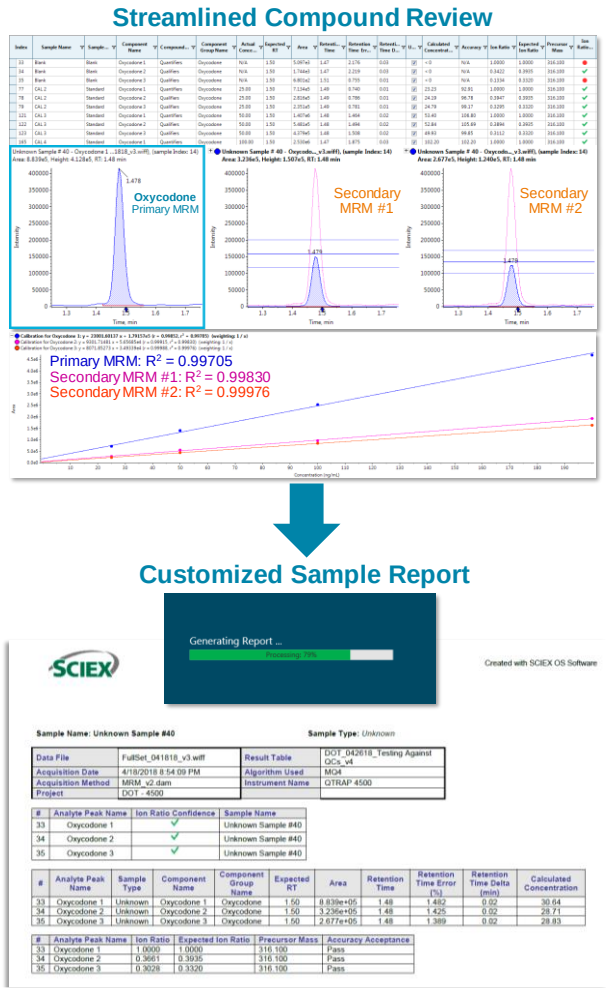

Figure 6. Streamlined data processing through customized sample reports using SCIEX OS Software. Oxycodone was identified in an unknown forensic urine sample (top), and a sample report containing analyte information was efficiently generated using the software's reporting tool (bottom).

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