Implementing quantitative drug screening for workplace testing using high-resolution mass spectrometry

Using Information Dependent Acquisition (IDA) and MRM^{HR} on the SCIEX X500R QTOF System

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Drug use has been linked to increased accidents, greater absenteeism and overall lower productivity in the workplace. This behavior burdens organizations with legal liability that can potentially result in significant risks and costs. As a result, drug testing has become a popular practice in the workplace in an effort to identify chronic drug users among applicants and employees. For some organizations, subjecting employees to workplace testing is mandatory to remain compliant with certain regulations and ensure that employees restraint from drug use.

In the forensic laboratory, drug screening for workplace testing is typically performed by immunoassay or GC-MS. Immunoassays are commonly used as a first-line screening method for drugs. However, this screening technique suffers from low specificity and results in a high rate of false positives. Immunoassays often require multiple tests to cover the entire panel of targeted drugs, which slows the analytical and reporting process. On the other hand, GC-MS requires sample derivatization and suffers from lengthy chromatographic runs. As a result, there is a need for comprehensive and robust screening methods that allow accurate identification of a panel of drugs with high quantitative accuracy, specificity and sensitivity.

The use of high-resolution mass spectrometry (HRMS) for workplace testing enables toxicologists to rapidly screen the presence of these drugs by acquiring their complete chemical profile. The acquisition of full scan, high-resolution mass spectra in both MS and MS/MS modes allows for retrospective data analysis without the need to re-run the sample while also providing quantitative information about the drugs present in the sample.

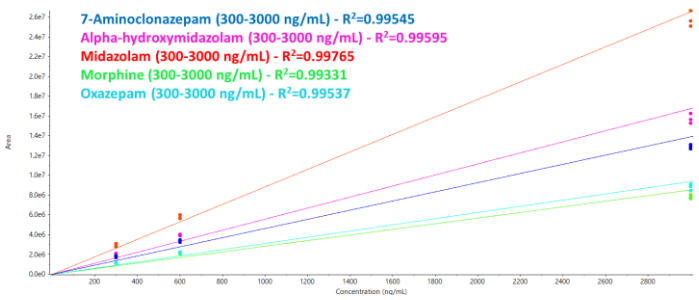

Figure 1. High linearity for the detection of drugs in human urine. Calibration curves resulting from the calibration series of 7-aminoclonazepam, alpha-drydroxymidazolam, midazolam, morphine, and oxazemap from 300 to 3,000 ng/mL.

Here, a rapid and comprehensive drug screening workflow for the analysis of workplace testing urine samples using the SCIEX X500R QTOF System is described. The targeted acquisition of accurate mass spectra using the developed LC-MS/MS drug screening method enabled identification and quantification of multiple drugs present in authentic forensic workplace testing urine samples.

Key features of HRMS drug screening workflow for workplace testing

- Simplified, dilute-and-shoot urine sample preparation provided an easily implemented procedure enabling rapid detection of drugs in real workplace testing case samples
- Robust data acquisition strategy enabled acquisition of high-quality MS/MS spectra, providing reliable compound MS/MS for comparison to library spectra for confident identification, greatly reducing the rate of false positives
- SCIEX OS Software (Analytics) provided a simplified interface for efficient data review based on the confidence criteria defined in the processing method
- Easy generation of a comprehensive and detailed sample report containing the name and concentration of the positively identified drugs in the workplace testing case samples for fast and efficient results reporting

Experimental details

Sample preparation: A standard mixture containing the 39 drugs used in this workplace testing panel was prepared by diluting with methanol: water (20:80, v/v). Three calibrator solutions ranging from 5 to 5000 ng/mL were prepared. The highest calibrator solution was used for initial method development and to determine the retention time of the 39 targeted drugs. Table 1 lists the name of the drugs used in this panel and includes their accurate mass information, chemical formula, retention time, limits of detection (LOD), and R^2 values.

A dilute-and-shoot sample preparation method was used for the detection of the 39 drugs in urine. Blank urine samples were spiked with the three calibrator solutions and diluted 10-fold with a solution of methanol: water (10:90, v/v). The spiked urine samples were thoroughly vortexed and centrifuged for 10 min at 12,000 rpm. The clear supernatent was transferred to autosampler vials and 10 µL of each sample was injected to build a data processing method. Three replicates for each of the calibrator solutions were analyzed to evaluate the reproducibility and sensitivity of the method.

Forensic workplace testing case samples and controls were prepared using the same dilute-and-shoot sample preparation method and subjected to the same data processing method as the spiked urine sample. These case samples were used to validate the robustness of the method.

Chromatography: HPLC separation was performed on an ExionLC™ System using a Phenomenex Phenyl-Hexyl column (50 × 2.1 mm, 2.6µm, 00B-4495-E0) held at 45ºC. Mobile phases were water and methanol with appropriate additives. The injection volume was 10 µL and the total LC runtime was 8.5 minutes.

Mass spectrometry: MS and MS/MS data were collected for each sample using the SCIEX X500R QTOF System. Information Dependent Acquisition (IDA) was used in positive ion mode. MRM^{HR} workflow with the *Apply TOF start/stop* mass feature activated was used in negative ion mode. Both detection methodologies contain a TOF MS experiment.

Data analysis: A targeted data processing method was developed using SCIEX OS Software 1.5 for positive analyte identification based on criteria previously described.[1] The four main confidence criteria used include mass error (M), retention time (R), isotope ratio difference (I), and library score (L). Subsequently, a combined score (C) was calculated based on these four confidence categories (MRIL) with custom weightings.

Developing a comprehensive screening workflow applied to forensic urine samples

Blank human urine samples were spiked with the standard mixture containing the 39 drugs at various concentrations ranging from 5 to 5000 ng/mL. These standard solutions were injected to build a data analysis processing method.

Two different data acquisition strategies were used to streamline the detection of the different classes of compounds making up the panel of the 39 drugs. This was performed to ensure collection of high quality data and to achieve desirable limits of detection (LOD) for all 39 compounds. Information Dependent Acquisition (IDA) was used as the acquisition strategy to detect compounds that ionize in positive ion mode. This acquisition method enables collection of high-quality TOF MS and TOF MS/MS spectra of the most abundant precursor/candidate ions. The resulting MS/MS spectra was used for confident drug identification using spectra library matching. MRM^{HR} workflow was used as the acquisition strategy to detect the compounds that ionize in negative ion mode. This data acquisition method is often used to increase detection specificity and sensitivity, and again confident compound identification is achieved by MS/MS spectral library matching.

Figure 2 shows the extracted ion chromatograms (XICs) for the 39 drugs at LOD concentrations analyzed with both positive (A) and negative (B) electrospray ionization modes in control urine samples. The optimized LC conditions in combination with the appropriate choice of column chemistry and mobile phase composition enabled baseline separation of all the analytes.

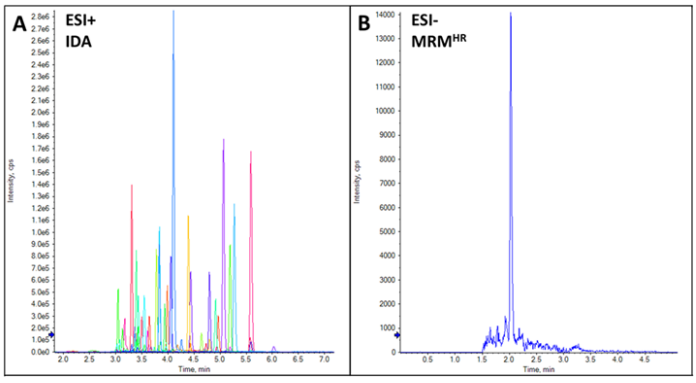

Figure 2. Chromatographic profiles of the 39 drugs targeted in this study. (A) Extracted Ion Chromatogram (XIC) showing a rapid (6 min) separation of 38 drugs of interest spiked in urine at LOD concentrations and detection in positive electrospray ionization (ESI+) mode. (B) Extracted Ion Chromatogram (XIC) showing the detection of Propofol β-D-Glucuronide spiked in urine at LOD concentrations, the only drug detected in negative electrospray ionization (ESI-) mode using MRM^{HR} workflow.

Table 1. List of the 39 compounds used in the panel along with their retention times, chemical formula, precursor mass, adduct and charge, cutoff concentration (LOD) and correlation coefficients (R^2 values).

Compound Name	Retention Time	Chemical Formula	Precursor Mass (Da)	Adduct & Charge	LOD (ng/mL)	R^2 Values
6-Monoacetylmorphine	3.42	$C_{19}H_{21}NO_4$	328.15433	[M+H]+	10	0.99492
7-Aminoclonazepam	4.06	$C_{15}H_{12}ClN_3O$	286.07417	[M+H]+	300	0.99545
Alpha-Hydroxyalprazolam	4.80	$C_{17}H_{13}ClN_4O$	325.08507	[M+H]+	300	0.99562
Alpha-Hydroxymidazolam	4.44	$C_{18}H_{13}ClFN_3O$	342.08039	[M+H]+	300	0.99595
Alprazolam	5.07	$C_{17}H_{13}ClN_4$	309.09015	[M+H]+	300	0.98805
Beta-Naltrexol	3.37	$C_{20}H_{25}NO_4$	344.18563	[M+H]+	50	0.99695
Buprenorphine	4.48	$C_{29}H_{41}NO_4$	468.31084	[M+H]+	5	0.99834
Carboxyzolpidem	3.61	$C_{19}H_{19}N_3O_3$	338.14992	[M+H]+	25	0.99743
Carisoprodol	4.94	$C_{12}H_{24}N_2O_4$	261.18088	[M+H]+	100	0.98250
Codeine	3.30	$C_{18}H_{21}NO_3$	300.15942	[M+H]+	300	0.99017
Cyclobenzaprine	4.70	$C_{20}H_{21}N$	276.17468	[M+H]+	50	0.97114
Diazepam	5.59	$C_{16}H_{13}ClN_2O$	285.07892	[M+H]+	300	0.99023
Hydrocodone	5.58	$C_{18}H_{21}NO_3$	300.15942	[M+H]+	100	0.99792
Hydromorphone	3.50	$C_{17}H_{19}NO_3$	286.14377	[M+H]+	100	0.99888
Ketamine	3.13	$C_{13}H_{16}ClNO$	238.09932	[M+H]+	50	0.99558
Lorazepam	3.64	$C_{15}H_{10}Cl_2N_2O_2$	321.01921	[M+H]+	300	0.99734
Meperidine	4.96	$C_{15}H_{21}NO_2$	248.16451	[M+H]+	50	0.99720
Meprobamate	3.98	$C_9H_{18}N_2O_4$	219.13393	[M+H]+	500	0.97382
Methylphenidate	4.18	$C_{14}H_{19}NO_2$	234.14886	[M+H]+	10	0.99671
Midazolam	3.87	$C_{18}H_{13}ClFN_3$	326.08548	[M+H]+	300	0.99765
Morphine	4.38	$C_{17}H_{19}NO_3$	286.14377	[M+H]+	300	0.99331
N-Desmethyltapentadol	3.06	$C_{13}H_{21}NO$	208.16959	[M+H]+	100	0.99087
Naltrexone	3.77	$C_{20}H_{23}NO_4$	342.16998	[M+H]+	50	0.99162
Norbuprenorphine	3.41	$C_{25}H_{35}NO_4$	414.26389	[M+H]+	5	0.97693
Nordiazepam	4.04	$C_{15}H_{11}ClN_2O$	271.06327	[M+H]+	300	0.99615
Norhydrocodone	5.19	$C_{17}H_{19}NO_3$	286.14377	[M+H]+	100	0.98979
Norketamine	3.43	$C_{12}H_{14}ClNO$	224.08367	[M+H]+	50	0.98193
Normeperidine	3.54	$C_{14}H_{19}NO_2$	234.14886	[M+H]+	50	0.99862
Norhydrocodone	3.93	$C_{17}H_{19}NO_4$	302.13868	[M+H]+	100	0.99673
O-Desmethyltramadol	3.38	$C_{15}H_{23}NO_2$	250.18016	[M+H]+	100	0.99207
Oxazepam	3.40	$C_{15}H_{11}ClN_2O_2$	287.05818	[M+H]+	300	0.99537
Oxycodone	4.91	$C_{18}H_{21}NO_4$	316.15433	[M+H]+	100	0.99247
Oxymorphone	3.42	$C_{17}H_{19}NO_4$	302.13868	[M+H]+	100	0.99548
Ritalinic Acid	3.08	$C_{13}H_{17}NO_2$	220.13321	[M+H]+	100	0.99081
Tapentadol	3.54	$C_{14}H_{23}NO$	222.18524	[M+H]+	100	0.99540
Temazepam	3.83	$C_{16}H_{13}ClN_2O_2$	301.07383	[M+H]+	300	0.99219
Tramadol	5.28	$C_{16}H_{25}NO_2$	264.19581	[M+H]+	100	0.99495
Zolpidem	3.82	$C_{19}H_{21}N_3O$	308.17574	[M+H]+	300	0.98921
Propofol β-D-Glucuronide	2.12	$C_{18}H_{27}O_7$	353.16185	[M-H]-	50	0.99767

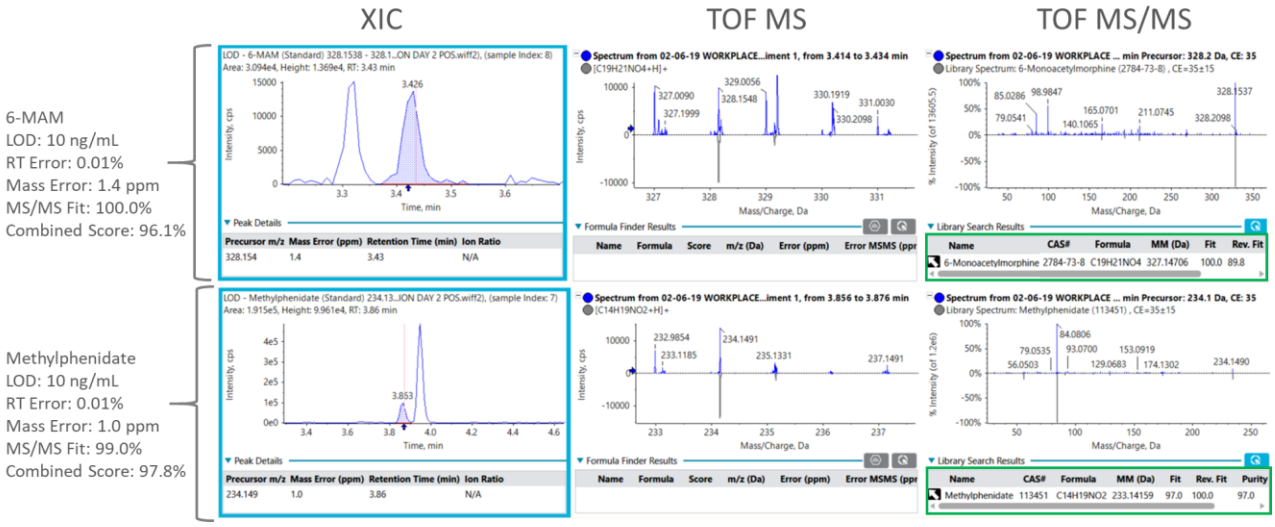

Figure 3. Confident identification of drugs based on the confidence criteria. XICs, TOF MS and TOF MS/MS spectra collected provide detailed and confident identification of 6-MAM (top) and methylphenidate (bottom) spiked in urine samples at their corresponding LODs (10 ng/mL).

The overall quality of the data collected was investigated by monitoring the combined scores for all the 39 drugs based on the four confidence criteria defined in the processing method. Assessing the quality of the collected data is a critical part of the method development process to ensure correct drug identification in a sample while minimizing false positives and/or false negatives. As part of this process, the XICs, TOF MS and TOF MS/MS spectra, along with the combined scores determined by the four confidence criteria (retention time, mass, isotope ratio error, and mass spectral library search) were reviewed for each of the 39 drugs spiked in urine at the LOD. Figure 3 shows the XIC, TOF MS and TOF MS/MS spectra with library search match for 6-MAM and methylphenidate at their corresponding LODs (10 ng/mL). The mass errors (less than or equal to 1 ppm), mass spectra library scores (above 97%) and the combined scores (above 96%) provided excellent measures of the confident identification of these two compounds in spiked urine samples.

Analytical performance of the workflow for the detection of drugs in urine samples

Developing a robust workflow that produces accurate and reproducible results is critical for its full implementation into routine laboratory testing. In this experiment, three replicate injections of the spiked urine samples at three concentration levels were performed to evaluate the linearity and the quantitation performance of workflow. These three-point calibration curves covered clinically-relevant concentrations ranging from LOD to 10x LOD for each of the drugs used in this panel. Three replicates for each concentration were used to build the calibration curves.

Figure 1 shows the calibration curves for selected drugs from the panel. The three-point calibrarion curves showed excellent correlation and linearity with correlation coefficient (R^2 values) above 0.97 for all the 39 drugs targeted in this study. Table 1 lists the correlation coefficient (R^2 values) resulting from the calibration curves of the 39 targeted drugs used in this study.

The reproducibility and robustness of the data was also investigated. The four confidence criteria were used to calculate the combined score for each of the 39 drugs targeted in this study. Th reproducibility and accuracy for the three concentration levels were also determined to assess the overall robustness of the assay. Table 2 summarizes the results obtained for the detection of the 39 analytes spiked in blank urine samples at the highest calibrator level. The table includes the calculated overall score (%), %CV, and accuracy. Overall, the assay showed excellent reproducibility, accurary, and linearity, proving the robustness of the developed workflow.

Accurate identification and quantitation of drugs in workplace testing case samples

The robustness of the combined acquisition workflow was further investigated by analyzing ten workplace testing urine samples. These biological specimens were prepared using the aforementioned dilute-and-shoot sample preparation method and analyzed using the X500R QTOF System to generate comprehensive and high-quality spectra. The high-resolution data enabled accurate detection of the drugs present in the case samples through extraction of specific accurate mass fragment ions that were matched to a spectral library database for confident identification.

Table 2. Average (n=3) combined scores, %CV and accuracy for the 39 drugs detected in urine samples using the SCIEX X500R QTOF System.

Compound Name	Combined Score (%)	% CV	Accuracy (%)	Compound Name	Combined Score (%)	% CV	Accuracy (%)
6-Monoacetylmorphine	97.94	2.48	89.54	Morphine	97.04	2.45	91.64
7-Aminoclonazepam	95.13	1.60	92.84	N-Desmethyltapentadol	93.654	2.04	90.26
Alpha-Hydroxyalprazolam	97.21	1.21	92.62	Naltrexone	97.64	0.91	85.93
Alpha-Hydroxymidazolam	96.88	3.07	93.93	Norbuprenorphine	96.48	5.80	81.72
Alprazolam	95.30	2.56	87.94	Nordiazepam	96.99	1.79	91.79
Beta-Naltrexol	98.19	0.13	93.46	Norhydrocodone	97.53	2.61	91.21
Buprenorphine	97.32	3.63	101.39	Norketamine	95.82	1.21	93.85
Carboxyzolpidem	97.97	2.79	94.42	Normeperidine	95.962	1.30	96.50
Carisoprodol	98.72	5.33	86.49	Norhydrocodone	98.49	2.70	94.57
Codeine	93.02	0.43	89.97	O-Desmethyltramadol	93.65	1.12	90.12
Cyclobenzaprine	96.84	13.78	112.78	Oxazepam	91.47	3.67	93.98
Diazepam	95.61	3.62	89.46	Oxycodone	46.62	4.22	93.22
Hydrocodone	97.95	2.42	97.78	Oxymorphone	96.65	1.97	94.40
Hydromorphone	98.22	3.16	97.35	Ritalinic Acid	96.44	2.24	91.04
Ketamine	98.16	4.17	95.73	Tapentadol	85.04	2.13	91.32
Lorazepam	100.00	3.31	95.39	Temazepam	93.84	1.01	88.00
Meperidine	95.01	3.23	96.95	Tramadol	89.67	0.78	91.13
Meprobamate	97.04	12.39	68.21	Zolpidem	86.84	3.67	88.78
Methylphenidate	98.56	1.96	92.50	Propofol β-D-Glucuronide	100.00	0.70	101.13
Midazolam	93.07	3.07	97.18				

Figure 4 shows the successful detection of hydrocodone, hydromoprhone, lorazepam and norhydrocodone from one of the tested workplace testing case samples at concentration of 63.07, 99.08, 574.20 and 311.10 ng/mL, respectively. The displayed results table in the SCIEX OS Software includes detailed information about the positively identified analytes filtered using the “traffic lights” based on the confidence criteria. SCIEX OS Software also provides the ability to review the XIC, TOF MS and TOF MS/MS spectra for each of the positively identified analytes. The library matching scores (>97%) together with the excellent calculated combined scores (>95%) for the four detected analytes provides a confident metric for the accurate identification of the drugs in this urine case sample. Table 3 summarizes the results of the workplace testing urine sample detailed in Figure 4 and includes the list of positively identified drugs along with their concentration, library score and combined score.

Comprehensive sample reporting of positively identified drugs in case samples

The data analysis component of SCIEX OS Software allows efficient review of the results and enables users to generate a customizable report to summarize the findings of the sample analysis. The confidence criteria (mass error, retention time, isotope ratio difference, and library score) used to positively identify drugs present in forensic case samples are automatically calculated and displayed in the report using green check symbols. The report can also be customized to include the XIC, TOF MS and TOF MS/MS spectra for each of the detected drugs along with their calculated concentration, library matching and combined scores. This comprehensive report provides the ability to streamline sample reporting and greatly improves laboratory sample throughput.

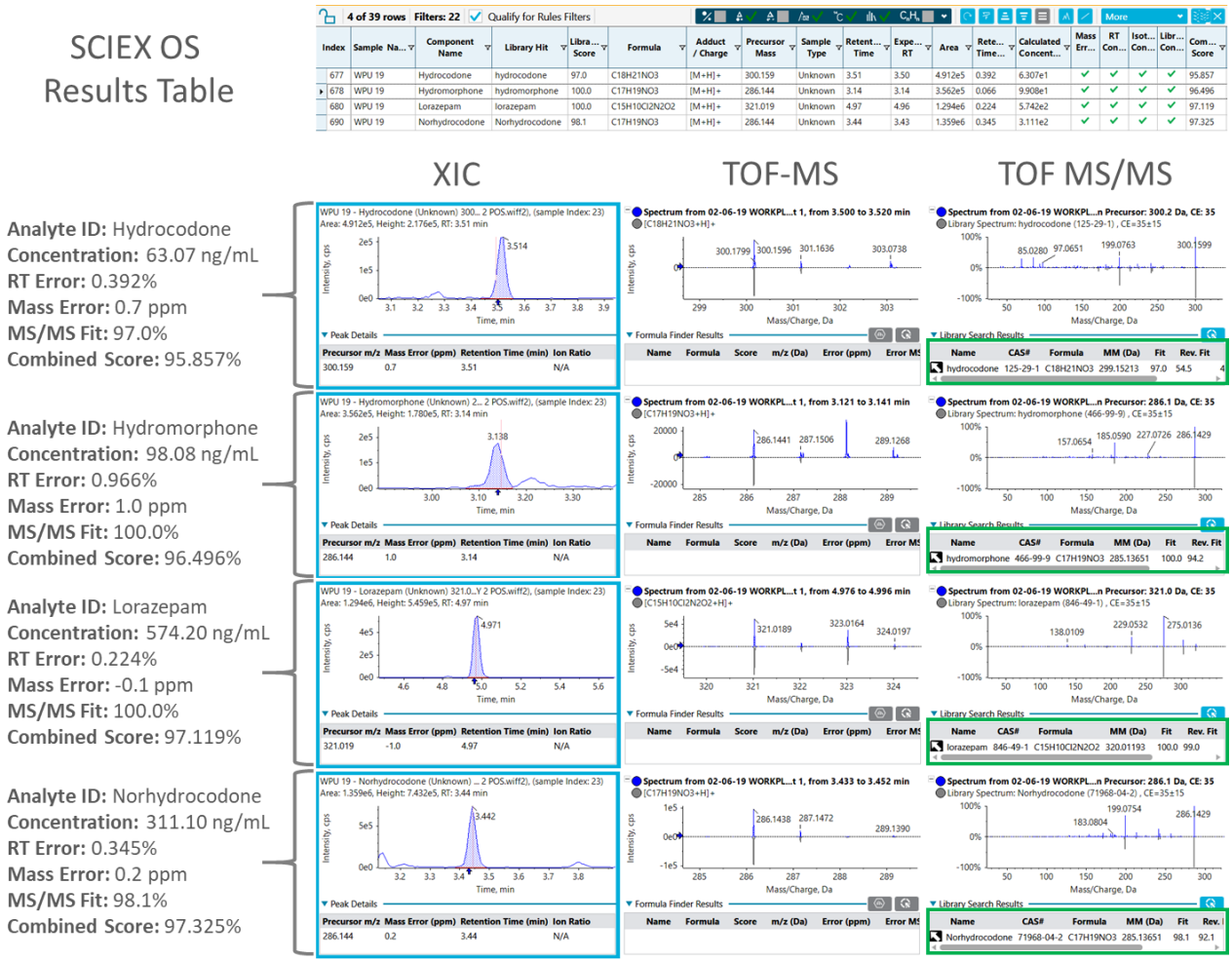

Figure 4. SCIEX OS enables streamlined data review of drugs positively identified in a urine case sample. (Top) Results table in SCIEX OS Software showing the drugs positively identified in a urine case sample along with calculated concentrations, library score and combined score using the confidence criteria. (Bottom) XICs, TOF MS and TOF MS/MS spectra collected provide detailed and confident identification of hydrocodone, hydromorphone, lorazepam and norhydrocodone in a urine case sample.

Figure 5 shows a customized report generated by SCIEX OS Software following the processing of a workplace testing urine sample. The report includes the total ion chromatogram (TIC) of the detected drugs along with a results table including the concentration, the library search score and combined score for each of the positively identified drugs present in the urine sample based on the acceptance criteria. Detailed XIC, TOF MS and TOF MS/MS spectra are also included to support the positive identification of buprenorphine and norbuprenorphine in the reported urine case sample at concentration of 41.65 and 492.30 ng/mL, respectively. A variety of report templates are available in SCIEX OS Software to accomodate different types of reporting needs. Those sample reports can also be modified to include custom reporting fields such as analyte retention time, retention time error, precursor mass, found at mass, mass error and concentration acceptance, among many others.

Table 3. Summary table for urine case sample. Summary of drugs positively identified in the urine case sample detailed in Figure 4 along with concentration, library score and combined score.

Drug Detected	Concentration (ng/mL)	Library Score (%)	Combined Score (%)
Hydrocodone	*63.07*	*97.0*	*95.857*
Hydromorphone	*98.08*	*100.0*	*96.119*
Lorazepam	*574.20*	*100.0*	*97.119*
Norhydrocodone	*311.10*	*98.1*	*97.325*

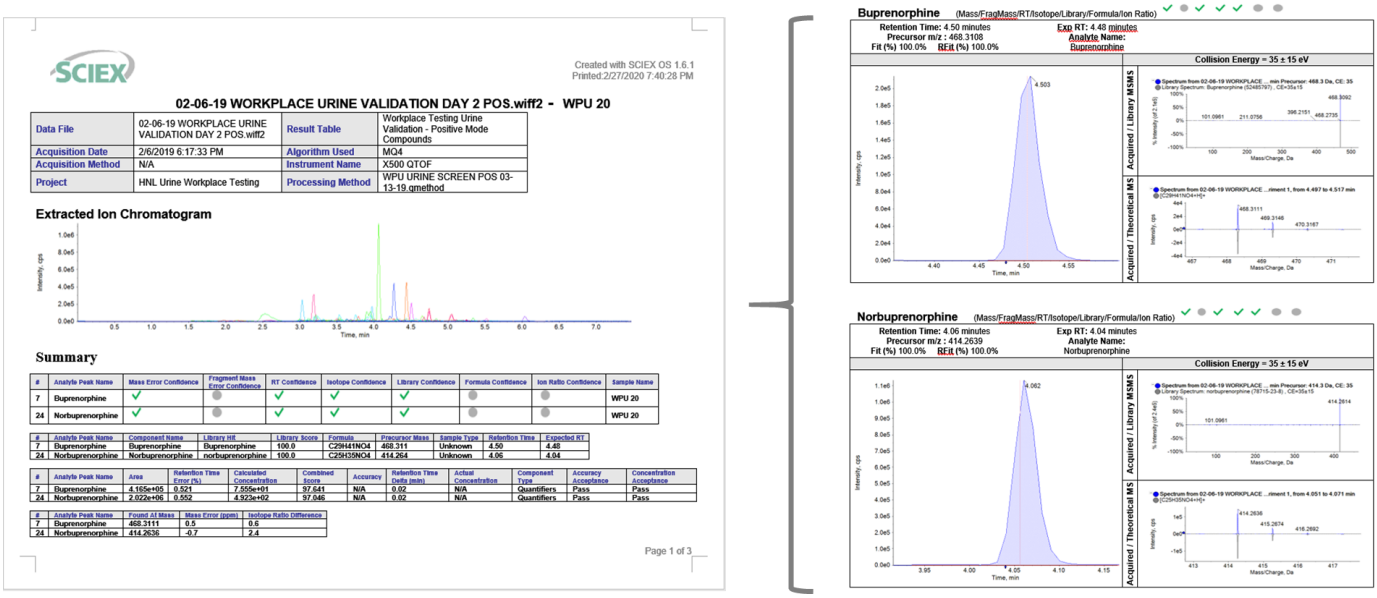

Figure 5. Detailed and comprehensive sample reporting using SCIEX OS Software. (Left) Sample information table, Total Ion Chromatogram (TIC) and results tables following the processing of a workplace testing urine sample. (Right) Extracted Ion Chromatogram (XIC), TOF MS and TOF MS/MS spectra of the positively identified analytes based on the acceptance criteria and displayed using the green check symbols.

Conclusions

A rapid and comprehensive drug screening workflow for the analysis of workplace testing urine samples was successfully developed using the SCIEX X500R QTOF System. The developed screening method enabled identification and quantitation of multiple drugs present in authentic forensic workplace testing urine samples.

- A simple dilute-and-shoot sample preparation method combined with a robust data acquisition strategy enabled collection of high-quality MS/MS spectra, allowing reliable compound quantitation and identification through spectral library matching
- Positive drug identification was confirmed using a combination of confidence criteria including mass accuracy, RT, %difference in isotope ratio, MS/MS library matching and the associated combined score
- The data analysis component of SCIEX OS software provided an intuitive and efficient data processing platform for confident analyte identification and efficient sample reporting process
- Adaptation of this efficient LC-MS method enabled quantitative drug screening and confident analyte identification from workplace testing urine samples

References

1. vMethod™ Application – Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System.

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