High sensitivity drug analysis using dried blood spots

Using the SCIEX Triple Quad 7500 system, powered by SCIEX OS software

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The ability to accurately measure low levels of drugs and their metabolites is critical for a wide variety of toxicology applications, including roadside testing (driving under the influence of drugs, or DUID cases), postmortem investigations, drug-facilitated sexual assault cases, follow-up of drug and alcohol addicts. As some drugs are rapidly metabolized in the body, comprehensive drug analysis approaches are critically needed to confirm the presence of these substances and provide the necessary drug concentration evidence to support their toxicity level with a high level of sensitivity and specificity.

Drug monitoring is typically performed using serum or plasma obtained by venous blood sampling. However, there is a growing interest in dried blood spots (DBS) as an alternative sampling strategy. Compared to traditional venous blood sampling, DBS have many advantages including (1) minimally invasive sample collection procedure, (2) small sample volume requirement, (3) increased analyte stability, and (4) convenient sample storage and transport with minimal chance of sample adulteration. Given the small amount of sample available for testing (usually in the 5-50 µL range), accurate quantification of low levels of drugs and their metabolites requires the use of a sensitive analysis technique.

In this technical note, an optimized sample extraction procedure was used in combination with the SCIEX 7500 system for picogram/mL detection of a panel of 24 drugs and metabolites extracted from DBS. This robust and comprehensive drug monitoring workflow is shown to provide the required sensitivity levels for accurate quantification of low levels of analytes with a wide range of physical and chemical properties.

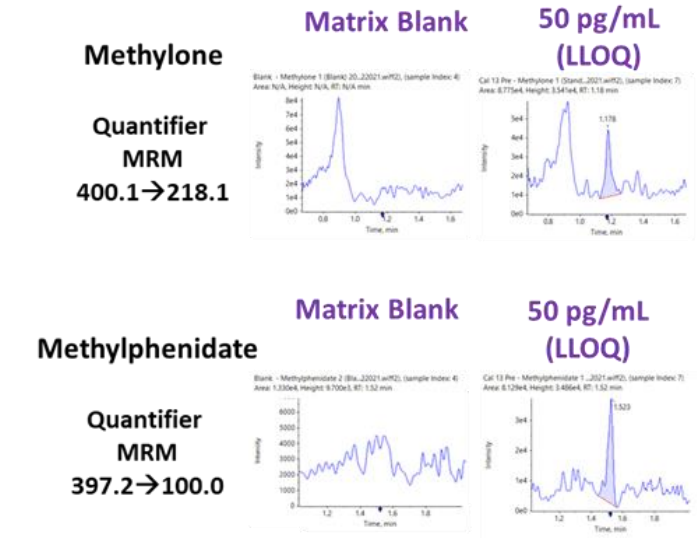

Figure 1. High sensitivity detection of drugs and metabolites extracted from DBS. Extracted ion chromatogram (XIC) traces for methylone (top) and methylphenidate (bottom) showing the quantifier ion traces for the matrix blank (left) and at the LLOQ at 50 pg/mL (right).

Key advantages of drug monitoring method for DBS analysis on the SCIEX 7500 system

- Optimized sample extraction procedure in combination with a robust detection method using the Scheduled MRM algorithm in SCIEX OS software enables pg/mL detection levels for a wide diversity of drug classes
- Ion ratio difference was <20% for the quantifier and qualifier ions of the targeted analytes, showing the quantitative robustness of the developed workflow
- Overall performance of the system resulted in excellent correlation (R^2 >0.98) with optimal precision (below 20%) and accuracy (with bias ±15%) across the calibration range
- Combination of low pg/mL LLOQs with acceptable analyte recoveries provides a sensitive and robust method fit for rapid implementation of DBS analysis for routine drug monitoring

Experimental details

Target analytes and solutions: A total of 24 drugs and 10 deuterated internal standards were purchased from Cerilliant Corporation (Round Rock, TX). Two solutions were prepared in water: a standard mixture containing the 24 target analytes and an internal standard mixture containing the 10 deuterated internal standards. Table 1 lists the name, the calibration range, linear correlation value (R^2), LLOQ, accuracy and precision reported at the LLOQ, as well as the recovery values calculated at two concentrations levels (1 and 5 ng/mL) for each of the 24 target analytes targeted in this panel.

Calibrator preparation: Thirteen levels of calibrators were prepared by spiking the standard mixture containing the 24 target analytes in human whole blood to final concentrations ranging from 1 pg/mL to 50 ng/mL. A 10 ng/mL IS standard stock solution containing the 10 deuterated internal standards was prepared in methanol/acetonitrile (3:1, v/v) and used as the extracting solvent to extract the analytes from the DBS cards.

Sample preparation and DBS sample extraction procedures: Protein saver cards (also known as DBS cards) were purchased from Whatman (Piscataway, NJ). Human whole blood calibrator samples spiked with various concentrations of the 24 analytes were spotted onto the DBS cards and the analytes were extracted using the sample extraction procedure summarized in Figure 2.

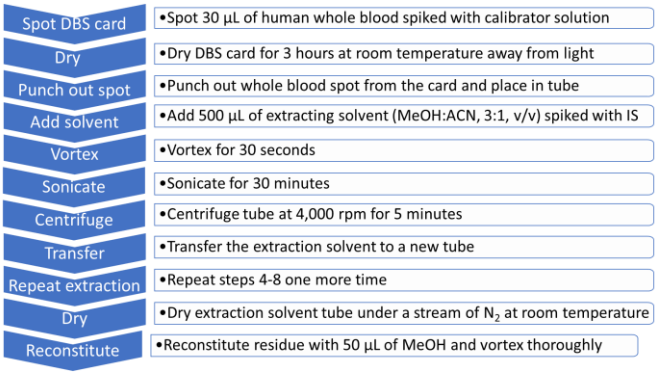

Figure 2. Analyte extraction workflow from DBS cards. An 11-step sample extraction protocol was optimized to selectively extract the 24 analytes from DBS cards for analysis using the SCIEX 7500 system.

Liquid chromatography: HPLC separation was performed on an ExionLC system using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6 µm, 00B-4495-AN). The separation conditions were identical to those previously described in a technical note.[2] Mobile phases were ammonium formate in water (MPA) and formic acid in methanol (MPB). The injection volume was 10 µL and the LC runtime was 6.5 min.

Mass spectrometry: A SCIEX 7500 system was equipped with an OptiFlow Pro ion source using an electrospray ionization (ESI) analytical probe and E Lens probe and was operated in positive mode. A single acquisition method consisting of 68 MRM transitions (48 for the drugs and 20 for the internal standards) was created using the Scheduled MRM algorithm in SCIEX OS software 2.0. Two MRM transitions were monitored for each of the targeted analytes and each sample was injected in triplicate to build a data analysis processing method.

Data analysis: Data processing was performed using SCIEX OS software. Rapid and automated quantitative data analysis was performed using the MQ4 algorithm in the Analytics module to streamline data processing. Peak area values, calibration curves, concentration calculations, assay precision and accuracy statistics were automatically generated in the Analytics module of the software.

Method development and optimization using the Scheduled MRM algorithm

A diluted, 10 ng/mL neat standard mixture containing the 24 analytes was used for initial method development. The Scheduled MRM Algorithm was used to automatically compute an optimized acquisition method based on user supplied analyte retention times based on MRM concurrency.[3] As a result, data sampling was optimal across each peak, maintaining good dwell times and desired cycles times. Most MRM transitions had 15 or more data points across each of the LC peaks, with 10 being the minimum number of data points observed on a peak for the 24 target analytes in this panel. Figure 3 shows the chromatographic profile of the 24 targeted analytes resulting from the optimized data acquisition method using the 10 ng/mL neat standard mixture.

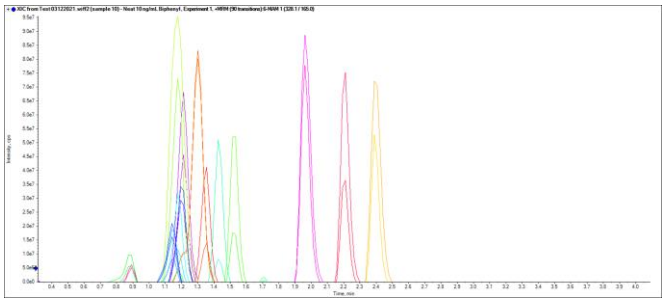

Figure 3. Chromatographic profile of the 24 analytes included in the panel. Extracted ion chromatograms (XICs) resulting from the optimized data acquisition method, obtained from the 10 ng/mL neat standard mixture containing the 24 targeted analytes. Method was built using the Scheduled MRM algorithm Pro in SCIEX OS software.

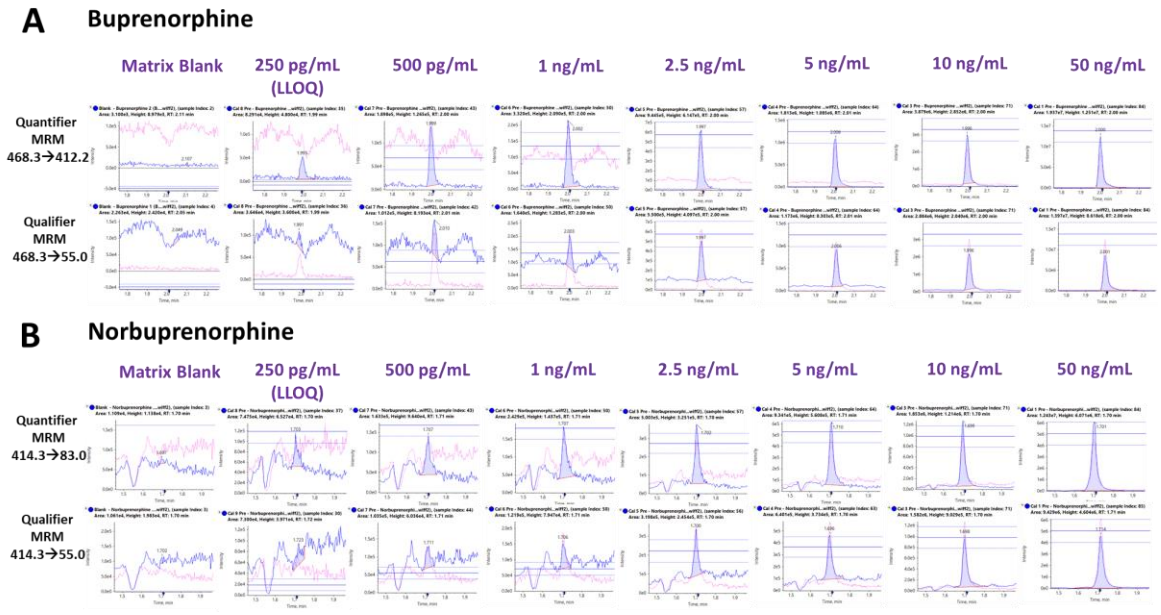

Figure 4. Representative extracted ion chromatograms (XICs) for selected drugs in the forensic panel. XICs for buprenorphine (A) and norbuprenorphine (B) from 0.25 to 50 ng/mL, including the blank injection. Both the quantifier and qualifier traces are shown. Ion ratios were also monitored across the dataset and tolerance lines are shown. The ion ratio difference was <20% for the quantifier and qualifier ions of each of the targeted analytes across the calibration range.

Robust detection method leads to accurate analyte quantification

Reliable measurements of drug concentrations are key to the successful implementation of drug monitoring workflows in testing laboratories. To that extent, reproducible and accurate quantification of drugs and metabolites extracted relies on the use of a robust detection method. Human whole blood calibrator samples spiked with concentrations ranging from 1 pg/mL to 50 ng/mL were injected to evaluate the quantitative performance of the system and its ability to accurately measure various levels of drugs and metabolites extracted from DBS with a high level of precision and accuracy.

Figure 4 shows representative extracted ion chromatograms (XICs) for the two MRM transitions monitored for buprenorphine and norbuprenorphine, two of the drugs targeted in this study. The XIC traces display overlays of both the quantifier and qualifier ion transitions monitored for each drug, for a blank injection (left) and for concentrations ranging from 250 pg/mL to 50 ng/mL. The confirmatory ion ratio lines between the two transitions are also displayed showing the tolerance limit. The lower limits of quantification (LLOQ) for the drugs and metabolites targeted in this workflow ranged from 50 to 250 pg/mL (Table 1). Figure 1 shows the XIC traces for methylone and methylphenidate, two drugs with LLOQs of 50 pg/mL. Overall, the quantifiable concentration ranges showcased in this workflow are well within the range of concentrations relevant for drug monitoring.

The ability to accurately quantify low levels of drugs and metabolites extracted from DBS is important, but the ability to consistently deliver high levels of data quality with high precision and accuracy is critical. The quantification performance of the SCIEX 7500 system was demonstrated with calculated precision compliance reported as CV% (values below 20%) and accuracy reported as bias% (values in the interval ±15%) across the calibration range for all 24 targeted analytes across the calibration range (Table 1).

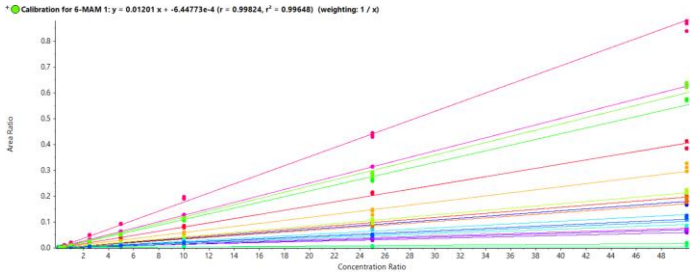

Figure 5. Excellent linearity for the 24 analytes extracted from DBS. Calibration curves generated using the two MRM transitions monitored for each of the 24 analytes targeted in this study. The assay showed excellent linearity with R^2 values greater than 0.99 for all the analytes.

Calibration curves were quickly generated using the two MRM transitions monitored for each analyte. Figure 5 shows the resulting regression lines plotted across the calibrator levels. The calibration curves demonstrated excellent linearity with R^2 values greater than 0.98 for all the drugs and metabolites in the panel (Table 1).

Optimized extraction procedure leads to acceptable levels of analyte recovery

One of the critical aspects of DBS analysis is the efficiency of the extraction method for analytes with a wide range of physical and chemical properties such as those included in this panel. An inefficient extraction method typically yields low analyte recovery, which can result in poor linear response, limits of quantification (LOQ) and assay reproducibility. The efficiency of the DBS extraction procedure used in this workflow was investigated by calculating the analyte recovery at two concentration levels (1 and 5 ng/mL). The recovery values were calculated by expressing ratio of the average (n=3) peak areas of each analyte spiked before and after the extraction procedure as a percentage. The recovery values at the two concentration levels ranged between 21% and 56% for the drugs and metabolites targeted in the panel. The range of recovery values can be explained in part by the generic sample preparation procedure used for the 24 analytes and the wide chemical diversity of the drugs and metabolites making up the panel. Overall, the recovery values were acceptable given the high reproducibility of the assay and the range of LLOQ values achieved for the analytes. In this scenario, the use of deuterated internal standards is recommended to compensate the analytes loss. The recovery values at the two concentration levels for each analyte are summarized in Table 1.

Conclusions

A highly sensitive workflow for the detection of 24 drugs and metabolites in DBS has been described using the SCIEX 7500 system. The broad applicability of the optimized sample extraction procedure in combination with the sensitivity of the system enabled accurate quantification at low levels of a panel of 24 chemically diverse drugs and metabolites.

- An 11-step optimized sample extraction procedure was developed to efficiently extract a broad panel of 24 drugs and metabolites from DBS
- Analyte extraction recovery values were found to be between 21% and 56% for the panel of 24 analytes
- The use of the Scheduled MRM Algorithm optimized data acquisition and ensured high data quality for all analytes in the fast 6.5-minute runtime
- The high sensitivity of the SCIEX 7500 system enabled accurate quantification with low levels of drugs and metabolites, with LLOQ values ranging from 50 to 250 pg/mL
- Excellent precision (CV%< 20%), accuracy (bias ±15%) and correlation (R^2>0.98) were observed across the calibration range, proving the robustness of the workflow and the quantification performance of the SCIEX 7500 system
- The method can be easily implemented by testing laboratories in routine drug analysis for low-level detection of drugs and their metabolites extracted from DBS

References

1. Enabling new levels of quantification — using the SCIEX Triple Quad 7500 system, powered by SCIEX OS software. SCIEX technical note, RUO-MKT-02-11886-A.
2. High sensitivity and dynamic range for 93-compound forensic panel analysis in urine. SCIEX technical note, RUO-MKT-02-9914-A.
3. Using Scheduled MRM algorithm in SCIEX OS software. SCIEX community post, RUO-MKT-18-11941-A.

Table 1. Statistical results for the 24 analytes targeted in this workflow. The table includes calibration range, linear correlation coefficient (R^2 Value), and the LLOQ, as well as the accuracy and precision measured at the LLOQ. The analyte recovery values at 1 and 5 ng/mL are also reported.

Compound	Calibration range (ng/mL)	Linear correlation (R^2)	LLOQ (ng/mL)	Accuracy at LLOQ (%)	Precision at LLOQ (%)	Recovery at 1 ng/mL (%)	Recovery at 5 ng/mL (%)
6-MAM	*0.1-50*	*0.99648*	*0.1*	*111.91*	*1.85*	*20*	*18*
Acetyl fentanyl	*0.1-50*	*0.99101*	*0.1*	*106.23*	*6.77*	*45*	*36*
Buphedrone	*0.25-50*	*0.99561*	*0.25*	*93.85*	*11.04*	*29*	*27*
Buprenorphine	*0.25-50*	*0.99075*	*0.1*	*89.69*	*5.20*	*26*	*28*
EDPP	*0.01-50*	*0.99168*	*0.01*	*105.62*	*4.77*	*37*	*23*
Fentanyl	*0.05-50*	*0.98625*	*0.05*	*91.35*	*8.94*	*47*	*56*
Hydromorphone	*0.25-50*	*0.98176*	*0.25*	*112.41*	*1.92*	*24*	*21*
Imipramine	*0.05-50*	*0.99323*	*0.05*	*87.28*	*7.23*	*49*	*45*
MDEA	*0.1-50*	*0.99210*	*0.1*	*93.50*	*8.97*	*39*	*42*
MDPV	*0.05-50*	*0.99817*	*0.05*	*92.51*	*6.45*	*30*	*21*
Mephedrone	*0.05-50*	*0.99224*	*0.05*	*95.24*	*0.89*	*31*	*31*
Methadone	*0.05-50*	*0.99299*	*0.05*	*96.87*	*8.33*	*44*	*40*
Methamphetamine	*0.1-50*	*0.99619*	*0.1*	*91.42*	*8.17*	*45*	*56*
Methedrone	*0.05-50*	*0.99346*	*0.05*	*89.73*	*13.13*	*45*	*38*
Methylone	*0.05-50*	*0.99687*	*0.05*	*94.97*	*13.09*	*37*	*38*
Methylphenidate	*0.05-50*	*0.99515*	*0.05*	*85.84*	*17.27*	*44*	*46*
Morphine	*0.1-50*	*0.99563*	*0.1*	*105.94*	*3.05*	*37*	*34*
Norbuprenorphine	*0.25-50*	*0.98456*	*0.5*	*104.60*	*9.76*	*43*	*36*
Norfentanyl	*0.05-50*	*0.99842*	*0.05*	*106.44*	*9.12*	*47*	*42*
Norhydrocodone	*0.1-50*	*0.99796*	*0.1*	*95.98*	*3.29*	*38*	*37*
Noroxycodone	*0.1-50*	*0.99426*	*0.1*	*97.35*	*10.08*	*46*	*39*
Oxycodone	*0.05-50*	*0.99230*	*0.05*	*94.33*	*12.94*	*51*	*58*
Oxymorphone	*0.1-50*	*0.99765*	*0.1*	*103.91*	*9.18*	*28*	*24*
Sufentanil	*0.01-50*	*0.99772*	*0.01*	*112.79*	*12.58*	*42*	*43*

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