Application Note

Forensics

Drug Screening in Whole Blood Using the Agilent 6546 LC/Q-TOF and the LC Screener Tool with Automated Sample Preparation

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Abstract

This application note describes a method for the routine screening of drugs and their related metabolites in whole blood. An Agilent 6546 LC/Q-TOF was used with the LC Screener Tool component of Agilent MassHunter Quantitative Analysis 10.1. Automated sample preparation was performed with an Agilent Bravo automated liquid handling platform. This method showcased the ability of the LC Screener Tool to be applied for routine drug screening of forensic toxicology samples.

Introduction

Mass spectrometers are gaining traction in forensic and toxicology labs for their high sensitivity, specificity, and ability to screen large numbers of drugs simultaneously. Due to the nature of the drug market, new entities are emerging constantly, especially illicit drugs. Therefore, these labs need to update their methods to have a more comprehensive panel offering as the market evolves. At some point, the number of analytes being tested is beyond the targeted capabilities of a triple quadrupole method and every time a new analyte is added to the method, a revalidation is required. Furthermore, the targeted nature of a triple quadrupole method removes the possibility of retrospective data analysis for emerging analytes, for instance a new designer drug. Using an LC/Q-TOF avoids these problems, as full-spectrum data in MS mode is acquired, allowing the reanalysis of the sample for new drugs without the need to acquire data again. This is achieved with a data-independent acquisition mode such as All Ions, which collects signal for every ion and its fragment. Additionally, the 6546 LC/Q-TOF provides high-resolution data (R >30,000 at *m/z* 118) and isotopic fidelity at fast acquisition speeds. This leads to excellent chromatographic integration with superb identification from the accurate mass and isotope pattern. With this instrument's high resolution, mass accuracy, and isotopic fidelity, analytes can be confidently identified without the need of constant comparison to an analytical grade standard. All these Q-TOF characterizations are advantageous for identifying drugs with confidence.

However, Q-TOF data analysis, especially in data-independent mode, can be time consuming for routine testing. With a triple quadrupole method, a lot of time is spent on the method development. With All Ions acquisition, the acquisition setup is very fast, but considerable time is typically spent on the data analysis. This analysis burden is removed with a new software tool in MassHunter Quantitative Analysis 10.1, the LC Screener Tool. The workflow is much more simplified: an analysis method with precursor and fragment information is created from a personal compound database and library (PCDL) containing the analytes of interest. Parameters like signal-to-noise, mass accuracy, and adduct pattern are then set for the analytes using a method setup workflow. When data is analyzed, the software extracts the information for these analytes and applies the analysis parameters to the extracted data. The LC Screener Tool displays all the pertinent information for data review and allows the user to filter results in a manner that makes analyzing hundreds or even a thousand analytes fast and simple.

Described here is a method that analyzed over 150 drugs and their metabolites in whole blood. The workflow is described from sample preparation through data reporting. The chromatography was fast and achieved excellent separation in 10 minutes. Experiments were performed to test the sample preparation, reproducibility, sensitivity, carryover, and longevity of the method. Ten unknown samples from a crime lab were also tested. All data analysis was performed with the LC Screener Tool to demonstrate its routine and fast analysis capabilities when analyzing Q-TOF data from forensic toxicology samples.

Materials

Instrumentation and software

For sample preparation, an Agilent Bravo automated liquid handling platform (p/n G5563AA) with the on-deck vacuum filtration station was used. The system was operated with accompanying software, Agilent VWorks automation control software. A Biotage 96-well plate nitrogen dryer was used to dry sample extracts. An Agilent PlateLoc thermal microplate sealer (p/n G5585BA) was used to seal the plates and an Eppendorf centrifuge 5804R, 15 amp, with a plate rotor, spun the plates. An Agilent 1290 Infinity II LC was used for liquid chromatography (LC) and the Agilent 6546 LC/Q-TOF was used with Agilent MassHunter Acquisition 10.1 for data acquisition. The LC included a high-speed pump (p/n G7120A), a multisampler (p/n G7167B), and a multicolumn thermostat (p/n G7116B).

For data analysis, MassHunter Quantitative Analysis 10.1 was used with the embedded LC Screener Tool. The method was built using the Agilent Forensic Toxicology PCDL for TOF or Q-TOF LC/MS systems. This spectral library contains nearly 4,000 analytes with expertly curated spectra, but only a subset of analytes from this library was used for this study. If spectra needed to be added, MassHunter Qualitative Analysis 10.0 was used. A Hewlett-Packard Z4 G4 Workstation with 64 GB RAM was used for the data acquisition and analysis.

Consumables, chemicals, standards, and samples

Pooled and individual lots of blank bovine whole blood were procured from BioIVT. Analytical-grade chemical standards were purchased from Agilent, Cerilliant, and Cayman Chemical. A full list of the analytes tested can be found in the Appendix. All organic solvents and modifiers were HPLC grade and purchased from Agilent Technologies or Sigma-Aldrich. Agilent HPLC-grade methanol was used for the mobile phase (p/n 5190-6896). Ultrapure water was from a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Captiva EMR—Lipid 96-well plates (p/n 5190-1001) with 1 mL collection plates (p/n A696001000) were used for sample preparation. Both standard and wide-bore 250 µL pipette tips (standard p/n 19477-002, wide-bore p/n 19477-032) were used for liquid transfer with the Bravo platform. A shallow-well Agilent 96-well plate (p/n 5043-9310) was used to hold the final sample, and the microplate was sealed using an Agilent PlateLoc thermal microplate sealer with a peelable aluminum seal (p/n 24210-001). This seal is pierceable and compatible with the autosampler needle. An Agilent InfinityLab Poroshell 120 EC-C18 column (p/n 695775-902) was used for separation. Reference ions, purine and HP-921, were purchased from Agilent (p/n G11969-85003). De-identified human whole blood samples were provided by collaborators.

Methods

Sample preparation

Analytical-grade standards were diluted to the desired concentrations in methanol:water (1:1). To prepare a spiked blood sample at 1x concentration, a 20x standard mix was created and then diluted 20x in blank whole blood. For example, 50 µL of 500 ng/mL standard in solvent was spiked into 950 µL blank blood to create a 25 ng/mL standard in whole blood. For most experiments, the working mixture was 160 analytes spiked at 25 ng/mL in whole blood. During method development and testing, six analytes were confirmed to have degraded and a new standard mix could not be acquired. One analyte, pregabalin, did not have a fragment detected reproducibly at the highest concentration tested (25 ng/mL) due to matrix interference, so this was removed from analysis. The analysis reporting, therefore, was based on 153 analytes.

Whole blood samples were processed on the Bravo platform with a Captiva EMR—Lipid 96-well plate. The protocol is described in Figure 1. VWorks, the Bravo automation control software, used a custom program to complete all the Bravo liquid transfer and vacuum steps automatically. Some offline steps were required (i.e., nitrogen dry down). Automation not only provides precision of measurement, but also allows labs to process a large number of samples with minimal human involvement.

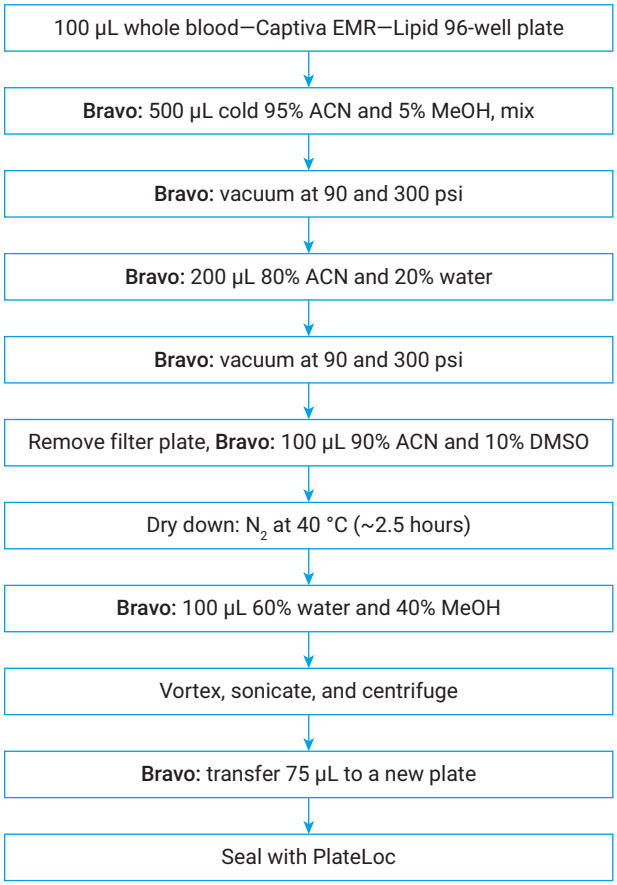

Figure 1. Procedure for whole-blood drug extraction and matrix removal using the Agilent Captiva EMR—Lipid 96-well plates. The pipetting of solvents and vacuum steps was automated with the Bravo platform. Offline steps include drying down the extraction, sonicating, and centrifuging for reconstitution.

LC/Q-TOF acquisition

After samples were processed, they were immediately placed in the Multisampler for analysis using the 1290 Infinity II LC (conditions in Table 1) and 6546 LC/Q-TOF with Jet Stream source (parameters in Table 2). Data-independent All Ions acquisition in positive mode was used. With this method, the Q-TOF cycled through three different MS-only scans at a rate of 8 Hz: one with 0 CE, one with 20 CE, and one with 40 CE. Purine (*m/z* 121.050873) and HP-921 (hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazene, *m/z* 922.009798) were used as reference ions during the analysis to achieve the best mass accuracy. SureMass, a data file format derived from profile data, was enabled and online data conversion was set as a postrun script. After an initial system tune, the instrument was only calibrated periodically throughout the data acquisition (approximately once a week). LC solvents and reference ion solution were refilled as required. No other maintenance was needed.

Data analysis

A data analysis method was created in MassHunter Quantitative Analysis 10.1 using a PCDL that contained spectra and retention times for the analytes of interest. The PCDL import workflow created the method. The intuitive LC Workflow dialog box guided input of the data analysis parameters. The parameters set in the LC Workflow are what the LC Screener tool used to flag data. They can be set to be the same for all the analytes in the method, or for a subset, or a single analyte, which allows flexibility and customization of the parameters for each analyte. This is beneficial when dealing with difficult analytes that pose potential to be false negatives or false positives. For analytes with the tendency to have false positives, stricter thresholds or chromatographic filters can be employed. For analytes where there is no tolerance for false negatives, looser parameters can be used with the tradeoff that more review for that analyte may be needed. The parameters used are listed in Table 3. The complete method was saved and used for all data analysis. At any point, to add a new analyte, the method can be appended with new analytes from a PCDL or created manually.

Table 1. Conditions applied with the Agilent 1290 Infinity II LC.

Parameter	Value
Analytical column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, narrow bore
Column temperature	55 °C
Injection volume	1 µL
Autosampler temperature	7 °C
Needle wash	Standard wash, 10 s, 80% methanol, 20% water
Mobile phase A	Water + 0.1% formic acid, 5 mM ammonium formate, 0.5 mM ammonium fluoride
Mobile phase B	Methanol + 0.1% formic acid, 5 mM ammonium formate, 0.5 mM ammonium fluoride
Flow rate	0.5 mL/min
Flow rate gradient	Time (min) % A % B 0 95 5 0.5 92 8 1.2 89 11 2 75 25 6 55 45 7.5 30 70 8.5 2 98 9.5 2 98 9.51 95 5
Stop time	10 min
Post time	1 min

Table 2. Agilent Jet Stream source and 6546 LC/Q-TOF data acquisition parameters.

Parameter	Value
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Gas temperature	275 °C
Gas flow	8 L/min
Nebulizer	35 psi
Capillary voltage	4000 V
MS tune	*m/z* 750, SureMass optimization enabled
MS mode	Positive
Acquisition	MS only with 0, 20, 40 CE segments
MS range	*m/z* 40 to 1,000
Divert to MS	0.5 to 9.2 min
Reference mass ions	*m/z* 121.050873 ([M+H]+ for purine) and *m/z* 922.009798 ([M+H]+ for HP-921) in 95% acetonitrile and 5% water

Table 3.

Parameter	Value
Mass Extraction	10 ppm (left and right)
Retention Time	0.3 min (left and right)
Retention Time Outlier	10%
Signal-to-Noise	3
Coelution Score	80
Mass Accuracy	5 ppm
Number of Verified Ions	2

Experimental

A series of experiments was designed to demonstrate matrix effects, analyte recovery, reproducibility, robustness, linearity, carryover, and sample variability.

Matrix effects were tested by comparing the analyte signal from a post-sample preparation spiked blood sample to a spiked solvent sample (n = 8). The matrix effect calculation was:

[1 – (Area of postspike of matrix/Area of solvent spike)] × 100

For recovery, a blank blood sample was processed next to a spiked blood sample (25 ng/mL, n = 8). After processing, the blank sample was spiked to yield a 25 ng/mL concentration. The calculation for percent recovery was:

Area of prespike/Area of postspike × 100

Spiked bovine blood samples were prepared at various concentrations ranging from 0.5 to 25 ng/mL (n = 6).

Each sample was injected to assess the limit of detection (LOD) and relative standard deviation (RSD) at the LOD.

A longevity study was performed to assess the robustness of the method and instrument. A 10 ng/mL spiked blood sample was injected over 1,400 times on the same column. It is worth noting that this column had already endured nearly 1,500 injections of processed blood prior to beginning this study. Over the 11-day experiment, the only maintenance steps that were taken were refilling mobile phases and reference mass solution as needed, and a mass calibration twice.

Quantitative capabilities were tested with a subset of analytes. A calibration curve ranging from 1–250 ng/mL was prepared in blood then analyzed with the method (n = 3).

To assess carryover, a 10,000 ng/mL sample was injected followed by blank blood injections. This was done for several mixtures containing a total of 66 analytes. If a peak was detected in the blank, the percent carryover was calculated with this equation: carryover blank area/10,000 ng/mL sample area. Since this concentration was so high, when analyte saturation was suspected, the ^{13}C isotope was used to calculate the percent carryover. Finally, eight individual blood samples were spiked with 25 ng/mL drugs and tested on the method to assess reproducibility when the matrix varied. These were processed and injected in replicates of six.

Results and discussion

Chromatography

The chromatography showed good separation for all the analytes in whole blood in under 10 min (Figure 2). There are several isobaric analytes that require chromatographic separation, and baseline separation was achieved for these analytes in this method (Figure 3).

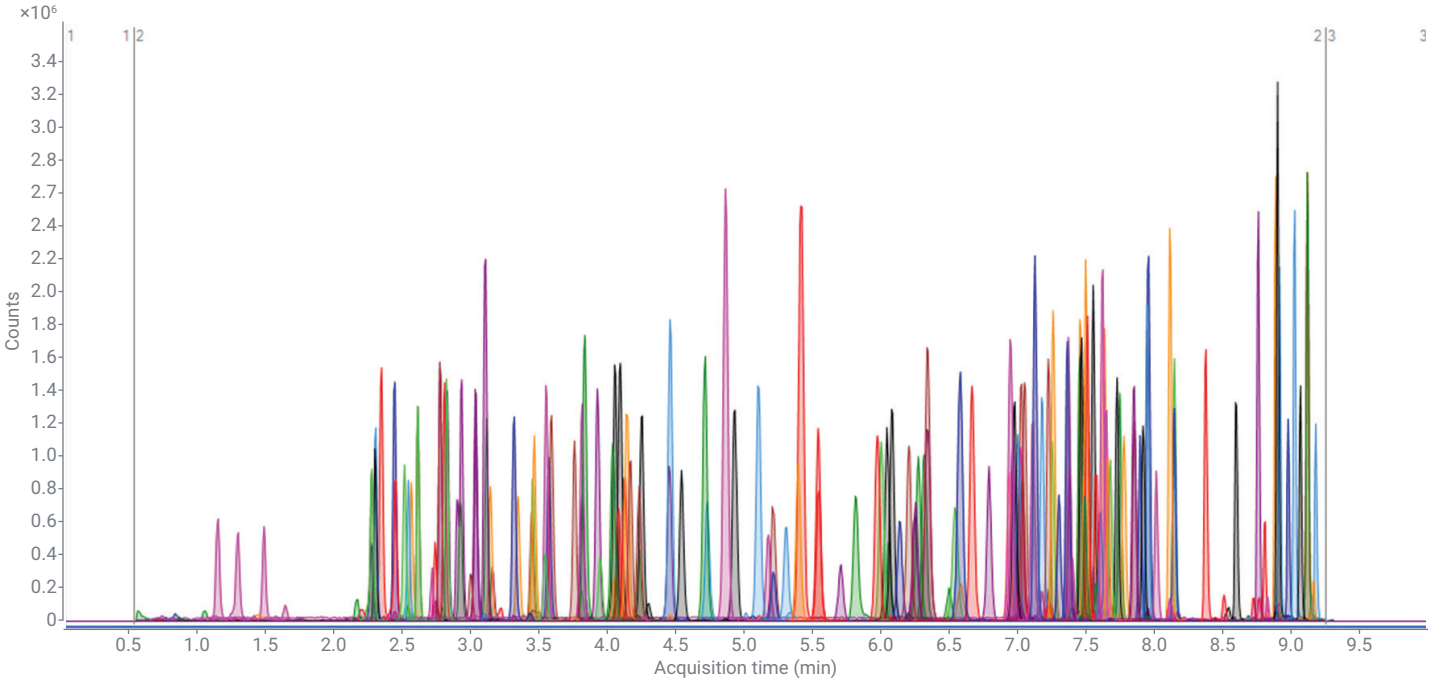

Figure 2. Chromatographic separation of 153 analytes at 25 ng/mL in whole blood over a 10 min gradient. Good separation and peak shape were achieved.

The fast acquisition rates allowed for ample points across the chromatographic peak (Figure 4). Furthermore, the Q-TOF's resolution performance at this faster speed held the same as it would at lower speed. The codeine peak, shown in Figure 4, had 12 precursor ion scans (CE 0), which had an average resolution greater than 48,000. In-between these data points, the Q-TOF was also collecting data at two other collision energies. These nonzero CE scans acquired the fragment information for codeine with the same resolution quality. This sufficient number of data points across the chromatographic peak ensures robust integration of every peak above the detection limits. For all experiments, no manual integration was needed due to this feature.

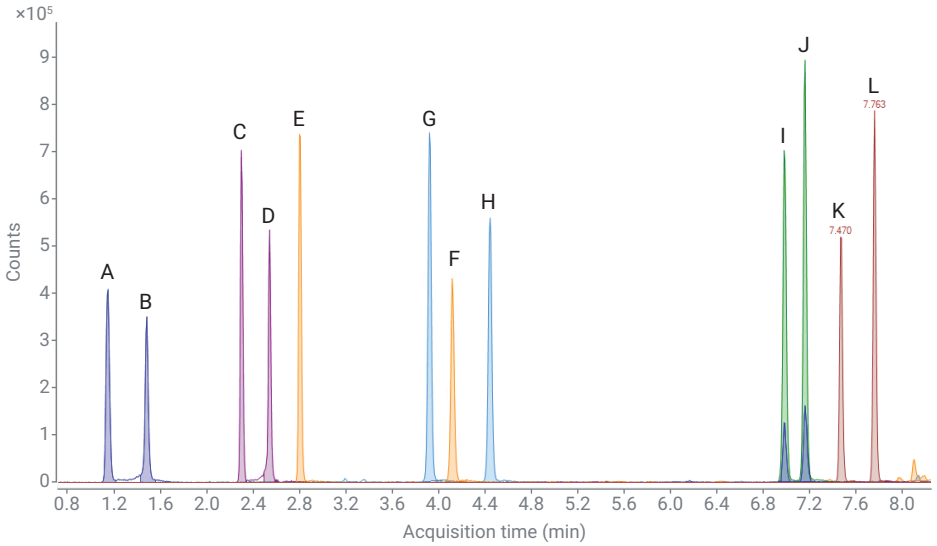

Figure 3. Extracted ion chromatograph of six sets of isobaric analytes that all have baseline separation with this LC method. Morphine (A), hydromorphone (B), codeine (C), hydrocodone (D), O-desmethyl-tramadol (E), N-desmethyl-tramadol (F), methylphenidate (G), normeperidine (H), promethazine (I), promazine (J), temazepam (K), and clonazepam (L).

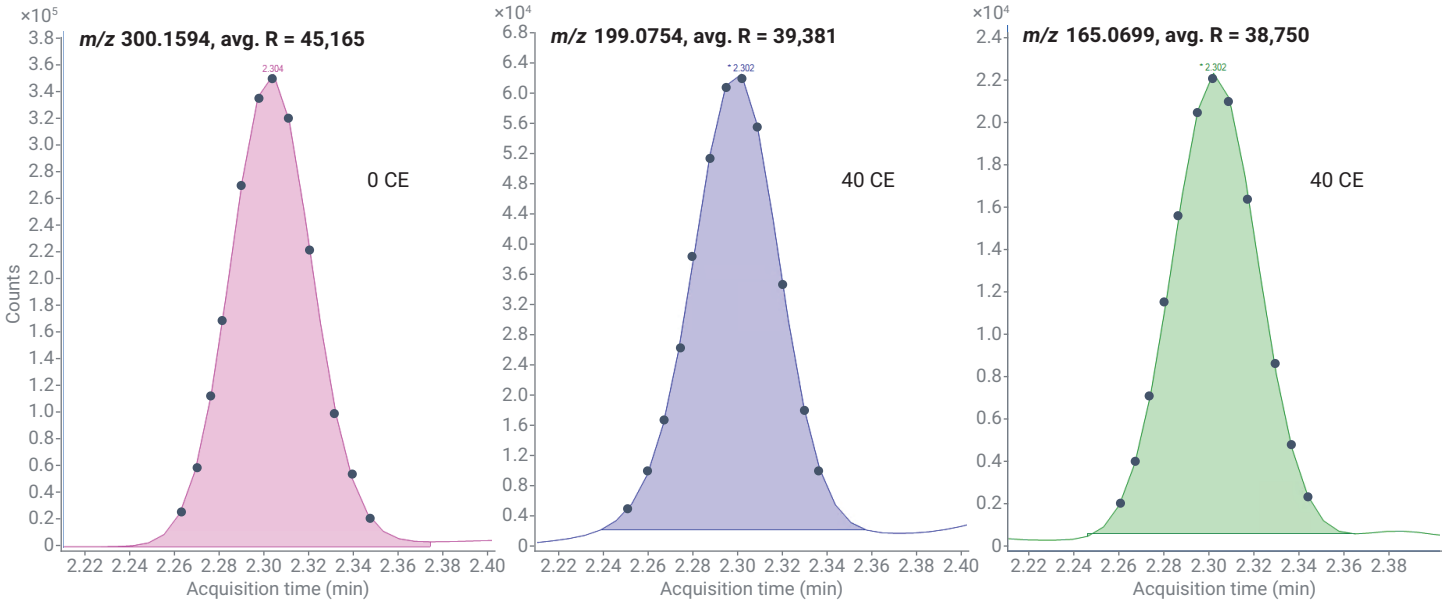

Figure 4. Chromatographic peaks of codeine's precursor (*m/z* 300.1594, CE 0) and two fragments (*m/z* 199.0754 and 165.0699, CE 40). There were 12 data points collected across their peak when using an acquisition rate for 8 spectra/sec, which provides good integration. Even at this fast acquisition, the resolution of the precursor averaged to 48,712 and the fragments had a resolution of 39,381 and 38,750 for this analyte. This data was from a blood sample spiked with drugs at 25 ng/mL.

Sample preparation

The matrix effects were very low after processing the blood through the Captiva EMR—Lipid 96-well plates. 81% of analytes had matrix effects below 10, and 97% were below 20% matrix effects (Figure 5). The recovery of the analytes with this sample protocol was also very good: 78% of the analytes fell between 80% and 120%, and 91% fell between 70% and 130% recovery. Those that fell below 70% recovery were late-eluting analytes such as cannabinoids, which are very hydrophobic and have similar structures to the lipids that were removed in the sample preparation (Figure 6).

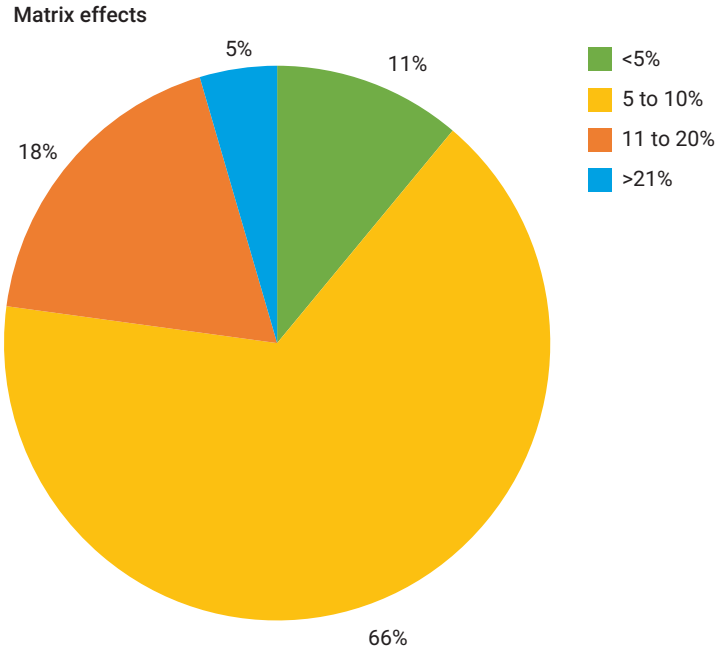

Figure 5. Pie chart showing matrix effect for 153 analytes. The matrix effects were calculated as stated in the experimental section. The majority of the analytes had less than 10% matrix effects, indicating good matrix cleanup was achieved with the Agilent Captiva EMR—Lipid sample preparation.

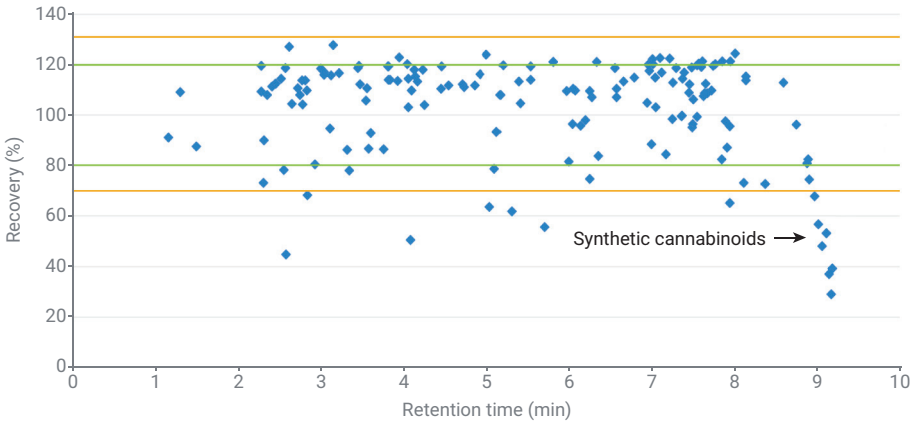

Figure 6. Percent recovery plotted against the retention time (min) of the analyte. The calculation for percent recovery was presented in the experimental section above. The green line indicates 80–120% recovery and the orange line indicates 70–130% recovery.

Spiked samples

The LOD was experimentally determined. This was a concentration where the parameters yielded a positive identification for the analyte with the LC Screener Tool. The LOD for the LC Screener was determined when the S/N of the precursor and one fragment was greater than 3, the RSD of the precursor was <20%, the mass accuracy of the precursor and fragment <5 ppm, and the coelution score was >80. The results for the LOD and the RSD at the LOD are summarized in Figure 7 and Figure 8.

Most of the analytes (63%) had an LOD of 1 ng/mL or lower. This level of sensitivity is acceptable for most analytes of interest in forensic toxicology labs. If more sensitivity is needed, the injection volume can be increased, or the samples can be reconstituted in less solvent to concentrate the analytes. The RSD of the precursor at the LOD was, by definition, below 20%. However, many of these (46%) were below 5%. This suggests a very reproducible method even at low concentrations and in complex matrix.

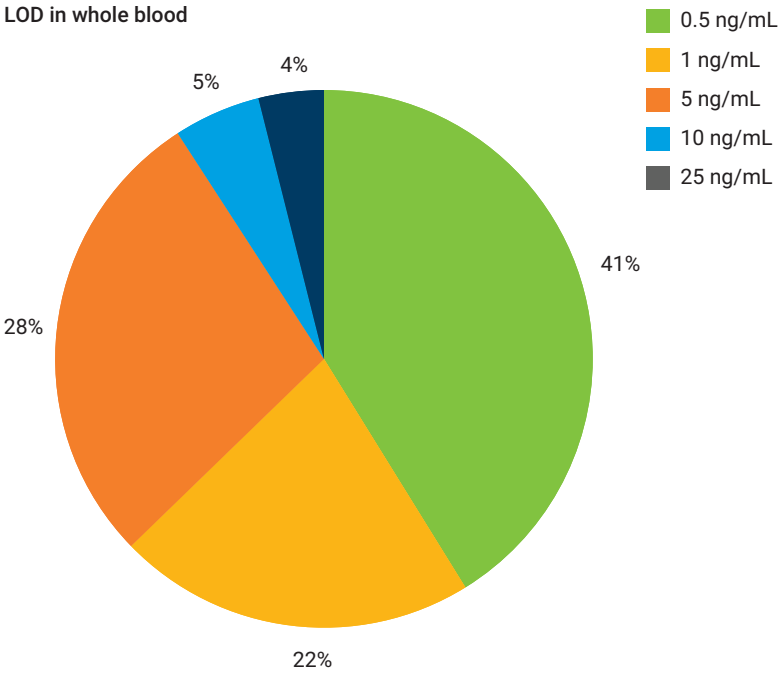

Figure 7. The LOD was determined from replicates of a mixture of analytes spiked into blank blood at different concentrations (n = 6).

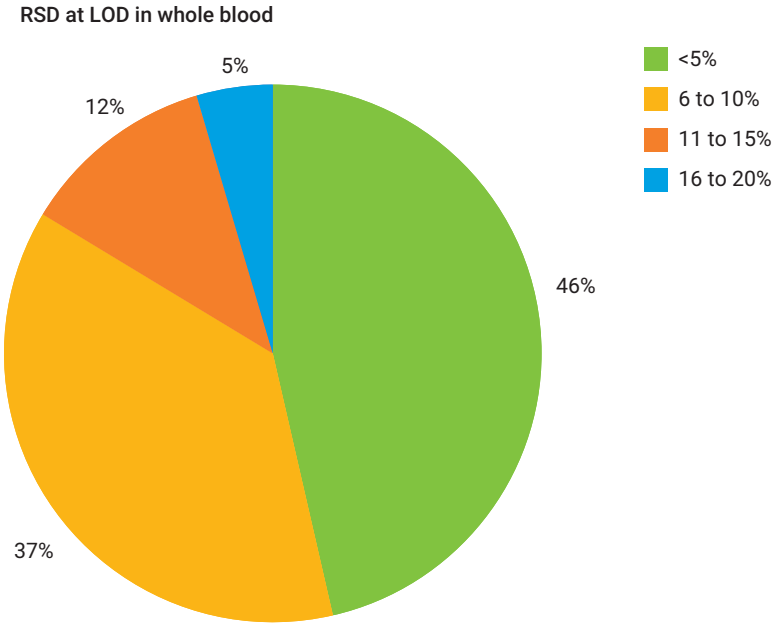

Figure 8. Pie chart displaying the RSD for 153 analytes in whole blood at their respective LOD (n = 6).

Longevity

For the method to be high throughput and deployed in a routine environment, it must produce robust and reproducible data on an instrument that does not require extensive maintenance. A longevity study was performed by injecting a 10 ng/mL spiked sample 1,465 times over an 11-day period. The area, retention time, and mass accuracy of all the analytes were stable. The data for morphine, the earliest eluter, is plotted in Figure 9.

The area was very stable for the analytes, indicating a robust method. The late eluters, cannabinoids, are not stable in the vial and decreased over time due to analyte loss and not due to sensitivity loss. The column used for this study had already received over 1,500 injections

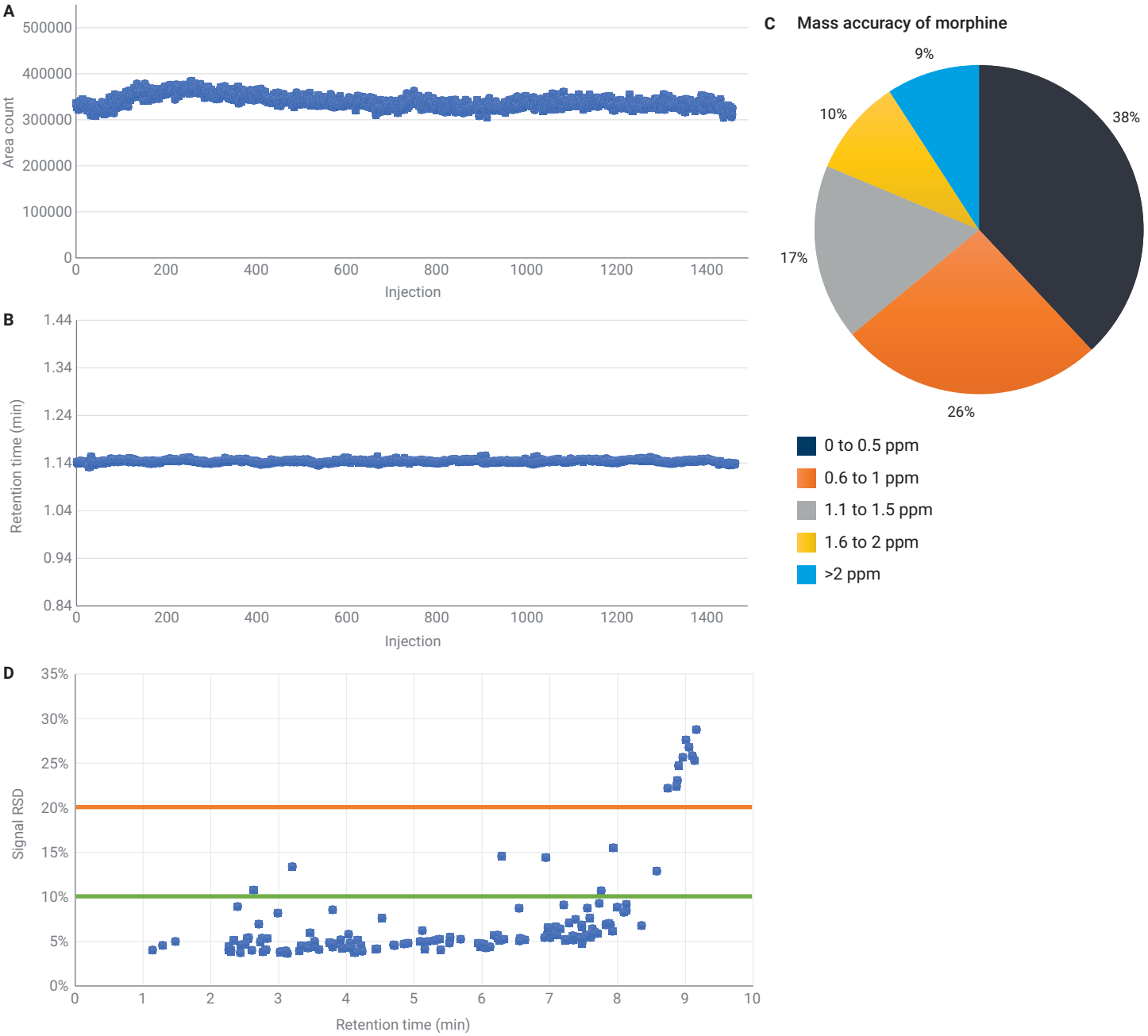

Figure 9. Area (A) and retention time (B) of morphine plotted over 1,465 injections. The mass accuracy (ppm) for morphine is reported in the pie chart (C). The sample was 10 ng/mL drugs in whole blood. The RSD of the 1,465 injections was plotted along with the retention time for all the drugs in the mix (D). The green line indicates 10% RSD and orange 20%. The high RSD of the cannabinoids is due to the sample degradation over time and not a loss of sensitivity of the Q-TOF. The maintenance included refilling the mobile phase and reference mass solution as needed and calibrating the Q-TOF twice over the eleven-day experiment.

from other tests. Additionally, the study ended at 1,465 injections due to time constraints and not because of performance. There was no indication that the system could not continue to produce high-quality data.

Carryover

Most analytes did not have any carryover and those that did were a very low percentage, which would not affect most analysis. Twenty-eight analytes had carryover that averaged <0.1%. The ^{13}C isotope was used for this calculation, since saturation occurred for the ^{12}C signal. An example of what the carryover looked like compared to the sample is shown in Figure 10.

Matrix reproducibility

To check for matrix variability, the analytes were spiked into eight different blood samples and each was tested in replicates (n = 6). All the analytes were detected in the eight individual samples. The signal for analytes spiked into the individual bovine samples was largely stable, with 75% having an RSD <5% (n = 48) (Figure 11). Five analytes had a higher RSD. This is because one of the individual blood samples had a coeluting analyte, which caused matrix suppression. The analyte of interest was still detected, but the reported area was lower, driving the RSD higher than 20%.

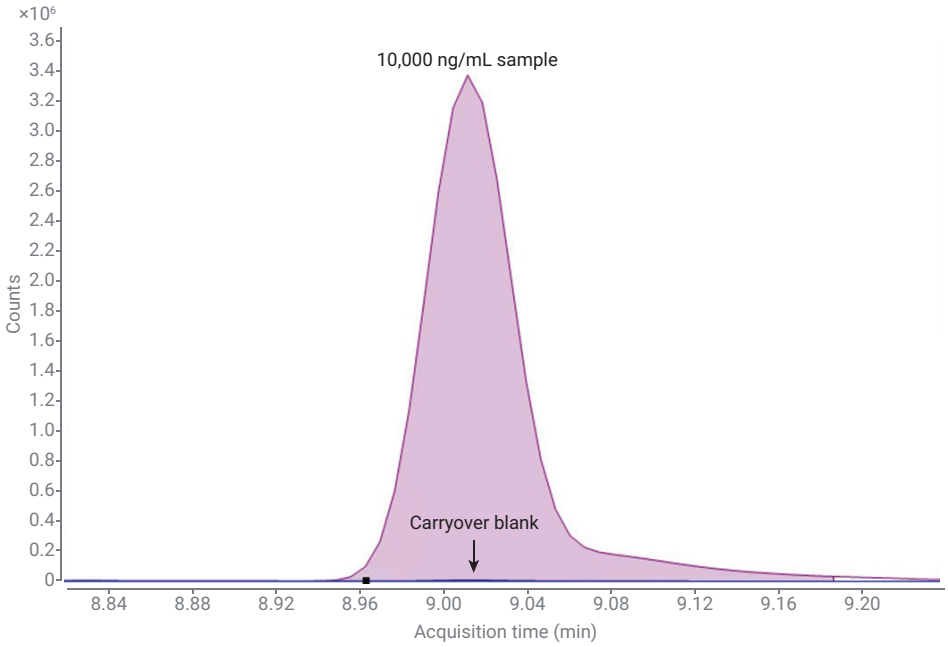

Figure 10. Extracted ion chromatogram of JWH 018 for the 10,000 ng/mL sample overlaid with the carryover solvent blank.

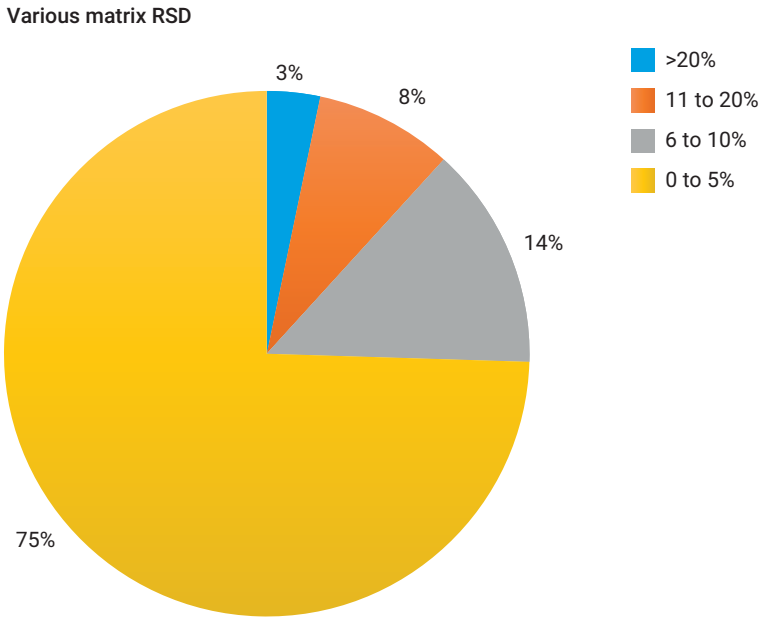

Figure 11. Pie chart showing the RSD of analytes spiked into eight individual bovine blood samples at 10 ng/mL and analyzed in replicates of six (n = 48).

LC screener analysis

With the MassHunter Quantitative Analysis software, the combined Quant-My-Way user interface (UI) and the LC Screener Tool created a powerful and easy-to-understand analysis experience (Figure 12). The LC Screener Tool displayed positively identified analytes in a sample (green), analytes that needed review (orange), and ones that were not identified (red) (Figure 13). The software determined how to identify each analyte based on the outlier parameters defined in the method (reported in the experimental section). These are flexible for each analyte, allowing a customizable method to be created. When an analyte was selected, related results were displayed for confirmation along with the mass accuracy and fragment information in the Quant-My-Way UI and/or Screener Tool (Figure 12 and Figure 13).

An analyte needed review (orange) when one analysis parameters was an outlier (i.e., mass accuracy). If this outlier was corrected in the data review process, the analyte moved to the positively identified list (green). This tool filtered and displayed data in a manner that makes reviewing hundreds or a thousand analytes in a sample easy and fast.

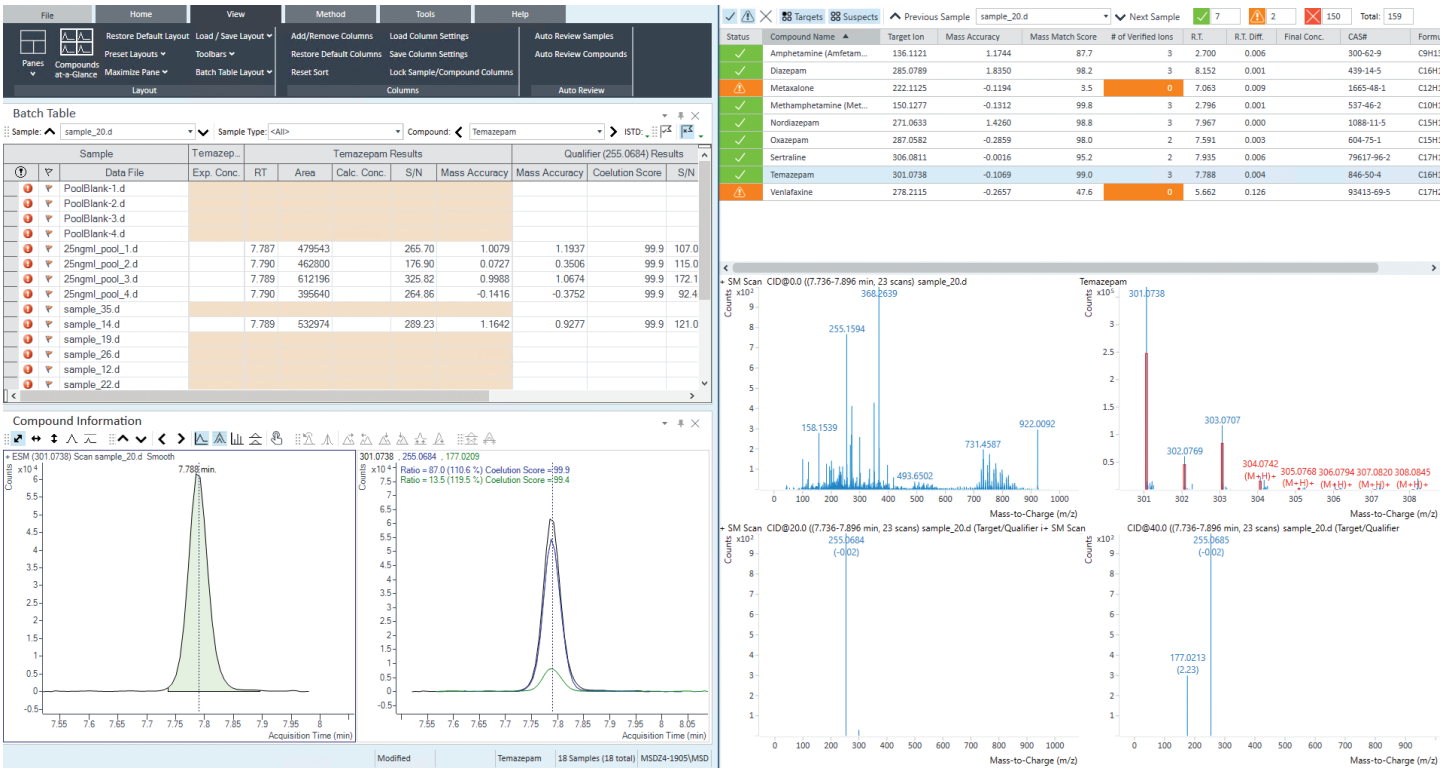

Figure 12. Quant my Way UI (left) and the LC Screener Tool (right) are complementary windows for fast LC/Q-TOF analysis. When an analyte is selected in either window, its complementary data appears automatically in the other.

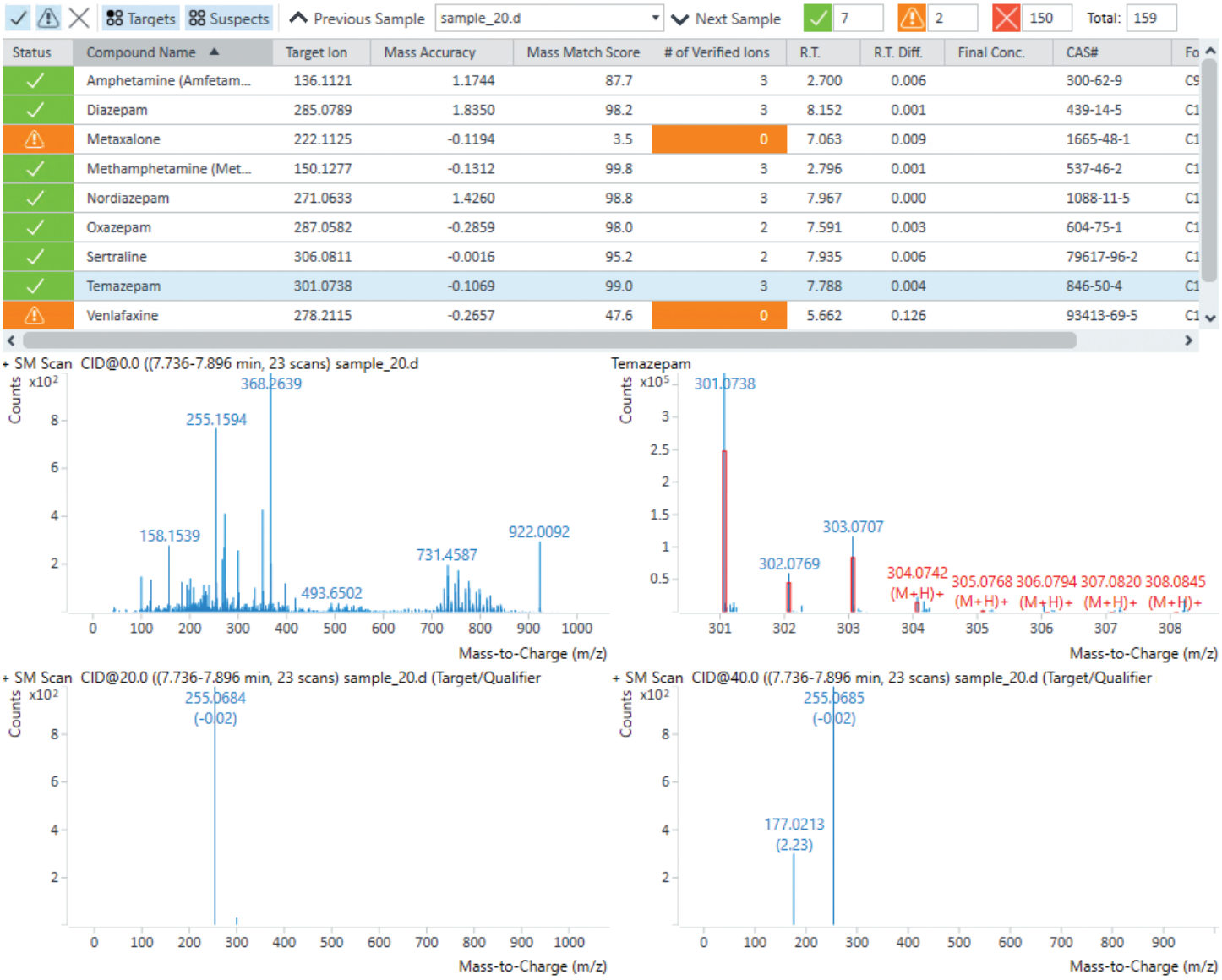

Figure 13. The LC Screener Tool showed a list of analytes that are positively identified or needed review in the selected sample. Unidentified analytes were filtered out. Important information like mass accuracy and number of detected analytes was shown in the top table. Temazepam was selected here, so its results were shown in the analysis panels. The middle left panel shows an average full spectrum at the time when temazepam elutes. The middle right panel shows the theoretical isotopes for temazepam (red) overlaid with the experimental data (blue). If adducts were present in the data, they would be displayed here as well. The lower left shows the analytes fragments at CE 20 and the lower right shows the fragments at CE 40.

Unknown samples

The samples from a collaborator were tested on the method. Drugs and their metabolites were detected (Table 3). Figure 13 shows the positively identified sample for temazepam in sample 2. To report the results, the simple screener report template (Figure 14) was used. This lists the identified analytes in the sample and the criteria by which they were positively identified in a PDF. Other reporting options showing chromatograms are also available. With the developed method, analyzing and reporting this data took no more than twenty minutes.

Simultaneous quantitation

If a lab also wanted to quantify all or a subset of analytes, this can be done easily with this platform and simultaneously analyzed in this software with the screening results. When an analyte calibration curve is added, the software labels it as a target, allowing for additional LC Screener filtering. A calibration curve is displayed in the Compound Information tab of the Quant-My-Way UI (Figure 15). Twenty-six analytes were calibrated and analyzed, while the remainder were suspects for screening. The calibration curves had an average R^2 >0.99 when using wither linear or power fits (n = 3). When a target analyte is detected, it is quantified, and the reported concentration is displayed in the LC Screener Tool (Figure 13) and the report (Figure 14).

Table 3. Table of positively identified analytes found in ten unknown samples provided by a collaborator.

Sample	Drugs Detected
1	Methamphetamine
2	Dihydrocodeine, oxycodone, hydrocodone, oxazepam, temazepam, nordiazepam, diazepam
3	Methamphetamine
4	Diphenhydramine, diazepam, nordiazepam
5	Amphetamine, methamphetamine, oxazepam, temazepam, sertraline, diazepam, nordiazepam
6	None
7	None
8	Amphetamine, methamphetamine, sertraline
9	Amphetamine, methamphetamine
10	Gabapentin, 7-aminoclonazepam, EDDP, clonazepam, methadone, lorazepam

LCQTOF Sample Screening Summary

Agilent Trusted Answers

Sample Name # of Hits 7

Status	CompoundName	CAS#	Formula	R.T.	R.T. Diff.	Final Conc.	Mass Match Score	Target Ion	Mass Accuracy	# of Verified Ions
+	Amphetamine (Amfetamine)	300-62-9	C9H13N	2.700	0.006			136.1121	1.17 PPM	3
+	Methamphetamine (Metamfetamine) (Deoxyephedrine)	537-46-2	C10H15N	2.796	0.001			150.1277	-0.13 PPM	3
+	Oxazepam	604-75-1	C15H11ClN2O2	7.591	0.003			287.0582	-0.29 PPM	2
+	Temazepam	846-50-4	C16H13ClN2O2	7.788	0.004			301.0738	-0.11 PPM	3
+	Sertraline	79617-96-2	C17H17Cl2N	7.935	0.006			306.0811	0.00 PPM	2
+	Nordiazepam	1088-11-5	C15H11ClN2O	7.967	0.000			271.0633	1.43 PPM	3
+	Diazepam	439-14-5	C16H13ClN2O	8.152	0.001			285.0789	1.83 PPM	3

Figure 14. One page of the batch report showing the unknown sample 2 results. Only positively identified analytes are listed in the report summary, with the pertinent information as to why it was identified as positive. If a calibration curve was added, the concentration would also be reported.

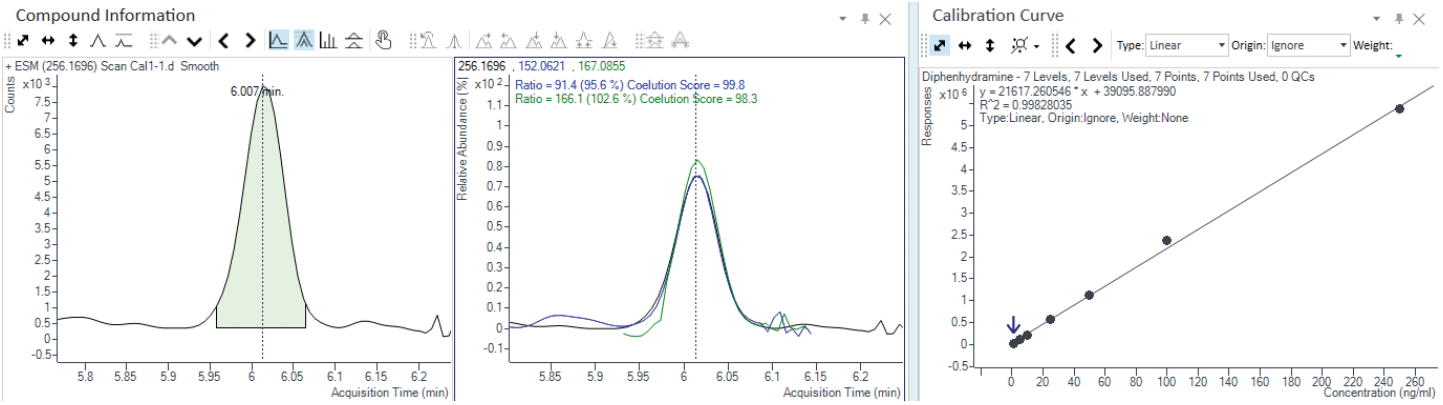

Figure 15. Calibration curve for diphenhydramine from 1–250 ng/mL displayed in the Compound Information table of the Quant-My-Way UI. This chromatogram on the left is for 1 ng/mL of the analyte in blood and its two fragments are overlaid in the center panel. R^2 = 0.9982 with linear fit and no weighting.

Conclusion

Sample preparation with the Bravo liquid handling platform is automated and facilitates good matrix removal and analyte recovery. The cannabinoid recoveries need to be improved, but the detection limits are much better with the solid phase extraction compared to just a liquid extract, where a large amount of signal suppression of the analytes was observed (data not shown). Overall, automation is beneficial, as it improves lab efficiency and consistency of results by removing manual pipetting steps.

The 6546 LC/Q-TOF and the LC Screener Tool make routine drug screening with a Q-TOF possible. The excellent data quality, reproducibility, and robustness of the 6546 LC/Q-TOF make it ideal for high-throughput routine laboratories. Confidently detecting drugs in a batch of unknown samples was very straightforward. The fast acquisition rates of the Q-TOF allow for excellent integration of a 0.1 min chromatographic peak while collecting fragment information—all with high resolution. The mass accuracy of the analytes and reproducibility of the area was maintained over the longevity study, demonstrating the robustness of the hardware. This performance held over 1,400 injections with minimal maintenance required, which gives confidence in the data collected over time. Furthermore, carryover was either not found or kept to a minimum with the LC method. When quantifying analytes, good linearity can be achieved and the analysis occurs in the same software, which improves data analysis efficiency. Labs can quantitate commonly found analytes and screen for a much larger and more uncommon, but still important, set of analytes.

The data, which contains all the analytes precursor and fragment information, is easy to analyze with the LC Screener Tool. The whole analysis method setup took under 10 minutes using the wizards. Once testing and development was completed, the analysis method was saved and was reused for analyzing new batches of data. This analysis workflow is very analogous to using MassHunter Quantitative Analysis software for triple quadrupole data analysis, but the data here has high resolution, greater mass accuracy, and isotopic information. The LC Screener Tool only shows you information required to identify for the analyte of interest, which simplifies the data review. Furthermore, how it flags an analyte for review and filters data makes batch review and reporting fast. Although only 153 analytes were in the analysis method here, data analysis for over a thousand analytes would not be overwhelming with this software.

Finally, because the data acquired are data independent, retrospective analysis for new or emerging analytes is possible for research purposes. For this, an analyte is added to the method and the resolution, isotopic pattern, mass accuracy, and fragments are used to find suspect identifications. If a PCDL entry doesn't exist (no standard at hand), then precursor and predicted fragment masses can be added manually, and the RT window kept open for the whole chromatographic run. This capability is unique to Q-TOFs and can be used for testing emerging drug trends without collecting new data.

Appendix

List of all tested analytes

- 10-Hydroxycarbazepine
- 2-Hydroxyethylflurazepam
- 3,4-Dimethylmethcathinone
- 3,4-Methylenedioxypyrovalerone (MDPV)
- 4-Methylmethcathinone (Mephedrone)
- 6-Acetylmorphine
- 7-Aminoclonazepam
- 7-Aminoflunitrazepam
- α-Hydroxyalprazolam
- α-Hydroxymidazolam
- α-Hydroxytriazolam
- Alprazolam
- AM2201
- Amisulpride
- Amitriptyline
- Amoxapine
- Amphetamine
- Aripiprazole
- Atropine
- Benzatropine
- Benzoylecognine
- Bromazepam
- Brompheniramine
- Buprenorphine
- Bupropion
- Cannabidiol
- Carbamazepine
- Carisoprodol
- Chlordiazepoxide
- Chlorpheniramine
- Chlorpromazine
- Chlorprothixene
- Citalopram
- Clobazam (Urbadan)
- Clomipramine
- Clonazepam
- Clonazolam
- Clozapine
- Cocaethylene
- Cocaine
- Codeine

- Cyclobenzaprine
- Desalkylflurazepam
- Deschloroketamine
- Desipramine
- Dextromethorphan
- Diazepam
- Diclazepam
- Dihydrocodeine
- Diltiazem
- Diphenhydramine
- Dothiepin
- Doxepin
- Doxylamine
- EDDP
- Fentanyl
- Fluconazole
- Flumazenil
- Flunitrazepam
- Fluoxetine
- Flupentixol
- Flurazepam
- Fluvoxamine
- Gabapentin
- Haloperidol
- Heroin
- HU-210
- Hydrocodone
- Hydromorphone
- Hydroxybupropion
- Imipramine
- JWH-018
- JWH-019
- JWH-073
- JWH-081
- JWH-122
- JWH-200
- JWH-203
- JWH-210
- JWH-250
- Ketamine
- Lamotrigine
- Levetiracetam
- Lorazepam
- MDA
- MDEA
- MDMA
- Medazepam
- Meperidine
- Meprobamate
- Metaxalone
- Methadone
- Methamphetamine
- Methcathinone
- Methocarbamol
- Methylone
- Methylphenidate
- m-Hydroxybenzoylecognine
- Mianserin
- Midazolam
- Mirtazapine
- Morphine
- Naloxone
- Naltrexone
- N-DM-Tramadol
- N-ethylamphetamine
- N-ethylcathinone
- Nitrazepam
- Norbuprenorphine
- Norclozapine
- Nordiazepam
- Norfentanyl
- Norketamine
- Normeperidine
- Nortriptyline
- O-Desvenlafaxine
- O-Desmethyl-*cis*-tramadol
- Olanzapine
- Oxazepam
- Oxycodone
- Oxymorphone
- Paliperidone
- Paroxetine
- PCP
- Pentazocine
- Phenazepam
- Phentermine
- Pipamperone
- Prazepam
- Primidone
- Promazine
- Promethazine
- Propoxyphene
- Protriptyline
- Pseudoephedrine
- Quetiapine
- RCS-4
- Risperidone
- Ritalinic acid
- Sertraline
- Tapentadol
- Temazepam
- Thioridazine
- Topiramate
- Tramadol
- Trazodone
- Triazolam
- Venlafaxine
- Verapamil
- Zaleplon
- Zolpidem
- Zonisamide
- Zopiclone

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