Poster Reprint

ASMS 2020
ThP 125

Drug Screening in Whole Blood Using a High-Resolution LC/Q-TOF and Novel Software Screener Tool

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Introduction

Drug Screening is Routine with LC/Q-TOF.

New drugs are continuously being introduced to the market. Whether it is a new illegal, prescription, or over-the-counter substance, laboratories need to test for these analytes. Targeted methodologies, like immunoassays or triple quadrupole mass spectrometer (MS) methods, do not allow the flexibility to quickly add these analytes to the method. Quadrupole Time-of-Flight (Q-TOF) MS methods allow for new analytes to be added without redeveloping the method because they can operate in a data independent acquisition (DIA) mode.

In this method, the 6546 LC/Q-TOF (Fig 1) was used for data acquisition. This instrument was chosen because it's high resolution (>30,000 at *m/z* 118), isotopic fidelity, and extended dynamic range produced confident identifications even when using fast chromatography with a whole blood sample. The extended dynamic range also made it possible to detect analytes at low levels even when there are often co-eluting analytes at higher abundances.

Figure 1. Bravo Automated Liquid Handling Platform (left) and the 6546 LC/Q-TOF (right) were the two instruments used in this method

In the past, Q-TOF data analysis has been complicated and challenging to implement in a high throughput way. This process is now routine and designed with the analyst in mind with a novel software tool. The LC Screener, in the MassHunter Quantitative Analysis 10.1 extracts the information for analytes of interest, applies identification criteria set in the method, and presents the data in an easy to understand manner. This software makes the analysis fast and simple.

Additionally, the Bravo Automated Liquid Handling Platform (Fig 1) was utilized with the Captiva EMR-Lipid 96 well plates to make extracting drugs from a whole blood sample routine. This instrument required minimal user intervention which lowers error and increases reproducibility.

Experimental

Sample Preparation and Data Independent Acquisition Methodology.

A solid phase extraction was performed on blood samples spiked with 153 analytes and ten deidentified samples from a crime lab. The sample prep steps are outlined in Figure 2. The 10 minute liquid chromatography (LC) method is described in Table 1.

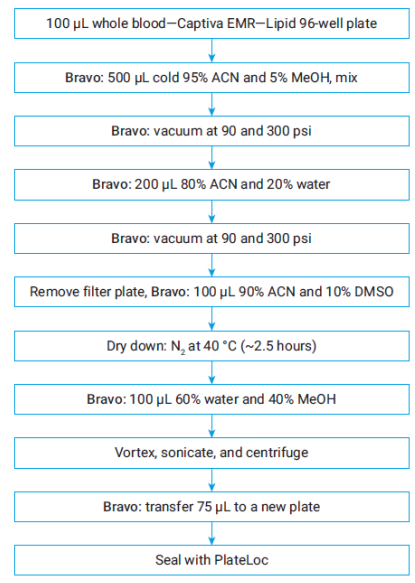

Figure 2. This workflow describes the steps for drug extraction and matrix removal for whole blood using the Captiva EMR-Lipid 96 well plates. Steps performed by the Bravo are labeled. Note, no sample concentration was performed for this method.

Table 1. LC method using an Agilent 1290 Infinity II.

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

An Agilent 6546 LC/Q-TOF with an AJS ion source were used to acquire molecular ion and fragment data in positive mode. The instrument operated from *m/z* 40-1000 at 8 Hz and used collision energies (CE) 20 and 40 to fragment molecular ions. Two reference ions were used to ensure mass accuracy.

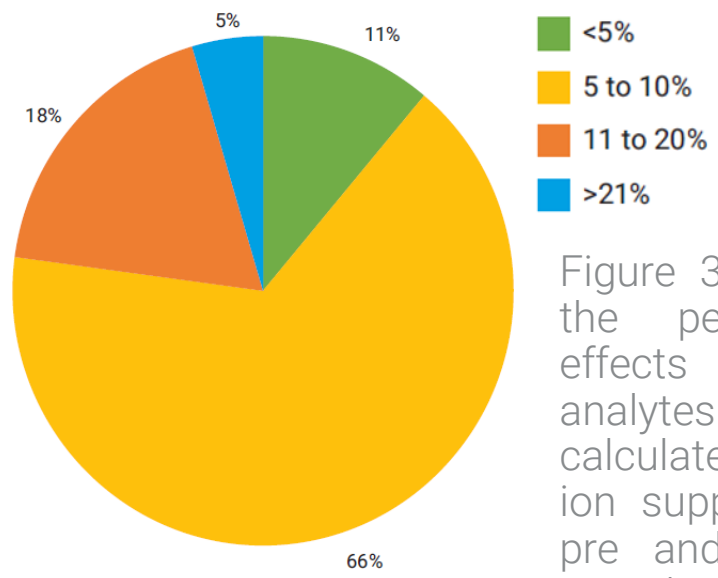

Figure 3. Pie chart of the percent matrix effects for the 153 analytes. This calculated the matrix ion suppression using pre and post spiked samples (n = 6).

Low Matrix Effects and High Recoveries were Achieved. The Fast Q-TOF Acquisition Speed Gave Robust Integration Without a Decrease to Mass Resolution.

The Captiva EMR-Lipid solid phase extraction procedure removed much of the matrix leaving a clean extract for injection. The matrix effects were very low with 77% of analytes have less than 10% matrix effects (Fig 3). Additionally recoveries were high with 91% of analytes falling between 70% and 130% recovery. Cannabinoids had lower recoveries with this solid phase chemistry.

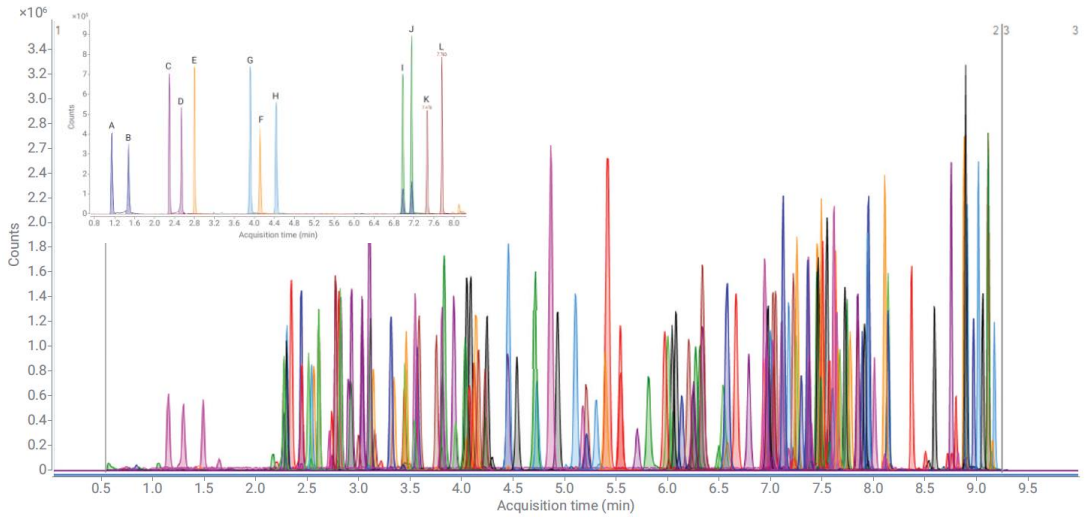

Figure 4. Separation of 153 analytes over 10 min. Inset chromatogram shows the baseline separation of isobaric analytes: morphine (A), hydromorphone (B), codeine (C), hydrocodone (D), O-desmethyl- tramadol (E), N-desmethyl-tramadol (F), methylphenidate (G), normeperidine (H), promethazine (I), promazine (J), temazepam (K), and clonazepam (L).

Chromatography was under 10 min and achieved good separation of all analytes tested. Baseline separation was achieved for six pairs of isobaric analytes in the method (Fig 4). Due to the Q-TOF's fast acquisition rate, plenty of data points were taken across the chromatographic peak allowing for robust integration of the molecular ion and fragment ions. With this instrument, the mass accuracy and resolution was maintained (Fig 5).

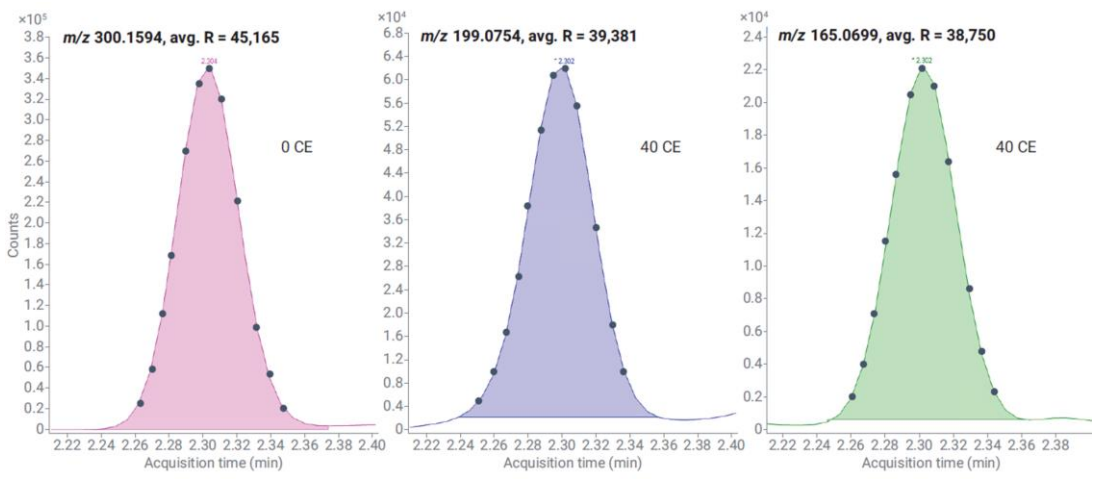

Figure 5. Chromatographic peaks for cocaine precursor (left) and two fragments (center and right). Using an acquisition speed of 8 Hz produced twelve data points (black dots) to be taken across the peaks which gave robust integration. With the Q-TOF, at these higher acquisition rates the resolution was not lowered.

MassHunter Quantitative Analysis and the LC Screener Make Q-TOF Analysis and Reporting Routine and Easy.

A screening method was built using a method creation wizard and a Personal Compound Database and Library containing MS/MS spectra for each analyte of interest. Criteria were set for identifying an analyte as positive namely, mass accuracy (5 ppm), signal to noise (3), retention time difference (10%), and at least two overlapping ions found (coelution > 80). The software filtered that data and labeled the analytes as positively identified (green) when all criteria were met, needs review (orange) if one criteria is out of bounds, or negatively identified (red) if more than one criteria are out of bounds.

The LC Screener displays all the analytes in the sample, filters the results based on identification category, and shows the isotope and chromatograms in a digestible manner (Fig 6). This allows for hundreds of analytes to be tested in a high throughput manner.

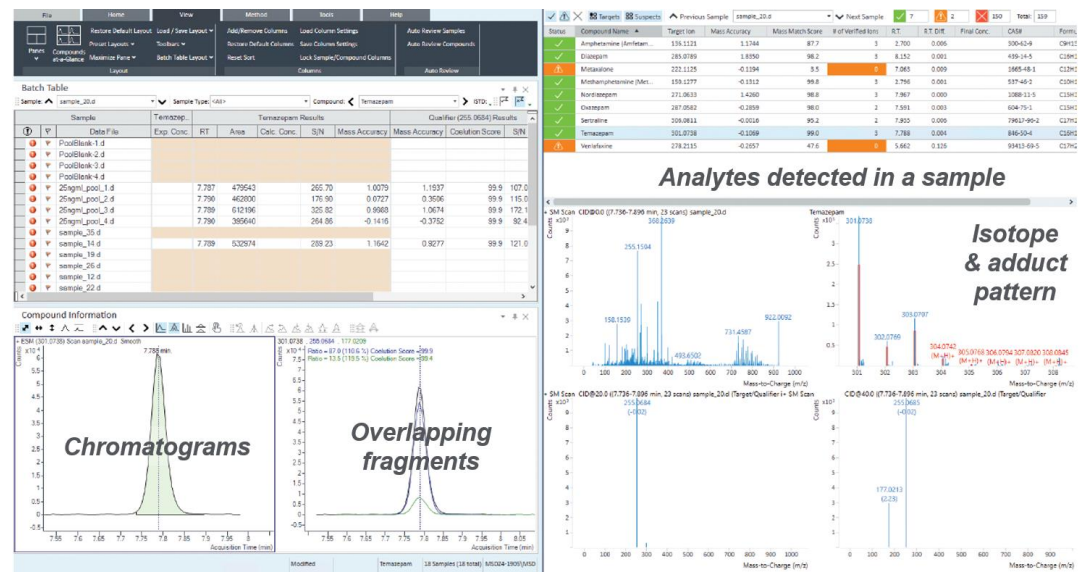

Figure 6. Analysis software display of the quant-my-way user interface and LC Screener Tool. Analytes in a sample, which are labeled as positively identified, needs review, or negatively identified, are displayed in the top right panel where they can be filtered. Each panel gives pertinent information for reviewing and confirming an identification.

Because the LC Screener Tool is located in the Quantitative Analysis software, simultaneous quantitation is possible for some or all analytes when you test calibration samples made from analytical standards (Fig 7). This means commonly found analytes may be quantified on the first injection while less common ones screened for only.

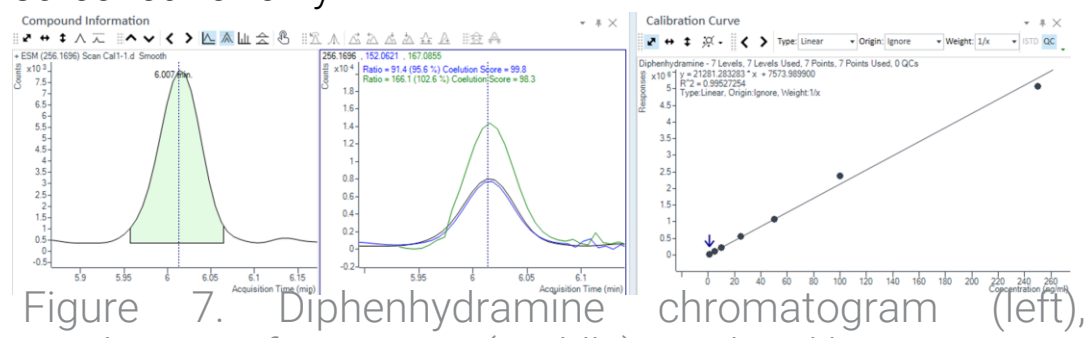

Figure 7. Diphenhydramine chromatogram (left), overlapping fragments (middle), and calibration curve (right) from 1 – 250 ng/mL where R^2 = 0.995 when weighting is 1/x.

Results and Discussion

Analytes were Detected as Positively Identified at Low Concentrations and Found in Crime Lab Samples.

The concentration at which an analyte can be reproducibly detected as positive was evaluated. Even without concentrating the sample, 91% were detected at 5 ng/mL or less in six replicate samples (Fig 8).

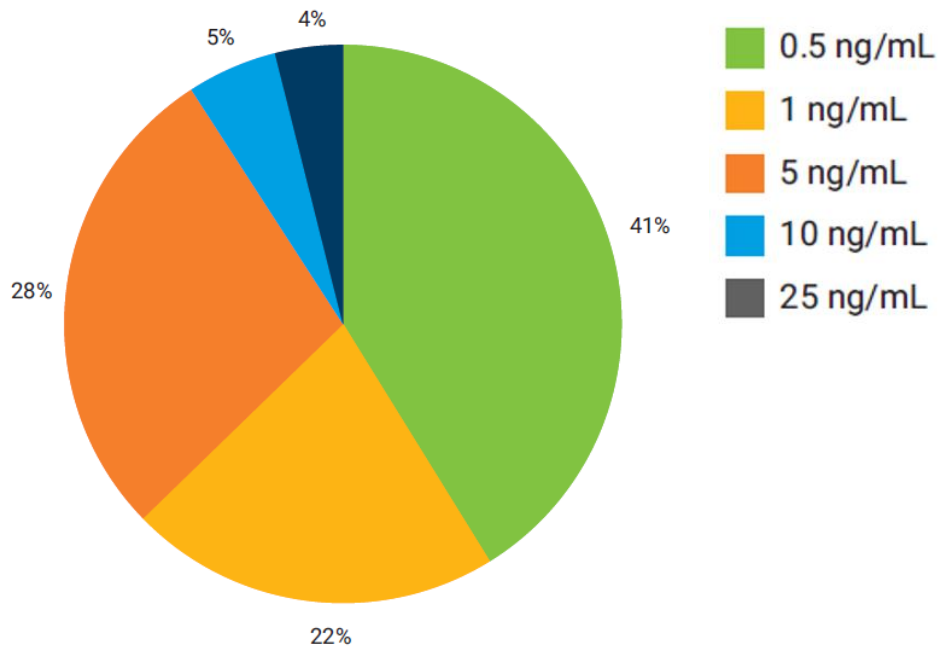

Figure 8. Pie chart of the concentration at which 153 analytes were detected as positively identified by the LC Screener Tool in six replicate samples. A positive identification was made when the molecular ion and one fragment had a mass accuracy = 5 ppm, RT difference <10%, S/N = 3, co-elution >80, and RSD of molecular ion <20%.

Ten deidentified samples from a crime lab were tested with the method. Many were found to be positive with the parent drug and, in some cases, a related metabolite (Table 2).

Table 2. Drugs detected in ten deidentified human samples.

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

To evaluate the robustness of the method and instrument, a 10 ng/mL sample extract was injected over 1400 times. During this time, minimal maintenance was performed- LC solvents refilled and calibration every few days. The data showed stable retention times, mass accuracy, and area counts for analytes. The data for morphine is showed in Fig 9. The study stopped after 1465 injections due to project timeline and not because of a decrease in the instrument's performance.

Data Quality Remained High for Over 1400+ Injections of Whole Blood Sample with Minimal Maintenance.

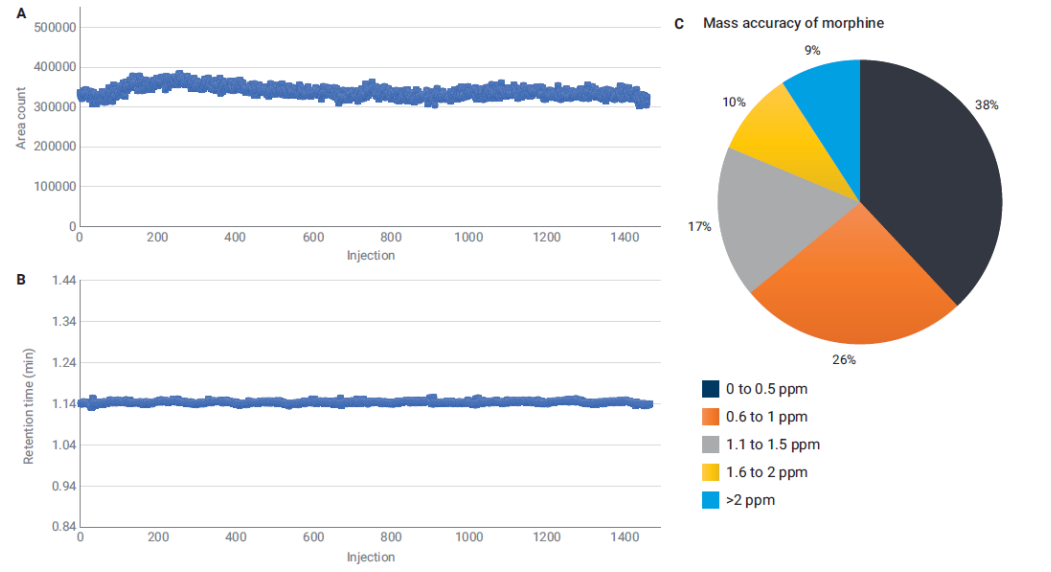

Figure 9. Morphine results for 1465 injections of a blood extract sample. The area counts (A), RTs (B), and mass accuracy (C) were all stable over this experiment.

Because this data is collected with a Q-TOF using DIA, at any point, if a new drug needs to be tested, it can be added to the analysis method with no effect on existing analytes and no redevelopment of the acquisition method needed.

Conclusions

Robust Routine Drug Screening with Automated Sample Prep is Achieved with the Bravo Liquid Handler, the 6546 LC/Q-TOF, and the LC Screener Tool.

- Sample prep with the Bravo Liquid Handler and Captiva EMR-Lipid plates was reproducible, removed matrix, and gave good analyte recoveries.
- 6546 LC/Q-TOF is a robust platform that consistently yielded high resolution results with excellent isotopic fidelity. In a complex sample, it could detect analytes at low concentration even over long periods of time with minimal maintenance.
- LC Screener tool made analyzing data fast and simple with the capability of quantitating some of the most commonly found drugs at the same time.

References

Yannell, K.E. and Gomes, M. Drug Screening in Whole Blood Using the Agilent 6546 LC/Q-TOF and the LC Screener Tool with Automated Sample Preparation. Agilent Technologies. March 20, 2020.

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Published in USA, June 1, 2020

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