# The Best of Both Worlds: LC/Q-TOF as a Method to Detect a Targeted List of 35 Drugs and Metabolites in Urine with Retrospective Data Mining Capabilities

## Application Note

Clinical Research


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### Abstract

Compliance testing continues to be an essential component of the drug testing market. As an alternative to conventional immunoassay screens with reflex to confirmation, we developed a dual identification strategy for drug screening and confirmation with one injection for clinical research purposes. Making use of the quadrupole-time-of-flight mass spectrometer's (Q-TOF) quadrupole, we combined fragmentation pattern with accurate mass, retention time, and isotope fidelity to identify analytes of interest with increased accuracy.


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## Introduction

High-resolution accurate mass liquid chromatography mass spectrometry is ideally suited for rapid analysis of common drugs of abuse and other illicit drugs. A highly sensitive and specific method has been developed for the qualitative determination of 35 drugs and metabolites in urine. The target compounds were detected and confirmed at their respective cutoffs using an Agilent LC/Q-TOF system. An Agilent 6550 LC/Q-TOF coupled to an Agilent 1290 Infinity UHPLC stack provided analyte analysis in a fast 3.3 minute method. This method was developed for common drugs of abuse in urine to screen and confirm target compounds in a single run, with the ability to retrospectively review scan data for possible unknowns at a later date.

Compound identification is based on accurate mass, retention times, isotopic spacing and abundance, MS/MS spectral library matching, and overall score. MassHunter Qualitative Analysis uses peak picking algorithms to generate an overall score based on how the observed identification criteria correspond with theoretical and empirical values. Semiquantitation of compounds is determined with a one-point calibration forced through the origin based on the respective drug cutoffs designated by a previously published method[1].

### Internal standards

- 6am-d6
- αOH Alprazolam-d5
- Diazepam-d5
- Meperidine-d4
- Methamphetamine-d5
- Morphine-d3
- Norfentanyl-d5

## Experimental

### LC Configuration and parameters

| Configuration | | |
|---|---|---|
| Agilent 1290 Infinity II high speed pump (G7120A) | | |
| Agilent 1290 Infinity II multisampler (G7167B) | | |
| Agilent 1290 Infinity II multicolumn thermostat (G7116B) | | |
| Analytical column | Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm<br>LC column (699775-902) | |
| Column temperature | 60 °C | |
| Injection volume | 2 µL | |
| Mobile phase A | 5 mM ammonium formate in water, pH 3.5 | |
| Mobile phase B | Optima grade methanol | |
| Flow rate | 1 mL/min | |
| Gradient | Time (min) | %B |
| | 0.0 | 10 |
| | 0.5 | 10 |
| | 3.0 | 95 |
| | 3.3 | 95 |

### Q-TOF Mass spectrometer configuration and parameters

| Configuration | |
|---|---|
| Agilent 6550 Quadrupole Time of Flight Mass Spectrometer | |
| Electrospray, Positive ionization | |
| AutoMSMS mode, using a preferred list | |
| Drying gas temperature | 250 °C |
| Drying gas flow | 15 L/min |
| Nebulizer pressure | 30 psi |
| Sheath gas temperature | 400 °C |
| Sheath gas flow | 12 L/min |
| Nozzle voltage | 0 V |
| Capillary voltage | 3,500 V |
| Fragmentor voltage | 125 V |
| Skimmer voltage | 65 V |
| Octopole RF | 750 V |
| Mass range | MS 100–1,000 *m/z*<br>MS/MS 50–500 *m/z* |
| Acquisition rate | MS 8 spectra/s; MS/MS 4 spectra/s |
| Collision energies | 10, 20, and 40 V |
| Detector rate | Extended dynamic range, high sensitivity slicer position |

## Chemicals and reagents

All of the drug and metabolite compounds, as well as the deuterated internal standards, were purchased from Cerilliant (Round Rock, Texas). Methanol was purchased from ThermoFisher Scientific (Waltham, MA), and β-glucuronidase was purchased from IMCS (Columbia, SC).

## Sample preparation

Samples, single point calibrator, and QC materials in matrix were prepared using the following procedure. First, 200 µL of sample was added to a collection plate. Next, 180 µL of sodium bicarbonate buffer containing seven deuterated markers, 34 µL resorufin glucuronide solution, and 16 µL of β-glucuronidase were added and the sample was gently mixed. Following a 15-minute room temperature incubation, 300 µL of the sample mixture was transferred to a Biotage Isolute SLE+ 96-well plate (Charlotte, NC, USA) for further purification. Positive pressure was applied and the sample was allowed to absorb for 5 minutes. Samples were eluted with 0.8 mL ethyl acetate, dried under nitrogen, then resuspended using a 90:10 blend of mobile phase A and B.

## Data analysis

System control and data acquisition were performed by Agilent MassHunter Quadrupole Time of Flight Acquisition Software (B.06) with Qualitative Analysis (B.07 SP2) Software. Data were analyzed using Find by Formula and library search software functions. Acceptance criteria included a match to retention time (within 0.015 minures), isotope spacing and abundance, accurate mass (within 5 ppm), MS/MS spectral library matching, and overall score (>85).

## Drug and metabolite cutoffs (ng/mL)

| Compound | Cutoff limit |
|---|---|
| 6-Monoacetyl-morphine | 10 |
| 7-Aminoclonazepam | 40 |
| *alpha*-Hydroxyalprazolam | 20 |
| Alprazolam | 40 |
| Amphetamine | 100 |
| Buprenorphine | 5 |
| Clonazepam | 20 |
| Codeine | 40 |
| Diazepam | 50 |
| Fentanyl | 2 |
| Hydrocodone | 40 |
| Hydromorphone | 40 |
| Lorazepam | 60 |
| MDA (3,4-methylendioxyamphetamine) | 200 |
| MDEA (3,4-methylenedioxyethamphetamine) | 200 |
| MDMA (methylendioxymethamphetamine) | 200 |
| Methamphetamine | 400 |
| Midazolam | 20 |
| Morphine | 20 |
| Norbuprenorphine | 20 |
| Nordiazepam | 50 |
| Norfentanyl | 2 |
| Norhydrocodone | 100 |
| Normeperidine | 50 |
| Noroxycodone | 100 |
| Noroxymorphone | 100 |
| Oxazepam | 50 |
| Oxycodone | 40 |
| Oxymorphone | 40 |
| Phentermine | 100 |
| Ritalinic acid | 100 |
| Tapentadol | 100 |
| Tapentadol-O-sulfate | 200 |
| Temazepam | 50 |
| Zolpidem | 20 |

## Results and Discussion

De-identified samples (n = 160), previously analyzed with an Agilent 6230 Time of Flight mass spectrometer (TOF) were re-analyzed using the LC/Q-TOF system. Samples were prepared by adding sodium bicarbonate buffer with internal standard mix, resorufin glucuronide, and β-glucuronidase. Resorufin glucuronide acted as a hydrolysis control. After a short incubation period, the sample mixture was transferred to a supported liquid extraction plate for further purification. Samples were eluted with ethyl acetate, nitrogen dried, and resuspended in mobile phase. Samples were then analyzed using the LC/Q-TOF system and a reversed-phase C18 column. A single-point calibrator at the compound cutoffs was analyzed along with quality control samples.

The Agilent 6550 Q-TOF and 6230 TOF results showed excellent correlation within the 160-sample data set. Most discrepancies, such as finding additional metabolites, were due to the higher analytical sensitivity achieved with the 6550 Q-TOF instrument. In four cases, zolpidem and 7-aminoclonazeapm were detected by the 6550 QTOF system that were not originally reported. These samples had concentrations below the cutoff defined with the 6230 TOF assay.

Five times within the data set, the 6550 Q-TOF system identified highly abundant drugs based on known parent drug metabolites. Alternatively, these samples could have been diluted for confirmatory identification of the parent drug.

Within the original 160-sample data set, nine samples required additional confirmation using a liquid chromatography triple quadrupole mass spectrometer (LC/MS/MS) system. These nine samples either had an unusual drug metabolite pattern or suboptimal quality metrics required for acceptance with the 6230 TOF assay. In contrast, the 6550 Q-TOF was able to identify and obtain matching fragmentation spectra for all of the analytes present in those nine samples without any additional testing.

## Conclusions

The presented LC/Q-TOF method is highly specific and analytically sensitive, providing a fast and accurate way of identifying analytes. It eliminates the need for LC/MS/MS confirmation of suboptimal results because of the ability to fragment and match to a library. The method uses the ion transmission abilities of a TOF with the enhanced specificity of high-mass-accuracy detection of fragments, providing better specificity in comparison to conventional LC/TOF and LC/MS/MS methods. This method can be applied to a targeted screen, while the simultaneous collection of TOF data provides a mechanism for retrospective data analysis.

## Reference

1. McMillin, G. A.; *et al.* A hybrid approach to urine drug testing using high-resolution mass spectrometry and select immunoassays. *Am. J. Clin. Pathol*. **2015 Feb**; *143(2)*, 234-40.

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