Fast, Robust LC-MS/MS Method for Determination of Multiple Drug Classes for Therapeutic Drug Monitoring

Amanda Rigdon\(^1\), Chris Denicola\(^1\), Sharon Lupo\(^1\), Ty Kahler\(^1\)

\(^1\)Restek Corporation, Bellefonte, PA, USA

Introduction

As demand for therapeutic drug monitoring rises, laboratories are under increased stress to implement streamlined, cost-effective testing procedures. As with any high-volume application, methods developed for therapeutic drug monitoring must be fast, robust, easy to implement, and minimize costs associated with laboratory consumables and technician time. The method presented in this poster is suitable for the quantification of 29 drug compounds in urine across four drug classes in a total run time of 5.5 minutes.

Chromatographic Method Development

The initial chromatographic method was developed through screening 5µm, 100mm x 2.1mm Aqueous C18, Biphenyl, and IBD columns on a Shimadzu 2012 LCMS with mobile phases employing either acetonitrile or methanol. 0.1% Formic Acid was added to all mobile phases. A simple 0 – 100% gradient over 10 minutes was used to screen the columns. Mobile phase flow rate was 0.2mL/min, and injection volume was 5µL. All compounds for the final method were included in the screening, but special attention was paid to the isobaric compounds in the analyte list. Chromatograms from the mix containing the isobaric compounds are shown in Figure 1. The PFPP column was excluded from the analyte list. Chromatograms from the final chromatographic method are optimized for speed and compound resolution. Employing a fast 100mm column. Final chromatographic conditions are listed in Figure 3, and a list of MRM transitions may be used, but detuning these transitions is recommended for methadone overloaded the detector at higher concentrations, and because methadone was a very high responder, the first two transitions for methadone overlaid the detector at higher concentrations, and only the third transition was used for quantification. The first two transitions may be used, but detuning these transitions is recommended to reduce the response of these transitions. Since norbuprenorphine is a poor responder, further sample preparation is required to analyze this drug at levels less than 25 ng/mL. In addition to partial validation experiments, lot-to-lot reproducibility was evaluated for the Ultra Biphenyl column under the same conditions used to analyze all samples. The maximum retention time variation between these lots of Ultra Biphenyl columns was 0.126 minutes.

Optimization and Partial Validation of LC-MS/MS Method

After column scanning, the initial chromatographic method was optimized for speed and compound resolution. Employing a fast gradient allowed for the use of a more efficient 100mm column. Final chromatographic conditions are listed in Figure 3, and a list of MRM transitions can be found at: http://www.restek.com/chromatogram/view/LC_CFS35.

All samples analyzed during this work were prepared by spiking blank urine with a mix of the 29 drug compounds. The spiked urine was then hydrolyzed and diluted 10x in starting mobile phase containing 4ng/mL internal standard. To evaluate linearity and sensitivity, an 11-point calibration curve was prepared over a concentration range from 1 – 1000ng/mL.

Results and Discussion:

Results for each drug compound were quantified using one of three internal standards: codeine-d3, diazepam-d5, and doxepin-d3. The data from the triplicate analysis of the calibration curve was used to determine LOQ, LOD, linearity, precision, accuracy, and instrument injection reproducibility. Table 1 shows results for LOD, LOQ, linearity, precision, and accuracy. LODs were determined by evaluating signal-to-noise ratios for each of the three transitions used for each compound.

Because methadone, the quantifier ion for each compound had a signal-to-noise ratio of ≥ 10, and each qualifier ion had a signal-to-noise ratio of ≥ 3. The LOD for each compound was considered to be the next calibrator below the LOQ. Table 1 contains a summary of the results.

Table 1: Partial Validation Results for Therapeutic Drug Monitoring Compounds in Hydrolyzed Urine

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>LOQ (ng/mL)</th>
<th>Linearity (r)</th>
<th>% Accuracy at LOQ</th>
<th>%CV at LOQ</th>
<th>%SN at LOD</th>
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<tbody>
<tr>
<td>morphine</td>
<td>5.0</td>
<td>0.9996</td>
<td>99</td>
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Figure 3: 10ng/mL Spiked Urine Sample Analyzed Under Optimized Conditions