Quantitation of 77 Therapeutic and Clinical Toxicology Drugs in Dried Blood Spots using the Fully Automated Transcend DSX-1 System

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ABSTRACT

Purpose: Demonstrate a complete and fully automated workflow for dried blood spot analysis of the therapeutic and clinical toxicology drugs across multiple classes for research.

Methods: A 4-3 min analytical method was developed on the Thermo Scientific™ Transcend™ DSX-1 system consisting of a dried matrix spot module coupled with Thermo Scientific™ TurboFlow™ technology featuring a three-way isotope mass spectrometer to quantify 77 analytes from 6 µL DBS in 364.9 min transitions with retention time scheduling.

Results: All analytes were successfully quantitated at or below ng/mL concentration levels up to 400 ng/mL, meeting the screening sensitivity needs of analytical methodologies in forensic clinical laboratories.

INTRODUCTION

The dried blood spot (DBS) sampling technique is advantageous over the traditional liquid blood collection due to its minima/invasiveness, small sample volume, improved analyte stability, and ease of storage and transportation, resulting in its increasing usage in forensic drug monitoring and clinical toxicology research. Here we describe a fully automated workflow to rapidly extract and quantify a wide range of therapeutic and drugs of abuse in DBS using the Thermo Scientific™ Transcend™ DSX-1 system (Figure 1).

MATERIALS AND METHODS

Sample Preparation. The certified reference material of each synthetic standard and their stable-isotope labeled internal standards were purchased from Cerilliant® (Cerilliant Corporation, Round Rock, TX), I2-Q-EDTA stabilized normal human whole blood was obtained from BioIVT (Denver, NV) and stored at 4 °C. The analytes were spiked into the whole blood at 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10, 25, 50, 100, 200, 300, 400 ng/mL, and 6 µL of the mixture was spotted to the dried blood spot (DBS) cards. The DBS cards were dried at room temperature for at least 3 hours and placed directly on the biochip in the dried-matrix spotting.

Automated DBS Extraction. The analytes were extracted from DBS cards with a 6 mm sharp via the flow-through desorption (FTD™) technology using the Loading Pump solution A (Figure 2). Internal standards (25 ng/mL) in water were introduced using the built-in syringe in the DSM module that overflowed a 20 µL syringe to ensure reproducible 15 µL extraction (automated 15 µL addition, ASAP™). Every sample spot was positioned the sample spot in the center of the extraction module prior to and after each run for sample tracking and traceability. The "Full Spot" mode located the extracted analytes and isocratically positioned the sample spot in the center of the extraction clamp (Figure 4).

Online Sample Cleanup and Chromatography. Automated online cleanup and chromatographic separation were performed on a Thermo Scientific™ Transcend™ Transcend system utilizing TurboFlow™ technology. The Transcend system was controlled by Thermo Scientific™ Aria™ software and configured in "Focus mode". After loading the extracted samples onto the TurboFlow column, the analytes were eluted using the optimized high organic gradient shown in the "turboflow" and rebanding on the analytical column. The analyte separation was performed on the analytical column while the TurboFlow column was washed. To prepare for the subsequent analysis, the transfer loop was filled with eluent while the analytical column was washed and equilibrated. The gradient, mobile phases, clamp washes, and columns used are described in Figure 2.

Data Analyst. Post-acquisition data analysis was carried out using Thermo Scientific™ TraceFinder software (v 5.1).

RESULTS

A total of 77 therapeutic and clinical toxicology drugs from 11 classes, including antidepressants, anticonvulsants, antipsychotics, anxiolytics, benzodiazepines, cocaine, dissociatives, opioids, and stimulants, were quantified in a single injection from DBS cards using a rapid automated method on a Transcend DSX-1 system. DSX-1 combines a dried spot autosampler for direct analyte extraction with Thermo Scientific™ Transcend™ UPLC for online sample separation using TurboFlow™ technology. The method only takes 4.3 minutes from analyte extraction to MS detection. The survey of the extracted chromatograms of the analytes is shown in Figure 4. Analyte canopy was estimated to be below 0.5% by measuring analyte concentrations in a blank sample after the highest calibration sample.

CONCLUSIONS

A comprehensive LC-MS based method was set up to extract and quantify multiple classes of drugs from DBS using a fully automated and integrated system. 77 therapeutic and clinical toxicology drugs across 11 classes were reliably quantified from 6 µL DBS in a 4.3 min method.

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TRADEMARKS/LICENSES

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REFERENCES


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