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 for online sample cleanup and a triple quadrupole mass spectrometer to quantify 77 analytes from 6 µL DBS in 364 SRM 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 routine clinical laboratories.

INTRODUCTION

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

MATERIALS AND METHODS

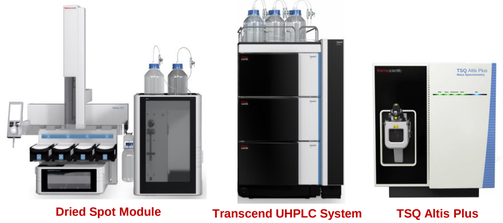
Sample Preparation. The certified reference material of each synthetic standard and their stable-isotope-labeled internal standards (IS) were purchased from Cerilliant® (Cerilliant Corporation, Round Rock, TX). K2-EDTA stabilized normal human whole blood was obtained from BioIVT (BioIVT, Westbury, NY) and stored at 4 ºC. 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 onto the cardholder in the dried matrix spot module.

Automated DBS Extraction. The analytes were extracted from DBS cards with a 6 mm clamp via the flow-though desorption (FTD™) technology using the Loading Pump solution A (Figure 2). Internal standards (25 ng/mL in water) were introduced using the built-in IS pump in the DSM module that overfilled a 20 µL IS loop to ensure reproducible IS addition (automated IS addition, AISA™). Every sample spot was photographed with the Intelligent Vision Camera (IVC™) in “Full Spot” sample recognition mode prior to and after each run for sample tracking and traceability. The “Full Spot” mode located the actual positions of the DBS samples and accurately 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™ system utilizing TurboFlow™ technology. The Transcend system was controlled by Thermo Scientific™ Aria™ MX software and configured in “Focus mode”. After loading the extracted samples onto the TurboFlow column, the analytes were eluted using the optimized high organic eluant stored in the “transfer loop” and refocused 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 eluant while the analytical column was washed and equilibrated. The gradient, mobile phases, clamp washes, and columns used are described in Figure 2.

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

Figure 1. Fully Automated Transcend DSX-1 System



Dried Spot Module | Transcend UHPLC System | TSQ Altis Plus

Figure 2. TurboFlow and analytical conditions controlled by Aria MX.

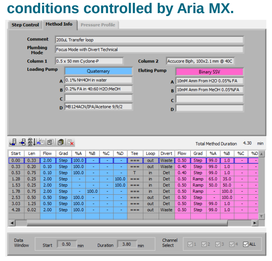


Table 1. TSQ Altis Plus mass spectrometer source parameters and SRM properties.

Capillary voltage	4000 (+)/2500 (-)	**Cycle Time (s)**	0.5
Sheath Gas (Arb)	50	**Q1 Resolution (FWHM)**	0.7
Aux Gas (Arb)	15	**Q3 Resolution (FWHM)**	1.2
Sweep Gas (Arb)	1	**Source Fragmentation**	5
Ion Transfer Tube Temp. (ºC)	320	**Chromatographic Peak Width (sec.)**	6
Vaporizer Temp. (ºC)	350	**CID Gas (mTorr)**	1.5

Mass Spectrometry. Analyte detection was performed using a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer (MS) equipped with a heated electrospray ionization probe (HESI) and operated in the Selected Reaction Monitoring (SRM) mode. The MS parameters are shown in Table 1. The SRM transitions were imported from Tox Explorer™ (TSQ Quantis Plus™ platform)[2] and mzCloud (https://www.mzcloud.org/). Analytes and their stable-isotope-labeled internal standards were monitored in a total of 364 SRM transitions with retention time scheduling. The dwell time per transition and the number of transitions per cycle is shown in Figure 4.

RESULTS

A total of 77 therapeutic and clinical toxicology drugs from 11 classes, including anticonvulsants, antidepressants, antihistamines, antipsychotics, benzodiazepines, cocaine, dissociative, opioids, and stimulants, are 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™ UHPLC for online sample separation using TurboFlow technology. The method only takes 4.3 minutes from analyte extraction to MS detection. The overlay of the extracted chromatograms of the analytes is shown in Figure 4. Analyte carryover was estimated to be below 0.5% by measuring analyte concentrations in a blank sample after the highest calibration sample.

Figure 3. Representative quantification results of doxylamine and its internal standard in DBS, and the calibration curve of 0.1 to 400 ng/mL

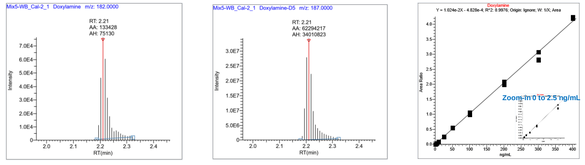


Table 2. Calibration results of the 77 analytes in 6 µL DBS.

Drug Classes	Compound	t_R (min)	IS	R^2	LOQ (ng/mL)
Opioids	6-Acetylmorphine	1.95	6-Acetylmorphine-$^2H_6$	0.9973	0.25
	Buprenorphine	2.39	Buprenorphine-$^2H_4$	0.9918	1.00
	Codeine	1.94	Codeine-$^2H_6$	0.9972	0.10
	Fentanyl	2.34	Fentanyl-$^2H_5$	0.9980	0.25
	Hydrocodone	2.00	Hydrocodone-$^2H_3$	0.9979	0.25
	Hydromorphone	1.80	Hydromorphone-$^2H_3$	0.9963	0.25
	Methadone	2.49	Methadone-$^2H_3$	0.9957	0.25
	Morphine	1.77	Morphine-$^2H_6$	0.9971	0.25
	Norbuprenorphine	2.24	Norbuprenorphine-$^2H_3$	0.9938	2.50
	Norfentanyl	2.10	Norfentanyl-$^2H_5$	0.9965	0.10
	O-Desmethyltramadol	1.95	O-Desmethyltramadol-$^2H_6$	0.9977	0.25
	Oxycodone	1.98	Oxycodone-$^2H_3$	0.9959	0.25
	Oxymorphone	1.79	Oxymorphone-$^2H_3$	0.9982	0.25
	Tramadol	2.13	Tramadol-$^{13}C,^2H_3$	0.9973	0.10
Anticonvulsants	10-OH-carbazepine	2.25	10-OH-carbazepine-$^{13}C_6$	0.9960	1.00
	Carbamazepine	2.45	Carbamazepine-epo-$^{13}C_6$	0.9942	0.50
	Gabapentin	1.87	Gabapentin-$^2H_{10}$	0.9913	10.00
	Lamotrigine	2.10	Methamphetamine-$^2H_5$	0.9971	0.10
	Levetiracetam	1.92	Levetiracetam-$^2H_6$	0.9972	0.25
	Primidone	2.12	Primidone-$^2H_5$	0.9981	0.50
	Topiramate	2.28	Topiramate-$^2H_{12}$	0.9951	5.00
Antidepressants	Amitriptyline	2.45	Amitriptyline-$^2H_3$	0.9950	1.00
	Bupropion	2.18	Bupropion-$^2H_9$	0.9966	0.25
	Citalopram	2.29	Citalopram-$^2H_6$	0.9960	0.50
	Clomipramine	2.50	Clomipramine-$^2H_3$	0.9935	0.10
	Desipramine	2.41	Desipramine-$^2H_3$	0.9961	1.00
	Doxepin	2.33	Doxepin-$^2H_3$	0.9963	0.50
	Fluoxetine	2.30	Fluoxetine-$^2H_6$	0.9956	0.25
	Imipramine	2.43	Imipramine-$^2H_3$	0.9962	0.10
	Mirtazapine	2.27	Mirtazapine-$^2H_3$	0.9983	0.25
	Nortriptyline	2.43	Nortriptyline-$^2H_3$	0.9957	1.00
	O-Desmethylvenlafaxine	2.03	O-Desmethylvenlafaxine-$^2H_6$	0.9971	0.25
	Paroxetine	2.40	Paroxetine-$^2H_6$	0.9960	2.50
	Sertraline	2.48	Sertraline-$^2H_3$	0.9939	10.00
	Trazodone	2.47	Trazodone-$^2H_6$	0.9980	0.10
	Venlafaxine	2.22	Venlafaxine-$^2H_6$	0.9959	0.25
Antihistamines	Brompheniramine	2.28	Risperidone-$^2H_4$	0.9957	0.10
	Chlorophenylpiperazine	2.12	Chlorophenylpiperazine-$^2H_8$	0.9983	0.50
	Chlorpheniramine	2.24	Chlorpheniramine-$^2H_6$	0.9985	0.25
	Diphenhydramine	2.30	Diphenhydramine-$^2H_3$	0.9975	0.10
	Doxylamine	2.23	Doxylamine-$^2H_5$	0.9976	0.25
	Hydroxyzine	2.41	Hydroxyzine-$^2H_8$	0.9952	0.10
	Methorphan	2.39	Methorphan-$^2H_3$	0.9938	1.00
	Norchlorcyclizine	2.40	Paroxetine-$^2H_6$	0.9912	25.00
	Promethazine	2.40	Promethazine-$^2H_3$	0.9964	1.00
Cocaine	Benzoylecgonine	2.16	Benzoylecgonine-$^2H_8$	0.9983	2.50
	Cocaethylene	2.25	Cocaethylene-$^2H_3$	0.9972	0.25
	Cocaine	2.19	Cocaine-$^2H_3$	0.9967	0.10
Dissociatives	Ketamine	2.18	Ketamine-$^2H_4$	0.9991	0.10
	Norketamine	2.13	Norketamine-$^2H_4$	0.9992	0.25
	Phencyclidine	2.38	Phencyclidine-$^2H_5$	0.9971	1.00
Benzodiazepine	7-Aminoclonazepam	2.24	7-Aminoclonazepam-$^2H_4$	0.9976	0.25
	Alprazolam	2.68	Alprazolam-$^2H_5$	0.9982	0.25
	Clonazepam	2.52	Clozapine-$^2H_4$	0.9950	5.00
	Diazepam	2.75	Diazepam-$^2H_5$	0.9979	0.25
	Lorazepam	2.47	Lorazepam-$^2H_4$	0.9987	0.50
	Nordiazepam	2.59	Nordiazepam-$^2H_5$	0.9987	0.50
	Oxazepam	2.50	Oxazepam-$^2H_5$	0.9991	0.50
	Temazepam	2.64	Temazepam-$^2H_5$	0.9990	0.25
	Zolpidem	2.43	Zolpidem-$^2H_6$	0.9994	0.10
	α-Hydroxyalprazolam	2.57	α-Hydroxyalprazolam-$^2H_5$	0.9988	0.50
Antipsychotics	9-Hydroxyrisperidone	2.27	9-Hydroxyrisperidone-$^2H_4$	0.9981	0.25
	Chlorpromazine	2.50	Chlorpromazine-$^2H_3$	0.9982	0.50
	Clozapine	2.32	Clozapine-$^2H_4$	0.9965	0.50
	Olanzapine	2.12	Benzoylecgonine-$^2H_8$	0.9857	2.50
	Quetiapine	2.45	Quetiapine-$^2H_8$	0.9974	0.25
	Risperidone	2.38	Risperidone-$^2H_4$	0.9962	0.10
Stimulants	Amphetamine	1.91	Amphetamine-$^2H_5$	0.9978	1.00
	Methamphetamine	1.97	Methamphetamine-$^2H_5$	0.9974	0.10
	MDA	1.98	MDA-$^2H_5$	0.9972	0.50
	MDMA	2.02	MDMA-$^2H_5$	0.9968	0.25
Miscellaneous	Carisoprodol	2.34	Carisoprodol-$^2H_7$	0.9954	1.00
	Cyclobenzaprine	2.43	Cyclobenzaprine-$^2H_3$	0.9970	2.50
	Meprobamate	2.16	Meprobamate-$^2H_7$	0.9985	0.50
	Zolpidem carboxylic acid	2.23	Zolpidem carboxylic acid-$^2H_4$	0.9962	0.50
	Zopiclone	2.31	Zopiclone-$^2H_4$	0.9974	0.50
OTC	Acetaminophen	1.79	Acetaminophen-$^2H_4$	0.9990	5.00

RESULTS (Cont’)

Calibration curves were built using a weighting factor of 1/x from a lower limit of quantification of 0.1 ng/mL to an upper limit of quantification of 400 ng/mL. All calibration curves achieved R^2 values greater than 0.98. The LOQ values were defined with % RSD and % CV < 15, |% Diff| < 20, and relative ion ratio < % 20, and are reported in Table 2. The extracted chromatograms of doxylamine and its internal standards at the LOQ level and its calibration curves are shown in Figure 3. The LOQ values are all in the low ng/mL levels, which largely meet the screening sensitivity needs of analytical methodologies in routine clinical laboratories.

Figure 4. Representative combined chromatogram for all analytes monitored in this method. Dwell time of each SRM transition and the number of transitions per cycle (0.5 s) during the chromatographic separation (left inserts). For each cycle, the minimum dwell time for any transition was >2.5 ms. Images of a 6 µL dried blood spot before and after extraction (right inserts). The Intelligent Vision Camera (IVC) recognizes the actual location of the blood spot and positions the clamp to the center of the sample spot.

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

REFERENCES

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2. Van Natta, K. et. al. Thermo Fisher Scientific, 2022. TN001256

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TRADEMARKS/LICENSING

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