Technical note | 002226

Clinical toxicology

Simultaneous quantitation of 77 therapeutic and clinical toxicology drugs in dried blood spots using the fully automated Transcend DSX-1 system

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Keywords

Transcend DSX-1 system, TSQ Altis Plus triple-stage mass spectrometer, therapeutic drug monitoring, drugs of abuse, clinical toxicology, TraceFinder software, Aria MX software, dried blood spot (DBS) analysis, TurboFlow technology, online SPE, 2D-LC-MS

Goal

To demonstrate a complete and fully automated workflow to quantitate 77 therapeutic and clinical toxicology drugs from 11 classes in dried blood spots using a Thermo Scientific™ Transcend™ DSX-1 system. The system combines direct analyte extraction, online sample cleanup, and chromatographic separation that can be coupled to a triple-stage quadrupole or Orbitrap-based mass spectrometer.

Application benefits

- A complete and fully automated workflow for dried blood spot analysis with integrated software for hands-free sample analysis
- Flow-through desorption (FTD™) technology delivers analyte extraction directly to 2D-LC, eliminating manual punching of dried spot discs
- DBS extract is loaded directly onto the TurboFlow column to start the 2-D sample cleanup for streamlined analysis
- Thermo Scientific™ TurboFlow™ technology provides automated online sample preparation and LC separation for fast matrix cleanup and analyte separation

Introduction

Dried blood spot (DBS) is an alternative sampling method where capillary blood is collected via a finger or a heel prick and dried on a paper card. DBS has been used in the screening of inborn error of metabolism in newborns since the early 1960s. Compared to the traditional venous liquid blood collection method, the DBS technique is advantageous for its minimal invasiveness, smaller sample volume, improved analyte stability, and ease of storage and transportation, which prompted its increasing usage in therapeutic drug monitoring (TDM),[1,2] clinical toxicology,[3] and sports anti-doping.[4,5]

Traditional preparation of DBS samples starts with punching a fixed volume disc out of a DBS card, which is subjected to the downstream workflow, including analyte extraction, transfer, drying, and reconstitution. The whole process can take 1–3 hours to complete. The Transcend DSX-1 system combines a dried spot autosampler with innovative flow-through desorption (FTD) technology and a UHPLC system capable of 2-dimensional sample preparation via TurboFlow technology. It automates the analyte extraction and cleanup processes, eliminating all manual preparation steps in the traditional DBS workflow.[2,4,5,6]

Here, 11 classes of common drugs of toxicological and therapeutic interest with broad chemo-physical properties were chosen to showcase the capability of the Transcend DSX-1 system. An automated workflow was developed to extract and quantitate 77 drugs commonly tested in clinical research (including anticonvulsants, antidepressants, antihistamines, antipsychotics, benzodiazepines, cocaine, dissociatives, opioids, and stimulants) from dried blood spots (DBS) using a Transcend DSX-1 system (Figure 1). The workflow allows for the rapid sample preparation and quantification of drugs in a 4.3-minute method with linearity from sub ng/mL up to 400 ng/mL, meeting the quantification needs for most laboratory purposes.

Experimental

Sample preparation

The certified reference material of each synthetic standard and their 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 °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 and 400 ng/mL, and 6 µL of the mixture was spotted to the Ahlstrom-Munksjö AutoCollect™ dried blood spot cards (Ahlstrom 226 grade paper, N = 3). The DBS cards were dried at room temperature for at least 3 hours and placed directly onto the cardholder in the dried spot module (DSM).

Automated DBS extraction

The analytes were extracted from DBS cards with a 6 mm clamp via the flow-through desorption (FTD) technology using Loading Pump solution A (0.1% ammonia in water). Internal standards (IS) (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 (Figure 3).

Online sample cleanup and chromatography

Automated online cleanup and chromatographic separation were performed on a UHPLC system utilizing TurboFlow technology. The Transcend system was controlled by Thermo Scientific™ Aria™ MX software (v. 2.7) and configured in "Focus mode." Analytes and matrix were extracted from the DBS sample spot and loaded directly to the TurboFlow column (Thermo Scientific™ Cyclone-P™, 0.5 x 5 mm), where many interfering molecules were washed away while analytes of interest were retained. The analytes were eluted from the TurboFlow column using 0.2% formic acid in 60% methanol in water stored in the "transfer loop" and refocused on the analytical column (Thermo Scientific™ Accucore Biphenyl™, 2.1 x 100 mm, 2.6 µm). The analytical separation was performed with 10 mM ammonium formate with 0.05% formic acid in water as mobile phase A and 10 mM ammonium formate with 0.05% formic acid in methanol as mobile phase B at a flow rate of 0.5 mL/min. The gradient, mobile phases, clamp washes, and columns used are described in Table 1.

Figure 1. Transcend DSX-1 system (dried spot module and Transcend UHPLC) and TSQ Altis Plus triple quadrupole mass spectrometer

Table 1. LC conditins for the online sample cleanup and separation controlled by Aria MX software

Time (min)	TurboFlow column						Analytical column				
	Flow rate (mL/min)	%A	%B	%C	Tee	Loop	Divert	Flow rate (mL/min)	Grad	%A	%B
0.00	2.0	100	-	-	====	Out	Waste	0.5	Step	99	1
0.33	0.1	100	-	-	====	Out	Waste	0.4	Step	99	1
0.53	0.1	100	-	-	T	In	Det*	0.4	Step	99	1
1.28	2.0	-	-	100	====	In	Det*	0.5	Ramp	65	35
1.53	2.0	-	-	100	====	In	Det*	0.4	Ramp	30	50
1.78	2.0	-	100	-	====	In	Det*	0.5	Ramp	-	100
2.53	0.5	100	-	-	====	Out	Det*	0.5	Step	-	100
3.03	0.5	100	-	-	====	Out	Det*	0.5	Step	99	1
4.28	2.0	100	-	-	====	Out	Waste	0.5	Step	99	1
4.30	2.0	100	-	-	====	Out	Waste	0.5	Step	99	1
Clamp washes	Wash 1: 0.1% formic acid in water Wash 2: 0.1% formic acid in acetonitrile Wash 3: Isopropanol/acetonitrile/acetone, 9/9/2 (v/v/v)										
Mobile phases	A: 0.1% ammonia in water B: 0.2% formic acid in 60% methanol C: Isopropanol/acetonitrile/acetone, 9/9/2 (v/v/v)						A: 10 mM ammonium formate, 0.05% formic acid in water B: 10 mM ammonium formate, 0.05% formic acid in methanol				
Columns	Cyclone-P TurboFlow column, 50 x 0.5 mm at room temperature						Accucore Biphenyl, 100 x 2.1 mm, 2.6 µm, 40 °C				
Data window	Start 0.5 min						Duration 3.8 min				

* Det: flow diverts to TSQ Altis Plus MS

Mass spectrometry

Analyte detection was performed using a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer equipped with a heated electrospray ionization probe (HESI) and operated in the Selected Reaction Monitoring (SRM) mode. The MS parameters are shown in Table 2. The SRM transitions were imported from Thermo Scientific™ Tox Explorer™ (TSQ platform)[2] and mzCloud (https://www.mzcloud.org/). The offline mzCloud integration tool in method setup reduces the cost of purchasing pure standards and the time spent on method optimization and development. Analytes and their isotope-labeled internal standards were monitored in a total of 364 SRM transitions with polarity switching and retention time scheduling (RT ± 0.1 min). The dwell time per transition and the number of transitions per cycle are shown in Figure 2.

Table 2. MS parameters and SRM properties

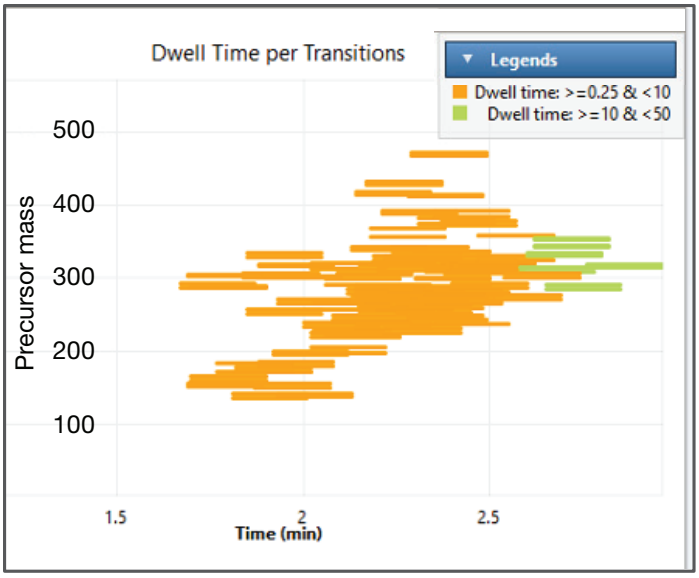
Capillary voltage	4,000 (+) 2,500 (-)	Cycle time (s)	0.5
Sheath gas (Arb)	50	Q1 resolution (FWHM)	0.7
Aux gas (Arb)	15	Q3 resolution (FWHM)	1.2
Sweep gas (Arb)	1	Source fragmentation	5
Ion transfer tube temp. (°C)	320	Chromatographic peak width (s)	6
Vaporizer temp. (°C)	350	CID gas (mTorr)	1.5

Data analysis

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

Results and discussion

A total of 77 therapeutic and clinical toxicology drugs from 11 compound classes were quantified in a single injection from DBS cards using a rapid automated method on a Transcend DSX-1 system. The overlay of the extracted chromatograms of the analytes is shown in Figure 3. Calibration curves were built using a weighting factor of 1/x. All calibration curves achieved R^2 values greater than 0.98. The LOQ values, all in the low ng/mL levels, were defined with % RSD and % CV < 15, |% Diff| < 20, and relative ion ratio < 20%, and are reported in Table 3. The extracted chromatograms of doxylamine, carbamazepine, and trazodone at the LOQ levels, the IS with % RSD demonstrating their reproducibility, and the calibration curves are shown as examples in Figure 4.



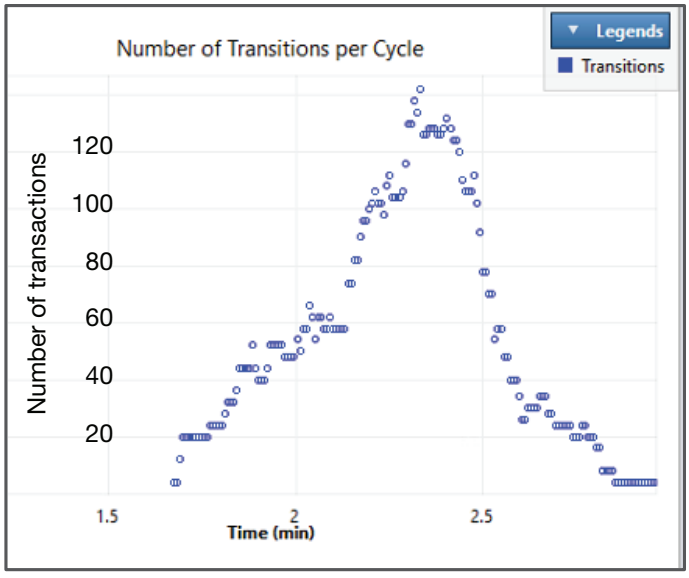


Figure 2. Dwell time of each SRM transition and the number of transitions per timed acquisition window (0.5 s) during the chromatographic separation. For each time segment, the minimum dwell time for any transition was >1.7 ms.

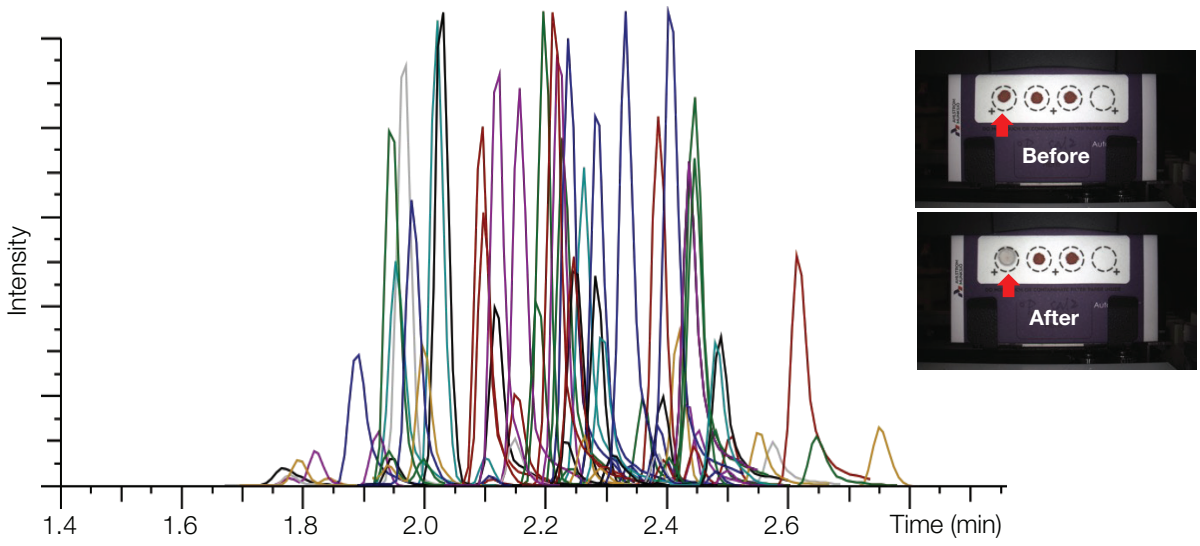


Figure 3. Representative combined chromatogram for all analytes monitored in this method. Images of a 6 µL dried blood spot before and after extraction are shown on the right inserts. The Intelligent Vision Camera (IVC) recognizes the actual location of the blood spot and positions the clamp to the center of the sample spot.

Table 3. Calibration results of the 77 analytes in a 6 µL DBS. LOQ is defined as the lowest concentration with % RSD and % CV < 15, |% Diff| < 20, and relative ion ratio < % 20 (N = 3).

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
	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	0.25
	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
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
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

Table 3. (continued)

Drug classes	Compound	t_R (min)	IS	R^2	LOQ (ng/mL)
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-$^2H_3$	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
	Acetaminophen	1.79	Acetaminophen-$^2H_4$	0.9990	5.00

The DSX-1 system combines the DSM for direct analyte extraction via flow-through desorption with a UHPLC for online sample cleanup and separation using the TurboFlow technology. The DSM removes the laborious manual disc-punch step of DBS. The employment of the TurboFlow column reduces the time and cost associated with sample handling by eliminating offline sample cleanup and analyte extraction, and extends the lifetime of the analytical column. The method only takes 4.3 minutes from analyte extraction to MS detection. Analyte carryover was estimated to be below 0.5% by measuring analyte concentrations in a blank sample analyzed immediately after the highest calibration sample. The established method demonstrated excellent sensitivity, robustness, and throughput, which meet the screening sensitivity needs of analytical methodologies in routine toxicology laboratories.

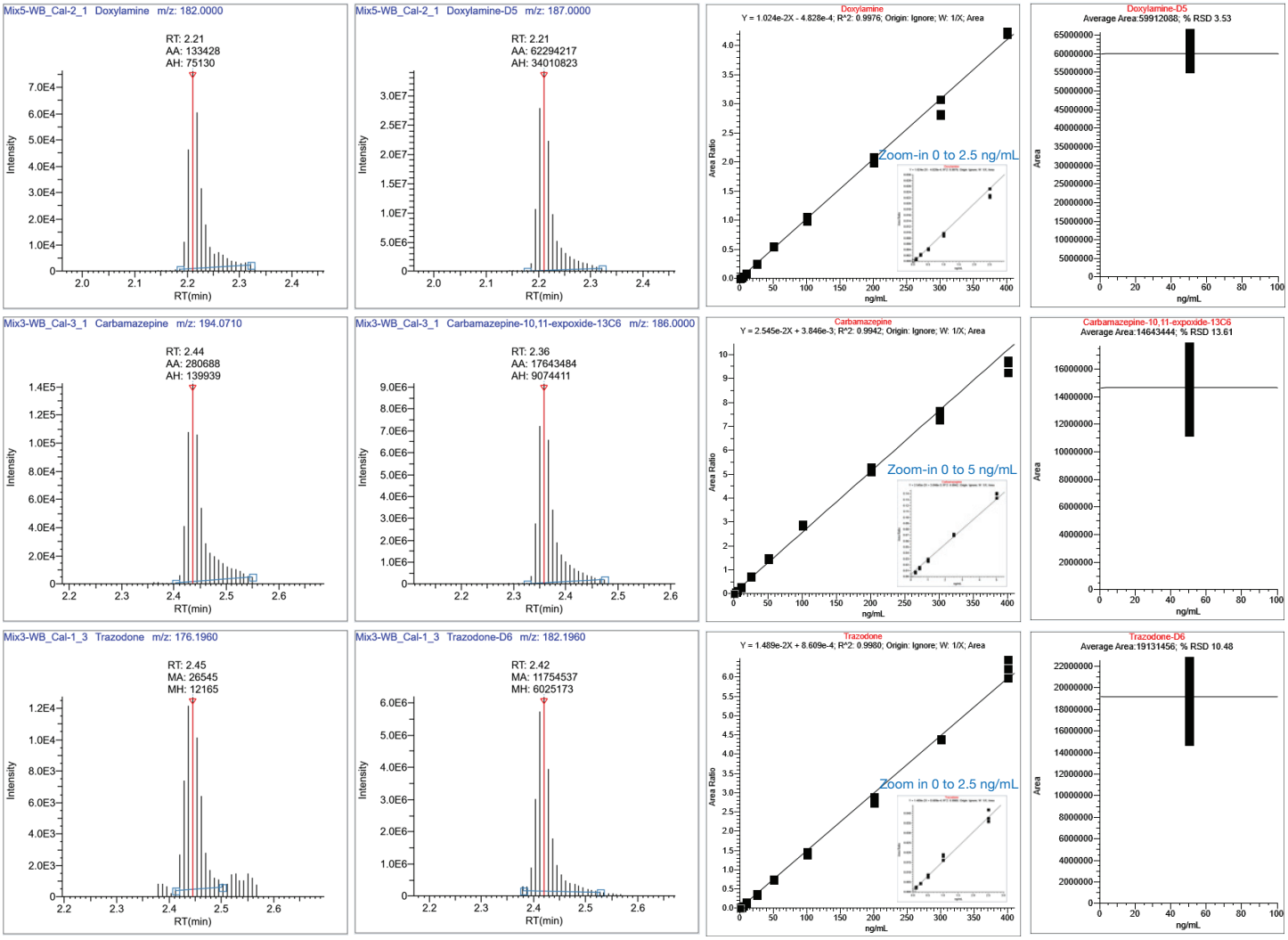


Figure 4. Representative quantification results of doxylamine, carbamazepine, trazodone, all at LOQ levels, and their IS in DBS, demonstrating peak area, peak height, number of scans across the peak, reproducibility of IS, and the calibration curve of LOQ to 400 ng/mL with the lower part of the curve zoomed-in shown in the insert figures.

Conclusions

A comprehensive, 4.3-min LC-MS-based method was set up to extract and reliably quantify 77 drugs of abuse across 11 classes from DBS using a fully automated and integrated Transcend DSX-1 system complete with sample tracking and IS addition.

References

1. Wilhelm, A. J.; den Burger, J. C. G.; Swart, E. L. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. Cin. Pharmacokinet. 2014, 961-973. DOI: 10.1007/s40262-014-0177-7
2. Gibson, R.; Guo, J.; Samra, S. Thermo Fisher Scientific, Technical Note 001740: Direct quantitation of five immunosuppressant drugs in volume-controlled dried whole blood spots by a fully automated Transcend DSX-1 system, 2023.
3. Sadones, N.; Capiau, S.; De Kesel, P. M. M.; Lambert, W. E.; Stove, C. P. Spot them in the spot: analysis of abused substances using dried blood spots. Bioanalysis. 2014, 2211-2227. DOI: 10.4155/bio.14.156.
4. Wu, I.; Guo, J.; Zhu, Y.; Samra, S.; Hassell, K.; Correa, T.; Takarewski, J.; Lafontaine, C. Thermo Fisher Scientific, Technical Note 000301: Fully automated dried spot analysis for the rapid quantitation of tramadol and its metabolites in various matrices, 2021.
5. Jing, J.; Shan, Y.; Liu, Z.; Yan, H.l.; Xiang, P.; Chen, P.; Xu, X. Automated online dried blood spot sample preparation and detection of anabolic steroid esters for sports drug testing. Drug Test Anal. 2022, 1–13. DOI: 10.1002/dta.3226
6. Guo, J.; Gibson, R. J.; Wu, I.; Samra, S.; Hassell, K.; Correa, T.; Goucher E. Thermo Fisher Scientific, Technical Note Direct quantitation of phosphatidylethanol (PEth) in volume-controlled dried whole blood spots using the fully automated Transcend DSX-1 system. Thermo Fisher Scientific, 2022. TN000625
7. Van Natta, K.; Samra, S.; Thibert, V.; Hassell, K.; Wijerantne, N. Thermo Fisher Scientific, Technical Note 001256: All-in-one LC-MS/MS toxicology solution for drugs of abuse quantitation using the TSQ Quantis Plus mass spectrometer, 2022.

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