Automated Dried Spot Analysis for Rapid Quantitation of Tramadol and Metabolites

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ABSTRACT

Purpose: Demonstrate an integrated and automated workflow for dried spot analyses of tramadol and metabolites in various biological matrices.

Methods: The analytical method was developed on the Thermo Scientific™ Transcend™ DSX-1 system, which consisted of a dried spot autosampler coupled with TurboFlow™ technology and mass spectrometer.

Results: High capacity (96 cards), and high-throughput analyses (8-minute for individual run) for quantification of tramadol, O-desmethyltramadol, and N-desmethyltramadol in dried spot (blood, serum, saliva, urine) were achieved with linearity (R^2>0.99) across 5 ng/mL to 400 ng/mL, RSD and CV <20% to satisfy different cut-off needs in clinical settings.

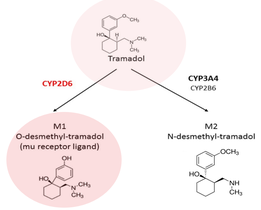
INTRODUCTION

Tramadol is a centrally acting analgesic widely prescribed to relieve acute or chronic pain. Pain management is achieved by a dual mechanism of inhibiting neurotransmitter re-uptake and weak opioid receptor activation.[1] Tramadol has increased popularity amongst athletes in the sports as it demonstrates lower risk of addition and flexible tolerability profile.

Recently, Union Cycliste Internationale (UCI) has identified tramadol as a doping agent and has banned the use in professional cycling[2] with recommended guidance to collect blood droplets of in-competition athletes randomly throughout entire competition and submitted for laboratory analysis. Therefore, implementation of dried blood spot (DBS) sample collection coupled with high throughput analysis to determine the presence of tramadol and metabolites that are used as indicators for the drug intake is needed.[3] However, conventional dried spot analyses utilize laborious and time-consuming sample extraction procedures, as well as manual punchouts of spot discs with risk of contamination.

A fully automated platform for dried spot analysis is introduced here with innovative integration of sample preparation into on-line analysis utilizing a revolutionary dried spot autosampler capable of Flow-through Desorption (FTD™), followed by a two-dimensional TurboFlow UHPLC-MS/MS for sample cleanup from biomatrix, separation, and identification.

Figure 1. Metabolic pathway of Tramadol



MATERIALS AND METHODS

Sample Preparation

Analytes were spiked into blood, serum, urine, and saliva samples. A 6-µL aliquot of sample was spotted on DBS card and dried at room temperature.

Fully Automated Sample Extraction

Extraction was performed using 0.1 % formic acid at a flow rate of 1 mL/min for 30 sec with HotCap enabled at 100 ℃. Internal standards (IS) were delivered using the built-in IS pump in the autosampler module to apply an accurate amount to each spot. Individual spots were photographed with the intelligent camera prior and after each run to check the presence of a spot, adjust the position for extraction, and verify the occurrence of extraction. After the desorption process, the clamp head was rinsed with the 0.1% formic acid and 30% acetonitrile/30% isopropanol/40% acetone.

TurboFlow UHPLC-MS/MS

Analytes were separated by a Cyclone™-P column followed by a biphenyl analytical column, coupled with a selected reaction monitoring (SRM) method. 10 mM ammonium formate in water and 10 mM ammonium formate in methanol were used as mobile phases.

Data Analysis

Data were analyzed using Thermo Scientific™ TraceFinder™ software.

Figure 2. Transcend DSX-1 system and TSQ Altis™ MD MS

Figure 3. Innovative Technologies for Dried Spot Autosampler

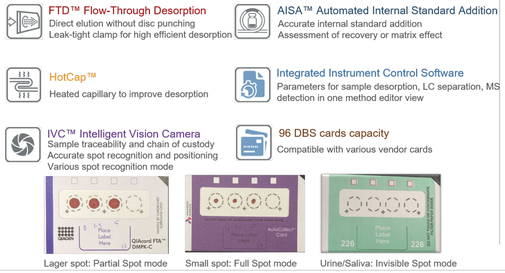
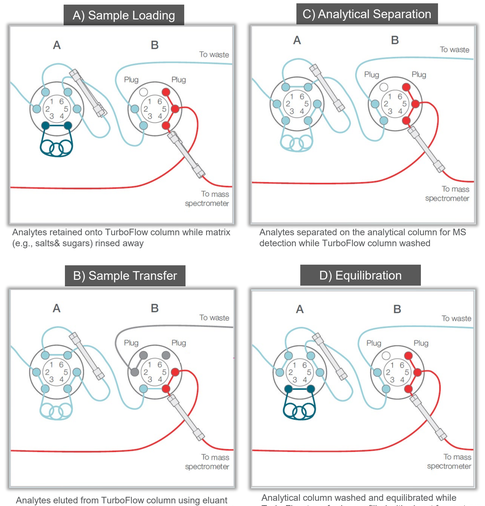


Figure 4. TurboFlow Technology for Sample Cleanup



RESULTS

Quantitation of individual analyte was performed using automated internal standards delivery module. Spiked samples from five matrices (blood, saliva, serum, urine, neat solution) were spotted in replicates onto various vendor cards to evaluate the automated workflow and card compatibilities. Accuracy and precision data compiled were listed in Table 1, with all analytes within acceptance limits (% RSD and % CV below 20%). Reproducibility (with % RSD <15%) was demonstrated using the peak response area of tramadol internal standard over 70 injections from various matrices over long run times (Figure 5).

Calibration curves for each analyte were built using a weighting factor of 1/x from lower limit of quantification (LLOQ) of 5 ng/mL to an upper limit of quantification (ULOQ) of 400 ng/mL with R^2 values greater than 0.99, internal standards % RSD < 20% in all experiments.

Dataset from dried blood spot were shown in Figure 6 for individual analyte and its internal standard. Representative chromatograms from the LLOQ and the demonstrated captured images prior and after the run were shown in Figure 7.

Table 1. Precision and Accuracy data in different dried matrices spot

Conc. (ng/mL)	Whole Blood			Saliva			Serum			Urine			Standard		
	% Diff	% RSD	%CV	% Diff	% RSD	%CV	% Diff	% RSD	%CV	% Diff	% RSD	%CV	% Diff	% RSD	%CV
Tramadol															
5	18.5	5.6	7.8	3.1	8.8	6.9	4.3	5.0	5.4	-9.1	4.7	3.5	11.2	6.3	8.0
10	-3.1	15.4	18.6	3.0	10.3	9.1	0.9	5.4	5.6	9.6	13.8	12.1	0.5	4.4	4.9
25	-3.0	5.2	5.6	-1.6	6.8	6.5	0.1	5.8	5.9	8.9	12.3	11.7	-7.5	5.4	5.7
50	-6.2	8.5	8.8	1.3	8.3	8.1	-3.2	7.7	7.7	8.7	9.1	8.8	-4.4	7.0	7.2
100	-4.2	6.5	6.6	-0.3	7.4	7.3	0.2	9.6	9.7	10.9	4.8	4.7	-1.4	8.5	8.6
200	-3.5	7.9	7.9	-1.4	8.1	8.0	-1.3	5.5	5.5	0.9	8.0	7.9	0.9	8.0	8.0
400	3.6	7.9	7.9	2.3	7.6	7.6	1.0	8.7	8.7	-4.8	5.8	5.8	0.8	7.1	7.1
O-Desmethyltramadol															
5	16.1	6.1	9.2	7.8	6.9	6.4	2.8	6.3	7.2	-5.0	4.3	3.6	6.6	6.1	7.3
10	-3.3	14.9	18.7	4.2	9.1	8.7	-0.5	4.9	5.2	3.8	13.6	12.5	-0.6	4.2	4.6
25	-1.6	4.9	5.3	-4.7	4.6	4.5	-0.1	4.7	4.8	4.6	11.1	10.7	-4.9	4.7	4.9
50	-5.5	8.1	8.5	-3.9	6.1	6.0	-1.5	7.6	7.7	5.5	5.4	5.3	-2.1	6.4	6.6
100	-4.0	6.0	6.1	-1.9	7.5	7.4	1.1	9.4	9.5	8.7	4.4	4.3	-0.4	5.9	6.0
200	-3.1	7.9	7.9	-0.9	9.0	9.0	-1.0	5.7	5.7	-0.9	7.9	7.8	1.6	5.3	5.3
400	3.3	7.4	7.5	3.1	7.9	7.9	0.4	8.0	8.0	-2.6	5.5	5.5	-0.2	6.0	6.0
N-Desmethyltramadol															
5	18.4	5.8	9.1	8.6	7.4	7.9	4.1	5.8	6.8	-4.5	6.0	5.4	12.6	5.9	9.0
10	-2.3	15.1	19.3	2.4	7.3	7.6	0.7	5.7	6.2	1.2	2.9	2.7	0.5	5.0	6.1
25	-3.3	6.0	6.6	-4.1	4.1	4.2	-0.3	5.4	5.6	-2.0	1.1	1.1	-8.3	6.9	7.5
50	-6.4	8.6	9.0	-1.1	7.0	7.0	-2.7	8.0	8.1	3.7	2.2	2.2	-5.5	8.8	9.2
100	-4.5	6.0	6.1	-2.4	7.2	7.2	0.7	9.9	10.0	8.9	0.5	0.5	-1.3	8.6	8.8
200	-3.5	8.5	8.6	-1.6	7.8	7.8	-1.7	5.9	5.9	1.5	5.1	5.0	1.2	7.6	7.7
400	3.7	8.0	8.0	3.9	8.2	8.2	1.0	9.2	9.2	-3.2	3.5	3.5	0.8	8.5	8.6

Figure 5. Peak Area Response of Tramadol Internal Standard Over Runs

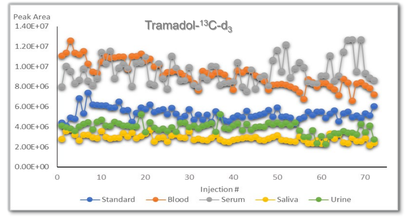


Figure 6. Calibration Curves and Internal Standards in Dried Blood Spots

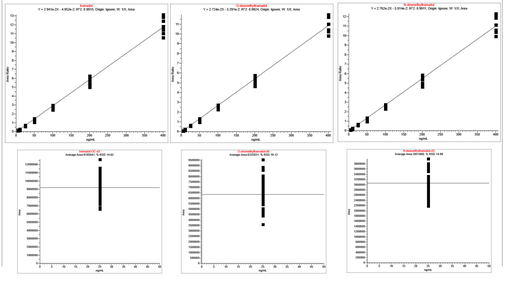
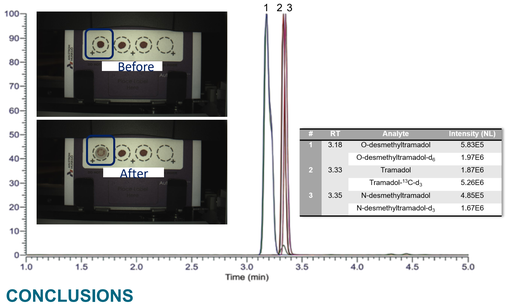


Figure 7. Chromatograms of LLOQ in Dried Blood Spot and Captured Images



CONCLUSIONS

The Transcend DSX-1 Dried Spot Autosampler (DSA) combined with TurboFlow LC-MS provides a fast, robust, and highly integrated on-line sample extraction, clean-up, separation, and data analysis workflow for quantification of tramadol and metabolites in dried spot matrices.

- Suitable for various matrices, for example urine, saliva, serum, blood
- Convenient on-line internal standard loop enables easier evaluation of desorption recovery or matrix effect
- Enabled delivery of sample traceability, minimal sample preparation and high-throughput LC-MS/MS analysis.

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TRADEMARKS/LICENSING

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