Phytronix

Application Note 1102

LDTD-MS/MS Method validation for Quantitative analysis of small molecule in DBS

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Introduction

A complete method validation was performed for the quantification of Dextrorphan extracted from dry blood spot (DBS). A high-throughput LDTD-MS/MS method was used for quantification of Dextrorphan. The following validation parameter was tested:

- Accuracy and precision of intra and inter-assay
- Matrix selectivity
- Matrix effect
- Recovery
- Stability tests.

Instrumentation

- Phytronix Technologies LDTD ion source (model T-960);
- TSQ® Vantage, Thermo Fisher Scientific.

LDTD ionization process

The LDTD ion source uses an infrared laser diode to desorb sample that have been dried onto a well of a LazWell™ (96-well plate). The desorbed gas phase molecules are carried into a corona discharge region to undergo APCI, and then they are transferred directly into the mass spectrometer for detection.

Samples Preparation

Blood spiking
- 1 ml Human Blood (Na. Citrate)
- 10µL of stock solution DT in methanol for STD and QC preparation.
- Vortex 0.5 min.

Blood spotting
- 15 µl Blood on card.
  - FTA-DMPK-C
- Dry at room temperature for 2h (minimum).

Punch extraction
- Add 3mm punch in eppendorf tube (0.5ml)
- 25 µL of NaCl (sat) solution in water
- 50 µL of Internal standard (DT-d3, 5 ng/ml in acetonitrile). Use acetonitrile for Blank.
- Vortex 0.5 min. and centrifuge 2 min. at 14000g.
- Transfer 5.0 µL of upper phase onto LazWell™

MS Parameters

Mode	APCI (+)
Collision gas	1.5 mTorr (Argon)
S-Lens	30 V
Collision energy	95 V
Scan time	0.020 sec
Needle current	3 µA
Dextrorphan	258.2 → 199.1 amu
Dextrorphan-d3	261.2 → 199.1 amu

LDTD Parameters

Laser power pattern: 0 to 45% in 3.0 sec.

Carrier gas flow: 3 L/min (Air)

Results and Discussion

Calibration Curves

Quantitative determination of Dextrorphan extract for DBS on card FTA-DMPK-C can be achieved over a nominal concentration range of 2 to 500 ng/ml for DT with FTA-DMPK-C card (Figure 1). An excellent linearity is obtained over the concentration range (R^2 > 0.99) in three different run.

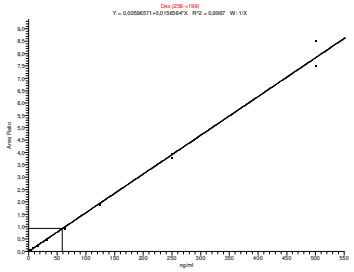

Fig. 1 Calibration curve of FTA-DMPK-C

	Day 1	Day 2	Day 3
R^2	0.9967	0.9985	0.9935
Slope (ratio area / concentration)	0.0157	0.0074	0.0068
y-Abciss	0.0060	0.0025	0.00095

Table 1 Calibration curve parameter.

Results and Discussion

Accuracy and Precision (Intra and Inter-assay)

Five levels of QC samples were analyzed in sixplicate to evaluate the LDTD-MS/MS method accuracy and precision for the intra-assay. The accuracy was evaluated to be within 94.25 and 111.04 % and the precision was within 3.95 and 8.61 % (**Table 2**)

Intra-assay	LLOQ	QC (Low)	QC (med)	QC (High)	ULOQ
Nom. Conc (ng/ml)	2	8	63	250	500
N	6	6	6	6	6
Mean (ng/ml)	2.2	8.1	59.4	261.9	499.9
RSD (%)	8.6	6.2	4.0	4.0	6.1
%Nom.	111.0	101.0	94.3	104.8	100.0

Table 2 Intra-run accuracy and precision for cards

Three levels of QC samples were analyzed in sixplicate in three different run to evaluate the LDTD-MS/MS method accuracy and precision for the inter-assay. The accuracy was evaluated to be within 95.16 and 101.76 % and the precision was within 5.38 and 8.99 % (**Table 3**).

Inter-assay	QC (Low)	QC (med)	QC (High)
Nom. conc. (ng/ml)	8	63	250
N	18	18	18
Mean (ng/ml)	8.1	60.0	254.4
RSD (%)	9.0	6.5	5.4
%Nom.	100.6	95.2	101.8

Table 3 Inter-run accuracy and precision for cards

Recovery

The percentages of recovery were evaluated for card FTA-DMPK-C.

FTA-DMPK-C	*QC (Low)*	*QC (High)*
%Recovery	98.9%	84.0%

Table 3 Recovery of DT at low and high level.

Matrix selectivity

Six different human blood matrix (2 females and 4 males) was evaluated. The percentage of interference of each blank was evaluated blank peak area compare to the mean peak area value of LLOQ. All blank are lower 20%.

Blank ID	%Interference LLOQ
B1-F	18,8
B2-F	10,0
B3-M	2,0
B4-M	10,9
B5-M	14,1
B6-M	16,7

Table 4 Matrix selectivity evaluation of six different blank

Matrix effect

Six different matrix were spiked at low QC level and extracted in triplicate. The accuracy was evaluated to be within 91.10 and 104.28 % and the precision was within 6.73 and 14.50 % (**Table 5**).

Matrix ID / Type	Nom.conc. (ng/ml)	N	Mean (ng/ml)	RSD (%)	%Nom. conc.
M1-F	8	3	8.3	14.5	104.3
M2-F	8	3	7.3	6.7	91.1
M3-M	8	3	7.9	11.2	98.9
M4-M	8	3	8.0	6.8	100.1
M5-M	8	3	8.3	8.3	104.1

Table 5 Matrix effect evaluation

Stability test result

A pourcentage of deviation from initial value was evaluated for different stability test and a mean value of %RSD was reported (**Table 6**).

Stability test	Dextrorphan	
	QC (Low)	QC (High)
Bench top in blood (94h, 4°C)		
Mean (%Difference)	7.9	9.6
Precision (Mean %RSD)	6.8	3.3
Dry on FTA-DMPK-C (120h, RT)		
Mean (%Difference)	-0.7	1.3
Precision (Mean %RSD)	9.2	4.3
Extraction solution (88h, 4°C)		
Mean (%Difference)	11.0	7.6
Precision (Mean %RSD)	7.2	4.9
Dry in LazWell (66h, RT)		
Mean (%Difference)	2.5	--
Precision (Mean %RSD)	13.8	--

Table 6 Stability result

Conclusions

A complete method validation was performed using dry blood spot (DBS) extract and a LDTD-MS/MS method. LDTD-MS/MS allows high-throughput quantification of Dextrorphan from DBS on card FTA-DMPK-C with a sample-to-sample run time of 10 seconds.

