

## Dry Blood spot of Dextrorphan on FTA-DMPK-A and FTA-DMPK-C cards: LDTD-MS/MS Analysis in 10 seconds

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### Overview

- High-throughput determination of Dextrorphan (DT) in dry blood spot (DBS) card is performed by LDTD-MS/MS;
- FTA-DMPK-A (with lyse cell and denature protein agent) and FTA-DMPK-C (without denature agent) cards are use;
- Sample-to-sample run time of 10 seconds;
- Calibration range from 2 to 500 ng/ml with  $r^2 > 0.99$ ;
- Accuracy within 94.3 and 104.8 % for Dextrorphan;
- Precision within 3.95 and 11.68 % for Dextrorphan

### Instrumentation

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



Figure 1. LDTD-MSMS system

### 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 (0.2, 0.4, 0.8, 1.6, 3.2, 6.3, 12.5, 25 and 50 µg/ml)
- Vortex 0.5 min.

#### Blood spotting

- 15 µl Blood on card.
  - FTA-DMPK-A
  - 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™
- Perform LDTD-MS/MS analysis

### 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-A and FTA-DMPK-C can be achieved over a nominal concentration range of 2 to 500 ng/ml for DT with FTA-DMPK-A card (**Figure 1**) and with FTA-DMPK-C card (**Figure 2**). An excellent linearity is obtained over the concentration range ( $R^2 > 0.99$ ).

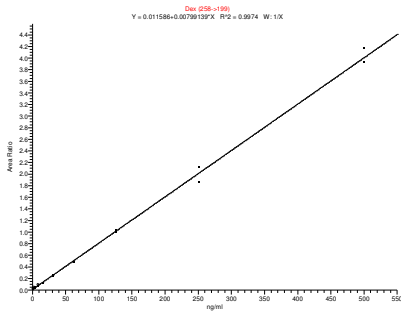


Fig. 1 Calibration curve of FTA-DMPK-A

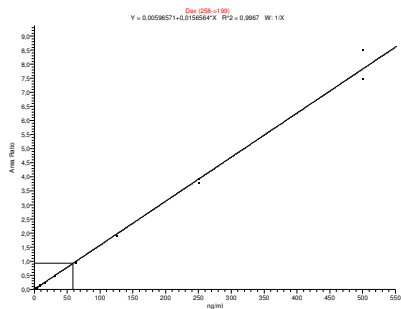


Fig. 2 Calibration curve of FTA-DMPK-C

	FTA-DMPK-A	FTA-DMPK-C
$R^2$	0.9974	0.9967
Slope (ratio area / concentration)	0.0080	0.0157
y-Abciss	0.0116	0.0060

Table 1 Calibration curve parameter.

### Accuracy and Precision

Three levels of QC samples were analyzed in sixuplicate to evaluate the LDTD-MS/MS method accuracy and

precision. The accuracy was evaluated to be within 94.3 and 104.8 % and the precision was within 3.95 and 11.68 % (**Table 2**)

FTA-DMPK-A	QC (Low)	QC (med)	QC (High)
Nominal conc. (ng/ml)	8	63	250
N	6	6	6
Mean (ng/ml)	8.4	61.3	260.6
RSD (%)	11.7	5.7	6.1
%Nom.	104.6	97.3	104.2

FTA-DMPK-C	QC (Low)	QC (med)	QC (High)
Nominal conc. (ng/ml)	8	63	250
N	6	6	6
Mean (ng/ml)	8.1	59.4	261.9
RSD (%)	6.2	4.0	4.0
%Nom.	101.0	94.3	104.8

Table 2 Within-run accuracy and precision for cards

### Recovery

The percentage of recovery were evaluated for card FTA-DMPK-A and C.

FTA-DMPK-A	QC (Low)	QC (High)
%Recovery	70.8%	81.0%

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

Table 3 Recovery of DT at low and high level.

### Conclusions

LDTD-MS/MS allows high-throughput quantification of Dextrorphan from DBS on card FTA-DMPK-A (with denaturing agent) and C (without denaturing agent) with a sample-to-sample run time of 10 seconds.

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