Phytronix

Application Note 1105

LDTD-MS/MS Method Validation According to FDA Regulation

Jean Lacoursière, Patrice Tremblay, Pierre Picard and Serge Auger

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Introduction

A full GLP method validation according FDA rules was performed for the quantification of Dextromethorphan (DM) in plasmas. A high-throughput LDTD-MS/MS method was used for quantification of Dextromethorphan. The following validation parameter was tested:

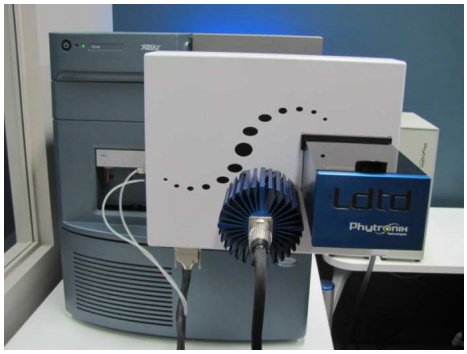
- Accuracy and precision of intra and inter-assay
- Matrix selectivity
- Matrix effect
- Recovery
- Stability tests (with and without metabolite).

Instrumentation

- Phytronix Technologies LDTD ion source (model WX-960);
- Xevo® TQMS, Waters.

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

Protein precipitation

- Add 25 µL sample in eppendorf tube (0.5ml)
- 100 µL of Internal standard (DM-d3, 50 ng/ml in acetonitrile). Use acetonitrile for Blank.
- Vortex 0.5 min. / centrifuge (2 min. /14000g).
- Transfer 2.0 µL onto LazWell™

MS Parameters

Mode	APCI (+)
Cone	40 V
Collision energy	40 V
Scan time	0.078 s
Needle current	3 µA
Dextromethorphan	272-> 171 amu
Dextromethorphan-d3	275-> 171 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 Dextromethorphan extract can be achieved over a nominal concentration range of 12.5 to 800 ng/ml.(Figure 1). An excellent linearity is obtained over the concentration range (R^2 > 0.99) in three different run.

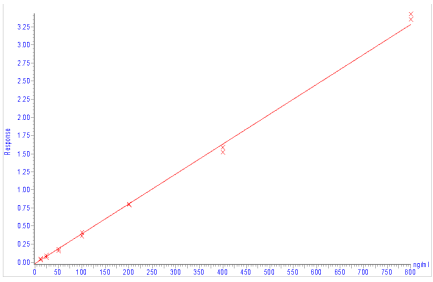


Fig. 1 Calibration curve

	Day 1	Day 2	Day 3	Day 4
R^2	0.9972	0.9961	0.9969	0.9989
Slope (ratio area / concentration)	0.0041	0.0044	0.0045	0.0041
y-Abciss	0.0189	0.0219	-0.0355	-0.0092

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 93.36 and 117.60 % and the precision was within 3.13 and 8.01 % (**Table 2**)

Intra-assay	LLOQ	QC (Low)	QC (med)	QC (High)	ULOQ
Nom. Conc (ng/ml)	12.5	25	100	400	800
N	6	6	6	6	6
Mean (ng/ml)	14.7	26.1	101.0	373.4	822.2
RSD (%)	7.9	8.0	5.7	3.1	3.9
%Nom.	117.6	104.3	101.0	93.4	102.8

Table 2 Intra-run accuracy and precision

Three levels of QC samples were analyzed in sixplicate in four different run to evaluate the LDTD-MS/MS method accuracy and precision for the inter-assay. The accuracy was evaluated to be within 99.91 and 106.09 % and the precision was within 5.37 and 13.30% (**Table 3**).

Inter-assay	QC (Low)	QC (med)	QC (High)
Nom. conc. (ng/ml)	25	100	400
N	23	23	24
Mean (ng/ml)	26.5	101.8	399.6
RSD (%)	8.2	13.3	5.4
%Nom.	106.1	101.8	99.9

Table 3 Inter-run accuracy and precision for cards

Recovery

The percentages of recovery were evaluated and a recovery higher than 80% were obtain.

	QC (Low)		*QC (High)*	
	Drug	*IS*	*Drug*	*IS*
%Recovery	80.9	87.1	80.9	81.7

Table 3 Recovery of DM at low and high level.

Matrix selectivity

Six different human plasmas (2 females and 4 males) were evaluated. The percentage of interference of each blank was evaluated blank peak area compare to the mean peak area value of LLOQ. All blank had a percentage of interference lower than 20%.

Blank ID	%Interference LLOQ
B1-F	0.0
B2-F	5.5
B3-M	9.8
B4-M	18.5
B5-M	4.8
B6-M	19.9

Table 4 Matrix selectivity evaluation of six different blank

Matrix effect

Six different matrixes were spiked at low QC level and extracted in triplicate. The accuracy was evaluated to be within 100.27 and 114.13 % and the precision was within 3.95 and 11.64 % (**Table 5**).

Matrix ID / Type	Nom.conc. (ng/ml)	N	Mean (ng/ml)	RSD (%)	%Nom. conc.
M1-F	25	3	26.6	7.4	106.3
M2-F	25	3	25.1	4.0	100.3
M3-M	25	3	26.5	6.4	105.9
M4-M	25	3	28.5	3.3	114.1
M5-M	25	3	26.5	11.6	106.1

Table 5 Matrix effect evaluation

Stability test result

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

Stability test	DM		DM + Metab.	
	QC-L	QC-H	QC-L	QC-H
Freeze-Thaw (4 cycles)				
Mean (%Difference)	-7.7	3.4	3.2	-2.9
Precision (Mean %RSD)	9.7	4.1	7.2	3.9
Bench top (24h, RT)				
Mean (%Difference)	12.3	-5.2	-0.3	-4.5
Precision (Mean %RSD)	8.5	3.4	9.4	3.2
Extraction solution (66h, 4°C)				
Mean (%Difference)	6.3	0.2	-2.4	-5.5
Precision (Mean %RSD)	6.7	7.3	7.3	3.7
Dry in LazWell (66h, RT)				
Mean (%Difference)	4.8	-8.1	5.5	-7.1
Precision (Mean %RSD)	8.4	2.8	4.7	6.3

Table 6 Stability result.

Conclusions

A full method validation according FDA rules was performed using a protein precipitation extract and a LDTD-MS/MS method. All acceptance criteria were followed.

Phytronix Technologies
Parc technologique du Québec métropolitain
4535, boulevard Wilfrid-Hamel, suite 120, Québec (Qc) Canada G1P 2J7
www.phytronix.com