

OVERVIEW

Purpose

- Dextromethorphan quantitation in human plasma following GLP requirement
- Laser Diode Thermal Desorption technology (LDTD) tandem mass spectrometry

Method

- Dextromethorphan spiked into human plasma
- Protein precipitation with acetonitrile (ACN)
- Nominal calibration range : 12.5 to 800 ng/ml
- LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer

Results

- Excellent linearity over the calibration range ($R^2 > 0.99$)
- Excellent accuracy ranging from 93.5 and 117.6% using area value
- Excellent precision ranging from 3.1 and 13.3 % using area value
- No carryover and good recovery
- All validation parameters followed the FDA guidelines for industry... Therefore, to run under GLP environment in LDTD-MS/MS we just need to follows the FDA guidelines

INTRODUCTION

The Food and Drug Administration (FDA) provides guidance for industry in developing bioanalytical method validation in human clinical pharmacology, bioavailability and bioequivalence studies requiring pharmacokinetic (PK) evaluations. The guidance generally applies to bioanalysis procedures such as high-performance liquid chromatography combined to mass spectrometry. The reported general recommendations for bioanalytical method validation can be adjusted or modified depending on the specific type of analytical method used. This study proposes to apply the FDA guidance (May 2001) for the high throughput bioanalysis of human plasma using Laser Diode Thermal Desorption (LDTD)-MS/MS. The characteristics of the LDTD system are :

LDTD (Figure 1)

- Plug-and-play ionization source interface to most popular mass spectrometer
- Thermal desorption induced by a laser diode
- The sample is carried by a carrier gas to a corona discharge region for APCI
- Loader capacity up to 10 LazWell™ plates

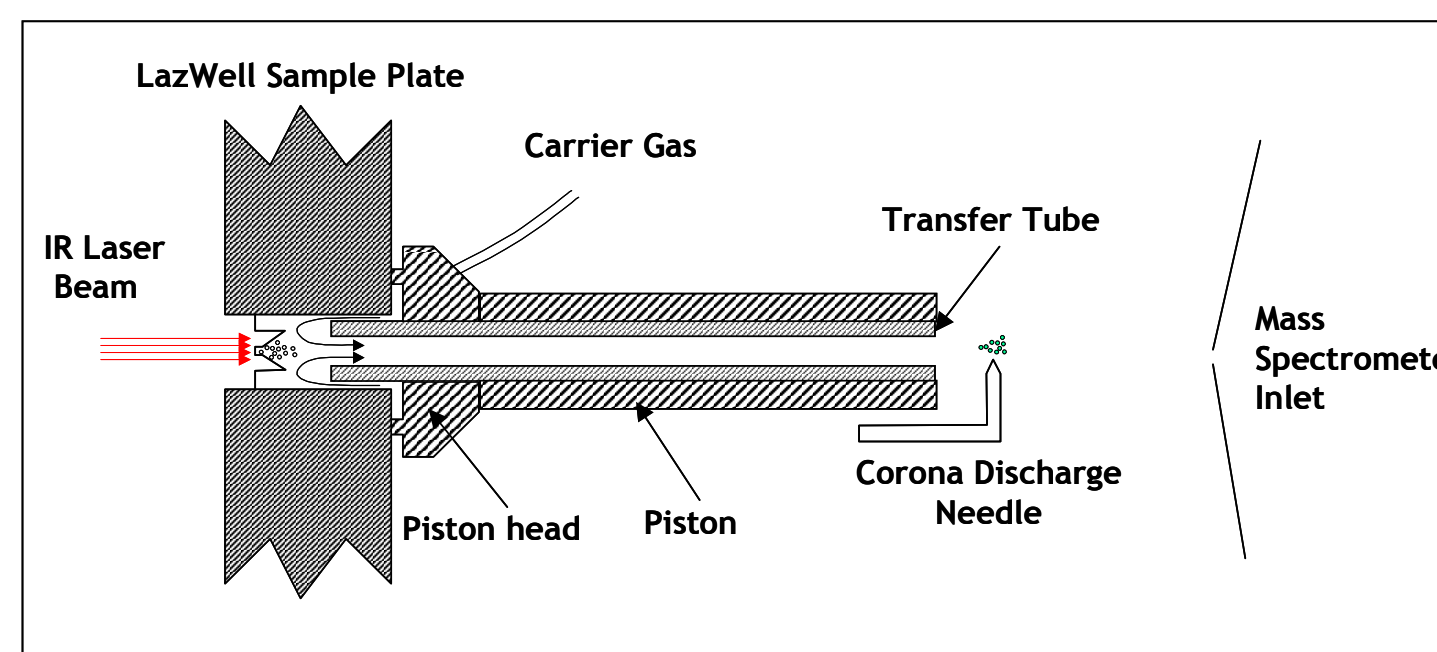


Figure 1 Schematic of the LDTD ionization source.

LazWell™ Plate (Figure 2)

- Standard 96-well plate format
- Low volume delivery (from 1 to 10 µL of sample per well)
- No carryover
- No enhancement matrix needed
- No sample desalting needed
- No liquid mobile phase needed
- Sample dried at room temperature

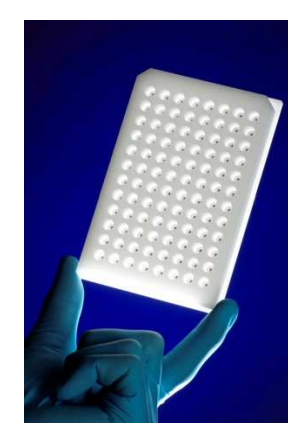


Figure 2 LazWell™ sample plate

METHOD

Instrumentation (Figure 3)

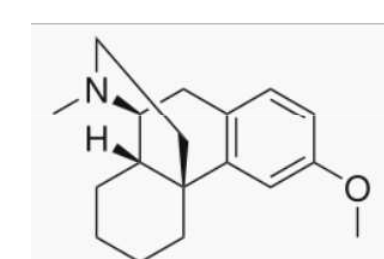
- LDTD model W-960, Phytronix Technologies
- Xevo, Waters



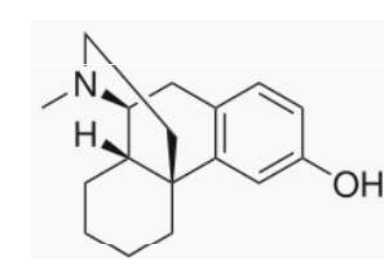
Figure 3 LDTD-MS/MS analytical system.

MS Parameters

PC	
Mode	APCI (+)
Cone	40 V
Collision energy	40 V
Scan time	0.078 s
Needle current	3 µA
Transition	272-> 171 amu
Transition(IS)	275-> 171 amu Dextromethorphan-d3



A) Dextromethorphan



B) Dextropran

Figure 4 Chemical structure

LDTD Parameters

- Laser power pattern :
 - Increase laser power to 45 % in 3.0 s
 - Hold at 45 % for 2.0 s
 - Shut down laser power to 0 %
- Carrier gas flow : 3 L/min (Air)
- Deposited sample volume: 2 µL

Sample Preparation

- Spiking
 - 10 µL of working solution
 - 990 µL of Human plasma (Na citrate)
 - Vortex 1 min.
- Protein precipitation extraction
 - 25 µL of spiked plasma in sampling tube (1.5 ml)
 - 100 µL of Internal standard (Dextromethorphan-d3 at 50 ng/ml in ACN) or ACN for Blank.
 - Vortex 1 min and centrifuge 3 min at 14000 g.
 - Transfer 2.0 µL of into LazWell™
 - Evaporate solvent at room temperature
 - Perform LDTD-MS/MS analysis

RESULTS

Calibration Curves

The calibration curve were evaluated over a nominal range of 12.5 to 800 ng/ml (Figure 5). The linearity were excellent (Table 1).

Table 1 Calibration curves parameters (Inter-days)

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

Weighting factor 1/x

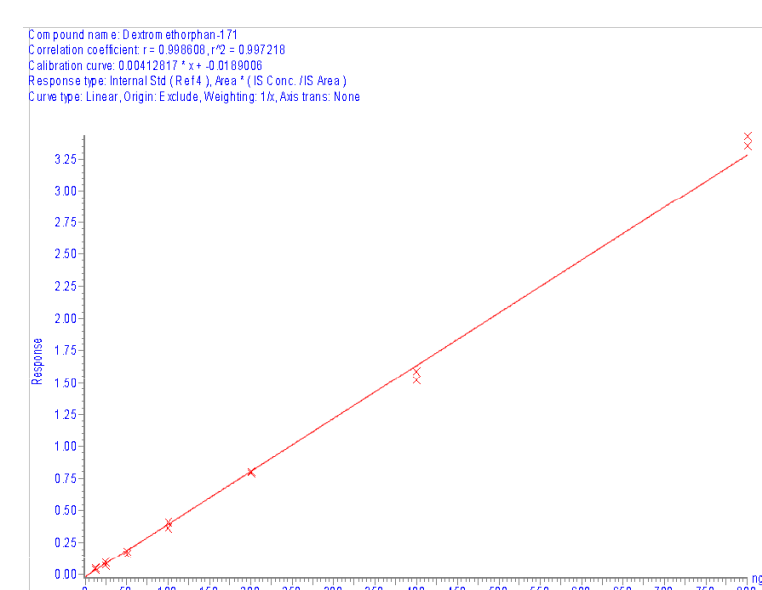


Figure 5 Calibration curve of Dextromethorphan

Within-run Accuracy and Precision

The within-run accuracy and precision were evaluated with quality control at 5 different levels. The LDTD shows an excellent accuracy of 93.4 to 117.6 %. The precision was excellent with values from 3.1 to 8.0 % (RSD).

Table 2 Within-run accuracy and precision

	LLOQ	QC (Low)	QC (med)	QC (High)	ULOQ
Nominal 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. conc.	117.6	104.3	101.0	93.4	102.8

Inter-run Accuracy and Precision

The inter-run accuracy and precision were evaluated with quality controls at 3 different levels. The LDTD shows an excellent accuracy of 99.9 to 106.1 %. The precision was excellent with values from 5.4 to 13.3 % (RSD).

Table 3 Inter-run accuracy and precision

	QC (Low)	QC (med)	QC (High)
Nominal 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. conc.	106.1	101.8	99.9

Recovery

The Recovery as defined by the FDA has been evaluated to be the detector response obtained from an amount of the analyte/IS added to and extracted from the biological matrix (3-QC levels) and compared to the detector response of the pure standard. The extraction method recovery has also being evaluated. The results shows that both recovery are within acceptable limits.

Table 4 Recovery

	Recovery					
	QC (Low)		QC (Med)		QC (High)	
	Drug	IS	Drug	IS	Drug	IS
FDA Recovery	72.1	61.8	84.4	71.6	76.8	66.6
Extraction Recovery	79.3	70.5	87.0	68.4	71.9	60.9

Selectivity test

The % of Interferences at lower standard for six different matrix were evaluated. All matrix had an interference lower than 20%.

Table 5 Matrix selectivity

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

Matrix effect evaluation

Matrix effect were evaluated for six different matrix at lower QC level. All matrix followed the FDA acceptance criteria.

Table 6 Matrix effect

Matrix ID / Type	Nominal 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
M6-M	25	3	28.3	7.5	113.2

Stability test evaluation

Stability test were evaluated with and without the metabolite (Dextropran). The mean difference between the original sample and the « aged-sample » was calculated as well as the corresponding CVs and they are reported in Table 7. For both QCs the results show an excellent sample stability within ± 13 %. Moreover, the data shows that the metabolite (Dextromethorphan) does not interfere (backconversion) during the analysis of Dextropran.

Table 7 Stability results

Stability		Deviation from nominal concentration (%)			
		Dextromethorphan (ng/ml)		Dextromethorphan + Metabolite (ng/ml)*	
		25	400	25	400
Freeze-Thaw (4 cycles)	Mean (%Difference)	-7.7	3.4	3.2	-2.9
	Precision (%RSD)	9.7	4.1	7.2	3.9
Bench top (24h, RT)	Mean (%Difference)	12.3	-5.2	-0.3	-4.4
	Precision (%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 (%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 (%RSD)	8.4	2.8	4.7	6.3
Longterm (52 days, -20°C)	Mean (%Difference)	ND	3.0	ND	-2.9
	Precision (%RSD)	ND	7.7	ND	9.1

*Dextropran (major Metabolite) was spiked in stability sample at a concentration of 800 ng/ml

Exogenous formulation agent effect evaluation

To evaluate the potential effect of Twin 80 as exogenous formulation agent frequently use for IV and PO test in PK we have spiked into "blank animal" to created a Biological-QC. The Twin 80 concentration under evaluation correspond to the maximal concentration well tolerated by rat in IV study. The results shows that for animal studies the LDTD can be used and would not be affected by Tween 80 in terms of matrix effect (Table 7).

Table 7 Biological-QC for formulation effect evaluation

Biological-QC	Nominal conc. (ng/ml)	N	Mean (ng/ml)	RSD (%)	%Nom. conc.
QCL-DMSO*	25	6	23.0	13.6	92.2
QCL-Twin 80**	25	6	24.5	7.3	97.9

*DMSO (6.4mg/ml) concentration correspond to IV injection of 200 mg/Kg in rat.

**Twin 80 (3.2mg/ml) concentration correspond to IV injection of 100 mg/Kg in rat.

CONCLUSIONS

- All FDA validation criteria's were evaluated successfully showing that the LDTD can be use in regulated environments.
- Not metabolite conversion was observed during analysis and stability.
- No matrix interference and no matrix effect.
- Good Recovery, Accuracy and Precision.
- No interference from Twin 80 if used in animal PK.
- What should be done ? Just follow the FDA guidelines.....