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Overview

- LC-MS/MS with Electrospray Ionization (ESI) has been extremely powerful methodology for quantitative analysis of compounds in complex matrices at low level of detection and high specificity ($m/z \leq 2000$).
- Fast LC/MS/MS validated quantitative methods of (~ 1 min run time) with clean extracted samples (SALLE & LLE) have been shown to be rugged and cost validated, dependent on the specificity and selectivity of MRM MS data acquisition.
- Ambient surface chemistry ionizations for mass spectrometry such as matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) have been applications of increasing interest by which mass-specificity is utilized for high throughput analysis.
- Quantitative measurements of one Abbott Compound extracted from biological matrices by MALDI-MS/MS using Sciex Quant Flash showed acceptable accuracy and precision at a run time of ~ 4 sec as compared to LC/MS/MS run time of ~ 3 min, Land O'Lake Poster 2009.
- The LDTD-MS/MS and LC-MS/MS quantitative measurements of ABT-X123 class compound in human plasma were cross validated with acceptable accuracy and precision.

Objective

- To evaluate the quantitative measurements of Abbott-X123 (Mol. Wt. 456 amu) and ABT-X123-d4 (IS) using positive ionization by LDTD/MS/MS as compared to the validated assay by LC/MS/MS and to assess the impact on determined concentrations of unknown clinical study samples.

Methods

Sample Preparation Salt Assisted Liquid/Liquid Extraction - A 96 well Plate format preparation process by Manual Pipetting or Hamilton Nimbis And AT ICS

- Extraction Procedures:**
 - LC-MS/MS (Run time ~ 1.5 min), Mobile Phase & Autosampler Rinse:
 - 1:1 (v/v) Acetonitrile [15 mM Ammonium Acetate and 0.005% Acetic Acid in Water] @ 0.6 mL/min
 - Waters Symmetry C18 5 μ m 2.1 x 50 mm @ RT
 - Add 0.05 mL of d-IS + 0.05 mL of plasma sample + 0.05 mL of 2 M NH₄OAc + 0.225 mL of Acetonitrile and mix well.
 - Centrifuge the extraction plate at approximately 3400 rpm for 5 minutes at approximately 10°C.
 - Transfer 0.1 mL of Organic layer into clean plate then add 0.1 mL of Water.
 - Inject 5 μ L into LC-MS.
- LazWell Plate Prep & Analysis Conditions:**
 - Spot 2 μ L of the extracted samples in 1:1 organic/water for LC/MS onto the LazWell plate* and analyze in singlet using 3-35-0 Pattern and Air gas flow rate of 3 L/min.

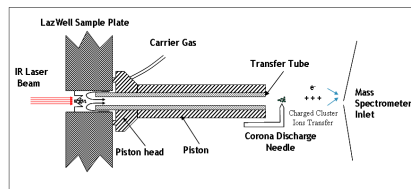
LDTD LazWell Plate Prep & Liquid Handling by "Nimbus"

LDTD-MS/MS Run time ~ 15 Sec.



Methods

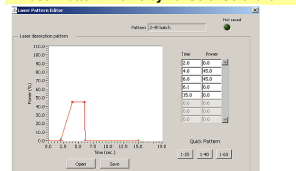
LDTD Ionization



LDTD Source Key Features

- A shotgun analysis approach; no mobile Phase required.
- Each Source holds up to ten shallow 96-LazWell plates with a metal sheet insertion.
- Sample size (~ 1 to 10 μ L), dried onto the bottom of each well.
- Samples are introduced to MS by indirect action of a laser diode (980 nm), a shot at the back of each well for thermal desorption, not interacting with the sample.
- Gaseous neutral species transferred by a carrier gas (Air).
- Ionization occurs into the corona discharge region.

A Laser Pattern Profile by LazSoft® Software



Results

Table 1. Reproducibility of Extracted Calibration Standards of ABT-639 in Human Plasma by LDTD-MS/MS

Standards (L ₁ → L ₄)	Correlation Coefficient (r ²)	Slope	Intercept
Mean ^a 1.11 → 3070	0.9958	0.0188	0.005
SD 0.0173 → 131	0.0018	0.0002	0.0003
%Bias -0.9% → -0.9%	NA	NA	NA
%CV 1.6 → 4.3	0.2	1.6	9.6

^an = 3
Standards (24 of 24) are within ± 15%.

Results

Table 2. Statistical Summary of LLOQ and ULOQ Measurements for ABT-639 in Human Plasma by LDTD-MS/MS

	STD L ₁ 1.12 ng/mL		STD L ₄ 3370 ng/mL	
	Run 1	Run 2	Run 1	Run 2
Mean	1.12	1.11	3370	3250
S.D.	0.0575	0.0801	0.134	88.2
%CV	5.1	7.1	11.9	2.6
%Bias	0	-13.8	0.9	-3.9
Count (n)	6	6	6	6

	Intra-Array Variability		Inter-Array Variability	
	Run 1	Run 2	Run 1	Run 2
Mean	1.12	1.11	3370	3250
S.D.	0.0935	0.083	80.8	88.2
%CV	8.3	7.5	2.5	2.6
%Bias	0	0	-4.2	-3.6
n	18	18	18	18

Figure 1. Typical Calibration Curve of ABT-X123 in Human Plasma by LDTD-MS/MS

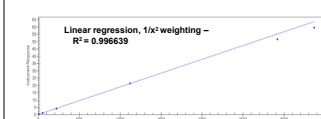


Table 3. Statistical Summary ABT-X123 QCs Measurements in Human Plasma by LDTD-MS/MS

Run Date	Curve Number	LLOQ	QC 3	QC 2	QC 1	ULOQ
8/20/2009	4	1.12	2.4	142	2640	3250
Interarray SD	0.0575	0.0801	1.07	49.4	88.2	88.2
Interarray %CV	5.1	7.1	11.9	18.2	2.6	2.6
Interarray %Bias	0	-13.8	0.9	-3.9	-3.6	-3.6
8/20/2009	1	1.12	2.4	142	2640	3250
Interarray SD	0.0881	0.0240	1.40	76	88.2	88.2
Interarray %CV	7.8	1.0	1.0	28.4	2.6	2.6
Interarray %Bias	1.8	5.8	4.2	-5.5	-3.6	-3.6
8/20/2009	2	1.12	2.4	142	2640	3250
Interarray SD	0.112	0.109	1.40	75	88.2	88.2
Interarray %CV	10.0	9.8	4.3	2.8	2.6	2.6
Interarray %Bias	0	0.3	5.1	-4.1	-4.1	-4.1
8/20/2009	3	1.12	2.4	142	2640	3250
Interarray SD	0.112	0.109	1.40	75	88.2	88.2
Interarray %CV	10.0	9.8	4.3	2.8	2.6	2.6
Interarray %Bias	0	0.3	5.1	-4.1	-4.1	-4.1

Table 4. Statistical Summary ABT-X123 QCs spotted by Nimbus

	LLOQ	QC 3	QC 2	QC 1	ULOQ
Mean	1.12	2.77	138	2770	3370
SD	1.18	2.89	144	2640	3230
%CV	0.123	0.109	1.14	62.6	100
%Bias	10.4	3.8	0.8	2.4	3.4
%Bias	5.4	4.3	4.3	-4.7	-4.2
n	5	5	5	6	6

2 mL Spot, 4% LLOQ, 16% QC, 66 ULOQ < 15%

Figure 2. Representative Peaks of ABT-X123 & IS in Human Plasma by LDTD/MS/MS

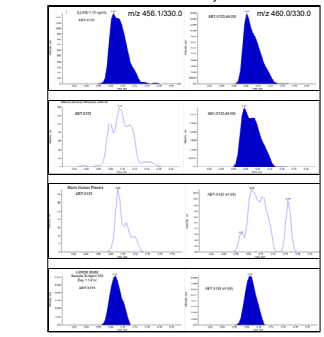


Table 5. PK Parameters of ABT-X123 in Pooled First in Man Clinical Study Samples

Pharmacokinetic Parameters	Units	LC-MS/MS	LDTD-MS/MS
C _{max}	ng/mL	1295	1260
C _{max} Dose	ng/mL/mg	43.2	42.0
T _{max}	hr	1.75	1.25
T _{1/2}	hr	5.75	5.75
AUC _{0-∞}	ng*hr/mL	12570	12520
AUC _{0-t} Dose	ng*hr/mL/mg	419	418

ESI-MS (91 samples) batch analysis time = 2 hr 31 min. LDTD-MS (91 samples) batch analysis time = 40 min 27 pooled human plasma samples analyzed by LDTD-MS/MS. % Bias ranged between -6.6% and 7.1%

Figure 3. Comparative Mean Pooled Human Plasma Concentration-Time Profile

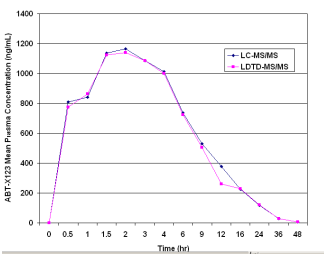


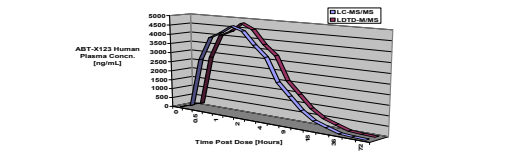
Table 6. Comparative Pharmacokinetic Results of ABT-X123 of Bioavailability Clinical Study

Pharmacokinetic Parameters	Units	LC-MS/MS		LDTD-MS/MS	
		Two 50 mg ABT-X-123 Formulation A (N = 12)	One 100 mg ABT-X-123 Formulation B (N = 12)	Two 50 mg ABT-X-123 Formulation A (N = 12)	One 100 mg ABT-X-123 Formulation B (N = 12)
C _{max}	ng/mL	5000 ± 734	5090 ± 990	4793 ± 945	5408 ± 1135
C _{min}	ng/mL	1.63 ± 0.38	1.25 ± 0.61	1.43 ± 0.44	1.24 ± 0.39
T _{max}	hr	6.72 ± 1.76	6.64 ± 1.55	6.48 ± 1.35	6.47 ± 1.16
AUC _{0-∞}	ng*hr/mL	39600 ± 8200	36000 ± 9100	35141 ± 10522	39900 ± 8678
AUC _{0-t}	ng*hr/mL	39700 ± 8220	36100 ± 9120	35175 ± 10569	39908 ± 8687

Number of Clinical Study Samples for ABT-X123	
Analyzed by LDTD-MS/MS	412
AOL (Confirm with Original Results)	38
BQL (Lower or higher than Original Results)	2
>20% Bias *	4
Max % Bias	-8.2%
Max % Bias	13.9%
Total LC/MS/MS Run Time (hr:mins)	13:15:15
Total LDTD/MS/MS Run Time (hr:mins)	2:32:48

AOL: Above Quantitation Level
BQL: Below Quantifiable Level
* 2% of all analyzed samples failed to meet acceptance criteria
- Suspect empty well or low reconstituted volume sample due to the length of extracted samples storage
- Extracted 96-well plates were stored @ -20°C for 30 Days prior to the Analysis by LDTD-MS/MS

Figure 4. Comparative Mean Concentration-Time Profile of Bioavailability Study



Conclusions

- We were able to optimize and operate the LDTD Source on Sciex API 5000 in placement of the Electrospray source using Analyst[™] & LIMS.
- Analysis of Clinical study samples showed no interference from endogenous compounds and/or metabolites peaks that share MRM transition ions of ABT-X123 and the determined concentrations and PK Parameters were acceptably comparable for measurements by LDTD-MS/MS.
- LLOQ @ ~ 1.12 ng/mL is achievable with LDTD, lower value may be a challenge for this compound, which is highly dependent on optimal sample preparation, MRM transitions and Laser Pattern.
- The high speed analysis can contribute to cost saving with reagents, supplies, and LC/MS usage, repair and maintenance in addition to the environmental impact & hazardous waste reduction.

Acknowledgment

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