

Effect of Phospholipids and Formulation Agents in LDTD-MS/MS Analysis of Dextropropofol in Human and Rat Plasma

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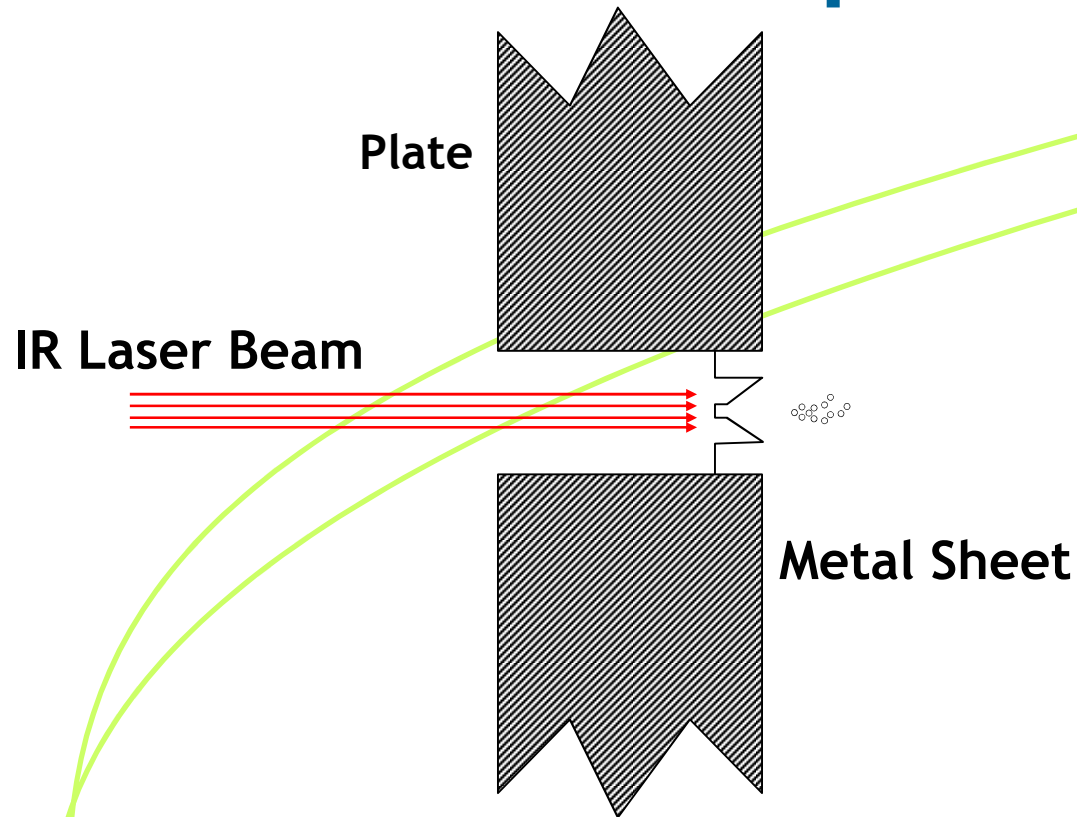
Phytronix



Presentation Highlights

- What is LDTD-APCI ?
- Matrix effect evaluation
 - Phospholipids in human plasma samples
 - Formulation agents in mouse plasma samples
 - Dry Blood Spot (DBS) card extracts
- Conclusions

What is Laser Diode Thermal Desorption ?

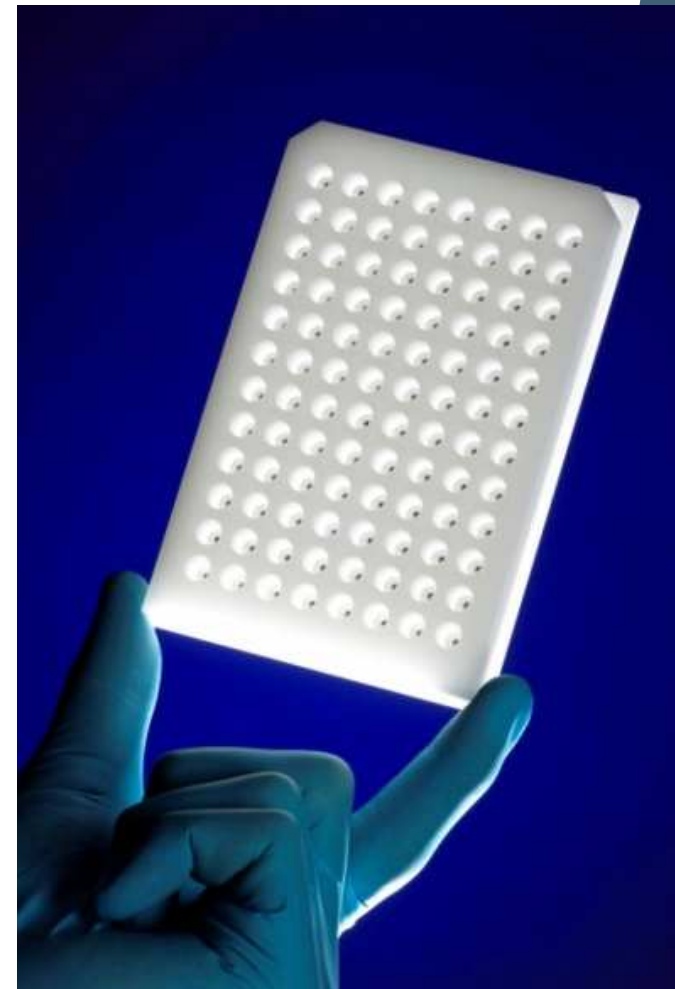


Sample dried into the well cavity

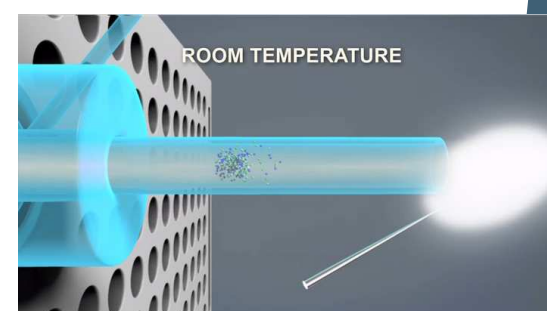
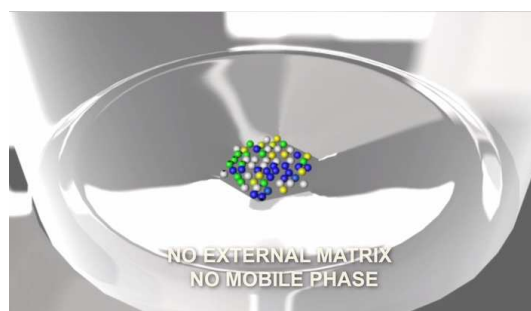
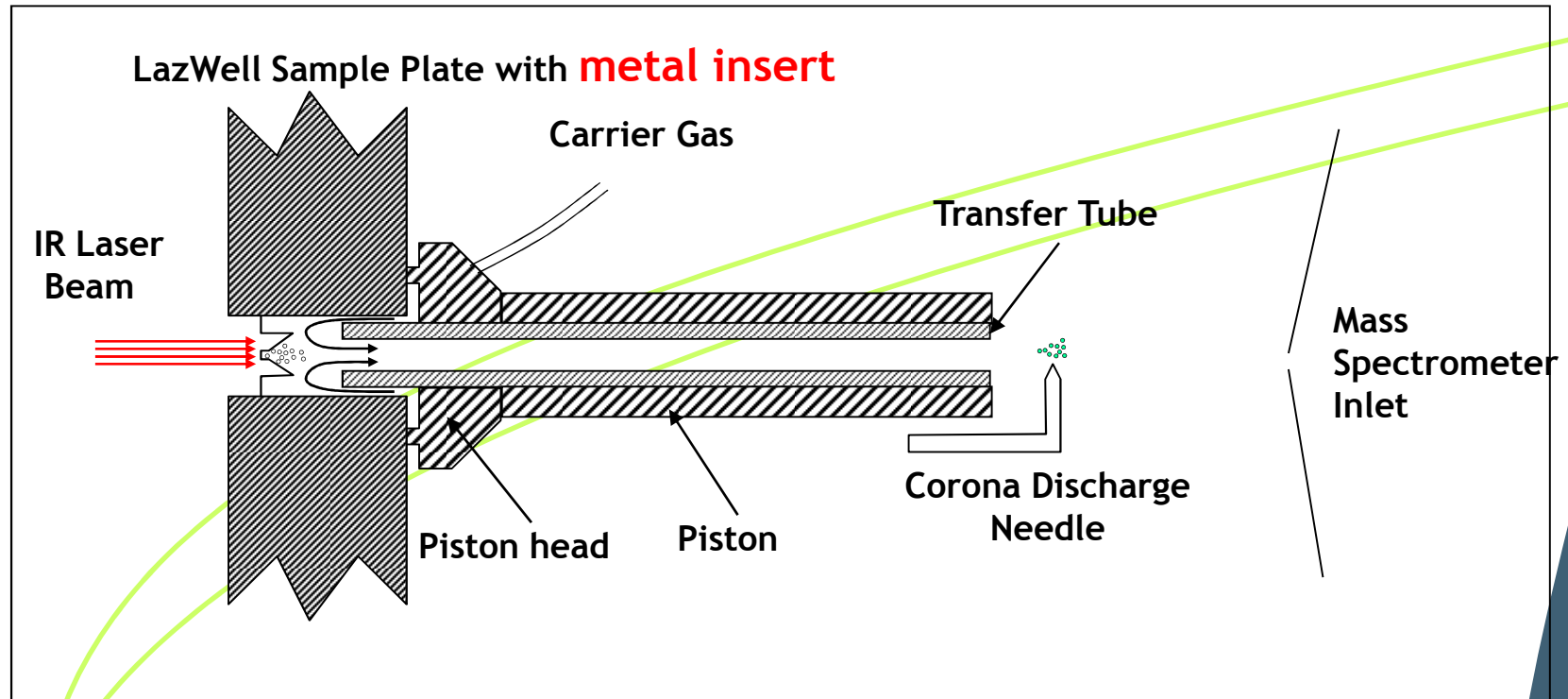
No photon interactions with the sample

Ultra fast heating transfer

Quantitative sample desorption



LDTD schematics



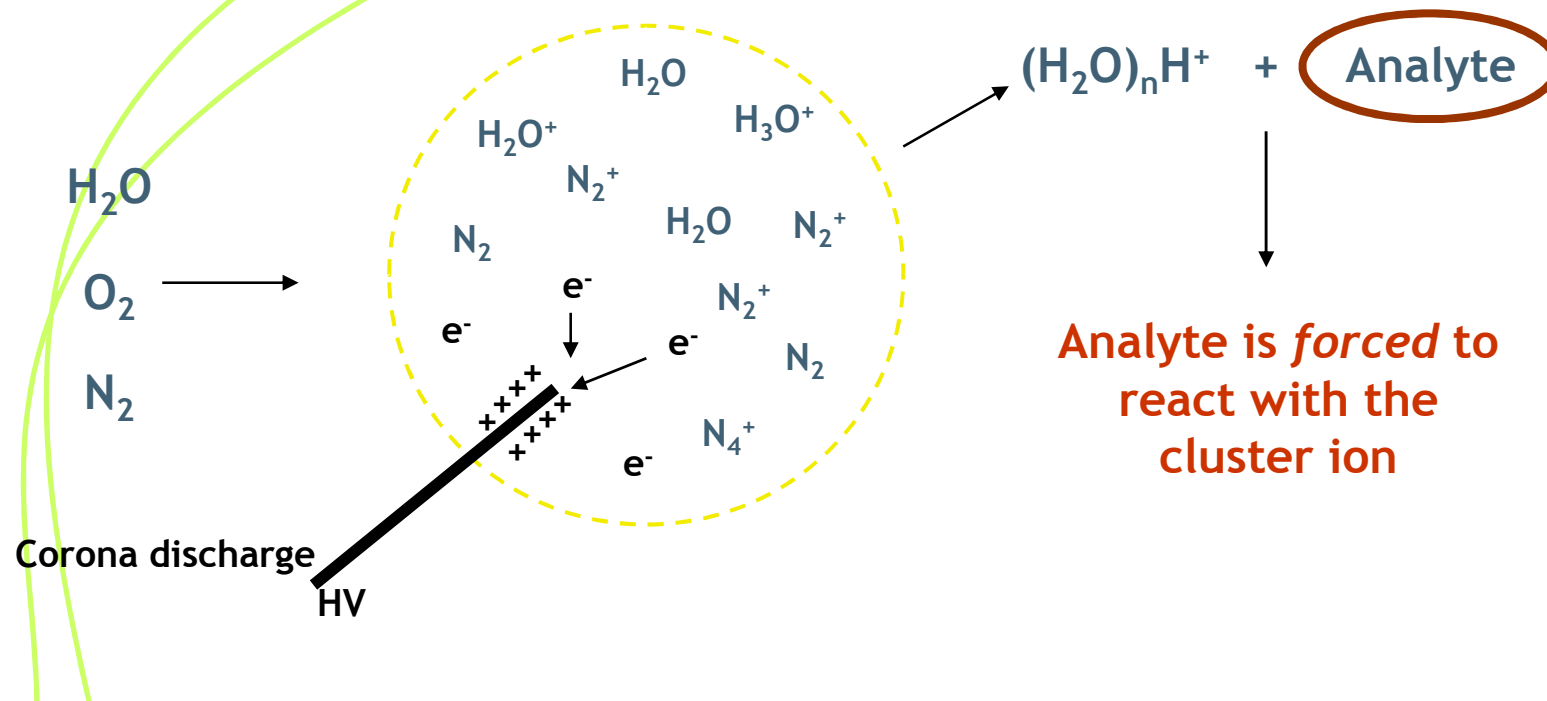
LDTD-APCI

LDTD-APCI : Ionization Source

Gas-phase atmospheric pressure chemical ionization

No solvent and no salt or acid/base added

Water clusters are the source of H⁺



LDTD-APCI vs LC-ESI

- The LDTD operates without chromatographic separation
- The LDTD analyzed dry samples
- APCI-type ionization known to be more robust to ionic suppression
- But who the LDTD-APCI(MS/MS) behaves with :
 - Phospholipids variation from matrix-to-matrix
 - Formulation agents present in high concentration
 - DBS sampling cards : Treated or Not

Phospholipids in LDTD analysis

- Dextrorphan analysis in human plasma
 - Calibration curve from 1 to 800 ng/mL
 - Dextrorphan-D3 used as ISTD
- Phospholipids spiked into human plasma
 - 1 mg/mL (calculated from human plasma concentration) into 6 plasma from different lots set as QC samples (200 ng/mL)
 - Most common phospholipids in human : 18:0-18:2 (PC), 16:1 (PE), 18:1 (PS) and 18:1 (PA)
- Sample preparation
 - Protein precipitation
 - Liquid-liquid extraction

Sample preparation

Protein precipitation

20 μ l of spiked plasma
+
100 μ l Acetonitrile

Vortex 10 seconds

Centrifuge 12 minutes

Deposit of 2 μ l
supernatant onto
Lazwell plate

Dry completely

Liquid-liquid extraction

20 μ l of spiked plasma
+
100 μ l Ethyl Acetate

Vortex 10 seconds

Centrifuge 12 minutes

Deposit of 2 μ l supernatant
onto Lazwell plate

Dry completely

Protein precipitation results

- Without ISTD correction

- No statistical effect on the area count as compared to the reference

Area difference = $\frac{100\% \times (\text{reference} - \text{fortified})}{\text{reference}}$

Matrix	PE	PS	PC	PA
M1	7.3	-9.2	3.2	5.8
M2	-5.2	-1.5	-5.8	-6.2
M3	-7.6	0.3	-6.3	1.2
M4	8.0	1.4	4.1	-2.3
M5	-10.3	-5.3	-3.2	7.2
M6	-5.5	7.7	7.2	3.9

- With ISTD correction

- No statistical effect on the calculated concentrations
- FDA accuracy criteria (15-20%) respected in all cases

Protein precipitation mid QC 200 ng/ml nominal (n=3)

Matrix	Reference		PE		PS		PC		PA	
	Measured (ng/ml)	CV (%)	Measured (ng/ml)	CV (%)	Measured (ng/ml)	CV (%)	Measured (ng/ml)	CV (%)	Measured (ng/ml)	CV (%)
M1	188	0.9	183	1.0	205	0.5	189	4.2	202	1.1
M2	211	3.8	202	1.9	185	1.1	210	3.1	197	0.8
M3	205	1.6	217	1.9	203	3.4	185	1.7	212	0.7
M4	197	1.4	201	0.1	188	0.8	210	2.0	190	1.4
M5	195	2.8	187	4.0	200	1.2	201	1.9	205	3.5
M6	203	5.7	193	2.4	195	2.0	184	1.4	193	0.2

Liq-liquid extraction results

- Without ISTD correction

- No statistical effect on the area count as compared to the reference (except M6-PC)

Area difference = $\frac{100\% \times (\text{reference} - \text{fortified})}{\text{reference}}$

Matrix	PE	PS	PC	PA
M1	-9.3	-17.7	7.2	-6.3
M2	8.3	-15.4	-5.9	-15.2
M3	-9.5	0.9	-8.5	5.1
M4	-7.6	14.6	12.2	4.6
M5	-11.9	10.8	-7.2	13.9
M6	-6.6	7.9	-19.3	3.8

- With ISTD correction


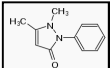
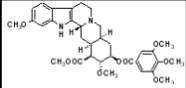
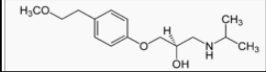
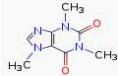
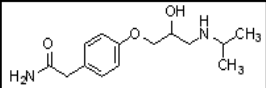
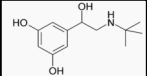
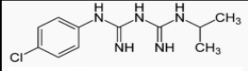
- No statistical effect on the calculated concentrations
- FDA accuracy criteria (15-20%) respected in all cases

Liquid-Liquid QC 200 ng/ml nominal (n=3)

Matrix	PE Measured (ng/ml)	PS Measured (ng/ml)	PC Measured (ng/ml)	PA Measured (ng/ml)
M1	199	206	208	206
M2	199	191	198	199
M3	201	187	201	192
M4	202	200	207	198
M5	200	197	205	198
M6	196	194	193	194

Effect on other drugs

- Protein precipitation plasma extract
 - Spiked with Dextrophan (no ISTD) at 200nM splitted :
 - Phospholipids at 200 µg/mL
 - Equivalent in volume of methanol

Name	Structure	Log D (pH 7)	Log P	MW	Area Variation
Carbamazepine		3.16	3.16	236.3	2.4%
Antipyrine		1.43	1.43	188.2	3.3%
Reserpine		0.98	4.42	608.7	-1.2%
Metoprolol		-0.97	1.85	267.4	-0.5%
Caffeine		-1.76	-0.46	194.2	-4.4%
Atenolol		-1.93	0.76	266.3	8.8%
Terbutaline		-2.42	0.43	225.3	33.0%
Proguanil		-3.92	1.58	253.7	7.1%

Formulation agent in LDTD analysis

- Specimen : Male Sprague-Dawley rats
- Administration : single oral at 30 mg/kg
- Formulation : PEG200/Tween80/PBS at pH 7.4 (10/5/85; v/v/v)
- Sampling time (hr) : 0.25, 0.50, 1, 2, 4, 6, 8 and 24 hours
- Sample preparation : Protein precipitation
- Calibration curve : Rat plasma without formulation agents into it (with ISTD)
- Sample analysis :
 - LC-MS/MS
 - LDTD-MS/MS

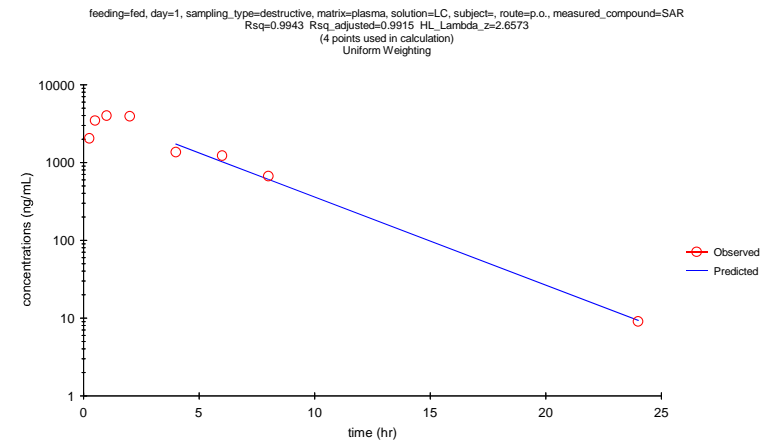
Samples prepared by Sanofi-Aventis, Montpellier, France

Results : Concentrations

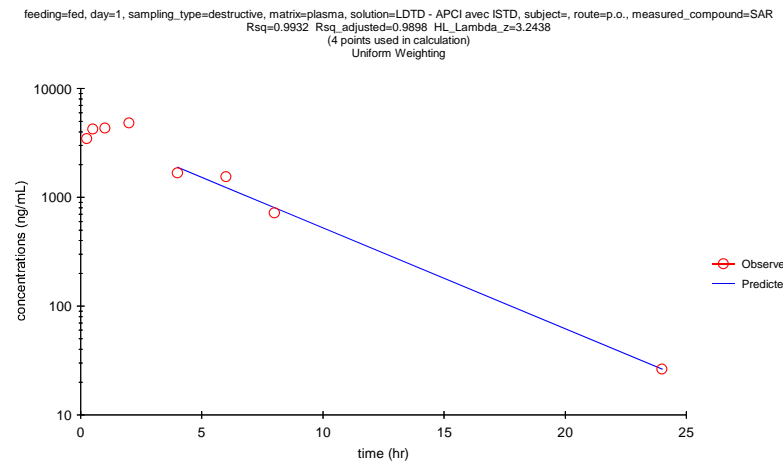
Animal number	Theoretical sampling time (hr)	LC MS/MS (ESI) Concentration (ng/mL)	LDTD MS/MS Concentration (ng/mL)
1	0.25	1680	1499
2		2110	2875
3		2340	3412
4	0.5	3130	2900
5		3530	3863
6		3730	6683
7	1	4490	5400
8		3370	3387
9		4140	4898
10	2	3910	3468
11		3490	3937
12		4380	5070
13	4	991	1525
14		1310	1871
15		1780	2028
16	6	1030	1628
17		922	1543
18		1710	2342
19	8	1200	1469
20		439	621
21		361	494
22	24	8.45	37
23		17.9	27
24		0.651	23

- Acceptable concentration match
- Predicted match observed results

LC-MS/MS analysis



LDTD-MS/MS analysis



Results : Pharmacokinetic parameters

- Good match on the Pharmacokinetic parameters
- Same biological information extracted from the PK study using the LDTD-MS/MS while formulation agents were present

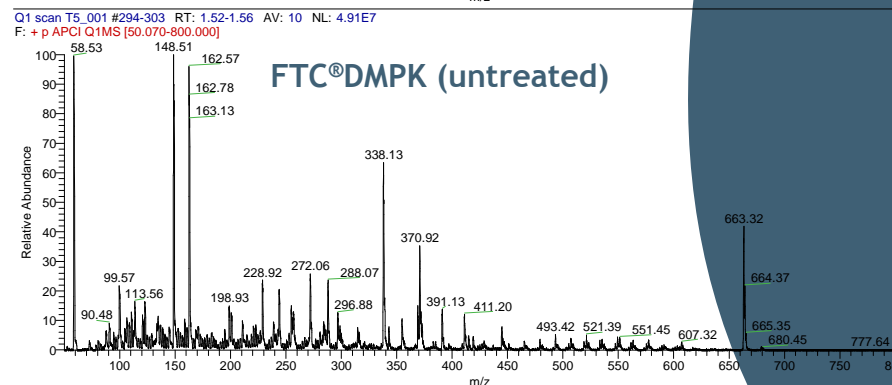
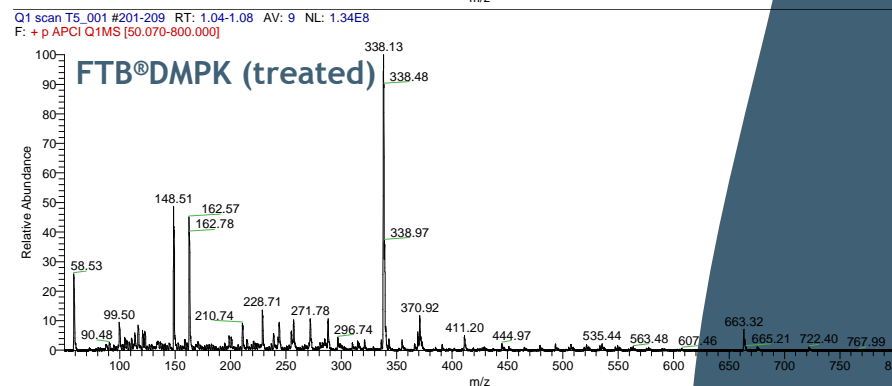
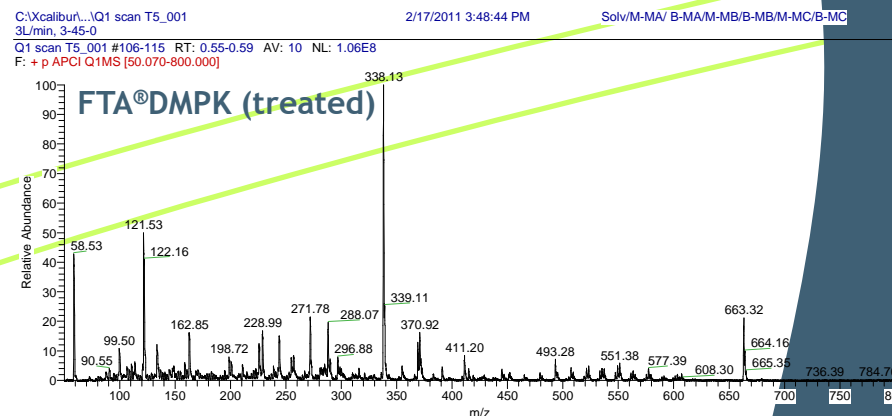
solution	No_points lambda_z	HL_Lambda z	Tmax	Cmax	Tlast	AUClast	AUCall	AUCINF	AUC %Extrap
LC-MS/MS	4	2.7	1.0	4000	24.00	22000	22000	22000	0.2
LDTD-MS/MS	4	3.2	2.0	4820	24.00	26000	26000	26000	0.5

Dry Blood Spot (DBS) sampling cards

- DBS sampling card might be treated or not for protein denaturation
- According to the manufacturer : Chemicals might interfere with the mass spectrometric detection
 - Ionic suppression
 - Isobaric interferences
- Experience
 - Blood spiked with dextroproporphane
 - 3mm punch extracted
 - 50 μ L NaCl_{sat} in water, Sonication 30 sec
 - 50 μ L acetonitrile (with ISTD), vortex 30 sec

Dry Blood Spot (DBS) sampling cards

- Full Q1MS scan from 100-800 amu
- Not much difference in the peak profile between the 3 sampling cards
- More material desorbed from the 2 treated cards
- Impact on ionization ?
- Impact on thermal desorption ?



Dry Blood Spot (DBS) sampling cards

- QC-samples (250 ng/mL) signal compared to a standard solution

	Recovery (%)	Signal Suppression (%)	S/N
FTA [®] DMPK (<i>treated</i>)	78	+ 3.4	173
FTB [®] DMPK (<i>treated</i>)	68	-79	110
FTC [®] DMPK (<i>untreated</i>)	66	0	161

- Comparable recovery and S/N over the cards
- No signal suppression for FTA[®] and FTC[®] DMPK cards
- Treated FTB[®]DMPK card shows strong signal suppression
- Accuracy on Qc's of 105 %

Conclusions

•Phospholipids

- No impact observed on raw signal intensity
- Both sample preparations show same results
- Same behaviour observed for many drugs
- Selectivity test using 6 plasmas from different lots can be used in LDTD (FDA requirement)

•Formulation agents

- Calibration curve without formulation agents used to quantify samples
- Reported sample concentrations match between LC and LDTD
- Comparable pharmacokinetic parameters
- Same biological prediction from LDTD-MS/MS results as compared to traditional LC-MS/MS technology

Conclusions

• DBS treated/untreated cards

- More material extracted/desorbed from treated sampling cards
- Signal suppression observed on treated card FTB[®] DMPK
- No signal suppression observed for treated/untreated FTA[®] and FTC[®] DMPK card
- Internal standard behaves as the drug leading to an accurate drug quantization following all cards extraction

Questions

