

“ I need BASi  
for impeccable  
scientific output.”



*Don Gray, Senior Scientist  
October, 29, 2009*

## Laser Diode Thermal Desorption (LDTD) an Alternative to the LC for High-Throughput Quantitation: Some Early Case Studies

## Why LDTD at BASi?

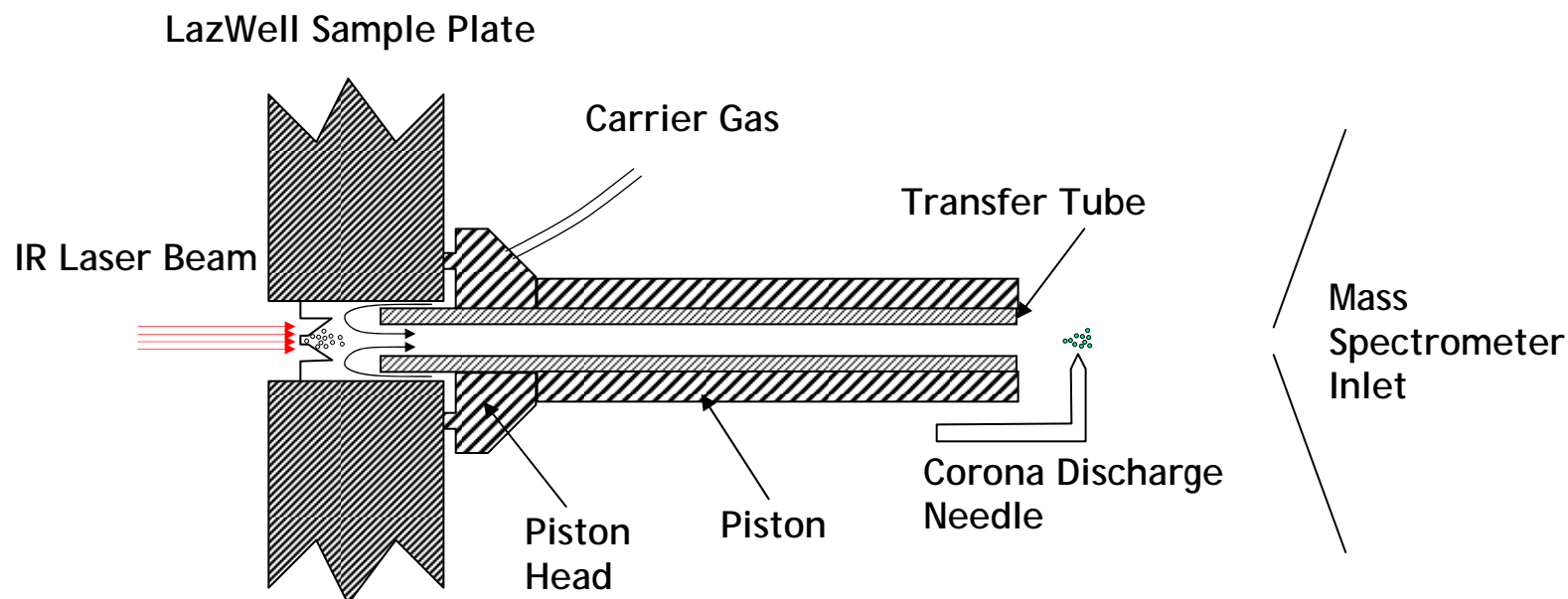
- Speed: 40 minutes / plate
- Resources: no column or mobile phase
- Familiar sample preparation
- No carryover
- Barcoded plates/Integration with LIMS
- Easily interchangeable with standard source

## Caveats

- Not universal: Sulfoxides, Proteins, Peptides don't work
- Thermal Lability a possible issue
- Isobaric Compounds are out
- Destructive analysis



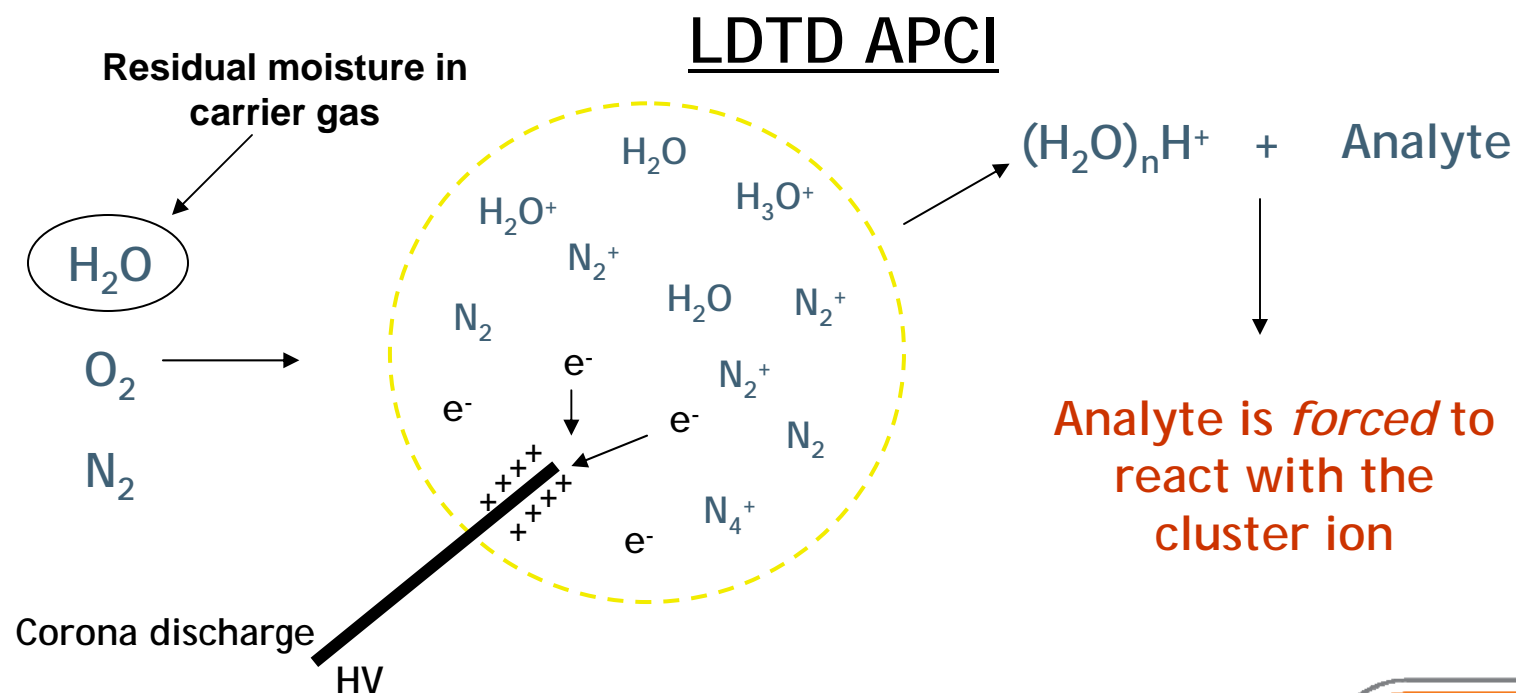
# LDTD Ionization Source



- Sample is dried onto the bottom of a well from a standard 96-well plate with a metal sheet insertion.
- Thermal desorption induced by a laser at 980 nm (no photon-sample interactions).
- Gaseous neutral species transferred by a carrier gas.
- Ionization occurs into the corona discharge region.

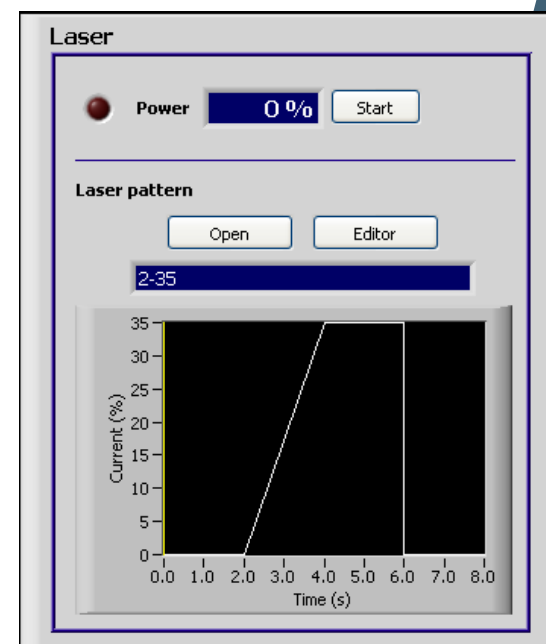
# Theoretical Aspects of the Ionization

- The ionization process that occurs in the LDTD source is an Atmospheric pressure chemical (APC) type of ionization without the presence of solvent (no mobile phase or enhancement matrix.)



## Key Features

- Low volume sample analysis (1 to 10  $\mu\text{L}$ ).
- 96-well plates are designed to be compatible with conventional sample preparation systems.
- No extra sample pre-treatment needed.
- The absence of enhance matrix and mobile phase lower the noise signal.
- Elimination of cross contamination / carryover due to LC.
- Wells are individually isolated during the thermal desorption.
- The thermal desorption process takes seconds.

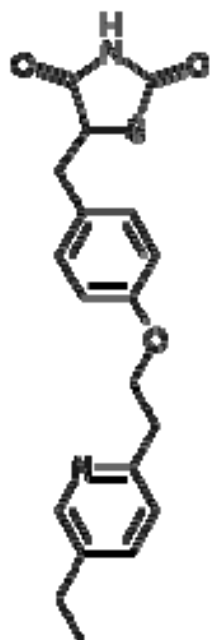


A1: Empty

A2: 2 $\mu$ L droplet

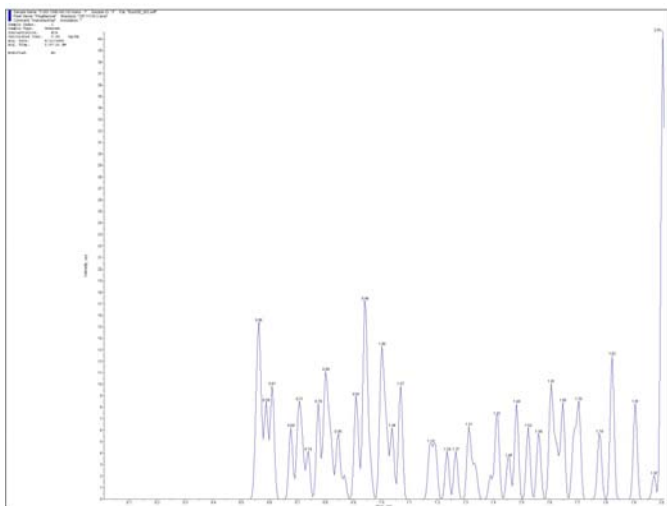


## Extraction Method



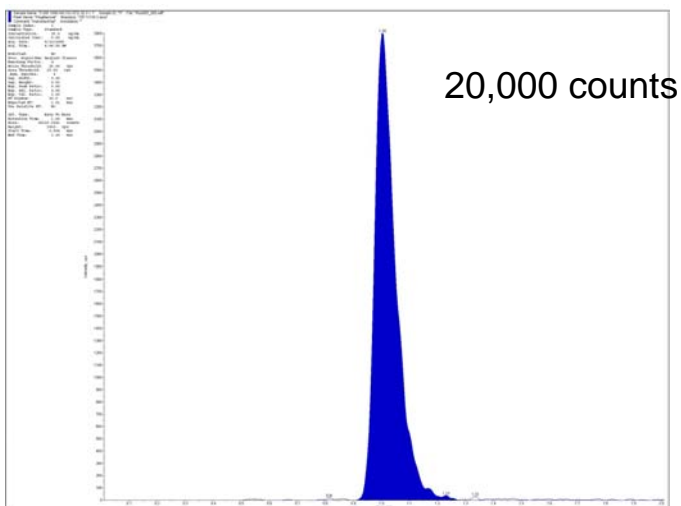
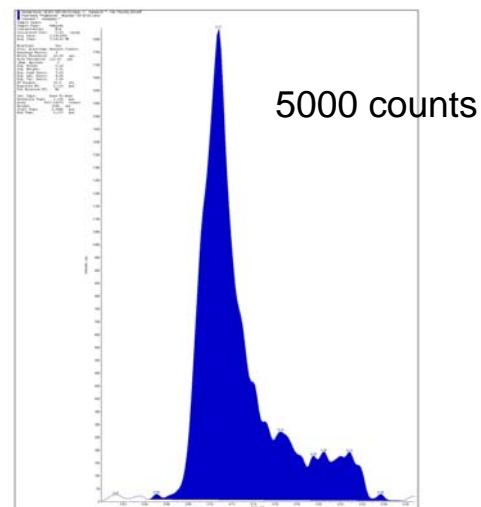
- 100  $\mu\text{L}$  sample + 50  $\mu\text{L}$  of IS + 600  $\mu\text{L}$  of acidified water
- Tomtec automated extraction procedure
- Solid phase extraction with Phenomenex® Strata™ X (30mg/well)
- Pioglitazone-d4 internal standard
- Evaporated samples reconstituted in 250  $\mu\text{L}$  of a water/acetonitrile/acetate buffer mixture
- Method range is 25.0- 2500 ng/mL in human serum

**HPLC**

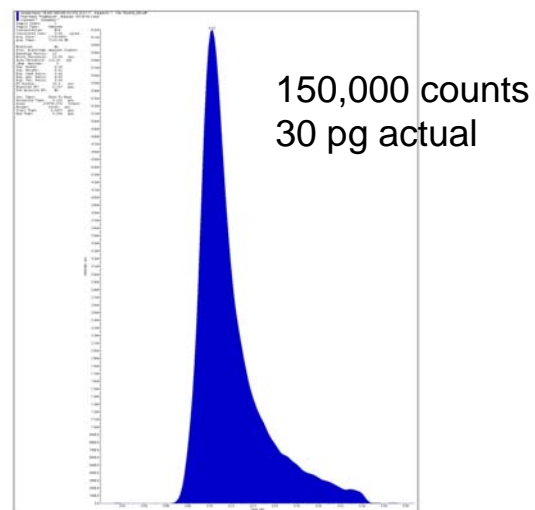


**Matrix Blank**

**LDTD**



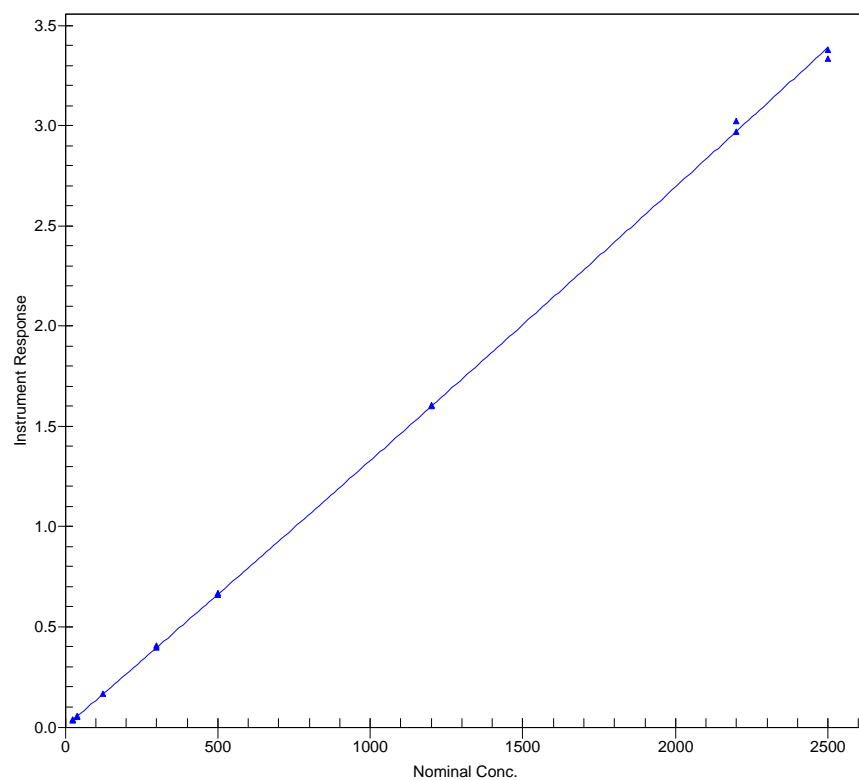
**LLOQ**



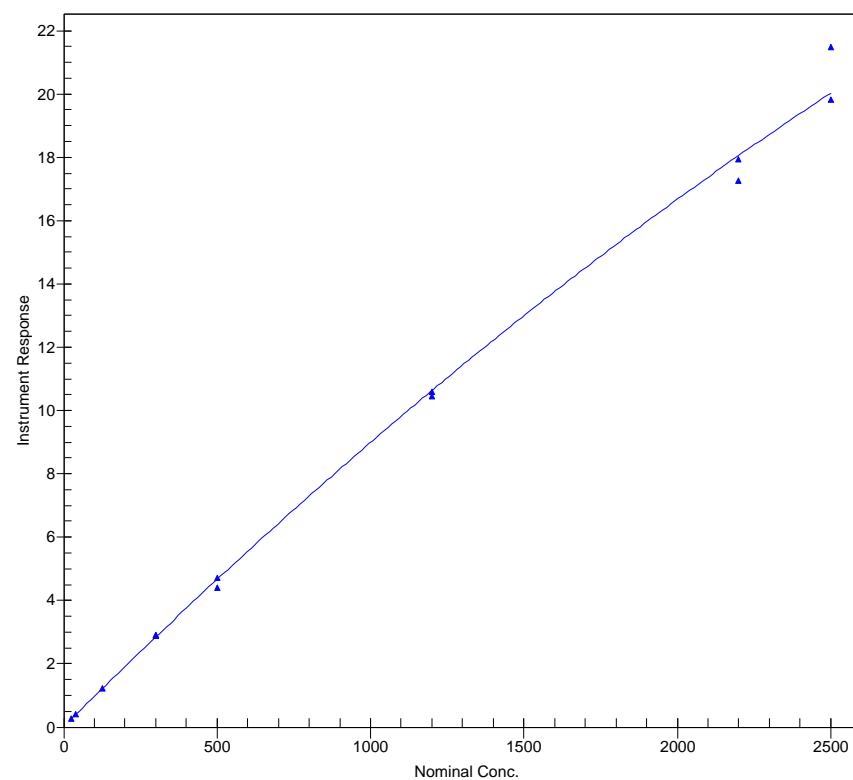
← 2.0 min →

← 0.29 min →

HPLC



LDTD

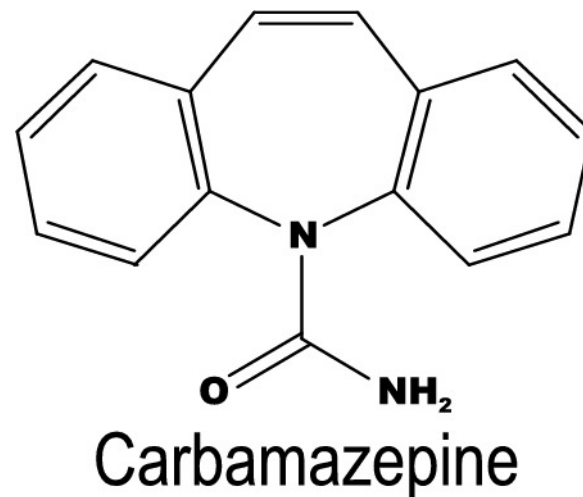


	HPLC Data					LDTD Data				
	25 ng/mL	50 ng/ml	1000 ng/ml	2000 ng/ml		25 ng/mL	50 ng/ml	1000 ng/ml	2000 ng/ml	
16-Aug-08	1.5%	1.4%	1.1%	1.3%	% CV	2.5%	1.7%	3.1%	4.5%	14-Jan-09
	103%	99%	102%	101%	% Nominal	102%	104%	99%	98%	
16-Aug-08	4.0%	3.2%	1.4%	1.2%	% CV	0.7%	1.4%	4.3%	8.9%	14-Jan-09
	107%	99%	102%	102%	% Nominal	102%	105%	96%	98%	
17-Aug-08	2.5%	1.8%	0.9%	0.9%	% CV	0.6%	0.8%	4.0%	4.7%	14-Jan-09
	109%	100%	101%	102%	% Nominal	103%	103%	97%	101%	
20-Aug-08	1.9%	2.6%	1.1%	0.7%	% CV	1.4%	0.8%	3.8%	5.3%	16-Jan-09
	103%	100%	102%	101%	% Nominal	100%	103%	98%	101%	

## Extraction Method

- Sample + internal standard + buffer + ethyl acetate
- Seal, mix, and centrifuge
- Transfer ethyl acetate and blow down
- Reconstitute (60% aqueous, no buffer)
- Split extract
- Dilute LDTD aliquot 1:1 with methanol
- Spot 2  $\mu\text{L}$  onto LazWell plate and allow to evaporate
- Analyze
- Method range is 5 to 5000 ng/mL

Compound	Precursor ion (Q1 m/z)	Product ion (Q3 m/z)
Carbamazepine	237.1	194.1
2-Hydroxy carbamazepine	253.1	209.8
Carbamazepine 10,11-epoxide	253.1	179.6
10,11-Dihydro-10-hydroxycarbamazepine	255.1	194.1
rac trans-10,11-Dihydro-10,11-dihydroxy carbamazepine	271.1	180
Carbamazepine-d10	247.1	204
Carbamazepine-10,11-epoxide-d10(rings-d10)	263.1	189.8



Compound	Precursor ion (Q1 m/z)	Product ion (Q3 m/z)	
<b>Carbamazepine</b>	<b>237.1</b>	<b>194.1</b>	
2-Hydroxy carbamazepine	253.1	209.8	<b>Isobaric with common product ions</b>
Carbamazepine 10,11-epoxide	253.1	179.6	
<b>10,11-Dihydro-10-hydroxycarbamazepine</b>	<b>255.1</b>	<b>194.1</b>	
<b>rac trans-10,11-Dihydro-10,11-dihydroxy carbamazepine</b>	<b>271.1</b>	<b>180</b>	
<b>Carbamazepine-d10</b>	<b>247.1</b>	<b>204</b>	
Carbamazepine-10,11-epoxide-d10(rings-d10)	263.1	189.8	

## Carbamazepine metabolites

### HPLC: 10,11-Dihydro-10-hydroxycarbamazepine

	(DFAC=50)				
Nominal	5	15	400	3800	20000
%CV	8.4	3.5	2.2	2.1	2
%Nominal	99.7	91.6	97.8	102	98.7

### LDTD: 10,11-Dihydro-10-hydroxycarbamazepine

	(DFAC=50)				
Nominal	5	15	400	3800	20000
%CV	9.7	8.4	5.7	2.3	4.4
%Nominal	78.3	92.7	101.8	94.3	97.2

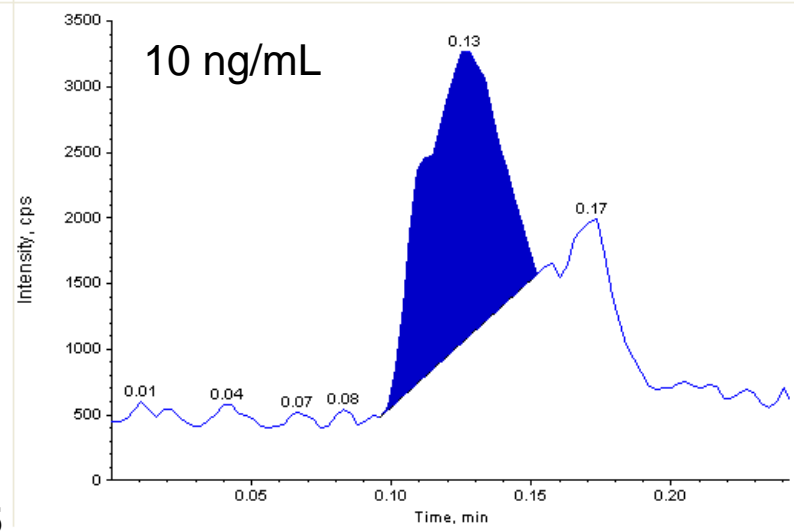
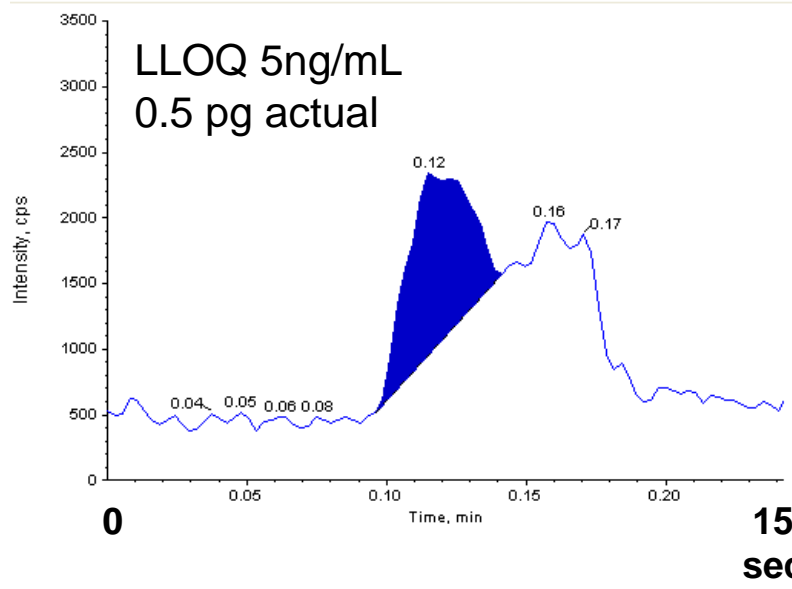
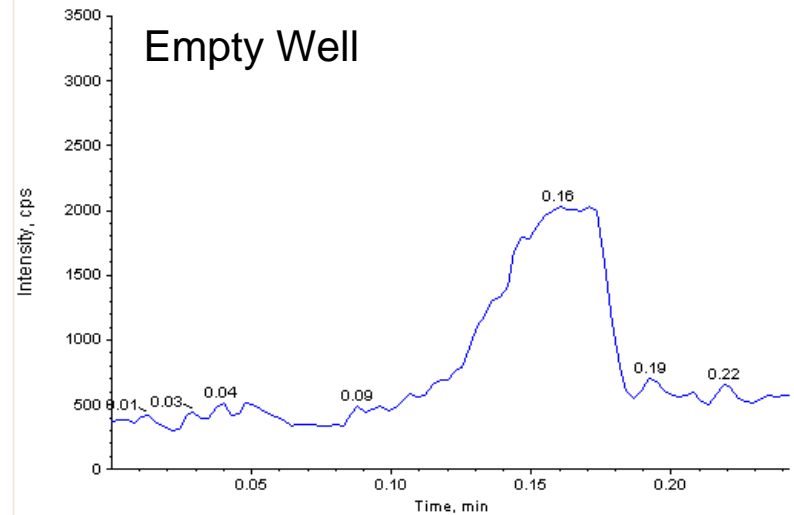
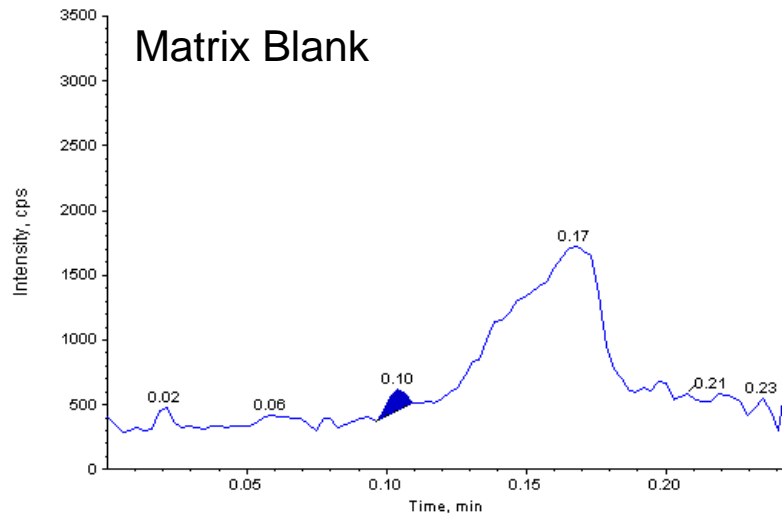
### HPLC: rac trans-10,11-Dihydro-10,11-dihydroxy carbamazepine

	(DFAC=50)				
Nominal	5	15	400	3800	20000
%CV	7.2	4.3	2.7	2	4.7
%Nominal	104.5	91.2	93.8	102.2	95.3

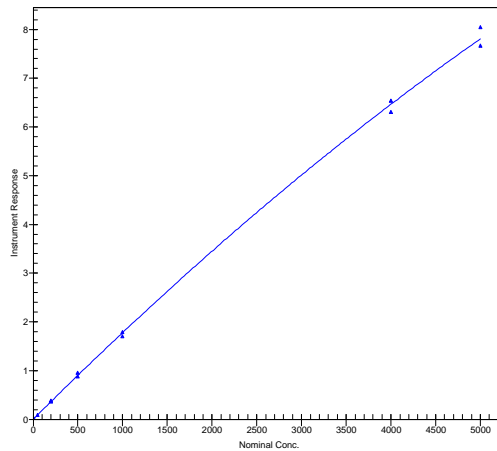
### LDTD: rac trans-10,11-Dihydro-10,11-dihydroxy carbamazepine

	(DFAC=50)				
Nominal	5	15	400	3800	20000
%CV	17.7	15.7	19.5	10.9	12
%Nominal	137.5	87.6	91	99.5	87

Compound	Precursor ion (Q1 m/z)	Product ion (Q3 m/z)	
<b>Carbamazepine</b>	<b>237.1</b>	<b>194.1</b>	
2-Hydroxy carbamazepine	253.1	209.8	
Carbamazepine 10,11-epoxide	253.1	179.6	<b>Isobaric with common product ions</b>
10,11-Dihydro-10-hydroxycarbamazepine	255.1	194.1	<b>Executive decision</b>
rac trans-10,11-Dihydro-10,11-dihydroxy carbamazepine	271.1	180	<b>ISTD does not track well</b>
<b>Carbamazepine-d10</b>	<b>247.1</b>	<b>204</b>	
Carbamazepine-10,11-epoxide-d10(rings-d10)	263.1	189.8	

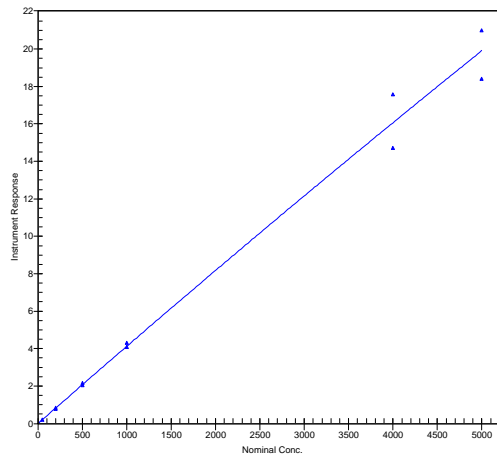


**HPLC Data**



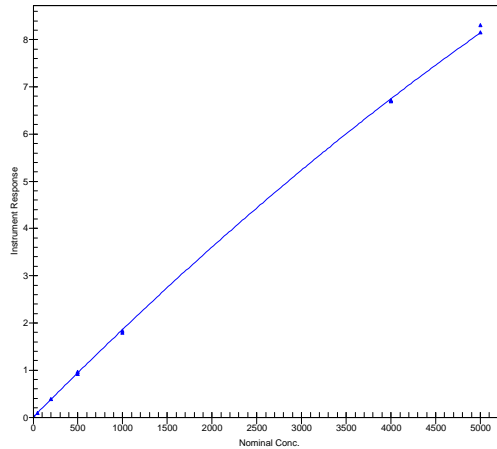
	5	15	BSPA 400	400	3800
<b>Nominal</b>	5	15	400	400	3800
<b>Mean</b>	4.7	14.1	404	393	3795
<b>S.D.</b>	0.211	0.417	6.906	2.559	53.653
<b>%CV</b>	4.5	3	1.7	0.7	1.4
<b>%Nominal</b>	94.5	94.1	100.9	98.3	99.9
<b>n</b>	6	6	6	6	6

**LDTD Data**



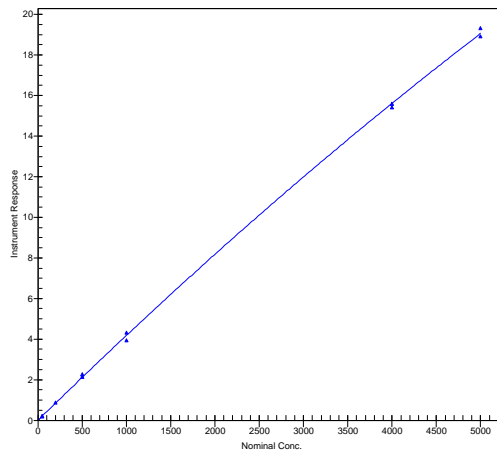
	5	15	BSPA 400	400	3800
<b>Nominal</b>	5	15	400	400	3800
<b>Mean</b>	4.3	13.4	406	391	3572
<b>S.D.</b>	0.985	1.461	8.718	13.708	112.97
<b>%CV</b>	23.2	10.9	2.1	3.5	3.2
<b>%Nominal</b>	85	89.2	101.6	97.7	94
<b>n</b>	6	6	6	6	6

**HPLC Data**



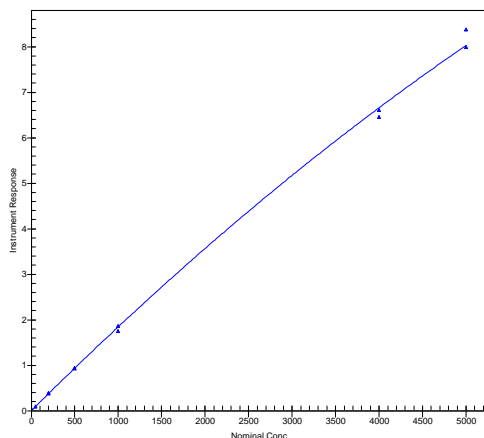
<b>Nominal</b>	<b>5</b>	<b>15</b>	<b>400</b>	<b>3800</b>
<b>Mean</b>	<b>5.4</b>	<b>14.0</b>	<b>391</b>	<b>3700</b>
<b>S.D.</b>	<b>0.606</b>	<b>0.485</b>	<b>2.163</b>	<b>70.474</b>
<b>%CV</b>	<b>11.2</b>	<b>3.5</b>	<b>0.6</b>	<b>1.9</b>
<b>%Nominal</b>	<b>107.8</b>	<b>93.2</b>	<b>97.7</b>	<b>97.4</b>
<b>n</b>	<b>6</b>	<b>6</b>	<b>6</b>	<b>6</b>

**LDTD Data**



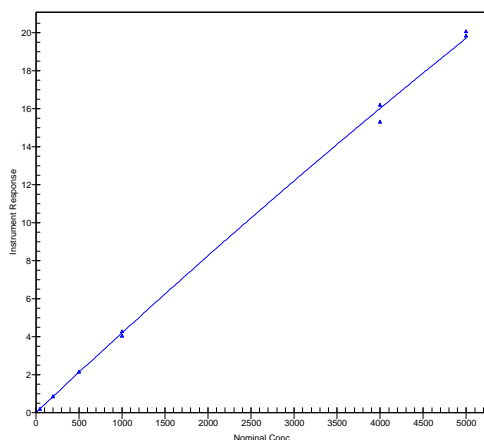
<b>Nominal</b>	<b>5</b>	<b>15</b>	<b>400</b>	<b>3800</b>
<b>Mean</b>	<b>4.4</b>	<b>13.8</b>	<b>412</b>	<b>3828</b>
<b>S.D.</b>	<b>0.693</b>	<b>0.677</b>	<b>12.757</b>	<b>109.41</b>
<b>%CV</b>	<b>15.6</b>	<b>4.9</b>	<b>3.1</b>	<b>2.9</b>
<b>%Nominal</b>	<b>88.8</b>	<b>91.8</b>	<b>103.1</b>	<b>100.7</b>
<b>n</b>	<b>6</b>	<b>6</b>	<b>6</b>	<b>6</b>

### HPLC Data



	5	FT 15	RT 15	15	400	FT 3800	RT 3800	3800
<b>Nominal</b>	5	15	15	15	400	3800	3800	3800
<b>Mean</b>	5.0	13.9	14.3	14.0	397	3794	3704	3808
<b>S.D.</b>	0.418	0.348	0.379	0.542	6.528	31.930	131.79	44.906
<b>%CV</b>	8.4	2.5	2.6	3.9	1.6	0.8	3.6	1.2
<b>%Nominal</b>	99.9	92.8	95.5	93.7	99.3	99.9	97.5	100.2
<b>n</b>	6	6	6	6	6	6	6	6

### LDTD Data



	5	FT 15	RT 15	15	400	FT 3800	RT 3800	3800
<b>Nominal</b>	5	15	15	15	400	3800	3800	3800
<b>Mean</b>	4.2	13.3	14.1	13.3	392	3703	3432	3605
<b>S.D.</b>	1.479	1.416	0.440	0.680	6.985	199.54	124.69	108.16
<b>%CV</b>	35	10.6	3.1	5.1	1.8	5.4	3.6	3
<b>%Nominal</b>	84.6	88.9	94	88.6	98	97.4	90.3	94.9
<b>n</b>	6	6	6	6	6	6	6	6

## Carbamazepine Conclusions

- Not quite there using HPLC extraction
- Want more signal.
  - Recon with smaller volume, different composition
  - Skipping the evaporation/recon, plate organic directly
  - Better optimize system
- Stable labeled internal standards are good

## Moving Forward

- Investigate the 'peak in the blank'
- MIST Guidance – conjugated metabolites, N-oxides
- Optimize extraction for LDTD
- Generic extraction
- Validate methods for non-proprietary drugs
- Analyze incurred (pre-clinical) samples

Thanks to:

- Patrice Tremblay – Phytronix
- Hasantha Jayaratna
- Michael Pugh
- Tim Shoaf
- Ben Slentz
- Management at BASi