

# Speeding Up Drug Discovery with DART-based Mass Spectrometry

Brian D. Musselman, IonSense, Inc.

Land O Lakes Conference

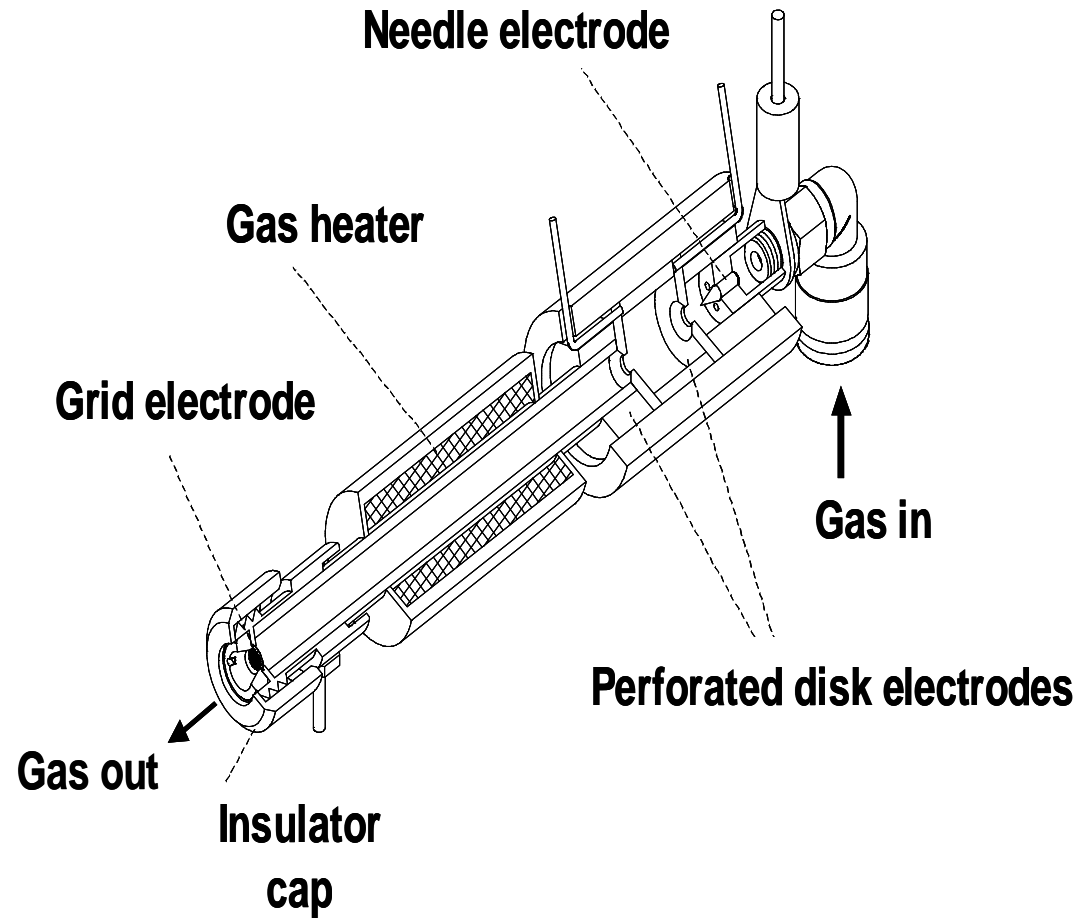
September 2008

# What is DART?

- DART stands for Direct Analysis in Real Time
- Generates ions directly from gases, liquid, and solids by using gas containing heated either metastable atoms or molecules to transfer energy into the sample environment.
- Provides a non-contact surface sampling technique for use in mass spectrometry based analysis
- Samples are presented for analysis in open air at atmospheric pressure in this case by using a robot sample handler.
- Developed by J. Laramée and R. Cody at JEOL USA, Inc.

# DART Schematic

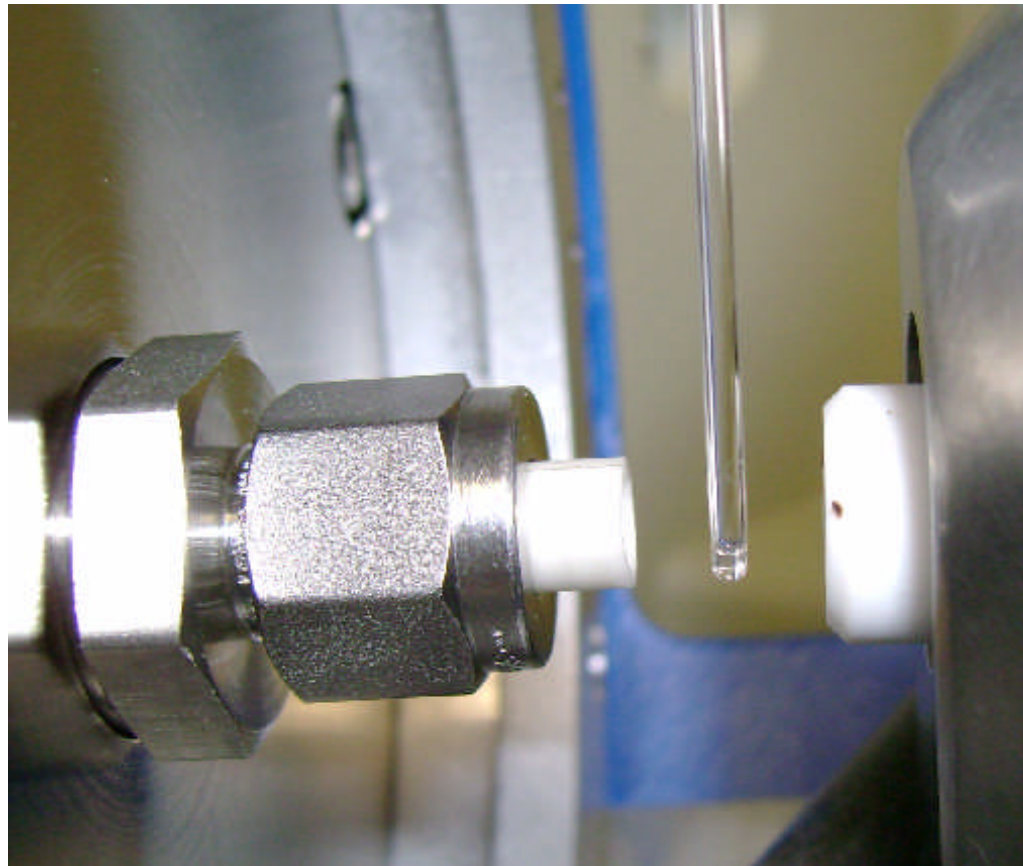
- Gas flows through DART
- Electrical discharge creates a plasma
- Lenses remove charged particles
- Grid prevents ion-ion recombination at exit, and other functions
- No exposed high voltages
- Operated at ambient pressure in open air



# Experimental Protocol

- Dip into the biological matrix and analyze

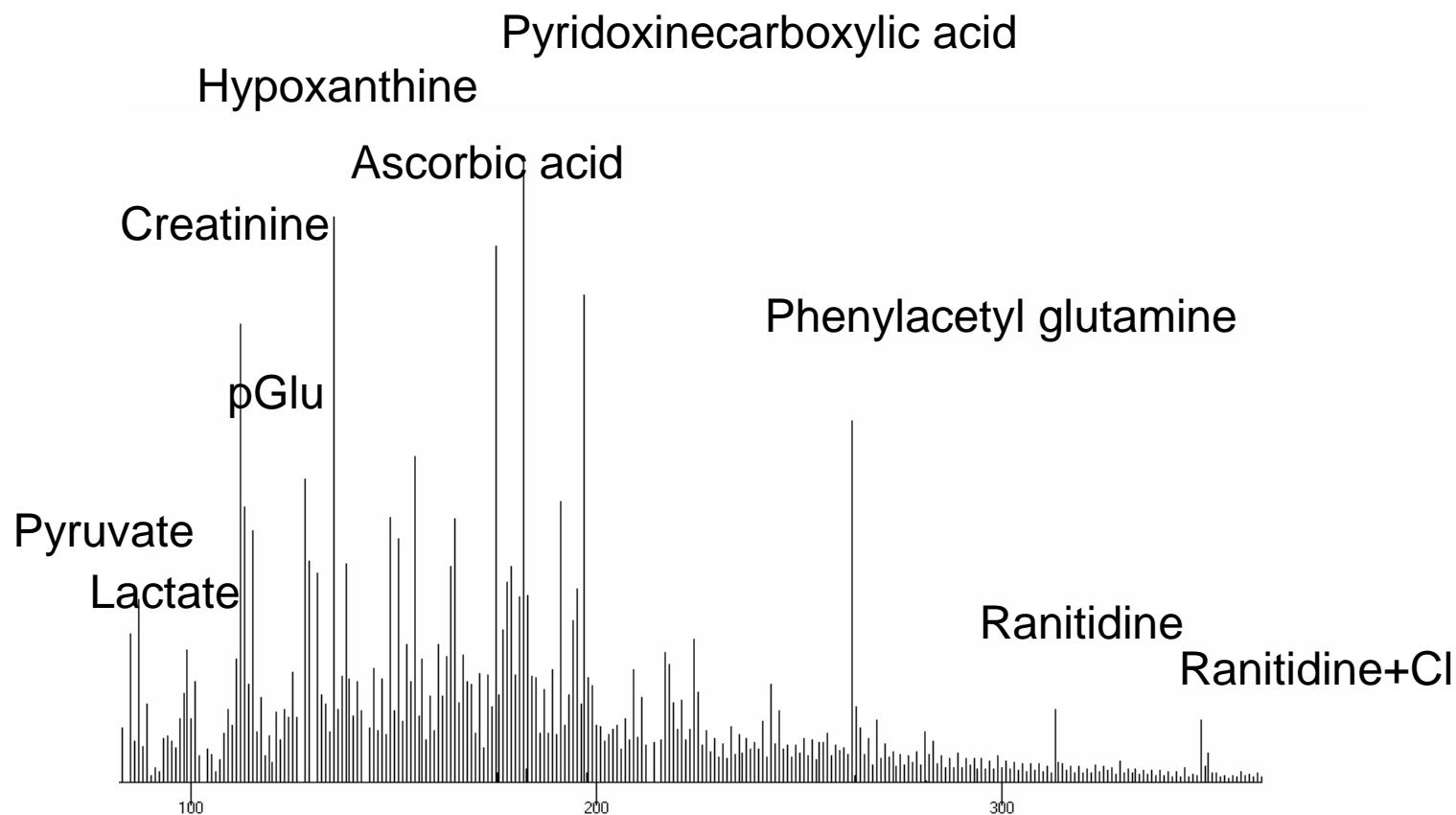
MS



DART  
Source

# DART of Urine

High Resolution Time-of-Flight MS

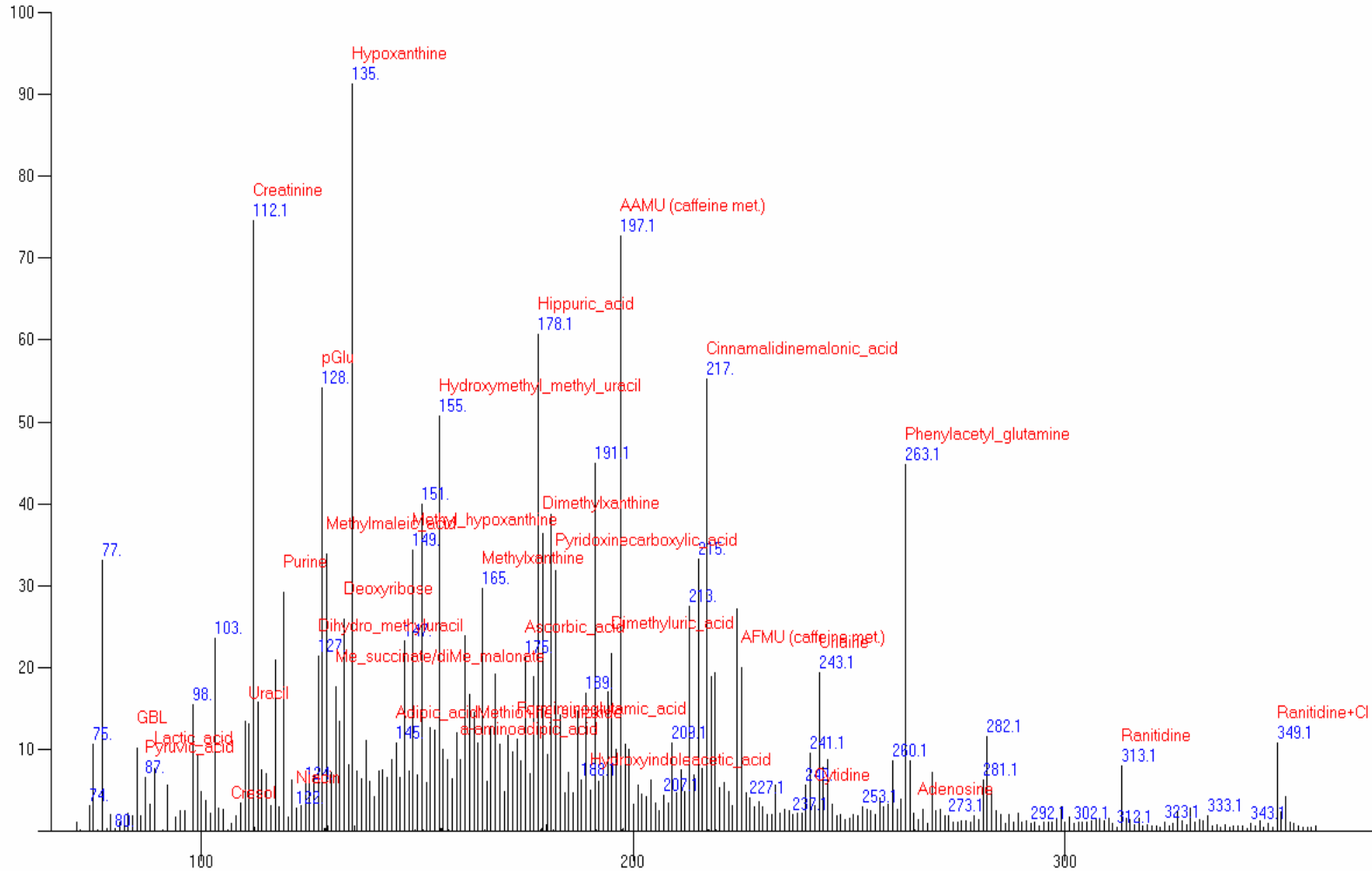


Data courtesy of Dr. Cody, JEOL USA

# Computer Analysis of Spectrum

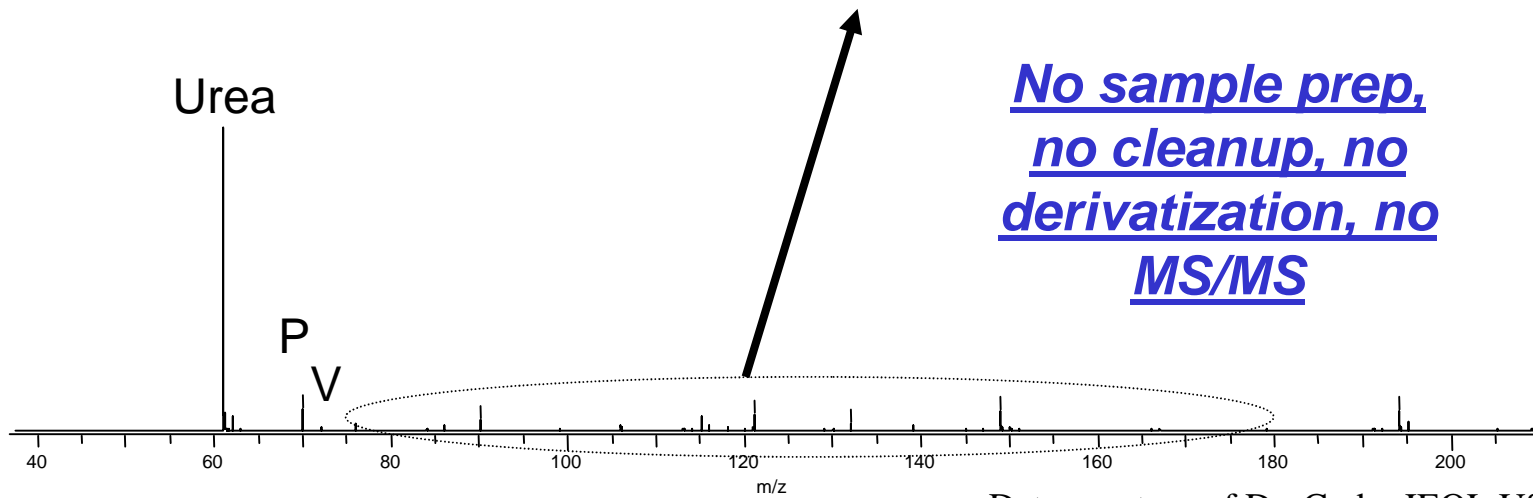
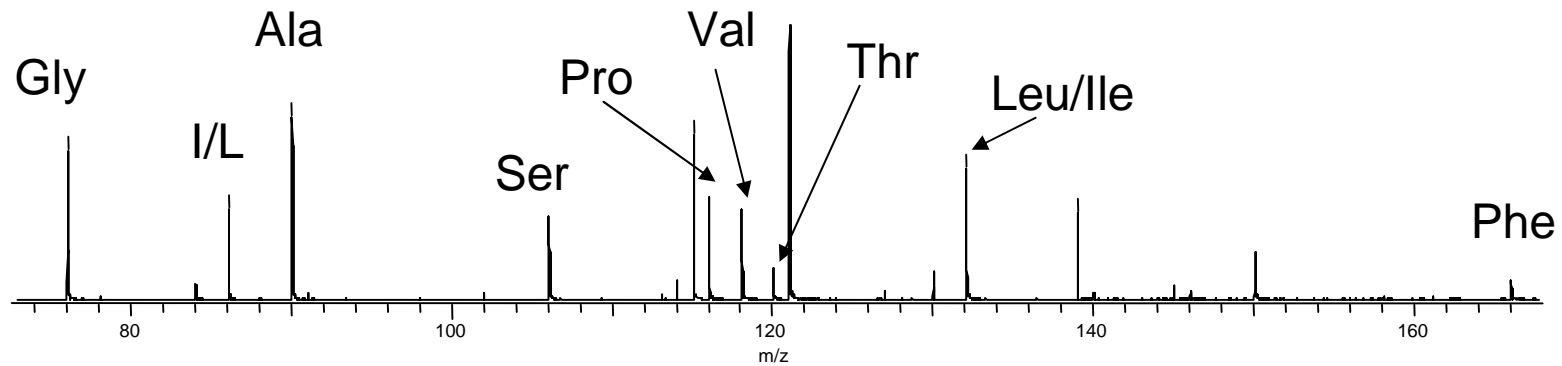
JEOL AccuTOF-MS

Rel. Abund.



Data courtesy of Dr. Cody, JEOL USA

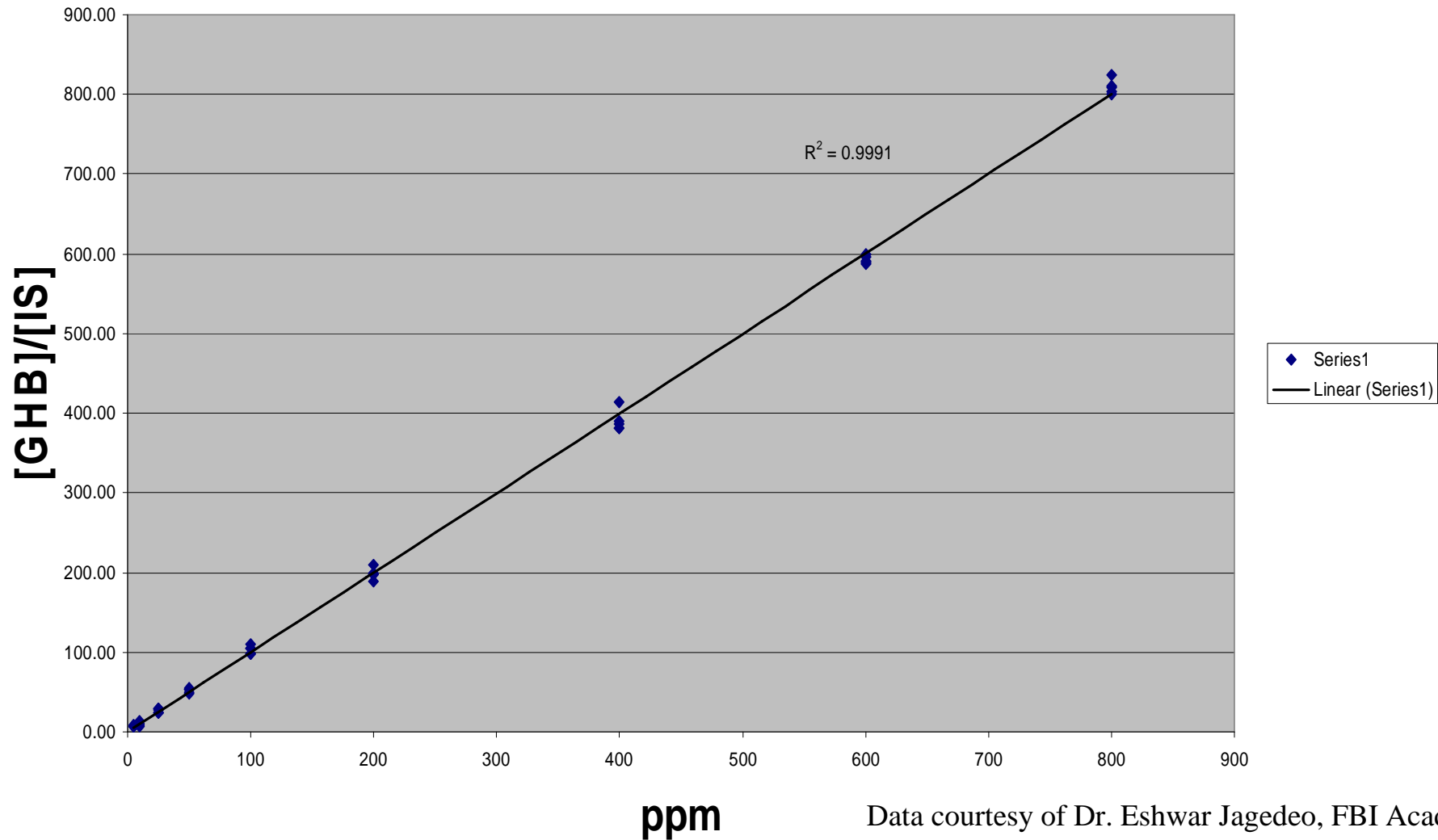
# Blood droplet: Amino Acids (Positive Ions)



**No sample prep,**  
**no cleanup, no**  
**derivatization, no**  
**MS/MS**

Data courtesy of Dr. Cody, JEOL USA

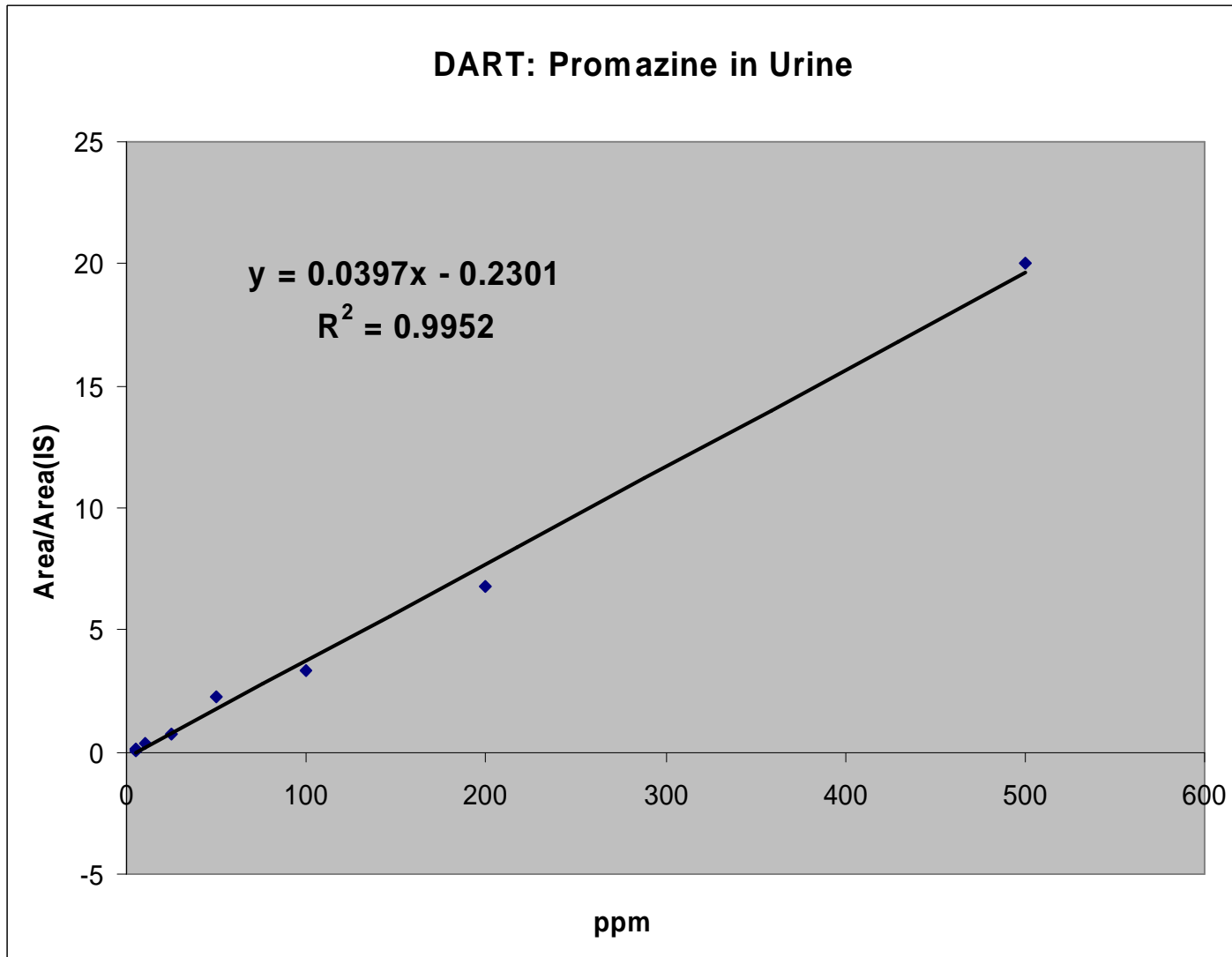
# Gamma Hydroxybutyrate (GHB) in Urine (Deuterated I.S.)



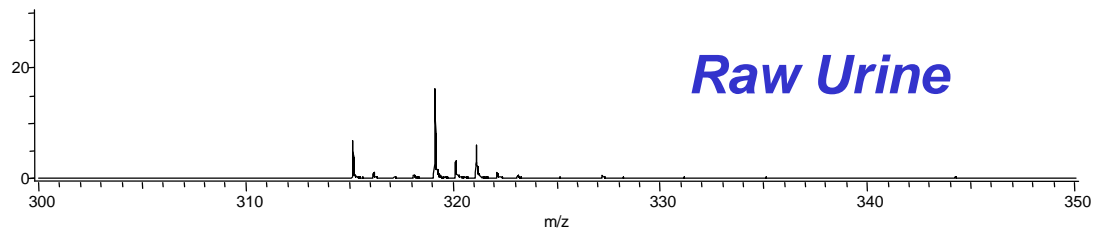
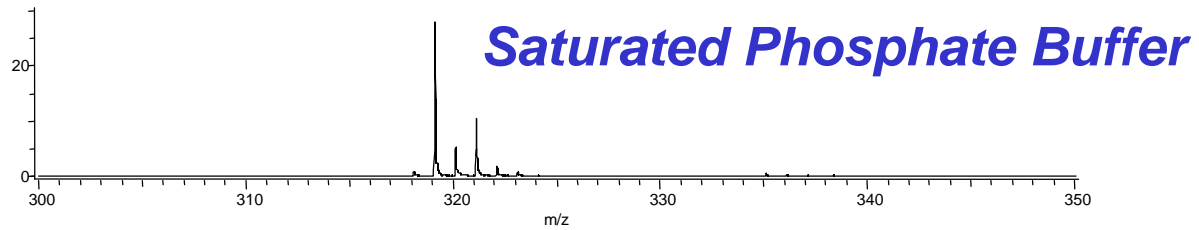
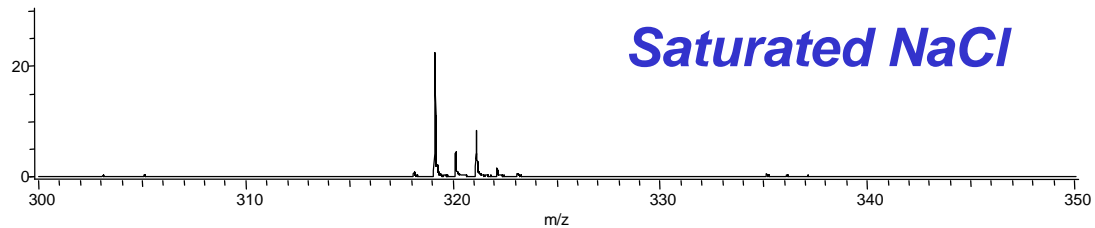
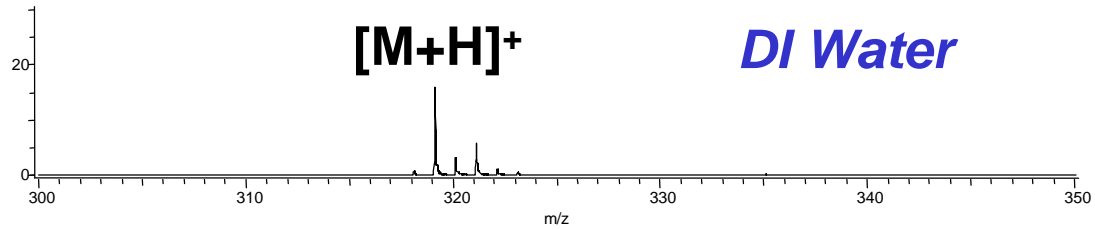
Data courtesy of Dr. Eshwar Jagdeo, FBI Academy

# Promazine in Urine

## Chlorpromazine internal standard



# What makes DART different?



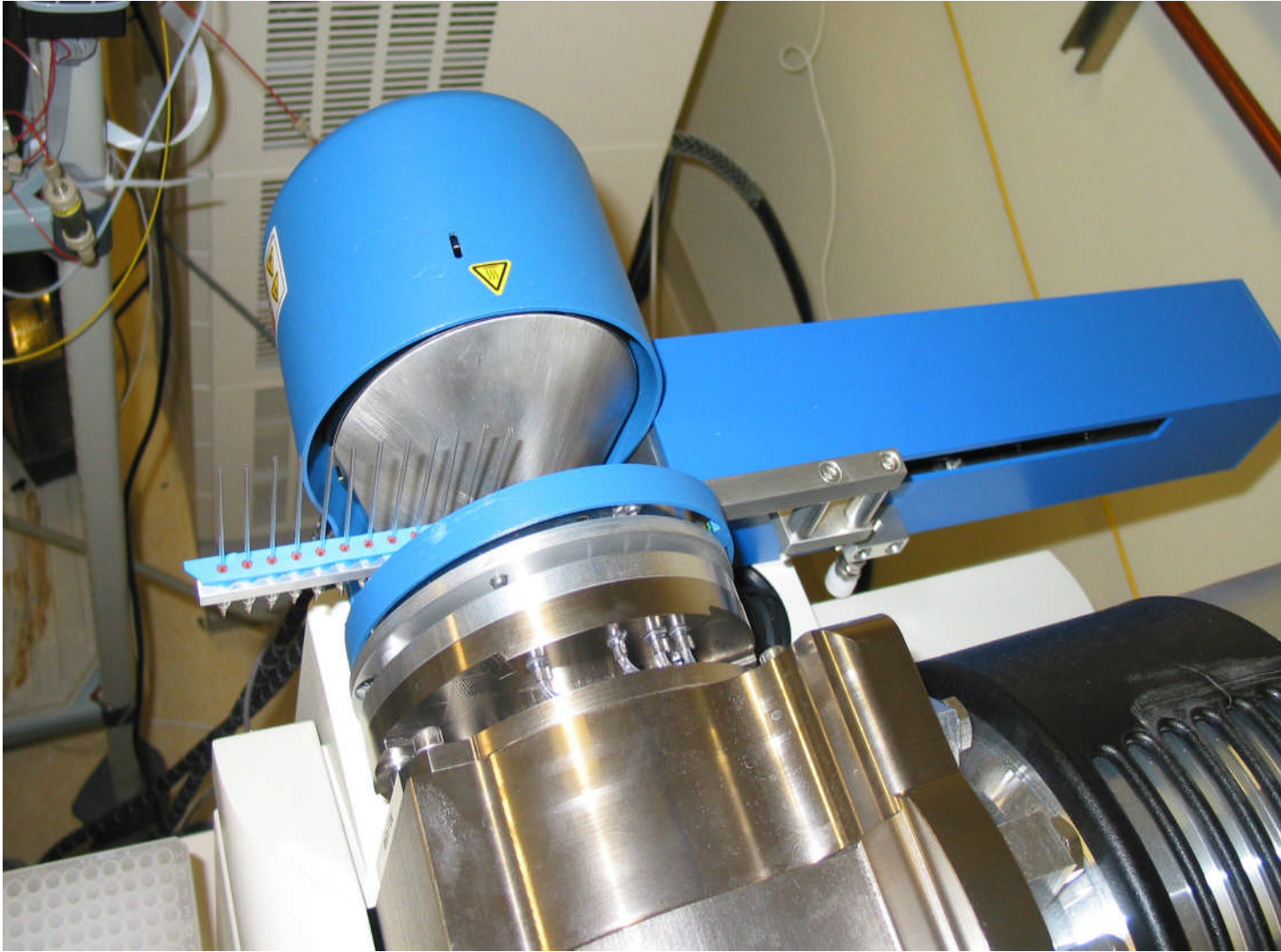
*Chlorpromazine*  
*[M+H]<sup>+</sup>*

*No alkali metal*  
*cation adducts*

*No multiple*  
*charging*

*No apparent*  
*suppression*

# Initial Evaluation Instrument Configuration



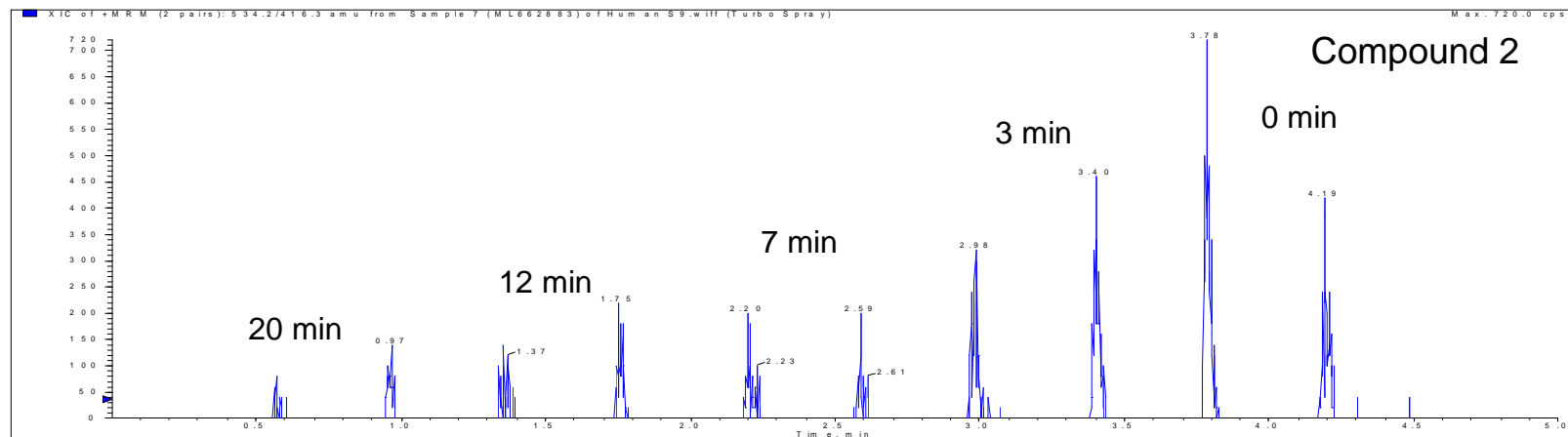
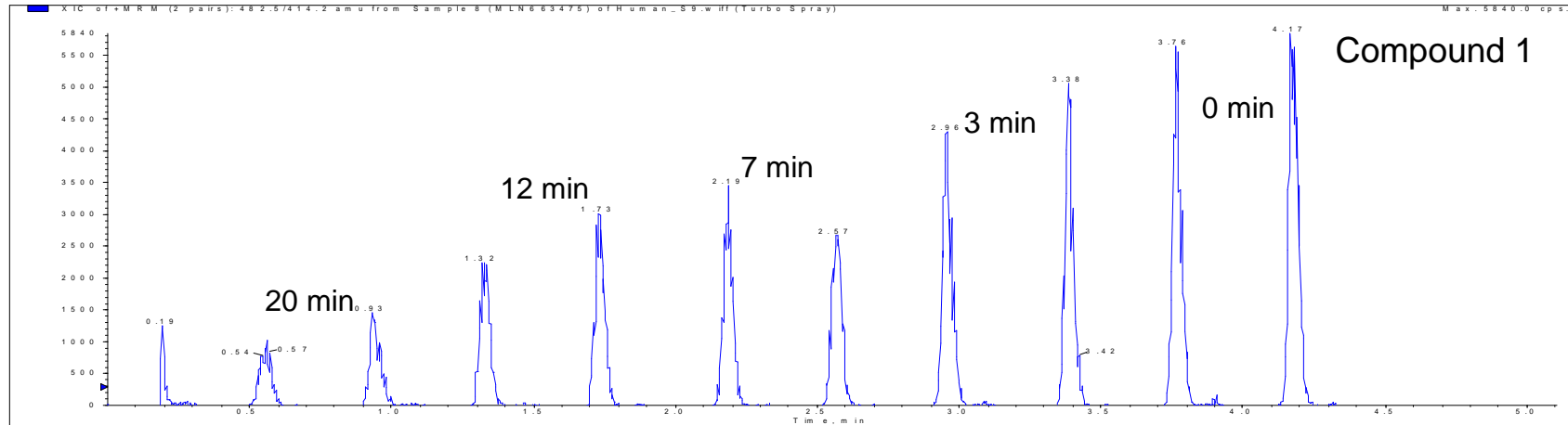
# In Vitro Intrinsic Clearance Samples

Initial Effort – May 07

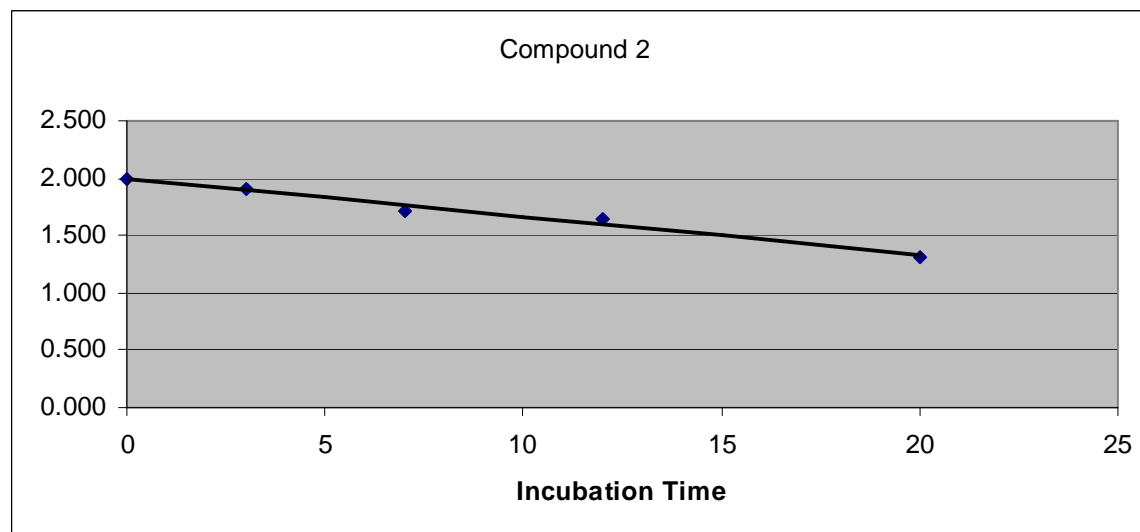
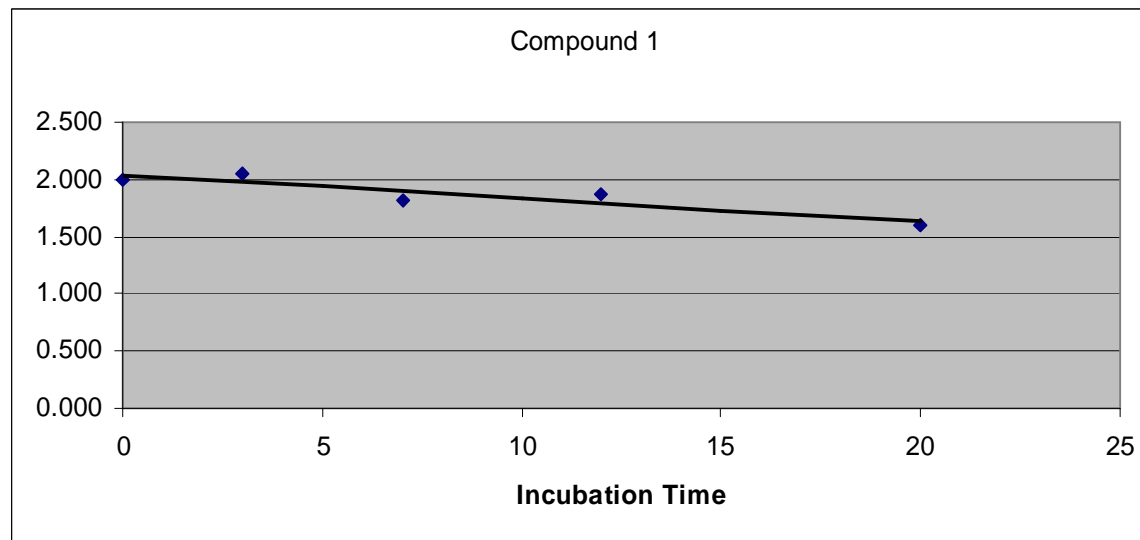
Jing-Tao Wu, Shaoxia Yu, Millennium Pharma

- Experimental
  - Incubate compounds in human liver S9. After quenching, split the samples for LC/MS and DART analysis
  - Compare the intrinsic clearance values obtained by each method for comparison
  - Can a simple protocol be established?

# DART for In Vitro Samples



# DART for In Vitro Samples



# DART for In Vitro Samples

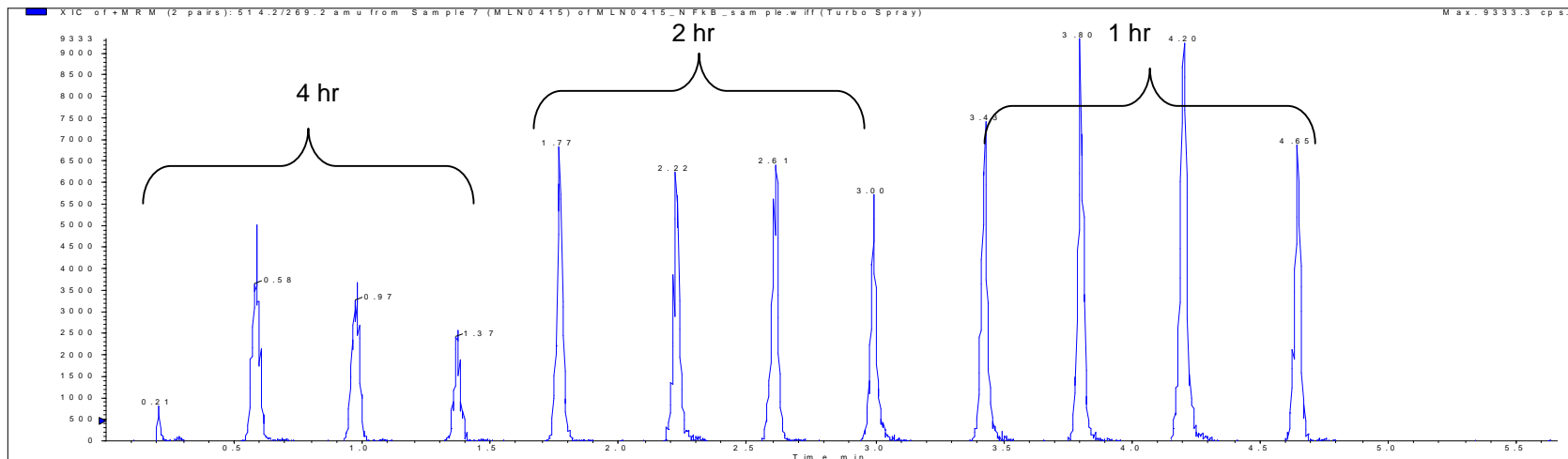
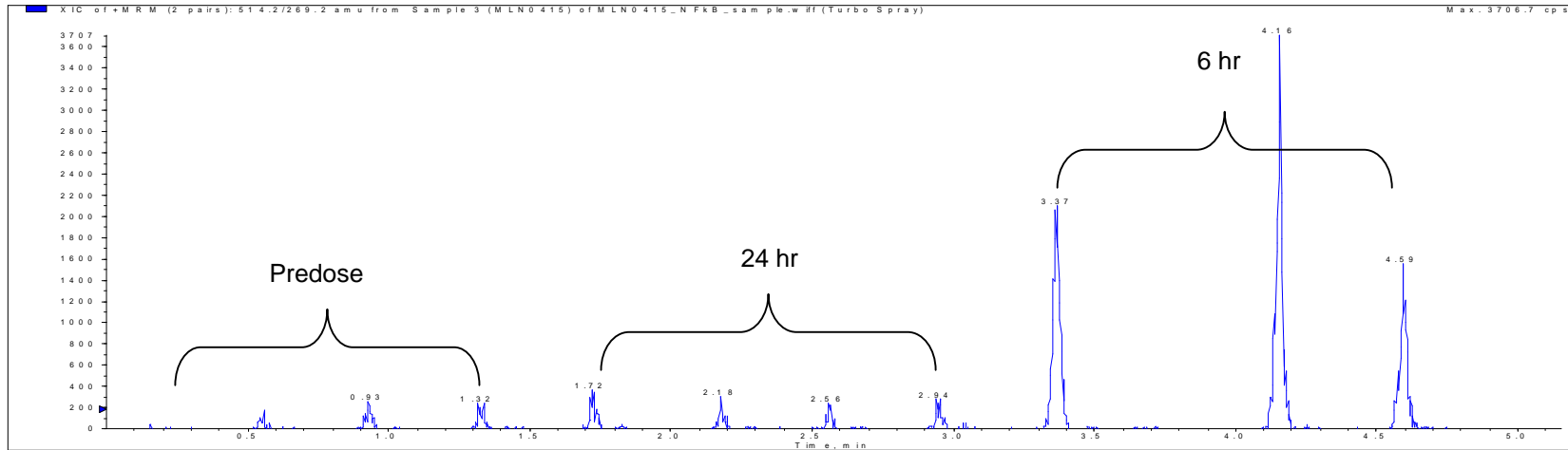
Comparison of LC/MS and DART data

	$CL_{int}$ (L/hr/kg) LC/MS	$CL_{int}$ (L/hr/kg) DART
Compound 1	1.41	2.67
Compound 2	3.17	3.66
Compound 3	1.02	1.68
Compound 4	0.61	2.08

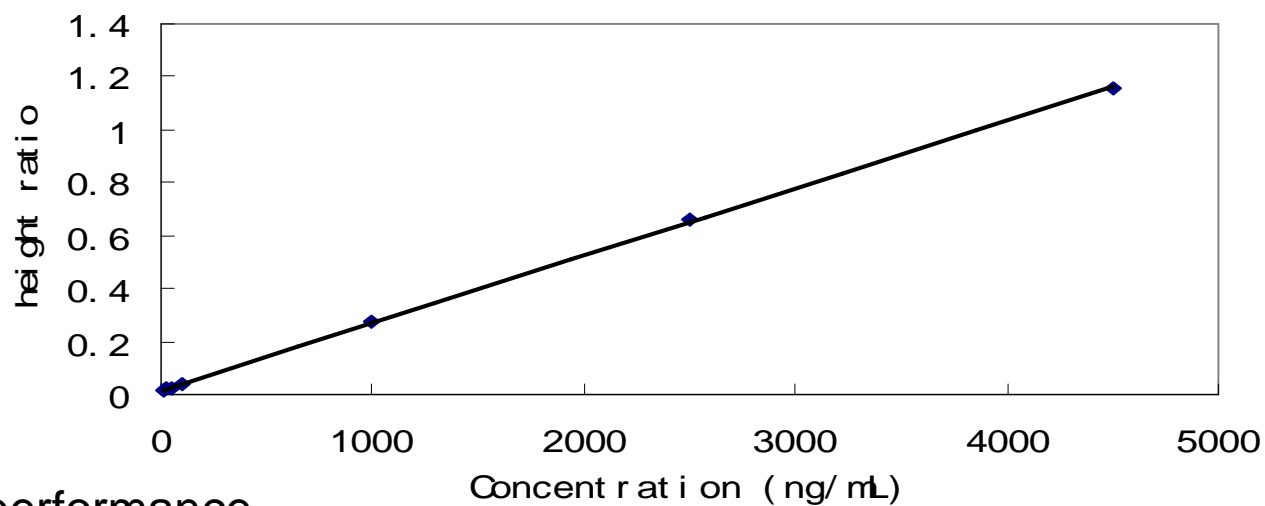
# DART for PK Samples

- Experimental (Compound A)
  - Rat PK study, 4 animals at 10 mg/kg PO
  - Plasma samples were extracted and analyzed by LC/MS
  - Plasma were re-aliquoted and mixed with high aqueous IS before subject to DART analysis
  - Comparison between DART and LC/MS results

# Rat PK Study for Compound A



# Rat PK Study for Compound A



QC performance

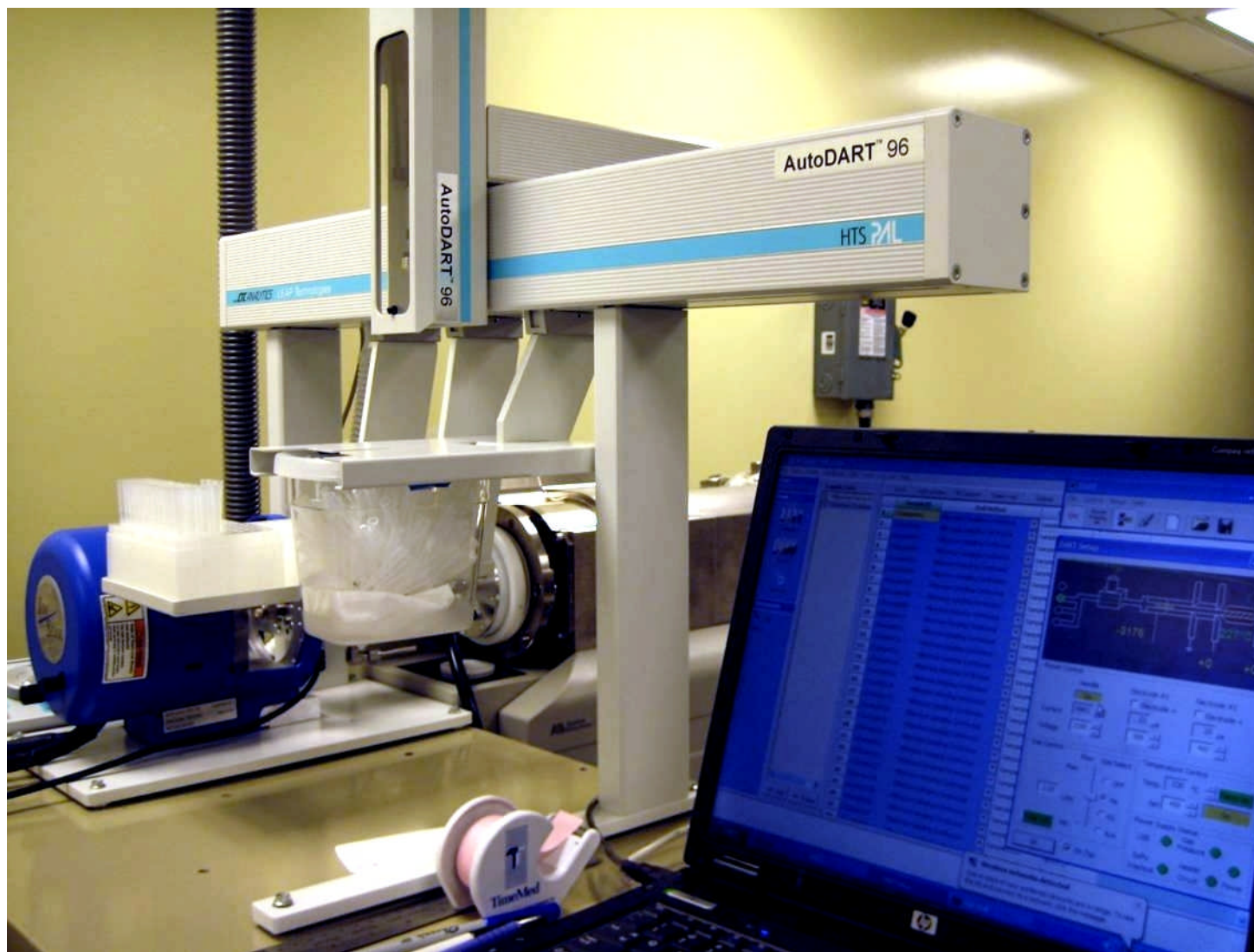
Norminal Concentration (ng/mL)	Calculated Concentration (ng/mL)	Bias%
30	25.4	-15.2
2400	2820	17.4
4000	4670	16.7

# Conclusions 07'

- Evaluation for Bioanalysis indicated that matrix effect were not overwhelming
- Applications for in vivo and in vitro ADME studies suggested that additional effort was necessary to achieve more optimal results
- Improvement in precision and sensitivity required

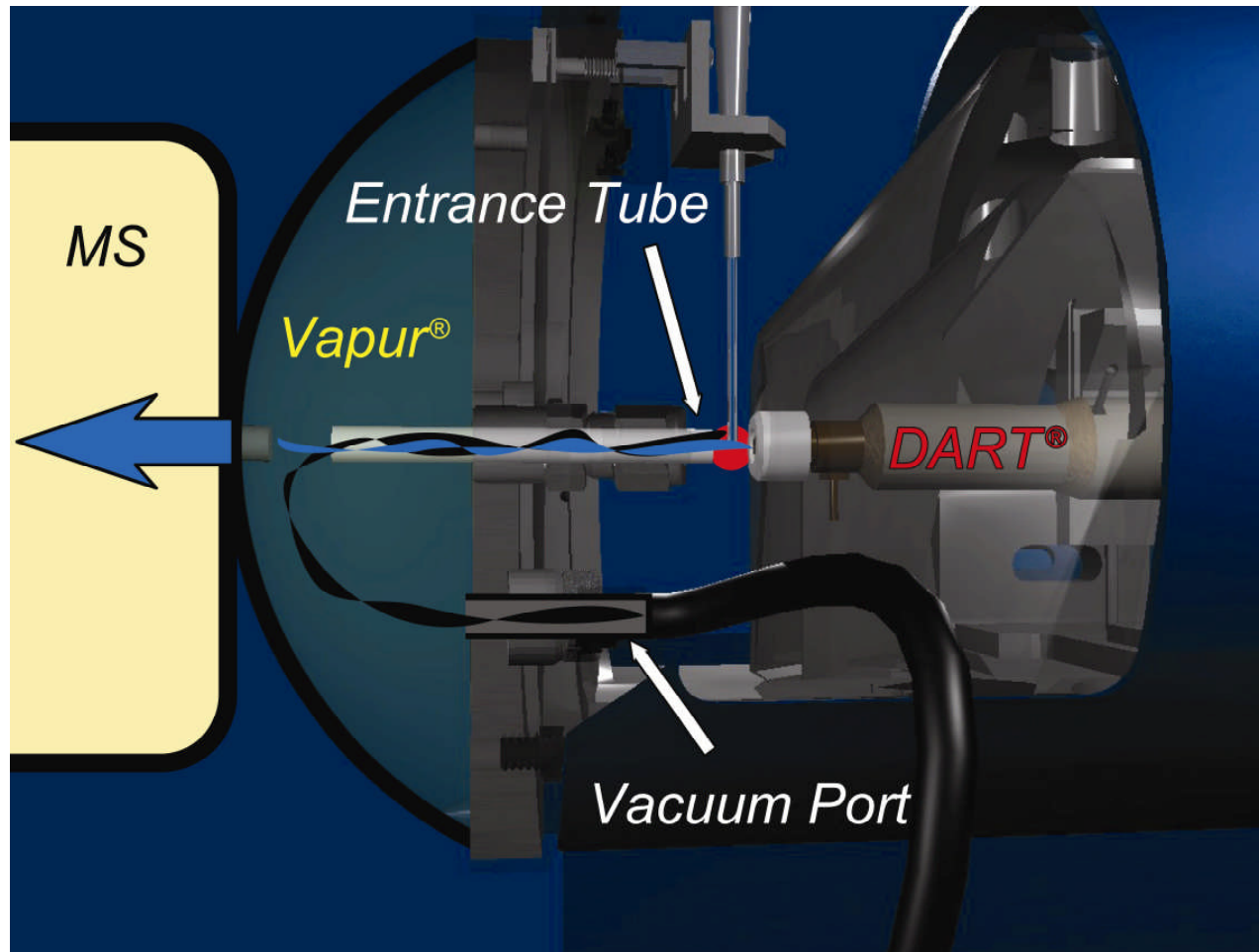
Jing-Tao Wu, Millennium Pharma  
APA Boston 9/2006

# Instrument Configuration – Circa 2008

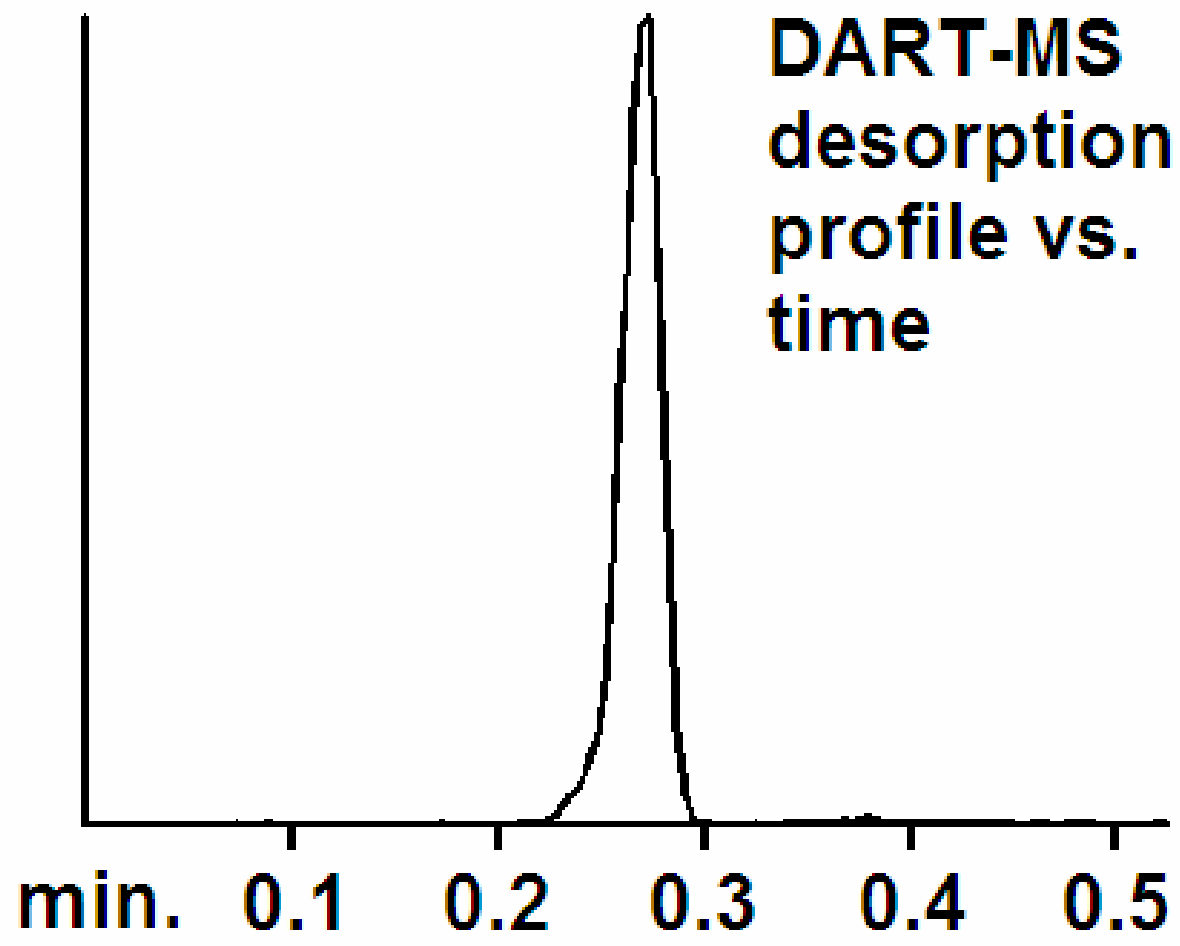


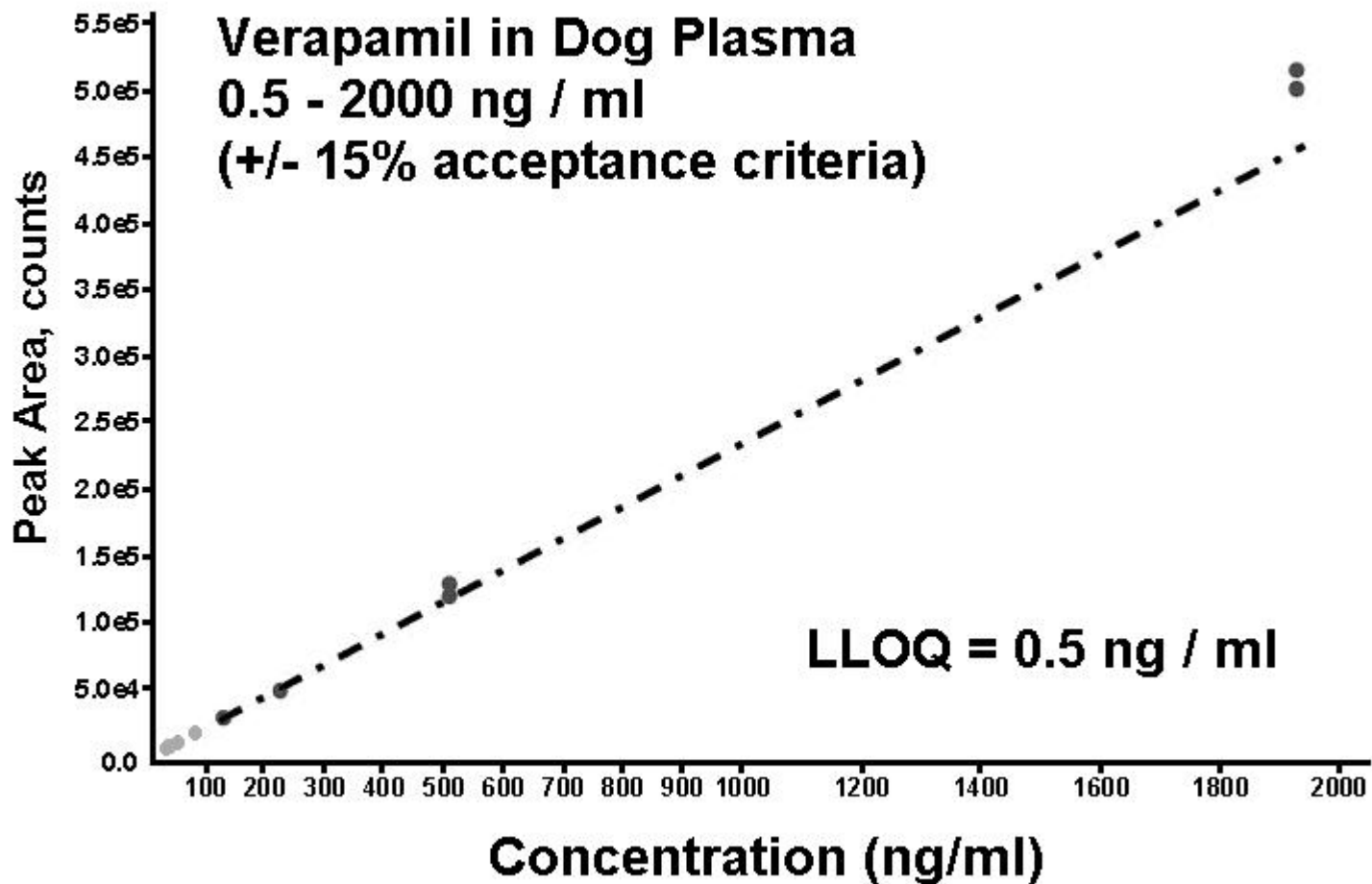
# Gas Ion Separator Developed 2008

MS People are Plumbing People



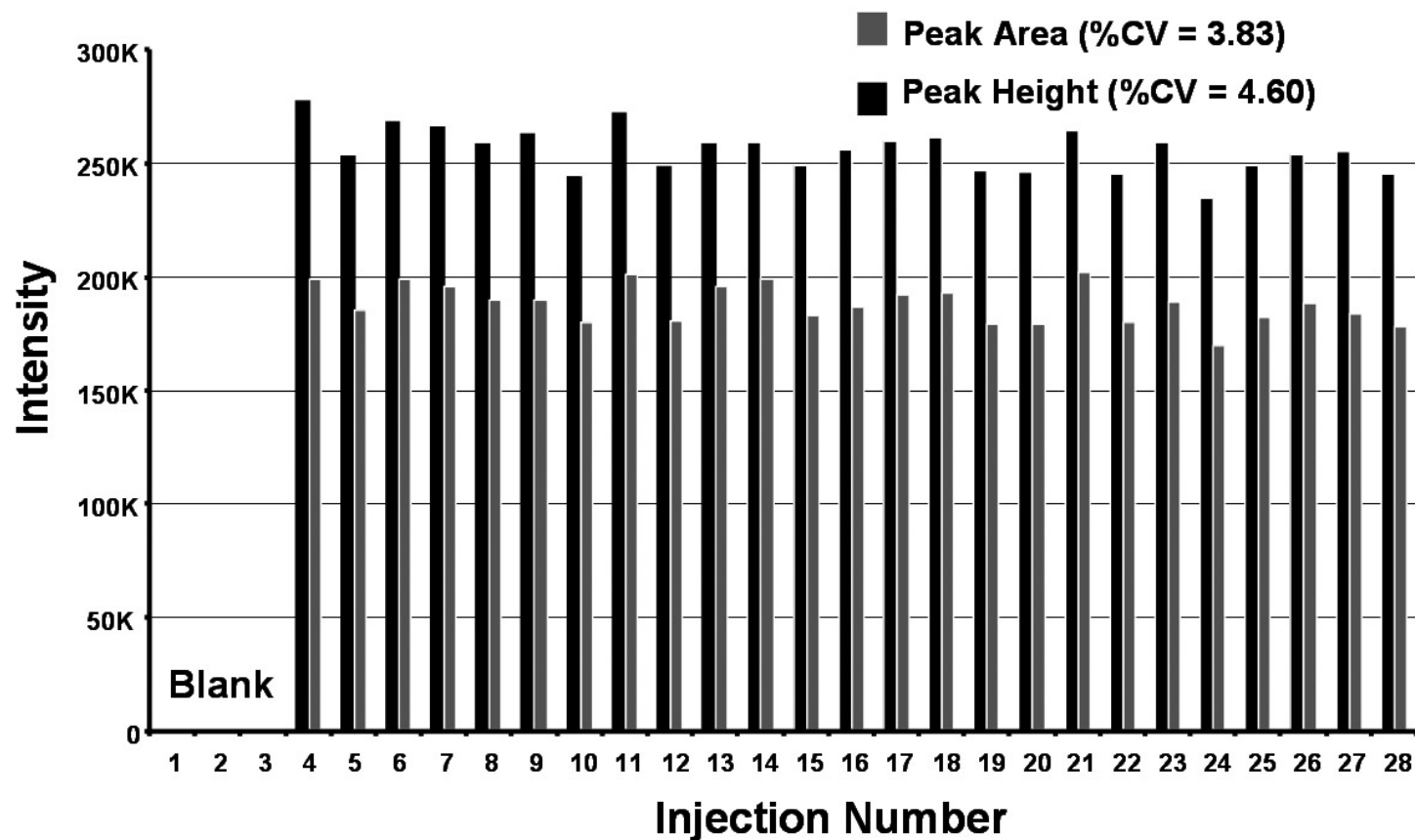
# DART DESORPTION PROFILE





Data courtesy of Dr. Shaoxia Yu, Takeda Pharmaceuticals

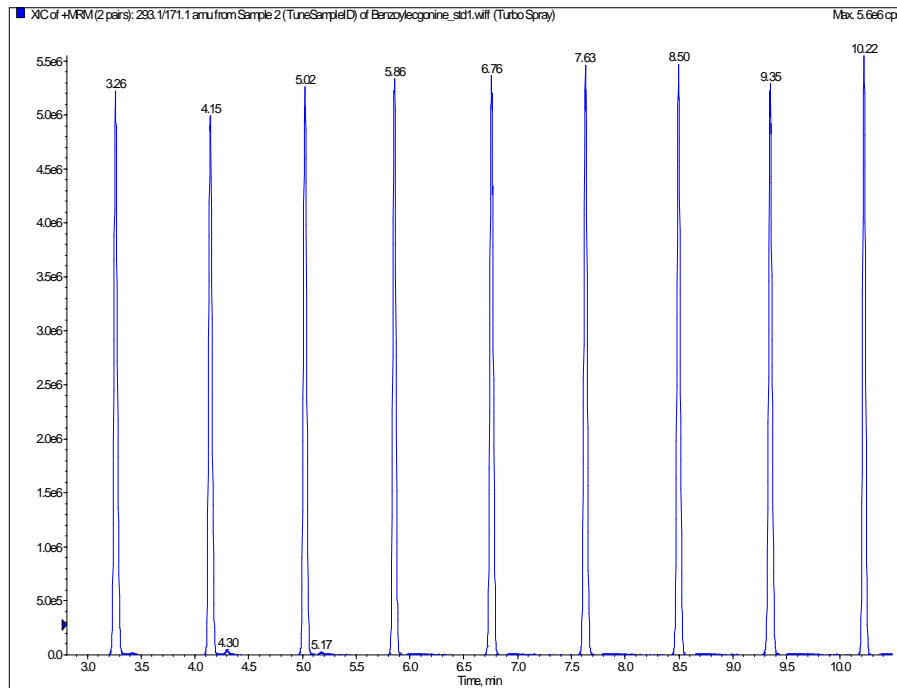
# The System Reproducibility of DART/MS/MS (Verapamil, 1 $\mu$ g/mL in dog plasma, n=25)



Data courtesy of Dr. Shaoxia Yu, Takeda Pharmaceuticals

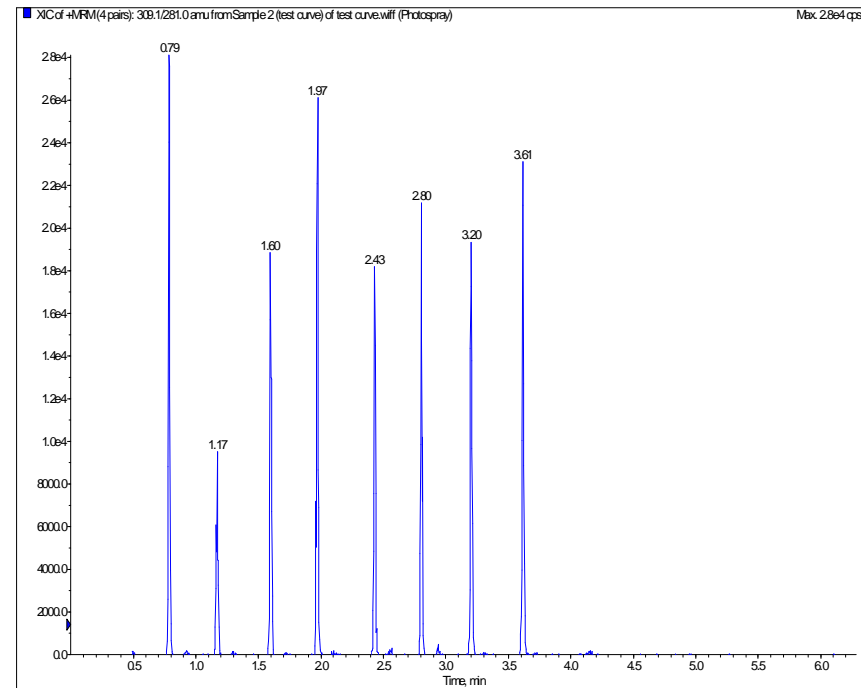
# Improved Reproducibility

Now



N=9, CV=3.1%

Before



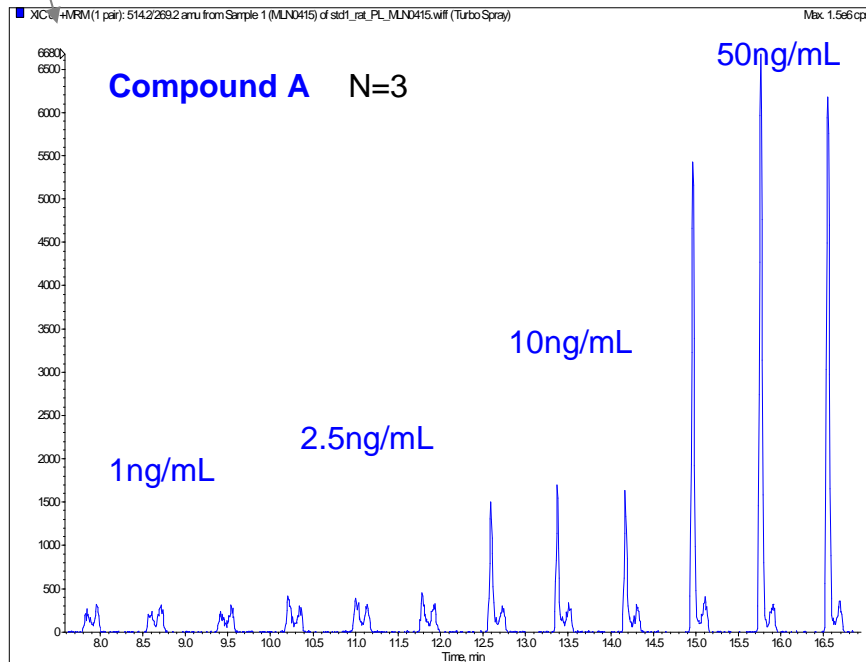
N=8 CV=27.7%

Data courtesy of Dr. Shaoxia Yu, Takeda Pharmaceuticals

# Improved Sensitivity

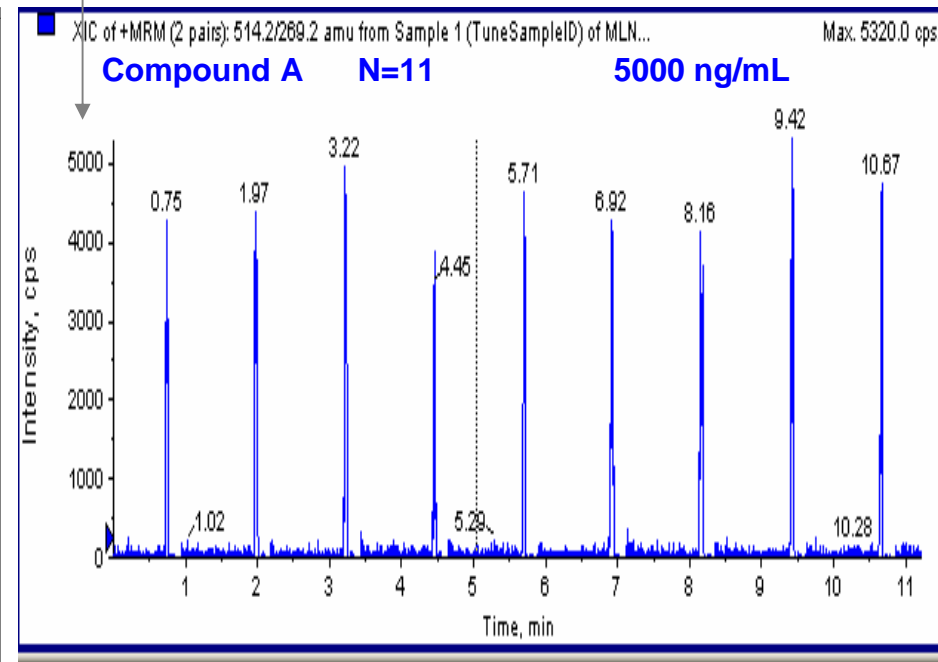
6680

W/GIST



5000

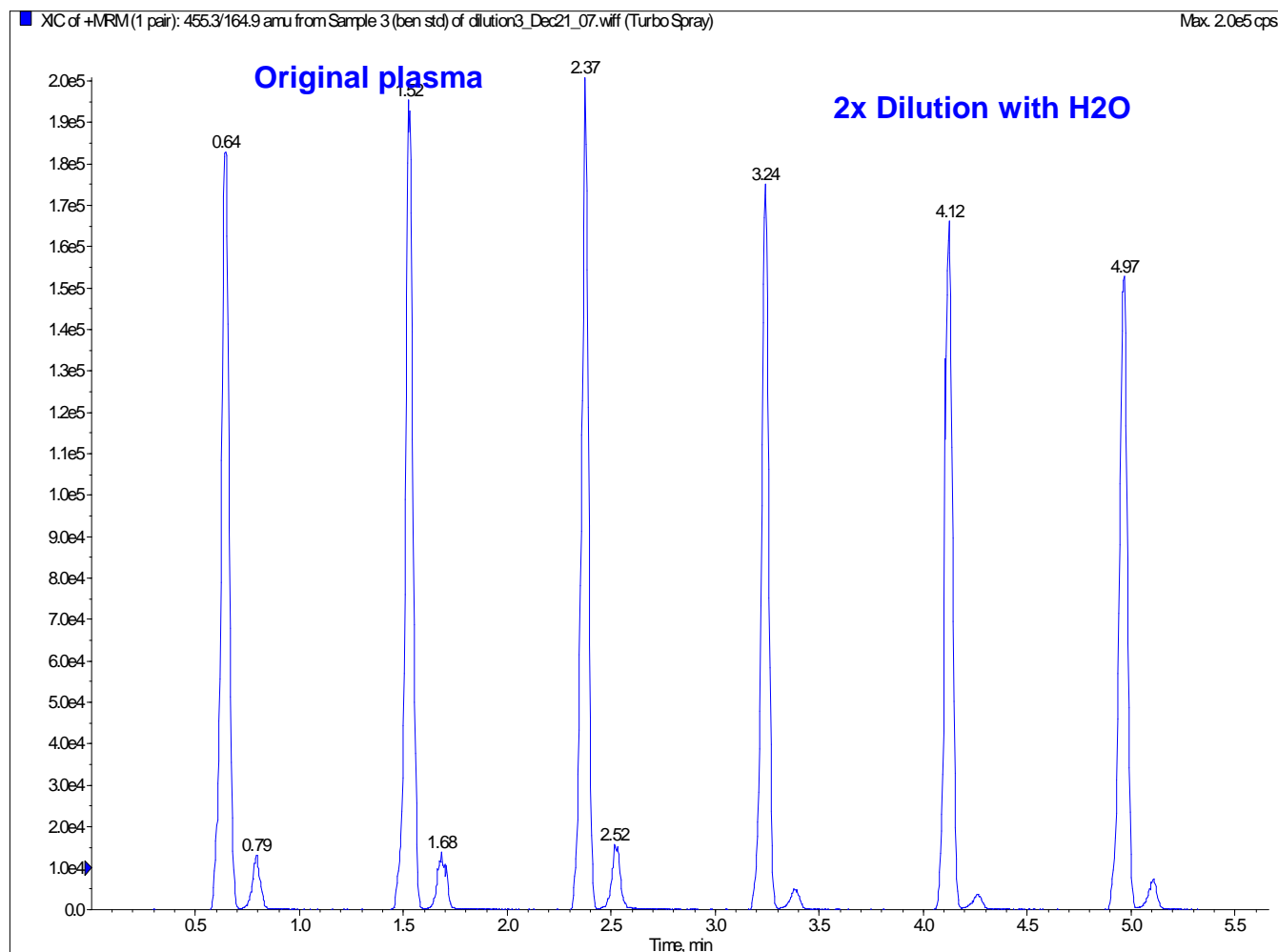
W/out GIST



Data courtesy of Dr. Shaoxia Yu, Takeda Pharmaceuticals

Reevaluate for Bioanalysis

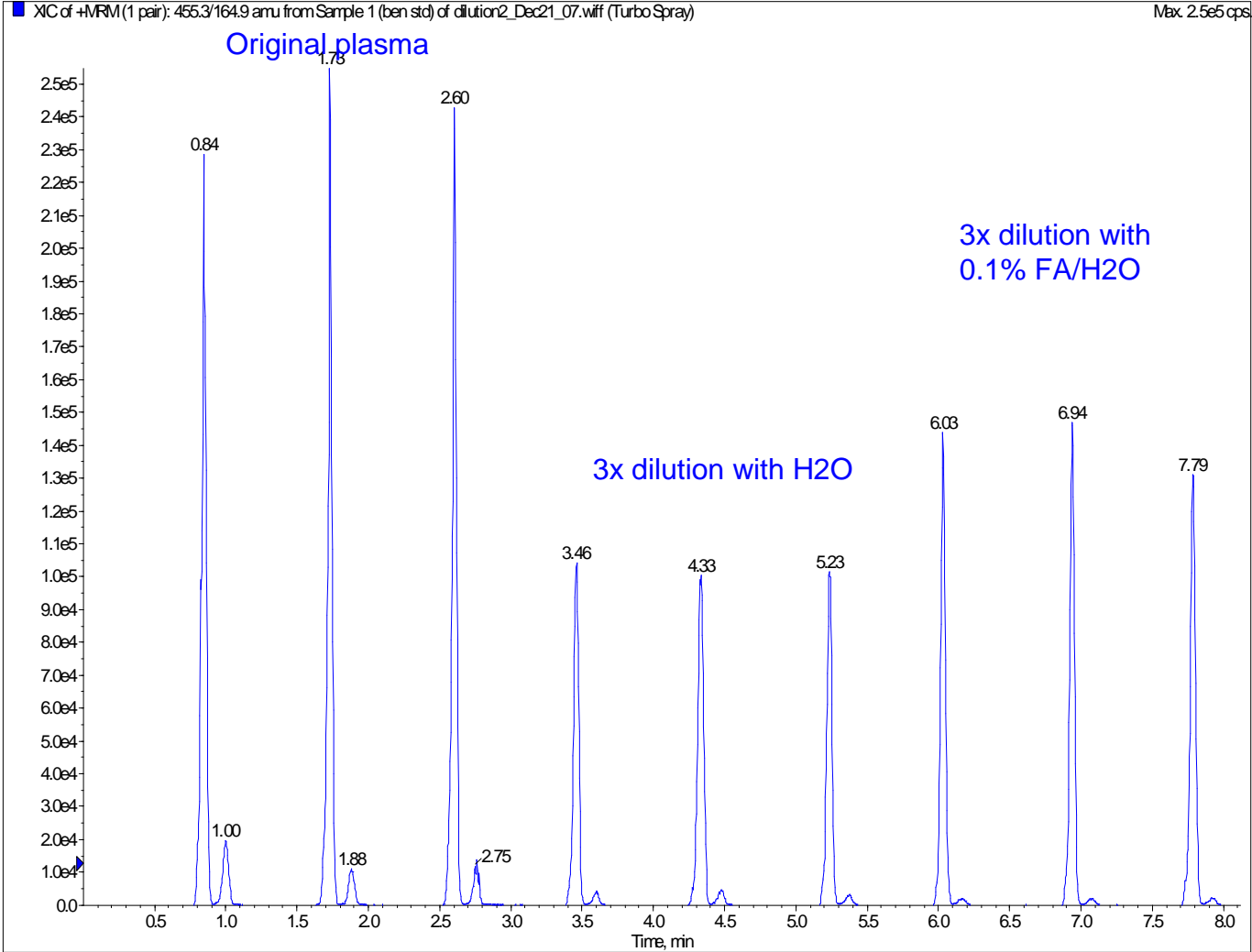
# Dilution Effect of Verapamil



Signal reduction due to the dilution is canceled out by less matrix effect.  
This is particularly useful when sample is in low volume.

Data courtesy of Dr. Shaoxia Yu, Takeda Pharmaceuticals

# Dilution Effect of Verapamil



Further dilution is not beneficial for sensitivity

# Matrix Effects in rat plasma

	<b>Methyacina</b>	<b>Verapamil</b>	<b>Alprozolam</b>	<b>Praparacaine</b>	<b>Compound E</b>
Plasma	6.9%	37.1%	68.4%	5.4%	43.2%
1:1 (plasma:H2O)	17.2%	56.9%	122.7%	8.0%	87.3%

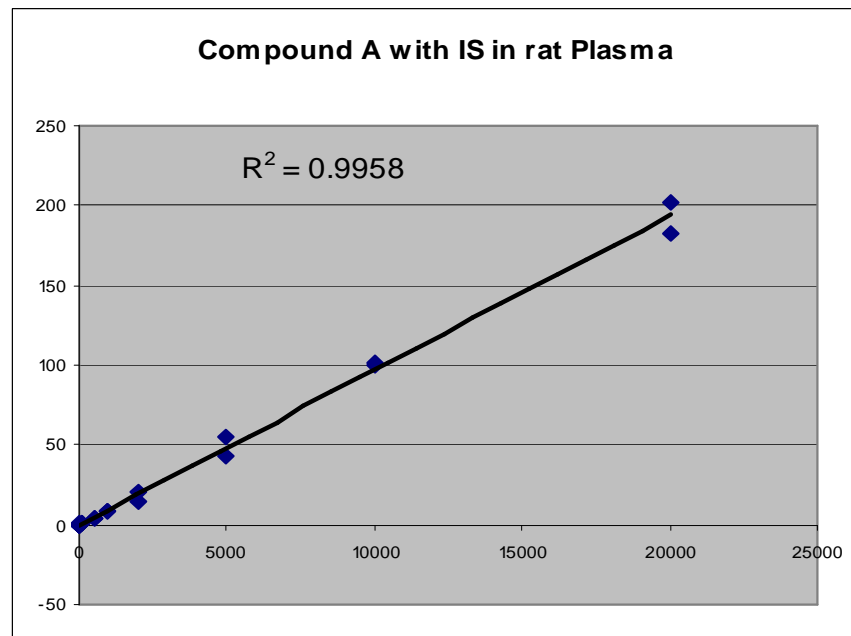
Matrix effect is reflected by the analyte response in matrix over that in neat solvent

# Compound A with Internal Standard

## Direct Ionization of Rat Plasma

STD Concentration with IS (ng/mL)	5.00	20.0	100	500	1000	2000	5000	10000	20000
1	5.20	*29.3	108	441	945	2250	6070	11300	22200
2	5.00	17.4	78.7	498	921	1680	4740	11000	20200
Mean	5.10	17.4	93.4	470	933	1970	5410	11200	21200
%Bias	2.0	-13.0	-6.6	-6.0	-6.7	-1.5	8.2	12.0	6.0
n	2	1	2	2	2	2	2	2	2

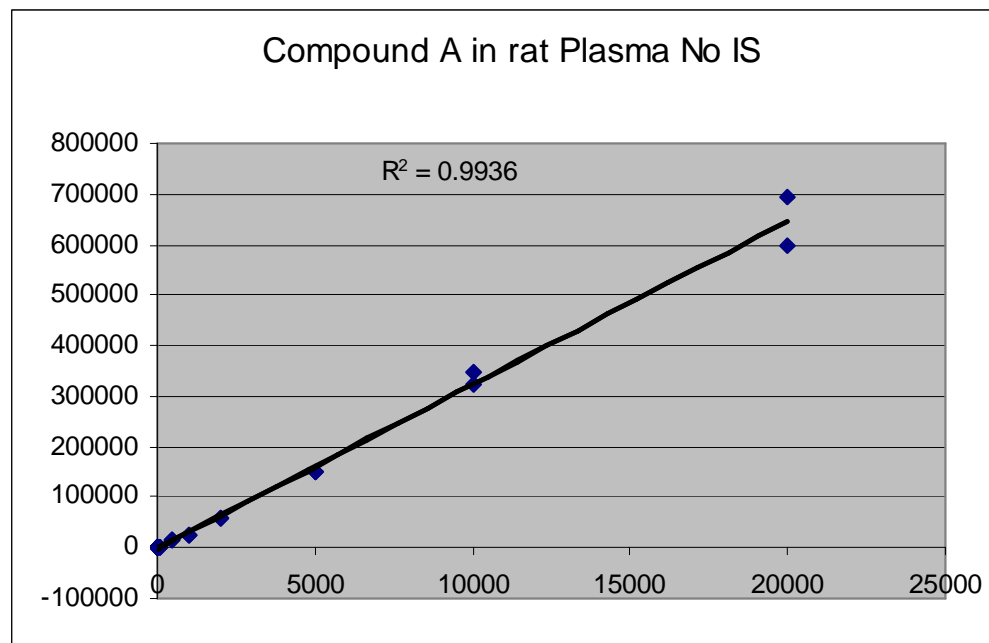
QC concentration (ng/mL)	Low (10.0)	Mid (2500)	High (16000)
1	12.1	2430	17500
2	8.88	2550	17900
3	~16.6	2570	16900
4	11.1	2470	17300
5	12.3	2660	19000
6	10.1	2710	11400
Mean	11.8	2570	16700
%CV	22.5	4.2	16.0
%Bias	18.0	2.8	4.4
n	6	6	6



# Compound A without IS in Rat Plasma

STD Concentration No IS (ng/mL)	5.00	20.0	100	500	1000	2000	5000	10000	20000
1	5.21	26.2	104	486	884	1890	5030	11600	23200
2	4.67	16.1	91.5	449	854	1980	4960	10900	20000
Mean	4.94	21.2	97.8	468	869	1940	5000	11300	21600
%Bias	-1.2	6.0	-2.2	-6.4	-13.1	-3.0	0.0	13.0	8.0
n	2	2	2	2	2	2	2	2	2

QC concentration (ng/mL)	Low (10.0)	Mid (2500 )	High (16000 )
1	10.4	2650	16300
2	9.33	2370	18500
3	~14.0	2590	15300
4	12.5	2670	18900
5	11.7	2470	17300
6	12.9	2570	13000
Mean	11.8	2550	16600
%CV	14.5	4.5	13.3
%Bias	18.0	2.0	3.8
n	6	6	6



# Indomethacin in Rat Plasma

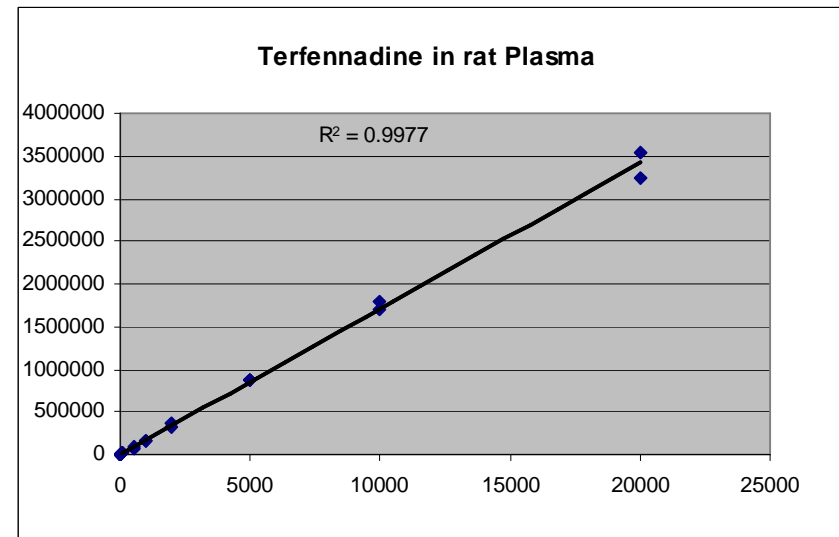
STD Concentration (ng/mL)	10.0	50	250	500	1000	2500	5000	10000
1	8.75	36.1	212	493	999	2510	5550	10800
2	10.6	59.4	253	434	857	2580	5420	10800
3	10.9	52.0	206	427	1050	2560	5360	12400
Mean	10.1	49.2	224	451	969	2550	5440	11300
Mean %Bias	1.0	-1.6	-10.4	-9.8	-3.1	2.0	8.8	13.0
%CV	11.5	24.2	11.4	8.0	10.3	1.4	1.8	8.2
n	3	3	3	3	3	3	3	3



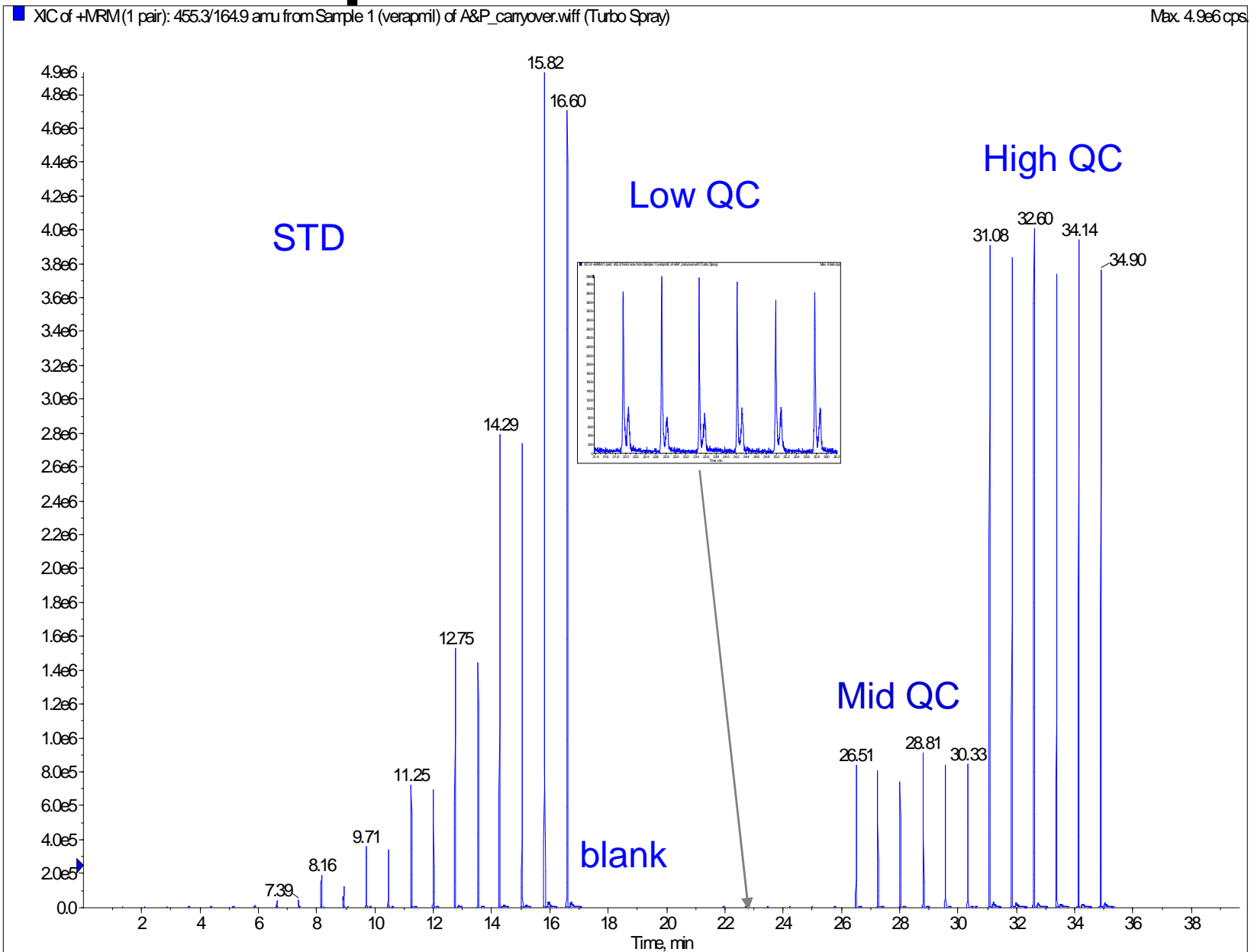
# Terfenadine in Rat Plasma

STD concentration (ng/mL)	2.00	5.00	20.0	100	500	1000	2000	5000	10000	20000
1	2.15	4.72	17.7	92.8	457	1050	2270	5280	10400	21500
2	2.01	4.51	19.0	89.7	498	1000	1990	5280	11000	19800
Mean	2.08	4.62	18.4	91.3	478	1030	2130	5280	10700	20700
%Bias	4.0	-7.6	-8.0	-8.7	-4.4	3.0	6.5	5.6	7.0	3.5
n	2	2	2	2	2	2	2	2	2	2

QC Concentration (ng/mL)	Low (10.0)	Mid (2500)	High (16000)
1	9.78	2680	11700
2	9.81	2830	14000
3	9.72	2710	15300
4	9.19	2740	14300
5	8.90	2540	15600
6	9.07	2920	15300
Mean	9.41	2740	14400
%CV	4.3	4.7	10.1
%bias	-5.9	9.6	-10.0
n	6	6	6



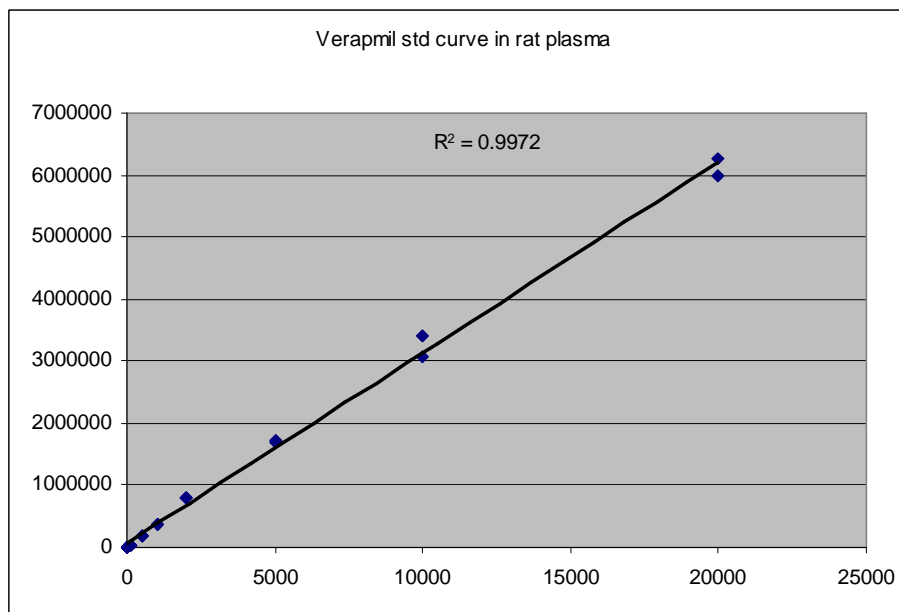
# Verapamil in Rat Plasma



# Verapamil in Rat Plasma

STD concentration (ng/mL)	2.00	5.00	20.0	100	500	1000	2000	5000	10000	20000
1	1.76	6.00	21.1	107	523	993	2160	4600	9330	17100
2	2.04	*7.30	21.9	111	523	1010	2190	4690	8440	16400
Mean	1.90	6.00	21.5	109	523	1000	2180	4650	8890	16800
%Bias	-5.0	20.0	7.5	9.0	4.6	0.0	9.0	-7.0	-11.1	-16.0
n	2	1	2	2	2	2	2	2	2	2

QC Concentration (ng/mL)	Low (10.0)	Mid (2500)	High (16000)
1	9.71	2300	14200
2	10.7	2270	13600
3	7.95	2230	13900
4	8.29	2490	13200
5	7.72	2520	12800
6	9.13	2300	13600
Mean	8.92	2350	13600
%CV	12.9	5.2	3.7
%bias	-10.8	-6.0	-15.0
n	6	6	6



# Applications for in Vivo and in Vitro ADME Studies

1. Mouse PK Study for Compound A (plasma samples)
  - 7 groups, 3 animals at 25 mg/kg PO
    - a) Plasma samples were extracted and analyzed by LC/MS/MS
    - b) Plasma samples were directly subjected to DART analysis

# Mouse PK Study

## Measured Plasma Concentrations By LC/MS/MS & DART

Sample ID	LC/MS /MS Concentration (nM)	DART Concentration (nM)	% MPD
C1 30min	3990	4170	2.2
C2 30min	3930	4412	5.8
C3 30min	2300	2918	11.8
C4 1 hr	3500	4297	10.2
C5 1 hr	4200	5846	16.4
C6 1 hr	2480	3011	9.7
C7 2hr	3500	4430	11.7
C8 2hr	1890	2340	10.6
C9 2hr	2900	3073	2.9
C10 4hr	2940	2674	-4.7
C11 4hr	3480	3672	2.7
C12 4hr	4510	4766	2.8
C13 8hr	3110	2851	-4.3
C14 8hr	1900	2111	5.3
C15 8hr	3270	3130	-2.2
C16 16hr	BQL	BQL	
C17 16hr	2.00	BQL	
C18 16hr	BQL	BQL	
C19 24hr	BQL	BQL	
C20 24hr	BQL	BQL	
C21 24hr	1.39	BQL	

# Applications for in Vivo and in Vitro ADME Studies

## 2. Mouse PK Study for Compound B (whole blood samples)

- 16 groups, 3 animals at 60 mg/kg SC
  - a) Whole blood samples were extracted and analyzed by LC/MS/MS
  - b) Whole blood samples were directly subjected to DART analysis

# Mouse PK Study

## Measured Whole Blood Concentrations Between LC/MS/MS & DART

Sample ID	LC/MS/MS Concentration (nM)	DART (nM)	%MPD	Sample ID	LC/MS/MS Concentration(nM)	DART (nM)	%MPD
AA1	BQL	BQL		II1	BQL	BQL	
AA2	BQL	BQL		II2	BQL	BQL	
AA3	BQL	BQL		II3	BQL	BQL	
BB1	19300	23271	9.3	JJ1	12900	15927	10.5
BB2	15900	17162	3.8	JJ2	14700	14677	-0.1
BB3	22200	23836	3.6	JJ3	14500	13027	-5.4
CC1	21300	23724	5.4	KK1	17700	16409	-3.8
CC2	24100	19131	-11.5	KK2	18900	22208	8.0
DD3	35700	29939	-8.8	KK3	20200	18168	-5.3
EE1	BQL	BQL		LL1	22400	23978	3.4
EE2	BQL	BQL		LL2	31700	27722	-6.7
EE3	BQL	BQL		LL3	31800	38092	9.0
FF1	20600	19858	-1.8	MM1	BQL	BQL	
FF2	21200	18763	-6.1	MM2	BQL	BQL	
FF3	15500	17434	5.9	MM3	BQL	BQL	
GG1	22300	32876	19.1	NN1	11400	13606	8.8
GG2	21600	20113	-3.6	NN2	16100	14609	-4.9
GG3	23800	24292	1.0	NN3	16000	17126	3.4
HH1	33200	30853	-3.7	OO1	16600	19852	8.9
HH2	44600	28290	22.4	OO2	18100	17639	-1.3
HH3	33200	34187	1.5	PP1	34800	33839	-1.4

# Applications for in Vivo and in Vitro ADME Studies

## 3. Intrinsic Clearance Study for In vitro Samples

	Matrix	Hepatic Extraction Ratio LC/MS/MS	Hepatic Extraction Ratio DART
Compound C	Human S9	<0.19	<0.19
Compound C	Rat S9	<0.16	<0.16
Compound D	Mouse Microsomes	0.91	0.92

# Other Speedy Methods

- Rapid Fire LC/MS (BioTrove)
  - UPLC/MS
  - MALDI / QTOF
  - DESI / DAPSI
- Laser Diode Thermal Desorption  
LDTD MS

# Conclusions

- The reproducibility, Sensitivity and Practicability of DART has been significantly improved with new instrumentation.
- DART was found to meet the general requirement for bioanalysis without sample preparation.
- Compared to results generated with LC/MS/MS, DART has produced comparable results for PK and in vitro ADME studies.
- Speed is on its way, one way or another

# Acknowledgements

Shaoxia Yu, Jing-Tao Wu, Gordon Justin, Chuang Lu, Ji Zhang, Cicely Berg, and members of the Takeda bioanalytical group for their help in this work.