

Quantification of Small Molecules in Biological Fluids with “Direct Analysis in Real Time” Tandem Mass Spectrometry (DART/MS/MS), Without Sample Preparation and LC Separation



Pharmaceuticals

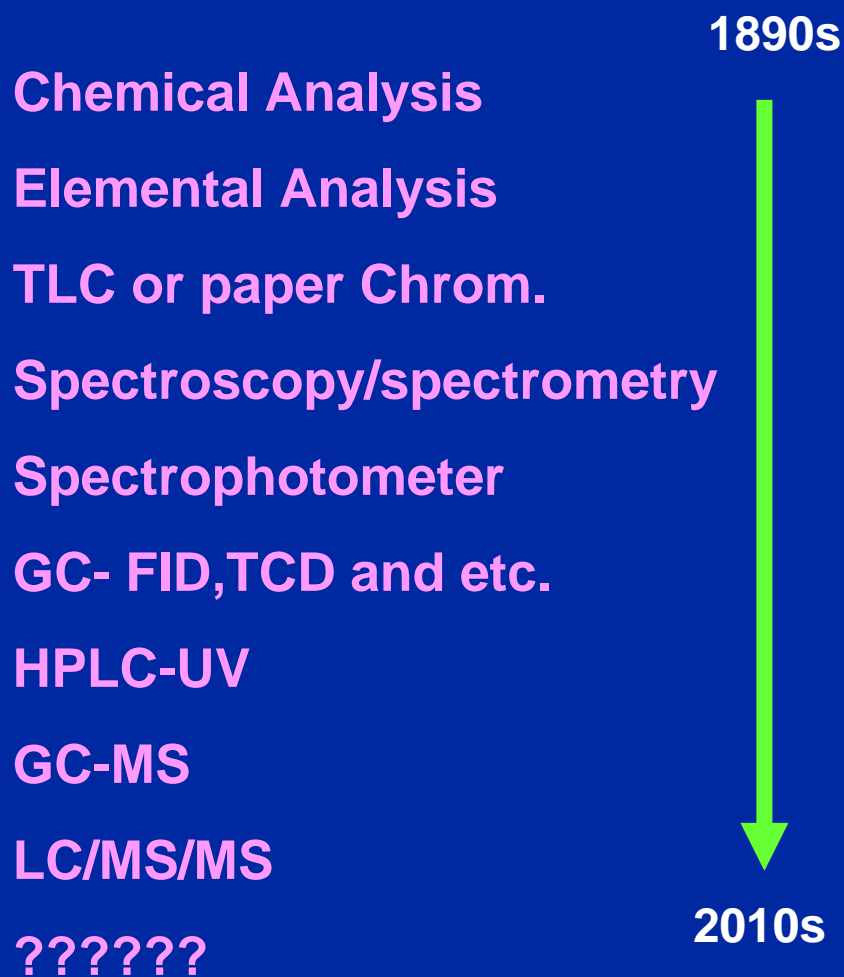
Yeping Zhao*, Michelle Lam, Rowena Mak and Danlin Wu

DMPK, Roche Palo Alto,
3431 Hillview Ave., Palo Alto, CA 94304
yeping.zhao@roche.com, 650-855-6073 (O)



Y. Zhao

The History about Major Quantification Tools in Bioanalytical/Analytical Chemistry



What is the next generation tool for Quantitative bioanalysis ?

C&E News Cover Story,

October 8, 2007



Taking Mass Spec Into The Open

Open-air ionization methods minimize sample prep and widen range of mass spectrometry applications
by [Celia Henry Arnaud](#)

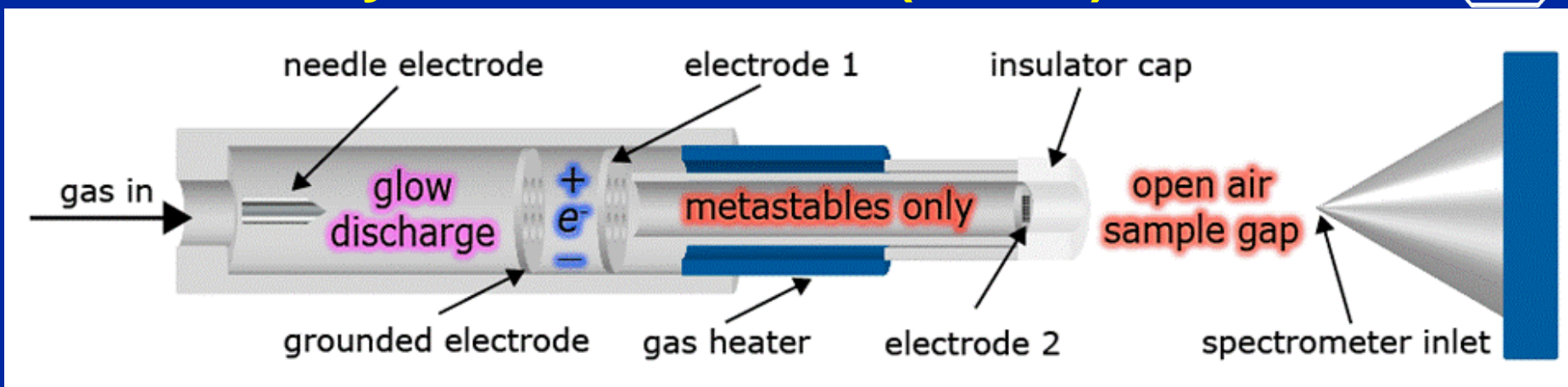


JEANETTE ADAMS gets excited talking about all the things she can do with mass spectrometry at the Library of Congress. A scientist in its [Preservation Research & Testing Division](#), Adams uses mass spectrometry to look for early signs of degradation in documents, photographs, and microfilm from the library's collections. In most cases she does so without even taking a sample, thanks to an ionization method called DART (direct analysis in real time).

Pharmaceuticals

Y. Zhao

Direct Analysis at Real Time (DART)

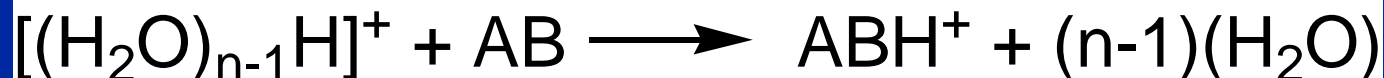
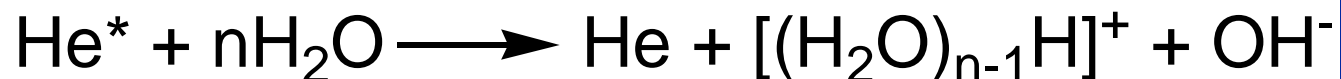


DART source operates by exposing the sample to a dry gas stream (typically helium or nitrogen) that contains long-lived electronically or vibrationally excited neutral atoms or molecules (or "metastables"). Excited states are typically formed in DART source by creating a glow discharge in a chamber through which the gas flows. Potentials applied to electrostatic lenses remove charged particles from the gas stream. A grid at the exit of the DART source acts as a source of electrons and reduces positive-ion/negative-ion recombination. The excited-state species can interact directly with the sample to desorb and ionize the sample.

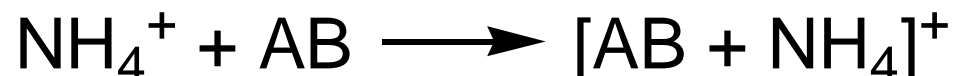
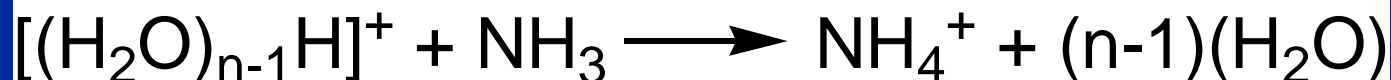
DART Ionization Mechanisms



- Gas-phase proton transfer reactions



- Gas-Phase Chemical Ionization



- Gas-phase electron transfer reactions

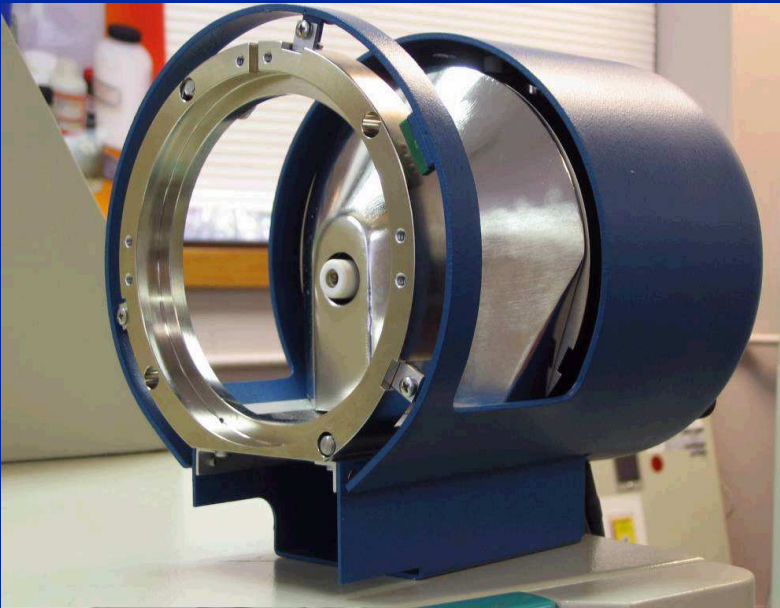


which reacts with the analyte to produce anion



Direct Analysis at Real Time (DART)

has been used to analyze gases, liquid, solids and materials on surfaces qualitatively.





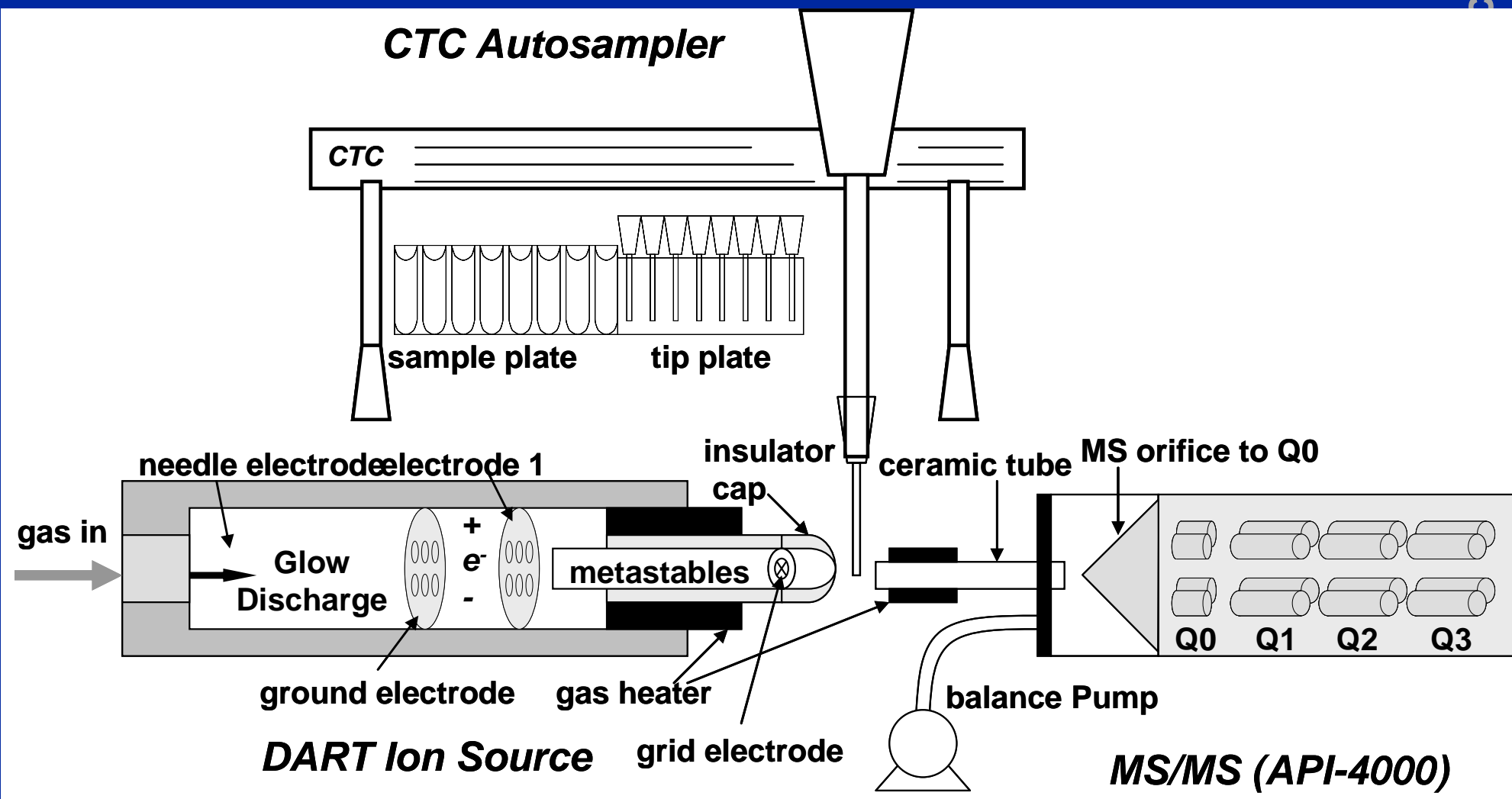
DART/MS/MS (API-4000)

We have connected a DART with API-4000 MS/MS system to replace LC/MS/MS for quantization of small molecules in biological samples.

This new system dose not need LC system, mobile phase, no west, sample preparation or purification.



DART/MS/MS at DMPK, Roche Palo Alto

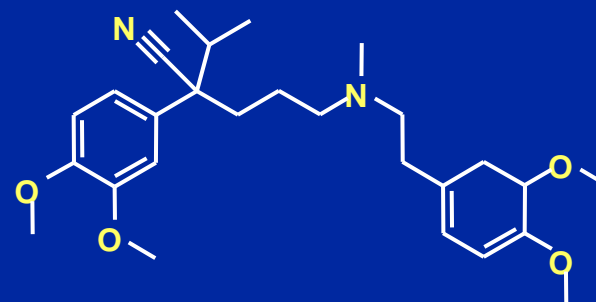
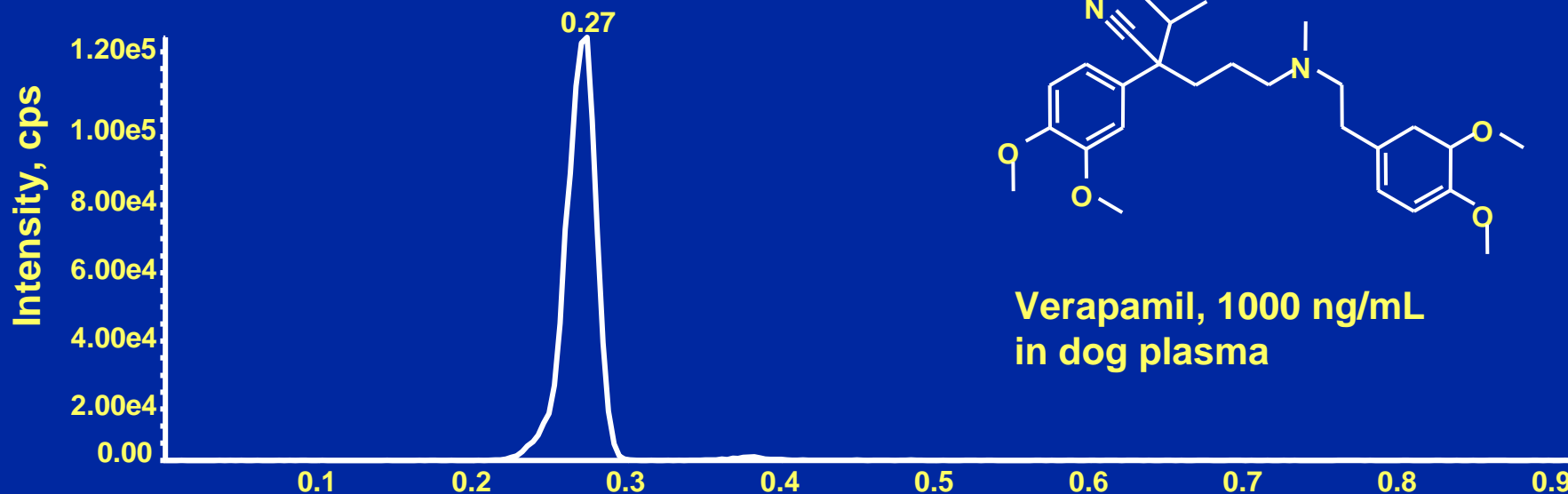


A Quantitation Profile of DART/MS/MS

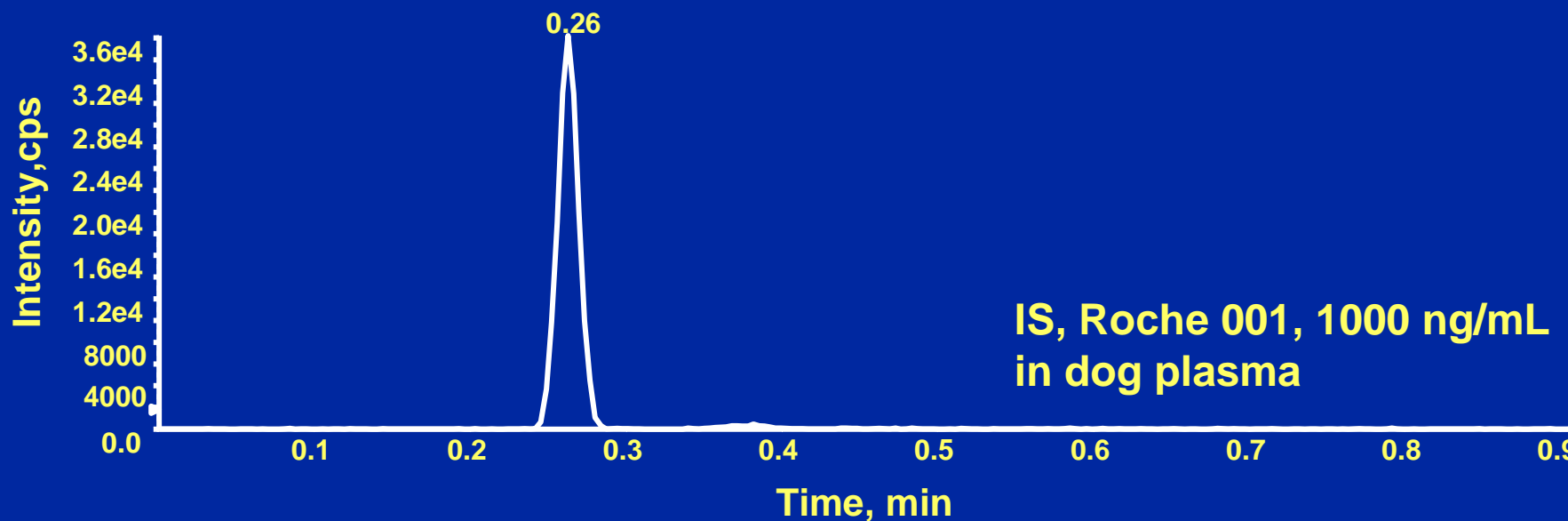
(Verapamil in dog plasma)



Pharmaceuticals



Verapamil, 1000 ng/mL
in dog plasma

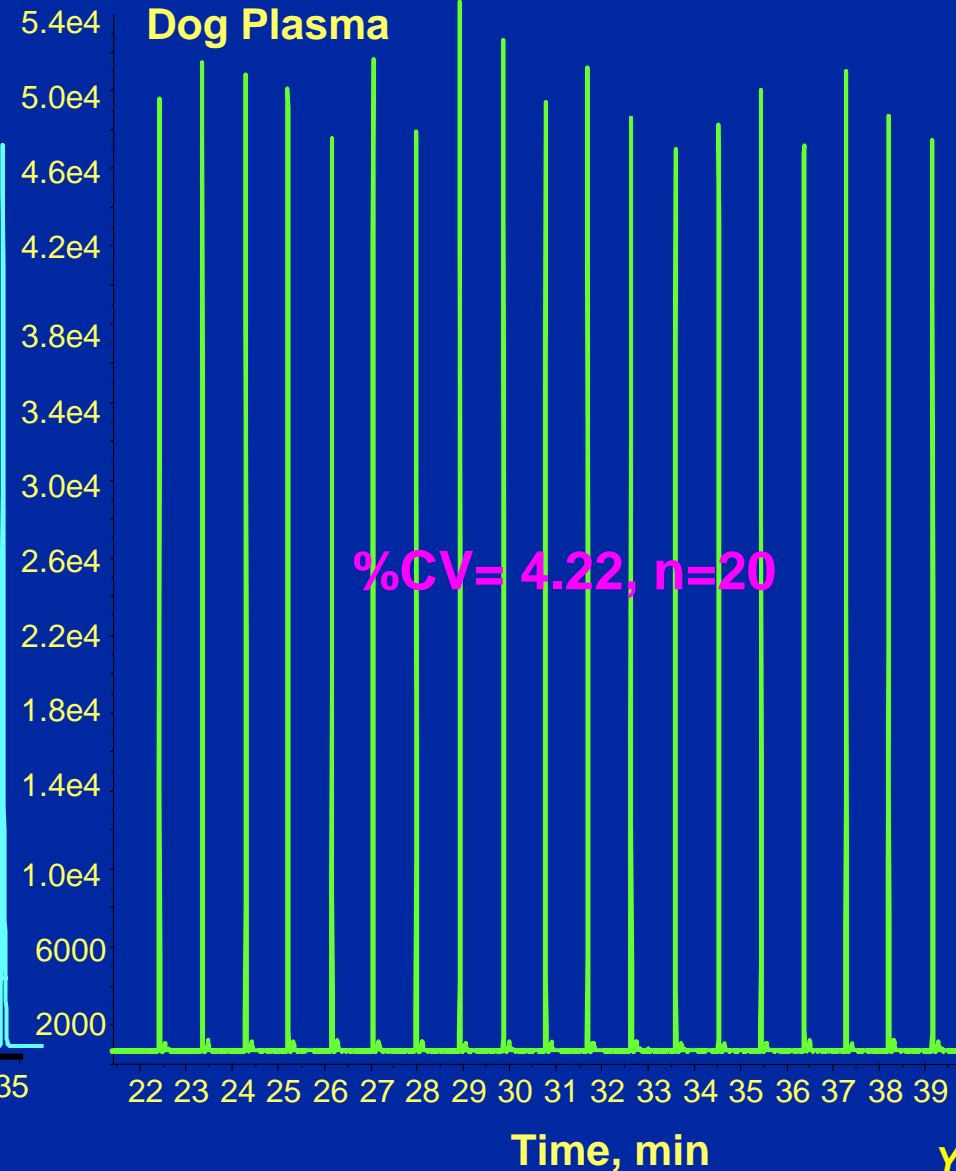
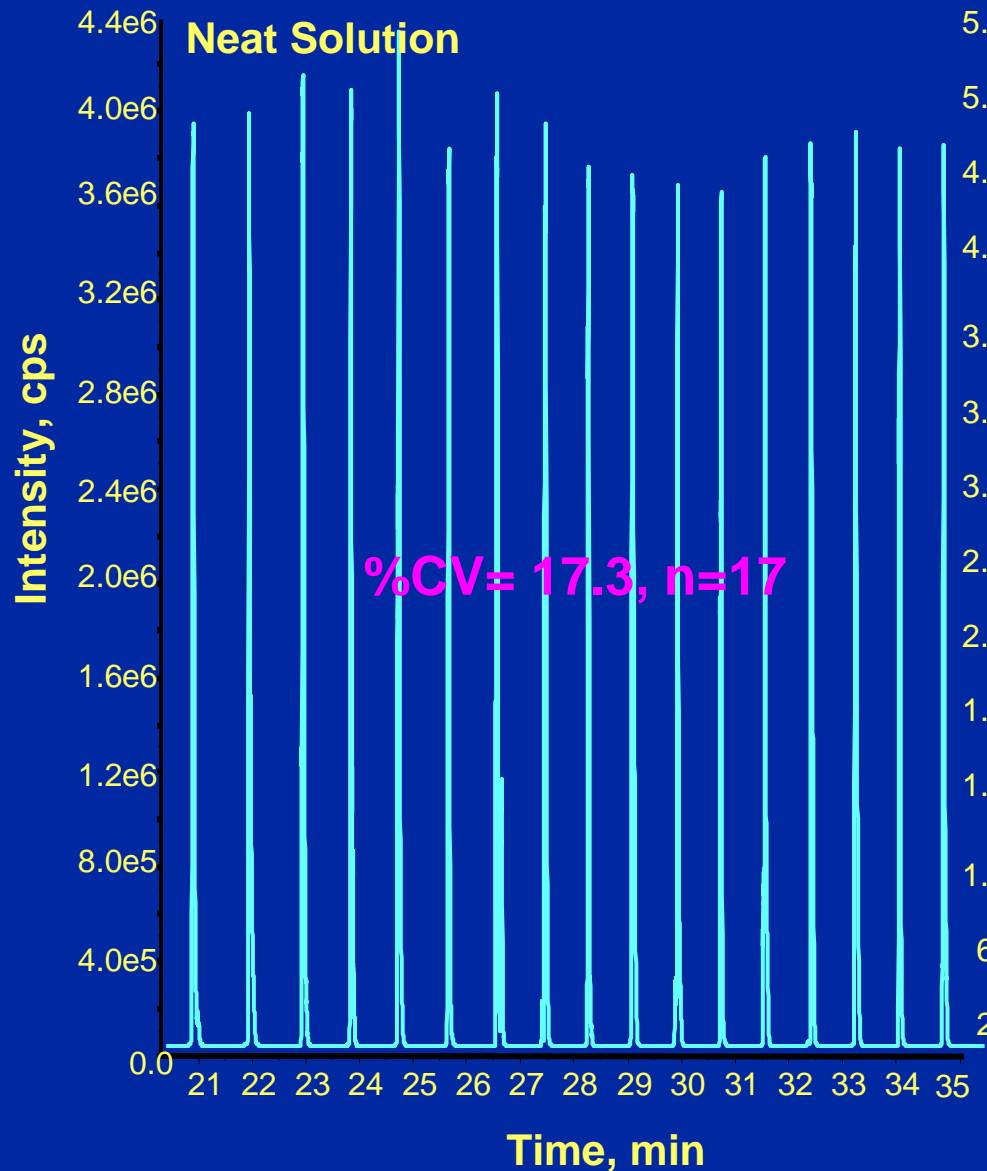


IS, Roche 001, 1000 ng/mL
in dog plasma

Reproducibility of DART/MS/MS for Compound A in a neat solution or a plasma sample



Pharmaceuticals

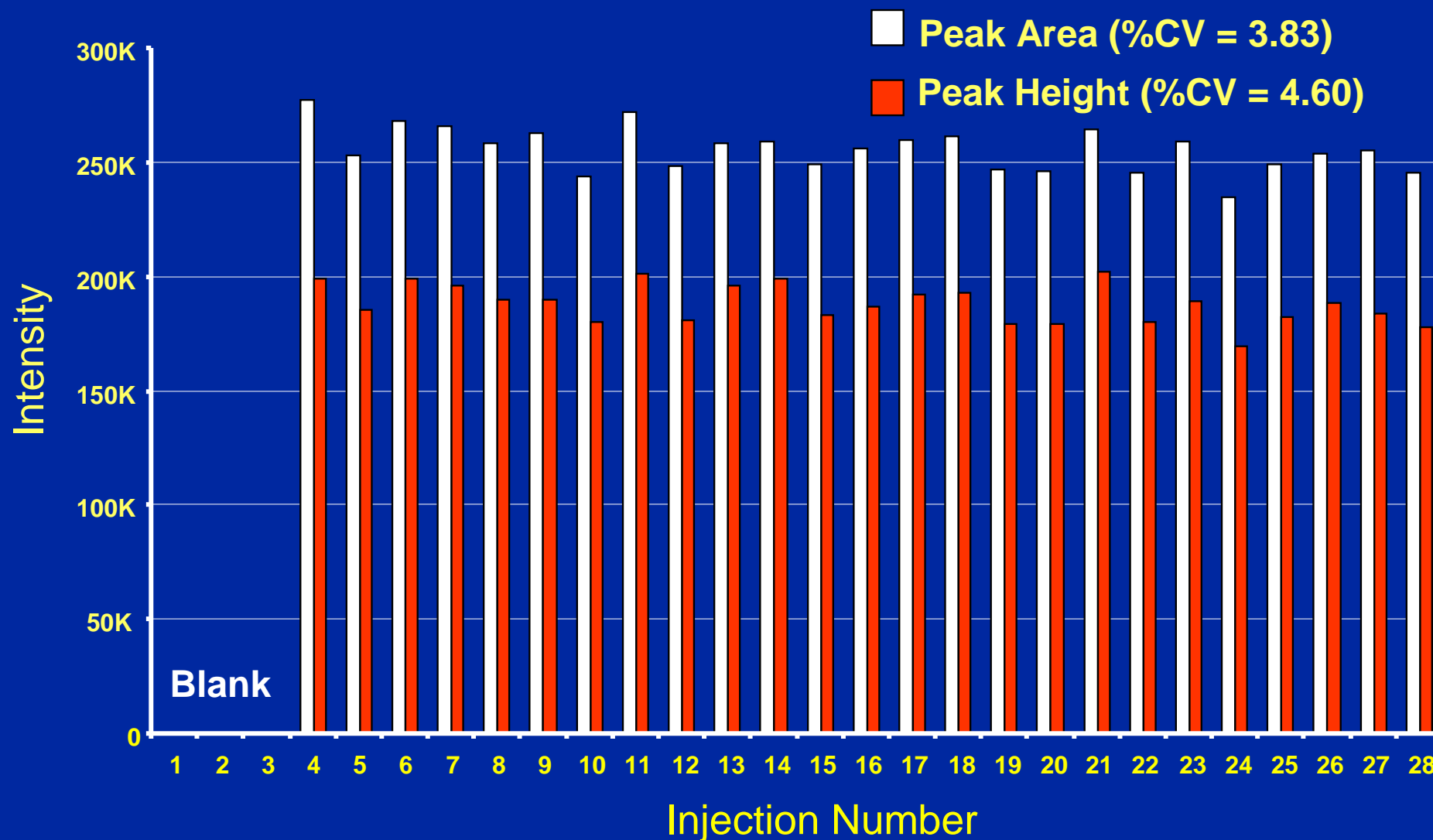


The System Reproducibility of DART/MS/MS

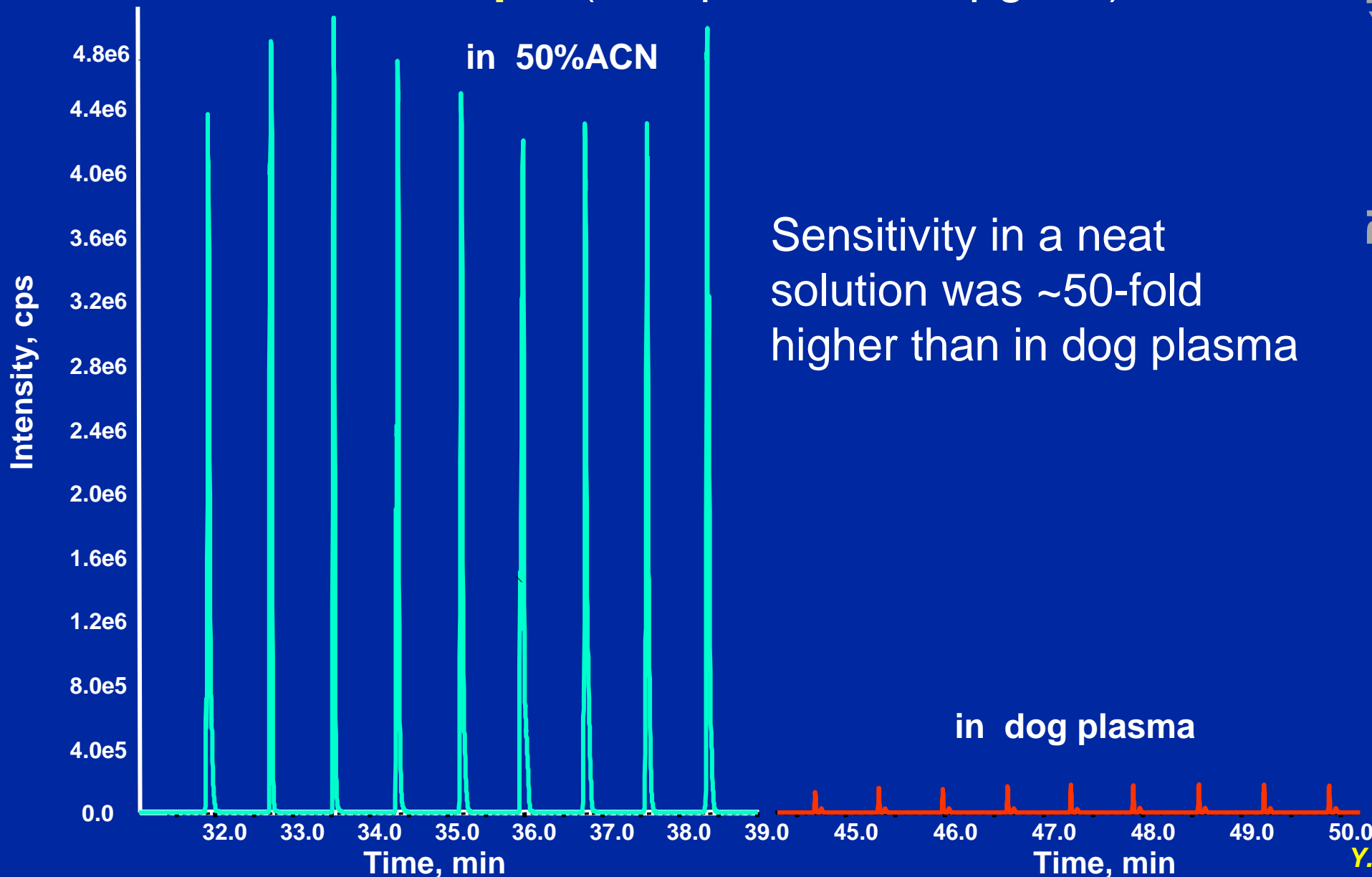


(Verapamil, 1 $\mu\text{g}/\text{mL}$ in dog plasma, n=25)

Pharmaceuticals

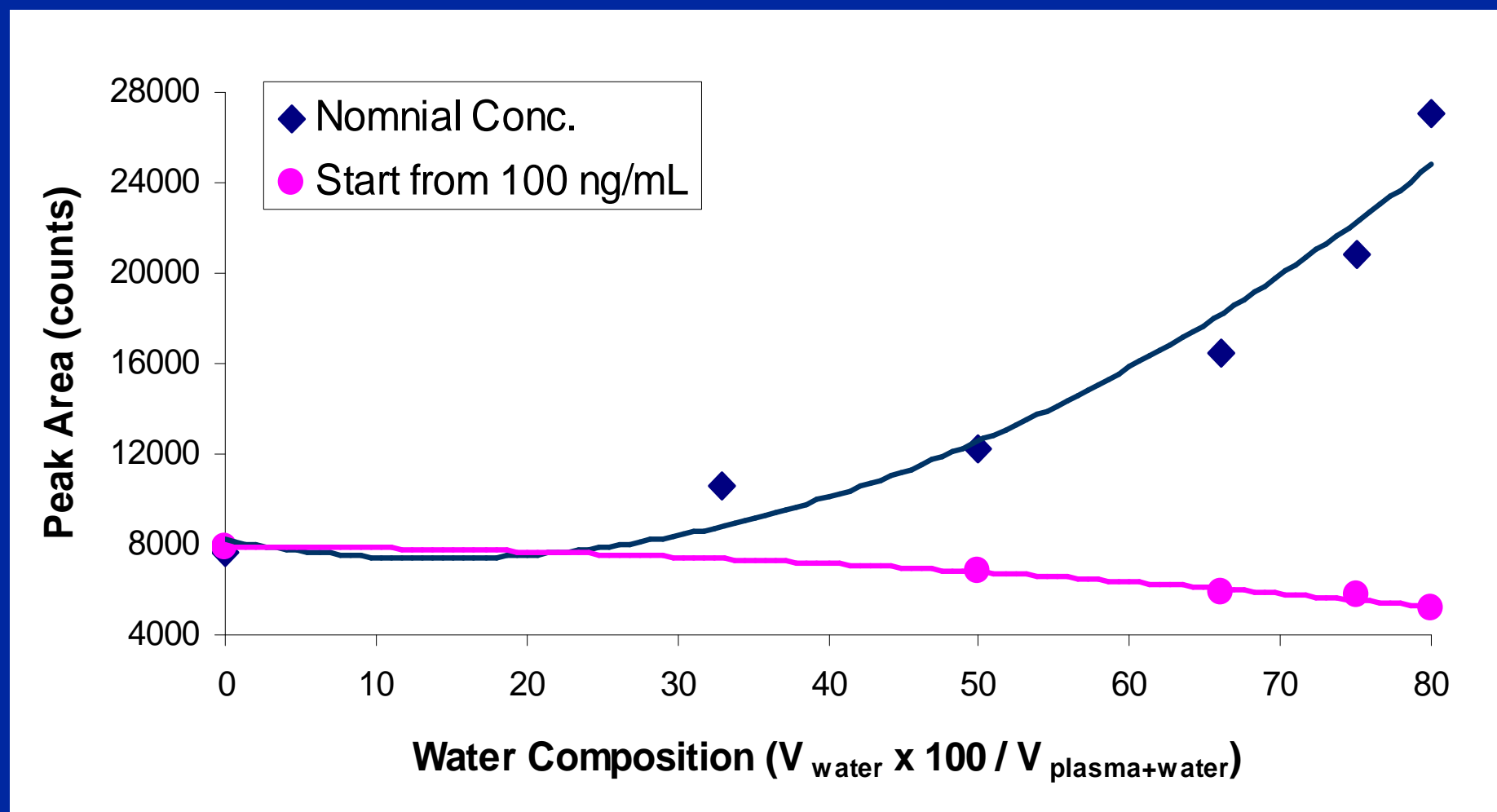


Sensitivity of DART/MS/MS for a Neat Solution or a Plasma Sample (Compound A, 10 μ g/mL)

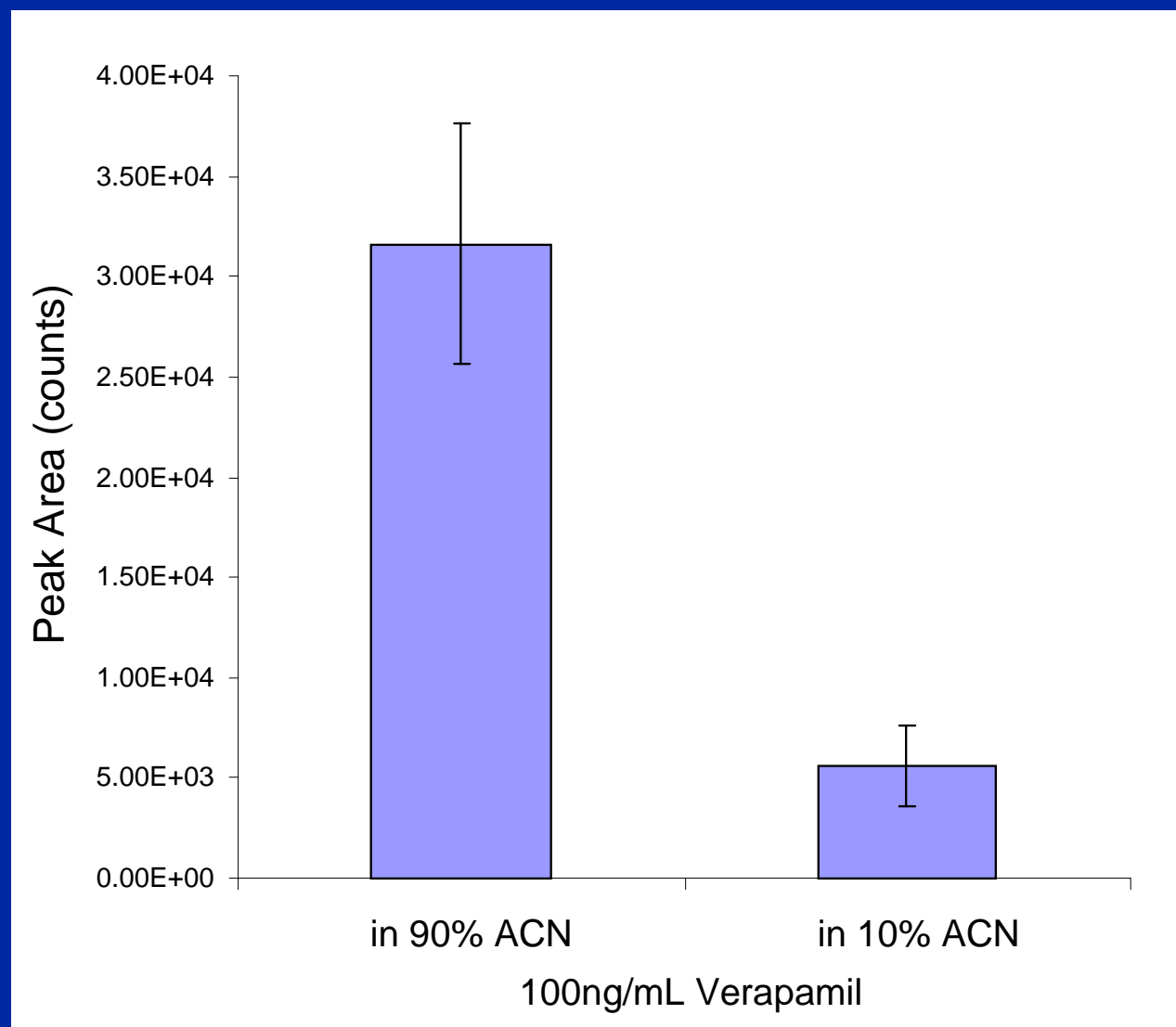


Influence of Matrix Composition in Plasma Sample to Sensitivity of DART/MSMS

(Verapamil, 100ng/mL in dog plasma)



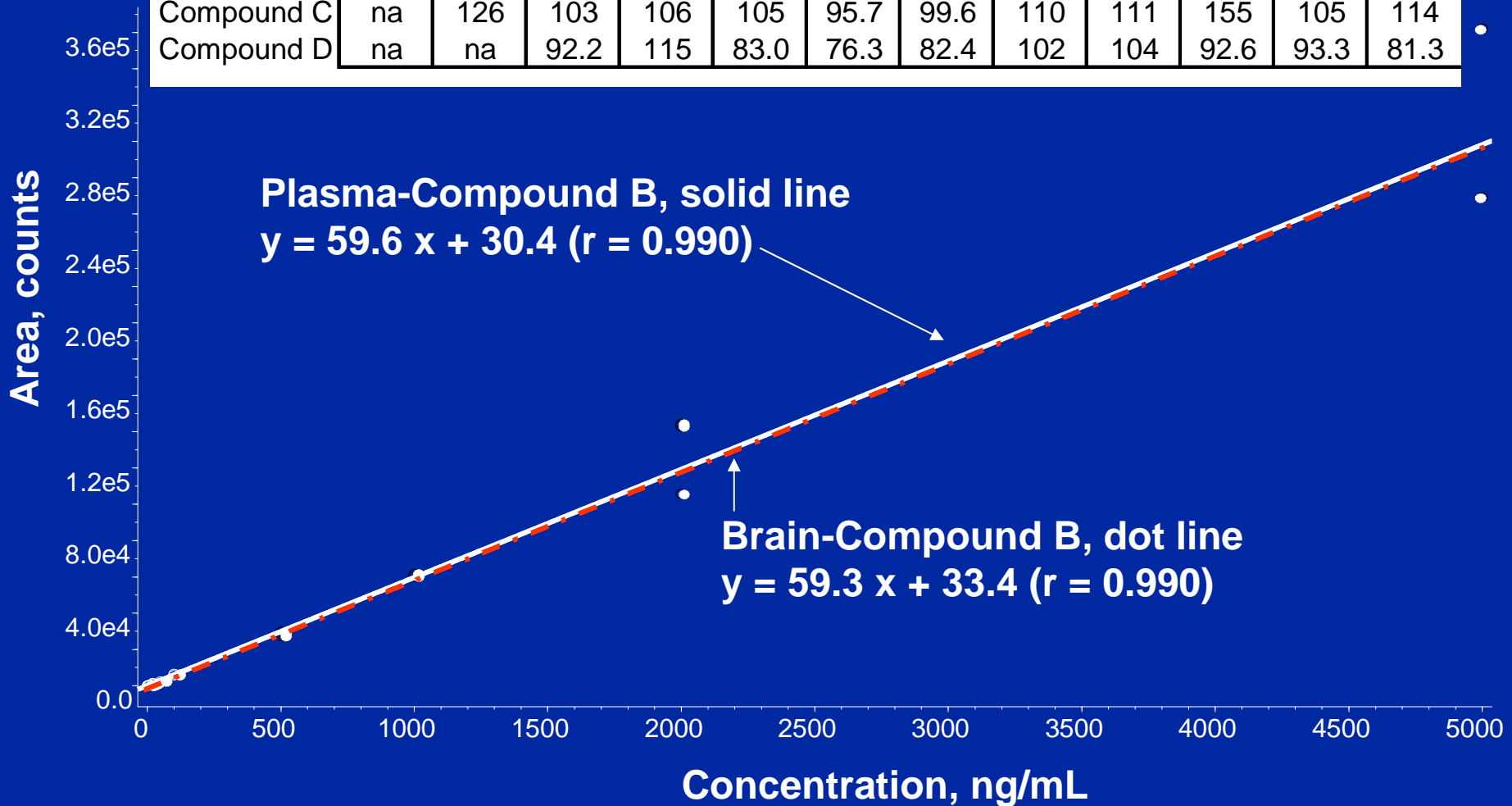
Influence of Sample Volatility to Sensitivity of DART/MS/MS



Matrix Effects of DART for both plasma and brain samples



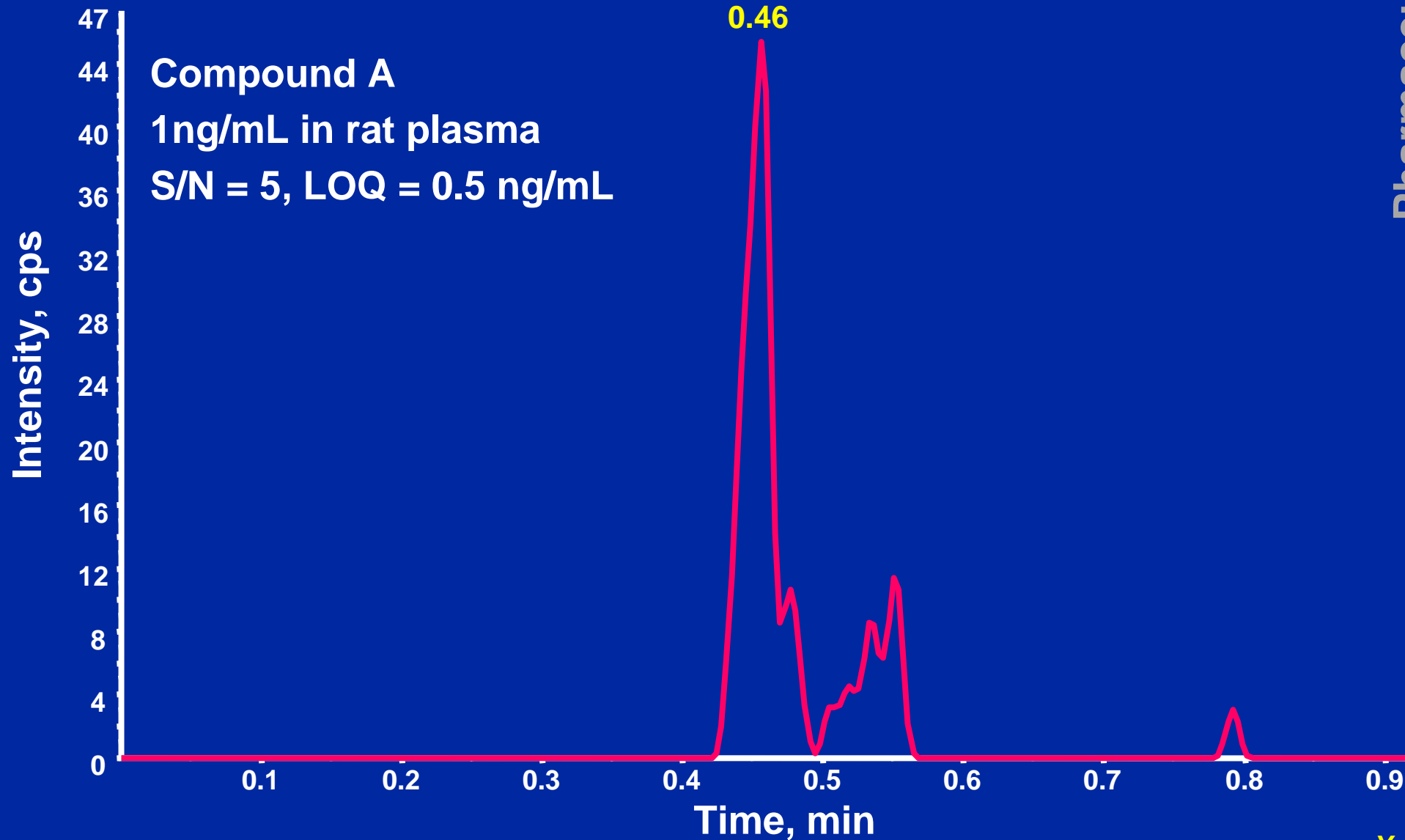
Compounds	Accuracy (%) against to plasma calibration curves											
	Samples, Nominal Concentration (ng/mL)											
	0.5	1	2	5	10	20	50	100	500	1000	2000	5000
Compound B	121	111	85.5	105	86.2	83.7	112	83.4	77.8	99.0	89.1	85.9
Compound C	na	126	103	106	105	95.7	99.6	110	111	155	105	114
Compound D	na	na	92.2	115	83.0	76.3	82.4	102	104	92.6	93.3	81.3



A Quantitation Profile of DART/MS/MS (Compound A in rat plasma)



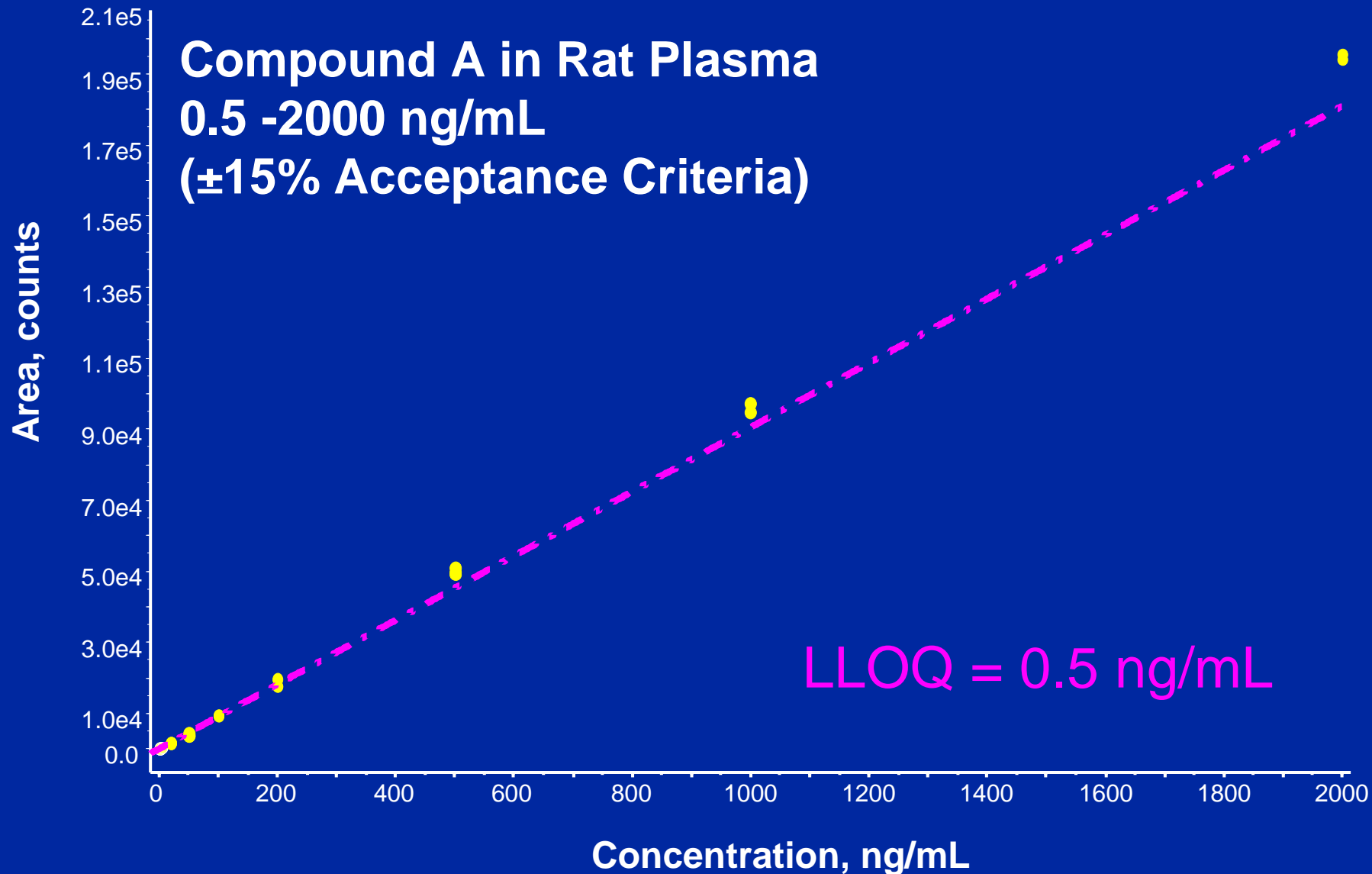
Pharmaceuticals



System Dynamic Range of DART/MS/MS



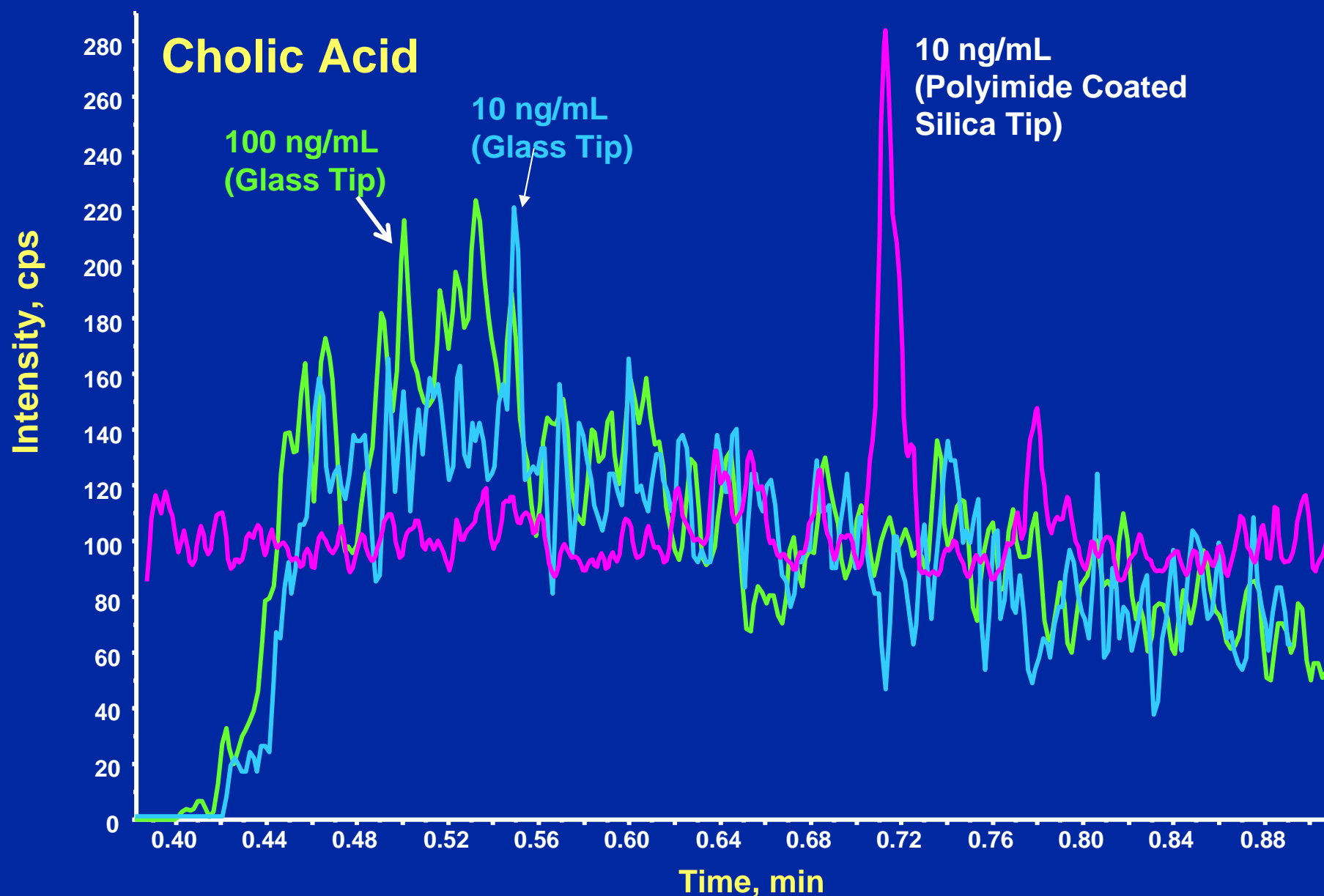
Pharmaceuticals



Comparison of Different Tip Materials



Pharmaceuticals



Commercial Compounds Tested in Dog Plasma

(80% LOD < 5 ng/mL, 90% LOD < 10 ng/mL)



icals

Test Compound	MW	LOD (ng/mL)	Ion Charge	Test Compound	MW	LOD (ng/mL)	Ion Charge
SULCONAZOLE	461	5	+	TERBUTALINE	274	2	+
ATROPINE	289	5	+	WARFARIN	308	5	-
ESTRADIOL	272	1	+	PIROXICAM	331	2	-
PYRIMETHAMINE	249	2	+	CHLORTHALIDONE	339	10	-
DARICON(R)	381	5	+	PROGLUMIDE (NA SALT)	356	5	-
CIMETIDINE	252	5	+	CHOLIC ACID	409	5*	+
SOTALOL HYDROCHLORIDE	309	5	+	4-PHENYPIPERIDINE	161	10*	+
PROPANOLOL HCL	196	2	+	CORTISOL	362	2*	+
LEVASOLE(R)	241	1	+	PROPRANOLOL	259	5	+
VERAPAMIL	454	0.5	+	MIDAZOLAM	325	1	+
QUINIDINE	324	5	+	TERFENADINE	471	0.5	+
ATENOLOL	266	20	+	EZETIMIBE	409	10	+
CAFFEINE	194	5	+	SN-38	392	10**	+
FINLEPSIN	236	10	+	HYDROXY-MIDAZOLAM	341	5	+
CLOZAPINE	327	0.5	+	DEXTROMETHORPHAN	271	5	+
HYDROXYZINE DIHYDROCHLORIDE	448	2	+	BUFURALOL	298	0.5	+
LABETALOL HCL	365	2	+	HYDROXY-TESTOSTERONE	304	50***	+
LOMEFLOXACIN HCL	388	5	+	OXIDIZED-NIFEDIPINE	344	20***	+
LOPERAMIDE HYDROCHLORIDE	514	0.25	+	RALOXIFENE	474	NS	+

NS = No Signal at 100 ng/mL

*Polymide Coated Silica Tip

** Added Acetonitrile to Plasma (2:1)

***in buffer/ACN

Test Compound	Project	MW	LOD (ng/mL)	Ion Charge	Test Compound	Project	MW	LOD (ng/mL)	Ion Charge
RO1102981	P38	417	5	+	RO5258355	P2X3	404	5 ^{##}	+
RO5166606	5HT6	392	0.5	+	RO5236829	JNK	582	5	+
RO5041184	5HT6	385	<0.5	+	RO5200628	TRI	287	5	+
RO5252101	CCR5	616	<0.5	+	RO5248911	P2X7	436	5	+
RO5170011	CCR5	612	0.5	+	RO5211402	HIVRT	419	5* **	+
RO4879188	TRI	513	0.5	+	RO5247425	P2X7	448	5	-
RO5015657	TRI	555	5**	+	RO5263013	CCR5	595	0.5***	+
RO4995855	HCV (6)	259	5	+	RO5257935	CCR5	591	0.5***	+
RO5046887	HCV (6)	329	2	+	RO5262563	CCR5	476	5	+
RO5202679	TRPV1	430	5*	-	RO5210888	CCR5	595	2***	+
RO4402257	JNK	406	0.5	+	RO5259716	TRI	301	10***	+
RO5208272	P2X7	489	0.5	+	RO5261658	P2X7	422	0.5	+
RO5255180	CCR5	553	2.5	+	RO5211863	JNK	555	5***	+
RO4925954	HIVRT	436	2.5	+	RO5190591	HCV	732	50*	-
RO5246746	CCR5	584	2.5	+	RO5165657	5HT6	399	5***	+
RO5247908	TRI	271	5**	+	RO1048297	HCV(2)	284	5 [#]	+
RO5251952	5HT6	569	2.5	+	RO5211890	HIVRT	441	NS	+
RO5255165	P2X7	436	10	+	RO5247445	HCV	767	NS	+
RO5254337	P2X7	429	2.5	+	RO5025181	5HT6	362	5***	+
RO5252541	CCR5	575	0.5	+	RO5219652	TRI	369	2***	+
RO5093296	TRPV1	419	5	+	RO5045044	HIVRT	634	5***	+
RO5257913	CCR5	568	5	+	RO5285867	CCR5	554	5	+

NS= No Signal at 100 ng/ml

*Polyimide Coated Silica Tip

**Added water to plasma (1:1)

***Added Acetonitrile to plasma (2:1)

Added 2% NH₄OH to plasma

Dipped the tip to plasma 10 times

Roche Compounds Tested in Dog Plasma
(90% LOD < 5 ng/mL, 95% LOD < 10 ng/mL)

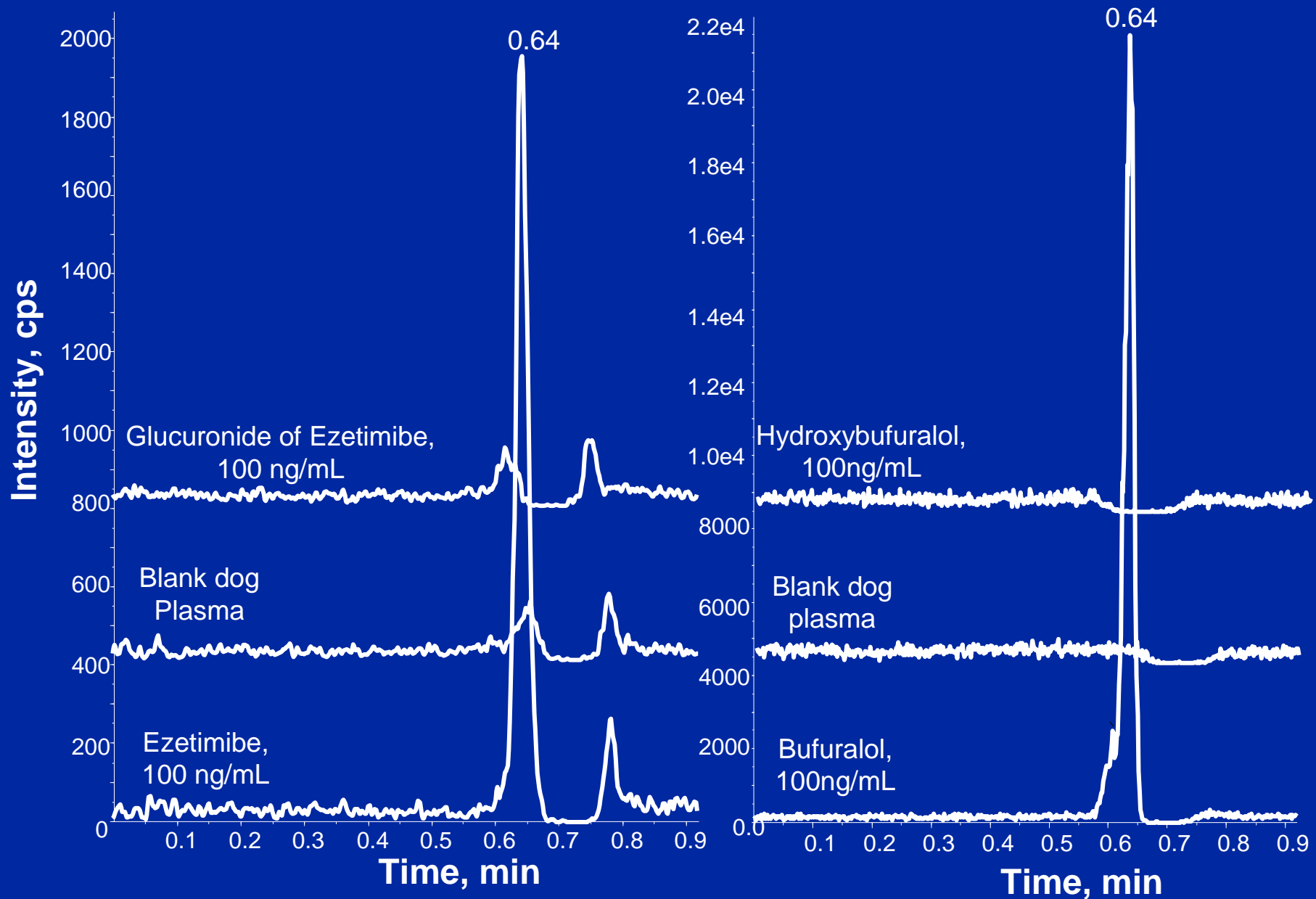
Roche

Pharmaceuticals

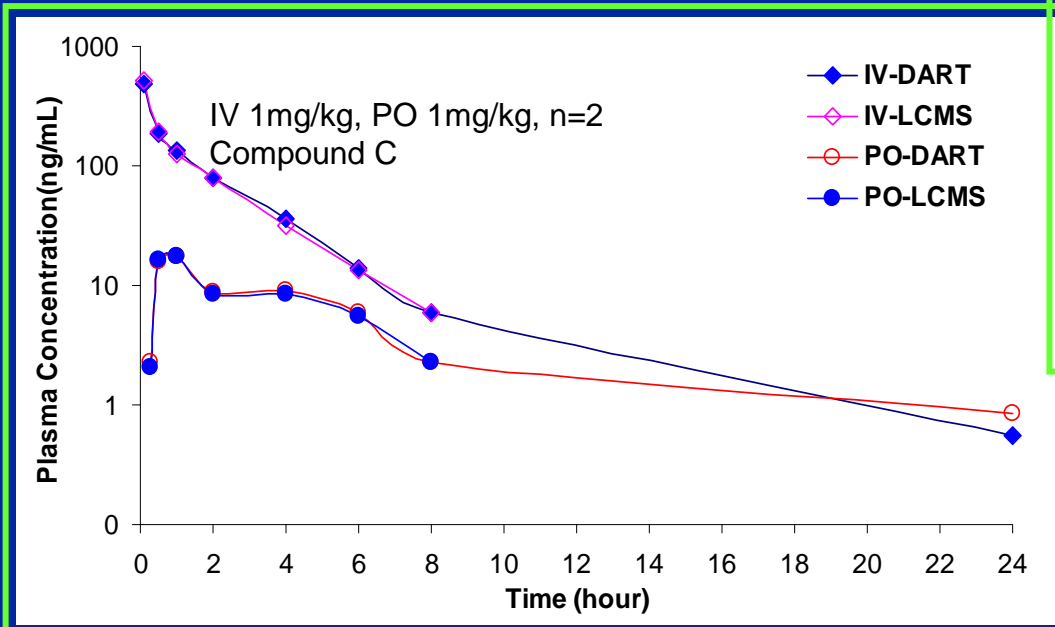
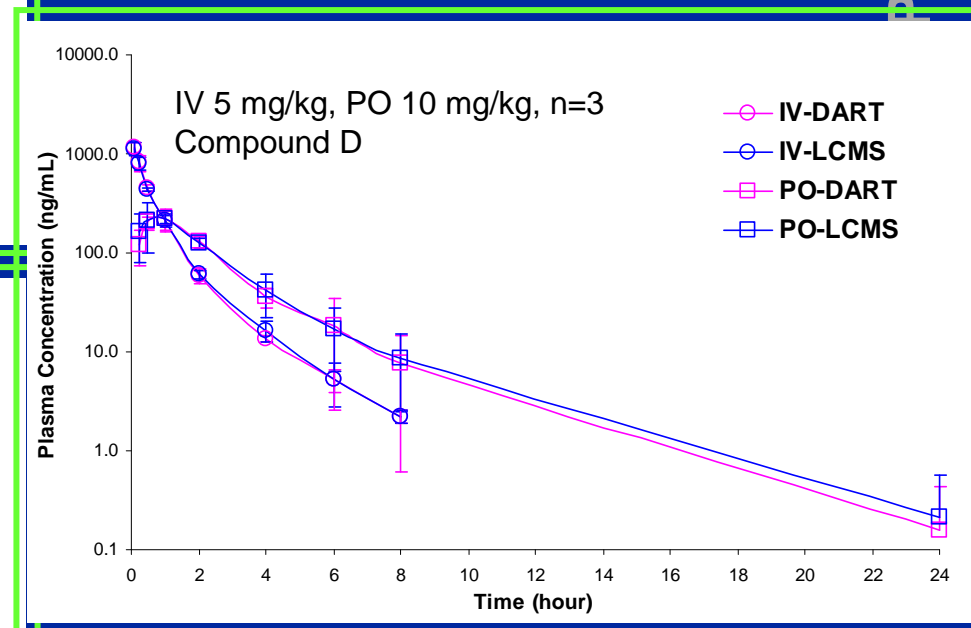
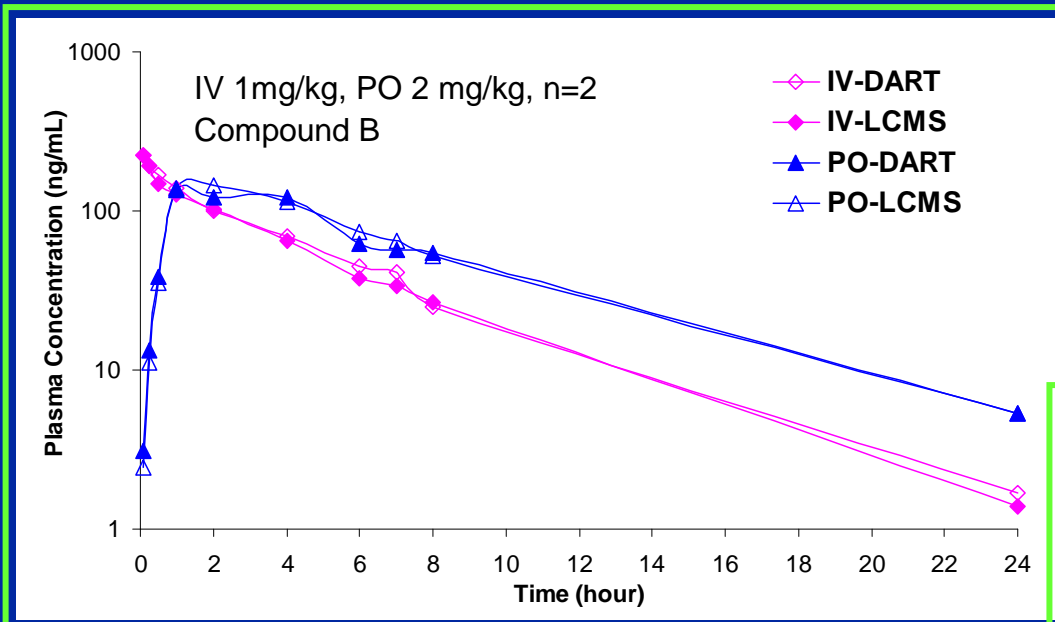
Ion Source Stability of Metabolites in Dog Plasma with DART



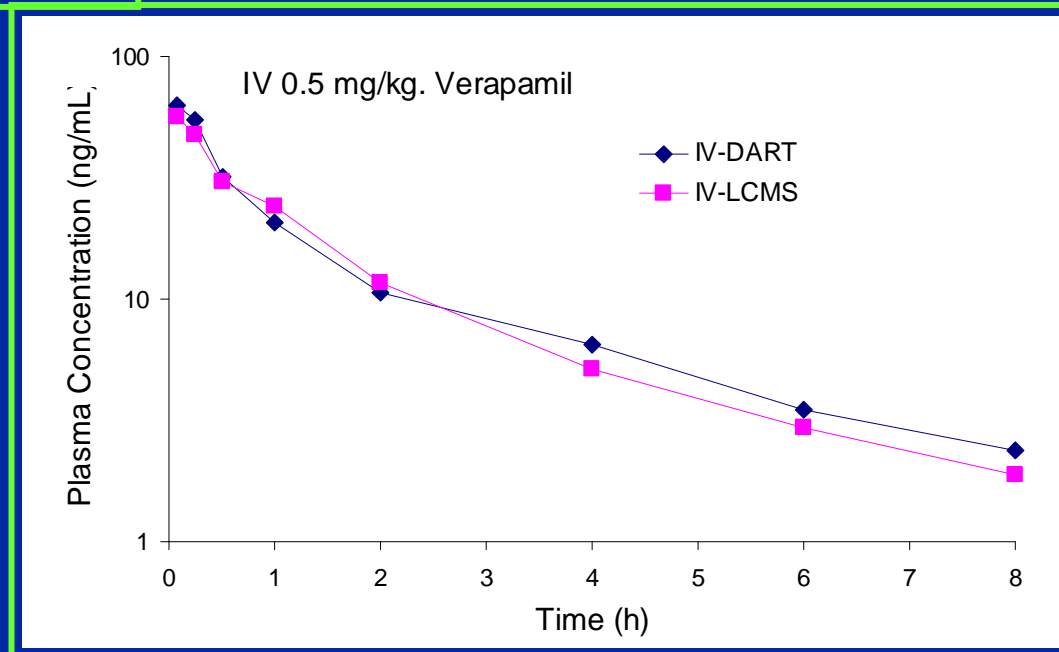
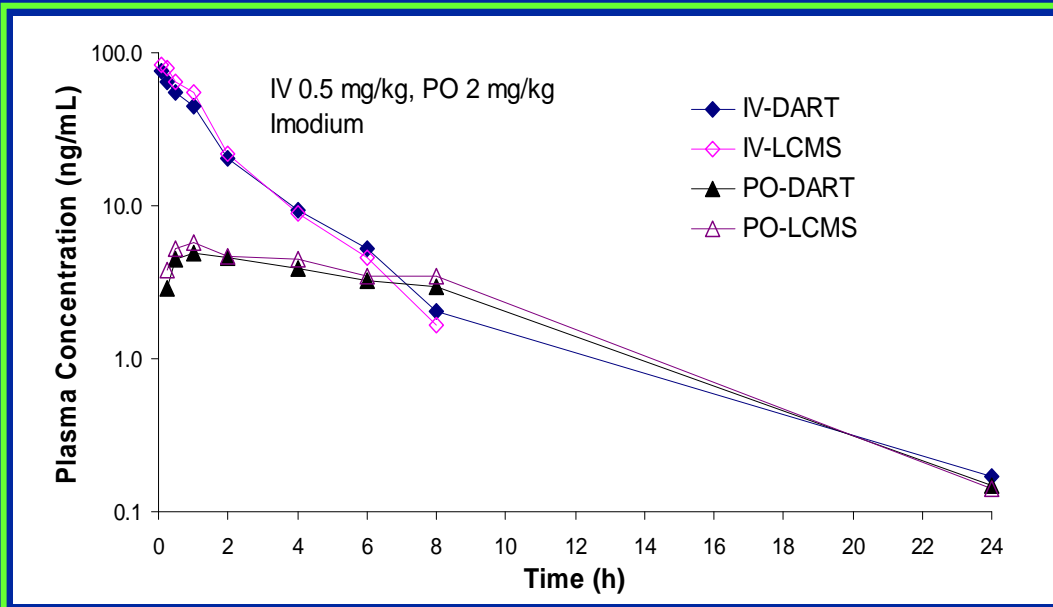
Pharmaceuticals



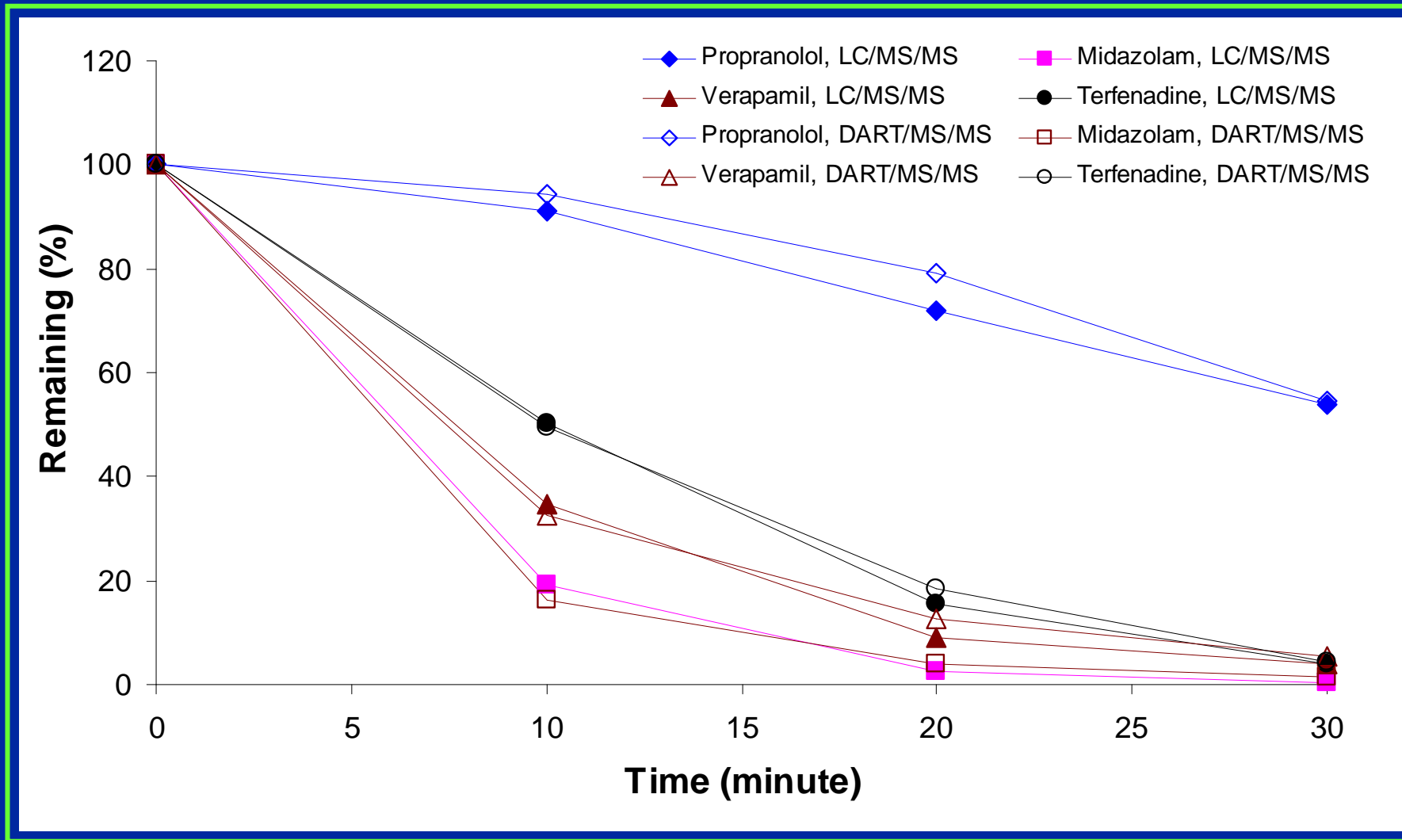
PK Profiles from DART/MS/MS and LC/MS/MS



PK Profiles from DART/MS/MS & LC/MS/MS



Comparison of metabolic stability data obtained from LC/MS/MS and DART/MS/MS



Conclusions:

The DART/MS/MS system has demonstrated significant advantages over conventional LC/MS/MS in drug research; especially in bioanalytical support of discovery and early development programs where simplicity and operating speed are a premier. The eradication of sample extraction and LC separation allowed rapid turn over. An added environmental benefit is the elimination of LC mobile phase use. Some common difficulties associated with LC/MS/MS such as carryover, contamination and peak shape integrity are also removed.

Acknowledgement



- Roche Palo Alto, LLC

Pam McLawhon, Liming Ho, Jae Cheng, Bill Fitch

- Ionsens, Inc.

Brain Musselman, Elizabeth Crawford, Joseph Tice

- Millennium Pharmaceuticals, Inc

Jing-Tao Wu, Shaoxia Yu