

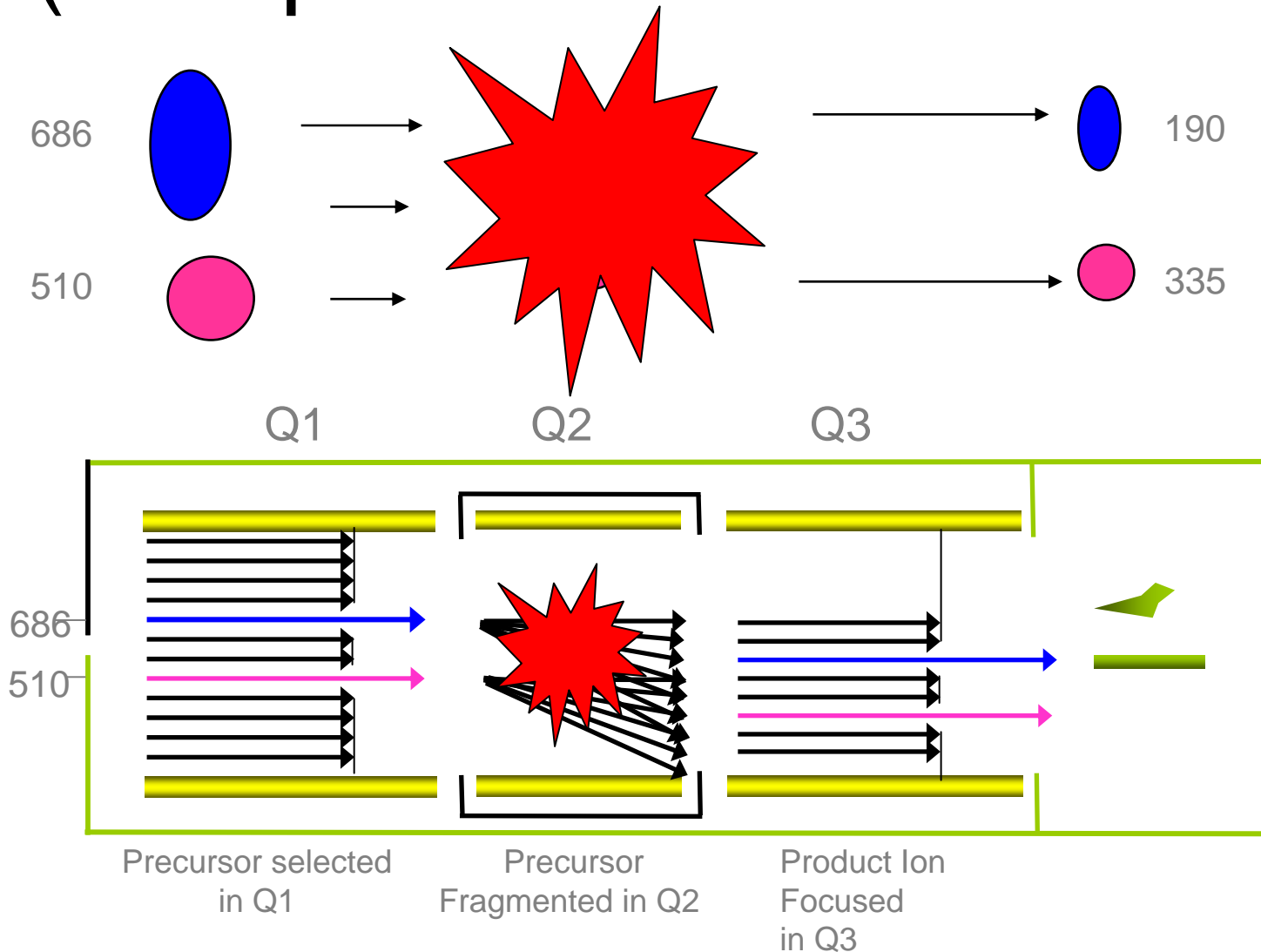
The Need for Resolution (sometimes) in Bioanalytical LC-MS/MS Methodology

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Resolution

- “For mass spectroscopists, the LC is just how your samples get into the instrument.”
- Mass spectroscopists rely on specificity of MRM scan.

MS/MS – MRM (Multiple Reaction Monitoring)



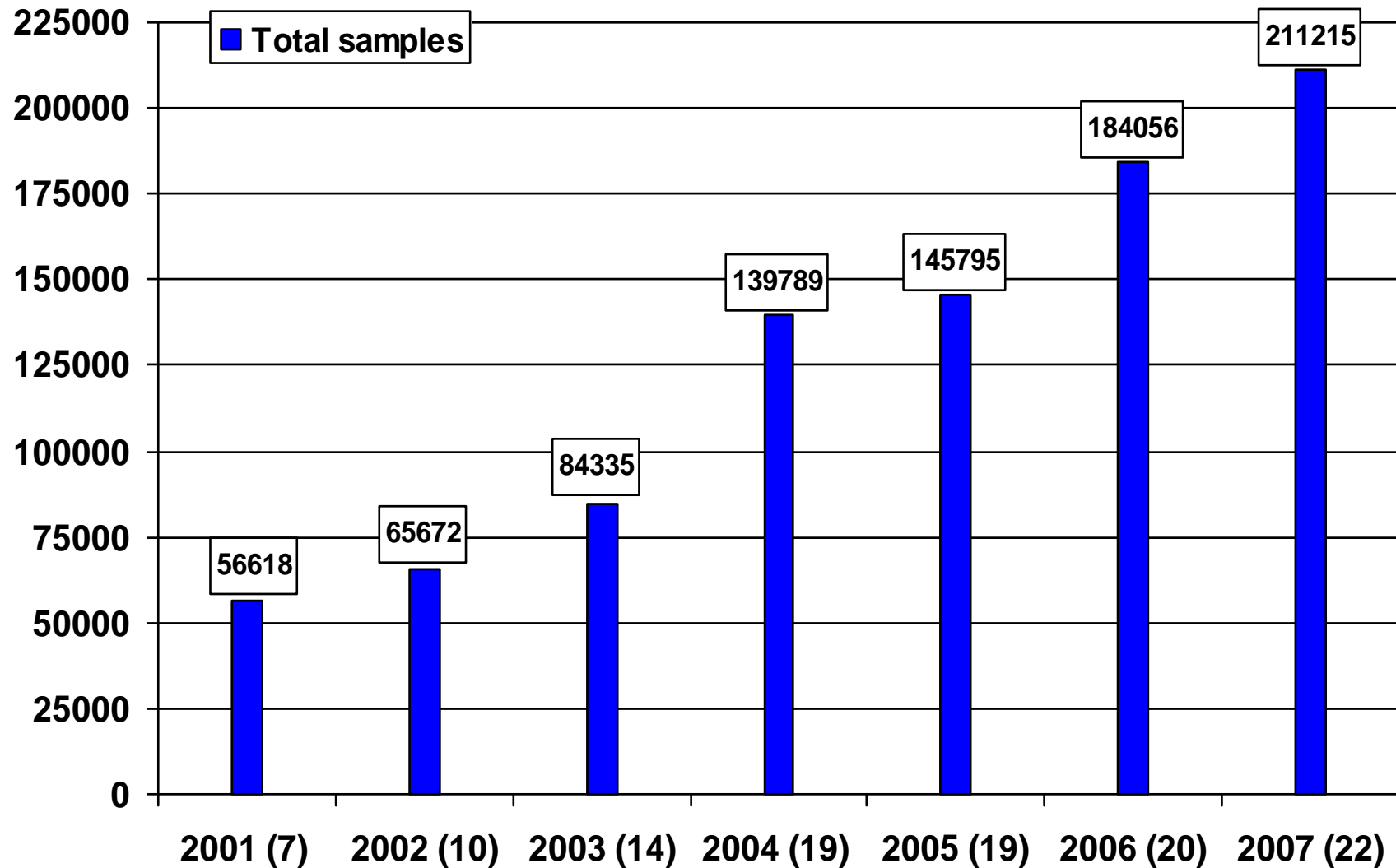
Confessions of a Reformed Mass Spectroscopist

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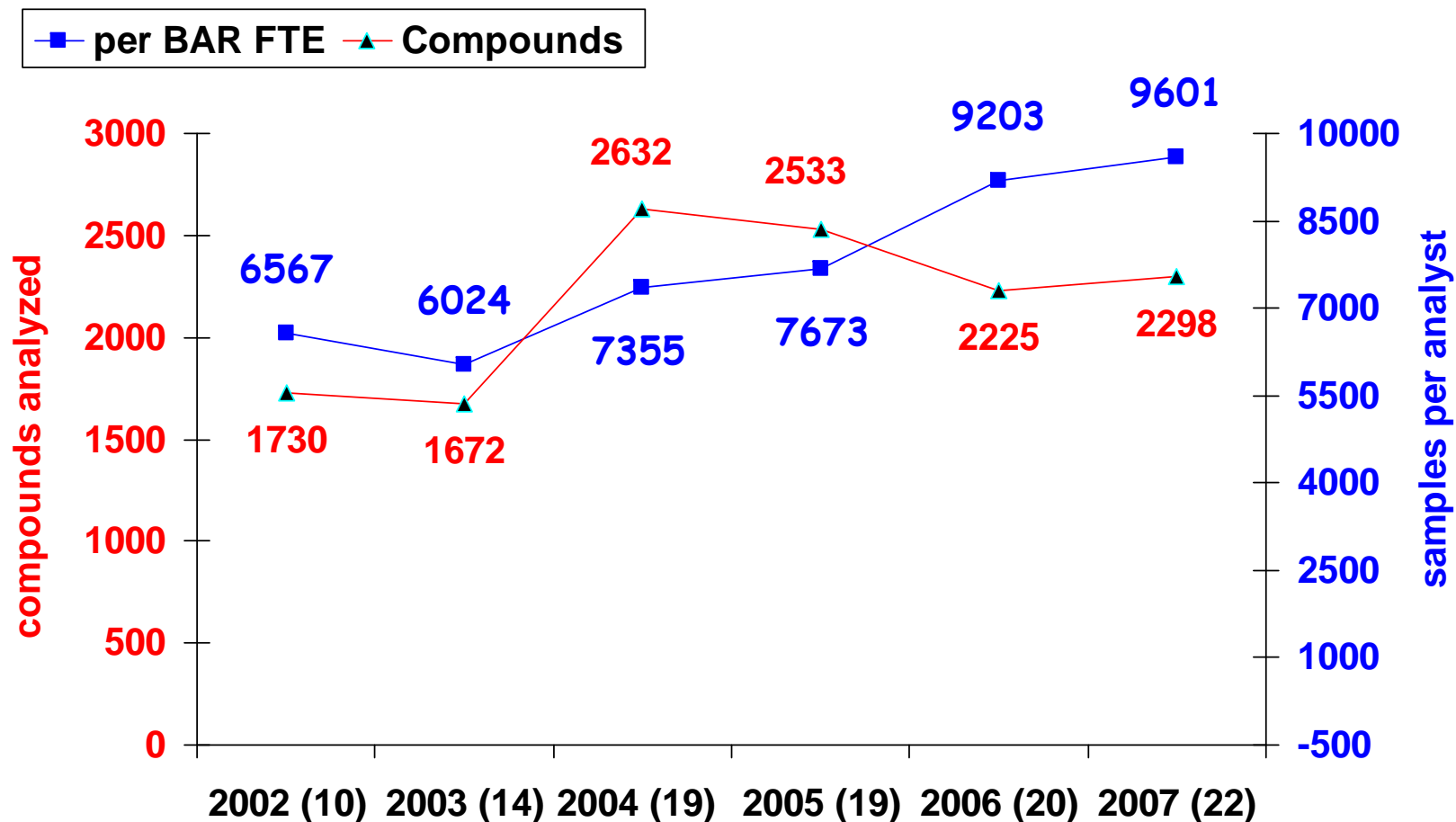
Introduction

- Why do we need to improve throughput, sensitivity and resolution?

Total Number of Samples analyzed by BAR (FTE) in support of Discovery



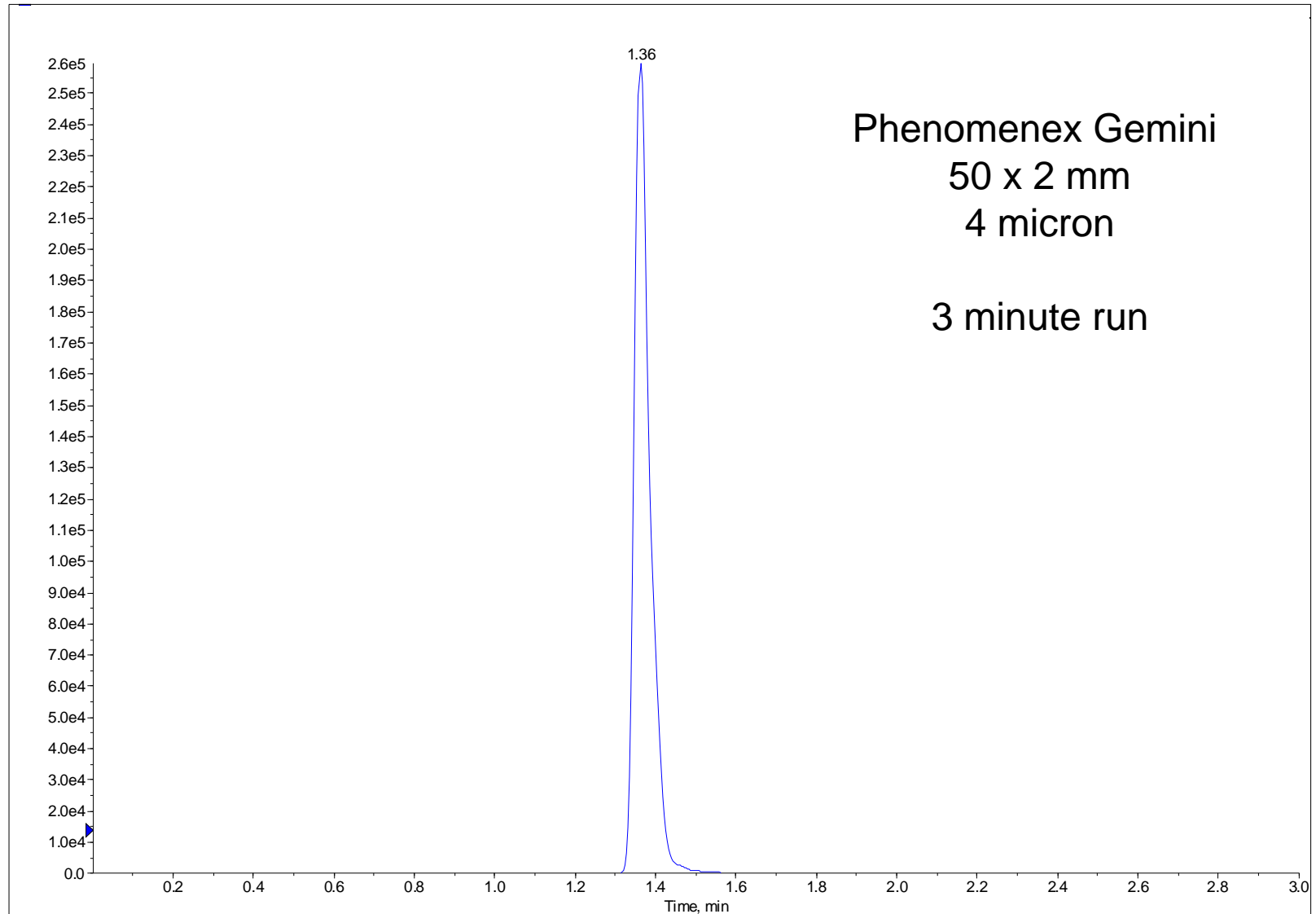
BAR-Discovery Annual Sample Count



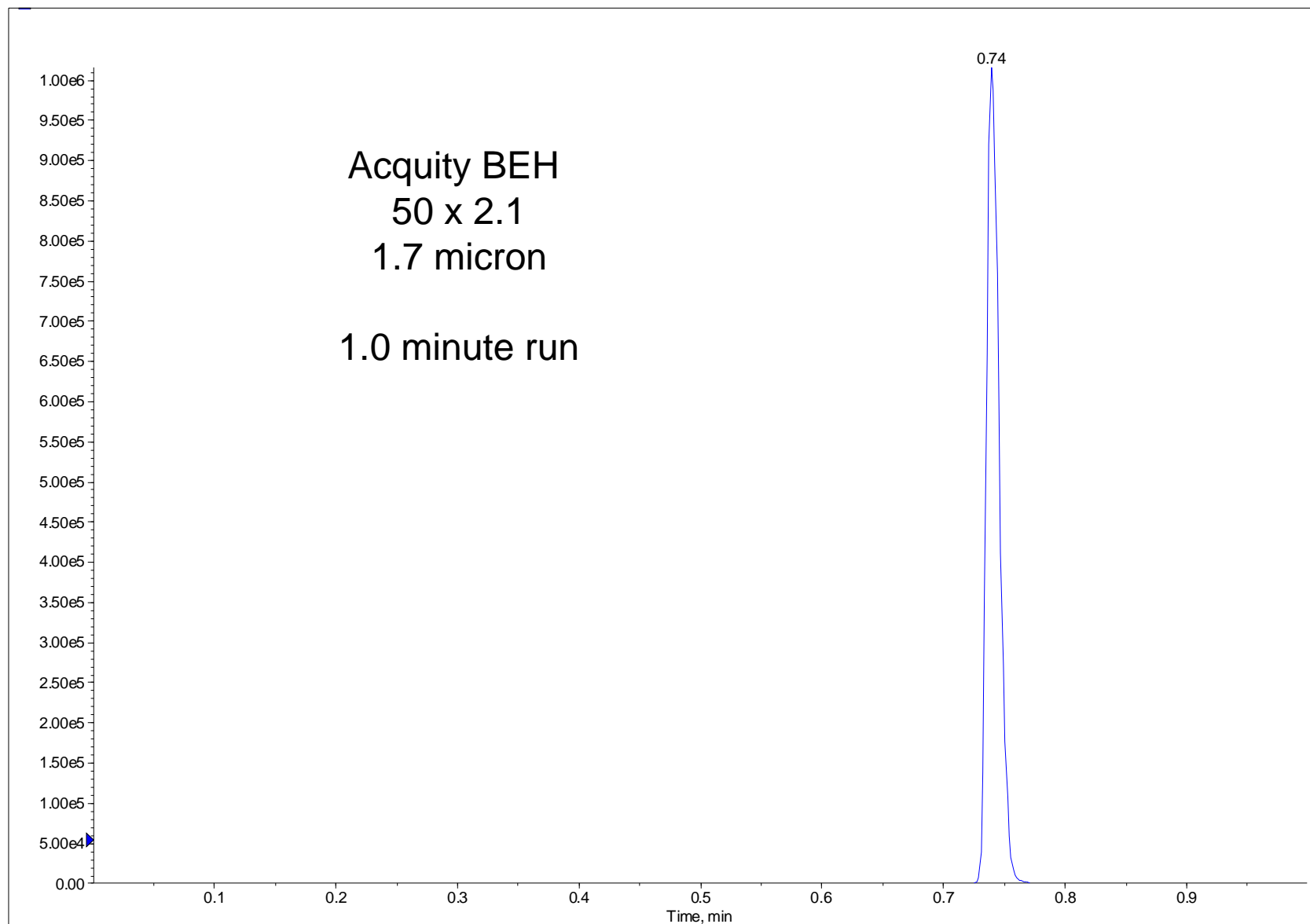
Introduction

- Why do we need to improve throughput, sensitivity and resolution?
- Sensitivity is something we are always trying to push...the use of UPLC provides us better S/N values and pushes LOQ lower.
- Resolution...lets take a look at why we need better resolution

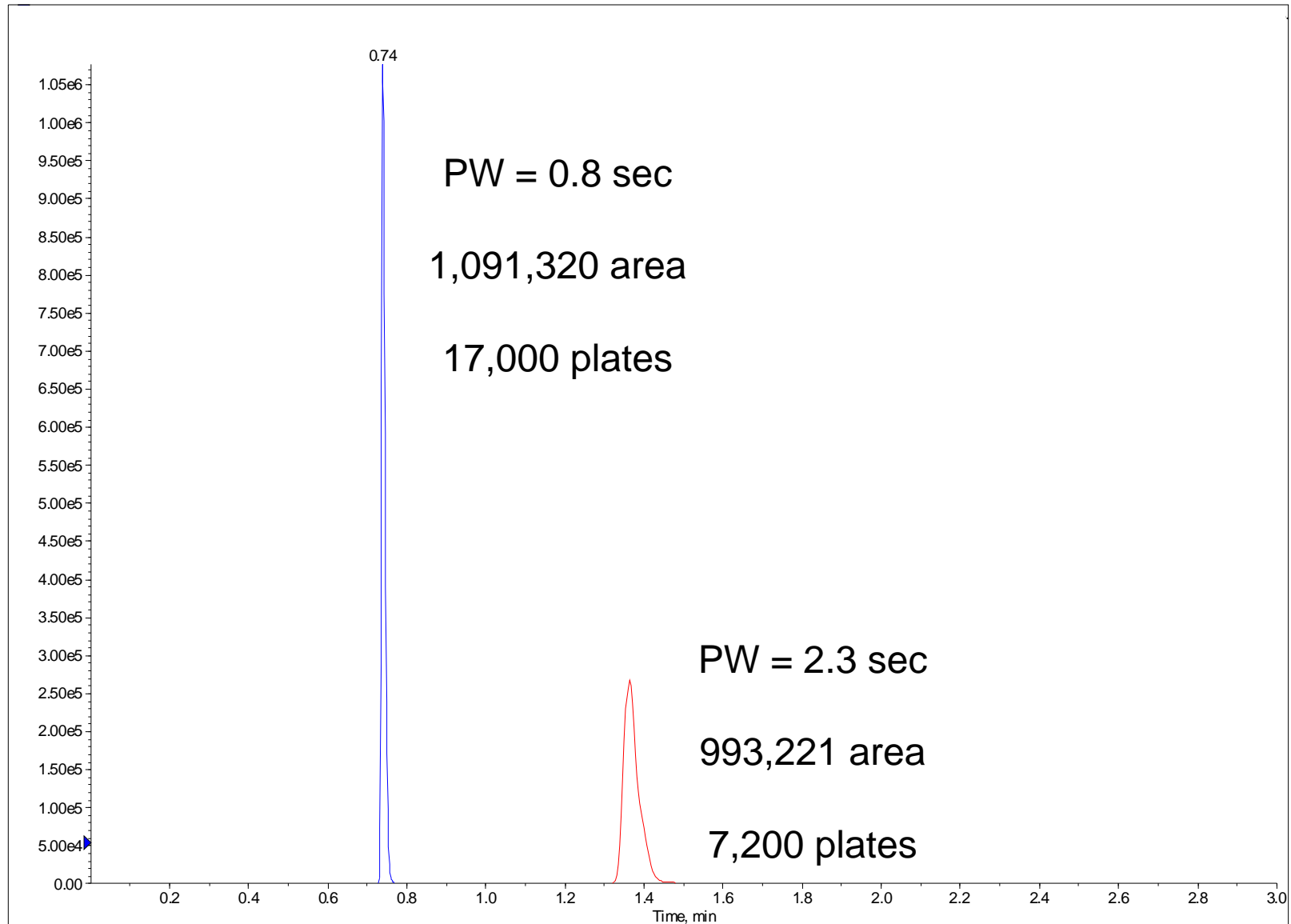
HPLC/MS/MS



UPLC/MS/MS



UPLC vs HPLC



HPLC-UPLC Method Comparison

- Traditionally, bioanalytical methods developed in early drug discovery use ballistic HPLC gradients to rapidly analyze large numbers of samples.

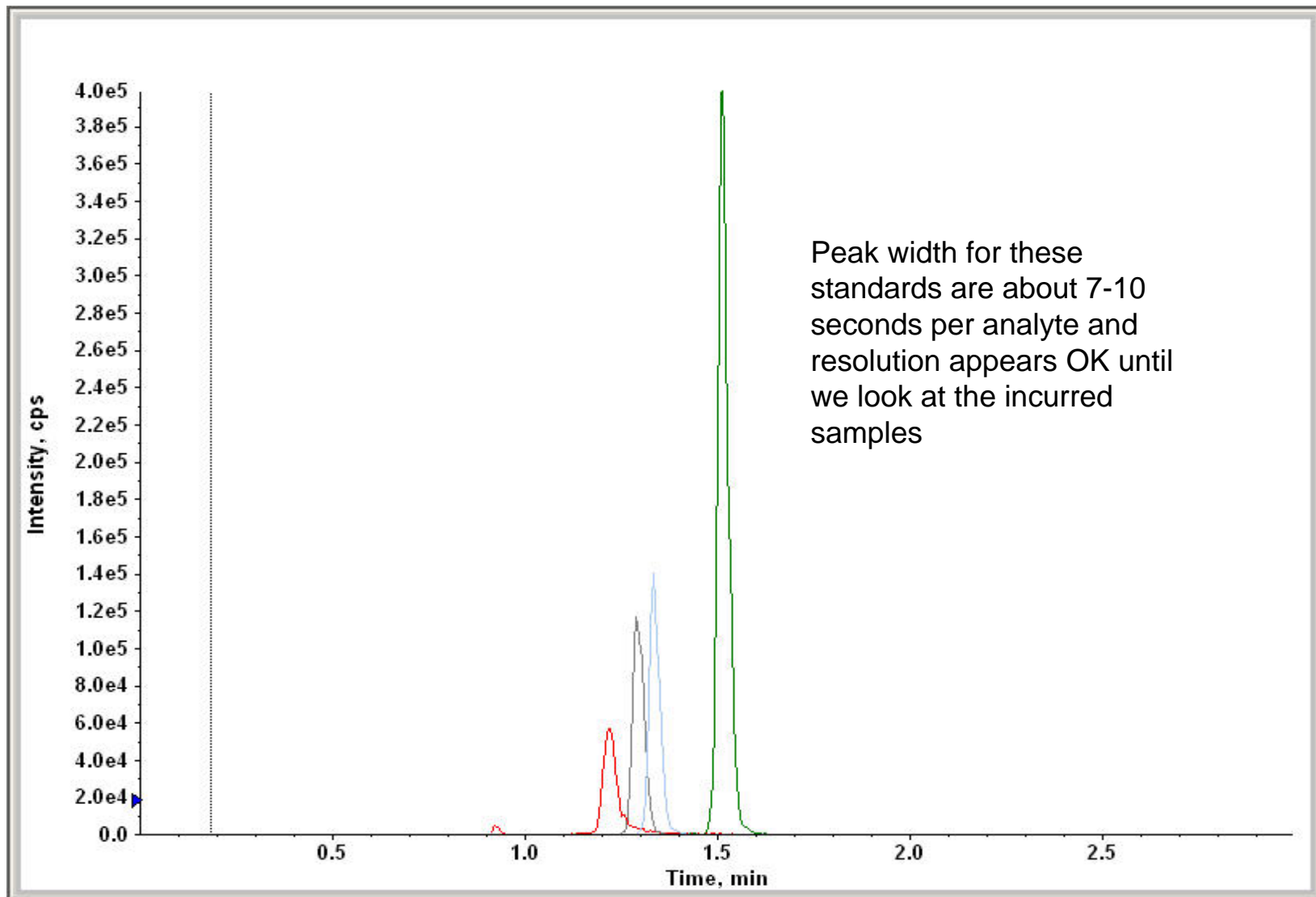
HPLC

Time (min)	%A	%B	Flow Rate (ml/min)
0	95	5	0.6
0.5	95	5	0.6
1	5	95	0.6
2.1	5	95	0.6
2.2	95	5	0.6
3	95	5	0.6

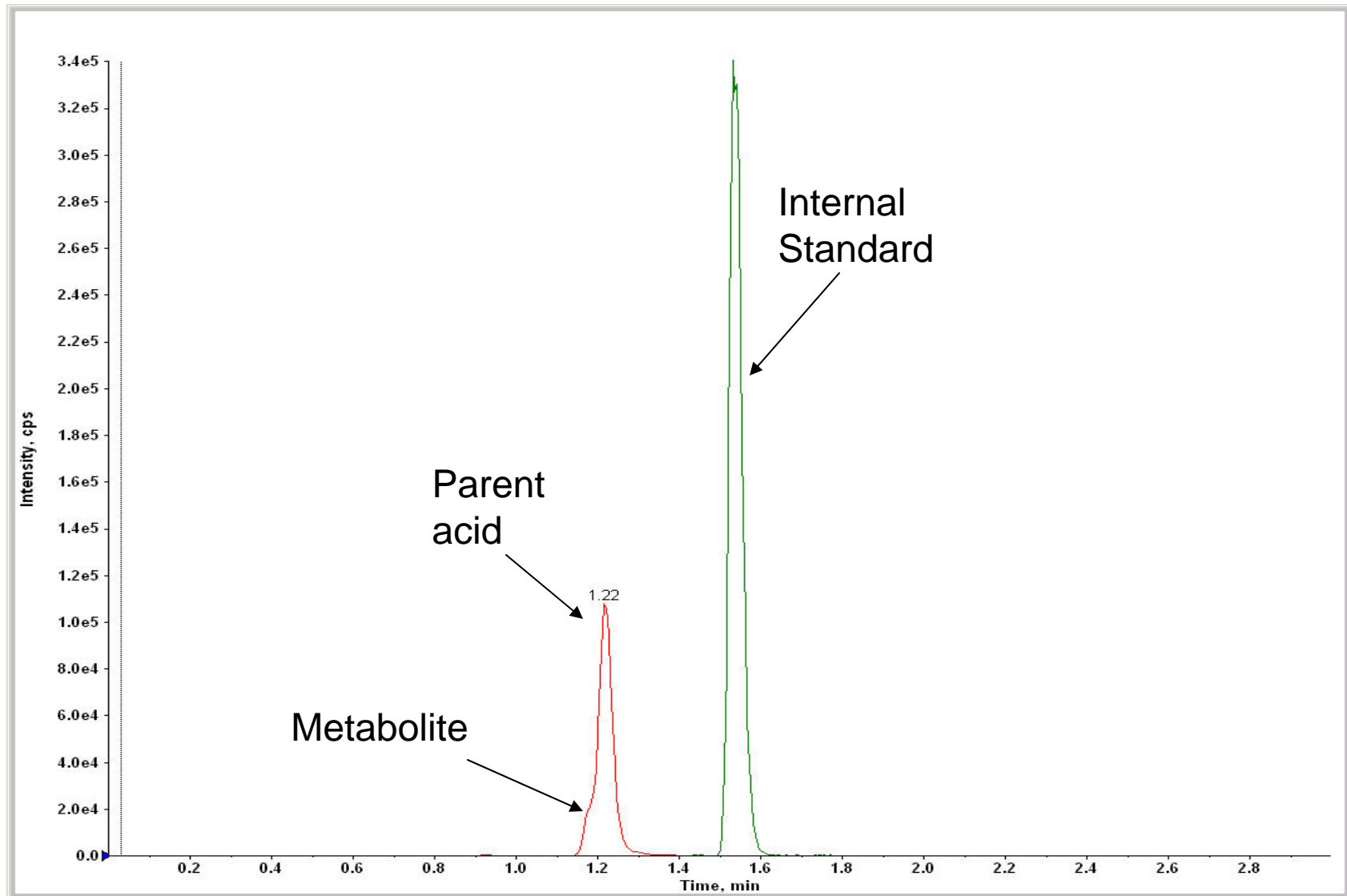
UPLC

Time (min)	%A	%B	Flow Rate (ml/min)
0	95	5	0.6
0.1	95	5	0.6
0.5	5	95	0.6
0.75	5	95	0.6
0.8	95	5	0.6
1	95	5	0.6

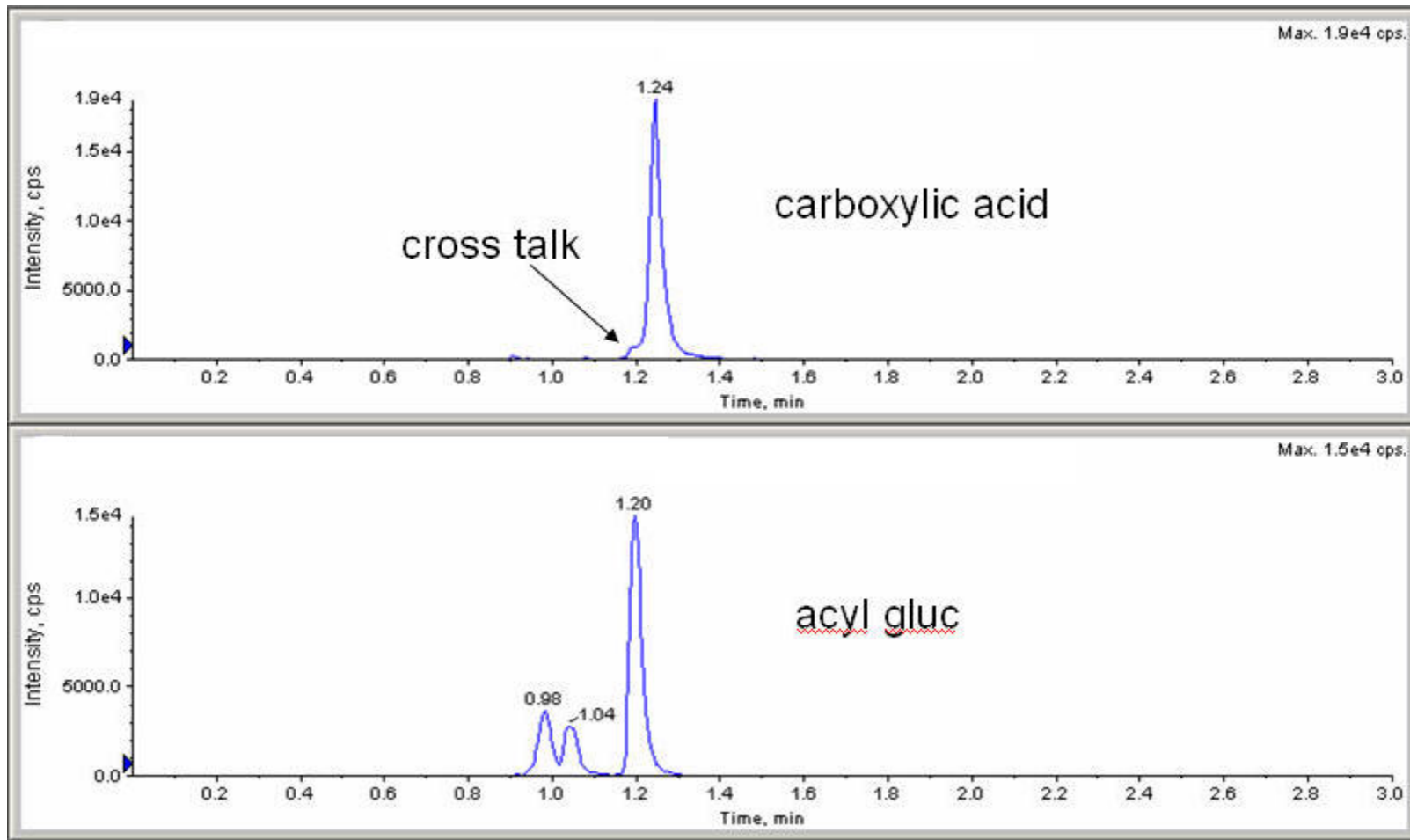
Conventional HPLC



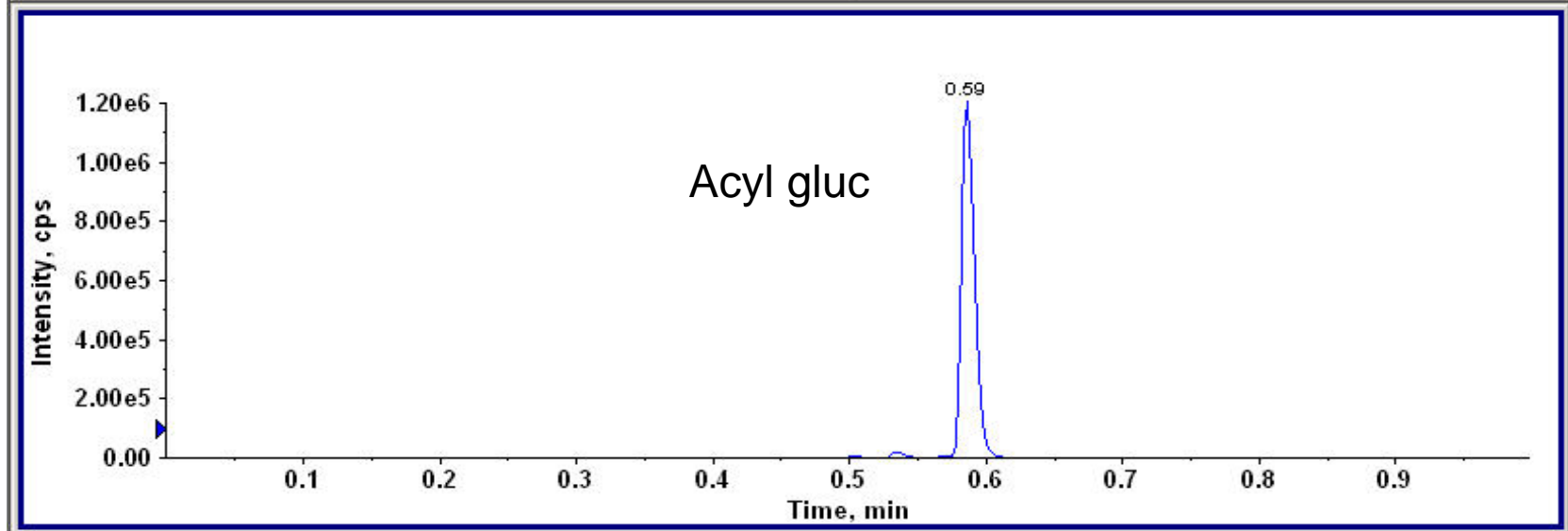
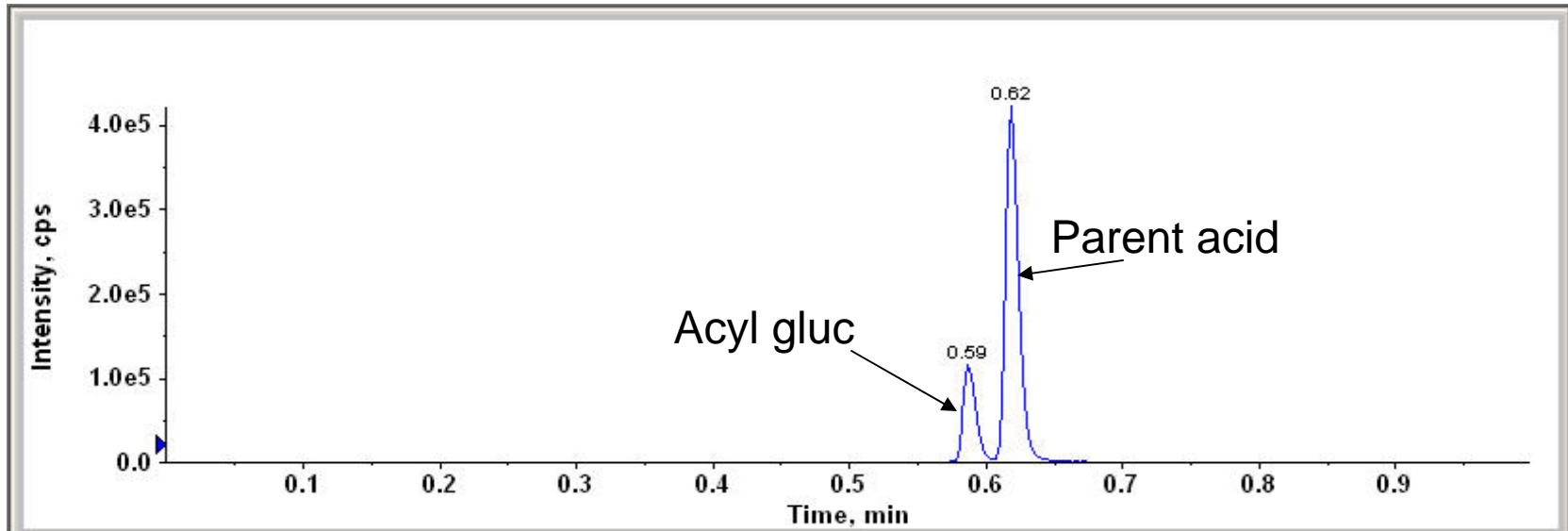
Parent acid and metabolite



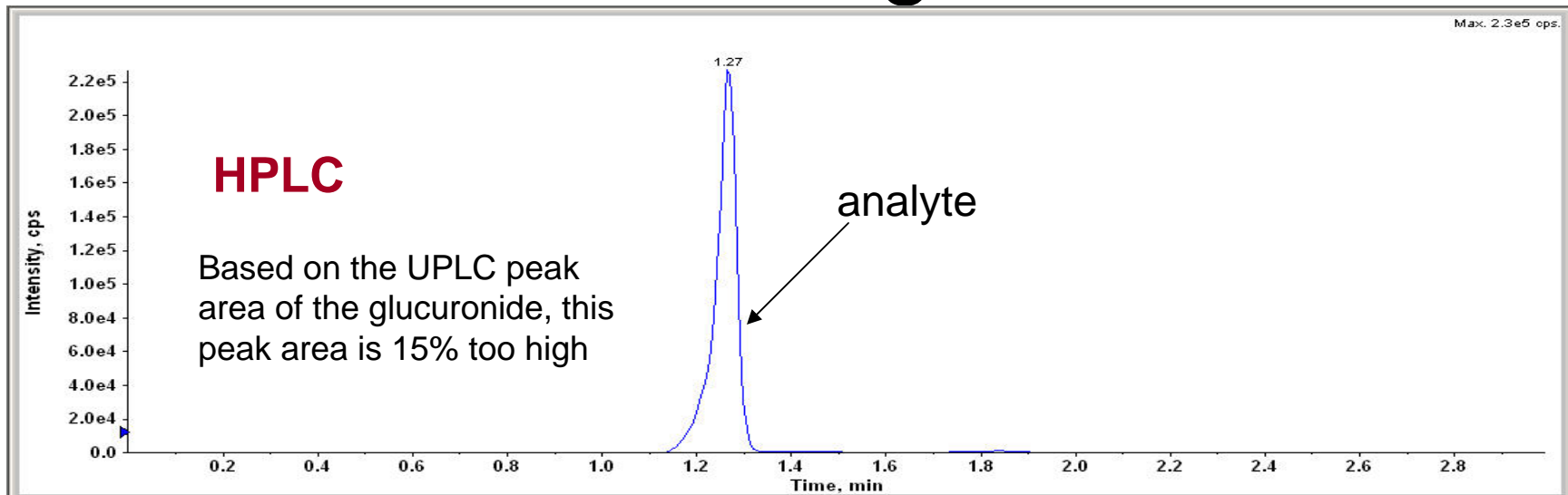
Metabolite Resolution-HPLC



Metabolite Resolution-UPLC



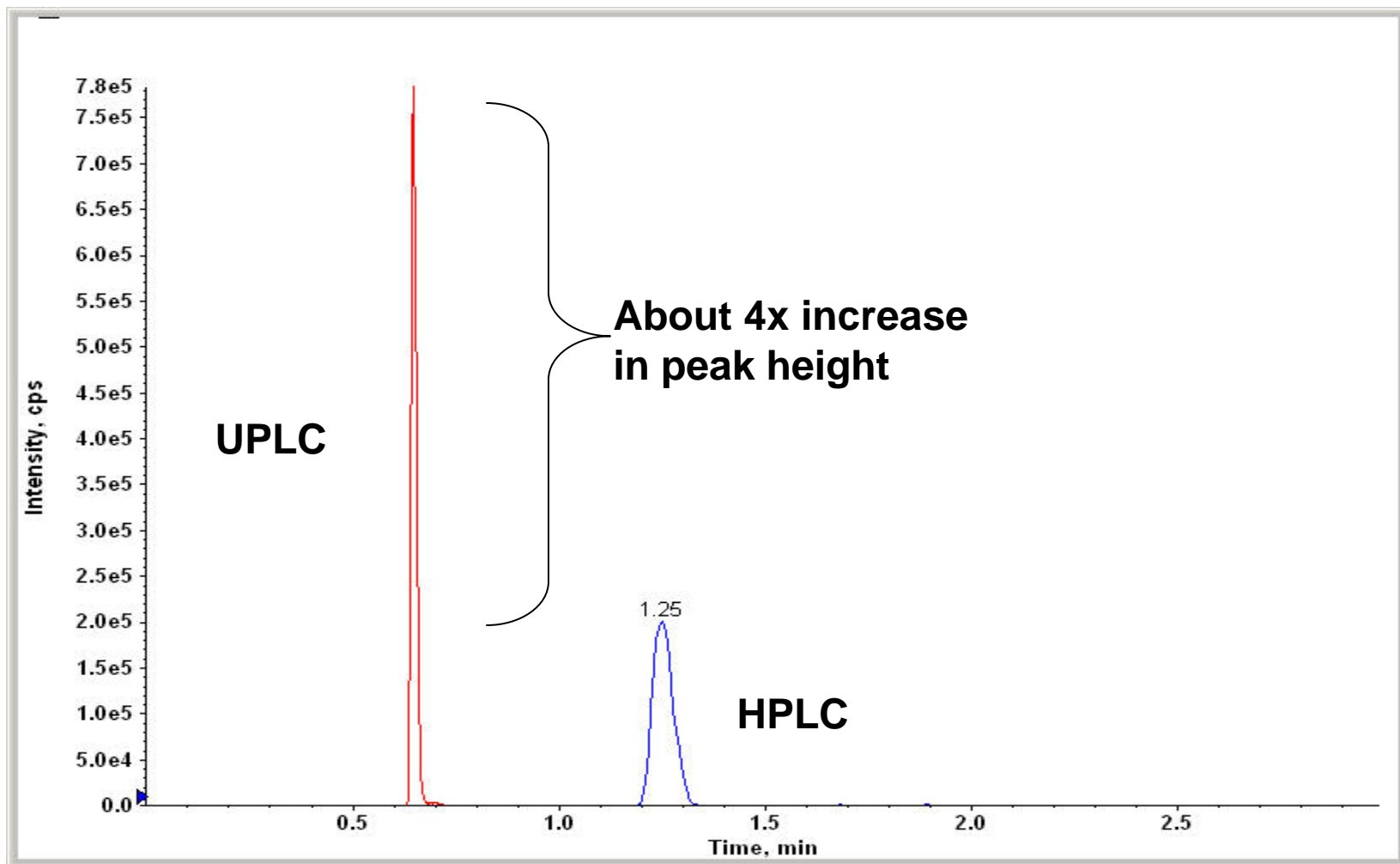
Metabolite Resolution-compound dosed in dog at 2 h



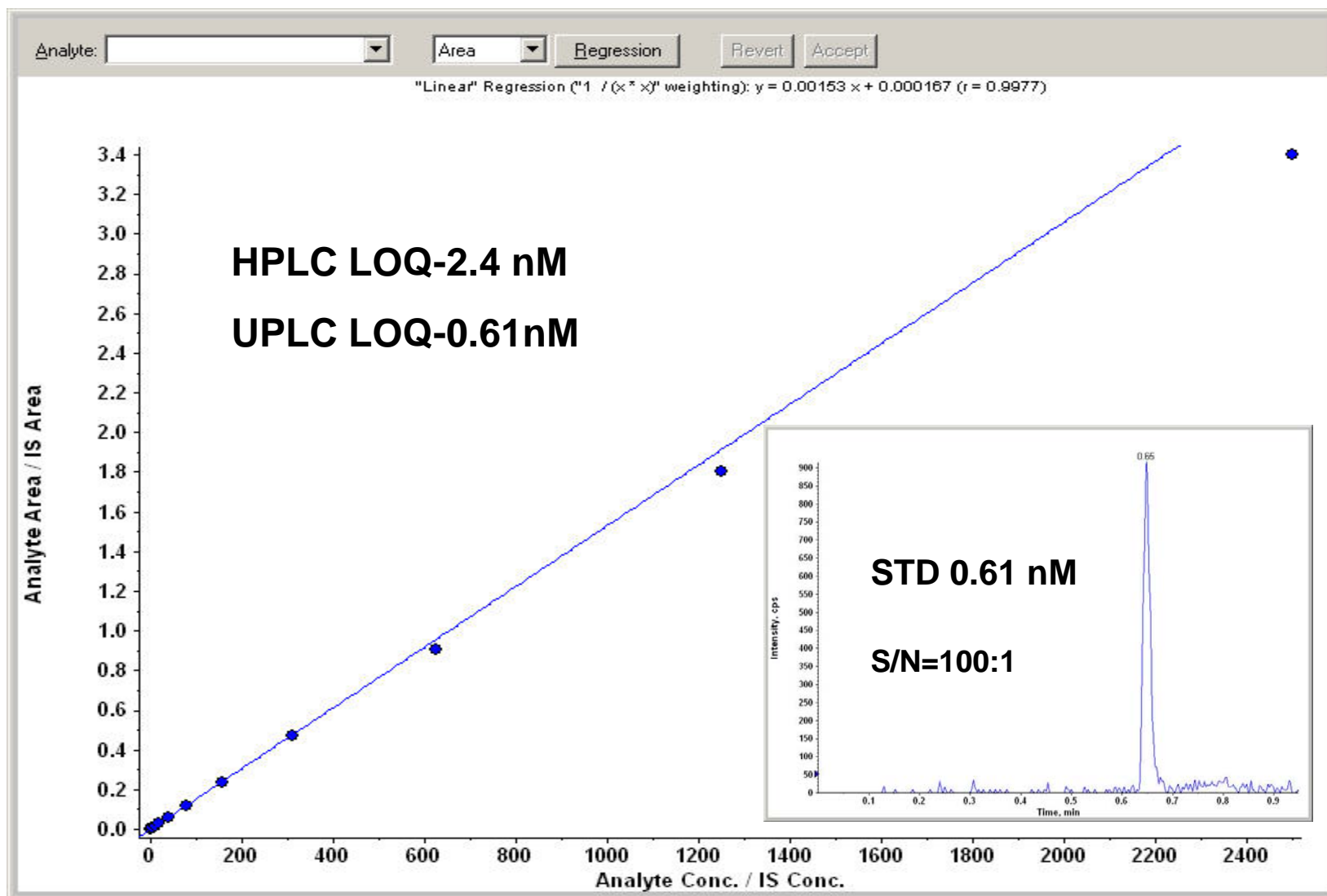
Accidental Inclusion of Metabolite

- Decrease Clearance value
- Increase AUC
- Potentially supply inaccurate data
- Could potentially misguide chemistry
- Could potentially move compound into development

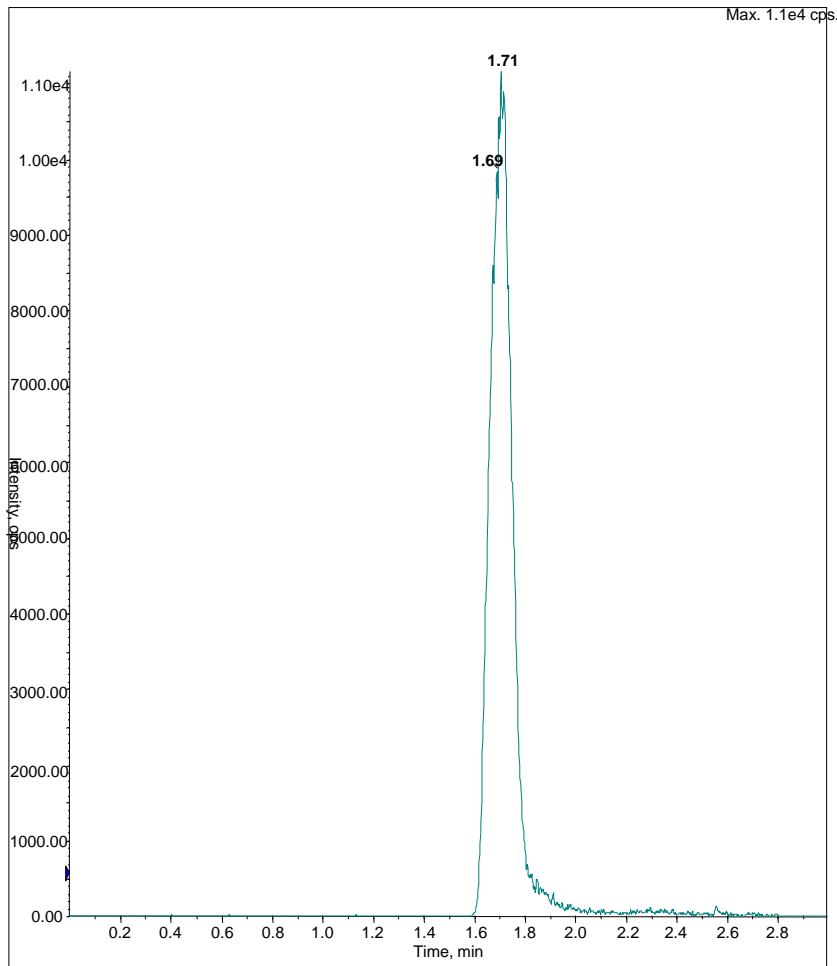
Increased Sensitivity-625 nM standard



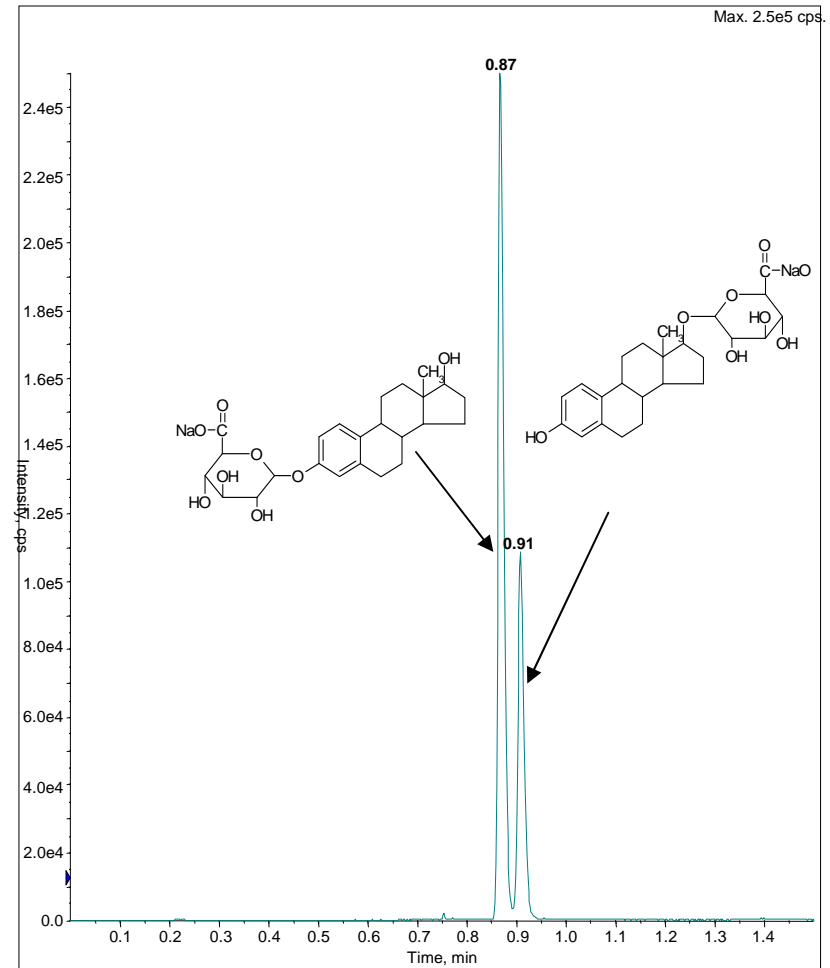
Linear Range-UPLC



Resolution of Isobaric Compounds



**HPLC resolution of
Estradiol-3-Glucuronide and
Estradiol-17-Glucuronide**



**UPLC resolution of
Estradiol-3-Glucuronide and
Estradiol-17-Glucuronide**

Acquity with API4000



Workflow-HPLC

Time/Injection minutes	Total Analysis minutes	Total Analysis hours
10	1100	18.3
8	880	14.7
5	550	9.2
4	440	7.3
3	330	5.5
1.5	165	2.8
1.1	121	2.0
0.5	55	0.9

Workflow-UPLC

Time/Injection minutes	Total Analysis minutes	Total Analysis hours
10	1100	18.3
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Dwell Time (msec)

		Number of Compounds				
		1	2	3	4	5
Peak Width (seconds)	10	1000	500	333	250	200
	5	500	250	167	125	100
	2.5	250	125	83	63	50
	1	100	50	33	25	20
	0.75	75	38	25	19	15
	0.5	50	25	17	13	10

Based on 10 points across the peak
Does not include cycle time

Conclusions

- The smaller particle size columns used in UPLC produced highly resolved chromatographic peaks with shorter run times.
- Increased peak heights obtained from the UPLC data resulted in a higher signal to noise ratio for all standards and yielded a 3-5x gain in sensitivity over HPLC.
- Mass spectrometer dwell times were reduced to 25 msec to accommodate the narrow UPLC peaks.
- The UPLC integrates directly into the Analyst software configuration of the Sciex mass spectrometer.
- Metabolite interferences that could lead to inaccurate quantitation are easier to resolve with UPLC

Acknowledgements

- Timothy Olah
- Adrienne Tymiak
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- Sarah Taylor
- Kimberly Snow

