

High Resolution Mass Spectrometry for Qualitative and Quantitative Toxicology Analysis

William Clarke, PhD, MBA, DABCC
Associate Professor, Pathology
Johns Hopkins University School of
Medicine

Disclosures

- Research funding: NIH, FDA, Thermo Fisher, Nova Biomedical, Saladax Biomedical
- Consulting/Advisory Boards: Thermo Fisher, Nova Biomedical, Roche Diagnostics, Instrumentation Laboratories

Historic Johns Hopkins Hospital



Historic Johns Hopkins Hospital



- Johns Hopkins Hospital founded in 1889
- Johns Hopkins SOM founded four years later
- First to use rubber gloves in surgery
- First to develop renal dialysis and CPR
- Discovery of restriction enzymes
- Birthplace of HeLa cells

Johns Hopkins Hospital Today



Johns Hopkins Hospital

- 1,059 patient beds with >2,000 full-time attending physicians
- JHH ranked #1 in the US for 21 of the past 22 years (including 2013)
- >120,000 inpatient admissions annually;
>350,000 emergency visits annually
- Johns Hopkins Medicine is #1 in federal research support, with >\$450M annually
- 18 Nobel Laureates are current or former JHM scientists

Clinical Mass Spectrometry Lab



Current Lab Members



Purpose/Mission Statement

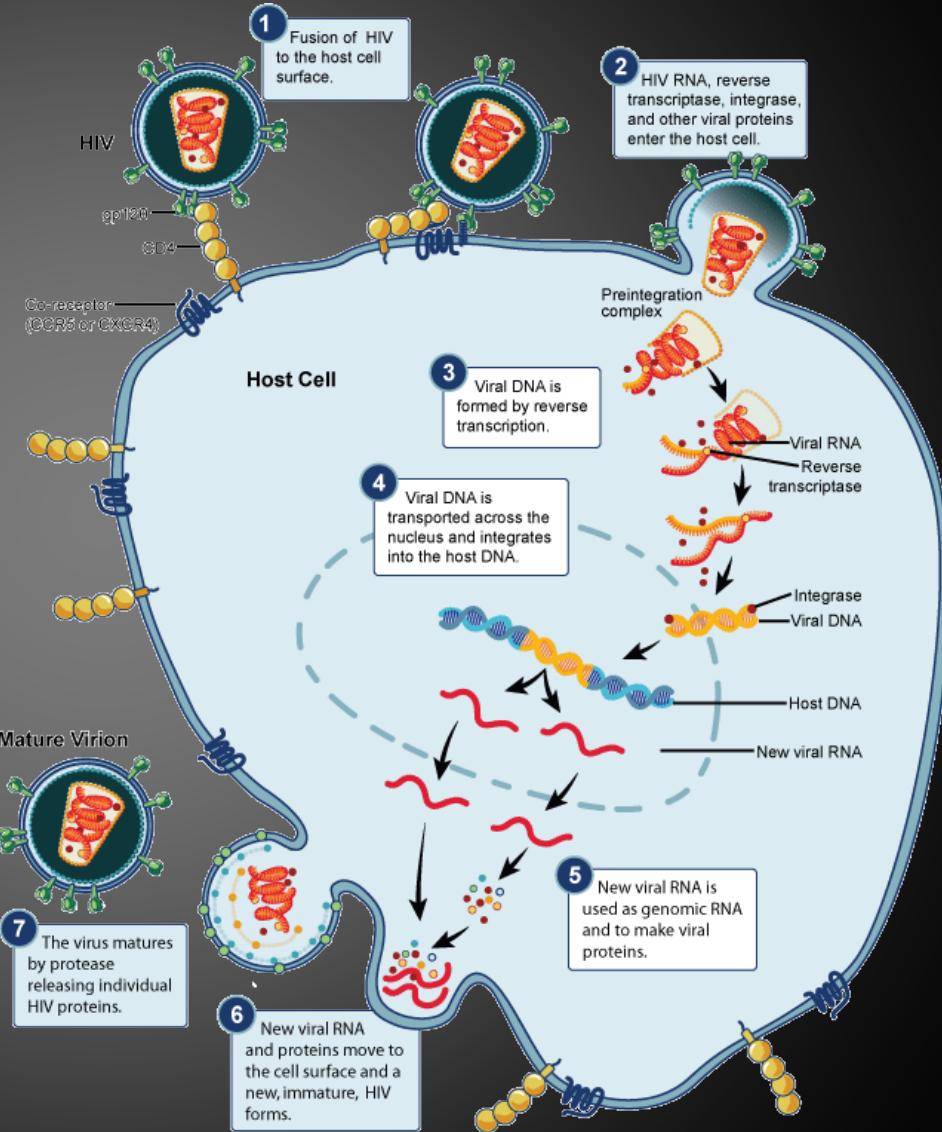
- Develop methods for clinical analyses where gaps are identified
- Custom assay design for support of clinical studies and trials
- Advance clinical mass spectrometry through research and consulting

HIGH RESOLUTION MASS SPECTROMETRY SCREENING AND ANTIRETROVIRAL ADHERENCE MONITORING

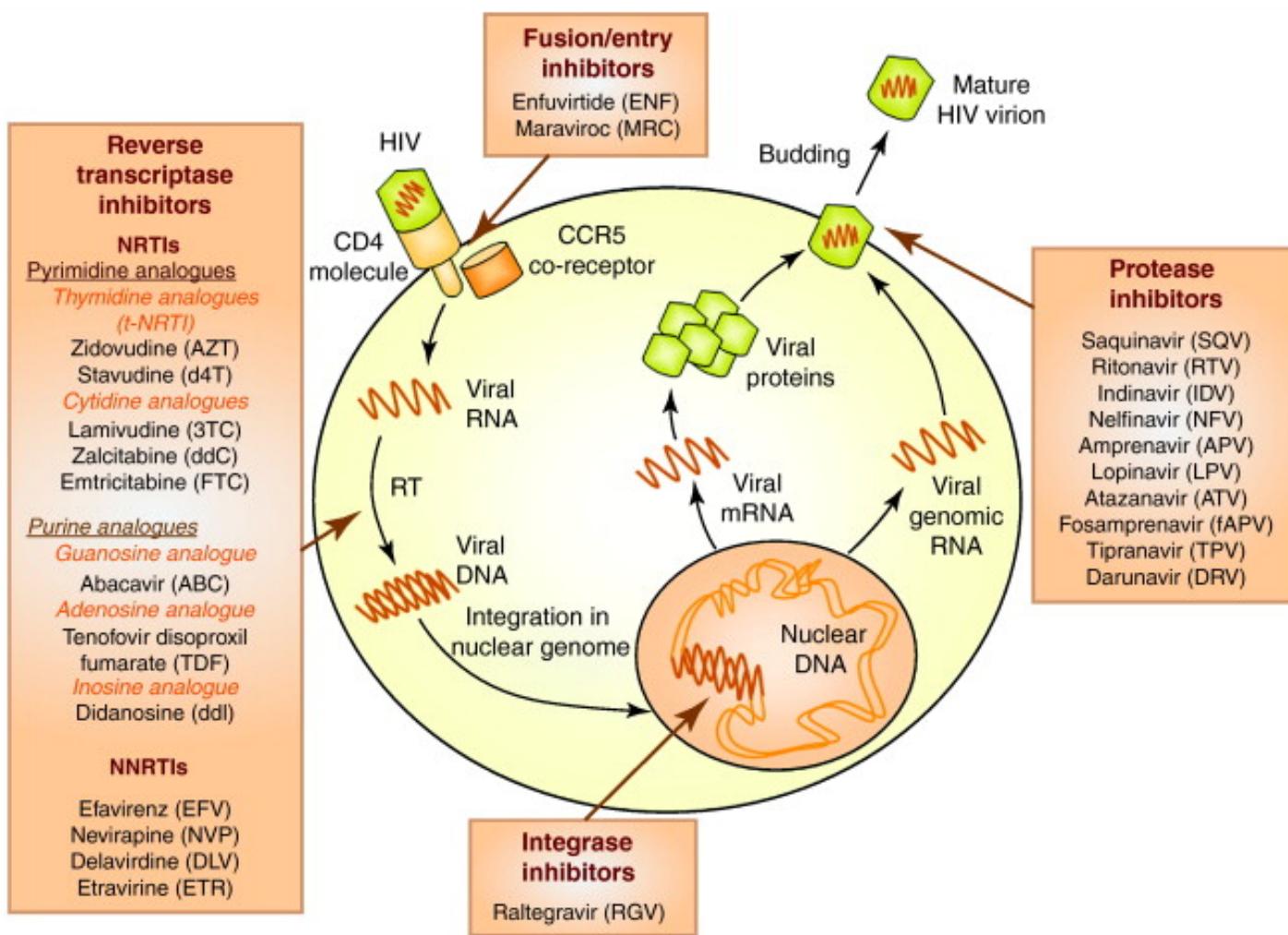
HIV Life Cycle

Major steps in HIV infection

- Adsorption of viral particles to CD4 receptor (via gp120)
- Release of HIV RNA, RT, Integrase
- Virion RT activation and viral cDNA synthesis
- Viral DNA incorporated into host genome
- Production of viral proteins
- Protease-mediated production of mature viral particles



Antiretroviral (ARV) Targets



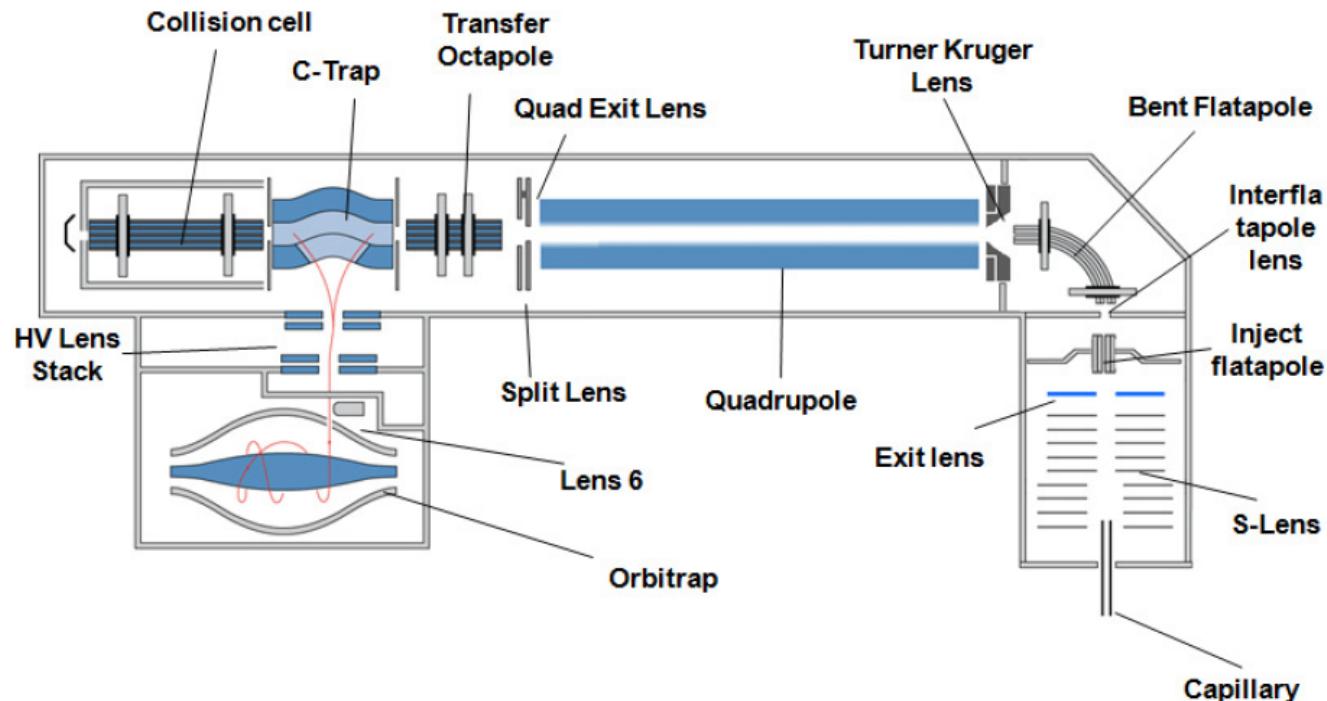
TRENDS in Pharmacological Sciences

Use of ARVs in HIV Prevention

- **HIV Prevention Trials Network (HPTN)** is a global collaborative network focused on non-vaccine interventions of HIV transmission prevention
- Data suggest a correlation between HIV viral load and HIV transmission (increased viral load leads to an increased likelihood of HIV transmission)
 - ART as Treatment
- Studies have demonstrated that administration of ARVs at birth (and prenatally) prevent HIV transmission
 - Pre-exposure Prophylaxis (PrEP)
- Several completed and ongoing trials are focused on preventative treatment with ARVs as pre-exposure prophylaxis in high risk populations

High Resolution Accurate Mass MS

Q Exactive: Overview

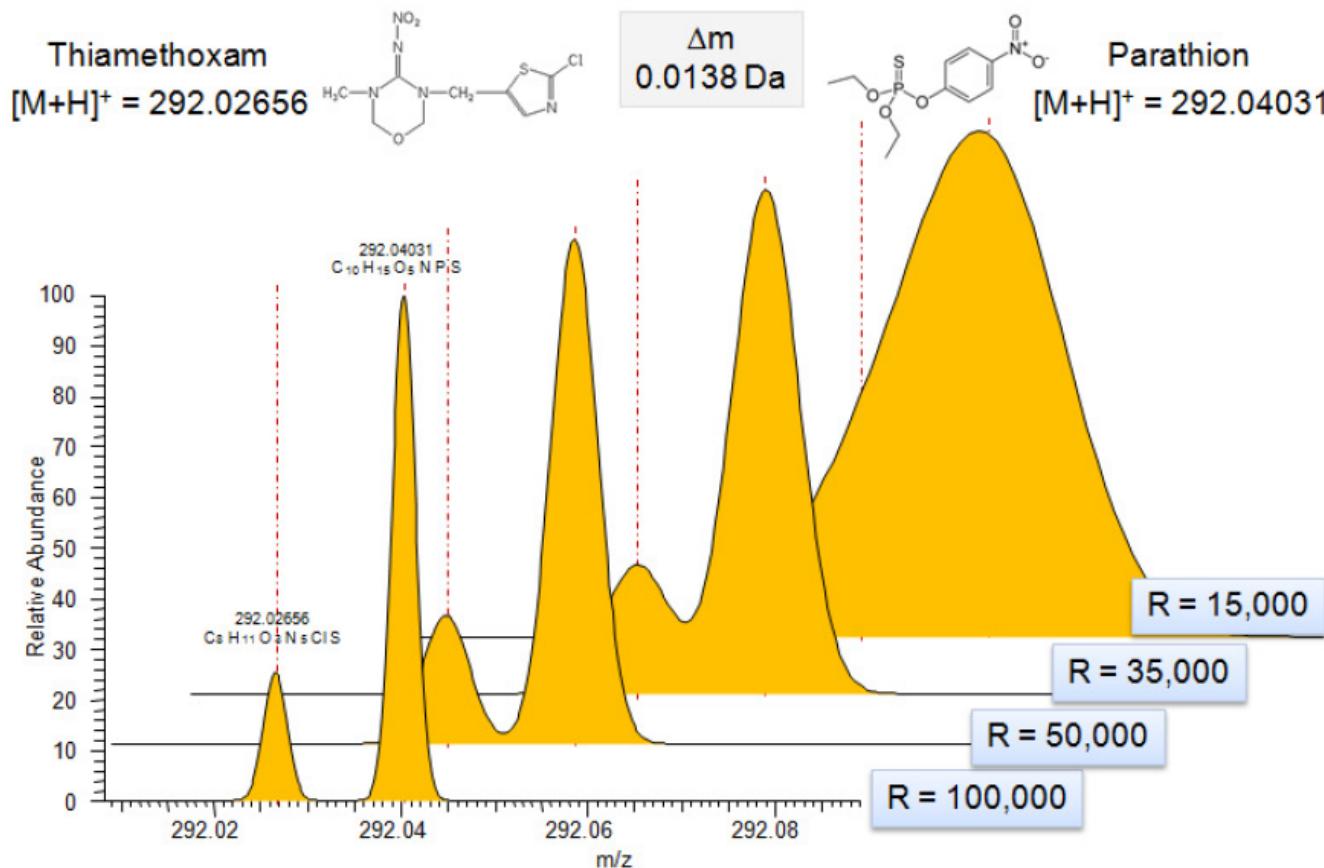


Q Exactive Specifications

Q Exactive	
Mass Accuracy	< 1ppm with internal and < 5 ppm with external calibration
Mass Range	m/z 50-4000
Scan Range	first mass < m/z < 15 x first mass
Resolution	140,000 @ m/z <u>200</u>
Scan Speed	12 scans / sec
Fragmentation	HCD with precursor and in-source CID
Dynamic Range	within one spectrum > 5000:1
Polarity Switching	One positive and one negative scan within 1 second at lowest resolution

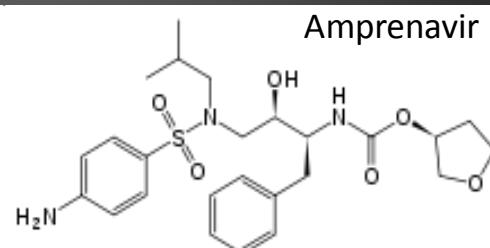
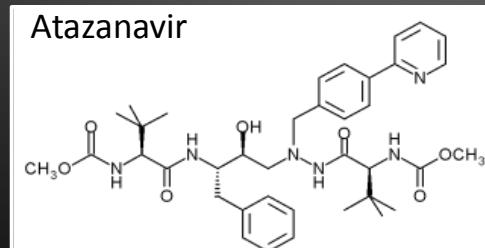
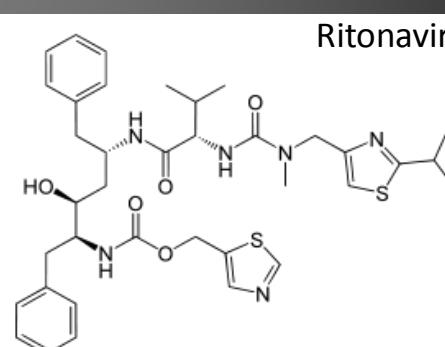
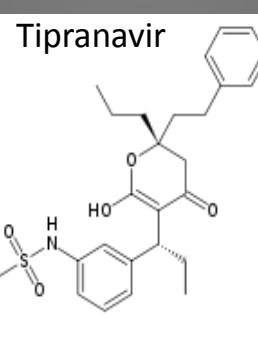
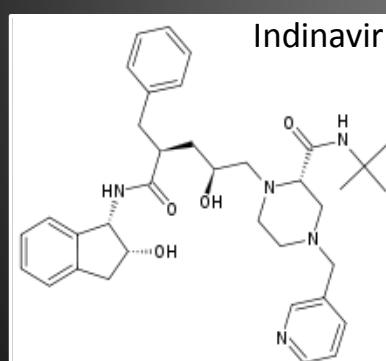
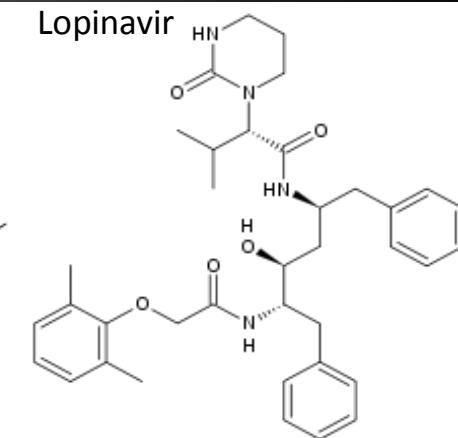
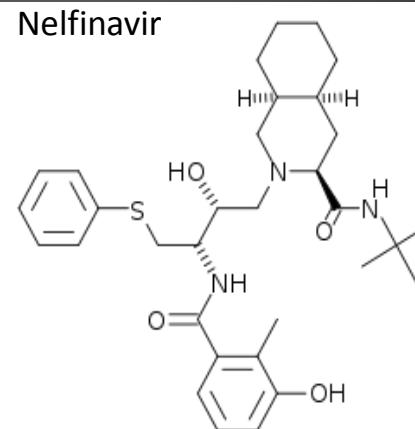
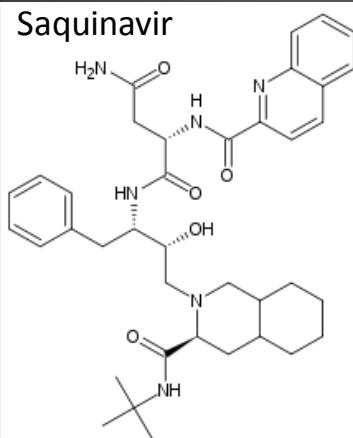
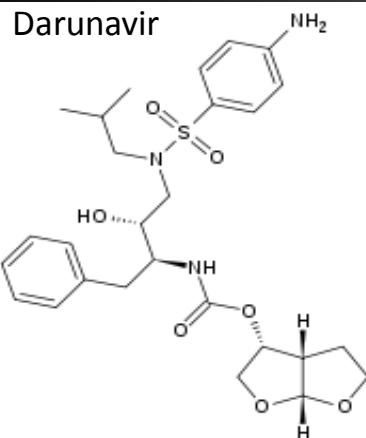
High Resolution

Isobaric Pesticides: Mix 1:3 Simulated



ARV Panel

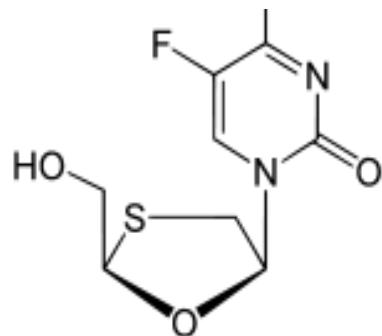
PIs



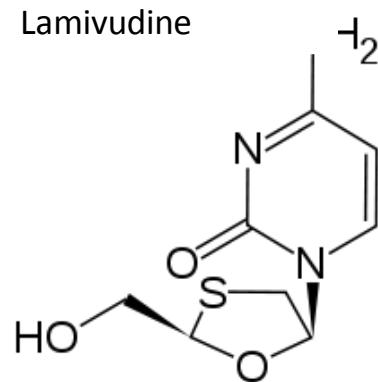
ARV Panel

NRTIs

Emtricitabine

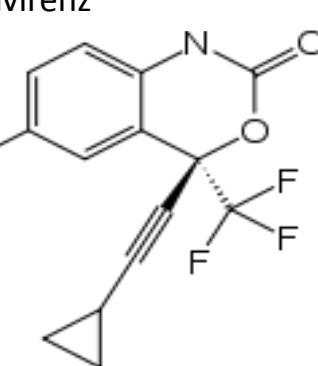


Lamivudine

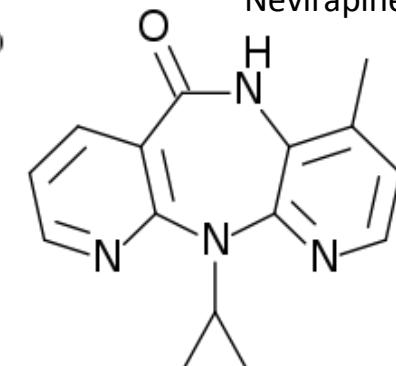


NNRTIs

Efavirenz

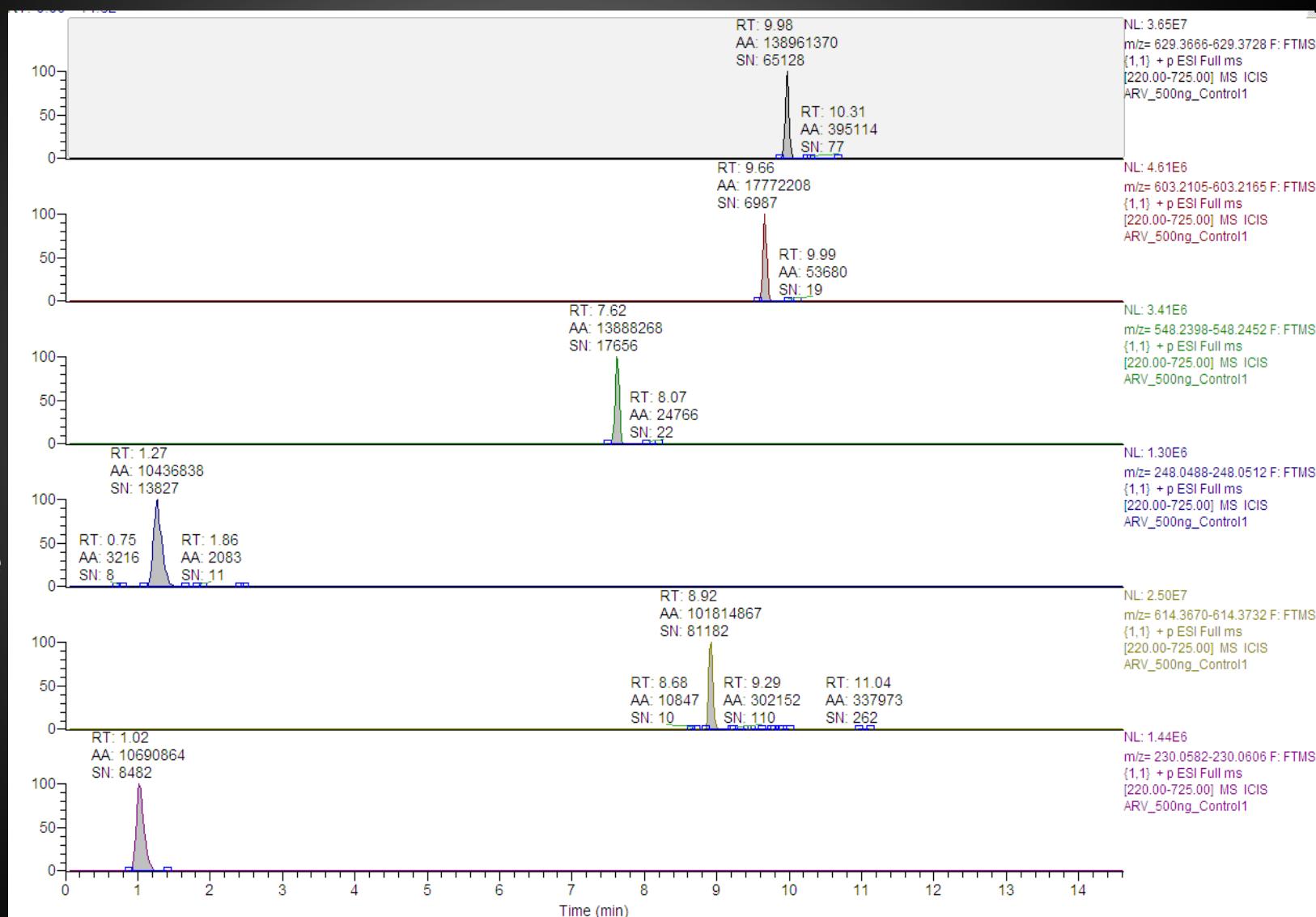


Nevirapine



LC-MS/MS Analysis of ARVs prepared in drug-free serum

Lopinavir



Tipranavir

Darunavir

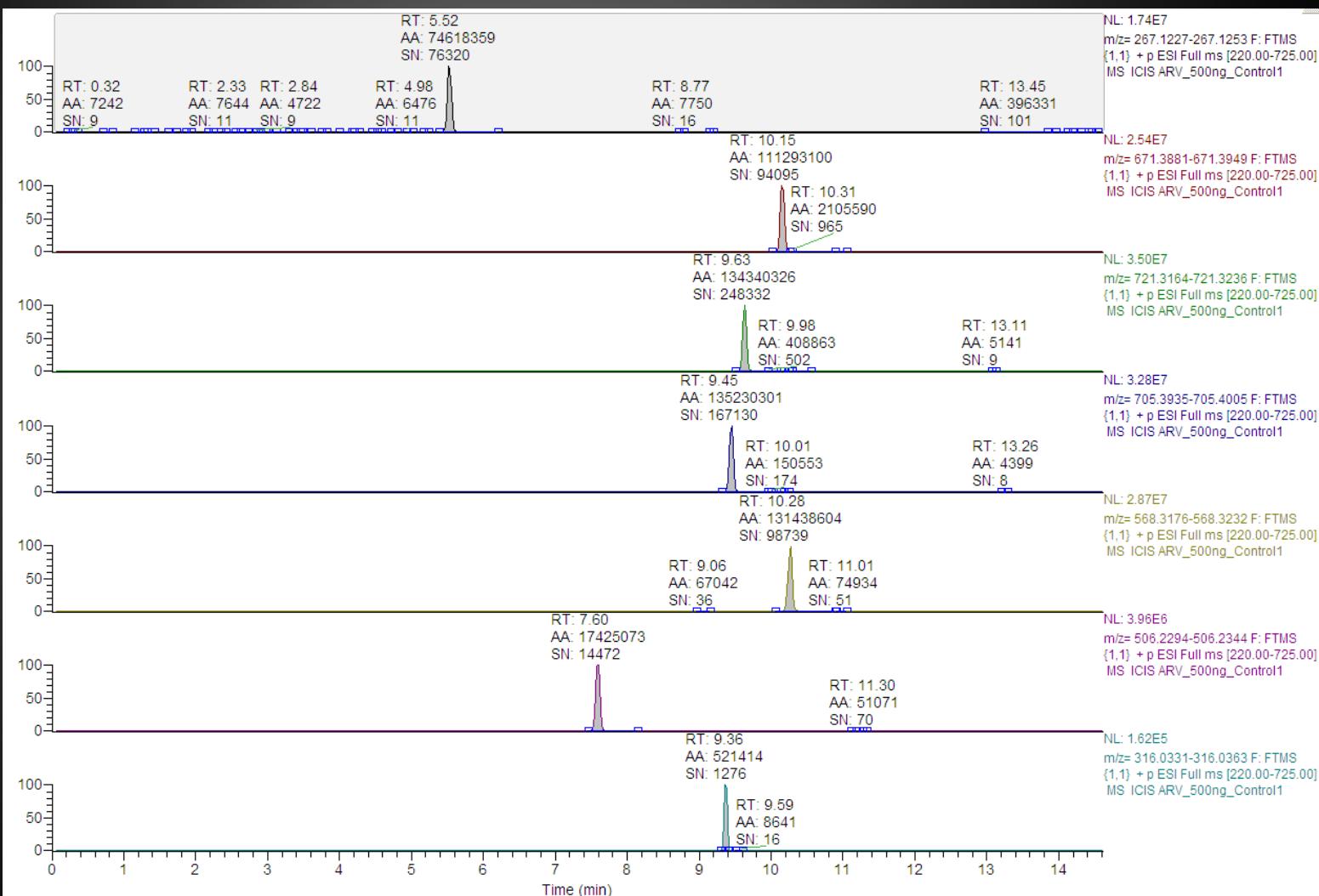
Emtricitabine

Indinavir

Lamivudine

LC-MS/MS Analysis of ARVs prepared in drug-free serum

Nevirapine



Saquinavir

Ritonavir

Atazanavir

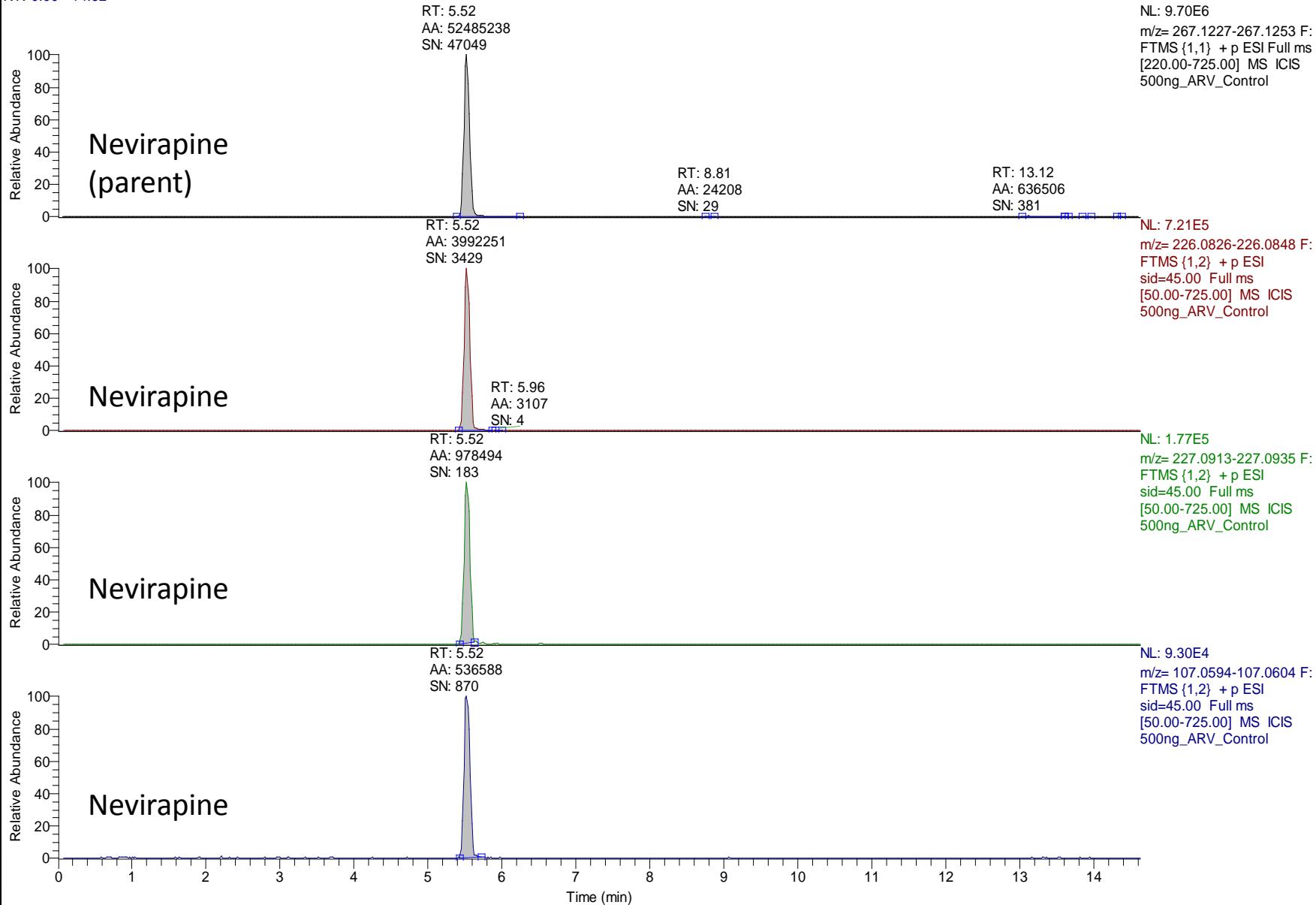
Nelfinavir

Amprenavir

Efavirenz

Nevirapine Fragmentation Analysis

RT: 0.00 - 14.62



Concordance with SRM-based LC-MS/MS Methods

Concordance of ARV Screening Panel with SRM-Methods

ARV	Total hits (either method)	% agree	% missed by Screen	% missed by Reference
Atazanavir	7	86	0	14
Efavirenz	22	73	9	18
Lamivudine	81	78	6	16
Nevirapine	76	78	6	16
Ritonavir	3	100	0	0

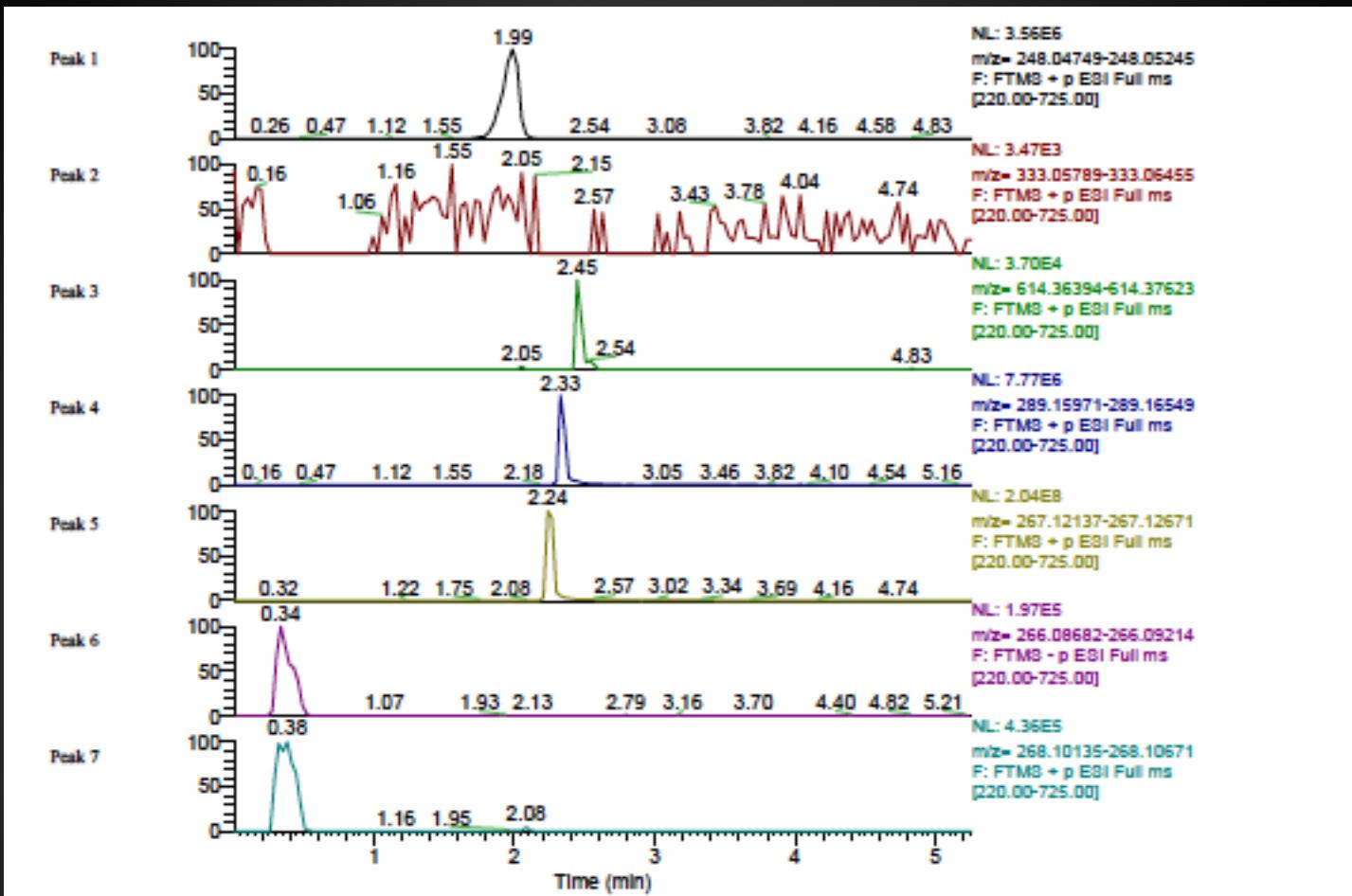
Concordance with SRM-based LC-MS/MS Methods

Concordance of ARV Screening Panel with SRM-Methods

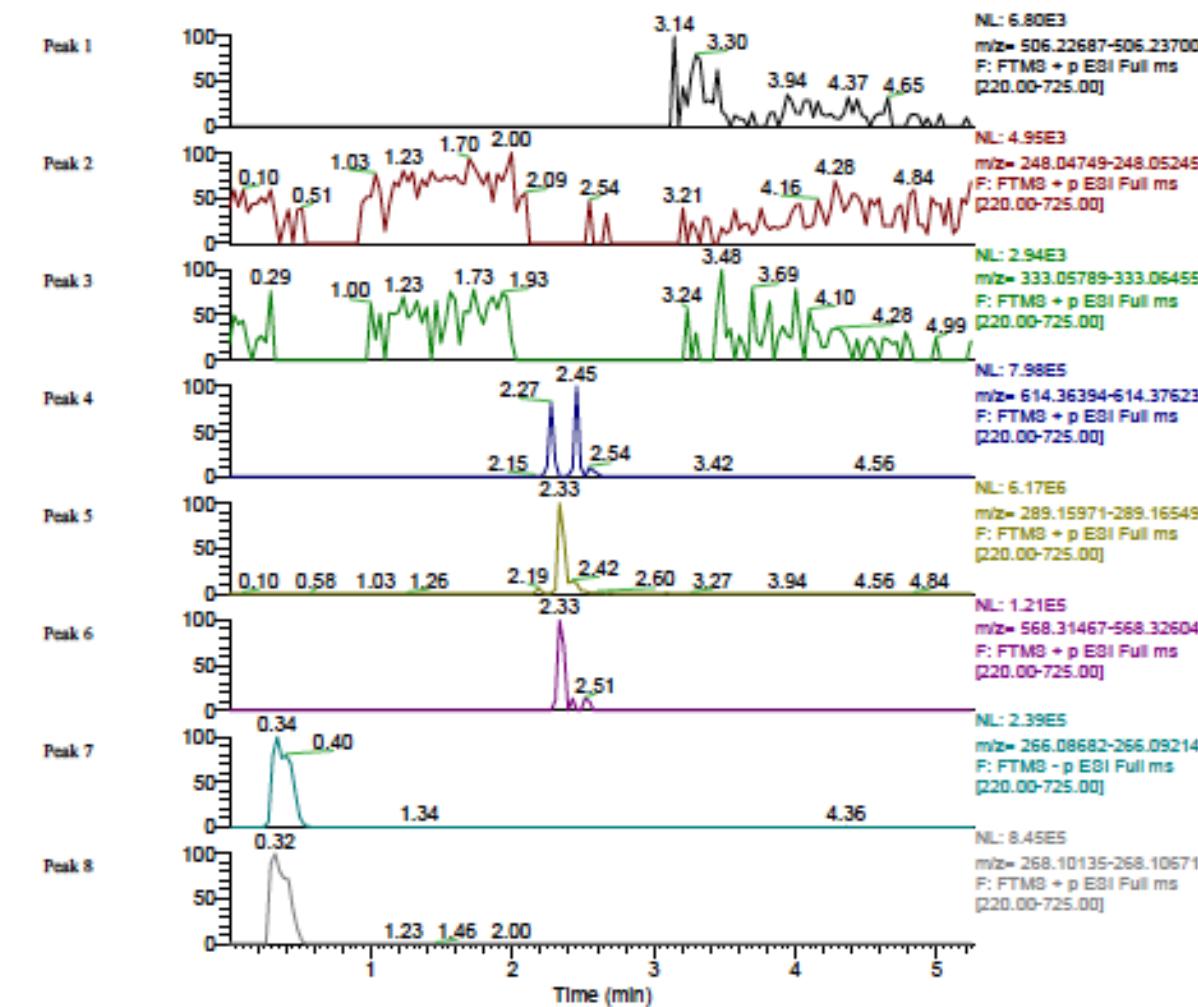
ARV	Total hits (either method)	% agree	% missed by Screen	% missed by Reference
Atazanavir	7	86	0	14
Efavirenz	22	73	9	18
Lamivudine	81	78	6	16
Nevirapine	76	78	6	16
Ritonavir	3	100	0	0

Performance Characteristics of ARV Screening Method

ARV	Atazanavir	Efavirenz	Lamivudine	Nevirapine	Ritonavir
Sensitivity (%)	100.0	88.9	92.6	92.2	100.0
Specificity (%)	99.5	98.0	91.7	92.0	100.0
PV (+, %)	85.7	80.0	82.8	83.1	100.0
PV (-, %))	100.0	99.0	96.6	96.5	100.0



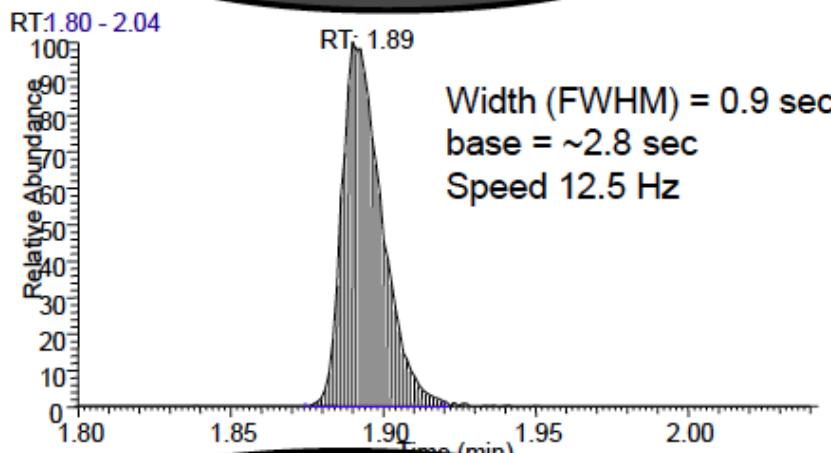
#	Comp. Index	Compound Name	Formula	Detected m/z	Delta (ppm)	Expected RT	Actual RT	Intensity	Adducts			Fragments		
									H+	NH4+	Na+	1	2	3
1	4	Emtricitabine	C8H10FN3O3S	248.04951	-1.8	2.54	1.99	3561269	Y*	-	-	Y	Y	Y
2	5	Efavirenz	C14H12ClF3N2	333.06070	-1.6	2.52	1.55	3448	Y*	-	-	N	N	N
3	6	Indinavir	C36H47N5O4	614.36700	-5.0	2.55	2.45	36951	Y*	-	-	Y	N	N
4	9	Morphine D3	C17H16D3NO3	289.16180	-2.8	2.20	2.33	7772808	Y*	-	-	N	N	N
5	11	Nevirapine	C15H14N4O	267.12344	-2.2	2.31	2.24	204083725	Y*	-	-	N	N	Y
6	16	Zidovudine Neg	C10H13N5O4	266.08847	-3.8	2.26	0.34	197142	Y*	-	-	-	-	-
7	17	Zidovudine	C10H13N5O4	268.10223	-6.7	2.26	0.38	435667	Y*	-	-	N	N	N



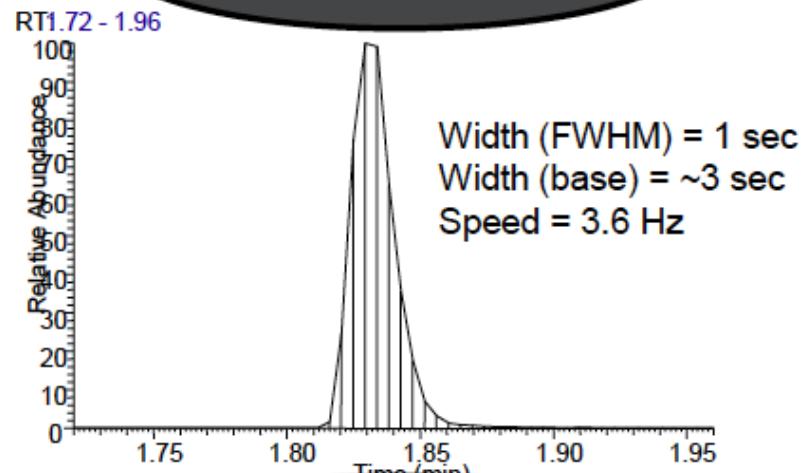
#	Comp. Index	Compound Name	Formula	Detected m/z	Delta (ppm)	Expected RT	Actual RT	Intensity	Adducts			Fragments		
									H+	NH4+	Na+	1	2	3
1	1	Ampranavir	C25H35N3O6S	506.23547	7.0	2.40	3.30	5273	Y*	-	-	N	N	N
2	4	Emtricitabine	C8H10FN3O3S	248.05070	3.0	2.54	2.00	4613	Y*	-	-	N	N	N
3	5	Efavirenz	C14H12ClF3N2	333.06079	-1.3	2.52	3.48	2879	Y*	-	-	N	Y	N
4	6	Indinavir	C36H47N5O4	614.37384	6.1	2.55	2.45	798053	Y*	-	-	Y	Y	N
5	9	Morphine D3	C17H16D3NO3	289.16208	-1.8	2.20	2.33	6165960	Y*	-	-	N	N	N
6	10	Nelfinavir	C32H45N3O4S	568.31805	-4.0	2.58	2.33	121154	Y*	-	-	N	N	N
7	16	Zidovudine Neg	C10H13N5O4	266.08868	-3.0	2.26	0.34	238567	Y*	-	-	-	-	-
8	17	Zidovudine	C10H13N5O4	268.10217	-6.9	2.26	0.32	844906	Y*	-	-	N	N	N

Loperamide

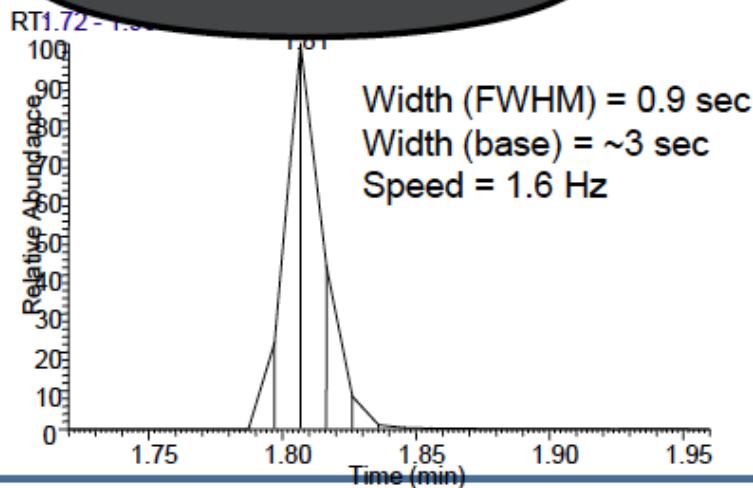
35 data points@ 17,500



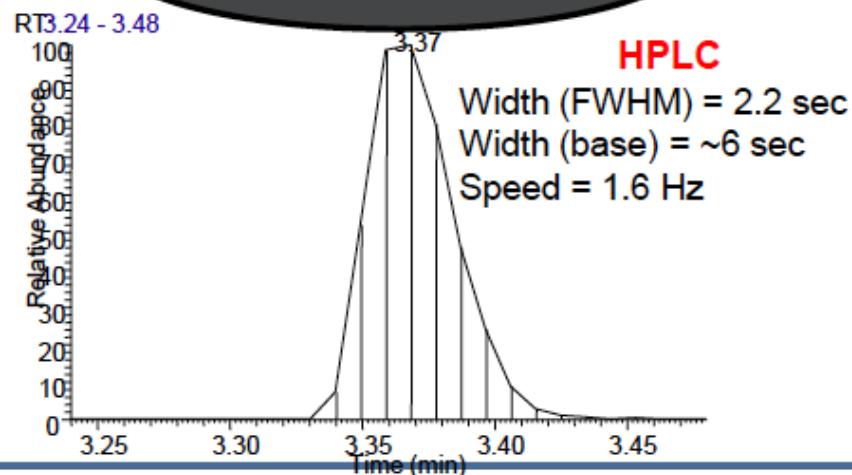
11 data points@ 70,000



5 data points@ 140,000



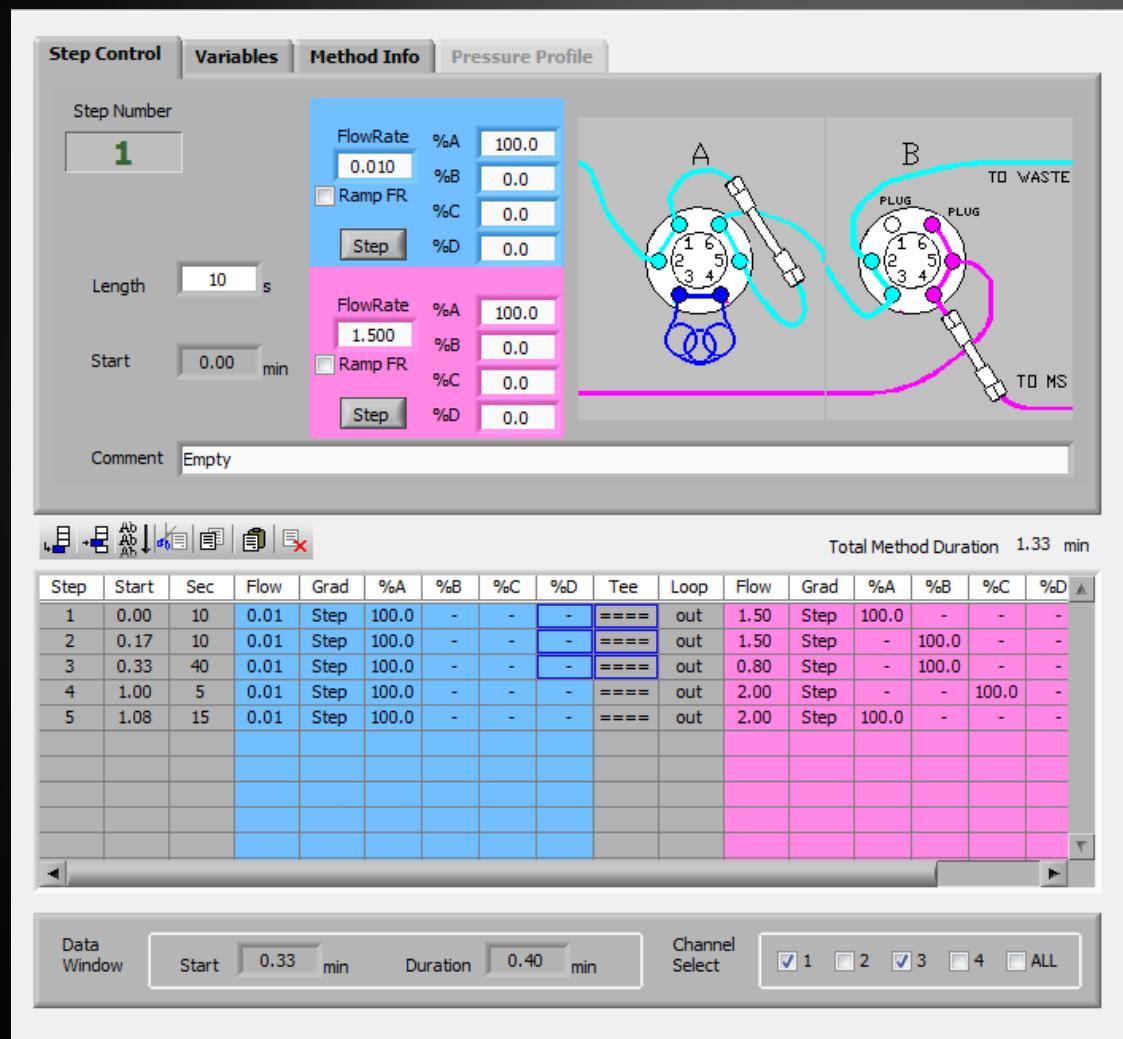
10 data points@ 140,000



Challenges and Goals

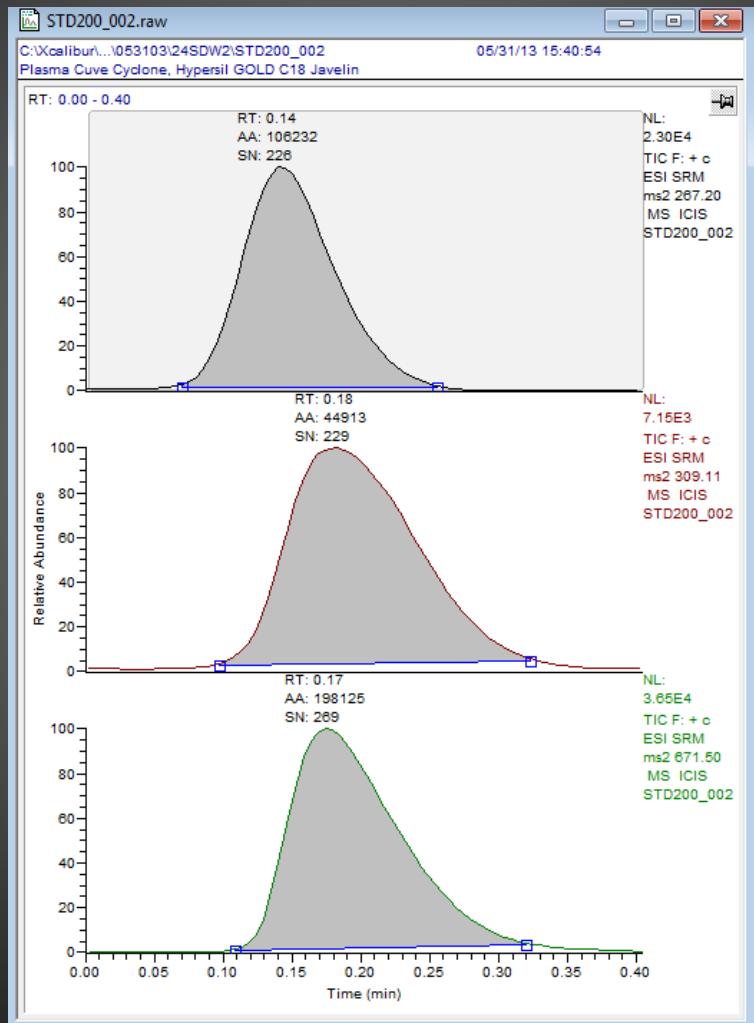
- Must have faster methods
 - >10K specimens in the queue; ~50K to come
- Automated data analysis – remove subjective interpretation
- Confirmatory testing selected specimens

Preliminary work on TSQ Endura



With .4 minute data
Collection window. Using
0.5*50 Cyclone P column
With 2.1*10 Hypersil gold
Javelin cartridge @ source

24 second data window



Warfarin - IS

Nevirapine

Saquinavir

Ongoing and Planned Studies

- Development of Rapid Targeted Screening method
- Programming for automated data review and compilation
- Automated sample preparation for traceability
- Ongoing studies in HIV Prevention
 - ART as Prevention
 - PrEP in IDU and SOA settings
- Additional screening from same extracted plate
 - Hormonal contraceptives
 - Substances of abuse
- Clinical trial for adherence monitoring in domestic (US) patient care

HIGH RESOLUTION MASS SPECTROMETRY FOR QUANTIFICATION OF COMPOUNDS IN ENVIRONMENTAL TOXICOLOGY

Background

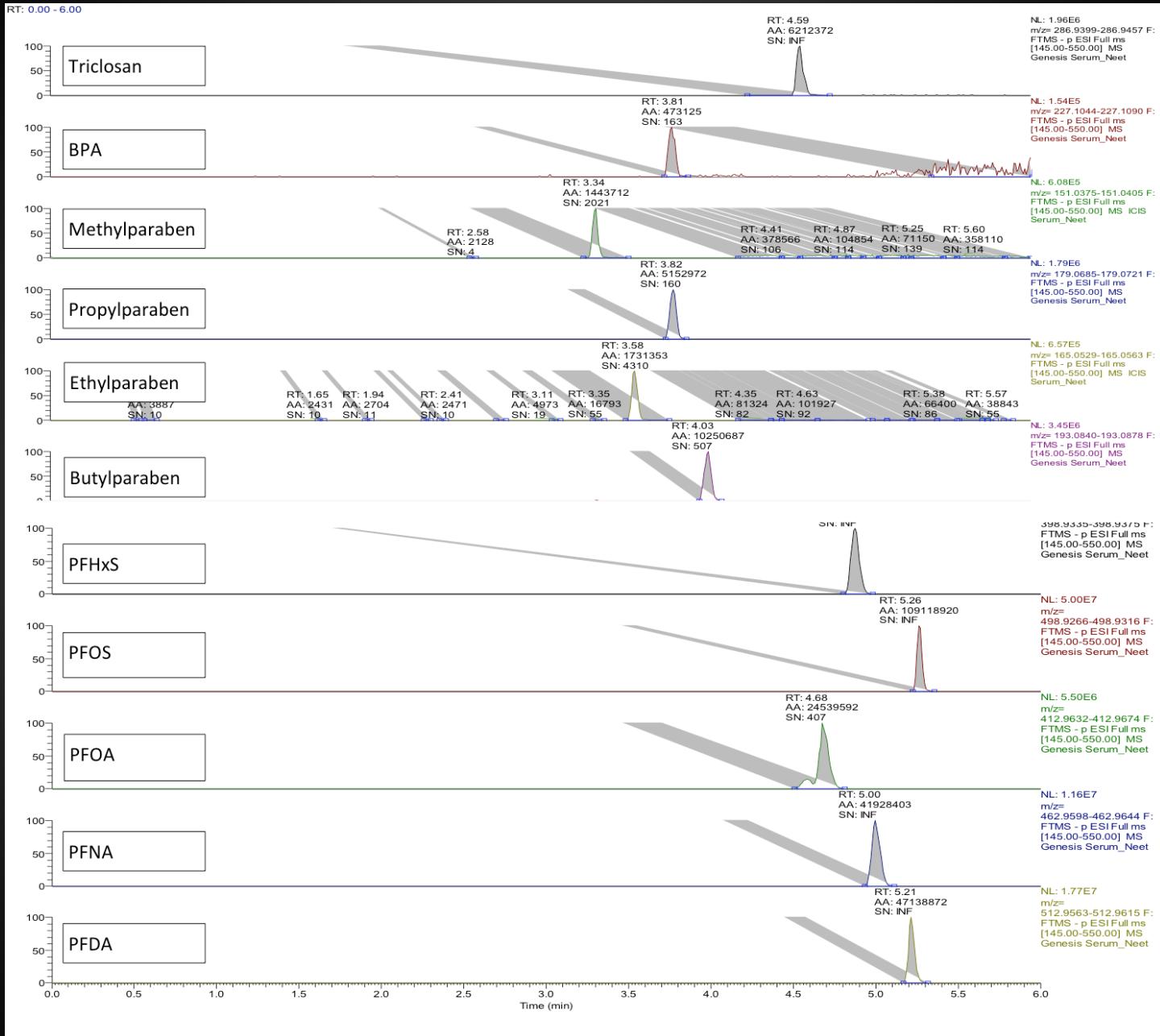
- Approached by SPH investigator for triclosan analysis
- Same group interested in parabens, BPA, and PFCs
- Sample types: urine, blood, cord blood, and water
- Offered to develop multiplex assay for all compounds to conserve sample
- Pilot project for HRMS quantification of small molecules in our lab

Data Collection Modes

- Qualitative
 - Full Scan AIF
 - Full Scan dd-MS2
 - SIM dd-MS2
 - Quantitative
 - Full Scan (no fragmentation)
 - Full Scan AIF
 - SIM
 - Parallel reaction monitoring (PRM); similar to MS/MS in QQQ methods
- 
- Extracted ion chromatogram

Analytical Method

- Calibrators prepared in drug free human serum, urine, or water
- Isotopically labeled IS for each compound
- Protein crash with MeOH (blood) or ACN (urine); dilute 1:1 with water
- Hypersil Gold C18, 50 x 2.1; 1.9 uM particles
- Solvent A: 0.01% acetic acid
- Solvent B: CAN w/0.01% acetic acid
- Full scan HRMS; R = 140K at 1 Hz



Validation

- Selectivity: pre- and post-extraction analyte addition compared to solvent spike
- Accuracy: recovery at 50, 5, and 1 ppb (n=20)
- Precision: %CV at 50, 5, and 1 ppb (n=20)
- Freeze-Thaw Stability: comparison of pre- and post-freeze-thaw cycle (1 cycle)
- LOQ: functional sensitivity (%CV<20; bias<15%)
- Carryover: analyses of high (500 ppb) and blank samples alternately.
 - Acceptable = residual signal<10% of lowest calibrator
- Linearity: each calibrator injected 1x daily for 5 days
 - Mean concentration must be w/in 15% of expected for each sample

	Serum					Urine					Water				
Analyte	Limit of Quantification (ppb)	% CV @ 5ppb	% Inaccuracy @ 5ppb	% CV @ 50 ppb	% Inaccuracy @ 50ppb	Limit of Quantification (ppb)	% CV @ 5ppb	% Inaccuracy @ 5ppb	%CV @ 50 ppb	% Inaccuracy @ 50ppb	Limit of Quantification (ppb)	% CV @ 5ppb	% Inaccuracy @ 5ppb	% CV @ 50 ppb	% Inaccuracy @ 50ppb
BPA	1	20	17	11	10	1	10	16	4	7	1	9	10	3	8
Butylparaben	1	12	8	3	2	1	2	4	1	2	1	2	9	1	2
Ethylparaben	1	20	18	3	4	1	8	7	3	3	1	3	5	2	2
PFHxS	5	4	4	2	1	1	1	4	<1	1	5	1	32	<1	6
PFOS	1	9	8	2	3	1	1	9	<1	5	1	3	5	<1	<1
Methylparaben	1	17	6	3	3	1	12	7	5	9	1	4	4	1	9
PFDA	1	6	6	7	3	1	1	14	1	1	1	3	70	1	<1
PFNA	1	4	6	2	2	1	1	5	<1	14	5	2	52	<1	9
Propylparaben	5	20	32	3	1	1	5	2	1	<1	1	2	10	1	2
PFOA	5	9	3	4	6	1	2	<1	1	1	5	1	22	1	5
Triclosan	1	20	8	7	6	5	20	16	2	4	5	17	20	5	1

RESULTS

Selectivity: The assay is selective for each analyte, as the pre- and post- extraction addition of analyte sample signal were within 20% of extraction solvent spike, across all matrix types.

Accuracy: The assay was found to have <20% inaccuracy across sample concentrations tested, and across both biological matrices. The PFC's were found to have >20% inaccuracy in water, with all other analytes <20% in this matrix.

Precision: Precision was found to be <20% for all analytes across matrices at 5ppb and ≤10% for all analytes across matrices at 50ppb, except in the case of PFDA in urine, with %CV=14.

Freeze-thaw Stability: There was <10% variability in analyte signal across matrix types and for all analytes after a freeze-thaw cycle, relative to newly-prepared samples.

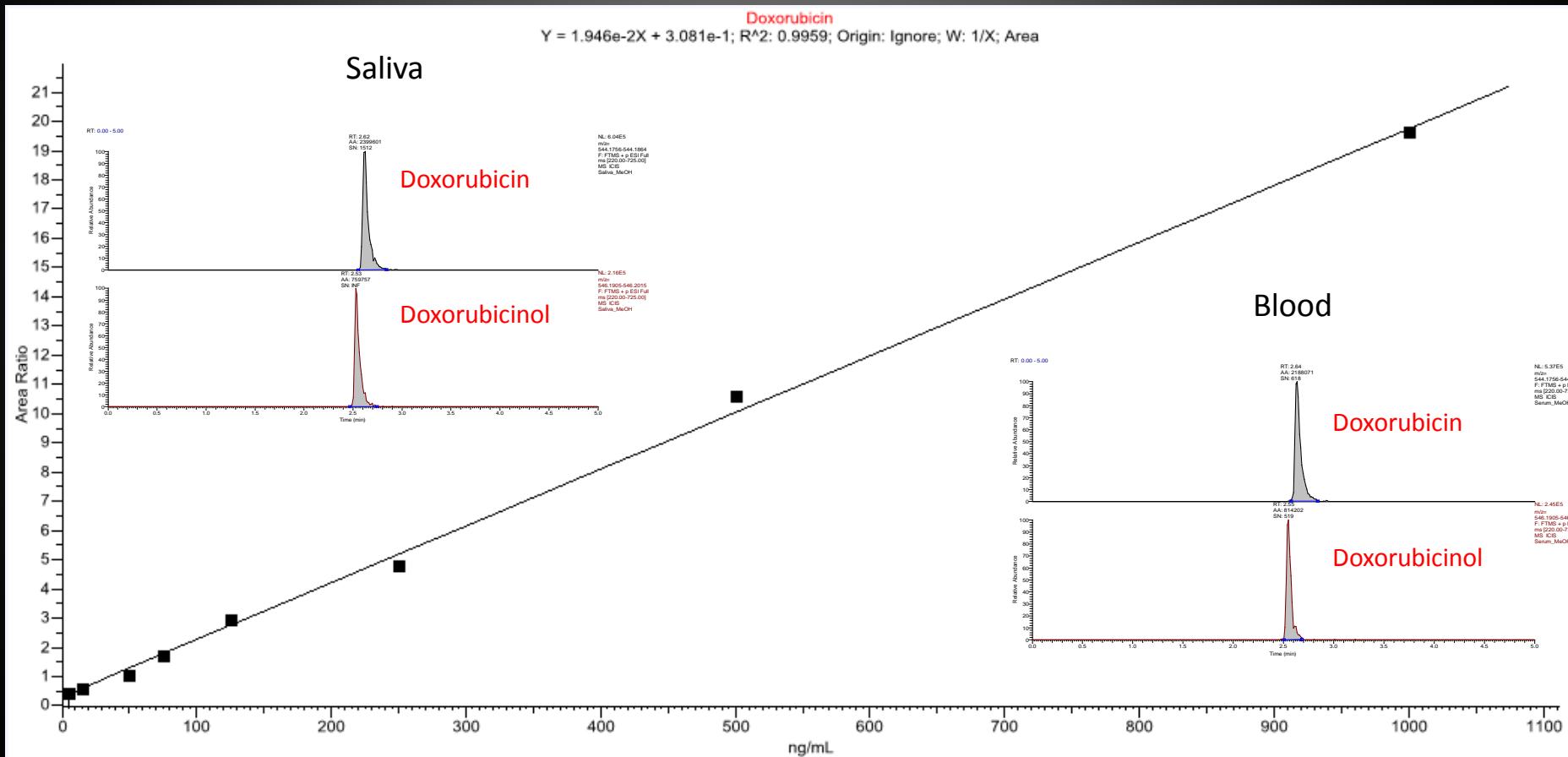
Limit of Quantification: Limits of quantification were found to be 1 to 5 ppb for all analytes, across the three matrix types.

Carryover: Carryover signal was found to be <10% of the lowest calibrator signal across all analytes and matrices.

Linearity: Linearity was found to be acceptable, with all calibrators having signal within 15% of specified values, across multiple runs of calibrator materials and across all analytes and matrices.

Detailed validation results are compiled in **table 1**.

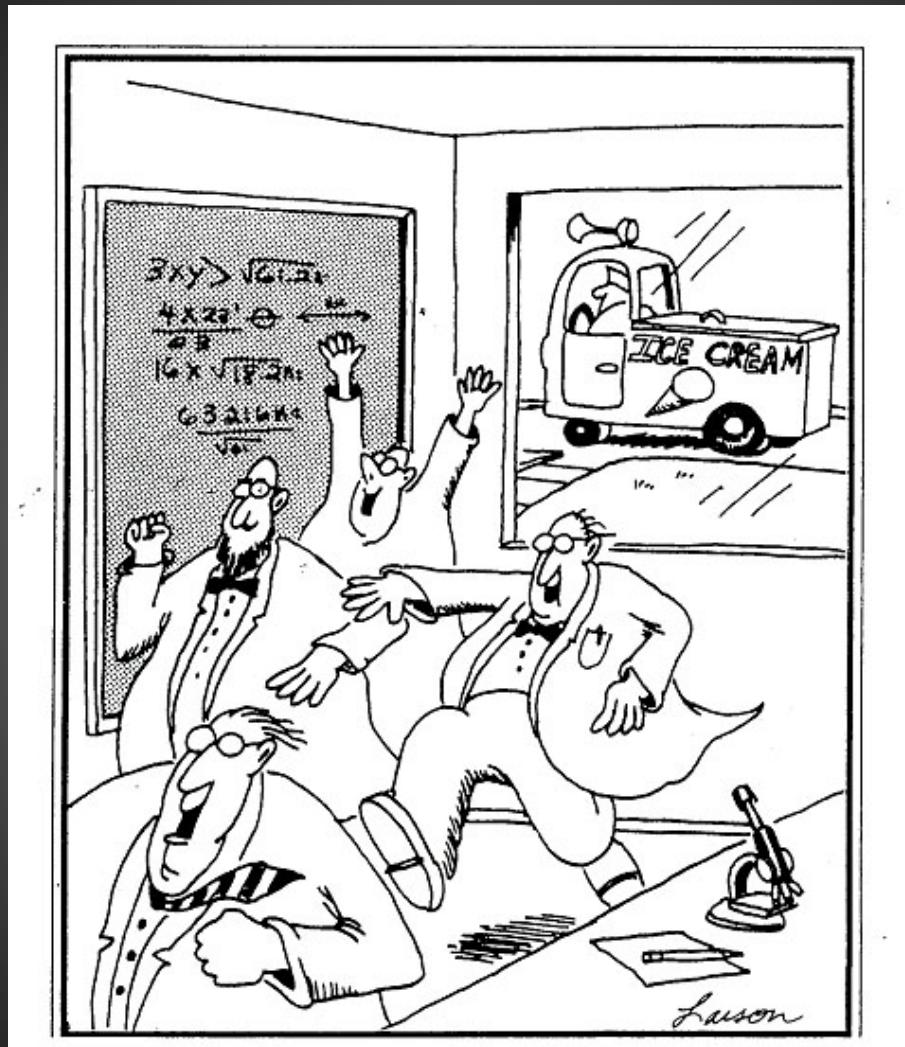
Alternate Application (Doxorubicin)



Acknowledgements

- Johns Hopkins School of Medicine
 - Mark Marzinke, PhD
 - Sue Eshleman, MD, PhD
 - Janelle Coughlin, PhD
 - Craig Hendrix, MD
 - Teresa Parsons, PhD
 - Matthew Olson, MD
 - Athena Petrides, PhD
- Clinical Mass Spec Lab
 - Autumn Bread
 - Sabitha Schools
 - Veronica Gantert
 - Josh Moskowitz
- Funding
 - NIH (UM1-AI068613)
 - NIAID, NIDA, NIMH under Cooperative Agreement # UM1 AI068619
 - Thermo Fisher

QUESTIONS??



wclarke@jhmi.edu