

Tandem Mass Spectrometry: Practicalities and troubleshooting

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The Camelia Botnar Laboratories at Great Ormond Street Hospital For Sick Children



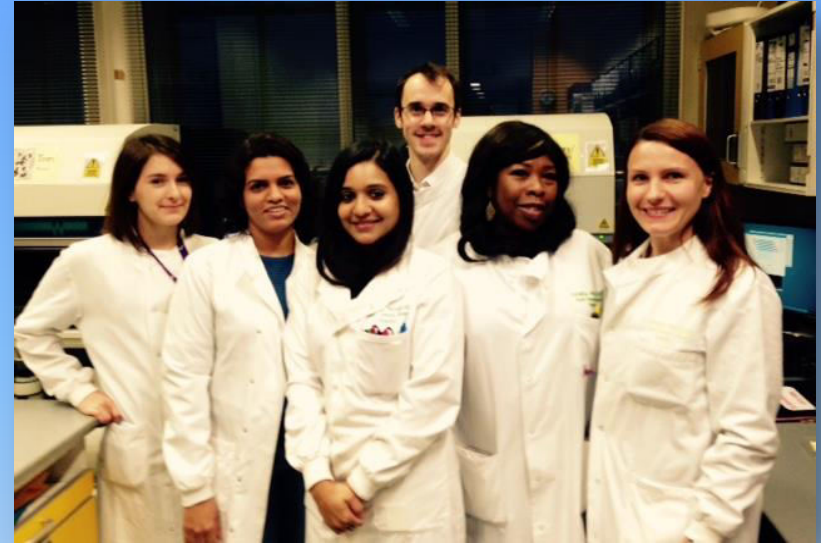
- Our world-class teams play an essential role in the diagnosis and treatment of childhood illness
- We are the largest Newborn screening centre in the UK and we screen 125,000 babies every year
- 1 of every 6 babies born is screened at GOSH



Experience

Biomedical Scientists

- **Dipti** – BMS1 at GOSH since December 2013.
- **Sarah** – BMS1 at GOSH since August 2014.




Department

- One of six pilot sites for the expanded newborn screening programme.





Analysis at GOSH

Inherited metabolic disorder screening using the AB SCIEX API 3200 Tandem Mass spec

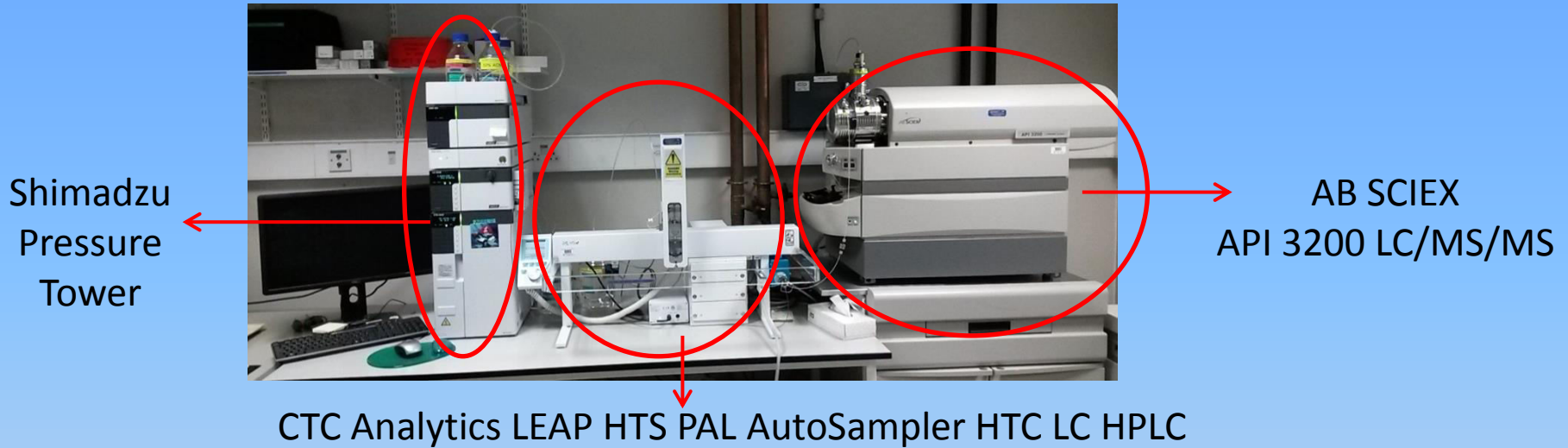
- Phenylketonuria-PKU (Phe and Tyr)
- Medium-chain acyl-CoA dehydrogenase deficiency-MCADD (C8:C10 Ratio)
- Isovaleric acidaemia -IVA (C5)
- Maple syrup urine disease -MSUD (Leu)
- Homocystinuria-HCU (Meth)
- Glutaric aciduria type 1-GA1 (C5DC)

Right	Do ✓
 <p>Circle filled and evenly saturated</p>	<p>Clean and dry the baby's heel before taking sample. This will avoid contamination of the sample</p> <p>Fill the circle completely with one drop of blood</p>

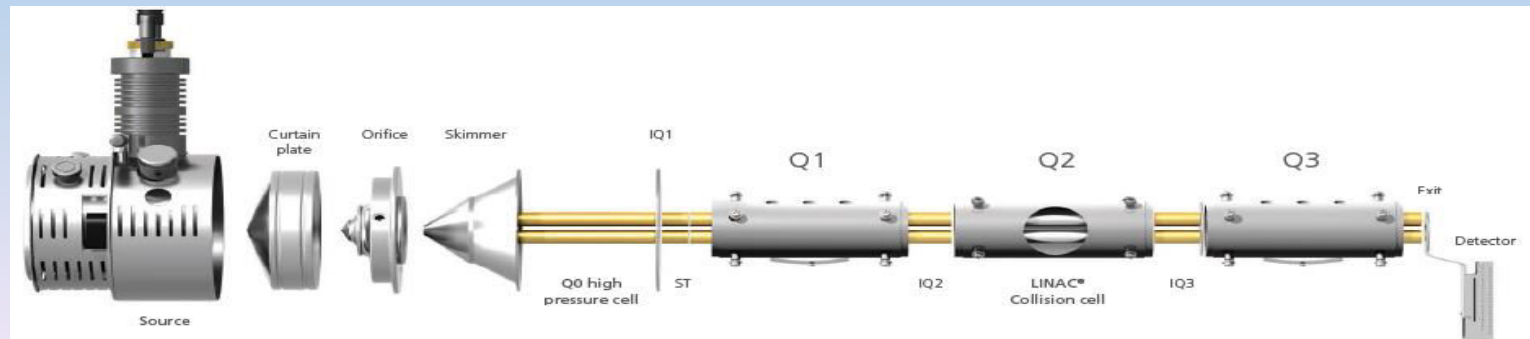


Wrong	Don't ✗
 <p>Insufficient, multiple spots</p>	<p>Take insufficient or multiple applications. This is unacceptable for testing and a repeat will be required</p>
 <p>Layering</p>	<p>Layer the blood. Too much blood can cause erroneous results</p>
 <p>Contaminated</p>	<p>Contaminate the sample (e.g. faeces, adult blood and touching the circles)</p>
 <p>Compressed</p>	<p>Compress the blood spot. Applying pressure reduces the density of blood on the sample and can lead to a 'suspected' result being missed</p>

Methodology



The triple Quadrupole API 3200 SYSTEM





Common Problems & Troubleshooting



Types of problems

- **Pre-analytical**

Sample contamination, insufficient, multi-spotted or compressed spots.

- **Analytical**

- **Post analytical**

Transcription errors.

Maintenance

Maintenance logs

Great Ormond Street
Hospital for Children



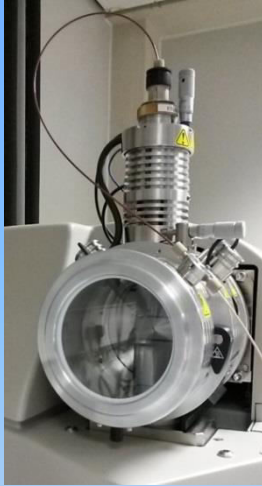
NHS Foundation Trust

AB SCIEX API 3200 Main				
Newborn Screening Laboratory				
Analyser	Jasmine API 2			
Daily	1	2	3	4
Check mobile phase level	✓	✓		
Check needle wash level	✓	✓		
Check waste level	✓	✓		
Check nitrogen generator	✓	✓		
Flush all lines	✓	✓		
Back-flush filter	✓	✓		
Flush needle	✓	✓		
Record LC pump pressure	272	280		
Vac. gauge pressure (running)	3.2	4.4		
Vac. gauge pressure (standby)	0.6	0.6		
Archive data files / zip	✓	✓		
Reboot PC	✓	✓		
Initials	DS	DS		

Weekly (or after ~ 10 plates)	1	2	3
Clean skimmer / orifice plate		✓	
Clean needle port / curtain		✓	
Replace port seal		✓	
Change filter (weekly)		✓	
Flush sample sprayer unit		✓	
Check/change 10% MeOH		✓	
Check rotary pump oil level		✓	
Initials		DS	
As required	1	2	3
Replace peek tubing			
Replace sample needle			
Replace syringe			
Clean/change rotor seal			
Top up rotary pump oil			
Initials			

Maintenance

Performing the weekly maintenance

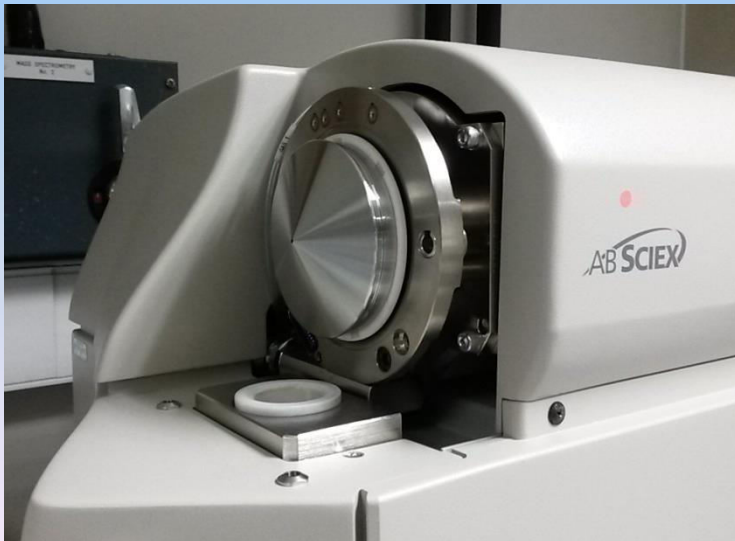


Curtain plate



Skimmer

Orifice Plate

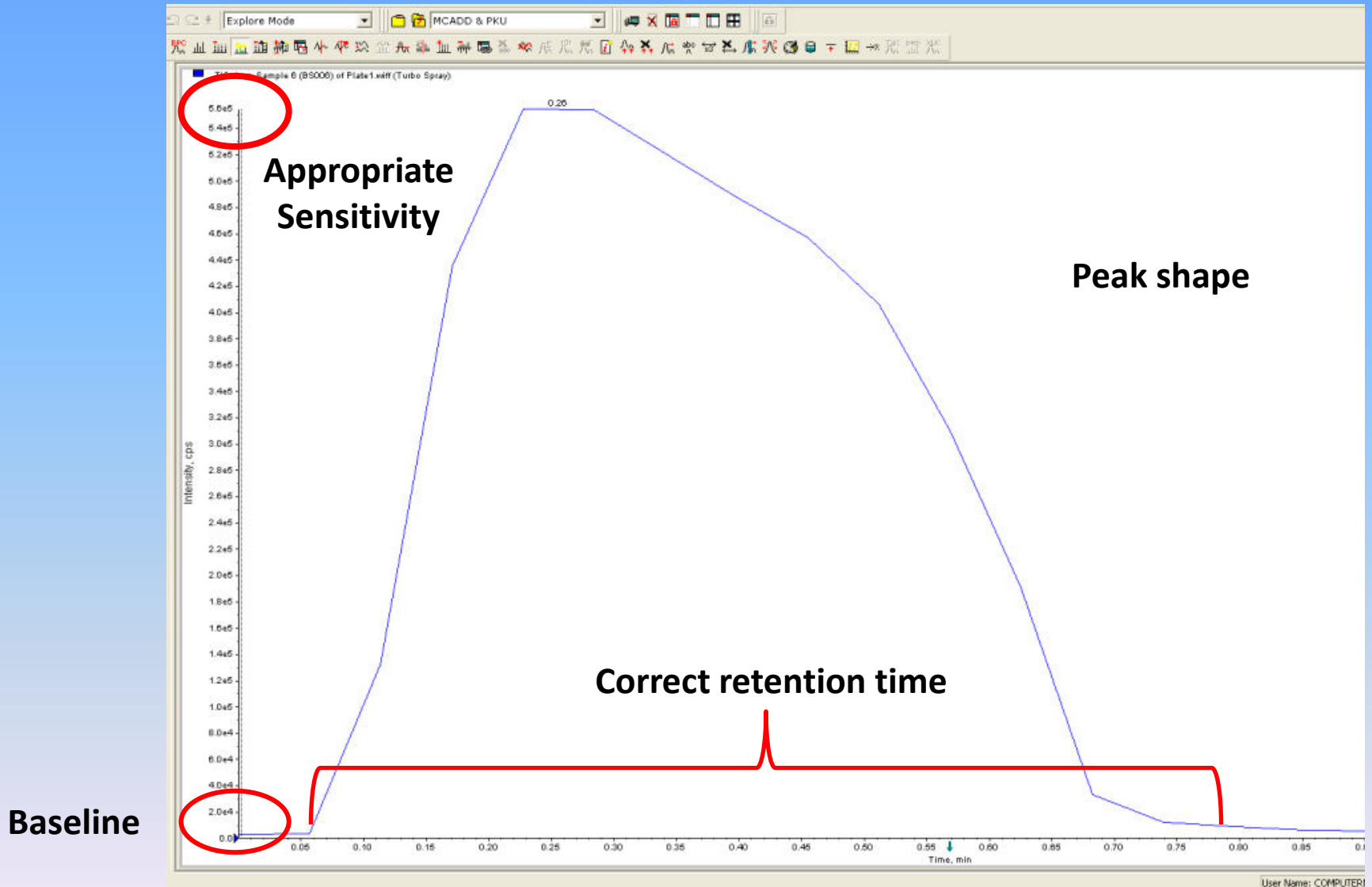


Maintenance

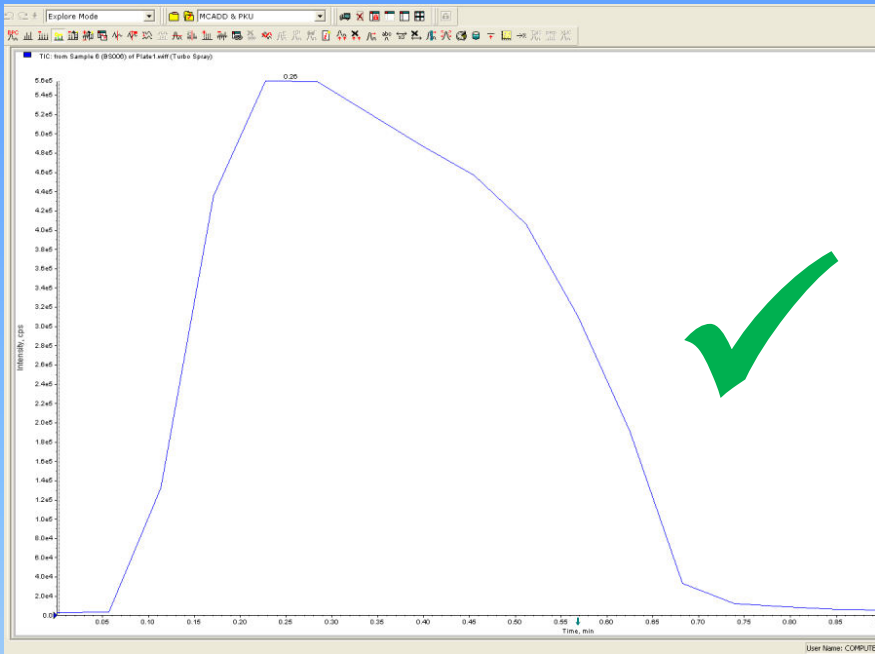
Delayed/Poor maintenance

- **Poor sensitivity/signal leading to failed internal standards caused by a drastic drop in Tandem performance**
- **Poor chromatograms caused by air in lines or leaks**
- **High pressures caused by blocked/pinched peek tubing or filters**
- **Low pressures caused by loose peek tubing connections or wrong solvent in use**
- **Discrepant results (Flyers)**

Good chromatogram



The child first and always



Normal/good chromatogram

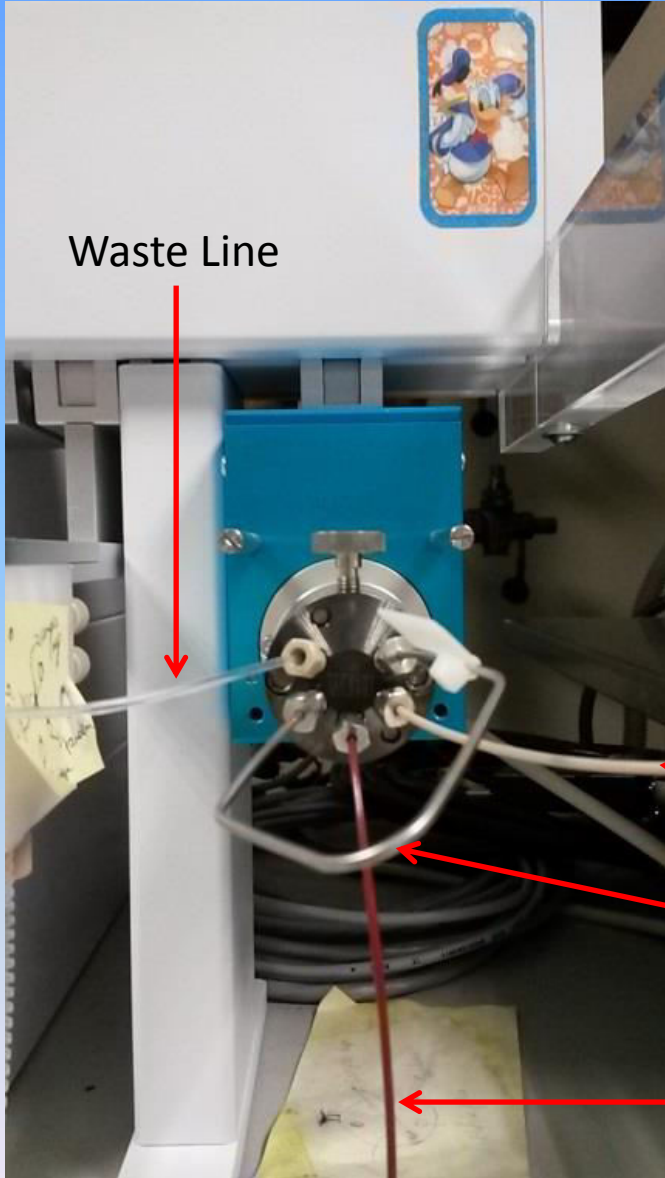


Failed injection

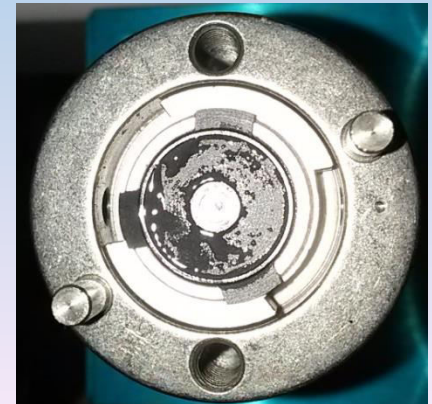
The child first and always

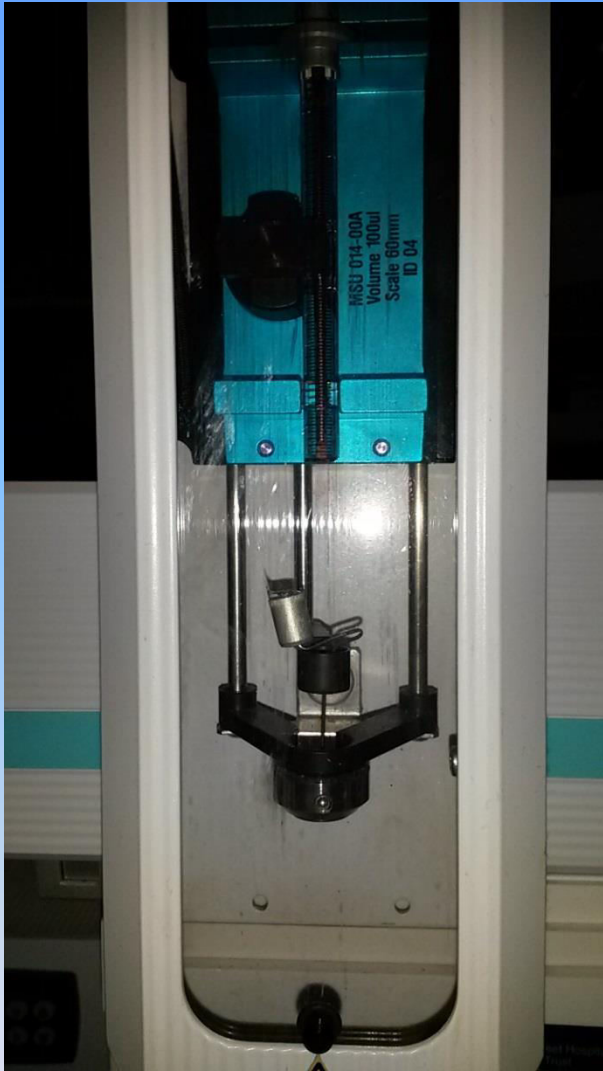
Failed injection

- Inaccurate pipetting of internal standard- Short Sampled
- Air in the lines
- Blocked lines
- Blocked rotor seal
- Broken syringe
- Slack bungee cord (operates syringe)
- Insufficient mobile phase
- Faulty chip on board sample manager



Rheodyne Area

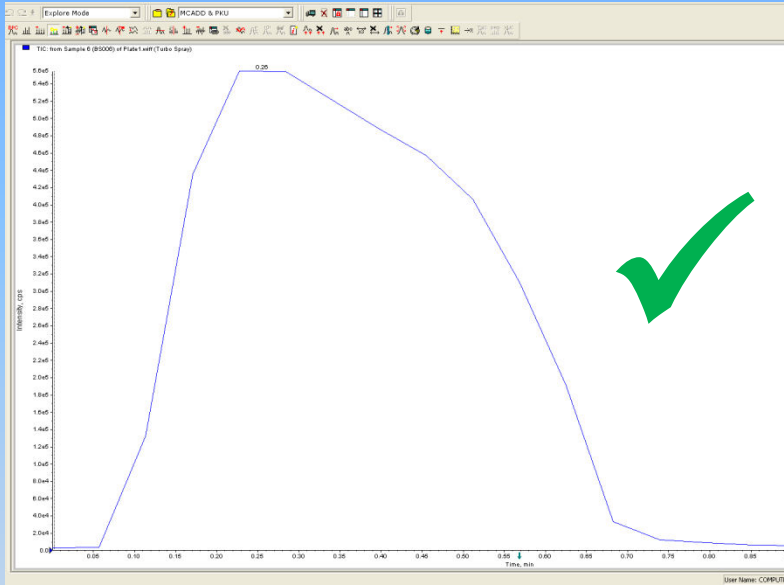




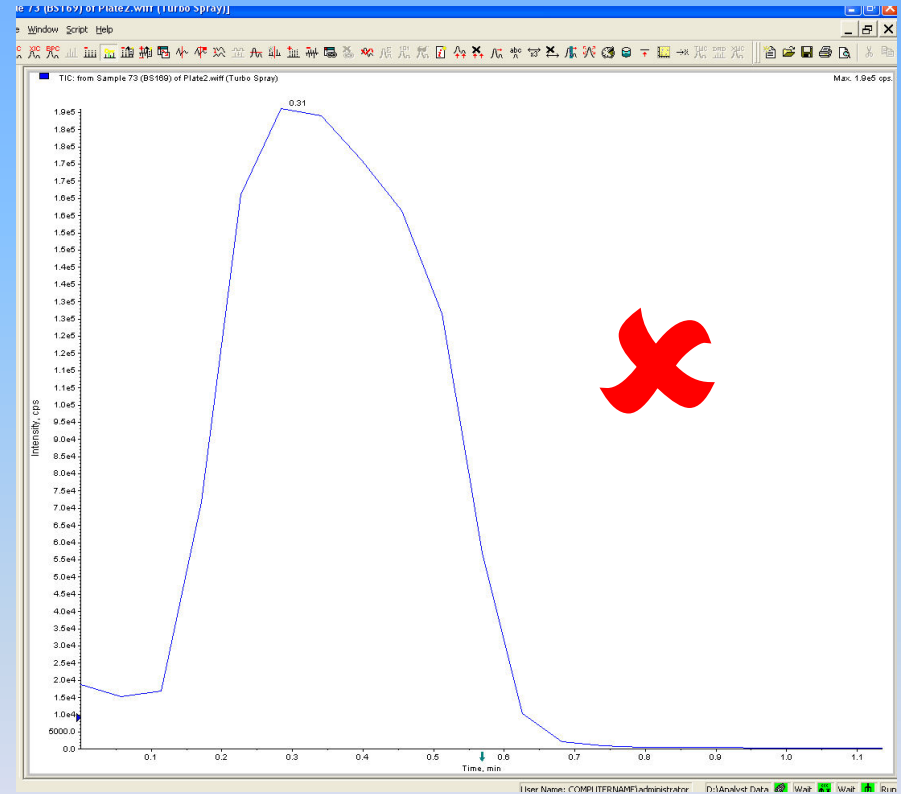
**A very broken
sample syringe!**



The child first and always



Normal/good chromatogram



**Carry-over
(False
Positives)**

The child first and always

Carry-over

Unreliable/biased results and false positives

- **Insufficient needle washing on board**
- **Blockage in needle wash or waste line causing back-flow**
- **PEEK tubing with loose connections**
- **Leaking syringe**

I.T Issues

- **Communication problems between software and instrumentation.**

Final thoughts

- **Always perform maintenance**
- **Keep records of pressure**
- **Record problems in log and follow up**
- **Call engineer if necessary**

Thank you for listening
Any Questions?

