An MSMS Method for Urine Cystine
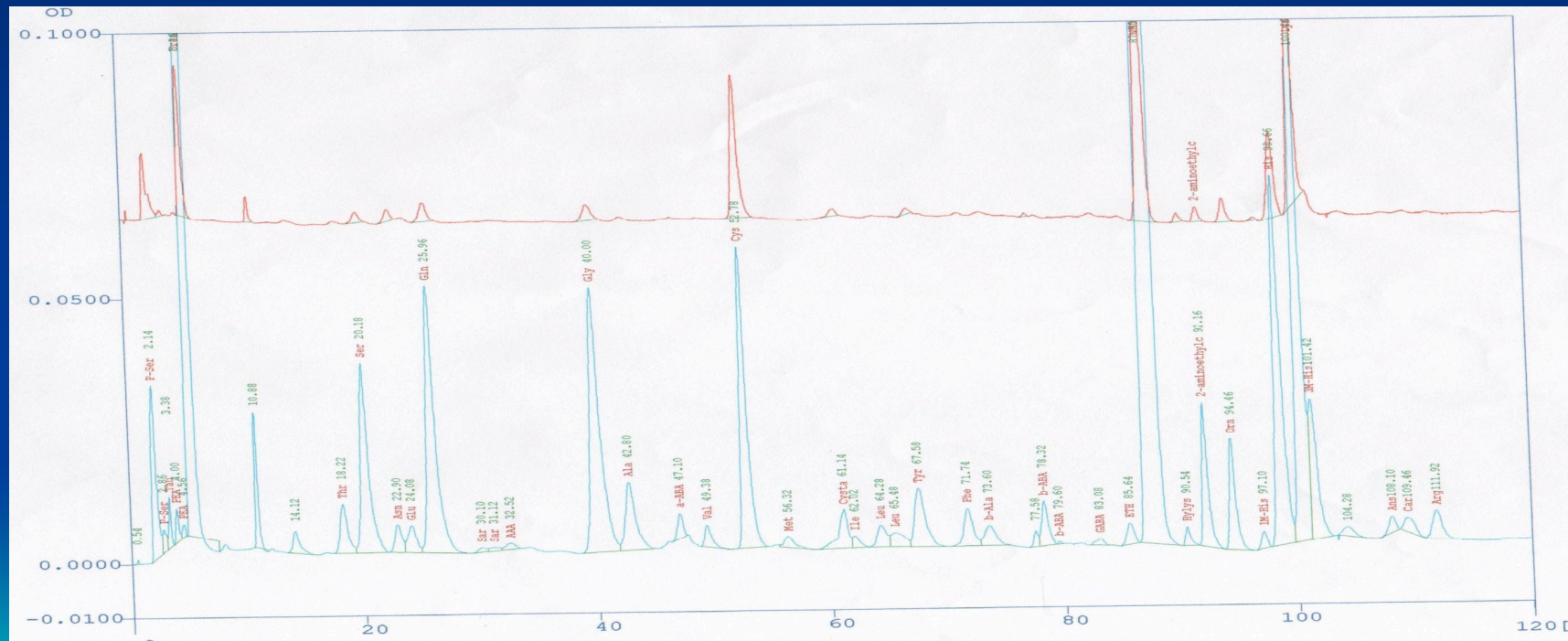
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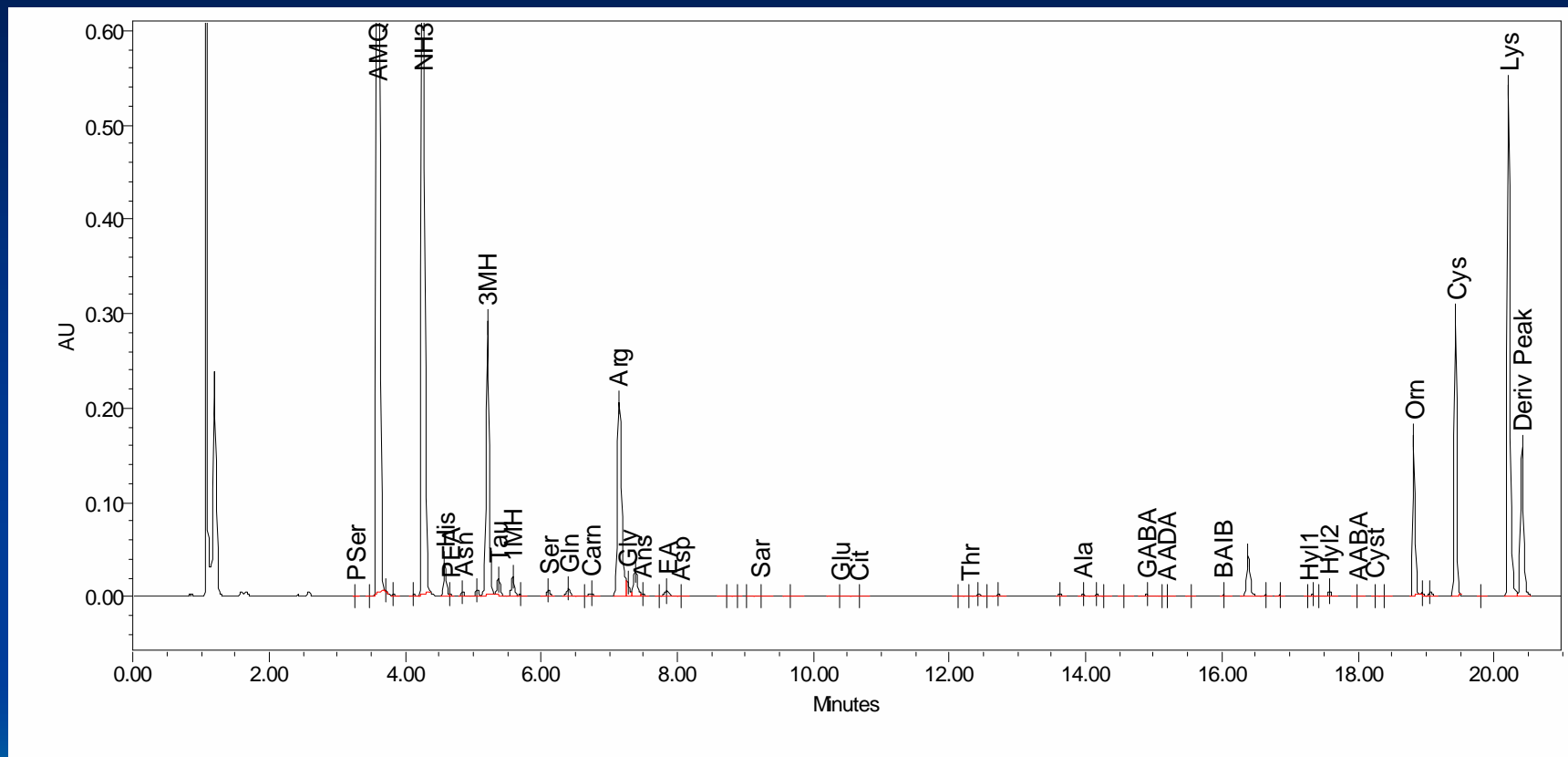
Amino acid Analyser

- 2.5 hours and expensive

UPLC

20 mins runtime for basic amino acids

UPLC MS/MS

- Uses HILIC technology
- Hydrophilic Interaction Chromatography
- 1.7um stationary phase particles allowing rapid separataion at low flow rates
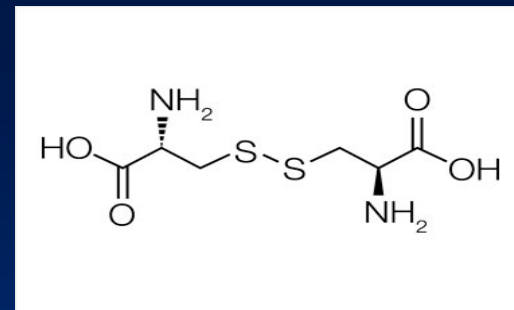- Small particle size leads to enhanced peak resolution

HILIC

- Consist of bare silica or polar bonded phase
- Mobile phases are mainly organic
- Suited to MS/MS
- Analytes eluted with water
- Water forms a layer on bonded phase and separation is by liquid-liquid interaction

METHOD

- Acquity HILIC BEH C18. 2.1 X 50mm column with BEH pre column

- Mobile Phase

  A: 50:50 ACN:H20, 0.2% H_3PO_4

  B: 95:5 ACN:H20, 0.2% H_3PO_4

Sample Diluent: 73:25:2 ACN:MeOH:H_2O

UPLC Programme

Time (Min)	%A	%B	Flow ml/min
0.00	0.1	99.9	0.529
1.65	0.1	99.9	1.000
2.50	99.9	0.1	0.529
4.00	0.1	99.9	0.529
5.00	0.1	99.9	0.529

- Seal Wash
  50:50 ACN:H_2O
- Strong Needle Wash
  90:10 ACN:H_2O
- Weak Needle Wash
  Mobile phase A

MS/MS Settings

Function	MS/MS settings
Cap. Voltage	4.00 kv
Source Temp	120C
Desolvation Temp	400C
Desolvation Gas flow	900 litres/hr
Cone gas flow	40 litres hr

	CYS	D4 -CYS
Dwell (sec)	0.1	0.1
MW	240.90	244.90
MRM	240.9 > 152	240.9 > 154
Cone V	20.0	20.0
CE	13.0 V	13.0 V
Interscan delay	0.05 sec	0.05 sec

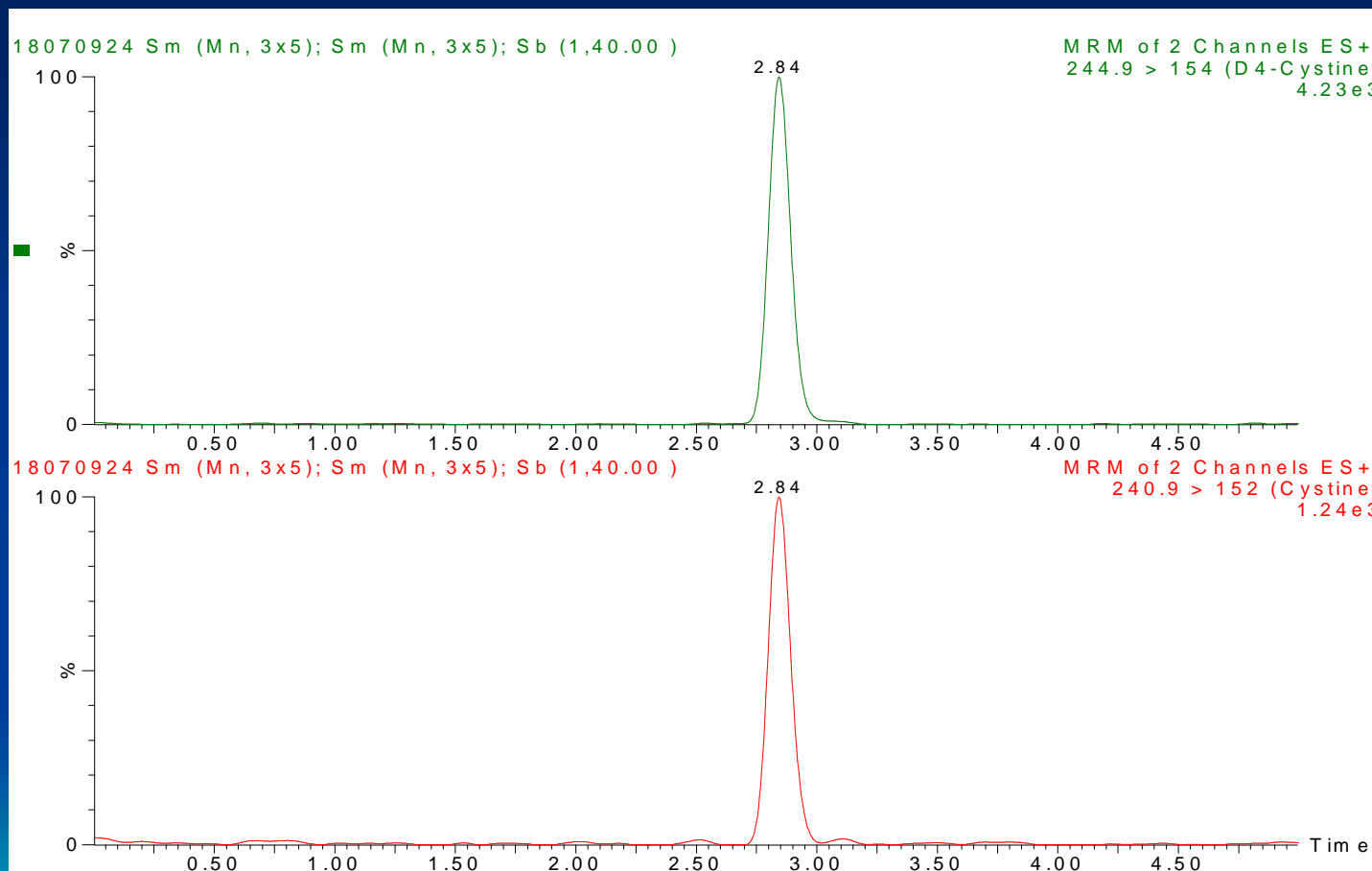**Diverter valve switched to waste from 0 – 2.5 minutes and 3.5 - 5 mins**

Sample Prep

- 20ul Urine
- 20ul IS
- 180 ul Sample diluent
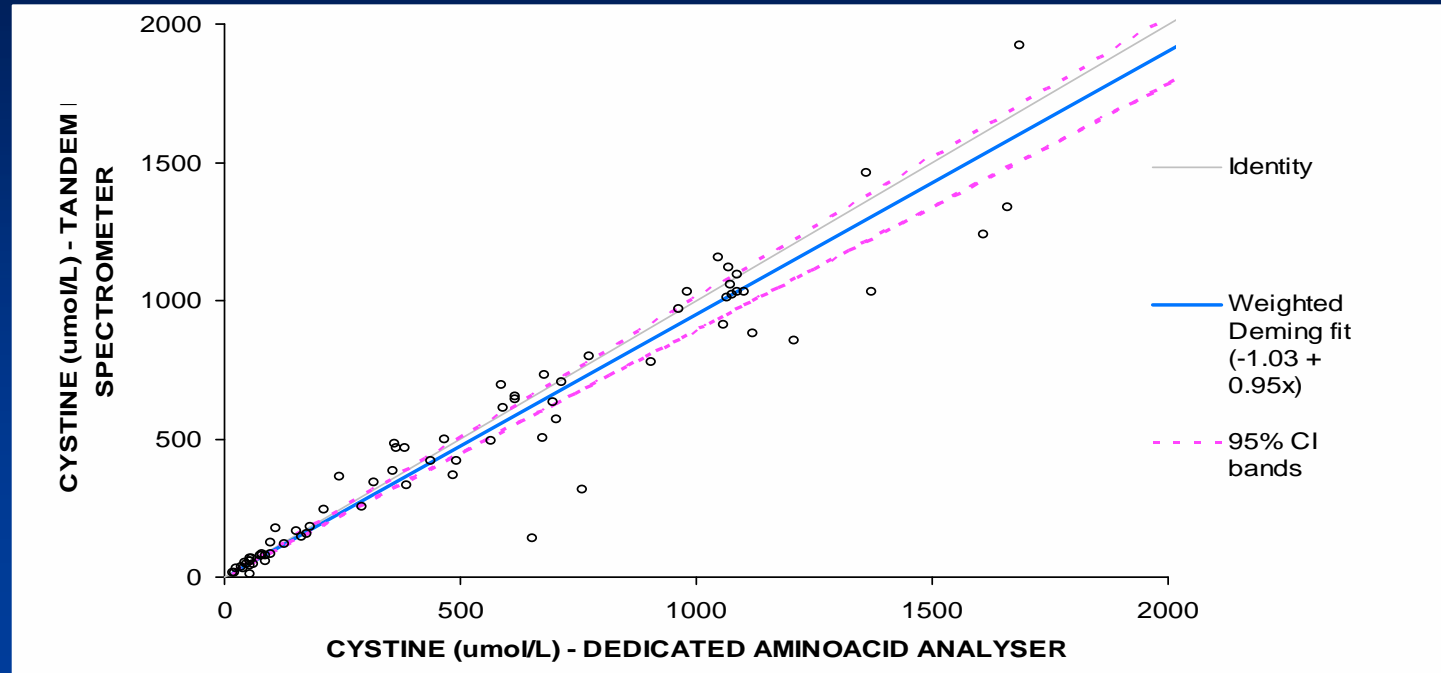- Centrifuge
- Inject 5ul

Typical Ion Chromatogram

Results

- Calibrated up to 1000 umol/l
- Linearity > 2000 umol/l
- LOQ 8umol/l
- Recovery 104.1 – 123%
- Stable at RT for 72 hrs

	High QC	Med QC	Low QC
Mean (n=50)	929.9	656.6	32.3
CV_W	7.5	6.3	12.7
CV_B	5.4	6.7	24.3
CV_T	9.2	9.2	27.4

Method Comparison

N	Regression Equation	95% CI	Bias at 1000 umol/l	95% CI
24	Y = 1.07x − 2.59	Slope 0.9 – 1.3 Intercept -14.1 to 8.9	+71.9 umol/l	-92 to 236.1

Feasibility of Serial Cystines in Random Urines

- Thymol added to random urine bottles as pH and antimicrobial perservative
- Sample acidified on receipt in lab
- Results suggest thymol was effective in stabilising cystine concentration (even with thiol binding drugs)
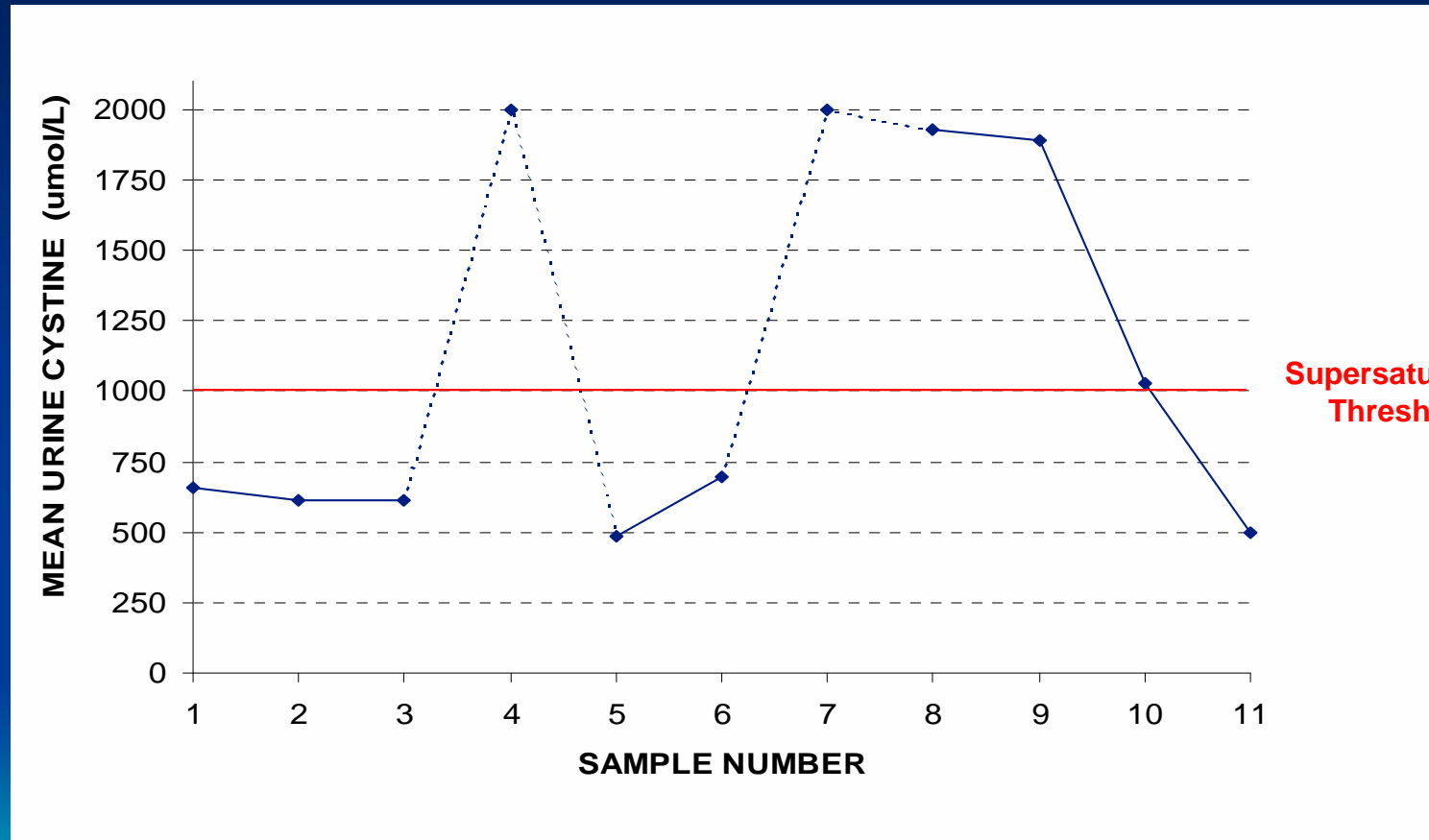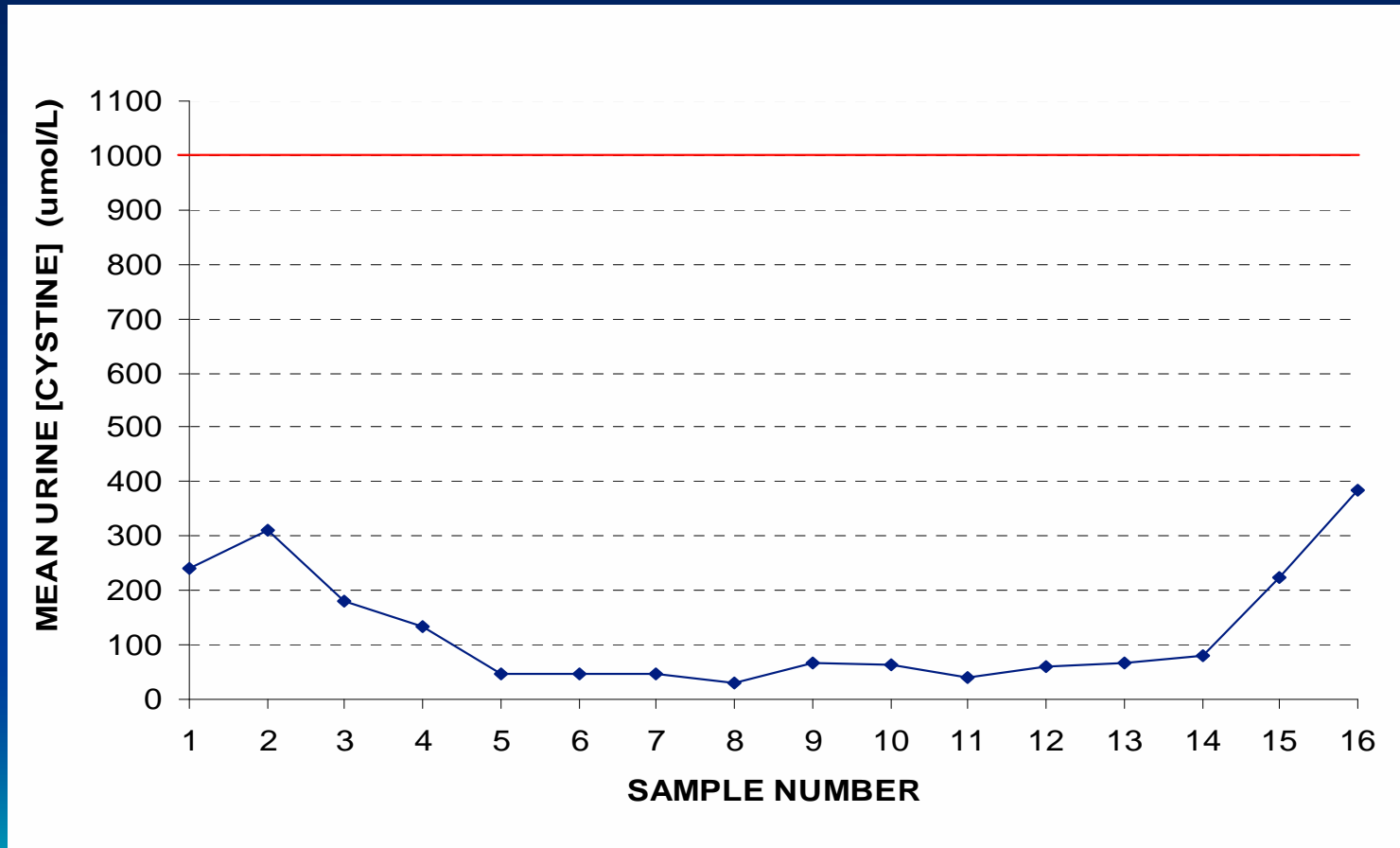
Serial Urine profiles
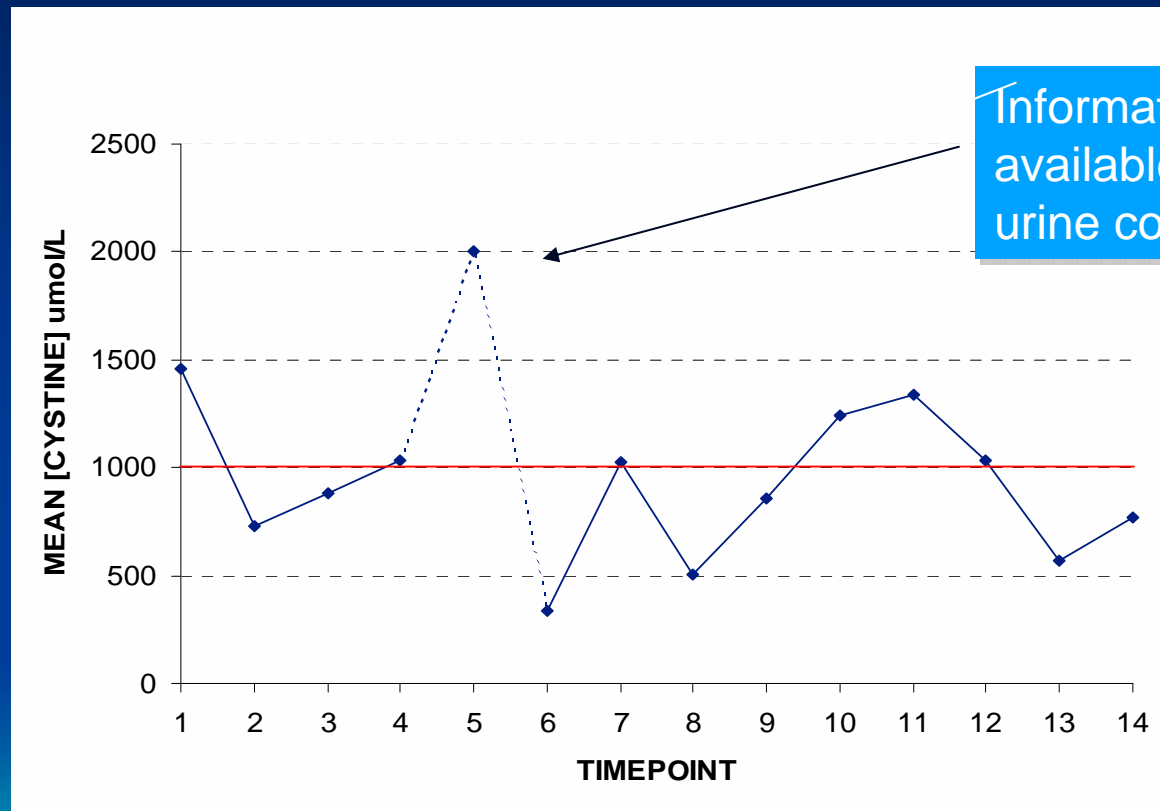
Serial Urine Profiles
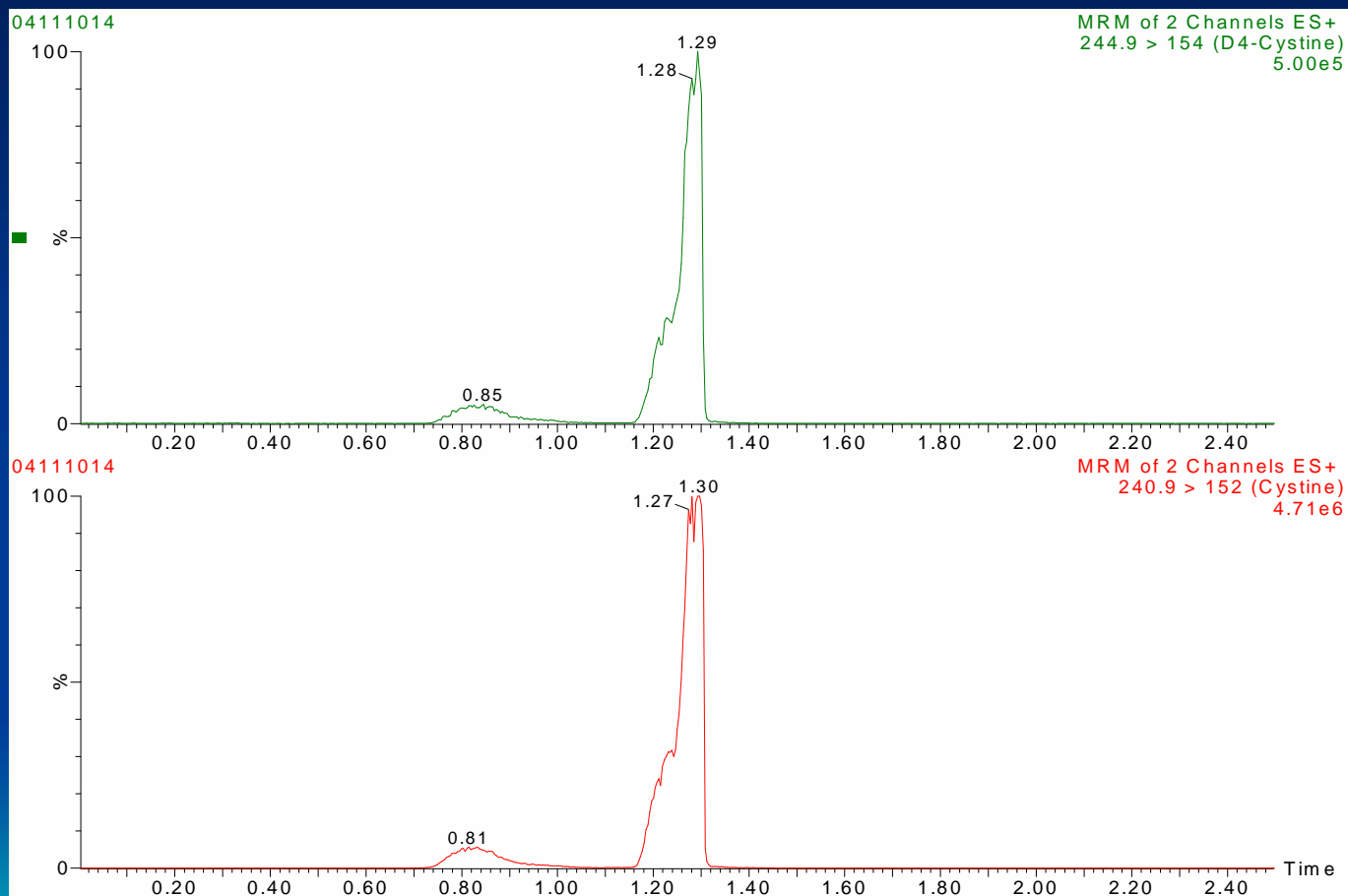
Serial Urine Profiles
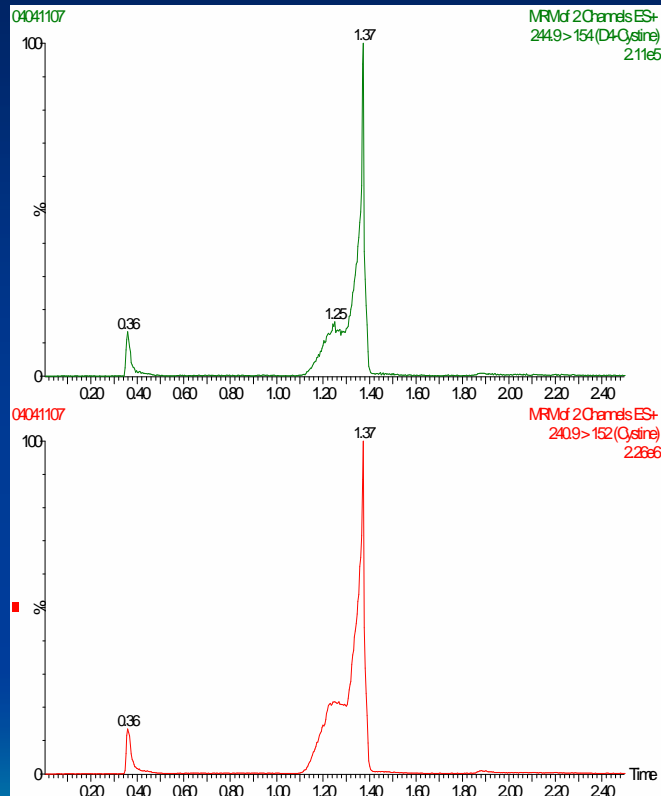
Technical Problems

- Phosphoric acid very corrosive for the source and caused significant problems for other assays

- Not viable to be introduced as a routine service

- Other mobile phase additive need to reduce pH<2.

- Trifluroacetic acid was main candidate

Current Chromatography

Current Chromatography

Are there any other mobile phase additives we could use?

Any experience at running MS/MS at low pH?

Could use high pH but would need to change urine collection method?