A Short Case Study – Sulphite Oxidase Deficiency picked up on Amino Acid Analysis

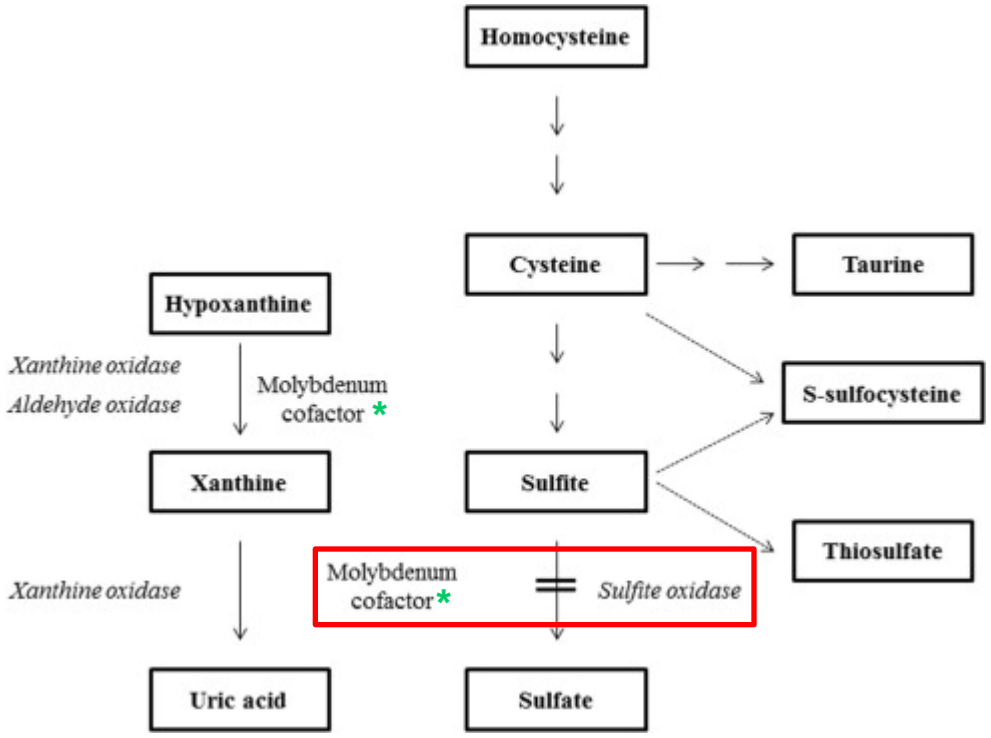
MetBio.Net BMS Training Group Annual Conference

14th November 2019

Sulphite Oxidase Deficiency

Metabolic Derangement:

- Autosomal recessive disorder caused by mutations in SUOX gene which encodes the sulphite oxidase enzyme.



* Sulphite oxidase requires Molybdenum co-factor which can also be defective in another metabolic disorder (Molybdenum co-factor deficiency (MoCo)).

Sulphite Oxidase Deficiency

Clinical Presentation:

- Neonatal:
  - Poor feeding
  - Hypotonia
  - Seizures
  - Followed by spasticity and severe development impairment
- Childhood:
  - Movement disorder
  - Stroke
  - Developmental regression

Sulphite Oxidase Deficiency

Diagnostic Tests:

- Plasma amino acids: ↑ Tau, Sulphocysteine, ↓ Cys
- Plasma: ↓ Total Homocysteine, (↓ Urate – in MoCo)
- Urine sulphite: Positive on dipstick (fresh urine sample required)
- Urine purine screen (xanthine elevated) – in MoCo
- Genetic analysis

Sulphite Oxidase Deficiency

Treatment and Prognosis:

- Poor prognosis for neonatal onset cases
- Low cysteine and methionine diet may help patients with mild form of sulphite oxidase deficiency

Biochrom Amino Acid Analysis

Ion Exchange Chromatography (IEC):

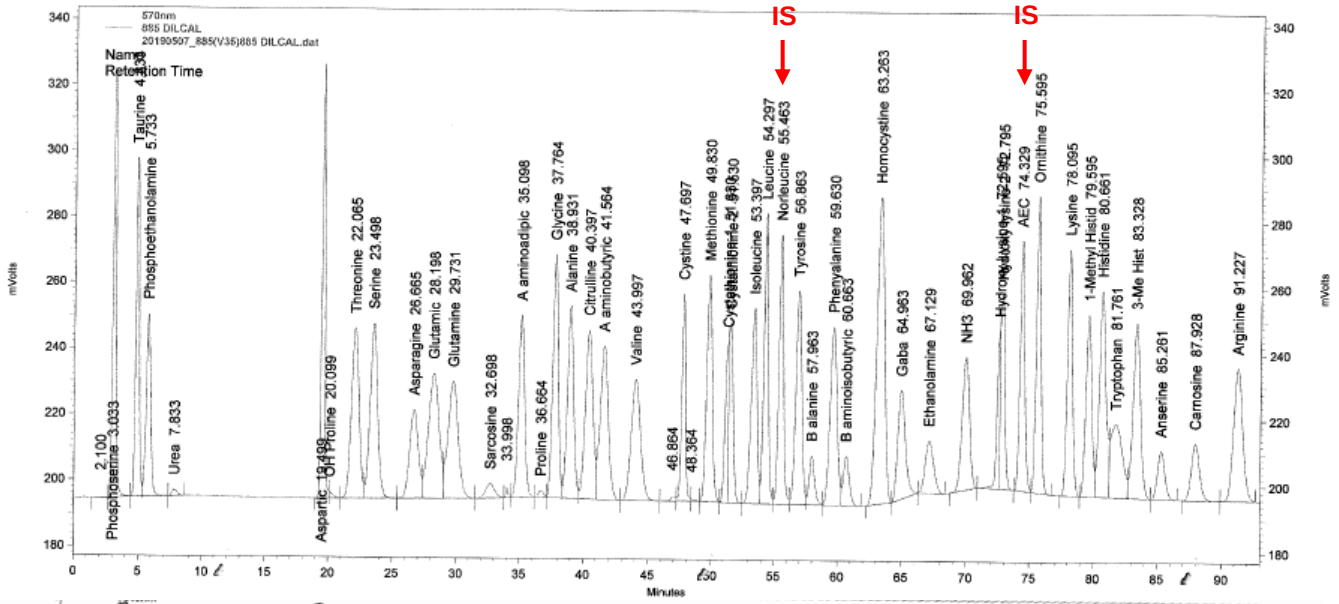
- Traditionally IEC referred to as the gold standard methodology.
- Routinely used in clinical laboratory.
- Cation exchange chromatography with post column ninhydrin detection
  - Stepwise elution with a series of lithium citrate buffers of increasing pH and ionic strength.
  - More acidic species elute first
- Dual detection:
  - 570nm (purple)
  - 440nm (yellow): better for detection of imino acids (proline, hydroxyproline)

Biochrom Amino Acid Analysis

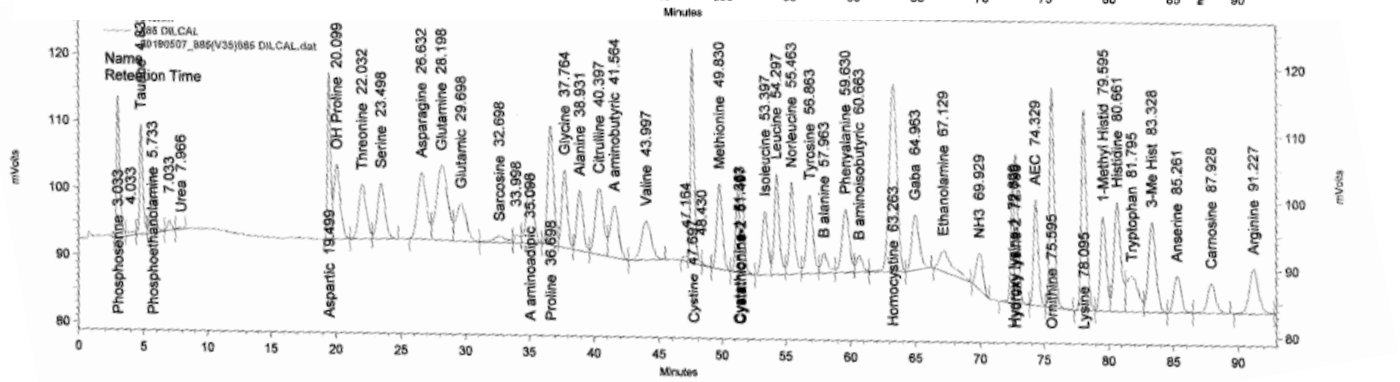
Ion Exchange Chromatography (IEC):

Standard:

570nm



440nm



Biochrom Amino Acid Analysis

Ion Exchange Chromatography (IEC):

Standard:

16	Proline	200 CAL	35.1	1862160 4.97
17	Glycine	200 CAL	36.7	52748 0.09
18	Alanine	200 CAL	37.8	2185885 6.43
19	Citrulline	200 CAL	38.9	1966673 6.57
20	A aminobutyric	200 CAL	40.4	2130421 4.94
21	Valine	200 CAL	41.6	2023232 6.04
22		200 CAL	44.0	1861393 6.18
23	Cystine	0	46.9	29809
24		100 CAL	47.7	1224033 1.91
25	Methionine	0	48.4	10009
26	Cystathionine-1	200 CAL	49.8	2075141 5.43
27	Cystathionine-2	100 CAL	51.3	809876 3.43
28	Isoleucine	100 CAL	51.5	1279892 4.06
29	Leucine	200 CAL	53.4	1870500 6.15
30	Norleucine	200 CAL	54.3	2114650 5.35
31	Tyrosine	200 CAL	55.5	2058942 5.48
32	B alanine	200 CAL	56.9	2113141 5.23
33	Phenyalanine	200 CAL	58.0	437570 4.00
34	B aminoisobutyric	200 CAL	59.6	2051648 5.56
		200 CAL	60.7	493377 5.96
35	Homocystine	200 CAL	63.3	3909184 3.39
36	Gaba	200 CAL	65.0	1335058 4.05

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Use channel ratio (570nm:440nm) for all standard compounds to compare against patient values to confirm where peaks are coeluting with interfering substances or presence of unusual (atypical) amino acids which can coelute (e.g. homocitrulline and methionine).

Biochrom Amino Acid Analysis

Ion Exchange Chromatography (IEC):

- Advantages:
  - Dedicated instrument
  - Minimal sample preparation required – no derivitisation step
  - Stable and precise
  - Identifies all analytes of interest
  - Identifies unusual (atypical) amino acids
  - Commercial kit
  - In routine use for a number of years

Biochrom Amino Acid Analysis

Ion Exchange Chromatography (IEC):

- Disadvantages:
  - Long analysis time ~130mins for standard profile
  - Structural analogue internal standard
  - Single point calibration, sporadic frequency
  - Expensive capital cost
  - Expertise in technology required
  - Significant maintenance
  - Often running to capacity
  - Method lacks specificity
    - Identification based on retention time alone (channel ratio used as a second marker for reassurance)
    - Co-eluting substances
    - Interferences from drugs
    - Poor resolution of some analytes e.g. sulphocysteine

Cases

Case 1

Clinical Presentation:

- Baby girl, first child of non-consanguineous parents of Polish origin.
- Born at term following normal pregnancy and delivery
- Presented at 90 minutes of life with seizures that were unresponsive to Phenobarbitone, Valproate, Keppra and Midazolam
- On examination she had increased tone in limbs
- Brain MRI showed appearances suggestive of hypoxic-ischaemic change

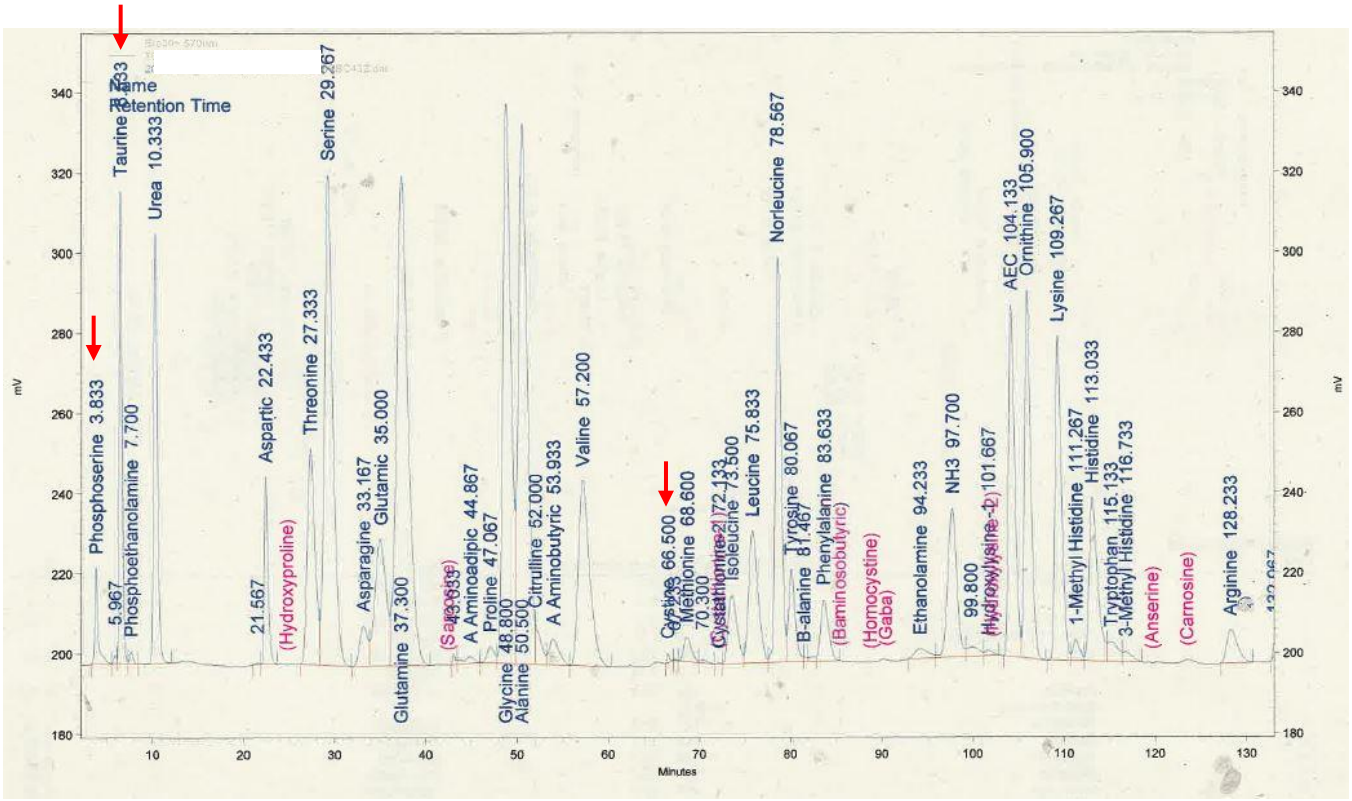
Case 1

Biochemistry:

- Plasma Ammonia: Normal
- Plasma Urate: Normal
- Acylcarnitines: Not suggestive of a Fatty Acid Oxidation Disorder.
- Urine Organic Acids: No specific abnormality detected.
- Amino Acid results….

Case 1

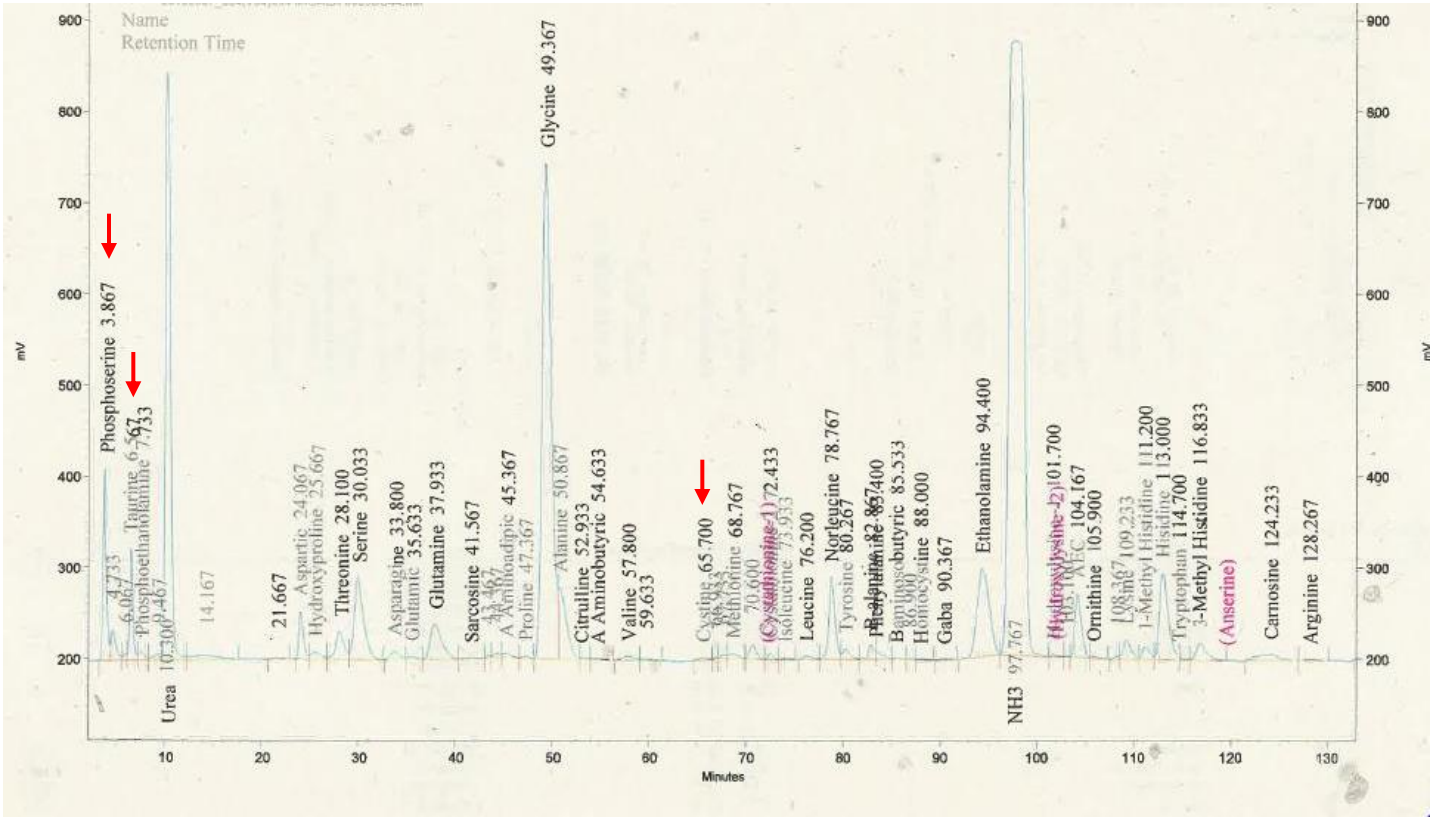
Plasma Amino Acids:



- Plasma Tau = 144umol/L (26-169)
- Plasma Cys = 2umol/L (21-53)
- Plasma Phosphoserine channel ratio: 1.37 (5.08) - **? sulphocysteine**

Case 1

Urine Amino Acids:



- Urine Tau = 121umol/mmol creatinine (8-266)
- Urine Cys = 4umol/mmol creatinine (24-78)
- Urine Phosphoserine channel ratio: 1.23 (5.37) - **? sulphocysteine**

Case 1

Biochemistry:

- Urine Sulphocysteine: 90.6 umol/mmol (0-10)
- Genetic Analysis: SUOX homozygous gene mutation c.90C>A (p.(Cys30Ter))

Case 2

Clinical Presentation:

- Baby girl, second child of consanguineous parents
- FH:
  - 10yr old brother with severe global developmental delay, ? undiagnosed metabolic disorder
- Presented at 2 months of age with:
  - 1 day history of irritability
  - Attended to local hospital with seizures and abnormal posturing that eventually progressed to apnoeas
  - Previously fit and well
  - Abnormal brain MRI

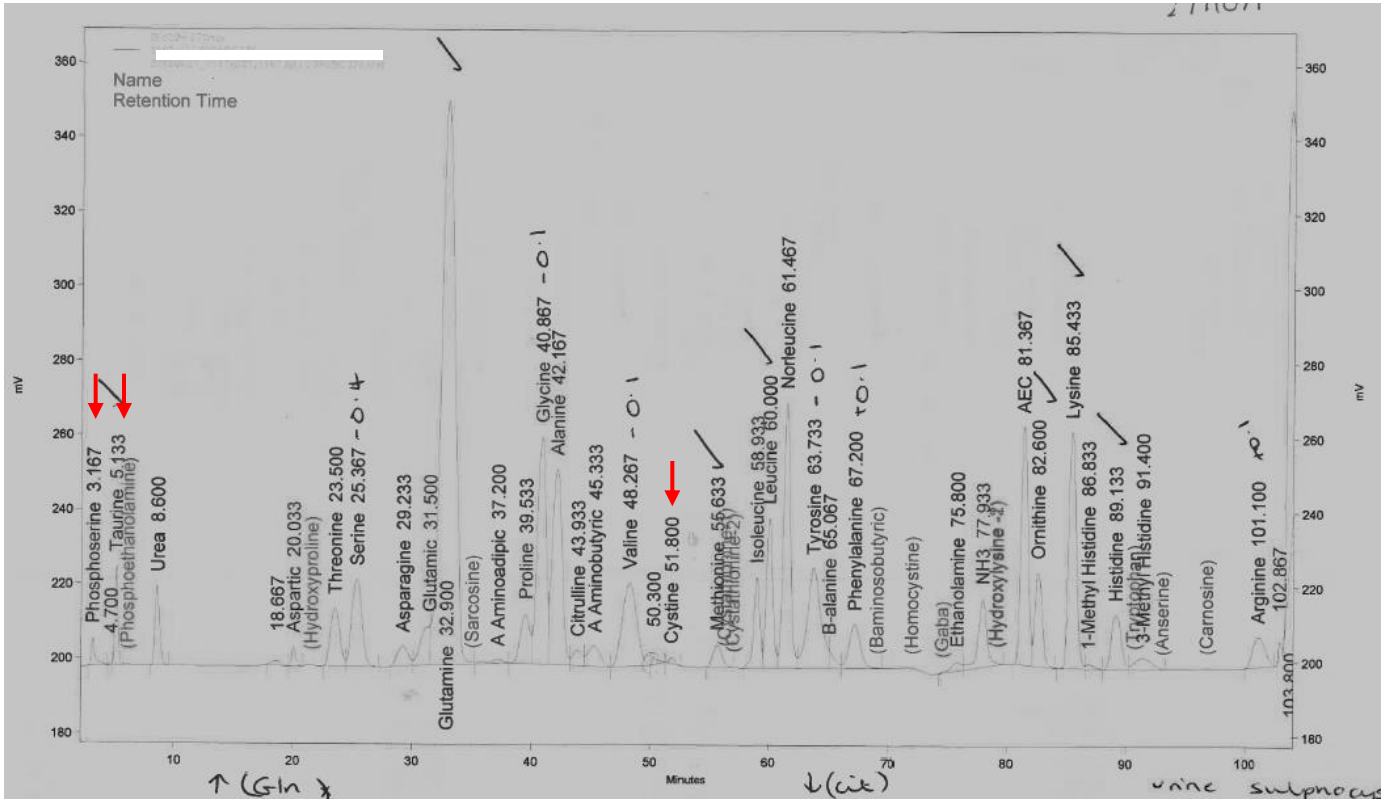
Case 2

Biochemistry:

- Plasma Ammonia: Normal
- Plasma Urate: Normal
- Acylcarnitines: Not suggestive of a Fatty Acid Oxidation Disorder.
- Urine Organic Acids: No specific abnormality detected.
- Amino Acid results….

Case 2

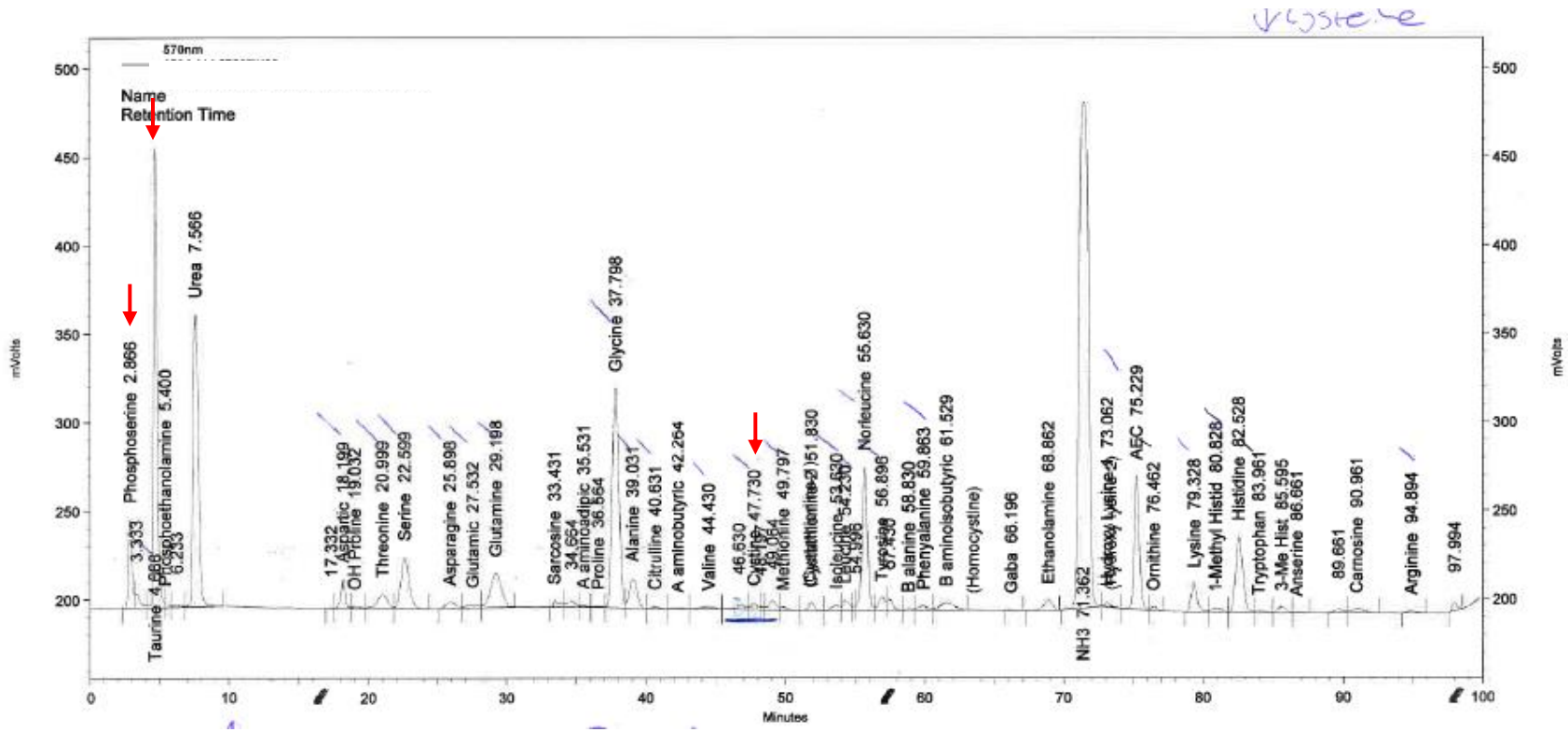
Plasma Amino Acids:



- Plasma Tau = 26umol/L (26-169)
- Plasma Cys = 3umol/L (21-53)
- Plasma Phosphoserine channel ratio: 1.44 (5.71) - **? sulphocysteine**

Case 2

Urine Amino Acids:



- Urine Tau = 1400umol/mmol creatinine (6-89)
- Urine Cys = 25umol/mmol creatinine (13-48)
- Urine Phosphoserine channel ratio: 2.03 (5.92) - **? sulphocysteine**

Case 2

Biochemistry:

- Urine Sulphocysteine: 112.8 umol/mmol (0-10)
- Genetic Analysis: SUOX homozygous gene mutation c.1097G>A (p.(Arg366His))

Summary

- Sulphide oxidase deficiency can be picked up on amino acid analysis using IEC (Biochrom).
  - Plasma/ Urine: ↑ Tau, Sulphocysteine, ↓ Cys
- However….
  - Sulphocysteine:
    - Difficult to detect as coelution with phosphoserine
      - Separation difficult to adjust as at the beginning of the run – immediately start with buffer 1 and there is no buffer or temperature change over the period in which it elutes.
    - Channel ratio can be an indicator that there is a coeluting substance present but not specific to sulphocysteine.
  - Taurine is not always raised.
  - Most reliable marker is absence of plasma cystine (although there is a buffer change in the region so often get a small peak).
  - Interpret results in conjugation with clinical details and other biochemical markers.