Birmingham Children's Hospital
NHS Foundation Trust

Tackle your AAA

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Birmingham Children's Hospital

So what do we mean by *tackle?*

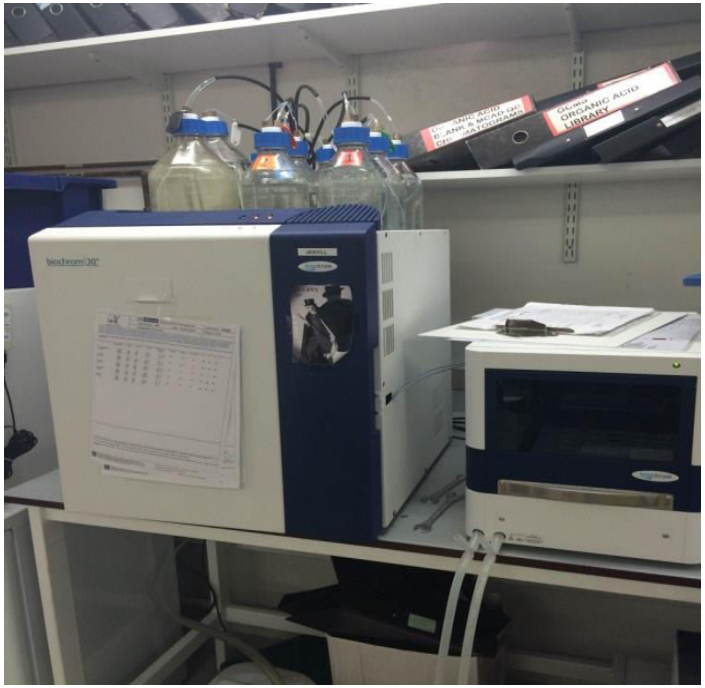
make determined efforts to deal with
(a problem or difficult task)

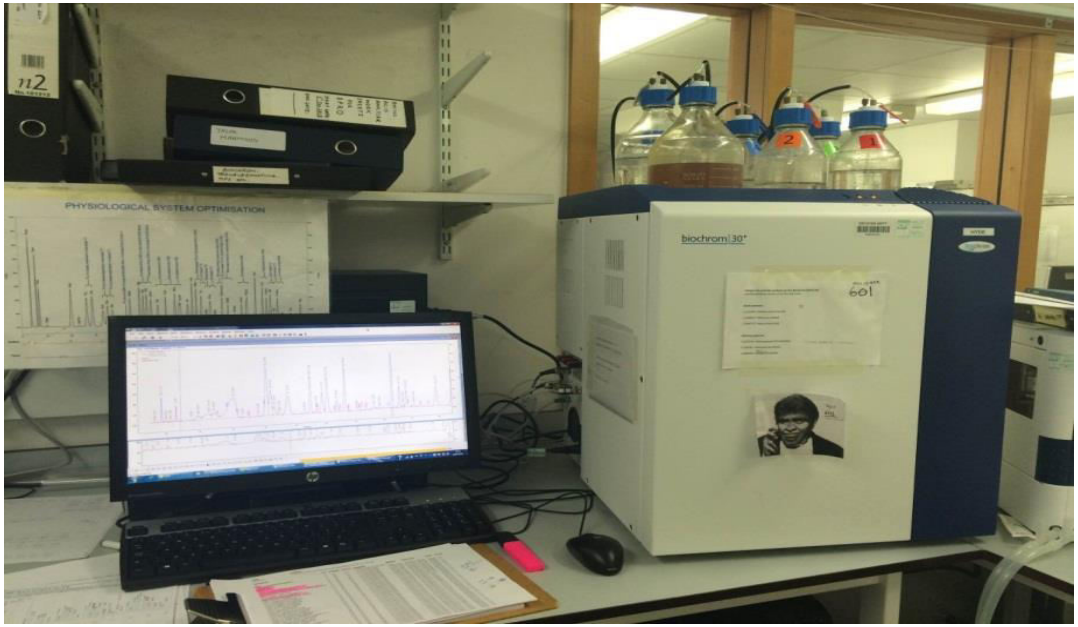
synonyms:

get to grips with, apply oneself to, address oneself to, address, set about, go about, get to work at, take forward, busy oneself with, set one's hand to, grapple with, approach, take on, attend to, see to, throw oneself into, try to solve, try to deal with, try to cope with, try to sort out!

Meet Jekyll and Hyde

- **Autosampler, Biochrom 30+ amino acid analyser, PC with instrument control software + EZ Chrom Elite integration software**





Our Biochrom 30 plus'

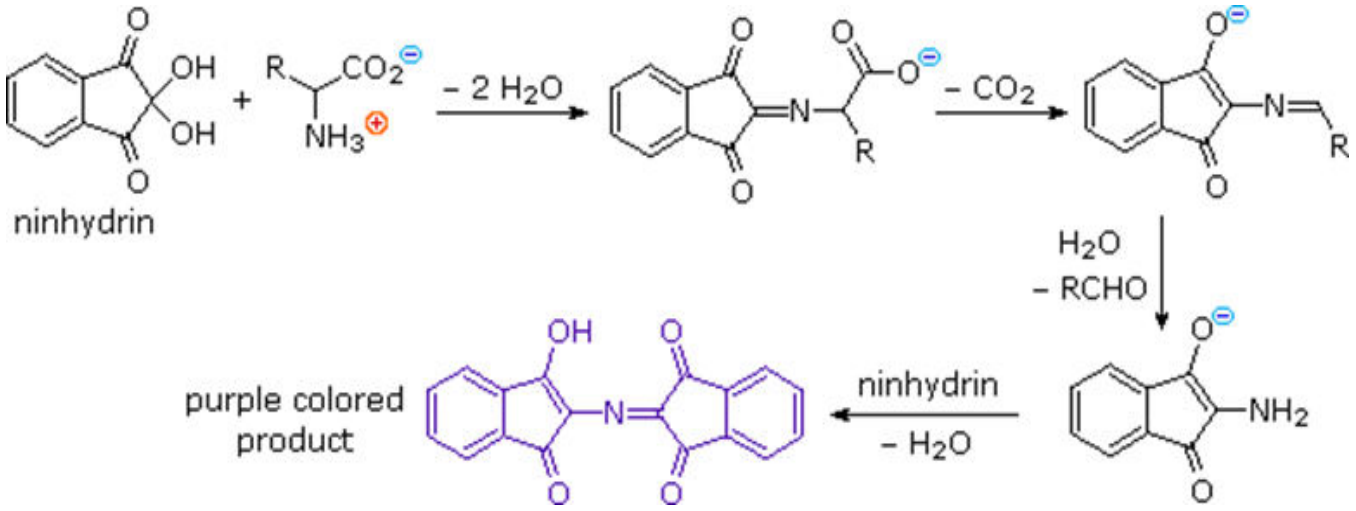
Jekyll and Hyde – named due to their slightly *unpredictable* nature

(And it's our local pub just across the road…..)

Briefly – How does it work?

- Sample prep – 1:1 with IS, containing deproteinising reagent SSA
- ?filter sample
- Plasma, CSF, urine
- Continuous flow cation-exchange.
- Lithium citrate buffers varying pH, ionic strength
- Accurately controlled temperature of the column
- Column resin – small beads of polystyrene with –ve charge
- Amino acids introduced at low pH – net positive charge
- Basic – most strongly bound, acidic least
- Separation is due primarily to differences in the pKA of the amino acids.
- Follows the general order: acidic, neutral, basic.
- Effluent that comes off the column mixed with the ninhydrin – passed through reaction coil at high temperature

- Ninhydrin reacts with the amino acids to form coloured compounds.
- Amount of coloured compound - directly proportional to the quantity of amino acid.
- The light absorption is measured at two wavelengths 570nm (purple) and 440nm (yellow)



Maintenance

Daily/Weekly

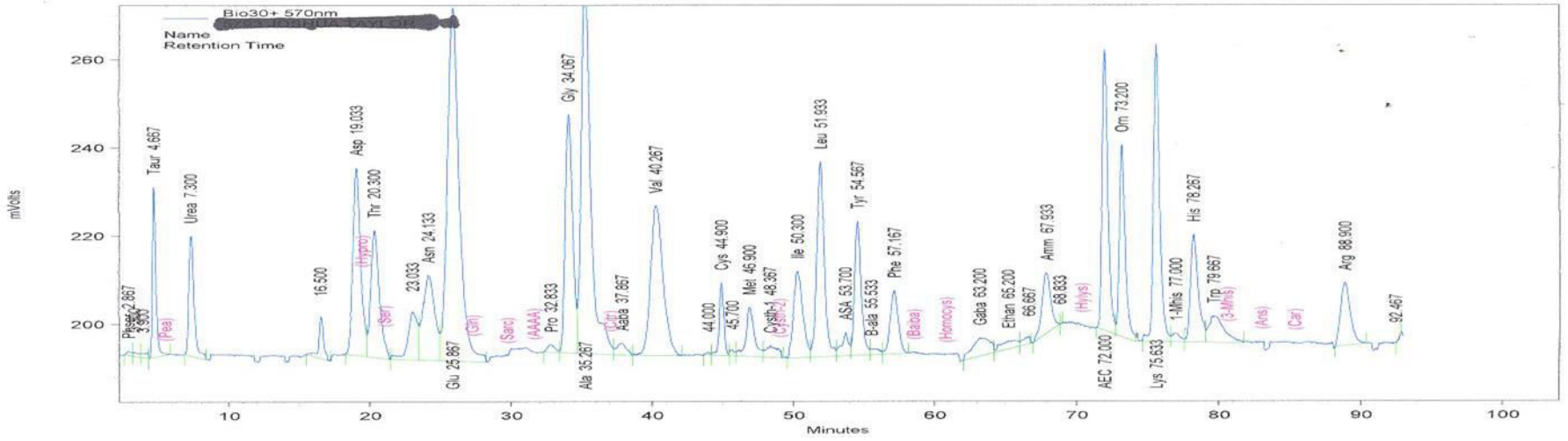
- Consumable levels (buffers, wash solutions) and waste

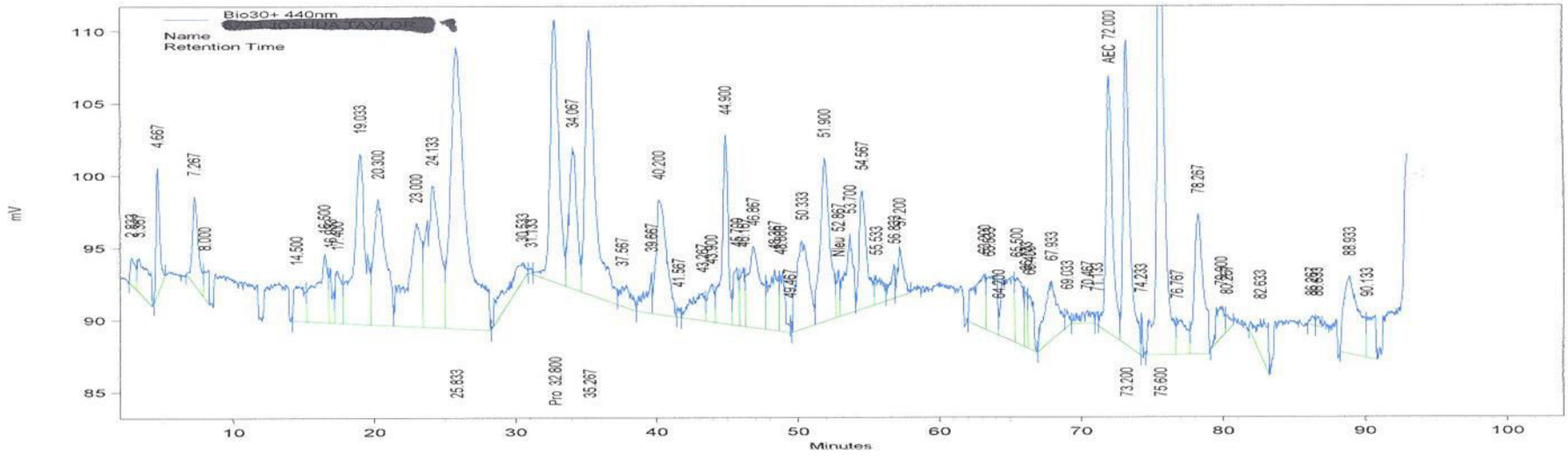
Monthly

- Isopropanol wash – reaction coil and back pressure valves
- Ninhydrin filter
- Reboot PC

Now it's your turn…..! Get ready

Audience participation section

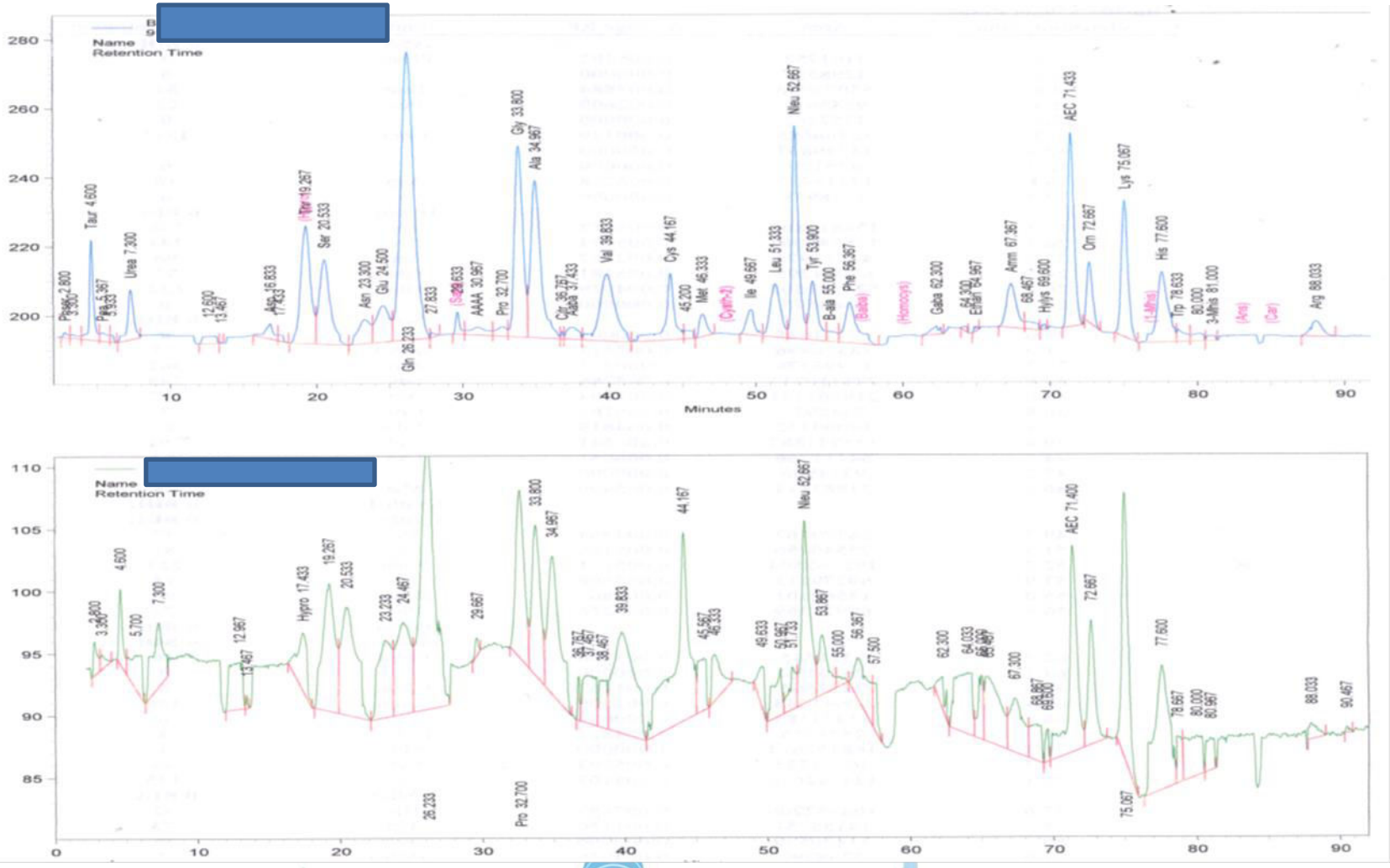

Bio30+ 570nm
Name
Retention Time
mVolts
Minutes
Taur 4.667
Urea 7.300
Asp 19.033
Thr 20.300
Asn 24.133
Glu 25.867
Gly 34.067
Ala 35.267
Val 40.267
Cys 44.900
Met 46.900
Ile 50.300
Leu 51.933
Tyr 54.567
Phe 57.167
Orn 73.200
His 78.267
Trp 79.667
Arg 88.900
Lys 75.633
AEC 72.000


Bio30+ 440nm
Name
Retention Time
mV
Minutes
4.667
7.267
19.033
20.300
24.133
25.833
34.067
Pro 32.800
35.267
40.200
44.900
51.900
54.567
AEC 72.000
73.200
75.600
78.267
88.933

Typical Lamp failure

- Electrical baseline noise
- Spikes caused by filament collapsing

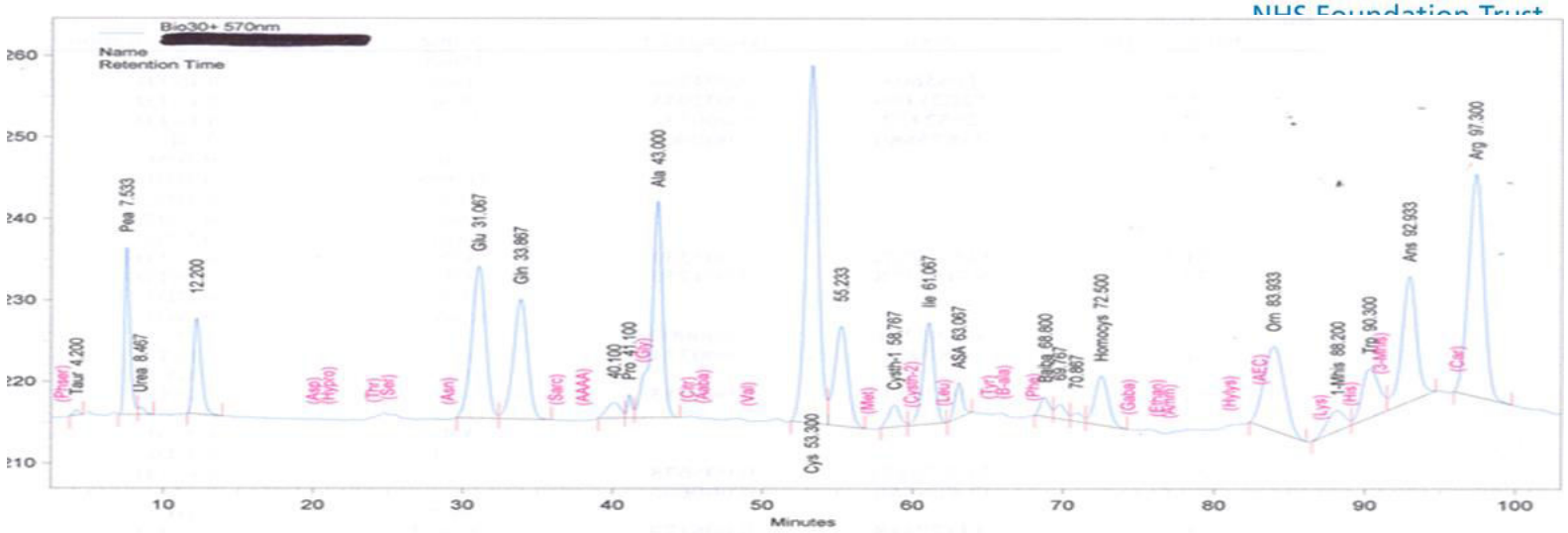
- Replace lamp
- Other sources of baseline noise – flow-cell may require cleaning

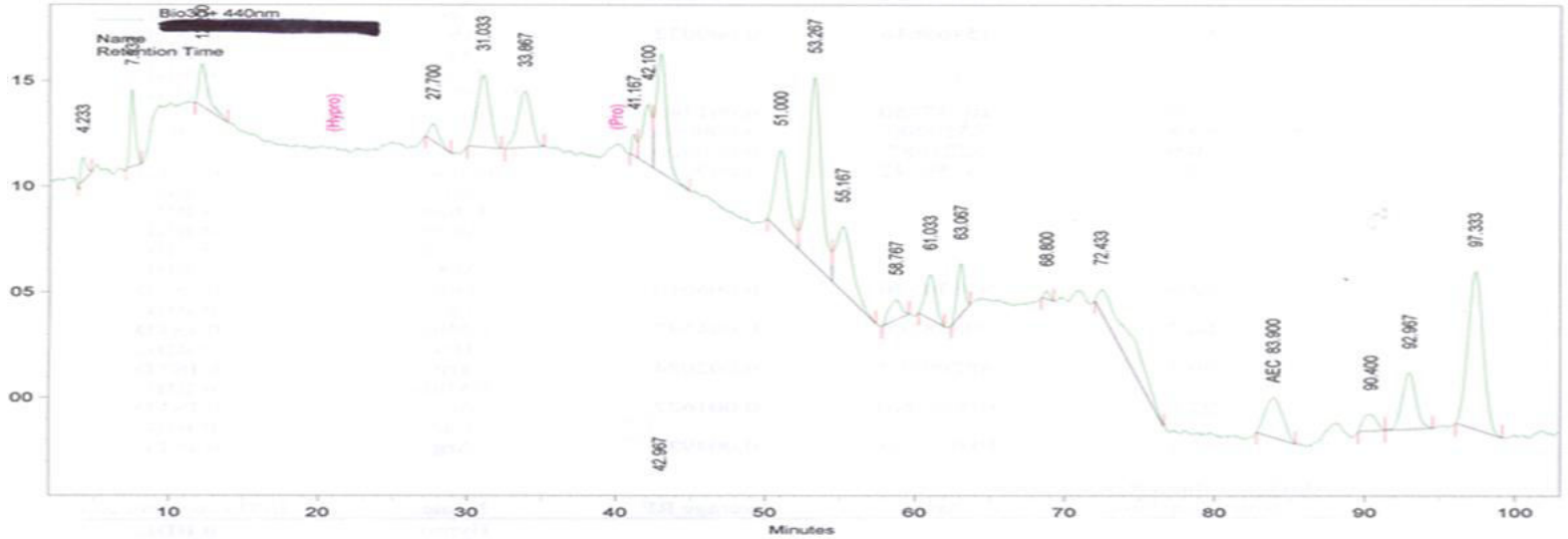

Name
Retention Time
Minutes
Hydro 17.433
Hyp 19.267
Ser 20.533
Gly 33.800
Ala 34.967
Leu 52.867
AEC 71.433
Lys 75.067
Pro 32.700
Gln 26.233

Actually…. Another Lamp failure

- Poor defined peaks ? Column fail, some spiking
- Trace sent to Biochrom
- Engineer visit

- Replace lamp
- Work through the obvious trouble-shooting first (lamp had only recently been replaced)

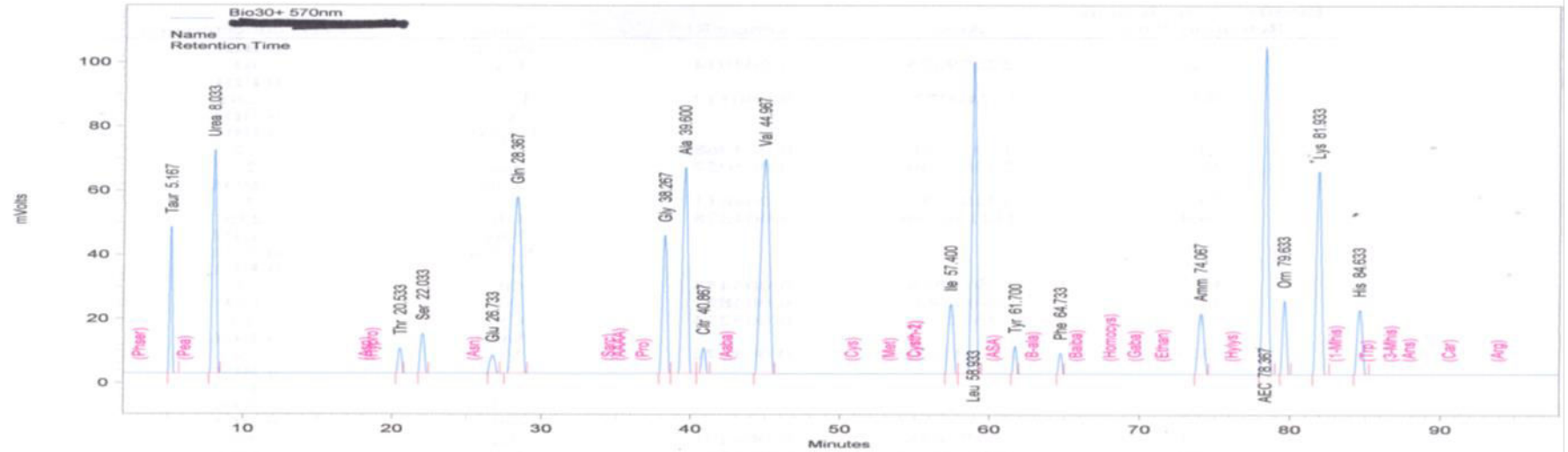

Bio30+ 570nm
[redacted]
Name
Retention Time
Pea 7.533
12.200
Glu 31.067
Gln 33.867
Ala 43.000
Cys 53.300
55.233
Cysth-1 58.767
Cysth-2
Ile 61.067
ASA 63.067
Homocys 72.500
Orn 83.933
Ans 92.933
Arg 97.300
Minutes

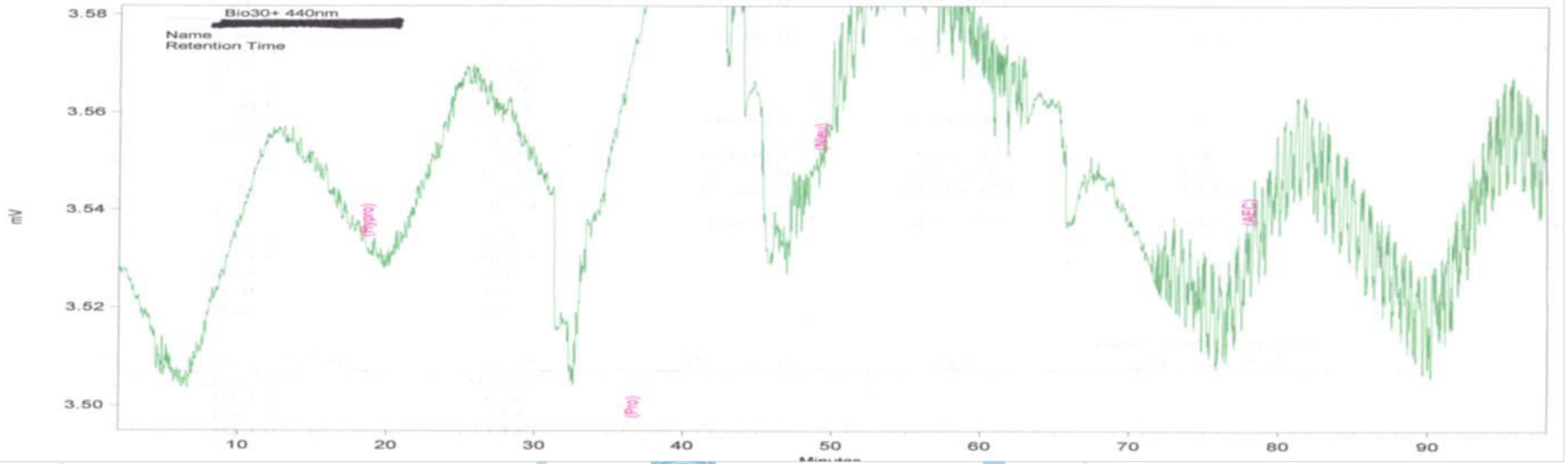

Bio30+ 440nm
[redacted]
Name
Retention Time
4.233
27.700
31.033
33.867
41.167
42.100
42.967
51.000
53.267
55.167
58.767
61.033
63.067
68.600
72.433
AEC 83.900
90.400
92.967
97.333
Minutes

Column heating peltier failure

- Significant change to peak retention times
- Check that the column temperature increases and decreases when changed on Biosys instrument control
- check that both Peltier elements are heating

- Replace peltiers (Engineer visit)


Bio30+ 570nm
Name
Retention Time
mVolts
Minutes
Taur 5.167
Urea 8.033
Thr 20.533
Ser 22.033
Glu 26.733
Gln 28.367
Gly 38.267
Ala 39.600
Cit 40.867
Val 44.967
Ile 57.400
Leu 58.933
Tyr 61.700
Phe 64.733
Amm 74.067
Orn 79.633
Lys 81.933
His 84.633


Bio30+ 440nm
Name
Retention Time
mV
Minutes

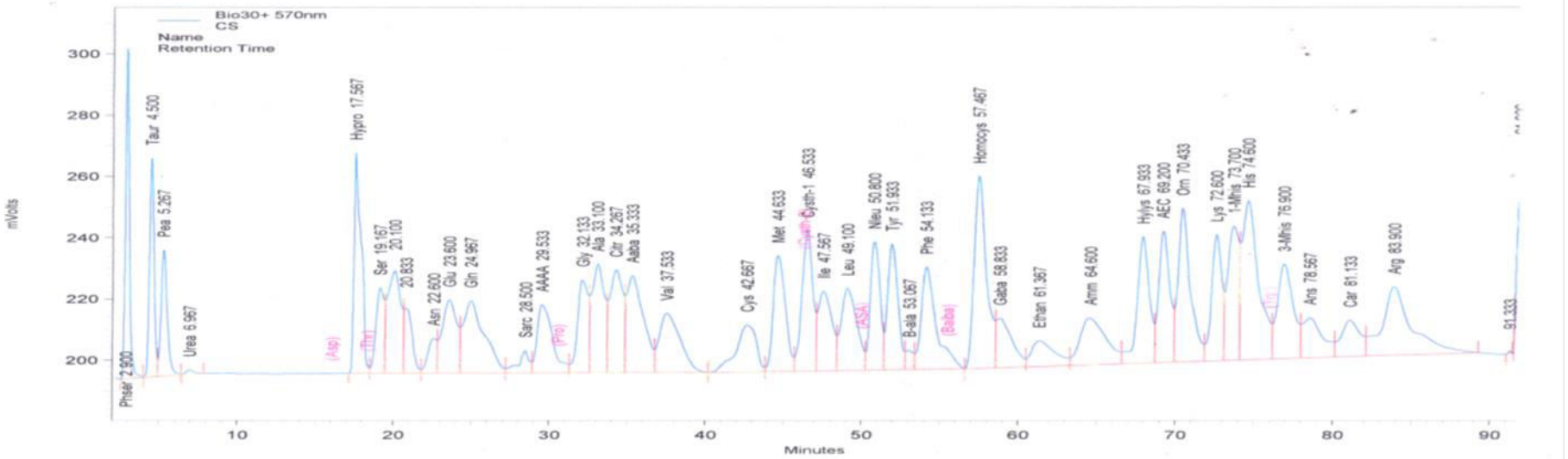
baseline hasn't started at the correct position

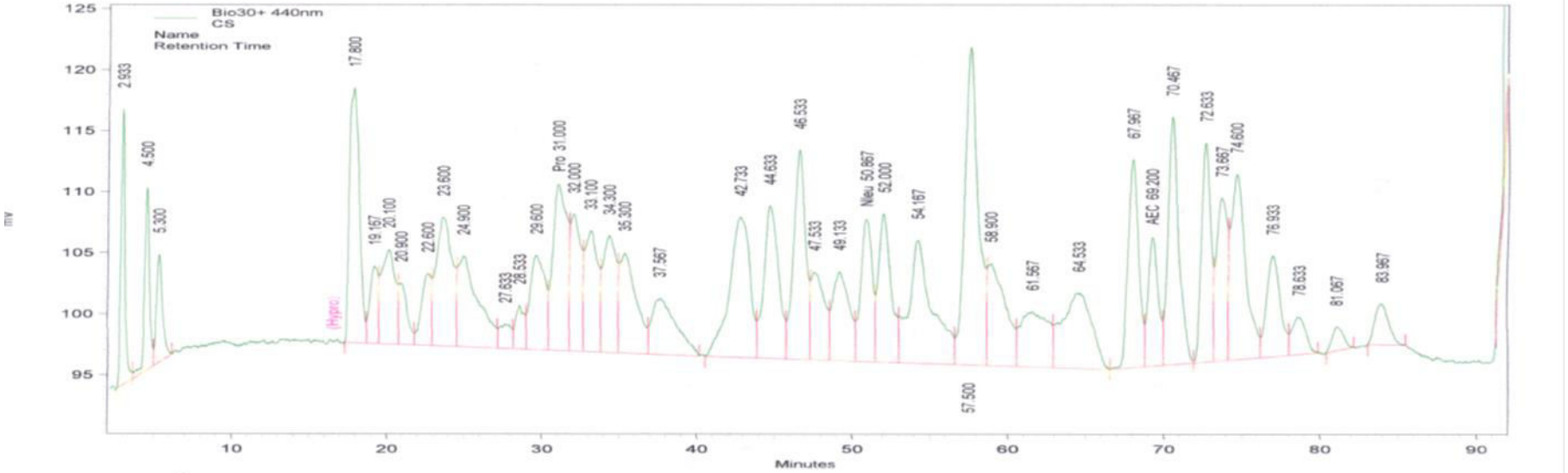
this could be caused by:-

1) the Ninhydrin pump not coming on for the last two steps of the Biosys program so then the mixture of Buffer and Ninhydrin isn't correct so the baselines on both channels start near to zero .
2) the baseline reset hasn't happened

Solutions

- shut everything down and wait for 10 minutes and then turn everything back on
- run an regeneration program and then run a blank (loading buffer) and make sure the chromatography shows the starting points are 200mv for the 570nm channel and 100 mv for the 440nm channel.
- Also check chromatography display options are set correctly I.E. Auto scale

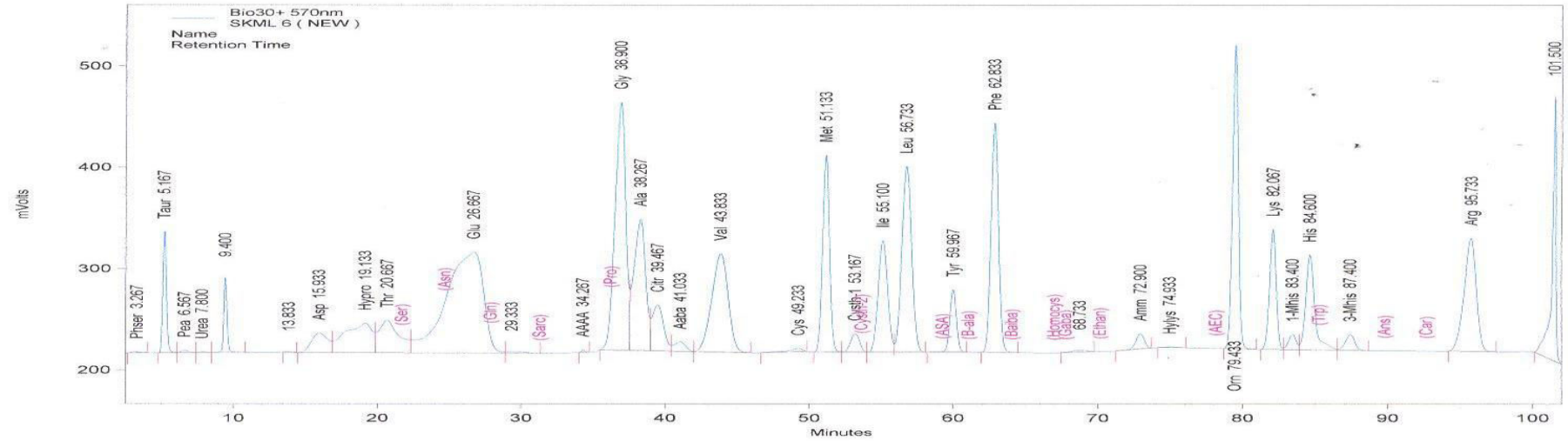

Bio30+ 570nm
CS
Name
Retention Time
mVolts
Minutes
Phear 2.900
Taur 4.500
Pea 5.267
Urea 6.967
(Asp)
Hypro 17.567
(Thr)
Ser 19.167
20.100
20.833
Asn 22.600
Glu 23.600
Gln 24.967
Sarc 28.500
AAAA 29.533
(Pro)
Gly 32.133
Ala 33.100
Citr 34.267
Aaba 35.333
Val 37.533
Cys 42.667
Met 44.633
Cystath 46.533
Ile 47.567
Leu 49.100
(ASA)
Nleu 50.800
Tyr 51.933
B-ala 53.067
Phe 54.133
(Baba)
Homocys 57.467
Gaba 58.833
Ethan 61.367
Amm 64.600
Hylys 67.933
AEC 69.200
Orn 70.433
Lys 72.600
1-MHis 73.700
His 74.600
(Tau)
3-MHis 76.900
Ans 78.567
Car 81.133
Arg 83.900
91.333

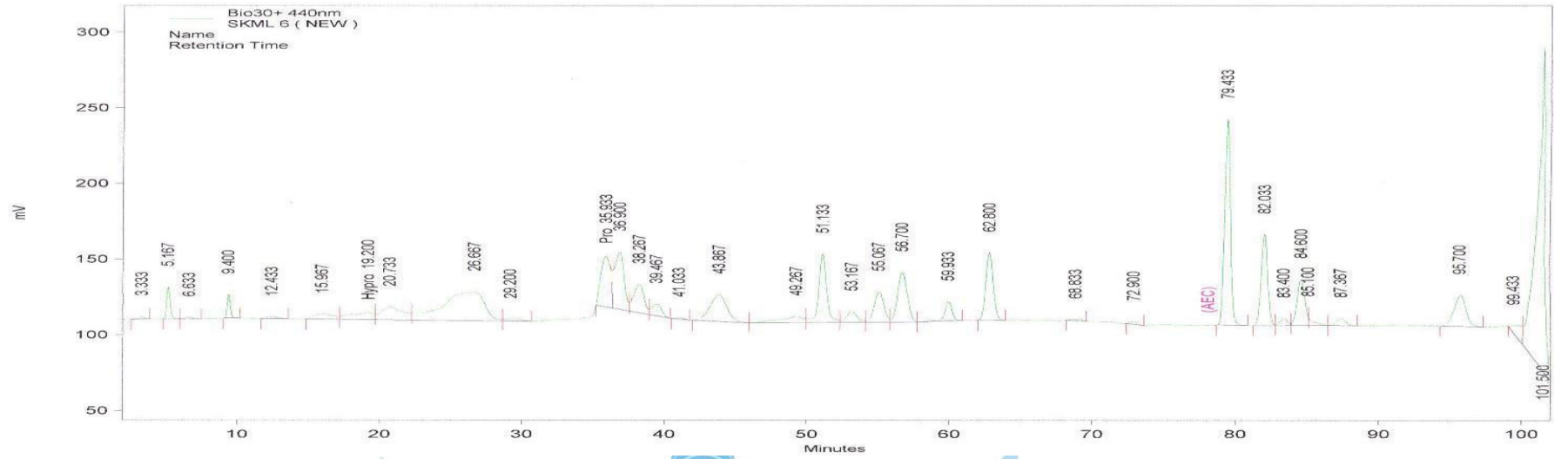

Bio30+ 440nm
CS
Name
Retention Time
mv
Minutes
2.933
4.500
5.300
(Hypro)
17.800
19.167
20.100
20.800
22.600
23.600
24.900
27.633
28.533
29.600
Pro 31.000
32.000
33.100
34.300
35.300
37.567
42.733
44.633
46.533
47.533
49.133
Nleu 50.867
52.000
54.167
57.500
58.900
61.567
64.533
67.967
AEC 69.200
70.467
72.633
73.667
74.600
76.933
78.633
81.067
83.967

Resin contamination

- Distorted peaks
- Poor separation
- Exaggerated valleys
- Headspace issue – affects separation at front of chromatography e.g up until Gly/Ala region
- High buffer pressure errors (early stage column failure)

- Reverse flush the analytical column (instructions available from Biochrom)
- Check head space and top up resin
- replace column and clean

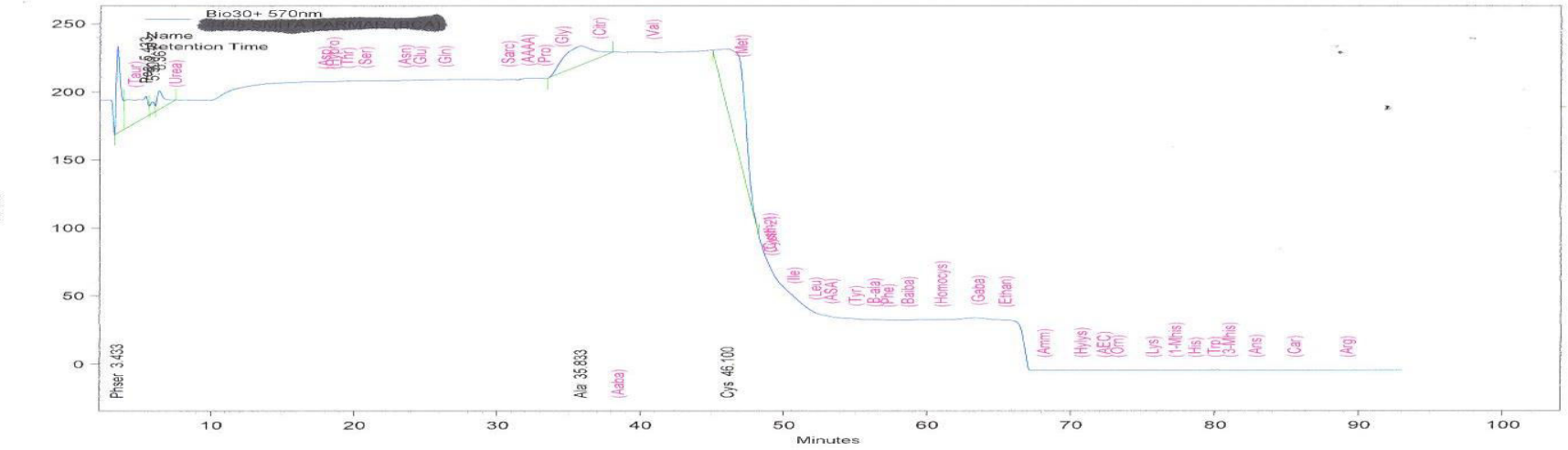

Bio30+ 570nm
SKML 6 (NEW)
Name
Retention Time
mVolts
Minutes
Phser 3.267
Taur 5.167
Pea 6.567
Urea 7.800
9.400
13.833
Asp 15.933
Hypro 19.133
Thr 20.667
(Ser)
(Asn)
Glu 26.667
(Gln)
29.333
(Sarc)
AAAA 34.267
(Pro)
Gly 36.900
Ala 38.267
Citr 39.467
Aabu 41.033
Val 43.833
Cys 49.233
Met 51.133
(Cystin1
(Cystin2)
53.167
Ile 55.100
Leu 56.733
Tyr 59.967
(ASA)
(B-ala)
Phe 62.833
(Baiba)
(Homocys)
(Gaba)
68.733
(Ethan)
Amm 72.900
Hylys 74.933
(AEC)
Orn 79.433
Lys 82.067
1-MHis 83.400
His 84.600
(Trp)
3-MHis 87.400
(Ans)
(Car)
Arg 95.733
101.500

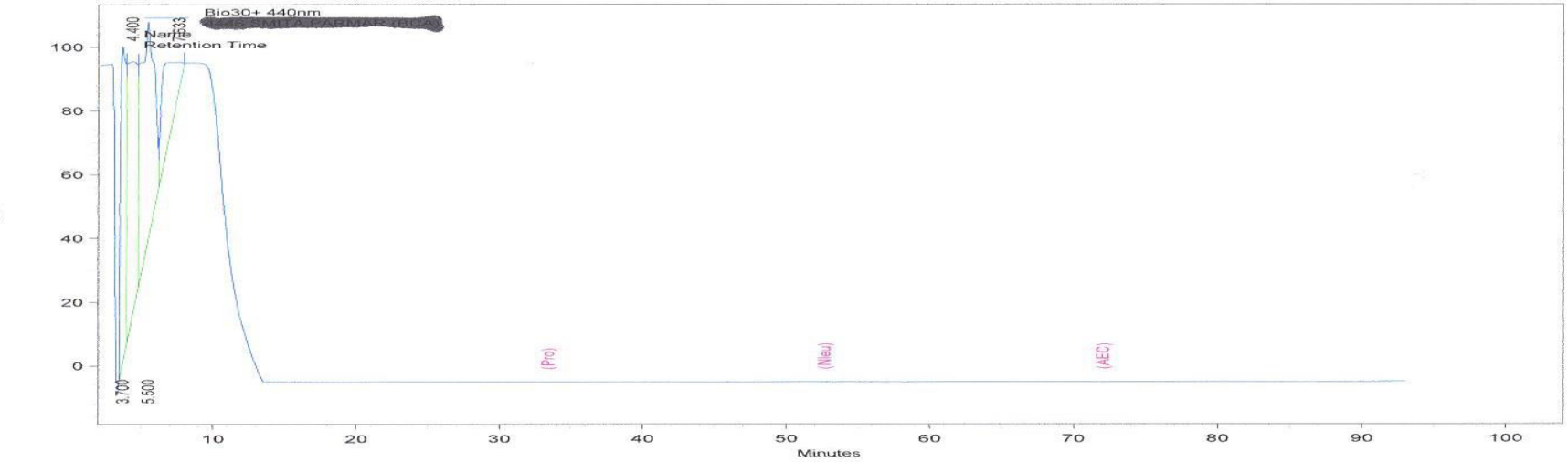

Bio30+ 440nm
SKML 6 (NEW)
Name
Retention Time
mV
Minutes
3.333
5.167
6.633
9.400
12.433
15.967
Hypro 19.200
20.733
26.667
29.200
Pro 35.933
36.900
38.267
39.467
41.033
43.867
49.267
51.133
53.167
55.067
56.700
59.933
62.800
68.833
72.900
(AEC)
79.433
82.033
83.400
84.600
85.100
87.367
95.700
99.433
101.500

Undeproteinised sample

- Make sure all samples are pre-treated correctly!
- Can try running Buffer 6 through

- Column required resin clean


Bio30+ 570nm
Name
Retention Time
mVolts
Phser 3.433
Ala 35.833
Cys 46.100
(Aaba)
(Taur)
(Urea)
(Asn)
(Glu)
(Gln)
(Sarc)
(AAAA)
(Pro)
(Gly)
(Citr)
(Val)
(Met)
(Cystathi2)
(Ile)
(Leu)
(ASA)
(Tyr)
(Phe)
(Baiba)
(Homocys)
(Gaba)
(Ethan)
(Amm)
(Hylys)
(AEC)
(Orn)
(Lys)
(1-MHis)
(His)
(Trp)
(3-MHis)
(Ans)
(Car)
(Arg)
Minutes


Bio30+ 440nm
Name
Retention Time
mV
3.700
4.400
5.500
(Pro)
(Nleu)
(AEC)
Minutes

Reaction coil heating failure

-Just seeing the buffer trace

- replace reaction coil (replaced in house)

Other errors

- Low buffer/ninhydrin pressure:-

- ensure reservoirs are not empty
- Ninhydrin filter changed – air in system?

- High ninhydrin pressure:-

- Ninhydrin flow rate too high – program accidently altered with flow rate increased for one step

Summary

- Low maintenance analyser
- Generally very reliable
- Always helpful advice from Biochrom

- Balance between the speed of the analysis and quality of trace
- Volume of work
- Consumable dependant – good stocks
- Look for the obvious!