TITLE: APPLICATIONS OF HPLC AMINO ACID DETERMINATIONS (1-85-0013)

INVESTIGATORS: Principal Investigator: Clay Goodman
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Claudia S. Robertson

Over the past year, we have used Moran Foundation funds for the measurement of serum amino acids in head injuried patients using high pressure liquid chromatography with post-column ninhydrin derivatization. Though technically demanding, this technique permits accurate detection of some 40 serum constituents including all the essential and nonessential amino acids.

Pickering Laboratories of Mountain View, California, was the source of columns and reagents for a post-column ninhydrin derivatization method of measuring amino acids in physiological fluids. Chromatographic conditions included a 3mm X 250mm 10µM lithium cation exchange column at 42°C with a guard column and a three component lithium salt mobile phase (flow rate 0.3 ml/min) gradient with an elution and regeneration cycle of 210 minutes (figure 1). Following separation, the amino acids were mixed with ninhydrin (flow rate 0.3 ml/min) at a mixing tee and subsequently reacted for 100 seconds at 120°C in a Pickering CRX390 post column reactor resulting in chromophores monitored at 546 nm in a 12µl cell (figure 2). An injection of standard and a serum sample on the system in its current configuration are shown (figures 3 & 4). Sample preparation involved simple deproteinization followed by autoinjection of a 20 µl sample.

We have injected 20 standards and 80 serum samples with good preservation of constituent resolution and peak areas. At this point, we

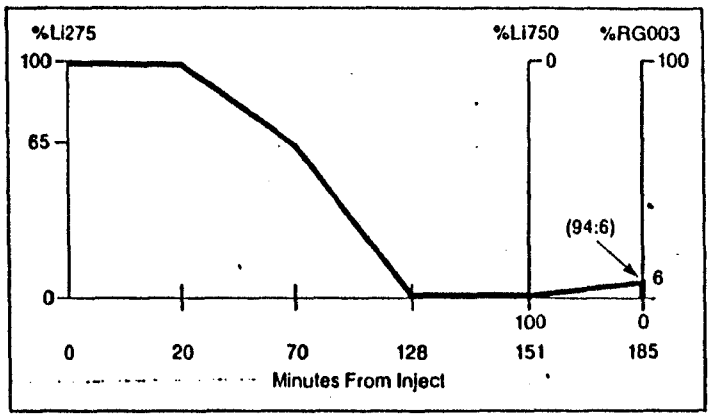
have about 20 more serum samples to process through the first week in December 1985 as well as the analysis of standards containing a few substances not contained in our original standard solution, but which are present and of interest in serum. Analysis of all of the data cannot be completed until these additional standards are used, but we anticipate completion of all data analysis by the end of December 1985. We have demonstrated already, however, that gamma-aminobutyric acid is not elevated in comatose head injuried patients as was suggested by preliminary experiments in which the amino acid analyses were performed outside of the medical center.

We have demonstrated that this method can be implemented in a clinical laboratory with gradient high pressure liquid chromatographic capability. The pre-mixed reagents are reasonably inexpensive further facilitating service laboratory implementation. A major drawback of the method is the analysis time of 210 minutes which limits the total number of samples which can be processed to about 7/day. We hope to complete our data analysis in the next few weeks in time for submission of abstracts to national clinical chemistry and critical care meetings. Additionally, the data from the amino acid experiment will be linked to cerebral metabolic and systemic nitrogen balance data we have on the patients in the study.

SUMMARY: The serum amino acid profile in metabolic disturbances following head injury and other critical illnesses is not used widely in clincal practice in part due to the lack of rapid, reliable methods for measuring amino acids in physiological fluids which could be implemented in service clinical laboratories. Recent developments in chromatographic technology described here may now permit the amino acid analyses to be

performed in a matter of hours in any clinical laboratory with competence in high performance liquid chromatography.

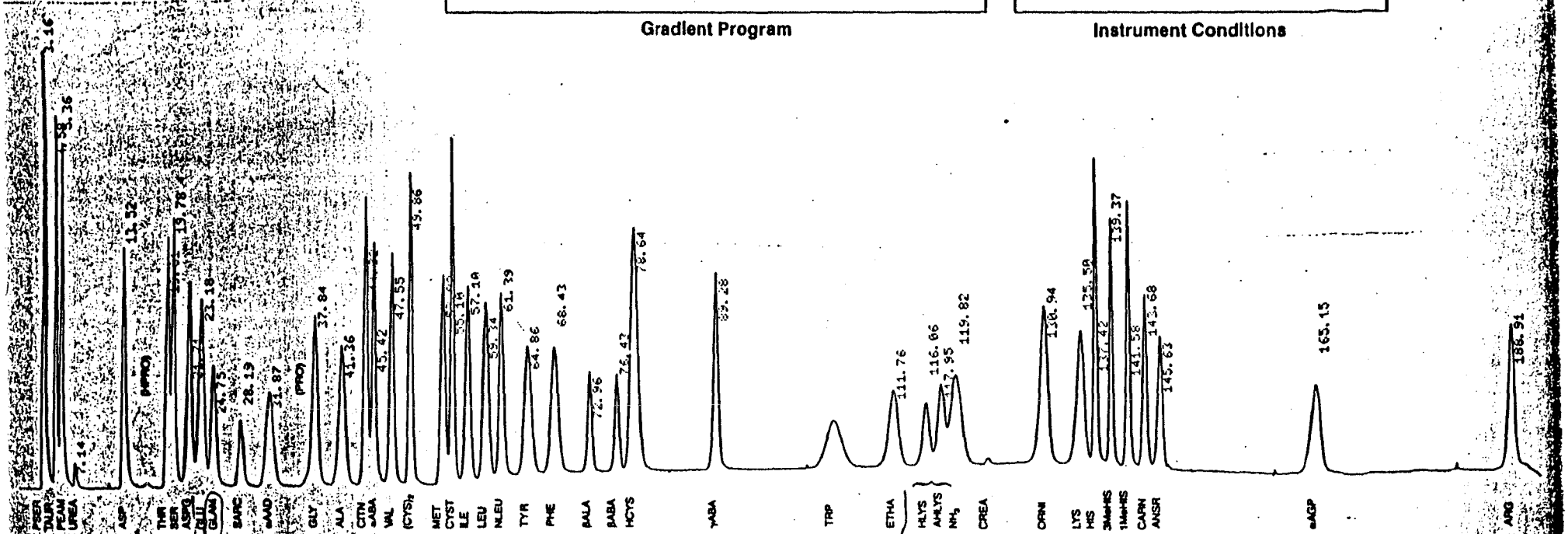
GRADIENT LC, AMINO ACIDS: Physiologic Fluid



Gradient Program

Sample:	2.5 nM/Constituent
Flow Rate:	0.3cc/min, Eluent 0.3cc/min, Trione
Temperature:	42°C Isothermal
Development:	50 sec at 130°C
Detector:	0.5 AUFS 25µL × 12mm

Instrument Conditions

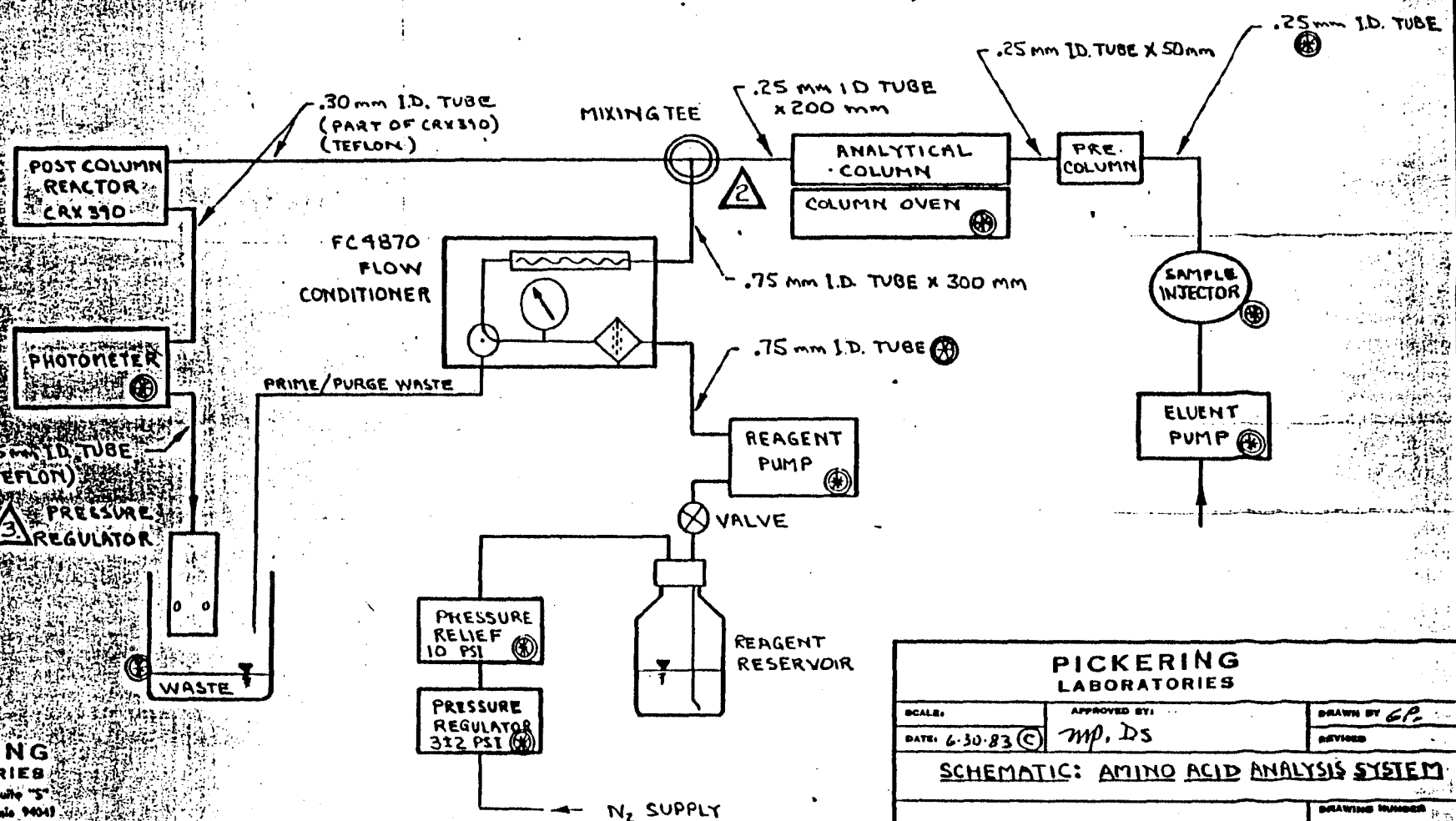


PICKERING LABORATORIES

NOTES: 1. ⊛ ITEMS NOT SUPPLIED BY PICKERING LABORATORIES.

2. ⚠ WARNING: DO NOT PERMIT REAGENT TO BACKFLOW INTO ANALYTICAL COLUMN. REAGENT WILL DAMAGE COLUMN.

3. ⚠ WARNING: REAGENT AND ELUENT BOIL AT 100°C. REACTOR TUBE WILL PLUG AND BURST IF OPERATED ABOVE 100°C WITHOUT SPECIFIED REGULATED BACK-PRESSURE.



PICKERING LABORATORIES
1951 Colony Street, Suite "S"
Mountain View, California 94043

PICKERING LABORATORIES		
SCALE:	APPROVED BY: mp, DS	DRAWN BY GP
DATE: 6-30-83 ©		REVISED
SCHEMATIC: AMINO ACID ANALYSIS SYSTEM		
		DRAWING NUMBER 1100-0180

ABSORBANCE AT 5.4-0-0-M
PSER
TAUR
PEAM
UREA
ASP
HPRO
THR
SER
ASPG
GLU
SARC
AABD
PRO
GLY
ALA
GLTN
AABA
VAL
(CYS)2
CYST
MET
ILEU
LEU
NLEU
TYR
PHE
BALA
BABA
HCYS
GABA
TRP
ETHA
HLYS
AHLYS
NH3
CRTA
ORNI
LYS
HIS
3MEHIS
1MEHIS
CARN
ANSR
AAGP
ARG
STANDARD MIXTURE
RETENTION TIME (min)
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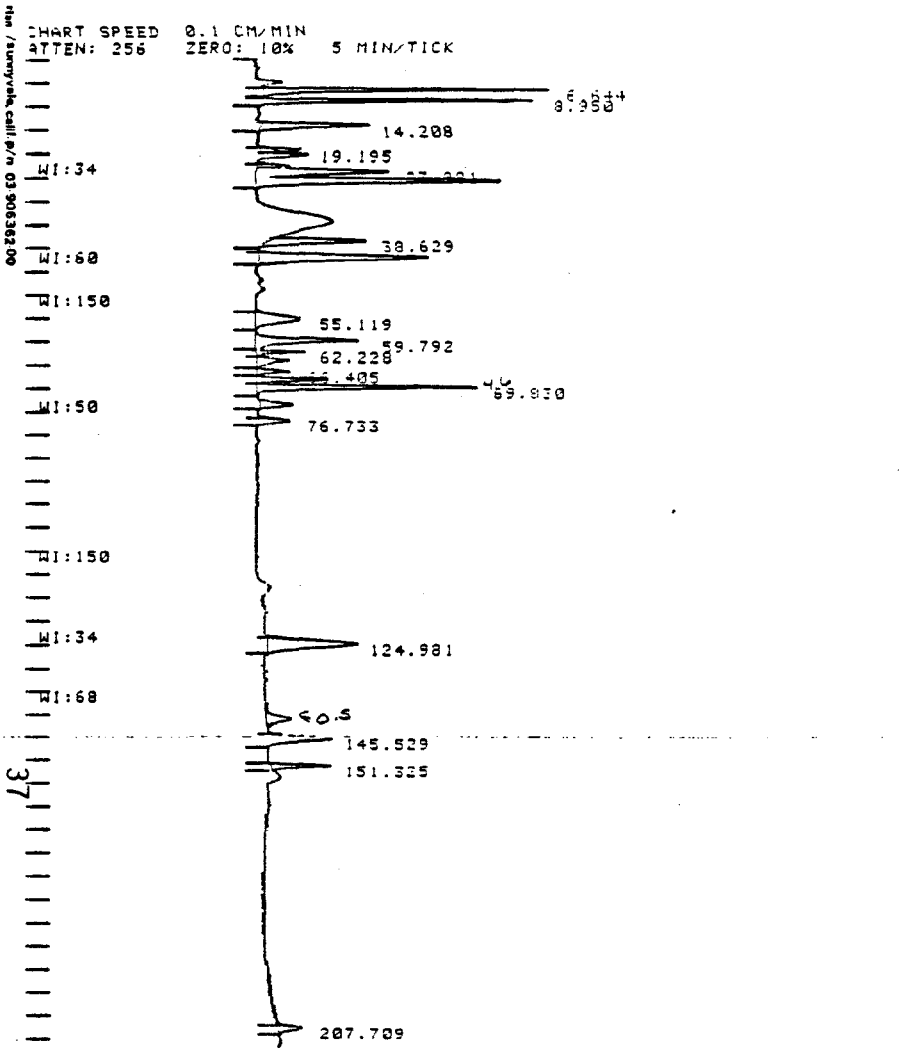
30
45
60
75
90
105
120
135
150
165
180
195
210



RECALC
TITLE: AMINO ACID ANALYSIS 11:42 20 JUL 85

CHANNEL NO: 1 SAMPLE: 6772A METHOD: AA

PEAK NO	PEAK NAME	RESULT UM/L	TIME (MIN)	TIME OFFSET	AREA COUNTS	SEP CODE		WI/2 (SEC)
1	TAUR	285.8	6.644	-0.306	3337030	BB		32.00
2	UREA	4238.6	9.950	-0.330	3797610	BB		38.15
3		166.8	14.209		2005550	BB	?	45.40
4		30.8	19.195		370912	BB	?	
5	SER	37.9	22.551	-0.231	449469	BV	?	26.20
6	ASPG	283.0	23.961	-0.175	2903170	VV	?	50.70
7	GLU	383.1	25.900	-0.270	4717050	VB		56.85
8	PRO	1489.6	38.629	-0.229	18571000	BB		53.95
9	ALA	325.6	42.161	-1.189	3865260	BB	?	70.45
10	VAL	138.1	55.119	-1.101	1577140	BV		100.00
11		178.8	59.792		2150430	VV		55.50
12	(CYS)2	33.0	62.228	-0.232	509062	VV		24.90
13	MET	55.5	63.991	-0.319	646359	VV		52.60
14	ILEU	35.0	66.405	-0.195	426842	VV		38.15
15	LEU	80.8	68.120	-0.170	961979	VV		39.70
16	NLEU	INT STD	69.830	-0.200	3006110	VV		40.05
17	TYR	54.2	73.383	-0.287	629925	VB	?	53.30
18	PHE	52.8	76.733	0.062	604969	BB	?	55.05
19	HLYS	163.8	124.981	0.071	2980900	BB	?	107.75
20	LYS	112.2	145.529	0.244	1643460	BB		72.40
21	HIS	60.6	151.325	0.105	802279	BB		40.25
22	ARG	50.2	207.709	0.589	567241	BB	?	71.50
	?ORN?	34.9						
TOTALS:		8256.2		-1.131	38710000			

DETECTED PKS: 22 REJECTED PKS: 0

DIVISOR: 1.00000 AMT STD: 250.000 MULTIPLIER: 1.00000

NOISE: 110.5 OFFSET: 8028