

AminoQuant II

Amino Acid Analyzer

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Pre-column Method Derivatization with OPA/FMOC

Fluorescence Detection

340/450 nm: primary amines (OPA)

266/305 nm: secondary amines (FMOC)

Equipment/Procedure

- HP 1090 HPLC
- HP 1046A Fluorescence Detector
- HP Chemstation (Pascal)
- Manual Hydrolysis
- Derivatization in Autosampler
- Manual Data Processing

Samples

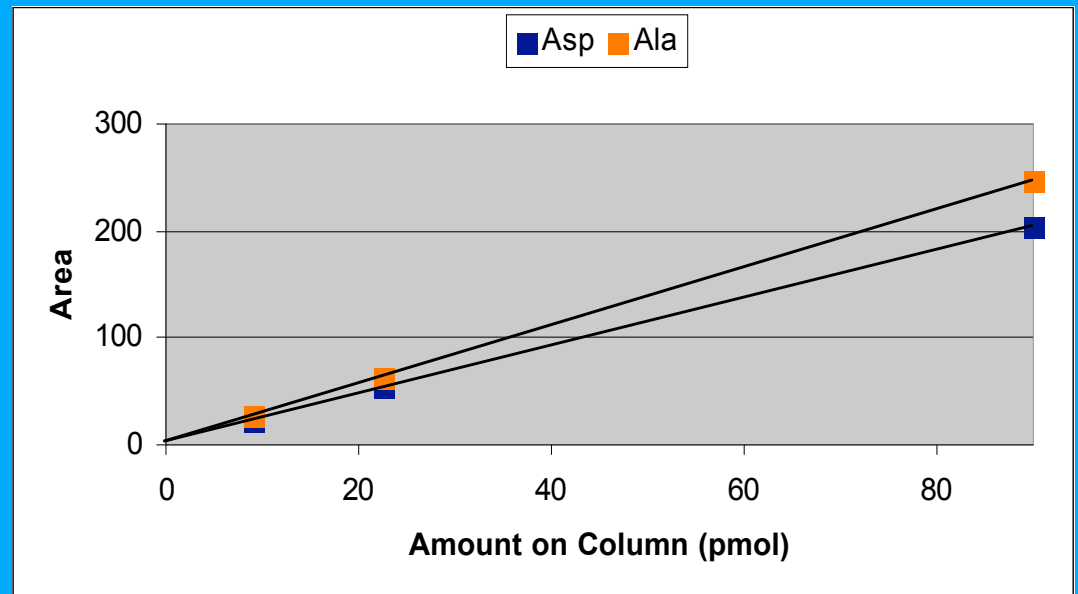
- Protein/Peptide Hydrolysate
- Free Amino Acids (Culture Media, Nectar, Lake Water)

Amino Acids

- All standard amino acids
 - Cys: as $CySO_3$, subtraction method
 - Trp: not from hydrolysates
- OH-Pro, methyl-Arg, -Lys, acetyl-Lys
- Some D/L-Amino acids
- Glutathione

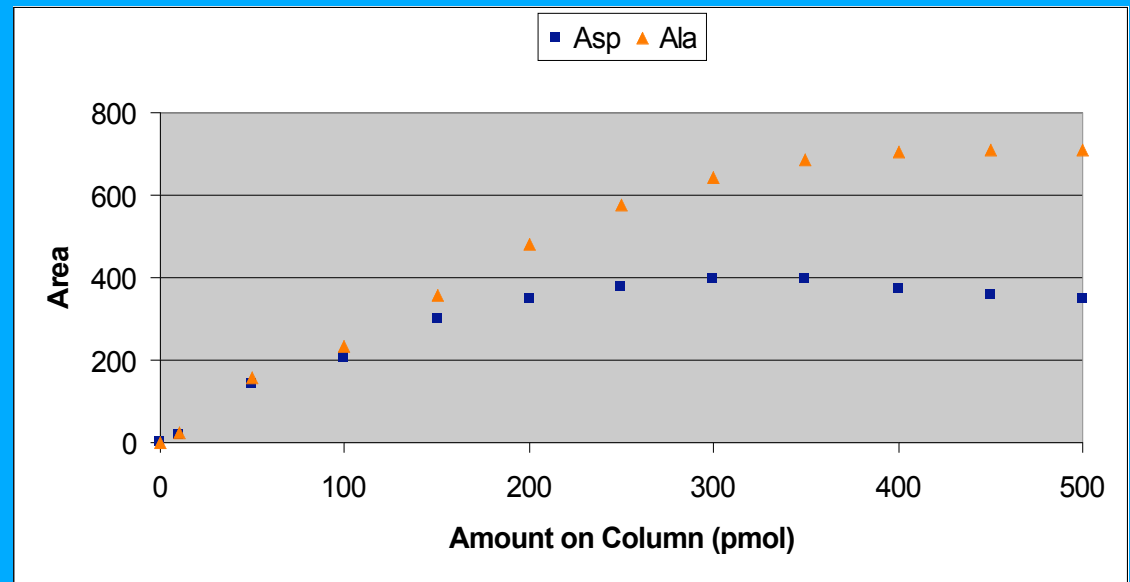
Calibration

- Three point calibration (9, 22.5, 90 pmol)
- Internal standard (Nval, Sar)



Dynamic Range

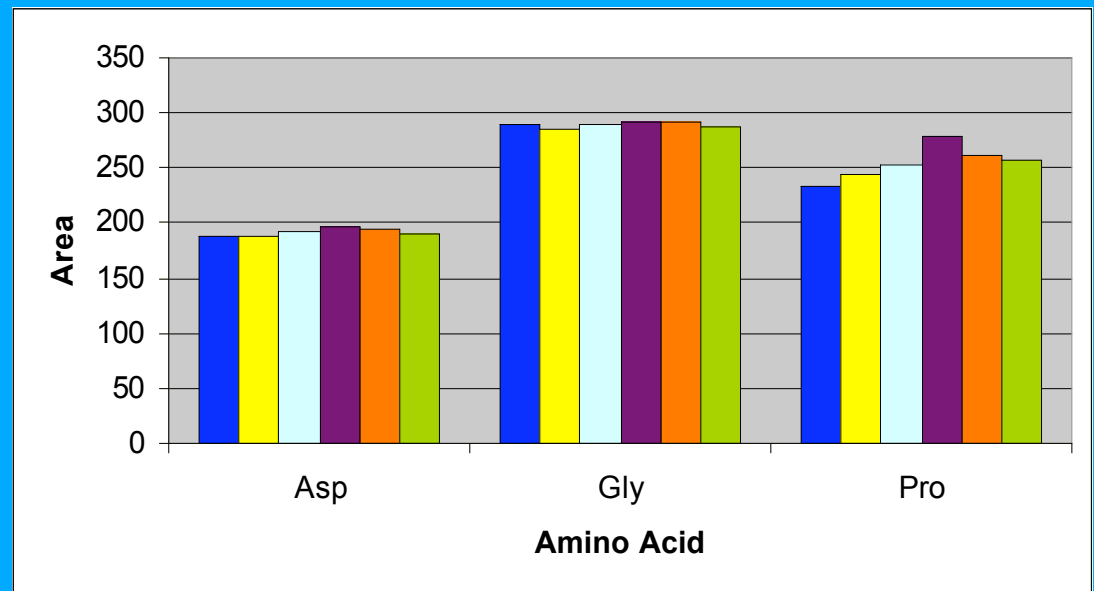
Linear response
up to 150 - 250
pmol on column
(depends on
amino acid)



Accuracy (RSD)

Primary amines:
1.11 - 1.63 %

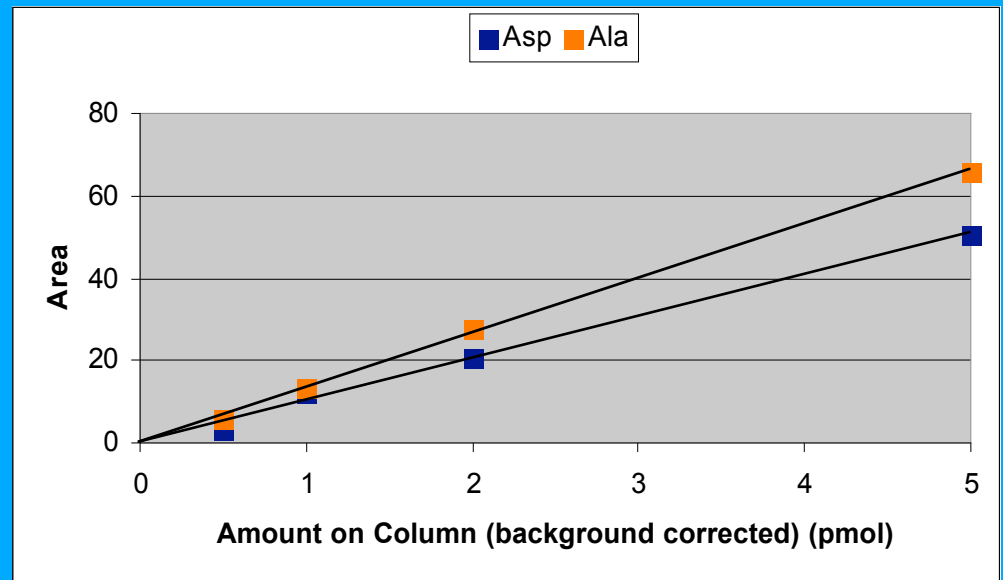
Secondary amines:
5.54 %



Limit of Detection

< 500 fmol on column

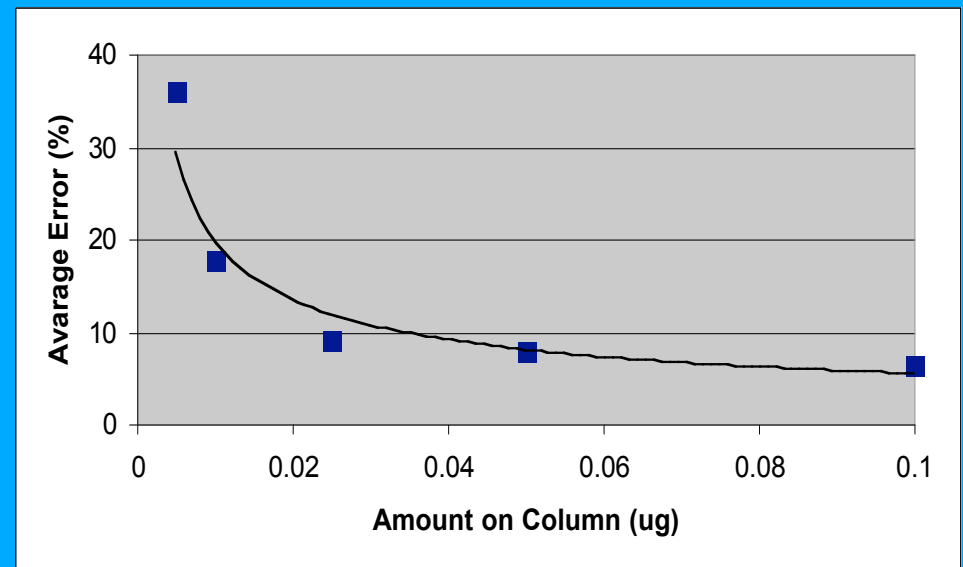
Depends on
fluorescence gain



Limit of Quantitation

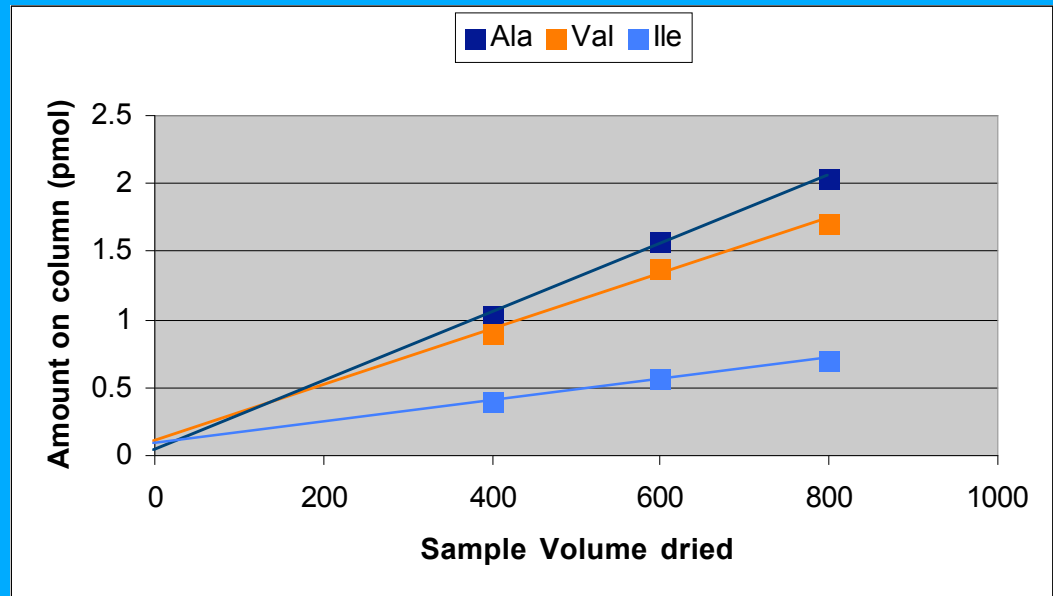
Hydrolysate:
Not determined

Routine analysis:
>25 ng on column
>0.5 ug for hydrolysis



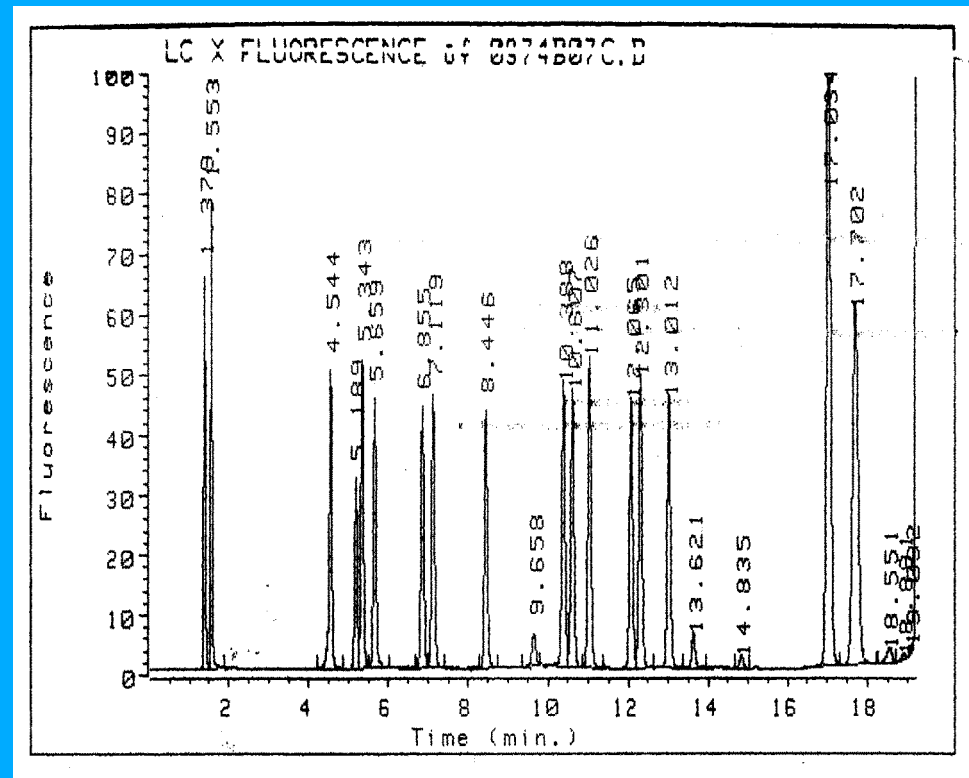
Limit of Quantitation

Free amino acids:
< 500 fmol on
column



Run Times

25 min
from
injection to
injection



Labor Intensity

For up to 18 samples

- Sample preparation • 0.5 - 1 hour
- Data Evaluation • 0.5 - 4 hours

Cost per Sample

(Average of 550 Samples/Year)

(Live Time of Instrument 10 Years)

- Consumables • 5 - 6 \$
- Salaries • 16 - 19 \$
- Maintenance • 2 - 2.5\$
- Instrument • 13 - 15 \$
- Total • 36 - 42.5 \$

Disadvantages

- Sensitive chemistry
- Only 1/20 of sample is analyzed
- Short column life (approx. 750 runs)
- No support for software

Advantages

- No special reagents and chemicals
- Turn-on-and-Analyze
- No need for long setup
- AminoQuant chemistry should also work on an Agilent 1100