application note

Automated Amino Acid Analysis of Peptide Hydrolysates

Abstract

The amino acid analyses of two peptides, Angiotensin II and Neurotensin, by AMINOMATE are described. Each derivatisation has been performed on 10 pmol of hydrolysate and analysis on 5 pmol of derivatised sample. the results are in excellent agreement with the expected value including Histidine and Tyrosine.

Keywords:
Amino Acid, AMINOMATE, Angiotensin II, Neurotensin, Peptide, Hydrolysate

'...sensitivity of the system is at the femtomole levels...'

AMINOMATE, GBC Automated Amino Acid Analyser,1 has been developed with the accurate amino acid determination of peptide and protein2 hydrolysates in mind. Sensitivity of the system is at the femtomole levels, allowing analyses to be performed with as little as 5 pmol of derivatised samples and 10 pmol of the peptides or proteins.

Human Angiotensin II is an oligopeptide with eight amino acid residues (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and a molecular weight of 1047. It has been chosen to demonstrate AMINOMATE's accuracy in the analysis of tyrosine, histidine and aspartic (Figure 1) which have been found to be problematic with existing methods3. The results (Table 1) are in excellent agreement with the expected values.

Table 1: Amino Acid Composition of Human Angiotensin II

Peak No.	Amino Acid	Molar Ratio	Expected Value determined
1	Asx[a]	1.2	1.0
2	His	0.8	0.9
3	Pro	1.1	1.0
4	Tyr	1.0	0.9
5	Arg	1.0	1.0
6	Val	0.9	0.9
7	Ile	0.9	0.9
8	Phe	1.0	1.1

a: Asp + Asn

Neurotensin is a basic tridecapeptide (Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro -Tyr-Ile-Leu) with a molecular weight of 1673. It is found in mammalian brain and gut, having a wide variety of hormone-like activities. The analysis results (Table 2) is again in excellent agreement with the expected values.

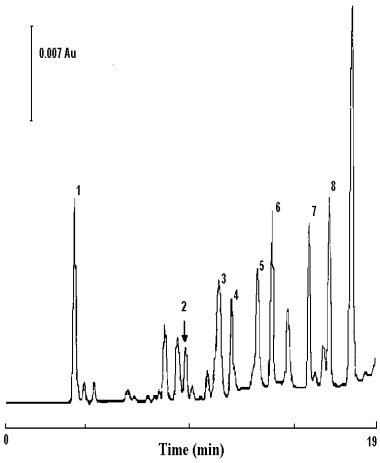

Figure 1 *Separation of Angiotensin II Hydrolysate (5 pmol)*

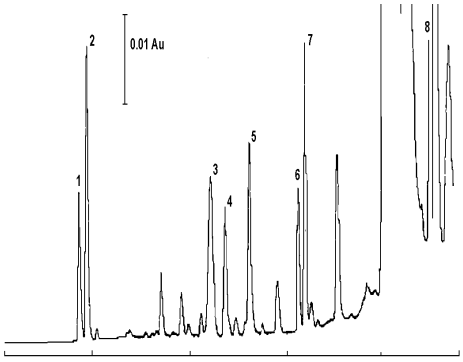

Figure 2 *HPLC Separation of Neurotensin Hydrolysate (5 pmol)*

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Table 2: Amino Acid composition of Neurotensin

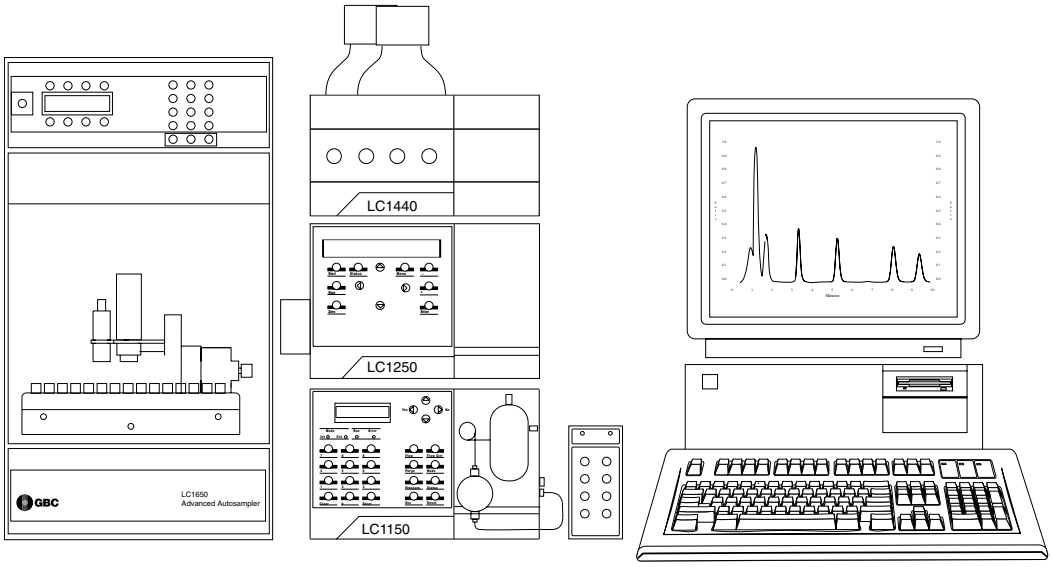
Peak No.	Amino Acid	Molar Ratio determined	Expected Value
1	Asxa	1.1	1.0
2	Glxb	2.0	2.0
3	Pro	2.0	2.0
4	Tyr	2.0	2.0
5	Arg	2.0	2.0
6	Ile	1.0	1.0
7	Leu	2.0	2.0
8	Lys	1.0	1.0

a: Asp + Asn

b: Glu + Gln

GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1250 Fluorescence Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option



Instrumentation

Hydrolysis Procedure: see Reference 4.

References

1. 'AMINOMATE: Automated Amino Acid Analysis by Precolumn Derivatisation', GBC HPLC Application B11.

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