Application Note B30-25:

Quantitative Amino Acid Analysis of Desmopressin

Introduction

Desmopressin is a synthetic, peptide-based hormone medication. It attaches to vasopressin V2 receptors on the cells of the kidney's collecting ducts, increasing water reabsorption and stimulating the release of clotting factors from the vascular endothelium. Common uses include treatment for cranial diabetes insipidus, nocturnal enuresis (bedwetting), and certain bleeding disorders like haemophilia A and von Willebrand disease.

Peptide analysis

With the continuing increase in peptide-based pharmaceuticals on the market, 3 samples of Desmopressin Acetate in powder form were analysed in order to demonstrate the performance of the Biochrom 30+ amino acid analyser.

The analytical method used is based on Cation Exchange Chromatography coupled with post column ninhydrin derivatization for the detection of amino acids. This method ensures high reproducibility and accuracy in the results thanks to high tolerance of complex sample matrices.
The Instrument used was the Bio30+ Sodium Hydrolysate System running a set of Sodium Hydrolysate Buffers (see below), coupled with ninhydrin detection using EZ Nin Reagent. This system delivers a maximum runtime of 60min injection to injection and allows the separation of 18 protein amino acids.

Method

A Bio30+ instrument was set up with the following configuration:

Buffer Set	Buffer 1: Sodium Hydrolysate Buffer 1 [80-2037-68] Buffer 2: Sodium Hydrolysate Buffer 2 [80-2038-08] Buffer 5: Sodium Hydrolysate Buffer 5 [80-2038-09] Buffer 6: Sodium Regeneration Buffer 6 [80-2037-57] Reagent: EZ Nin Reagent [80-6000-13]
Analytical Program	Standard NaHP program.
Column type	Sodium Column High-Performance 20cm x 4.6mm
Buffer Flow rate	35ml/h
Ninhydrin flow rate	25ml/h
Reaction coil	138ºC
Total run time	60min

All amino acids were measured at 570nm except Proline at 440nm.

Standard Solutions

ISO 17034-certified reference standard: 1 volume of Biochrom Physiological Amino Acid Standard (80-6002-80) is used to prepare the working standard by mixing it with 1 volume of Norleucine stock solution, completed with 8 volumes of Lithium Loading Buffer. The final concentration for each amino acid is 0.25mmol/L, except Cystine at 0.125mmol/L. 20µL of standard is injected into the analyser.

Sample Preparation

Open reflux acid hydrolysis using 6N HCl for 22h was performed to liberate the amino acids prior to analysis.

Around 3mg of each sample was weighed accurately into a round bottom flask and mixed with approx. 10mL of hydrolysis solution (6N hydrochloric acid containing 0.1% of phenol). The flasks were attached to a condenser with continuous water cooling. The flasks were heated on a graphite bath to boiling point for 22h.

After 22h, the flasks were removed and the liquid evaporated under vacuum until dryness. The residue was suspended in 5mL of Sodium Citrate Loading Buffer pH2.2.

20µL of this solution was injected into the analyser.

Results

Desmopressin contains a single S-S bridge between Cysteine and the Mercaptopropionyl group on the N-terminal of Tyrosine: the structure being Mercaptopropionyl(1)-Tyr-Phe-Gln-Asn-Cys(1)-Pro-D-Arg-Gly-NH2.

Simple acid hydrolysis breaks the protein into its constituent amino-acids. Concomitantly Asn, Gln and GlyNH2 are converted to Asp, Glu and Gly respectively and any Cysteine formed presents as Cystine. The amino acids quantified were therefore: Tyr, Phe, Glu, Asp, Cys, Pro, Arg and Gly.

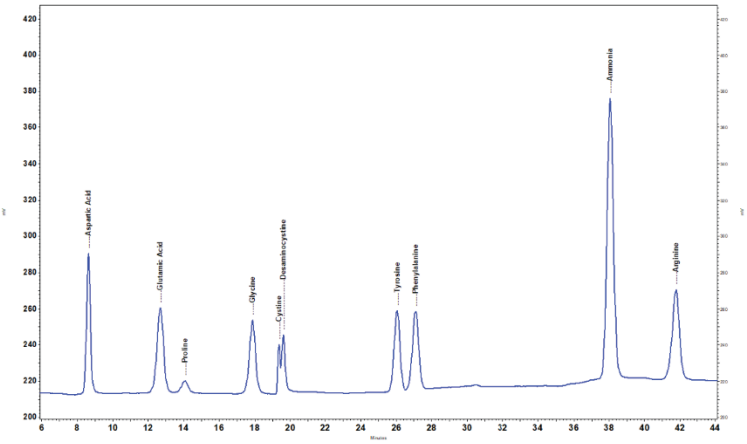
It is interesting to note that the propionyl-amide bond on the Tyrosine cleaves much faster than does the S-S bond to the mercaptopropionyl moiety. Consequently, only a small quantity of Cysteine is formed, whereas a substantial amount of another ninhydrin-positive compound was detected, eluting close to Cystine. This is presumably S-(Carboxyethylthio)-L-Cysteine the amount of which, in the absence of an authentic sample, can only be estimated.

Below are presented the amount of amino acids detected expressed in mole % for each sample.

Sample 1		
Name	nmol Analysed	Mole %
Asp	11.0	14.0%
Glu	10.7	13.7%
Pro	10.7	13.7%
Gly	11.0	14.0%
Cystine	2.1	2.7%
Tyr	10.7	13.7%
Phe	11.0	14.0%
Arg	11.0	14.1%
TOTAL	**78.3**	**100%**

Sample 2		
Name	nmol Analysed	Mole %
Asp	9.8	13.9%
Glu	10.0	14.1%
Pro	9.9	14.0%
Gly	9.7	13.7%
Cystine	2.0	2.8%
Tyr	9.9	13.9%
Phe	10.0	14.2%
Arg	9.5	13.5%
TOTAL	**70.8**	**100%**

Sample 3		
Name	nmol Analysed	Mole %
Asp	10.6	13.8%
Glu	10.5	13.7%
Pro	10.7	14.0%
Gly	10.9	14.2%
Cystine	2.0	2.7%
Tyr	10.5	13.7%
Phe	10.9	14.2%
Arg	10.6	13.8%
TOTAL	**76.8**	**100%**



Conclusions

The Bio30+ separated and identified all the amino acids presents in the samples. The results show

very similar results amongst the 3 batches analysed. The results also correlate with the known formula of desmopressin. No baseline interferences were observed.

When used in conjunction with Good Laboratory Practice and Phoenix CFR21 part 11 software for the Biochrom 30+ Amino Acid Analyser systems it is a suitable method of analysis in labs compliant with global pharmacopoeia.

Further Information

For detailed guidance on Biochrom 30+ setup, maintenance, and alternative quantification workflows visit:

www.support.biochrom.co.uk

support@biochrom.co.uk