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Somatonorm® (somatrem) and Genotropin® (somatropin): Two case studies

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1. Introduction

Production, isolation and purification of human proteins in recombinantly engineered bacteria or other organisms is complicated, and while great success has been achieved in both the technology and use of the new biopharmaceuticals to improve human health, the manufacturing processes remain complicated and need to be individualised to the protein in question.

The protein when made in a recombinant organism far removed from the normal cellular environment which ensures correct sequence and folding, is subject to modifications and contaminants which are only understood on an empirical basis. It is therefore imperative that any company wishing to produce products by this technique has access to state of the art analytical technology and not only highly qualified trouble shooting, problem solving personnel but personnel who have had a good deal of experience with the protein and the manufacturing process in question.

Despite this unexpected events can occur, and I have chosen to illustrate my talk with two examples from the vast in house experience of Pharmacia.

The first example, from Somatonorm® (methionyl growth hormone, Somatrem), shows what happened when a very minor change was made in a filter washing procedure early in the process; batches from a whole quarter's production had to be rejected; the second demonstrates how improvements in analytical technology revealed a hitherto unknown type of impurity, this time in human growth hormone, Genotropin® (somatropin, rhGH).

2. Somatonorm® (somatrem, met-hGH)

Somatonorm® received marketing approval for the treatment of Growth Hormone deficiency in October 1985 withdrawn from most markets 1988–1989 (marketing, not product reasons).

An example of manufacturing problems arising from a very minor change in a filter washing procedure (nothing that required notice to the FDA). A very faint band, apparent MW 18 kDa, was observed on SDS gels by an eagle eyed analytical chemist in a number of sequentially produced batches. Although the batch quality was within approved limits, an investigation was started to find out what the band was, its origin and more importantly how it could be removed.

The figure shows how the 18 kDa component co-chromatographs with rhGH. The component, found in Somatonorm batches, was isolated to homogeneity by electrophoretic elution from polyacrylamide gels.

The following molecular properties were found:

- MW 18,000 Da,
- Isoelectric point: 6.1,
- Best separation from hGH is in pH range 5.6–6.5,
- N terminal sequence: Ser-Ala-Val-Leu-Thr-Ala-Glu-Gln-Ala-Leu-Lys-Leu-Val-Gly-Glu-(Val)-Met-Phe-Val-Tyr,
- Disulphide bonds,
- No reaction with the used host cell protein antisera,
- No sequence homology to hGH,
- A specific antibody was made to be able to monitor batches,
- Removal was achieved by a change in manufacturing procedure.

3. Genotropin® (somatropin, rhGH)

A more hydrophobic variant of rhGH was found on analysis by HIC. Tryptic peptide purification and MS analysis showed a molecular weight increase of 32; two oxygen atoms or a sulphur atom.

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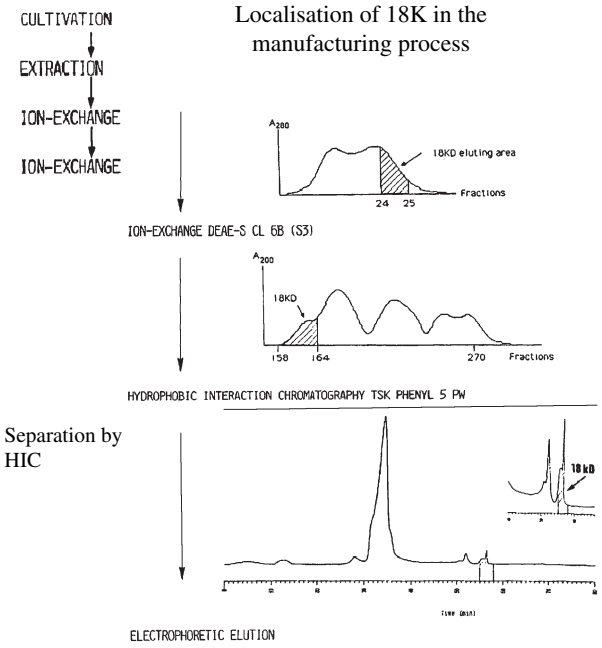

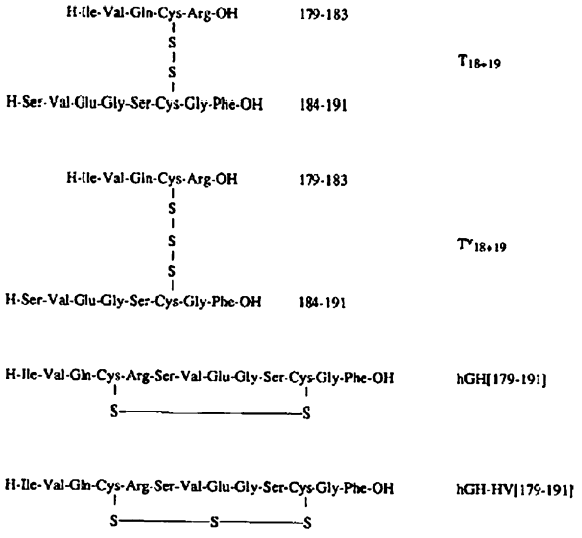

Structures of the tridecapeptides T_{18+19}, T'_{18+19}, hGH[179–191] and hGH-HV[179–191].

The explanation was the presence of a trisulphide bond – an artefact due to manufacturing conditions in the fermentation broth [1]. Surprisingly the bond is very stable, and the protein active. However for these very reasons, trisulphide containing proteins should be removed.

4. Conclusions

Selected examples show what problems can arise when innocuous changes are made in a manufacturing method, or when new analytical tools reveal unexpected deviations from purity.

Contamination problems can be solved BUT:

- Need experienced personnel to recognize the problem,
- Need analytical resources and instruments to solve the problem,
- Need money,
- Need time,
- 18 K and the trisulphide issues each needed 6 months work and a team of 6 PhDs to solve together with QA and Manufacturing resources. Notification to the regulatory authorities was needed once the improvements were to be introduced.

Reference

[1] Andersson C, Edlund P-O, Gellerfors P, Hansson Y, Holmberg E, Hult C, et al. Isolation and characterization of a trisulphide variant of rhGH formed during expression in *E. coli*. Int J Pept Protein Res 1996;47: 311–21.