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Identification of growth hormone releasing hexapeptide (GHRP-6) in an unknown pharmaceutical preparation

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Background

In December 2007, our laboratory was approached by the Norwegian Customs to help identify a shipment of an unknown pharmaceutical preparation which had been stopped at the border. The shipment of Chinese origin consisted of unmarked glass vials and was suspected to contain a doping agent. Several previous shipments seized by the Norwegian Customs had been shown by our laboratory to contain prohibited substances. Among them were preparations of anabolic-androgenic steroids cleverly disguised as massage oils, recombinant human growth hormone (rhGH) in unmarked vials and the tanning agent Melanotan-II deliberately mislabelled as the antifungal butenafine.

Experimental

Upon inspection, each vial contained a small amount (approximately 3 mg) of a colourless crystalline compound. Initially, the contents were thought to be sodium chloride intended for preparing a physiological saline solution for reconstituting lyophilised peptide doping agents. However, crude melting point and flame photometric tests showed this was not the case. The contents of one vial were dissolved in methanol and analysed by direct infusion mass spectrometry on a Thermo TSQ Quantum triple-quadrupole mass spectrometer. The instrument was operated in positive electrospray ionisation (ESI+) mode, and both full-scan and product spectra of the most intense precursor ions were acquired. Spray voltage was set to 4000 V, capillary temperature was 250 °C and sheath gas pressure was 10 arbitrary units. Product ion spectra were acquired at a collision voltage of 38 V.

Results

The full-scan mass spectrum, shown in Fig. 1, indicated a monoisotopic mass of 872.4, with a presumed quasimolecular ion $[M+H]^+$ at *m/z* = 873.4, and a doubly charged $[M+2H]^{2+}$ at *m/z* = 437.3.

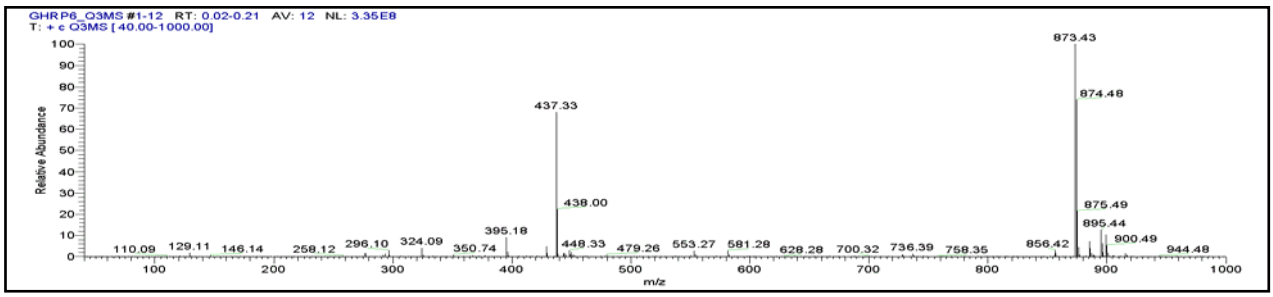

Fig. 1: Full-scan mass spectrum of the unknown substance.

It was noticed that the presumed molecular weight coincided with that of growth hormone releasing hexapeptide (GHRP-6); a peptide with the following amino acid sequence [1]: **His – D-Trp – Ala – Trp – D-Phe – Lys-NH2**. The product ion spectrum of *m/z* = 873.4 is shown in Fig. 2.

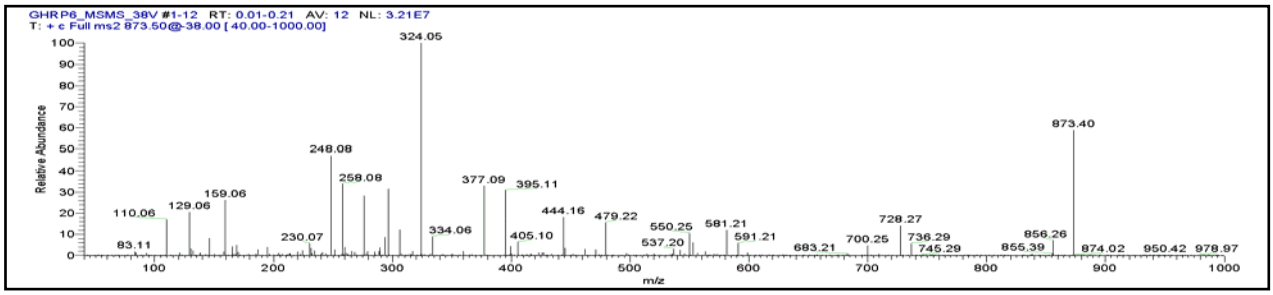

Fig. 2: Product ion spectrum of *m/z* = 873.4

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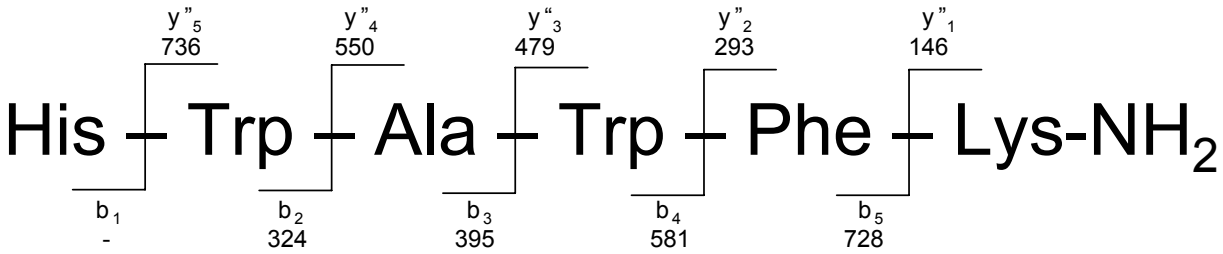
Interpretation of the mass spectrum confirmed the peptide sequence His – Trp – Ala – Trp – Phe – Lys-NH_2. The observed fragmentation pattern can be explained as follows:



Discussion

The amino acid sequence of the unknown substance corresponds to that of GHRP-6. However, mass spectrometry alone is incapable of distinguishing between the enantiomeric forms of each amino acid residue. Due to lack of certified reference material at this time, it was not possible to unequivocally confirm the stereochemistry of the peptide by chromatographic methods. Nevertheless, the unknown substance has been identified beyond reasonable doubt as GHRP-6.

GHRP-6 is normally administered by subcutaneous injection, and it mediates the release of growth hormone (GH) from the pituitary [2]. It qualifies as a Prohibited Substance under section S2 of the WADA Prohibited List [3]. Although its mechanism of action is not completely understood, it has been shown to act primarily at the hypothalamus [4]. According to sources in the bodybuilding community, GHRP-6 is used as a lower-priced alternative to recombinant human growth hormone (rhGH).

Conclusion

It is apparent that GHRP-6 is readily available and that it has the potential to be misused as a performance enhancing agent. It remains to be investigated to which extent GHRP-6 is excreted into urine and whether it could be monitored in routine doping analysis.

References

[1] Smith, R.G. (2006) Development of Growth Hormone Secretagogues. *Endocrine Reviews* **26** (3), pp 346-360.

[2] Raun, K., Hansen, B.S., Johansen, N.L., Thøgersen, H., Madsen, K., Ankersen, M., Andersen, P.H. (1998) Ipamorelin, the First Selective Growth Hormone Secretagogue. *Eur. J. Endocrinol.* **139**, pp 552-561.

[3] The World Anti-Doping Agency. The 2008 Prohibited List. International Standard, Montreal. http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf (access date 30.05.2008)

[4] Popovic, V., Damjanovic, S., Micic, D., Djurovic, A., Dieguez, C., Casanueva, F.F. (1995) Blocked Growth Hormone-Releasing Peptide (GHRP-6) Induced GH Secretion and Absence of the Synergic Action of GHRP-6 Plus GH-Releasing Hormone in Patients with Hypothalamopituitary Disconnection: Evidence that GHRP-6 Main Action is Exerted at the Hypothalamic Level. *J. Clin. Endocrinol. Metab*. **80** (3) pp 942-947.