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Unknown peptide preparations analysed in 2010: An overview

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Introduction

In recent years, there has been an increasing focus within the anti-doping community on peptides, partly because endogenous peptides may serve as markers for the misuse of prohibited substances or methods, but also because several new peptide pharmaceuticals with potential performance enhancing effects have emerged. This reality was reflected in the 2010 WADA Prohibited List, as section S2, "Peptide hormones, growth factors and related substances" was greatly expanded. The emergence of new peptide doping agents may in part be attributed to recent advances in genomics and proteomics, which have led to an increased emphasis in the pharmaceutical industry on the development of peptide drugs. However, simplified manufacturing techniques, disregard for patent protection and a growing market for non-approved drugs are contributing factors, and several peptide drugs are made available to the public before clearing or even entering clinical trials.

In collaboration with the Norwegian police and customs authorities, our laboratory routinely performs the analysis of pharmaceutical preparations suspected to contain peptide doping agents. Shipments of peptide drugs are typically falsely declared, the vials are unmarked and the various peptide preparations are visually indistinguishable from one another. Identification of the drugs is necessary in order to determine whether their importation, possession and distribution constitute a criminal offence.

Experimental

In 2010, a total of 21 seized peptide preparations were analysed by our laboratory. All were suspected to contain human growth hormone (hGH), and all were supplied in the form of white powder contained in unmarked glass injection vials with coloured plastic caps.

The contents of each vial were dissolved in water/methanol (50:50) containing 0.1% formic acid and diluted to a nominal concentration of 10 µg/mL. The solutions were analysed by direct infusion on a Thermo LTQ Orbitrap XL mass spectrometer. The instrument was operated in positive electrospray ionisation (ESI+) mode, and high resolution mass spectra were acquired in profile mode in the Orbitrap analyser with the resolution set at 30,000, followed by product ion scans of the most abundant precursor ions in the linear ion trap. The amino acid sequences of the peptides were elucidated by manual interpretation of exact mass measurements and MS/MS fragmentation patterns. The peptide constituents of the preparations were identified by comparison of the proposed amino acid sequences with known peptides marketed as non-approved drugs.

Results

Out of the 21 preparations analysed, only five were found to contain human growth hormone (hGH). The others were identified as CJC-1295 (five findings), sermorelin (one finding), GHRP-6 (three findings), ipamorelin (one finding), AOD9604 (one finding) and melanotan-II (three findings), respectively. Their high-resolution mass spectra are shown in Figures 1 through 3. The final two preparations were found to contain a mixture of several oligopeptides, each at very low abundance. They were not identified.

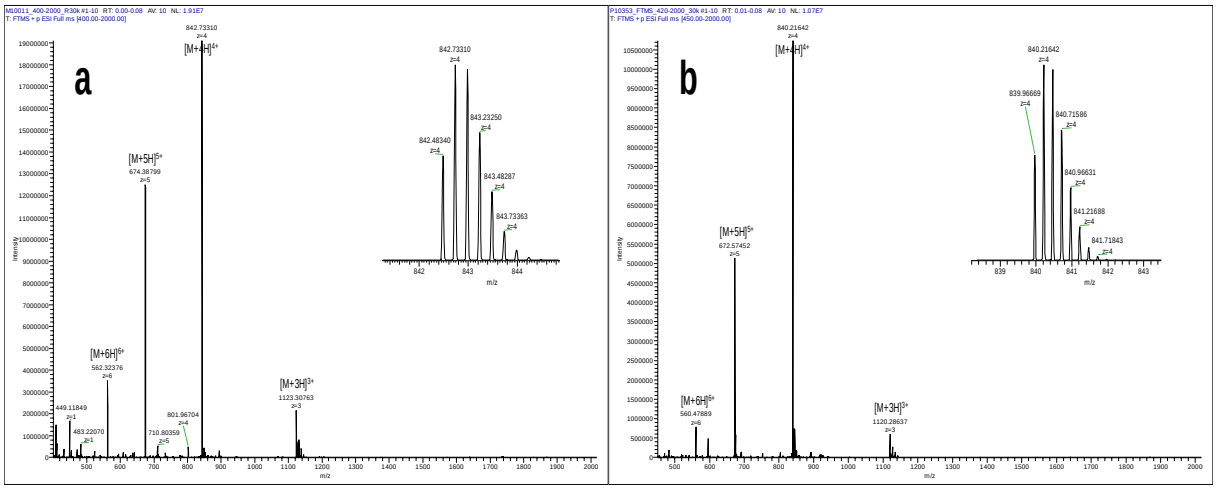

Figure 1: High resolution mass spectra of the unknown peptides identified as CJC-1295 (**a**) and sermorelin (**b**).

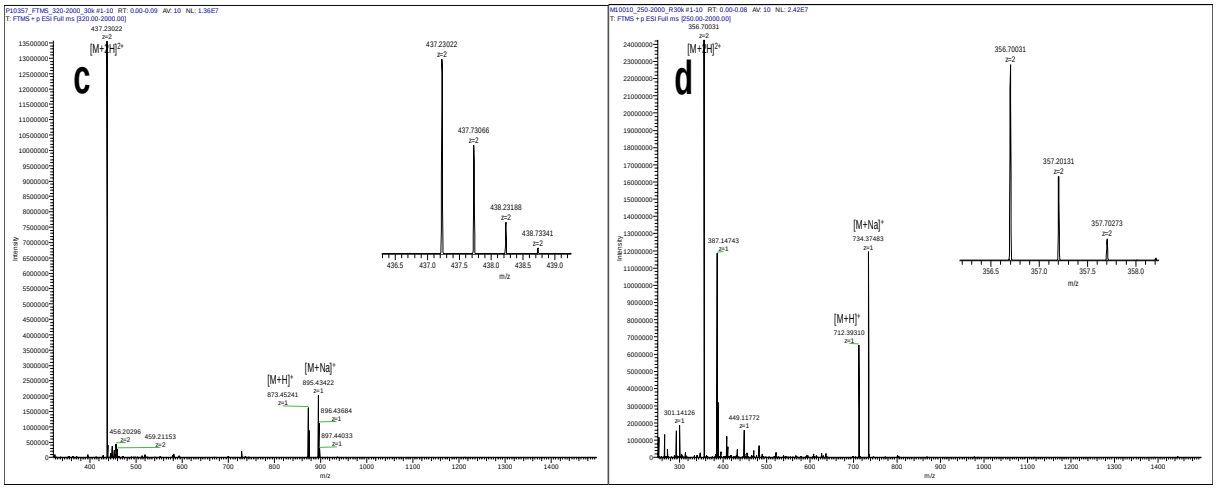

Figure 2: High resolution mass spectra of the unknown peptides identified as GHRP-6 (**c**) and ipamorelin (**d**).

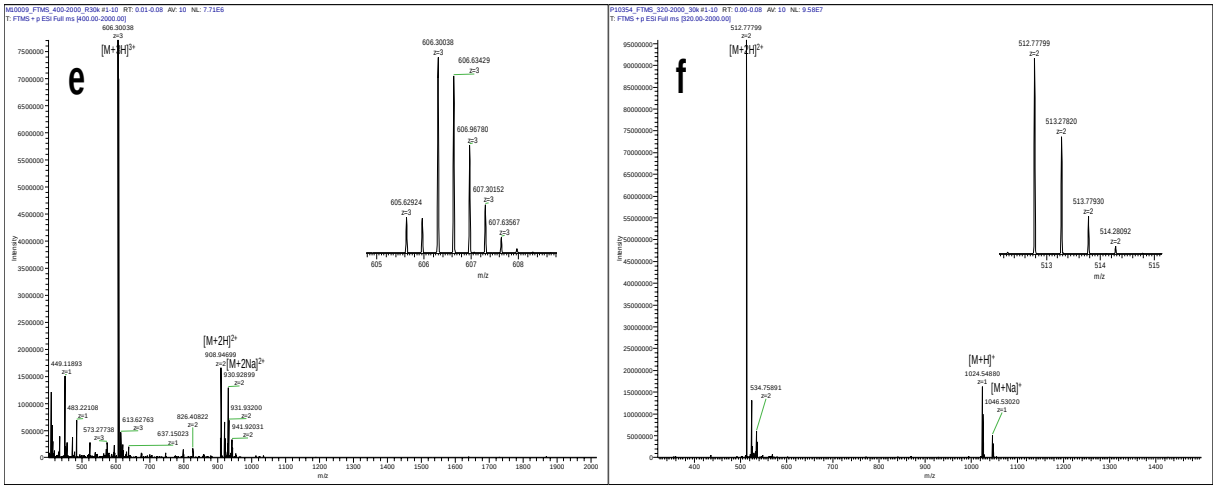

Figure 3: High resolution mass spectra of the unknown peptides identified as AOD9604 (**e**) and melanotan-II (**f**).

Discussion

CJC-1295 and sermorelin are synthetic analogues of growth hormone releasing hormone (GHRH) [1,2,3]. GHRP-6 and ipamorelin are ghrelin mimetics and have been shown to act as growth hormone secretagogues *in-vivo* [4,5]. Their mechanism of action is distinct from that of the GHRH analogues [6]. AOD9604 is a peptide which corresponds to the amino acid 177-191 fragment of hGH, with an additional tyrosine at the N-terminal end. It is claimed to retain the lipolytic properties of hGH [7]. Melanotan-II is a synthetic analogue of alpha melanocyte stimulating hormone (α-MSH), which is involved in skin tanning [8]. With the exception of melanotan-II, all of the encountered peptides have potential performance enhancing effects and should be regarded as Prohibited Substances under section S2 of the WADA Prohibited List. Key information on the identified peptides is shown in Table 1.

Table 1: Amino acid sequence, elemental composition and monoisotopic mass of the identified peptides.

Peptide	Amino acid sequence	Monoisotopic mass, calc.	Monoisotopic mass, exp.	Δ (ppm)
CJC-1295	Tyr-D-Ala-Asp-Ala-Ile-Phe-Thr-Gln-Ser-Tyr-Arg-Lys-Val-Leu-Ala-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Leu-Ser-Arg-NH_2	3365.8934	3365.9016	2.4
Sermorelin	Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH_2	3355.8186	3355.8336	4.5
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	872.4445	872.4444	0.1
Ipamorelin	Aib-His-D-Nal-D-Phe-Lys-NH_2	711.3856	711.3846	1.4
AOD9604	Tyr-Leu-Arg-Ile-Val-Gln-Cys-Arg-Ser-Val-Glu-Gly-Ser-Cys-Gly-Phe	1813.8603	1813.8637	1.9
Melanotan-II	Ac-Nle-*cyclo*[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2	1023.5402	1023.5400	0.1

Abbreviations: Aib = 2-aminoisobutyric acid, D-Nal = D-(2-naphthyl)alanine, Ac = acetic acid, Nle = norleucine

Conclusion

Of the 21 unknown peptide preparations analysed in 2010, only five were found to contain hGH. More than half were found to contain other peptides with potential performance enhancing effects. It is apparent that growth hormone releasing factors and mimetics are currently seeing widespread use within the bodybuilding community, and we believe that they have the potential to be misused in as performance enhancing agents in organised sports.

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