Determination of growth hormone-releasing hexapeptide in bovine urine by LC-MS/MS

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Background

The type of growth promoters used in animal husbandry changed over the years. Besides by using well-known steroids and anabolic agents, muscle growth within cattle can also be altered by administration of growth hormone-releasing peptides (GHRP's). There is not much known about analysis and excretion of GHRP's in cattle urine. In this study growth hormone-releasing hexapeptide (GHRP-6) was chosen as a model compound for this class of compounds

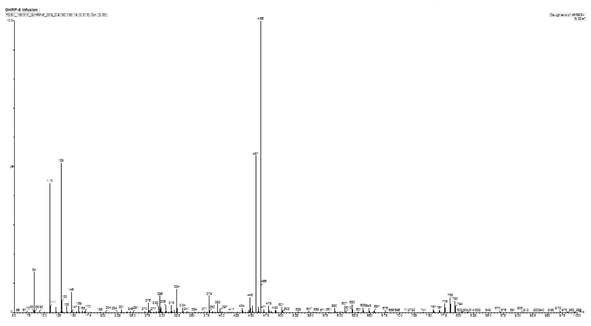
Growth hormone releasing peptides (GHRPs)

Growth hormone releasing peptides are secretagogues that act on a specific receptor on the hypothalamus. They are small peptides that induce the growth hormone secretion. It can be administered intravenously, subcutaneously, intranasally and orally. The GHRP-6 is small, stable, soluble and has a low toxicity, making it a good candidate to be used as a doping agent. See table below for the primary structure of peptides with growth hormone releasing activity

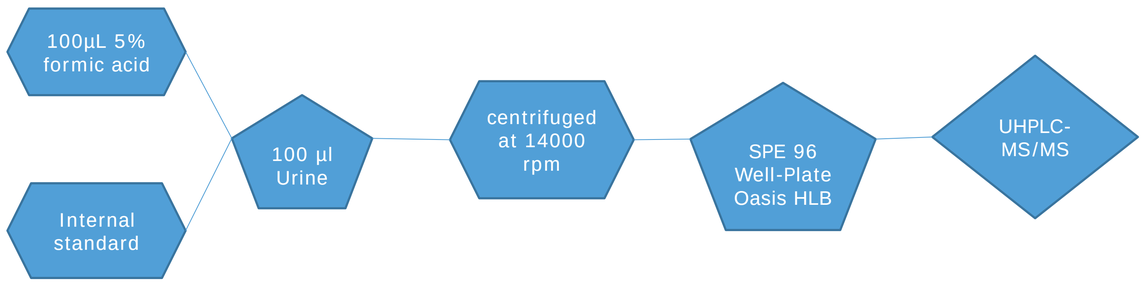
Compound	*Sequence*
GHRP-1	Ala-His-D-bNal-Ala-Trp-D-Phe-Lys-NH_2
GHRP-2	D-Ala-D-bNal-Ala-Trp-D-Phe-Lys-NH_2
GHRP-4	D-Trp-Ala-Trp-D-Phe-NH_2
GHRP-5	Tyr-D-Trp-Ala-Trp-D-Phe-NH_2
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2
Hexarelin	His-D-Mrp-Ala-Trp-D-Phe-Lys-NH_2

MS Measurements

Since GHRPs are small peptides they are double charged what makes selecting selective product ions more easy compared to small molecules. In the figure below the product spectra of GHRP-6 is shown for the doubly charged m/z 465.7.



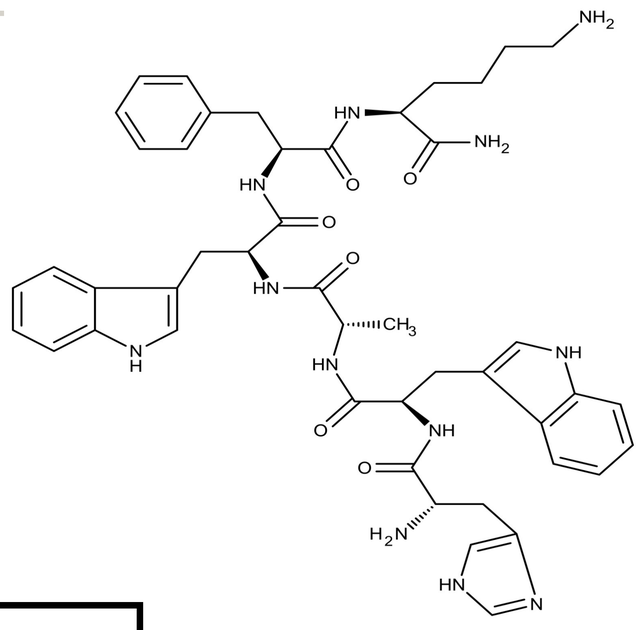
Sample clean-up

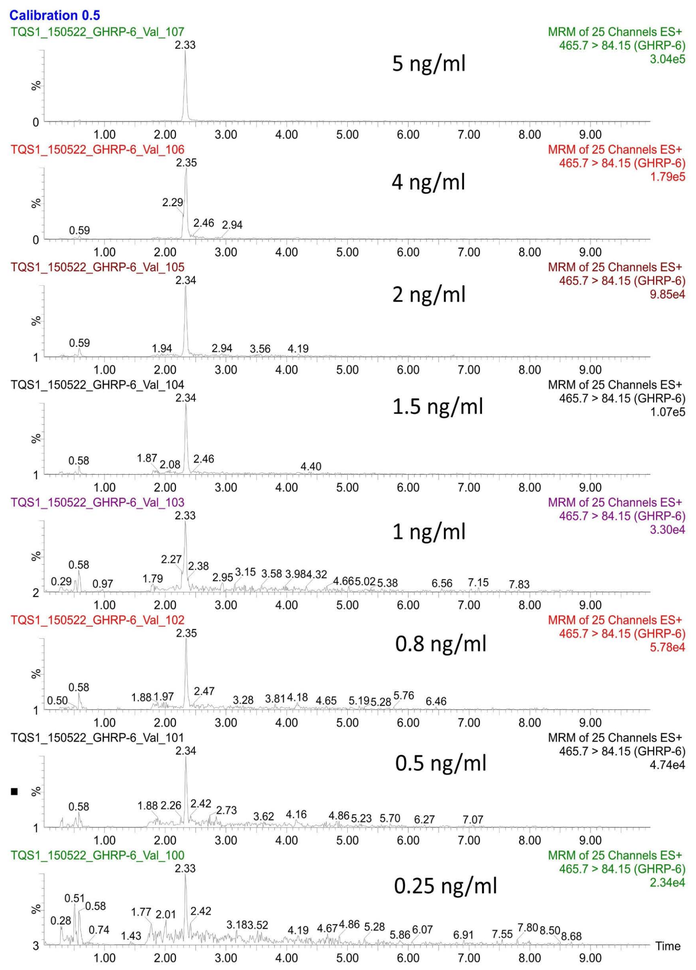


Results

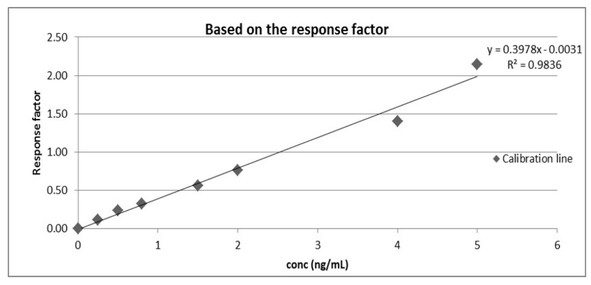
→ Example of spiked samples of urine at the level 0.25-5 ng/ml.

↓Structure of GHRP-6.

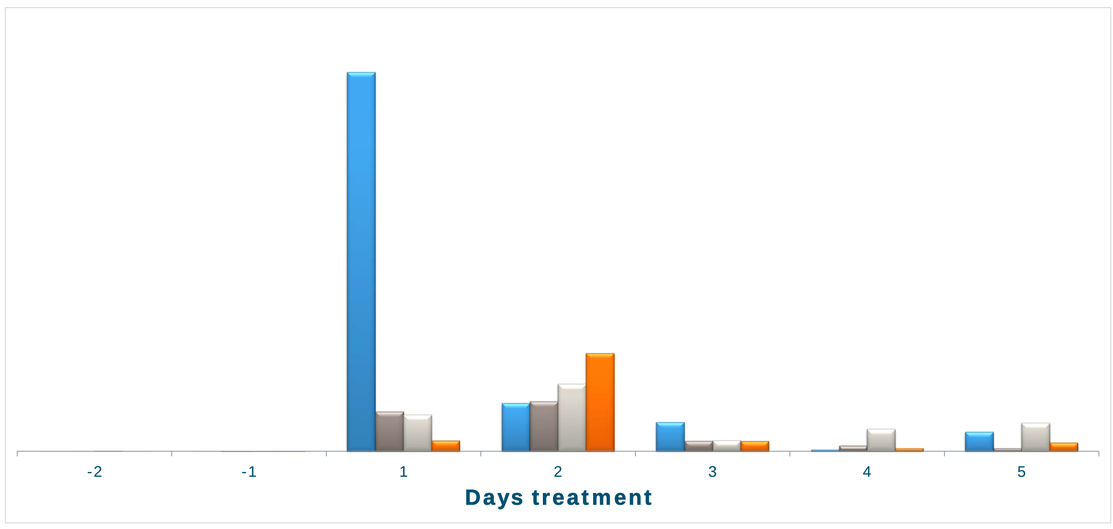




→ Corresponding calibration curve.



↓Preliminary results of the excretion of GHRP-6 after administration to 4 veal calves



Conclusion

The CCα was 0.37 ng/ml. The developed method can be used for confirming and quantifying GHRP-6 in bovine urine with 93% accuracy and 3.4% uncertainty. GHRP-6 can be detected in samples of treated animals

Performance characteristic	Validation level	Validation level	Validation level
Level	0.5	1	1.5
Unit	ng/mL	ng/mL	ng/mL
Accuracy	96.9%	87.4%	94.4%
Covariation reproducibility	1.7%	2.2%	1.5%
Covariation Repeatability	1.7%	1.8%	1.4%

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