DETECTION AND QUANTIFICATION OF IGF-1 AND IGF-2 IN BOVINE PLASMA BY LC-MS/MS

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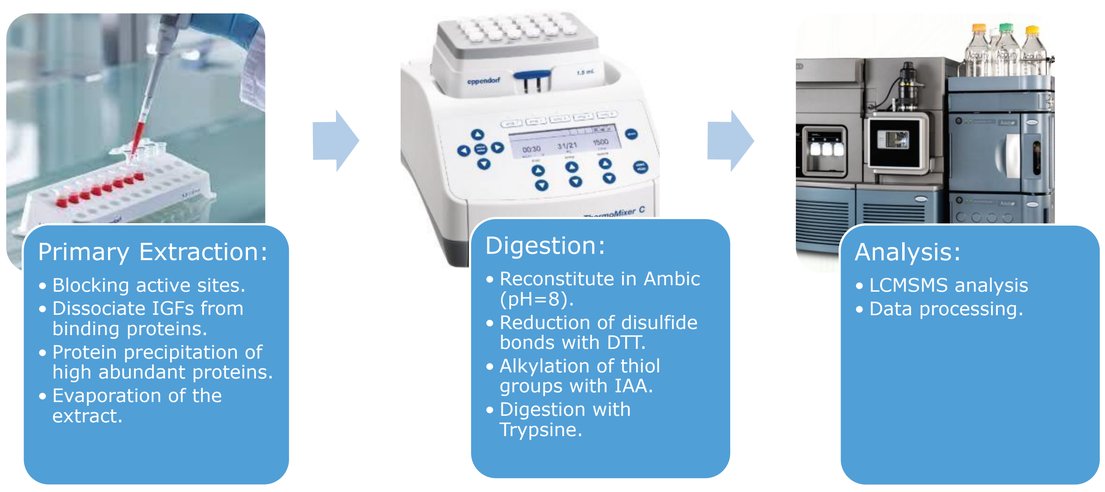
Introduction

The insulin-like growth factors (IGFs) are proteins with high sequence similarity to insulin. IGFs are part of a complex system that cells use to communicate with their physiologic environment. This complex system (often referred to as the IGF "axis") consists of two cell-surface receptors (IGF1R and IGF2R), two ligands (Insulin-like growth factor 1 (IGF-I) and Insulin-like growth factor 2 (IGF-2), a family of six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6), as well as associated IGFBP degrading enzymes. Insulin-like growth factor 1 and 2 (IGF-1, IGF-2) are key mediator of growth hormone action and a biomarker for growth hormone abuse and can therefore be used illegally by either athletes or farmers for enhancing muscle growth.

We developed a method for quantification of endogenous hormones IGF-1 and IGF-2 in bovine or human serum samples by selected reaction monitoring LC-MS/MS.

Method



Results

Human recombinant IGF-1, and IGF-2 are spiked into bovine plasma at different concentrations in order to create a matrix matched calibration curve (Figure 1) that covers the levels of IGF-1 and IGF-2. Due to the endogenic concentration of the protein in serum the calibration curves need to be corrected, to use these curves for quantification. Synthetic stable isotope labelled peptide homologues are used as an internal standard.

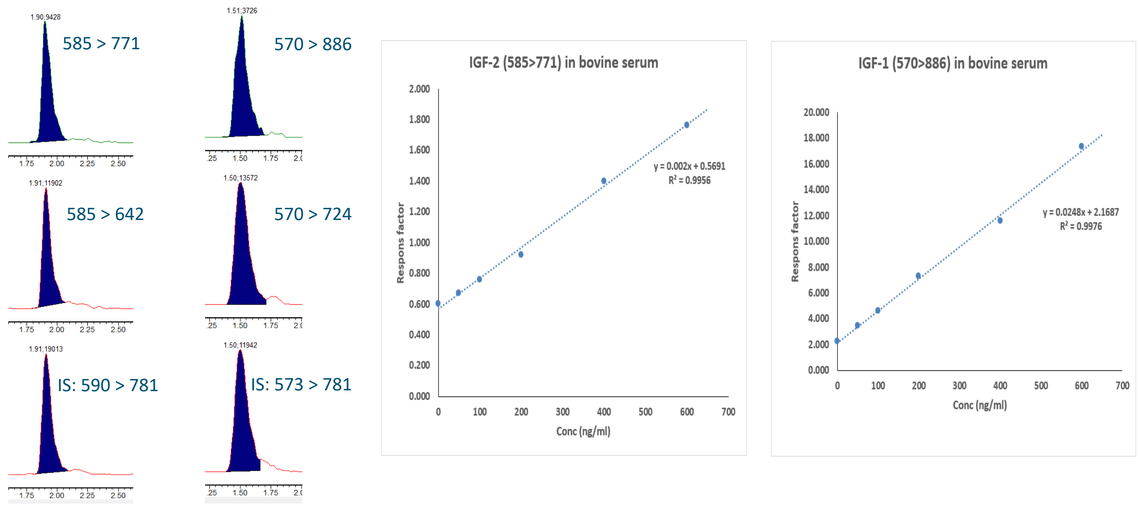


Figure 1: Representative peaks and calibration curves of human recombinant IGF-1 and IGF-2 in bovine plasma.

In Table 1 the selected peptides for the targeted LC-MSMS are presented:

Analyte Code	Precursor chain	Precursor	Product ion	Product chain
IGF-1 RT4 3+, B7+	RAPQTGIVDECCFR	570.3	724.6	RAPQTGI
IGF-1 RT4 3+, Y6+	RAPQTGIVDECCFR	570.3	886.3	RFCCED
IGF-1* RT4 (3+, Y6+ (IS)	RAPQTGIVDECCFR*	573.8	896.2	R*FCCED
IGF-2 T3 2+, Y4+	GIVEECCFR	585.3	642.2	RFCC
IGF-2 T3 2+, Y5+	GIVEECCFR	585.3	771.3	RFCCE
IGF-2* T3 2+, Y5+ (IS)	GIVEECCFR*	590.3	781.3	R*FCCE

Note: the Cysteines (C) are S-Carboxymethylated and for the internal standard: R*= (13C6, 15N4)

Table 1. Selected MRM transitions for IGF-1 and IGF-2 are indicated.

In Table 2 the performance characteristics of the method are shown. Confirmation of the identity of IGFs, based on EU criteria (2021/808), are met.
Due to the broad variation in the endogenous concentration of the IGFs in serum, the trueness (80-120%), the repeatability (<20%) and within lab reproducibility (<30%) could not meet the requirements from 2021/808 for all levels.

Component	Validation level (ng/ml)	Trueness (%)	Repeatability (%)	within lab reproducibility (%)	Confirmation
IGF-2	100	159.6	39.6	40.9	21 out of 21
	200	134.5	27.2	27.8	21 out of 21
	300	129.6	21.0	21.1	21 out of 21
IGF-1	100	100.6	27.9	28.2	21 out of 21
	200	81.4	17.5	18.7	21 out of 21
	300	78.6	12.5	14.2	21 out of 21

Table 2. Performance characteristics for IGF-1 and IGF-2 in bovine serum.

When an identical sample is processed in 5-fold (see Table 3), the repeatability does meet the specified criteria.

#	Sample	Concentration (ng/ml) IGF-2	IGF-1
1	Serum E	463.9	345.9
2	Serum E	401.3	362.9
3	Serum E	451.9	385.0
4	Serum E	454.7	361.5
5	Serum E	398.2	379.3
	AVG:	434.0	366.9
	STDEV:	31.6	15.6
	RSD (%):	7.3	4.2

#	Sample	Concentration (ng/ml) IGF-2	IGF-1
1	Serum A + 200 µg/l	734.4	240.7
2	Serum A + 200 µg/l	755.1	237.3
3	Serum A + 200 µg/l	763.0	248.2
4	Serum A + 200 µg/l	758.2	219.2
5	Serum A + 200 µg/l	710.9	238.8
	AVG:	744.3	236.8
	STDEV:	21.6	10.7
	RSD (%):	2.9	4.5

Table 3. Within sample repeatability for IGF-1 and IGF-2.

Conclusions

- This high-throughput method gives information for both IGF-1 and IGF-2 in bovine plasma in a single analysis.
- Confirmation of the identity of IGFs is based on EU criteria (2021/808) and those requirements were met.
- For quantification of IGFs in serum standard addition method is recommended.

Acknowledgements

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