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MDPI

Determination of endogenous (ala-bst) and recombinant protein (met-rbst) in serum samples by LC-Mass spectrometry

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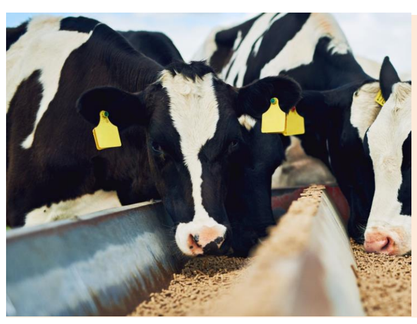
INTRODUCTION & AIM



Recombinant somatotropin (rbST) is a synthetic hormone that mimics endogenous bovine growth hormone. The treatment of diary cows with rbST during lactation leads to increase in milk production up to 25% (1), which approved in some countries such as the US. In EU, however, concerns for animal welfare, consumer health and milk production policies protection led to its ban in 1999 (2). To protect consumers and farmers rights against illicit treatment, a reliable method for detection of rbST in animals, milk and diary products is essential.

The aim of this study is the development of an LC-MS/MS method for rbST identification in serum samples and the discrimination between endogenous (ala-bST) and recombinant protein (met-rbST)

METHODS

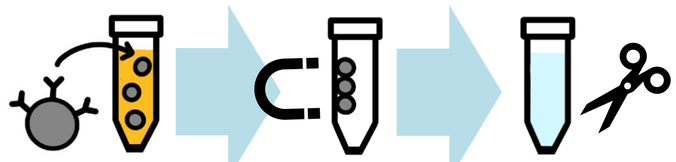
Analyses were performed with an Exion LC Sciex UHPLC system coupled with a 6500 QTRAP mass spectrometer (SCIEX, Milan, Italy), triple quadrupole with electrospray ionization source (ESI).

- **Stock solutions** (1000 µg/mL in water) of endogenous and recombinant somatotropin peptides were prepared and stored at 4 °C. Daily dilutions were used to assess linearity and repeatability of the LC-MS/MS method using synthetic peptides at various concentrations.
- **Serum samples** (n=48) from six Holstein cows in field in Piedmont were subjected to extraction, purification, and digestion prior to LC-MS analysis.

Focuses of the study :
- Protocol optimization of extraction, purification and digestion of rbST from serum samples
- Optimization of LC separation parameters (mobile phase, elution and gradients)
- MS condition setup, with MS acquisition parameters refined using 1 mg/mL standard solutions of synthetic peptides.
- Construction of calibration lines for quantification in a concentration range between 0.1ppb and 500ppb

peptide	Sequence (N->C)	aa	Modifications	Purity, counter ion
ALA-rbST	AFPAMSLSGLFANAVLR	17	-	>80%, tfa salt
MET-rbST	MFPAMSLSGLFANAVLR	17	-	>80%, tfa salt
ALA-rbST-isotope	AFPAMSLSGLFAN[A*][V*]LR	17	[A*]= (13C3 ; 15N)Ala ; [V*]=(13C5 ; 15N)Val , 99% stable heavy isotopes labeled. Total mass shift: +10	>80%, tfa salt
MET-rbST-isotope	MFPAMSLSGLFANA[V*]LR	17	[V*]=(13C5,15N)Val, 99% stable heavy isotopes labeled, mass shift: +6	>80%, tfa salt

Extraction - Purification - Digestion



Serum sample 5ml + Internal Standard + mAb coated magnetic beads (2h)

Trypsin 10µL (overnight)

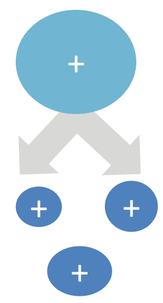
LC optimization

Mobile phase - A: Formic acid 0.1% aquoeus solution

B: Formic acid 0.1% in Acetonitrile

Column: Phenomenex, Kinetex 1.7um C18 100Å 50*2.1mm

MS/MS optimization



- **curtain gas**: 35 psi
- **Ion spray voltage**: 3000
- **Temperature**: 350°C
- **gas 1**: 35°C
- **gas 2**: 45°C

RESULTS & DISCUSSION

The HPLC-MS/MS method showed excellent performance: linearity (R^2 = 0.999, 1–250 ppb), repeatability (RSD <10% at 1, 5, and 50 ppb), and recovery (95–106%).
The analyte concentration in the analysed samples was below of the quantification limit of the method.

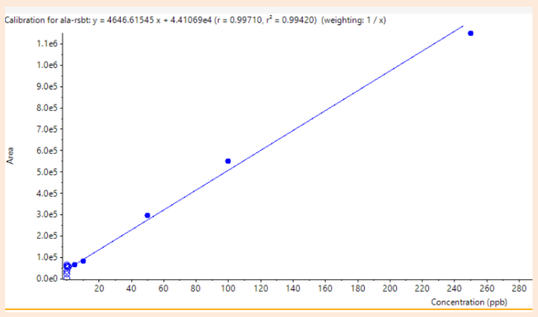


Fig. 1: Calibration line Hormone ALA-rbST 5-250ppb

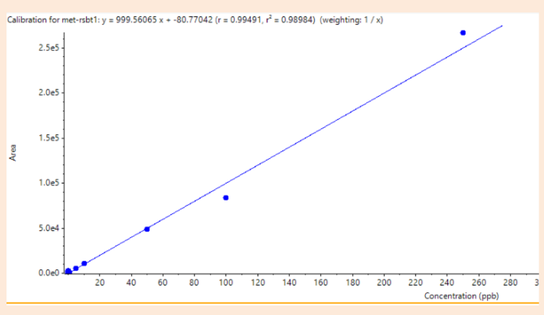


Fig. 2:Calibration line Hormone MET-rbST1-250ppb

Table 2 *:* Optimized MS/MS conditions for the selected compounds analyzed in the positive electrospray ionization (ESI) mode *MS/MS*

Precursor ion Q1	Product ion Q3	Analyte	identifier	DP	EP	CE	CXP
589.5	120.1	ala-rbst	quantifier	15	6	66	13
883.3	960.6	ala-rbst1	qualifier	15	6	41	17
883.3	1047.5	ala-rbst2	qualifier	15	6	41	22
913.7	1047.5	met-rbst1	quantifier	8	10	57	21
609	251.1	met-rbst2	qualifier	8	10	28	11
592.6	120.3	ala-rbst-isotope	quantifier	34	5	79	32
592.6	169.3	ala-rbst-isotope3	qualifier	34	5	47	10
592.6	191.3	ala-rbst-isotope1	qualifier	34	5	25	10
592.6	653.5	ala-rbst-isotope2	qualifier	34	5	31	15
611.4	120.1	met-rbst-isotope2	qualifier	49	7	85	10
916.3	1053.7	met-rbst-isotope1	quantifier	48	7	46	18

Collision energy (CE). decluster potential (DP), entrance potential (EP), collision cell exit potential (CXP)

CONCLUSION

Development and application of suitable analytical tools to quantify the presence of recombinant hormones in biological samples is crucial for the detection of illicit treatments and for ensuring the protection of consumer and farmer's rights.
In this study we have developed an LC- method for the endogenous (ala-bST) and recombinant protein (met-rbST) identification in serum. The process of extraction and digestion of somatotropin from serum is very complex, therefore new strategies will be evaluated to optimize the processes in order to reanalyze the samples and effectively verify the absence of the analyte even if the animals were not treated and therefore the somatotropin levels should be undetectable

FUTURE WORK / REFERENCES

[1] Butler, Leslie James. "The profitability of rBST on US dairy farms." (1999).
[2] Council Decision 1999/879/EC of 17 December 1999.

Acknowledgements
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