The Quantitation of Recombinant Bovine Somatropin by Q TRAP® LC-MS/MS Operated in MRM and MRM^3 Mode

Stephen Lock[1], Loic Beyet[2], Gaul Dervilly-Pinel[3], Malgorzata Olejnik[3], Fabrice Monteau[3], and Bruno Le Bizec[3]
[1]AB SCIEX Warrington (UK); [2]AB SCIEX Paris (France); [3]Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes (France)

Overview

A rapid, robust, sensitive and specific LC-MS/MS assay has been developed for the detection of recombinant bovine somatropin (rbST) using an MRM^3 approach. This growth hormone, which can be present in low levels in milk, has been shown to be detected at low parts-per-billion (ppb) levels by this method.

Introduction

In dairy farming rbST is used to treat cows in order to increase their milk output or as a growth promoter.[1,2] This growth hormone is banned in many countries[3] but is commonly used in the United States since it's authorization by the FDA in 1994.[4] To date most methods used to detect this hormone involve immunoassays[5,6] but the problem is that the native and the recombinant version of this hormone can not be differentiated by this approach. However, both native and recombinant forms do differ by one amino acid at the N-terminal end.[2] This slight difference means that a method based on mass spectrometry is a viable alternative and would have several advantages including specificity and sensitivity. rbST is usually only present at low ppb (ng/mL) amounts[1,2] in milk so any technique developed should be able to detect the hormone at this level.

Previously methods[1,7,8] have been developed to detect this hormone in plasma, but in this work we show the detection of this hormone at this level in milk, a more complex matrix, and also show how MRM^3 can help reduce the effect of the matrix on the results obtained.

Experimental

Sample Preparation

Internal standard [equine hormone (reST) used as a measure of whole protein recovery was added to bovine milk (10 mL) which was loaded onto a C4 SPE cartridge. The cartridge was washed with water containing 0.1% trifluoroacetic acid (TFA) followed by 30/70 mixture of acetonitrile / 0.1% TFA. The rbST was then eluted with an 80/20 mixture of acetonitrile / 0.1% TFA (7mL) and evaporated down to a volume of 1 mL. Cold methanol was

added to induce precipitation, the sample was centrifuged, and the supernatant dried down. This residue was reconstituted with ammonium bicarbonate buffer (120 µL) and digested overnight, at 37°C, with trypsin. The digest was evaporated to dryness and reconstituted with 30/70 acetonitrile / 0.2% formic acid and a C13 internal standard for the N-terminal peptide for rbST was added prior to injection.

LC-MS/MS Analysis

Final extracted samples were separated over a 25 minute gradient from 90% water / 10% acetonitrile to 10% water / 90% acetonitrile with both phases containing 0.1% formic acid. The separation occurred by reversed-phase HPLC on a 150x2.1 mm C18 Interchrom QS Uptisphere 3HDO HPLC column, at ambient temperature running at a flow of 300 µL/min on a Shimadzu UFLC$_{XR}$ system. MS detection was performed on an AB SCIEX QTRAP® 5500 system equipped with Turbo V™ source and electrospray ionization probe set at an IonSpray voltage of 3500 V. The conditions of the Multiple Reaction Monitoring (MRM) and MRM^3 experiments are shown in Table 1.

Table 1. MS conditions used for each peptide in MRM and MRM^3 mode

		MRM			MRM^3			
Hormone	N-Terminal Peptide sequence	Transitions	DP (V)	CE (V)	Transitions	DP (V)	CE (V)	AF2 (mV)
rbST	MFPAMSLSGLFANAVLR	913.2/774.0 913.2/1047.6	35	37	913.2/774.0/791.0 913.2/774.0/961.0	35	37	0.2
reST	MFPAMPLSSLFANAVLR	933.2/794.2	35	38				
rbST $^{13}C_6$	MFP(A^{13}C)MS(L^{13}C)SG(L^{13}C)F(A^{13}C)N(A^{13}C)V(L^{13}C)R	916.2/777.0	35	37				

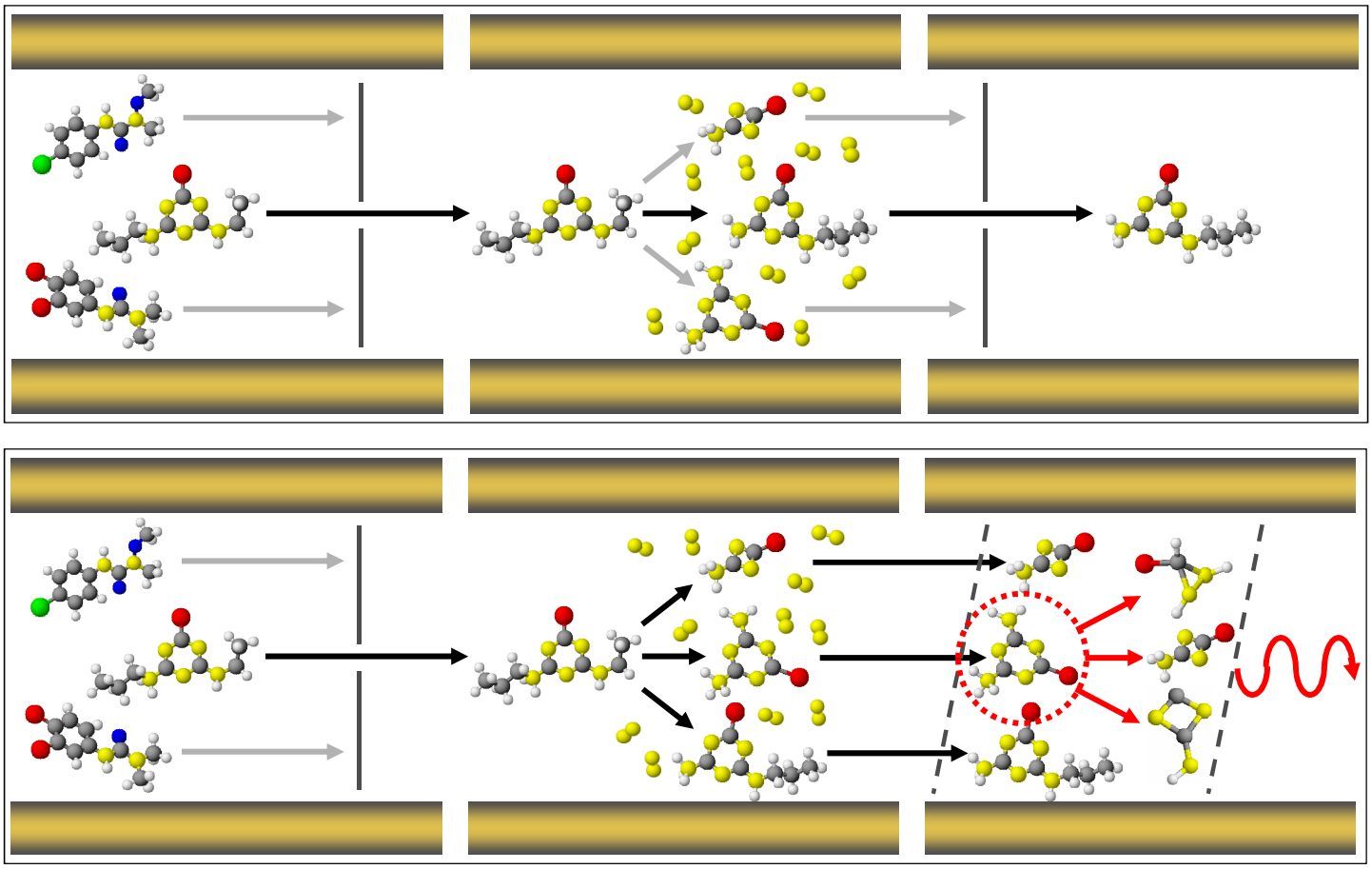

Figure 1. Operation of a QTRAP® system in MRM (top) and MRM^3 (bottom) modes

Results and Discussion

MRM^3 is a unique detection mode of hybrid triple quadrupole linear trap (QTRAP®) technology which is especially useful on the QTRAP® 5500 system because of sensitivity and speed enhancements in comparison to legacy instruments.

The QTRAP® 5500 system enables MRM^3 quantitation with a cycle time of 100 ms per scan providing scan speeds faster than LC demands and gives the ability to run in parallel several of these experiments in a single run or to combine MRM^3 and MRM experiments.

MRM^3 experiments enable higher specificity by first fragmenting precursor ions in the collision cell (Q2) and detecting the first set

of product ions (as in MRM experiment) but then the most intense of these product ions is trapped, isolated and fragmented again in the linear ion trap (Q3). These second generation fragment ions are then used for quantitation (Figure 1).

MRM^3 has a special advantage when analyzing dirty or complex samples for example food extracts. The increased selectivity allows the removing of matrix interferences and thus improves the signal-to-noise (S/N) ratio.

For this work MRM was directly compared to MRM^3 using a low ppb spike of rbST in milk (Figure 2). From this example it can be seen that MRM^3 has reduced matrix interference and an improved S/N for quantitation at low concentrations.

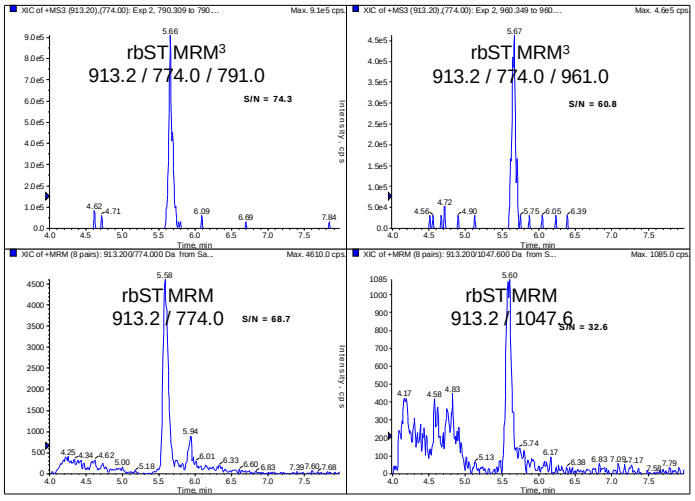

Figure 2. XIC of milk samples spiked with 10 ppb of rbST and purified using solid phase extraction: MRM^3 data (top) and MRM data (bottom)

As the MRM^3 experiment was shown to have advantages over the MRM experiment in this instance, milk was spiked at several different concentrations to generate a calibration line. The calibration line obtained can be seen in Figure 3.

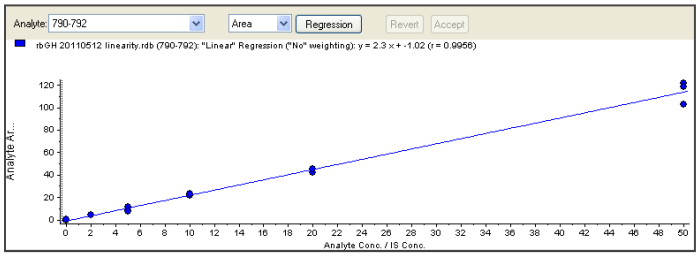

Figure 3. Calibration line for extracts of milk samples spiked with rbST at different levels. The calibration line is from the MRM^3 experiment 913.2/774.0/791.0

A typical 2 ppb spike into milk (a level which can be seen in milk) is shown in Figure 4. The top pane shows the total ion chromatogram, the middle pane shows the chromatograms of the MRM experiments for the internal standards and the bottom two panes show the two MRM^3 transitions for rbST.

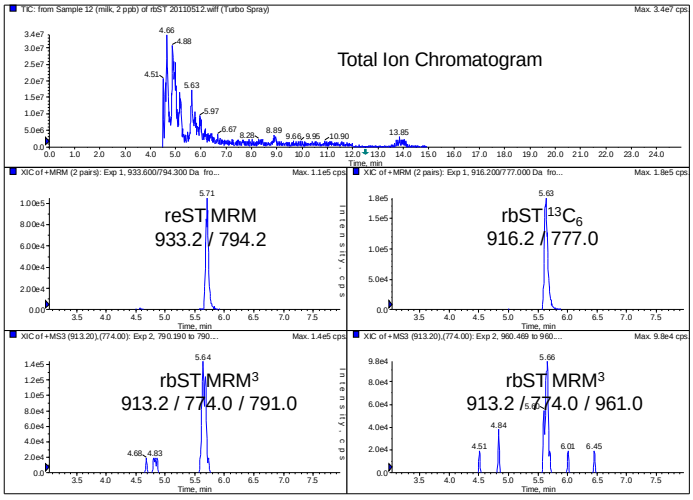

Figure 4. Typical chromatogram for a 2 ppb spiked milk calibration standard

Summary

From the results presented it can be seen that the application of more selective techniques for both sample preparation and mass spectrometric determination have improved the performance of the method for the detection of rbST in milk. These results show that we can unambiguously detect rbST in milk unlike the conventional methods which use immunoassay based technologies. This is now the first step to the development of a sensitive method for the efficient control of somatotropin abuse in milking cows.

Moving forward further improvements are still necessary, especially in the sample preparation as non-specific loss of rbST has been seen to occur, probably resulting from instability and adsorption issues, which have reduced the % recovery. Further to this additional optimization of the trypsin digestion step may well increase recoveries and provide even lower limits of detection. With regards to MS detection technologies the use of differential ion mobility is still to be investigated as a way to further increase specificity and improve detection limits.

Acknowledgement

We would like to acknowledge Prof. Bruno Le Bizec and his group at LABERCA for their continued contribution to this research area.

References

[1] M.-H. Le Breton, S.Rochereau-Roulet, S. Chereau, G. Pinel, T. Delatour, and B. Le Bizec: J. Agric. Food Chem. 58 (2010) 729-733

[2] M.-H. Le Breton, A. Beck-Henezelin, J. Richoz-Payot, S. Rochereau-Roulet, S. Chereau, G. Pinel, T. Delatour, and B. Le Bizec: Anal. Chim. Acta. 672 (2010) 45-49

[3] Council Decision 1999/879/EC: 'Concerning the placing on the market and administration of bovine somatropin (BST)' (1999)

[4] Food and Drug Administration Regulation FDA 58 FR 59946 (1993)

[5] C. M. Zwickl, H. W. Smith, and P. H. Bick: J. Agric. Food Chem. 38 (1990) 1358-1362

[6] S. Rochereau-Roulet, I. Gaudin, S. Chereau, S. Prévost, G. André-Fontaine, G. Pinel, and B. Le Bizec: Anal. Chim. Acta 700 (2011) 189-193

[7] M.-H. Le Breton, S. Rochereau-Roulet, G. Pinel, N. Cesbron, and B. Le Bizec: Anal Chim. Acta 637 (2009) 178-184

[8] L. Bailley-Chouriberry, G. Pinel, P. Garcia, M. A. Popot, Y. Bonnaire, and B. Le Bizec: Anal. Chem. 80 (2008) 8340-8347

Publication number: 4650211-01

Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
www.absciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices