# Detection of Recombinant Bovine Somatotropin in Milk by LC-ESI-MS/MS

Application

**Food Safety**

## Authors

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## Abstract

**Recombinant bovine somatotropin (rbST), also called growth hormone, is a protein hormone used in dairy farming to enhance milk production. A method has been developed for the detection of rbST in milk by ESI(+)-LC-MS/MS. This method allowed a detection limit of 20 pg of tryptic N-terminal peptide rbST in standard solution injected on-column and was successfully applied to extracts obtained from milk samples spiked with 50 ng/mL$^{-1}$ (2.3 pmol/mL$^{-1}$) rbST.**

## Introduction

Recombinant bovine somatotropin (rbST), also called growth hormone, is used in lactating cows to increase milk production. Different regulations exist regarding its use, but the lack of confirmatory methods [1] for its detection makes it difficult to apply these regulations. It turns out to be an international issue in terms of animal doping and in food safety as well. Indeed, residues of rbST can be present in the milk of dairy animals treated with this hormone.

In order to detect residues of rbST in milk, the choice has been made to focus the analysis on the tryptic N-terminal peptide of the protein, specifically the difference between the endogenous and recombinant forms. The N-terminal amino acid alanine that is present in the endogenous form is replaced by a methionine in the recombinant one [2].

This application describes a method for the detection of rbST by ESI(+) LC-MS/MS. The method was successfully applied to extracts from milk samples spiked with rbST.

## Experimental

### Standards of Proteins and Peptides

Protein standards of rbST and recombinant equine somatotropin, reST (EquiGen-5), were obtained from the Harbor-UCLA Medical Center, National Hormone and Pituitary Program (Torrance, CA, USA) and Bresagen Limited (Thebarton, Australia), respectively.

The peptides used as standards, with the following amino acid sequence MFPAMSLSGLFANAVLR (N-terminal tryptic rbST), MFPAMPLSSLFANAVLR (N-terminal tryptic reST), and AFPAMSLSGLFAN-AVLR (N-terminal tryptic bST) were synthesized from Millegen (Labege, France).

### Instrumentation

The detail of the instrumentation used for the detection of the N-terminal peptides is described in the following tables.

**LC**

| | | |
|---|---|---|
| Instrument | Agilent 1200 | |
| Column | Column Interchrom ModuloCart QS Uptisphere 3HDO 150 mm × 2 mm | |
| Mobile phase | A: Acetonitrile + 0.1% formic acid<br>B: $H_2O$ + 0.1% formic acid | |
| Flow rate | 0.3 mL/min | |
| Injection volume | 20 µL | |
| Gradient | Time (min) | %A |
| | 0 | 10 |
| | 5 | 55 |
| | 10 | 60 |
| | 15 | 100 |
| | 17 | 10 |
| | 20 | 10 |

**MS**

| | |
|---|---|
| Instrument | Agilent 6410 LC/MS Triple Quadrupole |
| Ionization mode | ESI (+) |
| Capillary | 5000 V |
| Nebulizer | 55 psi |
| Gas flow | 13 L/min |
| Gas temperature | 300 °C |

### Selected Reaction Monitoring (SRM) Method Parameters

In order to obtain a better specificity, the detection was performed in SRM mode. The transitions monitored are displayed in Table 1.

**Table 1. SRM Method Parameters**

| Compound | RT | Charge | Transitions monitored | Collision energy (V) |
|---|---|---|---|---|
| Nterm rbST | 8.33 | z = 2 | 913.2 → 1047.7 | 30 |
| | | | 913.2 → 774.1 | 20 |
| | | z = 3 | 609.3 → 774 | 10 |
| | | | 609.3 → 643.5 | 20 |
| Nterm reST | 8.39 | z = 2 | 933.2 → 1287.9 | 30 |
| | | | 933.2 → 794.1 | 20 |
| Nterm bST | 8.20 | z = 2 | 883.2 → 1047.8 | 20 |
| | | | 883.2 → 774.1 | 20 |

## Results and Discussion

### Application of Triple Quadrupole MS-MS and Electrospray Ionization Mode Methodology

In this method, the choice has been made to use electrospray ionization in positive mode. Indeed, this ionization mode presented as a “soft” ionization technique is optimal for peptides. The ionization of the N-terminal peptide rbST leads to two main forms (z = 2 and z = 3).

This use of a triple quadrupole based methodology enabled very good sensitivity and selectivity and also a possible quantification of the monitored signals.

### Separation of the Different Compounds

The detection method was developed for the detection of the tryptic N-terminal peptide of rbST and also for the tryptic N-terminal peptide of endogenous pituitary bovine somatrotropin (bST) and reST as well. Due to the high homology in the amino acid sequence with rbST, reST was used as the internal standard.

The three compounds were separated chromatographically and analyzed utilizing the transitions described in Table 1. The chromatogram corresponding to the injection of 0.2 ng of N-terminal peptide bST, rbST, and reST is shown in Figure 1.

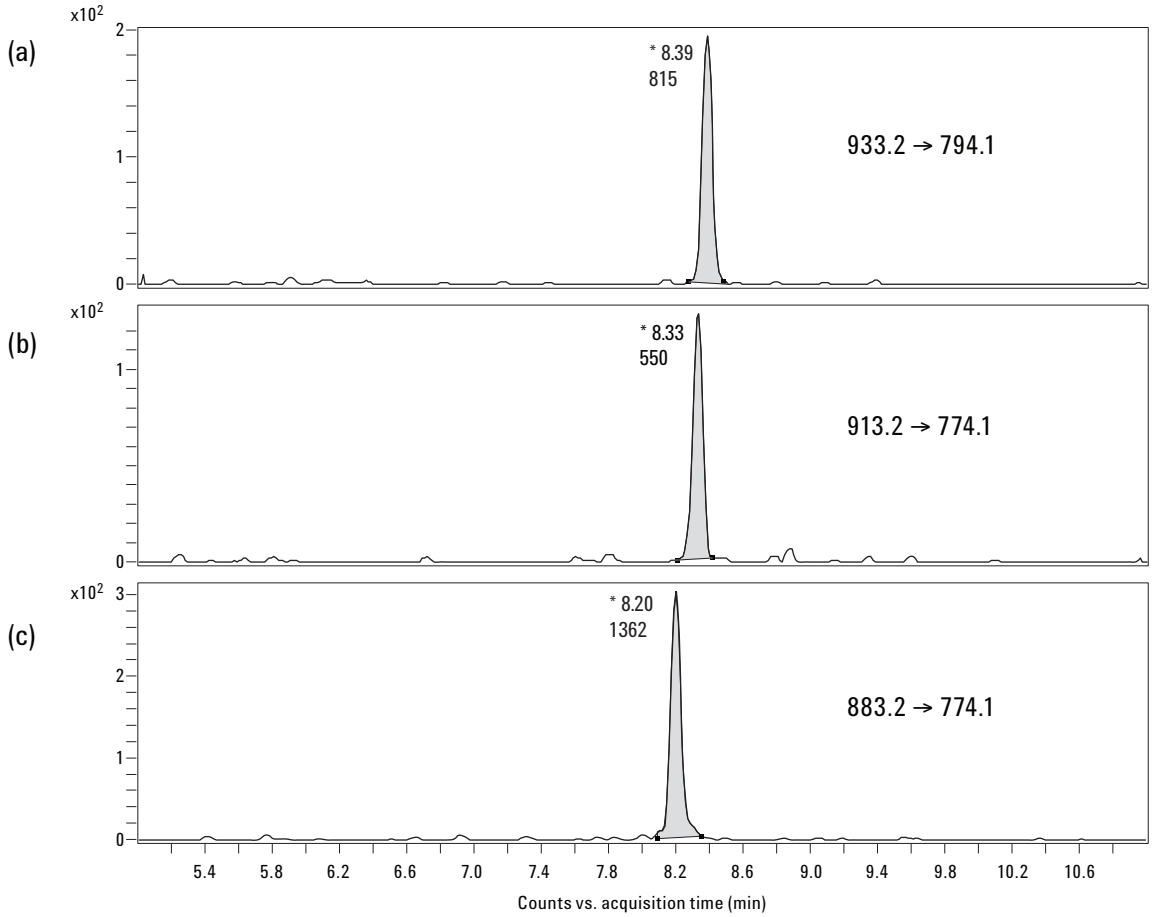


**Figure 1. SRM ion chromatograms of standard solutions of tryptic N-terminal peptide of (a) reST, (b) rbST and (c) bST. The injection aliquot used corresponded to 0.2 ng on-column.**

Even with an optimized gradient, due to their high homology in terms of sequence, the three compounds eluted with very similar retention times.

### Linearity and Sensitivity of the Method

The method described allowed detection of the three peptides with very good sensitivity. A limit of detection of 20 pg injected on-column (~900 femtomole) was reached. Quantification was possible as shown by the good linearity of the calibration curves (Figure 2).

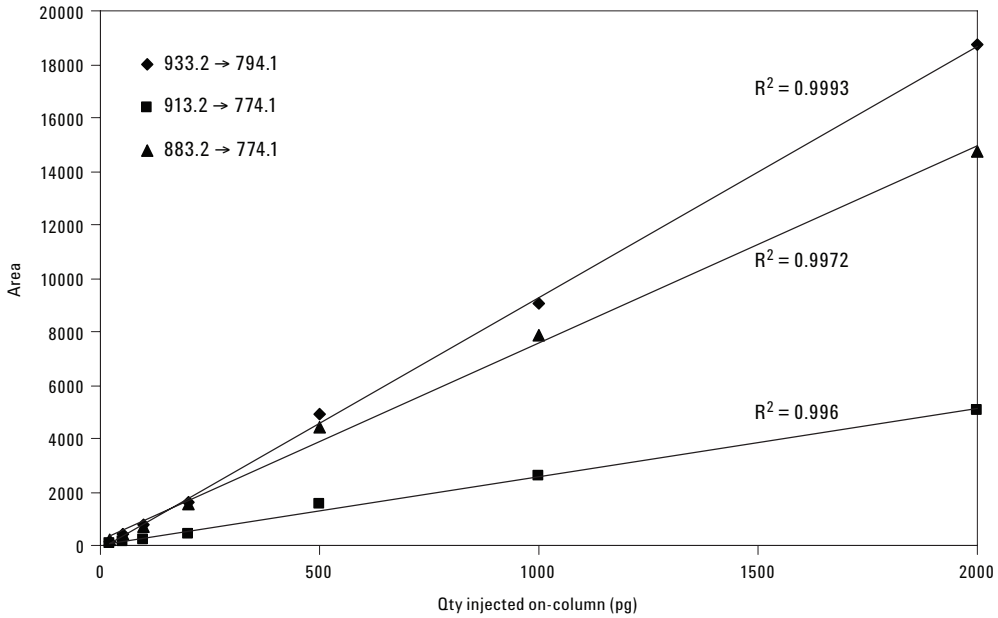


**Figure 2. Calibration curve of tryptic N-terminal peptides rbST (913.2 → 774.1), reST (933.2 → 794.1) and bST (883.2 → 774.1).**

### Results of Spiked Samples

The detection method was applied to extracts obtained from milk samples spiked with rbST. The purification procedure used is described in [3].

Figure 3 shows the chromatogram of a milk sample spiked with 50 ng.mL$^{-1}$ of rbST, in accordance with guidelines for the identification of rbST according to 2002/657 criteria [4]. The chromatogram shows excellent peak shape, and above all, nearly null

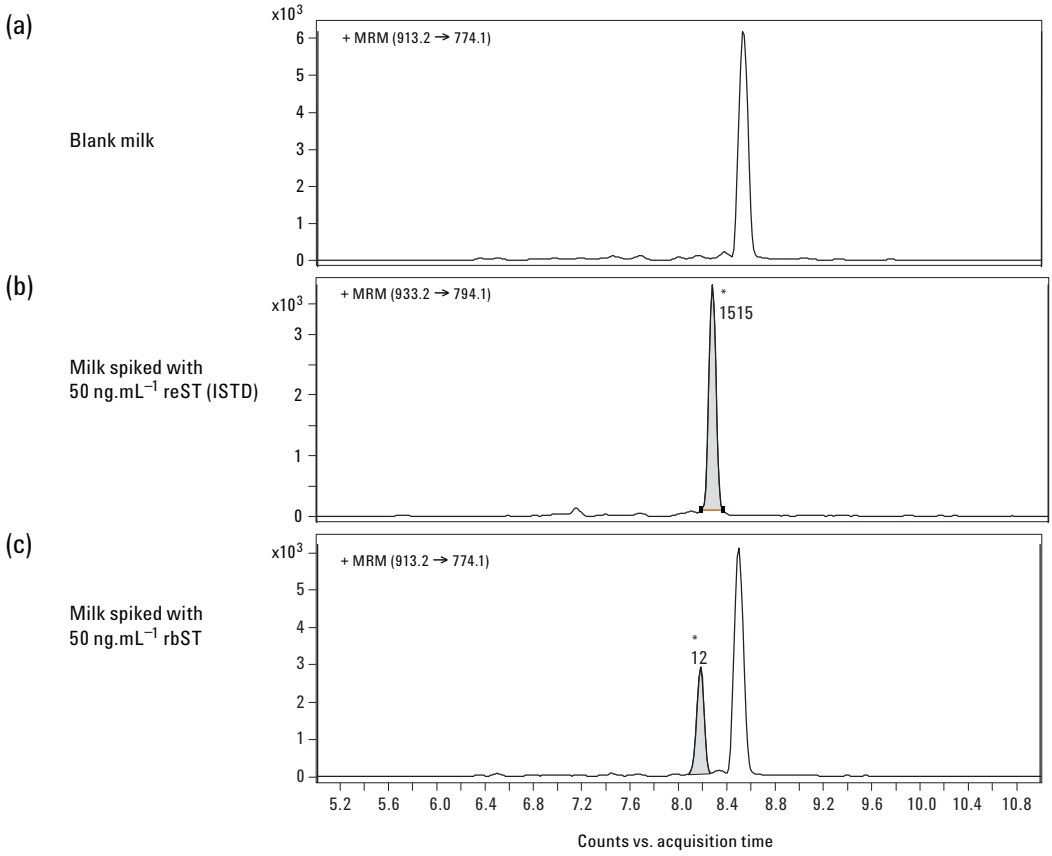


**Figure 3. SRM ion chromatograms obtained from milk samples. The different signals correspond to (a) blank milk, (b) the same milk spiked with 50 ng.mL$^{-1}$ reST (internal standard), and (c) 50 ng.mL$^{-1}$ rbST.**

background noise, demonstrating the selectivity of the method. The intensity of the signal, although lower than the internal standard, is, however, significant, and shows a clear and distinct signal. The method clearly allows for unambiguous identification of rbST in milk.

## Conclusions

The detection of rbST in milk was performed with detection by ESI(+) LC-MS/MS. The method showed very good sensitivity, specificity, and robustness. It was successfully applied to milk samples spiked with rbST at 50 $ng.mL^{-1}$, in accordance with criteria outlined by the 2002/657 Council Directive.

## References


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4. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Commun. 2002/657/EC, 2002


## For More Information

For more information on this application, you may also contact Agilent Technologies, Inc., Attention: Lea Bonnington, European Field Support Centre, Hewlett-Packard Strasse 8, 76337 Waldbronn, Germany.

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