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Rapid, High Sensitivity Detection of Recombinant Bovine Somatotropin in Cattle

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Abstract

This application note describes the use of Waters Xevo TQ MS for the specific detection of rbST in plasma samples collected from an animal treated with the recombinant hormone.

Benefits

The results presented are amongst the very first allowing unambiguous detection of the administration of recombinant bovine somatotropin some days after injection.

· The high sensitivity offered by the Xevo TQ MS allows the unambiguous detection of rbST in biological matrices according to 2002/657/EC requirements.

· Sensitivity of the detection is perfectly adapted to the detection of the circulating hormone at trace level in blood over several days after the administration to the animal.

· The robustness of the Xevo TQ MS shows good repeatability of results, with very low matrix effect, which is an important aspect for routine analysis in the framework of monitoring control plans

Introduction

Controlling safety with respect to food products of animal origin is a priority for governments, international regulatory bodies and organizations that process and handle products prior to consumption. Food safety issues arising from commodity products often become globally reported and have the potential to impact consumer confidence and trade at international levels.

Recombinant bovine somatotropin (rbST), also called growth hormone, is used in some countries as a general growth promoter in pigs and cattle but also in lactating cows to increase milk production[1,2]. This practice is strictly regulated, especially in the European Union, with a complete ban of this substance[3,4]. However, this regulation faces a lack of analytical tools able to detect the (mis)use of this hormone. The difficulty of analysis is due to the protein nature of the hormone, its low level of concentration in biological fluids, and the complexity of the matrices of interest. Up to now, methods have been limited to immunoassays with the problem that native and recombinant forms were not differentiated. A few attempts have been published but they were all unable to specifically detect rbST at physiological levels[5,6].

Only recently, a successful strategy has been developed for the direct detection of rbST in biological matrices [7,8]. The analysis is based on the detection of the tryptic N-terminal peptide, specific of the recombinant form of the hormone. Indeed, the Alanine N-terminal amino acid present in the natural form is replaced by a methionine in the case of the recombinant hormone as present in formulations for injection.

This application note describes the use of Waters Xevo TQ MS for the specific detection of rbST in plasma samples collected from an animal treated with the recombinant hormone. The results presented are amongst the very first allowing unambiguous detection of the administration of recombinant bovine somatotropin some days after injection.

Experimental

Standards of recombinant hormones

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

Sample preparation

Sample extraction and purification procedure for recombinant bovine somatotropin in plasma has already been described[7,8].

LC conditions

LC system:	ACQUITY UPLC System
Runtime:	8.00 min
Column:	ACQUITY BEH C_{18}, 1.7 μm, 2.1 x 100 mm
Porosity:	130 A
Mobile phase A:	0.1% formic acid dissolved in water
Mobile phase B:	CH_3CN + 0.1% formic acid
Flow rate:	0.6 mL/min
Injection volume:	8.0 μL

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate	%A	%B
1	Initial	0.600	95	5
2	0.6	0.600	95	5
3	1	0.600	95	5
4	4	0.600	5	95
5	6.5	0.600	5	95
6	7	0.600	95	5
7	8	0.600	95	5

Table 1. ACQUITY UPLC mobile phase gradient.

MS conditions

MS system: XEVO TQ MS

Ionization mode: ESI positive

Capillary voltage: 3 kV

Source temp: 150 °C

Desolvation temp: 550 °C

Desolvation gas: 800 L/hr

Collision gas flow: 0.15 mL/min

MRM method parameters

MRM transitions were first generated using Waters' IntelliStart[9]. The various parameters were then optimized individually for each diagnostic signal. These can be found in Table 2.

Compound	Charge (z)	Precursor	Product	Cone (V)	Collision Energy (V)
N-terminal rbST	2	913	774	34	26
		913	1047	34	25
N-terminal reST (I.S)	3	623	795	20	13
		623	530	20	14

Table 2. Xevo TQ MS MRM mode acquisition parameters.

Results and Discussion

Optimization of the LC-MS/MS conditions

The ACQUITY UPLC System allowed a good retention and separation of the tryptic N-terminal peptides with a total run time of 8 min, and retention times of 2.74 and 2.79 min for the N-terminal peptides rbST and reST, respectively (Figure 1).
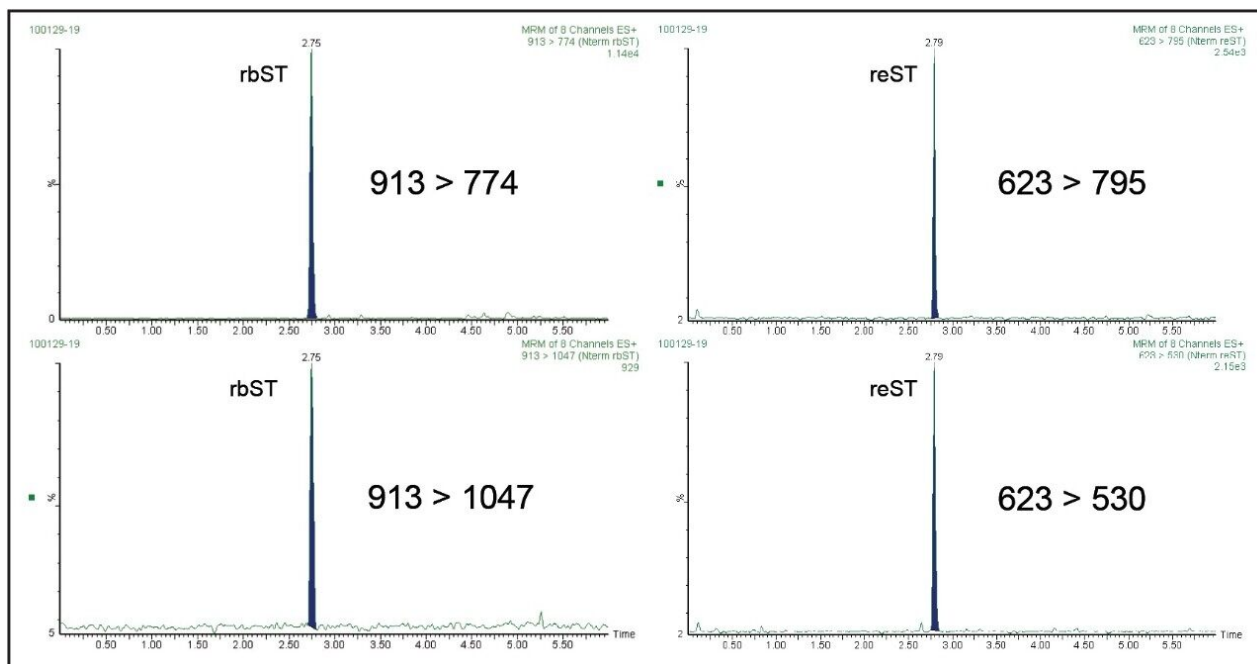
Figure 1. UPLC (ESI⁺)-MS/MS MRM diagnostic signals of N-terminal rbST (913 > 774 and 913 > 1047) and N-terminal reST (623 > 795 and 623 > 530)obtained from the injection of a standard solution at 10 ng mL⁻¹.

The positive electrospray ionization leads to a doubly-charged form for N-terminal peptide rbST: $[M+2H]^{2+}$ =913 which was selected as precursor ion. According to the Commission Decision 2002/657/EC[10]. Two diagnostic signals were selected in the MRM method for identification purposes. Recombinant equine somatotropin (reST) was used as internal standard with a fortification at 100 ng mL⁻¹ in samples. The ionization of its N-terminal peptide lead to a main triply-charged form $[M+3H]^{3+}= 623$, which was selected as precursor ion.

Detection of rbST in samples collected from a treated animal

This method has been applied to samples collected from a treated animal. A lactating cow was treated once subcutaneously with a dose of 500 mg of Lactatropin.

The chromatograms corresponding to plasma samples collected before treatment(D_0) and 2 days (D_2) after treatment are shown in Figure 2.
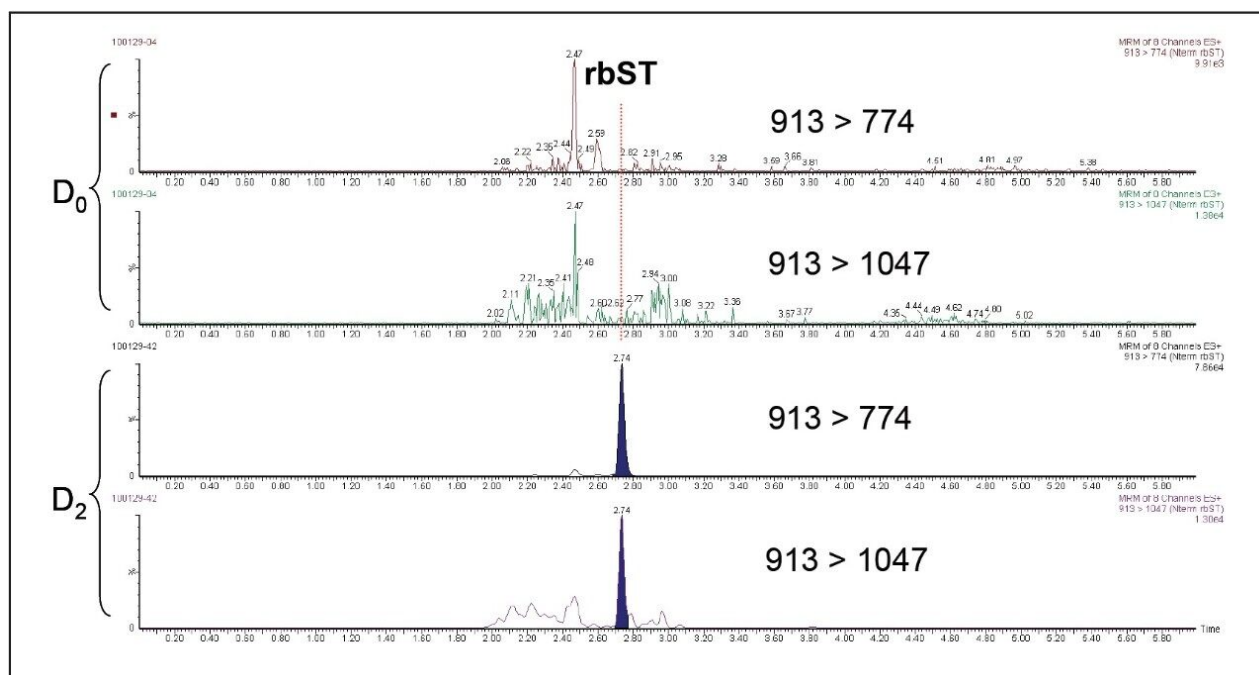
Figure 2. UPLC-ESI$^+$-MS/MS MRM diagnostic signals for rbST obtained from a plasma sample collected before treatment (D$_0$) and 2 days after treatment (D$_2$).

The results shown in Figure 2 show that it's possible to detect rbST in plasma two days after its administration to the animal. Analysis of the signals acquired leads to unambiguous identification of the protein according to 2002/657/EC requirements[10]. This confirms the suitability of the detection method's sensitivity in accordance with expected levels of the protein in animal plasma. Little evidence of matrix effects was observed and very good linearity in matrix allowed further relative quantification of rbST up to around 50 ppb in plasma two days after the administration of the hormone, which is in accordance with previous results[7,11]. The robustness of Xevo TQ MS allowed good repeatability at low levels in complex samples as shown in Table 3.

Injection #	Area	Height
1	394	11865
2	374	12323
3	376	12471
4	365	12848
5	373	11824
6	364	12012
7	311	10105
8	428	13203
9	393	11965
10	347	10667
Mean	372.5	11928.3
s.d.	30.8	934.5
%RSD	8.3	7.8

Table 3. Repeatability data for a 10-ppb fortified plasma sample.

Conclusion

- The high sensitivity offered by the Xevo TQ MS allows the unambiguous detection of rbST in biological matrices according to 2002/657/EC requirements.

- Sensitivity of the detection is perfectly adapted to the detection of the circulating hormone at trace level in blood over several days after the administration to the animal.

- The robustness of the Xevo TQ MS shows good repeatability of results, with very low matrix effect, which is an important aspect for routine analysis in the framework of monitoring control plans.

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