DETERMINATION OF POLYPEPTIDE ANTIBIOTIC RESIDUES IN MUSCLE AND MILK SAMPLES BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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INTRODUCTION

Bacitracin and colistin are a members of the polypeptide antibiotics with a variety of actions against many Gram-negative and Gram-positive bacteria. The therapeutic use of polypeptide antibiotics in food producing animals must be assessed not only in terms of good clinical efficacy but also considering the risk of the presence of residues in edible tissues. Therefore, to ensure human food safety, the EU has set a tolerance level for these compounds as the maximum residue limits (MRLs) for bacitracin at the level of 100 µg/kg and for colistin at the level of 50 µg/kg in milk. In muscle tissues both polypeptides have MRL of 150 µg/kg.

The aim of this work was to develop a reliable LC-MS/MS method with fast and simple sample pre-treatment, suitable for extraction of bacitracin and colistin residues from muscle and milk samples.

MATERIALS AND METHODS

A liquid chromatography coupled with tandem mass spectrometry method for determination and quantification of polypeptide antibiotics in muscle and milk samples has been developed. Prior to instrumental analysis, sample preparation technique involved extraction with solution of ammonia in acetonitrile followed by further evaporation, reconstitution and filtration steps. The chromatographic separation was performed on C18 column with gradient elution mode. The mass spectrometer was operated in electrospray positive ionisation mode and multiple reaction monitoring mode was used to quantify the analytes.

Sample preparation

2 g of sample + IS

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8 mL ACN:ammonia solution 25% (9:1, v/v)

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Vortex mixing

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Centrifugation, 4500 rpm, 4°C

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evaporation, 45°C

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Reconstitution, 0.5 ml of mobile phase

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Filtration, 0.22 µm PVDF

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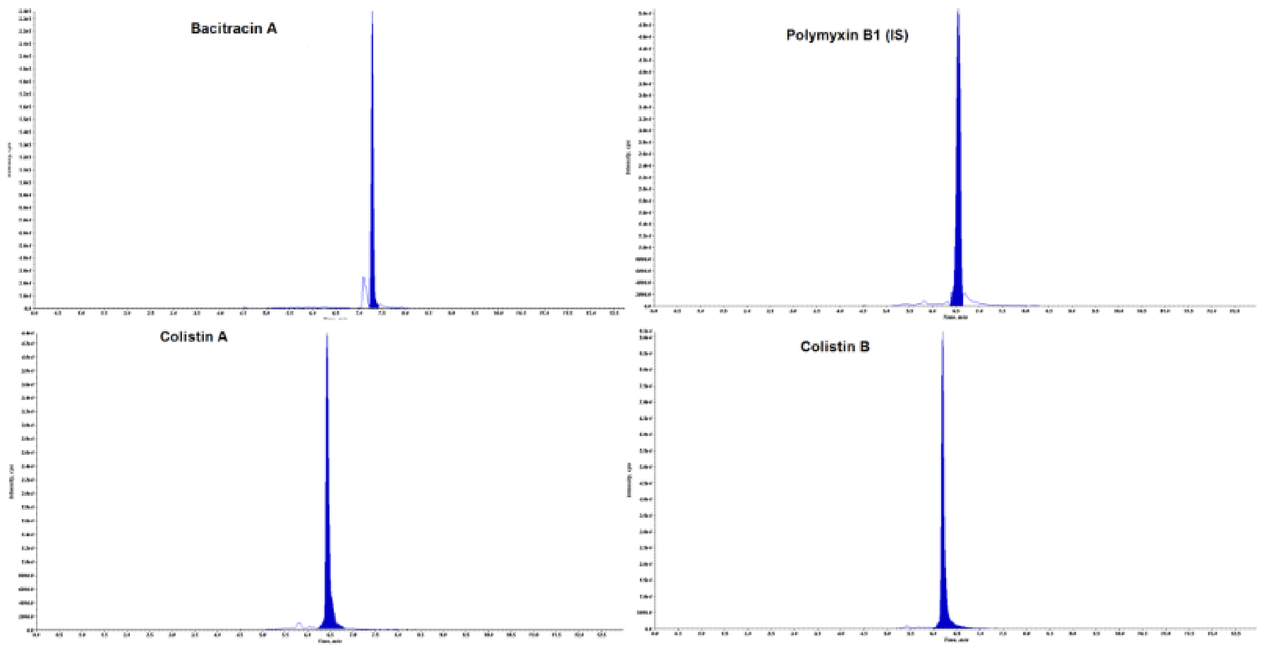
LC-MS/MS

LC-MS/MS conditions

Equipment: Agilent 1200 HPLC system
QTRAP 5500 mass spectrometer
Column: Kinetex XB-C18 (100 x 2.1 mm; 2.6 µm)
Mobile phase: 1% HCOOH in ACN : 1% HCOOH in H_2O, gradient mode
Flow rate: 0.3 ml/min
Column temperature: 45°C
Injection volume: 5 µl
MRM transitions:
Bacitracin A (m/z) 475 > 199; 475 > 227
Colistin A (m/z) 390.7 > 384.6; 390.7 > 101.1
Colistin B (m/z) 386 > 380; 386 > 101.1
Polymyxin B1 (IS) (m/z) 402 > 396.3

RESULTS



Chromatograms of muscle sample fortified with polypeptide antibiotics at the concentration level of 150 µg/kg

Validation results

Muscle	Bacitracin A			Colistin A			Colistin B		
Concentration, µg/kg	75	150	225	75	150	225	75	150	225
Recovery, %	99.6	102.2	99.1	99.3	101.4	99.1	98.4	102.3	98.5
Repeatability, CV %	7.8	7.3	5.6	8.4	7.4	5.7	8.8	7.6	5.7
Reproducibility, CV %	8.8	7.9	6.2	9.1	8.3	6.1	9.3	8.7	6.3
CCα, µg/kg	174			175			175		
CCβ, µg/kg	196			196			198		

Milk	Bacitracin A			Colistin A			Colistin B		
Concentration, µg/kg	50	100	150	25	50	75	25	50	75
Recovery, %	95.9	97.4	97.7	96.8	98.2	99.3	96.7	99.0	99.2
Repeatability, CV %	9.2	7.5	5.7	9.9	9.0	8.1	9.6	8.9	7.9
Reproducibility, CV %	10.8	9.1	6.1	11.6	10.2	9.5	10.9	9.9	9.2
CCα, µg/kg	116			58			58		
CCβ, µg/kg	130			68			69		

CONCLUSION

An analytical method for the determination of polypeptides antibiotics in muscle and milk samples has been successfully developed. A simple sample preparation and 13-min single chromatographic run allow to perform multiple analyses within one working day. The method validation according to Commission Decision 2002/657/CE demonstrates its reliability with high recovery and precision and ability to determination and confirmation polypeptide antibiotics in food matrices.

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