Polypeptide antibiotics in food control

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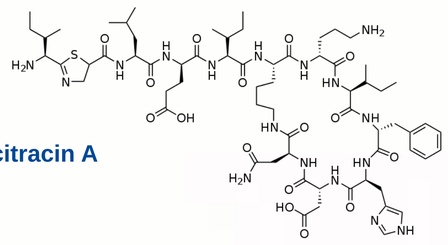
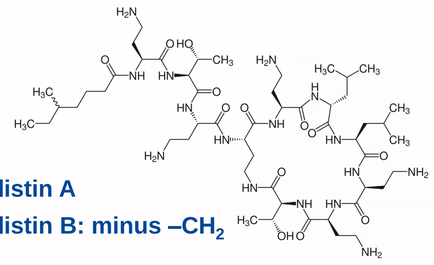
Introduction

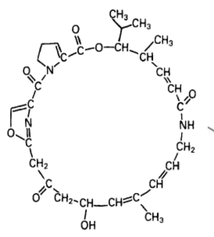
Bacitracin, Colistin, Polymyxin B, Virginiamycin and Enramycin are well-known polypeptide antibiotics. Most of them could be used in a sub-therapeutic level as antimicrobial growth promoters to improve growth rate and feed conversion efficiency, but they are not allowed as feed additives [1].
For a long time they have played only a minor role in human medicine because of their toxicity. With increasing drug resistance of pathogenic bacteria the mentioned antibiotics regain importance and a control of the limits in foodstuff established by the European Commission is required.
In the Commission Regulation (EU) No 37/2010 [2] maximum residue limits (MRL) are set for the sum of Bacitracin A, B and C at 150 µg/kg in meat and 100 µg/kg in milk, as well as for the sum of Colistin A and B at 150 µg/kg in meat and 50 µg/kg in milk . Polymyxin B, Virginiamycin and Enramycin are not regulated and therefore not allowed as veterinary medicine.
A method for the determination of Bacitracin A+B, Colistin A+B, Polymyxin B1+B2, Virginiamycin M1 and Enramycin A+B in meat and milk was developed. After extraction and purification by SPE, the substances were measured by LC-MS/MS.



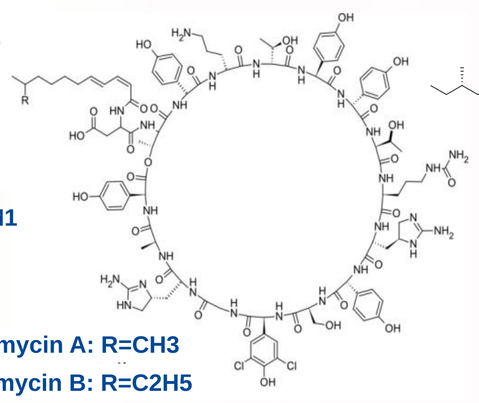
Bacitracin A



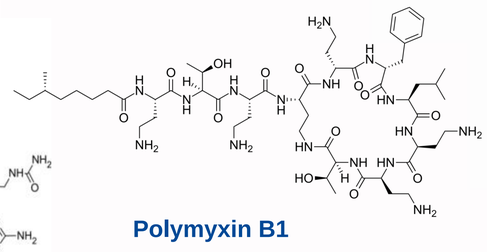
Colistin A
Colistin B: minus –CH_2



Virginiamycin M1



Enramycin A: R=CH_3
Enramycin B: R=C_2H_5



Polymyxin B1
Polymyxin B2: minus –CH2

Method

Sample Preparation

weigh sample
↓ spike with IS/Std
add water and methanol
↓ extraction
add HCl
↓ mix and centrifugate
SPE: MN HR-X
↓ elution
concentrate
↓
LC-MS/MS

HPLC

HPLC: Agilent 1200
Column: ThermoScientific HyPurity, 5 µm, 150 x 3 mm
Temp.: 30°C
Inj.Vol.: 20 µl
Eluent: A: H2O / 0.1% HCOOH
B: ACN / 0.1% HCOOH
Flow: 500 µl/min

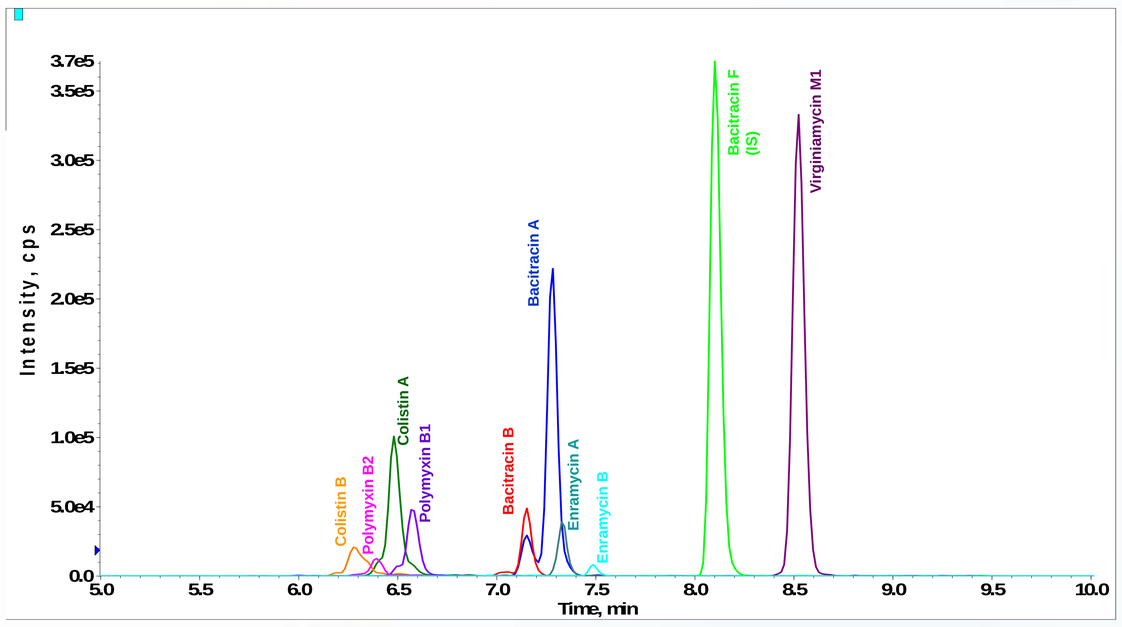
MS: AB Sciex API 4000 QTrap® TurboIonSpray® -Source (ESI), positive MRM mode

MS/MS

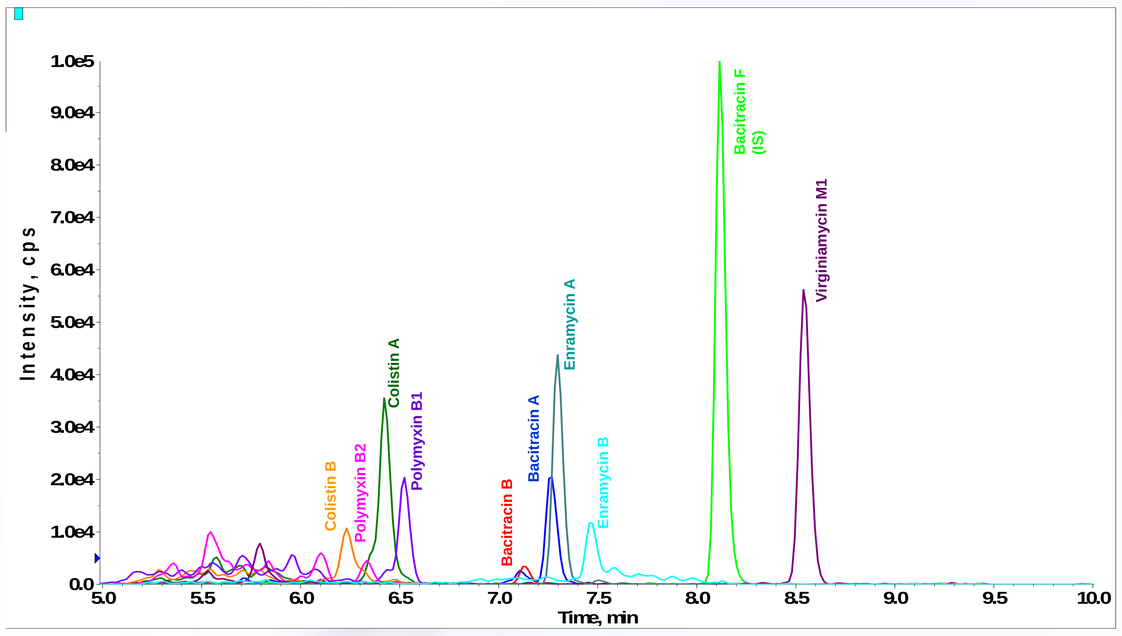
In positive ESI mode polypeptide antibiotics form single, two and triply charged molecular ions as parent ions. For quantification only the fragments of the triply charged parent masses are used, excluding for Virginiamycin and Bacitracin F (IS). As no isotopically labeled standard is available, the concentration is calculated with the recovery of a spiked sample. Therefore each sample is weighed twice. One is spiked with the internal standard (IS) only, the other with a standard solution containing all targeted analytes and the IS. Since experience has revealed that each matrix has a different effect on the sensitivity of the MS/MS signals, the recovery rate of each sample is determined individually.

Analyt	Polarity	Precursor Ion [M/Z]	Product Ion [M/Z]
Bacitracin A	[M+3H]3+	475.3	86.1 / 356.2 / 670.1
Bacitracin B	[M+3H]3+	470.3	662.6 / 669.6
Bacitracin C	[M+3H]3+	465.4	227.0 / 662.4
Bacitracin F (IS)	[M+2H]2+	710.6	280.9 / 309.2
Colistin A	[M+3H]3+	390.7	241.3 / 384.9 / 465.6
Colistin B	[M+3H]3+	386.1	101.0 / 374.5 / 380.2
Polymyxin B1	[M+3H]3+	402.1	101.2 / 390.5 / 396.1
Polymyxin B2	[M+3H]3+	397.2	385.6 / 391.8
Virginiamycin M1	[M+1H]1+	526.6	260.2 / 337.3 / 355.4 / 508.5
Enramycin A	[M+3H]3+	786.1	122.2 / 1089.5
Enramycin B	[M+3H]3+	790.7	122.2 / 1089.6

Results



XIC-Chromatogram of a standard mix, containing Bacitracin (A+B), Colistin (A+B), Polymyxin (B1+B2), Virginiamycin M1, Enramycin (A+B) and IS (200 µg/l each).



XIC-Chromatogram of a meat sample, spiked with Bacitracin (A+B), Colistin (A+B), Polymyxin (B1+B2), Virginiamycin M1 and IS (100 µg/kg each) and Enramycin (A+B) (500 µg/kg).

Conclusion

The method was validated in accordance with the requirements of the Commission Decision 2002/657/EC [3]. For Bacitracin and Colistin the Laboratory Performance Limit (LPL) was set to the half of the MRL, whereas Polymyxin B was handled like Colistin and the LPL for Virginiamycin was set to 20 µg/kg and to 35 µg/kg for Enramycin A. For all analytes the validated LOQ corresponds to these criteria. The validation for the matrix meat shows a good linearity of the method with a correlation coefficient R^2 greater than 0.995. A summary of the validation data for the matrix meat is shown in the table below.

Validation data for the matrix meat

	MRL (µg/kg)	LPL (µg/kg)	LOQ (µg/kg)	cca (µg/kg)	ccb (µg/kg)	Repeatability CV (%)	Within-laboratory reproducibility CV (%)	Trueness (%)
Bacitracin A	150		55	170	190	7	13	98
Bacitracin B	150		25	167	183	11	16	95
Colistin A	150		60	174	201	11	9	97
Colistin B	150		18	157	165	8.9	11	100
Polymyxin B1		150	67	166	221	9.6	15	96
Polymyxin B2		150	87	168	208	20	53	80
Virginiamycin M1		20	15	24	29	2	8	95
Enramycin A		35	42	42	51	6	11	104

References

[1] European Union Register of Feed Additives, pursuant to Regulation (EC) No 1831/2003, List of additives, released 20.12.2012.
[2] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits, Official Journal of the European Union (L15).
[3] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC), Official Journal of the European Union (L221/8).





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