Determination, Confirmation, and Quantification of Trace ß-Lactam Antibiotics in Milk by LC/MS/MS

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Application Brief

Food Safety

The ß-lactam antibiotics are widely used in veterinary medicine for the treatment and prevention of disease. This use can result in the presence of residues in milk and edible tissues which can lead to problems in the fermentation processes and to health problems for individuals who are hypersensitive to ß-lactams.

To protect the consumer, MRL values were laid down in EU regulation 2377/90. For milk these values range from 4 µg/kg for penicillin G, ampicillin, and amoxicillin to 30 µg/kg for dicloxacillin, cloxacillin, and oxacillin.

In practice, screening is performed by microbiological and immunological methods. In this way it is possible to give, for a positive sample, the specification of the group to which the residue belongs. Confirmation, however, has to be performed by an independent physicochemical technique. Therefore, a highly sensitive LC/MS/MS method was developed for the detection of penicillins, etc., in milk samples.

Highlights

- Ease of use for method optimization
- Good linearity $R^2 \geq 0.98$ in real milk samples
- Good separation to get almost all the compounds separated well within 9 minutes

Experimental

Sample Preparation

An aliquot of 2 mL of milk (half skimmed consumption milk or raw milk) was mixed with 4 mL of acetonitrile for protein precipitation. The sample was vortexed and then centrifuged for 10 minutes at 3500 rpm. After filtration, 20 µL of the supernatant was injected into the LC/MS/MS system.

LC Conditions

Instrument	Agilent 1200SL
Column	Agilent ZORBAX SB-C18, 2.1 × 150 mm, 3.5 µm (p/n 830990-902)
Mobile phase	A: Water/0.3% acetic acid B: Acetonitrile/0.3% acetic acid
Flow rate	0.3 mL/min
Gradient	0–2 min/A 90%; 2.01–8 min/A 35%; 8.01–9/A 5%
Column compartment temperature	30 °C
Stop time	9 min
Post time	6 min
Injection volume	10 µL

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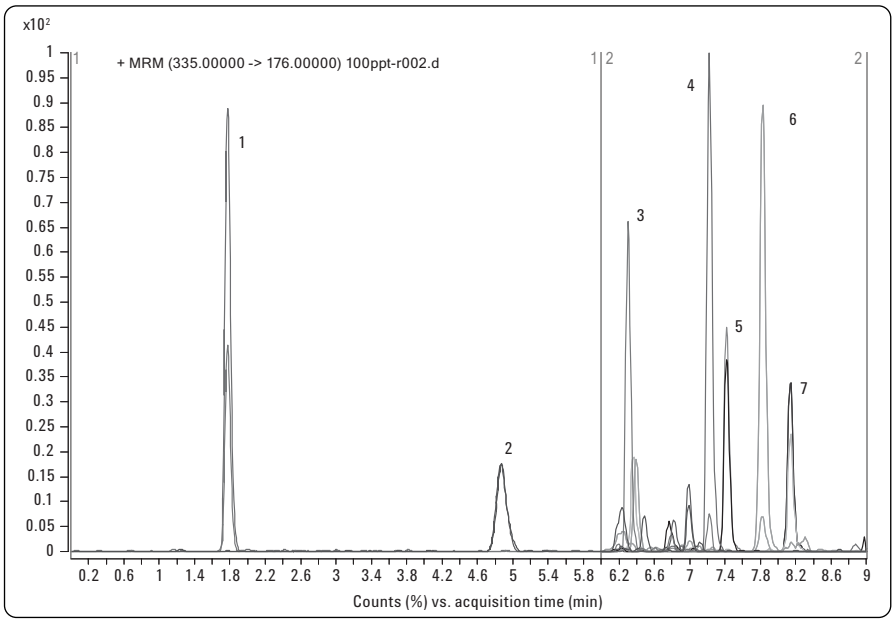
MS Conditions

Instrument	Agilent 6410A triple quadrupole LC/MS system
Source	ESI +
Drying gas temperature	350 °C
Drying gas flow	10 L/min
Nebulizer pressure	45 psi

MRM Setting

TS	Compound	Precursor	Product	Dwell (ms)	Frag (V)	CE (V)
0	Amoxicillin	366	114	100	110	15
			208	100	110	5
	Ampicillin	350	160	100	100	5
			192	100	110	10
6	Dicloxacillin	470	311	70	110	10
			160	70	110	10
	Nafcillin	415	256	70	110	15
			160	70	110	10
	Oxacillin	402	243	70	110	10
			160	70	110	10
	Penicillin V	351	192	70	100	5
			160	70	100	10
	Penicillin G	335	176	70	100	10
			160	70	100	5

Results



1. Amoxicillin
2. Ampicillin
3. Dicloxacillin
4. Nafcillin
5. Oxacillin
6. Penicillin V
7. Penicillin G

Compounds	S/N (Conc = 0.1 pppb)	R^2
Amoxicillin 366-114	224	0.992
Ampicillin 350-160	61.6	0.984
Dicloxacillin 470-160	48.5	0.981
Nafcillin 415-199	52.6	0.998
Oxacillin 402-160	70.9	0.993
Penicillin V 351-160	225.9	0.998
Penicillin G 335-160	33.2	0.981

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