



Brussels, **XXX**
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to the

Commission Delegated Regulation

**supplementing Regulation (EU) 2019/4 of the European Parliament and of the Council
by establishing specific maximum levels of cross-contamination of anti-microbial active
substances in non-target feed and methods of analysis for these substances**

ANNEX

Reference methods of analysis for the quantification of the levels of cross-contamination of antimicrobial active substances in feed in accordance with Article 3

Chemical class	Name of substance	CAS number ¹	EU number ²	Multi-analyte method ^{a,b,c}	Reference analytical method ^{3,4,5}	Maximum acceptable method quantification limit (LOQ) (µg/kg)
Polymixins (polypeptide antibiotics)	Colistin	1264-72-8	-	(b)	LSE - A - C - SPE - E - LC-MS/MS	150 (Colistin A) 300 (Colistin B)
Pyrimidine inhibitor of dihydrofolate reductase	Trimethoprim	738-70-5	212-006-2	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25
Macrolides	Tylvalosin	63409-12-1	-	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
	Tilmicosin	108050-54-0	639-676-2	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
	Tylosin	1401-69-0	215-754-8	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
Lincosamides	Lincomycin	154-21-2	205-824-6	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25

Pleuromutilins	Tiamulin	55297-96-6	259-580-0	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	10
	Valnemulin	101312-92-9	-	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	50
Penicillins	Amoxicillin	26787-78-0	612-127-4	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	150
	Penicillin V	1098-87-9	-	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	50
Aminoglycosides	Apramycin	65710-07-8	265-890-7 253-460-1	(a)	LSE - A - C - SPE - LC-MS/MS	50
	Neomycin	1404-04-2	1404-04-2	(a)	LSE - A - C - SPE - LC-MS/MS	50
	Paromomycin	1263-89-4	-	(a)	LSE - A - C - SPE - LC-MS/MS	50
	Spectinomycin	1695-77-8	-	(a)	LSE - A - C - SPE - LC-MS/MS	500
Amphenicols	Florfenicol	73231-34-2	642-986-0	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	150
	Tiamfenicol	15318-45-3	239-355-3	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	200
Coccidiostats	Amprolium	137-88-2	204-458-4	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
				(a)	LSE - A - C - SPE - LC-MS/MS	5

Fluoroquinolones	Flumequine	42835-25-6	255-962-6	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25
	Oxolinic Acid	14698-29-4	238-750-8	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25
Sulfonamides	Sulfamonomethoxine	1220-83-3	624-483-8	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25
	Sulfadimethoxine	122-11-2	204-523-7	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	25
Tetracyclines	Chlortetracycline	57-62-5	200-341-7	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
	Doxycycline	564-25-0	209-271-1	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
	Tetracycline	60-54-8	200-481-9	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100
	Oxytetracycline	79-57-2	-	(c)	LSE - US - A - C - F - LC-MS/MS or LC-HRMS	100

(1) Chemical Abstracts Service

(2) European Union number – not available for all substances

(3) Extraction methods:

- LSE – liquid solid extraction

- US – Ultrasonication
- A – Agitation

(4) Clean-up methods

- C – Centrifugation
- SPE – Solid Phase Extraction
- E – Evaporation, re-dissolution
- F – Filtration

(5) Analytical methods:

- LC-MS/MS – Liquid chromatography tandem mass spectrometry
- LC-HRMS – Liquid chromatography coupled to high resolution mass spectrometry

(a): multi-analyte method for the aminoglycosides and amprolium

(b): multi-analyte method for the polymixins colistin A and B

(c): multi-analyte method for trimethoprim, amprolium, the macrolides, the penicillins, the amphenicols, the fluoroquinolones, the sulphonamides and the tetracyclines