Residues

Definition

Pesticides

1. The designation pesticide applies to any substance or mixture of substances intended to prevent, destroy, or control any unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of pure articles. The designation includes substances intended for use as growth regulators, defoliants, or desiccants, and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.
2. Pesticides are commonly used in agriculture. Pesticides may stay in small amounts (called residues) in or on fruits, vegetables, grains, and other foods. To make sure the food is safe for consumption, official bodies like the United States Environmental Protection Agency (EPA) regulates the amount of each pesticide that may remain in and on foods.
3. Pesticides are categorized into four main substituent chemicals: herbicides; fungicides; insecticides and bactericides.

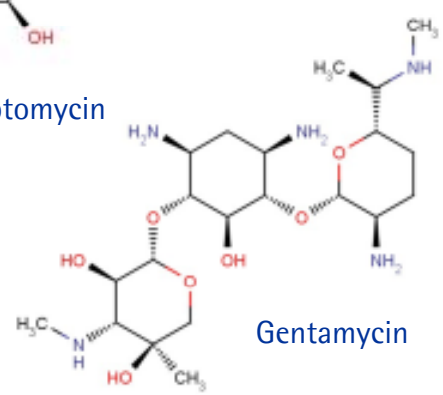
Antibiotics

1. During their lifetime animals may have to be treated with different medicines for prevention or cure of diseases. In food producing animals such as cattle, pigs, poultry and fish this may lead to residues of the substances used for the treatment in the food products derived from these animals (e.g. meat, milk, eggs). The residues should however not be harmful to the consumer. To guarantee a high level of consumer protection, legislation requires that the toxicity of potential residues is evaluated before the use of a medicinal substance in food producing animals is authorized. If considered necessary, maximum residue limits (MRLs) are established and in some cases the use of the relevant substance is prohibited.

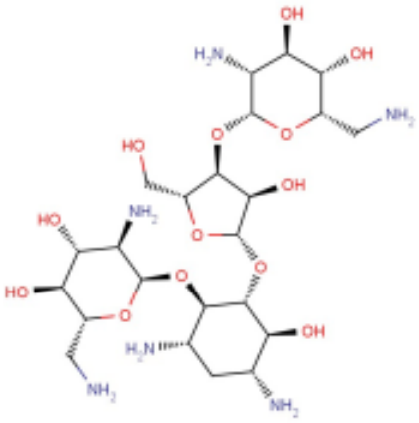
Further reading on pesticide and antibiotic residues:
http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/default.htm
http://www.epa.gov/pesticides/index.htm
http://www.agf.gov.bc.ca/pesticides/
http://ec.europa.eu/food/food/index_en.htm
http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm
http://ec.europa.eu/sanco_pesticides/public/index.cfm
http://en.wikipedia.org/wiki/Pesticide

Aminoglycosides

Streptomycin



Gentamycin



Neomycin

Aminoglycosides are bactericidal antibiotics which have amino-modified sugar in their molecules. This particular group of antibiotics is widely used as clinical and veterinary medicines to treat infections caused by gram-negative or some gram-positive bacteria, and are classified as bactericidal agents because of their interference with bacterial replication. However, these antibiotics can also cause varying degree of ototoxicity and nephrotoxicity.

Overuse of antibiotics and exposure from the animal food are the two major routes attributed to the antibiotic resistance. Therefore, it is important to develop sensitive and reliable analytical methods for determining and monitoring aminoglycosides residuals in different sample matrices. Aminoglycosides are normally very hydrophilic and carry several amino groups, which mean they are very positively charged at neutral pH condition.

In this compilation, an application for determination of streptomycin, gentamycin, and neomycin illustrate the benefit of combining HILIC with mass spectrometric detection.

Determination of Streptomycin, Gentamycin, and Neomycin Using HILIC-MS

SeQuant® ZIC®-HILIC

Column:	SeQuant® ZIC®-HILIC (3.5 µm, 100Å) PEEK 100×2.1 mm	(1.50441.0001)

Recommended solvents and reagents

Acetonitrile:	Hypergrade for LC-MS LiChrosolv®	(1.00029)
Water:	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q® water purification system	(1.15333)
Ammonium acetate:	for analysis EMSURE® ACS,Reag. Ph Eur	(1.01116)
Formic acid:	98-100% for analysis EMSURE® ACS,Reag. Ph Eur	(1.00264)

Recommended filtration tools

Mobile phase filtration:

PTFE coated with funnel, base, stopper clamp	(XX1004720)
Omnipore PTFE membrane filter 0.45µm	(JHWP04700)

Determination of Streptomycin, Gentamycin, and Neomycin Using HILIC-MS

SeQuant® ZIC®-HILIC

Column: SeQuant® ZIC®-HILIC (3.5 µm, 100Å) PEEK 100x2.1 mm (1.50441.0001)

Mobile phase A: Acetonitrile and formic acid (99:1)
B: Ammonium acetate (100 mM) and 3% formic acid (100%)

Time (min)	Solution A (%)	Solution B (%)	Elution
0	50	50	isocratic
4-8	50→5	50→95	gradient
8-16	50	50	Equilibration

Flow rate: 0.4 mL/min

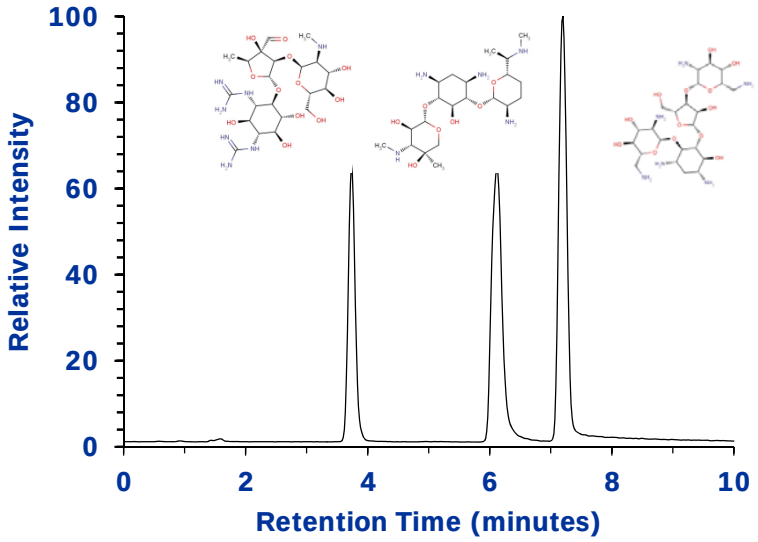
Detection: Shimadzu LCMS-2010EV; ESI in positive mode; Heat block and CDL temperature 250 ℃;
Spray gas: nitrogen at 1.5 L/min; Detector voltage: 2 kV.
SIM in positive mode: m/z 582 (Streptomycin), 464 (Gentamicin), and 615 (Neomycin)

Determination of Streptomycin, Gentamycin, and Neomycin Using HILIC-MS

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (3.5 µm, 100Å) PEEK 100x2.1 mm	1.50441.0001
Injection:	5 µL	
Detection:	Shimadzu LCMS-2010EV; ESI in positive mode; Heat block and CDL temperature 250 ℃; Spray gas: nitrogen at 1.5 L/min; Detector voltage: 2 kV. SIM in positive mode: m/z 582 (Streptomycin), 464 (Gentamycin), and 615 (Neomycin)	
Mobile Phase (v/v):	A: Acetonitrile and formic acid (99:1) B: Ammonium acetate (100 mM) and 3% formic acid (100%)	
Flow Rate:	0.4 mL/min.	
Gradient:	See Table.	
Temperature:	50 °C	
Diluent (v/v):	Acetonitrile/Milli-Q® water (30:70)	
Sample:	5 µg/mL STR, 25 µg/mL GEN and NEO in diluent	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (USP)
1	Streptomycin (STR)	3.7	1.1
2	Gentamycin (GEN)	6.1	1.2
3	Neomycin (NEO)	7.2	1.1

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/chromatography
chromatography@merckgroup.com