Determination of 14 aminoglycosides in foodstuffs by LC-MS/MS using molecularly imprinted polymer solid phase (SPE)

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Aminoglycosides are an important class of antibiotics frequently used to treat bacterial infections. In veterinary medicine, aminoglycosides are indicated to treat various infections in cattle, pigs, sheep, goats or horses. The presence of aminoglycosides in foodstuffs represents a risk to consumer health and is thus regulated through the enforcement of maximum residue limits (MRLs).

This poster describes the screening of 14 aminoglycosides in foodstuffs of animal origin using the Unspiked-Spiked approach. The method includes an extraction under acidic aqueous conditions followed by a selective clean-up using AFFINIMIP® SPE Aminoglycosides, a molecularly imprinted polymer - solid phase extraction (MIP-SPE) cartridge. Analytes are detected by LC-MS/MS. This procedure is part of the AOAC Official Method – Final Action 2020.04 [1].

Screening of 14 Aminoglycosides using AFFINIMIP® SPE Aminoglycosides

The Unspiked-Spiked approach

The unspiked-spiked approach is a confirmatory screening method of aminoglycosides in various matrices. The method allows the rapid identification of suspect samples and the validation of negative samples.

Each sample is extracted twice (one as such, and one spiked at a screening target concentration (STC) with 14 aminoglycosides) and analyzed. Each sample's matrix effect is therefore considered to give reliable results. An area ratio measured and is compared with a cut-off value determined during upstream validation.

Advantages of the Unspiked-Spiked approach :

- Quick and simple control of the presence/ absence of aminoglycosides in foodstuffs.
- Circumvent unpredictable matrix effects and improve response stability
- Helpful for testing new matrices

① Sample extraction

1. Weigh two 2.00 ± 0.05 g test portions into two separate 50mL polypropylene tubes. The first test portion is left as such (**= unspiked test portion**). The second test portion is spiked at the STC level (**= spiked test portion**).
2. Add 0.5mL of 0.5% EDTA aqueous solution, a ceramic homogenizer, 20mL of 2% trichloroacetic acid aqueous solution.
3. Shake at 1500rpm for 3 min (Until total dispersion of the sample).
4. Centrifuge at 4000 x g at room temperature for 10 min.
5. Transfer 4mL of the supernatant into a new 50mL polypropylene tube and add 4mL of ammonium carbonate 80mM in water. Vortex for a few seconds.
6. Centrifuge at 4000 x g at room temperature for 10 min.

Table : Maximum residue limits (MRL) and STC validation data[1]

Analyte	MRL (µg/Kg)	STC (µg/Kg)
Amikacin	-	50
Apramycin	50	50
Dihydro-streptomycin	200 & 600	50
Gentamicin C1	50 & 100	13.5
Gentamicin C1a		12.5
Gentamicin C2 + C2a		24
Hygromycin B	-	50
Kanamycin A	100 & 150	50
Neomycin B	150 & 500	50
Paromomycin	500	50
Sisomycin	-	50
Spectinomycin	100 to 500	50
Streptomycin	200 & 600	50
Tobramycin	-	50

② SPE protocol

Unspiked test portion | **Test portion spiked at 1x STC level**

SPE protocol with AFFINIMIP® Aminoglycosides

Conditionning
1. 1 mL methanol
2. 1 mL 50mM potassium phosphate pH = 7

Loading
3 mL of sample extract

Washing
1. 3 mL ultrapure water
2. 3 mL water/acetonitrile (60+40, v/v)
3. 1 mL Dichloromethane/methanol (50+50, v/v)
4. *Apply full vacuum for 30 seconds*

Elution
1 mL 30mM HFBA in Water/acetonitrile(75+25 v/v)

Analyze by LC-MS/MS in polypropylene vials.

③ Analysis by LC-MS/MS

Table : LC-MS/MS conditions used for the analysis of the 14 aminoglycosides

LC Agilent 1200 series

Delay column : SecurityGuard ULTRA Cartridges UHPLC C18 2.1mm ID
Column : Kinetex® 2.6µm C18 100A column 100*2.1mm at 40°C
Injection : 20µL **Flow rate :** 0.4mL/min

Time (min)	20mM HFBA (in water)	Acetonitrile
0	78%	22%
0.5	78%	22%
2	60%	40%
4	60%	40%
4.5	5%	95%
6	5%	95%
6.1	78%	22%
8	78%	22%

API 5000 Sciex ESI+ MS/MS

Curtain gas : 20 **/ CAD :** Medium / **IS :** 4500V
Temperature : 600°C / **GS1/GS2 :** 40/40

Analyte	Time (min)	Q1/Q3 (m/z)	CE (V)	Analyte	Time (min)	Q1/Q3 (m/z)	CE (V)
Streptomycin	1.89	582.2/263.3	46	Gentamicin C1a	3.31	450.3/322.4	21
	1.89	582.2/246.1	55		3.31	450.3/160.2	33
Hygromycin B	1.72	528.2/177.1	42	Gentamicin C2 & C2a	3.35	464.4/322.3	21
	1.72	528.2/352.2	34		3.35	464.4/160.2	32
Dihydro-streptomycin	1.94	584.4/263.4	44	Paromomycin	3.17	616.3/163.2	49
	1.94	584.4/246.3	54		3.17	616.3/293.2	34
Spectionmycin	1.29	351.2/97.9	43	Neomycin B	3.43	308.4/161.1	18
	1.29	351.2/207.2	32		3.43	308.4/163.2	28
Kanamycin A	2.78	485.3/163.1	37	Amikacin	2.68	293.7/163.1	28
	2.78	485.3324.4	25		2.68	293.7/264.3	20
Apramycin	3.17	540.3/217.2	39	Sisomicin	3.29	448.3/254.2	32
	3.17	540.3/378.2	26		3.29	448.3/271.2	27
Gentamycin C1	3.37	478.5/157.3	30	Tobramycin	3.23	468.3/163.1	34
	3.37	478.5/322.5	22		3.23	468.3/324.2	23

Interpretation of the Results

After analysis, a ratio between the areas of the two portions is calculated for each analyte using the following formula :

$$\text{Ratio} = \frac{\text{Area unspiked}}{\text{Area 1xSTC}}$$

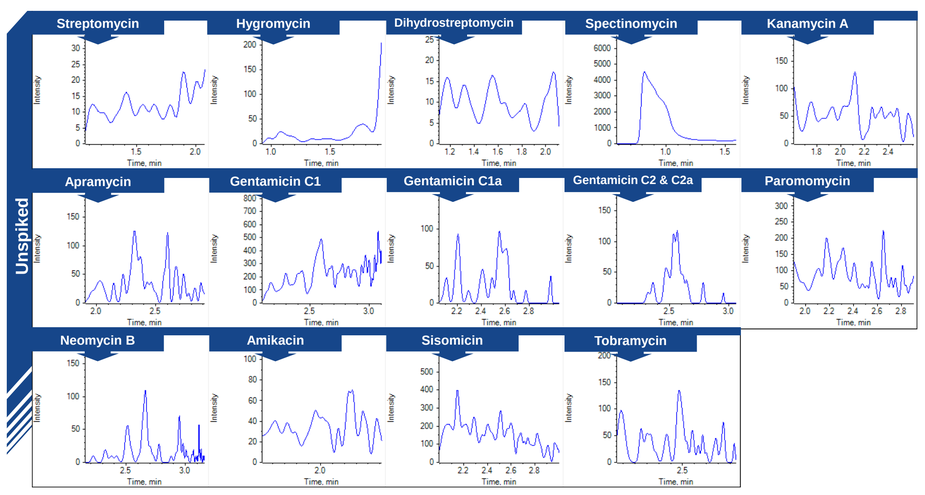
The ratio is compared with a cut-off value (that was determined during validation)

- **Negative :** Ratio below cut-off value
- **Suspect :** Ratio above cut-off value

The procedure was validated according to the European Community Reference Laboratories Residues Guidelines giving false-negative and false-positive rates ≤3% for the 14 aminoglycosides (validation by two laboratories on 148 samples)

Some of the matrices tested

Hydrolyzed infant formula, Skimmed milk powder, Fermented whole milk powder, Infant formula, Chicken meat powder, Chicken fat, Chicken cube, Honey, White chocolate



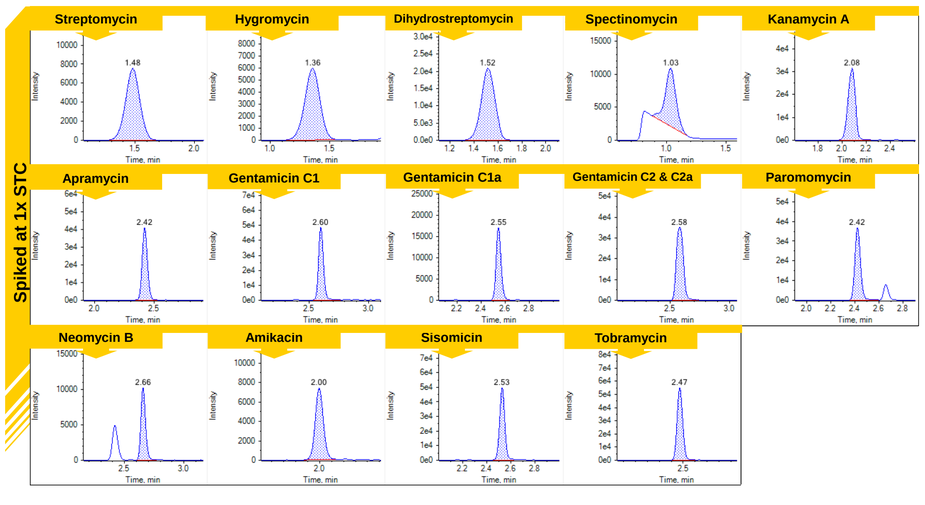


Figure : LC-MS/MS chromatograms obtained in unspiked (Up) and 1xSTC level spiked (down) skimmed milk powder using AFFINIMIP® SPE Aminoglycosides

CONCLUSION

The MIP-SPE clean-up using AFFINIMIP® SPE Aminoglycosides allows the monitoring of the 14 aminoglycosides from raw material to finished products composed of various matrices (milk, meat, fish, seafood, egg and/or fat).
For each analyte/matrix combination, the STC value is set at 50µg/Kg which is fully compliant with worldwide regulatory limits.
The procedure was validated according to the European Community Reference Laboratories Residues Guidelines giving false-negative and false-positive rates ≤3% for the 14 aminoglycosides (validation by two laboratories on 148 samples). This procedure is included in the AOAC Official Method – Final Action 2020.04.

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References :

1. Desmarchelier et Al (2024) Screening of 152 Veterinary Drug Residues in Animal Source Foods by LC–MS/MS, Multilaboratory Validation Study: Final Action 2020.04. *Journal of AOAC INTERNATIONAL*, 2024, 1-15.