Simultaneous quantification of aminoglycoside antibiotics in milk using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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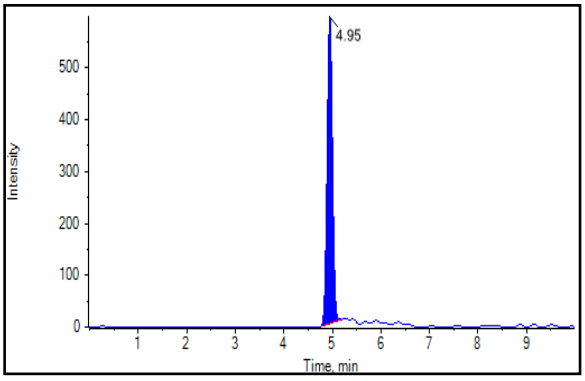
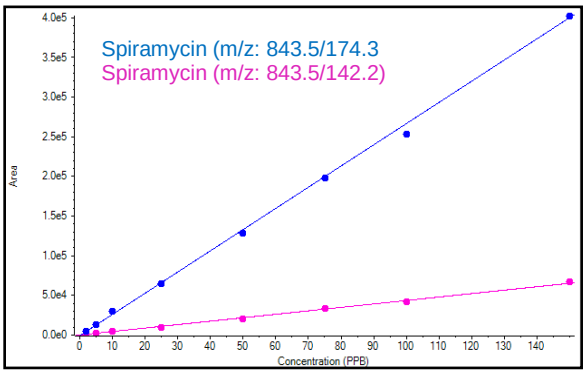
Monitoring of veterinary drug residues is mandatory in all regions to ensure food safety. Aminoglycosides (AGs) are broad spectrum antibiotics that have been extensively used in both human and veterinary medicine. The European commission and Export Indian Council had described maximum residue levels (MRLs) for aminoglycosides in milk and its products. The presence of AGs substances above the MRL concentration in a food may cause a risk to consumer health.

To measure AGs residues in milk (Spiramycin, Kanamycin, Neomycin) a targeted quantification method in milk matrix using the SCIEX Triple Quad 3500 LC-MS/MS system was developed.





Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
SPIRAMYIN_01	2.00	6 of 6	5.493e3	2.762e2	5.03

Figure 1. Quantification of Spiramycin at 2 ppb. (Top) Chromatogram of Spiramycin at 2 ppb in extracted milk sample. (Middle) Concentration curve from 2 – 150 ppb showed very good linearity, r = ≥0.99. MRL is 200 ppb. (Bottom) Reproducibility for quantification of Spiramycin at 2 ppb in extracted milk samples (LOQ), 5.03% CV for 6 replicates.

AGs residues were ionized using electrospray ionization in positive polarity using the Turbo V™ Ion Source. Two MRM transitions per analyte were used for confidence in detection.

Key features of targeted quantification method for pesticide residues in milk

- SCIEX Triple Quad 3500 System achieved a quantitative and qualitative determination at ~100x below the MRL level
- A simple sample preparation procedure was developed to extract the AGs residues from complex milk matrix
- Good linearity was found for all three analytes in extracted milk matrix
- Automatic MRM ratio calculation in MultiQuant™ Software was used for the confirmation of compounds
- The method performed in accordance with 2002/657/EC directive commission

Methods

Sample Preparation: All aminoglycosides (AGs) standards were purchased from Sigma Aldrich ≥99% Purity. The LC-MS grade chemicals and solvents were used, which were commercially available in market. Milk samples were collected from local markets of Delhi and Gurgaon, India and stored at 2−8 °C until end of the analysis.

1 mL milk sample was mixed with 0.4 mL of 10 % of TCA and vortexed followed by centrifugation at maximum rpm. The supernatant was collected and filtered using a 0.22 micron filter. The filtered samples were transferred into autosampler vials for further analysis.

Chromatography: LC separation was performed using a Shimadzu Nexera XR System and a Phenomenex Luna C18 (2) column (3.0 x 150 mm, 100 Å, 3 µm). A 20 µL injection volume was used. The gradient flow program is shown below in Table 1.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.00	*800*	*90*	*10*
6.00	*800*	*5*	*95*
7.50	*800*	*5*	*95*
8.00	*800*	*90*	*10*
10.00	*800*	*90*	*10*

Mobile phase A: water + 0.2% HFBA
Mobile phase B: acetonitrile

Mass spectrometry: The SCIEX Triple Quad 3500 System was operated in multiple reaction monitoring (MRM) mode. The Turbo V™ Ion Source was used with an electrospray ionization (ESI) probe in positive ionization mode using an ion spray voltage of 5000V. Two selective MRM transitions were monitored by software for compound identification. Analyst® Software 1.6.2 was used for method development and data acquisition. The MRM transitions quantifier and qualifier of AGs residues were showed in Table 2.

Table 2. MRM transitions of aminoglycosides residues.

Compound	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)
Spiramycin	*843.5*	*174.3*	*142.2*
Kanamycin	*485.0*	*163.0*	*324.2*
Neomycin	*615.3*	*161.3*	*293.3*

Data processing: LC-MS/MS data was processed using the MultiQuant™ Software 3.0.2. Peak integration, computation of concentration curves, and ion ratios for the confirmation of analytes was all done using MultiQuant Software.

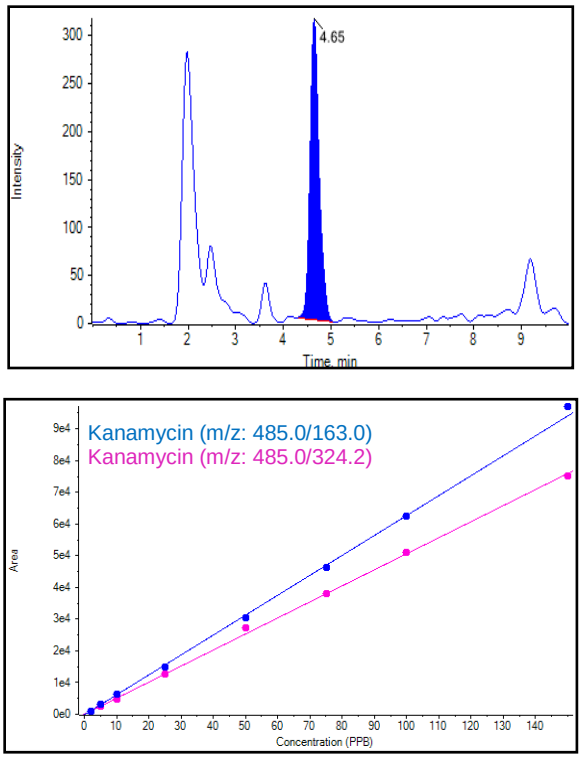
Results

A partial validation procedure was conducted in accordance with 2002/657/EC to develop a method for the quantification of aminoglycosides in milk extract. The major objective of the work was to develop a simple, fast and robust sample preparation based on buffer extraction that did not require any clean-up step. The matrix-based linearity for spiramycin (2.0 to 150 ppb), kanamycin and neomycin (3.0 to 100 ppb) was assessed and the regression coefficient was ≥ 0.99 using $1/x^2$ was used as a weighing factor (Figure 1-3). The recovery for each analyte was found to be in the range of 90 to 110% and the repeatability %CV was less than 10% (n=6) at LOQ level for each AG analytes.

Next, the developed method was tested on different milk samples collected from a local market (Delhi, Gurgaon in India), and no concentration of AGs antibiotic residues were found at the analyte retention time (Table 3).

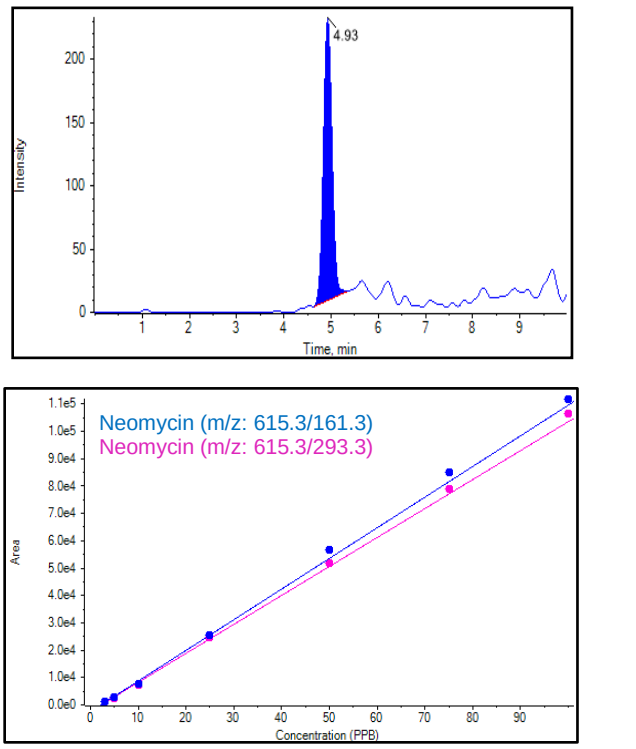
Table 3. %CV and % recovery at LOQ level (n=6) in extracted samples.

Compound	LOQ (ppb)	%CV at LOQ (n=6)	% Recovery at LOQ (n=6)
Spiramycin	*2.00*	*5.03*	*108.75*
Kanamycin	*2.00*	*5.14*	*109.27*
Neomycin	*5.00*	*2.37*	*97.20*



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
KANAMYCIN_01	2.00	6 of 6	1.302e3	6.701e1	5.14

Figure 2. Quantification of kanamycin at 5 ppb. (Top) Chromatogram of kanamycin at 2 ppb (quantifier ion) and 5 ppb for qualifier in extracted milk sample. (Middle) Concentration curve from 5 – 150 ppb showed very good linearity for both the transitions, r ≥0.99. MRL is 150 ppb. (Bottom) Reproducibility for quantification of kanamycin at 2 ppb in extracted milk samples (LOQ), 5.14% CV for 6 replicates.



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
NEOMYCIN_01	5.00	6 of 6	3.040e3	7.197e1	2.37

Figure 3. Quantification of neomycin at 5 ppb. (Top) Chromatogram of neomycin at 5 ppb in extracted milk sample. (Middle) Concentration curve from 5 – 100 ppb showed very good linearity, r ≥0.99. MRL is 1500 ppb. (Bottom) Reproducibility for quantification of neomycin at 5 ppb in extracted milk samples (LOQ), 2.37% CV for 6 replicates.

Conclusions

The method provided sensitive and accurate results for the quantification of aminoglycosides antibiotic residues in milk samples by the SCIEX Triple Quad 3500 System. The method developed as per 2002/657/EC directive recommendations which showed an acceptable recovery (90-110%). The correlation coefficient r ≥0.99 for both the transitions (quantifier and qualifier ions), repeatability %CV (n=) was ≤10 for all analytes at LOQ level. The method allows high throughput, rapid and sensitive LC-MS/MS identification and simultaneous quantification of aminoglycosides residues at levels ~100x below the MRL levels of these analytes.

References

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