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Application News

No. C175

LC/MS

Fast Quantitative Analysis of Aminoglycoside Antibiotic Residues in Meat, Eggs and Milk and Identity Confirmation with MRM Spectrum Mode

Aminoglycoside (AGs) are an antibiotic family widely used for the treatment of bacterial infections in cattle, sheep, pigs and poultry. They have a broad-spectrum activity and are used against Gram-positive and Gram-negative bacteria.

AGs possess oto- and nephrotoxicity which did not hinder the widespread use of AGs in veterinary applications because of their low cost.

Due to their high affinity for tissues, They may occur in meat, milk or eggs if the withholding period has not been observed or if used improperly. Therefore, eating food containing aminoglycosides can be potentially hazardous for human health.

Regulatory agencies have set maximum residue limits (MRL) for these compounds with veterinary use.

Aminoglycosides are very polar compounds poorly retained by reversed-phase liquid chromatography. Ion-pairing reagents are not desirable as they can easily contaminate the analytical system and interfere in other methods.

A Method Package has been developed to overcome these problems. It comprises a protocol to generate clean extracts in a variety of commodities and a rapid quantitative method using hydrophilic interaction liquid chromatography (HILiC) combined with triple quadrupole mass spectrometry detection. When necessary, a second method for formal peak identification using MRM Spectrum Mode can be applied without changing reagents.

In this document, we report the use of the method package to assess the safety level of several meat samples and milk.

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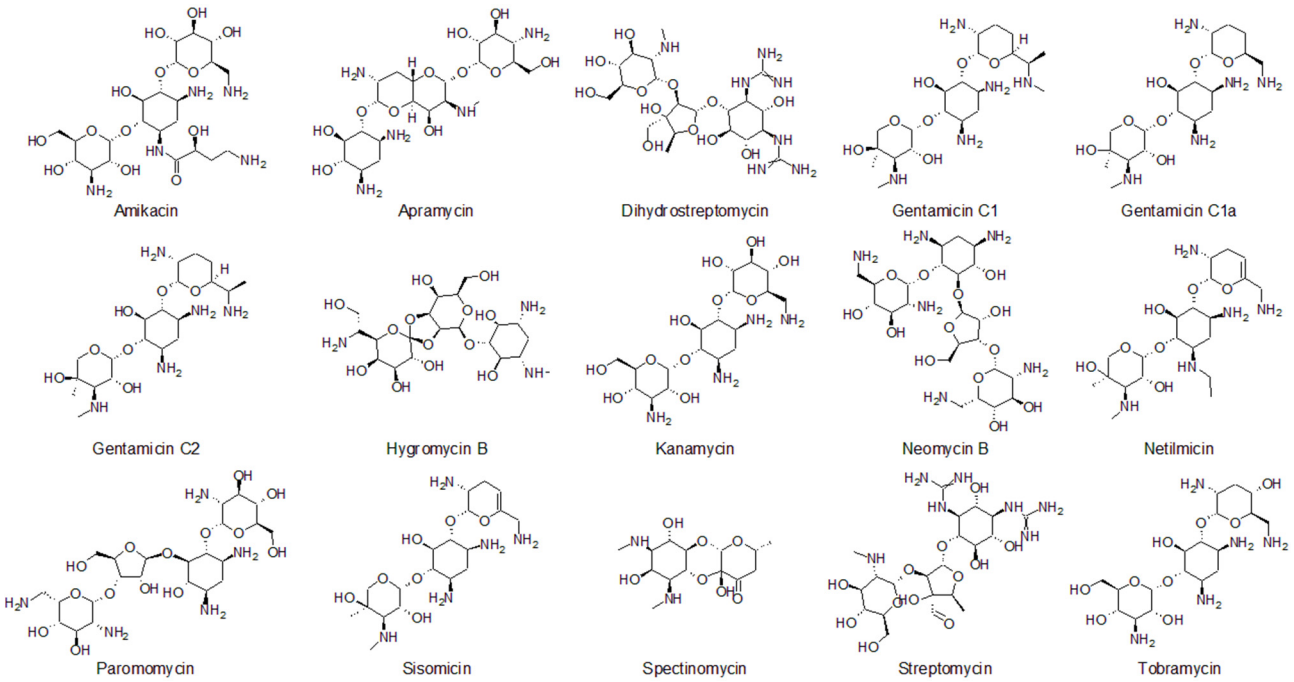

Fig. 1 Targeted aminoglycosides

Sample Preparation

Meat samples (Kobe style beef muscle, chicken breast and liver, pork cutlet) and cow milk were purchased from local supermarket. After grinding, 5 g of sample were treated as described in Method Package. Briefly, after addition of internal standard (Ribostamycin), compounds were extracted twice with acidic buffer. Extracts were then purified by weak-cation exchange and diluted by a factor of 5 before injection (5 µL). Each sample was also spiked at 0.5 times and 1.5 times the MRL defined by Japanese Ministry of Health, Labour and Welfare.

All samples were prepared once except the beef sample spiked at 0.5 × MRL, which was prepared in 6 replicates.

LC-MS/MS Analysis

Purified extracts were assayed using LC-MS/MS conditions and ready-to-use methods included in the Method Package. A calibration curve prepared in mobile phase was used to quantify samples.

Samples were first assayed using a fast quantitative method. This method use HILiC conditions to elute compounds with a gradient of acetonitrile and a formate buffer. Cycle time for analysis is 4.5 minutes. Detection was performed in Multiple Reaction Monitoring (MRM) mode with 2 transitions acquired per compound.

For positive samples (i.e. over the MRL), a second injection of purified extracts was performed to assess peak identity. For this purpose, a second method with same column and mobile phases but alternative gradient and 15 MRM per compound (except ISTD) was used.

The analytical system was a Nexera™ X2 UHPLC coupled with LCMS-8060 triple quadrupole mass spectrometer. Data processing was made with LabSolutions Insight™ v.3.1 with Screening option.

Results

Depending on the species and commodities, MRL are different. According to current rule in Japan, if no MRL has been officially defined for a veterinary drug residue, a 'default' MRL of 10 µg/kg should be considered for any chemical tested. Then, for Apramycin, Dihydrostreptomycin, Gentamicin, Kanamycin, Neomycin, Spectinomycin and Streptomycin, the calibration range was set to cover from 10 % of the lowest MRL to 150 % of the highest one. For other compounds without official MRL, the calibration range was set from 20 % to 150 % of 10 µg/kg. Calibration values can be found in Table 1. Seven calibration levels, regularly dispatched within the range were prepared. Calibration standards with an accuracy within 85 - 115 % were selected. Representative calibration curves are shown in Fig. 2.

Samples without spiking revealed to be free of aminoglycoside residues. Then recovery was calculated in spiked samples using the calculated concentrations. Results can be seen in Table 2. Recoveries were in the acceptable range of 70 - 120 % for all compounds and all type of samples. Repeatability have been assessed in beef sample spiked at 0.5 × MRL. Results are presented in Table 3. The % RSD was less than 20 % which is suitable for such application.

Mass chromatograms example is presented in Fig. 3.

Table 1 Maximum residue limits in Japan for the selected samples and corresponding calibration ranges

			Calibration Range			
	Low MRL (µg/kg)	High MRL (µg/kg)	LLOQ (µg/kg)	LLOQ (ng/mL)	ULOQ (µg/kg)	ULOQ (ng/mL)
Amikacin	No value	Default (10)	2	0.1	15	0.75
Apramycin	60	500	6	0.3	750	37.5
Dihydrostreptomycin	200	600	20	1.0	900	45.0
Gentamicin (sum)	100	200	10	0.5	300	15.0
Hygromycin	No MRL	Default (10)	2	0.1	15	0.75
Kanamycin	40	500	4	0.2	750	37.5
Neomycin	500	500	50	2.5	750	37.5
Netilmicin	No MRL	Default (10)	2	0.1	15	0.75
Paromomycin	No MRL	Default (10)	2	0.1	15	0.75
Sisomicin	No MRL	Default (10)	2	0.1	15	0.75
Spectinomycin	200	2000	20	1.0	3000	150.0
Streptomycin	200	600	20	1.0	900	45.0
Tobramycin	No MRL	Default (10)	2	0.1	15	0.75

Table 2 Calculated recoveries in spiked samples

		AMI	APRA	DHSTP	GENT C1a	GENT C1	GENT C2/C2a	HYGRO	KANA
Recovery at 0.5 × MRL	Milk	91.9 %	88.7 %	108 %	76.6 %	89.4 %	83.3 %	94.3 %	100 %
	Beef	107 %	89.0 %	117 %	90.4 %	94.2 %	95.2 %	107 %	102 %
	Pork	88.3 %	98.9 %	114 %	80.4 %	86.3 %	87.6 %	96.5 %	88.7 %
	Chicken Breast	82.2 %	90.3 %	97.4 %	98.7 %	92.4 %	90.3 %	105 %	94.8 %
	Chicken Liver	70.9 %	91.5 %	103 %	91.3 %	80.8 %	86.1 %	99.4 %	101 %
Recovery at 1.5× MRL	Milk	83.0 %	99.0 %	106 %	85.8 %	91.0 %	101 %	91.8 %	98.1 %
	Beef	89.9 %	95.9 %	96.9 %	98.8 %	91.2 %	95.5 %	104 %	96.1 %
	Pork	86.3 %	89.5 %	98.5 %	95.1 %	102 %	96.9 %	112 %	97.2 %
	Chicken Breast	82.2 %	90.3 %	97.4 %	98.7 %	92.4 %	90.3 %	105 %	94.8 %
	Chicken Liver	87.8 %	90.7 %	90.7 %	99.5 %	85.5 %	88.8 %	91.6 %	83.8 %

		NEO	NETIL	PARO	SISO	SPC	STP	TOB
Recovery at 0.5 × MRL	Milk	81.2 %	101 %	73.3 %	75.3 %	94.0 %	111 %	91.0 %
	Beef	91.4 %	101 %	88.1 %	88.4 %	110 %	114 %	91.5 %
	Pork	85.7 %	91.0 %	90.7 %	76.4 %	101 %	111 %	85.8 %
	Chicken Breast	94.1 %	90.5 %	78.4 %	84.9 %	92.7 %	102 %	107 %
	Chicken Liver	78.6 %	90.8 %	76.5 %	78.8 %	101 %	108 %	92.5 %
Recovery at 1.5 × MRL	Milk	96.7 %	93.6 %	86.9 %	99.4 %	94.8 %	105 %	102 %
	Beef	113 %	91.1 %	103 %	106 %	86.9 %	93.1 %	105 %
	Pork	106 %	90.4 %	94.8 %	94.3 %	95.2 %	105 %	108 %
	Chicken Breast	94.1 %	90.5 %	78.4 %	84.9 %	92.7 %	102 %	107 %
	Chicken Liver	109 %	82.4 %	89.5 %	95.3 %	75.3 %	90.0 %	98.1 %

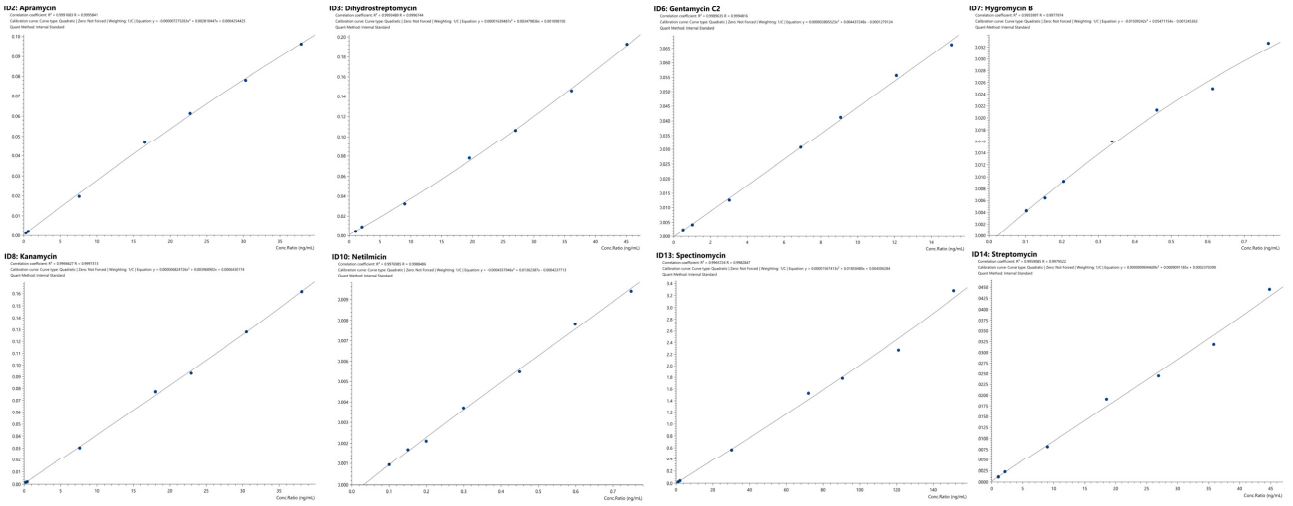

Fig. 2 Representative calibration curves

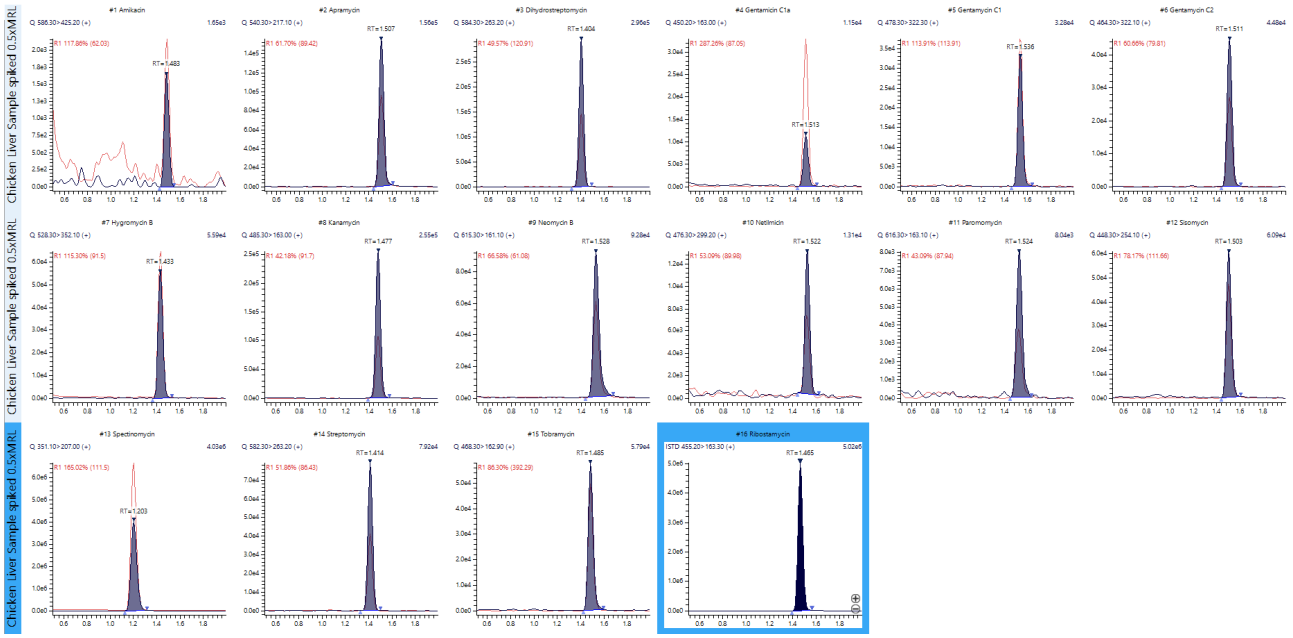

Fig. 3 Chicken liver sample spiked at 50 % of the MRL for each compound

Table 3 Repeatability in beef sample at 0.5× MRL

	AMI	APRA	DHSTP	GENT C1a	GENT C1	GENT C2/C2a	HYGRO	KANA
Mean Conc. (µg/kg)	5.38	225	350	45.6	47.5	48.0	5.32	21.1
Recovery	107 %	89.0 %	117 %	90.4 %	94.2 %	95.2 %	107 %	102 %
%RSD	19.9 %	7.7 %	10.0 %	10.8 %	10.2 %	6.9 %	7.1 %	12.0 %

	NEO	NETIL	PARO	SISO	SPC	STP	TOB
Mean Conc. (µg/kg)	228	5.03	4.39	4.47	275	348	4.66
Recovery	91.4 %	101 %	88.1 %	88.4 %	110 %	114 %	91.5 %
%RSD	8.8 %	10.0 %	8.1 %	4.4 %	11.0 %	11.9 %	6.2 %

Results (continued)

For increased confidence in identification of compounds exceeding the MRL, additional injection of the extracts can be done using a second method with elongated gradient time and acquisition of 15 MRM transitions per compound. MRM signals are then merged to create a spectrum in which every fragment is acquired at optimum collision energy.

An example of search result by LabSolutions Insight with Screening option was illustrated below (Fig. 4). The samples can be processed and the library search can be automatically done in batch mode. In this case, high identification score can be obtained. Dihydrostreptomycin got a score of 95 while the second hit (Streptomycin, a very close compound) got a score of only 51.

Conclusion

A newly developped Method Package was succesfully applied to real meat and milk samples. The quantitative method gave good recoveries and accuracies, even for non-regulated compounds at trace levels. It can be applied to a variety of samples without using matrix-matched calibration curves.

A complementary method gives increased confidence in identification for over-the-limit compounds using MRM Spectum mode.

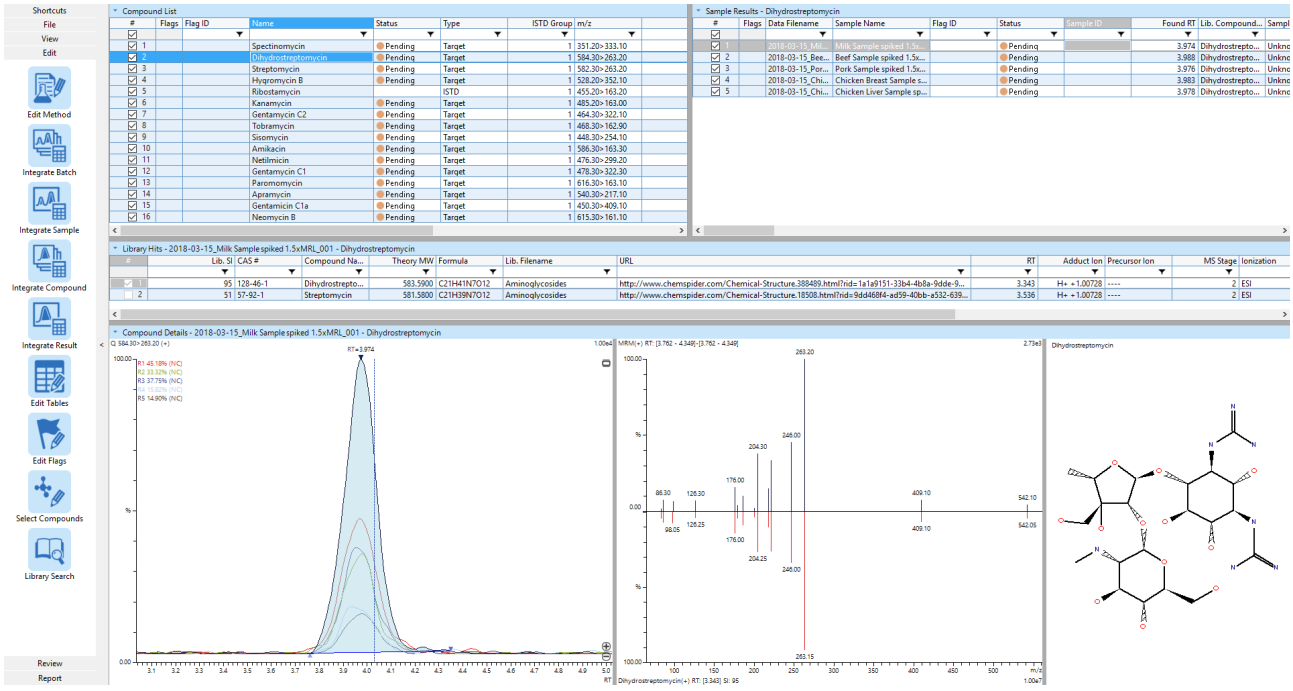

Fig. 4 Library search result of dihydrostreptomycin MRM spectrum in milk sample spiked at 1.5× MRL

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