Analysis of Streptomycin and its Metabolite in Milk Using the SCIEX Triple Quad™ 3500 System

Santosh Kapil.G[1]; Chandrasekar[1]. M; Aman Sharma[1]; Anoop Kumar[1]; Manoj G Pillai[1] & Jianru Stahl-Zeng[2]
[1]SCIEX, Udyog Vihar, Phase IV, Gurgaon - 122015, Haryana, India; [2]SCIEX, Darmstadt, Germany

Overview

A Liquid chromatography tandem mass spectrometry (LC-MS/MS) method for quantification of streptomycin and dihydrostreptomycin residues in milk was developed. A simple sample preparation was followed by the LC-MS/MS analysis. The method presented adequate linearity with correlation coefficients above r≥0.99 for both analytes in the dynamic range of 10–1000 ng/ml, with average accuracies for matrix based recovery calibration was between 85–105%. Method selectivity was verified by the absence of interfering peaks in the retention time of the analytes, the results qualified the method for the quantification and confirmation of the analytes in milk at concentrations inferior to the established Maximum Residue limit (200ng/ml).

Introduction

Antimicrobial agents are widely used in dairy cattle management. Improper administration for disease therapy and as growth promoting agents can result in antibiotic residues in milk and dairy products and can contribute to the development of microbial drug resistance and the spread of resistant bacteria, including those with serious health consequences in animals. Aminoglycosides like Streptomycin and dihydrostreptomycin are protein synthesis inhibitors. These are widely used in veterinary medicine for the treatment of gram-negative bacterial infection in clinical and sub-clinical mastitis in cattle. These are administered with combination of penicillin and tetracycline. The Maximum Residual limit (MRL) for streptomycin and dihydrostreptomycin in milk was 200ng/ml (1). Due to the harmful effects of veterinary medicinal residues, surveillance systems are enforced in the European Union pursuant to the requirement (EU).

The accurate detection of low levels of aminoglycosides residues in milk is of great importance for the dairy industry and also for farmers; the development of highly selective method for the detection of Streptomycin and dihydrostreptomycin using the SCIEX Triple Quad™ 3500 was performed

The SCIEX Triple Quad™ 3500 system takes the best features and enhances them with modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The

Figure 1. SCIEX Triple Quad™ 3500

advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

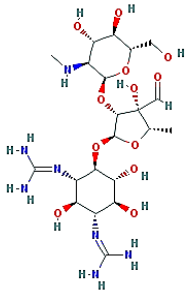

Figure 2: Structure of Streptomycin

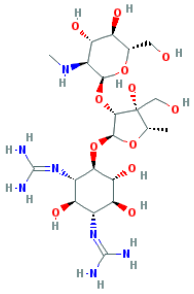

Figure 3: Structure of Dihydrostreptomycin

Unique Features

1. A sensitive, specific, rugged and reproducible LC-MS/MS method was developed for Streptomycin using simple extraction technique for the sample preparation.

2. Streptomycin analysis at 10.0 ng/ml level gave an S/N ratio > 142:1 with good accuracy and precision (n=6) in milk.

3. Dihydrostreptomycin analysis at 10.0 ng/ml level showed S/N ratio > 191.8:1 with good accuracy and precision (n=6) in milk.

4. Accuracy and Precision for Streptomycin and Dihydrostreptomycin in milk samples (matrix based) found to be between 80-120%.

5. Reproducibility of matrix based results for Streptomycin and Dihydrostreptomycin in terms of % CV in milk samples is less than 5%.

6. Average recovery of Streptomycin and Dihydrostreptomycin in milk using the developed extraction method is more than 88%.

Materials and Methods

Chemicals

Standard Streptomycin and Dihydrostreptomycin was purchased from Sigma Aldrich ≥99% Purity. All other chemicals used were of LC-MS grade, commercially available.

Milk samples

Milk samples were procured from the local market of Delhi & Gurgaon in India and were kept at 2 - 8 °C until end of analysis.

Sample Preparation

Milk sample (1ml) was taken and 10% Trichloroacetic acid (0.4ml) added and vortexed for 5 minutes followed by centrifugation at 4000 rpm for 5 minutes. Supernatant collected and filtered through 0.2µ syringe filter and injected for LC-MS/MS analysis.

LC Conditions

LC separation was achieved using the ExionLC™ AC with a Phenomenex, Synergi Hydro RP 150 x 4.6 mm, 4u column with a gradient of pump (A): Water: hepta fluoro butyric acid and pump (B): Acetonitrile at flow rate of 0.8 mL/min. The injection volume was set to 20 µl.

Time (min)	Mobile phase A %	Mobile phase B %
0.01	95	5
0.50	95	*5*
1.00	*70*	*30*
2.00	*70*	*30*
3.00	*95*	*5*
3.50	*95*	*5*

Table 1: LC conditions

MS/MS Conditions

The SCIEX Triple Quad™ 3500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored for Streptomycin and Dihydrostreptomycin, The LC-MS/MS data was processed using Analyst 1.6.2 software and MultiQuant™ software version 3.0.1.

Analyte	Q1	Q3 (Quantifier	Q3 (Qualifier)
Streptomycin	582.3	246.2	*263.4*
Dihydrostreptomycin	584.3	*263.1*	*246.2*

Table 2: MRM transitions

Results and Discussions

The SCIEX Triple Quad™ 3500 system was found to perform at lower levels than the required MRL. For this method matrix based calibration at 10ng/ml for Streptomycin had S/N>142 and for Dihydrostreptomycin showed an S/N>191.8 which is a much lower concentration than the MRL.

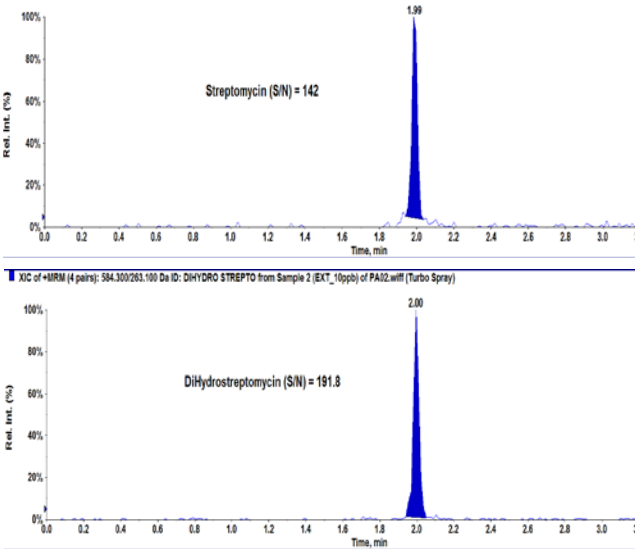

Figure 4: Signal to Noise (S/N) of Streptomycin and Dihydrostreptomycin (10ng/ml)

Matrix based calibration curves were made with standard levels ranging from 10.0ng/ml to 1000 ng/ml spiked concentration. Linear graph was obtained with regression co-efficient (r): 0.9993 and 0.9994 by using weighing factor $1/X^2$ for Streptomycin and dihydrostreptomycin respectively.

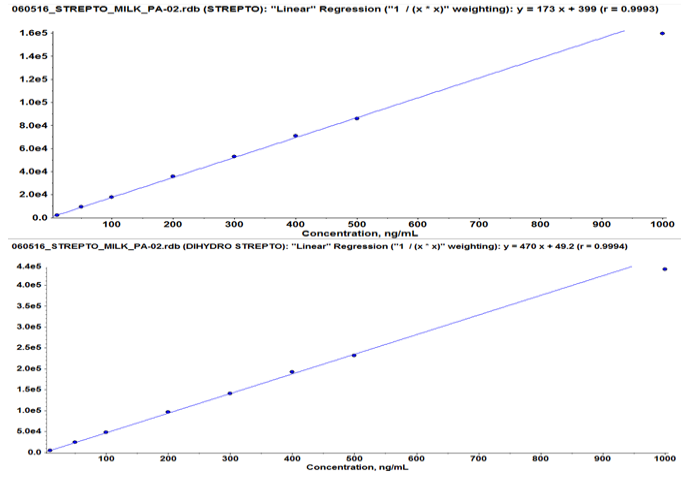

Figure 5: Linear range of the detection of Streptomycin and Dihydrostreptomycin from 10.0 to 1000 ng/mL (r = 0.9993, 0.9994)

Results of accuracy data obtained for Streptomycin and Dihydrostreptomycin in the milk matrix is given in Table 3

	Sample Name	Sample ID	Sample Type	Analyte Peak Area (counts)	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)	[Custom] Qualifier	MRM Ratio
1	EXT_10ppb	Streptomycin	Standard	2110.	10.0	9.92	99.2	1040.	0.493
2	EXT_50ppb	Streptomycin	Standard	9310.	50.0	51.7	103.	5090.	0.547
3	EXT_100ppb	Streptomycin	Standard	17700.	100.	100.	100.	9120.	0.516
4	EXT_200ppb	Streptomycin	Standard	35700.	200.	204.	102.	19500.	0.547
5	EXT_300ppb	Streptomycin	Standard	52900.	300.	304.	101.	28100.	0.531
6	EXT_400ppb	Streptomycin	Standard	71100.	400.	410.	102.	40200.	0.565
7	EXT_500ppb	Streptomycin	Standard	86900.	500.	496.	99.1	45100.	0.525
8	EXT_1000ppb	Streptomycin	Standard	160000.	1000.	923.	92.3	84600.	0.530

Table 3: Accuracy data obtained for Streptomycin in the milk matrix

	Sample Name	Sample ID	Sample Type	Analyte Peak Area (counts)	Analyte Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)	[Custom] Qualifier	MRM Ratio
1	EXT_10ppb	Dihydro Streptomycin	Standard	4710.	10.0	9.92	99.2	1820.	0.387
2	EXT_50ppb	Dihydro Streptomycin	Standard	24200.	50.0	51.4	103.	7900.	0.327
3	EXT_100ppb	Dihydro Streptomycin	Standard	47900.	100.	102.	102.	15300.	0.319
4	EXT_200ppb	Dihydro Streptomycin	Standard	96200.	200.	205.	102.	30600.	0.318
5	EXT_300ppb	Dihydro Streptomycin	Standard	141000.	300.	299.	99.7	44200.	0.314
6	EXT_400ppb	Dihydro Streptomycin	Standard	192000.	400.	409.	102.	62600.	0.326
7	EXT_500ppb	Dihydro Streptomycin	Standard	231000.	500.	493.	98.5	73600.	0.318
8	EXT_1000ppb	Dihydro Streptomycin	Standard	438000.	1000.	932.	93.2	140000.	0.320

Table 4: Accuracy data obtained for Dihydrostreptomycin in the milk matrix

Recovery was assessed by performing tests where fortified milk Samples at 0.5, 1 and 1.5 times the MRL level were analyzed (Six replicates, respectively). The Recovery of Streptomycin in matrix based was ≥ 90% and Dihydrostreptomycin ≥ 90%

	Streptomycin		
Replicates (n=6)	**50% of MRL**	**MRL**	**150% of MRL**
1	105.00	196.00	*295.00*
2	100.00	*191.00*	*283.00*
3	*107.00*	*197.00*	*284.00*
4	*105.00*	*198.00*	*271.00*
5	*102.00*	*205.00*	*264.00*
6	*104.00*	*200.00*	*273.00*
Average Conc (ng/mL)	*103.88*	*197.83*	*278.33*
Target Conc (ng/mL)	*100.00*	*200.00*	*300.00*
% Recovery	*103.88*	*98.92*	*92.78*

Table 5: Recovery of Streptomycin in the milk matrix

	Dihydrostreptomycin		
Replicates (n=6)	**50% of MRL**	**MRL**	**150% of MRL**
1	*102.000*	*198.000*	*292.000*
2	*102.000*	*188.000*	*274.000*
3	*102.000*	*192.000*	*274.000*
4	*107.000*	*199.000*	*262.000*
5	*105.000*	*197.000*	*265.000*
6	*103.000*	*206.000*	*266.000*
Average Conc (ng/mL)	*103.500*	*196.667*	*272.167*
Target Conc (ng/mL)	*100.000*	*200.000*	*300.000*
% Recovery	*103.50*	*98.33*	*90.72*

Table 6: Recovery of Dihydrostreptomycin in the milk matrix

Streptomycin and Dihydrostreptomycin eluted at RT of 1.99 and 2.00 minutes with minimum background noise in 3.50 minutes chromatographic run.

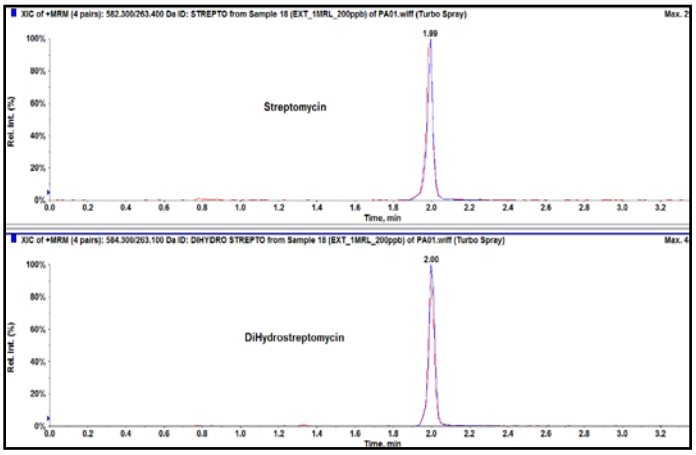

Figure 6: Representative chromatogram of Streptomycin and Dihydrostreptomycin at MRL (200ng/ml)

Conclusions

- The developed method on SCIEX Triple Quad™ 3500 was simple, sensitive and reproducible which can meet the regulatory requirements.
- Trueness (Average recovery %) for this method found to be ≥ 90%.

Summary

The method and data presented here showcase the fast and accurate solution for the quantitation and identification of Streptomycin and Dihydrostreptomycin in milk samples by LC-MS/MS. The SCIEX Triple Quad™ 3500 system provide excellent sensitivity and selectivity, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

References

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4. EMEA (2002b): Streptomycin, summary report (3) committee for veterinary medicinal products, EMEA. EMEA/MRL809/01 final, February 2002.

5. Renata Cabrera de Oliveira1,*, Jonas Augusto Rizzato Paschoal1, Marcela Sismotto1, Flávia Pereira da Silva Airoldi2, and Felix Guillermo Reyes Reyes1 Determination of Streptomycin and Dihydrostreptomycin Residues in Milk

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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