



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

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Overview

Streptomycin and Dihydrostreptomycin quantification method was developed in honey samples using SCIEX Triple Quad™ 3500 Liquid chromatography tandem mass spectrometry (LC-MS/MS) system. A simple sample preparation protocol was used for method development. The method showed adequate linearity with correlation coefficients above r≥0.98 for both analytes with the dynamic range of 0.25–20 ng/ml. The average accuracies for both the analytes were between88 to 117%. The Minimum Required Performance Limit (MRPL) for Streptomycin and Dihydrostreptomycin in Honey was 10µg/kg.

Introduction

Streptomycin (STR) and its metabolite dihydrostreptomycin (DHSTR) are the aminoglycoside antibiotics which work against gram-negative systemic bacterial infections. These antibiotics are commonly applied for crop protection and by bee keepers to eliminate disease among honeybees.

Safety of food and feed is one of the main objectives in consumer health policy. Honey is widely used as food and medicine. Many different antibiotics are used in Apiculture to keep bees away from various bacterial infections. Accumulation of antibiotic residues in the honey leads to adverse health effects during human consumption. Hence it become necessary for analyzing antibiotic residues in Honey as a part of its quality check.

There are several reports of Streptomycin (STR) and its metabolite dihydrostreptomycin (DHSTR) residue analysis in honey using LC-MS/MS. Streptomycin (STR) and its metabolite dihydrostreptomycin (DHSTR) belong to Aminoglycosides class of antibiotic. Aminoglycosides are a broad class of antibiotic having more than two amino sugars linked by glycosidic bonds to an aminocyclitol component.

Therefore, streptomycin and dihydrostreptomycin are highly soluble in water so the sample extraction, cleanup and

chromatographic method become very challenging in honey sample.



Figure 1. SCIEX Triple Quad™ 3500

SCIEX Triple QuadTM 3500 LC-MS/MS system takes the best features and enhances them with modern engineering and advanced eQTM electronics. The proven design of Turbo VTM source and Curtain GasTM interface provide exceptional robustness and ruggedness.

In this application note, we describe a sensitive, reproducible quantitative method for streptomycin (STR) and dihydrostreptomycin (DHSTR) in honey sample.



Molecular Formula: $C_{21}H_{39}N_7O_{12}$ Monoisotopic Mass: 581.265 Da

Figure 2. Structure of Streptomycin

Molecular Formula: C₂₁H₄₁N₇O₁₂ Monoisotopic Mass: 583.28 Da

Figure 3. Structure of Dihydrostreptomycin

Materials and Methods

Chemicals

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

Honey samples

Honey samples were procured from local market of Delhi and Gurgaon, India and were stored at room temperature until end of analysis.

Sample Preparation

Accurately weighed 1.0g of honey sample, mixed with 3ml of 100mM Ammonium acetate, vortexed for 5min and loaded into Cleanert (PWCX-SPE 30mg/ml) cartridge. Washed with 2ml of Methanol: water (80:20) and eluted with 1ml of methanol containing 5% formic acid. The eluent was evaporated to dryness under Nitrogen. Reconstituted in 1ml of Acetonitrile: water: Formic acid (5:95:0.2%) and subjected to LC-MS/MS analysis.

LC Conditions

LC separation was achieved using the Shimadzu prominence system with ZORBAX SB-C18 (4.6×150 mm) 5 μm column with a gradient (Table 1) of 0.1% Heptafluorobutyric acid (A) and Acetonitrile (B) at flow rate of 0.8 mL/min. The injection volume was 25 μL .

Time (min)	Mobile phase A%	Mobile phase B%
0.01	75	25
1.50	75	25
4.00	90	10
5.30	75	25
10.00	Controller	Stop

Table 1. Gradient Time Program

MS/MS Conditions

The SCIEX Triple Quad™ 3500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored (Table 2).



Compound	Precursor ion	Product ion Quantifier	Product ion Qualifier
Streptomycin	582.3	263.4	246.2
Dihydrostreptomycin	584.3	263.1	246.2

Table 2. MRM Transition for Streptomycin and Dihydrostreptomycin

Results and Discussions

The matrix based calibration curve for Streptomycin and its metabolites Dihydrostreptomycin showed excellent linearity (0.25 x MRPL to 2 x MRPL level), with a correlation coefficient r≥0.99 using linear regression and weighing factor 1/X2. The developed method in honey was found to be capable of analyzing concentrations well below the MRPL (10µg/Kg) required by EU.

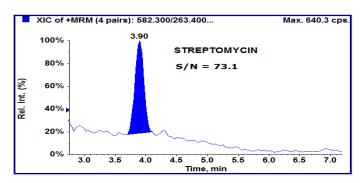
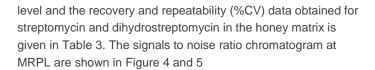


Figure 4. Signal to Noise (S/N) of Streptomycin at 10 ppb in matrix based samples.



Analyte	Repeatability (%CV, n=6)		Recovery (n=6)
	½ MRPL (5.0ppb)	MRPL (10.0ppb)	MRPL (10.0ppb)
STR	9.34	6.96	101.75
DHSTR	10.21	9.51	99.73

Table 3. Recovery and Repeatability (%CV) statistics of Streptomycin and Dihydrostreptomycin in the honey matrix (10ppb).

Recovery experiment was performed in honey samples at 10ppb MRPL level (Six replicates). The Recovery of streptomycin and dihydrostreptomycin were 101.75 and 99.73% respectively. The retention time of streptomycin and dihydrostreptomycin were 3.90 min and 3.91 min, respectively. A representative chromatogram obtained from a standard mixture of the Streptomycin and Dihydrostreptomycin is given in Figure 6 showing the qualifier and quantifier.

Repeatability experiment was evaluated at the MRPL level of 10 ppb (n=6) gives %CV of \leq 10.0.

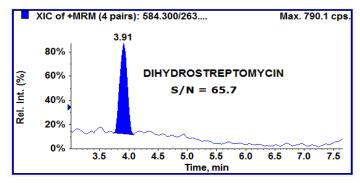


Figure 5. Signal to Noise (S/N) of Dihydrostreptomycin at 10 ppb in matrix based samples.

The signal to noise ratio for streptomycin and dihydrostreptomycin is \geq 73.1 and \geq 65.7, respectively at MRPL



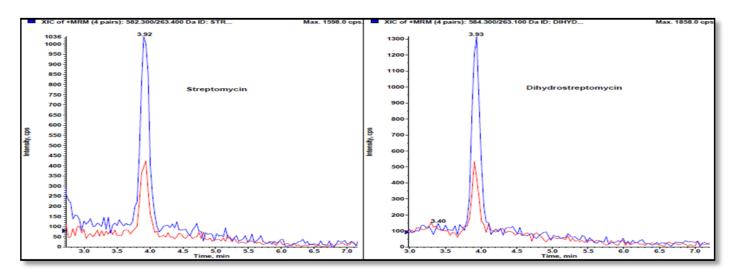


Figure 6. Representative chromatogram of Streptomycin and Dihydrostreptomycin (Quantifier & Qualifier) at 20ppb

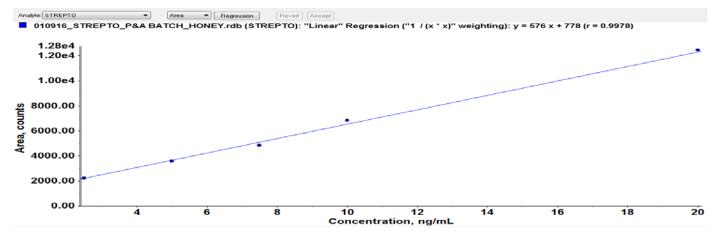


Figure 7. Linear range of the detection of Streptomycin from 2.50 to 20.0 ppb (r \geq 0.99).

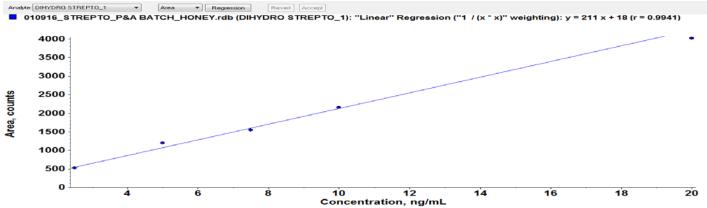


Figure 8. Linear range of the detection of Dihydrostreptomycin from 2.50 to 20.0 ppb (r ≥ 0.99).



Conclusions

- The developed quantitation method on SCIEX Triple QuadTM 3500 was simple, sensitive, linear, and reproducible for Streptomycin and its metabolite
- Better separation and reproducibility of Streptomycin and Dihydrostreptomycin was achived using ion pairing reagent in Honey sample.
- Average recovery for this method found to be $\geq 95\%$ meeting the requirement of EU/SANCO regulation of 70-120%.
- This method exhibited excellent linearity from 0.25x MRPL to 2x MRPL. with a correlation coefficient r≥0.99.
- Method developed on SCIEX Triple Quad™ 3500 can be used to check the presence of streptomycin and dihydrostreptomycin in honey sample for quality control purpose.

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