Quantitation of Chloramphenicol in Milk using SCIEX Triple Quad™ 3500 LC-MS/MS System

Aman Sharma[1]; M.Chandrasekar[1]; Santosh Kapil[1]; Anoop Kumar[1], Manoj Pillai[1] & Jianru Stahl-Zeng[2]
[1]SCIEX, 121, Udyog Vihar, Phase IV, Gurgaon - 122015, Haryana, India, [2]Sciex, Darmstadt • Germany

Overview

Chloramphenicol (CAP) is a Broad-Spectrum antibiotic used for the treatment of a number of bacterial infections. The use of CAP for the treatment of food producing animals is prohibited in several countries (e.g. European Union, Canada, United States, and most Asian counties). In India, the judgement referred to "Executive summary on National survey on milk adulteration" released by FSSAI said that at national level, 68.4% of milk being sold is adulterated. The Commission Decision 2002/657/EC Annexure II requires control of CAP residues in edible tissues, meat, seafood, eggs, honey, milk, and milk products. The Minimum Required Performance Limit (MRPL) for CAP in milk was 0.3µg/kg. An LC MS/MS method for the quantitation of Chloramphenicol in milk which meets the regulatory requirements is described in this article.

Introduction

The presence of antibiotics in food of animal origin is of concern due to the potential of increasing bacterial resistance and to hypersensitivity for some individuals. Tolerance limits and MRPL have been established around the world, and agencies monitor the food supply to ensure that antibiotic residue concentrations do not exceed these levels. The accurate detection of low levels of antibiotic residues in milk is of great importance for the dairy industry. The development of sensitive and selective method for the quantitation of Chloramphenicol in milk which meets the regulatory requirements was done using SCIEX Triple Quad™ 3500. The SCIEX Triple Quad™ 3500 system has the legendary Turbo V™ ion source, efficiently ionizes compounds and virtually eliminates cross-contamination for reliable quantitation over a wide range of flow rates. The proprietary Curtain Gas™ interface reduces the need for routine maintenance and ensures maximum productivity by protecting your mass spec from contamination. In addition the Curved LINAC® collision cell design improves data quality and helps in achieving optimal sensitivity for all compounds. The method development was performed as per the regulatory guidelines described in EU/SANCO/12495 directive recommendations

Figure 1: SCIEX Triple Quad™ 3500

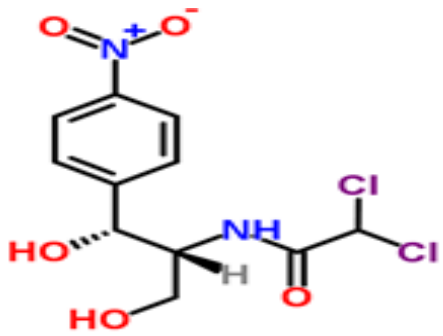

Figure 2: Structure of Chlormaphenicol (C11H12Cl2N2O5; MW:322.012)

Materials and Methods

Standard Chloramphenicol was purchased from Clearsynth. All other chemicals used were of LC-MS grade, commercially available. Milk samples were purchased from the local market of Delhi, and Gurgaon and stored in refrigerator at 2 to 8ºC till the analysis was completed.

Sample Preparation

A generalized extraction procedure was performed in which, 1ml of milk was vortexed with acetonitrile, water mixture (4:1) v/v), followed by the addition of NaCl and mixed well. This solution was centrifuged, supernatant was evaporated to dryness, reconstituted, filtered and 20µl was used for the LC MS/MS analysis.

LC Conditions

LC separation was performed on a ExionLC™ AC instrument using Synergy Fusion RP 18e (50 X 2.6) mm 2.5µ and a fast gradient of water (Mobile Phase A) and acetonitrile (Mobile Phase B) from 85% aqueous to 85% organic in 5 minutes at a flow rate of 0.4ml/min and injection volume of 20µl is used to obtain a good peak shape.

Time (min(	Mobile phase A%	Mobile phase B%
0.01	85	15
0.30	85	15
0.50	75	25
1.00	70	30
1.50	15	85
3.00	15	85
4.00	85	15
5.00	85	15

Table 1: Mobile Phase Gradient

MS/MS Conditions

The SCIEX Triple Quad™ 3500 was operated in Multiple Reaction Monitoring (MRM) mode. The TurboV™ source was used with an Electrospray Ionization (ESI) probe in negative ionization mode at 2800 ion spray voltage, with Declustering potential (DP) -85V and Collision Energy(CE) -15V and -23V for the MRM transitions 320.8/151.8 and 320.8/256.8 respectively. Analyst 1.6.2™ software was used for method development and data acquisition. LC-MS/MS data was processed using the MultiQuant™ software version 3.0.2.

Results and Discussions

Sensitivity, Reproducibility, Linearity and Accuracy

The SCIEX Triple Quad™ 3500 system showed very good sensitivity for chloramphenicol analysis in milk. The experimental data was acquired in accordance with EU SANCO/12495 directive recommendations. The matrix based method for the chloramphenicol analysis was set for a Minimum Required Performance Limit (MRPL) of 0.3µg/kg level. Chloramphenicol eluted at Retention time 2.90 min with minimal background noise in 5 minutes gradient run. The signal-to-noise ratio obtained was 88.8 for extracted 0.1µg/kg spiked sample. Repeatability at MRPL level (0.3ug/kg) was evaluated (n=6) and %CV was found < 5.0.

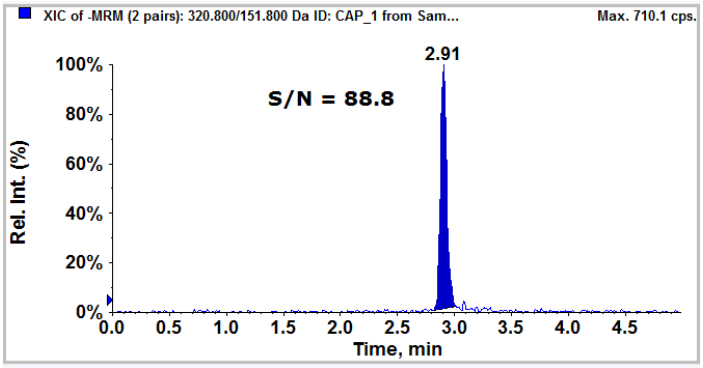

Figure 3: Chromatogram showing S/N ratio at 0.1µg/kg concentration

Extracted matrix based linearity curve plotted with a linear dynamic range of 3 orders was made from a set of standard dilutions in the range from 0.1µg/kg to 10.0µg/kg correlation regression co-efficient r is found > 0.99 for both quantifier and qualifier ions by using weighing factor of 1/ X2. Recovery of the extracted method is evaluated by spiking the milk samples at different concentrations.

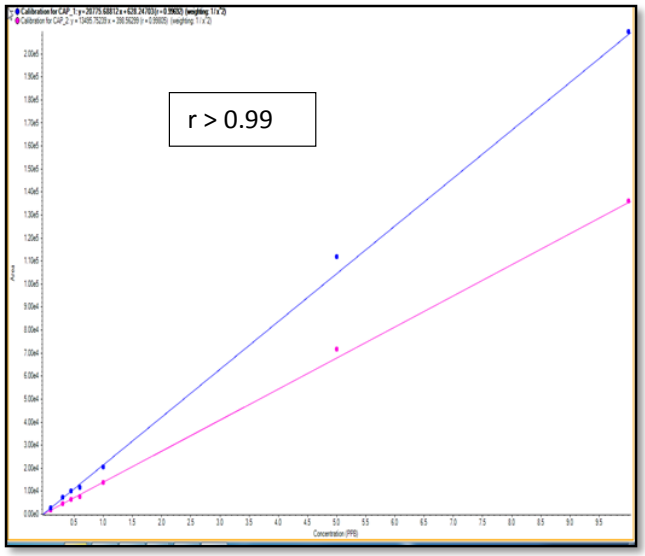

Figure 4: Matrix Based Calibration Curve

Sample Name	Sample Type	Component Name	Mass Info	Used	Accuracy	MRM Ratio
CAP_EXT_BLK	Blank	CAP_1	320.8 / 151.8	☑	N/A	0.589
CAP_EXT_0.1PPB	Standard	CAP_1	320.8 / 151.8	☑	99.70	0.650
CAP_EXT_0.3PPB	Standard	CAP_1	320.8 / 151.8	☑	106.42	0.631
CAP_EXT_0.45PPB	Standard	CAP_1	320.8 / 151.8	☑	101.75	0.640
CAP_EXT_0.6PPB	Standard	CAP_1	320.8 / 151.8	☑	87.81	0.659
CAP_EXT_1PPB	Standard	CAP_1	320.8 / 151.8	☑	96.55	0.672
CAP_EXT_5PPB	Standard	CAP_1	320.8 / 151.8	☑	107.15	0.642
CAP_EXT_10PPB	Standard	CAP_1	320.8 / 151.8	☑	100.62	0.649
CAP_EXT_BLK	Blank	CAP_1	320.8 / 151.8	☑	N/A	0.753
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	104.33	0.636
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	107.79	0.660
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	99.95	0.659
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	106.97	0.651
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	109.92	0.620
CAP_EXT_0.3PPB	Quality Control	CAP_1	320.8 / 151.8	☑	107.86	0.624
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	85.95	0.674
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	95.96	0.649
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	101.11	0.694
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	105.01	0.643
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	107.29	0.655
CAP_EXT_0.6PPB	Quality Control	CAP_1	320.8 / 151.8	☑	108.06	0.626
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	102.15	0.649
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	111.06	0.656
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	107.67	0.652
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	106.52	0.656
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	110.29	0.642
CAP_EXT_5PPB	Quality Control	CAP_1	320.8 / 151.8	☑	113.05	0.639

Table 2: Accuracy and MRM Ratio using Multiquant™

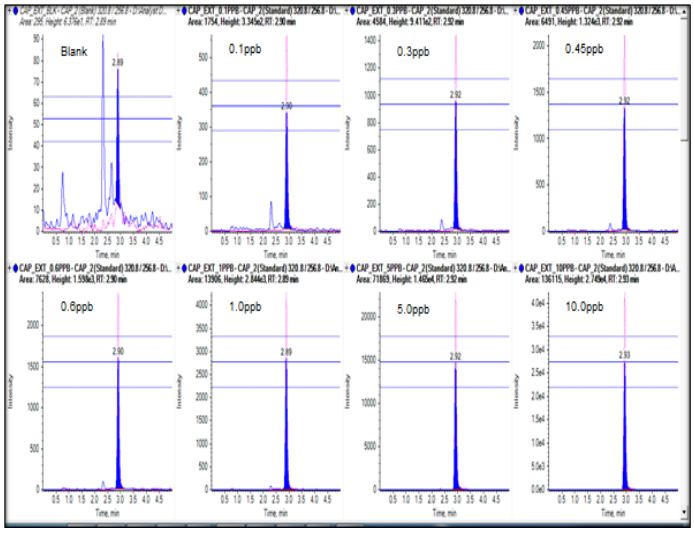

Figure 5: Ion Ratio Tolerance Chromatograms

Findings in Milk Samples

The samples (1) and (4) obtained from the market were found to contain chloramphenicol which is evident from the retention time as well as ion ratio, however samples (2) and (3) are possible false positives. The analysis was done in duplicate for each sample. Figure 6 shows chromatogram of different milk samples analyzed for the quantitation of chloramphenicol.

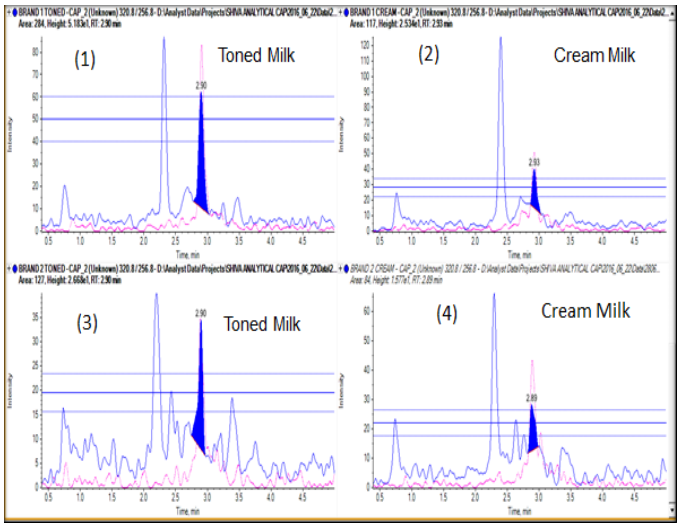

Figure 6: XIC's of different commercial Milk samples

Replicate injections	Chloramphenicol(320.8/151.8) 0.3µg/kg
1	0.313
2	0.323
3	0.300
4	0.321
5	0.330
6	0.324
Average conc	0.318
Original conc	0.300
%Recovery	106.14
%CV	3.32

Table 3: Recovery data for Matrix based samples

Conclusions

The method developed as per EU /SANCO/12495 directive recommendations showed acceptable accuracies (85%-120%) for matrix based recovery samples, linearity with r > 0.99 for both the transitions, repeatability was< 5. No significant matrix interferences observed. The method allows high throughput, rapid and sensitive LC-MS/MS identification and quantitation of banned antibiotic Chloramphenicol meeting EU MRPL of 0.3µg/kg level.

Summary

1 The method and data presented here shows fast and accurate solution for the quantitation and confirmation of Chloramphenicol in milk samples by LC-MS/MS.

2. The SCIEX Triple Quad™ 3500 system provides excellent sensitivity and selectivity for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of a bigger batch of samples in a short time period.

3. Automatic MRM ratio calculation in MultiQuant™ software can be used for confirmation of compound.

References

1. FAO-Dairy Production and products: milk production facts, Food and Agriculture Organization of United Nations, Rome.- 2013 http://fao.org/agriculture/ dairy-gateway/milk-production/en.

2. FSSAI manual of methods of analysis of food. http://fssai.gov.in/Portals/0/Pdf/15Manuals/ANTIBIOTICS%20AND%20RESIDUES.pdf

3. A. Schreiber: 'MultiQuant™ Software Version 3.0 - Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples' Application Note AB SCIEX (2013) #8160213-01

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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