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Food and Environmental

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# Rapid, Sensitive, Quantitation method for Chloramphenicol in Meat using SCIEX Triple Quad™ 3500 LC-MS/MS System

Aman Sharma[1]; Santosh Kapil[1]; M.Chandrasekar[1]; Anoop Kumar[1], Manoj Pillai1 and Jianru Stahl-Zeng[2]
[1]SCIEX, 121, Udyog Vihar, Phase IV, Gurgaon - 122015, Haryana, India, [2] SCIEX, Darmstadt, Germany

## Overview

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been widely used for the identification and quantification of trace amount of antibiotic residues like chloramphenicol in complex food matrices due to the specificity and sensitivity associated with this technique. The SCIEX Triple Quad™ 3500 system enables labs performing antibiotic testing in foods to upgrade to LC-MS/MS and capitalize on its many benefits like achieving the low level sensitivity along with reproducibility and method ruggedness. The Minimum Required Performance Limit (MRPL) set by the European Union for Chloramphenicol in meat and meat products was 0.3µg/kg. The method of analysis for chloramphenicol in meat described here was developed and performed according to the criteria established by the EC Commission Decision 657/2002.

## Introduction

Chloramphenicol (CAP) is a highly effective bacteriostatic compound. Due to the findings of chloramphenicol residues in animal derived foods intensive surveillance had been conducted by regulatory authorities like EU or USFDA. Most of the countries like EU, USA, Canada and Asian countries etc. have banned the usage of CAP in food products and implemented stringent regulations to keep a check on the entry of such products contaminated with Chloramphenicol into supermarkets. The purpose of this study was to develop a quantitative method for the determination of chloramphenicol in meat as per the regulatory requirements using the SCIEX Triple Quad™ 3500 system.

The SCIEX Triple Quad™ 3500 system takes the best features and enhances the response with modern engineering and electronics. The proven legendary design of Turbo V™ source and Curtain Gas™ interface provides exceptional robustness and ruggedness. The 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-residue analysis. Compound identification was based on the



Figure 1: SCIEX Triple Quad™ 3500

criteria of directive 2002/657/EC9 (retention time tolerance of ± 2.5% and maximum tolerances for ion ratios of ± 20 to 50% depending on the ratio).
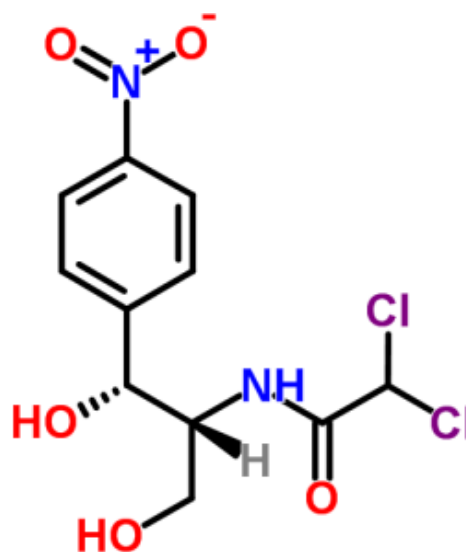


Figure 2: Structure of Chloramphenicol (C11H12Cl2N2O5 MW: 322.012)

## Materials and Methods

### Chemicals

Standard Chloramphenicol Purity(≥99%) was purchased from Clearsynth, India. All other chemicals used were of LC-MS grade.

### Sample Preparation

Accurately weigh 2.0g of homogenized chicken meat sample, 6ml of Ethyl acetate was added vortexed, centrifuged and the supernatant was evaporated to dryness. Add 1ml of Hexane and Carbon Tetrachloride (1:1 v/v), mix well and 1ml of Mobile phase, filtered using 0.22 µM PTFE filter and 20µl was used for LC-MS/MS.

### LC Conditions

LC separation was performed on an 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.

| Time (min) | Mobile phase A % | Mobile phase B % |
|---|---|---|
| 0.01 | 85 | 15 |
| 0.30 | 85 | 15 |
| 0.50 | 72 | 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 Turbo V™ 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 320.8/151.8 and 320.8/256.8 respectively. Two selective MRM transitions were monitored and ion ratio is calculated automatically by software for compound identification. Analyst 1.6.2™ software was used for method development and data acquisition. LC-MS/MS data was processed using the MultiQuant™ software version 3.0.1

## Results and Discussions

### Sensitivity, Reproducibility, Linearity and Accuracy

The sensitivity for the chloramphenicol analysis in meat was achieved below the required limit set by the regulations using the SCIEX Triple Quad™ 3500. The sensitivity of the method using matrix matched recovery study showed S/N ratio of 110.2 at 0.01µg/kg chloramphenicol level.
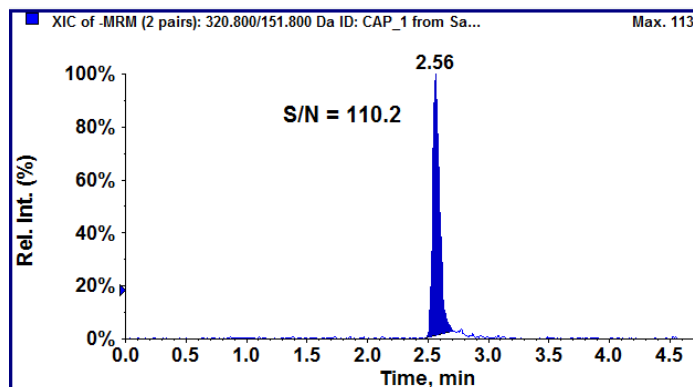


Figure 3: Chromatogram showing S/N at 0.01µg/kg level

Matrix matched Calibration curve plotted was found linear in the range of 0.01µg/kg (ppb) to 1.0µg/kg (ppb) with 2 orders of linear dynamic range and correlation regression co-efficient r > 0.99 for both quantifier and qualifier ions by applying weighing factor of $1/X^2$.
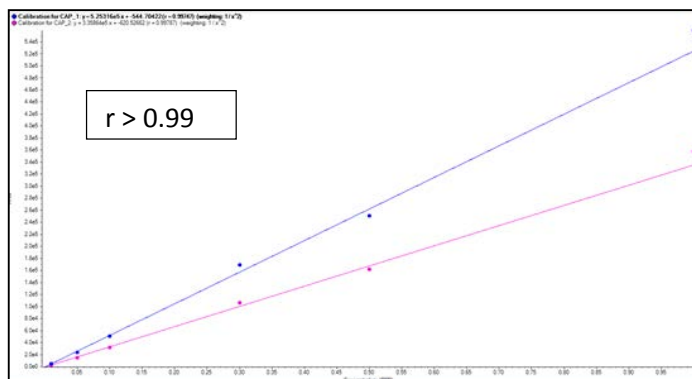


Figure 4: Matrix Matched Calibration curve ranging 0.01µg/kg to 1.0 µg/kg with r > 0.99 for both transitions

Repeatability at 0.01µg/kg level was evaluated for 6 replicate injections and percentage relative standard deviation (%CV) was observed to be < 5. Accuracies observed were in the range from 85% to 120%. Chloramphenicol eluted at RT of 2.56 min in 5

minutes run. Quantifier and qualifier ion ratio shown by MultiQuant™ software in figure attached below.
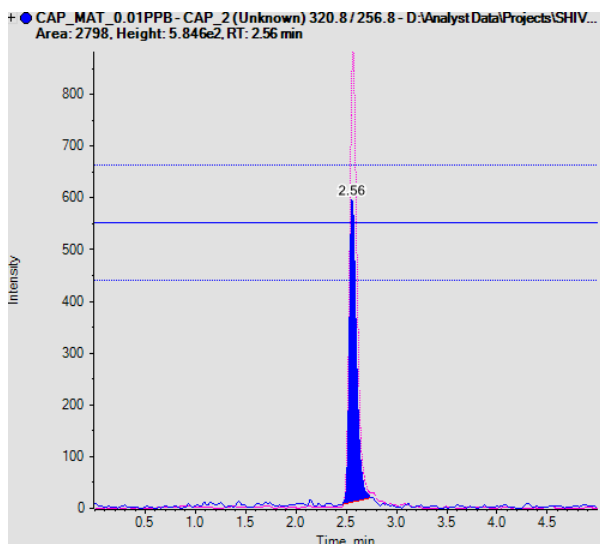


**Figure 5: Representative Chromatogram at 0.01µg/kg level showing Ion Ratio Tolerance set at ± 20%.**

| Replicate Injections | Chloramphenicol(320.8/151.8) | | |
|---|---|---|---|
| | 0.01µg/kg | 0.1µg/kg | 0.3µg/kg |
| 1 | 0.011 | 0.094 | 0.311 |
| 2 | 0.010 | 0.091 | 0.314 |
| 3 | 0.010 | 0.092 | 0.304 |
| 4 | 0.011 | 0.094 | 0.308 |
| 5 | 0.011 | 0.094 | 0.309 |
| 6 | 0.011 | 0.092 | 0.317 |
| Average conc. (µg/kg) | 0.011 | 0.093 | 0.310 |
| Original conc. (µg/kg) | 0.010 | 0.100 | 0.300 |
| %Recovery | 109.57 | 93.02 | 103.50 |
| %CV | 3.44 | 1.56 | 1.44 |

**Table 2: Recovery data for matrix matched samples spiked at MRPL and Lower than MRPL (0.01µg/kg & 0.1µg/kg)**

## Conclusions

The quantitation method described here meets the regulatory

requirements and the lowest point in the calibration curve was below the limits set by the EU to 0.01µg/kg with adequate linearity and reproducibility. The method showed acceptable accuracies, linearity with r >0.99 for both quantifier and qualifier, repeatability (%CV) observed was < 5. This method utilizes a generic sample preparation protocol which meets the validation parameters as per the recovery % requirements set by the global regulations (85%-120%). The method allows high throughput, selective, rapid and sensitive LC-MS/MS identification and quantitation of banned antibiotic Chloramphenicol in meat samples.

## 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/meat-production/en.

2. Method validation and quality control procedures for pesticide residue analysis in food and feed: SANCO/12495/2011

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

4. Determination of the antibiotic chloramphenicol in meat and seafood products by liquid chromatography – electrospray ionization tandem mass spectrometry. Pascal Mottier, Veronique Parisod, Eric Gre,aud, Philippe A Guy, and Richard H. Stadler, Journal of Chromatography A, 994 ( 2003) 75-74.