

Trace-Level Quantitation of Chloramphenicol Using the TSQ Quantum Discovery

Kevin J. McHale, Thermo Fisher Scientific, Somerset, NJ, USA

Key Words

- TSQ Quantum Discovery™
- Antibiotic
- Environmental Analysis
- LC/MS/MS

Introduction

Chloramphenicol is an attractive antibiotic due to its low cost, high potency and activity against both gram-positive and gram-negative bacteria. But, chloramphenicol has a serious drawback in that it causes the potentially lethal condition aplastic anemia in humans in approximately one in 30,000 cases. Since it is not known what dosage level of chloramphenicol causes the onset of aplastic anemia, many government agencies around the world have prohibited the use of chloramphenicol in the treatment of animals producing food products for human consumption.

Recently, government agencies testing food samples, particularly seafood and honey, originating from parts of East Asia have discovered significant levels of chloramphenicol. This has prompted an initiative to develop new analytical methodologies to screen food samples for chloramphenicol at sub-ppb concentrations. While gas chromatography coupled with electron capture detection (GC-ECD)^{1,2} and mass spectrometry (GC/MS)³⁻⁶ methods exist for measuring chloramphenicol in food samples at or below 1 ppb, these methods require chemical derivatization prior to analysis. However, LC/MS/MS methods, do not require derivatization and therefore require less sample preparation time and incur less sample losses. Several reports have been published in recent years which report LC/MS/MS assays for chloramphenicol with sub-ppb levels in food samples using a triple quadrupole mass spectrometer.⁷⁻¹¹

This report illustrates the sensitivity of an LC/MS/MS method for chloramphenicol using the Thermo Scientific TSQ Quantum Discovery. In accordance with methods developed by government agencies, multiple selected reaction monitoring (SRM) transitions for chloramphenicol ions were used for quantitative and conformational purposes.

Experimental Conditions

Chemicals and Reagents

HPLC grade methanol and water were purchased from Burdick and Jackson (Muskegon, MI, USA). Glacial acetic acid (99.99%) and chloramphenicol (98%) were obtained from Aldrich (Milwaukee, WI, USA).

Sample Preparation

A stock solution of chloramphenicol at 1.0 mg/mL was prepared in methanol and stored at 4 °C until needed. Prior to LC/MS/MS analyses, the chloramphenicol stock solution was diluted with 100% water to the target concentrations in a serial manner.

Sample Analysis

LC experiments were conducted using a Thermo Scientific Surveyor™ HPLC System (Thermo Fisher Scientific, San Jose, CA, USA). Separations of chloramphenicol were achieved using a 2.1 × 50 mm Thermo Scientific BDS Hypersil™ C18 column packed with 3 μm particles (Thermo Fisher Scientific, Bellafonte, PA, USA). Mobile phase compositions were [A] 0.1% (v/v) acetic acid in water and [B] methanol. A linear gradient method was employed where the percent B mobile phase was varied from 15% at t = 0.0 min to 75% at t = 3.0 min. After a one minute hold at 75% B, the column was recycled to 15% B and equilibrated for 3 minutes. The flow rate during the gradient separation (t = 0.0 - 4.0 min) was 0.25 mL/min and increased to 0.3 mL/min during column equilibration. The injection volume for all LC experiments was 20 μL.

A TSQ Quantum Discovery was used for detection of chloramphenicol. Prior to tuning with chloramphenicol, the mass scale and electron multiplier on the mass spectrometer were calibrated in negative ion mode using a solution of polytyrosine 1,3,6. The optimal mass spectrometer parameters for the LC/MS/MS analysis of chloramphenicol were as follows:

Source: ESI
Ion polarity: Negative
ESI Needle Voltage: -3000 V
Sheath Gas Pressure: 35 arb units
Auxillary Gas Pressure: 5 arb units
Ion Transfer Capillary Temperature: 350 °C
Tube Lens Offset: -90 V (at *m/z* 321)
Source CID Offset: 7 V
Scan Mode: Selected Reaction Monitoring
Q2 Pressure: 1.0 mTorr argon

SRM Transitions	Collision Energy	Scan Time
321 → 152	18 eV	0.1 s
321 → 194	13 eV	0.1 s
321 → 257	12 eV	0.1 s

Scan Width: 1.0 u
Q1, Q3 Resolution: Unit (0.7 u FWHM)

Results & Discussion

Figure 1 illustrates the sensitivity for chloramphenicol on the TSQ Quantum Discovery by LC/MS/MS. Clearly identifiable and quantifiable peaks were observed for all three SRM transitions of chloramphenicol at a retention time of three minutes. The chromatogram in Figure 1 represents the 50 pg/mL chloramphenicol standard and equates to the injection of 1 pg chloramphenicol on column.

Many of the reported LC/MS/MS methods for chloramphenicol use multiple SRM transitions for detection. Typically these methods use one or two SRM transitions for quantitation of chloramphenicol in food samples and use the other SRM transition(s) for confirmational purposes.^{7,9,11} In this report, the quantitation of chloramphenicol was performed by acquiring the two most abundant SRM transitions, namely m/z 321 \rightarrow 152 and m/z 321 \rightarrow 257. Additionally, to improve the statistical results, the responses from these two SRM transitions were summed.

The chromatogram for the 10 pg/mL chloramphenicol sample (Figure 2) shows the summation of the ion current from the two SRM transitions (top) yields an improved S/N (where the noise is the root mean square (RMS) variance over the time range of 2.25-2.85 min) over that of both the m/z 321 \rightarrow 152 and m/z 321 \rightarrow 257 transitions (center and bottom, respectively). The higher overall signal and area response for the summed SRM transitions improves the precision and accuracy statistics to allow the 10 pg/mL chloramphenicol standard, with 200 fg chloramphenicol injected, to be considered the limit of quantitation (LOQ).

Acquiring five replicate injections using chloramphenicol standards at 10, 25, 50, 100, 500, 1000, 5000 and 10,000 pg/mL produced a 1/x weighted linear calibration curve based on the summed area responses of the

m/z 321 \rightarrow 152 and m/z 321 \rightarrow 257 SRM transitions (Figure 3). The correlation coefficient for the chloramphenicol calibration curve was $R^2 = 0.9994$ for the concentration range of 10-10,000 pg/mL, equating to 0.2-200 pg chloramphenicol injected on column. The statistical results for the chloramphenicol standards are illustrated in Table 1. Note that the percent accuracies and precisions, listed as percent coefficient of variance (%CV), are within the US government agency guidelines for a LC/MS/MS validated method.¹²

To test the absolute sensitivity of chloramphenicol on the TSQ Quantum Discovery, the m/z 321 \rightarrow 152 SRM transition was monitored while 50 fg injections of chloramphenicol were made onto the LC column under isocratic conditions (50% B at 0.25 mL/min). The chromatogram in Figure 4 shows triplicate injections of 50 fg chloramphenicol. Based on the response under these experimental conditions, the estimated limit of detection for the m/z 321 \rightarrow 152 chloramphenicol SRM transition is 20 fg.

Conclusions

An LC/MS/MS assay for chloramphenicol standards on the TSQ Quantum Discovery yields sub-picogram limits of quantitation, which translates to sub-ppb sample concentrations. By summing the response from multiple SRM transitions for chloramphenicol, a greater S/N is achieved resulting in improved statistical results. A calibration curve with a linear regression fit for chloramphenicol over the concentration range of 10-10,000 pg/mL yielded accuracy and precision values well within the guidelines of U.S. government for analytical method development and validation.¹² Based on the most sensitive SRM transition for chloramphenicol, the estimated limit of detection is 20 fg on the TSQ Quantum Discovery.

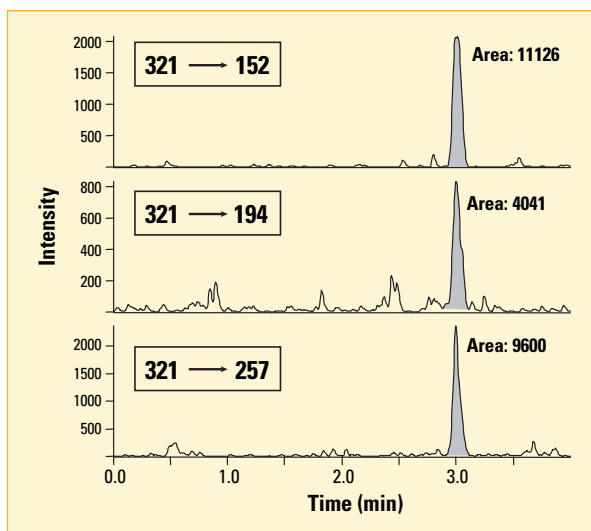


Figure 1: SRM chromatograms for 50 pg/mL chloramphenicol

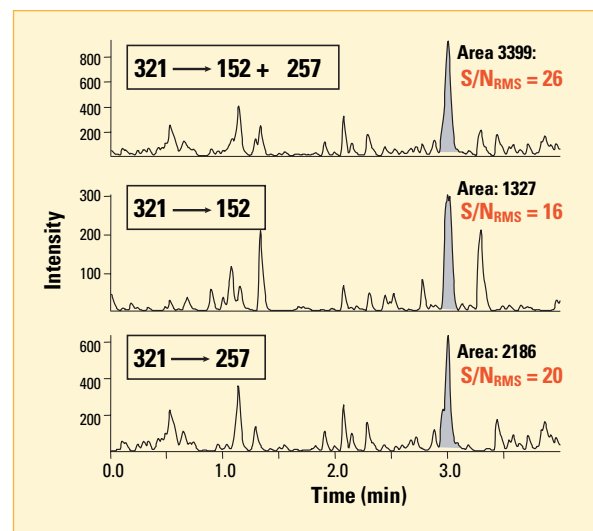


Figure 2: SRM chromatograms for 10 pg/mL chloramphenicol

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Nominal Conc. (pg/mL)	Mean Conc. (pg/mL)	% Accuracy	% CV
10.0	8.58	85.8	12.5
25.0	25.7	102.7	8.9
50.0	53.5	107.0	4.5
100	97.8	97.8	6.3
500	518	103.6	2.1
1,000	1,025	102.5	4.2
5,000	5,108	102.2	0.8
10,000	9,848	98.5	1.0

Table 1: Statistical data for the calibration curve of chloramphenicol (n = 5)

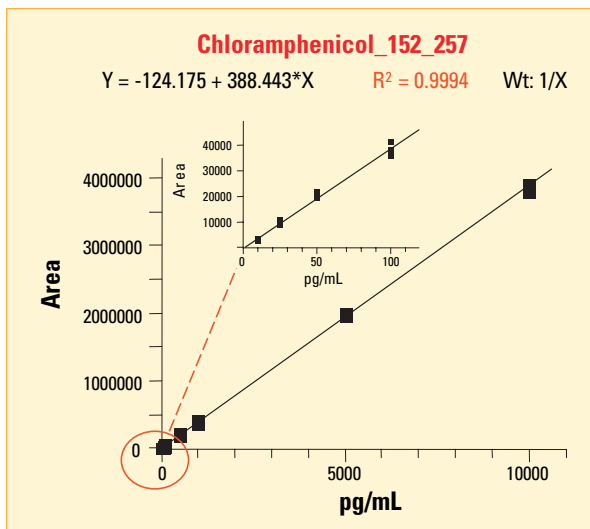


Figure 3: Linear calibration curve for chloramphenicol using the summed areas from the SRM transitions m/z 321→152 and m/z 321→257

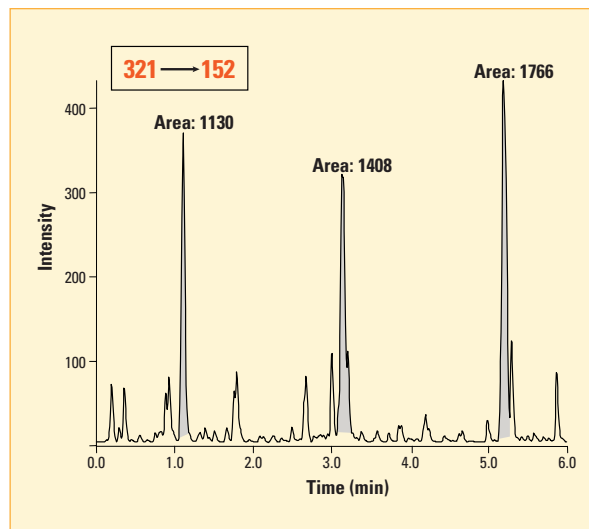


Figure 4: Triplicate injections of 50 fg chloramphenicol monitoring the m/z 321→152 transition

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