

A Rapid iMethod™ Test for Quantitation of Chloramphenicol in Seafood and Dairy Products

iMethod™ Test for Chloramphenicol Version 1.0 for Cliquant® Software

Chloramphenicol (CAP), an antibiotic, was formerly widely used in veterinary medicine, due to its broad range of activity and low cost. The treatment of animals used for production of food with CAP is prohibited in the European Union and United States because of the side effects in humans; in particular it can potentially cause fatal aplastic anemia. Thus, it is necessary to control residues of CAP in edible tissues, like milk and milk products.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Chloramphenicol in Seafood and Dairy products when using an AB SCIEX 3200 Series instrument. This method has also been developed and verified for use with the

4000 Series instrumentation. More in-depth sample preparation and instrument configuration/setting information is included as part of the standard operating procedure provided with this iMethod test, upon purchase.

Example sample preparation procedures are provided for milk, milk powder, honey, fish and seafood. While the actual protocol used is dependent upon the matrix, most protocols are based upon a simple sample homogenization, centrifugation, extraction and dilution. Deuterated chloramphenicol was added as an internal standard of known concentration during sample preparation to monitor sample recovery.

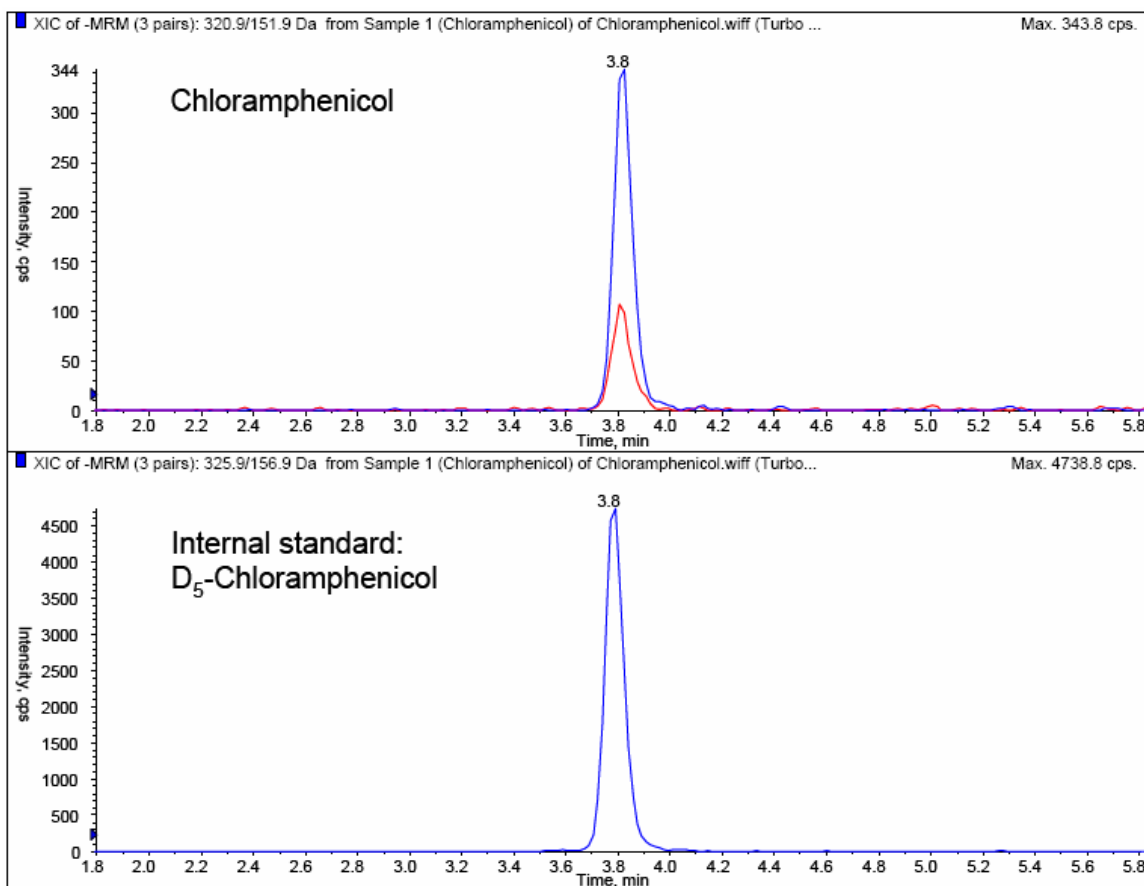


Figure 1: Chromatogram of a 0.1ng/mL chloramphenicol standard

Results

An example chromatogram of chloramphenicol is shown in Figure 1 and retention times are listed in Table 1. The excellent sensitivity of the method is highlighted by the limits of detection shown.

Table 1: MRM Transitions, Retention Times, and Limit of Detection

Compound	MRM 1	MRM 2	RT (min)	LOD (ng/mL)
Chloramphenicol	321 / 160	321 / 257	3.8	0.1
D ₅ -Chloramphenicol	326 / 157		3.8	

Calibration

The following calibration curves using the calibrator, low and high level controls are provided as examples, showing the range and linearity expected for the assay.

Please note that the results were obtained using a single instrument and single set of standards and samples, and the results here may not be typical for all instruments. Prior to production use, the method should be fully validated with real samples. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

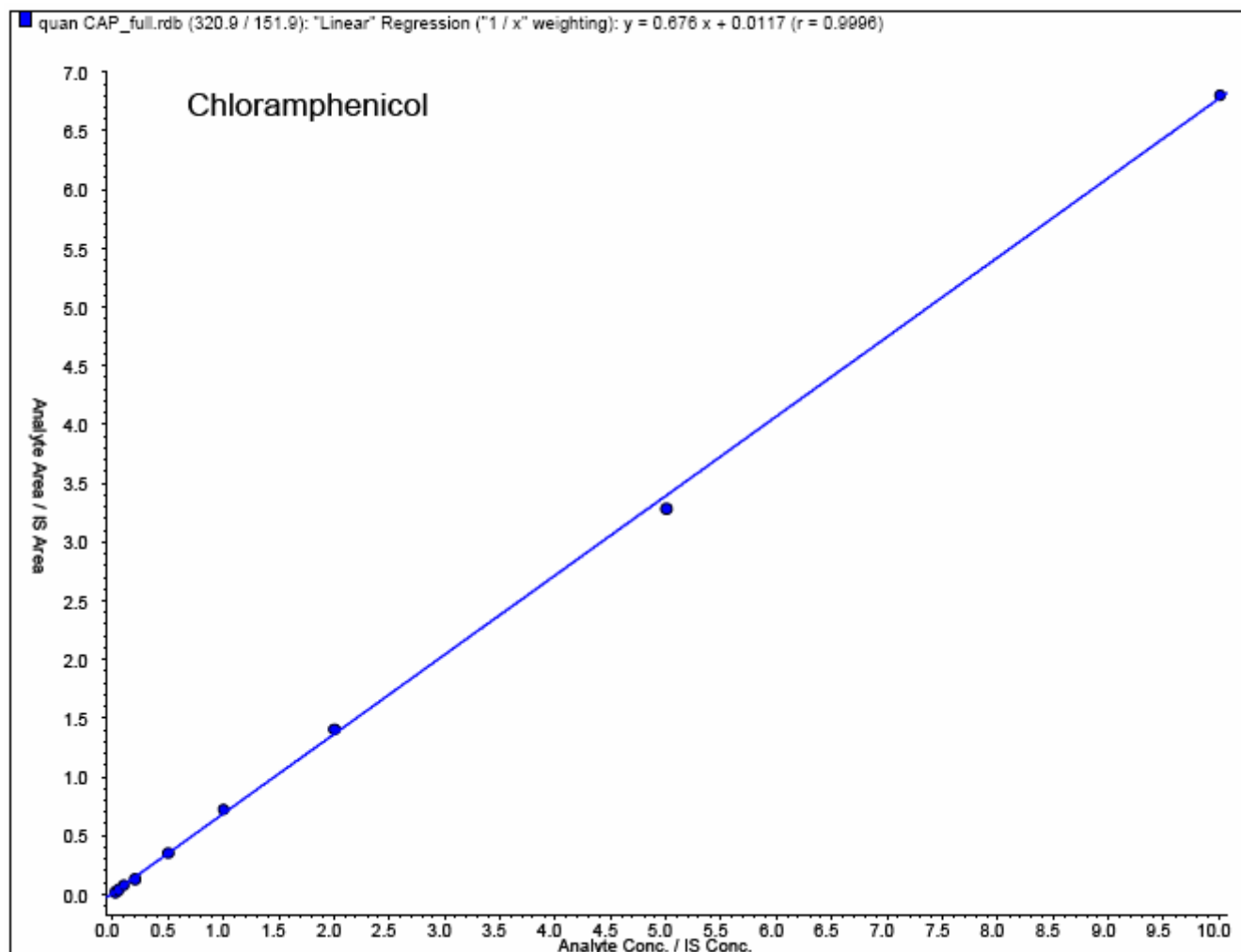


Figure 2: Representative calibration curve for chloramphenicol from 0.1 to 10 ng/mL.



System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP[®] or API 3200[™]) or 4000 Series (4000 QTRAP[®] or API 4000[™]) LC/MS/MS System
- A Shimadzu Prominence 20A LC System with reservoir tray and bottles, system controller CBM-20A, 100 µL mixer, 2 isocratic pumps LC-20AD, 3-channel degasser autosampler SIL-20AC, column oven CTO-20AC or Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate autosampler, and thermostated column oven
- Chloramphenicol standard (www.sigmaaldrich.com)
- Chloramphenicol-D₅ internal standard (www.sigmaaldrich.com)
- LC/MS-grade water, methanol, formic acid, and ammonium acetate
- 1.5 mL Eppendorf tubes
- A Phenomenex Aqua 5 µm C18 125 A 50 x 2 mm column (included with the iMethod[™] test)
- A centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- A Phenomenex Strata-X 33u Polymeric Reversed Phase cartridges, 30 mg/3 mL
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod [™] Test for Chloramphenicol Version 1.0 for Cliquant [®] Software	1034370

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod[™] Test for the Analysis of Chloramphenicol, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod test and are recommended for inexperienced users. Please consult your local sales representative for more details.

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Important Note

The iMethod[™] test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It is up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst[®] Software may be required to do so.

The purchase and use of certain of the chemicals listed below may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod procedure.

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