

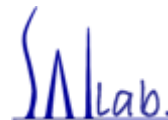
A Rapid iMethod™ Test for the Analysis of Penicillin in Meat

iMethod™ Test for Penicillin Antibiotics Version 1.0 for Cliquid® Software

Meat and other animal products need to be routinely monitored for veterinary drug residues that are used to fight disease and infection in animals, but are harmful to humans if present upon ingestion. However, with wide availability, variable legislation and variable animal husbandry practices from country-to-country, high drug residue levels in produce continue to be an issue. Over-use and inappropriate use of antimicrobial drugs can lead to increased antimicrobial resistance, reducing the ability to fight human infection. On top of this, exposure to such drugs can affect human reproduction, cause cancer and have a toxic effect.

The following description outlines the instrument requirements and expected results obtainable from the iMethod™ test for the quantification of eight penicillin antibiotics and metabolites when using an AB SCIEX 3200 QTRAP® LC/MS/MS instrument.

Sample preparation is based on extraction with acidified methanol, followed by filtration, centrifugation and dilution with



acidified water. This user-contributed iMethod™ test for penicillins includes method documentation, MS and LC method files, processing method files and report templates, all deployed as a new iMethod test in your Cliquid® software. More in-depth sample preparation and instrument parameter information is included as part of the standard operating procedure provided with the method, as are the required analytical columns; solvents, standards and any supplies required for sample preparation are not included.

The mobile phase consists of water with ammonia and acetonitrile with separation on Phenomenex Gemini C18 3µm 4.6 x 150 mm HPLC column. An example chromatogram of the separation achieved is shown below in figure 1.

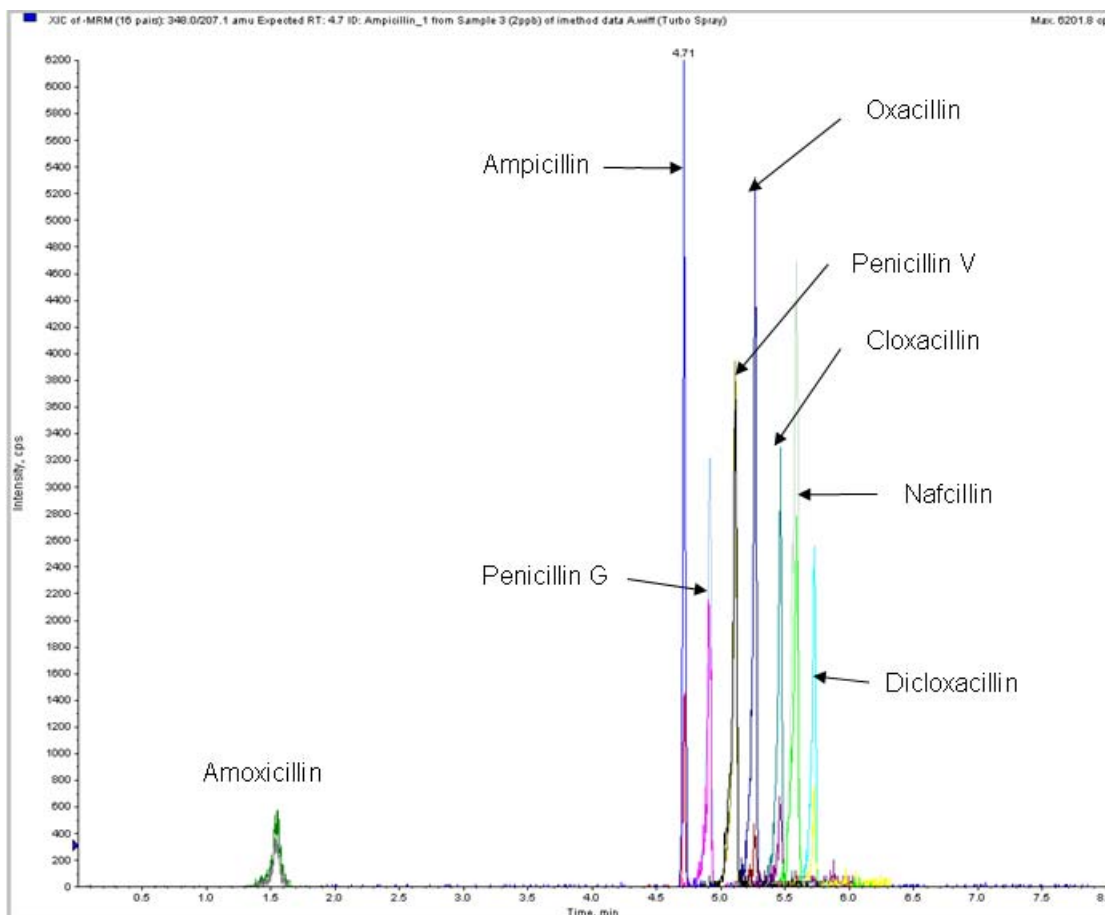


Figure1. Example chromatogram for an injection of a 2 ppb standard, showing all the penicillins overlaid in one window

Results

Figures 2 and 3 illustrate the performance of the method for two transitions for all eight analytes.

Please note that the results presented 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.

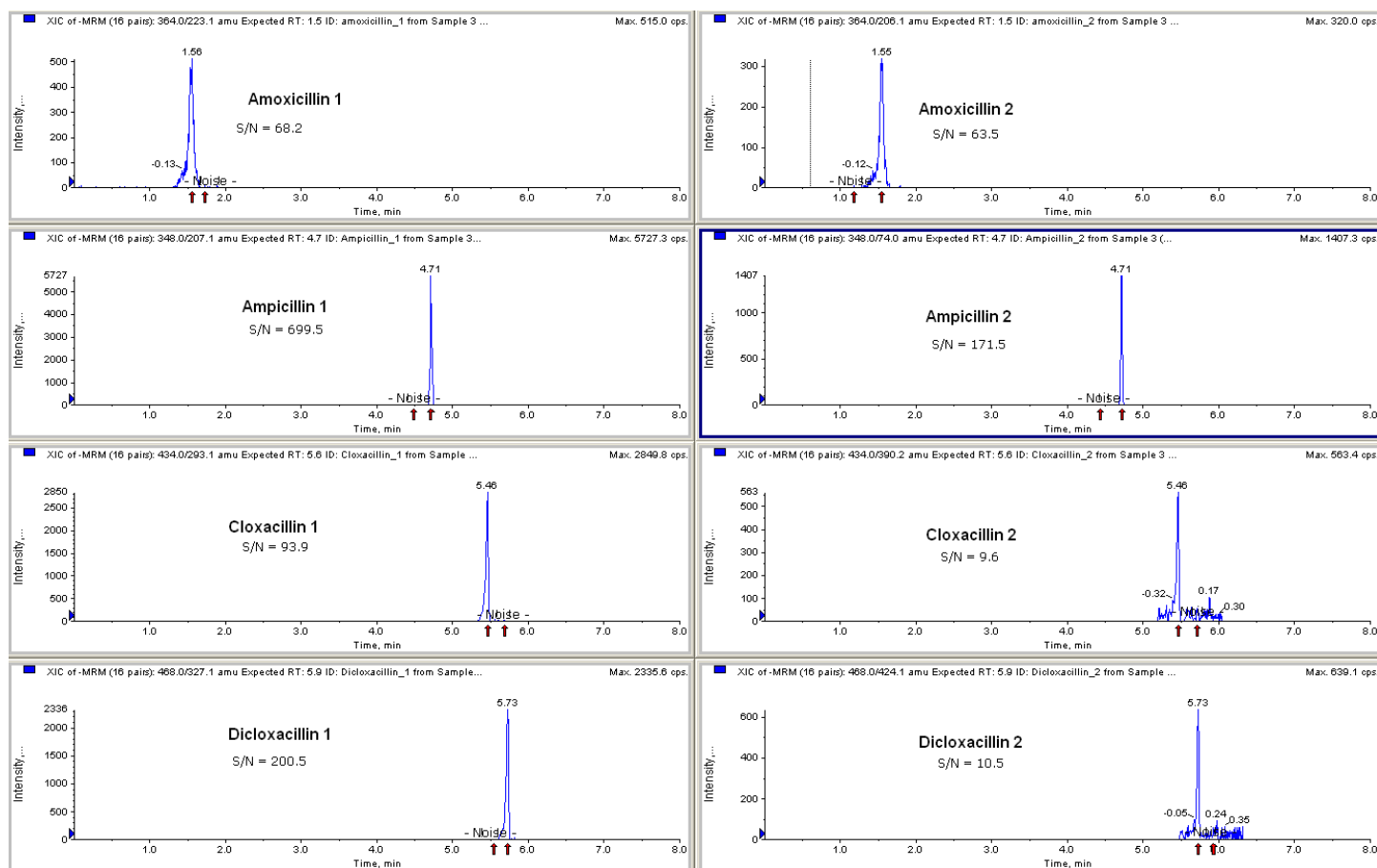


Figure 2. Example chromatograms for two MRM transitions of 2 ppb standards of amoxicillin, ampicillin, cloxacillin, and dicloxacillin (signal-to-noise measured using PEAK:PEAK script on smoothed data).

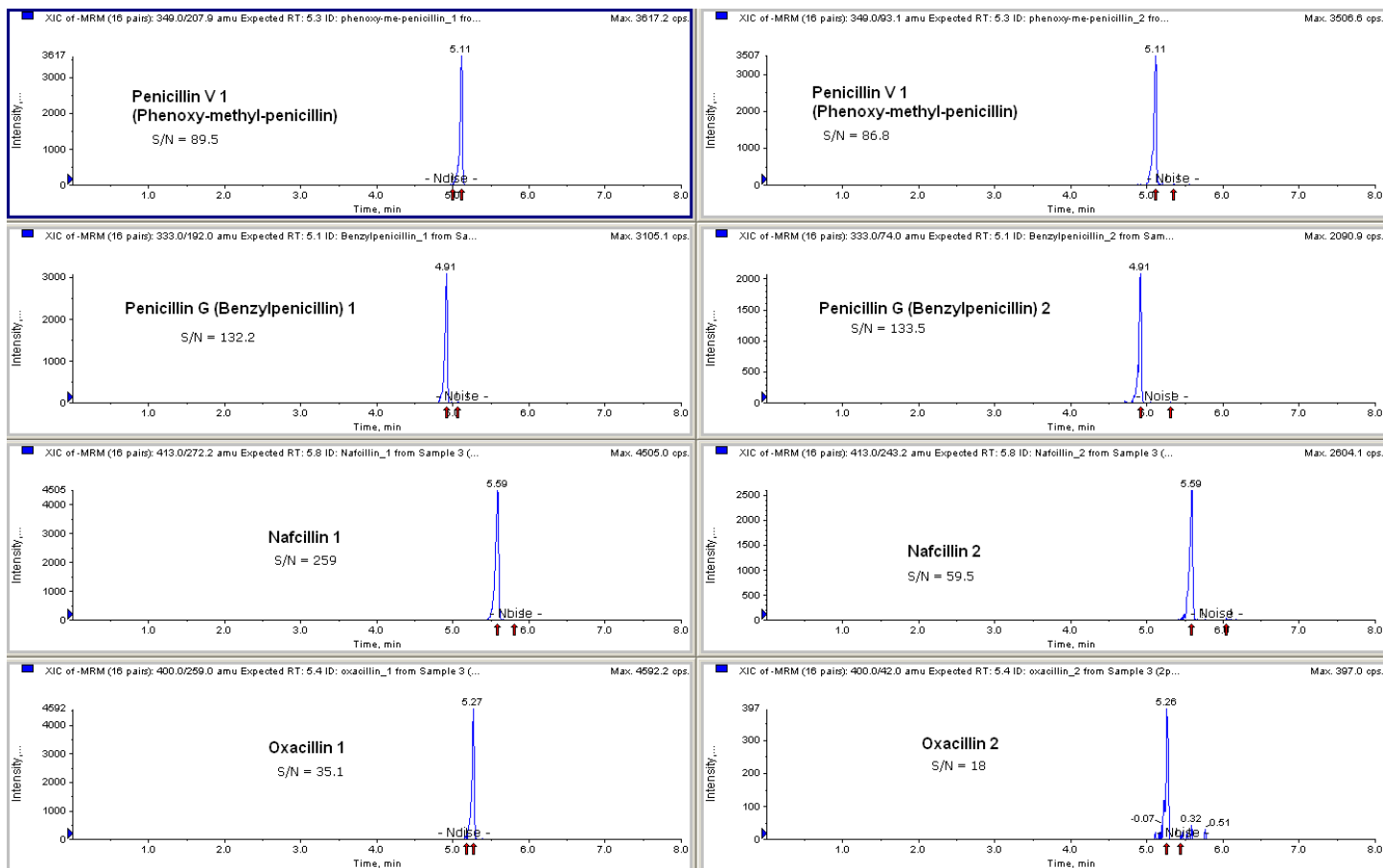


Figure 3. Example chromatograms for two MRM transitions of 2 ppb standards of penicillin V, penicillin G, nafcillin, and oxacillin (signal-to-noise measured using PEAK:PEAK script on smoothed data).

The following two calibration curves are representative of the performance obtained on the instrument using the method described here. No internal standards were used in the analysis reducing the overall cost per sample.

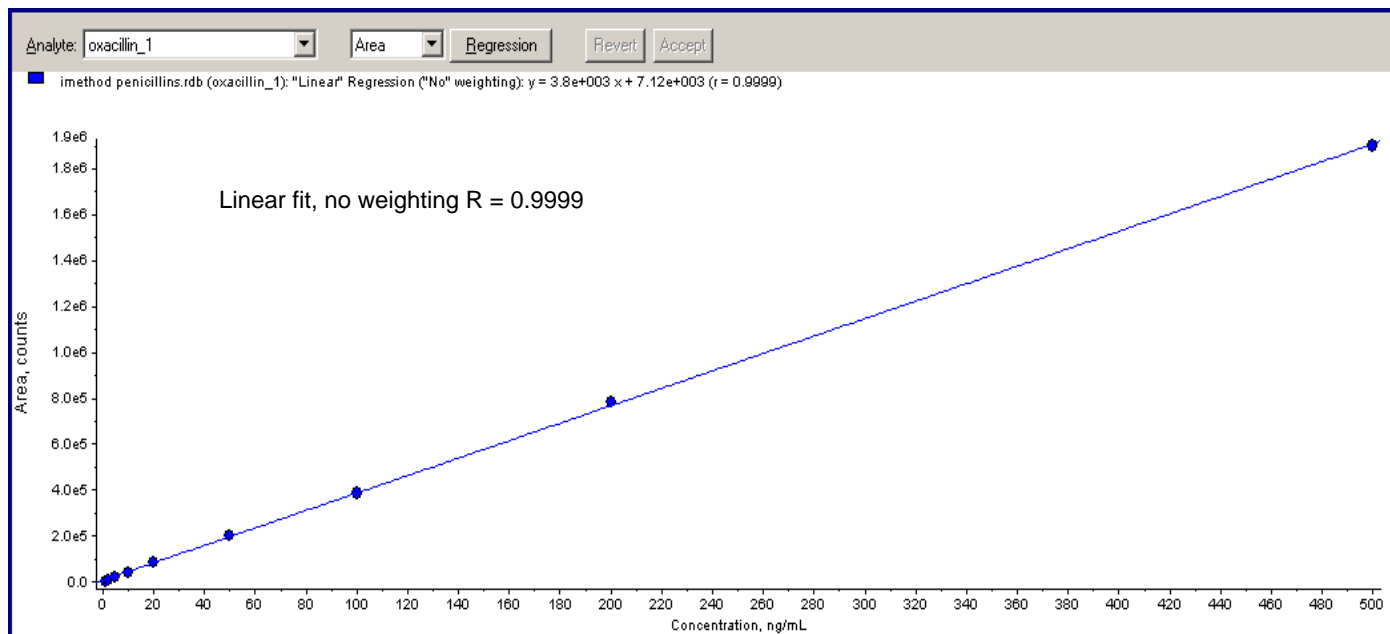


Figure 4. Calibration curve for oxacillin 1, solvent standards in water 1 to 500 ppb (no internal standard used).

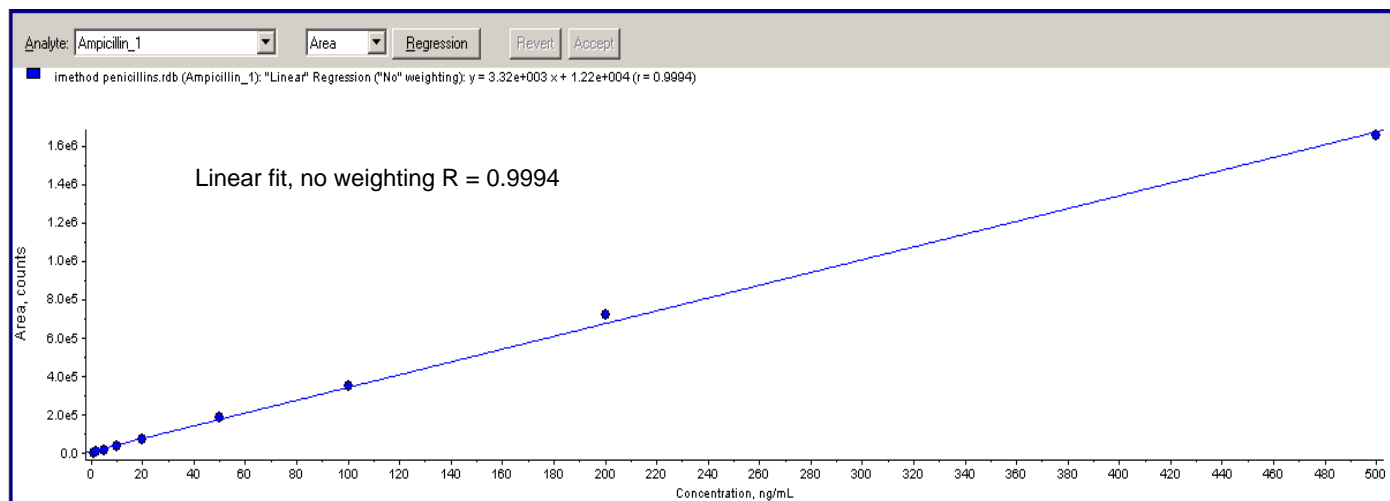


Figure 5. Calibration curve for ampicillin 1, solvent standards in water 1 to 500 ppb (no internal standard used).

System Requirements

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

- An AB SCIEX 3200 QTRAP[®] LC/MS/MS System
- Penicillin standards (www.sigmaaldrich.com)
- LC/MS-grade water and acetonitrile
- Shimadzu XR HPLC system or equivalent
- A Phenomenex Gemini 150 X 4.6 mm, 3 µm, HPLC column
- Phenomenex Strata C18 SPE cartridge (200 mg) 8B-S001-FBJ
- Pipettes and standard laboratory glassware

Please note that the Phenomenex HPLC column is required but not included with this iMethod[™] test. This method can also be run on other HPLC systems, given that they are supported for use by Cliquant[®] Software and the retention times are updated to reflect the configuration used.

Product Name	Part Number
iMethod [™] Test for Penicillins for Cliquant [®] Software	Free download at absciex.com

Important Note

The purchase and use of certain of the chemicals listed above 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.

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.

For Research Use Only. Not for use in diagnostic procedures.

© 2010 AB SCIEX. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX[™] is being used under license.

Publication number: 1860610-01