A Rapid iMethod™ Test for the Quantitation of Nitrofuran Metabolites in Meat and Shrimp, Liver and Honey

iMethod™ Test

While Nitrofuran based antibiotics like Furazolidone, Furaltadone, Nitrofurazone, and Nitrofurantoine have been commonly used to prevent infections like that of gastrointestinal infections in cattle and poultry in the past. However, these antibiotics were banned by the European Union since 1995 due to mutagenic and carcinogenic concerns. Given this, any meat, poultry or shellfish products entering the European Union must be free of all such compounds. Since the in-vivo half-life time of the parent compounds is on the order of a few hours, the analysis carried out to screen for such compounds is for the metabolites, namely, 3-Amino-2-oxazolidinone **(AOZ)**, 3-Amino-5-morpholinomethyl-2-oxazolidinone **(AMOZ)**, Semicarbazide **(SEM)**, and 1-Amino-hydantoin **(AHD).**

The following description outlines the instrument requirements and expected results obtainable from AB SCIEX iMethod™ Test for the Analysis of Nitrofuran Metabolites in Meat and Shrimp when using an AB SCIEX 3200 Series instrument. This method has also been developed and verified for use with 4000 Series instrumentation. More in depth sample preparation, and instrument configuration / setting information is included as part of the standard operating procedure that is included with this iMethod Test upon purchase.

Example sample preparation procedures are provided for Meat, Shrimp, Liver and Honey. While the actual protocol outlined is dependent upon the matrix to be used most protocols are based upon a homogenization, derivitization, extraction and dilution. Deuterated and C13 labeled internal standards of known concentration are added during sample preparation to monitor sample recovery.

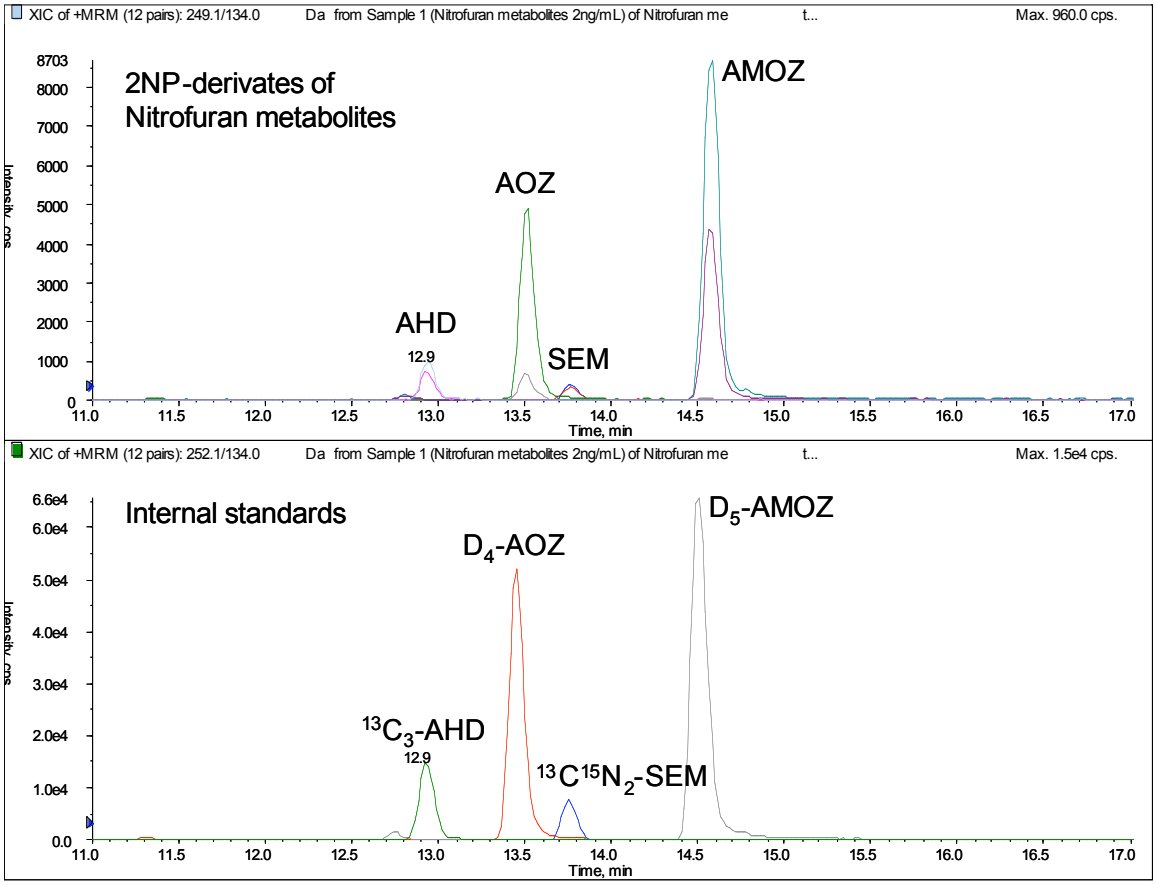

Figure 1: Chromatogram of a Nitrofuran Metabolites (2ng/mL) analyzed by LC/MS/MS

Results

An example chromatogram of all Nitrofuran Metabolites is shown in Figure 1 and retention times of all substances are listed in Table 2. The superior sensitivity of the method is highlighted by the limits of detection shown. Signal-to-Noise varies depending on the ionization and fragmentation efficiency of each analyte. Thus Limits of Quantitation (LOQ) are different for each compound and are dependent upon both sample preparation as well as the efficiency of derivitisation.

Table 1: Multiple Reaction Monitoring (MRM) transitions, retention times, limit of detection (LOD) in ? based on the qualifier MRM transition, and coefficient of variation (n=3) of all detected substances

Compound	MRM1	MRM2	RT (Min)	LOQ (ng/mL)*
2NP-SEM	209.1/ 192.1	209.1 / 166	13.7	0.1
2NP-AOZ	236.1 / 134.0	236.1 / 192.1	13.5	0.02
2NP-AHD	249.1 / 134.0	249.1 / 104.0	12.9	0.02
2NP-AMOZ	335.1 / 291.1	335.1 / 262.1	14.6	0.01

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.

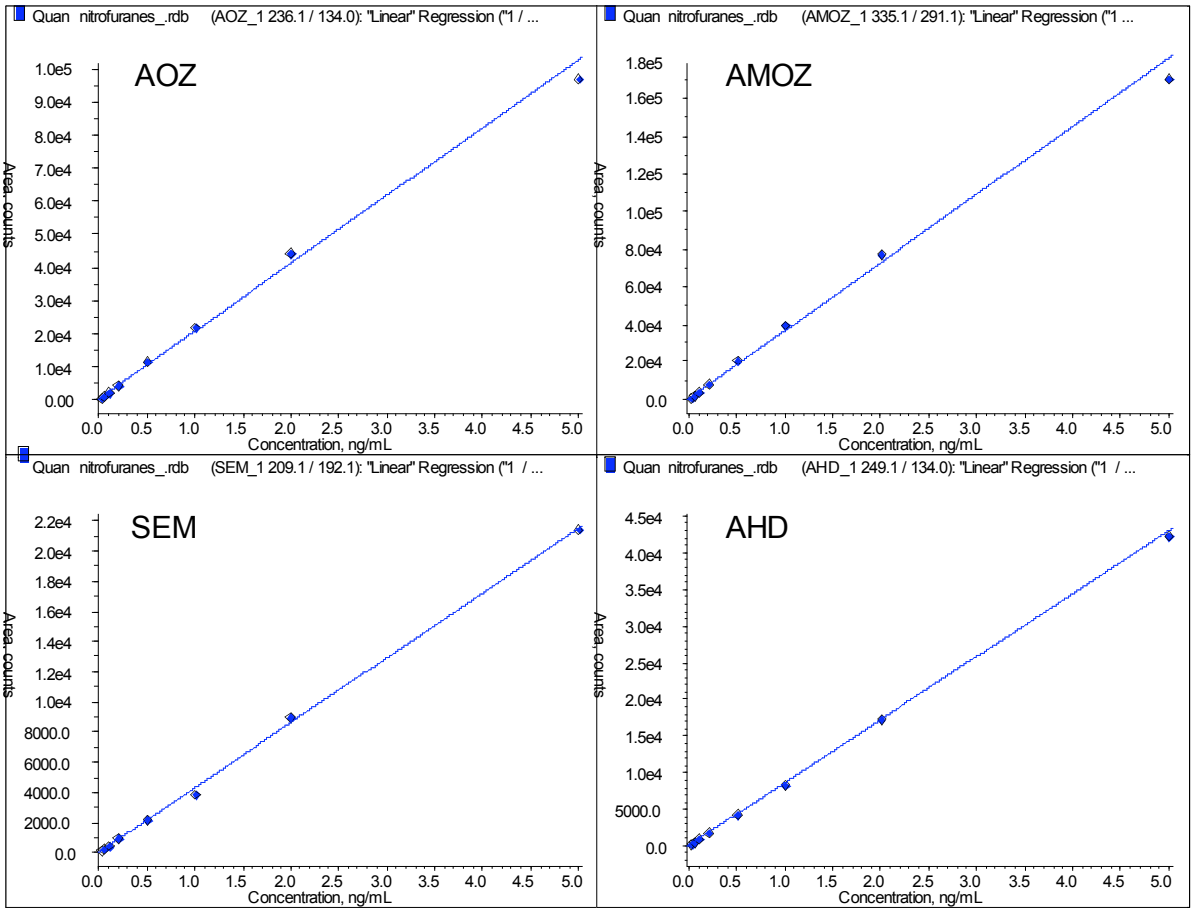

Figure 2: Representative calibration curves for Nitrofuran Metabolites that demonstrate the linear dynamic range from 0.01-5 ng/ml

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. 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.

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 auto sampler, and thermostated column oven.
- Nitrofuran Metabolite standards from Sigma (www.sigmaaldrich.com)
- Internal standards for AHD, AMOZ, AOZ and SEM (www.sigmaaldrich.com)
- 2-Nitrobenzaldehyde Derivitization Reagent (www.sigmaaldrich.com)
- LC/MS Grade Water, Methanol and Ammonium Acetate
- 1.5 mL Eppendorf tubes
- A Phenomenex, LUNA 3u C18 (2), 150 x 2 mm Column
- SPE Sample preparation catridges (clean protocol dependent)
- A centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod™ Test for Nitrofuran Metabolites Version 1.0 for Cliquid® Software	1034365

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 us 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 below 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 method transfer parameters shown in the table above are provided for the convenience of users with instrumentation different from the instrumentation for which this iMethod™ Test was developed. These parameters are generic and not derived experimentally for this test. In some cases, we provide the iMethod™ Test for a wider combination of instruments and during installation you may be able to install the correct iMethod™ Test for your configuration. The MS and LC portions of the method described herein are for specific instrument configurations, but are generic enough that they can be easily translated for other configurations. It is the responsibility of the end user to validate assays and transfer parameters and to comply with any regulatory requirements that pertain to their procedures and uses of the methods AB SCIEX offers both off and on-site support to assist you in translation of the iMethod™ test for your specific configuration. Please contact your local AB SCIEX Sales representative for more information about the services available.

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