A Rapid iMethod™ Test for the Analysis of Fluoroquinolone Antibiotics in Food

iMethod™ Test for Fluoroquinolones Screening version 2.0 for Cliquid® Software

Fluoroquinolones are broad-spectrum antibiotics used in both human and veterinary medicine. The presence of fluoroquinolone residue in meat has been implicated in the development of resistant pathogens. Because of these concerns, the FDA has banned the extra-label use of these products in food producing animals.

The method included is for the routine analysis of 20 fluoroquinolones and quinolones and their metabolites in animal tissue at or below 25 ppb.The method used three MRM transitions per compound to provide an extra degree of confirmation. This method is based upon USDA-FSIS protocol no# CLG-FLQ2.00.

Sample preparation is based upon a simple homogenization, extraction, centrifugation and reconstitution.

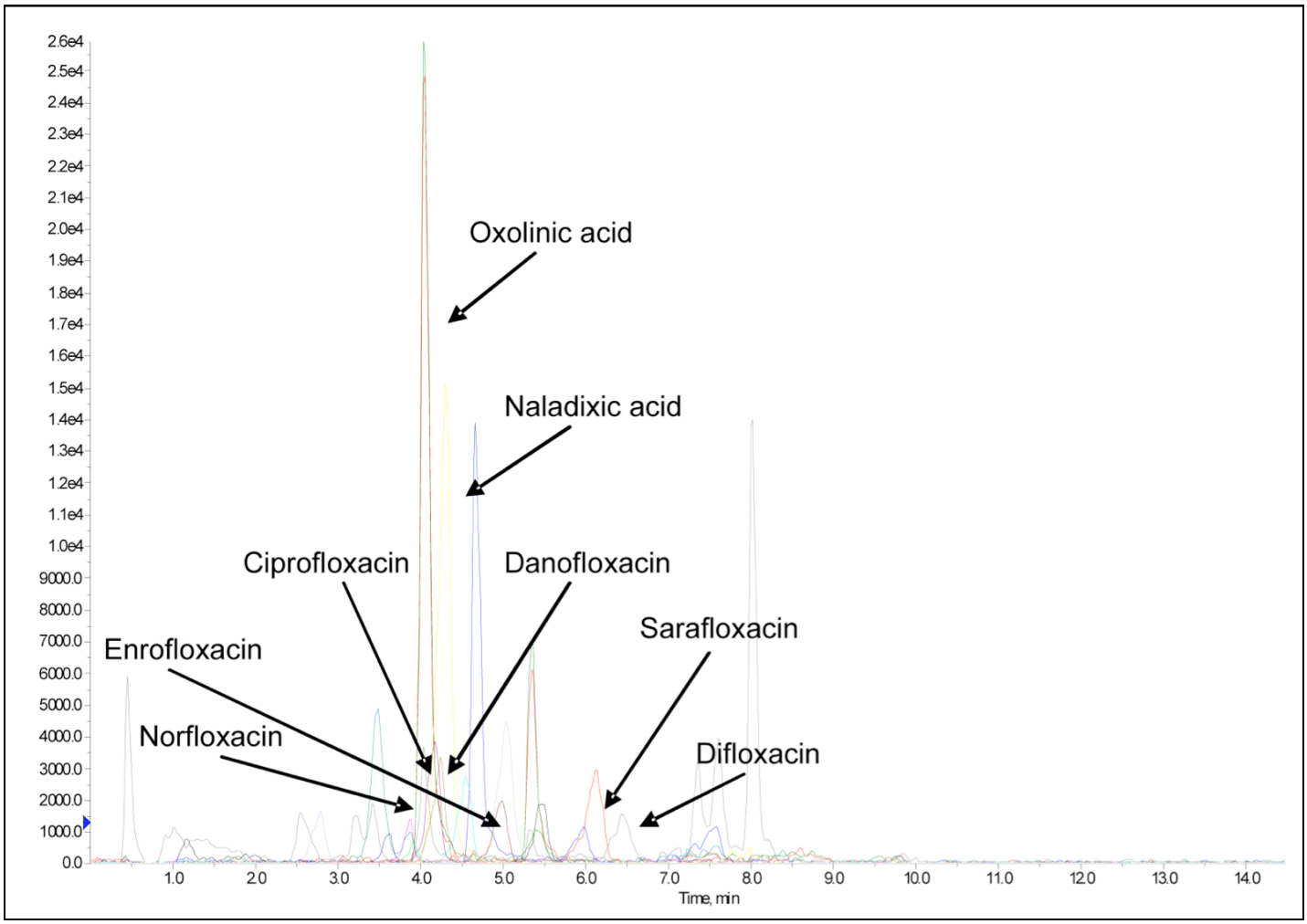

Figure 1: Chromatogram of fluoroquinolones and quinolones and their metabolites at 25 ppb in a spiked meat sample.

Table 1. Compound names, MRM Transitions and retention times for compound included in this method

Compound	MRM 1	MRM 2	MRM 3	RT
Ciprofloxacin	332 / 314	332 / 288	332 / 245	4.37
Enrofloxacin	360 / 316	360 / 342	360 / 245	5.31
Norfloxacin	320 / 276	320 / 302	320 / 233	4.04
Sparfloxacin	393 / 349	393 / 292	393 / 251	6.45
Danafloxacin	358 / 340	358 /314	358 / 283	4.52
Enoxacin	321 / 303	321 / 277	321 / 234	3.67
Lomefloxacin	352 / 308	352 / 265	352 / 237	4.78
Difloxacin	400 / 356	400 / 299	400 / 382	6.80
Marbofloxacin	363 / 320	363 / 345	363 / 72	4.12
Clinafloxacin	366 / 305	366 / 348	366 / 264	5.23
Oxolinic Acid	262 / 244	262 / 216	262 / 160	4.07
Flumequine	262 / 244	262 / 202	262 / 174	5.37
Nalidixic	233/ 215	233 / 187	233 / 159	4.69
Pipemidic Acid	304 / 217	304 / 286	304 / 189	2.93
Cinoxacin	263 / 245	263 / 217	263 / 189	3.41
Rifloxacin	364 / 263	364 / 320	364 / 235	3.81
Sarafloxacin	386 / 342	386 / 299	386 / 368	6.26
Orbifloxacin	396 / 295	396 / 352	396 / 267	5.71
Gatifloxacin	376 / 261	376 / 289	376 / 332	5.77
Fleroxacin	370 / 326	370 / 269	370 / 352	4.41

Calibration

The following example calibration curves for danofloxacin and sarafloxacin demonstrate the linear dynamic range achievable for this method.

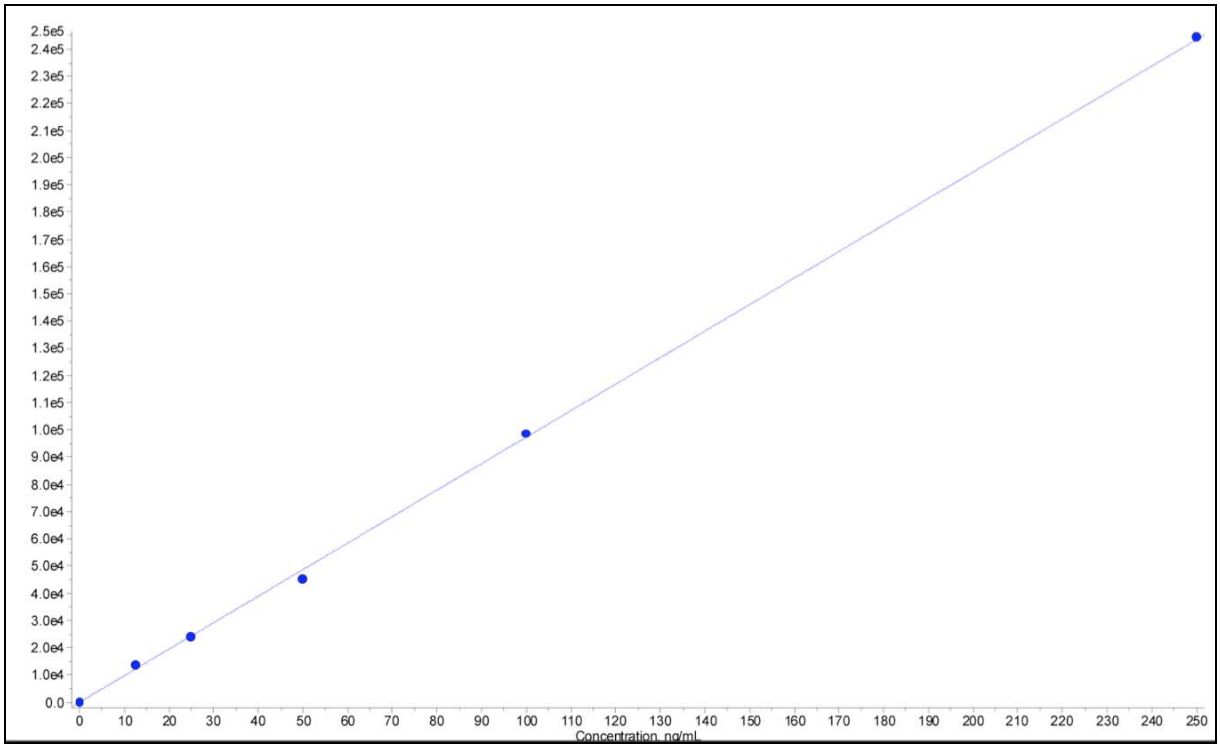

Figure 2: Representative calibration curves for danofloxacin and sarafloxacin at a concentration range from 12.5 to 250 ppb.

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.
- Fluoroquinolones and quinolones standards from Sigma Aldrich (www.sigmaaldrich.com)
- LC/MS grade water, acetonitrile and formic acid
- 1.5 mL Eppendorf tubes
- A Restek Allure PFP Propyl Column, 150 x 4.6 mm, 5 µm particle size (included in the iMethod Test)
- 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 Fluoroquinolones Screening version 2.0 for for Cliquid® Software	free download at www.absciex.com

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of fluoroquinolones, 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.

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.

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Publication number: 1046010-01

Headquarters
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

International Locations
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