phenomenex®
...breaking with tradition℠

# Analysis of Antibiotics in Meat for Human Consumption

YunYun Zhou*, Dave Lavorato*, Terrell Mathews† and Sky Countryman†
* Applied Biosystems/MDS Analytical Technologies, 71 Four Valley Drive Concord, Ontario, L4K 4V8, Canad
† Phenomenex, Inc., 411 Madrid Ave, Torrance, CA 90501 USA (www.phenomenex.com)

PO66690109_L_1

# Abstract

In modern mass production operations, many animal feeds, which are used in beef, pork, chicken and turkey production, contain antibiotics as prophylactics.[1] The beta-lactam, macrolide, sulfonamide, tetracycline, and other antibiotics are an indispensable part of food animal production and help to maintain the optimal health of the animals. However, the residues of antibiotics remaining in animal-derived human foods may pose potential human health hazards toxicologically, microbiologically or immunopathologically.[1, 2, 3, 4] Many countries have built a series of regulations to the use, dosage, and withdrawal times for many of these antibiotic classes in animal production.

While there are several methods to determine antibiotic residues including bioassays, immunoassay, thin layer, and so on, LC/MS/MS is much more compelling due to its higher specificity and sensitivity, which lead to better detection and confirmation.[1, 2]

The method described here is for screening of seven classes of antibiotics: beta-lactam, tetracycline, sulfonamide, macrolide, amphenicol, fluoroquinolone, and flunixin. HPLC method develoment issuues are explored and a newly introduced high efficiency core-shell paricle is used as HPLC media to optimize retention and provide higer sensitivity of analysis.

# Experimental Conditions

**Agilent 1100 system**

Vacuum degasser

Binary pump

Auto sampler

Column oven

**Separation Column:**

Phenomenex

Analytical column, Synergi™ Polar-RP 2.5 µm 90 Å, 50 x 2.0 mm (Part No. 00B-4336-B0)

Analytical column, Gemini®-NX C18 3 µm 110 Å, 50 x 2.0 mm (Part No. 00B-4439-B0)

Analytical column, Kinetex™ Core-Shell 2.6 µm 100 Å, 50 x 2.1 mm (Part No. 00B-4462-AN)

# Experimental Conditions

**Gradient – Agilent 1100 Series**

**Flow Rate:** 0.5 mL/min
**Column oven temperature:** 40 °C
**Injection Volume:** 10 µL

| Total Time (min) | Flow Rate (µL/min) | A (%) | B (%) |
|---|---|---|---|
| 0.00 | 500 | 98 | 2 |
| 0.30 | 500 | 98 | 2 |
| 7.27 | 500 | 20 | 80 |
| 7.37 | 500 | 1 | 99 |
| 9.50 | 500 | 1 | 99 |
| 10.00 | 500 | 98 | 2 |
| 13.00 | 500 | 98 | 2 |

# Experimental Conditions

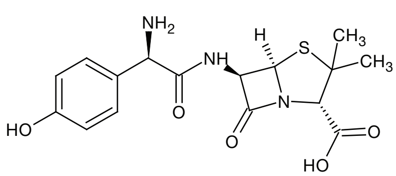
## Gradient – Agilent 1100 Series

- **Optimized for 4000 QTRAP® LC/MS/MS systems**
- **Turbo V™ Ion Source**
- **Positive polarity and negative polarity separately**

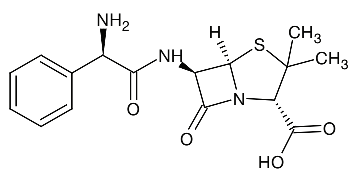
# Experimental Conditions

### Beta-Lactams

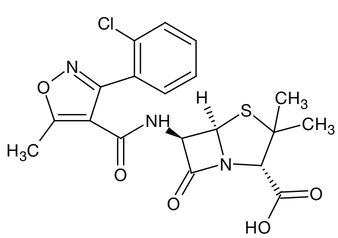
Amoxicillin: $C_{16}H_{19}N_3O_5S$
CAS: 26787-78-0 MW: 365.10



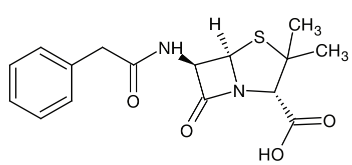
Ampicillin: $C_{16}H_{19}N_3O_4S$
CAS: 69-53-4 MW: 349.11



Cloxacillin: $C_{19}H_{18}ClN_3O_5S$
CAS: 61-72-3 MW: 435.07

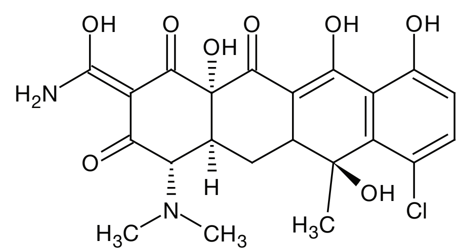


Penicillin G: $C_{16}H_{18}N_2O_4S$
CAS: 61-33-6 MW: 334.10

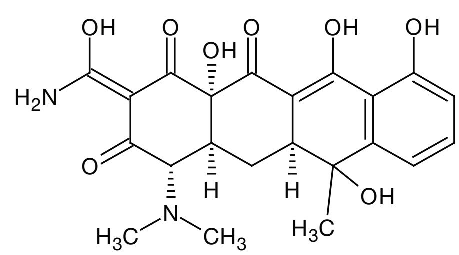


### Tetracyclines

Chlorotetracycline: $C_{22}H_{23}ClN_2O_8$
CAS: 57-62-5 MW: 478.11



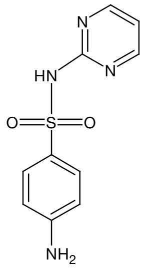
Tetracycline: $C_{22}H_{24}N2O_8$
CAS: 60-54-8 MW: 444.15



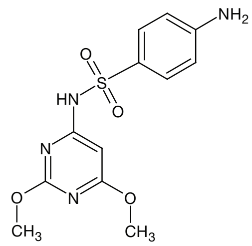
# Experimental Conditions

## Sulfonamides

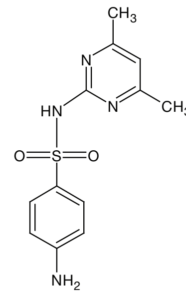
Sulfadiazine: $C_{10}H_{10}N_4O_2S$
CAS: 68-35-9 MW: 250.05



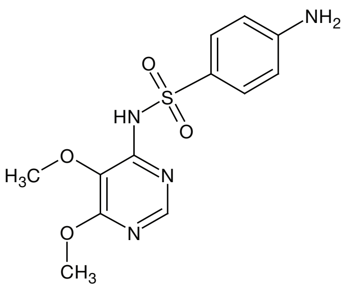
Sulfadimethoxine: $C_{12}H_{14}N_4O_4S$
CAS: 122-11-2 MW: 310.0736



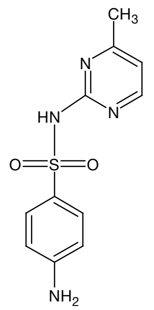
Sulfamethazine: $C_{12}H_{14}N_4O_2S$
CAS: 57-68-1 MW: 278.08



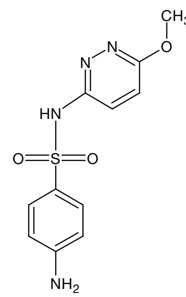
Sulfadoxine: $C_{12}H_{14}N_4O_4S$
CAS: 2447-57-6 MW: 310.07



Sulfamerazine: $C_{11}H_{12}N_4O_2S$
CAS: 127-79-7 MW: 264.07



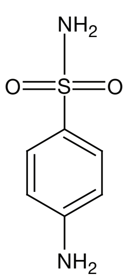
Sulfamethoxypyridazine: C11$H_{12}N_4O_3S$
CAS: 80-35-3 MW: 280.06



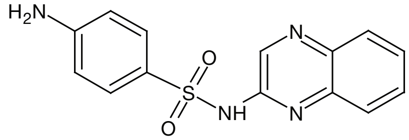
# Experimental Conditions

## Sulfonamides

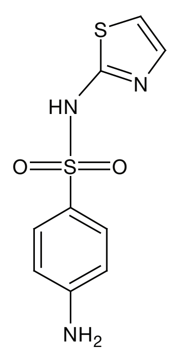
Sulfanilamide: $C_6H_8N_2O_2S$
CAS: 63-74-1 MW: 172.03



Sulfaquinoxaline: $C_{14}H_{12}N_4O_2S$
CAS: 59-40-5 MW: 300.07



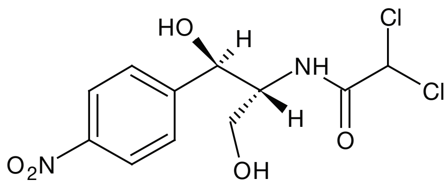
Sulfathiazole: $C_9H_9N_3O_2S_2$
CAS: 72-14-0 MW: 255.01



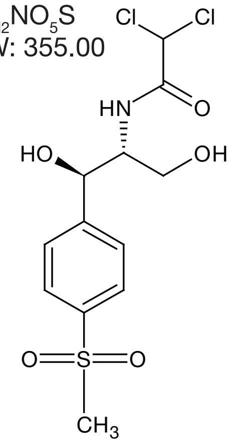
# Experimental Conditions

## Amphenicols

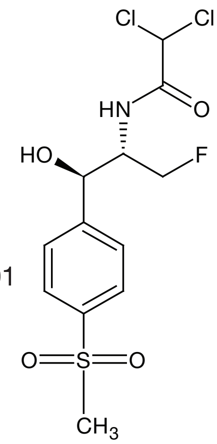
Chloramphenicol: $C_{11}H_{12}C_{l2}N_{2}O_{5}$
CAS: 56-75-7 MW: 322.01



Thiamphenicol: $C_{12}H_{15}C_{l2}NO_{5}S$
CAS: 15318-45-3 MW: 355.00



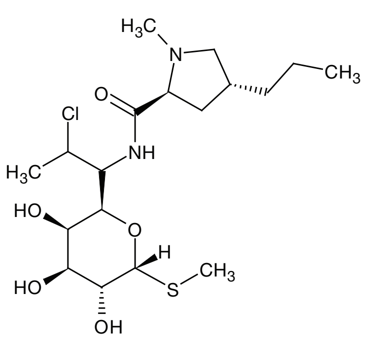
Florfenicol: $C_{12}H_{14}Cl_{2}FNO_{4}S$
CAS: 73231-34-2 MW: 357.01



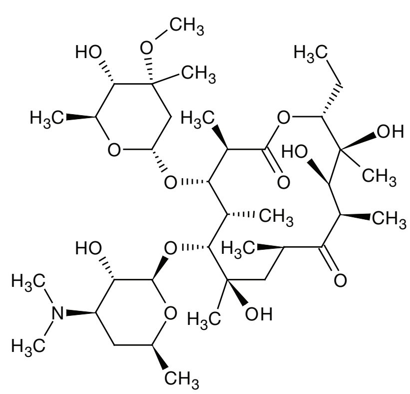
# Experimental Conditions

## Macrolides

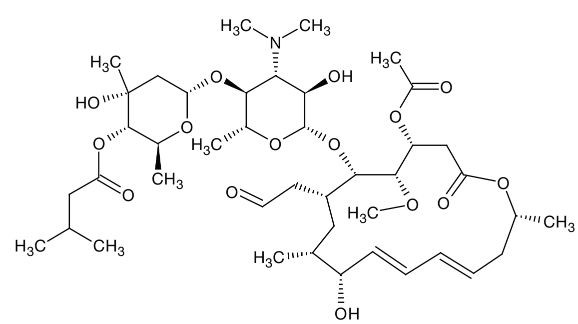
Clindamycin: $C_{18}H_{33}ClN_{2}O_{5S}$
CAS: 18323-44-9 MW: 424.18



Erythromycin: $C_{37}H_{67}NO_{13}$
CAS: 114-07-8 MW: 733.46



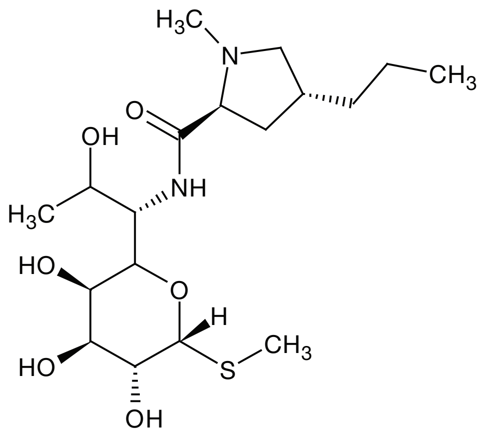
Josamycin: $C_{42}H_{69}NO_{15}$
CAS: 16846-24-5 MW: 827.47

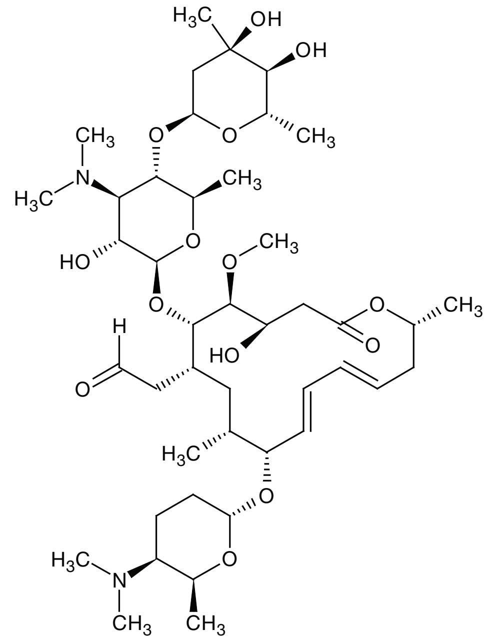


# Experimental Conditions

## Macrolides

Lincomycin: $C_{18}H_{34}N_2O_6S$
CAS: 154-21-2 MW: 406.21

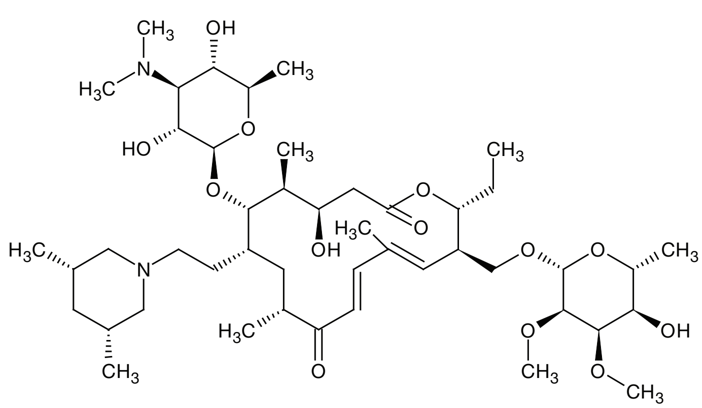




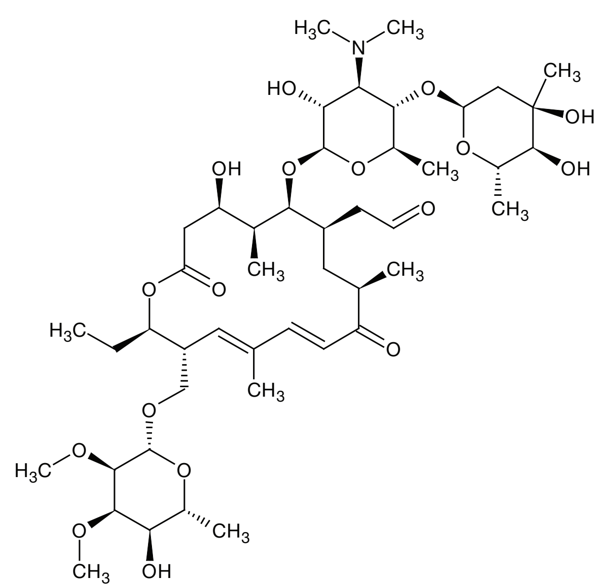
# Experimental Conditions

## Macrolides

Tilmicosin: $C_{46}H_{80}N_2O_{13}$
CAS: 108050-54-0 MW: 868.57



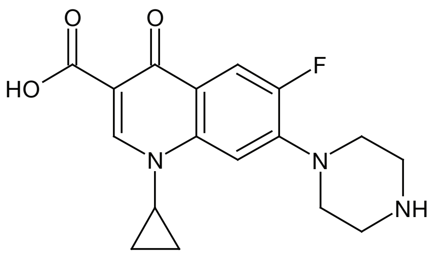
Tylosin: $C_{46}H_{77}NO_{17}$
CAS: 1401-69-0 MW: 915.52



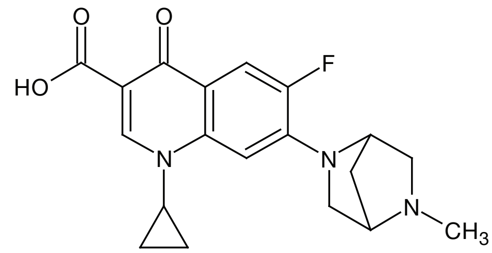
# Experimental Conditions

## Fluoroquinolones

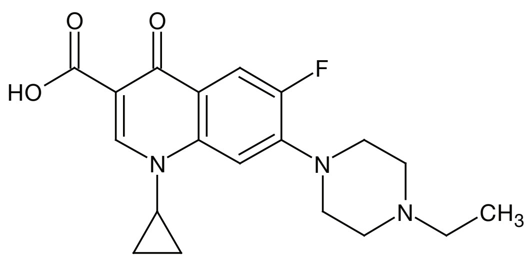
Cipprofloxacin: $C_{17}H_{18}FN_3O_3$
CAS: 852721-33-1 MW: 331.13



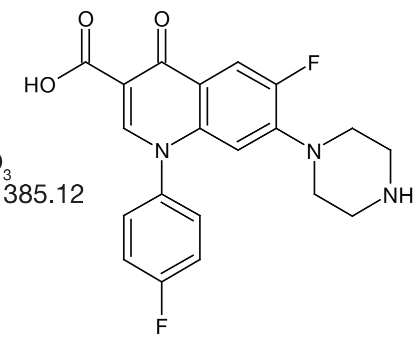
Danofloxacin: $C_{19}H_{20}FN_3O_3$
CAS: 112398-08-0 MW: 357.15



Enrofloxacin: $C_{19}H_{22}FN_3O_3$
CAS: 93106-60-6 MW: 359.16



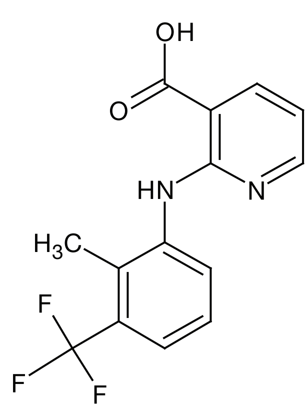
Sarafloxacin: $C_{20}H_{17}F_2N_3O_3$
CAS: 98105-99-8 MW: 385.12



# Experimental Conditions

## Flunixin

Flunixin: $C_{14}H_{11}F_3N_2O_2$
CAS: 38677-85-9 MW: 296.08



# Experimental Conditions

## Extended Sample Preparation

***Method for analysis of antibiotics in beef kidney/juice or serum 5:***

Weigh 1 g of homogenized beef kidney sample, or kidney juice or serum and add it to a 50 mL FEP (fluorinated ethylene propylene) tube. Alternatively you can use a disposable polypropylene Corning tube.

Add 2 mL water and 8 mL acetonitrile.

Vortex briefly and shake for 5 minutes.

Centrifuge at 3450 RCF for 5 minutes.

Decant the supernatant into a 50 mL tube with 500 mg C18 sorbent.

Vortex briefly and shake for 30 seconds.

Centrifuge at 3450 RCF for 1 minute.

Place 5 mL aliquot of the supernatant into a graduated tube.

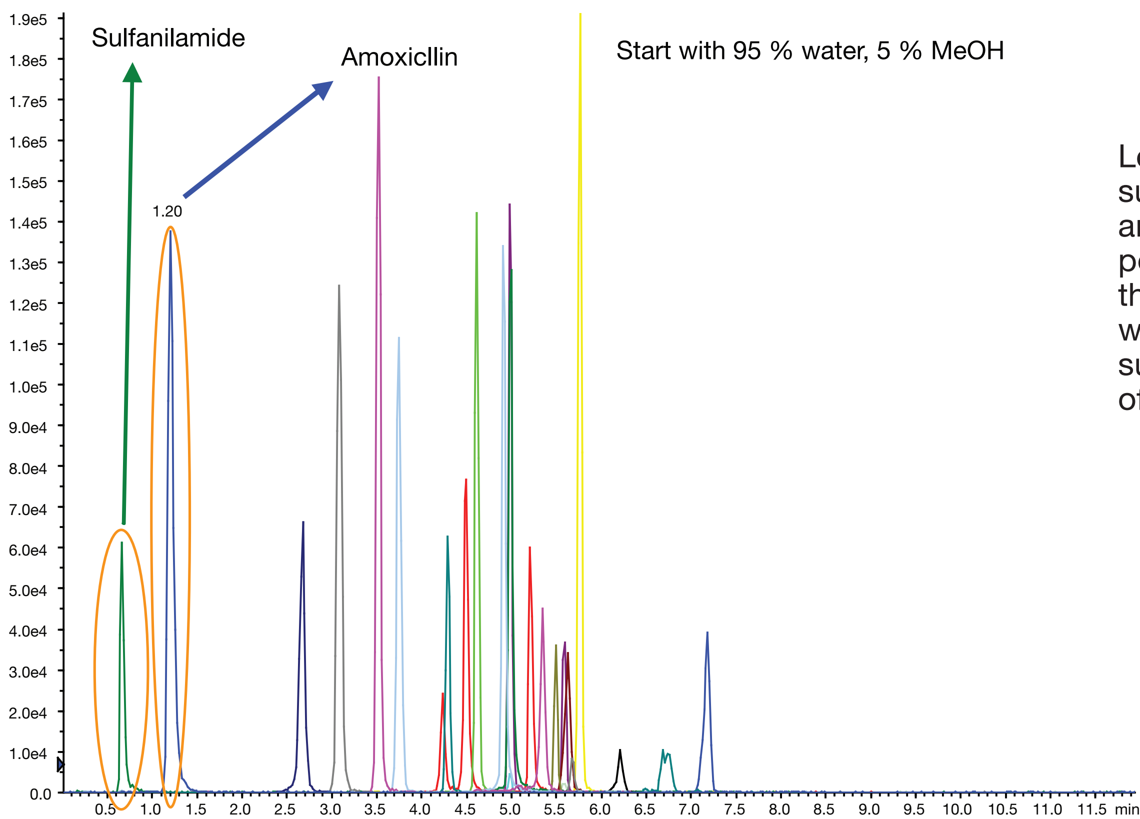
Evaporate down to less than 1 mL.

Make up the volume to 1 mL with water.

Filter the extracts (PVDF, 0.45 mm).The extracts are now ready for LC/MS/MS analysis.

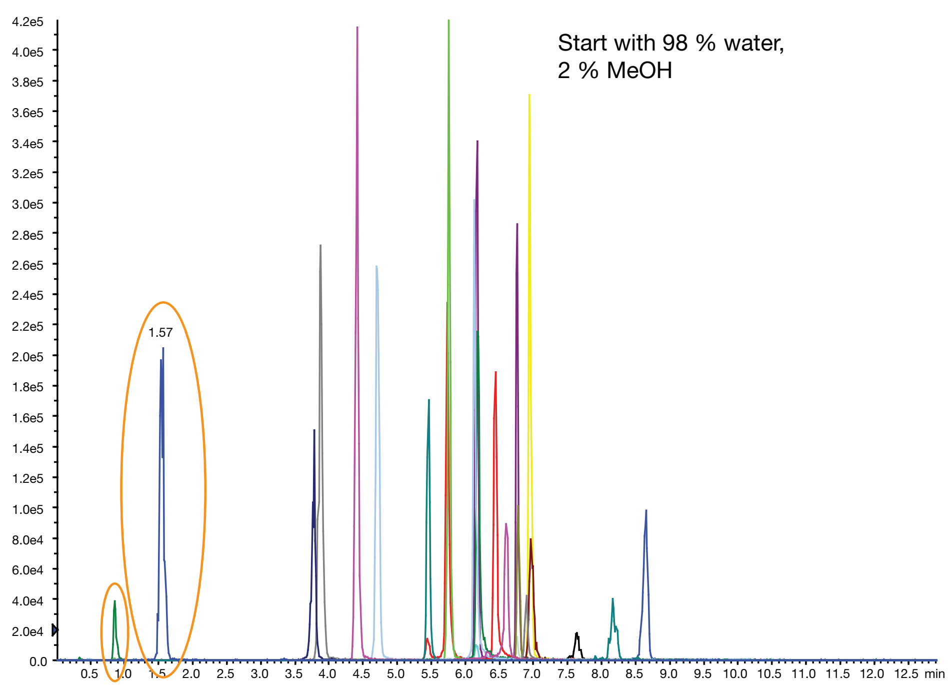
# LC Method Development

**Column: Phenomenex Synergi 2.5 µm Polar-RP**
**Mobile phase: $H_2O$ (Water) / MeOH (Methanol) + 0.1 % F.A. (Formic Acid), Gradient**




Low retention of sulfanilamide and amoxicillin are a potential problem as they are eluting in what may be the ion suppression zone of meat samples.

# LC Method Development

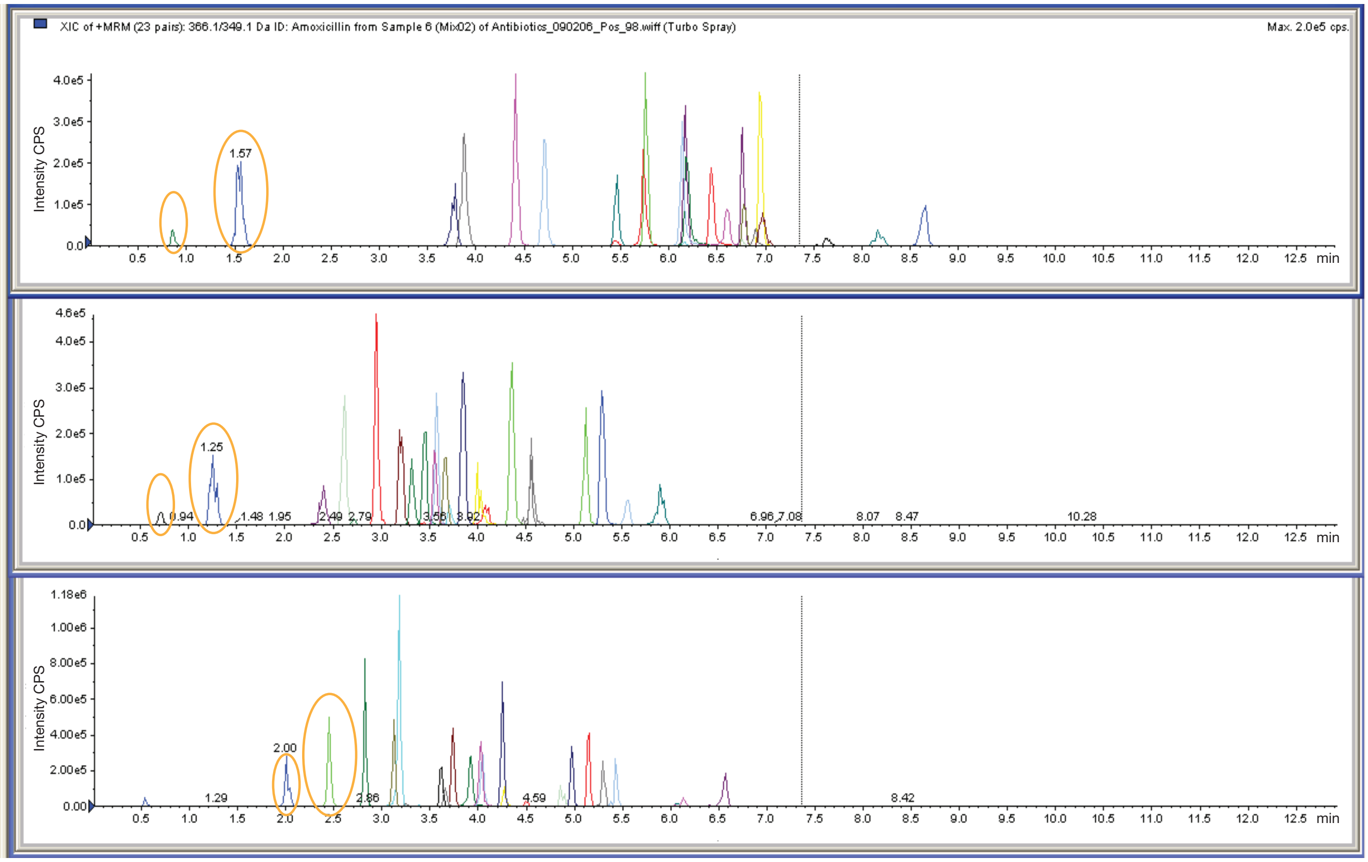



Decreasing the organic content seems to have little effect on increasing retention of these compounds.

# LC Method Development

## Positive Ion mode

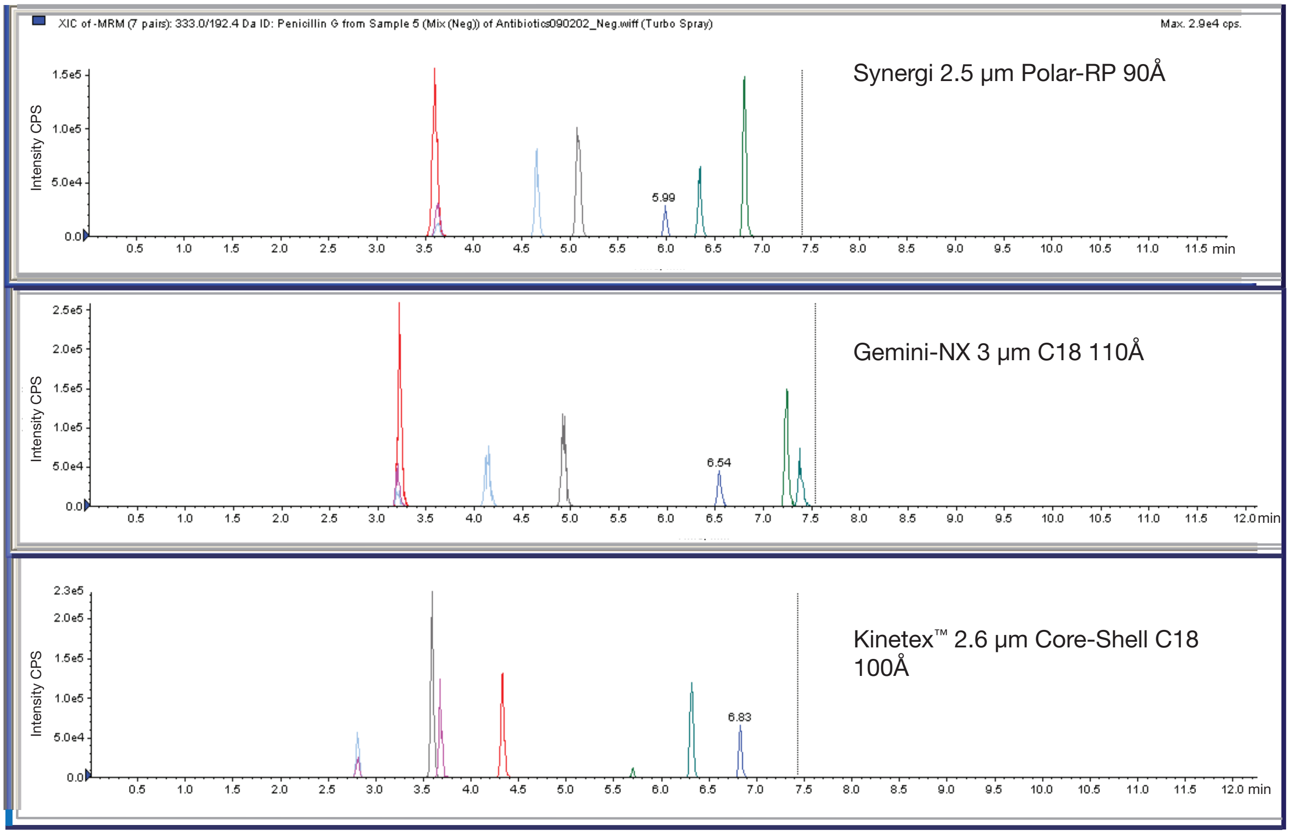
### Extracted Ion Chromatogram of Antibiotics Standard Mixture Working Solution (at 100 ng/mL), obtained on Agilent 1100 system




Column screening provides critical information leading to selection of the Kinetex™ core-shell C18 column. The core-shell particle provides longer retention of the compounds of interest. Narrow Peak width, on Kinetex™ core-shell C18 produces higher peak intensities.

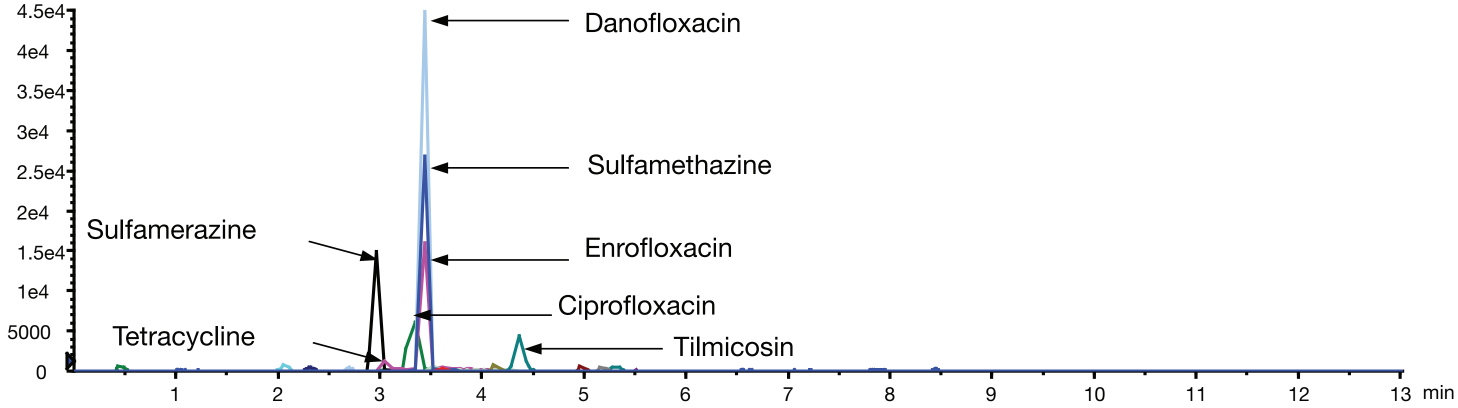
# LC Method Development

## Negative Ion mode



# Conclusions

**Extracted Ion Chromatogram of Spiked sample extracted from beef kidney (supplied by USDA), obtained on Agilent 1100 system with positive ion mode**




LC/MS/MS has the capabilities of screening multiple classes of antibiotics quickly and accurately from real world samples.

Kinetex™ Core-Shell columns deliver ultra high efficiencies on the Agilent 1100 used for this separation. For the separation of these complex mixtures, the ultra-high efficiencies manifest themselves as tighter peaks, providing a 2-fold increase in signal intensity that ensures a corresponding increase in sensitivity.

Kinetex™ 2.6 µm core-shell columns and API 4000™ Qtrap® LC/MS/MS systems provide a high efficiency high sensitivity platform for screening complex mixtures of antibiotics. This platform is optimal for use in regulatory screening of antibiotics in animal production.

# References


1. Francis F. J., Encyclopedia of Food Science and Technology Second Edition, Vol. 1. A Wiley-Interscience Publication, (2000)
2. Martins-Júnior H. A., Kussumi T. A., Wang A. Y., Lebre D. T., A Rapid Method to Determine Antibiotic Residues in Milk using Liquid Chromatography Coupled to Electrospray Tandem Mass Spectrometry. J. Braz. Chem. Soc., (2007), 18 (2), 397-405
3. Fagerquist C. K., Lightfield A.R., Lehotay S.J., Confirmatory and Quantitative Analysis of Beta-Lactam Antibiotics in Bovine Kidney Tissue by Dispersive Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry. Anal. Chem., (2005), 77 (5), 1473-1482
4. Daeseleire E., De Ruyck H., Van Renterghem R., Confirmatory Assay for the Simultaneous Detection of Penicillins and Cephalosporins in Milk Using Liquid Chromatography/Tandem Mass Spectrometry. Rapid Commun. Mass Spectrom. (2000), 14, 1404–1409
5. Mastovska K., Lightfield A.R., Streamlining Methodology for the Multiresidue Analysis of Beta-Lactam Antibiotics in Bovine Kidney Using Liquid Chromatography–Tandem Mass Spectrometry. J. Chromatogr. A, (2008), 1202 , 118–123