


# TN-1117

# APPLICATIONS

## Antibiotic Screen from Bovine Kidney Juice using Septra™ C18-E Bulk Sorbent and Gemini® C18 HPLC Columns

Antibiotics are successfully isolated from bovine kidney juice using a non-retentive clean up step and are then analyzed via LC/MS/MS.

 For AB SCIEX mass spectrometer users, this method can be instantly implemented by installing the iMethod™ Test. iMethod Tests are verified across several laboratories and contain everything you will need to start running samples including sample preparation recommendations, consumables, LC running conditions, optimized MRM parameters, reporting templates, and complete method documentation.

Visit [www.phenomenex.com/iMethod](http://www.phenomenex.com/iMethod) for more information about available iMethod Tests.

### Sample Preparation

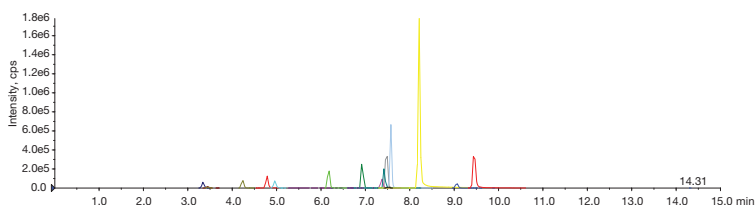
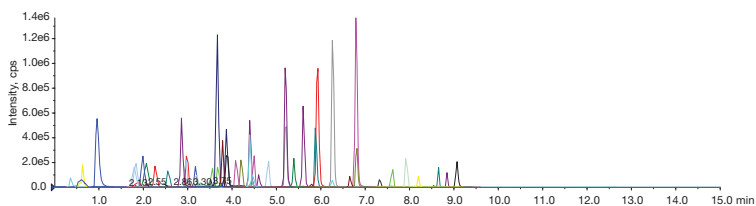
#### Method for analysis of antibiotics in beef kidney juice or serum<sup>1</sup>

1. Weigh 1 g of homogenized beef kidney sample, kidney juice, or serum into a 50 mL FEP (fluorinated ethylene propylene) tube. Alternatively you can use a disposable polypropylene Corning® tube.
2. Add 5 µL of the internal standard work solution.
3. Add 2 mL of water and 8 mL of acetonitrile
4. Mix briefly using a vortex mixer, and then shake for five minutes.
5. Centrifuge at 3450 rcf for five minutes.
6. Decant the supernatant into a 50 mL tube with 500 mg of Septra C18-E sorbent.
7. Mix briefly using a vortex mixer and shake for 30 seconds.
8. Centrifuge at 3450 rcf for one minute.
9. Place a 5 mL aliquot of the supernatant into a graduated tube.
10. Evaporate down to less than 1 mL.
11. Make up the volume to 1 mL with water.
12. Filter the extract through a 0.45 µm Phenex™ RC syringe filter (part no. AF0-8103-12) and then transfer to a Verex™ autosampler vial (part no. AR0-9925-13).

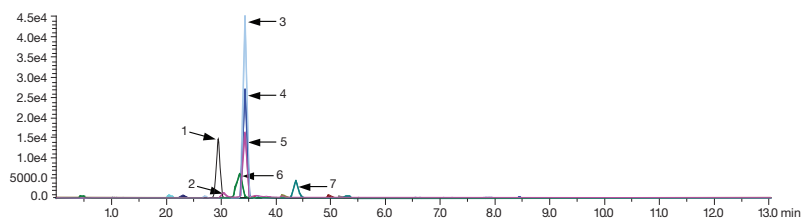
**Note:** The extracts are now ready for LC/MS/MS analysis.

### HPLC Methodology

Extracted Ion Chromatogram of antibiotics standard mixture working solution (at 200 ng/mL), obtained on an Agilent® 1200SL system



Extracted Ion Chromatogram of spiked sample extracted from beef kidney (supplied by USDA), obtained on an Agilent 1200SL system with positive ion mode



### LC/MS/MS Conditions

Column: Gemini 3 µm C18  
 Dimensions: 50 x 2.0 mm  
 Part No.: 00B-4439-B0  
 Mobile Phase: A: 0.1 % Formic acid in Water  
 B: 0.1 % Formic acid in Methanol  
 Gradient: Time (min) % B  
 0 2  
 7.27 80  
 7.37 99  
 11 2  
 15 2  
 Flow Rate: 500 µL/min  
 Temperature: 40 °C  
 Sample: 1. Sulfamerazine  
 2. Tetracycline  
 3. Danofloxacin  
 4. Sulfamethazine  
 5. Enrofloxacin  
 6. Ciprofloxacin  
 7. Tilmicosin

### MS/MS Detection

Optimized for 4000 QTRAP® LC/MS/MS systems  
 TurboV™ ion source with ESI probe  
 Positive polarity and negative polarity

### Source/Gas Parameters (Positive polarity)

CUR:	25 psi
IS:	5500 V
TEM:	600 °C
GS1:	30 psi
GS2:	60 psi
Ihe:	On
CAD:	High

### Compound-Dependent Parameters, Scheduled MRM™ Setting and Retention Times (Positive polarity)

DP:	Compound-dependent
EP:	Compound-dependent
CXP:	Compound-dependent
MRM Detection Window:	120 sec
Target Scan Time:	0.5 sec
Q1 Resolution:	UNIT
Q3 Resolution:	UNIT

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The retention times were collected using the the Shimadzu® Prominence® LC system and Agilent® 1200SL system

Analyte Name	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent 1200SL (min)
Amoxicillin	366.1	349.1	1.00	1.02
Carbadox	263.1	231.1	3.93	4.58
Chlortetracycline	479.3	444	3.45	4.02
Ciprofloxacin	332.2	314.2	2.79	3.35
Clindamycin	425.4	126.1	3.85	4.42
Danofloxacin	358.2	340.2	2.93	3.49
Desethylene Ciprofloxacin	306.2	288.1	2.61	3.07
Difloxacin	400.2	356.3	3.01	3.56
Doxycycline	445.2	428.3	3.99	4.97
Enrofloxacin	360.3	342.2	2.92	3.47
Erythromycin	734.6	158.2	4.80	5.29
Florfenicol Amine	248.1	230.2	0.33	0.36
Josamycin	828.7	109.1	5.38	5.77
Lincomycin	407.4	126.1	1.84	1.88
Lomefloxacin	352.2	265.2	2.91	3.45
Minocycline	458.2	441.3	2.10	2.46
Norfloxacin	320.2	276.2	2.69	3.26
Ofloxacin	362.2	318.2	2.62	3.17
Orbifloxacin	396.2	352.3	2.99	3.53
Oxolinic Acid	262.1	244	4.75	5.18
Oxytetracycline	461.2	426.1	2.85	3.47
Sarafloxacin	386.3	368.1	3.08	3.63

Analyte Name	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent 1200SL (min)
Spiramycin	422.5	174.1	3.39	3.90
Sulfacetamide	215.1	156.1	1.69	1.78
Sulfachloropyridazine	285	156.1	3.45	4.07
Sulfadiazine	251.1	156	2.20	2.54
Sulfadimethoxine	311.1	156.2	4.51	5.18
Sulfadoxine	311.2	156.2	3.76	4.38
Sulfaguandine	215	156.1	0.59	0.62
Sulfamerazine	265.1	92.2	2.71	3.17
Sulfamethazine	279.2	92.1	3.14	3.66
Sulfamethizole	271.2	156.1	3.16	3.76
Sulfamethoxazole	254.1	156.1	3.54	4.19
Sulfamethoxypyridazine	281.1	155.9	3.29	3.87
Sulfanilamide	173.1	92.1	0.66	0.57
Sulfapyridine	250.1	156.1	2.51	2.96
Sulfaquinoxaline	301.1	156.1	4.71	5.36
Sulfasalazine	399.3	381.3	7.68	8.55
Sulfathiazole	256.1	156.1	2.45	2.93
Sulfisoxazole	268.1	156.1	3.79	4.46
Tetracycline	445.2	410.1	2.72	3.24
Tilmicosin	435.6	695.7	3.95	4.44
Tylosin	916.7	174.3	4.91	5.43
Sulfamethazine-D4	283.1	186.1	3.13	3.65

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Source/Gas Parameters (Negative polarity)	
CUR:	25 psi
IS:	-4500 V
TEM:	600 °C
GS1:	30 psi
GS2:	60 psi
Ihe:	On
CAD:	High

Compound-Dependent Parameters, Scheduled MRM™ Setting and Retention Times (Negative polarity)	
DP:	Compound-dependent
EP:	-10 V
CXP:	-6 V
MRM Detection Window:	120 sec
Target Scan Time:	0.5 sec
Q1 Resolution:	UNIT
Q3 Resolution:	UNIT

The retention times were collected using the Shimadzu® Prominence® and Agilent® 1200SL LC system

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent 1200SL RT (min)
Ampicillin	348	207	2.77	3.32
Chloramphenicol	321.1	152	4.25	4.95
Cloxacillin	434.1	292.9	7.04	7.51
Florfenicol	356.1	185	3.59	4.22
Flunixin	295.1	191	6.90	7.36
Penicillin G	333	192.4	6.00	6.82
Thiamphenicol	354	289.9	2.91	3.44

### Ordering Information

 For AB SCIEX mass spectrometer users, this method can be instantly implemented by installing the iMethod™ Test. iMethod Tests are verified across several laboratories and contain everything you will need to start running samples including sample preparation recommendations, consumables, LC running conditions, optimized MRM parameters, reporting templates, and complete method documentation.

Visit [www.phenomenex.com/iMethod](http://www.phenomenex.com/iMethod) for more information about available iMethod Tests.

### Complete iMethod Kit\*

Description	Part No.
iMethod Test for Veterinary Antibiotics Screening Version 1.3	KHO-8982

\*Kit contains Gemini® 5 µm C18 HPLC column, SecurityGuard™ cartridges and holder, in-line filter, Septra™ C18-E Bulk Sorbent, Phenex™ Syringe Filters, Verex™ Vial kit and Sure-Lok™ Fingertight Nut.

### Septra Bulk Sorbent

Description	UOM	Part No.
Septra C18-E Bulk Sorbent	10g	04D-4348

### Gemini C18 HPLC Columns

#### 3 µm Microbore, Minibore and Narrow Bore Columns (mm)

Phase	50 x 1.0	20 x 2.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard Cartridges (mm)
C18	00B-4439-A0	00M-4439-B0	00A-4436-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	4 x 2.0* AJ0-7596 for ID: 2.0-3.0 mm

#### 3 µm Analytical Columns (mm)

Phase	20 x 4.0	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard Cartridges (mm)
C18	00M-4439-D0	00A-4439-E0	00B-4439-E0	00D-4439-E0	00F-4439-E0	00G-4439-E0	4 x 3.0* AJ0-7597 for ID: 3.2-8.0 mm

#### 5 µm Minibore and Narrow Bore Columns (mm)

Phase	30 x 2.0	50 x 2.0	150 x 2.0	250 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	250 x 3.0	SecurityGuard Cartridges (mm)
C18	00A-4435-B0	00B-4435-B0	00F-4435-B0	00G-4435-B0	00B-4435-Y0	00D-4435-Y0	00F-4435-Y0	00G-4435-Y0	4 x 2.0* AJ0-7596 for ID: 2.0-3.0 mm

#### 5 µm Analytical Columns (mm)

Phase	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard Cartridges (mm)
C18	00A-4435-E0	00B-4435-E0	00D-4435-E0	00F-4435-E0	00G-4435-E0	4 x 3.0* AJ0-7597 for ID: 3.2-8.0 mm

\*SecurityGuard Analytical Cartridges require holder, Part No.: KJO-4282

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## Australia

t: 02-9428-6444  
f: 02-9428-6445  
auinfo@phenomenex.com

## Austria

t: 01-319-1301  
f: 01-319-1300  
anfrage@phenomenex.com

## Belgium

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
beinfo@phenomenex.com

## Canada

t: (800) 543-3681  
f: (310) 328-7768  
info@phenomenex.com

## Denmark

t: 4824 8048  
f: 4810 6265  
nordicinfo@phenomenex.com

## Finland

t: +358 (0)9 4789 0063  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## France

t: 01 30 09 21 10  
f: 01 30 09 21 11  
franceinfo@phenomenex.com

## Germany

t: 06021-58830-0  
f: 06021-58830-11  
anfrage@phenomenex.com

## India

t: 040-3012 2400  
f: 040-3012 2411  
indiainfo@phenomenex.com

## Ireland

t: 01 247 5405  
f: +44 1625-501796  
eireinfo@phenomenex.com

## Italy

t: 051 6327511  
f: 051 6327555  
italiainfo@phenomenex.com

## Luxembourg

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

## Mexico

t: 001-800-844-5226  
f: 001-310-328-7768  
tecnicomx@phenomenex.com

## The Netherlands

t: 030-2418700  
f: 030-2383749  
nlinfo@phenomenex.com

## New Zealand

t: 09-4780951  
f: 09-4780952  
nzinfo@phenomenex.com

## Norway

t: +47 810 02 005  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## Puerto Rico

t: (800) 541-HPLC  
f: (310) 328-7768  
info@phenomenex.com

## Sweden

t: 08 611 6950  
f: 08 611 6951  
nordicinfo@phenomenex.com

## United Kingdom

t: 01625-501367  
f: 01625-501796  
ukinfo@phenomenex.com

## All other countries: Corporate Office USA

t: (310) 212-0555  
f: (310) 328-7768  
info@phenomenex.com



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