

# TN-1119

# APPLICATIONS

## LC/MS/MS Analysis of Mycotoxins and Other Fungal/Bacteria Metabolites using a Gemini<sup>®</sup> C18 HPLC Column

Mycotoxins and other fungal/bacteria metabolites are successfully analyzed by LC/MS/MS from several matrices.



For AB SCIEX mass spectrometer users, this method can be instantly implemented by installing the iMethod<sup>™</sup> 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

General method by liquid/liquid extraction.

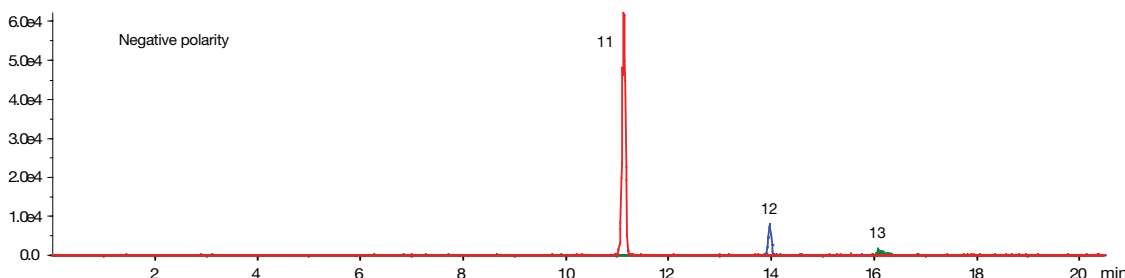
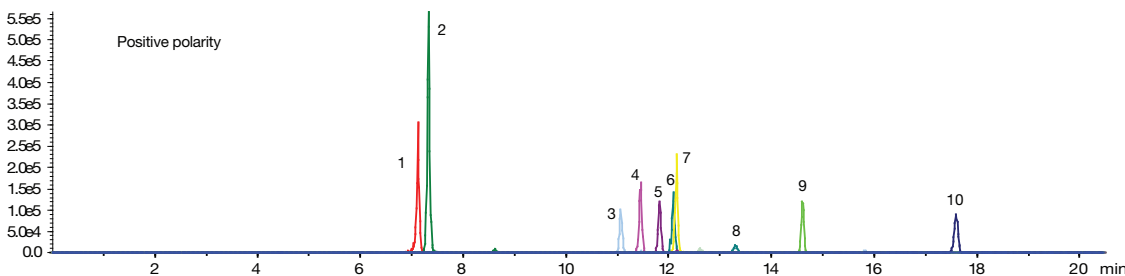
1. Add 4 mL of extraction solvent (acetonitrile/water/acetic acid (79:20:1), v/v/v) per 1 g sample.

The representative sample size may vary, depending on the type of sample and target analytes (a few grams may be sufficient in the case of Fusarium toxins on grains, whereas aflatoxin determination in nuts may require several tens of kilos). The stated extraction solvent has been shown to quantitatively extract most of the toxins from grain samples<sup>1, 2</sup>, from processed grain products<sup>3</sup>, and from nuts and dried fruits (unpublished results). It is also applicable for a semi-quantitative screening of other foodstuffs<sup>4</sup>.

2. Extract for 90 minutes on a rotary shaker.
3. Place 350  $\mu$ L of clear extract into an autosampler vial (after filtration, if necessary) and dilute with 350  $\mu$ L of dilution solvent (acetonitrile/water/acetic acid (20:79:1), v/v/v).

### HPLC Methodology

#### Extracted ion chromatogram of mycotoxins standard mix solution (at 100 ng/mL)



App ID 20027

# TN-1119

## APPLICATIONS

### LC/MS/MS Conditions

**Column:** Gemini® 5 µm C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4435-E0

**Mobile Phase:** A: Water/Methanol (90:10) containing 5 mM Ammonium acetate and 1 % Acetic acid  
 B: Water/Methanol (3:97) containing 5 mM Ammonium acetate and 1 % Acetic acid

Gradient:	Time (min)	% B
	0	0
	14	100
	18.01	0
	20.5	0

**Flow Rate:** 1 mL/min  
**Temperature:** 25 °C

**Sample:**

1. Lincomycin	8. Ergocryptinine
2. Ergometrine	9. Ochratoxin A
3. Aflatoxin G2	10. Nigerin
4. Aflatoxin G1	11. Chloramphenicol
5. Aflatoxin B2	12. beta-Zearalenol
6. Ergocryptine	13. Rapamycin
7. Aflatoxin B1	

### MS/MS Detection

Optimized for 4000 QTRAP® and QTRAP 5500 LC/MS/MS systems

TurboV™ source with ESI probe

Positive polarity and negative polarity

### Source/Gas Parameters (Positive polarity)

CUR:	20 psi
IS:	5000 V
TEM:	550 °C
GS1:	50 psi
GS2:	50 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:	180 sec
Target Scan Time:	0.5 sec
Q1 Resolution:	Unit
Q3 Resolution:	Unit

# TN-1119

## APPLICATIONS

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu® RT (min)	Agilent® RT (min)
15-Acetyl-deoxynivalenol	339.1	321.3	9.6	10.4
16-Ketoaspergillimide	374.2	315.2	10.7	11.5
2-Amino-14, 16-dimethyloctadecan-3-ol	314.3	296.5	15.3	15.9
3-Acetyl-deoxynivalenol	339.2	231.2	9.6	10.4
3-O-Methylviridicatin	252.1	236.1	13.5	14.2
AAL-TA1 Toxin	522.3	328.5	11.1	11.9
Aflatoxin B1	313	285.2	11.4	12.2
Aflatoxin B2	315.1	287.2	11.1	11.9
Aflatoxin G1	329	243.1	10.7	11.5
Aflatoxin G2	331.1	313.2	10.3	11.1
Aflatoxin M1	329.1	273.2	10.4	11.2
Aflatoxin M2	331	273.1	9.9	10.7
Agroclavine	239.1	183.2	8	8.9
Alamethicin F30	775.5	282.3	15.4	16
Altenuene	293.2	275.2	11.4	12.2
Anisomycin	266.3	121.3	6.9	7.8
Ascomycin	809.6	774.6	15.2	15.8
Aspergillimide	360.2	332.3	7.1	8
Asperlactone	185.2	141.2	6.1	7.1
Asperloxine A	394.1	123.1	12.6	13.3
Aspinonene	206.1	127.2	4.9	5.9
Aspyrone	185.1	125	7.3	8.2
Aureobasidin A	1101.7	665.5	16	16.6
Aurofusarin	571.2	556.3	14.2	14.8
Austocystin A	373.2	283.2	14.8	15.4
Avenacein Y	319.2	287.2	12.9	13.6
Bacitracin	712.1	669.5	10.8	11.6
Bafilomycin A1	645.6	443.5	15.8	16.4
Beauvericin	801.5	244.2	15.5	16.1
Brefeldin A	281	245.3	12.6	13.3
Brevicompanine B	368.2	130.1	13.7	14.4
Cephalosporin C	416.3	143	5.9	6.9
Cerulenin	224.1	179.1	12.3	13
Chaetoglobosin A	529.4	130.2	14	14.7
Chanoclavine	257.1	168.2	6.9	7.8
Chetomin	711.2	298.2	14	14.7
Chlamydosporol	227.1	167.1	9.3	10.1
Citreoviridin	403.4	297.2	14.2	14.8
Citrinin	251	233.2	13.9	14.6
Citromycetin	291	245.1	10.6	11.4
Cycloaspeptide A	642.3	150.1	14	14.7
Cycloheximide	282.2	246.1	10.7	11.5
Cyclophenin	295.1	146.1	11.2	12
Cyclopeptide	281.1	120.1	12.1	12.8
Cyclosporin C	610.2	100.1	15.8	16.4
Cyclosporin D	609.3	100.2	16.4	17

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent RT (min)
Cyclosporin H	602.3	100.3	16	16.6
Cytochalasin A	478.2	460.5	14.3	14.9
Cytochalasin B	480.2	462.5	12.9	13.6
Cytochalasin C	525.2	430.4	13.2	13.9
Cytochalasin D	525.2	430.5	12.7	13.4
Cytochalasin E	513.3	416.4	13.5	14.2
Cytochalasin H	494.2	434.5	12.9	13.6
Cytochalasin J	452.2	416.5	12.4	13.1
Decarestrictine	217.1	121.1	6.2	7.2
Dechlorogriseofulvin	319.1	181.2	11.8	12.5
Diacetoxyscirpenol	384.2	307.2	11.2	12
Dihydroergosine	550.2	270.1	10.3	11.1
Dihydroergotamine	584.3	270.3	10.6	11.4
Dihydrolysergol	257.1	167.2	6.2	7.2
Dinactin	782.8	121.2	15	15.6
Elymoclavine	255.1	181.2	6.4	7.3
Elymoclavine fructoside	417.2	255.2	5.7	6.7
Enniatin A	699.4	210.1	15.7	16.3
Enniatin A1	685.4	210.1	15.5	16.1
Enniatin B	657.5	196.3	15.3	15.9
Enniatin B1	671.4	196	15.4	16
Enniatin B2	643.5	214.3	15.1	15.7
Enniatin B3	629.4	196.3	14.7	15.3
Ergine	268.1	223.2	5.6	6.6
Ergocornine	562.2	223.3	10.6	11.4
Ergocorninine	562.3	544.3	10.6	11.4
Ergocristine	610.4	223.3	11.2	12
Ergocristinine	610.4	592.5	12.6	13.3
Ergocryptine	576.4	208.2	11.1	11.9
Ergocryptinine	576.4	558.4	12.2	12.9
Ergometrine	326.2	208.2	6.2	7.2
Ergometrinine	326.2	208.3	7.5	8.4
Ergosine	548.4	223.2	10.2	11
Ergotamine	582.2	223.2	10.5	11.3
Ergovaline	534.2	223.2	9.7	10.5
Festuclavine	241.2	154.2	8.1	9
FK 506	821.7	768.8	15.1	15.7
Fumagillin	459.2	177.1	14.6	15.2
Fumigaclavine A	299.3	167.2	7.5	8.4
Fumitremorgin C	380.3	212.3	13.1	13.8
Fumonisin B1	722.5	334.4	12.2	12.9
Fumonisin B2	706.5	336.4	13.4	14.1
Fumonisin B3	706.5	336.3	12.8	13.5
Fumonisin B4	690.5	338.3	13.9	14.6
Fusaproliferin	445.2	367.3	15.2	15.8

# TN-1119

## APPLICATIONS

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu® RT (min)	Agilent® RT (min)
Gibberellic acid	364.3	239.2	9.6	10.4
Gliotoxin	327.1	263.2	11.6	12.3
Griseofulvin	353.2	165.2	12.4	13.1
HT-2-Toxin	442.2	263.1	12.4	13.1
hydrolyzed Fumonisin B1	406.3	370.3	11.3	12.1
K252a	468.2	293.3	14.5	15.1
K252b	454.2	269.3	15	15.6
Kojic acid	143	113.2	2.2	3.3
Lincomycin	407.2	126.2	6.7	7.6
Lysergol	255.1	240.2	6.4	7.3
Marcfortine A	478.2	450.4	10.8	11.6
Meleagrins	434.3	403.3	10.5	11.3
Methysergide	354.2	237.2	8.5	9.4
Mevastatin	391.2	185.1	15	15.6
Mevinolin	405.4	199.2	15.2	15.8
Mitomycin C	335.2	242.3	8.2	9.1
Monactin	768.8	185.2	14.7	15.3
Monoacetoxyscirpenol	342.2	265	10.3	11.1
Mycophenolic acid	338.1	207.2	13.1	13.8
Myriocin	402.4	104	14.4	15
Neosolaniol	400.2	215	8.3	9.2
Nigericin	742.6	675.8	16.6	17.2
Nonactin	754.6	185.1	14.5	15.1
Ochratoxin A	404	239	14	14.7
Ochratoxin B	370.1	205	13.1	13.8
O-Methylsterigmatocystin	339	306.1	13.3	14
Ophiobolin A	401.2	365.1	14.5	15.1
Oxaspirodion	251.1	161.1	10.5	11.3
oxidized Elymoclavine	259.1	184.2	4.5	5.5
oxidized Luol	291.5	259.3	5.1	6.1
Parahequamide A	494.2	419.3	10.1	10.9
Paspaline	422.3	130.1	16.9	17.4
Paspalinine	434.2	130.1	15.3	15.9
Paspalitrem A	502.3	198.2	16	16.6
Paspalitrem B	518.3	214.2	14.5	15.1
Paxilline	436.4	182.2	15.3	15.9
Penicillic acid	171.2	125.2	8.3	9.2
Penicillin G	335.1	160.2	12.4	13.1
Penicillin V	351.2	160	13.2	13.9
Penitrem A	634.4	558.5	15.1	15.7
Pentoxifylline	279.2	181.1	9.9	10.7
Pestalotin	215.1	153.1	11.2	12
Phomopsis A	789.2	226.1	10	10.8
Phomopsis B	755.3	192.2	9.7	10.5
Puromycin	472.4	150.2	9.2	10

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent RT (min)
Pyrenophorol	313.1	139.1	9.8	10.6
Pyripyropene A	584.3	148.1	13.5	14.2
Roquefortine C	390.2	193.2	11.8	12.5
Roridin A	550.4	249.2	13.2	13.9
Satratoxin G	562.4	249.2	12.5	13.2
Satratoxin H	546.4	157.1	12.7	13.4
Secalonic acid	639.3	561.4	14.8	15.4
Setosusin	532.4	299.3	12.7	13.4
Stachybotrylactam	386.3	178.2	14.8	15.4
Staurosporine	467.3	130.2	11.9	12.6
Sterigmatocystin	325.1	310.2	14.3	14.9
Sulochrin	333.2	209.1	11.2	12
T2-Tetraol	316.2	215.3	4.9	5.9
T2-Toxin	484.3	215.2	13	13.7
T2-Triol	400.2	281.3	11.7	12.4
Territrems	527.3	291.1	13.5	14.2
Tetracycline	445.1	410.3	7.7	8.6
Thiolutin	228.9	211	9.6	10.4
Trichodermin	293.1	143.1	13.2	13.9
Trichostatin A	303.2	148.1	12.2	12.9
Tryprostatin A	382.3	160	12.7	13.4
Ustiloxin A	674.3	187.2	5.3	6.3
Ustiloxin B	646.2	181.2	2.8	3.9
Ustiloxin D	495.2	192.2	5.5	6.5
Valinomycin	1128.8	172.2	17	17.5
Vancomycin	724.9	100.1	5.5	6.5
Verrucarins	520.2	249.1	13.1	13.8
Verrucarol	267	249.1	9.5	10.3
Verrucofortine	410.2	130.1	13.7	14.4
Verruculogen	512.3	352.3	14.4	15
Viomellein	561.3	530.3	14.2	14.8
Viridicatin	238.1	165.2	13.4	14.1
Wortmannin	429.1	355.2	11.6	12.3

# TN-1119

## APPLICATIONS

Source/Gas Parameters (Negative polarity)	
CUR:	20 psi
IS:	-4500 V
TEM:	550 C
GS1:	50 psi
GS2:	50 psi
Ihe:	On
CAD:	High

Compound-Dependent Parameters, Scheduled MRM™ Setting and Retention Times (Negative polarity)	
DP:	Compound-dependent
EP:	Compound-dependent
CXP:	Compound-dependent
MRM Detection Window:	180 sec
Target Scan Time:	0.5 sec
Q1 Resolution:	Unit
Q3 Resolution:	Unit

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu® RT (min)	Agilent® RT (min)
3-Acetyl-deoxynivalenol	397.3	59.2	9.7	10.5
3-Nitropropionic acid	118	46	1.9	3
A23187	522.2	122.1	16.3	16.9
Actinomycin D *	1253.6	829.3	14.9	15.5
alpha-Zearalenol	319.2	160.1	13.9	14.6
alpha-Zearalenol-4-O-glucoside	541.3	319	12.1	12.8
Altenusin	289	245.2	11.7	12.4
Alternariol	257	212.9	13.3	14
Alternariolmethylether	271.1	256	14.7	15.3
Altersolanol	319	301.2	10.6	11.4
Altertoxin-I	351.1	315.3	12.7	13.4
Altertoxin-II	349	303.1	12.5	13.2
Amphotericin B	922.4	697.3	14	14.7
Apicidin	622.4	462.2	14.6	15.2
Aspercolorin	463.2	354	13.1	13.8
Asterric acid	347	149.1	12.5	13.2
Atpenin A5	363.9	292.3	15	15.6
Austdiol	237	147	7.8	8.7
beta-Zearalenol	319.2	160	13.2	13.9
beta-Zearalenol-4-O-glucoside	541.3	319.1	11	11.8
Calphostin C	789.3	459.3	15	15.6
Chaetocin	695	631	13.5	14.2
Chloramphenicol	320.9	151.9	10.2	11
Chromomycin A3	1181.6	269.2	14.6	15.2
Cochliodinol	505.1	224	15.5	16.1
Curvularin	291.1	122.9	12.3	13
Cycloechinulin	350.1	335	12.2	12.9
Cyclopiazonic acid	335.2	139.9	14.8	15.4
Cyclosporin A	1200.8	1088.8	16	16.6

\* Actinomycin D is excluded from the method for QTRAP® 5500 LC/MS/MS system because of mass range.

# TN-1119

## APPLICATIONS

Analyte	Q1 (m/z)	Q3 (m/z)	Shimadzu RT (min)	Agilent RT (min)
Deepoxy-deoxynivalenol	339.1	59.1	8.3	9.2
Deoxybrevianamide E	350.1	153.1	12.7	13.4
Deoxynivalenol	355.1	265.2	6.8	7.7
Deoxynivalenol-3-glucoside	517.3	427.1	6.8	7.7
Emodin	269	224.9	15.7	16.3
Equisetin	372.2	342.3	16	16.6
Erythromycin	792.5	498.2	11.1	11.9
Fulvic acid	307	203	10.4	11.2
Fusarenone-X	413.3	59.1	8.1	9
Fusidic acid	516.3	455.2	15.8	16.4
Geldanamycin	559.3	280	14.3	14.9
Geodin	397	337.9	13.7	14.4
HC-Toxin	435.2	184	9.8	10.6
Ionomycin	707.5	167.1	16.1	16.7
Lolitrein B	684.3	440.2	16.3	16.9
Macrosporin	283	268.1	15.5	16.1
Malfornin C	528.3	494.2	13.9	14.6
Mithramycin	1083.5	269.1	14.6	15.2
Moniliformin	96.9	41.2	2.2	3.3
Neoxaline	434.1	346	9.7	10.5
NG012	661.2	381	13.5	14.2
Nidulin	441	390	16.8	17.3
Nivalenol	371.1	281.1	4.8	5.8
Nornidulin	427	346.9	15.8	16.4
Ochratoxin alpha	254.9	210.9	12.5	13.2
Oligomycin A	789.6	109.1	15.7	16.3
Oligomycin B	803.5	547.5	15.4	16
Ophiobolin B	401.3	355.4	15.1	15.7
Patulin	152.9	108.9	4.8	5.8
Penigequinolone A	466.2	394.2	14.6	15.2
Physson	283	239.9	16.9	17.4
Pseurotin A	430.1	269.9	11.1	11.9
Radicicol	363	182.9	11.8	12.5
Rapamycin	912.6	166.8	15.2	15.8
Rubellin D	541.1	360.1	14.5	15.1
Rugulosin	541.1	269	14.3	14.9
Taxol	912.4	525.2	13.8	14.5
Tentoxin	413.3	141	12.7	13.4
Tenuazonic acid	196	138.9	11.4	12.2
Terphenyllin	337.1	306.9	11.9	12.6
Zearalenone	317.1	131.1	14	14.7
Zearalenone-4-glucoside	479.2	317.1	12.1	12.8
Zearalenone-4-sulfate	397.1	317.1	13.8	14.5

### References

1. Sulyok M, Berthiller F, Krska R, Schuhmacher R (2006) *Rapid Commun Mass Spectrom* 20:2649-2659
2. Sulyok M, Krska R, Schuhmacher R (2007) *Food Add Contam* 24:1184-1195
3. Sulyok M, Krska R, Schuhmacher R (2007) *Anal Bioanal Chem* 389:1505-1523
4. Sulyok M, Krska R, Schuhmacher R (2010) *Food Chem* 119: 408-416

# TN-1119

## APPLICATIONS

### 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 Mycotoxin Screening	KH0-8980

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

### 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* (10/pk) 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* (10/pk) 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* (10/pk) 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* (10/pk) AJ0-7597

for ID: 3.2-8.0 mm

\* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and comparable dimensions, return the product with comparative data within 45 days for a FULL REFUND.

#### Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

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# TN-1119 APPLICATIONS

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