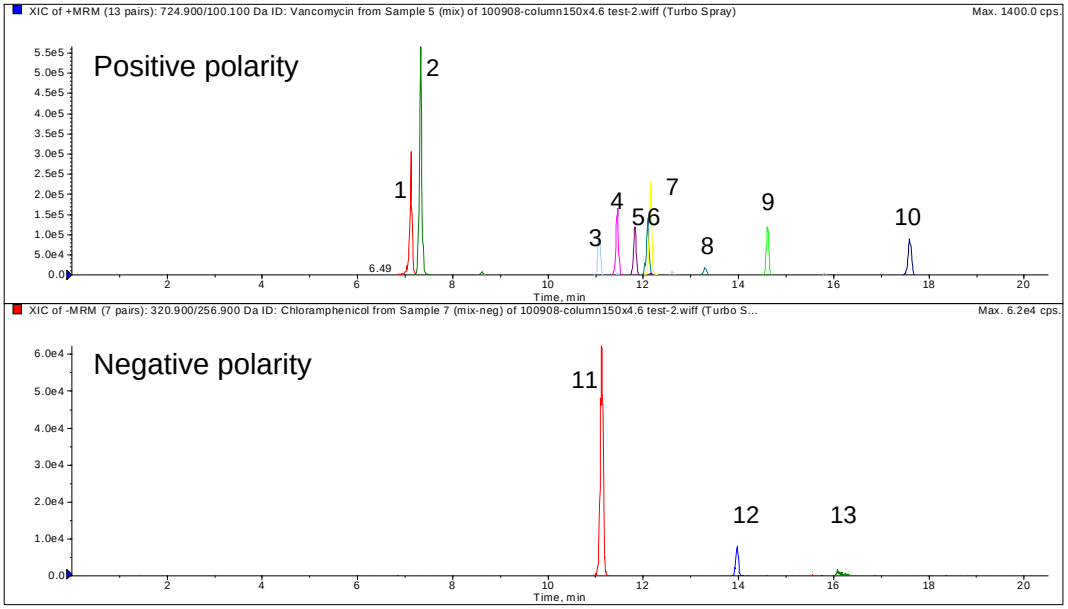
A Rapid iMethod™ Test for Mycotoxin Screening

iMethod™ Test for Mycotoxin Screening Version 1.0 for Cliquid® Software

It has long been known that the presence of mycotoxins in food products, specifically grain, cereals and groundnuts are known to cause health issues in humans. As a result, this has caused government agencies to legislate increased testing and strict limits on their presence. While analytical techniques like GC or GC/MS have traditionally been used for mycotoxin analysis, LC/MS/MS has quickly become the technique of choice due to the ability to analyze a more diverse set of analytes, faster with better sensitivity and less sample preparation.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Mycotoxin Screening using an API 4000™ or 4000 QTRAP® System. This iMethod™ test consists of two pre-configured methods to screen for 171 mycotoxins and plant metabolites in positive ion modes and 72 in negative ion mode.

Both methods use a C18 Guard Column and a 150 x 4.6 mm, 5 µm Gemini C18 analytical column from Phenomenex. Sample preparation is based on a liquid extraction, concentration and injection. Several references, showing that the approach is appropriate for quantitative analysis from grains, grain products, nuts and dried fruit, are provided. A third method has also been included for the quantitation of eight common analytes using two labeled internal standards. This method uses an Aquasil C18 150 x 4.6 mm 3 µm analytical column. All methods have been verified for use on the AB SCIEX Triple Quad™ 5500 system and the AB SCIEX QTRAP® 5500 system. More in-depth sample preparation and instrument parameter information is included as part of the standard operating procedure provided with the method, as are the required analytical columns. Solvents, standards and any supplies required for sample preparation are not included.



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

Figure 1: Chromatogram of a standard mix of 13 analytes at 100 ng/mL using a 4000 QTRAP® LC/MS/MS system.

Please note that the use of QTRAP® system technology is recommended for use with the screening method provided and that the associated library is not included and must be purchased separately. Table 1 provides a list of compounds for the positive ion screening method and Table 2 lists the compounds for the negative ion method.

Table 1: Analytes included in the positive ion screening method

15-Acetyl-deoxynivalenol	Beauvericin	Dihydroergosine	Fumonisin B1	Ochratoxin A	Setosusin
15-Monoacetoxyscirpenol	Brefeldin A	Dihydroergotamine	Fumonisin B2	Ochratoxin B	Stachybotrylactam
16-Ketoaspergillimide	Brevicompanine B	Dihydrolysergol	Fumonisin B3	O-Methylsterigmatocystin	Staurosporine
2-Amino-14,16-dimethyloctadecan-3-ol	Cephalosporin C	Dinactin	Fumonisin B4	Ophiobolin A	Sterigmatocystin
3-Acetyl-deoxynivalenol	Cerulenin	Elymoclavine	Fusaproliferin	Oxaspirodion	Sulochrin
3-O-Methylviridicatin	Chaetoglobosin A	Elymoclavine fructoside	Fusaric acid	oxidized Elymoclavine	T2-Tetraol
AAL-TA1 Toxin	Chanoclavine	Enniatin A	Fusarielin A	oxidized Luol	T2-Toxin
Aflatoxin B1	Chetomin	Enniatin A1	Gibberellic acid	Paraherquamide A	T2-Triol
Aflatoxin B2	Chlamydosporol	Enniatin B	Gliotoxin	Paspaline	Territrem B
Aflatoxin G1	Citreoviridin	Enniatin B1	Griseofulvin	Paspalinine	Tetracycline
Aflatoxin G2	Citrinin	Enniatin B2	HT-2-Toxin	Paspalitrem A	Thiolutin
Aflatoxin M1	Citromycetin	Enniatin B3	hydrolyzed fumonisin B1	Paspalitrem B	Trichodermin
Aflatoxin M2	Cycloaspeptide A	Ergine	K252a	Paxilline	Trichostatin A
Agroclavine	Cycloheximide	Ergocornine	K252b	Penicillic acid	Tryprostatin A
Alamethicin F30	Cyclopenin	Ergocorninine	Kojic acid	Penicillin G	Ustiloxin A
Altenuene	Cyclopeptine	Ergocristine	Lincomycin	Penicillin V	Ustiloxin B
Anisomycin	Cyclosporin C	Ergocristinine	Lysergol	Penitrem A	Ustiloxin D
Ascomycin	Cyclosporin D	Ergocryptine	Marcfortine A	Pentoxyfylline	Valinomycin
Aspergillimide	Cyclosporin H	Ergocryptinine	Meleagrin	Pestalotin	Vancomycin
Asperlactone	Cytochalasin A	Ergometrine	Methysergide	Phomopsin A	Verrucarin A
Asperloxine A	Cytochalasin B	Ergometrinine	Mevastatin	Phomopsin B	Verrucarol
Aspinonene	Cytochalasin C	Ergosine	Mevinolin	Puromycin	Verrucofortine
Aspyrone	Cytochalasin D	Ergotamine	Mitomycin C	Pyrenophorol	Verruculogen
Aureobasidin A	Cytochalasin E	Ergovaline	Monactin	Pyripyropene A	Viomellein
Aurofusarin	Cytochalasin H	Festuclavine	Mycophenolic acid	Roquefortine C	Viridicatin
Austocystin A	Cytochalasin J	FK 506	Myriocin	Roridin A	Wortmannin
Avenacein Y	Decarestrictine	Fumagillin	Neosolaniol	Satratoxin G	
Bacitracin	Dechlorogriseofulvin	Fumigaclavine A	Nigericin	Satratoxin H	
Bafilomycin A1	Diacetoxyscirpenol	Fumitremorgin C	Nonactin	Secalonic acid	

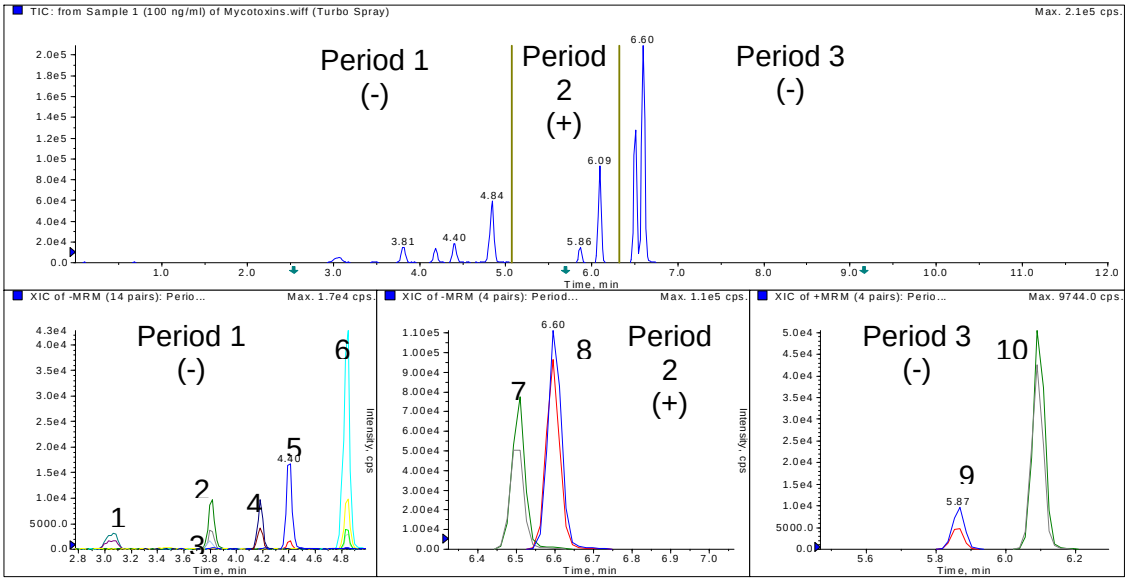
Table 2: Analytes included in the negative ion screening method

3-Acetyl-deoxynivalenol_neg	Amphotericin B	Cochliodinol	Fulvic acid	Neoxaline	Pseurotin A
3-Nitropropionic acid	Apicidin	Curvularin	Fusarenon-X	NG012	Radicicol
A23187	Aspercolorin	Cycloechinulin	Fusidic acid	Nidulin	Rapamycin
Actinomycin D	Asterric acid	Cyclopiazonic acid	Geldanamycin	Nivalenol	Rubellin D
alpha-Zearalenol	Atpenin A5	Cyclosporin A	Geodin	Nornidulin	Rugulosin
alpha-Zearalenol-4-O-glucoside	Austdiol	Deepoxy-deoxynivalenol	HC-Toxin	Ochratoxin alpha	Taxol
Altenusin	beta-Zearalenol	Deoxybrevianamide E	Ionomycin	Oligomycin A	Tentoxin
Alternariol	beta-Zearalenol-4-O-glucoside	Deoxynivalenol	Lolitrem B	Oligomycin B	Tenuazonic acid
Alternariolmethylether	Calphostin C	Deoxynivalenol-3-glucoside	Macrosporin	Ophiobolin B	Terphenyllin
Altersolanol	Chaetocin	Emodin	Malformin C	Patulin	Zearalenone
Altertoxin-I	Chloramphenicol	Equisetin	Mithramycin	Penigequinolone A	Zearalenone-4-glucoside
Altertoxin-II	Chromomycin A3	Erythromycin	Moniliformin	Physcion	Zearalenone-4-sulfate

Quantitation Method

The following information outlines the list of compounds that are included in the quantitation method.

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; 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.



1	NIV
2	DON
3	^{13}C-DON
4	FUS X
5	DOM
6	AcDON
7	HT-2
8	T-2
9	ZAN
10	ZON

Figure 2: Chromatogram of a standard mix of 10 analytes using a 3200 QTRAP® LC/MS/MS system.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 4000 Series (4000 QTRAP® system or API 4000™ system) or 5500 Series (AB SCIEX QTRAP® 5500 system or AB SCIEX Triple Quad™ 5500 system) 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.
- Mycotoxin Standards (www.sigmaaldrich.com)
- LC/MS-grade water, methanol, acetonitrile, ammonium acetate, and acetic acid
- Phenomenex Analytical Column, 5 µm 110 Å, Gemini C18 Column, 150 x 4.6 mm with SecurityGuard Cartridge Kit with C18 4 x 3.0 mm cartridge for the mycotoxin screening method
- Aquasil C18 150 x 4.6 mm 3 µm analytical column for the mycotoxin quantitation method
- Pipettes and standard laboratory glassware

Please note that both Phenomenex Fusion HPLC columns come standard with this iMethod™ Test Kit. This method can also be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated to reflect the configuration used.

Ordering Information

Product Name	Part Number
iMethods™ Tests for Mycotoxin Screening Version 1.0 for Cliquid® Software	5011851

While the information provided outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Mycotoxin Screening, 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 purchase and use of certain chemicals listed above 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.

Legal Acknowledgements/Disclaimers

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions. It is the responsibility of 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.

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