



# INDIAN FOOD SURAKSHAA (SAFETY) APPLICATION BOOK

USING WATERS ADVANCED TECHNOLOGIES

Develop, extend, and validate new methods for  
a wide range of food products and ingredients



## INTRODUCTION

The Food Safety and Standards Authority of India (FSSAI) is responsible for ensuring that food items sold are safe for consumers. FSSAI data shows that of the 240,000+ packaged food products that have been tested since 2011, approximately 13 % of samples have failed laboratory tests.

In light of the recent food crisis in India, which started when unsafe levels of lead and undeclared MSG were discovered in a popular food brand, current practices to ensure quality and safety of food products are now under scrutiny. Products have been taken off the shelves due to unsafe levels of prohibited substances. The food crisis has resulted in a dramatic increase in sample submissions to contract testing laboratories (up to ten-fold in some cases). Samples that are being submitted are varied and include grains, cereals, nuts, fruits, vegetables, and animal products. To comply with the FSSAI these packaged food products need to be tested for a number of different classes of potential contaminants, as well as food additives. These compounds include:

- Pesticides
- Mycotoxins
- Veterinary drugs
- Food dyes
- Antioxidants
- Vitamins

In order to address the demand, food testing laboratories must increase their testing capacity as well as the scope of analysis. Rapid method development for additional compounds and matrices is required along with an increase in sample throughput. Responding to this demand will help to protect the reputation and brands of Indian food companies and foreign food companies selling their products in the Indian market.

The purpose of this application book is to address the current challenges Indian food testing laboratories are facing. It outlines how to develop, extend and validate new methods for a wide range of food products and ingredients. It includes Waters application notes that highlight methods to address India's current food safety requirements.



## TABLE OF CONTENTS

- 4 Workflow Tools for Method Development
- 5 Method Validation and Extension to Scope
- 8 PESTICIDES**
  - 9 LC-MS/MS Analysis of Pesticide Residues in Rice and Unexpected Detection of Residues in an Organic Rice Sample
  - 10 Multi-Residue Pesticide Analysis in Dried Chili Powder: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis
  - 11 Routine UPLC-MS/MS Quantification of Pesticide Residues in Okra with Simultaneous Acquisition of Qualitative Full-Spectrum MS and MS/MS Data
- 12 BEVERAGES**
  - 13 Analysis of 2- and 4-Methylimidazole in Beverages using Alliance HPLC with Mass Detection
  - 14 Rapid Detection of Pesticide Residues in Fruit Juice Without Sample Extraction Using UPLC-MS/MS
  - 15 Screening for Melamine, Cyanuric Acid, and Dicyandiamide in Powdered Milk and Infant Formula Using Mass Detection
  - 16 Qualitative and Quantitative Analyses of Water Soluble Vitamins and Flavonoids in Pomegranate Aril Juice, Skin, and Commercially Available Fruit Juice
  - 17 Selective Quantitative Determination of Water Soluble Vitamins in Various Food Matrices
- 18 MYCOTOXINS**
  - 19 Rapid Quantitative Analysis of 12 Mycotoxins in Processed Maize
  - 20 Rapid Analysis of Aflatoxins in Corn, Cereal and Almonds
  - 21 The Development of a Sensitive Multi-Residue LC-MS/MS Method for the Quantitative Determination of Mycotoxins in Animal Feedstuffs and Silage
- 22 VETERINARY DRUGS**
  - 23 A Simple Cleanup Protocol Using a Novel SPE Device for UPLC-MS/MS
  - 24 Quantification of Chloramphenicol in Chicken Using Xevo TQD with RADAR Technology
  - 25 UPLC-MS/MS Determination of Aminoglycoside Antibiotics in Meat and Milk
- 26 DYES**
  - 27 Rapid Analysis of Sudan and Other Prohibited Dyes in Chili Powder
  - 28 Analysis of Artificial Food Dyes Using UPLC and PDA Detection
  - 29 A Determination of Triphenylmethane Dyes and their Metabolites in Shrimp Using QuEChERS Extraction and the ACQUITY UPLC H-Class System

## WORKFLOW TOOLS FOR METHOD DEVELOPMENT

With ever growing demands being placed on food testing laboratories to ensure product quality and consumer safety, accessible workflows for robust method development, validation, sample analysis, and reporting are essential for efficient throughput. Whether running multi-class, multi-residue, or single analyte methods, Waters® MassLynx® MS Software offers an intuitive interface for intelligent instrument control and software features that are built around the focus of your application for more efficient sample analysis. MassLynx has evolved into a powerful software package, driven by input from our extensive user base. It delivers the versatility and flexibility required so that all system operators can quickly and confidently develop methods and generate reproducible and high quality MS/MS results in an accredited environment. Utilizing the accessible package of Application Managers within MassLynx, the burden of complicated operation and method development, often associated with mass spectrometry is reduced.

### Standards

- The LC Multi-Residue Pesticide Standards Kit (P/N 186007574 ) is a unique collection of 204 standards in 10 mixes, with an initial concentration of 100µg/mL.
- The kit eliminates the need for sourcing individual pesticide standards and measuring each one manually.
- Allows easier set up, verification and validation of pesticide-screening methods in less time with proven confidence and at a significantly reduced cost.

### IntelliStart™

- With the click of a button, quickly and easily confirm that your MS/MS instrument is ready for qualitative and quantitative analysis.
- Automated operation allows for the instrument to be calibrated and resolution set, while routine performance checks can be recorded, and any system alerts resolved.

### QuanOptimize™

- Unattended instrument efficiently and accurately optimizes MS conditions such as cone voltage, collision energy, ionization type, and ionization mode. Generates MS methods for multiple analytes.

### Quanpedia™

- Fully search, store, and generate analytical LC-MS/MS methods from the compound centric Quanpedia database for simplified method development and extension.
- Currently available with over 50 methods, Quanpedia contains LC, MS, and processing methods on over 1100 compounds.

### RADAR™

- Obtain a complete picture of the sample, not just the analytes, by acquiring a full scan MS function along with MRM transitions.
- Track interferences and contaminants that may co-elute with analytes of interest influencing matrix effects (ion suppression and/or enhancement).

# METHOD VALIDATION AND EXTENSION TO SCOPE

## 1. Accreditation

Accreditation is a formal, third-party recognition of competence to perform specific tasks. It provides a means to identify a proven, competent evaluator so that the selection of a laboratory, inspection, or certification body is an informed choice. For India, the National Accreditation Board for Testing and Calibration Laboratories (NABL) is an autonomous body under the aegis of the Department of Science & Technology, Government of India. NABL has been established with the objective to provide government, regulators, and industry with a scheme of laboratory accreditation through third-party assessment for formally recognizing the technical competence of laboratories. <http://www.nabl-india.org/>

## 2. International standards, guidelines, and regulations for residue analysis

Accreditation is granted to organizations who have demonstrated that they fully comply with the requirements of relevant national and international standards. The following ISO Standards and official documents should be implemented by laboratories conducting residue analysis.

A prerequisite for a laboratory accreditation is to have a documented quality management system. The usual contents of the quality manual follow the outline of the ISO/IEC 17025 standard.

- ISO/IEC 17025:2005 – General requirements for the competence of testing and calibration laboratories
- ISO 9001 – Generic management standard covering all aspects of the management of quality
- CODEX Alimentarius Commission Guideline CAC/GL 40-1993 – Guidelines on Good Laboratory Practice in Pesticide Residue Analysis
- CODEX Alimentarius Commission Guideline CAC/GL 71-2009 – Guidelines for the design and implementation of national regulatory food safety assurance program associated with the use of veterinary drugs in food producing animals
- CODEX Alimentarius Commission Guideline CAC/GL 71-2009 amended in 2014 (Appendix C) – Guidelines on Performance Characteristics for Multi-Residue Methods
- Codex Alimentarius Commission Guideline CAC/GL 59-2006 – Guidelines on estimation of uncertainty of results
- European Commission Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed – SANCO/12571/2013
- European Commission Decision 2002/657/EC implementing Council Directive 96/23/EC – Performance of analytical methods and the interpretation of results
- Community Reference Laboratories Residues (CRLs) 20/1/2010 Guidelines For The validation of screening methods – For residues of veterinary medicines (Initial Validation and Transfer)

### 3. Flexible scope accreditation

A flexible scope (or schedule) offers laboratories the necessary flexibility to introduce new, or change existing methods of analysis without referring to the accreditation body. This will allow the laboratory to react more rapidly to constantly changing economical demands. Accrediting a flexible scope is subject to specific quality requirements, as defined by the accreditation body. A laboratory can only apply for a flexible scope if it can provide evidence that the analytical techniques to which the request applies are sufficiently mastered, and if experience in development and validation of new analytical methods can be demonstrated.

### 4. Method validation

Total validation should be performed to provide evidence that methods are fit-for-purpose for the procedure for which they will be applied. Method validation is a requirement of accreditation bodies, and must be supported and extended by method performance verification during routine analysis (analytical quality control and secondary method validation). Where practicable, all procedures undertaken in a method should be validated and the performance characteristics determined. The experimental requirements vary depending on whether the method is intended for quantitative or qualitative (screening) analysis. International guidelines and European regulations relevant for the different application areas are listed in Section 2.

For example, quantitative methods for pesticide residues must be tested to assess sensitivity, mean recovery (as a measure of trueness or bias), precision (as repeatability %RSD), and method Limit of Quantitation (LOQ). A minimum of five replicates are required (to check the recovery and precision) at the targeted LOQ or reporting limit of the method, and at least one other higher level, e.g. 2-10x the targeted LOQ or the Maximum Residue Limit (MRL) for the relevant compound/matrix combination. Where the residue definition includes two or more analytes, the method should be validated for all analytes included in the residue definition, wherever possible.

In the case of quantitative, confirmatory methods for veterinary drug residues, the following performance parameters must be determined in accordance with Commission Decision 2002/657/EC; intra-laboratory repeatability, reproducibility, Decision Limit ( $CC\alpha$ ), Detection Limit ( $CC\beta$ ), specificity, robustness, precision, recovery and trueness, and storage stability of the analyte(s) in extract.

#### 4.1 Total validation

##### Substance groups

When a laboratory applies for flexible scope accreditation for a substance group, e.g. pesticide residues, a total validation shall be performed for the most frequently found compounds both permitted and prohibited for the matrices in question. A total validation needs to be performed every time a new substance class is added under the flexible scope.

## Matrices

In the case of pesticide residues, when a laboratory applies for a flexible scope for one (or more) commodity group(s) (e.g. high water content), a total validation in a representative matrix from one sub-commodity (e.g. pome fruit) shall be performed. 'Representative' means the most frequently analyzed matrix in the applicant laboratory.

## 4.2 Secondary validation

When a laboratory wishes to add a new analyte or matrix for which it already has a flexible scope, a secondary validation is considered sufficient. A secondary validation involves the determination of the method efficiency and the specificity. This can be performed using at least one known blank material and two spiked samples or samples containing a known concentration (with permitted level of concentration LOQ up to 10 x MRL). Typically, this validation can be performed within the same analytical batch as the unknown samples for efficiency.

## 5. Analytical Quality Control (AQC) and proficiency testing

AQC refers to all those processes and procedures designed to ensure that the results of laboratory analysis are consistent, accurate, and within the specified limits of precision defined by validation. The control chart is a quality control tool that provides a statistical approach to the study of variation for the purpose of improving the effectiveness of the process. These methods are based on continuous monitoring of process variation. Control charts provide information about method performance over time, diagnostic information, and are a proven technique for improving productivity and avoiding method creep.

Proficiency testing provides an important means for verifying the general accuracy of results generated by a method and provides information on the between laboratory variability of the results.

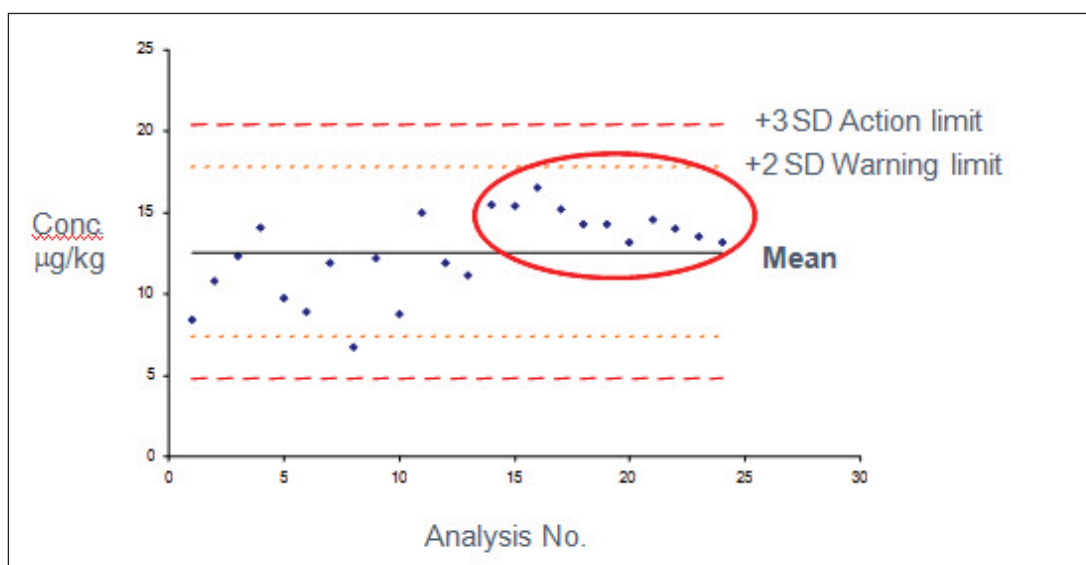


Figure 1. A control chart showing a systematic method bias indicating action is required.

## MULTI-RESIDUE PESTICIDE ANALYSIS

Protecting the consumer is the number one requirement for every food company. If an unsafe product makes it into the market, not only are lives at risk, but so is the reputation of the company. Food product recalls are not only costly but also damaging to brands, which may also negatively impact sales of other product lines.

Increasing the scope of residue screening allows for more residues to be tested for each sample and increases the chance of detecting an unexpected contaminant. There are a number of challenges associated with multi-residue screening. Expansion of matrix types and validation of methods can be costly. Sample preparation adaptation for various food products can be time consuming. Obtaining a blank matrix for certain residues can also be difficult, and in some cases impossible. Achieving reproducible results during routine testing at the levels required to meet regulations may prove challenging. Robust, sensitive instrumentation is essential.

Water's world-leading ACQUITY UPLC® Systems coupled with the Xevo family of tandem quadrupole mass spectrometers allow low level reproducible results to be achieved. The Waters 204 pesticide mix allows screening methods to be set up and validated in less time and at a significantly reduced cost. Quanpedia, which is included with all Waters tandem quadrupole MS systems, is a compound-centric UPLC-MS/MS method compendium allowing rapid, customized analysis. When method development is required, IntelliStart provides simple setup and selection of the appropriate tuning. RADAR, which enables simultaneous acquisition of full spectrum background information aids in method development and allows for matrix interferences to be determined. Established, easy to follow extraction methods using DisQuE™ Dispersive Sample Preparation products that deploy the QuEChERS methodology facilitate the rapid extraction and cleanup of pesticides and other residues from complex food matrices.





## LC-MS/MS Analysis of Pesticide Residues in Rice and Unexpected Detection of Residues in an Organic Rice Sample

- Method for the detection of over 200 pesticides at, or below current regulations in food (0.01 mg/kg).
- Turnkey method available using Quanpedia for rapid method generation.
- Established reproducible multi-residue UPLC method.
- Easy and robust sample preparation using DisQuE Dispersive Sample Preparation products (AOAC QuEChERS) methodology, providing recoveries within the SANCO guidelines.
- Automated data processing with TargetLynx™ XS reduces sample turnaround times.
- Standard addition capabilities for the most accurate determination of incurred residues in food samples.

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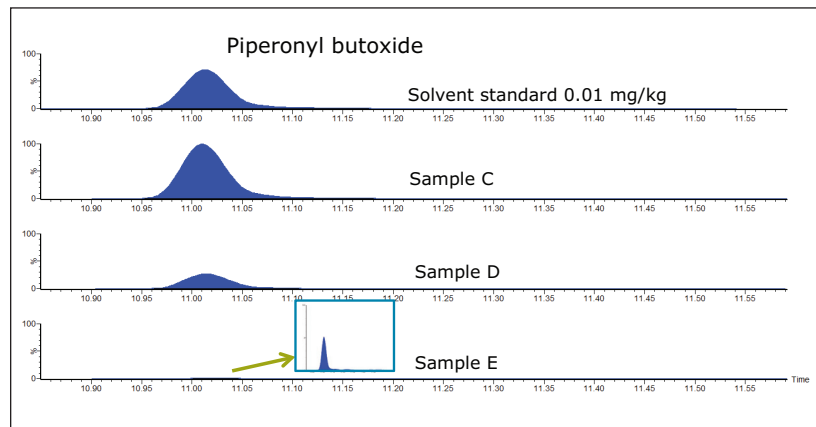


Figure 2. MRM chromatograms of piperonyl butoxide detected in Samples C, D, and E along with the solvent standard at 0.01 mg/kg.

## Multi-Residue Pesticide Analysis in Dried Chili Powder: Optimized Cleanup After QuEChERS Extraction for UPLC-MS/MS and GC-MS/MS Analysis

- Optimized extraction protocol for pesticides in chili powder which is a highly resinous, oily, and a complex matrix.
- Established reproducible multi-residue UPLC method.
- DisQuE with additional d-SPE cleanup removes heavy matrix interferences making analysis more robust for UPLC.
- LC and GC amenable pesticides analyzed on the same MS platform maximize instrument utility.
- Rapid conversion of the MS from LC to GC without venting allows a full suite of pesticides to be analyzed.

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Figure 3. SPE cleanup for LC-MS. Vial on left shows a chili powder sample prepared with no dSPE cleanup; vial on right shows a sample prepared with dSPE cleanup.

## Routine UPLC-MS/MS Quantification of Pesticide Residues in Okra with Simultaneous Acquisition of Qualitative Full-Spectrum MS and MS/MS Data

- A multi-residue analysis method for the detection of 212 pesticides in okra with limits of detection between 1 and 10 ppb for all compounds.
- The QuEChERS method using DisQuE products was used for rapid extraction of pesticides.
- Simultaneous acquisition of MRMs and RADAR full-scan data provides quantitative and qualitative information in single injection allowing rapid method development.
- Incurred residues were calculated using the standard addition method automatically within TargetLynx XS.

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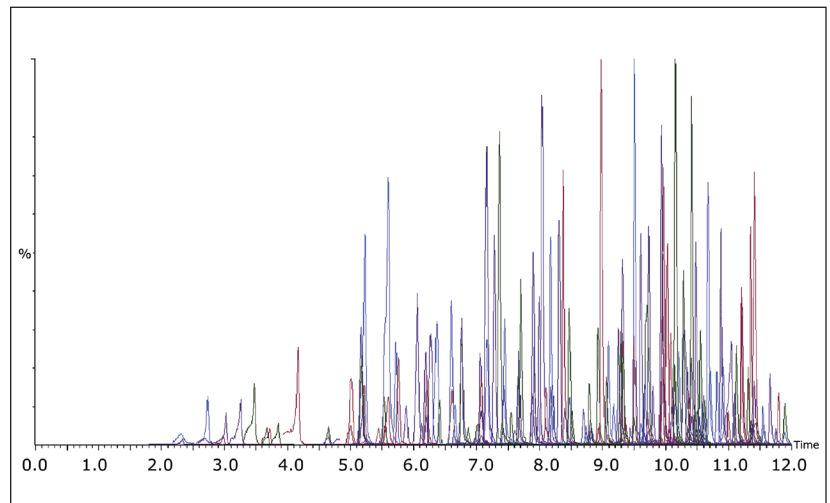


Figure 4. Overlay of MRM chromatograms of all pesticides at 10 ppb (0.01 mg/kg) in okra.

## SAFETY AND QUALITY TESTING OF BEVERAGES

FSSAI testing of beverages is mandatory to determine the label claims for contents including vitamins, antioxidants, and preservatives. Beverage companies are also responsible for ensuring their products are safe to consume and meet the label claims. The recent safety concerns in India have also affected the beverage market with the removal of flavored syrups from coffee shops.

In the past, quality testing of beverage products has typically involved many methods that may include only one, or a few analytes. Not only do the labeled ingredients in complex matrices need to be determined, but residue screens must also be performed. In order to address these requirements throughout, consolidation of methods is desirable wherever possible. Some compounds are not suitable for UV analysis and require MS detection which can be daunting for inexperienced users.

Waters ACQUITY UPLC H-Class and ACQUITY® Arc™ UHPLC systems provide versatility for food testing applications with a full range of detectors. UPLC provides unsurpassed peak resolution, which enables improved separation from matrix components and increased throughput. Recent advances focused on improving instrument usability and robustness have culminated in the ACQUITY QDa® Detector. This detector offers laboratories the opportunity to capture the benefits of mass detection without the challenges associated with the adoption of mass spectrometers. The ACQUITY QDa offers the ability to consolidate methods that in the past have required multiple detectors and chromatographic methods.



## Analysis of 2- and 4-Methylimidazole in Beverages using Alliance HPLC with Mass Detection

- Analysis of complex mixtures of caramel colorings for carcinogenic by-products at low levels (below 250 mg/kg).
- This analysis provides a baseline resolution of 2- and 4-methylimidazole using selected ion recording (SIR) mass analysis for UV transparent analytes.
- Retention of highly polar analytes using CORTECS® HILIC Columns.
- A sensitive and quantitative method without the need for sample cleanup or pre-concentration.

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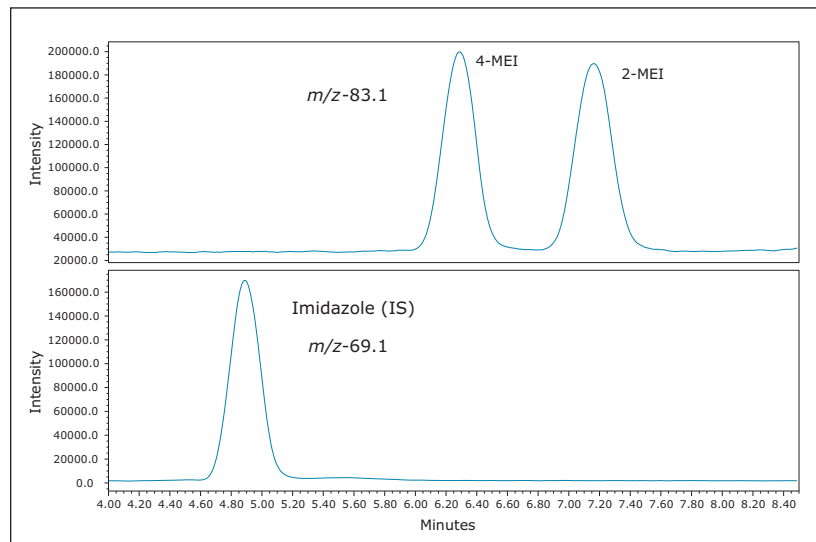


Figure 5. Chromatogram of Standard 6 (100 ppb).

## Rapid Detection of Pesticide Residues in Fruit Juice Without Sample Extraction Using UPLC-MS/MS

- Analysis of 375 pesticides with two MRM transitions from Quanpedia allowing simultaneous screening, identification, and quantification in 17 minutes.
- Simple dilute-and-shoot method reduces sample preparation time improving laboratory efficiency.
- Excellent repeatability for more than 150 injections of orange juice.
- Levels below legislative limits were achieved with ease using the ACQUITY UPLC coupled with Xevo TQ-S.

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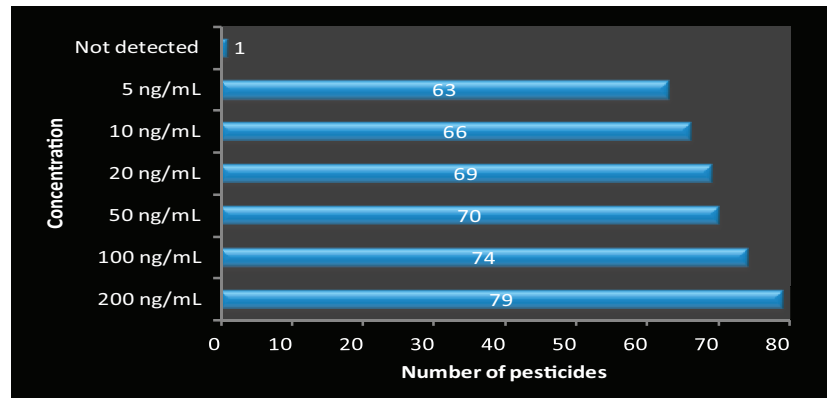


Figure 6. Total number of pesticides detected in orange juice (blue bars) across the range of concentrations tested (Y axis).

## Screening for Melamine, Cyanuric Acid, and Dicyandiamide in Powdered Milk and Infant Formula Using Mass Detection

- A rapid screening method for analysis of melamine, cyanuric acid, and dicyandiamide in less than three minutes.
- A simple and economical sample preparation procedure for various types of milk samples such as infant formula, soy, and milk powder.
- Quantification of weak UV active compounds with excellent recovery and repeatability using the ACQUITY UPLC H-Class coupled with ACQUITY QDa Detector.

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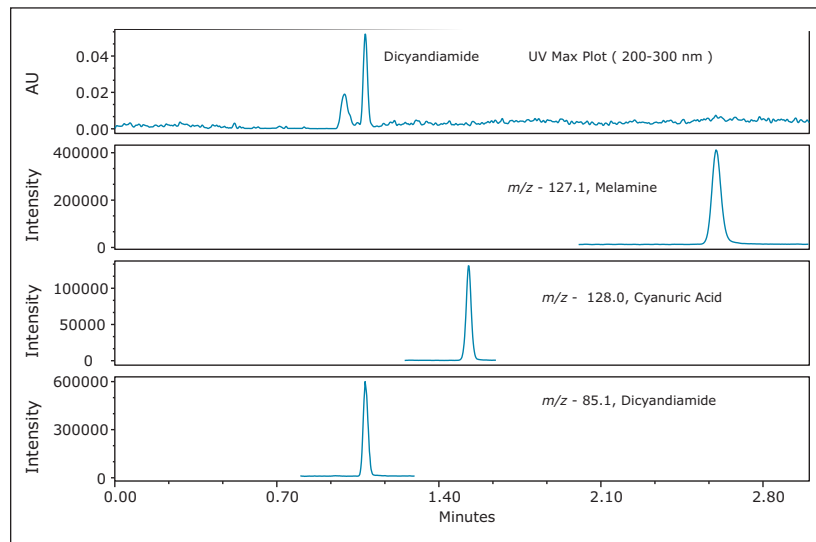


Figure 7. Overlay of UV Max Plot\* with SIR channels for melamine, cyanuric acid and dicyandiamide for Standard 1.

\*UV max plot is a 2D chromatogram plot derived from the 3D PDA data in which each data point is plotted at its maximum absorbance.

## Qualitative and Quantitative Analyses of Water Soluble Vitamins and Flavonoids in Pomegranate Aril Juice, Skin, and Commercially Available Fruit Juice Using the ACQUITY UPLC H-Class with PDA Detector

- The quantitative analysis of polyphenols in pomegranate aril juice, skin and commercially available fruit juice in less than 12 minutes.
- Rapid, reliable, and robust separation of antioxidants and preservatives for routine analysis to meet label claims.

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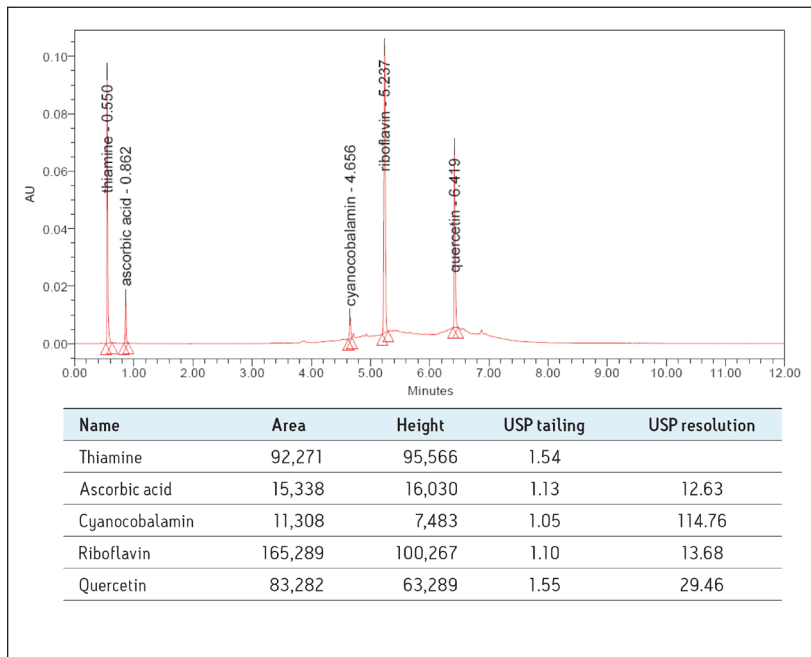


Figure 8. Reference mix of thiamine, ascorbic acid, cyanocobalamin, riboflavin, and quercetin standards.

Compound name	Content (pomegranate fruit)*		Linearity range (in ppm)
	mg/mL	ppm	
Ascorbic acid	0.102	102	12.5,25.0,50.0,100.0,200.0,400.0
Quercetin	0.002-0.01	2-10	1.0,2.0,4.0,8.0, 16.0,32.0
Riboflavin	165,289	100,267	1.10
Quercetin	83,282	63,289	1.55

\*Based on U.S. FDA Nutritional Database 10, 2010).  
Table 2. Concentration of standards used for linearity graph.

Table 1. Concentration of standards used for linearity graph.



## Selective Quantitative Determination of Water Soluble Vitamins in Various Food Matrices Using the ACQUITY UPLC H-Class System and the ACQUITY QDa Detector

- A single analysis method for 12 water soluble vitamins fortified at low levels in complex matrices in less than 18 minutes.
- Quick sample preparation methods for powdered vitamin beverage, multi-vitamin supplement tablet, and vitamin water.
- Mass detection enables the quantification of compounds that co-elute but have different masses.
- Consolidate water soluble vitamin methods into a single LC-MS method, reducing the need for the use of multiple methods and improving laboratory efficiency.

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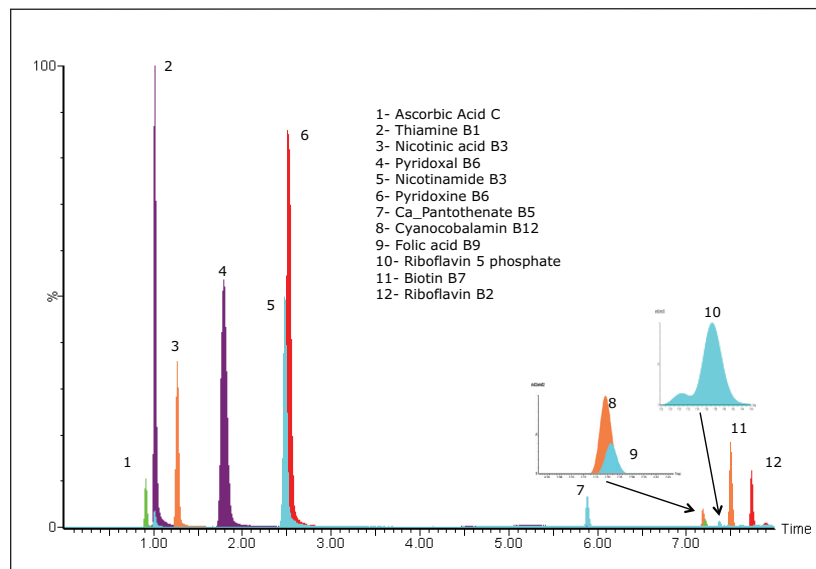


Figure 9. Overlay of SIR chromatograms of 12 water soluble vitamins separated in 8 minutes.

## DETECTION OF NATURAL TOXINS IN GRAIN AND GRAIN PRODUCTS

Grains are an important part of India's national diet and rice is a major export globally. In a large number of Indian packaged food products, including pasta, noodles, and snacks, grains are the key ingredient. Grain must be analyzed for not only pesticides, but also mycotoxins that are carcinogenic, estrogenic, and have immunotoxic effects. Extensive global regulations are enforced to ensure grain products are suitable for consumption.

Due to the variability and complex nature of grains, extensive sample preparation is typically required. This can lead to long method development and extended turnaround times. In order to address increased sample submissions, more efficient methods are required. In addition current methods need to be extended to encompass further types of grain products.

Waters overcomes the difficulties of sample preparation and analysis of grains through a range of solutions. Sensitive analysis of aflatoxins can be achieved without derivitization using the ACQUITY H-Class System and ACQUITY Fluorescence Detector with the large volume flow cell. Mycotoxin confirmation and quantification for all regulated mycotoxins with one detector is possible with the ACQUITY QDa Detector. Waters ACQUITY UPLC systems coupled with Xevo tandem quadrupole mass spectrometers enable identification and quantification of mycotoxins according to the regulatory requirements for confirmatory methods. With methods already available within the Quanpedia database, method development times can be vastly reduced, and routine methods can be rapidly implemented. The following highlights key applications for the detection of mycotoxins in grain.



## Rapid Analysis of Aflatoxins in Corn, Cereal, and Almonds using the ACQUITY UPLC H-Class System with Fluorescence Detection

- Detection of aflatoxins (B1, B2, G1, G2, and M1) at levels below the current European requirements in under four minutes.
- Using ACQUITY fluorescence (FLR) detection with the large volume flow cell eliminates the need to derivatize the aflatoxins which reduces the sample preparation time and UPLC runtime.
- A simple AflaTest® cleanup procedure which involves filtering the samples and passing them through an affinity column was used on three different matrices with excellent recoveries and the ability to achieve regulatory limit.
- Waters Aflatoxin Analysis Application Kit provides an easy-to-use solution that reduces method development time.

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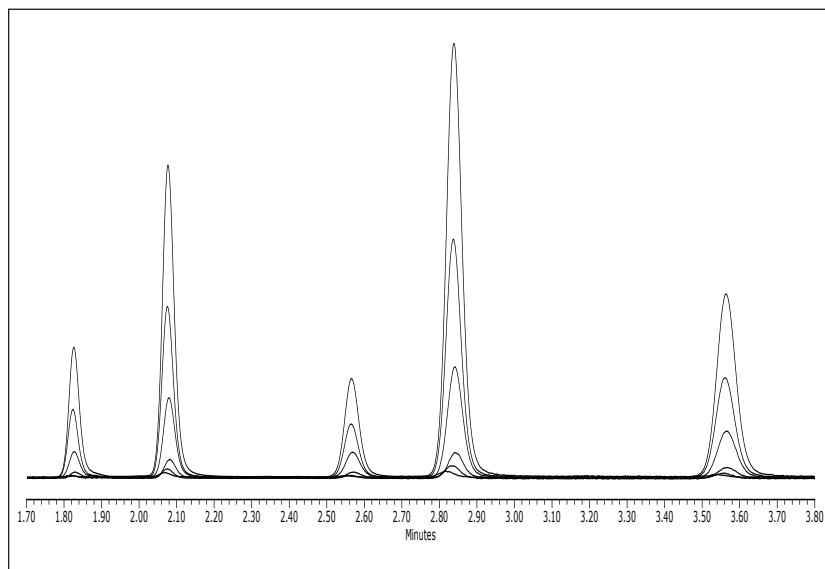


Figure 10. Representative overlay of standard mixes 1–6.

## Rapid Quantitative Analysis of 12 Mycotoxins in Processed Maize Using Myco6in1+ Immunoaffinity Clean-Up and the ACQUITY QDa Detector

- Analysis of multiple processed food types with varying matrices for reliable rapid screening of mycotoxins.
- Sensitive detection of 12 mycotoxins at regulatory limits in complex cereal-based foods.
- Ability to extract multiple mycotoxins using a single sample preparation method thereby reducing sample turnaround times.
- Added sensitivity and selectivity over UV detection using the ACQUITY QDa Detector.

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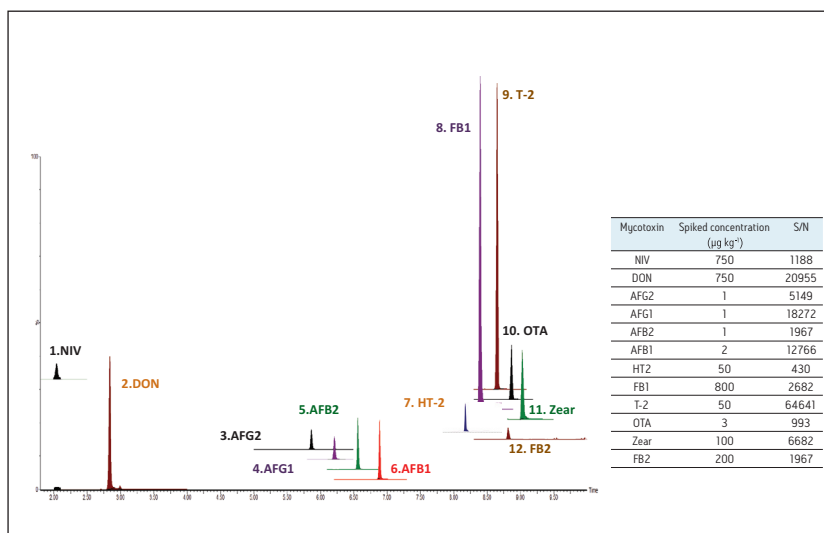


Figure 11. Processed maize food sample fortified at the displayed concentrations (EU regulatory limits). Chromatographically resolved peaks (normalized) were detected with excellent signal-to-noise ratios at legally permitted levels.

## The Development of a Sensitive Multi-Residue LC-MS/MS Method for the Quantitative Determination of Mycotoxins in Animal Feedstuffs and Silage Using Xevo TQ-S

- Quantitative LC-MS/MS method for the simultaneous determination of 33 mycotoxins in animal feed stuffs and silage.
- ACQUITY UPLC I-Class System coupled to a Xevo TQ-S was used for rapid, high quality, and ultra-sensitive analysis.
- Reduced complex matrix effects by incorporating a simple extract dilution step prior to analysis.
- Mean recovery for 17 mycotoxins in silage was found to be 96%.
- Simultaneous acquisition of MRMs and RADAR full-scan data provides allowed matrix effects to be determined.

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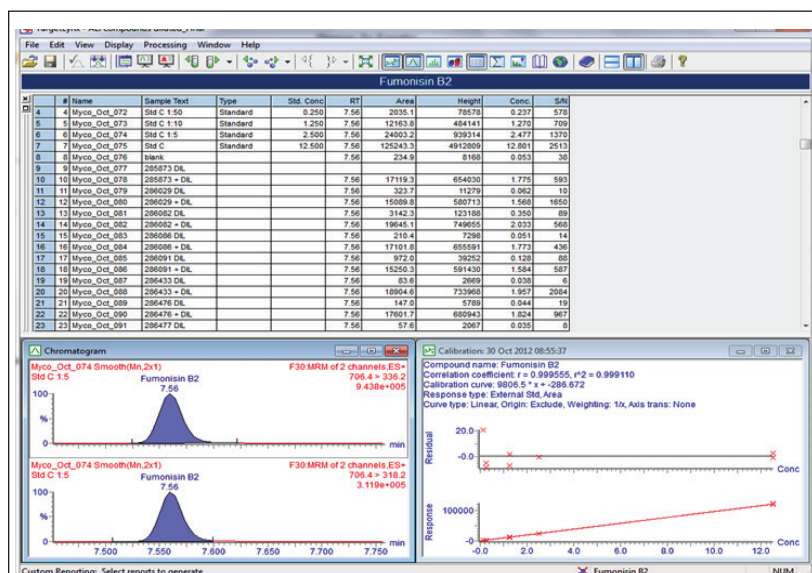


Figure 12. TargetLynx report showing linearity and sensitivity of standards down to 0.25 µg/kg for fumonisin B2 using the Xevo TQ-S.

## QUANTITATIVE METHODS FOR DETECTION OF VETERINARY DRUG RESIDUES

Livestock is often treated with veterinary drugs to ensure the health of the animal and prevent disease transmission. Certain veterinary drugs that are banned for use, such as chloramphenicol, are still utilized due to their low cost and efficacy. These drugs can often make it into the food chain and have adverse effects on consumers.

Food testing laboratories must test animal products for these prohibited substances in a timely manner. Freshness of the product is critical, and if results are delayed the product may not be suitable for consumption, even if it is drug free. Tests must be performed on both unprocessed and processed products, which involves validation of methods for a wide range of matrices.

Waters addresses these issues with world-leading technology. Fast ACQUITY UPLC methods coupled with the Xevo tandem quadrupole MS family provides low level reproducible results. Quangepedia provides methods for analysis of multiple classes of veterinary drugs. Extension of the methods to new matrices is aided by RADAR, which allows matrix interferences to be monitored in different types of animal products. Oasis® PRiME HLB Cartridges provide the most effective cleanup of extracts for both fat and phospholipids with a simple one-step pass through cleanup.



## A Simple Cleanup Protocol Using a Novel SPE Device for UPLC-MS/MS Analysis of Multi-Residue Veterinary Drugs in Milk

- A robust method for an analysis of 72 veterinary drugs in milk.
- A simple, effective, and suitable sample preparation approach using Oasis PRiME HLB Cartridge suitable for large numbers of samples in routine analyses.
- Removal of fatty/non-polar materials and phospholipids with Oasis PRiME increases the column lifetime and reduces the need for instrument maintenance.
- Recoveries were between 50% to 130% with RSD <20% for all compounds.

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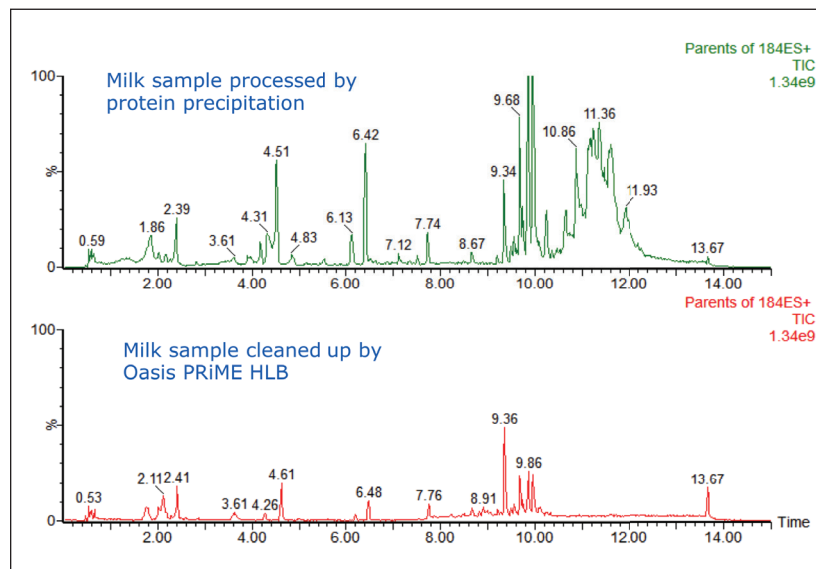


Figure 13. Chromatograms of phospholipid removal between milk samples processed by protein precipitation and cleanup by Oasis PRiME HLB.

## Quantification of Chloramphenicol in Chicken Using Xevo TQD with RADAR Technology

- Rapid quantitative analysis of the restricted antibiotic chloramphenicol in chicken.
- SPE sample preparation to concentrate the analyte and remove matrix interferences.
- Simultaneous acquisition of MRMs and RADAR full-scan data to determine matrix interferences while performing quantification.
- UPLC run times under three minutes provide faster sample turnaround times.

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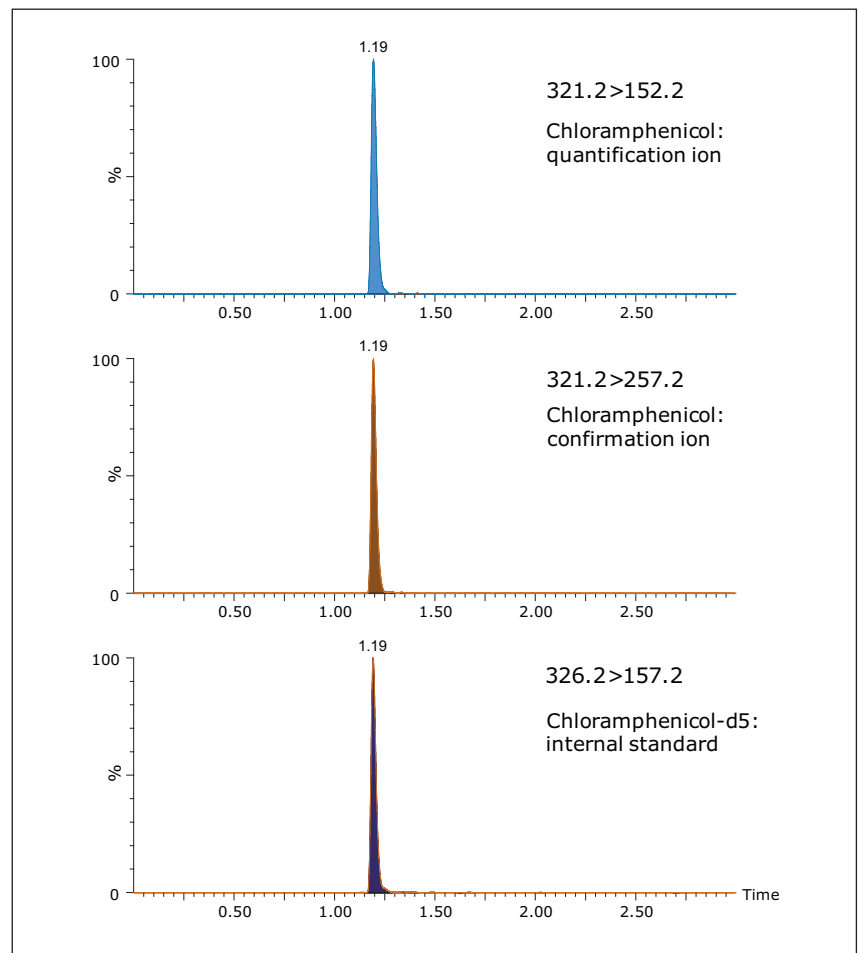


Figure 14. MRM chromatograms of standard chloramphenicol at 3 ng/mL (equivalent to 0.3 µg/kg in chicken) and internal standard at 5 ng/mL in water.



## UPLC/MS/MS Determination of Aminoglycoside Antibiotics in Meat and Milk

- Analysis of veterinary drugs used in the treatment of animals bred for meat using LC-MS at low ppb detection limits.
- An optimized SPE protocol using an Oasis HLB 96-well Plate to improve sample throughput.
- Two established MRM transitions for seven common veterinary drugs allows identification and quantification of prohibited compounds according to legislative requirements.
- Same extraction procedure for both milk and muscle tissue reduces the need for additional method development.

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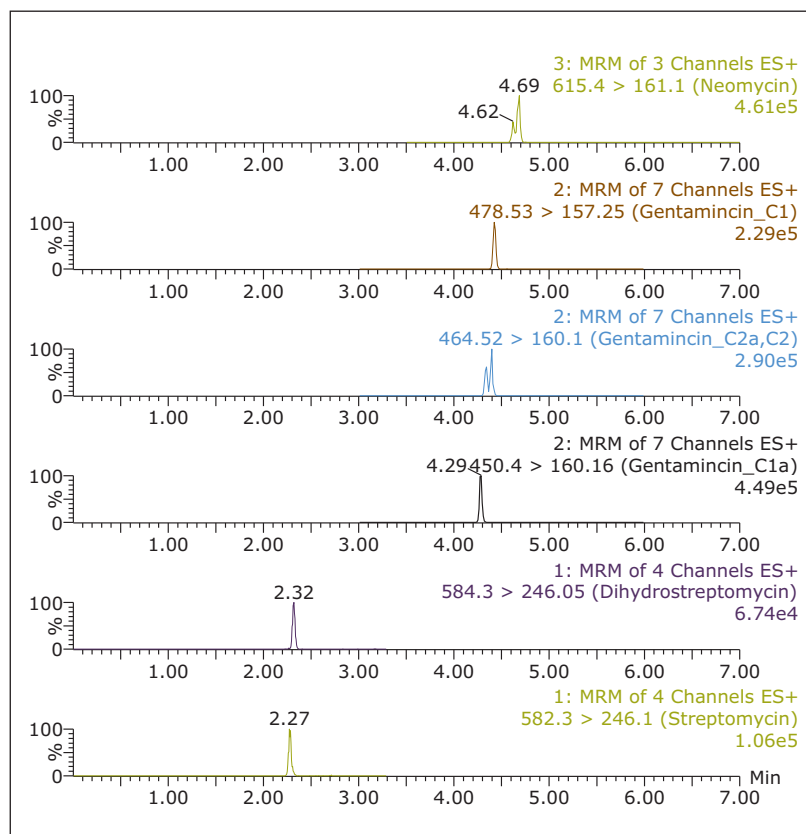


Figure 15. UPLC-MS/MS chromatogram obtained from bovine milk spiked at 10 µg/kg (ppb).

## DETECTION AND QUANTIFICATION OF PERMITTED AND BANNED COLORANTS

Synthetic food colors are added to a wide range of spices and other food products to enhance their appeal to consumers. Economic adulteration has led to a number of prohibited carcinogenic colorants such as Sudan dyes to be found in spices. This has led to rejection of consignments for global export.

Spices are one of the most challenging matrix types encountered in residue analysis. The matrix interferences are prominent even after extensive sample preparation. Methods and instrumentation must be robust to handle the large number of complex samples. Instrumentation must also be able to hit the low limits of detection required by legislations even in the presence of significant co-extracted interferences.

Waters offers multiple solutions for the routine analysis of both permitted and banned dyes. Simple screening techniques for food colorants with the extended ACQUITY UPLC Photodiode Array (PDA) eλ Detector allows for multiple compounds in the UV and visible spectrum to be quantified in food and beverage products. Low level quantitative analysis using the Xevo tandem quadrupole MS family provides reliable and robust methods for quantification of banned substances at or below regulatory limits following simple DisQuE Dispersive Sample Preparation. The rapid analysis times that can be achieved with ACQUITY UPLC improve lab productivity over traditional HPLC methodologies.



## Rapid Analysis of Sudan and Other Prohibited Dyes in Chili Powder Using the ACQUITY UPLC H-Class System with Xevo TQD

- Detection of genotoxic Sudan dyes in chili, paprika, tumeric, garam masala, and curry powder.
- Low limits of detection achieved in complex matrices using a combination of DisQuE Dispersive Sample Preparation with the QuEChERS methodology and the selectivity of MRM analysis.
- 60% to 105% recoveries for all analytes with a simple QuEChERS extraction.
- Linear calibration curves, even in heavy matrix, providing accurate, reproducible, and confident quantification.

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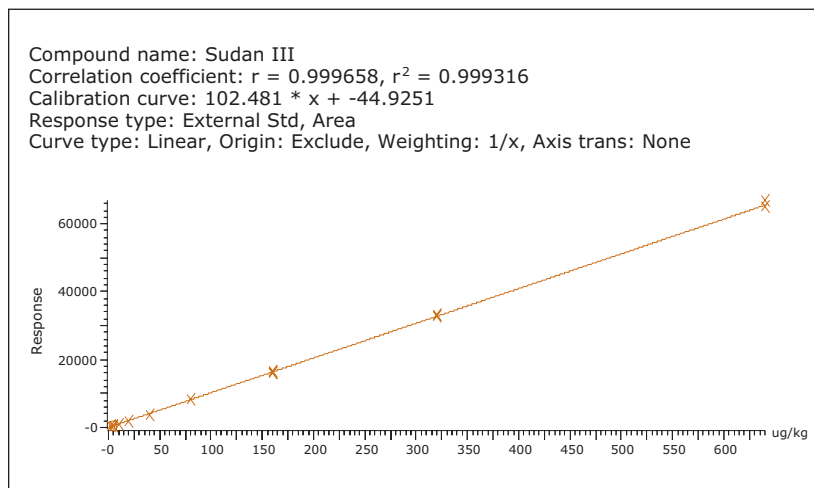


Figure 16. Matrix match spiked calibration curve of Sudan III from 0.5 ppb to 128  $\mu\text{g/L}$  ppb (equivalent to 2.5 to 640  $\mu\text{g/kg}$  in sample).

## Analysis of Artificial Food Dyes Using UltraPerformance Liquid Chromatography and an Extended Wavelength Photo Diode Array Detector

- An efficient and accurate quantitative method for the analysis of synthetic color additives in foods and beverages.
- A variety of real samples such as drink mixes, popsicles, powdered dessert mixes, jelly beans, hard candy, jams, and smoked salmon were analyzed in a shorter timeframe compared to traditional methods.
- The ACQUITY UPLC PDA eλ Detector provides excellent selectivity and sensitivity when analyzing more complex matrices.

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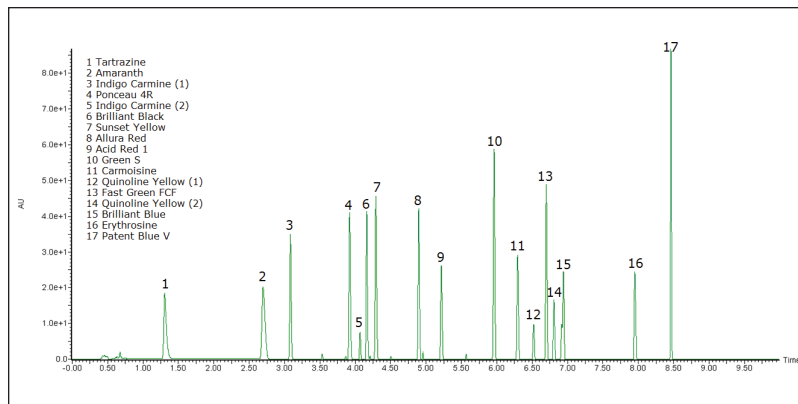


Figure 17. Separation of 15 artificial dye standards and selected isomers using the ACQUITY UPLC H-Class System and eλ PDA Detector.

## Determination of Triphenylmethane Dyes and their Metabolites in Shrimp Using QuEChERS Extraction and the ACQUITY UPLC H-Class System with Xevo TQD

- Selective quantitative determination of triphenylmethane dyes in aquaculture products using the ACQUITY UPLC H-Class System and the Xevo TQD to levels below FDA and EU regulations.
- Sample preparation using DisQuE Dispersive Sample Preparation with a modified QuEChERS methodology provides a fast and efficient method for seafood analysis.
- Rapid screening for multiple triphenylmethane dyes in under six minutes.
- Excellent linearity and low level quantification to 0.1 ppb providing confident results in difficult matrices.

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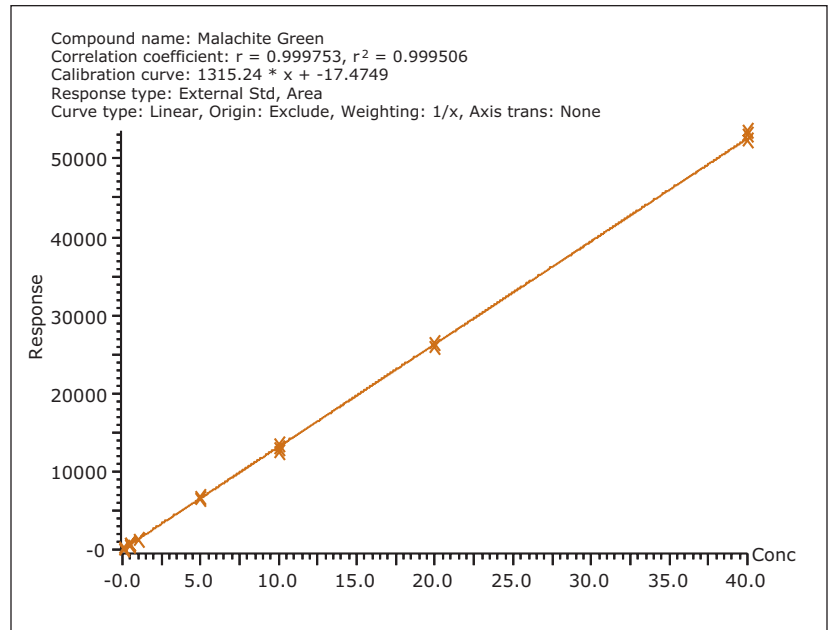


Figure 18. Matrix matched calibration curve for Malachite Green (0.1 to 40 ppb).

## LC Multiresidue Pesticide Standards Kit

A fit-for-purpose collection of 204 standards designed to eliminate the need for sourcing individual pesticide standards and measure each one manually. It allows scientists to setup, verify and validate their pesticide screening methods in less time with proven confidence and at a significantly reduced cost.



- Accurately detect and quantify pesticides of global food safety concerns from a wide range of samples by LC-MS/MS
- Formulated and grouped into 10 vials for maximum long-term stability
- Well-balanced chromatographic performance, even for early eluting compounds
- Quantitatively tested to confirm composition
- Manufactured and QC tested in ISO accredited lab
- Ideal for a wide range of fruits, vegetables and other commodities.

**Part Number [186007574](#)**

# Waters

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**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)