

AOAC Standard Methods Requirements for Non-Targeted Test Methods: Applying New Rules to Open-Ended Methods.

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Here We Go.....

- Are NTT methods looking for authenticity or the presence of an adulterant?
- Define authenticity
- Understand known and suspected adulterants
- Use Learnings from Targeted Testing to Create Non-Targeted Methods
- AOAC Non-Targeted Standard Method Performance Requirements
- AOAC Int'l Food Adulteration Methods Program (FAM)



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Typical NTT Application: Food Fraud

A deliberate act designed to increase the apparent value of an ingredient or finished product.

This is also referred to as economically motivated adulteration (EMA).



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Test Selection

- **Appropriate test selection to define “authentic”**
 - Proving authenticity is like a defense proving someone’s innocence in a court of law
 - Is this olive oil? Virgin olive oil? EVOO?
 - Is this ginseng? In general or Chinese vs. American?
 - Proving adulteration is like proving guilt
 - Is this oregano cut with another plant?
 - Does this milk have melamine in it?
- **Classes of testing**
 - Targeted
 - Non-Targeted
 - Combinations of the above



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Biggest Hurdle: Obtaining Authentic Material

- Any method validation requires authentic material
- You must control getting it
- Document all aspects of obtaining the authentic material
 - Source
 - Transport
 - Storage
 - Blockchain helps
- Verifying with already established methods builds confidence
- Defining Authentic is key!



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Targeted Versus Non-Targeted

Targeted methods

- Look for defined compounds for authenticity or a defined adulterant.
- Use sample preps best isolating these compounds.



Non-targeted methods

- Obtain authentic material.
- Create a comprehensive fingerprint.
- Typically uses a simple sample preparation to include as many matrix components as possible.
 - Some methods are performed on whole samples.



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Authenticity Testing

- **Target components in the ingredient**
 - Example: Does this turmeric sample have curcumin, demethoxycurcumin, and bisdemethoxycurcumin present? If so, at the right levels?
 - DNA authentication
 - Other targeted tests
- **Non-Targeted Test Sets a Profile**
 - Create fingerprint
 - HPTLC, LC-MS/MS, XRF, many others
 - Next Generation Sequencing



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Adulterants

- **High-Concentration Adulterants**
 - Replacements / Bulking Agents / Similar Products
 - Medium and Low Sensitivity Method Available
 - Many tests to choose from:
 - HPLC and GC with various detectors
 - Visual examination
- **Low-Concentration Adulterants**
 - Colorants / Enhancers (e.g., melamine)
 - Higher Sensitivity Methods Needed
 - Melamine issue



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Targeted Adulterant Testing

- Targeted testing requires the identification of adulterants.
 - Remember: These are only the ones we know about!
 - But these known ones are creating havoc now!
- Several analytical technologies are being used and developed for routine monitoring of food ingredients targeting specific adulterants likely to be used in food ingredients
 - Techniques include but not be limited to: LC-MS/MS, NMR, GC-MS/MS, FAME, NIR, Raman/SERS, FT-IR, Microscopy, PCR, UV, ELISA/LFD, and wet Chemistry
 - Each technique has power & risks
 - Known vs. unknown adulterants



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Targeted Testing for Two Types of Adulterants in Saffron Powder



Analytical Parameter	Acceptance Criteria for Colour Adulterants	Acceptance Criteria for Non-Colour Adulterants
Analytical Range	1 – 30 ppm	1 – 30 %
LOQ	≤1 ppm	1 %
Recovery (%)	80 – 120	80 – 120
Accuracy (%)	± 20	± 20
Repeatability, RSD _r	15	15
Reproducibility, RSD _R	20	20
*Beet, pomegranate fibers, red dyed silk fibers, safflower and marigold to red stigmas of saffron, dyed corn stigmas, stigmas of other saffron types, <i>curcuma</i> , acid orange II, metanil yellow, sudan I, Ponceau 4R and 6R		*Gardenia, meat fibers, gelatin fibers, sandalwood, Campeche wood powder, starch and glucose used as fillers or bulking agents

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But what if you
don't know what
may have been
added?



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Non-Targeted Testing

Concept

Create a standardized fingerprint for an ingredient.

Compare new lots of the ingredient to the fingerprint.

Chemometric examination to reveal if differences outside normal variability

– Binary Result: Adulterated or Authentic



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Non-Targeted Testing (NTT)

- Non-Targeted testing requires the creation of a baseline “fingerprint” to assess degree of difference of a tested lot
- This occurs prior to identification of adulterants likely to be present
- This is employed to assess true value of the ingredient/food, and to reduce the risk of a non-safe ingredient or food.



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Non-Targeted Screening

- **Variety of methodologies being explored**
 - LC-MS/MS
 - NMR
 - Spectroscopic
 - XRF and other ones for certain matrices
 - Others
- **Data analysis**
 - Chemometrics
 - Principle Component Analysis
 - Customized software



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Standard Method Performance Requirements

SMPR Section	Targeted Testing	Non-Targeted Testing
Applicability	ID & Measure specific analyte	Assess if something is "different"
Definitions	Defines targeted analyte. Includes reference standards	Define "authentic" List known adulterations
Method Performance Requirements	Analytical range Accuracy Repeatability Reproducibility	1) Can determine if food has known adulterants 2) Performance on food with unknown adulterant(s)
System Suitability / Quality Control	CRMs in each batch	Adulterated samples in batch; but unknown?
Reference Materials	CRM / SRM	????
Validation Guidance	Established	Newer
Maximum Time to Results	Variable	Variable



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Requirements for Saffron (Table 1b)

Test	Adulterant	% Adulterant in Test Materials	Number of Samples to be Tested ¹	Number of Test Results Qualified as Adulterated
Baseline	Authentic Saffron	0%	Establish Baseline Fingerprint ²	
Authentic Samples³	None	0%	30	0
Validation⁴	Pomegranate fibers	10%	30	30
Validation⁴	Beet	10%	30	30
Validation⁴	Gelatine fibers	10%	30	30
Validation⁴	Sandalwood	10%	30	30
Validation⁴	Campeche wood powder	10%	30	30
Validation⁴	Gardenia	10%	30	30
Validation⁴	Meat fibers	10%	30	30
Validation⁴	Starch	10%	30	30
Validation⁴	Glucose	10%	30	30
Validation⁴	Corn Silk	10%	30	30
Validation⁴	Red dyed silk fibers	10%	30	30

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NTT Requirements for Saffron (Table 1a)

Test	Adulterant	% Adulterant	Number of Samples to be Tested ¹	Number of Test Results Qualified as Adulterated
Baseline	None (Authentic Saffron)	0%	Establish Baseline Fingerprint ²	
Validation using Authentic Samples ³	None	0%	30	0
Validation ⁴	Metanil Yellow CAS 587-98-4	1 ppm	30	30
Validation ⁴	Acid Orange II CAS 633-96-5	1 ppm	30	30
Validation ⁴	Sudan 1 CAS 842-07-9	1 ppm	30	30
Validation ⁴	Ponceau 4R CAS 2611-82-7	1 ppm	30	30
Validation ⁴	Ponceau 6R CAS 2766-77-0	1 ppm	30	30

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Non-Targeted Testing Approach

▪ **AOAC NTT SMPRs employ a novel two-stage approach**

- SLV will be performed on common known adulterants in the specific commodities. Recipes will be included in SMPR.
- MLV will be performed on blind samples created by a third-party.

▪ **Submissions must also**

- Describe authentic sample collection and verification
 - Defines NTT method scope
- Include protocol on authentic fingerprint generation



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NTT Scope Evolution

Now: Establish NTT method with:

- Defined authentic scope
- Proven to “catch” known, common adulterants

In the Future: Evaluate the method on new or suspected adulterants

- If successful, add those adulterants to “proven to work method scope
- If not successful:
 - Adjust method, or
 - Create a 2nd method with different scope



Hurdles

- **Obtaining Authentic Material**
 - Are reference materials available?
 - North American Chemical Residue Workshop (NACRW)
 - AOAC SMPR defines matrix scope as what was used to create base fingerprint.
- **Standard Reference Material with Unknown Adulterant(s)**
 - Not available
 - My thought: Can we, on our own, use Authentic Material spiked with know adulterants and add “other stuff”?
 - Only determining if something else is present. NOT QUANTIFYING IT!



Food Authenticity Methods Program, FAM

- **Three Active Working Groups**

- Non-targeted testing, Targeted Testing, Molecular Applications
- Current commodity focus: Spices and botanicals (vanilla, turmeric, saffron)
- Previously milk, honey, EVOO

- **Emergency Response Guidance:**

- Where EMA and public health intersect
- A mechanism for implementation of standards/method development principals in emergency situations.

- **Development Systematics Tools to Identify EMA**






- Predictive tools incorporating AI,
- Traceability: pre- and post-production using integrated technologies,
- Mass balance assessment



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FAM Active Calls for Method Validation Plans

- **AOAC SMPR® 2020.006** Nontargeted Testing (NTT) of Ingredients for Food Authenticity/Fraud Evaluation of Honey 
- **AOAC SMPR® 2020.007** Nontargeted Testing (NTT) of Ingredients for Food Authenticity/Fraud Evaluation of Extra Virgin Olive Oil 
- **AOAC SMPR® 2020.008** Nontargeted Testing (NTT) of Ingredients for Food Authenticity/Fraud Evaluation of Pasteurized Whole Liquid Bovine Milk 
- **AOAC SMPR® 2020.009** Targeted Testing (TT) of Barley and Malt Extract, Beet Sugar Syrup, Corn and Cane Sugar Syrup, C-4 Plant Sugar, and High-Fructose Corn Sugar for Adulteration of Floral and Acacia Honey 
- **AOAC SMPR® 2020.010** Targeted Testing (TT) of Other Vegetable Oils and Low-Quality Olive Oils as Adulterants for Evaluation of Extra Virgin Olive Oil (EVOO) 
- **AOAC SMPR® 2020.011** Targeted Testing (TT) of Formaline/Formaldehyde, Starch, and Soy Protein as Adulterants for Evaluation of Liquid Raw Bovine Milk 



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Food Authenticity Methods Program for 2022



Food Authenticity Methods Program

AOAC's Food Authenticity Methods (FAM) Program focuses on identifying analytical tools to better characterize the intentional and economically motivated adulteration of foods.

- Publication of TT method gap-analysis assessment
- Training on NTT method development
- Establish **premarket intelligence** parameters i.e., standards and consensus mechanisms for the integration of environmental metagenomic testing, artificial intelligence, machine learning and database curation to proactively identify emerging EMA and subsequent method gaps,
- Finalize emergency response guidance



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Summary

- **Define authenticity**
- **Use Learnings from Targeted Testing to Create Non-Targeted Methods**
- **Non-Targeted Testing**
 - Create profiles
 - Prove Non-Targeted Test Identifies Sample Has Used Adulterants
 - Improve Method Scope as Needed
- **AOACI Food Adulteration Methods Program**



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