



Global Expertise  
Trusted Standards  
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# Standardization of Non-Targeted Approaches for Food Fraud Detection

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- ▶ **Who is USP?**
- ▶ What are non-targeted methods and why are they being used?
- ▶ Why aren't non-targeted methods more widespread?
- ▶ The USP non-targeted method guidance



# MISSION

To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.



A global resource for **food integrity and safety** solutions including science-based standards, tools, and services to improve confidence in the global food supply chain.



Training

**USP** Food Fraud Database  
U.S. Pharmacopeial Convention

[www.foodfraud.org](http://www.foodfraud.org)

**USP** Food Fraud Mitigation Guidance  
U.S. Pharmacopeial Convention



# Why Food Fraud

PREVALENCE



UP TO **10%** FOOD SUPPLY IS AFFECTED

IMPACT



**Public Health**  
(In some cases)



**Economic**



**Compliance**



**Brand Damage**



**Consumer Confidence**

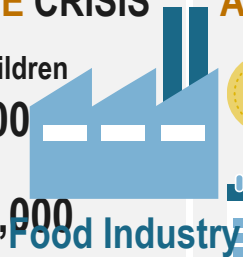
WHO IS AFFECTED

## MELAMINE CRISIS

6 Children  
7,000



300,000  
Children



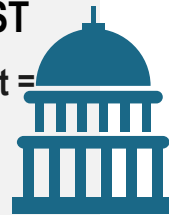
## AVERAGE COST



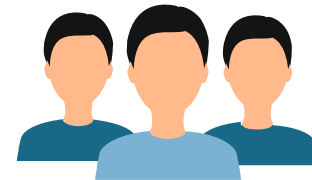
1 Incident =  
2-15%



Annual  
Business  
Revenue Loss



**Government**



**Consumer**

# The Challenge of Detecting Food Fraud

Criminal “designs”  
adulterant to evade  
existing QA system

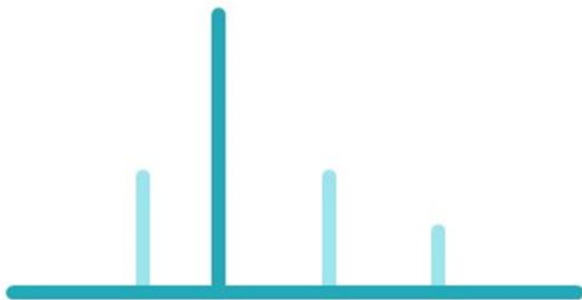


QA system reacts  
by developing  
new tests

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# Non-Targeted Methods?

**TARGETED  
ANALYSIS**



**NON-TARGETED  
ANALYSIS**



Infographics © Carmen Diaz-Amigo 2015



# A Way to Get Ahead of Fraud Perpetrators

Instead of looking for  
*what should not be there...*

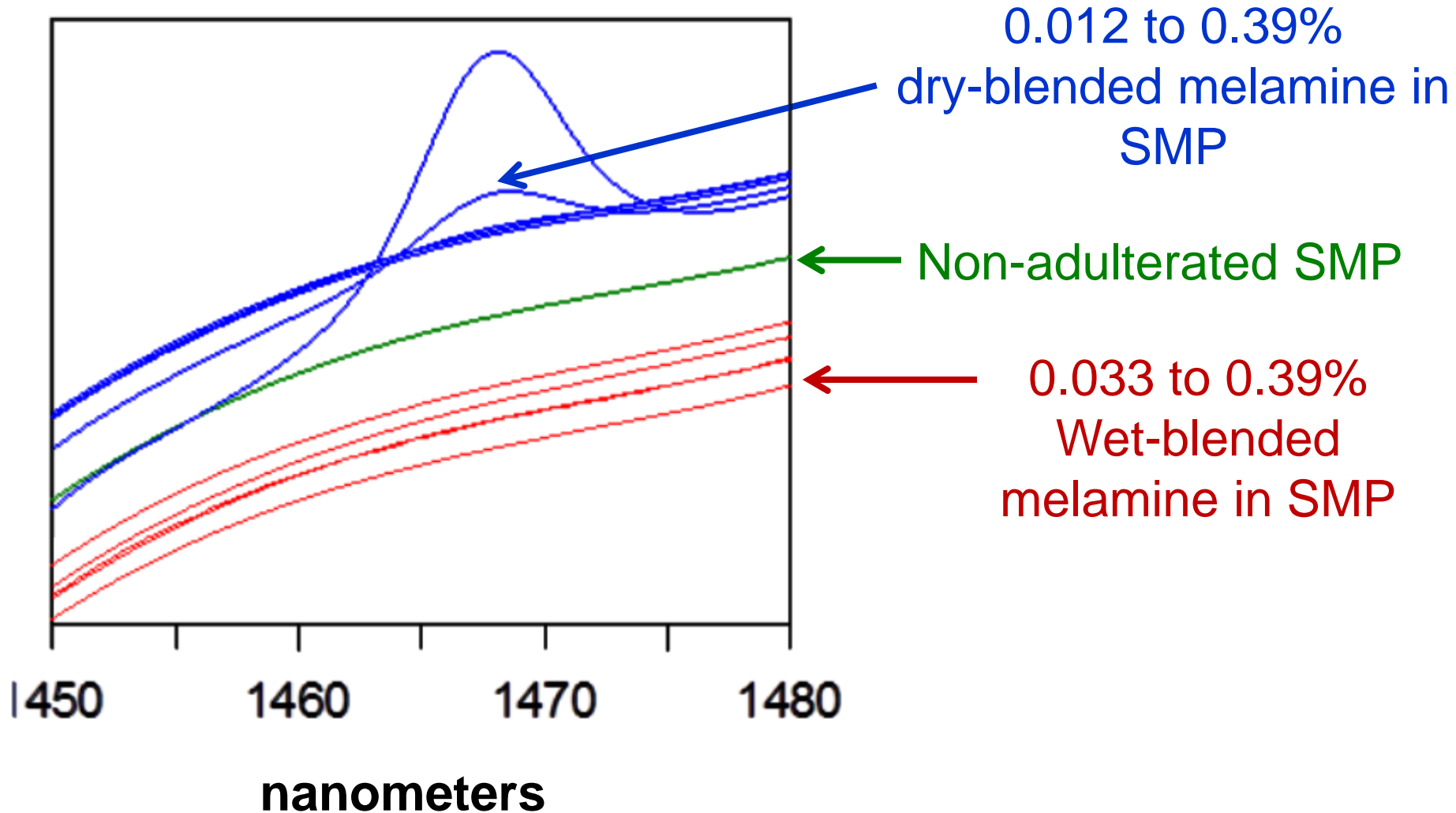


Define very carefully the  
characteristics of  
*what should be there*

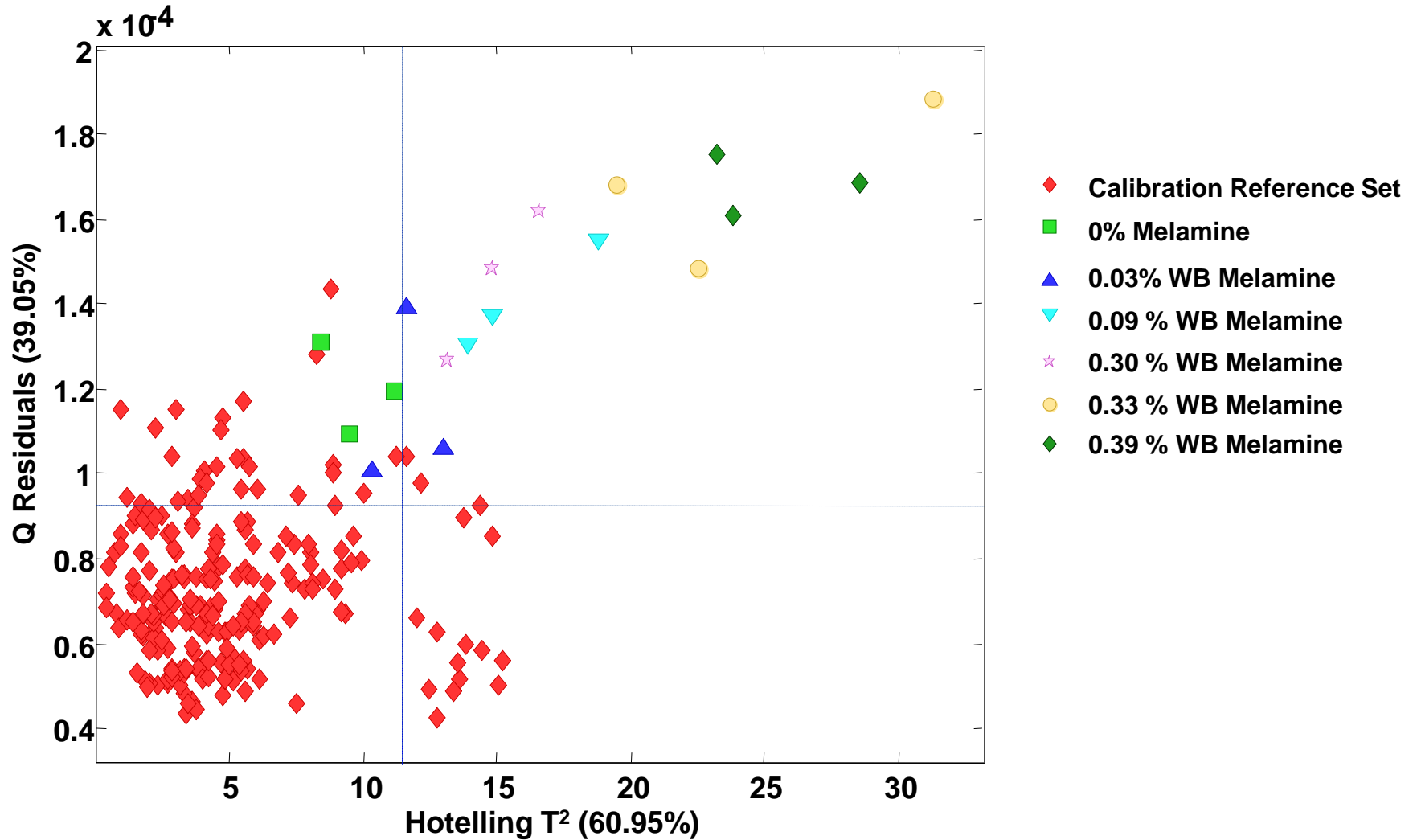


Exclude anything that  
*deviates significantly*  
*from those characteristics*

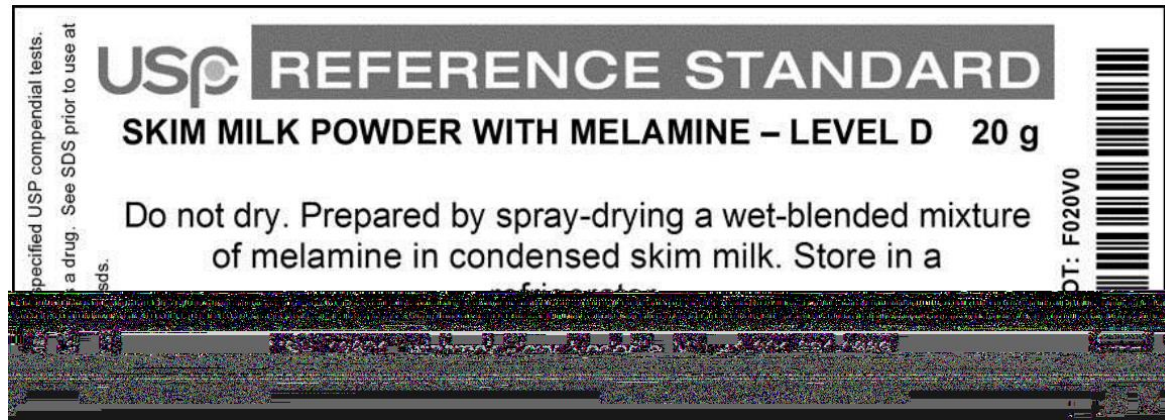
## Rapid detection on melamine in SMP by NIR



## SIMCA Model



- Non-targeted methods beneficial even when you know what adulterant you are looking for
- RM's for “genuinely fake” adulterated foods needed to provide confidence in rapid test methods



- ▶ Instrumentation and chemometrics advances -> to non-targeted methods.
- ▶ Used in routine testing as screening methods, followed by more targeted confirmatory methods for “abnormal” samples
- ▶ Continued significant investments by major food companies, Testing Labs, and European funded R&I projects

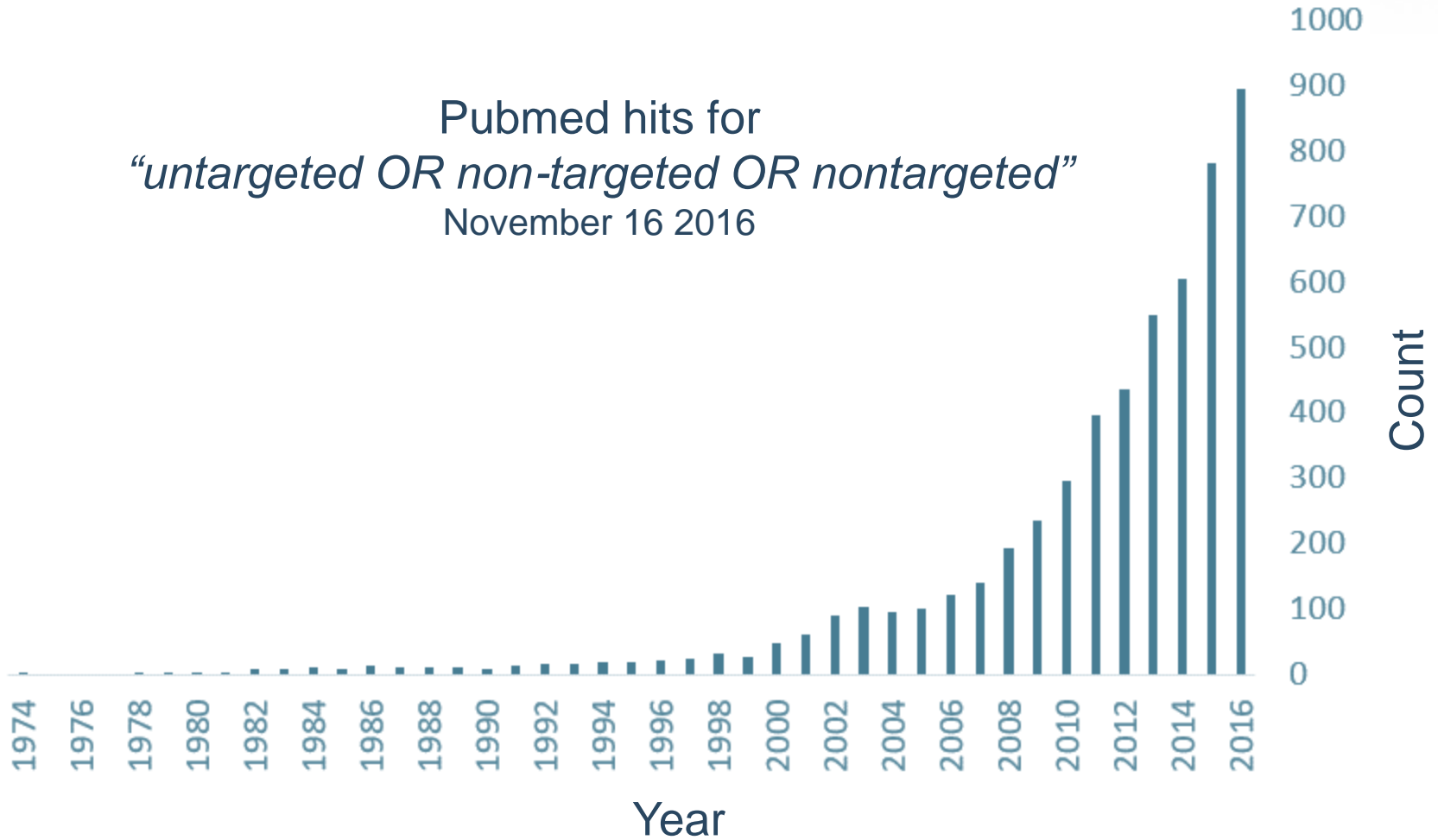


Horizon 2020



# Scientific Publications: Increasing Trend

Pubmed hits for  
*“untargeted OR non-targeted OR nontargeted”*  
 November 16 2016



Advances in, and maturation of, analytical technologies and data processing allowing rapid broad spectrum analysis

## Charge to 2009 Advisory Group on Milk Powder:

To develop and validate a “tool-box” of methods and specifications for skim milk powder that will help protect against economically adulterated materials, including the next melamine

### **Nonprotein Nitrogen Determination for Skim Milk Powder and Nonfat Dry Milk**

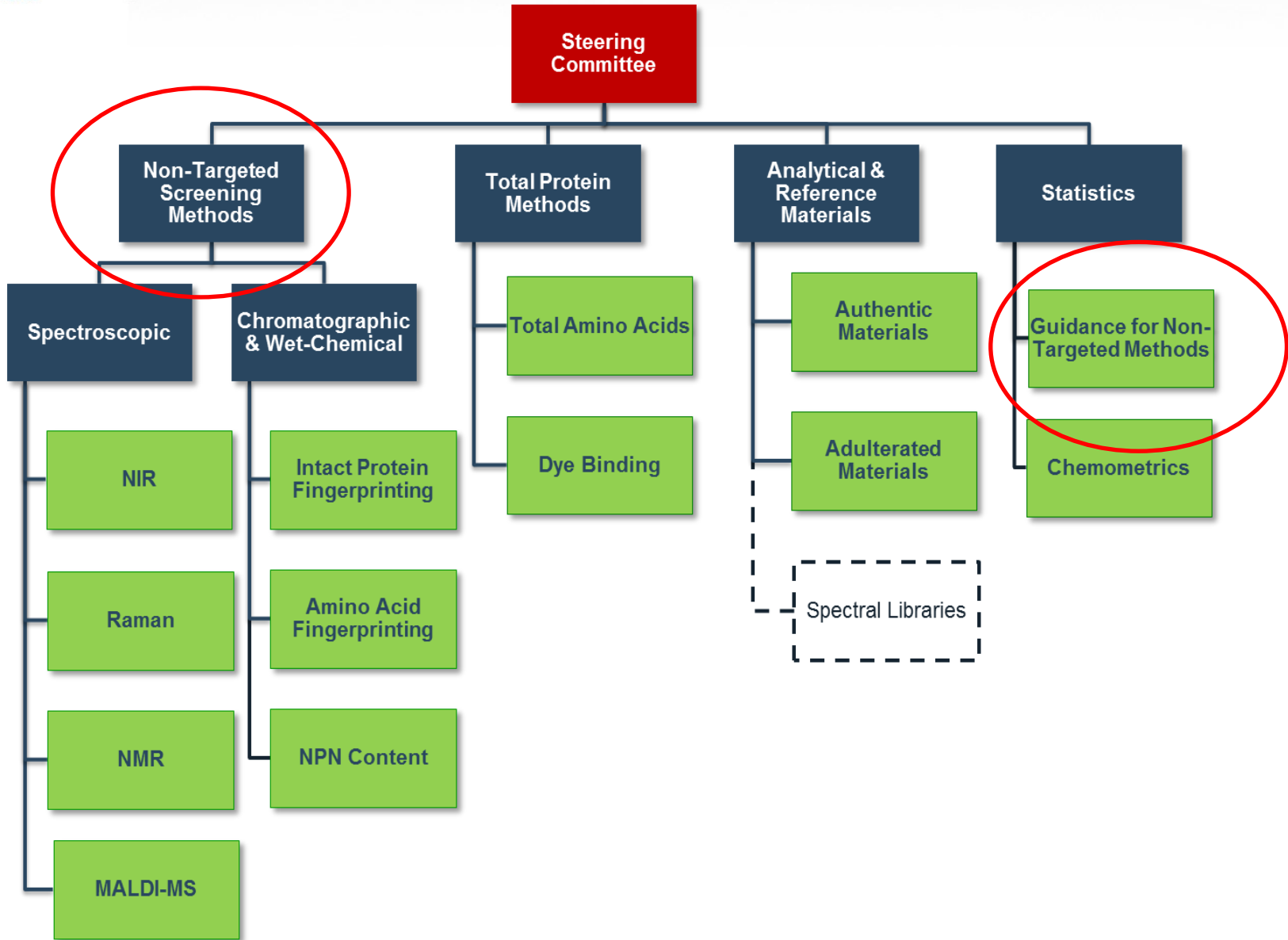
#### **Principle**

Biologically derived protein-based food ingredients inherently contain nonprotein nitrogen (NPN) compounds the

**Table 7. Estimated Detection Capabilities<sup>a</sup>**

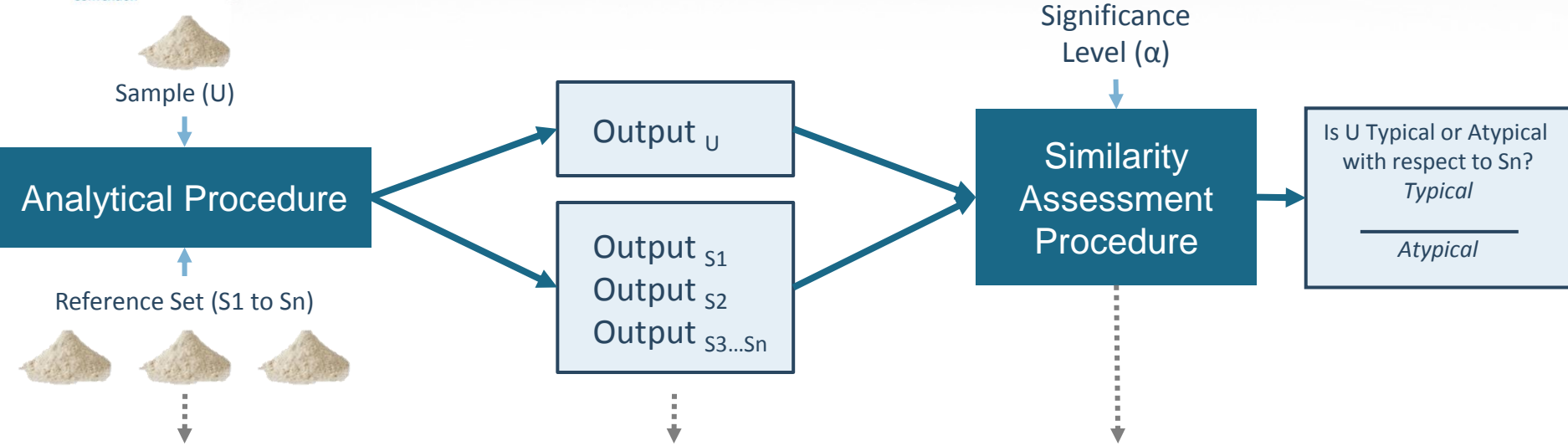
<b>Adulterant</b>	<b>Molecular Size Filtration</b>	<b>Tannic Acid Precipitation</b>
Melamine	0.06%	0.07%
Urea	0.09%	0.04%
Ammonium phosphate	0.22%	0.05%
Isobutylidene diurea (IBDU)	0.16%	0.09%
Aminotriazole	0.11%	0.05%
Dicyandiamide	0.07%	0.05%
L-Arginine	0.15%	0.06%

<sup>a</sup> The minimum level (% , w/w basis) of adulterant, that when added to a sample, increases the NPN content above the 95% confidence level for nonadulterated materials.





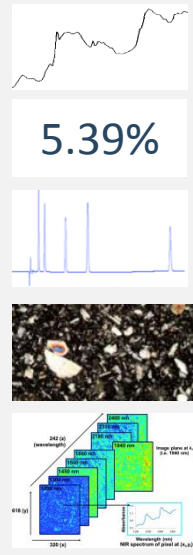
# Essential Elements



## Example Analytical Procedures

- Spectral
- Chemical
- Wet-chemical

## Example Output types

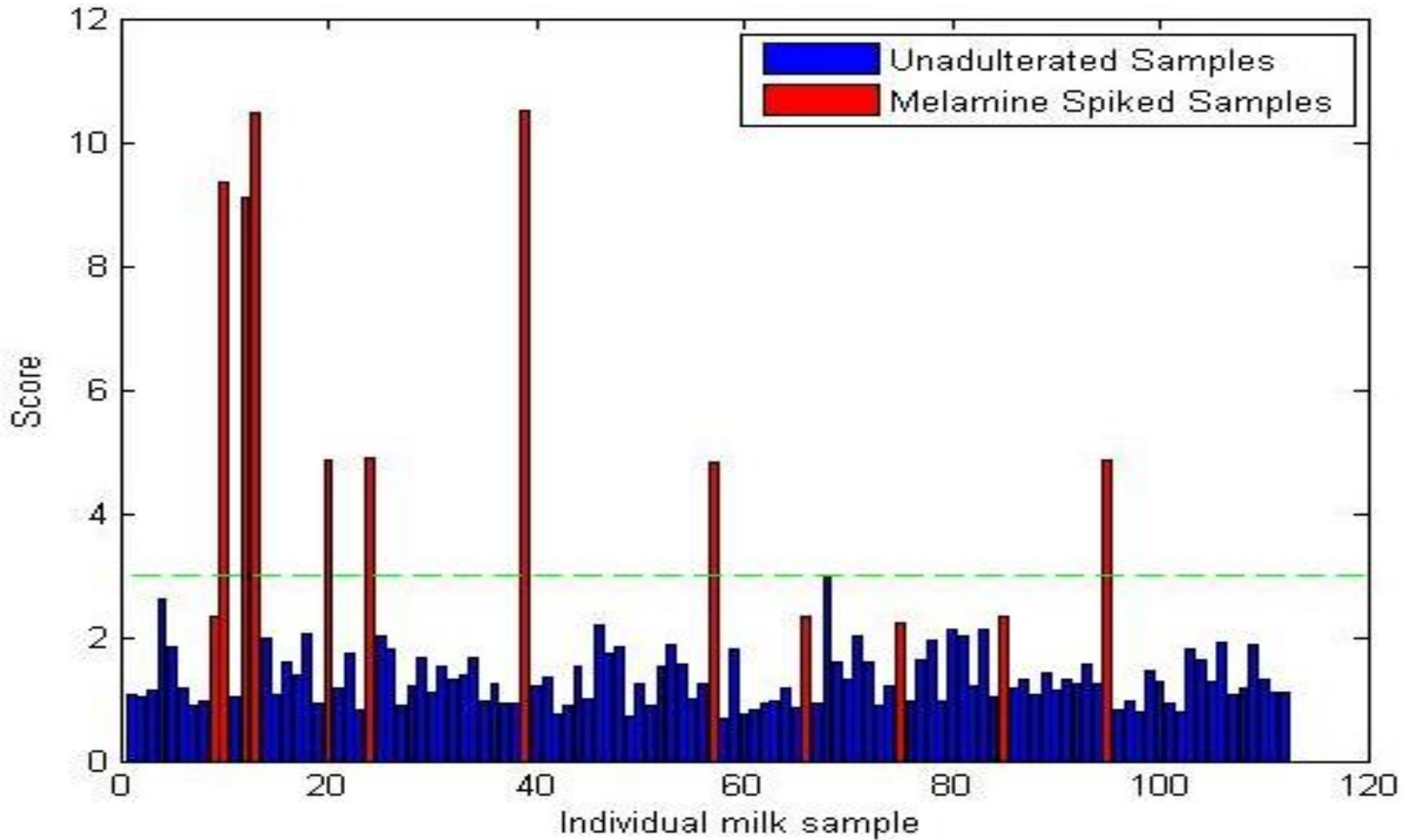


- Spectra
- Quantitative measurements
- Chromatograms
- Images, e.g. micro-photographs
- Hyperspectral images

## Example Similarity Assessment Procedures

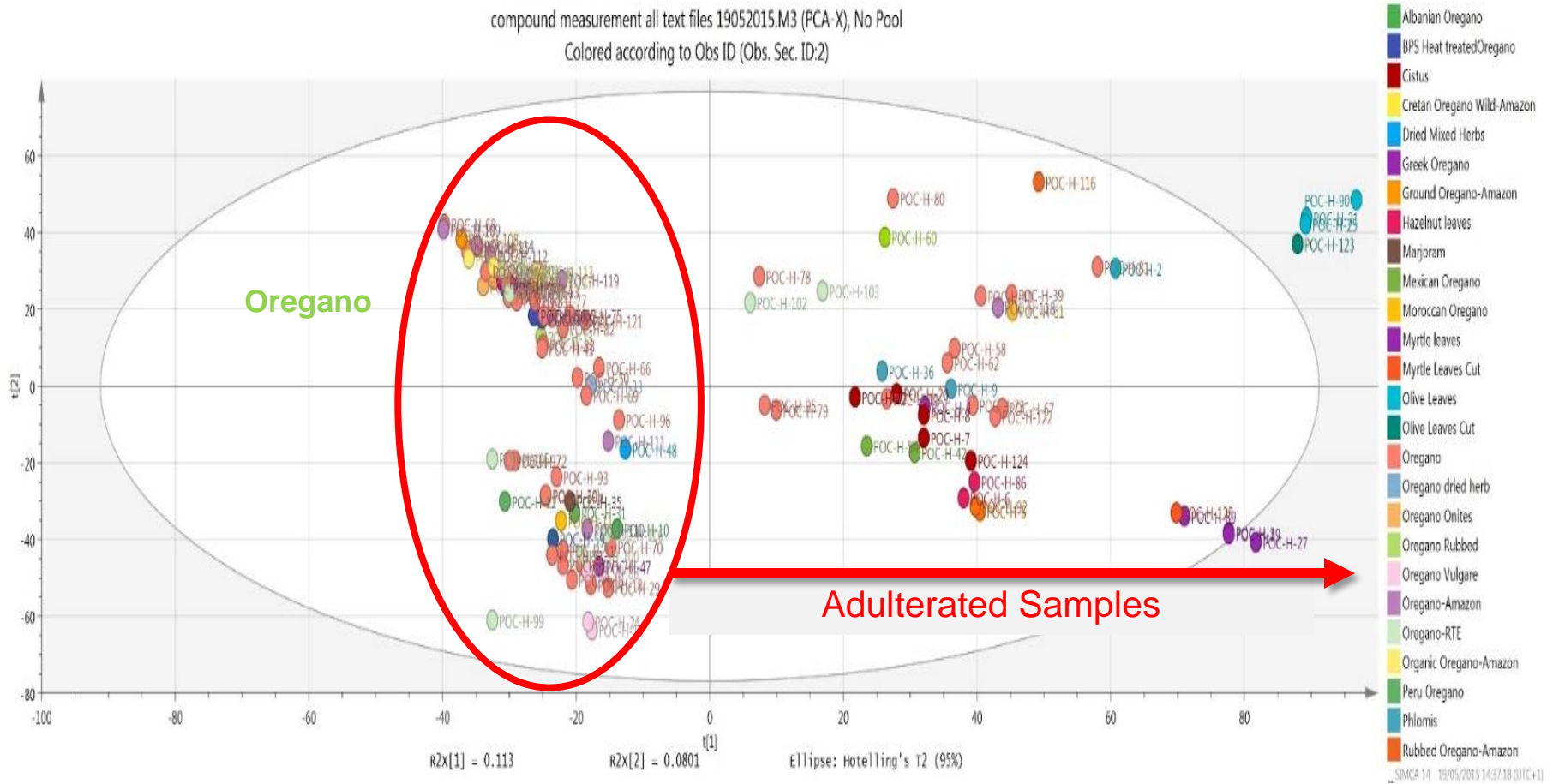
- Multivariate model (e.g. SIMCA)
- Univariate model (e.g. CI, HQI)
- Subjective criteria (e.g. “principle spots”)

# NT Adulterants Detection: Liquid Milk by FTIR (Fonterra)



Slide courtesy of  
Steve Holroyd, Fonterra

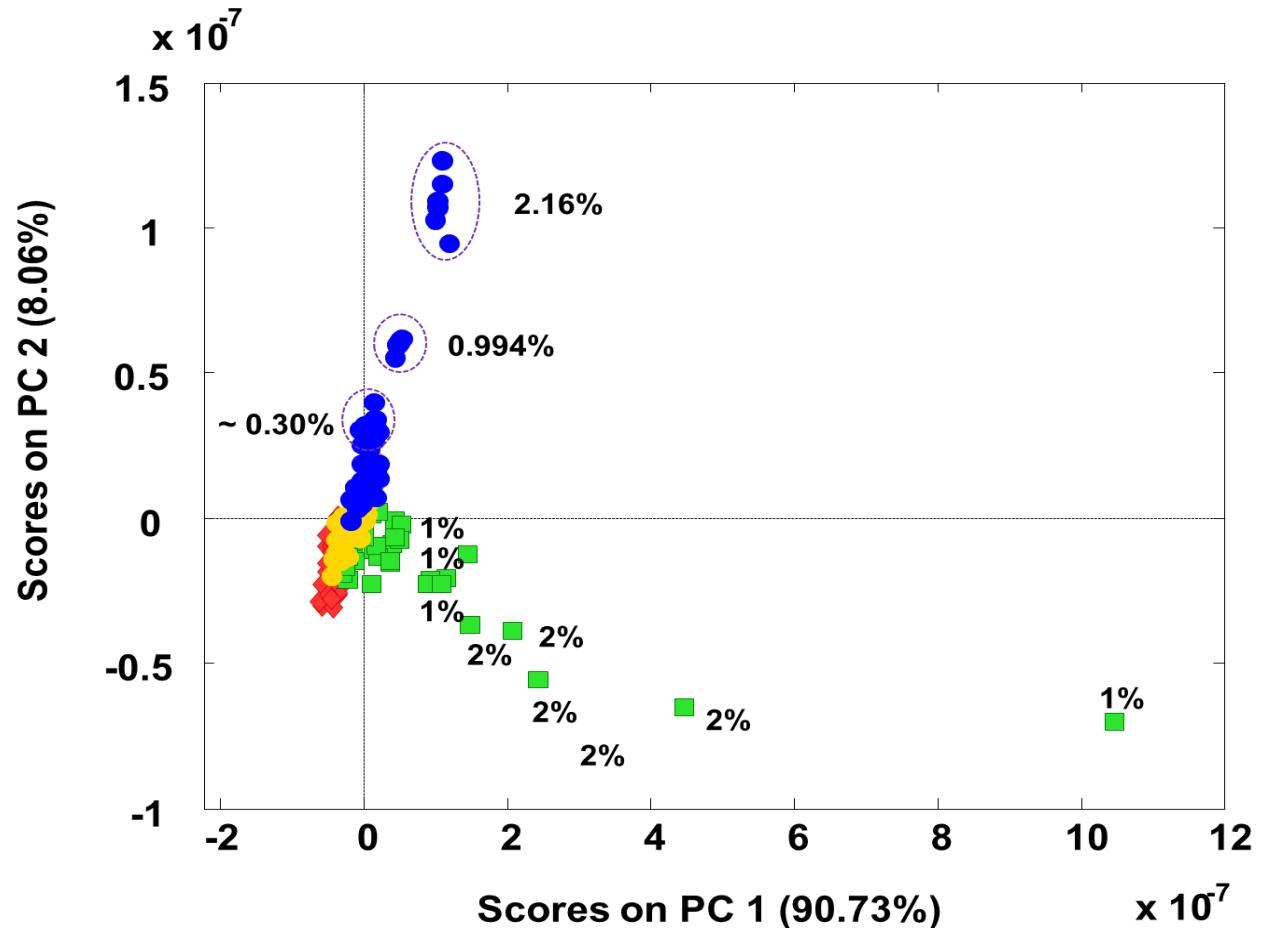
## Use of FTIR and LC-MS for non-targeted adulterant detection in oregano.



## Raman

*PCA Scores Plot,  
36 commercial  
SMPs*

Lowest  
concentration  
detected:  
WB: 0.30 %  
DB: 0.50%



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# Filling the Knowledge Gap

- ▶ Many companies already have the infrastructure, but may not know how to implement this novel approach
- ▶ There is a lack of solid information about the generation and application of NT methods, and very little support

- ▶ “Non-targeted” not defined
- ▶ In use, but inconsistent development
  - ▶ Organizational risk tolerance is not always taken into account when developing non-targeted methods
  - ▶ Representativeness of reference / calibration model
- ▶ Confusing terminology, e.g. false positive vs false negative; specificity vs sensitivity
- ▶ How to validate non targeted methods?

- ▶ **Name:** USP “Guidance On Developing and Validating Non-Targeted Methods For Adulteration Detection”
- ▶ **Aim:** Adaptable “framework”, encourage use of NT methods, reduce confusion
- ▶ **Elaboration:** 10 experts since early 2015
- ▶ **Stage:** Open for public comment until March 31, 2017
- ▶ **Where to find:**

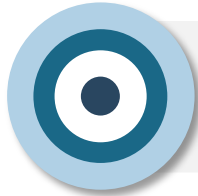
<http://www.usp.org/guidance-developing-and-validating-non-targeted-methods-adulteration-detection>

Send **comments** to: Dr. Kenny Xie, [KYX@usp.org](mailto:KYX@usp.org)

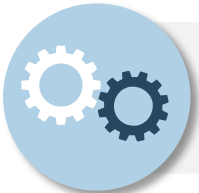


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# Logic flow of USP's NT Guide



Applicability statement



Method development using reference set



Application of test set to method



Method performance evaluation



Interpret results, monitor and maintain method

## Background...

- ▶ Largest fluid milk processor
- ▶ Needed a rapid non-specific adulterant detection method across 14 countries with very different risk profiles
- ▶ FTIR development application from Foss was used
- ▶ The method was to be validated for specific at-risk adulterants
- ▶ Note: Developed to be used as non-targeted ***in conjunction with*** targeted testing and selective testing as appropriate

A suitable applicability statement might have read:

“Rapid non-targeted method for detecting the adulteration of raw liquid milk with nitrogen-rich compounds added at economically motivating levels (e.g. **risk threshold = 0.05% for *melamine*** which is a food safety risk) with a **sensitivity rate of 99% and a specificity rate of 95%**”

- ▶ Reference set composed of ~10,000 verified unadulterated samples from a wide variety of local suppliers
- ▶ Model derived by PCA and spectral residuals with normalized spectra
- ▶ Boundary drawn around the data to achieve required sensitivity, with flexibility to adjust in response to model performance
- ▶ Highly structured and documented response to repeat alerts and other alert patterns

## Test set:

- ~50 verified unadulterated samples, not used in the reference set
- ~50 adulterated (spiked) samples for each of 11 different adulterants

**Sensitivity** = Ability to correctly recognize unacceptable samples/material as *Atypical*

$$= \frac{\text{Correct Atypicals}}{\text{Total Atypicals}}$$

**Specificity** = The ability to correctly recognize acceptable samples/material as *Typical*

$$= \frac{\text{Correct Typical}}{\text{Total Typical}}$$

# Compare Output to the Applicability statement

- ▶ Alone, the non-targeted method was promising, but a hybrid of targeted and non-targeted approaches proved to be extremely effective:
- ▶ Sensitivity= 99.9%
- ▶ Specificity= 99%

This compares favourably to the initial expectations, and the combined FTIR method was deployed



## Interpretation of results, monitoring and maintenance of the method

- ▶ Results are taken as indicative, and alerts are followed up by further investigation as appropriate
- ▶ Validation can be achieved via selectively spiked samples
- ▶ Method is monitored via statistical monitoring of actual alerts
- ▶ In reality, the method is constantly undergoing updates, to account for dynamic nature of the natural product



- ▶ Can be rapid, inexpensive, and powerful tools for mitigating risks in food ingredient supply chains, even for known adulterants (e.g. wet-blended melamine)
- ▶ Combined with targeted can be more effective than either individually
- ▶ USP helping to address standardization gap, seeking public comments on its proposed Guidance for Non-Targeted Methods

- ▶ USP Expert Panel on Non-Targeted Methods for Milk Ingredients
- ▶ Steve Holroyd (chair, sub-team on NT guidance)
- ▶ Anthony Hanlon
- ▶ Kenny Xie (scientific liaison for NT guidance)
- ▶ Carmen Diaz-Amigo



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A light gray silhouette of a world map is centered in the background of the slide.

# Thank You