# Checking What is Not There

## Non-targeted methods are the first step in detecting adulteration; one that could then be followed by targeted methods for confirmation of food fraud.

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In the food industry, it is estimated that up to 10 percent of the food supply is affected by food fraud at any time. The challenge for food safety professionals is to think one step ahead of fraudsters. While the norm for the food industry has been to develop tests to identify known adulterants, more recently many food ingredient manufacturers have begun to develop what are known as non-targeted methods.

A non-targeted method for detecting adulteration in food ingredients is one which models the properties of the authentic material rather than the properties of the known adulterants or any of the adulterant's characteristics. Instead of trying to find some chemical which may or may not be present, the focus is on assessing whether or not something that differs from the authentic source material is detected. It is the first step in detecting adulteration; one that could then be followed by targeted methods for confirmation of food fraud.

### Limited Adoption

The promise of non-targeted methods is great, but confusion in terminology and lack of guidance on procedures to develop and validate non-targeted methods for food fraud detection has limited their wider adoption by food companies and regulatory agencies.

In 2017, USP (a.k.a. The US Pharmacopeia) developed a guidance document to address some of the questions related to the use of non-targeted methods to detect food fraud. The guidance, published in the third supplement to the Food Chemicals Codex (FCC), 10th edition, addresses the collection and analysis of reference samples, development and validation of non-targeted testing statistical models, their monitoring and maintenance, as well as advice on the handling of abnormal samples.

After a full review and addition of updated information, a revised version of the document was proposed and made available for public comments on December 31, 2018. New comments may be submitted on the revised guidance document until March 31, 2019. The final document is scheduled to be published in the third supplement to FCC 11th edition (September 2019), after review of the comments and approval by USP's Food Ingredient Expert Committee.

### Finding the Unknown?

USP's Guidance on Developing and Validating Non-Targeted Methods for Adulteration Detection uses a step-through logic flow that starts with an applicability statement. The statement defines criteria that will determine whether a proposed non-targeted method has acceptable risk profiles to match the need for a test. Once it's been determined that the development of a specific test is feasible, the actual method development uses analysis of a reference set of "typical samples," adopting a pre-selected analytical procedure and pre-established criteria.

One example that can illustrate this is the analysis of paprika by paper chromatographic fingerprinting. Ground paprika spice procured from a supplier is screened to ensure that it is not adulterated with unknown colors. Colors, used with the intention of committing food fraud, would likely be added at concentrations sufficient to enhance the color of the authentic spice.

A non-targeted method would use an extract from the paprika sample, which is applied to a thin-layer chromatographic (TLC) plate and developed. The chromatogram would be visually compared to a reference set of chromatograms generated from samples of paprika determined to be genuine (by careful control and documentation of the sample's provenance and secondary or reference methods) using a predefined set of qualitative characteristics (e.g., the size and locations of the spots in the chromatograms of the sample would correspond to those of the reference set).

The outcome would be either "typical" for samples conforming to the predefined criteria, which implies a lower probability of adulteration; or "atypical" for samples not conforming to the criteria which indicates an increased probability of adulteration. A sample with an atypical outcome might be a sample adulterated with a color (e.g. Sudan IV or some undeclared food dye, etc.), or it might be a genuine, unadulterated sample with processing or compositional parameters outside those represented by the reference set.

A boundary between typical and atypical is determined by the variability present in the reference set. Figure 1 illustrates this. The authentic paprika samples from different sources had characteristic bands between Retention Factor (Rf) 0.8-0.95 under white light so this is the predefined characteristic. A sample showing color bands outside of the RF range will be seen as out of the boundary and the sample is "atypical" or adulterated.

After defining what would be an "authentic" product, a "test set" is made up of both knowingly adulterated samples (atypical) and unadulterated samples (typical). If the method appears capable of differentiating between atypical and typical samples, then it is validated using a separate, but similar, test set. From this series of analyses, the sensitivity (how well the method detects atypical samples) and specificity (how well the method responds to typical samples) of the test can be assessed.

If the method meets the requirements of the applicability statement, then it is released, monitored, and revised as required.

### The Challenges

Non-targeted methods to aid in the detection of food fraud – when appropriately developed and validated – have shown promise as a primary screening tool versus the more expensive targeted methods.

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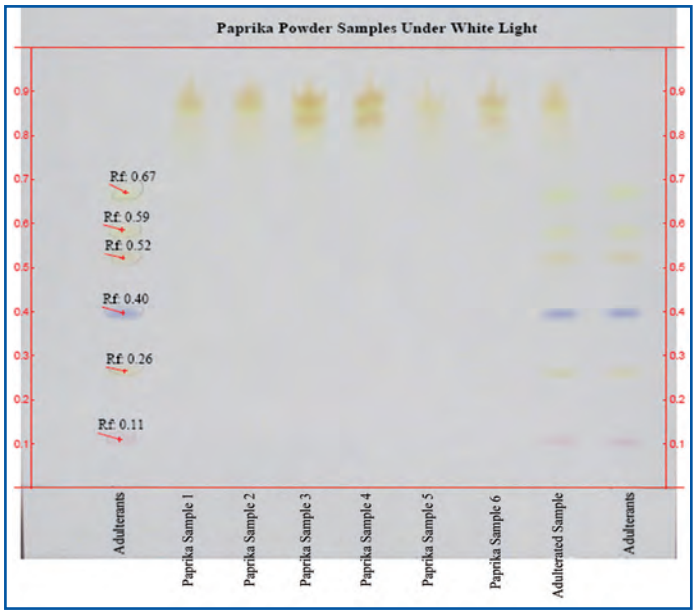
**Figure 1: Boundary between typical and atypical samples as determined by the variability present in the reference set**




Many companies already have the infrastructure to develop and use these methods more broadly, but they may still have questions about how to implement this novel approach.

A lack of consistent information about the development and application of non-targeted methods, and lack of standardization for terms and validation may be among the reasons for the somewhat slow adoption of these techniques.

A shift in food fraud detection paradigms may also be a deterrent to applying non-targeted methods more broadly. Traditional thinking on detection of food fraud focuses on whether a known adulterant is present or absent. For non-targeted methods, finding food fraud is a probability mindset. The non-targeted method needs to define the probability of detecting adulterated as atypical (sensitivity rate), and non-adulterated as typical (specificity rate). For an ingredient supplier, the sensitivity rate requirement will be high, as the supplier would not want any safety hazards.

The specificity rate requirement may be lower, however. This means that the supplier will accept that some portion of non-adulterated samples will be flagged as potentially adulterated, requiring the supplier to retest those flagged samples with a targeted method for confirmatory testing.

Finally, another challenge to the development and validation of non-targeted methods to detect food fraud might be the lack of sufficient data points to create reference sets. Testing facilities may only receive products originating from a certain region, a certain grade of an ingredient, products from a certain season, etc. They may not have sufficient data points to develop a robust calibration model to determine whether an incoming product is authentic or not. USP's guidance document attempts to address some of these challenges and provide food ingredients and food products manufacturers with consistent information that adds confidence to the process of developing and validating non-targeted methods.

## A Practical Example

Applying the principles of USP's guidance document, Fonterra created a non-targeted method for the detection of adulterants in liquid unprocessed milk, using high-capacity mid-IR instruments situated in a large milk payment laboratory that tests incoming raw milk to ensure the quality and safety prior to further processing.

This method was then assessed using samples of known levels of contamination with various adulterants. An assessment trial was then developed to test the non-targeted method with 12 potential dairy adulterants at various concentrations. Among those known adulterants were melamine, urea, and hydroxyproline, which are potential milk adulterants that may be used for economic gain.

Fonterra decided that the applicability statement would be a rapid non-targeted method for detecting adulteration of unprocessed liquid bovine milk with nitrogen-rich compounds added with a sensitivity rate of 99 percent, a specificity rate of 95 percent and a confidence interval of 95 percent.

The method was developed and tested for all 12 common dairy adulterants. A reference set was built using unprocessed liquid milk that had a high degree of variability, including differences in geographical origin as well as age, composition and breed of dairy cattle. The test set was built using milk from the same pool and the samples were deliberately adulterated with known concentrations of the chosen adulterants.

The boundary was set using a combination of spectral and statistical variables in the reference set. Using certain algorithms, these variables produced a numerical score. Any numerical score equal or higher than 3 (the boundary) was reported as "atypical," while below 3 was reported as "typical." The method was validated using different concentrations of adulterants deliberately diluted into liquid milk.

The sensitivity and specificity were tested and compared against the applicability statement. The results were overall satisfying in that the method was able to detect multiple adulterants meeting the sensitivity and specificity requirements.

## The Next Steps

USP's draft guidance document is now open for public comment until March 31, 2019. USP aims to assist the food industry in adopting the development of non-targeted methods to detect food fraud more broadly and consistently.▼

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