NON-TARGETED ANALYSIS (NTA) OF FOODS

WHITE PAPER

NOVEL ANALYTICAL METHODS FOR FOOD AUTHENTICATION

Globalization of complex food supply chains and consumer demand for food fraud prevention advance new research developing innovative approaches to more efficient, cost-effective tools for screening and regulatory programs in the food and beverage industry.

Non-Targeted Analysis (NTA) of Foods

What is this Technology?

Non-targeted analysis (NTA) is a powerful methodology used to classify samples and/or identify new and emerging contaminants, or unspecified or unknown compounds/classes of interest. These methods are especially useful for food applications including safety, quality, authentication, and nutrition, among others.

The figure below graphically presents the principles of targeted versus non-targeted authentication together with a conceptual presentation of primary versus secondary analytical markers.

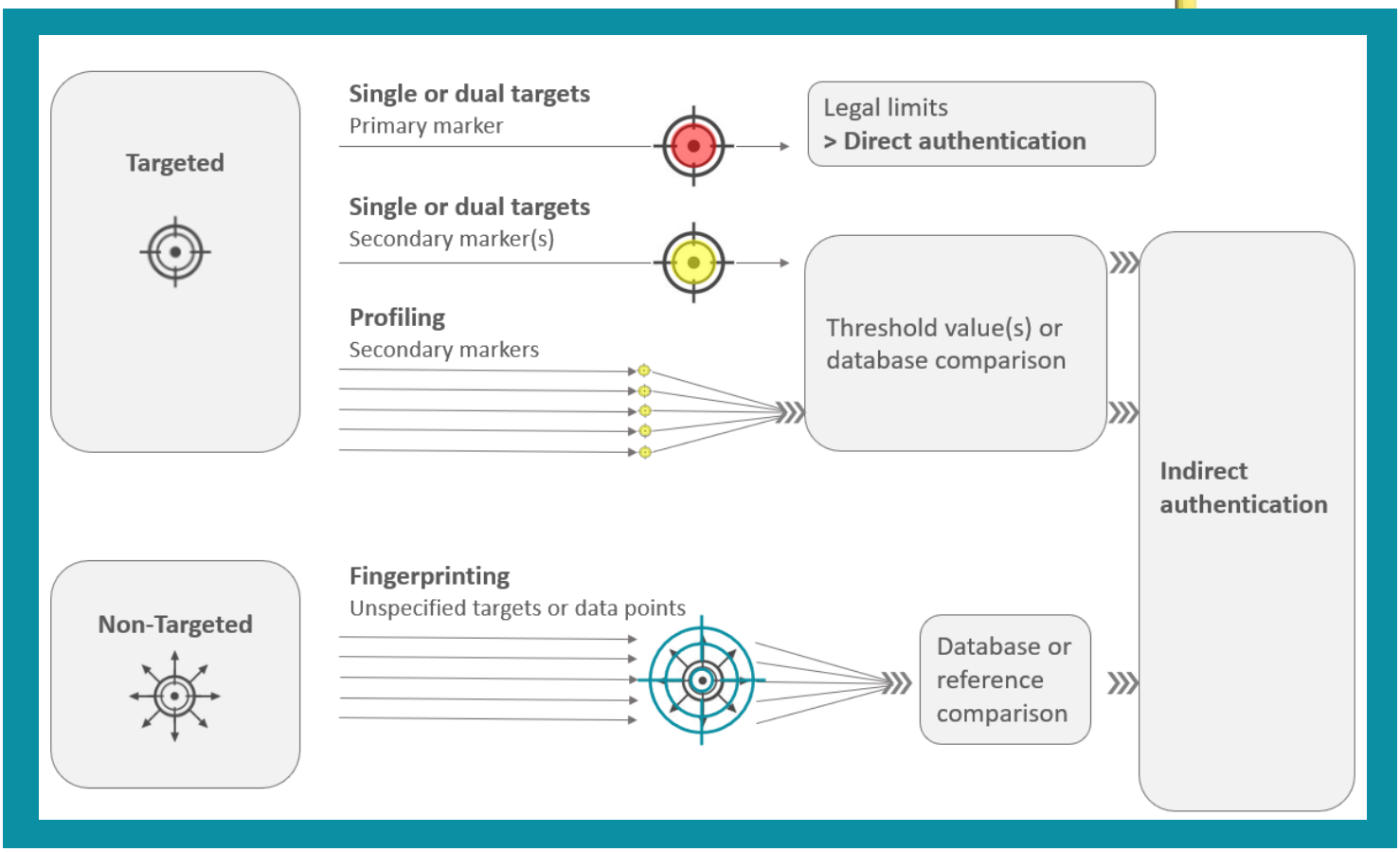


FIGURE 1. THE PRINCIPLES OF TARGETED VERSUS NON-TARGETED ANALYTICAL FOOD AUTHENTICATION.[1]

Background

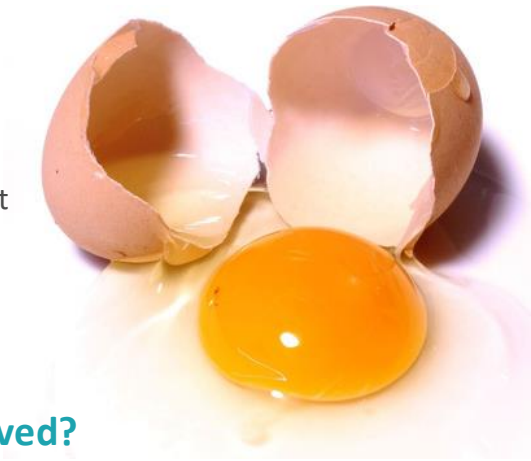
Tens-of-thousands of chemicals are registered in the U.S. for use in countless processes and products. Recent evidence suggests that many of these chemicals are measurable in environmental and/or biological systems, indicating the potential for widespread exposures. Traditional public health research tools, including in vivo studies and targeted analytical chemistry methods, have been unable to meet the needs of screening programs designed to evaluate chemical safety.

Background (continued)

Routine food safety analysis did not reveal the tainted milk scandal in China 2008 since melamine was not on the target list for control at that time.[2] As a consequence of the melamine adulteration, almost 300,000 babies were taken ill and 6 died as reported by the Ministry of Health, China, at the end of that year.[3,4] More recent examples of unexpected food-related contamination of public concern are PFAS and related compounds in drinking water in several countries in Europe and the USA, e.g., in Sweden in 2012,[5,6] and fipronil in Dutch eggs spread in Europe in 2017.[7] Again, the compounds were unexpected and therefore not screened for until it was too late to prevent them from being widely spread, and some contaminated Swedish sources of raw water can still (year 2017) not be used for drinking water production.

Food fraud has been gaining more and more attention in recent years because of an apparently never-ending series of scandals and incidents of fraudulent food labelling and of other food fraud activities that impacted the entire food chains. There are diverse reasons why food fraud is still an issue and why incidents are even increasing. Drivers of food fraud are:

- competition, particularly competitive and increasing price pressure
- globalization and increasing complexity of supply chains
- differences in official control systems of trading countries, and
- increasing consumer interest in and appreciation of distinctive product characteristics, e.g., regional (geographical) origin of products, means of production (such as sustainable, organic, fair trade)



Why is NTA Important and What is the Problem to be Solved?

Non-targeted methods can **detect and identify new, unknown, and/or unexpected compounds and can be instrumental in pinpointing unsafe contaminants in food**. They are often used in screening applications, such as olive oil authentication.[8] They can also be used for retrospective analyses where previous data can be mined for newly discovered compounds of concern to determine if these chemicals have been detected previously, for example, retrospective analysis for transformation products of detected pesticides and veterinary drugs.[9] Non-targeted methods are also useful in nutrient analyses and foodomics (the comprehensive, high-throughput approach for the exploitation of food science in the light of an improvement of human nutrition)[10] because they offer a more complete view of sample composition.

The challenges faced today are not novel or different from the ones faced a decade ago, but they have become more common – partly because of the globalization of the food supply which enables the year-round availability of a highly diverse array of foods, regardless of the local climate/weather conditions. Currently, the FDA is using targeted methods for food sample analyses to verify that foods, both domestic and imported, meet all applicable food safety standards. However, when targeted methods are not available, non-targeted analyses allow the detection and identification of new, unknown, and/or unexpected compounds.

True NTA (also called "untargeted" analysis) studies are those in which **chemical structures of unknown compounds are postulated without the aid of suspect lists**.[11] [12] NTA studies are gaining in popularity, but the rapid and accurate characterization of large suites of chemical unknowns remains challenging. Appropriate resources and efficient methods must be identified to propel NTA methods away from a niche field and into mainstream public health laboratories.

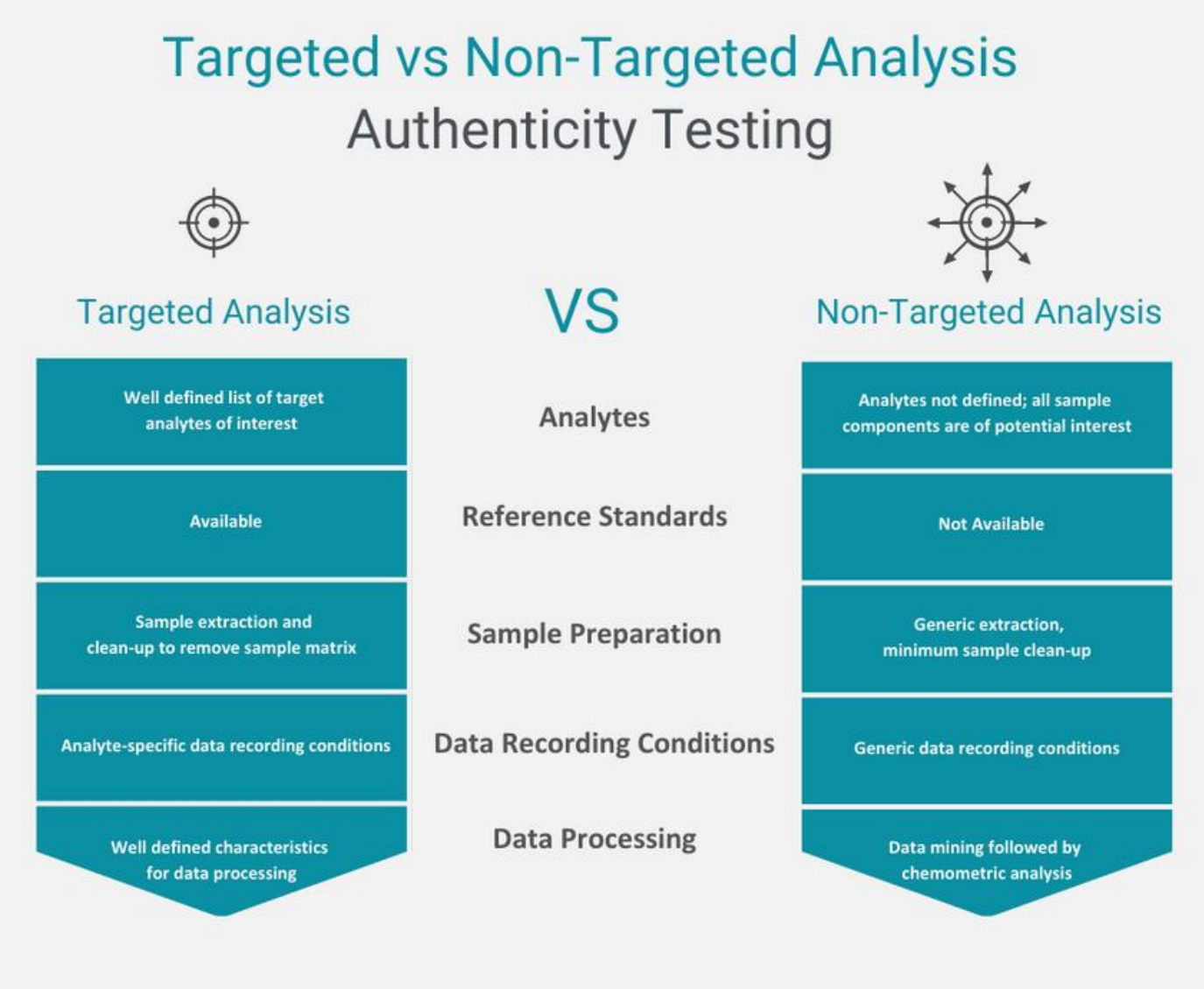
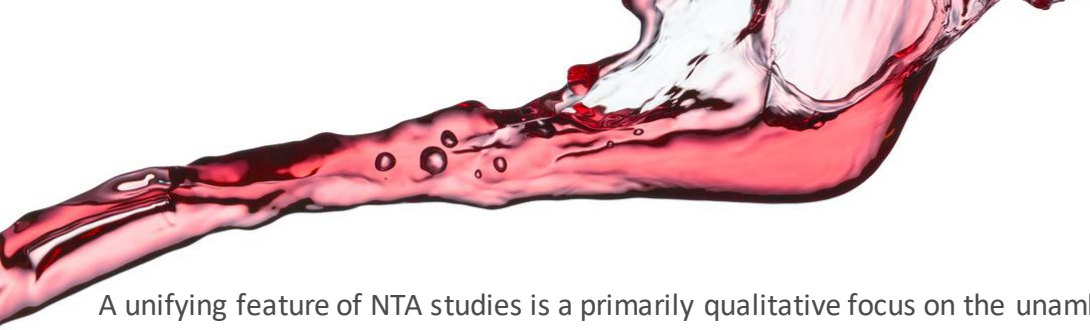


TABLE 1. TARGETED V. NON-TARGETED ANALYSIS [13]



A unifying feature of NTA studies is a primarily qualitative focus on the unambiguous identification of individual species—and there are large concerted efforts to improve and formalize chemical identification processes.[14][15][16] Quantitative interpretations of NTA data to date have been largely based on *relative quantitation*, where measured chemical responses are compared across two or more sample groups (e.g. fold-change comparison) for prioritization of chemicals, without attempt to link them directly to fixed concentration values. *Absolute quantitation* is rare, where direct measurements of compounds are translated to estimated sample (e.g., drinking water, blood) concentrations. Ideally, there would be approaches to convert measurements of feature response in NTA experiments to informative sample concentrations.

Examples of NTA

As of 2016, the use of NTA methods in routine analysis was limited to a few products (e.g. juice, wine, and honey – see below in characterizations by Bruker).[17] The reason for this is the lack of important prerequisites, such as:

- comprehensive method validation, effective proficiency tests, and quality assurance measurements, including:
  - -standardized protocols for sample collection
  - -standardized and valid analytical methods
  - -validated statistical methods for data analysis
- reliable databases of representative authentic samples and of genuinely adulterated material, and
- uniform data exchange formats for jointly usable databases

How is NTA Being Integrated Into Workflows?

There are several ongoing efforts to standardize and harmonize aspects of non-targeted analysis workflows, including those by several working groups such as the Metabolomics Quality Assurance and Quality Control Consortium (mQACC),[18] the NORMAN Network,[19] and Benchmarking and Publications for Non-Targeted Analysis (BP4NTA).[20] One of the biggest challenges for standardization is broad applicability of non-targeted analysis – even within a given field.

Metabolomics Quality Assurance and Quality Control Consortium (mQACC)

The consortium's mission is to engage the metabolomics community to communicate and promote the development, dissemination, and harmonization of best QA/QC practices in untargeted metabolomics.

The aims of the consortium are:

- To identify, catalog, harmonize and disseminate QA/QC best practices for untargeted metabolomics
- To establish mechanisms to enable the metabolomics community to adopt QA/QC best practices
- To promote and support systematic training in QA/QC best practices for the metabolomics community
- To encourage the prioritization and development of reference materials applicable to metabolomics research

The NORMAN Network

The NORMAN network enhances the exchange of information on emerging environmental substances and encourages the validation and harmonization of common measurement methods and monitoring tools so that the requirements of risk assessors and risk managers can be better met. It specifically seeks both to promote and to benefit from the synergies between research teams from different countries in the field of emerging substances. The mission of the NORMAN network is:

- Enhance the exchange of information and collection of data on emerging environmental substances
- Encourage the validation and harmonization of common measurement methods and monitoring tools so that the demands of risk assessors can be better met
- Ensure that knowledge of emerging pollutants is maintained and developed by stimulating coordinated, interdisciplinary projects on problem-oriented research and knowledge transfer to address identified needs

Benchmarking and Publications for Non-Targeted Analysis (BP4NTA)

Benchmarking and Publications for Non-Targeted Analysis is a working group formed to address challenges in non-targeted analysis studies using mass spectrometry. To date, group members have aimed to establish a consensus on NTA-related terms and concepts and to create consistency in reporting practices by providing resources on their public website; including consensus definitions, reference content, and lists of available tools. [21]

Within food analysis, workflows used to investigate food safety, quality, and authenticity are often distinct from those used to investigate foodomics and nutrition. Further, no single method can detect and identify everything, as demonstrated by the EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) [22,23] so standardizing a single method will inherently exclude some of the chemical space that may be of interest. It is also important to consider that standardizing non-targeted workflows may result in stifled creativity and advancement of the field.

Recording of Sample Fingerprints	Data Processing	Data Analysis	Identification of Features of Interest
Relevant Sample Set	Data-Mining of Fingerprints for Features Across Sample Set	Advanced Chemometric Techniques	HR MS/MS and NMR Data
Sample Preparation	Removal of Redundant Signals	Differential Analysis Between Two and More Sample Groups	Spectral Databases and Interpretation Software
Quality of Data	Normalization and Scaling	Selection of Characteristic Features for Sample / Sample Group	Unequivocal Identification Based on Analysis of Reference Standard
Reproducible Measurements	Advanced Software Tools	Classification Models	

TABLE 2. REPRESENTATIVE NON-TARGETED ANALYSIS WORKFLOW [13]

FIGURE 2. TYPICAL WORKFLOWS FOR TARGETED v. NON-TARGETED LC-MS ANALYSIS [24]

Non-targeted screening and the determination of unknowns

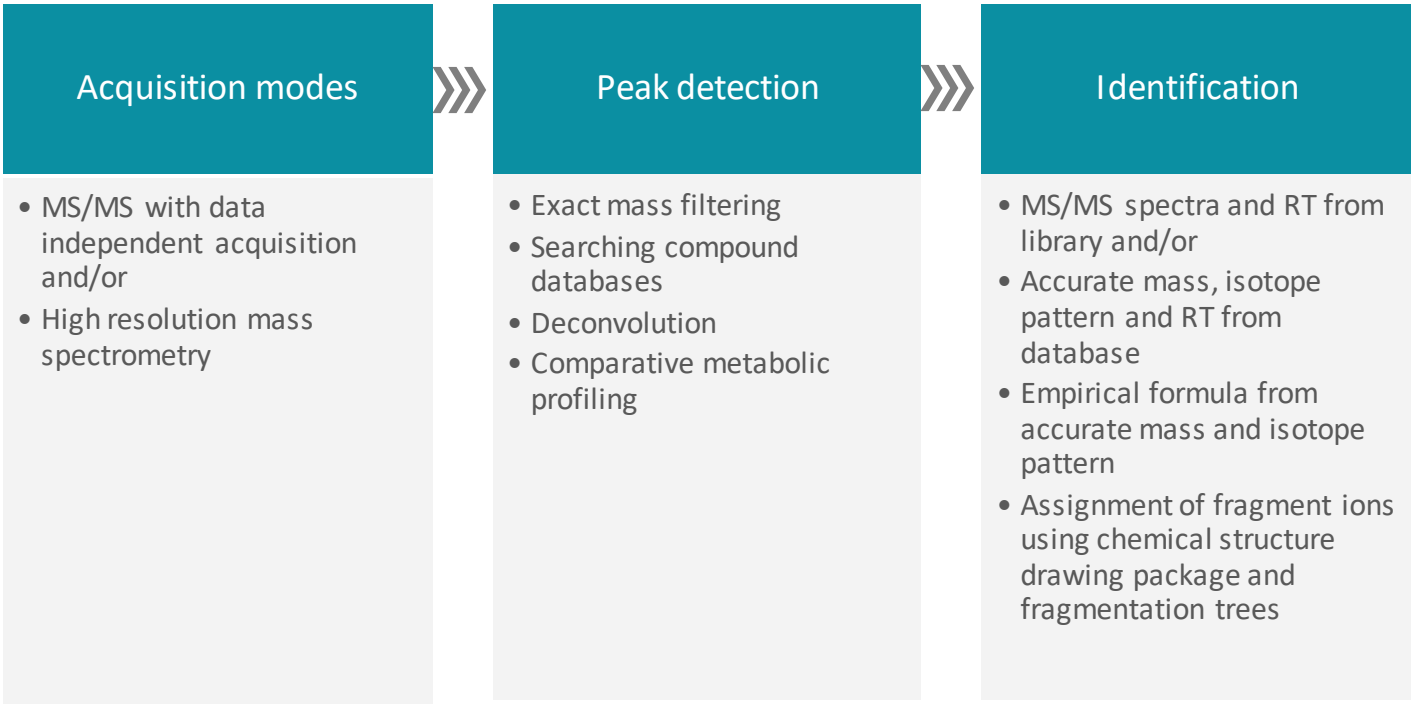


FIGURE 2. TYPICAL WORKFLOWS FOR TARGETED v. NON-TARGETED LC-MS ANALYSIS[24]

Examples of improving different aspects of non-targeted workflows have been described by Christine Fisher at the FDA. Dr. Fisher has been engaged in developing a method for introducing calibrant ions into the source of their instrument during heated electrospray ionization (HESI) to enable automatic lock-mass calibrations to improve the measured mass accuracy of observed compounds, especially during queues lasting several days to weeks. It is hoped that this could also lead to better putative compound identifications. Dr. Fisher is also working to further develop a standard mixture that can be used to evaluate non-targeted method performance, which is hoped to ultimately be commercialized to increase accessibility for other non-targeted analysis researchers.[25]

What are the analytical platforms?

While nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy play roles in NTA, the most prevalent analytical platforms involve high-resolution mass spectrometry (HRMS). HRMS methods (often coupled with separation techniques like liquid chromatography (LC) or gas chromatography (GC)) utilize ionization sources paired with high resolving power mass detectors to isolate and identify chemicals based on their observed accurate masses, isotopic fingerprints, and MS/MS fragments.

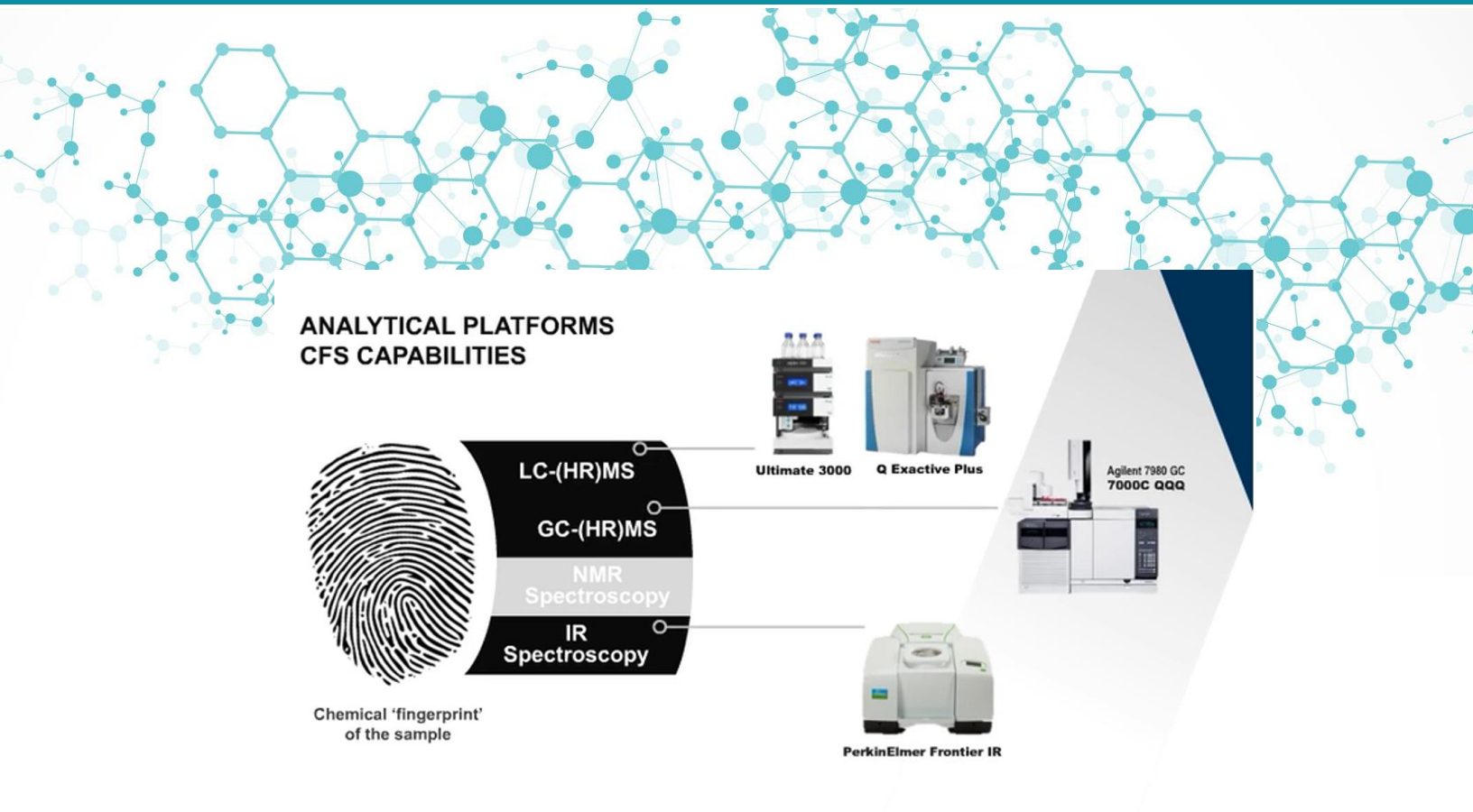


FIGURE 3. REPRESENTATIVE ANALYTICAL PLATFORMS IN NON-TARGETED ANALYSIS [13]

Who are Representative Equipment / Service Providers?

Agilient [26] Increasingly complex global food supply chains challenge the food industry to continuously identify new and emerging contaminants. Non-targeted testing approaches are especially useful for analysis of unspecified or unknown compounds. Sample profiles are compared to authentic "fingerprints" to quickly assess food authenticity, safety, and quality. Agilent's **Quadrupole time-of-flight (Q-TOF)** instruments have exquisite sensitivity and resolution, combined with powerful software and curated databases to enable rapid detection and identification of unknown or unexpected compounds within a food or drink sample without the need to pre-select the chemicals of interest.

Representative Equipment / Service Providers (continued)

Bruker [27] Customers who submit samples for non-targeted analysis through the Bruker Analytical Services Food Center demand results of the highest quality and reliability, requiring continuous validation of analyses performed. An intensive in-house validation is undertaken as well as participation in official and international ring tests.

Platform: FoodScreener. The FoodScreener enables the detection of unpredicted and even unknown fraud. Complex statistical models allow the testing of origin authenticity, production process control, false labeling, sample similarity, and species purity.

- Case Study: Honey Profiling
- Case Study: Wine Profiling
- Case Study: Juice Profiling
- Case Study: Olive Oil-Profiling

Waters [28] The ChromaLynx™ Application Manager automates the processing of LC/MS, GC/MS, LC/MS/MS, or GC/MS/MS data. ChromaLynx helps you rapidly detect, identify and semi-quantitatively determine all the components in your complex mixtures. ChromaLynx provides you with the ability to:

- Detect and locate the maximum number of components in the sample
- Identify the maximum number of components in the sample
- Estimate component concentrations
- Compare samples with controls to identify common and unique components ChromaLynx XS software facilitates the identification of compounds in complex mixtures in non-targeted analysis by utilizing automated chromatographic peak detection, mass spectral deconvolution, library searching, accurate mass measurements, and rigorous sample comparison algorithms.

SwRI [29] Recent advances in mass spectrometry coupled with large data collection in cheminformatics are benefiting many fields, including non-targeted analysis (NTA) and exposomics, by providing the potential to perform deep analysis and gain unprecedented insights into chemical properties. Floodlight™ provides high-throughput screening (HTS) capabilities for high-quality signal interpretation. Floodlight leverages machine learning algorithms for rapid pattern matching, allowing scientists to make faster data-informed decisions with non-targeted analysis and other complex datasets.

Floodlight solves many challenges associated with complex data analysis including:

- Chemical Data Analysis
- High-throughput Screening (HTS)
- Non-Targeted Analysis
- Targeted Analysis
- Pattern matching
- Signal interpretation

Instrument Applications:

- Mass Spec Data Analysis
- GC/MS, LC/MS, MSI, Optical Spectroscopy, Spectroscopy, Tandem MS

Representative Equipment / Service Providers (continued)

Tentamus [30] The Tentamus Center for Food Fraud (TCF^2) not only bundles the various analytical capabilities of the international network of Tentamus laboratories, but they also use their internal auditor network to support customers in identifying weak points in supply chains and introducing preventive measures.

Non-targeted NMR analytical fingerprint analysis is used in the analysis of honey, agave syrup and olive oil. Analytical fingerprints provide information on adulteration by admixture as well as on the origin of a sample. [31]

Campden BRI [32] Campden BRI is a leader in global food and drink science and research, possessing both the knowledge and practical expertise to deliver results that safeguard businesses and unlock opportunities. From 2016-2018, a member funded research project was undertaken to grant members access to non-targeted analysis for "unknown hazards" to provide more confidence in the safety of their food and drink products. The aim was to develop a robust non-targeted screening approach across a range of analytical instruments, demonstrating that this is a practical and useful tool for the detection and identification of unknown contaminants.

- Sample Preparation: final sample extraction method needs to be unselective and be able to solubilize and recover a large range of chemical compound classes.
- Pre-Separation Techniques: gas chromatography or liquid chromatography would be employed to reduce the complexity of the matrix, to help separate out any isomers and to concentrate the analytes of interest. Mass spectrometry is one analytical technique that has been identified as a powerful tool in non-targeted screening primarily because prior knowledge of chemical content isn't required, and it has a relatively large dynamic range.
- Data Processing and Interpretation: Often the bottleneck in a non-targeted workflow is data analysis and interpretation. The mass spec output data from these high-resolution instruments is very information-rich, and a powerful data mining tool needs to be employed to enable identification and interpretation. [33]

Webinar and Videos

Webinar

Non-Targeted Analysis for Authenticity Testing
Presenter: Lukas Vaclavik

Videos

LC/MS-based suspect and non-targeted screening in foods & food packaging
Presenter: Stéphane Bayen

Finding a Needle in a Haystack: Using High Resolution Mass Spectrometry in Targeted and Non-Targeted Searching for Food Contaminants
Presenter: Erik Verschuuren

Subject Matter Experts

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If you are interested in Food and Beverage Industry Innovation, Ezassi can help your R&D teams with technology discovery and analysis, SME identification, and startup partnership vetting to build collaborative connections.

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LET'S TALK ABOUT INNOVATION

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