Journal of Chromatography A, 1428 (2016) 86–96

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review article

Non-targeted screening approaches for contaminants and adulterants in food using liquid chromatography hyphenated to high resolution mass spectrometry

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ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 14 August 2015
Accepted 27 August 2015
Available online 31 August 2015

Keywords:
Non-targeted screening
Food safety
LC/MS
HR-MS
Unknown analysis

ABSTRACT

The majority of analytical methods for food safety monitor the presence of a specific compound or defined set of compounds. Non-targeted screening methods are complementary to these approaches by detecting and identifying unexpected compounds present in food matrices that may be harmful to public health. However, the development and implementation of generalized non-targeted screening workflows are particularly challenging, especially for food matrices due to inherent sample complexity and diversity and a large analyte concentration range. One approach that can be implemented is liquid chromatography coupled to high-resolution mass spectrometry, which serves to reduce this complexity and is capable of generating molecular formulae for compounds of interest. Current capabilities, strategies, and challenges will be reviewed for sample preparation, mass spectrometry, chromatography, and data processing workflows. Considerations to increase the accuracy and speed of identifying unknown molecular species will also be addressed, including suggestions for achieving sufficient data quality for non-targeted screening applications.

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Contents

1. Introduction

Monitoring and ensuring food safety is critical for public health. Chemical hazards in food have included agrochemicals, environmental and industrial contaminants, and toxins, among others [1]. Furthermore, adulteration and food fraud continues to be problematic [2]. The globalization of the food supply has also necessitated that analytical methods be developed for potential health hazards. Research is continually performed to identify emerging risks to food safety [3], but analytical methods need to be developed in tandem to be able to analyze samples for the presence of unexpected hazardous compounds. Traditionally, employed methods focus on the detection and identification of a particular compound or class of compounds. However, this becomes problematic if other hazardous compounds are present within a sample. For example, melamine was used to adulterate pet food and milk products to increase the apparent protein content, but was not

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http://dx.doi.org/10.1016/j.chroma.2015.08.059
0021-9673/Published by Elsevier B.V.

previously monitored; this adulteration was responsible for multiple illnesses and deaths among children and pets [1]. If a particular food sample has been found to make people sick or elicit a response, how does one go about identifying the responsible compound(s)?

Non-targeted analysis aims to identify compounds from samples where the molecular content is unknown. This is required in disciplines other than food safety, such as identifying potential biomarkers for disease using metabolomics [4] or determining potential hazards for environmental analysis [5,6]. In particular, the field of metabolomics aims to identify all compounds present within a biological sample, typically to examine a particular phenotype. Many different aspects of this area of research have been investigated, including sample preparation [7] and data treatment [8,9]. Non-targeted strategies developed in other research areas like metabolomics can be used as a model for food analysis. However, these analyses have different challenges than food analysis. For example, the inherent diversity of food sample types may require different sample preparation strategies. Food matrices will likely be more chemically complex.

One approach utilized for non-targeted screening is mass spectrometry (MS). MS is particularly useful because no prior knowledge of chemical content is needed and it has a relatively large dynamic range. High resolution mass spectrometry (HR-MS) has the ability to separate similar masses and yields accurate mass information, which can be used to generate molecular formulae; this approach is already being applied in food analyses [10]. A prior chemical separation step, such as liquid chromatography (LC), can reduce chemical complexity, separate isomers, provide orthogonal information (*i.e.*, retention time), and concentrate analytes. LC/MS is commonly used for applications in food safety [11,12] and is advantageous because derivatization of the sample is typically unnecessary.

A generalized approach for non-targeted screening with LC/HR-MS is listed in Fig. 1. Eluting compounds are extracted from the data and the monoisotopic peak is assigned for detected ions, where the mass-to-charge value, isotopic distribution, and any associated molecular species, such as adducts or neutral losses, are determined. This information can then be used to generate a molecular formula for each compound of interest, which can be searched against available chemical databases. Statistical analyses can be incorporated to narrow down the list of potential compounds, rather than analyzing the entire molecular content of the sample. Alternatively, or in parallel, tandem mass spectrometry (MS/MS) can dissociate the compound where product ions can be used to aid in the elucidation of the compound and its structure. The identity can be confirmed by analyzing an analytical standard, if one is available. However, implementing this envisaged approach requires optimization and development to be capable of high-throughput applications using non-targeted LC/HR-MS analysis of foods.

The aim of this review is to discuss current sample preparation and analytical strategies, as well as limitations and challenges, in implementing non-targeted workflows to analyze multiple compound classes in complex sample matrices. The reader is also referred to other reviews that have focused on particular compound types for non-targeted screening in food, such as unknown pesticides [13,14], food packaging contaminants [15], and veterinary drugs [16]. Furthermore, data collection and processing will also be addressed; often, evaluating the data is the rate-limiting step in this type of analysis due to sample complexity and the number of compounds that may be present in a given food matrix. The quality of the data can be impacted by how the data is collected, both in terms of the chromatographic separation and MS detection, and impacts the design of automated, high-throughput data analysis workflows.

2. Sample extraction

Non-targeted screening requires comprehensive sample preparation strategies to extract a wide range of chemical classes. However, the vast majority of extraction methods for food analyses tend to be for specific groups of compounds, such as pesticides [17], veterinary drugs [18], or antibiotics [19]. Sample preparation strategies have also been developed and optimized for metabolomic investigations, where a number of criteria have been established [7]. Some of these criteria also apply to non-targeted screening in food analyses. This includes developing an unselective extraction method that can solubilize and recover a wide range of compound classes. The method must also be capable of extracting compounds in low abundance and be compatible with LC/MS detection. Reproducibility is also imperative, especially if collected data will be statistically treated. Additionally, automated and high throughput sample handling would be beneficial. However, developed methods in metabolomics have been examined for a relatively limited number of biological sample types compared to the sample diversity encountered in food analysis.

Specific metabolomic extraction methods for applications in food have been previously described [20]. To ensure hazardous compounds in food matrices are detected, the method(s) should be able to extract compounds that differ in size, charge, acidity/alkalinity, and a range of polarities [21]. Developed methods for plant analysis could be more readily applied, since they are more similar to some food matrices that may be encountered. Protein removal from samples may aid in better analyte coverage for smaller molecular weight species [22,23], although these may co-precipitate with bound compounds [20]. This would also remove larger molecular weight protein toxins [24–26]; however, the removed proteins could be additionally analyzed. Sample storage is also an important consideration [27], but may not be as critical compared to the reduced analyte stability encountered in biological samples.

Metabolomic extraction methods for foods remain an area for future development. Common extraction methods for food include "dilute-and-shoot," solid–liquid extraction, solid-phase extraction, and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) [28,29], where liquid and solid samples can be handled differently [30–32]. These methods should be examined for use in non-targeted screening, and some research has begun in this area [33–35]. In particular, QuEChERS has been successively applied to different classes of compounds, including polar and non-polar compounds, with adequate recoveries [30,33,36,37]. Furthermore, QuEChERS has been successfully applied to diverse sample types including fruits and vegetables [38,39]; thus this sample preparation may be sufficient or easily modified for non-targeted screening.

At a minimum, some sample processing will be required, such as filtering sample extracts. This will ensure longer column lifetimes and increased time between instrument maintenance and cleaning. However, the incorporation of excessive clean-up steps in the applied method may result in the removal of hazardous compounds from the sample. Sample clean-up procedures also reduce ion suppression effects, which is a reduction in the measured ion abundance of a compound due to the ionization of a highly abundant coeluting compound. If ion suppression is too great, lower abundant coeluting compounds will not be detected. While it may seem counterintuitive, dilution of a sample can actually reduce ion suppression, resulting in better detection of compounds of interest [40]. This is illustrated in Fig. 2, where an undiluted orange extract results in a lower measured peak area for a detected pesticide compared to its 10- and 100-fold diluted counterparts.

Ultimately, the sample preparation chosen must be fit-for-purpose for the particular matrix and molecular class, if known. Obviously, if hazardous compounds are not extracted from a