Journal of Chromatography A, 1382 (2015) 136–164

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review

Current approaches and challenges for the metabolite profiling of complex natural extracts

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ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 23 October 2014
Accepted 26 October 2014
Available online 31 October 2014

Keywords:
Metabolite profiling and fingerprinting
Metabolite annotation and identification
Natural products
Liquid chromatography
Mass spectrometry and NMR
Metabolomics

ABSTRACT

Metabolite profiling is critical in many aspects of the life sciences, particularly natural product research. Obtaining precise information on the chemical composition of complex natural extracts (metabolomes) that are primarily obtained from plants or microorganisms is a challenging task that requires sophisticated, advanced analytical methods. In this respect, significant advances in hyphenated chromatographic techniques (LC–MS, GC–MS and LC–NMR in particular), as well as data mining and processing methods, have occurred over the last decade. Together, these tools, in combination with bioassay profiling methods, serve an important role in metabolomics for the purposes of both peak annotation and dereplication in natural product research. In this review, a survey of the techniques that are used for generic and comprehensive profiling of secondary metabolites in natural extracts is provided. The various approaches (chromatographic methods: LC–MS, GC–MS, and LC–NMR and direct spectroscopic methods: NMR and DIMS) are discussed with respect to their resolution and sensitivity for extract profiling. In addition the structural information that can be generated through these techniques or in combination, is compared in relation to the identification of metabolites in complex mixtures. Analytical strategies with applications to natural extracts and novel methods that have strong potential, regardless of how often they are used, are discussed with respect to their potential applications and future trends.

Contents

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http://dx.doi.org/10.1016/j.chroma.2014.10.091
0021-9673/

1. Introduction

Metabolite analysis, particularly metabolite profiling in complex biological matrices, is essential in many fields of the life sciences. The concept of **metabolite profiling** is not new [1]. In 1971, Horning and Horning first introduced the concept of metabolic profiling using mass spectrometry (MS) [2], and in that same year, Pauling et al. reported analyses of urine vapour and breath by gas chromatography (GC) and related the obtained profiles to the effects of a defined diet. Since then, many powerful analytical methods, both chromatographic and spectroscopic, have been developed or improved [3], and metabolite analyses in various types of matrices, has become necessary in various research fields. While many methods have reached a high level of resolution and sensitivity for such analyses, the unambiguous identification of metabolites remains challenging [4].

This review is primarily focused on crude extracts of natural origins as matrices of interest (primarily plant and microbial extracts that are defined as "natural extracts"). Such biological matrices are challenging to analyse due to the concomitant presence of metabolites with high chemodiversity [5–7], which makes them particularly interesting to study. In natural product research, the term "**metabolite**" is widespread and refers to compounds (small molecules with molecular weights (MW) of <1000 Da) that are either essential to sustain the life of a given organism via normal metabolic processes ("**primary metabolites**") or non-essential but necessary for survival in a given environment ("**secondary metabolite**"). Primary metabolites include amino acids, lipids, and carbohydrates; secondary metabolites are related to defence and signalling mechanisms and include polyphenols, alkaloids, terpenes, polyketides, and hormones [8]. These latter compounds are also referred to as natural products and have historically been a significant source for lead compounds for drug discovery due to their action on pharmacological targets [9]. The profiling of such extracts in classical natural product research has been underway for years, especially for dereplication purposes [10]. **Dereplication** is the process of testing sample mixtures that are active in screening in order to differentiate the novel compounds from active substances that have already been studied [11]. With the significant development that is occurring in metabolomics for biology and natural product research, this type of analysis is gaining more and more importance from both targeted and untargeted analytical perspectives [12].

Metabolomics is defined as a non-selective, universally applicable, comprehensive analytical approach for the identification and quantification of metabolites in a biological system. This area of research strives to obtain complete metabolite fingerprints, detect differences between metabolites and generate hypotheses to explain these differences. In natural product research, metabolomics is considered the large-scale analysis of metabolites of a given organism during various physiological states [13]. The advent of powerful modern analytical methods generate large data set which can be Interpreted from a holistic manner only by reducing their dimensionality using multivariate data analysis (MVDA) approaches. This highlights the significant variations that occurs at the metabolome level. The results can then be correlated with new biological knowledge. To achieve this goal, the metabolites of complex natural extracts must be comprehensively analysed by metabolite profiling and fingerprinting, which is a significant analytical challenge, as several extraction methods [14] and complementary sophisticated analytical platforms must be used [15].

In metabolomics, different analytical strategies are used to determine the chemical composition of a given biological matrix or extract. The profiling of different constituents can be achieved on several levels, such as "metabolite (or metabolic) fingerprinting," "metabolite profiling" and "metabolite target analysis." The term "**metabolic**" is more often used in drug research; it is frequently used to describe the metabolic fate of an administered drug [16]. Alternatively, in the analysis of complex mixtures, these approaches are defined according to Fiehn [16]:

(i) "**Metabolite fingerprinting**" is the rapid classification of samples. In these high-throughput analyses, extensive metabolite identification and quantitation are generally not used. MVDA is applied in fingerprinting to determine differences and classify samples. The purpose of this method is not to identify each individual metabolite, but to compare patterns or "fingerprints" of metabolites that change in a given biological system. In the frame of metabolomics, it is aims to be untargeted and is used as an hypothesis-generating approach.

(ii) "**Metabolite profiling**" focuses on the analysis of a large group of metabolites that is either related to a specific metabolic pathway or a class of compounds [17]. In most cases, metabolite profiling is more targeted than metabolite fingerprinting and follows specific hypotheses in the approach. Therefore specific analytical methods are developed for analyte determination. Metabolite profiling is the oldest and most established approach and is considered the precursor of metabolomics.