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Recent advances in liquid and gas chromatography methodology for extending coverage of the metabolome

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The metabolome is the complete complement of metabolites (small organic biomolecules). In order to comprehensively understand the effect of stimuli on a biological system, it is important to detect as many of the metabolites within that system as possible. This review briefly describes some new advances in liquid and gas chromatography to improve coverage of the metabolome, including the serial combination of two columns in tandem, column switching and different variations of two-dimensional chromatography. Supercritical fluid chromatography could provide complimentary data to liquid and gas chromatography. Although there have been many recent advancements in the field of metabolomics, it is evident that a combination, rather than a single method, is required to approach full coverage of the metabolome.

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Introduction

Metabolomics

Metabolites are small organic molecules that undergo biochemical modifications during metabolic reactions and are necessary for the correct growth, maintenance and function of living cells [1]. They are the direct result of regulatory processes and as such their concentrations serve as an indication of biochemical activity, and hence phenotype, which can be used to determine cellular response to stimuli [1,2]. Although metabolic analysis had been carried out previously, Oliver *et al.* were the first to describe the full complement of small molecules synthesised by an organism as the metabolome [3]. Ideally, metabolomics is the study of metabolic reactions through the identification and quantitation of all the metabolites, the metabolome, within a biological system [4]. The ability to directly link metabolite concentrations to molecular activity has meant that metabolomics has become a powerful tool in cell and systems biology research [5].

Coverage of the metabolome

In order to comprehensively understand the effect of stimuli on a biological system, it is important to detect as many of the metabolites within that system as possible. It is estimated that there are around 2000 metabolites present in mammals and 200 000 in the plant kingdom [6,7]. The diversity in mass, concentrations, polarity, volatility, solubility, pK_a and charge of these metabolites creates analytical problems [8•,9]. García-Cañaveras *et al.* described the metabolomics concept as 'the unbiased determination of all the metabolites present in a sample independently of their chemical structure' [10]. However, the analytical platforms used to detect these compounds tend to exploit specific chemical characteristics of different classes of metabolites.

Metabolomics techniques

The most widely utilised analytical platform in metabolomics is based on mass spectrometry (MS) detection [11]. To improve sensitivity and resolution of metabolite detection, liquid chromatography (LC) and gas chromatography (GC) separation techniques are commonly coupled to a mass spectrometer [1,11].

LC–MS can be applied to the analysis of the majority of chemical species. Innovations in LC technology, instrumentation, and column chemistries have led to wider coverage of the metabolome. Although reverse phase (RPLC) is better suited for the analysis of nonpolar compounds, due to its ease of use and wide ranging applicability, it is the most commonly used method in LC for metabolic analysis. To improve the retention of polar metabolites ion-pairing agents are often added to the mobile phase, but these can have significant ion suppression effects on mass spectrometry instrumentation, leading to a far more restricted range of available ion pairs than for chromatography as a whole [12].

GC–MS has long been used in the analysis of metabolites and metabolite profiling due to its separation capacity, sensitivity and selectivity [8•,13]. GC–MS requires chemical derivatisation to improve the volatility and thermal stability of polar compounds [14,15]. Developments in column stationary phases (SPs) and methods for sample preparation have increased the number of metabolites detectable by GC–MS [8•]. For example, ionic liquid SPs

exhibit a 'dual-nature' allowing the separation of polar and nonpolar compounds as well as extending the temperature range at which the column can be operated [16].

This review will focus on the recent advancements in LC–MS and GC–MS to extend the coverage of the metabolome, with a focus on the innovation of dual-column methods in LC and GC.

LC–MS methods

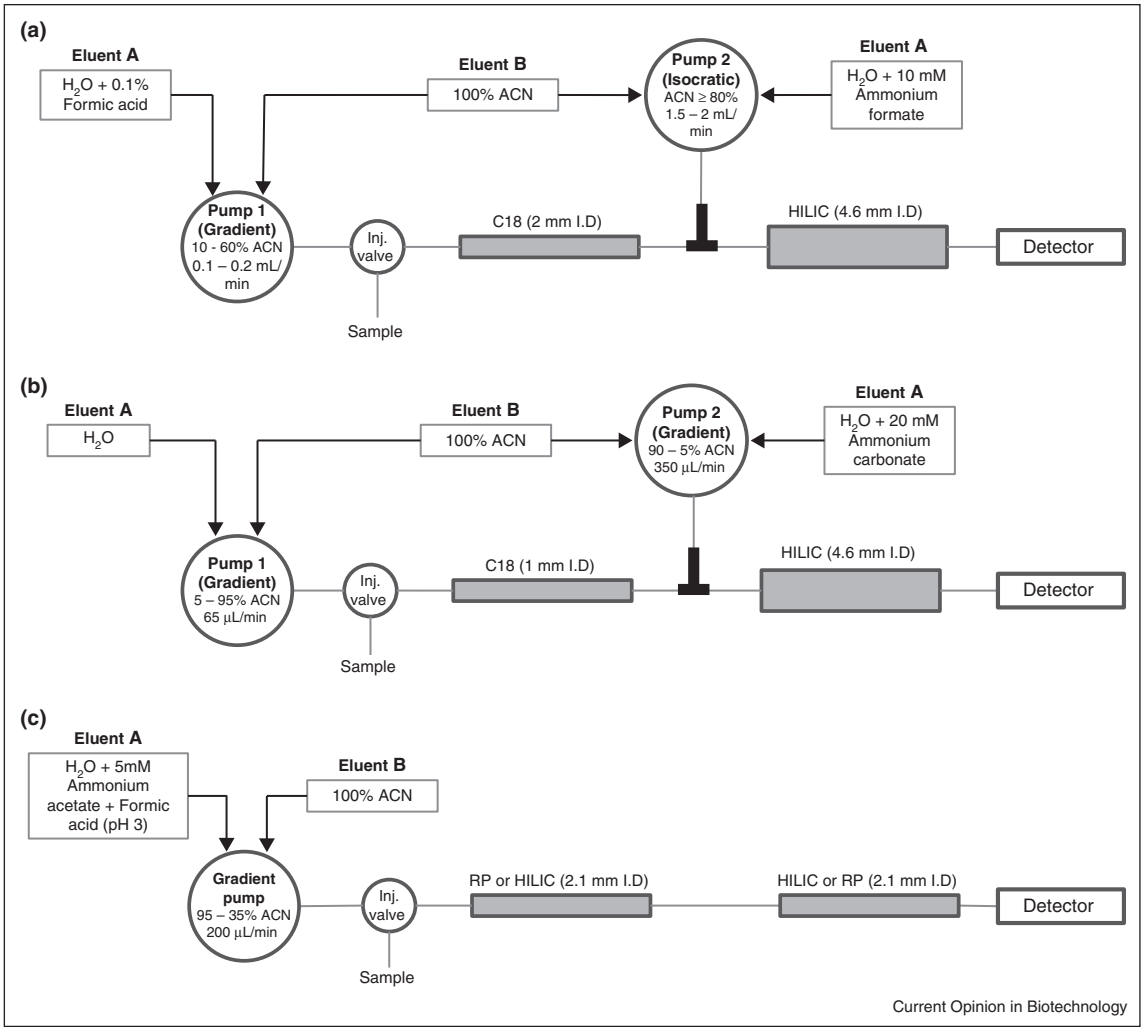
In any chromatographic analysis, a compromise must be achieved between column efficiency and analysis time. There have been many recent developments that improve efficiency and/or throughput in LC, either through optimisation of single columns or through the combination of multiple columns. Many of these can be applied to analysis of metabolites.

Single column methods

Although there have been many advancements in single column technologies, this review will focus on dual column methods as an in-depth review discussing the most recent approaches using single columns has been published by Fekete *et al.* [17••].

Although there have been extraordinary advancements, it is likely that a single column method is insufficient for the analysis of the entire metabolome due to the chemical diversity exhibited by metabolites. The combination of orthogonal columns can increase the coverage of the

Figure 1



Examples of serially combined (SCC) methods. **(a)** Isocratic pump (pump 2) joined via a t-piece to the system to deliver a high concentration of organic solvent before the HILIC column. This is run at a higher flow rate to increase the organic concentration of the eluent before the HILIC column [19]. **(b)** Two gradient pumps incorporated into the system for individual control of the mobile phase compositions [21]. **(c)** Two orthogonal columns serially combined without the addition of a second pump. The gradient pump is run under 'HILIC-style' conditions which allow the coupling of two orthogonal columns. The columns can be combined in any order [24].