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The potential of ion mobility–mass spectrometry for non-targeted metabolomics

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Non-targeted analysis of metabolites in hypothesis-generating workflows has proven its potential to answer essential questions that arise when dealing with complex biological systems. Nevertheless, tracking changes in perturbed systems via accurate quantification and the identification process itself represent the most critical challenges in these workflows. Recent advances in ion mobility–mass spectrometry have enabled this technique to increase the confidence of metabolite annotation by introducing a complementary conditional molecular descriptor, that is collision cross section.

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Introduction

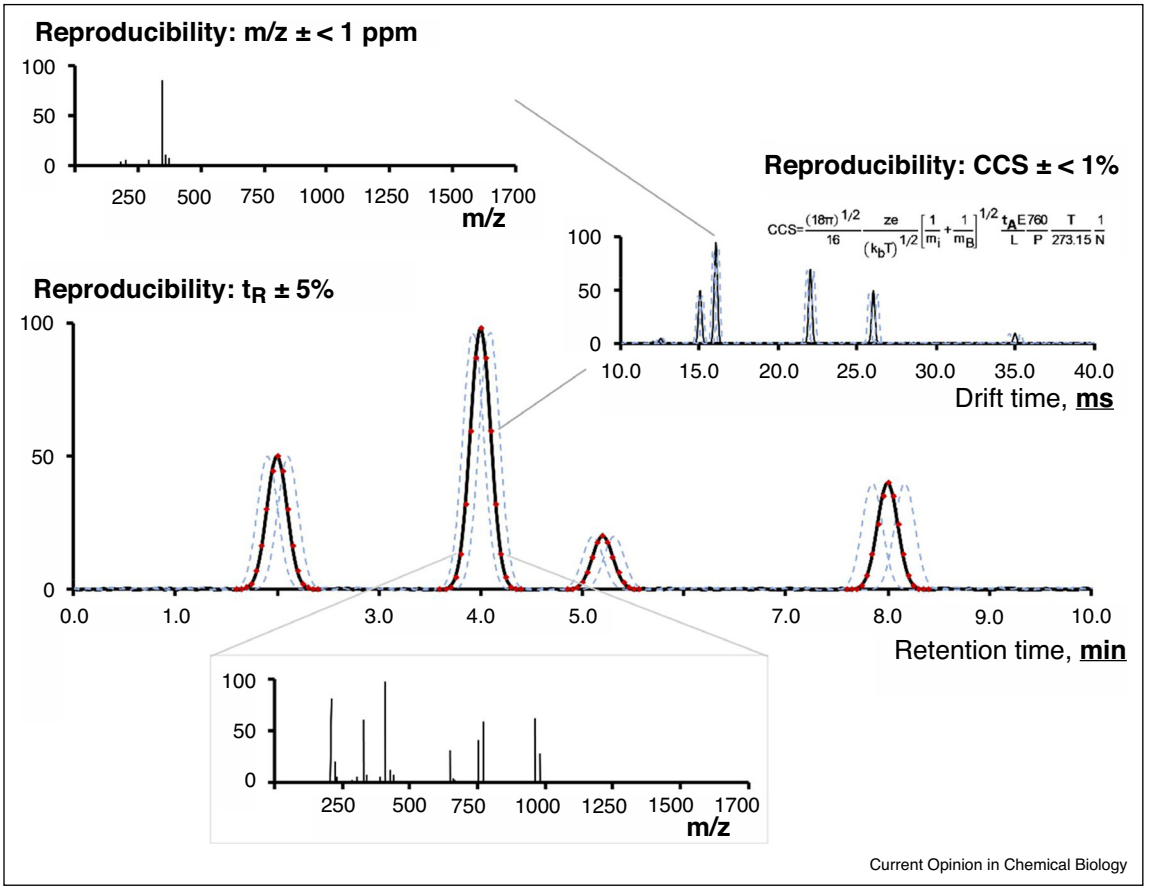
Metabolomics is considered to be the link between genotype and phenotype [1] and aims at elucidating the chemical structure as well as tracking changes in concentrations of small molecules within a biological system. Following this approach allows an unprecedented insight into regulation mechanisms as well as studying responses to different perturbations. Over the last decades, this 'omics' area has found its way into routine application across various fields, ranging from clinical diagnostics to biotechnological questions.

There are two principal analytical approaches, in which metabolomics can be broadly distinguished, that is 'targeted' and 'non-targeted' workflows. In contrast to targeted analytical approaches, the study of metabolites in a hypothesis-free (or hypothesis-generating) experimental setup, that is non-targeted analysis, aims at expanding the analysis coverage to provide a comprehensive metabolic fingerprint with a maximum number of detected and subsequently identified metabolites [2•]. However, the analysis of metabolites in such a comprehensive way is confronted with several challenges, mostly due to the fact that the metabolome comprises compounds highly diverse in terms of their physicochemical properties as well as substantial variation in concentrations. While a comprehensive analysis of metabolism on one single analytical platform is virtually impossible, the combination of orthogonal analysis techniques within one analytical method is a pragmatic way to extend metabolite coverage in a time- and cost-effective way [2•].

Recent commercial developments have brought ion mobility–mass spectrometry (IM–MS) into focus for a greater number of bioanalytical laboratories and, when incorporating this additional degree of separation in between chromatography-based separation and mass spectrometric detection, an effective multidimensional separation can be exploited [3•,4•]. As depicted in a schematic workflow in Figure 1, the rapid time scale of ion mobility separation, typically in the ms range, makes this combination with other front-end separation techniques (such as chromatography) ideal since comprehensive sampling of each dimension of separation is still ensured [5]. It has also been demonstrated that the combination of LC–IM–MS leads to an overall increase in peak capacity [6–8].

Different ion mobility separation approaches can be distinguished in terms of applied electric field and stationary state of the buffer gas, and are either based on dispersive (drift tube IMS (DTIMS) [9], travelling wave IMS (TWIMS) [10]), spatial (differential mobility analyzer (DMA) [11], field asymmetric IMS (FAIMS) [12]) or confinement-and-selective-release (trapped IMS [13]) principles [4•]. If the IM separation is performed under low-field conditions and care is taken that other assumptions (such as negligible ion heating) are also fulfilled, the measured drift times are exclusively a function of the experimental parameters, namely drift tube length, buffer gas pressure, temperature, electric field strength, the drift gas species and mass and shape of the analyte ion. As a consequence, drift times can be directly converted into a collision cross section (CCS) via the fundamental low field IM relationship, referred as the Mason–Schamp equation [14]. In fact, the CCS value represents a unique physicochemical property of an ion and gives an indication of the compound's chemical structure and three-dimensional conformation.

Figure 1



Schematic workflow of liquid chromatography (LC) combined with ion mobility (IM) separation and mass spectrometric (MS) detection including the precision under reproducibility conditions of measurement (i.e. inter-laboratory or inter-instrument) for retention time, CCS and *m/z*. The three measurands are employed in identity confirmation processes. The peak width in liquid chromatography is in the range of seconds, whereas IM separation for small molecules is performed within in the range of tens of ms.

Commercially available DTIMS and TWIMS instruments are currently the most developed for generic IM–MS operation (i.e. full spectral acquisition). As a consequence, TWIMS and DTIMS instruments have been employed for non-targeted screening experiments [15–19]. Excellent reviews on principles of IM separation as well as current developments in research and commercially-available IM–MS instrumentations have been recently published [3•,4•,20,21••,22].

DTIMS instruments utilize a uniform electrostatic field and enable direct derivation of CCS values via the Mason–Schamp equation [14] from the measured drift time using the so-called 'stepped field' method [23••]. Since this approach relies on acquiring data over multiple electric fields steps, this method is not compatible with the time scale of front-end separation techniques. To realize accurate CCS determination also for IM separation nested between LC and MS, a universal 'single-field' approach can be chosen [23••]. This is accomplished by setting up a regression of standardized CCS values of a tune mix calibrant under the same IM–MS measurement conditions as the sample. In a recently published inter-laboratory study, Stow *et al.* comprehensively compared these two approaches to conditional CCS values of a reference system for a broad set of compounds (Figure 2). For the single field method, which is mandatory for LC–IM–MS setups, the study revealed an overall average inter-laboratory RSD of 0.38 ± 0.19% for three laboratories applying identical conditions of measurement for a wide range of biological classes and charge states.

Travelling wave instruments employ an electrodynamic field, that is a travelling wave potential, for mobility separation [10]. Since the relationship between CCS and measured drift times in TWIMS is rather more complex, a calibration step using compounds with known CCS values (typically obtained on DTIMS instruments) is required [24•,25–27]. However, it has been demonstrated recently by Hines *et al.* that a structural match/