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Theoretical evaluation of peak capacity improvements by use of liquid chromatography combined with drift tube ion mobility-mass spectrometry

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ABSTRACT

In the domain of liquid phase separations, the quality of separation obtainable is most readily gauged by consideration of classical chromatographic peak capacity theory. Column-based multidimensional strategies for liquid chromatography remain the most attractive and practical route for increasing the number of spatially resolved components in order to reduce stress on necessary mass spectrometric detection. However, the stress placed on a chromatographic separation step as a second dimension in a comprehensive online methodology (*i.e.* online LC × LC) is rather high. As an alternative to online LC × LC combinations, coupling of HPLC with ion mobility spectrometry hyphenated to mass spectrometry (IMS-MS) has emerged as an attractive approach to permit comprehensive sampling of first dimension chromatographic peaks and subsequent introduction to an orthogonal IMS separation prior to measurement of ions by a mass spectrometer. In the present work, utilization of classical peak capacity and ion mobility theory allows theoretical assessment of the potential of two- (LC × IMS-MS) or even three-dimensional (LC × LC × IMS-MS) experimental setups to enhance peak capacity and, therefore, the number of correctly annotated features within the framework of complex, non-targeted analysis problems frequently addressed using HPLC-MS strategies. Theoretical calculations indicate that newly-available drift tube IMS-MS instrumentation can yield peak capacities of between 10 and 40 using nitrogen drift gas for typical non-targeted metabolomic, lipidomic and proteomic applications according to the expected reduced mobilities of components in the respective samples. Theoretically, this approach can significantly improve the overall peak capacity of conventional HPLC-(MS) methodologies to in excess of 10^4 depending upon the column length and gradient time employed. A more elaborate combination of LC × LC × IMS-MS would improve the ion suppression limitation and possibly allow access to theoretically even higher peak capacities, but such a combination may render the IMS separation practically redundant as well as imparting the well-known dilution problems associated with LC × LC. Finally, some predictions for the separation of co-eluted isobaric compounds can also be made by considering the required peak-to-peak resolution for acceptable IMS separation. The here-described theoretical predication approach can be used to aid method development for HPLC × IMS-MS and is also accompanied by some practical considerations that should be contemplated in associated non-targeted analysis workflows.

1. Introduction

The limitations of one-dimensional, column-based high performance liquid chromatography (HPLC) are well-established and have been exhaustively described, modelled and discussed within numerous excellent studies [1–6]. Fundamental physical limits calculated and practically demonstrated for this technique indicate that obtaining above 1,000,000 theoretical plates within 24 h using conventional packed columns is extremely unlikely [7] and can only be realistically achieved with alternative column technology [8], which may take some years to match the market significance of analytical-scale columns packed with spherical particles. A practical and easily comprehended concept for describing this limitation is to consider the number of components (peaks) that can be resolved in a single separation (*i.e.* the number of fully separated peaks that can be placed side-by-side within a defined

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chromatographic elution window is termed "peak capacity", n_c). In the case of HPLC, use of modern columns packed with sub-3 μm diameter particles with varying porous structures (*i.e.* non-porous, superficially porous or completely porous) in combination with modern instrumentation now provides a realistic means to achieve high peak capacities (*i.e.* 300–1000) for gradient reversed-phase liquid chromatography (RPLC) [9–12]. Such calculations, of course, assume a perfectly ordered sample where peaks are placed side-by-side filling the entire separation space. In reality, the disorder observed in real samples leads to a more sobering reality for this approach. In fact, it can be calculated that only 18–37% of peaks from this calculated peak capacity value can be properly resolved with such an approach [13].

In this regard, the pioneering thinking of early chromatographers including Giddings [14] foresaw this problem and thus pursued a multiplicative increase in peak capacity by subjecting all components from a single-dimensional analysis to a second separation step (*i.e.* a multidimensional analysis). In such a comprehensive two-dimensional system, the peak capacity can be theoretically estimated as the product of the two individual peak capacity values (*i.e.* ${}^1n_c \times {}^2n_c$). In this way, a peak capacity value of 500 from a powerful single-dimensional analysis can be complemented with a fast, second separation step with lower peak capacity values of 50–100 to yield total theoretical peak capacity values of 2500–50,000. Indeed, much practical research has been dedicated to this approach with a focus on developing methodologies capable of resolving many 100s of compounds within a single two-dimensional analysis (*e.g.* LC × LC) [9,15–18]. While practical implementations of the comprehensive approach have demonstrated some spectacular peak capacity improvements, certain practically challenging issues still remain. For example, the lifetime of the chromatographic column used in the second dimension is known to be relatively limited due to uneven pressure within the column during valve switching [19]. Furthermore, the solvent systems chosen for each dimension must fulfil compatibility requirements for chromatographic selectivity, miscibility and retention characteristics in order for high peak capacities to be practically realized with a given column combination [20]. Finally, it still remains challenging (although not impossible) for extremely high peak capacity generation (peaks per minute) to be realized for online LC × LC-MS systems [21]. One approach to address this final issue could be the implementation of spatial separations with two or three dimensions to allow simultaneous analysis of fractions from the preceding separation step(s) [22,23]. While such an approach could provide a means for unprecedentedly high peak capacities within a practically feasible analysis time, the technology required for such an undertaking is not yet available.

In considering the requirements for a rapid, second dimensional analysis, one can look to various alternative approaches already explored for comprehensive multidimensional liquid-phase analysis. For example, the possibility of using capillary electrophoresis (CE) as a second dimension was first demonstrated by the group of Jorgensen [24], whereby a rapid second dimension with an orthogonal separation mechanism (based on the size/charge ratios of analytes) is coupled to a first dimension RPLC system to yield extremely high peak capacity values. The use of a rapid, second-dimension separation based on a size to charge ratio is very attractive for generating high peak capacity values because of the high sampling frequency possible and the orthogonality with separation mechanisms associated with RPLC.

Another approach for yielding separation based on size to charge ratio is the use of ion mobility spectrometry (IMS). In this technique, gas phase ions undergo differential migration in a drift tube pressurized with carrier (buffer) gas in the presence of an electric field of low strength. Under these conditions, the drift time of an ion will depend upon the size and charge of the ion, ion-neutral interaction potential and the rotationally-averaged shape (collisional cross-sectional area, CCS) of ions meaning that this approach displays some orthogonality to mass spectrometry. For this reason, various forms of ion mobility spectrometry have been coupled with mass spectrometry (IMS-MS) with some commercially-available instrumentation becoming available in the last 10 years [25,26].

In addition to calculations made for HPLC-MS implementations [27–29], peak capacity calculations for standalone IMS-MS and IMS-IMS systems were described for the analysis of peptides in the early 2000s [30–33] illustrating the additional resolving power that such combinations can provide. These calculations do not consider any preliminary chromatographic separations, but IMS-MS instruments that can be routinely combined with HPLC have now become commercially available and a number of research groups are already exploring combinations of this approach for various applications. While IMS cannot provide a classical "chromatographic" peak capacity considered to be high in comparison with a CE or HPLC analysis as a second dimension of a multidimensional system, it has the major advantage of a rapid cycle time. For example, the potential of a differential IMS-MS combination to permit "real-time" 2D separations was described by Varesio et al. [34] using HPLC coupled with differential mobility spectrometry hyphenated to mass spectrometry (LC × DMS-MS). Although this approach will not improve ion suppression issues associated with HPLC-MS, the use of the IMS system between the HPLC and MS might be nevertheless considered as a second dimension separation with rapid cycle times readily enabling comprehensive sampling of peaks from conventional one-dimensional HPLC separations. While these concepts already appear advantageous for such systems over conventional HPLC-MS methodologies, it has not yet been explored from a theoretical or practical perspective how this combination compares to conventional multidimensional approaches or HPLC-MS in terms of overall peak capacity, which directly addresses the goals of non-targeted analysis, particularly for increased feature identification.

Thus, the aim of the present work is to theoretically explore the potential of this combination by considering IMS(-MS) as a second chromatographic-type separation that might provide gains in peak capacity in comparison to conventional HPLC-MS for non-targeted analysis strategies. Such a comparison requires both a theoretical framework and practical considerations in order to afford a meaningful description of advantages and limitations that HPLC coupled with IMS-MS can provide. In this study, we use the non-targeted analysis of proteomic, lipidomic and metabolomic samples as example analytical problems to yield practically relevant results for interpretation and assessment.

2. Theory

2.1. Peak capacity for HPLC

The concept of peak capacity (n_c) was introduced by Giddings for isocratic chromatography in 1965 [35] and extended to gradient elution by Horvath and Lipsky in 1967 [36]. Now representing the most practically-relevant form of HPLC, gradient peak capacity for a given total gradient time (t_G) is most simply approximated by assuming the peak spacing of four standard deviations [2,4]:

$$n_c \approx 1 + \frac{t_G}{4\sigma} \tag{1}$$

In addition to describing the theoretical maximum peak capacity for gradient HPLC, the amount of the separation space actually utilized for the separation was explicitly considered by Dolan et al.