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Instrument contributions to resolution and sensitivity in ultra high performance liquid chromatography using small bore columns: Comparison of diode array and triple quadrupole mass spectrometry detection

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ABSTRACT

UHPLC with DAD-UV detection or in combination with mass spectrometry (MS) has proven to be a robust and widely applicable platform for high sensitivity analyses of many types of chemical compounds. The majority of users employ narrow bore columns with 2.1 mm internal diameter (ID) typically exhibiting very high efficiencies (>200,000 plates/m). This ultimately sets stringent demands upon the chromatographic system as the separation efficiency can be compromised by external contributions to dispersion caused by connection capillaries, auto-sampler and/or the detection device. Sample limited applications often use reduced column diameters down to capillary- or even nano-column format. Capillary (ID ≤ 0.5 mm) or small-bore columns (ID ≤ 1 mm) can be a good compromise between system robustness and enhanced sensitivity. Yet in this case, extra-column dispersion gains additional importance due to reduced peak volumes. To design an optimized system configuration for specific column dimensions and applications it is crucial to understand the dispersion contributions of individual extra-column components. This was subject to many studies done within our group and by others. Here, we employed a fully optimized UHPLC/UV system to investigate the contribution to peak dispersion obtained from columns ranging from capillary to narrow bore (0.3, 0.5, 1, 2.1 mm) using a set of small molecules that were analyzed in gradient mode. Further UV detection was replaced by a triple quadrupole (QQQ) MS in order to evaluate its contribution to band broadening. In this context the impact of column-ID upon MS sensitivity when interfaced with an Agilent Jet Stream source was investigated. Data obtained from our test suite of compounds shows mostly mass-sensitive behavior of this advanced electrospray technology.

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1. Introduction

Over the past years the need for faster and more efficient separations in (U)HPLC has driven the trend toward smaller particles and smaller column dimensions. A decade ago the typical HPLC column contained 5 μm particles, was of dimension 250 mm × 4.6 mm, and could produce 20,000 plates in 20 min. Today, sub-2-μm (STM) materials packed into short UHPLC columns allow to achieve about the same separation efficiency at analysis speed in the range of 1.5–3 min [1–3].

The majority of users of UHPLC or LC/MS employ narrow bore columns with 2.1 mm internal diameter (ID). Sample limited applications may benefit from the use of columns with smaller ID to boost detection sensitivity. Due to on-column dilution, the sensitivity is inversely proportional to the square of the column ID for concentration dependent detectors. Theoretically a factor of 50 can be gained in sensitivity using a 0.3 mm ID compared to a 2.1 mm ID column [4–6].

An additional advantage obtained from the reduction of column-ID is the minimization of radial temperature gradients resulting from frictional heating associated with high column back-pressures in UHPLC. These lead to a heterogeneous distribution of eluent velocity, which in the case of non-adiabatic column operation can seriously deteriorate column performance [7,8].

An important parameter that has to be considered when using smaller ID-columns is the extra-column volume of the system and the associated peak dispersion, whose importance has already been recognized in the early days of HPLC by Kirkland [9] and Lauer et al.

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Table 1
Peak volumes for different column dimensions (N = 25,000, k = 5).

Column ID [mm]	Column length [mm]	Particle size [μm]	Peak volume [μL]
4.6	100	1.8	38
2.1	100	1.8	8
1	100	1.8	2
0.5	100	1.8	0.5
0.3	100	1.8	0.2

[10]. In recent years the topic has gained increasing interest in the view of the development of extremely efficient small bore columns [11–20]. The decrease in column-ID combined with increased efficiency leads to a significant decrease in peak volume. Such volumes can be on the order of a few microliters for short 2.1 mm columns. Table 1 gives typical values for different column dimensions. Thus, the performance of small bore columns can be seriously compromised if instrument contributions to dispersion are not minimized. This is especially important in isocratic separations of compounds with little retention or in fast gradient separations. While older generation HPLC instruments typically show system variances on the order of 40 μL^2 or more, this value has be reduced to <10 μL^2 in modern UHPLC instruments. To operate small bore ultra-high efficiency columns without major loss in performance, this value has to be even further reduced. In particular the introduction of superficially porous STM materials is driving the need for further minimization of extra-column dispersion in modern UHPLC instruments [21–25].

Several authors have studied extra-column contributions in standard HPLC and possibilities to counteract [26–28]. Also the need for further optimization of state-of-the-art UHPLC instrumentation has been discussed [29–32]. These investigations mainly focused on LC system with UV-detection.

Despite excellent mass resolution that can be obtained with modern MS-instruments (e.g. time-of-flight (TOF)) it remains crucial to optimize time-resolution prior to detection. This is particularly true for complex samples where band-broadening (either system or column induced) can result in an overall loss in sensitivity due to decreased peak heights. In addition, unresolved or partially resolved compounds (e.g. co-eluting with background matrix from the sample) can experience ionization suppression (or enhancement) effects in the ion source, which can also markedly affect sensitivity.

Studies comparing extra-column contributions of UV detectors with MS detectors have demonstrated that MS could add significantly to band broadening [33]. Eugster et al. [34] determined an extra-column contribution for UHPLC/TOF-MS of ca. 60 μL^2 vs. ca. 5 μL^2 for the system where time-of-flight was replaced by a UV detector. The highest peak capacities with MS were obtained from a 3 mm ID column while significant losses were reported using 2.1 and 1 mm columns. Spaggiari et al. [35] compared a range of modern MS systems (QQQ, Q-TOF and TOF) connected to various UHPLC instruments. They concluded that the major source of instrument dispersion originated from the connection tubing interfacing the LC and the MS systems.

In the current article we present data from a study comparing chromatographic performance as well as detection sensitivity obtained with columns that varied in ID (in the range 0.3–2.1 mm) and were packed with 3.5 μm and 1.7 μm particles. Post injector connections of capillary-LC and UHPLC systems were made with short, 50 μm ID capillaries and the UV detector was equipped with a low dispersion flow cell in an effort to minimize extra-column band broadening effects.

Part-1 of this paper embraces the impact of such rigorous system volume reduction on separation performance. This assessment used a multi-component sample containing low-molecular mass solutes. These were separated at the same linear velocity applying gradients of different durations (t_G = 15–120 min). The UV-benchmark was then compared to data acquired with QQQ MS.

In this context we examined volume dependent contributions to dispersion as well as time dependent contributions. Volume dependent contributions relate the dispersion variance of the system to the peak variance (in volume units) and become more relevant with decreasing column-ID. Time dependent contributions are related to detector acquisition rate and potentially applied smoothing peak filters and do not depend on the peak width in volume but on the width in time. The latter are independent of column-ID.

Part-2 investigates the impact of column-ID and corresponding flow rates upon MS sensitivity when interfaced with Agilent Jet Stream (AJS) thermal gradient focusing ionization technology [36].

2. Theoretical considerations

2.1. Impact of external band broadening in isocratic and gradient separations

The total variance of a peak generated by a chromatographic system is given as the sum of the contributions to variance from the column (σ^2_{column}) and from the system (σ^2_{ext}).

$$\sigma^2_{total} = \sigma^2_{column} + \sigma^2_{ext} \quad (1)$$

where σ^2 can be expressed either in volume or in time units.

To estimate the impact of extra-column contributions the variance generated by the column alone needs to be calculated.

For isocratic separations the variance in volume units it is given by:

$$\sigma^2_{v,column} = \frac{V_0^2}{N_{column}} \cdot (1+k)^2 \quad (2)$$

where V_0 is the dead volume of the column, N the plate number generated by the column and k the retention factor of the solute.

In gradient separations the column variance is given by:

$$\sigma^2_{v,column} = \frac{V_0^2}{N_{column}} \cdot (1+k_{elution})^2 \quad (3)$$

where $k_{elution}$ is the retention factor of solute at the point of elution during a gradient and is given by [37].

$$k_{elution} = \frac{k_{init}}{b \cdot (k_{init} - (V_d/V_0)) + 1} \quad (4)$$

For $V_d/V_0 > k_{init}$ the solute elutes under isocratic conditions and $k_e = k_{init}$ For $k_{init} \gg 1$ and $V_d = 0$ Eq. 4 reduces to

$$k_{elution} = \frac{1}{b} \quad (5)$$

k_{init} is the k value of a compound at the starting composition of the gradient, V_d is the dwell volume of the instrument, and b is the slope of the gradient defined as

$$b = S \cdot \Delta\varphi \cdot \frac{V_0}{V_g} \quad (6)$$

where S is the slope in the LSSR model [38], $\Delta\varphi$ is the composition range of the gradient and V_g is the gradient volume.

The peak volume is determined by the gradient slope and (in contrast to isocratic mode) is very similar for all components in a sample (provided the S values are not too different).

The ratio of the observed resolution $R_{observed}$ and the intrinsic resolution of a column R_{column} is proportional to the inverse ratio of the respective peak standard deviations.

$$\frac{R_{observed}}{R_{column}} = \frac{\sigma_{v,column}}{\sigma_{v,total}} \quad (7)$$

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