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On-tubing fluorescence measurements of the band broadening of contemporary injectors in ultra-high performance liquid chromatography

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ABSTRACT

We report on a detailed study of the injection contribution to band broadening in contemporary UHPLC-instruments, using either flow-through needle or fixed loop injection (full loop). Using on-tubing fluorescence measurements at the outlet of the injector valve, very localized and undisturbed measurements were obtained. Varying both the flow rate and the injected volume allowed to split the injection variance ($\sigma_V^2{}_{,inj}$) in a volumetric component (related to the amount injected) and a hydrodynamic component (related to the flow rate). For the flow-through needle injector and for the small injection volumes (<2 μL) typically used in UHPLC, it was found that the volumetric contribution (i.e. the part of $\sigma_V^2{}_{,inj}$, that increases with increasing injection volume) is given by a value of $\sigma_V^2{}_{,inj,vol} = 0.8$ to $1 \cdot V_{inj}^2$ rather than by the value of 0.125 to $0.2 \cdot V_{inj}^2$ that is normally assumed in literature. For the hydrodynamic contribution to $\sigma_V^2{}_{,inj,}$ (i..e, the part which remains present even for very small injection volumes), a clear increase in dispersion with flow rate is found, reaching a plateau around 0.8ml/min of 0.6 μL^2 or 1.2 μL^2 for the 75 μm and 120 μm needle seat capillaries respectively. The difference between both shows the clear advantage of using a low dispersion 75 μm injection needle seat capillary. For a loop-type injector operated in a full-loop mode, the increase in peak variance with the injection volume is much less pronounced, leading to a total injector variance given by $\sigma_V^2{}_{,inj} = 0.34\ \mu L^2 + 0.12 \cdot V_{inj}^2$ over the entire range of investigated injection volumes of 1.1 μL up to 4.5 μL when using 120 μm or narrower ID loops. This expression was nearly completely independent of the flow rate. For larger ID sample loops, a clear increase of peak variance with flow rate at fixed injection volume was observed ($\sigma_V^2{}_{,inj}$ increases with 20% for a 170 μm ID loop and with 70% for a 220 μm ID loop from 0.3 to 1 ml/min).

1. Introduction

In recent years, a large number of studies have been undertaken to characterize the dispersion taking place in the fluidic connections of commercial ultra-high performance chromatographic instruments [1–22], since it contributes significantly to the total band broadening when using short, narrow inner diameter (ID) columns packed with sub-3μm particles. In general, the extra-column contribution is measured by replacing the column with a zero-dead volume (ZDV) connection [7–16] or by extrapolating the volumetric dispersion $\sigma_V^2{}_{,tot}$ for a homologous series of compounds with increasing retention towards $(1+k)^2 = 0$ [17–22]. Most studies only focus on the combined contribution of the different parts of the chromatographic system (injector, connection tubing, preheaters, valves, detector), because they only need the total extra-column dispersion to correct the measured total dispersion to determine the "column-only" band broadening. Some studies went a step further and attempted to separate the effect of pre- and post-column contributions, or the effects of individual aspects, such as injection volume [1,3,4,12,23,24]. Understanding extra-column band broadening and, more specifically, the variance contribution of injector valves and sample loops is also critical for the design of improved multi-dimensional LC systems [25,26]. In most cases, the different contributions to band broadening are considered to be

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independent and additive and the total peak variance in volumetric units is usually written as [1,4,8,9,11,12,16–19,21,23]:

$$\sigma^2_{V,tot} = \sigma^2_{V,pre} + \sigma^2_{V,col} + \sigma^2_{V,post} \quad (1)$$

with the subscript 'col' corresponding to the column variance, and 'pre' and 'post' representing the fluidic path before (injector to column inlet) and after the column (from column outlet up to and including detector cell) respectively. Using the ZDV method, it is assumed the extra-column variance given by Eq. (1) with $\sigma_V{}^2{}_{,col} = 0$. However, the assumption that the pre and post column contributions are additive is not entirely true. This is due to the fact that, for the typical combinations of tubing length, ID and flow rates used in (U)HPLC, the dispersion in the inlet tubing has not reached its long time limit yet when it reaches the ZDV connector, whereas the additivity of variances only holds for systems in their long time dispersion limit [5,27–28]. A recent study indicates that, for this reason, the ZDV method overestimates the extra-column dispersion contribution by about 1.5 μL^2 on a total system contribution of 2.5 μL^2 [5].

Both the pre- and post-column contributions can be further subdivided into different parts, distinguishing the different pieces of connection tubing, the injection volume and the injector valve, preheaters or post-column coolers (e.g. for high temperature LC) and the detector cell. For the pre-column train, this subdivision can be written as [9,12,23]:

$$\sigma^2_{V,pre} = \sigma^2_{V,inj} + \sigma^2_{V,tub,pre} \quad (2)$$

wherein $\sigma_V{}^2{}_{,tub,pre}$, is the combined effect of the hydraulic circuitry connecting the injector valve to the column, which in the presence of a pre-column heat-exchanger, consists of different pieces of tubing, connectors, as well as of the internal channel leading through the heat exchanger.

In the present study, we only focus on the very first contribution in Eq. (2), i.e., on the variance of the bands produced by the injector ($\sigma_V{}^2{}_{,inj}$), prior to entering the pre-column tubing. In a practical way, this value can be split up in two parts. First, one has the peak variance that persists when the volume is decreased to almost zero, because the sample anyhow has to pass through the groove and bores of the injection valve (and also through the needle seat and tubing in case of a flow-through injector). This contribution is always present, even if only an infinitely thin slice of sample would be injected and is further referred to as the "hydrodynamic contribution", $\sigma_V{}^2{}_{,inj.hydro}$. Secondly, one has a contribution that becomes increasingly larger with increasing injection volume, referred to here as the "volumetric contribution", $\sigma_V{}^2{}_{,inj.vol}$. Together, both can be added to yield:

$$\sigma^2_{V,inj} = \sigma^2_{V,inj.vol}(V_{inj}) + \sigma^2_{V,inj.hydro} \quad (3)$$

Although Eq. (3) is helpful in a practical sense, it should be realized both contributions are difficult to separate completely, as the volumetric part in practice inevitably also always depends on the flow rate, while the length of the hydrodynamic tract (and hence the hydrodynamic injector dispersion) also depends on the injection volume. It is thus important to note that $\sigma_V{}^2{}_{,inj.vol}$ will hence also include a hydrodynamic contribution. Nevertheless, Eq. (3) still provides a convenient representation of the minimal amount of hydrodynamic dispersion all injected peaks have been subjected to ($\sigma_V{}^2{}_{,inj.hydro}$) as well as of the variance contribution that increases with increasing injection volume ($\sigma_V{}^2{}_{,inj.vol}$). A similar differentiation between those two contributions was made by Claessens et al. when investigating injection systems for open-tubular liquid chromatography [29], for normal and narrow bore column HPLC by Coq et al. Coq [30] and Sanchez et al. [31].

The "V_{inj}" between the brackets in Eq. (3) has thus been added to emphasize that we explicitly define $\sigma_V{}^2{}_{,inj.vol}$ here as the part of the injection band broadening that varies with V_{inj}, and can hence be eliminated by injecting ever smaller and smaller injection volumes.

The dispersion volumetric contribution ($\sigma_V{}^2{}_{,inj.vol}$) is generally related to the square of the injection volume via a dummy factor $1/\theta_{inj}$ [1,3,7,9,12,16,17,23,29,32–34] (also denoted as $1/D^2$, $1/K^2$, $1/k^2$).

$$\sigma^2_{V,inj.vol} = \frac{V^2_{inj}}{\theta_{inj}} \quad (4)$$

Ideally, i.e., if a perfectly rectangular injection band could be injected, this factor would be equal to 1/12 (variance of a rectangular plug), whereas a perfect mixer (without dead zones) yields a value of $1/\theta_{inj} = 1$ [7,9,29,30,32,34–37]. In most literature, a range of $1/8 < 1/\theta_{inj} < 1/5$ is proposed [30–32,34,37]. In practice, however, a much wider variety of $1/\theta_{inj}$-values has been reported, ranging from 1/12 over 1 [12,17,23,30,32,34,35,38] to even 50 [38]. In part, the wide variation in reported $1/\theta_{inj}$-values in literature may be explained by the fact that the distinction between $\sigma_V{}^2{}_{,inj.vol}$ (for which the $1/\theta_{inj}$-factor has been originally introduced) and $\sigma_V{}^2{}_{,inj}$ is not always made. Another reason for the wide range of reported values is that the dispersion also depends on the type of injector, i.e. can be expected to be different for a full loop, a partial loop or a flow-through needle injector.

An alternative method to represent $\sigma_V{}^2{}_{,inj}$ instead of Eq. (3) would be to assume that the injection variance always has a minimum value of $V_{inj}{}^2/12$ (i.e., the variance of a perfectly rectangular plug) and to treat all additional dispersion caused by the non-equilibrium dispersion as hydrodynamic dispersion. This would however depend on both the operating flow rate and injection volume, making the discussion of the effect of V_{inj} on $\sigma_V{}^2{}_{,inj}$ cumbersome.

In the present study, we measured $\sigma_V{}^2{}_{,inj}$ and its constituent contributions for a number of state-of-the art injection systems using an on-capillary LED-Light Induced Fluorescence detector (abbreviated as LIF in this work), offering the unique possibility to measure the dispersion as close to the injector as possible (in practice, typically around 5–10 cm away from the valve port). Varying the injection volume, it was also attempted to study the two different contributions to Eq. (3) separately. Only the flow-through needle and the full loop injection mode were considered. The flow-through needle injector consists of a sample needle which is moved into the sample vial to load the sample into the needle and the sample loop connected to it, according to the FILO principle (First in Last out) [39]. Subsequently they are placed back in line via a needle-seat connection in which the needle is pushed to seal against the operation pressure. After a valve switch, the loop and needle are placed back in line and the sample is injected by eluting from the needle, through the needle seat and needle seat capillary and the injector valve. For a fixed loop injector, the sample is first drawn into a needle (with loop) and subsequently this is injected in a sample loop. It is required to draw a larger volume than the sample loop volume to compensate for the volume of the flow path between needle and loop and to ensure full filling of the loop (in full loop mode this requires at least 2 times the loop volume) [39]. The loop is directly connected to the valve and the sample plug therefore does not need to travel through an additional capillary (see Fig. 1).

Partial loop injections are more complex and are hence more difficult to systematically investigate and model. This is due to the fact that most instruments also introduce a small air bubble in the loop before and/or after the sample to optimize the delivered sample plug, but technical aspects of this methodology vary from one vendor to the other. Another reason why the partial loop method was left outside the scope of the study is that it can be assumed to display a behavior that is intermediate between that of a fixed loop and a flow-through needle injector. In fact, if the loop is considered

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