



# Peak distortions arising from large-volume injections in supercritical fluid chromatography



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This paper is dedicated to the memory of Georges Guiochon.

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## ABSTRACT

Preparative separations in supercritical fluid chromatography (SFC) involve the injection of large volumes of the solute. In SFC, the mobile phase is typically high pressure CO<sub>2</sub> + modifier and the solute to be injected is usually dissolved in the modifier. Two-types of injection methods, modifier-stream and mixed-stream, are common in commercial preparative SFC systems. In modifier-stream injection, the injection is made in the modifier stream which is later mixed with the CO<sub>2</sub> stream, while in the mixed-stream injection, the injection is made in a mixed CO<sub>2</sub> + modifier stream. In this work a systematic experimental and modelling study of the two techniques is reported using single-enantiomers of flurbiprofen on Chiralpak AD-H with CO<sub>2</sub> + methanol as the mobile phase. While modifier-stream injection shows non-distorted peaks, mixed-stream injection results in severe peak-distortion. By comparing the modelling and experimental results, it is shown that the modifier “plug” introduced in the mixed-stream injection is the primary cause of the peak distortions. The experimental results also point to the possible existence of viscous fingering which contributes to further peak distortion.

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## 1. Introduction

Preparative chromatography, in both the single and multicol-umn modes, is a proven tool for the separation of highly value added products, e.g., enantiomers [1–5]. In the last few years preparative supercritical fluid chromatography (SFC) has gained significant attention in the pharmaceutical, food and fragrance industries [6–8]. Supercritical fluids, compared to liquid solvents, are less vis-uous and show higher diffusion coefficients for solutes. This allows the operation of SFC at higher flow rates without loss of resolu-tion. The physical properties of the supercritical fluid allows the use of near micron-sized particles, also for preparative separations, contributing to further increase in resolution. From a preparative perspective, this results in productivity improvement compared to high performance liquid chromatography. Further, since the solute can be concentrated rather easily by depressurizing the fluid, evaporation costs can be significantly reduced. Both academic and industrial studies show the potential of SFC to reduce organic

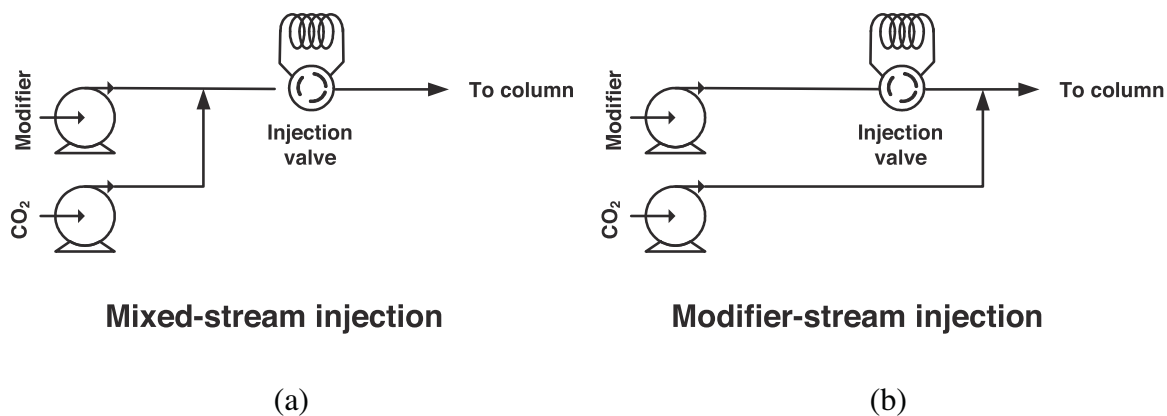
solvent consumption offering significant opportunities towards greening preparative chromatography [9,10].

Most modern SFC applications use CO<sub>2</sub> as the major component of the mobile phase, owing to its mild critical properties, benign, non-toxic nature and low-cost. The major bottleneck in employing CO<sub>2</sub>, a non-polar substance, is its poor solvation power for polar solutes. This limitation is overcome by the use of organic modifiers, such as alcohols. Addition of a modifier affects the density and vis-cosity of the mobile phase. Modifiers also compete for adsorption sites hence reducing the solid phase capacity for the solute [11,12]. The effect of adding a polar modifier on the retention properties of polar solutes has been documented in the literature [13,14]. For the case of polar solutes, the addition of a modifier weakens the retention of the solute.

Sample injection plays an important role in deciding the efficiency of separation. Improper injection can lead to band-broadening resulting in reduced resolution and productivity. This aspect is important both in analytical injections where very small amounts (μL scale) are injected and in preparative separations where large injections (100s of mL scale) are common. The impact of large-volume injections on the band-broadening in SFC has not been particularly studied. This paper addresses this topic based on the combination of experiments and modelling. In particular, two types of injection modes, mixed-stream and modifier-stream,

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**Fig. 1.** Two injection schemes considered in this study. (a) Mixed-stream injection: sample is injected into a mixed  $\text{CO}_2$  + modifier stream. (b) Modifier-stream injection: sample is injected into the modifier stream that is mixed with the  $\text{CO}_2$  stream downstream of the injection valve.

which are the most commonly found methods in commercial SFC systems are investigated.

## 2. Injection techniques in SFC

Recent literature has expounded on the key differences between HPLC and SFC [6,7]. One aspect that has received less attention, but has high practical relevance is that of the sample injection. In HPLC, barring exceptional situations, the sample to be separated is typically dissolved in the mobile phase and injected using an automated valve. In SFC, since the mobile phase is a mixture of high pressure  $\text{CO}_2$  and modifier it is not straightforward to dissolve the sample in this mixture. Hence, in most practical situations, the solute (typically a solid or a liquid) is dissolved in the modifier and this solution is injected into the column. In some cases for the purpose of injection, a third solvent, other than  $\text{CO}_2$  and the modifier, is used to dissolve the solute.

Two types of injection methods are available in most commercial SFC instruments: mixed stream and modifier-stream injection. The schematic of the two schemes is shown in Fig. 1. In the mixed stream, which is almost the standard configuration in analytical SFC units, the modifier and  $\text{CO}_2$  streams are mixed upstream of the injection valve. In this mode, when an injection is performed the mixed solvent ( $\text{CO}_2$  + modifier) pushes the sample “plug”, which is a mixture of modifier and the solute (no  $\text{CO}_2$ ) into the column. For a given injection volume and mobile phase flow rates, this mode has a shorter injection time, as the entire mobile phase passes through the injection valve. The modifier-stream injection is a patented technique in which only the modifier stream flows through the injection valve and the  $\text{CO}_2$  stream is mixed downstream of the valve [15]. Hence, the sample plug that was present in the mixed-stream injection is avoided. However, since only the modifier stream flows through the injection valve, the time required to push the sample is longer than in the mixed-stream injection. When modifier compositions are low, this can result in longer injection times, reducing process productivity. Many commercial prep-SFC units are equipped with the modifier-stream injection.

The propagation of chromatographic bands in case of the mixed-stream injection is worth considering [7,16,17]. Let us consider a case where modifier is weakly retained compared to the solute. Fig. 2 shows the propagation of the bands in the physical (length–time) plane and the corresponding elution profiles. Note that this figure is constructed under the assumption of local equilibrium, i.e., in the absence of mass transfer or dispersion effects. In the example shown in Fig. 2(a) and (b), the column is initially saturated with  $\text{CO}_2$  + modifier and a mixed-stream injection is made.

The modifier is assumed to be non-adsorbing, it travels through the column unaffected, and exits the column in the same shape as it was injected. Now, consider the solute band. Since the solute typically has a weaker retention within the modifier band than in a mixed  $\text{CO}_2$  + modifier stream, the trailing edge of the solute band travels slower as it is only exposed to the environment of  $\text{CO}_2$  + modifier stream, while the leading edge travels faster as it travels within the modifier band. If the injection is “small” enough (c.f. Fig. 2(a)), the leading edge eventually moves from the modifier band to the  $\text{CO}_2$  + modifier environment. Note that, in this case, even in the absence of dispersion effects, the solute band is broadened compared to the injected band. If the injection volume is rather large (c.f. Fig. 2(b)), then the leading edge of the solute band is eluted under the modifier band leading to peak distortion. This rather simple analysis points to the fact that band broadening and peak distortion can occur in the case of the mixed-stream injection, even under local-equilibrium conditions, i.e., in the absence of mass transfer and solubility effects. In contrast, in the case of the modifier-stream injection (c.f. Fig. 2(c)) since there is no modifier plug, the entire solute band travels in the  $\text{CO}_2$  + modifier environment. Hence, independent of the injection volume, and the absence of dispersion effects, no band broadening occurs in the chromatographic column.

In addition to the band broadening discussed above, which is caused due to the different retention characteristics in the modifier plug and the mixed mobile phase, other reasons have been attributed to peak distortion in mixed-stream injections. Berger and Fogelman attributed peak distortions to issues related to solubility [15]. They anticipated precipitation of the solute at the column head as the pressure could suddenly change or as the solute starts to interact with the solid phase. In order to avoid this, they proposed the modifier-stream injection where the formation of the injection plug is avoided and the solute band propagates consistently within the mixed stream. Miller and Sebastian reported an experimental study of the two techniques and observed that the mixed-stream injection usually led to reduced efficiency and peak distortions compared to the modifier-stream injection [18]. The primary aim of the current study is to compare these injection techniques under controlled conditions and model their behaviour to understand the sources of band broadening.

## 3. Experimental characterization

Pure enantiomers of Flurbiprofen with a purity  $\geq 99\%$  and tert-butylbenzene (TTBB) were purchased from Sigma–Aldrich (Singapore). A 250 mm long 4.6 mm diameter Chiralpak AD-H column with 5  $\mu\text{m}$  particles was obtained from Chiral Technologies

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