Injection Sequence for Optimizing Performance in UHPLC Separations

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Abstract

Over the last 7 to 10 years there has been a lot of work done in bringing forth ultra-high performance liquid chromatography (UHPLC) separations. Initially these UHPLC separations were performed using totally porous particles with sub-2 µm particle size media. To use these sub-2 µm particles, special instrumentation had to be developed to deliver higher pressure and lower band dispersion as compared to previous generation instrumentation. Recently, the use of core-shell materials has become a very popular alternative to the use of small particles for UHPLC separations. By using these new 2.6 µm core-shell materials, performances equal to the sub-2 µm materials are obtained, but at operating pressures compatible with 400 bar instrumentation. On both the older 400 bar and the newer 1000+ bar generation instruments, there is still significant band dispersion (extra column effects) generated within the injection system. In this poster we will demonstrate a Performance Optimizing Injection Sequence (POISe) that effectively eliminates the dispersion due to the injector, allowing for higher performance UHPLC separations on both 400 and 1000+ bar generation instruments.

Introduction

In recent years, there has been much interest in the performance benefits of UHPLC separations. UHPLC technology has given chromatographers the ability to obtain significantly higher plates per unit column length. By leveraging these higher plates and optimizing column length, it has been possible to significantly reduce analysis times while retaining or even improving the resolving power of the separation. When this technology was first introduced, it was based upon columns packed with totally porous particles that were < 2 µm in diameter. These sub-2 µm columns generate significantly higher backpressures than traditional columns. In order to facilitate their use, a new generation of higher pressure and lower dead volume instrumentation was introduced at the same time. More recently a new generation of core-shell particles have been introduced. These particles consist of a non-porous core coated with a porous shell. These new generation particles have extremely tight particle size distributions leading to column efficiencies similar to sub-2 µm totally porous columns but with the benefit of generating backpressures only slightly higher than 3 µm columns.[1-5] The combination of higher efficiency and lower backpressure leads to the ability to obtain UHPLC separations without the need for new instrumentation. When pursuing very high separation efficiencies, not only does the column need to provide high performance, but the instrument must be capable of maintaining very narrow analyte bands. To achieve and maintain very narrow analyte bands, there have been efforts to develop methods to optimize and maintain instrumentation that provide as little extra-column dispersion as possible.

One of the proposed optimization strategies is the use of a band compression injection technique.[5-6] In order to accomplish the band compression, a band of weak-eluting solvent is injected onto the column following the analyte band. As the analyte and weak solvent bands migrate towards the column, minute mixing occurs such that the analytes are subsequently dissolved in a non-eluting solvent when they enter the column, leading to isocratic band compression. This Performance Optimizing Injection Sequence (POISe) has been briefly introduced previously.[5-6] In this poster we will explore the broader applicability of the POISe injection strategy on different UHPLC and HPLC column types, as well as different HPLC and UHPLC systems.

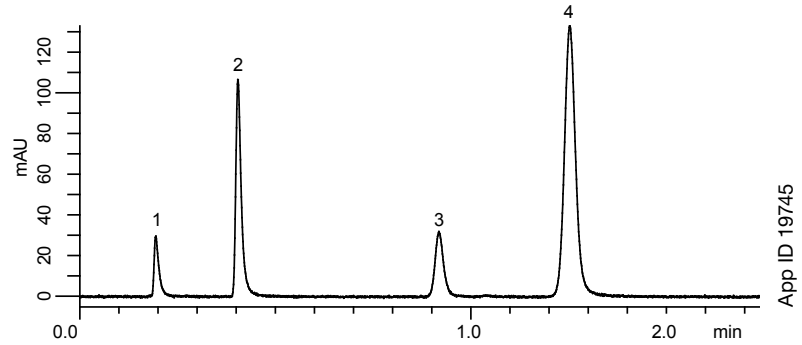
Figure 1. Isocratic Test Chromatograms

Same conditions for all columns:

Column: Kinetex® 2.6 µm XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4496-AN
Mobile Phase: Water / Acetonitrile (50:50)
Flow Rate: 0.5 mL/min

Temperature: Ambient
Detection: UV
Injection Volume: 1.0 µL
Instrument: Agilent® 1100
Sample: 1. Uracil
2. Acetophenone
3. Toluene
4. Naphthalene

A without the POISe injection sequence



B with the POISe injection sequence (4 µL weak solvent plug)

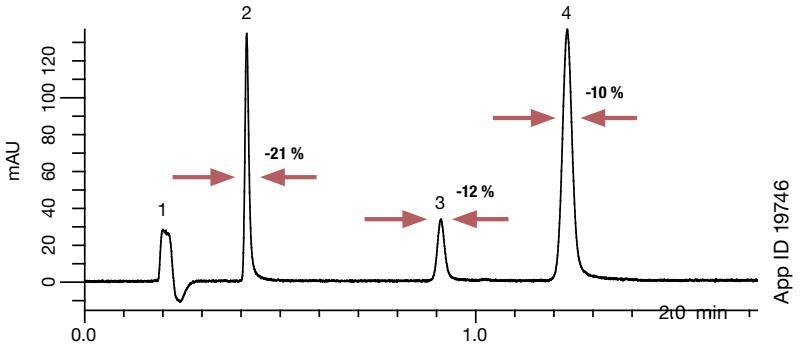


Table 1. Chromatographic performance obtained on a Kinetex 2.6 µm XB-C18 50 x 2.1 mm column with and without the POISe injection technique

Instrument: Agilent® 1100 HPLC system

Compound	Injection Style	Width	Plates	% Decrease in Width
Acetophenone	Normal	0.024	4823	
Toluene	Normal	0.040	9217	
Naphthalene	Normal	0.052	9902	
Acetophenone	POISe	0.019	**8105**	**20.57 %**
Toluene	POISe	0.035	**11639**	**12.27 %**
Naphthalene	POISe	0.047	**11577**	**9.72 %**

Figure 2. Isocratic Test Chromatograms

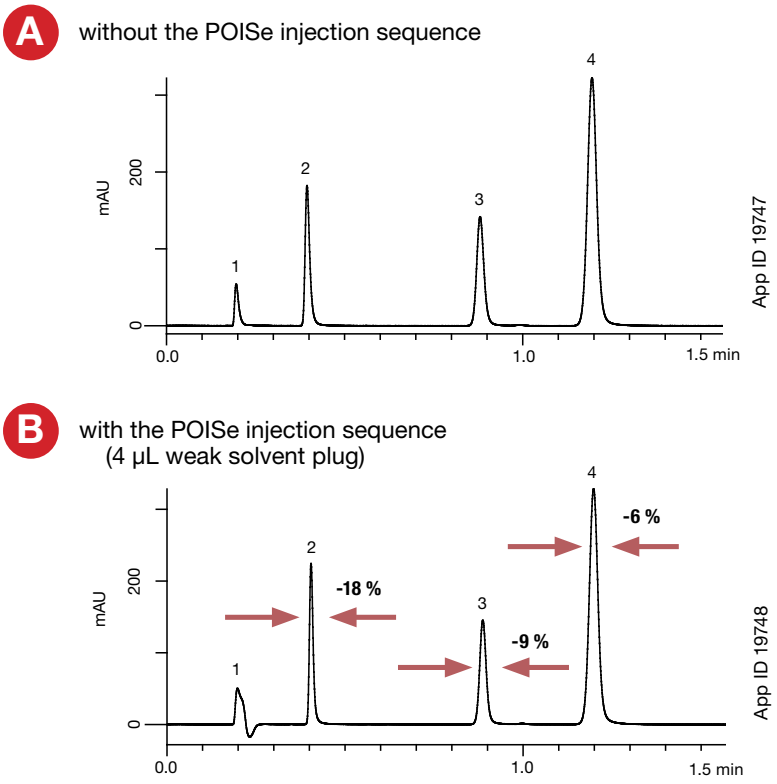


Table 2. Chromatographic performance obtained on a Kinetex 2.6 µm XB-C18 50 x 2.1 mm column with and without the POISe injection technique

Instrument: Agilent 1200 SL UHPLC system

Compound	Injection Style	Width	Plates	% Decrease in Width
Acetophenone	Normal	0.023	4916	
Toluene	Normal	0.037	9527	
Naphthalene	Normal	0.049	10196	
Acetophenone	POISe	0.019	**7757**	**17.63 %**
Toluene	POISe	0.034	**11630**	**8.95 %**
Naphthalene	POISe	0.046	**11662**	**5.98 %**

Injection Sequence

For autosamplers with a needle wash feature:

Make sure that the injection loop is 3 – 4 times the volume of sample you intend to inject. Next, select a weak needle wash solvent—generally either 100 % water or 95 % water with 5 % organic, preferably weaker than your initial mobile phase strength. The sample loop will initially fill with weak wash solvent before the sample is aspirated into the sample loop. Whatever excess volume of weak solvent that is not displaced by the aspirated sample subsequently follows the sample onto the column providing the POISe effect. With this new technique it is important to turn off any overfill option, which would replace all the weak solvent with sample, negating the desired effect.

For autosamplers without an automatic needle wash sequence, you need to program the injection sequence. A sample of this program is shown below for an Agilent instrument.

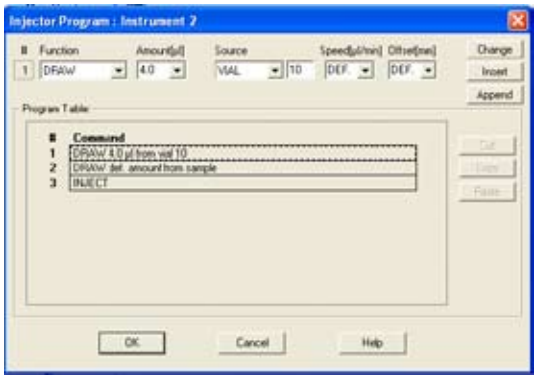


Figure 3. Chromatographic performance obtained on a Kinetex 2.6 µm XB-C18 50 x 2.1 mm column with varying volumes of weak solvent using the POISe injection technique

Instrument: Agilent 1100 HPLC system

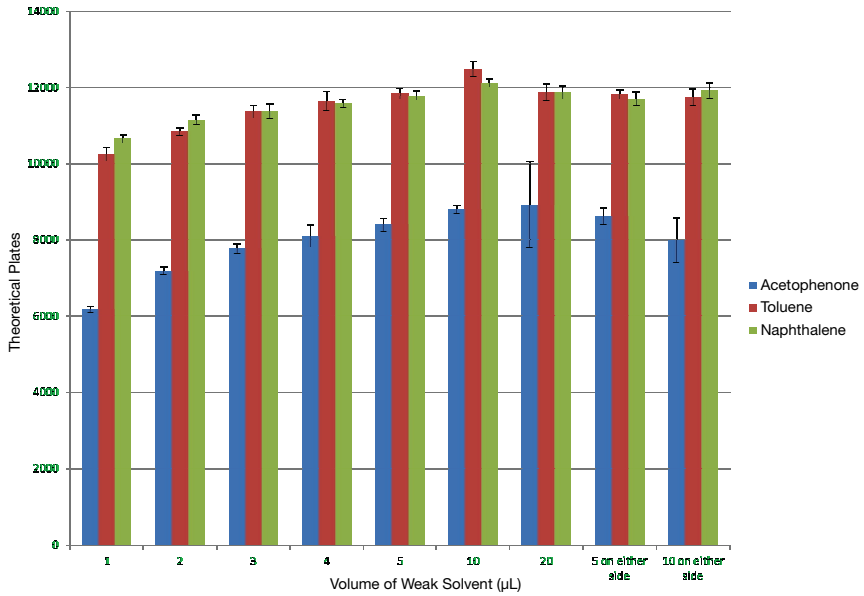


Table 3. Chromatographic performance obtained on a Kinetex 2.6 µm XB-C18 50 x 2.1 mm column with the POISe injection technique on different UHPLC systems

Instrument	POISe	Peak	Plates	Width	% Decrease in Width
Agilent® 1200SL	None	Uracil	1565	0.020	
		Acetophenone	4857	0.023	
		Toluene	9321	0.037	
		Naphthalene	10140	0.048	
	4 µL	Uracil	304	0.043	-109.87 %
		Acetophenone	**7757**	0.019	**17.63 %**
		Toluene	**11630**	0.034	**8.95 %**
		Naphthalene	**11662**	0.046	**5.98 %**
Agilent 1290	None	Uracil	2152	0.016	
		Acetophenone	6582	0.020	
		Toluene	10414	0.035	
		Naphthalene	10567	0.048	
	4 µL	Uracil	1206	0.021	-28.74 %
		Acetophenone	**9536**	0.017	**15.63 %**
		Toluene	**12258**	0.033	**6.78 %**
		Naphthalene	**12137**	0.045	**4.91 %**
Waters® ACQUITY®	None	Uracil	1067	0.025	
		Acetophenone	3865	0.027	
		Toluene	9196	0.039	
		Naphthalene	10059	0.051	
	4 µL	Uracil	293	0.041	-64.00 %
		Acetophenone	**13532**	0.015	**44.44 %**
		Toluene	**13736**	0.032	**17.95 %**
		Naphthalene	**12736**	0.046	**9.80 %**

Table 4. Chromatographic performance obtained on HPLC/UHPLC systems with a variety of totally porous and core-shell columns with the POISe injection technique

Column	Instrument	POISe	Peak	Plates	Width	% Decrease in Width
Kinetex 1.7 µm XB-C18 core-shell	Agilent 1200 SL	0	Uracil	1422	0.022	
			Acetophenone	5307	0.024	
			Toluene	10563	0.040	
			Naphthalene	11412	0.053	
		4	Uracil	402	0.041	-85.59 %
			Acetophenone	**8343**	0.020	**18.14 %**
			Toluene	**12725**	0.037	**6.74 %**
			Naphthalene	**12680**	0.051	**3.89 %**
ACQUITY 1.7 µm BEH™ C18 (fully porous)	Agilent 1200 SL	0	Uracil	2272	0.020	
			Acetophenone	7036	0.025	
			Toluene	10302	0.050	
			Naphthalene	10735	0.068	
		4	Uracil	678	0.032	-56.81 %
			Acetophenone	**9879**	0.022	**12.46 %**
			Toluene	**11003**	0.048	**2.93 %**
			Naphthalene	**11114**	0.067	**2.10 %**
ZORBAX® 1.8 µm SB-C18 (fully porous)	Agilent 1200 SL	0	Uracil	1798	0.019	
			Acetophenone	6601	0.024	
			Toluene	11177	0.052	
			Naphthalene	11583	0.073	
		4	Uracil	326	0.041	-110.40 %
			Acetophenone	**8256**	0.023	**5.63 %**
			Toluene	**11991**	0.051	**2.95 %**
			Naphthalene	**11977**	0.072	**0.78 %**

Table 4. Chromatographic performance obtained on HPLC/UHPLC systems with a variety of totally porous and core-shell columns with the POISe injection technique *con't*

Column	Instrument	POISe	Peak	Plates	Width	% Decrease in Width
Gemini®-NX 3 µm C18 (fully porous)	Agilent 1100	0	Uracil	1410	0.022	
			Acetophenone	4424	0.033	
			Toluene	6552	0.070	
			Naphthalene	6502	0.098	
		4	Uracil	157	0.066	-195.38 %
			Acetophenone	**5902**	0.030	**8.81 %**
			Toluene	**6975**	0.069	**2.31 %**
			Naphthalene	**6886**	0.097	**1.49 %**

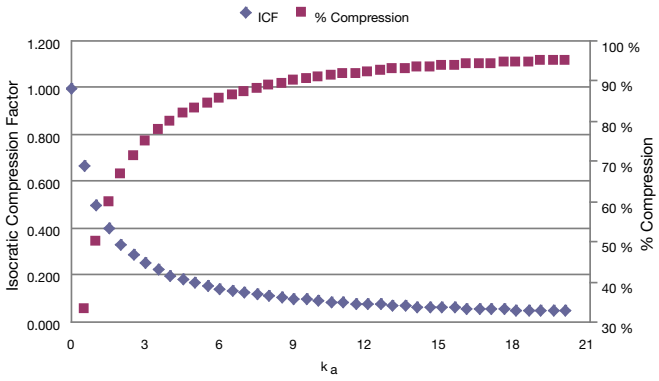
Results and Discussion

In **Figure 1**, chromatograms are shown with and without the POISe injection sequence. These chromatograms were both produced using a Kinetex 2.6 µm XB-C18 column on an Agilent 1100 HPLC system. By using the POISe technique to eliminate the band dispersion due to injection, the peaks were 10 – 20 % more efficient as is shown in **Table 1**. The same experiment was repeated on an UHPLC system (Agilent 1200 SL), as is shown in **Figure 2**. Even with the newer decreased band dispersion systems, there was still a 6 – 18 % increase in the efficiencies of the peaks by using the POISe technique, shown in **Table 2**. This demonstrates that even the injection systems on the new generation systems still introduce a significant amount of band dispersion. We investigated the increase in efficiency (plates) with increasing volumes of weak solvent (**Figure 3**). It was determined that using 3 – 4 times the injection volume of weak solvent provided the most benefit. Further, increasing the volume of weak solvent did not significantly increase the peak efficiencies. Also, sandwiching the injection plug between two plugs of weak solvent did not provide any further increase in efficiencies over just having the weak solvent behind the injection plug.

We investigated the effect of POISe on several different UHPLC systems. Of the three systems tested, the Waters ACQUITY® system saw the largest performance benefit from using the POISe technique. It should be noted, however, that after the Waters system saw the performance enhancement due to the injection sequence, it provided slightly higher efficiencies than the other systems tested.

In order to determine the broad applicability of the POISe technique for fully porous materials as well as core-shell materials, a series of UHPLC and HPLC columns were tested using the POISe injection technique. This data is presented in **Table 4**. In every case there was an improvement in the peak efficiency when using the POISe technique. It is interesting to note that, in the data presented in **Table 4**, the amount of efficiency improvement observed was directly related to the retention factor of the compound, as was seen in earlier examples. Early eluting compounds experienced a much greater efficiency improvement than their more retained counterparts. This enhancement for the earlier eluting compounds is due to the isocratic compression. By using the POISe technique the effective elution strength of the sample plug solvent is significantly reduced, allowing the analytes to focus on the head of the column. Compounds with lower retention factors travel further and spread more without this isocratic compression. The more the initial spreading is reduced or eliminated, the larger the gains in performance. Alternatively, analytes with higher retention factors experience a portion of the isocratic compression in the mobile phase already without POISe, leading to lower compression ratios when POISe is implemented. The relationship between retention factor and peak compression is shown in **Figure 4**. Even with highly retained compounds there is a benefit for the POISe technique. The POISe technique is also effective in gradient elution for compounds eluting early under gradient conditions.

Figure 4. **Peak compression percentage and isocratic compression factor as a function of retention factor in isocratic HPLC**



Conclusions

- The implementation of the POISe technique effectively eliminates band broadening contributions due to the injection system
- The efficiency improvements are realized regardless of system
- The efficiency improvements are realized regardless of the HPLC/UHPLC column
- The POISe technique is simple to implement and requires no changes to the system configuration

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