# Influence of Injector Bypass on Lifetime of Small-Particle Liquid Chromatographic Columns*

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## Key Words

Liquid chromatography
High-speed liquid chromatography
Small-particle LC columns
Injector bypass
Column lifetime in LC

## Summary

In the case of liquid chromatography columns packed with small (3–5 $\mu$m) particles and utilized at relatively high flow rates, pressure pulses associated with the sample injection process (caused by a short-duration interruption in mobile phase flow to the column during injection) may result in a reduction of column lifetime. This detrimental effect can be eliminated by the utilization of specially constructed sample injection valves incorporating properly designed bypass loops. The paper investigates in detail the effect of pressure pulses, describes the criteria and performance of the bypass systems, and documents column life time and performance.

## Introduction

The general trend in liquid chromatography in recent years has been towards the use of smaller particle packings in order to increase the efficiency generated per unit time [1, 2]. Today, the use of three and five $\mu$m particles is widespread resulting in substantial column efficiencies on the order of 10,000 theoretical plates being generated in time frames of only 1–2 minutes. These high efficiencies in rather short analysis times are feasible due to the inherently lower HETP minimum, higher optimum linear velocities, and lower mass transfer effects associated with the use of smaller particles [3, 4].

The combined use of smaller particle supports and higher flow velocities results in substantially higher operating backpressures than typically encountered using conventional columns packed with larger particles. Operation at 20–30 MPa (3000–4500 psig) backpressures is not uncommon with these new "high-speed columns". These higher operating backpressures can lead to drastically reduced column lifetimes under certain conditions. Our investigation shows that these premature column failures are caused by pressure pulses associated with the injection process caused by an interruption in mobile phase flow to the column. While this phenomenon appears to have been of general knowledge, its effects have never been clearly documented and are certainly of increased importance now due to the rapidly increasing use of these smaller-particle columns. In this report we document the magnitude of these pressure pulses and their deleterious effects on columns. Additionally, the construction of bypass loops to circumvent these pressure pulses is described.

The use of smaller particle columns with their inherently higher efficiencies and lower void volumes imposes additional constraints on the design and construction of injection valves as extra-column band broadening must be minimized if the full potential of these columns is to be realized. Therefore, the injector bypass must be constructed so as to eliminate pressure pulses yet not substantially contribute additional extra-column band broadening. When properly designed and constructed the injector bypass extends the lifetime of a "high-speed column" from an untolerably short period to an infinitely long one.

## Experimental

A Perkin-Elmer SERIES 3B liquid chromatograph equipped with a Model LC-85 variable wavelength UV/VIS detector (190–600 nm) was used. A 2.4-$\mu$l flowcell was used in the detector along with 0.007 in. (0.18 mm) i.d. connecting tubes. A Perkin-Elmer SIGMA 15 data station, Model 056 recorder or a Bascom-Turner (Newton, MA, U. S. A.) Model 8120 recorder capable of data acquisition rates of up to 1000 points per second were used for various experiments. Manual injections were accomplished using a Rheodyne Model 7125 injector equipped with a 6 $\mu$l sample loop made of 0.007 in. (0.18 mm) i.d. tubing. Connecting tubing in the sample flow path was also 0.007 in. (0.18 mm) i.d. with a total of 80 cm used throughout the system. All connecting tubing was stainless steel 1/16 in. (1.59 mm) o.d. A 250 mm × 9 mm i.d. column filled with 55–105 $\mu$m high surface area silica was installed between

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* Dedicated to Dr. L. S. Ettre on the occasion of his sixtieth birthday

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the pump and injector and served as a presaturation column for some experiments [5]. For automated sample injections during column life testing, either a Perkin-Elmer Model 420B Auto Sampler having a Rheodyne 7413 injection valve with a 1.0-μl sample loop or a UHP series Valco valve with a 0.2-μl internal loop equipped with a Valco two-position electric valve actuator (Valco Instrument Co., Inc; Houston, TX, U. S. A.) was used. For the measurement of pressure surges during the injection process, a pressure transducer (Perkin-Elmer part number 0254-0980) was installed between the injector and the column inlet and used in conjunction with the Bascom-Turner recorder at a high sampling rate (300 data points per second).

The columns used had an internal diameter of 4.6 mm and a length of 100 mm or 125 mm. The 100-mm length columns (Perkin-Elmer/HS-3 C-18, part number 0258-1501) were packed with a 3-μm $C_{18}$ bonded phase material. The 125 mm length column (Perkin-Elmer/HS-5 C-18, part number 0258-1001) was packed with a 5-μm $C_{18}$ bonded phase material. The column characteristics have been described in more detail elsewhere [3].

### Procedure

### Construction of injector capillary bypass

Injector bypasses were constructed using two union-tees, stainless steel 1/16 in. (1.59 mm) o.d. tubing and the appropriate fittings. Three types of union-tees were used in this study: a brass tee having a 0.007 in. (0.18 mm) hole, and SSI tees having 0.043 in. (1.09 mm) and 0.015 in. (0.38 mm) holes (Scientific Systems, Inc. State College, PA. U. S. A.; part number 01-0164 and 01-0165 respectively). Details of the construction are shown in Fig. 1.

### Measurement of instrumental bandwidths

Instrumental bandwidth measurements were performed by injecting a solute (uracil) into the LC system in which the column is replaced by a zero-dead volume connecting union. Measurements of instrumental bandwidth (4 σ peak volume) were obtained by measuring peak widths at half-height and multiplying by a factor of 1.7 [4].

### Column life testing

Isocratic column life testing was performed using the equipment described above. The flow rate was 2.0 ml/min using a mobile phase consisting of 60 : 40 acetonitrile-water or 80 : 20 methanol-water. Test solutes were either a dilute solution of toluene in the mobile phase or a seven-component test mixture. Efficiency measurements were obtained by using the peak width at half-height method. Peak assymmetry was measured at 10 % of peak height [6]. Gradient column life tests were performed using an 11-component test mix according to conditions described previously [7].

## Results and Discussion

### General Bypass Construction

Fig. 1 shows a diagram of the general valve construction incorporating a bypass loop. The valve utilized is a standard six-port Model 7125 Rheodyne valve with external modifications on the valve's inlet and outlet lines to incorporate the bypass. Tees are utilized in the inlet and outlet lines along with tubing to permit flow either through the valve, through the bypass loop or through both simultaneously. Therefore, when an injection is performed and flow is interrupted within the valve, flow to the column is still maintained through the bypass. The construction of the bypass with respect to tubing bore and length is critical with respect to eliminating pressure surges and minimizing extra-column band broadening as described below.

The magnitude and duration of the pressure surges caused by an interruption in mobile phase flow during an injection is shown in Fig. 2. The top tracing indicates the changes in pressure which occur at the top of a column during the injection process when a bypass is not used. In this experiment pressure is monitored just before the top of the column using a very sensitive pressure transducer with the analog output being sampled at a rate of 300 data points per second using the Bascom-Turner high-speed recorder.

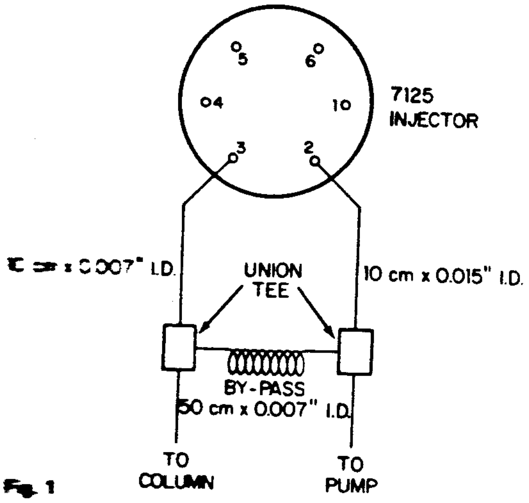

Fig. 1

Schematic of Rheodyne Model 7125 injector with external bypass connection

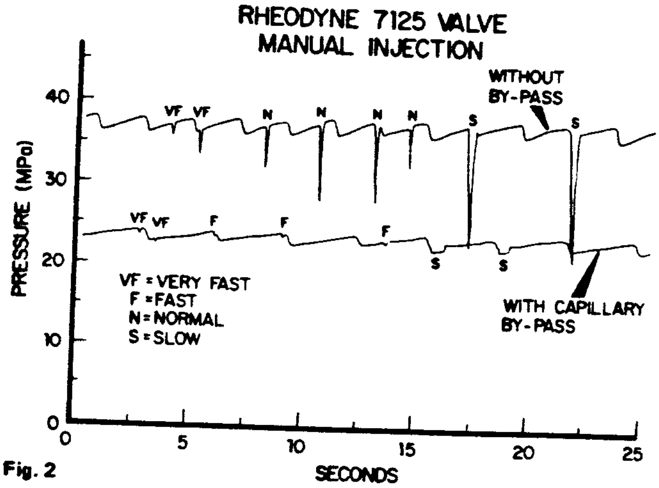

Fig. 2

Plot of pressure (MPa) versus time (seconds) showing the magnitude and duration of pressure surges both without (top tracing) and with (bottom tracing) bypass. Manual injections were performed turning the injector handle at various rates as indicated.

[illegible] depends a great deal on the manner in which the injection valve is rotated as indicated in the figure. For what is considered a normal type of manual injection the pressure at the top of a column drops as the mobile phase flow is interrupted within the valve during rotation from the LOAD port and then the pressure rises up to the original operating level after the valve reaches the INJECT port. The pressure drop experienced is on the order of 25 % of the operating backpressure and the duration is about 100 ms. For quicker injections the magnitude is less and the duration is shorter, while for slower injections the magnitude is greater and the duration is longer, as expected. This phenomenon is very detrimental to a column and the effect is exacerbated as the operating backpressure increases. Proper incorporation of the bypass completely eliminates these pressure surges as shown in the lower tracing of Fig. 2.

Construction of the injector bypass is critical as the pressure surges must be eliminated but, at the same time, extra-column band broadening should not be increased significantly as this will also adversely effect the column's performance.

Table I describes various system configurations tested along with the measured split ratio (flow to injector/flow to bypass) and the instrumental bandwidth at various flow rates. The instrumental bandwidth is a measurement made on the complete LC system of injector through detector. but without a column. An injection of a sample is monitored on a recorder thus revealing the magnitude of extra-column band broadening [3, 4]. An example of such a measurement is shown in Fig. 3. The basic system utilized originally did not contain a bypass, therefore, all of the flow went throughout the injector. The measured instrumental bandwidth under these conditions was approximately 21 µl at a flow rate of 1.0 ml/min and about 27 µl at a flow rate of 3.0 ml/min. The basic system consisted of a pump, detector, and injector, as indicated with about 80 cm of 0.007 in. (0.18 mm) i.d. connecting tubing. Injections of 1 µl were made into the 6-µl sample loop using either water or acetonitrile as the mobile phase. The addition of a zero-dead-volume tee alone had no influence on the system, however, we found that the non-zero dead volume tee (0.043 in. i. d. hole) adds substantially to band

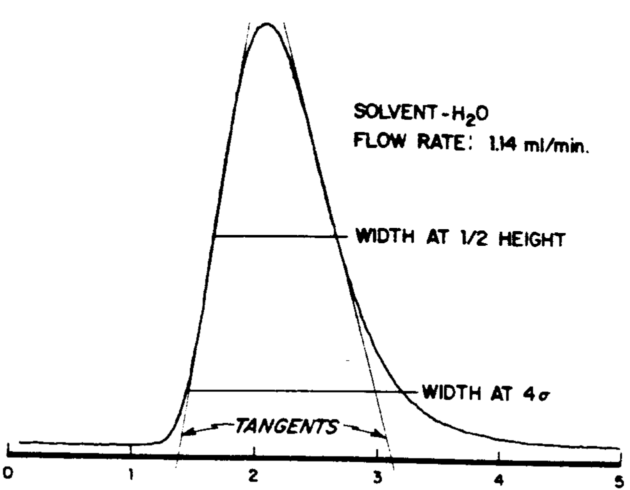

Fig. 3

Instrumental bandwidth measurements. A 6-µl sample of uracil was injected into the liquid chromatograph with the column removed, at a flow rate of 1.1 ml/min of water. The measurement of the recorder output was made using the peak width at half height and multiplying by 1.7 to obtain the 4σ peak width.

broadening. Increasing the length of the bypass tubing causes the flow split ratio (injector/bypass) to increase. A split ratio of greater than unity is necessary in order to prevent excessive increases in extra-column band broadening. Increasing the split ratio to approximately 5–7 appears to give excellent long-term results with respect to eliminating pressure surges and not adding incrementally to band broadening.

Fig. 4 demonstrates the deleterious effect which pressure surges can have on a column. A new high-speed column (100 X 4.6 mm i.d.) packed with 3 µm particles was capable of generating in excess of 13,000 theoretical plates under the conditions utilized. However, after only 60 injections under these conditions, which produced an operating backpressure of 18 MPa (2650 psig) a great loss in column efficiency is observed if an injector bypass is not utilized. This loss in performance appears to occur rather precipitously after numerous injections and then gradually worsens. A sampling of many commercially available columns found none to be immune to this phenomenon. As seen in Table II

Table I. Effect of flow rate, bypass length and union tee on instrumental bandwith*

| No. | System | Split ratio (inj./bypass) | Flow rate, ml/min 0.5 | 1.0 | 3.0 | 5.0 |
|---|---|---|---|---|---|---|
| | | | Instrumental bandwidth**, µl | | | |
| 1 | Basic system[1] (no bypass) | – | 17.8 | 21.1 | 26.7 | 30.6 |
| 2 | Basic system + ZDV tee[2] (plugged) | – | 20.6 | 21.2 | 25.9 | 32.6 |
| | Basic system + ZDV tee | | | | | |
| 3 | + 15 cm x 0.007 in. i.d. bypass | 0.7 | 27.3 | 27.3 | 31.8 | 38.4 |
| 4 | + 30 cm x 0.007 in. i.d. bypass | 1.4 | 20.3 | 21.0 | 28.4 | 35.4 |
| 5 | + 50 cm x 0.007 in. i.d. bypass | 3.0 | 20.9 | 21.5 | 28.4 | 35.4 |
| 6 | + 120 cm x 0.007 in. i.d. bypass | 7.0 | 18.1 | 21.2 | 26.7 | 34.8 |
| 7 | Basic system + non-ZDV tee[3]<br>+ 100 cm x 0.007 in. i.d. bypass | 6.5 | 35.6 | 36.2 | 38.6 | 41.8 |
| 8 | Basic system + SSI ZDV tee[4]<br>+ 100 cm x 0.007 in. i.d. bypass | 6.5 | 22.6 | 24.0 | | |

*Conditions: LC-85 UV detector with 2.4-µl cell; 80 cm x 0.007 in. i.d. tubing mobile phase: acetonitrile; Model 7125 injector; 1 µl sample injection

**Calculated using the peak with at half height method.

[1] See Fig. 1 for bypass configuration.

[2] Specially designed union tee with 0.007-in. hole (used in systems nos. 2–6).

[3] Scientific Systems, Inc. (SSI) union tee with 0.043-in. hole.

[4] Scientific Systems, Inc. (SSI) union tee with 0.015-in. hole.

and in Fig. 5, the incorporation of the injector bypass eliminates this problem completely and column lifetime becomes extended greatly. Both experiments were terminated voluntarily as no indication of column degradation was obvious. Fig. 5 shows run number 2187 on the high-speed 5-μm column, where the column efficiency is essentially unchanged from the initial run. A high-speed 3-μm column was similarly tested and found to be essentially unaltered in performance after more than 3000 analyses. Peak symmetries were also maintained as indicated in Table II.

The design of injection valves incorporating a bypass loop or a split stream injector of some type in which only part of the mobile phase flow is directed through the sample loop is certainly not novel [8–12]. However, in all instances the design intent was generally to improve column efficiency by controlling to some degree the sample flow characteristics at the top of the column. Fortuitously, these designs would also serve to extend column lifetimes if the proper split ratios are achieved. Observations of the pressure surges which can be created by injectors and their deleterious effects on columns has previously not been documented.

The question of column lifetime has rarely been addressed for many reasons as many factors can contribute to reduced column lifetimes. The role of silica dissolution particularly at high pH has been well documented [5] and there is no question that certain samples can contribute to column failure. In these instances guard columns can be utilized to extend the lifetime of the analytical columns. In this study we have described another type of column failure caused by momentary interruptions in mobile phase flow to the column and the resultant mechanical (pressure) shock. This failure mode can be eliminated by the construction of a simple injector bypass.

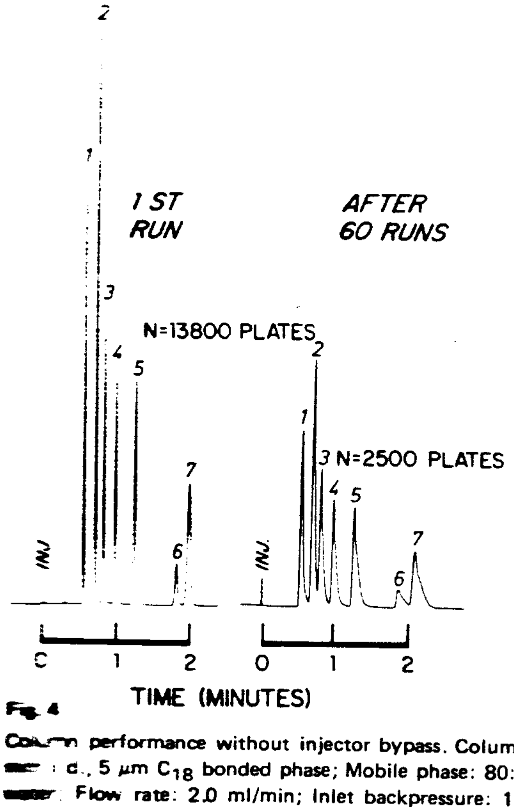

**Fig. 4**
Column performance without injector bypass. Column: 125 X 4.6 mm i.d., 5 μm $C_{18}$ bonded phase; Mobile phase: 80:20 methanol-water; Flow rate: 2.0 ml/min; Inlet backpressure: 18 MPa (2650 psig); 10 μl injection; ambient temperature.

Peaks: *1* uracil (2,4-dioxopyrimidine), *2* phenol, *3* nitrobenzene, *4* toluene, *5* ethylbenzene, *6* isopropylbenzene, *7* tert. butylbenzene

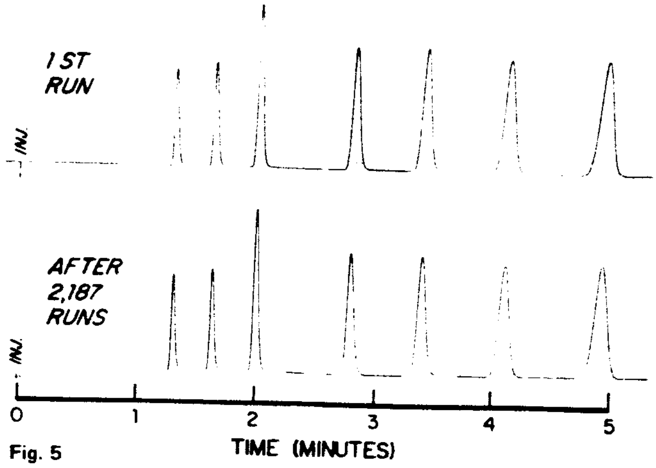

**Fig. 5**
Column performance using injector bypass Column: 125 X 4.6 mm i.d., 5-μm $C_{18}$ bonded phase; Mobile phase: 80 : 20 methanol-water; Flow rate: 0.8 ml/min; Inlet backpressure: 7 MPa (1050 psig); ambient temperature. Sample as in Fig. 4.

**Table II.** Column life performance using injector bypass*

| Column | Number of injection | Efficiency (theoretical plates) | Asymmetry (As) |
|---|---|---|---|
| Perkin-Elmer HS-5 C-18** | 1 | 10,700 | 1.16 |
| | 585 | 11,800 | 1.10 |
| | 1,294 | 11,700 | 1.36 |
| | 2,187 | 11,200 | 1.24 |
| Perkin-Elmer HS-3 C-18*** | 1 | 13,850 | 1.18 |
| | 3,201 | 13,262 | 1.07 |

*See text concerning details of test conditions.
**LC conditions: 80:20 methanol-water, 2.0 ml/min; backpressure: 18 MPa.
***LC conditions: 60:40 acetonitrile-water, 2.0 ml/min; backpressure: 18 MPa.

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