Journal of Chromatography A, 659 (1994) 255–259
Elsevier Science B.V., Amsterdam

CHROM. 25 603

Comparison of two injection systems to be used with 5 μm I.D. open-tubular columns

Antonio L. Crego, Manuel V. Dabrio and José C. Díez-Masa*

Instituto de Química Orgánica General (CSIC), Juan de la Cierva 3, 28006 Madrid (Spain)

(First received June 25th, 1993; revised manuscript received September 29th, 1993)

ABSTRACT

The split injection system and the pressure pulse-driven stopped-flow injection system (PSI) were compared in terms of band broadening and sample injection volume reproducibility for 5 μm I.D. open-tubular columns. It was found that the PSI injector has a better injection profile factor than the split injector, maximum column efficiency being obtained with higher injection volumes (ten times) using the PSI injector. The repeatability of the injected volumes obtained with the PSI injector is twice as good as that with the split injector. The peak efficiency reproducibility is similar with both injectors (R.S.D. = 3–4%).

INTRODUCTION

The use of open-tubular columns (OTCs) in high-performance liquid chromatography (HPLC) is promising in microseparation techniques. Plate counts higher than $0.5 \cdot 10^6$ m^{-1} [1], plate generation velocities around 1000 s^{-1} [1,2] and enhanced mass sensitivity [3] have been obtained using such columns. Despite their advantages, OTCs are not as popular in HPLC as their counterparts in gas chromatography. This may be due to the demanding features of the injection and detection systems associated with these columns.

The maximum sample volume (V_{inj}) to be injected into an HPLC column for a relative loss in the plate number smaller than Θ^2 is given by [4]

$$V_{inj} = (\pi/4)\Theta^2 K\varepsilon(1 + k')(L/\sqrt{N})d_c^2 \qquad (1)$$

where N is the column plate number, k' is the solute capacity factor, L is the column length, ε is the porosity of the packed bed ($\varepsilon = 1$ for OTCs), K is the injection profile factor and d_c is the inner diameter (I.D.) of the column. As OTCs should have very small d_c (<10 μm) to achieve a very high plate generation velocity [1], the injection volume should be as small as a few picolitres to achieve a very small efficiency loss ($\Theta^2 < 0.05$). The injection of such a small amount of sample in a reproducible way without excessive band broadening is a major challenge in OTC instrumentation design.

Several injection systems, including stopped-flow [5,6], sample tube technique [7], in-column injection [7,8], split injection [9], groove injection [10], heart-cut injection [11], moving injection technique (MIT) [12,13] and pressure pulse-driven stopped-flow injection (PSI) [14] have been used with OTCs. However, only a few of them (split injector, MIT and PSI) can introduce subnanolitre-range sample volumes accurately. It has been shown [15,16] that, for fairly large I.D. (>40 μm) columns, the MIT system shows poor reproducibility (R.S.D. > 24%) when the sample volume injected approaches the subnanolitre range.

In this work, we compared the simple and

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1034-W

cost-effective split injection system with the more sophisticated and expensive PSI system in terms of band broadening and injection volume reproducibility when used with 5 μm I.D. OTCs.

EXPERIMENTAL

Samples and chemicals

The mobile phase [water–methanol (30:70, v/v)] was prepared by mixing water purified with a Milli-Q system (Millipore, Bedford, MA, USA) with HPLC-quality methanol (Scharlau, Barcelona, Spain). The mobile phase was degassed by sparging with helium for 30 s. In all experiments, the sample was phenanthrene (Fluka, Buchs, Switzerland) dissolved in methanol.

Instrumentation

The OTCs utilized were prepared following the method described elsewhere [1] using fused-silica capillary tubing of 5 μm I.D. × 365 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA). In the determination of the injection profile factors, empty open-capillary tubing (without stationary phase) was employed instead of the column.

The chromatographic system was composed of a Model 590 pump (Waters, Milford, MA, USA) and a Waters Model 440 detector. This detector was modified in-house for on-column monitoring, featuring an illuminated volume of *ca.* 6 pl. The split injection system consisted of a VICI CI4W injection valve with an internal 60-nl sample loop (VICI, Valco Europe, Schenkon, Switzerland), a Swagelock SS-1FO-36C T-piece (Crowford Fitting, Solon, OH, USA) and fused-silica capillaries of several sizes (I.D. and length) (Polymicro Technologies) used as restrictors. The PSI system was similar to that described by Claessens *et al.* [16] and was kindly lent by Dr. Van Tilburg (Valco Europe). This injection system consisted of two VICI C6W valves, a VICI C3W valve (all from VICI, Valco Europe) and a digital valve sequence programmer (DVSP) from Valco. Each valve was rotated using a Valco A4C10WT helium actuator, two of which were equipped with Model 125A pilot valves (Humphrey Products, Kalamazoo, MI, USA) for fast valve switching. The actuation sequence of the valves was controlled by the DVSP and six three-way solenoid valves. The gas pressure [4 atm (1 atm = 101 325 Pa)] used to pulse the sample into the column, wash the system and move the actuators was obtained from a C55-quality helium cylinder (Carburos Metalicos, Madrid, Spain).

Procedures

The sample volume injected (V_{inj}) using the PSI system depends on the pressure applied (P_i) during the pulse, the pulse time (t_i), and the characteristics of the column used. It has been calculated that

$$V_{inj} = \frac{\pi}{4} \cdot \frac{d_c^4}{\eta L \phi} \cdot P_i t_i \quad (2)$$

where η is the viscosity of the mobile phase, ϕ is the column flow resistance (32 for an OTC) and the other parameters are as defined above. For a given P_i, the volume injected can be calculated using eqn. 2 if the injection time is known.

The volume injected with the split injector was estimated using data obtained from the PSI injector. A straight line ($r = 0.9996$) for the plot of the peak area *versus* the volume injected was obtained using the PSI injector. Using the same sample, the volume injected from the split injector was deduced by interpolating the peak area obtained in each instance. The area used in these calculations was an average of six injections for each point.

The area under the peak was calculated by multiplying the peak height by the peak width at half-height, which was measured using a magnifying glass. As, the peaks were symmetrical in this work, this was an easy and exact method for area measurement.

Peak efficiency was calculated from the peak width at half-height on the recorder trace obtained at increased paper speed. The peak width at half-height was also used to calculate the peak variance in those instances where the injection profile factor (K) of the injector was measured.

RESULTS AND DISCUSSION

Performance

The efficiency (N) obtained depends on the efficiency of the column itself (N_{col}) and the contribution of the extra-column effects to the band broadening. If it is accepted that here the contribution of the detector to the band broadening is very small ($\Theta^2 < 5\%$), the efficiency obtained is given by the equation

$$\frac{1}{N} = \frac{1}{N_{col}} + \frac{V_{inj}^2}{K} \cdot \frac{1}{V_R} \quad (3)$$

where V_{inj} is the sample volume, K is the injection profile factor and V_R is the retention volume of the sample. According to Eqn. 3, the contribution of the injector to the band broadening can be considered to be negligible when the efficiency obtained does not change with the volume injected. The variation of $1/N$ with the sample volume using the split and PSI systems is shown in Fig. 1. In both instances, as predicted by Eqn. 3, the efficiency decreases with increasing sample volume from a given V_{inj} value. Fig. 1 also shows that to avoid a significant efficiency loss (<5%) using the split injector, a sample volume smaller than 1 pl should be injected into the column, whereas the same efficiency loss is obtained when as much as 10 pl are injected with the PSI injector. As deduced from Eqn. 3, for a given sample retention, the maximum injected volume without considerable efficiency loss depends on the injection profile factor (K) of the injector used.

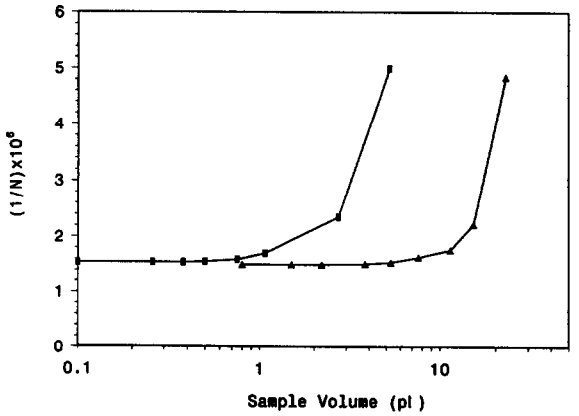

Fig. 1. Semi-logarithmic plot of the inverse of the column efficiency *versus* sample volumes injected for (■) the split injector and (▲) the PSI injector. Conditions, splitting ratio and pulse times correspond to Tables I and II.

We measured the K^2 value for both injection systems studied utilizing an empty capillary tube (100 cm × 5 μm I.D.) instead of the column. The plot of the phenanthrene peak variance *versus* the square of the injection volume gave a good straight line ($r = 0.999$) for a wide range of injection volume (2–35 pl) in both instances. From the slopes of these lines, values of $K^2 = 3.6$ and 7.0 were obtained for the split and the PSI injector, respectively. These values demonstrate that the PSI injector gives a better quality injection (more like ideal plug injection for which $K^2 = 12$) than the split injector. Consequently, larger sample volumes can be injected without any significant efficiency loss using the PSI injector. Data from Claessen *et al.* [16] suggest that the range of injection volumes to give $K^2 > 6$ with the PSI injector decreases with decreasing column I.D. This could justify why, when using 5 μm I.D. columns, sample volumes in the picolitre range should be injected to obtain a similar K^2 value. Further, Claessen *et al.* also observed that K^2 increases with increasing volume injected. This result was not observed in this work, probably because we studied a much smaller sample volume range.

Reproducibility

Another important feature of the injection system is its reproducibility, because of its effect on the precision in quantitative analysis. We compared the two injection systems in terms of sample injection volume and efficiency reproducibility. The results obtained for the split injector and the PSI injector are given in Tables I and II, respectively. Sample reproducibility was deduced from the peak area obtained for the sample peak. The sample injection volume estimated and calculated for the split injector and the PSI injector, respectively, are given in the second column in each table. In both experiments the area irreproducibility was not due to flow variations because the flow stability was better than 0.1%, as was deduced from the R.S.D. for sample retention. It can be concluded

TABLE I

SAMPLE AND EFFICIENCY REPRODUCIBILITY OBTAINED USING THE SPLIT INJECTOR

Open-tubular column, 120 cm × 5 μm I.D.; stationary phase, chemically bonded silica gel C_{18}; mobile phase, water–methanol (30:70, v/v); mobile phase linear velocity, 1.1 mm/s; sample, phenanthrene ($k' = 0.1$) in methanol (1 mg/ml); detection, on-column at 254 nm.

Estimated splitting ratio	V_{inj} [a] (pl)	R.S.D.$_{V_{inj}}$ [b] (%)	Efficiency (plates)	R.S.D.$_N$ [b] (%)
60 000	5.2	8.0	200 000	4.0
120 000	2.7	10.5	424 000	4.5
360 000	0.8	11.6	627 600	3.5
607 000	0.5	15.0	645 600	4.0
1 215 000	0.3	18.0	646 850	3.7

[a] The sample injected was estimated using the area under the phenanthrene peak (see Experimental).
[b] Relative standard deviation ($n = 6$).

from Tables I and II that the injection volume reproducibility decreases with increasing volume injected with both injectors. For the same volume injected (*ca.* 5 pl), the reproducibility of the PSI injector (R.S.D. ≈ 4%) is better than that obtained with the split injector (R.S.D. ≈ 8%). Further, the reproducibility of the PSI injector is roughly three times better than that of the split injector at maximum column efficiency with the highest volumes injected. These results show that the PSI injector is better than the split injector in terms of sample injection volume reproducibility, particularly when the maximum column efficiency has to be achieved with the maximum sample volume. This could be the case in trace analyses of complex samples. In terms of peak efficiency reproducibility, which could be of some interest in theoretical studies for OTCs, both injection systems give similar reproducibility (R.S.D. = 3–4%). This value is fairly good when compared with the reproducibility obtained for injections in packed columns using sample valves (R.S.D. ≈ 2.5%).

TABLE II

SAMPLE AND EFFICIENCY REPRODUCIBILITY OBTAINED USING THE PSI INJECTOR

Conditions as in Table I.

Pulse time (s) (at 4 atm)	V_{inj} [a] (pl)	R.S.D.$_{V_{inj}}$ [b] (%)	Efficiency (plates)	R.S.D.$_N$ [b] (%)
7.03	22.7	1.7	206 400	2.7
3.51	11.4	2.4	566 300	3.1
1.64	5.3	3.8	646 900	3.3
1.17	3.8	4.3	660 600	3.4
0.69	2.2	5.4	663 000	3.2

[a] Sample volume calculated using eqn. 2.
[b] Relative standard deviation ($n = 6$).

CONCLUSIONS

This study has demonstrated that the PSI injector presents a better injection profile factor than the split injector. Subsequently, although maximum efficiency of the 5 μm I.D. columns can be achieved using both injectors, larger injection volumes (ten times) can be injected with the PSI injector without a substantial efficiency loss. The sample injection volume reproducibility obtained using the PSI injector is twice as good as that obtained with the split injector using 5 μm I.D. columns. This PSI injector feature is important with regard to quantitative analysis using OTCs. Finally, in terms of cost effectiveness, the PSI injector is about five times more expensive than the less sophisticated split injector.

ACKNOWLEDGEMENTS

This work was supported by CICYT (grant No. PB91-0077-C03-02). Thanks are due to M. van Tilburg (VICI, Valco Europe) for the loan of the PSI injector and to M. Campos for technical assistance.

REFERENCES

1 A.L. Crego, J.C. Díez-Masa and M.V. Dabrio, *Anal. Chem.*, 65 (1993) 1615.
2 C.A. Moning, D.M. Dohmeier and J.W. Jorgenson, *Anal. Chem.*, 63 (1991) 807.
3 M. de Frutos, S.K. Paliwal and F. Regnier, *Anal. Chem.*, 65 (1993) 2159.
4 M. Martin, C. Eon and G. Guiochon, *J. Chromatogr.*, 108 (1975) 229.

5 Y. Hirata and M. Novotny, *J. Chromatogr.*, 186 (1979) 521.
6 D. Ishii and T. Takeuchi, *J. Chromatogr. Sci.*, 18 (1980) 462.
7 M.J. Capacci and M. Sepaniak, *J. Liq. Chromatogr.*, 9 (1986) 3365.
8 T. Tsuda, K. Tsuboi and K. Nakagawa, *J. Chromatogr.*, 214 (1981) 283.
9 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
10 V. Berry and K. Lawson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 121.
11 V.L. McGuffin and M. Novotny, *Anal. Chem.*, 55 (1983) 580.
12 M. Harvey and S. Stearns, *J. Chromatogr. Sci.*, 21 (1983) 83.
13 M. Harvey and S. Stearns, *Anal. Chem.*, 56 (1984) 837.
14 A. Manz and W. Simon, *J. Chromatogr.*, 387 (1987) 187.
15 V. Berry and K. Lawson, *J. Liq. Chromatogr.*, 10 (1987) 3257.
16 H.A. Claessens, A. Burcinona and C.A. Cramers, *J. Microcol. Sep.*, 2 (1990) 132.