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Short communication

Modification of a conventional high-performance liquid chromatography autoinjector for use with capillary liquid chromatography

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Abstract

This paper will discuss the conversion of a conventional HPLC autoinjector to a format which is suitable for use with capillary HPLC. In addition to the actual modification procedure, the paper will also present data which characterize the performance of the modified injector. These data include items such as precision (approximately 1%), optimal valve loop loading volume (approximately 35–40 μl), and absence of sample carryover between injections.

1. Introduction

Capillary HPLC has several characteristics which make its use attractive in a variety of applications. These attractive features include significantly reduced solvent consumption, enhanced sensitivity when dealing with limited sample volume [1,2], and easy direct interfacing with a mass spectrometer [3–5]. However for any technique to realize its full potential, it must be capable of unattended automated operation. At the present time few autoinjectors are commercially available for use specifically with capillary HPLC, thus slowing its acceptance as a viable and practical technique. Alternatively, individuals performing an initial evaluation of the use of capillary HPLC as related to their specific application may not be willing or able to justify the expense of purchasing a dedicated capillary system autoinjector. This paper will describe the conversion of a conventional HPLC autoinjector to a configuration which is compatible with capillary HPLC, as well as the characterization of the modified autoinjector's performance based upon various experimental parameters.

2. Experimental

2.1. Equipment and supplies

The conventional HPLC autoinjector modified in this procedure was a Model 506 unit (Beckman Instruments, Palo Alto, CA, USA). The microinjector valve (with 0.5- or 1-μl loop volume) was a Model 7526 pneumatically activated unit (Rheodyne, Cotati, CA, USA) connected to a Model 7163 12 V double three-way solenoid valve (Rheodyne). The solenoid valves were activated by the chromatographic system hardware with contact closures directed through a

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Model 201A 12 V d.c. solenoid interface (Autochrom, Milford, MA, USA) operated in the step-input mode. Manual injections were performed with a Model 7520 valve with a 0.5-μl loop volume (Rheodyne). The capillary HPLC columns (150 × 0.32 mm 3-μm C_{18} and 300 × 0.32 mm 5-μm C_{18}) and guard cartridges were purchased from LC Packings (San Francisco, CA, USA). Ultraviolet detection was at 254 nm and was performed using a Model 783 detector (Applied Biosystems, Ramsey, NJ, USA) fitted with a capillary flow cell (LC Packings). The mobile phase was delivered to the system by a L6200A/L6000 binary solvent-delivery system (Hitachi Instruments, Danbury, CT, USA) through an AccuRate flow splitter (LC Packings). For all studies, the mobile phase was delivered to the column at a flow-rate of 4 μl/min. The mobile phase consisted of water–CH_3CN (35:65) for precision and peak height studies and water–CH_3CN (50:50) for column efficiency studies. The mobile phase used for comparison of isocratic and gradient elution consisted of water and CH_3CN (80% CH_3CN for isocratic elution and a linear ramp of 80–90% CH_3CN over a 10-min period for the gradient elution mode). The test probe for peak height and precision studies consisted of acetophenone in water at a concentration of 10 μg/ml with an injection volume of 1 μl. Column efficiency studies were conducted using a commercially available reversed-phase test mixture (Supelco, Bellefonte, PA, USA) diluted 1:1 with water and using a 0.5-μl injection sample loop. The comparison of isocratic and gradient elution modes was performed using a commercially available reversed-phase test mixture in methanol (Hitachi) containing (in elution order) naphthalene (80 μg/ml), anthracene (2.5 μg/ml) and chrysene (3 μg/ml), diluted 1:4 with water and using a 1-μl injection sample loop. Polyether ether ketone (PEEK) tubing and Fingertight fittings were supplied by Upchurch Scientific (Oak Harbor, WA, USA). Chromatographic data were collected and processed using Access Chrom software (PE/Nelson, Cupertino, CA, USA).

2.2. Autoinjector modification

The actual modification of the standard autoinjector is a relatively straightforward procedure. The standard injection valve is disconnected from the system. The microvalve itself is attached directly to the top of the autoinjector housing. The liquid flow from the pumps is directed into the microvalve by a short length (15–20 cm) of 50 μm I.D. fused-silica capillary tubing between the flow splitter and liquid inlet port on the microvalve. The tubing from the autoinjector syringe is disconnected from the standard valve and connected instead to the waste port of the microvalve. Connection of the microvalve sample inlet (needle) port to the autoinjector needle tower assembly requires removal of the needle port tube assembly from the valve. In its place is inserted a short (approximately 10 cm) length of 0.010 in. I.D. × 1/16 in. O.D. PEEK tubing (1 in. = 2.54 cm), with a Rheodyne ferrule pre-swaged onto the tubing. The PEEK tubing is retained in the microvalve sample inlet port by means of the reduced-diameter compression screw/spring assembly previously used to hold the needle port tube assembly in position. The remaining end of the PEEK tubing is connected to the autoinjector needle tower assembly using a PEEK 1/4·28 Fingertight fitting.

Operation of the system is controlled primarily by contact closure signals from the autoinjector. The valve is initially configured in the "LOAD" position. Upon completion of the sample loading into the sample loop, the autoinjector provides two simultaneous contact closure signals to initiate the chromatographic run. One signal goes to the 12 V solenoid interface which in turn activates the solenoid valves for rotation of the microvalve from the "LOAD" position to the "INJECT" position, thus introducing sample onto the head of the column. The second contact closure signal is directed to the data system to initiate acquisition of chromatographic data. Due to the presence of only two contact closure terminals on the autoinjector, the remaining signals must be generated by the data system and

solvent-delivery system. Immediately upon initiation of data acquisition the data system provides a contact closure signal to start the solvent delivery system timer cycle. At the end of the chromatographic analysis (if the assay used isocratic elution) or column re-equilibration period (if the assay used gradient elution) the solvent delivery system transmits a contact closure signal to the 12 V solenoid interface to return the microvalve to the "LOAD" position and wait for initiation of the next injection.

3. Results and discussion

Initial attempts to use the autoinjector in its standard configuration proved to be unsuccessful. Upon injection of 1 μl of the acetophenone test probe, extreme peak tailing was observed, presumably due to unacceptably large dead volume within the standard valve. Even use of a small volume (5 μl) sample loop did not significantly improve the peak shape to an acceptable degree. An example of the peak obtained with the conventional valve is shown in Fig. 1. The column performance was independently evaluated using the same conditions, but using a manual microvalve, and found not to be the source of the extremely poor peak shape. Thus it became apparent that modification of the autoinjector would be necessary in order to obtain satisfactory performance.

As described above, a simple exchange of the conventional injection valve with the microvalve was not performed. Although such an approach would have been easier it was not possible due to the different angles of travel for the two valves (45° for the microvalve and 60° for the standard valve). However as assembled the modified system retains the same flow path design as in the original system. During sample loop loading, the valve is in the "LOAD" position and the needle is inserted into the sample vial. The syringe withdraws the specified volume from the vial, pulling the sample through the needle, PEEK transfer line, and sample loop. Thus the injector may be described as operating in a "PULL" mode for sample loading. Upon injection (i.e. rotation of the microvalve to the "INJECT" position) the microvalve, PEEK transfer line, and needle are automatically rinsed by the autoinjector wash solvent, just as in the standard autoinjector configuration, thus decreasing or eliminating sample carryover between injections.

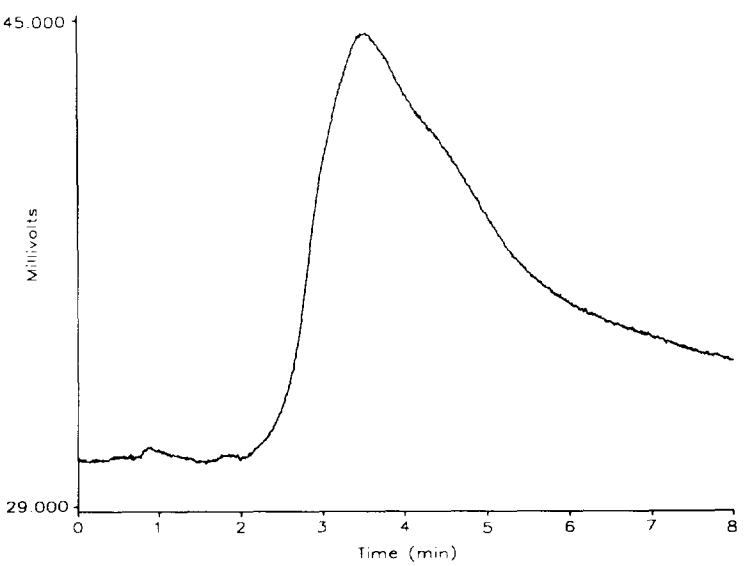

Fig. 1. Chromatographic peak obtained for the acetophenone test probe using the conventional valve on the autoinjector. Mobile phase: water–CH_3CN (35:65) at 4 μl/min. Column: 150 × 0.32 mm 3-μm C_{18} with guard.

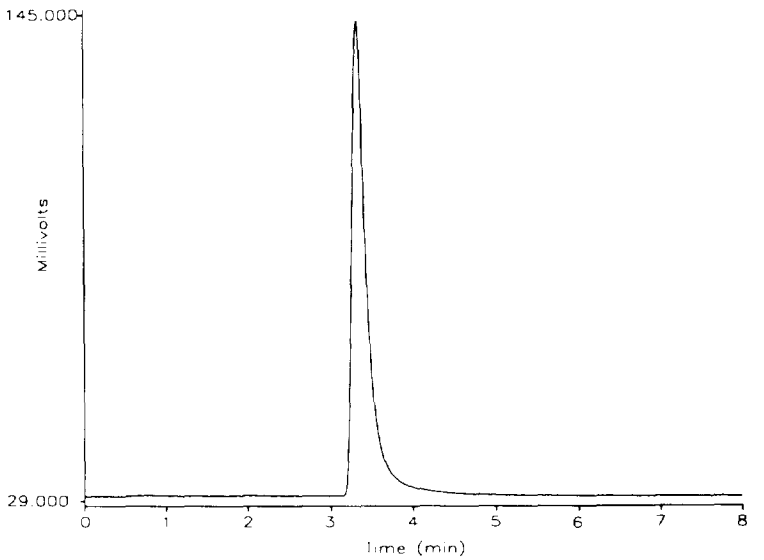

Fig. 2. Chromatographic peak obtained for the acetophenone test probe using the modified autoinjector with the microvalve. Mobile phase: water–CH_3CN (35:65) at 4 μl/min. Column: 150 × 0.32 mm 3-μm C_{18} with guard.

As previously stated, the valve is not returned from the "INJECT" position to the "LOAD" position until just prior to the initiation of a sample injection sequence. This was done specifically to accommodate gradient elution operation. This timing sequence enables use of gradient elution without trapping strong elution solvents in the sample loop which could interfere with the separation of the following sample. Since this event occurs at the end of any column re-equilibration period, use of the HPLC pump system to provide the required contact closure as a timed event is the easiest approach to ensure the valve switch occurs at the very end of any re-equilibration step.

Although only the two initial contact closure signals to initiate sample injection and data acquisition are from the autoinjector, such an arrangement is dictated by the fact that there are only two contact closure switches on the output terminal of the autoinjector used in this study. If more contact closure outputs were available on the autoinjector, it could provide all signals required for operation of the system. Following the above modifications, the acetophenone test probe was again injected onto the system, but

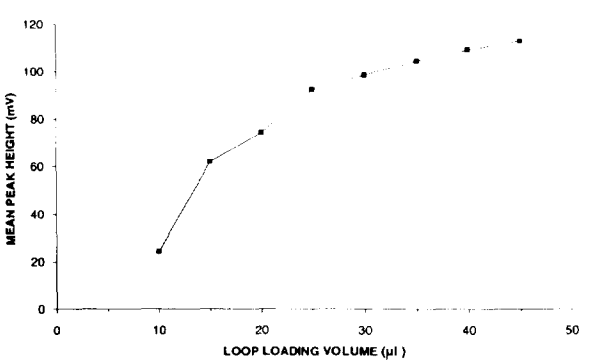

Fig. 3. Average peak height as a function of loop loading volume.

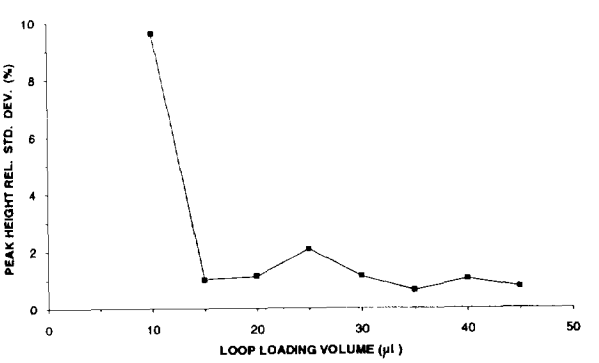

Fig. 4. Peak height relative standard deviation (%) as a function of loop loading volume.

using the modified autoinjector. The peak obtained for a 1-μl injection of the acetophenone probe is shown in Fig. 2. Upon comparison with the results obtained with the standard valve (Fig. 1), the improvement in performance is easily noted.

In order to characterize the performance of the modified autoinjector, a variety of experiments were performed. The first study was designed to determine the optimal loop loading volume (i.e. sample volume withdrawn by the syringe) to fill a 1-μl loop. The study involved making 10 injections at each of a series of loop loading volumes ranging from 10 to 45 μl and monitoring the peak heights obtained. The test analyte used in this study was acetophenone in water (10 μg/ml). The mean peak heights obtained at each loop loading volume are plotted in Fig. 3. As seen in the plot, the response increases dramatically up to loop loading volumes

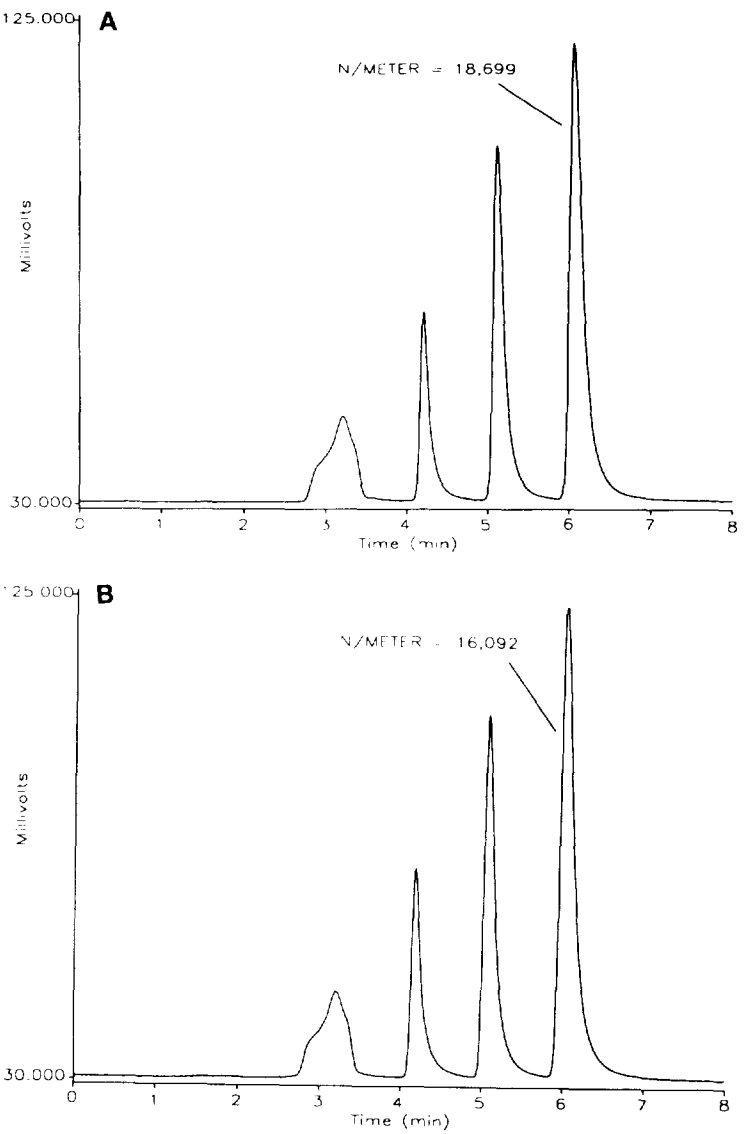

Fig. 5. Chromatographic system efficiency obtained using (A) modified autoinjector and (B) manual microvalve. Mobile phase: water–CH_3CN (50:50) at 4 μl/min. Column: 150 × 0.32 mm 3-μm C_{18} with guard.

of 30–35 μl, with only modest gains beyond that. With a measured needle and PEEK transfer line dead volume of 13 μl (i.e. dead volume between sample vial and sample loop), these data indicate that an approximately 3-fold flush volume of sample is sufficient to give acceptable on-column sample loading.

Not only is sample loading an issue, but so is reproducibility. Fig. 4 presents a plot of the relative standard deviation (R.S.D.) of the peak heights obtained in the loop loading study above. As illustrated in the figure, the variability drops to a fairly constant level in the range of 0.5 to 1% at loop loading volumes of 15 μl or greater. These data in conjunction with those from the loop loading volume study indicate that a loop loading volume of 35–40 μl should provide acceptable performance in terms of adequate sample loading and reproducibility.

Another important issue to consider is whether use of the modified autoinjector will have any adverse effect on column efficiency. Fig. 5 presents two chromatograms of a commercially available reversed-phase test mixture injected using the modified autoinjector and also by a manually operated microvalve. Although the chromatographic conditions used for the separation are not necessarily optimal for the capillary column used, the efficiency data generated indicate that use of the modified autoinjector results in system efficiency equivalent to that obtained with a manual micro-injection system.

The issue of sample carryover was also examined. As stated earlier, the modifications described permit the normal wash cycle of the standard autoinjector to be employed in the modified version. Fig. 6 shows the chromatogram of a blank injection of mobile phase following an injection of the reversed-phase test mixture. As is readily apparent, there is no evidence of any significant sample carryover in the chromatogram. Thus the modified autoinjector exhibits behavior comparable to the normal configuration.

Finally, the use of the modified autoinjector in gradient separations was examined. Fig. 7 shows a comparison of the separation of a three-component test mixture using both isocratic and gradient elution modes following a 1-μl injection by the modified autoinjector. As can be seen in the figure, the modified autoinjector performs well in either mode, thus allowing the automated capillary system to be operated with the same

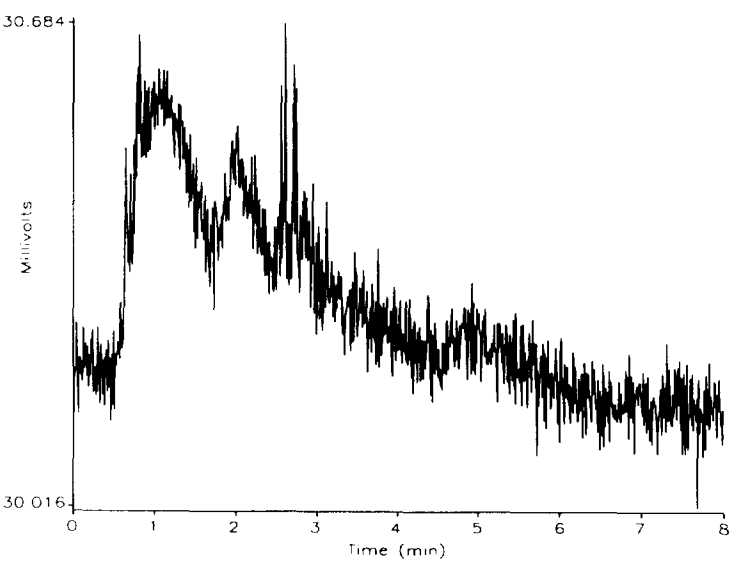

Fig. 6. Blank mobile phase injection following injection of reversed-phase test mixture. No significant sample carryover is observed. Mobile phase: water–CH_3CN (35:65) at 4 μl/min. Column: 150 × 0.32 mm 3-μm C_{18} with guard.

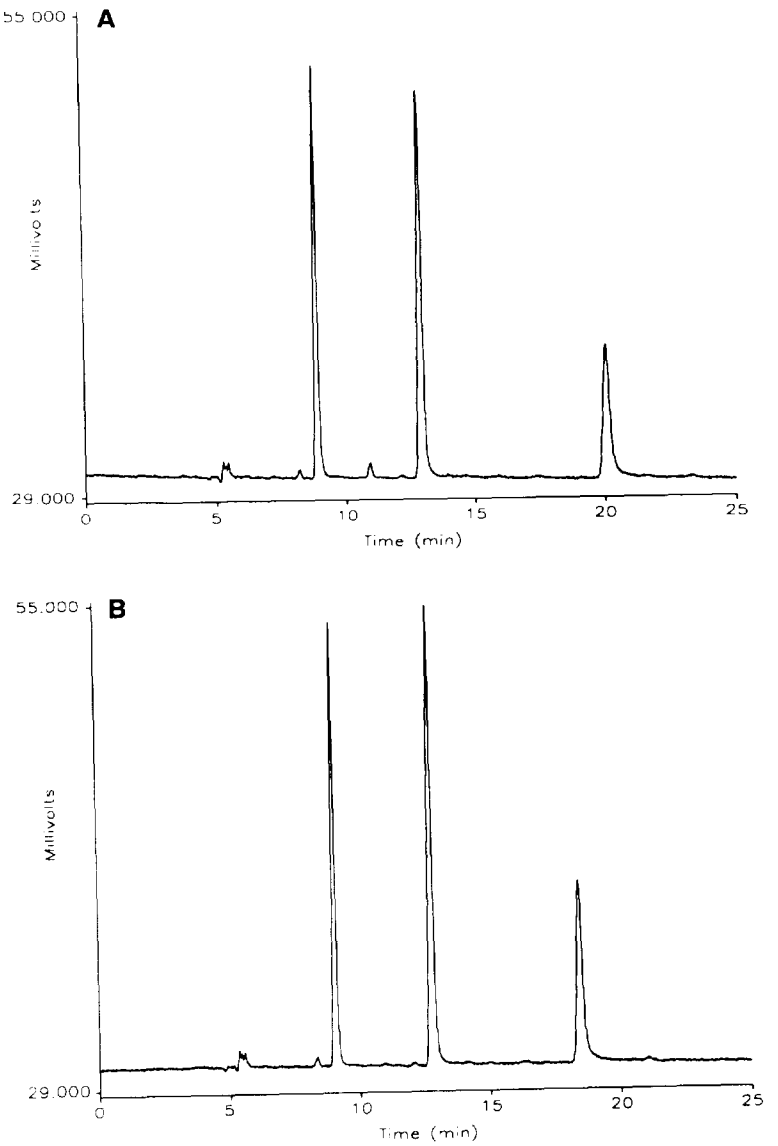

Fig. 7. Comparison of (A) isocratic and (B) gradient separations of a three-component test mixture following injection with the modified autoinjector. Mobile phase solvents: A = water, B = CH_3CN; flow-rate: 4 μl/min. Column: 300 × 0.32 mm 5-μm C_{18} with guard. Isocratic conditions: A–B (20:80). Gradient conditions: 80–90% B linearly over 10 min.

degree of flexibility as can be expected when using an automated conventional HPLC system.

4. Conclusions

This paper has described a straightforward procedure for the modification of a conventional autoinjector to a format acceptable for use with capillary HPLC. Various experimental parameters were investigated to characterize and demonstrate the suitability of the modified system for use in capillary HPLC applications. Although the modifications were applied to a specific model of autoinjector in the current paper, the basic approach should also be generically applic-

able to autoinjectors supplied by different vendors.

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