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AUTOMATIC DEVICE FOR INJECTION AND MULTIPLE COLUMN SWITCHING IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A fully automated high-performance liquid chromatographic loop sampler is described with capability for multidimensional column switching. The apparatus consists of a modified Hewlett-Packard gas chromatographic liquid sampler with a programmable laboratory data system or with a programmable event timer which allows precise switching of the high-pressure pneumatically operated valves. Besides the multidimensional chromatographic separation, this device offers possibilities of on-column concentration, for different kinds of cutting fractions (front, heart and end cut) and for backflushing.

INTRODUCTION

In column switching the effluent from a primary column is transferred by means of valves for finite periods of time of a chromatographic run to one or a number of secondary columns[1]. By introducing new solvent systems or new stationary phases in the secondary columns, multiple dimensions are introduced into the chromatographic separation system[2]. The differences in column selectivity achievable by multidimensional chromatography result in a significant improvement in resolution and in a reduction of interfering components relative to the analyte[3,4]. This gradual enrichment of the analyte relative to the interfering matrix constituents in a multistage process generally does not necessitate extensive clean-up of samples of biological origin in trace analysis[5,6].

To determine low concentrations of analyte, *e.g.*, in residue and environmental analysis, or to overcome low sensitivities of detectors, the column must be loaded with sufficient sample. On-column concentration techniques[7–9] reduce band broadening effects by combinations of stationary phases and mobile phases in such a way that the retention times of the analytes are extremely long during the injection of large sample volumes or during the transfer time onto a secondary column. Column life is increased by bypassing the analytical columns when the bulk of interferences is eluted from a previous separation column, or by backflushing the column to flush out the remainder of the sample concentrated on the top of the column[10,11].

An important criterion for on-line column switching is exact and reproducible

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timing of switching valves. This is particularly important as the analytes are identified from their retention times. The use of column switching, on-column concentration and backflushing in a fully integrated system allows the full advantages of liquid chromatography to be realized in the analysis of trace compounds. Many examples of on-line coupled columns have been published, ranging from a simple two-column straightforward system to a real multidimensional column network for pesticide residue and environmental analysis[1,6,8–17], polymer separation[15,19–21] and the analysis of biological plasmas[5,22].

This paper describes the modification of a commercial gas–liquid chromatographic sampler for multidimensional column switching with on-column concentration and backflushing of the precolumn and the first of two analytical columns.

Instrumentation

Flow switching in high-performance liquid chromatography (HPLC) is achieved by use of pneumatically actuated switching valves controlled by sequence timers or time programmable event switches of the laboratory data system. Erni and Bosshard[23] describe the adaptation of the Hewlett-Packard (HP 7671 or 7672) liquid sampler for HPLC loop sampling. Together with the Hewlett-Packard laboratory data system HP 3354, a fully automated liquid chromatographic system is realized with random access to the samples and control of the chromatographic results by the data system. Most of the pneumatic functions used to handle the syringe for injection into the gas chromatograph are not necessarily needed for loop injection in liquid chromatography. The system described herein uses the now functionless solenoid valves to actuate the pneumatic switching valves of the multidimensional chromatographic system.

Fig. 1 shows the column network and switching valves used. Large sample volumes, up to 1.5 cm^3 given by the sample vials used, are concentrated on a short precolumn or cartridge. Pump 1, a low pressure type (10^6 Pa), rinses this concentration column with a solvent of low elution power. The analyte is retained on the column, whereas part of the impurities is eluted through the waste exit of switching valve 1. After the washing period, the flow of pump 2 is directed for a finite period of time by means of switching valve 1 onto the precolumn. At the same time the eluate flow from the precolumn is connected to the first analytical column. The higher elution power of the eluent delivered by pump 2 elutes the analyte from the concentration column (precolumn) and resharpens the analyte zone on the top of the first analytical column. Pump 2 is a high-pressure, constant-flow pump as during the transfer periods the backpressure to be overcome by pump 2 changes. After this transfer to the first analytical column, the switching valve 2 is returned to the initial position. The analyte is separated in the first analytical column (first dimension).

The section of interest in the resulting chromatogram is transferred via switching valve 2 onto the second analytical column using the mobile phase of pump 2. After the transfer, valve 2 returns to its initial position and the mobile phase of pump 3 resharpens and separates the analyte zone (second dimension). The flow of the precolumn and the first column is reversed by switching the backflush valve and valve 1 after the second transfer period. The mobile phase of pump 2 rinses both columns in the reverse direction. Slowly moving compounds are now backflushed from the top of the columns. After a preset time the switching valve 1 returns to the normal flow

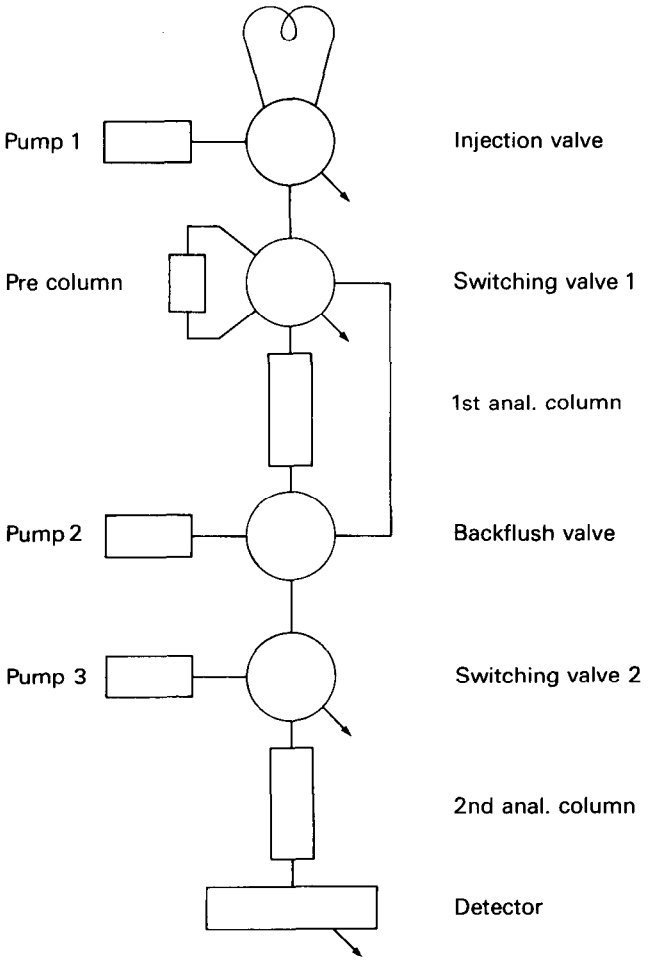

Fig. 1. Schematic diagram of the coupled column system.

direction. Reconditioning of the precolumn by the mobile phase of pump 1 then starts. The first analytical column needs no reconditioning and therefore the flow returns to its normal direction only just at the start of the next injection cycle. The analyte bands are resharpened by the choice of the stationary phases and mobile phases in the order of increasing elution power from the precolumn to the second analytical column. Seven time sequences are required to move the analyte through this column network to the detector. Fig. 2 shows the transferline tubing connections between columns and switching valves.

Materials

All six-port valves are pneumatically actuated models, Rheodyne Type 7126 automatic sample injector (Rheodyne, Berkeley, CA, U.S.A.) The injection valve was used without modification. The rotor seals of the switching valves 1 and 2 and the

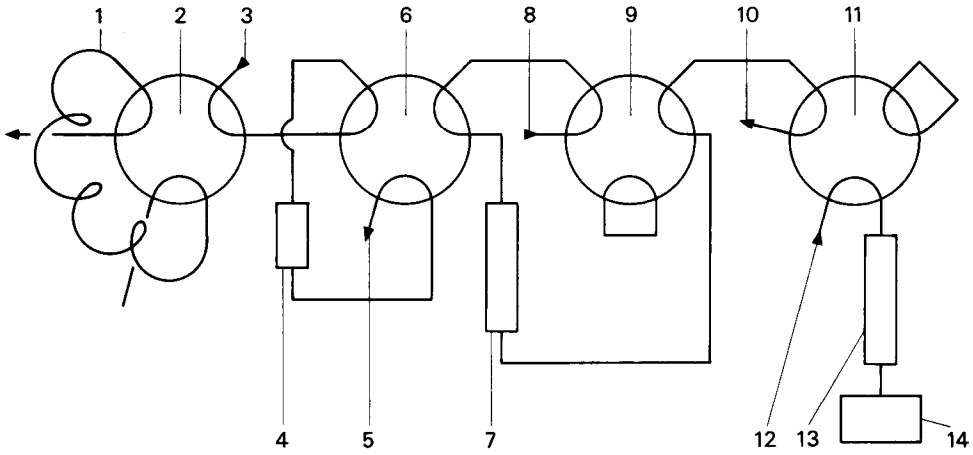

Fig. 2. Column and connection tubes. All valves are in the load position. 1 = Sample loop; 2 = injection valve; 3 = connection from pump 1; 4 = precolumn; 5 = waste exit from pump 1; 6 = switching valve 1; 7 = first analytical column; 8 = connection from pump 2; 9 = backflush valve; 10 = waste exit from pump 2; 11 = switching valve 2; 12 = connection from pump 3; 13 = second analytical column; 14 = detector.

backflush valve were replaced by rotor seals of the injection valves Model 7010 (rotor seal No. 7010-039). A magnetic micro gear pump was used to fill the sample loop (Micropump Series Paragon P-11-361-500, 0–24 V d.c., flow range 0–6 cm^3/min; Micropump Co., Concord, CA, U.S.A.). Pumps 1 and 3 are reciprocating membrane pumps (Model DMP-SK 15-15/3, Orlita, Giessen, G.F.R.). Pump 2 is a constant-flow pump (Model AX-110; Altex, Berkeley, CA, U.S.A.). The spooling valve (Kuhnke 44-220) and pneumatically actuated timer (Kuhnke 87.017) (Fig. 3) were from Kuhnke (Malente, G.F.R.).

One additional three-way solenoid valve, R7, and the spooling valve, S, are installed to perform all switching functions with one electronic control module. Fig. 3 shows the pneumatic connections of the switching valves and solenoid valves.

The laboratory data system[24] controls the liquid sampler via an electronic control module (ECM), a general purpose interface. This control module provides a binary input of the bottle number and seven a.c. power outputs. These outputs may be switched independently by the system software (chromatographic methods) or by postanalytical BASIC programs initiated by the chromatographic methods. The methods contain all parameters for peak integration and also the time events to control external devices by the ECM during the chromatographic run.

Programmable sequence timers may also be used to control the switching time and sample transport without restriction of the chromatographic behaviour of the system.

Each solenoid valve may be electrically connected in parallel with a 110 VAC relay to control external devices or functions. Output 1 of the electronic control module (ECM) controls the start/stop function of the analog to digital converter and the injection marker.

Output 2 of the ECM starts the injection cycle with solenoid R2 and the relay connected in parallel switches the power supply of the micro gear pump.

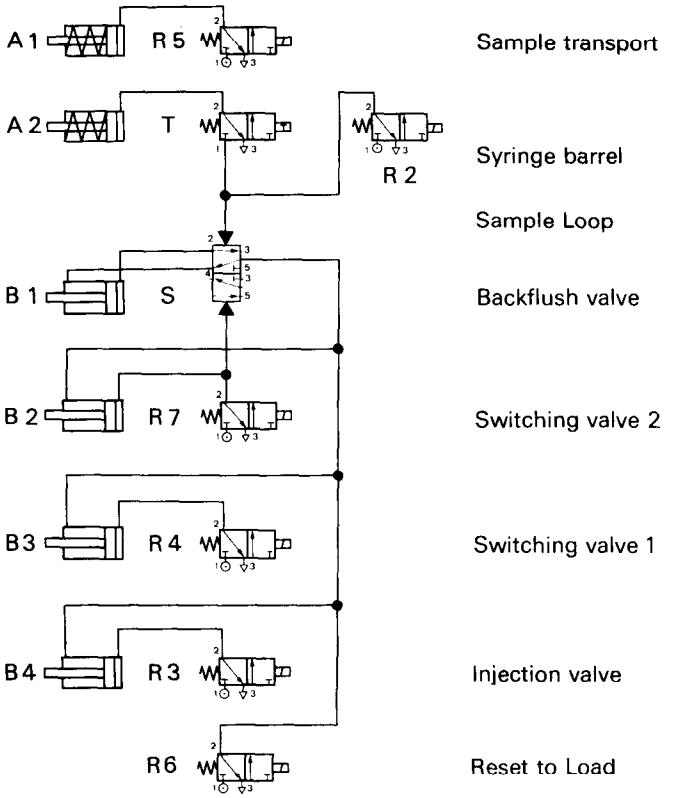

Fig. 3. Pneumatic flow connections. A1, A2 = Cylinders with spring return; B1–B4 = double acting cylinders; R2–R7 = solenoid valves (= relay no. of the data system); T = pneumatic timing element; S = spooling valve (pneumatically actuated with memory mode); ⊙ 1 = pressure inlet; 2, 4 = consumer; 3, 5 = vent exits.

Time sequences

Table I shows the valve positions during the chromatographic run.

First period. The sample loop is filled by a micro gear pump for a preselected time. The loop is flushed with an air segment (1–20 sec) before the needle penetrates the septum of the sample vial.

Second period. The sample is injected and concentrated on the precolumn. The precolumn is rinsed.

Third period. The analyte elutes from the precolumn and is transferred onto the first analytical column (end cut mode[11]).

Fourth period. Separation occurs in the first dimension (first analytical column).

Fifth period. The analyte zone is transferred and concentrated onto the second analytical column (heart cut mode[11]).

Sixth period. The analyte elutes into the second dimension (second analytical column). The first analytical column and the precolumn are backflushed with the mobile phase of pump 2.

TABLE I

VALVE POSITIONS AND TIME PERIODS

I = Inject position; L = load position.

Valve	*Time period*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Injection valve	L	I	I	L	L	L	L
Switching valve 1	L	L	I	L	L	I	L
Switching valve 2	L	L	L	L	L	I	I
Backflush valve	L	L	L	L	I	L	L

Seventh period. The precolumn is reconditioned with the mobile phase of pump 1.

Fig. 4 shows the positions of the switching valves during the corresponding time periods and the actuating periods of the solenoid valves.

CONCLUSIONS

This modified liquid sampler with column switching valves is of a highly flexible design. It is applicable to backflush mode and many cutting techniques, and is fully automated. It ensures good reliability of operation and reproducibility of retention time, peak height or peak area sufficient for quantitative work in residue analysis. The only strict condition to be observed is that preconcentration must be effective.

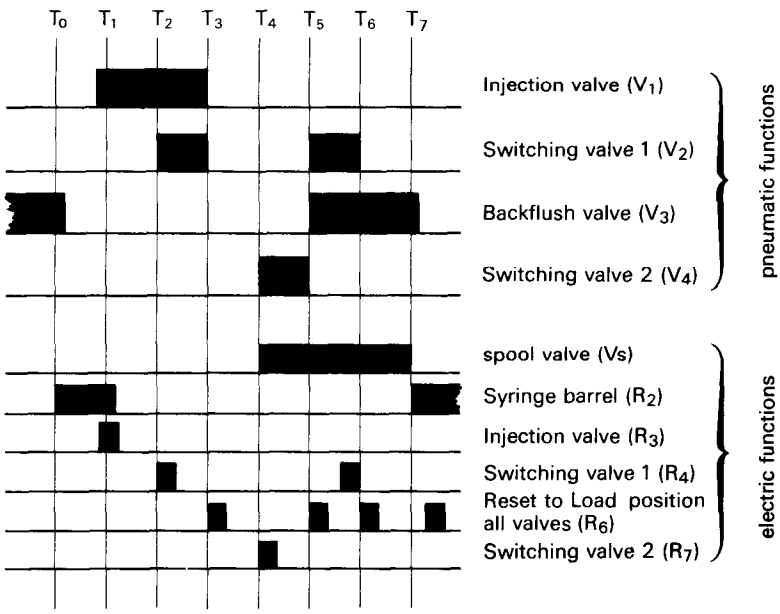

Fig. 4. Time sequences of the pneumatic valves and electric relay functions. —, Valve in the load position or relay or solenoid valve off; ■, valve in the inject position or relay or solenoid valve on.

TABLE II

PRECISION OF PEAK HEIGHTS AND PEAK AREAS AFTER A THREE-COLUMN SWITCHING CYCLE

Sample (ng)	*N*	*Standard deviation (%)*	
		Peak height	*Peak area*
225	10	4.7	6.6
225	7	3.1	3.9
450	8	2.6	3.5
450	8	2.2	1.6
450*	12	3.9	3.2

* Aliquot of 2 g soil injected and fortified with 450 ng sample.

This sharpens the peaks and increases the independency from the previous chromatographic dimension. Coupling with the laboratory data system also permits efficient handling of the chromatographic data[26]. Basic programs allow easy and fast modifications of switching cycles.

The range of application is not restricted to the column network presented in this paper. The freely programmable time and valve sequences allow a wide variety of column networks and switching cycles. Straightforward two-column systems[21,22,25,27] may easily be realized, as may size exclusion chromatography[15] with a trapping step[6,14,15] and subsequent two-dimensional liquid chromatographic separation. Phase exchange[18] from aqueous mobile phases to organic phase and reverse with an intermediate purge-and-dry sequence may also be realized with the described system.

Post-column reaction may also be carried out between the first and second analytical columns and introduces a new chemical dimension. During the transfer (fifth) period only the derivatization reagent is mixed with the column effluent and transferred to the second column. For all other time sequences the derivatization reagents are directed to waste, therefore the dosage may be stopped to save reagents without disturbing the chromatographic separation and detection. Routine application of this column switching technique showed no column deterioration after analysis of several hundred biological samples, indicating an effective backflush of the precolumn and the first analytical column.

This fully automated system reaches a level of precision sufficient for trace analysis. Table II shows the precision obtained on injection of 0.5-ml samples using a 3-cm precolumn and 12.5-cm first and 25-cm second analytical columns (all 4.6 mm I.D.). Reproducibility of retention time is excellent, with per cent relative standard deviations of less than 0.12% ($n = 17$).

The substitution of the six-port valves by ten-port multifunctional valves[28] increases the possibilities of the chromatographic system without hardware modification.

Electric wiring diagrams and column connections for other switching modes are available from the authors.

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