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# SINGLE-VALVE, SINGLE-PUMP, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN-SWITCHING ANALYSIS OF HYDROLYZED WOOD COMPONENTS

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## SUMMARY

A novel column-switching system using a single pump and a single ten-port valve has been developed for the analysis of both water soluble and lipophilic organics in reaction products of wood hydrolysis. Specifically, furfural and hydroxymethyl furfural are determined in addition to the water soluble sugars and aliphatic acids using a Bio-Rad HPX-87H series resin column. The unique switching arrangement reduces analysis time by a factor of three as compared with unswitched analysis on the same column, without sacrificing the necessary chromatographic resolution. The technique is generally applicable to resin-based high-performance liquid chromatographic columns which separate by a combination of size exclusion and ion moderated partition effects, particularly when highly retentive lipophilic components are responsible for long run times. The geometry of the ten-port valve switch minimizes pressure shock, ensuring long column lifetimes.

## INTRODUCTION

In recent years, the use of resin-based high-performance liquid chromatographic (HPLC) columns has increased significantly, particularly in biological or natural product type analysis. Many resin-based columns which operate in the ion moderated partition (IMP)–size exclusion (SEC) mode are very effective for water soluble organics, but are strongly retentive for more lipophilic organics; this phenomenon leads to very long retention times for aqueous eluant IMP-based columns which, in many cases, renders a column unusable due to excessive run times. Although these problems can be moderated by the addition of a co-solvent such as acetonitrile to the aqueous eluant, such additives can compromise the overall separation.

Hydrolyzed wood samples are extremely complicated mixtures of sugars, phenolics, organic acids, furfurals, and many other compound classes. Analysis of the sugar fractions is complicated by the complex array of additional compound classes present. Sugars have been analyzed on bonded-phase columns containing amino or propyl amino groups[1,2]. The presence of aldehydes or ketones in the sugar samples can significantly change the surface of the amino-bonded phases by Schiff base for-

mation. Several IMP–SEC-based resin columns are effective for the analysis of wood sugar solutions[3]. These resin-based columns have demonstrated greater stability in use than bonded-phase amino columns when used for the analysis of carbohydrates[1]. Hydrolyzed wood sugar solutions and polyols (polyols are obtained upon reduction of sugars) have been successfully analyzed in this laboratory on Bio-Rad HPX-87H and HPX-87P IMP–SEC-based resin columns, but analysis on either column suffered from long run times (60 min/sample) due to the presence of strongly retained hydroxymethyl furfural (HMF) and furfural in the sugar solutions. The goal of the present study was to develop a simple, efficient, isocratic HPLC system capable of separating and quantitating the major components of hydrolyzed and reduced wood sugar solutions.

Few column-switching systems designed to reduce long analysis time have been reported in the literature for resin-based HPLC columns[4]. The reason for such a lack of papers no doubt stems from the sensitivity these columns exhibit toward pressure shocks. Pressure shocks associated with valve switching can result in column bed compaction with resultant resolution loss; column lifetime can be significantly reduced. Two-column switching systems, a conventional single-pump, two-valve system and a new single-pump, single-valve system are described in the present paper. The effectiveness of each system for the separation of wood sugars has been evaluated.

## EXPERIMENTAL

All standards were obtained from commercially available suppliers. Quantitation of components was obtained by either peak heights or peak areas using the external standard technique. Stainless-steel (316 ss, 0.010 in. I.D.) tubing was used for all sample lines downstream from the injector. All samples were passed through a 0.45-$\mu$m filter prior to injection.

### *Two-valve, single-pump column switching*

The system shown in Fig. 1 consisted of a Waters M-45 pump with external pulse dampener, a Rheodyne 7125 injector with 20-$\mu$l sample loop, a Rheodyne 7040 four-way switching valve, a Jones Chromatography column oven maintained at 55°C, one 300 × 7.8 mm I.D. Bio-Rad HPX-87H Organic Acid Analysis Column, two 40 × 4.6 mm I.D. Bio-Rad Aminex HPX-85H Micro-Guard Columns with holders, an Altex Model 156 refractive index detector, 0.005 *M* sulfuric acid in Milli-Q purified deionized water (eluent, 0.6 ml/min), and a Spectra-Physics SP-4100 integrator. The injector was actuated automatically by the SP-4100, while the switching valve was actuated manually.

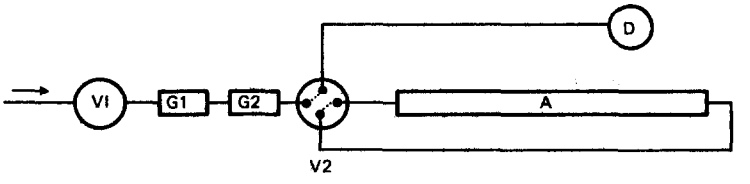


Fig. 1. Two-valve, single-pump column switching. V1 = Injector; G1, G2 = guard columns; V2 = four-way switching valve; A = analytical column; D = detector.

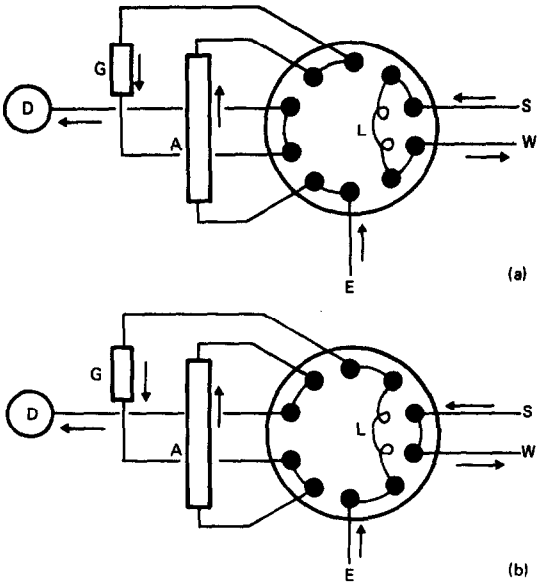


Fig. 2. Single-pump, single-valve, Boxcar-type column switching. (a) Load and bypass mode; (b) inject mode. A = Analytical column; G = two guard columns in series; D = detector; L = sample loop; S = sample in; W = to waste; E = eluent in.

*Single-valve, single-pump column switching*

The system shown in Fig. 2 consisted of a Waters M-45 HPLC pump with external pulse dampener, a Valco AC10W (air actuated) ten-port valve (body of Nitronic 60, ferrules and gland nuts of 316 ss) fitted with 20-$\mu$l sample loop, fillport assembly and pneumatic actuator. The chromatographic columns, column heater, integrator and eluent were described earlier. Either the Altex Model 156 or LDC Model 1109 refractive index detector was used. Sample injection and column switching were totally automated by the SP-4100 integrator.

## RESULTS AND DISCUSSION

A chromatogram of a synthetic mixture of major, water soluble components found in acid hydolyzed wood and several polyols is shown in Fig. 3. Bio-Rad's HPX-87H Organic Acid Column with 2 Aminex $H^+$ cation guard columns (in series) was used to achieve the separation. Note that the sugars, including oligosaccharides, elute within 11 min and acetic acid within 15 min. HMF and furfural, however, require 37 min and 55 min, respectively, to elute. To reduce analysis time for the sugars on the HPX-87H column while maintaining information on the late eluting peaks, a column-switching system was developed. Due to the large void volume associated with the HPX-87H column, totally non-retained peaks elute after 6 min. In order to take full advantage of this lengthy void volume time, late eluting peaks were switched to the void volume portion of the chromatogram. Two separate column-switching systems were investigated.

*Two-valve, single-pump column switching*

Initial column switching experiments with the hydrolyzed wood sugar solution

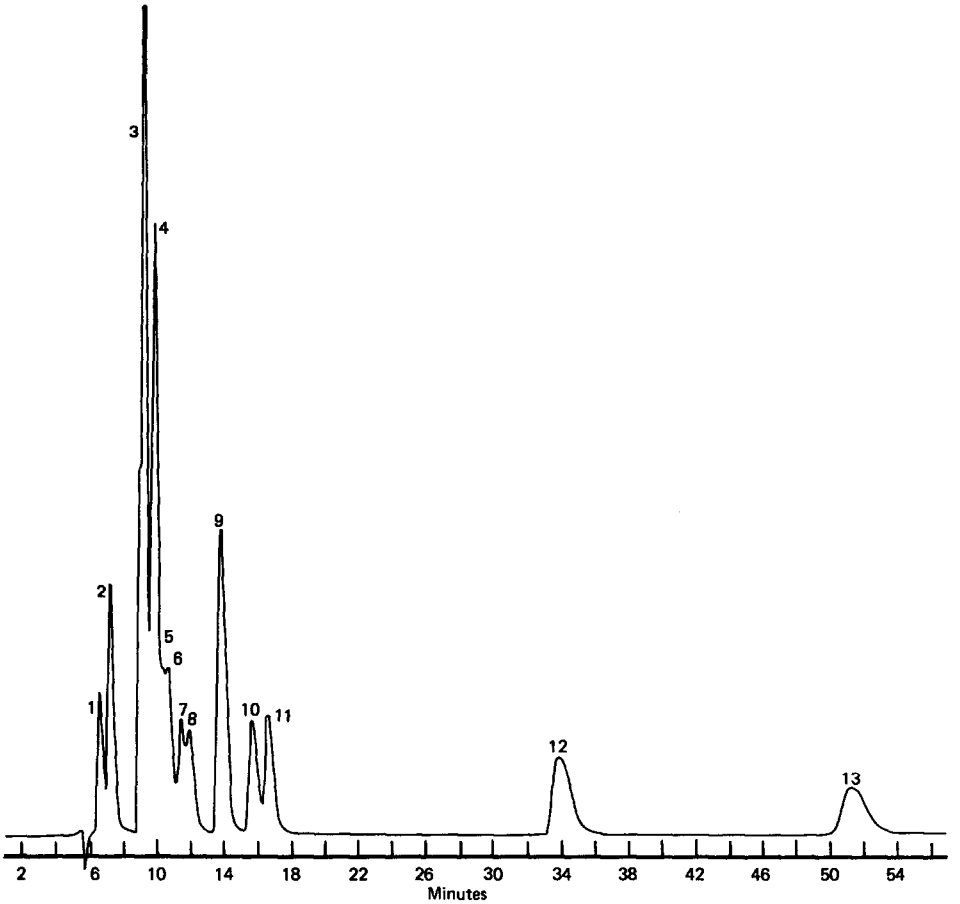


Fig. 3. Unswitched separation of a synthetic sugar standard with ten-port valve. Peaks: 1 = maltotriose; 2 = cellobiose; 3 = glucose; 4 = xylose (co-elutes with galactose and mannose); 5 = sorbitol; 6 = arabinose; 7 = xylitol; 8 = erythritol; 9 = glycerol; 10 = ethylene glycol; 11 = acetic acid; 12 = HMF; 13 = furfural.

were accomplished with the system shown in Fig. 1. The Rheodyne four-way valve was used for the actual column switching. This column–valve configuration is similar to a system described by Snyder and Kirkland[5]. All early eluting peaks, including acetic acid, were allowed to pass through the guard columns and enter the analytical column; the late eluters, furfural and HMF, were still retained on the guard columns. The four-way switching valve was actuated, diverting the late eluters directly to the detector, thus bypassing the analytical column. Upon elution of the furfural and HMF, the switching valve was actuated again restoring flow to the analytical column; the early eluters were separated on the analytical column and subsequently detected. Note that during the bypass mode, eluant in the analytical column is stagnant. A chromatogram of a synthetic mixture of sugars, polyols, furfural and HMF obtained from the above column switching system is shown in Fig. 4. Note that the HMF peak is quite broad and is not baseline resolved from furfural. The above switching

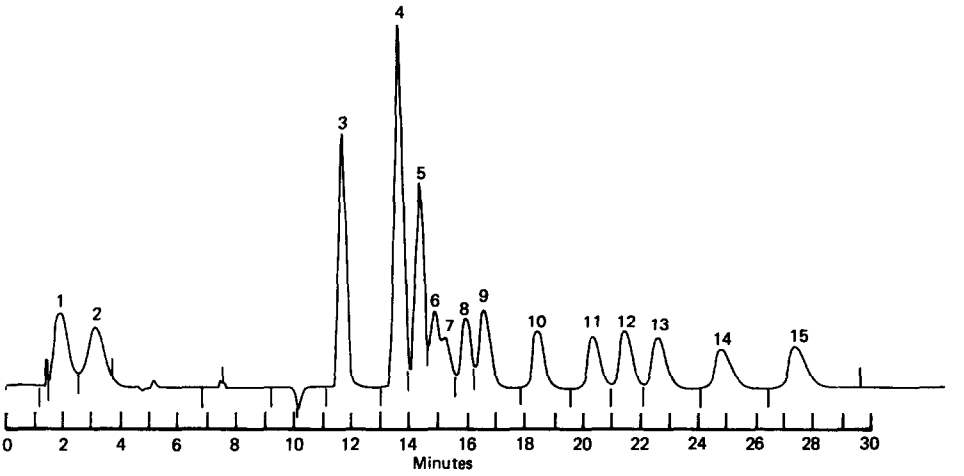


Fig. 4. Separation of a synthetic sugar standard with two-valve, single-pump column switching. Peaks: 1 = HMF; 2 = furfural; 3 = cellobiose; 4 = glucose; 5 = xylose; 6 = sorbitol; 7 = arabinose; 8 = xylitol; 9 = erythritol; 10 = glycerol; 11 = acetic acid; 12 = ethylene glycol; 13 = propylene glycol; 14 = 1,3-butanediol; 15 = 1,4-butanediol.

configuration suffers significantly from a dramatic pressure change during switching to the bypass mode (Fig. 5a). Total system pressure (guard columns plus the analytical column) is about 1000 p.s.i.g. In the bypass mode, system pressure is about 200 p.s.i.g. and represents an 800 p.s.i.g. pressure differential. During switching to the bypass mode, the guard columns are subjected to an 800 p.s.i. pressure shock

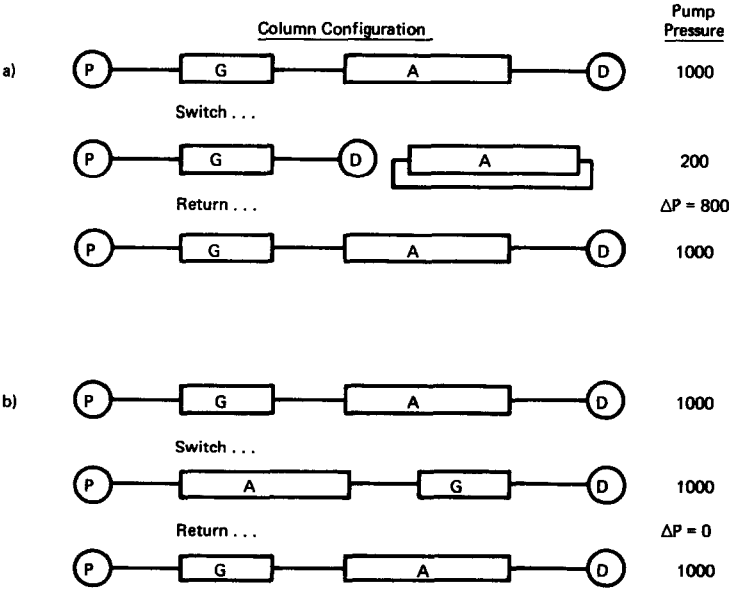


Fig. 5. A schematic comparison of system pressure changes during six-port four-way and ten-port valve column switching. (a) Two-valve, single-pump column switching; (b) single-valve, single-pump column switching. P = Pump; G = guard columns; A = analytical column; D = detector.

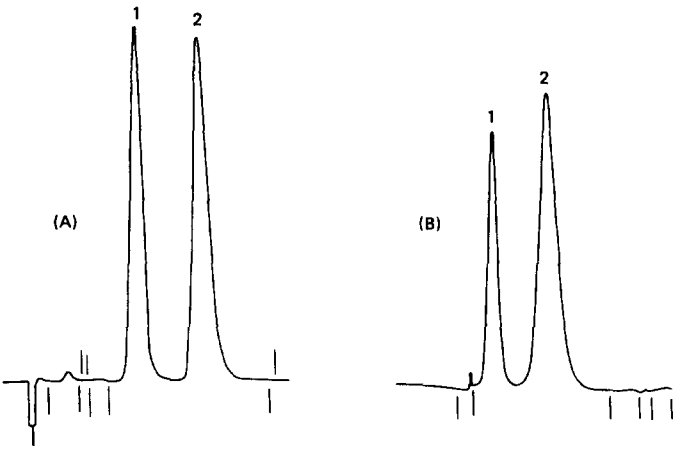


Fig. 6. Effect of pressure shock on the separation of HMF and furfural on two guard columns in series. (A) Separation on two guard columns at 200 p.s.i.g. pump pressure; (B) separation by two guard columns during single-pump, two-valve column switching (800 p.s.i.g. pressure shock). 1 = HMF; 2 = furfural.

which seriously degrades the efficiency of guard columns for separation of HMF and furfural. Analysis of a standard solution of HMF and furfural on two guard columns in series at 200 p.s.i. system pressure with no switching resulted in the chromatogram shown in Fig. 6A. Baseline resolution of the two components is achieved. Effective plate count calculations on the furfural peak yield 550 effective plates ($N$). When the same sample is analyzed with switching to bypass the analytical column, the chromatogram in Fig. 6B is obtained. The 800 p.s.i.g. differential pressure shock to the guard columns results in a reduction in effective plates from 550 to 200. In addition, the retention times for furfural and HMF are reduced by 24% and 29%, respectively. Clearly, the column-switching system shown in Fig. 1 causes a loss of guard column efficiency and will be detrimental to the resin column lifetime due to pressure shocks. Reliable quantitative data for furfural and HMF will be difficult to obtain with this switching system. An alternate switching system was investigated.

*Single-pump, single-valve column switching*

In an effort to reduce pressure shock to the columns, a valve was sought which would effectively perform the switching operation described schematically by Fig. 5b. The concept is similar to "Boxcar" column switching described by previous workers[6]. The Valco C10W ten-port valve, when configured as in Fig. 2, effected the desired switching operation. Note that the total system pressure is always 1000 p.s.i.g. Numerous additional HPLC column switching examples utilizing the ten-port valve are described in ref. 7. As an added bonus, the valve doubles as the injection valve, thereby reducing the overall cost and complexity of the system. A chromatogram of a synthetic mixture of sugars and polyols using the ten-port system is shown in Fig. 7; HMF and furfural are baseline resolved. The pressure shock resolution problem found with the two-valve switching system has been overcome with the single-pump, ten-port valve system. In the column switching mode, baseline resolution is maintained and the overall analysis time has been reduced by a factor of three. Switching

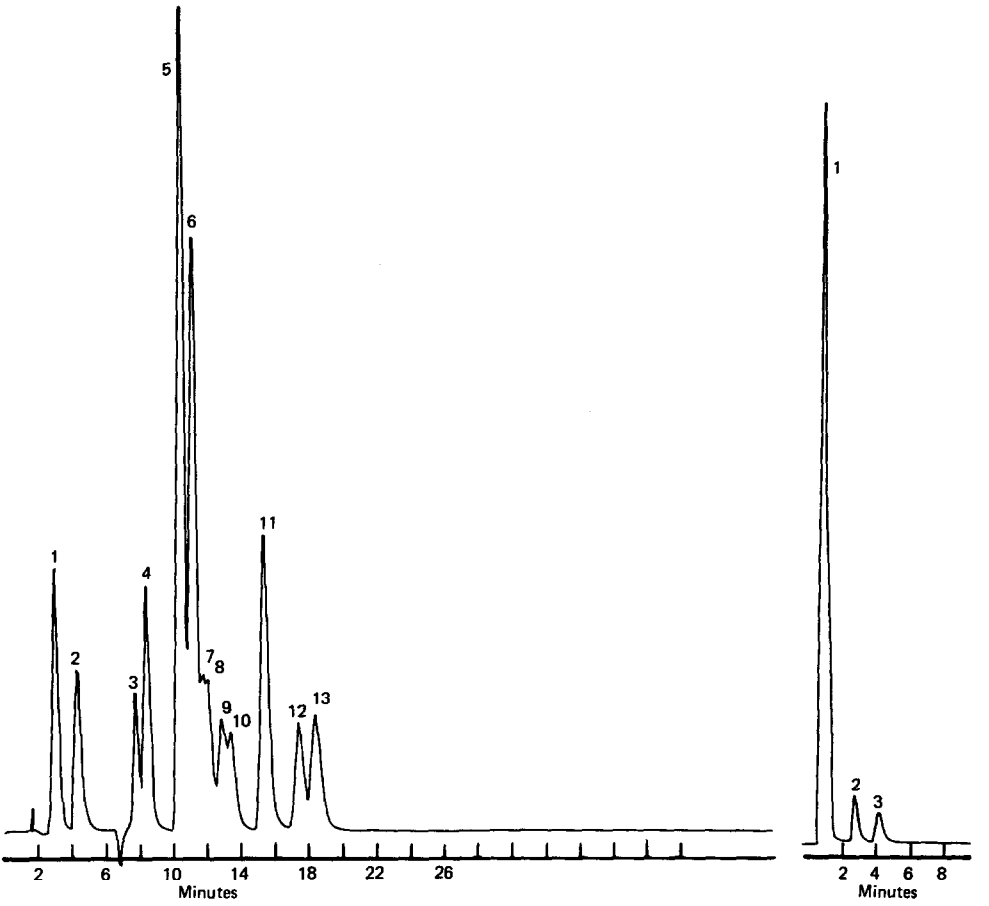


Fig. 7. Separation of a synthetic sugar standard with single-pump, single-valve column switching. Switching time to bypass mode is 1.6 min. Peaks: 1 = HMF; 2 = furfural; 3 = maltotriose; 4 = cellobiose; 5 = glucose; 6 = xylose; 7 = sorbitol; 8 = arabinose; 9 = xylitol; 10 = erythritol; 11 = glycerol; 12 = ethylene glycol; 13 = acetic acid.

Fig. 8. Separation of a synthetic sugar standard on guard columns only with single-pump, single-valve column switching. Switching time to bypass mode is 0.33 min. Peaks: 1 = mono-, di- and trisaccharides and acetic acid; 2 = HMF; 3 = furfural.

time is very critical to obtain accurate quantitative results. When the same synthetic mixture above is chromatographed on two guard columns only, the chromatogram shown in Fig. 8 is obtained. All the sugars and acetic acid co-elute, while HMF and furfural are separated and are baseline resolved from each other. Since the combined guard columns generate only 550 effective plates, the switching times must be chosen such that all the early eluters (retention time less than the start of the HMF peak) have a chance to enter the analytical column and the HMF/furfural peaks still reside in the guard columns. To better illustrate the sensitivity to switching times, a sugar sample containing 1,4-butanediol (1,4-BD), 2,5-tetrahydrofurfuryl alcohol (2,5-THFA) and furfural was analyzed in the unswitched mode and at various switching

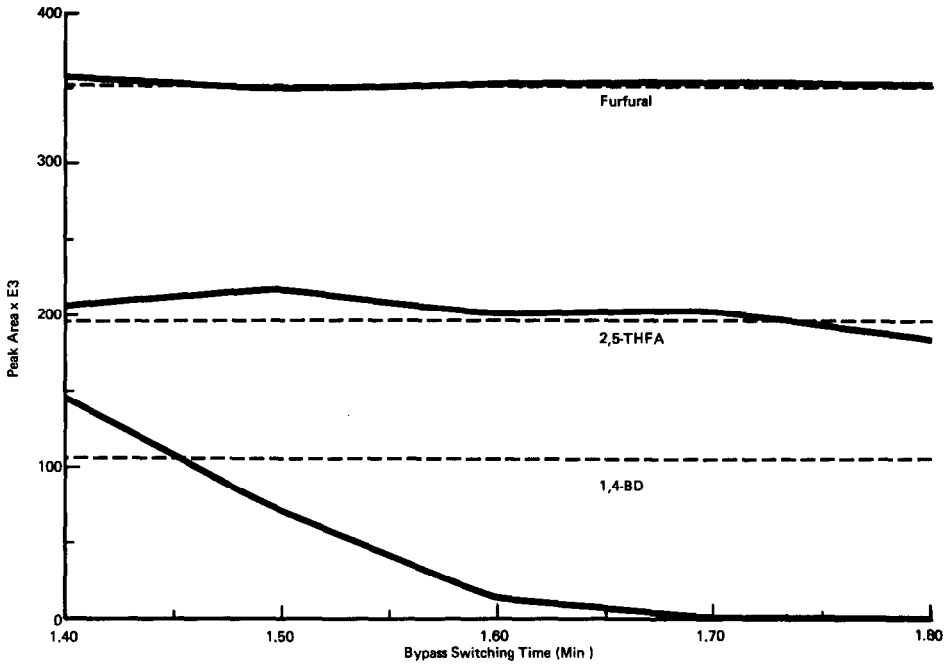


Fig. 9. Raw peak area *vs.* switching time for single-pump, single-valve column switching: (----) unswitched raw areas; (——) 1,4-BD, 2,5-THFA and furfural switched peak, raw areas. Unswitched retention times for 1,4-BD, 2,5-THFA and furfural are 25, 30, and 53 min, respectively.

TABLE I

RECOVERY DATA FOR SYNTHETIC SUGAR MIXTURE USING THE TEN-PORT VALVE COLUMN SWITCH

| *Sample No.* | | *Cellobiose* | *Glucose* | *Xylose* | *Arabinose* | *Acetic acid* | *HMF* | *Furfural* |
|---|---|---|---|---|---|---|---|---|
| 1 | Actual (wt.%) | 0.0098 | 0.611 | 0.289 | 0.198 | 0.122 | 0.048 | 0.051 |
| | Found (wt.%) | 0.0090 | 0.591 | 0.280 | 0.211 | 0.114 | 0.048 | 0.051 |
| | Recovery (%) | 91.8 | 96.7 | 96.9 | 106.6 | 93.4 | 100.0 | 100.0 |
| 2 | Actual (wt.%) | 0.025 | 0.971 | 0.485 | 0.029 | 0.206 | 0.039 | 0.104 |
| | Found (wt.%) | 0.025 | 0.947 | 0.479 | 0.030 | 0.199 | 0.039 | 0.105 |
| | Recovery (%) | 100.0 | 97.5 | 98.8 | 103.5 | 96.6 | 100.0 | 101.0 |
| 3 | Actual (wt.%) | 0.307 | 1.389 | 0.674 | 0.387 | 0.382 | 0.097 | 0.249 |
| | Found (wt.%) | 0.290 | 1.375 | 0.661 | 0.340 | 0.365 | 0.093 | 0.250 |
| | Recovery (%) | 94.5 | 99.0 | 98.1 | 87.9 | 95.6 | 95.9 | 102.0 |
| 4 | Actual (wt.%) | 0.107 | 1.562 | 1.074 | 0.132 | 0.422 | 0.078 | 0.243 |
| | Found (wt.%) | 0.102 | 1.544 | 1.046 | 0.123 | 0.415 | 0.077 | 0.247 |
| | Recovery (%) | 95.33 | 98.9 | 97.4 | 93.2 | 98.3 | 98.7 | 101.7 |
| 5 | Actual (wt.%) | 0.060 | 0.300 | 0.199 | | 0.356 | 0.069 | 0.154 |
| | Found (wt.%) | 0.057 | 0.295 | 0.193 | | 0.343 | 0.065 | 0.153 |
| | Recovery (%) | 95.0 | 98.3 | 97.0 | | 96.4 | 94.2 | 99.4 |
| | $\bar{X}$ | 95.3 | 98.1 | 97.6 | 97.6 | 96.0 | 97.8 | 100.8 |

times in the switched mode. Unswitched retention times of the above components were 25, 30 and 53 min, respectively.

A plot of the areas for each component in the unswitched mode *vs.* the switched mode at various switching times is shown in Fig. 9. These areas are measured for the switched peaks at the front end of the chromatogram. The bypass switching time was varied from 1.4–1.8 min; furfural and 2,5-THFA showed little sensitivity to switching time while 1,4-BD was profoundly affected. Optimum bypass switching time is about 1.7 min for the above sample. Furfural and 2,5-THFA should be quantitated after separation on the guard columns only while 1,4-BD and all the other earlier eluters should be chromatographed on both the guard and analytical columns. Note that the earlier eluters actually pass through the guard columns a second time prior to detection without adversely affecting total analysis time or resolution.

A recovery study was conducted with a synthetic series of sugar solutions; bypass switching time was 1.60 min. The results are shown in Table I. Recoveries greater than 95% can be expected.

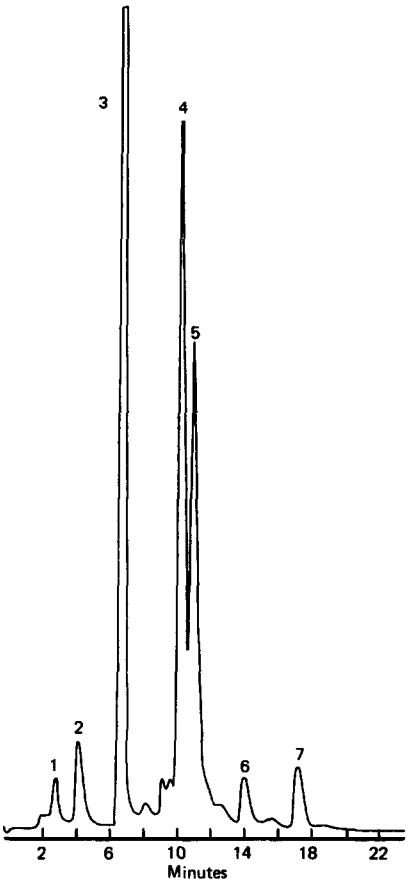


Fig. 10. Separation of wood sugar solution (acid hydrolysate) with single-pump, single-valve column switching. Switching time to bypass is 1.5 min. Peaks: 1 = HMF; 2 = furfural; 3 = sulphuric acid; 4 = glucose; 5 = xylose, galactose, mannose; 6 = unidentified, possibly formic acid; 7 = acetic acid.

A precision study was also conducted and yielded a relative precision ($2s$) of 5.1, 4.8, 5.2, 5.2, 5.3, 5.1, 5.6 and 5.3%, respectively, for HMF (0.40 wt.%), furfural (0.61 wt.%), maltotriose (0.46 wt.%), cellobiose (0.49 wt.%), glucose (1.37 wt.%), arabinose (0.18 wt.%), xylose (0.45 wt.%), and acetic acid (0.74 wt.%). All recovery and precision data were obtained with complete automation of injection and bypass switching time.

A typical chromatogram of an actual wood sugar solution in the switched mode is shown in Fig. 10. Column switching significantly sharpens the otherwise broad, diffuse HMF and furfural peaks while significantly reducing the analysis time. There is one caveat, however. If the baseline around the HMF peak has significant low-level impurities, column switching may enhance the peak area of the HMF since these impurities will likely no longer separate from HMF on the low effective plate count guard columns. Quantitation of the sugars and furfural, however, was excellent.

## CONCLUSIONS

Two-column switching systems designed to reduce the analysis time of hydrolyzed wood solutions on the Bio-Rad HPX-87H resin column have been evaluated. The two-valve, single-pump switching system did not adequately resolve HMF and furfural due to significant pressure shocks during switching; the large pressure changes during switching were deleterious to column longevity. Although analysis time was reduced, the accuracy of the data generated for the late eluting peaks was not good.

The single-pump, single ten-port switching valve system allows reduction of the overall analysis by a factor of three. Quantitation of both the late and early eluters is very good; better detection is obtained for late eluting peaks. The addition of a multi-wavelength UV detector to the system would aid significantly in identification of organic acids and other UV absorbers found in hydrolyzed wood. In addition, the switching operation subjects the resin columns to minimal pressure changes, ensuring good column longevity. The ten-port valve acts as both the sample injection and switching valve, thereby reducing the complexity and cost of the system. Although the ten-port valve system was designed mainly for switching, normal unswitched analyses can still be run without any mechanical modifications.

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