Simultaneous Multicolumn Operation of the UV-Analyzer for Body Fluids

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At the current state of development, high-resolution liquid chromatographs for analyses of complex physiologic fluids require relatively long analysis times and consequently the rate at which analyses can be done is limited. This rate can be increased by decreasing the analysis time or by use of multiple columns to process several samples in unison. An automated multicolumn high-resolution chromatograph for separating ultraviolet-absorbing molecular constituents of body fluids is being developed. The sample-handling capacity was increased several-fold with minimum added cost by using many of the components of the single-column analyzer. Results from routine use of a UV-Analyzer with two columns in parallel show that parallel operation is not only feasible but highly advantageous. The design of a high-pressure, multiple-column, sample injection valve for simultaneous injection of samples on different columns is discussed.

It has been suggested that future clinical laboratories should be able routinely to analyze hundreds, or even thousands, of physiological samples for hundreds of constituents (1). At least two approaches are being investigated to meet this requirement: (a) the development of automated, high-speed, multiple analyzers capable of analyzing a large number of samples for a single constituent or, at most, only a few constituents (2); and (b) the development of automated, high-resolution analytical systems capable of analyzing a single sample for hundreds of constituents (3–10). At present, neither of these two types of systems, used separately, will meet the above requirement: the first is incapable of analyzing for hundreds of constituents, and the second cannot be used for large numbers of samples. Obviously, integration of the two systems, combining high resolution with high sample handling capacity, is desirable. This paper describes a first attempt to combine the approaches, with a high-resolution analyzer as the starting point. A later paper will describe a similar attempt, in which the high-speed analyzer is the starting point.

Development of high-resolution systems for analyzing physiological fluids has been under way for more than 10 years (11–14). One such system, the UV-Analyzer (14), which is used to separate and quantify the ultraviolet-absorbing constituents of body fluids, has been developed during the past four years. A model of this Analyzer (the Mark II-A), although not suitable for general use in clinical laboratories, has been in routine operation in various medical research facilities for as long as three years (7–9). To increase sample handling capacity, significant development effort has been aimed at decreasing the analysis time from 60 to 40 h, and then finally to 20 h. However, in the light of present technology, the prospects of decreasing analysis time to less than 7 to 10 h without significant loss of resolution appear to be rather poor for the near future.

Another approach to the problem of increasing sample handling capacity is the use of parallel systems (15). Pursuing this approach by simply using duplicate high-resolution analyzers would be prohibitively expensive; on the other hand, it is possible to build multicolumn liquid chromatographs with which several samples can be analyzed in parallel without the complete duplication of equipment (16). We have extended this technique to high-pressure, ion-exchange chromatography with gradient elution, attempting to minimize duplication of equipment.

Experimental System

The high-resolution UV-Analyzer has been previously described in detail (17). It consists primarily of a heated, high-pressure anion-exchange column; a sample injection valve; an acetate concentration-gradient generating and pumping sys-

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tem; a two-wavelength, dual-beam uv photometer; and a strip-chart recorder. The high-pressure anion-exchange column is a 150-cm length of type 316 seamless stainless-steel tubing (0.22 to 0.54 cm i.d.) packed with strongly basic anion-exchange resin ("Aminex A-27"; Bio-Rad Laboratories, Richmond, Calif. 94804). The column is jacketed to provide heating to uniform temperature. A 0.05- to 2.5-ml sample (depending on the inside diameter of the ion-exchange column and the nature of the sample) is applied to the column by a 6-port injection valve (18), mounted as near to the top of the column as possible to minimize peak broadening. The chromatograms of ultraviolet-absorbing constituents of body fluids are developed by eluting the constituents with an ammonium acetate-acetic acid buffer solution (pH 4.4), the acetate concentration of which is gradually increased from 0.015 to 6.0 mol/liter. The eluent is pumped through the ion-exchange column at the rate of about 250 ml cm⁻² h⁻¹ with a pressure drop of 10.1 × 10⁴ to 20.2 × 10⁴ N/m²(100 to 200 atm). The absorbances of the column effluent at 254 and 280 nm, referenced (at the same wavelengths) to the stream entering the column, are monitored by a two-wavelength dual-beam flow photometer (19) and recorded on a strip chart.

At one period during the construction of Mark III-A prototypes (20), two 150-cm-long folded columns (one 0.15 cm i.d. and one 0.22 cm i.d.), two sample injection valves, and two dual-beam flow photometers were on hand but not immediately needed. This situation provided an excellent opportunity for us to perform some preliminary tests on the concept of parallel-column operation of the UV-Analyzer. Thus the two columns, sample injection valves, and photometers were placed in parallel with an existing Mark III-A UV-Analyzer, which had a 0.22 × 150 cm folded column. Several standards and urine samples were analyzed simultaneously on the three columns, using a single-gradient generation system and pump, and the results were recorded on three strip-chart recorders. The resulting chromatogram triplets showed that parallel operation of the UV-Analyzer was not only feasible but highly advantageous. They also showed that columns of different cross-sectional areas would produce equivalent chromatograms if the sample volumes were proportional to the cross-sectional area of the chromatographic columns.

In the light of these encouraging results, we decided that a dual-column UV-Analyzer should be designed and built for proof of principle and routine use. The final design (shown schematically in Figure 1) was based on the Mark II-A UV-Analyzer design, but incorporated two recent improvements and a dual-column sample injection valve (Figure 2). The valve, which essentially consists of two ganged 6-port sample-injection valves, enables simultaneous injection of two different samples onto two columns. The same design principle could be used to construct multicolumn injection valves. The improvements were: (a) replacement of the constant-temperature circulator with a more reliable thermistor-controlled, resistance heater wound around the outside of the column jacket (Figure 3); and (b) replacement of the metal two-chamber gradient generator with a redesigned clear-plastic two-chamber device that produces a more gentle transition into the gradient.

To convert the existing Mark II Analyzer into a dual-column system, we replaced the single 0.62 × 150 cm ion-exchange column in the Mark II unit initially with two 0.45 × 150 cm columns, then later with two 0.30 × 150 cm columns. Each of the smaller pairs of columns contained one-fourth the volume of the single large column; thus the amount of resin and eluent required for the two columns...
are equal to one-half of the amounts needed for the single column in the Mark II prototype. The effluent from each column was monitored with a two-wavelength, dual-beam photometer, and the absorbances at 254 and 280 nm of each were recorded on a single strip chart.

The operating procedure for the dual-column UV-analyzer is identical with that used for the Mark II UV-Analyzer except that, in the dual-column system, two samples are loaded into the dual-column sample injection valve and are simultaneously injected, one onto each chromatographic column.

Results

The dual-column analyzer yields chromatograms that are almost superimposable when identical samples are injected onto each column (Figure 4); however, the patterns show striking dissimilarities when different samples are injected. The dual-column analyzer is particularly useful for comparing samples of body fluids taken before and after therapy. Figure 5, which shows the first portions of chromatograms obtained from urine excreted by an individual before and after ingestion of 3 g of nicotinamide, dramatically illustrates the effects of drug ingestion on the excretion of metabolites of that particular drug. Figure 6, which shows chromatograms of the urine excreted by an individual suffering from cancer of the colon, provides another example of the comparison of results obtained before and after therapy. In this case, the therapy was upper body irradiation. It is obvious that the amounts of hypoxanthine, xanthine, vanillloylglycine, and p-hydroxyhippuric acid are increased and that the amounts of creatinine, 7-methylxanthine, 3-methylxanthine, quinaldine acid, and o-hydroxyhippuric acid are decreased.

Discussion

To date, the results obtained from routine use of the dual-column UV-Analyzer have demonstrated the usefulness of the multicolumn mode of operation. In addition to the higher sample capacity, the capability for comparing samples simultaneously in different columns is particularly useful.
neously is a significant improvement over single-column operation. With relatively minor modification, the existing Mark II UV-Analyzers could be adapted to dual-column operation. However, to take full advantage of multiple-column operation, as many columns as possible should be operated in parallel. For example, with the gradient generator and eluent pump currently used for dual-column operation, four 0.22-cm (i.d.) columns or eight 0.15-cm (i.d.) columns could be operated in parallel. It then becomes necessary to develop a multicolon column monitor, since the space requirements and cost of individual monitors for each of four, or more, parallel columns would be prohibitive. Further, the detailed manual interpretation of the chromatograms produced by such a large number of columns would probably require several man-days and thereby defeat the purpose of multicolumn analysis. Therefore it would be highly desirable, if not necessary, to provide a means for simultaneous computer analysis of the multiple sets of chromatograms.

References


