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Note

Automated short column chromatography for high-speed routine analysis

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In the past routine automatic analysis has been dependent largely on the use of specific reagents. Except in the case of substances such as creatinine where flow dialysis is possible, a complex mixture had to be analysed directly, and this drawback applied to both flow methods and discrete sample analysis. Many analyses which would be desirable in clinical laboratories are often not undertaken because tedious chromatographic methods are necessary to isolate the compounds before estimation. When a complex mixture has to be analysed for several similar components such as amino acids, long columns are used, the eluate being monitored continuously. Even when short columns are used it is usually impossible to isolate and estimate more than a very limited number of samples each day.

There is a need for an automatic short column system so that the column can be buffered, loaded, eluted and regenerated at such a rate that the eluate can be monitored either by discrete sample analysis or by a flow system.

Corisano *et al.*[1] have approached the problem by using a series of valves to control the flow of buffers etc., through a single column, the output being monitored in a flow system. This was applied to the estimation of creatinine. If the cycle of operation of the column involves six steps (buffer, wash, sample, wash, elution, regeneration), then clearly some 5/6th of the time is wasted. By coupling six columns in a suitable manner each one could be made to undergo progressively a different step in the chromatographic cycle and the output from successive columns could be measured continuously.

EXPERIMENTAL

Apparatus

Two six-way taps (Springham & Co.) (Fig. 1) were mounted coaxially in opposite directions, 14 cm apart. The polytetrafluoroethylene keys were fixed to a steel spindle so that they could be rotated simultaneously. The spindle was driven by a rotary solenoid (Ledex) via a free wheel. The rotation of the taps was controlled by a timing device consisting of a synchronous motor with a cam-operated microswitch, and a 60° rotation occupying about 1 sec was arranged to take place every minute. Six columns of plastic tubes fitted with adjustable plungers and sintered polyethylene filter discs were suspended between the taps, connected to top and bottom by flexible tubing (Fig. 2).

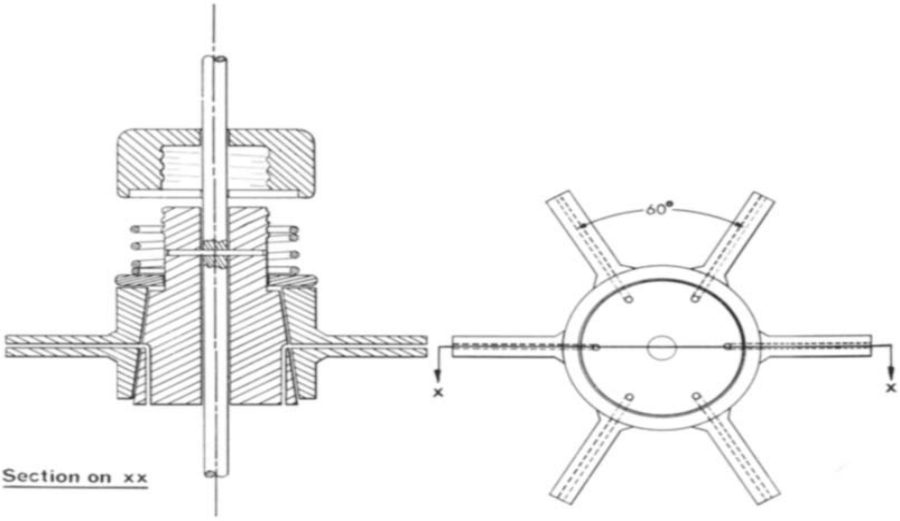

Fig 1. Six-way tap.

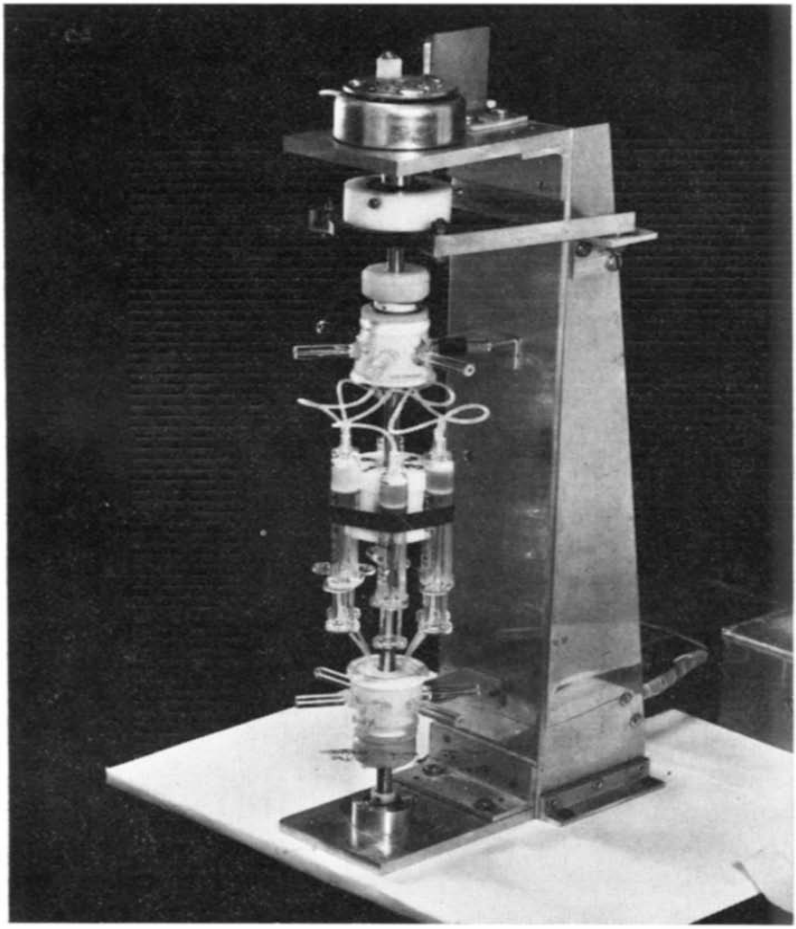

Fig. 2. Assembled apparatus.

Mode of operation

Accurately weighed column packing was put in the columns with filter discs above and below. Appropriate buffers were pumped upwards through the columns by means of a peristaltic pump. Use of suitable diameter pump tubing enabled different flow-rates for the various solutions to be used in different columns.

The samples were introduced at the appropriate stage in the cycle via the pump. In order to ensure that the sample addition was reproducible, the sampling time was reduced to 30 sec, the remaining 1/2 min being used to wash the sample on to the column. This also prevented carry-over on to the next column. The output from the appropriate stage was monitored for the required components.

Test system —analysis of xanthurenic acid

Columns (0.9×0.9 cm diameter) were packed with Zerolit 225 (SRC6, 0.4 g). The columns were pumped as follows: Column 1, 0.3 *M* NH_4OH at 2.9 ml/min; column 2, water at 2.0 ml/min; column 3, 0.2 *N* HCl at 2.9 ml/min; column 4, sample at 2.0 ml/min (*i.e.* sampling for 1/2 min followed by 0.2 *N* HCl 1/2 min); column 5, 0.2 *N* HCl at 2.0 ml/min; column 6, water at 2.9 ml/min.

Xanthurenic acid standards added to column 4 were 5, 15, 20 and 25 μg/ml. The effluent from column 6 was buffered with Tris/maleic acid (1.0 *M*, pH 6.6) in a flow system and reacted with dichloroquinone–chloroimide (2:6) (ref. 2). The

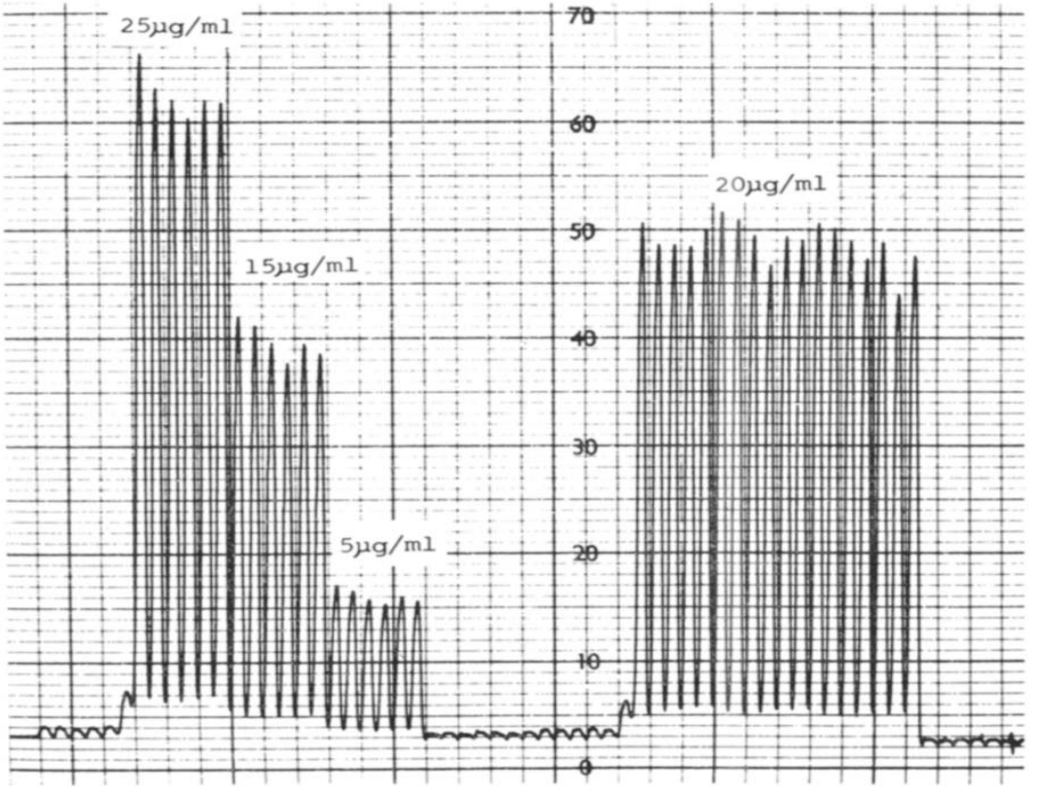

Fig. 3. Analysis of xanthurenic acid at the rate of 60 samples per hour.

colour was produced at 622 nm. The system was further evaluated by measuring the recovery of xanthurenic acid added to urine.

RESULTS

A typical series of recordings are shown in Fig. 3. The standards were estimated with a coefficient of variation of ±4.5%. Standards added to urine were recovered with a mean of 88 ± 6.9% (six experiments).

DISCUSSION

There are several possible ways of switching the flow of liquids through the six columns but it seemed likely that six-way taps of the kind used here would be preferable to solenoid operated pinch valves. There were no signs of leakage from the taps after initial adjustment. Whilst six-way taps provided a fair choice of steps, greater versatility would be available in an eight-way tap.

Air bubbles introduced during sampling tended to disrupt the structure of the column packing. This was avoided by the use of a pinch valve during the change-over from sample addition to wash. Water, which had been boiled to remove air, was also found to be advantageous but not essential. The addition of detergent to the solutions was not necessary. The usual characteristics of column packings, such as bead size and degree of cross-linking exerted an influence on the separations, although it seemed that the flow-rates of the solutions were of more importance. Using a resin of low cross-linking (SRC6, 2% divinylbenzene), the volume of the column had to be arranged so that under conditions of maximum swelling it was just filled. Subsequent shrinkage left a small space at the bottom of the column. The swelling/shrinking cycle also helped to expel air bubbles and hence to maintain the integrity of the columns.

The eluate from the column could be measured either by a flow system or with suitable timing devices by means of a discrete sample analyser. Analyses were performed in a straightforward manner at the rate of 60 samples per hour.

REFERENCES

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