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APPLICATIONS OF A MICROPROCESSOR-CONTROLLED VALVE-SWITCHING UNIT FOR AUTOMATED SAMPLE CLEANUP AND TRACE ENRICHMENT IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Various methods and principles of column switching are described with particular emphasis being given to their use in automated sample cleanup and trace enrichment. Commercial equipment is defined which enables all the methods described to be carried out automatically using microprocessor control.

INTRODUCTION

Conventional chromatographic methods now comprise a wide range of techniques including adsorption (normal-phase), reversed-phase, ion-suppression, ion-pairing, ion-exchange, partition, size-exclusion and several other popular forms of chromatography.

There are potential advantages in combining several of these methods[1–4]. For example, adsorption or size exclusion is often used to cleanup a sample prior to chromatographic analysis or other analytical methods. Prior chromatography is sometimes used to separate a complex mixture into different groups of compounds, followed by more specific analysis.

In some complex mixtures, it is necessary to carry out two separations using different methods such as adsorption followed by ion pairing or size exclusion followed by reversed phase or many other combinations of methods. In high-performance liquid chromatography (HPLC) this combination of methods is often referred to as multidimensional HPLC.

The principles just described, form the basis of sample cleanup using valve

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TABLE I

METHODS OF VALVE SWITCHING

1	Sample cleanup
2	Trace enrichment
3	Method development
4	Sample identification
5	Boxcar chromatography
6	Multi-column chromatography
7	Incremental gradient elution

switching methods. Here valves re-route the chromatographic eluent flow from one column to another. Other valves change the solvents. Both operations must be carried out precisely and accurately.

In trace enrichment, dilute aqueous samples are selected and routed through small reversed-phase pre-columns. Concentration of the solute arises from non-elution from the pre-column and only when the solvent is changed by re-routing the pre-column in-line with an analytical column, will the enriched solute species be eluted from the pre-column. This approach enables very low levels of pollutants to be determined.

In addition to these methods, there are several others which benefit from a valve-switching approach, as indicated in Table I.

DESCRIPTION OF VALVE SWITCHING

In order to carry out the methods indicated in the introduction it is necessary to utilize several valve-switching techniques. A list of these techniques is given in Table II, some of which will be described in greater detail.

Principles of valve switching

There are two types of valve used in the Kontron valve-switching unit. These are Rheodyne 6-port, 2-way valves (Model 7000) which operate at high pressure and Rheodyne 6-way selector valves (Model 5012) which operate at low pressure.

TABLE II

USES OF SWITCHING

1	Column selection
2	Column switching
3	Solvent switching
4	Solvent selection
5	Zone cutting
6	Auxilliary pump on/off
7	Trap solute in detector
8	Recycle
9	Fraction collection
10	Sample injection
11	Detector selection

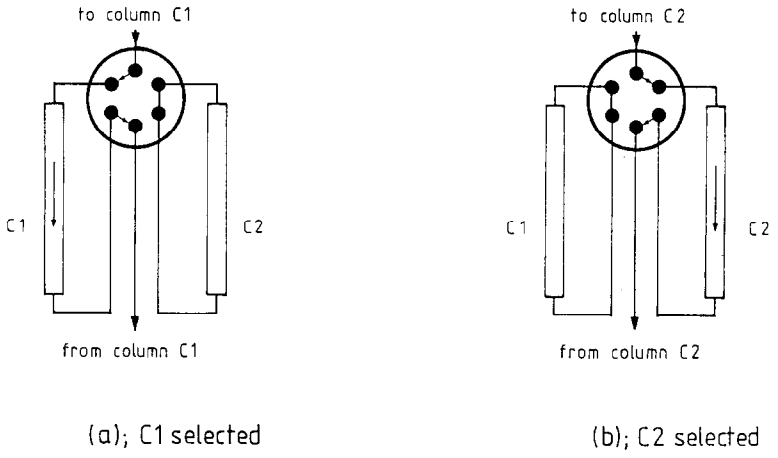


Fig. 1. Description of column selection using a 6-port, 2-way valve.

Essentially, the 2-way valves (Model 7000) have two internal pathways connecting ports 1 and 2, 3 and 4, 5 and 6, in the non-switched position, and in the switched position, ports 2 and 3, 4 and 5, 6 and 1 are connected. Depending on the valve position, different external connections to the valve are selected.

The 6-way valves, however, do not return directly to their starting position but step sequentially through ports 1 to 6, thus enabling the selection of different samples for trace enrichment and when connected in reverse order, for the collection of fractions from semi-preparative seperations.

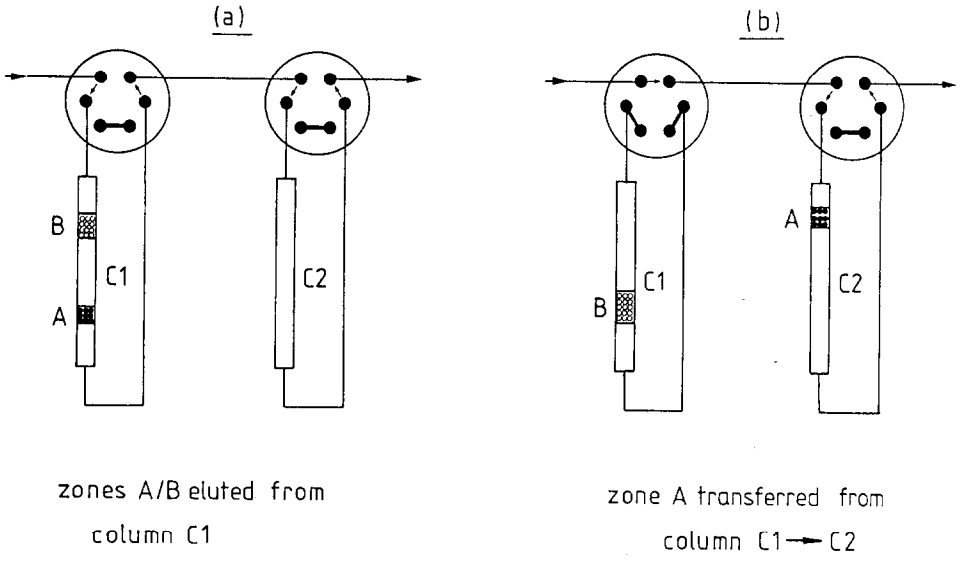


Fig. 2. Description of zone cutting by column switching.

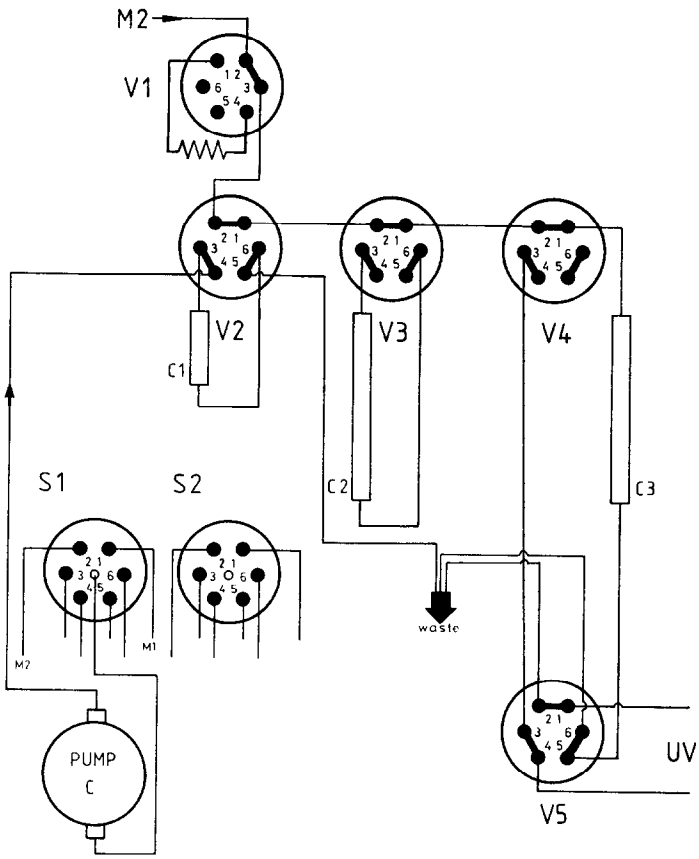


Fig. 3. Kontron MCS 670 Tracer configuration suitable for sample cleanup using zone cutting methods and automatic solvent change for regeneration of pre-columns.

Column selection

Fig. 1 shows how a 2-way valve can be used to select one of two columns. The procedure is used in this paper for valve V4 in order to select the detector flow cell. Switching V5 when a solute is passing trough the detector enables the trapping of the solute for wavelength scanning etc. (see Fig. 3).

Column switching

Fig. 2 indicates how the eluent can be re-routed from one column to another. This procedure is very important and is used to:

(a) Carry out zone transfer and zone cutting.
(b) Optimise analysis time.
(c) Change the selectivity of the system.
(d) Increase column length.

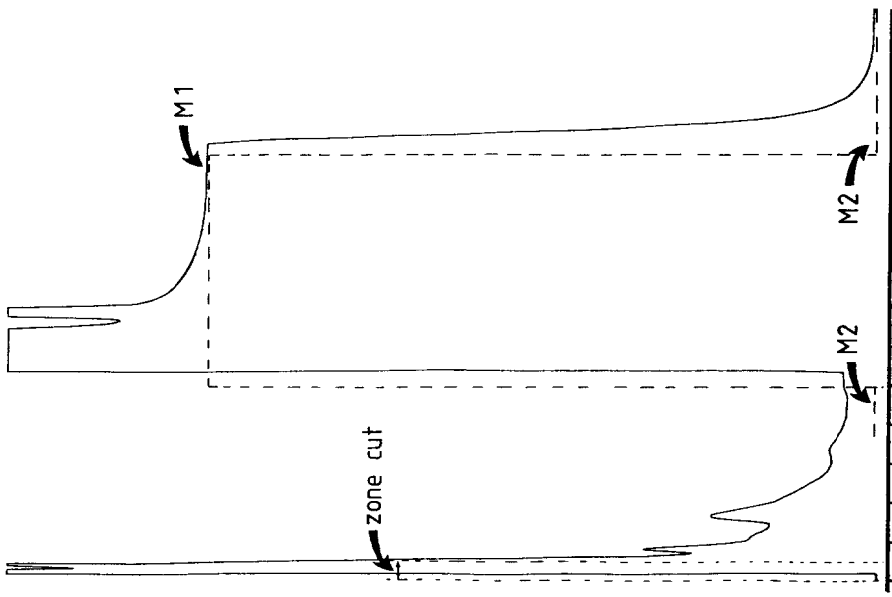


Fig. 4. Chromatogram of red wine illustrating the need for sample cleanup by zone cutting. Column: 25 cm × 4.6 mm I.D. S5 ODS. Solvents: A, 0.01 *M* NaH_2PO_4, pH 2.4; B, methanol. Detection: UV at 214 nm.

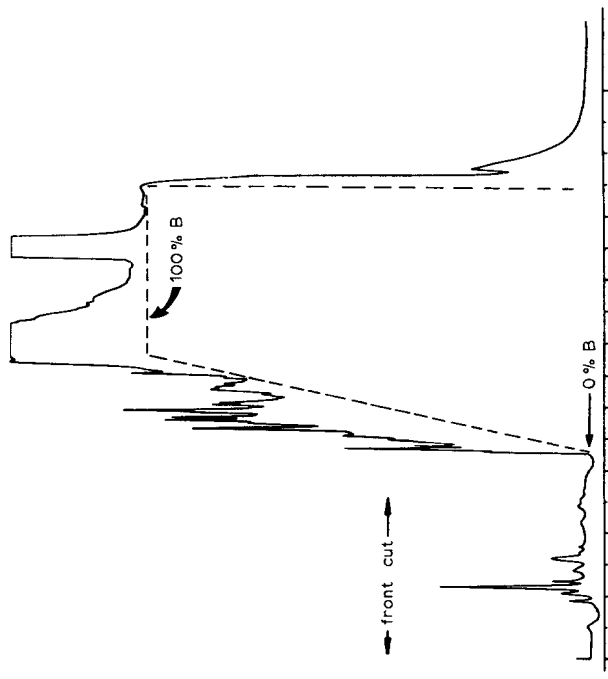


Fig. 5. Chromatogram of red wine on Brownlee pre-column illustrating timing of the front cut taken and the cleaning and re-equilibration requirements.

Zone transfer and zone cutting

Zone transfer is illustrated in Fig. 2 and represents the basic principles of column switching, where a zone is allowed to elute from one column to another. To minimise extra column band broadening, the tubing connecting the column to the valve should be made from the finest bore capillary tube (*e.g.* 0.007 in. I.D. tube).

Zone cutting is an extension of this principle and is probably the most useful and versatile of all the column switching techniques. The figure shows how a heart cut can be taken from column C1 and transferred to column C2. Once the cut has been made, C1 can be isolated from the system. If further zones are required, C1 can be used to store the remainder of the chromatogram until elution from C2 is complete. Further zone cuts can be made in the same way as the first.

Column C1 can be isolated from the analytical system and regenerated by means of a secondary pumping system. This is the principle used to carry out sample cleanup and is one of the most useful and versatile techniques of column switching.

Solvent selection

Selector valves S1 and S2 (Fig. 3) enable solvent changes to be made either sequentially or arbitrarily. If the common outlet is connected to a pump, several applications arise.

(a) Solvent change for column cleaning and re-equilibration, as used in the examples of sample cleanup.

(b) Incremental gradient elution necessitates a series of mobile phases of in-

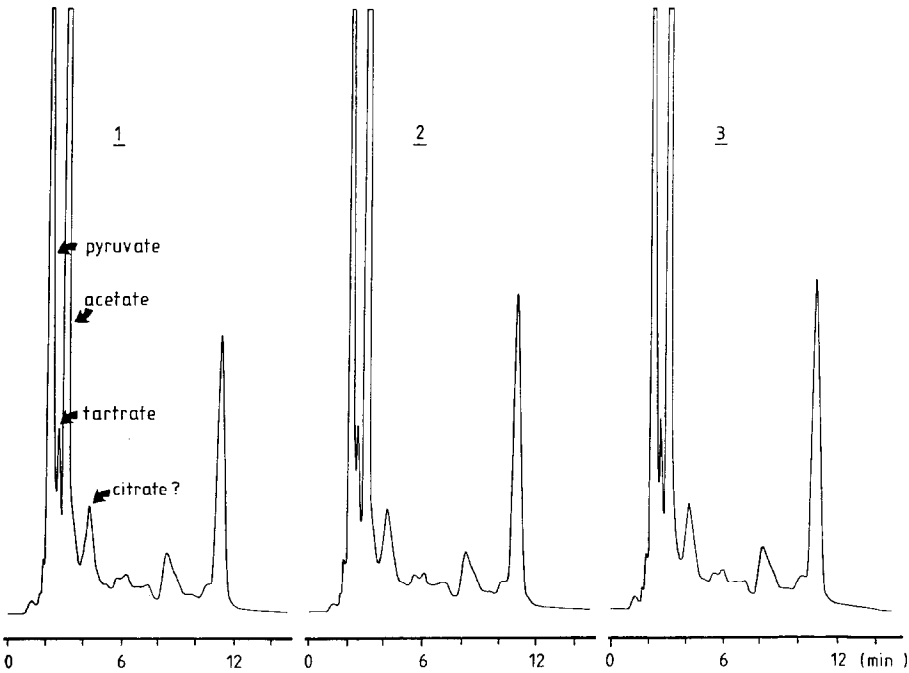


Fig. 6. Chromatogram of organic acids in red wine using automatic sample cleanup by zone cutting. 1, 2 and 3 are replicates.

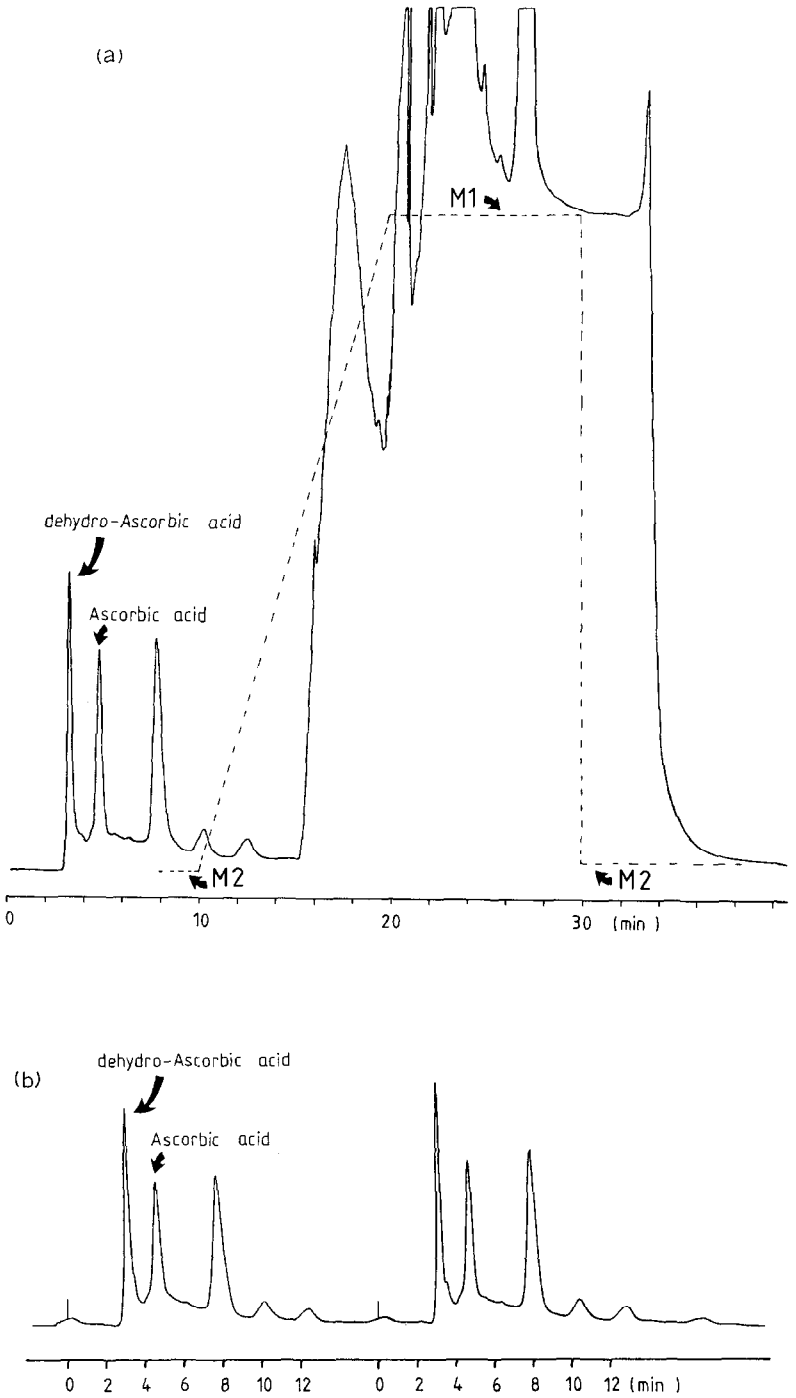


Fig. 7. Chromatogram showing the separation of blackcurrant syrup without (a) and with (b) sample cleanup

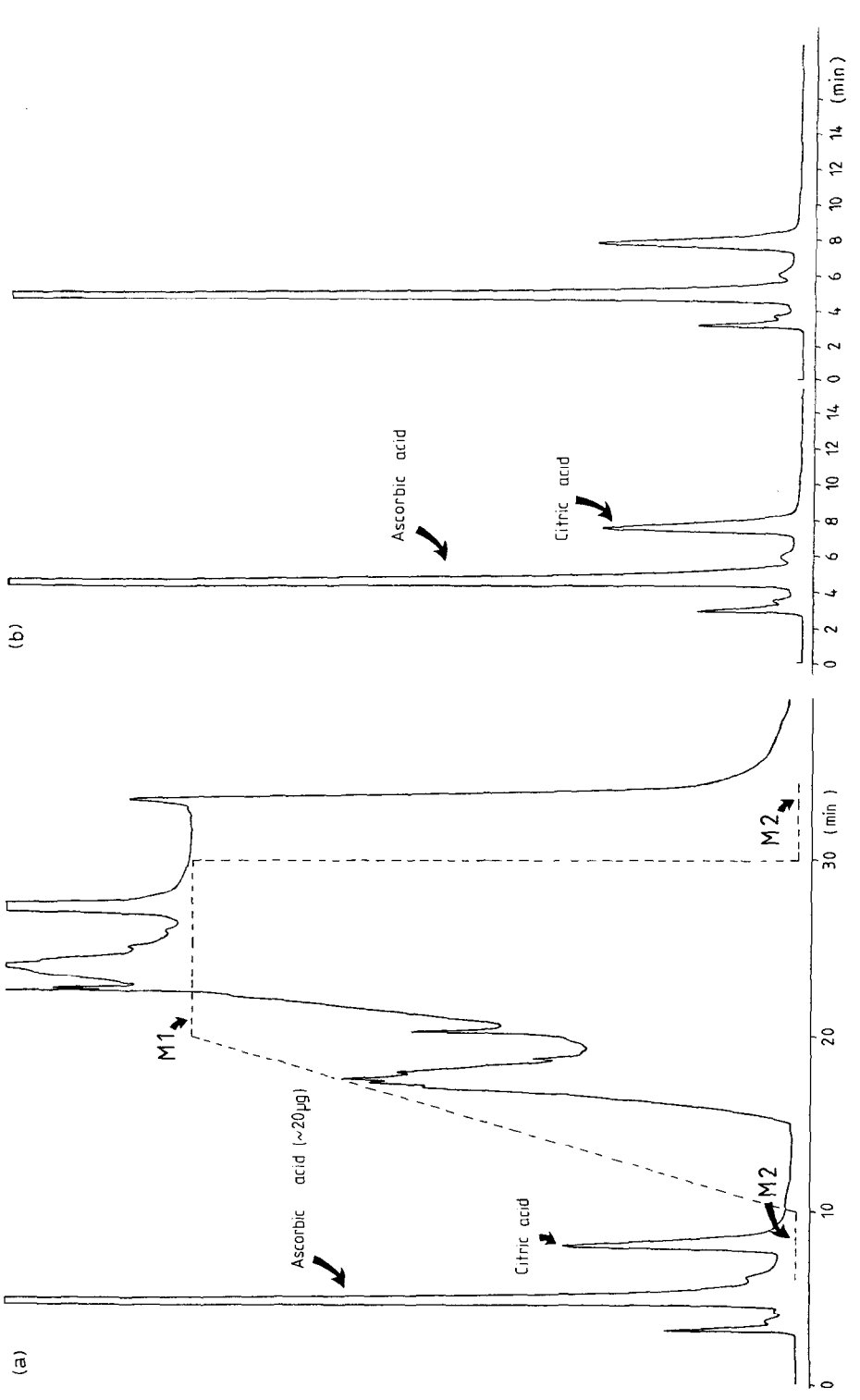


Fig. 8. Chromatogram showing the separation of rose hip syrup without (a) and with (b) sample cleanup.

creasing eluting power. As changes are made sequentially it is obvious that solvents adjacent in the series, must be mutually miscible.

(c) Arbitrary solvent selection for different methodologies is possible. As the Tracer recognizes position No. 1 it is best the reset to position 1 and switch the valve x times to select the required solvent; *i.e.* switch twice to select position No. 3.

Sample selection

Each outlet can be connected to a dilute aqueous sample. After selection, the sample can be pumped through a pre-column for trace enrichment.

Fraction collection

By reversing the connections so that the common outlet is connected to the column end; up to six fractions can be collected of any volume per fraction. This is useful in semi-preparative chromatography, particularly when multiple runs are envisaged.

EXPERIMENTAL

Apparatus

A Kontron Model 640 gradient elution chromatograph with automatic sampler, Kontron MCS 670, "Tracer", valve-switching unit, and Uvikon 720 LC detector (Kontron) were used. All units were controlled through the Kontron Model 200 or 205 programmer. The Model 205 programmer has dedicated keys for valve switching, detector control and autosampler control and is therefore the easiest method for control; however, other methods of control include (a) manual switching, (b) Model 210 Tracer Timer unit and (c) computer control via built-in RS232 interface.

Reagents

All solvents were HPLC grade (Rathburn) and water was freshly double-distilled. Sodium dihydrogen orthophosphate and phosphoric acid were of AnalaR grade (BDH).

Columns

Kontron Spherisorb S5 ODS column and Brownlee S5 ODS pre-columns were used throughout.

RESULTS

Two areas of application were considered in this paper: (a) sample cleanup, and (b) trace enrichment.

Automatic sample cleanup

The fundamental concept of valve switching which relates to sample cleanup is "zone cutting" and has previously been described. The configuration shown in Fig. 3 is extremely versatile and flexible, allowing two successive zone cuts prior to final analysis. In the following applications column C1 is used as the pre-column where the cleanup takes place. In each application, a single zone cut is sufficient to allow subsequent high resolution analysis. Once the zone cut has been made, C1 is then cleaned and

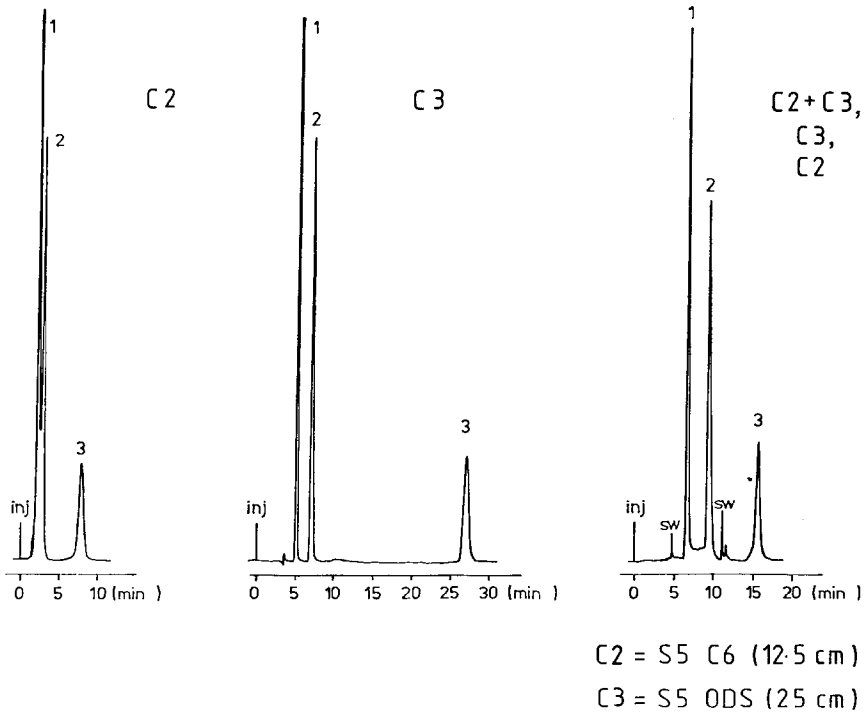


Fig. 9. Application of muticolumn chromatography to the optimization of the chromatogram. Peaks: 1 = acetone; 2 = methyl ethyl ketone; 3 = octaphenone. Detection, UV at 254 nm, 0.05 a.u.f.s., flow-rate, 1 ml/min.

re-equilibrated through the second flow path through the system, driven by pump C. Cleaning is generally made in the foreflush mode to protect the column lifetime and the mobile phase changes are made by selector valve S1.

Acids in wine, fruit juices, beverages, etc.

Organic acids in wine are important for quality control purposes for the control of the fermentation processes. Vitamin C control of fruit juices and extracts is also of significance.

Fig. 4 shows a chromatogram of red wine injected directly onto the analytical column C3. The compounds of interest elute in the front cut shown. The principle of sample cleanup is further demonstrated in Fig. 5, where the same sample is eluted from C1. The zone containing the acids of interest is a tight band eluting at the front of the chromatogram. Subsequent cleaning with methanol (Ml) and re-equilibration with buffer (M2) establishes the necessary times for regeneration. Consequently, C1 and C3 are switched in-line at the start of the analysis such that the zone cut is transferred to C3. At the point, C1 is switched out of line and is regenerated as described. Analysis proceeds on C3 as shown in Fig. 6.

Under similar conditions, but where C3 is a 20-cm Hypersil-5 ODS column, similar applications are shown for the direct analysis of (a) vitamin C in blackcurrant syrup (Fig. 7) without and with cleanup by column switching; (b) vitamin C in rose hip syrup (Fig. 8) without and with cleaning by column switching.

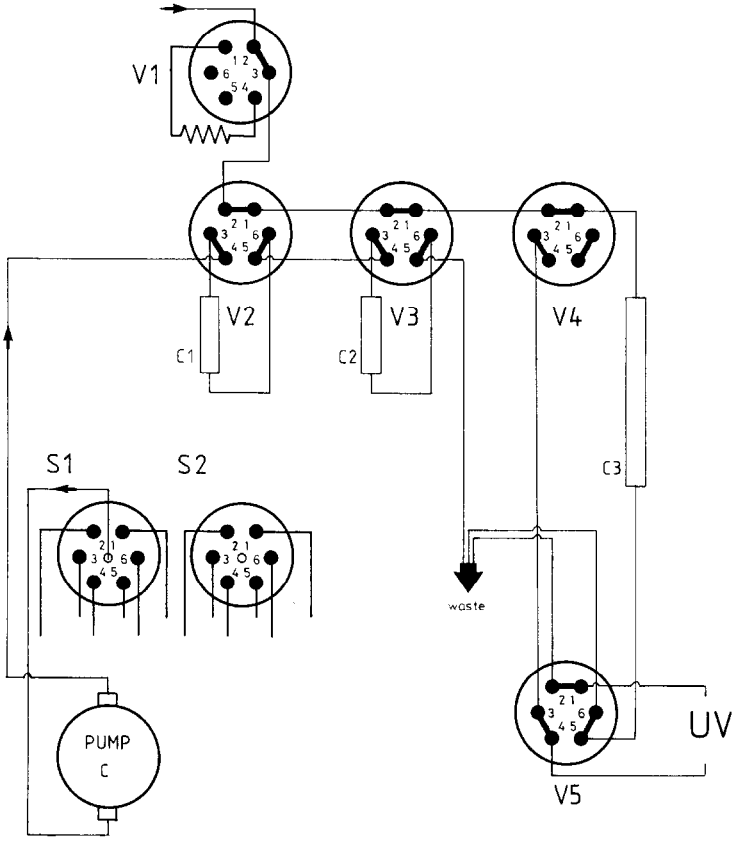


Fig. 10. Kontron MCS 670 Tracer configuration suitable for the trace enrichment. Alternative loading of pre-columns eliminates much lost time.

Multicolumn optimisation of resolution

A further aspect of column switching which is pertinent to sample cleanup is illustrated by Fig. 9.

After the primary zone cut has been made for cleanup, the zone of interest may chromatograph as shown on columns C2 and C3 (C2, C3 as indicated on Fig. 9). In order to optimise this separation, columns C2 and C3 are placed "in-line" to allow peaks 1 and 2 to elute from C2 onto C3. Then C2 is switched "off-line" while higher resolution of peaks 1 and 2 occurs on C3. After elution from column C3, column C3 is switched "off-line" and C2 switched "in-line" allowing elution of peak 3. This is a powerful approach to optimization of chromatographic elution. The example given is for a mixture of ketones.

Automatic trace enrichment

The simplest forms of trace enrichment have been described in the introduction. This instrument (Tracer) facilitates the automation of this process as shown in Fig. 10.

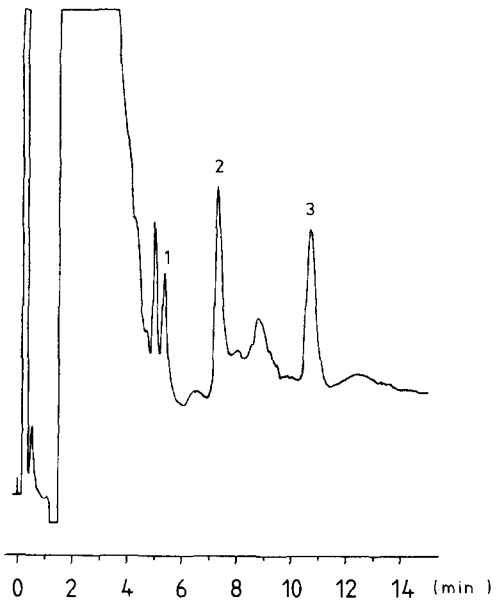


Fig. 11. Application of trace enrichment to chlorophenols in tapwater. Volume concentrated (50 ml water acidified to pH 2.4). Pre-column: Brownlee RP18 cartridge (3 cm). Analytical column: 25 cm × 4.6 mm I.D. S5 ODS (Kontron). Eluent; methanol–$10^{-3}M$ H_3PO_4 (75:25). Flow-rate: 2 ml/min. Detection: UV at 220 nm, 0.01 a.u.f.s.. Peaks: 1 = 3,4,5-trichlorophenol (0.4 ppb); 2 = 2,3,4,5-tetrachlorophenol (0.4 ppb); 3 = pentachlorophenol (0.3 ppb).

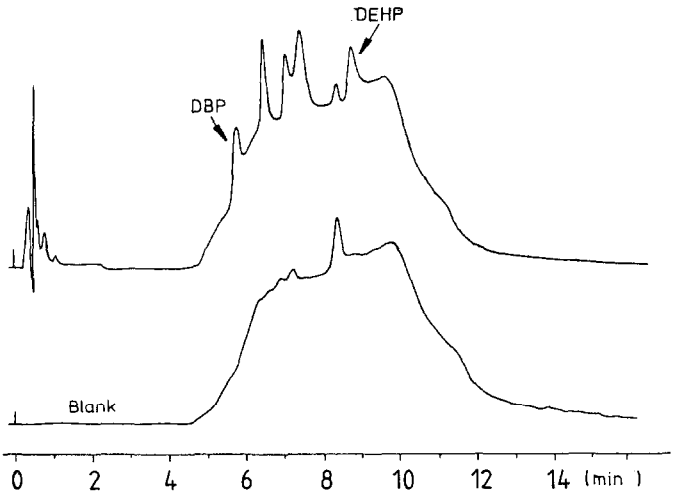
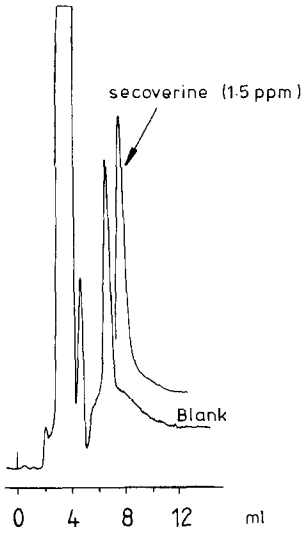


Fig. 12. Application of trace enrichment to plasticisers in aqueous extracts (Evian mineral water). Volume concentrated (50 ml at 10 ml/min). Pre-column: Brownlee RP18 cartridge (3 cm). Column: 10 cm, RP18, 10 mm (Kontron). Flow-rate: 4 ml/min. Eluent: methanol–water gradient (65–100 % methanol gradient over 2-min period, starting at time 4 min, then isocratic for 4 min and then 2-min gradient back to 65% methanol. Detection: UV at 224 nm. DBP = dibutyl phthalate (0.11 ppb); DEHP = diethylhexylphthalate (0.13 ppb).



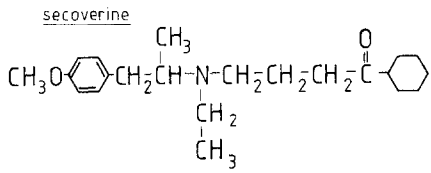


Fig. 13. Trace enrichment of secoverine from deproteinised serum. Volume concentrated; 1 ml serum. Pre-column: 2.2 mm × 4.6 mm I.D., Polygosil-CN (10 μm). Column: 25 cm × 4.6 mm I.D., Sil 60-D5-CN. Eluent: dioxan–0.1 *M* aqueous phosphate pH 3.2 (15:85). Detection: UV at 274.5 nm.

Sample loading occurs through pump C which is directly connected to selector valve S1 or S2 or S1 + S2 (connected together).

The selector valves enable selection of the required dilute aqueous sample which is then routed through one of the pre-columns (*e.g.* C1) typically 50 ml of the sample is pumped through the column. While this is taking place column C2 is "in-line" with the analytical column C3. The appropriate mobile phase (*e.g.*, methanol–water, 75:25) elutes the enriched species (and impurities) from C2 onto C3. After elution, solvent change to water is necessary in order to equilibrate C2. While this re-equilibration is taking place, C3 has been by-passed to preserve stable isocratic conditions. The next stage of the automated process is to switch C2 "off-line" and C1 "in-line". The procedure continues only this time C2 is being loaded while C1 is being eluted.

Chlorophenols in tapwater. Control of phenolic species in tapwater is critically important for environmental control[5–7]. The application of the Tracer to these compounds is very well demonstrated in Fig. 11. The power of trace enrichment is emphasized by the very low levels that can be determined by this method.

Plasticisers. We live in a plastic world which is constantly contributing to environmental pollution by the liberation of toxic plasticisers. The application shown (Fig. 12) indicates that many of the plastic bottles containing beverages, contaminate that beverage with plasticisers such as dibutyl phthalate and diethylhexyl phthalate.

Deproteinated serum. Many drugs can be determined from deproteinated serum but occur at levels too low for easy determination. Enrichment similar so that previously described allows detection at low levels. In these applications shown in Fig. 13, 1 ml deproteinated serum was concentrated on a pre-column and eluted as described previously. However, the loading took place via an autosampler set to load 1 ml samples. Using this method, secoverine at a concentration of 1.5 ppm in plasma was determined.

CONCLUSIONS

The enormous power of valve switching has been indicated and demonstrated for automatic sample cleanup and trace enrichment. It is obious that in trace enrichment, impurities are also concentrated, thus limiting the degree of concentration possible. The Kontron "Tracer" has been shown to be able to carry out all the requirements of this methodology with precision and accuracy. It is versatile, flexible and indicates the way in which much of the future chromatography will be carried out.

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