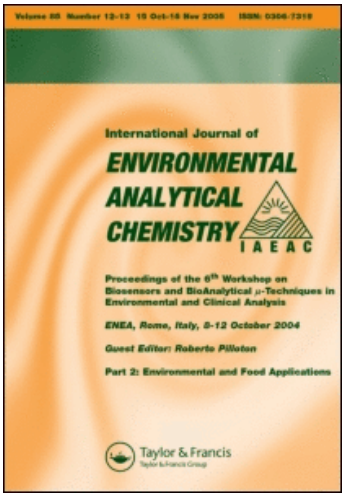


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

The Role of Column Switching in Analysing Complex Samplest

C. J. Little[a]; O. Stahel[b]; K. Hales[c]
[a] Anachrom Ltd., Slough, Berks, U.K. [b] Kontron Ltd., Zurich, Switzerland [c] Toxicology Lab., Birmingham, U.K.

To cite this Article Little, C. J. , Stahel, O. and Hales, K.(1984) 'The Role of Column Switching in Analysing Complex Samplest', International Journal of Environmental Analytical Chemistry, 18: 1, 11 — 23
To link to this Article: DOI: 10.1080/03067318408076988
URL: http://dx.doi.org/10.1080/03067318408076988

Intern. J. Environ. Anal. Chem., 1984, Vol. 18, pp. 11–23
0306-7319/84/1802-0011 $18.50/0

Printed in Great Britain

The Role of Column Switching in Analysing Complex Samples†

C. J. LITTLE

Anachrom Ltd., PO Box 366, Slough, Berks, U.K.

O. STAHEL

Kontron Ltd., Bernerstrasse Sud 169, CH8048 Zurich, Switzerland.

and

K. HALES

Toxicology Lab., Dudley Hospital, Birmingham, U.K.

(*Received November 25, 1983*)

Our objective in using column switching is primarily to achieve the desired separation in the minimum analysis time. Complimentary to this aim is the need for sample and column cleanup followed by column re-equilibration. Finally, all operations should be capable of automation.

Fundamental to column switching methodology is the concept of Zone cutting, where part of the chromatogram is transferred to another column. This forms the basis of sample cleanup and is a very versatile and powerful method. Multiple zone cutting is also possible to further increase the scope of cleanup or to minimise analysis time. Zone cutting is also complimentary to the techniques of trace enrichment and recycling.

Examples will be given involving the use of these techniques in the analysis of complex matrices such as urine, plant extracts, wine and serum. The latter will be used to propose a novel approach to the quantitative analysis of anti-convulsants in serum using hexobarbital as internal standard.

KEY WORDS: Column switching, sample clean-up, trace enrichment.

†Presented at the International Workshop on Handling of Environmental and Biological Samples in Chromatography, Lausanne, 24–25 November, 1983.

INTRODUCTION

Column switching is a means of re-routing the chromatographic eluent from one column to another by means of high pressure, in-line, switching valves. In addition, low pressure valves can be used to change the solvents (samples in the case of trace enrichment)[1]

These techniques enable us to carry out a number of procedures such as Sample Cleanup, Trace Enrichment, Method Development, Sample Identification, Incremental Gradient Elution, Boxcar Chromatography etc.,[2] automatically and on-line.

In order to carry out these procedures, it is necessary to utilise several valve-switching techniques. A list of these is given in Table I. Usually, combinations of these techniques are used, especially in the more complex applications.

TABLE I

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

Obviously these operations must be carried out reproducibly but with suitable electronic control, they are more reliable than manual, off-line procedures.

Fundamental to the majority of column switching methods is the concept of ZONE CUTTING.[1] When the eluent is re-routed from one column to another, the timing of the operation is planned so that only the chromatographic zone containing the peaks of interest is transferred to the next column. At this stage, the conditions can be altered, if necessary, such that a different mode of chromatography is used (i.e. multi-dimensional), or the column can be more retentive either by changing the functionality or the surface area of the support.

Probably the major uses of column switching at the present time are in automatic SAMPLE CLEANUP and as an alternative approach to gradient elution.

EXPERIMENTAL

Equipment used

All column switching was carried out using the Kontron "TRACER", MCS 670 column switching unit. The chromatographic equipment used comprised the Kontron model 640 chromatograph with gradient elution and automatic sampler (model MS1 660) built in, the Kontron "Uvikon 720 LC" scanning UV detector and the whole system was controlled through the Kontron model 205 programmer.

Reagents used

The solvents were of HPLC grade (from Rathburn Chemicals) and water was used after double distillation.

Buffers were prepared from analar grade sodium dihydrogen orthophosphate and phosphoric acid (from BDH Chemicals).

Columns

All columns were Kontron Spherisorb type with varying functionality, such as C8 and ODS.

Valve configuration

Perhaps the most useful and generally applicable configuration in column switching is shown in Figure 1, which has been used for the first 3 applications shown in this paper. The fourth is a modification of the configuration and is shown later for continuity. Otherwise, the only variables are columns and solvents.

RESULTS AND DISCUSSION

The following examples demonstrate how the concepts previously described can be used advantageously.

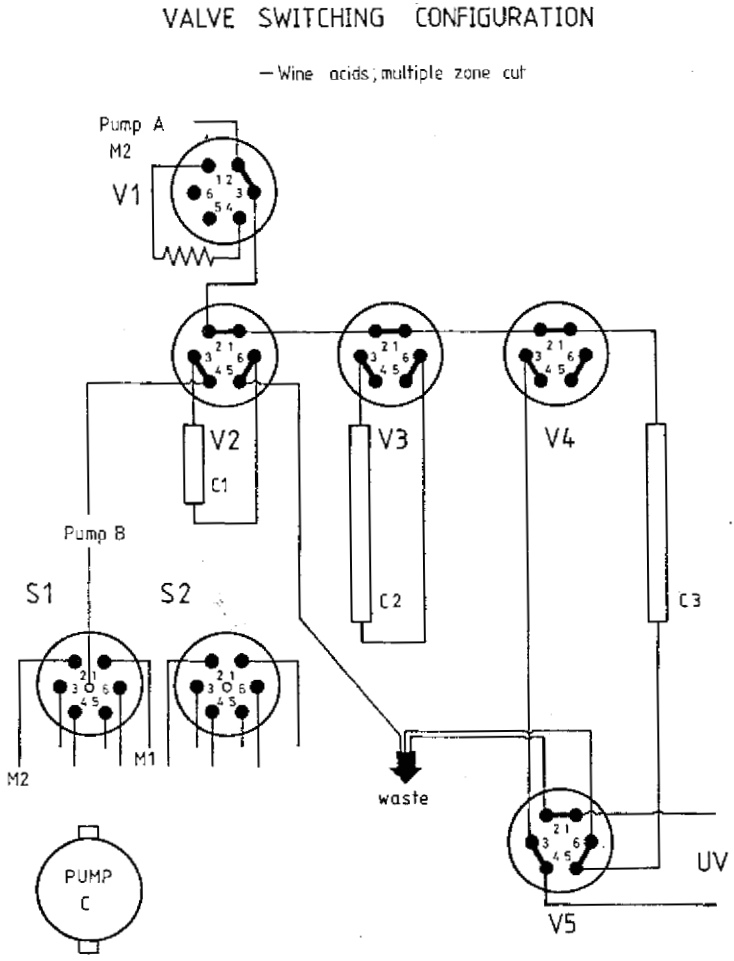


FIGURE 1 Valve configuration for applications 1–3.

a) Nicotinic acid in urine

Urine is one of the main forms in which the body gets rid of unwanted foodstuffs, metabolites and toxic contaminants of the body such as drugs and poisons. As such it is a highly complex matrix containing hundreds of different solutes, the majority of which are water soluble. Consequently, many of the solutes elute fairly close

together, making it necessary to (a) elute with the weakest solvent and (b) use a column with high resolving power.

The net effect of these requirements is a long analysis time, even for solutes which elute early, because the column has to be cleaned and re-equilibrated between samples.

Column switching offers an alternative to the gradient elution approach. A zone containing all the peaks of interest is transferred from a pre-column to an analytical column where it is eluted isocratically.

This allows for a rapid cleanup of the pre-column and time to re-equilibrate it whilst the analytical separation proceeds. Not only is the time saving considerable but the separation can also be improved by this method.

Figure 2 shows an isocratic elution of urine such that the compounds of interest, nicotinic acid and nicotinamide are separated. In order to clean the column sufficiently, the analysis must be allowed to proceed for about 40 minutes. This could be

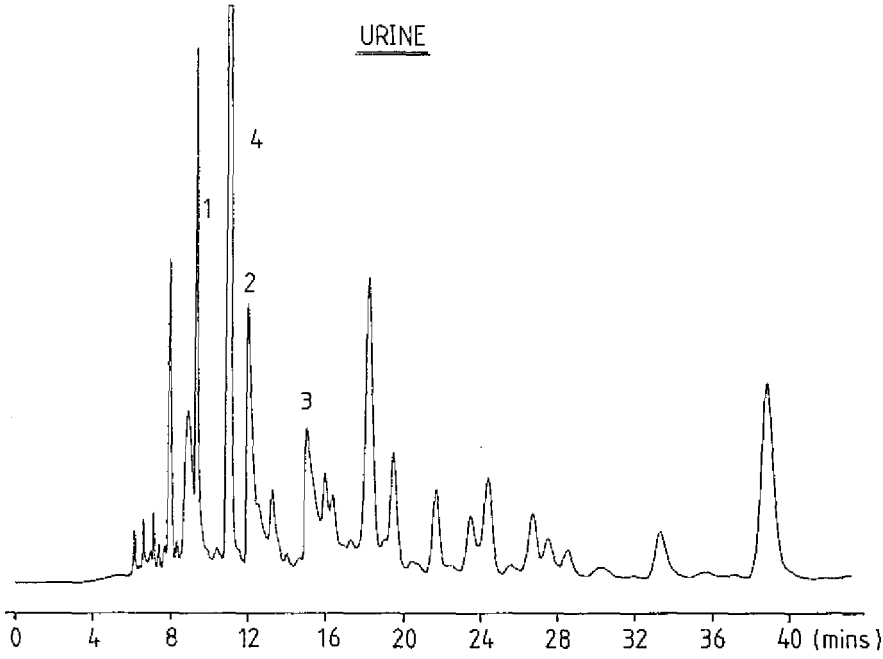


FIGURE 2 Nicotinic acid in urine without sample cleanup.

speeded up using gradient elution, but then the column would have to be re-equilibrated.

Using a zone-cut cleanup procedure the separation can be completed in 15 minutes (Figure 3) which includes a pre-column

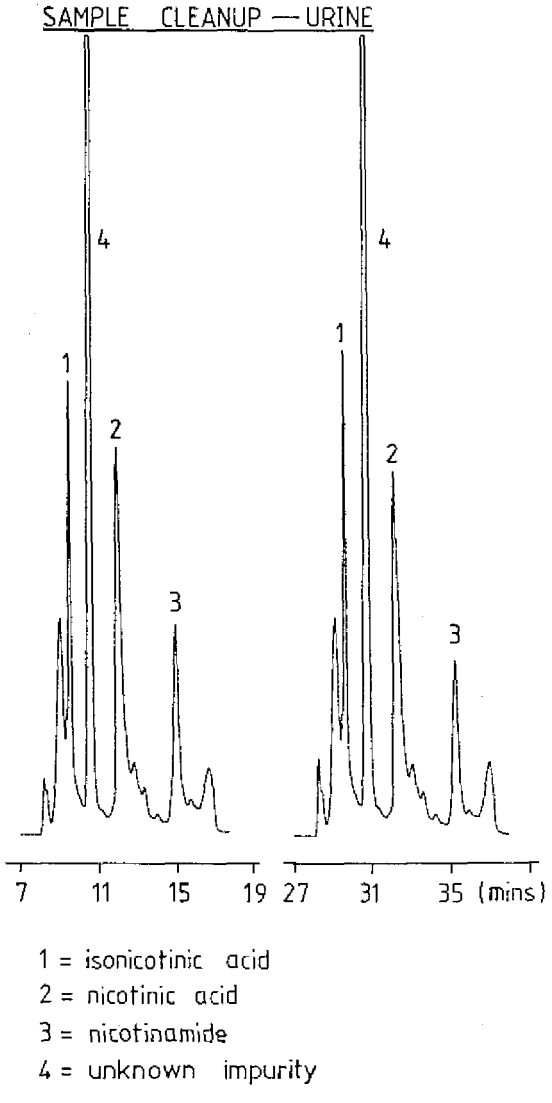


FIGURE 3 Nicotinic acid in urine with sample cleanup.

cleaning and equilibration. An improvement in the resolution of nicotinamide can be observed.[3]

b) Stevioside in leaves

Stevioside is a promising new natural sweetener[4] extracted from the leaf of the plant "Stevia rebaudiana".

Its analysis from leaf extract is improved using a similar approach to that previously described. In this case, if the zone-cut is carefully timed, almost complete separation from the rest of the extract is achieved. (Figures 4, 5). This approach can be successfully scaled up for preparative extraction of the sweetener.

c) Organic acids in wine

This analysis again demonstrates the power of column switching to effect an on line sample cleanup with considerable saving in time.[2, 5] However, it has proved necessary to increase resolution at the front of the chromatogram.

In order to achieve this goal[6] a second zone-cut is taken from the second column (C2) such that the leading part of this chromatogram is transferred to the column (C3) the remainder being stored on C2 and eluted after completion of the separation from C3.

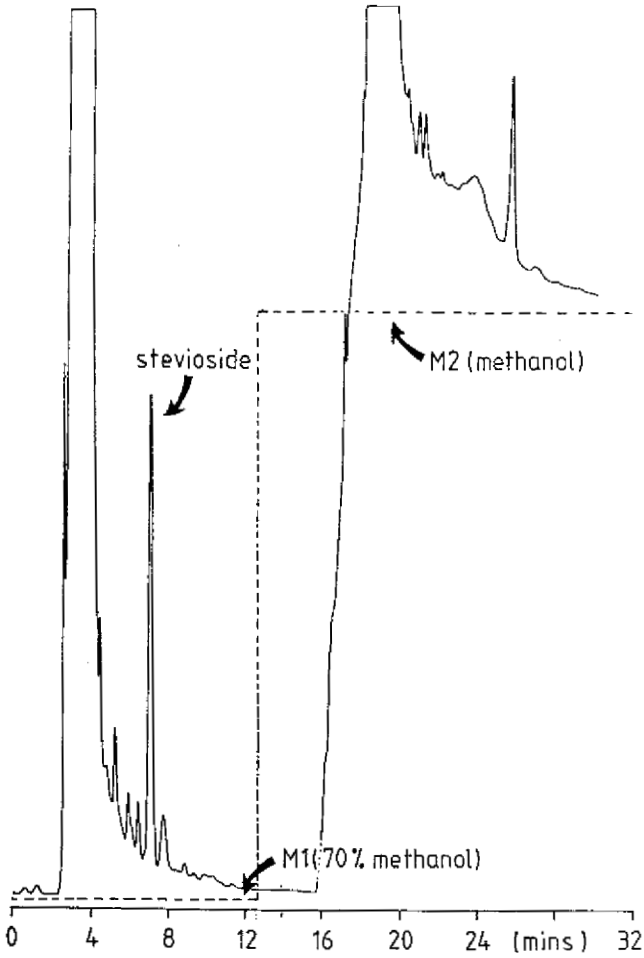
Multiple zone-cutting is a powerful technique which requires all four of the high pressure switching valves on the "Tracer". It maintains all the advantages of automatic sample cleanup and enables optimisation of the chromatogram under isocratic conditions (Figure 6).

d) Anticonvulsants in serum

Serum is a difficult matrix for direct injection on to an HPLC column because of the high molecular weight lipids, etc., which form a significant part of the serum. They are important because they (a) interfere with drug availability and (b) elute only very slowly from the column, which requires extensive cleanup after each injection.

The principles described in this paper for overcoming these problems are:

i) *Load serum onto pre-column using water as eluent.* The advantages of this approach are several such as concentration of



Chromatogram of STEVIOSIDE from a leaf extract (without sample cleanup)

FIGURE 4 Stevioside in leaf extract without sample cleanup.

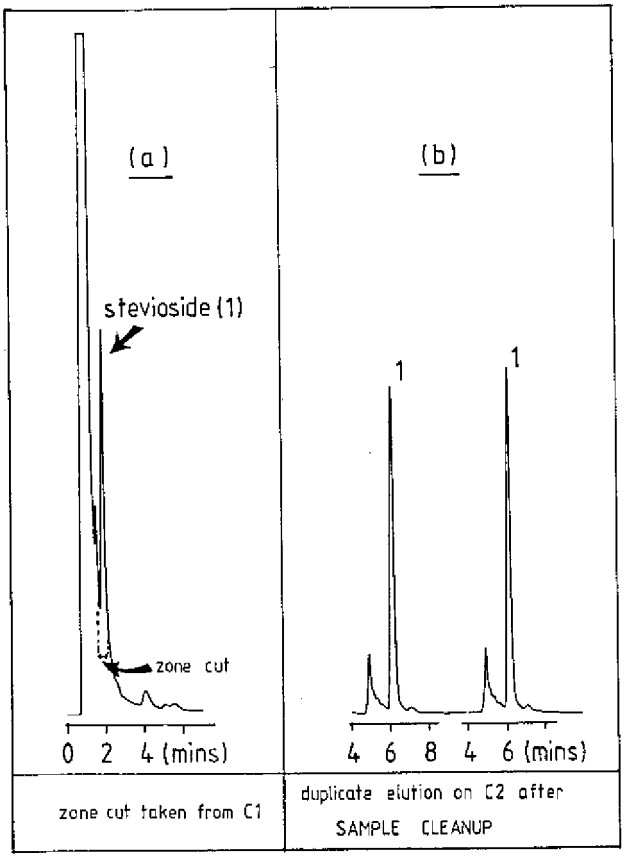


FIGURE 5 Stevioside in leaf extract with sample cleanup.

trace species and the elution of highly water soluble species to waste, thus effecting a partial cleanup and freeing of bound drug from proteins.

ii) *Change eluent to elute anticonvulsants.* About 30% of organic modifier is necessary in order to elute the drugs while still retaining unwanted lipids etc., on the top of the pre-column.

iii) *Transfer this zone onto an analytical column* where higher resolution is achieved.

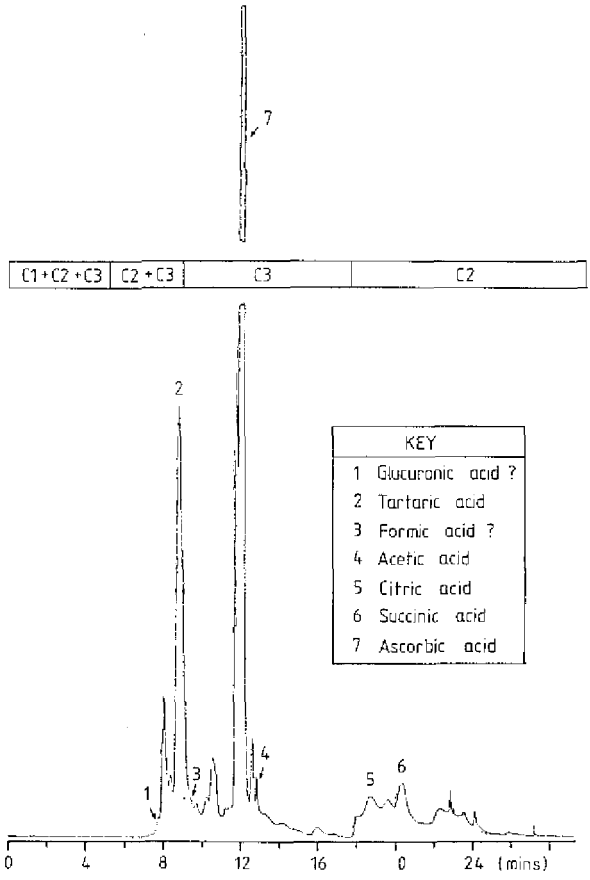


FIGURE 6 Organic acids in wine using a multiple zone cut method.

iv) *Meanwhile clean and re-equilibrate pre-column* by back-flushing first with methanol and then water. Back-flushing is important because the lipids are still trapped at the top of the pre-column and it would take hours to wash them through the column in a foreflush mode.

The valve configuration required for this technique is shown in Figure 7, where one of the valves has been used as a secondary injection device to add a fixed amount of internal standard with each sample. Added in this manner, it goes through all the switching processes and is subject to the same errors as is the sample. The single direction valve, placed at the open end of the internal standard loop is necessary in order to prevent loss of sample, through seepage, once the loop is filled.

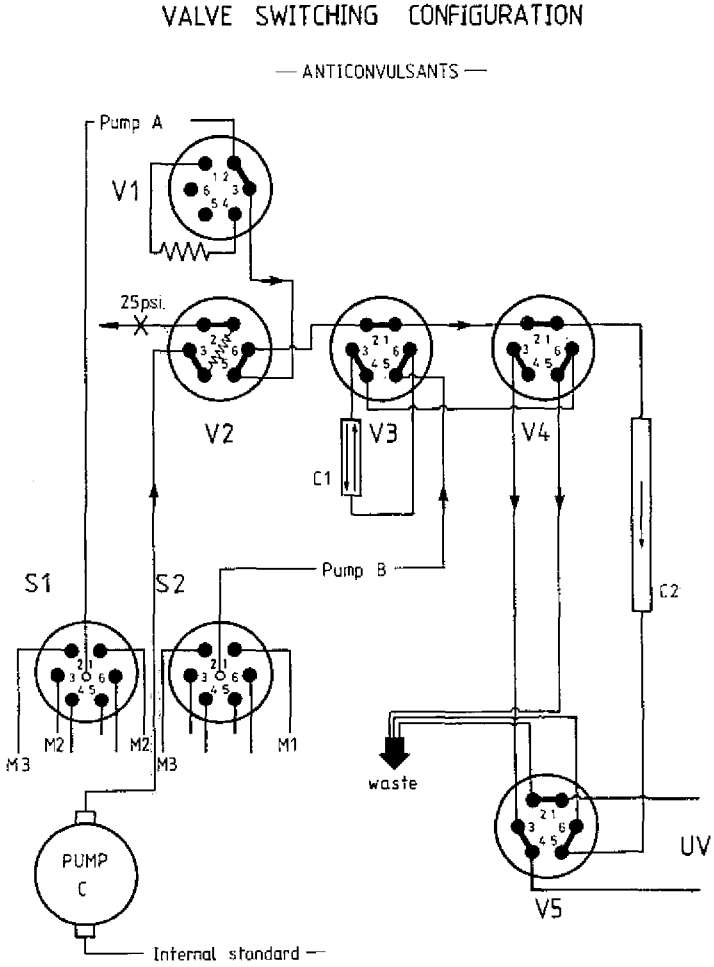
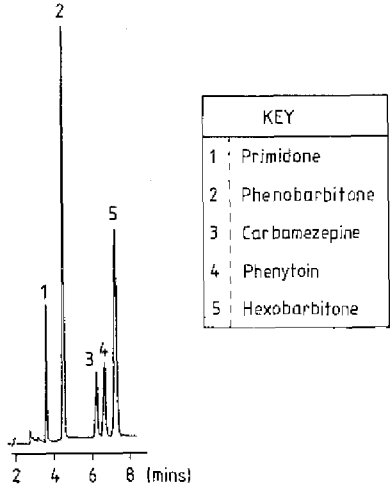


FIGURE 7 Valve configuration for anticonvulsants in serum.

The chromatography has been developed with speed of analysis uppermost in mind. The procedure is suitable for routine use in hospital toxicology laboratories where speed is of the essence (a) to provide a rapid information service and (b) to cope with the high number of samples involved. (Figure 8)



Chromatogram of ANTICONVULSANTS in serum

Pre column :- Brownlee RP 18 (20μm)

Column :- - - Spherisorb S5 C8 (12·5 cm)

Eluents M1 = MeOH: CH_3CN (8:2) / water 35 / 75

M2 = Water

M3 = Methanol

FIGURE 8. Anticonvulsants in serum with sample cleanup.

CONCLUSION

We have shown some of the ways in which column switching can be used in HPLC. Obviously there are many more ways such as recycling and trace enrichment. The authors are of the opinion that

now there is commercial instrumentation available for carrying out complex valve switching techniques, there will be a dramatic change in approach to the separation problem during the next 5 years, and that the major limitations will often be those of the imagination of the user.

References

1. C. J. Little, O. Stahel, W. Lindner, and R. W. Frei, *International Lab.* 1984 (in press).
2. C. J. Little, D. J. Tompkins, O. Stahel and R. W. Frei, Werkhoven-Gouwie, C.E., *J. Chromatog.* **264**, 183, (1983).
3. Kontron HPLC report, Application 383.
4. A. D. Kingman, N. P. D. Nanayakkara, D. D., Soejarto, P. J. Medon and S. Kamarth, *J. Chromatog., chrom* **14**, 757 (1982).
5. Kontron HPLC report, Application 182.
6. Kontron HPLC report, Application 1783.