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ROLE OF COLUMN SWITCHING IN SEMI-PREPARATIVE LIQUID CHROMATOGRAPHY

ISOLATION OF THE SWEETENER STEVIOSIDE

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SUMMARY

Column switching has been used specifically to carry out semi-preparative liquid chromatography. A fully automated instrument is described which includes facility for repetitive sampling and fraction pooling. The combination of two cleanup steps is shown to give a good throughput of high-purity product. Whilst the second stage progresses, the first column can be cleaned and re-equilibrated in readiness for the next loading.

INTRODUCTION

Semi-preparative liquid chromatography (SPLC) is similar in concept to high-performance liquid chromatography (HPLC) but has been scaled up in size to enable the isolation of weighable quantities of solute. These compounds are often required for further studies by NMR, mass spectrometry and other techniques which allow the compound to be characterised. Occasionally, it is necessary to isolate enough compound in high purity for use as an analytical standard and at the limit of definition of SPLC, purification is often carried out on an intermediate in an organic synthesis. Depending on the nature of the problem, there are often conflicting priorities, such as high purity and throughput of sample. Quite often high throughput is achieved at the expense of purity. High throughput is also related to the level of sample loading, which, in turn, is affected by column dimensions and eluent flow-rate. An excellent review of definitions, strategy, and practice in preparative liquid chromatography is given in ref. 1.

In a recent review[2] the concepts and scope of column switching in modern HPLC have been described, and it was pointed out that some of the techniques used in column switching can be beneficially used in the scale up to SPLC.

In HPLC, column switching is most important in the context of sample clean-up. An unresolved or only partially resolved zone, eluted from a pre-column or first

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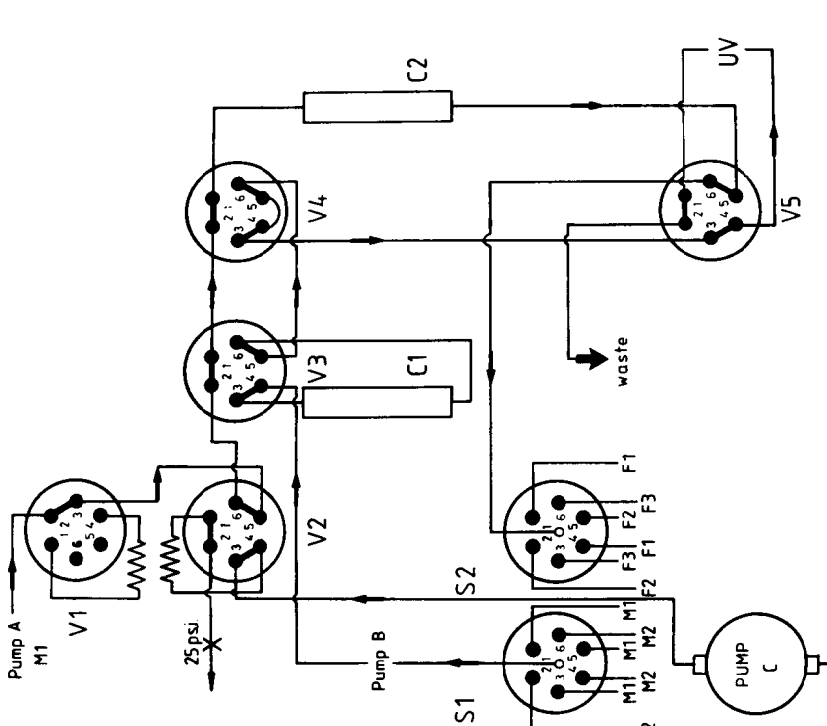

Fig. 1. Chromatogram of an extract from *Stevia rebaudiana*. Column: 25 cm Kontron Spherisorb S5-ODS; eluent: methanol–water (70:30) at 2 ml/min; detection: UV at 214 nm, 0.5 AUFS.

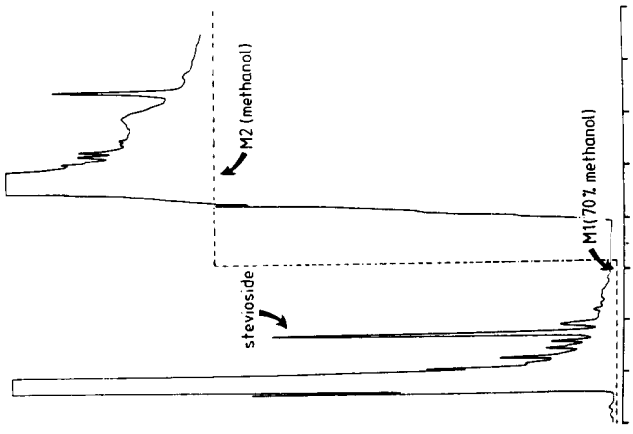

Fig. 2. Column-switching system for semi-preparative liquid chromatography.

stage column is transferred to a second column for further separation. This zone transfer is effected by the use of switching valves, which re-route the chromatographic eluent. Whilst the secondary separation is proceeding, the first stage column can be cleaned and equilibrated independently. Often, this results in a considerable saving of time.

The use of column switching should be more obvious in SPLC, because it is common practice to effect purification by taking a heart cut, especially when the column is overloaded. Usually, the operator is watching the chromatogram developed and physically changes the collecting vessel. Alternatively, fixed-volume fractions can be collected and suitable ones are pooled to give the required product.

When the sample is very dirty, a preliminary off-line cleanup can be carried out and further purification achieved by using on-line SPLC methods.

It is only an extension of these already well established methods to use column switching completely on-line. This paper illustrates the modified strategy necessary for column switching and uses as an example the isolation of the sweetener stevioside from dried leaves[3].

EXPERIMENTAL

Column switching was carried out with the Kontron "TRACER" MCS 670 column switching unit. One of the pumps used was a Kontron Model 414, modified with a preparative head, which gives a higher flow-rate. Other pumps used were the standard HPLC Model 414 units. The detector used was the Kontron Uvikon 720 LC, which is a UV detector with wavelength-scanning capability. Control of the complete system, including column switching was through the Kontron programmer, Model 205.

Kontron semi-preparative reversed-phase columns were used which were packed with 10 μm Spherisorb ODS material.

HPLC-grade methanol was obtained from Rathburn. The water used was freshly double-distilled. It was *not* subjected to ion-exchange purification.

Dried leaves from the plant *Stevia rebaudiana* (5 g) were macerated (5 min) with 20 ml of an aqueous methanol solution (methanol–water, 80:20). After filtration, the green extract was concentrated and then used directly.

RESULTS AND DISCUSSION

Any preparative separation must be developed from thin-layer experiments and/or from analytical HPLC studies.

Fig. 1, the reversed-phase HPLC separation of an extract from the dried leaves of *Stevia rebaudiana*, shows that the sweetener can be reasonably well separated from other material, but that substantial quantities of long-retained species have to be washed from the column before a second analysis can be carried out.

At this stage, the system can be usefully transferred to a column-switching unit, as shown in Fig. 2. This figure describes what is essentially a fully automated semi-preparative chromatograph. Method development can be carried out with manual injection through the valve V1, and repetitive loading is carried out through the valve V2. In this case, the sample loop is filled by using the built-in pump C such

that sample feed is returned to the reservoir after flushing the sample loop between loadings. A one-way valve is included in-line to prevent sample syphoning from the loop. Column selection and switching are carried out with valves V3, V4 and V5[2,4,5].

Solvent selection is carried out with the selector valve S1 and fraction collection is carried out by using the selector valve S2. The latter is extremely useful, as it does not require fixed sample volumes, but is only limited by the size of the vessel used. Each fraction can be of vastly different volume.

Using semi-preparative columns, the analytical HPLC system can be duplicated and scaled up. In Fig. 3, we can see how larger loadings can give a result very similar to the analytical chromatogram. Fig. 3a indicates the zone that was transferred from the primary column C1. By careful timing of the switching a high recovery of purified material can be transferred to the secondary column, where further separation takes place. A second zone cut is then taken (Fig. 3b) which is collected as fraction F2. Whilst this fractionation is proceeding, column C1 is cleaned after an eluent change to methanol (using S1) and is subsequently re-equilibrated, after a second eluent change, in readiness for the next loading. This facility for running two parts of the system independently saves much time and increases sample throughput significantly[6].

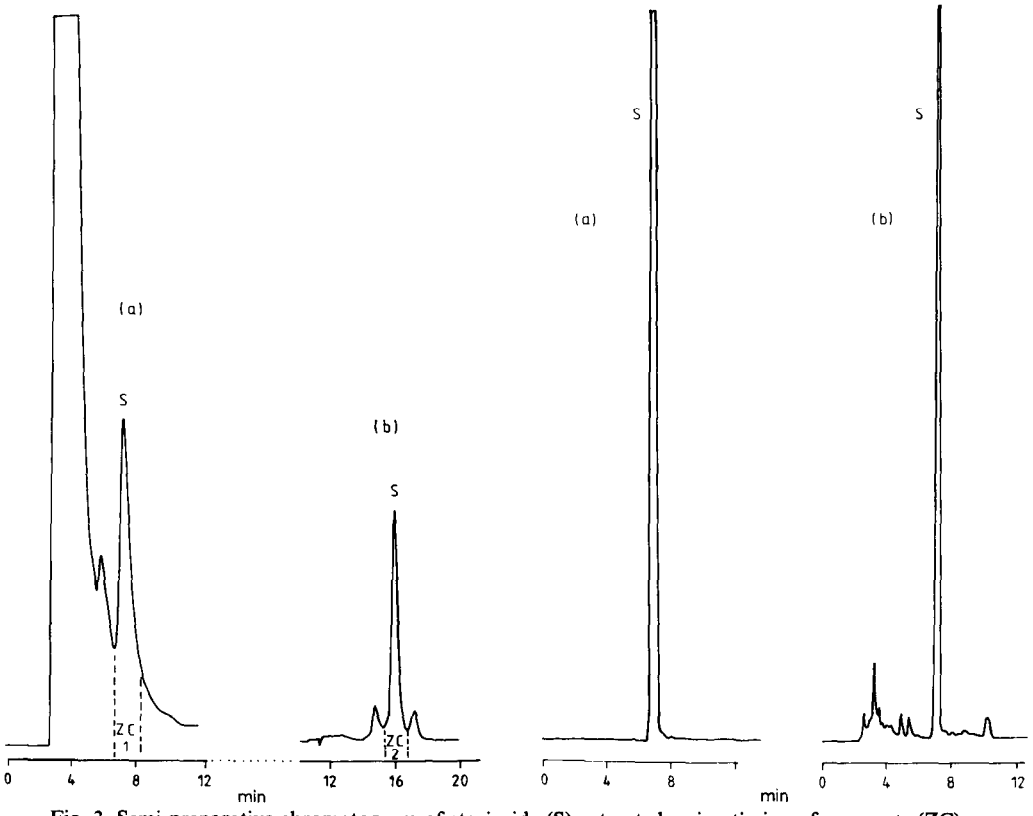

Fig. 3. Semi-preparative chromatogram of stevioside (S) extract showing timing of zone cuts (ZC).

Fig. 4. Chromatogram showing the purity of the isolated stevioside (S) (a) in comparison with a conventional isolate (b).

TABLE I

TYPICAL PROGRAM FOR SEMI-PREPARATIVE ISOLATION OF STEVIOSIDE

Chromatography details			*Program details*				
Configuration	*Valve*	*Position*	*File*	*Time*	*Function*	*Value*	
Reset tracer			55	0	670	Reset	
Reset detector				0.1	720	Reset	
Initial flow				0.12	Flow	4	
				0.12	% B	50	
Initial valve settings	V3	b		0.14	V3	670	
	V4	b		0.16	V4	670	
	V5	b		0.18	V5	670	
Inject sample	V2	b		0.2	V2	670	
Reload sample loop	V2	a		2.0	V2	670	Clear
				3.0	T.Pump	670	
				4.0	T.Pump	670	Clear
Zone transfer	V4	a		6.8	V4	670	Clear
	V3	a		8.3	V3	670	Clear
	S1	2		8.4	S1	670	
Flow-rate change				„	Flow	5	
				„	% B	60	
C1 equilibration	S1	3		14	S1	670	
Collect fraction 2	S2	2		15	S2	670	
	S2	3		16.8	S2	670	
				22	End		

The quality of separation that can be achieved is shown in Fig. 4, which compares the purity of stevioside isolated by the above procedure with a product isolated by conventional extraction procedures.

In practice, the interpretation of column switching can be difficult, and it is hoped that Table I and Fig. 5 showing the program, as entered into the programmer (Model 205), and the schematic of what each item in the system is doing at any one time will help in this matter.

The column-switching approach to SPLC is very flexible because the primary cleanup enables gross overloading of the column. Normally, gross overloading results in a less pure product due to resolution loss on the column. If pure product were then required, the pooled sample would have to be concentrated and re-chromatographed. This is the technique often used when stage 1 is carried out off-line by using open column chromatography and large-particle disposable columns.

The advantage of column switching is that this stage can be carried out on-line and the zone is transferred to the second column where higher resolution is achieved under conditions where the columns are frequently not grossly overloaded.

This approach is demonstrated in Fig. 6, which shows the timing of suitable zone cuts to produce a high yield of pure material.

FUNCTION ACTIVATED	4	8	12	16	20	
Inject(Load) Zone cut(ZC)		ZC 1		ZC 2		nb ZC, 2 = F2
C1	PA – M1		PB–M2		PB – M1	
C2	PB – M1		PA–M1			
Pump A (PA)	2 ml / min					
Pump B (PB)	2 ml/min		3ml / min			
Pump C (PC)	1					
Valve V2 position	b	a				
Valve V3 position	b		a			
Valve V4 position	b		a			
Valve V5 position	b					
Selector Valve S1 position	1=M1		2=M2		3=M1	
Selector Valve S2 position		1 = F1		2	3= F3	
Detector	Uvikon 720 LC, 220nm, 1AUFS					
Recorder	0·5 cm / min					
	4	8	12	16	20	

COLUMNS		MOBILE PHASES			
C 1	25 cm × 3/8 inch S 10 – ODS	M 1	Methanol–Water (70 : 30)	F 1	
C 2	25 cm × 3/8 inch S 10 – ODS	M 2	Methanol	F 2	Stevioside - main peak
C 3		M 3		F 3	
C 4		M 4			
		M 5			
		M 6			

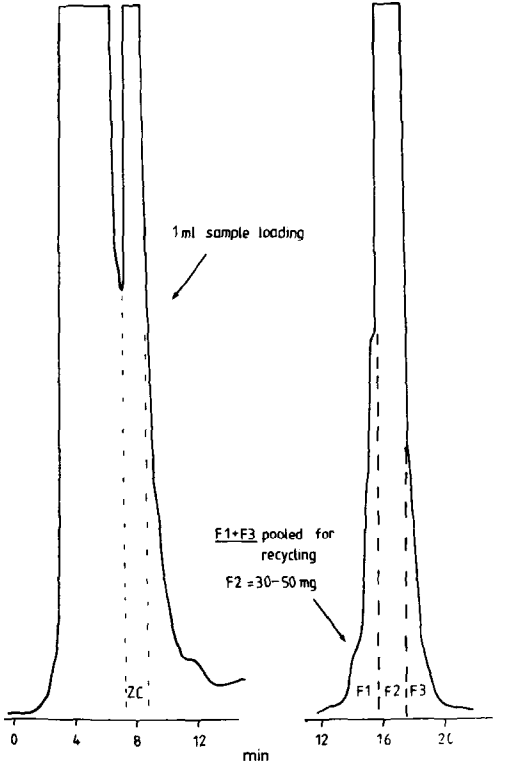

Fig. 5. Column-switching schematic for semi-preparative isolation of stevioside.

Fig. 6. Semi-preparative chromatogram of stevioside under conditions of gross overloading. Throughput of stevioside is between 30 and 50 mg per run.

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