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Review

Column-switching techniques for high-performance liquid chromatography of drugs in biological samples

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ABSTRACT

In recent years, an increasing number of publications have demonstrated the potential of column-switching techniques for the chromatographic separation, determination and preparative isolation of analytes from biological matrices. Column-switching systems greatly facilitate drug analysis, by on-line sample clean-up and trace enrichment, or by improving the analytical separative process. In this paper, the main applications of column-switching techniques to drug analysis in biological samples, are reviewed.

CONTENTS

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1. INTRODUCTION

The analysis of drugs and their metabolites or endogenous compounds in biological fluids by high-performance liquid chromatography (HPLC) is usually difficult, owing to the large number of substances present in the samples and because the compounds of interest are often present at very low concentrations. Therefore, both sample clean-up and enrichment of the investigated compounds must be carried out prior to analysis.

Sequential separation techniques using switching devices are well suited to such problems. Switching valves permit variable combinations of columns, carriers and/or detectors, which cooperatively contribute to the overall system selectivity [1]. The term "column-switching" or "coupled column chromatography" includes, in the widest sense, all techniques by which the direction of the flow of the mobile phase is changed by valves, so the effluent from a primary column is passed to a secondary column for a defined period of time. An unlimited number of columns can be theoretically incorporated in a chromatographic network. However, in each successive step, the transferred fraction must be reconcentrated to reduce the dispersion of the analyte onto the chromatographic system, and the dead volumes in the connections between columns and in any switching valves must be minimized to achieve maximum separation efficiency.

Switching within these networks can be effected manually or by automated controllers. In particular, electrically controlled valves greatly facilitate the full automation of the chromatographic process, thus increasing the speed and the work capacity of the HPLC system.

The operating modes of switching systems have been recently reviewed [2,3], and several studies indicate that, by changing the technique used to achieve the transfer of a fraction of the eluent from the primary to the secondary column, and/or by changing the switching function, versatile and powerful networks can be designed [4–6].

This paper reviews the main applications of column switching for the analysis of drugs in biological samples by HPLC.

2. APPLICATIONS OF COLUMN SWITCHING

In clinical analysis, column switching has been mainly applied to on-line sample clean-up and trace enrichment. However, in recent years, many other applications of column switching techniques have been reported. In addition, because of their flexibility, switching systems can be used to achieve various objectives within a chromatographic network. Different set-ups are required for each application, involving different levels of apparatus cost. The simplest configuration consists of two columns connected by a switching valve, and this has been successfully applied in many sample clean-up procedures. However, relatively sophisticated set-ups, involving additional switching valves, pumping systems, columns and/or detectors, may be required if highly selective separations are to be achieved.

2.1. Sample clean-up

The sample preparation step remains the most serious problem for the automation of HPLC [7]. Biological samples usually require some form of preparation before injection into the liquid chromatographic system. Two main problems are associated with direct injection: tight adsorption of some proteins to the column and rapid pressure build-up at the head of the column owing to protein denaturation and adsorption. As a result, resolution decreases and the column lifetime is greatly shortened.

Liquid extraction, followed by solvent evaporation, is the traditional method for sample preparation in drug analysis. However, liquid–liquid extractions usually involve several complicated steps for sample clean-up, which are time-consuming and often imprecise. In addition, this technique has proved difficult to automate efficiently [8]. The use of solid-phase extraction on disposable cartridges has been reported to increase laboratory throughput and to replace many liquid–liquid extraction procedures [9].

TABLE I

PROCEDURES FOR SAMPLE CLEAN-UP BY LIQUID–LIQUID EXTRACTION, SOLID-PHASE EXTRACTION ONTO A DISPOSABLE CARTRIDGE, AND COLUMN SWITCHING

Liquid–liquid extraction	Solid-phase extraction	Column-switching
Pipetting out the sample	Pipetting out the sample	Possibly centrifugation, filtration, and/or sample dilution
Addition of an internal standard	Addition of an internal standard	Injection
Addition of an organic solvent	Cartridge conditioning	Switching-valve rotation
Agitation	Elution of the sample	
Centrifugation	Matrix elimination by washing the cartridge	
Separation	Elution of the analyte	
Possibly re-extraction	Solvent evaporation	
Solvent evaporation	Redissolution	
Redissolution	Filtration	
Filtration	Injection	
Injection		

In recent years, an increasing number of HPLC methods incorporating on-line sample clean-up by solid-phase extraction using column switching have been developed. Switching devices permit the off-line multi-step methods to be transformed into single-step procedures by the on-line purification of the samples. The principle of column switching for sample clean-up is to trap, in a primary column or pre-column, the fraction of the sample that contains the analytes. The compounds of the biological matrix are eluted to waste, whereas the cut of effluent containing the analytes is diverted to the secondary column or analytical column, where they are separated for identification and/or quantification. The fraction of effluent to be transferred may elute at the front (front-cut technique), in the middle (heart-cut technique) or at the end (end-cut technique) of the chromatogram of the primary pre-column. Zone cutting probably is one of the most useful and versatile of all the column-switching techniques [4].

In Table I, the usual sample-preparation steps required in classical liquid–liquid extraction and solid-phase extraction onto disposable cartridges and those involved in a conventional sample clean-up process by column-switching are compared. As can be seen, classical preparation techniques involve numerous steps, which can cause loss of the compounds of interest. With column-switching techniques, the manual sample preparation steps are drastically reduced, or even eliminated. As a result, the accuracy and precision that can be obtained in the determination of drugs in biological fluids is generally improved [9]. In addition, the time required for processing the samples with this latter technique is greatly shortened, and the selectivity that can be reached is comparable with, and sometimes better than, that obtained with traditional sample pre-treatments. This is illustrated in Fig. 1, which shows typical chromatograms of urine samples processed by a liquid–liquid or solid-phase extraction and by column switching.

There are additional advantages of column switching over classical procedures for sample clean-up. It makes unnecessary the use of an internal standard, because both accuracy and precision are increased [10–12]; however, the introduction of an internal standard can be useful when long sequences of samples are processed [13]. On the other hand, sample clean-up by column switching protects light-sensitive analytes from light during the analysis [12,14]. Table II summarizes the main advantages and disadvantages of column-switching techniques for the sample clean-up.

TABLE II

MAIN ADVANTAGES AND DISADVANTAGES OF COLUMN-SWITCHING TECHNIQUES FOR SAMPLE CLEAN-UP

Advantages	Disadvantages
Minimal sample handling	Requirement of switching valves and additional columns and/or pumping systems
On-line sample processing	Requirement of compatible mobile phases
Considerable time reduction	Periodic replacement of pre-column
Possibly of fully automation	
Greater precision and accuracy	
Improvement in the selectivity by combining different chromatographic modes	
Avoiding of an internal standard	
Protection of photo-labile analytes	
Minor consumption of organic solvents	

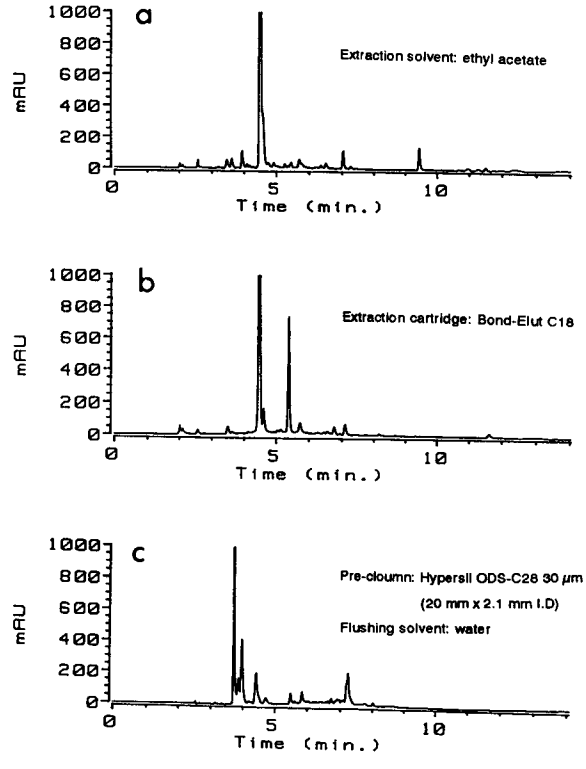

Fig. 1. Typical chromatograms obtained from a urine sample by (a) liquid–liquid extraction, (b) solid-phase extraction onto a disposable cartridge, and (c) on-line solid-phase extraction by column switching. Conditions: detection wavelength, 230 nm; analytical column, Hypersil ODS C_{18} (5 µm, 250 mm × 4 mm I.D.); mobile phase, acetonitrile–0.05 *M* phosphate buffer (pH 3); (In all cases, the amount of sample injected is equivalent).

The success of a sample preparation depends on a variety of factors: the type of sample, the pre-column, the eluent and the column-switching configuration.

2.1.1. Type of sample and protein binding

In principle, any liquid biological sample free from suspended particles can be injected into a switching system. Solid samples can be processed after prior solubilization and homogenization [15]. The type of sample matrix and the amount of sample injected determine the number of injections that can be processed before replacing the pre-column. The most problematic fluids are those that contain a large fraction of proteins: blood, plasma and serum. Cerebrospinal and interstitial fluids, as well as urine, are in general more compatible with liquid chromatographic systems owing to their low protein content [16], and therefore a simple filtration can provide suitable stability on the pre-column [17]. Different approaches have been suggested to increase pre-column lifetime, mainly the centrifugation [18,19], dilution [20–22] or filtration [23,24] of the samples, as well as the prior off-line extraction of the analyte [25]. Proteins can also been precipitated and removed before injection into the chromatographic system [26–28]. Routine laboratory use for several thousand injections has been reported [9,29].

Efficiencies lower than expected can be obtained if the drug is tightly bound to the sample proteins. In such a case, significant differences in the recovery can even be observed for the same kinds of sample [24]. The main ways to displace a drug from its binding site on a protein are to dilute samples or to add an organic solvent before injection into the pre-column [14,30–32].

2.1.2. Pre-columns

Clean-up effects mainly depend on the quality of separation and efficiency of the pre-column.

2.1.2.1. Dimensions. The pre-column dimensions must be chosen such that it has a high loading capacity to prevent losses of the analytes by break-through during the flushing step. These parameters should be selected taking into account the volume of sample to be injected, and the degree of protein binding. For most practical purposes, pre-columns much shorter than the analytical column provide acceptable results. Short pre-columns are preferable because the duration of the flushing needed to remove undesirable matrix components is minimized; moreover, they are easier to pack and need less packing material. However, if the analyte is tightly bound to the sample proteins, the length of the pre-column must be increased to avoid analyte break-through. Pre-column length can also be increased to improve the selectivity of the clean-up process. For example, primary columns as much as three times longer than the analytical column have been proposed for the pre-separation of different drugs in plasma [33]. The internal diameter of both pre-column and analytical column should be the same to minimize extra-column band broadening. A vast majority of authors employ pre-columns with an internal diameter in the 2–4.6 mm range [34].

2.1.2.2. Packings. The pre-column packing should have a relatively large particle size in comparison with the analytical column, otherwise clogging problems can be significant. For a majority of the applications, particle sizes in the 10–40 μm range provide suitable stability and loading capacity [9,35].

On the other hand, the retention capability of the pre-column should be lower than that of the secondary column; in such a way, the analyte is re-concentrated at the top of the analytical column and therefore the band broadening is minimized. For most biological samples, non-apolar packings in the pre-column retain the analytes of low or intermediate polarity, whereas the polar components of the matrix are discarded. Lecaillon *et al.* [35] studied the influence of the polarity of the drugs on the choice of the switching system. For compounds of low or medium polarity, reversed-phase chromatography can be well adapted. The best results were obtained using pre-columns packed with cyano, ethyl or diol materials, the analytical separation being achieved on octyl or octadecyl columns. A third column of lower polarity can be added to improve the specificity of the system. For compounds of high polarity, normal-phase chromatography was recommended, using amino or dimethylamino pre-columns, and a more polar material for the analytical column.

Simple column-switching assays often suffer from decreased selectivity in comparison with liquid–liquid extraction [13]. Using common reversed-phase materials, only proteins, salts and other highly polar sample constituents are flushed to waste, while several endogenous compounds are retained on the pre-column and transferred to the analytical column together with the substances of interest. The combination of different chromatographic modes during the overall process by selecting an alternative packing for the pre-column has been reported to provide enough selectivity for many applications. For example, different column-switching applications are based on the employment of gel-permeation [33,36] or ion-exchange materials [18,22,37] in conjunction with reversed-phase columns. Gel chromatography permits the separation of higher-molecular-mass matrix components, whereas the analytes and lower-molecular-mass compounds are retained on the pre-column. Low-molecular-mass matrix compounds frequently elute from the pre-column in the desorption step as a single peak, and therefore they are well resolved from the analytes; however, the

TABLE III

METHODS PROPOSED FOR THE ANALYSIS OF DRUGS AND THEIR METABOLITES BY COMBINING DIFFERENT CHROMATOGRAPHIC MODES

Pre-column	Analytical column	Analyte	Sample	Ref.
Gel chromatography packing				
TSK gel G2000 SW	ERC-ODS ERC-ODS μBondapak C_{18} μBondapak C_{18}	Cefmetazole Ketamine Warfarin Carboquone	Plasma	33
Ion-exchange packing				
Cation-exchange Bondapack Cx Corasil	Nova-Pak C_{18}	Amifloxacin and metabolites	Plasma and urine	22
Cation-exchange Bondapack Cx Corasil	Nova-Pak C_{18}	Cicletanine	Plasma	37
ISPR packing				
ISRP glycine-phenylalanine-phenylalanine	Waters Radial Compression C_{18}	Dopamine antagonist	Plasma	24
ISRP glycine-phenylalanine-phenylalanine	Brownlee C_{18}	Phenytoin	Plasma	39
ISPR	Nucleosil C_{18} TSK gel ODS 80-TM	Anticonvulsants Methylxanthines	Serum	41
Protein-coated				
Protein-coated ODS	TSK gel ODS 80-TM	Indole ring compounds	Plasma	42
Protein-coated CN	TSK gel ODS 80-TM	Clocapramine and metabolites	Plasma	43
Protein-coated ODS	Chemcrosorb ODS	Tryptophan and metabolites	Plasma and serum	44
Protein-coated CN	Ultron ES-OVM	Ketoprofen	Plasma	45
Copolymers				
XAD-2	LiChrosorb RP 18	Metaqualone	Plasma	46
PRP-1	Phenomenex ODS	Barbiturates Benzodiazepines Amphetamines Antidepressants Opiates	Urine	47
Immunoaffinity				
Sepharose-immobilized[a] polyclonal antibodies C_{18}[b]	LiChrospher 60 RP	Clenbuterol	Urine	49
Sepharose-immobilized[a] polyclonal antibodies C_{18}[b]	LiChrosorb RP 18	Oestrogen	Urine	50

[a] First on-line pre-column.
[b] Second on-line pre-column.

combination of two different pre-columns permits the sequential separation of low- and high-molecular-mass compounds [38]. Gel chromatography has also been recommended, because the selection of the mobile phase is simpler than for pre-columns with other separation modes. Ion-exchange columns do not require organic solvents for elution, and therefore they are slightly less subject to protein adsorption and denaturation compared with the reversed-phase columns. Table III gives some examples representative of the different combinations of chromatographic modes reported for biological samples.

A new packing material specially designed for the direct injection of biological fluids into chromatographic systems is the internal-surface reversed-phase (ISRP) silica support. Introduced by Pinkerton and co-workers [39,40], the fundamental principle behind the ISRP concept is to confine the hydrophobic partitioning phase exclusively to the internal particulate region of a porous silica support, while keeping the external surface of the support hydrophilic and non-adsorptive to proteins. When biological samples are injected, all proteinaceous substances are excluded and recovered in the column void volume, while the small hydrophobic analytes penetrate the particles and interact with the hydrophobic partitioning phase. Switching devices greatly facilitate the ISRP supports to be applied to drug analysis in biological fluids, as the protein fraction is directly wasted whereas the drug fraction is retained and subsequently switched to a conventional analytical column. Examples of application of this technique [24,39,41] are given in Table III.

A number of methods are based on the use of protein-coated silica in the pre-columns. In this approach, small-pore silica is coated with denatured plasma proteins [42]. The resulting packing has reversed-phase characteristics for small molecules, but has no affinity for proteins. Applications of protein-coated silica with almost complete recoveries of analytes from plasma and serum samples have been reported [43–45] (see Table III).

The special copolymers of polystyrene and divinylbenzene, XAD-2 and PRP-1, have successfully been applied for the analysis of different kinds of sample [46,47] (see Table III). The main advantage of using these packings is the possibility of using eluents with a high pH.

In recent years, different assays in which immunoaffinity pre-columns (usually, polyclonal antibodies immobilized on Sepharose) are used for selective pre-concentration and clean-up of biological samples have been reported. Owing to their high selectivity, immunoaffinity pre-columns yield high clean-up efficiencies [48–50] (see Table III).

2.1.3. Flushing eluent

The flow-rate of the flushing solvent and the duration of the wash-step must be adjusted to obtain suitable efficiencies. The time required for the washing step depends on the sample volume and on the pre-column loading capacity. These parameters must be adjusted to prevent loss of the analytes by break-through, as has been previously indicated. Moreover, the mobile phase composition should re-concentrate the analytes before injection into the analytical column, to reduce band broadening (effect of "peak compression"). Peak compression can best be obtained if the analytes are introduced into a column in a solvent with a low elution strength [51]. Conventional reversed-phase chromatography typically uses water as non-eluting solvent and, for most applications, aqueous eluents are used to avoid band broadening when analytes are transferred from the pre-columns to the analytical columns. In the case of strong acidic or basic analytes, the employment of an appropriate buffer instead of water is recommended [9]. If other chromatographic modes are involved, the mobile phase composition must be adjusted to provide the same effect [52].

In recent years, the employment of surfactants as mobile phase modifiers (micellar chromatography) has been reported to allow the direct injection of biological fluids through the solubilization of sample proteins. However, micellar chromatography shows only moderate efficiency owing to poor mass transfer, and it is inadequate

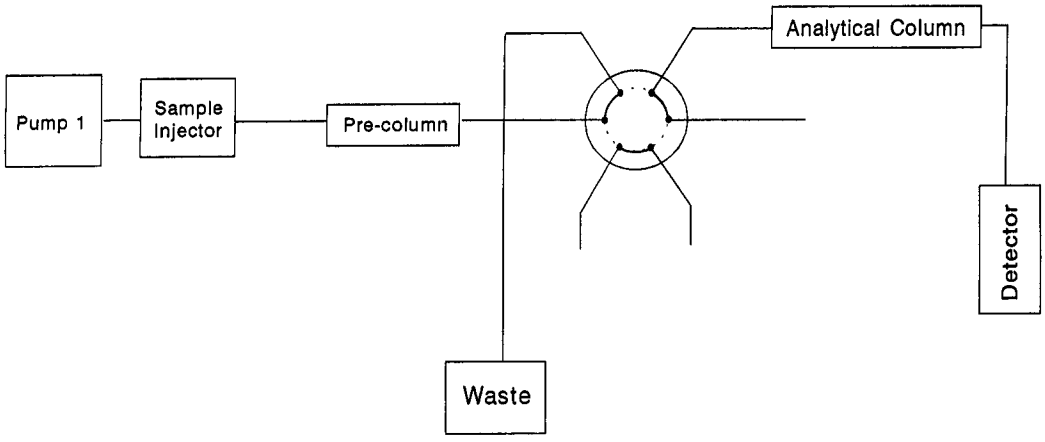

Fig. 2. Chromatographic system used for sample clean-up in straight-flush mode. Valve positions: injection and pre-separation (——); analytical separation (---).

for many applications. Column-switching techniques combine the advantages of micellar chromatography (for direct sample injection) and the conventional reversed-phase process (for the analytical separation) [53]. Posluszny *et al.* [54,55] illustrated the applicability of this approach by determining different therapeutic compounds in plasma and serum. When the analytes are tightly bound to sample proteins, the recovery can be increased if the surfactant is directly added to the samples [56].

If very sensitive detectors (*e.g.* electrochemical) are to be used, the composition of the mobile phases used for elution in the primary and in the secondary columns should be as similar as possible to prevent baseline drift [24]. With diode-array technology, timed programmed-wavelength switching can correct or eliminate baseline shifts [55]. In recent years, the use of column-switching has been reported to be very useful in conjunction with mass spectrometric (MS) detectors. Switching valves are used to remove aqueous eluents used in the clean-up and separation steps. After that, the trapped analytes are eluted in a solvent suitable for the HPLC–MS [57–59].

2.1.4. Column-switching configuration

The simplest configuration described for processing biological samples by column switching is shown in Fig. 2. Samples are initially chromatographed onto the pre-column, the unwanted components being directly vented to waste. By rotating a six-port switching valve, the cut fraction of eluate containing the analytes is directed to the analytical column for further separation (straight-flush mode). This scheme has been used for the analysis of several drugs, such as oxiracetam [21], ofloxacin [27], aminopyrine [60] or adriblastin [61], in different kinds of sample.

A more powerful system can be achieved by operating in back-flush mode (Fig. 3). This configuration is obtained by changing the external connections to the valve. The back-flush technique reverses the flow of the primary column, so the analytes retained at the head of the pre-column are directly transferred to the analytical column. The pre-column can also be back-flushed to remove the components of the matrix that are strongly retained, after the fraction of interest has been eluted [20]; thereby, the analytical column is protected from contamination by late-eluting matrix components. The back-flush configuration also minimizes peak-broadening. However, additional pumping systems are often required to obtain suitable stability, and the pre-column packing can be disturbed by applying mobile phases alternately in opposite directions [16]. The back-flush mode has been used for the separation and determination of several antiarrhythmic [23,28], gastrointestinal [37], antihyper-

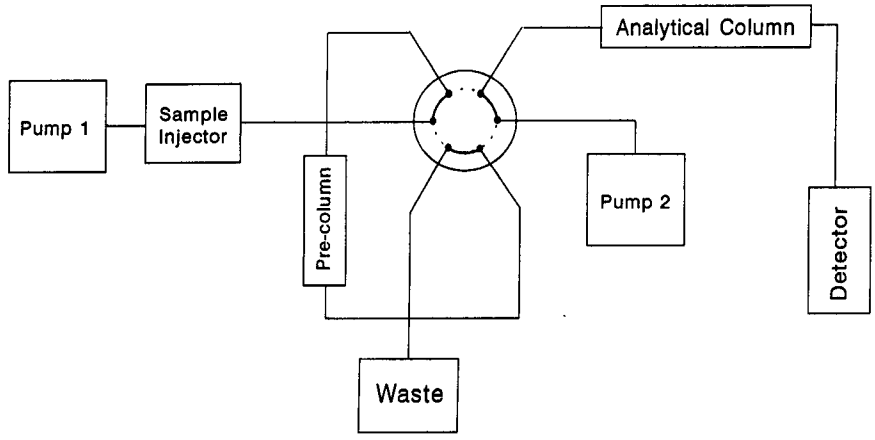

Fig. 3. Chromatographic system used for sample clean-up in back-flush mode. Valve positions: injection and pre-separation (——); analytical separation (---).

tensive [20,25,36] or antidepressant [62] drugs and related compounds.

The installation of additional switching valves offers the possibility of combining cut techniques and the back-flush mode. For example, Wyss and Bucheli [14,30] have reported the determination of several retinoids in plasma using a device containing three switching valves. This system permits the straight and back-flush purging of the pre-column and the capillaries between the automatic sample injector and the detector, then increasing the lifetime of both the pre-column and the analytical column. Timm *et al.* [13] have also described a system of three switching-valves for the analysis of non-benzodiazepine anxiolytics in plasma.

Many authors have reported the combination of switching devices with automated disposable cartridge-exchange modules, for on-line sample handling. The main advantage of a disposable cartridge system is that the retention conditions are maintained constant from one sample to another, as one disposable cartridge is used per sample. This technology has been applied to the analysis of retinoids in plasma [13], antiepileptic drugs in serum, anticancer agents in plasma, barbiturates in urine [63] or catecholamine metabolites in plasma and urine samples [64, 65].

2.2. Sample enrichment

In biological samples, interesting compounds are often present at very low concentrations. Therefore, enrichment of investigated compounds has to be carried out. Sample enrichment by column-switching is based on the fact that the analytes are trapped in a narrow zone on the top of a pre-column when a large volume sample is pumped. The eluent is subsequently changed, and the enriched solute species are switched to the analytical column. Unfortunately, sample enrichment also concentrates components other than the analytes, thus limiting the degree of concentration possible.

According to the basic principles indicated in previous sections, the optimization of the chromatographic conditions should include careful choice of the type of pre-column (dimensions and packing), eluent conditions and system design, to obtain good recovery and minimum peak-broadening of the compounds of interest [66]. The best enrichment factors can be achieved by back-flushing the analytes from the pre-column to the analytical column. The back-flush configuration permits samples up to 50 ml to be directly injected without significant loss of resolution, resulting in enrichment factors of *ca.* 200 [32].

The first application of column switching for

sample enrichment was reported by Roth *et al.* [67]. They proposed a functional alternating pre-column sample enrichment device, consisting of a programable automatic sample unit, which was connected via two alternating working pre-columns to an analytical column. Then, work capacity was greatly increased, because while one pre-column is eluted in back-flush mode onto the analytical column, the other pre-column is reconditioned. This system was applied to the determination of different antiplatelet and cardiotonic drugs in plasma, urine and saliva by reversed-phase or ion-exchange chromatography. A similar system has been used by Juergens [68] for the determination of different anti-epileptic drugs and their metabolites in serum.

Nielen *et al.* [69] described a micro-pre-column inserted within the axis of a common six-port switching valve, which was applied for the determination of clobazam and its active metabolite in plasma. In this way, extra-column broadening can be reduced, and the sensitivity greatly increased. The same authors have proposed a dual-pre-column approach for the determination of ethenyl steroids in urine at trace levels [10]. In the first pre-column (prepacked with a styrene–divinylbenzene copolymer) the analytes were separated from inorganic and water-soluble constituents, and subsequently eluted to the second pre-column (an Ag(I) oxine column), on which they were selectively trapped in a narrow zone by complexation. Finally, the ethenyl steroids were back-flushed to the C_{18} analytical column, on top of which peak compression occurs. The detection limits obtained were in the sub-nanogram range, with UV absorbance detection.

Aerts *et al.* [70,71] have studied the potential of dialysis coupled on-line with trace enrichment by column-switching, for complex biological samples containing very low analyte concentrations. The sample is dialysed on-line through a flat cellulose membrane, to eliminate large proteins or lipid macromolecules, and the dialysate containing the drug is fed into a concentration pre-column. After that, the enriched sample is back-flushed to the analytical column. The combination of dialysis and the pre-column technique can be very useful in the successive removal of high- and low-molecular-mass matrix components, in a fully automated process.

2.3. Improvement of the separation process

Column-switching systems are often used, after conventional off-line clean-up or pre-concentration of the samples, to improve the analytical separation. Highly selective separations can then be achieved by changing the column packing, the total column length, the eluent composition and/or the detector within a chromatographic process. For example, Yamashita *et al.* [72–75] have applied column-switching techniques for the UV determination in plasma of several basic compounds, which show significant absorbances only in the short-wavelength region, where most of the matrix compounds also absorb. The proposed assays involve the liquid–liquid extraction of the analytes and their pre-separation on a primary column by ion-pair chromatography. Finally, the heart-cut fraction of interest is switched for further separation onto a reversed-phase secondary column. In this way, selective and sensitive analysis of a wide variety of basic compounds can be achieved without prior derivatization.

A special area of interest of column-switching is the resolution of enantiomeric drugs in biological fluids, by coupling chiral systems to conventional reversed-phase columns. The order in which the columns are combined can be varied to obtain adequate resolution and peak compression. Walhagen *et al.* [76], using a set-up similar to that shown in Fig. 4, reported the isolation of terbutaline, metoprolol, oxazepam, bipivacaine and metoprolol enantiomers in plasma. The first column is packed with a chiral stationary phase for separation of the enantiomers, which are then trapped and compressed on two separate achiral columns, and subsequently transferred to a fourth column for final separation. The overall effect was an increase in efficiency and selectivity. The set-up includes a ten-port switching valve. Instead, an arrangement consisting of three six-port switching valves can be used with the same

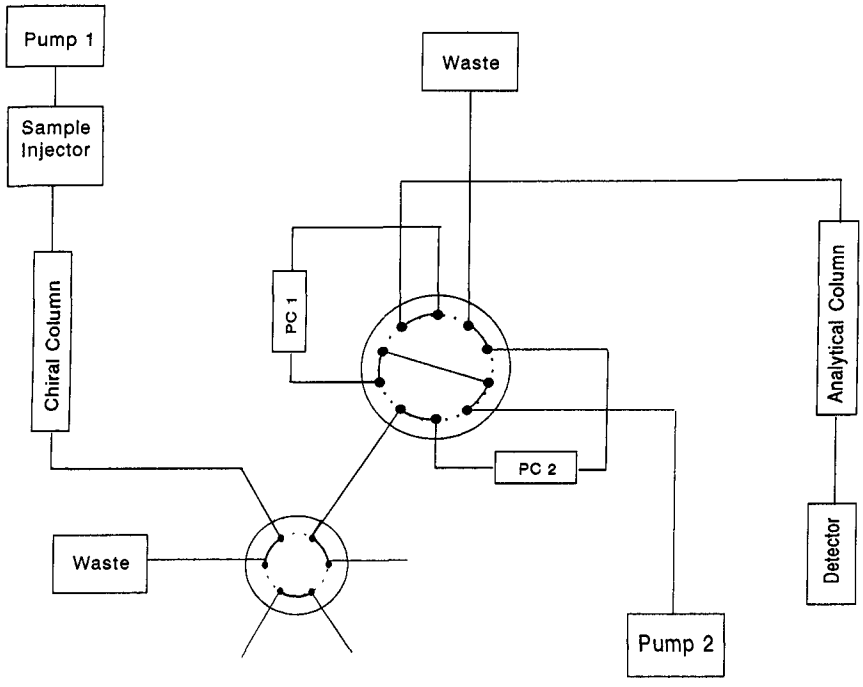

Fig. 4. Chromatographic system used for separation of chiral drugs by column-switching. The ten-port valve directs the previously separated enantiomers to different pre-columns (PC 1 and PC 2), and subsequently to the analytical column.

objective [58]. The drugs were previously extracted from the matrix with C_{18} solid-phase extraction cartridges or after a liquid–liquid extraction onto hexane. Hsieh and Huang [77] described a separation of the enantiomers of the major metabolite of phenytoin in different biological samples. In such an assay, the racemate is first trapped on a reversed-phase pre-column and subsequently transferred to a chiral ligand-exchange column, where the enantiomers were separated. A similar scheme has been applied to the resolution of verapamil enantiomers in plasma [78].

On the other hand, selection of a suitable column length by column-switching has been reported to be particularly useful in the reduction of the time required for the analysis of groups of compounds of very different polarities. For example, the major difficulty in the HPLC analysis of catecholamines and metabolites is related to their chemical heterogeneity, which results in large differences in polarity and consequently in retention times. Julien *et al.* [79] reported a sophisticated system, consisting of four switching valves that connect four columns packed with the same stationary phase, but with different lengths. After off-line sample pre-treatment, analytes of major polarity were eluted through the four columns, whereas analytes of minor polarity are eluted only through the first one. This allows the total analysis time to be reduced from 60 to 35 min.

Column-switching devices have been used to eliminate unwanted products after off-line derivatization [80], or to facilitate the on-line derivatization of many drugs by trapping the solutes on a reaction pre-column. Fig. 5 illustrates a simple set-up for sample derivatization by column-switching. This scheme has recently been applied to the determination of amphetamine in plasma, using a derivatization column packed with a fluorescent resin-based reagent [81].

A very interesting form of column-switching is the so-called boxcar chromatography, developed by Snyder *et al.* [82]. The boxcar configuration

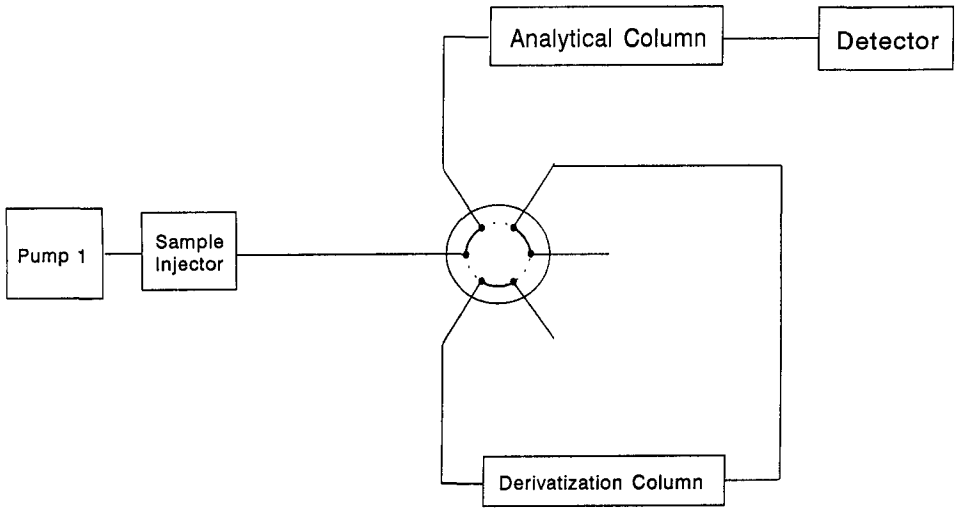

Fig. 5. Chromatographic system used for sample derivatization by column-switching. Valve positions: derivatization and pre-separation (——); analytical separation (---).

involves the partial separation of one or a few compounds of interest on a primary column, with diversion of the resulting fraction to a secondary column, in which these fractions are injected with the maximal frequency permitted by the resolution of the separation column. This technique can be used on either an analytical or a preparative scale, and permits effective separations with a considerable reduction of the total time of analysis. As an example, a boxcar configuration was developed for different antiepileptic drugs and their metabolites in serum [83].

3. CONCLUSIONS

The use of column-switching techniques has become a major area of interest, and its application to drug analysis has been the subject of an increasing number of publications. Switching devices have been proved to simplify the HPLC analysis of drugs in biological samples, by facilitating the total automation of the chromatographic process, then increasing the speed and work capacity.

By careful selection of the chromatographic parameters, flexible chromatographic systems with varying levels of apparatus cost can be designed. These systems can be used for the on-line clean-up of samples, the pre-concentration of the analytes, the highly selective separation of similar compounds, and/or the reduction of the time required for the analytical separation. Switching devices also avoid the use of some materials (such as extraction solvents), which can compensate for the prices of the required switching valves, additional pumping systems or chromatographic columns.

4. ACKNOWLEDGEMENT

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