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FULLY AUTOMATED SAMPLE HANDLING SYSTEM FOR LIQUID CHROMATOGRAPHY BASED ON PRE-COLUMN TECHNOLOGY AND AUTOMATED CARTRIDGE EXCHANGE

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SUMMARY

The design of an automated cartridge exchange module for on-line sample handling in liquid chromatography is described. When combined with a low-cost purge pump, a solvent selection valve and an auto-sampler, a fully automated sample handling system is obtained. Samples are sorbed on a disposable cartridge packed with 40 μm octyl-bonded silica, purged for clean-up and eluted on-line to the analytical column. Unattended operation of the system is demonstrated for various examples, *i.e.*, the determination of anti-epileptic drugs in serum, an anti-cancer drug in plasma, barbiturates in urine, phenylurea herbicides in river water and caffeine in a soft drink.

INTRODUCTION

Nowadays, sample handling is often the time-determining step in liquid chromatography (LC). Despite the use of automated LC systems and auto-samplers, for many LC analyses sample preparation is almost invariably carried out manually. However, on-line coupling of sample handling steps to a liquid chromatograph via pre-column technology is very attractive[1]. In this instance, basically solid-phase extraction procedures are employed, *i.e.*, the sample is loaded on a suitable sorbent, purged for clean-up and finally eluted on-line to the analytical column.

Recently, McDowall *et al.*[2] evaluated liquid–solid sample preparation in drug analysis. The advantages of solid-phase extraction over traditional (manual) liquid–liquid extraction procedures were pointed out and an overview was given of

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the state-of-the-art of manual and automated procedures. In addition, off-line and on-line (referred to in their paper as "column switching") procedures were compared. They observed that the development of a new generation of fully automated sample preparation systems is essential in order to exploit fully the advantages of solid-phase extraction.

The impact of sample handling via solid-phase extraction procedures has been clearly recognized by several companies. Table I summarizes some different approaches which are commercially available. Except for the (pre)column switching methods and the automated sample handling (PROSPEKT) system described in this paper, none of the other systems is fully automated. For the PROSPEKT approach, we designed an inexpensive disposable cartridge and a simple cartridge transport system, which can be controlled by, *e.g.*, the built-in microprocessor of an autosampler.

General considerations

Several workers have described the routine use of on-line pre-columns for automated solid-phase extraction prior to LC[7–12]. A trend towards the use of relatively small pre-columns (length 2–10 mm, I.D. 2–4.6 mm) packed with large particles (d_p between 25 and 60 μm), equipped with stainless-steel sieves instead of porous frits and connected with relatively wide-bore (0.5 mm I.D.) capillaries, can be clearly recognized. Obviously, prevention of blockage of the system by protein fragments has been an important aspect of the design of these pre-columns which, in most instances, are used for a number of subsequent analyses. Most workers apply 5–250 μl of serum or plasma samples to such pre-columns, but usually after initial dilution, filtration, centrifugation and/or pH adjustment, which are necessary for the proper functioning of the system. Depending on the clean-up achieved via these off-line manipulations, up to a total of 20 ml plasma or serum can be applied, independently of the individual injection volumes, before the pre-column (which is cleaned after each analysis) and the guard column have to be replaced.

On the other hand, if one intends to take full advantage of the potential for automation, off-line manipulations should be reduced to the absolute minimum. This unavoidably will result in a serious reduction of the pre-column lifetime. In addition, when the concentration of the analyte(s) varies over a wide range, the pre-column should be exchanged frequently in order to prevent memory effects. In essence, from

TABLE I

AUTOMATION POTENTIAL OF COMMERCIALLY AVAILABLE SOLID-PHASE EXTRACTION SYSTEMS

Stage	*Baker-10*[3]	*Bond-Elut*[4]	*Du Pont Prep*[5]*	*AASP*[6]	*PROSPEKT***	*(Pre)column switching*[1,7]
Sampling	Off-line	Off-line	On-line	Off-line	On-line	On-line
Purging	Off-line	Off-line	On-line	On-line	On-line	On-line
Elution	Off-line	Off-line	Off-line	On-line	On-line	On-line

* No longer available.

** This work.

the point of view of quality assurance, it is preferable to use a fresh pre-column for each analysis. Finally, there is another important aspect, *viz.*, the use of more selective pre-column sorbents, such as ion exchangers[13,14] and metal-loaded phases[15]. Regeneration of these pre-column materials is often tedious, time consuming and sometimes even impossible.

For the above reasons, there is obviously a need for a sample handling system that combines the advantages of pre-column/cartridge exchange (with its inherent quality assurance) and the automation aspects of pre-column technology. In this paper we describe such a system, which consists of a microprocessor-controlled auto-sampler, a simple cartridge exchanger, an inexpensive purge pump and a low-pressure solvent selection valve. The retention on the cartridges, extra-column band broadening, repeatability and analyte recovery were studied and the feasibility of the approach was demonstrated for five different matrices, *viz.*, serum, plasma, urine, river water and a soft drink.

EXPERIMENTAL

Apparatus

A Kipp & Zonen (Delft, The Netherlands) Model 4140 pump, equipped with a Kontron (Zürich, Switzerland) pulse damper, was used in combination with a Kratos (Ramsey, NJ, U.S.A.) Spectroflow 757 variable-wavelength UV absorbance detector. The automated sample handling system consisted of a Spark Holland (Emmen, The Netherlands) Promis auto-sampler and a prototype of a Spark Holland PROSPEKT system, consisting of a cartridge exchange module, an inexpensive purge pump (minipump VS; LDC-Milton Roy, Riviera Beach, FL, U.S.A.) and an electrically operated low-pressure six-port solvent selection valve (Latek TMV, Heidelberg, F.R.G.). Chromatograms were recorded and processed using an Anacomp Model 220 computer (Kontron). The sample handling system and the computer were controlled and started by the auxiliary contact closures of the Promis auto-sampler.

For comparison purposes, some experiments were performed with an Advanced Automated Sample Processor (AASP; Varian, Sunnyvale, CA, U.S.A.), which was loaded with 20 × 2 mm I.D. 40 μm octyl-bonded silica cartridges from Analytichem (Harbor City, CA, U.S.A.).

Stationary phases and columns

The analytical column was a 10 or 20 cm × 3.0 mm I.D. ChromSep (Chrompack, Middelburg, The Netherlands) 5 μm octadecyl-bonded silica column, equipped with a 10 × 2 mm I.D. guard column, pre-packed with pellicular C_{18} material. The pre-columns/cartridges were manufactured by Chrompack to our requirements; they are shown in Fig. 1. These cartridges (length 10 mm; I.D. 2 mm; O.D. 10 mm), which are constructed from poly(vinyldifluoride) (PVDF), are pressure-resistant up to at least 400 bar. They were slurry-packed with 40 μm octyl-bonded silica (Baker, Deventer, The Netherlands) and sealed with two 25 μm stainless-steel sieves (Dinxperlo, Dinxperlo, The Netherlands).

Chemicals

LC-grade methanol, LC-gradient grade acetonitrile and analytical-reagent

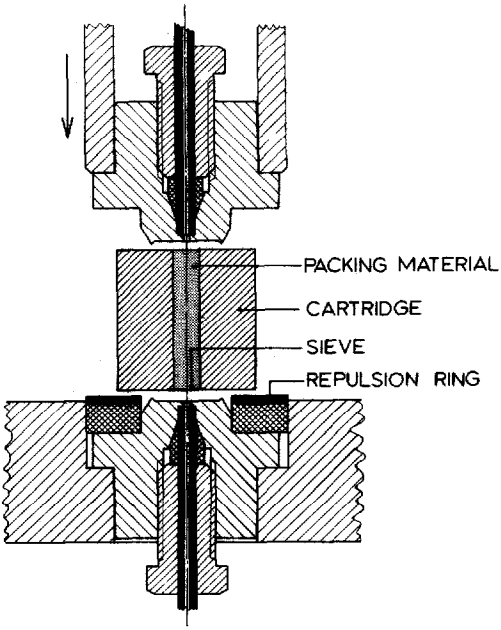


Fig. 1. Design of the pressure-resistant cartridge and the pneumatically controlled connection system.

grade phosphoric acid, potassium dihydrogen phosphate and acetic acid were obtained from Baker and 3,5-dichlorophenol and caffeine from Aldrich (Beerse, Belgium). The anti-epileptic drugs primidone, phenobarbital, phenytoin and carbamazepine (Katwijk Farma, Katwijk, The Netherlands) were kindly provided by Dr. B. Tuyl (Katwijk, The Netherlands). The anti-cancer drug etoposide (VP-16) and pharmaceutical-grade barbiturates, butabarbital, hexobarbital and secobarbital were a gift from the Free University Academic Hospital (Amsterdam, The Netherlands). The phenylurea herbicides chlorobromuron, diuron, chlorotoluron, monolinuron and monuron were obtained as a gift from the Food Inspection Service (Amsterdam, The Netherlands). Serum was obtained from Nyegaard (Oslo, Norway) and human plasma was obtained by collecting whole blood in heparinized tubes with subsequent centrifugation.

Demineralized water was purified in a Milli-Q (Millipore, Bedford, MA, U.S.A.) filtration system to obtain LC-grade water for use in eluents and standard solutions. Eluents were degassed in an ultrasonic bath under vacuum prior to use.

Procedures

Stock solutions of the model compounds were prepared by weighing and dissolving in methanol and stored at −20°C. The solutions were diluted with either water or blank serum, plasma, urine, river water or soft drink.

The reproducibility of the retention on the cartridges was studied for the model compound 3,5-dichlorophenol using aqueous 0.05% acetic acid as the mobile phase at a flow-rate of 1 ml min^{-1}. The influence of wetting of the cartridge by methanol prior to conditioning (with water or buffer, in order to remove the excess of methanol)

and sampling was investigated by storing the cartridges in methanol or, alternatively, by on-line treatment with methanol via loop injection.

The contribution of the cartridge exchange module to extra-column band broadening was studied using the 20-cm analytical column and a mobile phase consisting of acetonitrile–0.11% acetic acid (55:45) at 0.4 ml min^{-1}. Under these conditions, 3,5-dichlorophenol showed a k' value of 3. The total system efficiency was evaluated by comparing a 10-μl direct loop injection of 1 mg ml^{-1} of 3,5-dichlorophenol dissolved in the mobile phase with a pre-concentration experiment of the model compound (1 ml of the same sample, after its 100-fold dilution with 0.05% acetic acid). The cartridges were wetted on-line via a 1 ml loop injection of methanol and conditioned with about 5 ml of 0.05% acetic acid prior to the actual pre-concentration. Pre-concentration of the analyte and purging with 5 ml of 0.05% acetic acid were carried out at 0.5 ml min^{-1}.

Design of the automated sample handling system

The heart of the automated sample handling system is the pressure-resistant cartridge that can be pneumatically mounted and connected to a high-pressure switching valve (Fig. 1). Because of the small inner diameter of the cartridges, they are also compatible with narrow-bore (2–3 mm I.D.) LC systems. In addition, the length of the cartridge (10 mm) allows the introduction of highly protein-bound drugs, which will be released from the proteins with a recovery close to 100%, if an appropriate flow-rate is used[7]. The cartridges are placed in a transport strip, which is placed in a reservoir (Fig. 2). The self-adjusting transport mechanism is shown in more detail in Fig. 3. The complete fully automated system, shown in Fig. 4, further includes a purge pump, a solvent selection valve and an auto-sampler.

For actual operation, a cartridge is first wetted with methanol via the purge

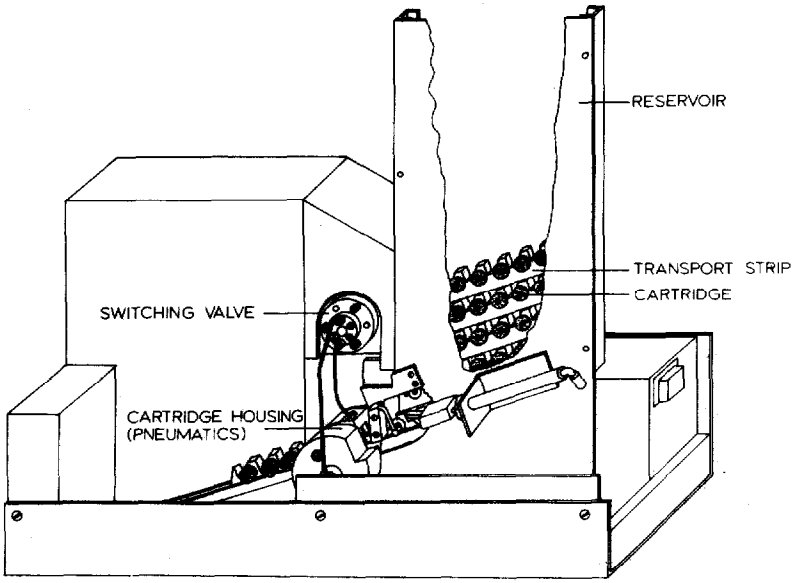


Fig. 2. Design of the automated cartridge exchange module.

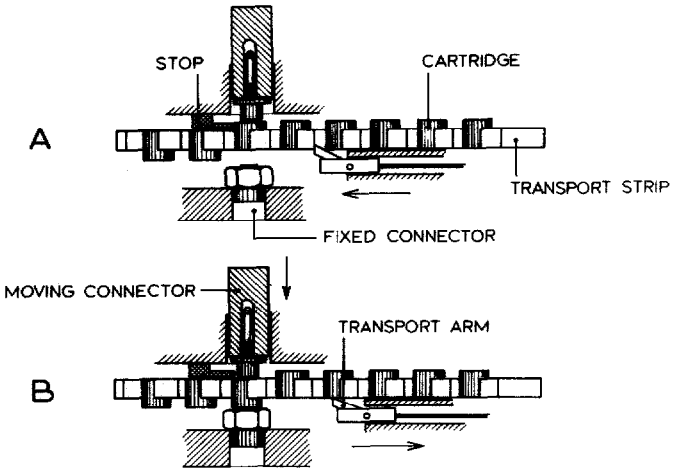


Fig. 3. Detail of the self-adjusting cartridge transport mechanism. (A) No connection, allowing cartridge transport; (B) closed, leak-tight connection with the switching valve.

pump, then the solvent selection valve switches to water or a buffer solution in order to condition the cartridge and to transfer the sample from the loop of the auto-sampler towards the cartridge. After sampling, the cartridge may be purged with the transfer solution or with another solution for clean-up. Next, the analytes are eluted from the cartridge in the forward-flush mode and the cartridge is disposed of and replaced prior to the next sample. Because of the forward-flush elution, the cartridge also acts as a filter that prevents precipitates, proteins or other particulate matter from clogging the connective outlet capillary.

The needle of the auto-sampler, and all capillaries through which the untreated sample is transferred, have inner diameters of at least 0.4 mm, to prevent clogging of the system.

The injection valve of the auto-sampler, the switching valve of the automated

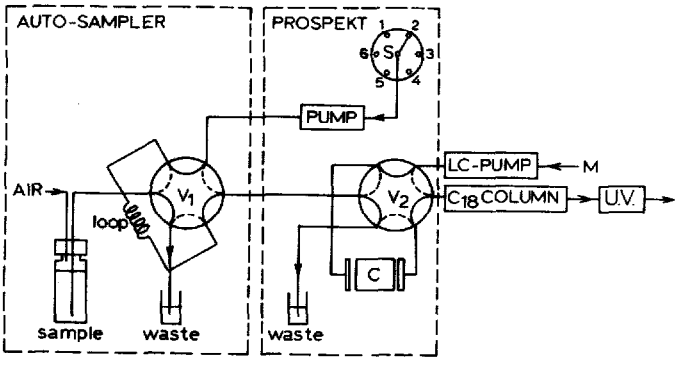


Fig. 4. Design of the automated sample handling (PROSPEKT) system. V_1, Injection valve; V_2, high-pressure switching valve; S, six-port solvent selection valve; M, mobile phase; C, 10 × 2 mm I.D. cartridge, packed with 40 μm octyl-bonded silica.

cartridge exchanger, the purge pump and the solvent selection valve are all controlled via the auxiliary programme of the Promis auto-sampler. A flow-chart of the system is given in the Appendix.

RESULTS AND DISCUSSION

Retention on the cartridges

The retention of 3,5-dichlorophenol on the cartridges expressed in terms of its breakthrough volume was found to be strongly dependent on the wetting of the sorbent prior to use (*cf.*, Table II). In accordance with general findings, wetting by methanol was an essential step in the solid-phase extraction procedure. However, the volume of methanol used was found not to be very critical, which allowed on-line wetting via a loop injection by using, *e.g.*, a vial filled with methanol in the satellite position of the Promis auto-sampler. However, we generally performed the on-line wetting via the purge pump and the solvent selection valve in our experiments. For our purpose the breakthrough volume and the relative standard deviation (R.S.D.) of the polar model compound are acceptable. It should be noted that in practical applications the total volume of sample and purge solvent is selected to be (far) below the actual breakthrough volume.

Contribution to extra-column band broadening

The influence of the cartridge system on the total system efficiency was investigated according to the procedure described under Experimental. The results are shown in Table III. Obviously there is no significant decrease in the total system efficiency when the PROSPEKT system is compared with a direct 10 μl loop injection. On the other hand, under comparable conditions the AASP showed a relatively poor performance, which resulted in a 30% decrease in plate number and serious fronting of the 3,5-dichlorophenol peak. Possibly the relatively long connecting capillaries from the cartridge to the switching valve of the AASP and the longer cartridges (2 cm) are responsible for this result.

Generally, it is disadvantageous to use long pre-columns when elution is carried out in the forward-flush mode[16], *i.e.*, in the same direction as the sample application, but of course this mode is to be preferred in order to maintain the protective filter aspect of the disposable cartridges.

TABLE II

BREAKTHROUGH VOLUMES (V_B) OF 3,5-DICHLOROPHENOL ACCORDING TO THE PROCEDURE DESCRIBED UNDER EXPERIMENTAL

Conditions: 10 × 2 mm I.D. cartridges pre-packed with 40 μm octyl-bonded silica; mobile phase, 0.05% acetic acid at 1 ml min^{-1}; injection volume, 33 μl (1.7 μg of dichlorophenol); detection, UV at 220 nm.

Cartridge pre-treatment	*n*	*V_B (ml)*	*R.S.D. (%)*
Not wetted	6	0.4	14
Stored in methanol	6	7.6	29
Wetted on-line with 33 μl of methanol	6	7.5	9

TABLE III

INFLUENCE OF THE CARTRIDGE EXCHANGE SYSTEM ON TOTAL SYSTEM EFFICIENCY ACCORDING TO THE PROCEDURES DESCRIBED UNDER EXPERIMENTAL

Method	*No. of analyses*	*Plate number*
10 µl loop injection	5	7900 ± 600
1 ml pre-concentration using the PROSPEKT system	6	7300 ± 600
1 ml pre-concentration using the AASP system**	6	5500 ± 400*

* Fronting.
** 20 × 2 mm I.D. cartridges.

Filter action of the cartridge

The filter aspect was clearly demonstrated by the following experiment. A 1 ml volume of whole blood, *i.e.*, including red blood cells, etc., was introduced into a cartridge in order to induce clogging of the system. This resulted in a serious increase in back-pressure, which was attributed to the cartridge and not to clogging of valves, connective tubing, guard or separation column. The hypothesis of the cartridge being the only part responsible was shown by exchanging the cartridge, which resulted in immediate restoration of the original back-pressure.

TABLE IV

CONDITIONS FOR APPLICATIONS USING THE AUTOMATED SAMPLE HANDLING (PROSPEKT) SYSTEM

Parameter	*Anti-epileptic drugs*	*VP-16*
Matrix	Serum	Plasma
Length of analytical column (cm)	10 + 1	10 + 1
Eluent composition	CH_3OH–H_2O (45:55)	CH_3CN–H_2O (25:75)
Flow-rate (ml min^{-1})	0.8	0.5
UV detection	195	230
Sample composition	1 ml stock solution of drugs + 1 ml 2 *M* phosphate (pH 3.5) + 18 ml blank serum	1 ml stock solution of VP-16 + 1 ml 2 *M* phosphate (pH 3.5) + 2 ml blank plasma
Sample volume (µl)	20	100
Concentration (µg ml^{-1})	5	0.2
Solvents selected	(a) CH_3OH; (b) H_3PO_4 (pH 2); (e) eluent	(a) CH_3OH; (b) H_3PO_4 (pH 2); (e) eluent
Flow-rate of purge-pump (ml min^{-1})	1	1
Timed events (solvent, ml):		
Wetting	(a) 0.5	(a) 0.5
Conditioning	(b) 2	(b) 2
Sampling	Loop	Loop
Purging	(b) 1.2	(b) 1.2
Desorption re-set	(e) 0.55	(e) 0.33

Applications

In order to demonstrate the range of applicability and analytical performance of the system, unattended analyses of real samples were carried out on five different model systems, *viz.*, anti-epileptic drugs in serum, an anti-cancer drug in plasma, barbiturates in urine, on-line trace enrichment of phenylurea herbicides from river water and the determination of caffeine in a soft drink.

Manual (off-line) sample preparation was restricted to the absolute minimum. For serum and plasma samples only buffer solution was added; the cola soft drink was degassed only and river water samples were filtered over a 0.8 μm membrane. With the urine samples, the most complex matrix under investigation, the elimination of interfering endogenous compounds caused some problems. In the final procedure we acidified urine with phosphoric acid to pH 3.5 and filtered the suspension thus obtained through a filter paper. Finally, the urine was diluted 1:1 with LC-grade water.

With most applications the samples were placed in auto-sampler vials and the reservoir of the cartridge exchange module was filled with the disposable cartridges. However, in the on-line trace enrichment of the phenylureas, the samples were introduced via the solvent selection valve and the purge pump. The analytical conditions for the different applications are summarized in Table IV. Figs. 5–9 show the chromatograms obtained with the system described above for the different matrices under investigation. Each chromatogram shows (a) a direct loop injection, (b) an analysis of a standard solution via the automated sample handling system and (c)

Barbiturates	*Herbicides*	*Caffeine*
Urine	River water	Cola
20 + 1	20 + 1	10 + 1
CH_3CN–H_3PO_4 (pH 2.7) (3:7)	CH_3OH–0.02 *M* phosphate buffer (pH 7) (45:55)	CH_3CN–H_2O (15:85)
0.5	0.4	0.5
254	245	280
1 ml stock solution of drugs + 19 ml filtered urine (pH 3.5) + 20 ml water	1 ml stock solution of herbicides + 99 ml river water	Degassed cola
20	10 000	20
5	0.01	Blank 60; spike 120
(a) CH_3OH; (b) H_2O; (c) 10% CH_3CN; (e) eluent	(a) CH_3OH; (b) H_2O; (c) sample; (d) H_2O; (e) eluent	(a) CH_3OH; (b) H_2O; (e) eluent
1	1	1
(a) 0.5 (b) 2 Loop (b) 5.5; (c) 0.5 (e) 1.0	(a) 0.5 (b) 1 (c) 10 (d) 2 (e) 2.4	(a) 0.5 (b) 1 Loop (b) 1 (e) 0.5

the automatic analysis of a real sample using the same conditions as in (b). Only in Fig. 8, the blank already contained the compound to be determined. In that case, chromatogram (c) represented a standard-addition experiment. Table V summarizes the analytical data obtained with the PROSPEKT system. Data for the repeatability and the recovery are the mean values of twenty experiments and for the memory effect the means of five experiments. Recoveries were calculated by comparing the peak areas of chromatograms (a) and (b) and of (b) and (c), in order to be able to distinguish between the influence of the PROSPEKT system and the influence of the matrix on the final recovery. The memory effect was studied by the injection of a blank solution directly after the analysis of a real sample and performing the automated analysis at a 10-fold increased sensitivity of the UV-absorbance detector. In general, the analytical data for the fourteen analytes in five different matrices as shown in Table V are satisfactory, with a repeatability ranging from 1.5 to 5.1% R.S.D. (n = 20), recoveries (n = 20) ranging from 87 to 115% (except for VP-16; see below) and a memory effect (n = 5) within the specification of the auto-sampler itself, *i.e.*, $\leqslant$0.5% (except for two compounds).

As regards the anti-epileptic drugs (*cf.*, Fig. 5), the strong protein binding usually observed for phenytoin and carbamazepine did not influence the recovery. However, the samples and the purge solvent had to be acidified in order to obtain a good recovery for phenobarbital. This phenomenon and the recoveries for the other anti-epileptic drugs are in excellent agreement with the results obtained by other workers[10,12]. To control the reliability of the system for the determination of these drugs in serum, a series of 96 samples were run unattended overnight. Chromatograms were evaluated by computer and the repeatability was calculated from the individual peak-height data. The repeatability was better than 3.4–5.2% R.S.D. for the anti-epileptic drugs studied. No blocking of the guard or analytical column occurred and the memory effect was less than 0.5% for all but one of these drugs (1%).

The recovery of VP-16 in plasma (*cf.*, Table V and Fig. 6) was relatively poor (61%) but the repeatability was still acceptable for an unattended analysis of real samples. This relatively low recovery was only observed for real samples, which is in

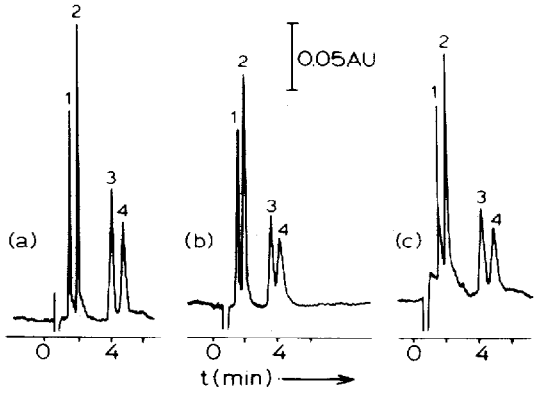


Fig. 5. Chromatograms of the anti-epileptic drugs primidone, phenobarbital, phenytoin and carbamazepine. (a) Direct loop injection; (b) as (a), but using the system described in Fig. 4; and (c) spiked serum sample, analysed as (b). For conditions and peak identification, see Tables IV and V.

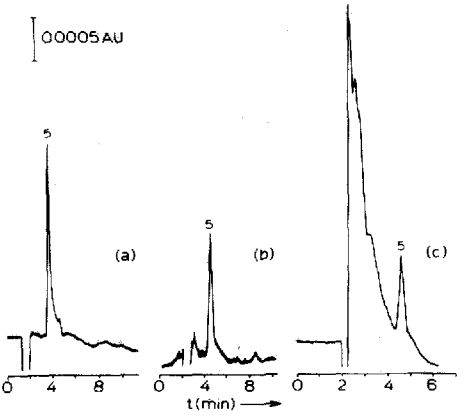


Fig. 6. Chromatograms of the anti-cancer drug VP-16. (a) Direct loop injection; (b) as (a), but using the system described in Fig. 4; and (c) spiked plasma sample, analysed as (b). For conditions and peak identification, see Tables IV and V.

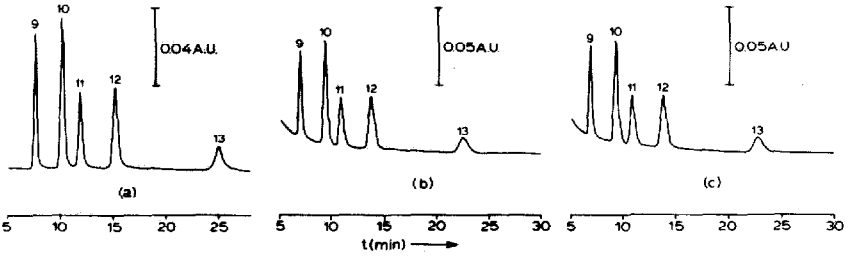


Fig. 7. Chromatograms of the phenylurea herbicides monuron, monolinuron, chlorotoluron, diuron and chlorobromuron. (a) Direct loop injection; (b) 10 ml on-line trace enrichment using the system described in Fig. 4; and (c) spiked river water sample, analysed as (b). For conditions and peak identification, see Tables IV and V.

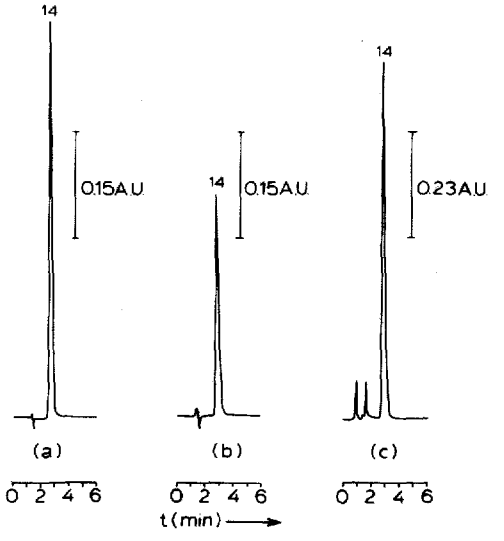


Fig. 8. Chromatograms of caffeine. (a) Direct loop injection, 60 μg ml^{-1}; (b) cola, analysed using the system described in Fig. 4, 60 μg ml^{-1}; (c) spiked cola, 60 + 120 μg ml^{-1}, analysed as (b). For conditions and peak identification, see Tables IV and V.

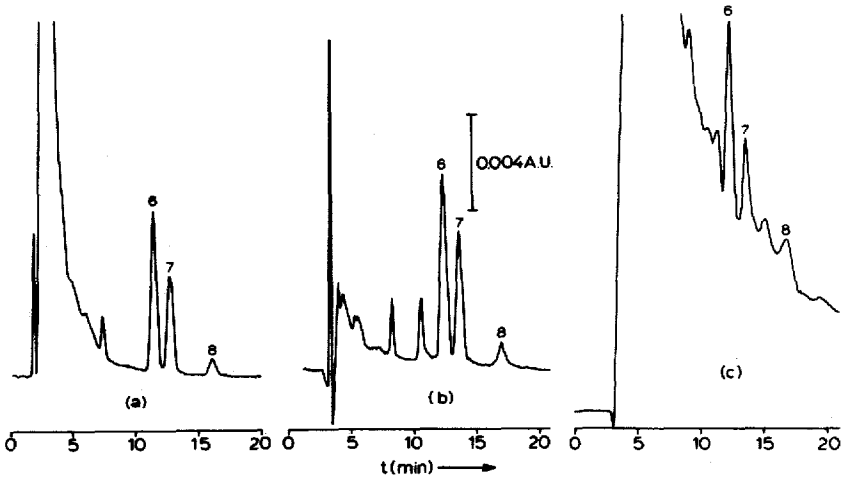


Fig. 9. Chromatograms of the barbiturates butabarbital, hexobarbital and secobarbital. (a) Direct loop injection; (b) as (a), but using the system described in Fig. 4; and (c) spiked urine sample, analysed as (b). For conditions and peak identification, see Tables IV and V.

TABLE V

ANALYTICAL DATA FOR APPLICATIONS WITH THE AUTOMATED SAMPLE HANDLING (PROSPEKT) SYSTEM

Conditions as in Table IV; the system used is further described under Experimental. Data based on peak-area measurements; memory effect specified for the auto-sampler, ≤0.5%.

Peak no.	*Analytes*	*Repeatability (R.S.D., %) (n = 20)*	*Recovery (%)*		*Memory (%) (n = 5)*
			Standard vs. loop (n = 20)	*Sample vs. standard (n = 20)*	
1	Primidone	2.5	112	89	≤0.5
2	Phenobarbital	4.6	104	89	≤0.5
3	Phenytoin	2.9	98	98	≤0.5
4	Carbamazepine	4.3	103	101	1.0
5	VP-16	5.1	92	61	≤0.5
6	Butabarbital	4.2	99	98	≤0.5
7	Hexobarbital	5.1	96	86	≤0.5
8	Secobarbital	3.5	104	115	≤0.5
9	Monuron	2.4	89	95	≤0.5
10	Monolinuron	1.5	89	104	1.0
11	Chlorotoluron	3.1	97	94	≤0.5
12	Diuron	3.2	96	95	≤0.5
13	Chlorobromuron	4.8	96	97	≤0.5
14	Caffeine	3.2	87	97	≤0.5

good agreement with earlier findings for a 5 × 1.1 mm I.D. pre-column packed with the same material[17].

The on-line trace enrichment of phenylurea herbicides from river water samples (Fig. 7) and the determination of caffeine in cola (Fig. 8) showed a good overall performance with repeatabilities of 1.5–4.8% R.S.D., recoveries ranging from 89–104% and a memory effect of less than 0.5% for all but one of the herbicides (1%).

The determination of barbiturates in urine (Fig. 9) was found to be more critical. Although the analytical data are still acceptable (*cf.*, Table V) at this realistic concentration level, it is obvious that when a lower detection limit is desired, the selectivity will have to be improved. This can be achieved by using a more selective detection mode or via the on-line dual pre-column approach described by De Jong *et al.*[18]. They used a pre-column packed with a hydrophobic resin in series with an ion-exchange pre-column in order to eliminate selectively organic and inorganic interferences, thus allowing the LC determination of barbiturates in urine with (non-selective) UV absorbance detection.

CONCLUSIONS

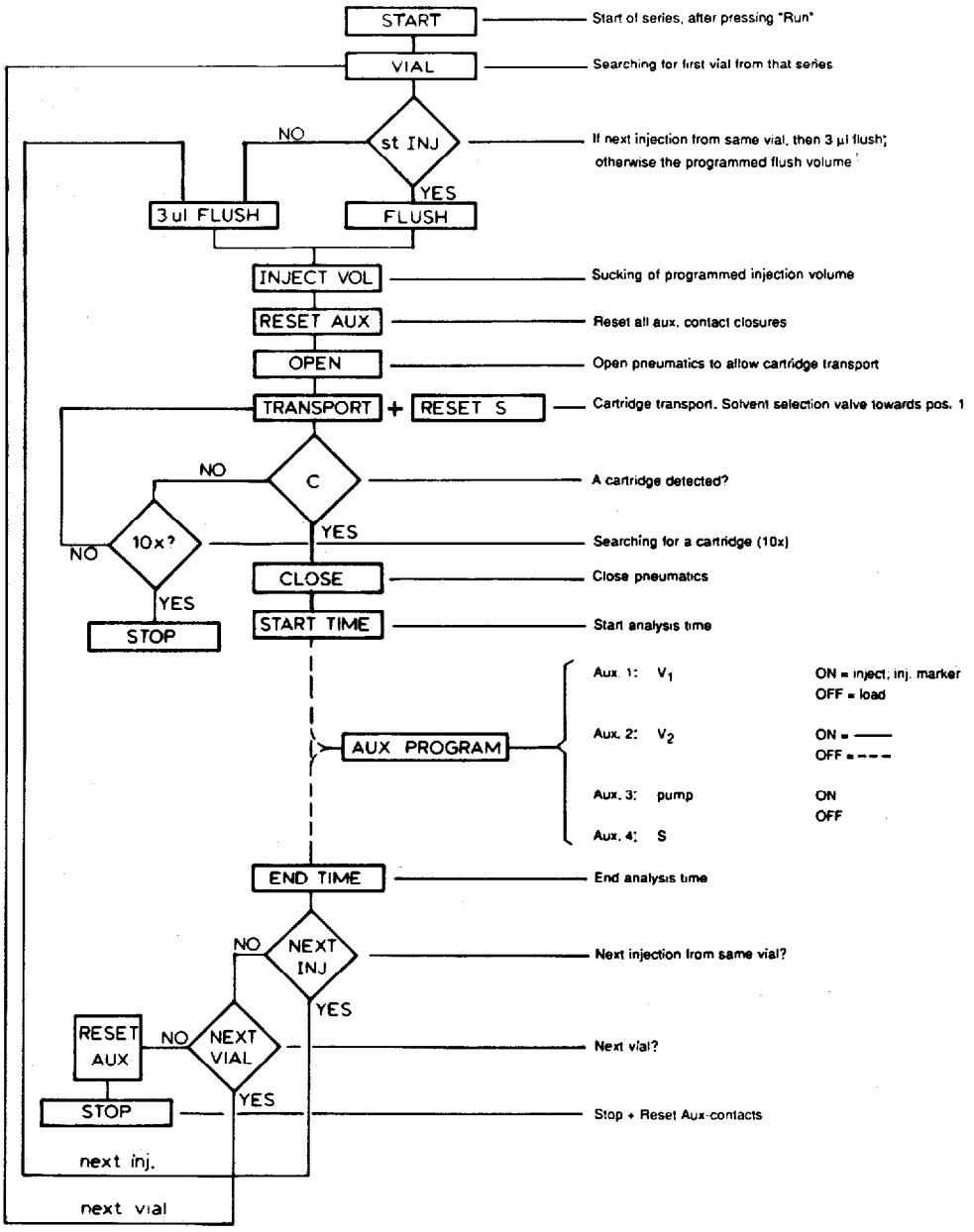
A fully automated sample handling system has been developed for liquid chromatography that combines the advantages of a disposable cartridge system with its inherent constant quality assurance and pre-column technology with its high automation potential. The present design represents a prototype of a new generation of automated sample handling systems in which, in principle, the samples only have to be applied to the instrument.

In order to achieve this end, an inexpensive pressure-resistant cartridge packed with 40 μm octyl-bonded silica was developed; this packing shows acceptable reproducibility, as was demonstrated by the retention for a polar model compound. Extra-column band broadening caused by this system was found to be negligible when combined with a 20 cm analytical column.

Five different sample matrices were investigated in order to demonstrate the potential of the system for unattended routine analyses. The repeatability and recovery were found to be satisfactory for the determination of drugs in serum, plasma and urine, for the trace-level determination of herbicides in river water and for the determination of caffeine in cola. In addition the memory effect was found to be the same as the specification for the auto-sampler used ($\leqslant 0.5\%$) for all but two compounds (1%).

It is obvious that the potential of an automated sample handling system based on solid-phase extraction can only be fully exploited if a wide range of cartridges packed with selective sorbents become commercially available. In addition, it is the intention to provide interesting additional options such as switching valves, allowing the use of a dual-pre-column approach[18], *e.g.*, the combination of a non-selective reversed-phase type of pre-column with a selective ion-exchange cartridge, solvent selection valves for the on-line trace enrichment from large sample volumes and an inexpensive sampler, instead of the sophisticated auto-sampler used in this study.

APPENDIX


START
Start of series, after pressing "Run"
VIAL
Searching for first vial from that series
NO
st INJ
If next injection from same vial, then 3 µl flush;
otherwise the programmed flush volume
YES
3 ul FLUSH
FLUSH
INJECT VOL
Sucking of programmed injection volume
RESET AUX
Reset all aux. contact closures
OPEN
Open pneumatics to allow cartridge transport
TRANSPORT + RESET S
Cartridge transport. Solvent selection valve towards pos. 1
NO
C
A cartridge detected?
YES
NO
10x?
Searching for a cartridge (10x)
CLOSE
Close pneumatics
YES
STOP
START TIME
Start analysis time
AUX PROGRAM
Aux. 1: V1
ON = inject; inj. marker
OFF = load
Aux. 2: V2
ON = ——
OFF = - - -
Aux. 3: pump
ON
OFF
Aux. 4: S
END TIME
End analysis time
NO
NEXT INJ
Next injection from same vial?
YES
RESET AUX
NO
NEXT VIAL
Next vial?
YES
STOP
Stop + Reset Aux-contacts
next inj.
next vial

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