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REVIEW

AUTOMATION OF LIQUID CHROMATOGRAPHIC TECHNIQUES FOR BIOMEDICAL ANALYSIS

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1 INTRODUCTION

The analytical techniques employed in biomedical laboratories are dictated by the need for the bulk of samples to be analysed and reported the same day or within a few hours of receiving the specimens To achieve this the majority of analyses are performed by large high-capacity dedicated automatic multichannel analysers which employ homogeneous colorimetric methodologies. Although separation techniques, such as liquid chromatography (LC) would undoubtedly yield more accurate results, their application in biomedical service laboratories is limited by their inherent low speed and low work capacity

For LC techniques to be accepted in an environment dominated by multichannel analysers, it is necessary to increase their speed and work capacity Whilst little improvement can be made to the analytical speed of LC methods, the work capacity can be greatly increased if the system is completely automated to the extent of analysing raw samples without operator intervention [1] With the advent of highly flexible laboratory robots such as the Zymate (Zymark, Hopkinton, MA, U S A) and the MasterLab (Perkin-Elmer, Norwalk, CT, U S A) in the early to mid 1980s it became possible to realise this goal [2–5] Recently however, other manufacturers have begun to address this problem by employing more dedicated but less flexible forms of automation and now there are several instruments available that can be used to completely automate LC

This article aims to review the various approaches to automation, how these may be considered when designing an ideal system and how currently available equipment can be utilised in an automated LC system

2 THE AIMS OF AUTOMATION

The justification for automation should be both economic and strategic [6] In biomedical laboratories automation is economically justified by means of decreased operator time, increased work capacity and hence increased capitalisation on the investment in analytical equipment It can be argued that strategic justification for the automation of LC is realised by the increased accu-

racy achievable over the currently used colorimetric and immunological analysis techniques

The first step in designing an automated system is to define its required performance, not only in terms of accuracy, precision and sensitivity but also capacity and speed The capacity of an analytical system is measured by the number of samples it can process per unit of time once it is running, i.e. its work rate. A fast system is one in which the time interval between the specimen entering the system and a useful report being produced is short.

Unfortunately, system speed and capacity are likely to be inversely related and so, too, are speed and reliability It follows that the automation of LC is indicated only when the system is required to have a high capacity for handling non-urgent specimens with minimum operator attention Additionally, in medical laboratories, urgent or priority specimens need to be analysed and reports made within minutes of receipt and therefore are usually performed 'manually' on fast analysers dedicated to specific analytes In an attempt to combine both speed and capacity in one instrument, some large, high-capacity analysers have the facility to handle individual priority specimens during a main analytical run. Although this is of benefit only if the priority specimen arrives while the other specimens are being analysed, it is a feature to be considered when designing an automated LC

3 OPERATIONS IN AN LC SYSTEM

Zenie [6] has defined a system as a group of interdependent, interacting elements combined to perform a unified purpose and has coined the term "laboratory unit operation" (LUO) to describe the smallest steps of which the system is composed. Fig 1 shows the separate operations involved in an LC system and the LUOs in each operation To automate the system, the various operations need to be coordinated so that the system as a whole will function with precision and reliability, completely unattended

However, the equipment employed to perform a single procedure may vary considerably in terms of its optimisation or dedication to that operation For example, the absorbance of the eluate from an LC column may be monitored either by collecting 3 ml aliquots and measuring the absorbance in a 1 cm path length cuvette or it may be passed through a small-volume flow-through cell in a photometer The former approach uses a standard unmodified photometer but would require some form of automation to collect the aliquots of eluate and transfer them in and out of the cuvette In contrast, the photometer incorporating a flow-through cell is highly optimised for monitoring eluates but is of little use for anything else

It is generally the case that the flexibility of automation employed varies inversely with the degree of specialisation of the operations An extreme example of this is seen when the automated system is designed around a highly

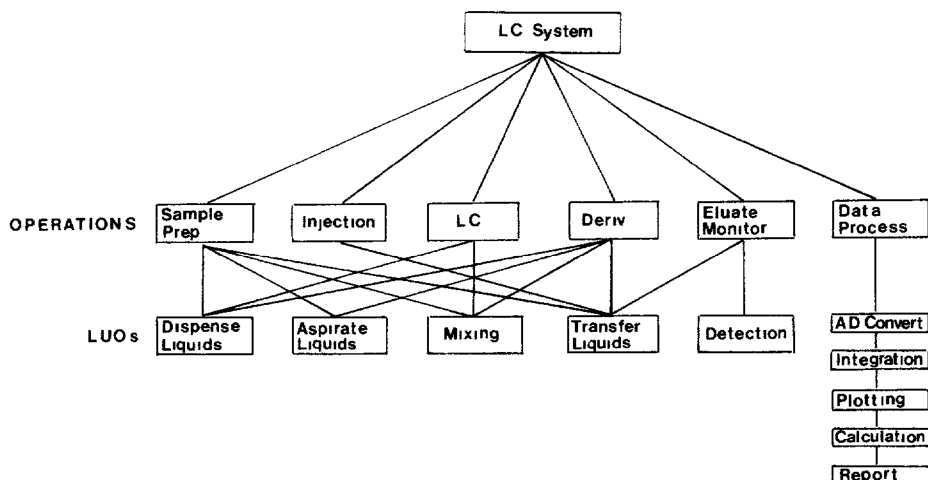

Fig 1 Operations and laboratory unit operations (LUOs) that may comprise an automated LC

flexible robot, e g. the Zymate The equipment employed to perform the necessary operations, e g weighing or mixing, are basic manual bench-top models which have required only slight modification to interface with the robot.

3 1 *Sample identification*

It should always be possible to identify the sample in some way so that the analytical result form the LC can be unequivocally linked with the original specimen when the final report is made The final report should at least contain the analytical result and some form of identification with the original specimen In its simplest form, the number and sequence of specimens and results can be used If the numbering system is started at the first specimen and at completion of the run the number of results equals the number of specimens, then results are matched with specimens according to their positions in the sequence and reports are made However, this simple procedure requires the operator to place the specimens onto the sampler in the correct sequence. It also assumes (a) that the specimens were sampled in the sequence that was intended and (b) that each specimen was sampled once and only once

An option provided on a number of instruments is for the sampler to send to the processing computer data concerning the sequence position of the specimen currently being sampled. This information can be used by the system to generate automatically reports having specimen identification However, as with the previous option, the same two assumptions have to be made The data the sampler transmits to the data processing computer is only proof that a particular point in the sequence has been reached and that a command to take

a sample has been given Furthermore, it does not prove that a sample has been taken from the specimen indicated. Because of the apparent validity of the reports, detection of sampler failures may not be obvious.

Positive sample identification overcomes some of these deficiencies provided that the reading of the identifying information is implicitly linked with the specimen during the act of taking a sample from it. Since reading the specimen identification can only occur during the sampling process, receipt of this information positively identifies the sample as originating from a particular specimen. Typically, positive sample identification is achieved by attaching machine-readable identification (e g bar code [7]) directly to the specimen in a position such that it can be read during the sampling process The main advantage of positive sample identification is that successful sampling sequence and frequency need not be assumed Indeed it is not even necessary for the operator to put the specimens onto the sampler in a particular sequence because their identification is always carried with them. Unfortunately, most commercial analysers with positive sample identification cannot read the sample identification at the same time as the sample is taken and therefore do not meet these ideals

3 2 Liquid handling

All the analytical operations in LC involve the handling of liquids, i e dispensing, aspirating and transferring For dispensing and aspirating liquids a range of pumps are available which are based on either positive displacement or peristalsis Which type to use for a particular application depends on a number of specific requirements operating pressure, accuracy, precision, priming volume and carry-over (e g. when a series of liquids are to be passed through the same system, as in a pipette)

However the process of quantitatively transferring liquids, especially in small volumes, is less clear There are only two ways in which liquids can be transferred (a) the liquid is retained in a vessel, e g a tube or a pipette tip, and the vessel moved to the next part of the system, or (b) the liquid is pumped through a static tube to the next part of the system The former approach is employed in most manual techniques but it is only with the advent of robots that it has become utilised in automated systems Apart from the obvious advantage that an automated system based on a working manual method is almost certain to work, there are other advantages Using this approach, very small volumes of liquids can be handled with great accuracy, precision and with little or no carry-over Although transferring a liquid by pumping it through a tube is simple and easy to incorporate into an automated system, its usefulness is limited to relatively high volumes, or where the transfers need not be quantitative because of the dispersion of the liquid as it travels along the tube

3 3 Mixing

A summary of the techniques for mixing liquids is given in Fig. 2. In all of these approaches, turbulence is created and therefore dispersion of the liquids occurs. High turbulence gives efficient mixing but also leads to an increased dispersion. Since dispersion either reduces the quantitative recovery or increases the dilution of a sample when it is transferred, any mixing technique is a compromise between either high mixing efficiency with high dispersion or low mixing efficiency with low dispersion Although dilution with purge liquid can be eliminated by using a separate test tube for each sample and mixing by inversion or vortexing, automating the handling of such a tube is difficult. It is also the case that the maximum number of samples that can be processed in one run is limited by the number of disposable tubes that can be accessed by the system. Alternatively, the mixing tube can be re-used, e.g. in the multiple aspiration and dispensing, high-pressure dispensing or stirrer techniques. This, however, requires the tube to be washed and dried between samples.

Mixing devices based on the dispersion of liquids as the sample is transferred through a tube are simple to automate but many do not give complete mixing In practice this is not a problem in automated systems because, although mixing is incomplete, each sample is treated in precisely the same way However, this can be a limitation when high analytical recoveries are required

3 4 Sampling

Sampling is fundamentally a transfer operation which, depending on the design of the instrument, may involve aspiration and dispensing, or aspiration alone The percentage sampling efficiency of a system can be expressed as

$$\frac{V_1 - (V_1 - V_2)}{V_1} \times \frac{V_3}{V_2} \times 100$$

where V_1 = the volume of specimen placed on the sampler, V_2 = the volume of sample aspirated and V_3 = the volume equivalent of sample injected after transfer, i e. the amount lost due to dispersion.

Fig. 3 shows the type of effect on sampling efficiency that can be expected, depending on the sample volume to be transferred and the dispersion encountered Obviously, in an ideal system there would be no dispersion and 100% efficiency would be obtained regardless of the sample volume aspirated. However, for most samplers, the greater the volume of sample taken the more efficient sampling becomes and vice versa The recovery of quantitative transfer will also directly affect sampling efficiency Any liquid movements through tubes will involve dispersion and sample losses and the smaller the sample volume the greater the losses that will be observed

Many aspects of the sampler design can influence the sampler efficiency

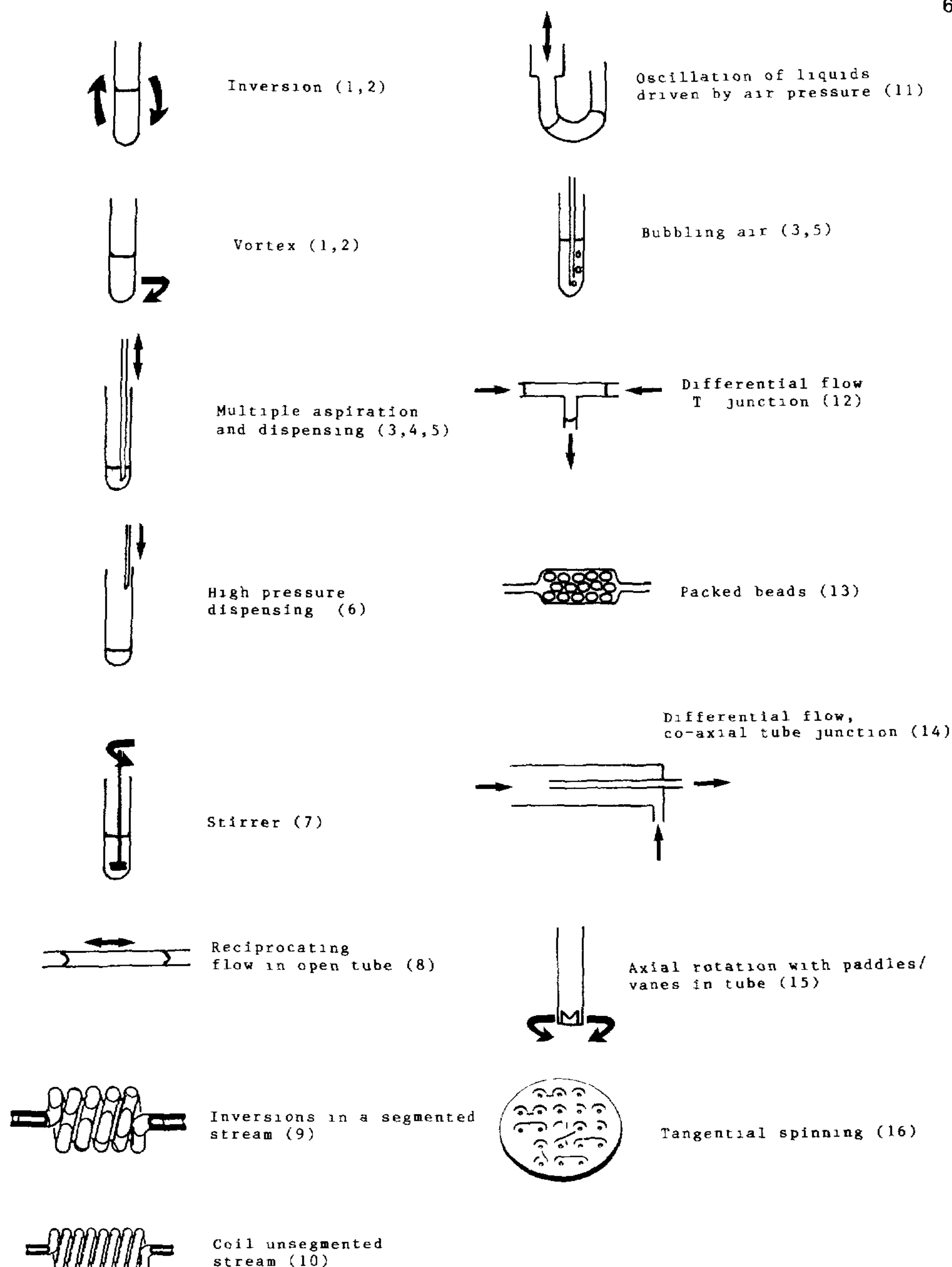

Fig 2 Mixing techniques Examples of instruments using the various mixing techniques are as follows 1, Zymate (Zymark), 2, MasterLab (Perkin-Elmer), 3, Millilab (Millipore U.K), 4, ASTED (Gilson Medical Electronics), 5, ASPEC (Gilson Medical Electronics), 6, M300 (Vickers Medical, Basingstoke, U K), 7, Parallel (American Monitor, Burgess Hill, U K), 8, 1090 Series M (Hewlett-Packard, Bracknell, U K), 9, Fast LC (Technicon Instruments), 10, Chromospec (Hilger Analytical, Margate, U K), 11, ACP and EPOS (Eppendorf, Hamburg, F R G), 12, SpH 125 PCD and PROMIS II (Spark Holland), 13, WISP sampler operating pre-column derivatisation (Waters Assoc), 14, UK Patent No 2124370B (not used commercially), 15, 2086 reaction rate analyser (LKB, Bromma, Sweden), 16, Visco Jet (The Lee Co) used in Model 1025D (Drew Scientific, Chiswick, U K)

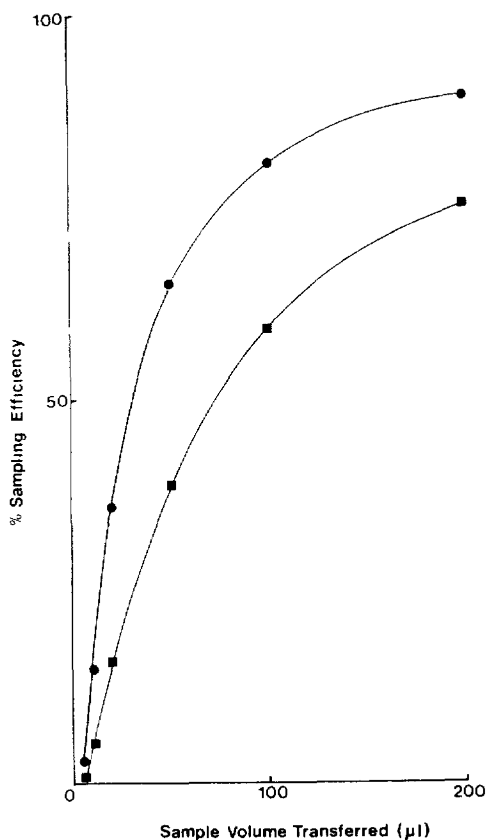

Fig 3 Hypothetical sampling efficiencies when transferring different volumes of sample (●) Sampling efficiencies obtained when 10 μl of sample are always left in the specimen vial, the volume of the transfer tube is 200 μl and assuming that there is a 2% dispersion of the sample each time it travels through a length of transfer tubing equal to its own volume (■) Sampling efficiencies obtained when 50 μl of sample are always left in the specimen vial, the volume of the transfer tube is 600 μl and assuming that there is a 2% dispersion of the sample each time it travels through a length of transfer tubing equal to its own volume In each case the injection loop and sample volume are assumed to be equal

The volume of specimen left in the specimen vial after the sample has been taken depends on the positional accuracy of the sampler probe and the internal shape and dimensions of the vial Any dispersion of the sample occurring during sample transfer might reduce the proportion of the sample that is loaded into the next part of the system Dispersion will increase in proportion to the area of tubing with which the sample is in contact during the transfer operation When there is no facility to wash and purge the sampler probe between sampling, some means has to be applied to eliminate carry-over effects Usu-

ally this is achieved by purging the remains of the previous sample in transfer tubing with part of the current sample.

Generally therefore, samplers that aspirate the sample from a vial into a tube physically transfer the tube to the injection valve and then dispense the sample have a higher sampler efficiency than samplers which aspirate directly from the vial through the injection valve

3.5 Sample preparation

Biological samples such as blood, serum or plasma usually require some form of preparation before injection onto the LC to remove compounds that would otherwise interfere with the separation or reduce the performance of the analytical column A variety of methods have traditionally been found to be successful, but some are easier to automate than others

3.5.1 Liquid–liquid extraction

In liquid–liquid extraction, compounds of interest are selectively transferred, on the basis of their different partition coefficients, from one liquid phase into a second immiscible liquid phase A portion of this second liquid phase can either be injected directly onto the LC or, more usually, the solvent evaporated and the solutes re-dissolved in a small volume of solvent that is compatible with the LC separation The volume ratio of the second liquid/first liquid phase (the sample) should be large in order to maximise the extraction recovery and the two liquids should be mixed well for the process to operate quickly. The sampling efficiency of liquid–liquid extractions are poor due to losses incurred during extraction and over reconstitution of the dried extract [8]

Without the use of highly flexible robots that emulate the manipulations involved in manual technique, liquid–liquid extraction has proved very difficult to automate efficiently The most successful dedicated automation of liquid–liquid extraction has been that based on the continuous-flow principle developed by Skeggs [9] Although this approach proved very successful for the automatic analysis of biological samples using aqueous reagents, its performance fell when immiscible liquids were used The liquid stream was segmented with air bubbles to minimise dispersion as it moved through the tubing This is very effective when the liquid is aqueous in nature but when two immiscible liquids are used the effect of the bubble is much reduced and dispersion increases In continuous-flow systems mixing devices such as coiled tubes inevitably increased dispersion

The two immiscible liquids are separated using a specially designed T junction This type of phase separator is extremely sensitive to flow-rate fluctuations for various possible reasons The sensitivity may be due to the peristaltic pump tubing connections or the compressibility of the air bubbles Of these,

the latter phenomenon causes more dispersion than all the other parts of the system put together, even when things are working well In addition the solvents used are restricted to those which do not solubilise the plasticisers in the peristaltic pump tubing. In spite of all these limitations, continuous-flow liquid–liquid extraction was used with success in the Fast LC (Technicon Instruments, Tarrytown, NY, U.S A) [10,11] which could also automatically evaporate and redissolve the extract

3 5 2 Solid-phase extraction

Solid-phase extraction requires lower volumes of solvents than liquid–liquid extraction and when the sorbent is contained in disposable cartridges, carry-over is not a problem because the small area of tubing in contact with the sample is easily purged There are numerous articles reporting the use of these procedures [12–17] Most systems using disposable cartridges have employed 'off-line' dedicated techniques for applying the sample to the cartridge and elution of unwanted components The AASP (Varian Assoc , Sunnyvale, CA, U S A) automatically elutes unwanted components and injects the retained analytes from sorbent-packed cartridges that have been previously charged with sample This instrument involves a specially designed mechanism for transporting the cartridges and sealing them into the liquid flow of the LC A similar approach is employed in the PROSPEKT (Spark Holland, Emmen, The Netherlands) but in this case, by the use of a second switching valve, operation is 'on-line' with automatic loading of the sample onto the cartridge, elution of unwanted components and injection of analytes [18]

An alternative approach is used in the Millilab (Millipore U K , Harrow, U K) where the tip of the probe is equipped with a pneumatic collar which is used either as a seal or to move cartridges and the ASPEC (Gilson Medical Electronics, Villiers-le-Bel, France) Both of these systems and the PROSPEKT can be said to offer complete automation

3 5 3 Column switching

Many reports have appeared describing many different forms of column switching techniques [19–23] All column switching techniques use combinations of multi-port valves Sample preparation methods using column switching methods include trace enrichment, on-line solid-phase extraction, heart-cutting and box car techniques Although the advent of column switching techniques permitted the preparation of biological samples in an 'on-line' manner using the benefits of solid-phase extraction, complete automation of such systems is compromised by the nature of the sample matrix Most biological samples are not free from particulate matter and require filtration if a usable extraction column life time is required

3 5 4 *Precipitation*

Automating the separation step is difficult Filtration or centrifugation can be used but these techniques are not easily incorporated into dedicated automation. Consequently, the only systems capable of this are based on robots, e g Zymate, which emulate the manual procedure

3 5 5 *Ultrafiltration*

As with the precipitation techniques, automation of ultrafiltration methods is difficult. If disposable ultrafiltration units are used a dedicated piece of automation is required to move and connect the units in the system Conversely, if the ultrafiltration unit is re-usable, connections into the system are simplified but very efficient purging must be employed to eliminate carry-over Off-line ultrafiltration techniques have been used for sample preparation [24,25]

3 5 6 *Dialysis*

This technique is simple to automate using Auto Analyser dialysers (Technicon Instruments) or their equivalent In a continuous-flow system and using membranes with molecular mass cut-offs as low as 10 000–15 000 relative molecular mass, all the compounds usually responsible for reducing column performance are eliminated from the sample However, when considering dialysis for 'on-line' sample preparation for LC it has two major limitations· (a) it is a slow process because the dialysable compounds pass across the membrane by simple diffusion which is proportional to the concentration gradient, (b) the recovery of analyte is low and is obtained in the dialysate at high dilution [26,27] Consequently, dialysis can only be used to prepare samples in which the analytes are at high concentration or where the detection is very sensitive, e g fluorescence detection of derivatised amino acids using *o*-phthalaldehyde [28]

3 5 7 *Hybrid sample preparation systems*

The 'on-line' nature of the dialysis approach and the fact that the membranes are re-usuable make this approach economically viable for automation However, to make full use of dialysis for many applications, it is necessary to trace-enrich the dialysates This technique, the automated trace enrichment of dialysates (ASTED) has been described previously [29–32] and has recently become commercially available (Gilson Medical Electronics) [1] The advantages of such a system are complete automation and much reduced running costs due to the efficiency of sample clean-up using dialysis and the many hundreds of cycles that the trace enrichment material can be regenerated

3 6 *Derivatisation*

3 6 1 *Post-column derivatisation*

Post-column derivatisation is used to improve the sensitivity or selectivity of detection Since the chromatographic eluate emerges from the column at a constant rate, it is simple to perform the derivatisation using continuous-flow techniques When long incubation times are required, air segmentation can be used to minimise dispersion and the liquids metered by a peristaltic pump Alternatively, when using unsegmented streams, reagents can be introduced by an inexpensive positive displacement pump At the same time, the metering of the reagent and its mixing with the eluate must be very precise, since any variation in flow will cause quantification errors which are very difficult to detect and cannot be corrected by the use of internal standards Good mixing can be achieved either by passing the liquid stream through a coil, as in an Auto Analyser, or through specifically designed mixers, e g the Visco-Jet (The Lee Co , Westbrook, CT, U S A.) However, mixing always causes dispersion and therefore undesirable band broadening Therefore mixing should be performed with the minimum of dispersion and in as small a volume as possible Numerous reviews on post-column derivatisation techniques have appeared [33,34] An alternative to the use of liquid reagents is to pass the eluate through a reactor containing an immobilised reagent such as an enzyme [35,36]. Although usually no mixer is required when using a reactor, for the reaction to occur the analyte must come into contact with the immobilised reagent This inevitably causes dispersion Thus the problem of dispersion in a reactor is identical with that in the design of a mixer

In practice, post-column derivatisations are limited to those reactions that require simple conditions and only one or two reagents. This is necessary because it is difficult to maintain precise operation of complex derivatisation systems.

3 6 2 *Pre-column derivatisation*

Pre-column derivatisation is used to improve the sensitivity or selectivity of detection or to change the physical nature of the analytes so as to alter their chromatographic mobility It is only in recent years that the automation of on-line pre-column derivatisation has been addressed [37–39] It is a discontinuous process, being required only to operate just before the start of each chromatographic run The sample enters the system as a discrete volume and not as a constantly flowing eluent stream as in post-column derivatisation Continuous-flow techniques take many minutes of running from start up before a stable flow-rate is achieved This is due to the compressibility of the segmenting bubbles. Therefore, continuous-flow techniques have limited use for this application Omitting the air segments from a conventionally configured continuous-flow system results in very high dispersion of the sample which is often

unacceptable when the volume of sample is limited A more reliable procedure to totally mix the sample and reagent(s) using differential flows in a double-lumen probe has been described [38]

3 7 *Data handling*

With any automated LC system reliable data handling is of prime importance to the end result Data handling may conveniently be discussed as integration and data storage and its retrieval Both may be incorporated in a single controller device that can dictate the overall level of automation of the LC system It is necessary that the more complex computer controllers have sufficient external input/outputs to control events that occur during the automation of the sample through the LC

3 7 1 *Integration*

Numerous integrators are commercially available that convert the analog signal of the detectors to a digital form and permit quantification of the analyte peak emerging from the LC column If maximum flexibility is required such that the user needs to define the automation process required, then it is advantageous to be able to have some programmable capabilities within the system that can interact with the main running integrator and controller

3 7 2 *Data storage and retrieval*

To permit accurate quantification of the peaks during the automated process it is usually necessary to have some means of storing the raw data and to be able to retrieve the same in a rapid manner This is essential for some chromatographic separations, e g gradient applications, where the peak widths will vary so much that reliable integration cannot be completely performed and the user will be required to re-integrate the peaks at a later stage

4 TYPES OF PROCESSING

The operations comprising an analytical process have to be performed in the same sequence for each sample and in an identical manner However, when a number of samples are to be analysed, they can be either batch- or sequentially processed. Refinements of these to give shorter process times are parallel batch and concurrent sequential processing

4 1 *Batch processing*

In batch processing each single operation in the analytical process is carried out on all the samples before the next operation is performed In this way all the samples to be analysed pass along the sequence of operations as a batch

(Fig. 4a). Priority is given to the completion of a single operation on all the samples. Most manual techniques employ batch processing due to the convenience of performing a single operation multiple times The total time taken to process a group of samples is equal to the sum of the times for each unit operation in the analysis multiplied by the number of samples in the batch

The disadvantages of batch processing are: (a) when the times taken to perform each operation are not identical then the time interval between the operations performed on each sample will be different, i e each sample does not receive identical treatment with respect to time, (b) when the analytical process fails all the samples in the batch are lost; (c) a priority sample cannot be analysed preferentially since all the samples are treated in one batch.

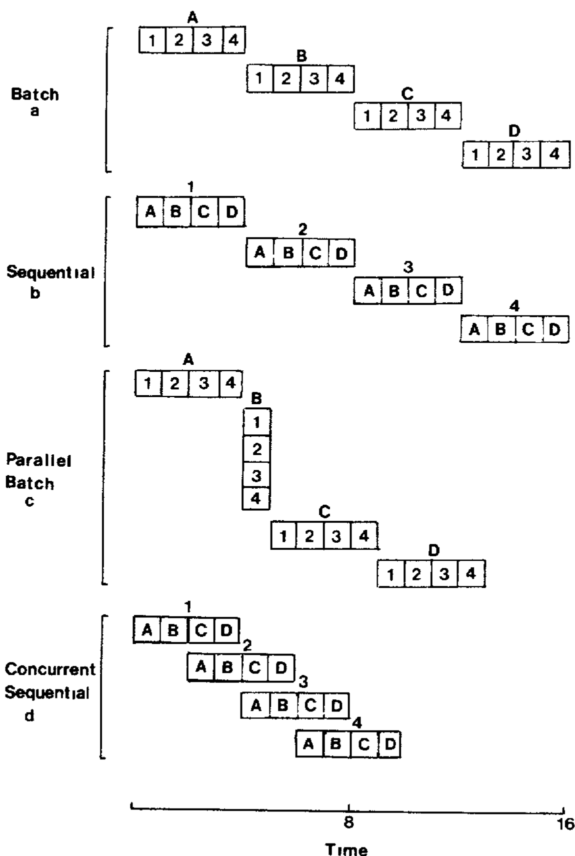

Fig 4 Analysis of four samples using different types of processing The total process comprises four operations (A, B, C and D) that have an equal duration of one unit of time The samples are identified by numbers 1, 2, 3 and 4 In the example of parallel batch processing, only operation B is performed in parallel In concurrent sequential processing, only operations B and C are performed concurrently

4 2 *Sequential processing*

In sequential processing each sample individually receives treatment from all of the operations before the next sample in the sequence is analysed (Fig 4b) Priority is given to the completion of all operations on a single sample and at any time only one sample is ever present in the analytical process The total time taken to process a group of samples is equal to the sum of the times for each unit operation in the analysis multiplied by the number of samples in the sequence, i e the same as for batch processing The disadvantage of sequential processing is that the timing of the operations must be very precise to ensure identical treatment of each sample.

4 3 *Parallel batch processing*

When employing batch processing it is usual, in some of the operations, for the batch of samples to be treated in parallel (Fig 4c), e.g centrifugation, incubation with reagents, mixing or passing liquid through solid-phase extraction columns with the assistance of vacuum or compressed gas Parallel processing considerably reduces the total process time. The time saving increases with the number of samples in the batch

When the duration of a treatment is important, any errors due to variations in the length of treatment are eliminated because, by parallel processing, all the samples in one batch are treated simultaneously. Nevertheless, in these cases it is important to ensure that the treatment actually starts at the beginning of the parallel treatment and finishes at the end of it For example, in a procedure where a volume of a derivatisation reagent is added to each sample in a batch, the batch is incubated in parallel at an elevated temperature to accelerate the reaction Then a second reagent is added to precipitate the derivative Each sample would then have been reacting for different lengths of time and temperatures before it is stopped by the addition of the precipitant A further problem with parallel batch processing is monitoring the function of the operation With the other types of processing, if an operation fails to function, all the samples are affected In contrast, if the failure occurs with a parallel operation, e g a multiple pipette with eight syringes, one of which had failed, only one in eight samples would be affected

4 4 *Concurrent sequential processing*

With concurrent sequential processing, as a sample passes along the chain of operations, further samples enter the system so that multiple operations function simultaneously (Fig 4d) Once the system is running, the number of samples being treated at any one time is equal to the number of operations running concurrently and it is only these that will be lost in the event of a

failure In contrast to parallel batch processing, the reduction in total processing time is proportional to the number of operations running concurrently and is independent of the number of samples being analysed

Of the four different types of processing, concurrent sequential processing requires the most complex system controls to coordinate the concurrent operations Additionally, if the duration of the concurrent operations is different, then, with respect to time, the first sample receives different treatment than do the subsequent samples [40]

5 SYSTEM CONTROL

The two major objectives of the system control are to make the system operate efficiently when unattended and simple to operate when it is attended For efficient unattended operation, the control system needs to coordinate the individual operations and at the end of a run, or in the event of a failure, to stop taking specimens, consuming reagents and power When it automatically stops, the system should leave itself in a state from which it is easy to re-start Similarly, the system should be simple to operate with the minimum of pre-start checks and commands

Different control configurations will give varying degrees of coordination, validation of operations, fault detection and failsafes To illustrate this Fig 5 shows the features of four control configurations for a system consisting of a sampler or sample preparation unit, a gradient LC unit and an integration unit

As the analytical process starts with the samples entering the system, one obvious configuration is to have the sampler as the controller (Fig 5a) When an injection is made, the sampler starts the LC and integration units simultaneously Clocks in the LC and integration units control the duration of their individual operations The clock in the sampler determines the interval between taking each sample and must be set to a time greater than either that of the LC or integrator operations

Whilst this configuration is simple to construct and the number of cycles in the run is determined only by the number of samples on the sampler, each operation requires an individual timing program that must be set-up at the start of a run If either the LC or integrator fails, the system will not stop because the sampler, which is the controller, cannot validate their function Additionally, the LC will not stop at the end of the analytical run This problem can be overcome by using the gradient controller to switch off the LC Nevertheless it does require a specific program together with the number of samples in the run to be entered

The configuration of a system that will stop if any of the operations fails is shown in Fig 5b This is based on the principle that since the system is a series of linked operations, only adjacent operations need to be coordinated In this example each operation is started by the previous one When this occurs it

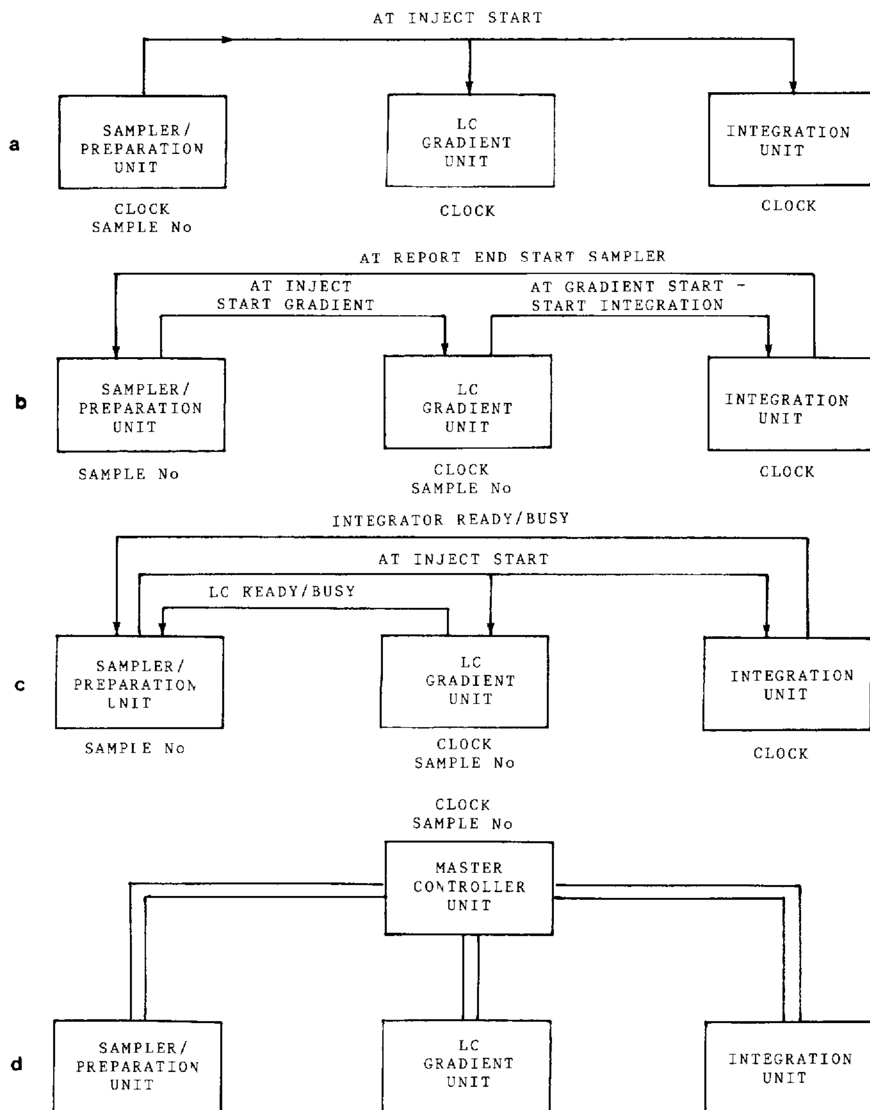

Fig 5 System control using four different configurations

validates the function of the previous operation and if it does not occur the system will stop Unlike the previous configuration (Fig 5a), this requires clocks only in the LC and integrator units to time the duration of their operation However, the gradient controller still needs a program to start the integrator after each injection and to stop the LC at the end of the run

The system shown in Fig 5c is controlled by the sampler which also monitors the status of the LC and integrator units via the READY/BUSY lines When

the sampler is ready to make an injection it examines the status of the LC and integrator. If both units are READY the sampler sends the signal to start and re-examines their status Only if both the LC and integrator units go BUSY is the injection then made Thus in the event of a major failure no further samples are injected Unlike the system in Fig. 5a, this configuration has the added advantage that the coordination of the operations is independent of time, with the sampler making the injection immediately the other operations are ready and not after a constant pre-set time interval

A single master unit that controls and coordinates all the operations is shown in Fig. 5d Although the system is complex it has many advantages over the other configurations Time-dependent operations are controlled by a single clock and the status of each unit, together with its functioning, is monitored Because the master unit controls each operation, the system can automatically shut down if a failure is detected This will facilitate an easy re-start once the fault is rectified The ultimate test of the operation of the system is the quality of the analytical report Irrespective of the nature or position of a fault, its existence will be manifested by a fall in performance The master controller can monitor this and if it falls below a pre-set level will stop the system An example of a crude but effective failsafe is to shut down the system if the internal standard is not located. Alternatively, the controller can be programmed to take a range of actions based on the performance assessments made on the functioning of different parts of the system and quality of the analytical results, e g if an analyte concentration exceeds the linear range of the detector, the system can dilute the sample and re-assay it

6 DESIGNING AN AUTOMATED SYSTEM

As described earlier (Section 2), the first step in designing an automated system is to define its required performance The accuracy, precision and sensitivity needed will determine the analytical methodology and instrumentation to be employed The required capacity and speed will influence how the individual operations in the analytical process are best controlled and configured into an automated system.

The appropriate chromatographic method should be selected because this sets the chromatography time which is a fixed parameter It is usually found that the chromatographic operation is the rate-limiting step, regardless of what form of automation is used.

Batch processing is usually applied to manual methods because users find it more convenient to perform multiple identical operations rather than multiple different operations However, when automated, batch or sequential systems give identical process times (Fig 6) When the system contains an operation that can only process samples separately, e g chromatography, there is no advantage to be gained from batch processing Moreover, for batch processing,

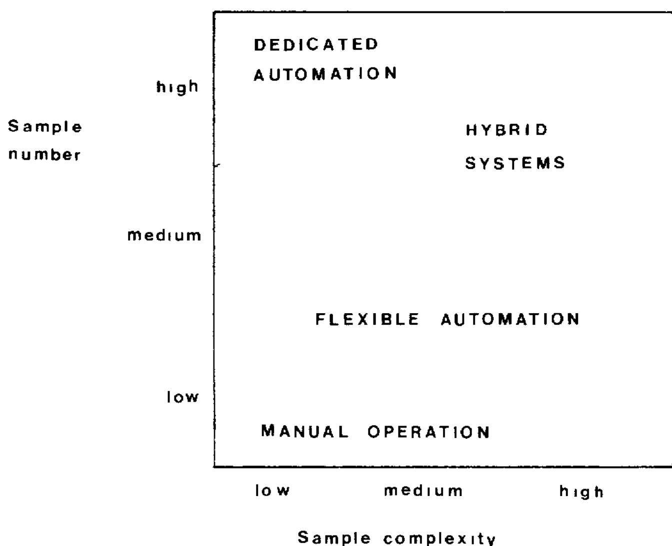

Fig 6 Domains of manual operation and different automated systems (Modified from F H Zenie, Laboratory Robotics Handbook, Zymark)

extra space and equipment, such as racks, are needed to accommodate the batch of samples as they pass through the system Generally therefore, for these applications sequential processing is optimal

When the system is required to have a higher capacity, then parallel batch or concurrent sequential processing should be considered over the simpler but slower batch or sequential techniques Additionally, the system must be reliable in order to attain high capacities and hence the benefits of automation. This is a function of the instrumentation and methodologies used and the ability of the system to detect failures and take appropriate action Some methods are intrinsically more reliable than others For example, transferring samples by aspirating and then dispensing is less susceptible to blockages than when using aspiration alone The system control should be able to validate operations, monitor functions and contain some sort of failsafe mechanism(s). This enables failures to be detected promptly and wasteful consumption of further samples prevented It is inevitable that if economic use is made of an automated system, i e it is always run unattended, then when it breaks down there is no one present to correct the fault. But once the fault has been rectified it is essential to re-start the system rapidly so as to minimise further loss of time This is facilitated if, on detection of a failure, the system automatically shuts down rather than switches off Good system control cannot improve on reliable instrumentation and methodologies but it can minimise the waste of time and samples in the event of a failure

Occasionally, with very complex separations, shifts in analyte retention times

occur due, for example, to variations in ambient temperature [41] In these cases, the resolution and integration of the peaks can be unaffected but can cause the integrator to mis-identify them To overcome this problem the raw data should be stored during the unattended run so that it can be re-processed later if mis-identification has occurred Although this is only a partial failure it is still necessary for the reprocessing to be fast and involve little operator time

After setting the defined capacity, it is desirable to define the maximum length of an expected unattended run, overnight (16 h) or over a weekend (64 h) Whether or not all the available time can be used productively will depend on the time for a single analysis and the specimen capacity of the sampler or limiting consumables such as solvents, reagents or extraction columns The result of this might indicate that a large capacity sampler is required or that a 'regeneratable' sample preparation method should be considered Alternatively, if the time for a single analysis is short, multiple assay runs might be considered At most, this would require the system controller to be able to switch columns and solvents, change chromatographic and detector conditions, change integrator files and finally sample the correct specimens for the particular assay engaged

If it is impossible to use all the projected available analytical time and automation is still economically justifiable, then re-assess the time taken for each operation Since speed and reliability are inversely related it would be expedient to slow down some operations, e.g chromatography times, that may improve the accuracy of the technique Additionally, if the target capacity for the system cannot be met, then parallel processing of the rate-limiting step should be considered, e.g using two or three chromatographs simultaneously

Frequently the volume of specimen is limited, often to the point where there is only sufficient for a single assay In these cases it is imperative that samples should not be loaded into a system that is not functioning correctly A failsafe device that is based on monitoring a product of the entire system can be used to prevent further samples pointlessly entering a malfunctioning system At the same time, all the other samples in the system at the moment the failure is detected will be lost If batch processing is used, then the entire batch of samples will be lost

To minimise the magnitude of such a loss, the size of the batches can be decreased but this would be self defeating and would reduce the benefit of automation In contrast, with concurrent sequential processing, the number of samples lost is equal to the number of operations that are operating concurrently With sequential processing, only one sample is lost It is also more appropriate to sequentially process, rather than batch process, when the system is required to assay a priority sample during a main analytical run The main run can be interrupted, the priority sample entered into the system and the result available in the time taken for a single analytical cycle Although it is

possible to interrupt a batch process for a priority assay, in practice the problems associated with this mean that it is rarely justifiable or even attempted

The degree of flexibility of the system should be based on anticipated future applications Systems based on laboratory robots are the most flexible while those using dedicated automation are the least flexible Generally, as flexibility of operation increases so speed of operation decreases This trend is even seen within the different types of laboratory robots (Fig 6) Those based on the cylindrical or flexible-arm robots are highly flexible but slower than the less flexible cartesian robots. Thus speed of operation and economic justification must be balanced with flexibility of operation and strategic justification. In the final analysis, the performance of the system should not be compromised by increased flexibility in the interests of future applications that cannot be immediately identified

7 AUTOMATED SYSTEMS AND MODULES

7 1 Commercially available systems

All the commercially available, fully automated LC systems are based on laboratory robots. The Perkin Elmer MasterLab system uses a flexible arm, Zymark's Zymate system uses a cylindrical robot while Water's Millilab and Gilson's ASTED and ASPEC all use cartesian robots. The MasterLab and Zymate are highly flexible systems having the potential to automate any laboratory procedure The Waters and Gilson systems, on the other hand, are directed solely towards chromatographic techniques with ASTED currently applicable to only LC applications. MasterLab, Zymate and Millilab can operate all the classical sample preparation techniques, while ASPEC is restricted to solid-phase or liquid–liquid extraction System control of the MasterLab, Zymate and Millilab is by a central computer as in Fig. 5d. Although this is possible in ASPEC and ASTED the usual system configuration is as shown in Fig. 5c with the sample preparation and LC operating independently of each other, communicating only at the time of injection The systems can run all types of processing on- or off-line (with the exception of ASTED which cannot operate off-line or, at the present, a batch process) All these are available as complete automated systems containing all the necessary controls and validations

7 2 In house system design and construction

There is a wide range of commercially available modules that can be utilised to construct a fully automated LC system. Most laboratories will already contain hybrid LC systems from different manufacturers that will create difficulties when automation of the whole is attempted. Users can also attempt to

electrically re-design redundant equipment, e.g samplers, so that they can be managed externally by a master controller

The control and configuration that is selected for the system will determine the operation management and communication features that are required in the process modules If, as in Fig. 5d, a single master controller is to be used, then each module, e g sampler, injector, LC, detector or integrator needs to have communication facilities such that each step of their operation can be controlled and their function and status monitored. There is no need for each module to manage its particular operation. Alternatively, when a master controller is not going to be used, as in the configurations shown in Fig. 5a–c, each module does have to manage its own operation and usually with respect to time. Also, depending on the configuration, at least one of the modules needs to be able to stop the system after the last sample has been analysed.

Nearly all recent LC modules such as samplers, injection valves, pumps, detectors and integrators have inputs and outputs that can be used to control them by external commands Many can also output their status i e READY or BUSY to an external device as well as being able to control gradients, etc without the necessity for a master controller. Finally it would appear that there is virtually no limit to the degree of automation that can be achieved, even, for example, to conserving lamp lives of detectors by switching them off after the analytical run is completed

8 CONCLUSION

A biomedical assay service demands high working specimen capacity and fast response time from an analytical technique. Accuracy appears to be of secondary importance to both objectives. It is therefore not surprising that the inherently slow LC techniques have not become widely accepted The exception has been the analysis of small molecules such as amino acids and some drugs together with their metabolites, where a plurality of analytes need to be quantified and where there is no obvious alternative technique

By increasing the work capacity of LC through automation it is now possible to justify its use both economically and strategically The cost savings of assaying drugs by automated LC instead of specific homogeneous immunoassays are substantial. When the immunoassays are run on a dedicated fast analyser, the automated LC techniques are slower However, if the immunoassays are batch-processed on a high-capacity analyser, the response time is long [42] and a concurrent sequentially operating LC can produce a result faster As well as being economically justifiable, the superior accuracy of separation techniques has been shown to be an advantage compared with the traditional homogeneous methods that are all prone to random interferences [43]

The evolution of commercially available fully automated LC systems has swung between extremes of design philosophies The first system was the

Technicon Fast LC. This employed dedicated automation for sample preparation and an isocratic high-performance liquid chromatograph (HPLC). It had a high capacity but the flexibility of both the sample treatment and the HPLC was restricted The next advance came with the application of laboratory robots to automating LC methods. These systems emulated manual methods, were highly flexible but had a low capacity compared with the Fast LC At the same time modular instruments began to appear that embodied dedicated automation of specific operations such as pre-column derivatisation (SpH 125 PCD, Spark Holland), post-column derivatisation (PCRS 520, Kratos, Westwood, NJ, U S A), trace enrichment/column switching (PROMIS II and MUSIC, Spark Holland) and the analyte elution and injection step of solid-phase extraction (AASP, Varian Assoc.) These instruments could easily be incorporated to great advantage into automated systems

Although solid-phase extraction was being increasingly used for sample preparation, it proved impossible to fully automate without resorting to the use of laboratory robots. By employing specifically adapted cartesian robots, the Gilson ASPEC and Waters Millilab systems provide fully automated solid-phase extraction that is more economic and have a higher capacity than systems based on the more flexible laboratory robots such as Zymate and MasterLab.

Arguably the ASPEC and Millilab are hybrid systems (Fig 6) since they embody features of both flexible and dedicated automation. A clear example of a hybrid system is the Gilson ASTED In this system specimens are prepared by a dedicated piece of automation, employing dialysis and trace enrichment, with the sample transfers being performed by a cartesian robot In this way the advantages of flexible and dedicated automation are combined whilst their disadvantages are minimised

Future developments will probably take place in the field of hybrid systems in which the capacity and speed is provided by dedicated automation with the operations that are difficult to specifically automate being performed by a robot

9 SUMMARY

The complete automation of liquid chromatographic (LC) techniques increases their work capacity and makes them more acceptable for biomedical applications The ability to analyse priority specimens is also advantageous. The areas to be considered in a fully automated LC are reviewed in terms of the analytical operations, the types of processing and the various control systems that can be adopted In particular, sample preparation and liquid handling are described in detail together with techniques and equipment that can be utilised for their automation. Strategies are given for the rational design of a fully automated LC that can analyse raw samples completely unattended

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