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Advances in automation of pharmaceutical analysis*

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Abstract: The development of a new drug substance and its dosage forms demands the establishment and implementation of suitable analytical methods. Through the application of chemometrics, "intelligent" laboratory systems and integrated data handling systems, considerable progress is being made in automating both the development and routine use of analytical methods in pharmaceutical analysis.

Keywords: *HPLC method development; robotics; information management.*

Introduction

All new drug substances intended for therapeutic use, together with their formulations, have to be subjected to extensive investigations to demonstrate their safety and efficacy. The analytical chemist is intimately involved with the total development of a drug, from its initial discovery through to product licence applications. In particular, the pharmaceutical analyst is concerned with the characterization and control of the drug substance and its dosage forms (tablet, cream, injection, etc). For a new drug substance or dosage form, many tests will be precedented in national pharmacopoeia but new methods will also be required. However, all methods will comprise the basic elements of the usual measurement process illustrated in Fig. 1.

In the search for increased accuracy, precision and productivity, the pharmaceutical analyst seeks to select optimal measurement processes and to automate as appropriate. The use of mathematical and statistical methods to design or select such optimal procedures and to obtain maximum information from them is defined as chemometrics. Chemometrics in pharmaceutical analysis has been the subject of recent reviews [1–3] but to provide a concrete example of chemometrics in use, its application in the automation of chromatographic method development and optimization will be considered.

In automating a method, the choices are usually between dedicated automation and flexible methods, such as robotics. There are many factors to be taken into account in the decision, and whilst the successes of robotics are frequently discussed, the drawbacks and pitfalls are less often described.

Finally, successful automation results in the generation of large amounts of data. A well designed and implemented laboratory information management system (LIMS) is

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then a "must" to capture the data effectively and integrate it into the corporate information handling process. The selection, implementation and validation (including automated validation) of LIMS in a multinational organization poses many challenges.

Automation and Method Development in HPLC

High-performance liquid chromatography (HPLC) is one of the most widely used techniques for the analysis of both the drug substance and its dosage forms. However, the development, optimization and validation of HPLC methods is often time consuming and is still frequently carried out by inefficient processes.

The first two steps (Fig. 1) in HPLC method development will probably have already been taken (in part) before the analyst begins the development and optimization process. Nevertheless it is essential to ensure that the problem has been explicitly defined and that HPLC is indeed the most appropriate method. The selection of HPLC as the method of choice then initiates many other choices, such as the chromatographic mode (Fig. 2) and detection method. Only very recently have these choices been tackled by chemometric methods, particularly expert systems [4, 5]. A major EEC funded project (ESCA — expert system for chemical analysis) has focused on many of the development

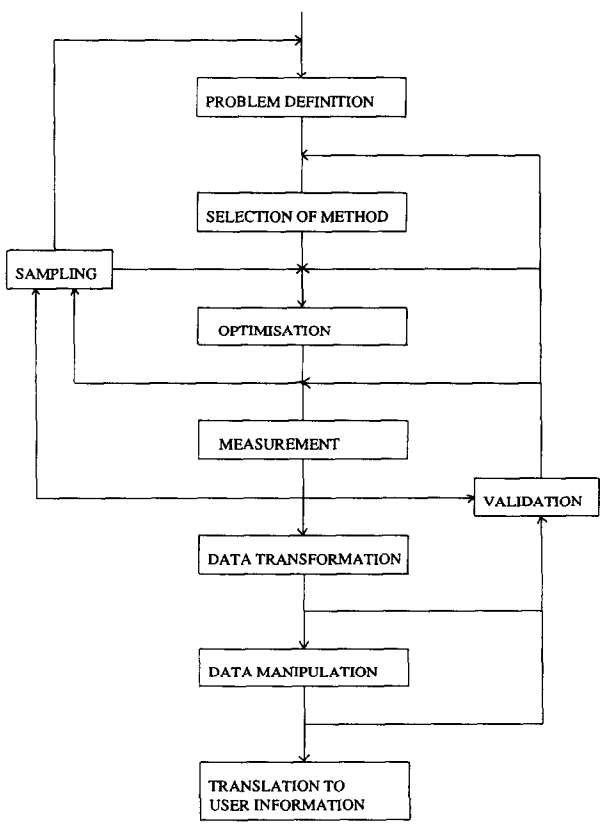

Figure 1
The analytical measurement process.

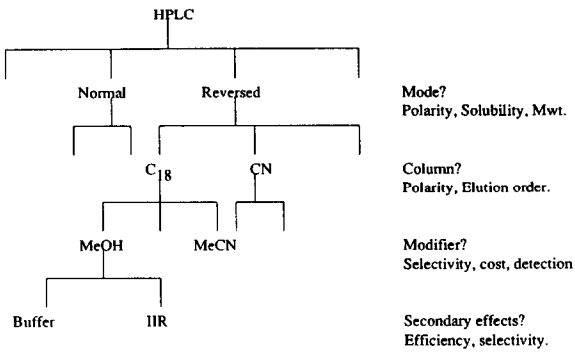

Figure 2
Decision tree representation of HPLC method development.

decisions that are required in HPLC [6] while other workers have investigated expert systems to relate structures to chromatographic properties.

Few of the expert systems so far developed would claim to yield optimum separation conditions and the next step is thus to fine tune or optimize the separation. Expert systems are again being used at this stage to assist in the selection of an appropriate optimization criterion [7] or experimental design [8]. The process of HPLC separation optimization is far from trivial and many methods have been described [9, 10].

Despite the assertion of manufacturers who may insist that their method is *the* one for rational separation optimization there is no single ideal system. Many factors have to be taken into account before an optimization method is selected: for example (1) the number and complexity of process related or degradation products to be separated (2) interference from sample matrix, (3) sensitivity, (4) speed and/or cost, (5) robustness and ease of transfer to other laboratories. Progress is being made with the development of mathematical expressions which can be used as optimization criteria in these situations [11] and with chemometric methods to handle such multi-criteria decision making processes [12].

The experimental design which may be chosen for the final separation optimization will fall into one of two broad categories, namely a sequential or simultaneous procedure. Once again there are compromises to be effected. The simplest sequential procedure is to carry out a stepwise search of pre-defined variable space. Such procedures are easily automated on many contemporary computer controlled chromatographs and a propriety system, complete with data handling, is available [13]. Another easily automated sequential method uses the sequential simplex procedure [9, 14] and fully automated commercial implementations of this have also been offered [15].

Simultaneous designs require that experiments be conducted according to a pre-defined experimental plan and the data thus acquired are used to describe the separation performance within the regions examined. Most of the currently available procedures have their origins in the solvent selectivity triangle concept of Snyder and Glajch [16, 17]. Whilst such mixture design approaches are valuable methods, their successful automation has proved difficult due to the need to track the retention of solutes in each separation. Peak tracking can be accomplished if standards are available but, even with a fully automated chromatograph, the procedure is time consuming. However, the

availability of diode-array detectors enables peak tracking to be achieved without standards. Wright *et al.* [18] showed that simple multiwavelength data could be used to track components reliably during multiple peak overlap and extensive peak cross-over allowing automated solute recognition and calculation of optimum separation conditions. Other chemometric techniques have also been used successfully in peak tracking [19] and will enable alternate approaches to the automation of simultaneous designs.

The variety of problems arising in the development of a new HPLC method for pharmaceutical analysis results in neither sequential nor simultaneous methods providing a universal experimental design. For example, Berridge *et al.* [20] found it necessary to combine factorial, mixture and sequential simplex designs in the development of an HPLC assay capable of quantifying a drug substance and its potential impurities in a single run. Even then, further optimization of detection conditions was required to provide the desired selectivity and sensitivity without having to resort to extended analysis times [21].

Having established optimum experimental conditions, the method requires validation and should also be tested to establish its ruggedness or robustness. Many factors impact upon the robustness of a method but by use of a partial factorial design the major influences can be determined and, with the use of a microcomputer controlled chromatograph, the whole process can be automated [22].

Automation and Robotics

The introduction of the laboratory robot heralded a new era of automation in the analytical laboratory. Previously, the wide diversity of products and tests had made the automation of all but the highest volume methods (e.g. HPLC) impractical. The laboratory robot was introduced as a potentially cost-effective solution to automating lower volume tests and providing the necessary flexibility to cope with changing needs.

In the author's laboratories, the most successful applications of robotics have been with single techniques which are applied to many products, such as the dissolution test [23]. Here the robot is used to mimic the functions of a human operator, filling the apparatus with the dissolution medium, adding the dosage form, sampling at set time points and then washing the apparatus in readiness for the next cycle. It is interesting to compare "a day's work" of the robot against its human counterpart (Fig. 3). Two facts are immediately obvious: the robot is able to process twice as many samples, and the reproducibility is better. However, while the robotic method offers many advantages in reducing peak workloads, its implementation has not been without problems. In the example quoted, the dissolution aliquots are pumped through a UV spectrophotometer for measurement: this requires that the instrument is switched on permanently. With normal lamp lifetimes in the range 100–300 h, changing the lamp becomes a 2-weekly requirement, with the appropriate checks and validation too. Thus automation of one part of a method is placing high demands on peripheral equipment. The system is now 4 years old, but over the last 9-month period, approximately 50% of the available utilization time was lost primarily due to failures in peripheral equipment, equipment not designed for 24 h/day usage.

The flexibility of the dissolution system is increased by using HPLC as the analytical method. The complexities of autosamplers having moving carousels results in the simplest method for transferring dissolution aliquots to the HPLC being a procedure for filling and capping vials for subsequent off-line analysis. However, with a cartesian

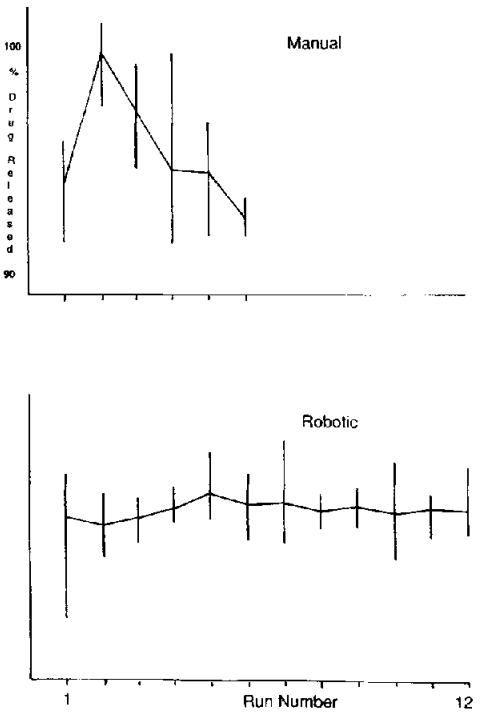

Figure 3
Comparison of manual and robotic dissolution results over a single day (reproduced from ref. 3 with permission).

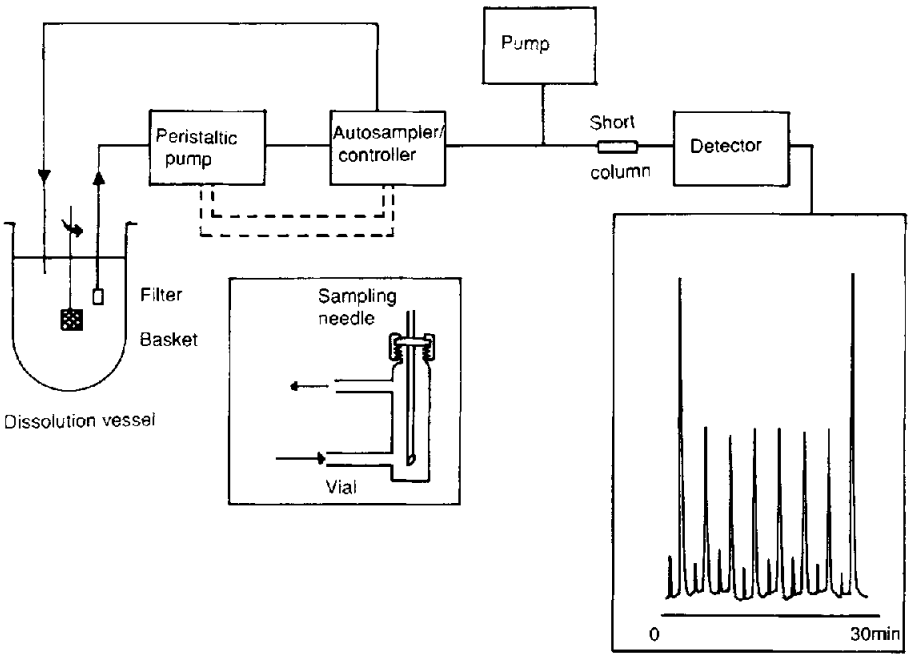

Figure 4
Flow through automated dissolution system with HPLC analysis.

sampler ("XYZ") the samples themselves do not move, allowing the use of flow-through autosampler vials. By using high-speed chromatography and injecting standards and samples sequentially during a single run, the analysis can be achieved in real time (Fig. 4) and the whole process is simply automated.

The system described above is dedicated to dissolution testing since it is impractical to consider its use for other tasks. The time required to set up and program the robot is far greater than the time required to carry out the task manually. However, ways of improving the flexibility of robotic systems, their throughput and the speed of new application implementation are being developed. For example pre-programmed, pre-located segments allow for more rapid changes of chemistries and operations [24]. Such a system has been developed for sample preparation but, with sequential operations only, is restricted to processing just two samples per hour. This limitation stems from the waiting times occurring during steps such as vortex mixing or centrifuging. By invoking sequencing software, which will allow operations to be conducted in parallel, sample throughput can be increased to eight per hour.

At present, developing first the sequential and subsequently the parallel procedure is a slow and laborious process. Expert systems are now becoming available [25] which can aid in the temporal optimization of robotic task sequences (TORTS) and have the advantage that the robotic system itself is not required. With such systems more rapid changes in task, and hence greater flexibility, should result. Other approaches to increased flexibility with robots is to build more generic automated sample preparation (GASP) systems [26]. All these approaches offer potentially elegant solutions to increasing robotic flexibility but they are not without their own challenges, not the least of which is the validation for each new method developed.

Robots are not the only route to flexible automation. As indicated above, XYZ autosamplers are versatile and can form the basis of a variety of automated systems. An example is in the use of an unmodified sampler (Gilson Model 231, Anachem, Luton, UK) as an automated sample application device for thin-layer chromatography (TLC). The manufacturers now offer a modified injector needle designed for TLC but, even without it, adequate precision is achievable. Table 1 illustrates the precision obtained with four different dosing volumes as measured using scanning densitometry of developed plates. Such precision brings with it the possibility of using TLC as a single method providing semi-automated results for quantitative determination of the drug substance (e.g. content uniformity of dosage forms) and its related substances. However, it is evident that the detection response is far from linear due to sample overload.

Since XYZ autosamplers also have the capability to dilute and dispense reagents, they can form the basis of a comprehensive liquid handling and sample preparation system. When interfaced to additional sample processing instruments capable of carrying out

Table 1
Reproducibility data for single component with spot application by unmodified HPLC autosampler (n = 16)

Application volume (μl)	Height mean	CV (%)	Area mean	CV (%)
10	698	0.9	42464	1.7
5	675	1.1	35526	3.4
2	523	1.5	23926	1.4
1	375	3.0	15434	3.1

solid phase extractions, the combination produces a powerful, automated sample preparation system capable of handling biological samples [27] and pharmaceutical samples having complex matrices, for example medicated feeds. Such is the versatility of these autosamplers that they are now being built into an increasing range of instruments and can function both for sample application and for machine maintenance, for example in capillary electrophoresis [28].

Data Handling

A consequence of increasing method automation is the increasing demands for automated data capture, handling and archiving. The tools to handle these include LIMS [27, 29].

New drug applications (NDAs) and product licence applications (PLAs) may be regarded as end products emerging from analytical development laboratories. These NDAs and PLAs contain large quantities of data, data generated and validated at least according to good laboratory practice (GLP). The resource involved in manually capturing, transcribing, validating and collating the data is large but, while many production and quality control laboratories have been using LIMS for many years, the implementation of LIMS has been slow in analytical development laboratories. However, developments in computer technology now mean that LIMS can be tailored with sufficient flexibility to cover the evolutionary demands of research and development analytical laboratories.

The benefits of LIMS have been defined [27] as data storage, data integrity, data manipulation and productivity and it is easy to see how removal of the need to transcribe, hand calculate and check data can dramatically improve laboratory productivity. To fully realise the benefits of LIMS requires that the stored data are readily available to all who need them. The analytical development groups of Pfizer Central Research recognized the need to share data between their respective groups (based in Sandwich, UK, and Groton CT, USA) and are jointly developing an integrated approach to information handling. This approach covers all aspects of analytical data from capture from instruments to their ultimate filing with regulatory authorities as, for example, a computer aided new drug application (CANDA).

A joint, integrated approach ensures that all data are accessible irrespective of geographical location and that they are stored in compatible structures and formats. A joint approach also enables development costs to be minimized since, for example, aspects of the system validation can be shared. Validation of analytical methods and computer systems within the pharamceutical industry is currently very topical [30, 31]. One advantage which accrues from LIMS is that such systems can be used to monitor, online, the performance of an analytical method. For example, chromatographic assays may be required to satisfy system suitability criteria [32]. The appropriate criteria can be stored within the LIMS and checked by the data acquisition system for each sample to ensure the method is still valid. If a criterion should fail to be met the analysis can be halted to avoid sample loss, or, if appropriate ruggedness information is available, it may be possible to adjust the chromatographic parameters to enable the suitability criteria to be met again.

The LIMS and data acquisition system also need validation, both on installation and at periodic intervals. This validation can be very demanding on resources and there is the incentive to share it between groups and automate it as much as possible with computer

driven validation protocols. While a never ending line of computers validating each other is not envisaged, there is little doubt that ancilliary computers can be used, particularly for automated revalidation, and that such exercises need not be limited by national boundaries. Thus through the implementation of international LIMS, the framework of total laboratory automation can be completed. The analyst can, in principle, interact with an automated procedure irrespective of their geographical location (Fig. 5). Local, national and international management, can use the systems for resource management and planning at all levels.

The development of this integrated approach to total laboratory automation places new demands on the pharmaceutical analyst. The analyst has still to be conversant with the fundamental chemistry of the sample and the principles of the methods or instruments being used. Automation demands an additional understanding of electronic and mechanical engineering. Eventually, the data generated will reside in databases which may be located physically thousands of miles from the original experiment. Handling and manipulating these databases therefore demands new levels of computer literacy.

Conclusion

Some 125 years ago it was suggested: "the only apparatus required for the bulk of pharmaceutical substances are: one burette, two or three pipettes, three graduated flasks, a few beakers and dishes, filter funnels and glass rods, and a balance carrying about 300 grains" [33].

Since that time there have been dramatic increases in the nature and extent of tests required and in our ability to automate them. Advances in automation have provided systems capable of automated method development and with complex sample preparation capabilities. Robots are also in increasingly widespread use. As data production rates increase, more laboratories are seeking help through information management

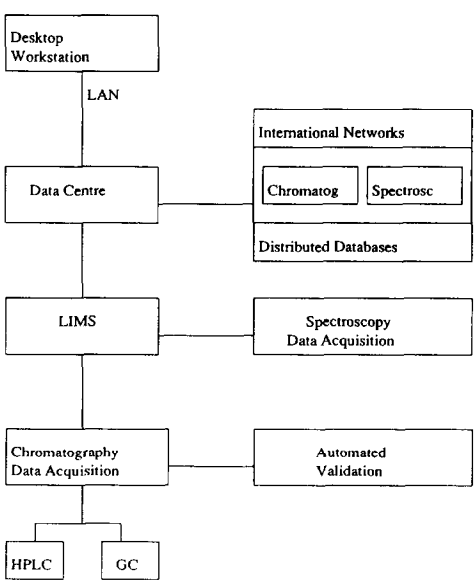

Figure 5
Schematic representation of the international information management system.

systems which are now practicable on an international scale. The diversity of these developments places new demands on the pharmaceutical analyst, demanding skills not only in classical analytical chemistry but also in engineering and information technology.

In the next few years there will be increasing emphasis on newer techniques such as flow injection analysis, on the use of chemometric techniques to avoid complex sample preparation, e.g. *in situ* testing using near-infrared, and in the use of expert systems for aiding the analyst to minimize method development times.

References

[1] J. C. Berridge, *Analyst* **112**, 385–389 (1987).
[2] D. L. Massart and L. Buydens, *J. Pharm. Biomed. Anal.* **6**, 535–545 (1988).
[3] J. C. Berridge, *Analytica Chim. Acta* **233**, 149–159 (1989).
[4] M. de Smet and D. L. Massart, *TrAC* **6**, 266–271 (1987).
[5] H. Cunningham, B. Srinivasan and A. Anada, *Analytica Chim. Acta* **182**, 193 (1986).
[6] D. Goulder, T. Blaffert, A. Blokland, L. Buydens, G. Kateman, H. van Leewan, D. Massart, M. Mulholland, G. Musch, P. Naish, A. Peeters, G. Postma, P. Schoenmakers, M. de Smet, B. Vandeginste and J. Vinke, *Chromatographia* **26**, 237–243 (1988).
[7] A. Peeters, L. Buydens, D. L. Massart and P. J. Schoenmakers, *Chromatographia* **26**, 101–109 (1988).
[8] A. F. Fell, T. P. Bridge and M. H. Williams, *J. Pharm. Biomed. Anal.* **6**, 555–564 (1988).
[9] J. C. Berridge, *Techniques for the Automated Optimisation of HPLC Separations*. Wiley, Chichester (1985).
[10] P. J. Schoenmakers, *Optimisation of Chromatographic Selectivity*. Elsevier, Amsterdam (1986).
[11] P. J. Schoenmakers, P. J. Naish and R. J. Hunt, *Chromatographia* **24**, 579–587 (1987).
[12] A. K. Smilde, C. H. P. Bruins, D. A. Doornbos and J. Vink, *J. Chromatogr.* **410**, 1 (1987).
[13] M. W. Doug, R. D. Conlon and A. F. Poile, *Am. Lab.* May 48–56, June 50–60 (1988).
[14] A. G. Wright, A. F. Fell and J. C. Berridge, *Chromatographia* **24**, 533–540 (1987).
[15] T. O'Dwyer, P. DeLand and R. Smith, *Am. Lab.* June 40–48 (1988).
[16] L. R. Snyder, *J. Chromatogr.* **16**, 223–234 (1978).
[17] J. L. Glajch, J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.* **199**, 57–79 (1980).
[18] A. G. Wright, A. F. Fell and J. C. Berridge, *J. Chromatogr.* **458**, 335–353 (1988).
[19] J. K. Strasters, H. A. H. Billiet, L. de Galan, B. G. M. Vandeginste and G. Kateman, *J. Chromatogr.* **385**, 530 (1987).
[20] A. G. Wright, A. F. Fell and J. C. Berridge, *J. Chromatogr.* **464**, 27–38 (1989).
[21] A. G. Wright, J. C. Berridge and A. F. Fell, *Analyst* **114**, 53–56 (1989).
[22] M. Mulholland and J. Waterhouse, *J. Chromatogr.* **395**, 539–551 (1987).
[23] R. M. Sample, *Anal. Proc.* **23**, 266–267 (1986).
[24] W. J. Hurst, K. C. Kuhn and R. A. Martin, in *Advances in Laboratory Automation, Robotics* (J. R. Strimatis and G. L. Hawk, Eds). Zymark, Hopkinton, MA (1988).
[25] J. L. Isenhour and P. B. Harrington, *J. Chem. Inf. Comput. Sci.* **28**, 215–221 (1988).
[26] R. L. Sharp, R. G. Whitfield and L. E. Fox, *Analyt. Chem.* **60**, 1056A–1062A (1988).
[27] R. D. McDowall, *J. Pharm. Biomed. Anal.* **6**, 547–553 (1988).
[28] R. G. Brownlee and S. W. Compton, *Int. Biotechnol.* **7**, 13–19 (1989).
[29] R. D. McDowall, *Laboratory Information Management Systems*. Sigma Press, Wilmslow (1987).
[30] G. Masters and P. Figarole, *Pharm. Tech.* **10**, June 45–46 (1986).
[31] J. Guerra, *Pharm. Tech.* **12**, September 142–152 (1988).
[32] United States Pharmacopeia, XXI 1229. USP Convention Inc., Rockville, MD (1985).
[33] F. W. Sutton, *Quantitative Analysis*. Churchill, London (1863).

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