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Use of high-performance liquid chromatography in the pharmaceutical industry

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SUMMARY

Requirements for new pharmaceutical products and their impact on applications of high-performance liquid chromatography (HPLC) are discussed. The strengths and weaknesses of HPLC in this context are evaluated and compared with current trends and expectation in separation science.

INTRODUCTION

In recent years, a significant change in the techniques available for the chemical analysis of drugs has been observed. More and more sophisticated analytical instrumentation with highly automated sample handling, control and data-processing systems are playing an increasingly important role in analytical laboratories for drug development and quality control. The main goal of this trend is to obtain more reliable results with less effort in a shorter time. This should lead to faster drug development, better quality of the final products and a higher degree of safety for the patient.

This paper reviews the role of high-performance liquid chromatography (HPLC) in the pharmaceutical industry and analyses trends based on new technologies, on new regulatory requirements for new pharmaceutical products and on modern ways of exploring and developing new drugs.

HPLC AS A TOOL IN THE PHARMACEUTICAL INDUSTRY

In the drug discovery process, HPLC is used as both an analytical and a semi-preparative method for the isolation of new natural products. These natural products from plants, fungi and animals are important starting materials ("lead compounds") for chemical modifications which lead to new drugs. In biotechnology, HPLC has many applications for the isolation, characterization and preparation of proteins and peptides. These compounds are thermally and chemically unstable. Therefore, HPLC techniques are ideally suitable. Because of the polarity and the ease of sample preparation, reversed-phase (RP) HPLC is preferred to normal-phase systems in most instances.

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In the course of drug development, HPLC is applied for the characterization of new drugs, especially purity determinations and assays. Automated HPLC is used for the analysis of various dosage forms, for process validation and in process control. HPLC has increasing importance as an analytical technique for raw materials that are used as pharmaceutical excipients for new drug delivery systems, and it will supplement or even replace many simple pharmacopoeial methods. For the determination of drugs, the characterization of metabolites and pharmacokinetic studies HPLC is an important method for the analysis of biological materials. Modern sample preparation methods, precolumn derivatization[1], column switching[2] and the use of robotic systems[3] can simplify complex assay procedures and can improve reproducibility and accuracy.

In the production and quality control of final products and raw materials, HPLC is used as a quality assurance tool. However, HPLC is not only an analytical technique, but it is also applied on a preparatory scale or production scale for the final purification of drugs. HPLC purification is an alternative to multiple crystallization, because the loss of expensive drugs can be minimized by HPLC purification.

In all of these applications, HPLC is found to be a simple and reliable method. The total analysis costs are competitive with those of other analytical methods, *e.g.*, gas (GC) supercritical fluid (SFC) or thin-layer chromatography (TLC) or spectroscopic methods.

CHALLENGES TO THE PHARMACEUTICAL INDUSTRY

The pharmaceutical industry faces challenges in which HPLC plays an important role. It is hoped that modern biotechnology will generate new drugs that fulfil medical needs. New biotechnological products are also important tools for pharmacology. Modern synthetic drugs are no longer marketed as racemates but whenever possible as optically active compounds. Therefore, the optical purity has to be checked[4]. Economic pressure and limiting patent protection call for the fast development of new drugs in order to shorten the time between the discovery of a drug and its introduction into the market. Good manufacturing practice (GMP) and good laboratory practice (GLP) require careful validation of methods and documentation of method development, system suitability[5] and the results of the analysis. All these challenges will lead to a more frequent application of HPLC.

STRONG POINTS OF HPLC

In the pharmaceutical industry, most of the samples are aqueous solutions, which can be analysed efficiently, by RP-HPLC with or without sample preparation. The selectivity of HPLC helps to resolve optically active compounds, *e.g.*, in purity determinations[4] or in pharmacokinetic studies[6]. HPLC is very fast[7]. Automation, including the use of robot samplers, helps to increase the throughput and will be increasingly used in process control.

WEAK POINTS OF HPLC

Most of the currently available HPLC instruments have severe limitations with regard to dead volumes and speed for the use of modern 3-μm HPLC columns. Many

gradient instruments have delay volumes and mixing volumes 10–100 times larger than the column volume of a 3-μm column of 3 cm × 2 mm I.D. This is the main reason why efficient HPLC columns with 3-μm and smaller particles have not yet made a breakthrough. The reliability of HPLC instrumentation is another weak point in routine applications. The mean time to failure (MTF) of samplers and pumps should be increased. The stability and reproducibility of HPLC columns are not yet good enough for complicated separations where the selectivity plays an important role, and finally there is still no sensitive universal detector, such as the flame ionization detector in GC. Compared with other separation techniques, HPLC lacks the separation efficiency of capillary GC, capillary zone electrophoresis (CZE)[8] and capillary SFC[9].

TRENDS IN HPLC

HPLC will be very much influenced by other separation techniques, especially CZE and capillary SFC. These new techniques have boosted instrument development in HPLC. It can therefore be expected that micro-HPLC systems will soon become important. This will help in overcoming the present limitations and open new areas for more efficient LC with smaller particles and shorter columns.

Fig. 1 shows a new development in this respect. The new micro-HPLC UV detector[10] with a capillary flow cell has an optical path length of *ca.* 20 mm and can significantly improve the signal-to-noise ratio, as shown in Fig. 2. On-column fluo-

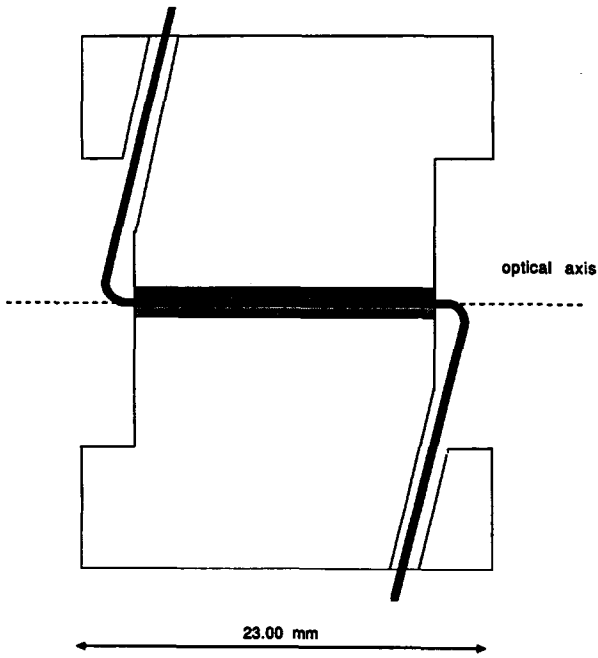

Fig. 1. Cross-sectional view of the logitudinal capillary flow cell (reproduced with permission of LC Packings, Amsterdam).

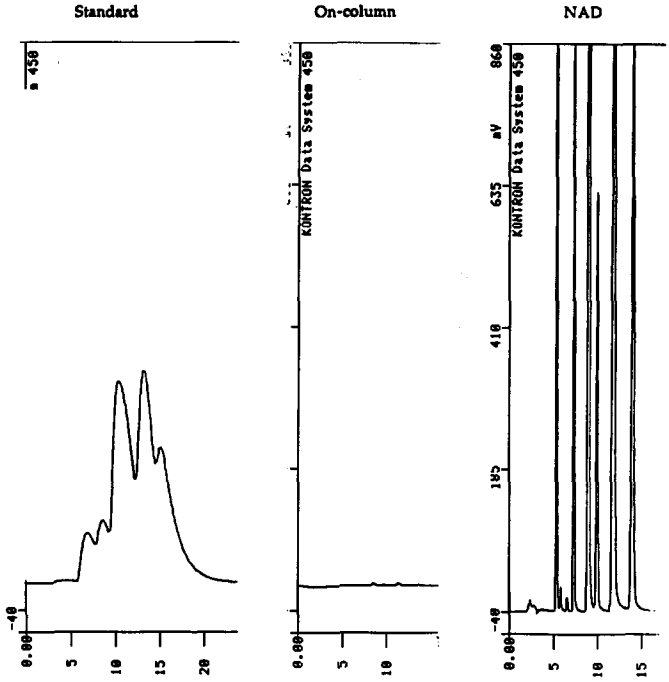

Fig. 2. Resolution and sensitivity in different flow cells (reproduced with permission of LC Packings, Amsterdam, The Netherlands). Test conditions: column, FuC-150, 15 cm × 320 μm I.D.; mobile phase, acetonitrile–water (7:3, v/v); flow-rate, 3 μl/min; temperature, ambient; pressure, 4.6 MPa (46 bar); sample, PAHs; injection volume, 60 nl; detection, UV (254 nm), 0.1 a.u.f.s., t = 2.0 s; chart speed, 0.25 cm/min.

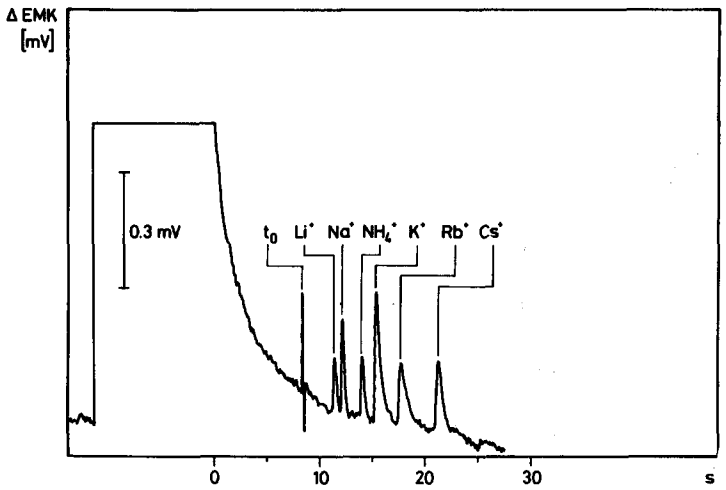

Fig. 3. Separation of ions by open-tubular HPLC (reproduced with permission of Prof. W. Simon, ETH, Zurich, Switzerland). Sample, 10 pl chloride in water, 40–2000 μmol/l; column, 0.33 m × 4.6 μm I.D. coated with 3-sulphopropylsilane; mobile phase, 20 mM formic acid; flow-rate, 10 nl/min.

rescence and UV detectors used in CZE can also be used for open-tubular HPLC systems. Fig. 3 shows an example[11] of electrochemical detection with an ion-selective electrode[12] in an open-tubular LC system for separating ions in a few seconds[13,14].

It is expected that elevated temperature will play an increasingly important role in HPLC. It has been known for years that in packed-column chromatography temperatures above room temperature can improve the performance dramatically, as shown in Fig. 4. From the *H versus u* curve it can be seen that for higher temperatures the minimum shifts to higher flow velocities and also the slope has a much lower value. The loss of efficiency at higher flow-rates is significantly smaller than at room temperature. This is important for the chromatography of large molecules where diffusion can be a limiting factor. As shown by Antia and Horvath[15], elevated temperature also has a beneficial effect on the viscosity and consequently the pressure drop across the column. The elevated temperature is not only beneficial for packed-column chromatography. HPLC in open tubes is feasible at higher temperature even when the inner diameters are 20–100 μm (Fig. 5a). Fig. 5b shows that the strong dependence on k' is much more favourable at higher temperatures. Liquid mobile phases at elevated temperatures have physical properties very similar to those of supercritical fluids and have the same beneficial effects on the kinetics of the separation processes. The question is whether there is any significant difference between high-temperature HPLC and SFC.

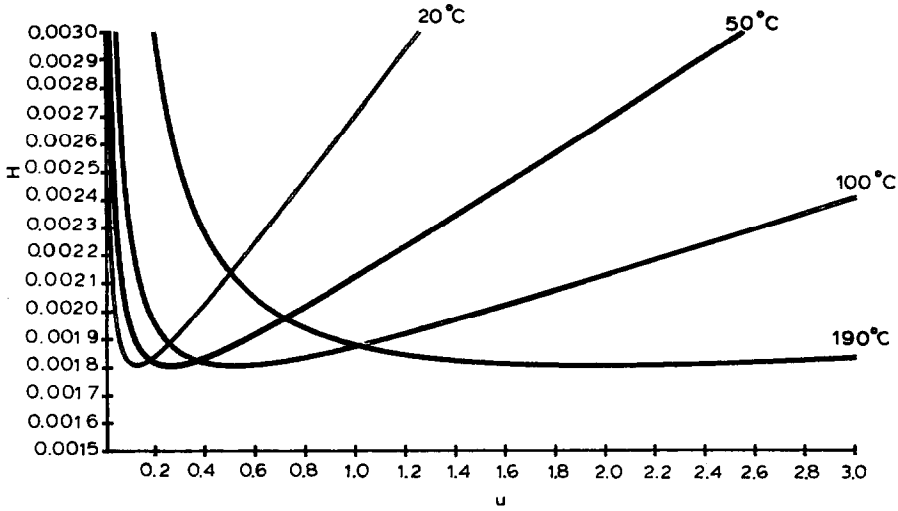

Fig. 4. Simulations of H (cm) *versus* u (cm/s) u curves for packed columns at 20, 50, 100 and 190°C, as indicated. Packed columns, 5 μm.

NEW TECHNIQUES

CZE and SFC are two techniques that have emerged in recent years and will compete with HPLC[16] but are also inspiring new instrumental developments that are important for HPLC techniques. A comparison of SFC, CZE and HPLC techniques in their application to pharmaceutical analysis has been described[16]. In our experi-

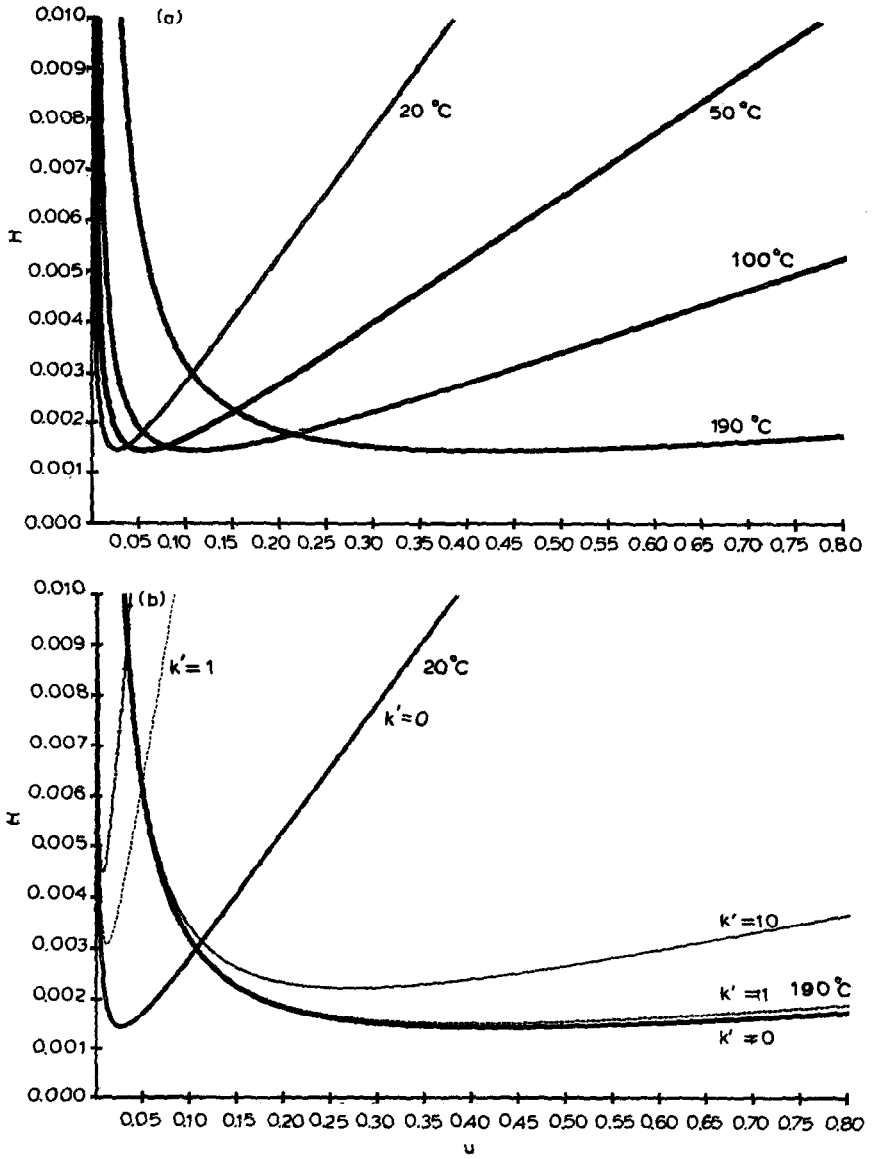

Fig. 5. Simulations of H (cm) *versus* u (cm/s) curves for open-tubular HPLC at 20, 50, 100 and 190°C, as indicated. $k' = 0$. (b) Influence of k' at 20 and 190°C. Capillary, 50 μm I.D.

ence, capillary SFC systems are suitable for the investigation of polymeric excipients for drug delivery systems. These materials, based on natural products, are often complex mixtures that can be analysed of SFC techniques without derivatization or complicated sample preparation.

CZE is a method that has had an important impact not only on the analysis of peptides, proteins and other high-molecular-weight compounds but also on small

molecules[16], as shown in Fig. 6. CZE in open tubes has some limitations, however. The separation of isomeric compounds or, generally, compounds with similar molecular weights and similar structures is often difficult, even with the enormous efficiency of CZE. Secondary interactions, *e.g.*, with gels or micelles, can improve the selectivity of the separations without decreasing the resolution. The development of CZE into electrokinetic chromatographic systems or electroendosmotically driven LC systems (compared with pressure-driven HPLC systems) makes it possible to combine the advantages of HPLC (selectivity) with the advantages of CZE (high efficiency)[8].

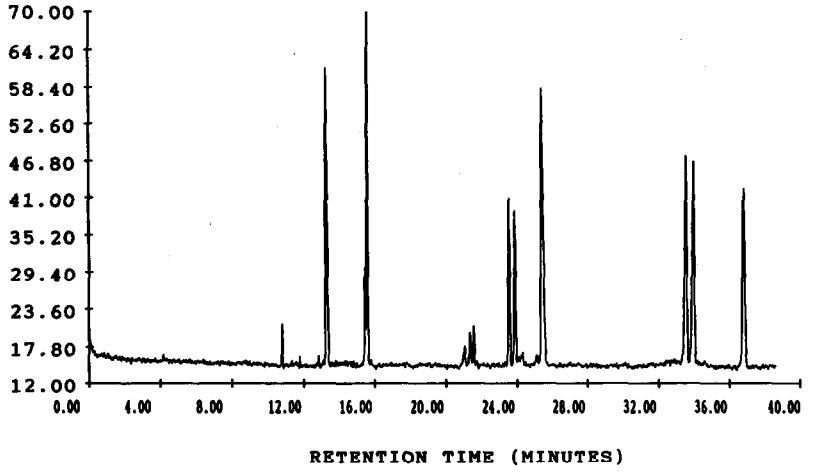

Fig. 6. Separation of terbinafin and its by-products by CZE. Capillary, 87 cm × 50 μm I.D.; buffer, 30 m*M* Na_2HPO_4–40% acetonitrile; voltage, 25 kV; detection, UV on-column (200 nm), 0.006 a.u.f.s.

AUTOMATION

The role of automation in HPLC in saving time, making better use of the instrumentation and obtaining better results has been discussed elsewhere[5]. Automatic sample injectors, intelligent sample processors and the use of robotic systems for sample preparation are part of the mechanization of HPLC systems which dramatically improve the cost efficiency (throughout per instrument) and the quality of the results. Fast LC systems are valuable alternatives to flow-injection analysis (FIA) or continuous flow analysers (CFA) and direct UV determination. The advantage of fast LC systems with very short columns (1–3 cm) compared with FIA, CFA and UV methods is that much less interference and artefacts can be expected and validation of the methods is very simple. More sophisticated HPLC methods for assay and purity determinations can be scaled down to simple and fast HPLC systems by just shortening the columns from 15 cm to 1–3 cm for a high throughput in dissolution-rate testing, content uniformity and process control.

EXPERT SYSTEMS AND METHOD DEVELOPMENT

In addition to automation of sample preparation, sample injection and data evaluation, expert systems are beginning to play a role in HPLC. These expert systems not only help to solve problems efficiently, but are also very useful for training and may become a knowledge base for an organization (knowledge-based expert systems). A few expert systems are already commercially available, *e.g.*, the HPLC Doctor[17] from PiTechnology and DryLab[18] simulation software from LC Resources. The main application of expert systems lies in method development. Many strategies have been discussed in the literature[19]. The most valuable are those which give information about the critical parameters of a separation, also indicating potential problems and the ruggedness of a method[20]. A good documentation of method development also makes method transfer easier from one laboratory to another. Fig. 7 shows an example of DryLab for the optimization of a gradient run with the minimal resolution plot. This and a multi-dimensional presentation of the chromatographic response functions not only give a survey of absolute or local minima but also indicate how sensitive a separation is to variations in separation parameters[5], such as pH, ionic strength or content of organic modifier in reversed-phase systems.

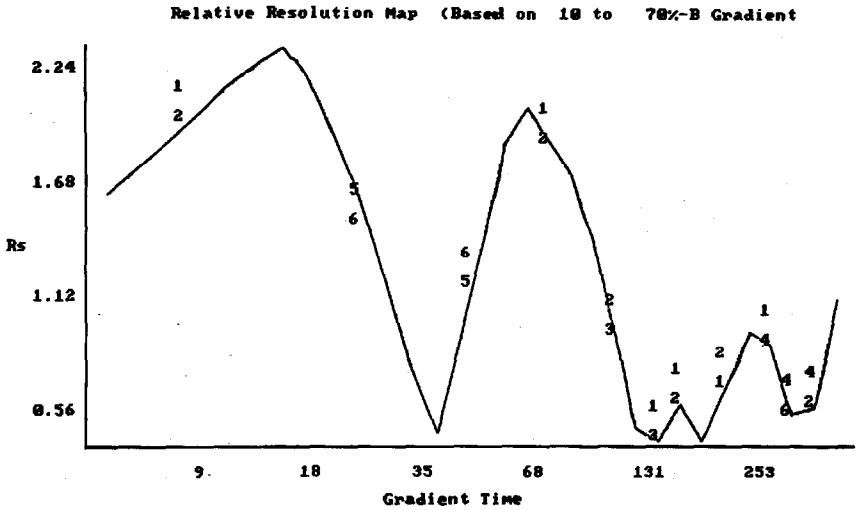

Fig. 7. Simulations of minimal resolution with Drylab.

Electronic data processing (EDP) is playing an increasingly important role in the documentation of HPLC experiments. Laboratory information and managment systems (LIMS) help to organize the work and include office automation systems and scientific spreadsheet programs, such as RS/1[21], statistical evaluation packages, such as SAS[22], and other decision-support software or desk-top publishing tools for making presentations and slides.

CONCLUSIONS

Despite the advent of new techniques, such as SFC and CZE, HPLC is still a growing analytical method in analytical laboratories in the pharmaceutical industry. It can be expected that capillary technology will inspire new developments in HPLC such as smaller, faster and more efficient systems and that future instrument generations will be more reliable and therefore suitable for applications in process control and on-stream analysis. Automation of HPLC systems will continue to make sample preparation easier and method development more efficient. In the future, expert systems will help both in data interpretation and in decision support.

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