Journal of Chromatography, 507 (1990) 125–140
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1678

Comparison of high-performance liquid chromatography, supercritical fluid chromatography and capillary zone electrophoresis in drug analysis

W. STEUER*, I. GRANT and F. ERNI[a]
Analytical Research and Development, Pharma Division, Sandoz Ltd., Basle (Switzerland)

SUMMARY

High-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE) are compared with respect to their usefulness in drug analysis. Factors discussed include efficiency, performance, sensitivity, optimization parameters, method development time, sample preparation, technical difficulties, orthogonality of the information obtained and the possible application to various substance groups. It is concluded that HPLC can be applied successfully in virtually all areas of pharmaceutical analysis. CZE has a promising future in the analysis of drugs and in the field of biotechnological analysis, where a high number of plates is required together with a short analysis time. Nevertheless improvements in detection are still necessary for most applications. SFC is particularly suitable for moderately polar compounds or substances for which mass-sensitive detection is required. SFC and CZE can be considered as complementary to HPLC owing to the orthogonality of the acquired data, and as a result more information can be obtained from the analysis.

INTRODUCTION

High-performance liquid chromatography (HPLC) is a well established method for the purity control and assay of drugs in galenical forms or in biological matrices. Over the last 4 years, the use of supercritical fluid chromatography (SFC) has greatly increased. At present, in addition to non-polar compounds, strongly polar substances and large molecules can be separated. Recently, SFC[1] has been extended to include applications involving ionic compounds. During the last few years, capillary zone electrophoresis (CZE), a high-efficiency separation technique, has attracted considerable attention, mainly for the separation of proteins. However, there has been comparatively little interest in the analysis of pharmaceuticals.

This paper compares the utility of HPLC, SFC and CZE in drug analysis. The advantages and limitations of each technique are discussed, in most instances, on the basis of our own experience in the laboratory. Most of the example separations using

0021-9673/90/$03.50

SFC and CZE were carried out using laboratory-constructed apparatus, which has been described elsewhere[1,2].

Drug analysis can be divided into the following subgroups: drug substance analysis, stability investigations of galenical forms, analysis of excipients, determination of drugs in biological fluids and biotechnological analysis. Each of these subgroups possesses an additional profile of requirements in terms of efficiency, performance, selectivity, sensitivity and sample preparation, which must be taken into account when deciding on the suitability of a separation method.

EVALUATION CRITERIA

This comparative study is based on the following evaluation criteria: efficiency (N); performance (N/t) (N = number of plates; t = time); sensitivity; parameters available for modification of selectivity; method development time; sample preparation; and orthogonality of information.

Efficiency and performance

Davis and Giddings[3] and Martin *et al.*[4] demonstrated, using a statistical method, that a very high peak capacity is necessary in order to obtain, with 90% probability, a baseline separation of a small number of components in a single experiment. For the separation of eight compounds more than half a million plates would be necessary[5]. This indicates that in many analytical problems a high number of plates is desirable. Table I compares the necessary analysis times in HPLC, SFC and CZE for the separation of a given number of species with a given number of plates. A limiting pressure drop of 300 bar was assumed for the chromatographic techniques. In chromatography the optimum particle diameter for a given set of conditions can be determined from the kinetic optimization procedure of Knox and Saleem[6]. For SFC and HPLC the calculated optimum particle diameters range from 0.3 to 7.6 μm. In all instances the most appropriate commercially available particle size was used, which means that for N = 1000–50 000 optimum kinetic conditions are not possible (see Appendix). For N > 50 000 the required times were calculated under kinetically optimized conditions. In HPLC the maximum available pressure of 300 bar can be used for bringing about the flow of the mobile phase. In SFC, however, a post-column

TABLE I

ANALYSIS TIMES REQUIRED FOR HPLC, SFC AND CZE

Assumptions: ΔP = 300 bar; coefficients for the Knox hv curve were taken as; A = 1.0, B = 2.0, C = 0.1, which leads to h_{min} = 2.4 and v_{min} = 2.7; for the calculation of diffusion coefficients of pharmaceutical compounds, a typical value for the effective hydrodynamic radius of 4 Å was assumed.

No. of components	*N*	*Times required (s)*			
		HPLC (20°C)	*HPLC (90°C)*	*SFC*	*CZE*
5	1000	4.2	1.1	0.8	–
10	10 000	56	15.2	10.6	–
20	50 000	480	140	95	60
50	500 000	32 000	10 300	7100	600

restrictor must be employed to maintain the desired fluid density at the column outlet, which often means an outlet pressure of 200 bar. Hence, two thirds of the available pressure is consumed merely to sustain the supercritical conditions, leaving only 100 bar for generation of fluid flow. The separation of drug substances by SFC requires, owing to their often polar nature, high fluid densities and the use of polar modifiers[7]. Taking this into consideration, a diffusion coefficient of $0.3 \cdot 10^{-4}$ cm^2 s^{-1} and a viscosity of $7.2 \cdot 10^{-2}$ cP were calculated[8] and used together with a maximum pressure drop of 100 bar in the calculations for SFC. Ideally, the efficiency of CZE increases indefinitely as the analysis time is reduced[9]. However, when factors such as wall adsorption and ohmic heating are taken into account, diffusion-limited efficiency can be realised in only a few instances. In our experience, typical efficiencies of 500 000 plates per metre are attained with an electric field gradient of 30 kV m^{-1} and an ionic strength of 0.030 mol dm^{-3}. If the electric field gradient is held constant while varying the column length, the retention time and the plate number will be directly proportional to the column length, at least to a first approximation. Instrumental difficulties, namely extra-column broadening, impose a practical limit of no less than about 10 cm for the column length. Hence capillary zone electrophoresis with a desired plate number of less than 50 000 cannot be considered practical.

Table I demonstrates that HPLC should be used when a small number of components have to be separated in a short time. This is a typical requirement for the stability analysis of galenical forms. When a high number of plates is required, SFC and CZE are to be preferred. Calculations using the values estimated above show that SFC has a three times greater performance than HPLC. However, if HPLC is carried out at high temperatures[10], performances approaching those of SFC can be obtained. For the determination of drug purity, where often 10–15 peaks have to be separated, in some instances SFC may be a useful alternative to HPLC. CZE is the most appropriate technique for the separation of very complex mixtures, where more than 100 000 plates

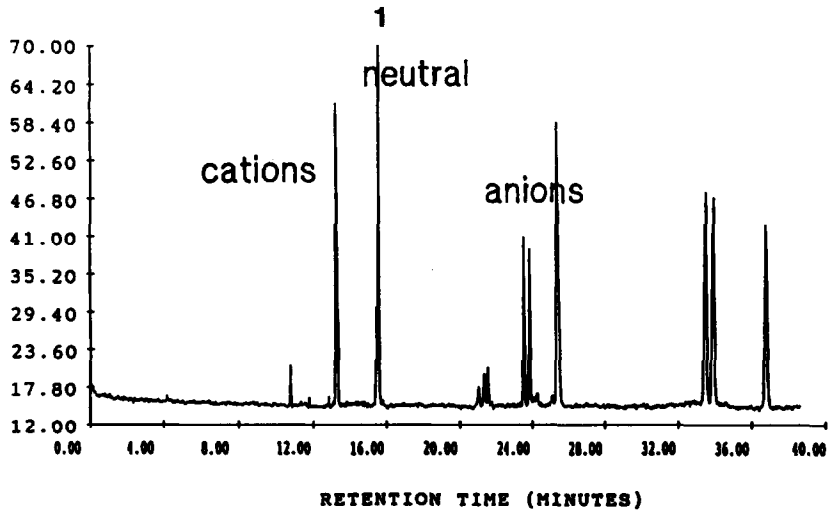

Fig. 1. Analysis of a stressed pharmaceutical alkaloid (1) by CZE. Applied voltage, 25 kV; buffer, water–acetonitrile (60:40)–30 m*M* Na_2HPO_4; capillary, 90 cm × 50 μm I.D.

are required (Fig. 1). Typical examples are the purity control of drugs and biotechnological analyses such as the separation of protein digests (Fig. 9).

Sensitivity

The sensitivity of a separation method depends on both the loading capacity of the system and the nature of the available detectors. In addition to concentration-dependent detectors such as UV absorbance and fluorescence types, the mass-sensitive flame-ionization detector can be implemented in SFC, provided that the addition of modifiers to the carbon dioxide can be avoided. Unfortunately, this is seldom the case for polar drugs. In contrast to HPLC and SFC, CZE can only be used in a miniaturized form, resulting in constraints in terms of loading capacity and sensitivity. It should be emphasized that for absorbance and fluoresence detection it is the concentration sensitivity and not the minimum detectable amount that is relevant in drug analysis.

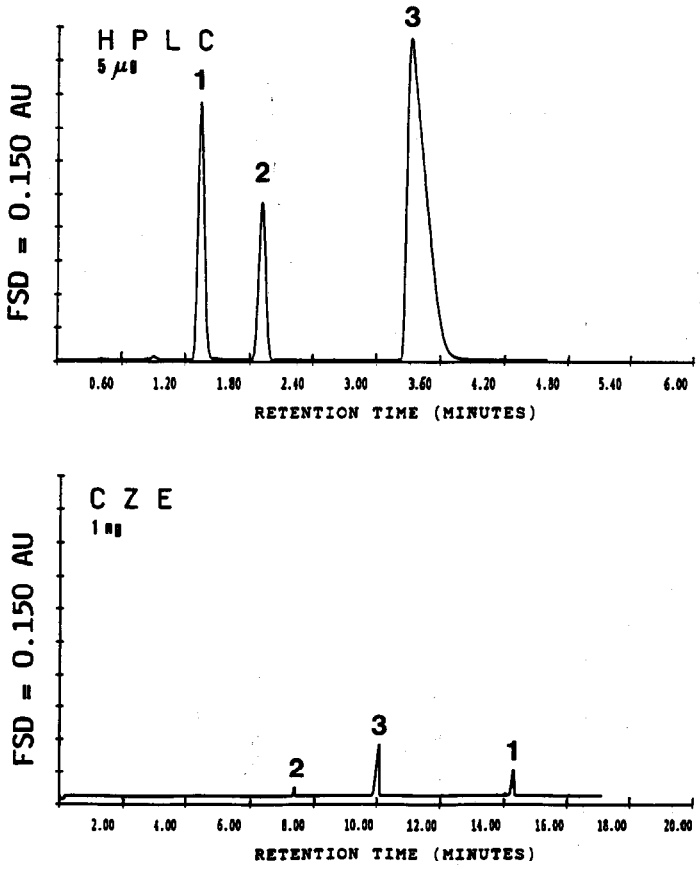

Fig. 2. Sensitivity comparison for HPLC and CZE; separation of spirapril and its degradation products. HPLC: column, Brownlee RP-18, 5 µm, 100 mm × 4.6 mm I.D.; eluent, acetonitrile–water (50:50)–0.5 m*M* tetramethylammonium hydroxide, adjusted to pH 2; temperature, 70°C; UV detection at 217 nm. CZE: UV detection at 214 nm; buffer, acetonitrile–10 m*M* disodium tetraborate (15:85), adjusted to pH 11.7 with sodium hydroxide; capillary, 90 cm × 50 µm I.D.; applied voltage, 25 kV ($i = 14$ µA).

The latter can often be deceptive in capillary SFC and CZE. When working with packed conventional columns, UV detection in SFC and HPLC is comparable in terms of sensitivity and loading capacity, although the noise level may be greater in SFC owing to density-related refractive index changes in the highly compressible supercritical fluid. UV detection in capillary SFC demands an on-line detection technique[11,12], with detector volumes of less than 100 nl and optical path lengths of less than 250 μm, resulting in a sensitivity loss, relative to HPLC, of a factor 40.

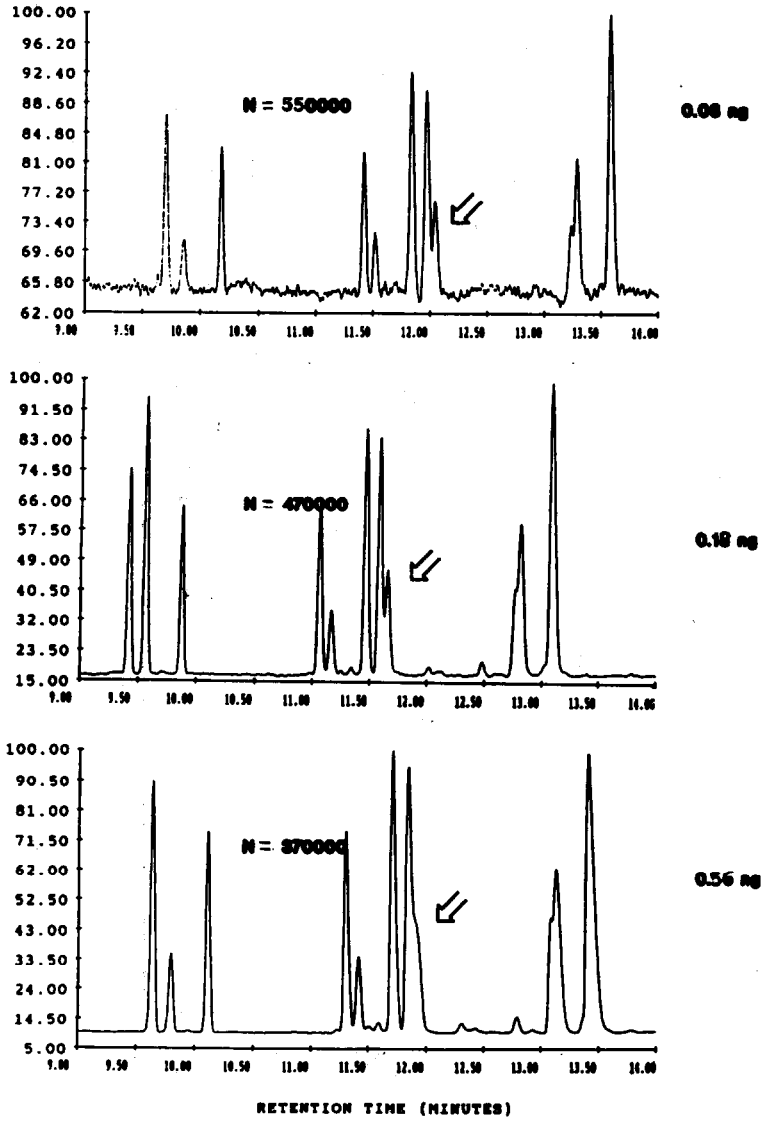

Fig. 3. Loading capacity study for the separation of terbinafine and its by-products by CZE. UV detection at 214 nm; volume injected held constant; buffer, 60% acetonitrile in 12 m*M* KH_2PO_4–12 m*M* Na_2HPO_4, adjusted to pH 2.4 with phosphoric acid; capillary, 90 cm × 50 μm I.D.; applied voltage, 30 kV ($i = 14$ μA).

From our experience with the separation of spirapril an angiotensin converting enzyme inhibitor co-developed by Sandoz and Schering-Plough and its degradation products (Fig. 2), it can be concluded that the concentration sensitivity in HPLC is at least ten times better than that in CZE. In both instances the same sample concentration was injected (1 mg/ml). As shown in Fig. 3, for the separation of terbinafine and its by-products, the poor sensitivity of CZE cannot be totally compensated for by an increase in the amount injected. In our study of the loading capacity in CZE, the efficiency was observed to decrease by a factor of four following a 100-fold increase in the sample concentration (Fig. 4), which leads to an unacceptable loss of resolution. Additionally, the signal-to-noise ratio does not increase linearly with the amount injected because of the additional peak broadening, which results in a less than linear increase in peak height. From the above, it is clear that, in addition to the optimization of selectivity, the amount injected can also be optimized, subject to resolution requirements. The loading capacity can be increased by increasing the ionic strength, but the applied voltage then has to be reduced, which results in a longer analysis time. In general, it can be concluded that CZE has a limited application in trace analysis, where one component is usually present in a large excess.

Parameters available for modification of selectivity

The number of parameters and the extent to which they influence selectivity are indications of the optimization potential of the separation technique. However, it does not imply that the method that has the most available parameters influencing

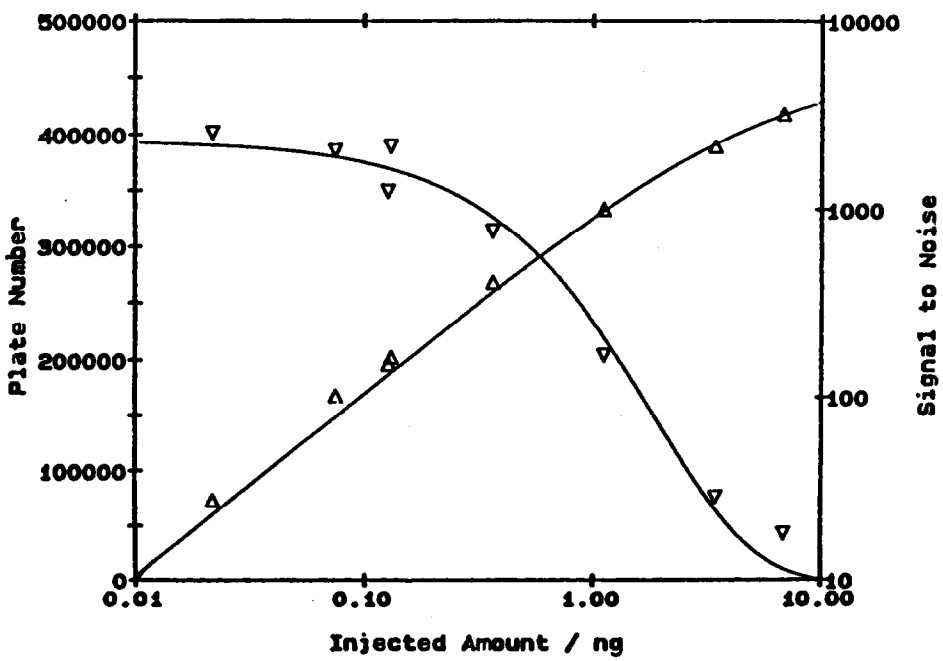

Fig. 4. Effect of amount injected on efficiency and signal-to-noise ratio in the CZE of terbinafine with constant injection volume. Conditions as in Fig. 3. ∇ = Number of theoretical plates; Δ = signal-to-noise ratio.

TABLE II

USEFULNESS OF PARAMETERS INFLUENCING THE SELECTIVITY

Scale from 0 to 10, where 10 = most significant effect.

Parameter	*HPLC*	*SFC (packed)*	*CZE*
Stationary phase	10	5	0
pH	10	1	10
Ionic strength	7	2	1
Modifier	10	5	4
Ion pairing agent	5	5	5
Pressure	0	10	0
Temperature	1	4	0
Gradients	8	9	0
Total	51	41	20

selectivity can be optimized in the shortest time. Table II compares the parameters that may be capable of modifying the selectivity. The parameters are evaluated according to their relative significance for each technique; the number 10 indicates that the parameter is of the greatest importance on a scale from 0 to 10 and 0 indicates that the effect of changing the parameter is negligible. The greatest advantage of HPLC over SFC is that a large number of stationary phases and mobile phases are available with a wide range of polarities. Organic modifier gradients can be run in HPLC and SFC but not, so far, in CZE. In SFC the density can be used to change the selectivity rapidly and very effectively. This effect can be exploited by the use of pressure gradients, which can even be combined with organic modifier gradients allowing the simultaneous separation of polar and non-polar species (Fig. 5). Hence the lack of efficiency in HPLC and SFC, compared with that of CZE, can be compensated for, to a certain extent. In CZE the most important factor for bringing about selectivity changes is the pH. In pure CZE the electrolyte must possess, at least to a certain extent, an aqueous character, which limits the choice of buffer composition[13]. The separation of neutral substances can be achieved only through the use of micellar solutions[14]. In electrically driven chromatography (electrochromatography)[15], the selectivity can also be influenced by the choice of the stationary and mobile phases as in HPLC. Based on Table II, HPLC is the most versatile method. The fact that SFC possesses more possibilities for selectivity modification than CZE must not be overestimated in drug analysis, as it should be taken into account that compounds such as strongly basic amines and large peptides cannot be considered for analysis by SFC owing to the limited polarity range of the mobile phase.

Method development time

The time required to develop a separation method depends mainly on the time required for column equilibration, the performance (N/t) and the efficiency (N). CZE shows the most promise in this area because of its very high performace and efficiency

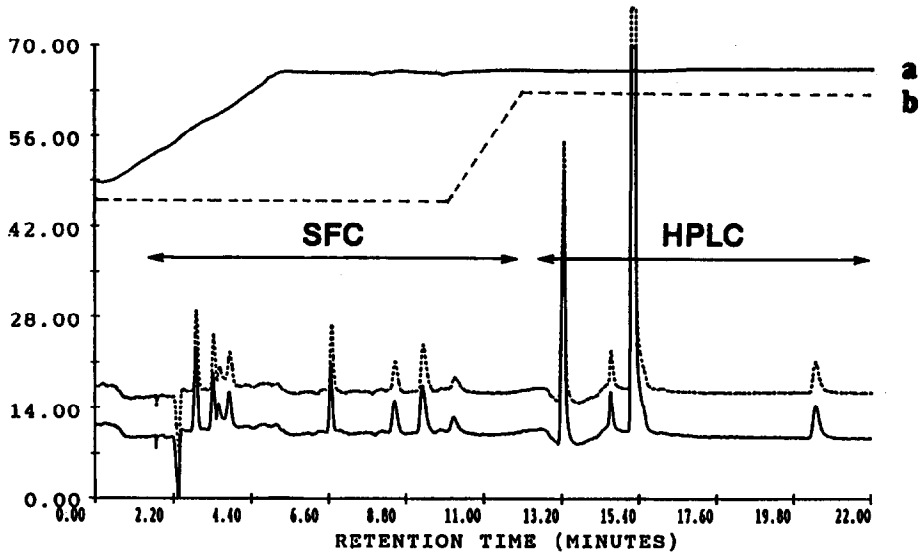

Fig. 5. Combined pressure and modifier gradient in SFC. Column, Brownlee diol-phase, 5 μm, 500 mm × 4 mm I.D.; eluent, carbon dioxide–methanol–20 m*M* dimethyloctylamine; UV detection at 220 nm. (a) Pressure gradient 183–322 bar; (b) modifier gradient 8–25%.

(Fig. 1) compared with SFC and HPLC. SFC offers a higher performance than HPLC and often shorter equilibration times[7,16], because following changes of the supercritical fluid density a stable system is rapidly obtained. From the above it can be concluded that HPLC requires the longest method development time.

Sample preparation

The sample preparation should be minimal and should not be influenced by the separation step. The separation should not be sensitive to the nature of the extraction solvent or to the presence of matrix components. On-line sample pretreatment procedures such as column switching should be available. Because, in most instances, the extraction of drugs from their galenical forms requires the use of a polar solvent, the subsequent separation step should not be affected by its presence, *i.e.*, the eluent should have at least the same eluotropic strength as the extraction solvent. When comparing HPLC, SFC and CZE, only reversed-phase HPLC comes close to fulfilling these requirements. In this instance only minimum constraints are imposed on the extraction solvent and it may even contain large amounts of water. The choice of extraction solvent in SFC is, in contrast, severely restricted. Polar modifiers containing water would cause spurious artefacts or at the very least a loss of efficiency. Therefore, SFC is often not suitable when polar solvents must be used for the extraction of polar substances from an aqueous excipient matrix, or from biological matrices. This problem can be alleviated by the use of an on-line extraction set-up, in which the eluent is also used as the extraction solvent[17], or by column switching, permitting the replacement of the polar solvent. These problems are less severe in capillary SFC.

In CZE, the sample solvent and the electrophoresis buffer are preferably the same, otherwise disturbances of the electric field or precipitation of the extracted components could occur. The problem can be avoided by preventing electroosmosis and using electromigration as the injection principle, although care must be taken to ensure that the analytes are ionized in the sample solvent. When the separation requires buffers that are vastly different from the extraction solvents, such as extreme pH values, or pure water, the extraction may be disturbed. This is demonstrated for the

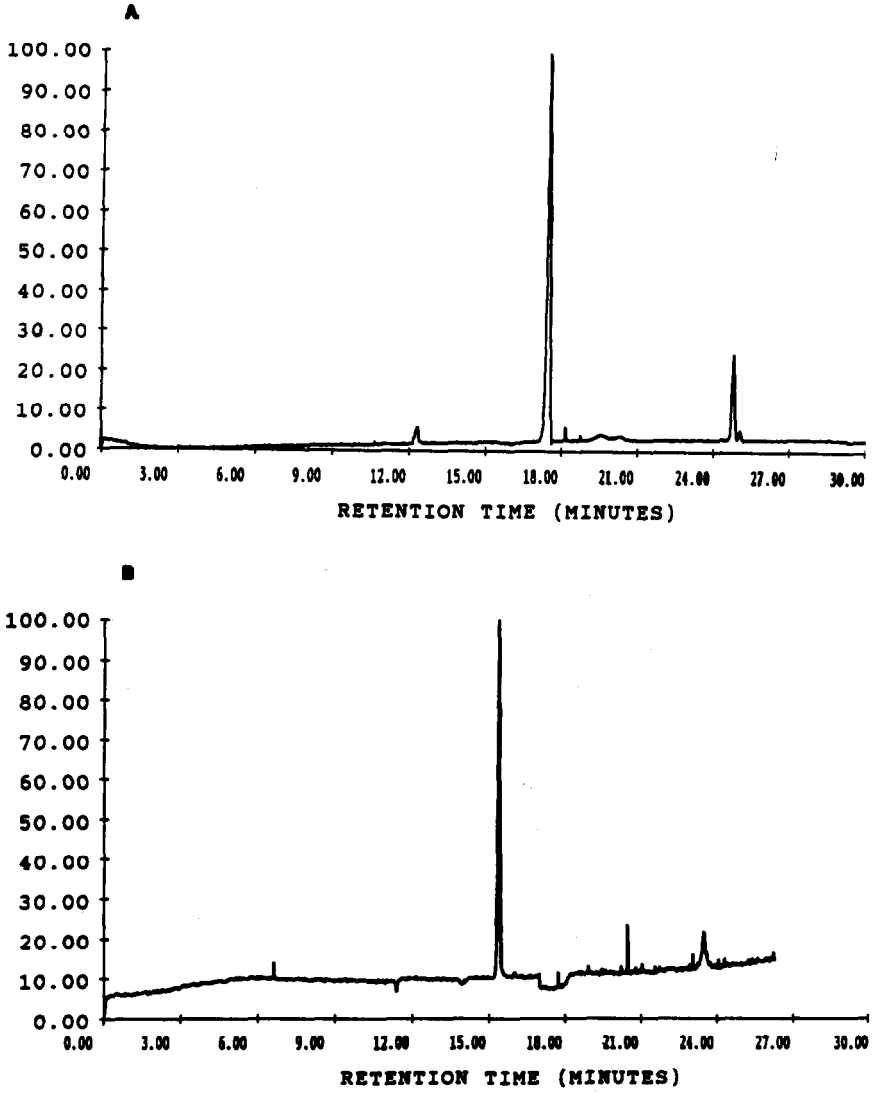

Fig. 6. Interaction between the preparation of the sample and the subsequent separation step. Extraction of spirapril from dosage forms: (A) CZE, buffer of pH 11.7; (B) standard HPLC procedure. Extraction buffer for HPLC, acetonitrile–water (50:50); buffer for CZE, see Fig. 2. HPLC buffer causes disturbances of the electric field.

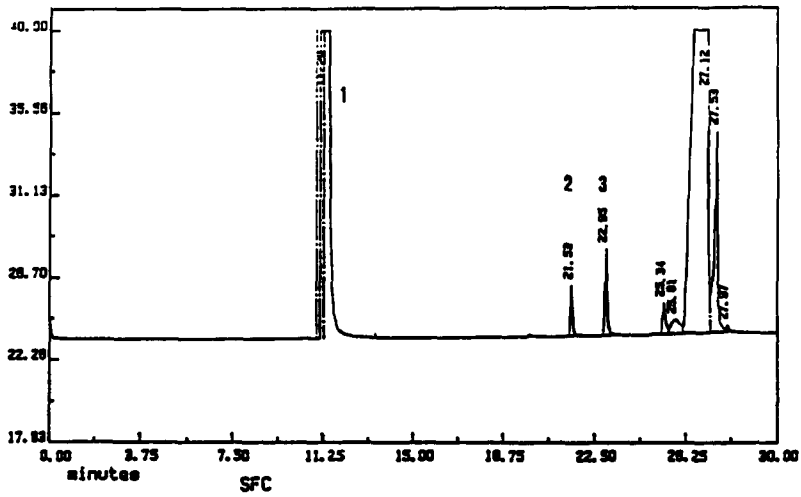

Fig. 7. Determination of cetyl alcohol in cetyl palmitate by capillary SFC. Cyano column, 10 m × 50 μm I.D.; film thickness, 0.25 μm; density gradient, 0.2 → 0.5 g/ml; FID. Peaks: 1 = methylene chloride; 2 = cetyl alcohol; 3 = stearyl alcohol (internal standard).

extraction of spirapril and its degradation products from the capsule mixture (Fig. 6). At the necessary pH of 12 the excipients were partly dissolved as colloids, which hindered the centrifugation. In most instances these problems are less critical in CZE than in SFC.

Technical feasibility

During the last decade, HPLC with packed columns has been developed to a high level of sophistication. Injection systems are reliable with a reproducibility of 0.5% (relative standard deviation). There are no severe problems with the delivery of eluent in conventional HPLC. Flow-rate relative standard deviations are less than 0.5%. Problem with solvent delivery are only encountered with micro-HPLC systems (column diameter < 1 mm).

The injection precision of SFC is relatively poor in comparison with HPLC. For the analysis of the excipient cetyl palmitate (Fig. 7), relative standard deviations, on injected amount, of up to 5% were observed, mainly as the result of non-constant splitting ratios or sample precipitation caused by density changes occurring during the injection[18]. The use of an internal standard is therefore recommended. The maintenance of a constant density independent of the flow-rate requires precise restrictors and a sophisticated pressure–flow regulating system. No such systems are commercially available for packed or capillary SFC. The lack of such a device leads to a significant loss of efficiency in capillary SFC during the latter stages of a density gradient, as the flow-rate, and consequently the plate height, increase with increasing density.

In CZE, concentration-dependent detectors, such as UV absorbance of fluorescence types, are 10–20 times less sensitive than their counterparts in HPLC or SFC and the coupling to mass-sensitive detectors is difficult. Depending on the injection method (electromigration, gravitational or pneumatic), relative standard deviations on peak

TABLE III

TECHNICAL FEASIBILITY

Scale from 0 to 10, where 10 = most reliable.

Operation	*HPLC*	*SFC*	*CZE*
Detection	8	8	1
Injection	10	3	6
Delivery of eluent	8	5	10
Pressure limitation	6	2	10
Total	32	18	27

areas of up to 3–4% are typical[19], and bias effects are unavoidable with electrokinetic injection[20]. For this reason in CZE, as in SFC, the use of an internal standard is advisable. If the capillaries are washed with sodium hydroxide between runs, the fluctuations in electroosmotic flow are less than 0.5%.

As shown in Table III, for technical evaluation HPLC is the most reliable technique, followed by CZE, for which fully automated systems are now on the market. To a large extent the detector still represents an unsolved problem in CZE.

Orthogonality of information

The objective of combined analytical separations is to obtain non-redundant information from independent systems[21–23]. In the ideal case different systems can be coupled together to give an on-line multi-dimensional set-up[24]. For comparison of our data, the analysis times for each technique were normalized to give "retention parameters" defined according to the expression

$$\chi_i = \frac{t_i - t_0}{\Delta t}$$

where t_i represents the time for the i_{th} component, t_0 the time for the first component and Δt the total range of analysis times. This procedure was carried out for several drugs and their by-products and degradation products, wich represent a range of substances with vastly different chemical properties. χ_{CZE} and χ_{SFC} are plotted against χ_{HPLC} in Fig. 8. Comparison of HPLC with CZE and HPLC with SFC for several separations suggests that HPLC and CZE are usually orthogonal systems (Figs. 1, 8 and 9). As HPLC and SFC often show only a slight correlation, the on-line coupling of these techniques would be of considerable benefit.

Influence of substance properties on choice of technique

An obvious prerequisite for the analysis of a substance by SFC is an adequate solubility in the supercritical medium. This can often be increased by the addition of an organic modifier. However, this leads to a considerable deterioration of the favourable kinetic properties of the supercritical carbon dioxide when the modifier content exceeds *ca.* 20%. For this reason, substances such as isradipine (calcium antagonist), Sandimmune (immunosupressive agent) or non-polar excipients (waxes, polymers such as cetyl palmitate) (Fig. 7), which are readily soluble in acetonitrile, chloroform

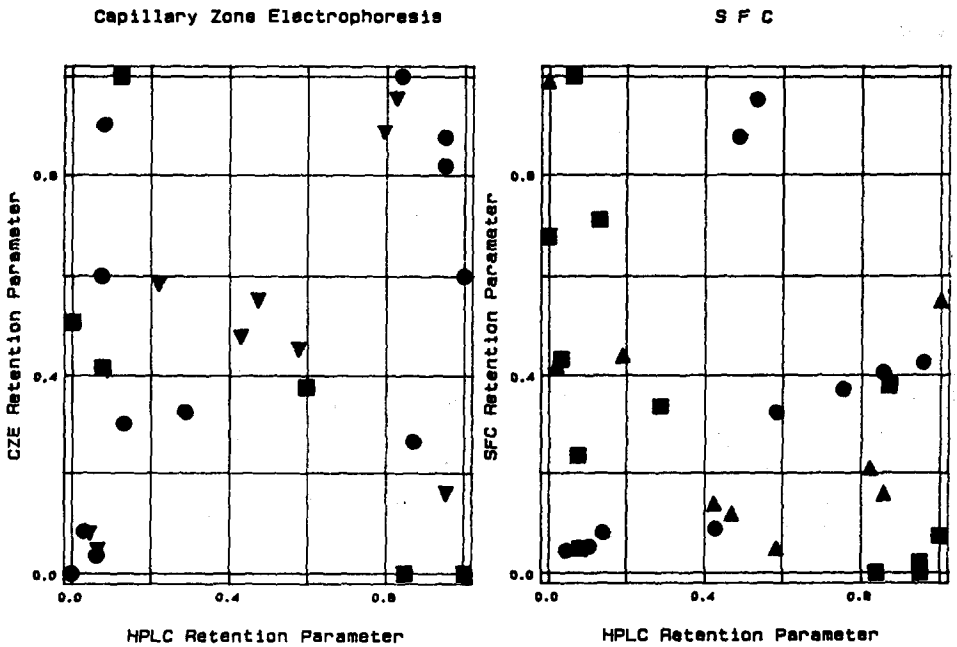

Fig. 8. Demonstration of the orthogonality between HPLC and CZE and between HPLC and SFC. No obvious correlation arises between the retention parameters in HPLC and CZE; some correlation between those of HPLC and SFC may arise (isradipine). All HPLC separations were carried out under reversed-phase conditions, with the exception of isradipine (normal-phase). Compounds: (left) ▼ = terbinafine; ■ = spirapril; ● = AH 21 132; (right) ▲ = terbinafine; ● = isradipine; ■ = AH 21 132.

and similar aprotic solvents (comparable with supercritical carbon dioxide in terms of eluotropic strength), are easily separated, whereby only small amounts of polar modifiers (*ca.* 5%), *e.g.*, methanol, are called for. Moderately basic ionic substances such as alkaloids[25], indole derivatives, α-amino alcohols[7] or barbiturates, and weak acids, *e.g.*, benzoic acid or spirapril[7] and PTH-amino acids[26], can also be separated, although in many instances suitable ion-pairing agents are required. Large peptides such as octreotide and calcitonine are not soluble in carbon dioxide–methanol mixtures.

CZE in its pure form is suitable only for substances that can be ionized in solution, and therefore requires the use of polar eluents of an aqueous nature, although large amounts of organic modifiers can be added if required (Fig. 3). The separation of neutral substances can be achieved using micellar solutions provided that they have an adequate solubility in the buffer mixture. The addition of organic solvents normally increases the critical micelle concentration, which limits the amount of modifier that can be added.

CZE can be applied both for the separation of low-molecular-weight drugs such as terbinafine, propranolol and spirapril and also for peptides and proteins.

Owing to the broad scope of possible eluents and stationary phases, HPLC has the fewest constraints. Polar, non-polar, ionic, small and even large molecules, *e.g.*, antibodies, can be separated. Detection problems arise for molecules without chromophores, because of the lack of a simple mass-sensitive detector.

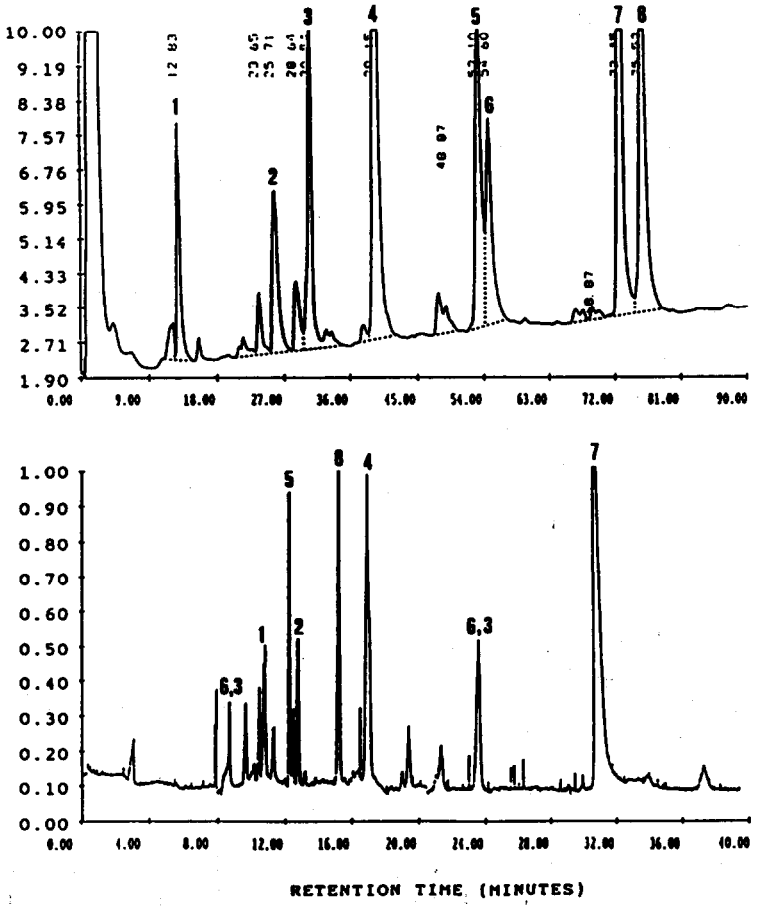

Fig. 9. Comparison of the separation of a protein digest by HPLC (top) and CZE (bottom). HPLC: column, Vydac C_{18} (with Aquapore pre-column); eluent, 0–58% acetonitrile in water in 130 min (0.1% trifluoracetic acid); UV detection at 215 nm. CZE: capillary, 50 cm × 50 μm I.D.; applied voltage, 14 kV (i=24 μA); buffer, 20 mM citrate buffer (pH 2.5); UV detection at 200 nm.

Optimum use of HPLC, SFC and CZE

HPLC can be utilized in the determination of drugs in biological matrices, where in addition to a high performance a high sensitivity is required. It can also be implemented for drug stability investigations in galenical forms, where a high performance is required, for the determination of drug purity and in the analysis of biotechnological products (Fig. 9), in which event the use of gradient elution gives rise to a high peak capacity.

CZE is favoured for drug purity control and also for the separation of biotechnological products. It is particularly suitable for the separation of protein digests (Fig. 9) owing to its enormous peak capacity.

Of the three methods, SFC possesses the narrowest range of application in pharmaceutical analysis. Because mass sensitive detection [flame ionization detection (FID)] can be employed, capillary SFC is suitable for the analysis of excipients (Fig. 7).

With a more limited scope than HPLC or CZE, SFC can be used for the purity control of drugs because of its high performance (Fig. 5).

Strengths and weaknesses of HPLC, SFC and CZE

The strength of HPLC is its high technical level, selectivity and versatility according to substance groups and task. The lack of performance and efficiency is partly compensated for by the possibility of using gradient elution. A weak point is the lack of a simple, universal, sensitive method of detection equivalent to FID in gas chromatography and SFC.

CZE displays an enormous efficiency and therefore separation methods can be rapidly developed. The main drawbacks are the poor sensitivity and the fact that elution and extraction buffer have to be similar.

SFC exhibits a higher performance than HPLC and often shorter method development times. An advantage is the possibility of using both modifier and density gradients or a combination of both. Owing to technical problems, SFC with packed columns is far from being at a routine working level. The method is limited to only a small range of eluents and accordingly to a small number of substance groups.

CONCLUSIONS

From the comparison of the three techniques in drug analysis according to the evaluation parameters discussed above, it can be concluded that HPLC can be used successfully in nearly all areas of pharmaceutical analysis. At higher temperatures, performances approaching those of SFC are possible.

CZE has a promising future in drug analysis, where a high number of plates is required within a short time. The method is especially suitable for ionic species. For most applications the detection sensitivity must be improved.

SFC is useful for non-polar and moderately polar compounds or substances for which mass-sensitive detection is required. It is a suitable method for purity control and for the analysis of excipients, although the technical level requires further improvement.

Owing to their orthogonality, CZE and SFC are worth developing, not in competition or as an alternative to HPLC, but as an additional method in order to augment the information obtained from the analysis.

In the future, each of the three techniques will have its place in pharmaceutical analysis, its particular share of application depending on the product range concerned.

APPENDIX

Time optimization under non-ideal conditions: Calculation of optimum time for a given plate number, pressure drop and particle size

The shortest time for a given number of plates with a predetermined particle size will be obtained when the full available pressure is used. Hence the length must be calculated such that the desired number of plates is realized at this pressure drop.

Linear velocity is governed by the equation

$$u = \frac{\Delta P d_p^2}{\varphi \eta L} \tag{1}$$

Substituting $L = Nhd_p$ and $u = vD_m/d_p$ yields

$$vh = \frac{\Delta P d_p^2}{\varphi \eta N D_m} \tag{2}$$

If h is then substituted by the Knox hv curve, following minor rearrangement the equation

$$f(v) = Cv^2 + Av^{4/3} + B - \left(\frac{\Delta P d_p^2}{\varphi \eta N D_m}\right) = 0 \tag{3}$$

is obtained. This equation is then solved for v using a Newton–Raphson iterative scheme in which values of coefficients $A = 1$, $B = 2$ and $C = 0.1$ were assumed:

$$v_{n+1} = v_n - \frac{f(v)}{f'(v)} \tag{4}$$

where $f'(v) = 2Cv + (4/3)Av^{1/3}$

$$v_{n+1} = v_n - \frac{Cv_n^2 + Av_n^{4/3} + B - (\Delta P d_p^2)/(\varphi \eta N D_m)}{2Cv_n + (4/3)Av_n^{1/3}} \tag{5}$$

Successive iterations are carried out until no significant change in v is observed. The reduced velocity obtained from solution of eqn. 3 is then used to calculate h by substitution into eqn. 2. The required column length and the analysis time for an unretained component are given by

$$L = Nhd_p \tag{6}$$

and

$$t_m = Ld_p/vD_m \tag{7}$$

respectively. The time required for the nth component is calculated using the standard expression for the peak capacity:

$$\log\left(\frac{t_n}{t_m}\right) = \frac{n-1}{0.6\sqrt{N}} \tag{8}$$

GLOSSARY OF SYMBOLS

u	Mobile phase linear velocity
d_p	Particle diameter
ΔP	Pressure drop
φ	Dimensionless flow resistance parameter (typically = 500)

η	Mobile phase viscosity
L	Column length
h	Reduced plate heigth
v	Reduced velocity
D_m	Diffusion coefficient
A, B, and C	Knox equation coefficients
N	Number of theoretical plates
v_n, v_{n+1}	(n)th and $(n+1)$th iterates
f(v), f'(v)	see eqn. 3
t_m	Column dead time
t_n	Time for the (n)th component

REFERENCES

1 W. Steuer, M. Schindler, G. Schill and F. Erni, *J. Chromatogr.*, 447 (1988) 287.
2 J. H. Knox, *Chromatographia*, 26 (1988) 329.
3 J. M. Davis and J. C. Giddings, *Anal. Chem.*, 55 (1983) 418.
4 M. Martin, D. P. Herman and G. Guiochon, *Anal. Chem.*, 58 (1986) 2200.
5 F. Erni, W. Steuer and H. Bosshardt, *Chromatographia*, 24 (1987) 201.
6 J. H. Knox and M. Saleem, *J. Chromatogr. Sci.*, 7 (1969) 614.
7 W. Steuer, J. Baumann and F. Erni, *J. Chromatogr.*, 500 (1990) 469.
8 P. J. Schoenmakers, in R. M. Smith (Editor), *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London, 1988, Ch. 4, p. 102.
9 J. W. Jorgenson and K. D. Lukas, *J. Chromatogr.*, 218 (1981) 209.
10 F. D. Antia and Cs. Horváth, *J. Chromatogr.*, 435 (1988) 1.
11 P. A. Peaden and M. L. Lee, *J. Chromatogr.*, 259 (1983) 1.
12 D. W. Later, B. J. Bornhop, E. D. Lee, J. D. Henion and R. C. Wiebolt, *LC · GC, Mag. Liq. Gas Chromatogr.*, 5 (1987) 804.
13 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.
14 S. Terabe, K. Otsuka, K. Ichikava, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
15 J. H. Knox and I. H. Grant, *Chromatographia*, 24 (1987) 135.
16 W. Steuer, M. Schindler and F. Erni, *J. Chromatogr.*, 454 (1988) 253.
17 E. D. Ramsey, J. R. Perkins, D. E. Games and J. R. Startin, *J. Chromatogr.*, 470 (1989) 353.
18 M. L. Lee, B. Xu, E. C. Huang, N. M. Djordjevic, H. C. K. Hang and K. E. Markides, *J. Microcolumn Sep.*, 1 (1989) 7.
19 D. J. Rose and J. W. Jorgenson, *Anal. Chem.*, 60 (1988), 642.
20 X. Huang, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 60 (1988) 375.
21 J. F. K. Huber, E. Kenndler and G. Reich, *J. Chromatogr.*, 172 (1979) 15.
22 H. Vink, *J. Chromatogr.*, 69 (1972) 237.
23 D. L. Massart and R. Smits, *Anal. Chem.*, 46 (1974) 283.
24 F. Erni and R. W. Frei, *J. Chromatogr.*, 149 (1978) 561.
25 J. L. Janicot, M. Caude and R. Rosset, *J. Chromatogr.*, 437 (1988) 351.
26 T. A. Beyer, J. F. Deye, M. A. Khorassani and L. T. Taylor, *J. Chromatogr. Sci.*, 27 (1989) 105.