# Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay

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## Abstract

Capillary zone electrophoresis (CZE) was used to study the migration behaviour of selected 1,4-benzodiazepines and metabolites over the pH range 2–12, exhibiting the ability to determine $pK_a$ values using this technique. The selectivity of capillary electrophoresis was then demonstrated for the separation of four benzodiazepines using capillary zone electrophoresis with 20 m$M$ citric acid + 15% methanol, and micellar electrokinetic capillary chromatography with 75 m$M$ sodium dodecyl sulphate in 6 m$M$ sodium tetraborate–12 m$M$ disodium hydrogen phosphate + 5% MeOH, and compared with other topical analytical techniques in terms of retention times, capacity factors and efficiencies. Capillary zone electrophoresis was also applied to the assay of a variety of pharmaceutical formulations which contain 1,4-benzodiazepines, omeprazole and metronidazole, and was compared with alternative analytical techniques such as reversed-phase high-performance liquid chromatography, capillary gas chromatography and automated differential pulse polarography. Limits of detection of CZE and the alternative techniques are also compared for these molecules.

*Keywords:* Capillary electrophoresis; Micellar electrokinetic chromatography; Electrophoretic mobility

## 1. Introduction

The quality of medicines is controlled by a variety of official compendia such as The British Pharmacopeia, and tests for pharmaceuticals must be suitable for detection and determination of the active drug molecule, as well as synthetic intermediates, impurities of manufacture, decomposition products and excipients. For these purposes, capillary zone electrophoresis (CZE) is now gaining increasing acceptance as a complementary analytical technique to other separation methods such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) [1–3]. In fact, being based on different physicochemical mechanisms, CZE can provide a new dimension in separation science, thus being an ideal complement to chromatography.

Chlordiazepoxide, diazepam, flurazepam and nitrazepam (Fig. 1) are members of the 1,4-benzodiazepine series of drugs (**I**) and have three main uses: as anxiolytics, anti-convulsants and muscle relaxants–all acting on specific receptors which enhance the effect of inhibitory neurotransmitters in the brain and spinal cord. Pharmacodynamically the benzodiazepines are
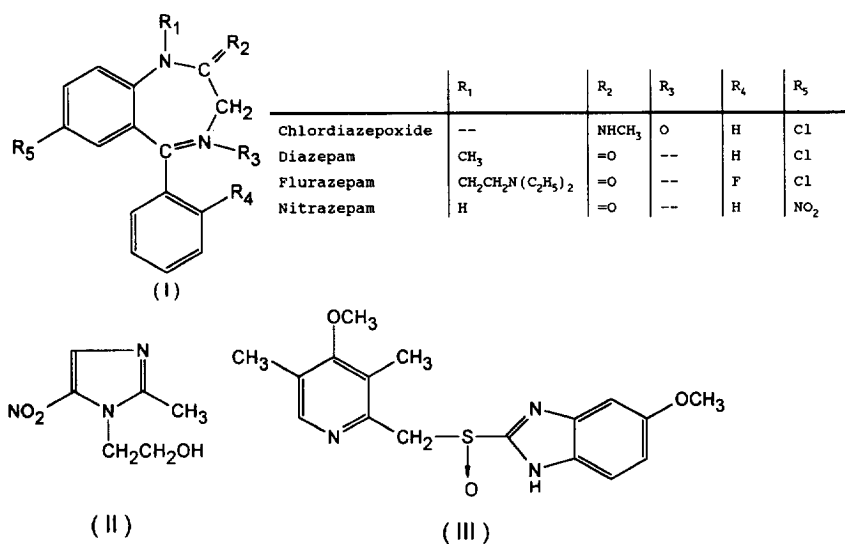
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| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| Chlordiazepoxide | -- | NHCH₃ | O | H | Cl |
| Diazepam | CH₃ | =O | -- | H | Cl |
| Flurazepam | CH₂CH₂N(C₂H₅)₂ | =O | -- | F | Cl |
| Nitrazepam | H | =O | -- | H | NO₂ |

Fig. 1. Structure of 1,4-benzodiazepines (**I**), metronidazole (**II**) and omeprazole (**III**).

virtually identical–the differences between them being due to their different pharmacokinetics.

Metronidazole (**II**) is active against bacteria and protozoa with primarily anaerobic metabolism. It enters the organism and is biochemically reduced to a derivative which binds to DNA and inhibits further nucleic acid synthesis. It is used in the treatment of genital infections, gingivitis and amoebic dysentery.

Omeprazole (**III**)–a benzimidazole sulphoxide–is an anti-ulcer agent.

This paper investigates the CZE behaviour of selected 1,4-benzodiazepines and their metabolites. The separation of four 1,4-benzodiazepines by CZE, micellar electrokinetic capillary chromatography (MECC) and other topical analytical techniques is then carried out. Finally, CZE formulation assay for these drugs, metronidazole and omeprazole is compared with those of automated differential pulse polarography (DPP), HPLC and capillary GC.

## 2. Experimental

### 2.1. Reagents and analytes

All solvents used were of HPLC grade or better. Citric acid, sodium hydroxide, potassium dihydrogen phosphate, sodium acetate and sodium dodecyl sulphate were purchased from Aldrich (Gillingham, Dorset, UK); sodium tetraborate, sodium acetate, acetic acid and disodium hydrogen phosphate were purchased from BDH. Benzodiazepine drug samples were obtained from Roche Products (Welwyn Garden City, Herts, UK), a pure sample of metronidazole was obtained from Sigma (Poole, Dorset, UK) and the formulation obtained from May and Baker (Dagenham, UK). A pure sample and the formulation of omeprazole was obtained from Astra Hassle AB (Molndal, Sweden).

### 2.2. Preparation of analytical solutions

(1) CZE: a $10^{-3}$ mol/l stock solution of each drug was prepared in methanol, and dilutions (up to $10^{-6}$ mol/l) obtained from this solution for limits of detection studies. For formulation assay, the formulation was dissolved in methanol, and the same mass of drug as was present in its formulation was dissolved in an equal volume of methanol. The same volume of internal standard (a different benzodiazepine) was added to both. All solutions were filtered and sonicated before use. Mesityl oxide was used as a neutral marker in the mobility studies. In all experiments, 0.3 ml of methanolic drug solution was added to 1 ml of

water before injection. (2) Automated DPP: a $10^{-3}$ mol/l stock solution of each drug was prepared by dissolving the appropriate mass of drug in ca. 5 ml of methanol and made up to 100 ml with the appropriate electrolyte. From this, $10^{-6}$ to $10^{-7}$ mol/l solutions were prepared. The formulation solution was prepared similarly. (3) HPLC: as for CZE, with the same mass of drug as was present in its formulation being dissolved in mobile phase (10 ml) and diluted by a factor of 10. The formulations were treated similarly. (4) Capillary GC: solutions prepared as for CZE with the methanolic drug solutions being injected onto the column.

## 2.3. Apparatus and procedures

### 2.3.1. Capillary electrophoresis

All experiments were performed on a SpectraPhoresis 1000 (ThermoSeparation Products, Stone, Staffordshire, UK) equipped with UV–Vis diode fast-scanning detector. All equipment control and data handling were performed using SpectraPhoresis software. Separations were carried out in a 70 cm × 50 $\mu$m I.D. uncoated fused-silica capillary (Composite MetalServices, Hallow, Worcs., UK), with an effective length ($l$) of 63 cm. Before CE separations, new capillary columns underwent a conditioning/equilibration cycle which consisted of three phases: (1) purging with 1 mol/l NaOH at 60°C for 5 min, with 0.1 mol/l NaOH at 60°C for 5 min (to deprotonate the silanol groups), and with water at 60°C for 5 min. (2) Equilibration with run buffer for 10 min. (3) A series of "no-injection" injections whereby a voltage was applied for 5 min. The capillary column was washed with buffer before each injection for 5 min.

Samples were injected using the hydrodynamic mode via a vacuum controlled system using a pressure difference of 10.34 kPa (1.5 p.s.i.), with an injection time of 7 s (42 nl) in the formulation assay study and 2.5 s (15 nl) in the other studies. To prevent capillary blockage, buffers and sample solutions were filtered using 13-mm diameter filter discs with a pore size of 0.45 $\mu$m (Gelman Sciences, Ann Arbor, MI, USA). Capillary electrophoresis was carried out at an applied voltage

of 20 kV (with a typical current being 10 $\mu$A), at a temperature of 30°C.

The buffer for the CZE studies was 20 m$M$ citric acid (pH 2.5) with 15% methanol. MECC separations were carried out with 75 m$M$ sodium dodecyl sulphate (SDS) in 6 m$M$ sodium tetraborate–12 m$M$ Na$_2$HPO$_4$ with 5% methanol. Scans between 200 and 300 nm were used to obtain spectra of the eluting compounds. Quantitative determinations were carried out with the internal standard method, using ultraviolet detection at 200 nm.

### 2.3.2. Automated differential pulse polarography (DPP)

Automated polarographic analyses were carried out on solutions of the pure drugs and the formulations using a Metrohm (Herisau, Switzerland) 646VA processor, 647 stand, 675VA sample changer and 665 Dosimat. The mercury multimode electrode with a mercury drop size of 0.40 mm$^2$ was used as the indicator electrode. The differential pulse polarographic mode was used with a sweep rate of 12 mV s$^{-1}$.

The formulations were assayed by the method of standard addition whereby 20 ml of the methanolic drug–buffer solution was degassed with oxygen-free nitrogen for 5 min. This was followed by DPP analysis and then two standard additions of a standard solution of the drug, thus enabling calculation of the concentration of the unknown. Analysis of each formulation was performed 10 times and the standard deviation and accuracy calculated.

For the analysis of the mixture of 1,4-benzodiazepines a pH 4.7 sodium acetate–acetic acid buffer was used.

### 2.3.3. High-performance liquid chromatography

HPLC, carried out under isocratic conditions, was performed on a system consisting of a Pye Unicam PU4010 pump (Cambridge, UK), a Rheodyne sample injector (20 $\mu$l) Model 7125 (Cotati, CA, USA), a Pye Unicam PU4020 variable-wavelength ultraviolet detector and a C$_{18}$ Novapack column (Waters Millipore, Milford, MA, USA). The mobile phase consisted of acetonitrile–water (50:50, v/v) for the determination of diazepam, chlordiazepoxide, nit-

razepam, omeprazole, and metronidazole, and a methanol–phosphate buffer pH 7.6 (85:15, v/v) for flurazepam, with the phosphate buffer consisting of a mixture of 90.7 ml 0.0667 $M$ disodium hydrogen phosphate dihydrate and 9.3 ml of 0.0667 $M$ potassium dihydrogen phosphate–diluted by a factor of 10. This mobile phase was also used for the separation of four benzodiazepines. The operating pressure was $1.034 \times 10^7$ Pa (1500 p.s.i.), and the flow-rate was 5 ml/min. Formulation assay was carried out using the calibration plot method of quantitation.

### 2.3.4. Capillary gas chromatography (GC)

The chromatographic analysis of the formulations and the mixture of four benzodiazepines was carried out on a Perkin-Elmer 8600 gas chromatograph (Beaconsfield, Buckinghamshire, UK), incorporating a flame ionisation detector, equipped with a 12-m BP1 column (0.32 mm I.D.) manufactured by Perkin-Elmer. The injection mode was splitless using a Perkin-Elmer splitless (2 mm I.D.) type sleeve. Nitrogen was used as the carrier gas, with a head pressure of $1.60 \times 10^5$ Pa (23.2 p.s.i.g) and a flow-rate of 4 ml/min. The instrument settings were as follows: injection port and detector 250 and 300°C, respectively, initial temperature 140°C for 3 min, increasing to 245°C at 20°C/min, remaining at this temperature for a further 5 min. As stated previously, the injection mode was splitless with the solvent venting off after 1 min (split flow-rate 12 ml/min and septum purge rate 3 ml/min). The column was injected with methanol between runs to avoid sample contamination. Samples of 1 $\mu$l of the methanolic drug solutions were injected using a 1-$\mu$l Hamilton syringe. As for CZE, quantitation was carried out using the internal standard method.

## 3. Results and discussion

### 3.1. CZE study of the migration behaviour of selected 1,4-benzodiazepines and metabolites

In capillary electrophoretic studies of ionisable compounds, pH plays a fundamental role as it determines the extent of ionisation of a solute,

and this can be used for the determination of $pK_a$ by measuring the ionic mobility of a solute as a function of pH. Conventional methods of $pK_a$ determination such as UV–Vis spectrophotometry have disadvantages in that the drugs often have low solubility in water, and more importantly, the differing ionic states of the solute may not exhibit different spectra.

In previous studies [4,5], other groups have demonstrated the ability to determine $pK_a$ values of compounds using CZE. However, the formulae which they often use contain parameters, such as ionic radii and activity coefficients, which are not readily available for the majority of compounds, making their application in these cases difficult or even impossible. Here, we have studied the migration behaviour of selected drugs and metabolites, using CZE, with reference to $pK_a$ determination, in simpler terms.

A generalized acid–base equilibrium involving a solute $BH^+$ and its conjugate base B is shown in Eq. 1:

$$BH^+ \rightleftharpoons B + H^+ \qquad (1)$$
$$c(1 - \alpha) \quad c\alpha \quad c\alpha$$

where $c$ is the concentration and $\alpha$ is the degree of dissociation. In a buffer solution, the relative amounts of B and $BH^+$ are given by the Henderson–Hasselbach equation, where $K_a$ is the acid dissociation constant of $BH^+$:

$$pH = pK_a + \log(\alpha/1 - \alpha) \qquad (2)$$

In the mixture of $BH^+$ and B, the former species only dictates the effective electrophoretic mobility $\mu_{ef}$, as B is uncharged:

$$\mu_{ef} = (1 - \alpha)\mu_{BH^-} \qquad (3)$$

Changes in $\mu_{ef}$, which are associated with a change in the state of ionisation of a solute, can be predicted using Eqs. 2 and 3, by plotting $(1 - \alpha)$ against pH for each of the drugs, as shown in Fig. 2, using known $pK_a$ data determined either using conventional UV–Vis spectrophotometry [6,7] or CZE (only for $pK_2$ of flurazepam). A CZE mobility study of the drugs over the pH range 2–11.5 gives similar type graphs (Fig. 3), in agreement with the predicted
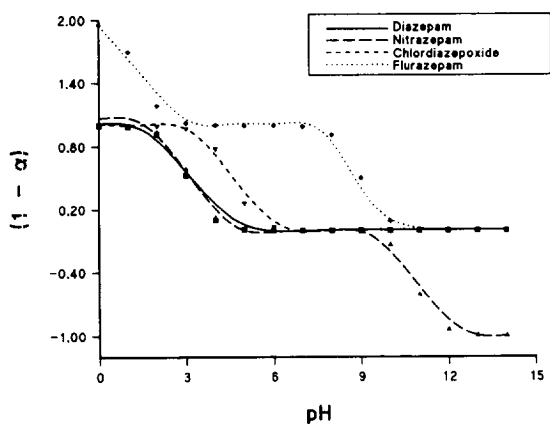
Fig. 2. Plot of $(1 - \alpha)$ against pH for four benzodiazepines.

changes in $\mu_{ef}$ shown in Fig. 2. Electrophoretic mobilities, $\mu_{ef}$, were calculated using:

$$\mu_{ef} = \mu_o - \mu_{eo} = Ll/V\{1/t_r - 1/t_{eo}\} \qquad (4)$$

where $\mu_o$ is the overall mobility, $\mu_{eo}$ the electro-osmotic mobility, $t_r$ the migration time of solute, $t_{eo}$ the migration time for an uncharged solute (neutral marker), $L$ the total length of the capillary, $l$ the capillary length between injection and detection, and $V$ the applied voltage.

Flurazepam (IV) has a known $pK_a$ of 1.42 [7], due to protonation of the azomethine group (Fig. 4). It was also claimed that a $pK_a$ of 8–9 was possible, but this was not observable spectrophotometrically since the aliphatic nitrogen was well removed from the UV absorbing part of the molecule. Fig. 5 shows $\mu_{ef}$ values for flurazepam
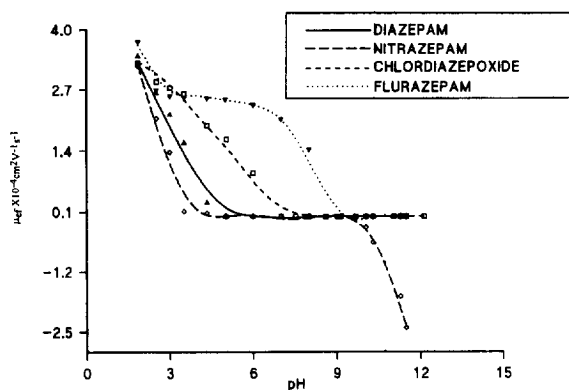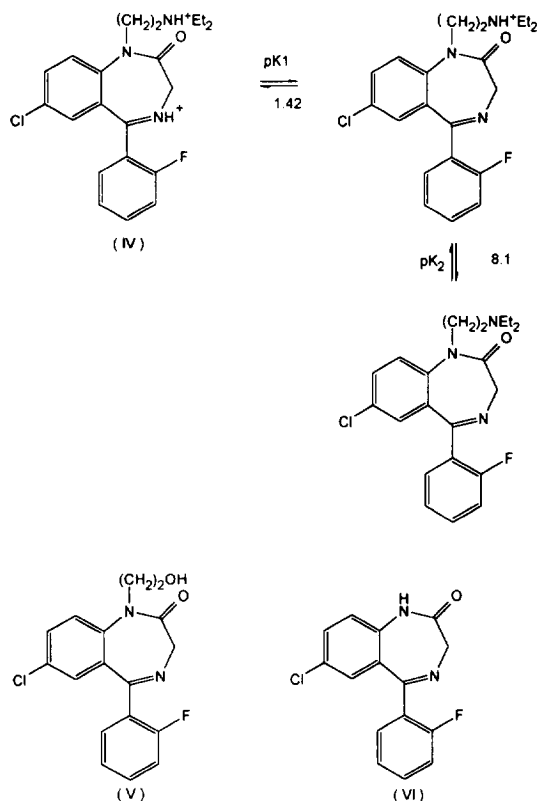


Fig. 4. Sites of protonation and deprotonation on flurazepam (IV), and structures of N-1-hydroxyethyl flurazepam (V) and N-1-desalkyl flurazepam (VI).



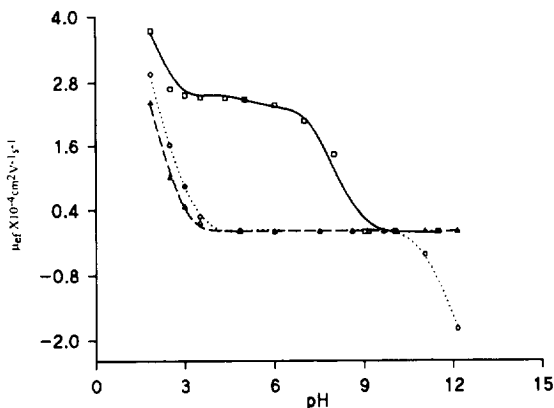Fig. 3. Plot of electrophoretic mobility $(\mu_{ef})$ against pH for four benzodiazepines.



Fig. 5. Plot of electrophoretic mobility $(\mu_{ef})$ against pH for flurazepam (IV) (full line) and its metabolites (V) (dashed line) and (VI) (dotted line).

over the pH range 2–12, and it can be seen that between pH 2–3, $\mu_{ef}$ decreases due to deprotonation of the azomethine group. However, it still has a positive $\mu_{ef}$ value due to the protonated nitrogen on the 1-position of the benzodiazepine ring. The $\mu_{ef}$ value begins to decrease again at pH ca. 7.0, decreasing to zero by pH 9, indicating the presence of a second $pK_a$ at 8.1. Fig. 5 also shows $\mu_{ef}$ values over the pH range 2–12 for the flurazepam metabolites N-1-hydroxyethyl flurazepam (**V**) and N-1-desalkyl flurazepam (**VI**). In the pH range 2–3, these molecules have smaller $\mu_{ef}$ values than (**IV**) since they lack the protonated aliphatic nitrogen. However, they also show a decrease in their $\mu_{ef}$ values as their azomethine groups are deprotonated (spectrophotometrically determined $pK_{a1}$ values of (**V**) and (**VI**) are 2.26 and 2.57, respectively [7]). Compound **VI** also shows formation of an anion with a $pK_a$ value of 11.76 [7]. The optimum pH for the separation of **IV**, **V** and **VI** was in the pH range 2–3.

### 3.2. A study of the separation of some 1,4-benzodiazepines.

Retention times, capacity factors and efficiencies for the chromatographic techniques are shown in Table 1.

In both capillary electrophoretic methods, the individual compounds could be identified by taking UV spectra at the time slice where the compound passes through the detector window, and comparing this spectrum with a standard library. Separation of the four benzodiazepines and the resultant UV spectra for CZE and MECC are shown in Figs. 6 and 7, respectively.

Separation under CZE conditions was carried out at pH 2.5 using 20 mM citric acid + 15% methanol, with chlordiazepoxide (a) eluting first, followed by flurazepam (b), diazepam (c) and nitrazepam (d). Methanol was used as an organic modifier, to increase the viscosity of the buffer, thus reducing electroosmotic flow, increasing separation, and also improving the solubility of the drugs. CZE had the shortest analysis time of 8 min with efficiencies in the 40 000–50 000 region. The capacity factors for CZE in this case

Table 1
Retention times ($t_R$), capacity factors ($k'$) and efficiencies ($N$) for CZE, MECC, HPLC and capillary GC separation of four benzodiazepines

| | $t_R$ | $k'$ [a] | $N$ [b] |
|---|---|---|---|
| **(a) Chlordiazepoxide** | | | |
| CZE | 5.57 | −0.62 | 39 762 |
| MECC | 18.02 | 2.35 | 274 038 |
| HPLC | 6.06 | 0.98 | 966 |
| CGC | 10.1 | 32.67 | 391 718 |
| **(b) Flurazepam** | | | |
| CZE | 5.73 | −0.61 | 38 595 |
| MECC | 20.12 | 2.75 | 252 436 |
| HPLC | 10.56 | 2.45 | 1 715 |
| CGC | 12.3 | 40 | 580 954 |
| **(c) Diazepam** | | | |
| CZE | 6.41 | −0.57 | 52 771 |
| MECC | 19.61 | 2.64 | 326 530 |
| HPLC | 6.78 | 1.22 | 1 081 |
| CGC | 9.6 | 31 | 353 894 |
| **(d) Nitrazepam** | | | |
| CZE | 7.75 | −0.48 | 46 752 |
| MECC | 17.21 | 2.20 | 296 579 |
| HPLC | 4.32 | 0.41 | 685 |
| CGC | 11.9 | 38.67 | 847 856 |

Experimental conditions for the chromatographic separations are given in the Experimental section.
[a] $k' = (t_R - t_o)/t_o$.
[b] $N = 16(t_R/w)^2$.

are negative, indicating that, as there is a positive charge on the benzodiazepines, they elute from the column before the neutral marker.

MECC separation used 75 mM sodium dodecyl sulphate, with nitrazepam (d) eluting first, followed by chlordiazepoxide (a), diazepam (c) and finally flurazepam (b). The addition of the micelles causes zone sharpening resulting in the great increase in efficiency as compared to CZE. However, the total analysis time of 21 min is almost three times that of CZE, and this is reflected in the relatively high capacity factors.

DPP analysis of a sample is determined by the ability of a functional group on that compound to be oxidized or reduced at a particular potential. In many cases, however, the same functional group is present on more than one analyte–causing problems with resolution. Such is the
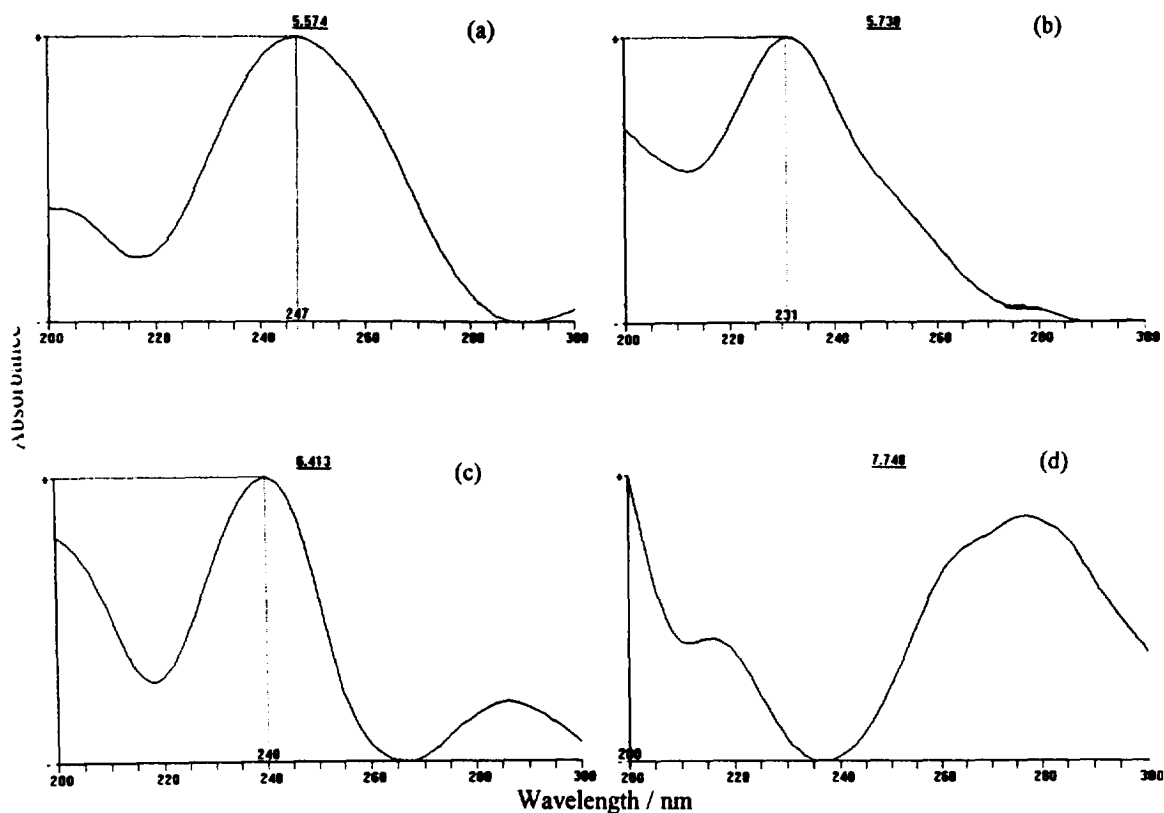
Fig. 6. CZE separation and resultant UV spectra of four benzodiazepines at pH 2.5 using 20 mM citric acid with 15% methanol as an organic modifier.

case for the analysis of benzodiazepines, shown in Fig. 8. From Fig. 1, it can be seen that all four contain the C = N moiety present in the diazepine ring, which reduces at ca. −860 mV, thus making their differentiation using this signal impossible. Chlordiazepoxide also contains NO and N = C groups in the diazepine ring, which are reduced at −525 and −1278 mV, respectively. The NO₂ group of nitrazepam becomes reduced at −285 mV. Polarography is not suitable for the application of chromatographic calculations and is thus not included in Table 1.
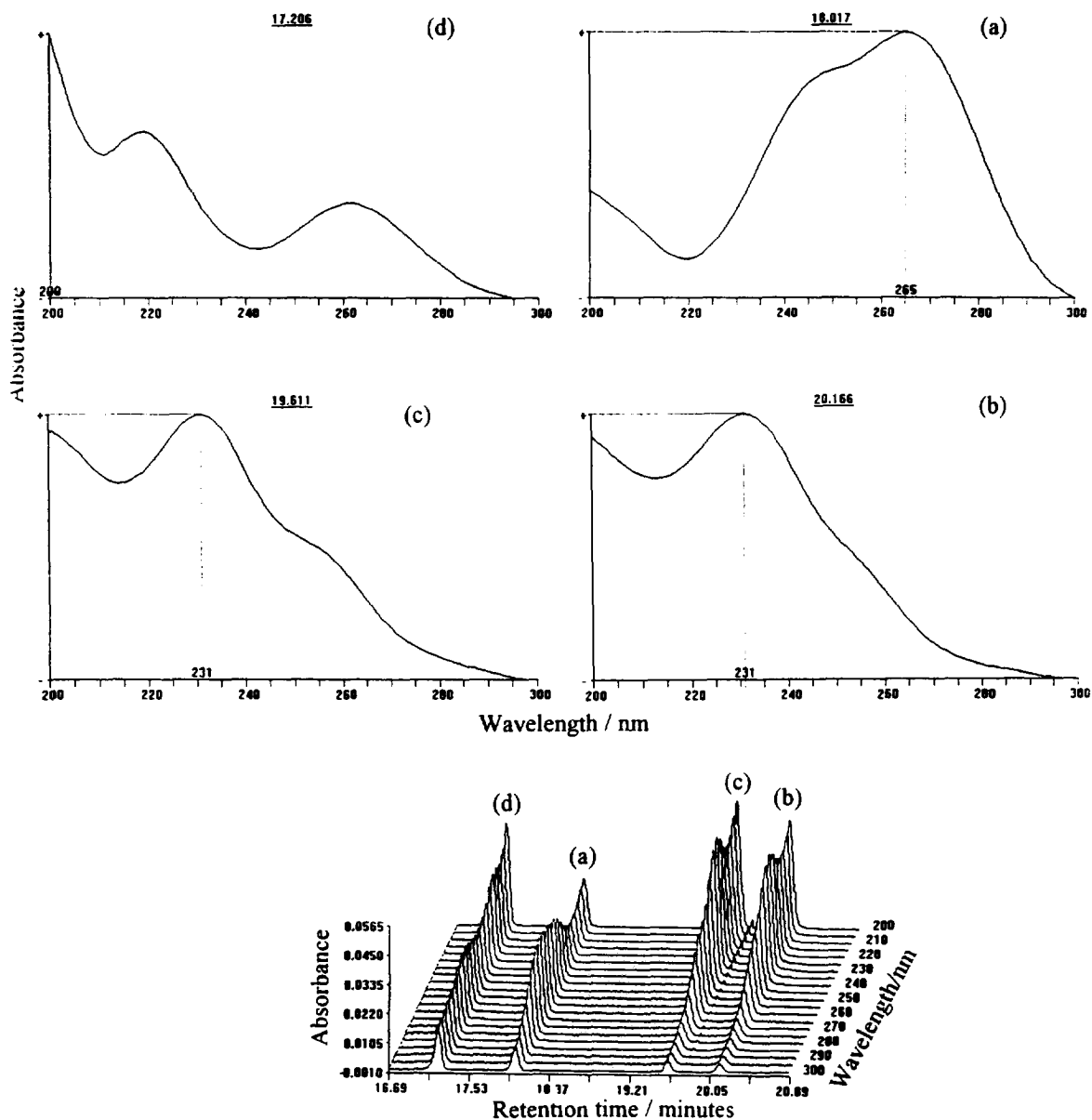
Fig. 7. MECC separation and resultant UV spectra of four benzodiazepines at pH 8 using 75 mM sodium dodecyl sulphate in 6 mM sodium tetraborate–12 mM disodium hydrogen phosphate with 5% methanol.

The order of elution using HPLC (Fig. 9) followed that obtained for MECC, where the micelles can be considered to be a pseudo-stationary phase. Chlordiazepoxide (a) and diazepam (c) were not baseline resolved, having an $R_s$ value of 0.92 compared to an $R_s$ value of 1.5 which indicates baseline resolution. The total

analysis time was relatively fast, at 12.5 min, with capacity factors ranging between 0.41 and 2.45. However, efficiencies for this technique were poor — at ca. 1000.

Analysis by capillary GC (Fig. 10) yielded the most efficient separation with plate numbers of up to 850 000 and very sharp peaks, with peak
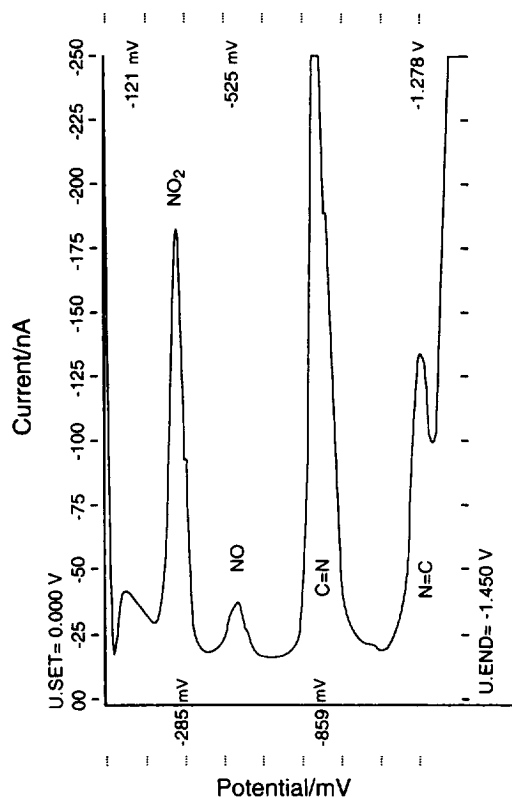
Fig. 8. Polarographic analysis of four benzodiazepines using pH 4.7 acetate buffer.



Fig. 9. HPLC separation of four benzodiazepines using the mobile phase used for the analysis of flurazepam (Apparatus and Procedures, Section 2.3).

width only ranging between 6 and 11 s, contributing to the high plate count. All peaks were completely resolved, with diazepam (c) emerging from the column first, followed by chlordiazepoxide (a), nitrazepam (d) and flurazepam (b).

## 3.3. Limits of detection

In general terms, the limit of detection of an analyte may be described as that concentration which gives an instrument signal ($y$) significantly different from the "blank" or "background signal" ($y_B$). A commonly used definition of this significant difference is 3 standard deviations of the blank:

$$y - y_B = 3S_B$$

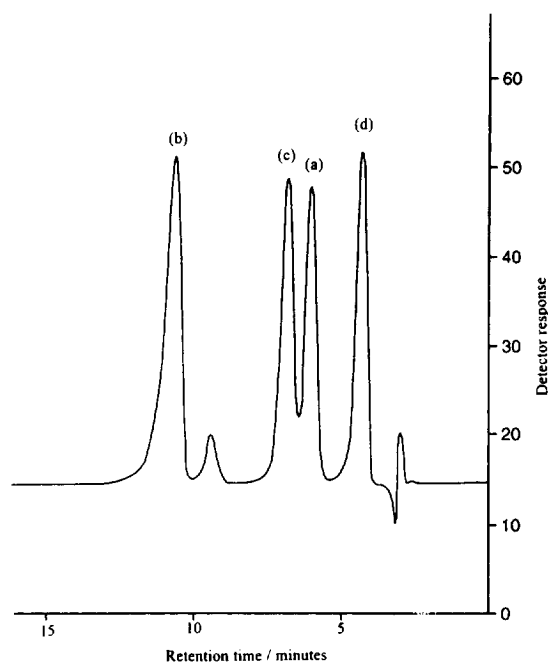Regression line equations were calculated for each of the drugs with each of the instruments,

using drug solutions of differing concentrations (including the "zero level"). Limits of detection (LODs) were then calculated by running the blank six times and obtaining the average signal and standard deviation ($S_B$) of the blank. The $S_B$ value of the blank was trebled, added to the average signal of the blank and then inserted as the $y$ value in the regression equation in order to obtain an $x$ concentration term for the LOD. LODs using HPLC were obtained using a separate visual protocol whereby the concentration of analyte which gives a signal equivalent to three times the peak-to-peak noise is the LOD. The results are shown in Table 2. CZE and capillary GC have moderate LODs of the order of $10^{-6}$ mol/l, with HPLC LODs being lower, at around $10^{-7}$ mol/l. LODs are the lowest of all the analytical techniques at $10^{-7}$ to $10^{-8}$ mol/l for automated DPP. CZE LODs can be improved by either changing the path length using a Z-shaped capillary flow cell, a rectangular capillary or a bubble cell. LODs can also be improved using electrokinetic injection or field amplified tech-
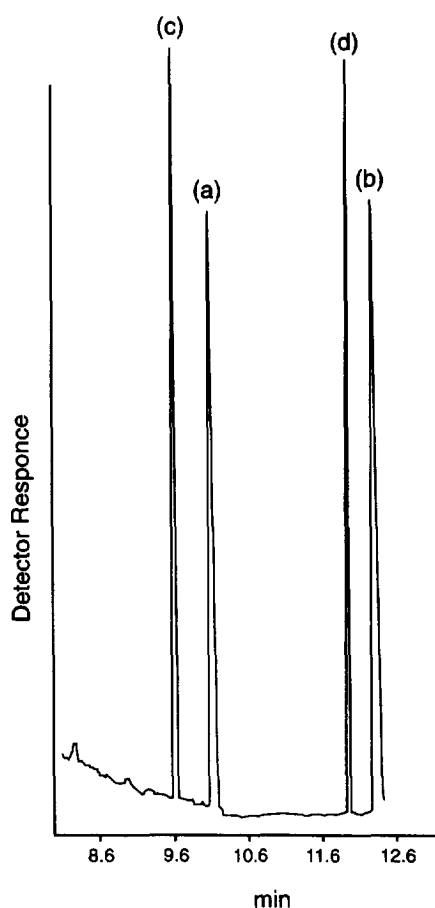
Fig. 10. Capillary GC separation of four benzodiazepines.

niques to increase the amount of sample loaded into the capillary without spoiling the efficiency [8], and recent advances include large-volume sample stacking for positive species, which actually reverses the electroosmotic flow and pumps water out of the system, before applying a reversed polarity voltage to the system [9].

### 3.4. Drug formulation assay

In this paper, four analytical techniques have been compared for their drug formulation results, CZE, automated DPP, HPLC and capillary GC. Table 3 shows precisions and accuracies for these assays. Linearity was achieved in the range $10^{-3}$ to $10^{-6}$ $M$ for each of the techniques, and precisions and accuracies were calculated at about the mid range of the calibration plot for each formulation.

The 1,4-benzodiazepine formulations were assayed by the four techniques with comparable percent accuracies and precisions, generally of the order of a few percent. A notable exception was the automated DPP analysis of the diazepam formulation (valium), which underestimated drug formulation content by 17%. This result is difficult to explain, considering the percent accuracies of CZE, HPLC and capillary GC of 2.92, 2.34 and 2.04, respectively, for this formulation. Flagyl (metronidazole) also gave poor assay results by automated DPP (percent accuracy 6.07, percent precision 14.84) and also by capillary GC (percent accuracy 12.81, percent precision 5.74), but percent accuracies and percent precisions of the order of a few percent when assayed by CZE and HPLC. The omeprazole formulation gives poor percent accuracy results when assayed by automated DPP and HPLC, and CZE was found to be inappropriate for omeprazole assay since it undergoes on-capillary acid catalysed degradation [10], and capillary GC

Table 2
LODs for drugs using CZE, automated DPP, HPLC and capillary GC

| Compound | CZE | Polarography | HPLC | CGC |
|---|---|---|---|---|
| Chlordiazepoxide (Librium) | $3.42 \cdot 10^{-6}$ | $1.06 \cdot 10^{-8}$ | $1 \cdot 10^{-7}$ | $1.05 \cdot 10^{-6}$ |
| Diazepam (Valium) | $1.03 \cdot 10^{-6}$ | $3.52 \cdot 10^{-8}$ | $5 \cdot 10^{-8}$ | $8.2 \ \cdot 10^{-6}$ |
| Flurazepam (Dalmane) | $4.78 \cdot 10^{-6}$ | $5.39 \cdot 10^{-8}$ | $1 \cdot 10^{-7}$ | $2.9 \ \cdot 10^{-6}$ |
| Nitazepam (Mogadon) | $1.28 \cdot 10^{-6}$ | $8.40 \cdot 10^{-9}$ | $1 \cdot 10^{-7}$ | $1.9 \ \cdot 10^{-6}$ |
| Metronidazole (Flagyl) | $4.66 \cdot 10^{-6}$ | $1 \cdot 10^{-7}$ | $1 \cdot 10^{-7}$ | $4.7 \ \cdot 10^{-6}$ |
| Omeprazole (Losec) | – | $7.99 \cdot 10^{-8}$ | $1 \cdot 10^{-7}$ | – |

Table 3
Precisions[a] and accuracies[b] for the formulation assays using the four techniques

| Formulation | CZE | | DPP | | HPLC | | CGC | |
|---|---|---|---|---|---|---|---|---|
| | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) | Accuracy (%) | Precision (%) |
| Librium 5 mg (Chlordiazepoxide) | −3.22 | 3.59 | +4.38 | 5.47 | −0.44 | 1.27 | −1.14 | 3.30 |
| Valium 2 mg (Diazepam) | −2.92 | 2.60 | −17.00 | 0.54 | +2.34 | 0.73 | −2.04 | 3.35 |
| Dalmane 20 mg (Flurazepam) | +0.87 | 3.40 | +0.73 | 1.27 | +0.07 | 0.40 | +1.04 | 2.60 |
| Mogadon 5 mg (Nitrazepam) | +1.60 | 2.52 | +2.63 | 3.41 | +1.61 | 0.35 | +1.66 | 3.90 |
| Flagyl 400 mg (Metronidazole) | −2.09 | 2.52 | +6.07 | 14.84 | +0.10 | 1.69 | +12.81 | 5.74 |
| Losec 20 mg (Omeprazole) | – | – | −15.00 | 2.38 | +9.73 | 1.11 | – | – |

[a] Percent precision = (standard deviation/mean value) · 100.

[b] Percent accuracy = ((calculated value − actual value)/calculated value) · 100.

gave a selection of peaks, indicating thermal on-column degradation at the elevated gas chromatographic temperatures.

Overall, CZE and HPLC would appear to be suitable analytical techniques for the assay of a range of formulations in terms of both percent accuracy and percent precision. They can therefore be used with confidence as complementary analytical techniques in drug formulation assay.

## 4. Conclusions

The conclusions which can be drawn from this paper are that CZE is a new and versatile technique with many applications. CZE can be used to determine physicochemical constants such as $pK_a$ values of selected 1,4-benzodiazepines, and with its excellent selectivity, can be used to separate mixtures of 1,4-benzodiazepines and their metabolites. LODs are still inferior to those obtained by HPLC and automated DPP. CZE, with its fast analysis time and superior efficiency, can be used as a complimentary tech-

nique to HPLC for drug formulation assay with percent accuracies and percent precisions of a few percent.

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