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Quantitative analysis of fosinopril sodium by capillary zone electrophoresis and liquid chromatography

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Abstract: A capillary zone electrophoresis (CZE) method was developed for the quantitative analysis of fosinopril sodium. Validation parameters of the CZE method were evaluated and compared to an existing LC method. In terms of precision and sensitivity, LC performance was superior to that of the CZE method for this application. The CZE method achieved better selectivity for several degradants of interest within a much shorter analysis time than did the LC method. Effects of detection wavelength, applied voltage and buffer concentration on optimization of the CZE method are presented. Effects of diluent composition on capillary loading and peak behaviour are also discussed.

Keywords: *Fosinopril sodium; capillary zone electrophoresis; reversed-phase LC.*

Introduction

Fosinopril sodium (Monopril™) is an antihypertensive agent belonging to the class of angiotensin-converting-enzyme inhibitors [1]. It is a phosphinic acid ester prodrug which undergoes *in vivo* hydrolysis to the active diacid moiety, fosinoprilat (Fig. 1). In fosinopril sodium tablet formulations containing magnesium stearate as a lubricant, two magnesium-catalysed degradants, SQ-27451 and SQ-33232, have been identified (Fig. 1). The existing method for potency and degradant analysis of fosinopril sodium tablets is an isocratic LC separation.

Capillary electrophoresis (CE) has the potential to provide new selectivities which may extend or complement the capabilities of liquid chromatography for pharmaceutical analysis [2]. While many literature reports have discussed the useful separations which can be achieved [3–6], relatively little has been written about the ability of CE methods to perform routine quantitative work [7–11].

To explore this capability, a free solution CE, or capillary zone electrophoresis (CZE) method was developed for the quantitative analysis of fosinopril sodium and its related compounds. Diluent composition and the optimization of detection wavelength, applied voltage and run buffer concentration were the main focus of method development. Key validation parameters including linearity, precision and sensitivity of the CZE method were evaluated and compared to the existing LC method.

Experimental

Chemicals

Fosinopril sodium, fosinoprilat, SQ-27451 and SQ-33232 were produced by Bristol–Myers Squibb Co. (New Brunswick, NJ, USA). LC-grade acetonitrile and methanol were obtained from Burdick & Jackson (Baxter Healthcare, Muskegon, MI, USA). Analytical-grade sodium tetraborate and phosphoric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water was further purified through a Milli-Q System (Millipore, Bedford, MA, USA). Buffer solutions were filtered through a 0.2-μm membrane filter and were degassed.

Capillary electrophoresis system

CZE was performed in a 57 cm × 75 μm i.d. fused-silica capillary (50 cm to the detector), using a P/ACE 2050 CE instrument (Beckman Instruments, Fullerton, CA, USA). Analyses were run with 50 mM sodium tetraborate

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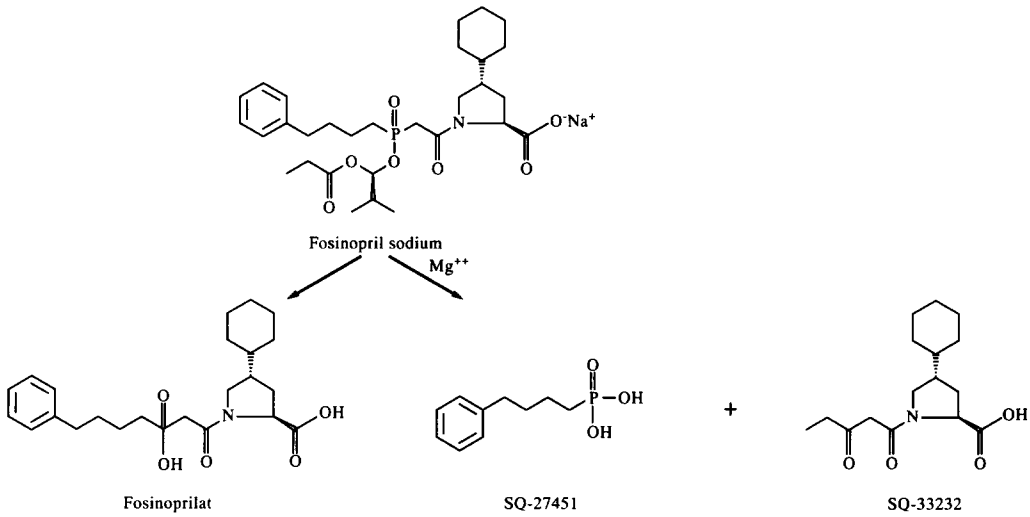

Figure 1
Chemical structures and degradation scheme for fosinopril sodium and related compounds.

buffer (pH 8.3). Samples were injected hydrodynamically using 10-s pressure injections, followed by a 1 s injection of buffer prior to voltage application. The optimum running voltage was 30 kV. Capillary temperature was maintained at 25°C, unless otherwise noted. Detection was by UV absorption at 200 nm, with a range of 0.05 AUFS. Additional studies were also performed at 214 nm. The optimum sample diluent was water–acetonitrile (80:20, v/v). Migration times and peak areas were measured using a VG Multichrom data acquisition system.

LC system

Reversed-phase LC was performed using a Spectra-Physics 8810 pump (Thermo Separation Products, Fremont, CA, USA), an ISS-100 autosampler (Perkin–Elmer, Norwalk, CT, USA), an Applied Biosystems Kratos 783 programmable absorbance detector (Perkin–Elmer, Norwalk, CT, USA), and a 30 cm × 4.0 mm i.d. alkylphenyl 10-μm column (Column Resolution Inc., San Jose, CA, USA). The mobile phase was methanol–0.2% phosphoric acid (72:28, v/v) at a flow rate of 2.0 ml min^{-1}. The nominal injection volume was 50 μl. Detection was by UV absorption at 215 nm. Data were collected on a VG Multichrom system (VG Instruments, Danvers, MA, USA).

Results and Discussion

LC analysis

Figure 2 is a typical chromatogram of a standard mixture of fosinopril sodium (*ca* 100 μg ml^{-1}), fosinoprilat, and the two magnesium-catalysed degradants, SQ-27451 and SQ-33232. Adequate baseline resolution of the three degradants was achieved, but the long retention of fosinopril required a 13-min run time. Quantitation limits were estimated using the following calculations: (a) the standard deviation of peak area responses was determined from replicate injections of a standard solution, at a concentration near the suspected quantitation limit; (b) the slope of a calibration line for peak area response vs concentration (50–150 μg ml^{-1}) was determined; (c) the quantitation limit is then estimated to be equal to 10 times the standard deviation divided by the slope. For fosinoprilat, the estimated quantitation limit was 0.09 μg ml^{-1}, or 0.09% (w/w) of the fosinopril sodium working concentration (100 μg ml^{-1}). The degradants SQ-27451 and SQ-33232 were not examined in detail here. Linearity of the peak area response for fosinopril sodium was demonstrated over a range between 50 and 150% of the working concentration, i.e. 50–150 μg ml^{-1}. Precision of the peak area response from replicate injections of fosinopril sodium was 0.4% RSD. Precision of the peak retention time for replicate injections of fosinopril sodium was 0.4% RSD.

CZE separation

Figure 3 is an electropherogram showing the separation of the mixture of fosinopril sodium (*ca* 25 μg ml^{-1}) and the three related degradants by CZE. As compared to the LC method,

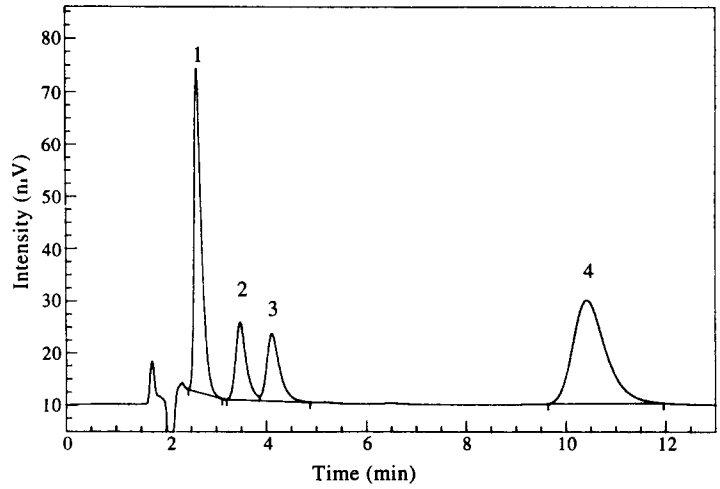

Figure 2
LC chromatogram of a spiked mixture of fosinopril sodium and related degradants. (1) SQ-27451, (2) SQ-33232, (3) fosinoprilat, (4) fosinopril sodium. Conditions are described in Experimental.

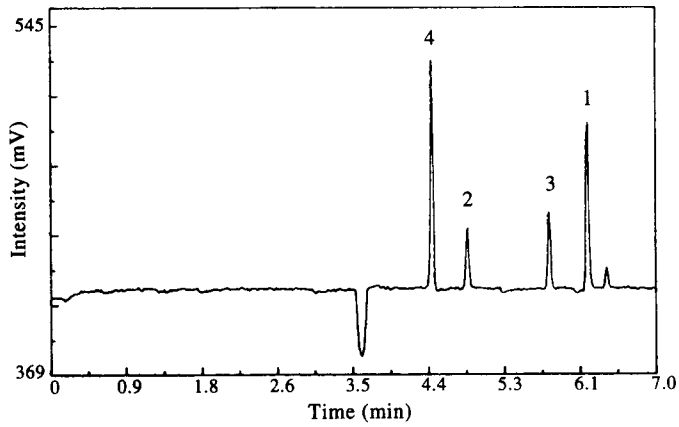

Figure 3
CZE electropherogram of a spiked mixture of fosinopril sodium and related degradants. (1) SQ-27451, (2) SQ-33232, (3) fosinoprilat, (4) fosinopril sodium. Electrolyte, 50 mM borate buffer (pH 8.3); temperature, 22°C; applied voltage, 20 kV; 4-s injection; UV detection at 214 nm.

the CZE method resulted in improved baseline resolution of the three degradants within a shorter run time of 7 min.

Effect of detection wavelength

Detection by CZE of a mixture of fosinopril sodium (0.4 mg ml^{-1}) and fosinoprilat (1.0 μg ml^{-1}) was compared at 200 vs 214 nm (Fig. 4). Detection at 200 nm was more favorable for quantitative analysis, as it produced increased peak heights, particularly a two-fold increase in fosinoprilat peak height, with no appreciable increase in baseline noise.

Effect of applied voltage

Electropherograms of the fosinopril sodium–fosinoprilat mixture were compared using 20 vs 30 kV applied voltage (Fig. 5). The higher voltage shortened migration times by about 40% and sharpened both peaks. In general, when applied voltage is increased, column efficiency and selectivity will reach a maximum and then decrease as the voltage is further increased, due to inefficient heat dissipation within the capillary and shorter migration times of the solutes [12]. In this application, resolution between the fosinopril sodium and fosinoprilat peaks was satisfactory at either voltage.

Effect of run buffer concentration

Electropherograms of the fosinopril

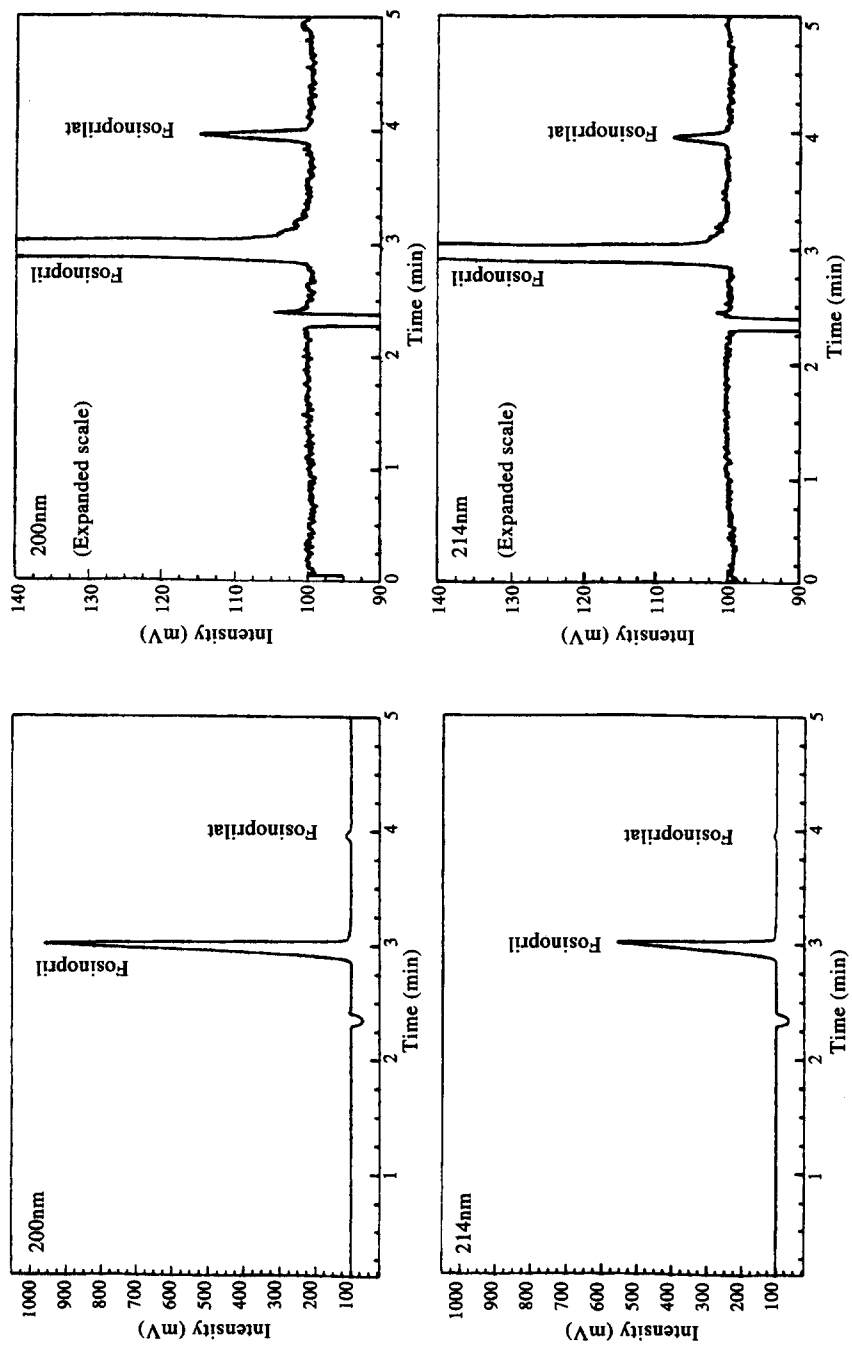

Figure 4
Electropherograms of spiked mixture of fosinopril sodium and fosinoprilat comparing detection wavelengths (200 vs 214 nm). Electrolyte, 50 mM borate buffer (pH 8.3); temperature, 25°C; applied voltage, 30 kV; 10-s injection.

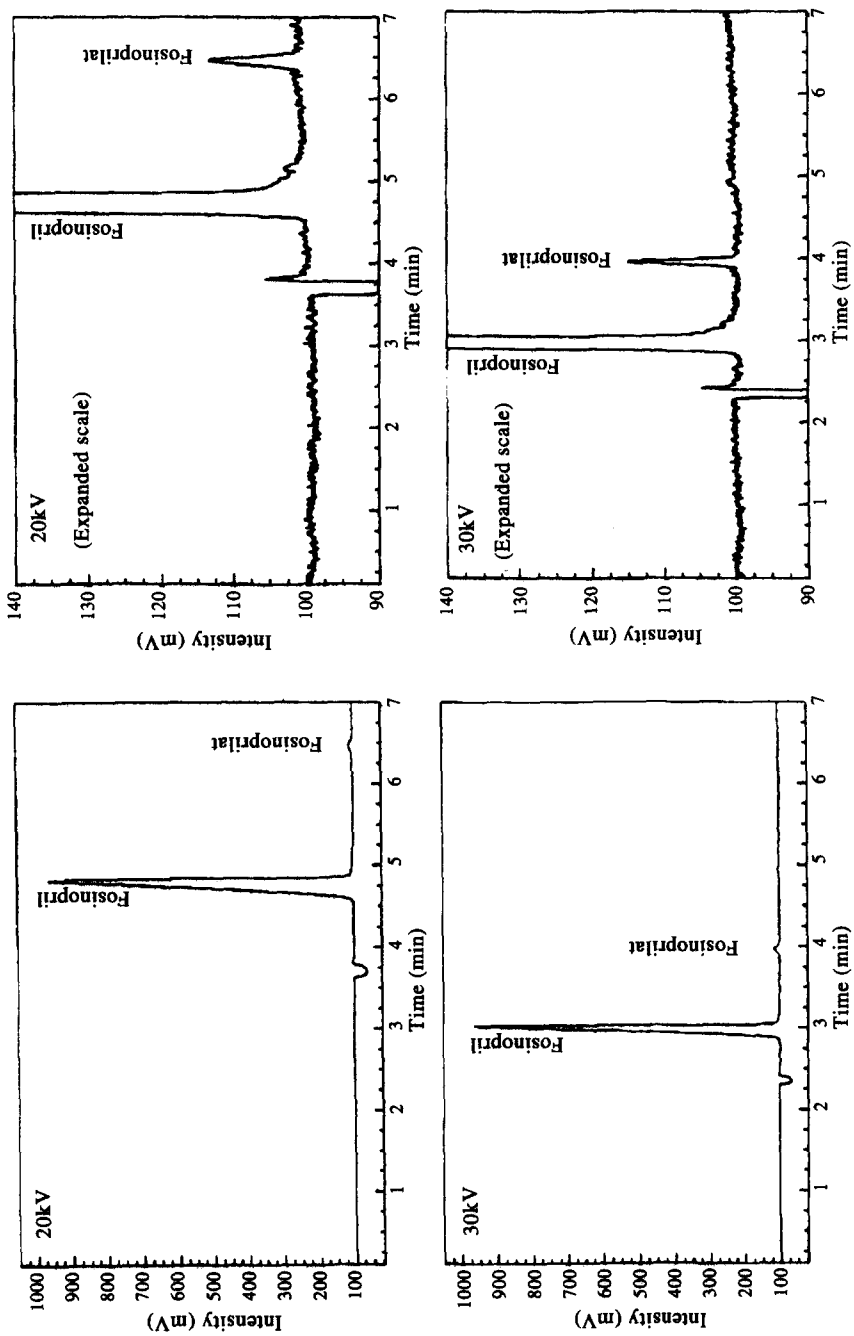

Figure 5
Electropherograms of spiked mixture of fosinopril sodium and fosinoprilat comparing applied voltages (20 vs 30 kV). Electrolyte: 50 mM borate buffer (pH 8.3); temperature: 25°C; 10-s injection; UV detection at 200 nm.

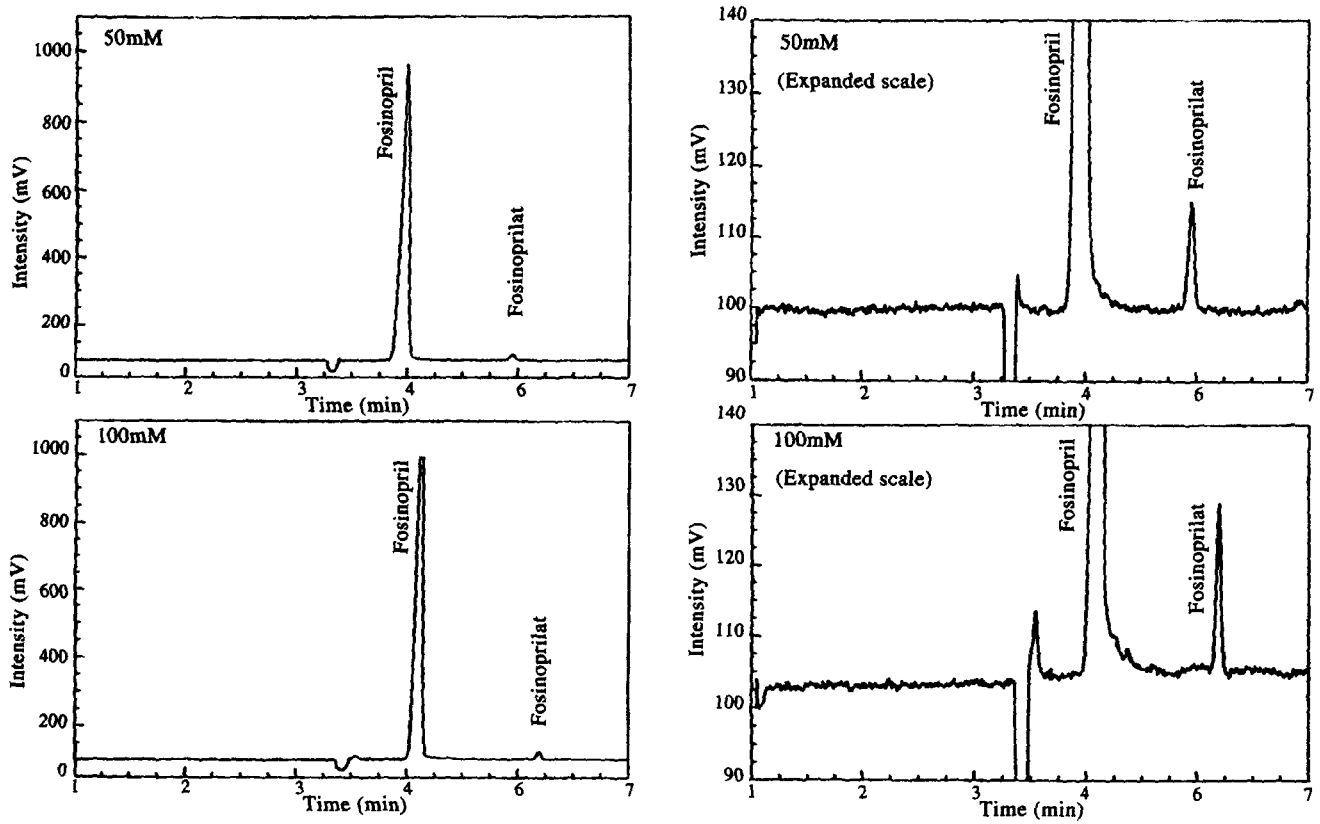

Figure 6
Electropherograms of spiked mixture of fosinopril sodium and fosinoprilat comparing concentrations (50 vs 100 mM) of borate buffer (pH 8.3) as electrolyte. Temperature, 25°C; applied voltage, 30 kV; 10-s injection; UV detection at 200 *nm*.

sodium–fosinoprilat mixture were run using 50 vs 100 mM borate buffer, pH 8.3 (Fig. 6). Although the higher buffer concentration slightly increased migration times and provided a 20% increase in the fosinoprilat peak height, it also caused the fosinopril peak to saturate the detector limit, giving a flat-top peak. The effect of run buffer concentration on linearity of the fosinopril peak response is discussed below. Resolution between the two peaks remained satisfactory at either buffer concentration.

Effect of diluent composition

The inclusion of 20% acetonitrile in the sample diluent was required to maintain the solubility of fosinoprilat. The effect of sample diluent (water–acetonitrile vs borate buffer–acetonitrile) on CZE analysis was investigated. Sample loading studies of fosinopril sodium run with 50 mM borate buffer were conducted. The injection time for sample loading was set at either 2, 10 or 20 s. When water–acetonitrile (80:20) was used as the sample diluent, the precision of peak response improved and

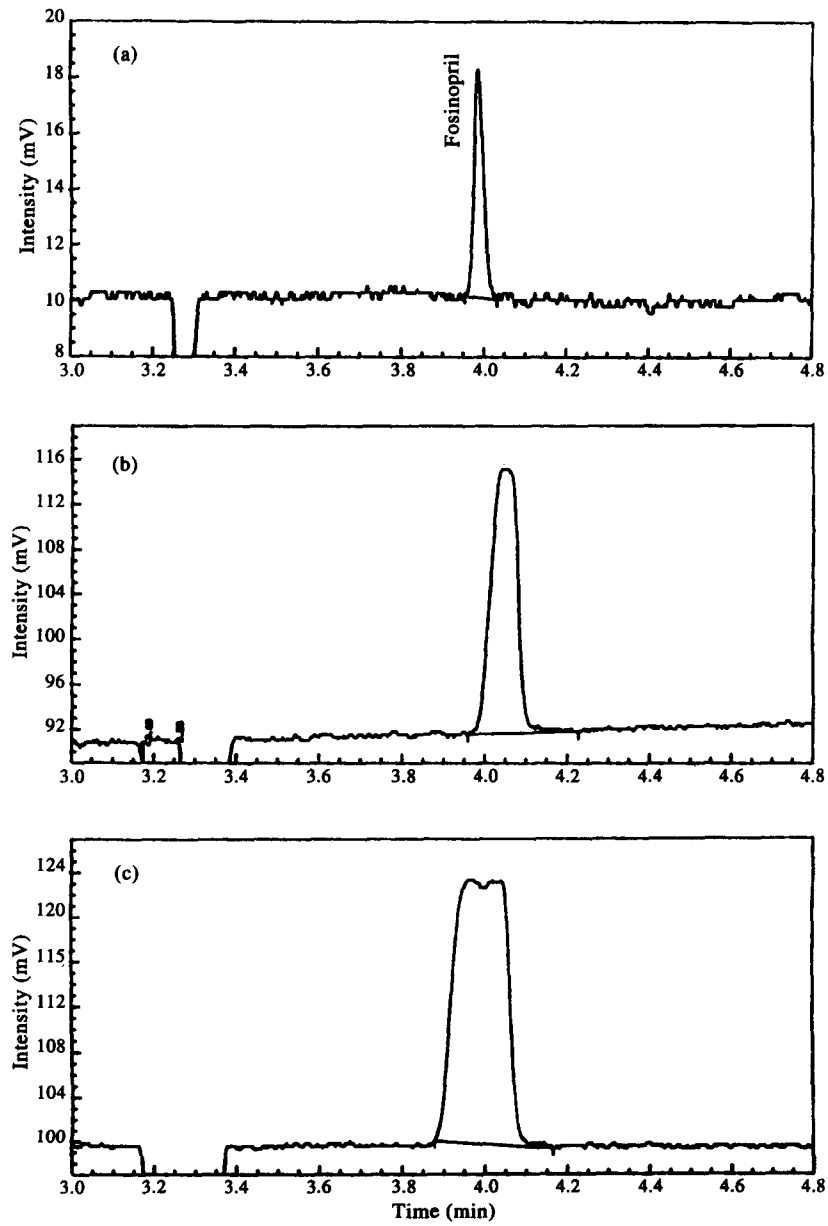

Figure 7
Electropherograms of fosinopril sodium dissolved in 50 mM borate buffer–acetonitrile (80:20), comparing sample injection times (a) 2 s; (b) 10s; (c) 20 s. Electrolyte, 50 mM borate buffer (pH 8.3); temperature, 25°C, applied voltage, 20 kV; UV detection at 214 nm.

migration time decreased slightly as the sample load increased. Fluctuation and disturbance of the instrument current were minimal during the 2-s injection run, but increased as the sample load time increased to 20 s. For analysis, a 10-s load time appeared to be a satisfactory compromise between current disturbance and precision of peak response. In contrast, electropherograms obtained with 50 mM borate buffer–acetonitrile (80:20) as the sample diluent are shown in Fig. 7. As the sample load increased from 2 s (Fig. 7a) to 10 s (Fig. 7b) to 20 s (Fig. 7c), the fosinopril peak became distorted. There was little effect of sample load on either migration time or instrument current. The buffer–acetonitrile diluent was, therefore, unacceptable, due to loss of the stacking effect necessary for peak sharpening.

To determine how the composition of the buffer–acetonitrile diluent affected the fosinopril peak shape, further studies were conducted in which either the percentage of acetonitrile or the concentration of borate buffer was varied in the diluent. When the percentage of acetonitrile in the diluent (50 mM borate buffer–acetonitrile) was set to 5, 10 or 20%, the fosinopril peak response

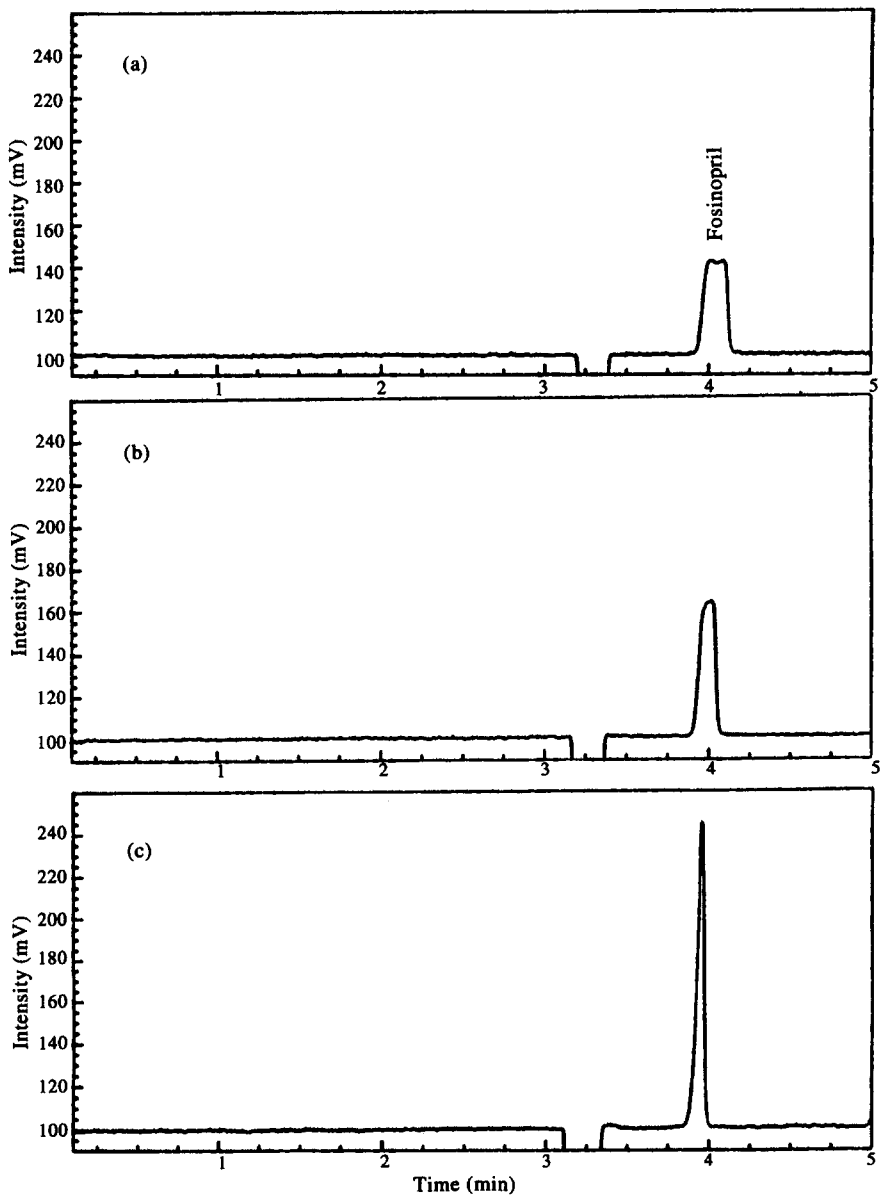

Figure 8
Electropherograms of fosinopril sodium dissolved in 50 mM borate buffer–acetonitrile (80:20), comparing buffer concentrations in the diluent. (a) 50 mM; (b) 37.5 mM; (c) 12.5 mM. Conditions as in Fig. 7.

increased as the acetonitrile percentage increased, but the peak shape remained distorted (as it was in Fig. 7c). However, when the concentration of borate buffer in the diluent (buffer–acetonitrile [80:20]) was set to 50 mM (Fig. 8a), 37.5 mM (Fig. 8b) or 12.5 mM (Fig. 8c), the peak became sharper as the buffer concentration decreased. Therefore, buffer concentration, and not the presence of acetonitrile in the sample diluent, controlled peak sharpness. To benefit from the stacking effect, the diluent ionic strength should be less than that of the run buffer.

Linearity

Under the optimized conditions (temperature, 25°C; applied voltage, 30 kV; 10-s injection; UV detection at 200 nm; sample diluent, water–acetonitrile [80:20]), and using 50 mM borate as the run buffer, acceptable linearity was obtained over a concentration range of 1–400 μg ml^{-1}. However, when 100 mM borate buffer was used, the response became non-linear above 200 μg ml^{-1}.

Precision

Precision of the peak migration time for replicate injections of fosinopril sodium was typically about 0.4% RSD. Reproducibility of peak area responses for fosinopril sodium at concentrations between 5 and 400 μg ml^{-1} was typically 1–5% RSD.

Sensitivity

The quantitation limit for the CZE analysis was calculated as described above for the LC analysis. Under optimized conditions, the CZE quantitation limit for fosinoprilat was estimated to be 0.26 μg ml^{-1}. The quantitation limit can also be expressed as a percentage (w/w) relative to the amount of primary analyte (fosinopril sodium) in the sample. Therefore, the quantitation limit can be controlled by adjusting the sample concentration. If the sample were diluted to give the LC working concentration of 100 μg ml^{-1} fosinopril sodium, the CZE quantitation limit for fosinoprilat would be *ca* 0.3% (w/w). If the sample were made less dilute to give a fosinopril sodium concentration of 0.26 mg ml^{-1}, then the quantitation limit for fosinoprilat would be *ca* 0.1% (w/w). Thus, with a modified sample preparation (i.e. a more concentrated sample), the CZE method was capable of attaining a quantitation limit similar to that of the LC method.

The quantitation limit can be affected by the concentration of the run buffer. As discussed above, use of the 100 mM borate buffer (as compared to 50 mM) would favour quantitation of fosinoprilat by enhancing sensitivity (due to greater peak height, as shown in Fig. 5). However, the 100 mM buffer might also be detrimental to quantitation of the main analyte, fosinopril sodium, due to its limitations on linearity of peak response.

Conclusions

The inherent sensitivity and reproducibility of current capillary electrophoresis systems generally does not match what LC instruments normally attain. The tradeoffs between sample loading, linearity, efficiency and reproducibility are quite different for the two techniques. For the quantitative analysis of fosinopril sodium by the methods described here, the CZE method had no significant advantages over the LC method. The CZE method did achieve superior analyte selectivity within a much shorter run time. However, the LC method gave superior performance in terms of sensitivity and precision of peak area responses. Factors such as sample diluent composition and sample loading are critical to electrophoretic as well as chromatographic methods.

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