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Short Communication

Reductions in sample pretreatment requirements by using high-performance capillary electrokinetic separation methods*

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Introduction

Pharmaceutical compounds must be analysed to prove stability and purity prior to their approval by the regulatory authorities. Often samples of formulated drugs require extensive sample pretreatment prior to analysis. This sample preparation is both time and material consuming. Consequently, new analytical techniques and automated methods are increasing in importance. We have investigated the use of the high-performance capillary electrokinetic (HPCE) techniques of capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) for such purposes. Both techniques exploit an electroendosmotic (EEO) flow of solution, which, in effect, acts as a pump sweeping solutes towards the detector.

Following the development of CZE and MECC in the early 1980s by Jorgenson and Lukacs [1] and Terabe *et al.* [2], respectively, both are becoming viable analytical techniques. Equipment is now commercially available from a number of suppliers, the majority of which incorporate sensitive on-column detection, temperature control, multiple injection modes and autosamplers.

To date, there have been a limited number of reports on the use of HPCE methods for pharmaceutical analysis, but this number is expected to increase rapidly as more pharmaceutical companies investigate this area. Researchers in this group have found both MECC and CZE to be suitable for the determination of related impurity levels in drug substance [3] and in the resolution of closely related impurities which were difficult to achieve using HPLC [4].

Experimental

Apparatus

In this work both an ABI270A and a Dionex CES1 instruments were used. Fused silica capillaries of dimensions either 75 cm × 75 μm i.d. or 70 cm × 50 μ i.d. were supplied by Polymicro Technology. Sample introduction was achieved by electrokinetic, vacuum or gravimetric means and on-line UV detection was employed throughout.

Chemicals and reagents

Sodium dodecyl sulphate, di-sodium tetraborate and sodium dihydrogen orthophosphate were purchased from BDH. Sodium citrate (pH 2.5) buffer was supplied by ABI. Ranitidine syrup, GR43175 and cefuroxime axetil were obtained internally within Glaxo Group Research.

Results and Discussion

Pharmaceutical formulations

The wide range of pharmaceutical formu-

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lations developed give rise to a diversity of matrices from which the drug must be extracted prior to analysis. If no sample pretreatment is carried out, the component of interest is often masked by peaks attributable to excipients. In addition, excipient and contaminant adsorption onto the chromatographic column would lead to a decreased efficiency and hence reduced column lifespan. However, the open tube nature of HPCE coupled with repeated caustic washing procedures, greatly reduces this adsorption, thus allowing complex matrices to be investigated.

An ester of cefuroxime, cefuroxime axetil (cefax), is an established antibiotic currently sold in a tablet form (Zinnat). A paediatric suspension dosage form is currently under development. HPLC is used as a stability indicating assay method for the suspension but extensive sample pretreatment is required because of the complex nature of the product. However, when analysing samples using a MECC method the sample is simply dissolved in methanol prior to analysis. Figures 1 and 2 show the separation of the two cefax diastereoisomers where the cefax B isomer elutes before

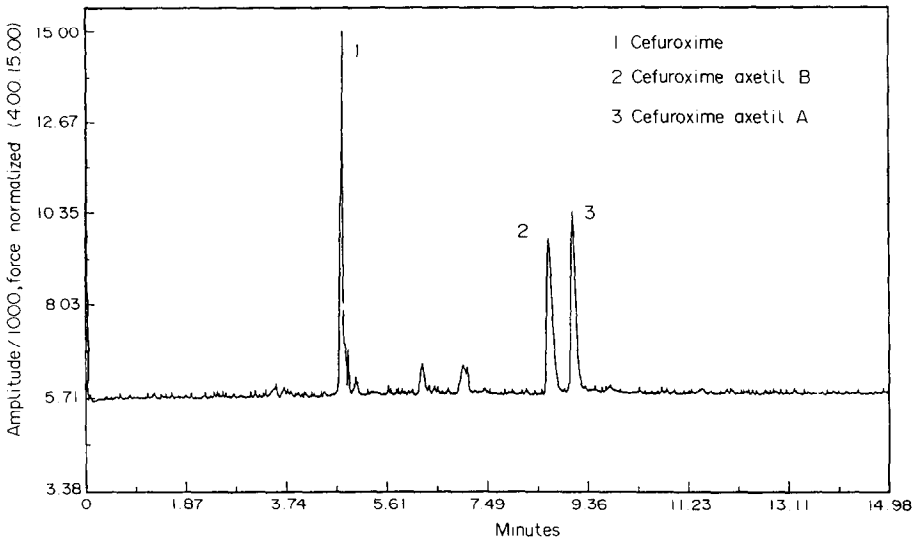

Figure 1
Degraded cefuroxime axetil sample analysis by MECC. Operating conditions: 0.01 M Na_2HPO_4, 0.06 M $Na_2B_4O_2$ and 0.05 M sodium dodecyl sulphate, V = 10 kV, T = 30°C, UV detection at 276 nm.

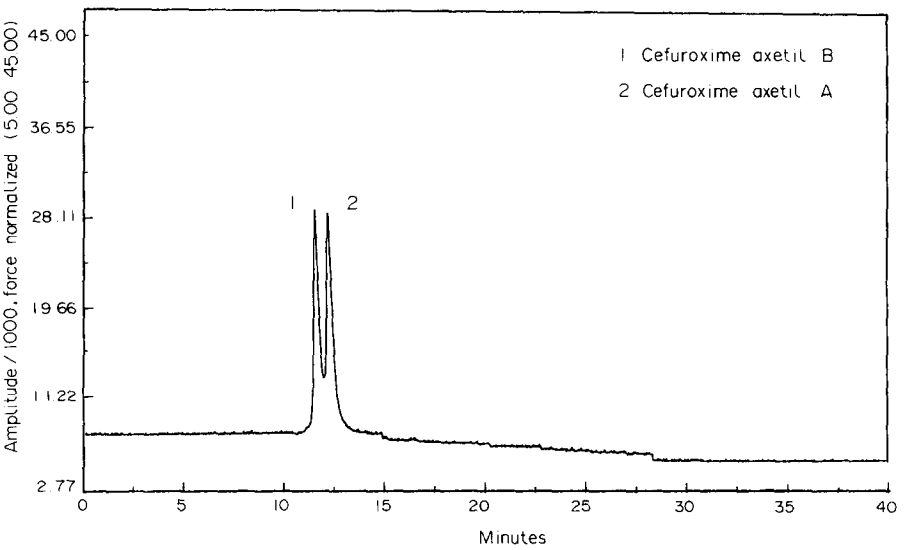

Figure 2
Paediatric suspension analysis by MECC. Operating conditions: as Fig. 1.

cefax A. This elution order is the same as that obtained using reversed phase HPLC [5]. The MECC method is stability indicating as Fig. 1 shows an early eluting peak which is cefuroxime. The cefuroxime is a major degradation impurity arising from hydrolysis of the ester. Attempts at this separation using non-micellular carrier electrolytes had been unsuccessful.

Ranitidine (Zantac) is a widely prescribed drug for the treatment of peptic ulcers. Several pharmaceutical formulations are available, one of which is a syrup. HPLC is used to ascertain ranitidine content following quantitative dilution of the syrup. Direct analysis of the syrup is possible (Fig. 3) using acidic CZE operating conditions and electrokinetically introducing the sample.

Drug-metabolism studies

Typical samples encountered in metabolism studies often contain analytes at concentrations of 1 μg ml^{-1} or less. Considerable sample work-up is needed prior to HPLC analysis. This need may be avoided in CZE by suitable choice of carrier electrolyte and sample introduction procedure. Electrokinetic introduction

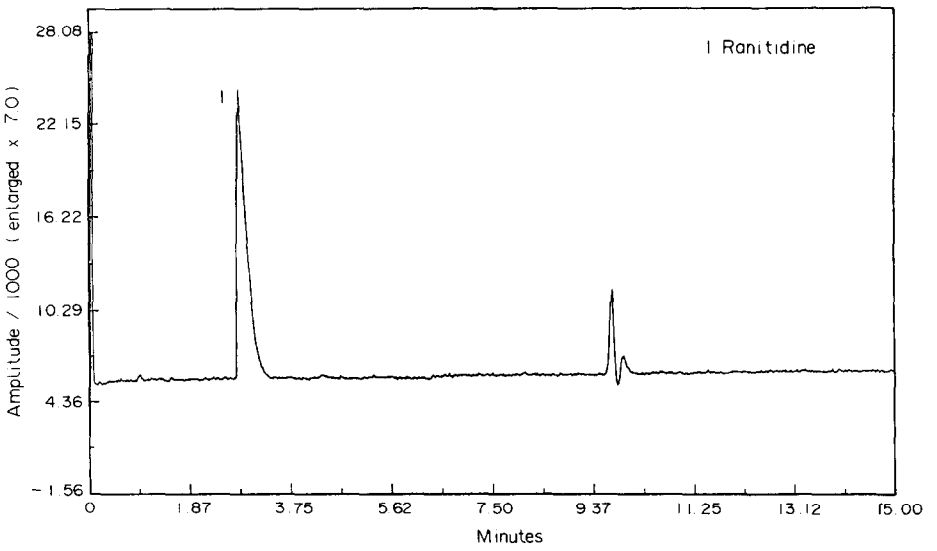

Figure 3
Direct analysis of Zantac syrup. Operating conditions: 20 mM sodium citrate (pH 2.5), V = 30 kV, T = 30°C, UV detection at 210 nm.

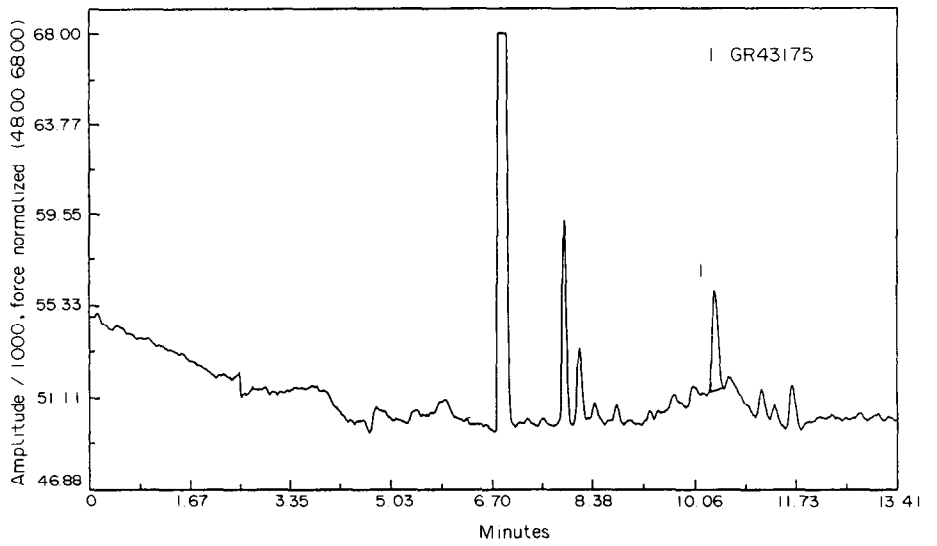

Figure 4
Analysis of 1 μg ml^{-1} GR43175 in urine. Operating conditions: 20 mM sodium citrate (pH 2.5), V = 11 kV, T = 25°C, UV detection at 210 nm.

of sample into the capillary involves placing the capillary into the sample solution and briefly applying a high voltage. Sample enters the capillary by virtue of its electrophoretic movement and EEO flow [6]. The EEO flow is greatly reduced at low pH [7]. Therefore, when performing electrokinetic sampling using buffers with low pH values only cations will enter the capillary when a positive voltage is applied. This selective sampling can be used to reduce interference from neutral species and anions from within the sample matrix.

Using a pH 2.5 carrier electrolyte and electrokinetically introducing sample it was possible to analyse a potential anti-migraine compound (GR43175) at ~1 ppm levels directly in untreated urine (Fig. 4).

Conclusions

The HPCE techniques can offer a viable option in reducing the sample pretreatment needs for pharmaceutical products. Increased interest in HPCE will highlight these advantages.

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