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The determination of the geometric isomers and related impurities of dothiepin in a pharmaceutical preparation by capillary electrophoresis*

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Abstract: The application of capillary zone electrophoresis in the assay of the tricyclic antidepressant drug, dothiepin, in tablets is discussed. The method developed for dothiepin which exists as the *cis*- and *trans*-isomers and contains two major related impurities, an 11-oxo compound and a propanamine, utilizes inclusion complexation with β-cyclodextrin. For optimization of the method the structured procedure of factorial design was used; the electrolyte solution was 50 mM disodium hydrogen phosphate with 10 mM β-cyclodextrin–propan-1-ol (90:10, v/v). Good precision (RSD = 1.06%, n = 6), linearity ($y = 26.84x + 2.25$), and correlation ($r = 0.999$, $n = 7$) was obtained for *trans*-dothiepin. The reproducibility of tablet extraction was also acceptable (RSD = 0.77%, $n = 6$); the recovery of the *trans*-isomer was 98% (w/w) and the level of *cis*-isomer in tablets of dothiepin (75 mg) was 5.58% (w/w).

Keywords: *Capillary zone electrophoresis; geometric isomers; dothiepin; related impurities; inclusion complexation.*

Introduction

Applications of capillary electrophoresis (CE) in the assay of drugs in pharmaceutical and biological samples are still somewhat restricted. The examples that have been published have mainly concentrated on the use of micellar electrokinetic capillary chromatography (MECC) and coated silica capillaries [1]. MECC has been used in a number of fields: in chiral separations, primarily by Terabe *et al.* [2]; in forensic samples to detect trace impurities in street heroin [3]; and in bioanalysis where applications have included the therapeutic drug monitoring of xanthines for screening and confirmation of drugs of abuse [4, 5] and anticancer drugs and their major metabolites [6]. In the assay of biological fluids the major problem has been the detection of trace analyte levels and much of the emphasis has been on laser-induced fluorescence, which has given such impressive lower limits of detection of zeptamole levels as described by Wu and Dovichi [7].

However in the CE assay of bulk drugs and pharmaceutical preparations the need for high sensitivity is generally not as critical and determinations using UV-detection for related impurities should be achievable for many compounds in concentrations of less than 1% (w/w). In the development of a suitable procedure, the most significant methods of MECC and coated columns have attempted to avoid the effects of electroendosmosis which is so dominant and causes general bulk flow towards the negative electrode in CE. This effect can reduce the possibility of resolution of similar charged components. In macromolecule separations this is compounded by the regular adsorption effects with the charged groups on the capillary wall. These effects have led to the suggestion that poor precision and accuracy can result from electroendosmotic effects.

Nevertheless, it is possible to demonstrate that electroendosmosis can be used in quantitative assays of drugs in pharmaceutical preparations [8], particularly when distinctive differences in the chemical structures exist. Here the use of capillary zone electrophoresis (CZE) is focused on the resolution of the tricyclic antidepressant drug, dothiepin, from its two major related impurities in both the bulk drug and in tablets. Moreover the presence of geometric isomerism has been

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addressed and the method incorporates inclusion complexation for separation of the *cis*- and *trans*-components.

Experimental

Instrumentation

An Applied Biosystems Model 270A capillary electrophoresis system (ABI, Foster City, CA, USA) was used with 72 cm × 50 μm i.d. fused silica capillaries. The integrator was a Hewlett–Packard Model 3396A.

On-column UV-detection was used, 50 cm along the capillary, and the wavelength was 220 nm; the temperature of the capillary was held at 30°C and the applied voltage at 25 kV. The samples were injected by the vacuum technique for 1.5 s which is equivalent to an introduction volume of 5.2 nl of sample.

Assay of dothiepin

The electrolyte solution was 50 mM disodium hydrogen phosphate with 10 mM β-cyclodextrin (pH 4.7)–propan-1-ol (90:10, v/v). The capillary was initially washed with 1 M sodium hydroxide for 30 min, prior to use, at the beginning of the day. The subsequent conditioning cycle was: washing (0.1 M sodium hydroxide) for 2 min; buffer for 3 min; and sample for 1.5 s.

Reagents and materials

The reference standards, dothiepin and its related impurities 11-(3-dimethylaminopropylidene)-6*H*-dibenzo[*b*,*e*]thiepin-5-oxide (propanamine) and 6H-dibenzo[*b*,*e*]thiepin-11-one, (11-oxo) were obtained from the British Pharmacopoeia Laboratory (Middlesex, UK). The *cis*- and *trans*-isomers were not available and were used as a (35:65, w/w) mixture, obtained from Approved Prescription Services Ltd, (APS Ltd, Cleckheaton, Yorkshire, UK). Tablets of dothiepin (75 mg) (Prothiaden) were also kindly supplied by APS.

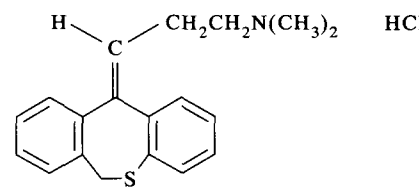
Tablet extraction procedure

Twenty tablets (75 mg) were weighed, ground and the weight equivalent to one tablet was extracted with 25 ml of acetonitrile–water (60:40, v/v) under sonication; after shaking the sample was diluted to 100 ml with the electrolyte, before centrifugation. A sample of about 30 ml was filtered and 500 μl was placed in a vial for assay. The sample had a final concentration of about 750 μg ml^{-1}.

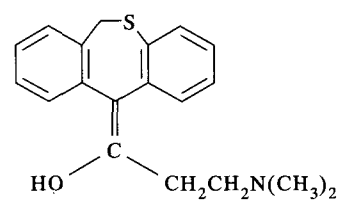
Results and Discussion

In the British Pharmacopoeia (BP 1988) [9], the assay method for the detection and quantitation of the related impurities (propanamine and 11-oxo) (Fig. 1) of dothiepin is based on a reversed-phase procedure. For the geometric isomers a gas chromatographic method is described, where the *cis*- isomer is treated as the impurity and limited to 7.5% (w/w). Another method has been reported for the assay of the *cis*- and *trans*-isomers by LC, which utilizes a column packed with porous graphitic carbon and is operated in a normal-phase mode [10]. But this method is again restricted to the isomers and of more help to the analyst would be a combined method for the isomers and their related impurities.

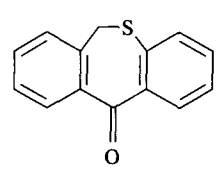
In CZE separation of the analytes is principally through electroendosmosis; differences result from electrophoretic migration effects. The electrosmotic effect of the electrical double layer, formed from the negatively charged silica and positively charged buffer components, is generally appreciable and in-



Dothiepin HCl



11-(3-dimethylaminopropylidene)-6H-dibenzo [b,e] thiepin-5-oxide (propanamine)



6H-dibenzo [b,e] thiepin-11-one (11-oxo)

Figure 1
Structures of dothiepin and its propanamine and 11-oxo related impurities.

fluences the absolute velocities of even the most highly negatively charged species towards the negative electrode. In the assay of dothiepin it was proposed to use this overall effect of electroendosmosis in both a qualitative and a quantitative manner. In this respect it is usually considered that the reproducibility of migration times depends on capillary column conditioning and on the operating pH [11]. Therefore to reduce pH hysteresis the capillary used in this program was washed twice during the day with 1 M sodium hydroxide (30 min) and between samples with 0.1 M sodium hydroxide (3 min).

The initial experiments involved choice of buffer, pH, organic additive, voltage and temperature. In order to optimize these parameters in a more rational process than that of trial and error or a stepwise procedure, the structured technique of factorial design was utilized. From this the major factors contributing to resolution of the related impurities were pH, the buffer and its concentration and the organic additive; voltage and temperature had a more minor influence on the resolution of dothiepin from the propanamine and 11-oxo components. At this stage the emphasis was not on the resolution of the geometric isomers but on establishing a basic methodology for the assay. The pH was particularly crucial as electrophoretic peak shape relied on an accurate value of pH 4.7. For resolution of the parent compound and the impurities, the inclusion of an organic modifier was more significant and acetonitrile, methanol, propan-2-ol and finally propan-1-ol were all considered. It is reasoned that this effect of the organic additive results from changes in the zeta potential around the analyte molecules together with reduction in electrosmosis because of viscosity changes that occur with a number of organic additives [12]. Particularly with the more viscous propanols, this was confirmed by a reduction in migration times and a subsequent improvement in resolution of the components. Propan-1-ol gave the best results (Fig. 2).

However, the goal was to resolve the related impurities and the *cis*- and *trans*-isomers of dothiepin. To achieve this aim the approach adopted was to examine the inclusion complexation capabilities of cyclodextrins and their derivatives for resolution of these geometrically orientated isomers. β-Cyclodextrin, methylated-β-cyclodextrin and hydroxypropyl-

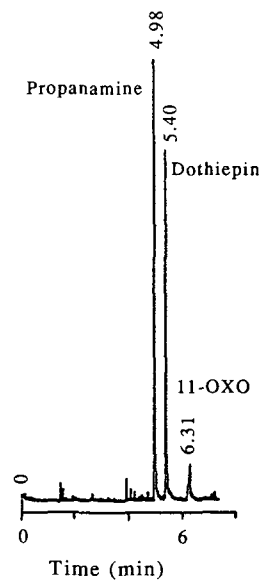


Figure 2
Separation of dothiepin and the propanamine and 11-oxo related impurities after application of factorial design to optimize buffer concentration, pH and the propan-1-ol concentration. The optimized electrolyte mixture was 40 mM disodium hydrogen phosphate (pH 4.8)–propan-1-ol (95:5, v/v). The temperature was 30°C, the applied voltage 25 kV and the detection wavelength 220 nm.

β-cyclodextrin were all examined over a range of concentrations; the best results were achieved with β-cyclodextrin at a concentration of 10 mM as shown in Fig. 3. The parameters used in the initial separation without β-cyclodextrin had to be slightly modified to achieve this optimized separation.

The precision of the assay was determined on a sample of *trans*-dothiepin (400 μg ml^{-1}) in the phosphate buffer (RSD was 1.06%, $n = 6$) and also on the propanamine impurity (250 μg ml^{-1}) in the phosphate buffer (RSD was 0.94; $n = 7$). In both these determinations the peak areas were normalized to one of the migration times in each experiment, since in CZE small changes in electroendosmosis can lead to changes in the peak areas [13]; this effect is in contrast to that of LC where the peak area is constant with changes in flow rate. Linearity was also acceptable and over a range of 100–1000 μg ml^{-1} for *trans*-dothiepin, the regression equation was $y = 26.84x + 2.25$ ($r = 0.999$; $n = 6$) and for the propanamine impurity (100–800 μg ml^{-1}), $y = 22.56x + 3.87$ ($r = 0.998$; $n = 7$). The lower detection limit (SNR = 3) for *cis*- and *trans*-dothiepin and for propanamine was 4.65 μg ml^{-1} and 2.79 μg ml^{-1}, respectively. The propanamine

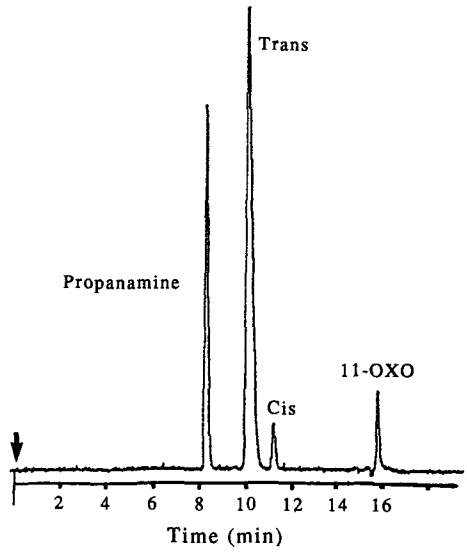


Figure 3
Resolution of the *cis*- and *trans*-isomers of dothiepin and the propanamine and 11-oxo related impurities by CZE with inclusion complexation to resolve the geometric isomers. The optimized operating parameters were: electrolyte, 40 mM disodium hydrogen phosphate with 10 mM β-cyclodextrin (pH 4.7)–propan-1-ol (90:10, v/v). The temperature was 30°C, the applied voltage 25 kV, the detection wavelength 220 nm and the absorbance range 0.01 aufs.

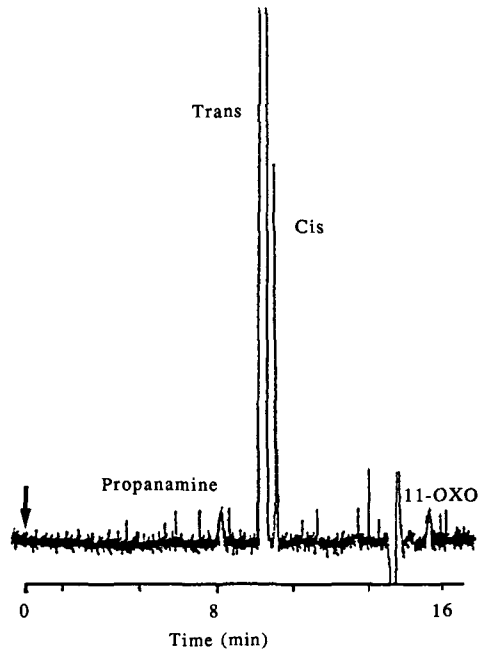


Figure 4
The examination of the extract from Prothiaden tablets (75 mg) (at 750 μg ml^{-1}) for levels of the two major related impurities. The parameters are as in Fig. 3, except that the absorbance range was 0.001 aufs.

was chosen as representative of the two related impurities.

Application of the developed method to tablets (75 mg) led to a reproducibility (RSD) of 0.77% (n = 6) and a recovery of 98% (w/w) for the *trans*-dothiepin. In the tablets the mean value of the *cis*- impurity was 5.58% (w/w) which was comfortably under the BP limit. In both the tablet preparation (Fig. 2) and the bulk drug the related impurities, propanamine and 11-oxo components, gave mean values of <0.2% (w/w). The robustness of the assay was tested by measurements of the day-to-day precision on a 400 μg ml^{-1} *trans*-dothiepin sample; the RSD of the mean values over 5 days was 1.87%.

Conclusions

The single method based on inclusion complexation for the *cis*- and *trans*-isomers and the combination of an organic additive with a buffer offers a stable and robust CZE assay for the determination of the isomers and their major related impurities. The success of the method can be attributed in part to the controlled operating mode of the instrumental system, where precise sample take-up under vacuum, careful conditioning of the capillary and close temperature control of the silica capillary are prerequisites for reliable quantitative results in CZE.

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