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Short Communication

Assay of dothiepin hydrochloride and its geometric isomers by liquid chromatography*

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Introduction

For some years HPLC has played an important part in the development of stability-indicating assay methods for pharmaceuticals. Dothiepin hydrochloride, a tricyclic antidepressant, is included in the British and European Pharmacopoeias in monographs relating to the raw material and capsule preparations. Unfortunately, the assay methods described are non-specific, involving chloroform extraction of the drug, followed by non-aqueous titration with perchloric acid. HPLC methods for identification and quantitation of forensic and clinical samples have previously been developed but procedures specifically developed for the stability testing of solid dosage formulations have not been reported.

In this work, a fully validated ion-pair reversed-phase HPLC method is described, which has been optimized for column packing material and mobile phase conditions. In addition to this method, further development has yielded a suitable method for the determination of the geometric isomers of dothiepin. This is based on the novel use of a PGC column material in normal-phase mode [1].

Experimental

The chromatographic system consisted of a dual reciprocating pump (Model 420, Kontron, Milan, Italy) connected to an autosampler with variable loop volume (Model 460, Kontron) and a variable wavelength UV detector (Model 430, Kontron). The HPLC instrumentation was interfaced to an IBM–AT microcomputer and instrument control was through resident Kontron chromatographic software. For constant chromatographic column temperature control a column oven was incorporated into the system (Jones Chromatography, Hengoed, Mid-Glamorgan, UK).

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Chromatographic conditions

Analysis of dothiepin. A 250 × 4.9 mm i.d. stainless-steel column packed with 5 μm cyanopropyl–Hypersil (Hichrom, Reading, UK) was used with a mobile phase of acetonitrile–0.15 M sodium hexane sulphonic acid (Fahrenheit Laboratories, Leeds, UK) adjusted to pH 3.0. The composition was 40:60, v/v for the determination of related impurity levels and 60:40, v/v for the parent compound determination in the pharmaceutical preparations. A flow rate of 2.0 ml min^{-1} was used and the column temperature maintained at 30°C, with a detection wavelength of 260 nm.

Analysis of dothiepin isomers. A 100 × 4.6 mm i.d. stainless-steel column packed with 7 μm PGC (Shandon Scientific, Runcorn, Cheshire, UK) was used. The mobile phase consisted of ethyl acetate–methanol–3% v/v ammonia solution (sg 0.880) (75:30:1, v/v) which was degassed with helium prior to use. The flow rate was 1.0 ml min^{-1}. The column oven was not used in this application. Detection wavelength was 260 nm.

Reagents and materials

Dothiepin and its related impurities 6H-dibenzo[b,e]thiepin-11-one, (11-oxo) and 11-(3-dimethylaminopropylidene)-6H-dibenzo[b,e]thiepin-5-oxide (propanamine) were obtained from the British Pharmacopoeia Laboratory (Middlesex, UK) as reference standards. The *cis–trans* isomers were not available as individual components and were used as a 65:35, w/w mixture, obtained from the manufacturer. The tablet pharmaceutical preparation (75 mg tablet) was obtained from A.P.S. Ltd (Approved Prescription Services Ltd, Bradford) a subsiduary of Rhône–Poulenc, Paris, France.

Dothiepin extraction from tablets

For the assay of the 75 mg tablets, at least 20 tablets were weighed and their average weight per tablet calculated. A sample of the ground and powdered tablets equivalent to 100 mg of dothiepin hydrochloride was accurately weighed in duplicate into 100 ml volumetric flasks. Approximately 70 ml of acetonitrile–water (60:40, v/v) was added and the mixture sonicated and shaken to extract the drug. The solution was them made up to volume with the same solvent, centrifuged and diluted 20-fold with mobile phase to obtain a final solution containing 0.05 mg ml^{-1} dothiepin prepared in mobile phase, using the sample preparation steps described above.

Results and Discussion

The development of the HPLC method initially considered a C-18 column material. Later this was replaced by other column materials and finally a cyanopropyl column material was selected as the stationary phase, since this exhibited the most suitable retention characteristics and symmetrical peak shapes with the mobile phase initially used. This was followed by a step-wise optimization of the chromatographic mobile phase operating parameters, namely: organic modifier, pH and concentration of the ion-pairing agent. This resulted in the resolution of dothiepin ($k| = 2.51$) from its principal related impurities, the 11-oxo ($k| = 1.62$) and propanamine component ($k| = 0.51$), as illustrated in Fig. 1.

From this optimized method the linearity of chromatographic response for the parent compound was determined over the range 0.025–1.00 mg ml^{-1} ($y = 1.02x + 5.14 \times$

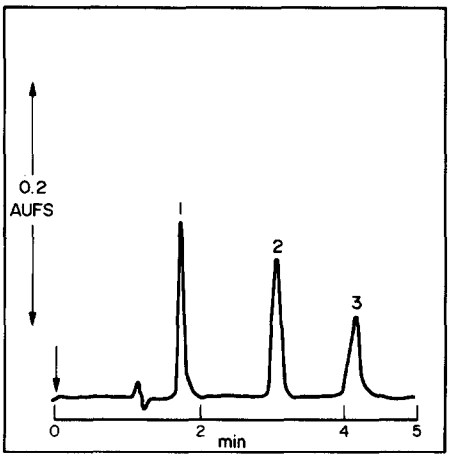

Figure 1
Chromatographic profile of the manufacturing impurities of dothiepin hydrochloride: (1) dothiepin hydrochloride; (2) 11-oxo impurity; (3) propanamine impurity. For chromatographic conditions, see text.

10^{-3}) ($r = 0.999$; $n = 6$). The reproducibility for replicate injections was good (RSD = 0.97%; $n = 9$ at 0.05 mg ml^{-1}).

On application of the method to a tablet dosage form of dothiepin (75 mg tablets), typical recovery figures for extraction of the dothiepin of 100% w/v were observed. Replicate assay values for the ground tablet powder were also acceptable (RSD = 0.96%, $n = 10$ at 0.05 mg ml^{-1}).

When considering a 1 mg ml^{-1} solution of dothiepin in the bulk drug and pharmaceutical preparation for the presence of related compounds with mobile phase: acetonitrile–0.15 M sodium hexane sulphonic acid (40:60, v/v) at high detector sensitivity, it was found that no appreciable impurity levels were present (Fig. 2) (<0.1% w/v for each impurity peak) when measured against the parent component peak. The levels of impurity were also observed not to have increased significantly when long term storage trials of the pharmaceutical preparation were undertaken under various conditions of heat and humidity.

In addition to the conventional assay described above, a method was developed for the resolution and quantitation of the *cis–trans* isomers of dothiepin [2]. The *cis*-isomer is limited in the British Pharmacopoeia to 7.5% w/v and it is thus regarded as an impurity. The method is based on normal-phase mode using a PGC column [3], in contrast with the GLC method described in the BP 1988 [4]. So far as the authors are aware normal-phase methods on PGC have not been reported previously.

Porous graphitic carbon is a strongly hydrophobic stationary phase which contrasts with the polar properties of silica when used in the normal-phase mode. By adapting and optimizing a method [5] reported on silica it was found possible to obtain baseline resolution of the *cis–trans* isomers of dothiepin (resolution factor, $R_s > 2.5$), with an analysis time of 8 min and fast equilibration times. It was observed that the reported GLC method gave incomplete resolution of the isomers (R_s 1.0) compared with that for the PGC method. However, both methods yielded very similar results. For the raw material tested, the average concentration of *cis* isomer found by both methods was 3.5% w/v, which agreed with the manufacturer's stated impurity level.

This method was validated for the solid dosage form (75 mg tablets) and the following results were obtained. The method was linear over the range up to 2.0 mg ml^{-1} ($y =$

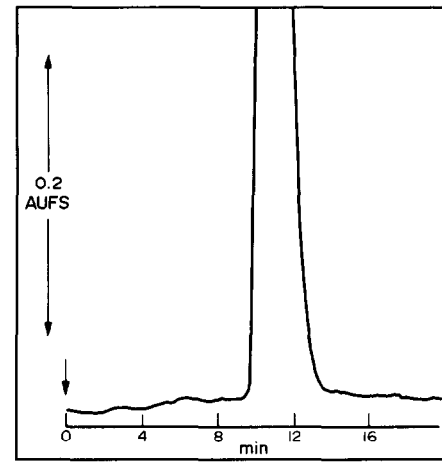

Figure 2
Chromatographic profile of a bulk drug sample of dothiepin hydrochloride containing 1 mg ml^{-1} of the drug.

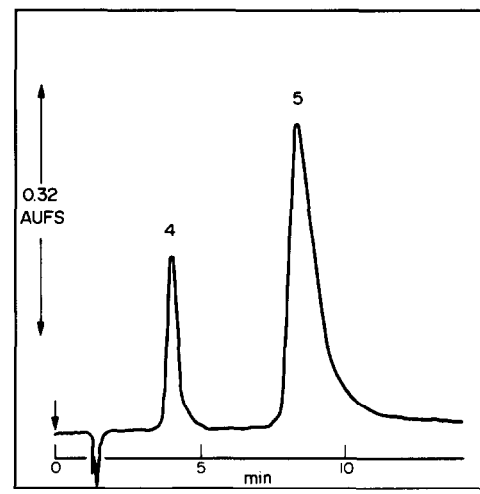

Figure 3
Resolution of the geometric isomers of dothiepin hydrochloride using a PGC column material in normal-phase mode. Sample solutions containing 1 mg ml^{-1} as 65:35, w/w of the *trans–cis* isomers, respectively; (4) *cis*-isomer and (5) *trans*-isomer. Resolution factor $R_s > 2.5$. For chromatographic conditions, see text.

$338x - 6.44$; $r = 0.999$, $n = 7$) for the *cis*-isomer with a limit of detection of 1.9 μg ml^{-1}. Reproducibility for replicate injections for *cis*-isomer determinations was acceptable at a concentration of 5.3 μg ml^{-1} (RSD = 0.90%, $n = 9$). A check on possible interference from tablet excipients did not reveal any co-elution with the chromatographic peaks of interest.

Conclusion

A double column method has been developed for the determination of dothiepin, its related impurities and geometric isomers. The method involves a novel application of PGC as a stationary phase in the resolution of the isomers in a solid dosage form and the

bulk drug. It has been shown in this work that these methods are suitable for the routine quality control of dothiepin hydrochloride tablets and are considered to be accurate, precise and reproducible.

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