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Development of a liquid chromatographic method for the control of related substances in chlorprothixene hydrochloride

G.M. Duignan[a], J.H. McB. Miller[b,*], G.G. Skellern[a]

[a]*Department of Pharmaceutical Sciences, University of Strathclyde, 204 George Street, Glasgow G11XW, UK*
[b]*European Pharmacopoeia Laboratory, 226 Avenue de Colmar, B.P. 907, F-67029 Strasbourg Cedex 1, France*

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Abstract

The development of a reversed-phase liquid chromatographic method, using a mobile phase containing a mixture of anion and cation ion-pairing agents and a base-deactivated octyldecylsilyl column as stationary phase, is described for the control of all known impurities in (*Z*)-chlorprothixene hydrochloride (bulk drug). Validation of the method showed it to be reproducible, selective for both (*Z*) chlorprothixene hydrochloride and its *E*-isomer, accurate and linear over the concentration range of analysis with a limit of detection of 0.3 μg ml^{-1}.

Keywords: Chlorprothixene hydrochloride; Drug impurities; Reversed-phase liquid chromatography

1. Introduction

Chlorprothixene hydrochloride, 3-(2-chlorothioxanthen-9-ylidene)-*N*,*N*-dimethylpropylamine hydrochloride, is a thioxanthene that exists as cis (*Z*) and trans (*E*) isomers [1] (Fig. 1), with pharmacological properties similar to those of the phenothiazine chlorpromazine.

Investigations of the structure–activity relationships of these isomeric forms have shown that the cis isomer is far more potent than the trans isomer [2]. Consequently, formulations of the chlorprothixene · HCl contain the more active *Z*-isomer [3], and are administered both intramuscularly and orally for the treatment of psychoses. On exposure to light, this isomer is rapidly converted into the *E*-form until an equilibrium is reached [4]. In the presence of air it is converted into 2-chlorothioxanthone (**III**), which is the major degradation impurity [4]. As the photochemical equilibrium ratio of the *Z*- to *E*-isomers and the batch composition may differ considerably, possible changes should be monitored using isomer-specific quality control procedures.

The method described in the European Pharmacopoeia (Ph. Eur.) monograph for controlling the concentration of the *E*-isomer in the substance is a thin-layer chromatographic method

* Corresponding author.

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where the test substance is compared with the Ph. Eur. CRS which contains 2% (m/m) of the *E*-isomer. The CRS, therefore, provides control of this isomer while at the same time acting as a performance indicator for the method. All other known potential impurities of chlorprothixene hydrochloride arise from the manufacturing process, which has 2-chlorthioxanthone (**III**) as a starting material. Chlorprothixene hydrochloride is formed via a chlorthioxanthenol intermediate (**V**) (Fig. 1) [5]. Moreover, it is this intermediate (**V**) that is undetected by the related substances test described in the Ph. Eur. monograph.

The known manufacturing impurities are 2-chlorothioxanthone (**III**), (*Z*)-3-(2-chloro-9H-thioxanthen-9-ylidene)-*N*-methylpropylamine(**IV**), 3-(2-chloro-9H-thioxanthen-9-ol)-*N*,*N*-dimethylpropylamine (**V**), (*Z*)-3-(4-chloro-9H-thioxanthen-9-ylidene)-*N*,*N*-dimethylpropylamine (**VII**) and 3-(9H-thioxanthen-9-ylidene)-*N*,*N*-di-methylpropylamine (**VI**) (Fig. 1).

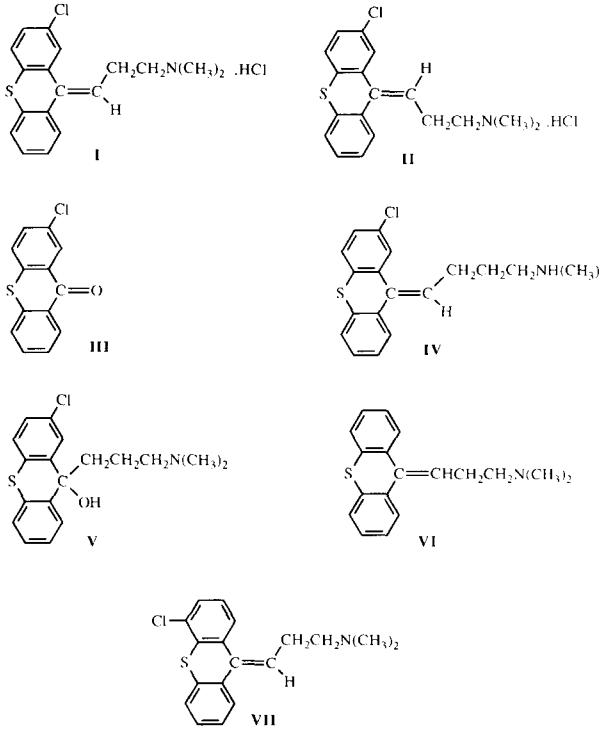

Fig. 1. Structural formulae of chlorprothixene hydrochloride (*Z*-isomer, **I**) and impurities.

The liquid chromatographic (LC) separation of chlorprothixene hydrochloride and phenothiazines, in general, uses reversed-phase columns of ODS-silica and mobile phases of various compositions, with and without an ion-pairing reagent [6,7]. The separation of impurity **III** and the *E*-isomer from (*Z*)-chlorprothixene hydrochloride in the bulk drug by LC has been reported [8].

This paper describes the development and validation of an ion-pair, reversed-phase LC method for the separation not only of the Z-isomer of chlorprothixene from the *E*-isomer but from all its known impurities in the bulk drug.

2. Experimental

2.1. Materials

(*Z*)-Chlorprothixene hydrochloride (**I**), (*E*)-chlorprothixene hydrochloride (**II**), 2-chlorthioxanthone (**III**), (*Z*)-3-(2-chloro-9H-thioxanthen-9-ylidene)-*N*-methylpropylamine (**IV**), 3-(2-chloro-9H-thioxanthen-9-ol)-*N*,*N*-dimethylpropylamine (**V**), (*Z*)-3(4-chloro-9H-thioxanthen-9-ylidene)-*N*,*N*-dimethylpropylamine (**VII**) and 3-(9H-thioxanthen-9-ylidene)*N*,*N*-dimethylpropylamine (**VI**) were kindly donated by H. Lundbeck (Copenhagen, Denmark).

Chlorprothixene hydrochloride CRS was prepared by European Pharmacopoeia Laboratories and contained 2.0% (m/m) of the *E*-isomer.

Acetonitrile, methanol, potassium dihydrogenphosphate (KH_2PO_4), sodium lauryl sulphate (SLS), methylene chloride and Kieselgel $60F_{254}$ (0.25 mm) thin-layer chromatographic plates were supplied by Merck (Darmstadt, Germany). Tetra-*n*-butylammonium bromide 99% ($Bu_4N^+Br^-$) was supplied by Aldrich Chemie (Steinheim, Germany).

2.2. Instrumentation

An LC system (Spectra Physics Analytical, Fremont, CA, USA), comprising a P1000 isocratic pump, an AS1000 autosampler with a fixed-volume loop injector and UV100 detector was used. Chromatograms were recorded on a Data Jet integrator (Spectra-Physics Analytical).

A computer-controlled system from Waters Chromatography (division of Millipore) was also employed. The Waters system comprised a Waters 600E system controller, Waters TM717 autosampler, Waters TM996 photodiode-array detector and Millennium 2010 Chromatography Manager from Millipore. A Hewlett-Packard Deskjet 500C printer was used.

A column (100 × 4 mm i.d.) packed with 3 μm Hypersil-C_{18}-BDS was supplied by Shandon Southern Products (Cheshire, UK). A mobile phase consisting of water–acetonitrile–methanol (55:40:5, v/v/v) containing potassium dihydrogenphosphate (6 g l^{-1}), sodium lauryl sulphate (2.9 g l^{-1}) and tetra-*n*-butylammonium bromide (9 g l^{-1}) was delivered at a flow rate of 1.5 ml min^{-1}.

Thin-layer chromatography (TLC) was performed using a Desaga AS30 TLC applicator with a Marathon autosampler and a Desaga densitometer (Desaga, Saststedt Gruppe, Heidelberg, Germany). The TLC plates were developed with *n*-propanol–concentrated ammonia solution–toluene–acetone (2:2:40:60, v/v/v/v).

2.3. Preparation of standard solutions

A mixture of impurities **III**, **IV** and **V**, **VI** and **VII** was prepared by dissolving 2 mg of each in the mobile phase and diluting to 100.0 ml (impurity stock solution). Chlorprothixene hydrochloride CRS (10 mg) (containing 2% (m/m) of *E*-isomer) was dissolved in 10.0 ml of the mobile phase. A sample of chlorprothixene hydrochloride (20 mg) was dissolved in a small volume of mobile phase, to which was added 3.0 ml of the impurity stock solution, and diluted to 20.0 ml with the mobile phase.

Quantities between 5.1 and 5.3 mg of chlorprothixene hydrochloride and all six impurities, including the *E*-isomer, were dissolved together in the mobile phase and diluted to 50.0 ml in mobile phase. A portion (10.0 ml) of this solution was then transferred into a 20.0 ml volumetric flask and diluted to volume with the mobile phase. Injections of this mixture were made initially on the Waters chromatographic system, with diode-array detection, and spectral information for each analyte was obtained for the determination of their respective response factors.

A solution containing chlorprothixene hydrochloride (1.0 mg ml^{-1}) and all six impurities at their limiting concentrations was prepared. Aliquots of this solution were injected ten times using the Spectra-Physics chromatographic system. Retention times and peak areas were recorded for each analyte and their respective relative standard deviations for each analyte peak were calculated.

Impurity **V** (2.0 mg) was accurately weighed and diluted to 20.0 ml with mobile phase. A portion (10.0 ml) of this solution was then further diluted to 100.0 ml to give a solution concentration of 0.01 mg ml^{-1}. An internal standard solution was prepared by weighing accurately 2.0 mg of impurity **VI** and diluting to 20.0 ml with the mobile phase (i.e 0.1 ml^{-1}). Calibration standards of 1.0, 2.0, 3.0, 4.0 and 5.0 μg ml^{-1} were prepared by pipetting 5.0, 10.0, 15.0 20.0, 25.0 ml, respectively, of 0.01 mg ml^{-1} stock solution into 50.0 ml volumetric flasks and then adding 2.0 ml of internal standard solution to each before diluting to volume with the mobile phase.

Sample solutions ($n = 2$) were prepared by weighing 2.4 and 2.1 mg of impurity **V** and diluting to 20.0 ml with mobile phase. Portions (1.5 ml) of each solution were then pipetted into separate 50.0 ml volumetric flasks containing internal standard solution (2.0 ml), which were diluted to volume with the mobile phase. Each calibration solution was injected on to the chromatograph and peak-area ratios were recorded and plotted against the concentration of impurity **V** in each solution. Sample solutions were also analysed, peak areas recorded and sample concentrations determined from the calibration graph. The samples were also quantified using the calibration graph obtained, by directly plotting the peak areas of impurity **V** against concentration, disregarding the peak areas of the internal standard.

Resolution was calculated from replicate chromatograms ($n = 7$) according to the method described in the European Pharmacopoeia [9].

The method described in the United States Pharmacopeia [10] was used to determine limit of detection (LOD) and limit of quantitation (LOQ).

Replicate injections ($n = 6$) of the mobile phase were made. The analysis time for each injection was 6 min. The average noise level for the six injections (i.e. the average peak area over the six injections) and the standard deviation of the noise level were calculated. The standard deviation value, which was in absorbance units (peak area), was converted into its equivalent concentration value using the peak area of a known concentration of impurity **IV** as a standard and multiplied by factors of 3 and 10 to give proposed concentration limits of detection and quantitation, respectively.

These limits were then confirmed by injecting two samples of impurity **IV**, prepared at both the detection limit (0.3 μg ml^{-1}) (equivalent to 0.03% impurity in the substance) and quantitation limit (0.9 μg ml^{-1}), in the presence of both the *Z*-isomer (1 mg ml^{-1}) and *E*-isomer (0.02 mg ml^{-1} of chlorprothixene hydrochloride.

Impurity **IV** was chosen for these determinations since it eluted between the *Z*- and *E*-isomers. Thus, the effects of peak tailing were considered in determining the correct limits of detection and quantitation.

The developed LC method was compared with the TLC test for related substances described in the monograph for chlorprothixene hydrochloride of the European Pharmacopoeia, using a standard solution of chlorprothixene Ph. Eur. CRS in methylene chloride (20 mg ml^{-1}).

3. Results and discussion

Whilst the TLC method described in the adapted monograph of the European Pharmacopoeia separated the *Z*- and *E*-isomers of chlorprothixene, impurity **V** co-eluted with the *Z*-isomer (Fig. 2). As an alternative method, LC was considered as a possible means of separating the *Z*-isomer from its known six likely impurities.

Addition to the mobile phase of an ion-pairing reagent, which is protonated and carries the same charge as the analyte, is known to improve the chromatography and to reduce peak tailing. Preliminary studies had shown that the inclusion of either a cationic or anionic ion-pairing reagent in the mobile phase failed to separate all the impurities from chlorprothixene. For example, the effect of adding the cationic ion-pairing reagent tetra-*n*-butylammonium bromide ($Bu_4N^+Br^-$) at various concentrations was examined. Increasing concentrations of $Bu_4N^+Br^-$ resulted in shorter retention times for all analytes, with the exception of impurity **III**, which is the only non-basic analyte. However, because the inclusion of both cationic and anionic ion-pairing reagents in the same mobile phase had previously provided the successful separation of atenolol from a complex mixture of related substances [11], this stratagem was considered to be potentially effective for the separation of chlorprothixene from its impurities. A ternary mobile phase composition of water–acetonitrile–methanol (55:40:5, v/v/v) containing SLS (2.9 g l^{-1}), $Bu_4N^+Br^-$ (9 g l^{-1}) and KH_2PO_4 (6 g l^{-1}) adequately separated all the impurities and (*Z*)-chlorprothixene with an analysis time of approximately 20 min (Fig. 3). The concentrations of impurities in the chromatogram shown correspond to the maximum limited concentrations (2.0% of the *E*-isomer and 0.3% of each of the other impurities). Unfortunately, there is no single wavelength at which all substances exhibited a similar detector response. However, a detection wavelength of 254 nm was selected since good uniformity of response was observed for all but one substance, impurity **III**. At 254 nm, impurity **III** has a response factor three to four times greater than that for (*Z*)-chlorprothixene. All other substances give response factors between 62% and 105% of the value for the *Z*-isomer and could be considered sufficiently similar for the purposes of the limit test for the drug substance.

There was very little variation in retention time between replicate injections ($n = 10$) of a solution containing all the analytes, with a maximum repeatability standard deviation (RSD) of 1.4%. Peak-area repeatabilities were less than 2% at the 2% limit for the *E*-isomer and less than 5% for the other impurities at a level of 0.3%.

Linearity was confirmed as satisfactory when using an internal standard ($y = 0.143x - 5.29 \times 10^{-3}$; $r^2 = 0.999$), where y is the peak-area ratio of **V** to **VI** using impurity **V** since it exhibits the lowest response factor, and without an internal

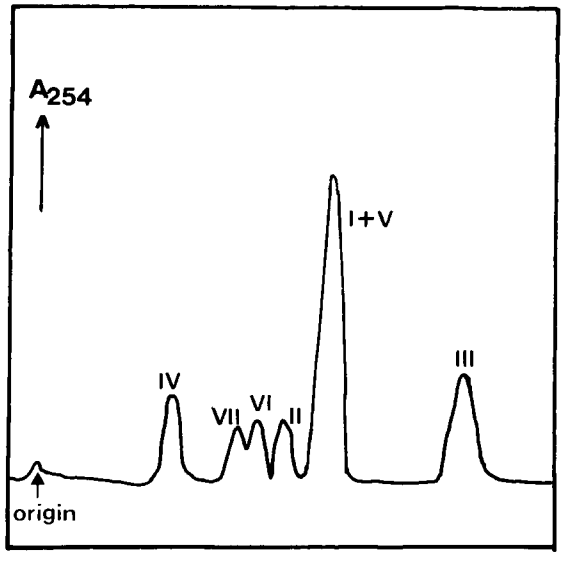

Fig. 2. Densitometric scan of a thin-layer chromatogram of a solution of (*Z*)-chlorprothixene (**I**) and its impurities (see Fig. 1 for structures of analytes). R_F values. **IV**, 0.27; **VII**, 0.37; **VI**, 0.40; **II**, 0.45; **I** and **V**, 0.53; **III**, 0.73.

standard ($y = 4.17 \times 10^4 x - 986.8$; $r^2 = 0.997$). For this reason, it was also chosen as a critical impurity for the determination of recovery. The amount of impurity **V** in 'spiked' samples was calculated using the regression equation for each calibration graph. Recoveries obtained using both the external and internal standard methods at the concentration limit of the impurity were 101.4% and 101.1%, respectively, for one sample and 107.9% for the second sample using both techniques. Hence there is no advantage in using an internal standard procedure.

Impurity **IV** was chosen to estimate the limits of detection and quantitation because it eluted between the *Z*- and *E*-isomers of chlorprothixene (Fig. 3) and as such could be influenced by the peak tailing effect of the first-eluting isomer (*Z*) and its separation from the *E*-isomer. Response factor data also showed that impurity **IV** has a lower response than the other impurities apart from **V**. The limits of detection (0.3 μg ml^{-1}) and quantitation (0.9 μg ml^{-1}) were also determined for this impurity because its chromatographic properties could influence these parameters considerably. (*Z*)-chlorprothixene hydrochloride (1 mg ml^{-1}) and its *E*-isomer (0.02 mg ml^{-1}) were also included in the sample to confirm that the estimated limits of detection and quantitation are accurate for any sample of the drug substance with the maximum tolerated content of the *E*-isomer.

There was good agreement between the values of the content of the *E*-isomer in two batches of raw material using the TLC method described in the Ph. Eur. monograph and quantified with a Desaga CD60 densitometer and those obtained by the LC method. The concentrations of *E*-isomer in the substance analysed on two different occasions by TLC and LC were 0.21% and 0.22%, and 0.23% and 0.21%.

During the study, it became apparent that the preparation of the mobile phase was critical for ensuring the consistent separation of all analytes. A slight variation in the composition of the mo-

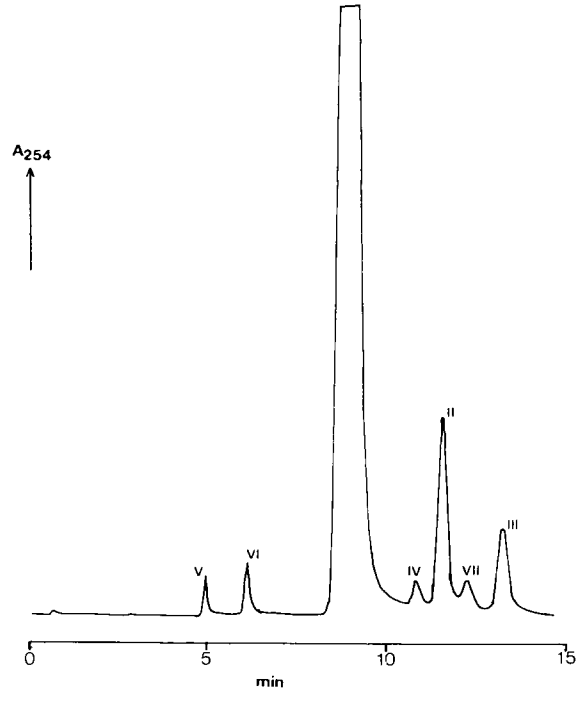

Fig. 3. Liquid chromatogram of a solution of (*Z*)-chlorprothixene containing the *E*-isomer (2.0%, m/m) and impurities (each 0.30%, m/m). Retention times (min): **V**, 4.97; **VI**, 6.14; **I**, 8.66; **IV**, 10.9; **II**, 11.6; **VII**, 12.5; **III**, 13.5. Mobile phase: water–acetonitrile–methanol (55:40:5, v/v/v) containing SLS (2.9 g l^{-1}), $Bu_4N^+Br^-$ (9 g l^{-1}) and KH_2PO_4 (6 g l^{-1}).

bile phase caused the impurities **III** and **VII** to co-elute. Moreover, variations in retention times were observed using mobile phases prepared on different days. On no occasion, however, did this affect the separation of the principal peak and the *E*-isomer from all other impurities. That the separation of analytes is sensitive to small changes in mobile phase composition highlights the importance of including a performance requirement in the test of the monograph which would enable the analyst to discern whether or not complete separation of all potential impurities is likely to be achieved. This would ideally be carried out using a reference solution containing the impurities of interest. However, as reference standards of the chlorprothixene impurities are not readily available, the resolution requirement between the *E*- and *Z*-isomers is considered to be satisfactory.

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