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Short Communication

Analysis of ICI 118551, a new β_2 blocking drug, and related compounds by RP-HPLC–DAD*

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Introduction

ICI 118551 [1] is one of the first highly selective β_2-blockers to be claimed in literature. The most interesting aspect of this compound is effective β_2 blocking action which is not accompanied by β_1 blocking activity. In general, other selective β_2 blockers not only block periferal receptors, but also cardiac receptors. This drug has been suggested for the therapy of the vasomotor migraines and somatic troubles related to anxiety states.

The synthesis of ICI 118551 (**I**) has recently been scaled up from laboratory output to full plant operation [2]. Its manufacturing procedure consists of six stages (Scheme 1) and produces a mixture of *erythro* isomer (the active compound; *ca* 85%), traces of *threo* isomer and oxidized compounds.

These oxidized compounds are all derived from an intermediate, the hydroperoxide as shown in Scheme 2, which could be formed during the epoxidation (stage 5). Notwithstanding its purification the crude material production batches contain the compounds **II** (indanone), **III** (indanol) and *threo* isomer as impurities.

ICI 118551 has two chiral centres and thus its complete analytical profile must consider chiral separation of all four stereoisomers. The chiral discrimination of ICI 118551 is the subject of another paper [3].

Many papers regarding the activity of ICI 118551 as β_2 blocker can be found in the literature, but at the moment no articles have been published about the determination of this compound and its impurities. Only two papers on solid-phase extraction [4] and TLC separation [5] of basic drugs refer to the chromatographic behaviour of ICI 118551.

Therefore a suitable RP-HPLC method with diode array detection is proposed for the analysis of ICI 118551 and related substances: the *threo* isomer, indanone and indanol, which can be present in bulk material as impurities. The suitability of the use of a fluorescence detector for ICI 118551 quality control also has been investigated.

Experimental

Apparatus

A Waters high-performance liquid chromatograph Model 6000 A (Waters, Milford, MA, USA), alternatively equipped with a diode array detector HP 1040M or a programmable fluorescence detector HP 1046A, controlled by a computer HP 9000 Model 310 (Hewlett–Packard, Washington, USA), was used in the investigation. Standard and sample solutions were injected via a Rheodyne Model 7125 valve using a 6 μl sample loop.

Reagent and chemicals

Pure standard of ICI 118551 and related compounds, ICI 118551 raw material were kindly supplied by ICI-Pharma, Pharmaceutical Division of ICI Italia S.p.A. (Milan,

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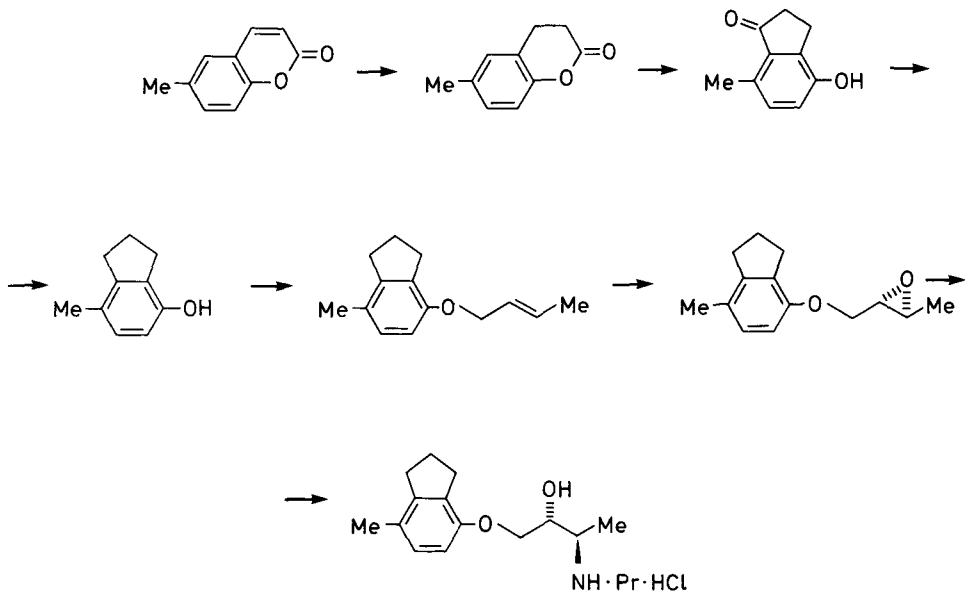
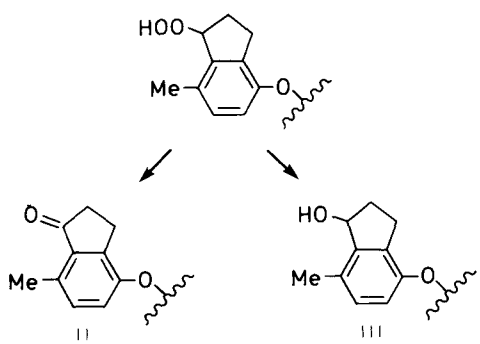


I

Scheme 1



Scheme 2

Italy). All other chemicals and solvents, were obtained from Merck (Darmstadt, Germany) and were of analytical or HPLC grade and were used without further purification.

Chromatographic conditions

The chromatographic separation of ICI 118551 and its impurities was achieved by means of a 5 μm Hypersil ODS (100 × 4.6 mm i.d.) column. The eluent was methanol–water (9:1, v/v) with 0.005 M sodium heptanesulphonate, as ion-pair reagent (pH 3.5 with acetic acid) at a flow rate of 1 ml min^{-1}. UV detection was carried out at 273 nm for ICI 18551 and indanol, and 250 nm for the indanone. The fluorescence parameters for the ICI 18551 and indanol determination were 270 nm (excitation), 297 nm (emission).

Procedure

Standard solutions. (a) 40 mg of ICI 118551 standard were weighed exactly, transferred to a 10 ml volumetric flask and diluted to volume with methanol. Separate volumes of 2 ml were diluted to different volumes with methanol to obtain a range of calibration standards.

(b) 10 mg of each impurity was transferred to a 100 ml volumetric flask and made up to mark with methanol. Dilution of this solution enabled calibration standards for indanone and indanol to be prepared.

Qualitative and quantitative analysis

The qualitative analysis and quantitative determination of ICI 118551 and relative impurities was carried out on 50 mg of raw material weighed and diluted to different volumes for the analysis of active or related compounds.

The quantitative analyses of bulk material samples were carried out using the calibration curves previously constructed.

Results and Discussion

The chromatogram illlustrated in Fig. 1 shows that the chromatographic conditions enable the satisfactory resolution of ICI 118551 (peak 3), indanone (peak 2) and indanol (peak 1) — the last two being the main impurities of the drug. The *threo* isomer cannot be separated

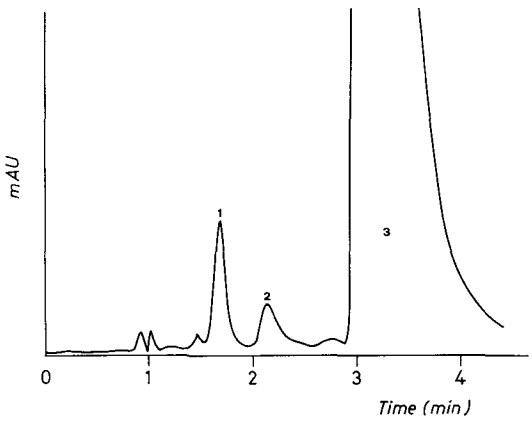


Figure 1
Chromatogram of ICI 118551 bulk material (peak 3) containing as impurities indanol (peak 1) and indanone (peak 2). Operating conditions are as given in the text.

from the *erythro* isomer with the chromatographic system used. However the discrimination of two diastereoisomers is possible if a chiral stationary phase (CSP) is used [3]. The use of a suitable CSP allows a partial discrimination of two diastereoisomers. In fact Fig. 2, obtained by the overlap of two chromatograms which refer to the separation of *erythro* and *threo* isomers, respectively, shows three well-separated peaks. Examination of the chromatogram reveals that while two enantiomers have the same retention time the others are well separated. The second peak (5.9 min) refers to the second separated enantiomer of the *erythro* isomer and the third one (7.8 min) is the second enantiomer of the *threo* isomer.

The purity control of chromatographic peaks showed that the peak at retention time of 1.8 related to two coeluted substances of which the main one is indanone (Fig. 3). The UV spectra of the unknown substance shows two absorbance maxima: one at the same λ value of indanone (250 nm) and the other one at 273 nm where the indanone shows a minimum absorbance value. However, the peak suppression method allows a correct determination of the impurity in question to be made.

Following a consideration of the UV spectra of the analytes (Fig. 4) the quantitative analysis of the active compound and indanol was performed at 273 nm, whilst the determination of indanone was carried out at 250 nm.

The determination of **I** and impurities **II** and **III**, was performed using an external standard method. The calibration curves of drug and impurities standards were found to be linear over the range 0.2–1 mg ml^{-1} for the ICI 118551, 0.001–0.05 mg ml^{-1} for indanone and 0.004–0.07 mg ml^{-1} for indanol. The correlation coefficients were 0.996, 0.996 and 0.980, respectively.

The analyses of bulk material samples showed the presence of indanone and indanol, but not *threo* isomer.

The use of the ion-pair reagent was suggested by the possibility of an ionic interaction between the basic drug (positively

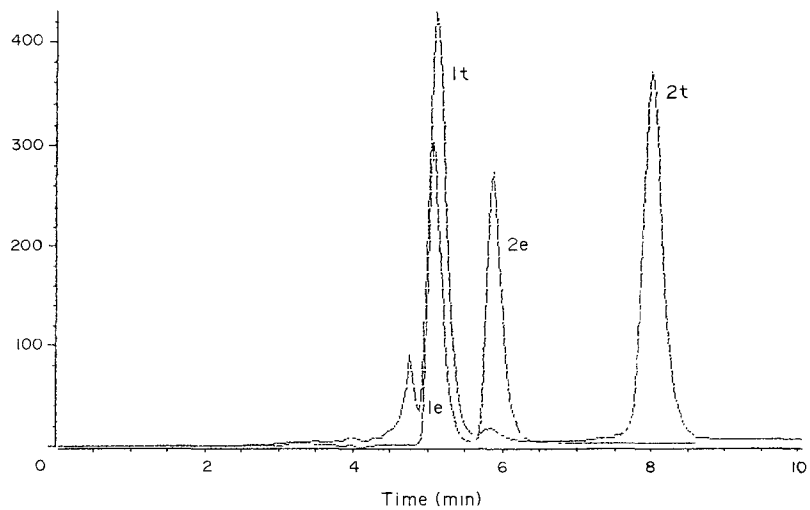


Figure 2
The overlapped chromatograms referred to enantiomer separation of *erythro* isomer (peaks 1e and 2e) and *threo* isomer (peaks 1t and 2t). Operating conditions as given in the text.

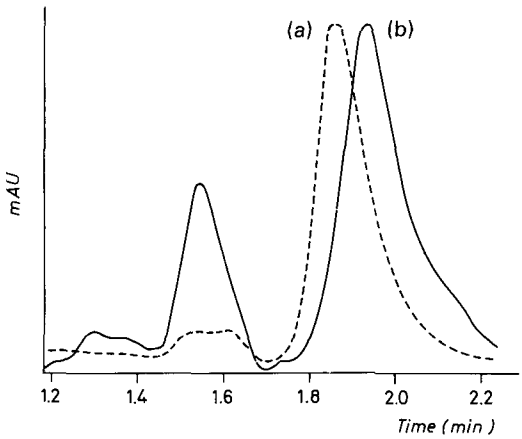


Figure 3
The normalized chromatograms of peak at the retention time 1.8 min. a, λ at 250 nm; b, λ at 273 nm. Operating conditions as given in the text.

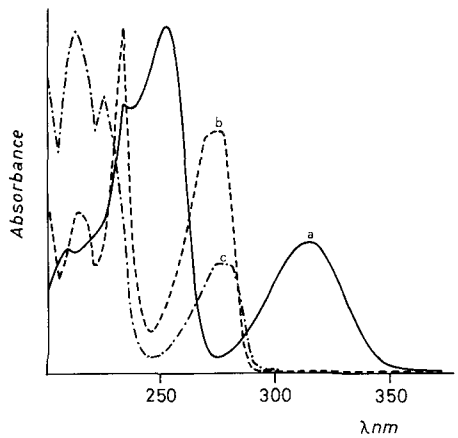


Figure 4
The UV spectra of β_2 blocker agent and its impurities. a, indanone; b, ICI 118551; c, indanol.

charged) and the few silanol groups (negatively charged) which can remain in the C18 phase.

The suitability of the proposed chromatographic method was checked using a working standard prepared by adding convenient amounts of standard compounds *threo* isomer, **II** and **III** to ICI 118551 standard.

While the minimum detectable amount of indanone was 4 ng, that of indanol was 18 ng. This different sensitivity was justified by absorbance value differences at the chosen wavelengths.

Since ICI 118551 and indanol are fluorescent molecules, they were also determined by using a fluorescence detector and the impurity minimum detectable amount obtained was compared to them with DAD. Undoubtedly the sensitivity of fluorescence detection is much higher than UV detection. The detection limit of indanol is about 1 ng, but the non-fluorescence of indanone suggested the need to use an RP-HPLC–DAD analysis which enables the determination of the active compound and both impurities.

The proposed method gives a good separation of ICI 118551 from its indanone and indanol impurities. Repeated analyses of the same samples confirmed the reproducibility, sensitivity and selectivity of the method.

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