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Short communication

High-performance liquid chromatographic determination of 1-benzyl-1*H*-indazol-3-ol in benzydamine in pharmaceutical formulations

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1. Introduction

Benzydamine hydrochloride, or *N*,*N*-dimethyl-3-[[1-(phenylmethyl)-1*H*-indazol-3-yl]oxy]-1-propanamine hydrochloride, is an indazole non-steroidal anti-inflammatory drug with specific action on local inflammatory processes [1,2], used both topically and systemically. The drug also possesses local anaesthetic and analgesic properties [3,4].

1-Benzyl-1*H*-indazol-3-ol, which is also one of the major metabolites of benzydamine [5], is a potential impurity of the drug, derived from its synthetic pathway [6]. Its formula is shown in Fig. 1.

So far, no method available for the determination of this substance in dosage forms containing benzydamine or in the bulk material has been described.

This paper describes a high-performance liquid chromatographic method for the determination of 1-benzyl-1*H*-indazol-3-ol whether in the bulk material or in dosage forms. This method is rapid and specific for 1-benzyl-1*H*-indazol-3-ol in the presence of benzydamine and excipients, and provides accurate and precise results.

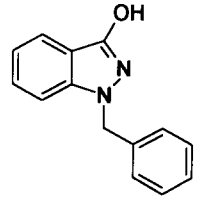

Fig. 1. Chemical structure of 1-benzyl-1*H*-indazol-3-ol.

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2. Experimental

2.1. Chromatographic system and conditions

The liquid chromatograph comprised a model 510 pump and a tunable absorbance variable-wavelength model 486 detector (Waters Assoc., Milford, MA, USA) set at 311 nm. Samples were injected using a model 7125 injector equipped with a 20 μl loop (Rheodyne, Cotati, CA, USA), and chromatograms were recorded and integrated with use of a model HP-3396-II instrument (Hewlett-Packard, Rome, Italy). Separations were carried out on a 250 mm × 4.6 mm i.d. column packed with 5 μm Hypersil ODS (Violet, Rome, Italy). The mobile phase consisted of methanol–0.01 M acetic acid (pH 3.5) (65:35, v/v), filtered under vacuum before use through a 0.2 μm membrane filter (Nucleopore). The flow rate was 1.5 ml min $^{-1}$.

2.2. Chemicals and reagents

Benzydamine hydrochloride and 1-benzyl-1*H*-indazol-3-ol were kindly supplied by Angelini Farmaceutici (Ancona, Italy), and 2-[4-(2′-furoyl)phenyl]propionic acid (internal standard) was supplied by the Department of Pharmacology of the University of L'Aquila. Methanol was HPLC grade (Fluka Chemika–BioChemika, Buchs, Switzerland). Acetic acid was analytical grade (Farmitalia–Carlo Erba, Milan, Italy). Water was purified and deionized using a Milli-Q ion exchange filtration system (Millipore, Bedford, MA, USA). The water was filtered through WCN 0.45 μm filters, while methanol was filtered through WTP 0.5 μm filters (Whatman, Maidstone, UK).

2.3. Standard solutions

A stock solution of the internal standard was prepared by dissolving 20 mg of compound in 100 ml of methanol. Standard solutions were prepared in methanol by varying the concentration of 1-benzyl-1*H*-indazol-3-ol in the range 0.1–1.0 μg ml^{-1}, and maintaining the concentration of benzydamine hydrochloride at a constant level of 150 μg ml^{-1}; an aliquot (30 μl) of the internal standard stock solution was added.

2.4. Sample preparation

Bulk material equivalent to about 150 mg was accurately weighed, transferred to a 100 ml volumetric flask, and the flask was then filled to volume with methanol. After filtration, if necessary, 1 ml of this solution was transferred to a 10 ml volumetric flask, spiked with the internal standard solution (30 μl), and the flask was filled to volume with methanol. A 20 μl portion was then analyzed by HPLC by using the calibration curve.

A 1 ml portion of the pharmaceutical formulation (collutory, vaginal washing or spray) was transferred to a 10 ml volumetric flask, spiked with the internal standard solution (30 μl) and the flask was filled to volume with methanol. A 20 μl portion was then analyzed by HPLC using the calibration curve.

3. Results and discussion

The essential criteria that the developed method was required to meet were that it should be suitable for a wide variety of dosage forms, that it should be free from interference from excipients, and that it should be able to indicate the stability. No major interference from any excipients or extraction solvents was observed. Peak shapes and areas were not influenced by the various proportions of the organic solvents used for extraction. Excipients carried through the extraction procedure were not detected or gave peaks at or near the solvent front and so did not interfere with the analysis.

Fig. 2 shows a typical HPLC chromatogram of a sample solution containing benzydamine hydrochloride with its impurity 1-benzyl-1*H*-indazol-3-ol and the internal standard. The retention times of 1-benzyl-1*H*-indazol-3- ol and the internal standard were 4.2 min and 2.7 min, respectively. The benzydamine hydrochloride peak does not influence the determination, because the substance is eluted at the column dead-time. The

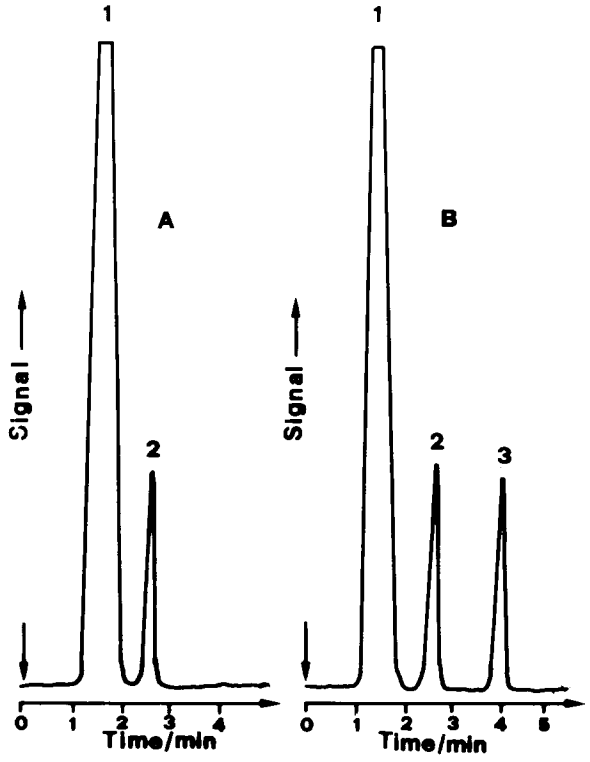

Fig. 2. Chromatograms of 1-benzyl-1*H*-indazol-3-ol in a collutory. (A) Sample solution containing benzydamine hydrochloride (150 μg ml^{-1}) spiked with internal standard (0.6 μg ml^{-1}). (B) Sample solution containing benzydamine hydrochloride (150 μg ml^{-1}) spiked with internal standard (0.6 μg ml^{-1}) and 1-benzyl-1*H*-indazol-3-ol (1 μg ml^{-1}). Peak 1, benzydamine hydrochloride; peak 2, internal standard; peak 3, 1-benzyl-1*H*-indazol-3-ol.

calibration curve was obtained by plotting the peak-area ratio of 1-benzyl-1*H*-indazol-3-ol to internal standard against its concentration.

The equation, obtained through regressional analysis of the data for ten standard solutions (each datum represented the average of a minimum number of five determinations), was $y = 219 + 2.09 \times 10^4 x$ with a correlation coefficient $r = 0.9997$, where y is the peak-area ratio in the arbitrary units of the HP-3396-II system used, and x is the 1-benzyl-1*H*-indazol-3-ol concentration (μg ml^{-1}). The mean relative standard deviation was less than 2%.

The detection limit of 1-benzyl-1*H*-indazol-3-ol, defined as three times the level of the baseline noise, was 50 ng ml^{-1}. The assay validation was carried out by adding known amounts of 1-benzyl-1*H*-indazol-3-ol to pharmaceutical formulations that had been proved to be thoroughly free of this impurity. These results are summarized in Table 1.

Table 1
Assay validation with pharmaceutical formulations free of impurity

Formulation	Benzydamine content (mg ml^{-1})	Bl[a] added (μg ml^{-1})	Bl[a] found (μg ml^{-1})
Collutory I	1.5	10.0	10.0
Collutory II	1.5	10.0	9.6
Vaginal washing I	1.0	8.0	8.2
Vaginal washing II	1.0	9.0	8.9
Spray	1.5	8.0	8.2

[a]Bl, 1-benzyl-1*H*-indazol-3-ol. The data are the average of five determinations for each sample. Relative standard deviation, approximately 1.5%.

The reported method for the quantitation of the impurity in the bulk material or in pharmaceutical formulations is very simple and rapid, and gives accurate and precise results.

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