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Short Communication

A study of the photodegradation of benzydamine in pharmaceutical formulations using HPLC with diode array detection

JIAN WANG and DOUGLAS E. MOORE*

Department of Pharmacy, The University of Sydney, Sydney 2006, Australia

Keywords: *Benzydamine; reversed-phase liquid chromatography; pharmaceutical formulations; photodegradation; stability-indicating assay.*

Introduction

Benzydamine is a tertiary amine indazole derivative (Fig. 1) which is a unique non-steroidal anti-inflammatory drug (NSAID) with local anaesthetic and analgesic properties [1, 2]. It has been shown to have beneficial effects after both topical and systemic administration by acting locally to inhibit the response to, and release of, inflammatory mediators [3]. Benzydamine is predominantly used in the form of a cream or gel containing 3% (w/w) of benzydamine hydrochloride for the relief of symptoms associated with inflammatory conditions of the musculo-skeletal system. A clear green mouthwash containing 0.15% (w/w) of benzydamine hydrochloride is also available for the relief of painful conditions of the mouth and throat.

Although no reports have indicated that benzydamine is unstable, some cases of photodermatitis associated with the use of the topical formulation have been reported [4–7]. This fact suggests that there is an action of sunlight on benzydamine leading to the initiation of a phototoxic response. Since one of the possible mechanisms of this adverse response involves the formation of toxic photoproducts [8], it is relevant to study the stability of benzydamine in its various formulations when exposed to sunlight.

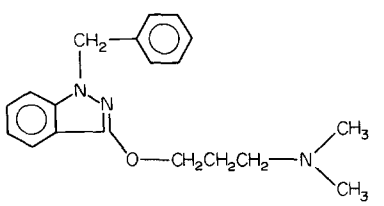


Figure 1
Benzydamine: 1-benzyl-3-[3-(dimethylamino)propoxy]-1H-indazole.

Previously published methods for the analysis of benzydamine in biological fluids and topical pharmaceutical preparations have involved the use of [^{14}C]-benzydamine and liquid scintillation counting [9] or high-performance liquid chromatography (HPLC) with either ultraviolet (UV) [10] or fluorescence detection [11]. However, no study has examined the possibility of benzydamine degradation. Preliminary experiments in the authors' laboratories showed benzydamine to be thermostable in aqueous solution at temperatures up to 70°C for 2 h, but photodegradation was detected within 5 min on exposure to UV light. The present paper reports an HPLC method which is capable of monitoring the stability of benzydamine in formulations when subjected to irradiation by sunlight or UV light and of detecting the photodegradation products.

Experimental

Reagents and materials

Benzydamine hydrochloride (pure substance), Difflam Cream, Difflam Gel and

*Author to whom correspondence should be addressed.

Difflam Mouthwash were generously provided by Riker Laboratories-3M Australia Pty Ltd. Water used in the preparation of solutions was double-distilled from an all-glass apparatus. All other chemicals and solvents were of analytical reagent grade (Ajax Chemicals, Sydney, Australia).

Apparatus and methods

Chromatography. The HPLC system comprised an LKB model 2150 HPLC pump and an SSI injector with a 10-μl loop. Detection was performed with a Spectra Physics model 100 variable wavelength detector or with a Hewlett–Packard model 1040A diode-array spectrophotometric detector. The eluent was monitored at 230 nm. Chromatograms were recorded on a Spectra Physics Data Jet integrator. Separation was achieved using a 100 × 4.6 mm i.d. column packed with 5-μm Merck Lichrospher 60 RP-Select B with a mobile phase of 0.05 M ammonium acetate (pH 7.0)–acetonitrile (45:55, v/v) at a flow rate of 0.9 ml min^{-1}. The mobile phase was prepared daily from double-distilled water and spectroscopically pure solvents, filtered through a 0.45-μm Millipore filter and degassed under vacuum for 10 min before use.

Spectroscopy. Ultraviolet absorption spectra were recorded at various times during irradiation with a Perkin–Elmer Lambda 5 spectrophotometer. The UV spectra of gel and cream formulations were measured in a cuvette of 0.1-mm pathlength (Starna, Sydney).

Irradiation. Laboratory UV irradiation was performed using a Hanovia 100 W medium-pressure mercury lamp and the apparatus previously described [8]. The cylindrical Pyrex glass reaction vessel (30 ml) was placed 2 cm from the arc lamp, such that the UVA irradiance at its plane surface was 100 W m^{-2} at 365 nm measured with an International Light 1500 Radiometer. The entire apparatus was immersed in a water bath maintained at 30.0 ± 0.5°C. After appropriate time intervals samples of the irradiated solution were taken and analysed immediately by HPLC.

For irradiation by sunlight, samples were exposed to direct spring sunshine in Sydney between the hours of 10 am and 3 pm.

Sample preparation.

Drug substance. An accurately weighed amount of benzydamine hydrochloride was dissolved in water to obtain a 10^{-3} M stock solution. The stock solution was protected from light at all times and no degradation occurred during 1 month. For each experiment a 10-ml aliquot of the stock solution was diluted to 100 ml. The resulting 10^{-4} M solution was irradiated by the UV lamp.

Cream and gel formulations. A fixed amount (57.5 mg) of gel or cream was applied to glass slides (7.5 × 25 mm) in a smooth layer about 1-mm thick. Some samples were exposed to the UV lamp and some to direct sunlight for different time intervals. After irradiation, the samples were dissolved completely in 10 ml of acetonitrile–water (1:1, v/v) for the gel or isopropanol–tetrahydrofuran (3:2, v/v) for the cream, before injection into the chromatograph.

Mouthwash. The mouthwash solution was placed in a silica cuvette and exposed to the UV lamp or to direct sunlight. Samples were injected directly into the chromatograph.

Results and Discussion

Basic drugs often show poor resolution with considerable peak tailing in reversed-phase HPLC [12]. The addition of an ion-pair reagent, such as heptane sulphonate, or a competing amine, such as *N,N*-dimethyloctylamine, can usually overcome this problem. In degradation studies this raises a difficulty in relation to the identification of the product by combined LC–MS as the reagent will interfere with sample vapourization in the MS. The HPLC system described here was designed to overcome that difficulty and can be used directly in LC–MS for the purpose of identifying the products. The critical requirement for the stationary phase is that the packing be completely end-capped to avoid acid–base interactions. The composition of the mobile phase was varied to enhance the resolution of photoproducts eluting both before and after benzydamine.

Benzydamine hydrochloride at a concentration of 10^{-4} M in water and stored in the dark for 1 week displayed no change in its UV absorption spectrum and only one peak in HPLC analysis. Upon exposure to UV light, the UV spectrum showed a steady broadening of the spectrum with a loss of peak definition at

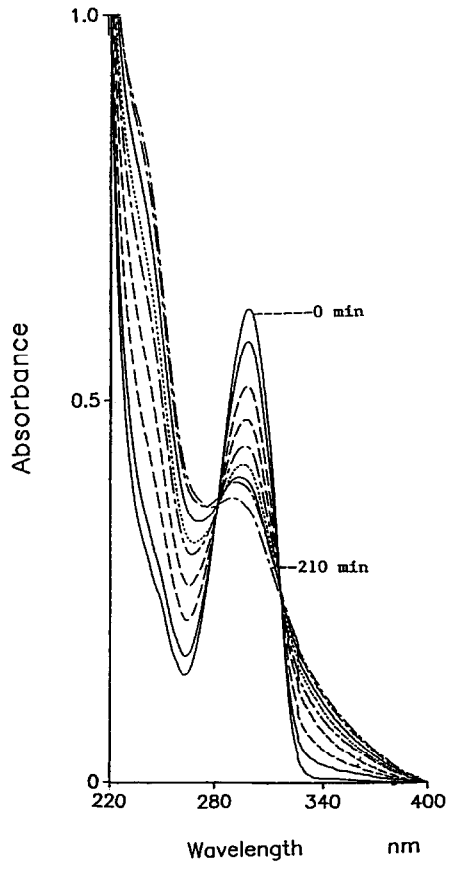
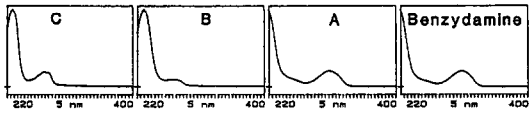


Figure 2
UV absorption spectra of UV irradiated benzydamine hydrochloride solution (5×10^{-5} M in water) taken at 30 min intervals up to 210 min.



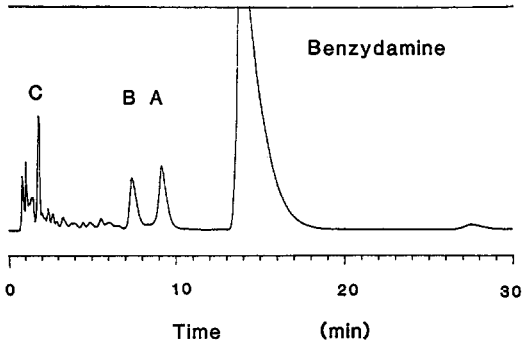


Figure 3
Chromatogram of benzydamine hydrochloride solution (10^{-4} M in water) after exposure to the UV lamp for 1 h. For chromatographic conditions see text.

306 nm (Fig. 2). Figure 3 illustrates a typical chromatogram of 10^{-4} M benzydamine hydrochloride in water before and after exposure to the UV lamp for 1 h. The HPLC profile indicates a complex degradation pathway with at least five degradation products. After irradiation for 1 h, 20% of the benzydamine was photolysed. The pH of the solution after this time of irradiation had shifted from 6.5 to 5. The three main product peaks (labelled A, B and C) were all eluted from the reversed-phase column before benzydamine, suggesting the presence of one or more additional hydrophilic groups. Figure 4 shows the time course of the relative peak areas of the three main photoproducts and the parent drug. Peak A reached a maximum after irradiation for 3 h and then underwent a slow decrease, indicating that the product generated from the primary reaction was subject to secondary breakdown.

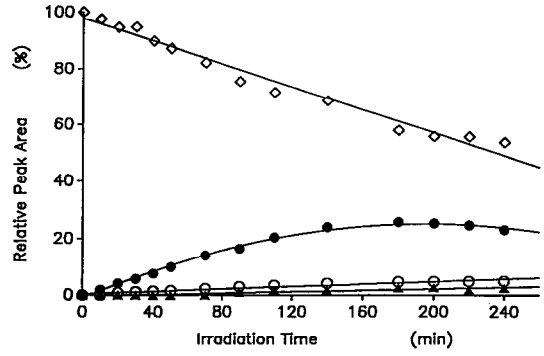


Figure 4
Relative peak area as a function of time of irradiation for benzydamine and the principal products following irradiation of benzydamine hydrochloride solution (10^{-4} M in water). Key: ◇, Benzydamine; ●, Product A; ○, Product B; ▲, Product C.

UV absorption spectra of the product peaks were obtained by means of the diode-array spectrophotometric detector and are shown in Fig. 3. Peak purity analysis clearly showed that each peak, including that of the parent drug, was spectroscopically homogeneous. Compound A has a maximum absorbance at 308 nm. Compounds B and C both have a maximum absorption wavelength of 230 nm making this the most appropriate wavelength for detection of degradation. Compound A has the same absorption bands as the parent drug but with different relative intensities, indicating the closeness of their structures, whereas in compounds B and C the chromophore has been changed significantly. The identities of

the products have not yet been established; consequently their molar absorptivities are not known and the amounts of each of the products cannot be calculated.

Nevertheless, the sensitivity of the assay can be stated in terms of the smallest peak that can be detected under a specified condition. For a solution of initial concentration 10^{-4} M benzydamine (35 μg ml^{-1}), the photodegradation products could be detected when their peak area individually represented 0.015% of the benzydamine peak area in the chromatogram recorded at 230 nm.

Benzydamine is commercially available in a variety of formulations. The photolability of benzydamine in Difflam Gel, Cream and Mouthwash (Riker-3M Australia) was examined. The UV spectra of the three formulations are shown in Fig. 5. The gel and cream were measured without dilution in a cuvette of 0.1-mm pathlength; the mouthwash was diluted 1 in 10 with water without affecting the shape of the spectrum. The maximum wavelengths of the three formulations are slightly different owing to the different matrix or solvent effects but it is clear that the major absorption above 300 nm is due to benzydamine. Representative chromatograms of degraded benzydamine formulations are given in Fig. 6. Clearly the principal reaction in the formulations is the photodegradation of benzydamine. When the gel and cream were spread as thin films on glass slides, they were exposed to the direct output of the mercury arc lamp (including 254 nm short wavelength UV light) and the degradation was detected within

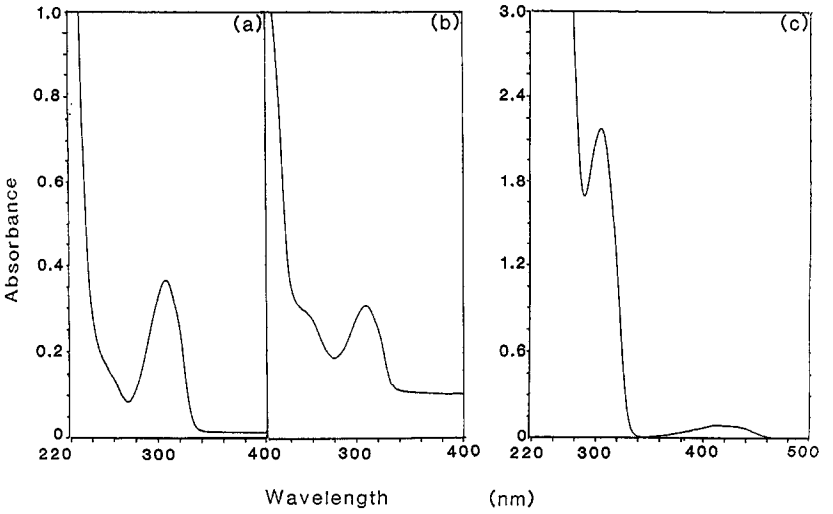


Figure 5
UV spectra of (a) Difflam Gel; (b) Cream; (c) Mouthwash. The gel and cream were measured in a cuvette of 0.1-mm pathlength and the mouthwash was diluted 10-fold with water and measured in a 10-mm cuvette.

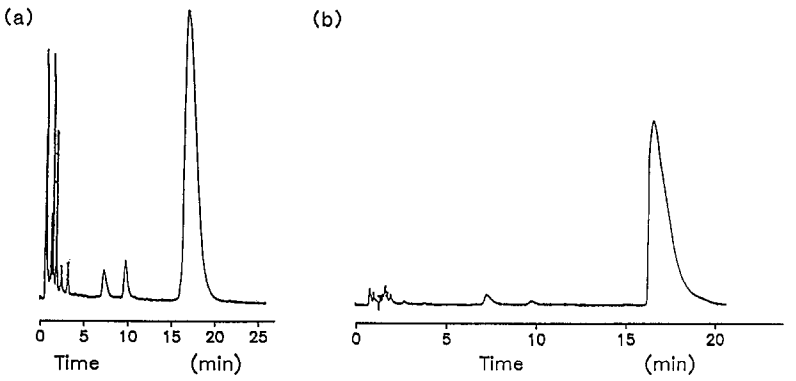


Figure 6
(a) Chromatogram of Difflam Mouthwash after irradiation by the UV lamp for 1 h. (b) Chromatogram of Difflam Cream after irradiation by sunlight for 1 h.

5 min of irradiation. When similar samples were exposed to direct sunlight ($\lambda > 290$ nm) a similar degradation profile was produced, but on a slower time scale. The mouthwash was contained within a silica cuvette when irradiated by UV or sunlight and therefore experienced similar wavelength conditions to the other formulations.

The comparative rates of the degradation process for the formulations under UV and sunlight are shown by semi-logarithmic plots in Fig. 7. The benzydamine in the gel decomposed faster than that in the cream and mouthwash by both methods of irradiation. Since both contained the same concentration of benzydamine (3%), the difference may be due to the opacity of the cream which does not allow as much light absorption by benzydamine as does the gel. However there may also be effects related to the different reactivity of the cream or gel constituents. The slower rate for the mouthwash solution (0.15% benzydamine) could be due to the photoreaction being slower in the aqueous medium. In each formulation, the concentration of benzydamine is high enough to absorb all of the incident radiation within the appropriate 306 nm range.

The principal degradation products from all formulations were found to be similar to those from benzydamine solution because the same retention times and spectra were observed in the chromatograms. In the spectrum of the mouthwash, there is an additional absorption with a maximum of 418 nm. This absorption is due to the colouring agents used in the mouthwash, a mixture of patent blue (0.003 mg ml^{-1}) and quinoline yellow (0.02 mg ml^{-1}) which have absorption spectra in the visible region. However, when the mouthwash was irradiated under the same lamp but with a yellow glass filter that cut off wavelengths below 400 nm, no decomposition was observed; this indicated that the colouring agent did not photosensitize the degradation of benzydamine although the colour did bleach.

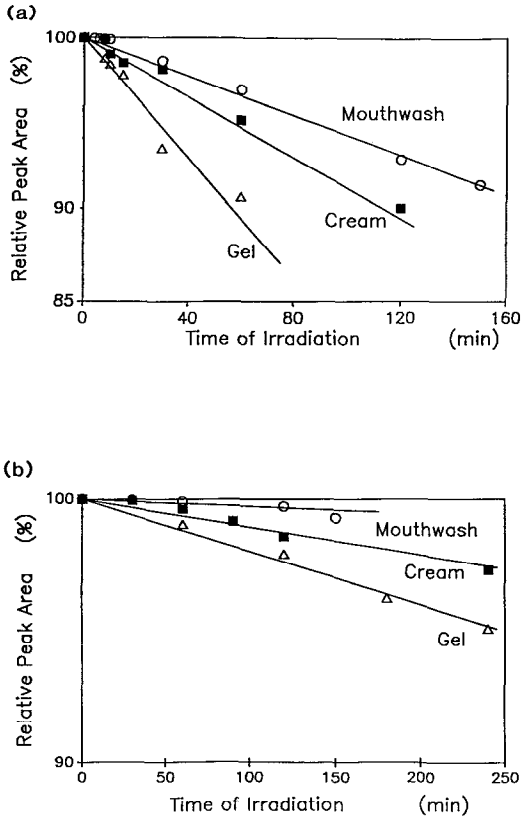


Figure 7
(a) Semi-logarithmic plot of relative peak area of benzydamine as a function of time of irradiation by UV lamp for three formulations. (b) Semi-logarithmic plot of relative peak area of benzydamine as a function of time of irradiation by sunlight for three formulations.

Conclusions

The results of these studies show that benzydamine is unstable under UV light. Any wavelength range that overlaps the absorption spectrum of benzydamine is capable of inducing photodegradation, as demonstrated by the initial experiments in which benzydamine solution was irradiated in a glass vessel with an effective wavelength cut-off of 310 nm. The irradiation conditions used in the experiments are far more severe than those likely to be encountered in everyday use. Nonetheless, they serve as an accelerated test to illustrate that photodegradation of benzydamine formulations will occur when exposed to sunlight under inappropriate storage conditions. The HPLC assay described here is capable of detecting photodegradation of the drug at its very early stages. Work is continuing on the identification of the photodegradation products of benzydamine.

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