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Note

High-performance liquid chromatography assay for the measurement of benzydamine hydrochloride in topical pharmaceutical preparations

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Benzydamine or N,N-dimethyl-3-{[1-(phenylmethyl)-1H-indazol-3-yl]oxy}-1-propanamine is a non-steroidal anti-inflammatory drug. Available data suggest that benzydamine interferes selectively with the localized phenomena of inflammation by stabilizing the cellular membrane and selectively inhibiting prostaglandin systems[1]. Benzydamine has been shown to be active after both topical[2,3] and systemic administration[4], and to be relatively devoid of both local and systemic side effects[5]. It has been reported that a high concentration of benzydamine is maintained in inflamed tissues for a much longer period of time when the drug is administered topically as opposed to orally[6]. In one study it was shown that 6 h after topical application, the concentration of benzydamine in inflamed tissues was approximately four times higher than the concentration observed 6 h after oral administration[6].

Due to these properties, benzydamine is widely used in the form of Difflam® cream [3% (w/w) benzydamine hydrochloride] for the relief of symptoms associated with painful inflammatory conditions of the musculo-skeletal system. A gel preparation, containing 3% benzydamine hydrochloride has also been formulated for specific purposes (*e.g.* use with ultrasound), but is not yet commercially available.

Few methods have been described for the measurement of benzydamine. Assays reported involve ^{14}C-labelled drug[3], fluorescence[7] and high-performance liquid chromatography (HPLC) with fluorescence detection[8].

In order to determine benzydamine concentration, within both the gel and cream formulations, a new analytical method based on HPLC with UV detection was devised. This procedure provides a simple, quick, reproducible method of quantification of benzydamine.

EXPERIMENTAL

Apparatus

The HPLC equipment used consisted of an Altex 110A pump and Kontron MS1660 Autosampler with a Spectroflow 773 variable-wavelength UV detector set to 305 nm (the measured λ_{max} of benzydamine hydrochloride in mobile phase). Chromatograms and peak area values were recorded on a Shimadzu Chromatopac C-R3A recording data processor. The column used was a Novapak C_{18} (5-μm particle size,

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150 × 3.9 mm I.D.; Waters). Both the analytical column and the guard column (5 cm) packed with Pellicular ODS C_{18} (Millipore) were enclosed in a block heater (Anachem) and maintained at 30°C.

Mobile phase

The mobile phase was acetonitrile–water–acetic acid (62:37.5:0.5, v/v) and contained 5 m*M* sodium dodecylsulphate. Incorporation of the acetic acid maintained the mobile phase pH at 4.00. The mobile phase was filtered through a 0.45-μm HA membrane filter (Millipore) and thoroughly degassed in an ultrasonic bath prior to use. The flow-rate of the mobile phase was 0.9 ml/min.

Internal standard

Indomethacin was used as the internal standard for the assay and peak area ratios were utilised in preparation of calibration curves and in determination of unknown benzydamine concentrations.

Sample preparation

Dissolution of benzydamine gel samples was carried out using a solvent mixture composed of acetonitrile–water (1:1, v/v). Aliquots of gel were dissolved in 10 ml of the solvent by vortexing for 30 s, and injected directly onto the column. Indomethacin solution (0.1 ml of 1 mg/ml) was added to each of the 10-ml samples prior to vortexing.

Dissolution of Difflam cream was carried out in a solvent mixture consisting of tetrahydrofuran–isopropanol (30:60). Difflam cream samples were weighed into centrifuge tubes, and dissolved in 10 ml of the solvent mixture by vortexing for 5 min. All samples were centrifuged (1100 *g*, 15 min) prior to injection directly onto the column. Indomethacin solution (0.1 ml of 1 mg/ml) was added to each of the 10-ml samples prior to vortexing. An injection volume of 20 μl was used at all times.

Calibration graph

The normal quantity of gel or cream used in preparing the 10 ml of solvent mixture for injection on to the HPLC system was 200 mg. Since a range of gels or creams containing different concentrations of benzydamine were not available and since no blank gel or cream was available, calibration graphs were prepared after dissolving a range of amounts of the product in 10 ml of the appropriate solvent mixture.

RESULTS AND DISCUSSION

Reversed-phase HPLC with UV detection was an effective method for quantifying benzydamine in both cream and gel formulations. Typical chromatograms obtained from gel formulation and cream formulation are shown in Figs. 1 and 2 respectively.

Under the assay conditions described, the indomethacin internal standard and benzydamine had elution times of 3.2 and 5.0 min respectively.

A calibration curve was prepared for benzydamine gel using data points over a concentration range of 0.625 to 20 mg gel/ml of solvent (equivalent to 18.75–600

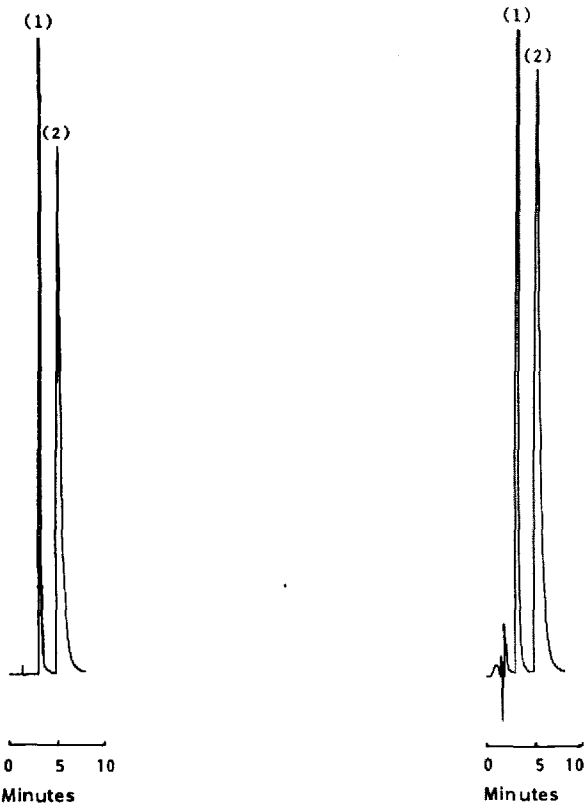

Fig. 1. Chromatogram of benzydamine gel: indomethacin internal standard (1) and benzydamine (2). Correlation coefficient = 0.999 (concentration range 0.625–20 mg gel per ml of solvent equivalent to 18.75–600 μg benzydamine hydrochloride per ml of solvent). Coefficient of variation = 1.15% (ten injections of 20 mg gel per ml of solvent equivalent to 600 μg benzydamine hydrochloride per ml of solvent).

Fig. 2. Chromatogram of Difflam cream: indomethacin internal standard (1) and benzydamine (2). Correlation coefficient = 0.999 (concentration range 0.625–20 mg cream per ml of solvent equivalent to 18.75–600 μg benzydamine hydrochloride per ml of solvent. Coefficient of variation = 1.60% (ten injections of 20 mg cream per ml of solvent equivalent to 600 μg benzydamine hydrochloride per ml of solvent).

μg/ml benzydamine hydrochloride). The correlation coefficient for the curve was 0.999. The coefficient of variation for ten sample replicates (20 mg gel/ml of solvent equivalent to 600 μg benzydamine hydrochloride per ml of solvent) assayed as above was 1.15%.

A calibration curve was also prepared for Difflam cream using data points over a concentration range of 0.625 to 20 mg cream/ml of solvent (equivalent to 18.75–600 /ml benzydamine hydrochloride). The correlation coefficient for the curve was 0.999. The coefficient of variation for ten sample replicates (20 mg cream/ml of solvent equivalent to 600 μg benzydamine hydrochloride per ml of solvent) assayed as above was 1.60%.

Using this method we have analysed the percutaneous absorption of benzyd-

amine from the gel preparation, in order to investigate the effectiveness of phonophoresis *i.e.* movement of drugs through intact skin into soft tissue by ultrasonic perturbation[9], in a double-blind placebo controlled clinical trial in ten healthy volunteers. The surface recovery method, which involves determination of the loss of drug from the vehicle as it penetrates into the skin, was used. The results obtained for percutaneous absorption of benzydamine (expressed as a percentage of the amount applied) following treatment with a range of continuous ultrasound frequencies (0.75 MHz, 1.5 MHz and 3.0 MHz) at an intensity of 1.5 W cm^{-2} for 5 min are given in Table I. A placebo control involving massage of the applied benzydamine gel without ultrasound for 5 min was included in the protocol. Statistical comparison of the results (analysis of variance) showed that there were no significant differences ($P > 0.05$) in absorption between control data and those using ultrasound at the frequency–intensity combinations noted.

TABLE I

COMPARISON OF BENZYDAMINE ABSORBED FOLLOWING TREATMENT WITH CONTINUOUS OUTPUT ULTRASOUND (INTENSITY 1.5 W cm^{-2} AT DIFFERENT FREQUENCIES)

S.E.M = Standard error of the mean. Ten determinations.

Ultrasound frequency (MHz)	*Benzydamine absorbed (%) ± S.E.M.*
0	24.33 ± 1.75
0.75	23.69 ± 1.94
1.5	24.97 ± 2.05
3.0	24.60 ± 1.25

CONCLUSION

A simple and rapid technique for the determination of benzydamine hydrochloride in topical formulations has been described. The use of direct injection and UV detection enables a limit of detection of 0.1 mg gel/ml of solvent (equivalent to 3 μg benzydamine hydrochloride per ml of solvent) and 0.26 mg cream/ml of solvent (equivalent to 7.8 μg benzydamine hydrochloride per ml of solvent). The method would be suitable for quality control of benzydamine content of topical formulations and also for studies on the percutaneous absorption of the drug. We are currently using this assay for further investigation of the influence of ultrasound on the percutaneous absorption of benzydamine both *in vitro* and *in vivo*.

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