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High-performance liquid chromatographic determination of dyclonine hydrochloride in the presence of its degradation products

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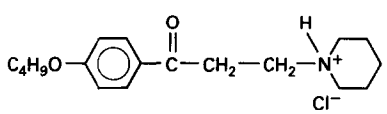
Abstract: A stability-indicating reversed-phase high-performance liquid chromatographic method for the determination of the topical anaesthetic dicyclonine hydrochloride in various oral spray and gel formulations is described.

Keywords: *Reversed-phase high-performance liquid chromatography; dyclonine hydrochloride; topical anaesthetic; stability indicating method of analysis.*

Introduction

Dyclonine hydrochloride, 4′-butoxy-3-piperidinopropiophenone hydrochloride, is a local anaesthetic [1] that is currently marketed in various pharmaceutical dosage forms including aqueous solution, gel, and a lozenge. The superiority of dyclonine hydrochloride as a local anaesthetic has been well documented and appears to be the most effective "non-caine" type of local anaesthetic that is free from acute toxicity [2, 3].



Dyclonine hydrochloride has been analysed using a lengthy, tedious and non-specific method, involving a chloroform extraction, evaporation, and non-aqueous titration of the dried residue in glacial acetic acid with perchloric acid [4]. A general method of analysis for local anaesthetics using gas chromatography has been described [5] but was deemed to be unsuitable for dyclonine because it would require an extraction which could affect its stability.

Three HPLC methods for the analysis of dyclonine hydrochloride have been reported. Gill *et al.* [6] described the HPLC analysis of dyclonine hydrochloride in biological fluids

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with a custom packed 5-μm ODS-Hypersil column and a mobile phase consisting of 50% methanol in a 73.7 mM, pH 2.8, *n*-hexylamine-orthophosphoric acid buffer. The current edition of USP (USP XXI) [7] reports a non-stability-indicating HPLC analysis for dyclonine hydrochloride in a gel and in a topical solution, with a 5-μm C3-bonded packing and a mobile phase consisting of 60% acetonitrile in 1.5 mM, pH 3.0, potassium phosphate–heptylamine buffer. In order to properly evaluate the stability of a pharmaceutical form, a method that separates degradation products from pure substance is preferred with readily available packed commercial columns. The C-3 packing is of limited availability. A commercially packed C-18 column (Radial-pak, 10 μm 4 mm × 8 cm, Waters Assoc., Milford, MA, USA) was initially evaluated. However, because this type of column is not end-capped, dyclonine was found to be strongly adsorbed to the stationary support and required a mobile phase consisting of 80% tetrahydrofuran in water to achieve reasonable retention times. More recently, Palermo [8] described a method for the analysis of dyclonine and its degradation products. However, this method requires a sample clean-up step with a Florisil column before conducting the HPLC analysis, as well as other precautions in preparing injection solutions and mobile phase in order to maintain precision.

This paper, therefore, reports an improved single step stability-indicating HPLC assay method with a phenyl-bonded column and a mobile phase consisting of acetonitrile–tetrahydrofuran–0.02 M, pH 6.0 ammonium acetate buffer (46:9:45%, v/v/v) for the analysis of dyclonine hydrochloride in pharmaceutical preparations.

Experimental

Reagents

All reagent grade chemicals and HPLC grade solvents were obtained from Fisher Scientific Co. (Fairlawn, NJ, USA). Silica chromatogram sheets were obtained from Eastman Kodak Co. (Rochester, NY, USA). Dyclonine hydrochloride was obtained from Ganes Chemical Co. (New York, USA).

Instrumentation

The HPLC system consisted of a Waters Model 6000A LC pump and Model U6K loop injector (Waters Associates, Milford, MA, USA), Model LC-55 variable wavelength UV detector (Perkin–Elmer, Norwalk, CT, USA), and Hewlett–Packard Model 3390A integrator-plotter (Hewlett–Packard, Avondale, PA, USA). The HPLC column was a chemically bonded phenyl column (μ-Bondapak Phenyl, 10 μm, 300 × 3.9 mm i.d., Waters Associates, Milford, MA, USA).

Mobile phase

The mobile phase consisted of acetonitrile–tetrahydrofuran–0.2 M, pH 6.0 ammonium acetate buffer (46:9:45%, v/v/v). The aqueous and organic solvents were filtered separately using HAWP and FHUP filters, respectively (Millipore Corp., Bedford, MA, USA), and then mixed. The mobile phase was degassed using a Model W-185 sonifier (Branson, Sonic Power Co., Danbury, CT, USA).

Chromatographic conditions

The column temperature was ambient and the detector was set at 282 nm, the wavelength of maximum absorbance for dyclonine hydrochloride. The integrator was set

at an attenuation of 5 (0.64 AUFS) and threshold of 3 with a chart speed of 0.5 cm min^{-1}. The injection volume was 20 μl and the mobile phase flow rate was 2.0 ml min^{-1}.

Preparation of standard and assay solutions

A standard solution of dyclonine hydrochloride was prepared in distilled water to give a concentration of 0.5 mg ml^{-1}. Fresh aqueous solutions of dyclonine hydrochloride containing 49% acetonitrile and 21% tetrahydrofuran were prepared from this standard solution to give concentrations of 80, 120, 180, 200 and 360 μg ml^{-1}. The standard solutions were injected in triplicate and the detector response was measured as peak area using an integrator-plotter. The peak area was regressed versus concentration to obtain a calibration plot for standard dyclonine hydrochloride solutions.

Sample solutions were prepared for analysis by diluting 1.0 ml of the sample in a 25-ml volumetric flask using 18 ml of a mixture of 30% tetrahydrofuran in acetonitrile. A sufficient quantity of ammonium hydroxide solution (1.0 M) was added to pH 6.0, and the final volume was adjusted to 25.0 ml with 0.1 M, pH 6.0 ammonium acetate buffer. The samples were injected immediately after preparation.

Degradation of dyclonine hydrochloride

Dyclonine hydrochloride solution (40 ml, 0.5% w/v) was degraded at pH 3.5 and temperature of 55°C for a 6-month time period [9]. The degradation products separated as oily droplets which were redissolved with 90 ml of a 6:1 mixture of acetonitrile and tetrahydrofuran. The solution was then evaporated under a stream of nitrogen at a temperature not exceeding 40°C to a semi-solid residue. This residue was dissolved in 5 ml of chloroform and washed with 5 ml of 0.1 N hydrochloric acid. The chloroform fraction was recovered, dried, and chromatographed on a silica gel column, prepared with 60 g of silicic acid dispersed in chloroform in a 4 × 60 cm glass tube. The degradation products were eluted with chloroform (0.2 ml min^{-1}) to obtain a mixture free of dyclonine, as confirmed by TLC on silica chromatogram sheets with 2% methanol in chloroform as the developing solvent. No spot corresponding to the F_f value of dyclonine was observed.

Preparation of degradation product spiked standard

Table 1 shows the concentrations and the mean peak areas of dyclonine hydrochloride that were spiked with the degradation product.

Table 1
Effect of the addition of dyclonine degradation products on the linearity response for dyclonine hydrochloride

Concentration of dyclonine hydrochloride added (μg ml^{-1})	Concentration of degradation mixture added (μg ml^{-1})	Peak area (× 10^7)*
306.0	0	2.785 ± 0.039
240.0	0	1.830 ± 0.014
200.0	0	1.525 ± 0.010
180.0	20	1.401 ± 0.049
120.0	80	0.935 ± 0.019
80.0	120	0.612 ± 0.023
0.0	0	0.0 (r = 0.9999)†

*Mean standard deviation of three determinations.
†Correlation coefficient determined by linear regression analysis.

Results and Discussion

Figure 1 shows the chromatograms obtained for the standard solution of dyclonine hydrochloride (A), dyclonine-free degradation product mixture (B), dyclonine hydrochloride spiked with the degradation products mixture (C), and an oral spray formulation containing dyclonine (D), with a phenyl-bonded column and a mobile phase as previously described. It can be seen from Fig. 1A–D that dyclonine eluted at 5.77 min and was well separated from its degradation products and other added flavouring and colouring agents.

The method was validated to be stability-indicating by chromatographing dyclonine with its degradation products. At dyclonine hydrochloride concentrations between 80–180 μg ml^{-1}, sufficient amounts of the dyclonine-free degradation residue (20–120 μg ml^{-1}; Table 1) was added to the standard solution to represent an initial dyclonine hydrochloride concentration of 200 μg ml^{-1}. The standard plot of these concentrations of dyclonine, with and without degradation products, showed a linearity of response with a correlation coefficient (r) of 0.9999 indicating that the degradation products, if found, will not interfere in the analysis of dyclonine stability studies. The mean per cent recovery of dyclonine as determined by the method of standard addition was 99.58 ± 2.42%.

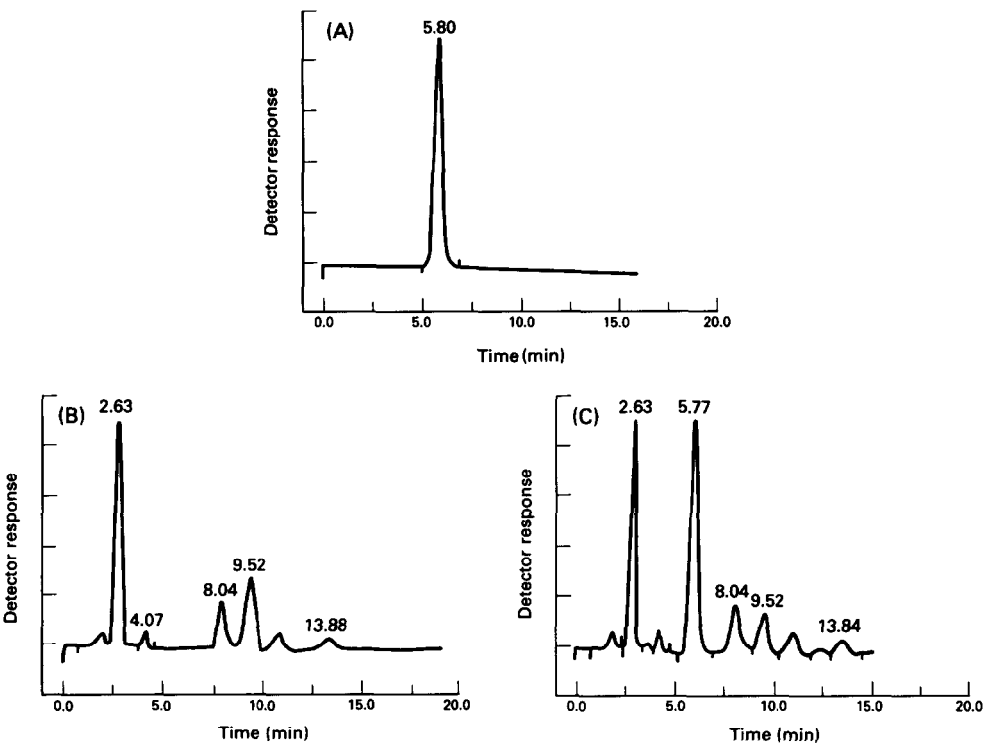

Figure 1
Representative chromatograms for dyclonine HCl standard (A), purified dyclonine degradation product mixture (B), and dyclonine spiked with degradation product mixture (C), with a phenyl-bonded column and mobile phase as described in the Experimental section.

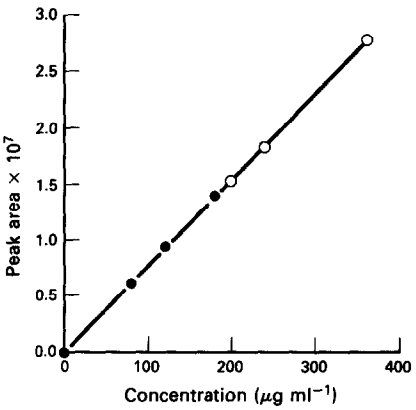

Figure 2
Standard plot for dyclonine hydrochloride. ○, Dyclonine hydrochloride standard; ●, dyclonine hydrochloride standard spiked with degradation products mixture.

Although an attempt was made to identify the degradation products separated on the silica gel column, IR and NMR studies were unable to readily confirm the identity of any products other than that they all possessed a 4-butoxyphenone nucleus [9].

An investigation of the kinetics of degradation for dyclonine hydrochloride showed a pH-dependency of degradation. Dyclonine hydrochloride was found to be stable between pH 1.0–2.2. However, the rate of degradation steeply increased between pH 2.9–4.5, and the degradation rate constant at pH 4.5 was found to be 47.5 times faster than at pH 2.9. Also, the rate of degradation increased with an increase in temperature, with an energy of activation (E) of 24.04 kcal mol^{-1}. Thus, for every 10°C rise in temperature, the rate of degradation increased 3.7 times.

Based on the degradation studies, stable oral spray and gel formulations, containing appropriate pharmaceutical excipients such as colour, flavour, etc. were prepared. The formulations with pH of 2.5 exhibited good chemical stability, with predicted shelf-life of more than 3 years.

Table 2 shows the recovery results (mean 99.74 ± 2.0%) for dyclonine hydrochloride from various gel and oral spray formulations. The presence of flavouring and colouring agents did not interfere with the HPLC analysis of dyclonine. This method therefore allows for the direct, rapid, and accurate analysis of topical local anaesthetic solutions

Table 2
The recovery results for dyclonine hydrochloride from various gel and oral spray formulations

Type of sample	Description	Recovery of dyclonine (%)
Preformulation	Dyclonine with peppermint flavour	101.89
Preformulation	Dyclonine with alcohol	99.61
Preformulation	Dyclonine with propylene glycol	102.61
Preformulation	Dyclonine with sorbitol solution	101.24
Preformulation	Dyclonine with colour solutions	100.52
Preformulation	Dyclonine with benzoic acid	96.71
Oral spray	Oral spray, pH 1.5	96.97
Oral spray	Oral spray, pH 2.0	100.06
Oral spray	Oral spray, pH 2.5	100.32
Oral gel	Gel, pH 2.5	96.83
Oral gel	Gel, pH 2.5	100.34
		99.74 ± 2.0%

containing dyclonine hydrochloride using a commercially available phenyl-bonded column without a sample clean-up step. The method is also suitable as a stability-indicating method for the measurement of dyclonine hydrochloride stability in other pharmaceutical preparations.

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