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Short Communication

High pressure liquid chromatographic determination of dyclonine hydrochloride and its degradation products in cough lozenges and liquids

PHILIP J. PALERMO

The Procter & Gamble Company, Sharon Woods Technical Center, 11511 Reed Hartman Highway, Cincinnati, Ohio 45241, USA

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Introduction

The local anaesthetic properties of dyclonine hydrochloride, 1-(4-butoxyphenyl)-3-(1-piperidinyl)-1-propanone have been known for some time [1]. Its use in a lozenge formulation has also been described [2]. Yet there is a paucity of analytical procedures for this drug reported in the literature. Thin layer chromatographic (TLC) systems [3, 4] and a gas chromatographic (GC) system [5] have been presented but without quantitation. Polarographic characterization procedures [6, 7] have also been presented. No decomposition studies or stability results for dyclonine have been reported.

Analytical methodology was required to assess the stability of dyclonine hydrochloride and determine its degradation route and products in pharmaceutical preparations used as cough lozenges and syrups. Both GC and high pressure liquid chromatographic (HPLC) approaches were investigated and found feasible. However, the GC approach necessitated pH sensitive extractions as the free base prior to injection. This paper presents HPLC systems and procedures developed for monitoring the stability of dyclonine hydrochloride in formulations and the results of the stability studies. In addition, the structures of some of the probable degradation products have been elucidated and described herein.

Experimental

Apparatus

The HPLC system consisted of a high pressure solvent delivery system (Constametric I pump, Laboratory Data Control, Riviera Beach, Florida, USA or Waters Model 6000A

pump, Waters Associates, Milford, Massachusetts, USA), a high pressure injection port (Rheodyne Model 7210, Bio Rad Laboratories, Richmond, California, USA), a 280 nm variable or fixed wavelength detector (Micromeritrics Model 785, Micromeritics Inst. Corp., Norcross, Georgia, USA or Waters Model 440, Waters Associates), a 10 mV recorder (Omniscribe, Cat. No. 5113-5, Houston Instruments, Austin, Texas, USA) and a chromatographic computer integrating system (HP 3353, Hewlett–Packard Instruments, Avondale, Pennsylvania, USA). The analytical column was a 300 × 3.9 mm i.d. stainless steel column packed with 10-μm C_{18}-microBondapak (Waters Associates, Cat. No. 27324) and the semi-preparative column was a 500 × 9.4 mm i.d. magnum 9 column packed with 10 μm partisil o.d. (Whatman Inc., Cat. No. 4230-225, Whatman Inc., Clifton, New Jersey, USA).

Materials

Unless otherwise stated AR grade solvents and chemicals were used throughout. Sample clean-up was accomplished with column chromatographic magnesium silicate (Florisil, 100/200, Cat. No. 92432, Floridan Co., Pittsburgh, Pennsylvania, USA). The optional internal standard, 4-*n*-butoxybenzoic acid (Aldrich Chemical Co., Milwaukee, Wisconsin, USA) was certified 98+% pure and showed no chromatographic interferences. Pharmaceutical grade dyclonine hydrochloride (Siegfried Ltd., Zofingen, Switzerland) was used without further purification.

Mobile phase

The HPLC mobile phase for both analytical and preparative chromatography, methanol–0.05 M ammonium acetate (pH 5), was prepared as follows. 7.7 g ammonium acetate was dissolved in 700 ml of distilled water in a 2 l volumetric flask. 50 ml glacial acetic acid were added and diluted to volume with methanol. The solution was filtered through a polyvinyl chloride 0.6 μm filter (Millipore Corp. BDWP, Cat. No. BDWP 04700, Millipore Corp., Bedford, Mass., USA) and degassed with vacuum before use.

Preparation of standard stock solution

Dyclonine hydrochloride standard stock solution. 60 ± 5 mg of the standard was weighed to the nearest 0.1 mg and transferred to a 100 ml volumetric flask. This was dissolved and diluted to volume with distilled water.

Preparation of solutions for analysis

Procedure for liquid samples. Due to the presence of preservatives which interfere in the HPLC, liquid samples required sample clean-up.

Florisil Eluent No. 1: 0.77 g ammonium acetate were dissolved in 500 ml distilled water. 400 ml of methanol were added and the pH adjusted to 4 ± 0.2 with glacial acetic acid. This was diluted to 1 l with methanol and mixed well.

Florisil Eluent No. 2: 50 ml of 37% HCl were diluted to 1 l with methanol and mixed well.

Florisil column preparation

A small glass wool plug was inserted at the bottom of a 10 × 300 mm chromatographic column. 3–3.5 ml of Florisil was measured into a 10 ml graduated cylinder. The Florisil was slurried in about 10 ml of Florisil Eluent No. 1 and poured into the column, allowing it to settle and drain. The residue of the slurry was rinsed into the column with a small

additional portion of Florisil Eluent No. 1. The column was then drained just to the top of the florisil bed.

Dyclonine hydrochloride standard solution. 3.0 ml of the dyclonine hydrochloride stock solution was transferred to a 50 ml volumetric flask and diluted to volume with Florisil Eluent No. 2.

Sample preparation

The sample was diluted with Florisil Eluent No. 1 to an expected concentration of 0.5 mg/ml dyclonine hydrochloride. 2.0 ml of diluted sample was transferred to the head of the Florisil column. The column was drained to the top of the bed and the sample was washed from the sides onto the head with about 2 ml of Florisil Eluent No. 1. The column was then eluted with 8–10 ml of Florisil Eluent No. 1 and the eluate was discarded.

The column was eluted to volume into a 25 ml volumetric flask with Florisil Eluent No. 2. Initially the eluent was introduced in two 1 ml portions to ensure that all of the dyclonine hydrochloride was deposited onto the column.

Procedure for lozenge samples

Dyclonine hydrochloride standard solution. 5.0 ml of the dyclonine hydrochloride stock solution was transferred to a 50 ml volumetric flask and diluted to volume with the HPLC mobile phase.

Sample preparation. The number of weighed lozenges or the weight of crushed lozenges equivalent to 3 mg of dyclonine hydrochloride was transferred to a 50 ml glass-stoppered Erlenmeyer flask. 40 ml of HPLC mobile phase was added and shaken to completely dissolve the sample. The solution was quantitatively transferred to a 50 ml volumetric flask and diluted to volume with HPLC mobile phase.

HPLC analytical conditions

All solutions were filtered through a 5 μm Teflon filter (Millipore Corp. LSWP, Cat. No. LSWP 01300, Millipore Corp., Bedford, Mass., USA) before injection using the parameters as follows:

Flow rate,	1.5 ml/min
Detector,	UV
Wavelength,	280 um
Sensitivity,	0.1 AUFS
Injection volume,	20 μl
Chart speed,	0.2 in/min
Voltage full scale,	0.01 V.

Preparation of degradation products

Dyclonine hydrochloride (100 g) was dissolved in water and subjected to continuous steam distillation/extraction (Aldrich Chemical Co., Cat. No. Z10,408-6, Milwaukee, Wisconsin) for 16 h using dichloromethane as the extractant. The dichloromethane was distilled off at room temperature at about 10 mm Hg. The resulting yellow liquid was stored under nitrogen for subsequent analysis.

Purification and isolation of degradation products

The yellow liquid produced by steam distillation was injected onto the preparative column at a mobile phase flow rate of 6.0 ml/min using an approximate 0.6 ml loop constructed of 1 mm i.d. stainless steel tubing. The injection was repeated in order to collect enough of the low yield product. Most of the methanol was evaporated from each fraction using a stream of nitrogen. Then 20 ml chloroform and 50 ml distilled water were added to each fraction. The chloroform layer was collected, washed once more with water and taken to dryness under a stream of nitrogen. The resulting residues were subjected to nuclear magnetic resonance (NMR), mass spectrometric and elemental analysis. The NMR analysis was performed using deuterated chloroform as solvent on an 80 MHz spectrometer (Brucker WP-80, Brucker Inst. Inc., Billerica, Massachusetts, USA). Mass spectrometry was carried out using methane reagent gas in the chemical ionization mode (Associated Electronics Inst., Model MS-902) and also by solid probe electron impact analysis (Finnigan Instruments, Model 3300).

Results and Discussion

The injection solvent was found to affect the peak response (especially heights) and peak efficiency of dyclonine hydrochloride. Therefore, it was necessary to prepare the standard solution in the same solvent as the sample. The response characteristics of the analytical system were measured by injecting 20 μl aliquots of various dilutions of the dyclonine hydrochloride stock solution. A linear regression analysis produced a correlation coefficient of 0.9999 and a standard error of estimate of 0.6% at about 1 μg injected.

The precision of the lozenge method was determined by analysing a sample of ground lozenges (label value 1.25 mg/g dyclonine hydrochloride) nine times varying the sample weight between 75 and 125% of that prescribed in the method. The relative standard deviation was 1.9%. Similarly, a liquid sample (label value 1 mg/ml dyclonine hydrochloride) was repetitively analysed at sample sizes 80–120% of that prescribed and a relative standard deviation of 0.6% was calculated. Initial studies were performed using 4-*n*-butoxybenzoic acid as an internal standard to increase analysis precision but its use was discontinued because of the simplicity of sample handling and the high precision of the loop injector which has a repeatability of about 0.5%. Moreover, the internal standard eluted close to the degradation products of dyclonine hydrochloride.

Accuracy for the lozenge method was determined by wet addition recovery of a dyclonine stock solution to the weight of lozenge placebo corresponding to two lozenges. Nine recoveries, three each at 80, 100 and 120% of theoretical dosage level were performed and the average recovery was 100.4 ± 0.4%. Similarly, accuracy data over the same range was acquired for the liquid by spiked addition to placebo. These data revealed a recovery of 100.2 ± 1.3%.

The effect of pH of Florisil Eluent No. 1 was studied over the range of pH 3–8 by multiple analysis of a liquid sample after separately collecting 25 ml of each Florisil Eluent. The recovery of dyclonine in each eluent showed an optimum pH of *ca* 4.0. Both higher and lower pHs cause incomplete retention of dyclonine and thus low recoveries.

The elution profile of the Eluent No. 1, at a pH of 4.0 was determined by analysing fractions of the Eluent No. 1 for dyclonine liquid samples. This study revealed that no dyclonine was eluted in the first 50 ml of Eluent No. 1. The other components of the liquid, sweeteners, dyes and preservatives are all eluted in Eluent No. 1, which can be

saved for their analysis. In addition, all impurities and degradation products not containing the piperidine moiety are eluted in the first 10 ml of Eluent No. 1.

A chromatogram of a lozenge sample subjected to high temperature is shown in Fig. 1. The dyes, sweeteners and flavour components elute shortly after the solvent breakthrough. It was decided to study the nature of the components eluting at 7.2 and 8.6 min since these did not appear in normal lozenge samples. It was postulated that dyclonine hydrochloride when subjected to heat could undergo a classical reverse Mannich reaction to yield piperidine and 4-*n*-butoxyphenyl vinyl ketone. This compound has been previously reported [8]. Consequently, dyclonine hydrochloride was subjected to the steam distillation procedure described. The chromatogram of 0.5 g of the yellow liquid on the preparative column using a flow rate of 6 ml/min is shown in Fig. 2. Using multiple injections, fractions of the major components were collected and combined. Fraction 1 was taken from 10 to 12 min, fraction 2 from 17.5 to 21 min and fraction 3 from 25 to 35 min. Only one injection was required to collect enough material for analysis of fraction 3. The fraction 1 material was immediately shown to be unreacted dyclonine by analysis on the analytical HPLC system. Fractions 2 and 3 corresponded to the chromatographic peaks at 7.2 and 8.6 min, respectively. Silica gel TLC was also used to show that the fractions 2 and 3 materials corresponded to the same impurity peaks observed in heat stressed lozenges.

The material from fraction 3 was studied most extensively. The chemical ionization mass spectrum showed peaks at *m/e* 205 and 233 suggesting a molecular weight of 204 presuming that these are the $M + 1$ and $M + 29$ peaks. This was substantiated by the solid probe electron impact mass spectrum whose major fragments are presented in Table 1 which also shows the peak assignments. Again a MW of 204 is suggested which is correct for 4-*n*-butoxyphenyl vinyl ketone.

The NMR spectrum of the purified fraction 3 material is shown in Fig. 3B and the spectrum of dyclonine for comparison purposes. Table 2 summarizes the chemical shifts (δ) relative to the internal standard TMS and peak integrations for dyclonine and the fraction 3 material. The spectrum of the fraction 3 material is consistent with that expected for 4-*n*-butoxyphenyl vinyl ketone. Figure 3C shows the NMR spectrum of dyclonine itself.

Elemental analysis further substantiated the structure of the fraction 3 degradation product. The material showed 76.5% C, 8.0% H and 0.06% N. Theoretical values for 4-*n*-butoxyphenyl vinyl ketone are 76.4% C, 7.9% H and 0% N. The fraction 3 material was chromatographed on the analytical system and was found to be 99+% pure by internal normalisation with a retention time of 8.6 min, the major impurity being a trace of fraction 2 material. 4-*n*-Butoxyphenyl vinyl ketone polymerizes within a few days unless stored under nitrogen and protected from light.

Study of the fraction 2 material was less extensive, being limited to NMR analysis only. The NMR spectrum is also presented in Fig. 3A. Comparison of the spectra and study of Table 2 suggests the structure of the fraction 2 material to be 4-*n*-butoxyphenyl acetophenone.

Crude 4-*n*-butoxyphenyl acetophenone was received (Bodman Chemicals, Media, Pennsylvania, USA) and purified by distillation. Its NMR spectrum was identical to the fraction 2 material. The fraction 2 material had a retention time on the analytical system of 7.2 min.

Formulations of aqueous dyclonine hydrochloride are most stable at low pH. Formulations at pH 4 begin to show degradation at one or two months at room

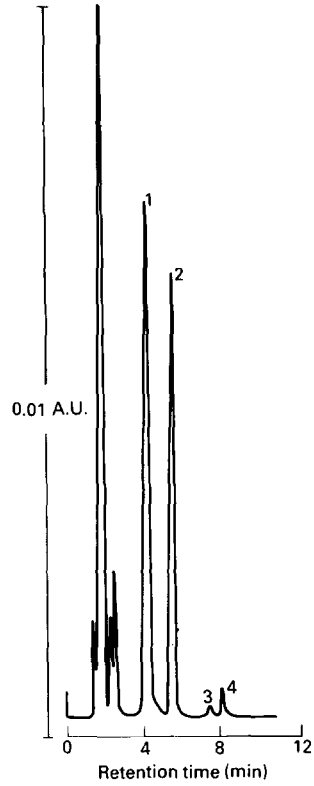


Figure 1

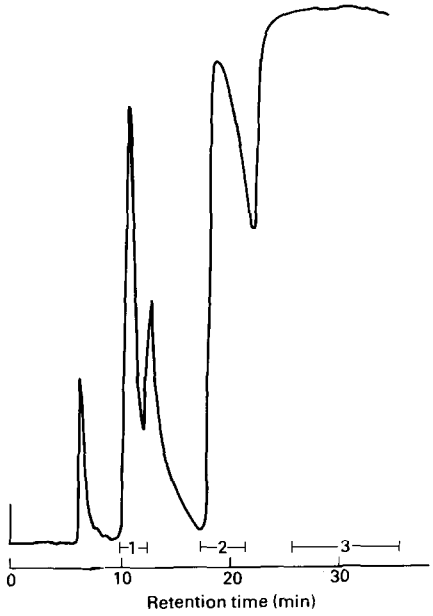
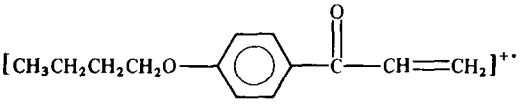
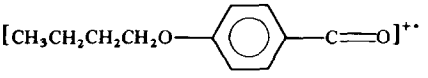
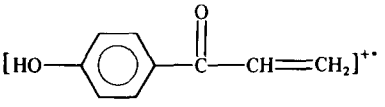
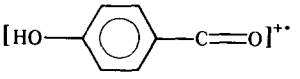
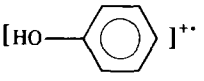


Figure 2

Table 1
Electron impact mass spectrum of 4-*n*-butoxyphenyl vinyl ketone

m/e	% intensity relative to *m/e* 121	Structural assignment
204	22.0	
177	4.5	
148	21.9	
121	100	
93	10.2	

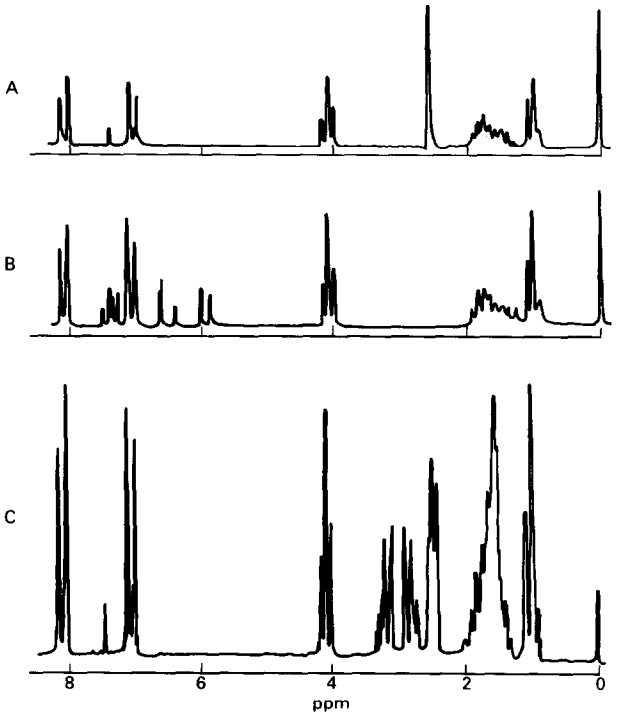
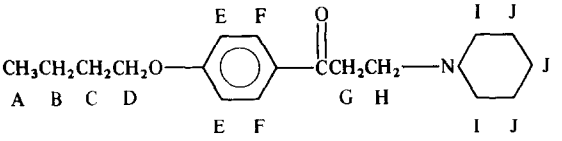
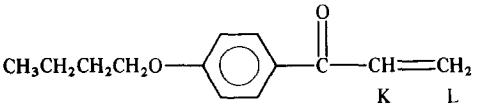
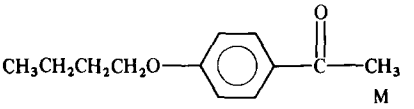


Figure 3

Table 2
NMR spectra of dyclonine and degradation products

Dyclonine	
4-*n*-butoxyphenyl vinyl ketone	
4-*n*-butoxyphenyl acetophenone	

δ	Proton designation	No. of protons integrated	Peak type
1.0	A	3	Triplet
1.3–1.9	B,C	4	A_2B_2
4.0	D	2	triplet
7.0	E	2	A_2X_2
8.0	F	2	
3.2	G	2	A_2B_2
2.8	H	2	
2.5	I	2	apparent triplet
1.6	J	3	$A_2B_2C_2$
7.2	K	1	ABX
5.9–6.4	L	2	
2.6	M	3	singlet

temperature. At pH 2 no degradation is observed except at high temperatures. With certain dyes such as FD&C Red No. 40 and in the presence of paraben preservatives, dyclonine precipitates out of aqueous solutions as a salt. The structure of the salt is not yet known and may be variable depending upon the concentrations of the reactants. One such salt after dissolution in the HPLC mobile phase consisted of about 2 moles of dyclonine, one mole of FD&C Red No. 40 and 1 mole of propyl paraben by HPLC analysis. Dyclonine hydrochloride in aqueous solutions above pH 7 is very unstable, yielding a multitude of peaks in the HPLC system probably including the two degradation products elucidated in this work and piperidine.

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