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First derivative spectrophotometric and high-performance liquid chromatographic determination of cinchocaine hydrochloride in presence of its acid degradation product

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Abstract

Two methods are presented for the determination of cinchocaine HCl in presence of its acid-induced degradation product using first (^{1}D) derivative spectrophotometry and high-performance liquid chromatography. Cinchocaine HCl was determined by measurement of its first derivative amplitude at the zero crossing point of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide as its acid degradation product (at 333.5 nm). The HPLC method depends upon using a μBondapak C_{18} column at ambient temperature with a mobile phase consisting of acetonitrile—0.01 M sodium acetate trihydrate (45:55, v/v) containing 0.06% (w/v) heptane sulphonic acid sodium salt and adjusted to apparent pH 4.5 with acetic acid at a flow rate 2 ml min^{-1}. Quantitation was achieved with UV detection at 254 nm based on peak area. The HPLC method was applied for simultaneous determination of cinchocaine HCl, methylparaben and propylparaben. The two proposed methods were successfully applied to the determination of the cinchocaine HCl in laboratory-prepared mixtures in the presence of its acid degradation product and in cream. Moreover, the proposed methods were utilized to investigate the kinetics of the acid degradation process at different temperatures and the apparent pseudo first-order rate constant, half-life and activation energy calculated.

Keywords: Cinchocaine HCl; HPLC; First-derivative spectrophotometry; Cream; Kinetics of degradation

1. Introduction

Cinchocaine HCl, 2-butoxy-*N*-(2-diethylaminoethyl)quinoline-4-carboxamide hydrochloride, is a long-acting local anaesthetic of the amide type used for surface anaesthesia in creams,

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ointments and suppositories for relief of pain and itching or for spinal anaesthesia as an injectable preparation. The official methods for determination of cinchocaine HCl are non-aqueous titration using standard perchloric acid as titrant and crystal violet indicator [1], and HPLC [2] using a C_{18} column and methanol–water containing sodium lauryl sulfate, sodium acetate, triethylamine and glacial acetic acid at pH 5.6 as mobile phase. Various methods have been reported for the determination of cinchocaine, including titrimetric procedures using sodium tetraphenylborate [3], perchloric acid [4], 6-hydroxy-3,4-xylenesulphonic acid [5] as titrants. Many methods have been applied for the determination of cinchocaine: spectrophotometry [6–9], first-derivative spectrophotometry [10], measuring its maxima in chloroformic solution at 336 nm for correction of interference from non-specific irrelevant absorption originated from the formulation matrix, fluorimetry [11], polarography [12] and acid-dye techniques [13]. The chromatographic techniques of analyses, TLC [14,15], GC [16,17] and HPLC, have been employed either in urine [18] using a Cosmosil 5C18 column and aqueous 60% methanol containing 30 mM triethylamine (pH 7.5) as mobile phase, or in spinal anaesthetics [19] using an ODS column.

Methyl- and propylparaben were added to cream and ointment as preservatives. Review of the literature reveals that there is no method described for the determination of such a combination. Therefore, it was thought necessary to develop a simple, fast and accurate method for the determination of such a mixture in pharmaceutical formulations.

The present work presents two methods for determining cinchocaine HCl in the presence of its acid-induced degradation product using first-derivative spectrophotometry and high-performance liquid chromatography. Furthermore, the developed methods were used to investigate the kinetics of the acid drug degradation at different temperatures. The proposed HPLC method was used for simultaneous determination of cinchocaine HCl, methylparaben and propylparaben in commercial cream without any interference from the excipients normally used in cream formulations.

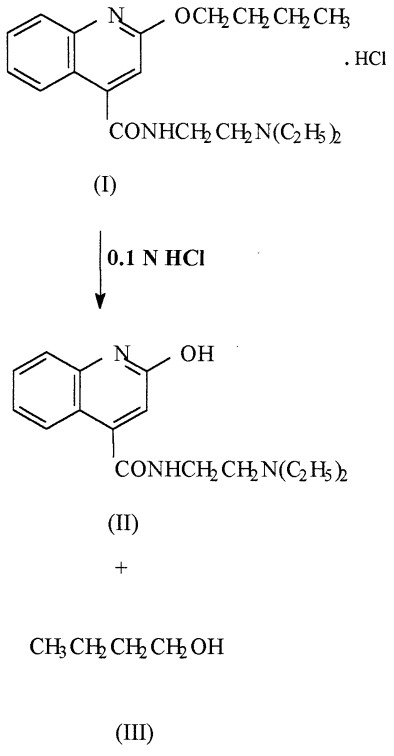
2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV-Visible spectrophotometer, model UV-1601 PC, connected to an promax computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) and a Hewlett-Packard Deskjet 600 printer was used. The spectral band width was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.

The first derivative curves of the spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 400–270 nm with $\Delta\lambda = 4$ nm and scaling factor = 6.

The HPLC (Waters Associates, Milford, MA) instrument was equipped with a model 501 pump, U6K universal injector, model 441 fixed-wavelength UV absorbance detector (at 254 nm) and model 740 Waters data module. Separation and



Scheme 1.

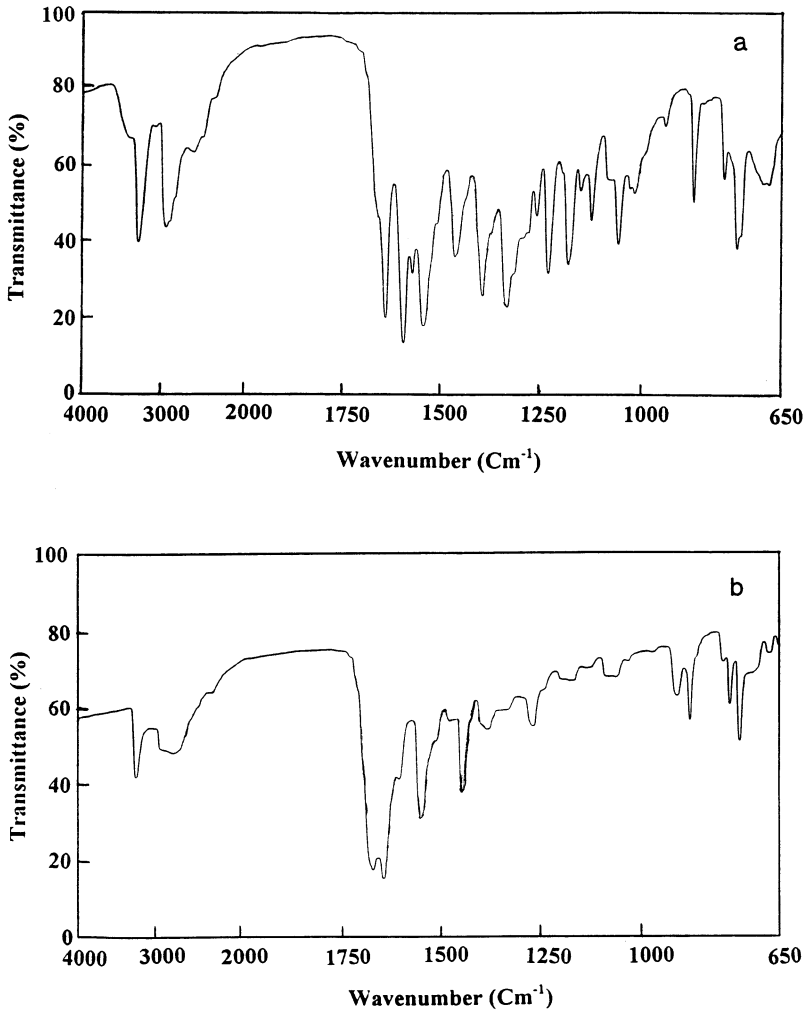


Fig. 1. IR spectrum of cinchocaine (a) and its degradation product (2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide) (b) in KBr.

quantitation were made on a 30-cm × 3.9-mm (i.d.) column from Waters μBondapak C_{18} (10 μm). Detection was made at 254 nm and sensitivity was set at 0.1 a.u.f.s. The samples were injected (20 μl) with a 25-μl Waters analytical syringe (Scientific Glass Engineering, Australia). The chart speed was 0.5 cm min^{-1}.

Glass TLC plates (20 × 20 cm) were covered with a 0.2-mm layer of silica gel 60 GF 254 (E. Merck, Darmstadt, Germany) and activated at 100–105°C for at least 1 h before use. The mobile phase used consisted of diethyl-ether–chloroform–methanol (20:60:20, v/v). The spots were examined at 254 nm using a UV source for TLC (Desaga, Heidelberg, Germany).

The melting point was recorded on Stuart Scientific melting point apparatus (UK) using open glass capillaries.

The infrared spectrophotometer used was a Shimadzu IR-408.

PMR spectra were recorded on a Varian Gemini 200, using tetramethylsilane as the internal standard.

A Shimadzu mass spectrometer model GCMS-QP 1000 EX was used.

2.2. Materials and reagents

Cinchocaine HCl (Orgamol, Switzerland), methylparaben and propylparaben (Nipa, Britain) were kindly Supplied by Alexandria Company for Pharmaceutical and Chemical Industries (Egypt), and their purity was certified and analyzed to be 99.90%. The water for HPLC was prepared by double glass distillation and filtration through a 0.45-μm membrane filter. The acetonitrile and methanol used for the chromatographic separation were HPLC grade (Romil Chem, UK). Sodium acetate trihydrate, heptane sulphonic acid sodium salt, acetic acid and hydrochloric acid were analytical grade. The commercial product, Neocaine cream, was supplied by Alexandria Company for Pharmaceutical and Chemical Industries. Each 100 g of the cream contains cinchocaine HCl equivalent to 1 g cinchocaine, 60 mg methylparaben and 40 mg propylparaben, with a washable matrix base consisting of bees wax, cetostearyl alcohol, light paraffin oil, anhydrous lanoline, vaseline, borax and water.

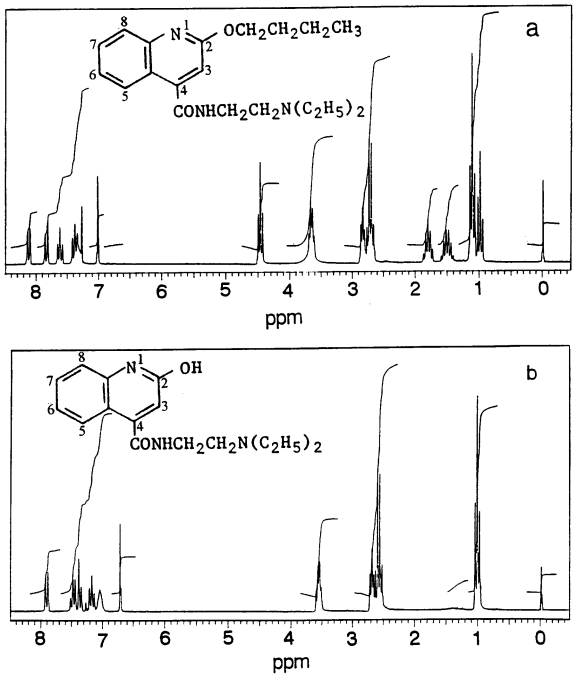


Fig. 2. PMR spectrum of cinchocaine (a) and its degradation product (2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide) (b) in deuterated chloroform.

Table 1
PMR spectral assignment for 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide

Chemical Shift δ (Ppm)	*Multiplicity*	*No. of Protons*	*Assignment*
1.00 (J = 7.2 Hz)	triplet	6	-N(CH2CH3)(CH2CH3)
2.59 (J = 7.2 Hz)	quartet	4	- CH2-N(CH2-)(CH2-)
2.68 (J = 6 Hz)	triplet	2	- CH2-N(CH2-)(CH2-)
3.51 - 3.59	Multiplet	2	-C(=O)-NH-CH2-CH2-
6.71	Singlet	1	aromatic C_3-H
7.16 (J = 8 Hz)	triplet	1	aromatic C_6-H
7.30 - 7.53	Multiplet	2	aromatic C_7-H and C_8-H
7.88 (J = 8 Hz)	doublet	1	aromatic C_5-H

2.3. Chromatographic conditions

The mobile phase was prepared by mixing acetonitrile and 0.01 M sodium acetate trihydrate in a ratio of 45:55, followed by addition of 0.06 g heptane sulphonic acid sodium salt to every 100 ml mobile phase mixture, and the apparent pH was adjusted to 4.5 using acetic acid. The mobile phase was filtered using a 0.45-μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The samples were also filtered using 0.45-μm disposable filters. The flow rate was 2 ml min^{-1}. All determinations were

Table 2
PMR spectral assignment for cinchocaine

Chemical Shift δ (Ppm)	*Multiplicity*	*No. of Protons*	*Assignment*
0.98 (J= 7.2 Hz)	triplet	3	-OCH_2-CH_2-CH_2-$C\underline{H_3}$
1.10 (J= 7.2 Hz)	triplet	6	-N$<^{CH_2CH_3}_{CH_2CH_3}$
1.40 - 1.60	Multiplet	2	-OCH_2-CH_2-$C\underline{H_2}$-CH_3
1.70 - 1.88	Multiplet	2	- OCH_2-$C\underline{H_2}$-CH_2-CH_3
2.72 (J=7.2Hz)	quartet	4	-CH_2-N$<^{CH_2-}_{C\underline{H_2}-}$
2.83 (J= 6Hz)	triplet	2	-$C\underline{H_2}$-N$<^{CH_2-}_{CH_2-}$
3.58 - 3.73	Multiplet	2	-C(=O) - NH - $C\underline{H_2}$-CH_2-
4.46 (J= 6.6Hz)	triplet	2	-O-$C\underline{H_2}$-CH_2-CH_2-CH_3
7.00	singlet	1	aromatic C_3-H
7.37 (J= 8Hz)	triplet	1	aromatic C_6-H
7.61 (J= 8.4 Hz)	triplet	1	aromatic C_7-H
7.81 (J= 8.4 Hz)	doublet	1	aromatic C_5-H
8.09 (J= 8.4 Hz)	doublet	1	aromatic C_8-H

performed at room temperature. The data module attenuation was set at 32.

2.4. Preparation of the acid degradation product

Accurately weighed cinchocaine HCl (100 mg) was dissolved in 100 ml 0.1 N hydrochloric acid and boiled under reflux for 10 h. Subsequently, the pH of the solution was adjusted to 8.5 using 1 N sodium hydroxide to precipitate the degradation product. The precipitate was filtered, washed with water and crystallized from aqueous methanol. It was filtered, dried under vacuum; the melting point was 245°C with decomposition ($R_f = 0.27$ using the above discussed TLC system). The analysis was calculated for $C_{16}H_{21}N_3O_2$: C 66.88%; H 7.37%; N 14.62%; the percentages of elements found were: C 67.16%; H 7.40%; N 14.36%.

The stock solution of acid degradation product was prepared by dissolving 25 mg of the acid degradation product prepared as above in 25 ml 0.1 N hydrochloric acid (for the ^{1}D method) or methanol (for the HPLC method).

2.5. Standard solutions and calibration graphs

2.5.1. First-derivative method

Stock solution was prepared by dissolving cinchocaine HCl in 0.1 N HCl to obtain a concentration of 1000 μg ml^{-1}. The standard solutions were prepared by dilution of the stock solution with 0.1 N HCl to reach concentration ranges of 10–80 μg ml^{-1}. The ^{1}D curves of the working standard solutions were scanned in the range of 400–270 nm against 0.1 N HCl as a blank. The values of the ^{1}D amplitudes at 333.5 nm were measured, and the concentrations versus their absolute first-derivative amplitudes were plotted in order to obtain the calibration graph.

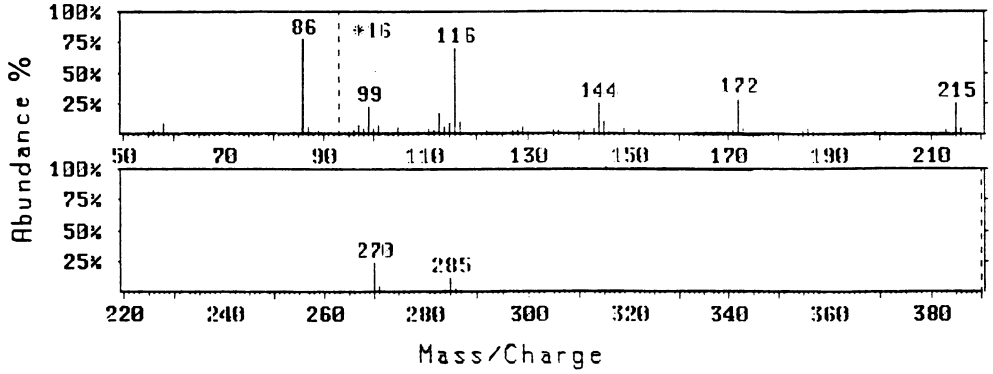
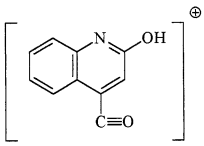
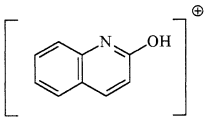
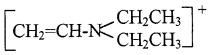
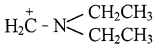
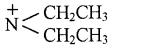


Fig. 3. Mass spectrum of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide.

Table 3
Fragmentation pattern of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide

m/z	Fragment	Abundance%
287	$M^{+\bullet}$	0.1
286	$[M-1]^+$	0.1
285	$[M-2H]^+$	0.6
270	$[M-OH]^+$	1.5
215	$[M-N(C_2H_5)_2]^+$	1.4
186	$[M-CH_3CH_2N(C_2H_5)_2]^+$	0.2
172		1.7
144		1.6
116	$[144 - CO]^+$	4.7
99		1.2
86		100.0
72		1.0

2.5.2. HPLC method

Stock solutions were prepared by dissolving cinchocaine HCl, methylparaben and propylparaben in methanol to obtain concentrations of 1000, 600 and 600 μg ml^{-1}, respectively. The standard solutions were prepared by dilution of the stock solutions with mobile phase to reach concentration ranges of 10–100, 0.6–6 and 0.6–6 μg ml^{-1} for cinchocaine HCl, methylparaben and propylparaben, respectively. Triplicate 20-μl injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph for each compound.

2.6. Sample preparation

2.6.1. First-derivative method

An accurately weighed amount of the cream, equivalent to about 5 mg cinchocaine HCl, was sonicated for 15 min with 50 ml hot 0.1 N HCl. The solution was cooled to room temperature and completed to 100 ml with 0.1 N HCl. The final solution was centrifuged at 7000 rpm for 10 min. The ^1D amplitude was measured for the supernatant solution directly as described under the calibration graph and the concentration of cinchocaine HCl in sample was calculated from the regression equation.

2.6.2. HPLC method

An accurately weighed amount of the cream, equivalent to about 10 mg cinchocaine HCl, 0.6 mg methylparaben and 0.4 mg propylparaben, was sonicated for 15 min with 50 ml hot methanol. The solution was cooled to room temperature and completed to 100 ml with methanol. The final solution was centrifuged at 7000 rpm for 10 min and a 25-ml aliquot of the supernatant solution was diluted in a volumetric flask to 50 ml with mobile phase. The solution was filtered through a 0.45-μm membrane filter. A 20-μl volume was injected into the HPLC, in triplicate, concurrently with the appropriate standard solution (50, 3 and 2 μg ml^{-1} for cinchocaine HCl, methylparaben and propylparaben, respectively) and chromatographed under the conditions described above. The peak area was used for determination of the three compounds in the sample.

2.7. Percent recovery study

This study was performed by adding different amounts of intact and acid-degraded cinchocaine HCl, methylparaben and propylparaben to a known concentration of the commercial cream (standard addition method). The resulting mixtures were assayed and the results obtained were compared with expected results (Table 7).

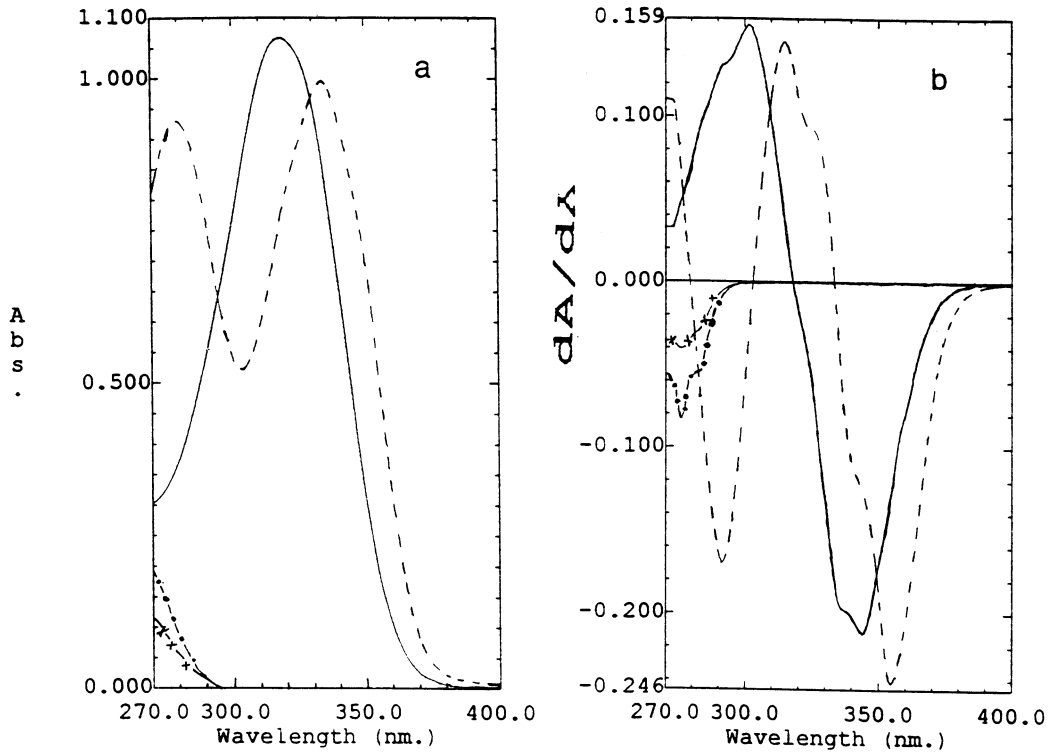


Fig. 4. Zero-order absorption spectra (a) and first derivative spectra (b) of 50 μg ml^{-1} cinchocaine HCl (——), 50 μg ml^{-1} 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (- - -), 3 μg ml^{-1} methylparaben (· · ·) and 2 μg ml^{-1} propylparaben (× × ×) in 0.1 N hydrochloric acid.

2.8. Kinetic investigation

Accurately weighed cinchocaine HCl (80 mg) was transferred into a 250-ml volumetric flask, dissolved and diluted to volume with 0.1 N hydrochloric acid for each temperature investigation. Separate 25-ml aliquots of this solution were transferred into separate stoppered conical flasks. The flasks were placed in a thermostatic oven at different temperatures (90, 80, 70, 65, 60°C) for different time intervals (1–10 h). For the ^{1}D method: at the specified time interval the contents of the flasks were transferred into 100-ml volumetric flasks and diluted to volume with 0.1 N hydrochloric acid. The ^{1}D amplitude was measured for the solution directly as described under the calibration graph and the concentration of the remaining cinchocaine HCl was calculated at each temperature and at time interval. For HPLC method: at the specified time interval the contents of the flasks were neutralized to pH 7 using predetermined volumes of 1 N sodium hydroxide solution. The contents of the flasks were transferred into 100-ml volumetric flasks and diluted to volume with mobile phase. Aliquots of 20 μl of each solution were chromatographed under the conditions described above and the concentration of the remaining cinchocaine HCl was calculated at each temperature and at time interval.

3. Results and discussion

3.1. Identification of the acid degradation product

According to Morch [20], when cinchocaine HCl (I) was boiled with 2 N hydrochloric acid for 4 h, 2-hydroxyquinoline-4-carboxylic acid diethy-

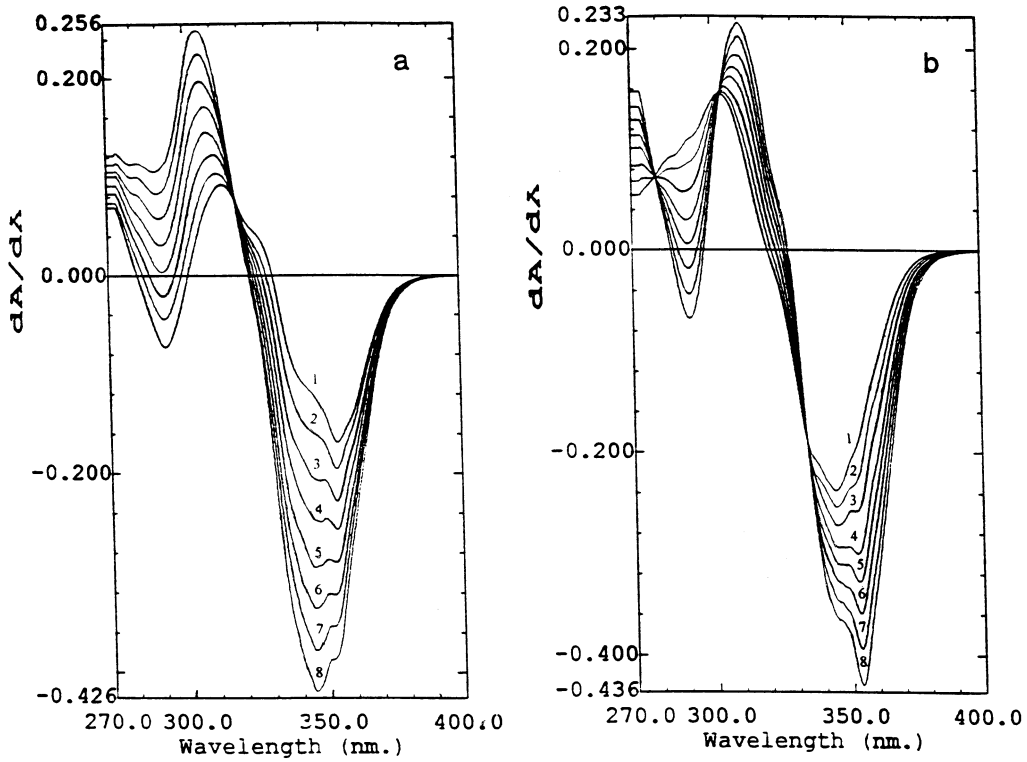


Fig. 5. (a) First derivative spectra of mixtures containing identical concentration of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (40 μg ml^{-1}) and different concentrations of cinchocaine HCl in 0.1 N hydrochloric acid. (b) First derivative spectra of mixtures containing identical concentration of cinchocaine HCl (50 μg ml^{-1}) and different concentrations of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide in 0.1 N hydrochloric acid. Key: (1) 10; (2) 20; (3) 30; (4) 40; (5) 50; (6) 60; (7) 70; (8) 80 μg ml^{-1}.

laminoethylamide (II) and 2-hydroxyquinoline-4-carboxylic acid were produced. In our hands, we were able to separate these products by the application of the proposed HPLC system on the reaction mixture at 1.7 and 1.3 min, respectively. However, in the present work, when (I) was boiled with 0.1 N hydrochloric acid for 10 h, only 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (II) could be isolated as pure product (as well as by TLC and HPLC) from the reaction mixture. The degradation of (I) to (II) would indicate that *O*-debutylation of the (I) is easier than hydrolysis of its amide side chain under our adopted reaction conditions. The suggested pathway for degradation of cinchocaine HCl (I) in 0.1 N hydrochloric acid is presented in Scheme 1. Using the above-mentioned TLC system, the R_f values were found to be 0.78 and 0.27 for (I) and (II), respectively.

The assignment of the degradation product (II), as 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide, was based on its elemental analysis and comparison of the IR and PMR spectral data of purified specimens, separated from the degradation reaction, with those of the intact compound. The IR spectrum of (II) (KBr) (Fig. 1b) revealed two bands with medium intensity at the 3250- and 2500–3000-cm^{-1} regions which can be attributed to the NH and OH absorptions. The spectrum showed the amide I band as a strong band split at 1640 and 1670 cm^{-1}, and a shoulder at 1600 cm^{-1}. The amide II band appeared at 1545 cm^{-1} with medium intensity. The spectrum lacked the characteristic

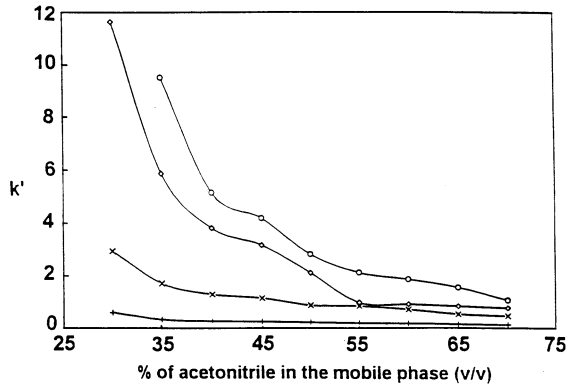


Fig. 6. Plots of the capacity factor, K', versus acetonitrile concentration in the mobile phase. Key: 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (+), methylparaben (×), propylparaben (◇) and cinchocaine HCl (○).

=C–O–C absorption of (I) at 1240 and 1060 cm^{-1}. By contrast, the IR spectrum of (I) (KBr) (Fig. 1a) showed the NH absorption as a strong band at 3250 cm^{-1}, the aliphatic and aromatic CH absorptions at 2940 cm^{-1}, and the amide I band as a sharp band at 1640 cm^{-1}. The strong band split at 1595 and 1540 cm^{-1}, and the shoulder at 1570 cm^{-1}, are attributed to the mixed absorptions of the C=C, aromatics and amide II band. The spectrum also revealed two bands at 1240 and 1060 cm^{-1} assigned to =C–O–C absorption of the butoxy residue.

The PMR spectrum of (II) in deuterated chloroform (Fig. 2b) lacked the proton signals of the *O*-butyl substituent of (I). The spectral assignment for (II) is shown in Table 1, whereas the PMR spectrum of (I) in the same solvent is shown in Fig. 2a and its assignment is shown in Table 2.

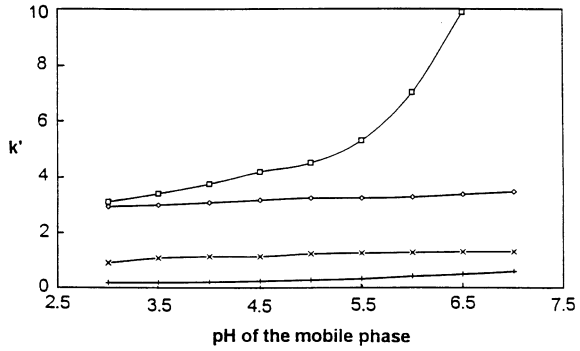


Fig. 7. Plots of the capacity factor, K', versus apparent pH of the mobile phase. Key: 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (+), methylparaben (×), propylparaben (◇) and cinchocaine HCl (□).

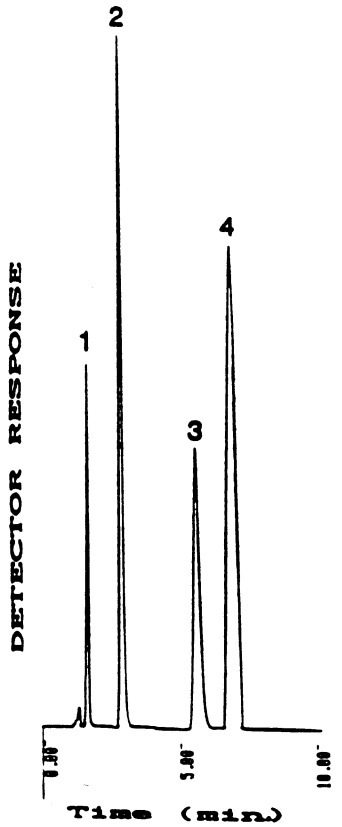


Fig. 8. A typical chromatogram of a 20-μl injection of synthetic mixture of 50 μg ml^{-1} intact cinchocaine HCl (4), 5 μg ml^{-1} degradation product II (2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide) (1), 3 μg ml^{-1} methylparaben (2) and 2 μg ml^{-1} propylparaben (3).

The mass spectrometric data of 2-hydroxyquinoline-4-carboxylic acid diethylaminoethylamide (II) was recorded (Fig. 3). The possible major fragments and their mass/charge ratios were illustrated in Table 3. The spectrum was characterized by the M–OH fragment at m/z 270, which would be indicative of *O*-dealkylation of (I) during the applied reaction conditions.

3.2. Assay parameters

Cinchocaine HCl in 0.1 N hydrochloric acid has maximum absorption at 319 nm, while its

Table 4
Analytical data and regression characteristics of the proposed methods

Parameters	Compounds			
	Cinchocaine HCl		Methylparaben (HPLC)	Propylparaben (HPLC)
	^{1}D	HPLC		
Beer's law limit (μg ml^{-1})	10–80	10–100	0.6–6	0.6–6
Detection limit (μg ml^{-1})	0.51	0.27	8.64×10^{-3}	8.84×10^{-3}
Regression equation (Y)[a]:				
Slope (b)	3.63×10^{-3}	1.92×10^{4}	1.28×10^{5}	1.17×10^{5}
Standard deviation of the slope (S_b)	1.26×10^{-5}	0.30×10^{2}	1.06×10^{2}	1.00×10^{2}
Variance of the slope	1.58×10^{-10}	0.88×10^{3}	1.13×10^{4}	9.99×10^{3}
Relative standard deviation of the slope (%)	0.35	0.15	0.08	0.09
Confidence limit of the slope (95% confidence limit)	3.60×10^{-3}	1.91×10^{4}	1.27×10^{5}	1.16×10^{5}
	-3.66×10^{-3}	-1.93×10^{4}	-1.28×10^{5}	-1.17×10^{5}
Intercept (a)	1.11×10^{-3}	4.32×10^{2}	6.19×10^{2}	8.72×10^{2}
Standard deviation of the intercept (S_a)	6.34×10^{-4}	1.82×10^{3}	3.91×10^{2}	3.67×10^{2}
Variance of the intercept	4.02×10^{-7}	3.31×10^{6}	1.53×10^{5}	1.35×10^{5}
Confidence limit of the intercept (95% confidence limit)	(-4.47×10^{-4})	(-4.02×10^{3})	(-0.34×103)	(-0.28×10^{2})
	-2.66×10^{-3}	-4.88×10^{3}	-1.58×10^{3}	-1.77×10^{3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	2.88×10^{-4}	0.8×10^{3}	1.72×10^{2}	1.61×10^{2}
Relative standard deviation (%)[b]	0.69	0.54	0.41	0.43

[a] $Y=a+bC$, where C is the concentration in μg ml^{-1} and Y is the ^{1}D amplitude at 333.5 nm or peak area for ^{1}D or HPLC method, respectively.
[b] Eight replicate samples of pure compound.

degradation product (II) has maximum absorption at 280 and 334 nm in the same solvent (Fig. 4a). The first derivative spectra of cinchocaine HCl and its degradation product (II) in 0.1 N hydrochloric acid (Fig. 4b) showed significant differences in some areas that permits the determination of cinchocaine HCl in the presence of its degradation product (II). The ^{1}D values at 280, 303 and 333.5 nm of the degradation product (II) are equal to zero. An analytical wavelength of 333.5 nm has been used for the quantitation of intact cinchocaine HCl in cream for more sensitivity and complete elimination of any interference resulting from degradation product (II), parabens and excipients. Intact cinchocaine HCl was determined by measuring the absolute ^{1}D amplitude at 333.5 nm. Thus, the measurements of the absolute value of the first derivative of the zero-order sum curves of intact cinchocaine HCl (in concentration ranging from 10 to 80 μg ml^{-1}) and its degradation product II (in constant concentration 40 μg ml^{-1}) at the zero-crossing point of the first derivative of the degradation product II (at 333.5 nm) afforded the linear response to the cinchocaine HCl concentration (Fig. 5a) with slope value equal to that of the pure cinchocaine HCl, and this value was not affected by the presence of the degradation product II.

The typical set of first derivative spectra (Fig. 5b) of laboratory mixtures with constant concentration of cinchocaine HCl (50 μg ml^{-1}) and different concentrations of degradation product II (ranging from 10 to 80 μg ml^{-1}) are shown. The absolute value of first derivative at 333.5 nm were found to be constant and completely independent of the degradation product (II) concentration.

The plots of the absolute value of first derivative at 333.5 nm against concentration of cinchocaine HCl showed a linear relationship.

Table 5
Determination of synthetic mixtures of cinchocaine HCl (I), its degradation product (II), methylparaben (IV) and propylparaben (V)

Mix. no.	Mixture compositions (μg ml^{-1})				% recovery of			
	I	II	IV	V	I		IV (HPLC)	V (HPLC)
					^{1}D	HPLC		
1	30	30	1	3	99.1	100.3	99.9	100.5
2	50	20	3	2	100.5	100.1	100.0	100.1
3	60	25	2	5	101.1	99.6	100.7	99.4
4	40	30	5	1	99.3	100.7	100.1	100.2
5	80	20	4	6	100.7	99.3	99.2	99.6
6	70	30	6	3	100.8	100.8	99.6	99.3
7	80	25	3	4	100.9	99.1	100.5	100.3
Mean					100.3	100.0	100.0	99.9
R.S.D. (%)					0.80	0.67	0.51	0.47

The developed HPLC method has been applied to the simultaneous determination of cinchocaine HCl in the presence of its degradation product (II), methylparaben and propylparaben in cream form. To optimize the HPLC assay parameters, the effect of acetonitrile composition and apparent pH of the mobile phase on the capacity factor (K') were studied (Fig. 6). A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–0.01 M sodium acetate trihydrate mixture (45:55, v/v). Increasing acetonitrile concentration to more than 65% led to inadequate separation of the four compounds. At lower acetonitrile concentration, separation occurred but with excessive tailing and increased retention time for propylparaben and cinchocaine HCl peaks. Variation of apparent pH of the mobile phase resulted in maximum K' value at apparent pH 6.5, with loss of peak symmetry for cinchocaine HCl peak. At lower apparent pH values (3–3.5) bad resolution was observed for propylparaben and cinchocaine HCl peaks. At apparent pH 4–5 improved resolution was observed; however, at apparent pH 4.5 optimum resolution with reasonable retention time was affected (Fig. 7). The addition of 0.06% (w/v) heptane sulphonic acid sodium salt was essential to improve the sharpness of the cinchocaine HCl peak.

The specificity of the HPLC method is illustrated in Fig. 8 where complete separation of the four compounds was noticed. The average retention time $\pm$ standard deviation for cinchocaine HCl, its degradation product II, and methylparaben and propylparaben, were found to be 7.1 ± 0.009, 1.7 ± 0.004, 2.9 ± 0.003 and 5.6 ± 0.005 min, respectively, for 10 replicates. The peaks obtained were sharp and have clear baseline separation.

To determine the linearity of the HPLC detector response, calibration standard solutions of cinchocaine HCl, methylparaben and propylparaben were prepared as described in the text. Linear correlation was obtained between peak area versus concentration for each compound. Each measurement represented the average of three replicates.

For the two proposed methods: Beer's law limit, detection limit [21], regression equation and correlation coefficient obtained by least-squares treatment of these results are given in Table 4. The intercept value was not statistically ($P < 0.05$) different from zero for each compound.

The ^{1}D and HPLC methods for determination of cinchocaine HCl were compared. The calibration graph of the HPLC method was found to be more linear than that of the ^{1}D method, based on relative standard deviation of the slope. Relative sensitivity, based on detection limit, was calculated. The HPLC method was found to be 1.9 times more sensitive than the ^{1}D method.

The two proposed methods are highly selective towards cinchocaine HCl (I) and 2-hydroxyquino-

Table 6
Determination of cinchocaine HCl (I), methylparaben (IV) and propylparaben (V) in Neocaine cream

Exp. no.	Concentration (μg ml^{-1})			% found of			
	I	IV	V	I		IV (HPLC)	V (HPLC)
				[1]D	HPLC		
1	30	1.8	1.2	99.8	99.2	100.2	100.4
2	40	2.4	1.6	100.8	100.5	100.4	99.6
3	50	3.0	2.0	99.6	100.0	99.6	99.2
4	60	3.6	2.4	100.9	100.8	100.7	100.4
5	70	4.2	2.8	99.9	99.4	99.2	99.6
6	80	4.8	3.2	100.7	100.6	99.4	100.2
Mean				100.3	100.1	99.9	99.9
R.S.D. (%)				0.58	0.66	0.60	0.50

line-4-carboxylic acid diethylaminoethylamide (II); regarding *n*-butanol (III), it is assumed that its ultraviolet absorption characteristics are relatively low to be detected by the assay conditions used in this work.

The accuracy of the two proposed methods were checked by analyzing seven laboratory-prepared mixtures of cinchocaine HCl, its degradation product (II), and methylparaben and propylparaben, at various concentration ratios (Table 5). Satisfactory recoveries with small relative standard deviations (R.S.D.) were obtained, which indicated the high repeatability and accuracy of the two methods.

3.3. Cream analysis

The two proposed methods were applied to the determination of the cinchocaine HCl in commercial cream. Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding different amounts of intact (I) and acid-degraded (II) cinchocaine HCl, and methylparaben and propylparaben, to the previously analyzed cream. The recovery of the added compounds were calculated by comparing the concentration obtained from the spiked mixtures with those of the pure compounds. The results of analysis of the commercial cream (Table 6) and

Table 7
Recovery of cinchocaine HCl (I), its degradation product (II), and methylparaben (IV) and propylparaben (V) added to commercial cream

Exp. no.	Conc. in sample (μg ml^{-1})			Conc. added (μg ml^{-1})				% Recovery of added			
	I	IV	V	I	II	IV	V	I		IV (HPLC)	V (HPLC)
								[1]D	HPLC		
1	20	1.2	0.8	30	30	1.8	1.2	100.2	100.4	99.9	100.6
2	30	1.8	1.2	20	20	1.2	0.8	99.4	99.1	100.2	99.5
3	40	2.4	1.6	10	25	0.6	0.4	100.4	99.6	99.8	100.2
4	50	3.0	2.0	20	30	1.2	0.8	100.9	100.3	100.7	100.3
5	10	0.6	0.4	40	20	2.4	1.6	99.6	100.6	100.4	100.8
6	60	3.6	2.4	20	30	1.2	0.8	99.1	99.8	99.1	99.2
Mean								99.9	100.0	100.0	100.1
RSD (%)								0.68	0.57	0.56	0.63

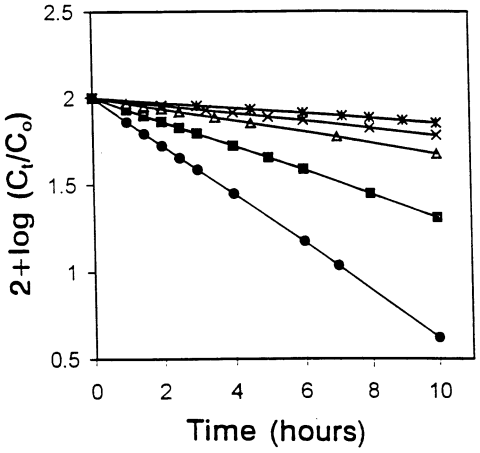


Fig. 9. Pseudo first-order plots for the degradation of cinchocaine HCl in 0.1 N hydrochloric acid at various temperatures using HPLC. Key: 90°C (●); 80°C (■); 70°C (△); 65°C (×); and 60°C (*); C_t, concentration at time t, and C_o, concentration at time zero.

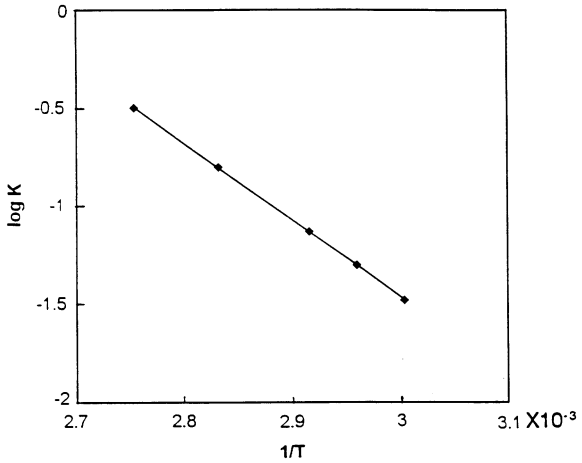


Fig. 10. Arrhenius plot for the degradation of cinchocaine HCl in 0.1 N hydrochloric acid using HPLC.

the recovery study (standard addition method) (Table 7) suggested that there is no interference from any excipients which are normally present in cream.

The results of determination of cinchocaine HCl in cream obtained from the ^{1}D method were compared with the HPLC method. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F-ratio test at 95% confidence level. The calculated Student's t-test and F-ratio values were found to be 0.56 and 1.32 ($n = 6$), while the theoretical values are 2.23 and 5.05, respectively. It is clear that there is no significant difference between the two proposed methods with regard to accuracy and precision.

Expired batches of Neocaine cream stored at ambient temperature under normal conditions were analyzed by the proposed HPLC method, the degraded cinchocaine (II) was clearly found.

Table 8
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for cinchocaine HCl

Temperature (°C)	K_{obs} (h^{-1}) determined by		$t_{1/2}$ (h) determined by	
	^{1}D	HPLC	^{1}D	HPLC
90	0.318	0.317	2.18	2.19
80	0.159	0.158	4.35	4.38
70	0.073	0.074	9.44	9.36
65	0.050	0.050	13.83	13.94
60	0.033	0.033	21.13	21.00

3.4. Kinetic investigation

To assess the specificity and selectivity of the method for the assay of intact cinchocaine HCl without interference from its acid degradation product, acid degradation of cinchocaine HCl was carried out under the previously described experimental conditions. A regular decrease in the concentration of intact cinchocaine HCl with increasing time intervals was observed. The influence of temperature on the degradation process in 0.1 N hydrochloric acid is shown in Fig. 9. At the selected temperatures (90, 80, 70, 65 and 60°C), the acid-degradation process followed pseudo first-order kinetics. From the slopes of the straight lines it was possible to calculate the apparent first-order degradation rate constant and the half-life at each temperature, determined by ^{1}D and HPLC methods (Table 8). Plotting log K_{obs} values vs. $1/T$, the Arrhenius plot (Fig. 10) was obtained, which was found to be linear in the temperature range 60–90°C. The activation energy was calcu-

lated to be 18.13 and 18.08 kcal mol^{-1}, determined by the ^{1}D and HPLC methods, respectively.

4. Conclusion

The proposed ^{1}D and HPLC methods provides simple quantitative analysis for the assay of cinchocaine HCl in pharmaceutical preparations and in the presence of its acid-degradation product. The proposed HPLC method can be conveniently used for the routine determination of cinchocaine HCl, methylparaben and propylparaben in pharmaceutical cream.

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