ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 13–23

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Spectrophotometric determination of loperamide hydrochloride by acid-dye and charge-transfer complexation methods in the presence of its degradation products

Z.A. El Sherif [a,*], A.O. Mohamed [a], M.I. Walash [b], F.M. Tarras [c]

[a] *National Organization for Drug Control and Research, Giza, Egypt*
[b] *Department of Pharmaceutical Analytical Chemistry Faculty of Pharmacy, Mansoura University, Mansoura, Egypt*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

Received 3 April 1998; received in revised form 20 August 1999; accepted 28 August 1999

Abstract

Two simple, sensitive and accurate spectrophotometric methods for the determination of loperamide hydrochloride (lop. HCl) are described. The first method is based on the formation of ion-pair association complex (1:1) with bromothymol blue (BTB), bromophenol blue (BPB) and naphthol blue black B (NBB). The coloured products are extracted into chloroform, and measured spectrophotometrically at 414 (BTB), 415 (BPB) and 627 nm (NBB). Beer's law was obeyed in the ranges of 5–35, 5–30 and 0.8–11.2 μg ml^{-1} for BTB, BPB and NBB methods, respectively. The method was found to be specific for the analysis of the drug in presence of its degradation products which can be detected by HPLC procedure. The second method is based on the reaction of the basic loperamide with iodine in chloroform to give molecular charge-transfer complex with intense bands at 295 and 363 nm. Beer's law was obeyed in the ranges 2.5–17.5 and 2.5–22.5 μg ml^{-1} for the method at 295 and 363 nm, respectively. Optimization of the different experimental conditions are described for both methods. The proposed methods have been applied successfully for the analysis of the drug in pure form and in its dosage forms. The methods have the advantage of being highly sensitive and simple for the determination of a small dose drug, which is also a weak UV-absorbing compound.

Keywords: Loperamide hydrochloride; Spectrophotometry; Ion-pair; Charge-transfer; Degradation products

1. Introduction

Loperamide hydrochloride (lop. HCl) 4-(4-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidine butanamide monohydrochlo-

* Corresponding author. Present address: National Organization for Drug Control and Research, P.O. Box 29, 6 Abou-Hazem St, Pyramids Avenue, Cairo, Egypt. Tel.: +20-2-851-299/278; fax: +20-2-585-5582.

0731-7085/00/$ - see front matter
PII: S0731-7085(99)00239-3

ride is a potent long acting antidiarrhoea and smooth muscle relaxant agent [1]. The biomedical, pharmacology and physico-properties of the drug have been reviewed [2]. Several methods have been reported for the quantification of the drug either in pure form or in its dosage forms including HPLC [3], GC-MS [4] colorimetric [5], spectrofluorimetric [6], non-aqueous titration [7] and derivative spectrophotometric [6,8]. Nevertheless, the paucity of literature references on the analysis of the drug in presence of its degradation product is surprising. The weak UV-absorption of lop. HCl (A 1%, 1 cm is 13.2 in ethanol [9]) in addition to the small dose (2 mg per capsule, 1 mg per 5 ml syrup) means that the direct spectrophotometric assay is susceptible to interference from formulation excipient and this necessitates the development of alternative methods for the analysis.

An extractive-spectrophotometric method for the determination of different organic base has been described [10]. The method is based on the reaction of basic nitrogenous compounds with an acidic dye, to yield ion-pair salts that may be extracted into organic solvent such as chloroform and dichloroethane [11]. The separated ion pair is determined colorimetrically. The acid-dye technique is used for the British pharmacopoeial assays of formulations containing amines salts such as biperidine lactate injection and clonidine hydrochloride injection.

This paper introduces three spectrophotometric procedures which involves ion-pair formation with bromothymol blue (BTB), bromophenol blue (BPB) and naphthol blue black B (NBB) followed by extraction of the ion pair with chloroform as well as a charge-transfer complexation method using δ-acceptor iodine in chloroform. The proposed procedures were applied in presence of degradation products which can be prepared by alkaline oxidation with $KMnO_4$ and detected by HPLC. No interference were observed in the presence of two fold excess of degradation by the acid-dye method.

2. Experimental

2.1. Instruments

All spectral measurements were carried out using Beckman Du64 spectrophotometer with 1 cm cells. For chromatographic (HPLC) procedure the system consisted of Waters (Model 684, Milford, USA), Tunable absorbance detector, Water 600 pump, Waters 600 controller. The peak area integration was performed using Waters 746 detector. The column used was Nucleosil 10 C18 (ET 250/8/4), methanol as mobile phase, at a flow rate of 2 ml min^{-1} and chart speed 0.5 cm min^{-1}.

2.2. Materials and reagents

Lop. HCl obtained from Solchem Italiano S.P.A Lot 126 591 501 certified to contain 100.12% BTB (BDH, Pool, UK), 1×10^{-3} M in ethanol:water, 1:4 (stable for several weeks). BPB obtained from Kock light laboratories, 2×10^{-3}M in ethanol:water, 1:4 (stable for several weeks). NBB from Fluka AG, CH-9470 Buchs, 1×10^{-3} M aqueous solution (stable for several weeks). Sulphuric acid solution, 0.2% v/v in water. Iodine solution, 1×10^{-3} M in chloroform. Chloroform and ethanol (BDH, Pool, UK) were used.

2.3. Dosage forms

Imodium capsules (Galaxo Wellcome Egypt-S.A.E., Cairo, Egypt under licence from Janssen Pharmaceutica, Beerse, Belgium), batch no. 51583A labelled to contain 2 mg per capsule. Loperazine capsule (Alex. for pharmaceuticals, Alexandria, Egypt), batch no. 5 050 009 labelled to contain 2 mg per capsule. Lopodium capsules (The Memphis Chemicals Co., Cairo, Egypt), batch no 394 196 labelled to contain 2 mg per capsule. Standard drug solution; stock solution 1 mg ml^{-1} of lop. HCl was prepared in ethanol and further diluted to contain 50–350 μg ml^{-1} for procedure A and B and 20–280 μg ml^{-1} for procedure C.

2.4. Procedures

2.4.1. Acid-dye method

2.4.1.1. Procedure A using BTB. A 1 ml lop. HCl solution containing 50–300 μg ml^{-1} was measured accurately in a 50 ml separating funnel. A 2 ml of BTB solution was added and completed to 10 ml with water. The mixture was extracted with two 5 ml portions of chloroform. The chloroformic extracts were dried over anhydrous sodium sulphate, collected in 10 ml volumetric flask and completed to volume with chloroform. The absorbance was measured at 414 nm against a blank reagent prepared simultaneously. The concentration of lop. HCl was calculated from the corresponding regression equation (Table 1).

2.4.1.2. Procedure B using BPB. A 1 ml lop. HCl solution containing 50–300 μg ml^{-1} was measured accurately in a 50 ml separating funnel. A 2.5 ml of BPB solution was added. The aqueous solution was completed to 5 ml with water. The mixture was extracted with two 5 ml portions of chloroform. The chloroformic extracts were dried over anhydrous sodium sulphate, collected in 10 ml volumetric flask and completed to volume with chloroform. The absorbance was measured at 415 nm against a blank reagent prepared simultaneously. The concentration of lop. HCl was calculated from the corresponding regression equation (Table 1).

2.4.1.3. Procedure C using NBB. A 1 ml lop. HCl solution containing 20–280 μg ml^{-1} was measured accurately in a 50 ml separating funnel. Then 1 ml of sulphuric acid solution and 2 ml of NBB solution was added. The aqueous volume was adjusted to 10 ml with water. The mixture was extracted with two 10 ml portions of chloroform. The chloroformic extracts were dried over anhydrous sodium sulphate, collected in 25 ml volumetric flask and completed to volume with chloroform. The absorbance was measured at 627 nm against a blank reagent prepared simultaneously. The concentration of lop. HCl was calculated from the corresponding regression equation (Table 1).

2.4.1.4. Analysis of pharmaceutical preparations. Ethanol (25 ml) was added to a quantity of the mixed content of 20 capsules equivalent to 7.5 mg of lop. HCl, shaken for 10–20 min and filtered. The filter paper was washed with ethanol, the filtrate diluted and washed to 50 ml with the same solvent. Procedure A, B or C was then followed.

2.4.2. Charge-transfer method

2.4.2.1. Standard drug solution. An accurately weighed amount of lop. HCl salt (about 25 mg) was transferred into a 100 ml separating funnel and dissolved in ≈ 2 ml ethanol then completed to 100 ml with water. The solution was rendered alkaline with 10 ml of 1N NaOH solution and extracted successively with five 20 ml portions of chloroform. Each chloroformic extract was filtered through a filter paper containing a few crystals of anhydrous sodium sulphate. The chloroformic extracts were collected in a 100 ml volumetric flask and diluted to volume with chloroform. This solution was further diluted with chloroform to give a solution containing equivalent to 2.5–25 μg ml^{-1}. In a 10 ml volumetric flask 1 ml of the drug solution was placed and 3 ml of iodine solution was added. The solution was kept in the dark for 30 min, then completed to volume with chloroform. The absorbance was measured at 295 and/or 363 nm. The concentration of lop. HCl was calculated from the corresponding regression equation (Table 1).

2.4.2.2. Preparation of degradation products of lop. HCl. A mixture of 2 mg of $KMnO_4$ and 82.4 mg of disodium hydrogen phosphate dissolved in 20 ml water was added to 100 mg of the drug dissolved in 10 ml of 4% aqueous methanol, dropwise with continuous sonication for an additional 30 min, at room temperature (25 ± 2°C). The whole mixture was extracted with 3×20 ml chloroform. The aqueous layer was reacidified with 40 ml dilute sulphuric acid, followed by 4 ml methanol and warmed gently to decompose excess $KMnO_4$ and dissolve the formed manganese dioxide. The aqueous layer was further extracted with 2×20 ml chloroform and rendered just alkaline (pH ≈ 7.5) using dilute aqueous solution of

Table 1
Analytical parameters for the determination of lop. HCl using BTB, BPB, NBB and iodine charge-transfer methods

Method	Wavelength of maximum absorbance (nm)	Concentration range (μg ml^{-1})	Number of exp.	Linear regression					ε^b 1 mol^{-1} cm^{-1}
				Intercept (a)	RSR (%)	Slope (b)	RSD[a] (%)	Correlation coefficient (r)	
BTB	414	5–35	7	−0.014	0.02	0.036	0.42	0.9994	1.80×10^4
BPB	415	5–30	6	−0.005	0.09	0.034	0.28	0.9998	1.77×10^4
NBB	627	0.8–11.2	9	−0.016	0.05	0.068	0.64	0.9995	3.47×10^4
Iodine charge-transfer	295	2.5–17.5	7	−0.017	0.07	0.058	1.5	0.9991	2.78×10^4
	363	2.5–22.5	8	−0.011	0.08	0.038	1.53	0.9992	1.83×10^4

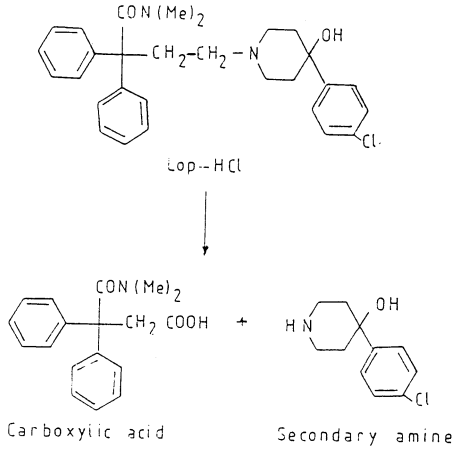
[a] Relative standard deviation.
[b] Apparent molar absorptivity.

sodium hydroxide, then reextracted with 20 ml chloroform. The organic extracts were combined and evaporated under vacuum until complete dryness. Into a 25 ml volumetric flask the solid residue was transferred quantitatively, then dissolve in ethanol containing 0.1 ml N/10 hydrochloric acid. The resulting solution was filtered to remove any undissolved solid. The stock solution was diluted quantitatively to obtain degraded sample of the required concentrations.

3. Results and discussion

As shown in Scheme 1, two main centers in the structure of lop. HCl, amide group and basic piperidine nitrogen represents the bases of search for an analytical method that can be used for its determination.

The reported methods for the analysis of some amide derivatives are time consuming with low sensitivity levels [13]. Therefore the piperidine nitrogen could be considered as good target for our purpose. Our attention was focused on ion-pair extraction technique on the fact that the cited drug is relatively basic as indicated by its pK_a (8.66). The acid-dye method has been applied to the determination of lop. HCl using $(NH_4)_2Co(SCN)_4$ [14], bromocresol purple [15] and tropaeolin 00 [5]. However none of the mentioned methods gave any attention regarding their possible application for the analysis of its degradation products. Recently, methyl orange used for the analysis of the drug in presence of its degradation products [12].



Scheme 1.

3.1. Acid-dye method

BTB, BPB and NBB were chosen to give the most stable and sensitive acid-dye procedure in the presence of the degradation products. The reaction stoichiometry of the associated ion-pair was found to be 1:1 using the three acid dyes. This confirms our finding as the piperidine nitrogen is the only basic center in this drug.

The absorption spectra of the ion-pair complexes formed between lop. HCl and each of BTB, BPB and NBB were measured at 250–700 nm against a blank reagent and shown in (Fig. 1).

3.2. Effect of pH

For BTB and BPB phthalate buffer was the buffer of choice, which did not interfere and gave the highest sensitivity. Different pH (2–6) were tested and the absorbance reading of the drug-dye ion-pair was examined (Fig. 2). The results showed that the most efficient extraction of the ion-pair with chloroform was obtained at pH 3.2–5.5 using BTB and pH 2.0–4.0 using BPB, where maximum absorbance were achieved. It was observed that the pH of the mixture of the drug solutions and each of the chosen dyes lies in these ranges of pH so the method can be applied without using the buffer. While in case of NBB it was observed that absorbance increase with decreasing pH of the solution, so acidification with 1 ml of 2% sulfuric acid was used for giving the highest sensitivity.

3.3. Dye concentration

The effect of the dye-concentration on the intensity of the colour developed at the selected wavelengths was ascertained using different millilitres of the reagent (0.5–4 ml), 1.5–2.5 ml of 1×10^{-3} M BTB solution, 2.5–3.5 ml of 2×10^{-3} M BPB solution and 1–3 ml of 1×10^{-3} M NBB solution were optimum.

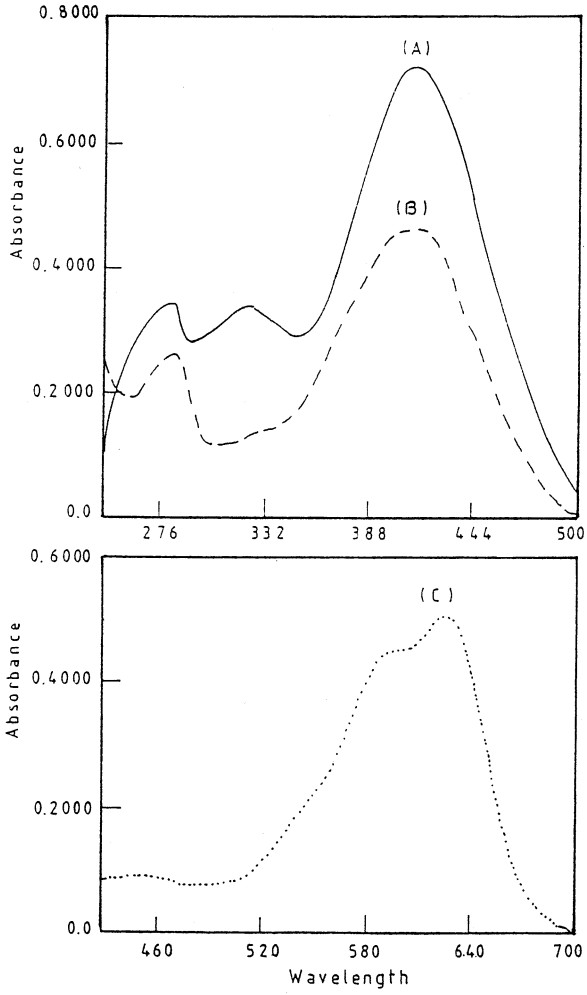


Fig. 1. Absorption spectra of lop-ion-pair in chloroform (A) BTB 1×10^{-3} M, (B) BPB 1×10^{-3} M, (C) NBB 1×10^{-3}.

3.4. Phase ratio

The ratio of aqueous to organic phase was ineffective in the case of BTB and NBB and the ratio 1:1 was used, while the ratio of 2:1 was chosen for the method using BTB for efficient extraction of the coloured species.

3.5. Stoichiometric relationship

The composition of ion-pair was determined by Job's method using equimolar solution. In all cases the plots reached maximum value at a mole fraction of 0.5, indicating the formation of 1:1 ion-pair (Fig. 3).

3.6. Iodine charge-transfer complexation method

Lop. HCl molecules, being n-electron donors, react with the δ-acceptor iodine to give a characteristic complex. Upon reaction of the drug with a chloroformic solution of iodine, the iodine colour fades to a pale pink colour. The absorption spectra of the product exhibit intense bands at 295 and 363 nm.

The effect of iodine concentration and reaction time selected was studied. By varying the concentration of iodine in the range 0.5–6 ml of 1×10^{-3} M solution, the optimum concentration was found to be 3 ml of 1×10^{-3} M. Although the complex forms rapidly, constant absorbance readings are obtained only after the solution has stood for 30 min in the dark after which the absorbance remains constant for about 1 h.

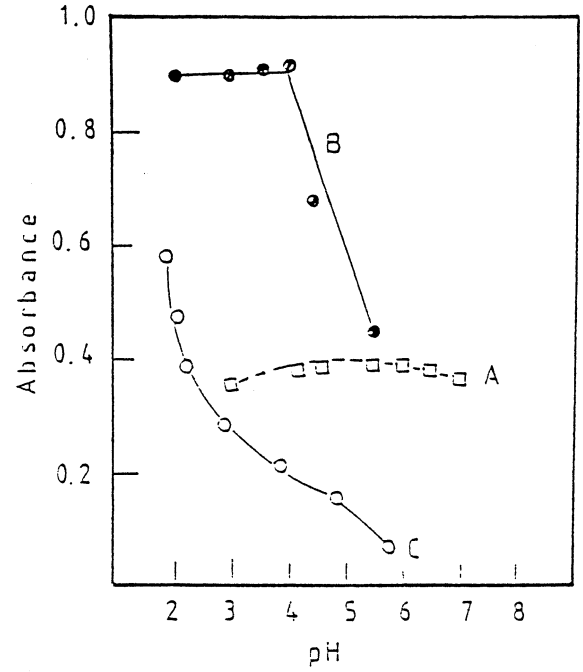


Fig. 2. Effect of pH on the absorbance of lop. acid-dye (A) using BTB (lop. HCl 12.5 μg ml^{-1}), (B) using BPB (lop. HCl 25 μg ml^{-1}), (C) using (lop. Hcl 10 μg ml^{-1}).

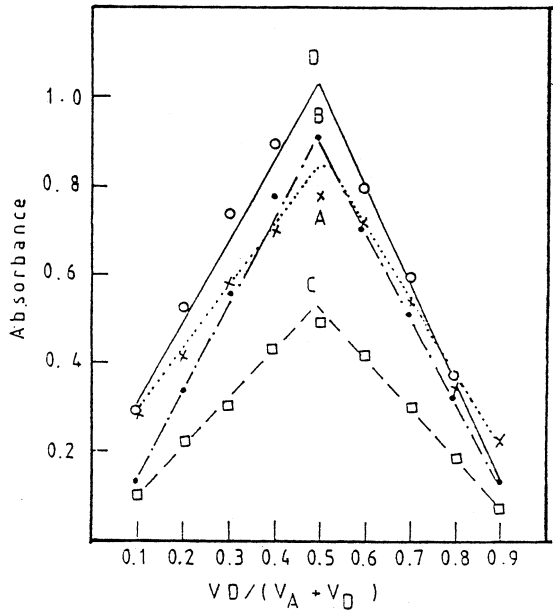


Fig. 3. Job's method of continuous variation of lop. HCl by acid-dye charge-transfer (0.5×10^{-3} M) methods. (A) BTB, (B) BPB, (C) NBB, (D) iodine.

3.7. Stoichiometric relationship.

The molar ratio of the reactant (loperamide:iodine) was determined by the method of continuos variation (Job's method), and found to be 1:1 (Fig. 3).

4. Validation data

4.1. Linearity

Under the experimental conditions described calibration graphs were constructed for both methods the acid-dye method and iodine charge-transfer method (Table 1) over the concentration ranges cited. Regression analysis indicated linear relationship between absorbance and concentration. The correlation coefficients were between 0.9994 and 0.9998.

4.2. Reproducibility, repeatability and precision of the proposed methods

Replicate determination of different concentration levels was carried out and their concentrations calculated from the respective regression equations. The mean percentage recoveries range from 99.26 to 100.77 with relative standard deviation values $< 1\%$ (Table 2). Also, LOD values were calculated for the proposed methods and were found to be 2, 0.9, 0.5 and 1 μg ml^{-1} for the methods using BTB, BPB, NBB and iodine. LOD values were calculated and were found to be 4.1, 3.8, 0.6 and 2.0 μg ml^{-1} for the proposed procedures, respectively.

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration and phase ratio. The capacity remain unaffected by small deliberate variations.

Method ruggedness was expressed as %RSD of the same procedure applied by two different operators in different laboratories on different days. Means and %RSD for the proposed method were 0.71, 0.58, 0.53 and 1.2 for BTB, BPB, NBB and iodine charge-transfer methods, respectively.

The proposed methods have been successfully applied to the determination of lop. HCl in some commercial preparations. The results were assessed by the standard addition technique and *t*- and *F*-values were calculated compared with the official colorimetic method [5]. The results obtained were found to be in good agreement with the proposed methods, In addition the proposed methods are more simple and less time consuming (Table 3).

4.3. Analysis of lop. HCl in presence of its degradation products

Lop. HCl is a stable drug at room temperature, and when protected from direct light. The stability of the drug has been studied under vigorous conditions such as heat, light, alkali, acid, water and strong oxidants [2]. With the exception of strong oxidants, the drug is considered fairly stable. Oxidative deamination of some tertiary amines, having an hydrogen atom on a carbon, to nitrogens with a number of oxidising agents have

Table 2
Analysis of lop. HCl in bulk powder by acid-dye methods, charge-transfer method and the official method

Exp. no.	Acid-dye						Charge-transfer		Official method	
	BTB		BPB		NBB					
	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)	Taken (mg)	Recovery (%)
1	10	99.72	5	100.00	4	99.63	10	99.83	100	100.13
2	15	100.28	10	100.58	6	99.27	12.5	100.45	200	100.13
3	20	99.16	15	100.39	8	99.26	12.5	99.37	300	99.92
4	25	99.94	25	99.88	9.6	100.77	12.5	100.5	375	99.96
5	30	99.91	30	100.29	10	99.41	15	99.14	400	100.45
$\overline{X}$		99.8		100.23		99.67		99.86		100.12
S.D.		0.41		0.29		0.63		0.62		0.21
C.V.		0.41		0.29		0.63		0.62		0.21
t		1.55[a]		0.69[a]		1.52[a]		0.89[a]		
F		0.26[b]		1.91[b]		0.11[b]		0.11[b]		

[a] Theoretical value 2.206 ($P = 0.05$).
[b] Theoretical value 6.39 ($P = 0.05$).

Table 3
Determination of commercial capsules with the proposed methods and reference methods

Preparation	Recovery ± standard deviation (%)[a]				
	Acid-dye method			Iodine charge-transfer method	Reference[b] method
	BTB	BPB	NBB		
Imodium capsule[c]					
$\bar{X} \pm$ S.D.	100.75 ± 1.14	101.50 ± 0.42	101.24 ± 0.42	99.88 ± 0.96	102.79 ± 1.56
C.V.	1.13	0.41	0.42	0.96	1.52
t	1.83	13.8	13.8	2.77	
F	1.87	13.8	13.8	2.64	
Lopodium capsule[c]					
$\bar{X} \pm$ S.D.	100.35 ± 1.60	102.18 ± 0.39	100.86 ± 0.32		102.40 ± 0.96
C.V.	1.59	0.48	0.31		0.94
t	1.9	0.37	2.64		
F	0.36	6.06	9		
Loperazin capsule[c]					
$\bar{X} \pm$ S.D.	101.74 ± 0.70	100.15 ± 1.52	101.89 ± 0.93		102.56 ± 1.02
C.V.	0.69	1.51	0.91		0.99
t	1.15	2.28	0.84		
F	2.12	0.45	1.2		

[a] The average of three replicates. The theoretical *t*-value is 2.776 ($P = 0.05$). The theoretical *F*-values is 19 (95%).
[b] The U.S.P. XXI method.
[c] Labelled to contain 2 mg per capsule.

been studied and among the products observed were secondary amine, aldehyde or ketones [16]. Therefore the interest was focused on the oxidative cleavage of the cited drug using $KMnO_4$ to prepare the degraded sample [12]. Since the drug is present in the form of hydrochloride salt it is better to run the reaction in a alkaline medium. For this reason, alkaline $KMnO_4$ solution using disodiums hydrogen phosphate (pH 9);was used through out this investigation. Complete disappearance of the tested drug was indicated through TLC (used for the identification of lop. HCl in BP 1993) and by HPLC where the peak of loperamide disappears and another two sharp peaks appear (Fig. 4). This confirms the suggestion of formation of the suggested degradation (Scheme 1).

The main product can be suggested to be secondary amine (4-hydroxy-4-chlorophenyl-piperidine) and carboxylic acid (*N*,*N*-dimethylformamide-α,α-diphenyl acid) after oxidative cleavage at α,α-diphenyl butamide side chain (Scheme 1).

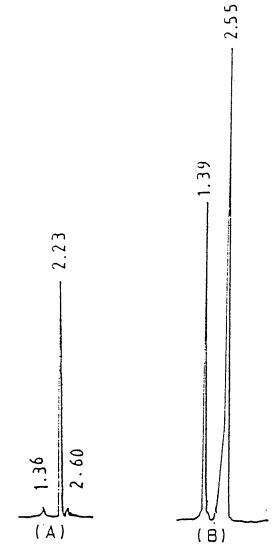


Fig. 4. Chromatograms of (A) lop. HCl 10 μg ml^{-1}and (B) degraded products of lop. HCl (40 μg ml^{-1}).

Table 4
Analysis of lop. HCl in the presence of its degradation products

Exp. no.	BTB method		BPB method		NBB method	
	Concentration of degradation product added[a] (μg ml^{-1})	Recovery (%)	Concentration of degradation product added[a] (μg ml^{-1})	Recovery (%)	Concentration of degradation product added[b] (μg ml^{-1})	Recovery (%)
1	4	97.32	4	97.82	2	99.4
2	8	97.47	8	98.11	4	100.74
3	16	100.7	16	102.47	10	101.19
4	24	102.39	24	103.49	20	100.89
5	32	101.83	32	104.51	–	–
6	40	103.29	40	104.65	–	–

[a] Each mixture contains 20 μg ml^{-1} of lop. HCl.
[b] Each mixture contains 10 μg ml^{-1} of lop. HCl.

Applying the proposed procedures to the degraded sample shows that no colour was observed using the acid-dye method; while the degradation product interfered with the charge-transfer method. Analysis of synthetic mixture each containing a fixed amount of the drug, plus a certain amount of degraded samples, indicates the absence of interference in the specified range for the three dyes used (Table 4). Therefore, the proposed methods are stability indicating and can be used for the routine analysis of lop. HCl formulations.

References

[1] J.E.F. Reynolds, Martindaile, The Extra Pharmacopoeia, 28 ed. The Pharmaceutical Press, London, 1982.
[2] K. Florey, Anal. Profile Drug Subst. 19 (1990) 341.
[3] K. Florey, Anal. Profile Drug Subst. 19 (1990) 360.
[4] H.J. Leis, H. Gleispach, J. Chromatogr. Biomed. Appl. 86 (1989) 324.
[5] The United States Pharmacopoeia, vol. XXI, Mack Printing Company, Easton, PA. 1985, p 605.
[6] I.I. Hewala, J. Pharm. Biom. Anal. 13 (6) (1995) 761.
[7] The United State Pharmacopoeia, vol. XXIII, NF XVIII, Rand McNally, Taunton, MA, Rockville, MD, USA 1995, p 900.
[8] P.Y. Kashaba, Alex. J. Pharm. Sci. 9 (1995) 1.
[9] E.G.C. Clark, Isolation and Identification of Drugs, The Pharmaceutical Society of Great Britain, 1986, p. 709.
[10] T. Higuchi, E. Brochmann-Hanssen, Pharmaceutical Analysis, Interscience, New York, 1961, p. 43.
[11] A.G. Davidson, in: A.H. Beckett, J.B. Stenlake (Eds.), Practical Pharmaceutical Chemistry, Part 2, Athalone Press, London, 1988, p. 304.
[12] P.Y. Khashaba, O.H. Abdelmageed, Egypt J. Anal. Chem. 4 (1) (1995) 41.
[13] M. Pesez, J. Bartos, Colorimetric and Fluorimetric Analysis of Organic Compound and Drugs, Marcel Dekker, New York, 1974, p. 319.
[14] D.M. Shingbal, V.R. Rao, Indian Drugs 23 (8) (1986) 472.
[15] K.M. Thomas, Indian Drugs 27 (11) (1990) 586.
[16] S. Patai, The chemistry of functional group, in: The Chemistry of Amino Group, Wiley, London, 1972, p. 429.