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Spectrophotometric Determination of Lornoxicam in Pure and in Pharmaceutical Formulations Using Ion-Associate Complex Formation

Alaa S. Amin[1]

[1]Chemistry Department, Faculty of Science,
Benha University, Benha, Egypt
Asamin2005@hotmail.com

Magda M. El-Henawee[2], Gamal H. Ragab[2], Amal F. Sultan[2]

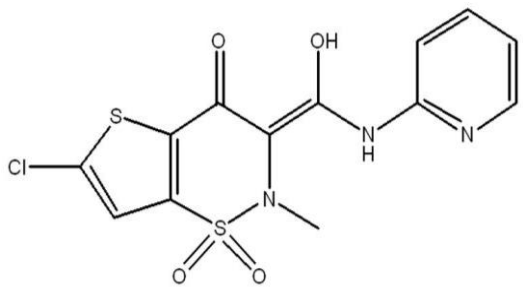
[2]Analytical Chemistry Department,
Faculty of Pharmacy, Zagazig University,
Zagazig, Egypt

Abstract: *Simple, rapid, and sensitive spectrophotometric methods for the determination of lornoxicam (LX) in pure form and in pharmaceutical formulations has been developed. The methods are based on the formation of colored ion-associate complexes between LX and three different reagents, brilliant blue G (BBG), bromocresol green (BCG), and bromocresol purple (BCP) using Britton-Robinson (B-R) buffer solutions. The colored complexes were measured exhibiting λ_{max} at 537, 616 and 650 nm for BBG, BCP and BCG, respectively. The analytical parameters and their effects were investigated. The ion- associate complexes are intensely colored and very stable at room temperature. The calibration graphs were linear over the concentration range of 1-12 µg/mL for BBG and BCG and 1.0 - 18 µg/mL for BCP. The stoichiometry of the ion-associate was found to be 1: 1 for all complexes. The proposed methods were successfully extended to pharmaceutical preparations in tablet dosage form. The results obtained by the proposed methods were comparable with those obtained by the official method.*

Keywords: *Lornoxicam determination, spectrophotometry, dasage forms, ion-associate complex formation*

1. INTRODUCTION

Lornoxicam is (3*E*)-6-chloro-3-[hydroxy(pyridin-2 ylamino) methylene] 2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one 1,1-dioxide. It is yellow or slightly yellow powder. It is poorly soluble in water, soluble in sodium hydroxide, slightly soluble in methanol [1=3]. Lx is a weak acidic NSAID of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. It is available in oral and parental formulations. It has an analgesic property by inhibition of prostaglandin synthesis (inhibition of the cyclo-oxygenase (Cox) enzyme). It used in the muscular skeletal and joint disorders such as osteoarthritis and rheumatoid arthritis. It has low gastrointestinal toxic effects [4, 5]. The chemical structure of LX is shown in Figure (1).



Literature survey revealed that various analytical methods such as HPLC [6-10], reversed phase HPLC [11-14], LC [15, 16], HPTLC [17,18], and voltammetric determination [19]. A few UV-spectrophotometric methods were reported for determination of LX in literature including UV-Spectrophotometry [20-24] and spectrofluorimetry [25,26]. The purpose of the present work was to develop simple, rapid, and sensitive direct spectrophotometric methods for the determination of LX in bulk drug and pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Apparatus

All spectral measurements were made using JASCO V-530 (UV-VIS) spectrophotometer (Japan) with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10-mm matched quartz cells. The pH measurements were performed by using (HI 8014, HANNA Instruments) pH-meter (Italy).

2.2. Materials and Reagents

All solvents and reagents used were of analytical grade and double distilled water was used throughout the work. Lornoxicam reference standard was kindly provided by (Eva Pharma, Egypt). Brilliant blue G, Bromocresol purple, and Bromocresol green were provided by BDH Chemicals Ltd. Company, Poole, England and used without further purification. A series of Britton-Robinson (B-R) universal buffer solutions were prepared according to the standard method [27]. A stock solution of lornoxicam (100 μg/mL) was prepared by dissolving 0.01 g of the reference standard in a 100-mL measuring flask and diluting up to the mark with (1:4) (dimethylformamide (DMF): methanol). Standard solutions of the reagents (1.0×10^{-3} M) were prepared by dissolving accurately weighted acid dyes in a few drops of ethanol and then diluting, separately, to the mark with water in a 100-mL measuring flasks.

2.3. Assay Procedure for Pure Drug

Aliquots of lornoxicam solution containing upto 180 μg/mL were transferred into a series of 10-mL separating funnels. Buffer solutions (5.0 mL) of various pH values (2.7, 6.7 and 6.4) were added to various volumes (3.5, 1.0 and 1.0 ml) of a fixed concentration (1.0×10^{-3} M) of BBG, BCP, and BCG, respectively. The absorbances of the blue-colored species for all reagents (except BCP, which forms a purple-colored product) were measured against a reagent blank at λ_{max} as recorded in Table (1). The calibration curves for the three proposed methods were constructed by plotting the absorbance of the colored product against the final concentration of lornoxicam.

2.4. Assay Procedure for Lornoxicam Formulations

Ten commercial tablets of Zeficam® (8 mg/tablet) were crushed and a weight equivalent to one tablet of lornoxicam was taken, dissolved in (1:4) (DMF: methanol), filtered into 100-mL measuring flask and then completed to volume with the same solvent ratios. Then follow the same procedures described for determination as in authentic sample. A standard addition technique was also used to confirm the accuracy and precision of the methods.

2.5. Procedure for Spiked Urine

Transfer 5.0 mL of urine into a 100 mL separating funnel. Spike with increasing quantities of LX to give a final drug concentration in the LX determination. Add 1.0 mL of $HClO_4$ and shake well. Extract with 3 × 5 mL of chloroform, and then pass the chloroform layer over anhydrous sodium sulfate. Evaporate the extract under reduced pressure till dryness. Dissolve the residue in 5.0 mL of (1:4) (dimethylformamide (DMF): methanol) and then proceed as described under "Assay Procedure for Pure Drug". Determine the nominal content of the drug from the corresponding regression equation.

2.6. Procedure for Spiked Plasma

Transfer 1.0 mL of plasma into a 100 mL separating funnel. Spike with increasing quantities of GLZ to give a final drug concentration in the range of determination. Add 1.0 mL of $HClO_4$ and shake well. Extract with 3 × 5 mL of chloroform, then pass the chloroform layer over anhydrous sodium sulfate. Evaporate the extract under reduced pressure till dryness. Dissolve the residue in 5.0 mL of (1:4) (dimethylformamide (DMF): methanol) and then proceed as described under "Assay Procedure for Pure Drug". Determine the nominal content of the drug from the corresponding regression equation.

3. RESULTS AND DISCUSSION

Anionic dyes form ion-association complexes with the positively charged pharmaceutical drug. The pharmaceutical drug- dye complex, with two oppositely-charged ions, behaves as a single unit held together by an electrostatic force of attraction [28]. Therefore, lornoxicam form ion-associate complexes in acidic medium with acidic dyes as BBG, BCP and BCG. These colored complexes were measured directly, against a reagent blank prepared similarly. The absorption spectra of the complexes were measured from 350 to 800 nm against blank solution containing the same reagent concentration as shown in Figure (2). The maximum absorbance was measured at λ_{max} of the different complexes are recorded in Table (1).

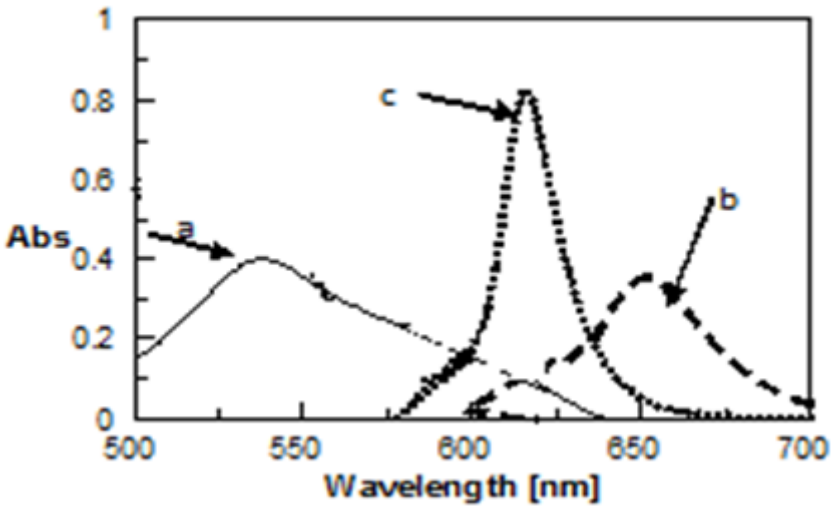


Fig 2. *Absorption spectra of 10 µg/mL lornoxicam complexed with (a)-BBG, (b) -BCG and (c) -BCP reagents.*

3.1. Optimization of the Reaction Conditions

The influence of each of the following variables on the reaction was tested to reach the maximum colour intensity.

3.2. Effect of pH

The effect of pH was studied by measuring the coloured complex directly using a series of B-R buffer solutions in the pH range 2.0 – 9.0. The maximum absorbance values and λ_{max} were obtained at pH 2.7, 6.7 and 6.4 for BBG, BCP and BCG, respectively and also recorded in Table (1). Moreover, the amount of buffer was studied by adding different volumes of optimum pH values (2.7, 6.7 and 6.4) to LX, then dyes stuff (BBG, BCP and BCG) was added and completed the volume with didistilled water to 10 mL then shaken well. The maximum absorbance was measured against the blank solution similarly prepared. Maximum absorbance corresponding to maximum complex formation was obtained at sing 5.0, 4.0 and 5.0 mL for BBG, BCP and BCG, respectively as shown in Figure (3)

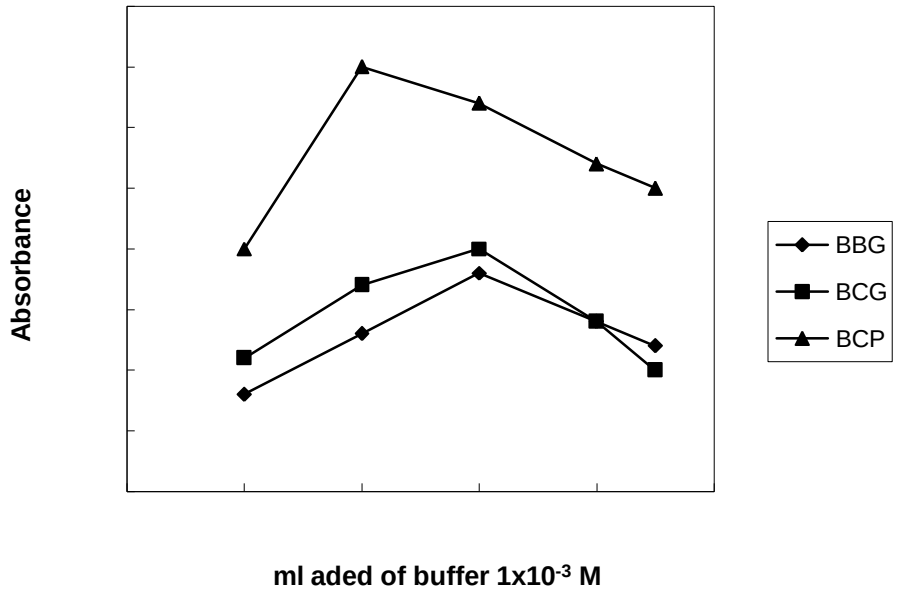


Fig 3. *Effect of ml added of optimum pH values on the absorption of LX complexes.*

Table 1. *Quantitative parameters for determination of Lornoxicam*

Parameters	**Reagents**		
	BCG	BCP	BBG
λ_{max},/ nm	537	616	650
pH	2.7	6.7	6.4
Volume of $1x10^{-3}$ M/ mL	3.5	1.0	1.0
Beer's law limits/ µg/mL	1.0 -12	1.0 -18	1.0– 12
Ringbom range/ µg/mL	1.8- 10.8	1.5 – 16.5	1.5 –11
Linear regression equation*			
Intercept (a)	0.051	0.043	0.033
Slope (b)	0.029	0.026	0.023
Correlation coefficient/ r	0.9990	0.9991	0.9990
Molar ratio	1:1	1:1	1:1
RSD	1.87	1.74	0.591
Molar absorptivity/ 10^3 L mol^{-1} cm^{-1}	10.78	9.667	8.553
Sandell's sensitivity/ ng cm^{-}	0.034	0.038	0.043
LOD,/ µg/mL	0.24	0.27	0.25
LOQ,/ µg/mL	0.8	0.93	0.75
Stability constant	4.09	6.98	6.67

*A = a + b C, where C = concentration of drug in µg/mL,

A = absorbance, a = intercept, b = slope

3.3. Effect of Reagent Concentration

The effect of the reagent concentration on the color intensities of different ion associate complexes was examined at constant concentration of LX (10 µg/mL at the optimum pH values. Maximum color development was obtained on using 3.5, 1.0 and 1.0 ml of 1.0×10^{-3} M for BBG, BCP, and BCG, respectively, as shown in Figure (4).

3.4. Effect of Sequence of Addition and Standing Time

The developed colored ion associate was stable for at least 24 hours at room temperature, while the optimum time was 5.0 minutes for all reagents. The most appropriate sequence of LX with proposed methods was (drug, buffer then dye)

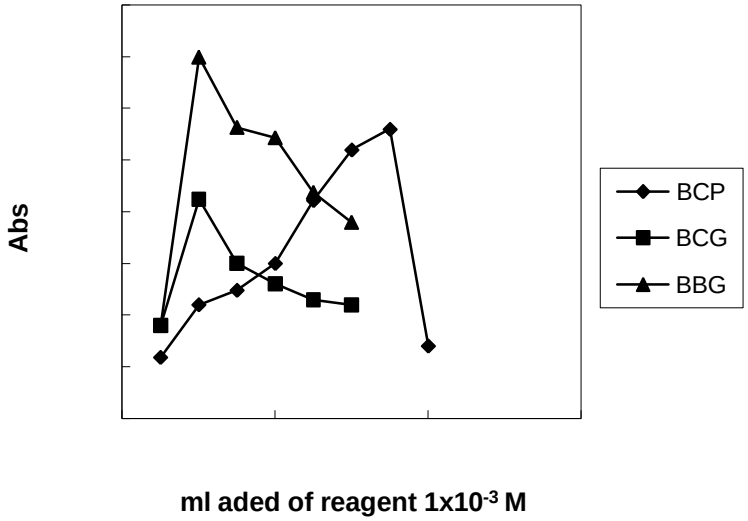


Fig 4. *Effects of ml added of reagents on the absorption of Lornoxicam-dye complexes.*

3.5. Effect of Temperature

The effect of temperature on the colored complexes was studied at different temperatures (25, 30, 35, 40, 45, 50 and 55 °C). It was found that the colored species were stable up to 40 °C. At higher temperatures, the developed color is decreased with increasing temperature which is due to the dissociation of the formed complexes.

3.6. Stoichiometric Ratio

The stoichiometric ratio was determined by Job's method (Figure 5). It was found to be 1: 1 for the three proposed methods.

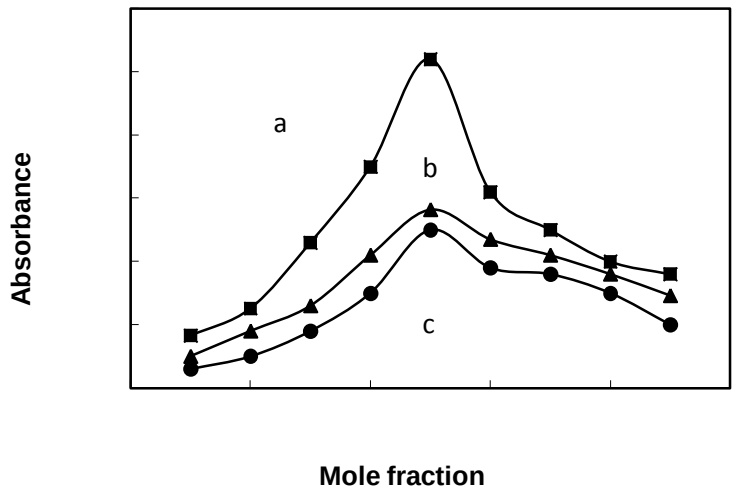


Fig 5. *Job's method of continuous variation of (1x10^{-3} M) LX with (a) BCP, (b) BBG and (c) BCG using 1x10^{-3} M dyes system.*

3.7. Quantification

The limits of the Beer-Lambert law, molar absorptivity, Sandell`s sensitivity, regression equations and correlation coefficients were calculated and given in Table (1). In order to determine the accuracy and precision of the proposed methods, three different concentrations of LX were prepared and analyzed in five replicates and satisfactory results were obtained as recorded in Table (2). The limits of detection (LOD) and limits of quantitation (LOQ) were determined [29] using the formula:

$$\text{LOD or LOQ} = \kappa SDa/b,$$

Where κ =3 for LOD and 10 for LOQ, SDa the standard deviation of the intercept and *b* is the slope. The LOD and values ranged from 0.24, 0.27 and 0.25, whereas LOQ values ranged from 0.8, 0.93, and 0.75 µg/mL, respectively.

3.8. Accuracy and precision

The accuracy of the proposed methods was evaluated by the standard addition method at three different concentrations levels. The recovery values of the added concentrations were 99.15–100.67 ± 0.67–1. 23% (Table 2). This indicated the accuracy of the proposed method. The precisions of the proposed methods were determined by replicate analysis of six separate solutions of the working standards at three concentration levels of LX. The intra-day precision was assessed by analyzing six replicates of each sample as a batch in a single assay run, and the inter-day precision was assessed by analyzing the same sample, as triplicate, in two separate runs. The method gave satisfactory results; the relative standard deviations did not exceed 1.55% indicating the good reproducibility of the proposed method. This precision level is adequate for the precision and routine analysis of the investigated drugs in quality control laboratories.

Table 2. *Evaluation of the accuracy and precision of the proposed methods*

Method	Taken (µg/mL)	Found[a] (µg/mL)	Recovery (%)	Confidence[b] Limits RSD (%)	RE (%)	
BCP	2	2.17	100.85	1.1339	1.0808	2.17 ± 0.0218
	4	3.98	99.50	0.8594	0.8191	3.98 ± 0.0326
	6	6.11	100.10	1.2429	1.1846	6.11 ± 0.0712
BCG	2	1.97	98.50	0.6604	0.6294	1.97 ± 0.0124
	4	4.23	100.50	1.2318	0.8300	4.23 ± 0.0332
	6	5.99	99.80	0.7461	0.7112	5.99 ± 0.0426
BBG	3	3.13	100.33	1.6208	1.169	3.13 ± 0.0352
	5	5.22	100.04	0.6939	0.940	5.22 ± 0.0472
	7	6.97	99.57	0.3191	0.4246	6.97 ± 0.0296

* a= Relative standard deviation for five determinations.

*b= 95% confidence limits and five degrees of freedom.

3.9. Robustness and Ruggedness

Robustness was examined by evaluating the influence of small variation of method variables including concentrations of analytical reagents, and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed where as the others were kept unchanged, and the recovery percentage was calculated each time. It was found that none of these variables significantly affect the method; the recovery values were 99.00–100.83 ± 0.55–1.23%. This provided an indication for the reliability of the proposed methods during its routine application for analysis of the investigated drugs. Ruggedness was tested by applying the proposed methods to the assay of LX using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day to-day variations were found to be reproducible, as RSD did not exceed 1.85 %.

3.10. Analytical Applications

The proposed methods have been successfully applied for the determination of LX in pharmaceutical formulations. For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed methods as given in Table (3), since the ion-pair complexes are stable for at least 24 h. The high percentage recoveries indicate that the excipients in pharmaceutical dosage forms of LX such as (talc, glucose, starch, lactose, sulfate, dextrose, and acetate) were not found to exhibit any interference in the analysis.

3.11. Analysis of Biological Fluids

The high sensitivity of the proposed method allowed the determination of LX in biological fluids. The proposed method was further applied to the in-vitro determination of LX in spiked human

urine and plasma. LX is readily absorbed from the gastro-intestinal tract. The half-life time is about 10–12 h. LX is extensively metabolized without significant hypoglycemic activity. The usual initial dose is 40–80 mg daily, gradually increased if necessary, up to 320 mg daily. Doses of more than 160 mg daily are given in two divided doses. Following oral ingestion of a single 80 mg LX dose gives a final plasma concentration of about 1.6 µg/mL. This value lies within the working concentration range of the proposed methods, thus it could be successfully applied to the determination of LX in spiked human urine and plasma over the specific concentration range. The results are abridged in Table 7. The mean percentage recoveries for LX in spiked urine and plasma are 99.20 to 100.04 ± 0.43 to 0.95 (n = 6). The method involved extraction of the drug using chloroform. The extraction procedure described by Wang et al [30] was adopted.

Table 3. *Evaluation of accuracy and precision of the standard addition methods*

Method	Taken µg/mL	Added µg/mL	Found µg/mL	Recovery (%)	RSD[a] %	RE%
BCP	4	0	3.9	99.75	1.668	- 0.010
	4	1	4.9	99.98	1.889	- 0.020
	4	2	5.9	99.97	0.49	- 0.033
BCG	5	0	5.06	100.12	1.012	0.012
	5	2	7.02	100.02	2.025	0.028
	5	3	7.9	99.98	0.796	- 0.016
BBG	7	0	7.04	100.05	1.638	0.057
	7	1	8.01	100.01	1.456	0.012
	7	2	8.9	99.98	1.673	- 0.011

* a= Relative standard deviation for five determinations.

4. CONCLUSION

The proposed methods are simple, precise, accurate and sensitive. Therefore, they can be used for routine analysis and quality control assay of LX in raw material and tablets dosage forms without interference caused by the excipients expected to be present in tablets.

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