Exploring the Use of Sodium Benzoate as Hydrotrope for the Estimation of Lornoxicam in their Marketed Formulation

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ABSTRACT

Increasing the aqueous solubility of insoluble drugs is of major importance for the estimation by spectrophotometric technique. Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs. Hydrotropic solubilization is one of them. In the present study, sodium benzoate was chosen as hydrotrope to enhance the solubility of Lornoxicam for its spectrophotometric estimation in their marketed formulation. Preliminary solubility of Lornoxicam, spectral study, method development and its validation was done as per ICH guidelines. Solubility study of Lornoxicam displayed a significant increment in the solubility due to 10% solution of sodium benzoate which will be sufficient to extract the drug from its dosage form. The sample solution has shown the λ_{max} at 376 nm and obeys the lambert beer law in the concentration range of 5-25 µg/ml with a correlation coefficient of 0.999. The accuracy of the method was proved by recovery study with mean recovery of 99.87. Precision study also shows no significant deviation from the mean value. A limit of detection and limit of quantitation result indicates the sensitivity of the method with the values 1.02 µg/ml and 3.39 µg/ml simultaneously. Hence, the methodology can be used safely and effectively for the routine estimation of Lornoxicam in the bulk drug and marketed formulation.

Keywords: Hydrotropic solubilization, sodium benzoate, spectrophotometric estimation, validation

INTRODUCTION

Solubility is an important physiochemical property of a drug substance. With the discovery of newer drugs, it has been observed that number of drugs possess poor aqueous solubility, limiting to their uses and leading to problems related to the formulation development and its effective, precise and accurate estimation. In the current scenario one utilizes, organic solvents like methanol, acetone, dimethyl sulfoxide for enhancing the solubility, especially for its spectrophotometric estimation. Therefore, it is of crucial importance to find out materials which may enhance the solubility of a drug significantly without producing any deleterious effect as in case of organic solvents. Hydrotropic solubilization is being overlooked as a better solution of the same.

Hydrotropes represents the compounds with both lipophillic and hydrophilic parts and possess short hydrophobic regions and differ from exemplified surfactants. Even after such property they have the ability to solubilize nonpolar compounds in water.[1]

These molecules have an amphiphilic molecular structure and enhance the solubility of organic molecules which are less soluble in water dramatically.[2,3] Sodium salt of salicylic acid, citric acid, benzoic acid and acetic acid, as well as urea and nicotinamide are some of the most examples which are used as hydrotropic agents to a large extent in order to increase the aqueous solubility of therapeutic agents.[4-7]

Solubility is the one of the critical factor during the analytical estimation of the poorly soluble drug in aqueous media and this can be minimized by using thee hydrotropic solutions. Baghel and Dhiman have explored the use of 8 M urea solution for the spectrophotometric estimation of diacerein.[8] Maheshwari *et al*. have explored the use of various hydrotropes as solubilizing agent to analyze a poorly water-soluble drug, cephalexin,[5] ketoprofen,[7] and Gatifloxacin.[9]

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Lornoxicam, chemically is 3E-6-chloro-3-{hydroxy[(pyridin-2-yl) amino]methylidene}-2-methyl-2H,3H,4H-1,l{6},5,2-thieno[2,3-e][1,l{6},2]thiazine-1,1,4-trione mainly used for the management of pain mainly associated with the joint (Figure 1).

Similar to the other non-steroidal anti-inflammatory drugs, it is very poor soluble drug. This presents difficulty in analytical estimation of its marketed formulation.[10]

Literature survey reveals that there are a number of methods reported for the estimation of Lornoxicam in single or combination dosage form. Some of the methods, reported are reverse phase-high performance liquid chromatography for simultaneous analysis and determination of Lornoxicam and Thiocolchicoside from tablets,[11] spectro-photometric simultaneous estimation of diacerein and Lornoxicam in pharmaceutical dosage form,[12] spectrophotometric estimation of Lornoxicam and paracetamol in a dosage form containing a combination of both,[13] ultraviolet (UV) spectroscopic method has had been deployed for the analysis of Lornoxicam in plasma.[14] Two different spectrophotometric methods were also developed by using 0.1 N NaOH[15] through the formation of color complex using ferric chloride and 2, 2 Bipyridine.[16] All the above methods possess one or other limitation. Hence efforts have been tried to develop a simple, accurate, eco-friendly, cost-effective, safe and sensitive methods for estimation of Lornoxicam through spectroscopy in both marketed formulation as well as in bulk. In previously reported methods, high concentrated solution of hydrotrope has been used for the estimation of poor water soluble drug. Present work displays attempts to overcome the problems associated using a 10% aqueous solution of sodium benzoate.

MATERIALS AND METHODS

Chemicals and instrument

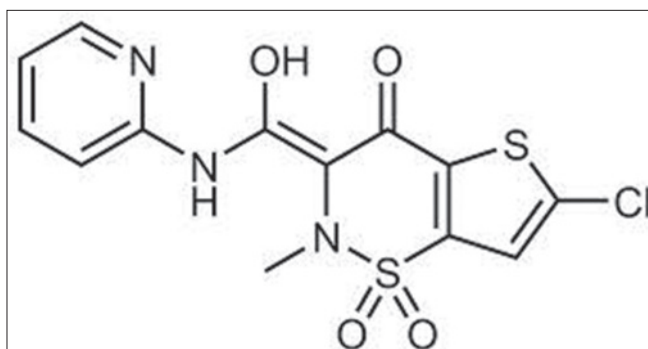Pure Lornoxicam was obtained as a generous gift from Glenmark Laboratory, Mumbai, India. Analytical grade sodium benzoate (Ranbaxy Fine Chemicals Limited) was purchased from local market. Commercial tablets, Lorsaid (Piramal Healthcare Private Limited) and Lornicam (Aristo Pharmaceutical Private Limited) of Lornoxicam were purchased from the local market.

Double beam UV spectrophotometer (Shimadzu model UV-1800, Japan), with 1 cm quarts cells was used in the study.

Before starting the validation of the method glassware calibration was performed to eliminate the errors. During the calibration of glasswares, it was taken in to care that variability due temperature should be minimum. Further these calibrated glasswares were used for the entire study.

Selection of hydrotrope

Sodium benzoate was selected on the basis of literature survey since its absorbance is below 270 nm and will not interfere with the λ_{max} of Lornoxicam spectra of sodium benzoate is mentioned in Figure 2.

Preliminary solubility study

Initial solubility study was randomly carried out for the selection of optimum concentration of sodium benzoate. During this 5%, 10%, 15%, and 20% of the sodium benzoate solution was taken for the study, and it was observed that the 10% solution of sodium benzoate was sufficient to solubilize the dose of Lornoxicam present in marketed formulation. Equilibrium solubility of the drug was determined at room temperature, with the addition of the excess amount of Lornoxicam to screw capped 30 ml glass vials containing distilled water and 10% of sodium benzoate solution, separately. The vials
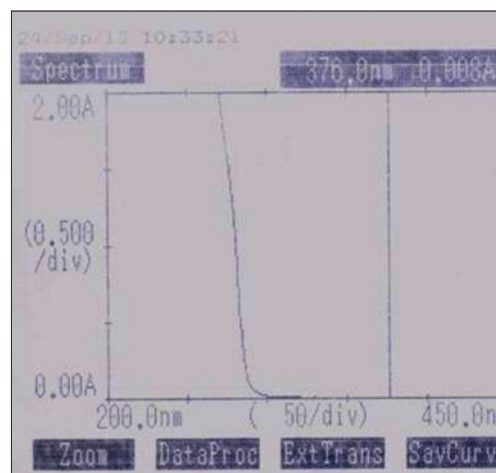

Figure 1. Molecular structure of Lornoxicam.

Figure 2. Spectra of sodium benzoate solution at 376 nm.

were mechanically shaken for a period of 12 h in the wrist action Shaker (Jyoti Laboratory Private Limited, Gwalior, India). The solutions were kept aside for a period of 24 h to attain equilibrium and then supernatants the vials were filtered through Whatman filter paper #41 separately. The saturated filtrates were diluted suitably with distilled water, and absorbances were noted against respective blanks.

UV spectral studies

In order to notice any interaction between drug and hydrotropic agent or interference due to hydrotropic agent, spectral analysis of Lornoxicam was performed in 10% sodium benzoate solution. Spectroscopic changes in the spectral structure of Lornoxicam in solution of sodium benzoate were subsequently investigated.

Preparation of stock solution

Ten milligram of Lornoxicam was weighed accurately and transferred into 25 ml of volumetric flask and dissolved using 25 ml 10% sodium benzoate solution. 5 ml of the resultant was taken with subsequent dilution with distilled water to form the stock solution of 100 μg/ml.

Method development

Analytical characteristics of the proposed methods
By using the proposed methods, the different optical characteristics of Lornoxicam in hydrotropic solution, such as absorption maxima, Beer's law limit, molar absorptivity, sensitivity, absorptivity (A1%, 1 cm) were calculated. The slope (m), intercept (c) and correlation coefficient (r^2) were calculated through regression analysis utilizing the method of least squares.

Selection of wavelength for analysis of Lornoxicam
Fresh aliquot of 20 μg/ml was prepared from stock solution. The same was scanned in the spectrum mode from 200-450 nm for obtaining maximum absorption wavelength (λ_{max}) on Shimadzu 1800 spectrophotometer. In spectrum, maximum absorbance was observed at 376 nm (Figure 3).

Validation of the method

Method validation is performed to ensure that the adopted analytical method is accurate, specific, reproducible and rugged over the specified range in which the analyte is to be analysed.[17] In the present study, the method was validated in order to substantiate linearity and range, precision, recovery, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

Linearity and range

To establish linearity of the proposed methods, accurately weighed 10 mg of the Lornoxicam drug was transferred into 25 ml of volumetric flask and the volume was make up to 25 ml with 10% of sodium benzoate solution. The aliquots of 5, 10, 15, 20, and 25 μg/ml were prepared from stock solution, and absorbance was noted at 376 nm against respective blank (Table 1). Calibration curve was plotted between concentration and absorbance (Figure 4).
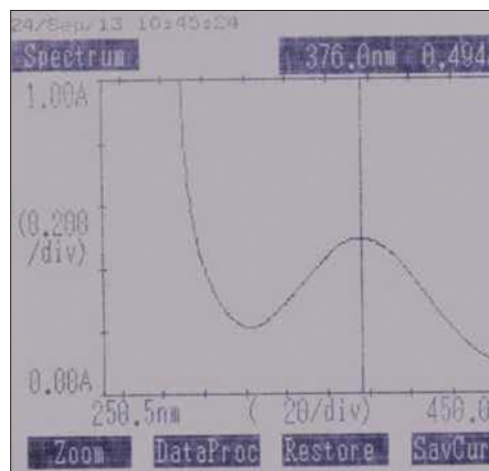

Figure 3. Spectra of Lornoxicam in 10% sodium benzoate solution at 376 nm.
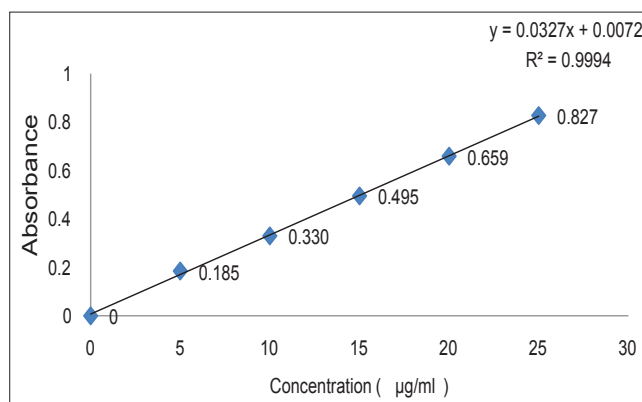

Figure 4. Graph showing linearity for Lornoxicam in 10% sodium benzoate solution.

Table 1 Linearity data for the Lornoxicam in the 10% sodium benzoate solution

Conc. (µg/ml)	Absorbance at 376 nm					
Rep.	0	5	10	15	20	25
Replicate-1	0	0.187	0.3351	0.476	0.645	0.806
Replicate-2	0	0.168	0.308	0.483	0.637	0.828
Replicate-3	0	0.199	0.347	0.526	0.696	0.847
Mean	0	0.185	0.330	0.495	0.659	0.827
S.D.	0	0.0128	0.0163	0.022	0.0261	0.017

S.D.: Standard deviation, Conc.: Concentration

Accuracy

The accuracy of the method represents the proximity of the measured value to the true value of a given sample. Accuracy of the adopted method was assessed by recovery studies. A standard addition method was also employed involving the addition of different concentrations of pure drug in order to grant an additional support (31.69, 46.59 and 60.18 µg/ml) or the addition of the same concentration of drug 11.26 µg/ml to a known pre-analyzed sample of formulation. Total concentration was determined using the adopted methods ($n = 3$). The percentage recovery for the added pure drug was calculated by using the equation, % recovery = $[(Ct - Cs)/Ca] \times 100$, where Ct, Cs, and Ca simultaneously represents to the total drug concentration measured after standard addition, concentration of the drug in the formulation sample, and concentration of drug added to formulation (Table 2).

Precision

Repeatability of the adopted method was determined by analyzing the different levels of drug concentrations prepared by different stock solutions. The intermediate precision of the adopted analytical methods was determined by studying the variation in different conditions such as inter- and intra-day using two different instruments. For intra-day variation, different levels of drug concentrations in triplicates were prepared and analyzed thrice (different times in a day). For inter-day variation ($n = 3$), similar procedure was adopted for three different days. UV spectrophotometer (Shimadzu-1700) was used for the analysis of one set of different levels of the concentrations. The another set was analyzed by shimadzu-1800. The percent relative standard deviation (% relative standard deviation) of the predicted concentrations from the regression equation was taken as precision and has been mentioned in Tables 3-5.

Robustness

The robustness was performed by making change of λ_{max} by 1 nm in wavelength. The results are shown in Table 5.

LOD and LOQ

The LOD and LOQ for Lornoxicam by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of the regression equation ($n = 5$). The details related to LOD and LOQ are mentioned in Table 6.

Table 2 Result of recovery studies of tablet formulation with statistical evaluation

Recovery method	Amount of drug in formulation (µg/ml)	Amount added (µg/ml)	Total drug found (µg/ml) (mean±SD) (*n*=3)	Percentage recovery
1	15.42	11.26	26.92±0.23	100.9±0.86
	32.53	11.26	43.72±0.67	99.84±1.53
	42.37	11.26	53.08±0.46	98.97±0.86
2	14.13	31.69	45.97±0.62	100.3±1.38
	14.13	46.59	60.44±0.68	99.53±1.17
	14.13	60.18	73.92±0.58	99.47±0.83

Table 3 Intra-day repeatability: Data obtained for precision study

Sr. no.	Concentration taken (µg/ml)	Estimated concentration±SD (*n*=3)		
		Day 1	Day 2	Day 3
1	10	10.15±0.12	9.97±0.18	9.88±0.14
2	15	15.09±0.45	15.25±0.11	15.19±0.14
3	20	20.06±0.16	19.84±0.13	20.25±0.14

SD: Standard deviation

Table 4(a) Inter-day repeatability: Data obtained for precision study

Sr. no.	Concentration taken	Estimated concentration	% estimated concentration±SD (*n*=3)
1	10	9.87±0.069	98.3±0.69
2	15	14.82±0.152	98.8±1.01
3	20	19.88±0.142	99.4±0.71

SD: Standard deviation

Table 4(b) Inter-instrument repeatability: Data obtained for precision study

Sr. no.	Concentration taken	Estimated concentration	% estimated concentration±SD (*n*=3)
1	10	9.91±0.087	99.1±0.87
2	15	14.87±0.167	99.13±1.11
3	20	19.81±0.186	99.05±0.93

SD: Standard deviation

In 10% sodium benzoate solution, LOD was found to be 1.02 µg/ml and LOQ was found to be 3.39 µg/ml.

Determination of Lornoxicam in marketed formulation

The concentrations of the drug were calculated from linear regression equation. 257 mg of tablet powder (equivalent to 5 mg of a drug) which was dissolved in the 10 ml of 10% sodium benzoate solution and further dilution of 10, 15, 20 µg/ml were prepared using water. One tablet of Lornoxicam contains 8 mg of drug (Total tablet weight = 411.2 mg). All the determinations were made in triplicate ($n = 3$).

RESULTS AND DISCUSSION

Lornoxicam is practically insoluble in water. In the present study during the solubility testing, it was found

Table 5 Data showing robustness of the method

Sr. no.	Concentration taken	Wavelength	Estimated concentration	% estimated concentration±SD (*n*=3)	% Deviation in conc. at different wavelength
1		377	9.84±0.072	98.4±0.72	
2	10	376	9.91±0.067	99.1±0.61	0.287±0.102
3		375	9.87±0.047	98.7±0.47	

SD: Standard deviation, conc.: Concentration

Table 6 Readings for the calculation of LOD and LOQ

Conc. (µg/ml)	Rep./absorbance at 376 nm					
	R1	R2	R3	R4	R5	Mean±SD (*n*=5)
0	0	0	0	0	0	0
5	0.172	0.185	0.187	0.168	0.199	0.185±0.011
10	0.325	0.330	0.3351	0.308	0.347	0.330±0.0.012
15	0.492	0.495	0.476	0.483	0.526	0.495±0.0.017
20	0.641	0.659	0.645	0.637	0.696	0.659±0.022
25	0.815	0.827	0.806	0.828	0.847	0.827±0.014
Slope	0.0323	0.0326	0.0317	0.03277	0.0337	0.0327±0.0007
Intercept	0.004	0.009	0.012	-0.015	0.014	0.007±0.0112

LOD: Limit of detection, LOQ: Limit of quantitation, Conc.: Concentration

that the solution of sodium benzoate significantly increases the solubility of Lornoxicam. Hence, sodium benzoate was chosen as a hydrotropic salt for the further study.

Linearity studies

Linearity study was performed by plotting a calibration curve (Figure 3) between absorption versus concentration using different series dilution, the data found during the study showed that the curve is following to the linear line equation, i.e., Y = mx + c with a regression coefficient of 0.999, slope 0.033 and intercept 0.006. The data for linearity study is mentioned in Table 1.

Accuracy

A standard addition method was employed, which involved the addition of different concentrations of pure drug (31.69, 46.59, and 60.18 µg/ml) or the addition of the same concentration of drug 11.26 µg/ml to a known pre-analyzed formulation sample and the total concentration was determined using the proposed methods (*n* = 3). The results as mentioned in Table 2 shows that in every study, the content of the drug was found in the range of 98.97- 100.9% with maximum standard deviation of 1.53%, which clearly indicates that the method is accurate.

Precision

Repeatability was determined by using different levels of drug concentrations, prepared from independent stock solutions and analyzed (*n* = 3). Inter-day, intra-day and inter-instrument variation were studied to determine intermediate precision of the proposed analytical methods. Repetitive results as mentioned in Tables 3-5, were found in the standard range, i.e., 98-102% which ensures the precision of the methodology.

Robustness

The robustness was performed by making change of λ_{max} by 1 nm in wavelength. The results are shown in Table 5. This deliberate alteration of wavelength results in 0.28% variation in the measured concentration, which is quite low as a comparison to the standard i.e., 1%. This demonstrates that the developed method was robust and remains unaffected by minor changes.

LOD and LOQ

The LOD and LOQ for Lornoxicam by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of the regression equation. During the validation of the methodology LOD and LOQ were also determined for the drug, and it was found to be 1.02 µg/ml and 3.39 µg/ml, respectively.

Determination of Lornoxicam in marketed formulation

During the drug estimation in the marketed formulation by the proposed method, the amount of drug in the two marketed formulation were evaluated. In the first formulation, i.e., Lorsiad (Piraml Healthcare), the amount of the drug was found in the range of 98.2-102% (Table 7). Similarly for the second formulation, i.e., Lornicam (Aristo) the amount of the drug was found in the range of 98.4-100.9% (Table 8).

CONCLUSION

The above method was developed in a view to provide a simple, precise, accurate, rapid, safe, and eco-friendly method for the estimation of Lornoxicam in their bulk drug sample and dosage form during its routine analysis. The proposed method will also be cost-effective due to

Table 7 Marketed formulation: Lorsaid (Piramal Healthcare)

Sr.no.	Tablets batch	Conc. used	Abs. of the tab. powder	Conc. µg/ml	(A) mg	(B) mg	% amount of drug in tab
1	A	10	0.341	10.15	5.08	7.96	99.6
2	B	10	0.349	10.39	5.2	8.16	102
3	C	15	0.492	14.72	4.91	7.86	98.2

Data obtained during the estimation of Lornoxicam using calibration graph equation.
Conc.: Concentration

Table 8 Marketed formulation: Lornicam (Aristo)

Sr.no.	Tablets batch	Conc. used	Abs. of the tab. powder	Conc. µg/ml	(A) mg	(B) mg	% amount of drug in tab
1	A	10	0.331	9.84	4.92	7.87	98.4
2	B	10	0.339	10.09	5.05	8.07	100.9
3	C	20	0.646	19.39	4.85	7.76	96.97

*Concentration of drug found in the tablet powder taken for preparing dilution
(A) = Concentration found in the sample×dilution factor. Concentration of drug in tablet
(B) = (A) × amount of drug in the label/drug taken for preparing dilution

easy availability of salt and use of lesser concentration of salt as compared to that used in earlier reported methods.

ACKNOWLEDGMENTS

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