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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC ASSAY METHOD FOR DETERMINATION OF LORNOXICAM IN SELF EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS) FORMULATION

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Keywords:

Lornoxicam, spray dryer Self Emulsifying Drug Delivery System (SEDDS) formulation Stability study, HPLC.

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ABSTRACT: The objective of the present work was to development and validation of stability indicating RP-HPLC assay by applying different stress degradation conditions on lornoxicam in Self Emulsifying Drug Delivery System (SEDDS) formulation. HPLC separation was achieved on analytical technique using C-18 column and mobile phase of Acetonitrile: Methanol (65:35, v/v) at a UV detector. The lornoxicam drug consist in SEDDS formulation was subjected to acid, alkali, oxidation, dry heat and photo degradation treatment apply as stress degradation condition. The method was linear in the drug concentration range of 10-60 µg/ml with a correlation coefficient 0.999. The stress degradation studies showed that Lornoxicam contains in Self Emulsifying Drug Delivery System (SEDDS) formulation undergoes degradation in acid, alkali, oxidation and dry heat condition respectively within the limit as per ICH guidelines and stable in photo-degradation condition.

INTRODUCTION: Lornoxicam (chlortenoxicam) is a non steroidal anti- inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. It is available in oral and parental dosage formulation. Lornoxicam is a yellow or slightly yellow powder. It is slightly soluble in water, soluble in methanol and ethanol [1].

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The low solubility of many new drug candidates is a substantial challenge facing the pharmaceutical industry [2]. The oral delivery of such drugs is frequently associated with implications of low bioavailability and high intra and inter subject variability. To overcome such problems we have formulate the Self Emulsifying Drug Delivery System (SEDDS) formulation of lornoxicam. It is an oral dosage form. It is a mixture of oils and surfactants that has the ability to form fine oil in water (o/w) emulsions or micro emulsions upon gentle agitation following dilution with the aqueous phase and improves drug dissolution through providing a large interfacial area for partitioning of the drug between the oil and GIT fluid.

Other advantages include increased stability of drug molecules and possibility of administering the final product as gelatin capsules [3-5]. The objective of the present study was to develop and validate a stability indicating HPLC method for the assay of lornoxicam in Self Emulsifying Drug Delivery System (SEDDS) formulation. It was a simple, economical analytical method that can be used for the quantitative assay of lornoxicam co-existing with the excipients and the potential degradation products.

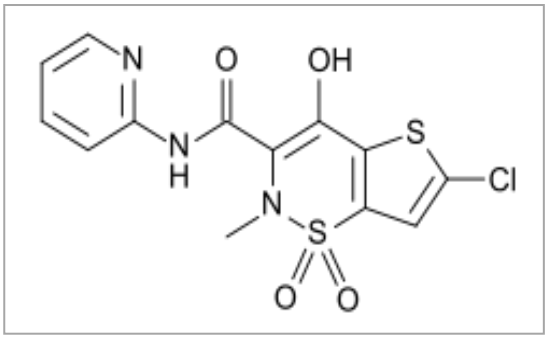

FIGURE 1: STRUCTURE OF LORNOXICAM. ((3*E*)-6-chloro-3-[hydroxy (pyridin-2-ylamino) methylene]-2-methyl-2, 3-dihydro-4*H*-thieno [2, 3-*e*] [1, 2] thiazin-4-one 1, 1-dioxide)

EXPERIMENTAL:

MATERIALS AND METHODS [6-11]: Lornoxicam was obtained as gift sample from Glenmark Pharma Ltd., MCT oil (Labrafac), Caprylic Capric Triglyceride and Mayasol (Labrasol) obtained as gift sample from Subhash Chemicals, Bhosari, Pune (M.S.) India. Capmul MCM, Captex 200 and Captex 350 were obtained as gift sample from Abitech Corporation, Mumbai, India. Cremophore RH and Cremophore EL obtained as gift sample from BASF Ltd, Mumbai. Tween 20, Tween 80, PEG 400, Soybean oil, Castor oil, Olive oil and Sesame oil was obtained from Prachi Enterprices, Pune, (M.S) India. The solvent used Acetonitrile (HPLC grade), Water (HPLC grade), NaOH (AR grade), HCl (AR grade), H_2O_2 (HPLC grade), were purchased from Merck Chemicals, Mumbai, India.

Preparation of SEDDS Formulation [12-14]: Lornoxicam (8 mg) was dissolved in 1 ml of the mixture of Capmul MCM, Tween 20 and PEG 400 as oil, surfactant and co-surfactant respectively. The ratio of S/CS (Smix) 1:1 was used for preparation of SEDDS.

A laboratory scale spray dryer (LABULTIMA; LU 222-ADV) was used for the preparation of solid SEDDS. 1 gm. of Aerosil 200 was suspended in 100 ml ethanol. 2 ml of liquid SEDDS was added to this solution and continuously stirred at room temperature for 30 min. This solution was then delivered to the nozzle (0.7 mm diameter) at a flow rate of 3 ml/min with peristaltic pump and spray dried at inlet temperature of 100 and 60oC and outlet temperature of 80 and 40oC respectively. The spray air pressure was used 4kg/cm2.

Selection of mobile phase and chromatographic conditions: Chromatographic separation studies were carried out on a C-18, column on the working standard solution of Lornoxicam (50µg/ml). Initially, trials were carried out using acetonitrile and methanol in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials, Acetonitrile: Methanol (pH adjusted to 4.5 with ortho phosphoric acid) (65: 35 v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Preparation of standard stock solution: Weighed accurately calculated SEDDS formulation containing 10 mg of Lornoxicam drug and transferred in 100 ml volumetric flask. Drug was dissolve in Acetonitrile: Methanol (65: 35 v/v) and volume was made up to 100 ml with same solvent which gives the concentration 100 µg/ml. 5ml standard stock solution of Lornoxicam was diluted in 10 ml Acetonitrile: Methanol (65: 35 v/v) to obtained working standard solution 50µg/ml.

Preparation of mobile phase: Mobile phase was prepared by mixing acetonitrile: methanol (pH adjusted to 4.5 with ortho phosphoric acid) (65: 35 v/v), filtered through 0.45µ membrane filter paper and sonicated on ultra-sonic water bath for 30 min.

Linearity: The standard stock solution containing 100 µg/ml of Lornoxicam used to prepare range of standard solutions containing six different concentrations of analyte. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 10-60µg/ml. The results obtained are shown in **Fig. 1.**

The peak areas were plotted against the corresponding concentrations to obtain the calibration curve **Fig. 2.**

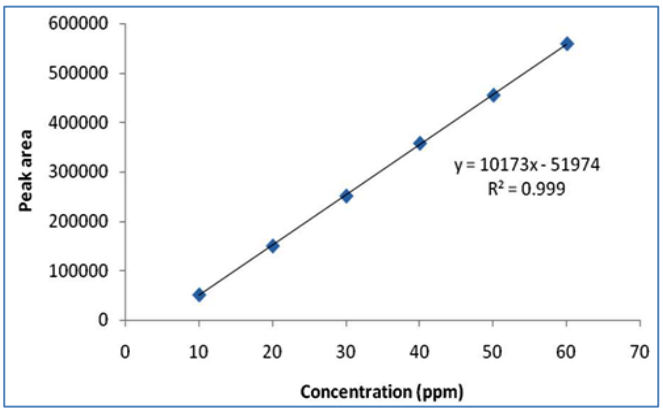

FIGURE 2: CALIBRATION CURVE FOR LORNOXICAM

Chromatogram of Lornoxicam SEDDS formulation: The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Solution of Lornoxicam SEDDS formulation was injected to get the chromatogram. The retention time was found to be 4.78 ± 0.02 min. Chromatogram shown in **(Figure 3)**

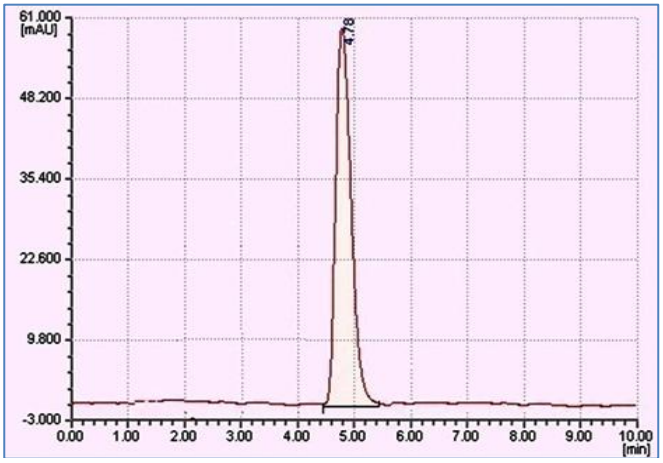

FIGURE 3: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML, RT = 4.78).

Recovery studies: Recovery studies were carried out by mixing standard drug solution into pre-analyzed sample of SEDDS formulation solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 25µg/ml of Lornoxicam formulation solution into which 20, 25 and 30µg/ml of Lornoxicam standard solution were added. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of Lornoxicam were calculated by using linearity equation. The results obtained are shown in **Table 1 and 2.**

Degradation Studies [15-17]: To perform the stress degradation study 5 ml of working solution (50µg/ml) of Lornoxicam SEDDS formulation subjected to acidic, alkaline, oxidizing, dry heat and photolytic conditions. For acid degradation, formulation was heated under reflux with 2 N HCl for 3 hours and neutralized with sodium hydroxide solution, for alkaline degradation the formulation was treated with 1.2 N sodium hydroxide solution for 3 hours neutralized with hydrochloric acid solution, for oxidation degradation formulation was heated under reflux with 6 % hydrogen peroxide solution for 2 hours, for thermal degradation the formulation was exposed at 65ºC for 48 hours and for photo degradation the formulation was exposed to UV light (366 and 254 nm) for 48 hours. Stressed sample was diluted upto 10 ml with acetonitrile: methanol (65:35) and injected in stable condition,

Acid degradation: 5 ml working solution of Lornoxicam SEDDS formulation was heated under reflux with 2N HCl for 3 hours and neutralized with sodium hydroxide solution and dilute with selected mobile phase and injected at stabilized condition, under this condition, degradation was observed (Deg-1, RT = 3.24) results are shown in **Table 2 and Figure 4.**

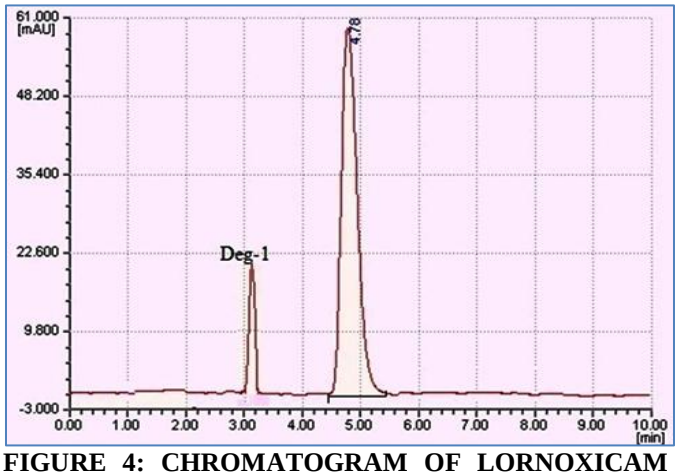

FIGURE 4: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER ACIDIC HYDROLYSIS INDUCED DEGRADATION WITH DEGRADATION PRODUCT AT RT 3.24

Alkali degradation: 5 ml of working solution of Lornoxicam SEDDS formulation was mixed with 2 ml of 1.2 N sodium hydroxide solution and kept for 3 hours. After 3 hours solution was neutralized with dilute HCl and solution was diluted upto 10 ml with acetonitrile: methanol (65:35) and injected at stabilized condition. Degradation was observed (Deg-2, RT = 3.94) results are shown in **Table 2 and Figure 5**

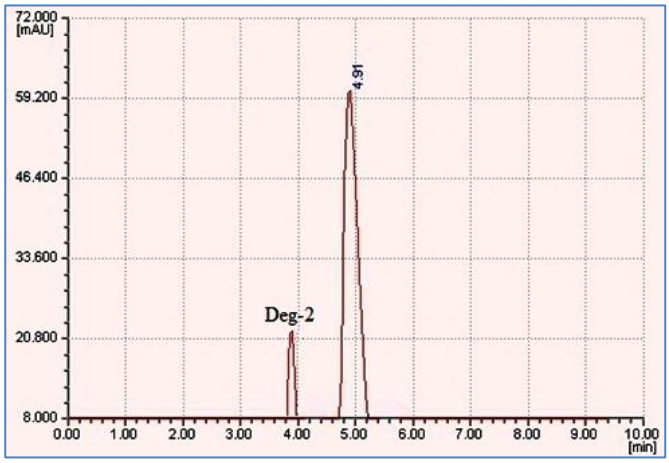

FIGURE 5: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER ALKALI HYDROLYSIS INDUCED DEGRADATION WITH DEGRADATION PRODUCT AT RT 3.94

Oxidation degradation: 5 ml of working solution of Lornoxicam SEDDS formulation was mixed with 3 ml 6 percent solution of hydrogen peroxide and reflux for 3 hrs. The solution was diluted upto 10 ml with Acetonitrile: Methanol (65:35). The solution was injected in stabilized conditions. Degradation was observed (Deg-3, 4, 5, RT =2.41, 2.50.2.98) results are shown in **Table 2 and Figure 6.**

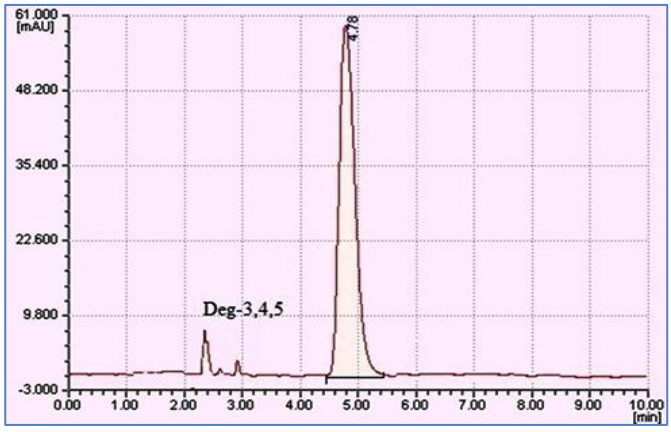

FIGURE 6: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER OXIDATIVE DEGRADATION WITH DEGRADATION PRODUCT AT RT 2.41, 2.50.2.98 RESPECTIVELY.

Dry heat Degradation: Dry heat studies were performed by keeping Lornoxicam SEDDS formulation sample in oven (65ºC) for a period of 48 hours, 10 mg exposed sample was weighed accurately and transferred in to 100 ml of volumetric flask and dissolved in Acetonitrile: Methanol (65:35), the volume was made up with Acetonitrile: Methanol (65:35) to get concentration of 100µg/ml to for stock solution. 5ml stock solution of Lornoxicam SEDDS formulation was diluted in 10 ml of Acetonitrile: Methanol (65:35) to get working standard solution 50µg/ml. The solution was injected in stabilized conditions. Results are shown in **Table 2 and Figure 7.**

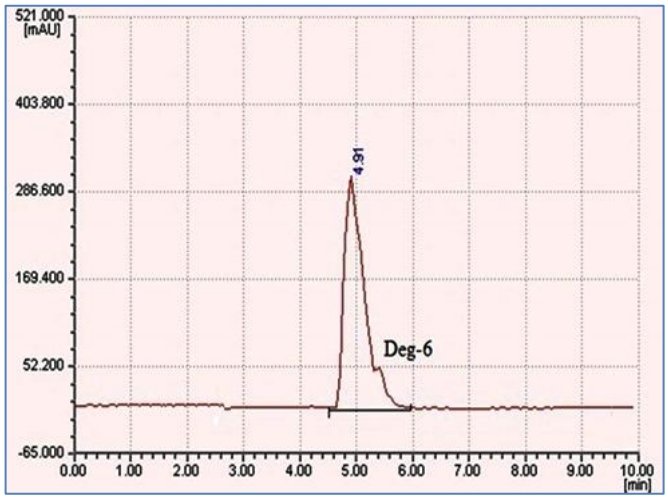

FIGURE 7: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER DRY HEAT DEGRADATION WITH DEGRADATION PRODUCT AT RT 5.4

Photo-degradation:

Long UV-Degradation at 366nm: The photo degradation study was perform by exposing the Lornoxicam SEDDS formulation sample in to long UV light (366 nm) for 48 hour. Weighed accurately sample contain 10 mg of Lornoxicam in SEDDS formulation after exposure, dissolved 10 mg of formulation in 100 ml of methanol to get concentration 100µg/ml (stock solution). 5 ml stock solution of Lornoxicam SEDDS formulation was diluted in 10 ml Acetonitrile: Methanol (65:35) to get working standard solution 50µg/ml and it was injected in stabilized conditions Results are shown in **Table 2 and Figure 8.**

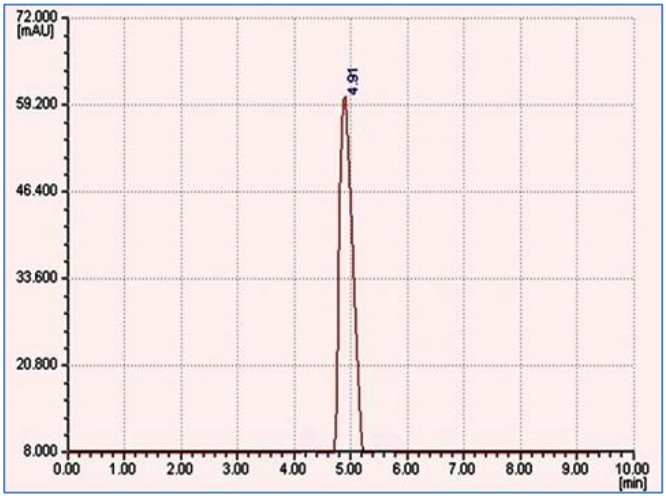

FIGURE 8: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER EXPOSURE OF 366 NM, NO DEGRADATION PEAK WAS FOUND

Short UV-Degradation at 256 nm: Weighed accurately sample contain 10 mg of Lornoxicam in SEDDS formulation after exposure, dissolved 10 mg of formulation in 100 ml of methanol to get concentration 100µg/ml (stock solution).

5 ml standard stock solution of Lornoxicam SEDDS formulation was diluted in 10 ml Acetonitrile: Methanol (65:35) to get working standard solution 50µg/ml and it was injected in stabilized chromatographic conditions Results are shown in **Table 2 and Figure 9.**

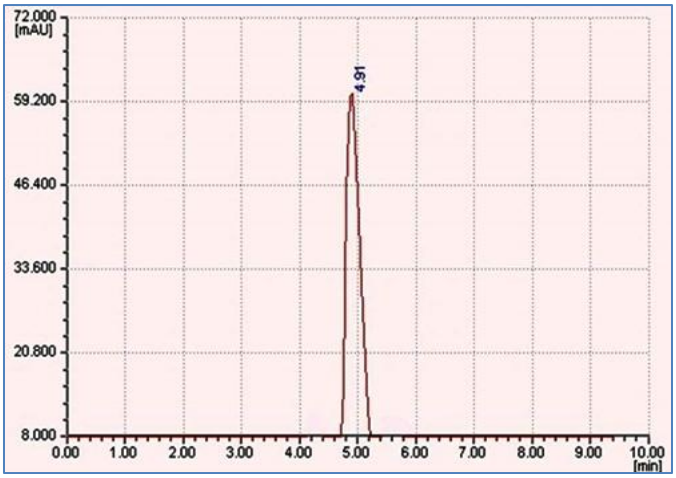

FIGURE 9: CHROMATOGRAM OF LORNOXICAM SEDDS FORMULATION (50MG/ML) AFTER EXPOSURE OF 256 NM, NO DEGRADATION PEAK WAS FOUND

TABLE 1: RECOVERY STUDIES OF LORNOXICAM SEDDS FORMULATION

Level	Conc. (µg/ml)	Area	Mean	Recovered	% Recovery
80	25 + 20	395631 395254 395825	395570	43.99	98.75
100	25 + 25	461039 461182 461833	461351.3	50.45	100.90
120	25 + 30	503126 502457 500364	501982.3	54.45	99.00

TABLE 2: SUMMARY OF VALIDATION PARAMETERS OF LORNOXICAM BULK DRUG AND SEDDS FORMULATION

Sr. no.	Validation Parameter	Lornoxicam
1.	Linearity Equation	Y=10173x - 51974
2.	(r^2)	0.999
3.	Range	10 – 60µg/ml
4.	Accuracy (% recovery)	98.7%, 100.9%, 99.9%
5.	Assay for standard lornoxicam	99.66 %
6.	Assay for acidic condition	95.38
7.	Assay for alkali condition	95.17
8.	Assay for oxidation condition	94.79
9.	Assay for dry heat condition	95.41
10.	Assay for photo degradation	99.66

DISCUSSION AND CONCLUSION: The developed method was found to be precise as the recoveries (98.75, 100.90 and 99.00% respectively) of the formulation were obtained at each added pure drug concentration, indicating that the formulation was accurate. The stress degradation studies showed that Lornoxicam in SEDDS formulation undergoes degradation in acid, alkali, oxidation and dry heat (4.80 %, 1.52 %, 2.16 %, and 1.81% respectively within limit as per ICH guidelines. The developed stability indicating high performance liquid chromatographic method was rapid and suitable for the estimation of degradation concentration of lornoxicam in SEDDS formulation.

It has a good ability to separate lornoxicam well from the degradation products. Stressed degradation studies were conducted to provide an indication of its stability indicating property of the formulation. All the above factors lead to the conclusion that the proposed method was accurate, precise, simple, sensitive, robust, cost effective and can be applied successfully for the estimation of Lornoxicam bulk and SEDDS formulation and percentage degradation. The proposed method is also useful for determination of Lornoxicam stability in sample of pharmaceutical dosage forms.

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