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Formulation and Evaluation of Parenteral Dosage Form of Lornoxicam Using Hydrotropic Solubilization Method

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ABSTRACT

Lornoxicam is comparatively a new non-steroidal anti-inflammatory drug, which is selective cyclooxygenase-1 and 2 (COX 1 and 2) inhibitors. Lornoxicam is a non steroidal anti-inflammatory drug that exhibits its anti inflammatory, analgesic and anti pyretic activities in animal models and it is presently available in the market only as tablet dosage form. It is preferred in the treatment of adults with osteoarthritis, acute pain rheumatoid arthritis, postoperative dental pain and primary dysmenorrhoea. The present study was undertaken with an intention to develop a stable and effective parenteral formulation, containing the drug Lornoxicam. Lornoxicam is a light sensitive and insoluble water soluble drug but unstable at higher temperature in water. So the effects of various co solvents in the solubility of Lornoxicam have been evaluated. Lornoxicam was tried with co solvents such as PEG-400, β-cyclodextrin and Sodium Lauryl sulphate. The drug was made into injection formulation for administered as a SVP. Various batches of Lornoxicam injection formulation were prepared in order to assess the influence of heat, light, atmospheric oxygen and antioxidant on the stability of the drug and the formulations were also subjected to accelerated stability test. Out of all trials, formulation containing Sodium Lauryl sulphate was found to be more soluble, stable and passed all tests satisfactorily.

Keywords: Lornoxicam, osteoarthritis, β-cyclodextrin, dysmenorrhoea, Sodium Lauryl sulphate.

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INTRODUCTION

Injections include a wide variety of therapeutic agents, e.g., for the treatment of cancer, infections, cardiovascular diseases, arthritis, inflammatory diseases, diabetes, hormonal deficiencies and many other disease states including life threatening emergency conditions. There are more than 400 injections products listed in the USP and, because of the huge number of biotechnology molecules in clinical study, this number will continue to grow rapidly over the next several years. About 80% or greater of all SVPs commercially available are prepared by aseptic processing. LVPs usually involve intravenous infusion, dialysis, or irrigation fluids containing electrolytes, sugar, amino acids, blood, blood products, and fatty lipid emulsions. SVP formulations are simple formulations compared with other pharmaceutical dosage forms, composed of active ingredients, solvent system (preferably aqueous), minimal number of excipients, in the appropriate container and closure packaging system. Formulation scientists have severe restrictions in number and choice of added substances because of safety considerations[1].

Lornoxicam is comparatively a new non-steroidal anti-inflammatory drug, which is selective cyclooxygenase-1 and 2 (COX 1 and 2) inhibitors. Lornoxicam is a non steroidal anti-inflammatory drug that exhibits its anti inflammatory, analgesic and anti pyretic activities in animal models. Lornoxicam is used in treatment of osteoarthritis, acute pain rheumatoid arthritis, postoperative dental pain and primary dysmenorrhoea. It has contraindication like hypersensitivity asthma after taking aspirin or other NSAIDS, GI bleeding and ulceration. The side effects of Lornoxicam are abdominal pain, dizziness, dyspepsia padal edema and upper respiratory infection and fatigue other effects are hypertension nausea diarrhea epigastric discomfort and heart burn. Lornoxicam is mainly available in dosage forms of tablets suspensions and dispersible tablets. For an oral dose Lornoxicam is extensively metabolized (less than 1% Lornoxicam is excreted unchanged in urine) in the liver and half life is 3-5 hrs. The objective of the present study is to formulate stable Lornoxicam injectables to have a rapid therapeutic action. The main problem is involved on the formulation of Lornoxicam injection is the extremely the hydrophobic nature of the drug. The aim of the present study is to formulate and evaluate the Parenteral dosage form containing Lornoxicam. The objectives of the study are, to study the solubility behavior of the drug in different solvents, to develop an analytical method for assay of, to design and formulate a stable parenteral formulation of Lornoxicam, to evaluate prepared parenteral formulations of Lornoxicam.

MATERIAL AND METHODS

Lornoxicam, PEG-400, Polyvinylpyrrolidone, Propylene glycol are obtained from KAPL, Bangalore.

Preformulation Studies [2,3,4,5,6]

Solubility studies of Lornoxicam in different solvents:

Excess of drug was added to different solvents in 10 ml stoppered volumetric flasks. Then Drug was made to dissolve in the solvent by placing the volumetric flask in the shaker bath at 25° C for 6 hours. The volumetric flasks were then placed at room temperature for 24 hours. The solutions were filtered and appropriate dilutions were made to measure absorbance at 378nm using UV visible spectrophotometer, and water as blank. The data are given in Table 2.

Effect of Temperature on Stability of Drug:

5% Lornoxicam solution in Sodium Lauryl sulphate is filled into vials. The vials were sealed and placed at refrigeration, room-temperature, 50°C, 75°C and 95°C for 1 week and observed for color change and crystal growth. The samples placed at refrigeration and room temperature served as controls. The data are given in Table 3.

Light Stability of Drug:

5% of Lornoxicam solution in Sodium Lauryl sulphate is filled in to 10ml glass vials (amber and clear).Also samples of drug substance are placed in an open Petridish to expose a large surface. Drug and dilutions placed in a light-resistant amber colored glass vials, foil wrapped and in a cardboard box as controls. This is carried out for 4 weeks with weekly examinations for visible color change or precipitation in solution in clear vials, the compound can be considered as potentially light sensitive and should be handled accordingly. The data are given in Table 4.

Effect of Oxygen on Drug:

5% of Lornoxicam in Sodium Lauryl sulphate is filled into vials and placed at 30°C and 40°C. One group is purged and another group is sealed with air. Solutions are observed for color change and drug content. The data are given in Table 5 and 6.

FORMULATION DEVELOPMENT

Attempts were made to develop a stable parenteral formulation using co solvent/s along with other excipients. The dose selected for formulation was 16 mg of Lornoxicam in 1ml solvent. The prepared formulations contain the following ingredients along with their concentrations are given in Table 1.

Table 1: Concentration of different ingredients used in various trial formulations

Ingredients	Formulation					
	A	**B**	**C**	**D**	**E**	**F**
Lornoxicam	20 gm	20 gm	20 gm	20 gm	20 gm	20 gm
Sodium Luryl Sulphate	-	25 gm	-	-	-	25 gm
PEG-400	-	24 ml	-	24 ml	-	24 ml
β-cyclodextrin	-	10 gm	15 gm	-	22 gm	22 gm
Benzyl alcohol	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Propyl Paraben	0.022 ml	0.022 ml	0.022 ml	0.022 ml	0.022 ml	0.022 ml
Sodium metabisulphite	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm
Water for Injection ml	q s	q s	q s	q s	q s	q s

Thus prepared formulations were assayed for drug content respectively and 10ml of these were placed at 5°C, room temperature (RT), 37°C, 40°C and 45°C for six weeks and observed for crystal growth, clarity, pH change, and drug content.

POST FORMULATION EVALUATIONS 7,8,9,10

Assay of Formulations:

Reference Solution Preparation-

100ml of stock reference solutions for each formulation was prepared. The composition of the reference stock solution was similar to that of the respective formulations excluding the drug and also they were diluted similarly as the formulations were diluted using water. This resulting solution is used as reference solution (blank) in comparison with the prepared formulations.

Sterilization Studies:

The injection samples were taken in glass syringe, the membrane filter holder was attached to the syringe. A prefilter of 1.5 micrometers was placed in this holder, after which filters of 0.22, 0.45, 1.2 and 1.5 micrometers were placed successively and tested whether the injection sample could pass through these membrane or not. The data are given in Table 7 and 12.

Stability Studies-

For any pharmaceutical dosage form stability of the prepared formulation is a very basic and important factor, from point of view of safety of the patient being treated with and to get a safe and maximum therapeutic response of the drug.

The provision of rapid means of quality control, which ensures that no unexpected changes in the stored product are occurred like: Crystal growth, Clarity and color change. The data are given in Table 8, 9 and 10.

Crystal Growth-

10 ml of the each prepared formulations B, F were placed at refrigeration, room temperature, 37°C, 40°C and 45°C respectively for six weeks and observed for crystal growth. The data are

given in Table 14.

pH Changes-

10ml of the each prepared formulations B, F were kept at different temperatures/conditions such as refrigeration, room temperature, 37°C, 40°C, 45°C and under light. At regular time intervals the samples were examined for pH changes for six weeks using a digital pH meter. The data are given in Table 13.

Clarity-

10ml of the formulations were placed at refrigeration, room temperature, 37°C, 40°C and 45°C for 6 weeks and observed for color change or turbidity. The data are given in Table 15.

% Drug Content-

The drug content of the formulations B, F were determined by following the same procedures as mentioned in assay. The estimates were done at intervals of one week up to six weeks. The data are given in Table 11, 17 and 18.

RESULTS AND DISCUSSION:-

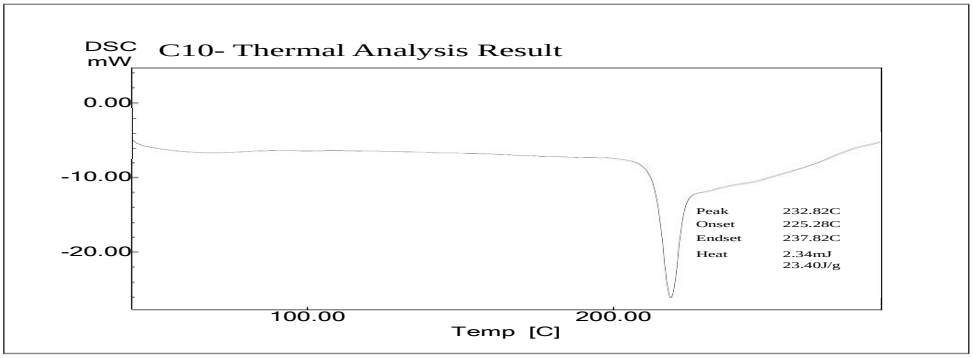

Figure 1: D.S.C. image of Lornoxicam. Lornoxicam showed a characteristic exothermic peak at 232.82^{0}C, which corresponds to its melting point.

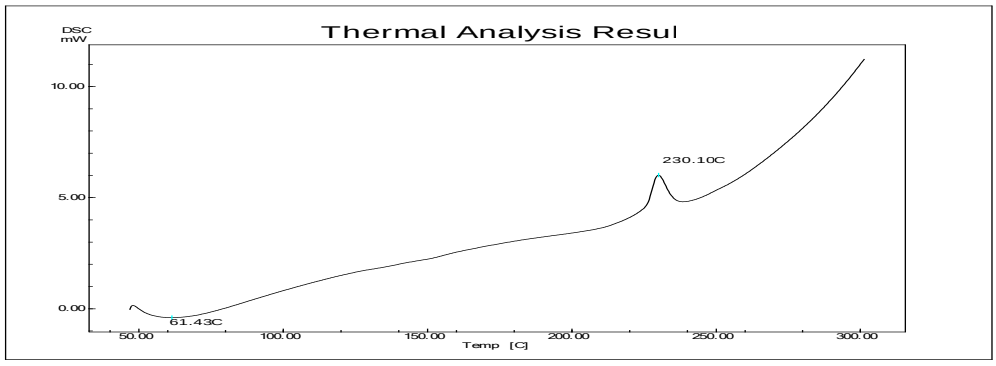

Figure 2: D.S.C. image of Lornoxicam and Sodium Luryl Sulphate . Lornoxicam showed a characteristic exothermic peak at 230.10^{0}C, which corresponds to its melting point.

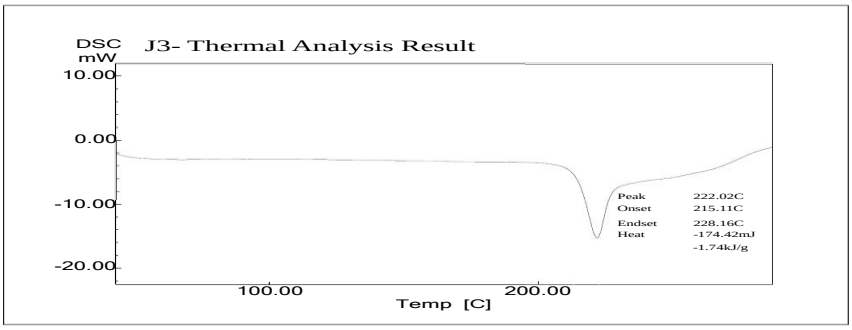

Figure 3: D.S.C. image of Lornoxicam and Sodium benzoate . Lornoxicam showed a characteristic exothermic peak at 222.02^{0}C, which corresponds to its melting point.

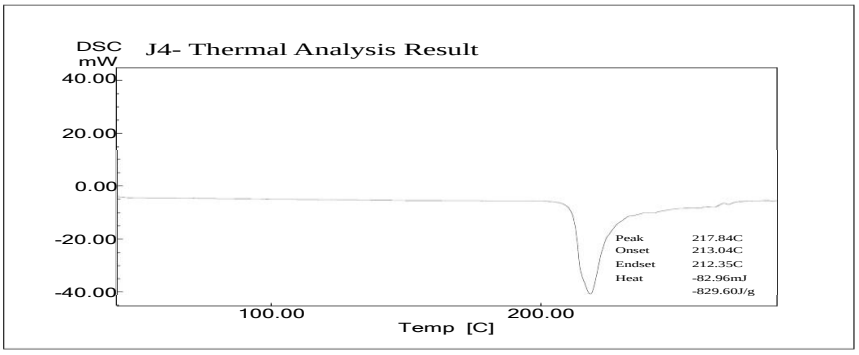

Figure 4: D.S.C. image of Lornoxicam and Urea . Lornoxicam showed a characteristic exothermic peak at 217.84^{0}C, which corresponds to its melting point.

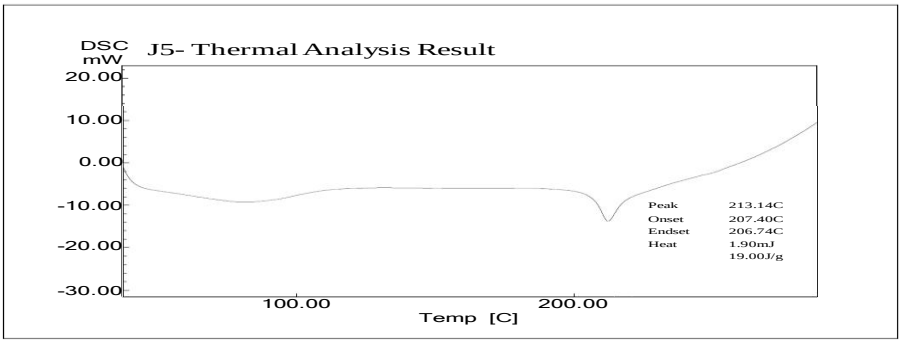

Figure 5: D.S.C. image of Lornoxicam and Sodium Salicylate . Lornoxicam showed a characteristic exothermic peak at 213.14^{0}C, which corresponds to its melting point.

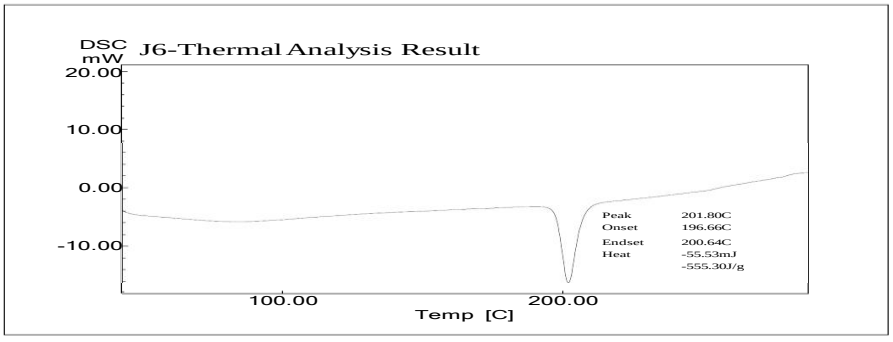

Figure 6: D.S.C. image of Lornoxicam and Nicotinamide . Lornoxicam showed a characteristic exothermic peak at 201.80^{0}C, which corresponds to its melting point.

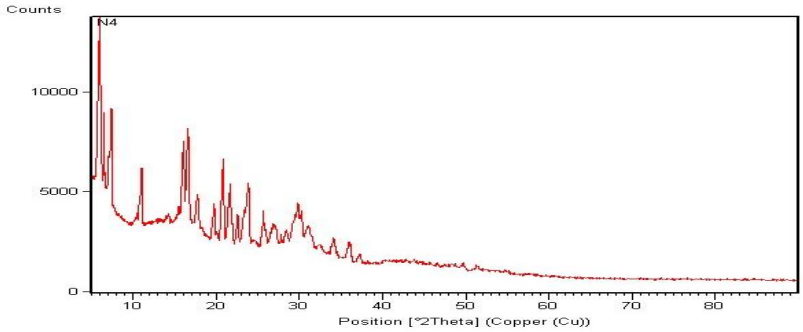

Figure 7: XRD image of Lornoxicam. Lornoxicam showed a characteristic peak at 20^o range, which range of 10^o to 60^o indicating that the powder was a high quality crystalline Powder.

Table 2: Solubility profile of Lornoxicam in different solvents.

Sl. No	Solvents	Concentration mg/ml	Standard deviation
1	DM Water	2.1439	± 0.108
2	0.1N Hcl	10.5571	± 0.003
3	Glycerin	9.9295	± 0.026
4	Polyvinylpyrrolidone	7.2613	± 0.009
5	10% PEG 400	19.9327	± 0.004
5	Cremophor EL	10.3129	± 0.006
6	Tween 80	17.0579	± 0.008
7	Span 20	13.0516	± 0.006
8	Lecithin	08.20031	± 0.007
9	10% Propylene glycol	14.6870	± 0.003
10	β-cyclodextrin	20.1455	± 0.003
11	0.1N NaOH	2.747	± 0.003
12	10% Sodium benzoate	11.33	± 0.026
13	10% Sodium salicylate	7.339	± 0.009
14	10% Resorcinol	10.354	± 0.006
15	10% Nicotinamide	4.363	± 0.008
16	10% SLS	36.311	± 0.007
17	10% Urea	12.617	± 0.003

Stability Evaluation

Various stress tests are performed on solid and solution samples to establish the effect of heat, light and oxygen on the drug substance stability.

1. Heat stability

Table 3: Heat stability profile of Lornoxicam

Temperature (°C)	Duration (weeks)			
	1	2	3	4
Refrigeration	-	-	-	-
Room temperature	-	-	-	-
40	-	-	-	-
50	+	+	+	+
75	+	+	+	+

+ Colour change, - No colour change

2. Light stability

Table 4: Light stability study of Lornoxicam

Withdrawal week	Observations	
	Clear	Amber
1	-	-
2	-	-
3	+	-
4	+	-

- Clear, + Turbidity

3. Effect of oxygen

Table 5: Test for color change after a week

Temperature(°C)	Air sealed vials	Perged vials
25	+	-
30	+	+

+ colour change, - no colour change

Estimation of drug content:

Table 6: Drug content in freshly prepared drug solution

Absorbance at 378nm	Conc. in µg/ml	Conc. in mg/ml
0.794	9940.53	9.9405

FORMULATION DEVELOPMENT

A stable parenteral formulation of water soluble drug Lornoxicam was formulated after performing trials with various solvents. Thus prepared formulations were subjected for various tests and results are discussed in the following section.

All the formulations were found to be easily passing through all the pore size filters and hence 0.22 µm pore size filter was selected to filter all the prepared formulations separately(Table 7). None of the formulations showed turbidity or signs of microbial growth (except the positive control) at the end of incubation period, indicating all the formulations were sterile and thus all the formulations are subjected to further evaluations.

Table 7: Filter pore size and filterability of the formulations of Lornoxicam

Formulation	Filter pore size(µm)	Observation
A	0.22	+
	0.45	+
	1.2	+
	1.5	+
B	0.22	+
	0.45	+
	1.2	+
	1.5	+

C	0.22	+
	0.45	+
	1.2	+
	1.5	+
D	0.22	+
	0.45	+
	1.2	+
	1.5	+
E	0.22	+
	0.45	+
	1.2	+
	1.5	+
F	0.22	+
	0.45	+
	1.2	+
	1.5	+

+ Injection passes through. - Injection does not pass through

POST FORMULATION STUDIES

Effect of different temperature on crystal growth:

Table 8: Effect of different temperature on crystal growth

Formulation	RT	40°C	Light
A	-	-	-
B	-	-	-
C	+	+	+
D	-	-	-
E	-	+	+
F	-	-	-

+ Crystal growth, - No crystal growth

In the formulations A, E, D and F no crystals were developed after two weeks. So A, E, D and F are stable at temperatures studied

Effect of different temperature on clarity:

Table 9: Effect of different temperature on clarity

Formulation	RT	40°C	Light
A	-	-	-
B	-	-	-
C	+	+	-
D	-	-	-
E	-	+	-
F	-	-	-

+ Turbid, - Clear

A, B, D and F are clear after two weeks. So A, B, D and E are stable at temperatures studied

Effect of different temperature on colour change:

Table 10: Effect of different temperature on colour change

Formulation	5°C	RT	40°C	Light
A	-	-	-	-
B	-	-	-	-
C	+	+	+	+
D	-	-	-	-
E	-	+	+	+
F	-	-	-	-

+colour change, - no colour change.

A, B and F show no colour change up to 40^0C after two weeks. So A, B and F are stable at temperatures studied.

SCALE UP STUDIES [11,12,13,14]

Assay of the formulations

Table 11: Drug content of B, F

Formulation	Drug content (mg/ml) *	% Drug content
B	16.89	101.156
F	16.658	104.663

* Each value is an average of three determinations

Sterilization studies and sterility testing filtration:

Table 12: Filter pore size and filterability of the formulations of Lornoxicam of B,F

Sl. No	Formulation	Filter pore size(µm)	Observation
1	B	0.22	+
		0.45	+
		1.2	+
		1.5	+
2	F	0.22	+
		0.45	+
		1.2	+
		1.5	+

+ Injection passes through. - Injection does not pass through.

The results of filterability show that both the formulations of Lornoxicam passes through all the four membrane filters. Hence they can be sterilized by filteration.

ACCELERATED STABILITY STUDIES

pH Changes

Table 13: pH changes of formulation B, F at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	Light
B	0	5.58	5.58	5.58
	1	5.55	5.55	5.58
	2	5.55	5.57	5.50

	3	5.59	5.59	5.50
	4	5.52	5.53	5.55
	5	5.54	5.52	5.54
	6	5.50	5.57	5.59
F	0	5.56	5.56	5.56
	1	5.53	5.52	5.55
	2	5.59	5.57	5.59
	3	5.55	5.54	5.57
	4	5.55	5.51	5.54
	5	5.53	5.51	5.50
	6	5.55	5.59	5.57

CRYSTAL GROWTH

Table 14: Crystal growth of formulation B, F at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C	Light
B	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
F	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-

+crystal growth, - no crystal growth

No crystal growth was observed in the formulations at different temperatures/conditions.

CLARITY STUDIES

Table 15: Clarity of formulation B, F at different temperatures/conditions on ageing

Formulation	Withdrawal Week	37°C	40°C	45°C	Light
B	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
F	0	-	-	-	-
	1	-	-	-	-

2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-

+ Turbid, - clear

All the formulations were clear at different temperatures/ conditions

DRUG CONTENT

Table 16: Percent drug content of formulation B at different temperatures/conditions on ageing.

Sample withdrawal (week)	% Drug Content		
	37°C	40°C	Light
0	101.116	101.156	101.156
1	101.015	100.973	101.000
2	100.873	100.761	100.728
3	100.758	100.537	100.569
4	100.569	100.365	100.296
5	100.470	100.107	100.100
6	100.017	99.897	99.901

Table 17: Percent drug content of formulation F at different temperatures/conditions on ageing.

Sample withdrawal (week)	% Drug Content		
	37°C	40°C	Light
0	104.006	104.066	104.066
1	103.857	103.410	103.839
2	103.028	103.698	103.725
3	103.501	103.527	103.400
4	103.089	103.271	103.317
5	103.109	103.106	103.118
6	102.961	102.894	102.996

CONCLUSION:

The concept of parenteral formulations containing Lornoxicam offers a suitable, practical approach to achieve desired stable parenteral preparation with solubility of drug in suitable solvent composition. In present work, parenteral formulation of Lornoxicam was prepared successfully by using different concentrations and combinations of PEG-400, β-cyclodextrin, Sodium lauryl sulphate in formulation design. These formulations were expected to be stable for sufficiently long time. The conclusions arrived from the above results indicated that the parenteral formulation containing Lornoxicam developed was found to be complying satisfactorily with all the evaluation tests performed and was stable for sufficiently longer duration of time.

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