



## Formulation Design and *In Vitro* Characterization of Etoricoxib Cream for the Treatment of Rheumatoid Arthritis

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### Abstract

Non-Steroidal Anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase (COX)-2 inhibitors, have come to play an important role in the pharmacologic management of musculoskeletal disorders. Clinical trials have established the efficacy of etoricoxib in Osteoarthritis, Rheumatoid Arthritis, Acute Gouty Arthritis, Ankylosing Spondylitis, Low back pain, acute postoperative pain, and primary dysmenorrhea. The present research has been undertaken with the aim to develop a novel topical cream formulation of etoricoxib, which would attenuate the gastrointestinal related toxicities associated with oral administration. Etoricoxib is a highly selective cyclooxygenase-2 (cox-2) inhibitor. In the present study, a fixed concentration of Etoricoxib cream (1%) was prepared by using a different combination of Active ingredient and Excipients. To assess the efficacy of formulated cream, *in vitro* evaluation including stability studies, tube extrudes ability, spread ability, pH, viscosity and rheological properties as well as drug diffusion studies were done. After *in vitro* evaluation of cream formulations, the formulation was evaluated for the anti-inflammatory and skin irritation study. The results obtained were encouraging and formulation containing Etoricoxib (1%) exhibited the most satisfying results of all the parameters.

**Key Words:** Anti-Inflammatory, Cream, Etoricoxib, Stability studies

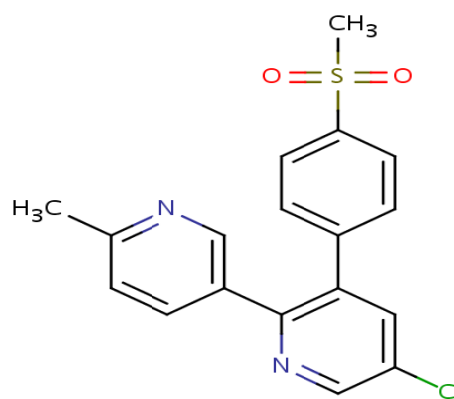
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### 1. Introduction

Non-Steroidal Anti-inflammatory drugs (NSAIDs) are widely employed in musculoskeletal disease, both for their anti-inflammatory as well as their analgesic properties [1]. Use of these agents may extend from the acute injury to the long-term chronic

disorder, and includes conditions considered to be 'degenerative' as well as those of a clearly inflammatory etiology [2]. Efficacy of various NSAIDs in different clinical settings has been extensively evaluated [3]. Musculoskeletal Disorders are degenerative diseases and inflammatory conditions that cause pain and impair normal activities. They can affect many different parts of the body including upper and lower back, neck, shoulders and extremities (arms, legs, feet, and hands) [4]. These disorders may include Rheumatoid Arthritis, Osteoarthritis, Tendinitis, Low back pain and Gout [5]. Musculoskeletal disorders can arise from the interaction of physical factors with economic, psychological, and social factors [6]. Pain is usually best relieved by treating the cause of musculoskeletal disorder. Non Steroidal Anti-inflammatory agents (NSAIDs) or if pain is severe opioids are recommended. Depending on the cause, applying cold or heat or immobilizing the joint may help relieve musculoskeletal pain [7]. Etoricoxib is a Non-Steroidal Anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities [8]. It is potent, highly selective which is described chemically as 5-chloro-6'-methyl-3-[4-(methylsulfonyl)phenyl]-2, 3'-bipyridine. However its use has been associated with a number of gastrointestinal disorders. These potential side effects may be overcome by the topical

administration of the drug [9]. *In vitro*, etoricoxib exhibits a greater selectivity for COX-2 over COX-1 compared with the COX-2 inhibitors rofecoxib, valdecoxib, and celecoxib. Etoricoxib binds competitively to COX-2 with 1:1 stoichiometry in a reversible, noncovalent manner [10]. In healthy volunteers, oral etoricoxib is rapidly and completely absorbed. It reaches  $C_{max}$  after approximately 1 hour and has up to 100% absolute bioavailability [11]. Etoricoxib is indicated in the management



**Figure 1.** Etoricoxib structural formula.

of Osteoarthritis, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, post operative pain, chronic low-back ache and Gout. The structural formula of Etoricoxib is given in Figure 1.

## 2. Materials and Methods

### 2.1. Chemicals

Etoricoxib was received as a gift from Global Pharmaceuticals (Pvt) Ltd, Cetostearyl Alcohol

(BDH Labs, England), White Petrolatum (Kukdong oils and chemicals, Korea), Methyl paraben and Propyl paraben (BDH labs, England), Liquid Paraffin (Kukdong oil and chemicals, Korea), Polyoxyethylene (80) Sorbitanmonooleate (Tween 80) (Merck, Germany), Triethanolamine (Merck, Germany), Carbopol 980 NF polymer (Lubrizol, USA), Glycerin (Merck, Germany) and De-ionized water (Medilines Diagnostic division).

### 2.2. Apparatus

Glass beaker 50ml, 100ml (Pyrex, England), Conical flask 50ml, 100ml (Pyrex, Germany), Pipette 10ml (Preciclor, Germany), White colored glass jar, Amber colored glass jar, aluminum collapsible tubes and aluminum foil.

### 2.3. Instruments

UV-Visible Spectrophotometer (Shimadzu,

Japan), Weighing balance (Analytical grade), Magnetic stirrer/ Hot plate (Made in Germany), pH meter (Model No: 3510, Germany), Homogenizer (Euro-Star, IKA D 230, Germany), Brookfield digital viscometer (model DV-III+, Brookfield Engineering Laboratory, INC. USA), Keshary-chien diffusion cell, Refrigerator (PEL, Pakistan), Soxhlet Apparatus, Incubator (Sanyo MIR-162, Japan), Oven (Schutzartdin 40050 IP-20, Germany).

### 2.4. Animal

The animals were Albino Rabbits.

### 2.5. Formulation of Etoricoxib Cream

1% by weight of Etoricoxib cream was made according to the formulation given in Table 1.

### 2.6. Preparation of Cream

Etoricoxib cream was formulated by the

**Table 1.** Formulation of Etoricoxib cream.

Serial No	Ingredients	%age Composition
1	Etoricoxib	1.0
2	Cetostearyl Alcohol	10.0
3	White Petrolatum	5.0
4	Methyl paraben	0.12
5	Propyl paraben	0.02
6	Liquid paraffin	5.0
7	Tween- 80	8.0
8	Triethanolamine	1.50
9	Carbopol 980	0.60
10	Glycerin	6.0
11	De-ionized water	62.76

method of Razi Ullah *et al* [12]. The oil and aqueous phases were taken into bakets and heated to 75°C over a water bath. The oil phase was comprised of Etoricoxib, Liquid Paraffin, White Petrolatum, Cetostearyl Alcohol, Tween-80 and Carbopol 980 while the aqueous phase was composed of Methyl parabens, Propyl parabens, Glycerin and Triethanolamine and De-ionized water. Drop wise addition of the aqueous phase to the oil phase was done with constant stirring at 2000rpm in a homogenizer for a period of 20 min. The homogenizer speed was then reduced to 1000rpm and homogenization was continued for another 5-10 min. The speed was further reduced to 500 rpm and the homogenization extended for 5min. The Etoricoxib cream was formulated.

## 2.7. Characterization of Etoricoxib Cream

### 2.7.1. Physical Evaluation of Cream

Physical evaluation including pH, Spread ability, Tube extrudes ability, viscosity and *In vitro* drug diffusion studies were carried out on Etoricoxib cream.

### 2.7.2. Determination of pH

The pH of the Etoricoxib cream was found by immersing pH meter to a depth 0.5 cm in a beaker containing cream [13]. The determinations were carried out in triplicate and the average of three reading is recorded.

### 2.7.3. Determination of Spread Ability

Spread ability was determined by the apparatus that consist of a wooden block, which was provided by a pulley at one end. By this method, spread ability was measured on the basis of 'Slip' and 'Drag' characteristics of creams [14]. A ground glass slide was fixed on this block. An excess of cream (about 2 gram) under study was placed on this ground slide. The cream was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the cream between the slides. Excess of the cream was scrapped off from the edges. The top plate was then subjected to pull of 80 grams. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spread ability. Spread ability was then calculated using the following formula:

$$S = M \times L / T$$

Where, **S** = is the spread ability, **M** = is the weight in the pan (tied to the upper slide), **L** = is the length moved by the glass slide and **T** = represents the time taken to separate the slide completely from each other.

#### 2.7.4. Determination of Tube Extrude ability

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by wood et al[15]. In the present study, the method adopted for evaluating Etoricoxib cream formulation for extrude ability was based upon the quantity in percentage of cream and cream extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of cream in 10 seconds. More quantity extruded better was extrude-ability. The measurement of extrude ability of each formulation was in triplicate and the average values are presented [16]. The extrude ability was than calculated by using the following formula [17]:

*Extrude ability* = Applied weight to extrude gel from tube (in gm) / Area (in cm<sup>2</sup>)

#### 2.7.5. Determination of Viscosity

The viscosity of cream formulation was determined by using Brookfield digital Viscometer. In a clean and dry 250ml beaker, take the test sample. Determine the viscosity of the test sample as per standard operating procedure of viscometer and the spindle T-D (Spindle code S 94) was used. The spindle was

rotated at speeds of 2.5, 4, 5 and 10 rpm. The reading near to 100% torque was noted [18]. The readings were taken as triplicate and average of readings was noted.

#### 2.7.6. In Vitro Drug Diffusion Studies

The diffusion studies were performed using a Keshary-chien diffusion cell. The cell was locally fabricated and had a 25 ml receptor compartment. The dialysis membrane was mounted between the donor and receptor compartments. The cream formulation was applied uniformly on the dialysis membrane and the compartments were clamped together. The receptor compartment was filled with the phosphate buffer (pH 7.4) and the hydrodynamics in the receptor compartment were maintained by stirring with a magnetic bead. The study was carried out for 24 hrs with the interval of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. 1ml of samples was withdrawn from the receptor compartment at pre-determined time intervals and an equal volume of buffer was replaced. The absorbance of withdrawn sample was measured spectrophotometrically against appropriate blank [19].

#### 2.8. Stability Studies of Etoricoxib Cream

Stability studies on Etoricoxib cream formulation were conducted over a period of three months at different conditions: (a) at 25 ± 1 °C (b) at 40 ± 1 °C. The cream was analyzed by

UV- Visible Spectrophotometer, immediately after preparation (at zero time) and after every month until three months period [20].

The active contents in cream formulation were determined by measuring the absorbance of sample solution on UV Spectrophotometer at 238nm wavelength at above mentioned time intervals and by calculating the remaining %age of active content by following formula:

*Remaining %age of active content in sample solution* = (Absorbance of Sample / Absorbance of Standard) × (Conc. of Standard / Conc. of Sample) × % age purity of Standard [21].

#### 2.8.1 Determination of Drug contents by Spectrophotometric Method

#### 2.8.2 Preparation of Standard Solution

50 mg of Etoricoxib was carefully weight on analytical balance and dissolved in ethanol and made the volume up to 100ml with ethanol. The solution was then filtered and 1ml was taken from that solution and made the volume of that solution up to 50ml with same solvent and it was taken to be the standard solution in UV Visible spectrophotometer.

#### 2.8.3 Preparation of Sample Solution

5 g of the Etoricoxib cream formulation was taken and dissolved in ethanol and made the volume up to 100ml with the same solvent. The solution was then filtered and 1 ml was taken made up to 50 ml with ethanol. The absorbance

was measured at 238nm using ethanol as blank solution.

#### 2.9 Anti-Inflammatory activity of Etoricoxib Cream

Anti-inflammatory [22] study was conducted using 10 albino rabbits (approved by Institutional Animal Ethical Committee, University of Sargodha, Sargodha, Pakistan) of either sex and divided into 2 groups. In these rabbits, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of carrageenan in normal saline in left hind paw of the rats. The medicated formulation (0.25g) was applied topically with gentle rubbing to the paw of each rat of respective group one hour before and one hour after the carrageenan challenge. The paw edema volume was measured using plethysmometer at 1, 2, 3 and 4 hour after injection of carrageenan. The average paw edema volume of all rabbits was calculated.

#### 2.10 Skin Irritation Study

In skin irritation study [23] three albino rabbits were selected for the study. 24 hours prior to the test, the test sites were depilated on both sides of the spine and demarcated for the application of the formulation. The measured quantity of cream was applied over the respective test sites. The test sites were observed

for erythema and edema for 24 and 48 hours respectively after the application.

### 3. Results and Discussion

The aim of the present study was to develop an Etoricoxib cream formulation, used for the management of musculoskeletal disorders and does not produce any undesirable side effects that are seen with the oral dosage form.

Etoricoxib cream was prepared using different concentration of excipients and active ingredient. Accelerated stability study was done at  $25 \pm 1^\circ\text{C}$  and  $40 \pm 1^\circ\text{C}$  as shown in table no 3, 4 and 5. Stability testing was done for the period of 3 months (90 days). It is evident from the results that cream formulation is best suitable at ( $25 \pm 1^\circ\text{C}$ ) as % age of drug remaining is not decreased by more than 10% [24]. It is also

**Table 2.** %Age remaining of drug content at zero time.

Absorption Standard	Absorption of Sample	% age of active drug in the sample	Mean	Standard Deviation
0.625	0.623	99.28%	0.624	0.001414

**Table 3.** %Age remaining of drug content after 1 month.

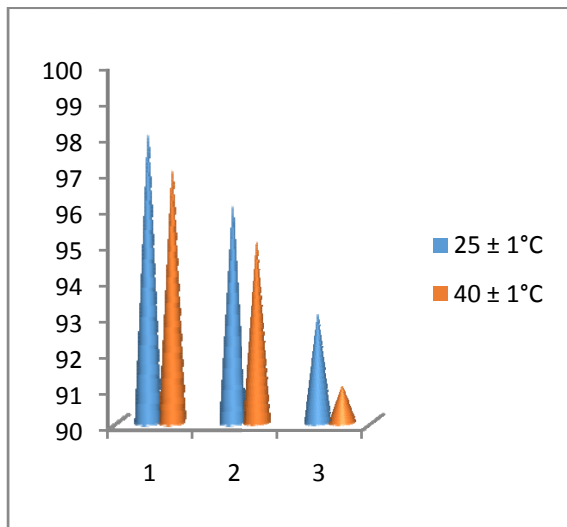
	Absorption Sample	% age of active drug in the sample	Mean	Standard Deviation
At $25 \pm 1^\circ\text{C}$	0.617	98.32%	0.621	0.005656
At $40 \pm 1^\circ\text{C}$	0.610	97.20%	0.6175	0.01060

**Table 4.** %Age remaining of drug content after 2 months.

	Absorption Sample	% age of active drug in the sample	Mean	Standard Deviation
At $25 \pm 1^\circ\text{C}$	0.604	96.25%	0.6145	0.014849
At $40 \pm 1^\circ\text{C}$	0.595	94.81%	0.61	0.021213

**Table 5.** %Age remaining of drug content after 3 months.

	Absorption Sample	% age of active drug in the sample	Mean	Standard Deviation
At $25 \pm 1^\circ\text{C}$	0.604	96.25%	0.6145	0.014849
At $40 \pm 1^\circ\text{C}$	0.595	94.81%	0.61	0.021213



**Figure 2.** A graphical representation between percentage of drug concentration (of drug in age %) and time in months.

evident from the results of standard deviation at the end of three months that at 25 ± 1°C standard deviation was least and it fell into acceptable range, but at 40°C the standard deviation is bit higher and away from normal and reasonable range. So it can be concluded, that at 25 ± 1°C, the cream formulation fulfils the criteria required for a pharmaceutical cream preparation to be acceptable concerning accelerated stability studies as shown in Figure 2.

pH evaluation is also important to check the stability of Etoricoxib cream formulation. Three reading of pH were taken at the time of preparation of cream and their average was determined. The pH of Etoricoxib cream was in the range of 6.6 ± 0.6 to 6.8 ± 0.351 and the average was found to be 6.73, which lies in the normal pH range of the skin and would not produce any skin irritation. The result of spread ability varies from 4.75 to 6.25 g/sec whereas the extrude ability of cream formulation from

**Table 6.** Evaluation data of Etoricoxib cream formulation.

Parameters evaluated	Study Period			
	1 <sup>st</sup> Reading	2 <sup>nd</sup> Reading	3 <sup>rd</sup> Reading	Average
<b>pH ± SD</b>	6.65± 0.6	6.74± 0.46	6.81± 0.351	6.73± 0.44
<b>Spread ability (g/sec)</b>	4.75	5.53	6.25	5.51
<b>Tube extrudability (g)</b>	185	190	195	190



**Table 7.** Viscosity of Etoricoxib cream formulation (cps) at different rpm.

Speed in rpm	1 <sup>st</sup> Reading	2 <sup>nd</sup> Reading	3 <sup>rd</sup> Reading	Average
2.5	65187	65235	65235	65219
4.0	34470	34697	34846	34671
5.0	25953	26123	26547	26208
10	14110	14735	15174	14673

the collapsible tube varies from 185 to 195. The viscosity of cream formulation varies from

of drug for longer period of time which will be helpful to avoid the more fluctuation and also

**Table 8.** *In Vitro* Drug Diffusion Study over period of 24 Hours.

Time (Hours)	% age of drug release
0.0	0.0
0.5	10.73
1.0	18.77
2.0	30.85
4.0	41.12
6.0	51.33
8.0	72.41
10.0	85.62
12.0	89.22
24.0	91.51

65219 cps to 14673 cps from 2.5 to 10 rpm.

reduces the cost of therapy.

### 3.1. *In Vitro* Drug Diffusion study

From the data we have found that the prepared topical cream formulation of Etoricoxib releases 91.51% of drug over a period of 24 hours. From the *In – vitro* drug diffusion study we have concluded that the cream formulation prepared, controls the release

### 3.2. *Anti-Inflammatory effect of Etoricoxib Cream*

Percentage increase in paw volume (inflammation) and percentage inhibition of inflammation in all the groups treated with test the product.

**Table 9.** Mean paw edema volume and %age inhibition of edema in albino rabbits.

Time (Hours)	Mean paw edema volume	%age inhibition of edema
0	0.204	24.36
1	0.163	37.59
2	0.103	63.08
3	0.097	70.72
4	0.096	73.41
5	0.099	74.54
6	0.105	75.56

### 3.3 Skin Irritation Test

In skin irritation test, no signs of erythema and edema were found after 24,48 and 72 hours of cream application in albino rabbits.

NSAIDs. The cream formulation contains Etoricoxib that is a potent, highly selective cyclooxygenase-2 (COX-2) inhibitor, that exhibits analgesic, anti pyretic and a strong anti-inflammatory activity. Etoricoxib is very



**Figure 3.** Rabbit Skin- on application of Etoricoxib cream.

### 4. Conclusion

The present cream formulation was developed by taking into consideration that in cream formulations there is present no direct contact of active drug with stomach wall. This can be a reason to remove the chances of gastric mucosal damage to a reasonable level that is caused by the use of solid dosage forms of

effective to mimic the pain and inflammation in Rheumatoid arthritis, Osteoarthritis and others musculoskeletal disorder patients. From the above results it can be concluded that the etoricoxib cream formulation containing 1% etoricoxib was suitable for topical application and it shows comparable results with that of marketed product.

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## References

- [1] Gabriel S.E, Matteson E.L. Economic and quality-of-life impact of NSAIDs in rheumatoid arthritis: A conceptual framework and selected literature review. *Journal of Indian Pharmaceutics* (1995) 8(6): 479–490.
- [2] Zochling. Non steroidal anti-inflammatory drug use in ankylosing spondylitis—A population-based survey. *J Clin Rheumatol* (2006)25(6): 794–800.
- [3] Hochberg M.C. COX-2 selective inhibitors in the treatment of arthritis: A rheumatologist perspective. *Journal of Current Tropical Medicinal Plants* (2005) 5(5): 443–448.
- [4] Kuorinka I. Standardised Nordic questionnaires for the analysis of musculoskeletal symptoms. *Journal of Applied Agronomics* (1987) 18(3): 233-237.
- [5] Sprig, C. A. Work characteristics, musculoskeletal disorders, and the mediating role of psychological strain: A study of call center employees. *J Appl Psychol* (2007) 9(5): 1456-1466.
- [6] Gatchel R. J., Kishino N. Pain. Musculoskeletal injuries and return to work. *Handbook of occupational health psychology*, 2<sup>nd</sup> edition, Washington, DC: American Psychological Association (2011).
- [7] Gallagher S, Heberger J. R. Examining the interaction of force and repetition on musculoskeletal disorder risk. A systematic literature review. *Human Factors* (2013) 55(1): 108-124.
- [8]Valenta C, Wanka M, Heidlas J. Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. *Journal of Control Release JCR* (2000) 63:165-173.
- [9] Rhee Y. Transdermal delivery of ketoprofen using micro emulsions. *International Journal of Pharmaceutics AIP* (2001) 2 (3):161-170.
- [10] Riendeau D, Percival MD, Brideau C. Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *Journal of Pharmacology and Experimental Therapeutics JPET* (2001)296:558–66.
- [11] Agrawal NG, Porras AG, Matthews CZ. Single- and multiple-dose pharmacokinetics of etoricoxib a selective inhibitor of cyclooxygenase-2 in man. *Journal of Clinical Pharmacology JCP* (2003)43:268–76.
- [12] M.RaziUllah Khan, Shahid Masood Raza and Musaddique Hussain. Formulation and *In-Vitro* evaluation of Cream containing Diclofenac Sodium and Curcuma Longa for the Management of Rheumatoid Arthritis. *International Journal of Pharma Sciences IJPS* (2014) 4(4): 654-660.
- [13] Gupta GD, Gaud RS. Release rate of Tenoxicam from acrypol gels, *Journal of Indian Pharmacist* (2005)69 – 76.
- [14] Chakole CM, Shende MA, Khadatkar SN. Formulation and development of novel combined halobetasol propionate and fusidic acid ointment, *International Journal of Chemical Technology Research* (2009)1: 103 – 116.
- [15]Rathore RPS, Nema RK. Formulation and evaluation of topical gels of Ketoprofen, *Asian Journal of Pharmaceutical and Clinical Research* (2008)1: 12 – 16.
- [16] Wood J. H., Catacalos G. Liberman S. V. Adaptation of commercial viscometers for special applications in pharmaceutical rheology-II, *Journal of Pharmaceutical Sciences* (1993) 52: 375-378.

[17] Umadevi SK. The effect of polymers and permeation enhancers on *in vitro* release of aceclofenac from topical gel formulations. *Biosciences Biotechnology Research Asia* (2005) 5(1): 237-244.

[18] Alexander S, Thyangarajapuram N. Formulation and Accelerated Stability Studies for an Extemporaneous Suspension of Amiodarone Hydrochloride. *International Journal of*

*Pharmaceutical Compounding* (2003) 3:34-36.

[19] Nakhat PD, Rathi LG, Yeole PG. Development and evaluation of sorbitanmonostearate organogels of ibuprofen. *Indian Drugs* (2006) 43 (5): 421-430.

[20] Kulkarni SK, Jain NK. Pharmacological and pharmacokinetic studies on marketed gel formulations of nimesulide. *Indian Drugs* (2001) 38 (2): 63-69.