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Research Article

# HPTLC AND HPLC METHOD FOR THE DETERMINATION OF ACECLOFENAC IN EMULGEL

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**ABSTRACT**
The aim of the current study was Determination of High Performance Thin Layer Chromatography and High-Performance Liquid Chromatography Method of Aceclofenac in Emulgel. The topical delivery system is generally used when other drugs cannot be administered or when local skin infections such as fungi are present. Local dermatological disorders can be effectively treated with drug delivery to the skin. The skin protects the body from invading pathogens with its formidable barrier functions. formulation of Aceclofenac containing emulgel were prepared and this prepared formulation was analysed as per the official guidelines. There is no doubt that aceclofenac emulgels will be the most commonly procured dosage form in the future due to their better therapeutic potential and fewer adverse effects on patients.

**Keywords**: HPTLC, HPLC, Aceclofenac, Emulgel

## INTRODUCTION

Drug and chemical analysis involves the use of analytical procedures for determining purity, safety, and quality. The majority of manufacturing industries use both qualitative and quantitative chemical analysis to ensure that raw materials meet certain specifications, and to ensure the final product is of high quality. There is a requirement to carry out an examination of raw materials to ensure that there are no unusual substances present in them that could adversely affect the manufacturing process or appear in the final product as a harmful impurity [1].

The final manufactured product is subjected to quality control procedures in order to verify that its essential components are present within a predetermined range of composition, and that any impurities have not exceeded certain limits set by the manufacturer [2].

Chromatography is defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases [3].

The technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase) [4].

Topical formulations are prepared in different consistency such as solid, semisolid, and liquid. The topical delivery system is failed in the administration of hydrophobic drug. In each formulation with the active ingredients many excipients are used. Sometimes more than one formulation can be combined to enhance the drug delivery; emulgel is such type of combination. It is the combination of emulsion and gel [5].

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## METHODOLOGY

### Experimental requirements

The following are the Equipment, Instrument, and Materials that were used for the formulation and evaluation of Drug. Aceclofenac, carbopol 934, Tween 80, Span 80, Propylene glycol, ethanol, clove oil, Methyl paraben. Digital balance, magnetic stirrer, Spectrophotometer, compound microscope, Dissolution test apparatus, pH meter

### Preformulation studies

Preformulation studies are performed for the improvement of Emulgel before the initiation of plan

advancement, and the significant objective of the investigation is to create or foster steady, safe, and restoratively powerful and effectual dose frames that are essentially identified with the portrayal of the physicochemical properties of the medication substance.

**Characterization of Aceclofenac**

For pre-formulation studies, the micronized form of Aceclofenac was subjected to physical tests.

**Drug – Excipients Compatibility Studies**

For the selection of suitable additives or excipients while developing a pharmaceutical formulation it's necessary to check the drug- excipients compatibility. Various organoleptic (macroscopic) properties were observed using this study. Drug excipients compatibility tests give the assurance of the stability of formulation. The active drug was mixed with Potassium bromide (KBr) and spectra were plotted on FT-IR.

Accordingly, the excipient and Potassium bromide mixed in the same ratio as 9:1 ratio and spectra were plotted. The FT-IR band of Aceclofenac was checked with FT-IR spectra of Aceclofenac with other additives used. Shifting or Disappearance of Aceclofenac peak in spectra was examined [6].

**Procedure**

Aceclofenac was mixed with various excipient used in the study in the ratio as given in table then filled in glass vials along with low-density polyethylene stopper with holes in the stopper and subjected to a different condition like room temperature, 60˚C and 2-8˚C for four weeks. After the completion of the specified period, blends were tested for their physical change and moisture content.

All the excipients were evaluated with the drug for compactability studies. The preformulation studies were performed according to the formula and results were recorded based on the data was obtained accordingly.

**Determination of λ max of Aceclofenac using a UV spectrometer**

Max. Absorbance (λmax.) of Aceclofenac was determined by UV visible spectrophotometer by scanning drug samples between 203-280 nm and spectra were found [7].

**Identification of Drug**

FT-IR spectroscopy technique was used for the identification and evaluation of drug and excipients. Drug KBR pellets were used to record the FT-IR spectrum with a Perkin-Elmer model.

**Formulation Development**

The material and method required for the formulation of the Emulgel and the associated evaluation parameter of the latter are explained in the following section.

**Method for the preparation of the Aceclofenac Emulgel**

A sum of 3 preparations were developed using various steps. The different formulation prepared for Aceclofenac Emulgel is given in the below table 2

**The steps used in the preparation of Emulgel were as follows**

Molecular dispersion technique with micronized active drug Aceclofenac by using excipients, the process will be the same for every trial batch from Batch No. F1 – Batch No. F3 for the manufacturing of Emulgel.

**Process -1: - Material Sifting**

Aceclofenac, Span 20, Tween 80, Carbopol 934, Liq. Paraffin, Propylene Glycol, HPMC K4M, Methyl Paraben shall be sifted through #20, #40 and filter through a suitable filter separately.

**Process– 2: - Manufacturing Process**

Here, two formulations were prepared by same method but they differed only in their type of gelling polymer used to formulate gel. The preparation method of emulsion was same. Gel was prepared by dissolving 1gm of Carbopol 934 and HPMC K4M separately in purified water (50 ml) with constant stirring at optimum speed by mechanical stirrer. The pH was adjusted by TEA (triethanolamine) to 6-6.5. The emulsion was prepared by following method, the Oil phase was prepared by dissolving span 20 in liquid paraffin. Then the drug was added to the above mixture. The aqueous phase was prepared by dissolving Tween 20 in purified water. Methyl paraben was dissolved in propylene glycol separately and this mixture was added to above mixture with constant stirring. Both oil and aqueous phases are heated to 70-80°C. Then oil phase is added to aqueous phase with constant stirring until to get cooled to temperature to form emulsion with constant stirring (Pravallika & Priyanka 2019).

**Process – 3: - Analysis**

Emulgel Formulations are analyzed as per the official guidelines for all parameters

**Evaluation Protocol of Emulgel**

**Physical tests**

The prepared Emulgel undergoes to determine the color, grittiness, appearance & viscosity

**Rheological Study**

The consistency was dictated by utilizing Brooke field viscometer DV II+ Pro, a cone and plate kind of viscometer with axle no 52. The instrument was collected and room temperature was kept up with at 25°C all through try. The emulgel whose consistency was to be estimated was weighed about 0.5 gm and set in plate and shut. Then the spindle is allowed to run and the viscosity was measured at 0.2 rpm

**Measurement of pH**

The pH was recorded using the digital pH meter under ambient & standard conditions [8].

**HPTLC studies on Aceclofenac**

**Preparation of Standard Solution and Construction of Calibration Plots**

The standard stock solution of aceclofenac were prepared by dissolving 10 mg of drug in 10 ml of methanol. From this solution, 1 ml of solution were taken and diluted to 10 ml with the same to get a solution containing 100µg/ml of each drug. A calibration curve was plotted between concentration against their respective area for aceclofenac [9].

**HPLC Analysis Of Emulgel**

**Materials**

Working standards of pharmaceutical grade ACF (batch no. 1103/09) obtained as a gift sample from Intas Pharmaceuticals. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India. High purity deionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) water purification system.

**Instrumentation and chromatographic conditions**

The HPLC system (Jasco corporation, Tokyo, Japan) which consisted of a Pump (model Jasco PU-2080 Plus) along with manual injector sampler programmed at 20 µl capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a HiQ-Sil™ HS C18 column (250×4.6 mm i.d., 5 µm particle size), kromatek, Essex CM6 1XN, Japan. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase consisted of 40: 60 (v/v); phosphate buffer (pH 6.0): methanol. The flow rate was set to 1.0 mL min−1 and UV detection was carried out at 270 nm at ambient temperature.

**Standard stock solution**

Stock standard solution containing ACF (1000 µg mL−1) was prepared by dissolving 50 mg of ACF in water: methanol; 50: 50 (v/v) (denoted 'diluent') in a 50 mL volumetric flask. This was further diluted with diluent to obtain working standard solutions in a concentration range of 40–160 µg mL−1 (i.e. 40, 60, 80, 100, 120, 140, and 160 µg mL−1) [10].

**Sample preparation**

To determine the content of ACF in emulgel, 100 mg of emulgel was taken and transferred into a 100 mL volumetric flask containing 20 mL methanol, sonicated for 10 min. Added 20 mL water and sonicated for 10 min. Then, diluted to 100 mL with diluent and sonicated for 20 min. with intermittent shaking. This solution was filtered through a 0.45 µm nylon syringe filter. 1 mL of the above solution was transferred to 10 mL volumetric flask and diluted to volume with diluent. The concentration achieved after the above dilution was 100 µg mL−1 of ACF. A constant 20 µL volume of sample solution was injected six times under the conditions described above. The peak areas were measured at 270 nm for ACF respectively.

**Method validation**

The optimized HPLC method was validated with respect to the following parameters. The validation was performed as per ICH guidelines determined by Precision, Robustness, Limit of detection (LOD) and limit of quantitation (LOQ), Specificity, System suitability [11].

**RESULTS**

**Preformulation studies**

**Analysis of Aceclofenac**

**Drug-excipient compatibility data**

The drug was evaluated along with all the excipients for compatibility studies and the results were recorded as per the data obtained after completion of the studies in following table

**UV Absorbance spectra of Aceclofenac**

The study of spectra showed that Aceclofenac have λmax at at 277.0 nm, respectively.

**FTIR Spectroscopy technique**

FT-IR spectroscopy method was used for the evaluation of the drug, there were several stretching's observed between C-H, C-N, and C-O. In FT-IR spectra the functional groups showing Characteristics peaks.

**Formulation of Emulgel**

A 3 formulation of Aceclofenac containing emulgel were prepared and this prepared formulation was analysed as per the official guidelines.

**Evaluation Protocol of Emulgel**

**Physical tests**

The prepared Emulgel was found optimum in terms of their color, grittiness, appearance & viscosity.

Emulgel formulations were observed for physical examinations as follows
Color: Yellowish white
Consistency: Viscous
Appearance: Glossy
Grittiness: No

**Rheological Study**

The different formulations of Emulgels were evaluated for their rheological properties. Formulation no. 1, 2 and 3 were analyzed at RPM 0.2. At shear stress 165.7 for F1 demonstrated the viscosity of 13950. For F2 at shear stress 169.4 the viscosity was noted as 15526. At last, for F3 at shear stress of 169.4 the viscosity was found as 14526. By exhibiting such viscosity strengths, all the formulation was found as suitable emulgel having the optimum level of rheological properties.

**Measurement of pH**

The pH was estimated for different preparations as below mentioned

**HPTLC studies on Aceclofenac**

Initially, ethyl acetate and toluene in the ratio of 4:4(v/v) was tried for both drugs. Then, toluene ethyl acetate and methanol in the ratio of 6:4:1 (v/v/v) was tried. The developed spots were diffused. To the above mobile phase, 0.1mL glacial acetic acid was added. The peaks were symmetrical in nature and tailing was observed. To improve resolution, the volume of glacial acetic acid was increased to 0.2 mL. The optimized mobile phase contained The analytes were monitored at 278 nm and the Rf were found to be 0.57 for aceclofenac, respectively. Figure 6 shows the HPTLC chromatogram of aceclofenac

**HPLC ANALYSIS**

The HPLC analysis of aceclofenac containing emulgel has done. The results discussed as follows

**Optimization of procedures**

The HPLC procedure was optimized with a view to develop a simultaneous assay method for ACF. The stock standard solution was diluted with diluent to a concentration of 100 µg mL−1 of ACF.

Then, the standard solution was injected into a HiQ-Sil™ HS C18 column (250×4.6 mm i.d., 5 µm particle size). Initially, acetate buffer was tried in acidic pH, ammonium acetate buffer (pH 4.0): methanol; 40: 60 (v/v), the ACF peak obtained with a retention time of more than 20 min. To decrease the $t_R$ of ACF at the same time, it was decided to increase the pH of the mobile phase more than 4.0 as suggested from the pKa values ACF (pKa=4.7).

The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that phosphate buffers gave better peak shapes than their acetate and citrate counterparts. Further to decrease the tR of ACF, gradient mobile phase consisting of phosphate buffer and methanol was also tried in various ratios but was ruled out because of the appearance of hump in baseline. Finally, in isocratic system different ratios of phosphate buffer and methanol at different pH were tried. To improve the peak shape, triethylamine was added. The optimum mobile phase was found to consist of 40: 60 (v/v); phosphate buffer (pH6.0): Methanol. Phosphate buffer (pH 6.0) was prepared by dissolving 1.56 g sodium dihydrogen ortho-phosphate dihydrate and 0.35 g disodium hydrogen phosphate dihydrate in 1,000 mL LC-grade water. Triethylamine (1 mL) was added and the pH adjusted to 6.0 by addition of ortho-phosphoric acid. The flow rate was set to 1.0 mL min−1 and UV detection was carried out at 270 nm. The retention time (tR) for ACF was found to be 14.650. Acceptable retention time (tR), plates, asymmetry and good resolution for ACF was obtained.

**Linearity**

Linear relationships were observed by plotting drug concentration against peak areas of ACF. ACF showed linear response in the concentration range of 40-160 µg/ml. The corresponding linear regression equation was y = 43113 x – 85483 with square of correlation coefficient ($r^2$) of 0.9998 respectively. Residual analysis was performed to ascertain linearity. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient. No significant difference was observed in the slopes of standard curves.

**Precision**

The % R.S.D. values depicted in table show that proposed method provides acceptable intra-day and inter-day variation of ACF. The repeatability of sample application and measurement of peak area were expressed in terms of % R.S.D. and were found to be 0.63, 0.49 for ACF.

**Robustness**

Each factor selected (except columns from different manufacturers) to examine was changed at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of chromatographic parameters (factors). Results, presented in Table 9, indicate that the selected factors remained unaffected by small variations of these parameters. The results from the two columns indicated that there is no significant difference between the results from the two columns.

**Limit of detection and Limit of quantitation**

The LOD and LOQ were found to be 0.69 and 2.09 µg mL−1, respectively for ACF

**Specificity**

There is no peak interference of blank and placebo at the retention time of ACF which indicates that the method is specific for the analysis in their pharmaceutical dosage form. The specificity of the method is illustrated in Fig. 3. The average retention time (tR) ± S.D. for ACF was found to be 14.567 ± 0.02 respectively for 6 replicates. Tailing factor for peaks of ACF was less than 2 (T≤2) and resolution was satisfactory (Rs≥2). The peaks obtained were sharp and have clear baseline separation.

**System suitability**

System suitability parameters including theoretical plates, peak asymmetry(T), capacity factor (K'), selectivity (α) and resolution of ACF peaks were calculated and summarized in table 10

**Table 1: Drug excipients profile**

| S.No | Ingredients | Drug: Excipients Ratio |
|---|---|---|
| 1 | Span 20 | 1:1 |
| 2 | Tween 20 | 1:1 |
| 3 | Liq. Paraffin | 1:1 |
| 4 | Propylene glycol | 1:1 |
| 5 | Methyl Paraben | 1:1 |
| 6 | Carbopol | 1:1 |
| 7 | Menthol | 1:1 |
| 8 | HPMC K4 M | 1:1 |
| 9 | Purified water | 1:1 |

**Table 2: Formulation of emulgel**

| S.NO | INGREDIENTS | UNIT FORMULA | | |
|---|---|---|---|---|
| | | F1 | F2 | F3 |
| 1 | Aceclofenac | 2 | 2 | 2 |
| 2 | Span 20 | 1 | 1 | 1 |
| 3 | Tween 20 | 0.5 | 0.5 | 0.5 |
| 4 | Liquid paraffin | 7.5 | 7.5 | 7.5 |
| 5 | PG | 5 | 5 | 5 |
| 6 | Methyl paraben | 0.03 | 0.03 | 0.03 |
| 7 | Menthol | 3 | 3 | 3 |
| 8 | Carbopol | 1 | 0 | 0.5 |
| 9 | HPMC K4 M | 0 | 1 | 0.5 |
| 10 | Purified water | 1.5 | 1.5 | 1.5 |
| Net wt/ tab in mgj | | 21.53 | 21.53 | 21.53 |

**Table 3: Characterization studies on Aceclofenac powder**

| Test | Observation |
|---|---|
| **Description** | **White colour powder** |
| **Odour** | **Odorless** |
| **Solubility** | **Highly soluble in water and partially soluble in methanol** |

**Table 4: Drug-excipient compatibility data**

| S. No | Drug + Excipient | Condition | | |
|---|---|---|---|---|
| | | Room temperature | Hot air oven | Freezing temperature |
| **1** | Aceclofenac | Unchanged | Unchanged | Unchanged |
| **2** | API + SPAN 20 | Unchanged | Unchanged | Unchanged |
| **3** | API + TWEEN 20 | Unchanged | Unchanged | Unchanged |
| **4** | API + CARBOPOL 934 | Unchanged | Unchanged | Unchanged |
| **5** | API + HPMC K4 M | Unchanged | Unchanged | Unchanged |
| **6** | API + Liq. PARAFFIN | Unchanged | Unchanged | Unchanged |

| | | | | |
|---|---|---|---|---|
| **7** | API + Propylene Glycol | Unchanged | Unchanged | Unchanged |
| **8** | API + Menthol | Unchanged | Unchanged | Unchanged |
| **9** | API + Methyl paraben | Unchanged | Unchanged | Unchanged |

**Table 5: Rheological properties of Emulgel**

| Formulation | Spindle No | RPM | Shear stress | %T | Viscosity |
|---|---|---|---|---|---|
| F1 | 32 | 0.2 | 165.7 | 90 | 13950 |
| F2 | 32 | 0.2 | 169.4 | 80 | 15526 |
| F3 | 32 | 0.2 | 169.4 | 80 | 14526 |

**Table 6: pH profile of formulation**

| S.No | Formulation | pH |
|---|---|---|
| **1** | **F1** | **6.2** |
| **2** | **F2** | **6.5** |
| **3** | **F3** | **6.4** |

**Table 7: Linearity analysis**

| S. No | Parameters | ACF |
|---|---|---|
| 1 | Linearity range | 40-160 µg/ml |
| 2 | Slope ± Standard error | 43113 ± 298.83 |
| 3 | Intercept ±standard error | -85483± 32185 |
| 4 | Confidence limit of slope | 42345 to 43881 |
| 5 | $r^2$ | 0.9998 |
| 6 | Correlation coefficient (r) | 0.999 |
| 7 | $Sy.x^b$ | 9025 |

**Table 8: Precision factors of ACF**

| Drug | Conc. (µg mL−1) | Repeatability (intra-day) | | | Intermediate precision (inter-day) | | |
|---|---|---|---|---|---|---|---|
| | | Found conc. ± S.D. | % R.S.D. | S.E. | Found conc. ± S.D. | % R.S.D. | S.E. |
| ACF | 40 | 39.57 ± 0.16 | 0.41 | 0.07 | 39.74 ± 0.28 | 0.70 | 0.11 |
| | | | | | 98.58 ± 0.18 | | |
| | | 99.19 ± 0.25 | | | | | |
| | 100 | | 0.25 | 0.10 | 161.02 ± 1.67 | 0.18 | 0.07 |
| | | 160.55 ± 1.34 | | | | | |
| | | | | | | 1.04 | 0.68 |
| | 160 | | 0.84 | 0.55 | | | |

**Table 9: Robustness evaluation of the method (n=6)**

| Factor | Level | Aceclofenac Retention time (tR) | Aceclofenac Asymmetry (T) |
|---|---|---|---|
| 0.9 | -1 | 15.336 | 1.27 |
| 1.0 | 0 | 14.648 | 1.27 |
| 1.1 | +1 | 13.950 | 1.26 |
| Mean ± S.D. | | 14.645 ± 0.69 | 1.27 ± 0.01 |

**Table 10: System suitability data**

| Parameters | ACF | Reference values |
|---|---|---|
| Theoretical plates (N) | 9511.86 | N>2000 |
| Peak asymmetry (T) | 1.33 | T≤2 |
| Capacity factor (K') | 4.53 | 1<K'<10 |
| Selectivity (α)[a] | 2.31 | α>1 |
| Resolution (Rs)[a] | 15.02 | Rs≥2 |
| HETP (H)[b] | 0.026 | - |

**Figure 1: UV- absorbance spectra for aceclofenac**

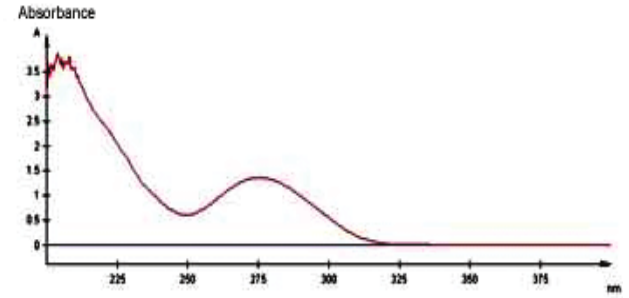



**Figure 2: FTIR spectroscopy**

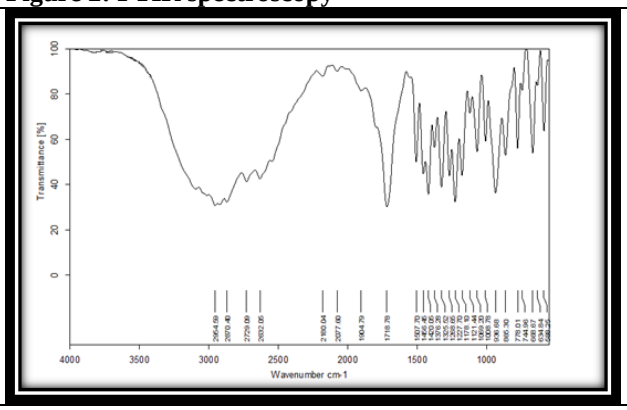



**Figure 3: Chromatogram of Standard Aceclofenac Rf (0.57)**

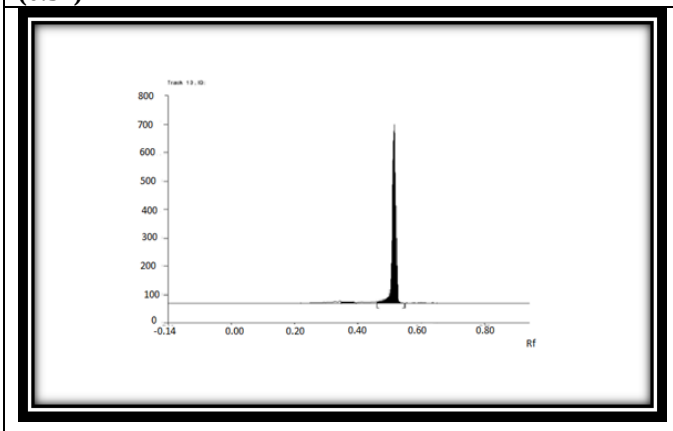



**Figure 4: Spectra of aceclofenac containing emulgel**

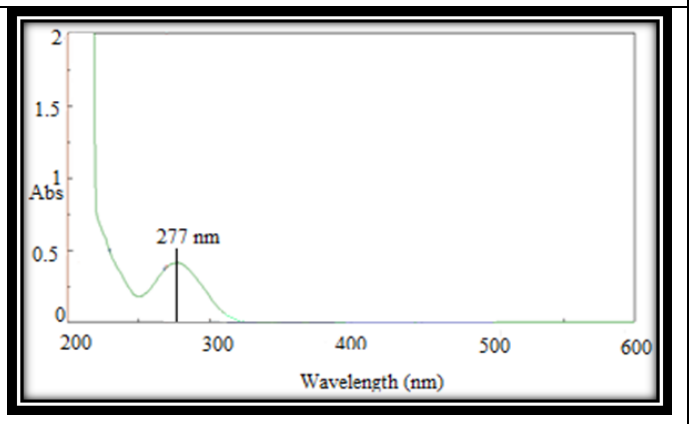
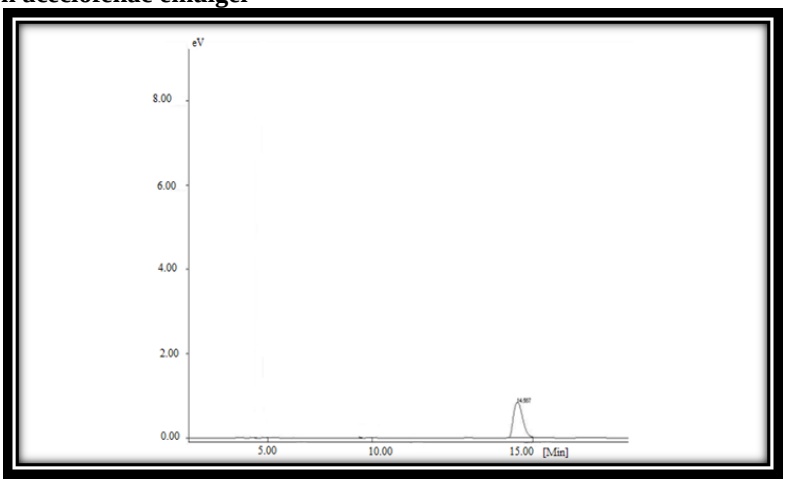



**Figure 5: HPLC study on aceclofenac emulgel**




## CONCLUSION

The future of pharmaceutical sciences of drug design and development will be focused on topical delivery systems in the face of huge drawbacks in oral, parenteral, and other routes showing high levels of compliance from patients across the globe. A major benefit of Emulgel is its high bio-adhesion, optimum viscosity, and long-term stability. The elegance and absorption of two of the formulations (out of three) was excellent. Research in this area has been conducted under the New Drug Delivery System (NDDS), which improves the new approach in frequent dermal delivery of Aceclofenac topical emulgel loaded with Aceclofenac. There is no doubt that aceclofenac emulgels will be the

most commonly procured dosage form in the future due to their better therapeutic potential and fewer adverse effects on patients. New technologies help in detection of the amount and effectiveness base on release of active drug from formulation.

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