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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF IBUPROFEN TOPICAL GEL FORMULATION

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ABSTRACT

Based on the study's findings, it can be concluded that the present RP-HPLC method worked successfully for estimating the Ibuprofen in the gel formulation. Accuracy, precision, specificity, repeatability, and sensitivity were all demonstrated by the approach. The designed and verified RP-HPLC method was used to analyse the gel formulation of Ibuprofen. Also the RP-HPLC process was easy to implement, precise, accurate, reproducible, and cost-effective. It can be used for regular IBU-only control analysis in gel formulation. Suitability of these methods on biological samples needs to be studied.These techniques cannot interact with additives, matrices, etc. These studies would be clarified by additional research on different useful formulations.

KEYWORDS: Ibuprofen, Topical gel, RP-HPLC, NSAID's, Analytical estimation.

INTRODUCTION:-

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of heterogeneous compounds of unrelated organic acids that have analgesic, anti-inflammatory and antipyretic properties. NSAIDS are the cyclo-oxygenase enzyme inhibitors, which results in the direct inhibition of the biosynthesis of prostaglandins and thromboxane from arachidonic acid.

There are two types of cyclo-oxygenase 1) COX-1 which is the constitutive form of the enzyme and 2) COX-2 which is the form induced in the presence of inflammation. Inhibition of COX-2 is therefore thought to be responsible for at least some of the analgesic, anti-inflammatory and antipyretic properties of NSAIDs whereas inhibition of COX-1 is thought to produce some of their toxic effects, particularly those on the gastrointestinal tract. Most of the NSAIDs currently available for clinical use inhibit both COX-1 and COX-2.

NSAIDs are used for the relief of mild to moderate pain, minor febrile conditions and for acute and chronic inflammatory disorders such as osteoarthritis, rheumatoid arthritis, juvenile chronic arthritis and ankylosing spondylitis.[1]

Gels

The word 'gel' is derived from gelatin and both gel and jelly can be traced back to the Latin gelu for frost and gel are, meaning 'freeze' or 'congeal'. This origin indicates that the essential ideal of liquid setting to a solid like material that does not flow, but it is elastic and retains some liquid characteristic.[8] Gels are semisolid systems consisting of either suspension made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system.

Classification of Gels:-

The definition in the BP states that gels consist of liquids gelled by means of suitable gelling agents and indicates that these are two classes, namely:

Hydrophobic gels.-The bases of hydrophobic gels (oleogels) usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminum or zinc soaps.[14]

Hydrophilic gels.:- The bases of hydrophilic gels (hydrogels) usually consist of water, glycerol, or propylene glycol gelled with suitable gelling agents such as tragacanth, starch, cellulose derivatives, carboxy vinyl polymers, and magnesium- aluminum silicates.[15]

High-Performance Liquid Chromatography (HPLC)

HPLC (High-Performance Liquid Chromatography) was created in the late 1960s and early 1970s. Today, it is widely used for separations and purifications in a number of industries, including pharmaceutical biotechnology, environmental, polymer, and food. Over the last decade, HPLC has emerged as the preferred technology for analysing a wide range of chemicals. Its key benefit over GC is that analytes do not need to be volatile, hence macromolecules can be analysed using HPLC. HPLC is achieved by injecting a small amount of liquid sample into a moving stream of liquid (referred to as the mobile phase) that passes through a stationary phase-packed column. A mixture's separation into its components is determined by the degree to which each component is retained in the column. In high-performance liquid chromatography (HPLC), the analyte is distributed between a stationary phase and a mobile phase (eluent), usually within the column packing material. The chemical structure of the analyte determines its movement rate through the stationary phase, which is the basis for separation. HPLC is a crucial analytical chemistry technology, especially in the pharmaceutical and chemical sectors, due to its ability to separate and analyse various substance.[30]

Validation

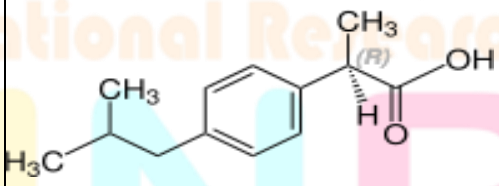
Validation is a systematic process that provides objective evidence that a product meets its intended usage requirements. The process comprises analysing a method's performance and demonstrating its capacity to meet particular requirements. Validation ensures that your analytical method, such as High-Performance Liquid Chromatography (HPLC), can deliver consistent results even under demanding conditions or with low doses.[50] Validation is laboratory testing that ensures an analytical method's performance meets the requirements of its intended application. Validation is necessary for new or updated methods to provide repeatable and trustworthy findings, regardless of whether they are used by many operators using the same equipment in various laboratories. Validation programs are necessary to ensure the analytical process is robust and suitable for varied circumstances, depending on the method and intended usage. Method validation evaluates the quality,

consistency, and reliability of analytical results, making it an essential part of any robust technique. Validation requires equipment that meets requirements, is calibrated accurately, and operates properly. The validation process assesses analytical procedures and either validates or invalidates them based on needed criteria. This ensures the precision and trustworthiness of analytical data in multiple applications the following are typical parameters recommended by the FDA, USP, and ICH.[51]

- Specificity
- Linearity
- Precision
- Accuracy
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Robustness

DRUG PROFILE: -

IBUPROFEN:

Name	**Ibuprofen**
Solubility	Practically insoluble in water but, very soluble in most organic solvent like ethanol 66.18 g/100ml at 40^0C
IUPAC name	(RS)-2-(4-(2-methyl propyl phenyl) propionic acid
Structural formula	
Molecular formula	$C_3H_{18}O_2$
Molecular weight	206.29 gm/ml
Melting point	75-77^0C
Category	Non-steroidal anti-inflammatory drugs
CAS number	15687-27-1
pka value	5.2
Mechanism of action	Ibuprofen inhibits cyclooxygenase (cox-1 and cox- 2) enzyme reducing the synthesis of prostaglandins, which are responsible for inflammation, pain and fever.
Indication	Mild to moderate pain headache, dental pain, menstrual cramps, muscle acne, inflammatory condition, arthritis, brucitus, tendinits, fever reduction, dysmenorrhea
Appearance	White powder

Side Effect	Gastrointestinal upset (Nausea, Heartburn, headache, Dizziness) Kidney dysfunction Increase cardiovascular risk
Contraindication	History of hypersensitivity to NSAIDS Active GI bleeding or ulcer severe renal or hepatic impairment Cardiovascular disease (used with caution) pregnancy (especially in third trimester)
Drug Interaction	Anticoagulant warfarin Increase bleeding risk ACE inhibitor / ARB reduce renal function
Diuretics Methotrexate	May reduce efficiency Increase toxicity
Monitoring parameter	Renal function (Especially in long term use) Sign of GI bleeding Blood pressure Liver function (In long term therapy)
Polymer used	Carbopol 934, Glycerin, Methyl paraben, Propyl paraben

Material and Method:

The drugs used for the present investigation were obtained from Yarrow Pharma.

Drug	**Supplied by**	**Quantity**	**Purity(Assay)**
Ibuprofen	Yarrow Pharma	100 g	99% w/w

Table No.1: Details of API

B. Reagents and Chemicals: All reagents and chemicals used were of AR grade and HPLC grade.

Reagent and Chemicals	**Grade**	**Supplied by**
Methanol	(HPLC grade).	Thermosil Fine Chem Industries
Acetonitrile	(HPLC grade)	Thermosil Fine Chem Industries
Distilled Water	(HPLC grade).	
Triethylamine	(HPLC grade).	Loba Chemie Pvt.Ltd
Ortho Phosphoric Acid	(HPLC grade).	Prayogina Laboratories India
Carbopol 934		Thermosil Fine Chem Industries
Propylene Glycol		Loba Chemie Pvt.Ltd

Methyl paraben		Prayogina Laboratories India
Propyl paraben		Prayogina Laboratories India
Glycerine		Prayogina Laboratories India

Table no 2: Details of reagent and chemicals

Instruments:

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Water 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104
5	Ultra sonicator	-	1.5 L 50

Table No 3: Instruments Used

PREFORMULATION:

One of the most important phases in the pharmaceutical and medication development process is pre-formulation research. These investigations offer a thorough comprehension of a drug candidate's physicochemical characteristics, directing further formulation and optimisation initiatives. The importance of pre-formulation studies in drug development, their essential elements, and their function in guaranteeing the success of pharmaceutical products are all highlighted in this overview.

Study of functional group by using Infrared Spectroscopy:

IBUPROFEN API: - Accurately weighed 50 mg of **IBUPROFEN** API was mixed properly with 300 mg of dried KBr, then carefully triturated in a mortar pestle. Keep this mixture in a die and IR spectrum was taken using the Diffused Attenuated reflectance mode.

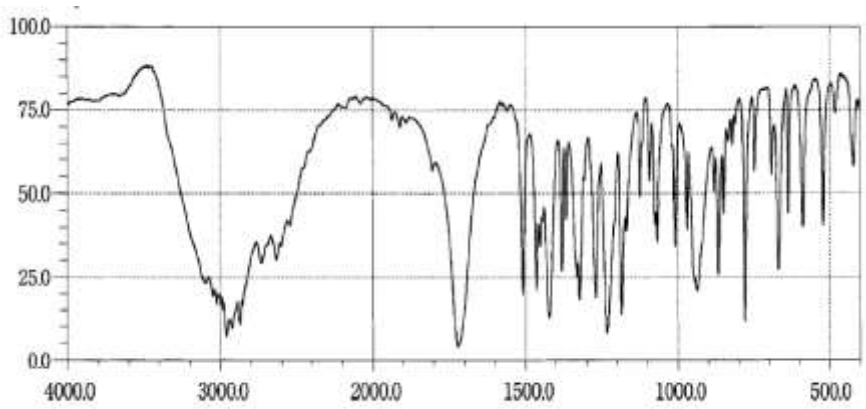


Fig.No.1.IR Spectra of Ibuprofen

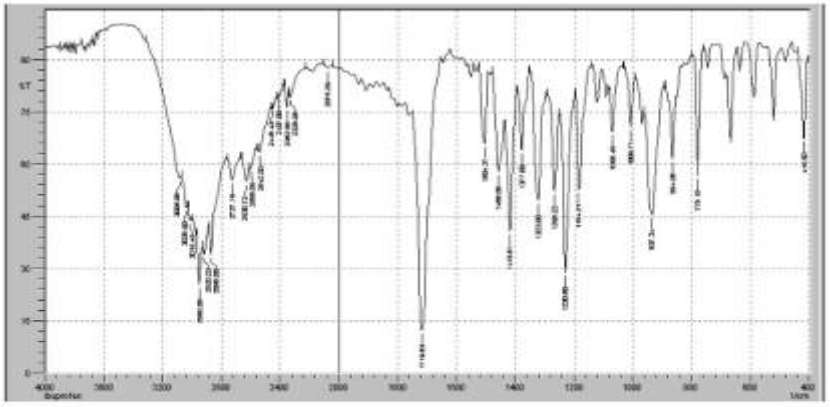


Fig No.2.Reference Spectra Of Ibuprofen

Determination of Wavelength Maxima

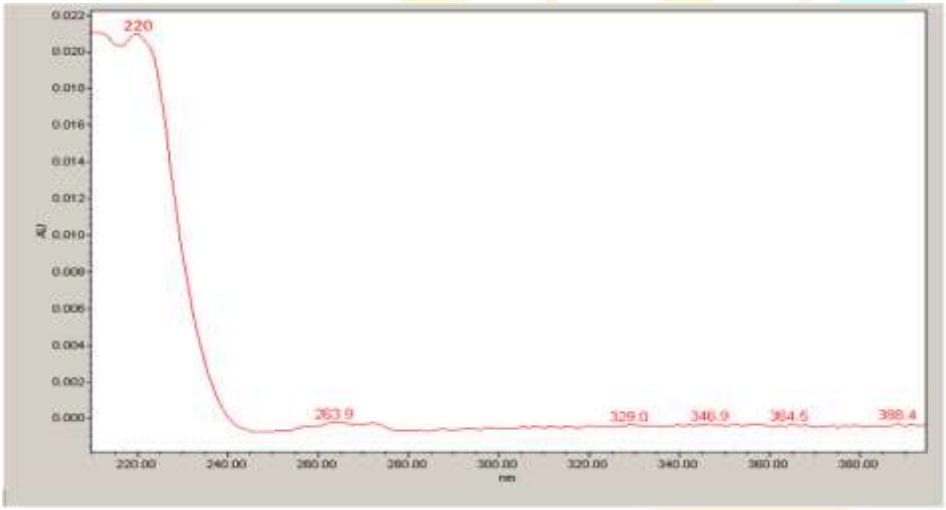


Fig. No. 3: Wavelength Maxima

Ibuprofen standard stock solution:

An accurately weighed quantity of **Ibuprofen** 5 mg was transferred to the 10 ml volumetric flask and dissolved in HPLC grade ACN. The volume was made up to the mark with the same to make (500 µg/ml).

The aliquot portions of stock standard solutions were diluted appropriately with HPLC grade ACN to obtain concentration 5 µg/ml of Ibuprofen The solutions were scanned in the range of 400–200 nm in 1 cm cell against blank. The UV absorbance spectrum of IBU were recorded and found to be 220nm.

Sr. No	Concentration(µg/ml)	Absorbance
1.	4.7	0.192
2.	8.5	0.305
3.	10.3	0.380
4.	12.8	0.455
5.	14.9	0.570

Table No 4: Observation for Calibration Curve

Development and validation of HPLC method for estimation of Ibuprofen

METHOD DEVELOPMENT STRATEGY:

Selection of Common Solvent (Diluents):

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in mobile phase. The selection was made after assessing the solubility of IBU in different solvents i.e. Acetonitrile and water.

Preparation of standard stock solution:

Accurately weighted IBU 50 mg was dissolved in 100 ml ACN. This solution was used as
Standard stock solution.

Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing IBU was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through Membrane filter (size 0.15µ). To achieve peaks with good symmetry various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

OPTIMIZED METHOD

Sr no	**CHROMATOGRAPHIC CONDITION**	
1	Mobile Phase	pH of 4 Modified by 1M OPA (WATER:ACN)
2	Concentration	50:50%v/v
3	pH	4
4	Column	Phenomenax Kintex C8
5	Run Time	20 min
6	Flow Rate	1ml/min

Table no:5. Chromatographic condition for Optimized method

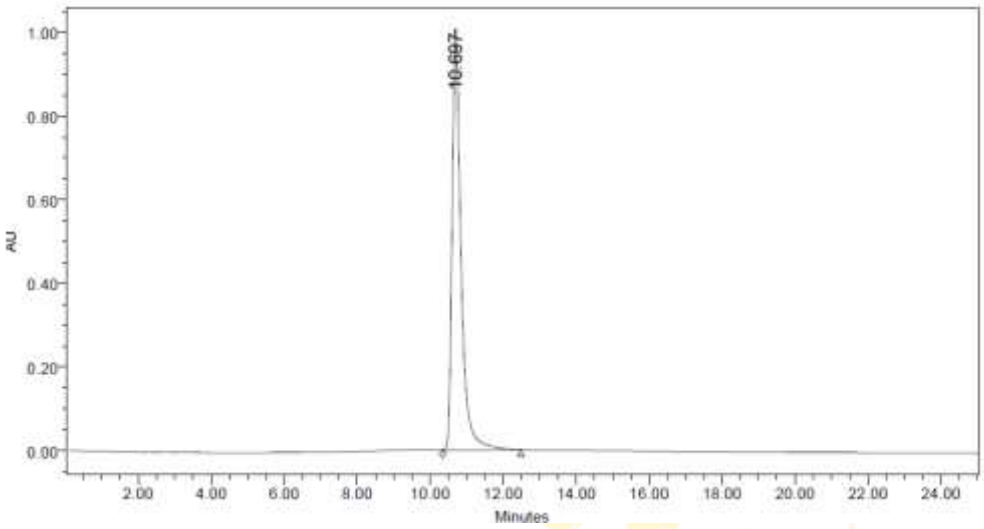


Fig.no.4.Chromatogram for Optimized Method

Chromatographic Parameters:

Column	:	C_8 (Phenomenex Hypersil gold) /4.6 x 250 mm, 5particle
Flow Rate	:	1.0ml/min
Wavelength	:	220 nm
Injection volume	:	20µl
Column oven Temperature	:	Ambient (25^0C)
Run Time	:	20 minutes
Mobile Phase	:	pH maintained up to 4.00 using 1M OPA solution and ACN (50:50 %v/v)

Preparation of (pH4) : Take 100 ml of volumetric flask and make up the volume up to the mark with HPLC water. The pH was maintained to 4.00 using 1M OPA.

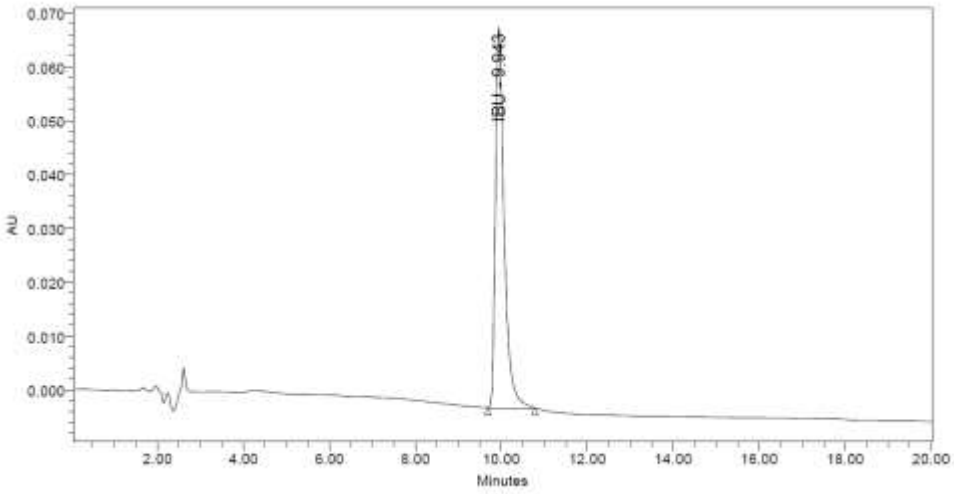


Fig No. 5: Separation of Ibu in selected mobile phase showing retention time at 9.943 min

VALIDATION PARAMETERS

Accuracy:

The accuracy samples were prepared by spiking the standard into the pre-analysed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

		IBU	
		Levels	
	80%	100%	120%
Amt added	40	50	60
(µg/ml)	40	50	60
	40	50	60
Amt taken	40	50	60
(µg/ml)	40	50	60
	40	50	60
Amt recovered	39.97	25.92	30.97
(µg/ml)	39.96	25.95	30.94
	39.94	25.96	30.99
% Recovery	99.8	99.5	99.87
	99.7	99.69	99.72
	99.5	99.76	99.96
Mean % recovery	99.66	99.65	99.85
% RSD	0.15	0.13	0.12

Table No 6: Accuracy studies by standard addition method

Precision:

System precision

Prepared the standard solution as per test method and inject into the HPLC system in three replicates. Calculate the % RSD for the area responses and record the observations into the following table.

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	0.6987	NMT 2.0
2	Theoretical plates	7586.03	NLT 2000
3	Tailing factor	1.74	NMT 2.0

Table No. 7: Results for System Precision showing system suitability

Were, NMT - Not More Than NLT – Not Less Than

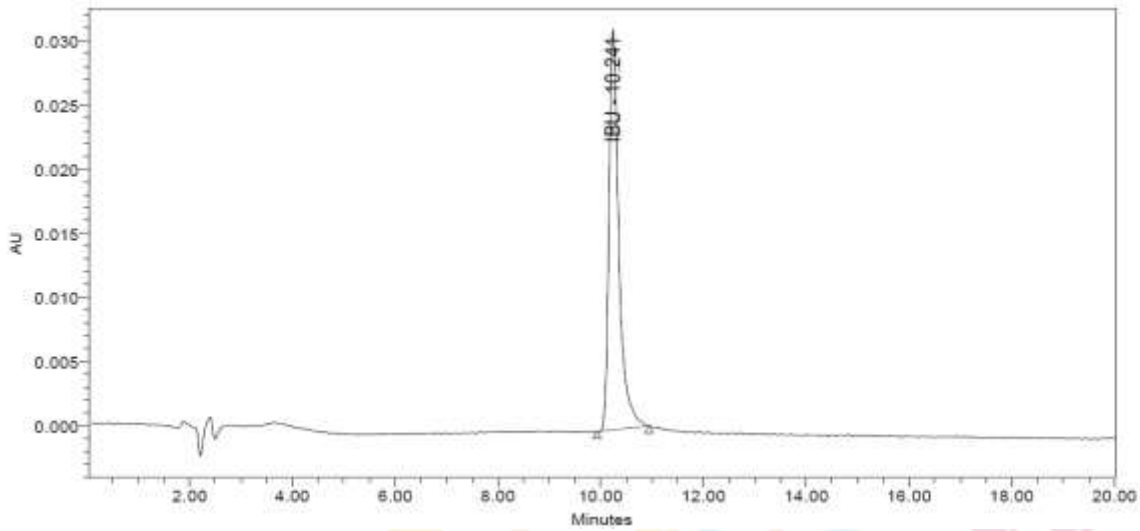


Fig.no 6: Chromatogram System precision Showing Repeatability

Method precision: Prepared three samples' solutions as per the test method and injected into the HPLC system by following the conditions prescribed in the Test method.

Procedure: Sample solution was prepared and injected into the HPLC system, the chromatograms were recorded for peak area response for the IBU.

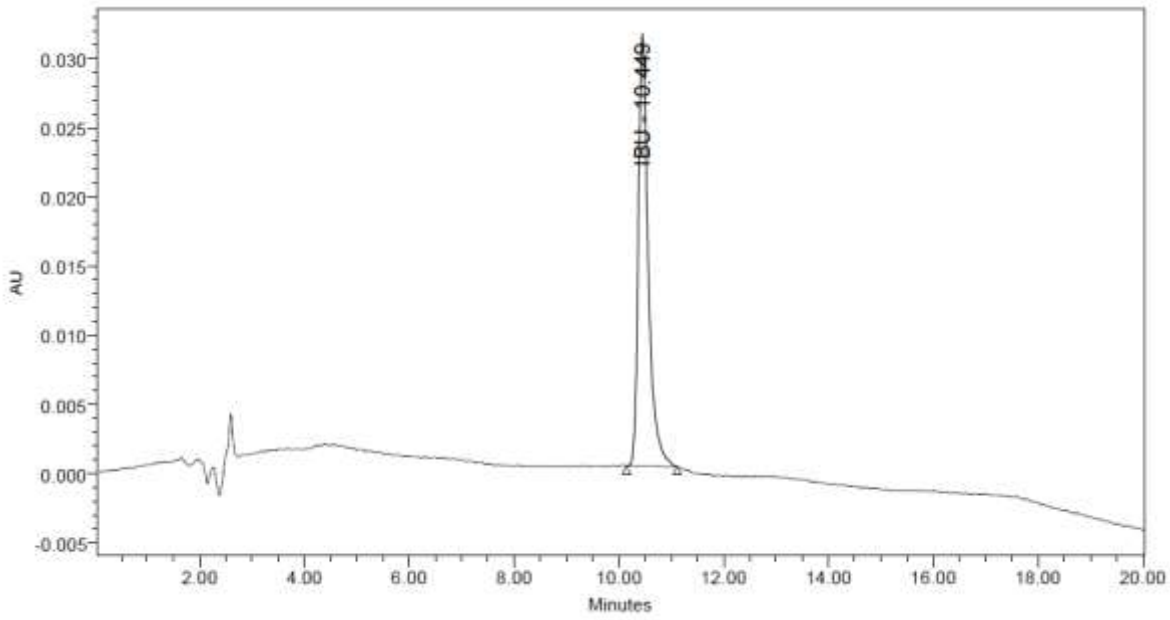


Fig.no 7: Chromatogram of Method precision

	IBU	
Sr.no.	**Assay (mg)**	**Assay % of LC**
1	49.5	98.3
2	49.8	99.3
3	49.7	99
Average	49.66	98.86

SD	0.15	0.513
% RSD	0.51	0.51

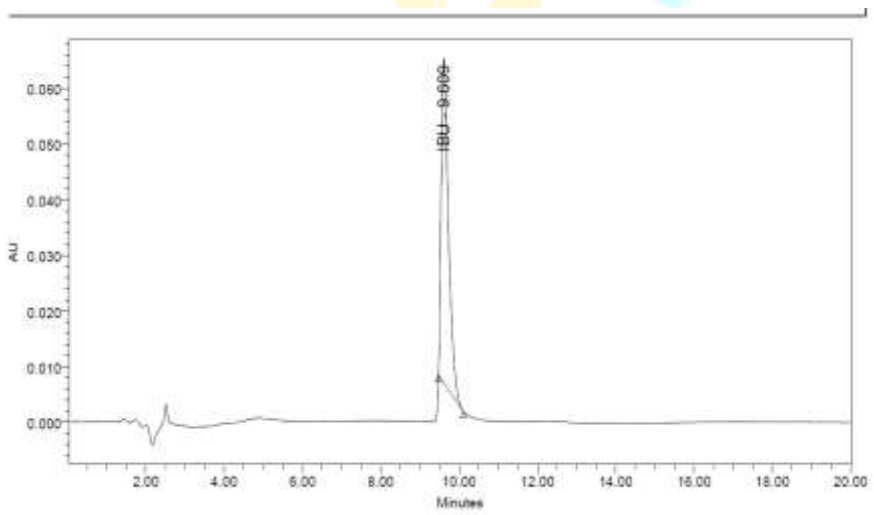
Table No.8: Method Precision Studies Set – I Acceptance

Robustness:

Effect of Variation in flow rate of mobile phase by ± 10%:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at –10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min) when compared with the Test method flow rate.

Procedure: Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

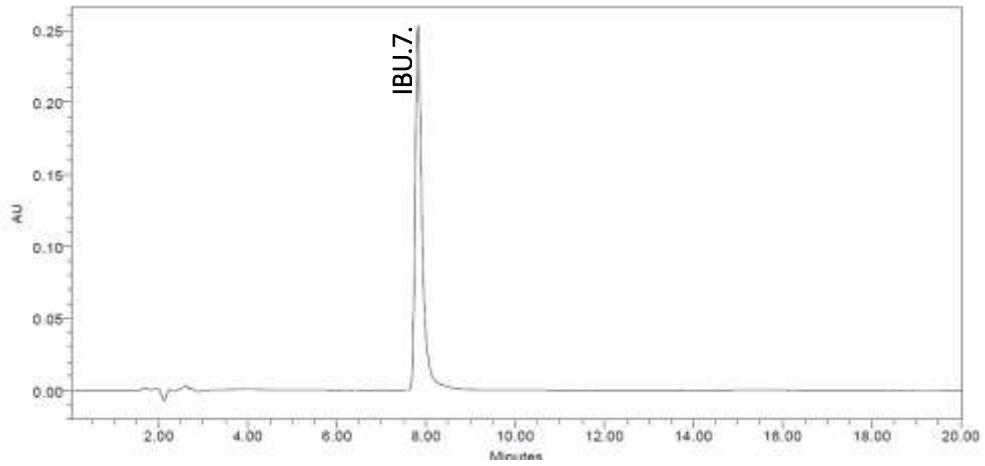


1.1 ml/min

Change in organic composition ± 10% (WATER: ACN)

System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.

-10% pH of 4 Modified by 1M OPA (WATER:ACN)



+10% pH of 4 Modified by 1 M OPA(WATER:ACN)

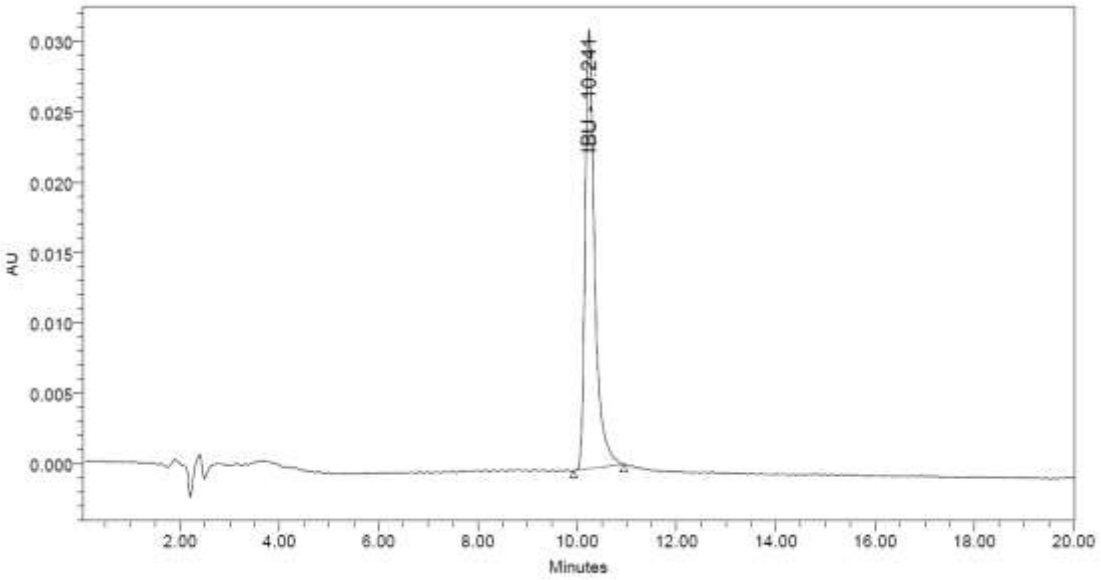


Fig. No. 9: Chromatograms of Change Organic Composition of mobile Phase

Linearity and range:

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of IBU. Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

Sr. No.	**% Level**	**IBU**	
		Conc. (µg/ml)	**Mean peak area ± SD (n=5)**
1	50	7.5	143251 ± 345.15
2	80	12	287891±215.60
3	100	15	376547±236.58
4	120	18	399423±568.48
5	150	22.5	412365±390.84

Table No.9: Observations of Linearity and range study for IBU

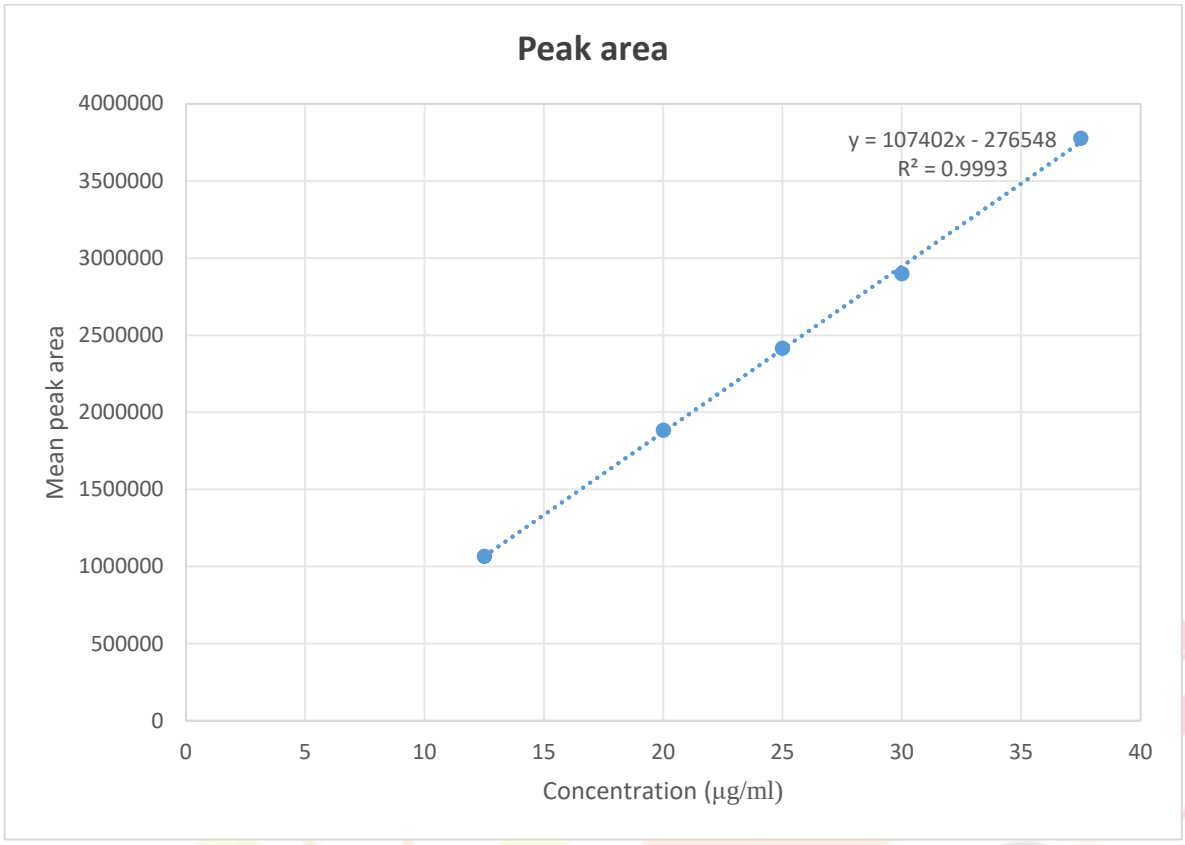


Fig. No. 10: Plot of linearity and range study for IBU

RESULT AND DISCUSSION:

PREFORMULATION STUDY:

Prior to Formulation, Pre formulation study was carried out on drug and polymer. In present work, total six formulation of topical gel were prepared. The detail composition is shown in table. Topical gel were evaluated for various parameters like appearance, PH, Viscosity, spreadability ,extrudability Drug content , moisture content, , drug diffusion study etc.

PREFORMULATION STUDY OF IBUPROFEN

a. Organoleptic Properties of Drug:

Ibuprofen was studied for organoleptic characters such as colour, odour, appearance and melting point. Results of organoleptic properties and melting point of IBUPROFEN were found to be similar as mentioned in literature.

Identification Test	**Observed Results**
Appearance	white powder
Colour	White
Odour	Characteristics

Table no:10. Organoleptic Characterization of drug

Solubility: Drug is highly soluble in Phosphate buffer pH 7.4 and also solvent like alcohol, ethanol, acetone etc.

b. Melting point:

The melting point was determined by open capillary method and the corrected melting point was found to be 77ºC.

Identification Test	Observed Result
Melting point	75-77º C

Table no:11. Melting point of IBUPROFEN

UV SPECTROSCOPY:

UV absorption spectrum of IBUPROFEN sample in ACN Shows Maximum at 220 nm specified in the range of 220-280 nm. Thus 220 were found to be in specification of drug. So it is further selected as ƛ max of IBUPROFEN.

Standard Calibration Curve: The present analytical method obeyed Beer's law in the concentration range 220 µg/ml and is suitable for the estimation of IBUPROFEN from Different solutions. The Correlation coefficient (r) was found to be 0.9996, indicating a positive correlation between the concentration of IBUPROFEN and the corresponding absorbance values.

Standard Calibration Curve- Ibuprofen

Table No:12. Data for standard calibration curve of IBU

Sr. No	Concentration(µg/ml)	Absorbance
1.	0.5	0.192
2.	0.8	0.305
3.	1	0.380
4.	1.2	0.455
5.	1.5	0.570

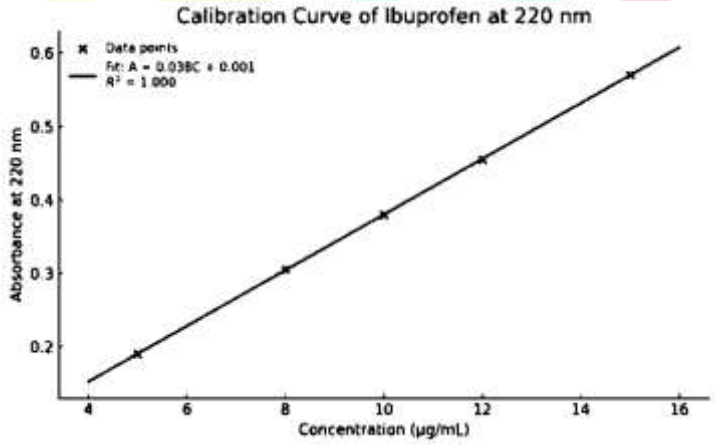


Fig no.11 : Standard Calibration Curve for Ibuprofen

: Physico-chemical evaluation of Topical gels.

Sr No	Batch	pH	Viscosity	Spredability (in minute)	Extrubality (weight required in gm)	Drug Contain %	Drug Diffusion
1	F1	5.4	46000	32.5	650	97.28	15.79
2	F2	5.6	49000	47.27	500	96.67	23.28
3	F3	5.3	44000	30.58	425	98.18	33.72
4	F4	5.1	42000	52	590	98.48	48.37
5	F5	5.6	51500	34.66	625	99.06	51.84
6	F6	5.3	50000	32.70	475	99.69	70.19

Table no:13. Physico-chemical evaluation of Transdermal films

In Vitro Drug Release Of Profile Ibuprofen

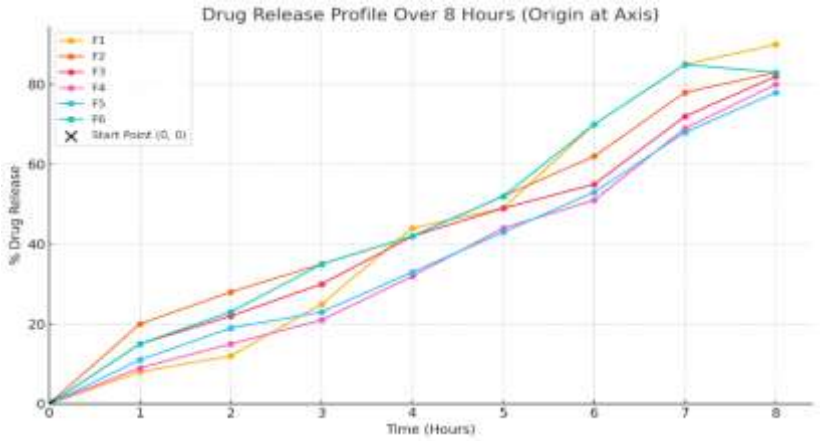


Fig.No.12: In Vitro Drug Release of Profile Ibuprofen

RP-High Performance Liquid Chromatography (HPLC) Method:

This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system.

HPLC Column Selected:

HPLC Waters 600 system with C_8 (Phenomenex Hypersil gold) /4.6 x 250mm, 5µ particle size column and PDA detector were used for the study.

Mobile Phase selected:

Mobile phase composed of water (4PH) and ACN (50:50 % v/v). An isocratic program was developed contributing a total run time of 20 min. The wavelength 220 nm was selected for the evaluation of the chromatogram of drugs.

VALIDATION

Validation of these methods was performed as per the USP guidelines for these following parameters:

Precision:

System Precision

Prepared the standard solution as per test method and injected into the HPLC system in three replicates. It was found that all system suitability parameters are well within the limits.

Method Precision Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed R.S.D. less than 2.

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	0.698701	NMT 2.0
2	Theoretical plates	7586.033	NLT 2000
3	Tailing factor	1.74	NMT 2.0

Table No. 14: Data showing system Precision

Linearity & Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 80% to 150%.

Accuracy

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results were well within the range **99.80-99.86%.** Thus the method was found to be accurate.

	IBU		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	40	50	60
	40	50	60
	40	50	60
Amt taken (µg/ml)	40	50	60
	40	50	60
	40	50	60
Amt recovered (µg/ml)	39.97	25.92	30.97
	39.96	25.95	30.94

	39.94	25.96	30.99
% Recovery	99.80	99.50	99.87
	99.70	99.69	99.72
	99.50	99.76	99.96
Mean % recovery	99.66	99.65	99.85
% RSD	0.15	0.13	0.12

Table No. 15: Result of Accuracy Studies

Robustness: Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of IBU. Thus, no interference was found at the Retention time of IBU.

SUMMARY

Gel formulation containing IBU is recently introduced in market, the drug along with other drugs is used to treat conditions of organ rejections. Literature survey revealed very few analytical methods for the estimation of IBU. The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for estimation of IBU in gel formulation.

In the developed RP-HPLC method the analyte was resolved using Mobile phase composed of water (4PH) and ACN in the ratio 50:50 % v/v. An isocratic program was developed contributing a total run time of 20 min. using HPLC auto-sampler system containing PDA detector with EMPOWER Software and C_8 (Thermo Hypersil gold) /4.6 x 250 mm, 5µ particle size column, the detection wavelength was 220 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.

CONCLUSION

From the results of the study it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the IBU in the gel formulation.

The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of gel formulation of IBU was done by the developed and validated RP-HPLC method. The RP-HPLC method was also simple, accurate, precise, reproducible and economical too. It may be adopted for routine control analysis of IBU alone in gel formulation.

No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. Suitability of these methods on biological samples needs to be studied.

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