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Development and Validation of HPLC Method for Estimation of Ibuprofen in an In-House Nano Sponges Formulation

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Abstract: *A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Ibuprofen in an in-house nanosponge formulation. Chromatographic separation was achieved using a C18 column with a suitable mobile phase comprising acetonitrile and aqueous buffer in optimized ratio under isocratic conditions. The flow rate was maintained at 1.0 mL/min, and detection was carried out using a UV detector at an appropriate wavelength for Ibuprofen analysis. The developed method showed good peak symmetry with a satisfactory retention time and no interference from formulation excipients or degradation products.*

The validated RP-HPLC method was successfully applied for routine estimation of Ibuprofen in the prepared nanosponge formulation and was found to be reliable, sensitive, and suitable for quality control and formulation development studies.

Keywords: *RP-HPLC*

I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.[1-6]

HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20th century analytical chemistry found wide applicationin forensic, environmental, industrial and medical field.

Importance of Analytical Chemistry:

It finds numerous applications in various disciplines of chemistry.

It finds wide applications in other fields of related sciences.

Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.

Qualitative analysis deals with the identification of elements, ions or compounds present in the sample.

Quantitative analysis

Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.[1-6]

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METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still verymuch a trial- and-error approach, expedited by a logical sequence of generic scouting runs and fine- tuning steps to achieve the requisite resolution and method performance.[7]

EXPERIMENTAL WORK

Characteristics of drug:

Description: Organoleptic properties of drug was observed and recorded by using descriptive terminology

Melting point: 75-77^{0}C

Solubility: Soluble in Ethanol, Methanol, Chloroform, Acetone.

Nature: White crystalline powder, Lipophilic in nature.

Formulation of Nanosponges:

Table No: 8. Ingredients of Nanosponges

Ingredients	Use
Ibuprofen	Drug (API)
B-Cyclodextrin	Polymer
DiPhenyl Carbonate	Cross linker
Ethanol/DMF	Solvent
PVA	Stabilizer
Water	Dispersion medium

Table No: 9. Composition of Nanosponges

Sr.no	Formulation	Ibuprofen (mg)	B-Cyclodext rin (mg)	DiPhenyl Carbonat e(mg)	Distilled water (ml)
1	NE-1	100	500	250	100
2	NE-2	100	1000	500	100
3	NE-3	100	1500	750	100
4	NE-4	100	2000	1000	100

The ingredients which are used to formulate Ibuprofen Nanosponges are as follows

Table No: 10. Ingredients of Nanosponges

Ingredients	Use	Manufacturer
Ibuprofen	Drug (API)	Yarrow Chem Products, Mumbai
B-Cyclodextrin	Polymer	HiMedia Laboratories Pvt. Ltd., Mumbai
DiPhenyl Carbonate	Cross linker	Sigma Aldrich, India
Ethanol/DMF	Solvent	Thermosil Fine Chem Industries, Pune

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PVA	Stabilizer	Loba Chemie Pvt. Ltd., Mumbai
Water	Dispersion medium	In-house Laboratory

Method for the preparation of Nanosponges:

Table No. 11: Composition of Nanosponges formulation of Ibuprofen

Sr.no	Formulation	Ibuprofen (mg)	B-Cyclod extrin (mg)	DiPhe nyl Carbo nate(m g	Ethanol (ml)	Distilled water (ml)
1	NE-1	100	18 +1	8 0	6 +1	100
2	NE-2	100	15 0	8 0	5 0	100
3	NE-3	1000	18 +1	8 0	5 0	100
4	NE-4	1000	15 0	8 0	6 +1	100
5	NE-5	100	21 +2	8 0	6 +1	100

List of ingredients:

Table No. 12: List of Ingredients

Ingredients	Use
Ibuprofen	Drug
B-Cyclodextrin	Polymer
Diphenyl Carbonate	Crosslinker
Ethanol	Solvent

List of Equipment's:

Table No. 13: List of equipment's

Sr. no	Equipment's	Manufacturer	Use
1	UV-Visible double beam spectrophotometer	Thermo Alpha Helios, Mumbai	To measure the absorbance of the sample
2	Digital Weighing Balance	Hanna Instruments	For weighing purpose
3	Magnetic Stirrer	Remi equipment, Mumbai.	For mixing
4	Diffusion Cell Apparatus	Magnified glass diffusion cell	To measure in Vitro release of drugs
5	High Shear Homogenizer	Ika laboratories	Nano-emulsions preparation
6	pH meter	p Hep Hanna Instruments, Italy	To measure the pH of the solution
7	Cooling centrifuge	Remi R-4C laboratory centrifuge	Phase separation study
8	FTIR	Shimadzu	Compatibility study
9	Particle size Analyzer	Malvern instruments Ltd	To measure the particle size
10	Stability Chamber	REMI, Mumbai	For stability studies

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Preparation of Nanosponges:

Oil phase: oil (oleic acid) and drug (Ibuprofen).

Aqueous phase: Water.

Emulsifying agent: Surfactant (Tween 80) and cosurfactant (Propylene glycol)

Make the oil phase by adding the Ibuprofen drug into oleic acid as an oil, and this is stirred with a magnetic stirrer to get a homogenous mixture, and then mild heating is done. Make an aqueous phase by taking water into a suitable beaker, then mild heating the water. Make an emulsifying agent by taking Tween 80 as a surfactant and propylene glycol as a cosurfactant in a suitable beaker, making them homogenous in a magnetic stirrer, and then mild heating is done.

Next, the oil phase is added dropwise to the aqueous phase, then an emulsifying agent is added dropwise, and all this mixture is homogenized under high-pressure homogenization at 17000 RPM for 1 hr. until a transparent Nanosponges is obtained.

Method for Preparation of Ibuprofen Nanosponges Preparing Polymer Phase

(β-Cyclodextrin)

↓

(Heating at mild temperature 60–70°C)

↓

Preparing Cross-linking Phase (Diphenyl Carbonate)

(Dissolve Diphenyl Carbonate properly and mix with β-Cyclodextrin by continuous stirring using magnetic stirrer)

↓

Formation of Nanosponges

(Heat the reaction mixture at 90–100°C for 4–5 hours with continuous stirring until cross-linking reaction completes)

↓

Cooling and Pulverization

(Allow the product to cool at room temperature, then crush the solid mass and sieve properly)

↓

Solvent Evaporation

(Evaporate solvent and dry the formulation until free-flowing Ibuprofen nanosponges are obtained)

↓

Final Product

(The resulting formulation obtained is Ibuprofen-loaded Nanosponges)

Material and instruments:

Materials:

The drugs used for the present investigation were obtained from Arrow Chem Mumbai.

Details of Pure drug:

Table No. 14: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Ibuprofen	Yarrow Chem Mumbai	10 g	99.3 % w/w

Marketed Preparation:

Table No. 15: Details of marketed Preparation

Brand Name	Mfd by	Content	Quantity
Ibuprofen Tablet	Mepro Pharmaceuticals	Ibuprofen	10 g

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The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

Reagents and cheIBUals:

All reagents and cheIBUals used were of AR grade and HPLC grade.

Methanol (HPLC grade).
Acetonitrile (HPLC grade)
Disodium hydrogen phosphate (AR grade).
Distilled Water (HPLC grade).
Ortho Phosphoric Acid (HPLC grade).

Instruments:

Table No. 16: Instruments Used

Sr. No	Instruments	Make	Model
1	**UV-Visible Spectrophotometer**	Shimadzu	UV 1900i
2	**HPLC**	Waters 600	996 PDA Detector
3	**pH Meter**	Hanna	-
4	**Balance**	Citizen	CY 104 (IBUro Analytical Balance)
5	**Ultra Sonicator**	-	1.5 L 50

Study of Functional Group by Using Infra-Red Spectroscopy:

Ibuprofen API: - Accurately weighed 3 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.

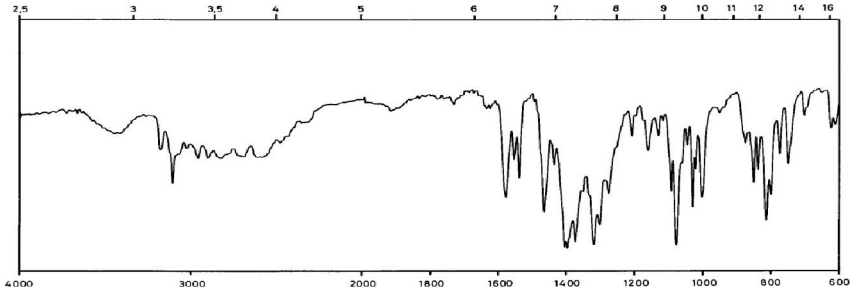

Fig. No. 11: IR Spectra of Ibuprofen

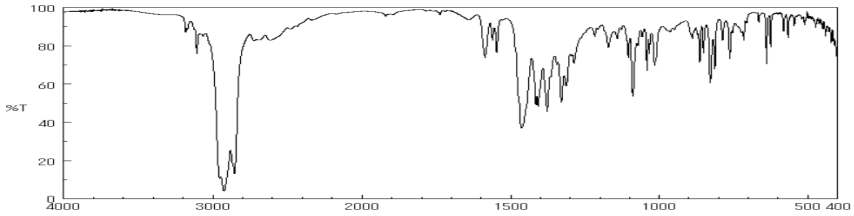

Fig. No.12: Reference IR Spectra of Ibuprofen

Conclusion:

The IR spectra of the given test drug matches with the IR spectra of reference.

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Determination of wavelength maxima Ibuprofen standard stock solution:

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

The aliquot portions of stock standard solutions were g/ml of diluted appropriately with HPLC grade ACN to obtain concentration 5 IBU. The solutions were scanned in the range of 400–200 nm in 1 cm cell against blank. The UV absorbance spectrum of IBU was recorded and found to be 214nm.

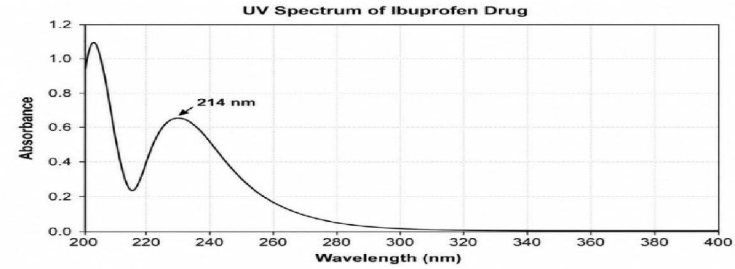

Fig. No. 13: UV Spectrum of Ibuprofen Drug at 214 nm

Development of HPLC method for estimation of Ibuprofen

Method Development Strategy:

Selection of Common Solvent (Diluents):

Acetonitrile of HPLC grade were 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 **Ibuprofen** in different solvents i.e Acetonitrile, methanol and water.

Preparation of standard stock solution:

Accurately weighted IBU 5 mg was dissolved in 100ml 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.

Chromatographic Parameters:

Column: C18 (Thermo Hypersil gold) /4.6 x 2 mm

Flow Rate: 1.0ml/min

Wavelength: 214 nm

Injection volume: 20µl

Column oven Temperature: Ambient (25^0C)

Run Time: 10 minutes

Mobile Phase: 0.1% OPA and ACN (30:70)

Preparation of 0.1% OPA: Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and makeup the volume upto the mark with HPLC water.

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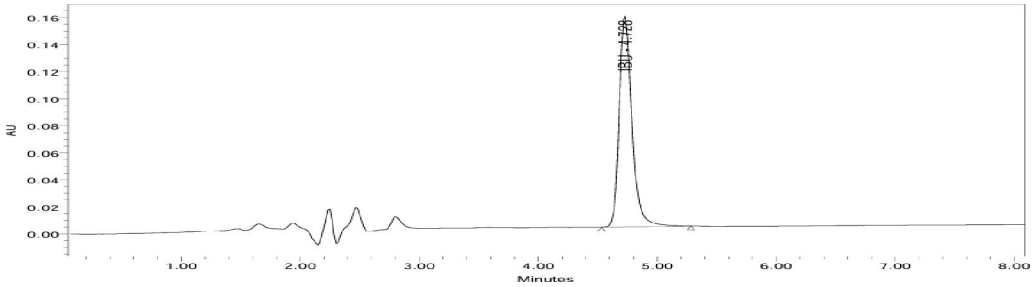

Fig. No. 14: Separation of IBU in selected mobile phase showing retention time at 4.728 min.

System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions. The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of IBU was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

B) Procedure:

Filtered mobile phase was allowed to equilibrate with L std. drug solution□stationary phase until steady baseline was obtained. A 20 was injected which was made in five replicates and the system suitability parameters were recorded.

Table No. 17: Result of System suitability test

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	IBU	IBU	IBU	IBU
1	150500	4.89	1.50	9530
2	150550	4.90	1.49	9525
3	150100	4.90	1.40	9622
4	149000	5.10	1.30	9667
5	149200	5.15	1.44	9545
Mean	149820	5.10	1.30	9578
S.D	414	5.15	0.05	63.36
%R.S.D.	0.25	0.81	2.4	0.67

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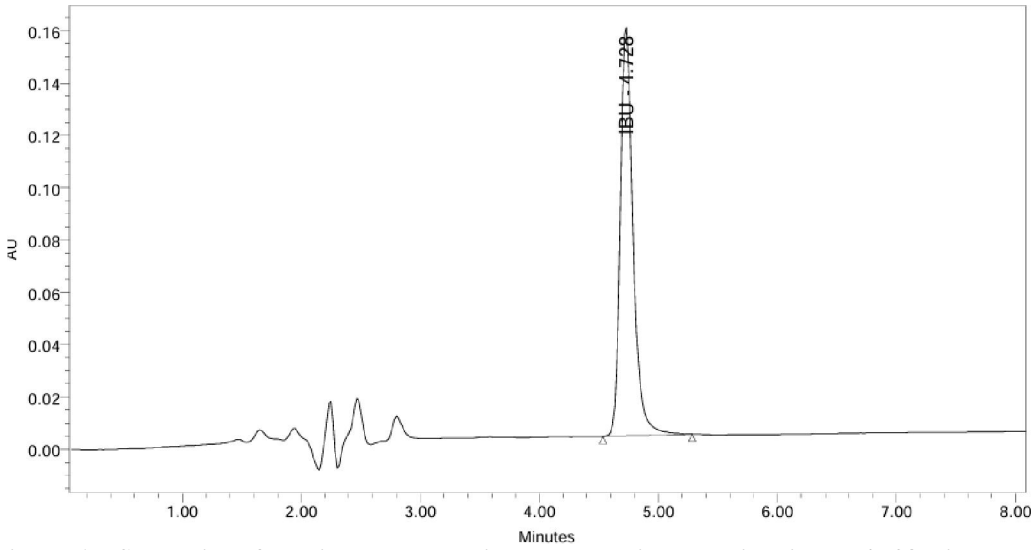

Fig. No 15: Separation of IBU in selected mobile phase showing retention time at 4.728 min.

Estimation of Ibuprofen from marketed Tablet formulation Standard stock solution:

a) Preparation of standard solutions:

Ibuprofen standard stock solution: Accurately weighed quantity of 100 mg IBU was dissolved in ACN and volume was made up to 100 ml mark by same to obtain 1000 µg/ml stock solution.

Ibuprofen standard working solution: Pipette out 1 ml from standard stock solution and dilute it with 10 ml ACN to obtain 100 µg/ml of IBU.

Sample solution preparation:

Entire content of IBU on cream 0.1% was transferred to a 1000 ml flask, the volume was made upto the mark with ACN, the resultant concentration was 1000 µg/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 µ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with ACN, the concentration of working sample solution was 100 µg/ml.

Procedure:

Equal volume L)□(20 of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response

i.e. peak area of major peaks were measured. The content of IBU was calculated by comparing a sample peak with that of standard.

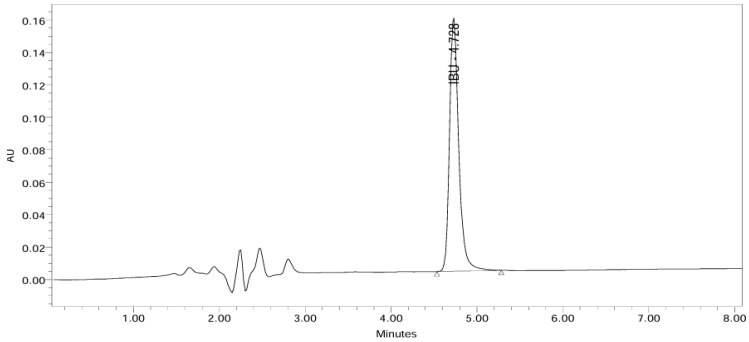

Fig. No.16: Chromatogram of IBU marketed formulation showing retention time 4.728 min.

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Brand name : Ibuprofen Tablet 0.1 %

Table No. 18: Results and statistical data for estimation of IBU in marketed formulation

	IBU	
Sr. No.	**Assay (mg)**	**% Purity**
1	99.90	99.90
2	99.95	99.95
3	99.90	99.90
4	99.90	99.90
5	99.95	99.95
Average	99.96	99.93
SD	0.005	0.005
% RSD	0.36	0.36

Validation parameters:

- Accuracy
- Ruggedness
- Robustness
- Linearity and range
- Specificity
- Placebo Interference study

Accuracy:

The accuracy samples were prepared by spiking the standard into the pre- analyzed 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.

The % Recovery was then calculated by using formula

$$\% \text{ Recovery} = A\text{-}B \times 100 / C$$

Where- A = Total amount of drug estimated.
B = Amount of drug found on pre analyzed basis.
C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

Table No 19: Accuracy studies by standard addition method

	IBU		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.95	99.80	119.85
	79.90	99.95	119.90
	79.90	99.95	119.85
	99.87	99.80	99.75
% Recovery	99.75	99.95	99.83

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	99.75	99.95	99.75
Mean % recovery	98.79	99.92	99.78
% RSD	0.06	0.05	0.04

Acceptance criteria:
The % RSD for the triplicate at each spike level shall be NMT 2.0.
The overall % RSD for % recovery for all spike levels shall be NMT 2.0.
The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

Ruggedness: Intermediate precision
Prepared three sample solutions as per the test method. Injected into the different HPLC system (preferably with different manufacturer or same manufacturer with different configuration) by using the different column and by the different analyst at different date.

Table No. 20 : Intermediate precision Studies (Ruggedness) Set – II

	IBU	
Sr. No.	**Assay (mg)**	**Assay % of LC**
1	99.8	99.8
2	99.2	99.2
3	99.9	99.9
Average	99.6	99.60
SD	0.034	0.034
% RSD	0.12	0.124

Acceptance criteria: The % RSD for the three determinations shall be NMT 2.0

Data analysis between method precision and Intermediate precision:
Compared the data obtained in this section verses the data obtained in method precision and evaluate the overall average, overall SD and overall % RSD and recorded the observation into the following table

Table No. 21: Intermediate precision (Ruggedness) evaluation of data

Sr.no.	**% Assay of LC**	
	IBU	
	Set – I	**Set - II**
1	99.95	99.8
2	99.90	99.2
3	100	99.9
Average	99.8	
SD	0.82	
% RSD	0.80	

SET – I: Method Precision data **SET – II:** Intermediate Precision data

Acceptance criteria: The overall % RSD for the twelve determinations shall be NMT 2.0

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.

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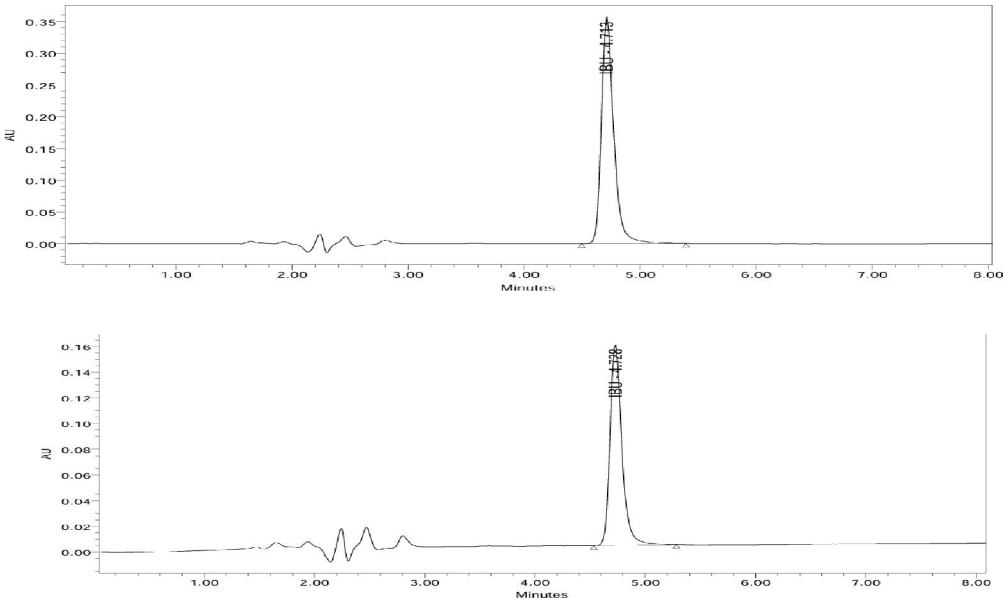
1.1 ml/min



0.9 ml/min

Fig. No. 17: Chromatograms of Change in Flow Rate

Checked the system suitability and recorded the results in the table.

Table No. 22: System suitability of change in Flow Rate

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	IBU	1.027	0.92	0.85	NMT 2.0
2	Theoretical plates	IBU	9600	9520	9557	NLT 2000
3	Tailing factor	IBU	2.30	2.41	2.30	NMT 2.0
4	Retention Time (Min)	IBU	4.2	4.6	4.8	

Observation: The allowable variation in flow rate of the method is from 0.9ml/min to 1.1 ml/min

Acceptance criteria: All the system suitability parameters shall pass

Change in organic composition of mobile phase + 10% (0.1%OPA: ACN) System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % (Organic phase) compared with the optimized method mobile phase concentration.

Procedure:

Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table.

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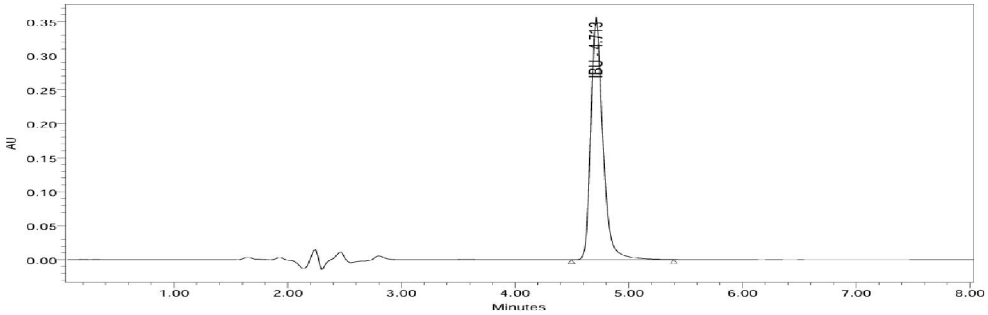
-10% ACN: (0.1%OPA: ACN 70:30)



+10% ACN: (0.1%OPA: ACN 33:67)

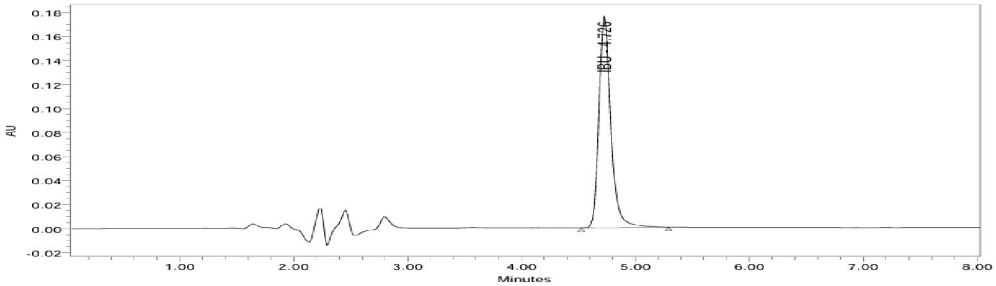


Fig. No. 18: Chromatograms of Change Organic Composition of mobile Phase

Table No. 23: System suitability of change in Organic Composition

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	IBU	1.017	0.655	0.046	NMT 2.0
2	Theoretical plates	IBU	9480	9520	9560	NLT 2000
3	Tailing factor	IBU	1.45	1.60	1.28	NMT 2.0
4	Retention Time (Min)	IBU	8.89	9.54	7.74	

Observation: The allowable variation in ACN composition of method is from 90% to 110%. **Acceptance criteria:** 1. All the system suitability parameters shall pass.

Effect of Variation in Wavelength by ± 2 units:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

Procedure:

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

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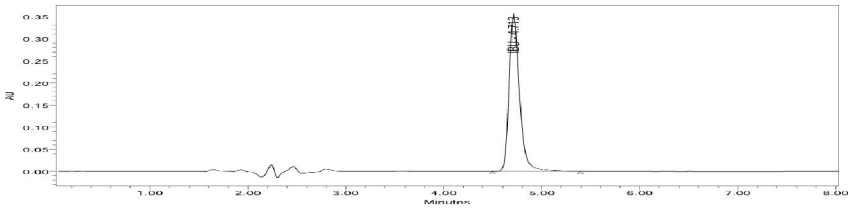
At 212nm wavelength



At 216 nm wavelength

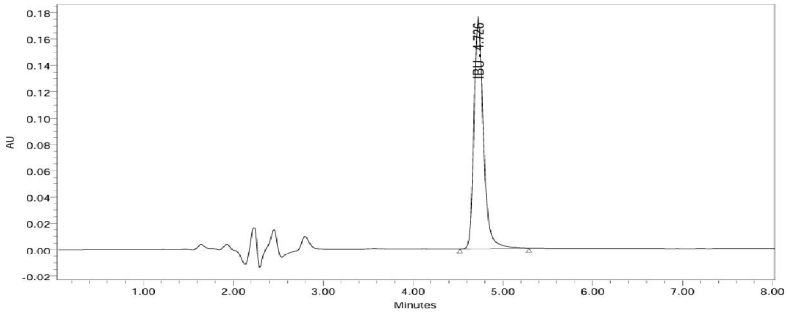


Fig. No. 19: Chromatograms of Change in wavelength.

Table No.24: System suitability of change in wavelength

Sr. No.	System Suitability parameter		Observations for wavelength			Limits
			Unchanged	278nm	282nm	
1	The % RSD of peak area response for five replicate injections	IBU	1.017	0.3638	0.141	NMT 2.0
2	Theoretical plates	IBU	9410	9690	9578	NLT 2000
3	Tailing factor	IBU	1.25	1.62	1.10	NMT 2.0
4	Retention Time (Min)	IBU	8.98	12.206	7.360	

Specificity:

5.4.6.1. Placebo Interference study:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

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Sample matrix

Placebo Preparation

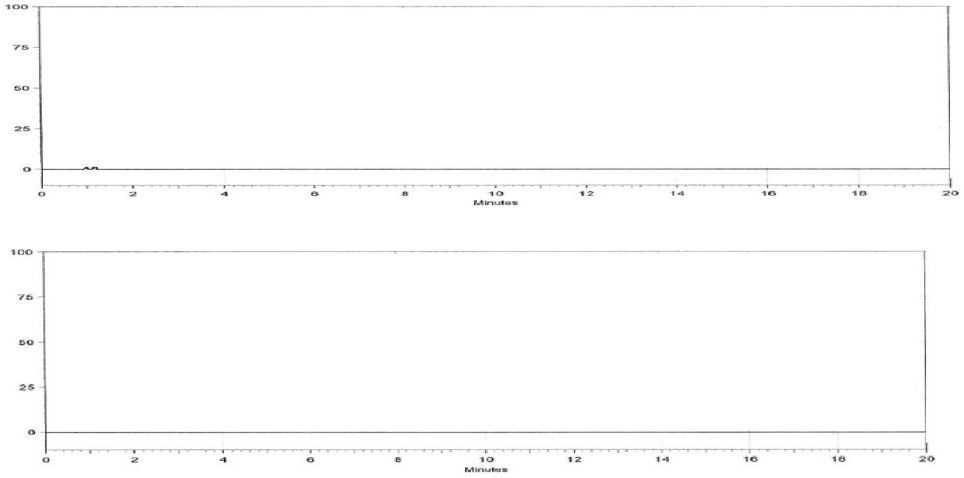

Fig. No. 20: Chromatograms of placebo interference study Table No. 25: Placebo Interference

Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

Acceptance criteria:

No interference should observe from placebo at the retention time of IBU.

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.

Procedure:

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for IBU peaks.

The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for IBU, the correlation coefficient and equation of regression were recorded.

Table No.26: Observations of Linearity and range study for IBU

Sr. No.	% Level	IBU	
		Conc. (µg/ml)	Mean peak area
1	50	50	90875
2	80	80	137100
3	100	100	150520
4	120	120	182430
5	150	150	245340

Acceptance criteria: The correlation coefficient shall be NLT 0.99

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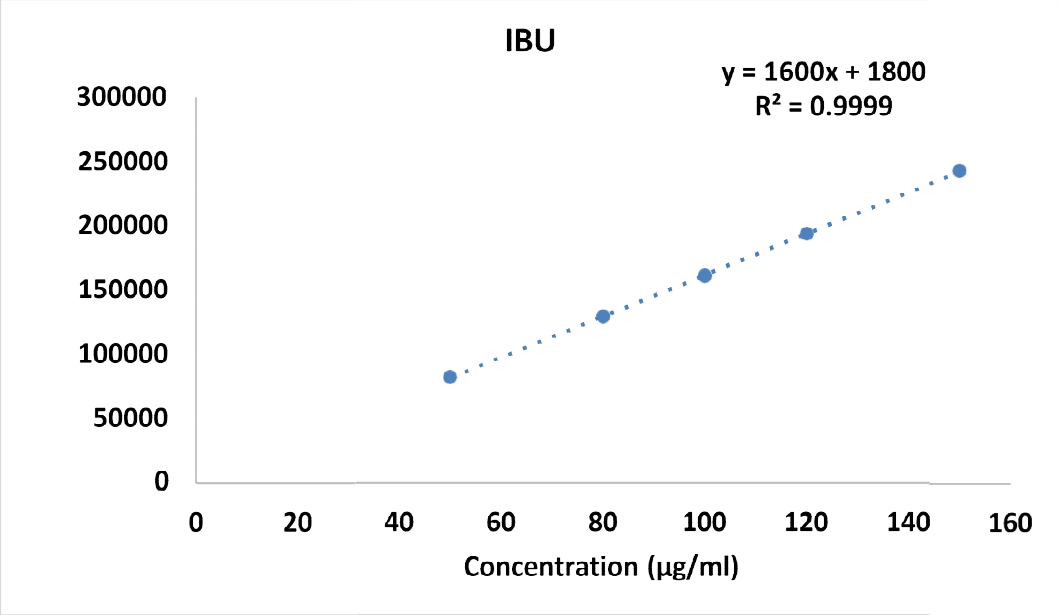

Fig. No. 21: Plot of linearity and range study for IBU

Formulation and HPLC evaluation of Ibuprofen nano-emulsion

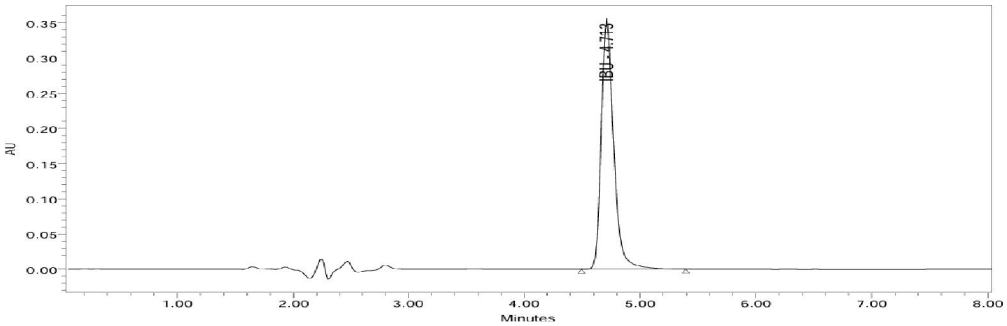

Fig No. 22: HPLC analysis of IBU nanosponges

II. RESULT AND DISCUSSION

Development of HPLC method for estimation of Ibuprofen

High Performance Liquid Chromatography which is a highly sophisticated technique, it is used for the determination of active molecules from their formulations. In the present study a HPLC method was developed for analysis of IBU from its in house formulated nano-emulsion.

It was undertaken to formulate nano-emulsion formulation of IBU and to perform HPLC analysis of the same.

Very few methods are so far reported for estimation of IBU. In the present investigation an attempt has been made to develop a simple HPLC method for estimation of IBU from its formulation. Pure standards of IBU were procured from the Arrow chem Mumbai. Percent purity of above-mentioned drug is reported by Supplier Company as follows.

Table No. 27: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Ibuprofen	Yarrow Chem Mumbai	10 g	99.3 % w/w

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The pure standard was not analyzed in our study and the % purity stated by the supplierswas taken as standard for comparison studies.

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. 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. This technique was employed in the present investigation for estimation of IBU from its in house formulated nano-emulsion. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

HPLC Column Selected:

HPLC Waters 600 system with C18 (Thermo Hypersil gold) /4.6 x 250 mm column and PDA detector were used for the study. The standard and sample solution of IBU was prepared in ACN. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

Mobile phase composed of water (0.1 % OPA) and ACN (30:70 % v/v). An isocratic program was developed contributing a total run time of 10 min. The wavelength 214 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were keptconstant throughout the method.

Table No. 28: Chromatographic Parameters:

Column	C18 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	214 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	Mixture of water (0.1% OPA) : ACN in ratio 30:70 % v/v

Mobile phase-preparation

Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and make up the volume upto the mark with HPLC water.

Preparation of diluent:

ACN of HPLC grade were 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. Thus, the results obtained for such method are given as follow:

Table No. 29: Summary of system suitability of Test results

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	IBU	IBU	IBU	IBU
1	150500	4.89	1.50	9530
2	150550	4.90	1.49	9525
3	150100	4.90	1.40	9622

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4	149000	5.10	1.30	9667
5	149200	5.15	1.44	9545
Mean	149820	5.10	1.30	9578
S.D	414	5.15	0.05	63.36
%R.S.D.	0.25	0.81	2.4	0.67

After establishing the chromatographic conditions, standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Table No. 30: Results and statistical data for estimation of IBU in marketed formulation

Brand name : : Ibuprofen

Sr. No.	**IBU**	
	Assay (mg)	**% Purity**
1	99.90	99.90
2	99.95	99.95
3	99.90	99.90
4	99.90	99.90
5	99.95	99.95
Average	99.96	99.93
SD	0.005	0.005
% RSD	0.36	0.36

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.

Table No. 31: Data showing system Precision

Sr. No.	**Parameter**	**Observations**	**Limits**
1	The % RSD of peak area response for three replicate injections of standard	0.60	NMT 2.0
2	Theoretical plates	9455	NLT 2000
3	Tailing factor	1.35	NMT 2.0

Table No.32: Method Precision Studies Set – I

Sr.no.	**IBU**	
	Assay (mg)	**Assay % of LC**
1	99.95	99.95
2	99.90	99.90

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3	100	100
Average	99.96	99.963
SD	0.58	0.58
% RSD	0.15	0.15

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 50% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y- axis. The R^2value was found to be 0.999 for IBU. The result show that an excellent correlation exists between concentration and mean peak areas within the concentration range. Thus, the method developed is accurate, precise, specific, & linear. Hence it can be said that, RP-HPLC is the most accurate, precise and reproducible among all methods.

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results werewell within the range **99-101%.** Thus the method was found to be accurate.

Table No. 33: Result of Accuracy Studies

	IBU		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.95	99.80	119.85
	79.90	99.95	119.90
	79.90	99.95	119.85
% Recovery	99.87	99.80	99.75
	99.75	99.95	99.83
	99.75	99.95	99.75
Mean % recovery	98.79	99.92	99.78
% RSD	0.06	0.05	0.04

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.

Following Parameters were covered under robustness parameter.

Effect of variation in flow rate of mobile phase by ± 10%

Organic phase composition (± 10%)

Change in Wavelength by ± 2 units

The results suggested all the system suitability parameters were within limits.

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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 foundat the Retention time of IBU.

Evaluation of Ibuprofen nanosponges

Organoleptic characteristics:

Appearance Test:

The appearance test was carried out visually, whereby it will include the observation of physical character of the nanosponges such as Colour, Texture and homogeneity of the nanosponges.

Table No. 34: Showing physical characteristics of the formulation F1-F5

Formulation Code	Colour	Texture	Remarks
F1	Yellow	Soft	Homogeneous
F2	Yellow	Soft	Homogeneous
F3	Yellow	Soft	Homogeneous
F4	Yellow	Soft	Homogeneous
F5	Yellow	Soft	Homogeneous

Colour:

No changes in colour were observed in the Formulation of F1, F2, F3, F4 and F5 Ibuprofen. This showed that Ibuprofen nanosponges were stable at different storage conditions up to 20 days.

Phase Separation:

All the formulations F1, F2, F3, F4 and F5 were stable under the 25°C (Room temperature).

Determination of Particle size and Zeta potential:

The size of dispersed particles in a nanosponges determines by Lite sizer DLS 500 instruments.

Determination of pH:

The pH values of the formulation were measured by immersing the electrode directly into the dispersion using calibrated pH meter.

Density measurements:

Density at certain temperatures can be determined by using high-precision hydrometer.

Viscosity measurements:

The Viscosity was determined by using Brook-field Viscometer.

Freeze Thaw Study:

Freeze-thaw testing is conducted by exposing the product to freezing temperatures (approximately -10 °C) for 24 hours, and then allowing it to thaw at room temperature for 24 hours.

The sample is then placed in a higher temperature (approximately 45°C) in freezing condition for 24 hours and then placed at room temperature for 24 hours. The sample is analyzed for significant changes.

In all the formulations F1 to F5 has no phase separation.

Drug content analysis:

1 ml Ibuprofen nanosponges was taken in 10 ml volumetric flask containing 1 ml DMF and volume was made up to 10 ml with phosphate buffer of pH 7.4. From the above solution, 1 ml was further diluted with 10 ml phosphate buffer. The resultant

solution was filtered through Whatman filter paper and absorbance of the solution was measured at 214 nm using UV spectrophotometer.

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Discussion:

The drug content in formulation fromF1, F2, F3, F4 and F5 was 52.5%, 10%, 29%, 37.5% and 70%, respectively. Based on these results, it can be concluded that batch F5 (70%) contain more drug than the other batch since they were taken for optimization.

Optical Transparency:

All the formulations F1, F2, F3, F4 and F5 were stable clear and transparent.

Table No. 35: Evaluation study of Ibuprofen nanosponges

Formulati on code	Particle size (nm)	pH	Densiy (g/cc)	Viscosity (cps)	Drug content (%)	Centrifugation study (5000 rpm)	Freeze thaw study
F1	811.6	6.93	1.03	40	52.5	Stable	Stable
F2	905.6	6.94	1.03	10.5	10	Stable	Stable
F3	937.0	6.97	1.03	30.1	29	Stable	Stable
F4	919.7	6.87	1.04	20.1	37.5	Stable	Stable
F5	258.8	6.88	1.05	50	70	Stable	Stable

III. SUMMARY AND CONCLUSION

SUMMARY

In house nano-sponges formulation containing IBU was formulated to treat anti inflammatory conditions. The formulation was to be analyzed using HPLC method.

The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for estimation of IBU and to apply it for the analysis of in-house nano-sponges formulation containing IBU.

In the developed RP-HPLC method the analyte was resolved using Mobile phase composed of water (0.1% OPA) and ACN in the ratio 30:70 % v/v. A isocratic program was developed contributing a total run time of 10 min. using HPLC auto- sampler system containing PDA detector with EMPOWER Software and C18 (Thermo Hypersil gold) /4.6 x 250 mm column, the detection wavelength was 214 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-house nano- sponges formulation.

The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of in-house nano-sponges 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.

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

REFERENCES

[1]. Khopkar S. M. Basic concepts of analytical chemistry, New Age International Ltd. Publishers, New Delhi (1998); 2:178-179.

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Impact Factor: 8.2

[2]. Settle F. Handbook of Instrumental techniques for analytical chemistry,Prentice Hall PTR, NJ (1997); 17(19): 56-57.
[3]. Skoog D. A. Holler F. J, Crouch S. R. Principle of Instrumental Analysis, Thomson Publications, India (2007); 6: 1-3, 145-147, 180.
[4]. Mendham J, Denney R. C, Barnes J. D, Thomas M. Vogel's Textbook of Quantitative Analysis, Pearson Education, Singapore (2003); 8-9.
[5]. Sharma B. K. Instrumental Methods of Chemical Analysis, Goel Publication, Meerut (1983); 25, 3, 6.
[6]. Christian G. D. Analytical Chemistry, John Wiley and Sons (2003); 5: 35-42, 131-132.
[7]. Beckett A. H, Stenlake J. B. Practical Pharmaceutical chemistry, CBSPublisher and Distributor, New Delhi (1997); 2:1-85.
[8]. Christianah M. A, Pui-Kai L. Analytical Profile of Drug Substances. Edi. By Klaus Florey, 124-141.
[9]. Dong M. W. Modern HPLC for Practicing Scientist. John Wiley and sons, (2006).
[10]. O. D. Ghatage et al. Development and validation of a simple RP-HPLC method for estimation of Fluconazole in pharmaceutical dosage forms. *2022*.
[11]. Nadia Bounoua et al. Development of a selective and sensitive stability-indicating HPLC method for analysis of Ibuprofen enantiomers using polysaccharide-based chiral columns. *2018*.
[12]. V. Ravi Shankar et al. Formulation and evaluation of Metronidazole cocoa butter suppositories containing Tween 80, SLS, and Span 20 for sustained drug release. *2012*.
[13]. Subhash Chandra Boss Penjur et al. Formulation and evaluation of Lansoprazole- loaded nanosponges for improved stability and controlled drug release. *2016*.
[14]. Swarupa Arvapally et al. Development and evaluation of Glipizide nanosponges using β-cyclodextrin for immediate-release tablet formulation. *2017*.
[15]. Anjali S. Kumar et al. Formulation and evaluation of Clotrimazole nanosponges incorporated into hydrogel for enhanced topical drug delivery. *2018*.
[16]. Nirosha Manyam et al. Development of Trimethoprim nanosponge-loaded extended-release tablets using ethyl cellulose and HPMC polymers. *2018*.
[17]. Rahul S. Solunke et al. Formulation and evaluation of Gliclazide-loaded nanosponges for sustained drug release and enhanced bioavailability. *2019*.
[18]. Shrishail M. Ghurghure et al. Preparation and evaluation of Itraconazole-loaded nanosponges incorporated into hydrogel using emulsion solvent diffusion method. *2019*.
[19]. Khanderao Rajaram Jadhav et al. Formulation and evaluation of nanosponge- based topical gel using ethyl cellulose and Eudragit RS100 polymers. *2020*.
[20]. Mane P. K. et al. Formulation and evaluation of Flurbiprofen nanosponges for topical delivery and extended drug release. *2021*.
[21]. Mohammed Muqtader Ahmed et al. Formulation and characterization of Butenafine-loaded nanosponges incorporated into Carbopol gel for antifungal delivery. *2021*.
[22]. Pushpalatha D. et al. Development and evaluation of Lovastatin-loaded nanosponges for enhanced bioavailability and controlled release. *2021*.
[23]. Shivansh Srivastava et al. Preparation and optimization of Econazole nitrate- loaded nanosponges using Box–Behnken design. *2021*.
[24]. Abhishek Soni et al. Formulation and evaluation of Luliconazole suppositories using glycerin, gelatin, and Carbopol bases. *2022*.
[25]. https://pubmed.ncbi.nlm.nih.gov/31194439/