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High Performance Liquid Chromatograph Method Development for the Simultaneous Estimation of Ibuprofen and Chlorpheniramine Maleate

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Abstract: This study presents a method development and analysis on HPLC for the simultaneous estimation of Ibuprofen and Chlorpheniramine Maleate in oral dosage forms. The method involved selecting a C18 Hypersil BDS column with dimensions of 4.6 x 150 mm and a particle size of 5 µm. Analytical wavelength selection was performed by preparing appropriate dilutions of each drug with the mobile phase and scanning across the range of 200 to 400 nm. A wavelength of 225 nm was identified as optimal for both Ibuprofen and Chlorpheniramine Maleate. The developed method offers a sensitive and efficient approach for the simultaneous estimation of these drugs in oral dosage forms, providing a valuable tool for pharmaceutical analysis and quality control.

Keywords: HPLC, Oral dosage form, Mobile phase

Introduction: High Performance Liquid Chromatography (HPLC) is a widely used analytical technique for the separation, identification, and quantification of chemical compounds in various samples. Method validation is a critical process that confirms the suitability and reliability of an analytical method for its intended application. It ensures that the method is capable of producing accurate, precise, and reproducible results consistently. The validation of an HPLC method involves a series of tests and experiments to evaluate its performance characteristics. These include parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability. Each of these parameters provides crucial information about the method's ability to detect and quantify analytes under specific conditions. The validation process typically follows established guidelines and protocols set forth by

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regulatory agencies or pharmacopeias, such as the International Conference on Harmonisation (ICH), United States Pharmacopeia (USP), or European Pharmacopoeia (Ph. Eur.). These guidelines ensure that the validated method meets the required standards for quality, reliability, and regulatory compliance.In summary, HPLC method validation is essential to demonstrate the analytical performance and suitability of the method for its intended use, providing confidence in the accuracy and reliability of the data generated for scientific research, quality control, or regulatory submissions.

Experimental Method- The stationary phase chosen for this method is C18 Hypersil BDS, with a column configuration of 4.6 x 150 mm and a particle size of 5 µm. For the selection of the analytical wavelength, the sensitivity of the HPLC method relies on choosing a wavelength that provides a robust response for the targeted drug compounds. To determine this, various concentrations of Ibuprofen and Chlorpheniramine Maleate HCl were separately diluted with the mobile phase. Each solution was then scanned across the spectrum mode within the range of 200 to 400 nm, and the resulting spectra were overlaid. The wavelength chosen for analysis was 225 nm, as both drugs exhibited significant absorbance at this wavelength. The UV spectra of Ibuprofen and Chlorpheniramine Maleate HCl in the mobile phase were overlaid to confirm this selection.

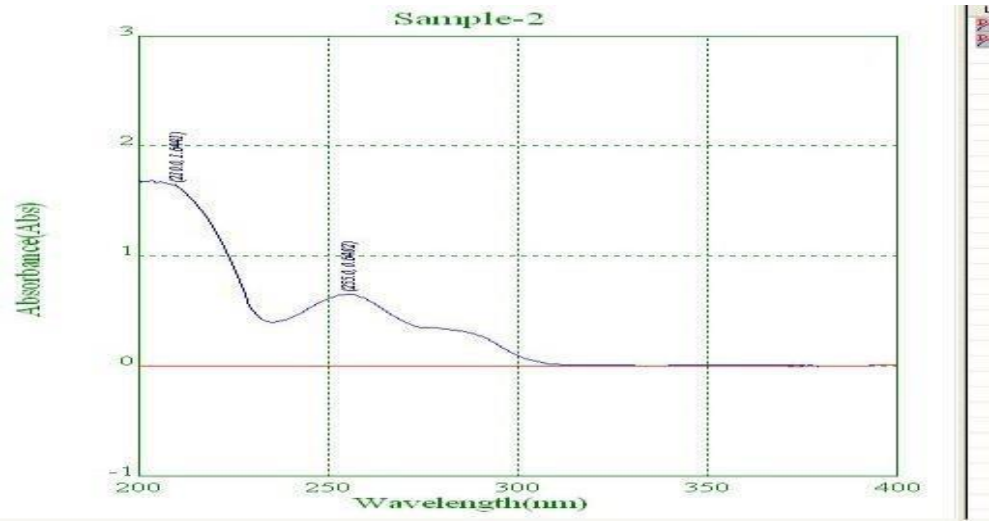

Fig. No.1 UV Spectra of Chlorpheniramine Maleate HCl

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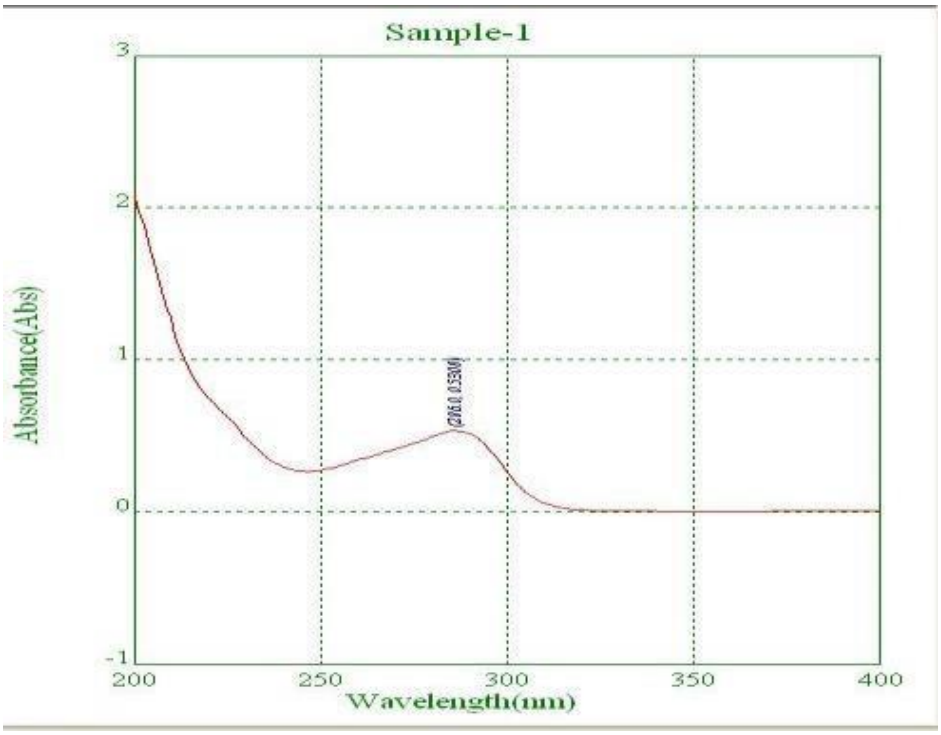

Fig No.2 Uv Spectra of Ibuprofen

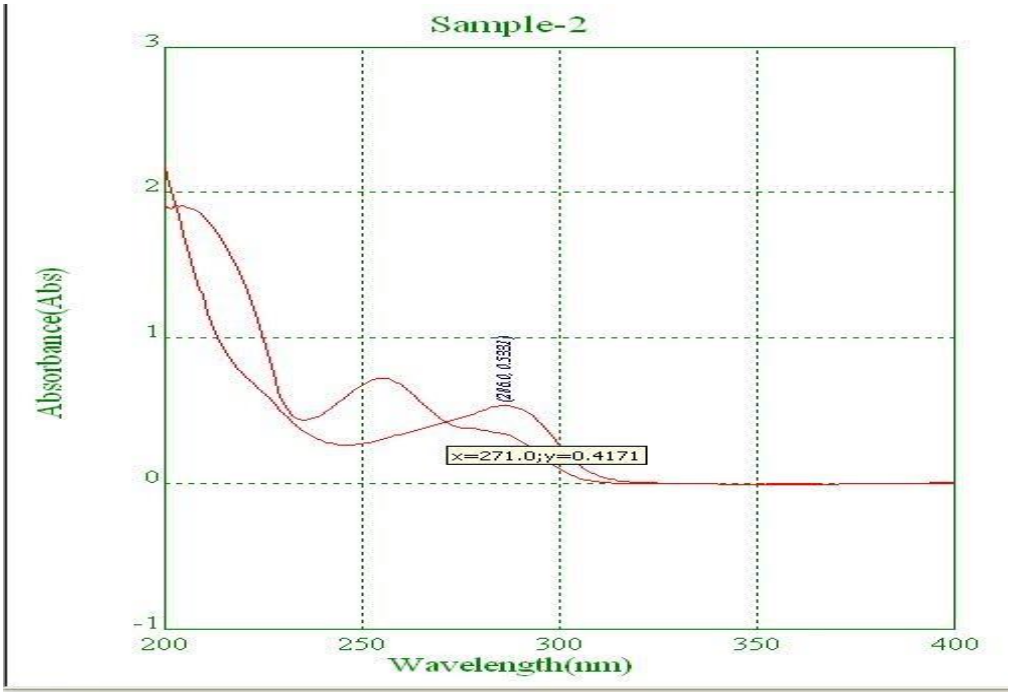

Fig.No.3.Iso-absorptive point of Ibuprofen and Chlorpheniramine Maleate

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Table No. 1 Chromatographic Conditionduring Different Trials Run

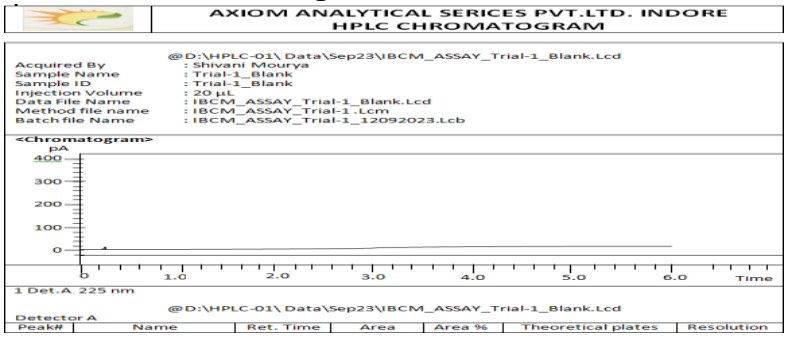
Fig. No.	Columnused	Mobilephase,FlowRateandWavelength	Inj. Vol.
1	Hypersil BDS C18 (250×4.6mm,5.0µ)	Methanol:waterpH3.0(80:20),225nm, Flowrate0.7ml	20 µl
2	Hypersil BDS C18 (250×4.6mm,5.0µ)	Methanol:waterpH3.0(70:30),225nm, Flowrate0.7ml	20 µl
3	Hypersil BDS C18 (250×4.6mm,5.0µ)	Methanol:waterpH7.0(60:40),225nm, Flowrate0.7mL	20 µl
4	Hypersil BDS C18 (250×4.6mm,5.0µ)	Methanol:waterpH3.0,(60:40)225nm, Flow rate 0.7ml	20 µl
5	Hypersil BDS C18 (250×4.6mm,5.0µ)	Acetonitrile:OPA0.05%inWater(60:40) 225nm,Flowrate 0.7ml	20 µl

Preparation of standard stock solutions:
Accurately weighed quantities of 30 mg Chlorpheniramine Maleate HCl and 40 mg Ibuprofen were individually dissolved in an adequate volume of methanol: water solvent (60:40 v/v) and transferred into separate 100 ml volumetric flasks. Each solution was then diluted to a final volume of 100 ml using the same solvent, resulting in solutions containing 300 ppm of Chlorpheniramine Maleate and 400 ppm of Ibuprofen, respectively.

Chromatographic Condition-Trial-1
HPLC: Waters Alliance Separation Module 2695 with UV detector 2487, Empower Software
HPLC Column :: Hypersil BDS C18 (250 ×4.6mm, 5.0µ) **Mobile Phase:** Methanol: water pH 3.0 with OPA:: (80:20) **Wavelegth :** 225nm,

Flowrate: 0.7ml
Injection Volume: 20µl

Chromatogram of Trial 1-Blank



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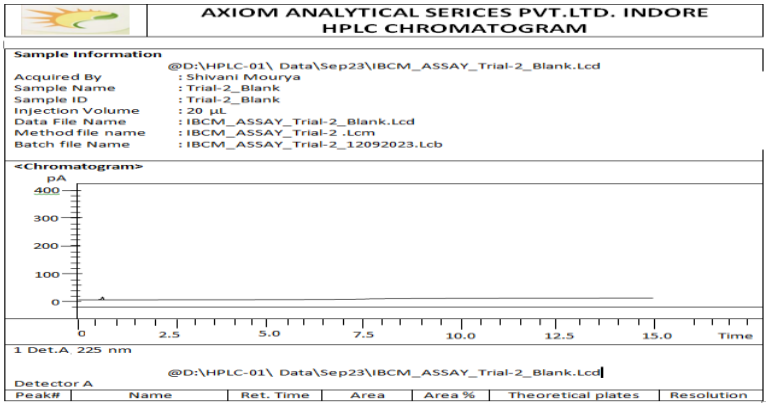
Chromatographic Condition-Trial-2

HPLC:Waters Alliance Separation Module 2695 with UV detector 2487, Empower Software
HPLC Column :: Hypersil BDS C18 (250 ×4.6mm, 5.0µ) **Mobile Phase:** Methanol : water pH 3.0 withOPA:: (70:30) **Wavelegth:** 225nm,

Flowrate: 0.7 ml
InjectionVolume:20µl

Chromatogram of Trial 2+Blank



Chromatogram of Trial2_Sample

Chromatographic Condition-Trial-3

HPLC: Waters Alliance Serration Module 2695 with UV detector 2487, Empower Software
HPLC Column :: Hypersil BDS C18 (250 ×4.6mm, 5.0µ) **MobilePhase:** Methanol : water pH 7.0 with OPA:: (60:40) **Wavelength:** 225 nm,

Flowrate: 0.7ml

InjectionVolume: 20µl

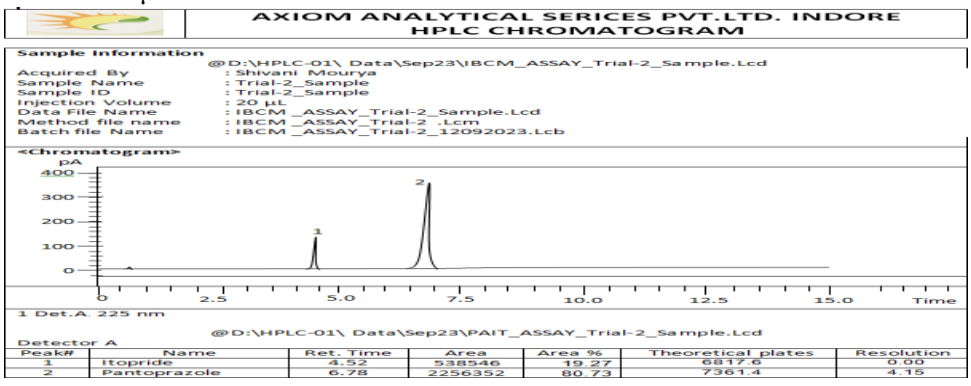


Fig.04. Chromatogram of Trial 3_Blank

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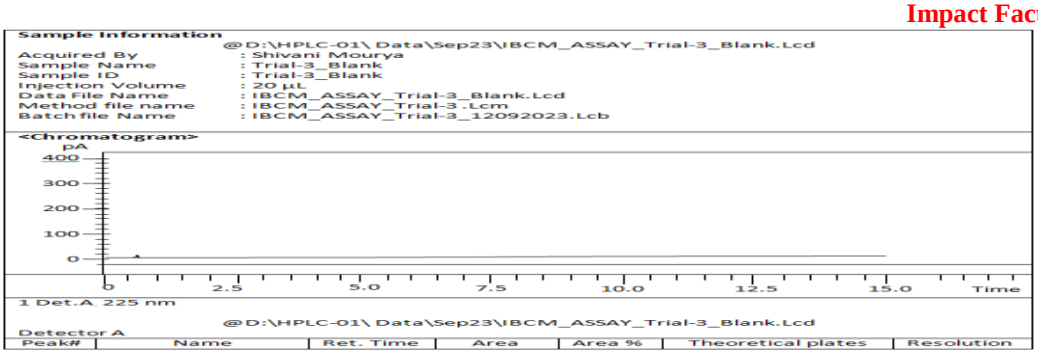
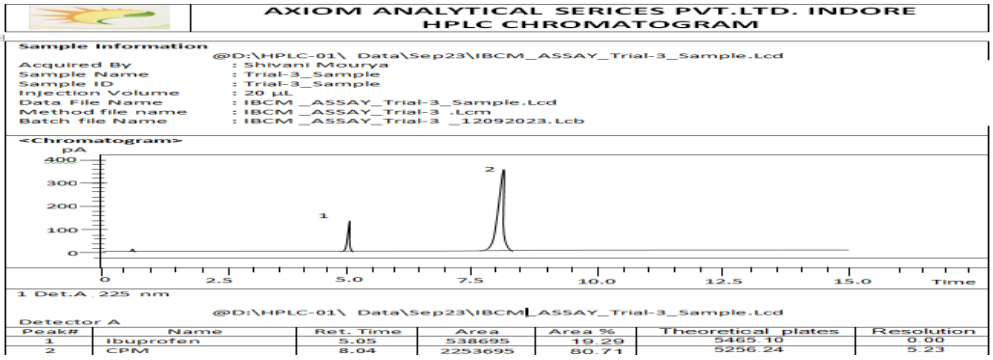


Fig.05. Chromatogram of Trial 3_Sample



Chromatographic Condition- Trial- 4

HPLC: Waters Alliance Separation Module 2695 with UV detector 2487, Empower Software

HPLCColumn: Hypersil BDS C18 (250×4.6mm,5.0µ)

MobilePhase: Methanol:waterpH3.0withOPA::(60:40) **Wavelength:** 225 nm,

Flowrate: 0.7ml

Injection Volume: 20µl

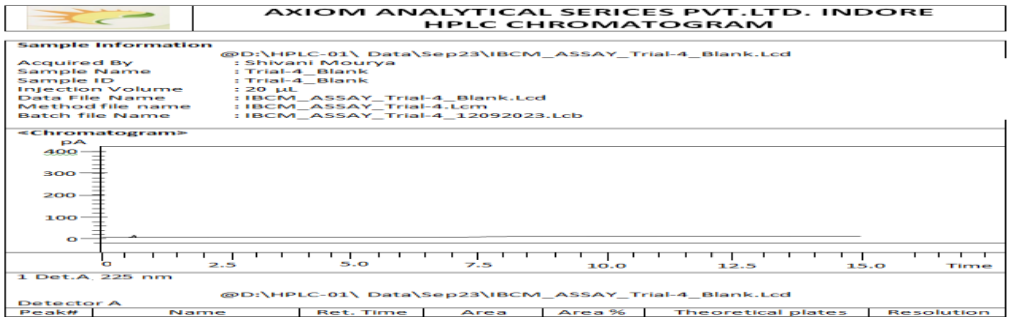


Fig.06.Chromatogram of Trial 4_Blank

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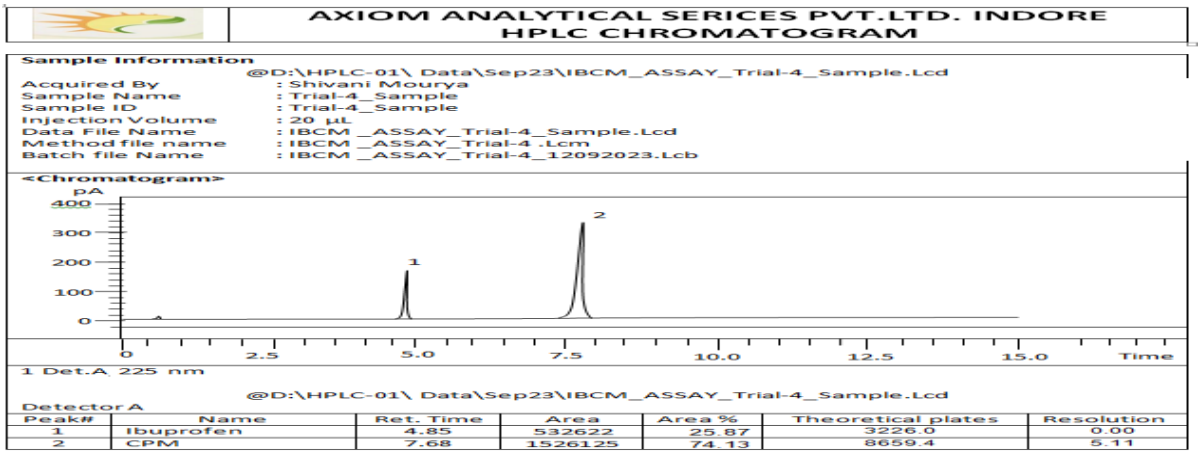

Fig.07.Chromatogram ofTrial4_Sample

Table No. 2: Observation table of Different Trials Run of Chromatographic Condition

Fig.No.	Columnused	Mobile phase, Flow Rate and Wavelength	Inj.Vol.	Observation	Conclusion
1	Hypersil BDSC18(250×4.6mm, 5.0µ)	Methanol : water pH3.0 (80:20), 225nm, Flow rate 0.7 ml	20µl	Well resolved peaks were not obtained.	Hence rejected
2	Hypersil BDSC18(250×4.6mm, 5.0µ)	Methanol : waterpH3.0(70:30),225nm , Flowrate0.7ml	20µl	Well resolved peaks were not obtained.	Hence rejected
3	Hypersil BDSC18(250×4.6mm, 5.0µ)	Methanol : water pH7.0 (60:40), 225nm, Flow rate 0.7 mL	20µl	Well resolved peaks were not obtained.	Hence rejected

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4	Hypersil BDSC18(250×4.6mm, 5.0µ)	Methanol : water pH 3.0, (60:40) 225 nm, Flowrate 0.7ml	20µl	Sharp and Well resolved peaks were not obtained.	Hence rejected
5	HypersilBDSC18(250×4.6mm, 5.0µ)	Acetonitrile:OPA0.05% in Water (60:40) 225nm, Flow rate0.7ml	20µl	Sharp well resolved peaks were obtained	Hence selected

Table No. 3 : Optimized parameters for HPLC method

Sr.No.	Parameter	Description
1	Stationary Phase	C_{18} column with 150 mmx4.6 mm i.d.and 5µm particle size
2	Mobile Phase	Acetonitrile:0.05% OPA in water (60:40)
3	Flow Rate	0.7ml/min
4	Detection wave length	225 nm
5	Detector	UV detector
6	Injector	Auto Injection
7	Injection volume	20µl
8	Column Temperature	Ambient
9	Run Time	10 min

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Preparation of Chlorpheniramine Maleate Standard Solution.

Precisely 30 mg of Chlorpheniramine Maleate HCl was meticulously weighed and added to a 100 ml volumetric flask. After dissolving it in an appropriate volume of methanol(60:40 v/v), the solution was made u p to 100 ml with the same solvent, achieving a concentration of 300 ppm Chlorpheniramine Maleate.

Preparation of Chlorpheniramine Maleate Standard Solution.

A precise measurement of 40 mg of Ibuprofen was transferred into a 100 ml volumetric flask and dissolved in an ample amount of methanol solvent (60:40 v/v). The solution was then diluted to 100 ml using the same solvent, resulting in a concentration of 400 ppm Ibuprofen.

Preparation of mix standard solutions: Accurate measurements of 30 mg of Chlorpheniramine Maleate HCl and 40 mg of Ibuprofen were individually transferred to separate 100 ml volumetric flasks. Each was dissolved in a sufficient volume of methanol solvent (60:40 v/v) and subsequently diluted to 100 ml with the same solvent. This resulted in solutions containing 300 ppm of Chlorpheniramine Maleate and 400 ppm of Ibuprofen, respectively.

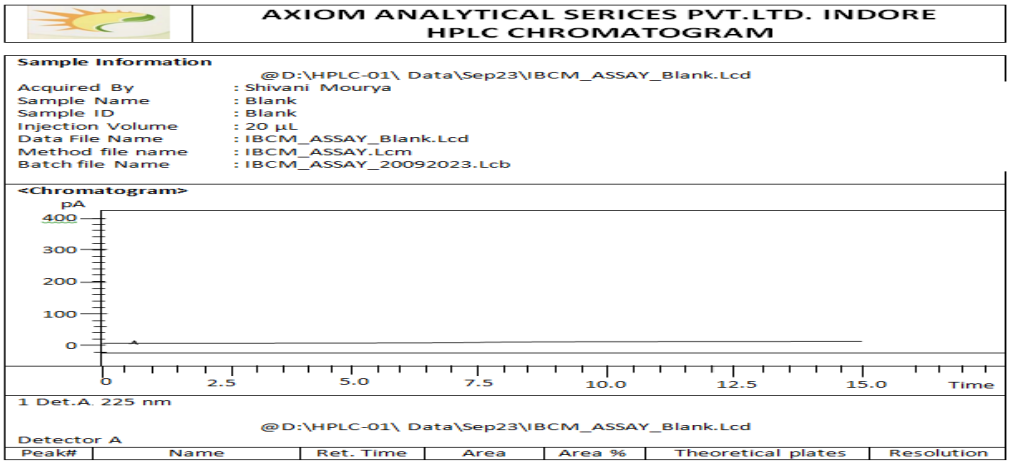

Fig.08.Chromatogram of Blank

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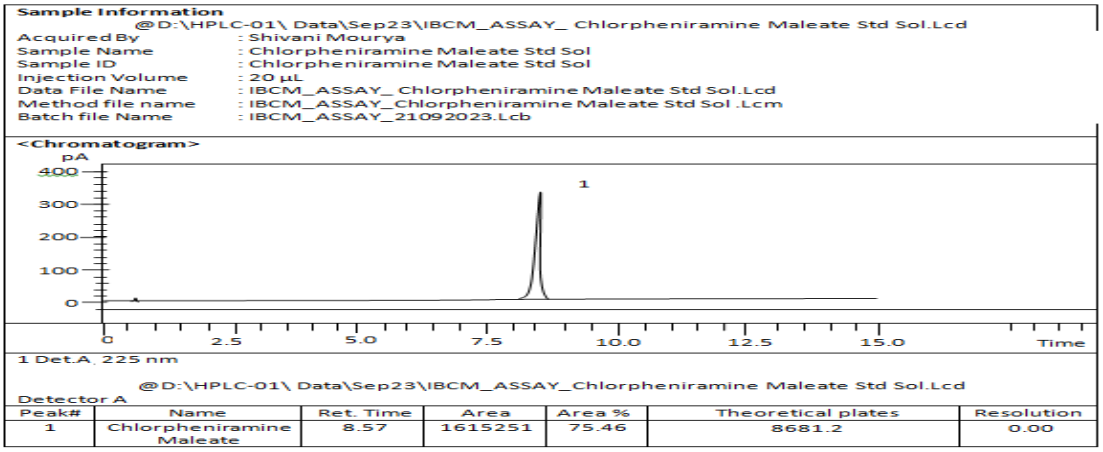

Fig.09.Chromatogram of standard Chlorpheniramine Maleate HCl

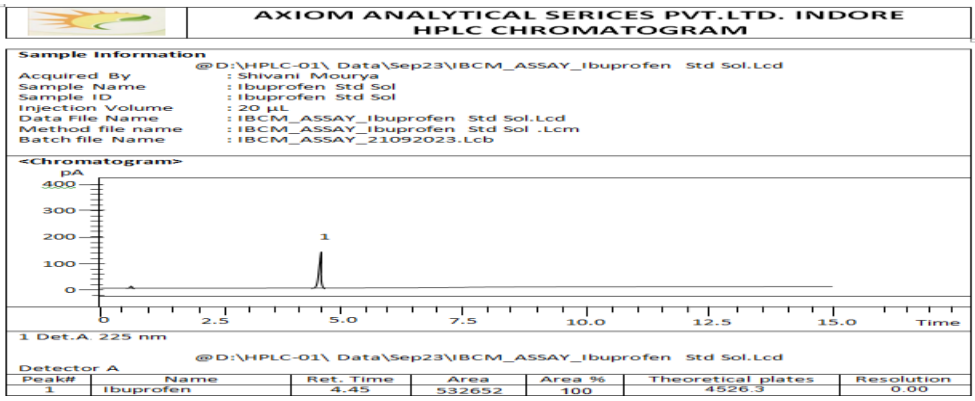

Fig.10.Chromatogram of standard Ibuprofen

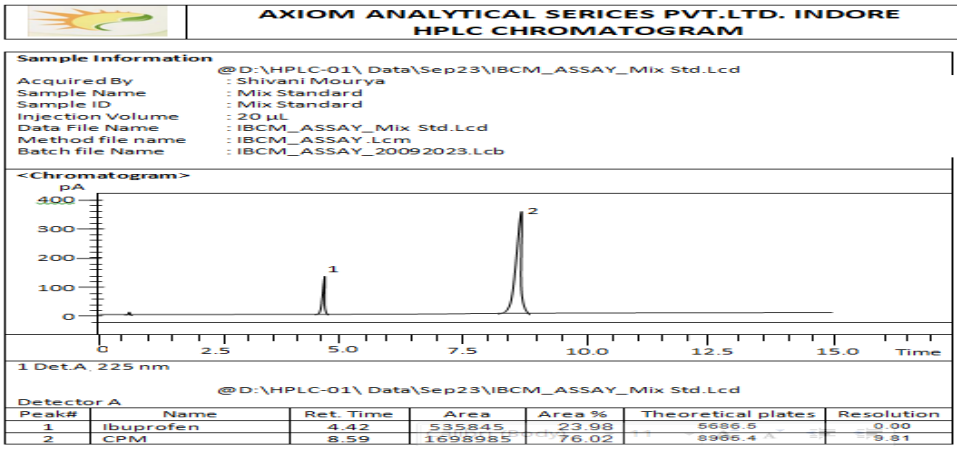

Fig.11.Chromatogram of standard mixture of Ibuprofen and Chlorpheniramine Maleate HCl

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Table No. 4 Details of chromatogram of standard mixture Ibuprofen & Chlorpheniramine Maleate HCl

Sr.No	Nameof drug	RT(min)	Area	T.Plates	Resolution
1	**Ibuprofen**	4.42	535845	5686.5	0.0
2	**Chlorpheniramine Maleate**	8.59	1698985	8965.4	9.81

Analysis of formulation:

Each capsule content: Chlorpheniramine Maleate HCl:30mg Ibuprofen: 40mg

Procedure: To analyze the tablet dosage form, 20 tablets were individually weighed, and their average weight was determined. Subsequently, the tablets were crushed into fine powders, and an amount equivalent to 30 mg of Chlorpheniramine Maleate and 40 mg of Ibuprofen from each tablet was transferred to a 100 ml volumetric flask. The powders were dissolved and diluted with a diluent composed of methanol (60:40 v/v). After sonication, the volume was adjusted to the mark with the same solvent mixture. The quantities of Chlorpheniramine Maleate and Ibuprofen per tablet were calculated by extrapolating their respective values from the calibration curve. This analysis procedure was repeated twice for the tablet formulation, and the results are presented in Table No. 5.

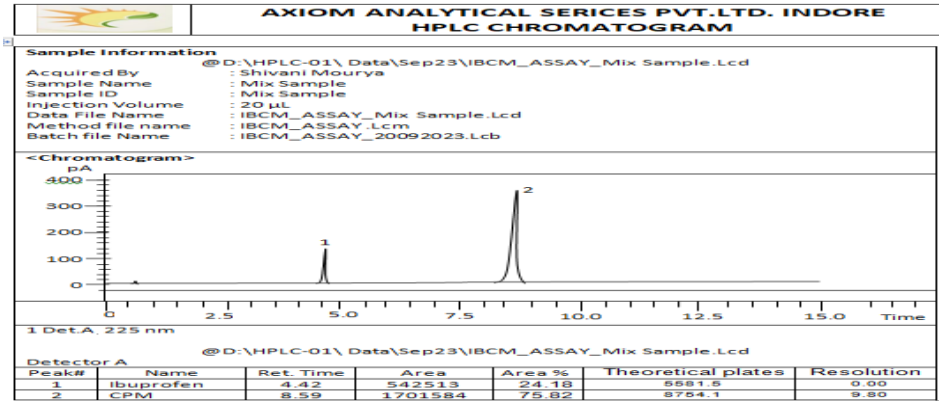

Fig.12. Chromatogram of Chlorpheniramine Maleate and Ibuprofen in tablet formulation Run-1.

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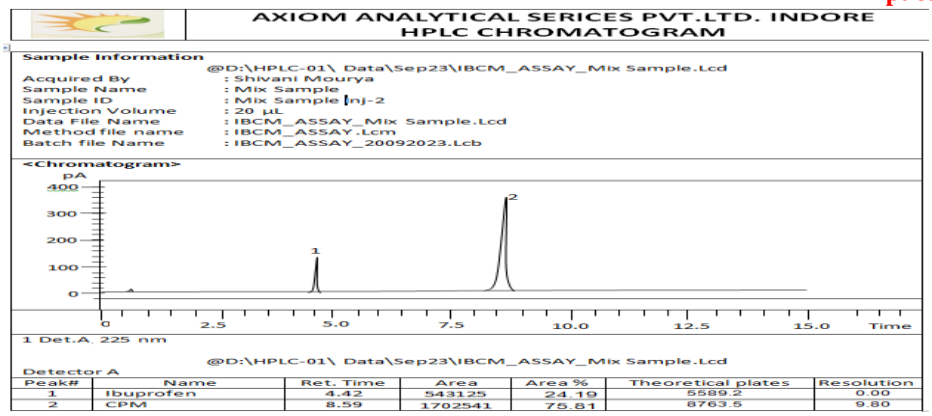

Fig.13. Chromatogram of Chlorpheniramine Maleate and Ibuprofen in tablet formulation Run-2.

Table No. 5 : Details of chromatogram of Itopirde &Ibuprofen in tablet formulation Run-1

Sr.no	Name ofdrug	RT(min)	Area	TheoreticalPlates	Resolution
1	Chlorpheniramine MaleateHCl	4.42	542513	5581.5	0.00
2	Ibuprofen	8.59	1702541	8754.1	9.80

Table No.6 : Details of chromatogram of Itopirde & Ibuprofen in tablet formulation Run-2

Sr.no	Name ofdrug	RT(min)	Area	TheoreticalPlates	Resolution
1	Chlorpheniramine MaleateHCl	4.42	543125	5589.2	0.00
2	Ibuprofen	8.59	1701584	8763.5	9.80

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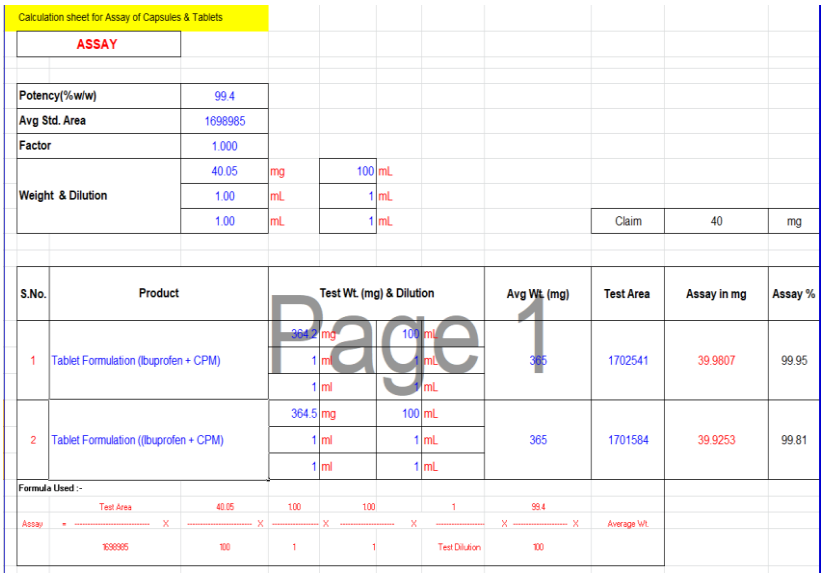
Calculation sheet for Assay of Capsules & Tablets

ASSAY

Potency(%w/w)	99.5							
Avg Std. Area	535845							
Factor	1.000							
Weight & Dilution	29.54	mg	100	mL				
	1.00	mL	1	mL				
	1.00	mL	1	mL		Claim	30	mg

S.No.	Product	Test Wt. (mg) & Dilution				Avg Wt. (mg)	Test Area	Assay in mg	Assay %
1	Tablet Formulation (Ibuprofen+ CPM)	364.2	mg	100	mL	365	542513	29.8234	99.41
		1	ml	1	mL				
		1	ml	1	mL				
2	Tablet Formulation (Ibuprofen+ CPM)	364.2	mg	100	mL	365	543125	29.8571	99.52
		1	ml	1	mL				
		1	ml	1	mL				

Fig.14. ASSAY Calculation Sheet: Chlorpheniramine Maleate



Calculation sheet for Assay of Capsules & Tablets

ASSAY

Potency(%w/w)	99.4							
Avg Std. Area	1698985							
Factor	1.000							
Weight & Dilution	40.05	mg	100	mL				
	1.00	mL	1	mL				
	1.00	mL	1	mL		Claim	40	mg

S.No.	Product	Test Wt. (mg) & Dilution				Avg Wt. (mg)	Test Area	Assay in mg	Assay %
1	Tablet Formulation (Ibuprofen + CPM)	364.2	mg	100	mL	365	1702541	39.9807	99.95
		1	ml	1	mL				
		1	ml	1	mL				
2	Tablet Formulation ((Ibuprofen + CPM)	364.5	mg	100	mL	365	1701584	39.9253	99.81
		1	ml	1	mL				
		1	ml	1	mL				

Formula Used :-

Assay = (Test Area / 1698985) × (40.05 / 100) × (1.00 / 1) × (100 / 1) × (1 / Test Dilution) × (99.4 / 100) × Average Wt.

Fig.15 ASSAY Calculation Sheet:Ibuprofen

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Table No. 7 Analysis of marketed formulation.

Sr.no	A mount presentinmg		A mount foundin mg		%Labelclaim	
	Chlorpheniramine Maleate	Ibuprofen	Chlorpheniramine Maleate	Ibuprofen	Chlorpheniramine Maleate	Ibuprofen
1	30	40	29.8234	39.9807	99.41	99.95
2	30	40	29.8571	39.9253	99.52	99.81
Mean	_	_	_	_	99.57	99.88
SD	_	_	_	_	0.08	0.10
%RSD	_	_	_	_	0.08	0.10

Summary & Conclusion: Efforts were undertaken to establish an RP-HPLC method for the simultaneous determination of Chlorpheniramine Maleate HCl and Ibuprofen. Through various trial injections with different solvent mixtures and pH adjustments of the mobile phase, optimal separation of peaks was achieved using a mobile phase composition of Acetonitrile: 0.05% OPA (60:40). This mobile phase ratio and pH were subsequently adopted for the method. Utilizing a Waters Gradient System with UV Detector and a C18 column (250mm x 4.6 mm i.d, 5µm particle size), the developed method demonstrated retention times of 4.42 min for Chlorpheniramine Maleate HCl and 8.59 min for Ibuprofen. With a detection wavelength set at 225 nm and a flow rate of 0.7 ml/min, this method is suitable for routine quality control analysis of Chlorpheniramine Maleate HCl and Ibuprofen in both bulk drug and formulations following comprehensive method validation.

Result and Discussion: The aim of the study was to devise methodologies for the quantification of Ibuprofen and Chlorpheniramine Maleate, lacking an established official procedure for their combined estimation. The developed RP-HPLC method proved to be swift, straightforward, and cost-effective for the regular assessment of these compounds in pharmaceutical formulations. Optimization of HPLC conditions ensured adequate separation of the eluted compounds, with mobile phase and flow rate selection guided by peak parameters such as height, capacity, theoretical plates, and symmetry factor, among others.

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The HPLC system employed for method development was a Waters model 2695, integrated with Empower-3 software ensuring 21CFR compliance, and equipped with a UV detector utilizing a deuterium lamp (model 2487) and a C-18 BDS column (250mm*4.6mm*5µ). The chosen mobile phase composition, acetonitrile(OPA 0.05%) in a ratio of 60:40, reliably produced symmetrical and well-resolved peaks for Ibuprofen and Chlorpheniramine Maleate. Retention times for Ibuprofen and Chlorpheniramine Maleate were observed at 8.59 and 4.42 minutes, respectively, with a flow rate maintained at 1ml/min and UV detection set at a maximum of 225nm. The HPLC method yielded substantial quantities of high-quality data, serving as a robust and convenient analytical tool.

