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Research

Method development and validation of taurolidine and heparin in tablet dosage form by using rp-hplc.

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Abstract

A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Taurolidine and Heparin in bulk and in combined pharmaceutical dosage form. The separation of Taurolidine and Heparin was achieved within 8 minutes on an Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column using Methanol: Acetate Buffer pH-3.8 (24:76v/v) as the mobile phase. Detection was carried out using wavelength at 262nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 100-500µg/ml and 30-70µg/ml for Taurolidine and Heparin, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The proposed method is suitable for simultaneous determination of Taurolidine and Heparin in bulk and pharmaceutical dosage form.

Keywords: Taurolidine and Heparin, RP-HPLC, Validation, Robustness, ICH Guidelines.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry,

physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

MATERIALS AND METHODS

Taurolidine,Heparin-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

HPLC method development

Trails

Preparation of standard solution: Accurately weigh and transfer 10 mg of Taurolidine and Heparin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 3ml of Taurolidine and 0.5ml of Heparin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-3.8 in proportion 24:76 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and X-Bridge. Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	37ºC
Column	:	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Mobile phase	:	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Flow rate	:	1ml/min
Wavelength	:	262nm
Injection volume	:	10 µl
Run time	:	8 min

Method validation

Preparation Of Mobile Phase: Accurately measured 240 ml (24%) of Methanol and 760 ml of Acetate Buffer (76%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	37ºC
Column	:	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Mobile phase	:	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Flow rate	:	1ml/min
Wavelength	:	262nm
Injection volume	:	10 µl
Run time	:	6 min

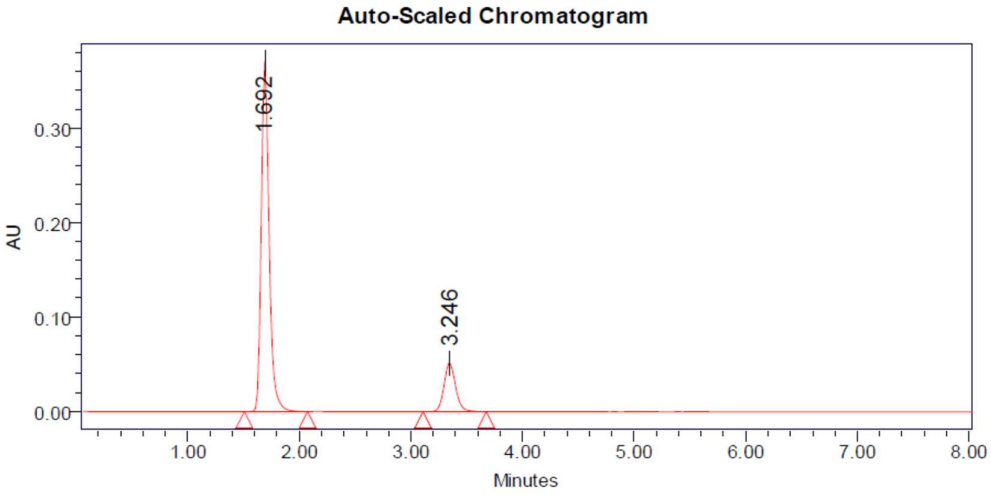

Fig 1: Optimized Chromatogram

Table 1: Observation of Optimized Chromatogram

S.No.	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Taurolidine	1.692	1658785	385669	1.69	7586	10.85
2	Heparin	3.246	425631	65245	1.58	6235	

1. *The Retention Time is decreased observed from chromatogram by increasing flow rate.*
2. *The retention time was Taurolidine and Heparin was found to be 1.692 and 3.246 respectively.*
3. *The tailing is not more than two and plate count observed is more than 2500. Pass all the system suitability parameters.*
4. *The peak shapes are good with good resolution and less Retention Time and more theoretical levels, pass the system suitability parameters.*

Optimized Chromatographic Conditions

Table 2: Shows Optimized Chromatographic conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Column :	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Flow rate :	1ml/min
Diluent	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Injection Volume	10 µl
Wavelength:	262 nm
Column temp:	37ºC
Run mode	Isocratic
Runtime	8minutes

From the above experiment it was found that Taurolidine and Heparin can effectively be analyzed by using the RP-HPLC method with Mobile phase at a flow rate of 1 ml/min and detection wave length of 262nm.
The retention time of Taurolidine and Heparin were found to be 1.692 and 3.246 minutes respectively.

Assay (Standard)

Table 3: Observation of standard Chromatogram-5

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Taurolidine	1.688	1659852	7695	1.69
2	Heparin	3.265	436598	6498	1.59

Assay (Sample)

Table 4: Observation of sample Chromatogram -3

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Taurolidine	1.694	1668985	7659	1.72
2	Heparin	3.234	436598	6347	1.61

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

System Suitability Results

1) Tailing factor obtained from the standard injection is 1.69.
2) Theoretical plates obtained from the standard injection are 7586.

Assay limits for Taurolidine and Heparin is 98-102%.

Table 5: Assay Result

Label claim	% purity
Taurolidine and Heparin	99.86%

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Azelnidipine and Telmisartan in pharmaceutical dosage form was found to be 99.89%

System Suitability Parameters

Table 6: Observation of system suitability parameters

S. No.	Parameter	Taurolidine	Heparin
1.	Retention Time (min)	1.688	3.282
2.	Theoretical Plates	7586	6235
3.	Tailing factor	1.69	1.58
4.	Area	1658768	426589
5.	Resolution	10.89	

The system suitability parameters were found to be within the specified limits for the proposed method.

Accuracy

Taurolidine

Table 7: Accuracy Observation of Taurolidine

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Heparin

Table 8: Accuracy Observation of Heparin

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

The accuracy studies were shown as % recovery for Taurolidine and Heparin at 50%, 100% and 150% the limits of % recovery should be in range of 98-102%. he results obtained for Taurolidine and Heparin were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Taurolidine 100.112%- and Heparin 100.16%. The limits of % recovery of drugs were 98-102 % and from the above results it indicates that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method. The chromatograms for accuracy shown in Figs 21-29 and results were shown in Tables 26-36.

Precision

System Precision

Table 9: Observation of System Precision

S. No	Sample Area 1	Sample Area 2
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515

Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

In the precision study %RSD was fond to be less than 2%. For Taurolidine 0.17% and Heparin 0.04% which indicates that the system has good reproducibility. For precision studies 5 replicated injections of Taurolidine and Heparin formulation was performed. %RSD was determined for peak areas of Taurolidine **and** Heparin. The acceptance limits should be not more than 2% and the results were found to be within the acceptance limits. The chromatogram of precision was showed in Figs: 29-33 results were reported in Table: 35

Ruggedness

Day 1

Table 10: Observation of Robustness Day 1

S. No.	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

- *%RSD of five different sample solutions should not more than 2.*

Day 2

Table 11: Observation of robustness Day 2

S. No.	Sample Area 1	Sample Area 2
1	1648598	415985
2	1642587	415267
3	1649852	415986
4	1648754	415265
5	1645289	415874
6	1647581	415632
Mean	**1647110**	**415668.2**
Std. Dev.	**2699.291**	**337.2106**
% RSD	**0.16388**	**0.081125**

- *%RSD of five different sample solutions should not more than 2.*

Linearity

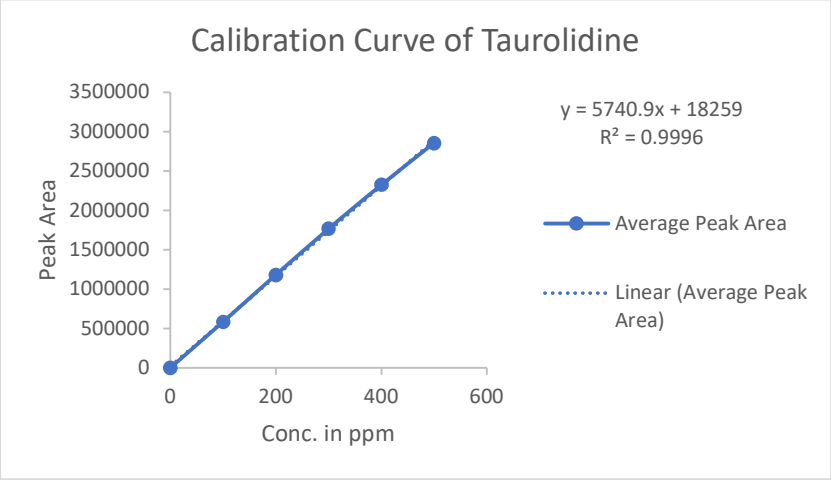

Fig 2: Calibration Curve for Taurolidine

Table 12: Linearity Observation of Taurolidine

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	100	585985
2.	II	200	1182468
3.	III	300	1768785
4.	IV	400	2326852
5.	V	500	2856874
Correlation coefficient			0.999

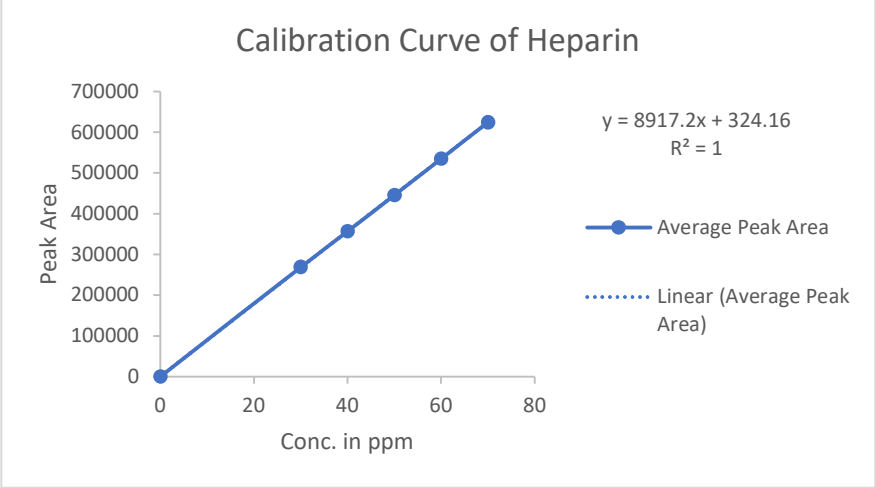

Fig 3: Calibration Curve for Heparin

Table 13: Linearity Observation of Heparin

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	30	268764
2	II	40	356958
3	III	50	445631
4	IV	60	535186
5	V	70	624698
Correlation coefficient			0.999

The linearity range was found to be 100-500 and 30-70µg/ml for both Taurolidine **and** Heparin respectively. alibration curve was plotted and correlated Co-efficient for both the drugs found to be 0.999. Hence the results obtained were within the limits. The linearity curves were shown in Figs: 52, 53. The linearity chromatograms recorded were shown in Figs: 47-51. The linearity results were reported in Table: 62, 63.

Table 14: Flow rate Observation of Taurolidine

System suitability Results for Taurolidine

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Results for actual flow rate have been considered from assay standard.

Table 15: Flow rate Observation of Heparin

System suitability Results for Heparin

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

On evaluation of the above results, it can be concluded that the variation in flow rate not affect the method significantly.

Organic Composition
Less organic Composition

Table 16: System suitability results Taurolidine

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	7269	1.61	1.868
Actual organic phase	55:45	7586	1.69	1.688
More organic phase	60:40	7496	1.64	1.675

Table 17: System suitability result Heparin

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Taurolidine and Heparin was done by RP-HPLC. The separation was optimized with mobile phase consists of Methanol: acetate buffer (pH-3.8) mixed in the ratio of 24:76%v/v. An Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column or equivalent chemically bonded to porous silica particles were used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Taurolidine and Heparin were found to be from 100-500µg/ml, 30-70µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Taurolidine and Heparin. LOD and LOQ were found to be within limits. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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