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HPLC METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF TIMOLOL MALEATE AND HYDROCHLOROTHIAZIDE IN THEIR COMBINED TABLET DOSAGE FORM

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Timolol Maleate, Hydrochlorothiazide, Reversed phase HPLC (RP- HPLC),validation

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ABSTRACT

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is able to separate macromolecules and ionic species, labile natural products, a wide variety of other high molecular- weight poly functional groups and polymeric materials Prepare a degassed mixture of methanol, water and TEA in the Ratio (85:15:0.25v/v).Drugs showed maximum absorbance both Timolol Maleate and Hydrochlorothiazide at 225nm. Timolol maleate is a beta1 and beta 2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic activity. Hydrochlorothiazide is a diuretic and antihypertensive agent. It affects the renal tubular mechanism of electrolyte reabsorption. Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. The mechanism of the antihypertensive effect of thiazides may be related to the excretion and redistribution of body sodium. Hydrochlorothiazide usually does not cause clinically important changes in normal blood pressure. The validation protocols usually followed is that defined by the International Conference on Harmonization (ICH) under their Validation for analytical procedures methodology.

1. INTRODUCTION

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is able to separate macromolecules and ionic species, labile natural products, a wide variety of other high molecular- weight poly functional groups and polymeric materials[1] HPLC is based on the mechanism of adsorption, mass distribution, ion exchange & stereo chemical interaction. Where, Normal phase HPLC consist a polar stationary phase and non-polar mobile phase, and polar mobile phase and non-polar stationary phase in case of reversed phase HPLC. RP-HPLC is most widely used technique due to Simplicity, Versatility and able to handle compound of diverse polarity and molecular mass.[2] The proposed analytical method development and simultaneous estimation of Timolol maleate and Hydrochlorothiazide and its validation by RP-HPLC is accurate, precise, simple, selective, sensitive and rapid can be applied successfully for routine analysis in quality control. The validation protocols usually followed is that defined by the International Conference on Harmonization (ICH) under their Validation for analytical procedures methodology.[5]

Introduction to drugs

1.1 TIMOLOL MALEATE:- Timolol maleate is a beta1 and beta 2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic activity.[3]

1.1.1 Description of drug

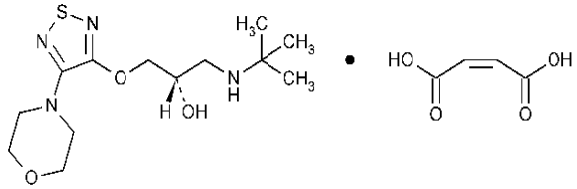

Figure no-I Structure of Timolol Maleate

1.1.2 EMPIRICAL FORMULA-$C_{13}H_{24}N_4O_3SC_4H_4O_4$

1.2 HYDROCHLOROTHIAZIDE- Hydrochlorothiazide is a diuretic and antihypertensive agent. It affects the renal tubular mechanism of electrolyte reabsorption. Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. The mechanism of the antihypertensive effect of thiazides may be related to the excretion and redistribution of body sodium. Hydrochlorothiazide usually does not cause clinically important changes in normal blood pressure.[4]

1.2.1 Description of drug

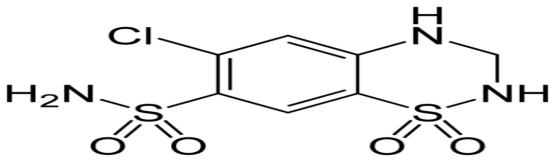

Figure no-II Structure of Hydrochlorothiazide

1.2.2 EMPIRICAL FORMULA-$C_7H_8ClN_3O_4S_2$

2. MATERIALS AND METHODS

Timolol maleate and Hydrochlorothiazide Supplied by Switzer Life sciences Pvt. Ltd., Ahmedabad.

2.1 Methods:-

2.1.1 Estimated Methodology for Timolol Maleate and Hydrochlorothiazide

2.1.1.2 Preparation of Mobile Phase

15ml of mill-Q water and 85ml of Methanol adjust the to pH 3.0

2.1.1.1 Preparation of sample solution:

Timolol Maleate and Hydrochlorothiazide are slightly soluble and freely soluble in water and methanol, so water methanol mixture was used.

3. RESULT AND DISCUSSION

3.1 HPLC method development, optimization and validation of Timolol Maleate and Hydrochlorothiazide

3.1.1 Selection of detection wavelength:

Drugs showed maximum absorbance both Timolol Maleate and Hydrocholorothiazide at 225nm.

3.1.2 Selection of Column Temperature

An inclusion of column temperature (40°C) has minimized day-to-day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

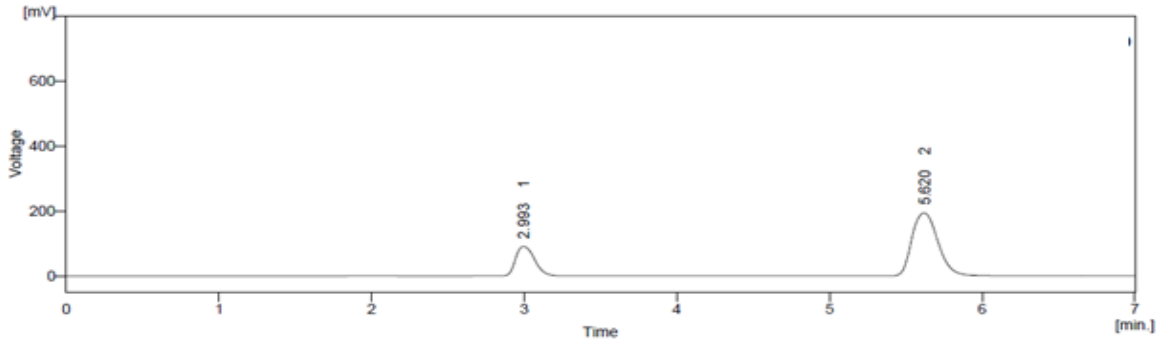

Fig. 1. Typical Chromatogram for Timolol Maleate and Hydrochlorothiazide

Column	Thermoscientific, Syncronis C_{18}, 250 mm × 4.60 mm, 5µ
Flow rate	1.0 ml/minute
Detection	AT 225nm
Injection Volume	10 µl
Run time	10 minutes
Diluent	Mobile Phase
Mobile phase Ratio	Methanol : Water : TEA (85:15:0.25v/v)
Mobile phase	Isocratic
Retention Time	2.993 (TIMO) and 5.620 (HCTZ)

Table no. I Optimized condition for Timolol Maleate and Hydrochlorothiazide

3.2 Method validation:

3.2.1 Accuracy:- The % Accuracy is within limit (98.0 – 102.0 %) with %RSD less than 2%.So the method is accurate.

Table No. II Accuracy…For Timolol

Level	Amount of Drug Added (ppm)	Amount of drug recovered (ppm)	% Recovery	% Mean	% RSD
80	8	8.09	80.99	100.19	0.87
	8	7.91	79.16		
	8	8.04	80.42		
100	10	10.09	100.91	100.19	0.80
	10	9.94	99.40		
	10	9.90	99.05		
120	12	12.05	100.46	100.33	0.62
	12	11.95	99.66		
	12	12.04	100.77		

Table No. III Accuracy…For HCTZ

Level	Amount of Drug Added (ppm)	Amount of drug recovered (ppm)	% Recovery	% Mean	% RSD
80	20	20.24	80.97	100.230	0.84
	20	19.80	79.22		
	20	20.10	80.43		
100	25	25.24	100.90	100.051	0.78
	25	24.84	99.39		
	25	24.77	99.10		
120	30	29.90	119.60	100.32	0.60
	30	30.22	120.88		
	30	29.90	119.60		

3.4.2 Precision:

Table No. IV Precision…Intraday

Set no.	Conc.ppm		Mean		SD		% RSD	
	TIMO	HCTZ	TIMO	HCTZ	TIMO	HCTZ	TIMO	HCTZ
1	10	25	654.676	2058.326	6.5949	18.7156	1.0	0.9
2	10	25						
3	10	25						

Table No. V Precision…Inter day

Set no.	Conc.ppm		Mean		SD		% RSD	
	TIMO	HCTZ	TIMO	HCTZ	TIMO	HCTZ	TIMO	HCTZ
1	10	25	664.772	2090.683	8.6022	28.0462	1.29	1.34
2	10	25						
3	10	25						

3.4.3 Linearity and Range

Table No. VI Linearity and Range Studies

Linearity Range	Stock solution to be taken in mL Stock A	Stock B	Dilute to volume (mL)with diluent	Final concentration in µg/mL (*TIMOLOL*)	Final concentration in µg/mL (*HCTZ*)
50%	5	5	100	5	12.5
75%	10	10	100	7.5	18.75
100%	15	15	100	10	25
125%	20	20	100	12.5	31.25
150%	25	25	100	15	37.5

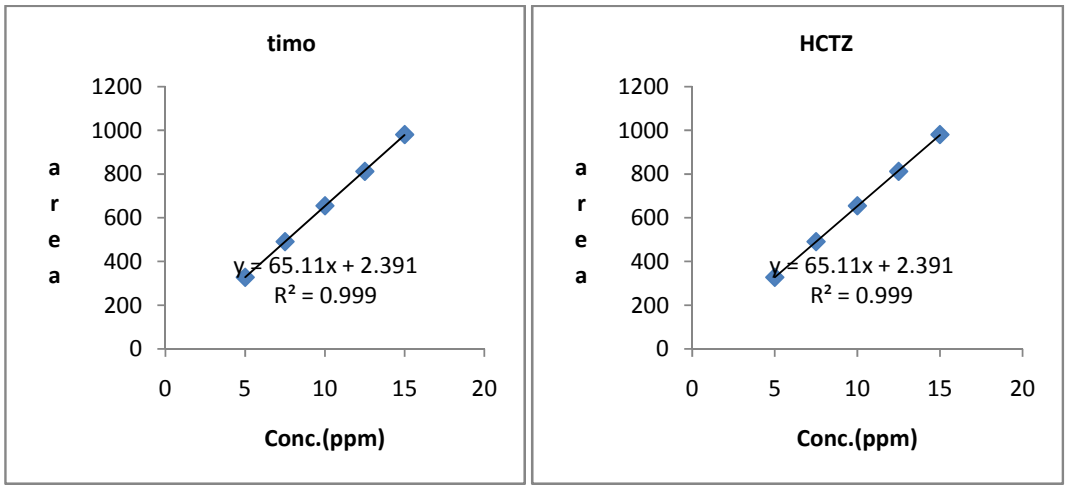

Fig. 2 Graphs of Linearity and Range Studies

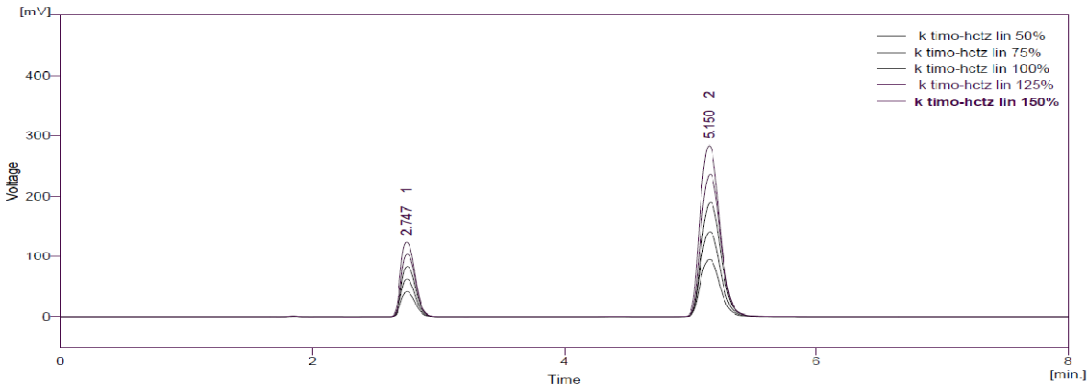

Fig. 3 Linearity Study…. Overlay Chromatogram

3.4.4 LOD and LOQ

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

LOD = 3.3 x (SD / Slope)

LOD = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

PARAMETER	TIMO	HCTZ
S.D. of Intercept	2.563	33.696
Slope of Calibration Curve	65.11	79.78
LOD (ppm)	0.129	1.393
LOQ (ppm)	0.393	4.223

Table No. VII LOD and LOQ

5. CONCLUSION

A novel RP- HPLC method has been developed for the simultaneous estimation of TIMO and HCTZ in marketed formulations. The method gave good resolution for both the drugs with a short analysis time of 10 minutes. The developed method was validated. It was found to be simple, precise and accurate. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of HCTZ and TIMO in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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