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Development and validation of a simultaneous HPLC method for Quantification of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride in Drug substance.

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Abstract
A Reverse phase method has been developed for the quantitative estimation of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride in Drug Substance.. The Quantification was carried out using RP stainless steel column ODS C18 250 x 4.6 x 5 µ L1 packing in Isocratic mode with mobile phase containing 0.03 M potassium buffer; Acetonitrile and Methanol in the ratio of 58 :21:21 and at pH3.5 ± 0.05. Flow rate was 1.0 ml/minute and the detection wavelength was set at 227 nm. The linearity was found to be in the range of 8-12 µg/ml for Atenolol and Metoprolol Tartarate and Propanolol Hydrochloride 4-6 µg/ml .The proposed method was found to be simple, precise, accurate, reproducible for the Estimation of the three drugs in presence of each other.

Key Words
Atenolol, Metoprolol Tartarate, Propanolol Hydrochloride, Method development, Validation, High performance liquid chromatography.

Introduction
Atenolol is chemically 2-[4-[(2RS)-2- hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl] acetamide It is official in U.S.P, B.P and European Pharmacopoeia . Atenolol (fig. 1) is a beta-adrenaceptor antagonist, or a commonly known as a beta-blocker. Beta-blockers are competitive inhibitors and interfere with the action of stimulating hormones on beta-adrenergic receptors in the nervous system. Beta-receptor blocking drugs were introduced in 1966, to treat, cardio vascular disorders. These drugs are efficient in cases of coronary failures, arterial hypertension & cardiac arrhythmia. Synthesis of Atenolol was reported in 1970 & pharmaceutical & clinical studies were made in 1973-74.

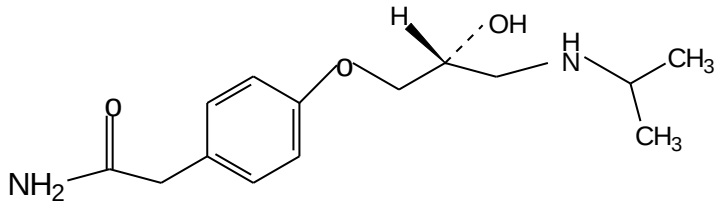

Fig. 1: Structure of Atenolol.

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Metoprolol tartrate (fig 2) USP is (±)-1-(Isopropyl amino)-3-[*p*-(2-methoxyethyl) phenoxy]-2-propanol L-(+)-tartrate (2:1) salt,

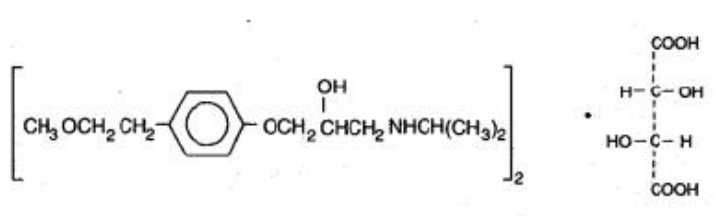
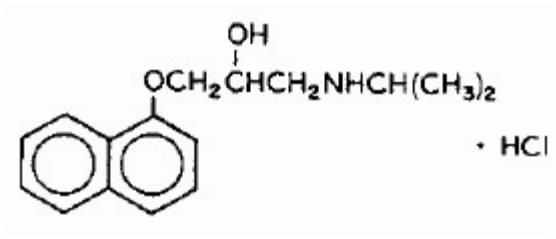


Fig. 2: Structure of Metoprolol.

Metoprolol tablets are indicated for the treatment of hypertension. They may be used alone or in combination with other antihypertensive agents. Metoprolol tartrate USP is a white, practically odorless, crystalline powder with a molecular weight of 684.82. It is very soluble in water; freely soluble in methylene chloride, in chloroform, and in alcohol; slightly soluble in acetone; and insoluble in ether. Metoprolol Tartrate is a beta-adrenergic blocking agent (beta-blocker). It works by reducing the amount of work the heart has to do (reduces chest pain) and the amount of blood the heart pumps out (lowers high blood pressure). It is also used to stabilize the heart rhythm in conditions in which the heart is beating too fast or at an irregular rhythm.

Propanolol hydrochlorides (fig. 3) a synthetic beta-adrenergic receptor blocking agent chemically described as 2-Propanol, 1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-, hydrochloride,(±)-.



$C_{16}H_{21}NO_2 \cdot HCl$

Fig. 3: Structure of Propanolol.

Propranolol hydrochloride is a stable, white, crystalline solid which is readily soluble in water and ethanol. Its molecular weight is 295.80.Literature survey reveals that there are some work related with method development of individual drug or combination of two, but no work is reported on the method development of these three drugs in presence of each other. Keeping in view above fact and as a part of our continuous work on method development by HPLC, I n this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride in Tablets. There are few papers related with method development of the drug understudy separately or in combination of some other drugs, but there is no work related with simultaneous determination of these three drugs in presence of each other[1-4]. Hence as a part of our work for method development[5-10], we describe a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination

Materials, Methods and Experimental work

Metoprolol are obtained from Cipla Ltd., Kurkumbh, and Atenolol from AMRI INDIA Pvt.Ltd Aurangabad & Propanolol Hydrochloride from Fine –Chem Laboratory Aurangabad. HPLC grade Acetonitrile, AR grade Ortho-Phosphoric acid Merck made and Bransted HPLC water were used wherever is required.

Equipments

Analysis was performed on a chromatographic system of Agilent 1100 series G1314B-UV Detector, G1310 an Isocratic Pump equipped with Auto sampler and Ezchrome software version 3.2.1

Results and Discussion

Chromatographic conditions

The chromatographic column used was RP stainless steel column ODS C18 250 x 4.6 x 5 µ L1 packing. The HPLC instrument operated at ambient temperature. The flow rate of the mobile phase was maintained at 1.0 ml/min. Detection was carried out at 227 nm and the injection volume was 5 µl .Retention time of Atenolol about 2.58 minutes. Metoprolol Tartarate 3.58 minutes Propanolol Hydrochloride 5.40. Run time 12 minutes.

Blank Preparation:

Blank was prepared, which consists of the mobile phase only

Atenolol standard solution

Accurately weighed 10 mg of Atenolol sample transferred into a 10-mL volumetric flask. 5 ml of mobile phase was added, thoroughly sonicated to dissolve and diluted to volume with mobile phase (Solution A).

Atenolol standard stock solution

1 ml of solution A taken into 100 ml volumetric flask and made up the volume with mobile phase.

Metoprolol Tartarate standard solution

Accurately weighed 10 mg of Metoprolol Tartarate taken into a 10-mL volumetric flask. 5 ml of mobile phase was added to it, thoroughly sonicated to dissolve and diluted to volume with mobile phase (Solution B).

Metoprolol Tartarate standard stock solution

1 ml of solution B taken into 100 ml volumetric flask and made up the volume with mobile phase.

Propanolol Hydrochloride standard solution

Accurately weighed 25 mg of Propanolol Hydrochloride taken into a 50-mL volumetric flask. 5 ml of mobile phase was added to it, thoroughly sonicated to dissolve and diluted to volume with mobile phase (Solution C).

Propanolol Hydrochloride standard stock solution

1 ml of solution C was taken in a 100 ml volumetric flask and made up the volume with mobile phase.

Mix standard solution

1 ml of each Solution A, Solution B & Solution C was taken in a 100 ml volumetric flask and made up the volume with mobile phase.

Method validation Parameter

Linearity

The developed method has been validated as per ICH guidelines Every 5 µL of the working Standard

solution of Atenolol & Metoprolol Tartarate in the concentration range of 8 to 12 µg/ml each were injected into the chromatographic system and Propanolol Hydrochloride in the concentration range of 4 to 6 µg/ml. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Atenolol (fig. 4), Metoprolol Tartarate (fig. 5) and Propanolol Hydrochloride (fig. 6) were obtained by plotting the peak area ratio versus the applied concentrations. As per linearity graph of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride is linear with co-efficient of Co-relation R^2 = 0.99.The linearity within the range of (80 % to 120%) the standard limits concentration is established.

Precision

Repeatability of the method was checked by injecting six replicate injections of the solution 5 µg/mL Each of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride respectively and the RSD was found to be 1.72 %, 0.31% and 0.63%. The relative standard deviation of Reproducibility and repeatability with respect to peak area and Retention time are well within the acceptance criteria. The Resolution between Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride are 7.7(table 1) which is more than 1.5 Hence the method is suitable.

Accuracy

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, Atenolol results are shown in table 2., Metoprolol Tartarate result shown in table-3 and Propanolol Hydrochloride shown in table-4. The Accuracy and recovery results obtained with all the three different concentration levels applied (80%,100% & 120%) are well within the acceptance criteria which shows that the method is accurate.

Specificity

The specificity of the method was checked for the interference of Retention time of a blank solution (without any sample) and then a drug solution of 5 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride As there was no interference of Blank, Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride in the retention time,

Resolution

The resolution between the peaks of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride should not be less than 1.5.The method was found to be specific and also confirmed. See Chromatogram of Specificity fig.7.

Limit of Detection & Limit of Quantitation

Limit of Quantitation (LOQ)

The smallest level of analyte that gives a measurable response & standard deviation and slope for the peak area responses were calculated and represented in table 5, 6and 7.

Solution Stability

Changes in the area response of the test material will be monitored throughout the validation. Each solution stability solution shall be injected after 8 hours and 40 hours (table 8).

Conclusion

The developed method was validated in terms of accuracy, Linearity and precision. A good linear relationship was observed for Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride. in the concentration ranges of 8–12 µg/mL for Atenolol and Metoprolol Tartarate and 4-6 µg/mL for Propanolol Hydrochloride. The correlation coefficient for Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride was found to be 0.99 .Selectivity experiment showed that there is no interference or overlapping of the peaks either due to diluents with the main peak of Atenolol , Metoprolol Tartarate and Propanolol Hydrochloride. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total runtime required for the method is only 12 mins for eluting Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Atenolol, Metoprolol Tartarate and Propanolol Hydrochloride.

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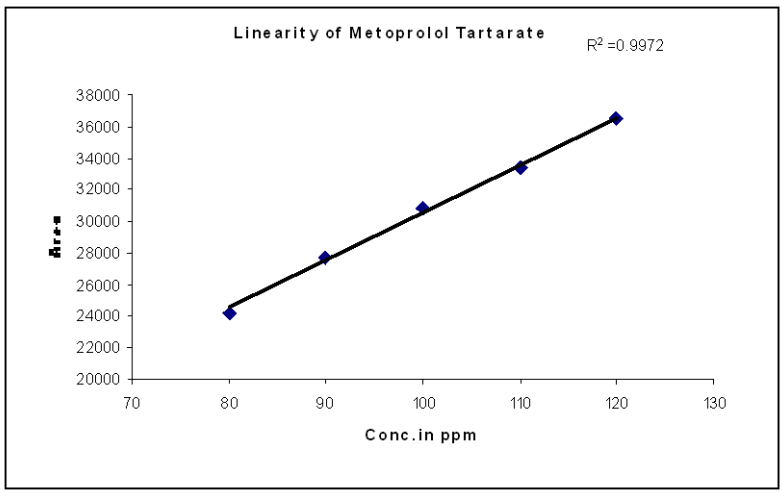

Fig. 5: Linearity graph of Metoprolol Tartarate.

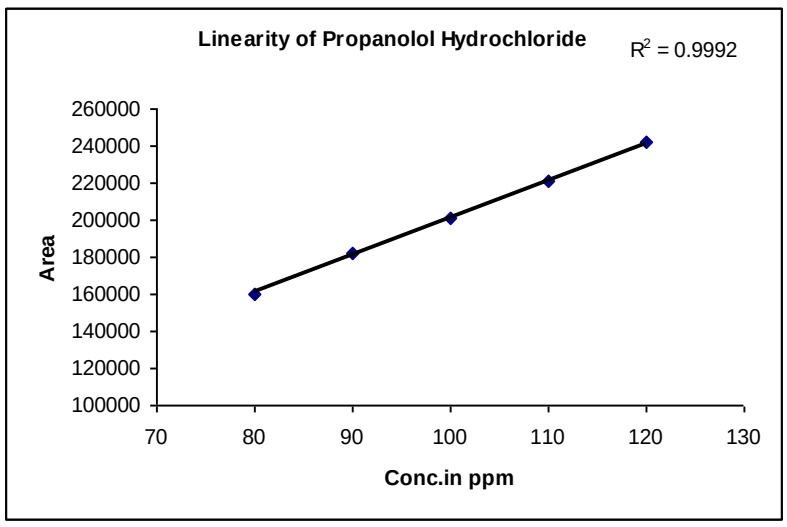

Fig. 6: Linearity graph of Propanolol Hydrochloride.

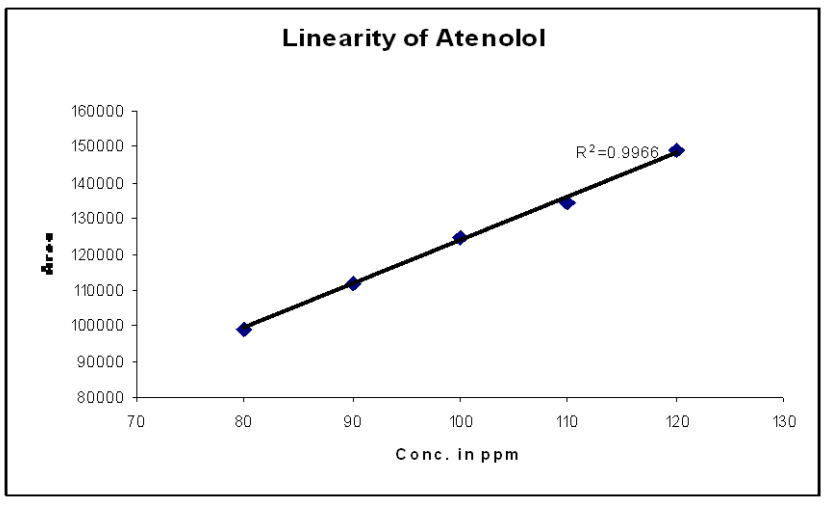

Fig. 4: Linearity graph of Atenolol.

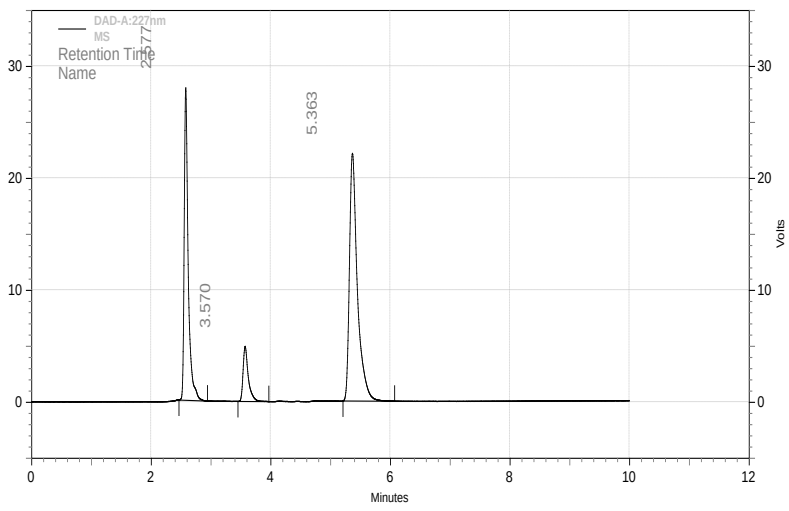

Fig 7: Chromatogram showing the resolution of the three drug**s.**

Table 1: Precision study of the three drugs.

Sr. No	Atenolol	Metoprolol Tartarate	Propanolol Hydrochloride	Remark.
1	127168	30413	209706	Within Limits
2	124342	30444	209050	
3	124648	30216	208049	
4	126898	30310	209813	
5	129711	30468	211850	
6	124224	30331	208677	
Average	126165	30363	209524.2	
RSD%	1.72	0.31	0.63	

Table 2: Atenolol Accuracy of the method.

Conc. Level	No. of Inj.	Area of un spiked sample	Area of spiked sample	Corrected area	Mean	% RSD	Accuracy %	Recovery%
80%	1	126165	223213	97048	95577	1.66	75.76	94.7
	2	126165	220057	93892				
	3	126165	221958	95793				
100%	1	126165	244153	117988	118402	1.46	93.85	93.85
	2	126165	243085	116920				
	3	126165	246465	120300				
120%	1	126165	273537	147372	147196	0.42	116.67	97.22
	2	126165	273862	147697				
	3	126165	272685	146520				
%RSD of all three levels							= 1.62	

Table 3: Metoprolol Tartarate Accuracy of the method.

Conc. Level	No. of Inj.	Area of unspiked sample	Area of spiked sample	Corrected area	Mean	% RSD	Accuracy%	Recovery%
80%	1	30363	52136	21773	21973	1.41	72.37	90.46
	2	30363	52013	22330				
	3	30363	52181	21818				
100%	1	30363	59089	28726	28727	0.66	94.61	94.61
	2	30363	59281	28918				
	3	30363	58900	28537				
120%	1	30363	65188	34825	35104	0.73	115.61	96.34
	2	30363	65691	35328				
	3	30363	65523	35160				
%RSD of all three levels							= 3.22	

Table 4: Propanolol Hydrochloride Accuracy of the method.

Conc. Level	No. of Inj.	Area of un spiked sample	Area of spiked sample	Corrected area	Mean	% RSD	Accuracy%	Recovery%
80%	1	209524	371790	162266	164868	1.47	78.69	98.36
	2	209524	376573	167049				
	3	209524	374813	165289				
100%	1	209524	407887	198363	198337	0.38	94.66	94.66
	2	209524	408607	199083				
	3	209524	407089	197565				
120%	1	209524	455688	246164	244881	0.80	115.80	96.50
	2	209524	455369	245845				
	3	209524	452159	242635				
%RSD of all three levels							= 1.58	

Table 5: RSD % of each concentration Level of Atenolol.

Inj. No.	RSD % of each concentration Level					
	Conc. In ppm	Sample ID	Peak area response	Mean	%RSD NMT 2.0%	Standard Deviation
1.	1.0ppm	Rep1	21800	21549	1.62	S.D = 21274
		Rep2	21697			
		Rep3	21151			
2.	2.0 ppm	Rep1	42594	43141	1.10	
		Rep2	43363			
		Rep3	43466			
3.	3.0ppm	Rep1	63295	64096	1.81	Slope =21273
		Rep2	62657			
		Rep3	63228			
		Rep2	65111			
		Rep3	64971			
			65314			

Table 6: RSD % of each concentration Level of Metoprolol Tartarate.

Inj. No.	RSD % of each concentration Level					
	Conc. In ppm	Sample ID	Peak area response	Mean	%RSD NMT 2.0%	Standard Deviation
1.	1.0ppm	Rep1	2728	2731	0.99	S.D = 2717
		Rep2	2760			
		Rep3	2706			
2.	2.0 ppm	Rep1	5423	5443	0.51	
		Rep2	5475			
		Rep3	5432			
3.	3.0ppm	Rep1	8157	8166	0.79	Slope =2717
		Rep2	8071			
		Rep3	8130			
		Rep2	8197			
		Rep3	8262			
			8180			

Table 7: RSD % of each concentration Level of Propanolol Hydrochloride.

Inj. No.	RSD % of each concentration Level					
	Conc. In ppm	**Sample ID**	**Peak area response**	**Mean**	**%RSD NMT 2.0%**	**Standard Deviation**
1.	0.5 ppm	Rep1	40078	47326	0.82	S.D = 46978
		Rep2	47774			
		Rep3	47128			
2.	1.0 ppm	Rep1	94482	94379	0.27	
		Rep2	94563			
		Rep3	94093			
3.	1.5 ppm	Rep1	940242	141282	0.57	Slope =46978
		Rep2	140554			
		Rep3	141236			
		Rep4	141294			
		Rep5	142045			
		Rep 6	142323			

Table 8: Solution stability data.

Hrs	Atenolol			Metoprolol				Propanolol			
	RT	**T.P**	**T.F**	**RT**	**T.P**	**T.F**	**Reso**	**RT**	**T.P**	**T.F**	**Reso**
16	2.58	9125	1.45	3.58	9567	1.38	7.91	5.41	9323	1.48	9.87
24	2.58	9104	1.39	3.58	9524	1.42	7.88	5.41	9201	1.47	9.84
32	2.58	9058	1.43	3.58	9387	1.45	7.84	5.42	9119	1.45	9.80
40	2.58	8772	1.54	3.57	9203	1.51	7.67	5.37	9048	1.56	9.59

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