Current Pharma Research
Vol. 1, Issue 1, October-December 2010

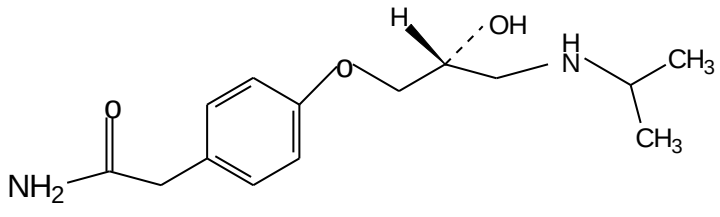
Simultaneous Determination of Atenolol and Metformin Hydrochloride by Reverse Phase High Performance Liquid Chromatography

[2]Sayyed Hussain Rashid R. Munjewar, [*1]Mazahar Farooqui
[1]P.G.Dept. of Chemistry, Sir Sayyed College, Aurangabad. (M.S.) India.
[2]Incharge P.G. & Research Center, Maulana Azad College, Aurangabad. (M.S.) India.

Abstract
A Reverse phase HPLC method has been developed for the quantitative estimation of Atenolol and Metformin hydrochloride in combination simultaneously. The quantification was carried out using RP stainless steel column (C18, 250 mm x 4.6 mm, 5 µm BDS Hypersil) in Isocratic mode with mobile phase containing 1.0 g of Octane-1-Sulphonic acid sodium salt and 0.4 g of Tetra – n - butyl ammonium hydrogen sulphate in a mixture of 20 volumes of Tetrahydrofuran, 180 volumes of methanol and 800 volumes of 3.4 g/L solution of Potassium dihydrogen phosphate and pH adjusted to 3±0.2 using dilute ortho-phosphoric acid. Flow rate 1.0 ml/minute and the detection wavelength was set at 226 nm and the linearity was found to be in the range of 3-20 µg/ml.The proposed method was found to be simple, precise, accurate, reproducible for the estimation of Atenolol and Metformin Hydrochloride.

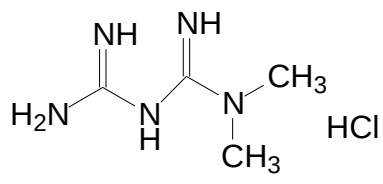
Key words
Atenolol, Metformin Hydrochloride, Method development,Validation, High performance liquid chromatography

Introduction
Atenolol (Atn) is chemically 2-[4-[(2RS)-2-hydroxy-3- [(1-methylethyl) amino] propoxyphenyl] acetamide It is official in U.S.P, B.P and E P. Atn 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. Atn is available in oral dosage forms viz. tablet, syrups and capsules. In the tablet dosage this drug is commonly available in three different strengths i.e. 25, 50 and 100 mgs. Atn is also available as an antihypertensive treatment in the form of compound preparations with diuretics.



***Corresponding Author:**
mazahar_64@rediffmail.com

Metformin Hydrochloride (mfm) is chemically N, N-Dimethyl biguanide hydrochloride**.** It is used alone or with other medications, including insulin, to treat type 2 (noninsulin-dependent) diabetes. Mfm helps to control the amount of glucose (sugar) in blood. It decreases the amount of



Metformin Hydrochloride

glucose from the food and the amount of glucose made by liver. Mfm also increases the body's response to insulin. If high blood sugar is not treated, it can cause serious problems, such as heart failure, blood vessel disease, eye disease or kidney disease. Mfm controls diabetes, but does not cure it. Mfm hydrochloride is available in oral dosage forms viz. tablet, and an extended-release (long-acting) tablet. In the tablet dosage this drug is commonly available in different strengths i.e. 250 mg, 500 mg and 850 mg. Although Atn and mfm combination in dosage form is rare, most of the patients take these two drugs to control sugar and blood pressure. There are

few work reported for single drug separately[1-3] but almost no work is related for the method development of Atn and mfm hydrochloride in combination. The methods for simultaneous determination of Atn and other drugs such as losartan[4], uerapamil[5], hydrochlorothiazide[6], amlodipine[7].

Materials and Methods

Working standards of Atenolol & Metformin Hydrochloride were obtained from Albany Molecular Research Inc. India Pvt .Ltd Aurangabad. HPLC grade Methanol, Tetrahydrofuran, Octane-1-Sulphonic acid sodium salt, Tetra – n - butyl ammonium hydrogen Sulphate and Potassium dihydrogen phosphate Merck grade and Bransted HPLC water. *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

Result and discussion

Chromatographic conditions: The chromatographic column used was a C18, 250 mm x 4.6 mm, (BDS Hypersil) i.d: stainless steel with 5 µm particle size **(**Make: Thermo scientific). The HPLC instrument operated at ambient temperature. The flow rate of the mobile phase was maintained at 1ml/min. Detection was carried out at 226 nm and the injection volume was 10 µl .Retention time of Atenolol about 8-9 minutes & Metformin Hydrochloride 6-7 minutes. Run time 30 minutes. Test Solution for Atn The substance to be examined (25 mg) was dissolved in 20 ml of the mobile phase and dilute to 25.0 ml with the mobile phase. It was mixed and labeled as solution A. Standard solution for Atn**:** From solution a (1 ml) was transferred in to a 100-mL volumetric flask and made up with mobile phase. Test Solution for Mfm HCl The substance to be examined (25 mg) was dissolved in 20 ml of the mobile phase and diluted to 25.0 ml with the mobile phase. Mixed well and labeled this solution as solution B. Standard Solution for Mfm HCl**:** solution B (1ml) was transferred in to a 100-mL volumetric flask and made up with mobile phase. Mix Standard Solution: With the help of pipette 1.0 ml of solution A & 1.0 ml of solution B was taken into a 100-mL volumetric flask and made up the volume with mobile phase. It was mixed and labeled as mix standard solution.

Method validation Parameter

Linearity The developed method has been validated as per ICH guidelines[8-10]. Every 10 µl of the working standard solution of Atn & Mfm Hydrochloride in the concentration range of 3 to 20 µg/ml. Each was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Atn and Mfm Hydrochloride were obtained by plotting the peak area ratio versus the applied concentrations of drug (Fig-1 & Fig-2)

As per linearity graph of Atn & Mfm HCl is linear with co-efficient of Co-relation R^2 = 0.99.The linearity within the range of (30 % to 200%) the standard limits concentration is established. Precision Repeatability of the method was checked by injecting six replicate injections of the solution 10 µg/ml Each of Atn and Mfm Hydrochloride respectively and the RSD was found to be 0.42% and 0.11%. 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 Atn and Mfm HCl are 6.9 which is more than 1.5 Hence the method is suitable for Equipment cleaning analysis. 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, Atn results are shown in Table 1. & Mfm HCl Result has shown in Table-2. The Accuracy and recovery results obtained with all the three different concentration levels applied (75%,100% & 120%) are well within the acceptance criteria i.e. the percent recovery should be not less than 70%., 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 10 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Atn and Mfm Hydrochloride. As there was no interference of blank, Atn & Mfm Hydrochloride in the retention time,

Resolution: The resolution between the peaks of Atn & Mfm HCl should not be less than a 1.5 .The

method was found to be specific and also confirmed. See Chromatogram of Specificity Fig.3.

Limit of Detection (LOD) & Limit of Quantitation (LOQ): For LOQ a sample was prepared in the same manner as in the linearity section, from this 3ml is diluted to 10 ml by using mobile phase and used for LOD. Limits of detection and Quantitation were determined from multiple injections of standard solutions that give signal to noise ratios of approximately 3:1 and 10:1 respectively. The data was evaluated for precision and the average of the signal to noise ratio is reported in table 3.

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 every six hours for 48 hours.

Mix standard stock solution for Accuracy**:** Dilute 1.0 ml of the test solution A & 1.0 ml of the test solution B to 10.0 ml with the mobile phase. Mix well and label this solution as Mix standard stock solution for Accuracy. See Table 4.

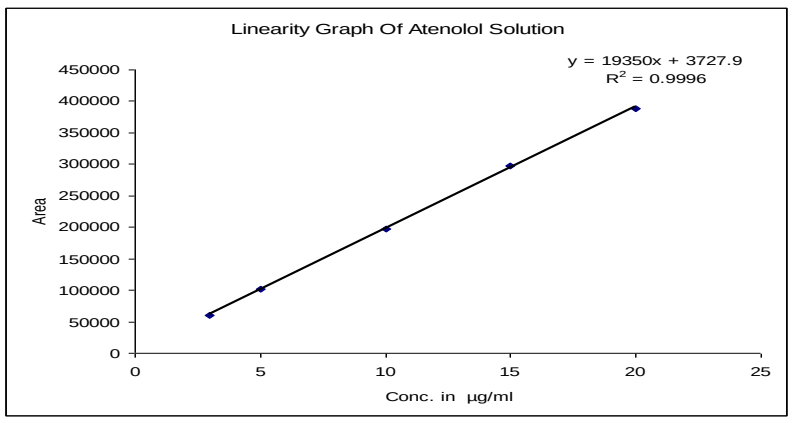


Fig. 1: Linearity graph of Atenolol

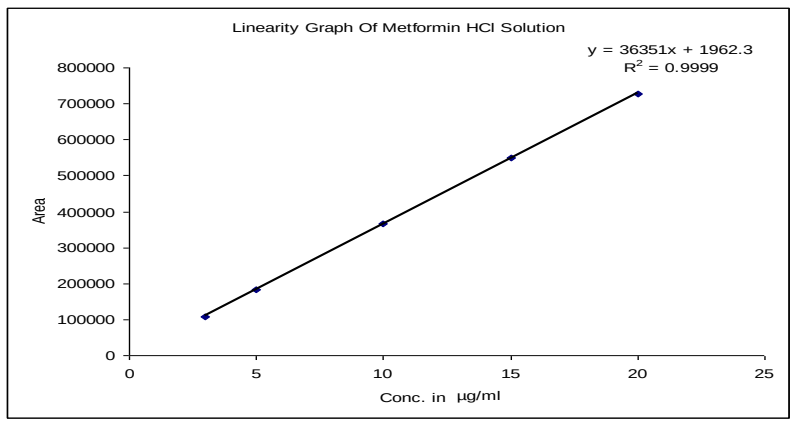


Fig.2: Linearity graph of Metformin Hydrochloride

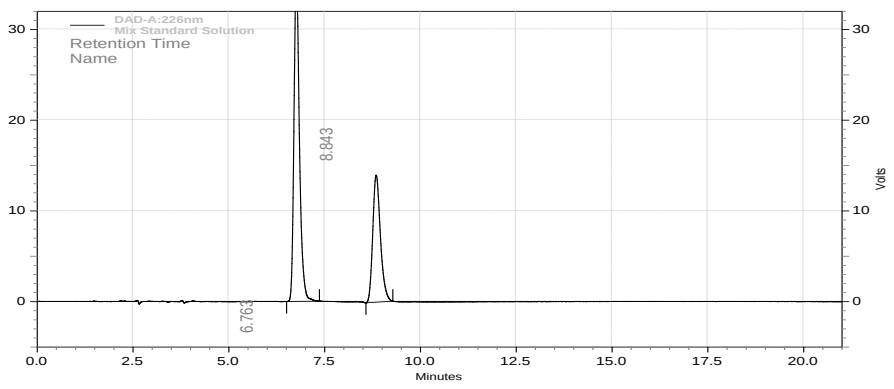


Fig.3: Specificity Chromatogram

Table 1: Atenolol Accuracy & Recovery Results

Conc. Of solution in %	Atenolol				Recovery NLT 70%
	Sample ID	Area	Accuracy	Recovery	
75%	Rep 1	140500	66.11	88.15	Within limit
	Rep 2	140510	66.12	88.16	Within limit
	Rep 3	139983	65.87	87.83	Within limit
100%	Rep 1	193142	90.88	90.88	Within limit
	Rep 2	193898	91.24	91.24	Within limit
	Rep 3	192504	90.58	90.58	Within limit
	Rep 4	192328	90.50	90.50	Within limit
	Rep 5	193259	90.94	90.94	Within limit
	Rep 6	192421	90.54	90.54	Within limit
125%	Rep 1	239191	112.55	90.04	Within limit
	Rep 2	239827	112.85	90.28	Within limit
	Rep 3	239532	112.71	90.17	Within limit

Table 2: RSD % of each concentration Level of Metformin HCl

Conc. Of solution in %	Metformin HCl				Recovery NLT 70%
	Sample ID	Area	Accuracy	Recovery	
75 %	Rep 1	260343	61.97	82.63	Within limit
	Rep 2	261610	62.27	83.96	Within limit
	Rep 3	262111	62.39	83.19	Within limit
100%	Rep 1	345515	82.24	82.24	Within limit
	Rep 2	344650	82.03	82.03	Within limit
	Rep 3	344361	81.96	81.96	Within limit
	Rep 4	345183	82.16	82.16	Within limit
	Rep 5	344783	82.07	82.07	Within limit
	Rep 6	345781	82.30	82.30	Within limit
125%	Rep 1	390931	93.05	74.44	Within limit
	Rep 2	390026	92.83	74.26	Within limit
	Rep 3	390303	92.90	74.32	Within limit

Table 3: RSD % of each concentration Level of Atenolol

Conc. of solution in %	Atenolol		Metformin HCl	
	Peak area	Signal to -noise	Peak area	Signal to -noise
LOD 10%	22528	14.38	33370	30.54
LOQ 30%			**LOQ 30%**	
Injection-1	63049	55.09	118021	115.63
Injection-2	63165	99.69	118044	116.51
Injection-3	63279	47.52	117952	119.31
Injection-4	63762	85.03	118401	121.16
Injection-5	62650	50.80	118253	65.368
Injection-6	64075	48.02	117919	124.33
Average	63330	Average	118098.33	
RSD% ≤ 15%	0.81	RSD% ≤ 15%	0.16	

.

The %RSD of peak area response for Atn LOQ Solution = 0.81 % & for Mfm HCl= 0.16%.

Table 4: Solution stability Data

Inj. Details	Atenolol	Metformin HCl
	Area	Area
Initial	170020	325527
6 hrs	169228	325871
12 hrs	168392	326218
18 hrs	168661	326326
24 hrs	169178	326247
30 hrs	169759	325497
36 hrs	168686	326862
42 hrs	169744	327556
48 hrs	169631	327326
Average.	169255.44	326381.11
RSD%	0.34	0.23

Conclusion

The developed method was validated in terms of accuracy, Linearity and precision. A good linear relationship was observed for Atn & Mfm Hydrochloride in the concentration ranges of 3–20 µg/ml. The correlation coefficient for Atn and Mfm 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 Atn and Mfm 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 30mins for eluting both Atn & Mfm Hydrochloride. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Atn and Mfm Hydrochloride.

Acknowledgement

The researchers are grateful to A.Vaidhyanatan, Director Quality Assurance Amri India Pvt Ltd for providing gift samples of Atenolol & Metformin Hydrochloride.

References

1. Naveen Kumar, Nishat Verma, Omveer Songh, Naveen Joshi and Kanwar Gaurav Sigh; E-J Chem 7(3) 962-966, 2010
2. D Sridharan, A Thenmozhi, S Sundaranadavalli; Asian J Pharm Clin Res 3(2) 92-94, 2010
3. Luis renato Pires de Abreu, Silvana Aparecida Calafatti de Castro, and Jose Pedrazzoli Jr; AAPS Pharm Sci2003, 5(2) article 21,(http:// www.pharmsci.org)
4. K.Abdus Salim, D.Boopathy, P. Perumal, Int J Pharm tech res 2(1) 471-474, 2010
5. A.Bright T.S.Renuga Devi,S.Gunashekharan Int.J chem. Tech res 2(2) 2010
6. A.Thomas, U.Chavan, R Nanda, L.Kothapally, S.Uagdale, S.Dhigle, S.Despande, Acta Chromatographica 22 (2)219-226, 2010
7. R.K.Bhrama, M.A. Islam, M.Ahmed, M.I.Ibnewaheb, R.Islam, A.Khan, Hossian, B.M.Rahmab Pak J Pharma sci 20(4) 274-279, 2007.
8. ICH-Q2B Validation of Analytical procedure: Methodology International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 1996BS 7501 EN 45001. General Criteria for the operation of Testing Laboratories 1989.
9. ICH, Q2A validation of analytical procedure, Methodology International Conference on Harmonization, Geneva, October 1994.
10. Validation of Analytical procedure Methodology, ICH Harmonized tripartite guidelines,1996,1-8

******