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Der Pharmacia Lettre, 2010, 2(6): 244-251
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ISSN 0975-5071
USA CODEN: DPLEB4

RP-HPLC method development for the determination of Atenolol related substance in bulk drug

Rashid R Munjewar[1], Mazahar Farooqui [2]and Sayyed Husain[1]

[1]P.G.Dept of Chemistry, Sir Sayyed College, Aurangabad, (MS) India
[2]Post graduate & Research Centre, Maulana Azad college, Aurangabad, (M.S.) India

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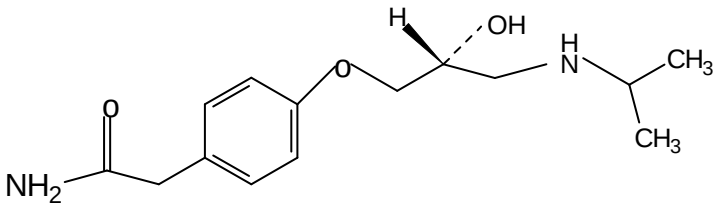
ABSTRACT

A simple Reverse phase HPLC method has been developed for the quantitative estimation of Atenolol Related substance in pure drug. The quantification was carried out using stainless steel column C_{18} 125 mm x 4.0 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. The method was linear in the range of 1.6-3.2 µg /ml. The proposed method was found to be simple, precise, accurate, and reproducible for the estimation of Atenolol Related substance.

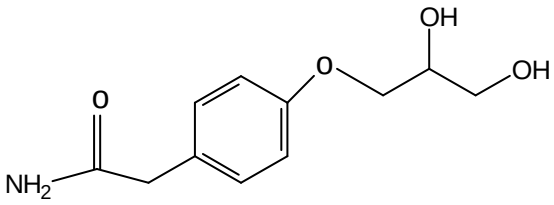
Key words: Method development, validation, High performance liquid chromatography, Atenolol Related substance.

INTRODUCTION

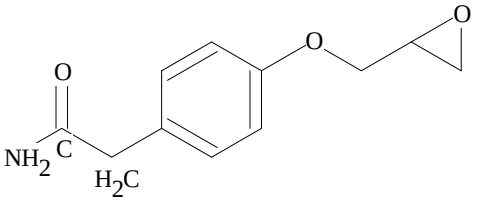
Atenolol is chemically 2-[4-[(2RS)-2- hydroxy-3- [(1-methylethyl) amino] propoxy] phenyl] acetamide.



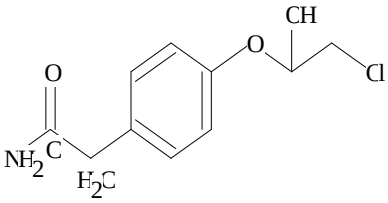
The related impurities are given as below



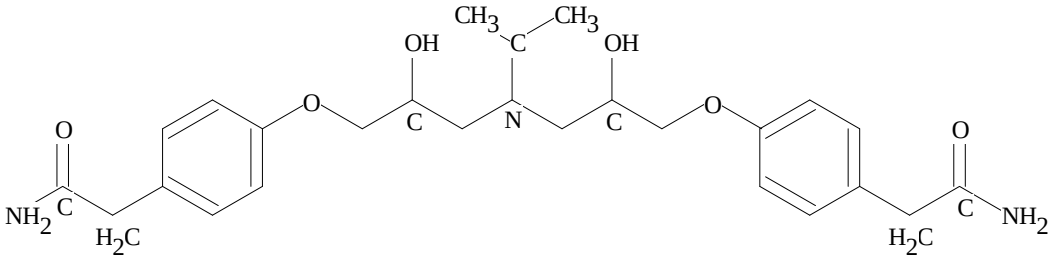
Impurity B: 2-[4-[(2RS)-2,3-dihydroxypropoxy] phenyl] Acetamide



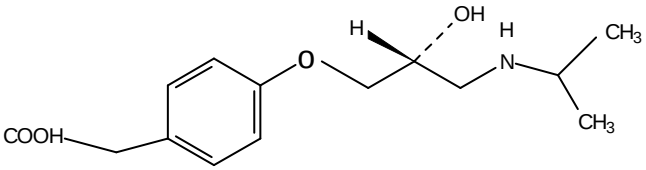
Impurity C: 2-[4-(2,3-epoxypropxy) phenyl] Acetamide



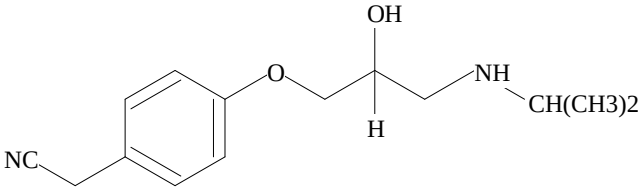
Impurity D: 2-[4-[(2RS)-3-chloro-2-hydroxy-propoxy] phenyl] acetamide



Impurity E: 2,2'-[(1-methylethyl) iminobis (2-hydroxypropan-3,1-diyloxy-4,1-phenylene)]-diacetamide



Impurity F : 2-[4-(2RS)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl] acetic acid



Impurity H: 2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] phenyl] acetonitrile

It is official in IP, USP, BP and EP[1-4]. Hence this HPLC method was developed which is simple, accurate, and precise for the determination of related substance in Atenolol bulk drug [5-8]. Atenolol 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.

Atenolol works by competing for receptor sites on cardiac muscle. This slows down the strength of the heart's contractions and reduces its oxygen requirements and the volume of blood it has to pump. Hypertension may be treated with these drugs because of their ability to increase the diameter of the blood vessels.

Atenolol 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 mg.

MATERIALS AND METHODS

*Chemicals***:** Working standards of Atenolol 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 were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. And Milli -QRO water purification system.

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

Chromatographic conditions: The chromatographic column was used C18, 125 mm x 4.0 mm, 5µm(BDS Hypersil) Make: Thermo scientific Part No.28205-254630. The HPLC instrument operated at ambient temperature. The flow rate of the mobile phase was maintained at 0.6 ml/min. Detection was carried out at 226 nm and the injection volume used was 10 µl throughout the experiment. Run time was adjusted to five times the retention time of Atenolol

Test solution preparation: A working test solution was prepared by dissolving 50 mg in 20 ml of the mobile phase and diluted to 25 ml with mobile phase, From this 1 ml of solution was diluted to 100 ml using mobile phase. Again 1 ml of diluted test solution was diluted to 10 ml using same mobile phase and labeled as reference a solution, Alenolol sample (5 mg) containing impurities was dissolved in 2.5 ml of the mobile phase. It was mixed well and labeled as reference-b solution. The chromatogram obtained with reference-b solution was used to identify the peaks due to impurities. The retention time for impurity are given in table 1.

Table 1 Retention time for different components

Sr No	Componeant	Retention time in minutes
1	Atenolol	8.0
2	Impurity B	0.3
3	Impurity J	0.7
4	Impurity I	0.8
5	Impurity E	2.0
6	Impurity F	3.5

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

The objective of the study was to develop simple and reproducible isocratic HPLC method using readily available chemicals and reagents. Mobile phase was 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 o- phosphoric acid was used. Separation of all impurities using a C_{18} 125 mm x 4.0 mm, 5 µm. BDS Hypersil column at a flow rate of 0.6 ml/min was acheied. .A typical chromatogram of seperation of all the impurities is shown in Fig.1

Validation of Method:

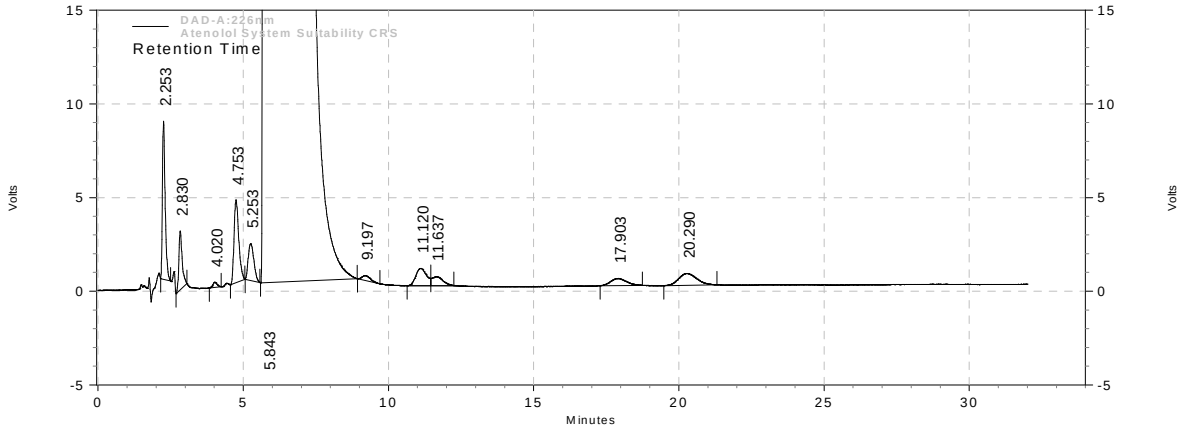
System suitability

System suitability criteria specified in test method EP 6.4 and BP 2009 was performed to provide information on the performance of the method. System suitability determination is required for all experiments carried out during the validation process. All the system suitability factors are well within the specified acceptance criteria. The Resolution between Impurity J and Impurity I are 1.54 and 1.52 which more than 1.4 Hence the method is suitable for the determination of related substance of Atenolol. Tailing Factor should not be more than 2.0 in the present study the tailing factor was for Impurity J= 1.35, Impurity I=1.21& Atenolol peak =1.39.Tailing factor of each component (Impurity J, Impurity I & Atenolol).Theoretical plate of Atenolol peak was 4047.

Specificity

The specificity of the method was checked for the non interference of impurities. There was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the analysis of Atenolol bulk drug.

Fig.1 Specificity chromatogram of Atenolol Related substance
Atenolol System Suitability CRS



DAD-A:226nm Results								
Pk #	Name	Retention Time	Width	Area	Area %	Theoretical plates (USP)	Resolution (DAB)	Asymmetry
1	IMP. B	2.253	0.34	56216	0.093	2683	0.00000	1.53
2	Unknown	2.830	0.37	24790	0.041	3282	3.20851	1.39
3	Unknown	4.020	0.41	2784	0.005	3492	5.19479	1.11
4	IMP.J	4.753	0.50	49930	0.082	4051	2.61657	1.33
5	IMP.I	5.253	0.50	26435	0.044	3374	1.51302	1.29
6	ATENOLOL	5.843	3.33	60421381	99.598	667	0.98358	4.68
7	Unknown	9.197	0.78	5234	0.009	4837	4.90594	1.35
8	IMP.E	11.120	0.82	23974	0.040	3662	3.04500	0.00
9	IMP.E (Pair of Peak)	11.637	0.79	12245	0.020	1791	0.00000	0.00
10	Unknown	17.903	1.45	14640	0.024	4809	0.00000	1.17
11	IMP.F	20.290	1.82	27340	0.045	4809	2.22071	1.17
Totals				60664969	100.00			

The specificity of the method was checked for the non interference of impurities. in the analysis of a blank solution (without any sample) and then a Related substance (Atenolol system suitability EP CRS) solution of 2 mg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of all related impurities i.e. Impurity B ,Impurity I, Impurity E ,Impurity F & Impurity J. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the analysis of Atenolol bulk drug.

*4.3 Precision System precision***:** System precision was determined using six injections for Retention time and area of all impurity. Observed RSD for area and retention are well within the limits. RSD Limits for area was NMT 2.0 % and for retention time RSD NMT 1.0 %. Repeatability of the method was checked by injecting replicate injections of the solution 2 mg/mL of Atenolol and the RSD of related impurities as follows.

Atenolol = 0.26, Impurity B=0.61, Impurity J=0.40, Impurity I=0.89,
Impurity E= 0.53 and Impurity F= 0.41.

*Method precision***:** Method precision was done by using working standard of different six preparations and inject. The related substances from the six determinations should be within the specified limits. %RSD results obtained for the impurities of Atenolol from the six determinations should be not more than 10.0%.

Intermediate precision: Three batches of previously analyzed Atenolol samples to be analyzed for related Substances analysis, by second analyst on a different day or HPLC system using the same HPLC conditions. Determine the % relative standard deviation (%RSD) and resolution between the impurities of all observed impurities. %RSD results between two analysts should not be more than 20.0%.The resolution between Impurity J & Impurity I should not less than 1.4

Observed Indudival test results and multiple samples relative standard deviation are well within the acceptance criteria.

4.4 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, results are shown in Table 2 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.

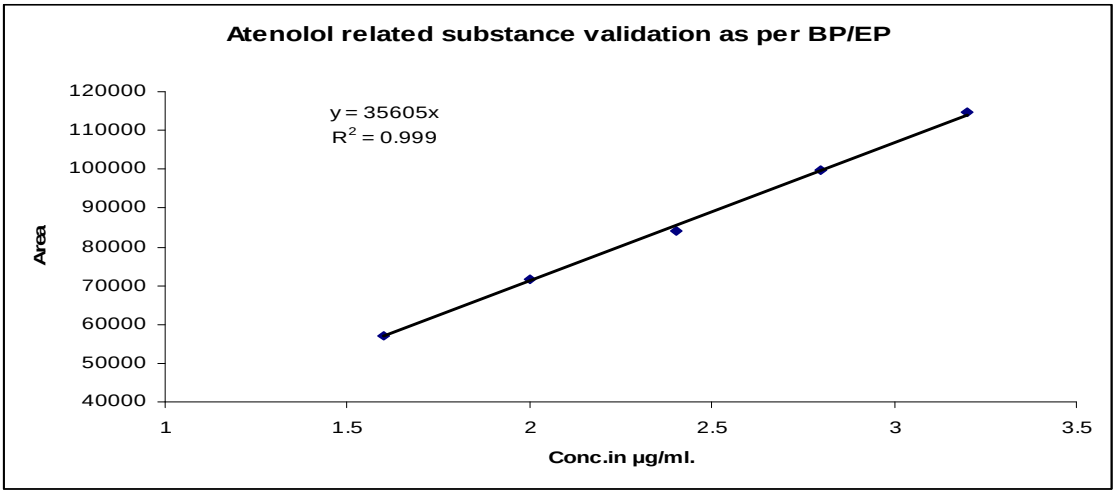
4.5 Linearity and Range The developed method has been validated as per ICH guidelines[16]. Inject 10 µL of the working Standard solution of Atenolol in the concentration range of 1.6 µg/ml to 3.2 µg/ml each were injected into the chromatographic system. To demonstrate the linearity of detector response of each component over the range from 80% to 160% of the nominal column loading employed during analytical procedure. Triplicate injections of each of the solutions are to be injected on to the chromatographic system and the average peak areas to be measured. The data should be presented in a table to show the linearity of the detector response to be acceptable, which provides the regression coefficient and data for linearity check in addition to the graph. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Atenolol were obtained by plotting the peak area ratio versus the applied concentrations of Atenolol.

Table-2 Accuracy & Recovery of Atenolol

Conc. Level	No. of Inj.	Area of impurity in un spiked sample	Area of impurity in spiked sample	Corrected area	Mean	%RSD	Accuracy %	Recovery %
80%	1	3815	74688	70873	70793.7	0.71	83.11	103.88
	2	3815	75072	71257				
	3	3815	74066	70251				
100%	1	3815	89107	85292	85408	0.13	100.26	100.26
	2	3815	89235	85420				
	3	3815	89327	85512				
120%	1	3815	107888	104073	104506.7	0.41	122.68	102.24
	2	3815	108322	104507				
	3	3815	108755	104940				
%RSD of all three levels							= 1.77	

Linearity of Atenolol related substance Validation

The range of Analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity



Linearity was determined in the range of 1.6 µg/ml to 3.2 µg/ml .The correlation coefficient value found 0.9995.Typically the regression equation for the calibration curve was found to be Y=35605 X -337.34.

4.5 Limit of Detection & Limit of Quantitation Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 *∂/S* and 10 *∂/S*, respectively as per ICH guidelines, where *∂* is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. Details of LOD & LOQ see in Table-2.

Based on the standard deviation of the response and slope forms the calibration curve of the analyte in the range of detection level I,e (1 ppm,1.5 ppm,2.0 ppm,2.5 ppm& 3.0 ppm) .The limit of detection for Atenolol is found as 3.87 ppm and the limit of Quantitation is found as 11.72 ppm.

4.6 Ruggedness The degree of reproducibility of results obtained under a variety of conditions, such as different days, different analysts, different instruments, environmental Conditions, operators and materials.

Table-3 RSD % of each concentration Level of Atenolol

Inj. No.	RSD % of each concentration Level				
	Conc. In ppm	**Peak area response**	**Mean**	**RSD NMT 2.0%**	**Standard Deviation**
1.	1.0ppm	33482 33475 33385	33447	0.16	39878
2.	1.5 ppm	50635 50551 50711	50632.3	0.16	
3.	2.0ppm	67801 67443 67774	67672.67	0.29	
4.	2.5 ppm	84989 84507 84481	84659	0.33	
5.	3.0 ppm	101157 101609 101500	101422	0.23	

Table-4 Analyst –I HPLC ID No. AMRI/QC/HPLC/06
Analyst –II HPLC ID No. AMRI/QC/HPLC/04

Details of Impurity	B.No.AM20110046			B.No.AM20110047			B.No.AM20110048		
	A-I	A-II	RSD NMT10%	A-I	A-II	RSD NMT10%	A-I	A-II	RSD NMT10%
Imp.BNMT 0.20%	0.072	0.068	2.86	0.093	0.088	2.76	0.13	0.13	0.00
Imp.I NMT 0.15%	0.047	0.045	2.17	0.042	0.037	6.33	0.042	0.036	7.69
Imp.FNMT 0.15%	0.11	0.098	5.8	0.082	0.071	7.19	0.023	0.019	9.52
Imp.GNMT 0.15%	0.035	0.038	4.11	0.023	0.02	6.98	0.023	0.021	4.55
Unspecified Imp. NMT 0.10%	0.049	0.043	6.52	0.044	0.037	8.64	0.083	0.07	8.50
Total Imp. NMT 0.5%	0.31	0.29	NA	0.28	0.25	NA	0.30	0.28	NA
Resolution Between Impurity J and Impurity I. Analyst-I =1.61									
Resolution Between Impurity J and Impurity I. Analyst-II =1.75									

To demonstrate the ruggedness of the method, three batches of Atenolol to be analyzed for related Substances, on two different HPLC system, by two Different analysts by using the same HPLC column. Tabulated results of each Analyst separately see in Table-4.

Three different batches of Atenolol analysed on two different system /Instrument by two different analyst are close to each other .The % RSD of results between two analyst is well within the acceptance criteria shows that the method applied for determination of related substance is rugged.

CONCLUSION

A simple specific, linear, precise and accurate Reverse phase method has been developed and validated for Quantitative determination of Atenolol Impurities. The method is very simple and specific as all peak were well separated from each other which makes it especially suitable for routine quality control analysis work.

Acknowledgement
The researchers are grateful to A.Vaidhyanatan, Director Quality Assurance AMRI INDIA PVT.LTD for providing gift samples of Atenolol.

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