Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2013, 4(2):76-84

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Development and validation of liquid chromatographic method for atenolol and its related substance

Nandini R. Pai* and Swapnali Suhas Patil

Department of Chemistry, D. G. Ruparel College, Mahim, Mumbai, Maharashtra, India

---

ABSTRACT

A simple isocratic, rapid and sensitive high performance liquid chromatographic method has been developed for quantitative determination of Atenolol and its three process related impurities. The method has been validated for determination of related substance in Atenolol. Analytical Column: A stainless steel column 150 mm long, 4.6 mm internal diameter filled with octadecylsilane chemically bonded to porous silica particles of 5 µm diameters (Use Chromatopak Peerless basic C 18, 5 µ, 150 mm x 4.6 mm). Pump mode : Isocratic wavelength: 226 nm, Flow rate : 1mL per minute, Injection volume : 50 µl, Run time: 30 min. Specificity, system suitability, linearity, precision, ruggedness, robustness along with limit of quantification and limit of detection have been carried out for Atenolol and its process impurities.

Keywords: HPLC, Atenolol, related impurities, validation

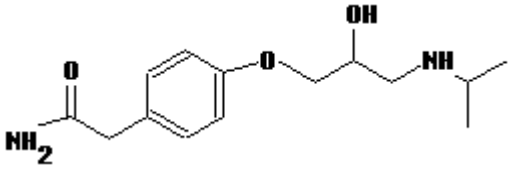
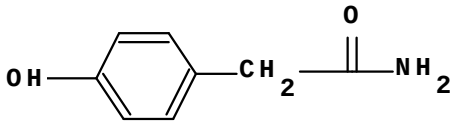
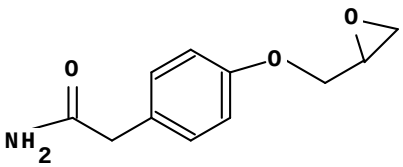
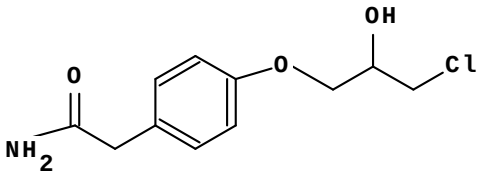
INTRODUCTION

Atenolol is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers a class of drugs used primarily in cardiovascular diseases. It's a off-white powder with chemical name benzeneacetamide,4 -[2'-hydroxy-3'-[(1- methylethyl) amino] propoxy].

Atenolol is in a group of drugs called beta-blockers. Beta-blockers affect the heart and circulation (blood flow through arteries and veins).Atenolol is used to treat angina (chest pain) and hypertension (high blood pressure). It is also used to treat or prevent heart attack.[12][13]

For the development and validation for liquid chromatographic method of Atenolol and its process impurities various parameters such as specificity, system suitability, accuracy, linearity, precision, ruggedness, robustness, limit of quantification and detection are determined according to USP and ICH guidelines..[9][10]

The impurities or unreacted precursors in Atenolol are following.

Compound	Structure
Atenolol	
(Imp A)	
(Imp C)	
(Imp D)	

MATERIALS AND METHODS

Reagents and Chemicals

Atenolol was prepared and well characterized with the help of various spectroscopic and chromatographic techniques. This was used as reference standard for further work. The reference standard samples of impurity A, C and D which are intermediates are obtained. They were characterized using various spectroscopic and chromatographic techniques and are taken as standards for further experiments. Analytical reagents such as sodiumhepta sulphonate was purchased from S.D.fine Chemicals Ltd.; tetra butyl ammonium hydrogen sulphate, tetrahydrofuran, methanol Potassium dihydrogen phosphate were purchased from Merck., phosphoric acid was purchased form Ranchem and HPLC grade Water from Thomas Baker.

Preparation of Solutions, Chromatographic Conditions and System Suitability Parameter-

Chromatographic Conditions-

Mobile phase was prepared by weighing accurately 1.0 g of Sodium heptasulphonate R and 0.4 g of tetra butyl ammonium hydrogen sulphate into a clean dry 1000 mL glass beaker. 20 volume of tetrahydrofuran, 180 volume of Methanol and 800 volume of 3.4 g/l solution of potassium dihydrogen phosphate R are added, pH adjusted to 3.0 with phosphoric acid R and filtered through 0.45µm membrane filter paper.

Analytical Column used was stainless steel column 150 mm long, 4.6 mm internal diameter filled with octadecylsilane chemically bonded to porous silica particles of 5 µm diameter (Inertsil C-18, 5 µ, 150 mm x 4.6 mm). Pump mode was isocratic. Wavelength used for analysis was 226 nm, flow rate was 1mL per minute, injection volume 50 µl and run time 30 min.[3]

Standard solution Preparation-

1. About 20 mg of **Atenolol Reference standard** was accurately weighed and transferred in 100 mL volumetric flask, dissolved in sufficient mobile phase and diluted to the mark. This solution was further diluted with mobile phase to obtain required ppm solutions. (200 ppm)
2. About 10 mg of **Impurity A** was dissolved with mobile phase upto the mark in 100 ml volumetric flask (100 ppm).
3. About 10 mg of **Impurity C** was dissolved with mobile phase upto the mark in 100 ml volumetric flask (100 ppm).
4. About 10 mg of **Impurity D** was dissolved with mobile phase upto the mark in 100 ml volumetric flask (100 ppm).

Method Validation:
The proposed method for estimation of related substances of Atenolol is validated as per the United States Pharmacopoeia and ICH guidelines .[1][2][11]

1) Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of component which may be expected to be present. Typically these might include impurities, degradation, matrix etc.[4]

In other words Specificity is a measure of relative separation of the individual components. The test is thus more useful for showing separation / estimation of impurity peaks from the principal peak.

Specificity is carried out to demonstrate that individual expected known peaks of the impurities are completely separated from Atenolol peak. Hence we conducted the test in the method validation program of the related substances / impurities.

Atenolol (100 ppm) and related impurities namely Impurity A, Impurity C and Impurity D (10 ppm each) are injected in combination to develop a chromatograph. (fig 1)

(2) Linearity
The Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample

The linearity of an analytical method is its ability to elicit test results that are directly, or by well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

From above mentioned stock solution, series of dilutions are made of different concentration levels and injected for Atenolol and its process related impurities.

(3) Precision-
The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. To ensure analytical system is working satisfactorily to give precise results, 100ppm solution of Atenolol and 10 ppm solution of its impurities were injected 5 times. RSD for retention time and area are calculated. [5][6]

Limit RSD: +/-2.0% [98.0% to 102.0%].

The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(4) Ruggedness-
Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. It is a degree of exactness of a measurement to its true value.
The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(5) Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found

From stock solution of 200 ppm further dilutions are made for the analysis of Atenolol and stock solution of 100 ppm of its process impurities were further diluted for analysis.

(6) Robustness-
Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides a indication of its reliability during normal usage

This was carried out by change in flow rate, change in mobile phase composition, change in wavelength and change in pH. It is observed that method is unaffected by small changes in experimental conditions and complies the robustness.

(7) Limit of Quantitation -
Limit of quantification is the lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions. Limit of quantification is calculated from signal to noise ratio. To determine limit of quantification, sample blank is injected first and noise is integrated at different intervals at different retention time near the peak of interest.

The quantitation limit (QL) is expressed as: $QL = \frac{10.0\delta}{S}$

Where,
δ = the standard deviation of the response
S = Slope of the calibration curve

(8) Limit of detection-
The detection limit is characteristic of limit test. It is the lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. Limit of detection is calculated from signal to noise ratio. To determine limit of detection, sample blank is injected and noise is integrated at different retention time near the peak of interest. It was observed that signal to noise ratio must be 3:1 as given in ICH guideline.[7][8][9]

The detection limit (DL) is expressed as: $DL = \frac{3.3\delta}{S}$

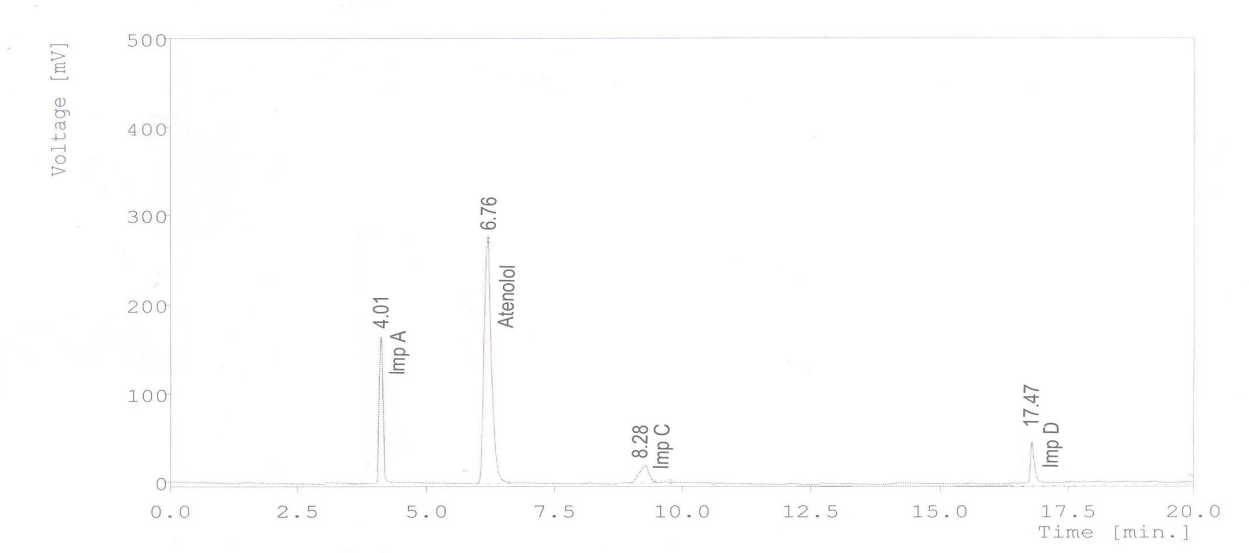
Where,
δ = the standard deviation of the response
S = Slope of the calibration curve

RESULTS

Main objective of this analytical method development was to separate Atenolol from Imp A, Imp C and Imp D. Different Mobile phases and different stationary phases were tried but effective chromatographic separation was achieved with a stainless steel column 0.15 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel. Flow rate of mobile phase was adjusted to 1.0 ml/min. Mobile phase was prepared by dissolving 1.0 g of sodium heptasulphonate and 0.4 g of tetrabutylammonium hydrogen sulphate in 1litre of a mixture of 20 volumes of tetrahydrofuran , 180 volumes of methanol and 800 volumes of a 3.4 g/l solution of potassium dihydrogen phosphate. pH was adjusted to 3.0 with phosphoric acid. UV detector was set at 226 nm with column temperature 30°C. Peak shapes and separation of Atenolol and impurities were as follows:

Sr.No	Name of API & its Impurity	Retention Time
1	Blank	--------
2	Atenolol (100 ppm)	6.76
3	Impurity A (10 ppm)	4.01
4	Impurity C (10 ppm)	8.28
5	Impurity D (10 ppm)	17.47

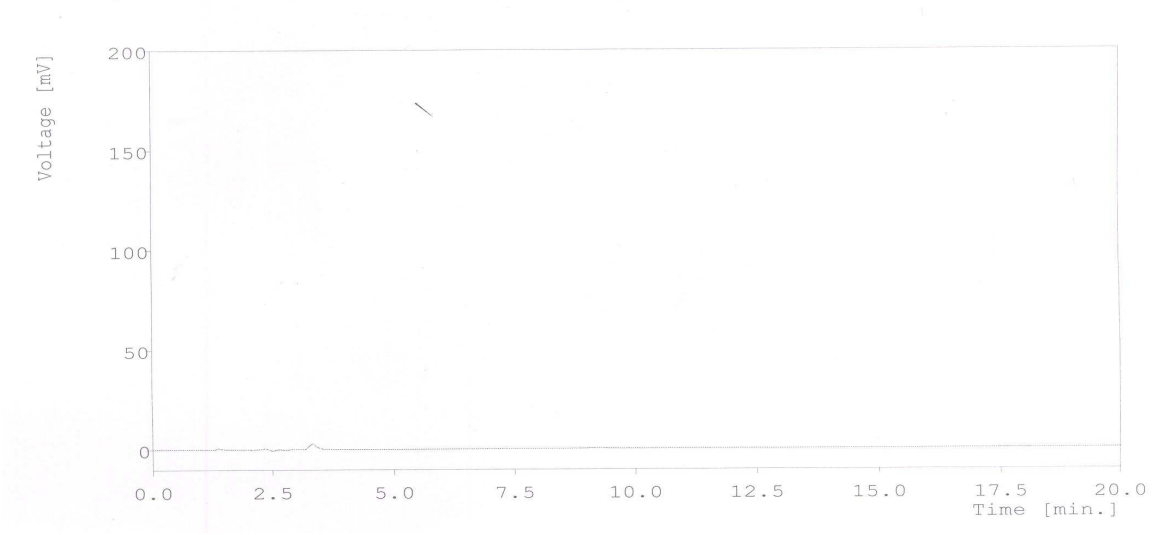
Fig 1 Typical chromatogram of specificity



Specificity and selectivity studies results

Selectivity of the method was performed by separately injecting individual impurities and none of these impurities were seen to interfere with the Atenolol peak with minimum resolution of 2.0 between any two peaks. No interference of blank was observed (fig 2)

Fig 2 Typical chromatogram of Blank



Linearity

Linear calibration plot for the method was obtained over the calibration ranges tested.

Stock solution: Dissolve 20 mg of **Atenolol** in 100 ml volumetric flask with mobile phase (200 ppm)

Volume of Stock Solution (ml)	Final dilution (ml)	Final Conc. (µg/ml)	Area			Mean Area	Relative standard deviation (%)
			1	2	3		
4	10	80	7646.06	7654.25	7660.08	7653.46	0.10
4.5	10	90	9327.99	9312.37	9335.42	9325.26	0.12
5	10	100	10242.03	10279.97	10236.98	10257.32	0.18
5.5	10	110	11235.44	11268.34	11306.84	11270.20	0.31
6.0	10	120	12283.47	12317.40	12365.39	12322.08	0.33
						Average	0.28
						Slope	112.82
						*Co-rell	0.989

Process Impurities Imp A C and D

Sr.No	Conc in ppm	Mean Area of Imp A	Mean Area of Imp C	Mean Area of Imp D
1	8	1223.98	678.97	203.15
2	9	1353.39	736.68	513.2
3	10	1470.80	874.79	739.87
4	11	1564.85	1079.95	817.83
5	12	1889.86	1247.56	1192.013
*RSD		0.43	0.90	1.15
Slope		154.32	148.04	228.23
*Co-rell		0.966	0.969	0.964

Co-rell : Correlation Coefficient
RSD : Relative standard Deviation

Precision

Five replicate injections of Atenolol (100 ppm) and process Impurity A, Impurity C and Impurity D each of 10 ppm was made.

The results for each impurity are summarized in the following table:

Injection Details	Standard Deviation	Relative standard Deviation
Atenolol	23.0	0.22
Impurity A	2.93	0.10
Impurity C	16.86	1.75
Impurity D	15.25	1.78

Limit of Detection and Limit of Quantifications
The results of each impurity are summarized in the following table:

	Average Standard Deviation	Slope of Calibration Curve	Detection Limit	Quantitation Limit
Atenolol	1.25	96.74	0.04	0.12
Impurity A	0.47	152.17	0.01	0.03
Impurity C	0.753	103.03	0.024	0.07
Impurity D	1.39	78.18	0.05	0.1

Accuracy
Results of percentage recovery of Atenolol and its process impurities are as follows:

Sr.No	Atenolol	Impurity A	Impurity C	Impurity D
1	100.27 %	99.78 %	99.52 %	99.84 %

Robustness
The robustness study was carried out by-
1. Changing mobile phase composition,
2. Mobile phase pH,
3. Wavelength and
4. Flow rate.

Sample of Atenolol and its process impurities were analyzed for the same. It was observed that the method is unaffected by small changes in the experimental conditions and the resolution is more than 2.0.which confirms robustness of the method.

Accuracy (Recovery study):
Standard Stock solution
Atenolol WS 10mg /50ml dilute with mobile phase (200 ppm)
Atenolol sample 10 mg /100ml with mobile phase (100 ppm)

Average Area of Atenolol (100 ppm) = 8986.98

Amount of Atenolol In Std stock Solution [Initial] ppm	Amount of Atenolol added (Sample Stock solution) ppm	Avg. Area of Atenolol	Amount Recovered in ppm	% Recovery = Amt found x 100 Amt added
100	10	9891.28	109.93	99.93
100	20	10760.21	119.73	99.77
100	30	11525.92	128.25	98.65
100	40	12730.95	141.65	101.1
100	50	13743.23	152.92	101.9
Mean				100.27
Standard Deviation				1.25
% Relative Standard Deviation				1.25

[Limit: RSD for recovery levels – NMT 2.0%]

Recovery of impurity A, C and D form Atenolol :
The recovery of added impurity form the drug (Atenolol) was performed at 80% to 120 % of impurity Concentration ie 8.0 , 10, 12 ppm. Each level was done in triplicate

[Limit: 98.0% to 102.0%]

% Recovery = Area of Impurity A or C or D with Atenolol X 100
Area of Impurity A or C or D without Atenolol

Standard stock solution: Atenolol 100 mg/ 50ml dilute with mobile phase (2000 ppm).
Sample stock solution: Impurity A 10.0 mg/100ml Diluted with mobile phase (100 ppm)

Recovery study (details of dilutions)

Sample	Amount of sample stock solution taken (ml) Impurity A or C or D	Amount of standard stock solution added (ml) Atenolol	Final dilution (ml)
1	0.8	0.0	10
2	0.8	5.0	10
3	1.0	0.0	10
4	1.0	5.0	10
5	1.2	0.0	10
6	1.2	5.0	10

Results of recovery study of (Impurity A)

Recovery level (%)	Conc. of Atenolol (µg/ml)	Amount of Imp A (µg/ml)	Area of Atenolol	Area of Impurity A	Mean Area of Impurity A	% Recovery
80	------	8.0	-----	1208.81	1212.93	99.85%
			-----	1220.78		
			-----	1209.20		
	100.0	8.0	10499.21	1207.24	1211.18	
			10492.21	1218.46		
			10482.21	1207.86		
100	-------	10.0	-------	1511.02	1516.16	99.59%
			-------	1525.98		
			-------	1511.50		
	100.0	10.0	10436.78	1495.76	1510.05	
			10425.72	1512.56		
			10425.72	1521.85		
120	-------	12.0	-------	1813.22	1819.25	99.90%
			-------	1831.17		
			-------	1813.38		
	100.0	12.0	10446.35	1812.49	1817.61	
			10429.76	1829.15		
			10435.81	1811.20		

Results of recovery study of (Impurity C)

Recovery level (%)	Conc. of Atenolol (µg/ml)	Amount of Imp C (µg/ml)	Area of Atenolol	Area of Impurity C	Mean Area of Impurity C	% Recovery
80	------	8.0	-----	350.46	352.41	99.41%
			-----	353.12		
			-----	353.67		
	100.0	8.0	8901.12	348.47	350.36	
			8909.45	350.51		
			8911.39	352.12		
100	-------	10.0	-------	443.24	445.13	99.36%
			-------	448.37		
			-------	443.78		
	100.0	10.0	8898.11	439.34	442.30	
			8881.93	446.18		
			8895.94	441.39		
120	-------	12.0	-------	532.00	532.70	99.81%
			-------	530.92		
			-------	535.18		
	100.0	12.0	8879.45	531.26	531.71	
			8885.36	529.81		
			8889.14	534.17		

Robustness:

The method was tested for capacity to remain unaffected by small variation in method parameters, such as

1. Change of Flow rate
2. Change of Wavelength
3. Change of pH
4. Change of Mobile phase concentration

For Atenolol and all its impurities(Impurity A , Impurity C and Impurity D) studied at different concentrations, the assay results demonstrated the reliability of the method, as expressed in the values of mean % recovery, standard deviation and relative standard deviation, and linearity correlation coefficient.

Results of recovery study of (Impurity D)

Recovery level (%)	Conc. of Atenolol (µg/ml)	Amount of Imp D (µg/ml)	Area of Atenolol	Area of Impurity D	Mean Area of Impurity D	% Recovery
80	------	8.0	-----	602.43	602.39	99.44
			-----	599.40		
			-----	605.36		
	100.0	8.0	9044.79	605.52	605.75	
			9049.82	603.14		
			9051.78	608.59		
100	-------	10.0	-------	752.13	751.53	99.38
			-------	746.85		
			-------	755.63		
	100.0	10.0	9043.69	756.91	756.15	
			9039.51	751.21		
			9047.59	760.36		
120	-------	12.0	-------	906.66	909.65	99.83
			-------	909.01		
			-------	913.30		
	100.0	12.0	9051.36	908.30	911.21	
			9049.14	910.11		
			9055.32	915.23		

Robustness of Atenolol and its process Impurity

Concentration of Atenolol is (100 ppm) and Impurities (10 ppm)

Change of flow Rate

Flow Rate	Atenolol (100ppm)		Impurity A (10 ppm)		Impurity C (10 ppm)		Impurity D (10 ppm)	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
0.8	9.26	11667.21	5.19	1942.15	11.17	899.91	24.05	826.38
	9.26	11657.67	5.19	1929.31	11.18	894.59	24.10	844.77
	9.25	11633.43	5.19	1925.48	11.18	899.91	24.12	861.36
1.0	7.35	10271.38	4.40	1469.98	9.58	865.28	19.37	729.54
	7.36	10248.88	4.40	1475.36	9.52	871.32	19.38	721.92
	7.36	10270.53	4.41	1479.52	9.54	869.15	19.38	725.12
1.2	5.12	9562.36	3.33	969.92	6.87	462.70	14.53	304.40
	5.12	9559.21	3.34	964.70	6.81	465.90	14.54	308.40
	5.13	9531.97	3.34	944.20	6.85	462.63	14.58	313.60

Change of Wavelength

Wave length	Atenolol		Impurity A		Impurity C		Impurity D	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
224	9.33	9670.98	4.23	1610.02	9.04	320.97	19.85	806.20
	9.35	9682.95	4.24	1615.90	9.09	323.98	19.87	818.63
	9.34	9695.17	4.24	1618.64	9.05	325.20	19.88	831.68
226	7.35	10248.88	4.40	1469.98	9.58	865.28	19.37	729.54
	7.36	10271.38	4.42	1475.36	9.57	871.32	19.35	721.92
	7.34	10270.53	4.43	1479.52	9.87	869.15	19.35	725.12
228	8.31	9235.98	4.21	1376.57	9.11	328.71	19.80	651.63
	8.32	9251.39	4.23	1368.79	9.11	323.99	19.82	643.11
	8.31	9263.26	4.22	1380.05	9.13	327.03	19.82	637.55

Change of pH

Wave length	Atenolol		Impurity A		Impurity C		Impurity D	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
2.8	6.80	9812.76	4.08	1144.85	8.41	230.84	17.34	699.97
	6.81	9824.04	4.09	1138.44	8.41	231.44	17.35	680.50
	6.80	9809.19	4.09	1131.18	8.42	235.11	17.35	693.70
3.0	7.35	10271.38	4.40	1469.98	9.58	865.28	19.37	729.54
	7.35	10248.88	4.42	1475.36	9.58	871.32	19.38	721.92
	7.35	10270.53	4.43	1479.52	9.59	869.15	19.38	725.12
3.2	6.90	7823.56	4.15	1443.33	8.65	219.86	18.62	664.87
	6.91	7829.75	4.15	1448.97	8.66	222.71	18.63	684.59
	6.91	7833.59	4.16	1454.14	8.66	229.10	18.63	693.33

Change of Mobile phase Concentration

Com-position	Atenolol		Impurity A		Impurity C		Impurity D	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
25 vol of THF 175 vol of MeOH	5.87	9469.61	3.90	995.72	7.97	257.42	17.04	589.22
	5.86	9482.40	3.91	1000.04	7.98	261.20	17.04	582.71
	8.87	9475.28	3.91	1004.16	7.97	265.37	17.05	579.90
20 vol of THF 180 vol of MeOH	7.35	10271.38	4.40	1469.98	9.58	865.28	19.37	729.54
	7.36	10248.88	4.41	1475.36	9.57	869.15	19.38	721.92
	7.36	10270.53	4.41	1479.52	9.57	879.32	19.37	725.12
15 vol of THF 185 vol of MeOH	7.76	6306.84	4.22	1086.54	9.25	232.18	19.46	599.04
	7.76	6314.86	4.23	1083.12	9.24	235.03	19.45	601.38
	7.77	6320.22	4.43	1077.77	9.25	238.18	19.45	622.70

CONCLUSION

Isocratic HPLC method has been developed for Atenolol in presence of its process related impurities. The proposed method is simple accurate, precise, specific and the method is suitable for use of routine quality control of drug, due to following reasons

A. Analytical method is found to be specific as proved by injecting known amount of component into the chromatogram.
B. Limit of quantification and limit of detection for Atenolol and process related
Impurities have been established and are found to be within the range.
C. Analytical method is found to be linear over a specific range.
D. Analytical method is found to be precise and accurate.
E. Analytical method is found to be robust.

Hence method is completely evaluated for its specificity linearity, precision, accuracy, robustness, ruggedness, limit of quantification and detection.

REFERENCES

[1] X. Zuo, F. Wang , P. Xu, R. Zhu, H. Li, *Chromatogr*., **2006**, 64, 387.
[2] K. Masanori, M. Yasuo, H. Yukihiro, O. Takahiko, *J. Chromatogr. B*., **2005**, 822, 294.
[3] K. Kirschbaum, M. Müller, G. Zernig, A. Saria, A. Mobascher, J. Malevani, C. Hiemke, *Clinical Chem*., **2005**, 51, 1718.
[4] H. Hui-Ching, L. Chin-Hung, L. Tsuo-Hung, H. Tsung-Ming, C. Hsien-Jane, W. Yu-Chun, *Chromatogr. B.*, **2007**, 856, 57.
[5] V. Shah, K. Kamal, D. Shrikant, J. Iain, P. Jerome, Y. Avraham, L. Thomas, C. ViswanathanC. Edgar, R. McDowall, A. Kenneth, S. Sidney, *J. Pharm. Sci.*, **1992**, 81, 309.
[6] Nandini R. Pai, *Der Pharmacia Sinica*, 2012, 3 (5):526-535
[7] International Conferences on Harmonization Q3A (R2). Draft Revised Guidance on Impurities in New Drug Substances,**(2006)**.
[8] International Conferences on Harmonization Q2 (R1). Validation of analytical procedures: Text and methodology, **(2005)**.
[9] www.wikipedia.org/wiki/Atenolol
[10] www.drugs.com/atenolol.html