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Validated ultra-high performance liquid Chromatographic method for estimation of Atenolol in bulk powder and Pharmaceutical dosage forms

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Abstract
The objective of proposed research work was to develop and validate new simple, selective, specific, accurate, precise and robust Ultra High Performance liquid chromatographic (UHPLC) method for estimation of Atenolol in bulk and pharmaceutical products. Method was developed by utilizing mobile phase composed of methanol: 0.1% orthophosphoric acid (50:50 v/v) with the use of BDS Hypersil C_{18}(250 × 4.6 mm, 5 µm) column as stationary phase. The flow rate of mobile phase was 1.5ml/min and the temperature was maintained at 20 ºC. Atenolol was detected at 224nm using UV detector. The retention time of Atenolol was found to be 8.9 min. It showed linear response between the concentration ranges 4-8 µg/ml with correlation coefficient 0.999. The method was validated as per current ICH guidelines in terms of specificity, selectivity, linearity and range, precision, accuracy and robustness and all the values of validation was found to be within the acceptance. Hence the method can be used for the routine quality control evaluation of Atenolol in bulk powder.

Keywords: Atenolol, UHPLC, ICH guidelines, Quality Control, UV Detector.

Introduction
Atenolol is a cardio-selective beta-blocker that is widely used in the treatment of hypertension and angina pectoris. Chemically (Fig.1) it is a benzene acetamide, 4-[21– hydroxy – 31 –[(1-methyl ethyl) amino] propoxy]. It acts as an antagonist to sympathetic innervation and prevents increases in heart rate, electrical conductivity, and contractility in the heart due to increased release of norepinephrine from the peripheral nervous system [1].Many branded formulation containing Atenolol available in the market. Hence the quality control analysis of Atenolol in bulk and its formulation play an important role. Literature survey revealed very few analytical methods for the estimation of Atenolol have been developed and validated. RP-HPLC [2-6] methods were reported for the estimation of Atenolol in bulk and pharmaceutical dosage forms. Literature search revealed that no ultra-high performance liquid chromatographic method reported for estimation of Atenolol. In the present research an attempt has been made to develop a new, simple, precise and accurate UHPLC method for estimation of Atenolol which will be helpful for routine analysis of Atenolol.

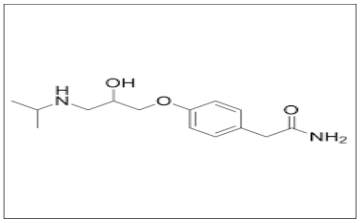

Fig 1: Chemical Structure of Atenolol

Material and Methods
Drug Sample: Atenolol
Chemicals and reagents: All the chemicals and reagents used for analysis were analytical grade and obtained from Merck laboratories. Milli-Q water was used and it was obtained from Basic Science Research Laboratory from KLE College of Pharmacy, Belagavi.

Instruments used: UHPLC of Shimadzu Prominence LC-30AD. The software used was Lab Solutions. BDS Hypersil C18 (250 x 4.6mm, 5µ) column was used as stationary phase. Analytical balance used was of Uni Bloc of Shimadzu make. Eutech Instruments pH meter was used to adjust the pH of mobile phase sonicated by Branson 1800 sonicator.

Method Development

Development of UHPLC method was started with the selection of mobile phase system and stationary phase selection. Literature survey was done and solubility of Atenolol in various solvents was checked and trials were carried out using different mobile phase composition.

Preparation of 0.1% orthophosphoric Acid: Transfer 500 ml of Millipore water in a 500 ml beaker. With the help of a calibrated digital pipette, pipette out 0.1 ml of Orthophosphoric acid and transfer into a beaker containing water. Filter the mobile phase by using 0.2 µ Nylon syringe filter.

Preparation of mobile phase: Methanol filtered through 0.2 µ Nylon syringe filter and degassed and 0.1% Orthophosphoric acid degassed and used for analysis in gradient mode.

Standard preparation: Weigh accurately 10 mg of Atenolol and transfer to 10 ml volumetric flask, make up the volume to 10 ml with methanol. It gives a stock solution of 1000 µg/ml. Pipette out 2.5ml from the above solution and transferred to 25 ml volumetric flask and make up the volume to 25ml with methanol to give stock solution of 100 µg/ml.

Working standard solution: From the stock of 100 µg/ml pipette out 0.8ml and transferred it to the 10 ml volumetric flask and make up the volume to 10 ml with methanol. This gives stock solution of 8µg/ml.

Determination of retention time: Working standard solution containing 8µg/ml of Atenolol was injected into UHPLC which was supported with Hypersil C-18 column as stationary phase and mobile phase composed of methanol: 0.1 orthophosphoric acid (50:50 v/v). The flow rate was adjusted to 1.5ml/min and the temperature was maintained at 20 ℃. UV detection was monitored at 224nm. The volume of the sample injected was 10 µl. The chromatogram was obtained and retention time was determined.

Validation [7, 8]

In order to check performance of developed UHPLC method, validation was performed as per ICH Guidelines using Specificity, selectivity, linearity, precision, accuracy and robustness parameters.

System Suitability: Six replicates of a solution containing analyte of working concentration were injected to determine the precision and accuracy of the system.

Specificity and selectivity: It was performed by injecting the triplicates of mobile phase and sample solution in to UHPLC. Standard solutions was prepared and injected into the UHPLC system and the Amount found, peak Area, Mean and % RSD were found within the limits.

Linearity: Preparation of linear dilution: From the stock solution of Atenolol 100µg/ml, pipette out 0.4ml, 0.8ml, 1.2ml, 1.6ml and 2.0ml and transfer each ml into separate 10 ml volumetric flask to get the final concentration of 4µg/ml, 8µg/ml, 12µg/ml, 16µg/ml and 20µg/ml. The freshly prepared linear dilutions were injected in to UHPLC in triplicates.

Precision: In order to prove precision of method, working standard solution containing Atenolol was injected into UHPLC in six replicates on two different days and on the same day at different time intervals.

Robustness: It was performed by changing the flow rate and temperature conditions and injecting the working standard solution containing Atenolol in to UHPLC.

Ruggedness: In order to prove the repeatability of developed analytical method six replicates of working standard Atenolol solution were injected by different analyst on different days.

Limit of Detection and Limit of Quantification: It was calculated from the standard calibration plot and statistical calculations.

Accuracy: Accuracy was performed by standard addition method at three different levels. Weigh 10 mg Atenolol and transfer to 10 ml volumetric flask and make up the volume to 10 ml with methanol to give stock solution of 1000 µg/ml. pipette out 2.5 ml from above stock solution and transfer to 25 ml volumetric flask make up the volume to 25 ml with methanol to give stock solution of 100 µg/ml. Pipette out 0.4 ml, 0.8 ml, 1.2 ml from stock solution and transfer each ml into individual 10 ml volumetric flask to get 4 µg/ml, 8 µg/ml, 12 µg/ml.

Accuracy of Atenolol at three different levels:

Level –I (50%): 8 µg/ml of stock solution of Atenolol is spiked with 50% i.e 4 µg/ml and injected. Level – II (100%): 8 µg/ml of stock solution of Atenolol is spiked with 100% i.e 8 µg/ml and injected. Level III (150%):8 µg/ml of stock solution of Atenolol is spiked with 150% i.e 12 µg/ml and injected.

Results and Discussion

Method Development: UHPLC method was developed for estimation of Atenolol in bulk powder using mobile phase composition Methanol: 0.1% orthophosphoric Acid (50:50 v/v) and the separation was achieved using BDS Hypersil C-18 (250mm x 4.6mm, 5µm) column. The flow rate was adjusted to 1.5ml/min and the temperature was maintained at 20 ℃. UV detection was monitored at 224 nm. The volume of the sample injected was 10µl. Retention time of Atenolol was found to be at 2.234 min. The chromatographic condition was presented in table 1 and chromatogram was showed in Fig. 2.

Table 1: Developed Method Parameters

Parameter	Description
Mobile Phase	Methanol:0.1%OPA (50:50 v/v)
Diluents	Methanol
Column	BDS Hypersil C_{18} (250x4.6mm,5µ)
Pressure	65kgf/c
Temperature	20℃
Run Time	1.5ml/min
Injection Volume	10µl
UV Wavelength	224nm

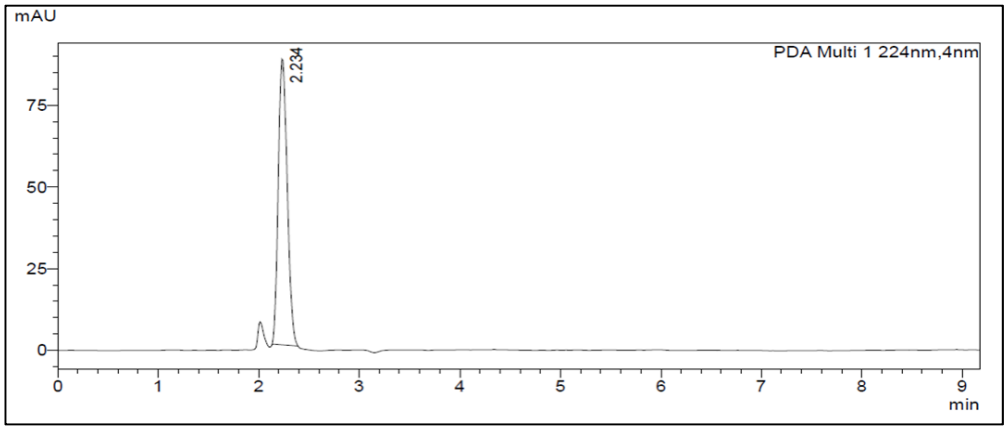

Fig 2: UHPLC Chromatogram of Atenolol

System suitability: System suitability parameter were performed and from the chromatogram obtained Plate count, tailing factor, Resolution and Reproducibility were analyzed. System suitability data were presented in table 2.

Table 2: System Suitability Data of Atenolol

Sample	Retention Time	Peak Area	Theoretical Plate	Tailing Factor
1	2.307 min	55011	2642	1.461
2	2.310 min	54670	2740	1.475
3	2.310 min	55307	2703	1.461
4	2.305 min	54357	2667	1.477
5	2.312 min	53981	2664	1.479
6	2.306 min	53215	2738	1.481
Mean	2.308 min	54423.5	2692	1.472
% RSD	0.04%	0.2%		

Method Validation

Specificity and Selectivity: Developed UHPLC method was found to be specific and selective as the mobile phase not eluting any components at the retention time of analyte.

Linearity and range: Linearity was carried out for Atenolol of working level concentration from 4.0 µg/ml to 20 µg/ml. The linearity regression correlation was within limits and found 0.999. The % RSD for the peak area and retention time was found within the limits. Data of linearity were showed in table 3 and calibration graph showed in Fig.3. The LOD and LOQ was found to be 0.0977and 0.2963 respectively.

Table 3: Linearity data of Atenolol by UHPLC

Concentration	Peak Area
4 µg/ml	24620
8 µg/ml	55781
12 µg/ml	83144
16 µg/ml	110734
20 µg/ml	142185
Mean	302716
Equation for Regression Line	Y= 7252.1 x -3732.1
Correlation Coefficient (r^2)	0.999

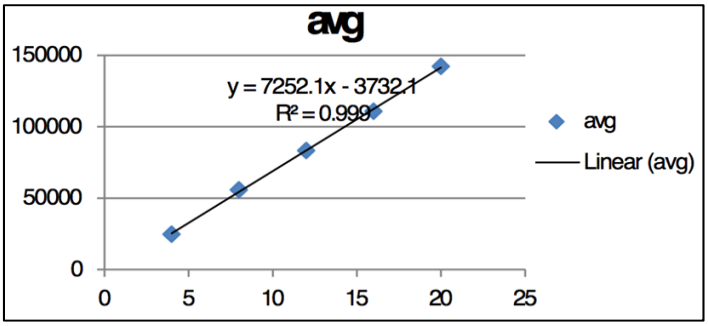

Fig 3: Standard calibration curve of Atenolol

Precision: Precision was carried out on different time intervals and on different days. Mean and % RSD were found within limits and data presented in Table 4.

Table 4: precision Data of Atenolol

Intraday precision				Interday Precision		
Sample	Peak Area 1	Peak Area 2	Peak Area 3	Day 1	Day 2	Day3
1	55781	54357	54505	54505	55781	53215
2	55011	54670	54699	54441	55165	53694
3	55416	54382	54866	54357	55011	53981
Mean	55402.6	54469.6	54690	54434.3	55319	53630
%RSD	0.69	0.31	0.33	0.13	0.73	0.72

Robustness: It was analyzed by modifying the method parameters. Variations in temperature, total flow were made and the outcome was analyzed and calculated. Robustness was presented in Table 5 and 6.

Table 5: Robustness data of Atenolol by change in flow rate

At 1.3ml/min Total Flow			At 1.7ml/min Total Flow	
Sample	Retention Time	Peak Area	Retention Time	Peak Area
1	2.653 min	62697	2.051 min	47814
2	2.645 min	63073	2.046 min	47082
Mean	2.649 min	62885	2.048 min	47448
%RSD	0.21	0.42	0.17	1.09

Table 6: Robustness data of Atenolol by change in the column oven temperature

At 15 ℃ temperature			At 25 ℃ Temperature	
Sample	**Retention Time**	**Peak Area**	**Retention Time**	**Peak Area**
1	2.325 min	58443	2.287 min	60044
2	2.321 min	58099	2.283 min	59852
Mean	2.323 min	58271	2.285 min	59948
% RSD	0.12	0.41	0.12	0.22

Ruggedness and Repeatability: It was carried out by injecting six replicates of the sample solution having target level of the analyte. Retention time, peak area was recorded. The mean, standard deviation and % RSD were found within the limits. Data were presented in Table 7.

Table 7: Ruggedness data of Atenolol

Samples	Retention Time	Peak Area
1	2.307 min	55011
2	2.310 min	54670
3	2.310 min	55307
4	2.305 min	54357
5	2.312 min	53981
6	2.306 min	53215
Mean	2.308 min	54423.5
%RSD	0.04	0.2

Accuracy: Samples were spiked at three different levels 50%, 100%, 150% of the target concentration. Three replicated for each level were analyzed. Theoretical plate, % recovery were calculated. Mean and % RSD were found within the limits. Accuracy data were presented in Table 8.

Table 8: Accuracy data of Atenolol

Levels	Conc (µg/ml)	Qty Added (µg/ml)	Amt Recovered (µg/ml)	% Recovered	%RSD
50%	8 µg/ml	4 µg/ml	6.11 µg/ml	101.8%	0.076
50%	8 µg/ml	4µg/ml	6.11 µg/ml	101.8%	0.004
50%	8 µg/ml	4 µg/ml	6.11 µg/ml	101.8%	0.07
100%	8 µg/ml	8 µg/ml	8.05 µg/ml	100.6%	0.027
100%	8 µg/ml	8 µg/ml	8.05 µg/ml	100.6%	0.025
100%	8 µg/ml	8 µg/ml	8.05 µg/ml	100.6%	0.053
150%	8 µg/ml	12 µg/ml	9.81 µg/ml	98.1%	0.070
150%	8 µg/ml	12 µg/ml	9.81 µg/ml	98.1%	0.12
150%	8 µg/ml	12 µg/ml	9.81 µg/ml	98.1%	0.058

Assay: In the proposed method the estimation of Atenolol has been done using UHPLC and is a new method as compared to previously performed works. The assay report was presented in table 9.

Table 9: Assay report of Atenolol

Conc.	Peak Area	Amt Found	% Label Claim
8 µg/ml	55781	8.20	102.05
8 µg/ml	54907	8.08	101.07
Mean	55344	8.14	101.56
SD	618.011	0.0848	0.6929
%RSD	1.11	1.04	0.68

Discussion: he work done using UHPLC shows better peak resolution and faster elution of peaks. The mobile phase that is used is Methanol: 0.1 Orthophosphoric acid and it shows a better peak elution and shows a peak retention at 2.234 min. The method shows theoretical plate count more than 2000and a tailing factor less than 2.

Conclusion

A new UHPLC method was developed and validated for estimation of Atenolol in bulk powder. The use of RP C-18 showed compatibility with analyte with good peak area, theoretical plate count, retention time and tailing factor. Validation results of analysis reveals all the parameters were within acceptable range which indicate method was specific, linear, precise robust, rugged, and accurate.

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