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RESEARCH ARTICLE

ANAYTICAL METHOD DEVEOPMENT AND VAIDATION OF BUTYLATED HYDROXYTOLUENE IN ROSUVASTATIN TABLETS BY USING RP-HPLC

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ABSTRACT

An RP-HPLC method was developed and validated for the estimation of Butylated Hydroxy toluene in Rosuvastatin Tablets. The chromatographic system was equipped with Hypersil BDS, C18, 250 X 4.6 mm, internal diameter with 5 micron particle size column and PDA detector set at 277nm, in conjunction with a mobile phase of Water and Methanol in the ratio of 100:900 at a flow rate of 0.8 ml/min. The retention time of Butylated Hydroxy toluene was found to be 7 minute. The separation was performed at ambient temperature. Linearity was observed in the concentration range of 2.5-7.5µg/ml with correlation co-efficient 0.999.Percentage recovery obtained 100.3%-100.8%. The percentage assay was found to be 99.8% .The proposed method is precise, accurate, selective and rapid for the determination of Butylated Hydroxytoluene in Rosuvastatin Tablets. The proposed method is optimized and validated as per the International Conference on Hormonization (ICH) Guidelines.

Key words: RP-HPLC, Butylated Hydroxytoluene , Rosuvastatin ,Validation

INTRODUCTION:

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Butylated hydroxytoluene (BHT), also known as butylhydroxytoluene, Chemical name is 2, 6-bis (1,1-dimethylethyl)-4-methylphenol. It is a lipophilic organic compound, chemically a derivative of phenol, that is useful for its antioxidant properties. Butylated hydroxytoulene has a solid crystalline appearance, with a phenolic or sweet and tarry odor. It does not have any taste, and its color ranges from white to yellowish. Since it is lipophilic, it readily dissolves in fat but not in cold water. It is soluble in methanol and acetone as well.

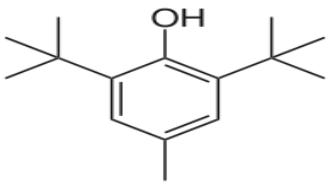

Figure 1: Chemical structure of Butylated hydroxy toluene (BHT)

Rosuvastatin chemical name is Bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methyl sulfonyl)amino] pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt, is a fully synthetic statin which has a potent cholesterol-lowering action than other drugs in its class. ROS is an inhibitor of 3-hydroxy-3methylglutarylcoenzyme A (HMG-CoA) reductase, used as the calcium salt in the treatment of hyperlipidemia.

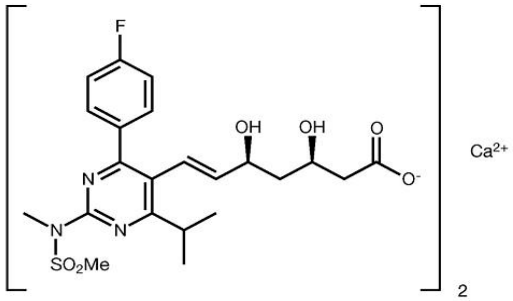

Figure 2: Chemical structure of Rosuvastatin Calcium

Experimental Section:

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Instrumentation and Chromatographic conditions:

The analysis was performed by using Hypersil BDS, C18, 250 X 4.6 mm internal diameter with 5 micron particle size column and PDA detector set at 277nm, in conjunction with a mobile phase of Water and Methanol in the ratio of 100:900 at a flow rate of 0.8 ml/min. The retention time of Butylated Hydroxytoluene was found to be 7 minute. The separation was performed at ambient temperature. The injection volume was 100µl.

Reagents and Solutions:

Methanol, Acetonitrile of HPLC grade and Milli Q water were used in analysis.

Mobile Phase Preparation:

Prepared a mixture of Water (Milli Q) and Methanol (HPLC Grade) in the ratio of 100:900% (v/v) mixed and sonicated.

Diluent Preparation:

Prepared a mixture of Water (Milli Q) and Acetonitrile (HPLC Grade) in the ratio of 100:900% (v/v) mixed and sonicated.

Preparation of Standard solution:

Standard Stock solution

Weigh accurately about 25 mg of Butylated Hydroxytoluene Working Standard and transferred it into a 100 ml volumetric flask. Add 60ml of diluent, sonicate to dissolve and make up to volume with diluent (250 µg/ml).

Standard solution

Transfer 2 ml of standard stock solution into a 100 ml volumetric flask and make up the volume with mobile phase. Filter the solution through 0.45µ filter and inject. (5 µg/ml)

Procedure for analysis of tablet formulation:

Weighed 20 tablets and triturate in mortar and pestle into fine powder. Weigh accurately the powdered tablet contents equivalent to 0.5 mg of Butylated Hydroxytoluene in to a 100 ml volumetric flask, add 60 ml of diluent, sonicate to dissolve the contents and make up the volume with diluent. Filter the solution through 0.45µ filter and inject.

Method Validation:

The method was validated for Specificity, linearity, accuracy, intra-day and inter-day precision, robustness and ruggedness in accordance with ICH guidelines.

Specificity:

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Butylated Hydroxy toluene. The specificity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Butylated Hydroxy toluene.

System suitability:

The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Table 1: System Suitability Test Parameter

System Suitability Parameters	Butylated Hydroxytoluene
Retention times (RT)	6.92min
No. of Theoretical plates (N)	4763
Resolution (RS)	10.73
Tailing Factor (As)	1.2
Peak area	276603

Linearity:

The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions were Prepared and inject into the HPLC and the chromatograms were recorded. Evaluation was performed with PDA detector at 277nm, peak areas were recorded for all the peaks. The peak areas show excellent correlation between peak area and concentration. The linearity graph is shown in the figure and the value obtained was shown in table no.2.

Table 2: Data for linearity of Butylated Hydroxy toluene

S. No	Linearity Level	Standard Stock Solution of B HT (ml)	Final Volume (ml)	Conc. (µg/ml)	Area
1	50%	1	100	2.5	160845
2	80%	1.6	100	4	257116
3	100%	2	100	5	321395
4	120%	2.4	100	6	385674
5	150%	3	100	7.5	491864

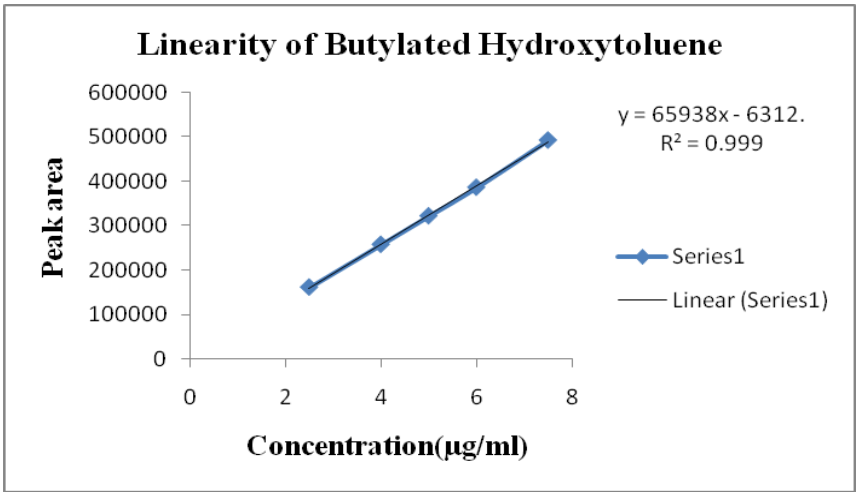

Figure 3: Calibration Curve for Butylated Hydroxy toluene

Recovery studies:

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre analyzed sample solution at three different levels 50%, 100%, 150%.Each level was injected 3 times. The percentages of recoveries were calculated. The value obtained was shown in table no.3.

Table 3: Data for Recovery Study

Level of % Recovery	Mean Recovery	Standard deviation	%RSD
50%	100.8	0.2	0.18
100%	100.4	0.3	0.29
150%	100.3	0.15	0.14

Precision:

The precision of the assay was studied with respect to both Intra-day and inter-day precision. Intra-day was calculated from six replicate injections of freshly prepared Butylated Hydroxy toluene test solution in the same equipment at a concentration value of 5 ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine inter-day precision. The peak areas were determined and % RSD was calculated for both series of analysis.

Ruggedness and Robustness:

In the robustness study, the influence of small, deliberate variations of the analytical parameters on the retention time of Butylated Hydroxy toluene was examined. The following two factors were selected for change: flow rate of the mobile phase (0.8±0.2ml/min) and a detection wavelength (277±3nm) at which chromatograms were recorded. Ruggedness of the method was determined by carrying out the assay by different analyst on different days. It was observed that there were no marked changes

in the chromatograms, which demonstrated that the RP-HPLC method developed is robust and rugged.

RESULTS AND DISCUSSION

A new RP-HPLC method has been developed and validated for the estimation of Butylated Hydroxy toluene in Rosuvastatin tablet formulation. The developed method was validated by using various parameters according to ICH guidelines. It was validated for specificity, linearity, precision, accuracy studies, robustness and ruggedness. All the validation parameters were found to be well within the acceptance criteria. The system suitability parameters reveals that the values within the specified limit for the proposed method. The theoretical plates for BHT were found to be more than 2000 and the tailing factor is NMT 2.0. From the linearity studies, the specified range for BHT was found to be 2.5µg/ml to 7.5µg/ml. The correlation co-efficient was found to be 0.999 and the results were found to be linear. The %RSD for Precision was found to be 0.19 % for intra-day and 0.21 % for Inter-day. The %RSD values indicate that the method had good precision. The average recovery of BHT was found to be 100.3 % - 100.8% and the results were found to be within the limits which indicate that the method is accurate. Ruggedness and Robustness test results were found to be with percentage RSD not more than 2.

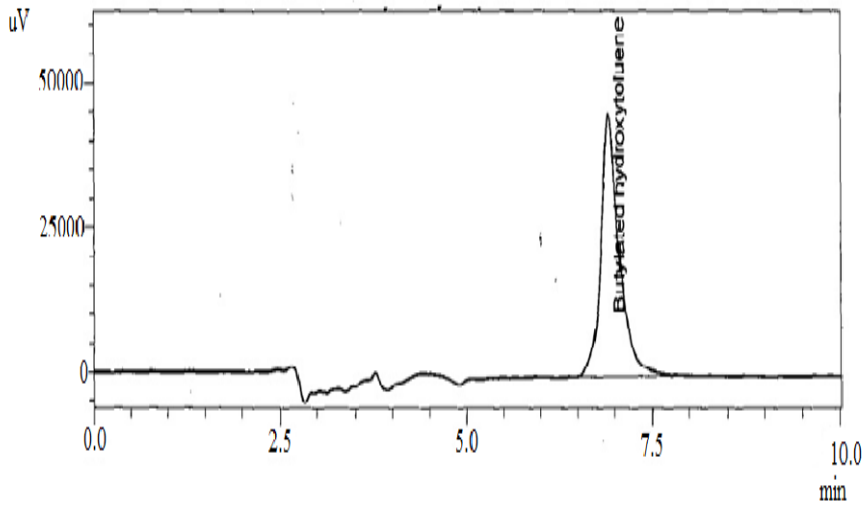

Figure 4: Chromatogram of Standard Butylated Hydroxytoluene

Table 4: Summary of validation parameters of proposed RP-HPLC method

S.No	Parameter	Observation
1.	Specificity	No Interference was found
2.	Linearity	0.999
3.	Accuracy (%Recovery)	100.3-100.8
	Precision(%RSD)	
4.	Intra-Day	0.19
5.	Inter-Day	0.21
6.	Ruggedness(%RSD)	0.43
	Robustness(%RSD)	
7.	Change in Wavelength	0.29
8.	Change in Flow Rate	0.15

CONCLUSION:

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of Butylated Hydroxytoulene isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of Butylated Hydroxytoulene without any interference. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

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