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Original Article

A FAST AND SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF HYDRAZINE IN ATAZANAVIR SULFATE DRUG SUBSTANCE

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ABSTRACT

Objective: To develop a fast and sensitive UV spectrophotometric method for the quantitative estimation of Hydrazine in Atazanavir Sulfate drug substances and validate as per ICH guidelines.

Methods: The method was based upon the observation, that a characteristic colour results upon addition of a solution of *p*-Dimethylaminobenzaldehyde in ethyl alcohol and hydrochloric acid to hydrazine and estimated at absorbance maximum λ 458 nm in Atazanavir drug substance.

Results: The developed method resulted in Hydrazine exhibiting linearity in the range 0.2 to 2.7 µg/g. The Intraday and interday precision is exemplified by relative standard deviation of 0.959 % and 0.947%. Percentage Mean recovery was found to be in the range of 97-101%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 0.2 µg/g and 0.6 µg/g respectively.

Conclusion: The present work was aimed to develop a visible spectrophotometric method, which is simple, sensitive, accurate and cost effective to evaluate the quality of the bulk and pharmaceutical formulations.

Keywords: UV Spectrophotometry, Hydrazine, Atazanavir Sulfate, Method development, Validation.

INTRODUCTION

Atazanavir Sulfate (ATV) methyl N′-[(1S)-1-{N-[(2S,3S)-2 - hydroxyl - 3 - [(2S) - 2 - [(methoxycarbonyl)amino] - 3,3 – dimethyl butanamido]-4-phenylbutyl]-N′-{[4-(Pyridin-2-yl) phenyl] methyl} hydrazine carbonyl}-2,2-dimethyl propyl]carbamate (Figure 1), an azapeptide is the 7th Protease Inhibitor[1] used in the treatment of human immunodeficiency virus (HIV) Type II infection. ATV is reported as poorly water soluble and a known substrate for both hepatic metabolizing enzyme Cytochrome 450 (CYP3A) and intestinal drug efflux pump, P-glycoprotein (Pgp) so have low oral bioavailability.[2] So co-administration of small dose of Ritonavir (RTV) is recommended as booster [1- 4].

The novelty of the developed method was that the reagent used are easily available and the mechanisms of reactions of the reagent are already well established. The reactions involved with these reagent are simple, rapid and sensitive. Spectrophotometric methods involve simple instrumentation which is cost effective as compared to other instrumental techniques. The present method involves the determination of hydrazine (Figure 2) which is in the form of t-Butyl carbazate [5] used as key raw material in the sysnthesis of Atazanavir sulfate.

The growing demand of analysing the reported genotoxic like Hydrazine and therefore the quantification by a cost-effective method inspired the development of the present method. The method was based on the coupling reaction between the hydrazine involved in Atazanavir drug substance and *p*-Dimethylaminobenzaldehyde (DMAB) in acidic medium. This method have advantage of sensitivity, selectivity, rapidity and low cost of analysis. Literature survey reveals many analytical methods include Capilllary Electrophoresis, HPLC, Gas Chromatography and UV Spectrophotometry for the determination of hydrazine in pollutants and biological samples [6-9]. Therefore, an attempt was made to develop a spectrophotometric method for the determination of Hydrazine in pharmaceutical drugs.

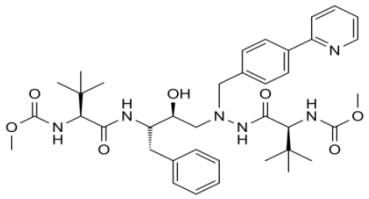

Fig. 1: Chemical Structure of Atazanavir sulfate.

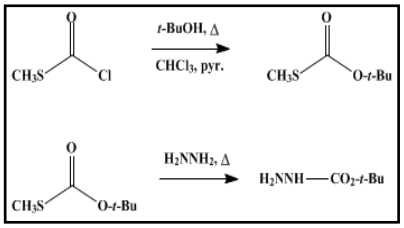

Fig. 2: Synthesis of t-Butyl carbazate

MATERIALS AND METHODS

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance.

Chemicals and Reagents

Pure drug Atazanavir sulfate was provided by our APL Research Centre-II. (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India.

Methods

Preparation of Solutions

Standard solution: Prepare 5 µg/ml hydrazine in water, transfer 14.4 ml of this solution into a 100 ml volumetric flask add 40 ml of methanol, dissolve completely and add 40 ml of *p*-Dimethylaminobenzaldehyde(DMAB) solution make upto 100 ml with 0.1 M HCl, keep 20 min aside at room temperature shake 5 min intervals which results in formation of a yellow coloured chromogens.

Sample solution

Weigh about 0.4 g of Atazanavir sulphate drug substance and transfer into a 10 ml of volumetric flask, initally add 4 ml of methanol and sonicate to dissolve completely. To this solution add 4 ml of DMAB solution, make upto 10 ml with 0.1 M HCl, keep 20 min aside at room temperature, shake 5 min intervals which results in formation of a yellow coloured chromogens.

Blank solution

Prepare blank solution as per above procedure without sample addition.

Procedure

The developed yellow coloured chromogen of standard and sample solutions against the reagent blank was scanned between 400 nm to 600 nm and the absorbance maxima at λ 458 nm. Figure 3 & 4.

Spectral Characteristics

Standard solutions of Hydrazine coupled with DMAB at different concentrations level were prepared. Calibration curve was constructed by plotting the concentration level versus corresponding absorbance at 458 nm. The results show an excellent correlation between absorbance and concentration level of hydrazine within the concentration range 0.2 to 27 µg/g show good agreement with Beer's law.

RESULTS AND DISCUSSIONS

Method development

The colour reagent employed had the following composition; *p*-Dimethylaminobenzaldehyde 0.4 g; ethanol, 20 ml; concentrated Hydrochloric acid, 2 ml. 4 ml of this reagent added to the aliquots of the standardized hydrazine solution selected so that the final hydrazine concentration would be within the range 0.2 to 27 ug/g.

At room temperature the yellow colour develops immediately and is stable for the period of 10 min. For a given concentration the per cent transmittance is unchanged if the hydrochloric acid concentration is less than 1 M but increases at acid concentration greater than 1 M. Addition of 10 ml colour reagent rather than 4 ml results in a decrease in per cent transmittance.

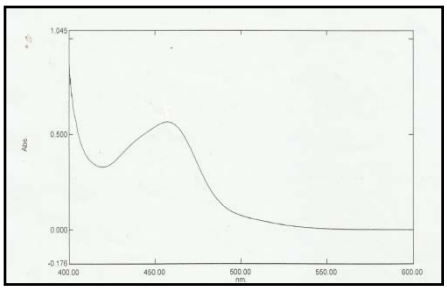

Fig. 3: UV Spectrum of Hydrazine coupling with DMAB

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines[10] for validation of analytical procedures.

The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

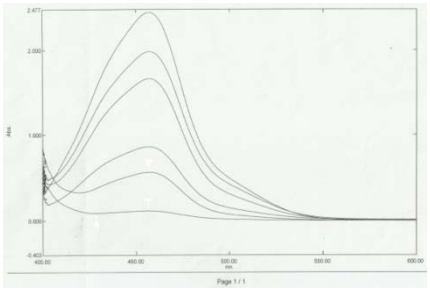

Fig. 4: Overlay spectrum of Hydrazine coupling with DBA at different concentrations

Precision

System precision

Six replicate recording of absorbance at 458 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 1 concerning absorbance for the hydrazine, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in (Table 1).

Table 1: System precision results of Hydrazine.

n	Absorbance
1	0.156
2	0.158
3	0.155
4	0.155
5	0.156
6	0.155
Mean	0.156
SD^	0.001
%RSD*	0.750

^ Standard deviation, * Relative standard deviation

Method precision

Method precision was determined by performing content of hydrazine by spiking with known concentration of hydrazine in the atazanavir drug substance under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 458 nm of the hydrazine from the same homogeneous mixture at working concentration showed % RSD less than 1, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 2).

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recording of absorbance at 458 nm of the hydrazine from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 1 within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

Table 2: Intra day precision results of Hydrazine (1.90 µg/g)spiked in Atazanavir drug substance.

n	Hydrazine Content(µg/g)
1	1.91
2	1.95
3	1.95
4	1.94
5	1.91
6	1.92
Mean	1.93
SD	0.019
%RSD	0.959

Table 3: Inter day precision results of Hydrazine (1.90 µg/g) spiked in Atazanavir drug substance.

N	Hydrazine Content(µg/g)		
	Day 1	Day 2	Day 3
1	1.91	1.90	1.91
2	1.90	1.94	1.93
3	1.95	1.90	
4	1.91	1.92	1.90
5	1.92	1.94	1.92
6	1.91	1.91	1.94
Mean	1.92	1.92	1.93
SD	0.018	0.018	0.019
% RSD	0.914	0.956	0.972

Linearity

Standard solutions of hydrazine coupled with DMAB at different concentrations level 0.2 µg/g to 27 µg/g were prepared. Calibration curve was constructed by plotting the concentration level of hydrazine versus corresponding absorbance at 458 nm. The results show an excellent correlation between absorbance and concentration level of hydrazine within the concentration range (0.2-27 µg/g) are given in (Table 4)**.** The correlation coefficients were greater than 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 0.2-27 µg/g.

Table 4: Calibration data for Hydrazine standard

N	Hydrazine standard (µg/g)	Absorbance
1	0.2	0.018
2	0.5	0.035
3	5.0	0.450
4	10.0	0.862
5	18.0	1.666
6	22.0	1.983
7	27.0	2.400
Regression equation	y = 0.090x – 0.004	
Correlation coefficient (r^2)	0.999	

Table 5: Recovery results from spiking of Atazanavir drug substance with Hydrazine.

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(µg/g)	0.604	1.91	2.90
Found(µg/g)	0.600	1.90	2.88
Recovery(%)	99.3	99.4	99.2
RSD(%)	2.04	1.29	1.54

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (LOQ-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in (Table 5). The accepted limits of recovery are 97% - 101% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the hydrazine content despite deliberate variations done concerning compositions of colored reagent and solvents.

Sensitivity

The sensitivity of measurement of hydrazine content by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 0.2 µg/g and 0.6 µg/g respectively. Optical characteristics results are summarized in (Table 6)

Table 6: Optical characteristics of Hydrazine content in Atazanavir drug substance.

Parameters	Results
Detection wavelength(nm)	458
Beer's law limits (µg/g)	0.2 – 27
Regression equation (y = mx+c)	y = 0.090x – 0.004
Correlation coefficient (r^2)	0.999
LOQ (µg/g)	0.6
LOD (µg/g)	0.2

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Hydrazine in Atazanavir drug substance as per ICH guidelines. The developed method resulted in Hydrazine exhibiting linearity in the range 0.2 to 2.7 µg/g. The Intraday and interday precision is exemplified by relative standard deviation of 0.959% and 0.947%. Percentage Mean recovery was found to be in the range of 97101%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 0.2 µg/g and 0.6 µg/g respectively. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of Hydrazine content in Atazanavir drug substances.

CONFLICT OF INTERESTS

Declared None

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