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Assay method development and validation for butamben drug substance by using high performance liquid chromatographic technique

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ABSTRACT

Stability indicating assay methodology was developed with UV detection for the simultaneous quantification of Butamben drug substance by HPLC. The separation between degradation impurities and the peak due to Butamben was achieved by using Universal C 18, (100 x 4.6) mm, 5.0-micron column. Mobile Phase was made up of Water, Methanol and Orthophosphoric acid by mixing 350:650:1 ml, respectively. Isocratic method was used by using flow rate of 1.0 ml/min, UV at 250 nm and column oven temperature at 50°C. Relative standard deviation for standard preparation under system precision was achieved within acceptance criteria. Relative standard deviation for retention time was achieved 0.11%, which shows reproducibility during replicate injections. After the successful development of this method, chemically forced degradation was performed by external acid, alkali and peroxide treatment. Not any degradation peak was interfering with any impurity. This newly developed innovative method is found suitable for the Assay analysis of Butamben API.



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INTRODUCTION

High-performance liquid chromatography technique is a specific form of column chromatography specifically used for analysis for impurity separation, identification and quantification from the active compound (Ramesh *et al.*, 2010). HPLC column has a stationary phase, which helps for separation, a pump that pushes the mobile phase through the column and a detector that shows the

retention times of the eluted compounds. Retention time may vary based on the interactions with the stationary phase, the molecules being analyzed, and the solvent used. The sample which is to be analyzed is injected in small volumes to the stream of mobile phase and is eluted by specific chemical or physical interactions with the stationary phase. The elution depends on the nature of the analyte and composition of both stationary and mobile phases. The time at which a specific analyte elutes is called the retention time. Common solvents used include any miscible combinations of water or organic liquids; the most common are methanol and acetonitrile. The separation between peaks has been achieved by changing the mobile phase composition during the analysis. This is called as gradient elution. The choice of solvents and gradient depends on the nature of the stationary phase and the analyte.

Butamben (Refer Figure 1) is a non-steroidal anti-inflammatory drug that works as local anesthesia. It blocks nerve conduction when applies locally to

nerve tissue in concentrations and acts on any part of the nervous system and on every type of nerve fiber. In contact with a nerve trunk, these anesthetics can cause both sensory and motor paralysis in the innervated areas and their action is completely reversible.

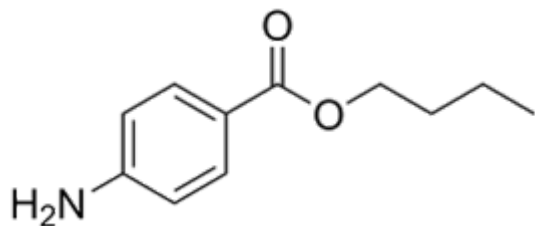


Figure 1: Structure of Butyl 4aminobenzoate (M.Formula= $C_{11}H_{15}NO_2$ and M.W.=193.24)

(Patel *et al.*, 2013) developed chromatographic method by HPLC, (Aswale *et al.*, 2015) developed and validated the analytical methods by HPLC for quantification of intermediate, unknown impurities and Z Isomer in Entacapone API. (Blessy *et al.*, 2014) extended their research for forced degradation and stability, indicating studies of drugs. (Ravi *et al.*, 2013) and (Koyasu *et al.*, 2016) aimed to develop and to validate a new HPLC method for Entacapone in Eantacapone tablet. (Naseef *et al.*, 2018) developed and validated an HPLC method to determine antidiabetic Drug Alogliptin Benzoate in drug substance and tablet formulation.

MATERIALS AND METHODS

HPLC grade methanol as a solvent (Spectrochem), orthophosphoric acid AR grade (Rankem) and HPLC grade water (Merck) were used. HPLC instrument of make Shimadzu LC 2010 C HT with LC-solution software was used.

For this development, a short length C18 column was selected to develop a method in less time and to achieve excellent separation with good sharp symmetric peak shapes. For this Universal C18 column was found suitable in terms of short length and carbon loading. In short length column compound eluted faster and C18 carbon load in column helps for separation and 5-micron particle size column gives sharp peaks, as well as more separation, also control column pressure.

Development of analytical method was initiated by using pure Water and Methanol as a mobile phase in ratio 60:40, flow towards column was used 1 ml/min, column oven compartment heated at 30°C and UV 250 nm wavelength selected based on maximum response. Short length C18 column, selected for development. In this condition, Butamben peak

eluted at about 2.6 minutes, where the satisfactory peak was not observed, and it shows fronting. Hence column oven temperature study was initiated to improve the peak shape and separation. It was observed that at 50°C, peak shape and separation is satisfactory.

During moving liquid phase study, an aqueous solution of 0.1% v/v orthophosphoric acid and Methanol used in the ratio 35:65, and also found satisfactory as mobile phase. In this mobile phase, peak shape and separation of impurities was found satisfactory.

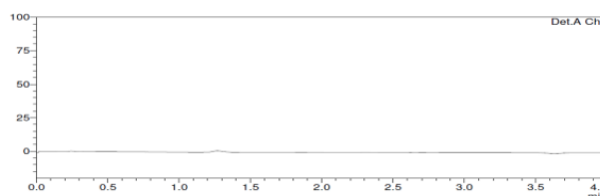


Figure 2: Blank Chromatogram

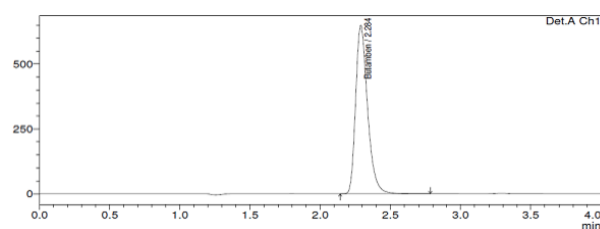


Figure 3: Standard Chromatogram

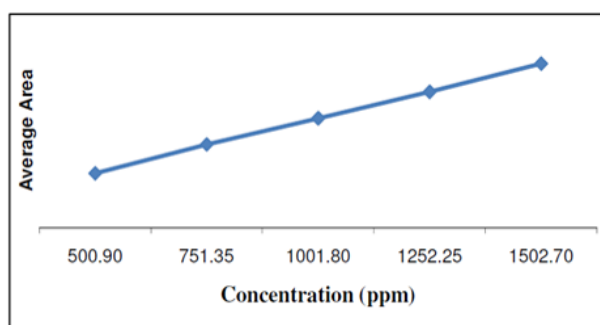


Figure 4: Linearity graph

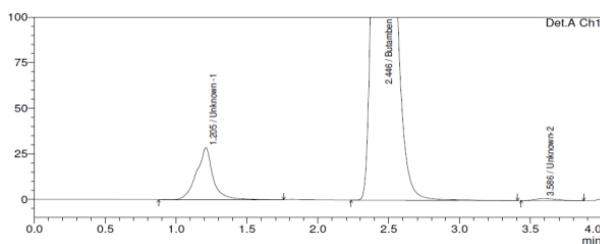
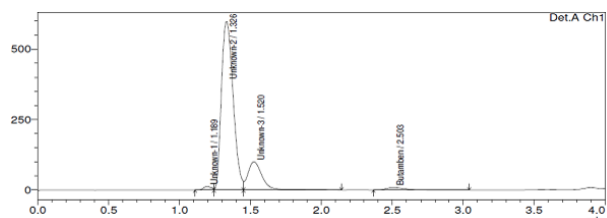


Figure 5: Acid degradation test chromatogram of Butamben API

In order to develop a stability indicative method, a forced degradation study was performed. In acid degradation study test sample was treated by

Table 1: Method and Intermediate precision for Assay of Butamben

Sr. No	Method Precision	Intermediate Precision
1	100.1	99.9
2	100.2	100.1
3	100.1	100.1
4	100.2	100.1
5	100.2	99.9
6	99.9	100.1
Average	100.1	100.0

**Figure 6: Alkali degradation test chromatogram of Butamben API**

adding 1 ml, 5 N hydrochloric acid and hold at RT (room temperature) for 3 hrs, after that it was analyzed. Formation of degradation impurities were found well separated from Butamben peak and the method was found suitable in terms of acid degradation sample. During acid degradation study, unknown impurity at retention time about 1.205 minutes is found as degradation impurity because, in acid degradation sample, it was observed about 6.125% (Refer Figure 5). In addition, there is no any interference from blank preparation.

In alkali degradation, the study test sample was treated by adding 1 ml, 5 N sodium hydroxide solution and held at room temperature for 3 hrs and after that, it was analyzed. Formation of degradation impurities were found well separated from Butamben peak and the method was found suitable in terms of acid degradation sample. During acid degradation study, unknown impurity at retention time about 1.32 minutes is found as degradation impurity because, in acid degradation sample, it was observed at about 80.374% (Refer Figure 6). Also, there is no any interference from blank preparation.

Chromatographic parameters

HPLC Column name - Universal C-18, (100 x 4.6mm), 5.0 micron

HPLC Flow - 1.0 ml / min

UV Wavelength - 250 nanometer.

HPLC Column Oven - 50° C

Acquisition time - 5 min.

Injection volume - 10 μ l

Diluent - Water: Methanol (50:50)

RESULTS AND DISCUSSION

Butamben drug substance has a low response in UV, hence standard and test concentration need to be increased to 1000 ppm for Assay test. Table 1 shows comparative data of precision study for Butamben drug substance. It was concluded that when standard preparation was injected, then a newly developed method shows exact reproducible area and retention time (RT) and variation of retention time and area not observed. Retention time for Butamben was 2.279 ± 0.05 minutes.

Figure 2 is a blank preparation chromatogram; from the standard chromatogram Figure 3, it was concluded that the Retention time for Butamben was 2.2 ± 0.05 minutes.

As per (Eldin, 2011), linearity was analyzed by preparing linearity solutions at five different concentration levels i.e., 500.90, 751.35, 1001.80, 1252.25 and 1502.70 ppm of Butamben. The peak response of the Butamben peak is found to be linear in the desired concentration range. Correlation coefficient 0.9999 was achieved (Refer Figure 4).

Reproducibility of the method was checked by performing method precision in which the same test was prepared six times and each of the preparations was injected. Results were calculated against the average area from five replicate injections of Butamben Standardized preparation, Table 1.

Degradation is also an important criterion in method development. In this process, the compound needs to be degraded by chemical treatment and formation of unknown impurities as well as increasing or decreasing trend of impurities to be monitored. From this study, it was concluded that there is not any interference to Butamben peak from any degradation impurity.

Robustness of method was checked by changing the

composition of the mobile phase, applied flow and oven temperature. Method is robust in all condition and there is no merging of any peaks and peak shapes is also found good. Robustness study should be performed at the development stage. It should show the reliability and reproducibility of an analysis with respect to deliberate variations in method parameters Figure 4.

CONCLUSIONS

Elution of Butamben peak was carried out on Universal C-18, (100 x 4.6mm), 5.0 micron HPLC column, at the flow of 1 ml/min under an isocratic condition with the liquid mobile phase, aqueous solution 0.1 % orthophosphoric acid and Methanol in the ratio 35:65. Linearity graph plotted against average area Vs. concentration is found linear.

The method can be used successfully for the identification and quantification of the active pharmaceutical ingredient Butamben from the pharmaceutical ingredient.

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