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Short communication

Forced degradation of timolol maleate on high temperature for verification of HPLC method for related substances in Timolol eye drop 0.5%

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Introduction

Timolol is a potent β-adrenergic blocker, useful in treatment of ocular hypertension or open-angle glaucoma. Many chromatographic analysis methods have been applied for the determination of pharmaceutical compounds containing heterocyclic rings (as timolol maleate), but the most commonly applied chromatographic technique is HPLC (Mandour et al., 2020).

Understanding the stability characteristics for both the active pharmaceutical ingredient (API) and for the medicinal product (MP) is crucial for the development of a safe and effective pharmaceutical agent. For this purpose, samples from API and MP during product development are disposed under strictly controlled storage conditions to assess stability testing. Forced degradation studies are being conducted to identify the degradation products that are likely to occur during long term storage as the worst-case scenario (Sengupta et al., 2018).

Materials and methods

The forced degradation studies were performed on API Timolol maleate (batch 005009102019) and MP Timolol eye drops 0.5% (batch 2112, expiry date: 12.2023) manufactured by Profarma Sh.a, Albania. Forced degradation was done only by exposing the API and MP on high temperature at 80°C for 48 hours to obtain degradation products quickly and to verify the method for determination of related substances listed in the British pharmacopoeia (BP, 2016).

Identification and quantification of degradation products was performed at room temperature, using an isocratic method and reverse phase HPLC stainless steel column (30 cm x 4.6 mm) packed with end-capped octadecyl silyl silica gel for chromatography (10 µm) (µBondapakTM C18 was used) on HPLC system (Agilent Technologies Infinity II 1260, DAD HS 1260: Serial No. DEAEK06706). Mobile phase was consisting of mixture of 0.02 M sodium octane-sulfonate and methanol in ratio 42,5:57,5; adjusted to pH 3.0 using glacial acetic acid. An isocratic method elution was used with flow rate of 1.5 mL per minute on ambient column temperature. UV detection was on wavelength of 295 nm. Injection volume was 20 µL of each solution and run time was 4 times of the retention time of the principal peak of timolol maleate.

Results and discussion

Verification of the HPLC method was done according to ICH guidelines Q2 (R1) Validation of Analytical Procedures (ICH Q2 (R1), 2022).

For forced degradation studies the chosen stress condition (temperature of 80°C for 48 hours) leads us to only one secondary peak at RRT 1,6 due to exposition of the product at higher temperature.

The parameter specificity, demonstrate good separation of all peaks derived from active substance

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(timolol maleate, secondary peak because of force degradation and maleic acid) and excipients (placebo).

The precision and reproducibility of the proposed method were evaluated by performing replicate analysis of the standard solution timolol maleate in concentration of 0.02 mg/mL (reference limit for impurities), to determine intraday and inter-day variability [within day (n=6) and between days (n=6)]. Relative standard deviations were calculated to obtain the precision of the method. Relative standard deviations were calculated to obtain the precision of the method. The results of precision, and reproducibility of the method demonstrate a good precision (RSD =1.05%).

Linearity was performed using timolol maleate standard solutions in concentration range from 0.005 mg/mL (limit of quantification-LOQ) to 0.03 mg/mL or 25%-150% from the reference limit for impurities. The response was linear over the range (R^2=0.9999).

Limit of Detection LOD and LOQ were determined by evaluation peak areas of timolol maleate at lower concentrations. Acceptance criteria were signal to noise ratio about 3:1 for LOD and about 10:1 for LOQ. According to results LOD for timolol was 0.05 µg/mL, and LOQ for timolol was 0.15 µg/mL.

Conclusion

From a regulatory perspective, the combined use of stress testing and accelerated degradation studies with an optimized separation technique and validation procedures is a useful approach to achieve a comprehensive understanding of API and MP stability, with regards to the nature of the relevant degradation products, within a reasonable timeframe.

The forced degradation studies and its application are mainly used for the determination of stability of molecule under accelerated conditions and for the development of stability indicating methods. In our case, forced degradation was done only by exposing the API and MP on high temperature at 80°C for 48 hours to obtain degradation products quickly and to verify the method for determination of related substances listed in the British pharmacopoeia. Testing of specificity of the method demonstrate good separation of all peaks derived from active substance and excipients in the formulation.

Verification of the HPLC method was done according to the ICH guideline Q2 (R1) to prove that monograph in BP for timolol eye drops is suitable for analyzing of degradation products in our formulation.

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