

RP-HPLC a valuable tool in monitoring dissolution test of fixed combination dosage forms

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Introduction

Fixed dosage forms usually contain two or more active ingredients which are responsible for a combined therapeutic activity. The combination of three well established antihypertensive agents: amlodipine, valsartan and hydrochlorothiazide in a dosage form, has been found successful for the treatment of moderate to severe hypertension (Deeks, 2009). Many analytical methods have been reported for simultaneous estimation of the active ingredients in this triple combination (Sharma et al., 2014) with HPLC being a method of choice. Different researchers have worked at applying developed and validated RP-HPLC methods in monitoring dissolution test of tablet formulations containing two or more active ingredients in combination (Celebier et al., 2010).

The aim of this work was finding a suitable HPLC method that can be used to monitor the dissolution test of two different solid dosage forms in our laboratory.

Materials and methods

Chemicals and reagent

Two commercially available tablet formulation containing 5mg amlodipine (AML), 160 mg valsartan (VAL) and 12.5 mg hydrochlorothiazide (HCT) (formulation A and formulation B) were used in this study. Working standard substances were kindly donated by TrePharm Pharmaceutical Co., Prishtina, Kosovo. Potassium dihydrogen phosphate,

orthophosphoric acid, monobasic potassium phosphate, sodium hydroxide, phosphoric acid and acetonitril (HPLC grade) were purchased from Krijon sh.p.k Tirana, Albania. Distilled water was obtained by using GFL Double Water Distillation 2102 and used within 4 hours.

Instrumentation and analytical method

Analysis were performed by a HPLC (Varian Prostar, equipped with a binary pump, manual injection, volume of the loop 20 μ L); on a Hipersil C18 ODS column (250 \times 4.6), 5 μ m particle size. The isocratic mobile phase consisted of acetonitrile and potassium dihydrogen phosphate buffer (pH 3, 0.05 M) in the ratio of 40:60 v/v. The mobile phase was filtered through 0.45 μ m membrane filter PTFE, degassed in an ultrasonic bath and pumped from the respective solvent reservoir to the column at a flow rate of 2 mL/min. All analyses were carried out at 25 °C and the detection wavelength set at 227 nm. The injection volume was 20 μ L.

Dissolution tests conditions

The dissolution test was performed in compliance with United States Pharmacopoeia (USP) (711) using apparatus 2 (Varian 705 DS) with paddles (USP, 2017). The medium selected was phosphate buffer of pH 6.8. Paddle speed was settled at 50 rpm as stated in product monograph. Media volume of 900 mL was filled in six baskets and two baskets were used as blank for replenishing. The medium, before processing, was degassed via sonication

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process, and temperature was set at 37 ± 0.5 °C. Samples (n=6) were drawn off at different time intervals (0–30 min).

Method Validation

The RP-HPLC method was validated for linearity, selectivity, accuracy, precision as per ICH guidelines (ICH, 2005). Four working standard concentrations were prepared from stock solution. The calibration curves were developed by plotting peak area versus concentration (n=5) for each of the active ingredients. The linearity of peak area responses concentrations was demonstrated by linear regression analysis.

Results and discussions

A chromatographic method reported in literature (El-Gizawy et al., 2014) for simultaneous estimation of the three active ingredients in a dosage form was chosen to monitor the dissolution test of two commercially available tablet formulations. The method was optimized: in terms of the flow rate used and column conditions adapted to suit our instrumentation, i.e. isocratic elution at 2 mL/min instead of 0.8 mL/min. Also, the mobile phase pH was adjusted to pH 3 instead of the reported pH value. The optimization of RP-HPLC method resulted in lower retention time of active ingredients. Regression analysis showed that the correlation coefficients for AML, VAL and HCT were found to be $0.998 < R^2 < 1$ and linear equations $y=16.01+23.885$ (AML), $y=33.095x-452.68$ (VAL), $y=60.534x-80.657$ (HCT).

The HPLC chromatograms obtained prove the selectivity of the method. The peaks of AML, VAL and HCT were confirmed by comparing retention time values of sample with those of standard solutions. The RSD values less than 2% for all active ingredients indicate the precision of the method. The chosen RP-HPLC method proved a high degree of accuracy, linearity, selectivity and precision.

The dissolution test of two tablet formulations containing AML, VAL and HCT was evaluated in 37 ± 0.5 °C for 30 minutes. At different time intervals [0, 5, 10, 20, and 30 min (n=6, samples were drawn off at each time interval)], the release rate of tablet dosage form having AML, VAL and HCT was noted. The claimed dissolution and experimental results have adequate similarity. Both formulations fully comply with the pharmacopeia requirements.

In 30 minutes 75% of amlodipine, 80% of hydrochlorothiazide and 80% of valsartan were released from both formulations.

Conclusion

The dissolution test and calculated profiles for each ingredient in formulations A and B can be considered satisfactory. The optimized RP-HPLC can be applied for simultaneous quantitative evaluation of amlodipine, valsartan and hydrochlorothiazide combined in a dosage form and in monitoring the dissolution test. The isocratic method is simple and has an affordable cost.

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