Abstract

Dipeptidyl peptidase-4 inhibitors (DPP-4 I) are being widely used worldwide either as monotherapy or as combination with other members of the family or with Biguanides for treatment of type-2 diabetes (T2D). Biguanide e.g. metformin hydrochloride is another family famous for its anti-hyperglycemic action and is being used since Middle Ages. Different formulations of these two anti-hyperglycemic families are beings synthesized and used. Pharmaceutical companies always look forward for an efficient and simple method in their QC and QA labs to evaluate qualitatively and quantitatively their formulations. An intensive survey of literature was done and found no simple and efficient method for simultaneous resolution of metformin, Trelagliptin succinate and Linagliptin. Therefore, a simple, fast, accurate, efficient and robust reverse phase chromatographic (RP-HPLC) method is developed and validated for simultaneous resolution of one biguanide and two gliptins i.e. Metformin hydrochloride and Trelagliptin succinate and Linagliptin respectively. Different conditions of mobile phase, flow rate, temperature, pH and other parameters were employed as well different columns were used to get resolution of mixture. Optimum chromatographic conditions on which good resolution of active ingredient of mixture in the presence of excipients and degradation products is obtained are as followings: mobile phase: phosphate buffer (pH 2.5) : ACN (85:15 v/v %), elution mode: linear gradient, diluent: same as mobile phase, flow rate: 1.0 mL/mint, injection volume: 20 µL, column: Hibar® RP-18 end-capped column (250 × 4.5mm), 5µm, column temperature: 25 °C, preferential wavelength: 208 nm, analysis time: 15 minutes and detector: diode array detector. Under these condition the sample mixture was efficiently resolved and further developed method was validated for parameters as per ICH guidelines with respect to specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. System specificity was analyzed by observing no emergence of any extra peaks at the time of principle peaks when it was compared with placebo containing solution and blanks. Regression analysis demonstrated efficient correlations with regards to $R^2 \geq 0.996$ over the range of 1-240 µg/mL. Method accuracy was analyzed by recovery experiment and 99.4 ± 0.03 % average recovery of all active analytes was observed. Inter-day precision and repeatability analysis demonstrated
a %age relative standard deviation (% RSD) of less than 2 % for all active ingredients. The LOD was found to be 0.7 µg/mL, 1 µg/mL and 0.5 µg/mL for metformin HCl, Trelagliptin succinate and Linagliptin respectively. Robustness of method was analyzed by performing deliberates changes in mobile phase flow rate (1 ± 0.2 mL/mint), mobile phase pH (2.5 ± 0.5) and by studying acid, base, thermal and photo degradation study of analytes. These altered parameters did not changed the results considerably, so the developed method is highly robust. Short analysis time, peak purities, resolution, precision and robustness made this method efficient for simultaneous analysis of cited analytes and hence, developed method can be used for standard solutions or drug dosage routine analysis in pharmaceutical and other QC or QA laboratories.