Abstract

In this work a method for determination of canagliflozin and its degradation products by HPLC with UV and MS detector was developed. The developed method was used to study the forced degradation of canagliflozin and to investigate the major degradation products resulting from exposure of canagliflozin to oxidative stress. Canagliflozin is a phenolic glycoside derivative and a glucose-sodium transporter 2 inhibitor that stimulates urinary glucose excretion by suppressing glucose reabsorption from the proximal tubule in the kidneys. Canagliflozin is used to control blood glucose levels in patients with type 2 diabetes. In an optimized method, an Agilent Poroshell 120 SB-Aq (2.1 × 100 mm, 2.7 μm) column was used and a mixture of buffer (10mM HCOOH adjusted with ammonium hydroxide to pH 3.5) and acetonitrile as a mobile phase. The method validation included testing of accuracy, repeatability, the limit of detection and quantification, linearity and linear dynamic range, the robustness of the method, and testing of sample stability. The limit of detection of the method was $8.9 \cdot 10^{-5}$ mg ml^{-1} ($2.0 \cdot 10^{-7}$ mol l^{-1}) and the limit of quantification was $3.0 \cdot 10^{-4}$ mg ml^{-1} ($6.8 \cdot 10^{-7}$ mol l^{-1}). At a concentration of 0.3 mg ml^{-1}, the repeatability (n = 7) was 0.17 % and 0.75 % for the retention time and the peak area, respectively. At a concentration of 5.10^{-3} mg ml^{-1}, the repeatability was 0.18 % and 1.58 %. The linearity coefficient of the calibration dependence was 0.9517, the determination coefficient was 0.9997 and the linear dynamic range of the method was $3.0 \cdot 10^{-4}$ – 0.5 mg ml^{-1}. The developed method was used for the forced degradation study of canagliflozin. For the study, the drug was subjected to chemical oxidation (3% H_2O_2) at 50 °C for 1– 3 days and at room temperature for 4 – 7 days. Based on the results, it was found that the rate of degradation is affected by the water content in the sample solvent and pH. The main degradation products were studied by MS. Due to oxidative stress, two oxygen atoms were bound to the molecule of canagliflozin. In the presence of hydrochloric acid, a chlorine atom was bound to the molecule of canagliflozin and formed thus an undesired unrealistic oxidation product. Therefore, H_2SO_4 was more suitable for testing the effect of acidic environment on the chemical oxidation rate. Degradation at room temperature was significantly slower than at 50 °C. A higher amount of degradation products was produced in the environment with

higher water content, while a lower content of degradation products was found with methanol used as an organic modifier.