Abstract

**Aim.** The purpose of this work was to develop a new method for determination of impurity content in Bromhexine Hydrochloride (BHX HCl) active pharmaceutical ingredient (API) using an Ultra High-Performance Liquid Chromatography (UPLC), in favor of shortening the analysis time and sparing the usage of large volumes of mobile phase while preserving a reliable chromatographic performance. In addition, the newly developed method had to be validated to ensure its suitability of use.

**Methods.** Two approaches were used to optimize the chromatographic conditions: 1. Adaption of different column temperatures (from 25–40°C) at flow rate 0.35 mL/min; 2. Adaption of different flow rates (from 0.35-0.6 mL/min) at 40°C column temperature. Injection volume was the same in both cases-1.6μL. Optimization of chromatographic conditions was based on the resolution between BHX HCl and Impurity C, the peak symmetry and column backpressure limit. The tests employed to validate the method were: linearity, limit of detection, limit of quantification, precision, accuracy and selectivity of analytical procedure. **Results.** The most optimal combination of chromatographic conditions was a flow rate of 0.5 mL/min at column temperature of 40°C. During method validation, linearity test showed a high correlation factor ($R^2=0.998$) between the peak areas and their corresponding concentrations. The detection limit was 0.006 % and the quantification limit was 0.02 %. The resulting relative standard deviation (RSD%) of six analyses of impurity content in precision testing was within the range guided by International Conference of Harmonization -ICH. The recovery of added Impurity C for accuracy test complied with the limit of 85–115% (by ICH guidelines). The selectivity of method showed no interference with placebo, in cases of BHX HCl preparation analysis. **Conclusion.** In comparison to the conventional method using HPLC, the analysis time was three times shorter, lower volumes of mobile phase were used and the efficiency of chromatographic performance was higher. Validation of this new method ensures its applicability in Pharmaceutical Analysis for determination of related substances in Bromhexine Hydrochloride (API).