ABSTRACT

Mephenoxalone is an active pharmaceutical ingredient which is used for muscle relaxation and anxiolytic treatment. This pharmaceutical ingredient is from the oxazilidinone group, which further belongs to group of β-blockers. β-blockers have an inhibitory action on the central nervous system and are used for the treatment of the anxiosity symptoms.

I developed a sensitive, selective and fast HPLC method for determination and quantification of impurities in mephenoxalone active ingredient as well as in pharmaceutical formulation, which can be used instead of TLC. To determine both polar and non-polar impurities, I developed a gradient HPLC method. For the separation of impurities, Kinetex C-18 non-polar column with 2.6 µm particles was used at temperature up to 35 °C, and mobile phase A: Milli Q water and mobile phase B: methanol. Quantification of impurities was performed by external standard method. Compounds were determined spectrophotometricaly by measuring the absorbance at wavelength 222 nm. Because one of evaluated impurities exceeded the ICH limit of identification, it was necessary to isolate it by preparative chromatography. Molecular mass of the impurity determined with LC-MS was 403 Da, with MS/MS we proposed the fragmentation. Using NMR spectroscopy, we determined its structure.

Evaluation of the method was performed by using validation parameters: system suitability test, intraday repeatability, ruggedness of the method, accuracy of the method, linearity of the method, selectivity/specificity of method, repeatability of measurements, stability of standard solutions, stability of sample solutions, determination of LOD and LOQ, confirmation of LOQ, robustness of the method (variation of flow rate and temperature) and determination of response factor.

Analytical method was found to be precise for repeatability of injections, accurate, linear and selective. Mephenoxalone and MEF-D responses were linear in range from LOQ (0.05 %) to 2.0 % of initial sample concentration. Guaiphenesin response was found to be linear in range from LOQ (0.05 %) to 0.6 % of initial sample concentration. Sample and standad solutions were stable for 48 h, if kept in cool auto sampler and protected from light, as defined in analytical procedure. They are stable for 48 h, if kept at room temperature, protected from light. It was confirmed, that the method is robust at column temperature 30 °C and 40 °C and at flow rate 0.8 mL/min and 1.2 mL/min. LOQ is determined at concentration of 0.00025 mg/mL, which corresponds to 0.05 % of mephenoxalone, guaifenesin and MEF-D by reference to the concentration of mephenoxalone in initial sample solution. LOD is determined at concentration of 0.00005 mg/mL for mephenoxalone, guaifenesin and MEF-D which corresponds to 0.02 % of mephenoxalone, guaifenesin and MEF-D by reference to the concentration of mephenoxalone in initial sample solution.

Key words: mephenoxalone, impurities, high performance liquid chromatography