ABSTRACT

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry

Candidate: Mgr. Tereza Bažantová
Supervisor: PharmDr. Ludmila Matysová Ph.D.
Title of rigorous thesis: Development and validation of HPLC method for analysis of pharmacopoeial eye drops with pilocarpine.

High-performance liquid chromatography method (HPLC) for the determination of pharmacopoeial pilocarpine-hydrochloride eye drops with a concentration of 1 and 2% was developed and partially validated.

A variety of HPLC methods have been described in the specialized literature for the determination of pilocarpine-hydrochlorid in eye drops. The methods use different stationary and mobile phases and the differences are in the time of analysis.

This work describes development of the method based on the work of authors El-Deeb S, Schepers U and Wätzig H.: Evaluation of Monolithic C18 HPLC columns for the fast analysis of pilocarpine-hydrochloride in the presence of its degradation products published in 2006 in the journal Pharmazie [17]. It was performed using a reverse phase liquid chromatography with a monolithic column Chromolith High Resolution RP 18 endcapped 100 x 4.6 mm, Merck, Germany. The used mobile phase was composed of buffer pH 3 and methanol in the ratio 98:2 (V/V) at the flow rate 1 ml / min. Results were analyzed by UV-VIS detector at a wavelength of 220 nm. The method of the internal standard was used for the quantitative determination. Phenylephrine hydrochlorid was chosen as an internal standard.

The method enables the determination of pilocarpine-hydrochloride and pilocarpic acid. The monitored substances were eluted in the order: isopilokarpin, pilocarpine, pilocarpic acid and isopilocarpic acid. The total time of the analysis is less than 10 minutes.

It was performed following validation parameters of the method: precision, accuracy, linearity, selectivity, stability pilocarpine-hydrochloride solution for 72 hours both at 5 ± 3°C and 20 ± 5°C and access to or protected from light and system suitability test.

The method is selective, precise and provides good results and is linear in range from 12.5 to 75.0 mg/100 ml of pilocarpine hydrochloride.

Only pilocarpic acid was closely studied from all four related substances, because its increasing in eye drops is mainly due to hydrolysis assumed. In the absence of pilocarpic acid standard only system suitability test and the test of the selectivity was possible to make.