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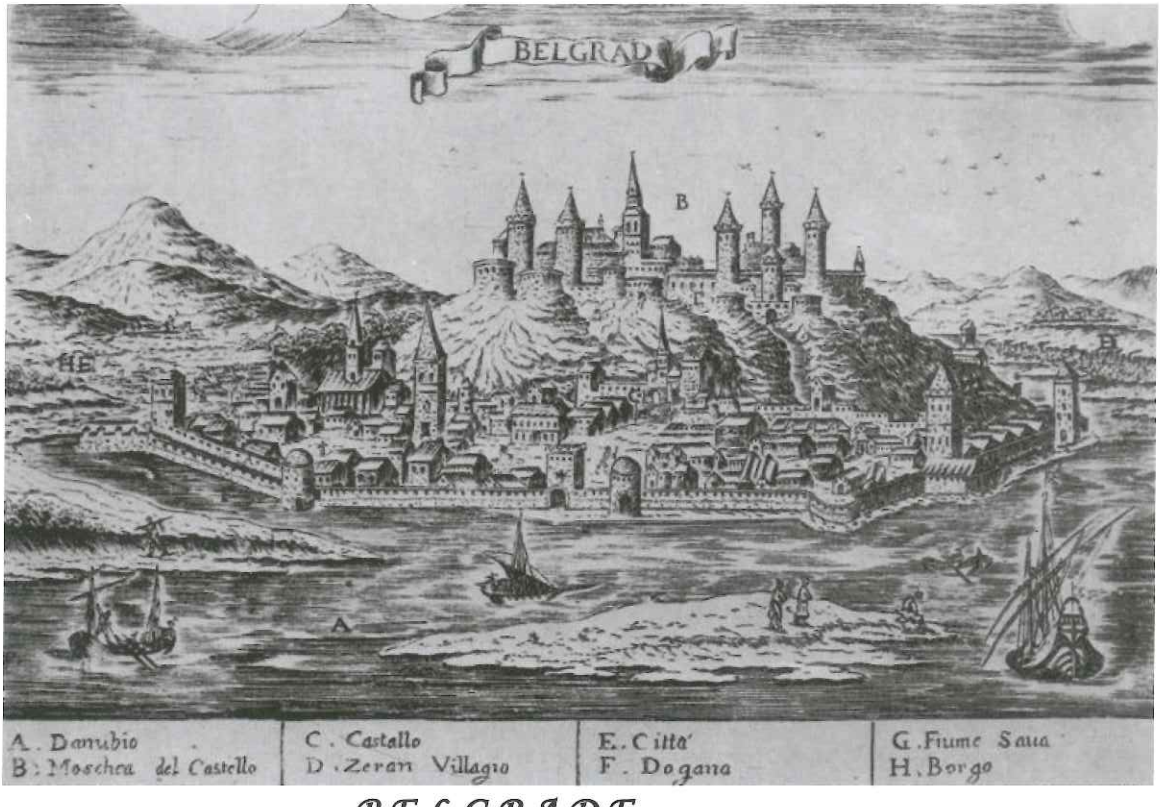

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OPTIMIZATION AND VALIDATION OF A HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF MOXONIDINE AND ITS IMPURITIES

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ABSTRACT

Fast and simple hydrophilic interactions liquid chromatography method was developed and validated for the analysis of moxonidine and its impurities in pharmaceutical dosage form. The separation was performed on Zorbax RX-SIL column (250 mm x 4.6 mm, 5 μm) using mixture of acetonitrile and 40 mM ammonium-formiat buffer (pH 2.8) in ratio 80:20 (v/v) as mobile phase at flow rate of 1 mL/min, detection at 255 nm and temperature of 25 °C. Under the selected chromatographic conditions separation and analysis of five examined compounds in the mixture is enable within 12 minutes. The validation criteria for selectivity, linearity ($r \geq 0.9976$), accuracy (*recovery*: 93.66 %-114.08 %), precision (RSD: 0.56%-2.55%) and robustness of the method were fulfilled. The obtained values of the limit of detection and quantifiqation revealed that the method can be used for determination of impurities levels below 0.1%.

INTRODUCTION

Moxonidine belongs to the second generation of centrally acting antihypertensive drug that exhibits high binding affinity for I_1-imidazoline receptor and minor activity at α_2-adrenoceptors. It is used in therapy as antihypertensive as well as to improve metabolic profile of patients with hypertension and the type 2 diabetes, or with an impaired glucose tolerance [1]. The European Pharmacopoeia lists 4 related substances of moxonidine (A, B, C and D) (Figure 1.) and prescribes their determination by high performance liquid chromatography using octylsilyl silica gel as stationary phase and the ion-pair reagents as a component of the mobile phase. Because moxonidine and its impurities possess basic character, they are completely ionized in an acid medium. In order to avoid application of ion-pair reagents hydrophilic interaction chromatography (HILIC) which allows the analysis of charged compounds could be also applied. HILIC is

described as a useful alternative to reversed-phase chromatography and in the HILIC mode, an aqueous–organic mobile phase is used with a polar stationary phase to provide normal-phase retention behavior. Silica and amino columns with aqueous–acetonitrile mobile phases offer potential for use in the HILIC [2].

EXPERIMENTAL

Moxonidine, 4-chloro-*N*-(imidazolidin-2-ylidene)-6-methoxy-2-methylpyrimidin-5-amine; **Impurity A**, 4,6-dichloro-*N*-(imidazolidin-2-ylidene)-2-methylpyrimidin-5-amine (6-chloromoxonidine); **Impurity B**,*N*-

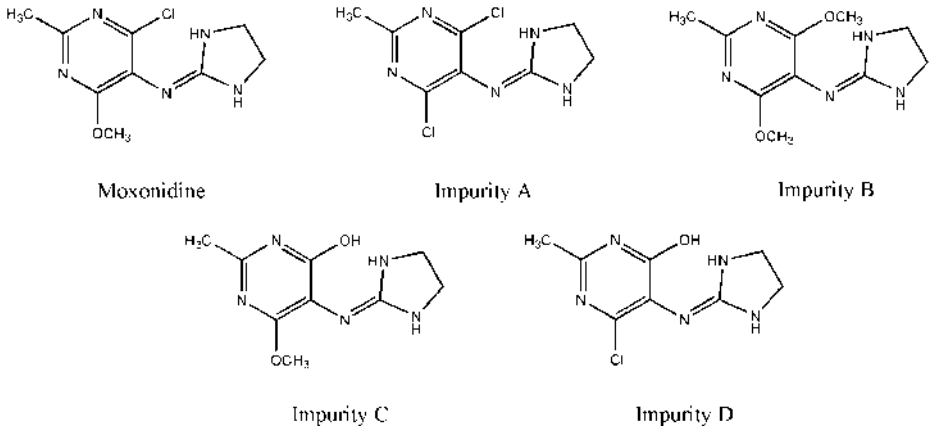

Figure 1. Chemical structure of moxonidine and its impurities

(imidazolidin-2-ylidene)-4,6-dimethoxy-2- methylpyrimidin-5-amine (4-methoxymoxonidine); **Impurity C**,5-[(imidazolidin-2-ylidene)amino]-6-methoxy-2-methylpyrimidin-4-ol (4-hydroxymoxonidine,); and **Impurity D,** 6-chloro-5-[(imidazolidin-2-ylidene)amino]-2-methylpyrimidin-4-ol (6 desmethylmoxonidine) were obtained from Chemagis (Bnei Brak, Israel) (Figure 1.). The Moxogamma® 0.4 mg film tablets were manufactured by Worwag Pharma (Böblingen, Germany).

All experiments were performed on the Agilent Technologies 1200HPLC system usingZorbax RX-SIL, 250 mm x 4.6 mm, 5 µm column as stationary phase and mixture of acetonitrile and ammonium-format buffer as a mobile phase. The acetonitrile content (70% to 80%), pH of the aqueous phase (2.8 to 4.2) and concentration of ammonium formate in aqueous phase (20 mM to 60 mM) were optimized according to the matrix of central composite design obtained by Design-Expert 7.0.0 programe (Stat-Ease, Minneapolis, MN, USA). Investigation of theinfluence of the examined factors on the retention behavior of the tested compounds was carried out by means of the soft independent modeling of class analogy SIMCA P+ 12.0 program.

RESULTS AND DISCUSSION

Moxonidine and its impurities are compounds with similar polarity (calculated logP values spread in the range 1.57 for the most polar impurity C to 2.49 for impurity A which has the most pronounced lipophilic properties due to the presence of 2 clorine atoms), thus separation of such compounds in a short period of time is a challenge for analysts. In order to select the chromatographic factors which will be examined during the optimization process a few preliminary experiments have been performed in which content of acetonitrile, pH and the concentration of the buffer, column temperature and flow rate were altered. Based on preliminary results it is observed that retention behavior of examined compounds was most affected by the components of mobile phase such as content of acetonitrile, pH and the concentration of the buffer which were further screened in CCD (Central Composite Design). During method optimization retention factors of analyzed compounds and resolution between critical peak pairs (A/B and C/D) were followed as responses. According to created PLS (Partial Least Squares) models the factors with the significant influence on resolution were identified. The optimal separation conditions were achieved using mobile phases consisting of acetonitrile : 40 mM buffer pH 2.8 (80:20 v/v) (T=25 °C, F =1 mL/min, λ=255 nm) which provide a total analysis time of 12 minutes. Under the selected chromatographic conditions validation of the method was conducted in accordance with ICH guidelines in order to ensure adequate selectivity, linearity, accuracy, precision and robustness. No intereference of tablet formulation was observed using the developed chromatographic method which confirms good selectivity of the method. The linearity was evaluated by analyzing nine working solution of impurities over the concentration range 0.0375-0.6 μg mL^{-1} for impurities A and B, and 0.075-1.2 μg mL^{-1} for impurities C and D while in case of moxonidine examination was performed in concentration range 0.05-0.15 μg mL^{-1}. The obtained correlation coefficients 0.9992, 0.9991, 0.9976, 0.9982 and 0.9976 for moxonidine and impurities A, B, C, and D respectively indicated high linearity over the examined concentration range. The assessment of method precision was done by calculating relative standard deviation (RSD): moxonidine (0.56%), impurity A (2.55%), impurity B (1.99%), impurity C (2.01%) and impurity D (2.19%). The obtained values fulfilled the required criteria (RSD 2% for active ingredients, and 10% for impurities C and D, and 15% for impurities A and B [2]. The accuracy of the proposed method was evaluated according to the obtained recovery values (concentration levels of 80%, 100% and 120% of target concentration for moxonidine, and LOQ, 100% and 120% of each

mpurity target concentration) which were in the range 93.66%-101.73%. Experimentally determined values of limit of detection were 0.011 μg mL^{-1} for impurities A and B, and 0.022 μg mL^{-1} for impurities C and D, while values of limit of quantification were 0.0375 μg mL^{-1} for impurities A and B, and 0.075 μg mL^{-1} for impurities C and D. No observable effects on resolution between moxonidine and its impurities was perceived during small variations of working conditions, showing that the proposed method is robustness. The validated method was successfully applied for the analysis of moxonidine purity in available moxonidine tablets (Moxogamma® 0.4 mg), Figure 2. The obtained results (97.5 % for content of moxonidine, 0.68 % for impurity C, 0.87% for impurity D, and below LOQ values for impurities A and B) were in accordance with manufacture specification (impurities A i B below 0.5%, and impurities C and D below 1%).

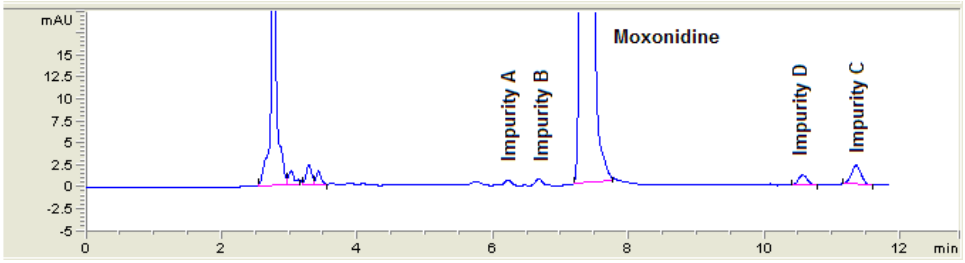

Figure 2. The representative chromatogram of sample solution

CONCLUSION

According to the obtained results the proposed method can be used as fast, simple and reliable for determination of moxonidine and its four impurities in pharmaceutical dosage forms.

Acknowledgement

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