Macedonian pharmaceutical bulletin, 69 (Suppl 1) 233 - 234 (2023)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2023.69.03.114
Short communication

Development and validation of a RP-HPLC method for simultaneous determination of Ciprofloxacin and Ornidazole in a solid dosage formulation

Irena Slaveska Spirevska*, Marjan Piponski, Tanja Bakovska Stoimenova, Kristina Grncharoska, Martina Miloshevska, Marijana Nikoloska, Maksim Osman Nikolov, Mimoza Sokleva Ivanova

Replek Farm Ltd., Kozle 188, 1000, Skopje, N. Macedonia

Introduction

Ciprofloxacin, chemically known as 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, is a board spectrum fluoroquinolone antibacterial agent, used to treat a number of bacterial infections. It produces its action by inhibiting a bacterial DNA gyrase and topoisomerase IV (Kawahara, 1998).

Ornidazole, 1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol, is used as an anti-infective agent. It is used in treatment of amoebiasis, giardiasis, trichomoniasis and anaerobic infections. The mechanism of action involves reactivation by reduction of the nitro group, and production of toxic derivatives and radicals (Chaudhari, 1997).

Ciprofloxacin and Ornidazole are indicated for treatment of infections caused by strains sensitive to the combination of ciprofloxacin and ornidazole. The combination of these two active substances is used in treatment of bacterial and parasitic infections. It is also used to treat diarrhea, gynecological and pelvic infections. It also helps treat mixed infections of teeth and gums, urinary tract infections, infectious and inflammatory diseases of pelvic minor organs including post- operative and post-abortion prophylaxis, bronchitis, chronic bronchitis exacerbation, pneumonia, infected bronchiectasis, lung abscess, pleural empyema, external otitis and otitis media, mastoiditis, skin, soft tissues, joint and bone infections and etc. (Chaudhari, 1997).

Ciprofloxacine is official in European Pharmacopoeia (European Pharmacopoeia, 11th ed., 2023), United States Pharmacopoeia (The United States Pharmacopeia / The National Formulary (USP 43-NF 38), 2020) and British Pharmacopoeia (British Pharmacopoeia, 2022). Ornidazole is not official in any pharmacopoeia. There are a few non-pharmacopoeial methods for their simultaneous determination in bulk, pharmaceutical dosage forms and biological fluids (Rote and Saudagar, 2015).

The aim of our work was to develop and validate a simple and rapid reversed-phase high performance liquid chromatography (RP-HPLC) method for simultaneous quantification of the active substances, Ciprofloxacin, as hydrochloride and Ornidazole, in a solid dosage form such as film coated tablets.

Materials and methods

The reagents that have been used are: methanol, *o*-phosphoric acid (H_3PO_4) and triethylamine purchased from Carlo Erba. All the chemicals used were of Ph. Eur grade. Reference standards batches: Ciprofloxacin Hydrochloride CRM and Ornidazole RS were purchased from Sigma-Aldrich. Solid dosage form, film coated tablets, which contains Ciprofloxacin, as hydrochloride/ Ornidazole (500+500) mg, as well as placebo were obtained from Replek Farm Ltd., Skopje, N. Macedonia. The syringe filters Nylon and RC, 0,45 µm, were purchased from Agilent Technologies (USA).

Chromatographic analyses were performed on a Shimadzu Prominence System and Shimadzu Nexera XR System, both with low pressure gradient (LPG) quaternary pump with degasser, autosampler, controller, column oven and PDA detector. Signals were monitored and processed using the Lab solutions software. Additional instrumental equipment was used: analytical balance Mettler Toledo AG285, pH meter Metrohm 827, US bath Branson 3510, and IKA orbital shaker KS 260 basic

Separation was performed on chromatographic column Purospher Star C18e, 125 mm × 4.0 mm, 5 µm.

irena.slavevska@replek.mk

The column temperature was maintained at 35°C. An isocratic elution was used, with flow rate of 1.0 mL/min. The injection volume was 10 μL and wavelength detection was at 300 nm. A mixture of Acetonitrile and 0.1% o-Phosphoric acid, adjusted to pH 6.0 with triethylamine was used as mobile phase. Methanol and 0.1% o-Phosphoric acid were used as diluent.

Results and discussion

In this study, the established method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guideline for validation of analytical procedures (International Conference on Harmonization, 2005), in respect to selectivity, linearity, precision and accuracy. During selectivity testing, no interference from the formulation excipients was observed.

The linearity of the method was proved in five concentration levels, for each substance of interest, in the range between 50% and 150% of the working concentration of Ciprofloxacin, as hydrochloride and Ornidazole in the test solution. The results confirmed the linear relationship between peak areas of the examines analytes and the respective concentrations. The results were evaluated by linear regression analysis and following results were obtained: correlation coefficient > 0.9990 (0.9999 for Ciprofloxacin, as hydrochloride and 1.0000 for Ornidazole) and relative standard deviation (RSD) of the response factors for each concentration level < 2 %, in all cases.

The precision of the RP-HPLC method was evaluated through repeatability (system and method repeatability) and intermediate precision. The system repeatability was evaluated by six determinations of the peaks areas of standard solutions and the obtained values for relative standard deviation (RSD) were below 1%. Method repeatability analysis performed on six different test solutions showed that RSD was not more than 1 %, for each active substance. The intermediate precision assessed on two consecutive days, by two different analysts, on two different HPLC systems showed that the RSD value obtained from analysis of test solutions is not more than 2 %.

Accuracy of the method was studied by recovery investigation. The accuracy of the method was tested using nine determinations over three concentration levels in the interval between 70% and 130% of the working concentration of Ciprofloxacin, as hydrochloride and Ornidazole in the test solution. The obtained recovery values were within the range of 100 ± 2 %, for each substance.

To determine the robustness of the analytical method for simultaneous determination of Ciprofloxacin, as hydrochloride and Ornidazole, the one factor at a time approach was used. The robustness testing showed that the obtained results are not adversely affected by small variations in method parameters.

Conclusion

The developed RP-HPLC method provides simple, specific, accurate, precise, robust and reproducible simultaneous quantitative analysis of Ciprofloxacin, as hydrochloride and Ornidazole from the solid dosage formulation. The established method was validated and proved as suitable for its intended use, in accordance to ICH guideline Q2 (R1), guideline for validation of analytical procedures. The proposed method, by use of simple sample preparation, low-cost reagents, and short run time, provides reproducible simultaneous quantification of the active substances of interest and can be successfully used for routine analysis of solid dosage formulations especially in pharmaceutical industry.

References

Rote, A.R., Saudagar, R.B., 2015. High performance liquid chromatographic determination of ciprofloxacin hydrochloride and ornidazole in human plasma. J. Pharm. Anal. Chem. 1(1), 1-4. doi: 10.4172/2471-2698.1000103

British Pharmacopoeia, 2022. British Pharmacopoeia Commission - The Stationery Office: London, UK.

Chaudhari, S.K., 1997. Quintessence of Medical Pharmacology. 1st ed. New Delhi: New Central Book Agency (P) Ltd., 484.

European Pharmacopoeia, 11th ed., 2023. European Directorate for the Quality of Medicines & Healthcare – Council of Europe: Strasbourg.

International Conference on Harmonization, 2005. Validation of analytical procedures: Text and methodology Q2(R1). ICH, Geneva, Switzerland.

Kawahara, S., 1998. Chemotherapeutic agents under study. Nippon Rinsho. Japanese journal of clinical medicine, 56(12), 3096-3099.

The United States Pharmacopeia / The National Formulary (USP 43-NF 38),2020. The United States Pharmacopeia Convention: Rockville.