Extended Abstract Journal of Clinical and Bioanalytical Chemistry 2021
Vol.5 No.1

High Performance Liquid Chromatographic Determination of Ciprofloxacin Hydrochloride and Ornidazole in Human Plasma

AR Rote

Sapkal College of Pharmacy, INDIA

A simple, rapid, sensitive selective chromatographic method has been developed for simultaneous determination of ciprofloxacin hydrochloride and ornidazole in human plasma by using internal standard. The method depends on reverse phase high performance liquid chromatography. The plasma sample was extracted using methanol: formic acid (5.5:0.5, v/v). A concentration range from 100-400 ng/ml for both drugs was used for calibration curve. The percent recoveries of ciprofloxacin and ornidazole were found to be 71.49-75.68 and 73.78-83.1 respectively. The mobile used consist of acetonitrile: methanol: water: triethylamine (40:20:40: 1% v/v/v) and flow rate 1 ml/min in isocratic mode. The separation was carried out by UV detector at wavelength 300 nm. The stability of ciprofloxacin hydrochloride and ornidazole in plasma were confirmed during three freeze-thaw cycles (-20°C), on bench during 12h, and post preparative stability study. The proposed method was validated statistically and by performing recoveries study for determination of ciprofloxacin hydrochloride and ornidazole in human plasma. Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4- oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] is broad spectrum fluoroquinolone antibacterial agent. It is effective in the treatment of a wide variety of infections including infections of bones and joints, particularly those caused by gram-negative pathogens. Grampositive bacteria are generally susceptible or moderately susceptible. Ciprofloxacin only treats bacterial infections; it does not treat viral infections such as the common cold. For certain uses including acute sinusitis, lower respiratory tract infections and uncomplicated gonorrhea. Ciprofloxacin are not considered a first-line agent. Ciprofloxacin is one of the few broad spectrum antibacterial available in both intravenous and oral formulations. The primary mechanism of action of ciprofloxacin is inhibition of bacterial DNA gyrase . Ornidazole, 1-chloro-3-(2-methyl-5nitro-1H-imidazol-1-yl) propan2-ol, used as an anti-infective agent. Use of ornidazole in combination with fluoroquinolone in the treatment of pelvic inflammatory disease and intra-abdominal infection. It is an antimicrobial agent used in treatment of susceptible protozoal infections and anaerobic bacterial infection. It is prescribed to treat different health conditions due to anaerobic infections, amoebic liver abscess, amoebic dysentery, hepatic amoebias; the drug is available in both intravenous and oral formulations. The primary mechanism of action of ofloxacin appears to be the specific inhibition of DNA gyrase (topoisomerase II). This enzyme is responsible for the negative super coiling of the bacterial DNA and consequently for its topological configuration, governing functions such as RNA transcription, protein synthesis, DNA replication and repair functions . The literature survey revealed that variety of analytical methods reported for estimation of ciprofloxacin in human plasma and other biological fluids , spectrophotometry , HPLC , HPTLC , and spectrophotometric methods have been reported for estimation of ornidazole alone as well as in combinations. RP-HPLC method was reported for estimation of Ciprofloxacin Hydrochloride and Ornidazole in Combined Pharmaceutical Dosage Form . However no method was reported for simultaneous determination of ciprofloxacin hydrochloride and ornidazole in human plasma by RP-HPLC using liquid-liquid . One of the most difficult parts during the method development was to achieve a low 200, mid 300 and high 400 ng/ml in triplicate and reproducible recovery from the solvent which is used for extraction of the drug and also difficult task to select such single extracting solvent from which both the drugs are extracted. Different solvents were tried for the extraction of ciprofloxacin hydrochloride and ornidazole from human plasma. Five ml each of hexane and toluene were tried for the precipitation of plasma but the recovery was very less. Ethyl acetate and chloroform were also tried up to 5.0 ml. It gave 50 to 55% of recovery because of less precipitation of protein from plasma. At the last methanol was tried and 70 to 80% of recovery was obtained. It was found that the addition of formic acid (0.5 ml) increases the precipitation of protein and also the recovery which is reproducible and high as compare to other solvents. So methanol and formic acid (5.5 ml: 0.5 ml) was kept as final solvent for extraction of ciprofloxacin hydrochloride and ornidazole. For HPLC the method demonstrated good chromatographic specificity with no plasma interference at the retention times of ciprofloxacin hydrochloride, ornidazole and internal standard, representative chromatogram of plasma spiked with ciprofloxacin hydrochloride ornidazole and internal standard tinidazole. Ciprofloxacin hydrochloride, ornidazole, tinidazole well resolved with good symmetry and retention time of 2.13, 4.91, 6.86. Initially plain solvent like acetonitrile, water, methanol were used but retention time for the all three drugs are above 10 min. Then methanol and water mixed in the ratio of (70:30 v/v) and vice-versa but no change was observed. Then addition of acetonitrile was done which leads to shorten the retention time and also shows good resolution between ciprofloxacin hydrochloride, ornidazole and tinidazole but the peak shape were not good. Selectivity should be assessed to show that the intended analytes are measured and that their quantitation is not affected by presence of biological matrix. There was no significant interference observed and no changes in retention time of ciprofloxacin and ornidazole which shows the method is selective. Sensitivity of the method is defined as the lowest concentration that can be measured with an acceptable limit of accuracy and precision which is lower than 20% [20]. The accuracy and precision at lower limit of quantitation (LLOQ) analyzed by using five replicates (n=5) of the sample (100 ng/ml) at the LLOQ concentration. The accuracy is determined by %RE at this LLOQ concentration. The lower limit

of quantitation which could be detected and were found to be % Relative Error=11 and %Relative Standard Deviation=6.54 for ciprofloxacin hydrochloride and % Relative error=10.80 and % Relative Standard Deviation=1.75 for ornidazole which is within acceptable limit. Absolute recoveries were calculated by comparing peak areas obtained from freshly prepared samples extracted with unextracted standard solutions of same concentration. Recovery data was determined in triplicate at three concentrations (low 200, mid 300 and high 400 ng/ml) as recommended by FDA guidelines [19]. Recovery was calculated with comparison of areas obtained with standard drug spiked with plasma before extraction (unextracted) at room temperature and area obtained of slandered drug with spiked plasma after extraction (extracted). The recovery at three concentrations 200, 300, 400 ng/ml was found to be 71.49, 77.23, 75.68% for ciprofloxacin hydrochloride and 73.78, 71.15, 83.1% for ornidazole. The mobile phase was selected as mixture of acetonitrile, methanol, water and triethylamine in the ratio of (40: 20: 40: 1%, v/v/v). The mobile phase was degassed prior to use under vacuum by filtration through Nylon 66 membrane of 47 mm size and 0.45 µm thicknesses with 20 µl injection. The detector was set at 300 nm. Flow rate was used 1 ml/min. An isocratic mode was used for the separation of the analyte. The calibration curve was constructed for each of ciprofloxacin hydrochloride and ornidazole 100, 150, 200, 250, 300, 350 and 400 ng/ml in triplicate by plotting the peak response ratio of ciprofloxacin hydrochloride to IS versus concentration of ciprofloxacin hydrochloride and ornidazole to IS versus concentration of ornidazole in plasma. Correlation coefficients were found to be 0.9995 and 0.9993 for ciprofloxacin hydrochloride and ornidazole respectively. Linearity's were found over the range 100-400 ng/ml. The lower limit of quantification was defined as lowest concentration in the calibration curve. The ciprofloxacin hydrochloride and ornidazole can be determined at LLOQ 100 ng/ml. The proposed method was validated statistically and by performing recoveries study for determination of ciprofloxacin hydrochloride and ornidazole in human plasma. Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4- oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] is broad spectrum fluoroquinolone antibacterial agent. It is effective in the treatment of a wide variety of infections including infections of bones and joints, particularly those caused by gram-negative pathogens. Grampositive bacteria are generally susceptible or moderately susceptible. Ciprofloxacin only treats bacterial infections; it does not treat viral infections such as the common cold. For certain uses including acute sinusitis, lower respiratory tract infections and uncomplicated gonorrhea. 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