Method Development for the Quantification of Ciprofloxacin in Human Plasma

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INTRODUCTION

The bactericidal fluoroquinolone ciprofloxacin has a broad spectrum of activity and is low in toxicity. It is used to treat a variety of infections ranging from urinary infections to skin and soft tissue infections. [1]

AIMS

This work describes the development of a simple alternative method for the quantification of ciprofloxacin in human plasma.

METHOD

Materials and reagents: Reagents used were ciprofloxacin, ofloxacin and sulfamethazine (Sigma Aldrich), acetonitrile, orthophosphoric acid and analytical grade type 1 water (Fisher scientific) and disodium hydrogen phosphate (Scharlau). A mobile phase made up of phosphate buffer (pH 3.0) and acetonitrile 70:30 v/v was prepared.

HPLC instrumentation: A Varian® ProStar HPLC unit with a UV– visible detector and a reversed– phase ACE 5 C18 column (250 x 4.6mm; 5µm particle size) were used.

Choosing a solvent for ciprofloxacin: Ciprofloxacin is insoluble in water and was therefore first dissolved in methanol. Upon analysis it could be noted that another peak was eluting together with that of ciprofloxacin and the solvent was changed to 0.2M hydrochloric acid.

With hydrochloric acid as the diluent, chromatographic peaks exhibited shouldering and ciprofloxacin was consequently dissolved in the mobile phase. Analysis of ciprofloxacin in plasma was then performed with ofloxacin as the internal standard. Protein precipitation was used for sample preparation.

Changing the internal standard: When observing peaks produced it could be noted that ciprofloxacin and ofloxacin were eluting at the same time. To increase resolution the amount of acetonitrile in the mobile phase was decreased from 30% to 23%. When this did not produce a shift in retention time of the internal standard, sulfamethazine was used instead.

RESULTS

The unknown peak which eluted when analysing ciprofloxacin in methanol was probably due to an esterification reaction which occurred between the carboxylic acid functional group and the alcoholic solvent. This finding agrees with that made in a study by Muchohi et al.[2] Peak shouldering observed when ciprofloxacin was dissolved in dilute hydrochloric acid was due to poor pH control (Figure 1) and this was resolved by dissolving the analyte of interest in the mobile phase (Figure 2). Since decreasing the amount of acetonitrile in the mobile phase did not result in any shift in the retention time of ofloxacin, a different internal standard, sulfamethazine was chosen. This has similar chromatographic behaviour to ciprofloxacin. Peaks produced using sulfamethazine were well resolved from those of the analyte of interest.

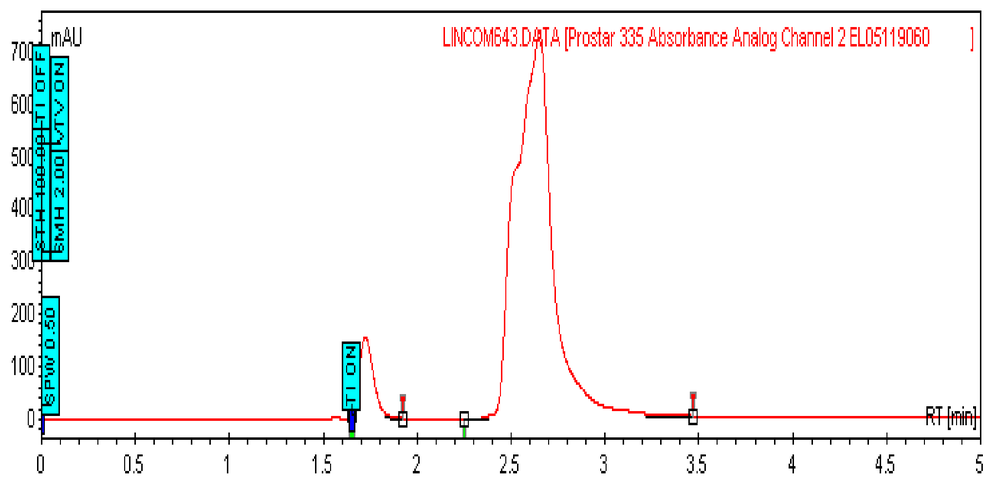

Figure 1: Ciprofloxacin in dilute hydrochloric acid

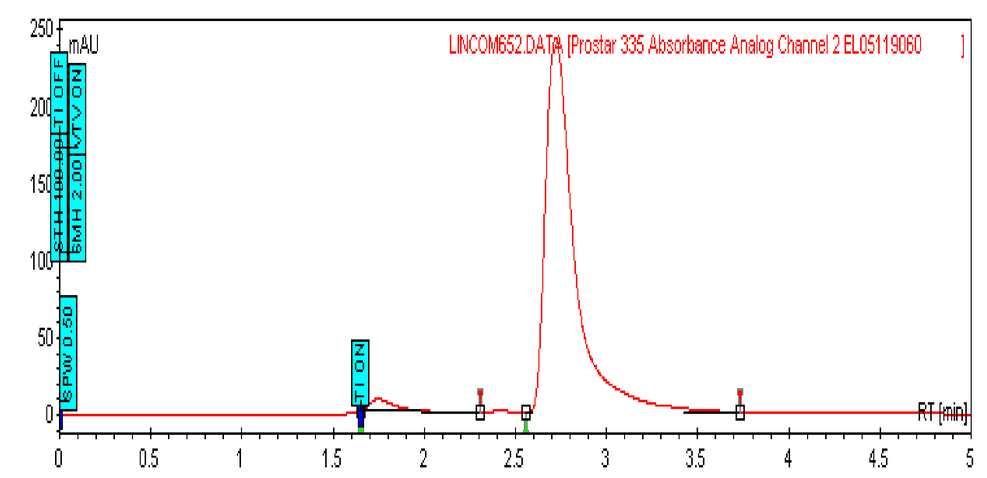

Figure 2: Ciprofloxacin in mobile phase (phosphate buffer and acetonitrile 70:30v/v)

CONCLUSION This developed method provides efficient analysis of ciprofloxacin in plasma with a total run time of less than 5 minutes. This method is being currently validated to be later used and applied in clinical and pharmacokinetic studies.

Reference(s) 1. Olivera ME, Manzo RH, Junginger HE, Midha KK, Shah VP, Stavchansky S et al. biowaiver monographs for immediate release solid oral dosage forms: ciprofloxacin hydrochloride. J Pharm Sci. 2011; 100(1): 22-33.

2. Muchohi SN, Thuo N, Karisa J, Muturi A, O. Kokwaro G, Maitland K. Determination of ciprofloxacin in human plasma using high– performance liquid chromatography coupled with fluorescence detection: application to a population pharmacokinetics study in children with severe malnutrition. J Chromatogr B Analyt Technol Biomed Life Sci 2011; 879(2): 146-52 .