

EVALUATION OF DIFFERENT EXTRACTION METHOD FOR THE ISOLATION OF CIMETIDINE AND RANITIDINE FROM HUMAN PLASMA FOR HPLC ANALYSIS

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Keywords: Cimetidine, ranitidine, plasma and HPLC

Abstract. Cimetidine and ranitidine are classified as the H₂-receptor antagonists (H₂ antagonist). These classes of drugs used to block the action of histamine on parietal cells in the stomach, decreasing the production of acid by these cells. H₂ antagonists are widely used in the treatment of gastro oesophageal reflux, gastric and duodenal ulceration. Malaysian government has implemented bio equivalence requirements for manufacturing all generic drugs, cimetidine and ranitidine are also listed with other high demand generic drugs. The study focused on the preliminary chromatographic detection and determination of cimetidine and ranitidine in human plasma which eventually can be used for bioequivalence study of generic formulation of cimetidine and ranitidine. Different sample extraction process were evaluated namely protein precipitation, solvent extraction and solid phase extraction (SPE). It was found for both cimetidine (89%) and ranitidine (87%) showed a better recovery using SPE. With solvent extraction cimetidine showed comparable recovery (85%) using ethyl acetate as a solvent of choice and dichloromethane for ranitidine (82%). For the protein precipitation technique both cimetidine (75%) and ranitidine (73%) showed a lower recovery compared to the other method. HPLC method was used for the analysis of the spiked plasma samples. Chromatographic separation was obtained using a C18 column and mobile phase was a combination of phosphate buffer and acetonitrile. Elution of cimetidine and ranitidine were detected with a UV-Vis detector at 230 nm wavelength.

Introduction

H₂ receptor antagonists' cimetidine and ranitidine are used to block the action of histamine on parietal cells in the stomach, which will results in decreasing the production of acid. H₂ antagonists are used in the treatment of dyspepsia which can cause abdominal pain, heart burn, bloating and nausea. It is also related to the gastro esophageal reflux or gastritis. Cimetidine and ranitidine suppress the parietal cells normal acid secretion and also reduce the stimulated secretion of acid due to food intake.

Cimetidine (C₁₀H₁₆N₆S) molecular mass is 253.34 g/mol. Pharmacokinetic data reveals that it shows 70% bioavailability and has a half life of 2 hours. It has hepatic metabolism and excreted through renal excretion. Ranitidine (C₁₃H₂₂N₄O₃S) molecular weight is 314.4 g/mol. It shows 88% bioavailability according to the pharmacokinetic findings. It also shows a hepatic metabolism. It has a half life of three hours and excreted almost 70-80% through renal excretion.

Analysis of cimetidine and ranitidine in dosage forms are available in pharmacopeia like USP and BP. Mostly they are following spectrophotometric methods or HPLC (High Performance Liquid Chromatography) methods for the qualitative and quantitative analysis of dosage forms [1]. There

are reports on the study of the cimetidine and ranitidine in different biological matrices like human plasma [2, 4, and 7] urine [3, 5] animal plasma [6]. One of the areas where these methods will be contributing is the pharmacokinetics and bio equivalence study. Generic drugs are now getting popular due to their cost and availability. It refers to pharmaceutical product, usually intended to be interchangeable with the innovator product, marketed after the expiry of patent or other exclusivity right. Due to the mass manufacturing of generic drugs the quality and efficacy of the generic drugs become questionable and WHO expresses the concern about the compatibility or interchangeability of generic product with patent product or pioneers. Bio equivalence is one of the tools to assure this interchangeability.

In Malaysia, bioequivalence of the generic drugs become the part of the drug registration process since more than a decade and starting from 2012 all generic oral dosage forms applying for registration must submit the bio equivalence results [8] to the drug control authority. So far reported study for the development of bioanalysis for different dosage forms in human plasma is very few. In that regard our present study is in line with the necessity of such methods for bioequivalence study. In this paper we are reporting our preliminary findings of the bio analytical method development of cimetidine and ranitidine.

Methodology

Chemicals and Reagents:

Unless and otherwise mentioned all reagents were of analytical grade. Acetonitrile was HPLC grade from fisher, sodium di hydrogen phosphate was from Merck. Triethylamine and NaOH were from Merck.

Standards:

Cimetidine and ranitidine were from Sigma USA. 0.1 mg/mL stock standard solutions were prepared for each of the drug. Human plasma samples were prepared by spiking appropriate amount of the working standard solutions into drug free human plasma.

HPLC Eluent:

Aqueous phase consist of 20 mM sodium di hydrogen phosphate and 1% triethyl ammine. The aqueous and acetonitrile ratio was 95: 5. The mixture was filtered through a 0.45 µm filter prior to the use.

Chromatographic condition:

Agilent 1100 HPLC system was used for the study. Data acquisition was performed with the Agilent Chemstation processor. The analytical column employed was a Waters (Nova Pak) C₁₈ (150 mm x 3.9 mm, i.d, 5µm.) with Waters (Nova Pak) guard column (20 mm x 3.9 mm i.d., 5µm) of the same packing material. The analytes were detected using a UV-Vis detector and detection wavelength was set at of 230 nm. 20µL sample was injected by the programme controlled auto injector. Chromatographic separation was performed at ambient temperature and flow rate was maintained at 1.0 mL/min.

Plasma Sample preparation:

Solid Phase Extraction (SPE): A SPE vacuum manifold was used for sample preparation. Satisfactory values for recovery of ranitidine and cimetidine were obtained with a single extraction using the RP solid phase cartridge (200 mg) for the isolation of the drugs from spiked plasma samples. The cartridge was conditioned sequentially by 2 ml of methanol and 2 ml water. 0.5 ml of plasma sample was introduced into the cartridge under vacuum at 5 psi. Water (3 ml) was used to

rinse the cartridge. The analytes were eluted with 2 ml methanol. The eluate was evaporated to dryness, under N₂ for about 20 min at 40 ° C. After reconstitution of the residue it was injected into the HPLC system.

Liquid Liquid Extraction (LLE): 0.5 ml of spiked plasma samples were extracted using ethyl acetate and dichloromethane for cimetidine and ranitidine respectively. 3ml of the solvent was added to the spiked plasma and then vortexes for one minute and then it was centrifuged to obtain the organic part. After separating the organic part it was dried under nitrogen and then reconstitutes using mobile phase prior to the introduction to the HPLC system.

Protein Precipitation (PP): Protein precipitation was done using acetonitrile as a solvent. 4 ml of acetonitrile was added to the 0.5 ml spiked plasma sample of cimetidine and ranitidine. After vortexing for a minute the samples were centrifuge and the supernatant was collected and injected in the HPLC system for analysis.

Results

It was found that for the SPE technique both the drugs showed a highest recovery of 89% and 87% for cimetidine and ranitidine respectively. This finding is similar with other earlier reported works [4]. For the solvent extraction method both the drug showed an excellent recovery yet lower than the SPE technique. In this study it was found that 85% recovery of cimetidine was achieved using ethyl acetate as an extracting solvent. On the other hand ranitidine showed a comparatively better results using dichloromethane (82%). For the protein precipitation technique both cimetidine (75%) and ranitidine (73%) showed a lower recovery compared to the other method. The recovery of the cimetidine and ranitidine following different extraction process are shown in Figure 1 and 2 respectively. In all the techniques ranitidine showed a lower percentage recovery than cimetidine. It was reported in one of the study that ranitidine can be detected with the fluorescence detector as ranitidine has a better sensitivity with fluorescence detector [6]. Compare to the other reported works the results obtained in this study is comparable with the reported findings.

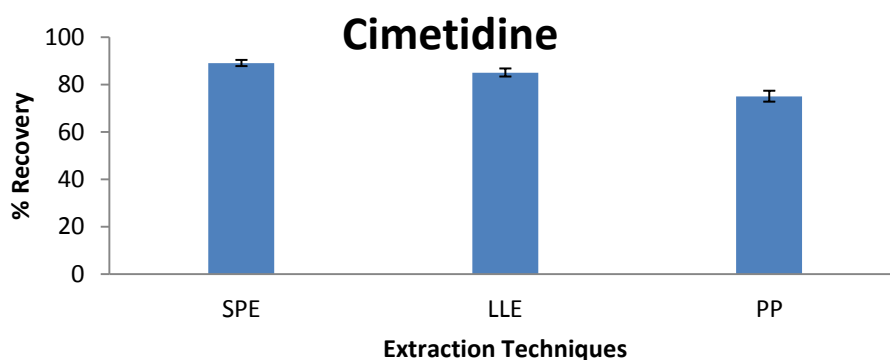


Figure 1: % Recovery of cimetidine following different extraction technique.

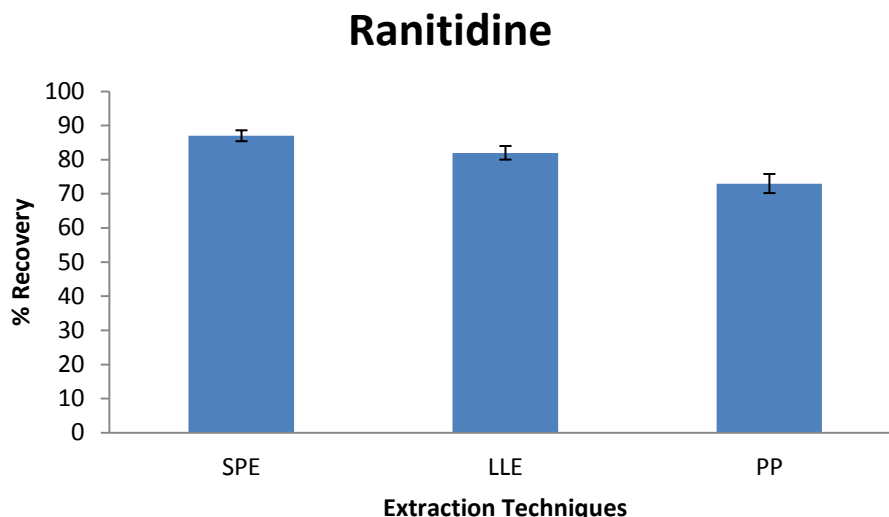


Figure 2: % Recovery of ranitidine following different extraction technique.

Conclusion

Preliminary study for the extraction of the cimetidine and ranitidine shows encouraging result for the recovery of the drugs from plasma. Different extraction procedure produces satisfactory recovery level for the drugs. Further investigation of the reproducibility of the recovery results will be performed to continue the full validation method. It could be concluded that SPE and LLE both the extraction is suitable for the detection and quantification of the drugs namely cimetidine and ranitidine.

Acknowledgement

Authors like to express their gratitude to all the academic and technical staff of Kulliyyah of Pharmacy, IIUM for their support. Authors are thankful to the research management centre (RMC) of IIUM for the financial support (EDW B1002-357).

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