In vitro Spectroscopic studies on drug interaction of cefpodoxime proxetil and H_2 receptor blockers

Sadia Iqbal[1], Sohail Hassan[2], Amir Hassan[2*], Mohsin Ali[3], Urooj Nazim[2], Erum Zaheer[4], Ateka Ahmed[2], Kanwal Hussain[2], Shereen[2] and Hina Furqan[2]

[1]Department of Pharmaceutical Chemistry, Dow College of Pharmacy, Dow Medical University, Karachi, Pakistan
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[3]Department of Chemistry, Faculty of Science, University of Karachi, Pakistan
[4]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Abstract: One of the relatively advance 3rd generation cephalosporins, cefpodoxime proxetil, is being used all-around. Generally, these are used for the cure of infections allied to urinary and respiratory tract. These cephalosporins have showed a remarkable *in vitro* activity against many strains of bacteria which are resistant to other orally used active medicinal substances. It is the first oral 3rd generation cephalosporin to be used in the cure of skin infections. The practice of H_2 receptor antagonists, concerning lots of treatments recommended in patients with different types of ulcers and allergic urticarial condition, is raising hazards of unwanted secondary outcomes and drug interactions. Learning of in-vitro interaction between cefpodoxime poxetil and H_2 blockers (Ranitidine, Famotidine and Cimetidine) were examined applying UV/Visible spectrophotometry and Infrared spectrometry. In the existence of H_2 receptor blockers, the cefpodoxime proxetil availability was found to be decreased *in vitro* only under specific conditions. Furthermore, complexes of Cefpodoxime proxetil-H_2 receptor antagonists were manufactured approving the interaction of these drugs. Finally, the above mentioned spectrophotometric techniques were employed to examine the complexes formed (Cefpodoxime proxetil-cimetidine, cefpodoxime proxetil-famotidine and cefpodoxime proxetil-ranitidine).

Keywords: Drug interaction, cefpodoxime proxetil, IR-Spectroscopy

INTRODUCTION

All H_2-receptor antagonists belong to the class of drugs which reduce acid secretion and used for the treatment of peptic ulcers (Brittain and Jack 1983, Jin *et al*., 2014); (Palacios *et al*., 1998). Comparison of H_2-receptor antihistamines demonstrates presence of three basic important units for activity: a substituted hetero-aromatic or aromatic ring, usually a four membered alkyl chain and a polar group like urea or amidine mostly acetamidine (Palacios *et al*., 1998) (Lumma Jr *et al*., 1982) (Mirossay *et al*., 1992) (Katsura *et al*., 1992).

Famotidine is a potent H_2-receptor antihistamine, it is structurally different from cimetidine and ranitidine as it contains thiazole ring while cimetidine and ranitidine has imidazole and furan ring, respectively (Campanero *et al*., 2001; Foye 2008). Most earliest of all H_2-antagonists is cimetidine having many drug interactions. In cimetidine, presence of the imidazole ring results in the inhibition of most of the CYP450 oxidative processes, while ranitidine and famotidine don't show this effect. So other agents of H_2-blockers with OTC status are more potent, and used widely for reduction of gastric acidity (Foye 2008).

There are four different classes of receptors of histamine; H_1, H_2, H_3 and H_4 (Gilman and Goodman 2006) (Howland and Mycek 2006). Antihistamines block the receptor mediated response of a target tissue. H_2 blockers compete with histamine at H_2 receptor of parietal cell and depending on the dose they abolish acid secretion. These are only selective to H_2 receptors. H_2 blockers include Cimetidine (fig. 1A), Famotidine (fig. 1B), Ranitidine (fig. 1C) and which are rather different in their pharmacokinetics and chemical structures. These drugs are clinically useful in prevention of stress-related gastritis bleeding, non-ulcer dyspepsia, peptic ulcer disease, gastroesophageal reflux disease (GERD), etc. (Gilman and Goodman 2006, Katzung 2007).

Cefpodoxime proxetil, (1RS)-1-[[(1-Methylethoxy) carbonyl]oxy]ethyl (6R,7R)-7-[[(2Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-3-(methoxymethyl) -8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate (fig. 1D), is a first oral and new broad spectrum antibiotic which belongs to the 3rd generation of cephalosporin (Rodrı´guez, Herna´ndez *et al*. 2003). It is highly active against both Haemophilus influenza and Moraxella catarrhalis including β-lactamase producing strains (equal potency as of cefixime and greater potency than cefaclor, cefuroxime (Chugh and Agrawal 2003). Cefpodoxime proxetil binds to penicillin binding proteins (PBPs) which inhibits peptidoglycan synthesis, finally results in interfering bacterial cell wall biosynthesis (Adam *et al*., 1991). Cefpodoxime proxetil was well tolerated without any seriously adverse drug reactions in *in vivo* study but *in vitro* reaction requires stirring and time. It is used for

**Corresponding author:* e-mail: ameerhassanhassan@yahoo.com

the treatment of chronic and acute bronchitis, sinusitis, pneumonia, acute and chronic bronchitis, tonsillitis, pharyngitis, respiratory tract infections, folliculitis, abscess, otitis media, etc. (Todd, 1994). This study aimed to conduct spectroscopic studies on drug interaction of cefpodoxime proxetil and H_2 receptor blockers.

MATERIALS AND METHODS

Cefpodoxime proxetil was gifted from PharmEvo (Pvt.) Ltd. (Karachi, Pakistan) and Bosch Pharmaceuticals (Pvt.) Ltd.(Karachi, Pakistan), GlaxoSmithKline Pharmaceutical (Pvt) (Karachi, Pakistan) gifted Ranitidine, Famotidine was taken from Mediate Pharmaceutical (Pvt.) Ltd. (Karachi, Pakistan) and cimetidine was given by Bosch Pharmaceuticals (Pvt.) Ltd. (Karachi, Pakistan).

Shimadzu UPVC version 3.9 software loaded in a P-IV computer with 1 cm rectangular quartz cells connected, UV/VIS spectrophotometer with range of 200 to 800 nm (Shimadzu, Japan, Model 1601) and methanol was used to observe UV-visible spectra, KBr pellets in Shimadzu Prestige-21 spectrophotometer to note FTIR spectra with range of 4000-400 cm^{-1}.

Preparation of solutions

100ml, 0.1M methanol solutions of cefpodoxime proxetil, cimetidine, Famotidine and Ranitidine were prepared.

Preparation of cefpodoxime proxetil-H_2 complexes

First of all purity of prepared solutions of cefpodoxime proxetil and cimitidine was checked separately with TLC by using CH_3OH:H_2O (8:2) as mobile phase. Cefpodoxime proxetil solution (10ml) and cimitidine solution (10ml) was taken in round bottom flask and pH of the mixture was adjusted by adding 1-2 drops of ammonia and a TLC was run at 0 min. The flask was placed on magnetic stirrer and refluxed for 5 hours and a TLC was taken and observed under UV lamp to check the complex formation of cefpodoxime proxetil-cimitidine. When complex formation was confirmed the mixture was left to dry and crystalize at room temperature. Same method was used to prepare cefpodoxime proxetil-famotidine and cefpodoxime proxetil-Ranitidine complexes. Complexes were characterized by their molecular masses (obtained by mass spectroscopy) solubility and respective melting points.

UV/Visible Spectrophotometry

The individual UV spectra of Ranitidine, Famotidine, Cimetidine, and cefpodoxime proxetil, as well as of complexes including cefpodoxime proxetil-ranitidine, cefpodoxime proxetil-famotidine and cefpodoxime proxetil-cimetidine were obtained in 200 nm to 800 nm range of wavelength. The solvent used for UV spectra was methanol.

Infrared spectroscopy

Individually IR spectra of all seven i.e. ranitidine, famotidine, cimetidine and cefpodoxime proxetil, cefpodoxime proxetil-Ranitidine, cefpodoxime proxetil-famotidine and cefpodoxime proxetil-cimetidine were scanned in the range of 4000-400 cm^{-1} using KBr pellets.

RESULTS

Physical examination

It include melting point of each drug and complex: Cimetidine (144°C), famotidine (168°C), ranitidine (70°C), cefpodoxime proxetil (111°C), cefpodoxime proxetil-cimetidine (90°C), cefpodoxime proxetil-famotidine (95°C) and cefpodoxime proxetil-ranitidine (83°C).

UV visible spectrophotometry

In the wavelength range of 200-800nm, UV spectra were obtained of selected drugs (ranitidine, famotidine, cimetidine and cefpodoxime proxetil), as well as of all three prepared complexes (Cefpodoxime proxetil-Ranitidine, Cefpodoxime proxetil-famotidine and cefpodoxime proxetil-cimetidine) using methanol as solvent. The maximum wavelength of ranitidine, famotidine, cimetidine and cefpodoxime proxetil was found to be 251nm, 252nm, 286nm and 223.4nm, respectively. Maximum wavelength of complexes (cefpodoxime proxetil-cimetidine, cefpodoxime proxetil-famotidine and cefpodoxime proxetil-ranitidine) was 252nm.

DISCUSSION

In our present study a cephalosporin drug, cefpodoxime proxetil was selected to determine its interaction (*in vitro*) with H_2 receptor antagonists (cimitidine, Famotidine and Ranitidine). Cefpodoxime proxetil, cimitidine, famotidine, ranitidine along with three complexes of cefpodoxime proxetil- H_2 receptor antagonists were evaluated by UV-Visible and IR spectroscopy.

Change in maximum wavelength of complexes (252nm) from the individual drugs showed that there is an interaction between cefpodoxime proxetil and H_2 receptor antagonists. The similar maximum wavelength of complexes may be due to containing similar portion of cefpodoxime proxetil in their structures.

In our elucidated infrared spectrum of cimetidine NH stretch and aliphatic CH including sp^2 and sp^3 appear in between 3224.98 and 2903.16 cm^{-1}. Although nitrile -C≡N band is at 2177.6 cm^{-1}, imine >C=N- is at 1624.06 cm^{-1} and C-N and C-C absorption band appears at 1583.3 cm^{-1}. CH_2 and CH_3 bands are at 1462.54 and 1392.61 cm^{-1} (table 1, fig. 1A). The obtained data was found comparable with the stated IR spectrum of cimetidine (Onoa *et al*., 2002; Sultana *et al*., 2010).

IR Spectroscopy

Table 1: IR absorption bands (cm^{-1}) of cimitidine, famotidine, ranitidine and cefpodoxime proxetil

Compound	IR absorption bands (cm^{-1})
Cimitidine	3224.98 - 2903.16, 2177.6, 1624.06, 1583.3, 1462.54, 1392.61
Famotidine	3395-3371, 3238.48, 3150, 2933.73, 1623.28, 1579.7, 1448.54, 1327.03, 1284.84, 1147.05, 900-600
Ranitidine	3265.84 - 2258.64, 1596.99, 1562.34, 1238.3-1008.27
Cefpodoxime proxetil	3437.15, 3331.07, 3213.41, 2945.81, 2930.52, 2823.7, 1761.01, 1678.07, 1627.92, 1535.34, 1454.33, 1375.25, 1219.01, 1140.5, 905.54, 785.9, 690.52

Table 2: IR absorption bands (cm^{-1}) of cefpodoxime proxetil-cimitidine complex, cefpodoxime proxetil-Famotidine complex and cefpodoxime proxetil-ranitidine complex

Complex	IR absorption bands (cm^{-1})
Cefpodoxime proxetil-Cimitidine	3437.15-3302.13, 2931.08, 2177.63-2160.06, 1761.01-1741.16, 1627.92-1662.64, 1624.06
Cefpodoxime proxetil-Famotidine	1761.01-1743.85, 1689.54 -1678.07, 1579.70 -1538.54, 690.52 - 634.44
Cefpodoxime proxetil-Ranitidine	1761.01- 1741.72, 1678.07 - 1663.00, 1645.28 to 1620.21, 1535.34 - 1576.36

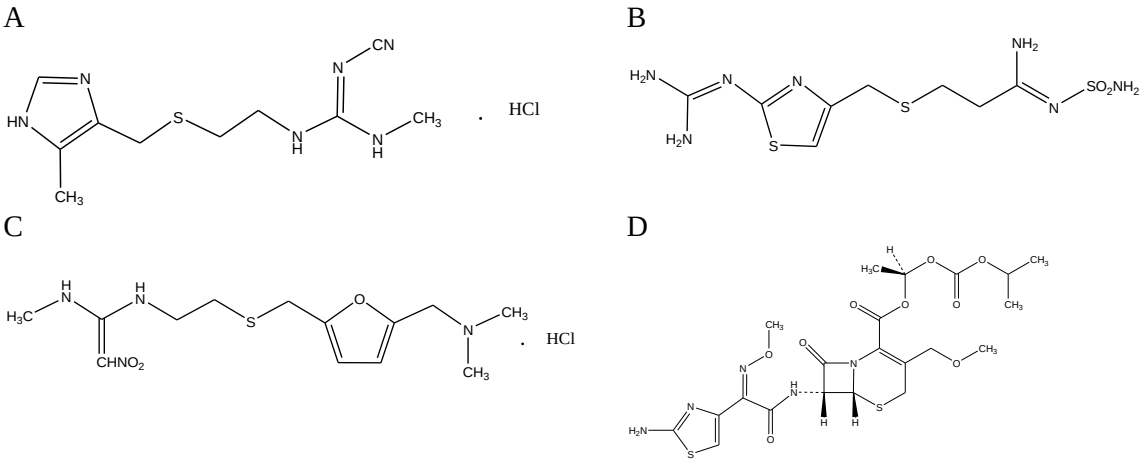


Fig. 1: Structures of Cimetidine (A) Famotidine (B) Ranitidine (C) and Cefpodoxime proxetil (D)

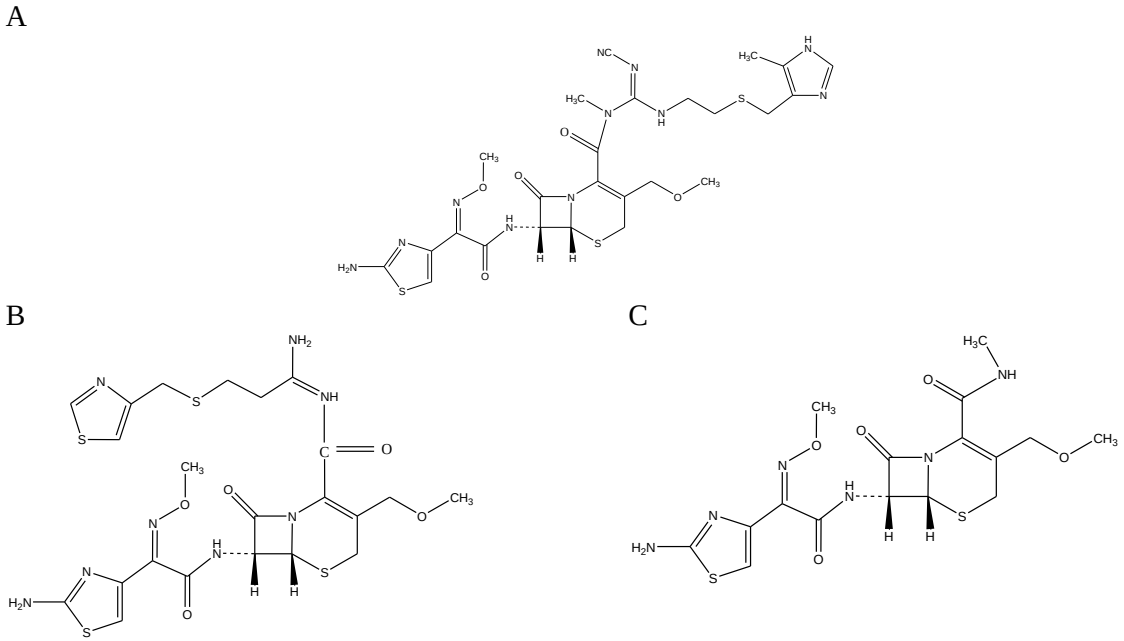
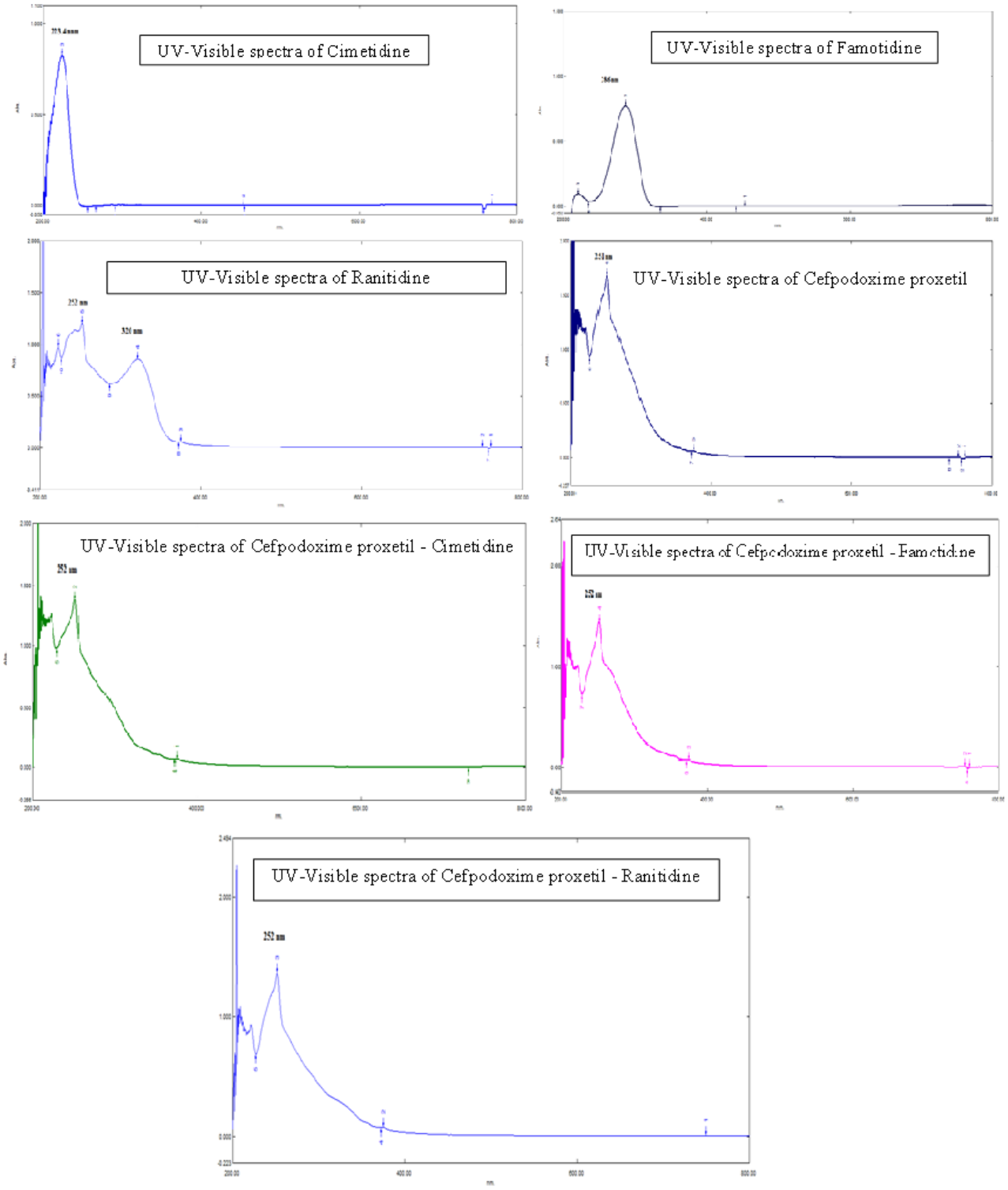
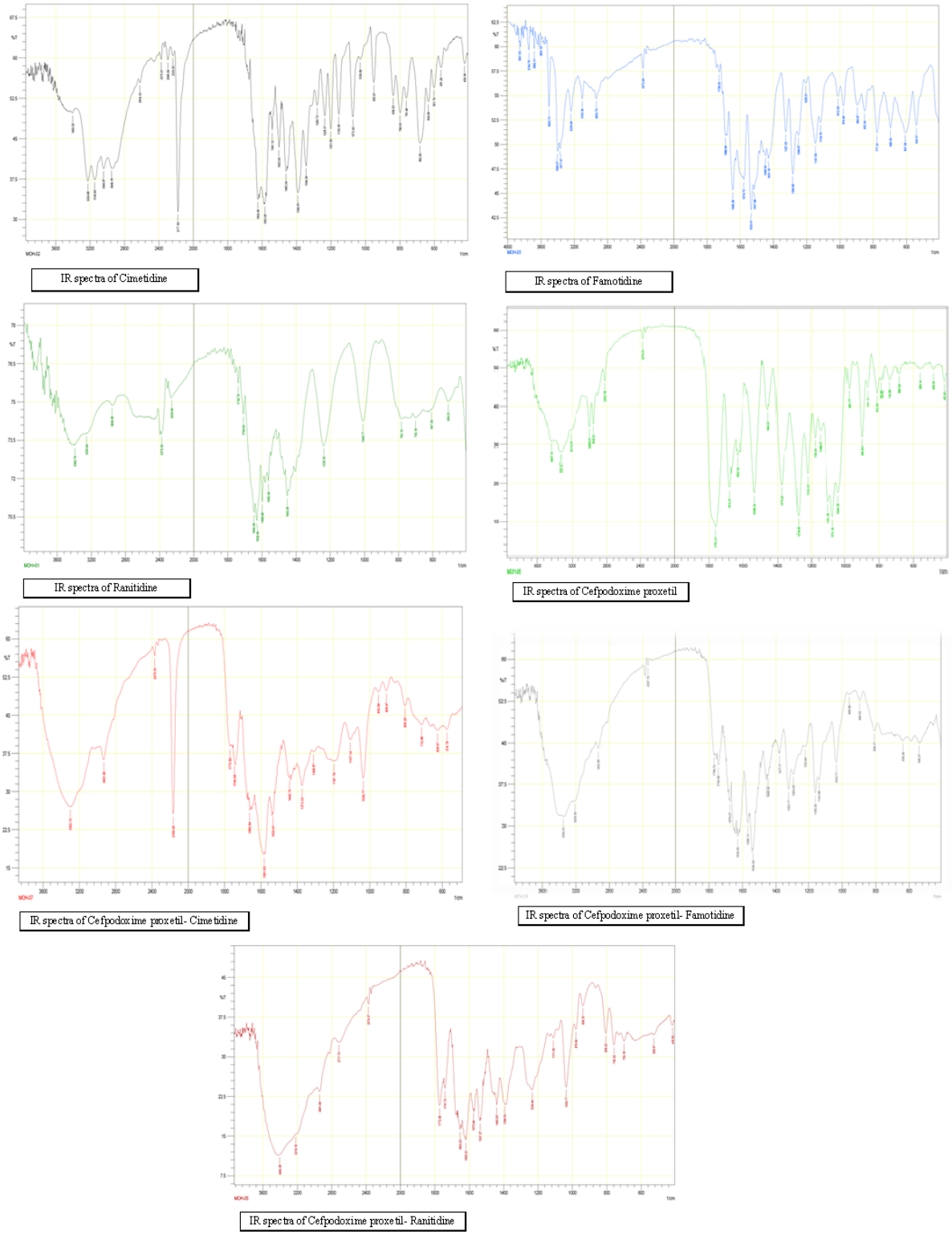


Fig. 2: Structures of Cefpodoxime proxetil-Cimetidine Complex (A) Cefpodoxime proxetil-Famotidine Complex (B) Cefpodoxime proxetil-Ranitidine Complex (C)



Previous study showed that Famotidine IR spectra demonstrated N-H stretching was between 3403 to 3212 cm^{-1}, CH stretching was recognized at 3092 cm^{-1}, sulfonyl amide peak for N-H absorption was appeared at 3196 cm^{-1}, thiazole ring peak was identified at 1527 cm^{-1} and stretching of C=N was showed at 1278 and 1450 cm^{-1} with two peaks of S=O that were reported at 1355 and 1283 cm^{-1} (Sultana, Arayne *et al*. 2010). In our Famotidine spectra, N-H stretching region is 3395-3371 cm^{-1} and sulfonyl amide peak is appeared at 3238.48 cm^{-1}. C-H stretching of sp^2 is at 3105 cm^{-1} and sp^3 is at 2933.73 cm^{-1}. C=C bond is at 1623.28 cm^{-1}, thiazole ring shows peak at 1579.

IR spectra of all compounds



70 cm^{-1} and two peaks of S=O are appeared at 1327.03 and 1147.05 cm^{-1}. Stretching of C=N is found at 1448.54 and 1284.84 cm^{-1}, NH_2 is out of plane, while sp^2 and sp^3 are also observed between 900-600 cm^{-1} (table 1, fig. 1B).

Stated infrared spectra of Ranitidine (Sultana, Arayne *et al.*, 2010) exhibited specific peak of tertiary amine at 2393 cm^{-1}, and two wide peaks of N-H bond at 2557 and 2635 cm^{-1}. Peaks of C-O-C stretching was at 1038 and

1245 cm^{-1}. In our interpreted IR spectrum, aliphatic CH stretching and N-H stretching emerged in the area of 3265.84 to 2258.64cm^{-1}. Nitro group stretching was developed at 1562.34 and 1596.99cm^{-1}. C-O-C and C-N stretching was in the region of 1238.30 to 1008.27 cm^{-1} (table 1, fig. 1C).

Our interpreted infrared spectra of cefpodoxime proxetil showed peak at 3437.15 and 3331.07 cm^{-1} for N-H stretch, and at 3213.41 cm^{-1} for C-H stretch (sp^2), while CH stretch for sp^3 is at 2945.81 cm^{-1} and 2930.52 cm^{-1}. C-H stretch for $–OCH_3$ is appeared at 2823.70 cm^{-1}, ester (C=O) and amide (C=O) stretches at 1761.01 cm^{-1}, imine peak is at 1678.07 cm^{-1}, C=C stretch is at 1627.92 cm^{-1} and primary NH bend is found at 1535.34 cm^{-1}. CH_2 and CH_3 bend is at 1454.33 cm^{-1} and 1375.25 cm^{-1}. C-N stretching is appeared at 1219.01 cm^{-1}. Ether C-O stretch comes into view at 1140.5 cm^{-1}. Out of plane of =C-H and NH emerges at 905.54 cm^{-1} and 785.90 cm^{-1}, and C-S stretching is originated at 690.52 cm^{-1} (table 1, fig.1D).

IR Spectrum of complex of cefpodoxime proxetil-cimetidine showed fusion peak of NH stretching, sp^2 and sp^3 at 3302.13cm^{-1} and 2931.80cm^{-1} w.r.t both cefpodoxime proxetil and Cimetidine. Peak shifting was easily seen w.r.t cefpodoxime proxetil where ester peak C=O was moved from 1761.01 cm^{-1} to 1741.16 cm^{-1}, CN stretching from 1219.01 cm^{-1} to 1034.37 cm^{-1}, NH stretching was shifted from 3437.15 cm^{-1} to 3302.13 cm^{-1}, C=C absorption was shifted from 1627.92cm^{-1} to 1662.64 cm^{-1}. W.r.t Cimetidine peak shifting of nitrile (-C≡N) from 2177.63 cm^{-1} to 2160.06 cm^{-1} and imine peak from 1624.06 cm^{-1} to 1662.64 cm^{-1} (table 2, fig. 2A).

In complex of cefpodoxime proxetil-famotidine, IR spectra displayed C=O for ester and amide from 1761.01 cm^{-1} to 1743.85 cm^{-1}, C-S stretch was shifted from 690.52 cm^{-1} to 634.44 cm^{-1} w.r.t cefpodoxime proxetil and sp^2 stretching combine with NH stretch in complex spectra of cefpodoxime proxetil-Famotidine. W.r.t Famotidine imine peak was shifted from 1689.54 cm^{-1} to 1678.07 cm^{-1}, bend for NH_2 was also moved from 1579.70 cm^{-1} to 1538.54 cm^{-1} (table 2, fig. 2B).

Cefpodoxime proxetil-Ranitidine complex showed absence of C-N stretch peak as in cefpodoxime proxetil at 1219.01 cm^{-1}. C=O peak for amide was shifted from 1761.01 cm^{-1} to 1772.58 cm^{-1} and C=O peak for ester was shifted from 1761.01 cm^{-1} to 1741.72cm^{-1}, imine peak was shifted from 1678.07 cm^{-1} to 1663.00 cm^{-1} and NH bend was changed from 1535.34 cm^{-1} to 1576.36 cm^{-1}. W.r.t ranitidine C=C bond peak was shifted from 1645.28 cm^{-1} to 1620.21 cm^{-1}, C-NO_2 peak was absent as in Ranitidine at 1596.99 cm^{-1} and 1562.34 cm^{-1} (table-3, fig. 2C).

In IR spectra of complexes some absorption peaks were found absent but they were present in individual spectra of the drugs. Similarly shifting of some peaks were observed in IR spectra of complexes as compare to IR spectra of pure drugs. It confirmed the interaction between cefpodoxime proxetil and H_2 receptor antagonists and it may reduce or change the desire therapeutic effect of the drugs.

Cefpodoxime proxetil was well tolerated without any seriously adverse drug reactions in *in-vivo* study but in *in-vitro* reaction requires stirring and time.

CONCLUSION

It was concluded that cefpodoxime proxetil and H_2 receptor antagonists (cimetidine, famotidine and ranitidine) have interaction between them. The interaction between them is confirmed by same maximum wavelength of complexes, formed by interaction, as compare to maximum wavelength of individual drugs. Changing in IR spectra of complexes with respect to individual drugs also indicates interaction between the drugs and it may change therapeutic effect of the drug. The new formed complexes may be tested for biological activity in future.

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