Study of Drug-Drug and Drug-Food Interactions of Mesalazine Through FTIR and DSC

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ABSTRACT: A well-established drug used in the management of inflammatory bowel disease is 5-aminosalicylic acid (e.g. Mesalazine or Mesalamine). For the treatment of mild to moderate flares of ulcerative colitis and Crohn's disease, Mesalazine has been used as the first line drug in both western and Asian countries due to its superiority over other drugs in terms of side effects and toxicities. Besides, some other drugs are also prescribed for total resolution of different symptoms of ulcerative colitis and associated diseases, which include Acetaminophen, Metronidazole and Vitamin D_3. Moreover, physicians instruct that Mesalazine should be taken at least one hour before meal. So, there are enough scopes of studying the drug-food interaction of Mesalazine to assess if there is any incompatibility present with food. Thus, in the present study, Mesalazine and physical mixtures of Mesalazine (1:1) in solid form along with the aforementioned drugs were prepared and analyzed to evaluate the compatibility among them using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). In addition, interactions of Mesalazine with food stocks such as chicken and vegetable broth, fruit juice, milk and soybean oil were studied using FTIR, considering these as the common sources of protein, vitamin, fiber, minerals and fat. From this study, it was interpreted that, major interactions of Mesalazine were present with food samples. Besides, FTIR and DSC data revealed subtle clues of incompatibilities between Mesalazine and the other two drugs except Vitamin D_3. So, the results may prove to be useful for related research works in future.

Key words: Mesalazine, interaction, compatibility, FTIR, DSC, ulcerative colitis, Crohn's disease

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel conditions, which are combinedly known as inflammatory bowel disease (IBD). The pathogenesis being still unknown, it is thought that this inflammatory process is peddled by an exaggerated immune response to antigenic stimulation by the gut microbiota on a background of genetic susceptibility.

In clinical practice, the disease activity of CD is typically described as mild to moderate (without manifestations of dehydration, abdominal tenderness or more than 10% weight loss), moderate to severe (more prominent symptoms such as fever, weight loss, abdominal pain, intermittent nausea and vomiting or significant anemia) and severe to fulminant disease (high fever, persisting vomiting or evidence of intestinal obstruction). Similar to CD, the severity of UC is classified as mild, moderate, severe and fulminant.[1]

Since the late 1970's, Mesalazine has been used as the gold standard first line treatment for IBD. It is an anti-inflammatory agent which is structurally related to the salicylates. Known as 5-aminosalicylic acid (5-ASA), it is monohydroxybenzoic acid substituted by an amino group at the C_5-position and the functional groups present are significantly IR active. It is odorless and textured as white to pinkish crystals or purplish-tan powder. The aqueous solution is acidic and pH is approximately 4.1 at 0.8

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mg/l water. Mesalazine is slightly soluble in water, alcohol and hydrochloric acid; more soluble in hot water. It may reduce inflammation through inhibition of cyclooxygenase and prostaglandin production. Following rectal or oral administration, only a small amount of Mesalazine is absorbed; the remainder acting topically, reduces diarrhea, rectal bleeding and stomach pain associated with IBD.[2,3]

The prevalence of Crohn's disease and ulcerative colitis has been on the rise in western countries and in the Asian countries as well. These are not uncommon in Bangladesh and are being diagnosed more commonly. On this view, the research work was accomplished by means of IR and DSC methods so as to establish a rapid, simple and precise interaction profile of Mesalazine with food and commonly prescribed drugs with it. [4]

MATERIALS AND METHODS

Chemicals and reagents. Analytical grade isopropyl alcohol (IPA) and acetone procured from RCI Labscan Ltd. (Thailand), were used to clean the FTIR machine and the required glass apparatus. Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from BDH Chemicals (England). Acetaminophen, Metronidazole and dry Vitamin D_3 were provided as generous gifts from Incepta Pharmaceuticals Limited and Mesalazine was obtained from Unimed and Unihealth Mfg. Ltd., Bangladesh. The chicken stock, vegetable stock and fruit juice were prepared at home. Soybean and milk were collected from a local market in Dhaka city. The drug samples were refrigerated under proper temperature.

Preparation of food stocks. Chicken stock was prepared by boiling bony chicken pieces in water along with bay leaf, cardamom, cinnamon, cloves, black cumin and garlic. Then the boiled water was used as the stock. Vegetable stock was prepared by boiling raw papaya, pointed gourd and snake gourd with a very small amount of mashed ginger in it. The boiled water was used as the stock. Fruit juice was prepared by squashing orange, grapes and with it, apple extract was added and filtered through Whatman filter paper (No. 1) to get the juice in proper form.

Preparation of phosphate buffer of pH 7.4. Eudragit coated Mesalazine dissolves at intraluminal pH 6, and Eudragit S-coated Mesalazine works optimally at pH 7 or above. So in this current experiment, we prepared a phosphate buffer solution of pH 7.4 to evaluate the IR spectra of Mesalazine. Disodium hydrogen phosphate solution of 0.01M was prepared in a volumetric flask (solution A) and then, potassium dihydrogen phosphate solution of 0.02 M was prepared in another volumetric flask (solution B), both measuring 500 ml. Afterwards, 290 ml of solution A was properly mixed with 66 ml of solution B. Finally, the solution was made 1000 ml by adding distilled water and pH was checked by a pH meter.[5]

***In vitro* IR studies of samples.** IR spectra of the drugs Mesalazine, Acetaminophen, Metronidazole and dry Vitamin D_3 were recorded in the solid state by putting them individually on the sample platform of the instrument. The bands were assigned in the range of 4000 - 600 cm^{-1}. Background was checked before every analysis. The force gauge was kept at 60. In the similar way, spectrum data of mixture of Mesalazine with Acetaminophen, Metronidazole and dry Vitamin D_3 each were taken by preparing a mixture of the drugs in the ratio 1:1.[6]

Since Mesalazine works best at pH 7 or above, the solution of the required pH was prepared in the lab and the IR data of the solution was taken individually. Afterwards, the IR spectrum of the solution in combination with Mesalazine was taken. In doing this, there required no force gauge and the liquid sample provided the necessary pressure to stay in contact with the crystal of the sample platform.

Individual IR data of soybean oil, vegetable and chicken stock, milk and fruit juice were obtained. Afterwards, data of the mixture of Mesalazine with the aforementioned foods were taken. These mixtures were made by blending Mesalazine (quantity sufficient) and the food samples in a petridish one at a time.

***In vitro* DSC studies of samples.** The sample Mesalazine was weighed and placed in an aluminum pan which was then hermetically sealed. Then it was put on a constantan disc on a platform in the DSC analysis cell with a chromel wafer below it. A chromel-alumel thermocouple under the constantan disc measured the sample temperature. An empty reference pan was put on a symmetric platform with its own underlying chromel wafer and chromel-alumel thermocouple. Heat flow was determined by comparing the difference in temperature between the sample and the reference chromel wafers. The inert nitrogen gas flow rate was maintained at 20 ml/min. Then from software, the thermogram of Mesalazine was obtained. Similar procedure was followed to get the thermograms of Acetaminophen, Metronidazole and dry Vitamin D_3. The DSC data of physical mixtures of Mesalazine with Acetaminophen, Metronidazole and Vitamin D_3 in the ratio 1:1 each were also obtained. The DSC runs ranged from the temperature 30-400°C and the increase in temperature was 10° C/min.

RESULTS AND DISCUSSION

Fourier transform infrared spctroscopy (FTIR). The interactions between Mesalazine and drug and food samples were determined by using FTIR spectroscopy between the scan range of 4000-600 cm^{-1}.

Compatibility study of Mesalazine. An IR spectra of pure Mesalazine illustrated the peaks of amine and carboxylic group because of the existence of amine (basic) and carboxylic acid (acidic) functional groups. The peaks could be considered as the characteristic peaks of Mesalazine (Table 1).[7] These peaks were also presented by other researchers in their studies which were practically similar to the peaks found in our study, thereby indicating the identity and purity (Figures 4,5) of Mesalazine that we were working with. The IR spectra of Mesalazine showed no matching peaks with Acetaminophen, Metronidazole, Vitamin D_3 or the food samples individually. On the contrary, the characteristic peaks of Mesalazine (2500.06, 1644.89, 1484.36, 1445.35, 1350.96, 806.18, 771.08 and 683.97 cm^{-1}) were significantly observed in the IR spectra (Figures 4L, 4 M) of combination of Mesalazine with Metronidazole and dry Vitamin D_3. However, substantial characteristic peaks of pure Mesalazine were absent in the spectra of combination of Mesalazine with the food samples such as soybean oil, milk, fresh fruit juice, vegetable broth and chicken broth.

From table 2, it was interpreted that, column (ii) showed the absence of three characteristic peaks of Mesalazine in the spectra of combination of Mesalazine and Acetaminophen in the fingerprint region, which might indicate the presence of possible interaction between them. The shift of the peaks might be attributed to the change in hydrogen bond strengths which, as a consequence, changed the vibrational frequency of the bands.[9] Shift to higher wavelength value represented the reduction in bond length. Difference in intensities might be due to the difference of concentration of molecules in the samples. Again, the spectra of Mesalazine and combination of Mesalazine with Metronidazole in column (iii) and combination of Mesalazine with Vitamin D_3 in column (iv) showed matching peaks,

Table 1. Characteristic peaks of Mesalazine.[8]

Peaks (cm^{-1})	Range (cm^{-1})	Functional Group
2500.06	3200-2500	Intramolecular hydrogen bonds, chelate compounds
1644.89	1650-1590	Primary amine (N-H bend)
1484.36	1485-1445	C-H bending (alkane $-CH_2$)
1445.35	1485-1445	C-H bending (alkane $-CH_2$)
1350.96	1350-1260	O-H bending and C-O bending of primary or secondary alcohol
806.18	900-675	C-H stretch in aromatic compound
771.08	1000-700	C-H out of plane bending vibrations
683.97	900-670	Aromatic C-H out of plane bending vibrations

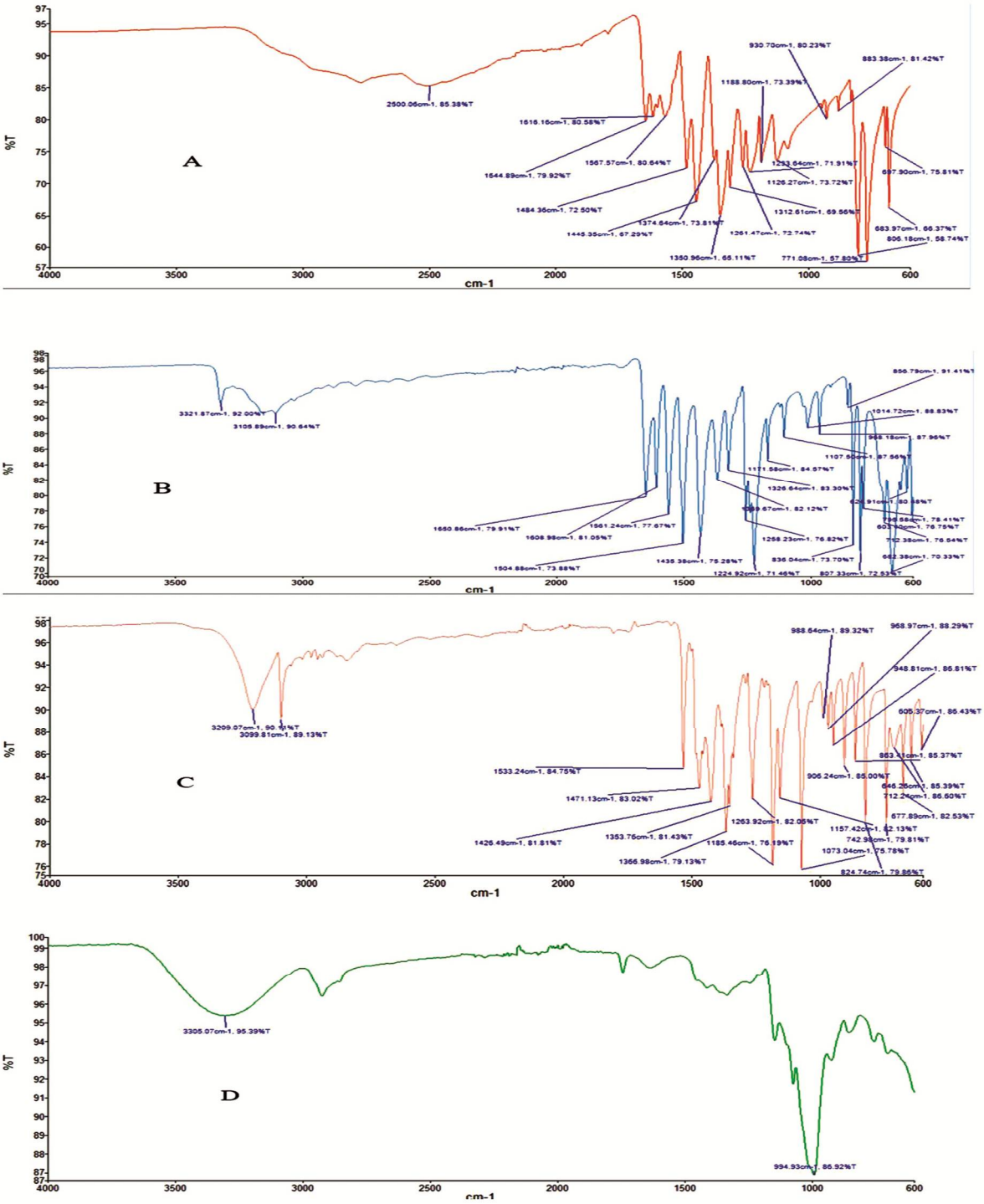


Figure 1. FTIR spectra of (A) Mesalazine, (B) Acetaminophen, (C) Metronidazole and (D) Vitamin D_3.

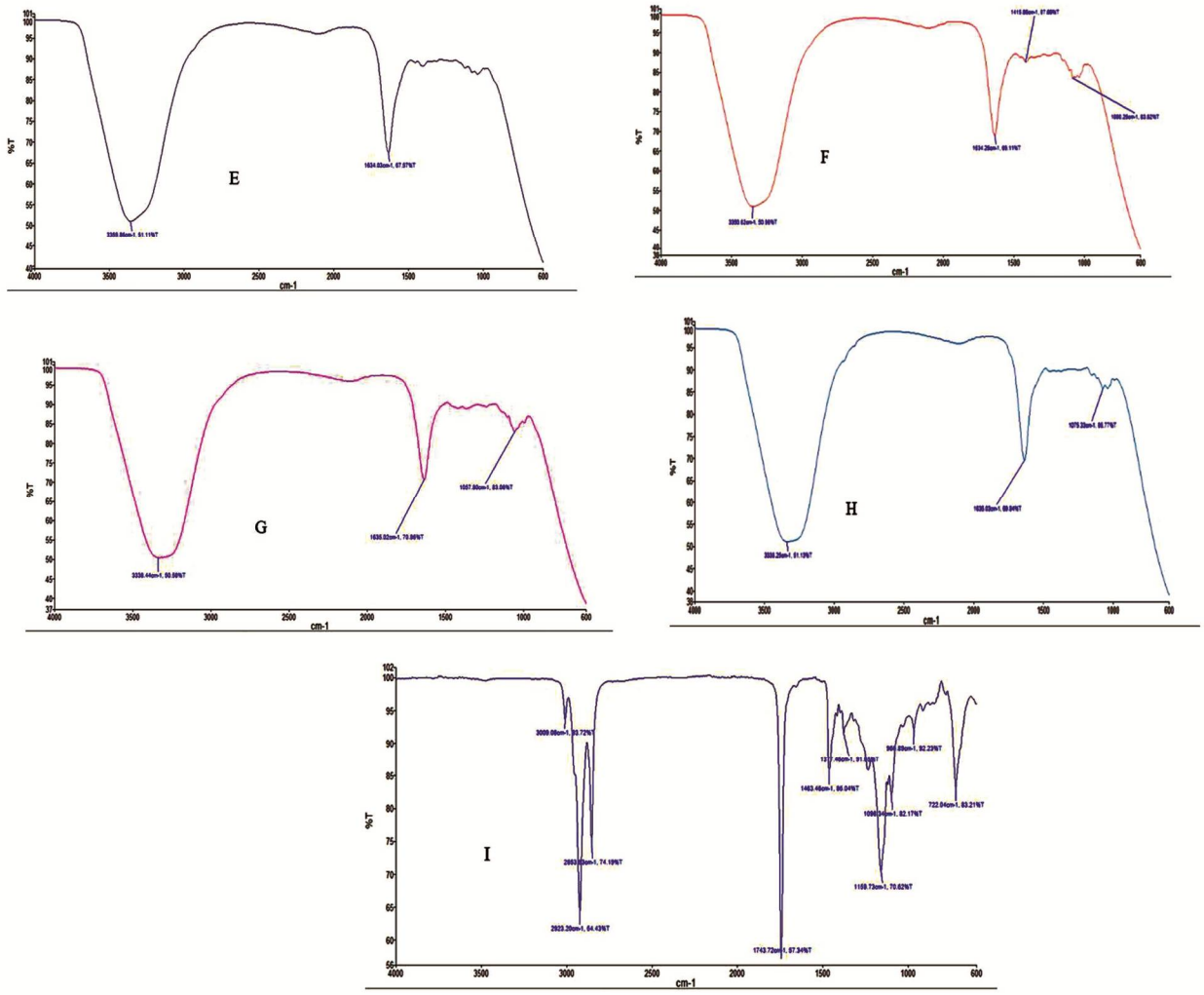


Figure 2. FTIR spectra of (E) Chicken stock, (F) Vegetable stock, (G) Fruit juice, (H) Milk and (I) Soybean oil.

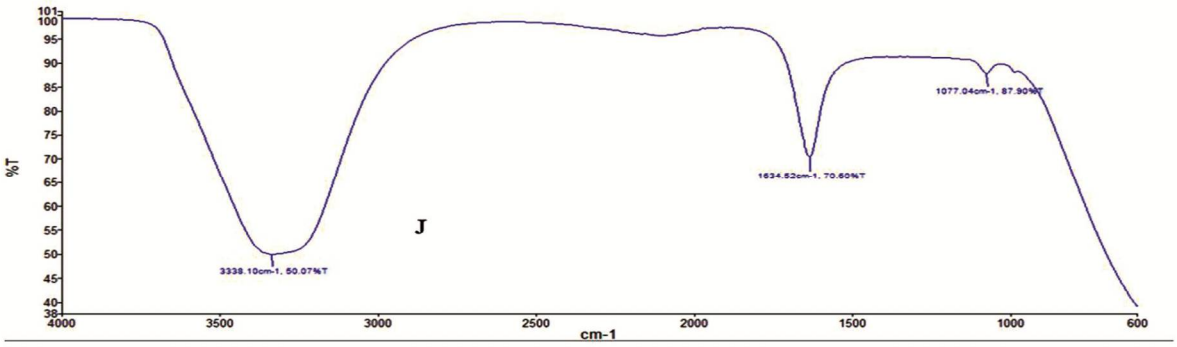


Figure 3. FTIR spectrum (J) of pH 7.4 buffer solution.

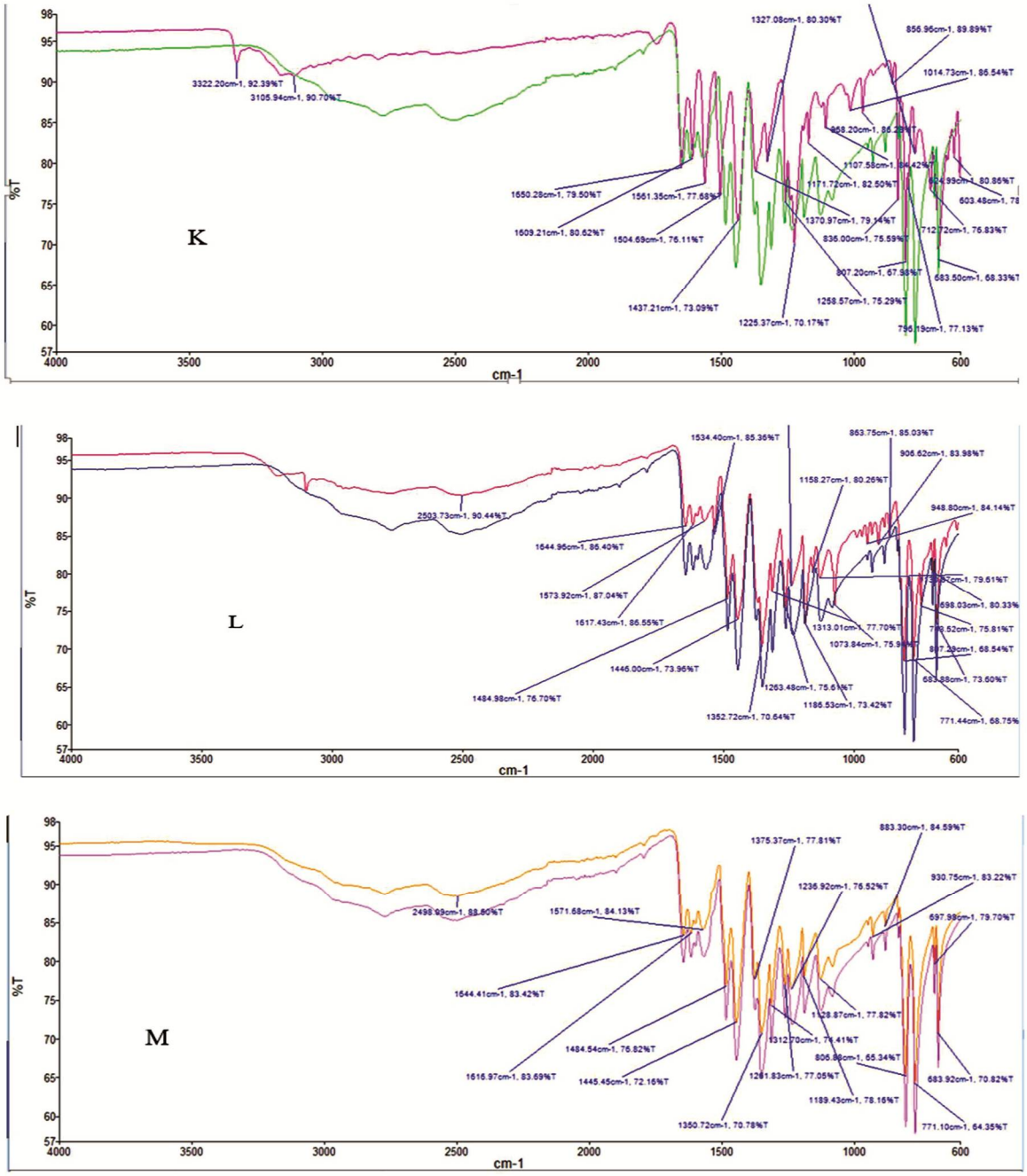
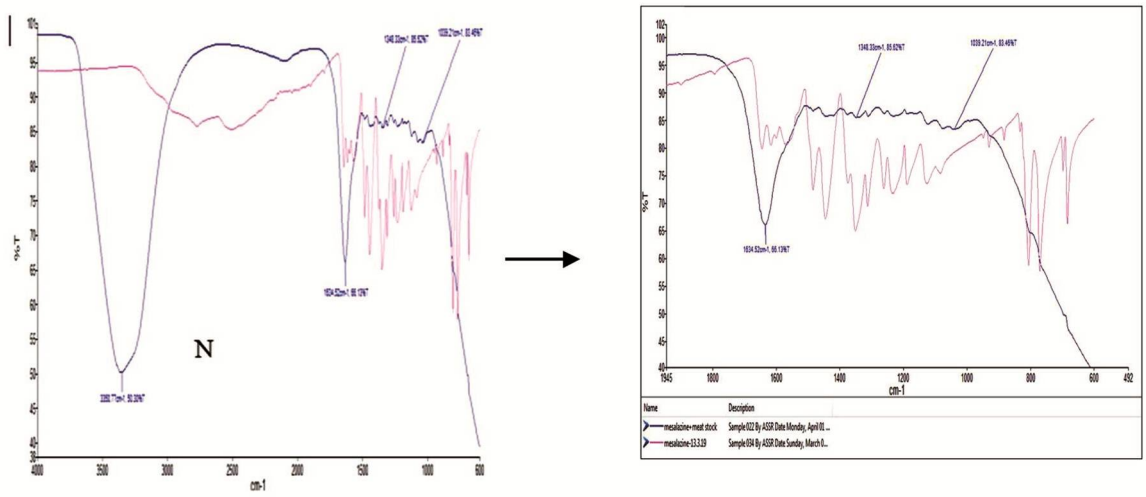
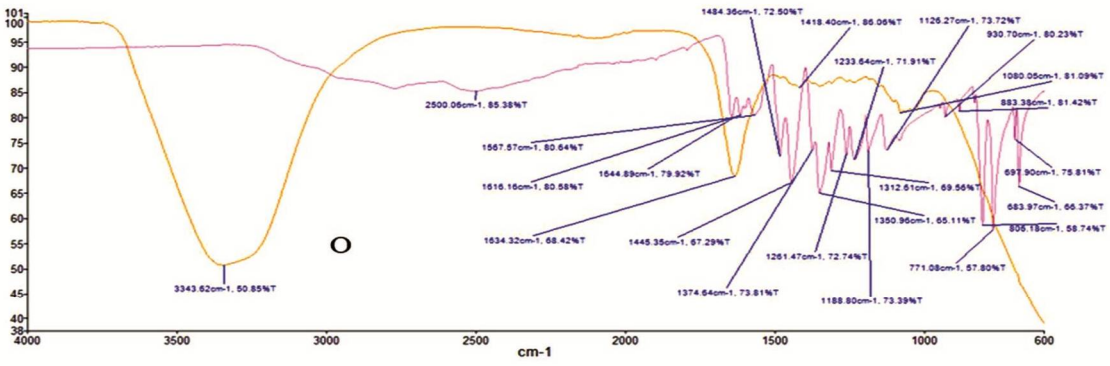
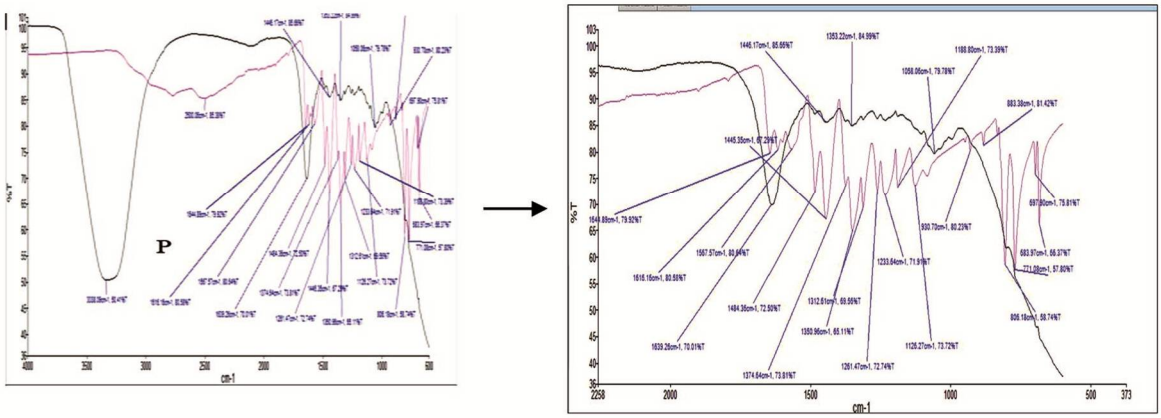


Figure 4. FTIR spectra of Mesalazine and (K) mixture of Mesalazine and Acetaminophen, (L) mixture of Mesalazine and Metronidazole and (M) mixture of Mesalazine and Vitamin D_3.



(a) **(b)** Expanded spectrum of N





(a) **(b)** Expanded spectrum of P

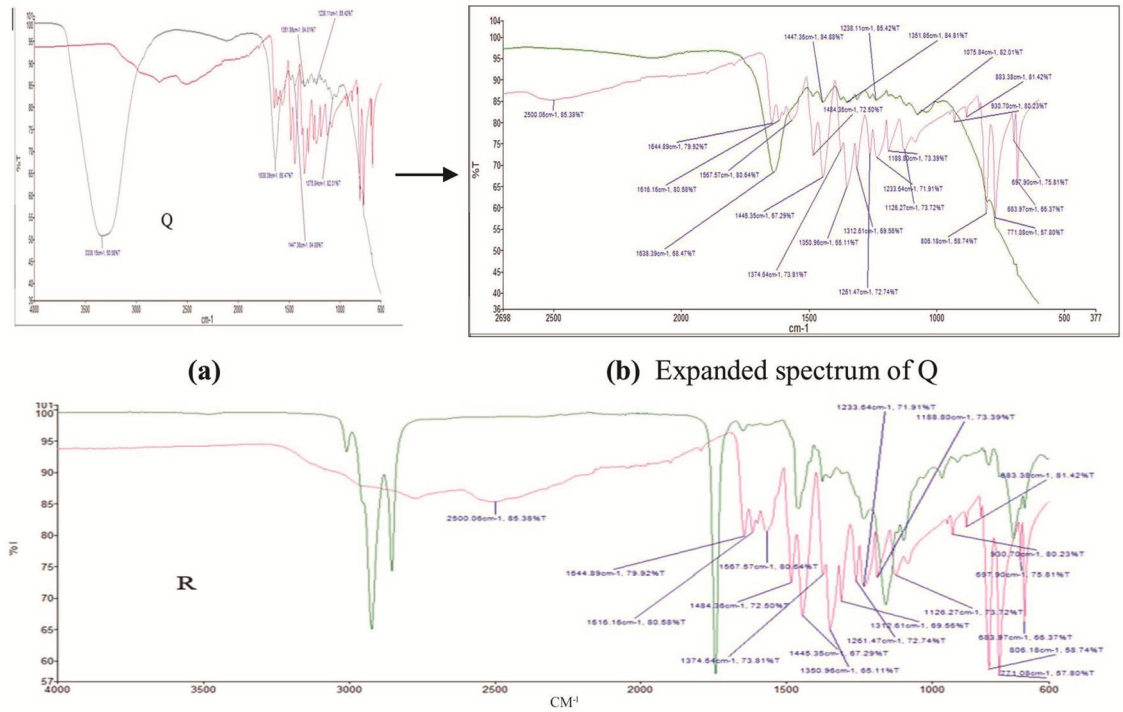


Figure 5. FTIR spectra of Mesalazine and (N) (a) mixture of Mesalazine and chicken stock, (b) close-up view of the fingerprint region with peak differences, (O) mixture of Mesalazine and vegetable stock, (P) (a) mixture of Mesalazine and fruit juice, (b) close-up view of the fingerprint region with peak differences, (Q) (a) mixture of Mesalazine and milk, (b) close-up view of the fingerprint region with peak differences and (R) mixture of Mesalazine with soybean oil.

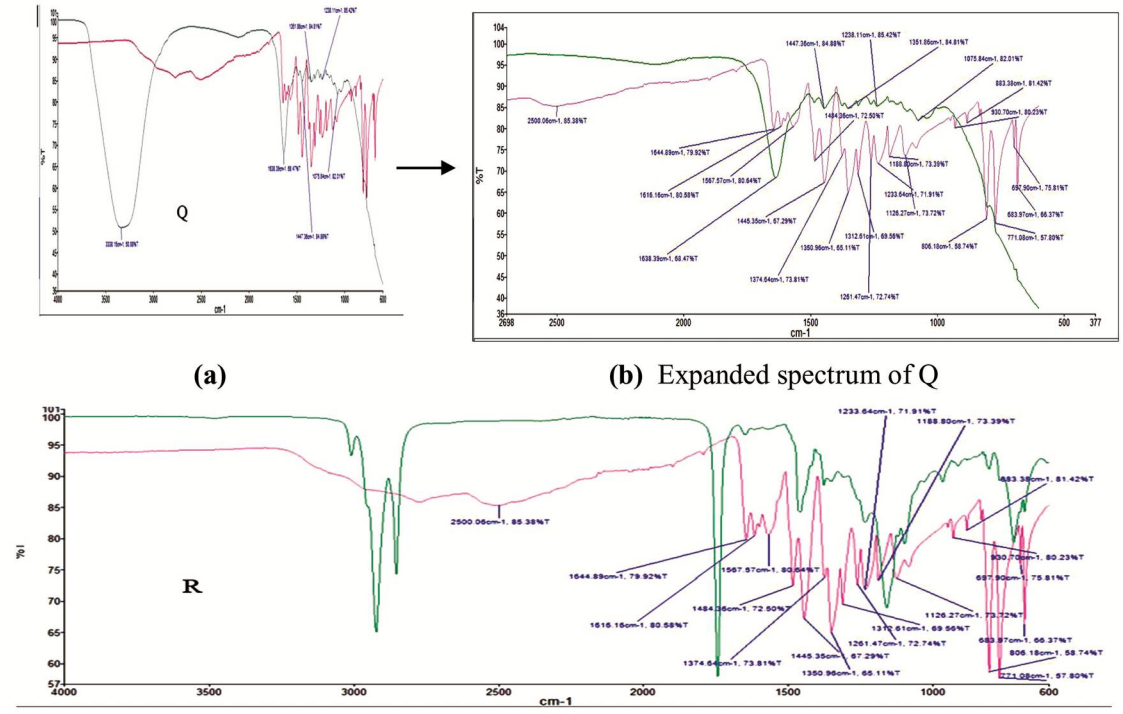


Figure 6. FTIR spectra of Mesalazine and (S) (a) mixture of Mesalazine and phosphate buffer of pH 7.4 and (b) close-up view of the fingerprint region with peak differences.

Table 2. Comparison of characteristic peaks (in cm^{-1}) of mesalazine and combinations of mesalazine with drug and food samples.

Peaks of Mesalazine	Peaks of mixture of Mesalazine and Acetamino-phen	Peaks of mixture of Mesalazine and Metronidazole	Peaks of mixture of Mesalazine and dry Vitamin D_3	Peaks of mixture of Mesalazine and chicken stock	Peaks of mixture of Mesalazine and vegetable stock	Peaks of mixture of Mesalazine and fruit juice	Peaks of mixture of Mesala-zine and milk	Peaks of mixture of Mesalazine and soybean oil	Peaks of mixture of Mesalazine in pH 7.4 solution
(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)	(ix)	(x)
2500.06	-	2503.73	2498.09	-	-	-	-	-	-
1644.89	1650.28	1644.96	1644.41	-	-	-	-	-	1644.76
1484.36	-	1484.98	1484.54	-	-	-	-	-	1485.19
1445.35	1437.21	1446.0	1445.45	-	-	1446.17	1447.36	-	1446.84
1350.96	-	1352.72	1350.72	1348.33	-	1353.22	1351.86	-	1350.56
806.18	807.2	807.29	806.88	-	-	-	-	806.25	-
771.08	771.29	771.44	771.1	-	-	-	-	-	-
683.97	683.5	683.88	683.92	-	-	-	-	684.13	-

which marked the absence of any interaction of the drug with Metronidazole and Vitamin D_3. However, the significant absence of characteristic peaks of Mesalazine in the spectra of combination of Mesalazine and chicken stock in column (v), Mesalazine and vegetable stock in column (vi), Mesalazine and fruit juice in column (vii), Mesalazine and milk in column and (viii) Mesalazine and soybean in column (ix) in the fingerprint region revealed the presence of possible interactions of the drug with foods. This also suggested that the properties of Mesalazine were also not retained, questioning its therapeutic advantage when taken simultaneously with the selected food items. Lastly, the absence of four characteristic peaks of Mesalazine in the spectra of combination of Mesalazine in buffer solution of pH 7.4 in column (x) might suggest the presence of possible interactions between them, but from previously conducted studies by other researchers, it was found that the luminal release of 5-ASA from Mesalazine was possibly not repressed by the active inflammatory condition in IBD patients[10] and worked satisfactorily to treat the flares of ulcerative colitis and Crohn's diseases. In addition, it can be perceived from the peaks that Mesalazine retained its own properties by keeping the amino functional group and carboxyl group undamaged and peaks that were absent did not take part in binding with the receptor sites, rather they only played role in securing the structural integrity of Mesalazine. Having been said this, we could assume that Mesalazine worked efficaciously in the pH above 7, which is generally the intestinal milieu of IBD patients.

Differential Scanning Calorimetry (DSC).

The DSC thermograms of the individual drugs and the binary mixtures (1:1) of Mesalazine with Acetaminophen, Metronidazole and dry Vitamin D_3 are shown in figure 7 and figure 8 respectively.

It can be easily found from figure 7 that the DSC curve of Mesalazine consisted of one endothermic peak at 286.21°C, related to the melting process. In the same way, Acetaminophen showed one endothermic peak at 170.78° C and Vitamin D3 showed an exothermic peak at 197.37° C. However, there was one sharp endothermic peaks of Metronidazole found at 164.75° C.

In the DSC curve of the binary mixtures of other drugs with Mesalazine, the following events were seen.

The appearance of a peak in DSC curves of the mixtures (Mesalazine/ Metronidazole) and peak broadening and a shift towards lower temperature (Mesalazine/ Acetaminophen) owing to the melting deviations from characteristic peaks of the individual drugs suggested interactions indication.[11] However,

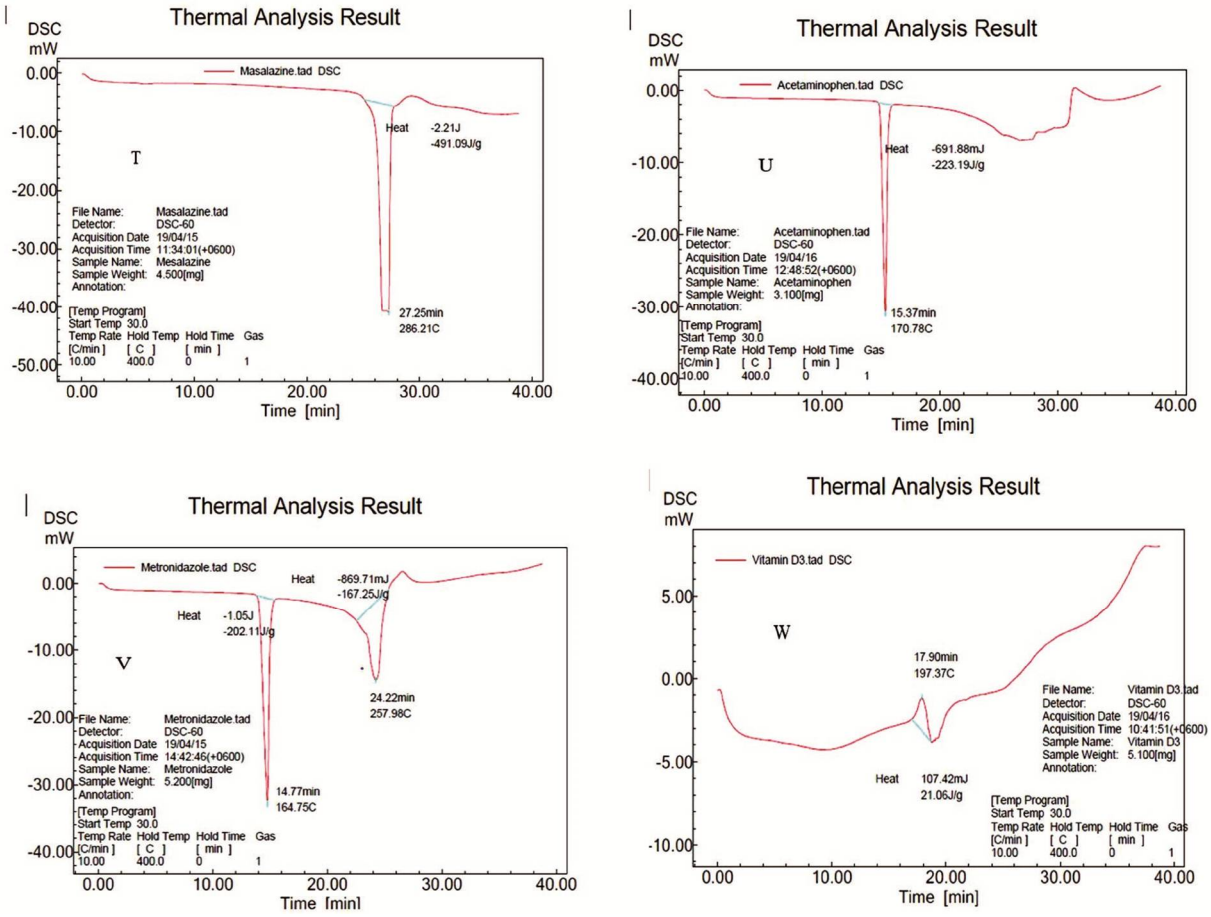
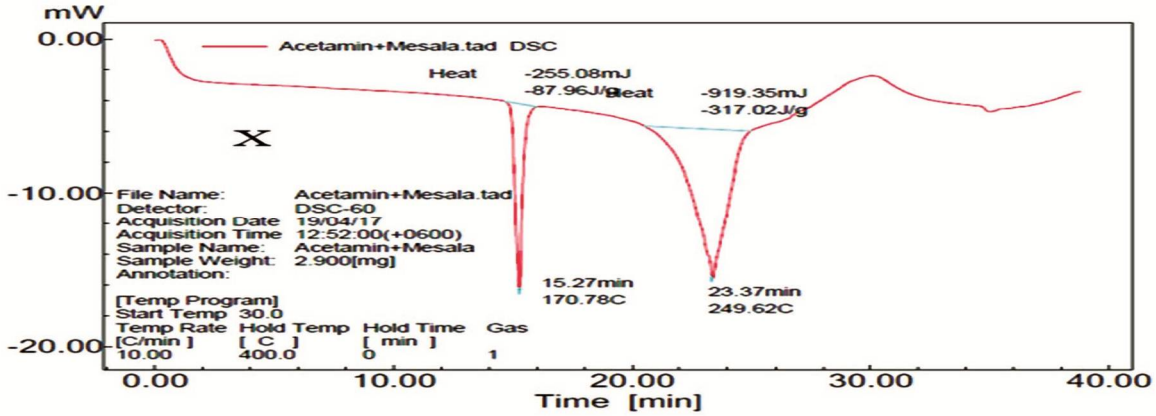


Figure 7. DSC thermograms of (T) Mesalazine, (U) Acetaminophen, (V) Metronidazole and (W) Vitamin D_3.



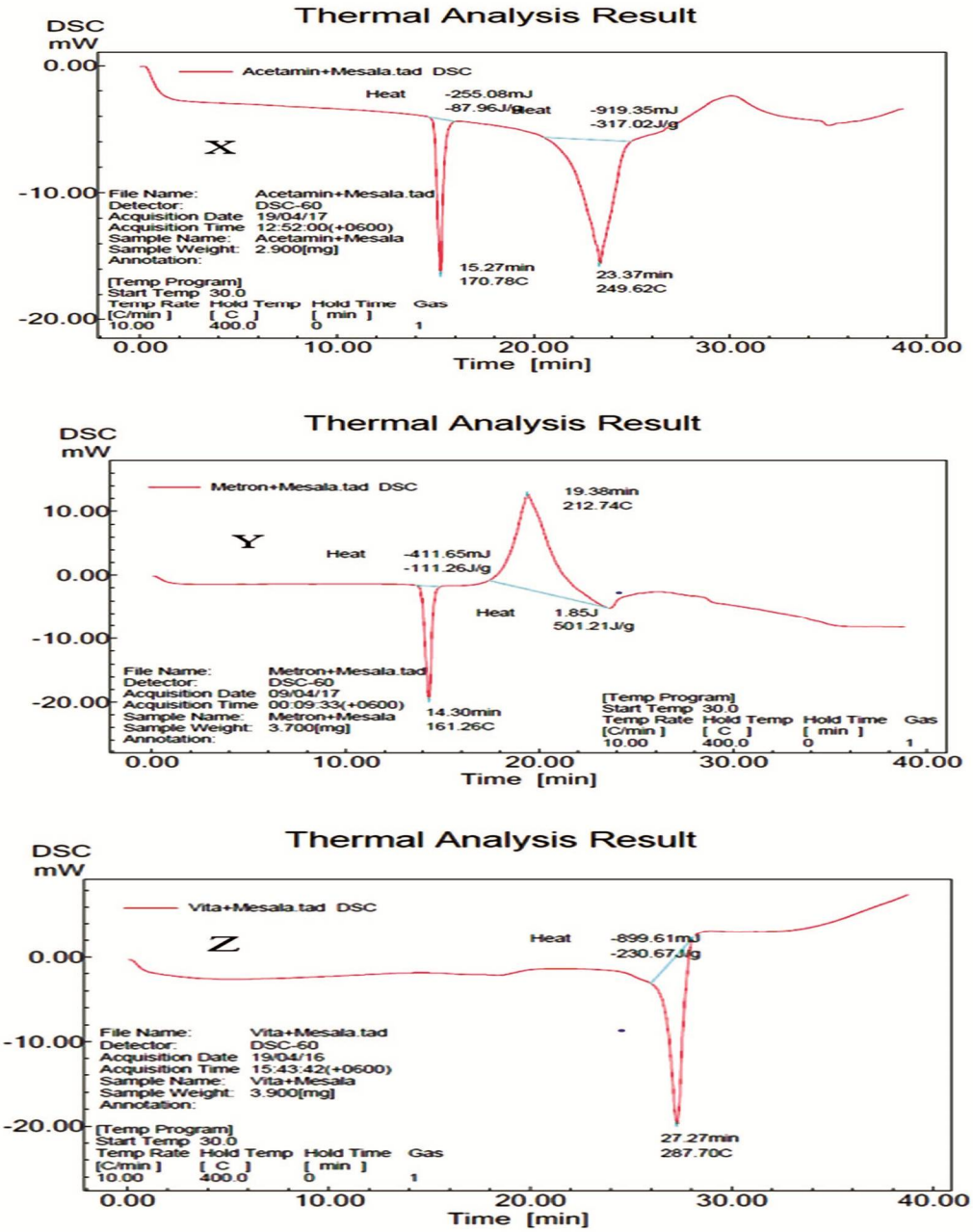


Figure 8. DSC thermograms of the 1:1 mixture of (X) Mesalazine and Acetaminophen, (Y) Mesalazine and Metronidazole and (Z) Mesalazine and Vitamin D_3.

Table 3. Comparison of DSC data of Mesalazine and binary mixtures (1:1) of Mesalazine with other drug substances.

Sample	Endothermic Peak	Exothermic Peak	Temperature (°C)	Peak considered for possible interaction
Mesalazine	Present	Absent	286.21	286.21
Mesalazine/ Acetaminophen	Present	Absent	170.78	
			249.62	249.62
Mesalazine/ Metronidazole	Present	Present	161.26	
			212.74	212.74
Mesalazine/vit. D3	Present	Absent	287.70	287.70

the change in peaks might be obliged to the interactions between the drugs and the gas used in DSC, difference of molecular weights between the studied compounds or due to insufficient mixing when the binary combinations of the drugs were made. Also, very small particles in the mixture might amalgamate with larger particles causing surface interactions, but such physical interactions can not cause any hamper in the biosystem, because in the presence of aqueous gastrointestinal fluid, the smaller particles get separated from the larger particles to dissolve and make the drug bioavailable.[12]

CONCLUSION

The study was undertaken with an aim to evaluate the drug-drug and drug-food interactions of Mesalazine using the commonly prescribed drugs with it and various food samples. FTIR study of pure Mesalazine and its mixtures with other drug samples showed that there were no interactions of Mesalazine with Metronidazole and Vitamin D_3, however, possible interactions may exist with Acetaminophen. On the other hand, from the FTIR study of Mesalazine and food samples, it was concluded that food shows major interactions with Mesalazine, if administered concurrently. So, it is better to avoid taking this medication with food.

Furthermore, the DSC study on the compatibility of Mesalazine with Acetaminophen, Metronidazole and dry Vitamin D_3 has shown that Vitamin D_3 fulfills its role as a compatible drug with Mesalazine, nonetheless, Acetaminophen and Metronidazole showed some clues of interactions, leaving us the space for further investigation with divergent analytical approaches.

Conclusively, it is worth mentioning that, Mesalazine may be prescribed with the above mentioned drugs concomitantly if the benefits outweigh the risks in specific patient groups but it should not be administered in conjunction with foods prerequisitely.

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AUTHORS' CONTRIBUTION

Alam, M.M. conceived the presented idea. Alam, M.M. and Tasneem, F. developed the theory and performed the computations. Kabir, A.K.L. and Rouf, A.S.S. verified the analytical methods and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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