4.0 Preformulation study

Preformulation study is one of the important pre-requisite in development of any drug delivery system. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence, preformulation studies on the obtained sample of drugs are required (1).

4.1 Confirmation of Drugs

Confirmation of drugs (TMZ and LND) was carried out by melting point determination, Fourier Transformed Infrared (FTIR) analysis, Differential Scanning Calorimetric (DSC) analysis and powder X-ray diffraction (pXRD).

4.1.1 Melting point method

Melting point determination is prime requirement for confirming purity of drug sample. In this method, drug (TMZ and LND) was filled into capillary tube and the capillary tube was placed in an electrically operated melting point apparatus (VEEGO, Mumbai) in which temperature was gradually increased. The temperature range at which the drug started melting and completely melted was noted (2).

4.1.2 FTIR analysis

FTIR spectrum of drugs (TMZ and LND) was measured with a FTIR spectrophotometer (IR Affinity -1S (Shimadzu, Japan) in range 400–4000 cm^{-1} using a resolution of 4 cm^{-1} (2).

4.1.3 DSC analysis

DSC analysis was carried out using a Differential Scanning Calorimeter (DSC-60, Shimadzu, Japan) at a heating rate of 10°C per minute in the range of 30°C to 250°C under inert nitrogen atmosphere at a flow rate of 40 ml/min. DSC thermograms were recorded for TMZ and LND (3).

4.1.4 pXRD analysis

X-ray diffraction patterns of both drugs (LND and TMZ) were obtained using X-ray diffractometer (RigakuUltima IV; Japan) in which Cu-Kα line used as a source of radiation by operating at the voltage 40 kV and the current applied was 40 mA. Both samples were measured in the 2θ angle range between 5°-50° with a scanning rate of 3°/min and a step size of 0.02° (2).

4.2 Drug excipient interaction study

The drug-excipients interaction study was carried out using FTIR analysis. In case of TMZ-excipients interaction studies, FTIR spectra of TMZ, BSA, HA, CS and physical mixture of excipients and drug were scanned while in case of LND-excipient interaction studies, FTIR spectra of LND, BSA, Lf and physical mixture of excipients and drug were scanned. In both the cases, The FTIR spectra of mixture was compared with that of the FTIR Spectra of pure drug and excipient, to study the interaction by identifying any changes occur or not in the principle peaks of spectra of pure drug and excipient.

4.3 Stability assessment of TMZ in different solvents

The stability of TMZ in various solvents were assessed by the UV-Visible spectrophotometry method in which stock solution of TMZ (1000 μg/ml) was prepared in different solvents like water, methanol, ethanol and isopropyl alcohol, From the stock solution, 10 μg/ml working stock was prepared and scanned by UV-Visible spectrophotometer at periodic time interval to assess the time dependent stability of TMZ in different solvents. In order to increase stability different stabilizers were also tried like sodium acetate buffer, citrophosphate buffer, sodium metabisulfite and tartaric acid. To assess the stability in presence of stabilizer TMZ were dissolved in different stabilizer solutions and from the stock 25 μg/ml working solution was prepared and analyzed by same method as described for solvents.

4.4 Results and discussion

4.4.1 Melting point

The melting point of TMZ was found to be 206°C – 208°C which was similar as reported melting point 212°C (2). In case of LND, melting point was found to be 269°C – 271°C which was similar as reported range 268°C – 270°C (4).

4.4.2 FTIR analysis

The FTIR spectrum of TMZ and LND is shown in figure 4.1 and figure 4.2 respectively and value obtained is given in table 4.1 and 4.2 respectively. The obtained data was compared with the standard spectrum of drugs which was found to be similar as the entire characteristic peaks were present in the obtained spectrum which authenticated the drug (2,5).

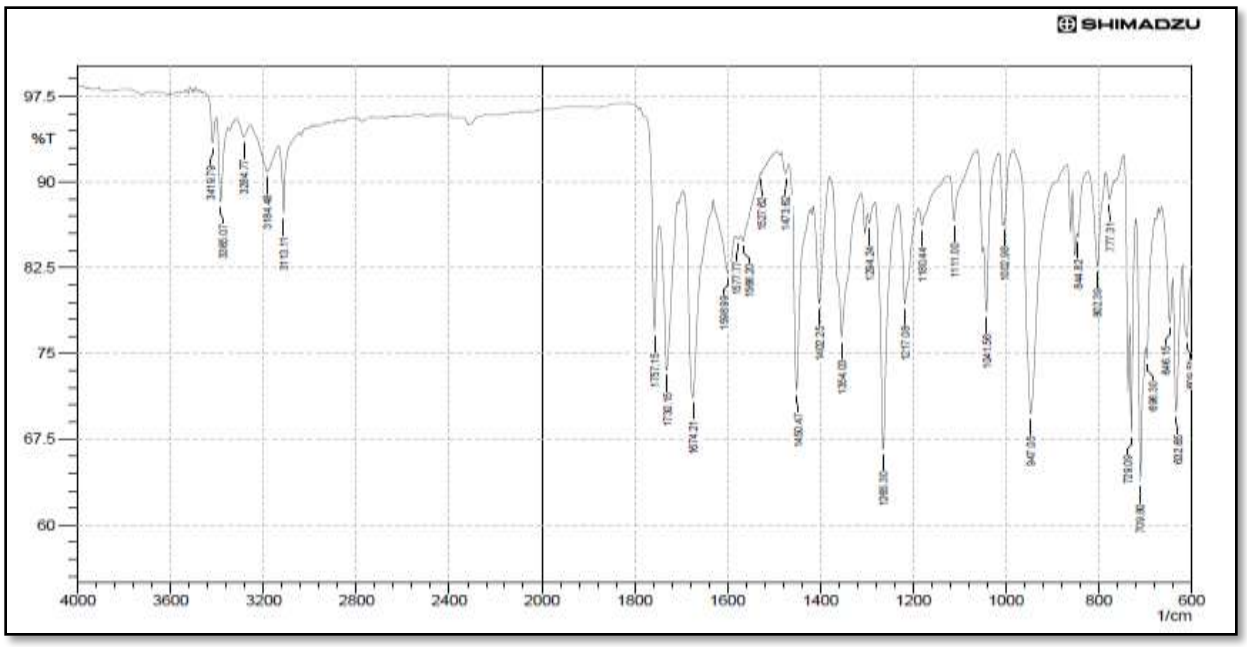

Figure 4.1 FTIR spectrum of TMZ

Table 4.1 Interpretation of FTIR spectrum of TMZ

Group present	Frequency reported	Frequency Observed
-N-H(stretching) for amine	3500-3100 cm-1	3419.79 cm^{-1} and 3385.07 cm^{-1}
=C-H (stretching) for alken	3150-3050 cm-1	3113.11 cm^{-1}, 3184.48 cm^{-1} and 3284.77 cm^{-1}
-C=O (stretching) for aldehyde, ketone or amide	1740-1720 cm-1	1757.15cm^{-1}, 1730.15cm^{-1} and1674.21 cm^{-1}
-N-H (bending)	1690-1640 cm-1	1690-1640 cm^{-1}
-C=C (stretching) for alken	1680cm^{-1} to 1600cm^{-1}	1598.99 cm^{-1} and 1674.21 cm^{-1}
-C-N (stretching) for amine	1360cm^{-1} to 1180cm^{-1}	1360cm^{-1} to 1180cm^{-1}

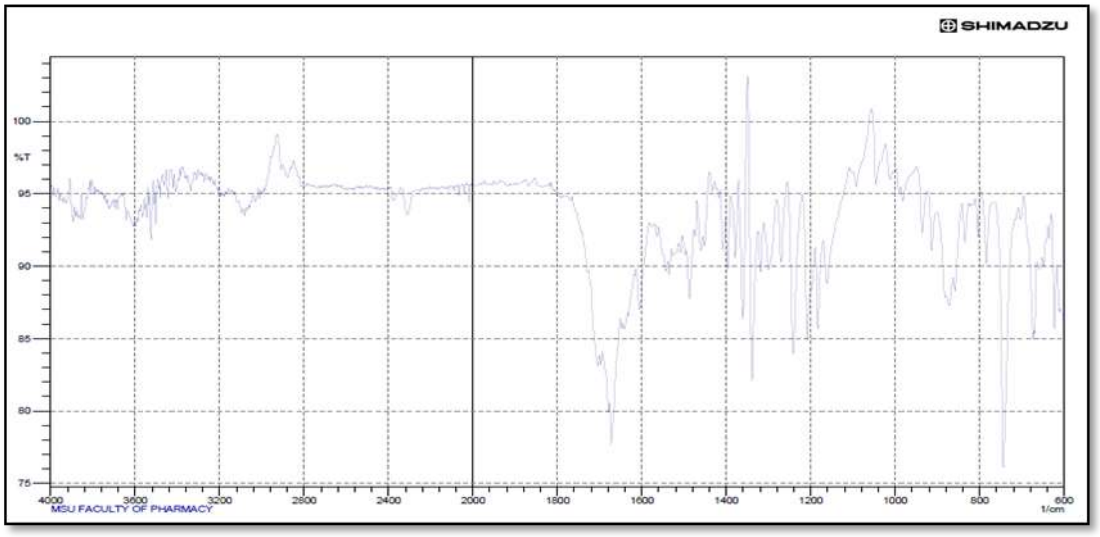

Figure 4.2: FTIR spectrum of LND

Table 4.2 Interpretation of FTIR spectrum of LND

Group present	Frequency reported	Frequency Observed
-N-H(stretching) for amine	3600-3200 cm-1	3408.22 cm^{-1}
-C-H (stretching) for aromatic alken	3100-3000 cm-1	3113.11 cm^{-1}, 3184.48 cm^{-1} and 3284.77 cm^{-1}
$-NH_2$ stretching	1600 cm-1	1670 cm^{-1}
-C=O (stretching) for carbonyl	1740-1720 cm-1	1720.18 cm^{-1}

4.4.3 DSC analysis

The melting point and the behaviour of the drug upon heating were studied through the DSC curve. In case of TMZ, a sharp exothermic peak at 208.81^{o}C was observed which indicates crystalline nature of TMZ (Figure 4.3) while in case of LND a sharp endothermic peak at 271.61^{o}C was observed which also indicates crystalline nature of LND (Figure 4.4). The observed values were also accordance with previous reported literature (2,6).

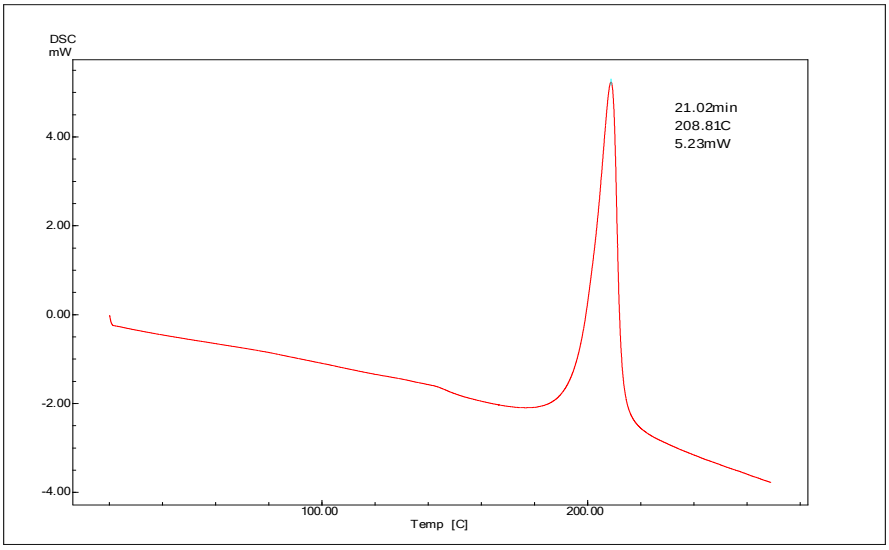

Figure 4.3: DSC thermogram of TMZ

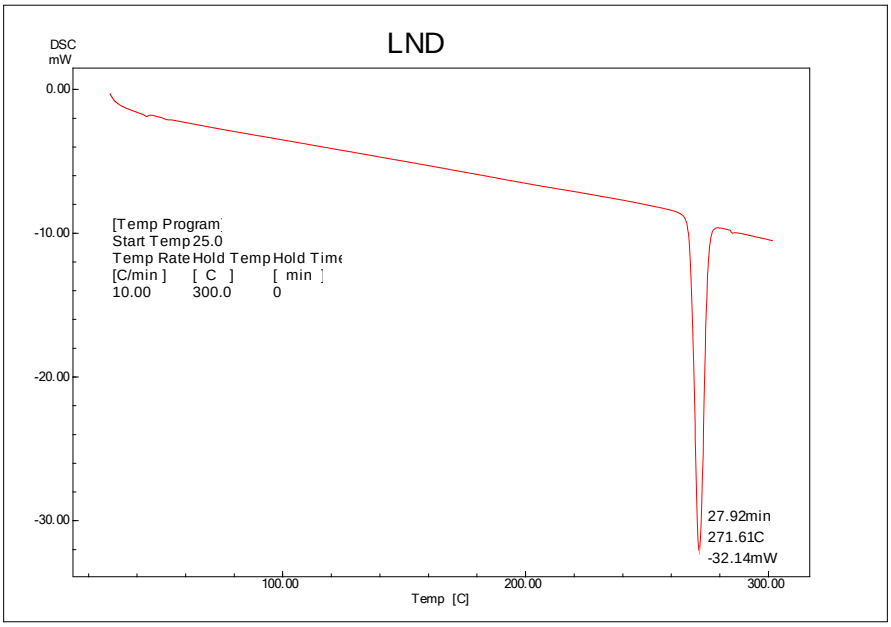

Figure 4.4: DSC thermogram of LND

4.4.4 pXRD analysis

pXRD diffractograms of pure TMZ and LND are shown in figure 4.5 and 4.6 respectively. TMZ showed characteristic sharp and intense peaks at 2θ values of 10.7°, 14.64°, 26.4° and 28.2° indicative of highly crystalline nature of drug. While LND showed sharp and intense peaks at 2θ

values of 7.78°, 14.34°, 17.64°, 20.58°, 24.01° and 26.08° which also indicated the crystalline nature of LND. The obtained results were similar as reported earlier (2,6).

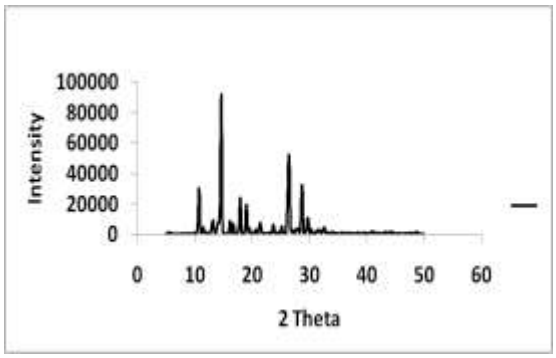

Figure 4.5: pXRD diffractogram of TMZ

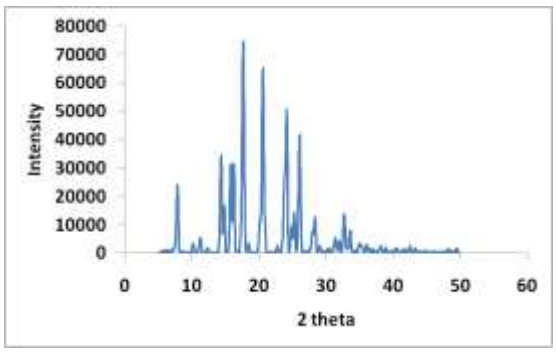

Figure 4.6: pXRD diffractogram of LND

4.4.5 Drug-excipient interaction studies

To assess TMZ-excipient interactions, different FTIR spectrum was generated and analysed (Figure 4.7). The FTIR spectrum of BSA showed characteristic peaks at 3277.06 cm^{-1}[–NH stretching vibration], 2873 -2950 cm^{-1} [-C-H and -C-H methoxy stretching vibration], 1641.42 cm^{-1}[-C=O stretching vibrations of amide] and 1535.34cm^{-1}[-N–H bending -C–N stretching vibration of amide]. FTIR spectrum of HA showed characteristic peaks at 3374cm^{-1} [-OH stretching], ~1550-1560 cm^{-1} [amide II -N-H band], 1604.77 cm^{-1} and ~1400 cm^{-1} [-C=O stretching vibration]. FTIR spectrum of CS showed characteristic peaks at 3340.71 cm^{-1} [-OH stretching], ~1562.34 cm^{-1} [amide II -N-H band], 1612.49 cm^{-1} and ~1408.04 cm^{-1} [-C=O stretching vibration]. In case of TMZ-BSA physical mixture spectrum, all the characteristic peaks of TMZ (mentioned in table 4.1) and BSA were retained and no sign of interactions was observed (no addition or deletion of peak) which indicated compatibility of TMZ and BSA. In

case of LND-excipient interactions studies also no signs of interaction was observed and results are depicted in figure 4.8. FTIR spectrum of Lf showed characteristic peaks at 1639.49 cm^{-1} [C=O stretching of amide I], 1525.69 cm^{-1} [N-H bending with contribution of C-N stretching of amide II], 3300 cm^{-1} [O-H stretching], 2872.01 cm^{-1}, 2972.31 cm^{-1} and 2980.02 cm^{-1} [C-H stretching vibration], 900 to 1200 cm^{-1} [C-O, C-C stretches and C-O-H, C-O-C deformation] and 611.43-659.66 cm^{-1} [-NH2 and-NH wagging] (7). The physical mixture (LND-BSA-LF) spectrum showed all the characteristic peaks of LND (mentioned in table 4.2), BSA and Lf (no addition or deletion of peak) which indicated compatibility of LND with BSA and Lf.

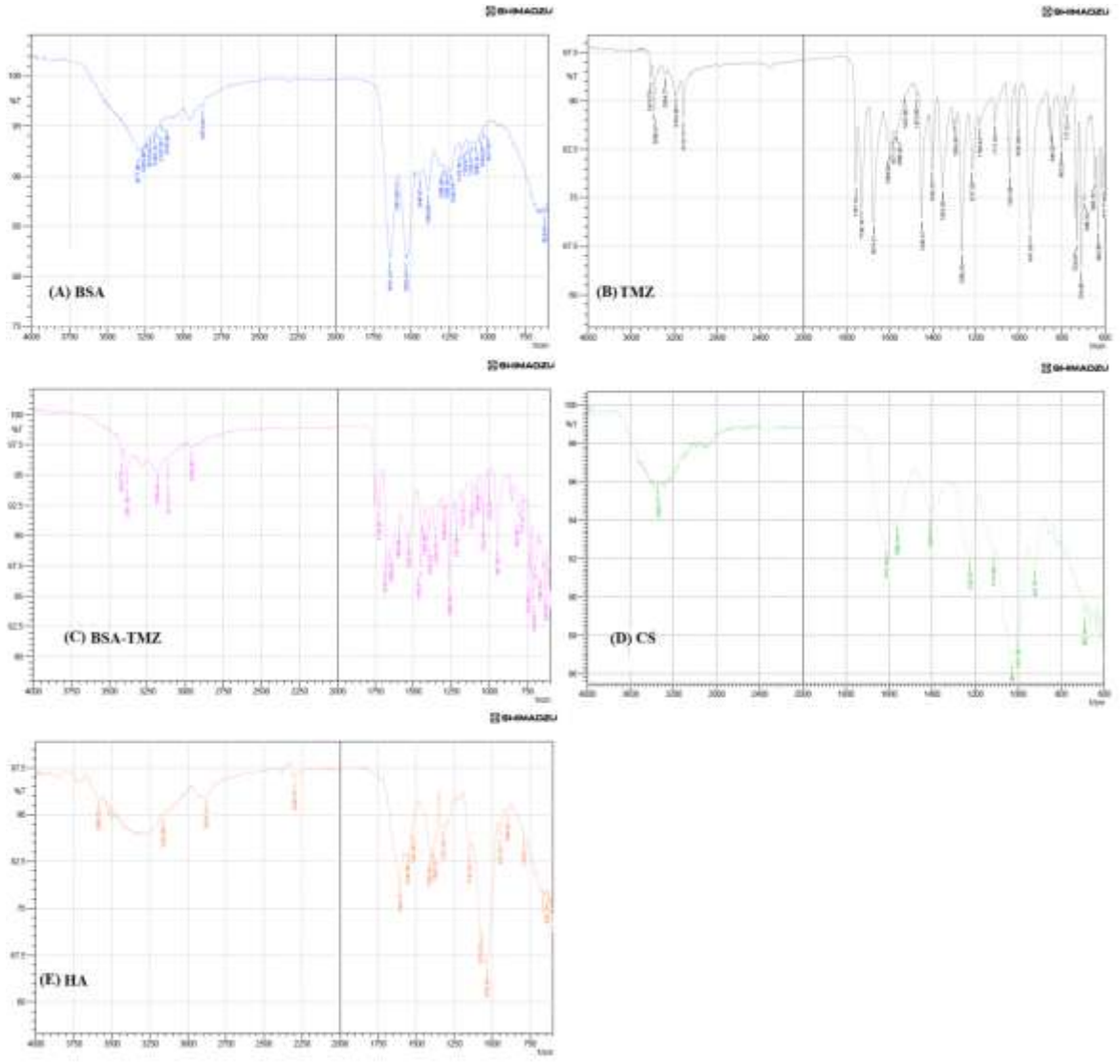

Figure 4.7: TMZ-excipients compatibility study by FTIR where (A) Spectrum of BSA, (B) Spectrum of TMZ, (C) Spectrum of Physical mixture of BSA-TMZ, (D) Spectrum of CS and (E) Spectrum of HA

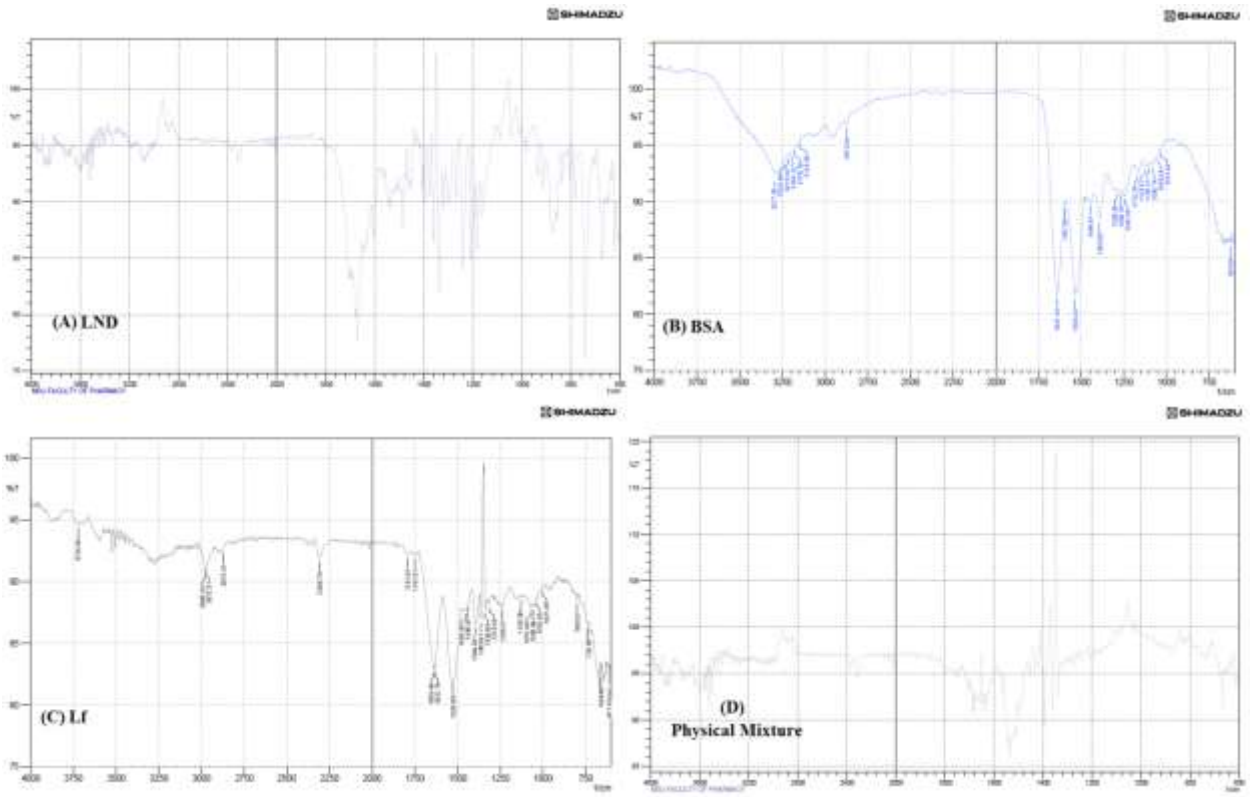

Figure 4.8: LND-excipients compatibility study by FTIR where (A) Spectrum of LND, (B) Spectrum of BSA, (C) Spectrum of Lf and (D) Spectrum of Physical mixture of BSA-LND - Lf

4.4.6 Stability of TMZ in different solvents

TMZ showed pH dependent stability. At neutral and alkaline pH it rapidly degraded and converted in AIC (5-amino-imidazole-4-carboxamide) which can be detected by the UV-Visible spectrophotometry because it shows characteristic absorption maximum at 265nm (8). So before selecting solvent for the development of formulation, stability of TMZ in various solvents were assessed by the UV-Visible spectrophotometry method and results are shown in table 4.3. The results indicated that in water, TMZ was stable up to 5-6 hrs and after that degradation of TMZ started as the absorbance at 265 nm increased which corresponded to AIC (Figure 4.9). In case to methanol, TMZ was stable only for 1 hr while in ethanol and IPA stability was higher (more than 15 hr and 7 hr respectively) (Figure 4.9). Stability of TMZ in presence of different stabilizers were also assessed and results (figure 4.10) indicated maximum stability of TMZ in citro phosphate buffer and tartaric acid so either of these two can be used in the formulation to enhance the stability.

Table 4.3: Stability of TMZ in different solvents

Sr. no.	Solvent	Stability	Time
1	Water	Stability decrease with time	5-6 hr
2	Ethanol	stable	15 hr and more
3	Methanol	unstable	1 hr
4	IPA	stable	7 hr and more

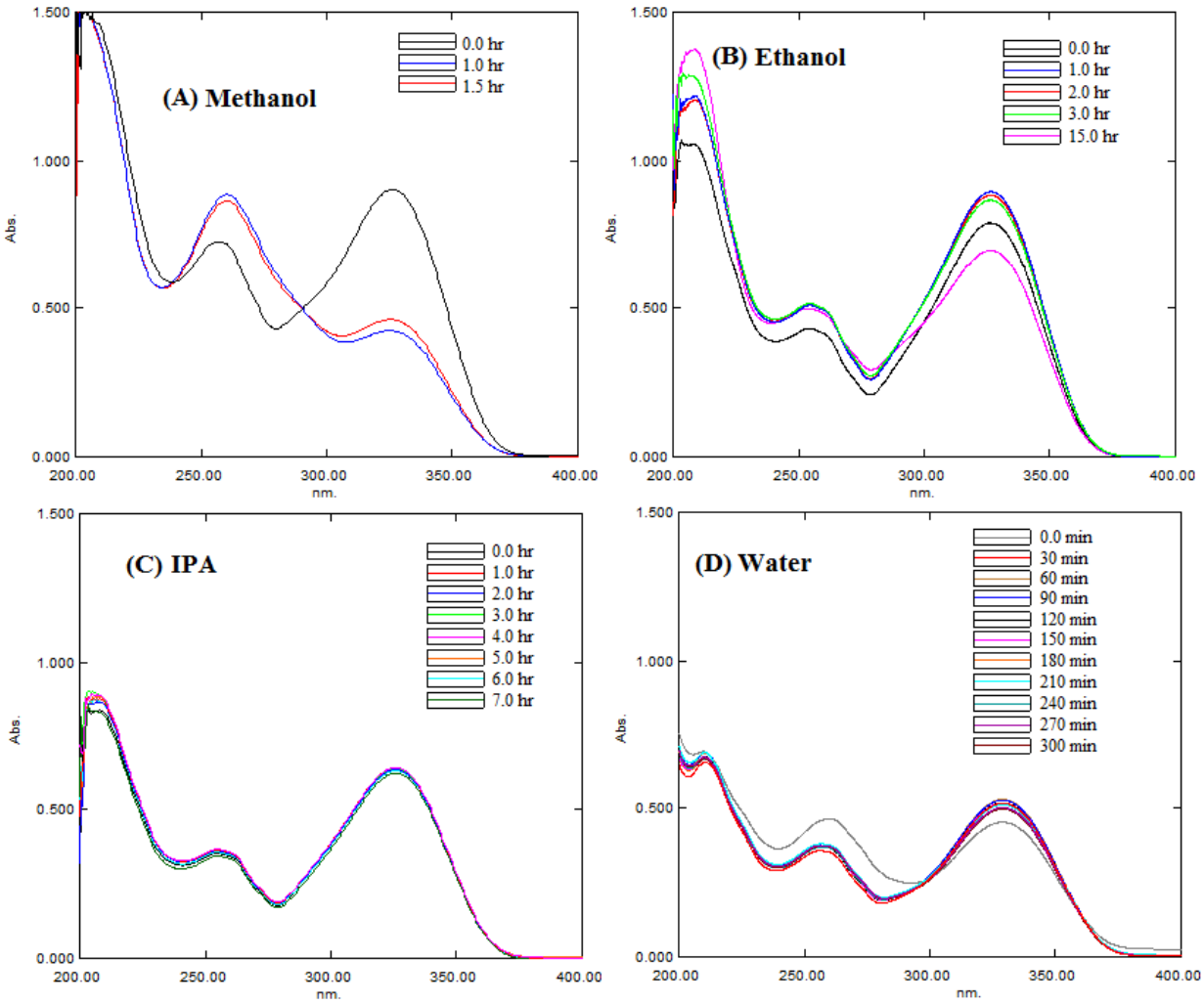

Figure 4.9: Stability of TMZ in different solvents where (A) Spectrum in methanol, (B) Spectrum in ethanol, (C) Spectrum in IPA and (D) Spectrum in water

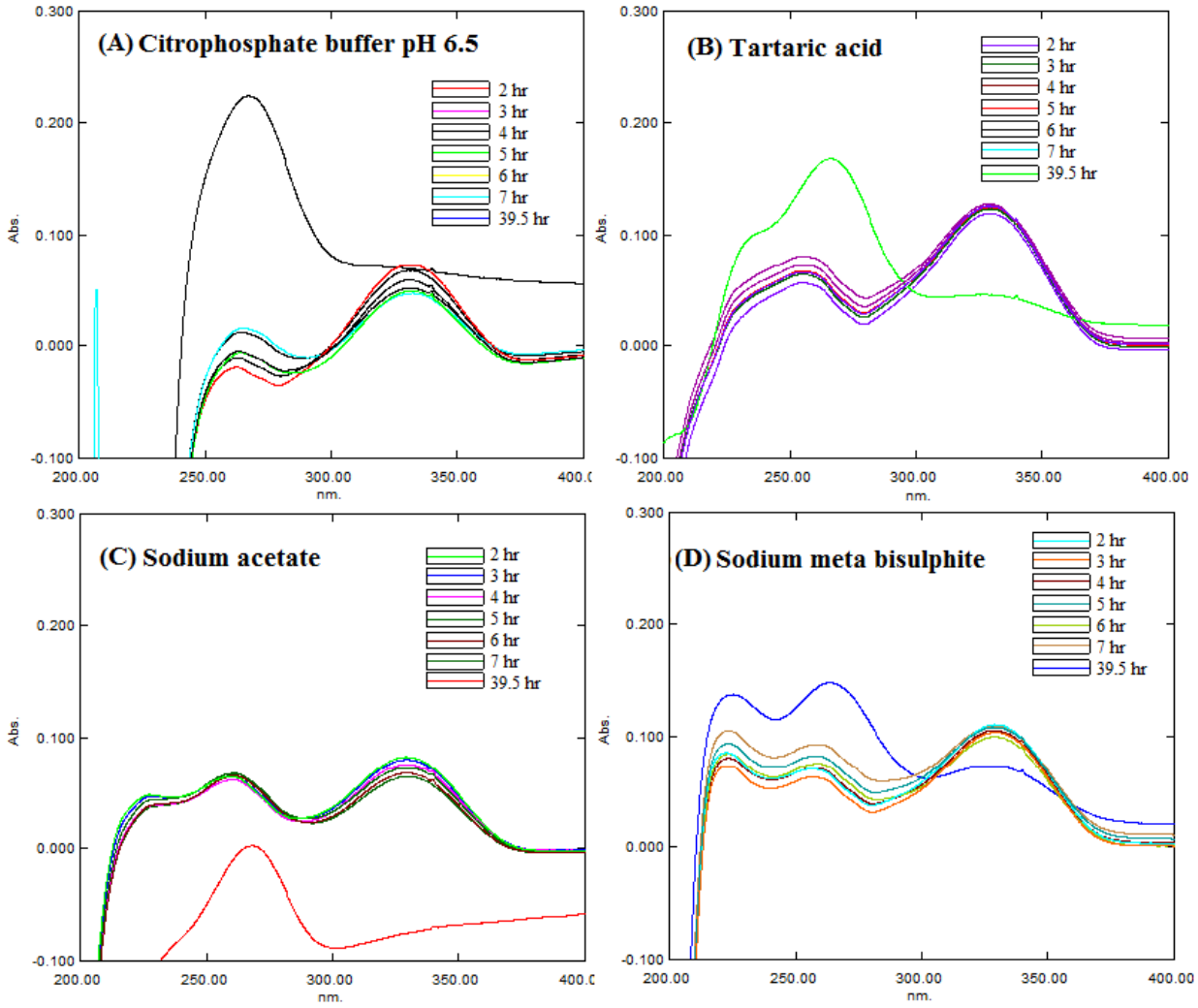

Figure 4.10: Stability of TMZ in different stabilizers where (A) Spectrum in citrophosphate buffer pH 6.5, (B) Spectrum in tartaric acid, (C) Spectrum in sodium acetate and (D) Spectrum in sodium meta bisulphite

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