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EVALUATION OF PHYSIOCHEMICAL AND ANTI-TUBERCULAR ACTIVITY OF CO-CRYSTAL OF ISONIAZID WITH METHYL PARABEN

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ABSTRACT

The aim of the present research work to apply crystal engineering for the selection of API and co-former with primary amide and to investigate the preparation and characterization of co-crystal preparation. Isoniazid (INH, Pyridine-4-carboxyhydrazide) is used as a first line anti-tubercular agent, in combination with other anti-tubercular drugs for the effective treatment of active diseases and also used for prevention of tuberculosis in individuals who have been exposed to active disease. The supra molecular interactions of isoniazid with carboxylic acid resulted in co-crystal based on the nature of carboxylic acid.co-crystals of (1:1 stoichiometric ratio) isoniazid with methyl paraben was reported. These prepared crystal forms I resolve the poor micrometric problems of isoniazid and shows improved flow and compaction property than isoniazid. From the anti-tubercular test performed it has confirmed that the co-crystal forms of methyl paraben and INH (S9) had shown increased activity compared to the pure drug.

KEY WORDS: Isoniazid, Methyl paraben, Anti-tubercular activity.

INTRODUCTION

The drugs therapeutic efficiency depends upon the bioavailability i.e. the amount of drug reaches the systemic circulation. Two main factors influence the bioavailability are Solubility, Permeability. Many other factors influence these bioavailability such as chemical stability, poor dissolution rate, purity, compaction behaviour of crystal, moisture uptake and crystal habit etc. Usually market value of a drug significantly lowered by these factors.

Various solid state modifications have been done to improve the bioavailability of drug without hanging its pharmacological activity. These approaches are described such as polymers, solvates, hydrates, salts, co-crystals, and amorphous solids which involves non covalent interactions. Usually developers and regulatory authorities prefer these types of crystal forms, because highly pure products that are superior with respect to reproducibility and scalability were afforded by crystallisation.

The physicochemical properties of each form differ individually which can influence the manufacturability, bioavailability, purification and stability of drugs. Among this the widely used approach is salt formation. However this salt formation has major limitation i.e. a suitable ionisable site should be possessed by a drug (acidic or basic drug). In comparison, the co-crystal formation (freely reversible multi component assemblies) may potentially be employed with all drugs, including acidic, basic and non ionisable molecules.

Isoniazid (INH,Pyridine-4-carboxyhydrazide)is used as a 1st line anti tubercular agent, in combination with other anti-tubercular drugsfor the effective treatment of active diseases and also used for prevention of tuberculosis in individuals who have been exposed to active disease. The supra molecular interactions of isoniazid with carboxylic acid resulted in co-crystal based on the nature of carboxylic acid.co-crystals of (1:1 stoichiometric ratio) isoniazid with

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methyl paraben was reported [1-3].

The scope of present research work aims to apply crystal engineering for the selection of API and co-former with primary amide and to investigate the co-crystal preparation and characterization of the same.

MATERIALS AND METHODS

Following materials were used for the present study,

1. Isoniazid (Loba chemie pvt. Ltd, Mumbai)
2. Methyl paraben (Hi media pvt . ltd, Mumbai)
3. Methanol, Ethanol & Potassium dihydrogen phosphate (Merck Pvt. Ltd, Mumbai)

Following Equipments were used for the present study,

1. Electronic Analytical Balance (Shimadzu,Japan),
2. UV-Visible spectrophotometer UV-1700 (Shimadzu, Japan),
3. FT-IR Spectrophotometer IR 200 (Thermo electron corporation)
4. Differential Scanning Calorimetry (NETZSCH DSC 204)
5. Scanning Electron Microscope (ZEISS Electron Microscope, EVO MA 15)
6. Single Crystal X-Ray Diffractometer (Enraf Nonius CAD4-MV31)

ANALYTICAL METHOD OPTIMIZATION

Number of analytical methods is available for quantification of Isoniazid such as ultra-violet spectroscopy, liquid chromatography with UV detection, gas chromatography and mass spectroscopy. The following method was optimized for further studies.

Standard Curve of Isoniazid (INH) with 0.1N HCl

10 mg of Isoniazid was dissolved in 100 ml of 0.1 N HCl, and further dilutions were made by using 0.1 N HCl to obtain concentrations ranging from 2 to 10 µg/ml. The absorbance of solution was measured at 266.5 nm using UV –Visible Spectrophotometer. The readings obtained are shown in figure no. 1.

Standard Curve of Isoniazid with pH 6.8 Phosphate buffer

10 mg of Isoniazid was dissolved in 100 ml of 6.8 Phosphate buffer, and further dilutions were made by using 6.8 Phosphate buffer to obtain concentrations ranging from 5 to 25 µg/ml. The absorbance of solution was measured at 262.5 nm using UV –Visible Spectrophotometer. The readings obtained are shown in figure no.2.

PREFORMULATION STUDIES

Selection of drug and Coformers

Isoniazid

It is the principal ingredient in "triple therapy" used to effectively treat tuberculosis from 1952 onwards and on the basis of previous literature studies it was evident that a potentially versatile supramolecular reagent to prepare novel supramolecular complexes. The pyridine ring of INH is excellent hydrogen bonding acceptor for carboxylic acids and the possible attaching point for any heterosynthons. The carbohydrazide group of INH has both good hydrogen bonding acceptor (O and N atoms) and donor (3 H atoms) functionality. Hence, it is a potentially versatile supramolecular reagent to prepare cocrystals. Therefore it was selected for further studies.

Coformers

Depend upon the functional group and possible supramolecular synthon of the Isoniazid, GRAS coformers which having carboxylic acid functional group like methyl paraben was selected to prepare novel multicomponent crystals of isoniazid by carboxylic acid pyridine heterosynthon. The solid state properties of Isoniazid and methyl paraben was determined by FTIR, DSC, SEM and XRD.

Infrared spectroscopy (FTIR)

IR spectroscopy was conducted using a FTIR Spectrophotometer (Thermo-IR 200) and Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. The spectrum of INH and methyl paraben was recorded in the wavelength region of 4000–400 cm−1 [4].

Differential scanning calorimetry (DSC)

Thermal analysis of INH and methyl paraben was recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 100C/min was employed with nitrogen pursing. Powder samples (15- 30 mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminum pan was used as reference [5].

Scanning electron microscopy (SEM)

The surface characteristics of INH and methyl paraben was studied by SEM (ZEISS Electron Microscope, EVO MA 15). The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode [6].

Powder X-Ray Diffraction (P-XRD)

The pXRD were undertaken to investigate the crystalline nature of INH and Methyl paraben. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 500 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s [7].

Anti-Tubercular activity

The anti- mycobacterial activity of compounds

were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA). In this method, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of untreated drug and prepared cocrystal were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

ISONIAZID (INH)

FTIR (Fourier Transform Infra-red Spectroscopy) Studies

The FT-IR spectrum of INH showed a strong C=O stretch (Amide) band around 1666.3 cm−1, free NH2 at 1221.3 cm−1, N-H bend at 1634.2 cm−1 and 1411.8 cm−1of pyridine. IR Spectrum and Interpretation of Isoniazid have shown in Figure no.3.

Differential scanning calorimetry (DSC)

DSC thermo grams of isoniazid shows sharp endothermic peak at 172.6C. This indicates pure crystal form. A DSC thermo gram of Isoniazid was shown in figure no.4.

Scanning electron microscopy (SEM)

SEM photography of Isoniazid demonstrates small rod like crystals. SEM photographs are shown in Figure no.5.

Powder X-Ray Diffraction

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The X-ray powder diffraction (XRD) spectra of INH (figure no.6) shows characteristic peak at 19.7^0 (100%), 16.75^0, 15.6^0, 14.35^0 and 12^0 indicates pure Isoniazid.

METHYL PARABEN

FTIR Studies:

IR Spectrum and Interpretation of Methyl paraben has shown in Figure no 7 and table no.2.

From this FTIR results indicated that the methyl paraben is pure and it was selected for further studies.

Differential scanning calorimetry (DSC)

DSC study of Methyl paraben shows endothermic peak at 126.91 0C, A DSC thermo gram of salicylic acid was shown in figure no. 8.

Powder X-Ray Diffraction

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification.

Preparation of novel multicomponent crystal forms of isoniazid

Crystallization can happen from the melt or from the solution, or from the vapor phase. Crystallization can produced from the solution by lowering the temperature, removing the solvent from solution (this is also called evaporation), by an anti-solvent addition method (drowning out), by reactive crystallization (precipitation), or by altering the solution pH (iso-electric precipitation method). In this study solution crystallization (slow evaporation) method has followed to prepare following crystal forms of isoniazid.

Preparation of Crystal form 1: INH-Methyl paraben (1:1) co-crystal by solvent evaporation

Isoniazid (0.137g, one m.mol) and, and methyl paraben (0.152.15g, one m.mol) were dissolved separately in 5 ml of methanol with warming and mixed together. Solution was cooled to room temperature and kept for slow evaporation for 6 h. The crystals were isolated by filtration through a membrane (0.45 µm) and dried in the air (102).

Preparation of Crystal form 1: INH-Methyl paraben (1:1) co-crystal by solvent drop method

INH(0,137g, one m.mol) and methyl paraben (0.152.15g, one m.mol) were taken in glass motor and pestle and grounded up to 10 min. then add solvent(ethanol) few drops in drop wise. And again grounded for 10 min. and keep it for drying.

Preparation of Crystal form 1: INH-Methyl paraben (1:1) co-crystal by co- grinding method

INH (0,137g, one m.mol) and methyl paraben (0.152.15g, one m.mol) were taken in glass motor and pestle And grounded up to 1 hr. and keep it for drying.

RESULT AND DISCUSSION

Crystal form II: INH- methyl paraben (1:1) co-crystal (solvent evaporation)

11.4.1. FTIR Studies

The IR spectra and interpretation for isoniazid, methyl paraben and crystal form **II** were presented in figure no.10.

The FT-IR spectrum of INH showed a strong C=O stretch (Amide) band 1666.3 cm−1 has changed to 1676.3 cm^{-1}, free NH2 at 1221.3 cm−1 has shifted to 1233.5 cm−1, and

1555.5cm−1 to 1556 cm−1of pyridine. The FT-IR spectrum of methyl paraben showed a strong O-H str. vibration at 3308.8 cm−1 that is totally absent and 1681 cm−1 of C=O altered to 1676.3 cm−1, from this result it is indicated that the formation of co-crystal of INH, methyl paraben by solvent evaporation method.

Differential scanning calorimetry (DSC)

DSC experiments were carried out to study the thermal behavior of the crystal form IV in relation to the individual components. DSC thermal data are shown in figure no.11. DSC study of INH and methyl paraben shows endothermic peak at 172.60^0C and 126.91^0C while DSC study of prepared co-crystal shows sharp endothermic value at 103.05^0C, the sharp endothermic values of crystal form IV and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form IV was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form IV of INH with methyl paraben (1:1 molar ratio). This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

Scanning electron microscopy (SEM)

SEM photography of prepared co-crystal shows uniform block like crystals while Isoniazid shows small rod like crystals shows small rod like crystals. SEM photographs of isoniazid, Methyl paraben and crystal form IV shown in figure no.12

Crystal form V: INH- methyl paraben (1:1) co-crystal (solvent drop)

FTIR Studies

The IR spectra and interpretation for isoniazid, methyl paraben and crystal form **V** were presented in figure no.13.

The FT-IR spectrum of INH showed a strong C=O stretch (Amide) band 1666.3 cm−1 has changed to 1677.9 cm^{-1}, free NH2 at 1221.3 cm−1 has shifted to 1233.4 cm−1, and 1555.5cm−1 to 1556.1 cm−1of pyridine. The FT-IR spectrum of methyl paraben showed a strong O-H str. vibration at 3308.8 cm−1 that is totally absent and 1681 cm−1 of C=O altered to 1677.9 cm−1, from this result it is indicated that the formation of co-crystal of INH, methyl paraben by solvent drop method.

Differential scanning calorimetry (DSC)

DSC experiments were carried out to study the thermal behavior of the crystal form V in relation to the individual components. DSC thermal data are shown in figure no.14. DSC study of INH and methyl paraben shows endothermic peak at 172.60^0C and 126.91^0C while DSC study of prepared co-crystal shows sharp endothermic value at 99.44^0C, the sharp endothermic values of crystal form V and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form V was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form V of INH with methyl paraben (1:1 molar ratio). This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

The X-ray powder diffraction (XRD) spectra of INH and methyl paraben co-crystal shows characteristic peak at 25.125^0 which is 100% relative intensity which is different from individual components of INH and METHYL PARABEN also at 16.681^0, 26.073^0, 27.181^0 new peaks were appeared. This indicates formation of new crystalline phase (Fig 15).

Crystal form VI: INH-Para methyl paraben (1:1) co-crystal (co- grinding)

FTIR Studies

The IR spectra and interpretation for isoniazid, methyl paraben and crystal form **VI** were presented in figure no.16.

The FT-IR spectrum of INH showed a strong C=O stretch (Amide) band 1666.3 cm−1 has changed to 1677.8 cm^{-1}, free NH2 at 1221.3 cm−1 has shifted to 1233.5 cm−1, and 1555.5cm−1 to 1556.2 cm−1of pyridine. The FT-IR spectrum of methyl paraben showed a strong O-H str. vibration at 3308.8 cm−1 that is totally absent, from this result it is indicated that the formation of co-crystal of INH, methyl paraben by co- grinding method.

Differential scanning calorimetry (DSC)

DSC experiments were carried out to study the thermal behavior of the crystal form VI in relation to the individual components. DSC thermal data are shown in figure no.17. DSC study of INH and methyl paraben shows endothermic peak at 172.60^0C and 126.91^0C while DSC study of prepared co-crystal shows sharp endothermic value at 99.37^0C, the sharp endothermic values of crystal form VI and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form VI was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form VI of INH with methyl paraben (1:1 molar ratio). This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

The X-ray powder diffraction (XRD) spectra of INH and methyl paraben co-crystal shows characteristic peak at 25.132^0 which is 100% relative intensity which is different from individual components of INH and methyl

paraben also at 16.72^0, 21.411^0, 27.222^0 new peaks were appeared. This indicates formation of new crystalline phase (Fig 18).

Drug content

Drug content of prepared crystal forms were determined in triplicate by spectrophotometrically. The practical yield was found satisfactory and ranged from 88.4% to 96.4% for those in pH 6.8 phosphate buffer and 74.8% to 100% for those in 0.1 N HCl. The values of prepared crystal forms were shown in table no.3.

Saturation Solubility

The solubility studies of Isoniazid and prepared novel multicomponent crystal forms in 0.1N HCl distilled water and pH 6.8 phosphate buffer were shown in table no. 17 and figure no.19. This indicates that pure drug shows high solubility compare with prepared crystal forms of isoniazid. In the prepared crystal forms, Crystal forms 2 have least solubility in 0.1N HCl distilled water and pH 6.8 phosphate buffer.

Measurement of flowability and compressibility

In order to achieve uniformity in tablet weight, the feed crystals must flow smoothly into the die cavity of the tablet machine. Therefore, it is an essential purpose to improve the flow properties of powders.

The micromeritic properties such as angle of repose, Carr's index and Hausner's ratio were calculated in triplicate for pure drug and prepared novel multi component crystal forms. The results of these micromeritic properties were given in table no. 4.

Pure drug isoniazid exhibited poor flowability and compressibility as indicated by Carr's index (0.84%), Hausner's ratio (1.31) and angle of repose (32.37). This could be due to the rod shape and small size of powder with stickiness, which put hurdles in the uniform flow of powder from the funnel. The prepared crystal form 2 showed poor flowability and compressibility as showed by low value of Carr's index (0.136%), Hausner's ratio (1.15) and angle of repose (43.4) when compared to pure drug. The crystal form 1 has shown improvement in the flowability with angle of repose showing 28.39 with Carr's index 0.20% and Hausner's ratio 1.20.

In vitro dissolution studies

Invitro dissolution studies were done in triplicate for isoniazid and prepared novel multicomponent crystal forms of isoniazid in 0.1 N HCl and pH 6.8 phosphate buffer. The powder dissolution profiles for Isoniazid, Crystal form 1, 2, 3, 4, 5 and 6 were shown in figure no.20 and 21 respectively.

Anti-tubercular activity

The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using micro plate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Table 1. Interpretation of IR spectra of INH

FTIR region cm^{-1}	Assignment
3303	N-H stretching
3055	C-H Asym. Stretching
1666.3	C=O stretching
1634.2	Ring Asym. Stretching
1602.4	Ring Asym. Stretching
1555.5	Pyridine nitrogen
1411.8	Ring sym. Stretching
1334.1	C-N stretching
1221.3	Ring C-C-H Asym. Bending
1138.9	N-X.stretching($X=NH_2$)
887.26	C-N-C bending
844.92	Ring C-C-H sym. bending

Table 2. Interpretation of IR spectra of Methyl paraben.

FTIR region cm^{-1}	Asignment
3308.8	O-H stretching vibration
1681	C=O stretching vibration

Table 3: Drug content of prepared crystal forms

	10 mg of crystal form contains (% Yield)	
	0.1N HCl	pH 6.8 phosphate buffer
Crystal form 1	83	94.8
Crystal form 2	74.8	91.6
Crystal form 3	96	88.4
Crystal form 4	100	91.6
Crystal form 5	90.3	96.4
Crystal form 6	88.9	96.4

Table 4. Micromeritic properties of isoniazid and prepared crystal forms

	INH	Methyl paraben	Crystal Form 1	Crystal form 2	Crystal form 3	Crystal form 4	Crystal form 5	Crystal form 6
Bulk density	0.64	0.45	0.4	0.6	2.0	0.61	0.39	0.39
Tapped density	0.84	0.75	0.5	0.85	2.54	0.81	0.57	0.53
Angle of repose	32.37	45.49	28.39	43.4	43.6	44.06	47.54	41.95
Carr's index	0.23	0.40	0.20	0.136	0.27	0.24	0.31	0.26
Hausner ratio	1.31	1.66	1.25	1.15	1.37	1.32	1.46	1.35
Compressibility %	23	40	20	13.6	27	24	31	26

Table 5. MABA results of isoniazid and prepared crystal forms

Sample	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
S1	S	R	R	R	R	R	R	R	R	R
S2	S	S	R	R	R	R	R	R	R	R
S4	S	S	R	R	R	R	R	R	R	R
S5	S	S	R	R	R	R	R	R	R	R
S6	S	S	R	R	R	R	R	R	R	R
S9	S	S	R	R	R	R	R	R	R	R

R-Resistant, S-Sensitive

Fig 1. Standard Curve of Isoniazid with 0.1N HCl

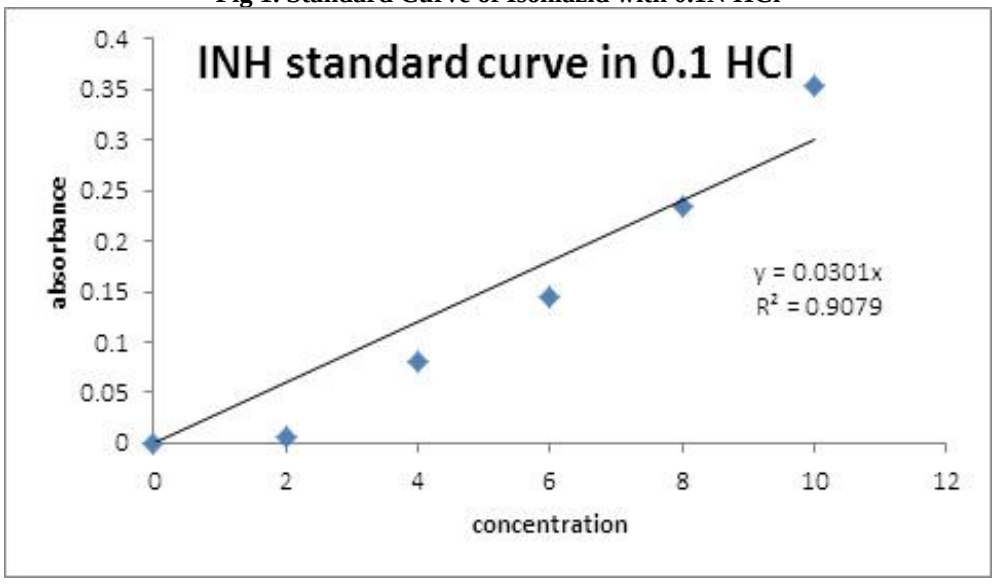


Fig 2. Standard Curve of Isoniazid with pH 6.8 Phosphate buffer

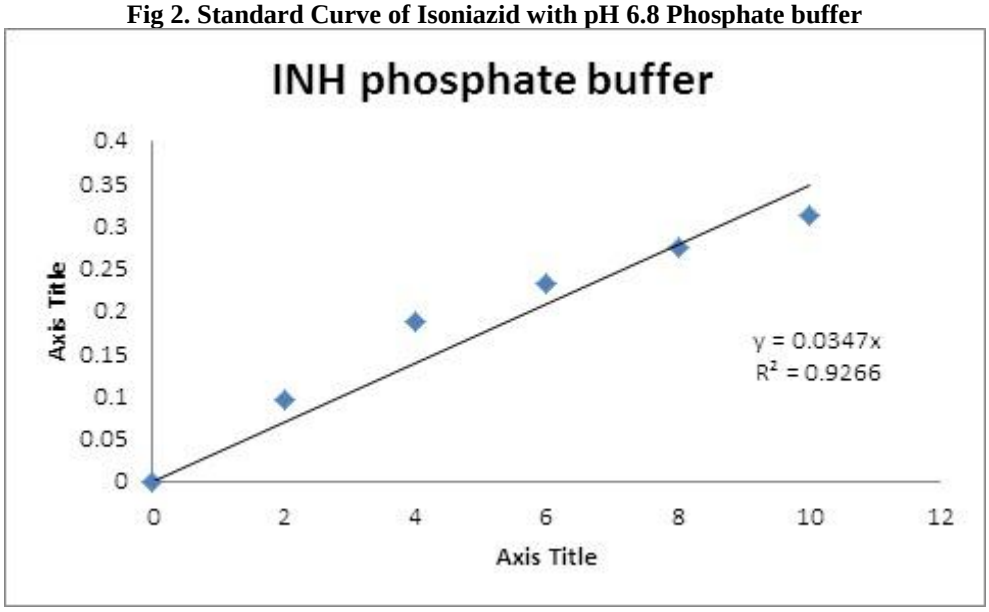


Fig 3. FTIR spectra of INH

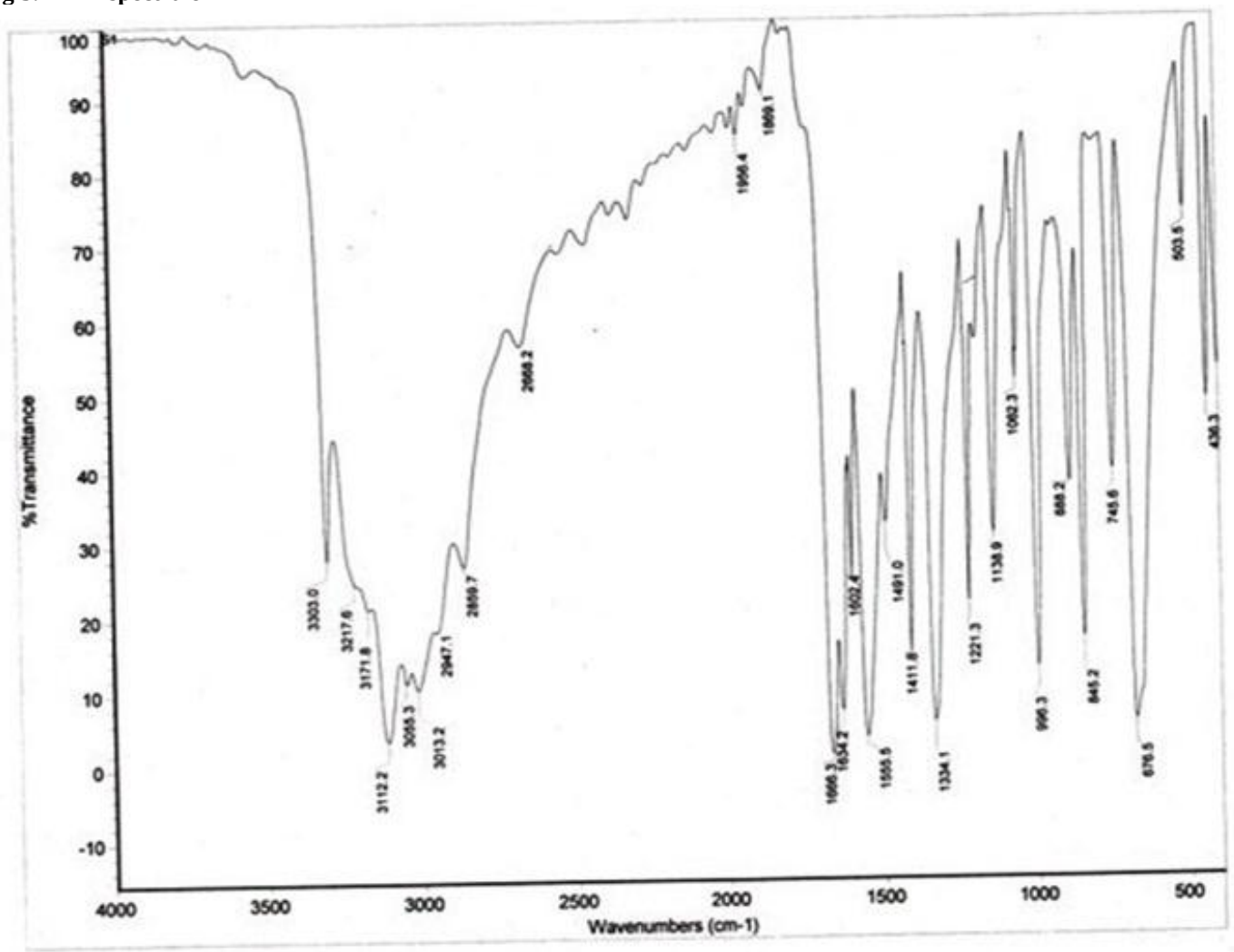


Fig 4. DSC thermo gram of INH

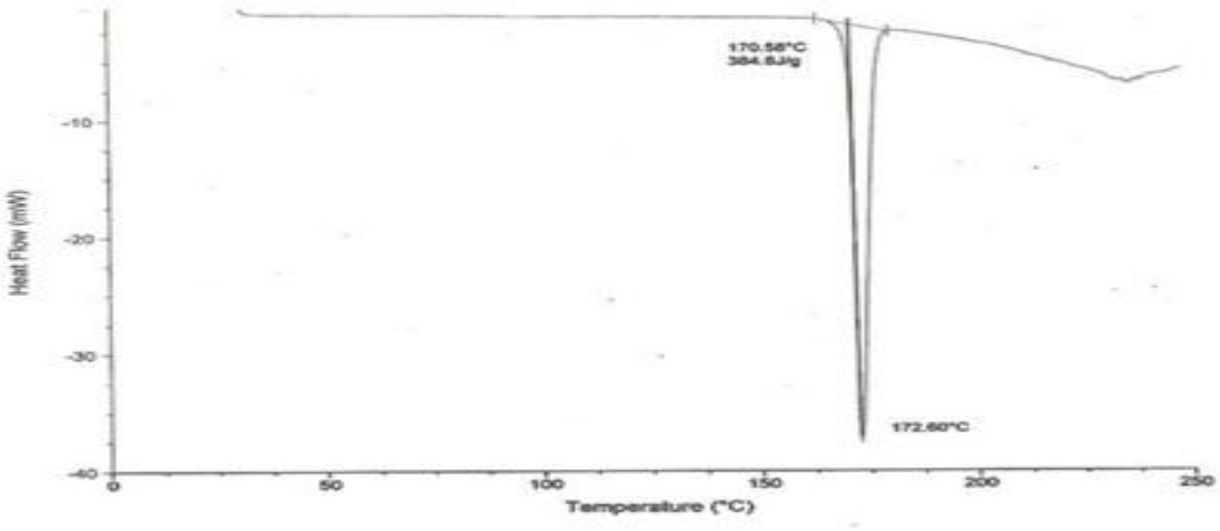


Fig.5. SEM photographs of INH

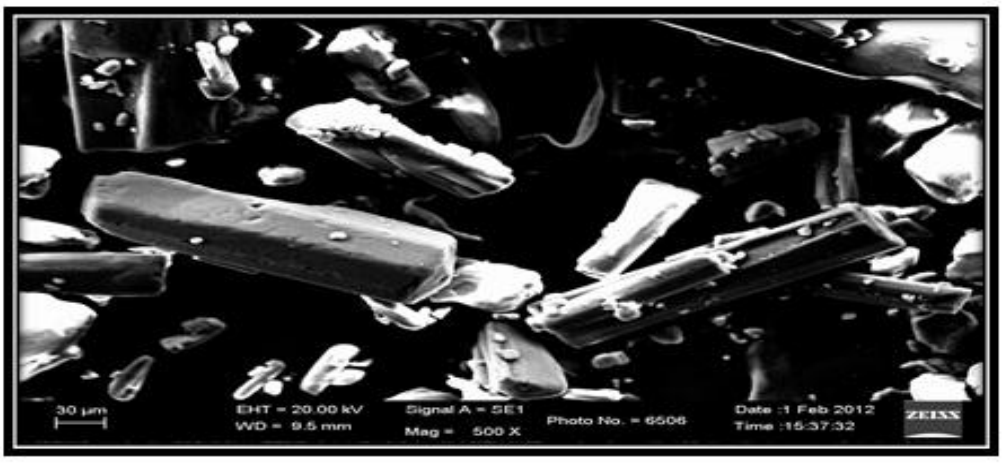


Fig 6. XRD of INH

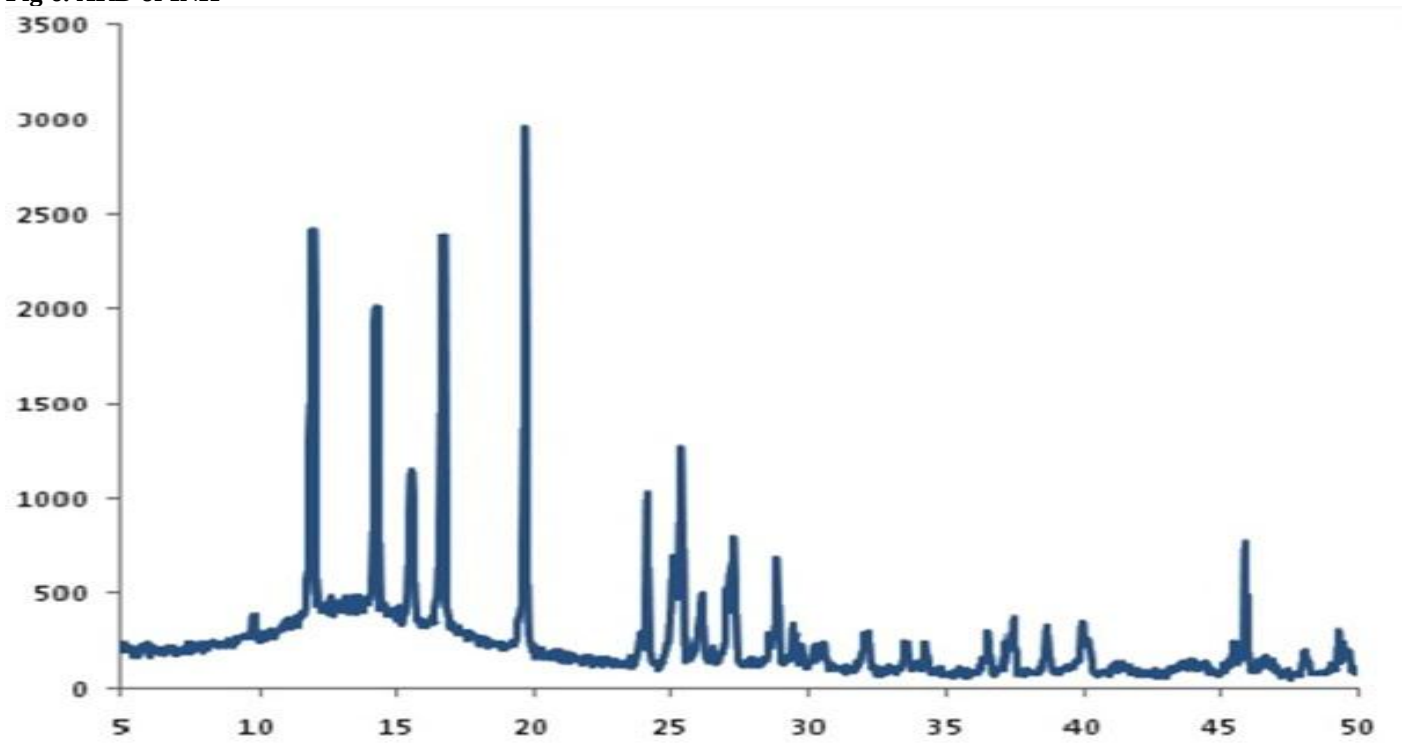


Fig.7. IR spectra of Methyl paraben.

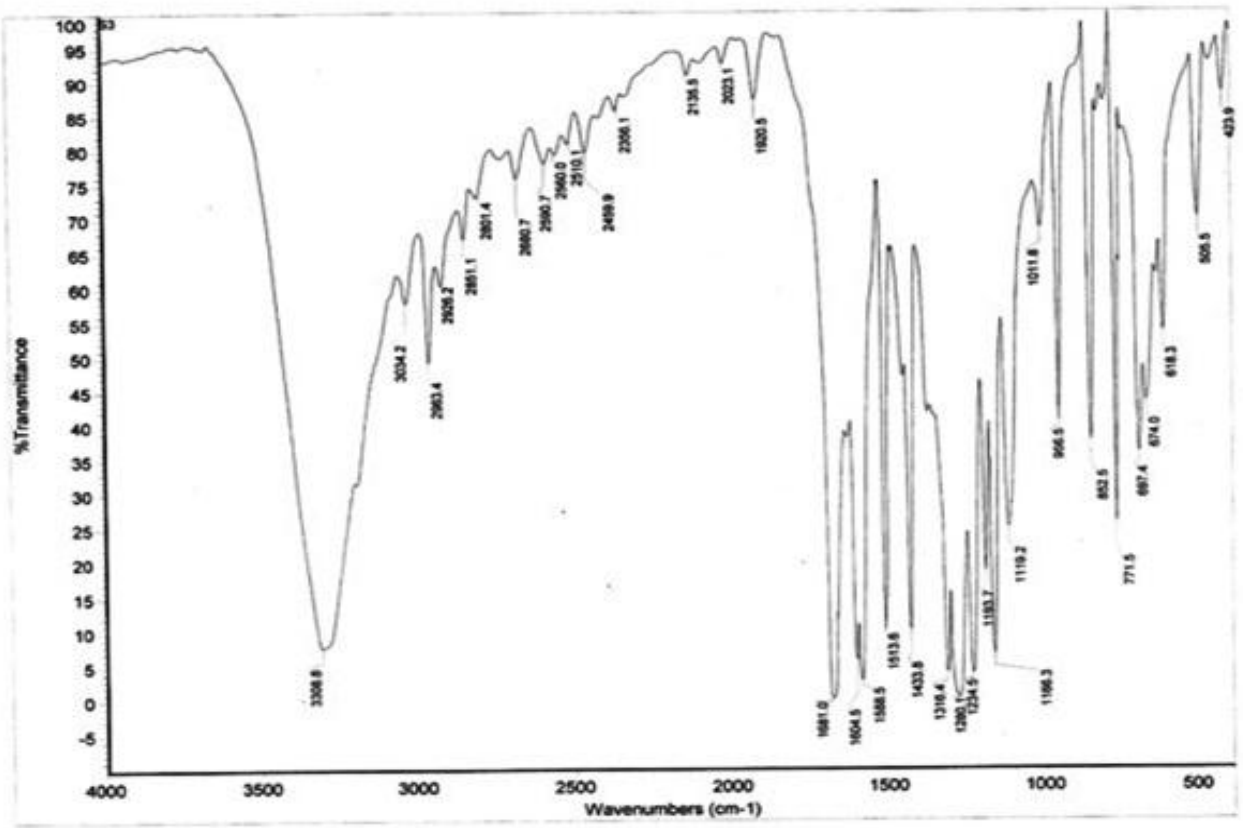


Fig. 8. DSC thermo grams of methyl paraben.

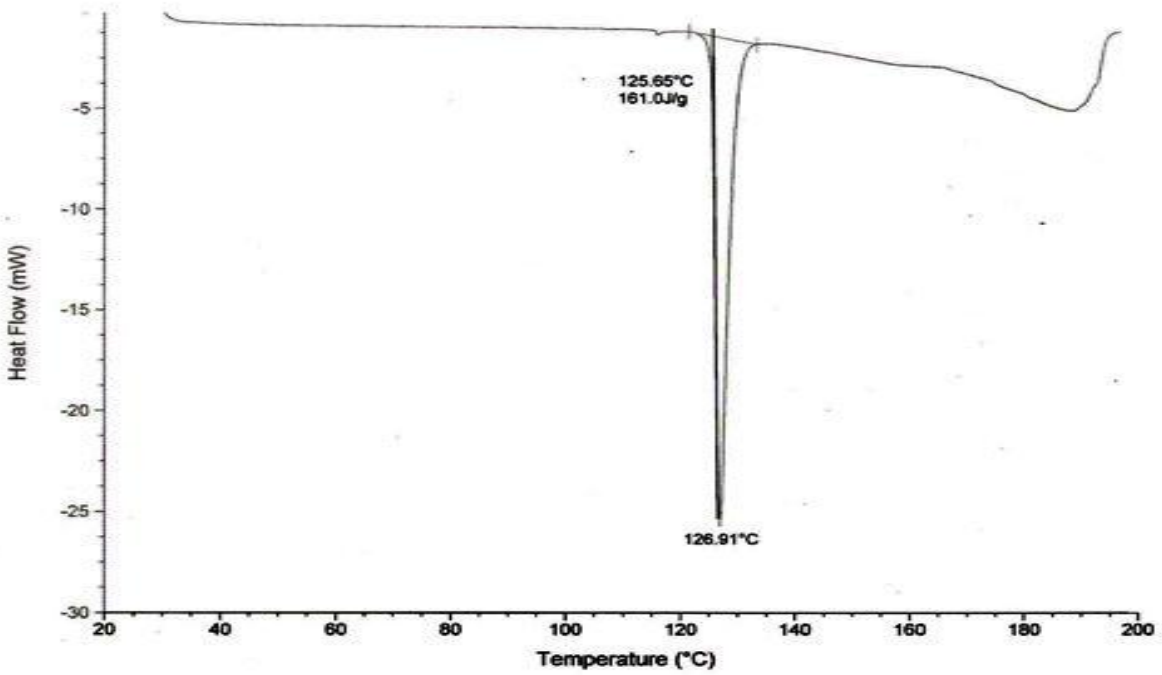


Fig. 9. XRD pattern of methyl paraben

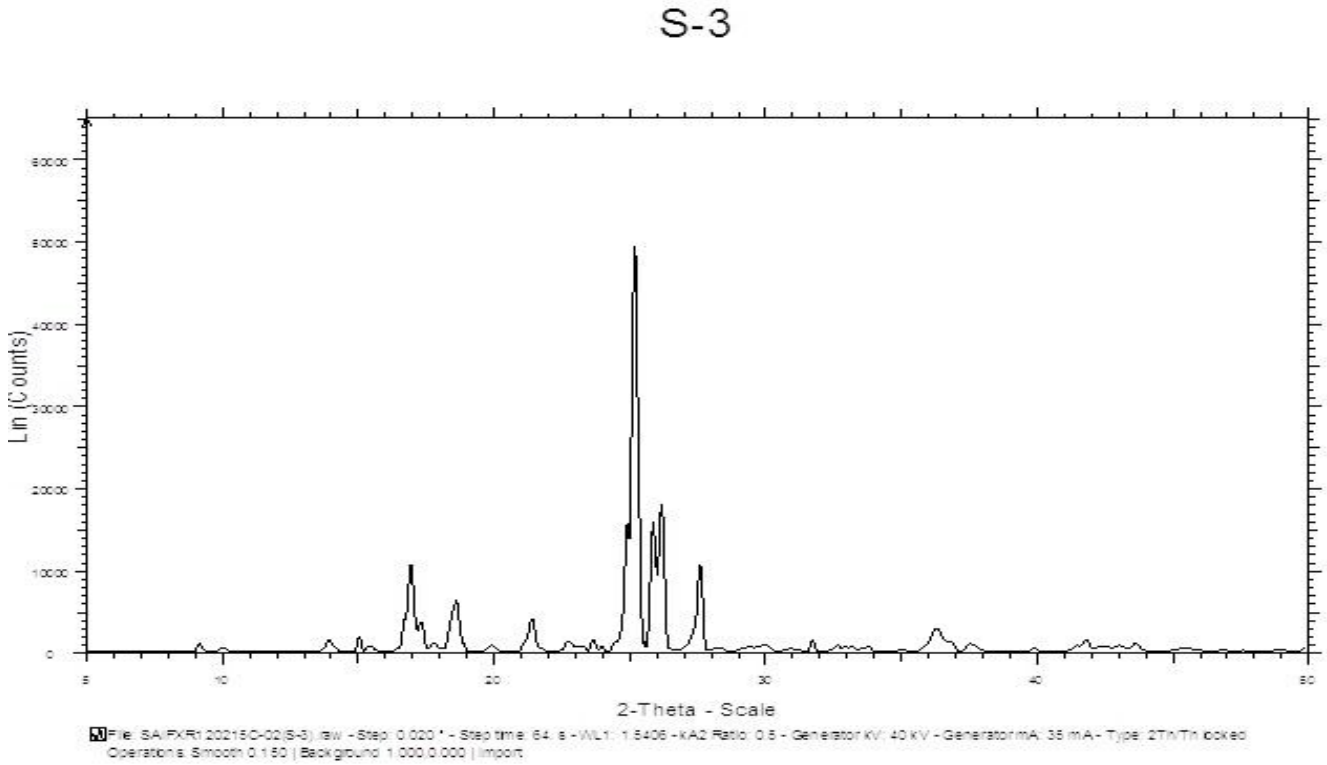


Fig 10. FTIR photographs of (S-1) Isoniazid, (S-3) Methyl paraben and (S-7)Crystal form 1V

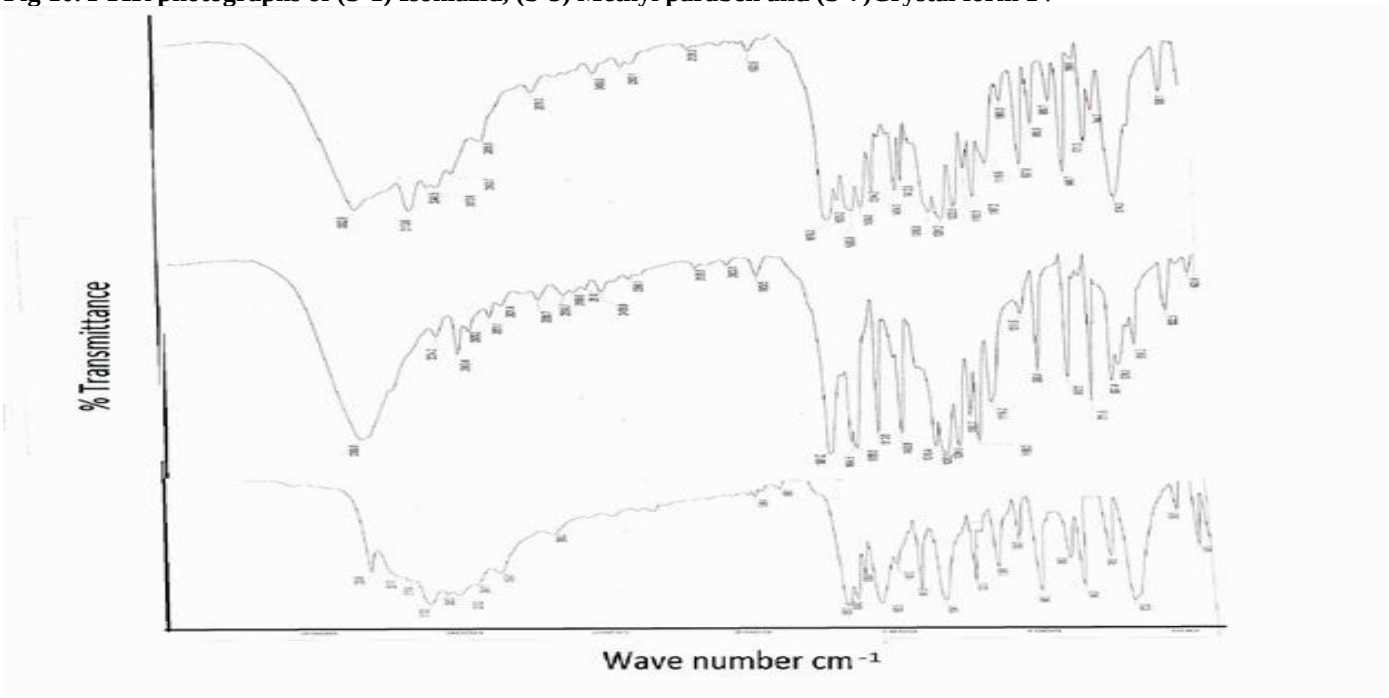


Fig11. DSC of (S-1) Isoniazid, (S-3) methyl paraben and (S-7)Crystal form IV

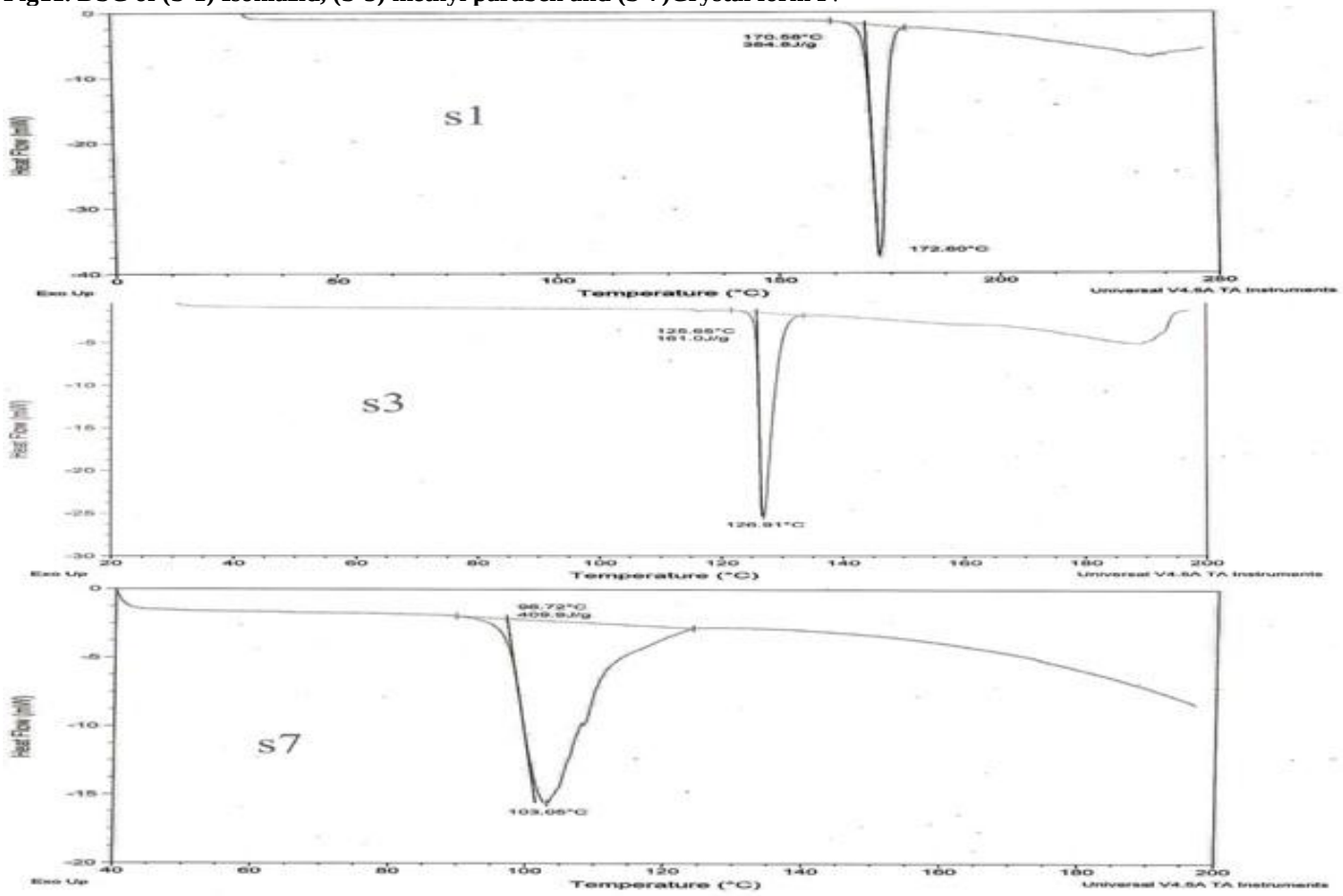
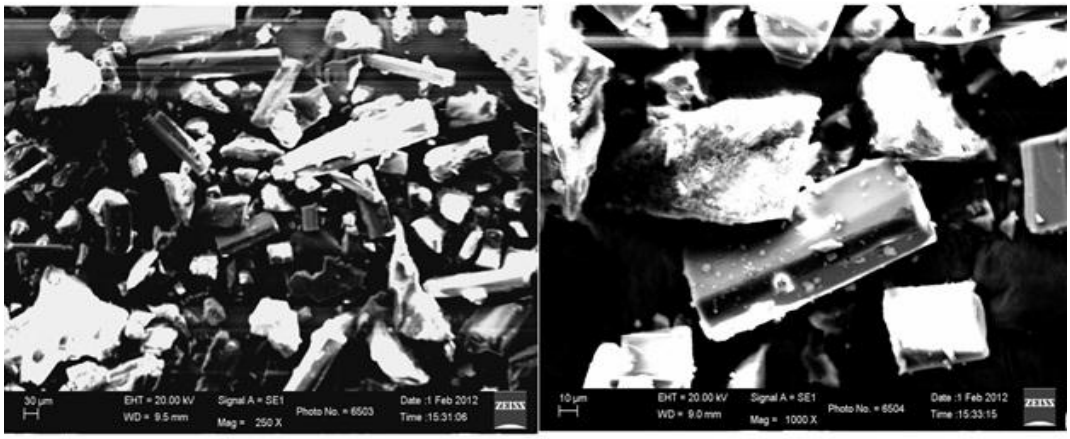


Fig12. SEM photographs of (S-1) Isoniazid, (S-3) methyl paraben and (S-7)

S- 1



S- 7

Fig13. FTIR photographs of (S-1) Isoniazid, (S-3) Methyl paraben and (S-8) Crystal form V

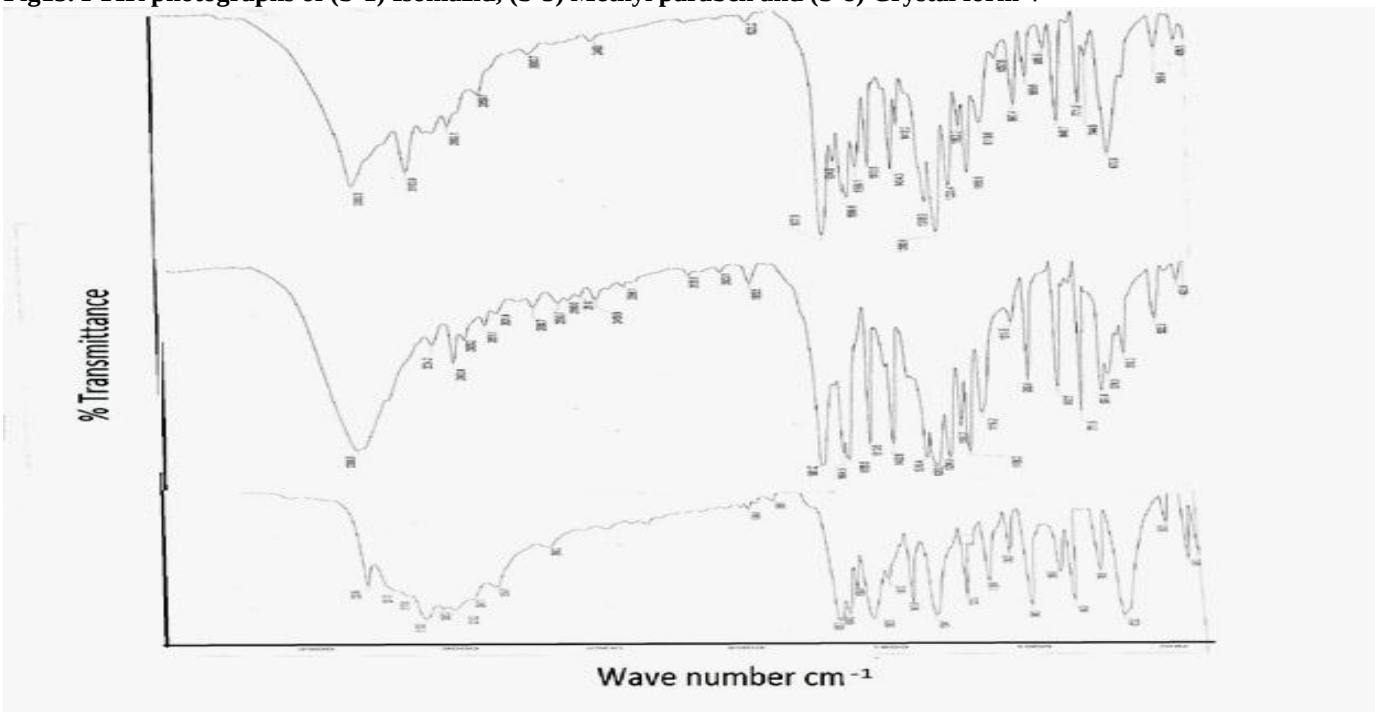


Fig14. DSC of (S-1) Isoniazid, (S-3) methyl paraben and (S-8) Crystal form V

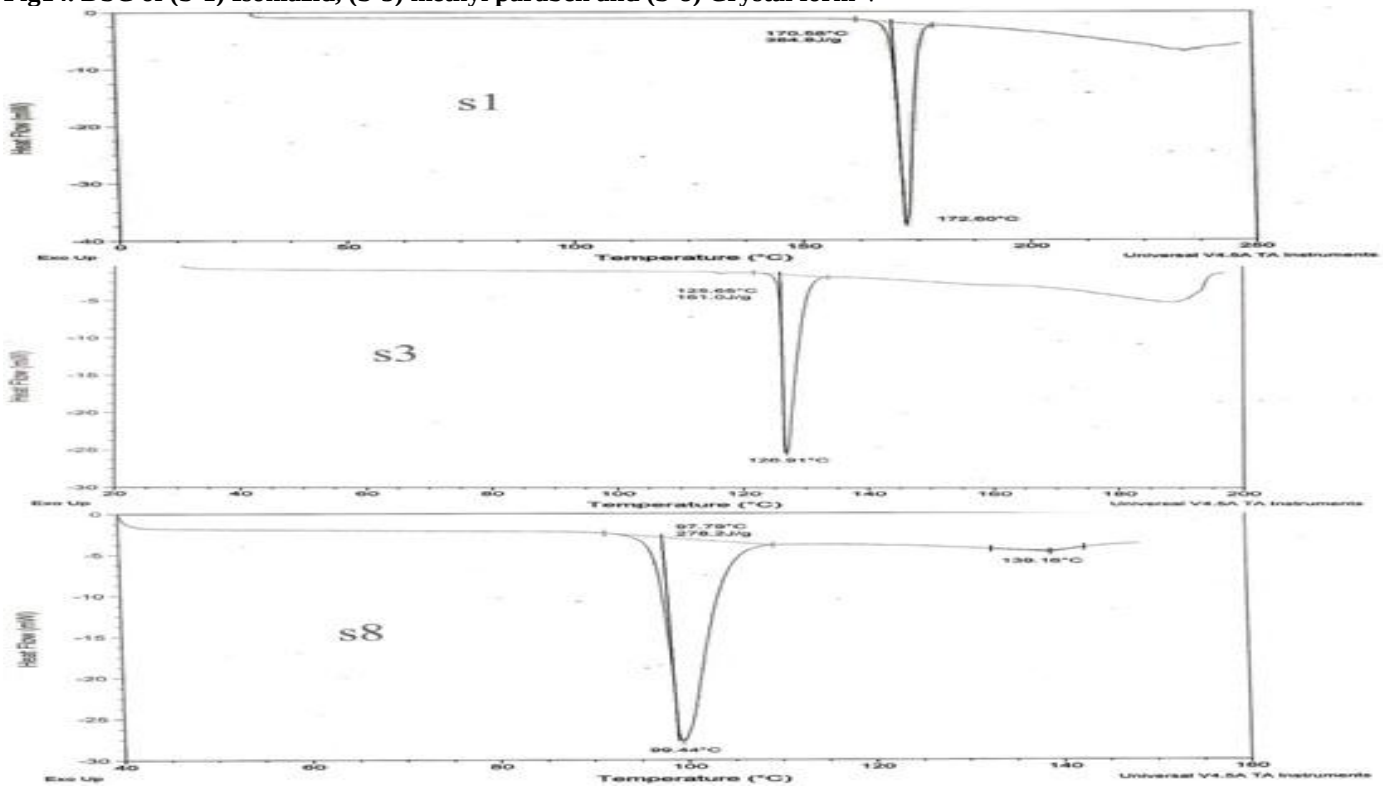


Fig 15. XRD photographs of (S-1) Isoniazid, (S-3) methyl paraben and (S-8) Crystal form V

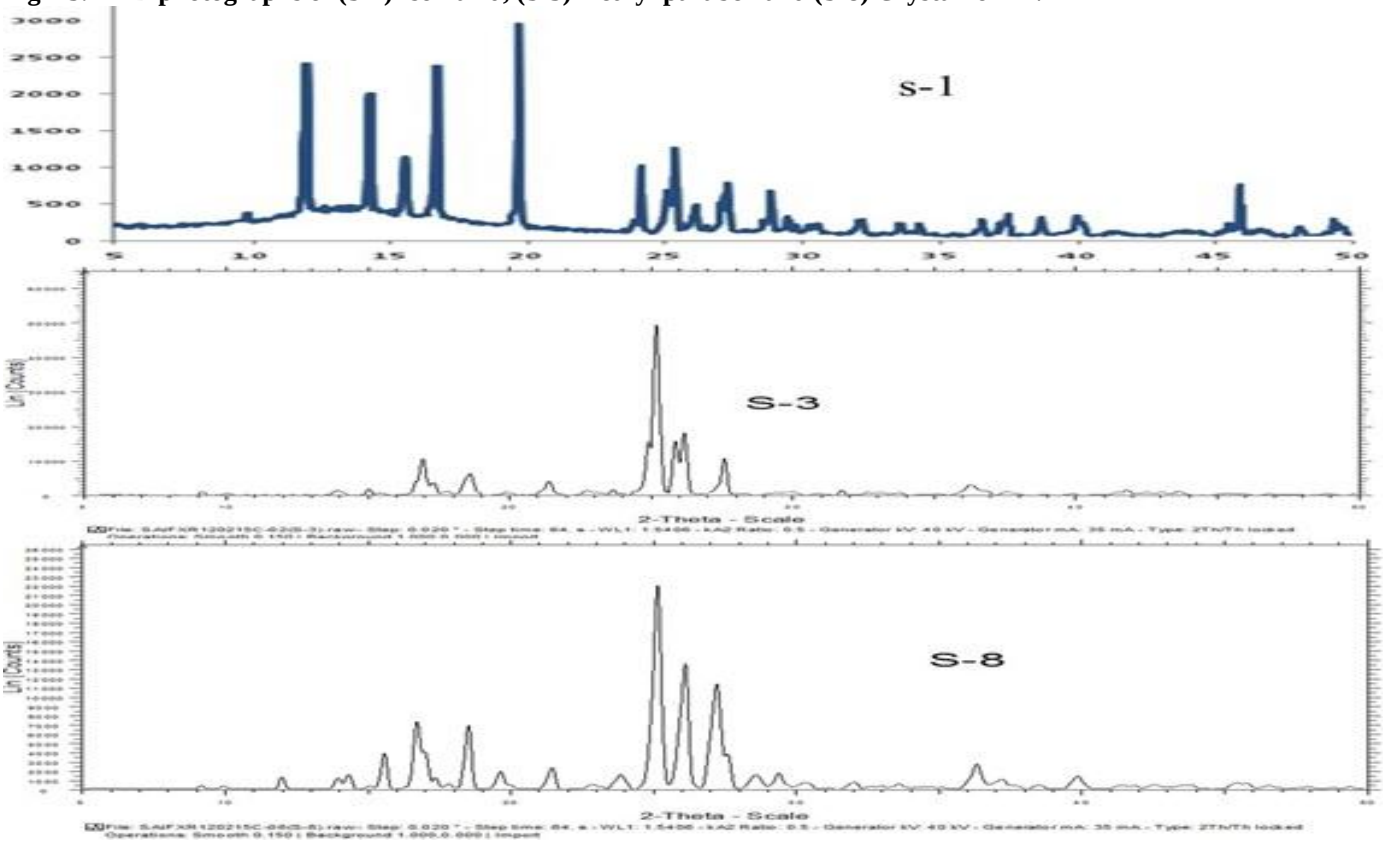


Fig 16. FTIR photographs of (S-1) Isoniazid, (S-3) Methyl paraben and (S-9)Crystal form VI

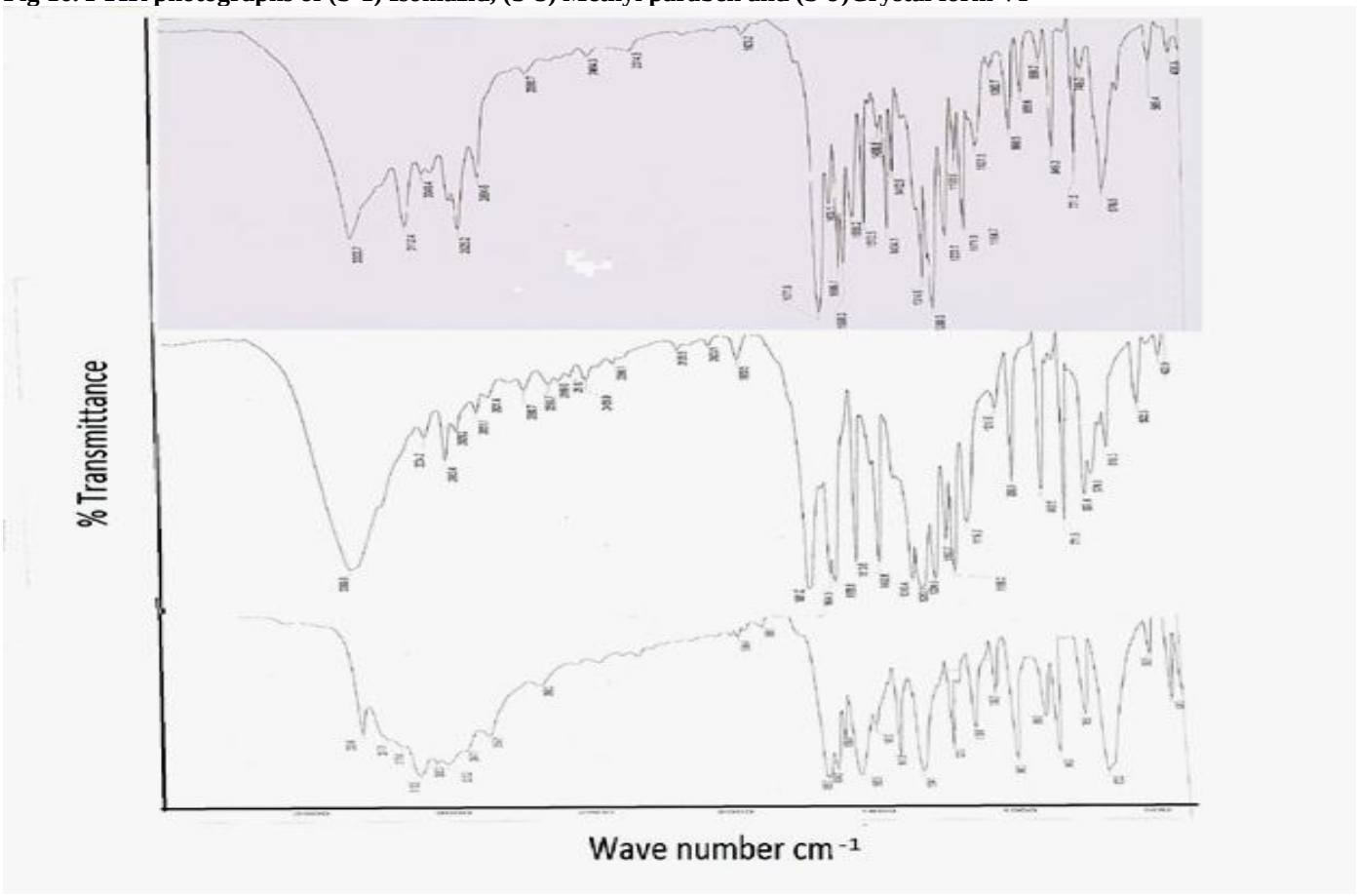


Fig 17. DSC of (S-1) Isoniazid, (S-3) methyl paraben and (S-9)Crystal form VI

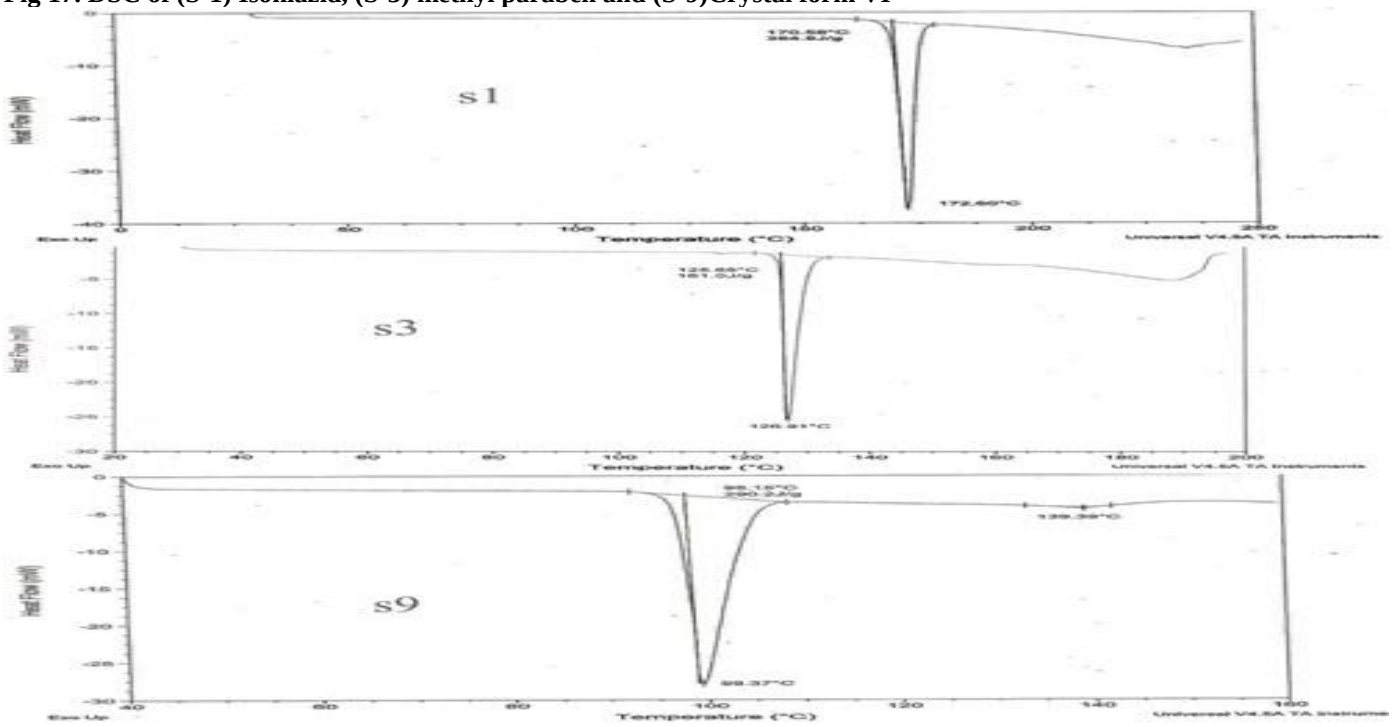


Fig 18. XRD photographs of (S-1) Isoniazid, (S-3) methyl paraben and (S-9)Crystal form VI

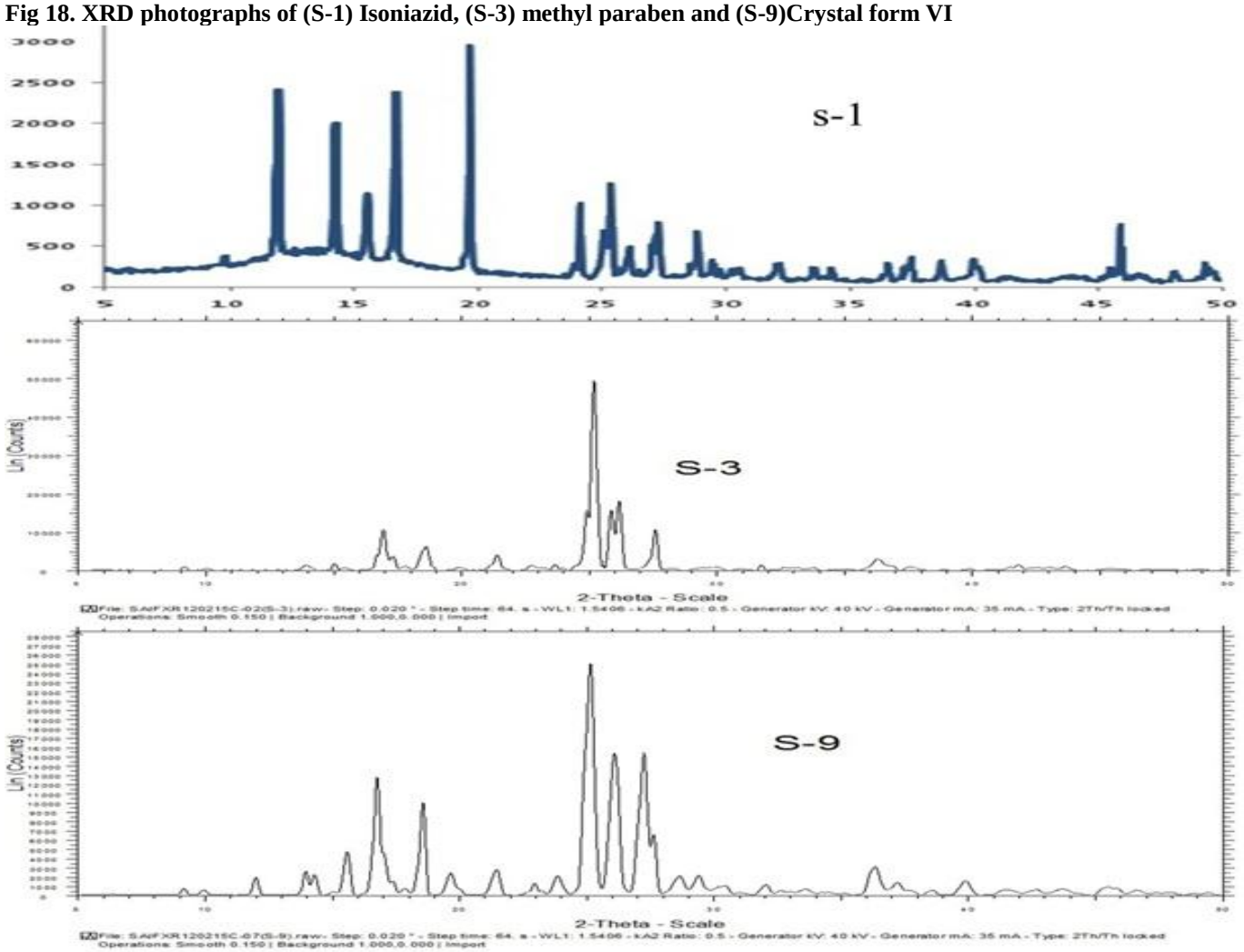


Fig 19. Solubility profile of INH and prepared co-crystals

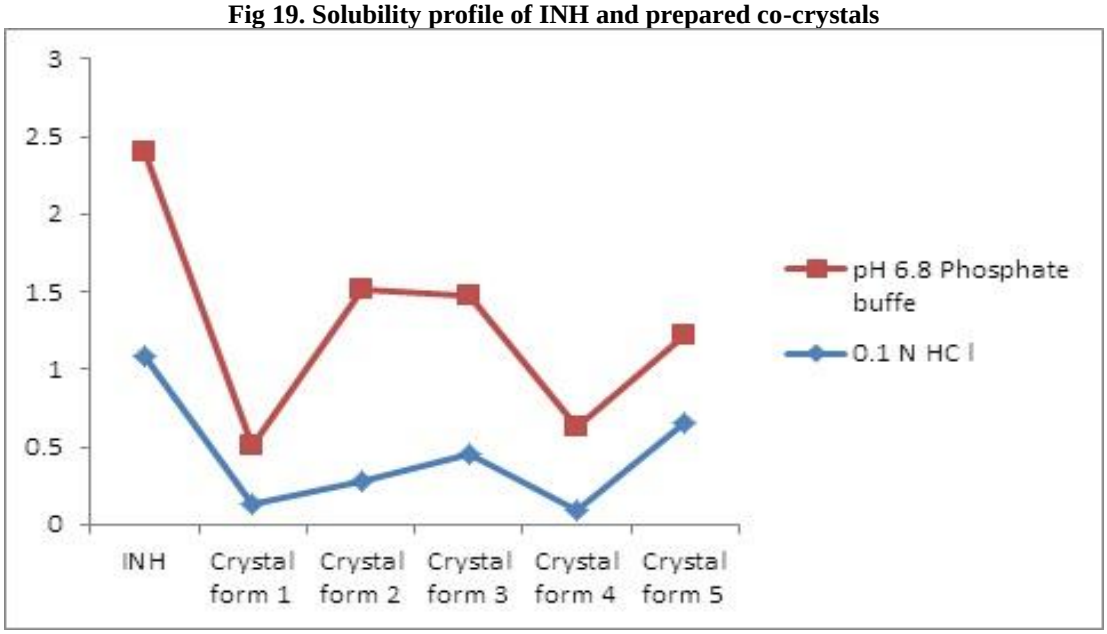


Fig 20. Dissolution profile of pure drug and prepared co-crystal forms in 0.1N HCl

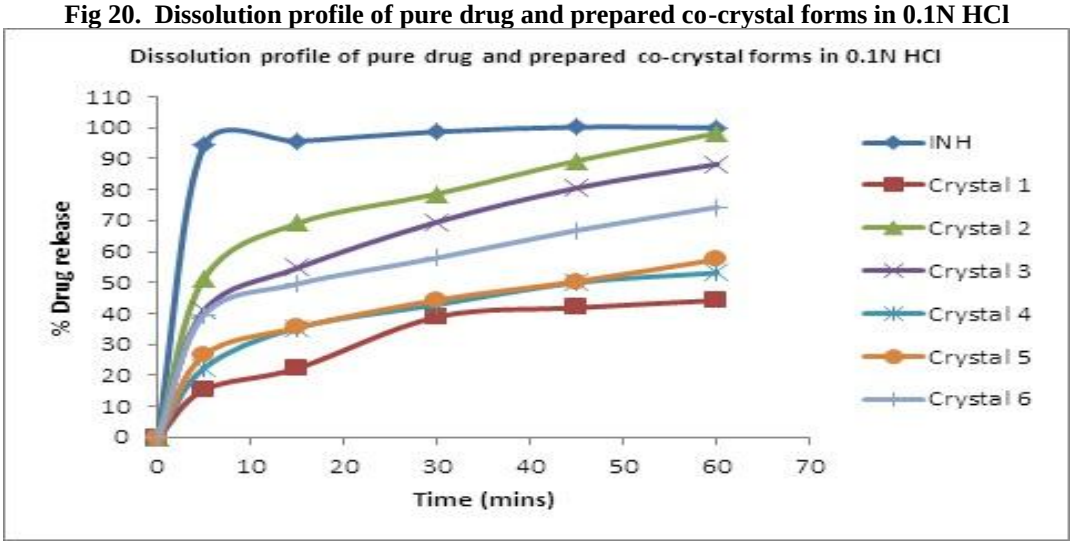


Fig 21. Dissolution profile of pure drug and prepared co-crystal forms in pH 6.8 phosphate buffer

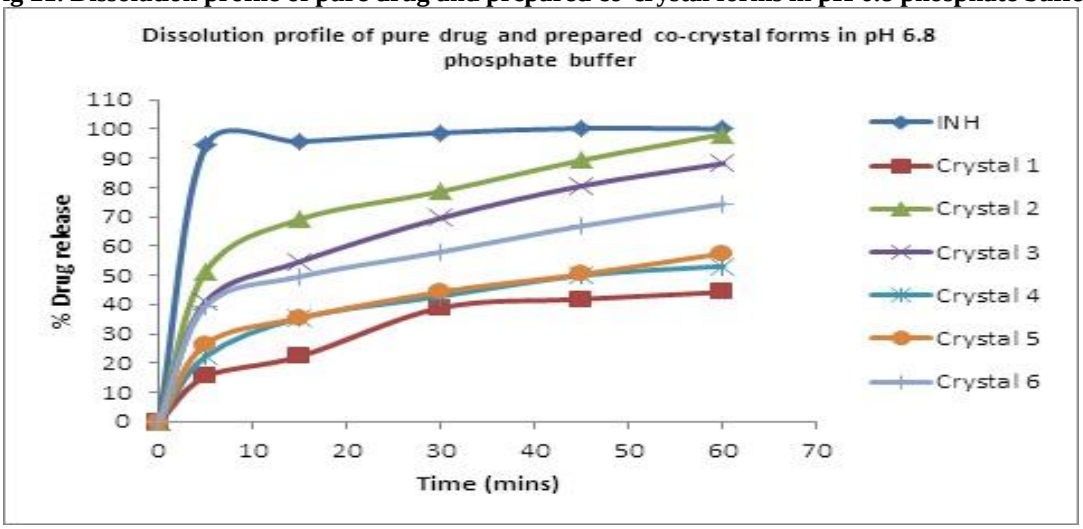
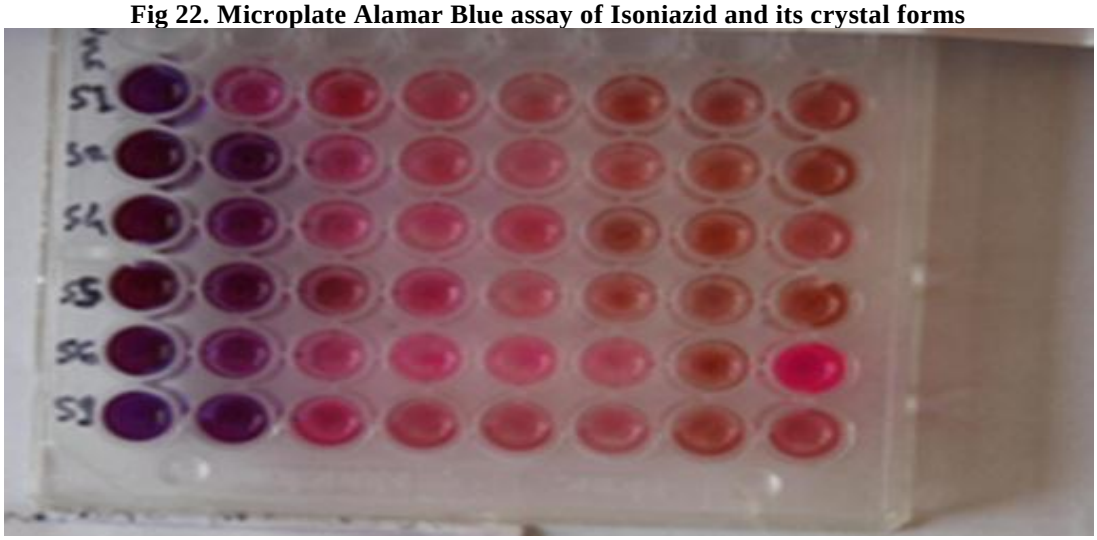


Fig 22. Microplate Alamar Blue assay of Isoniazid and its crystal forms



DISCUSSION AND CONCLUSION

Preformulation studies

From the preformulation studies the solid-state characterization of isoniazid by SEM, FTIR, DSC and XRD studies have clearly demonstrated that isoniazid is pure Crystalline phase. From the FTIR, SEM, DSC and XRD studies it can conclude that methyl paraben sample are pure and it was selected for the further investigations.

Crystal form IV: INH-Methyl paraben (1:1) co-crystal (solvent evaporation method)

Crystal form IV (1:1 molar ratio, Co-crystal) was prepared by solvent evaporation- solution crystallization method and confirmed by comparison of the melting points with those of the starting materials. It characterized in terms of SEM, FTIR, DSC, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, micromeritic studies, Invitro dissolution studies and Antitubercular activity.

IR, DSC and SEM support the formation of co-crystal between isoniazid and methyl paraben at 1:1 stoichiometric ratio. From the pharmaceutical characterization, Crystal form IV shows 100% of practical yield. It was showing decreased solubility in 0.1N HCl but drastic increase in the solubility in pH 6.8 phosphate buffer when compared to 0.1N HCl.

From micromeritic studies of Crystal form IV show, similar bulk (0.61) and tapped (0.81) densities, when compare with isoniazid bulk (0.64) and tapped (0.84) densities. Increase of angle of repose value of Crystal form IV (44.06) indicates poor flow, this is supported by Carr's Index (0.24) and Hauser Ratio (1.32), than isoniazid, angle of repose (32.37), Carr's Index (0.23) and Hauser Ratio (1.31).

From Invitro dissolution studies the crystal form IV seems to be satisfactory in its dissolution profile, with 88.19% at the end of 60 min where pure drug has 100% drug release.

Crystal form V: INH-Methyl paraben (1:1) co-crystal (solvent drop method)

Crystal form V (1:1 molar ratio, Co-crystal) was prepared by solvent drop method and confirmed by comparison of the melting points with those of the starting materials. It characterized in terms of SEM, FTIR, DSC, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, micromeritic studies, Invitro dissolution studies and Antitubercular activity.

The crystal structure determination of Crystal form V reveals that the two molecules (isoniazid and methyl paraben) are associated via a carboxylic acid-pyridine hydrogen bond heterosynthon.

IR, DSC and SEM support the formation of co-crystal between isoniazid and methyl paraben at 1:1 stoichiometric ratio. From the pharmaceutical characterization, Crystal form V shows 90.3% of practical yield. It was showing decreased solubility in 0.1N HCl but drastic increase in the solubility in pH 6.8 phosphate buffer when compared to 0.1N HCl.

From micromeritic studies of Crystal form V show, decrease bulk (0.39) and tapped (0.57) densities, when compare with isoniazid bulk (0.64) and tapped (0.84) densities. Increase of angle of repose value of Crystal form V (47.54) indicates poor flow, this is supported by Carr's Index (0.31) and Hauser Ratio (1.46), than isoniazid, angle of repose (32.37), Carr's Index (0.23) and Hauser Ratio (1.31).

From Invitro dissolution studies the crystal form V shows decrease in its dissolution profile, with 75.41% at the end of 60 min where pure drug has 100% drug release.

Crystal form VI: INH-Methyl paraben (1:1) co-crystal (co-grinding method)

Crystal form VI (1:1 molar ratio, Co-crystal) was prepared by solvent evaporation- solution crystallization method and confirmed by comparison of the melting points with those of the starting materials. It characterized in terms of SEM, FTIR, DSC, and XRD subjected to Preliminary pharmaceutical characterization such as solubility, micromeritic studies, Invitro dissolution studies and Antitubercular activity.

The crystal structure determination of Crystal form VI reveals that the two molecules (isoniazid and methyl paraben) are associated via a carboxylic acid-pyridine hydrogen bond heterosynthon.

IR, DSC and SEM support the formation of co-crystal between isoniazid and methyl paraben at 1:1 stoichiometric ratio. From the pharmaceutical characterization, Crystal form VI shows 88.9% of practical yield. It was showing decreased solubility in 0.1N HCl but drastic increase in the solubility in pH 6.8 phosphate buffer when compared to 0.1N HCl.

From micromeritic studies of Crystal form VI show, decrease bulk (0.39) and tapped (0.53) densities, when compare with isoniazid bulk (0.64) and tapped (0.84) densities. Increase of angle of repose value of Crystal form VI (41.95) indicates poor flow, this is supported by Carr's Index (0.26) and Hauser Ratio (1.35), than isoniazid, angle of repose (32.37), Carr's Index (0.23) and Hauser Ratio (1.31).

From Invitro dissolution studies a satisfactory result in dissolution profile were noticed, with 84.26% at the end of 60 min where pure drug has 100% drug release. But it has more Invitro antitubercular activity when compare with pure drug. However, the activity of Crystal form VI is effective in antitubercular activity compared to that of the pure drug.

The design of new multicomponent crystal phases of APIs with desired physicochemical properties by applying crystal engineering is an evolving novel

concept. Capability to design new multicomponent crystal structures will depend mostly on supramolecular chemistry and on viewing a crystal structure with interactions of various types and strengths. Crystal engineering approach involves identification of interactions or supramolecular synthons that will covers an entire family of structures with the object of identifying a set of new crystal phases of API.

The development of new supramolecular complexes, co-crystal and polymorphs of drugs by crystal engineering is becoming progressively more important as an alternative to salt formation, mainly for neutral or weakly ionizable compounds. Even though lack of priority in marketed products and concerns about the safety and toxicity of co-crystal forming agents.

This concept was applied to the co-crystallization of isoniazid with carboxylic acids like methyl paraben by solvent evaporation, solvent drop and co-grinding assisted crystallization. The carboxylic acid-pyridine hydrogen bond has again been used to successfully create a new pharmaceutical co-crystal of isoniazid, and crystal form 4, 5, 6 with methyl paraben. The supramolecular interaction of isoniazid (pyridine ring) with carboxylic acid of methyl paraben resulted in genuine co-crystals. The crystallization of isoniazid with methyl paraben is in 1:1 ratio.

These prepared crystal forms I resolve the poor micromeritic problems of isoniazid and shows improved flow and compaction property than isoniazid. From the anti-tubercular test performed it has confirmed that the co-crystal forms of methyl paraben and INH (S9) had shown increased activity compared to the pure drug.

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