WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 4, 914-925. Research Article ISSN 2277– 7105

PREPARATION AND SOLID-STATE ANALYSIS OF PIPERINE POLYMORPHS

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Article Received on
01 Feb. 2020,

Revised on 21 Feb. 2020,
Accepted on 12 March 2020,

DOI: 10.20959/wjpr20204-17041

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ABSTRACT

Pepper is a dietary phytochemical obtained from dried unripe fruit of 'piper nigrum'(black pepper) and piper longum belongs to the family of 'piperaceae' pepper is a mixture of three geometrical isomers of piperine [chavicine, isochavicine] and capsaicin is the main pungent principle. The present study relates to the preparation of different solid forms of piperine. Polymer induced heteronucleation is a powerful crystalline polymorph discovery method has revealed the novel polymorphs of low solubility bio enhancer of piperine the existence polymorphs can enhance the solubility when compared to input material there by potentially increasing the efficacy of piperine as a bio enhancer. The piperine polymorphs are prepared by different methods like solvent evaporation, cooling crystallization, vapor diffusion method, gradual cooling method, slurry method, binary solvent mixture method, Anti-solvent method. The developed polymorphs were characterized by various spectral methods like Fourier Transform Infrared Spectroscopy [FTIR] Powder X-ray diffraction [PXRD], Differential Scanning Colorimetry [DSC] and thus the polymorphic forms of piperine enhances the solubility of drug.

KEYWORDS: (Piper nigrum, polymorph, crystallization, PXRD, DSC, FT-IR).

INTRODUCTION

Predominance and importance of polymorphism occurring in pharmaceutical compounds are well recognized, especially in pharmaceutical community. Different crystal lattices or crystal arrangements are possible for any compound. Polymorphic changes can be induced by heat,

stress, pressure and humidity conditions or solvent mediated process. Different polymorphs of same substance can have the different physical properties such as melting point, chemical reactivities, dissolution rate and bioavailability due to differences in molecular packing.

The change in the polymorphic form frequently causes the clinical failure. Hence it is very important to prepare and select the right form from the beginning during drug discovery and development. The polymorphic transitions depend on the processing conditions and the relative stability of the polymorphs.[1,2] Piperine is extracted from the dried unripe fruits of 'Piper nigrum' [black pepper] and 'Piper longum' [white pepper] belongs to the family 'piperaceae' pepper is a naturally obtained alkaloid. It contains a chavicine, iso chavicine, capsaicin, and mainly piperine which are pungent principles for the pepper.

Taxonomically, pepper can be classified into

Kingdom : Plantae
Class : Equaisetopsida
Sub-class : Magnolidae
Super order : Magnolidae
Order : Piperales
Family : Piperaceae
Genus : Piper
Species : Nigrum[3]

The large number of studies demonstrated that the potential biological activity of piperine according to these studies, piperine exhibit anti- inflammatory, antioxidant[16], anti – carcinogenic[18], antipyretic, anti-spasmodic[19], anti-diarrhoeal[19], anxiolytic, anti-depressant, hepato protective[17], anti-thyroid and it is effective in the arthritis and pain. The structure of the piperine and its functional groups responsible for the biological activity are follows in the chemical structure of the piperine.[4,5]

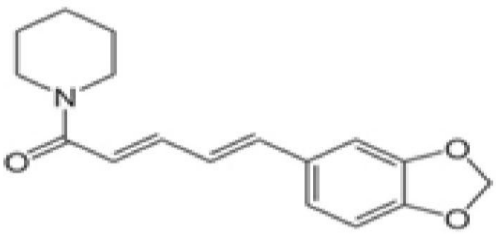

Fig. 1: Structure of Piperine.[6]

IUPAC name: -(2E,4E)5-(1,3 Benzodioxol 5-yl) 1-piperidine-1-yl penta-2,4 diene 1-one.

Fig. 2: Black pepper (Piper nigrum).

The problems associated with piperine i.e. low aqueous solubility and poor bioavailability may be addressed through the studies on development of new polymorphic forms. With this background, the present study mainly focuses on the polymorphic behavior of piperine.

METHODS AND MATERIALS

Piperine sample was gifted by Herba diet chemical Pvt Ltd, different solvents used in the study like ethanol, isopropanol, methanol, dimethyl sulfoxide [DMSO], dimethyl formamide [DMF], and acetone was procured from Mahadev scientific chemicals.

Preparation of solid forms by different techniques

1. Cooling crystallization: To 300 mg of piperine, acetone (60ml) was added until it completely gets dissolved, then it was heated on water bath at 100℃ for 15 min and immediately placed on ice bath or refrigerator till the formation of crystals. The crystals thus obtained were dried off and stored in desiccator till use.[7]

2. Crystallization from binary mixture of solvents

- **Ethanol-Water:** Weigh accurately 300 mg of piperine 30 ml of ethanol was added drop wise until saturated solution was formed, then to this solution 30 ml of water was added and heated up to the boiling point and kept at room temperature till the evaporation of solvent. The crystals thus obtained were filtered off, dried at room temperature and stored till use.
- **Acetone-ethanol:** Weigh accurately 300 mg of piperine 30 ml of acetone was added drop wise until the saturated solution was formed, to this solution 15 ml of ethanol was added heated up to the boiling point for 10 minutes and kept at room temperature till the evaporation of the solvent. The crystals thus obtained were filtered off, dried at room temperature and stored in desiccators till use.

2. Slurry method: It is a dynamic process where the more soluble (less stable) form dissolves and less soluble (more stable) form precipitates out of the solution. One set was stirred for 2 days and other for 2 weeks .3mg/ml was minimum solubility to minimize the time of the experiment.[8]

Characterization Methods

Fourier transform infrared spectroscopy [FTIR]

The FTIR spectra were acquired on a BRUKER model 65 with OPUS software at room temperature from 4000 to 400 cm^{-1}. standard KBr pellets were prepared from 100 mg of KBr pressed under 15000 Ibs and solid forms containing 2 -3 mg were used. Interpretation of the IR spectra was based on the identification of the functional groups represented by specific wave numbers.

Differential Scanning Calorimetry [DSC]

The thermal behavior of solid forms of piperine was measured with Thosivin DSC-60 module for thermal analysis; 2.211 mg of weighed samples [Mettler M3 micro balance] of each solid form was placed in crimped 40µL aluminum pans. Each sample was heated from 40- 500 at a ramp rate of 10℃/minute. The instrument was preventively calibrated with indium as a standard reference. A purge gas of nitrogen was passed over the pans with a flow rate of 50ml/minute. The temperature and enthalpies were calculated by the software [Thosivin DSC-60] by integrating the transition are as associated and normalizing the rate of each sample.

Powder X-ray diffraction [PXRD]

Powder X- ray diffraction patterns of solid forms of piperine were performed. The divergence and scattering and receiving slit were set at 1.0mm, and receiving slit was set at 0.3mm. Diffraction patterns within the 2θ range of $10\text{-}80^{0}$were recorded at room temperature using copper Kα radiation at following conditions tube voltage 40Kv, tube current of 30.0 [mA] continuous scan mode with step size of 0.02, 2θ and counting time 0.30sec/temp.[12]

RESULTS AND DISCUSSION

Characterization of solid forms by PXRD, DSC, FTIR

Input material of piperine: P-XRD is an important tool for the characterization of chemical nature of the molecule, and is used to distinguish the solid form of piperine, results are given below. The P-XRD diffraction lines recorded for input material is showed significant 2θ

values at 14.36[19], 14.60[21], 14.78[24], 14.96[100], 19.72[10], 22.04[11], 22.28[21], 22.50[21], 22.64[19], 22.82[13], 25.34[10], 25.40[18], 25.72[39], 26.00.[23] The DSC thermo gram of input material of piperine showed a single melting endotherm at 131℃ [ΔH 130.08 j/g] with onset temperature and end set temperatures at 133.77 respectively. When compared to form 1 there is difference in melting endotherm and end set temperatures. According to the USP general chapter on x-ray diffraction, the agreement in the 2 θ-diffraction angles between specimen and references is within $\pm 0.2^0$ for the same crystal form, while relative intensities between specimen references may vary considerable due to preferred orientation effects.[13]

1. Solid form A: [acetone-ethanol]

The solid form A is considered as acetone-ethanol system. The P-XRD diffraction lines of solid form A is displayed prominent 2θ values at 14.32[15], 22.16[32], 22.49[100], 22.76[14], 22.90[10]. And the matching peaks are 14.32[15], 22.49[100]. And new peaks obtained are 22.16[32], 22.76[14], 22.90[10]. Corresponding to piperine form 1 indicating that solid form A the P-XRD behavior of solid form A is similar to input material of piperine. On crystallization from acetone -ethanol was reported to be crystallized as solid form A. The DSC trace of solid form A depicts a single melting endotherm at 140℃. With onset temperature. And end set temperatures at 133.44 respectively. A melting endotherm was similar to the melting endotherm of solid form A [131℃] indicating that piperine exist as a form 1. From the above data it is confirmed that input material of piperine and piperine sample contains the form1.In this study, various solid forms of piperine were prepared by employing different processing conditions like crystallization of solvents with acetone-ethanol.

2. Solid form B: [ethanol-water]

Solid form B red is considered as ethanol-water system. The solid form B is considered as form I the P-XRD pattern of solid form B and significant **2** θ values 14.34 (17), 14.57 (29), 14.94 (17), 19.76 (100), 21.16 (12), 21.46 (19), 21.76 (12), 22.10 (25), 22.31(42), 22.62(26), 25.60(31), 25.74(45), 26.00(18). And new peaks obtained are 21.16(12), 21.46(19), 21.76(12), 25.60(31), 28.00(13) and matching peaks 14.34(17), 14.57(29), 14.94(17), 19.76(100), 22.10(25), 22.31(42), 22.62(26), 25.74(45), 26.00(18) corresponding to piperine input material and solid form A suggesting that solid form B exist as a form I. The crystalline nature of piperine was significantly reduced to form I on crystallization from ethanol-water.

Dsc trace of solid form B depicts is single broad melting endotherm at 120℃ with onset temperature 126.02℃ and end set temperature at 131.46℃ respectively. No significance changes in the melting endotherm were observed but the shape of the endotherm was broader than the melting endotherm of input material which may be due to partial transformation of piperine into form 1.

3. Solid form C

It is well known that grinding or milling is one of the manufacturing processes in pharmaceutical industry. Grinding processes can modify the physical and chemical properties of drugs, such as introduction of a significant lattice strain within the crystalline drugs, alteration of crystallinity of drug, reduction of particle size and induction of polymorphic transformation of drug polymorphs. Diffraction spectra of solid form C exhibited signature 2 θ values at 12.58(12), 12.86(13), 13.28(13), 14.44(59), 15.34(11), 18.94(14), 19.22(10), 21.15(48), 21.42(13), 21.94(31), 22.18(11), 27.35(19), 28.33(100). corresponding to piperinepolymorphs. The PXRD behavior of solid form C was similar to input material of piperine. The DSC behavior of solid form C furnished a melting endotherm at 139℃ with onset temperature and end set temperature at 130.59℃ and 135.98℃ respectively. The melting endotherm was similar to the endotherm and of input material. But it is relatively sharp when compared to input material.

4. Solid form D

Diffraction lines recorded for solid form D showed significant 2 θ values at 12.71(27), 12.89(23), 13.90(31), 14.24(31), 14.48(54), 14.67(90), 14.90(21), 15.27(77), 15.48(58), 15.82(14), 16.21(68), 16.57(30), 19.29(12), 19.54(10), 21.37(34), 22.16(74), 22.60(43), 23.92(65), 24.15(15), 25.45(100), 26.09(36), 28 42(14), 29.63(36), 30.2928), 31.37(11), 38.76(12), 38.40(19), 47.51(16). And matching peaks obtained at 14.67(100), 22.60(43), 25.45(100), 26.09(36) corresponding to piperine input material suggesting that solid form D exists as a form II. The crystalline nature of piperine was significantly reduced on slurry method by using of ethanol as a solvent. However, it was reported that piperine was transformed into form II by slurry method. The small broad melting endotherm was observed for solid form D in DSC trace at 118℃ onset temperature and end set temperature at 130.59℃ and 135.98℃ respectively. The boarding of endotherm may be due to the solid-solid transition of form I of piperine. This difference in thermal behavior suggests that piperine underwent as significant polymorphic change under slurry method conditions.

DSC thermo grams of all solid forms were shown in fig: 1

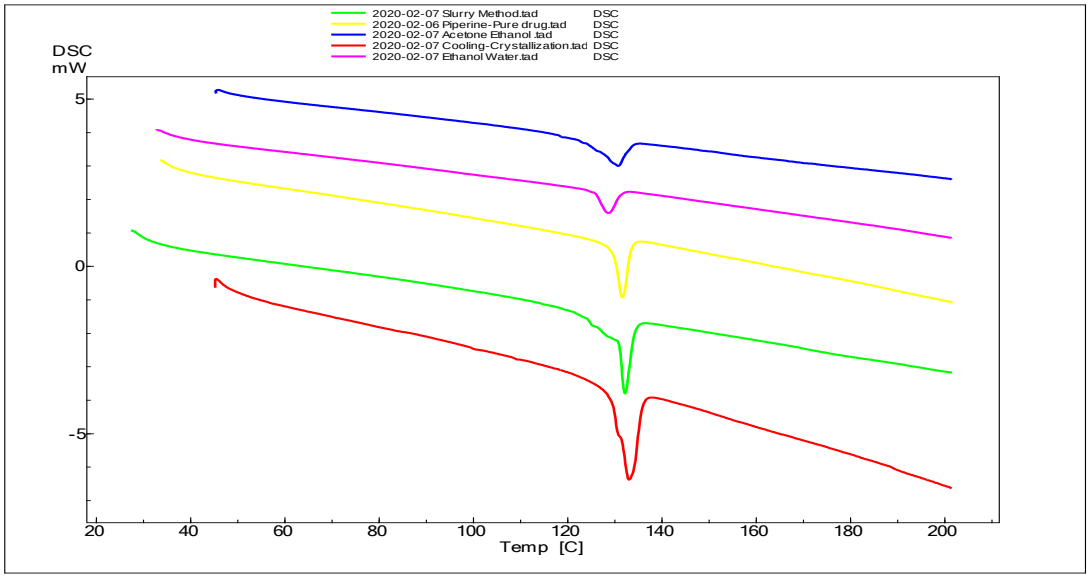

Fig 1: DSC thermo grams of all solid forms, input material, solid form A, solid form B, solidform C, solid form D.

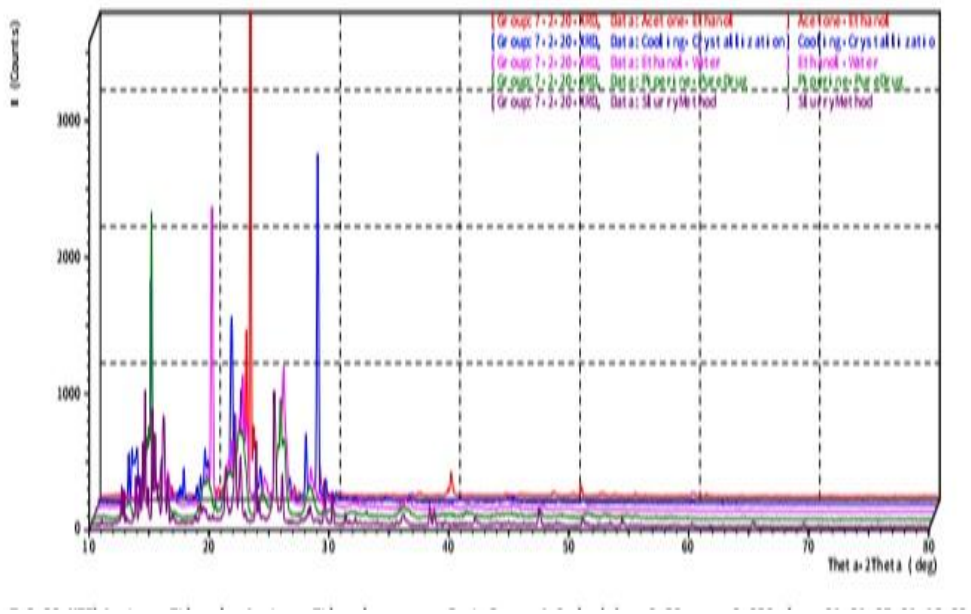

Fig 2: PXRD patterns of piperine, (I) input material, solid form A, Solid form B, Solid form C, Solid form D.

Characterization of solid forms by FTIR

- **Input material of piperine: -**IRspectroscopy is an important tool for the characterization of chemical nature of the molecule, and is used to distinguish the solid forms of piperine, results are shown in below. The IR spectrum of input material displayed all the characteristic bonds which were agreement with literature data of piperine. It exhibited a strong C=O

stretching vibration at 1632.53cm^{-1}, a medium intense absorption bond was observed at 1583.38cm.owing to the aromatic C=C stretching vibration at of piperine at 1442.83, 1490.70, 1583.38, a phenolic C-O stretching vibration was observed at 1027.31, 1131.07, 1195.45, 1250.89cm^{-1}, N-H bond stretching vibrations at 1583.38, 1632.58cm^{-1}.

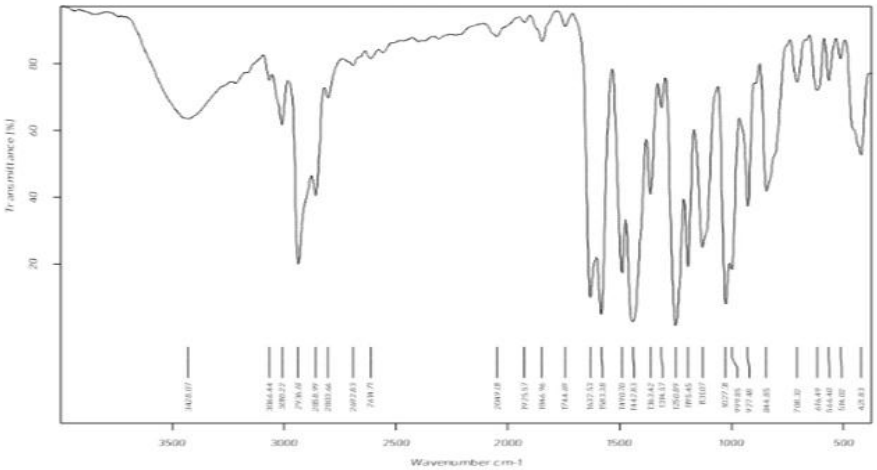

Fig no: 3 FT-IR spectra of input sample.

- **Solid form A:**The IR spectrum of solid form A, obtained from binary mixture solvent method using acetone - ethanol, displayed all the characteristic bands which were similar to the input material of piperine as shown in fig. it exhibited a strong C=C stretching vibration at1458.64, 1585.38cm^{-1} and aromatic C-H stretching vibrations at 3025.30, 3052.44cm^{-1}, aliphatic C-H starching vibrations at 2915.23cm^{-1}, a phenolic C-O stretching vibrations was observed at 1033.11, 1120.31, 1267.77cm^{-1} and also N-H stretching vibrationsat 1585.38cm^{-1}.

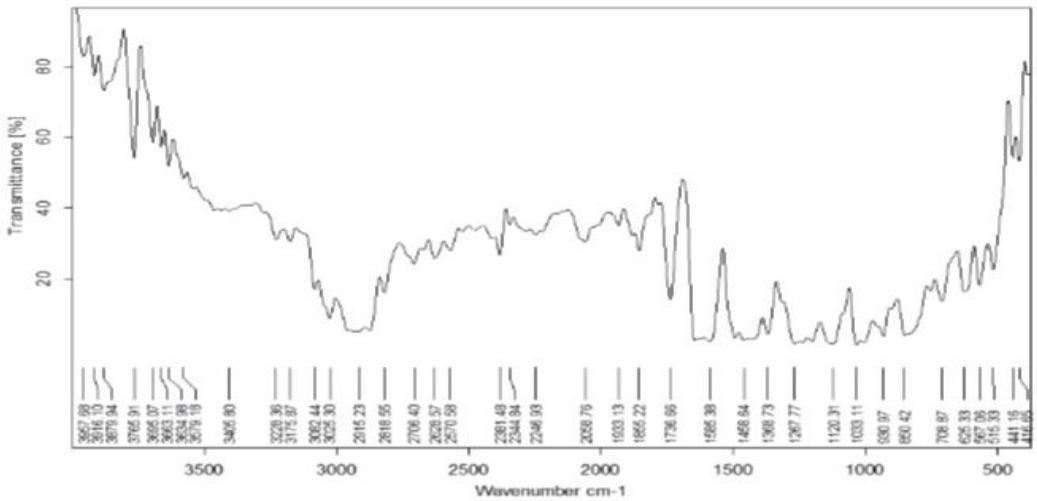

Fig no 4: FT-IR spectra of solid form –A.

- **Solid form B**

The IR spectrum of solid form B obtained from binary mixture of solvent method using ethanol-water, displayed all the characteristic bands very similar to input material piperine as shown in fig. it exhibited aromatic C-H stretching observations at 3008.03, 3066.48, aliphatic C-H starching vibrations at 2858.83, 2938.19cm^{-1}, a strong C=C stretching vibrations was observed at 1443.76, 1429.88, 1583.17cm^{-1}, C-O stretching vibrations at 1027.80, 1074.10, 1131.58, 1194.45, 1251.24cm-^{1}, and N-H stretching vibrations at 1583.17, 1612.38, 1633.95 cm.$^{-1}$

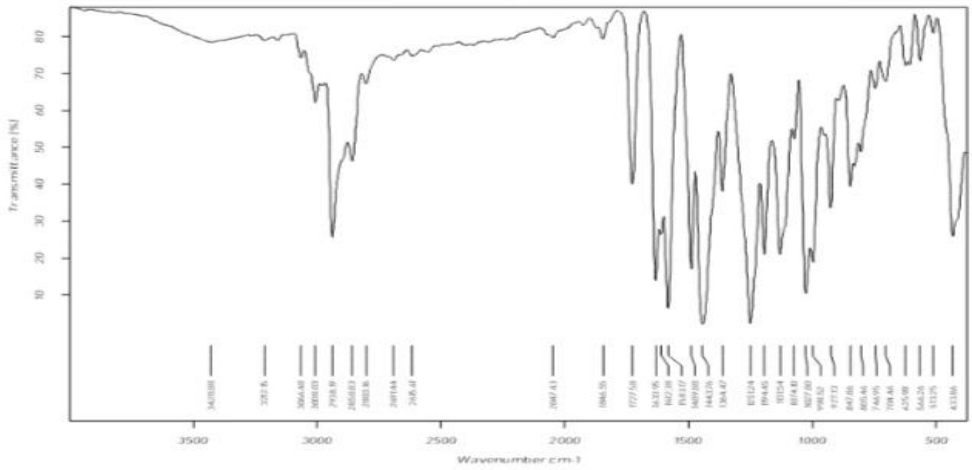

Fig No: 5 Ft-Ir Spectra Of Sold Form-B.

- **Solid form C**

The IR spectrum of solid form C obtained by cooling crystallization method displayed all the characteristic bands which are similar to input material. It displayed aromatic C-H stretching vibrations at 3009.01, 3066.02cm^{-1}, aliphatic C-H stretching vibrations at 2857.89, 2937.99cm^{-1}, strong aromatic C=C stretching vibrations 1443.71, 1490.32, 1583.57cm, phenolic C-O starching vibrations observed at 1028.34, 1132.78, 1194.97, 1251.27cm^{-1}, N-H starching vibrations at 1583.57, 1612,35, 1633.89cm^{-1}.

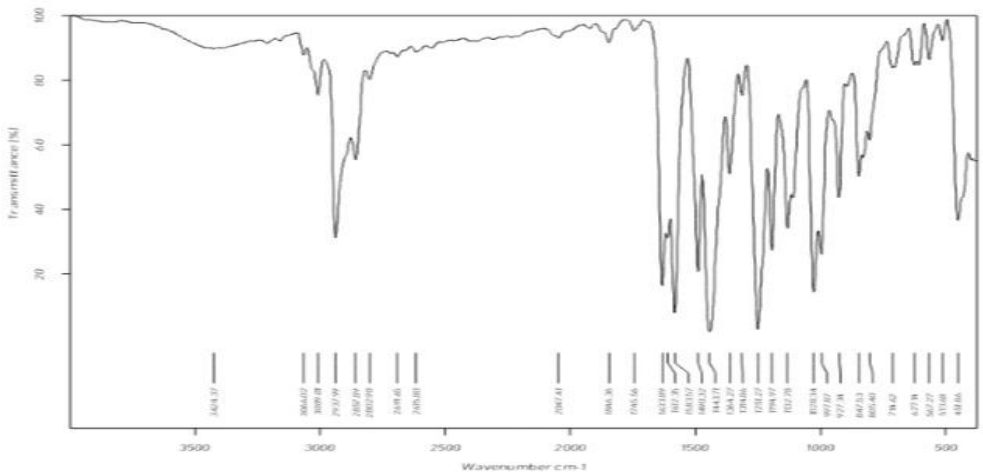

Fig No 6: Ft-Ir Spectra Of Solid Form C.

- **Solid form D**

The IR spectrum of solid form D was obtained from slurry method using ethanol as a solvent, displayed all the characteristic bands which were similar to input material. It exhibited strong absorbance bands aromatic C-H stretching vibrations at 3007.83, 3065.82cm^{-1}, aliphatic C-H stretching vibrations at 2858.22, 2936.97, strong C=C stretching vibrations at 1443.84, 1489.69, 1582.69cm^{-1}, a aliphatic C-O stretching vibrations was observed at 1026,92, 1130.69, 1194.28,1250.79cm^{-1} and N-H stretching vibrations at 1582.69, 1633.40 cm^{-1}.

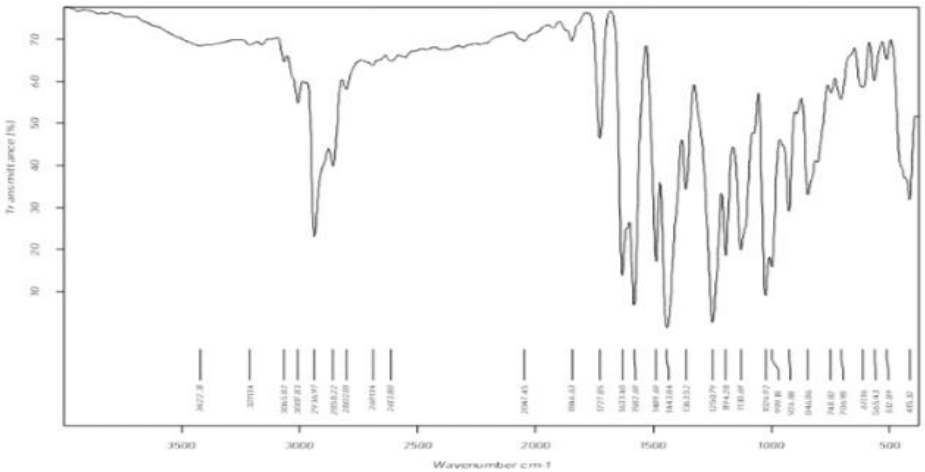

Fig.No 7: Ft-Ir Spectra Of Solid Form D.

CONCLUSION

Black pepper is the “king of spices” and “real gold” used traditionally from the ayurvedic periods. The active constituent of the black pepper is the piperine along with alkaloids, olefins, fibers and other constituents. The content of piperine in the black pepper is more than

95 % by showing numerous biological activities and used in clinical purposes. The solid-state characterization of Piperine demonstrated interesting polymorphic behavior of its constituents present in the form of a mixture. The input material of Piperine was similar to piperine solid form C. Piperine, on crystallization of binary solvent method from ethanol underwent polymorphic transformations resulting of forms 1. Solid form B (ethanol-water) upon binary solvent method also, similar transformation into form I. By developing solid forms solubility of piperine may be increased which shows impact on bioavailability.

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