POLYMORPHIC STABILITY EVALUATION OF DARUNAVIR AND RITONAVIR IN FIXED DOSE DRUG COMBINATION PRODUCT BY X-RAY DIFFRACTION AND DIFFERENTIAL SCANNING CALORIMETRY TECHNIQUES

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ABSTRACT

Evaluation of polymorphism for active ingredients and its stability during manufacturing and storage is a critical task for pharmaceutical firms. For solubility enhancement of ritonavir, hot melt extrusion process was selected through conversion of commercially available crystalline form to amorphous. XRD method (with normal and slow scan) and DSC method was considered for polymorphic evaluation in tablet formulation comprising darunavir 800 mg and ritonavir 100 mg. Specificity and precision of XRD method was justified by no interference of placebo and 5.6% RSD, respectively. The observed correlation coefficient of 0.9991 and average recovery of 98.7% (range 91.5 to 105.1) depicts linearity and recovery power of developed method. Studied DSC method shows exotherm for ritonavir crystalline form II at 127.4 °C and darunavir ethanolate at 105.7 °C, which were specific and sensitive to assess polymorphic form of available drug substances in ritonavir blend as well as in tablet formulation during release and stability period.

Keywords: Polymorphism, Hot melt extrusion, X-Ray diffraction, Differential scanning calorimetry, Method validation

INTRODUCTION

Polymorphism, being a key aspect in Active Pharmaceutical ingredient (API), is monitored as a part of API structural characterization. The use of particular polymorph for the manufacturing of pharmaceutical dosage form is linked with its manufacturing process, stability and its bio availability, as API with different polymorphism shows difference in pH Solubility[1-3]. There are multiple examples of APIs which exist in different polymorphs, out of which ritonavir is a classic example where polymorphism based solubility difference is observed. According to the literature, the solubility of ritonavir and hence its bioavailability is higher in case of amorphous form as compared to its crystalline form[4,5].

The immediate release tablet formulation having darunavir 800 mg and ritonavir 100 mg as fixed dose drug combination was considered for polymorphic evaluation study. The active ingredient darunavir having stable ethanolate form A[6] was selected showing characteristic 2theta value of 7.0, 13.8, 16.6, 17.3, 20.6 and 21.2° (± 0.2 °) by X-Ray diffraction analysis and the melting point between 94 °C to 107°C, by differential scanning calorimetry analysis. The X-Ray diffractogram of darunavir ethanolate form A is shown in Fig. 1.

The second active ingredient ritonavir, having different pH solubility is linked with multiple polymorphic forms[7,8]. The crystalline form II was selected showing characteristic 2theta values by X-Ray diffraction of 8.6, 10.8, 16.0, 18.3, 20.0 and 21.6 ° (± 0.2 °) where peak at 2theta value 8.6 is having highest intensity. The reported melting point of ritonavir crystalline form II is between 121 °C to 128 °C by differential scanning calorimetry. The X-Ray diffractogram of ritonavir form II is shown in Fig. 2.

The drug product formulation was manufactured using hot melt extrusion (HME) technique[9,10] to convert ritonavir crystalline form to amorphous. The manufacturing process includes ritonavir blend prepared by melting ritonavir with excipient at about 130-140 °C temperature which

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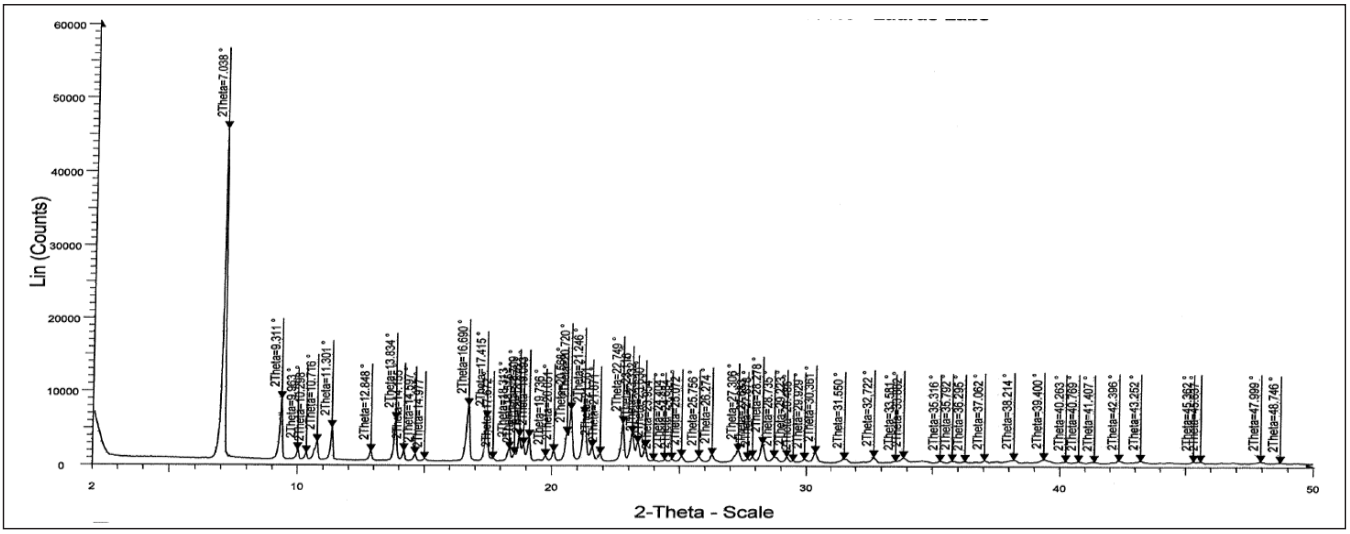

Fig. 1: X-Ray diffractogram of darunavir ethanolate form A

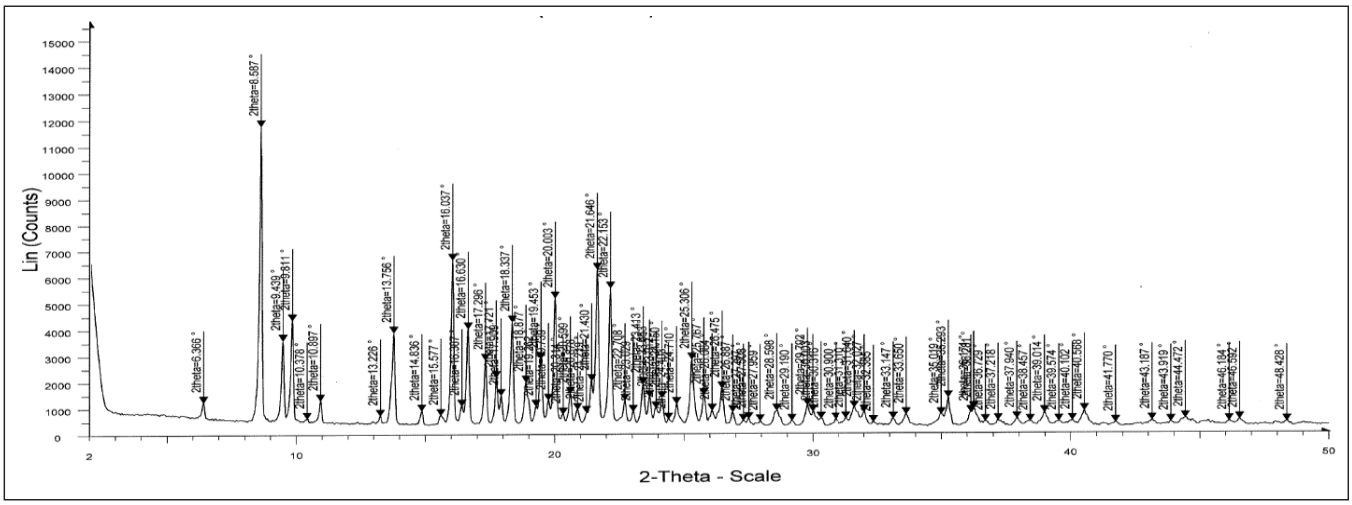

Fig. 2: X-Ray diffractogram of ritonavir crystalline form II

was further compressed into tablet along with darunavir ethanolate and excipients, resulting in fixed dose drug combination immediate release tablet.

Considering recent trends in pharmaceutical field, the study of polymorphic evaluation was carried out by hyphenated techniques like X-ray diffraction and differential scanning calorimetry[11,12]. For semi-quantitative application, slow scan X-ray diffraction technique was chosen due to importance of ritonavir polymorph control in fixed dose drug combination product linked with bio availability[13].

Another technique, DSC, provides an additional polymorphic characterization support where exposure of sample at controlled temperature program is done so that the heat flow into (endotherm) or out of a sample (exotherm) is measured as a function of temperature or time. The outcome is measured by energy transferred to or from a sample undergoing a physical or chemical change. The difference in behaviour of crystalline and amorphous material can be easily distinguished because crystalline materials undergo crystallization and melting at particular temperature recorded as exotherm, however amorphous materials do not exhibit other phase transition and hence no peak is observed.

This research work was focussed on application of XRD and DSC technique to evaluate polymorphism for darunavir ethanolate and ritonavir at in-process stage of product manufacturing and in finished formulation including stability of drug product under accelerated condition.

MATERIALS AND METHODS

Instrumentation

X-Ray diffraction study using normal scan and slow scan

The analysis was carried out using Bruker X-Ray diffractometer with operating parameters like X-Ray source: Cu, Detector used: LynX Eye, Voltage: 40 kV / 30 mA, Scan range: 2.000 to 50.000 degree, Scan speed 5 degree/min, Sampling width: 0.02 degree, scan type: continuous. This normal scan XRD method was applied for monitoring of both darunavir ethanolate and ritonavir polymorphism in finished product tablet sample.

For semi-quantitative method by slow scan XRD technique, the scan range followed: 8.2 to 9.0 degree, Scan speed 30 Second/step, Step Size: 0.02 °, scan type: continuous keeping remaining parameters same as normal scan XRD method. This slow scan method was applied for semi-quantitate evaluation of ritonavir polymorph in ritonavir blend manufactured using hot melt extrusion process as an in-process control for drug product manufacturing.

DSC (Differential scanning calorimetry) technique

The analysis was carried out using differential scanning colorimeter instrument following temperature program as equilibrate at 30 °C then raised at the rate of 10 °C per minute to 300 °C and held for '0' minutes. The outcome as thermogram was recorded for temperature in °C vs heat flow in mW and obtained values of onset point and peak were recorded. The method used about 2 to 4 mg of sample which was kept in aluminum cell and crimped. A pin hole was made in the lid. Reference pan was kept empty following rest procedure same as sample pan. This method was applied for monitoring of both darunavir ethanolate and ritonavir polymorphism in finished product tablet sample through monitoring of melting point as exotherm.

Method of preparation

X-Ray diffraction method

The procedure followed for blank-placebo, standard and sample preparation is described below.

The placebo powder having excipients without active was taken equivalent to the quantity of total excipients in one tablet derived from its average weight. For individual drug standard, ritonavir crystalline API form II and darunavir ethanolate form A API were used. The ritonavir blend and tablet test samples were prepared by gentle crushing of ritonavir blend equivalent to 100 mg ritonavir and finished product tablets, respectively. Tablet sample considered for study included initial sample of drug product and stability study sample after exposure to accelerated condition i.e. 6-month exposure in HDPE bottle at 40 °C/75 % RH condition.

For slow scan XRD study the spiked standard was prepared by gentle crushing to get homogeneous mixture of 10 mg (equivalent to 10 % of ritonavir label claim) of crystalline ritonavir form II standard and equivalent to one tablet weight placebo without active. Similarly, spiked test sample was prepared by using 10 mg of crystalline ritonavir form II standard and ritonavir hot melt extrusion process blend equivalent to 100 mg ritonavir. Each sample was transferred individually into sample holder for evaluation by normal scan and slow scan XRD as applicable. The normal scan XRD data was evaluated based on characteristic 2theta value of respective API and slow scan XRD data was evaluated considering characteristic and highest intensity 2 theta peak of ritonavir at 8.6, as reflected Fig. 2.

DSC method

The individual API standard, placebo, crushed powder of ritonavir blend and crushed powder of drug product tablet sample of initial and accelerated stability condition (about 2 to 4 mg each) were considered for evaluation of polymorphism by DSC technique. The crystallinity was monitored by referring exotherm for each active ingredient. The thermogram showing onset (° C) and peak (° C) for each sample was recorded.

Method validation of slow scan XRD method

Considering semi-quantitative application, the slow scan XRD method was validated in accordance with the ICH Harmonized guidance for selectivity, linearity and precision study[14,15].

The system precision was evaluated by analyzing six replicates of ritonavir spiked standard. The selectivity was evaluated by using ritonavir crystalline form II, excipient blend (placebo), ritonavir blend (after hot melt process) and ritonavir blend test spiked with crystalline ritonavir form II at 10 % level. The method linearity was evaluated by analyzing different concentrations of ritonavir crystalline form II standard in the range of 50 % to 150 % w/w.

RESULTS AND DISCUSSION

X-ray diffraction study using normal scan method

The input active drug substance X-ray diffractogram was recorded as shown in Fig. 1 and Fig. 2 for darunavir

ethanolate and ritonavir, respectively. Further evaluation was carried out by referring characteristic 2theta value of each active ingredient in drug product tablet sample of initial and accelerated stability along with placebo comparison. The detailed outcome with supportive XRD diffractogram and its interpretation is explained below.

The outcome of study indicates characteristic theta values observed for ritonavir API and darunavir ethanolate API which is linked with input material used in formulation. The placebo (excipient blend) did not show any interference at the characteristic theta value peaks of each API, confirming selectivity of the method.

The overlay diffractogram as per Fig. 3 indicates that the characteristic theta value based peaks of darunavir ethanolate were also observed in initial sample of drug product and no such peaks observed in plain placebo confirming method selectivity and stability of darunavir polymorph in drug product after manufacturing.

The observed absence of characteristic theta value peaks in hot melt extrusion process blend of ritonavir as compared to that observed in ritonavir crystalline form II API confirms polymorphic conversion of ritonavir from crystalline to amorphous form during manufacturing process as per overlay diffractogram shown in Fig. 4. Similarly, absence of characteristic 2theta related to

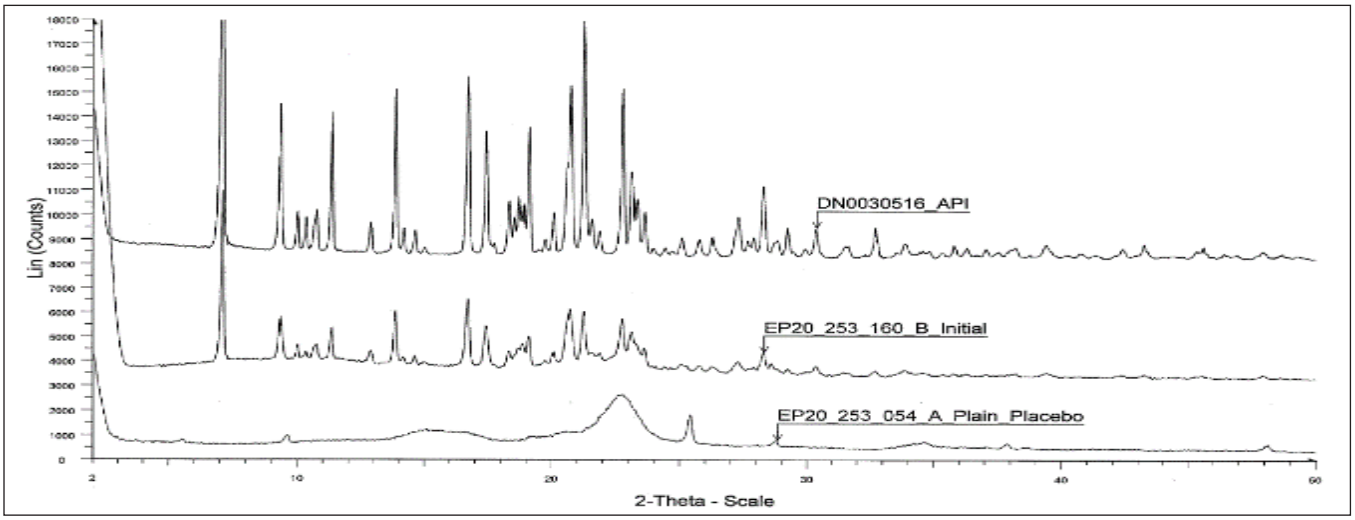

Fig. 3: Overlay X-Ray diffractogram of darunavir ethanolate API (DN0030516_API), placebo (excipient blend) (EP20_253_A_Plain_Placebo) and fixed dose drug combination tablet initial (EP20_253_160_B_Initial)

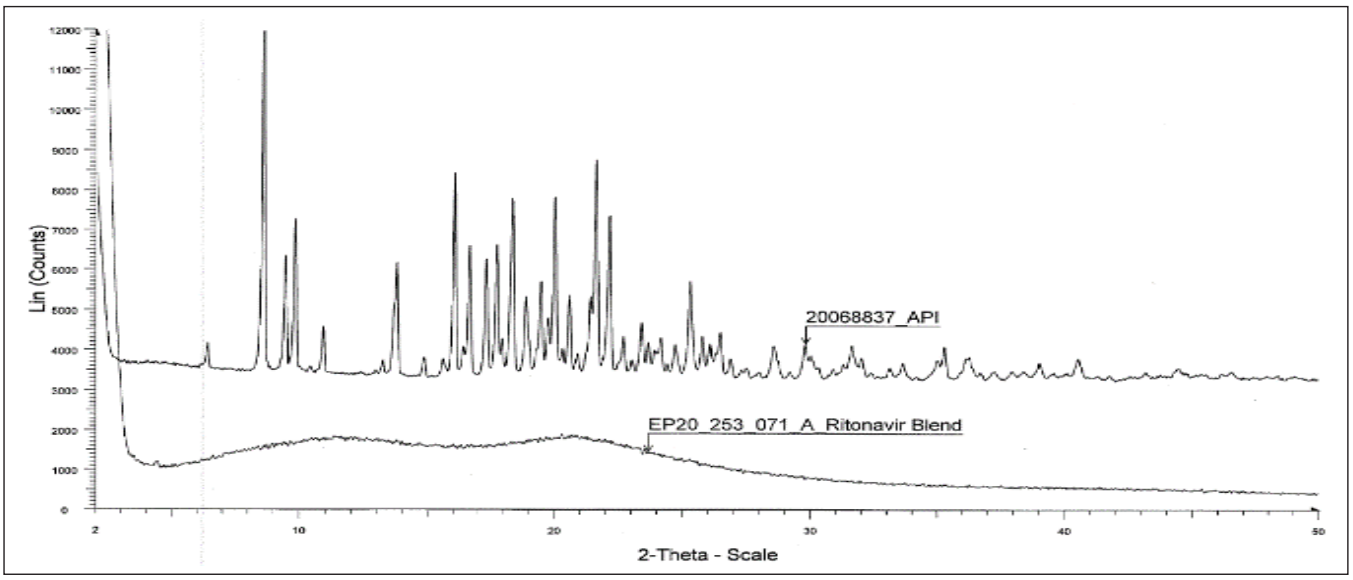

Fig. 4: Overlay X-Ray diffractogram of ritonavir crystalline form II API (20068837_API) and ritonavir blend (EP20_253_071_A_ritonavir blend)

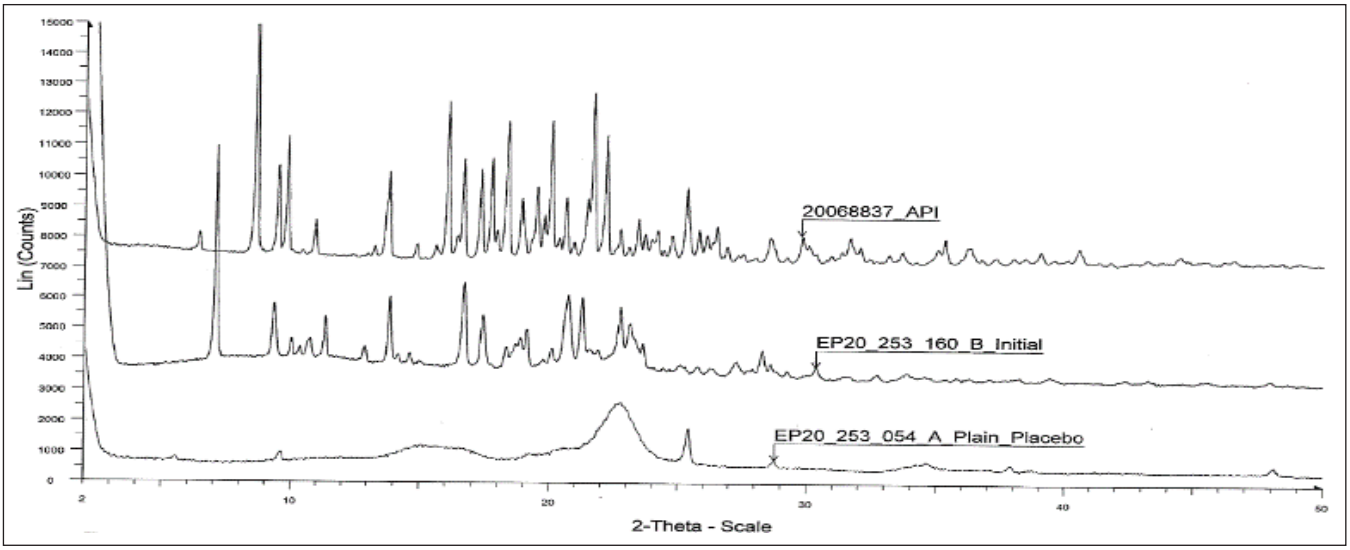

Fig. 5: Overlay X-ray diffractogram of overlay diffractogram of ritonavir API (20068837_API), placebo (excipient blend) (EP20_253_054_A_Plain_Placebo) and tablet initial (EP20_253_160_B_Initial)

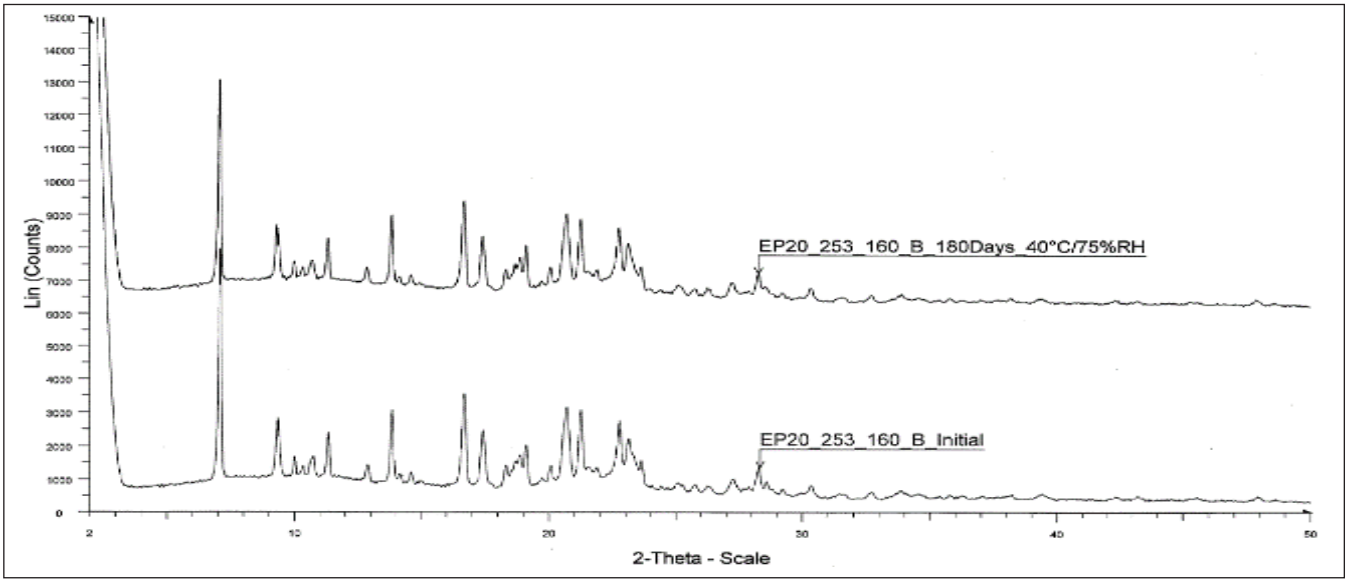

Fig. 6: Overlay X-ray diffractogram of darunavir and ritonavir tablet initial (EP20_253_160_B_initial) and stored at accelerated condition i.e. 40 °C/75 % RH for 180 days (EP20_253_160_B_180 days_40 °C/75 %RH)

crystalline ritonavir was also observed in drug product sample as per Fig. 5, confirming that ritonavir amorphous form was retained in tablet sample.

Further, polymorphic stability of active ingredients in drug product was evaluated as reflected in Fig. 6. Observed similar X-ray diffractogram for tablet sample of initial and accelerated condition i.e. 40 °C/75 % RH for 6 months stored in HDPE bottle confirms polymorphic stability of drug product.

X-Ray diffraction study using slow scan method

The outcome of X-Ray diffraction study using slow scan method was applied for ritonavir polymorphism evaluation at in-process stage of drug product manufacturing (hot melt extrusion process blend of ritonavir), where characteristic 2theta value of ritonavir i.e. 8.6 ± 0.2 having highest intensity was considered for semi-quantitative evaluation of ritonavir crystalline form.

The said study illustrated a semi-quantitative tool to monitor percentage of crystalline ritonavir presence in ritonavir hot melt process blend through comparison against crystalline standard of known concentration. The study was designed to have control of crystalline form at 10 % level as an indirect tool to ensure above 90 % conversion of ritonavir crystalline to amorphous form.

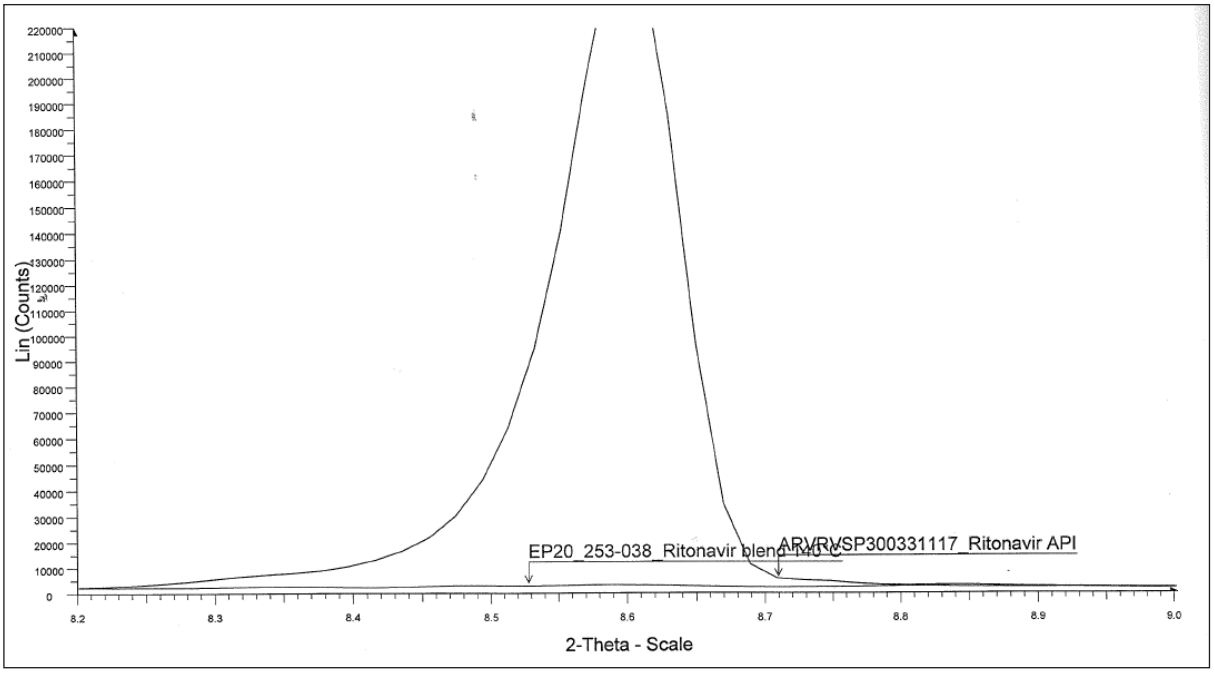

Fig. 7: Slow scan overlay XRD diffractogram of ritonavir crystalline form II API (ARVVSP300331117_ ritonavir API) and hot melt extrusion processed blend of ritonavir (EP20_253-038_ritonavir blend 140 °C)

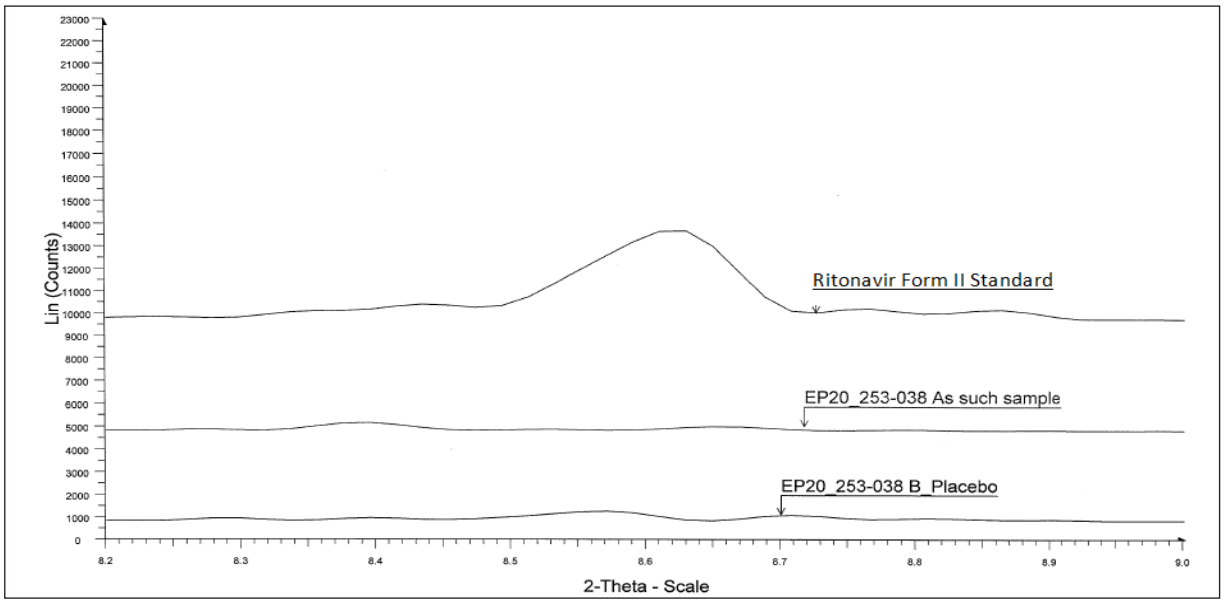

Fig. 8: Slow scan overlay XRD diffractogram of ritonavir crystalline form II standard at 10% w/w concentration (Ritonavir Form II standard), hot melt extrusion processed blend sample of ritonavir (EP20_253-038_as such sample) and placebo for ritonavir processed blend (EP20_253_038B_placebo)

As reflected in Fig. 7, the overlay diffractogram of ritonavir crystalline form II API and hot melt process blend sample of ritonavir shows comparative outcome of 8.6 theta peak. We observed absence of 8.6 theta peak in processed blend sample of ritonavir as compared to very high intense peak in ritonavir API.

The Fig. 8 shows comparative slow scan XRD diffractogram of ritonavir crystalline form II standard at 10% w/w level, ritonavir process blend as such sample and placebo for ritonavir processed blend. No crystalline peak at characteristic 2theta of ritonavir i.e. 8.6 ± 0.2 was observed in placebo and processed blend sample

of ritonavir as compared to that observed in ritonavir crystalline form II standard. This observation highlight that crystalline form of ritonavir gets converted to amorphous form and no any interference observed from placebo.

Method validation of slow scan XRPD method

The described X-Ray diffractogram method for the identification and estimation of % crystalline ritonavir was validated through selectivity, linearity, system precision and method precision study to ensure suitability for intended use. The compiled outcome for method validation study is described below.

System precision and selectivity

System precision was monitored by replicating XRD slow scan of ritonavir crystalline form II standard and the intensity of crystalline ritonavir peak was recorded and % RSD was calculated. Also, the selectivity of method was carried out by XRD scan of placebo blank and ritonavir blend test sample. The slow scan overlay XRD diffractogram as per Fig. 8 shows selectivity of method by comparison of ritonavir crystalline form II standard, placebo and processed blend sample. The outcome for system precision and selectivity study is presented in Table I.

Linearity

Linearity was carried out using ritonavir crystalline form II standard in the range of 50 % w/w to 150 % w/w correlated as 5 % w/w to 15 % w/w of ritonavir test concentration in tablet with respect to ritonavir label claim i.e. 100 mg. The outcome of linearity as average intensity in counts for average of triplicate determination and obtained correlation coefficient is presented in Table II and linearity graph is presented in Fig. 9.

Method precision

Method precision was carried out using sample preparation of ritonavir blend spiked with crystalline ritonavir standard at 100 % level (equivalent to 10 % w/w of ritonavir label claim). The outcome of method precision as average % crystallinity and % RSD presented in Table III. The observed % crystallinity from precision data was interpreted to get recovery against 10% w/w spiking, the obtained values were observed in the range of 9.15% to 10.51% confirms recovery of method as average 98.7% with the range 91.5% to 105.1% which lies well within 90.0% to 110.0% criteria.

Table I: System precision and selectivity study

Sr. No.	Sample detail	Intensity of ritonavir	Observation outcome
1	Placebo-(blank)	No peak observed	No interference at 8.6 ± 0.2 theta
2	Std-1	3973	Average: 3928 SD (±): 169.3 RSD: 4.31 % (Control Limit: Less than 10 %)
3	Std-2	3931	
4	Std-3	4173	
5	Std-4	3672	
6	Std-5	3822	
7	Std-6	3995	
8	Test-Unspiked	No peak observed	Absence of Crystalline ritonavir peak at 8.6 ± 0.2 theta

Table II: Linearity study using crystalline ritonavir

Sr. No.	Linearity level	Concentration in % (with respect to label claim of ritonavir i.e.100 mg)	Average intensity n=3 (counts)
1	50%	4.999	3388
2	80%	7.974	3765
3	100%	10.008	3995
4	120%	12.008	4215
5	150%	15.011	4607
Correlation Coefficient (Control Limit: Not less than 0.99)			0.9991

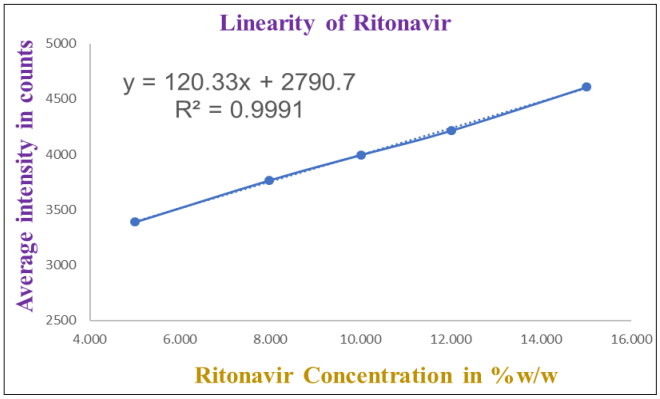

Fig. 9: Linearity graph for ritonavir concentration in % w/w vs average intensity in counts using slow scan X-ray diffraction method

Observation from method validation study

The slow scan X-Ray diffraction method was found selective for ritonavir crystalline form II quantitation in presence of placebo. Also, the method was found linear and precise for the estimation of crystalline ritonavir in ritonavir blend sample which confirms conversion of crystalline ritonavir to amorphous form. The outcome of precision study confirmed repeatability of method as well as recovery of method at 100 % level. Further, as shown in Fig. 1 for the X-Ray diffractogram of darunavir ethanolate form A, no peak was found at 2theta value of 8.6 ± 0.2, hence proposed method can also be extended for semi-quantitative estimation of ritonavir polymorph in fixed dose drug combination tablet formulation.

Polymorphism by DSC (Differential scanning calorimetry) study

The study of polymorphism by DSC includes generation of DSC thermogram which shows onset and peak for each component refer to respective melting range[16]. The obtained onset and peak value is interpreted to monitor polymorphism as compared to that observed in input API. The thermogram showing Onset (° C) and Peak (° C) for each sample is recorded. The detailed outcome is interpreted.

Fig. 10 shows overlay DSC thermogram of ritonavir crystalline form II, placebo (excipient blend), hot melt extrusion process blend of ritonavir and fixed dose drug combination tablet sample. It was observed that ritonavir crystalline form II shows exotherm with onset at 120.8 °C and peak at 127.4 °C, which is characteristic melting point of crystalline material. Further, the absence of exotherm at ritonavir melting point was observed in placebo, hot melt extrusion process blend of ritonavir and in drug product tablet sample.

Fig. 11 shows overlay DSC thermogram of placebo, fixed dose drug combination tablet sample and darunavir ethanolate form A API. It was observed that characteristic exotherm with onset at 99.3° C and peak at 105.7° C was found in darunavir ethanolate form A API, which was also found in fixed dose drug combination tablet sample, however it was not observed in placebo sample.

Further, to confirm polymorphic stability of drug product by DSC technique, DSC thermogram of fixed dose drug combination tablet initial sample was compared with that of accelerated stability condition tablet sample i.e. 6 months 40 °C/75 % RH HDPE pack as presented in Fig. 12. It was observed that darunavir ethanolate form A exotherm was present and ritonavir crystalline form II exotherm was absent in both initial and exposed samples of tablet formulation.

Table III: Method precision study using spiked sample preparation

Sr. No.	Sample (Sm) Sets	Intensity of crystalline ritonavir peak at 8.6 ± 0.2 theta	Observation outcome as % crystallinity
1	Sm-1	3875	9.9
2	Sin-2	4100	10.48
3	Sm-3	3683	9.41
4	Sm-4	4112	10.51
5	Sm-5	3833	9.76
6	Sm-6	3628	9.15
Average % crystallinity against 10% spiked			9.87
SD (±) for six determinations			0.552
% RSD for six determination (Control limit not more than 10%)			5.60

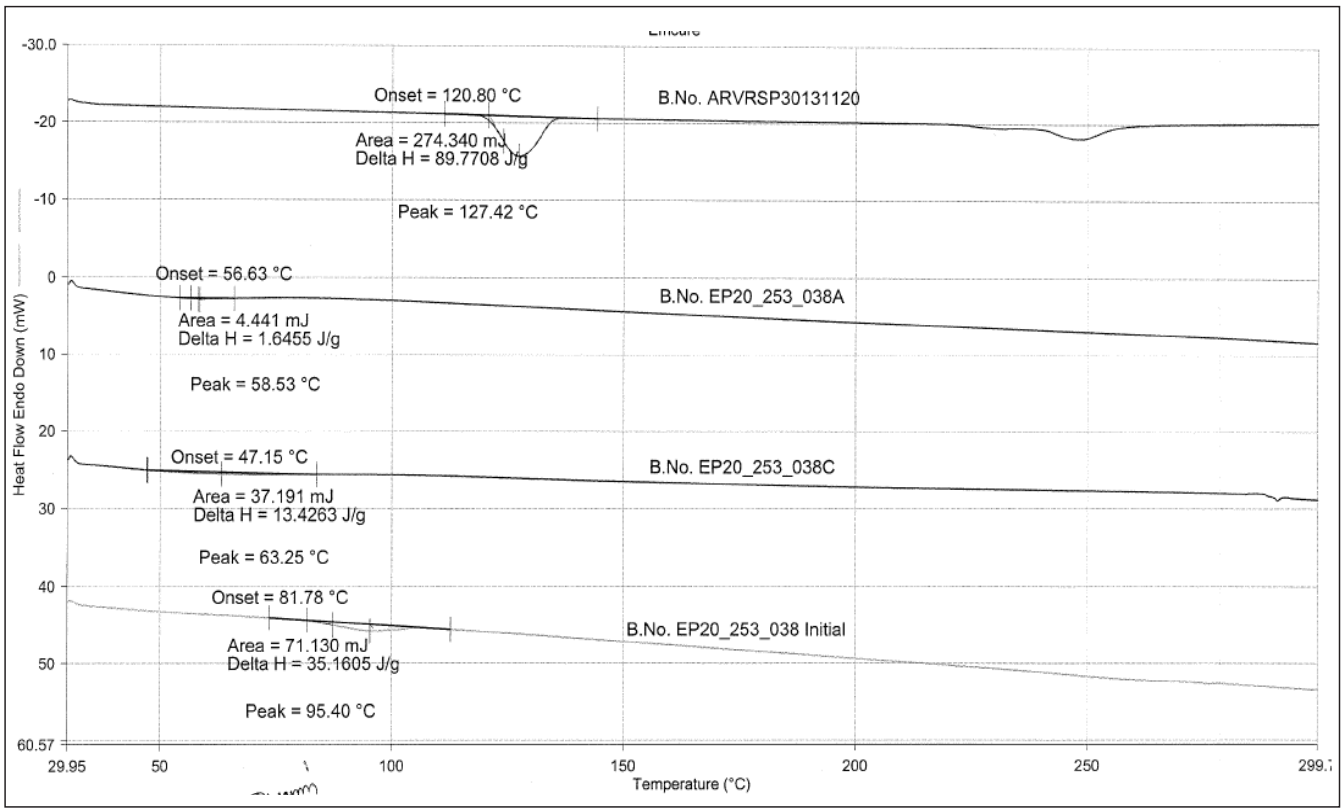

Fig. 10: Overlay DSC thermogram of ritonavir crystalline form II API (B. No. ARVRSP30131120), placebo (excipient blend) (B. No. EP20_253_038A), hot melt extrusion process blend of ritonavir (B. No. EP20_253_038C) and fixed dose drug product tablet sample of initial (B. No. EP20_253_038 Initial)

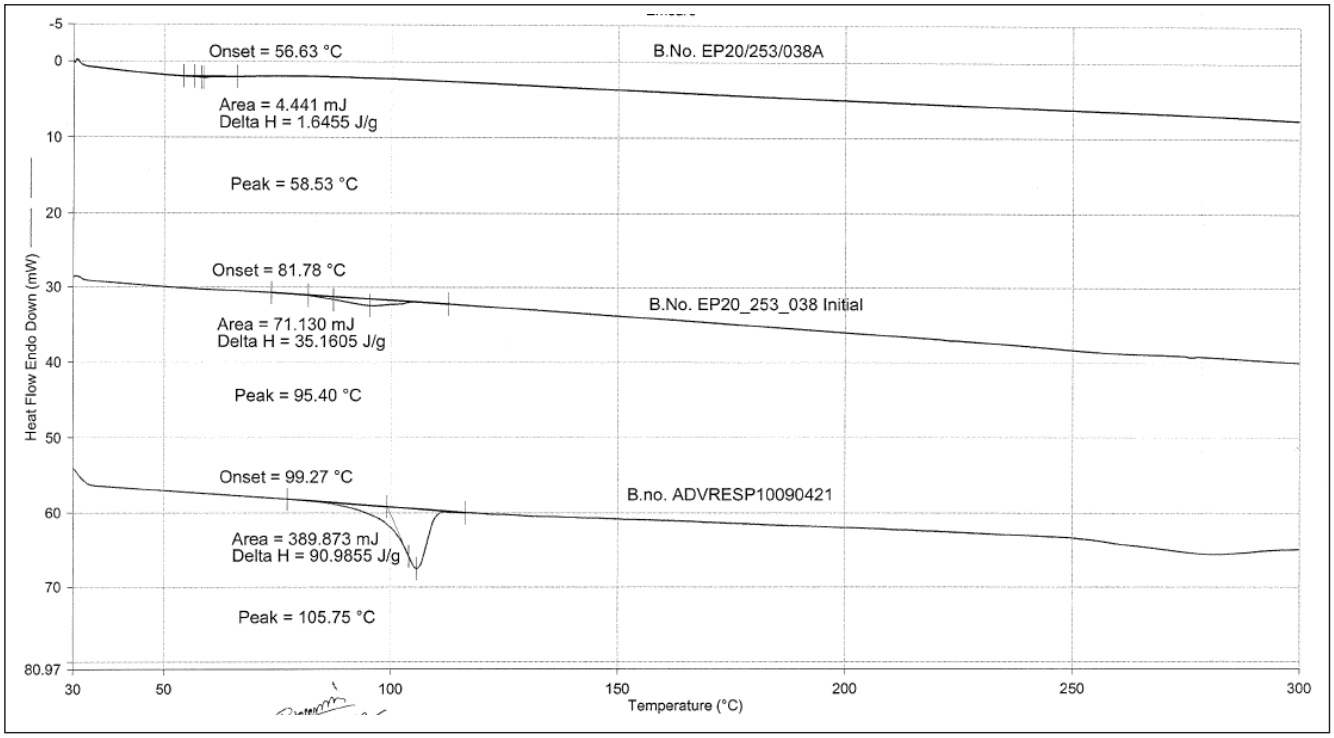

Fig. 11: Overlay thermogram of placebo (excipient blend) (B. No. EP20/253/038A), fixed dose drug product tablet sample of initial (B. No. EP20_253_038 Initial) and darunavir ethanolate form A API (B. no. ADVRESP10090421)

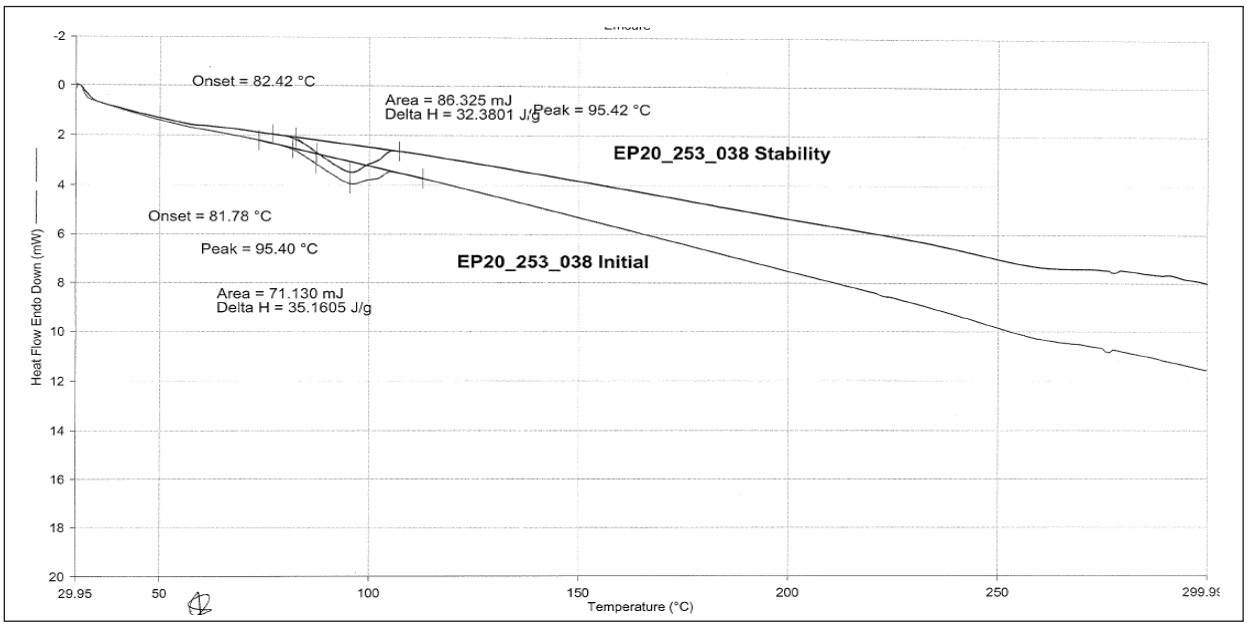

Fig. 12: Overlay DSC thermogram of fixed dose drug combination product sample of initial (EP20_253_038 Initial) and stored at accelerated condition i.e. 6 months 40 °C/75 % RH HDPE pack (EP20_253_038 stability)

Table IV: Data interpretation of DSC analysis

Sample (Sm)	Sample detail	Onset (°C)	Peak (°C)	Observation
Sm-1	Danmavir ethanolate API	99.3	105.7	Exotherm / peak observed at melting temperature of danmavir ethanolate
Sm-2	Ritonavir API	120.8	127.4	Exotherm / peak observed at melting temperature of ritonavir
Sm-3	Excipient blend / Placebo	56.6	58.5	No peak at melting temperature of darunavir ethanolate and ritonavir
Sm-4	Ritonavir blend	47.1	63.2	No peak at melting temperature of ritonavir
Sm-5	Tablet initial sample	81. 8	9 5.4	No peak at melting temperature of ritonavir & peak observed at melting temperature of danmavir ethanolate
Sm-6	Tablet accelerated stability study sample	82.4	95.4	No peak at melting temperature of ritonavir & peak observed at melting temperature of danmavir ethanolate

The outcome of each sample with observed peak and onset during DSC analysis corresponding to respective polymorph based observation is summarized in Table IV.

The above study indicates that selected DSC method can distinguish both active ingredients due to its characteristic melting temperatures. As no interference was observed in excipient blend at characteristic melting point of darunavir ethanolate form A and ritonavir crystalline form II, it confirms selectivity of the method. Also, absence of exotherm related to ritonavir crystalline in hot melt process blend confirmed amorphous form of ritonavir. The tablet sample of initial and stored sample showed peak related to melting temperature of darunavir ethanolate, confirming its polymorphic stability in fixed dose drug combination product and absence of ritonavir peak confirmed amorphous form of ritonavir and its stability.

The overall outcome confirmed suitability of the DSC method selected for polymorphic evaluation of both darunavir ethanolate and ritonavir in fixed dose drug combination product.

CONCLUSION

XRD method (with normal scan and slow scan) and DSC method was considered for evaluation of polymorph in fixed dose drug combination product containing darunavir ethanolate 800 mg and ritonavir 100 mg. The normal scan XRD method was found selective for evaluation of polymorphic form of both active drug substances in tablet form. The slow scan XRD method for analysis of ritonavir process blend was validated to confirm suitability for intended use. Specificity of method was proven by no interference due to placebo. The observed correlation coefficient value of 0.9991 assured linearity of method in the range of 50% to 150% w/w of ritonavir which was equivalent to 5% w/w to 15% w/w of ritonavir label claim. Also observed very precise outcome for system precision having % RSD value of 4.31 for 5 replicates of standard and 5.60% for six replicate of spiked sample analysis reported as % crystallinity. The recovery at 100% level interpreted from method precision data shows minimum 91.5%, maximum 105.1% and mean recovery 98.7%. Accordingly, the slow scan method was found suitable for semi-quantitative estimation of ritonavir polymorphism. The DSC as an alternate technique showed characteristic peak for ritonavir crystalline form II at 127.4 and darunavir ethanolate at 105.7 was also found specific and sensitive to evaluate polymorphism of both active drug substances in processed blend of ritonavir as well as in fixed dose drug combination tablets at initial as well as stability exposure. The selected methods by XRD and DSC were found capable to perform polymorphic evaluation of darunavir ethanolate and ritonavir in fixed dose drug combination product. The described techniques can be useful to characterize polymorphic form and its stability in fixed dose drug combination product which may have direct impact on *in vivo* clinical performance owing to difference in solubility profile of different polymorph which is very critical task for pharmaceuticals manufacturing and process control[17].

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