DRUG-EXCIPIENTS COMPATIBILITY TESTING PROTOCOLS AND CHARACTERIZATION FOR SELECTION TEZOSENTAN IN SOLID DOSAGE FORM

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Abstract

A simple HPLC method was developed and used to study of compatibility between Tezosentan and excipients. Proper formulation is an important aspect of any dosage form design. As a part of preformulation studies was used to investigate the physiochemical compatibility between Tezosentan and various excipients commonly used in tablet manufacturing such as povidone and pvp k-30. Differential scanning calorimetry studies indicated incompatibility with povidone and pvp k 30.HPLC method shows linearity range of 1-10 µg/ml, R^2 0.9997 and %RSD was found to be less than 2.The drug exipients results shows that suitability of Tezosentan with selected excipients when stored in elevated temperature condition.

Keywords: Tezosentan; DSC, FTIR, XRPD, HPLC, Tablet excipients, Compatibility

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INTRODUCTION

Tezosentan is dual Endothelin receptor anatgonist used in the treatment of pulmonary arterial hyperension (PAH). A thorough drug-excipient compatibility study is a very important part of QbD and in general for the development of a stable pharmaceutical formulation. In the present study, the compatibility of Tezosentan with 14 different pharmaceutical excipients of common use in the development of solid dosage forms was evaluated. The collection of real-time stability and compatibility data is time-consuming and expensive, so obtaining rapid and reliable information about possible drug–excipient interactions is highly desirable [1,5]. For this purpose, DSC measurements were carried out both in the pure form and the corresponding 1:1 (w/w) physical mixtures. The absolute value of the difference between the melting endothermic peak temperature of pure drug and that in each analyzed mixture [6,7,8]and the absolute value of the difference between the enthalpy of the pure Tezosentan melting peak and that of its melting peak in the different analyzed mixtures were chosen as indexes of the drug–excipient interaction degree. FT-IR spectroscopy[9,10] and X-ray powder diffraction were used as complementary techniques to adequately implement and assist in interpretation of the DSC results[11,12].

The aim of the study is to verify the compatibility between Tezosentan and the main excipients employed in the solid pharmaceutical dosage forms, using differential scannning calorimetry (DSC), infrared spectroscopy (FTIR), x-ray powder diffraction (XRD), and IST (HPLC)[13,14].

MATERIALS AND METHODS:

Tezosentan obtained as a gift sample from Elder Pharmaceuticals (India). Following excipients were purchased from commercial sources and used as such Crosspovidone, Lactose monohydrate, Di – calcium posophate, Starch, Magnesium stearate, Hydroxyl Propyl Methyl Cellulose (HPMC), Microcrystalline cellulose (MCC), Sodium cross carmellose, Xanthan gum, Talc, Povidone, Sodium Carboxy Methyl Cellulose (Na CMC), PVP K -30, Carbopol. S.D Fine Chemicals Ltd. Mumbai (India). Methanol, Acetonitrile & Doubled distilled water was used for mobile phase passed through the 0.45µ filter purchased from Merck Specialties Pvt. Ltd.,India.

Differential Scanning Calorimetry (DSC):

For thermal analysis of drug and drug excipient mixtures, a differential scanning calorimeter (DSC 821e, Mettler Toledo, Switzerland) was used. Individual components and there mixture were subjected to room temperature as well as an isothermal stress condition of 40°C for 4 weeks all samples were prepared by an arithmetic dilution method. Samples (5-10 mg) were accurately weighed and hermatically sealed in pierced DSC aluminium pans and scanned in the temperature range of 25-300°C under an atmosphere of dry nitrogen

FTIR (Fourier-transform infrared) Spectrosopy:

Tezosentan at room temperature and mixtures of drug and excipient kept at 40°C for 4 weeks were subjected to IR spectroscopy using a JASCO FTIR 4100 instrument. Pellets of samples were prepared after grinding and dispersing the powder in micronized IR grade KBr powder using an agate pestle and mortar, for 3 – 5 minutes The concentration of sample in potassium bromide should be in the range of 0.2% to 1% [29]. The pellets were placed in light path and spectrum was obtained and reviewed for evidence of any interactions scanned over a wave number range 4000 cm-1 - 400 cm-1.

XRD (X-Ray powder diffraction):

Individual components at room temperature and their mixtures kept at room temperature and at 45°C for 4 weeks were subjected to XRPD studies. The physical mixture of drug and excipient at room temperature was taken in the ratio 1:1. This ratio was used to maximize the likelihood of interaction taking place, and thus helps in easier detection of incompatibilities. The XRPD patterns of sample were recorded using a Bruker D8 Advance X-ray diffractometer using MoKα radiation (Zr filter on the diffracted beam, 50 kV and 40 mA) in a Bragg–Brentano θ:2θ configuration, with Soller and fixed slits and a NaI (Tl) scintillation detector. The measurements of 2θ ranged between 0◦ and 30◦. Data analysis and acquisition were performed using ORIGIN PRO 8 software from Bruker AXS.

ISOTHERMAL STRESS TESTING:

Three different samples were prepared and store at 40°C±5°C & 75% R.H for Four weeks before analysis. 1. 50 mg pure API. 2. 50 mg pure excipient. 3. 50 mg pure excipient with 50 mg pure API. For IST studies Drug and different excipients were weighed directly in 15 ml glass vials and mixed on a vortex mixer for 2 min. samples of each excipient, each mixture and the pure API were analysed initially, and following storage by HPLC. Incompatibility was identified by observation of

chromatographic changes compared to the changes in the chromatograms and in the recovery of the compound. Each vial was sealed using a teflon-lined screw cap and stored at 40°C±5°C & 75% R.H. These samples were periodically examined for any unusual colour change. After 4 weeks of storage at the above conditions, samples were quantitavely analysed using HPLC. Samples were diluted with mobile phase, sonicated for 10 min and centrifuged for 10 min at 4000 rpm before the HPLC analysis. The samples were centrifuged and the supernatant filtered through nylon membrane filters (0.45 _m pore size). After appropriate dilutions, samples were analyzed using HPLC and drug content determined from the calibration curve prepared within the expected range (1 - 10µg/ml). The method was found to be linear within the studied range (*R*2: 0.9997). For the HPLC analysis of drug – excipient mixtures Analysis performed on Thermo Hypersil ODS C18 Column. Specification – of (250mm length × 4.6 mm Internal diameter, 5µ Particle size). An isocratic HPLC method with a flow rate of 1.0ml/min & injection volume of 20 µl. Mobile phase consisted of Methanol: Acetonitrile: D.D.Water (60: 30: 10). Sample analysed by U.V Detection at 225 nm.

TABLE 1: THERMO ANALYTICAL DATA OF TEZOSENTAN AND EXCIPIENTS

Samples	**T onset (°C)**	**T peak (°C)**	**ΔHf corr (J/g)$^{-1}$**	**Nature of the process**
Tezosentan	102.45	109	-28.61	Decomposition
Povidone	29.92	64.93	-156.75	Dehydration:Decomposition
PVP K 30	56.23	100.79	-239.57	Dehydration:Decomposition

TABLE 2: THERMO ANALYTICAL DATA OF TEZOSENTAN AND DRUG:EXCIPIENT PHYSICAL MIXTURE

Samples	**Ratio (Drug : Excipient)**	**T onset (°C)**	**T peak (°C)**	**ΔHf (J/g)-1**
Tezosentan	-	102.45	109	-28.61
Boaentan + Povidone Initial	1:1	86.11	96.63	-15.22
Boaentan + Povidone Final	1:1	-	-	-
Tezosentan + PVP K 30 Initial	1:1	107.44	114	-2.28
Tezosentan + PVP K 30 Final	1:1	111.41	120.21	-8.58

TABLE 3: PHYSICAL AND CHEMICAL CHANGES OF TEZOSENTAN IN THE PRESENCE OF EXCIPIENTS

Physical and chemical changes of Tezosentan in the presence of excipients			
Sr. no	**Name of Excipients**	**Physical Change**	**Chemical change (% degradation)**
1	Crosspovidone	NC	96.12
2	Lactose monohydrate	NC	100.41
3	Di cal Phosphate	NC	97.96
4	Starch	NC	96.27
5	Mg stearate	NC	99.89
6	HPMC	NC	104.58
7	MCC	NC	94.36
8	Crosscarmellose Na	NC	97.13
9	Xanthan gum	NC	97.28
10	Talc	NC	104.54
11	Povidone	Wet mass	89.07
12	CMC Na	NC	100.93
13	PVP K 30	Wet mass	NA
14	Carbopol	NC	100.19%

TABLE 4: LINEARITY PARAMETERS

Parameters	
Linearity range meteµg/ml	1-10
R2	0.9997
Slope	49281
Intercept	7665.6

TABLE 5: RESULT OF ANALYSIS OF IST SAMPLES AFTER 4 WEEKS OF STORAGE AT STRESSED CONDITIONS

Sample	**Ratio (drug : excipient)**	**%Drug remaining**	
		Control samples	**Stressed samples**
Tezosentan	1:01	101.98	99.8
Tezosentan+Povidone	1:01	97.98	89.07
Tezosentan+PVP K 30	1:01	96.84	NA

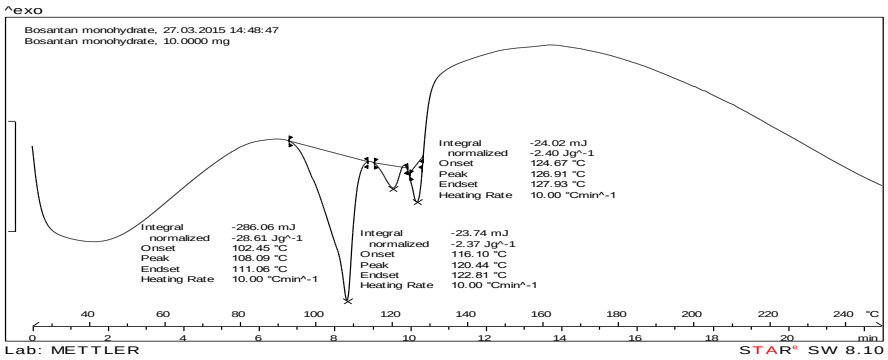


Fig. 1: Thermal behaviour of Tezosentan

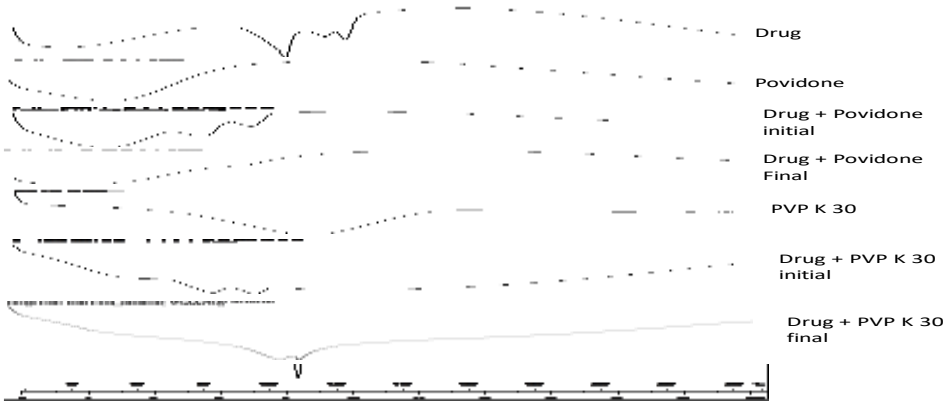


Fig. 2: DSC curves were comapred with those of their 1:1 physical mixtures

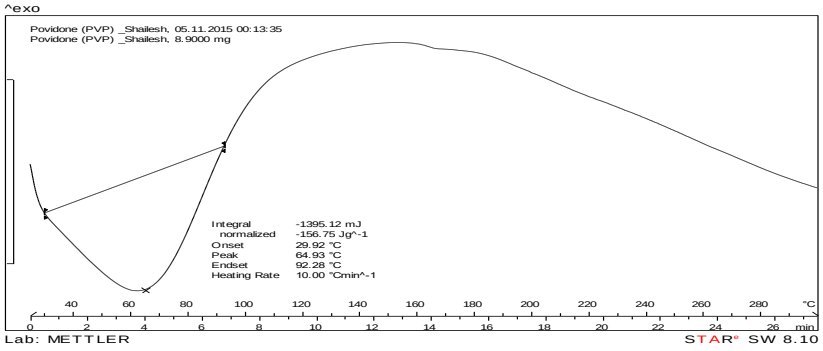


Fig. 3: DSC trace of povidone

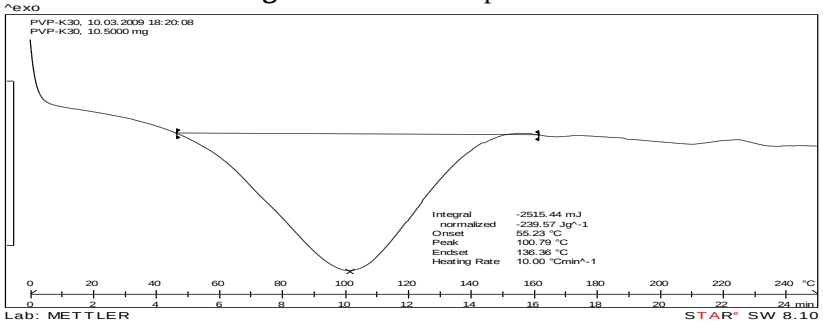
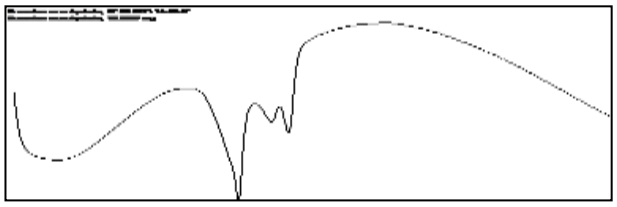
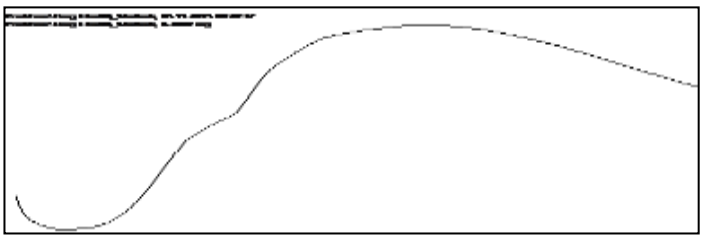


Fig 4: DSC trace of PVP K30



Tezosentan

Drug+Povidone



Drug+Povidone

Final

Fig 5: DSC curves Tezosentan, D+Povidone initial 1:1, D+Povidone final 1:1

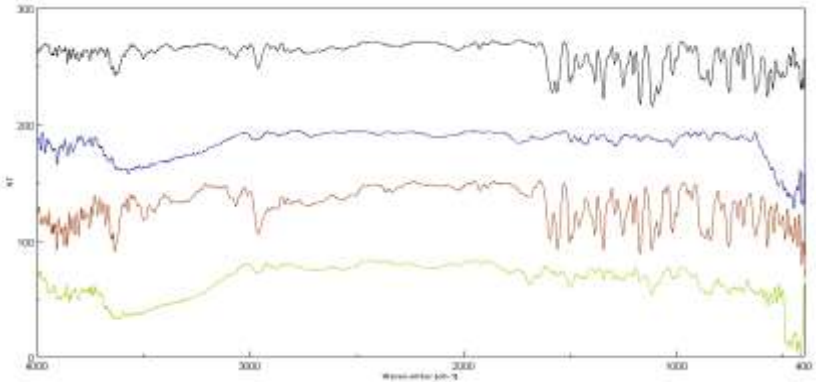


Fig 6: IR spectra : Tezosentan, Povidone, BT+Povidone initial, BT + Povidone After 4 weeks

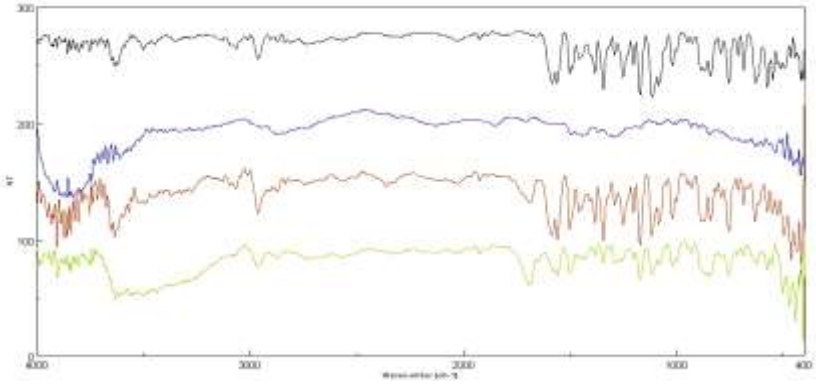


Fig 7: IR spectra : Bosenatan, PVP K 30, BT+PVP K 30 initial, BT+PVP K 30

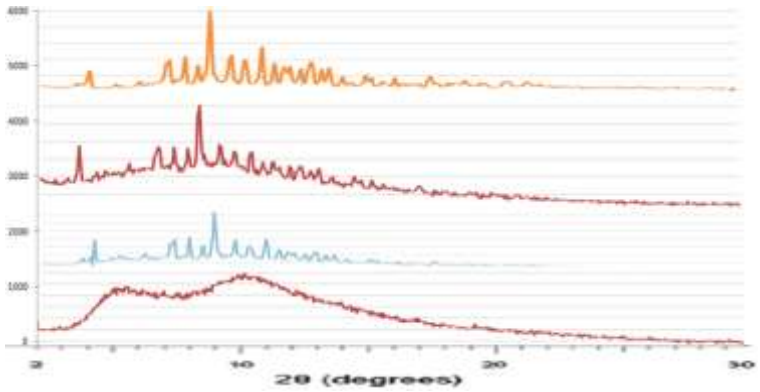


Fig 8: X- ray diffractogram: Tezosentan, Povidone, BT-Povidone initial, BT+Povidone

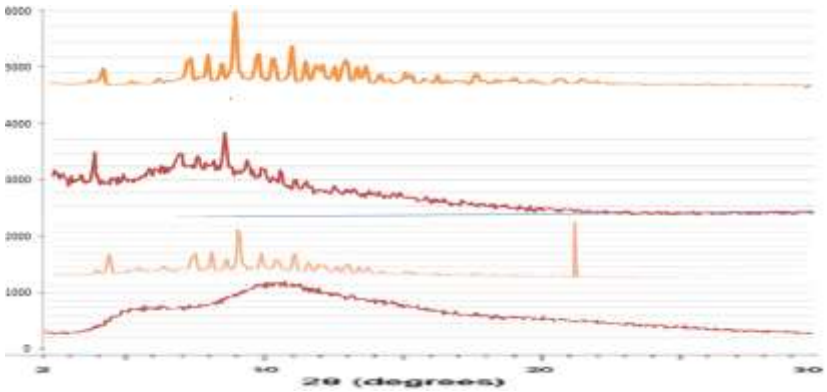


Fig 9: X-ray diffractogram: Tezosentan, PVP K 30, BT-PVP K 30 initial, BT-PVP K 30

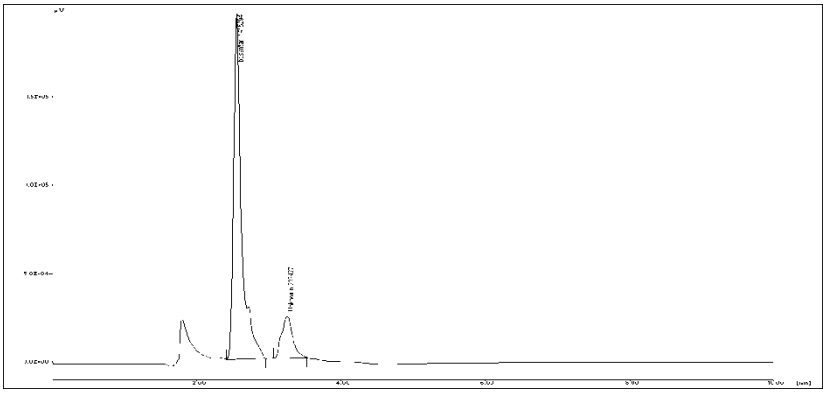


Fig: 10 *The decrease of peak area of Tezosentan was associated with appearance of very small peaks of degradation products.*

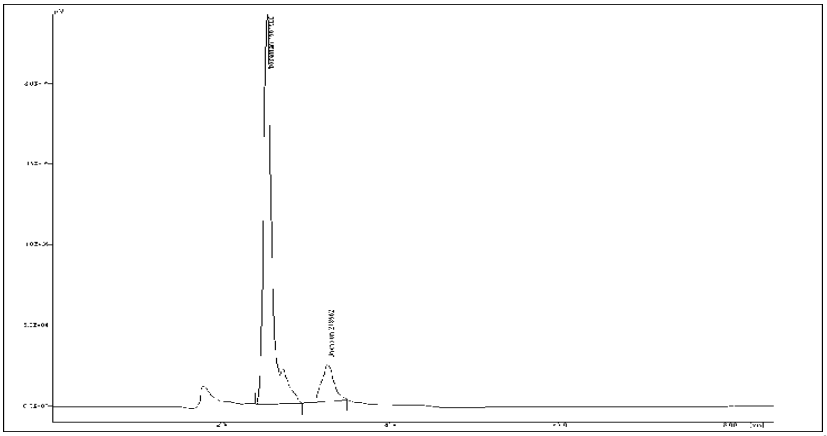


Fig 11: Tezosentan in the presence of Povidone was converted to single major product (16%) which was indicated unknown.

RESULT AND DISCUSSION:

Thermal behaviour of Tezosentan

The DSC curves obtained for TEZOSENTAN are presented in (Fig .1). The DSC curve of Tezosentan (for β= 10°C min-1) present sharp endothermic peak at 108°C. (T onset 102°C; ΔH fusion 28.61 J g−1). Indicating the melting and which corresponds to the values indicated in literature (107°C – 110°C). In this temperature range, The characteristic peak pattern indicates the β-form of Tezosentan underwent thermal transition at 120.44°C (melting enotherm of β-form) and reccrystallized into the α form. Which further melted into at 126.91°C. also the ΔH fusion for the for the β form (-2.37 J g−1). The melting endotherm of the drug was well preserved in majority of cases. However, there were slight changes[15,16] in the peak shape with little broadening or shifting towards the lower temperature, which could be attributed due to the mixing process that lowers the purity of each component in the mixture[17].

Compatibilty study with excipients

In fact, DSC has been proposed to be a rapid method for evaluating physico-chemical interactions between components of the formulation through the comparison of thermal curves of pure substances with the curve obtained from a 1:1 physical mixture[18,19] and therefore select adequate excipients with suitable compatibility. The thermoanalytical data of Tezosentan and tested excipients, obtained[20] from the thermal curves, are collected in Table 1. and for the Povidone and PVP K30 which are susceptible to presents some interactions, the characterization is made detailed. Because of the fact that the DSC curves were compared with and for the Povidone and PVP K30 which are susceptible to presents some interactions, the characterisation is made detailed. Because of the fact that the DSC curves were compared with those of their 1:1 [21,22](w/w) physical Mixtures, these are presented in Fig.2.

The DSC curves of a Povidone, below 150°C display on initial mass loss of ≈9%. This mass loss is accompanied by a broad endothermic phenomena 30-90°C (DSC peak =64.93°C) over an ill-defined baseline which makes evaluation of the dehydration enthalpy quite uncertain. The sample readily dehydrates and its initial mass depends upon the moisture content of the atmosphere. Fig. 3

Simultaneous DSC curves of PVP K 30 show several dehydration stages below 110°C. The first endothermic effect is due to release of small amount of surface water. Around 50°C begins the first dehydration stage of structural water which partially overlaps with the second stage at higher temperature. The overall mass loss due to surface water and to the first stage ≈3%, while the amplitude of the second stage is ≈1.5% of the initial mass. This mass loss is accompanied [23,24]by a broad endothermic phenomena 55.2 – 136.36°C (DSC peak 100°C) DSC curves of PVP K 30 as shown in Fig: 4

wide endothermic effect (Tpeak = 100 ◦C), representing dehydration in the range of 25 - 300°C. The possible interaction between components are derived or deduced from DSC curves by appearance, shift, or disappearance of DSC peaks, especially the melting peak, and /or variation in excpeced enthalpy (ΔH) [25,26]values. An interaction was assumed to result in decrease, respectively increase of the ΔH in the case of overlapping a more complex process. Modification in the peak shape, peak onset or peak maximum temperature may indicate an interaction, but it is necessary[27] to bear in mind that some broadening of the peaks is a result of the missing process, which lowers the purity of each component in the mixture. The DSC curves of Tezosentan and excipient mixture are shown in Fig. 5.

In the 1:1 physical mixtures when there is no any interaction between Tezosentan and excipient, the *T*peak value of melting event (DSC curve), remain practically unchanged, similarly when the drug is alone. In this case the thermal profiles of this mixture can be considered as the superposition of the curves of Tezosentan and adequate excipients. According to the thermal curves, especially DSC curves that provide the most complete information, majority of 1:1 (w/w) physical mixture of Tezosentan with each of these excipients, reflecting substantially the characteristic features of the respective individual components. Practically, the thermal curves of binary mixtures can be considered as a superposition of adequate curves of Tezosentan and excipients. Meaningful differences were found out only for the binary mixtures of Tezosentan with Povidone, respectively PVP K30 which were further discussed on.

Compared DSC curves with initial and after 4 weeks of storage at 40°C±5°C 75% R.H. The DSC curve of the Physical mixture of Tezosentan with Povidone presents a broad and weak peak which corresponds to the dehydration endothermic peak at endothermic values. Excipients peak at 57.59°C and drug peak at 96.63°C. The reason for this behavior could be because of shifting of

Tezosentan peak to lower temperature, which could have merged with the water loss peak of Povidone. The DSC curves of dug and excipients in the ratio of 1:1 after 4 weeks of storage shows no peak main finding of DSC peak shows disappearance of characteristic peak of Tezosentan fusion peak. DSC result showed that the higher water solubility of drug coupled with the increasing of the temperature during DSC experiment, because of which Tezosentan and excipient peak disappears[28]. It was a chemical interaction between these substances due to heating reason. The reason for this behavior could be because of shifting of Tezosentan peak to lower temperature, which could have merged with the water's loss peak of Povidone, Another possi-bility including that the water, which emerged from Povidone (in the temperature range of 30-90°C) resulted in dissolution of Tezosentan (higher water solubility of drug coupled with the increasing of the temperature during DSC experiment), because of which Tezosentan peak disappears. Another possibility included that water which could emerged from PVP K 30. The same behavior was described in the literature for the mixture of Povidone with other drugs such as naproxen, cetoprofen, ibuprofen, captopril, indicating the occurrence of a solid – solid interaction with heating. One considers that this way of interaction takes place by so called dissolution of the drug in the presence of humidity and at heating. By the comparison of the DSC curves (Fig. 5) of pure Tezosentan and PVP K 30 with there 1:1 physical mixture initial day the differences are well visible and these can be attributed to any incompatibility (interaction) between the two components. The endothermic peak of Tezosentan broadened or shifted towards to 114°C, excipient peak shifted towards to lower temperature 97°C in this case significant reduction in the enthalpy values was observed. It is suggested that before using this excipient in formulation compatibility confirmed by IST. Reason for shifting of the drug peak to lower temperature due to mixing with the excipients that lowers the purity of each component.

The physical mixture of Tezosentan and PVP K 30 (1:1) indicates chemical interaction between these substances due to heating reason, it was concluded that the thermal stability of Tezosentan in these mixture changed, it was established that the PVP K 30 accelerate the thermal decomposition of Tezosentan. Due to this modifications thermal stability of Tezosentan in presence of PVP K 30 was lower.

Generally, the melting peak of Tezosentan was preserved and the enthalpy values are reduced to half, less for the two binary mixtures mentioned. The slight lowering and/or broadening of the melting temperatures, respectively beginning and maximum temperature of decomposition may be attributed to the mixing process, which lower the purity of each component in the mixture. Appreciably decreasing or the absence of the melting temperature, respectively values of ΔH fusion, suggests a process which takes place with low intensity or even disappears (the case of binary mixture Tezosentan – Povidone). A higher value of ΔH fusion shows an overlapping of two processes (the case of binary mixture Tezosentan – PVP K 30, with melting and dehydration). The small variations in the enthalpy's values for the binary mixtures can be attributed to some heterogeneity of in the small samples used for the DSC experiments (3-4 mg). The FT-IR spectroscopy was used as a supplementary technique in order to investigate the possible chemical interaction between drug – excipient and to confirm the results obtained by the thermal analysis. It is the most suitable technique of the non-destructive spectroscopic methods and has become an attractive method in the analysis of pharmaceutical solids, since the material are not subjected to thermal or mechanical energy during samples's preparation, therefore preventing solid-state transformation's. The appearances of new absorption bands, broadening of bands, and alteration in intensity are main characteristics to evidence interactions between drug and excipients. Further it will be presented only the spectra for the cases where the thermal analysis indicates a possible. Interaction namely Tezosentan, Povidone, and the mixture of Tezosentan:Povidone (Fig. 6),

The DSC curves of dug and excipients in the ratio of 1:1 after 4 weeks of storage shows shifting of endothermic peak of drug to 120°C and excipients peak at 128°C. The reason for this behavior could be because of shifting of Tezosentan peak to higher temperature, which could have merged with the moisture loss peak of PVP K 30. The results obtained from the DSC curves for binary mixture are collected in Table 2.

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The main differences resulting from comparing the spectra are presented below. For the binary mixture Tezosentan-Povidone 1:1. The significant decrease (≈20%) of the band attributed to the OH group (3450 cm-1) with the movement of the absorption's maximum at 3614 cm-1;

The bands in the 3000-2800 cm-1 region appears as a broad band with a maximum absorption increased (≈20 %) at 2968 cm-1 accompanied by two shoulders in the right;

The intensity of main absorption bands (1925, 1776, 1690, 1643, 1578, 1502, 1442, 1343, 1253, 1116,1018, 928, 753 cm−1). is maintained.

2968, 1690, 1502, 1116 bands showed alterations in intensity.

The bands in the region from 1502-1643 no peak was observed after 4 weeks of storage.

Two maximums of absorption observed in the region of 1502 and 1116 cm-1.

The bands in the range 1000-1116 cm-1 region become single narrow band shown by 1018 value.

Based on the submitted aspects one can sustained chemical interaction between Tezosentan and Povidone, The main differences observed in the FT-IR spectrum of the binary mixture of Tezosentan and PVP K 30 were:

A significant increase (≈30 %) of the band attributed to the OH group (3631 cm-1), respectively for the bands from 2732 to 2543 cm−1 region (≈45%).

Bands in the region 3090 – 3732 cm−1 appears as a broad band.

The intensity of the absorption bands from (2965, 2875, 1917, 1692, 1560, 1501, 1383, 1343, 1171, 1115, 1019, 755 cm−1) is maintained or increased (≈15%).

On the basis of mentioned differences it may be considered that the Tezosentan interacts with Povidone & PVP K 30 appearance of new band is not possible.Alteration in peak intensities is observed in both Povidone and PVP K 30 physical mixtures. The X-ray powder diffraction pattern has been used for qualitative and quantitative identification of crystallinity in order to investigate the possible interaction of Tezosentan with Povidone and PVP K 30, besides the FT-IR spectroscopy which is a qualitative analysis technique.

The X-ray diffraction patterns of the Tezosentan, Povidone, and Tezosentan-Povidone mixture respectively Tezosentan, PVP K 30 and Tezosentan-PVP K 30 mixture are shown in Fig 8 and 9.

The additional prominent DSC peaks in the mixture of the drugs and excipients are a positive indication of chemical interaction of the drugs with excipients, such interaction should result in the partial or complete disappearances of the reactants and appearances of new phases, which can be inferred from X-ray diffraction patterns, X ray diffraction pattern of the mixture, prerpared at room temprerature, when compared with those of its individual components showed appearance of new lines and disappearance of some of the lines present in the individual components.

The X-ray patterns of Tezosentan-Povidone mixture prepared at room temperature did not show the lines in addition to those present in patterns of the individual components (Fig. 8), however the number of lines present in the XRD patterns of individual components was found missing in the similar pattern recorded for the mixture. The significant difference in the X-ray patterns of the drug-excipient mixtures compared to those of individual drugs and excipient indicates-possible incompatibility of the drugs with the excipients, even at room temperature. The presence of majority of lines of parent substances in the thoroughly ground mixture prepared at room temperature, corresponding to the significant

increase of the peak's intensities indicates the interaction of KT with PVP at room temperature, which could increase with increased temperature.
The diffractrogram of the binary mixture Tezosentan-PVP K 30 (Fig. 9) does not show the appearance of new lines. The same diffractogram indicates disappearance of some of the diffraction lines of higher, moderate and lower intensities in the mixture which are originally present in the X-ray diffraction patterns of the individual components. In the same time, the intensities of the majority peaks are appreciably increased. These differences indicate the interaction of Tezosentan with Povidone at room temperature, which could increased with the increased temperature.

Isothermal stress testing
Degradation studies
The drug-excipient mixtures were prepared in a ratio of 1:1 to accelerate the interaction process. 50 mg of each drug and excipients were accurately weighed and transferred to 15 ml glass vials in triplicate. The mixtures were thoroughly mixed using a capillary tube, which was broken inside the vial. The open vials were exposed to ICH recommended accelerated stability test conditions of 40°C and 75% RH for 4 weeks to calculate the increase in weight (due to absorbed water) during the testing period, parallel studies were also done on pure drug.

Physical changes and chemical analyses by HPLC
The samples were observed visually for any physical change and also analyzed by a validated HPLC method to determine chemical changes the content were dissolved in 50 ml methanol and filtered through 0.22 µ nylon filter before HPLC injection.

Physical and chemical changes
Pure Tezosentan was almost stable on storage for 1 month under accelerated stability test conditions. There was no physical change and chemical change was also very small (≈1.5 %). The decrease of peak area of Tezosentan was associated with appearance of very small peaks of degradation products. (Fig.10). The drug was stable both physically and chemically in the presence of most of the excipients, other than those containing Povidone & PVP K 30. In all the cases, there was no physical change. Also, the decomposition was <3% and very small peaks due to degradation products were seen on enlargement of the chromatograms. In combinations of drugs with povidone and PVP K 30 both physical and chemical changes were observed. No peak was observed in case of PVP K 30 after 4 weeks of storage at accelerated condition.
The formation of wet mass in case of mixture containing Tezosentan-povidone and Tezosentan-PVP K30 was due to moisture gain, which was verified. Through wet gain determinations, wherein an increase ~17mg and ~20mg observed for the two mixtures. As evident from the Fig. 1b,
Tezosentan in the presence of Povidone was converted to single major product (~16%) which was indicated unknown. As shown in Fig: 11 Incompatibility identified by observation of chromatographic changes, compared to the changes in the chromatogram and the recovery of the compound. After appropriate dilutions, samples were analysed using HPLC and drug content determined from the calibration curve prepared within the expected range (1-10 µg/ml). The method was found to be linear within the studied range R^2: 0.9997. As shown in Table. 4 Results of analysis of of IST samples after 4 weeks of storage at stressed condtions shown in Table. 5

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REFERENCES:
1. S. D. Holler, F. Crouch, "Principles of Instrumental Analysis," pp. 44-50, 2009.
2. B.K. Sharma, "Instrumental Methods Of Chemical Analysi".Goel Publication Co., Meerut, vol 25th edition, pp. 3-6, 1983.
3. A.H. Beckett, J.B. Stenlake, "Practical Pharmaceutical Chemistry" CBS Publishers and Distributors, New Delhi, Part-2, pp. 275-288, 2002.
4. M.C. Adeyeye, H.G Brittain, "Drugs and the Pharmaceutical Sciences," Preformulation in Solid Dosage Form Development, vo. 159, Informa Healthcare USA, CRC Press 2008.
5. ICHQ8 Pharmaceutical Development Draft No. 4.3 Dated 18 November 2004.
6. G.G.G. de Oliveira a, H.G. Ferraz a, P. Severino, "Compatibility studies of nevirapinein physical mixtures with excipients for oral HAART," Material Science and Engineering C, pp. 596-602, 2013.
7. R. Chadha, S. Bhandari, "Drug-excipient compatibility screening role of thermoanalytical and spectroscopic

techniques," Journal of Pharmaceutical and Biomedical Analysis, pp. 499-505, 2014.
8. P. Patel, K. Ahir, V. Patel, L.Manani et al, "Drug-excipient compatibility studies first stage for dosage form development," The Pharma Innovation Journal, pp. 14-20, 2015.
9. K. Hotha, S. Chowdhary, V. subramanian, "Drug-excipient interactions, case studies and overview of drug degradation pathways," American Journal of Analytical Chemistry, pp.107-140.
10. R. K. Verma, S. Garg, "Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extrended release formulations," Journal of Pharmaceutical and Biomedical Analysis, pp. 449-458, 2004,
11. V. Kumara, R. P. Shaha, S. Malikb et al., "Compatibility of atenolol with excipients LC–MS/TOF characterization of degradation/interaction products and mechanisms of their formation," Journal of Pharmaceutical and Biomedical Analysis, vol. 49, pp. 880–888, 2009.
12. A.M. Lira, A.A.S. Ara´ujo, I.D.J. Bas´ılio et al., "Compatibility studies of lapchol with pharmaceutical excipients for the development of topical formulations, Thermochimica Acta, pp. 1-6, 2007.
13. S.S. Marc´ılio CunhaFilho, R. M. Pacheco, M. Land, "Compatibility of the antitumoral β-lapachone with different solid dosage forms excipients," Journal of Pharmaceutical and Biomedical Analysis, vol. 45, pp. 590–598, 2007
14. R. K. Verma, S. Garg, "Selection of excipients for extended release formulations of glipizide through drug–excipient compatibility testing." Journal of Pharmaceutical and Biomedical Analysis, vol. 38, pp. 633–644, 2005.
15. K. Liltorp a, T. G Larsenb, B. Willumsenb et al., "Solid state compatibility studies with tablet excipients using non thermal methods." Journal of Pharmaceutical and Biomedical Analysis Journal, pp. 424–428, 2011.
16. I.A.Silvaa, L.C.L. Sá-Barretob, E.M. Limac et al., "Preformulation studies of itraconazole associated with benznidazole and pharmaceutical excipients." Thermochimica Acta. vol. 575, p.p 29– 33, 2014.
17. K. K Berzin¸ A. Actin, "The effect of excipients on the stability and phase transition rate of xylazine hydrochloride and zopiclone." Journal of Pharmaceutical and Biomedical Analysis, vol. 107, pp. 168–174, 2015.
18. M. Tomassettia, A. Catalanib, V. Rossib et al., "Thermal analysis study of the interactions between acetaminophen and excipients in solid dosage forms and in some binary mixtures." Journal of Pharmaceutical and Biomedical Analysis, vol. 37, pp. 949–955, 2005.
19. Y. Ganesh Kumar, J. Sreekanth, D. Satyavati, "Preparation and Evaluation of Sustained Release." Int. J. Pharm. Sci, vol. 26, pp. 155-161, 2015.
20. S.G Raja, G.D Dreyfus, "Current status of Tezosentan for treatment of pulmonary hypertension." Ann. Card. Anaesth, pp. 6-14, 2008.
21. L.J Rubin, "ACCP evidence-based clinical practise guidelines diagnosis and management of pulmonary arterial hypertension," Chest, pp. 1-92, 2010.
22. E. Gabbay, J. Fraser, K. Mc Neil, "Review of Tezosentan in the management of pulomonary arterial hypertension," HealthRisk Management, pp. 87-900, 2007.
23. L.M. Rus, I. Tomuta, C. Iuga, "Compatibility studies of Indapamide/Pharmaceutical Excipients Used in Tablet Formulation," Farmica, vol. 60, p.p 1, 2012.
24. A.B. Ahmed, L. K. Nath, "Drug Excipients Compatibility Studies of Nicorandil in Controlled Release Floating Tablet," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 6, pp. 0975-1491, 2014.
25. S.B Pehlivan, B. Suba, M. Vural et al., "Evaluation Drug Excipient Interaction in the Formulation of Celecoxib Tablets,"Acta Poloniae Pharmaceutica n Drug Research, vol. 68 No. 3 pp. 423-433, 2015.
26. P. Patel, K. Ahir, V. Patel, L. Manani et al., "Drug-Excipient compatibility studies," First step for dosage form development,The Pharma Innovation Journal, vol. 4(5), pp. 14-20, 2018.
27. A. Gupta, H. K. Kar, "Solid State Compatibility Studies of Miconazole Using Thermal and Spectroscopic Methods," Advances in Analytical Chemistry, vol. 5(3), pp. 51-55, 2021.
28. T.A. Júlio1, I.F. Zâmara, J.S. Garcia, et al., "Compatibility and stability of valsartan in a solid pharmaceutical formulation," Brazilian Journal of Pharmaceutical Sciences, vol. 49, n. 4, 2021.