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Co-Crystallization of Glipizide & Rosuvastatin Calcium and it's Characterization

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ABSTRACT

Co-crystals consists of API and a stoichiometric amount of a pharmaceutically acceptable co-crystal former. Pharmaceutical Co-crystals are nonionic supramolecular complexes and can be used to address physical property issues such as solubility, stability and bioavailability in pharmaceutical development without changing the chemical composition of the API. Maximum of the drugs belong to BCS class II, means these are drugs which have low solubility and high permeability. There are various methods of solubility enhancement such as salt formation, solvates, polymorphs, complexation, co-crystallization, etc. Co-crystallization mainly consists of two components the API and the coformer. Thse coformer is the one which acts as a main component for solubility enhancement. In case of ionization or salt formation there is a drawback as compared to co-crystallization. In case, of salt formation or ionization presence of an ionic center is required. This is not a requirement in case of co-crystallization.FDA has approved such combination called Juvisync[1] (Sitagliptin and Simvastatin) in 2011.Glipizide belongs to the anti-diabetic class of drug and Rosuvastatin calcium is a cholesterol reducing agent. As both these drugs are class II drug, solubility issues has to be solved. As Glipizide was available not in its salt form, its co-crystallization was decided to do. Hence, co-crystallization method was selected as the method to enhance the solubility. The co-crystals were characterized and showed the conformance of the presence of both the APIs.

Keywords: Glipizide, Rosuvastatin calcium**,** co-crystals, coformer.

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INTRODUCTION

Co-crystals consists of API and a stoichiometric amount of a pharmaceutically acceptable co-crystal former. Pharmaceutical co-crystals are nonionic supra molecular complexes and can be used to address physical property issues such as solubility, stability and bioavailability in pharmaceutical development without changing the chemical composition of the API. Cocrystal is a crystalline entity formed by two different or more molecular entities where the intermolecular interactions are weak forces like hydrogen bonding and π- π stacking. Co-crystallization alters the molecular interactions and composition of pharmaceutical materials, and is considered better alternative to optimize drug properties. Co-crystals offer a different pathway, where any API regardless of acidic, basic, or ionizable groups, could potentially be co crystallized. This aspect also helps complement existing methods by reintroducing molecules that had limited pharmaceutical profiles based on their nonionizable functional groups.

Glipizide is an anti-diabetic drug and Rosuvastatin calcium is cholesterol reducing agent. The rationale of using this combination was basically to reduce the chances of various cardiovascular disease which follows in patients having diabetes. As both these API's are class two dugs solubility enhancement is the prime hindrance to be overcome. Hence, it was decided to co-crystallize Glipizide and Rosuvastatin calcium by solvent evaporation method.[2]

The article gives the formulation of co-crystals of Glipizide and Rosuvastatin calcium using dichloromethane as the solvent for solvent evaporation. The characterization of co-crystals such as melting point, solubility, Ultraviolet spectra scan, IR, photomicroscopy, X-ray diffraction and DSC were carried out. Pharmaceutical co-crystallization is a reliable method to modify physical and technical properties of drugs.[3]

MATERIALS AND METHODS:

Objective:

The main objective of the study is to prepare co-crystallization of Glipizide and Rosuvastatin calcium and to perform its characterization. The article also gives the idea about the effect of compression force on the dug release. While selecting the method for co- crystallization, the effect of trituration time was also studied.

Experimental Work:

Ratio selection for co-crystal preparation

The minimum starting dose for both Glipizide and Rosuvastatin calcium was found to be 10 mg and hence equal ratio (1:1) was decided to take.

Selection of common solvent

The saturation solubility studies were done with three solvents water, methanol and dichloromethane. The studies were done by adding individual APIs in 10 ml of each solvent.

Method of preparation [4, 5]

1. Slurry Method

This is simple process which includes the addition of crystallization solvent in the API along with its acceptable former. The selection of this process mainly depends upon the physical stability of the crystallization solution to co crystals and its solid former. Both Glipizide and Rosuvastatin were taken in 1:1 ratio and then grinded until the powder appeared uniform. Then drop wise dichloromethane was added until a point where thick slurry of the powder was formed.

2. Solvent drop method

This is a modification of solid grinding technique, where two materials can be grinded by adding a small volume of solvent. As the solvent being added is in very minute quantity which when added acts as a catalyst but does not form a part of the end product. In this method again the ratio of 1:1 was taken grinded and 3-4 drops of dichloromethane was added. The mixture was lubricated with few drops of solvent and was triturated for homogeneous mixture. The samples (10mg) were withdrawn at different time points. The samples were then added in 10ml water and sonicated for half an hour, filtered and diluted (0.1 ml in 10 ml water). Theses samples were then scanned under UV at 240nm and 275 nm to see the drug content of Rosuvastatin and Glipizide respectively.

3. Solvent Evaporation

Solvent evaporation is the conventional method in case of crystallization. In this technique the material is mixed with the common solvent and evaporated completely. In evaporation stage the molecules in the solution are expected to undergo various hydrogen bonding reactions. But in case of co-crystallization which consists of API and conformers solubility of both in the selected solvent plays a great role. In this method, co-crystals were prepared by adding excess solvent. Solvent was evaporated on water bath and the other at room temperature to study the effect of rate and temperature effect on co-crystal formation. On carrying the solubility studies it was found that the solvent evaporation gave the best results.

Characterization of Crystals

The pure APIs and the prepared co-crystals were subjected to physical evaluation like solubility, UV and melting point. The APIs, their crystals and co-crystals were subjected to characterization techniques like IR, DSC, XRD and photomicroscopy.

1. Melting Point

Melting point was carried out in Bellstone Precision Melting Point Apparatus. The samples were filled in the capillaries closed at one end and then places in the three hole of the Teflon lid. The capillaries were placed in a way that they are half dip in the silicon bath. The instrument was switched on which allows the magnetic stirrers to rotate. The heater was adjusted and the melting point was noted. The co-crystals formation was confirmed when the melting point was obtained at the intermediate of the individual melting point of the APIs. The melting point of the co-crystals was carried out at different intervals until it confirms constant value. The melting point of Glipizide, Rosuvastatin calcium and Co-crystals was carried out.

2. Solubility

In order to carry out co-crystallization a common solvent is needed where both the API has solubility. In case of Glipizide and Rosuvastatin calcium 10 mg of individual API was weighed and individual solubility was checked in 10 ml water, methanol and dichloromethane.

3. FT-IR

The infra red spectrum for glipizide, rosuvastatin their cocrystals was recorded between 4000 cm^{-1} to 650 cm^{-1} using KBr pellet method on Perkin Elmer Spectrum RX.1 [6]

4. Wavelength

Both Glipizide and Rosuvastatin were individually scanned in UV spectrophotometer in order to find the maximum absorption wavelength. Once this was obtained the co-crystals were scanned to see whether or not it gives the absorption maxima of both the APIs. If absorption maxima of both the API's were present, indicates that the formed co-crystals have the presence of both Glipizide and Rosuvastatin Calcium without any changes in their structures.

5. Photomicroscopy

Photomicroscopy was carried out to analyze particle size, shape of the drug substance. Few milligrams of crystallized Glipizide and Rosuvastatin and cocrystals were taken individually and it was observed under Motic microscope of DBM series. By Photomicroscopy changes between the APIs and cocrystals were observed with respect to the shape of the particles.

6. Differential Scanning Calorimetry[7]

In order to cross verify the melting points of both the drugs as well as their cocrystals and to record their thermal behavior, thermograms were recorded.

7. X-Ray Diffractometry [8]

To confirm crystalline/amorphous nature of the drugs as well as their cocrystals, X-ray diffraction patterns were recorded.

8. Formulation

To study the effect of compression force on the formed co-crystals, tablets were punched.

Blend for direct compression was prepared by using co-crystals (20 mg) and other excipients (Starch and Lactose Monohydrate) as showed in Table 1. The co-crystals and excipients were thoroughly mixed and passed through 24# ASTM sieve. The tablets were compressed each weighing 150 mg.

Table 1: Formula

Sr. No.	Ingredients	Quantity per Tablet (mg/tab)
Dry Mix		
1	Co-crystals of Glipizide and Rosuvastatin Calcium (1:1)	20
2	Starch	7.50
3	Lactose Monohydrate	122.50
Total weight		150.00

Evaluation of tablets

The tablets were subjected to various quality control tests to see whether they are having the optimum characteristics like uniform weight, friability, disintegration time, hardness and dissolution. It was seen that the tablets passed all the tests as per Indian Pharmacopoeia 2010 (IP).

Assay [8]

Assay of the drug in the formulation was carried out on 10 Tablets as per IP procedure. Powder equivalent to 150 mg was taken in a 100 ml volumetric flask. The powder was dissolved in 50 ml of dichloromethane and kept for sonification for 30 minutes flask. The volume was made up to 100 ml by dichloromethane and labeled as stock solution. Pipette out 0.1 ml of stock solution and make up the volume up to 10 ml. The sample was scanned by using UV spectroscopy method at 275 nm and 240 nm wavelengths for Glipizide and Rosuvastatin calcium respectively.

In-vitro studies

In-vitro studies were done in order to find whether there is any effect of the compression force on the release when the co-crystals are formulated into Tablets or whether the release is hampered by the presence of any other excipients. The dissolution studies were done using USP Apparatus II (Paddle type) in 900 ml for 60 minutes in pH 6.8 phosphate buffer. The temperature was set at 37°C± 2°C with a 75 rpm.

RESULTS AND DISCUSSIONS:

Method of preparation:

Initial trial was taken by solvent drop method. Here the mixture lubricated with few drops of solvent was triturated and samples (10mg) were withdrawn at different time points. The samples were then added in 10ml water and sonicated for an hour, filtered and diluted (0.1 ml in 10 ml water). These samples were then scanned under UV at 240nm and 275 nm to see the drug content of Rosuvastatin calcium and Glipizide respectively. The results obtained are summarized in Figure.1.

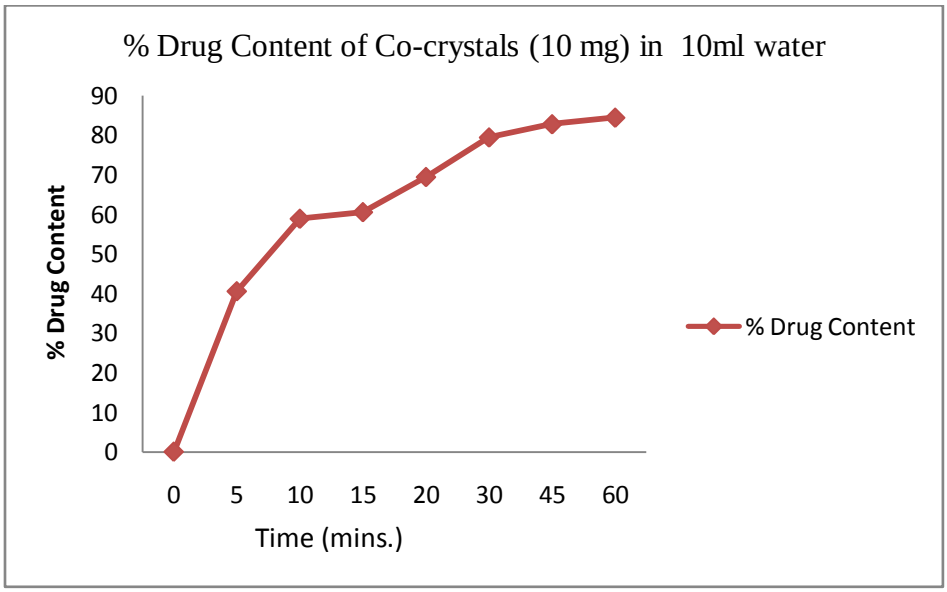

Figure.1: % Drug content of co-crystals in 10 ml water at different time points

Table 3: Selection of method of co-crystal preparation

Method	Saturation solubility (mg/10ml)
Solvent Drop	0.7
Slurry	0.1
Solvent Evaporation (Room Temperature)	0.2
Solvent Evaporation (Water Bath)	1

The co-crystals solubility studies was done in 10 ml water .Solubility studies results of the co-crystals obtained from all the three methods shown in Table No.3 led to the conclusion that the solvent evaporation should be selected as the method for co-crystallization.

Characterization of API and co-crystals

1. Melting Point

Table 4: Melting point of observations

Sr.No.	Condition	Sample	Range (°C)	Observation(°C)
1.	Initial	Glipizide	208-209	205
2.		Rosuvastatin calcium	151-156	154
3.		Co-crystals	151-209	175
4.	Intraday (after every hour for 7 hrs)			180
5.	Interday (after 24 hr)			179

The melting point determination of the co-crystals was carried out at different intervals until it confirms constant value. The melting point initially of Glipizide, Rosuvastatin calcium and Co-crystals was carried out. Table No.4 shows that the melting point of the co-crystals was in between the melting point of two APIs. This may be due to the denser packaging of the co-crystals. In order to achieve constant melting point range and to confirm the complete evaporation of the solvent, melting point was determined at different duration. In intraday the melting point was examined each hour (for 7 hours) and for interday the melting point was checked after 24 hours (next day).

2. Solubility

As a common solvent is needed for co-crystallization, solubility check was carried out. Glipizide and Rosuvastatin calcium 10 mg of individual API was weighed and individual solubility was checked in 10 ml water, methanol and dichloromethane. The observations seen are depicted in Table 5.

Table 5: Solubility Observations

Sr. No.	Solvent used	Glipizide	Rosuvastatin calcium
1.	Water	Insoluble	Soluble (Takes time)
2.	Methanol	Freely soluble	Soluble (Takes time)
3.	Dichloromethane	Freely soluble	Freely soluble

From the solubility studies observation it was decided to use Dichloromethane as the common solvent for co-crystallization for both the APIs.

3. FTIR

Separate IR of Glipizide, Rosuvastatin calcium and co-crystals were taken in order to see the changes in spectrum.

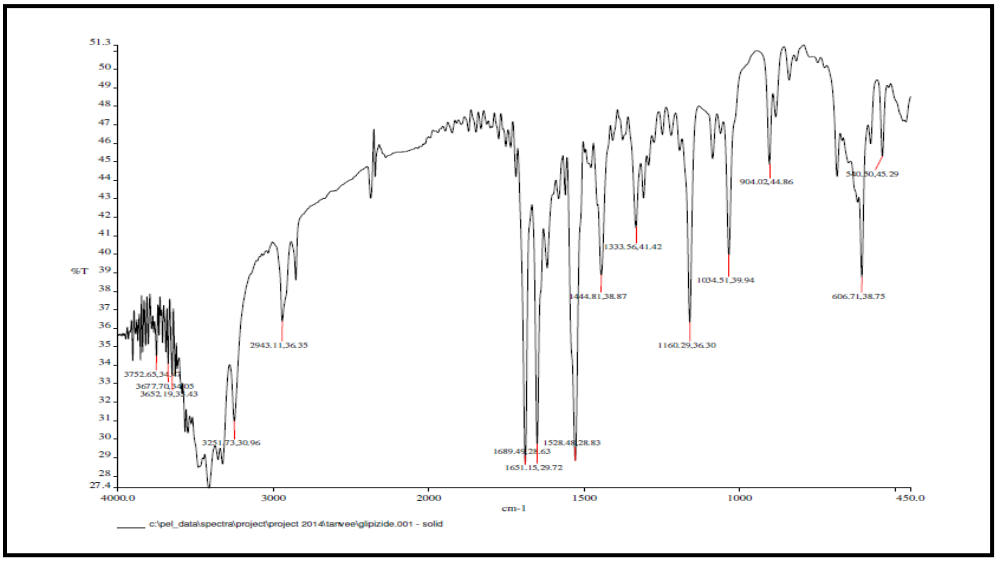

Figure.2: IR Spectra of Glipizide

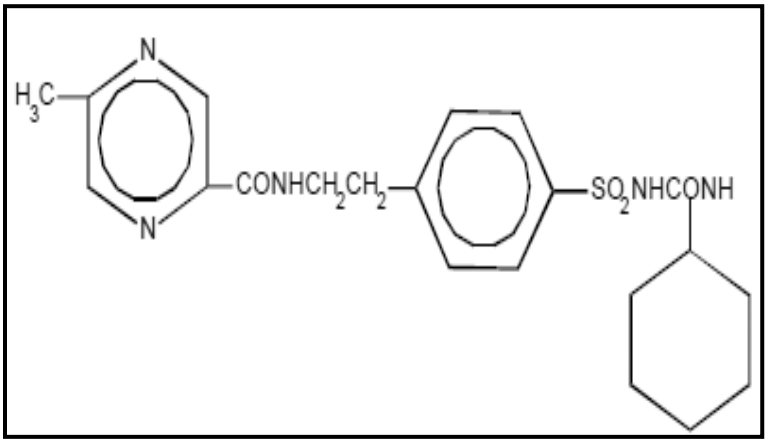

Figure.3: Structure of Glipizide

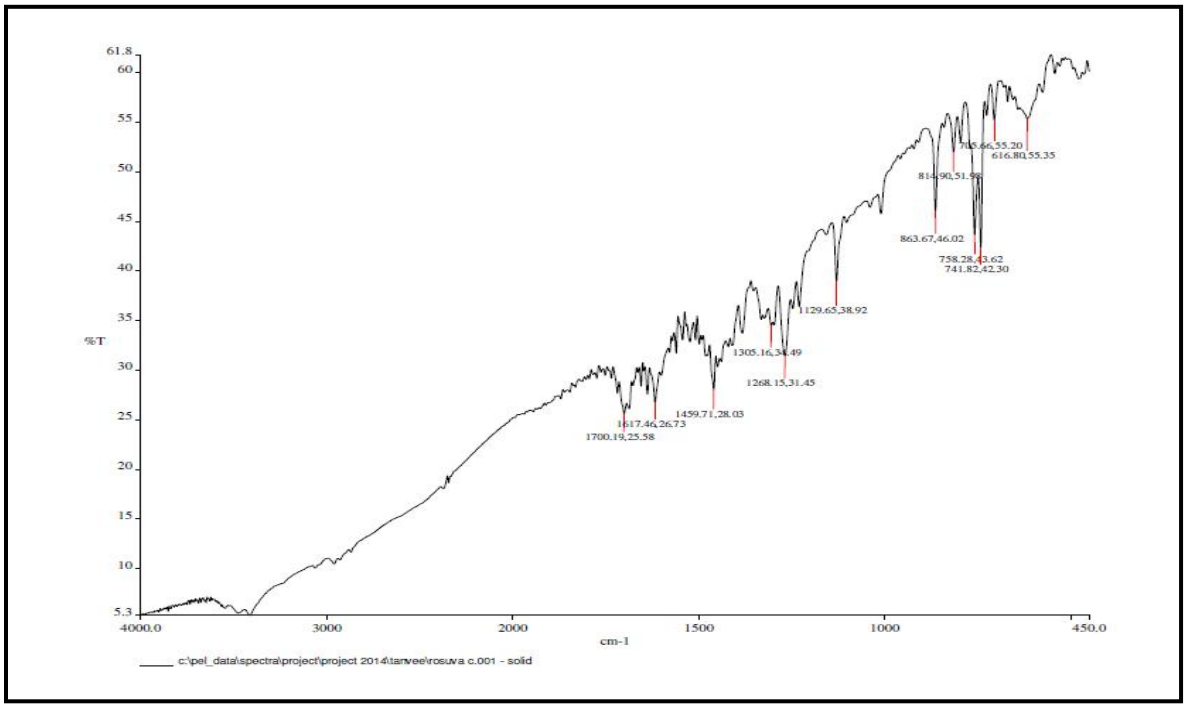

Figure.4: IR Spectra of Rosuvastatin calcium

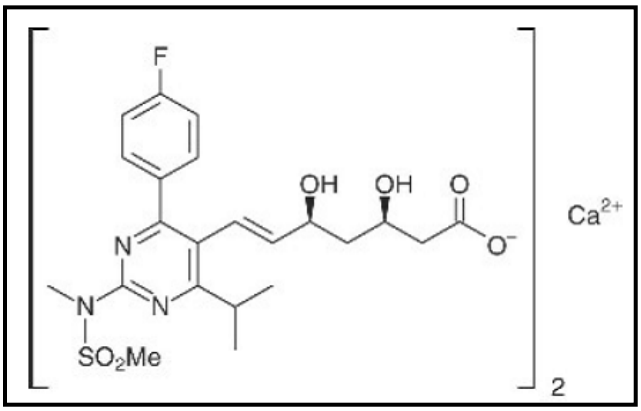

Figure.5: Structure of Rosuvastatin calcium

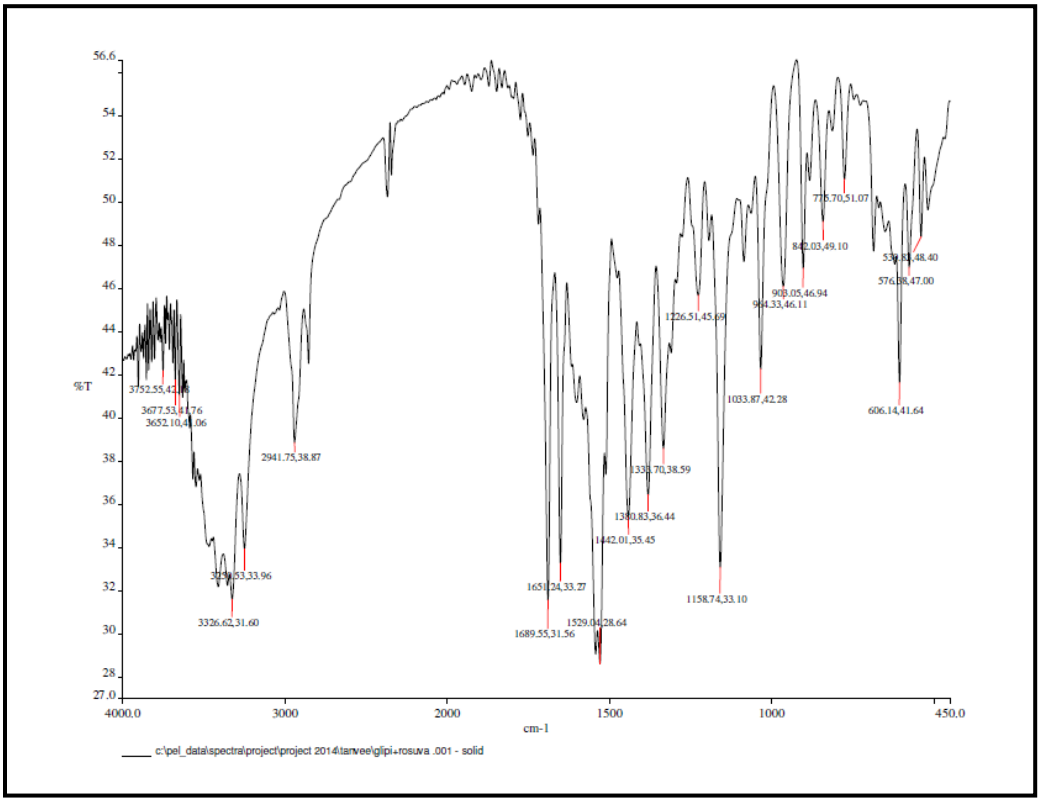

Figure.6: IR Spectra of Co-crystals

Table 8: Predicted functional groups of APIs &co-crystals

Functional Group	Wave number (cm^{-1})	Glipizide	Rosuvastatin	Co-crystals
C=O stretching	1687	Y	-	-
	1550, 1525, 1460	Y	-	-
	1732.13	-	Y	-
	3350-3190	-	-	Y
	1725-1695	-	-	Y
	1665-1635	-	-	Y
N-H Bending /stretching	1649	Y	-	-
	2968.55	-	Y	-
	3450-3330	-	-	Y
	1625-1575	-	-	Y
	1620-1685	-	-	Y
O-H stretching	3387.11		Y	
	3650-3595	-	-	Y
	3510-3200	-	-	Y
Aromatic ester	3500-3300	-	-	Y
C=C stretching	1546.96	-	Y	-

Y: the particular functional group is present

-: the particular functional group is absent

4. Wavelength

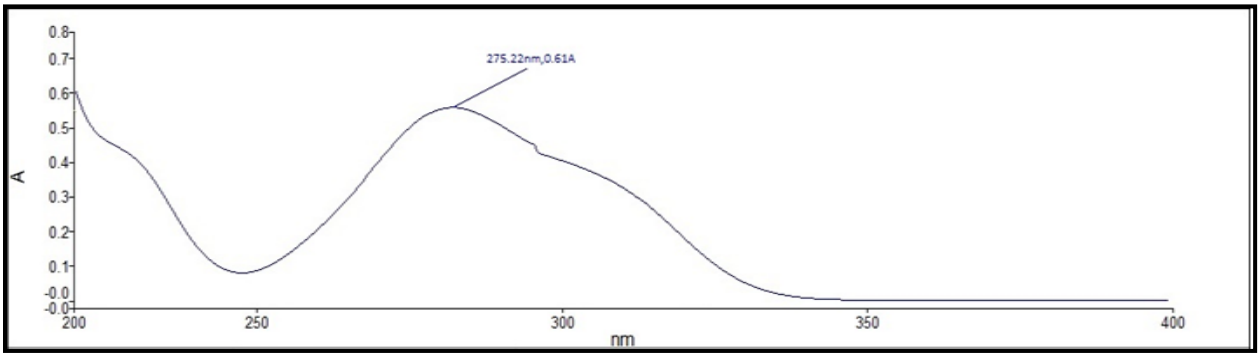

Figure.7: UV Scan for maximum wavelength of Glipizide

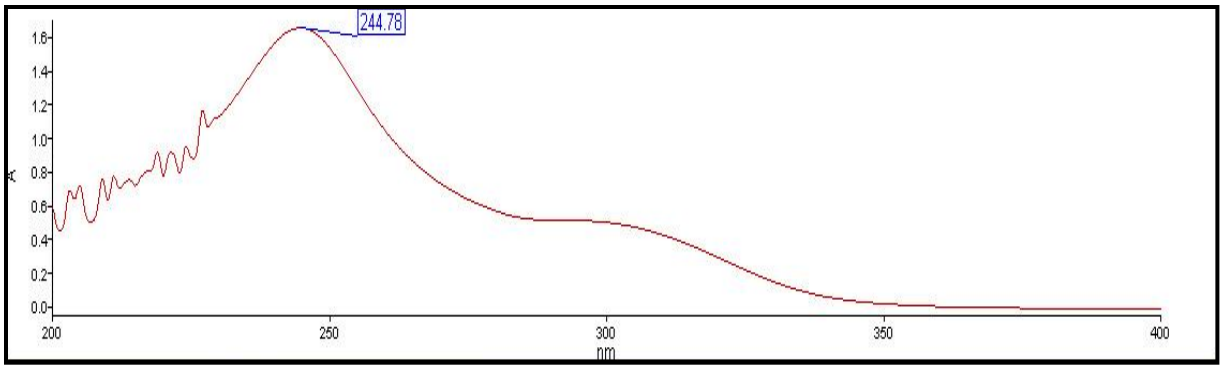

Figure.8: UV Scan for maximum wavelength of Rosuvastatin calcium

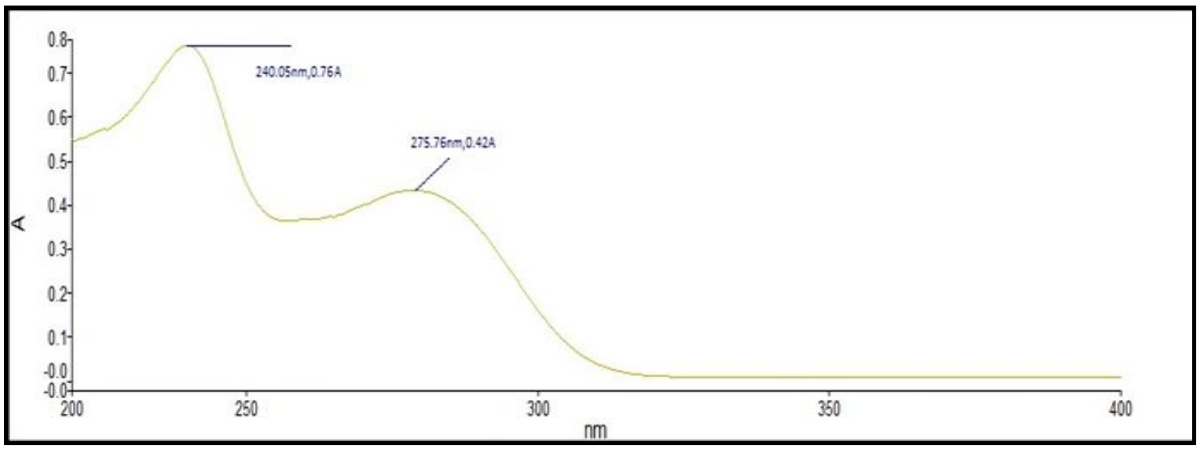

Figure.9: UV Scan for maximum wavelength of Co-crystals

Initially determination of UV absorption maxima for individual APIs were carried out using UV spectroscopy between 200 to 400 nm range. As co-crystal was a mixture of both Glipizide and Rosuvastatin calcium in equal ratio. Hence, the UV scan of the co-crystals gave two maximum wavelengths confirming that the co-crystals have the presence of both APIs.

Glipizide and Rosuvastatin calcium showed the absorption maxima at 275 nm and 244 nm respectively as depicted in Figure.7 and Figure.8 respectively. The co-crystals showed the lambda maxima at 275 nm and 240 nm as shown in Figure.9.

5. Photomicroscopy

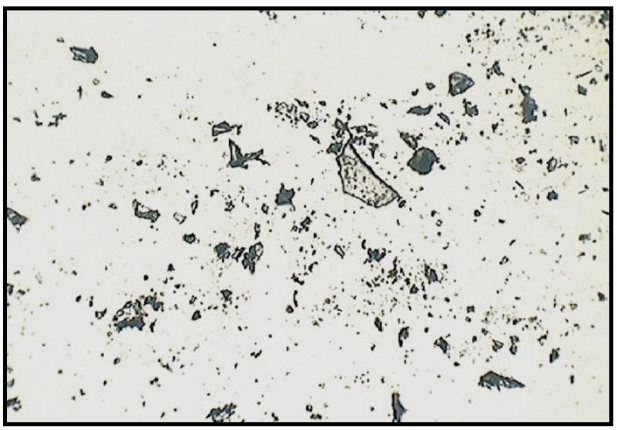

Figure.10: Motic image of Glipizide

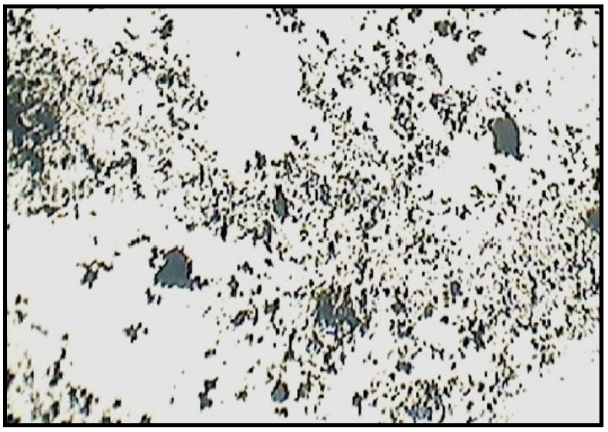

Figure.11: Motic image of Rosuvastatin calcium

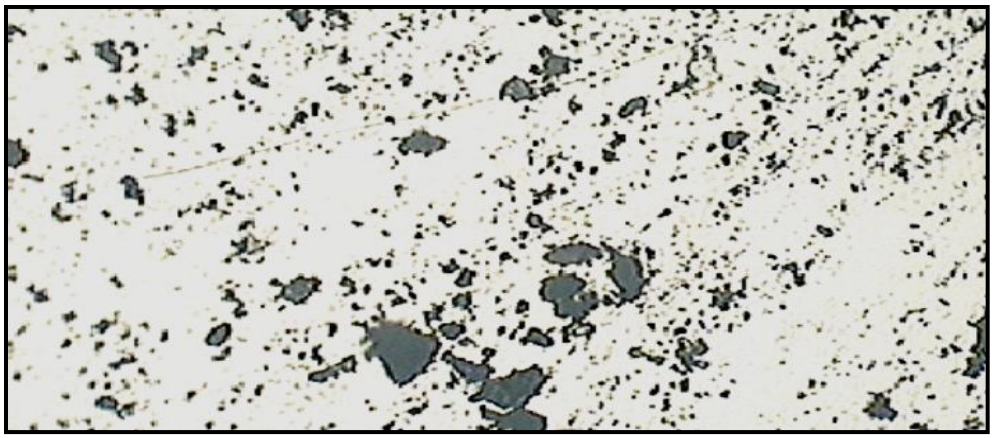

Figure.12: Motic image of Co-crystals

Figure 10 & 11 shows the motic images of Glipizide and Rosuvastatin calcium respectively. The characteristic particles shape and size observed in individual motic images of the APIs can be found in the co-crystal motic image shown in Figure 12.

6. DSC

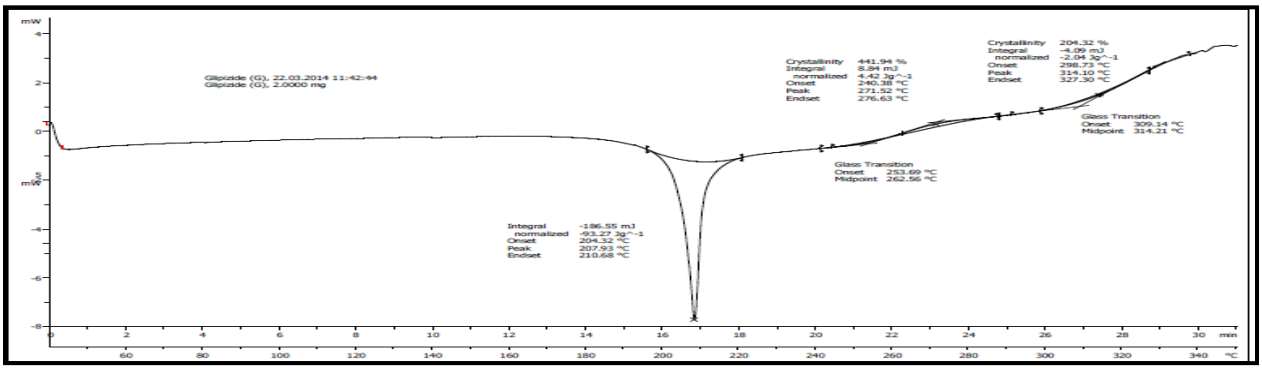

Figure.13: DSC of pure Glipizide API

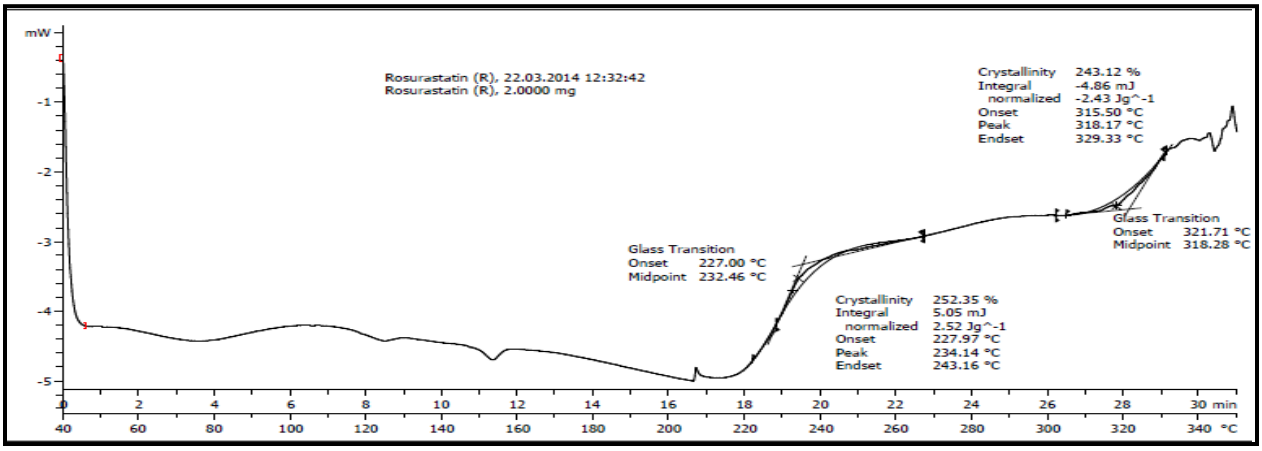

Figure.14: DSC of pure Rosuvastatin calcium API

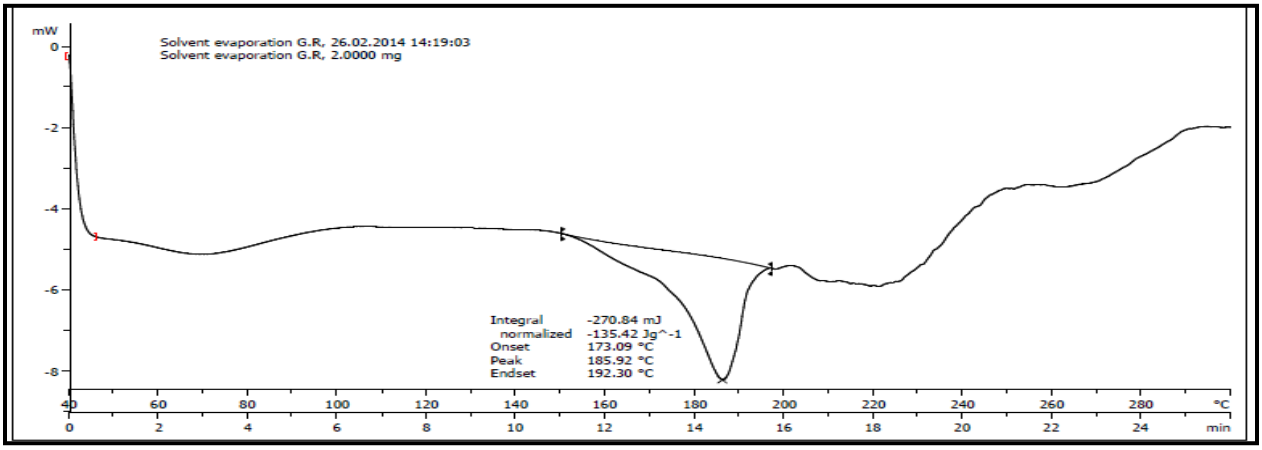

Figure.15: DSC of Co-crystals

Fig 13, 14 and 15 shows the DSC of Glipizide, Rosuvastatin and co-crystals. The endothermic peak is achieved at 207°C and 234°C respectively. The co-crystals 185°C are showing a downward endothermic shift.

7. XRD

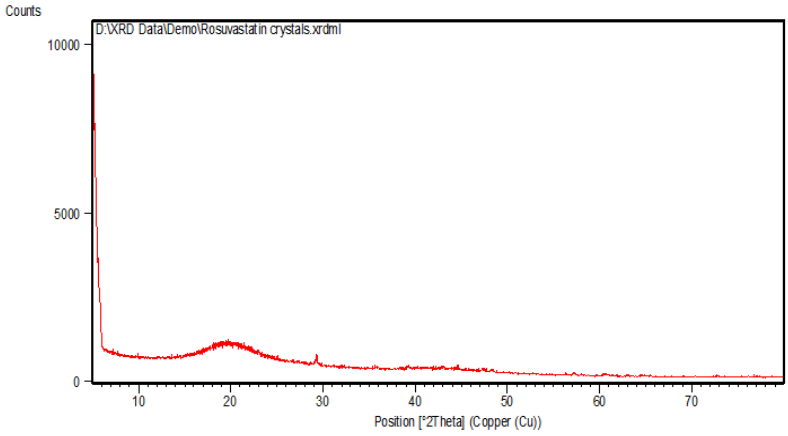

Figure.17: XRD of Rosuvastatin calcium crystals

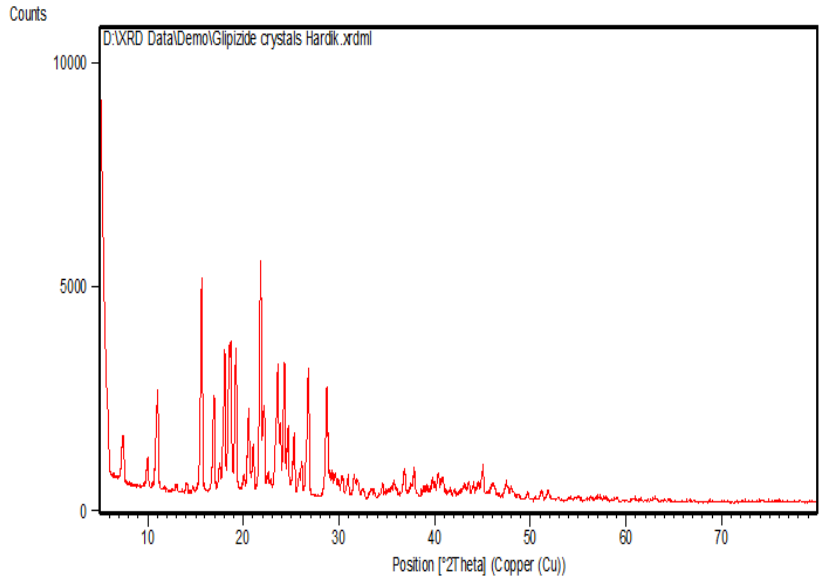

Figure.16: XRD of Glipizide crystals

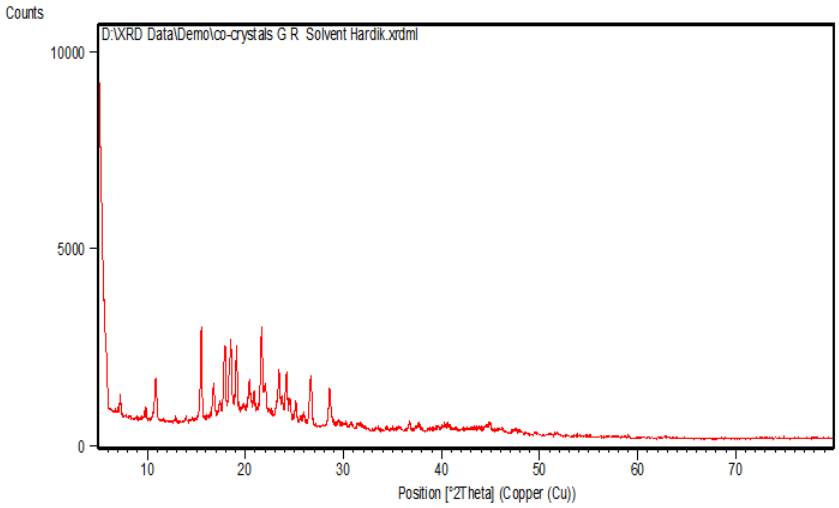

Figure.18: XRD of Co-crystals

Figure 16, 17 and 18 shows the XRD of Rosuvastatin, Glipizide and co-crystals respectively. From the XRD of Rosuvastatin the absence of sharp peaks shows that it has amorphous nature on the other hand in case of Glipizide there are sharp peaks showing the crystalline nature of the API. In case of XRD of co-crystals the peak intensity is decreased as compared to Glipizide. This may be because there is interference in the crystalline property of Glipizide or may be because the end product is amorphous in nature which ultimately gives an idea that the solubility has enhanced.

8. Formulation

IPQC tests were carried out as per IP.

Table 9: Observations of physical evaluation of Tablets

Parameters	Results
Average weight (mg)	150 ± 7.5% (138.75- 161.25)
Diameter (mm)	8
Thickness (mm)	3.15-3.20
Hardness	4-5 Kg/cm2
Friability	0.614 %w/w
Disintegration time	30-35 seconds

Chemical Evaluation of Tablets

Table 10: Assay results

	Glipizide	Rosuvastatin calcium
Standard Range (IP 2007)	90%-110% of the stated amount of API	
Results	98.06 ± 1.5	97.75 ± 1.9

N = 3

In-vitro drug release

Table 11: In-vitro drug release of Glipizide and Rosuvastatin calcium from co-crystals

Time (mins)	Average % release Glipizide	Standard Deviation	Average % release Rosuvastatin calcium	SD
0	0	0	0	0
10	79.94	2.50	80.06	0.86
15	82.99	2.16	81.00	1.69
20	84.79	1.77	83.25	1.49
30	85.07	1.89	88.13	1.41
45	84.33	0.82	90.00	1.69
60	87.60	0.82	89.25	0.86
	n=6		n=6	

Reproducible batch was punched and shown comparable results with initial batch indicating no effects of excipients and operating conditions on release of drugs from tablets

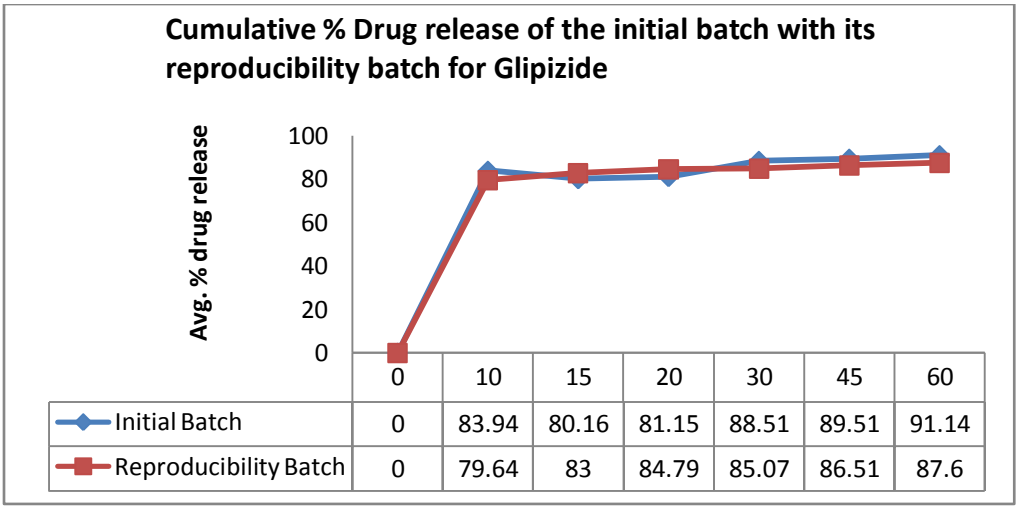

Figure.19: Comparative dissolution profile of initial batch with the reproducibility batch of Glipizide from co-crystals tablet

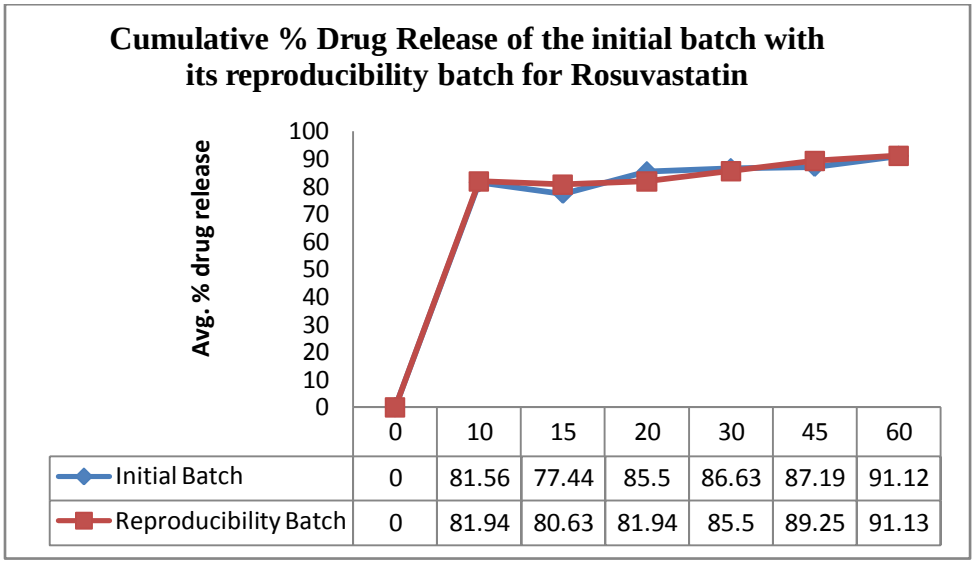

Figure.20: Comparative dissolution profile of initial batch with the reproducibility batch of Rosuvastatin from co-crystals tablet

CONCLUSION

The results of the study revealed that co-crystallization is a promising technique to improve solubility of the drugs. Case to case study needs to be carried out to check the effect of operation parameters to convert co-crystals in to formulation. As the methods employed for co-crystallization are same as that of solid dispersion or solid solutions, careful characterization of co-crystals is highly required.

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