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INFLUENCE OF NATURAL STABILIZERS ON ESOMEPRAZOLE NANOCRYSTALS PREPARED BY ANTISOLVENT PRECIPITATION METHOD FOR ENHANCED ORAL BIOAVAILABILITY

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ABSTRACT

Esomeprazole is a low-aqueous solubility proton pump inhibitor that also leads to a low oral bioavailability, solubility and dissolution rate to maximize therapeutic efficacy.The antisolvent precipitation method was used to prepare nanocrystals of esomeprazole using several stabilizers such as guar gum, karaya gum, and polyethylene glycol 400 (PEG 400). Eight formulations (F1-F8) had been formulated. The formulations were compared on the basis of size of particle, content uniformity and in vitro drug release. The optimized formulation along with drug and polymers were subjected to Characterizations studies. Results:This optimized formulation (F8) showed a 99 percent drug release in 5 minutes, much higher compared to pure drug. The FTIR test was done to prove the lack of drug stabilizer interactions and the excipient compatibility. The DSC results showed a decrease in crystallinity indicating the formation of nanocrystals. Improved dissolution was credited to smaller particle size, greater surface area and good stabilization.Conclusion:Antisolvent precipitation is an efficient and easy process to synthesize stable esomeprazole nanocrystals with increased dissolution rates, which may lead to an improved oral bioavailability.

Keywords: Esomeprazole, Nanocrystals, Antisolvent Precipitation, Guar gum, Karaya Gum and polyethylene glycol 400, FTIR, DSC, Dissolution Enhancement.

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Introduction

The most favored route of drug delivery is by mouth which is convenient, prevents lack of patient compliance and is cheap. Nevertheless, poor aqueous solubility is one of the safest factors to limit an agent oral bioavailability and, in particular, this phenomenon is very frequent among Class II drugs, are extremely permeable and poorly soluble in water.[1] esomeprazole magnesium is widely used to treat acid-related gastrointestinal conditions such as Zollinger-Ellison syndrome, GERD, and erosive oesophagitis. Although esomeprazole is clinically effective, it has poor aqueous solubility and is also not stable in acidic conditions hence it has patchy oral bioavailability and delayed commencement of its therapeutic action [2, 3].

The drug nanocrystals are the crystals of the drug materials, which are pure drug materials of a nanometer size which are generally stabilized by the polymers or the surfactants. [4] They provide a high surface area and high velocity of dissolution as stipulated in the Noyes Whitney equation since they are at nanosize and therefore drugs are released quickly and more absorbed. [5]

The antisolvent precipitation technique, although not the only method employed in producing nanocrystals, has been the method of choice that is highly popular because of its ease of use, scalability and production of uniform nanocrystals which does not involve expensive machines.[6] In such approach, a drug is emulsified in an appropriate given solution, undergoes by its rapid addition to a miscible antisolvent in which it is supersaturated due to low solubility and, as a result, it leads to prompt nucleation/growth of nanocrystals.[7,8] The solvent to antisolvent ratio, the rate and degree of crystallization are dependent on the stirring rate and existence of stabilizers.

The stabilizers need to be applied in order to avoid aggregation and provide the physical stability to nanocrystals during and after their synthesis. The common stabilizing agents used are surfactants and hydrophilic polymers like Guar gum, Karaya Gum, and polyethylene glycol 400 (PEG 400). These excipients prevent the agglomeration of the particles and they preserve the nanofunctions. [9, 10] Also, they improve wettability and increase better dissolution properties.

Eight formulations of esomeprazole nanocrystals were prepared in the present

study by the antisolvent precipitation method by varying the use of Guar gum, Karaya Gum and polyethylene glycol 400 (PEG 400) as the stabilizers. The overall aim was to have an optimal formulation that would maximize the dissolution. The formulations were tested on the particle size, drug release, and physicochemical interaction. Some of the formulae were optimised and showed high-rate drug release, with maximum release of 99% in 5 minutes, which is a sign of great increase in dissolution rates in comparison to pure esomeprazole.

To further support the physical stability and compatibility between the drug and the chosen excipients, FTIR and DSC analyses were done. FTIR analyses further established that there were no major drug-excipient interactions, which implies that they are chemically compatible, whereas DSC thermograms revealed that the drug has lost some of its crystallinity, which possibly will improve its dissolution profile additionally.[11,12] It is often seen that amorphous or semi-crystalline forms with the higher energy content are less solid and more soluble compared to their crystalline counterparts due to reduced crystallinity .[13]

These results indicate that the antisolvent precipitation technique, together with the appropriate stabilizers has a good potential in the development of stable nanocrystals of esomeprazole that could dissolve faster with possible increase of bioavailability. Further, this type of improvement might enable them to be used in lower doses and at a quicker rate of action and improved therapeutic results.[14]

Methodology

Esomeprazole a gift sample was purchased in Bangalore at Harmoni Analyticals Private limited Ltd. S.D Fine Chem, Ltd., Mumbai produced Guar gum, Karaya Gum, and polyethylene glycol 400 (PEG 400) on commercial basis.

Saturated solubility studies of Esomeprazole

Esomeprazole was tested using dissolution media with regard to solubility. The accurate quantity of 10 mg of weighed drug was added individually in 10 mL aliquots of different media which included distilled water, 0.1N hydrochloric acid, and phosphate buffers at different pH values of 6.8 and 7.2 in separate conical flasks. Flasks were sealed tightly and incubated on a

REMI shaker at 50 rpm and 37ºC and 24 hours was used to get them to saturation. The solutions were filtered using Whatman filter paper to eliminate the un-dissolved particles after incubation. The clean filtrates were diluted in the corresponding media and then the absorbance was determined at 296 nm against media as blanks.[15]

Method of Preparation

Preparation of organic phase:

- Weigh accurately esomeprazole, dissolve it in ethanol: DCM in a 1:1 ratio with constant stirring.
- Add to this solution, Gaur gum, Karaya Gum and PEG 400 separately.
- Stir until a clear homogenous solution is achieved.

Preparation of aqueous phase (Antisolvent):

- Make an aqueous phase consisting of distilled water and SLS.
- The aqueous solution should be stirred and Gaurgum and Karaya gum thoroughly dissolved to obtain a viscous medium.

Antisolvent precipitation:

- Organic phase is drop wise added to the aqueous phase under high speed or ultrasonic magnetic stirrer.
- Precipitation of nanocrystals is because of the decreased solubility of Esomeprazole to the antisolvent.

Stirring and homogenization:

- Keep on stirring the resulting dispersion on average 1- 2 hrs to obtain uniform particle formation and stabilization.
- Optional, reduce particle size by probe sonication or high pressure homogenization addition to block aggregation.

Solvent removal:

- Traces of organic solvents may be removed by either rotary evaporation or overnight, leaving solvent in a fume hood.

Drying:

- These nanocrystal dispersions may be freeze-dried or spray-dried to dry nanocrystals which may be further formulated or characterized.

Table 1. Composition of Esomeprazole nanocrystals by antisolvent precipitation technique

S.no	Ingredients	Formulation								
		PD	F1	F2	F3	F4	F5	F6	F7	F8
1	Esomeprazole	40	40	40	40	40	40	40	40	40
2	SLS (%)	-	1	1	1	1	1	1	1	1
3	Guar gum	-	--	1	2	-	-	-	-	1
4	PEG 400	-	-	-	-	1	2	-	-	1
5	Karaya gum	-	-	-	-	-	-	1	2	1
6	Ethanol: Dichloromethane	1	1	1	1	1	1	1	1	1
7	Water	1	1	1	1	1	1	1	1	1

Evaluation of Esomeprazole nanocrystals

Physical properties of Esomeprazole nanocrystals were measured according to the requirements of the official compendia. The respective findings are noted in Table 2.

Table 2. Physical parameters of Esomeprazole nanocrystals

S.NO	Formulation	Angle of Repose (º)	Carr's index (%)	Particle size (nm)	Drug Content (mg)
1.	PD	22.52	15.39	120	37.33
2.	F1	23.67	14.22	133	38.25

3.	F2	24.36	16.39	145	39.65
4.	F3	22.54	13.87	147	39.14
5.	F4	23.14	12.02	166	38.69
6.	F5	23.59	14.88	188	38.96
7.	F6	24.17	13.69	195	39.00
8.	F7	22.39	12.39	190	39.24
9.	F8	24.85	15.26	210	39.87

Estimation of Esomeprazole nanocrystals

A weighed amount of Esomeprazole nanocrystals which is 10 mg of drug was withdrawn. It was dissolved in phosphate buffer pH 6.8, and a sonication 10-15 minutes was done, to facilitate the solubilization of the drug. The dispersion was centrifuged at 10,000 rpm and 10 min or passed through the Whatman No. 1 filter paper to isolate undissolved excipients or particles. This supernatant or filtrate was diluted sufficiently with phosphate buffer of pH 6.8 to range constant. Absorbance of the final solution, at 296 nm, was measured on a UV-visible method, using pH 6.8 buffer as the blank. Quantitation of the drug was done by reference to a previously prepared calibration curve of esomeprazole in similar medium.

***Invitro* drug release studies of Esomeprazole nanocrystals**

Sample loading:

The labeled amount of esomeprazole nanocrystals was prepared by weighing a number of the particles into the dissolution vessel and adding 900 mL of phosphate buffer pH 6.8 to the vessel. The medium was prewarmed and this was adjusted to 37+ 0.5^0C after the sample has been added.

Dissolution run:

A 50 rpm was applied on the paddle, and the nanocrystals were left to dissolve under sink. After predetermined time intervals up to 20 minutes a syringe with 0.45 m filter was used to decant undissolved particles.

Sample replacement:

An amount of fresh buffer was added to bring the volume back up (5 mL) after every withdrawal so that the volumes were kept constant.

Drug estimation:

The samples withdrawn were estimated in the double beam spectrophotometer (ELICO SL-210) at 296 nm. A pre-existing calibration graph of esomeprazole, which was made using dissolution media pH 6.8, was used in determining the concentration of esomeprazole in each sampled material.

Data analysis:

The dissolved percentage of drug with respect to time was summed up and the result was plotted as a profile of dissolution.

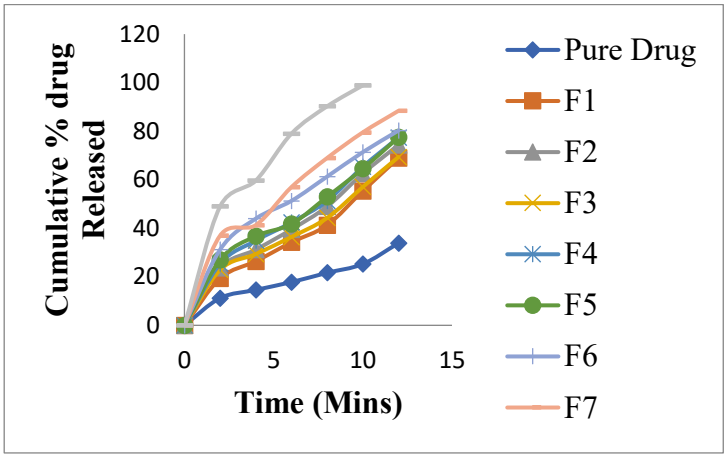

Fig. 1. Drug Release profiles of Esomeprazole nanocrystals

Table 3. *Invitro* dissolution parameters of Esomeprazole nanaocrytals

Formulation	T $_{50}$ (Mins)	T $_{90}$ (Mins)	K (Min^{-1})	R^2
PD	>15	> 15	0.0245	0.911
F1	9	14.5	0.0325	0.942
F2	8.5	14	0.0374	0.954
F3	8.5	13.5	0.0412	0.956
F4	7.8	14	0.0589	0.974
F5	7.4	13.5	0.0662	0.966
F6	5.8	13.3	0.0711	0.979
F7	5.5	10.5	0.0458	0.985
F8	3.5	8	0.0569	0.999

Characterization of Esomeprazole nanocrystals

Characterization Studies may be undertaken

by taking Stabilizers pure drug (Esomeprazole) pure stabilizers were taken in following forms i.e., Gaur gum and Karaya Gum were taken and characterized by FTIR studies / DSC studies.

FTIR

The FTIR of pure drug Esomeprazole, stabilizers used Gaur gum, Karaya Gum and formulation optimized (F8) were recorded using Brucker FTIR spectrophotometer. KBr-disks (a sample of 2 mg in 200 mg KBr) were used as samples and 400-4000 cm-1 with resolution of 1 cm-1 sampling range were noted. Figures 2 to 4 reflected the FTIR spectra.

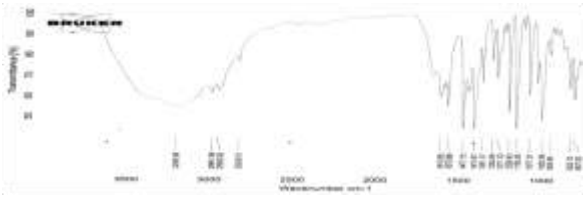

Fig. 2. Esomeprazole pure drug

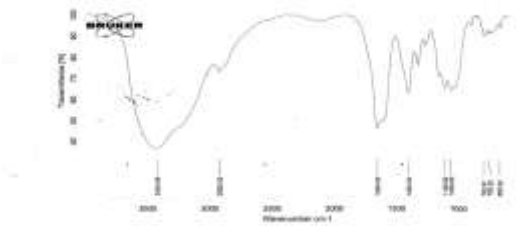

Fig. 3. Guar gum

Fig. 4. Karaya gum

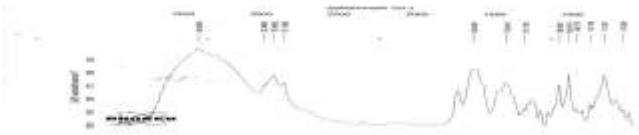

Fig.5. Optimized Formulation (F8)

DSC:

It is used to measure the amounts of the pure drug esomeprazole, stabilisers such as Gaur gum and Karaya gum, and formulation optimized (F8). The 10 mg samples were placed in a sealed aluminium crucible and heated at a rate of 20.59 C/min while maintaining a temperature differential of 25-250. 59^{0}C. they were displayed in numbers six through nine after DSC thermograms were recorded. Table 5 and Figure 6 to 9 were showed.

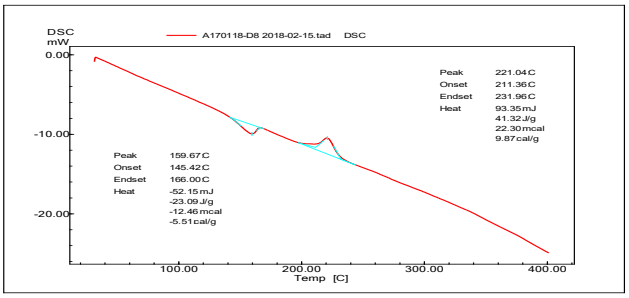

Fig. 6. Esomeprazole pure drug

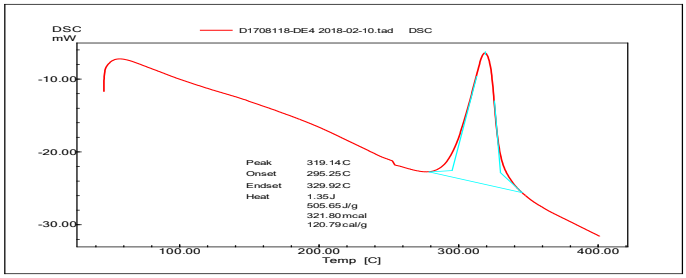

Fig.7. Guar gum

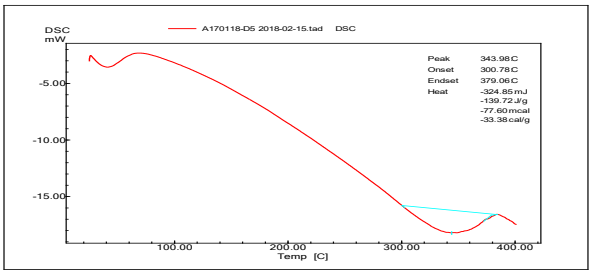

Fig. 8. Karaya gum

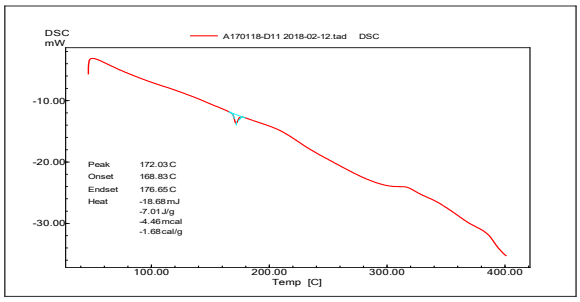

Fig. 9. Optimized formulation (F8)

Table 5. DSC Thermogram of Esomeprazole

Esomeprazole	Guar gum	Karaya gum	Optimized Formulation F8
160.75^{0}C & 231.1^0 C	200^0·07C	160.22^{0}C	168.44^{0}C
Broad Endothermic Peak	Broad Endothermic Peak	Broad Exothermic Peak	Sharp Endothermic Peak

Results

Selection of optimal dissolution medium based on pH solubility profile

Esomeprazole was most soluble in phosphate buffer pH 6.8 followed by pH 7.2 buffer (0.18 mg/mL) and distilled water (0.04 mg/mL), and least soluble in 0.1N HCl (0.01 mg/mL).

Role of SLS, Guar Gum, Karaya Gum and PEG in stabilizing Esomeprazole nanocrystals

Nanocrystals were prepared by means of antisolvent precipitation (eight different formulations F1-F8) with various stabilizers. Formulation F8 which included a mixture of 1% SLS, Guar gum and PEG exhibited the most solubility, stability, less aggregation, and enhanced physicochemical properties.

Assessment of flowability, compressibility, and drug content

Physical characteristics of esomeprazole nanocrystals were tested and compared with the characteristic of the pure drug. The angle of repose of the pure drug provided a satisfactory result of 22.52 degree implying

that the flowability of the pure drug was good. Angles of repose of nanocrystals, on the other hand, were slightly high, 23.67-24.36 as compared to the pure drug but still within acceptable limits indicating that the nanocrystals have good flow characteristics.

The Carr percent of the pure Esomeprazole was found to be 15.39 but the nanocrystals were found to lie between the range of 14.22 to 15.26 percent. These values allude to small change in compressibility and good flow and packing properties in general of the nanocrystal formulation.

The particle size measurement showed there was a substantial decrease in the particle size of the pure drug which was 120 nm to the nanocrystals which were 110nm to 133nm. This decrease in size is proof that nanosizing has been successful, which is good in the drug to increase the rate of its dissolution and bioavailability.

Concerning drug content, the pure Esomeprazole had a drug content measuring 37.33 mg as compared to the nanocrystal formulations that revealed a range between 37.33 mg and 39.87 mg. Such consistents of drug loading is an indication of the uniformity of the formulation and promising that the drug was entrapped well into the nanocrystals.

Comparative drug release kinetics (F1–F8)

Overall, the evaluated parameters demonstrate that Esomeprazole nanocrystals possess improved or comparable physical characteristics to the pure drug, making them a promising candidate for enhanced pharmaceutical performance. The in vitro drug release profiles of Esomeprazole from various nanocrystal formulations (F1–F8) were evaluated and compared to the pure drug (PD). The pure drug exhibited a prolonged release with T_{50} and T_{90} values greater than 15 minutes, a low release rate constant (K) of 0.0245 min^{-1}, and a correlation coefficient (R^2) of 0.911, indicating slower and less predictable release kinetics.

Formulation F1 showed a T_{50} of 9 minutes and T_{90} of 14.5 minutes, with a moderate increase in K (0.0325 min^{-1}) and R^2 of 0.942. A gradual improvement in release was observed across formulations F2 to F6, with decreasing T_{50} and T_{90} values and increasing K values, signifying faster drug release. Specifically, F6 demonstrated a T_{50} of 5.8 minutes, T_{90} of 13.3 minutes, and the highest K value of 0.0711 min^{-1} among the first six formulations, with an R^2 of 0.979, indicating good linearity.

Formulation F7 further reduced T_{90} to 10.5 minutes with a T_{50} of 5.5 minutes, while maintaining a high R^2 of 0.985, suggesting a reliable and faster release profile. Notably, F8 exhibited the most rapid release kinetics with a T_{50} of 3.5 minutes, T_{90} of 8 minutes, and a K value of 0.0569 min^{-1}, along with the highest R^2 value of 0.999, indicating near-perfect linearity and predictability in drug release behavior.

Spectroscopic analysis (FTIR)

The shift of N-H stretching band of 3102 cm^{-1} (pure drug) to F8 3259 cm^{-1} demonstrated the presence of hydrogen bonding or physical bonding between the drug and polymers. A slight shift of 2856 cm^{-1} to 2874 cm^{-1} in C=C in F8 indicated the alteration of molecular environment of aromatic structures, which was plausibly due to the embedding of the drug in polymer. The O-C stretching went downward in value, with the pure drug 2785 cm^{-1} to 2630 cm^{-1} in the F8 and inter-molecular interactions existed, possibly entrapment of the drug in its polymeric basis. A significant movement to the upside of the O-C -H stretching mode of 1485 cm^{-1} in the pure drug to 1455 cm^{-1} in the F8 describes compatibility of the components. F8 shows a drastic change between the S=O stretch at 1485 cm^{-1} and 1233 cm^{-1} indicating intermolecular interaction and stabilization of sulfoxide subunit in the nanocrystal network. The FTIR spectral shifts present in the optimized form (F8) evidences that no chemical incompatibility has occurred, and that there exist physical interactions between Esomeprazole and the excipients. These interactions play a very important role in the stability and performance of formulation.

Thermal Behavior and Solid-State Transformation in Esomeprazole Nanocrystals

The pure Esomeprazole revealed two broad endothermic peaks of 160.75 ^0C and 231.10 ^0C which were characteristic of a crystalline substance and melting behavior. Guar gum and Karaya gum revealed broad transition, characteristic of amorphous and semi-crystalline materials respectively. F8 exhibited sharp endothermic transition at 168.44 ^0C with complete absence of original Esomeprazole transitions.

Discussion

The solubility profile indicated that esomeprazole is acid-labile and is therefore very unstable in acidic media where solubility decreases and the solution turns

yellow. Better solubility at pH 6.8 and 7.2 indicates that ionization at close to the pKa of the drug (about 4.0) promotes dissolution and thus phosphate buffer PH 6.8 is the most appropriate solvent to be used in dissolution studies. The stabilizers had complementary functions in the stability of nanocrystals. An anionic surfactant, SLS, decreased the interfacial tension and inhibited agglomeration. Guar gum was used to stabilise and PEG was used to promote hydrophilicity and dispersion. Their combination in F8 resulted in an improved stabilization and increase in solubility. The flowability and compressibility tests ensured that nanocrystals had retained good powder characteristics like the pure drug and could be handled easily and further developed into a formulation. Small size of particles and uniform formulations indicated low dissolution rate in nanocrystals based on the fixed change in drug concentration. Kinetics of drug release indicated that nanocrystals released esomeprazole more quickly than the solitary drug and that F8 exhibited the quickest and the most predictable release (highest R2 value). The greater the dissolution rates are, the greater the bioavailability. FTIR showed the presence of physical interactions between the drug and stabilizers, including hydrogen bonds and polymer inclusion, but nothing to suggest that the two were chemically incompatible. The interactions increased the stability of the nanocrystals. DSC thermograms indicated a loss of crystalline drug peaks in F8, which indicated that the drug was partially amorphized using a solvent with high solubility. The observations, in their totality, confirm that optimized nanocrystal formulation F8 was able not only to provide a mechanism of enhanced solubility and a higher dissolution rate but also permitted stability in the presence of physical contact with stabilizers, providing a promising approach to restoring the pharmaceutical activity of esomeprazole.

Conclusion

The current concludes that could develop Esomeprazole nanocrystals using the antisolvent precipitation method to help boost the solubility and dissolution in vitro. The solubility experiments confirmed the phosphate buffer of pH 6.8 as the most appropriate medium to employ since Esomeprazole is a weekly basic medication. Among the eight formulations, that contain SLS, Guar gum, and PEG one formulation (F8) permitted the presence of smaller particles (110-133 nm), excellent flowable properties, and good drug content (up to

39.87 mg). Remarkable increase in the level of in vitro drug release was also observed with T 50 of 3.5 minutes, T90 of 8 minutes and R2 = 0.999 indicating that release is rapid and predictable. The FTIR analysis showed no chemical interaction, but DSC analysis of the results showed reduced crystallinity that was more stable and soluble. Overall Formulation F8 was the most successful in improving the pharmaceutical characteristics of Esomeprazole and warrants further investigation as a way of developing a stable and bioavailable oral dosage form.

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