# Enhancement of the Dissolution Rate and Bioavailability of Glipizide through Cyclodextrin Inclusion Complex

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**Glipizide (GZ) is a widely used oral hypoglycemic drug with very poor water solubility. The purpose of this study was to enhance the dissolution rate and bioavailability of this drug. To study the in vivo bioavailability, three formulations were administered in suspension form to three groups of mice. The results revealed a significant decrease in glucose levels with the formulation containing the GZ–β-CD complex with NaCMC. Also, a good in vivo–in vitro correlation was detected among the three formulations.**

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Glipizide (GZ) is a second-generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically perscribed to treat non-insulin-dependent diabetes mellitus. The drug is insoluble in water, and its dissolution is considered to be a rate-determining step (i.e., an effective factor) in its absorption from the gastrointestinal fluids (1).

Cyclodextrins (CDs) form a group of structurally related oligosaccharides with cylinder-shaped cavities that have the capacity to form inclusion complexes with many drugs by taking a whole drug molecule, or a part of it, into the cavity (2,3). CDs have widespread pharmaceutical applications mainly because of their effect on enhancing the solubility and bioavailability of many drug formulations. The interaction of ketoprofen and ibuprofen with β-CD in solution and in a solid state has been studied by Mura et al. (4). The nuclear magnetic resonance of the inclusion complexation of gliclazide with β-CD and the enhancement of its aqueous solubility also has been investigated (5,6). Many other drugs have been tested for CD inclusion to enhance solubility such as bropirimine, ibuprofen, tolbutamide, and doxorubicin and daunorubicin (7–10). The improved bioavailability of many drugs complexed with CDs has been documented in several articles (11–16).

The purpose of this study was to characterize the interaction between GZ and α- and β-CDs, to study the effect of the CDs on GZ's solubility, and to elucidate the effect of some additives on the drug dissolution rate. The authors also studied the influence of three tablet formulations, containing pure GZ, GZ–β-CD, and GZ–β-CD with sodium carboxymethyl cellulose (NaCMC), on the blood glucose levels of mice.

## Materials

The authors obtained GZ (Dar Al-Dawa Pharmaceutical Manufacturing Co. Ltd., Amman, Jordan), α- and β-CD hydrate (Acros Organics, Morris Plains, NJ), and NaCMC (BDH Chemicals Ltd., Poole, UK). All other chemicals used were analytical reagent grade.

**Table I: Composition of the prepared GZ tablets.**

| Materials | Control (pure GZ) | GZ–β-CD | GZ–β-CD + NaCMC |
|---|---|---|---|
| GZ | 5 | 25.47 (equivalent to 5 mg GZ) | 31.75 (equivalent to 5 mg GZ) |
| Anhydrous lactose | 72 | 51.53 | 45.25 |
| Avicel PH 102 | 120 | 120 | 120 |
| Aerosil | 1 | 1 | 1 |
| Talc | 2 | 2 | 2 |

**Table II: Physical properties of GZ tablets selected for in vivo study.**

| Formula | Tensile strength ($Ncm^{-1}$) | Uniformity of thickness (mm) | Uniformity of diameter (mm) | Friability value (loss %) | Disintegration time (s) | Uniformity of weight (mg) |
|---|---|---|---|---|---|---|
| GZ | 133.5 (2.742)* | 3.436 (0.596) | 8.01 (0.675) | 0.8101 | 31.7 (1.965) | 0.1812 (2.403) |
| GZ–β-CD | 148.55 (4.35) | 3.657 (0.596) | 7.973 (0.166) | 0.493 | 16.4 (3.002) | 0.2004 (1.847) |
| GZ–β-CD + NaCMC | 139.5 (7.889) | 3.876 (0.367) | 7.973 (0.166) | 0.284 | 2.10 (2.125) | 0.2057 (1.266) |

*Values in parentheses are the coefficient of variation percent (CV%).

**Table III: Blood glucose levels after oral administration of 1.0 mg GZ powder and GZ–β-CD complex with and without NaCMC (mean of six mice).**

| Time (min) | GZ | GZ–β-CD | GZ–β-CD + NaCMC |
|---|---|---|---|
| 0 | 127.25 (35.29)* | 68.50 (4.95) | 82.0 (14.14) |
| 30 | 84.75 (53.89) | 40.0 (0) | 49.50 (6.36) |
| 60 | 88.0 (52.62) | 45.0 (8.49) | 49.0 (12.73) |
| 90 | 93.0 (67.18) | 45.50 (7.78) | 45.00 (4.24) |
| 120 | 92.25 (48.79) | 43.50 (0.71) | 44.50 (2.12) |
| 150 | 89.0 (34.70) | 45.50 (13.44) | 41.50 (0.71) |
| 180 | 67.25 (27.79) | 41.50 (26.16) | 44.5 (16.26) |
| 210 | 57.25 (24.17) | 38.50 (14.85) | 44.0 (5.66) |
| 240 | 49.25 (16.70) | — | — |

*Values in parentheses are the standard deviation (±SD).

## Methods

**Preparation of GZ–CD complexes.** Complexes of GZ with α- and β-CDs in aqueous solution were prepared by a method similar to the one used by Mura et al. (4). The method consisted of adding an excess amount of GZ to sealed glass containers of serial concentrations of CD (from 1 to 12 mmol of each α- and β-CD) that were equilibrated with electromagnetic stirring at a constant rate at 37 ± 0.5 °C for 7 h. Aliquots were withdrawn, filtered (filter pore size was 0.22 μm), and analyzed spectrophotometrically at $\lambda_{max}$ 276 nm for GZ. The presence of CD did not interfere with the spectrophotometric assay of the drug. Each determination was performed in triplicate.

**The effect of various additives on the solubility of GZ–β-CD complex.** Serial concentrations of NaCMC, polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG6000) were dissolved in a complex prepared as previously described and contained GZ–β-CD (in 1:2 molar ratio). An equivalent amount of 5 mg of GZ was added to each formula as shown in Table I.

**Kneading method.** The GZ–β-CD inclusion complex was prepared by the kneading method. GZ–β-CD was weighed accurately in a molar ratio (1:2). The specified amount of GZ was added to a slurry of CD in ethanol, and the mixture was kneaded in a mortar for 1 h to produce a paste of suitable consistency. The paste was then dried at 30 °C for 24 h, milled in a mortar, and passed through a 355-μm sieve. The same procedure was used to prepare a formulation with NaCMC (24.6%). Also, a formula containing a physical mixture (i.e., mixing without complexation) of GZ–β-CD (1:2) was prepared.

**Characterization of GZ–β-CD complexes. Differential scanning calorimetry (DSC) studies.** Ten milligrams of pure GZ, GZ–β-CD inclusion complex, and the physical mixture of GZ–β-CD were subjected to DSC studies using PerkinElmer's DSC7 scanner (Bodenseewerk, GmbH, Überlingen, Germany). The scanning rate was 10°/min.

**In vitro dissolution study.** In vitro dissolution of the pure GZ and the GZ–β-CD inclusion complexes was carried out using a dissolution test apparatus (Six Cups Station, Hanson Co., Chatsworth, CA). The dissolution medium used was a buffer solution of pH 7.4 at 37 ± 0.5 °C (100 rpm). Aliquots were analyzed spectrophotometrically for drug content at λ = 276 nm. Each determination was performed in triplicate.

**Tablet preparation.** The ingredients of each formula, as presented in Table I, were sufficiently mixed using a cubic mixer (AR 400, Erweka GmbH, Heusenstamn, Germany) for 10 min. The mixture was compressed into 200-mg flat tablets (8 mm in diameter) using a constant-rate tableting machine (EK/O, Korsch GmbH, Heusenstamn, Germany).

**Evaluation of prepared tablets. Weight uniformity.** Twenty tablets were randomly chosen and weighed individually, and the average weight, the standard deviation, and the coefficient of variation percent (CV%) were calculated.

**Thickness and diameter uniformity.** Ten tablets were randomly cho-

**Table IV: In vivo availability data of GZ after the oral administration of 1.0 mg to mice.**

| Formula | AUC[a] | AAC[b] | Relative availability (from AAC data) | Standard error | Significance ($p > 0.05$) (from AAC data) |
|---|---|---|---|---|---|
| GZ | 274.47 | 75.53 | 1 | — | — |
| GZ–β-CD | 226.88 | 123.13 | 1.6302 | 59.42 | 0.482 |
| GZ–β-CD with NaCMC | 204.68 | 145.32 | 1.9240 | 20.69 | 0.043 |

[a]area under the blood sugar curve.
[b]area above the blood sugar curve.

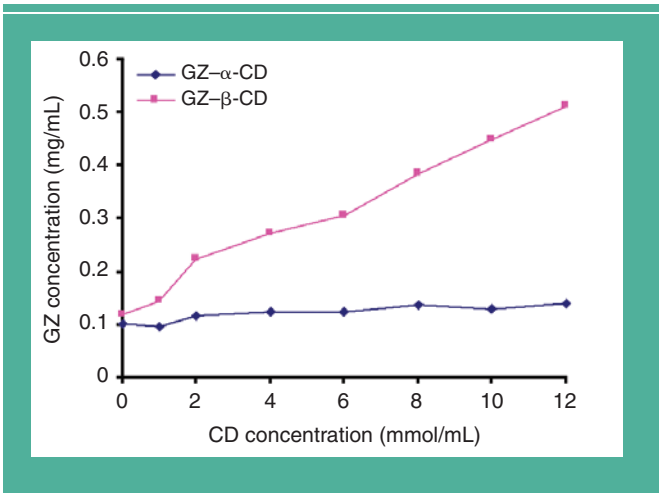


**Figure 1:** Comparison of GZ with the inclusion of α- and β-CD.

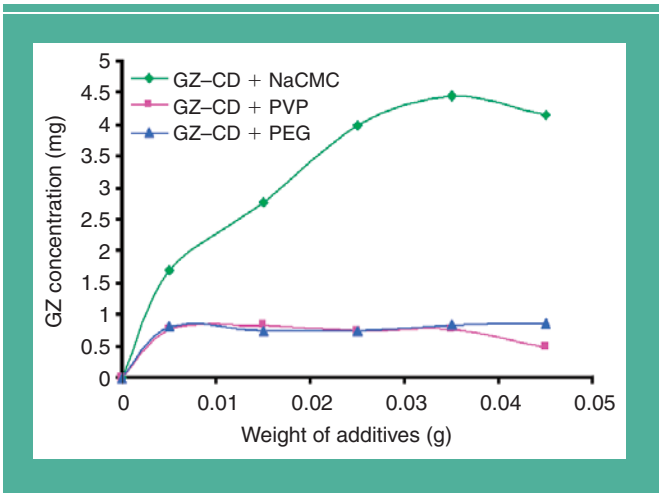


**Figure 2:** Effect of additives on the solubility of GZ–β-CD.

sen from each batch, and the thickness and diameter of each tablet were measured using a hardness tester (TBH 30, Erweka GmbH).

**Tensile strength.** The tensile strength ($T_S$) was calculated using the equation

$$T_s = 2H \div \pi TD \qquad [1]$$

in which $H$ is tablet hardness, $T$ is tablet thickness, and $D$ is tablet diameter.

The hardness, thickness, and diameter of 10 tablets randomly selected from each batch were determined using a hardness tester (TBH 30, Erweka GmbH).

**Friability.** The percentage weight loss was determined after rotating 20 preweighed tablets for 4 min at 25 rotations/min using a friabilator (TAR 20, Erweka GmbH).

**Disintegration time.** The disintegration time of one tablet from each batch was determined using a disintegrator (Pharmacia, Testo, Italy).

**Dissolution studies.** A *USP/NF 23* dissolution apparatus (Hanson Co.) with six baskets was used for dissolution studies. One tablet was placed in each basket and rotated at 100 rotations/min in 900 mL of the dissolution medium (phosphate buffer, pH 7.4) at 37 °C. The experiment was performed for 2.5 h, during which time samples were withdrawn at suitable time intervals and replaced by an equal volume of dissolution medium, which was kept heated at 37 °C. Samples were assayed spectrophotometrically at 276 nm for GZ. Each determination was performed in triplicate.

**In vivo study.** This method is similar to a method that was applied in a previous study (17). Three groups of mice were fasted (with water) at least 12 h before the experiment. Each group consisted of five mice weighing 16-20 g each. Before drug administration, a blood control sample was taken from each mouse from behind the eyeball through the angle of ocular cavity using small capillary tubes. The blood glucose level was determined using the glucose-measuring instrument Surestep (Lifescan, Inc., Johnson & Johnson Company, Milpitas, CA). The instrument was self-calibrated, and the samples were allowed to dry before the results were read to avoid contaminating the lens. The different formulations of GZ were administered orally to each group of mice using stomach intubations. A dose of 1.0 mg/0.5 mL was administered in a suspension form (freshly prepared for each time interval) for each mouse (a total of 15 mice), blood samples were collected, and the glucose determination test was performed immediately. Samples were withdrawn at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min. All in vivo experiments began at 8:00 a.m.

**Data analysis.** The student *t*-test was applied as the statistical method of analysis (SPSS computer program) in which $p < 0.05$ was considered as the least significant level.

## Results and discussion

**Solubility study.** Various CDs and CD derivatives have been shown to be powerful solubilizers for many poorly soluble drugs. To enhance GZ's solubility, the phase solubility diagram (see f 1) of GZ with α- and β-CD in distilled water was tested. The results revealed that β-CD was more effective as a solubilizing complex with GZ than α-CD. On the other hand, the effect of several additives, namely NaCMC, PVP, and PEG6000, on the solubility of GZ were studied. Figure 2 shows that NaCMC is the most-enhancing additive compared with PVP or PEG6000. However, no significant difference could be detected between the dissolution of PVP or PEG6000.

**DSC studies.** To study the physicochemical properties of the GZ–β-CD complex, DSC studies were performed for the pure GZ, GZ–β-CD inclusion complex, and the physical mixture that contained GZ–β-CD. The results presented in Figure 3 revealed that, in the case of the physical mixture, a broad peak at

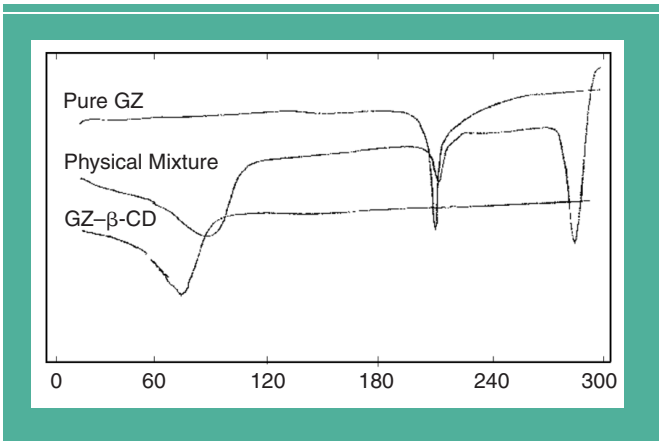


**Figure 3:** Differential scanning calorimetry of some GZ formulations.

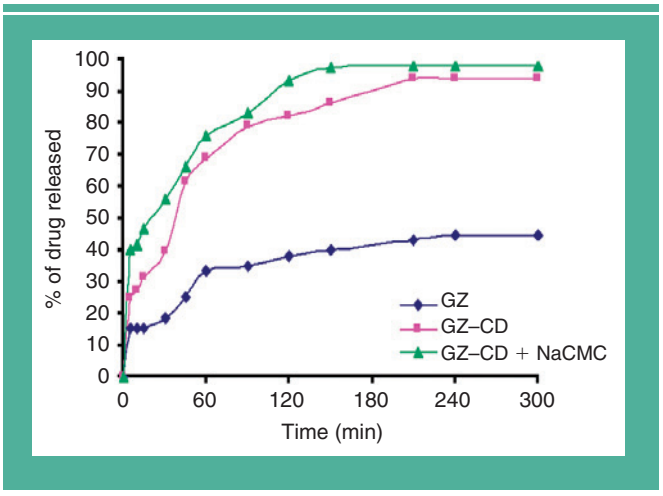


**Figure 4:** Release profiles of GZ from various formulations in a phosphate buffer at pH 7.4.

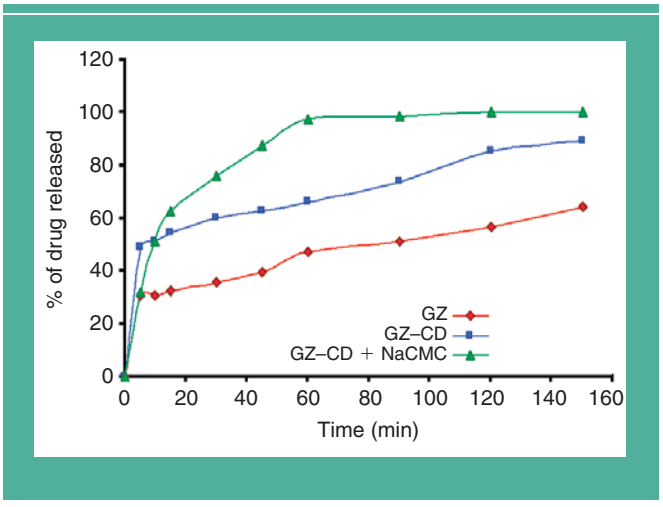


**Figure 5:** Release profiles of GZ from various tablet formulations in distilled water.

100° represented the CD melting and the endothermic peak of GZ at 210° with less intensity than that of pure GZ, which may be the result of a decrease in crystallinity from mixing. The latter peak disappears completely in a complex state, thereby confirming the formation of a GZ complex.

**GZ tablets.** Tablet formulations containing pure GZ, GZ–β-D complex, and GZ–β-CD complex with NaCMC were prepared. All the prepared tablet formulations showed acceptable physical properties (see Table II). The weight and thickness uniformity, the friability value, and the disintegration time (a very rapid disintegration, especially for those containing NaCMC) of the formulations fulfilled the *USP/NF* 1995 requirements. Also, acceptable tensile strength and thickness values were obtained.

The dissolution characteristics of pure GZ, GZ–β-CD complex, and GZ–β-CD complex with NaCMC in a powder state (before tableting) were studied in a phosphate buffer (see Figure 4) and distilled water (see Figure 5). The inclusion of NaCMC with the GZ–β-CD complex produced a pronounced enhancement of drug release. Also, GZ inclusion inside β-CD did improve the dissolution rate of GZ. The dissolution rate in the phosphate buffer (pH 7.4) was higher than in the distilled water because GZ is more soluble in an alkaline medium.

The release studies of GZ from the prepared tablets in distilled water (see Figure 5) and in a phosphate buffer (pH 7.4) (see Figure 6) showed that tablets containing NaCMC had a comparatively higher dissolution rate followed by those containing the GZ–β-CD complex, while the pure GZ showed the slowest dissolution rate in both media. The release of GZ was faster at pH 7.4 than in distilled water because the solubility of GZ increased as the alkalinity increased (1).

**In vivo study.** Because the dissolution of a dosage form in vivo is often the rate-limiting factor when determining the physiological availability of a drug, measuring the in vitro dissolution rate or a related parameter is more likely to offer a meaningful indication of physiological availability (17). Powdered GZ was administered to the mice in a suspension form. The decrease in glucose levels could be observed 0.5 h after administration (see Table III and Figure 7). This effect was gradually enhanced ⩽6 h. The decrease in glucose levels reflects an increase of GZ in the blood levels as a result of the drug's dissolution and absorption.

Formulations containing GZ–β-CD with NaCMC showed the lowest glucose level comparatively followed by that of the GZ–β-CD complex alone. After applying the *t*-test analysis for the data obtained from the in vivo areas above the curve (AAC), a significant difference was observed between the untreated GZ powder and the formulation containing GZ–β-CD with NaCMC ($p = 0.043$). This significant decrease (see Table IV) was attributed to the improvement of the bioavailability of GZ using the β-CD inclusion complex, especially when including NaCMC. The areas under the curve (AUC) and the relative bioavailability values confirm these results. However, the difference between the GZ powder and the formulation containing GZ–β-CD only was insignificant ($p = 0.482$). An excellent in vitro (after 10 min) versus in vivo (after 90 min) correlation was detected (see Figure 8) with a correlation coefficient of 0.987.

## Conclusion

The β-CD was more effective than the α-CD in enhancing the dissolution rate of GZ, and the addition of NaCMC enhanced the dissolution rate of the GZ–β-CD complex more than PVP or PEG6000.

Tablet formulations containing pure GZ, GZ–β-CD complex, and GZ–β-CD complex with NaCMC were prepared and all showed acceptable physical properties. Tablets containing NaCMC showed a comparatively higher dissolution rate, tablets

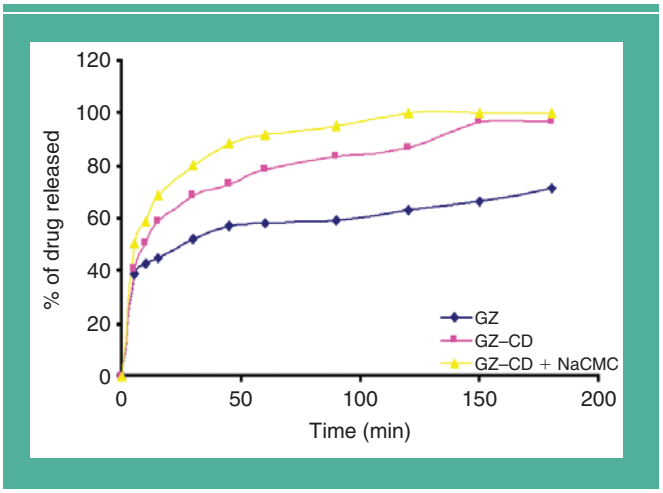


**Figure 6:** Release profiles of GZ from various tablet formulations in phosphate buffer at pH 7.4.

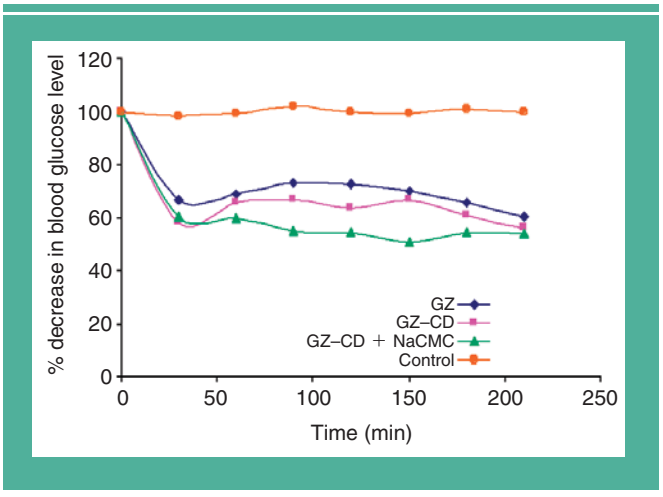


**Figure 7:** Effect of oral administration of 1.0 mg of GZ on the blood glucose levels of mice (mean of six mice given pure GZ and GZ–β-CD complex with and without NaCMC). Zero time value is taken only once.

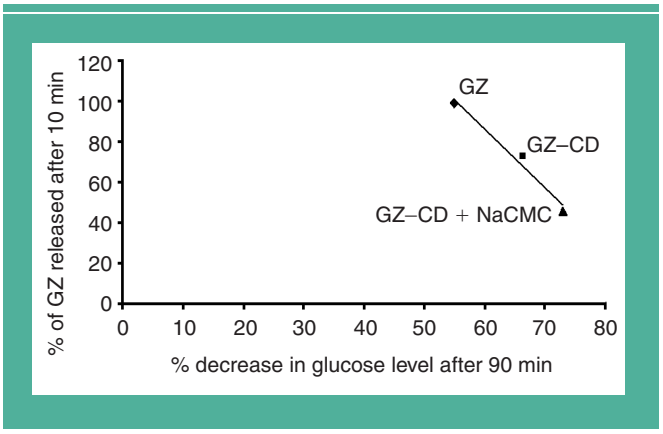


**Figure 8:** Correlation between percentage of GZ release after 10 min (in vitro) and percentage decrease in glucose levels after 1 h (in vivo) for the three tested formulations.

containing the GZ–β-CD complex showed the next highest dissolution rate, and the pure GZ formulation exhibited the lowest dissolution rate.

For the mice that were given the formulation with the GZ–β-CD complex and NaCMC, the results showed a significant decrease in their glucose levels compared with those that had been given the formulation of pure GZ. No significant difference was observed between the GZ–β-CD and GZ–β-CD with NaCMC formulations. A good in vivo–in vitro correlation was detected for the three tested formulations.

## Acknowledgment

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