

IN VIVO – IN VITRO CORRELATION – A STRATEGIC TOOL FOR DRUG DEVELOPMENT

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ABSTRACT

Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent, which is a time consuming and costly process. Correlation between *In Vivo – In Vitro* (IVIV) data is often used after proper validation. It predicts the bioavailability results from in vitro dissolution. In this paper, we have described an in vitro advanced gastric model to study the behavior of oral compounds under interactive kinetic conditions in the human stomach. This model simulates the motility and pressure forces in the different regions of stomach, the rate and composition of gastric secretion fluid. IVIVC is a mathematical model, among all the three

levels of correlation Level A is widely accepted by regulatory agencies.

KEYWORDS: In Vivo – In Vitro Correlation, validation, bioavailability, advanced gastric model, etc.

INTRODUCTION

Correlations between in vitro and in vivo data (IVIVC) are often used during pharmaceutical development in order to reduce development time and optimize the formulation. A good correlation is a tool for predicting in vivo results based on in vitro data. IVIVC allows dosage form optimization with the fewest possible trials in man, fixes dissolution acceptance criteria, and can be used as a surrogate for further bioequivalence studies; it is also recommended by regulatory authorities.

Many studies reported in the late '70s and early '80s established the basic concept of IVIVC. Various definitions of in vitro–in vivo correlation have been proposed by the International Pharmaceutical Federation (FIP), the USP working group, and regulatory authorities such as

the FDA or EMEA. The FDA defines IVIVC as “a predictive mathematical model describing the relationship between an *in vitro* property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response, e.g., plasma drug concentration or amount of drug absorbed”. As stressed in this definition, IVIVC is more an *in vitro*–*in vivo* relationship than a strict correlation. It should be kept in mind that a relationship does not imply a causality link between the *in vitro* data, in our case, and the *in vivo* data.^[1,2]

Levels of IVIVC

Bases on the type of data used to establish the relationship, three main levels are defined by the FDA:

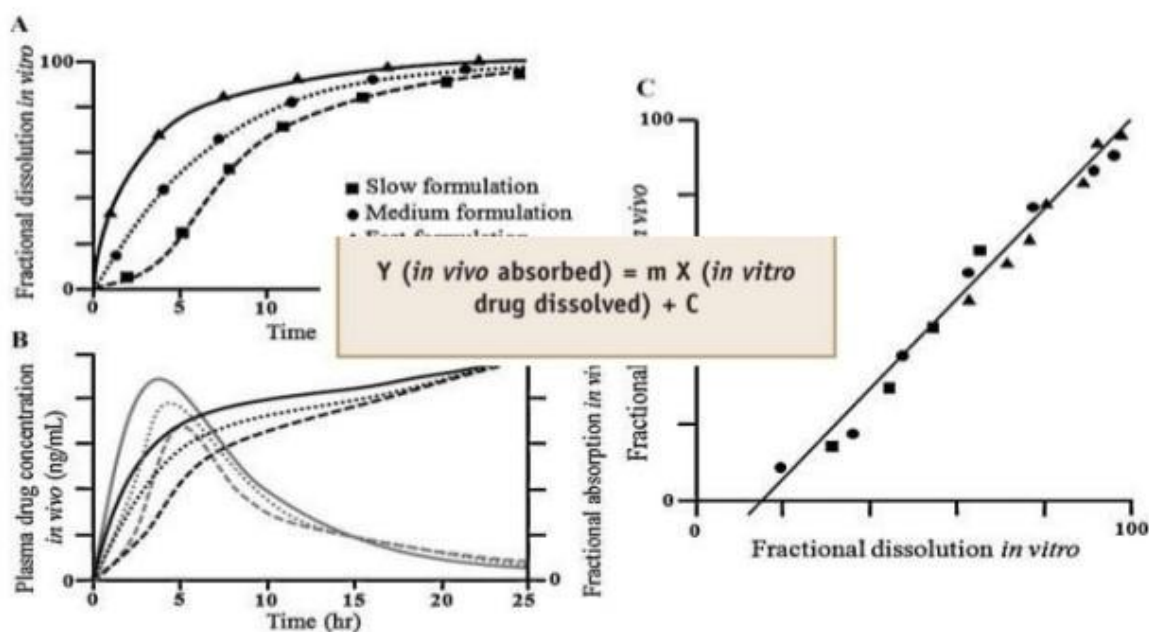


Fig.1. Levels of IVIVC.

Level A

A correlation of this type is generally linear and represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate (e.g., the *in vivo* dissolution of the drug from the dosage form). In a linear correlation, the *in vitro* dissolution and *in vivo* input curves may be directly super imposable or may be made to be superimposable by the use of scaling factor. Nonlinear correlations, while uncommon, may also be appropriate. Alternative approaches to developing a Level A IVIVC are possible. Whatever the method used to establish a Level A IVIVC, the model should predict the entire *in vivo* time course from the

in vitro data. In this context, the model refers to the relationship between in vitro dissolution of an ER dosage form and an in vivo response such as plasma drug concentration or amount of drug absorbed.

Level B

A Level B IVIVC uses the principles of statistical moment analysis. The mean in vitro dissolution time is compared either to the mean residence time or to the mean in vivo dissolution time. A Level B correlation does not uniquely reflect the actual in vivo plasma level curve, because a number of different in vivo curves will produce similar mean residence time values.

Level C

A Level C IVIVC establishes a single point relationship between a dissolution parameter, for example, $t_{50\%}$, percent dissolved in 4 hours and a pharmacokinetic parameter (e.g., AUC, C_{max} and T_{max}). A Level C correlation does not reflect the complete shape of the plasma concentration-time curve, which is the critical factor that defines the performance of ER products. In addition to these three levels, a combination of various levels C is also described: A multiple Level C correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile.

Level A correlations use all the information of the dissolution and absorption curves, in contrast to levels B or C. The establishment of a relationship implies the use of many formulations, each of them giving one pair of data (vitro and vivo). It is obvious that level B or C needs more data and, as they do not use all the information related to vitro and vivo behavior of the formulation, they are less powerful.

Ranking of levels

The FDA ranked the levels as follows^[2]:

A Level -A IVIVC is considered the most informative and is recommended, if possible. Multiple Level C correlations can be as useful as Level A correlations. However, if a multiple Level C correlation is possible, then a Level A correlation is also likely and is preferred.

Level C- correlations can be useful in the early stages of formulation development when pilot formulations are being selected.

Level B- correlations are least useful for regulatory purposes.

In vivo data are obtained from well-standardized fasted studies on healthy volunteers. In attempting to establish a Level A relationship, the major point to consider is the sampling schedule in the “absorption “phase in order to have an accurate representation of the input curve. Establishing an IVIVC is nothing more complicated than trying to reproduce all the complex phenomena that lead to the in vivo release and solubilisation of the API in the gut in a “simple” in vitro system like a vessel agitated with a paddle.

Table 1. Various Parameters used in IVIVC based on LEVEL.

| Level | In Vitro | In Vivo |
|-------|---|---|
| A | Dissolution curve | Input (absorption) curves |
| B | Statistical moments: MDT | Statistical moments: MRT, MAT, etc |
| C | Disintegration time, Time to have 10, 50, 90% dissolved, Dissolution rate, Dissolution efficiency | C _{max} , T _{max} , K _a , Time to have 10, 50, 90% absorbed, AUC |

Data Collection

In Vivo Data^[2]

The in vivo data are derived from the plasma concentration curve. Various phenomena can occur after administration of drug depending of the route of administration. The plasma concentration curve is a global representation; it depends on drug input within the blood, which depends on the dosage form and the properties of the drug and thereafter its pharmacokinetic input processes. The disposition of the drug afterwards depends only on the drug and patient.

As stated by the FDA, absorption curves can be calculated by various methods by estimating “the in vivo absorption or dissolution time course using an appropriate deconvolution technique for each formulation and subject (e.g., Wagner-Nelson, numerical deconvolution)”.

After the calculations are performed, the input curve, known as “absorption” curve, is presented as the percentage of the fraction of dose (%FD: 0–100%) absorbed versus time. This absorption curve represents the sum of all the phenomena such as release or dissolution but also the permeability through the intestinal membranes. To obtain a good estimation of the absorption curves, a sufficient number of well-positioned samples are required.

In Vitro Data^[6]

In the case of oral administration of a solid dosage form, the limiting factor for the appearance of the drug in the blood could be the pure permeability through the intestinal

membrane (mainly for Class III and IV of the BCS), the release from the drug dosage form, or the solubility of the active ingredient.

In case of solubility-limited product (Class II and IV of BCS), the absorption could be governed by physico-chemical characteristics of the API (such as solubility, dissolution rate, particle size, crystal shape, polymorphism, pKa, stability in GIT) and not by its permeability or formulation.

In the case of release-limited formulations, the absorption is limited by the drug release from the drug dosage form. As described above, to establish IVIVC, the release must be the limiting factor; in this case, solubility (or dissolution rate) and permeability must be faster than the release.

Correlations are always possible with Class I drugs and depend on the relative magnitude of the various factors versus the release rate for the other classes. The active substance is the core of any formulation; the physico-chemical characteristics and the stability of the API are the main points to be considered first. In the case of oral products, there are two types of formulations^[7].

The first is an immediate-release (IR) product that disintegrates and disperses rapidly after administration.

The second is an extended-release (ER) formulation, which keeps its integrity throughout much of the GIT. In the case of the IR product, the release of both the drug and excipients is rapid.

In the ER case, the excipients are the core of the formulation, and they control the release of drug within the body. For example, the HPMC matrices are biocompatible, do not exhibit any degradation by enzymes and GIT fluids and have no direct interaction with bile salts, but they interact with water. They swell, form a gel and then the API slowly diffuses through the formatted gel. In this second case, in addition to the characteristics of the API, the characteristics and quantities of excipients and the process itself are of importance. The release of drug from the dosage form can be studied *in vitro*, and the dissolution reflects all of the phenomena implied in the formulation.

In Vitro Data Evaluation^[8]

Various dissolution tests exist and can be used to generate the in vitro data. In order to compare the results, the same method must be used for all the formulations. The in vitro dissolution curves depend on –

- (1) The release characteristics of the formulation and manufacturing process
- (2) The characteristics of the API such as the solubility and dissolution rate
- (3) The apparatus, method parameters, dissolution medium

The dissolution curves are expressed as the percentage dissolved versus time and should usually reach 100% at infinity (or in practice at the end of dissolution). The best dissolution method for in vivo—in vitro correlation is, obviously, the method that describes what happens in vivo. All of these factors cannot be easily reproduced in vitro by a simple dissolution method. In addition, the relative importance of these factors is not similar if the subject is in fed or fasted state; in the fed state, food also has a direct influence on the API or formulation behavior.^[10]

In Vitro Media^[12]

Usually, aqueous based media are used, most of them being described in pharmacopoeias. To simulate gastro media, the simplest one is composed of 0.1 N HCl.

A more complicated medium called simulated intestine fluid (SIF) can also be used.

For other pHs, acetate or phosphate buffers are usually used (or sometime TRIS).

For specific applications (e.g., poorly soluble drugs), the pharmacopoeias recommend the addition of either enzymes or surfactant.

Noncompendial media are also described to simulate the fasted and fed states in vivo: fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF).

These media try to reproduce, not the direct food influence on the formulations, but rather the secretions occurring in either fasted or fed state in the small intestine.

In Vitro Apparatus^[4,6]

Any compendial method that can discriminate between the formulations may be used, but certain techniques are preferred (i.e., paddle, flow-through cell, basket—USP Apparatus 2, 4,

and 1, respectively). No in vitro system developed to date can maintain the following requirements that correspond to the physiological states in vivo:

- (1) Sequential use of enzymes in physiological amounts
- (2) Appropriate pH for the enzymes
- (3) Removal of the products of digestion
- (4) Appropriate mixing at each stage of digestion
- (5) Physiological transit times for each step of digestion
- (6) A peristaltic dynamic approach

TNO MODEL (TMI)^[8, 9, 12]

It is an in vitro system developed at TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the GI tract in man and fulfils the requirements mentioned above. In addition, this in vitro model offers the possibility of introducing a solid meal to investigate all food–drug interactions and food impact on dosage form behavior.

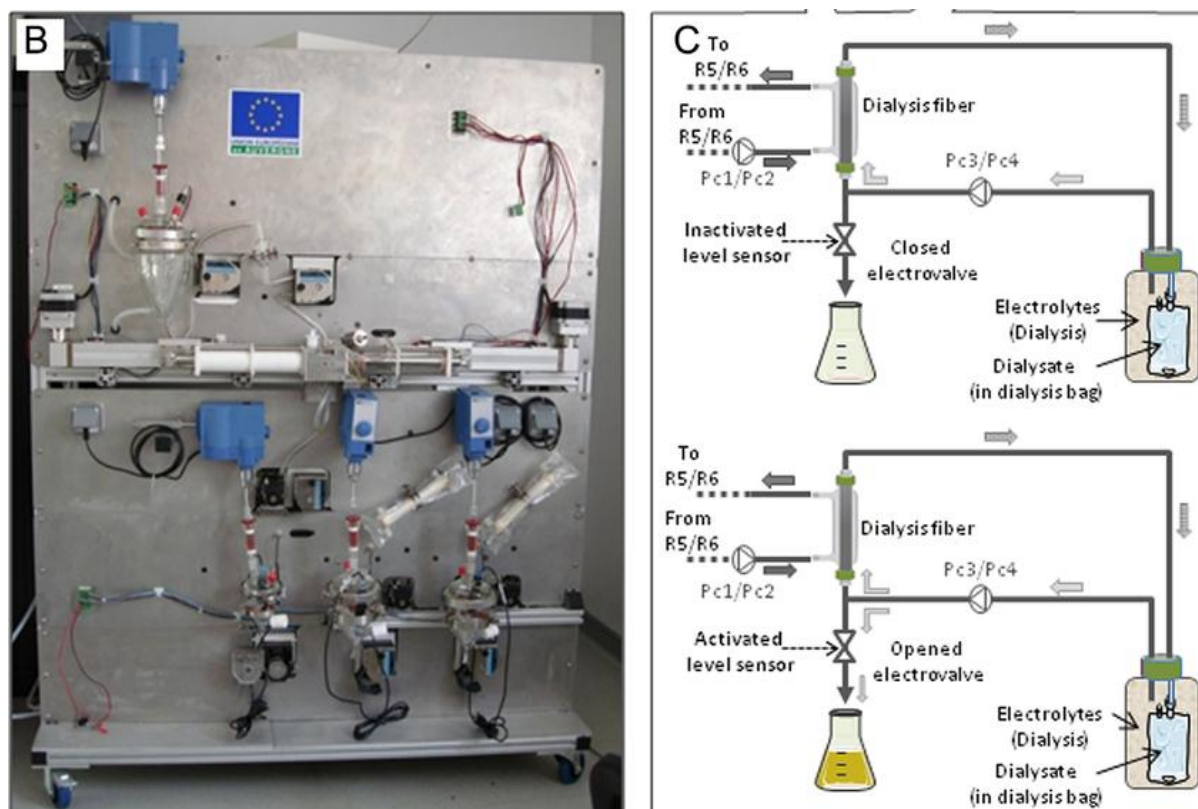


Figure 2 TNO Model.

It consists of

Four serial compartments simulating the stomach and the three segments of the small intestine: the duodenum, jejunum and ileum.

Each compartment is filled with water that is pumped from a water bath into the glass jackets around the flexible walls to control the temperature inside the units (37°C) and the pressure on the flexible walls.

Changes in the water pressure enable mixing of the chyme by alternate compression and relaxation of the flexible walls.

To control the transit of the chyme, an exponential formula is used for gastric and ilea delivery.

Chime transit is then regulated by opening or closing the peristaltic valves that connect the compartments.

The volume in each compartment is monitored by a pressure sensor connected to the computer.

The pH is computer monitored and continuously controlled by secretion of either water or 0.5 M HCl (0.25 mL/min) into the stomach and either electrolytes or 0.5 M NaHCO₃ (0.25 mL/min) into the small intestine.

Simulated gastric (0.5 mL/min), biliary (0.5 mL/min) and pancreatic (0.25 mL/min) secretions (i.e., pepsin, lipase, pancreatic and bile salts) are introduced into the corresponding compartments by computer-controlled pumps.

The model is equipped with hollow-fibre membranes connected to the two compartments representing the jejunum and the ileum, respectively.

Water and small molecules are removed from the lumen of the compartments by pumping dialysis fluid (10 mL/min) through the hollow fibres.

This system mimics GI motility and the mechanical destructive force on drug release, The drug concentration can be measured in jejuna and ileal dialysis fluids, following its passive diffusion through the hollow-fibre membranes connected to the two compartments representing the jejunum and the ileum, respectively.

Although this Artificial Digestive System has been widely used in nutrition studies, few examples of drug dosage forms have been studied. However, this type of system, even if it

could be used in drug development with success is not a quality control tool due to its complexity.

The classical methods do not allow all the characteristics of the *in vivo* interaction to be reproduced, especially the direct food interaction, but can help to study the mean characteristic of drug release.

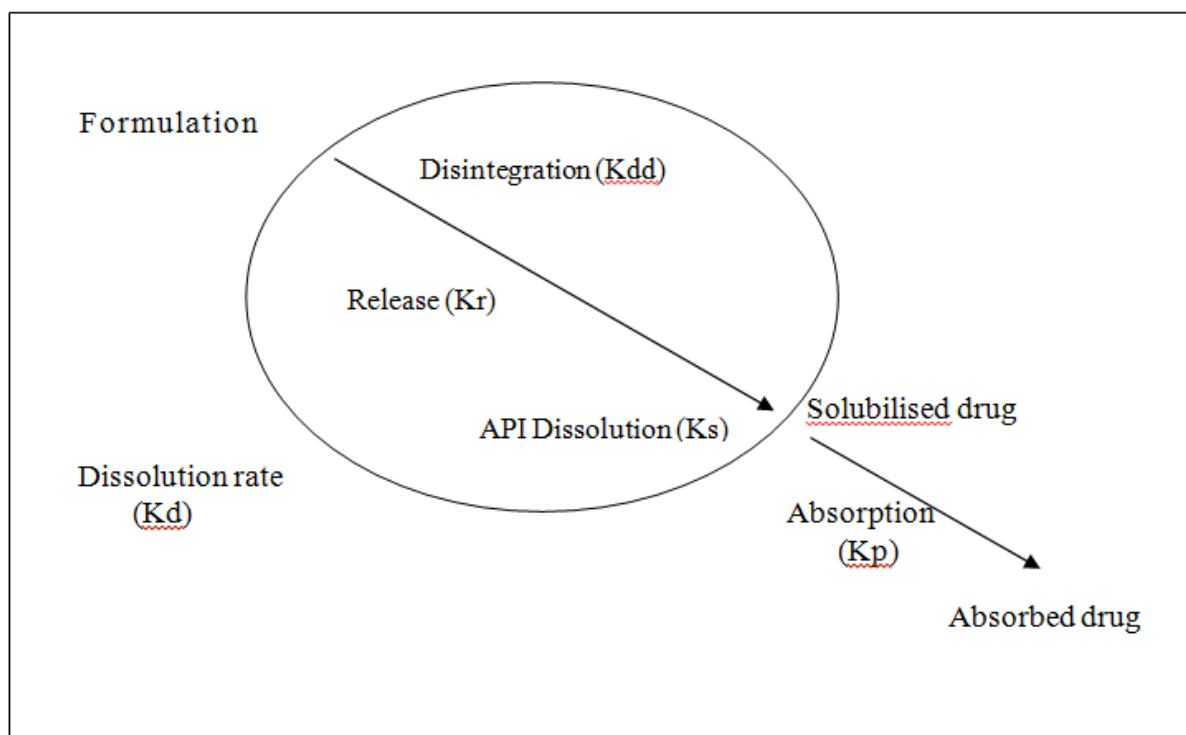


Fig. 3. Representation of the main phenomena leading to absorption *in vivo*, outside (macro) phenomena, inside dissolution (micro) phenomena.

The *in vivo* drug release can be simplified as a multifactorial process. Three main cases exist for the macro phenomena.^[13]

- (1) If k_d and k_p are fast, drug is well absorbed (class I of the BCS usually)
- (2) If k_d is greater than k_p , the drug is permeation-controlled (Class III)
- (3) If k_d lower than k_p , it is a dissolution-controlled release (Class II).

The various phenomena underlined in k_d (dissolution rate) are of three types, expressed by the micro phenomena (k_r , k_{dd} and k_s) can be split into 4 main categories.

First, if k_{dd} is greater than k_s , the apparent dissolution is driven by the dissolution of the API. In this case, the study of either the intrinsic dissolution as described in USP and EP or the

apparent dissolution determined with the specific USP 4 cell as described in EP is a key factor in dosage form development.

Second, if k_{dd} is lower than k_s , then the formulation is controlled by disintegration and the formulation is the key factor.

Third, if k_{dd} is close to k_s , then disintegration is the key factor.

Finally, a k_r that is lower than k_s indicates slow release and the critical attribute is the dissolution. Thus, a classical dissolution technique on the API and the dosage form provides relevant information on the key factors for the establishment of IVIVC, the fact that the release rate of the drug from the drug dosage form is the limiting factor (k_d lower than k_p , and k_r limiting factor of k_d).

Significance of IVIV correlation

Correlation defines a linear relationship between in vitro and in vivo data so that measurement of the in vitro dissolution rate alone is sufficient to determine the pharmacokinetic profile in vivo. After a proper validation, IVIVC predicts the in vivo bioavailability results from in vitro dissolution data, and this simulation reflects the in vivo behaviour of the various formulations. In the presence of an IVIVC, the FDA states In vitro dissolution testing is important for

- (1) Providing process control and quality assurance^[4]
- (2) Determining stable release characteristics of the product over time^[5]
- (3) Facilitating certain regulatory determinations (e.g., absence of effect of minor formulation changes or of change in manufacturing site on performance).^[2]
- (4) In certain cases, especially for ER formulations, the dissolution test can serve not only as a quality control for the manufacturing process but also as an indicator of how the formulation will perform in vivo.^[10]
- (5) The main objective of developing and evaluating an IVIVC is to establish the dissolution test as a surrogate for human bioequivalence studies. Bioequivalence studies highlight the significance of IVIVC and dissolution studies both during development and throughout the life of the product. For example, the establishment of dissolution limits could be based on IVIVC, as stated by FDA.^[3]
- (6) IVIVC can also be used to support bio waivers in two cases over the five categories described in the SUPAC guidance.^[3]

- (7) IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development.^[4]
- (8) The main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support bio waivers.^[11]
- (9) It supports and/or validates the use of dissolution methods and specification settings.^[6]
- (10) The IVIVC includes in vivo relevance to in vitro dissolution specifications.
- (11) It assists in quality control for certain scale-up and post-approval changes (SUPAC).^[4]
- (12) With the proliferation of modified-release products, it becomes necessary to examine the concept of IVIVC in greater depth.^[13, 11]
- (13) Investigations of IVIVC are increasingly becoming an integral part of extended release drug development.
- (14) There are some in vitro means of assuring that each batch of the same product will perform identically in vivo.^[15]
- (15) The FDA guidance, development, evaluation and validation of an IVIVC to grant bio waivers.^[2]
- (16) It set dissolution specifications for oral dosage forms, biopharmaceutics classification systems (BCS), BCS bio waivers, application of BCS in IVIVC development and concept of mapping.^[5]
- (17) The importance of dissolution media and methodology and pharmacokinetic studies in the context of IVIVC.^[14]
- (18) Formulation dependency of IVIVCs and IVIVCs of pure enantiomers versus race mate drugs. The same principles of IVIVC used for oral extended release products may be applied for non-oral products such as parenteral depot formulations and novel drug delivery systems as well.^[12]

CONCLUSION

IVIVC includes in vivo relevance to in vitro dissolution specifications and can serve as surrogate for in vivo bioavailability and to support bio waivers. Furthermore, IVIVC can also allow setting and validating of more meaningful dissolution methods and specifications. It can also assist in quality control for certain scale-up and post-approval changes. Both the regulatory agencies and pharmaceutical industries have, therefore, understood this value of IVIVCs. Therefore, the activity in the area of IVIVC for oral extended release dosage forms has increased.

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