In Vitro-In Vivo Correlation (IVIVC) and Determining Drug Concentrations in Blood from Dissolution Testing – A Simple and Practical Approach

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Abstract: Evaluating an IVIVC is a desirable feature for any drug dissolution test to establish relevance and confidence in assessing the quality and safety of solid oral dosage products, such as tablets and capsules. However, success in this area has been limited. One of the reasons for this lack of success may be that the approaches described in the literature to achieve IVIVC appear to be intuitive expectations rather than an objective end-point based on scientific rationale. For example, rather than predicting an *in vivo* response based on *in vitro* results, which is the objective of IVIVC, attempts are usually made to match *in vitro* results with *in vivo* results by adjusting experimental conditions for *in vitro* testing. This article provides a discussion and clarification on the underlying scientific principles to help in alleviating current difficulties in developing IVIVC. Further, it provides a simpler and practical approach based on experimental studies to achieve appropriate IVIVC by predicting blood drug levels from dissolution results.

INTRODUCTION

A dissolution test is employed for the assessment of the impact of formulation and manufacturing attributes on drug (active pharmaceutical ingredient, API) release characteristics of solid oral dosage products for both product development and quality control purposes. Dissolution tests are mostly conducted in vessel-based apparatuses, commonly known as the paddle and basket apparatuses [1] to demonstrate appropriate release characteristics of API. The core components of these apparatuses are a vessel and a stirrer. Such tests are commonly interchangeably referred to as *in vitro* dissolution tests or drug release evaluation tests for solid oral products such as tablets and capsules.

For a drug to be absorbed into the blood stream to reach its site of action it should be present in a solution form in the GI tract, more specifically in the intestine. The *in vitro* dissolution testing is conducted to estimate or predict dissolution of the drug in the GI tract or *in vivo*. Therefore, some form of relationship between these two dissolution types is desirable and it is commonly referred in the literature as *in vitro-in vivo* co-relationship (IVIVC). Developing and conducting a dissolution test based on such a relationship not only enhances the credibility of an *in vitro* test but also provides a number of ethical and economical benefits such as reduction of the required number of *in vivo* studies in humans, thus simplifying and expediting the development and modification of the drug products.

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Although, such IVIVC relationships are highly desirable and have been the focus of intense research work, limited success has been achieved in this regard [2]. Therefore, present day drug dissolution testing practice faces many challenges including whether the testing should be conducted at all.

It appears that this confusion and associated challenges are the result of some misunderstandings of the objectives and practices of developing IVIVC. By its nature, IVIVC requires predictability of *in vivo* dissolution results. However, the majority of the literature reflects a practice of fitting/matching, in retrospect, *in vivo* outcome with *in vitro* dissolution results [3]. Therefore, there appears a clear deviation from the objective of conducting IVIVC studies, which leads to lack of success.

This article describes the limitation of current practices and provides a practical and simple alternate to achieve the desire objective of IVIVC, i.e. predicting of drug levels in the blood.

IVIVC AND UNDERLYING SCIETIFIC PRINCIPLES

As stated above and a very well established fact, drug dissolution testing is conducted to establish quality, and the consistency in the quality, of a drug product. However, the fundamental question is what is this "quality" parameter, which is often referred to?

This quality aspect may be explained as follows. Efficacy or effect of a drug product depends on the API present in the product. However, seldom is a raw API in a powder form is administered to humans, in particular through the oral route. It is difficult, if not impossible, to routinely administer accu-

rate and consistent amounts of drug to patients in this manner. Thus, products, in this case solid oral dosage forms, are developed so that each tablet or capsule may be taken conveniently. Quality control tests such as potency, identity and content of uniformity are conducted for each product to establish that accurate and consistent amount of drug are present in each unit. However, these quality tests do not provide an answer to another critical aspect, i.e., whether or not the API present in the product would also be released as expected under the physiological conditions present in humans. How are these expected release characteristics in humans established? These drug release characteristics are established by conducting appropriate pharmacokinetic studies in humans.

These pharmacokinetic studies are conducted by administering the product to patients or healthy human volunteers. Blood samples are withdrawn at different time intervals to measure the availability of drug in the blood stream. These measured blood levels (also known as profiles, shown in Fig. **1**) are plotted against time and reflect release (or availability) of the drug from the product. If two products or batches of the same API show the same or similar profiles, it would show that the drug release characteristics are the same. The sameness of these profiles reflects the "quality" of a product that one refers to with regard to dissolution testing.

Such human pharmacokinetic studies, commonly referred to as bioavailability/bioequivalence studies, are routinely conducted and form the basis of the pharmaceutical product development and evaluation, for innovator and generic products manufacturing. To appreciate and understand the link of dissolution testing with blood drug concentration profiles, one must have some knowledge of the pharmacokinetics aspects. Although, numerous books [e.g. see 4] are available on the subject of pharmacokinetics, a discussion from an analyst point of view is essential, but is often limited or lacking, and this may be the cause of the lack of success in this respect. Therefore, a brief relevant discussion is provided here.

The Link of Dissolution Testing to Pharmacokinetics of a Drug

A pharmacokinetic study monitors the fate of a drug or API in the body. In practice, pharmacokinetics includes the study of the absorption, distribution and elimination of an administered drug. For the drugs administered orally, the absorption of drug occurs in the GI tract. The drug is then distributed in body through blood followed by elimination mostly *via* the kidney as a native drug or its metabolite(s). The combined effect of all these processes in the body are reflected in drug levels as shown in Fig. (**1**), producing what is commonly known as drug concentration-time profiles.

Let us consider a similar but slightly different blood profile, as shown in Fig. (**2**). The profile shown in the Fig. (**2**) represents a profile of the same drug as for Fig. (**1**), but after administration in solution form. Because the absorption is assumed to be fast (instantaneous), the blood profile (Fig. **2**) represents the distribution and elimination phases as if the drug was administered directly into the blood stream *via* intravenous injection. Mathematically such profiles may be described by an exponential equation such as $C = C_0 e^{-kt}$,

where "C" is the drug concentration at time "t", "k" is the rate constant, and "C_0" is the proportionality constant or drug concentration at time zero. Drugs follow similar pattern of distribution and elimination, however, the pattern may be represented by different type of exponential equations i.e. sums of two or more exponential components. As the drug was administered in solution, a dissolution step is not involved in process.
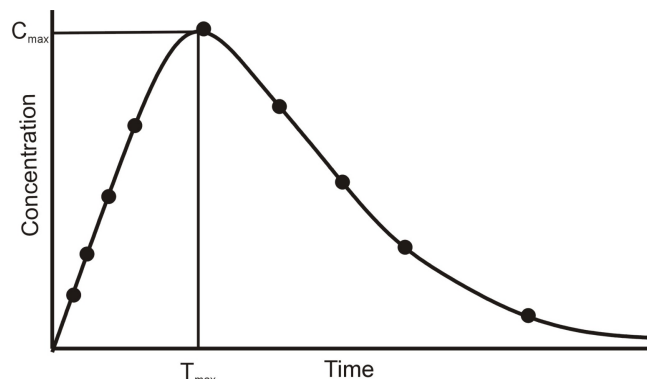

Fig. (1). A typical drug concentration (in blood) – time profile reflecting the fate of a drug in the human body following an oral dose (tablet/capsule).
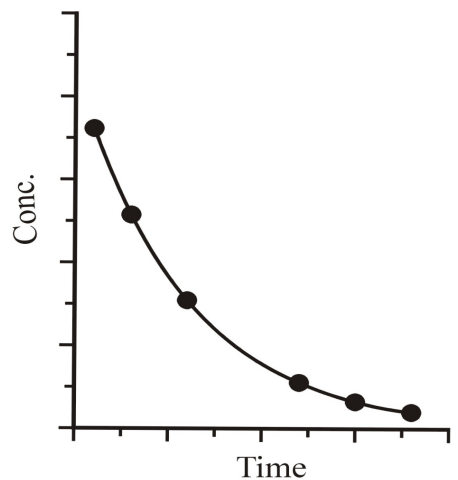

Fig. (2). A typical drug concentration (in blood) – time profile reflecting the fate of a drug in human body following an instantaneous absorption, i.e. negligible absorption phase or effect, following administration of a drug through oral route in solution form.

Consider a scenario in which the drug was administered in three discrete doses, in solution form, at half hour intervals, which happens to be the half-life of the drug (i.e. half of the drug in blood will be eliminated in every half hour interval). As the drug is assumed to be absorbed instantaneously, three overlapping curves will be observed as shown in Fig. (**3**), represented by open circles and dotted lines. However, as blood or blood compartment is the same, the drug concentration-time profile will reflect collective concentrations from all these three curves, as represented by solid circles with solid line in Fig. (**3**).
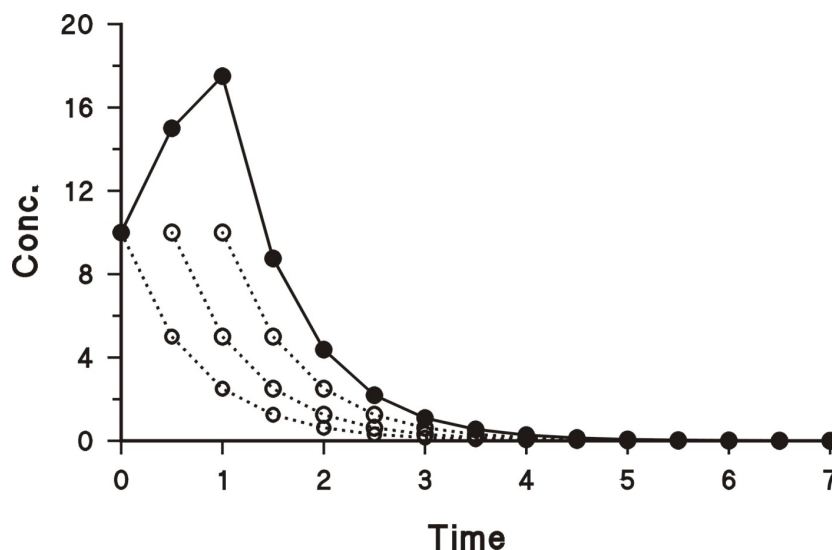
Fig. (3). The fate of a drug in a human body following three consecutive instantaneous doses (○----○) and their combined concentrations at times from these doses at different times (●——●).

Now consider that rather than giving intermittent doses in solution, one develops a mechanism in which the drug is administered in a format (tablet or capsules) which releases the drug into solution over time. Then effectively the tablet or capsules will serve the same process as continuous dosing which may be broken down in different, equal or un-equal, intervals. Therefore, in reality, the drug administered using tablets or capsules is a mechanism of delivering the drug in a controlled manner. The outcome is a reflection of the summation of drug levels at each time interval following individual pharmacokinetic profiles as described above.

What this means is that pharmacokinetics is dependent on the property of the drug and dissolution is the property of the product (tablet or capsule). Product dissolution does not usually change the pharmacokinetics (absorption, metabolism, elimination) of the drug which remains the same. However, based on dissolution (drug release and solution formation), and of the summation of different blood levels after discrete absorption, different drug concentration-time profiles will result. In short, blood drug concentration-time profiles from product to product may differ depending on the drug release (dissolution) pattern of the product, but pharmacokinetic characteristics of the drug will remain the same. For example, pharmacokinetic characteristics (e.g. half life) of diltiazem will remain the same, however, its drug concentration-time profile for fast release product will be significantly different compared to a slow-release product, because of the change in dissolution characteristics and the summation effect.

Therefore, it is critical to note that pharmacokinetics is a drug property and dissolution is a product property. There is no link of dissolution to pharmacokinetics but to drug concentration-time profile as a reflection of summation effect of numerous concurrent pharmacokinetic profiles, dependent on the product drug releasing characteristics. Further, permeability characteristic of a drug is another parameter which is often referred to when developing IVIVC, however, it is also a drug characteristic and not related to product. Whether a drug is of higher or lower permeability, at a constant dose/potency level, its absorption behaviour should

remain the same. This emphasizes the fact that when developing IVIVC, predicting blood concentrations with the product changes, evaluation should be done at the same dose level or in the range where drug absorption is linear, to avoid the negative effects of permeability or non-linear pharmacokinetic aspects.

The relationship of observed drug concentration-time profiles following administration of a tablet/capsule with drug dissolution and pharmacokinetics may be described graphically as shown in Fig. (**3**) (or Fig. **1**). It is important to note that dissolution and absorption characteristics of a drug are commonly shown interchangeably since it is generally assumed that absorption and dissolution have a linear relationship. Thus from Fig. (**3**), it is to be noted that one should be able to establish drug profiles with dissolution profiles combined with the pharmacokinetic characteristics of the drug as describe in the example above. This process of obtaining a drug profile from dissolution results is known as convolution. The opposite of this, i.e., obtaining or extracting a dissolution profile from a blood profile, is known as deconvolution (Fig. **4**).

Deconvolution *vs* Convolution Technique

The deconvolution technique which requires the comparison of *in vivo* dissolution profile obtained from the blood profiles with *in vitro* dissolution profiles. It is the most commonly cited and used method in the literature. Perhaps that is the reason for the lack of success of developing IVIVC, since this approach is conceptually weak and difficult to use to derive the necessary parameters for their proper evaluation [5]. For example: (1) Extracting *in vivo* dissolution data from a blood profile often requires elaborate mathematical and computing expertise. Fitting mathematical models are usually subjective in nature, and thus do not provide an unbiased approach in evaluating *in vivo* dissolution results/profiles. Even when *in vivo* dissolution curves are obtained there is no parameter available with associated statistical confidence and physiological relevance, which would be used to establish the similarity or dissimilarity of the curves. (2) A more serious limitation of
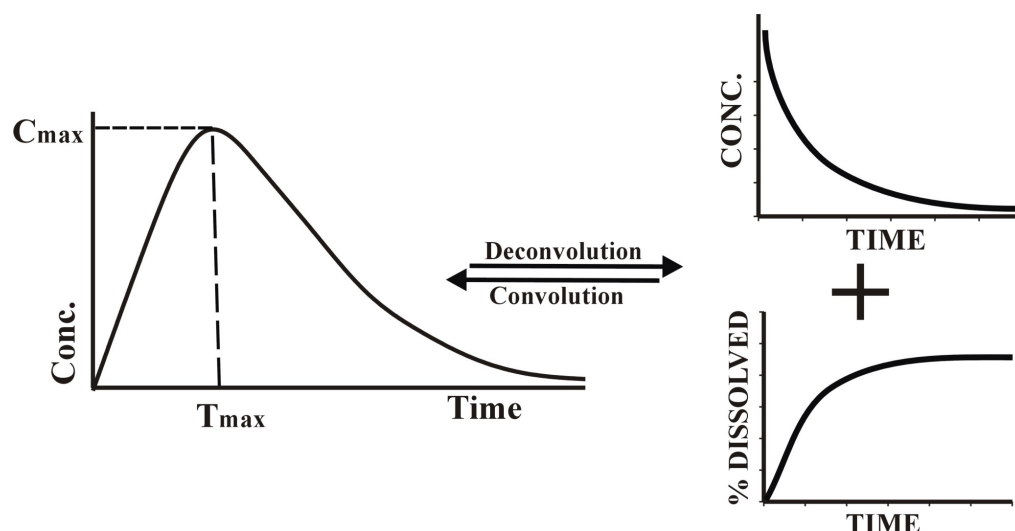
Fig. (4). Schematic representation of deconvolution and convolution processes. Convolution is the process of combined effect of dissolution and elimination of drug in the body to reflect blood drug concentration-time profile (right to left). On the other hand, extracting dissolution profiles from blood drug concentration-time profile is known as the deconvolution process (left to right).

this approach is that it often requires multiple products having potentially different *in vivo* release characteristics (slow, medium, fast). These products are then used to define experimental conditions (medium, apparatus etc.) for an appropriate dissolution test to reflect their *in vivo* behavior. This approach is more suited for method/apparatus develop-ment as release characteristics of test products are to be known (slow, medium, fast) rather product evaluation. (3) This technique requires blood data (human study) for the test products to relate it to *in vitro* results. Thus, it may not be used at the product development stage where the product is still to be developed based on dissolution testing.

On the other hand, convolution technique, less commonly reported in the literature, addresses all the above mentioned limitations and should provide simple and practical approach to develop IVIVC and product evaluation. A detail discussion of the scientific principles of this approach is described.

Developing IVIVC Based on Convolution Method

The convolution method uses *in vitro* dissolution data to derive blood drug levels using pharmacokinetic parameters of a test product. It is to be noted that the required pharmacokinetic parameters can be obtained from literature or from a standard text book of pharmacology [6]. However, before describing the methodology and practicality of the method, two additional pharmacokinetic parameters need to be explained.

Volume of Distribution

As stated earlier, once the drug is absorbed, it immedi-ately starts its distribution into the blood and different tissues throughout the body. The drug is administered in amounts or in mass units but measured in concentration units, therefore a volume parameter is necessary to relate the amount of drug administered and the observed concentration (concentra-tion=mass/volume). For this reason, the understanding of this volume parameter is critical.

Unlike the common practice, where volume is a physical space and measured experimentally e.g. using a measuring cylinder for liquids, in pharmacokinetics it is a virtual (imaginary) space. The volume of drug distribution in the body is often much larger than any physiological space or volume available. Therefore, most commonly, the concept of volume of distribution is described as an "apparent" volume of distribution and is denoted by V_d.

The concept of apparent volume of distribution may be understood with the help of the following analogy. Suppose, there is one litre of water in a container with one gram of drug dissolved in the water. The concentration of drug in a sample from the solution would be 1 mg/mL. However, if a small amount of charcoal is added to the container and it adsorbs 99% of the drug, only 1% (10 mg) of the drug will be left in solution form, with a measured concentration of 10 µg/mL. The original amount of drug in the container remains the same (1 gm) but at a measured concentration of 10 µg/mL. This means that a volume (apparent) must have in-creased from 1 L to 100 L. This charcoal analogy is often reflected in the body by different depots; for example fat tissue. Non-polar drugs usually reside in fat tissues, which result in low blood concentrations of such drugs. What this means is that even though large amounts of drug are deliv-ered to the body, the drug may not be available in the blood. Apparent volume of distribution is commonly reported as litre/kg of body weight.

Oral Bioavailability of a Drug

For a drug to be effective it has to be delivered into the blood stream (systemic circulation) so that it would reach the desired site of action to exert its therapeutic effect. The simplest form of drug delivery is by intravenous injection, that is directly into blood stream, so all the drug which is injected will be available, commonly termed as bioavail-ability. However, if the same amount of drug is administered in the GI tract, rarely will all of the drug be available in the blood, as some of the drug will be degraded in the GI tract

and still more will be metabolized (changed to other chemical entities) before appearing in the blood stream. Thus, usually a smaller amount of the drug will be available in the blood than that administered. The fraction of drug which is available in the blood after administration is called oral or absolute bioavailability and is denoted as "F". Again this is an API property and can be obtained from the literature.

Once one obtains the value of these parameters i.e., volume of distribution of the drug, oral bioavailability or F value, and the elimination rate equation one may be able to develop an *in vivo* drug profile, from the *in vitro* dissolution results.

Evaluating Blood Drug Concentration-Time Profiles

The ultimate objective of dissolution testing is to assess or compare the dissolution results from a test and reference products in a meaningful way reflecting its *in vivo* or physiological activity, which in this case are drug concentration-time profiles. There are well established methods for evaluating drug concentration-time profiles which are recognized by regulatory agencies around the world [7]. The most common approach used in this regard is based on two parameters, C_{max} (the maximum observed drug concentration) and AUC (area under the drug concentration-time profile).

Similarly, one can evaluate the calculated drug concentration-time profiles obtained from the *in vitro* dissolution results as one would do for the *in vivo* study using the C_{max} and AUC parameters.

To demonstrate the practical aspects of this approach, drug dissolution test were conducted for diltiazem products and a step-by-step procedure is provided for calculating expected drug levels.

EXPERIMENTAL METHODS AND MATERIALS

Pharmaceutical Products

Diltiazem products, 60-mg conventional- or immediate-released (IR) tablet and 120-mg extended-released (ER) capsule products were evaluated. These were obtained from the local Canadian market.

Dissolution Testing Instrumentation

The dissolution tests were conducted using a DISTEK 2100C system which comprised of a bath with six vessels and met the physical and mechanical specifications as noted in the USP.

The dissolution tests were conducted using the crescent-shaped spindles at 25 rpm in all cases [8].

Prior to use, the dissolution media were equilibrated at 37 °C overnight to deaerate the medium so that bubble formation, due to escape of dissolved gasses during the test, was minimized.

The dissolution medium (900 mL) used was distilled water. The amounts of diltiazem dissolved in each vessel were determined at various time intervals; up to three hours for the IR products and 24 hours for the ER products. The dissolution sampling was achieved using an automated

sampling system connected to an online UV diode-spectrophometer (Agilent 8453). The quantitation of diltiazem was done by ultraviolet absorbance at 240 nm, of filtered portions of the solutions under test, in comparison with a reference solution having a known concentration of diltiazem standard [9].

Data Analysis

The data were collated and analysed using Excel software (Microsoft, Seattle, WA)

RESULTS AND METHODOLOGY FOR CALCULATING BLOOD LEVELS

In the convolution approach, one needs to start with dissolution results or profiles and develops the *in vivo* or drug concentration-time profiles. In this regard, dissolution profiles of two diltiazem products, a 60 mg IR (immediate released type) and a 120-mg ER (extended released type), are shown in Fig. (**5**). These profiles are the typical outcomes of a dissolution test as described. As a first step towards developing the IVIVC, these profiles are to be converted into discrete dosage segments, which are shown in Table **1**, where at the end of each sampling time the amount of drug in mg is calculated, i.e., if 10% of the drug is released between sampling times then 6 or 12 mg of drug would be released for 60 or 120 mg products, respectively, using the formula (% drug release x product strength/100). As the bioavailability of diltiazem is about 44%, [6] then the blood should only see 44% of the dose. So, the observed amount of drug in the blood will be: amt = amt*bioavailability factor (F).
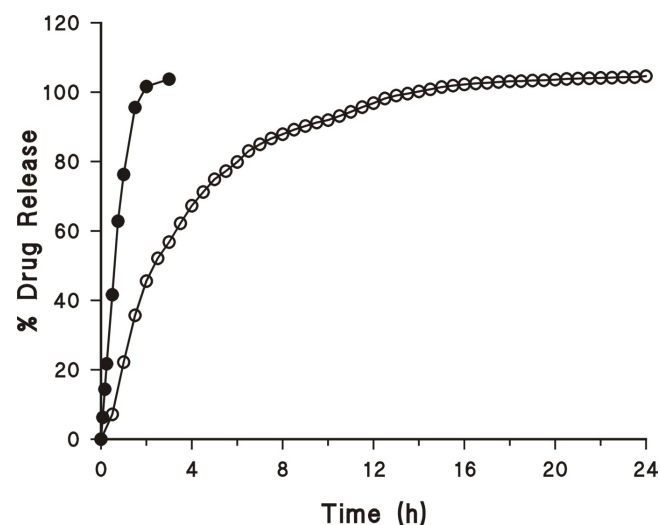
Fig. (5). Drug dissolution profiles of diltiazem products; (●——●, 60 mg IR tablets), (○——○, 120 mg ER capsules). The profiles were obtained using a vessel-based apparatus with a crescent-shaped spindle (25 rpm) using water (900 mL) as the dissolution medium.

As a next step and as described earlier, the amount after each sampling interval will immediately be absorbed and appear in the blood. Following absorption, the elimination phase starts with a first order elimination rate which is calculated from the half life of diltiazem [6] of 3.2 h using the

Table 1.   Percent Dissolution at Different Times from a 60 mg IR Tablet Product with Corresponding Percent and Amounts in mg Obtained within the Sampling Interval. Values Represent Averages of 6 Tablets

Time (h)	% Released (Cumulative)	% Released (within Sampling Interval)	Amt. (mg) Released (within Sampling Interval)
0.00	0.00		
0.08	6.28	6.28	3.77
0.17	14.40	8.12	4.87
0.25	21.73	7.33	4.40
0.50	41.62	19.89	11.93
0.75	62.82	21.20	12.72
1.00	76.28	13.46	8.08
1.50	95.58	19.30	11.58
2.00	101.62	6.04	3.62
3.00	103.74	2.12	1.27

formula ($k_e=0.693/t_{1/2}$). Each dosage, i.e., drug release in dissolution sampling interval, will have its individual profile as described in Table **2**. This example describes elimination based on single exponential equation, however, other equations consisting of multiple exponential components may equally be used by obtaining corresponding rate constants from the literature. The second last column in Table **2**, reflects total amount of drug present in the blood at different times after ingestion of tablet/capsule. The last column in Table **2**, reflects blood concentrations, equivalent to amounts shown in the previous column. This is obtained using the formula; (Conc.=Amt*F*1000/V_d*body weight). The amount is from the previous column, F (0.44, bioavailability factor), V_d (5.3 L/Kg, obtained from the literature [6]), body weight of 70 Kg, 1000 is conversion factor to report concentration in ng/mL rather μg/mL. For the ER product, the dissolution profile and amount released/absorbed at different times are shown in Fig. (**5**) and Table **3**, respectively. The process of obtaining the blood levels for ER product is exactly the same as that for the IR product, using the same pharmacokinetic parameters such as half life, V_d, bioavailability etc. The calculated drug levels in blood are shown in Table **4** and are drawn in the Fig. (**6**).

The pharmacokinetic parameters (C_{max}, t_{max}, and AUC) obtained from blood profiles were 58.13 ng/mL, 1.5 h, 307 ng/mL . h and 59.8 ng/mL, 4.0 h, 682 ng./mL . h, for IR and ER products, respectively.

DISCUSSION

Whenever an *in vitro* dissolution study is conducted, the explicit or implicit objective is to draw some inference regarding the drug's behaviour *in vivo*. This *in vivo* behaviour is represented by the drug concentration-time profile in humans. Therefore, predicting a drug concentration-time profile from dissolution results should indeed be considered as an endpoint for dissolution testing. There are established and recognized methods available to evaluate drug concentration-time profiles [2], based on C_{max} and AUC. These methods may also be used to evaluate drug concentration-time

profiles derived from dissolution results. No new or different mathematical or statistical approaches are needed.

The technique based on convolution, therefore, appears to provide a better endpoint than a deconvolution approach, in which a dissolution profile is extracted from an *in vivo* study and is compared with the *in vitro* dissolution profile. In the latter case, a separate set of criteria are required for evaluation of *in vitro* reflecting *in vivo* testing and these are not usually linked.

Although, elaborate mathematical and computing methods can be used to derive drug concentration-time profiles [4], an advantage of the convolution-based approach is that such profiles may be obtained with the use of simple spreadsheet software.

This report provides a step- by-step procedure for obtaining drug concentration-time profile from a dissolution curve using a commonly available spreadsheet program. It does, however, require some understanding of basic principles of pharmacokinetics, which are provided in this article as well.

Another advantage of using the convolution approach is that it does not require data from an *in vivo* study of the test product, and it is product independent. This indeed is a desired characteristic needed at the product development stage, where one needs to develop a product with desired *in vivo* characteristics based on only available *in vitro* results.

For the convolution approach, one would need only a dissolution profile, and a few simple pharmacokinetic parameters to obtain predicted blood drug levels. Once these blood levels are obtained, usual bioavailability parameters may be derived for these as for *in vivo* studies. These bioavailability parameters (C_{max} and AUC) would then be assessed using a well established protocol [2]. Therefore, there is no need for any other or new evaluation approaches.

The suggested method in the literature [4] to compare actual observed blood levels in human vs the calculated based on convolution technique is by using linear regression.

Table 2.   **Calculated Drug Levels at Different Times Following Absorption of Drug Released *In Vitro* During Sampling Intervals from 60-mg IR Tablets. Dissolution Values Represent Average of 6 Tablets**

Dissolution Sampling Time	0.08	0.17	0.25	0.50	0.75	1.00	1.50	2.00	3.00		
Amt. (mg) Equivalent	3.77	4.87	4.40	11.93	12.72	8.08	11.58	3.62	1.27		
Time after Absorption (h)	Blood Amt after Absorption									Total Blood Amt. (mg) after Absorption	Conc. (ng/mL) at Times
0.00											
0.08	3.77									3.77	4.47
0.17	3.70	4.87								8.57	10.16
0.25	3.63	4.79	4.40							12.82	15.20
0.50	3.44	4.54	4.17	11.93						24.07	28.55
0.75	3.26	4.30	3.95	11.30	12.72					35.52	42.13
1.00	3.09	4.07	3.74	10.71	12.05	8.08				41.72	49.48
1.50	2.77	3.65	3.35	9.61	10.81	7.25	11.58			49.01	58.13
2.00	2.48	3.28	3.01	8.62	9.70	6.50	10.39	3.62		47.60	56.45
3.00	2.00	2.64	2.42	6.94	7.81	5.23	8.36	2.92	1.27	39.59	46.95
4.00	1.61	2.12	1.95	5.58	6.28	4.21	6.73	2.35	1.02	31.86	37.79
5.00	1.30	1.71	1.57	4.49	5.06	3.39	5.42	1.89	0.82	25.65	30.42
6.00	1.04	1.37	1.26	3.62	4.07	2.73	4.36	1.52	0.66	20.64	24.48
7.00	0.84	1.11	1.02	2.91	3.28	2.20	3.51	1.22	0.53	16.62	19.71
8.00	0.68	0.89	0.82	2.34	2.64	1.77	2.83	0.99	0.43	13.38	15.86
9.00	0.54	0.72	0.66	1.89	2.12	1.42	2.27	0.79	0.35	10.77	12.77
10.00	0.44	0.58	0.53	1.52	1.71	1.15	1.83	0.64	0.28	8.67	10.28
11.00	0.35	0.46	0.43	1.22	1.38	0.92	1.47	0.51	0.22	6.98	8.27
12.00	0.28	0.37	0.34	0.98	1.11	0.74	1.19	0.41	0.18	5.62	6.66
13.00	0.23	0.30	0.28	0.79	0.89	0.60	0.95	0.33	0.15	4.52	5.36
14.00	0.18	0.24	0.22	0.64	0.72	0.48	0.77	0.27	0.12	3.64	4.31
15.00	0.15	0.20	0.18	0.51	0.58	0.39	0.62	0.22	0.09	2.93	3.47
16.00	0.12	0.16	0.14	0.41	0.46	0.31	0.50	0.17	0.08	2.36	2.80
17.00	0.10	0.13	0.12	0.33	0.37	0.25	0.40	0.14	0.06	1.90	2.25
18.00	0.08	0.10	0.09	0.27	0.30	0.20	0.32	0.11	0.05	1.53	1.81
19.00	0.06	0.08	0.08	0.22	0.24	0.16	0.26	0.09	0.04	1.23	1.46
20.00	0.05	0.07	0.06	0.17	0.20	0.13	0.21	0.07	0.03	0.99	1.17
21.00	0.04	0.05	0.05	0.14	0.16	0.11	0.17	0.06	0.03	0.80	0.94
22.00	0.03	0.04	0.04	0.11	0.13	0.08	0.14	0.05	0.02	0.64	0.76
23.00	0.03	0.03	0.03	0.09	0.10	0.07	0.11	0.04	0.02	0.52	0.61
24.00	0.02	0.03	0.03	0.07	0.08	0.05	0.09	0.03	0.01	0.42	0.49

Table 3.   Percent Dissolution at Different Times from a 120-mg ER Tablets Product with Corresponding Percent and Amounts in mg Obtained within the Sampling Interval. Values Represent Averages of 6 Tablets

Time (h)	% Released (Cummulative)	% Released (within Sampling Interval)	Amt. (mg) Released (within Sampling Interval)
0.00	0.00	0.00	0.00
0.50	7.17	7.17	8.60
1.00	22.20	15.03	18.04
1.50	35.68	13.48	16.18
2.00	45.55	9.87	11.84
2.50	52.12	6.57	7.88
3.00	56.80	4.68	5.62
.	.	.	.
.	.	.	.
.	.	.	.
23.00	104.32	0.14	0.17
23.50	104.33	0.01	0.01
24.00	104.62	0.29	0.35

Table 4.   Calculated Drug Levels at Different Times Following Absorption of Drug Released *In Vitro* During Sampling Intervals from 120-mg ER Tablets. Dissolution Values Represent Average of 6 Tablets

| Dissolution Sampling Time	0.5	1.0	1.5	2.0	2.5	3.0			23.0	23.5	24.0		
Amt. Released	8.60	18.40	16.18	11.84	7.88	5.62			0.17	0.01	0.35		
Time after Absorption (h)	Blood Amt (mg) after Absorption											Total Blood Amt (mg)	Conc. (ng/mL)
0.0							.	.					
0.5	8.60						.	.				8.60	10.20
1.0	6.92	18.40					.	.				25.32	30.03
1.5	6.21	14.81	16.18				.	.				37.20	44.12
2.0	5.57	13.29	13.02	11.84			.	.				43.72	51.86
2.5	5.00	11.92	11.68	10.62	7.88		.	.				47.11	55.87
3.0	4.49	10.70	10.48	9.53	7.07	5.62	.	.				47.88	56.79
.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.					.	.
23.0	0.06	0.14	0.14	0.12	0.09	0.07	.	.	0.17			4.26	5.05
23.5	0.05	0.13	0.12	0.11	0.08	0.07	.	.	0.15	0.01		3.83	4.55
24.0	0.05	0.11	0.11	0.10	0.07	0.06	.	.	0.14	0.01	0.35	3.79	4.49
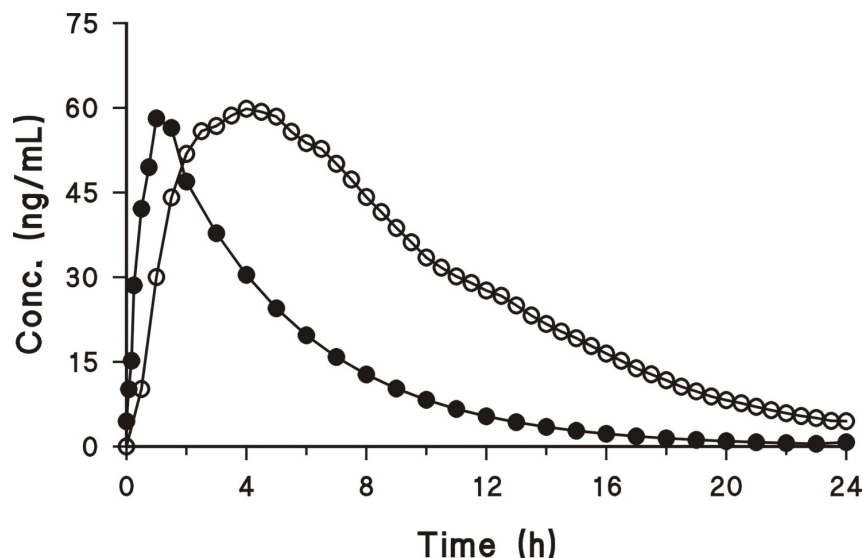
Fig. (6). Drug concentration profiles derived (calculated) from dissolution profiles for tested products: (●——●, 60 mg IR tablets), (○——○, 120 mg ER capsules).

Such an approach assumes similarity of variability of *in vitro* and *in vivo* systems, which may not be an appropriate assumption. *In vivo* systems are potentially highly variable compared to the *in vitro* systems, e.g. variabilities in vessel sizes, medium volumes and mixing rate may be far less variable than the corresponding physiological characteristics. Not only for the comparison between *in vitro* vs *in vivo* results, even within *in vivo* comparison for bioavailability/bioequivalence assessments, blood drug levels are not compared, because of the extreme variability within and between subjects. To address this high *in vivo* variability aspect, parameters such as C_{max} and AUC are used. These are in fact normalized parameters derived from drug concentration-time profiles. Therefore, rather than comparing blood levels for IVIVC, one should also evaluate C_{max} and AUC parameters.

The bioavailability parameters (C_{max} and AUC) obtained from the *in vitro* results (58 ng/mL and 307 ng/mL . h for 60 mg IR product and 60 ng/mL and 682 ng /mL . h for ER product), reported in this article, are similar to those reported in the literature for diltiazem from *in vivo* studies (45 ng/ml and 323 ng /mL . h for 60 mg IR product and 62 ng/mL and 671 ng/mL . h for ER product) [10]. This provides evidence for the validity of the approach. The dissolution characteristic observed for IR and ER products clearly differentiate blood levels representative of an IR and ER product with expected differences in t_{max} values. In addition, AUC differences reflect the differences in dosage strength (60 vs 120 mg) which again supports the validity of the approach.

If one would like to evaluate impact of formulation and/or manufacturing changes on the drug plasma levels of the product, one should evaluate dissolution characteristics of the test and reference products and corresponding blood levels should be calculated. These profiles would be evaluated based on bioavailability/bioequivalence assessment criteria as described earlier. If the profiles meet the bioequivalence criteria then it should be considered that the formulation/manufacturing changes had no negative

impact on the product quality otherwise they may. In the case of potential negative impact on bioequivalence, product manufacturing would require adjustment accordingly.

CONCLUSION

Based on the IVIVC concept, a simple and practical procedure is described here to obtain blood drug concentration-time profiles from dissolution results. The underlying principle of the convolution technique appears to provide a simple and practical approach for developing IVIVC. The drug concentration-time profiles obtained from dissolution results may be evaluated using criteria for *in vivo* bioavailability/bioequivalence assessment, based on C_{max} and AUC parameters.

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