Doctorado en Biomedicina y Farmacia

FACULTAD DE FARMACIA

UNIVERSIDAD DE VALENCIA

Validación de Procedimientos Poblacionales mediante Convolución para el Establecimiento de Correlaciones *In Vitro-In Vivo*

Ignacio González García

Directores:

Matilde Merino Sanjuán

María del Val Bermejo Sanz

Víctor Mangas Sanjuán

Doctorado en Biomedicina y Farmacia

FACULTAD DE FARMACIA

UNIVERSIDAD DE VALENCIA

Tesis doctoral

Validación de Procedimientos Poblacionales mediante Convolución para el establecimiento de Correlaciones *In Vitro-In Vivo*

Trabajo presentado por Ignacio González García para obtener el grado de Doctor en Farmacia

Fdo.: Ignacio González García

Dña. MATILDE MERINO SANJUÁN, Doctora en Farmacia y Catedrática de la Universidad de Valencia, Dña. MARÍA DEL VAL BERMEJO SANZ, Doctora en Farmacia y Catedrática de la Universidad Miguel Hernández de Elche y D. VÍCTOR MANGAS SANJUÁN, Doctor en Farmacia y profesor Ayudante Doctor en la Universidad de Valencia certifican que:

El presente trabajo: "Validación de Procedimientos Poblacionales mediante Convolución para el establecimiento de Correlaciones *In Vitro-In Vivo*", presentado por D. Ignacio González García para optar al grado de Doctor ha sido realizado bajo su dirección y una vez revisado, autorizan su presentación y su defensa.

Y para que así conste, firman el presente certificado en Valencia, a 21 de junio de 2018.

MERINO SANJUAN MARIA MATILDE - 73941910S
Firmado digitalmente por MERINO SANJUAN MARIA MATILDE - 73941910S
Fecha: 2018.06.21 11:53:51 +02'00'

Fdo.: Matilde Merino Sanjuán

Maria del Val Bermejo Sanz
Firmado digitalmente por Maria del Val Bermejo Sanz
Fecha: 2018.06.21 10:11:55 -04'00'

Fdo.: María del Val Bermejo Sanz

Victor Mangas Sanjuan
Firmado digitalmente por Victor Mangas Sanjuan
Fecha: 2018.06.21 12:09:37 +02'00'

Fdo.: Víctor Mangas Sanjuán

Doctorado en Biomedicina y Farmacia

FACULTAD DE FARMACIA

UNIVERSIDAD DE VALENCIA

Tesis doctoral

Validación de Procedimientos Poblacionales mediante Convolución para el establecimiento de Correlaciones *In Vitro-In Vivo*

Ignacio González García

Valencia, 2018

A todos a los que me habéis permitido aprender
de vosotros en estos cinco años

A mi familia

A Lucía

"Lo importante en la ciencia no es tanto obtener nuevos datos,
sino descubrir nuevas formas de pensar sobre ellos."
William Lawrence Bragg

Este proyecto ha sido parcialmente subvencionado por “MODELOS IN VITRO DE EVALUACION BIOFARMACEUTICA” SAF2016-78756 (AEI/FEDER, EU) financiado por la Agencia Estatal Investigación y la Unión Europea, a través de FEDER (Fondo Europeo de Desarrollo Regional).

LOS RESULTADOS OBTENIDOS EN ESTA TESIS DOCTORAL HAN DADO LUGAR A LAS SIGUIENTES PUBLICACIONES

- Gonzalez-Garcia I, Mangas-Sanjuan V, Merino-Sanjuan M, Bermejo M. In vitro-in vivo correlations: general concepts, methodologies and regulatory applications. Drug Dev Ind Pharm. 2015;41(12):1935-47.

- González-García I, Mangas-Sanjuan V, Merino-Sanjuán M, Álvarez-Álvarez C, Díaz-Garzón Marco J, Rodríguez-Bonnín MA, et al. IVIVC approach based on carbamazepine bioequivalence studies combination. Die Pharmazie - An International Journal of Pharmaceutical Sciences. 2017;72(8):449-55.

- Gonzalez-Garcia I, Garcia-Arieta A, Merino-Sanjuan M, Mangas-Sanjuan V, Bermejo M. Defining level A IVIVC dissolution specifications based on individual in vitro dissolution profiles of a controlled release formulation. Eur J Pharm Sci. 2018;119:200-7.

AGRADECIMIENTOS

En los 5 años que ha durado este proyecto he tenido la suerte cruzarme con diferentes personas, y de todas ellas he podido aprender cosas que me han hecho crecer tanto en el ámbito profesional como personal, por ello quisiera agradecer:

A mi directora Marival. El día que me propusiste el tema no tenía ni idea de qué me estabas hablando. Pero todo lo que sé sobre las IVIVC te lo debo a ti. Porque durante la carrera fuiste una gran profesora y te convertiste en una mejor directora.

A mi directora Mati. Porque, aún en la distancia, siempre estuviste pendiente de mí.

A mi director Víctor. Has sido el que más me ha sufrido en este tiempo. Al que primero llamaba cuando algo no me salía y el que primero me llamaba para ponerme las pilas. Hemos discutido mucho, pero creo que los dos hemos aprendido mucho con esas discusiones.

Al Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Valencia.

Al Área de Farmacia y Tecnología Farmacéutica de la Universidad Miguel Hernández de Elche. En especial, a Marta, Sarín, Alejandro, Vero, Isa Lozoya, Adrián, Mayte y Andrés. Con todos vosotros empecé esta aventura, y aunque mi paso por la Universidad Miguel Hernández fue breve, me llevo conmigo un poco de cada uno de vosotros.

A María, Ana e Hilda. Sois las que durante más tiempo me habéis aguantado. Desde la carrera hasta el doctorado. En Alicante y en Pamplona. Gracias por vuestra ayuda, vuestros consejos y por haberme cuidado tan bien en mi estancia por el norte.

Al Pharmacometrics and System Pharmacology Group y demás gente del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Navarra. En primer lugar, al Profesor Iñaki F. Trocóniz, por darme la oportunidad de haber formado parte de ese gran grupo de investigación. Y cómo no, a toda la gente que conocí allí, Itziar, Leire, Violeta, JD, Belén, María y Ana Margarita; y también a los que ya conocía, Barce, Simón y Yolanda. Muchas gracias por haberme hecho la estancia mucho más fácil. Son muy buenos los momentos que me llevo de mi estancia allí.

A Hugo y Guille, por ser unos magníficos caseros y compañeros de piso.

A Carlos, por ser también un gran casero y mejor compañero. Porque cuando te llamo con cualquier duda, me preguntas si ya soy doctor. He aprendido de ti más de lo que crees.

A Ana. Desde el minuto uno te volcaste en mí de la forma más desinteresada. En estos años, han sido muchas las ocasiones en las que te he asaltado con todo tipo de dudas, y tú con una paciencia infinita y la mejor de tus sonrisas has estado dispuesta a resolvérmelas.

A Isa. Te he dejado la última por un motivo. Porque gracias a ti hoy estoy aquí. Si no hubiera sido por vuestra dedicación en las clases y por la frase de: "si alguien está interesado en hacer prácticas con nosotras que se pase por el despacho", es muy pero que muy probable que jamás hubiera llamado a esa puerta. No has sido mi directora, pero te has comportado como tal.

ABREVIATURAS

Abreviatura	Definición
AUC	Área bajo la curva
BCS	Sistema de clasificación biofarmacéutica
BE	Bioequivalencia
CBZ	Carbamazepina
C_{max}	Concentración máxima
CV	Coeficiente de variación
EMA	Agencia Europea de Medicamentos
EP	Error de predicción
f_2	Factor de similitud
FDA	Agencia americana *U.S. Food and Drug Administration*
FTFF	Comprimido más rápido de la formulación más rápida
IVIVC	Correlación *in vitro – in vivo*
k_a	Constante de velocidad de absorción
k_d	Coeficiente de velocidad de disolución
k_{el}	Constante de velocidad de eliminación
MDT	Tiempo medio de disolución
MRT	Tiempo medio de residencia
PK	Farmacocinéticos
STSF	Comprimido más lento de la formulación más lenta

RESUMEN

Para desarrollar medicamentos que sean efectivos y seguros es necesario conocer su comportamiento tanto *in vitro* como *in vivo*. Uno de los desafíos en la investigación biofarmacéutica es correlacionar la información de liberación *in vitro* de un fármaco con los perfiles plasmáticos *in vivo*. Por lo tanto, durante los últimos años se ha incrementado el uso de modelos matemáticos que sean capaces de predecir el comportamiento *in vivo* de un fármaco a través de los datos de disolución *in vitro*, es decir, correlaciones *in vitro - in vivo* (IVIVC). Las IVIVC pueden acortar la duración del desarrollo de los medicamentos, así como reducir el gasto y mejorar la calidad del producto.

El principal objetivo de una IVIVC es predecir el comportamiento *in vivo* a partir de datos *in vitro*, como predictor de la biodisponibilidad *in vivo* y para permitir la solicitud de bioexenciones. Por este motivo, las IVIVC suelen utilizarse para cuantificar la liberación in vivo del fármaco y los efectos sobre la absorción relacionados con la formulación, establecer las especificaciones de disolución y la relevancia clínica de la disolución *in vitro* y para solicitar una bioexención.

Teniendo en cuenta el tipo y calidad de los datos obtenidos se pueden desarrollar diferentes niveles de correlación. La calidad de la IVIVC dependerá a su vez de su capacidad para predecir el comportamiento *in vivo*. El nivel más bajo de correlación (nivel B) únicamente es capaz de proporcionar un parámetro que refleje el comportamiento *in vivo* (generalmente el tiempo medio de residencia); mientras que el nivel más alto de correlación (nivel A) es capaz de predecir el perfil farmacocinético completo. Del mismo modo que existen diferentes niveles de correlación, también existen diferentes métodos matemáticos que pueden utilizarse para conseguir una IVIVC. Una descripción más detallada de los métodos y de las ventajas e inconvenientes de cada uno de ellos se puede encontrar en la sección correspondiente de este trabajo.

En esta Tesis Doctoral se ha llevado a cabo:

- Una revisión bibliográfica que ha permitido aunar en un único documento las diferentes definiciones y aplicaciones de las IVIVC, las recomendaciones de las diferentes guías regulatorias, una descripción minuciosa de los diferentes métodos existentes para el desarrollo de una correlación *in vitro - in vivo*, y las consideraciones a tener en cuenta a la hora de desarrollar el método de disolución *in vitro* cuando se intenta establecer una IVIVC.

- El desarrollo de diferentes niveles de correlación utilizando datos *in vivo* provenientes de dos ensayos de bioequivalencias diferentes y datos de disolución *in vitro* obtenidos en dos laboratorios diferente. Además, se hizo una comparación de la predictibilidad del modelo cuando se utilizan datos promediados o datos individuales.

- El desarrollo y aplicación de una nueva aproximación para el establecimiento de las especificaciones de disolución cuando previamente se ha desarrollado una IVIVC nivel A. Además, se compararon estos resultados con los obtenidos si se hubieran establecido las especificaciones de disolución siguiendo las recomendaciones de las guías.

ÍNDICE

Introducción

ANTECEDENTES

Una gran proporción de los medicamentos desarrollados y comercializados en los últimos años son medicamentos destinados a su administración por vía oral, ya que esta vía presenta ventajas frente a otras vías de administración. Entre las ventajas más importantes de la formulación oral resaltar la comodidad para el paciente, lo que favorece la adherencia al tratamiento, y por lo tanto mejora el cumplimiento del mismo; así como el menor coste de desarrollo para la industria farmacéutica. En la mayoría de formas farmacéuticas de administración oral, el medicamento debe disolverse en los fluidos intestinales como un paso esencial antes de su absorción a través de la membrana gastrointestinal. El impacto de la disolución sobre la biodisponibilidad oral quedó demostrado a partir de los estudios realizados en la década de 1950 (1), siendo posible relacionar los parámetros de disolución obtenidos mediante modelos *in vitro* y los obtenidos *in vivo* a partir de los perfiles de concentración plasmática de fármaco frente al tiempo mediante la utilización de diferentes modelos matemáticos.

Para la industria farmacéutica resulta de gran interés encontrar, en las primeras fases del desarrollo del medicamento, métodos *in sílico* o *in vitro* capaces de predecir el comportamiento en la disolución/absorción *in vivo,* ya que de esta forma en la fase inicial del desarrollo del medicamento sería posible disponer de información capaz de predecir una óptima absorción del principio activo, aspecto crucial en las últimas fases del desarrollo clínico del medicamento. Además, conocer de una forma más precisa los mecanismos involucrados en la disolución del fármaco permite una toma de decisiones más racional y eficiente durante el proceso de desarrollo clínico. Por ello, en las últimas décadas se han desarrollado diferentes métodos de disolución capaces de reproducir los procesos de disolución *in vivo* (2-19).

Las correlaciones *in vitro – in vivo* (IVIVC) se definen como un modelo matemático predictivo que relaciona una propiedad de un medicamento obtenida *in vitro* con una respuesta relevante al mismo obtenida después de su administración *in vivo.* Generalmente, la propiedad *in vitro* utilizada es la velocidad o la cantidad de principio activo disuelto o liberado, mientras que la respuesta *in vivo* es la concentración del mismo en plasma o su cantidad absorbida.

APLICACIONES DE LAS IVIVC

Diferentes trabajos describen los conceptos y aplicaciones de las IVIVC (20-25), así como los aspectos regulatorios recogidos en las guías publicadas tanto por la Agencia Europea de Medicamentos (EMA) como por la Agencia americana *U.S. Food and Drug*

Administration (FDA) (26-29). De acuerdo con estas, las principales aplicaciones de las IVIVC son:

- Cuantificar la liberación *in vivo* del fármaco y los efectos sobre la absorción relacionados con la formulación. Además, pueden servir como herramienta en el control de calidad para ciertos procesos de escalado y cambios post-aprobación permitidos en la guía de la FDA SUPAC-MR (29).

- Establecer las especificaciones de disolución y la relevancia clínica de la disolución *in vitro*. Las IVIVC se utilizan como soporte para establecer las especificaciones de disolución de la molécula, estableciendo un rango de porcentajes de principio activo disuelto a lo largo de todo el perfil de disolución.

- Estimar el comportamiento *in vivo* en base a los ensayos de disolución *in vitro* una vez ha sido establecida la IVIVC. Mediante esta aplicación, se podría solicitar una bioexención y de esta forma se evitaría la realización de nuevos estudios de bioequivalencia (BE) tras procesos de escalado y cambios post-aprobación, reduciendo el coste y el tiempo de desarrollo de medicamentos, además de las ventajas éticas asociadas.

NIVELES DE LAS IVIVC

En las guías de la FDA (27) y de la EMA (30) se describen los diferentes niveles de correlación en función de su capacidad de relacionar el componente *in vitro* con el componente *in vivo*.

- **Nivel A.** Es el nivel de correlación más alto y representa una relación punto a punto entre la velocidad de disolución *in vitro* y la velocidad de absorción *in vivo* de un fármaco desde la forma de dosificación que lo contiene (31). El objetivo de este nivel de correlación es predecir el perfil completo de concentración-tiempo *in vivo* a partir de la curva de disolución *in vitro*. Las correlaciones más habituales son las que muestran una relación lineal entre la disolución *in vitro* y la entrada al organismo *in vivo*, siendo además dos curvas superponibles (relación 1:1). Si esto no ocurre, se pueden introducir factores de escalado para hacerlas superponibles. Las IVIVC no lineales son menos frecuentes, pero se deben utilizar si la relación lineal no fuese capaz de explicar la relación existente entre los perfiles *in vitro* y los perfiles *in vivo* (20).

- **Nivel B.** En el nivel de correlación B se compara el tiempo medio de disolución del perfil promedio *in vitro* con el tiempo medio de residencia del perfil promedio

in vivo (32). La correlación nivel B utiliza todos los datos procedentes de los estudios realizados *in vitro* e *in vivo*; sin embargo, no se considera una correlación punto a punto, ya que la relación se obtiene entre un parámetro *in vitro* y un parámetro *in vivo*. Desde el punto vista regulatorio, es el nivel más bajo de correlación, ya que no es capaz de predecir el comportamiento *in vivo*. Es por ello que los datos *in vitro* de dicha correlación no pueden utilizarse para justificar comportamientos extremos en los estándares de control de calidad (27, 31).

- **Nivel C.** El nivel de correlación C se encuentra en un punto intermedio entre los dos niveles anteriores. En este caso, la correlación se produce entre un parámetro de disolución *in vitro* y un parámetro *in vivo*. Este nivel de correlación tampoco es capaz de predecir el perfil completo de concentración-tiempo, y por ese motivo tampoco puede usarse como herramienta predictiva en cambios de producto, lugar de fabricación, ni como elemento que justifique el comportamiento extremo en los controles de calidad estándares (31). Con mayor capacidad predictiva frente al nivel C se encuentra el **nivel C múltiple**, en el cual la relación se produce entre varios parámetros *in vitro* e *in vivo* a diferentes tiempos de muestreo del perfil de disolución. Estos tiempos de muestreo deben de cubrir la fase inicial, media y final del proceso de disolución. Una correlación nivel C múltiple puede servir como justificante de bioexención, aunque dicha correlación se debe establecer utilizando el perfil de disolución *in vitro* completo y varios parámetros farmacocinéticos (PK) obtenidos a partir del tratamiento de datos obtenidos *in vivo*. Al haberse establecido una relación entre los parámetros *in vitro* e *in vivo* a los mismos tiempos de muestreo, se podría evaluar el efecto *in vivo* ante cualquier modificación en el proceso de disolución (27). Generalmente, si puede establecerse una correlación nivel C múltiple, el desarrollo de una correlación nivel A es igualmente posible.

MÉTODOS PARA ESTABLECER UNA IVIVC

En la literatura se pueden encontrar diferentes métodos para establecer una IVIVC nivel A (33), pero por regla general todos comparten unas etapas comunes: desarrollar un modelo estructural capaz de relacionar las cantidades disueltas *in vitro* y las cantidades disueltas *in vivo*. Las primeras se utilizan como variables de entrada (disolución *in vitro*) y las segundas como variables de salida (disolución *in vivo*), para posteriormente estimar los parámetros del modelo (32). Cada uno de los métodos propuestos vienen mencionados en las guías de la FDA (27) y la EMA (30) y se dividen en métodos de dos etapas y métodos de una etapa.

Los métodos de dos etapas son los que se aplican con mayor frecuencia y son los requeridos por la FDA para establecer una IVIVC de nivel A. En la primera etapa se utiliza un método de deconvolución para estimar la absorción o disolución *in vivo* a lo largo del tiempo. En la segunda etapa, se establece un modelo que enlaza los perfiles de disolución *in vitro* con los perfiles de absorción *in vivo*. Posteriormente, utilizando este modelo, se predicen las concentraciones de plasma a partir de los datos de disolución *in vitro*.

Una de las desventajas que tiene este tipo de métodos es que únicamente se pueden aplicar a sistemas lineales, es decir, a sistemas que son superponibles y cumplen el requisito de invarianza en el tiempo. A su vez, los métodos de dos etapas pueden diferenciarse entre métodos de deconvolución modelo-independiente y métodos modelo-dependiente. La principal diferencia es que estos últimos asumen un modelo cinético previo sobre la disposición del fármaco (Wagner-Nelson para modelos farmacocinéticos monocompartimentales y Loo-Riegelmann para modelos bicompartimentales). Sin embargo, los métodos de deconvolución modelo-independiente no asumen ningún modelo cinético sobre la disposición del fármaco en el organismo.

Métodos de deconvolución modelo-dependiente

Wagner-Nelson

El método de Wagner-Nelson sólo puede aplicarse a fármacos monocompartimentales (1). La ventaja de este método (ECUACIÓN 1) es que los cálculos se pueden realizar a partir de las curvas de concentración plasmática-tiempo tras una administración oral sin necesidad de disponer de datos procedentes de una administración intravenosa, ya que se basa en la premisa de que la constante de velocidad de eliminación (k_{el}) se puede obtener a partir de la fase terminal de la curva oral, siempre y cuando la constante de velocidad absorción (k_a) sea mayor que la k_{el}. Además, se asume que no existen variaciones relevantes entre las cinéticas de disposición en las dos administraciones por cada sujeto.

$$F_{abs} = \frac{A_t}{A_\infty} = \frac{C_t + k_{el} \cdot AUC_0^t}{k_{el} \cdot AUC_0^t} \qquad (1)$$

ECUACIÓN 1. La ecuación de Wagner-Nelson representa la fracción absorbida de la dosis biodisponible a tiempo t.

Loo-Riegelmann

El análisis de datos utilizando el método de Loo-Riegelmann se aplica para fármacos con una cinética bicompartimental (34) y para realizar adecuadamente el balance de masas es necesario tener en cuenta la cantidad de fármaco en el compartimento periférico.

$$P_t = k_{12} \cdot e^{-k_{21} \cdot t} \cdot \int_0^t C \cdot e^{k_{21} \cdot t} \cdot \partial t \qquad (2)$$

ECUACIÓN 2. Solución exacta de la ecuación de Loo-Riegelman.

$$P_t = P_{t-1} \cdot e^{-k_{21} \cdot \Delta t} + \frac{k_{12}}{k_{21}} \cdot C_{t-1} \cdot \left(1 - e^{-k_{21} \cdot \Delta t}\right) + \frac{k_{12}}{2} \cdot \Delta C \cdot \Delta t \ (3)$$

ECUACIÓN 3. Solución aproximada de la ecuación de Loo-Riegelman.

La ECUACIÓN 2 es la solución exacta de la ecuación de Loo-Riegelman, que fue publicada por Wagner JG en 1967 (35), y la ECUACIÓN 3 es una solución aproximada que se puede utilizar cuando los intervalos de muestreo son cortos y los cambios entre las concentraciones entre dos puntos de muestreo sigan una función lineal.

Métodos de deconvolución modelo-independiente

Estos métodos de deconvolución no asumen ningún modelo cinético para explicar la disposición del fármaco. La deconvolución se aplica en sistemas lineales, esto es, la integral de convolución es la definición matemática de un sistema lineal.

Un sistema está caracterizado por una entrada o pulso (que corresponde a la zona de absorción) y una respuesta, considerando respuesta como la variable que se mide debido al impulso. La función de entrada más importante es el impulso unitario. En términos matemáticos es conocida como la función (δ). Un bolus intravenoso es una buena aproximación o descripción de un impulso unitario. La respuesta se denomina función respuesta al impulso unitario o $C_\delta \cdot T$

La respuesta al impuso unitario es el resultado de un impulso dividido por su magnitud. En términos prácticos es el perfil concentración-tiempo obtenido por una administración de un bolus intravenoso dividido por la dosis administrada (36, 37).

$$C(t) = \int_0^t f(t) \cdot C_\delta \cdot (t - \tau) \cdot d\tau \qquad (4)$$

ECUACIÓN 4. Integral de convolución.

La ECUACIÓN 4 muestra la integral de convolución donde C es la concentración de fármaco a tiempo t, C_δ es la respuesta al impulso unitario, y f es la velocidad de disolución.

La deconvolución puede ser empleada para estimar una función de entrada dada la correspondiente respuesta del sistema y la respuesta al impulso unitario del sistema. La respuesta al impulso unitario tiene que ser obtenida a través de la administración de una formulación de referencia. Habitualmente la formulación de referencia consiste en la administración de una formulación intravenosa, pero también puede utilizarse una solución oral o bien otras formulaciones orales de liberación inmediata.

Con cualquiera de los métodos anteriormente descritos, se puede obtener el perfil de absorción *in vivo*. A continuación, se debe establecer la correlación *in vitro-in vivo* con el objetivo de predecir, en una segunda etapa, las concentraciones de fármaco en plasma a partir de los datos *in vitro*. Las fracciones disueltas predichas *in vivo* tienen que ser convueltas de nuevo con los estimados finales de los parámetros farmacocinéticos obtenidos por los datos de la referencia para calcular los perfiles de concentración plasmática (38).

Métodos de convolución

Los métodos de convolución se basan en modelos en una sola etapa, en la cual directamente se relaciona la liberación *in vivo* con la liberación *in vitro*. La ecuación sobre la cual giran estas aproximaciones reside en la integral de convolución (37, 39). Las bases y las ecuaciones de este método han sido descritas con detalle en varias publicaciones (33, 37, 39, 40). Para estos métodos, se debería utilizar una formulación de referencia, aunque la correlación también puede desarrollarse sin disponer de estos datos. La ventaja frente a los métodos en dos etapas es que la relación entre la liberación *in vitro* y las concentraciones de fármaco en plasma se establecen en un solo

paso, por lo que el modelado puede centrarse en la capacidad predictiva del comportamiento *in vivo* (37, 39).

En este contexto, el principal objetivo de una IVIVC es establecer una dependencia funcional que relacione una velocidad entrada *in vivo* (liberación o absorción), F_{i2}, a una velocidad de disolución, F_{i1}. La opción más sencilla es la aproximación lineal (*ECUACIÓN 5*):

$$F_{i2}(t) = F_{i1}(t) \quad (5)$$

ECUACIÓN 5. Función de relación entre la velocidad de disolución *in vitro* e *in vivo*.

De acuerdo a lo descrito por Dunne et al. (40), la relación entre la disolución *in vivo* e *in vitro* se puede expresar en términos de una relación entre las funciones de distribución o equivalentemente a una relación entre funciones relacionadas, tales como la función Odds, la función Hazard o la función de Hazard inversa.

Modelos basados en ecuaciones diferenciales

Una de las asunciones de los métodos de convolución y deconvolución es que el sistema es lineal, pero no siempre ocurre de esa manera. Cuando el sistema es lineal, los métodos de convolución y los modelos de ecuaciones diferenciales son matemáticamente equivalentes (33). Pero se sabe que numerosos fármacos se absorben o eliminan por mecanismos que implican procesos saturables (no lineales) (41, 42). La aproximación compartimental, que utiliza ecuaciones diferenciales, puede ser la solución para este tipo de compuestos. Además, permite incorporar efectos aleatorios a la IVIVC, tales como fenómenos tiempo-dependientes, factores de escalado, etc. (38).

VENTAJAS Y LIMITACIONES DE LOS MÉTODOS PARA ESTABLECER UNA IVIVC

Existen varios trabajos que exponen de manera muy clara las limitaciones de los métodos en dos etapas (Wagner-Nelson y Loo-Riegelmann y métodos modelo-independiente) (36, 40, 43-46), y podrían resumirse en:

- Los datos observados son promediados a cada punto de muestreo, lo cual resulta una pérdida considerable de información.

- Los datos *in vivo* y los datos *in vitro* deben recogerse a los mismos tiempos, ya que únicamente los datos con tiempos comunes se pueden utilizar en el análisis.

- El proceso de deconvolución es por sí mismo inestable.

- La deconvolución predice la fracción de fármaco disuelto *in vivo* en lugar de las concentraciones plasmáticas, las cuales aportan una información mucho más relevante.

- Para poder predecir las concentraciones plasmáticas es necesario convolver de nuevo las fracciones absorbidas.

- Asume linealidad del sistema e invarianza en el tiempo.

- No se cumplen dos de las principales asunciones sobre mínimos cuadrados.

    - La variable independiente (generalmente la fracción disuelta *in vitro*) se mide sin error.

    - Se requiere que todas las observaciones no estén correlacionadas o sean independientes.

- La administración de una formulación intravenosa es imprescindible sobre todo para fármacos con una cinética no monocompartimental.

A pesar de estas limitaciones, en la literatura es posible encontrar gran cantidad de artículos donde se han utilizado los métodos de Wagner-Nelson y Loo-Riegelmann para relacionar la absorción *in vivo* con la disolución *in vitro* (5, 6, 8, 10, 14, 47-52).

Por otro lado, la deconvolución modelo-independiente ha ganado popularidad en los últimos años, ya que permite una mayor flexibilidad, ya que no se asume ningún modelo farmacocinético previo para describir la evolución temporal de las concentraciones plasmáticas del fármaco (3, 4, 10-12, 15-18, 40, 45, 53-65). No obstante, tiene la misma limitación que los métodos anteriores, y es que se utilizan datos promedios en lugar de los datos individuales obtenidos a partir de cada unidad del medicamento ensayado.

Por el contrario, la convolución no requiere que los datos estén recogidos a los mismos tiempos, predice las concentraciones plasmáticas directamente en un solo paso y utiliza los datos individuales. Sin embargo, al igual que en el método de deconvolución, asume linealidad del sistema e invarianza en el tiempo. Y, aunque de momento el número de

trabajos publicados utilizando este método no es muy relevante, en la actualidad existen numerosos programas informáticos que permiten implementar los métodos de convolución lo que puede considerarse un apoyo que emerge desde la comunidad científica dirigido a potenciar la utilización de estos métodos (3, 37, 38, 40, 48, 66-70).

Los métodos con ecuaciones diferenciales ofrecen una mayor flexibilidad permitiendo cinéticas no lineales e incluso, procesos de varianza en el tiempo. Además, propuestas semi-mecanicista permiten el uso de datos individuales con el fin de evaluar la variabilidad interindividual y residual del modelo (38, 46, 71).

EVALUACIÓN DE LA PREDICCIÓN DE LAS IVIVC

Una vez se ha establecido la IVIVC, es necesario evaluar su capacidad de predicción antes de su utilización como predictor del comportamiento *in vivo*. Generalmente, la capacidad de predicción de un modelo se determina a través del error de predicción (EP), el cual se calcula comparando los parámetros observados *in vivo* con los parámetros predichos. En último término, el objetivo de la evaluación de la IVIVC es determinar la magnitud del error de predicción de la biodisponibilidad *in vivo* a partir de los datos de disolución *in vitro* (20).

La validación interna consiste en evaluar los EP después de comparar los parámetros observados *in vivo* (utilizados para desarrollar la IVIVC) frente a los parámetros predichos *in vivo* (obtenidos a partir de la IVIVC). El % de EP se calcula según la *ECUACIÓN 6*:

$$\%EP = \frac{(Parámetro\ Observado - Parámetro\ Predicho)}{Parámetro\ Observado} \cdot 100 \qquad (6)$$

ECUACIÓN 6. Cálculo del error de predicción de las IVIVC.

De acuerdo con las guías de la FDA y la EMA, la capacidad de predicción de una IVIVC se considera adecuada cuando el EP medio absoluto de todas las formulaciones, expresado en porcentaje, es inferior al 10% y el % de error de predicción individual de cada formulación no supere el 15%.

La validación externa sirve para evaluar si las IVIVC pueden ser utilizadas como sustitutivos del ensayo de BE y se lleva a cabo utilizando un conjunto de datos que no haya sido utilizado para desarrollar la IVIVC. El EP (%) se calcula de la misma manera

que en la validación interna (*ECUACIÓN 6*). De acuerdo con las guías de la FDA y la EMA (26, 27):

- Los errores de predicción situados por debajo del 10% indican buena capacidad predictiva de la IVIVC.
- Los errores de predicción entre el 10-20% señalan una capacidad no concluyente y necesitan de estudios adicionales.
- Los errores de predicción superiores al 20% implican baja o mala capacidad predictiva de la IVIVC.

Aunque la EMA insiste en la aplicación de la validación externa como evaluación final de la IVIVC, la FDA no exige validación externa si la IVIVC ha superado con éxito la validación interna (26, 27).

RECOMENDACIONES DE LAS AGENCIAS REGULATORIAS SOBRE LAS IVIVC

La guía titulada "*Extended release oral dosage forms: development, evaluation and application of in vitro/in vivo correlations"* publicada por la FDA en septiembre de 1997 fue la primera guía sobre IVIVC por parte de una agencia regulatoria de impacto mundial. En octubre de 2012, la EMA publicó la guía titulada "*Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms".* Ambas incluyen numerosas recomendaciones sobre aspectos fundamentales para desarrollar una IVIVC, desde conceptos generales sobre los niveles de IVIVC aceptados, modelos matemáticos recomendados para establecer una IVIVC y consideraciones sobre la validación de la IVIVC, hasta aspectos sobre el número de individuos *in vivo* o vasos necesarios *in vitro*, el número y características de las formulaciones y las condiciones de los ensayos de disolución, entre otros.

Aunque la FDA y la EMA recomiendan al menos dos formulaciones con diferentes velocidades de liberación para poder desarrollar una IVIVC, la mayoría de los trabajos publicados utilizan tres formulaciones (disolución lenta, media y rápida). Además, desarrollar una IVIVC utilizando tres o más formulaciones aporta mayor robustez y precisión, y por tanto, pueden esperarse mejores resultados en la validación externa (20, 28).

ESPECIFICACIONES DE DISOLUCIÓN

Las especificaciones de disolución se establecen como rango de disolución que garantiza la consistencia entre lotes durante el proceso de fabricación y permiten detectar desviaciones que puedan afectar a la biodisponibilidad *in vivo* de la formulación. Según estas guías, existen diferentes métodos para calcular dichas especificaciones dependiendo del nivel correlación existente:

- **Cuando no se dispone de IVIVC**. Cualquier punto de la disolución no debe de tener una diferencia mayor a ±10% con respecto al perfil *in vitro* promedio.

- **Nivel A**. Las especificaciones deben establecerse utilizando los datos promedio, permitiendo una diferencia máxima de ±20% entre los parámetros predichos (área bajo la curva [AUC] y concentración máxima [C_{max}]).

- **Nivel C múltiple**. Se establecen las especificaciones de disolución a cada tiempo para que las diferencias entre el AUC y C_{max} no superen el ±20%. Además, en el último tiempo medido se debe haber disuelto, al menos, el 80% de la cantidad de fármaco que contiene la forma de dosificación.

- **Nivel C simple**. Únicamente se debe de utilizar un punto de la disolución *in vitro*, con el cual se deben obtener unos valores de AUC y C_{max} que no difieran más de un ±20% con respecto al perfil promedio. Además, el resto de puntos no debe diferenciarse más de un ±10%.

En todos estos casos se utiliza el perfil promedio de disolución. Sin embargo, independientemente del nivel de correlación existente, el tratamiento de datos habitual genera una pérdida de información que puede dar lugar a conclusiones sesgadas. Es por ello que resulta conveniente desarrollar un método capaz de establecer las especificaciones de disolución teniendo en cuenta tanto la variabilidad *in vitro* como la variabilidad *in vivo* de tal manera que se determine de forma más adecuada la bioequivalencia de una formulación o de sus lotes mediante una IVIVC de nivel A.

Objetivos

Los objetivos de esta Tesis Doctoral han sido:

- Realizar una revisión bibliográfica acerca de los diferentes métodos existentes para la obtención de una IVIVC, así como analizar las ventajas y limitaciones de cada uno de ellos, las recomendaciones que facilitan las guías y las principales aplicaciones de las IVIVC.

- Explorar la posibilidad de desarrollar una IVIVC (en sus distintos niveles) de carbamazepina (CBZ) utilizando las diferentes metodologías propuestas en las principales guías regulatorias con datos *in vitro – in vivo* promedios o individuales.

- Analizar la capacidad predictiva del método de disolución utilizado en los ensayos *in vitro* de CBZ para el establecer correlaciones *in vitro – in vivo* utilizando datos *in vivo* procedentes de diferentes estudios de bioequivalencia y datos *in vitro* procedentes de estudios de disolución independientes.

- Evaluar la capacidad discriminatoria del uso de datos individuales para declarar un nuevo lote bioequivalente basándose en una IVIVC de nivel A, así como valorar las diferencias existentes entre utilizar datos individuales o promediados a la hora de establecer los límites en las especificaciones de disolución.

Material y Métodos

DESCRIPCIÓN DE LOS DATOS DE CARBAMAZEPINA

Estudios de disolución de carbamazepina

Los estudios de disolución de CBZ se realizaron en dos laboratorios diferentes utilizando las mismas condiciones de disolución, es decir, utilizando el aparato de paletas giratorias PhEur/USP a 75 revoluciones por minuto y usando 900 mL de medio de disolución. El medio de disolución estaba compuesto por una solución acuosa de lauril sulfato de sodio al 1% (SLS).

Los lotes utilizados en ambos estudios de disolución fueron diferentes, y en ambos casos se obtuvieron 12 perfiles de disolución para cada una de las formulaciones ensayadas.

Para demostrar que los perfiles de disolución obtenidos eran semejantes, a pesar de que los ensayos se habían realizado en laboratorios diferentes, se calculó el factor de similitud (f_2) para todas las formulaciones ensayadas (dos referencias, cuatro test).

Curvas concentración plasmática-tiempo de Carbamazepina

Los perfiles *in vivo* de concentración de CBZ-tiempo utilizados procedían de dos ensayos de BE distintos. Estos ensayos de BE fueron ensayos ciegos, controlados, balanceados, aleatorizados y cruzados con dos períodos.

Desarrollo de la IVIVC y codificación de los datos

En la TABLA 1 se indica la codificación utilizada para identificar los datos *in vitro* e *in vivo* disponibles para desarrollar la IVIVC. La letra A o B hace referencia al tipo de datos: *in vitro* (A) o *in vivo* (B). El primer dígito identifica la procedencia de los datos *in vivo* (ensayo de BE) y datos *in vitro* (diferentes laboratorios). El último dígito identifica la formulación (referencia, test 1 o test 2). Como los ensayos de BE cuentan únicamente con dos formulaciones (test y referencia), para desarrollar una IVIVC con tres formulaciones, fue necesario combinar los datos de ambos estudios.

TABLA 1. Codificación utilizada para la identificación de las diferentes formulaciones utilizadas para desarrollar las IVIVC

	Laboratorio	Formulación	Código	IVIVC1	IVIVC2	IVIVC3	IVIVC4
***In vitro* (A)**	Laboratorio 1	Referencia	A11	A11	A11		
		Test 1	A12	A12	A12		
		Test 2	A13	A13	A13		
	Laboratorio 2	Referencia	A21			A21	A21
		Test 1	A22			A22	A22
		Test 2	A23			A23	A23
***In vivo* (B)**	Ensayo de BE 1	Referencia	B11	B11		B11	
		Test 1	B12	B12	B12*	B12	B12*
	Ensayo de BE 2	Referencia	B21		B21		B21
		Test 2	B23	B23*	B23	B23*	B23

Para evitar el efecto de las diferentes poblaciones seleccionadas en cada estudio de BE, los datos de las formulaciones de test se normalizaron en función de la relación entre las dos formulaciones de referencia. En cada tiempo de muestreo, se calcularon las proporciones B11 / B21 (las referencias de ambos ensayos *in vivo*) para obtener los perfiles de concentración individuales normalizados (análisis de datos individuales) o perfiles de concentración promedio normalizados (análisis de datos promedio) de la formulación test incluido en cada conjunto de datos IVIVC.

Los gráficos de Levy y la relación entre los tiempos de disolución *in vitro* e *in vivo* se obtuvieron mediante regresión lineal. Los gráficos de Levy se realizaron utilizando los tiempos *in vitro* a los que existen datos *in vivo* (fracción oral absorbida), que a su vez se correlacionaron con los tiempos *in vitro* en los que se había disuelto la misma fracción. En el caso de que no hubiera datos experimentales *in vitro* que coincidieran con esta fracción disuelta, se estimó el tiempo *in vitro* mediante una regresión no lineal.

Una vez establecida la IVIVC, se utilizaron los comprimidos extremos (el comprimido de disolución más rápida de la formulación más rápida [FTFF] y el comprimido de disolución más lenta de la formulación más lenta [STSF]) para realizar un análisis adicional. A través de estos perfiles de disolución *in vitro* y de la ecuación de enlace se calcularon las fracciones absorbidas *in vivo* y con ellas, las concentraciones plasmáticas. Los parámetros de AUC y C_{max} obtenidos se utilizaron para compararlos con los resultados de los ensayos de BE de ambas formulaciones test.

ESPECIFICACIONES DE DISOLUCIÓN

Simulación de una IVIVC

Se simularon tres formulaciones (disolución lenta, media y rápida), cumpliéndose la condición de que el valor de f_2 entre la formulación media y la rápida/lenta fuera menor de 50.

La IVIVC se desarrolló utilizando un modelo de ecuaciones diferenciales similar al desarrollado por Rossenu et al. (69). Se escogió un modelo monocompartimental con absorción y eliminación lineal y con una disolución tanto *in vitro* como *in vivo* que siguiera una función de primer orden. La función de enlace entre el comportamiento *in vitro* e *in vivo* se estableció en la relación entre el coeficiente de velocidad disolución *in vitro* ($k_{d,\ in\ vitro}$) y el coeficiente de velocidad de disolución *in vivo* ($k_{d,\ in\ vivo}$). Se asumió que el proceso limitante de la absorción *in vivo* y la biodisponibilidad era la disolución. Es por ello que la k_d de cada formulación era menor que la k_a. Con respecto a la relación entre los datos *in vitro* y los datos *in vivo,* se propusieron dos tipos de escenarios:

- Relación lineal entre $k_{d,\ in\ vitro}$ y $k_{d,\ in\ vivo}$ (Escenarios 1, 2, 3).

- Relación no lineal (basada en una función sigmoide) entre $k_{d,\ in\ vitro}$ y $k_{d,\ in\ vivo}$ (Escenarios 4, 5, 6).

Evaluación de la bioequivalencia de nuevos lotes

Una vez establecida la correlación, utilizando simulaciones de Monte Carlo (n = 1000), se generaron seis nuevos lotes. Tres de ellos presentaron una k_d inferior a la k_d de la formulación media, (lotes 1, 3 y 5), mientras que los otros tres lotes se obtuvieron a partir de una k_d superior a la k_d de la formulación media. En ambos casos, los valores de las k_d simulados se situaban dentro del rango de k_d con el que se estableció la IVIVC.

Utilizando los parámetros de esta correlación, se obtuvieron los perfiles de concentración *in vivo* de la siguiente manera:

- **Aproximación clásica**: Se utilizaron 1000 perfiles medios de disolución *in vitro* para calcular 1000 perfiles medios *in vivo*. Por último, se calcularon 1000 ratios de C_{max} entre la formulación de referencia y el lote simulado.

- **Aproximación individual**: Se obtuvieron 12000 perfiles *in vivo* a partir de 12000 perfiles *in vitro* para cada lote. Posteriormente, se seleccionaron los perfiles

correspondientes al: (i) STSF, el cual se define como el comprimido que se disuelve más lento del lote que presenta una k_d menor que la k_d de la referencia (lote 1), o (ii) FTFF, el cual se define como el comprimido que se disuelve más rápido del lote que presenta una k_d mayor con respecto a la k_d de la referencia (lote 2). Por tanto, se seleccionaron un total de 1000 perfiles *in vitro* e *in vivo* STSF o FTFF. Posteriormente, se calcularon las ratios entre la C_{max} de la formulación y la C_{max} del FTFF o STSF.

Especificaciones de disolución

Para la aproximación clásica, los límites de las especificaciones de disolución se establecieron utilizando los valores de disolución *in vitro* del lote (perfil promedio *in vitro*) cuya ratio estuviera más próximo al ±20% de diferencia en C_{max} con la referencia. Por otro lado, para la aproximación individual, las especificaciones de disolución se establecieron utilizando los valores de disolución *in vitro* de los comprimidos de disolución más rápida de la formulación más rápida (FTFF) y los comprimidos de disolución más lenta de la formulación más lenta (STSF) cuya ratio de la C_{max} fuera exactamente (cuatro dígitos significativos) de ±20% con respecto a la C_{max} de la referencia.

Simulación de ensayos de BE

Con el fin de establecer unas especificaciones de disolución que garantizaran que todos los comprimidos disueltos del nuevo lote fueran bioequivalentes, se simularon 1000 ensayos de BE cruzados con 24 individuos por estudio. Se administró a cada individuo 100 mg del medicamento correspondiente a la formulación de referencia y test con un período de lavado entre administraciones. Los individuos se distribuyeron en dos secuencias de 12 individuos cada una.

Los valores de las k_d *in vitro* de la formulación test se encontraban dentro del rango marcado por los FTFF y STSF, es decir, todos los comprimidos simulados cumplían las especificaciones de disolución.

Además, para estos ensayos de BE se propusieron tres escenarios de variabilidad intra-individual:

- Variabilidad en la k_a (30%).
- Variabilidad en el aclaramiento (CL) (30%).

- Variabilidad en la k_a (30%) y CL (30%).

Programas utilizados

Todos los perfiles *in vitro* e *in vivo* fueron simulados utilizando NONMEM versión 7.3 (72). Los análisis estadísticos y los gráficos se realizaron en R (http://cran. r-project.org, versión 3.3.2) y R Studio (versión 1.0.136).

Resultados y Discusión

CORRELACIONES *IN VITRO* – *IN VIVO* DE LOS DATOS DE CBZ

Análisis de los datos *in vitro*

El análisis de f_2 demostró que la referencia y el test 1 presentan una disolución similar ($f_2 > 50$) mientras que la disolución entre la formulación de referencia y el test 2 no es similar ($f_2 < 30$). Este resultado se obtuvo para ambos laboratorios. Del mismo modo, cuando se compararon entre sí los datos de las mismas formulaciones obtenidos en los dos laboratorios (referencia - referencia, test 1 - test 1 y test 2 - test 2) se comprobó que en los tres casos las formulaciones eran semejantes entre sí ($f_2 >50$).

Para ajustar los datos *in vitro* se seleccionó un modelo de disolución de primer orden. Los perfiles *in vitro* se ajustaron de manera individual. La TABLA 2 muestra los datos promedio y su coeficiente de variación (CV) para cada formulación.

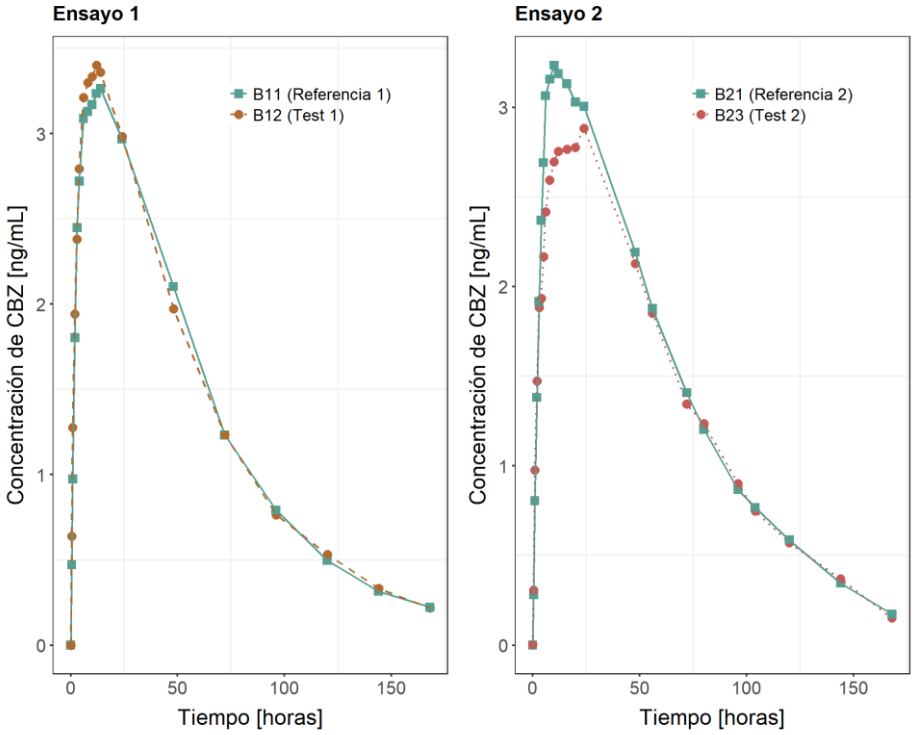
TABLA 2. Parámetros *in vitro* obtenidos para cada una de las formulaciones utilizadas en los ensayos de disolución y su CV (%); min, minutos.

	Referencia	Test 1	Test 2	Referencia	Test 1	Test 2
	A11	**A12**	**A13**	**A21**	**A22**	**A23**
AUC [%·min]	4462.2	4896.3	3997.6	9138.4	10158.9	8832
	(2.1)	(1)	(1)	(5.3)	(3.5)	(3)
MDT [min]	10.2	7.8	15.4	20.7	13.9	25.1
	(5.3)	(12.5)	(1.1)	(13.5)	(10.7)	(9.2)
T_{25} [min]	1.2	0.8	4	1	0.9	4.9
	(12.9)	(19)	(0.5)	(53.6)	(37.2)	(18.4)
T_{50} [min]	4.3	8.3	9.9	1	4	13.6
	(9)	(8.5)	(0.5)	(53.6)	(17.9)	(12.2)
T_{75} [min]	11.8	8.3	20.4	19.1	13.9	30.7
	(6.3)	(8.5)	(1.2)	(21.9)	(6.6)	(11.7)
T_{80} [min]	14.6	10.4	23.9	24.4	18.7	36.8
	(6.6)	(7.8)	(2.7)	(30.4)	(10.4)	(11.6)

Análisis de los datos *in vivo*

Los parámetros *in vivo* de ambos ensayos de BE de CBZ fueron similares y el CV fue bajo (7%). El doble pico que se observa en el perfil del ensayo 2 (FIGURA 1) puede deberse al ciclo enterohepático del fármaco (73). La no aparición de los picos mencionados en los perfiles del ensayo 1 se puede explicar por la ausencia de muestras plasmáticas en este intervalo de tiempo.

FIGURA 1. Perfiles promedio de las concentraciones de CBZ en ambos ensayos de BE.



Correlaciones *in vitro – in vivo*

La CBZ es un fármaco poco soluble y de alta permeabilidad, clase II, según el sistema de clasificación biofarmacéutica (BCS), y debido a sus propiedades biofarmacéuticas es un buen candidato para desarrollar una IVIVC.

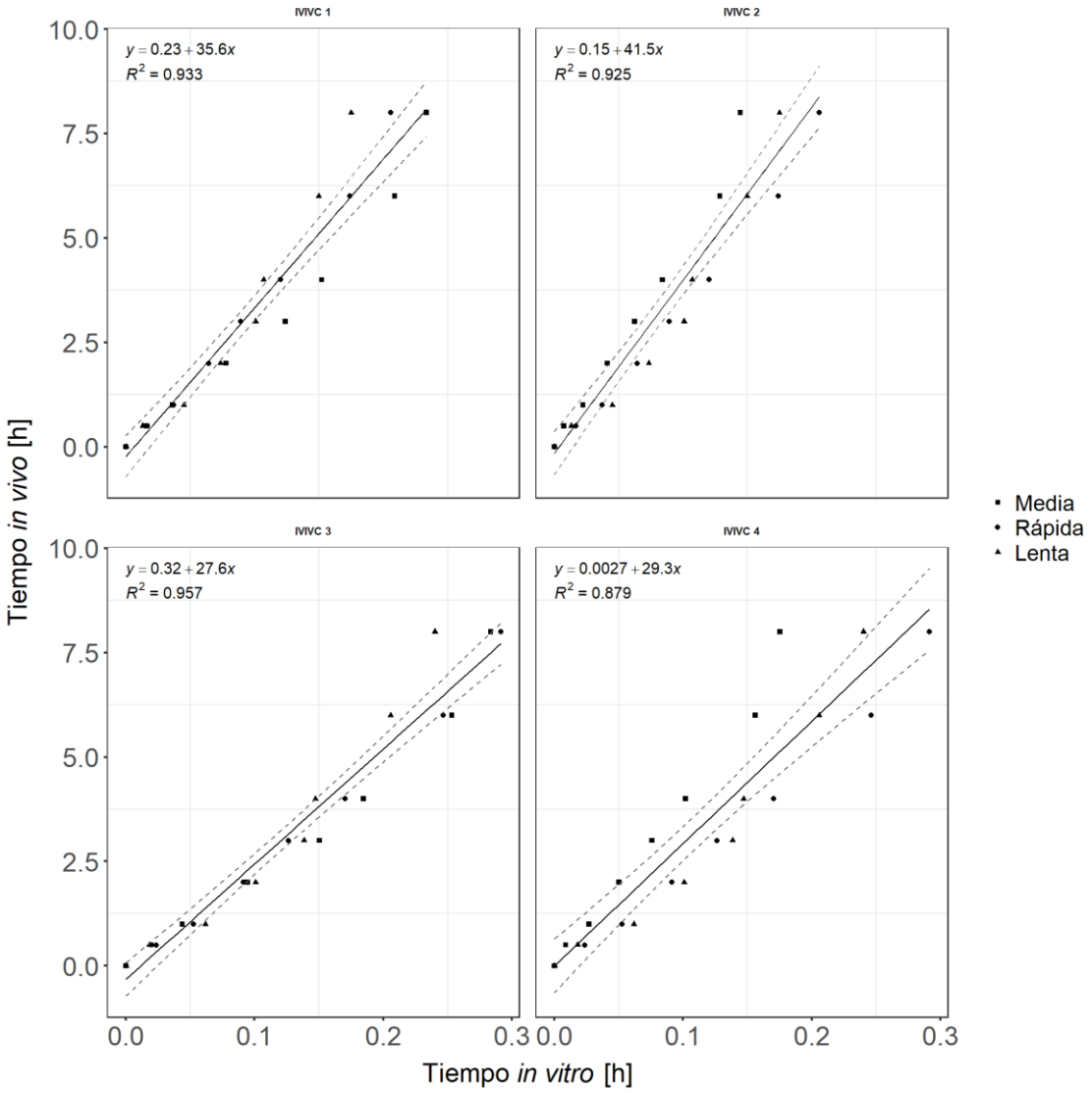
En este trabajo se han combinado datos de dos estudios de BE de CBZ con datos de dos ensayos de disolución independientes obteniéndose varios niveles de IVIVC:

- **El nivel B** de correlación se estableció entre el tiempo medio de disolución (MDT) *in vitro* y el tiempo medio de residencia (MRT) *in vivo*. Para los cuatro conjuntos de datos, el valor de R^2 fue siempre mayor de 0.75, mostrando así una buena correlación entre el MDT y el MRT.

- **El nivel C** de correlación en la que se enfrentaron diferentes parámetros de disolución *in vitro* (t_{25}, t_{50}, t_{75}, t_{80} y MDT) a diferentes parámetros PK *in vivo* (C_{max} y AUC). No se encontró relación entre el AUC y alguno de los parámetros de disolución *in vitro*. Sin embargo, se pudo establecer la correlación nivel C entre el parámetro C_{max} y los parámetros de disolución *in vitro*. Cuando se comparan los valores de R^2 obtenidos entre los distintos grupos de datos, se puede observar que el valor de R^2 de las IVIVC obtenidas con los datos *in vivo* B12*, B21 y B23 fue mayor.

- **Nivel A** de correlación. Dado que la disolución *in vivo* era mucho más lenta que la disolución *in vitro*, fue necesario primero encontrar una relación entre los tiempos de disolución *in vitro* e *in vivo*. El gráfico de Levy mostró que el tiempo necesario para obtener una determinada fracción disuelta *in vivo* era entre 20 y 40 veces mayor que el tiempo necesario para obtener la misma fracción disuelta *in vitro* (FIGURA 2). Para desarrollar este nivel de correlación se utilizó el método de deconvolución propuesto por Wagner-Nelson, ya que en la literatura existen varias referencias en las que se muestra que el modelo monocompartimental se ajusta de forma satisfactoria a los datos disponibles de CBZ (74-76). Además, ante la ausencia de datos tras administración intravenosa, el método de deconvolución de Wagner-Nelson era la opción más adecuada.

- *FIGURA 2. Gráfico de Levy que representa la relación entre los tiempos de disolución* in vitro *e* in vivo.



En los tres niveles de correlación se encontraron pequeñas diferencias en los R^2 debidas a las variaciones en los conjuntos de datos. Cuando se enfrentaron las fracciones disueltas *in vitro* a las fracciones disueltas *in vivo* se pudo observar que la correlación entre ellas (R^2) dependía en cierta manera del conjunto de datos *in vitro* e *in vivo* utilizado. Sin embargo, se encontraron mayores diferencias entre los R^2 cuando, para el mismo conjunto de datos, se utilizaron los datos individuales frente a los datos promediados.

Estos resultados están en concordancia con los obtenidos en otros trabajos publicados sobre IVIVC donde concluyen que la variabilidad entre los perfiles *in vitro* es mucho menor que la obtenida con datos *in vivo* (77, 78), y que al hacer la correlación con datos

promedios (estrategia comúnmente utilizada en los métodos de dos etapas) se produce una pérdida de información que podría producir un sesgo en los resultados.

Esto se contrasta al observar los resultados de la correlación nivel A. Cuando se utilizaron los datos individuales los errores de predicción eran menores que cuando se establecía la correlación con datos promedios. Además, la utilización de datos promedios generó, en alguno de los casos, valores de los errores de predicción superiores a los límites aceptados por la EMA y la FDA. Los resultados obtenidos en este ejercicio apoyan la recomendación de la guía de la EMA de utilizar datos individuales en vez de datos promedio para obtener una IVIVC.

Los resultados de los errores de predicción obtenidos en la validación interna de la correlación nivel A se detallan en la TABLA 3. Los errores de predicción obtenidos en la validación interna cuando se utilizaron los datos individuales cumplían los límites establecidos por la FDA y EMA. Sin embargo, cuando se hizo el análisis utilizando los datos promedios, en alguno de los casos se obtuvieron valores de los errores de predicción mayores a los aceptados en las guías.

TABLA 3. Resumen de los errores de predicción (%) obtenidos para todas las IVIVC desarrolladas, tanto para los datos individuales como datos promediados.

			B11/B21	B12	B23	MEDIA
W-N Individual	IVIVC 1	AUC	8.1	2.3	0.4	3.6
		C_{max}	3.1	6.0	12.8	7.3
	IVIVC 2	AUC	2.2	12.0	8.4	7.5
		C_{max}	5.0	14.1	4.8	8.0
	IVIVC 3	AUC	7.6	6.5	0.5	4.9
		C_{max}	5.6	1.0	13.3	6.7
	IVIVC 4	AUC	0.2	14.4	6.0	6.9
		C_{max}	8.1	9.5	4.2	7.3
W-N Promedio	IVIVC 1	AUC	4.2	1.7	6.2	4.0
		C_{max}	2.7	2.5	9.0	4.7
	IVIVC 2	AUC	15.0	0.8	3.1	6.3
		C_{max}	5.9	11.3	11.4	9.5
	IVIVC 3	AUC	6.9	0.8	2.7	3.5
		C_{max}	1.5	3.0	11.9	5.5
	IVIVC 4	AUC	15.5	0.9	1.5	6.0
		C_{max}	15.9	20.0	18.4	18.1

En el análisis de los comprimidos extremos (FTFF y STSF) se trató de comprobar si, para los perfiles de disolución *in vitro* generados por estos comprimidos, la ratio de AUC y C_{max} entre el comprimido extremo (FTFF o STSF) y la referencia se encontraba incluido dentro de los límites establecidos para la BE (intervalo de confianza [IC] al 90% de la ratio de AUC y C_{max}). Los perfiles de disolución *in vitro* obtenidos por estos dos comprimidos se convolvieron de nuevo utilizando el principio de superposición (79, 80) para transformar las fracciones absorbidas en concentraciones plasmáticas. El análisis de los comprimidos extremos mostró que la FTFF cumplía el criterio para los dos parámetros evaluados (AUC y C_{max}), mientras que el STSF no cumplía el criterio cuando el parámetro analizado fue la C_{max} (TABLA 4).

TABLA 4. Resultados del análisis de los comprimidos extremos. FTFF, comprimido que se disuelve más rápido de la formulación rápida; STSF, comprimido que se disuelve más lento de la formulación lenta.

	C_{max}	AUC
BE 90% CI	1.00 – 1.15	0.89 – 1.12
FTFF	1.09	1.06
STSF	0.72	0.94

ESPECIFICACIONES DE DISOLUCIÓN

Los resultados anteriores permiten proponer un nuevo método, basado en una aproximación individual, útil para establecer, tras el desarrollo de una IVIVC nivel A, las especificaciones de disolución. Se ha desarrollado un método en una etapa y se ha tenido en cuenta tanto la variabilidad interindividual tanto *in vitro* como *in vivo* de los lotes simulados. Esta aproximación ofrece unos límites en las especificaciones de disolución más amplios que los establecidos cuando se utiliza la aproximación clásica (valores promedios), pero asegura que cualquier perfil (individual) *in vitro* que esté incluido en los límites aceptados ofrecerá un comportamiento *in vivo* cuya ratio de C_{max} estará siempre dentro del rango establecido (±20%). El uso de la aproximación clásica, que asume una diferencia máxima del ±20% entre el valor de los parámetros AUC y C_{max} observados y predichos utilizando datos promedio, puede permitir que ciertos comprimidos no sean BE dentro del mismo lote. Tal y como explica Cardot et al, el hecho de promediar los datos conlleva una pérdida de información, y el hecho de utilizar la media aritmética puede que no sea la mejor aproximación cuando existen valores extremos. Es por ello que esta nueva aproximación individual permite asegurar la BE en el 100% de los comprimidos incluidos en un mismo lote, ya que establece los límites de

disolución con el comprimido más extremo dentro del propio lote, teniendo en cuenta la variabilidad *in vitro* e *in vivo*. Ello garantiza que las diferencias con respecto a la formulación/lote de referencia serán como máximo del ±20% para AUC y C_{max}.

Escenarios lineal y no lineal

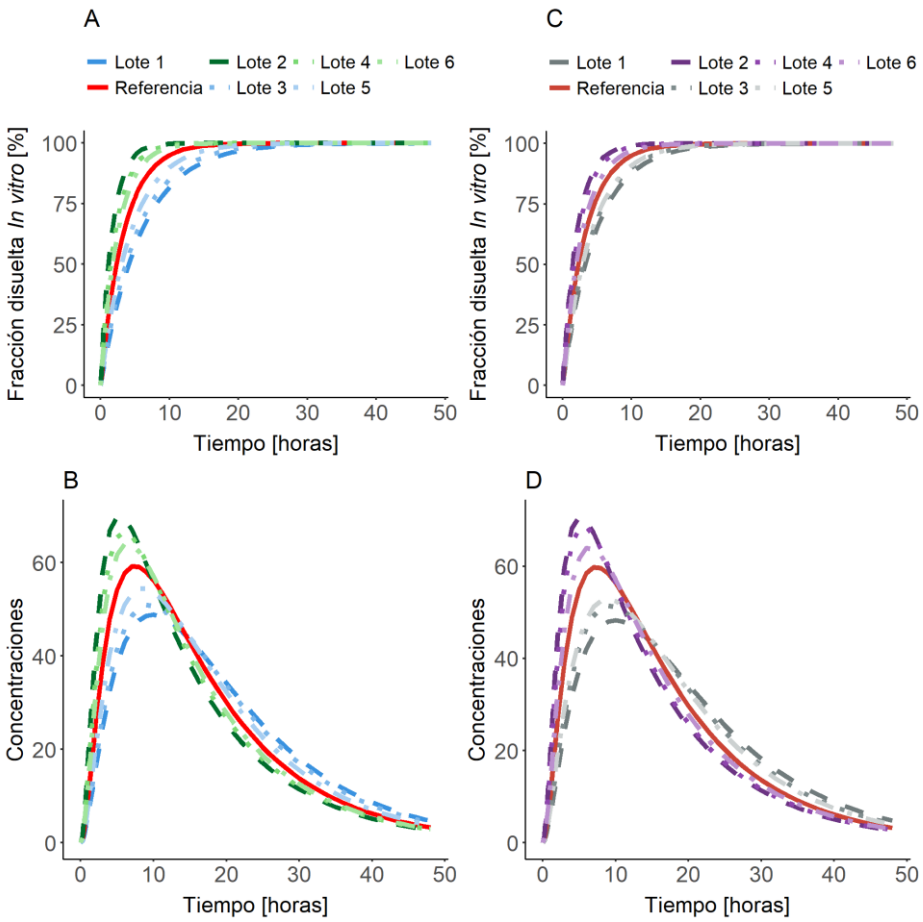
Cuando se comparan las especificaciones de disolución entre los escenarios lineal y no lineal se observa que la correlación no lineal ofrece unos límites más estrechos. Por lo general, un escenario no lineal es más restrictivo que un escenario lineal, debido a que cambios en la disolución *in vitro* provocan cambios de diferente magnitud en el perfil de concentración-tiempo *in vivo*. Este hecho se observa al comparar la probabilidad de obtener lotes bioequivalentes utilizando la aproximación clásica (valores promedios) o la propuesta en este estudio (valores individuales) en el escenario de IVIVC nivel A no lineal (FIGURA 4).

Evaluación de los nuevos lotes

La

Figura 3 representa el perfil PK medio *in vivo* obtenido a partir del perfil medio de disolución *in vitro* para la formulación de referencia y los seis lotes considerados. La relación entre la C_{max} de cada lote y el perfil promedio de la formulación de referencia se encuentran dentro del rango ±20% para ambas IVIVC (lineal y no lineal).

FIGURA 3. Perfiles *in vitro* (arriba) e *in vivo* (abajo) obtenidos a través de la ecuación de enlace de la IVIVC tanto para los escenarios lineal (izquierda) como no-lineal (derecha).

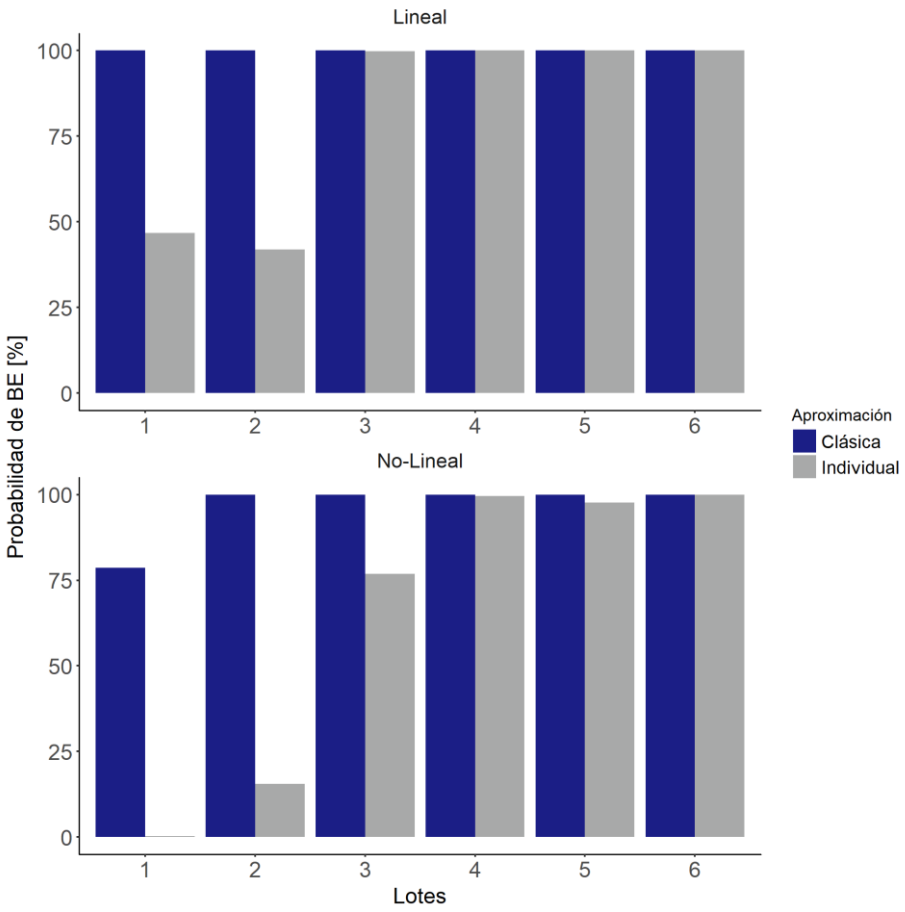


Se realizaron 1000 simulaciones de los perfiles de disolución *in vitro* y se obtuvieron los perfiles PK de los seis lotes tanto para la IVIVC nivel A lineal como para la IVIVC nivel A no lineal, siguiendo el criterio de la aproximación clásica y la aproximación individual. Cuando se aplicó la aproximación clásica, se obtuvieron 1000 perfiles *in vivo* a partir del perfil de disolución medio (12 unidades) *in vitro* de cada ensayo de disolución simulado (n = 1000). Por otro lado, la aproximación individual permitió generar 1000 perfiles PK de la unidad más lenta/rápida (STSF/FTFF) de cada ensayo de disolución simulado (n = 1000).

En la FIGURA 4 se representa el porcentaje de lotes considerados aptos (dentro del rango ±20%) cuando se aplicaron tanto la aproximación clásica como la aproximación individual. De acuerdo a los resultados obtenidos en el escenario lineal, cuando se compararon las ratios de C_{max} entre el nuevo lote y la referencia utilizando la aproximación clásica, todos los lotes cumplieron con los requisitos (es decir, estarían dentro del rango ±20%). Sin embargo, es posible observar que cuando se utilizó la aproximación individual tan solo el 46.7 y 41.9% de los lotes 1 y 2 cumplían con los mismos requisitos. Si, por el contrario, se comparan los resultados obtenidos cuando la IVIVC es de tipo no lineal, las diferencias entre las aproximaciones clásica (a partir del perfil de disolución medio) e individual se hacen más notorias. Observando el lote 1, la

aproximación clásica mostró que únicamente el 21.4% de los estudios se encontraban fuera del rango de ±20%. No obstante, la aproximación individual demostró que el 99.7% de esos estudios no hubiera cumplido con los requisitos establecidos por la FDA y la EMA.

FIGURA 4. Porcentaje de lotes considerados aptos obtenidos con la aproximación clásica (azul) como individual (gris) tanto en el escenario lineal (arriba) como no-lineales (abajo).



Establecimiento de las especificaciones de disolución a partir de una IVIVC

Los lotes 1 y 2 (cuya ratio de C_{max} era el más cercano a ±20%) fueron los lotes escogidos para establecer las especificaciones de disolución. La aproximación clásica, al estar basada en los perfiles de disolución promedios, ofrecía un rango más estrecho en las especificaciones de disolución frente a la aproximación individual: Esta situación se explica por el hecho de que las especificaciones de disolución utilizando la aproximación individual, establece los límites a partir de los perfiles de disolución individuales que generaban una diferencia C_{max} exactamente del ±20% con respecto a la formulación de referencia. Ello supone, por tanto, una ventaja a nivel de desarrollo de medicamentos y

regulatorio, dado que los límites son más amplios al garantizar diferencias con respecto a la formulación/lote de referencia igual es al 20%, pero que en ningún caso podrán producir diferencias superiores a los límites regulatorios establecidos.

Ensayos de bioequivalencia

Para cada uno de los escenarios pre-establecidos se generaron 1000 simulaciones de Monte Carlo. Los ensayos de BE simulados se generaron con el fin de evaluar el impacto de las especificaciones de disolución sobre los criterios de BE *in vivo.* Por tanto, los comprimidos utilizados cumplían las especificaciones de disolución propuestas por la nueva aproximación individual, obteniéndose como resultado que, en el 100% de las simulaciones de Monte Carlo y para cualquier escenario de simulación, el IC del 90% calculado entre la formulación de referencia y el nuevo lote simulado estaba dentro de los límites 0.8-1.25 establecidos para un ensayo de BE. Estos resultados confirmaron que la aproximación individual proporciona unas especificaciones de disolución por las cuales el 100% de los perfiles *in vivo* generados serán bioequivalentes, incluso para el peor escenario donde la variabilidad intra-sujeto de los parámetros PK (k_a y CL) es del 30%.

Lo anteriormente mencionado resalta el valor añadido de la nueva propuesta en el tratamiento de datos y analiza las deficiencias que proporcionan los métodos actuales que utilizan valores promedio de los parámetros para tomar decisiones de BE basados en una IVIVC. Además, estas deficiencias se magnifican cuando existe no linealidad entre la disolución *in vitro* y la disolución *in vivo.*

Limitaciones del estudio

Una de las limitaciones que se pueden encontrar en esta última parte del trabajo es que los escenarios y las condiciones de simulación son empíricas y no están relacionadas directamente con ningún fármaco específico. Sin embargo, en todas las simulaciones realizadas se asume que el fármaco es de clase II según el criterio BCS, donde la disolución *in vivo* es el paso limitante de la absorción y biodisponibilidad del fármaco. Con el fin de simplificar la comparación entre las dos aproximaciones presentadas en este trabajo, no se incluyeron modelos de disolución más complejos (81-84), pero estos modelos son fácilmente aplicables y pueden ser incluidos en análisis futuros que requieran cinéticas de disolución más complejas. Por otro lado, únicamente se utilizó el valor de C_{max} como parámetro comparador ya que se asumió una absorción completa del fármaco a lo largo de todo el lumen intestinal y por tanto no se esperaban cambios en el AUC.

Por último, indicar que este análisis es posible aplicarlo utilizando modelos cinéticos que restrinjan la absorción del fármaco en determinados tramos del intestino, al igual que se pueden incluir mecanismos no lineales en los procesos de absorción, distribución y eliminación del fármaco. Asimismo, es posible evaluar el efecto de la magnitud asignada a la variabilidad de los parámetros farmacocinéticos, aspecto que no se ha realizado en este trabajo ya que la variabilidad asignada a los parámetros PK (k_a y CL) se limitó a un único nivel (30%) con el objetivo de reducir el número de escenarios posibles.

Conclusiones

1. La utilización de datos *in vivo*, obtenidos de dos estudios de boequivalencia distintos, e *in* vitro, realizados en laboratorios distintos, ha permitido el desarrollo de IVIVC de nivel C, B y A de Carbamazepina, a partir de datos individuales y promedios, de forma satisfactoria.

2. El medio de disolución acuoso conteniendo un 1% de lauril sulfato sódico ha demostrado ser un medio de disolución biopredictivo para la CBZ, incluso cuando se utilizan diferentes lotes entre los estudios *in vitro* e *in vivo*.

3. El desarrollo de IVIVC de nivel A mediante la aproximación individual reduce los errores de predicción de los parámetros, lo que refuerza la utilización de datos individuales para desarrollar estas correlaciones y ofrece la posibilidad de revisar los métodos actualmente propuestos por las agencias regulatorias FDA y EMA.

4. La utilización del método de análisis individual propuesto en este trabajo establece las especificaciones de disolución a partir del perfil de disolución *in vitro* obtenido por el comprimido más rápido de la formulación más rápida (FTFF) o el comprimido más lento de la formulación más lenta (STSF), garantizando unas diferencias en los parámetros AUC y C_{max} iguales o inferiores al ±20% de la formulación de referencia.

5. El método basado en la utilización de datos individuales permite obtener límites de especificación de disolución más amplios que la aproximación clásica, la cual utiliza el perfil de disolución promedio de cada formulación, asegurando que las ratios *in vivo* obtenidos entre todas las unidades de cada lote de la formulación test versus referencia son exactamente del ±20%

6. El método de análisis individual propuesta en esta Tesis para el establecimiento de las especificaciones de disolución de una formulación a partir del desarrollo de una IVIVC de nivel A (lineal y no lineal) garantizan que la totalidad de los lotes que se disuelvan de acuerdo a los límites de disolución propuestos en las especificaciones de disolución serán bioequivalentes.

Referencias

1. Wagner JG, Nelson E. Per cent absorbed time plots derived from blood level and/or urinary excretion data. Journal of pharmaceutical sciences. 1963;52:610-1.

2. Balan G, Timmins P, Greene DS, Marathe PH. In-vitro in-vivo correlation models for glibenclamide after administration of metformin/glibenclamide tablets to healthy human volunteers. The Journal of pharmacy and pharmacology. 2000;52(7):831-8.

3. Balan G, Timmins P, Greene DS, Marathe PH. In vitro-in vivo correlation (IVIVC) models for metformin after administration of modified-release (MR) oral dosage forms to healthy human volunteers. Journal of pharmaceutical sciences. 2001;90(8):1176-85.

4. Corrigan OI, Devlin Y, Butler J. Influence of dissolution medium buffer composition on ketoprofen release from ER products and in vitro-in vivo correlation. International journal of pharmaceutics. 2003;254(2):147-54.

5. Eroglu H, Burul-Bozkurt N, Uma S, Oner L. Preparation and in vitro/in vivo evaluation of microparticle formulations containing meloxicam. AAPS PharmSciTech. 2012;13(1):46-52.

6. Ghosh A, Bhaumik UK, Bose A, Mandal U, Gowda V, Chatterjee B, et al. Extended release dosage form of glipizide: development and validation of a level A in vitro-in vivo correlation. Biol Pharm Bull. 2008;31(10):1946-51.

7. Guhmann M, Thommes M, Gerber F, Pollinger N, Klein S, Breitkreutz J, et al. Design of biorelevant test setups for the prediction of diclofenac in vivo features after oral administration. Pharmaceutical research. 2013;30(6):1483-501.

8. Honorio Tda S, Pinto EC, Rocha HV, Esteves VS, dos Santos TC, Castro HC, et al. In vitro-in vivo correlation of efavirenz tablets using GastroPlus(R). AAPS PharmSciTech. 2013;14(3):1244-54.

9. Kesisoglou F, Rossenu S, Farrell C, Van Den Heuvel M, Prohn M, Fitzpatrick S, et al. Development of in vitro-in vivo correlation for extended-release niacin after administration of hypromellose-based matrix formulations to healthy volunteers. Journal of pharmaceutical sciences. 2014;103(11):3713-23.

10. Khaled AA, Pervaiz K, Karim S, Farzana K, Murtaza G. Development of in vitro-in vivo correlation for encapsulated metoprolol tartrate. Acta Pol Pharm. 2013;70(4):743-7.

11. Kovacevic I, Parojcic J, Homsek I, Tubic-Grozdanis M, Langguth P. Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. Mol Pharm. 2009;6(1):40-7.

12. Macha S, Yong CL, Darrington T, Davis MS, MacGregor TR, Castles M, et al. In vitro-in vivo correlation for nevirapine extended release tablets. Biopharmaceutics & drug disposition. 2009;30(9):542-50.

13. Ostrowski M, Wilkowska E, Baczek T. In vivo-in vitro correlation for amoxicillin trihydrate 1000 mg dispersible tablet. Drug Dev Ind Pharm. 2009;35(8):981-5.

14. Rossi RC, Dias CL, Bajerski L, Bergold AM, Froehlich PE. Development and validation of discriminating method of dissolution for fosamprenavir tablets based on in vivo data. J Pharm Biomed Anal. 2011;54(3):439-44.

15. Rostami-Hodjegan A, Shiran MR, Tucker GT, Conway BR, Irwin WJ, Shaw LR, et al. A new rapidly absorbed paracetamol tablet containing sodium bicarbonate. II. Dissolution studies and in vitro/in vivo correlation. Drug Dev Ind Pharm. 2002;28(5):533-43.

16. Saibi Y, Sato H, Tachiki H. Developing in vitro-in vivo correlation of risperidone immediate release tablet. AAPS PharmSciTech. 2012;13(3):890-5.

17. Sirisuth N, Augsburger LL, Eddington ND. Development and validation of a non-linear IVIVC model for a diltiazem extended release formulation. Biopharmaceutics & drug disposition. 2002;23(1):1-8.

18. Sunesen VH, Pedersen BL, Kristensen HG, Mullertz A. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2005;24(4):305-13.

19. Yaro P, He X, Liu W, Xun M, Ma Y, Li Z, et al. In vitro-in vivo correlations for three different commercial immediate-release indapamide tablets. Drug Dev Ind Pharm. 2014;40(12):1670-6.

20. Chowdhury AK, Islam, S. In vitro-in vivo correlation as a surrogate for bioequivalence testing: the current state of play. Asian Journal of Pharmaceutical Sciences. 2011;6(3-4):176-90.

21. Cook JA. Development strategies for IVIVC in an industrial environment. Biopharmaceutics & drug disposition. 2012;33(7):349-53.

22. Emami J. In vitro - in vivo correlation: from theory to applications. J Pharm Pharm Sci. 2006;9(2):169-89.

23. Gonzalez-Garcia I, Mangas-Sanjuan V, Merino-Sanjuan M, Bermejo M. In vitro-in vivo correlations: general concepts, methodologies and regulatory applications. Drug Dev Ind Pharm. 2015;41(12):1935-47.

24. Hayes S, Dunne A, Smart T, Davis J. Interpretation and optimization of the dissolution specifications for a modified release product with an in vivo-in vitro correlation (IVIVC). Journal of pharmaceutical sciences. 2004;93(3):571-81.

25. Limberg J, Potthast H. Regulatory status on the role of in vitro dissolution testing in quality control and biopharmaceutics in Europe. Biopharm Drug Dispos. 2013;34(5):247-53.

26. EMA. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms. 2014.

27. FDA. Guidance for industry. Extended release oral dosage forms: development, evaluation and application of *in vitro/in vivo* correlations. Center for Drug Evaluation and Research (CDER): US Department of Health and Human Services; 1997.

28. FDA. Guidance for industry: Dissolution testing for immediate release solid oral dosage forms. Centre for Drug Evaluation and Research: US Department of Health and Human Services; 1997.

29. FDA. Guidance for Industry. SUPAC-MR: Modified Release Solid Oral Dosage Forms Centre for Drug Evaluation and Research (CDER): US Department of Health and Human Services; 1997.

30. EMA. Guideline on quality of oral modified release products. 2014.

31. USP. *In vitro* and *In vivo* Evaluations of Dosage Forms. 27th. ed. Easton, PA.: Mack Publishing Co.; 2004.

32. Lu Y, Kim S, Park K. In vitro-in vivo correlation: perspectives on model development. Int J Pharm. 2011;418(1):142-8.

33. Dunne A. Approaches to Developing in vitro-in vivo Correlation Models In: Chilukuri DS, G.; Young, D., editor. Pharmaceutical Product Development in vitro-in vivo Correlation. New York: Informa Healthcare USA, Inc; 2007. p. 47-70.

34. Loo JC, Riegelman S. New method for calculating the intrinsic absorption rate of drugs. Journal of pharmaceutical sciences. 1968;57(6):918-28.

35. Wagner JG. Method for estimating rate constants for absorption, metabolism, and elimination from urinary excretion data. Journal of pharmaceutical sciences. 1967;56(4):489-94.

36. Young D. In vitro in vivo correlations: Plenum Pub. Corp; 1997.

37. O'Hara T, Hayes S, Davis J, Devane J, Smart T, Dunne A. In vivo-in vitro correlation (IVIVC) modeling incorporating a convolution step. Journal of pharmacokinetics and pharmacodynamics. 2001;28(3):277-98.

38. Gaynor C, Dunne A, Costello C, Davis J. A population approach to in vitro-in vivo correlation modelling for compounds with nonlinear kinetics. Journal of pharmacokinetics and pharmacodynamics. 2011;38(3):317-32.

39. Gillespie WR. Convolution-based approaches for in vivo-in vitro correlation modeling. Adv Exp Med Biol. 1997;423:53-65.

40. Dunne A, O'Hara T, Devane J. A new approach to modelling the relationship between in vitro and in vivo drug dissolution/absorption. Stat Med. 1999;18(14):1865-76; discussion 77.

41. Gibiansky L, Gibiansky E. Target-mediated drug disposition model and its approximations for antibody-drug conjugates. Journal of pharmacokinetics and pharmacodynamics. 2013.

42. van Kuilenburg AB, Maring JG. Evaluation of 5-fluorouracil pharmacokinetic models and therapeutic drug monitoring in cancer patients. Pharmacogenomics. 2013;14(7):799-811.

43. Buchwald P. Direct, differential-equation-based in-vitro-in-vivo correlation (IVIVC) method. The Journal of pharmacy and pharmacology. 2003;55(4):495-504.

44. Costello C, Rossenu S, Vermeulen A, Cleton A, Dunne A. A time scaling approach to develop an in vitro-in vivo correlation (IVIVC) model using a convolution-based technique. Journal of pharmacokinetics and pharmacodynamics. 2011;38(5):519-39.

45. Gaynor C, Dunne A, Davis J. A comparison of the prediction accuracy of two IVIVC modelling techniques. Journal of pharmaceutical sciences. 2008;97(8):3422-32.

46. Soto E, Haertter S, Koenen-Bergmann M, Staab A, Troconiz IF. Population in vitro-in vivo correlation model for pramipexole slow-release oral formulations. Pharmaceutical research. 2010;27(2):340-9.

47. Bose A, Wui WT. Convolution and validation of in vitro-in vivo correlation of water-insoluble sustained-release drug (domperidone) by first-order pharmacokinetic one-compartmental model fitting equation. European journal of drug metabolism and pharmacokinetics. 2013;38(3):191-200.

48. Dutta S, Qiu Y, Samara E, Cao G, Granneman GR. Once-a-day extended-release dosage form of divalproex sodium III: development and validation of a Level A in vitro-in vivo correlation (IVIVC). Journal of pharmaceutical sciences. 2005;94(9):1949-56.

49. Liu Y, Schwartz JB, Schnaare RL. A multimechanistic drug release approach in a bead dosage form and in vitro predictions. Pharm Dev Technol. 2003;8(2):163-73.

50. Mirza T, Bykadi SA, Ellison CD, Yang Y, Davit BM, Khan MA. Use of in vitro-in vivo correlation to predict the pharmacokinetics of several products containing a BCS class 1 drug in extended release matrices. Pharmaceutical research. 2013;30(1):179-90.

51. Naeem Aamir M, Ahmad M, Akhtar N, Murtaza G, Khan SA, Shahiq uz Z, et al. Development and in vitro-in vivo relationship of controlled-release microparticles loaded with tramadol hydrochloride. International journal of pharmaceutics. 2011;407(1-2):38-43.

52. Parejiya PB, Barot BS, Patel HK, Chorawala MR, Shelat PK, Shukla A. In vivo performance evaluation and establishment of IVIVC for osmotic pump based extended release formulation of milnacipran HCl. Biopharmaceutics & drug disposition. 2013;34(4):227-35.

53. Egan TD, Lemmens HJ, Fiset P, Hermann DJ, Muir KT, Stanski DR, et al. The pharmacokinetics of the new short-acting opioid remifentanil (GI87084B) in healthy adult male volunteers. Anesthesiology. 1993;79(5):881-92.

54. Kakhi M, Marroum P, Chittenden J. Analysis of level A in vitro-in vivo correlations for an extended-release formulation with limited bioavailability. Biopharmaceutics & drug disposition. 2013;34(5):262-77.

55. Khaled AA, Pervaiz K, Khiljee S, Karim S, Shoaib QU, Murtaza G. In vitro to in vivo profiling: an easy idea for biowaiver study. Acta Pol Pharm. 2013;70(5):873-5.

56. Modi NB, Lam A, Lindemulder E, Wang B, Gupta SK. Application of in vitro-in vivo correlations (IVIVC) in setting formulation release specifications. Biopharmaceutics & drug disposition. 2000;21(8):321-6.

57. Mundin GE, Smith KJ, Mysicka J, Heun G, Kramer M, Hahn U, et al. Validated in vitro/in vivo correlation of prolonged-release oxycodone/naloxone with differing dissolution rates in relation to gastrointestinal transit times. Expert Opin Drug Metab Toxicol. 2012;8(12):1495-503.

58. Okumu A, DiMaso M, Lobenberg R. Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. Pharmaceutical research. 2008;25(12):2778-85.

59. Okumu A, DiMaso M, Lobenberg R. Computer simulations using GastroPlus to justify a biowaiver for etoricoxib solid oral drug products. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2009;72(1):91-8.

60. Parojcic J, Ethuric Z, Jovanovic M, Ibric S, Jovanovic D. Influence of dissolution media composition on drug release and in-vitro/in-vivo correlation for paracetamol matrix tablets prepared with novel carbomer polymers. The Journal of pharmacy and pharmacology. 2004;56(6):735-41.

61. Patel AR, Spencer SD, Chougule MB, Safe S, Singh M. Pharmacokinetic evaluation and in vitro-in vivo correlation (IVIVC) of novel methylene-substituted 3,3' diindolylmethane (DIM). European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2012;46(1-2):8-16.

62. Rietbrock S, Merz PG, Fuhr U, Harder S, Marschner JP, Loew D, et al. Absorption behavior of sulpiride described using Weibull functions. International journal of clinical pharmacology and therapeutics. 1995;33(5):299-303.

63. Sakuma S, Ogura R, Masaoka Y, Kataoka M, Tanno FK, Kokubo H, et al. Correlation between in vitro dissolution profiles from enteric-coated dosage forms and in vivo absorption in rats for high-solubility and high-permeability model drugs. Journal of pharmaceutical sciences. 2009;98(11):4141-52.

64. Shah VP, Konecny JJ, Everett RL, McCullough B, Noorizadeh AC, Skelly JP. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. Pharmaceutical research. 1989;6(7):612-8.

65. Yang Z, Teng Y, Wang H, Hou H. Enhancement of skin permeation of bufalin by limonene via reservoir type transdermal patch: formulation design and biopharmaceutical evaluation. International journal of pharmaceutics. 2013;447(1-2):231-40.

66. Csajka C, Drover D, Verotta D. The use of a sum of inverse Gaussian functions to describe the absorption profile of drugs exhibiting complex absorption. Pharmaceutical research. 2005;22(8):1227-35.

67. Drover DR, Angst MS, Valle M, Ramaswamy B, Naidu S, Stanski DR, et al. Input characteristics and bioavailability after administration of immediate and a new extended-release formulation of hydromorphone in healthy volunteers. Anesthesiology. 2002;97(4):827-36.

68. Pitsiu M, Sathyan G, Gupta S, Verotta D. A semiparametric deconvolution model to establish in vivo-in vitro correlation applied to OROS oxybutynin. Journal of pharmaceutical sciences. 2001;90(6):702-12.

69. Rossenu S, Gaynor C, Vermeulen A, Cleton A, Dunne A. A nonlinear mixed effects IVIVC model for multi-release drug delivery systems. Journal of pharmacokinetics and pharmacodynamics. 2008;35(4):423-41.

70. Veng-Pedersen P, Gobburu JV, Meyer MC, Straughn AB. Carbamazepine level-A in vivo-in vitro correlation (IVIVC): a scaled convolution based predictive approach. Biopharmaceutics & drug disposition. 2000;21(1):1-6.

71. Mistry B, Patel N, Jamei M, Rostami-Hodjegan A, Martinez MN. Examining the Use of a Mechanistic Model to Generate an In Vivo/In Vitro Correlation: Journey Through a Thought Process. Aaps J. 2016;18(5):1144-58.

72. Bauer R. NONMEM users guide: introduction to NONMEM 7.2.0. Elicott City2011.

73. Fleischman A, Chiang VW. Carbamazepine overdose recognized by a tricyclic antidepressant assay. Pediatrics. 2001;107(1):176-7.

74. Bondareva IB, Jelliffe RW, Gusev EI, Guekht AB, Melikyan EG, Belousov YB. Population pharmacokinetic modelling of carbamazepine in epileptic elderly patients: implications for dosage. J Clin Pharm Ther. 2006;31(3):211-21.

75. Graves NM, Brundage RC, Wen Y, Cascino G, So E, Ahman P, et al. Population pharmacokinetics of carbamazepine in adults with epilepsy. Pharmacotherapy. 1998;18(2):273-81.

76. Punyawudho B, Ramsay ER, Brundage RC, Macias FM, Collins JF, Birnbaum AK. Population pharmacokinetics of carbamazepine in elderly patients. Ther Drug Monit. 2012;34(2):176-81.

77. Gaynor C, Dunne A, Davis J. The effects of averaging on accuracy of IVIVC model predictions. Journal of pharmaceutical sciences. 2009;98(10):3829-38.

78. Cardot JM, Davit BM. In vitro-in vivo correlations: tricks and traps. Aaps J. 2012;14(3):491-9.

79. Langenbucher F. Handling of computational in vitro/in vivo correlation problems by Microsoft Excel: III. Convolution and deconvolution. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2003;56(3):429-37.

80. Qureshi S. In Vitro-In Vivo Correlation (IVIVC) and Determining Drug Concentrations in Blood from Dissolution Testing – A Simple and Practical Approach. The Open Drug Delivery Journal. 2010;4:38-47.

81. Abuhelwa AY, Mudge S, Hayes D, Upton RN, Foster DJ. Population In Vitro-In Vivo Correlation Model Linking Gastrointestinal Transit Time, pH, and Pharmacokinetics: Itraconazole as a Model Drug. Pharm Res. 2016;33(7):1782-94.

82. Locher K, Borghardt JM, Frank KJ, Kloft C, Wagner KG. Evolution of a mini-scale biphasic dissolution model: Impact of model parameters on partitioning of dissolved API and modelling of in vivo-relevant kinetics. Eur J Pharm Biopharm. 2016;105:166-75.

83. Ramteke HK, Dighe PA, Kharat AR, Patil SV. Mathematical Models of Drug Dissolution: A Review. Scholars Academic Journal of Pharmacy (SAJP). 2016;3(5):388-96.

84. Weiss M, Kriangkrai W, Sungthongjeen S. An empirical model for dissolution profile and its application to floating dosage forms. Eur J Pharm Sci. 2014;56:87-91.

APÉNDICE I

IN VITRO-IN VIVO CORRELATIONS: GENERAL CONCEPTS, METHODOLOGIES AND REGULATORY APPLICATIONS

IN VITRO-IN VIVO CORRELATIONS: GENERAL CONCEPTS, METHODOLOGIES AND REGULATORY APPLICATIONS

González-García, I.[a,b], Mangas-Sanjuán V.[a,b], Merino-Sanjuán, M.[b,c] and Bermejo M.[a,*]

[a] Department of Engineering, Area of Pharmaceutics and Pharmaceutical Technology. Miguel Hernández University, Elche (Spain).

[b] Department of Pharmaceutics and Pharmaceutical Technology. University of Valencia, Valencia (Spain)

[c] Centre for Molecular Recognition and Technological Development, Centre of Polytechnic University of Valencia-University of Valencia, Valencia (Spain).

Gonzalez-Garcia, I: PhD candidate at University of Valencia

Mangas-Sanjuan, V.: Post-doctoral Researcher at Miguel Hernandez University

Merino-Sanjuan, M.: Professor of Pharmacokinetics at University of Valencia

Bermejo, M.: Professor of Pharmacokinetics at Miguel Hernandez University.

CORRESPONDING AUTHOR FOOTNOTE

*Address for correspondence: Marival Bermejo, mbermejo@goumh.umh.es

Miguel Hernandez University. Carretera Alicante Valencia km 87, 03550. San Juan de Alicante (Alicante). Spain

Phone +34 965 919217

Fax +34 963544911

ABSTRACT

The major objective of IVIVC is to be able to use *in vitro* data to predict *in vivo* performance serving as a surrogate for an *in vivo* bioavailability test and to support biowaivers. Therefore, the aims of this review are: (i) to clarify the factors involved during bio-predictive dissolution method development; and (ii) the elements that may affect the mathematical analysis in order to exploit all information available.

This paper covers the basic aspects of dissolution media and apparatus used in the development of in vivo predictive dissolution methods, including the latest proposals in this field as well as the summary of the mathematical methods for establishing the *in vitro-in vivo* relationship and their scope and limitations.

The incorporation of physiological relevant factors in the *in vitro* dissolution method is essential to get accurate *in vivo* predictions. Standard quality control dissolution methods do not necessarily reflect the *in vivo* behaviour so they rarely are useful for predicting *in vivo* performance. The combination of physiological based dissolution methods with physiological-based pharmacokinetics models incorporating gastrointestinal variables will lead to robust tools for drug and formulation development nevertheless their regulatory use for biowaiver application still require harmonization of the mathematical methods proposed and more detailed recommendations about the procedures for setting up dissolution specifications.

KEYWORDS

IVIVC, FDA, EMA, BCS, Biorelevant media, Biowaiver, Dissolution methods, Two-stage methods, One-stage methods.

ABREVIATIONS

IVIVC: *in vitro-in vivo* correlation; FDA: Food and Drug Administration; EMA: European Medicines Agency; IR: immediate-release; ER: extended-release; USP: US Pharmacopeia; BCS: biopharmaceutics classification system; FaSSIF: Fasted state simulated intestinal fluid; FeSSIF: Fed state simulated intestinal fluid; FaSSGF: Fasted state simulated gastric fluid; FeSSGF: Fed state simulated gastric fluid; FaSIMS: Fasted state intestinal micellar solution; FeSIMS: Fed state intestinal micellar system; FeSIES: Fed state intestinal emulsion system; TIM-1: TNO gastroIntestinal Model; DGM: dynamic gastric model; ASDM: artificial stomach-duodenal model; k_{el}: elimination rate coefficient; k_a: absorption rate coefficient; PBPK: physiological-based pharmacokinetics; WN: Wagner-Nelson; LR: Loo-Riegelmann; BE: bioequivalence; ND: not declared.

1. INTRODUCTION

The consistent performance of drug products for the oral route, as the most used and preferred for patients, is essential for therapeutic effect and clinical success. Thus, validating *in vitro* and *in silico* methods to predict oral product performance in the development phase is a key step to ensure the maximal absorption in clinical phases.

Any dosage form for the oral route must dissolve on the intestinal fluids as an essential step before its absorption through the intestinal membrane. Decades of research have been devoted to the development of *in vitro* dissolution methods capable of reproducing the process of *in vivo* dissolution to guarantee the proper performance of the drug (Balan et al., 2000, Balan et al., 2001, Corrigan et al., 2003, Eroglu et al., 2012, Ghosh et al., 2008, Guhmann et al., 2013, Honorio Tda et al., 2013, Kesisoglou et al., 2014, Khaled et al., 2013a, Kovacevic et al., 2009, Macha et al., 2009, Rossi et al., 2011, Rostami-Hodjegan et al., 2002, Saibi et al., 2012, Sirisuth et al., 2002, Sunesen et al., 2005, Ostrowski et al., 2009, Yaro et al., 2014).

The science of dissolution methods has evolved in the last decades in parallel to the development of gastroenterology and it has led to the development of new *in vivo* biorelevant methods (Dressman and Reppas, 2000, Nicolaides et al., 2001) and *in vivo* predictive methods (Tsume et al., 2014, Tsume et al., 2013, Mudie et al., 2012). This new field appears thanks to the advances on intestinal physiology and it has numerous applications to optimize and to accelerate drug development and to ensure formulation bioequivalence.

Rate and extent of dissolution and absorption depend on the physicochemical characteristics of the drug (as pKa, crystalline habit, solubility, partition coefficient), as well as on the characteristics of the dosage form. On the other hand, the physiological

parameters such as gastrointestinal buffers, pH, ionic strength, bile salt concentration, gastric emptying rate, fluid volume and hydrodynamic conditions are also relevant. In spite of the use of the dissolution tests for quality control, there is not a single dissolution test or apparatus able to capture the complexity of *in vivo* relevant parameters that determine *in vivo* drug product dissolution on the gastrointestinal lumen. Due to the difficulty of developing a single dissolution system, it would be desirable to know which the relevant characteristics of the drug substance and the dosage form are and then design a dissolution method that incorporates them.

Since the beginning of 1950's, the effect of drug dissolution on bioavailability has been demonstrated (Wagner and Nelson, 1963) and, nowadays many efforts are employed from a biopharmaceutical perspective to establish a relationship between the *in vitro* drug release data (dissolution) and *in vivo* plasma profiles of new and marketed formulations. Setting this kind of relationship becomes a fundamental tool in drug development due to the increased knowledge on the behaviour of the drug product *in vivo*, which determines a more rational decision-making process. Moreover, ethical reasons promote the establishment of validated *in vitro-in vivo* correlations (IVIVC) in order to use *in vitro* dissolution data as a surrogate of the *in vivo* behaviour in order to avoid as much as possible the use of human volunteers, which reduces the cost and time of a drug to be marketed. For these reasons, the use of IVIVC has grown rapidly in the field of novel drug delivery systems.

Many references in the last decade can be found about the concept and application of IVIVC for pharmaceutical dosage forms (Chowdhury, 2011, Cook, 2012, Emami, 2006, Hayes et al., 2004, Limberg and Potthast, 2013). Academia, pharmaceutical industry, and regulatory sectors have focused on the use of IVIVC for different purposes. In fact, FDA published in 1997 three regulatory guidances to set the conditions for developing

IVIVC for *immediate-release (IR)* (FDA, 1997d), *extended-release (ER)* (FDA, 1997b) and *scale-up and post-approval changes: chemistry, manufacturing and controls, in vitro dissolution testing, and in vivo bioequivalence documentation for IR and ER* (FDA, 1997c). Several years after, in 2012, European Medicines Agency (EMA) published a draft guideline entitled: *Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms,* which describes the applications, study design considerations and IVIVC development and validation (EMA, 2012) and it has been finished in 2014 (EMA, 2014b). Both agencies developed a regulatory framework motivating the application of IVIVC and minimizing the need for *in vivo* bioavailability studies.

The major objective of IVIVC is to be able to use *in vitro* data to predict *in vivo* performance serving as a surrogate for an *in vivo* bioavailability test and to support biowaivers. Therefore, the aims of this review are: (i) to clarify the factors involved during bio-predictive dissolution method development; and (ii) the elements that may affect the mathematical analysis in order to exploit all information available.

2. BODY MANUSCRIPT

2.1 Applications

According to what is stated in the guidelines of the FDA and the EMA (EMA, 2012, FDA, 1997b), the main applications of IVIVC are:

-To quantify *in vivo* release and formulation-related effect on absorption.

-To establish dissolution specifications and clinical relevance of *in vitro* dissolution.

-To support biowaiver claims: once an IVIVC has been settled, *in vivo* performance may be estimated by *in vitro* dissolution tests. However, there are some exceptions detailed in the FDA guideline where an IVIVC may not support a biowaiver claim (FDA, 1997b):

- Approval of a new formulation of an approved extended-released drug product when the new formulation has a different release mechanism.

- Approval of dosage strength higher or lower than the doses that have been shown to be safe and effective in clinical trials.

- Approval of another sponsor's extended-released product even with the same release mechanism.

- Approval of a formulation change involving a non-release controlling excipient in the drug product that may significantly affect drug absorption

2.2 IVIVC Levels

Four levels of correlation are described in FDA guidance (FDA, 1997b), based on the predictive capability to reflect the concentration-time *in vivo* profile after administration of an oral dosage form (USP, 2004). The most relevant level in terms of predictability and regulatory application is the Level A. Nevertheless, level B, C and multiple C could be useful in formulation development.

Level A is the highest level of correlation and it represents a point-to-point relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from the dosage form (USP, 2004). The purpose of this level of correlation is to predict the entire *in vivo* profile from the *in vitro* dissolution curve. Usually, linear correlations are observed and *in vitro* dissolution and *in vivo* input curves may be directly superimposable (1:1 relationship) or may be made superimposable by the use of a scaling factor (point-to-point relationship). Non-linear correlations are uncommon, but may be also appropriate (Chowdhury, 2011). A change in manufacturing site, method of manufacture, raw material suppliers, minor

formulation modification, and even product strength using the same formulation can be justified without the need for additional human studies (USP, 2004).

2.3 Considerations for dissolution method development

Drug absorption from a solid dosage form following oral administration involves mainly three processes: release of the drug substance from the drug product, dissolution or solubilization of the drug under physiological conditions, and drug permeability across the biological layers of the gastrointestinal tract. *In vivo* performance is deeply dependent on the first two steps. Noyes-Whitney equation and subsequent modifications (Horter and Dressman, 2001, Dressman et al., 1998) provide an initial framework to compare the different factors affecting the *in vitro-in vivo* dissolution.

2.3.1 Physicochemical factors

Dissolution rate is directly proportional to the available surface. Particle size and the ability of the liquid to wet particles (wettability) determine this surface. For hydrophobic compounds, with low wettability, the presence of surfactants in the intestinal tract increases its wettability capacity, decreasing the solid-liquid contact angle and, therefore, the dissolution rate increases. The addition of surfactants in the dissolution media can reproduce *in vitro* this physiological factor. The thickness of the boundary layer depends on the hydrodynamic conditions in the gastrointestinal tract (flux rate, motility and mixing factors).

Temperature, molecular radius and medium viscosity determine the drug diffusivity. Drug solubility depends on physicochemical characteristics but may change by the presence of surfactants and/or pH in the intestinal liquid. For poorly soluble drugs, the addition of surfactant (e.g., 1% sodium lauryl sulphate) may be appropriate (FDA, 1997b, Shah et al., 1989, Sievert, 1998). Drug solubility may increase solubilizing the drug into

bile salts micelles and increasing drug ionization, which can be simulated *in vitro.* However, the addition of enzymes, salts and surfactants need also to be justified (FDA, 1997b, FDA, 1997d). In general, it becomes relevant to simulate all parameters involving the *in vivo* drug dissolution, although formulations containing highly soluble drugs are not strictly dependent on physicochemical factors.

2.3.2 BCS

Biopharmaceutics classification system is a framework for classifying drugs according to their solubility and intestinal permeability and establishes the basis and justification for IVIVC biowaiver (Amidon et al., 1995) based on drug solubility and permeability (Amidon et al., 1995). Although there is great concordance between FDA and EMA guidelines concerning the relevance of *in vitro* dissolution testing for BCS Class I drugs, however the BCS criterion does not capture the most significant physicochemical differences that are critical to dosage form design and performance for BCS Class II and IV (Tsume et al., 2014, Butler and Dressman, 2010). For these drugs, *in vivo* dissolution is the rate-limiting step of *in vivo* absorption and bioavailability because of its high dependency on the drug solubility, the acidic or basic nature of the drug, formulation factors and *in vivo* luminal environment. Therefore, bio-reflective *in vitro* dissolution methodologies are encouraged in order to assure the bioequivalence standards, and a new proposed BCS subclassification has been published in order to better characterize drug and dissolution properties, which may affect drug absorption (Tsume et al., 2014). A summary table, relating BCS class, advisable dissolution method and likelihood of developing an IVIVC is reported in Table 1.

2.3.3 Standard USP apparatus

USP (US Pharmacopeia) and FDA describe seven types of dissolution apparatus: rotating basket (Apparatus I), paddle method (Apparatus II), reciprocating cylinder (Apparatus III) and flow through cell (Apparatus IV) for oral solid dosage forms (FDA, 1997d, Sievert, 1998, USP, 2004). Initial recommendations involve the use of first two methods, more simple and easy to handle, prior to using the others unless shown unsatisfactory (FDA, 1997d, Sievert, 1998). Mainly, paddle and basket apparatus have demonstrated their application for BCS I and III drug products with modified-release (MR) mechanism, where the release rate is very robust to variations in gastrointestinal physiology (Kostewicz et al., 2013). Apparatus 4 may offer advantages for MR dosage forms that contain active ingredients with very limited solubility. Paddle over disk (Apparatus V) uses paddle and vessel from Apparatus II with a stainless-steel assembly to hold the transdermal on the bottom of the vessel. Cylinder (Apparatus VI) is based on Apparatus I but it replaces the basket shaft with a stainless-steel cylinder element. Reciprocating Holder (Apparatus VII), apparatus V and apparatus VI have been shown to be useful for evaluating and testing transdermal dosage forms at 32ºC (USP, 2004).

2.3.4 Volume

Another important issue that may be taken into account is the volume used during *in vitro* dissolution tests. Usually, apparatus I and II require volumes in the range of 500 to 1000 mL in order to reflect sink conditions. Only under fed conditions, the physiological volume in the gastrointestinal tract reaches those quantities. Under fasted conditions, a recent research indicates that a gastrointestinal volume of 80-100 mL may be more physiologically appropriate (Mudie et al., 2014). The commonly estimated volume of 250 mL can lead to an overestimation of the dissolution in the stomach *in vivo* for poorly

soluble drugs (Kostewicz et al., 2013). Another relevant aspect is the influence between the volumes used with USP apparatus I and II, sink conditions, drug solubility and drug permeability in the small intestine. For BCS class II drugs, sink conditions may reflect better *in vivo* drug dissolution because of their high permeability characteristics. Therefore, depending on BCS class, smaller volumes might be required in order to reflect the *in vitro-in vivo* behaviour (Table 1).

Table 1. Likelihood of IVIVC depending on the BCS category. Adapted from (Tsume et al., 2014). PIB: physiological intestinal buffer; PGB: physiological gastric.

Class	Solubility	Permeability	Gastric medium	Consider gastric compartment	Intestinal luminal medium	Consider absorption compartment	Likelihood of IVIVC
I	High	High	250 mL PGB	No (b)	900 mL PIB	No	IVIVC expected (if dissolution is rate-limiting step)
IIa	Low	High		Yes	100 mL PIB	Yes	IVIVC expected
IIb	Low	High		Yes	100 mL PIB	Yes	IVIVC expected
IIc	Low	High		Yes	100 mL PIB + bile acids/lipid	Yes	IVIVC expected
III	High	Low		No	100 mL PIB	No	Little or no IVIVC
IVa	Low	Low		Yes	100 mL PIB	Yes	Little or no IVIVC
IVb	Low	Low		Yes	100 mL PIB	Yes	Little or no IVIVC
IVc	Low	Low		Yes	100 mL PIB + bile acids/lipid	Yes	Little or no IVIVC

2.3.5 Stirring speed

Stirring speed may be a critical factor in *in vitro* dissolution test because it tends to become less discriminative when operated at faster speeds. Then, if apparatus I is selected, the common agitation is 50-100 rpm; with the apparatus II, it is 50-75 rpm and 25 rpm for suspensions (FDA, 1997b, Shah et al., 1992, USP, 2004). Inadequate stirring speed may produce coning effect, when particles with high density form a mound, inhibiting dissolution of those particles below the paddle. Peak vessels instead of paddle or increasing the stirring speed are the solutions proposed by USP.

2.3.6 Biorelevant media

Reproducing *in vitro* the *in vivo* dissolution process is not a straightforward task due to the complexity of the *in vivo* environment. Drug solubility is the driving force in the dissolution process, which has to occur for the drug to permeate. Thus, high permeability drugs may be in high gradient conditions. Moreover, the volume of fluids in the gastrointestinal tract changes along the gastrointestinal tract and is different in fed or fasted conditions (**¡Error! No se encuentra el origen de la referencia.**). Hydrogen carbonate ions, which are secreted by the pancreas and intestinal epithelial cells, buffer the intraluminal content physiologically. Other elements in the GI lumen show a variable and often limited effect on the pH and buffer capacity (Kalantzi et al., 2006, McConnell et al., 2008, Persson et al., 2005, Repishti et al., 2001).

Table 2. Physiological parameters in fasted or fed conditions in gastric, duodenum and jejunum. Adapted from (Bergstrom et al., 2014, Mudie et al., 2010)

		Gastric	Duodenum	Jejunum	Ileum
Human gastrointestinal fluid in the fasted state	pH	2.5	6.3	6.5	6.5
	Buffer capacity [mM·ΔpH^{-1}]	14.3	5.6	8.5	6.4
	Osmolarity [mOsm]	202	197	280	
	Surface tension [mN·m^{-1}]	36.8	37.5		
	Bile salt [mM]	0.28	3.25	2.52	2-10
	Phospholipid composition [mM]	0.029	0.26	0.19	
Human gastrointestinal fluid in the fed state	pH	5.6	6.0	6.3	7.5
	Buffer capacity [mM·ΔpH^{-1}]	19.5	27.5	13.9	
	Osmolarity [mOsm]	388	346	-	
	Surface tension [mN·m^{-1}]	30.5	31.3	30.0	
	Bile salt [mM]	0.17	11.8	2.52	0.5, 1
	Phospholipid composition [mM]	0.022	2.15	2.5	
	Monoglycerides [mM]		2.2		
	Free fatty acids [mM]		13.2		

In some cases, successful IVIVC have been achieved with simple dissolution media. In general, an aqueous test medium is preferred (FDA, 1997b, FDA, 1997d, USP, 2004). Looking at USP and FDA guidelines, pH recommendations differ slightly (FDA, 1997b, FDA, 1997d, USP, 2004). Generally, water is accepted by regulatory agencies (FDA, 1997b, FDA, 1997d, USP, 2004) or buffered solution preferably not exceeding pH 6.8 are recommended by FDA as the initial medium for the development of an IVIVC (FDA, 1997b, FDA, 1997d). As recommended by USP, deaerated water, a buffered solution (typically pH 4 to 8) or a dilute acid (0.001 to 0.1 N) may preferably be used as dissolution medium for MR dosage forms (USP, 2004). On the other hand, non-aqueous and hydro-alcoholic systems must be justified by a documented IVIVC (FDA, 1997b, Shah et al., 1989, Sievert, 1998, USP, 2004) and other extreme testing conditions (e.g. pH>8) should be justified (FDA, 1997b, FDA, 1997d). In order to simulate intestinal fluid or gastric fluid, dissolution medium of pH 6.8 or pH 1.2 are encouraged respectively (Bates et al., 1977).

The use of the so called "biorelevant" media have raised up in the last years (Table 3), because of the increased research and development of poorly soluble drugs. They may reflect better the *in vivo* drug dissolution conditions and therefore it creates a basis for a better IVIVC (Sunesen et al., 2005, Shono et al., 2009, Lue et al., 2008, Jantratid et al., 2009, Wei and Lobenberg, 2006, Okumu et al., 2008). In the fasted state, FaSSIF has demonstrated a successful approximation for Montelukast, Glibenclamide, Diclofenac sodium and Celecoxib obtaining IVIVC level A and B using USP apparatus II, III and IV (Guhmann et al., 2013, Okumu et al., 2008, Wei and Lobenberg, 2006, Jantratid et al., 2009, Sunesen et al., 2005). FeSSIF medium has also been selected for the *in vivo* prediction of Danazol dissolution with the addition of lipolysis products (Dressman and Reppas, 2000, Vertzoni et al., 2012). However, with other compounds reported, FeSSIF

required the combination with simulation packages to achieve level A IVIVC for Diclofenac sodium and Celecoxib (Kleberg et al., 2010).

Table 3 Biorelevant media composition published and IVIVC/IVIVR developed. Adapted from (Luner and Vander Kamp, 2001, Jantratid et al., 2008b). FaSSGF: Fasted state simulated gastric fluid; FeSSGF: Fed state simulated gastric fluid; FaSSIF: Fasted state simulated intestinal fluid; FeSSIF: Fed state simulated intestinal fluid; FaSIMS: Fasted state intestinal micellar solution; FeSIMS: Fed state intestinal micellar system; FeSIES: Fed state intestinal emulsion system.

	FaSSGF	**FeSSGF**	**FaSSIF**	**FaSSIF-V2**	**Early FeSSIF**	**Middle FeSSIF**	**Late FeSSIF**	**FeSSIF-V2**	**FaSIMS**	**FeSIMS**	**FeSIES**
Acetic acid [mM]		17.12			144						
Dodecanoic Acid									0.25	10	20
Glyceryl monooleate [mM]					6.5	5	1	5			
KCl			103		204						
KH2PO4			29								
Lecithin [mM]	20		0.75	0.2	3	2	0.5	2	0.25	0.6	3
Linoleic acid											
Linolenic acid											
Lysolecithin									0.75	2.4	3
Maleic acid [mM]				19.12	28.6	44	58.09	55.02			
Monocaprin										3	10
Sodium chloride [mM]	34.2	237.02		68.62	145.2	122.8	51	125.5	142	85	85
Sodium hydroxide [mM]				34.8	52.5	65.3	72	81.65			
Pepsin	0.1										
Sesame oil											70
Sodium acetate [mM]		29.75									
Sodium oleate [mM]					40	30	0.8	0.8			
Sodium Taurocholate [mM]	80		3	3	10	7.5	4.5	10			

Sodium Taurodeoxycholate [mM]									5	10	10
Milk/buffer		1:1									
pH	1.6	5	6.5	6.5	6.5	5.8	5.4	5.8	5-7.5	5-7.5	5
Osmolarity [$mOsm \cdot kg^{-1}$]	120.7±2.5	400		180±10	400±10	390±10	240±10	390±10			
Buffer capacity [$mmol \cdot L^{-1} \cdot \Delta pH^{-1}$]		25		10	25	25	15	25			
Visual description			Slightly cloudy		Clear				Clear	Peralescent pH 5; Clear pH 7.5	Pearlescent w/TG; Cloudy without TG
IVIVC	(Shono et al., 2009, Schamp et al., 2006)	(Schamp et al., 2006, Jantratid et al., 2008, Shono et al., 2009)	(Corrigan et al., 2003, Jantratid et al., 2008, Okumu et al., 2008, Okumu et al., 2009, Wei and Lobenberg, 2006)	(Shono et al., 2009)				(Schamp et al., 2006, Shono et al., 2009)			
IVIVR	(Kambayashi et al., 2013, Shono et al., 2010)	(Kambayashi et al., 2013)	(Dressman and Reppas, 2000, Nicolaides et al., 2001,	(Kambayashi et al., 2013, Shono et al., 2010)				(Berlin et al., 2014, Dressman and Reppas, 2000, Jueneman			

	Nicolaides et al., 1999, Jueneman n et al., 2011, Fotaki et al., 2009, Almukainzi et al., 2014, Berlin et al., 2014, Kambayas hi et al., 2013)	n et al., 2011, Nicolaides et al., 1999, Nicolaides et al., 2001, Shono et al., 2010)

2.3.7 New approaches for biopredictive method development

Because of the presence of bicarbonate ions in the GI lumen, the application of hydrogen bicarbonate buffer systems has become fundamental for IR solid oral dosage forms containing ionisable drugs and/or excipients (Garbacz et al., 2014). The dissolution process may be altered by the ionic composition, total ion concentration and buffer capacity of the dissolution media (Wagner and McGinity, 2002, Wagner and Gruetzmann, 2005). Some authors have also remarked the more discriminative ability of bicarbonate buffer than compendial phosphate buffers (Fadda et al., 2009, Liu et al., 2011), although Sheng and co-workers concluded that phosphate buffer had a higher intrinsic dissolution rate compared to bicarbonate buffer for drugs with pKa values below 5.5 (Sheng et al., 2009). The difficulties associated with maintaining constant pH of the media and avoiding loss of carbon dioxide (CO_2) over time have reduced their use in dissolution media (Merchant et al., 2014). However, Auto pH System™ and pHysio-grad® have been proposed with intrinsic differences to solve this limitation allowing a dynamic and constant pH control and CO_2 supply (Merchant et al., 2014, Garbacz et al., 2014).

Recently, the use of milk as fed state media and as a lipid source has been used as dissolution medium to simulate the fed gastric and intestinal environment (Christophersen et al., 2014, Klein, 2010). Also, the use of Ensure® Plus has also been proposed (Klein et al., 2004). Both are composed of standardized homogenized cow's milk with a fat content of 3.5% and pH range of 6.5-6.6. Although Ensure® Plus resembles more to the properties of the FDA breakfast, no IVIVC examples can be found in the literature. Therefore, the use of biorelevant media might be a good alternative to

classical media for the establishment of IVIVC, but more rational and conclusive work is still needed to ensure a good prediction of *in vivo* drug behaviour.

2.3.8 New physiologically dissolution methodologies proposed

Generally, a dissolution methodology, which is able to discriminate between the study formulations with different release patterns and best reflects the *in vivo* behaviour should be used to establish an IVIVC (Emami, 2006). Therefore, ideally it might replicate not only the gastrointestinal medium composition, but also the system hydrodynamics. Knowledge on gut motility, *in vivo* mechanical stresses, media flow shear stress and media contact have been improved in the last years, allowing the development of dynamic systems to better predict the *in vivo* drug behaviour such as artificial stomach-duodenal model (ASDM), TNO gastrointestinal model (TIM-1) and the dynamic gastric model (DGM) (Kostewicz et al., 2013) (Table 4). However, the movement of the drug along the gastrointestinal tract is something difficult to simulate *in vitro* (Galia et al., 1998, Dressman et al., 1998, Nicolaides et al., 1999) and, although all these new systems might reflect better the *in vivo* conditions, additional validation work is still needed. Once a discriminating system is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation and should be fixed before further steps towards correlation evaluation are undertaken (FDA, 1997b). Very extensive and detailed information concerning the factors that influence the dissolution process can be found in the review published by Kostewicz et al. (Kostewicz et al., 2013) and Gray (Gray et al., 2009)

Table 4. New proposed methods and physiological GI properties simulated. Adapted from (Kostewicz et al., 2013). DGM: dynamic gastric model; TIM-1: TNO gastrointestinal model; ASD: artificial stomach duodenal model

	Control on dosage form movement (C) or physiologically relevant transfer (P)	**Exposure of dosage form to biorelevant stresses**	**Constant flow conditions (C) or physiologically relevant flow conditions (P)**	**Dynamic changes of GI environment**	**Simulation of interrupted media contact**
Paddle-bead method	-	-	-	-	-
Rotating beaker	-	-	+ (C)	-	-
Stress test device	+ (C)	+	+ (P)	-	+
DGM	-	-	-	+	-
TIM-1	-	+	+ (P)	+	-
ASD	+ (P)	+	+ (P)	+	-

2.4 IVIVC Mathematical methodologies

As described in the previous section, an established IVIVC must predict *in vivo* performance from *in vitro* release data. There are different mathematical methods to establish an IVIVC and they can be classified into two classes as described in the FDA (FDA, 1997b) and EMA (EMA, 2012) guidelines.

2.4.1 Two-Stage methods

2.4.1.1 Model-dependent deconvolution methods

The two-stage methods are the most widely used and are the mathematical methodology required by the FDA to establish an IVIVC. In the first stage, a deconvolution method is used to estimate the *in vivo* absorption or dissolution time course, *i.e.* fraction absorbed *vs.* time. In the second stage, a link model is established between *in vivo* absorption-time profile and *in vitro* dissolution or release profile. Then, plasma concentrations are predicted from *in vitro* release data using the link model. Only linear systems allow this mathematical procedure. A system is linear if it has two features: superposition and time invariance (Veng-Pedersen et al., 2000).

2.4.1.1.1 Wagner-Nelson deconvolution

Wagner-Nelson (WN) analysis can be applied only to one-compartment drugs (Wagner and Nelson, 1963). This method is based on the mass balance theory, where no kinetic model for the absorption process is assumed. WN method does not require IV drug administration, because it assumes identical elimination rate coefficient (k_{el}) between intra- and extravasal administration and, therefore k_{el} can be estimated from the final stage of the oral curve. However, when flip-flop occurs, IV drug administration is therefore needed to estimate k_{el}.

$$F_{abs} = \frac{A_t}{A_\infty} = \frac{C_t + k_{el} \cdot AUC_0^t}{k_{el} \cdot AUC_0^\infty} \quad (1)$$

Equation 1 is the WN equation that represents the fraction absorbed of the bioavailable dose at time t, where F_{abs} is the fraction absorbed, A_t is the drug amount absorbed at time t, A_∞ is the drug amount absorbed at infinite time, C_t is the drug concentration at time t, k_{el} is the elimination rate coefficient, AUC_0^t is the area under the curve from time 0 to time t and AUC_0^∞ is the area under the curve from time 0 to infinity.

2.4.1.1.2 Loo-Riegelman

Loo-Riegelman (LR) analysis can be applied only to two-compartment drugs (Loo and Riegelman, 1968) and it is also based on the mass balance theory (Eq. 1).

Peripheral compartment concentration could be calculated by the following equations:

$$P_t = k_{12} \cdot e^{-k_{21} \cdot t} \cdot \int_0^t C \cdot e^{k_{21} \cdot t} \cdot \partial t \quad (2)$$

$$P_t = P_{t-1} \cdot e^{-k_{21} \cdot \Delta t} + \frac{k_{12}}{k_{21}} \cdot C_{t-1} \cdot \left(1 - e^{-k_{21} \cdot \Delta t}\right) + \frac{k_{12}}{2} \cdot \Delta C \cdot \Delta t \quad (3)$$

Equation 2 is the LR equation which was published by Wagner (Wagner, 1967) and Equation 3 is an approximate solution that can be applied when sampling intervals are small and linear.

2.4.1.2 Model-independent deconvolution methods

The deconvolution methods do not assume a pharmacokinetic model for drug disposition and can be applied to linear systems. A system is characterized by an input point or pulse (which corresponds to the absorption zone) and the response, as the

variable measured due to an impulse. The most important input function is the unit impulse (δ). An OR is a good approach or description of the unit impulse. The response is called the unit impulse response function or C_δ. The unit impulse response is the result of an impulse divided by its magnitude (Young, 1997, O'Hara et al., 2001).

$$C(t) = \int_0^t f(t) \cdot C_\delta \cdot (t - \tau) \cdot d\tau \quad (4)$$

Equation 4 shows the convolution integral where C is the actual drug concentration at time t, C_δ is the unit impulse response function, and f is the dissolution rate.

The deconvolution can be used to estimate an input function, given the corresponding system response and unit impulse response of the system. The unit impulse response must be obtained through the reference administration. Although OR is preferred, an intravenous (IV) administration is generally used and an immediate release (IR) dosage form can also be selected.

Below, three mathematical procedures for deconvolution are presented:

2.4.1.2.1 Analytical Laplace transform deconvolution

Laplace transforms simplify the resolution of the convolution integral. To convolve two functions:

1. The Laplace transform of each function is determined.
2. The transform functions are multiplied
3. The inverse transform is calculated.

Thus, the convolution of two functions is the inverse Laplace transform of the product of both functions in the Laplace domain. An example is shown below.

Laplace transform of the input function f (t) and the unit impulse response C_δ:

$$f(t) = F \cdot D \cdot k_a \cdot e^{-k_a \cdot t} \rightarrow \ l\,[f(t)] = \frac{F \cdot D \cdot k_a}{(s+k_a)} \quad (5)$$

$$C_\delta(t) = \frac{1}{D} \cdot \frac{D \cdot e^{-k_{el} \cdot t}}{Vd} \rightarrow l\,[C_\delta(t)] = \frac{1}{V_d \cdot (s+k_{el})} \quad (6)$$

Product of transformed and anti-transformed functions to obtain expression of the response function:

$$l[f(t)] \cdot l[C_\delta(t) = \frac{F \cdot D \cdot k_a}{V_d \cdot (k_a - k_{el})} \quad (7)$$

$$l^{-1}\left[\frac{F \cdot D \cdot k_a}{V_d \cdot (s+k_a) \cdot (s+k_{el})}\right] = \frac{F \cdot D \cdot k_a}{V_d \cdot (k_a - k_{el})} \cdot (e^{-k_{el} \cdot t} - e^{-k_a \cdot t}) \quad (8)$$

2.4.1.2.2 Deconvolution by curve-fitting

Assuming that the input function is a function of p parameters, the deconvolution problem is transformed into a regression problem in which the parameters are estimated by nonlinear regression. If the input function is considered as an exponential function, its convolution with the unit impulse response is the Bateman function.

2.4.1.2.3 Point-area deconvolution

In the point-area deconvolution approach, the main assumption is that in a short interval, the input function is constant (Vaughan and Dennis, 1978).

$$R_n = \frac{C_n - \sum_{i=2}^{n} R_{i-1} \cdot AUC_{\delta n-i+1}^{n-1+2}}{AUC_0^1} \quad (9)$$

2.4.1.3 Mathematical procedures for concentration estimation.

It is important to stand out that using LR and WN methods bioavailability fraction is obtained. Using model independent deconvolution-based methods, the *in vivo* function obtained depends on the reference dosage form used during the deconvolution process.

If IV administration is used as a reference, the unit impulse response of the system corresponds to the disposition of the drug and thus the input function incorporates all the processes, namely, drug dissolution, absorption and first-pass effect. In the case of an oral solution, the unit impulse response includes the absorption process and first-pass effect, so that by the deconvolution, the input function corresponds to dissolution and release rate. If the reference is an IR dosage form, unit impulse response incorporates the dissolution from the dosage form immediately, so that the input function represents the release rate of the dosage form.

With either of the methods described above, an *in vivo* absorption profile is obtained. The next step is to establish the *in vivo-in vitro* correlation. In the second stage, the aim is to predict the *in vivo* plasma concentrations from the *in vitro* data. The predicted *in vivo* fractions dissolved must be 'reconvolved' with estimates of the pharmacokinetic parameters obtained from the reference data to produce plasma concentration profiles (Gaynor et al., 2011).

Different approaches have been published in the last years. Langenbucher et al (Langenbucher, 2003) and Qureshi (Qureshi, 2010) proposed to use the superposition principle to obtain the predicted plasma concentrations. Gohel equation (Eq. 10) is an approach only valid for one compartment drugs (Gohel, 2005). The third method (Takka et al., 2003) consists of demonstrating that the mean *in vitro* dissolution rate constant (k_d) is correlated with the mean *in vivo* absorption rate coefficient (k_a). The purpose of this k_d-k_a correlation is to calculate predicted k_a in order to use the Bateman equation (Eq. 11) to estimate the predicted plasma concentrations.

$$C_{t-1} = \frac{\left(\frac{2 \cdot \Delta F_{abs} \cdot D}{V_d}\right) + C_t \cdot (2 - k_{el} \cdot \Delta t)}{(2 - k_{el} \cdot \Delta t)} \quad (10)$$

$$C = \frac{F \cdot D}{V_d} \cdot \frac{k_a}{k_a - k_{el}} \cdot \left(e^{-k_{el} \cdot t} - e^{-k_a \cdot t}\right) \quad (11)$$

2.4.2 One-Stage methods

2.4.2.1 Convolution-based methods

Conversely, convolution-based methods are one-stage modelling approaches, which directly relate the *in vivo* release to the *in vitro* release. The equation that forms the centre of these approaches relies on a convolution-type integral transform (Gillespie, 1997, O'Hara et al., 2001). The basis and equations for this method have been described in detail in several papers (Gillespie, 1997, Veng-Pedersen et al., 2000, Costello et al., 2011, Gaynor et al., 2008, O'Hara et al., 2001). For these methods, a reference administration could be useful, but it is not mandatory. The advantage of this method against two-stage methods is that the relationship between the *in vitro* release and plasma concentrations of the drug are set in one step, so that the modelling is focused on the ability to predict the *in vivo* behaviour (O'Hara et al., 2001, Balan et al., 2001).

Within this context, defining an IVIVC the main aim is to establish the functional dependence that relates the *in vivo* input (release or absorption) rate F_{i2} to the *in vitro* dissolution rate F_{i1}.

The simplest choice is a linear one:

$$F_{i2}(t) = F_{i1}(t) \quad (12)$$

As Dunne et al. published (Dunne et al., 1999), the relationship between the *in vivo* and *in vitro* dissolution may be expressed in terms of a relationship between the related

functions such as the odds functions (Eq. 13), the hazard functions (Eq. 14) or the reversed hazard functions (Eq. 15)

$$\frac{F_{i2k}(t)}{1-F_{i2k}(t)} = \alpha_{ik} \cdot \frac{F_{i1}(t)}{1-F_{i1}(t)} \quad (13)$$

$$1 - F_{i2k}(t) = (1 - F_{i1}(t))^{\alpha_{ik}} \quad (14)$$

$$F_{i2k}(t) = (F_{i1}(t))^{\alpha_{ik}} \quad (15)$$

where α_i is the constant of proportionality for the ith unit.

Following the mathematical development proposed by O'Hara et al. (O'Hara et al., 2001), equations 13-15 can be written as:

$$g\big(F_{i2k}(t)\big) = \log(\alpha) + g(F_1(t)) \quad (16)$$

Where g(-) is the so called link function which maps [0, 1] to [-∞, +∞], and is the logit, complementary log-log or log-log depending on which of the three models described above is being considered. The link function guarantees that both rates (*in vivo* absorption and *in vitro* dissolution) lie in the interval [0, 1].

Time scaling is frequently used to justify differences in time profiles for *in vitro* and *in vivo* release. The presence of a slight time-delay (time-shift or lag time), for the *in vivo* data is also a realistic assumption in most cases, because compared with *in vitro* in which dissolution may start instantaneously, *in vivo* absorption may be somewhat delayed.

$$g(F_{i2k}(t) = \theta_0 + \theta_1 t + g(F_1(t)) \quad (17)$$

Equation 17 assumes that time profiles of the *in vitro* and *in vivo* release are similar; θ_0 and θ_1 are the time scaling and the scaling factor.

However, these equations do not include random errors. As it is described in the literature (O'Hara et al., 2001, Rossenu et al., 2008, Dunne et al., 1999, Gaynor et al., 2008), the error associated with the *in vitro* dissolution and the error associated with the *in vivo* profiles are different. Therefore, it may be modelled independently,

$$g\big(F_{i2k}(t)\big) = \theta_0 + \theta_1 t + g\big(F_1(t)\big) + u_i + s_{ik} \quad (18)$$

Where u_i and s_{ik} are time invariant independent random effects that describe the variation *in vitro* between dosage units and *in vivo* between dosage unit-subject combinations, respectively.

2.4.2.2 Differential equations-based models

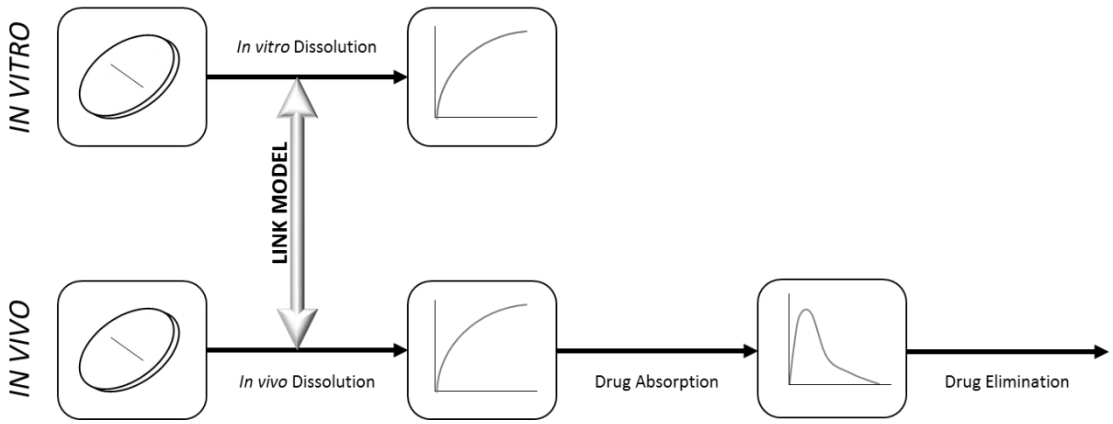
The need for a convolution or deconvolution step can be avoided employing direct numeric solution to differential equation-based models. Moreover, measurable plasma concentrations are directly related to *in vitro* fraction-dissolved data. It has been illustrated that the convolution-based and compartmental models are mathematically equivalent when the system being modelled is linear (Dunne, 2007). Nonetheless, differential equation allows a compartmental approach, while convolution-based methods does not.

One of the assumptions of convolution- and deconvolution-based methods is that the system that is being modelled is linear, but not always it occurs in that way. For example, several drugs are eliminated by mechanisms that imply saturable processes (Gibiansky and Gibiansky, 2013, van Kuilenburg and Maring, 2013). It has been demonstrated (Gaynor et al., 2008) that the convolution-based method is truthful but, the assumption of linearity is violated when data for a drug with nonlinear kinetics is analysed and therefore this approach could not be adequate.

In the study of nonlinear kinetic drugs, it is essential to use a method that allows modelling this nonlinearity. It is clear that both convolution and deconvolution-based models do not satisfy these requirements, but a compartmental approach, using differential equations, can be the solution for this type of drugs. Additionally, the IVIVC relationship can be specified by the user to incorporate random effects, time dependence, scale factors etc. as required by each particular set of data (Gaynor et al., 2011).

Gaynor et al (Gaynor et al., 2011) described a compartmental approach for a drug with nonlinear kinetics (Figure 1). Five compartments are used to establish the IVIVC where the first two compartments correspond to *in vitro* dissolution data and the last three to the *in vivo* data allowing a Michaelis-Menten elimination.

Figure 1. Schematic representation of in vitro-in vivo model development. Based on figures from **Gaynor, C.,** et al. JPKPD **38** 317-332, 2011.



2.5 Evaluation of IVIVC predictability

Once the IVIVC has been established, the last step before its use as a surrogate of the *in vivo* performance is to evaluate the predictability of the IVIVC. Usually the IVIVC is

evaluated by the prediction error that is calculated using the observed *in vivo* property (i.e. AUC and Cmax) and the estimated *in vivo* property.

2.5.1 Internal validation

Internal validation results from the evaluation of the prediction errors (PE) obtained after comparing the observed *in vivo* parameter used to develop the IVIVC versus *in vivo* predicted parameter from the developed IVIVC. The %PE is calculated by the following equation:

$$\%PE = \frac{(Observed\ Parameter - Predicted\ Parameter)}{Observed\ Parameter} \cdot 100 \qquad (19)$$

According to FDA and EMA guidelines, the mean absolute %PE for all formulations should be less than 10% and the %PE for an individual formulation should be less than 15%.

2.5.2 External validation

External validation must be established with a dataset not selected during the development of the IVIVC. In agreement with FDA and EMA guidelines (EMA, 2012, FDA, 1997b), %PE less than 10% means good predictability of the IVIVC, %PE between 10-20% is referred to inconclusive predictability and they need for further study using an aditional dataset and %PE higher than 20% implies inadequate predictability. Although EMA insists on external validation as a final evaluation of the IVIVC, FDA does not require external validation if the IVIVC has succesfully passed the internal validation (EMA, 2012, FDA, 1997b).

2.6 Recommendations on IVIVC from regulatory agencies

Athough, FDA and EMA recommend at least two formulations with different release rates to develop an IVIVC, most investigations included three formulations (slow,

medium and fast release rates) (Balan et al., 2001, Dutta et al., 2005, Kakhi et al., 2013, Soto et al., 2010, Kesisoglou et al., 2014, Modi et al., 2000). It is recognized that if three or more different formulations are employed for the *in vivo* and *in vitro* study, more robustness is obtained and better results might be obtained in the external validation.

For modified-release products, the release controlling excipient(s) in the formulation should either be identical or very similar, in order to develop an IVIVC. Dissolution datasets are recommended to be obtained in different test conditions to evaluate how dissolution factors influence drug release. The most relevant recommendations are summarized in Table 5.

EMA current guidance requires an individual one step convolution approach to account for interindividual and residual variability that many authors consider that is a more robust approach as it is dicussed in the next section.

Table 5. Comparison between FDA and EMA guidelines. Nd: not declared.

<table>
<tr><th></th><th>FDA</th><th>EMA</th></tr>
<tr><td rowspan="3">General</td><td>Correlation level: A or multiple C</td><td>Correlation level: A</td></tr>
<tr><td>Two-stages method approach is required</td><td>Two-stages method approach as exploratory Deconvolution, convolution and differential equation-based methods can be used</td></tr>
<tr><td colspan="2">Two or more formulations are required</td></tr>
<tr><td rowspan="3">*In vivo*</td><td colspan="2">Healthy volunteers</td></tr>
<tr><td>6-36 subjects for BE *in vivo* studies</td><td>≥ 12 subjects for BE *in vivo* studies</td></tr>
<tr><td colspan="2">Fasted state</td></tr>
<tr><td rowspan="3">*In vitro*</td><td>Preferred apparatus I or II</td><td>*Nd*</td></tr>
<tr><td>pH less than 6.8</td><td>*Nd*</td></tr>
<tr><td>12 dosage units for *in vitro* dissolution test</td><td>*Nd*</td></tr>
</table>

2.7 Advantages and limitations of IVIVC modelling approaches

Based on WN assumptions, an IV drug administration is not imperative for establishing IVIVC and this is one of the main advantages of the WN method in ethical and economical terms. Moreover, deconvolution model approaches are easier to develop experimentally and during data analysis. The use of averaged data and integrated equations may be implemented in many common and worldwide used packages. Those reasons may explain the great number of IVIVC examples in literature using WN method (Naeem Aamir et al., 2011, Honorio Tda et al., 2013, Malewar et al., 2013, Ostrowski et al., 2010, Khaled et al., 2013a, D'Souza et al., 2014, Liu et al., 2003, Rossi et al., 2011, Ostrowski et al., 2009, Yaro et al., 2014, Hou et al., 2012, Philip and Pathak, 2008, Emara et al., 2000). One of the major and more discussed limitation of WN and LR methods is the employment of averaged data in almost all cases. A significant handicap of these methods is that if raw data is averaged, they are not able to calculate dosage units and/or subjects' variability and intra-individual variation. For this reason, a relevant information is not considered. But if IVIVC fails, is it a consequence of the lost information? Cardot et al (Cardot and Davit, 2012) describe the differences in lost information when averaging *in vitro* or *in vivo* data. Averaging *in vitro* data for analysis is a common practice in tests such as the f1 and f2 tests (FDA, 1997a). Maybe, the major reason for average *in vitro* data is that the dissolution test is a reproducible technique with a controllable environment (pH, temperature, medium composition, etc.) that produce narrow results and low variability.

On the other hand, *in vivo* inter and intra-subject variability must be analysed. When a large intra-subject variability exists, two different profiles for a given subject after receiving two formulations could be due to either the large intra-subject variability or to the true differences between formulations. Then, the IVIVC could not determine the

reason of these different profiles. However, when intra-subject variability is low, it must be determined whether averaged curve reflects the individual behaviour or not.

In the recent years, the use of physiologically based pharmacokinetic (PBPK) approach has increased because its great potential to assist in the design, selection and development of drugs (Rowland et al., 2004). Current dissolution methodologies may not reflect, in some cases, the complexity of all processes affecting the *in vivo* performance. Thus, PBPK integrates parameters determined *a priori* from *in silico* predictions, *in vitro* experiments, or *in vivo* data when required (Bouzom et al., 2012). These advantages have enabled the implementation of PBPK by pharmaceutical companies in dossiers submitted to the regulatory agencies in the last years (Zhao et al., 2011). In fact, in the last version of the *Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms* published by EMA, recognizes the use and application of PBPK analysis for drug performance prediction. However, more confirmative work is still needed in order to assess the extrapolation of *in vitro* properties to *in vivo* performance. Table 6 lists a summary of free and designed packages for IVIVC establishment.

Table 6. Open and designed pharmacokinetic packages used for IVIVC establishment.

	Software	Source	IVIVC
Open software	acslX® (Aegis Technologies) ®	http://www.acslx.com	
	MATLAB-simulink® (The Mathworks Inc.)	http://www.mathworks.com	?
	ADAPT 5® (University of Southern California)	http://bmsr.usc.edu/	Yes
	Berkeley-Madonna® (University of California)	http://www.berkeleymadonna.com	Yes
	MCSIM®	http://www.gnu.org/software/mcsim/	
	SAAM II® (University of Washington)	http://tegvirginia.com/solutions/saam-ii/	
Designed software	Cloe PK® (Cyprotex Ltd)	http://www.cyprotex.com/cloepredict/	No
	GastroPlus® (Simulations Plus Inc.)	http://www.simulations-plus.com	Yes
	MEDICI-PK® (Computing in Technology)	http://www.cit-wulkow.de/	No
	NONMEM®	http://www.iconplc.com/technology/products/nonmem/	Yes
	PK-Sim® (Bayer Technologies Services)	http://www.systems-biology.com	Yes
	Simcyp Simulator (Simcyp Ltd)	http://www.simcyp.com	Yes
	STELLA®	http://www.iseesystems.com/softwares/Education/StellaSoftware.aspx	Yes
	WinNonlin Phoenix®	http://www.certara.com/products/pkpd/phx-wnl	Yes

As a model-independent approach, deconvolution have gained popularity in the last years because it refuses the assumption of model-dependency, allowing more flexibility (Balan et al., 2001, Corrigan et al., 2003, Dunne et al., 1999, Egan et al., 1993, Gaynor et al., 2008, Kakhi and Chittenden, 2013, Kakhi et al., 2013, Khaled et al., 2013a, Khaled et al., 2013b, Kovacevic et al., 2009, Macha et al., 2009, Modi et al., 2000, Mundin et al., 2012, Okumu et al., 2008, Okumu et al., 2009, Parojcic et al., 2004, Patel et al., 2012, Rietbrock et al., 1995, Rostami-Hodjegan et al., 2002, Saibi et al., 2012, Sakuma et al., 2009, Shah et al., 1989, Sirisuth et al., 2002, Sunesen et al., 2005, Yang et al., 2013, Tang et al., 2013) (see Table 7). However, the use of averaged data in some articles is still controversial and it has become itself an important research field. Gaynor et al (Gaynor et al., 2009) analysed and compared the same dataset using deconvolution-based method with individual data and averaged data. Gaynor concludes that "averaging the observed data before deconvolution leads to predictions which are even less accurate than those obtained when deconvolution takes place on the individual subject level".

Although FDA (FDA, 1997b) prefers two-stage methods (deconvolution and model dependent methods) to establish an IVIVC, several authors emphasized the limitations of deconvolution (Costello et al., 2011, Dunne et al., 1999, Gaynor et al., 2008, O'Hara et al., 2001, Soto et al., 2010, Buchwald, 2003), and even EMA (EMA, 2014a) recommends deconvolution methods only for exploratory analysis which can be used as basis to develop a one-stage method model.

Many of these limitations are described below:

- Like model dependent methods, very often, the observed data are averaged at each point before analysis resulting into an important loss of information.
- The *in vivo* and *in vitro* data had to be collected at the same time point, namely, only common times can be used
- The deconvolution process could be unstable depending on the methods and samples.
- Deconvolution predicts the fraction of the drug dissolved *in vivo* instead of drug plasma concentration, which has more interest and gives more information
- Two of the main least squares assumptions are violated: system's linearity and time invariance.

On the contrary, convolution methods do not require *in vitro* and *in vivo* data collected at the same point, predicts plasma concentration directly in one-stage, and uses individual observed data. However, as deconvolution-methods, convolution assumes linearity of the system and time invariance.

Differential equations offer greater flexibility and allow to model nonlinear kinetic drugs even when there is a time-variance. In addition, this semi-mechanistic approach allows the use of individual data in order to incorporate inter- and intra-subject variability. EMA includes the recommendation of convolution methods and differential equations to obtain more robust and precise IVIVC results (EMA, 2012). This view is supported by Gaynor et al (Gaynor et al., 2008), which explains a noticeable difference in the accuracy and precision between convolution- and deconvolution-based methods.

From a regulatory point of view another limitation is that IVIVC cannot be extrapolated outside the design space that has been investigated to develop the correlation. Nevertheless, when IVIVC is used as a development tool some degree of extrapolation can be used with caution to design new formulations. (EMA, 2014a, EMA, 2014b).

Table 7. Examples of IVIVC published based on different drug properties, drug formulation, software and IVIVC method selected. . CR: controlled release; IR: immediate release; ER: extended release; EC: Enteric coated, IRFA: Inmediate release fast absorption; Caps: capsule, PR: prolonged release; MR: modified release; DT: Dispersible Tablet IV: Internal Validation; EV: External Validation.

Author	BCS Class	Drug	Route	Formulation	IVIVC	Method	Software	Averaged	IV / EV
(Naeem Aamir et al., 2011)	I	Tramadol	Oral	CR	NO IVIVC	Wagner Nelson		YES	NO
(Balan et al., 2000)	II	Glibenclamide	Oral	IR, MR	A and C	Convolution			IV
(Balan et al., 2001)	III	Metformin	Oral	MR	A	Convolution	Sigma-Plot®		
(Bose and Wui, 2013)	II	Domperidone	Oral	MR	A	Wagner Nelson		YES	IV / EV
(Bredael et al., 2014)	IV		Oral	IR	C			NO	
(Corrigan et al., 2003)	II	Ketoprofen	Oral	ER	A	Deconvolution	PCDCON®	YES	
(Dutta et al., 2005)	III	Divalproex sodium	Oral	ER	A	Wagner Nelson		YES	IV / EV
(Eddington et al., 1998)	I	Metoprolol	Oral	ER	A				
(Emara et al., 2000)		Vincamine	Oral	PR	A	Wagner Nelson	WinNonlin®		
(Ghosh et al., 2008)	II	Glipizide	Oral	ER	A	Wagner Nelson		YES	IV / EV
(Honorio Tda et al., 2013)	II	Efavirenz	Oral	IR	A	Wagner Nelson	GastroPlus®	YES	IV
(Ilic et al., 2014)	II	Nifedipine	Oral	MR	A	Convolution			IV

(Jantratid et al., 2008)		RZ-50	Oral	Caps	A	Wagner Nelson	SigmaPlot®		
(Jantratid et al., 2009)	II	Diclofenac	Oral	MR	A	Deconvolution	WinNonlin®	YES	
(Kakhi et al., 2013)			Oral	IR	A	Deconvolution	WinNolin®	NO	
(Khaled et al., 2013a)	I	Metoprolol	Oral		A, B and C	Wagner Nelson			IV
(Kovacevic et al., 2009)	II	Carbamazepine	Oral	IR and CR	A	Deconvolution	GastroPlus®	YES	
(Liu et al., 2003)	I	Theophylline	Oral	IR, EC, CR	A	Wagner Nelson		NO	
(Lue et al., 2008)	II		Oral	IR	A	Deconvolution	PDxIVIVC®	YES	IV
(Macha et al., 2009)	II	Nevirapine	Oral	ER	A	Deconvolution	WinNolin®	YES	IV
(Mirza et al., 2013)	I		Oral	ER	A	Wagner Nelson	GastroPlus®	YES	IV / EV
(Mundin et al., 2012)	I	Oxycodone	Oral	PR					IV
(Okumu et al., 2008)	II	Montelukast	Oral	EC	A	Deconvolution	GastroPlus®	YES	
(Okumu et al., 2009)	II	Etoricoxib	Oral	IR	A	Deconvolution	GastroPlus®	YES	IV
(Ostrowski et al., 2009)	II	Amoxicilin	Oral	DT	A	Wagner Nelson	Excel®		IV
(Parojcic et al., 2004)	III	Paracetamol	Oral	Matrix tablet	A	Deconvolution			
(Pitsiu et al., 2001)	III	Oxybutynin	Oral	OROS	A	Convolution	NONMEM®	NO	IV/EV
(Rossi et al., 2011)	II	Fosamprenavir	Oral	IR	A	Wagner Nelson	Scientist®	YES	IV

(Rostami-Hodjegan et al., 2002)	III	Paracetamol	Oral	IR and IRFA	A	Deconvolution	Excel®		
(Saibi et al., 2012)	II	Ripesridone	Oral	IR	A	Deconvolution	GastroPlus®	YES	IV
(Singhvi et al., 2015)	I	Milnacipran	Oral	Matrix	A	Convolution		YES	
(Sirisuth and Eddington, 2000)	I	Metoprolol	Oral	ER	A				IV
(Sirisuth et al., 2002)	I	Diltiazem	Oral	ER	A	Convolution	Adapt II®	YES	IV
(Shono et al., 2009)	II	Celecoxib	Oral	Caps	A		STELLA®	YES	IV
(Soto et al., 2010)	I	Pramipexole	Oral	ER	A	Differential Eq	NONMEM®	NO	IV
(Sunesen et al., 2005)	II	Danazol	Oral		A	Deconvolution	PDx-IVIC®	YES	IV
(Tashtoush et al., 2004)	II	Glibenclamide	Oral	Caps	C			YES	
(Veng-Pedersen et al., 2000)	II	Carbamazepine	Oral	IR	A	Convolution	PC_IVIC®		CROSS
(Wei and Lobenberg, 2006)	II	Glyburide	Oral	IR	A		GastroPlus®	YES	

3. CONCLUSION

IVIVC approaches can be useful tools for speeding development and optimizing and even reducing cost by avoiding future *in vivo* testing. Nevertheless, the development and validation of *in vivo* predictive dissolution methods still require extensive research as there is not a single apparatus or media able to resemble the gastrointestinal system complexity and the different impact of the physiological environment on the different drugs and drug products. BCS has been a good starting point to define the design of *in vivo* predictive dissolution methods and it has open new fields of research as the physiological buffers or the multi-compartment dissolution apparatuses. The integration of the *in vivo* information including *in vitro* dissolution variability with the *in vivo* system characteristics and variability through PBPK models and the adequate statistical procedures for setting up dissolution specifications will ensure the fail-safe use of dissolution data as surrogate of *in vivo* assays but the mathematical methods also need further investigation through simulation approaches and validation against *in vivo* data. The classification of drugs needing more sophisticated mathematical approaches to ensure *in vivo* bioequivalence from *in vitro* data versus others for which the standard and simple two step method over average profiles are enough would be a step forward facilitating the use of the IVIVC approach as well as its regulatory acceptance.

4. ACKNOWLEDGEMENTS

The authors acknowledge financial support to projects: DCI ALA/19.09.01/10/21526/245-297/ALFA 111(2010)29: Red-Biofarma. Red para el desarrollo de metodologias biofarmaceuticas racionales que incrementen la competencia y el impacto social de las Industrias Farmaceuticas Locales. A grant from Ministry of Education and Science of Spain and Miguel Hernandez University was awarded to Victor Mangas Sanjuán (Grant FPU AP2010-2372).

DECLARATION OF INTEREST

The authors report no declarations of interest.

6. REFERENCES

ALMUKAINZI, M., OKUMU, A., WEI, H. & LOBENBERG, R. 2014. Simulation of In Vitro Dissolution Behavior Using DDDPlus. *AAPS PharmSciTech*.

AMIDON, G. L., LENNERNAS, H., SHAH, V. P. & CRISON, J. R. 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical research,* 12, 413-20.

BALAN, G., TIMMINS, P., GREENE, D. S. & MARATHE, P. H. 2000. In-vitro in-vivo correlation models for glibenclamide after administration of metformin/glibenclamide tablets to healthy human volunteers. *The Journal of pharmacy and pharmacology,* 52, 831-8.

BALAN, G., TIMMINS, P., GREENE, D. S. & MARATHE, P. H. 2001. In vitro-in vivo correlation (IVIVC) models for metformin after administration of modified-release (MR) oral dosage forms to healthy human volunteers. *Journal of pharmaceutical sciences,* 90, 1176-85.

BATES, T. R., PIENIASZEK, H. J., JR., SEQUEIRA, J. A. & RASMUSSEN, J. E. 1977. Gastrointestinal absorption of griseofulvin from corn oil-in-water emulsions: effect of amount of corn oil ingested in man. *Archives of dermatology,* 113, 302-6.

BERGSTROM, C. A., HOLM, R., JORGENSEN, S. A., ANDERSSON, S. B., ARTURSSON, P., BEATO, S., BORDE, A., BOX, K., BREWSTER, M., DRESSMAN, J., FENG, K. I., HALBERT, G., KOSTEWICZ, E., MCALLISTER, M., MUENSTER, U., THINNES, J., TAYLOR, R. & MULLERTZ, A. 2014. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 57, 173-99.

BERLIN, M., PRZYKLENK, K. H., RICHTBERG, A., BAUMANN, W. & DRESSMAN, J. B. 2014. Prediction of oral absorption of cinnarizine - A highly supersaturating poorly soluble weak base with borderline permeability. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 88, 795-806.

BOSE, A. & WUI, W. T. 2013. Convolution and validation of in vitro-in vivo correlation of water-insoluble sustained-release drug (domperidone) by first-order pharmacokinetic one-compartmental model fitting equation. *European journal of drug metabolism and pharmacokinetics,* 38, 191-200.

BOUZOM, F., BALL, K., PERDAEMS, N. & WALTHER, B. 2012. Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs? *Biopharmaceutics & drug disposition,* 33, 55-71.

BREDAEL, G. M., BOWERS, N., BOULINEAU, F. & HAHN, D. 2014. In vitro-in vivo correlation strategy applied to an immediate-release solid oral dosage form with a biopharmaceutical classification system IV compound case study. *Journal of pharmaceutical sciences,* 103, 2125-30.

BUCHWALD, P. 2003. Direct, differential-equation-based in-vitro-in-vivo correlation (IVIVC) method. *The Journal of pharmacy and pharmacology,* 55, 495-504.

BUTLER, J. M. & DRESSMAN, J. B. 2010. The developability classification system: application of biopharmaceutics concepts to formulation development. *Journal of pharmaceutical sciences,* 99, 4940-54.

CARDOT, J. M. & DAVIT, B. M. 2012. In vitro-in vivo correlations: tricks and traps. *The AAPS journal,* 14, 491-9.

COOK, J. A. 2012. Development strategies for IVIVC in an industrial environment. *Biopharmaceutics & drug disposition,* 33, 349-53.
CORRIGAN, O. I., DEVLIN, Y. & BUTLER, J. 2003. Influence of dissolution medium buffer composition on ketoprofen release from ER products and in vitro-in vivo correlation. *International journal of pharmaceutics,* 254, 147-54.
COSTELLO, C., ROSSENU, S., VERMEULEN, A., CLETON, A. & DUNNE, A. 2011. A time scaling approach to develop an in vitro-in vivo correlation (IVIVC) model using a convolution-based technique. *Journal of pharmacokinetics and pharmacodynamics,* 38, 519-39.
CHOWDHURY, A. K., ISLAM, S. 2011. In vitro-in vivo correlation as a surrogate for bioequivalence testing: the current state of play. *Asian Journal of Pharmaceutical Sciences,* 6, 176-190.
CHRISTOPHERSEN, P. C., CHRISTIANSEN, M. L., HOLM, R., KRISTENSEN, J., JACOBSEN, J., ABRAHAMSSON, B. & MULLERTZ, A. 2014. Fed and fasted state gastro-intestinal in vitro lipolysis: In vitro in vivo relations of a conventional tablet, a SNEDDS and a solidified SNEDDS. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 57, 232-9.
D'SOUZA, S., FARAJ, J. A., GIOVAGNOLI, S. & DELUCA, P. P. 2014. IVIVC from Long Acting Olanzapine Microspheres. *International journal of biomaterials,* 2014, 407065.
DRESSMAN, J. B., AMIDON, G. L., REPPAS, C. & SHAH, V. P. 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharmaceutical research,* 15, 11-22.
DRESSMAN, J. B. & REPPAS, C. 2000. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 11 Suppl 2, S73-80.
DUNNE, A. 2007. Approaches to Developing in vitro-in vivo Correlation Models *In:* CHILUKURI, D. S., G.; YOUNG, D. (ed.) *Pharmaceutical Product Development in vitro-in vivo Correlation.* New York: Informa Healthcare USA, Inc.
DUNNE, A., O'HARA, T. & DEVANE, J. 1999. A new approach to modelling the relationship between in vitro and in vivo drug dissolution/absorption. *Statistics in medicine,* 18, 1865-76; discussion 1877.
DUTTA, S., QIU, Y., SAMARA, E., CAO, G. & GRANNEMAN, G. R. 2005. Once-a-day extended-release dosage form of divalproex sodium III: development and validation of a Level A in vitro-in vivo correlation (IVIVC). *Journal of pharmaceutical sciences,* 94, 1949-56.
EDDINGTON, N. D., MARROUM, P., UPPOOR, R., HUSSAIN, A. & AUGSBURGER, L. 1998. Development and internal validation of an in vitro-in vivo correlation for a hydrophilic metoprolol tartrate extended release tablet formulation. *Pharmaceutical research,* 15, 466-73.
EGAN, T. D., LEMMENS, H. J., FISET, P., HERMANN, D. J., MUIR, K. T., STANSKI, D. R. & SHAFER, S. L. 1993. The pharmacokinetics of the new short-acting opioid remifentanil (GI87084B) in healthy adult male volunteers. *Anesthesiology,* 79, 881-92.
EMA 2012. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms. London.
EMA 2014a. Guideline on quality of oral modified release products.

EMA 2014b. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms.

EMAMI, J. 2006. In vitro - in vivo correlation: from theory to applications. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques,* 9, 169-89.

EMARA, L. H., EL-MENSHAWI, B. S. & ESTEFAN, M. Y. 2000. In vitro-in vivo correlation and comparative bioavailablity of vincamine in prolonged-release preparations. *Drug development and industrial pharmacy,* 26, 243-51.

EROGLU, H., BURUL-BOZKURT, N., UMA, S. & ONER, L. 2012. Preparation and in vitro/in vivo evaluation of microparticle formulations containing meloxicam. *AAPS PharmSciTech,* 13, 46-52.

FADDA, H. M., MERCHANT, H. A., ARAFAT, B. T. & BASIT, A. W. 2009. Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. *International Journal of Pharmaceutics,* 382, 56-60.

FDA 1997a. Guidance for Industry Nonsterile Semisolid Dosage Forms. *Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation.* Centre for Drug Evaluation and Research (CDER): U.S. Department of Health and Human Services.

FDA 1997b. Guidance for industry. Extended release oral dosage forms: development, evaluation and application of *in vitro/in vivo* correlations. Center for Drug Evaluation and Research (CDER): US Department of Health and Human Services.

FDA 1997c. Guidance for Industry. SUPAC-MR: Modified Release Solid Oral Dosage Forms Centre for Drug Evaluation and Research (CDER): US Department of Health and Human Services.

FDA 1997d. Guidance for industry: Dissolution testing for immediate release solid oral dosage forms. Centre for Drug Evaluation and Research: US Department of Health and Human Services.

FOTAKI, N., AIVALIOTIS, A., BUTLER, J., DRESSMAN, J., FISCHBACH, M., HEMPENSTALL, J., KLEIN, S. & REPPAS, C. 2009. A comparative study of different release apparatus in generating in vitro-in vivo correlations for extended release formulations. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 73, 115-20.

GALIA, E., NICOLAIDES, E., HORTER, D., LOBENBERG, R., REPPAS, C. & DRESSMAN, J. B. 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharmaceutical research,* 15, 698-705.

GARBACZ, G., KOLODZIEJ, B., KOZIOLEK, M., WEITSCHIES, W. & KLEIN, S. 2014. A dynamic system for the simulation of fasting luminal pH-gradients using hydrogen carbonate buffers for dissolution testing of ionisable compounds. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 51, 224-31.

GAYNOR, C., DUNNE, A., COSTELLO, C. & DAVIS, J. 2011. A population approach to in vitro-in vivo correlation modelling for compounds with nonlinear kinetics. *Journal of pharmacokinetics and pharmacodynamics,* 38, 317-32.

GAYNOR, C., DUNNE, A. & DAVIS, J. 2008. A comparison of the prediction accuracy of two IVIVC modelling techniques. *Journal of pharmaceutical sciences,* 97, 3422-32.

GAYNOR, C., DUNNE, A. & DAVIS, J. 2009. The effects of averaging on accuracy of IVIVC model predictions. *Journal of pharmaceutical sciences,* 98, 3829-38.
GHOSH, A., BHAUMIK, U. K., BOSE, A., MANDAL, U., GOWDA, V., CHATTERJEE, B., CHAKRABARTY, U. S. & PAL, T. K. 2008. Extended release dosage form of glipizide: development and validation of a level A in vitro-in vivo correlation. *Biological & pharmaceutical bulletin,* 31, 1946-51.
GIBIANSKY, L. & GIBIANSKY, E. 2013. Target-mediated drug disposition model and its approximations for antibody-drug conjugates. *Journal of pharmacokinetics and pharmacodynamics.*
GILLESPIE, W. R. 1997. Convolution-based approaches for in vivo-in vitro correlation modeling. *Advances in experimental medicine and biology,* 423, 53-65.
GOHEL, M. 2005. Simplified Mathematical Approach for Back Calculation in Wagner-Nelson Method *Pharmainfo,* 3.
GRAY, V., KELLY, G., XIA, M., BUTLER, C., THOMAS, S. & MAYOCK, S. 2009. The science of USP 1 and 2 dissolution: present challenges and future relevance. *Pharmaceutical research,* 26, 1289-302.
GUHMANN, M., THOMMES, M., GERBER, F., POLLINGER, N., KLEIN, S., BREITKREUTZ, J. & WEITSCHIES, W. 2013. Design of biorelevant test setups for the prediction of diclofenac in vivo features after oral administration. *Pharmaceutical research,* 30, 1483-501.
HAYES, S., DUNNE, A., SMART, T. & DAVIS, J. 2004. Interpretation and optimization of the dissolution specifications for a modified release product with an in vivo-in vitro correlation (IVIVC). *Journal of pharmaceutical sciences,* 93, 571-81.
HONORIO TDA, S., PINTO, E. C., ROCHA, H. V., ESTEVES, V. S., DOS SANTOS, T. C., CASTRO, H. C., RODRIGUES, C. R., DE SOUSA, V. P. & CABRAL, L. M. 2013. In vitro-in vivo correlation of efavirenz tablets using GastroPlus(R). *AAPS PharmSciTech,* 14, 1244-54.
HORTER, D. & DRESSMAN, J. B. 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced drug delivery reviews,* 46, 75-87.
HOU, J., HE, X., XU, X., SHI, X., XU, Y. & LIU, C. 2012. Application of an in vitro DDASS to evaluate oral absorption of two chemicals simultaneously: establishment of a level A in vitro-in vivo correlation. *Drug development and industrial pharmacy,* 38, 1305-12.
ILIC, M., ETHURIS, J., KOVACEVIC, I., IBRIC, S. & PAROJCIC, J. 2014. In vitro--in silico--in vivo drug absorption model development based on mechanistic gastrointestinal simulation and artificial neural networks: nifedipine osmotic release tablets case study. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 62, 212-8.
JANTRATID, E., DE MAIO, V., RONDA, E., MATTAVELLI, V., VERTZONI, M. & DRESSMAN, J. B. 2009. Application of biorelevant dissolution tests to the prediction of in vivo performance of diclofenac sodium from an oral modified-release pellet dosage form. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 37, 434-41.
JANTRATID, E., JANSSEN, N., CHOKSHI, H., TANG, K. & DRESSMAN, J. B. 2008. Designing biorelevant dissolution tests for lipid formulations: case example--lipid suspension of RZ-50. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 69, 776-85.

JUENEMANN, D., JANTRATID, E., WAGNER, C., REPPAS, C., VERTZONI, M. & DRESSMAN, J. B. 2011. Biorelevant in vitro dissolution testing of products containing micronized or nanosized fenofibrate with a view to predicting plasma profiles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 77, 257-64.

KAKHI, M. & CHITTENDEN, J. 2013. Modeling of pharmacokinetic systems using stochastic deconvolution. *Journal of pharmaceutical sciences,* 102, 4433-43.

KAKHI, M., MARROUM, P. & CHITTENDEN, J. 2013. Analysis of level A in vitro-in vivo correlations for an extended-release formulation with limited bioavailability. *Biopharmaceutics & drug disposition,* 34, 262-77.

KALANTZI, L., GOUMAS, K., KALIORAS, V., ABRAHAMSSON, B., DRESSMAN, J. B. & REPPAS, C. 2006. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharmaceutical research,* 23, 165-76.

KAMBAYASHI, A., BLUME, H. & DRESSMAN, J. 2013. Understanding the in vivo performance of enteric coated tablets using an in vitro-in silico-in vivo approach: case example diclofenac. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 85, 1337-47.

KESISOGLOU, F., ROSSENU, S., FARRELL, C., VAN DEN HEUVEL, M., PROHN, M., FITZPATRICK, S., DE KAM, P. J. & VARGO, R. 2014. Development of in vitro-in vivo correlation for extended-release niacin after administration of hypromellose-based matrix formulations to healthy volunteers. *Journal of pharmaceutical sciences,* 103, 3713-23.

KHALED, A. A., PERVAIZ, K., KARIM, S., FARZANA, K. & MURTAZA, G. 2013a. Development of in vitro-in vivo correlation for encapsulated metoprolol tartrate. *Acta poloniae pharmaceutica,* 70, 743-7.

KHALED, A. A., PERVAIZ, K., KHILJEE, S., KARIM, S., SHOAIB, Q. U. & MURTAZA, G. 2013b. In vitro to in vivo profiling: an easy idea for biowaiver study. *Acta poloniae pharmaceutica,* 70, 873-5.

KLEBERG, K., JACOBSEN, J. & MULLERTZ, A. 2010. Characterising the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies. *The Journal of pharmacy and pharmacology,* 62, 1656-68.

KLEIN, S. 2010. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *The AAPS journal,* 12, 397-406.

KLEIN, S., BUTLER, J., HEMPENSTALL, J. M., REPPAS, C. & DRESSMAN, J. B. 2004. Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts. *The Journal of pharmacy and pharmacology,* 56, 605-10.

KOSTEWICZ, E. S., ABRAHAMSSON, B., BREWSTER, M., BROUWERS, J., BUTLER, J., CARLERT, S., DICKINSON, P. A., DRESSMAN, J., HOLM, R., KLEIN, S., MANN, J., MCALLISTER, M., MINEKUS, M., MUENSTER, U., MULLERTZ, A., VERWEI, M., VERTZONI, M., WEITSCHIES, W. & AUGUSTIJNS, P. 2013. In vitro models for the prediction of in vivo performance of oral dosage forms. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences.*

KOVACEVIC, I., PAROJCIC, J., HOMSEK, I., TUBIC-GROZDANIS, M. & LANGGUTH, P. 2009. Justification of biowaiver for carbamazepine, a low soluble high

permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. *Molecular pharmaceutics,* 6, 40-7.
LANGENBUCHER, F. 2003. Handling of computational in vitro/in vivo correlation problems by Microsoft Excel: III. Convolution and deconvolution. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 56, 429-37.
LIMBERG, J. & POTTHAST, H. 2013. Regulatory status on the role of in vitro dissolution testing in quality control and biopharmaceutics in Europe. *Biopharm Drug Dispos,* 34, 247-53.
LIU, F., MERCHANT, H. A., KULKARNI, R. P., ALKADEMI, M. & BASIT, A. W. 2011. Evolution of a physiological pH 6.8 bicarbonate buffer system: application to the dissolution testing of enteric coated products. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 78, 151-7.
LIU, Y., SCHWARTZ, J. B. & SCHNAARE, R. L. 2003. A multimechanistic drug release approach in a bead dosage form and in vitro predictions. *Pharmaceutical development and technology,* 8, 163-73.
LOO, J. C. & RIEGELMAN, S. 1968. New method for calculating the intrinsic absorption rate of drugs. *Journal of Pharmaceutical Sciences,* 57, 918-28.
LUE, B. M., NIELSEN, F. S., MAGNUSSEN, T., SCHOU, H. M., KRISTENSEN, K., JACOBSEN, L. O. & MULLERTZ, A. 2008. Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 69, 648-57.
MACHA, S., YONG, C. L., DARRINGTON, T., DAVIS, M. S., MACGREGOR, T. R., CASTLES, M. & KRILL, S. L. 2009. In vitro-in vivo correlation for nevirapine extended release tablets. *Biopharmaceutics & drug disposition,* 30, 542-50.
MALEWAR, N., AVACHAT, M., POKHARKAR, V. & KULKARNI, S. 2013. Controlled release of ropinirole hydrochloride from a multiple barrier layer tablet dosage form: effect of polymer type on pharmacokinetics and IVIVC. *AAPS PharmSciTech,* 14, 1178-89.
MCCONNELL, E. L., FADDA, H. M. & BASIT, A. W. 2008. Gut instincts: explorations in intestinal physiology and drug delivery. *International Journal of Pharmaceutics,* 364, 213-26.
MERCHANT, H. A., GOYANES, A., PARASHAR, N. & BASIT, A. W. 2014. Predicting the gastrointestinal behaviour of modified-release products: Utility of a novel dynamic dissolution test apparatus involving the use of bicarbonate buffers. *International Journal of Pharmaceutics,* 475, 585-91.
MIRZA, T., BYKADI, S. A., ELLISON, C. D., YANG, Y., DAVIT, B. M. & KHAN, M. A. 2013. Use of in vitro-in vivo correlation to predict the pharmacokinetics of several products containing a BCS class 1 drug in extended release matrices. *Pharmaceutical research,* 30, 179-90.
MODI, N. B., LAM, A., LINDEMULDER, E., WANG, B. & GUPTA, S. K. 2000. Application of in vitro-in vivo correlations (IVIVC) in setting formulation release specifications. *Biopharmaceutics & drug disposition,* 21, 321-6.
MUDIE, D. M., AMIDON, G. L. & AMIDON, G. E. 2010. Physiological parameters for oral delivery and in vitro testing. *Molecular pharmaceutics,* 7, 1388-405.
MUDIE, D. M., MURRAY, K., HOAD, C. L., PRITCHARD, S. E., GARNETT, M. C., AMIDON, G. L., GOWLAND, P. A., SPILLER, R. C., AMIDON, G. E. & MARCIANI, L. 2014.

Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Molecular pharmaceutics,* 11, 3039-47.
MUDIE, D. M., SHI, Y., PING, H., GAO, P., AMIDON, G. L. & AMIDON, G. E. 2012. Mechanistic analysis of solute transport in an in vitro physiological two-phase dissolution apparatus. *Biopharmaceutics & drug disposition,* 33, 378-402.
MUNDIN, G. E., SMITH, K. J., MYSICKA, J., HEUN, G., KRAMER, M., HAHN, U. & LEUNER, C. 2012. Validated in vitro/in vivo correlation of prolonged-release oxycodone/naloxone with differing dissolution rates in relation to gastrointestinal transit times. *Expert opinion on drug metabolism & toxicology,* 8, 1495-503.
NAEEM AAMIR, M., AHMAD, M., AKHTAR, N., MURTAZA, G., KHAN, S. A., SHAHIQ UZ, Z. & NOKHODCHI, A. 2011. Development and in vitro-in vivo relationship of controlled-release microparticles loaded with tramadol hydrochloride. *International journal of pharmaceutics,* 407, 38-43.
NICOLAIDES, E., GALIA, E., EFTHYMIOPOULOS, C., DRESSMAN, J. B. & REPPAS, C. 1999. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. *Pharmaceutical research,* 16, 1876-82.
NICOLAIDES, E., SYMILLIDES, M., DRESSMAN, J. B. & REPPAS, C. 2001. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. *Pharmaceutical research,* 18, 380-8.
O'HARA, T., HAYES, S., DAVIS, J., DEVANE, J., SMART, T. & DUNNE, A. 2001. In vivo-in vitro correlation (IVIVC) modeling incorporating a convolution step. *Journal of pharmacokinetics and pharmacodynamics,* 28, 277-98.
OKUMU, A., DIMASO, M. & LOBENBERG, R. 2008. Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. *Pharmaceutical research,* 25, 2778-85.
OKUMU, A., DIMASO, M. & LOBENBERG, R. 2009. Computer simulations using GastroPlus to justify a biowaiver for etoricoxib solid oral drug products. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 72, 91-8.
OSTROWSKI, M., WILKOWSKA, E. & BACZEK, T. 2009. In vivo-in vitro correlation for amoxicillin trihydrate 1000 mg dispersible tablet. *Drug development and industrial pharmacy,* 35, 981-5.
OSTROWSKI, M., WILKOWSKA, E. & BACZEK, T. 2010. The influence of averaging procedure on the accuracy of IVIVC predictions: immediate release dosage form case study. *Journal of pharmaceutical sciences,* 99, 5040-5.
PAROJCIC, J., ETHURIC, Z., JOVANOVIC, M., IBRIC, S. & JOVANOVIC, D. 2004. Influence of dissolution media composition on drug release and in-vitro/in-vivo correlation for paracetamol matrix tablets prepared with novel carbomer polymers. *The Journal of pharmacy and pharmacology,* 56, 735-41.
PATEL, A. R., SPENCER, S. D., CHOUGULE, M. B., SAFE, S. & SINGH, M. 2012. Pharmacokinetic evaluation and in vitro-in vivo correlation (IVIVC) of novel methylene-substituted 3,3' diindolylmethane (DIM). *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 46, 8-16.
PERSSON, E. M., GUSTAFSSON, A. S., CARLSSON, A. S., NILSSON, R. G., KNUTSON, L., FORSELL, P., HANISCH, G., LENNERNAS, H. & ABRAHAMSSON, B. 2005. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharmaceutical research,* 22, 2141-51.

PHILIP, A. K. & PATHAK, K. 2008. Wet process-induced phase-transited drug delivery system: a means for achieving osmotic, controlled, and level A IVIVC for poorly water-soluble drug. *Drug development and industrial pharmacy,* 34, 735-43.

PITSIU, M., SATHYAN, G., GUPTA, S. & VEROTTA, D. 2001. A semiparametric deconvolution model to establish in vivo-in vitro correlation applied to OROS oxybutynin. *Journal of pharmaceutical sciences,* 90, 702-12.

QURESHI, S. 2010. In Vitro-In Vivo Correlation (IVIVC) and Determining Drug Concentrations in Blood from Dissolution Testing – A Simple and Practical Approach. *The Open Drug Delivery Journal,* 4, 38-47.

REPISHTI, M., HOGAN, D. L., PRATHA, V., DAVYDOVA, L., DONOWITZ, M., TSE, C. M. & ISENBERG, J. I. 2001. Human duodenal mucosal brush border Na(+)/H(+) exchangers NHE2 and NHE3 alter net bicarbonate movement. *American journal of physiology. Gastrointestinal and liver physiology,* 281, G159-63.

RIETBROCK, S., MERZ, P. G., FUHR, U., HARDER, S., MARSCHNER, J. P., LOEW, D. & BIEHL, J. 1995. Absorption behavior of sulpiride described using Weibull functions. *International journal of clinical pharmacology and therapeutics,* 33, 299-303.

ROSSENU, S., GAYNOR, C., VERMEULEN, A., CLETON, A. & DUNNE, A. 2008. A nonlinear mixed effects IVIVC model for multi-release drug delivery systems. *Journal of pharmacokinetics and pharmacodynamics,* 35, 423-41.

ROSSI, R. C., DIAS, C. L., BAJERSKI, L., BERGOLD, A. M. & FROEHLICH, P. E. 2011. Development and validation of discriminating method of dissolution for fosamprenavir tablets based on in vivo data. *Journal of pharmaceutical and biomedical analysis,* 54, 439-44.

ROSTAMI-HODJEGAN, A., SHIRAN, M. R., TUCKER, G. T., CONWAY, B. R., IRWIN, W. J., SHAW, L. R. & GRATTAN, T. J. 2002. A new rapidly absorbed paracetamol tablet containing sodium bicarbonate. II. Dissolution studies and in vitro/in vivo correlation. *Drug development and industrial pharmacy,* 28, 533-43.

ROWLAND, M., BALANT, L. & PECK, C. 2004. Physiologically based pharmacokinetics in drug development and regulatory science: a workshop report (Georgetown University, Washington, DC, May 29-30, 2002). *AAPS pharmSci,* 6, E6.

SAIBI, Y., SATO, H. & TACHIKI, H. 2012. Developing in vitro-in vivo correlation of risperidone immediate release tablet. *AAPS PharmSciTech,* 13, 890-5.

SAKUMA, S., OGURA, R., MASAOKA, Y., KATAOKA, M., TANNO, F. K., KOKUBO, H. & YAMASHITA, S. 2009. Correlation between in vitro dissolution profiles from enteric-coated dosage forms and in vivo absorption in rats for high-solubility and high-permeability model drugs. *Journal of pharmaceutical sciences,* 98, 4141-52.

SCHAMP, K., SCHREDER, S. A. & DRESSMAN, J. 2006. Development of an in vitro/in vivo correlation for lipid formulations of EMD 50733, a poorly soluble, lipophilic drug substance. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 62, 227-34.

SHAH, V. P., GURBARG, M., NOORY, A., DIGHE, S. & SKELLY, J. P. 1992. Influence of higher rates of agitation on release patterns of immediate-release drug products. *Journal of pharmaceutical sciences,* 81, 500-3.

SHAH, V. P., KONECNY, J. J., EVERETT, R. L., MCCULLOUGH, B., NOORIZADEH, A. C. & SKELLY, J. P. 1989. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. *Pharmaceutical research,* 6, 612-8.

SHENG, J. J., MCNAMARA, D. P. & AMIDON, G. L. 2009. Toward an in vivo dissolution methodology: a comparison of phosphate and bicarbonate buffers. *Molecular pharmaceutics,* 6, 29-39.

SHONO, Y., JANTRATID, E., JANSSEN, N., KESISOGLOU, F., MAO, Y., VERTZONI, M., REPPAS, C. & DRESSMAN, J. B. 2009. Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 73, 107-14.

SHONO, Y., JANTRATID, E., KESISOGLOU, F., REPPAS, C. & DRESSMAN, J. B. 2010. Forecasting in vivo oral absorption and food effect of micronized and nanosized aprepitant formulations in humans. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* 76, 95-104.

SIEVERT, B. A. S., M. 1998. Dissolution tests for ER products. *Dissolution Technology,* 5, 1-7.

SINGHVI, G., SHAH, A., YADAV, N. & SAHA, R. N. 2015. Prediction of in vivo plasma concentration-time profile from in vitro release data of designed formulations of milnacipran using numerical convolution method. *Drug development and industrial pharmacy,* 41, 105-8.

SIRISUTH, N., AUGSBURGER, L. L. & EDDINGTON, N. D. 2002. Development and validation of a non-linear IVIVC model for a diltiazem extended release formulation. *Biopharmaceutics & drug disposition,* 23, 1-8.

SIRISUTH, N. & EDDINGTON, N. D. 2000. Influence of stereoselective pharmacokinetics in the development and predictability of an IVIVC for the enantiomers of metoprolol tartrate. *Pharmaceutical research,* 17, 1019-25.

SOTO, E., HAERTTER, S., KOENEN-BERGMANN, M., STAAB, A. & TROCONIZ, I. F. 2010. Population in vitro-in vivo correlation model for pramipexole slow-release oral formulations. *Pharmaceutical research,* 27, 340-9.

SUNESEN, V. H., PEDERSEN, B. L., KRISTENSEN, H. G. & MULLERTZ, A. 2005. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 24, 305-13.

TAKKA, S., SAKR, A. & GOLDBERG, A. 2003. Development and validation of an in vitro-in vivo correlation for buspirone hydrochloride extended release tablets. *Journal of controlled release : official journal of the Controlled Release Society,* 88, 147-57.

TANG, X., TAI, L. Y., YANG, X. G., CHEN, F., XU, H. M. & PAN, W. S. 2013. In vitro and in vivo evaluation of gliclazide push-pull osmotic pump coated with aqueous colloidal polymer dispersions. *Drug development and industrial pharmacy,* 39, 67-76.

TASHTOUSH, B. M., AL-QASHI, Z. S. & NAJIB, N. M. 2004. In vitro and in vivo evaluation of glibenclamide in solid dispersion systems. *Drug development and industrial pharmacy,* 30, 601-7.

TSUME, Y., AMIDON, G. L. & TAKEUCHI, S. 2013. Dissolution Effect of Gastric and Intestinal pH fora BCS class II drug, Pioglitazone: New in vitro Dissolution System to Predict in vivo Dissolution. *Journal of Bioequivalence & Bioavailability,* 224-227.

TSUME, Y., MUDIE, D. M., LANGGUTH, P., AMIDON, G. E. & AMIDON, G. L. 2014. The Biopharmaceutics Classification System: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 57, 152-63.

USP 2004. *In vitro and In vivo Evaluations of Dosage Forms,* Easton, PA., Mack Publishing Co.

VAN KUILENBURG, A. B. & MARING, J. G. 2013. Evaluation of 5-fluorouracil pharmacokinetic models and therapeutic drug monitoring in cancer patients. *Pharmacogenomics,* 14, 799-811.

VAUGHAN, D. P. & DENNIS, M. 1978. Mathematical basis of point-area deconvolution method for determining in vivo input functions. *Journal of pharmaceutical sciences,* 67, 663-5.

VENG-PEDERSEN, P., GOBBURU, J. V., MEYER, M. C. & STRAUGHN, A. B. 2000. Carbamazepine level-A in vivo-in vitro correlation (IVIVC): a scaled convolution based predictive approach. *Biopharmaceutics & drug disposition,* 21, 1-6.

VERTZONI, M., MARKOPOULOS, C., SYMILLIDES, M., GOUMAS, C., IMANIDIS, G. & REPPAS, C. 2012. Luminal lipid phases after administration of a triglyceride solution of danazol in the fed state and their contribution to the flux of danazol across Caco-2 cell monolayers. *Molecular pharmaceutics,* 9, 1189-98.

WAGNER, J. G. 1967. Method for estimating rate constants for absorption, metabolism, and elimination from urinary excretion data. *Journal of pharmaceutical sciences,* 56, 489-94.

WAGNER, J. G. & NELSON, E. 1963. Per cent absorbed time plots derived from blood level and/or urinary excretion data. *Journal of pharmaceutical sciences,* 52, 610-1.

WAGNER, K. & MCGINITY, J. 2002. Influence of chloride ion exchange on the permeability and drug release of Eudragit RS 30 D films. *Journal of controlled release : official journal of the Controlled Release Society,* 82, 385-97.

WAGNER, K. G. & GRUETZMANN, R. 2005. Anion-induced water flux as drug release mechanism through cationic Eudragit RS 30D film coatings. *The AAPS journal,* 7, E668-77.

WEI, H. & LOBENBERG, R. 2006. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences,* 29, 45-52.

YANG, Z., TENG, Y., WANG, H. & HOU, H. 2013. Enhancement of skin permeation of bufalin by limonene via reservoir type transdermal patch: formulation design and biopharmaceutical evaluation. *International journal of pharmaceutics,* 447, 231-40.

YARO, P., HE, X., LIU, W., XUN, M., MA, Y., LI, Z. & SHI, X. 2014. In vitro-in vivo correlations for three different commercial immediate-release indapamide tablets. *Drug development and industrial pharmacy,* 40, 1670-6.

YOUNG, D. 1997. *In vitro in vivo correlations*, Plenum Pub. Corp.

ZHAO, P., ZHANG, L., GRILLO, J. A., LIU, Q., BULLOCK, J. M., MOON, Y. J., SONG, P., BRAR, S. S., MADABUSHI, R., WU, T. C., BOOTH, B. P., RAHMAN, N. A., REYNOLDS, K. S., GIL BERGLUND, E., LESKO, L. J. & HUANG, S. M. 2011. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clinical pharmacology and therapeutics,* 89, 259-67.

Apéndice II

IVIVC approach based on Carbamazepine Bioequivalence studies combination

IVIVC APPROACH BASED ON CARBAMAZEPINE BIOEQUIVALENCE STUDIES COMBINATION

Affiliation: [1]Department of Pharmaceutics and Pharmaceutical Technology. University of Valencia. Valencia (Spain). [2]Department of Engineering, Area of Pharmaceutics and Pharmaceutical Technology. University Miguel Hernández. Elche (Spain). [3]Molecular Recognition and Technological Development Institute, Joint Center Polytechnic University of Valencia-University of Valencia. Valencia (Spain). [4]Department of Pharmacy and Pharmaceutical Technology, Complutense University of Madrid. Madrid (Spain). [5]Pharmaceutical Technology and Biopharmaceutics, Johannes Gutenberg University Main. Mainz (Germany). [6]Head of Service on Pharmacokinetics and Generics, Division of Pharmacology and Clinical Evaluation, Department of Human Use Medicines, Spanish Agency for Medicines and Health Care Products. Madrid (Spain). [7]This manuscript represents the personal opinion of the authors and does not necessarily represent the views or policy of the Spanish Agency for Medicines and Health Care Products

Authors: González-García, I.[1]; Mangas-Sanjuan, V.[2]; Merino-Sanjuán. M.[1, 3]; Álvarez-Álvarez, C.[4]; Díaz-Garzón Marco, J.[4]; Rodríguez-Bonnín, M.A.[4]; Langguth, T.[5]; Torrado-Durán, JJ.[4]; Langguth, P.[5]; García-Arieta, A.[6, 7]; Bermejo, M.[2].

Corresponding Author: Marival Bermejo, mbermejo@goumh.umh.es

Facultad de Farmacia, UMH, Carretera Alicante Valencia km 87, 03550 San Juan de Alicante, Alicante, Spain

Phone +34 965 919217

Fax +34 963544911

ABSTRACT

The aim of the present study was to explore the feasibility of obtaining an IVIVC by combination of data from two Bioequivalence (BE) studies of CBZ in order to assess if the previously published dissolution media and conditions could be applicable to any other oral immediate release (IR) carbamazepine products with conventional excipients. Twenty-four healthy male subjects from two BE study received one IR dose of the test (test 1 or 2) or the reference formulation (Tegretol, 400mg). Dissolution studies of the IR CBZ tablets were performed in two different laboratories. In order to develop IVIVC, individual or average data analysis were considered. A level C, level B and Level A correlation have been successfully developed by combining data from different BE studies of CBZ immediate release drug products. A level A IVIVC was developed with all four datasets with a good R^2 for all the combinations of *in vivo* and *in vitro* data. A dissolution medium containing 1% SLS has demonstrated its suitability as the universal biopredictive dissolution medium, even if different batches and *in vivo/in vitro* studies were combined.

KEYWORDS

IVIVC, Biopharmaceutics classification system, pharmacokinetics, oral absorption, mathematical model, dissolution

ABBREVIATIONS

BCS: biopharmaceutics classification system; BE: bioequivalence; CBZ: carbamazepine: CV: coefficient of variation; EMA: European Medicines Agency; FDA: Food and Drug Administration; HPLC: high performance liquid chromatography; IR: immediate-release; IVIVC: in vitro-in vivo correlation; MDT: mean dissolution time; MRT: mean residence time; PE: prediction error; SLS: sodium lauryl sulfate

1. INTRODUCTION

In vitro-in vivo correlations (IVIVC) are a widely used tool in Biopharmaceutics research. FDA and EMA guidelines indicate that an IVIVC can be useful in product development for quantifying the *in vivo* release, evaluating formulation related effects on absorption, supporting in quality control for certain scale-up and post approval changes, and as a tool for setting *in vitro* dissolution specifications (FDA 1997, FDA 1997, EMA 2014). However, the major objective of a validated IVIVC is to use *in vitro* dissolution data to predict *in vivo* performance, serving as a surrogate for an *in vivo* bioequivalence (BE) study e.g. supporting a biowaiver approach.

There are several correlation levels depending on the quality of the established IVIVC. Level A correlation is the highest level of correlation and represents a point-to-point relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from the dosage form. Its purpose is to predict the entire *in vivo* profile from the *in vitro* dissolution curve (USP 2007). Level B correlation compares a summary parameter from the mean *in vitro* profile (i.e. Mean dissolution time, MDT) with a summary parameter from the mean *in vivo* profile (i.e. Mean Residence Time, MRT) (Lu, Kim et al. 2011). Level C correlation could be obtained using a single time point correlation between a dissolution parameter and an *in vivo* one (C_{max} or AUC). Level C and B correlations cannot be used to support product/site changes or for setting specification as they do not reflect the entire shape of the plasma concentration time profile (USP 2007).

Several authors (Gaynor, Dunne et al. 2009, Cardot and Davit 2012) have pointed out the relevance and impact of using individual versus average data in the establishment of an IVIVC. The IVIVC parameters (link function between *in vitro* and *in vivo*) and the validation results might be different if individual concentration profiles are used but this point is not always addressed on regulatory guidelines and there are no clear recommendations from regulatory authorities about all of the calculation steps.

Carbamazepine (CBZ) is an anticonvulsant and mood-stabilizing drug, classified as BCS Class II drug. Physicochemical and pharmacokinetic parameters are summarized in Supplementary Table I. Two different level A and a level C carbamazepine IVIVCs have been published (Lake, Olling et al. 1999, Veng-Pedersen, Gobburu et al. 2000,

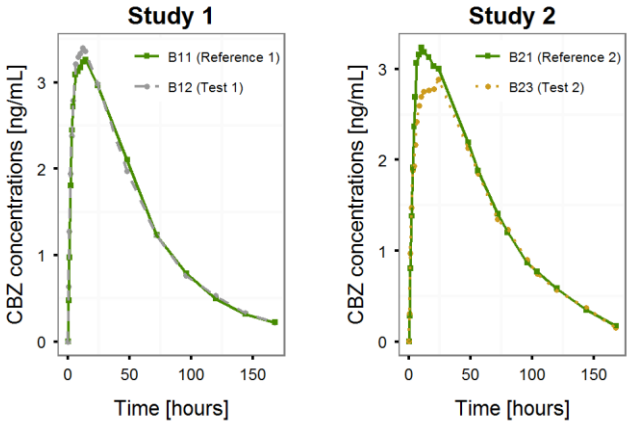
Kovacevic, Parojcic et al. 2009), using the same *in vitro* dissolution media and conditions. This level of correlation with the adequate internal and external validation is the warranty of similar drug absorption in terms of rate and extent and supports the claim of a biowaiver to the regulatory authority.

The aim of the present study was to explore the feasibility of obtaining an IVIVC by combination of data from two Bioequivalence (BE) studies of CBZ in order to assess if the previously published dissolution media and conditions (Veng-Pedersen, Gobburu et al. 2000, Kovacevic, Parojcic et al. 2009) could be applicable to any other oral immediate release (IR) carbamazepine product with conventional excipients. Therefore, it would be possible to assess the usefulness of *in vitro* dissolution data from different laboratories to predict *in vivo* concentration profiles. The second objective was to evaluate the variability associated to the use of data obtained in different laboratories as well as data from different *in vivo* studies and the influence of the use of individual or average data on the establishment of an IVIVC.

2. RESULTS

In vivo data: Figure 1 shows the average plasma profiles of CBZ test and reference formulations in both BE studies.

Figure 1. Carbamazepine in vivo profiles. B = in vivo data; first digit study (1; 2); second digit product (1=reference, 2=test, 3=test).



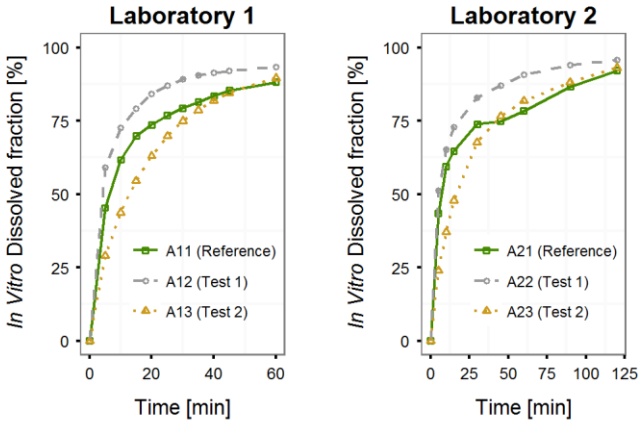
Mean pharmacokinetic parameters of each *in vivo* BE study obtained from individual parameters are shown in *Table 1*.

Table 1. In vivo parameters for both bioequivalence studies. Identification code B=in vivo data; first digit study (1;2); second digit product (1=Reference, 2=Test 1; 3=Test 2). Formulations labeled with the asterisk are the normalized formulations. CV is expressed in %. Left values belong to the mean parameters and right values were obtained from the averaged profiles.

	ID code	**AUC [mg·h/mL]**		**C_{max} [mg/mL]**		**MRT [h]**		**AUC [mg·h/mL]**	**C_{max} [mg/mL]**	**MRT [h]**
		Geom mean	CV	Geom mean	CV	Mean	CV	Value	Value	Value
Reference 1	B11	235.9	18.8	3.4	16.5	60.4	19.6	237.7	3.2	55.3
Test 1	B12	241.7	21.5	3.6	18.2	62.0	16.5	238.0	3.4	55.1
Reference 2	B21	253.2	21.4	3.5	18.9	66.9	15.5	242.8	3.2	54.9
Test 2	B23	241.4	19.2	3.2	23.3	65.4	16.6	230.4	2.9	56.0
*Test 1**	*B12**	*240.5*	*19.5*	*3.6*	*17.0*	*54.2*	*21.2*	*237.5*	*3.4*	*57.0*
*Test 2**	*B23**	*245.9*	*19.1*	*3.1*	*24.0*	*60.2*	*15.3*	*238.9*	*2.9*	*56.5*

In vitro data: Average dissolved fractions versus time from all the formulations obtained in two laboratories are represented in Figure 2. Sampling times were different between laboratories, but in both cases, the asymptote was reached.

Figure 2. Carbamazepine mean in vitro dissolution profiles from each laboratory. A = in vitro data; first digit study (1 = laboratory 1, 2 = laboratory 2); second digit product (1 = reference, 2 = test 1, 3 = test 2).



Several dissolution models were fitted to the data and the best one was selected from the standard goodness of fit criteria. Only the results from the best model are shown. The purpose of fitting dissolution data is to be able to estimate a fraction dissolved at any time apart from the sampling times. A first order dissolution model was the best to describe *in vitro* data based on Snedecor's F test. Fitting was done with the individual (tablet) data. *Table 2* shows the mean value of each parameter calculated from the individual estimated parameter (for each tablet) and its coefficient of variation (CV). *Table 3* represents the results of IVIVC level C, although f_2 analysis revealed that Reference and Test 1 profiles are similar while Reference and Test 2 profiles are not similar. This phenomenon occurs in both laboratories. When profiles of laboratory 1 and 2 are compared with each other, f_2 value was greater than 50 in all cases.

Table 2. In vitro First order model parameters for both laboratories. CV is expressed in %. Identification code A=in vitro data; first digit study (1=Laboratory 1, 2=Laboratory 2); second digit product (1=Reference, 2=Test, 3=Test). f2 test results based on EMA or FDA criteria between Ref-Test 1 or Ref-Test 2 in vitro dissolution profiles comparison and between References or Test for each laboratory. AUC: area under the curve of dissolved fractions (%) versus time (min).

	Identification code	**AUC (%·min)**		**MDT (min)**		**t_{25} (min)**		**t_{50} (min)**		**t_{75} (min)**		**t_{80} (min)**		**f_2 (intra-laboratory)**		**f_2 (inter-laboratory)**	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	EMA	FDA	EMA	FDA
Reference	A11	4462.2	2.1	10.2	5.3	1.2	12.9	4.3	9.0	11.8	6.3	14.6	6.6				
Test 1	A12	4896.3	1.0	7.8	12.5	0.8	19.0	8.3	8.5	8.3	8.5	10.4	7.8	51.06	51.57		
Test 2	A13	3997.6	1.0	15.4	1.1	4.0	0.5	9.9	0.5	20.4	1.2	23.9	2.7	44.93	47.3		
Reference	A21	9138.4	5.3	20.7	13.5	1.0	53.6	1.0	53.6	19.1	21.9	24.4	30.4			56.8	54.13
Test 1	A22	10158.9	3.5	13.9	10.7	0.9	37.2	4.0	17.9	13.9	6.6	18.7	10.4	54.57	51.62	57.71	58.75
Test 2	A23	8832.0	3.0	25.1	9.2	4.9	18.4	13.6	12.2	30.7	11.7	36.8	11.6	43.94	43.94	57.66	57.65

In vitro-in vivo correlations

Level B: Results of level B correlations between MDT and MRT were 0.98, 0.97, 0.96 and 0.77 for IVIVC 1, IVIVC 2, IVIVC 3 and IVIVC 4, respectively. As can be seen from the R^2 values, significant correlations were found between MDT and MRT for all IVIVC datasets.

Level C: Table 3 shows the R^2 values obtained when AUC and C_{max}, respectively, were correlated with $t_{25\%}$, $t_{50\%}$, $t_{75\%}$, $t_{80\%}$ and MDT. No correlation was found between AUC and any *in vitro* parameter. However, an IVIVC level C was successfully achieved between C_{max} and several *in vitro* parameters for each *in vivo* dataset.

Table 3. Correlation coefficient values for IVIVC level C. Formulations labeled with the asterisk are the normalized formulations

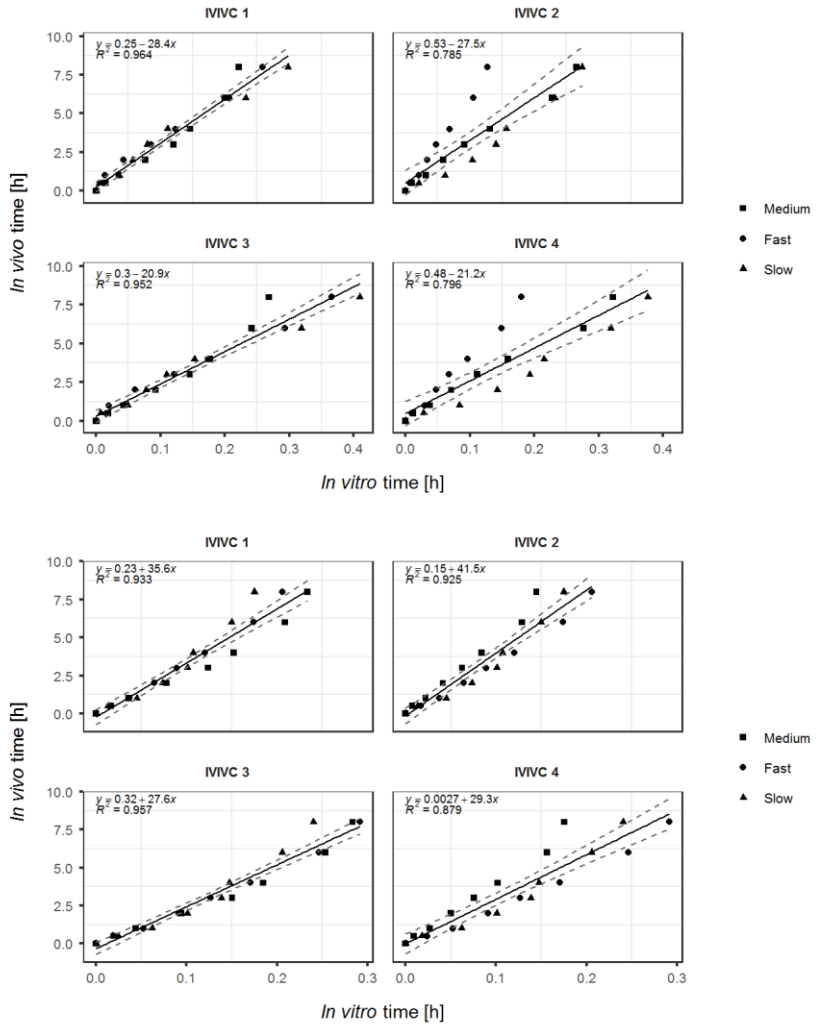
	R^2 Values			
In vitro	Group 1 (A11, A12, A13)		Group 2 (A21, A22, A23)	
In vivo	IVIVC1 B11, B12, B23*	IVIVC2 B12*, B21, B23	IVIVC3 B11, B12, B23*	IVIVC4 B12*, B21, B23
T25vsCmax	0.97	0.99	0.92	1.00
T50vsCmax	0.97	0.99	0.92	1.00
T75vsCmax	0.97	0.99	0.92	1.00
T80vsCmax	0.97	0.99	0.92	1.00
MDTvsCmax	0.98	0.97	0.96	0.77
T25vsAUC	0.97	0.02	0.10	<0.01
T50vsAUC	0.97	0.01	0.10	<0.01
T75vsAUC	0.97	0.01	0.10	<0.01
T80vsAUC	0.97	0.01	0.10	<0.01
MDTvsAUC	0.95	0.03	0.72	0.24

Slight differences in R^2 values of the level C correlations were observed between IVIVC1 versus IVIVC3 and IVIVC2 versus IVIVC4 i.e. when the source of *in vitro* data is changed from Laboratory 1 to Laboratory 2.

When the comparison was performed between IVIVC1 versus IVIVC2 and IVIVC3 versus IVIVC4 (i.e. same *in vitro* data versus different *in vivo* studies) there were also differences in the R^2 values. Better correlations were obtained with *in vivo* dataset B12*, B21 and B23.

Level A: Levy Plot results indicate that *in vivo* time is around 20 and 40 times larger than *in vitro* times i.e. dissolution *in vivo* is slower than *in vitro*. A linear correlation between *in vitro* dissolution time and *in vivo* absorption time is represented in Figure 3.

Figure 3 Levy plots shows the correlation between in vivo absorbed time and in vitro dissolved time (top: average method, bottom: individual method). Linear regression is represented by the solid line and dashed lines represent the 5% prediction confidence interval.



Dissolved and absorbed fractions (IVIVC) are shown in Figure 4. There are slight differences in the determination coefficient (R^2) depending on the *in vitro* or *in vivo* dataset that is used, but more pronounced differences in R^2 values are observed depending whether average or individual data are considered (Figure 5). Individual data analysis led to higher correlation coefficients for all IVIVC datasets.

Figure 4. IVIVC level A shows the correlation between in vitro dissolved fractions and in vivo absorbed fractions (top: average method, bottom: individual method). Linear regression is represented by the solid line and dashed lines represent the 95% prediction confidence interval.

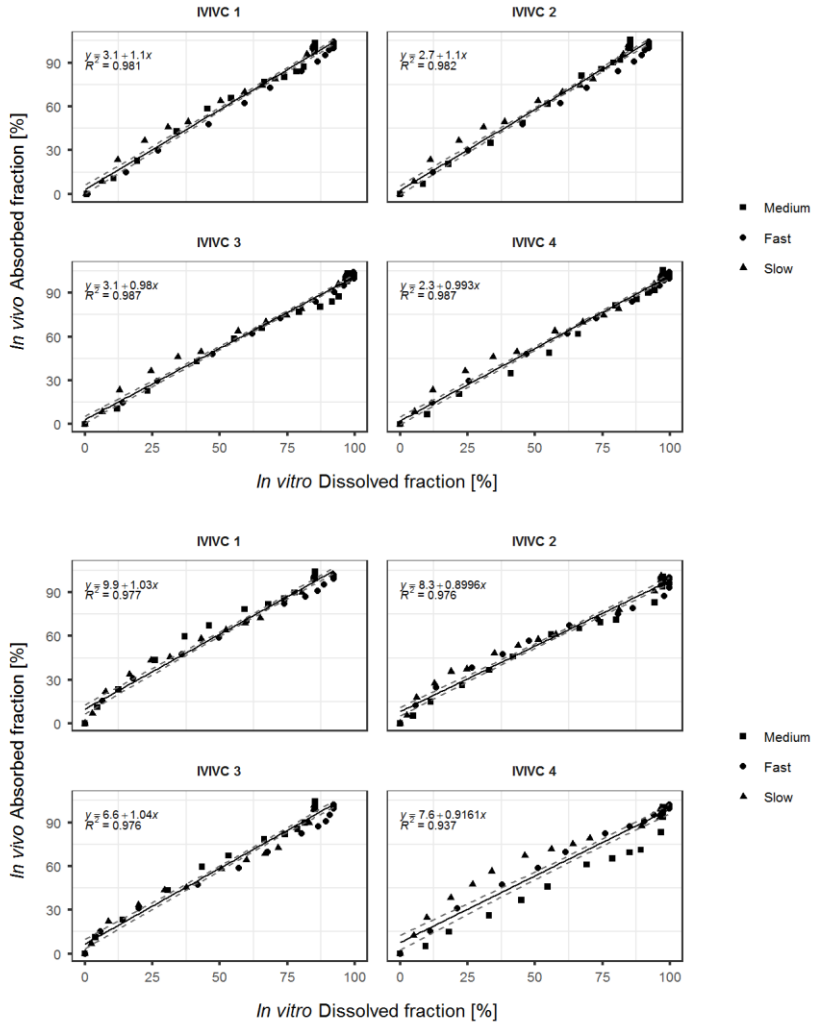
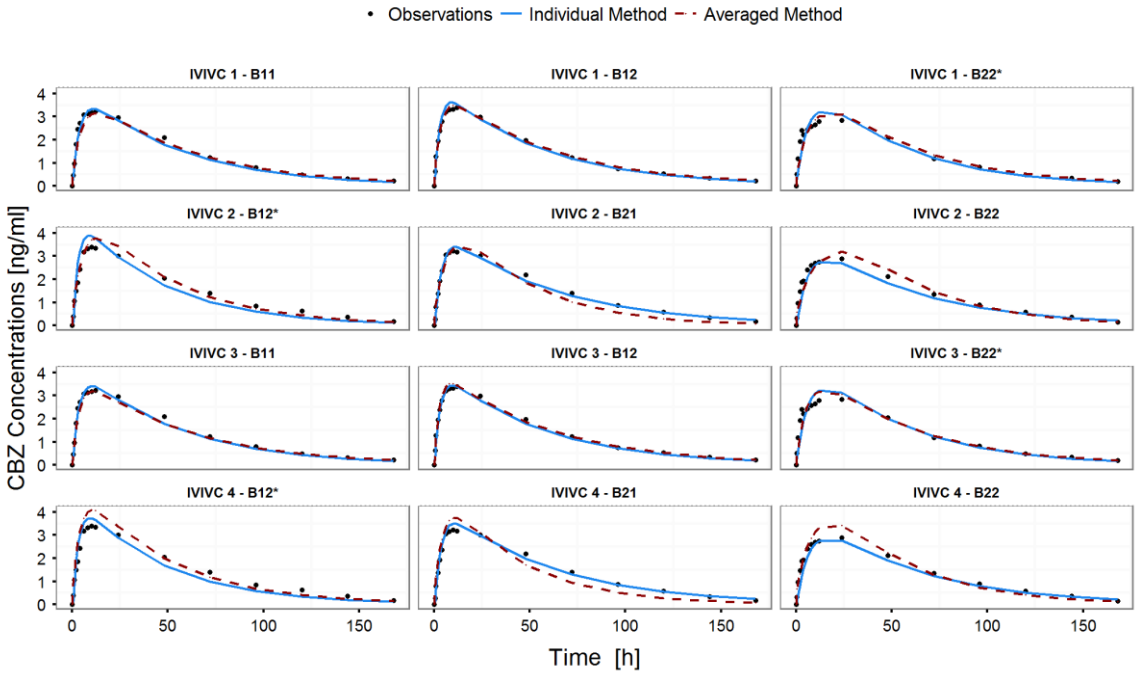


Figure 5. Solid and dashed lines represent predicted concentrations; dots represent the observed concentrations for each formulation. Blue solid lines represent predictions of individual analysis and red dashed lines predictions of average analysis.



The analysis of the extreme tablets (Table 4) i.e. those with the fastests and slowest dissolution rate showed that the fastest tablet of the fastest dissolving formulation (FTFF) fulfill the BE criteria, whereas the slowest tablet of the slowest dissolving formulation (STSF) did not satisfy the BE for Cmax, being smaller than the lower limit of the 90% confidence interval for the BE study.

Table 4. Results for the analysis of the extreme tablets based on the BE criteria. Identification code: FTFF = fastest tablet of the fastest formulation; STSF = slowest table of the slowest formulation.

	C_{max}	AUC
BE 90% CI	1.00 – 1.15	0.89 – 1.12
FTFF	1.09	1.06
STSF	0.72	0.94

Internal validation: AUC and C_{max} prediction errors are summarized in *Table 5*. Figure 5 shows the observed and the predicted plasma concentrations for all IVIVC groups.

Table 5. Summary of PE (%) obtained for all IVIVC dataset using individual or mean data analysis method with Wagner-Nelson deconvolution approach.

			B11/B21	B12	B23	MEAN
Individual W-N	IVIVC 1	AUC	8.1	2.3	0.4	3.6
		CMAX	3.1	6.0	12.8	7.3
	IVIVC 2	AUC	2.2	12.0	8.4	7.5
		CMAX	5.0	14.1	4.8	8.0
	IVIVC 3	AUC	7.6	6.5	0.5	4.9
		CMAX	5.6	1.0	13.3	6.7
	IVIVC 4	AUC	0.2	14.4	6.0	6.9
		CMAX	8.1	9.5	4.2	7.3
Average W-N	IVIVC 1	AUC	4.2	1.7	6.2	4.0
		CMAX	2.7	2.5	9.0	4.7
	IVIVC 2	AUC	15.0	0.8	3.1	6.3
		CMAX	5.9	11.3	11.4	9.5
	IVIVC 3	AUC	6.9	0.8	2.7	3.5
		CMAX	1.5	3.0	11.9	5.5
	IVIVC 4	AUC	15.5	0.9	1.5	6.0
		CMAX	15.9	20.0	18.4	18.1

3. DISCUSSION

Carbamazepine is a high permeability and low solubility drug, classified as Class II according to the BCS. Due to its biopharmaceutic properties it is a good candidate for developing an IVIVC. The capability to predict an *in vivo* property of a drug from *in vitro* data is an essential tool in the drug development process and IVIVC's help to reduce time and costs. In general, an IVIVC is developed for a drug and drug formulation with a particular release mechanism so its applicability is restricted to drug formulations manufactured by the company developing the IVIVC. Nevertheless, for immediate release oral drug formulations in which the dissolution rate depends on disaggregation characteristics and solid drug properties (as particle size) it might be possible to find a dissolution method of broader applicability. In this paper the combination of data from bioequivalence studies of CBZ has been explored as an approach to establish an IVIVC. This strategy could be used by pharmaceutical companies to assess the *in vivo* predictive ability of a dissolution method as a tool for formulation selection before further *in vivo* studies. A question that remains unanswered is whether it is possible to combine data from different BE studies to develop an IVIVC, a procedure that could be useful internally in pharmaceutical companies during the development process as least as informative tool. A dissolution method with 1% of SLS previously used to establish IVIVC for IR CBZ formulations was used to predict the *in vivo* behavior observed in two bioequivalence studies. As an added source of variability, the dissolution studies were performed with different batches from the selected in the *in vivo* test and in two different laboratories.

In vivo data

In vivo parameters from both BE studies of CBZ were similar among studies and the coefficients of variation were low (7%). The double peak that appears in Study B profile may be due to CBZ entero-hepatic cycle (Fleischman and Chiang 2001). The absence of those peaks in profiles from Study A might be explained because of the absence of sampling in this time interval. This phenomenon does not strongly affect the estimation of pharmacokinetic parameters because only a slightly greater AUC value of Reference B was obtained due to the double-peak. The normalization procedure used with the reference (bioequivalent) products was done in order to reduce between-studies variability.

In vitro

AUC, MDT, t_{25}, t_{50}, t_{75}, t_{80} parameters were obtained from *in vitro* dissolution profiles as the mean of each individual (tablet) parameter. Higher CV's were obtained from profiles A21, A22 and A23. These results may be explained by small differences in the medium composition, analytical variability, operator's influence and/or batch-to-batch variability. *In vitro* dissolution media in both laboratories were prepared including sodium lauryl sulfate (SLS). Differences in batches of SLS and its purity might explain in part the observed differences between laboratories. Despite these small differences, *in vitro* dissolution profiles from each laboratory present a rank order in accordance with the *in vivo* results. So even if the media composition and assay site may impact the variability among tablets, the average behavior still reflects the *in vivo* results.

IVIVC Level B

A significant correlation was obtained between MDT and MRT for any formulation assayed. Differences in the R^2 value may be explained due to the differences that exist between IVIVC datasets.

IVIVC Level C

The best level C correlations were obtained for C_{max}. One of the goals of this work was to explore the influence of the *in vivo* and *in vitro* data sources in the quality of the IVIVC obtained. The differences associated with the use of different *in vitro* data sets are very small (Table IV). This is in accordance with other publications on IVIVC which conclude that variability between *in vitro* profiles is much lower than from *in vivo* data (Gaynor, Dunne et al. 2009, Cardot and Davit 2012). Thus, the influence of laboratory/experimental conditions or inter-batch variability in the establishment of an IVIVC is much less evident, obtaining consistent IVIVC fittings among different laboratories, if experimental conditions are equally kept.

IVIVC Level A

As it can be observed in the Levy plot (Figure IV), a good relationship has been achieved between *in vitro* dissolution times and *in vivo* absorption times. It demonstrates the slower *in vivo* dissolution rate compared to the *in vitro* drug dissolution which could be

due to different agitation conditions and degree of sink conditions. Once *in vivo* fractions absorbed have been calculated based on the scaled *in vitro* times, good correlation coefficients (higher than 0.95) were obtained in both data analysis methods (mean or individual). As stated by Gaynor et al (Gaynor, Dunne et al. 2009) and Cardot et al (Cardot and Davit 2012), higher R^2 were observed based on individual data analysis, although it does not improve the internal validation predictions. Regarding the predicted profiles for the fastest and slowest tablets of test formulations using IVIVC1, the C_{max} predicted values are 1.09 (FTFF) and 0.72 (STSF). The first one (FTFF) is included on the 90%CI of the Reference formulation for the Bioequivalence study used to develop IVIVC1 but not the corresponding to the slowest tablet. Eventually that could mean that a tighter dissolution specification should be set to ensure bioequivalence of all the tested formulations.

CONCLUSION

In this work a level C, level B and Level A correlation have been successfully developed by combining data from different BE studies of CBZ immediate release drug products. Level C correlation is useful for screening formulations before the *in vivo* test. In addition, a level A IVIVC was developed with all four datasets with a good R^2 for all the combinations of *in vivo* and *in vitro* data. However, a slightly higher R^2 was obtained using the individual data analysis method. Internal validation predictions errors obtained with the individual data approach were inside the established limits by FDA and EMA but the average method of data analysis led to prediction errors outside the accepted limits. This result supports the EMA guidance recommendation on individual data analysis but it points out also the relevance of the calculation methods as for example the convolution method used that could lead to different results. Nevertheless, the comparison of the different convolution methods was not the objective of this paper. Our main conclusion in accordance with the main objective is that it was possible to develop successfully an IVIVC by combining data from two BE studies with the adequate normalization. A dissolution medium containing 1% SLS has demonstrated its suitability as a biopredictive dissolution medium of broader application to other immediate release formulations with conventional excipients, even if different batches and *in vivo/in vitro* studies were combined.

4. EXPERIMENTAL

In vivo studies

Study 1 and 2 were single-blind, controlled, balanced, randomized, two-period crossover BE studies developed independently, using different batches. Twenty-four healthy male subjects in each BE study received one IR dose of the test formulation (test 1 or 2, 400mg) and one dose of the reference formulation (Tegretol, 400mg) in the sequence determined by randomization. A washout period of twenty-one days was set between the study periods. Blood samples were taken at the following times in study 1: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 24, 48, 72, 96, 120, 144 and 166 hours after administration and in study 2: 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 26, 28, 32, 48, 56, 72, 80, 96, 104, 120,144 and 168 hours.

CBZ concentration in blood samples was determined by a validated HPLC method in both studies. The following parameters were derived from the average or individual plasma concentration time profiles: peak plasma concentration (C_{max}) and area under the curve ($AUC_{0-\infty}$).

AUC and MRT were estimated individually by non-compartmental methods (Hedaya 2012) from the *in vivo* observations. AUC and C_{max} were estimated as the geometric means of the individual AUC_i and $C_{max,i}$ (AUC_{mean} and $C_{max,mean}$: individual data analysis method) or based on the average concentration-time profile ($AUC_{average}$ and $C_{max,average}$: average data analysis method (Cardot and Davit 2012)).

In vitro study

Dissolution studies of the IR CBZ tablets were performed in two different laboratories in the PhEur/USP rotating paddle apparatus at 75 rpm using 900 mL of dissolution media. The dissolution medium was composed of a 1% sodium lauryl sulfate (SLS) aqueous solution. Dissolution studies were performed using the same formulations as the ones used in the *in vivo* studies but from different batches. Each laboratory used different batches of the formulations that were provided by Spanish community pharmacies.

CBZ concentrations on the dissolution samples were determined spectrophotometrically at 285 nm. All tests were performed with twelve tablets. Different *in vitro* dissolution kinetic models were tested (Zero Order, First Order and Weibull models) to describe the dissolution profiles as shown in Supplementary Table II. Similarity test (f_2) were carried out in order to compare the dissolution profiles. Dissolution profiles were compared intra-laboratory and between laboratories.

The curve-fitting of the dissolution models to the experimental *in vitro* data was performed using Solver tool in Microsoft Excel 2013®. Sum of squared residuals (SSR) were compared using the Snedecor's F test to determine the best model for the *in vitro* data.

In vitro–in vivo correlation

In order to identify which in vitro and in vivo data is used in each correlation the in vitro and in vivo experiments were identified with identification codes. The identification codes are summarized in Table I. Letter A or B refers *in vitro* (A) or *in vivo* (B) studies. First digit identifies to the *in vivo* study (study 1 or 2) or dissolution laboratory (Laboratory 1 and 2) and the last digit identifies formulation, reference, test 1 or test 2. As only two formulations (test and reference) were available from each *in vivo* study, in order to develop an IVIVC with three formulations, it was necessary to combine the data from both studies. In order to avoid the effect of different populations selected in each BE study, test formulations data were normalized based on the reference's ratios. Normalization was carried out using the average concentration time profiles from each reference's formulation. At each sampling time, B11/B21 ratios were calculated to obtain the normalized individual concentration profiles (individual data analysis) or normalized average concentration profiles (average data analysis) of the test formulation included in each IVIVC dataset. On the other hand, *in vitro* data for the three formulations (Reference, Test 1 and Test 2) were generated in two different laboratories. Therefore, as a result of this combination and normalization exercise, four datasets were generated (Table I). All the potential data combinations are explained in *Table 6*.

Table 6. Codification for in vitro and in vivo formulations. Four different datasets combinations generated to develop level A, B and C IVIVC. Formulations labeled with the asterisk are the normalized formulations.

	Laboratory	Formulation	Name	IVIVC1	IVIVC2	IVIVC3	IVIVC4
In vitro	Laboratory 1	Reference	A11	A11	A11		
		Test 1	A12	A12	A12		
		Test 2	A13	A13	A13		
	Laboratory 2	Reference	A21			A21	A21
		Test 1	A22			A22	A22
		Test 2	A23			A23	A23
In vivo	Study 1	Reference	B11	B11		B11	
		Test 1	B12	B12	B12*	B12	B12*
	Study 2	Reference	B21		B21		B21
		Test 2	B23	B23*	B23	B23*	B23

Oral CBZ plasma profiles were well described with a one-compartment model (Graves, Brundage et al. 1998, Bondareva, Jelliffe et al. 2006, Punyawudho, Ramsay et al. 2012). Wagner-Nelson deconvolution method was selected in order to develop the level A IVIVC because no intravenous CBZ data was available. In the individual data analysis, each individual profile was deconvolved to obtain the individual oral fractions absorbed. Mean *in vivo* absorbed fractions ($F_{a,mean}$) profile was estimated from the averaged individual *in vivo* absorbed fractions. In average data analysis, *in vivo* absorbed fractions ($F_{a,average}$) were calculated from the average concentration plasma profiles. Levy Plots and the *in vitro-in vivo* time relationship were obtained by linear regression. Levy Plots were performed using the *in vitro* times at which a particular oral fraction absorbed is obtained, which were correlated with the *in vitro* times at which the same fraction is dissolved. If there is no experimental *in vitro* data matching this dissolved fraction, the *in vitro* time was estimated by non-linear regression. In order to establish the IVIVC, *in vitro* dissolved fractions were estimated at the *scaled in vitro* times with the dissolution model previously selected. Once the IVIVC was accomplished, the extreme tablets (the fastest tablet of the fastest formulation (FTFF) and the slowest tablet of the slowest formulation (STSF)) were used to perform an extra analysis. Those *in vitro* profiles i.e. the dissolved fractions were used to obtain the corresponding absorbed fractions (by means of the established in vitro in vivo correlation). The absorbed fractions were transformed into plasma levels by convolution with the disposition function for CBZ

previously estimated. The individual plasma levels from test and reference were finally used to carry out a BE analysis.

Level B IVIVC were stablished using *in vivo* MRT and *in vitro* MDT and several level C IVIVC were developed using *in vivo* AUC and C_{max} and *in vitro* $t_{25\%}$, $t_{50\%}$, $t_{75\%}$, $t_{80\%}$ and MDT.

The determination coefficient (R^2) was estimated for each level of IVIVC combination datasets. Graphical and statistical analysis were performed using R software (http://cran.r-project.org, version 3.1.0), RStudio® and Microsoft Excel 2013®.

Internal validation

$F_{a,mean}$ and $F_{a,average}$ were convolved to obtain the predicted *in vivo* profiles using superposition principle to transform absorbed fractions into concentrations (Langenbucher 2003, Qureshi 2010). Those predicted profiles were utilized to obtain the predicted AUC and C_{max}. Internal predictability was calculated using Eq. 7. FDA (FDA 1997) and EMA (EMA 2014) guidelines validate the IVIVC when the mean prediction error (%PE) in AUC and C_{max} is less than 10%, and 15% for each formulation.

$$Prediction\ Errors\ (\%PE) = \frac{(Observed\ Parameter - Predicted\ Parameter)}{Observed\ Parameter} \cdot 100 \qquad (7)$$

ACKNOWLEDGEMENTS

The authors acknowledge financial support to projects: DCI ALA/19.09.01/10/21526/245-297/ALFA 111(2010)29: Red-Biofarma. Red para el desarrollo de Metodologías Biofarmacéuticas racionales que incrementen la competencia y el impacto social de las Industrias Farmacéuticas Locales. A grant from Ministry of Education and Science of Spain and Miguel Hernandez University was awarded to V.M.S. (FPU AP2010-2372).

REFERENCES

Amidon GL, Lennernas H, Shah VP, Crison JR A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of In Vitro Drug Product Dissolution and In Vivo Bioavailability, Pharm Res 12, 413–420, 1995—Backstory of BCS(2014). AAPS J 16(5): 894–898.

Bondareva IB, Jelliffe RW, Gusev EI, Guekht AB, Melikyan EG and Belousov YB (2006) Population pharmacokinetic modelling of carbamazepine in epileptic elderly patients: implications for dosage. J Clin Pharm Ther 31(3): 211-221.

Cardot JM and Davit BM (2012) In vitro-in vivo correlations: tricks and traps. AAPS J 14(3): 491-499.

EMA (2014). Guideline on quality of oral modified release products.

FDA (1997). Guidance for industry. Extended release oral dosage forms: development, evaluation and application of *in vitro/in vivo* correlations. Center for Drug Evaluation and Research (CDER), US Department of Health and Human Services.

FDA (1997). Guidance for Industry. SUPAC-MR: Modified Release Solid Oral Dosage Forms Centre for Drug Evaluation and Research (CDER), US Department of Health and Human Services.

Fleischman A and Chiang VW (2001) Carbamazepine overdose recognized by a tricyclic antidepressant assay. Pediatrics 107(1): 176-177.

Gaynor C, Dunne A and Davis J (2009) The effects of averaging on accuracy of IVIVC model predictions. J Pharm Sci 98(10): 3829-3838.

Graves NM, Brundage RC, Wen Y, Cascino G, So E, Ahman P, Rarick J, Krause S and Leppik IE (1998) Population pharmacokinetics of carbamazepine in adults with epilepsy. Pharmacotherapy 18(2): 273-281.

Hedaya MA (2012) Monocompartmental approach to pharmacokinetic data analysis. Basic Pharmacokinetics, CRC Press

Kovacevic I, Parojcic J, Homsek I, Tubic-Grozdanis M and Langguth P (2009) Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. Molecular pharmaceutics 6(1): 40-47.

Lake OA, Olling M and Barends DM (1999) In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers. Eur J Pharm Biopharm 48(1): 13-19.

Langenbucher F (2003) Handling of computational in vitro/in vivo correlation problems by Microsoft Excel: III. Convolution and deconvolution. Eur J Pharm Biopharm 56(3): 429-437.

Lu Y, Kim S and Park K (2011) In vitro-in vivo correlation: perspectives on model development. Int J Pharm 418(1): 142-148.

Punyawudho B, Ramsay ER, Brundage RC, Macias FM, Collins JF and Birnbaum AK (2012) Population pharmacokinetics of carbamazepine in elderly patients. Therapeutic drug monitoring 34(2): 176-181.

Qureshi S. (2010) In Vitro-In Vivo Correlation (IVIVC) and Determining Drug Concentrations in Blood from Dissolution Testing – A Simple and Practical Approach. The Open Drug Delivery Journal 4: 38-47.

USP (2007). In vitro and In vivo Evaluations of Dosage Forms. Easton, PA., Mack Publishing Co.

Veng-Pedersen P, Gobburu JV, Meyer MC and Straughn AB (2000) Carbamazepine level-A in vivo-in vitro correlation (IVIVC): a scaled convolution based predictive approach. Biopharm Drug Disp 21(1): 1-6.

APÉNDICE III

DEFINING LEVEL A IVIVC DISSOLUTION SPECIFICATIONS BASED ON INDIVIDUAL *IN VITRO* DISSOLUTION PROFILES OF A CONTROLLED RELEASE FORMULATION

DEFINING LEVEL A IVIVC DISSOLUTION SPECIFICATIONS BASED ON INDIVIDUAL *IN VITRO* DISSOLUTION PROFILES OF A CONTROLLED RELEASE FORMULATION

Authors names and affiliations: I González-García[1], A García-Arieta[2,a], M Merino-Sanjuan[1,3], V Mangas-Sanjuan[1,3 *], M Bermejo[4]

[1] Pharmacy and Pharmaceutical Technology Area. University of Valencia, Spain; [2]División de Farmacología y Evaluación Clínica, Departamento de Medicamentos de Uso Humano, Agencia Española de Medicamentos y Productos Sanitarios. [a]This manuscript represents the personal opinion of the authors and does not necessarily represent the views or policy of the Spanish Agency for Medicines and Health Care Products; [3]Institute of Molecular Recognition and Technological Development (IDM), Polytechnic University of Valencia and University of Valencia, Spain; [4] Department of Engineering, Pharmacy and Pharmaceutical Technology Area. Miguel Hernandez University, Spain.

Corresponding author: *Corresponding author: Victor Mangas-Sanjuan, victor.mangas@uv.es. Pharmacy and Pharmaceutical Technology Area, School of Pharmacy, University of Valencia. Av/Vicent Andres Estelles, s/n, 46100. Burjassot. Valencia (Spain). Phone +34 963543351.

ABSTRACT

Regulatory guidelines recommend that, when a level A IVIVC is established, dissolution specification should be established using averaged data and the maximum difference between AUC and C_{max} between the reference and test formulations cannot be greater than 20%. However, averaging data assumes a loss of information and may reflect a bias in the results. The objective of the current work is to present a new approach to establish dissolution specifications using a new methodology (individual approach) instead of average data (classical approach). Different scenarios were established based on the relationship between *in vitro-in vivo* dissolution rate coefficient using a level A IVIVC of a controlled release formulation. Then, in order to compare this new approach with the classical one, six additional batches were simulated. For each batch, 1,000 simulations of a dissolution assay were run. C_{max} ratios between the reference formulation and each batch were calculated showing that the individual approach was more sensitive and able to detect differences between the reference and the batch formulation compared to the classical approach. Additionally, the new methodology displays wider dissolution specification limits than the classical approach, ensuring that any tablet from the new batch would generate *in vivo* profiles which its AUC or C_{max} ratio will be out of the 0.8-1.25 range, taking into account the *in vitro* and *in vivo* variability of the new batches developed.

Keywords

Bioequivalence; *In vitro – in vivo* correlations; Controlled release; Dissolution specifications; NONMEM

Nonstandard abbreviations

AUC: Area under the curve; BE: Bioequivalence; CI: Confidence interval; CL: Clearance; C_{max}: Maximum plasma concentration; CR: Controlled release; CV: Coefficient of variation; f_2: Similarity factor; FTFF: The fastest tablet of the fastest dissolving formulation; k_a: *In vivo* absorption rate coefficient; k_d: *In vitro* or *in vivo* dissolution rate coefficient; IIV: Inter-individual variability; IVIVC: *In vitro-in vivo* correlation; PK: Pharmacokinetic; RUV: Residual unexplained variability; STSF: The slowest tablet of the slowest dissolving formulation; t_{max}: Time to maximum plasma concentration; V_c: Apparent central volume of distribution.

INTRODUCTION

Bioequivalence (BE) concepts have evolved during the last decades globally, allowing the authorization of changes during the development process, variations or post-approval changes, and line extensions of brand-name products and generic products. Bioavailability, measured as maximum plasma concentration (C_{max}), area under the curve (AUC), and time to maximum plasma concentration (t_{max}), is used as a surrogate to demonstrate equivalent biopharmaceutical quality between the test and the reference product [1, 2].

Regulatory authorities have set predefined regulatory requirements for bioequivalence studies and for waiving *in vivo* BE studies using *in vitro* dissolution data (e.g., BCS-based biowaiver and *in vitro-in vivo* correlation (IVIVC)-based biowaivers). In general, for low solubility drug substances where dissolution is the rate limiting step for bioavailability, the possibility of establishing a correlation between *in vitro* dissolution and *in vivo* absorption can be expected (Limberg and Potthast, 2013). An IVIVC is a mathematical model that defines the relationship between the *in vitro* dissolution data and the *in vivo* performance of drug product. The establishment of an IVIVC offers several advantages during the drug development process (Cook, 2012). One of the most relevant uses of the IVIVC is as a surrogate for human bioavailability studies to reduce the number of BE studies needed during the development process and later for post-approval changes (Chowdhury, 2011; Limberg and Potthast, 2013). Four levels of correlation (A, B, C, and D) have been described based on the predictive capability of the *in vitro* dissolution profiles to reflect the *in vivo* behavior. However, only a level A IVIVC represents a point-to-point relationship that can be used as a surrogate of *in vivo* studies for regulatory purposes (Chowdhury, 2011; FDA, 1997; Uppoor, 2001). This IVIVC could also be used to establish the dissolution specifications that guarantee BE (EMA, 2014a; FDA, 1997). Once an IVIVC is developed, dissolution specifications will ensure the safe space of *in vitro* dissolution data that guarantees *in vivo* BE according to the observed *in vitro/in vivo* variability.

Regulatory guidelines (EMA, 2014b; FDA, 1997) define different ways to calculate these dissolution specifications depending on the level of the IVIVC:

- No IVIVC. Any time point should not be greater than ±10% of the mean profile.
- Level A IVIVC. Specifications should be established based on average data, where the maximum difference allowed in the predicted AUC and C_{max} is 20%.

- Multiple Level C IVIVC. The maximum difference in the predicted AUC and C_{max} should not exceed ±20% from the mean dissolution profile obtained from the clinical/bioavailability batches (where the last time point should be at least 80% of drug dissolved).

- Single Level C IVIVC. Not more than a 20% difference in the predicted AUC and C_{max} is allowed at the time point used. At other time points, maximum recommended range should be ± 10% of label claim deviation from the mean dissolution profile obtained from the clinical/bioavailability batches.

The Dissolution Analytical Working group of the IQ Consortium also put forward another two approaches (Hermans et al., 2017):

- A clinically established "safe space" for dissolution can be established when formulation/process variants demonstrate acceptable PK performance, but the dissolution method can discriminate those variants.

- In silico IVIVe (in vitro - in vivo extrapolation). The link between the in vivo dissolution and the observed pharmacokinetic response is established via the use of a physiologically based absorption/pharmacokinetic model, and the model is used to identify dissolution profiles that are projected to ensure the desired clinical performance.

- According to the above-mentioned requirements, dissolution specifications are established based on the mean *in vitro* dissolution profile and all batches whose *in vivo* simulated profiles are within ±20% in C_{max} and AUC will be considered bioequivalent. However, there is evidence suggesting that the use of mean profiles instead of individual information could lead to a biased analysis and less accurate predictions (Cardot and Davit, 2012; Gaynor et al., 2009; González-García et al., 2017; Roudier et al., 2014). This issue is of special relevance for IVIVC-based biowaived batches where some individual profiles within the same batch could overcome the ±20% difference boundary in the predicted *in vivo* C_{max} and AUC parameters.

Therefore, the purpose of this work is to compare the classical approach (the use of mean data) with a new methodology in which we have used individual data in order to assess the probability of declaring bioequivalence for a new batch based on an IVIVC of a controlled release (CR) formulation. Furthermore, we have evaluated the impact of these two different methodologies on the establishment of dissolution specifications.

MATERIAL AND METHODS

2.1 IVIVC development

Slow, medium, and fast dissolving drug formulations were used to develop the IVIVC. Dissolution data sets were generated for 12 units (e.g. tablets) based on a first-order dissolution model (Gibaldi and Feldman, 1967) and forced to show a similarity factor (f_2) below 50 between the medium and fast/slow formulation.

A level A IVIVC using differential equations (Rossenu et al., 2008) was established using these three drug formulations, where the link between *in vitro* and *in vivo* performance of the drug products was related between *in vitro* and *in vivo* dissolution rate coefficients (k_d). It was assumed that the dissolution process was the rate limiting step of *in vivo* absorption and bioavailability, where the k_d of each formulation was lower compared to the absorption rate coefficient (k_a). Two types of scenarios were drawn:

- Linear relationship between $k_{d,\ in\ vitro}$ and $k_{d,\ in\ vivo}$ (Scenarios 1, 2, 3)
- Non-linear relationship between $k_{d,\ \textit{in vitro}}$ and $k_{d,\ \textit{in vivo}}$ based on a sigmoid function (Scenarios 4, 5, 6)

In vitro individual data for each formulation were simulated randomly, using inter-individual variability (IIV) on k_d through an exponential model. Residual unexplained variability (RUV) on the *in vitro* dissolved fractions was also considered.

Plasma profiles were generated using a one compartment model with first order dissolution, absorption, and elimination kinetics. Twelve individual units were considered for each formulation or batch. IIV on pharmacokinetic parameters was not included in order to avoid any influence on the dissolution performance of drug formulation or batch. *In vitro* dissolution and *in vivo* pharmacokinetic parameters used in the establishment of the level A IVIVC were the same in both linear and non-linear relationships, but the link equation parameters were different. Study design characteristics and parameters used in the development of the level A IVIVC are summarized in Table 1.

Table 1. Parameters used in establishment of the level A IVIVC

Study design characteristics		
	In vitro	***In vivo***
Sampling times (h)	0, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 16, 24, 28, 32, 48	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 24, 28, 32, 48
Nº of individuals	12	12
Study design parameters		
Parameter	**Value**	**IIV (%)**
k_d (slow formulation) (h^{-1})	0.1	5
k_d (medium formulation) (h^{-1})	0.3	5
k_d (fast formulation) (h^{-1})	0.8	5
K_a (h^{-1})	1.13	0
V_c (L)	1	0
CL (L/h)	0.08	0
In vitro RUV (%)	1	-
In vivo RUV (%)	3	-

CL, clearance; h, hours; k_a, i*n vivo* absorption rate coefficient; k_d: *in vitro* dissolution rate coefficient; IIV, inter-individual variability; L, liters; RUV, residual unexplained variability; V_c, central volume of distribution.

2.2 Batch suitability

Once the level A IVIVC was established, in order to assess the impact of the different mathematical approaches on concluding BE of a new batch, six additional batches (12 units each) were simulated following the same dissolution model according to the following rules: (i) three new batches with different k_d among them, but within the range of medium and slow formulations (Batch 1, 3, and 5), and (ii) three new batches with different k_d among them, but within the range of medium and fast formulations (Batch 2, 4, and 6) from the developed IVIVC.

For each batch, simulation (n=1,000) of a dissolution assay with 12 units was generated through the Monte Carlo simulation approach. With the aim of clarifying the conclusions, we assumed complete absorption of the dosage form. Thus, dissolution performance of each batch was assessed on C_{max} only. Twelve thousand *in vitro* dissolution profiles per batch were obtained. Then, using the IVIVC link, *in vivo* time course profiles were calculated as follows:

- Classical approach: 1,000 C_{max} ratios of the batch formulation and reference were obtained from the mean 1,000 *in vivo* profiles using 1,000 mean *in vitro* dissolution profiles.

- Individual approach: 12,000 *in vivo* profiles from the 12,000 *in vitro* dissolution profiles were generated. Then, according to rules i) and ii), the slowest tablet of the slowest dissolving formulation (STSF) or the fastest tablet of the fastest dissolving formulation (FTFF) for each dissolution assay (n=1,000) were selected. If the k_d of the new batch was within the range of medium and fast formulation the FTFF was selected, otherwise the STSF was chosen. Thus, 1,000 ratios from the mean C_{max} of the reference formulation and the C_{max} of the STSF or FTFF tablet were determined.

The percentage of BE batches was computed for each approach. BE of a new batch was concluded when the C_{max} ratio between new batch formulations and the reference was within ±20%.

2.3 Dissolution specification

For the classical approach (using mean data), *in vitro* dissolution limits of each formulation were computed using the batch whose ratio was the closest to ±20%. On the other hand, using the individual approach, the STSF and FTFF whose ratio was exactly (to four significant digits) ±20% were selected in order to establish the *in vitro* dissolution specifications.

2.4 Bioequivalence studies

In order to assess the influence of the two methodologies used, and to establish dissolution specification that would guarantee that all dissolved units from the new batch will be BE (its AUC or C_{max} ratio will be within of the 0.8-1.25 range), Monte Carlo simulations (n = 1,000) of crossover BE studies with 24 healthy simulated subjects per study were performed. Each simulated subject received an oral dose of 100 mg of the test and reference formulations, with a wash-out period between the administrations. Samples were collected at 0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 hours after a single dose administration of the drug. The simulated subjects were distributed into two sequence-groups of 12 volunteers each.

In vitro k_d of the test formulation ranged within the k_d values of the STSF and FTFF that previously set the dissolution specifications. Moderate (30%) intra-individual variability was applied to pharmacokinetic parameters in order to generate different conditions to allow the assessment of *in vivo* performance of the drug formulation: i) 30% intra-individual variability on k_a; ii) 30% intra-individual variability on clearance (CL); iii) 30% intra-individual variability on k_a and CL.

2.5 Software

The simulations were performed using NONMEM 7.3 (Bauer, 2011). Graphical and statistical analyses were performed using R software (http://cran. r-project.org, version 3.3.2) and RStudio® (version 1.0.136)

RESULTS

3.1 IVIVC development

Mean *in vitro* dissolved fraction versus time from the three formulations (fast, medium, and slow) are depicted in Figure *1*. Plasma concentrations were calculated using the *in vitro* dissolved fraction and according to the linear (Scenarios 1-3) or non-linear (Scenarios 4-6) IVIVC link model. Figure *2* represents the mean in vivo profiles for each type of formulation included in the development of the IVIVC.

Figure 1. Mean in vitro profiles for IVIVC formulations.

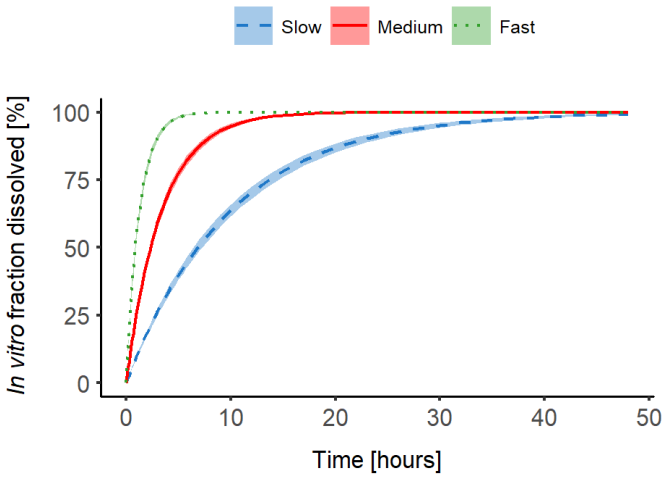


Figure 2 Plasma in vivo profiles obtained through IVIVC link (top linear IVIVC, bottom non-linear IVIVC)

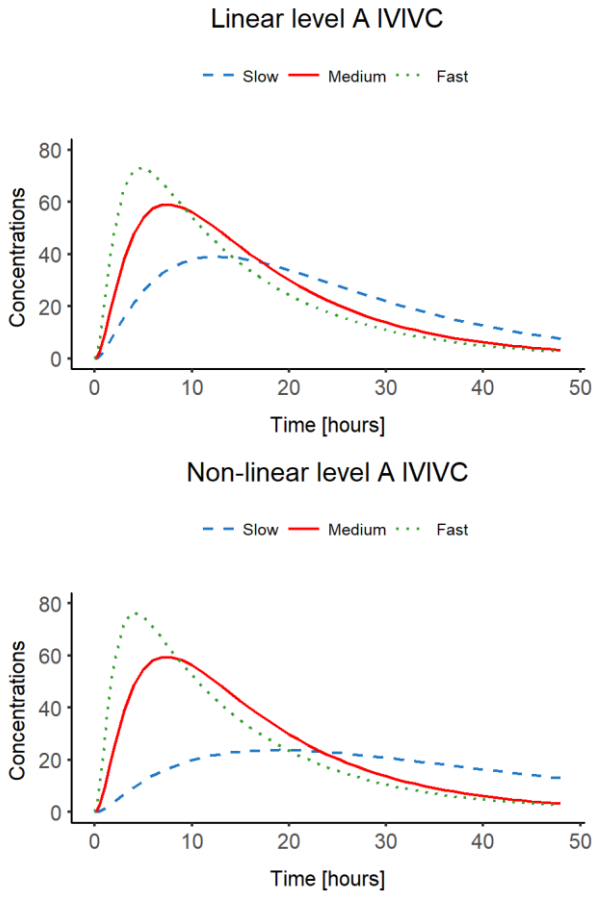


Table 2 includes the 90% confidence interval (CI) for C_{max} demonstrating that neither the slow nor the fast formulation were BE to the medium formulation.

Table 2. 90% CI for the BE studies performed comparing slow-medium and fast-medium formulations used in the establishment of the level A IVIVC.

Formulations	Linear level A IVIVC	Non-linear level A IVIVC
Slow formulation	63.52%-68.87%	38.07%-40.70%
Fast formulation	120.68-129.04%	127.84%-129.59%

3.2 Batch suitability

Figure 3 represents the mean in vivo PK profile obtained from the mean in vitro dissolution profile for the reference formulation and the six batches considered. The C_{max} ratio between each batch and the reference formulation from the mean *in vivo* PK profile are summarized in

Table 3, showing that all batches were within the regulatory threshold of ±20% for both types of level A IVIVC. One thousand simulations were performed using the mean *in vitro* dissolution profile (classical approach) and the STSF/FTFF (individual approach).

Figure 3. Mean in vitro (top) and in vivo (bottom) profiles of the new batches (left linear scenarios, right non-linear scenarios).

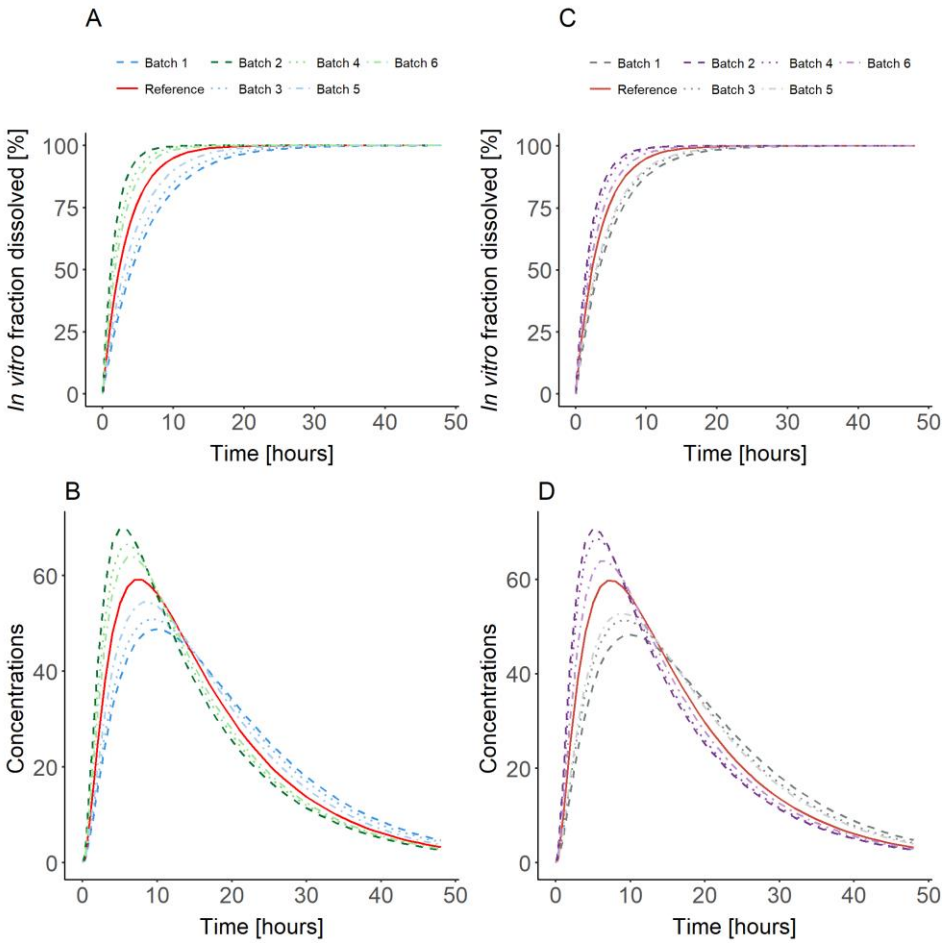


Table 3. C_{max} ratios obtained between the reference formulation used in the development of a level A IVIVC and the six new batches simulated (Batch 1-6).

	C_{max} Ratios	
	Linear level A IVIVC	**Non-linear level A IVIVC**
Batch 1 / Reference formulation	82.6%	80.8%
Batch 2 / Reference formulation	118%	118%
Batch 3 / Reference formulation	86.1%	86.0%
Batch 4 / Reference formulation	113%	115%
Batch 5 / Reference formulation	92.0%	88.4%
Batch 6 / Reference formulation	108%	107%

Figure 4 depicts the *in vivo* PK profiles of the reference and the six batches according to the classical and individual approaches obtained from the linear and non-linear IVIVC developed. When the classical approach was applied, 1,000 time-course *in vivo* profiles were obtained from the mean *in vitro* dissolution profile of each dissolution assay (12 units) simulated (n=1,000). Otherwise, the individual approach was allowed to generate 1,000 PK profiles from the slowest/fastest unit (STSF/FTFF) of each dissolution assay simulated (n=1,000).

Figure 4. In vivo plasma profiles obtained using a linear and non-linear level A IVIVC models when the classical and individual approaches were applied, where A represents linear IVIVC and classical approach, B represents linear IVIVC and individual approach, C represents non-linear IVIVC and classical approach, and D represents non-linear IVIVC and individual approach. Blue/grey (linear/non-linear) dashed areas represent batch 1 (left), batch 3 (middle), and batch 5 (right), respectively; red solid line represents the reference formulation; green/purple (linear/non-linear) dotted areas represent batch 2 (left), batch 4 (middle) and batch 6 (right), respectively. Horizontal lines represent the acceptance interval of 80.00-125.00%.

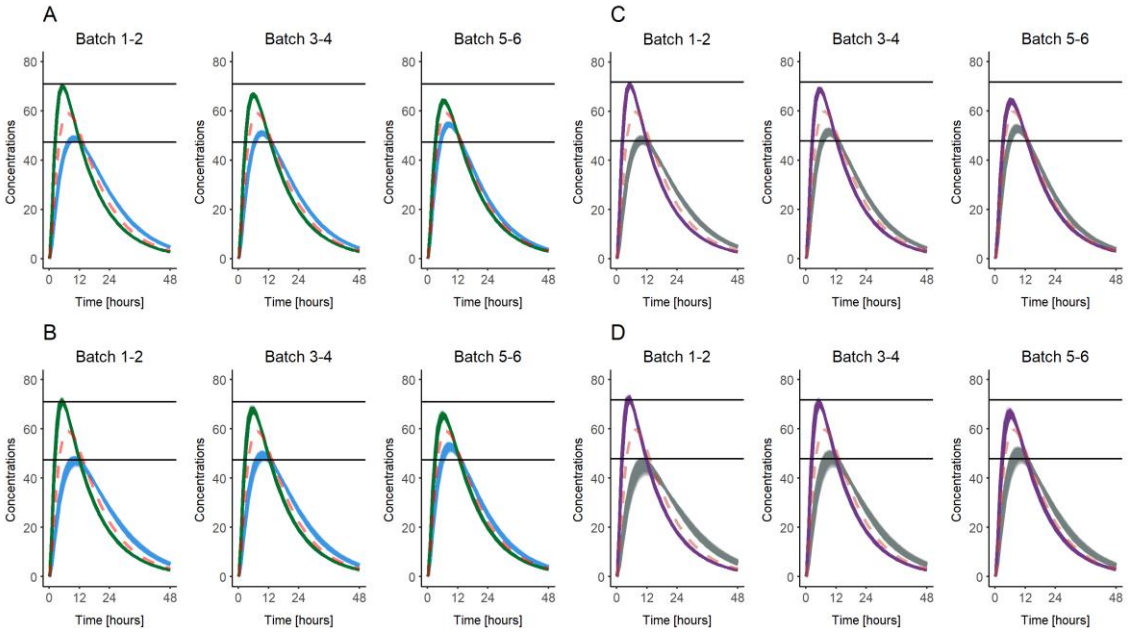
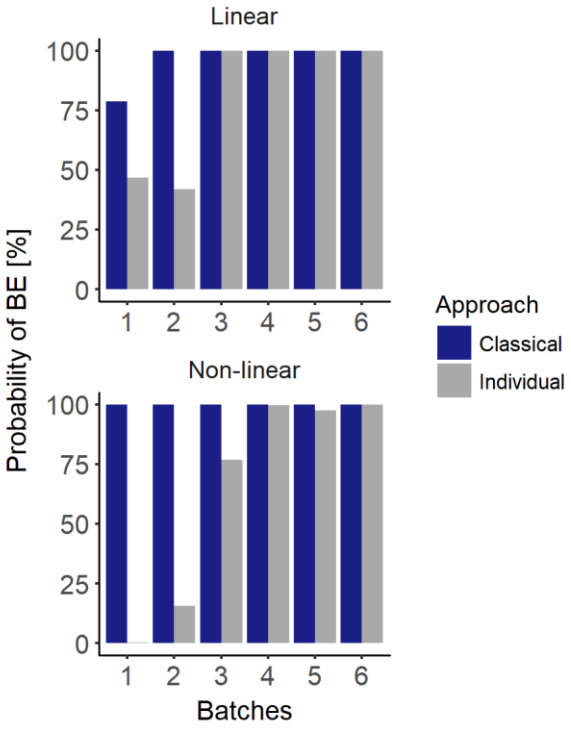


Figure 5 shows the results of the number of suitable batches (within ±20%) using the classical and individual approach. According to the results from the classical approach, the C_{max} ratio from the six batches fulfill the ±20% range under linear level A IVIVC. Similar results were observed for Batches 2-6 when non-linear level A IVIVC was developed, but only 78.6% of the simulations with Batch 1 achieved a C_{max} ratio within the ±20% difference. However, when the individual approach was applied under linear level A IVIVC, a significant number of simulations with Batches 1 and 2 were outside of the ±20% limits: 53.3 and 58.1%, respectively. Greater differences between the classical and individual approaches were observed for the non-linear relationship (scenarios 4-6), where the suitable number of batches of Batch 1 and 2 diminished to

0.3 and 15.5%, respectively. Additionally, 23.1% of the simulations with Batch 3 resulted in a C_{max} ratio greater than ±20% compared to the reference formulation. The dissolution performance of Batch 3 was more similar to the reference formulation than Batches 1 and 2, but differences were not detected when the classical approach was applied.

Figure 5. Suitable batches calculated by the classical and individual approach based on Monte Carlo simulations of a cross-over BE study (n=1,000).



3.3 Dissolution specification

The batches whose C_{max} ratios were closest to ±20% (Batches 1 and 2) were used to establish the dissolution limit specifications (Table *4*). The classical approach provides narrower specification limits because it is established based on the mean *in vitro* dissolution profile that is closest to ±20%, whereas the individual approach provides the dissolution specification limits that exactly achieved ±20% difference on C_{max} between reference and new batch.

Table 4. Dissolution specifications for the different methodologies

	Dissolved [%]	**Classical approach [min]**	**Individual approach [min]**
Linear level A IVIVC	25	30-120	30-120
	50	75-270	60-300
	85	165-780	165-840
Non-linear level A IVIVC	25	30-90	30-90
	50	90-210	75-225
	85	210-600	195-600

3.4 Bioequivalence studies

One thousand Monte Carlo simulations were generated using linear and non-linear IVIVC models and according to the different scenarios of *in vivo* variability in k_a and/or CL mentioned above. Based on the dissolution limits previously established using the individual approach proposed in this article, 100% of the 90% CI of C_{max} estimated between the reference formulation and the new batch simulated were within the 0.8-1.25 limits when linear and non-linear IVIVC models were applied. These results confirmed dissolution specification limits would guarantee bioequivalent units within the batch when 30% of intra-individual variability on PK and *in vitro* dissolution parameters were accounted for, even in the worst *in vivo* case scenario (30% in k_a and CL).

DISCUSSION

In this paper a new methodology based on an individual approach has been proposed and successfully applied to establish dissolution specifications from a level A IVIVC, which was developed using a one-stage method. It considers the *in vitro* and *in vivo* variability of batches and, as a consequence, it displays wider dissolution specification limits than the classical approach, ensuring that any tablet from the new batch would generate *in vivo* profiles which its C_{max} ratio will out of the 0.8-1.25 range. The widening of the dissolution specification limits could be accomplished because individual data is used instead of average data. The use of the classical approach, which assumes a maximal difference of 20% in the predicted AUC and C_{max} using average data, might result in considering non-BE units within the batch as BE. Averaging data implies loss of information and use of the geometric mean might not be an adequate approach due to extreme values (Cardot and Davit, 2012). For these reasons, this new approach makes use of the individual data to ensure BE for all tablets.

When comparing the linear and non-linear relationship between the *in vitro* and *in vivo* dissolution, it is observed that non-linear conditions narrow the dissolution specification limits. Nonetheless, 100% of the Monte Carlo simulations achieved a BE conclusion, even in the non-linear scenario when 30% intra-individual variability in k_a and CL was considered. The aforementioned highlights the shortcomings of the current methods that are employed to define the dissolution specification limits based on IVIVC, mostly when there is non-linearity between *in vitro* and *in vivo* dissolution or when the IIV is relevant.

The current constraint regarding the use of average data in the establishment of dissolution specifications has been highlighted in this analysis (Figure *5*), showing the regulatory and clinical implications of declaring BE batches that contain non-BE units. Based on the most different batches (Batch 1 and Batch 2), only 46.7% (Batch 1) and 41.9% (Batch 2) of the simulated batches in the linear level A IVIVC were declared BE compared to 100% simulated batches using the classical approach. These differences between the classical and the individual approach largely increase when a non-linear level A IVIVC is developed. On the other hand, when batches similar to the reference formulation are developed (Batches 3-5), the individual approach achieved equal results to the classical approach. This demonstrates the new methodology proposed is more restrictive and accurate to declare BE of a new batch based on a level A IVIVC.

As a limitation of the present work, the simulated conditions and scenarios are empirical and not related to any specific drug. However, the drug product conditions employed assumed a BCS class II drug, where the *in vivo* dissolution was the rate limiting step of

in vivo absorption and bioavailability due to the high dependency of the drug product on the drug solubility, formulation factors, and *in vivo* luminal environment (Gonzalez-Garcia et al., 2015). All these elements provide the ideal basis for the development of an IVIVC (Balan et al., 2000; Corrigan et al., 2003; Ghosh et al., 2008; Honorio Tda et al., 2013; Ilic et al., 2014; Jantratid et al., 2009; Kovacevic et al., 2009; Lue et al., 2008; Macha et al., 2009; Okumu et al., 2008, 2009; Ostrowski et al., 2010; Rossi et al., 2011; Saibi et al., 2012; Shono et al., 2009; Sunesen et al., 2005; Tashtoush et al., 2004; Veng-Pedersen et al., 2000; Wei and Lobenberg, 2006). *In vivo* variability on PK parameters was not considered during the development of both level A IVIVCs in order to only assess the influence of dissolution variability on the establishment of dissolution specifications, as IIV on PK parameters would have impacted equally to the methodologies compared. More complex *in vitro* dissolution models (Abuhelwa et al., 2016; Locher et al., 2016; Ramteke et al., 2016; Weiss et al., 2014) have not been included in the current analysis in order to simply compare the predictability of both methodologies, but future analyses should incorporate them in order to account for complex dissolution kinetics. On the other hand, the analysis included only the assessment of dissolution performance on C_{max} because we assumed complete absorption of the dosage form and, therefore, no differences in AUC would be expected. Additionally, moderate *in vivo* variability was considered in k_a and/or CL in order to reflect real conditions of a BE study. The influence of higher and lower *in vivo* variability on PK parameters was not assessed to reduce the number of simulated scenarios.

The establishment of an IVIVC offers several advantages during the drug development process (Cook, 2012), with one of the most relevant uses of the IVIVC being a surrogate for BE studies due to post-approval changes (Chowdhury, 2011; Limberg and Potthast, 2013). As described in the guidelines (EMA, 2012; FDA, 1997), a level A IVIVC is established by i) a two-stage procedure, where the *in vivo* absorption is obtained through deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved (Gonzalez-Garcia et al., 2015; Loo and Riegelman, 1968; Margolskee et al., 2016; O'Hara et al., 2001; Qiu et al., 2016; Suverkrup et al., 1989; Vaughan and Dennis, 1978; Wagner and Nelson, 1963, 1964; Young, 1997; Yu et al., 1996), and ii) one-stage procedures, which directly relate *in vivo* - *in vitro* data (Costello et al., 2011; Gaynor et al., 2011; Gillespie, 1997; O'Hara et al., 2001; Veng-Pedersen et al., 2000) and are mathematically more stable than two-stage methods (Dunne et al., 2006; Gaynor et al., 2008; Veng-Pedersen et al., 2000).

Roudier et al. proposed calculations based on the back-calculation of the 90% CI of C_{max} and AUC in order to solve the limitations of using average data when a level A IVIVC is developed as an *in vivo* surrogate of a new batch/formulation (Roudier et al., 2014). This

takes into consideration the intra-subject variability and leads to wider *in vitro* dissolution limits compared to the classical approach in the same line as the present work. However, the dissolution limits allow that 10% of the units from a batch overcome the BE limits, whereas the individual approach guarantees all units within the same batch are BE because dissolution limits are set based on the STSF and FTFF. This result was confirmed in this article when one thousand batches were simulated and used in cross-over BE studies, assuming different *in vivo* variability on PK parameters and none of the simulated batches generated a 90% CI outside of 80-125%.

FDA and EMA regulatory guidelines have been adapted to increase the applicability of IVIVC as a surrogate of the *in vivo* performance (EMA, 2012; FDA, 1997). However, both still consider the use of average data and allow an arbitrary limit of 20% in C_{max} and AUC. The *in vitro* and *in vivo* variability is an inherent element of the experimental studies and not taking it into consideration suggests a very simple vision of the *in vitro* and *in vivo* behavior of drug products.

4.1 Conclusion

In conclusion, an individual approach has been proposed to establish dissolution specifications using a level A IVIVC, ensuring BE of all units within the new batch developed. This methodology takes into consideration the *in vitro* and *in vivo* variability observed, providing dissolution specification limits that ensure *in vivo* ratios exactly between 80-125. Thus, the widening of dissolution specifications is a consequence of using individual data, but the approach ensures the BE of all tablets, which is not always achieved using the classical approach.

ACKNOWLEDGEMENTS

This work was partially funded by "MODELOS IN VITRO DE EVALUACION BIOFARMACEUTICA" SAF2016-78756 (AEI/FEDER, EU) funded by Agencia Estatal Investigación and European Union, through FEDER (Fondo Europeo de Desarrollo Regional)

REFERENCES

Abuhelwa, A.Y., Mudge, S., Hayes, D., Upton, R.N., Foster, D.J., 2016. Population In Vitro-In Vivo Correlation Model Linking Gastrointestinal Transit Time, pH, and Pharmacokinetics: Itraconazole as a Model Drug. Pharm Res 33, 1782-1794.
Balan, G., Timmins, P., Greene, D.S., Marathe, P.H., 2000. In-vitro in-vivo correlation models for glibenclamide after administration of metformin/glibenclamide tablets to healthy human volunteers. The Journal of pharmacy and pharmacology 52, 831-838.
Bauer, R., 2011. NONMEM users guide: introduction to NONMEM 7.2.0, in: Solutions, I.D. (Ed.), Elicott City.
Cardot, J.M., Davit, B.M., 2012. In vitro-in vivo correlations: tricks and traps. Aaps J 14, 491-499.
Cook, J.A., 2012. Development strategies for IVIVC in an industrial environment. Biopharmaceutics & drug disposition 33, 349-353.
Corrigan, O.I., Devlin, Y., Butler, J., 2003. Influence of dissolution medium buffer composition on ketoprofen release from ER products and in vitro-in vivo correlation. International journal of pharmaceutics 254, 147-154.
Costello, C., Rossenu, S., Vermeulen, A., Cleton, A., Dunne, A., 2011. A time scaling approach to develop an in vitro-in vivo correlation (IVIVC) model using a convolution-based technique. Journal of pharmacokinetics and pharmacodynamics 38, 519-539.
Chowdhury, A.K., Islam, S., 2011. In vitro-in vivo correlation as a surrogate for bioequivalence testing: the current state of play. Asian Journal of Pharmaceutical Sciences 6, 176-190.
Dunne, A., Gaynor, C., Davis, J., 2006. Deconvolution Based Approach for Level A In Vivo-In Vitro Correlation Modelling: statistical considerations. Clin Res Regul Aff 22.
EMA, 2012. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms, London.
EMA, 2014a. Guideline on quality of oral modified release products.
EMA, 2014b. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms.
FDA, 1997. Guidance for industry. Extended release oral dosage forms: development, evaluation and application of *in vitro/in vivo* correlations. US Department of Health and Human Services, Center for Drug Evaluation and Research (CDER).
Gaynor, C., Dunne, A., Costello, C., Davis, J., 2011. A population approach to in vitro-in vivo correlation modelling for compounds with nonlinear kinetics. Journal of pharmacokinetics and pharmacodynamics 38, 317-332.
Gaynor, C., Dunne, A., Davis, J., 2008. A comparison of the prediction accuracy of two IVIVC modelling techniques. Journal of pharmaceutical sciences 97, 3422-3432.
Gaynor, C., Dunne, A., Davis, J., 2009. The effects of averaging on accuracy of IVIVC model predictions. Journal of pharmaceutical sciences 98, 3829-3838.
Ghosh, A., Bhaumik, U.K., Bose, A., Mandal, U., Gowda, V., Chatterjee, B., Chakrabarty, U.S., Pal, T.K., 2008. Extended release dosage form of glipizide: development and validation of a level A in vitro-in vivo correlation. Biol Pharm Bull 31, 1946-1951.
Gibaldi, M., Feldman, S., 1967. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. J Pharm Sci 56, 1238-1242.
Gillespie, W.R., 1997. Convolution-based approaches for in vivo-in vitro correlation modeling. Adv Exp Med Biol 423, 53-65.
González-García, I., Mangas-Sanjuan, V., Merino-Sanjuán, M., Álvarez-Álvarez, C., Díaz-Garzón Marco, J., Rodríguez-Bonnín, M.A., Langguth, T., Torrado-Durán, J.J., Langguth,

P., García-Arieta, A., Bermejo, M., 2017. IVIVC approach based on carbamazepine bioequivalence studies combination. Die Pharmazie - An International Journal of Pharmaceutical Sciences 72, 449-455.
Gonzalez-Garcia, I., Mangas-Sanjuan, V., Merino-Sanjuan, M., Bermejo, M., 2015. In vitro-in vivo correlations: general concepts, methodologies and regulatory applications. Drug Dev Ind Pharm 41, 1935-1947.
Hermans, A., Abend, A.M., Kesisoglou, F., Flanagan, T., Cohen, M.J., Diaz, D.A., Mao, Y., Zhang, L., Webster, G.K., Lin, Y., Hahn, D.A., Coutant, C.A., Grady, H., 2017. Approaches for Establishing Clinically Relevant Dissolution Specifications for Immediate Release Solid Oral Dosage Forms. Aaps J 19, 1537-1549.
Honorio Tda, S., Pinto, E.C., Rocha, H.V., Esteves, V.S., dos Santos, T.C., Castro, H.C., Rodrigues, C.R., de Sousa, V.P., Cabral, L.M., 2013. In vitro-in vivo correlation of efavirenz tablets using GastroPlus(R). AAPS PharmSciTech 14, 1244-1254.
Ilic, M., Ethuris, J., Kovacevic, I., Ibric, S., Parojcic, J., 2014. In vitro--in silico--in vivo drug absorption model development based on mechanistic gastrointestinal simulation and artificial neural networks: nifedipine osmotic release tablets case study. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 62, 212-218.
Jantratid, E., De Maio, V., Ronda, E., Mattavelli, V., Vertzoni, M., Dressman, J.B., 2009. Application of biorelevant dissolution tests to the prediction of in vivo performance of diclofenac sodium from an oral modified-release pellet dosage form. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 37, 434-441.
Kovacevic, I., Parojcic, J., Homsek, I., Tubic-Grozdanis, M., Langguth, P., 2009. Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. Mol Pharm 6, 40-47.
Limberg, J., Potthast, H., 2013. Regulatory status on the role of in vitro dissolution testing in quality control and biopharmaceutics in Europe. Biopharm Drug Dispos 34, 247-253.
Locher, K., Borghardt, J.M., Frank, K.J., Kloft, C., Wagner, K.G., 2016. Evolution of a mini-scale biphasic dissolution model: Impact of model parameters on partitioning of dissolved API and modelling of in vivo-relevant kinetics. Eur J Pharm Biopharm 105, 166-175.
Loo, J.C., Riegelman, S., 1968. New method for calculating the intrinsic absorption rate of drugs. Journal of pharmaceutical sciences 57, 918-928.
Lue, B.M., Nielsen, F.S., Magnussen, T., Schou, H.M., Kristensen, K., Jacobsen, L.O., Mullertz, A., 2008. Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 69, 648-657.
Macha, S., Yong, C.L., Darrington, T., Davis, M.S., MacGregor, T.R., Castles, M., Krill, S.L., 2009. In vitro-in vivo correlation for nevirapine extended release tablets. Biopharmaceutics & drug disposition 30, 542-550.
Margolskee, A., Darwich, A.S., Galetin, A., Rostami-Hodjegan, A., Aarons, L., 2016. Deconvolution and IVIVC: Exploring the Role of Rate-Limiting Conditions. Aaps J 18, 321-332.
O'Hara, T., Hayes, S., Davis, J., Devane, J., Smart, T., Dunne, A., 2001. In vivo-in vitro correlation (IVIVC) modeling incorporating a convolution step. Journal of pharmacokinetics and pharmacodynamics 28, 277-298.

Okumu, A., DiMaso, M., Lobenberg, R., 2008. Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. Pharmaceutical research 25, 2778-2785.
Okumu, A., DiMaso, M., Lobenberg, R., 2009. Computer simulations using GastroPlus to justify a biowaiver for etoricoxib solid oral drug products. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 72, 91-98.
Ostrowski, M., Wilkowska, E., Baczek, T., 2010. The influence of averaging procedure on the accuracy of IVIVC predictions: immediate release dosage form case study. Journal of pharmaceutical sciences 99, 5040-5045.
Qiu, J., Martinez, M., Tiwari, R., 2016. Evaluating In Vivo-In Vitro Correlation Using a Bayesian Approach. Aaps J 18, 619-634.
Ramteke, H.K., Dighe, P.A., Kharat, A.R., Patil, S.V., 2016. Mathematical Models of Drug Dissolution: A Review. Scholars Academic Journal of Pharmacy (SAJP) 3, 388-396.
Rossenu, S., Gaynor, C., Vermeulen, A., Cleton, A., Dunne, A., 2008. A nonlinear mixed effects IVIVC model for multi-release drug delivery systems. Journal of pharmacokinetics and pharmacodynamics 35, 423-441.
Rossi, R.C., Dias, C.L., Bajerski, L., Bergold, A.M., Froehlich, P.E., 2011. Development and validation of discriminating method of dissolution for fosamprenavir tablets based on in vivo data. J Pharm Biomed Anal 54, 439-444.
Roudier, B., Davit, B.M., Beyssac, E., Cardot, J.M., 2014. In vitro- in vivo correlation's dissolution limits setting. Pharm Res 31, 2529-2538.
Saibi, Y., Sato, H., Tachiki, H., 2012. Developing in vitro-in vivo correlation of risperidone immediate release tablet. AAPS PharmSciTech 13, 890-895.
Shono, Y., Jantratid, E., Janssen, N., Kesisoglou, F., Mao, Y., Vertzoni, M., Reppas, C., Dressman, J.B., 2009. Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 73, 107-114.
Sunesen, V.H., Pedersen, B.L., Kristensen, H.G., Mullertz, A., 2005. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 24, 305-313.
Suverkrup, R., Bonnacker, I., Raubach, H.J., 1989. Numerical stability of pharmacokinetic deconvolution algorithms. J Pharm Sci 78, 948-954.
Tashtoush, B.M., Al-Qashi, Z.S., Najib, N.M., 2004. In vitro and in vivo evaluation of glibenclamide in solid dispersion systems. Drug Dev Ind Pharm 30, 601-607.
Uppoor, V.R., 2001. Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations. J Control Release 72, 127-132.
Vaughan, D.P., Dennis, M., 1978. Mathematical basis of point-area deconvolution method for determining in vivo input functions. Journal of pharmaceutical sciences 67, 663-665.
Veng-Pedersen, P., Gobburu, J.V., Meyer, M.C., Straughn, A.B., 2000. Carbamazepine level-A in vivo-in vitro correlation (IVIVC): a scaled convolution based predictive approach. Biopharmaceutics & drug disposition 21, 1-6.
Wagner, J.G., Nelson, E., 1963. Per cent absorbed time plots derived from blood level and/or urinary excretion data. Journal of pharmaceutical sciences 52, 610-611.

Wagner, J.G., Nelson, E., 1964. Kinetic Analysis of Blood Levels and Urinary Excretion in the Absorptive Phase after Single Doses of Drug. Journal of pharmaceutical sciences 53, 1392-1403.
Wei, H., Lobenberg, R., 2006. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 29, 45-52.
Weiss, M., Kriangkrai, W., Sungthongjeen, S., 2014. An empirical model for dissolution profile and its application to floating dosage forms. Eur J Pharm Sci 56, 87-91.
Young, D., 1997. In vitro in vivo correlations. Plenum Pub. Corp.
Yu, Z., Schwartz, J.B., Sugita, E.T., Foehl, H.C., 1996. Five modified numerical deconvolution methods for biopharmaceutics and pharmacokinetics studies. Biopharm Drug Dispos 17, 521-540.