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MARCELO GOMES DAVANÇO

CORRELAÇÃO IN VITRO - IN VIVO NO DESENVOLVIMENTO DE MEDICAMENTOS GENÉRICOS: APLICAÇÃO PARA FORMULAÇÕES DE DESVENLAFAXINA E CARBAMAZEPINA

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Orientador: Profa. Dra. Patrícia de Oliveira Carvalho

Coorientador: Dr. Daniel Rossi de Campos

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Profa. Dra. Patrícia de Oliveira Carvalho - Orientadora e Presidente
Universidade São Francisco

Profa. Dra. Andréia de Melo Porcari
Universidade São Francisco

Prof. Dr. Leonardo de Souza Teixeira
Instituto de Ciências Farmacêuticas de Estudos e Pesquisas Ltda

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Universidade Federal de São Paulo

Prof. Dr. Mario Angelo Claudino
Universidade São Francisco

Prof. Dr. Michel Leandro de Campos
Universidade Federal do Mato Grosso

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RESUMO

A disponibilidade de medicamentos genéricos tem importante papel socioeconômico no Brasil. O desenvolvimento assertivo de produtos desta categoria tem se tornado fundamental para as indústrias farmacêuticas que buscam ampliar seus portfólios e atuar neste mercado altamente competitivo. Nesse contexto, a correlação *in vitro – in vivo* (CIVIV) é de grande relevância para a pesquisa e desenvolvimento de medicamentos genéricos, uma vez que sua adequada aplicação permite selecionar os protótipos mais adequados para seguir para os estudos de bioequivalência. O objetivo deste trabalho foi, após uma ampla revisão dos estudos publicados nas últimas décadas sobre CIVIV, aplicar essa ferramenta na construção de modelos matemáticos para correlação dos dados de fração dissolvida e absorvida de duas formulações orais: succinato de desvenlafaxina monoidratado 50 mg comprimido revestido de liberação prolongada e carbamazepina 400 mg comprimido. Na primeira etapa deste trabalho, a avaliação de artigos científicos publicados nas últimas décadas sobre a aplicação da CIVIV para o desenvolvimento de formas farmacêuticas sólidas orais possibilitou elencar as principais características da área e discutir sobre as possibilidades de melhoria. Na segunda etapa, com os dados de fração dissolvida e absorvida de ambas as formulações, foram construídos modelos matemáticos para estabelecimento da CIVIV. Na avaliação da capacidade preditiva dos modelos foi possível observar erros de predição menores que 10%, o que demonstrou que as condições de dissolução avaliadas foram adequadas para predição dos parâmetros farmacocinéticos. Assim, os resultados gerados por este trabalho poderão servir como importantes ferramentas para auxiliar no desenvolvimento galênico, definição da especificação dos limites de dissolução e possíveis alterações pós-registro de formulações de desvenlafaxina e carbamazepina. Além disso, a revisão sistemática abordada neste trabalho possibilitou realizar uma importante "fotografia" do uso da CIVIV no contexto de desenvolvimento de medicamentos, bem como disponibilizar na literatura uma base de dados com 45 diferentes substâncias para pesquisadores interessados nesta área.

Descritores: Medicamentos Genéricos. Estudos de Correlação. Dissolução. Bioequivalência. Desenvolvimento de Medicamentos.

ABSTRACT

The availability of generics has an important socioeconomic role in Brazil. The assertive development in this category has become essential for pharmaceutical industries to expand their portfolios and to play in this highly competitive market. In this context, in vitro – in vivo correlation (IVIVC) is of great relevance for the research and development of generics since its adequate application allows selecting the most suitable prototypes for bioequivalence studies. The objective of this work was, after a broad review of studies published in recent decades on IVIVC, to apply this tool in the construction of mathematical models for correlation of dissolved and absorbed fraction data of two oral formulations: desvenlafaxine succinate monohydrate 50 mg extended-release film coated tablet and carbamazepine 400 mg tablet. In the first stage of this work, the evaluation of scientific articles published in recent decades on the application of IVIVC for the development of solid oral dosage forms made it possible to highlight the main characteristics of the area and discuss possibilities for improvement. In the second stage, with the data of dissolved and absorbed fractions of both formulations, mathematical models were constructed to establish the IVIVC. In predictive capacity assessment of the models, it was possible to observe prediction errors lower than 10%, which demonstrated that the dissolution conditions applied were adequate for predicting the pharmacokinetic parameters. Thus, the results showed in this work may serve as important tools to support galenic development, the definition of specification of dissolution limits and possible post-approval changes of desvenlafaxine and carbamazepine formulations. In addition, the systematic review addressed in this work showed an important "photograph" of the use of IVIVC in the formulation development and became available in the literature a database with 45 different drugs to researchers interested in this area.

Keywords: Generic drugs. Correlation studies. Dissolution. Bioequivalence. Drug Development.

LISTA DE SÍMBOLOS E ABREVIAÇÕES

ANVISA – Agência Nacional de Vigilância Sanitária

ASC – Área sob a curva

CETER – Coordenação de Equivalência Terapêutica

CIVIV - Correlação in vitro – in vivo

Cmáx – Concentração plasmática máxima

CMED – Câmara de Regulação do Mercado de Medicamentos

EMA – *European Medicines Agency*

FDA – *Food and Drug Administration*

FIP – *International Pharmaceutical Federation*

HCl – ácido clorídrico

ICH – *The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*

IFA – Insumo Farmacêutico Ativo

LSS – Lauril sulfato de sódio

PE – erro de predição

PNM – Política Nacional de Medicamentos

P&D – Pesquisa e Desenvolvimento

RDC – Resolução da Diretoria Colegiada

RE – Resolução

TGI – trato gastrointestinal

USP – *United States Pharmacopeia*

SUMÁRIO

1. INTRODUÇÃO

Nos últimos vinte anos, os requerimentos técnicos e a regulamentação para registro e pós-registro de medicamentos genéricos no Brasil tiveram alterações significativas, implicando positivamente na avaliação da qualidade, segurança e eficácia dos produtos comercializados no mercado brasileiro (LEE et al., 2015; PATEL et al., 2020; SOARES et al., 2015).

Dentre as provas necessárias para registro de um medicamento genérico, os estudos de bioequivalência representam parte significativa do montante financeiro alocado em um projeto dentro de uma indústria farmacêutica. Esses estudos fazem parte das últimas etapas de avaliação antes da petição de registro junto ao órgão regulador. Antes de chegarem a esse estágio, as formulações candidatas a medicamento genérico são criteriosamente avaliadas durante as etapas de desenvolvimento galênico e analítico a fim de se explorar suas características e desempenho (CAMPOS; GOMES, 2011; NIAZI, 2007; VIEIRA; CAMPOS, 2010).

Alguns ensaios *in vitro* são realizados com o objetivo de elencar o melhor protótipo de cada formulação para avançar para os estudos de bioequivalência, sendo de grande interesse por parte das indústrias farmacêuticas, uma vez que buscam minimizar falhas de desenvolvimento, como, por exemplo, a bioinequivalência e/ou a falha de estabilidade do produto. Neste contexto, podem ser destacados os ensaios de perfil de dissolução realizados em meios biopreditivos que visam mimetizar as condições físico-químicas dos fluidos biológicos, principalmente do trato gastrointestinal (TGI) quando se trata de formulações de uso oral (KLEIN, 2019; SOUZA; FREITAS; STORPIRTIS, 2007; SUAREZ-SHARP et al., 2018).

Através da correlação dos dados obtidos nos perfis de dissolução (*in vitro*) e aqueles obtidos através de estudos farmacocinéticos (*in vivo*), é possível estabelecer modelos matemáticos e, posteriormente, aplicá-los para predição do comportamento *in vivo* de uma formulação através dos seus dados *in vitro*. Denominada correlação *in vitro - in vivo* (CIVIV), esta ferramenta tem sido cada vez mais buscada por pesquisadores acadêmicos e de indústrias farmacêuticas com objetivo de dar mais celeridade, assertividade e, principalmente, redução nos custos de desenvolvimento de um medicamento genérico (CAMPOS et al., 2011; CARDOT; DAVIT, 2012; CARDOT; GARRAY; BEYSSAC, 2011; EMAMI, 2006).

1.1. Histórico, Bases Legais e Impacto Socioeconômico dos Medicamentos Genéricos no Brasil

Três episódios históricos podem ser, didaticamente, destacados como o ponto de partida para a evolução dos requisitos técnicos e regulamentação de medicamentos genéricos no Brasil. De maneira cronológica, o primeiro a ser citado é a criação da Política Nacional de Medicamentos (PNM) através da publicação da Portaria n° 3.916, de outubro de 1998 (BRASIL, 1998). Como segundo episódio, decorrente de uma das prioridades da PNM, a criação da Agência Nacional de Vigilância Sanitária (ANVISA), através da Lei N° 9.782, de janeiro de 1999, proporcionou ao país as condições legais para implementação de ações destinadas à defesa da saúde das pessoas, bem como conferiu autarquia em regime especial à Agência, assegurando as prerrogativas necessárias ao exercício adequado de suas atribuições (BRASIL, 1999a). Outro momento histórico foi a promulgação da Lei N° 9.787, de fevereiro de 1999, comumente chamada de "Lei dos Genéricos", que estabeleceu as bases legais para a instituição do medicamento genérico no país, bem como o prazo para a ANVISA regulamentar os critérios para o registro e controle de qualidade desta categoria, as provas de biodisponibilidade de produtos farmacêuticos, a aferição da equivalência terapêutica para caracterização de sua intercambialidade, entre outros (BRASIL, 1999b). Assim, o primeiro instrumento publicado neste sentido foi a Resolução N° 391, de agosto de 1999 (ARAÚJO et al., 2010; BRASIL, 1999c). Desde então, os requerimentos para as provas de biodisponibilidade relativa e bioequivalência para fins de registro e pós-registro de medicamentos genéricos evoluíram consideravelmente, muitos deles estando atualmente harmonizados aos mais elevados padrões internacionais.

Além do desenvolvimento regulatório e a criação dos requerimentos técnicos para assegurar a qualidade, eficácia e segurança dos medicamentos genéricos que todos estes importantes episódios históricos proporcionaram, é importante destacar o aspecto socioeconômico que a inclusão desta categoria trouxe ao país. A partir da criação da "Lei dos Genéricos", observou-se uma expansão significativa das indústrias farmacêuticas nacionais e, consequentemente, o aumento do número de apresentações disponíveis no mercado nesta categoria. Em função disso e, também, da possibilidade de intercambialidade com o respectivo medicamento de referência, o custo de tratamento aos pacientes foi reduzido significativamente, beneficiando, principalmente, aqueles que

dispensam quantias mensais dos seus rendimentos em tratamentos para doenças crônicas (exemplos: diabetes, hipertensão, hipercolesterolemia etc.) (BERTOLDI et al., 2016; 2019; FIUZA; CABALLERO, 2015). Por lei, o preço do medicamento genérico deve ser, pelo menos, 35% menor do que o valor do respectivo medicamento de referência (CMED, 2004), sendo que, diante da concorrência acirrada entre as empresas nesta categoria, essa diferença pode chegar até 80% (PRÓGENÉRICOS, 2019). De maneira complementar, a entrada no mercado da versão genérica de um medicamento pressiona o detentor do registro do medicamento de referência a reduzir o valor praticado para se manter competitivo (NISHIJIMA, 2008). Adicionalmente, vale destacar que o impacto econômico da entrada de medicamentos genéricos no mercado não se dá, exclusivamente, aos pacientes no momento de adquiri-los em uma farmácia, mas também ao Estado, que economiza nos processos licitatórios para distribuição através de programas assistenciais (BERTOLDI et al., 2016; EMMERICK et al., 2015). Nessa mesma linha, o Estado também se beneficia desta categoria atuando como fabricante de medicamentos considerados estratégicos para o sistema de saúde e, também, em classes terapêuticas que não são de amplo interesse comercial para as indústrias farmacêuticas (ex.: doenças tropicais negligenciadas) (OLIVEIRA; LABRA; BERMUDEZ, 2006).

Segundo anuário estatístico do mercado farmacêutico de 2018 publicado pela ANVISA, no mercado brasileiro, o crescimento e penetração dos medicamentos genéricos tem sido expressivo. A proporção desta categoria na quantidade total de apresentações comercializadas no país foi de 32,4%, 34,6% e 37,0% para os anos de 2016, 2017 e 2018, respectivamente. Com essa expressiva representação no mercado, os medicamentos genéricos atingiram a marca de, aproximadamente, 1,7 bilhão de embalagens vendidas em 2018, caracterizando-se, mais uma vez, como o tipo de medicamento mais comercializado no país. Segundo dados da Câmara de Regulação do Mercado de Medicamentos (CMED), em 2018, havia 91 empresas comercializando medicamentos genéricos no Brasil, envolvendo 499 princípios ativos e associações e 196 subclasses terapêuticas. Vale destacar que os dados mencionados são exclusivos da categoria de medicamentos genéricos. Quando esses dados são somados aos da categoria de medicamentos similares – hoje popularmente chamados de “genéricos de marca” (categoria esta que, atualmente, requer as mesmas provas de registro de um medicamento genérico), a proporção na quantidade total de apresentações comercializadas sobe para

68% (genéricos 37% e similares 31%) em 2018, representando o segundo maior faturamento do mercado farmacêutico brasileiro (ANVISA, 2019).

Considerando as projeções globais publicadas no relatório do IQVIA de janeiro de 2019, o Brasil ocupará, em 2023, a quinta posição no *ranking* dos maiores mercados farmacêuticos, atrás apenas dos EUA, China, Japão e Alemanha (IQVIA Institute, 2019). A tendência de expansão de mercado demandará da indústria farmacêutica de genéricos, nacional e multinacional, um grande empenho nos próximos anos a fim de atender a alta demanda oriunda, principalmente, do envelhecimento populacional de um país ainda muito desigual em termos de renda (CARDOSO; DIETRICH; SOUZA, 2021).

1.2. Regulamentação de Bioequivalência para Registro de Medicamentos Genéricos

Como mencionado anteriormente, a regulamentação de bioequivalência para registro de medicamentos genéricos no Brasil teve seu início em 1999. Após a promulgação da lei de criação da ANVISA, houve então a regulamentação da referida lei através da Resolução Nº 391/99. Nesse momento foram introduzidos os conceitos fundamentais para comprovação da equivalência terapêutica entre um medicamento genérico e o medicamento de referência, bem como regulamentou-se a necessidade da apresentação de um dossiê composto, entre outros requisitos, por provas de estabilidade, equivalência farmacêutica e bioequivalência para registro de um medicamento genérico no país (ARAÚJO et al., 2010; BRASIL, 1999c).

Abaixo foram listadas as definições vigentes dos termos biodisponibilidade, bioequivalência, medicamento de referência e medicamento genérico presentes no endereço eletrônico da ANVISA, publicadas em 21/09/2020 e atualizadas em 22/10/2020 (ANVISA, 2020):

> *Biodisponibilidade: indica a velocidade e a extensão de absorção de um princípio ativo em uma forma de dosagem, a partir de sua curva concentração/tempo na circulação sistêmica ou sua excreção na urina.*
>
> *Bioequivalência: consiste na demonstração de equivalência farmacêutica entre produtos apresentados sob a mesma forma farmacêutica, contendo idêntica composição qualitativa e quantitativa de princípio(s) ativo(s), e*

que tenham comparável biodisponibilidade, quando estudados sob um mesmo desenho experimental.

Medicamento de referência: é um produto inovador, registrado no órgão federal responsável pela vigilância sanitária e comercializado no País cuja eficácia, segurança e qualidade foram comprovadas cientificamente junto ao órgão federal competente por ocasião do registro, conforme a definição do inciso XXII, artigo 3º, da Lei n. 6.360, de 1976 (com redação dada pela Lei nº 9.787 de 10 de fevereiro de 1999). A empresa interessada em registrar medicamentos genéricos e/ou similares deverá utilizar obrigatoriamente o medicamento de referência constante nas listas vigentes disponíveis nesta página (lista A e lista B) de acordo com os requisitos específicos da RDC 35 de 15/06/2012, que dispõe sobre os critérios de indicação, inclusão e exclusão de medicamentos na Lista de Medicamentos de Referência.

Medicamento genérico: é aquele que contém o mesmo princípio ativo, na mesma dose e forma farmacêutica, é administrado pela mesma via e com a mesma posologia e indicação terapêutica do medicamento de referência, apresentando eficácia e segurança equivalentes à do medicamento de referência podendo, com este, ser intercambiável. A intercambialidade, ou seja, a segura substituição do medicamento de referência pelo seu genérico, é assegurada por testes de equivalência terapêutica, que incluem comparação in vitro, através dos estudos de equivalência farmacêutica e in vivo, com os estudos de bioequivalência apresentados à Agência Nacional de Vigilância Sanitária. Os medicamentos genéricos podem ser identificados pela tarja amarela na qual se lê "Medicamento Genérico". Além disso, deve constar na embalagem a frase "Medicamento Genérico Lei nº 9.787/99". Como os genéricos não têm marca, o que você lê na embalagem é o princípio ativo do medicamento.

Passados mais de vinte anos após a primeira Resolução sobre o tema, observa-se que diversas alterações foram realizadas na legislação, muitas delas através da revogação de resoluções e a publicação de outras com textos atualizados, além da criação de notas

técnicas, instruções normativas, manuais, "perguntas & respostas", entre outros documentos que suportam o setor regulado no desenvolvimento e registro de medicamentos genéricos. Muitas dessas atualizações buscaram tornar a legislação equiparável às melhores práticas internacionais sobre o tema (CHAROO, 2020). Vale destacar que a ANVISA, como agente regulador, e as empresas farmacêuticas, como agente regulado, tiveram papeis fundamentais e conjuntos durante estes anos para este aprimoramento da legislação nacional.

Recentemente foi publicada a Consulta Pública Nº 760/19 a fim de revogar algumas resoluções e notas técnicas (BRASIL, 2019), merecendo destaque a revogação integral da RE nº 1.170, de 19 de abril de 2006 (BRASIL, 2006). A proposta de RDC, que teve seu período de contribuições encerrado em 7 de abril de 2020, trouxe como pontos principais: 1) a revisão geral da RE N° 1.170/06 para atualizar os conceitos e critérios estabelecidos, 2) a inclusão de detalhes para condução dos estudos de adesividade e irritabilidade para medicamentos transdermicos, 3) o requerimento para condução de estudos farmacodinâmicos para corticoides de uso tópico, 4) o estreitamento do intervalo de aceitação dos critérios de bioequivalência para medicamentos de índice terapêutico estreito, 5) os critérios para ampliação do intervalo de aceitação dos critérios de bioequivalência para fármacos de alta variabilidade, 6) a necessidade de condução de estudos em doses múltiplas para formulações de liberação modificada com tendência a acumular, 7) a adoção do parâmetro área sob a curva parcial (ASCp) como desfecho primário para estudos de bioequivalência de formulações de liberação modificada, entre outros (BRASIL, 2019). O período de contribuições para esta proposta de RDC contou com diversas discussões técnicas a fim de convergir o entendimento do setor regulado, entidades farmacêuticas e a própria ANVISA, bem como buscar uma harmonização dos requisitos técnicos aos melhores padrões internacionais. Aliado a isso, considerou-se também o importante papel socioeconômico que os medicamentos genéricos representam para a população brasileira, principalmente considerando-se a possibilidade de acesso a tratamentos pioneiros oriundos de formulações de liberação modificada. Até o momento de redação desta tese, as contribuições do setor regulado e entidades farmacêuticas estavam sob avaliação da Coordenação de Equivalência Terapêutica (CETER) da ANVISA para finalização do texto da RDC e, posteriormente, o documento seria apreciado pela Diretoria Colegiada.

1.3. Pesquisa e Desenvolvimento de Medicamentos Genéricos

No contexto nacional, a instituição da "Lei dos Genéricos" teve papel fundamental no desenvolvimento da indústria farmacêutica brasileira. Pode-se dizer que, além do capital financeiro gerado durante estes mais de vinte anos, um dos principais ganhos foi o capital humano desenvolvido nacionalmente para atuar na indústria farmacêutica, principalmente, em atividades relacionadas às equipes de garantia da qualidade, assuntos regulatórios, analítica, galênica e pesquisa clínica/bioequivalência. Com o início dos medicamentos genéricos no país, as empresas farmacêuticas precisaram desenvolver seus profissionais para atender aos requerimentos técnicos ora regulamentados no país, bem como implementar equipes de Pesquisa & Desenvolvimento (P&D) para formular e avaliar seus produtos. Alinhado a isso, observou-se também a adequação da grade curricular dos cursos de graduação em farmácia (BRASIL, 2017) e pós-graduação de algumas universidades para atender a demanda crescente no país por profissionais qualificados para atuar nas diversas áreas da indústria farmacêutica e, também, em centros de bioequivalência e equivalência farmacêutica (ARAÚJO et al., 2010; FONSECA; SHADLEN, 2017; QUENTAL et al., 2008).

Conjuntamente ao desenvolvimento humano-profissional, o parque fabril farmacêutico sofreu uma significativa expansão quando comparado o cenário no início dos anos 2000 aos dias atuais. Nas últimas duas décadas foi possível observar o desenvolvimento expressivo dos complexos industriais farmacêuticos, principalmente, nas cidades de São Paulo (e região metropolitana), Campinas (e região) e Anápolis, regiões estas que, atualmente, comportam as plantas produtivas e centros de P&D de grande parte das indústrias farmacêuticas nacionais e multinacionais. Vale mencionar também que o rendimento financeiro proporcionado por meio da comercialização de medicamentos genéricos permitiu que elas expandissem seus portfólios nessa categoria e alocassem uma parte dos seus recursos em P&D não só no desenvolvimento de genéricos, mas também em inovações incrementais (novas formas farmacêuticas, novos sistemas de liberação etc.) e em casos pontuais de investimento em inovações radicais (novas moléculas) (SINDUSFARMA, 2018, 2020; TIGRE; NASCIMENTO; COSTA, 2016).

Pelo fato de a margem de lucratividade ser menor para os medicamentos genéricos, a competividade nesta categoria é determinada através do desconto praticado frente ao preço do medicamento de referência, do custo de produção, da estratégia comercial direcionada aos canais de distribuição (farmácias, drogarias, instituições públicas etc.) e,

principalmente, da posição de chegada no mercado (NISHIJIMA; BIASOTO JR; LAGROTERIA, 2014; SILVA; BORJA, 2019). Este último se refere ao momento em que uma indústria farmacêutica consegue lançar o produto e gozar de certa vantagem comercial por ser um dos primeiros *players* a chegar no mercado. Assim, as estratégias das áreas de negócios, marketing, industrial e P&D devem estar completamente alinhadas, tendo como premissas o prazo de expiração das patentes atreladas a um determinado produto (patente do IFA e/ou patente da formulação e/ou patente de processo), o tempo de desenvolvimento da formulação, o prazo para realização das provas obrigatórias de acordo com os requerimentos vigentes, tempo de análise pelo órgão regulador, a expectativa para lançamento do produto e os possíveis competidores que entrarão juntamente no mercado. No contexto de P&D, a celeridade, assertividade e custos vinculados são fundamentais para que a empresa tenha sucesso na chegada ao mercado como um dos primeiros *players*, visto que nesse processo pode haver resultados negativos que impactam diretamente na etapa final, o lançamento do produto no mercado.

Do ponto de vista técnico, a P&D de medicamentos genéricos deve permear em um ambiente multidisciplinar, composto por profissionais com visão técnica e do negócio. Cada empresa distribui seu efetivo de funcionários conforme a demanda das áreas e as especialidades requeridas de acordo com atividades a serem exercidas, podendo esse efetivo variar caso haja variação no número de projetos. De maneira geral, um centro de P&D de uma indústria de genéricos será composto, direto e indiretamente, pelo trabalho das equipes analítica, galênica, regulatória, gerenciamento de projetos, comercial, comércio exterior, suprimentos, pesquisa clínica/bioequivalência e garantia da qualidade. Dentre essas, as de desenvolvimento analítico, desenvolvimento galênico (ou desenvolvimento de produto) e de pesquisa clínica/bioequivalência devem, constantemente, trabalhar de maneira integrada, desde a concepção do projeto, até as provas finais de desempenho da formulação (NIAZI, 2019; SHARGEL; KANFER, 2014). A seguir foram resumidas as principais atividades desenvolvidas por estas equipes na P&D de medicamentos genéricos:

> Desenvolvimento Analítico: equipes responsáveis pelo desenvolvimento e validação de diversas metodologias analíticas relacionadas aos processos de avaliação de desempenho e controle de qualidade da formulação e do IFA, avaliação da estabilidade de longa duração e acelerada do produto acabado, compatibilidades fármaco-excipiente e excipiente-excipiente, investigação de

impurezas e produtos de degradação do IFA e produto acabado, entre outras. Destacam-se os resultados dos ensaios de teor, dissolução, perfil de dissolução comparativo, estabilidade, tamanho de partícula e polimorfismo do IFA como estritamente relacionados ao desempenho *in vitro* e *in vivo* do produto acabado e que requerem avaliação multidisciplinar desde o início do projeto.

Desenvolvimento Galênico (ou Desenvolvimento de Produto): equipes responsáveis diretamente pelo desenvolvimento das formulações candidatas à medicamentos genéricos. Atuam desde o planejamento da fórmula e equipamentos produtivos, passando pela formulação de protótipos com variações de excipientes e parâmetros dos equipamentos produtivos em escala piloto, até a definição da fórmula final e escalonamento para as plantas industriais. Destaca-se a atenção dada por essas equipes na escolha dos excipientes a fim de modular a liberação do fármaco no organismo, a necessidade de ter formulações estáveis, com adequado "tempo de prateleira", bem como na definição de processos produtivos escalonáveis e robustos a fim de garantir o ciclo de vida do produto.

Pesquisa Clínica/Bioequivalência: equipes responsáveis pelo desenho, planejamento, contratação, gerenciamento, monitoria e interpretação dos estudos de bioequivalência (e outros estudos clínicos) patrocinados pela indústria farmacêutica e conduzidos em centros de bioequivalência certificados pela ANVISA. Os desenhos adotados para estes estudos, em grande parte, possuem desfechos farmacocinéticos que visam caracterizar a velocidade e a extensão de absorção do fármaco, a partir da administração dos medicamentos teste (candidato à genérico) e referência em seres humanos, e compará-los estatisticamente. Vale destacar que estes estudos devem cumprir com as normativas nacionais e internacionais de boas práticas clínicas e de laboratório, bem como serem aprovados previamente por um comitê de ética em pesquisa. Em alguns casos essas equipes também são responsáveis pelos estudos de equivalência farmacêutica e bioisenção patrocinados pela indústria farmacêutica e realizados em centros de equivalência farmacêutica certificados pela ANVISA.

Fundamentalmente, o trabalho integrado destas equipes tem como propósito desenvolver uma formulação que tenha desempenho *in vivo* semelhante, ou muito próximo, ao medicamento de referência, garantindo assim que sua eficácia e segurança sejam intercambiáveis. Apesar de constar na bula do medicamento de referência os

excipientes de maneira qualitativa, por questões de confidencialidade, não há o quantitativo de cada um deles na fórmula. Há também, em algumas situações, a proteção patentária da fórmula do medicamento de referência para determinadas proporções de excipientes, ou até mesmo do processo produtivo, o que impossibilita que o medicamento genérico contemple tais composições e processos (GUPTA et al., 2010). Daí parte o desafio galênico em desenvolver uma formulação com o mesmo desempenho *in vitro* e *in vivo* do medicamento de referência sem ser, exatamente, uma "cópia" quali e quantitativamente. Esse desafio se torna ainda mais complexo quando se trata de formulações de liberação modificada (comprimido de liberação prolongada, adesivos transdérmicos, comprimido de liberação bimodal etc.) que contemplem polímeros e outros excipientes moduladores da liberação do ativo.

Assim, neste contexto em que a competitividade por lançar medicamentos genéricos é cada vez mais acirrada e há a necessidade de tomada de decisão cientificamente embasada em um curto espaço de tempo, ferramentas como a CIVIV (KAUR et al., 2015), *Quality by Design* (QbD) (PRAMOD et al., 2016; YU et al., 2014) e *Design of Experiment* (DoE) (POLITIS et al., 2017) vem sendo adotadas por empresas farmacêuticas a fim de tornar o desenvolvimento de produtos mais assertivo.

1.4. Regulamentação da CIVIV no Brasil

A regulamentação vigente no Brasil relacionada a CIVIV é a RE N° 482, de março de 2002, que conta com o anexo intitulado "Guia para Estudos de Correlação In vitro – In vivo", o qual aborda cinco tópicos: 1) Introdução, 2) Níveis de CIVIV, 3) Desenvolvimento de uma CIVIV, 4) Estabelecimento dos limites de especificação da dissolução e, 5) Considerações gerais sobre medicamentos de liberação imediata (BRASIL, 2002). Apesar de ter sido publicada já nos primeiros anos após a promulgação da "Lei dos Genéricos", não se observou no cenário nacional uma ampla aplicação deste guia e o uso da CIVIV para fins regulatórios. Duas hipóteses podem ser levantadas para ocorrência deste fato. A primeira seria relacionada ao desconhecimento ou falta de habilidade do setor regulado com esse tipo de ferramenta e, consequentemente, o desencorajamento da sua implementação na rotina de P&D de medicamentos genéricos. A segunda e, talvez, mais importante é que, diferentemente dos guias americano (FDA, 1997) e europeu (EMA, 2014), na regulamentação nacional não é mencionada a

possibilidade de uso de uma CIVIV nível A para fins de bioisenção em caso de alterações pós-registro, por exemplo, de formulações de liberação prolongada. A regulamentação nacional se restringe a indicar a aplicabilidade de uma CIVIV ao estabelecimento dos limites de especificação do ensaio de dissolução e para controle interno para prever os efeitos de modificações no processo de fabricação (ex.: alterações menores de formulação, local de fabricação, equipamento, fornecedor de excipientes e de dosagem do fármaco). Assim, caberia uma atualização da regulamentação nacional a fim de revisar os conceitos vigentes, implementar novas orientações e abordagens baseadas na evolução da literatura sobre o tema e, conjuntamente, encorajar o setor regulado na aplicação desta importante ferramenta para a P&D de medicamento genéricos, englobando, se aplicável, estes estudos no dossiê regulatório do produto.

A nível global, uma iniciativa partindo do *The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use* (ICH) para criação de um guia específico e harmonizado sobre CIVIV seria de grande valia para o setor farmacêutico. Além de beneficiar os países que já possuem orientações sobre o tema no seu arcabouço regulatório, ajudaria também aqueles que não contam com orientações sobre o uso desta ferramenta no desenvolvimento de medicamentos.

1.5. CIVIV aplicada a P&D de Medicamentos Genéricos

Os ensaios *in vitro* prévios, realizados com o objetivo de selecionar o melhor protótipo de cada formulação para avançar para os estudos de bioequivalência, podem servir como ferramentas poderosas para as indústrias farmacêuticas, uma vez que buscam minimizar falhas nas últimas etapas de desenvolvimento, levando à redução no número de estudos clínicos, horas de trabalho das equipes e demais custos das etapas prévias à petição de registro. Neste cenário, pode-se destacar a avaliação de desempenho de formulações em meios de dissolução biorrelevantes e/ou biopreditivos (CARDOT; GARRAY; BEYSSAC, 2011; KAUR et al., 2015; ZABORENKO et al., 2019).

A mimetização dos compartimentos gastrointestinais humanos, através da realização de ensaios *in vitro*, tem sido explorada nos últimos anos, principalmente, com o advento de programas avançados de simulações computacionais, que possibilitam explorar, concomitantemente, diferentes variáveis e, também, decorrente da customização de meios de dissolução e aparatos para elevar o poder de biopredição dessas

metodologias. Dada a complexidade dos fenômenos biológicos, físicos e químicos do TGI, a mimetização destes ambientes em sua integralidade de variáveis se torna praticamente impossível. Markopoulos e colaboradores (2016) propuseram quatro níveis de simulação da composição luminal do TGI:

> *Nível 0*: simples soluções aquosas cujo pH é ajustado, geralmente, através de um tampão para representar o pH de um compartimento específico do TGI. Neste nível, a capacidade tamponante pode ou não ser fisiologicamente relevante. O objetivo principal é manter o pH constante durante o curso do experimento;
>
> *Nível 1*: tanto o pH quanto a capacidade tamponante são configurados, na medida do possível, para serem fisiologicamente relevantes.
>
> *Nível 2*: componentes biliares, lipídios dietéticos e outras substâncias essenciais para digestão são adicionados ao meio para refletir a capacidade de solubilização e osmolaridade dos fluidos luminais, principalmente, para mimetizar as diferenças na composição entre estado jejum e alimentado.
>
> *Nível 3*: corresponde as composições mais complexas. Ou seja, aquelas que envolvem proteínas, enzimas e efeitos da viscosidade na liberação do fármaco.

A decisão pelo nível a ser aplicado dependerá, principalmente, da finalidade do uso dos resultados dos perfis de dissolução nos meios mencionados e, também, da disponibilidade de recursos financeiros, tempo e capacidade de recursos humanos para reproduzir tais condições. Nos casos em que há a necessidade de correlacionar os dados de perfil de dissolução às variáveis *in vivo*, condições fisiologicamente relevantes devem ser buscadas (KLEIN, 2019; MARKOPOULOS et al., 2016).

No âmbito da indústria farmacêutica, as metodologias de dissolução para controle de qualidade não, necessariamente, são fisiologicamente relevantes. Em sua grande maioria, os métodos de dissolução disponíveis em compêndios oficiais foram desenvolvidos para detecção de variações no desempenho *in vitro* para fins de controle de qualidade (ex.: detectar desvios no processo produtivo), são os chamados métodos discriminativos. Raramente estas metodologias são desafiadas quanto a sua relevância clínica para prever possíveis alterações no desempenho *in vivo* do produto. Ou seja, se o método de dissolução para controle de qualidade não possui relevância fisiológica, diferenças observadas nos resultados de perfis de dissolução das formulações não

necessariamente se correlacionam com o comportamento *in vivo* dos produtos. Ou o caso inverso, semelhança nos perfis de dissolução não, necessariamente, corresponderia a desempenho *in vivo* semelhante entre as formulações. Desse contexto parte a importância de se ter meios biopreditivos e de se estabelecer uma CIVIV para o desenvolvimento de um medicamento genérico, bem como para fins de investigação do impacto *in vivo* de alterações pós-registro. Se a metodologia de dissolução apresenta capacidade discriminativa e biopreditiva, isso servirá como uma poderosa ferramenta para detecção de lotes bioinequivalentes no controle de qualidade (GRAY, 2018; KLEIN, 2019; SUAREZ-SHARP et al., 2018).

De maneira conceitual, a CIVIV é definida como um modelo matemático preditivo capaz de descrever a relação entre uma propriedade *in vitro* (ex.: fração dissolvida) e uma resposta biológica relevante *in vivo* (ex.: fração absorvida do fármaco). Comumente, os dados para construção desses modelos matemáticos são provenientes de ensaios de perfis de dissolução (porcentagem dissolvida do fármaco vs tempo) e estudos farmacocinéticos (concentrações plasmáticas *vs* tempo). Os dados *in vivo* são tratados, geralmente, por técnicas de deconvolução (*Wagner-Nelson*, *Loo-Riegelman*, entre outros) para obtenção da fração absorvida; enquanto os dados de fração dissolvida são trabalhados para encontrar o modelo cinético (*Weibull, Higuchi, Hixson-Crowell*, entre outros) mais adequado para modelar o perfil de liberação *in vitro* do fármaco a partir da sua forma farmacêutica. Do ponto de vista de bioequivalência, a predição dos parâmetros *Cmáx* (concentração plasmática máxima do fármaco - representando a velocidade de absorção do fármaco no organismo) e *ASC* (área sob a curva - descrevendo a extensão de absorção do fármaco no organismo) é considerada de grande relevância para fins de comparabilidade *in vitro* entre o medicamento de referência e a formulação teste (candidata à medicamento genérico), uma vez que estes parâmetros são considerados como desfechos primários na avaliação estatística para conclusão sobre a bioequivalência entre formulações (CAMPOS et al., 2011; CARDOT; DAVIT, 2012; CARDOT; GARRAY; BEYSSAC, 2011; EMAMI, 2006).

Apesar da importância da aplicação da CIVIV no desenvolvimento de medicamentos genéricos, nota-se, na literatura, ainda pouca harmonização nos conceitos e critérios aplicados, bem como dados escassos para determinados fármacos e formas farmacêuticas. Esse cenário remete a necessidade de mais estudos e publicações nesta

área, principalmente aquelas oriundas dos ambientes de P&D das indústrias farmacêuticas.

Considerando o cenário supracitado, esse trabalho foi divido em duas etapas; a primeira foi identificar e avaliar estudos publicados nas últimas décadas sobre CIVIV para formas farmacêuticas sólidas orais, apontando as principais características da área, bem como as necessidades de melhoria; a segunda parte foi dedicada a demonstrar a aplicação da CIVIV para duas formulações com diferentes fármacos, diferentes sistemas de liberação, diferentes abordagens e modelos de deconvolução e diferentes meios de dissolução. Ambas as etapas geraram publicações científicas em periódicos internacionais e, por isso, a tese foi dividida em capítulos para contemplar os artigos publicados conforme formatação recomendada no Manual para Normalização de Teses e Dissertações do Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco (RUIZ et al., 2010; USF, 2015).

No Capítulo I é apresentada uma revisão sistemática da literatura sobre o uso da CIVIV no desenvolvimento de formulações orais. Através dessa revisão foi possível avaliar um número significativo de artigos científicos disponíveis na literatura e identificar as principais características destes estudos, bem como fornecer uma avaliação crítica sobre as possibilidades de melhoria na área.

Nos capítulos II e III, a tese é dedicada a apresentar duas aplicações da CIVIV, as quais envolveram as formulações succinato de desvenlafaxina monoidratado 50 mg comprimido revestido de liberação prolongada (*Capítulo II*) e carbamazepina 400 mg comprimido (*Capítulo III*). Estes dois trabalhos foram publicados em parceria com colaboradores de universidades nacionais (Universidade Federal do Rio de Janeiro e Universidade Estadual de Campinas) e internacional (Universidad Miguel Hernández de Elche) que possuem ampla experiência nessa temática.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Esse trabalho teve como objetivo, após uma ampla revisão de estudos publicados nas últimas décadas sobre CIVIV, aplicar essa ferramenta na construção de modelos matemáticos para correlação dos dados de fração dissolvida e absorvida de duas formulações orais: succinato de desvenlafaxina monoidratado 50 mg comprimido revestido de liberação prolongada e carbamazepina 400 mg comprimido.

2.2. OBJETIVOS ESPECÍFICOS

- Avaliar de forma sistemática os principais trabalhos no período de 1998 a 2018 a respeito do uso da CIVIV no desenvolvimento de formulações orais e discutir sobre o cenário das últimas décadas sobre a aplicação desta ferramenta no desenvolvimento de formulações (*Capítulo I*);

- Estabelecer uma CIVIV de nível A entre os dados de dissolução (*in vitro*) e farmacocinéticos (*in vivo*) de duas formulações de succinato de desvenlafaxina 50 mg comprimido revestido de liberação prolongada, bem como avaliar a capacidade preditiva do modelo construído (*Capítulo II*);

- Estabelecer uma CIVIV entre os dados de dissolução (*in vitro*) e farmacocinéticos (*in vivo*) de duas formulações de carbamazepina 400 mg comprimido, proporcionando evidências adicionais a respeito de um meio de dissolução biopreditivo contendo 1% do tensoativo lauril sulfato de sódio (LSS) para predição do desempenho *in vivo* de formulações de carbamazepina. Além disso, explorar os modelos *one-step* e *two-steps* na construção da CIVIV, bem como a aplicação da abordagem de *time-scaling* para mensurar a diferença nas taxas de dissolução *in vitro* e *in vivo* (*Capítulo III*).

3. ARTIGOS PUBLICADOS

3.1. CAPÍTULO I

DAVANÇO, M. G.; CAMPOS, D. R.; CARVALHO, P. O. *In vitro - In vivo* correlation in the development of oral drug formulation: A screenshot of the last two decades. **Int. J. Pharm**., v. 580, 119210, 2020.

Este trabalho teve como objetivo realizar um levantamento de artigos publicados nas últimas duas décadas a respeito do uso da CIVIV no desenvolvimento de formulações orais, avaliá-los de maneira sistemática a fim de extrair as principais características dos estudos e, assim, traçar um cenário retrospectivo do uso desta ferramenta no desenvolvimento de formulações nas últimas décadas. Uma busca sistemática nas bases de dados *PubMed* e *Web of Science* foi realizada para recuperar artigos relatando o uso da CIVIV no desenvolvimento de formulações orais no período de 1998 a 2018. Os estudos qualificados foram resumidos com relação as informações sobre o IFA, classificação biofarmacêutica, forma farmacêutica, dados *in vitro* e *in vivo* utilizados, nível da CIVIV estabelecida, número de formulações utilizadas, presença de validação e capacidade de predição do modelo. Uma robusta discussão foi suportada por esses dados, o que permitiu abordar de forma ampla os pontos fortes e fracos desta área considerando o período avaliado. Além disso, um banco de dados foi apresentado no artigo contemplando diferentes modelos de CIVIV, com diferentes IFAs e formas farmacêuticas, provendo, assim, uma fonte de informações para pesquisadores interessados na área.

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Mini review

In vitro – In vivo correlation in the development of oral drug formulation: A screenshot of the last two decades

Marcelo Gomes Davanço*, Daniel Rossi Campos, Patrícia de Oliveira Carvalho

Universidade São Francisco, Programa de Pós-graduação Stricto Sensu em Ciências da Saúde, Bragança Paulista, São Paulo, Brazil

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ABSTRACT

In vitro – in vivo correlation (IVIVC) allows prediction of the *in vivo* performance of a pharmaceutical product based on its *in vitro* drug release profiles and can be used to optimize formulations, set dissolution limits, reduce the number of bioequivalence studies during product development, and facilitate certain regulatory decisions. This review article aimed to assess papers published in the last two decades regarding the use of the IVIVC in the development of oral formulations, to demonstrate the scenario in this area, as well as to describe the main characteristics of the assessed studies. A systematic search of *PubMed* and *Web of Science* databases was conducted to retrieve articles reporting the use of the IVIVC in the oral formulation development in the period from 1998 to 2018. The qualified studies were abstracted regarding drug name, dosage form, BCS class, *in vitro* and *in vivo* data, level of IVIVC, number of formulations, presence of the validation and predictability. The discussion was supported by these data, which allowed to address broadly strengths and weaknesses in this area. Moreover, a large database has been described in this article containing different IVIVC models, with different substances, providing support to scientists interested in this area.

1. Introduction

Worldwide, the pharmaceutical industry and regulatory agencies have continuously worked to reduce the time for approval of a new pharmaceutical product, reduce the cost of development to maximize the return on investment, and to improve the access of the patients, especially for generic drug products. In the last two decades, the pharmaceutical industry has experimented and adopted several integrated and multidisciplinary approaches to achieve a more rational and assertive development flow. In the development of oral drug formulations, these efforts were mainly in the use of tools such as Quality by Design (QbD) (Yu et al., 2014; Pramod et al., 2016), Design of Experiments (DoE) (N. Politis et al., 2017), *In vitro – In vivo* Correlation (IVIVC) (Kaur et al., 2015), and the use of the Biopharmaceutical Classification System (BCS) in the adoption of biowaiver approaches to register some products (Davit et al., 2016).

The development and optimization of a pharmaceutical product involves varied levels of selection of excipients, processes, manufacturing equipment, development and validation of analytical methods to assess the *in vitro* performance and quality attributes, as well as expensive *in vivo* studies to asses efficacy and safety (Pramod et al., 2016). Considering that quantitative and/or qualitative changes in a formulation may affect drug release and its performance *in vivo*, impacting directly on its efficacy and safety (Yousefi et al., 2017), pharmaceutical development must be considered a complex process that requires a systematic approach based on multidisciplinary contributions. Moreover, with ever increasing pressures to reduce the timeline of product development, it is necessary an integrated work of scientists from the analytical, galenic, clinical and other pharma teams to ensure the success of pharmaceutical products in all stages of the development.

In this context, IVIVC has been used as a powerful tool for establishing a rational relationship between *in vitro* and *in vivo* characteristics. By definition, IVIVC is a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response, e.g. plasma drug concentration or amount of drug absorbed (González-García et al., 2015; Kaur et al., 2015; FDA, 1997). *In vitro* release is generally represented by dissolution profiles in biorelevant and/or bio-predictive media, and *in vivo* release is provided generally by pharmacokinetic studies. IVIVC constitutes an integral part of the development of a drug product, mainly for modified-release (MR) formulations, aiming to optimize prototypes, set dissolution limits, reduce the number of bioequivalence studies during the development and to support post-approval changes (components or composition,

* Corresponding author at: Universidade São Francisco, Programa de Pós-graduação Stricto Sensu em Ciências da Saúde, Avenida São Francisco de Assis, 214, Postal code 12916-900, Bragança Paulista, SP, Brazil.
E-mail addresses: davanco.marcelo@gmail.com (M.G. Davanço), camposrossi@gmail.com (D.R. Campos), patricia.carvalho@usf.edu.br (P.d.O. Carvalho).

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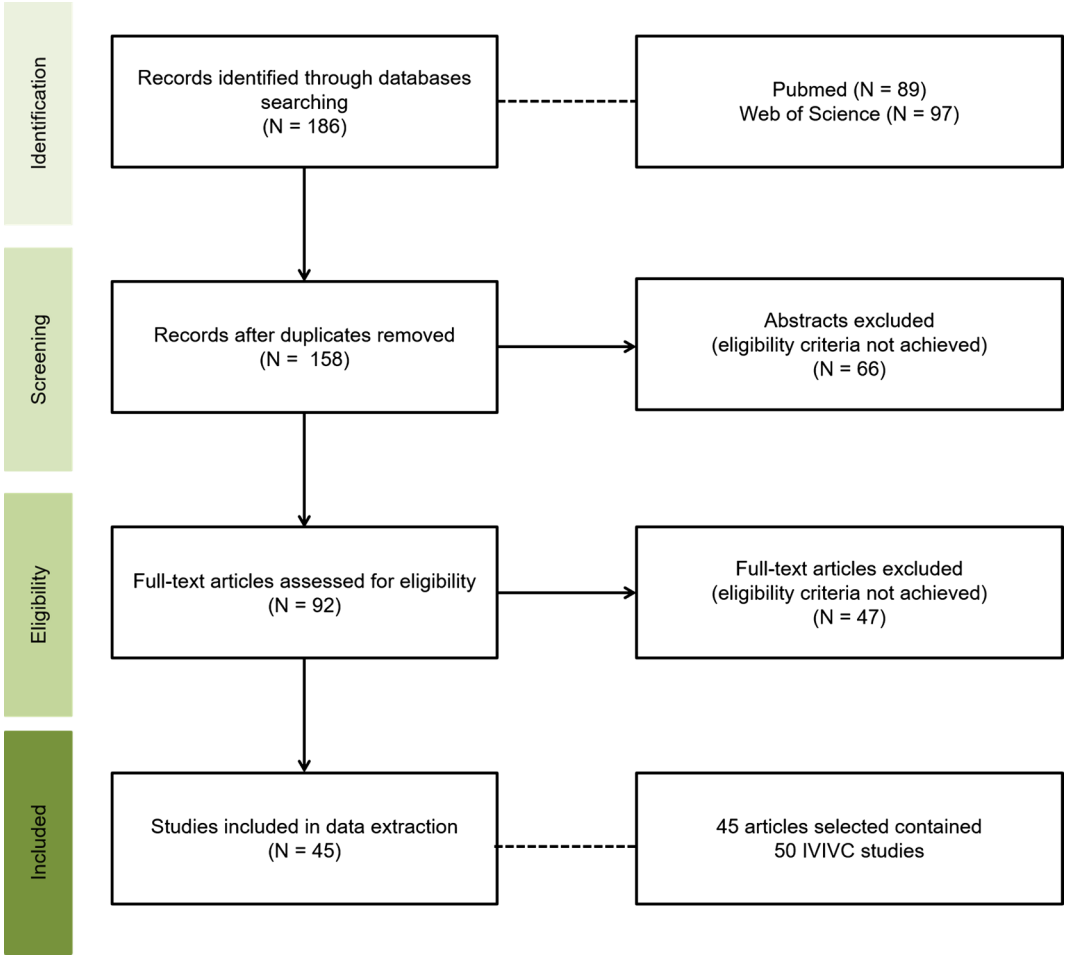

Fig. 1. PRISMA four-phase flow diagram for study retrieval and selection.

manufacturing site, scale-up/scale-down, manufacturing process or equipment) (Cardot et al., 2011).

Thus, the objective of this work was to assess how this tool has been used by scientists in the context of oral drug formulation development. For this purpose, papers published in the period from 1998 to 2018 related to the use of the IVIVC in the development of oral formulations were retrieved to compose a significant sample and demonstrate the scenario in this research field, as well as the main characteristics of these studies. Based on that, it was possible to carry out a broad discussion regarding the main concepts applied in the use of the IVIVC during the period searched. Finally, this article provides a large compilation of information on different drugs regarding the application of IVIVC, and it may be consulted by scientists interested in this approach for oral formulation development.

2. Methodology

2.1. Database search strategy

In April 2019, a systematic search of *PubMed* and *Web of Science* databases was conducted to retrieve all articles reporting IVIVC in the development of oral drug formulations considering the period from 1998 to 2018. Search terms including '*in vitro in vivo* correlation', 'oral dosage form', and 'product development' combined with the Boolean operator "AND", were applied for all database fields. Restrictions were applied to article language (only in English) and period (articles published from January 1998 to December 2018 were considered). The search was set to include the twenty-year period after the publication of the FDA Guidance for Industry 'Extended Release Oral Dosage Forms: Development, Evaluation, and Application of *In vitro/In vivo* Correlations' (FDA, 1997).

2.2. Eligibility criteria

The titles and abstracts of retrieved studies were firstly examined under a double check. In cases that it was not possible to identify the eligibility criteria only by evaluation of the title and abstract, the full text was evaluated for inclusion or rejection of the retrieved studies. Inclusion criteria were as follows: an article with original research, published from January 1998 to December 2018, in the English language, accessible in the database, and involving the application of IVIVC for development of oral drug formulations. The articles describing IVIVC application for non-oral formulations were rejected. Considering articles selected based on inclusion criteria, the full text was examined for an overall assessment and included in data extraction. In order to report numbers of studies screened, assessed for eligibility, and included in the review, a PRISMA four-phase flow diagram (Moher et al., 2009) of information was constructed through the different phases of systematic review.

2.3. Data extraction and analysis

The included articles were analyzed, and the following data extracted: drug/substance name, dosage form, BCS class of drug (when available in the article or consulted in the Drug Delivery Foundation database (available in http://www.ddfint.org/bcs-about), *in vitro* and *in vivo* data used for IVIVC, IVIVC level, number of formulations used in IVIVC, and presence of validation and predictability of IVIVC (internal

Table 1
Data extraction from the articles included involving IVIVC studies for oral formulations (N = 45 articles and 50 studies).

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
Alfuzosin hydrochloride	ER tablet	II	Data: fraction permeated Apparatus: USP II (paddle) at 50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8 Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method Design: PK study in beagle dogs (N = 6) under fasting condition.	A	One formulation (ER tablet marketed)	ND	ND	(Li, et al., 2018)
Aminophylline/ Theophylline	MR tablet	III/I	Data: fraction dissolved. Apparatus: USP II (paddle) at 50 rpm. Medium: pH 1.2 SGF without pepsin (SGF) (1 h) and pH 7.5 SIF without enzyme (2 – 8 h). Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method. Design: PK study in rabbits (N = 4).	A	Three tests and two reference formulations.	Internal validation	The %PEs of Cmax and AUC evaluated.	(Petrovic, et al., 2013)
Apremilast	ER tablet	IV	Data: fraction dissolved. Apparatus: USP II (paddle) at 50 rpm. Medium: pH 6.8 50 mM phosphate buffer. Volume: 900 mL.	Data: fraction absorbed. Deconvolution: numerical deconvolution and Wagner-Nelson method. Design: PK study in beagle dogs (N = 6) under fasting condition.	A	Two formulations: fast and slow.	Internal validation	The %PEs of Cmax and AUC evaluated.	(Tang, et al., 2016)
Arundic acid	Soft-gel capsule	ND	Data: fraction dissolved Apparatus: USP II (paddle). Media: 50 mM Na_2HPO_4 + 25 mM citric acid pH 8.0 and pH 6.8 dissolution medium + 2% SDS. Volume: 900 mL	Data: simulated fraction absorbed. Deconvolution: ND Design: plasma concentration data were deconvoluted by i.v. study.	A	Two formulations	Internal validation	The %PEs of Cmax and AUC evaluated.	(Nishimura, et al., 2007)
Capecitabine	ER tablet	I	Data: fraction dissolved (Higuchi modelling) Apparatus: USP II (paddle) at 50 rpm. Medium: water Volume: 900 mL	Data: 12 h *in vivo* release profile. Deconvolution: NA Design: PK study in patients (data from another article).	NA	One formulation	ND	ND	(Meulenaar, et al., 2014)
Capsaicin	Liposome	ND	Data: fraction dissolved. Apparatus: dialysis bag immersed in a single-neck flask and shaken at 70 rpm. Media: pH 7.4 phosphate buffer solution, pH 1.2 HCl solution and water. Volume: 100 mL	Data: absorbed *in vivo* input Deconvolution: ND Design: PK study in rats under fasting condition (N = 3).	A	One formulation	ND	ND	(Zhu, et al., 2015)
Carbamazepine	Nanodispersion	II	Data: fraction permeated. Apparatus: non-sink membrane permeation dissolution method. Media: Simulated gastric and intestinal fluids. Volume: 1 L	Data: cumulative AUC values. Deconvolution: Wagner-Nelson method. Design: PK study in in mice.	C	Three formulations: microamorphous, nanoamorphous, and nanocrystalline.	ND	ND	(Warnken, et al., 2018)
Cilostazol	IR tablet	II	Data: fraction dissolved in 4 h. Apparatus: USP IV (flow-through cell) apparatus closed-loop system. Medium: water (fasting condition) and 0.20% SLS (fed condition). Volume/flow: 20L at 4 mL/min.	Data: fraction absorbed in 4 h. Deconvolution: numerical deconvolution method. Design: bioavailability study in beagle dogs (N = 4) under fasting and fed condition.	C	Two formulations: wet-milled tablet and commercial tablet.	ND	ND	(Jinno, et al., 2008)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
Cyclosporine	Self-microemulsifying drug delivery contained in soft-gelatin capsule	II	Data: fraction dissolved Apparatus: USP II (paddle) apparatus at 50 rpm. Media: SGF without enzymes (pH 1.2), pH 4.5 phosphate buffer and SIF without enzymes (pH 6.8). Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method Design: PK study in male dog (N = 6 per group) under fasting condition.	A	Three formulations: test, reference I and reference II.	ND	ND	(Yang, 2010)
Diclofenac sodium	ER tablet	II	Data: fraction permeated. Apparatus: USP II (paddle) at 50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8 Volume: 900 mL	Data: fraction absorbed. Deconvolution: Wagner-Nelson or Loo-Riegelman methods (not specified). Design: PK study in beagle dogs (N = 6) under fasting condition.	A	One formulation: ER tablet marketed	ND	ND	(Li, et al., 2018)
Dipyridamole	MR tablet (floating osmotic pump system)	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: 0.1 N HCl solution Volume: 900 mL	Data: fraction absorbed. Deconvolution: ND. Design: bioavailability study in Beagle dogs (N = 6) under fasting condition.	A	One formulation: floating osmotic pump system.	ND	ND	(Zhang Z, 2009)
Emedastine difumarate	CR tablet	ND	Data: fraction dissolved. Apparatus: USP IV (flow-through cell) apparatus. Media: pH 1.2, 0–3 h and pH 6.8, 3 – 24 h. Volume/flow: 2.5 mL/h.	Data: fraction absorbed Deconvolution: Wagner-Nelson method. Design: data not showed.	A	Two formulations: CR tablet coated with different compositions)	ND	ND	(Morita, et al., 2003)
Etodolac	CR pellets	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 100 rpm. Media: phosphate buffer solution (pH 7.4). Volume: ND.	Data: fraction absorbed. Deconvolution: ND. Design: PK study in male beagle dogs (N = 6) under fasting condition.	A	One formulation: CR pellets	ND	ND	(Zhang, et al., 2018)
Felodipine	Nanocapsule	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: 40.0% v/v methanolic phosphate buffer pH 6.5. Volume: 250 mL.	Data: fraction absorbed. Deconvolution: Wagner-Nelson, GastroPlus mechanistic absorption, and numerical deconvolution. Design: PK study in Sprague Dawley rats (N = 6) under fasting condition.	A	One formulation (optimized formulation)	Internal validation	The %PEs of Cmax and AUC evaluated.	(Geroge, et al., 2017)
Fenofibrate	IR formulations	II	Data: fraction dissolved. Apparatus: custom-made biphasic dissolution-partition test (USP IV combined with USP II at 60 rpm). Media: FeSSIF-V2/octanol. Volume: 250 mL.	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: two PK study in human subjects under fasting and fed conditions.	A	Three formulations individually (capsule, nano-tablet and hot melt extrusion tablet).	ND	ND	(Xu, et al., 2018)
Fenofibrate	Capsule and suspension	II	Data: dissolution efficiency (%). Apparatus: USP II (paddle) at 75 rpm).	Data: oral bioavailability. Deconvolution: NA. Design: oral bioavailability	C	Three formulations (reference product, capsule and suspension prototypes)	ND	ND	(O'Shea, et al., 2017)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
			Medium: FaSSIF. Volume: 500 mL.	study in male Landrace pigs (N = 6) under fasting condition.					
Flurbiprofen	Lozenges (acting in oral cavity)	II	Data: mass loss of the lozenges. Apparatus: custom-made apparatus for simulating the oral cavity. Medium: simulated saliva fluid pH 6.8. Volume: saliva flow at 10 mL/min and artificial tongue at 180 dpm.	Data: mass loss of the lozenge after determined time of suction. Deconvolution: NA. Design: healthy volunteers (N = 12).	A	Two formulations (Prototype I and II)	ND	ND	(Tietz, et al., 2018)
Ginsenosides (NGR1, GRg1 and GRb1)	Bio-adhesive pellets	ND	Data: fraction dissolved. Apparatus: USP I (basket) apparatus. Medium: phosphate buffer pH 7.4. Volume: 500 mL.	Data: fraction absorbed. Deconvolution: numerical deconvolution. Design: PK study in Sprague Dawley rats (N = 6).	A	One formulation	ND	ND	(LI, et al., 2017)
Gliclazide	Push-pull osmotic pump tablet	II	Data: fraction dissolved (zero-order modelling) Apparatus: USP II (paddle) apparatus (with sinker) at 100 rpm. Medium: phosphate buffer pH 7.4 with NaCl. Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method Design: PK study in dogs (N = 6) under fasting condition	A	Reference and test formulations	ND	ND	(Tang, et al., 2013)
Glipizide	ER tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: potassium phosphate buffer pH 6.8. Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method. Design: PK study in male pigs (N = 6).	A	One formulation (ER tablet marketed)	ND	ND	(Kulkarni, et al., 2012)
Indapamide hemihydrate	ER tablet	I	Data: fraction dissolved. Apparatus: USP I (basket) apparatus at 100 rpm. Medium: buffer solution pH 1.2, 4.5 and 6.8. Volume: 500 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method. Design: pilot and pivotal BE study (under fasting condition).	A (non-linear)	Three formulations (2 test and reference).	Internal validation	The %PEs of Cmax and AUC evaluated.	(Antovska, et al., 2017)
Isosorbide-5-mononitrate	SR tablet (monolithic osmotic pump tablet system)	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Media: water, 0.1 N HCl pH 1.2, and phosphate buffer solution pH 6.8. Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method Design: PK study in healthy male beagle dogs (N = 3 per group) under fasting condition.	A	2 formulations: test and the marketed SR tablet	ND	ND	(Duan, et al., 2009)
Isosorbide-5-mononitrate	ER tablet	I	Data: fraction permeated Apparatus: USP II (paddle) at 50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8 Volume: 900 mL	Data: fraction absorbed Deconvolution: Wagner-Nelson method Design: PK study in beagle dogs (N = 6) under fasting condition.	A	One formulation (ER tablet marketed)	ND	ND	(Li, et al., 2018)
Ketoconazole	Tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm.	Data: Cmax. Deconvolution: NA. Design: bioequivalence study	C	2 formulations: test and reference.	ND	ND	(Viçosa, et al., 2009)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
			Media: buffer solution pH 4.5 without surfactant. Volume: 900 mL	in healthy adult volunteers (N = 28).					
Ketoprofen	Time-adjustable pulsatile release tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 100 rpm. Media: pH 1.2 0.1 M HCl solution (acid phase) and pH 6.8 solution (basic phase). Volume: 250 mL (acid phase) and 750 mL (basic phase).	Data: fraction absorbed. Deconvolution: ND. Design: PK study in beagle dogs (N = 6) under fasting condition.	ND	One formulation	ND	ND	(Wang, et al., 2017)
Ketorolac tromethamine	Colon-targeted tablet	II	Data: fraction dissolved (Koresmeyer-Peppas model) Apparatus: USP I (basket) apparatus at 50 rpm. Medium: 2 h in 0.1 N HCl, 2 h in buffer pH 5.5 and phosphate buffer pH 7.4 up to 24 h. Volume: ND	Data: AUC value in each collect-time. Deconvolution: NA Design: PK study in healthy volunteers (N = 12) under fasting condition.	NA	One formulation: prototype of colon-targeted tablet	ND	ND	(Vemula & Veerareddy, 2013)
Lamivudine	Gastroretentive tablets (floating-bioadhesive formulation)	I	Data: % Dissolved, Log %Dissolved, MDT, T60%, T70%, T80%, T90%, Log T70% and Log T80%. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: SGF pH 1.2. Volume: ND	Data: % Absorbed, Log %Absorbed, MRT, Log Tmax, Log AUC, Log Cmax/AUC. Deconvolution: Wagner-Nelson method (for IVIVC level A). Design: PK study in rabbits (N = 6) under fasting condition.	A, B, C and multiple C.	Two formulations individually.	ND	ND	(Singh, et al., 2012)
Loxoprofen	ER tablet	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 100 rpm. Medium: 0.01 N HCl pH 2.0 and increased to pH 6.8. Volume: 750 mL (acid phase) and 1000 mL (basic phase).	Data: *in vivo* dissolution parameters obtained by POP-PK model. Deconvolution: NA Design: PK study in beagle dogs (N = 4 per group) under fasting condition.	A	Three formulations: fast, medium and slow.	Internal validation	The %PEs of Cmax and AUC evaluated.	(Kim, et al., 2017)
Metaxalone	IR tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: pH 4.5 dissolution medium containing 0.5% NaCl with 0.2% SLS. Volume: 900 mL.	Data: direct plasma concentration (single step method). Deconvolution: NA. Design: bioequivalence study in healthy volunteers (N = 18) under fasting condition.	A	3 formulations: test A, test B, and reference.	Internal validation	The %PEs of Cmax and AUC evaluated.	(Vuletić, et al., 2018)
Metoclopramide hydrochloride	SR tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 and 75 rpm. Media: pH 1.2, 4.5, 5.5, 6.8 and 7.4. Volume: 900 mL	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in healthy volunteers (N = 6) under fasting condition	A	Three formulations: fast, medium and slow.	Internal and external validation	The %PEs of Cmax and AUC evaluated.	(Narayanasamy & Shabaraya, 2017)
Metoprolol tartrate	ER tablet	I	Data: fraction permeated Apparatus: USP II (paddle) at	Data: fraction absorbed Deconvolution: Wagner-	A	One formulation (ER tablet marketed)	ND	ND	(Li, et al., 2018)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
			50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8 Volume: 900 mL	Nelson method Design: PK study in beagle dogs (N = 6) under fasting condition.					
Metoprolol tartrate	ER tablet	I	Data: fraction dissolved. Apparatus: USP I (basket) and II (paddle) apparatus at 150 and 50 rpm, respectively. Medium: phosphate buffer pH 6.8 Volume: ND	Data: fraction absorbed Deconvolution: numerical deconvolution approach. Design: bioavailability study in healthy subjects (N = 9) under fasting condition.	A	Three formulations: slow, moderate and fast.	Internal validation	The %PEs of Cmax and AUC evaluated.	(Eddington, et al., 1998)
Niacin	ER tablet	I	Data: dissolution time-point. Apparatus: USP II (paddle) apparatus (speed not informed). Medium: phosphate buffer pH 6.8 Volume: ND	Data: Cmax, AUC and total urinary excretion (for parent drug and metabolite). Design: PK study in healthy volunteers (N = 36) under fasting condition.	Multiple C	Three different prototypes (varying HPMC percentage)	Internal validation	The %PEs of Cmax and AUC evaluated.	(Kesisoglou, et al., 2014)
Niacin	SR tablet	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: phosphate buffer pH 6.8 Volume: ND	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in healthy male volunteer subjects (N = 18) in fasting condition.	A	Three formulations: A, B and marketed product.	ND	ND	(Turner, et al., 2004)
Nifedipine	ER tablet	II	Data: fraction dissolved. Apparatus: USP III (reciprocating cylinder) at 12–16 dips/min. Medium: FaSSIF-V2 and FeSSIF-V2. Volume: 235 mL.	Data: fraction absorbed. Deconvolution: deconvolution-based approach using the Phoenix software. Design: unpublished *in vivo* data.	ND	Two formulations with different release mechanisms (osmotic-pump and matrix-type)	ND	ND	(Andreas, et al., 2016)
Olmesartan medoxomil	Solid and liquid self-nanoemulsifying tablet	II	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: SGF (pH 1.2) containing 0.5% SLS. Volume: 1000 mL	Data: fraction absorbed. Deconvolution: ND. Design: PK study in male Wistar rats (N = 6 per group).	A	Two formulations individually.	ND	ND	(Beg, et al., 2016)
Oxycodone hydrochloride	CR tablet	IV	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50–150 rpm. Medium: pH 6.8 phosphate buffer. Volume: 900 mL	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in healthy male volunteers (n = 18) under fasting condition.	A	One formulation.	ND	ND	(Kim, et al., 2015)
Pregabalin	Gastro-floating SR tablet	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus with sinker at 100 rpm. Medium: SGF pH 1.2 without pepsin. Volume: 900 mL.	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in male beagle dogs (n = 5) under fed condition.	A	One formulation	ND	ND	(Qin, et al., 2018)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
Propranolol hydrochloride	ER tablet	I	Data: fraction dissolved. Apparatus: USP apparatus I (basket) at 100 rpm. Medium: pH 1.2 varying to 6.8. Volume: 900 mL	Data: fraction absorbed. Deconvolution: numerical deconvolution approach. Design: PK study in male beagle dogs (N = 6) under fasting condition.	A	Two formulations: slow and fast.	Internal and external validation.	The %PEs of Cmax and AUC evaluated.	(Cheng, et al., 2014)
Ritonavir	Tablet	II	Data: fractional partition. Apparatus: custom-made biphasic dissolution partition test. USP IV apparatus (flow through cell) combined with USP II (dual paddle) apparatus at 60 rpm. Medium: aqueous/octanol. Volume: 200 mL of aqueous media and 200 mL of octanol.	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: mean plasma drug concentration − time profiles based on scientific literature.	A	One formulation	ND	ND	(Xu, et al., 2017)
Ritonavir	Tablet	II	Data: fractional partition. Apparatus: USP IV apparatus (flow through cell) combined with USP II (dual paddle) apparatus at 50 rpm. Medium: aqueous/octanol. Volume: 59 mL of aqueous media and 50 mL of octanol.	Data: Relative bioavailability and AUC. Deconvolution: NA Design: PK study in a dog model (data published in another article).	C	Three formulations: commercial generic products.	ND	ND	(Xu, et al., 2018)
Silybin	Capsule filled with calcium-phosphate microparticles	ND	Data: fraction dissolved. Apparatus: USP I (basket) apparatus with dialysis bag at 100 rpm. Media: phosphate buffer solution (pH 7.4) and HCl solution (pH 1.2). Volume: 900 mL	Data: fraction absorbed. Deconvolution: ND Design: PK study in male beagle dogs (N = 2) under fasting condition.	A	One formulation	ND	ND	(Zhu, et al., 2016)
Sinomenine	ER tablet	I	Data: fraction permeated. Apparatus: USP II (paddle) at 50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8. Volume: 900 mL	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in beagle dogs (N = 6) under fasting condition.	A	One formulation (ER tablet marketed)	ND	ND	(Li, et al., 2018)
Sulfur	Nanoparticle	NA	Data: fraction dissolved. Apparatus: ND. Media: gastric simulated HCl buffer (pH: 1.2; for 2 h) and intestine simulated phosphate buffer (pH: 6.8; for 6 h) Volume: ND	Data: fraction absorbed Deconvolution: ND. Design: PK study in female New Zealand white rabbits (N = 9) under fasting condition.	A	One formulation	ND	ND	(Choudhury, et al., 2013)
Tanshinone IIA	Capsule filled with SR pellets	ND	Data: percentage dissolved in 4, 6, 8, 10, 12, and 24 h. Apparatus: USP I (basket) apparatus at 100 rpm. Medium: distilled water containing 0.5% SDS. Volume: 900 mL.	Data: AUC_{4h}, AUC_{6h}, AUC_{8h}, AUC_{10h}, AUC_{12h} and AUC_{24h}. Deconvolution: NA Design: PK study in healthy male New Zealand rabbits (N = 6) under fasting condition.	C	One formulation	ND	ND	(Liu, et al., 2012)

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Table 1 (*continued*)

Drug/substance name	Dosage form	BCS Class*	*In vitro* data used for IVIVC/ *In vitro* methodology	*In vivo* data used for IVIVC/*In vivo* study	IVIVC level	Number of formulations used for IVIVC	Validation of the IVIVC	Predictability	Reference
Theophyline	ER tablet	I	Data: fraction permeated. Apparatus: USP II (paddle) at 50 rpm + dissolution/absorption simulating system. Medium: pH 2.0 and pH 6.8. Volume: 900 mL	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: PK study in beagle dogs (N = 6) under fasting condition.	A	One formulation (ER tablet marketed)	ND	ND	(Li, et al., 2018)
Tramadol hydrochloride	CR microparticle and IR formulation	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: initial 2 h in pH 1.2, next 2 h in pH 4.5, then 2 h in pH 6.8 and finally in pH 7.4 (phosphate buffer) for subsequent 18 h. Volume: 900 mL.	Data: fraction absorbed. Deconvolution: Wagner-Nelson method Design: PK study in healthy male human volunteers (N = 24) under fasting condition.	A	Three formulations individually (two CR and one IR formulation)	ND	ND	(Naeem Aamir, et al., 2011)
Tramadol hydrochloride	SR tablet	I	Data: fraction dissolved. Apparatus: USP II (paddle) apparatus at 50 rpm. Medium: acidic medium. Volume: ND.	Data: fraction absorbed Deconvolution: Wagner-Nelson method. Design: PK study in rabbits (N = 9) under fasting condition.	A	One formulation	ND	ND	(Kotta, et al., 2014)
Valsartan	Nanoparticles	II	Data: dissolution efficiency. Apparatus: USP II (paddle) at 50 rpm. Medium: HCl solution (pH 1.2) and acetate buffer (pH 4.0). Volume: 900 mL	Data: Cmax and AUC. Deconvolution: NA. Design: PK study in male Sprague-Dawley rats (N = 5 per group) under fasting condition.	C	Four formulations	ND	ND	(Kim & Baek, 2014)
Vincamine	Prolonged-release coated pellets	ND	Data: fraction dissolved. Apparatus: USP IV (flow-through cell) apparatus open-loop system. Medium: variable pH range (1.2, 4.5, 6.9 and 7.5). Volume and flow: ND.	Data: fraction absorbed. Deconvolution: Wagner-Nelson method. Design: comparative bioavailability study (N = 16) in human subjects.	A	Three formulations: two tests and reference.	ND	ND	(Emara, et al., 2000)

and/or external predictions).

3. Results

In total, 186 records were retrieved from the two databases searched, 89 from *Pubmed* and 97 from *Web of Science*. Within the articles retrieved, 28 were duplicated in the two databases. Then, 158 articles were assessed to meet the eligibility criteria under a double check for abstract and full-text. Finally, 45 studies (containing 50 IVIVC studies) were included in data extraction, as described in Fig. 1 (PRISMA four-phase flow diagram).

To demonstrate all relevant information from the included articles, Table 1 was designed to present the following data: name of drug/substance, dosage form, BCS class of drug, *in vitro* and *in vivo* data used for IVIVC, methodology applied for dissolution test or other used to assess the *in vitro* characteristics, deconvolution method used to obtain fraction absorbed (when applied), *in vivo* study design and population, IVIVIC level achieved (A, B, C, multiple C or D), number of formulations applied in the IVIVC and presence or absence of validation (internal and/or external) and predictability.

Glossary for Table 1: AUC: area under the curve, BCS: biopharmaceutics classification system, Cmax: maximum plasma concentration, CR: controlled-release, ER: extended-release, FaSSIF: fasted state simulated intestinal fluid, FeSSIF: fed state simulated intestinal fluid, HPMC: hydroxypropyl methyl cellulose, IR: immediate-release, IVIVC: *in vitro – in vivo* correlation, MR: modified-release, MDT: mean dissolution time, MRT: mean residence time, NA: not applied, ND: not described, PE: prediction error, PK: pharmacokinetic, SDS: sodium dodecyl sulfate, SGF: simulated gastric fluid, SIF: simulated intestinal fluid, SR: sustained-release, T50% (or TXX%): time to reach 50% (or XX %) of dissolution, Tmax: time to reach maximum plasma concentration, USP: United States Pharmacopoeia.

4. Data analysis and discussion

The aim of this work was to demonstrate a screenshot of the last two decades regarding the use of IVIVC for the development of oral drug formulations and to have a broad and critical analysis and discussion based on the scenario found. For this purpose, a systematic search was carried out to retrieve articles published from 1998 to 2018 related to use of this tool. Considering the results obtained in this review, the topics below are discussed, aiming to demonstrate the main characteristics of the studies selected and to provide a critical analysis on how this tool has been applied in the last twenty years.

4.1. Dosage form and biopharmaceutics classification system

Oral route is the most frequently used way of drug administration, as well as the most convenient, economic and preferred by patients (Viswanathan et al., 2017). In conventional oral drug products such as IR formulations (e.g. tablets and capsules), no deliberate efforts are made to modify the drug release rate. Thus, IR products generally result in relatively rapid drug absorption and onset of clinical effects. In the other hand, MR dosage forms are formulated to achieve a desired therapeutic objective or better patient compliance. Types of MR drug products include delayed-release (DR) (e.g. enteric-coated), extended-release (ER or XR), sustained-release (SR), controlled-release (CR), and others (Viswanathan et al., 2017; Shargel et al., 2012). These definitions may have slight variations between countries and regulatory agencies.

Biopharmaceutics classification system (BCS) is often used to predict the *in vivo* behavior of oral formulations, essentially based on drug solubility and intestinal permeability extension (Amidon et al., 1995). BCS class I drugs are highly permeably and soluble substances; therefore, they depend only on the release rate of the dosage form for dissolution on the gastro-intestinal (GI) fluids and, then, permeate intestinal or stomach mucosae. For a BCS class I drug contained in an IR dosage form, gastric emptying is the only limiting factor for drug absorption, which would not enable prediction of the *in vivo* behavior based on *in vitro* assays. Therefore, for this case, the acronym "IVIVC" (quantitative correlations) is inappropriate and the other acronym, as IVIVR (*in vitro in vivo* relationship – qualitative correlations), should be considered, since this approach only provides a formulation rank order based on dissolution profiles, and is not useful for regulatory purposes. In general, IVIVC for IR dosage forms is more difficult to be achieved (Qiu & Duan, 2017; FDA, 1997).

In addition to BCS class I, in the group of highly permeable substances, BCS class II are drugs with good permeability but with poor solubility. Thus, in this case, the dosage form plays a key role to improve drug solubility and to control the release rate to promote the best condition for dissolution and, consequently, permeability. Sub-classifications for BCS class II have been proposed recently (IIa, IIb and IIc) (Tsume et al., 2014), since these substances are highly dependent on the characteristics of the drug in the physiological pH range (acidic, basic or neutral drugs), formulation factors and luminal environment (e.g. presence of food). This approach considers BCS class IIa as weak acid drugs, with good solubility in the small intestine and low solubility in the acidic stomach pH, while BCS class IIb is the classification for weak base drugs with low solubility in the small intestine and good solubility in the acidic stomach pH. Finally, BCS class IIc should be considered neutral drugs as their solubility is not influenced by the physiological pH range (Tsume et al., 2014).

BCS classes III and IV drugs are poorly permeable, and this is related to molecule characteristics, with no regard to the formulation. Thereby, for these classes, any *in vitro* simulation aimed at predicting the *in vivo* behavior is generally more difficult or limited.

On the other hand, since only the dissolved drug in luminal fluids may permeate the mucosa at the absorptive sites of the GI tract, both the solubility of the drug and the release/dissolution rate of the dosage form are crucial for the *in vivo* input rate. Considering that release/dissolution is a limiting factor able to be simulated through *in vitro* tests (e.g. dissolution profiles), an IVIVC successful is expected mainly for BCS classes I and II, since for these classes drug permeability is not a limiting factor (Cardot & Davit, 2012). In this sense, Table 2 describes a relationship regarding the dosage form and probability to obtain a powerful IVIVC for each BCS class.

To understand whether IVIVC studies published in the literature

Table 2
Relationship between dosage form, BCS class and IVIVC probability.

Dosage form	BCS class	IVIVC probability	Justification
MR	I	High.	Release/dissolution rate is the limiting factor.
	II	High.	Release/dissolution rate is the limiting factor.
	III	Limited.	Permeability is the limiting factor.
	IV	Limited.	Permeability is the limiting factor.
IR	I	Limited.	Gastric emptying is the limiting factor.
	II	High.	Release/dissolution rate is the limiting factor.
	III	Limited.	Permeability is the limiting factor.
	IV	Limited.	Permeability is the limiting factor.

MR: modified release; IR: immediate release; BCS: biopharmaceutics classification system. IVIVC: *in vitro in vivo* correlation.

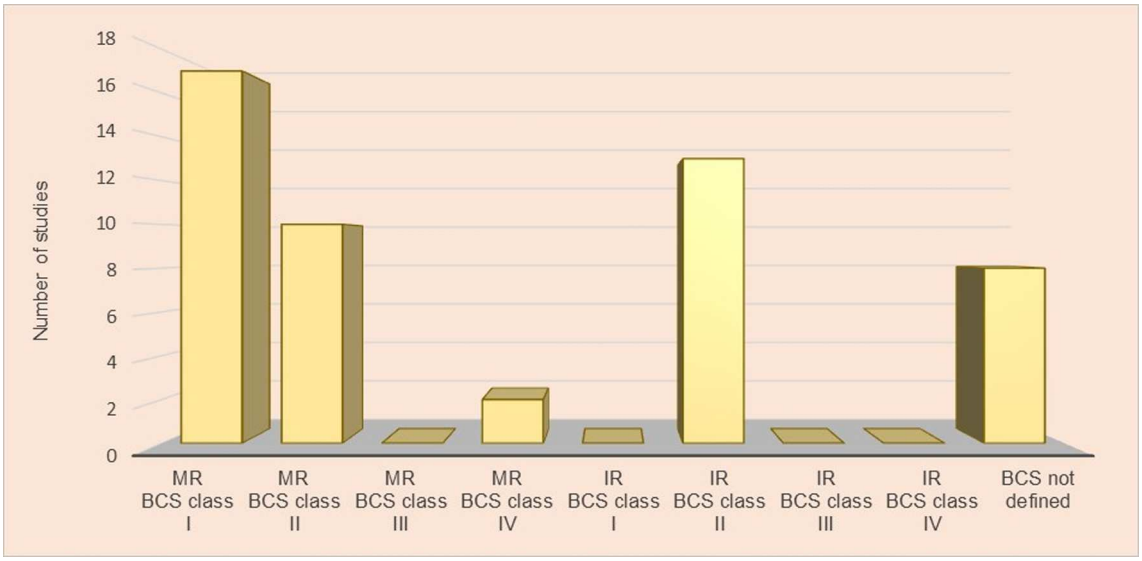

Fig. 2. Study distribution according to release dosage form and BCS class of drug (N = 50). BCS: biopharmaceutics classification system; MR: modified release; IR: immediate release.

(considering the period selected) match with the theoretical concepts to obtain a successful IVIVC, Fig. 2 was designed to show the distribution of dosage forms and BCS classes from the IVIVC studies assessed.

As demonstrated in Fig. 2, the MR dosage form has been the most applicable (54%) for IVIVC approaches, considering the articles assessed. In consensus with the IVIVC theoretical concepts (Qiu & Duan, 2017; Cardot & Davit, 2012; Kaur et al., 2015), BCS classes I (34%) and II (46%) have been the most usual classes used for IVIVC. Moreover, for IR dosage forms, only IVIVC studies with BCS class II drugs have been observed, and this is an important point that also matches with theoretical concepts described in the literature. In general, the articles assessed demonstrated that high permeability (BCS class I and II) drugs have been the main class applied for IVIVC approaches.

4.2. Dissolution media and apparatuses

For oral dosage form intended to drug absorption in GI tract, it is common to use dissolution media within the pH range of 1.2–6.8 to simulate the GI environment (stomach and intestine portions). Fasting and fed states also are important to set the adequate pH and dissolution media components (e.g. enzymes, salts, etc.) necessary to simulate these conditions. In addition, in the fed state, the delayed intragastrical dissolution caused by some food components may affect the absorption rates of drugs (especially poorly soluble drugs) and, subsequently, may influence its pharmacokinetics compared to the fasted state (Abrahamsson et al., 2004; Dressman et al., 2007). In this way, biorelevant and bio-predictive dissolution media for simulating stomach and small intestine, as well as conditions before and after meals, have been developed. The following examples may be cited: Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF), Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) (Nicolaides et al., 2001).

Among the retrieved articles, it was observed that the dissolution media in physiological pH range have been used as a single step or varying pHs in order to simulate different parts of GI tract (multiple steps). Single step dissolution medium was found in 24 studies (48%), while multiple steps approach was related in 13 of them (26%). Bio-predictive/bio-relevant media (SGF, SIF, FaSSIF and FeSSIF) were observed in 10 studies (20%) and water medium was related in 3 of them (6%). Even though single step approach has been found in the most of the studies and it could be related to the simplicity of this technique, multiple steps dissolution media in physiological pH range appeared in many studies, since this approach aims to simulate the different regions of the TGI tract and it could be more bio-predictive for some modified-release dosage forms (Li et al., 2018; Petrovic et al., 2013; Morita et al., 2003; Vemula & Veerareddy, 2013). The development and application of bio-predictive/bio-relevant media have been explored in the last two decades, mainly for a rational/assertive formulation development and, consequently, to save time and cost associated with pharmacokinetic and clinical studies (Klein, 2019; Klein, 2010).

Many articles have been published with different approaches regarding dissolution media and apparatuses to find the adequate condition to simulate the *in vivo* behavior for an oral dosage form. Conventional dissolution apparatuses such as USP I (basket) and USP II (paddle) are widely applied due to their practicality, availability in many laboratories, as well as the possibility to obtain expeditious results. However, in some cases, these apparatuses are not able to discriminate different formulations and to simulate conditions mimicking those *in vivo*. Thus, in some cases, USP III (reciprocating cylinder) and USP IV (flow-through cell) would be applied with rational protocols aiming to expose the dosage form to an environment potentially closer to that of the GI tract (Pezzini et al., 2015; Chevalier et al., 2009; Gao, 2009). USP IV has been widely recommended for poorly soluble drugs (Bhattachar et al., 2002), MR tablets (Andreas et al., 2015), and medical devices. Additionally, in the last years, non-conventional, custom-made and specific apparatuses have been developed to improve IVIVC approaches. TNO GI Model (TIM), a multi-compartmental model designed to realistically simulate conditions of the GI tract based on a computer simulation of the digestive conditions, is an example that demonstrates the advance of the dissolution apparatus in this area. Some systems have included a second stage in the apparatus, using an organic solvent or a membrane, for simulating the absorption process in the small intestine (Minekus, 2015).

Among the articles retrieved, the USP II (paddle) apparatus was the most applicable in the IVIVC studies assessed (52%, 26 of 50 studies) as shown in Fig. 3. Non-conventional or custom-made apparatuses were used in 24% of studies, followed by USP I (basket) in 14%, USP IV (flow-through cell) in 6% and USP III (reciprocating cylinder) in 2%.

These results demonstrated that the application of conventional basket (USP I) and paddle (USP II) apparatuses represents almost two thirds of the IVIVC studies assessed. As discussed previously, some characteristics allow these apparatuses to be widely applied for IVIVC, such as availability in many research laboratories and industries, easy handling, practicality and fast results. In many studies described in

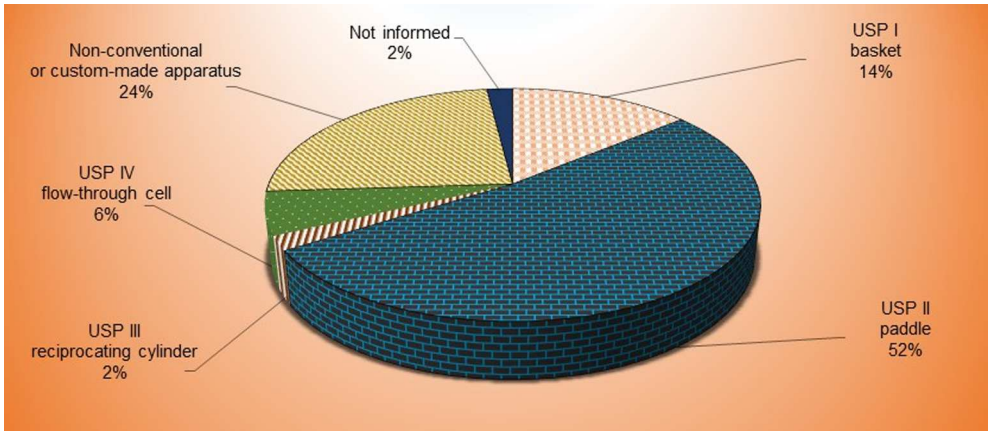

Fig. 3. Percentage distribution of dissolution apparatuses applied in the IVIVC studies retrieved (N = 50).

Table 1, it is possible to observe that the application of USP I and II is combined with dissolution media in the physiological pH range of GI tract. This approach has been used with physiological medium individually (Antovska et al., 2017), and as a combination of media with transition among physiological pH in the cube (e.g. acid phase for 1 h and basic phase for 24 h), mainly for MR formulations (Vemula & Veerareddy, 2013; Cheng et al., 2014).

Another point to be highlighted is the proportion (24%, 12 of 50 studies) of non-conventional and/or custom-made apparatuses applied for IVIVC. These systems are intentional changes made by scientists to mimic the *in vivo* condition. As examples, the article published by Li and collaborators described the application of a Drug Dissolution/Absorption Simulating System (DDASS) for IVIVC (Li et al., 2018); and the custom-made biphasic dissolution-partition test system, a combination of USP IV and II apparatuses, applied by Xu and collaborators in their works with different drugs (Xu et al., 2018; Xu et al., 2018; Xu et al., 2017).

By contrast, USP III and IV apparatuses were found only in 2 and 6% (Fig. 3), respectively, of the IVIVC studies assessed. It might indicate that these systems are not widely used either due to their unavailability in laboratories, or these methodologies are more complex and expensive than the others, since USP I and II are widely used also for quality control tests. Despite the low number of studies found with USP III and IV apparatuses, these systems have been extremely recommended for IVIVC approaches, mainly in the development of ER products (Andreas et al., 2015; Pezzini et al., 2015).

4.3. In vitro data

An important aspect in the development of pharmaceutical products is finding *in vitro* characteristics that reflect *in vivo* performance. Generally, the *in vitro* data used for an IVIVC are derived from curves obtained from dissolution tests performed in bio-predictive and bio-relevant media. Recent advances in dissolution methodologies coupled with the availability of sophisticated modeling software enabled dissolution testing to be used for both the IVIVC and quality control approaches (Klein, 2019; Cardot & Davit, 2012; Gray, 2018).

To study the *in vitro* release kinetics of a dosage form, it is recommended that data obtained from *in vitro* drug release studies have results at least 85% of drug released/dissolved, twelve individual values (with adequate variability between cubes) and sufficient number of points to elucidate the dissolution curve shape. Having these data, modeling the dissolution profile curve to characterize the rate of drug release/dissolution is possible by using a mathematical model. Moreover, for IVIVC purposes, in cases where the timepoints of dissolution profile are not the same as those obtained *in vivo*, data modeling allows reaching the results calculated in other timepoints to construct a point-to-point relationship. Mathematical models such as Weibull, Higuchi, Korsemeyer-peppas among others, are usually applied for dissolution profile modeling (Costa & Sousa Lobo, 2001; Siepmann & Siepmann, 2013).

In face of the data extracted from IVIVC studies selected, 35 (70%) used data from dissolution profiles (represented by the fraction dissolved) for correlation with *in vivo* data, while 7 applied the permeated fraction, since they used a dissolution/absorption simulating system. In three of them, dissolution timepoints were used individually to construct a IVIVC level with pharmacokinetic parameters (Kesisoglou et al., 2014; Singh et al., 2012; Liu et al., 2012). Two of them used fractional partitioning (based on an octanol/water dissolution medium) as *in vitro* data to correlate with *in vivo* fraction absorbed (Xu, et al., 2017; Xu, et al., 2017); and, in two other studies, dissolution efficiency data was used to correlate with *in vivo* predictions (Xu, et al., 2018; Kim & Baek, 2014). Finally, only in one study applied the *in vitro* mass loss of the lozenges to correlate with *in vivo* mass loss after determined time of mouth suction (Tietz, et al., 2018).

In conclusion, based on IVIVC studies assessed for oral formulations, the main *in vitro* parameter, which has been used to correlate with *in vivo*, is the fraction dissolved obtained from dissolution tests. This type of correlation seeks to establish a link between *in vitro* and *in vivo* dissolution, which would be directly related to the *in vivo* input rate of a drug, mainly for highly permeable substances.

4.4. In vivo data

Pharmacokinetic studies are the way to elucidate the behavior of a drug in the body when administered through a dosage form (Derendorf & Meibohm, 1999). Indeed, some characteristics of these studies should be adequately selected to have reliable results for IVIVC purposes. Adequate sample size, design, population, and bioanalytical method are fundamental characteristics to perform a pharmacokinetic study and to have useful data. Subjects should be standardized as much as possible and acceptable to minimize intra and inter individual variation (Nishant, et al., 2011; ATKINSON, 2007).

Generally, concentration-time profile generated from a pharmacokinetic study is treated previously to the IVIVC approach to relate directly to the *in vitro* release rate. First, *in vivo* data are converted to fraction of dose absorbed or fraction absorbed 'Fa', to have the "pure" absorption process and, consequently, to be possible correlate directly with *in vitro* release (fraction dissolved). In other words, it is necessary to "remove" the elimination process of the absorption curve (initial phase after administration), since the *in vitro* assay (e.g. dissolution test) does not predict the *in vivo* elimination rate of a drug. For oral formulations, the traditional deconvolution/convolution-based approach is the most common methodology to establish an IVIVC level A.

Wagner–Nelson (Wagner & Nelson, 1964) and Loo-Riegelman (Wagner, 1975) are model-dependent methods based on one and two compartments, respectively. Wagner-Nelson has the great advantage of not requiring additional *in vivo* data beyond oral plasma profile, while the Loo–Riegelman method requires intravenous dosing data. Numerical deconvolution is a model-independent method that requires *in vivo* plasma data from an oral solution, or intravenous, as the unit impulse response, UIR. All three methods have limitations, but the requirement of additional data in addition to oral plasma data (from tablet or capsule) significantly limits the application of the Loo-Riegelman and numerical deconvolution methods (Langenbucher, 2003; Margolskee et al., 2016). Wagner-Nelson, Loo-Riegelman and numerical deconvolution are considered conventional methods and widely applied for IVIVC models. Additionally, the mechanistic absorption method, based on physiologically *in silico* tools such as GastroPlus™, has been used to predict oral drug absorption, since this approach allows to estimate separately the different processes that are involved in drug systemic absorption: dissolution, permeation, GI transit time, gut wall metabolism and first pass metabolism (Sjögren et al., 2013; Lin & Wong, 2017; Pathak et al., 2017). Among the IVIVC studies assessed (N = 50), the most frequently applied model for deconvolution was Wagner-Nelson (26 studies), followed by numerical deconvolution (6 studies), Loo-Riegelman (one study) and mechanistic absorption model using GastroPlus™ software (one study). For the remaining studies, deconvolution model was either not described, or it was not applied. Considering the distribution of deconvolution models found in this review work, the choice for Wagner-Nelson reflects that this model has been widely applied for IVIVC purposes and it would confirm that scientists, when possible, opted for a simpler and more practical model to deconvolute *in vivo* data.

Regarding the population applied for IVIVC purposes, Fig. 4 represents the distribution of *in vivo* models found in the assessed studies. As observed, dogs and humans have been the main models applied for IVIVC, responding for 19 and 14 studies assessed, respectively (N = 50). These categories were followed by rats (6), rabbits (5), and pigs (2). In 4 of them, the *in vivo* model was not declared.

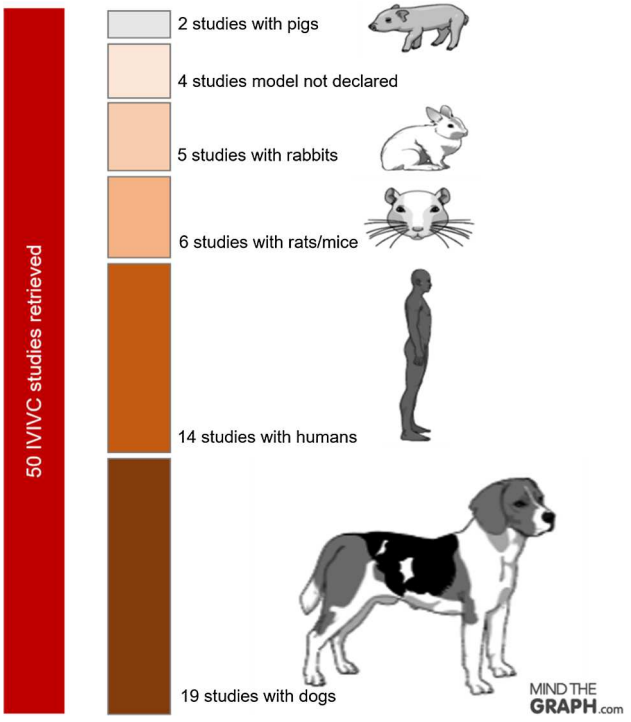

Fig. 4. Distribution of the *in vivo* models applied in the IVIVC studies retrieved (N = 50).

Unexpectedly, animal models (dog, rat, rabbit, and pig) represented 64% of the IVIVC studies assessed in the literature sampling assessed. Two main reasons may be discussed regarding this scenario. Firstly, the majority of IVIVC studies (published in literature) are being accomplished just for early formulation development, and not for regulatory purposes. Secondly, and in contrast, many studies with humans, with regulatory purposes and for clinical formulation development, are not published due to confidential and strategic reasons from the pharmaceutical industry, as well as lack of encouragement and/or interest of scientists for publication of the data. Other important reasons may be mentioned, such as high costs and large amounts of time to have a validated IVIVC in humans.

4.5. IVIVC level, number of formulations, validation and predictability

Levels A, B, and C IVIVC are clearly defined in some regulatory guidelines (EMEA, 1999; FDA, 1997). Level A, as a point-to-point correlation, represents the most informative class of correlation that considers complete *in vivo* and *in vitro* profiles. Based on FDA guidance (FDA, 1997), an IVIVC may be defined with a minimum of two formulations with different release rates (e.g. highest and lowest release rate formulations); three or more formulations with different release rates are recommended. In addition, the IVIVC should be evaluated to demonstrate its capacity to predict the *in vivo* performance of an oral drug formulation based on its *in vitro* dissolution characteristics and whether this model is maintained over a range of *in vitro* dissolution release rates and manufacturing changes (e.g. changes in excipients' proportion or manufacturing parameters). Predictability may be evaluated in two ways: internally or externally, depending on the intended application. Internal predictability is based on the initial data used to define the IVIVC model; in other words, it is based on a retrospective calculation of initial data. Differently, external predictability is assessed when an additional formulation (new dataset) is applied in the IVIVC model established (Cardot & Davit, 2012). In general, the combination of both internal and external assessments is recommended. For product development, to choose the best prototype for bioequivalence study, Cmax and AUC are essential parameters for testing IVIVC predictability. Moreover, these parameters must be assessed and established when IVIVC is applied for biowaiver in post-approval changes. Both internal and external predictabilities must be assessed after convolution process (application of *in vitro* data on IVIVC to obtain plasma concentration profile predicted), comparing the data observed versus data predicted. Average absolute percent prediction error (PE) between observed and predicted must not exceed 10% and individual PE for each formulation must not exceed 15%. If these criteria are not met, IVIVC is considered inconclusive, and should not be considered a surrogate for bioequivalence (FDA, 1997).

Fig. 5 demonstrates a schematic flow to establish a level A IVIVC, and its subsequent predictability assessments (internal and external).

Of the 50 studies applied in the data extraction (Table 1), 39 of them (78%) did not show any validation data and/or proof of predictability of the IVIVC. For these studies, only a point-to-point relationship between *in vitro* and *in vivo* data has been demonstrated. Although this approach (point-to-point relationship) is considered the highest IVIVC level to predict *in vivo* behavior though *in vitro* tests, a minimum number of formulations for validation and predictability evaluation should be considered to demonstrate if an IVIVC was constructed adequately. The findings of our work regarding the absence of validation and/or predictability assessment are consistent with the data published by Kaur et al. (2015). In this article, the authors reported a list of recurring common deficiencies related to IVIVC data in the abbreviated new drug application (ANDA) submissions based on FDA databases from 1996 to 2014. The list includes failure to assess external and/or internal predictability in the IVIVC model. In many cases, the model did not accurately predict plasma concentration profiles and pharmacokinetic parameters in the range of release rates tested.

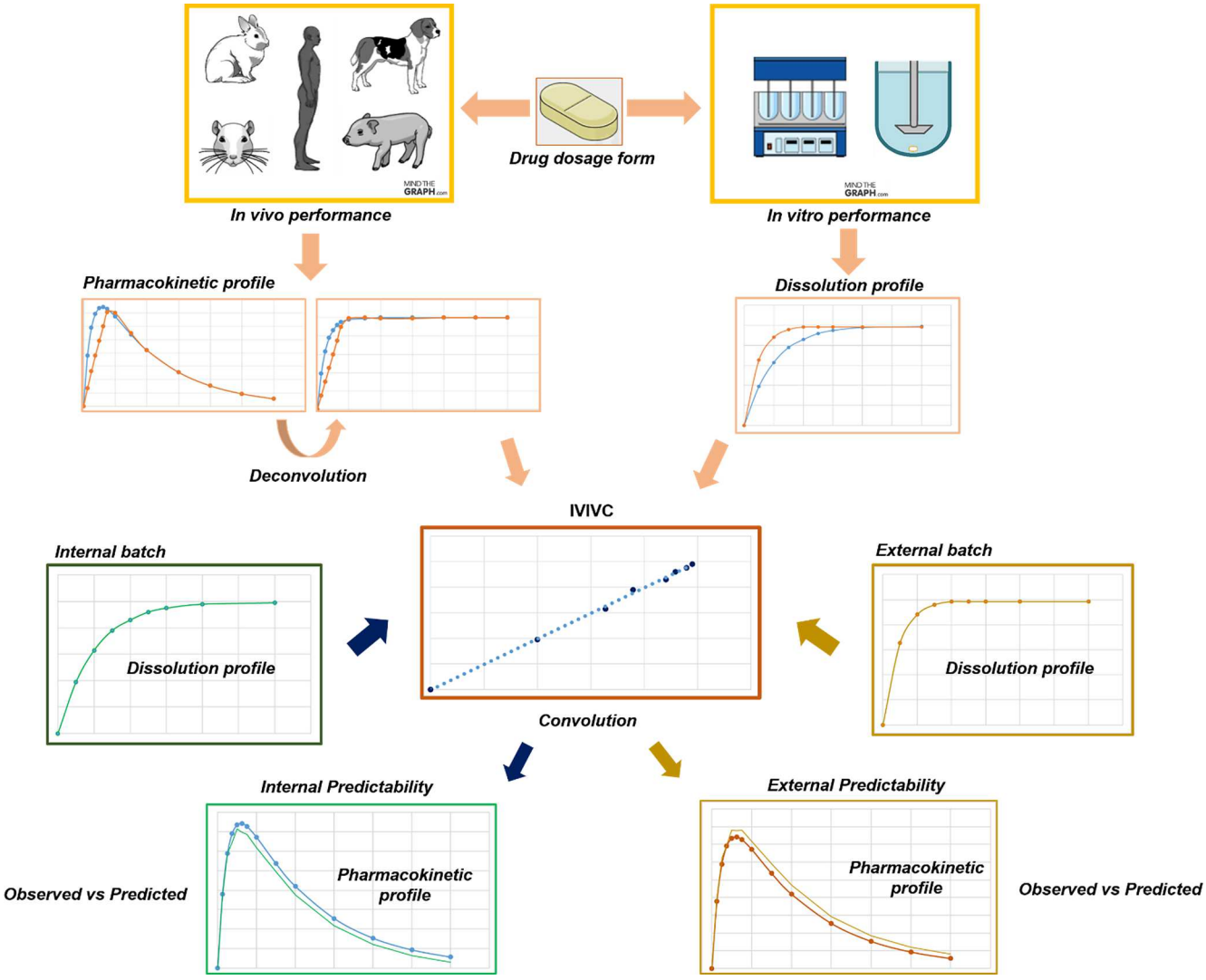

Fig. 5. Schematic flow to establish a level A IVIVC and its predictability assessments.

Considering the articles which showed validation data and/or proof of predictability of the IVIVC (11 of 50 studies examined), it is important to mention that most of them applied two or three formulations, with different release rates, for validation of IVIVC model. This approach is consistent with FDA guidance (FDA, 1997) that states IVIVC should be demonstrated consistently with two or more formulations with different release rates. Another relevant feature of those studies was the application of PE criteria between observed and predicted values in order to consider IVIVC model validated. Cmax and AUC parameters were considered in the predictability assessment as recommended by FDA guidance (FDA, 1997).

4.6. Distribution of articles published during the period assessed

To observe the distribution of articles published regarding the use of IVIVC for oral formulation development during the period assessed, Fig. 6 is presented.

Considering the last two years (2017 – 2018) of the period searched, it is possible to observe a significant growth in the number of publications of IVIVC studies for oral formulations. This fact might be related to the greater interest of scientists in the use of IVIVC for oral formulation development and in the publication of these data. In this way, if a constant growth in the number of publications of IVIVC studies is achieved in the next years, it can help not only academic scientists but also the pharmaceutical industry, which will be able to access more robust data.

5. Conclusion

Recent advances in the development of new methodologies that mimic GI conditions or other regions of the body has been observed, mainly with the development of multi-compartmental apparatuses that simulate environments and processes concomitantly (disintegration, dissolution and permeation). In the same sense, mathematical models for modeling have been implemented in the field of pharmacokinetics to create algorithms and applications in extrapolation of data to other populations or in specific pathological condition. In this context, IVIVC is currently supported by several tools that allow advancing in data correlation, as well as mimicking more accurately the conditions *in vivo* from *in vitro* assays/technologies.

Despite the observed advances of these tools, the sample of articles extracted from the two databases used in this review demonstrated that there are still very few published works with advanced technologies and that the application has been mainly in the early stage, and not for regulatory purposes. The following highlights have been described, based on discussion session, to summarize the main findings observed in the articles assessed:

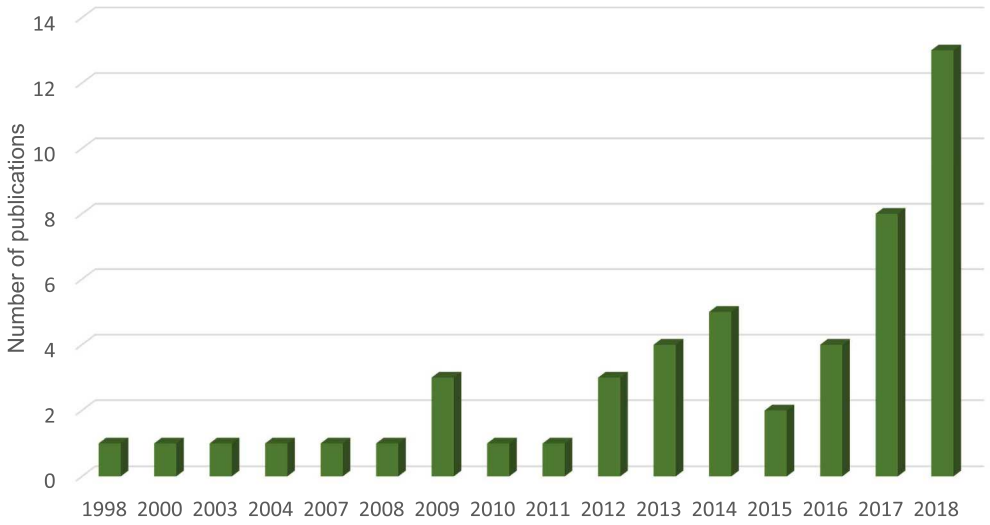

Fig. 6. Distribution of articles published regarding the use of IVIVC for oral formulation development during the period searched (1998–2018).

- MR dosage form has been the most applicable (54%) for IVIVC approaches;
- BCS classes I and II have been the most classes used for IVIVC;
- USP II (paddle) was the most (52%) applicable apparatus for IVIVC purposes;
- 70% of the IVIVC studies assessed used dissolution profile data, specifically fraction dissolved, for IVIVC approaches;
- Wagner-Nelson is the most frequently applied deconvolution model for IVIVCs (26 of 50 IVIVC studies);
- 78% of the IVIVC studies assessed did not show any validation and/or predictability data to prove the applicability of the IVIVC model; and
- The last two years (2017–2018) of the period searched showed a significant growth in the number of publications of IVIVC studies for oral formulations.

Based on the articles retrieved from both databases applied in our review, it was possible to gather a significant literature sample regarding the use of IVIVC in the development of oral formulations. Data analysis from retrieved articles showed the main characteristics of the studies, such as applied mathematical models, apparatus, main BCS classes, dosage forms, *in vivo* and *in vitro* models, among others. A discussion was completed based on these data, which allowed to address strengths and weaknesses in this area in a broad, comprehensive fashion. Additionally, a database of 45 different substances has been showed in this article and may serve as a consultation for researchers who intend to work with these drugs and dosage forms for IVIVC approaches.

Finally, this article contains an important screenshot of the use of IVIVC in the oral formulation development, as well as a view on trends and improvements needed in this area.

6. Perspectives

IVIVC is considered a tool of high potential to improve the success rate in bioequivalence studies and contributes to the registry applicants (e.g. pharmaceutical industry) to know precisely the quality attributes of their product for a rational/assertive development and possible post-approve changes. From this perspective, IVIVC approaches must be increasingly encouraged in R&D teams from pharmaceutical industries and research centers. Another important point to improve the use of this tool would be to create a harmonized and specific guideline. A possible way to do this may be to create a working group in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) to develop a guideline proposal for the evaluation of agencies around the world. Consequently, there would be an encouragement for teams to apply IVIVC in their product development, as well as harmonized concepts among the main regulatory agencies. Likewise, researchers must publish more articles in this area, so that a broad database would be available in the literature for consultation.

The ideal scenario for an IVIVC model is constructed based on a multidisciplinary team, very well-trained, with micro and macro views in pharmaceutical product development, as well as harmonized concepts for acceptability in different regulatory agencies. Galenic, analytical, quality assurance, clinical, biopharmaceutic, project management and business teams working together since the beginning of the project, and supported with tools such as IVIVC, QbD, DoE, etc., would bring significant time, cost and competitiveness to the product development process, promoting more pharmaceutical products on the market and greater access to medicines for the population. Therefore, the encouragement and initiatives in this area are extremely important, mainly from academic and pharmaceutical industry experts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3.2. CAPÍTULO II

DA SILVA, J. D.; DE SOUSA, V. P.; CABRAL, L. M.; DAVANÇO, M. G.; MEULMAN, J.; DE OLIVEIRA CARVALHO, P.; CAMPOS, D. R. *In Vitro-In Vivo* Correlation for Desvenlafaxine Succinate Monohydrate Extended Release Tablets. **AAPS PharmSciTech**., v. 21, n. 5, 195, 2020.

Este trabalho foi conduzido em parceria com pesquisadores da Universidade Federal do Rio de Janeiro (UFRJ) e Universidade Estadual de Campinas (Unicamp), com o objetivo de estabelecer um modelo de CIVIV para a formulação succinato de desvenlafaxina monoidratado 50 mg comprimido revestido de liberação prolongada. Os desempenhos *in vitro* das formulações teste e referência foram caracterizados através de ensaios de perfis de dissolução utilizando o aparato USP 1 (cesto), a 75 rpm, nos meios de dissolução HCl pH 1,2, tampão acetato de sódio pH 4,5 e tampão fosfato de sódio pH 6,8. As concentrações plasmáticas de desvenlafaxina, obtidas a partir de um estudo de bioequivalência, foram utilizadas para o cálculo da fração absorvida através do modelo de *Wagner-Nelson*. Os fatores de similaridade *f1* e *f2* foram utilizados para comparar os perfis de dissolução das formulações teste e referência em cada meio avaliado. O modelo de CIVIV foi estabelecido utilizando os dados de fração dissolvida e fração absorvida do medicamento referência (formulação *target* para bioequivalência). Na avaliação da capacidade preditiva da CIVIV, o erro de predição percentual (% PE) para a Cmáx foi de 7,63%, demonstrando que o modelo estabelecido foi adequado para predição deste parâmetro através dos dados de perfil de dissolução nas condições avaliadas.

Notas de correção:
1. Na página 5 de 9 deste artigo, onde se lê “The Weibull 1 α and β parameters (Fig 4a - f) ....”, considerar “The Weibull 1 α and β parameters (Fig 4a, c and e).
2. Na página 6 de 9 deste artigo, onde se lê “Weibull 2 α and β values, described in Fig. 4b – f ....”, considerar “Weibull 2 α and β values, described in Fig. 4b, d and f...”.

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Research Article

In Vitro–In Vivo Correlation for Desvenlafaxine Succinate Monohydrate Extended Release Tablets

Jéssica Domingos da Silva,[1] Valéria Pereira de Sousa,[1,4] Lucio Mendes Cabral,[1] Marcelo Gomes Davanço,[2] Jessica Meulman,[3] Patrícia de Oliveira Carvalho,[2] and Daniel Rossi Campos[2]

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Abstract. The objective of this study was to develop a dissolution test in order to establish an *in vitro–in vivo* correlation (IVIVC) model for desvenlafaxine succinate monohydrate (DVSM) extended release (ER) tablets. The *in vitro* release characteristics of the drug were determined using USP apparatus 1 at 75 rpm, with volume of HCl pH 1.2, acetate buffer solution (ABS) pH 4.5, or phosphate buffer solution (PBS) pH 6.8. *In vivo* plasma concentrations and pharmacokinetic parameters in healthy volunteers were obtained from a bioequivalence study. The similarity factors f_1 and f_2 were used to compare the dissolution data. The IVIVC model was developed using fraction dissolved and fraction absorbed of the reference product. For predictability, the results showed that the percentage prediction error (%PE) value of C_{max} was 7.63%. The observed low prediction error for C_{max} demonstrated that the IVIVC model was valid for this parameter.

KEY WORDS: desvenlafaxine succinate monohydrate; extended release tablets; dissolution; bioequivalence; *in vitro–in vivo* correlation.

INTRODUCTION

In vitro–in vivo correlation (IVIVC) is a powerful tool for establishing a rational relationship between *in vitro* and *in vivo* characteristics. In the last years, this tool has been experimented and adopted as an integrated and multidisciplinary approach to achieve a more rational and assertive flow in the development of drug products. In the development of modified-release formulations, IVIVC constitutes an important part of the process, aiming to optimize prototypes, set dissolution limits, reduce the number of bioequivalence studies during the development, and support post-approval changes (1,2).

Desvenlafaxine (DV), also known as *o*-desmethylvenlafaxine, is the major metabolite of venfalaxine. DV is a serotonin and norepinephrine reuptake inhibitor (SNRIs) and was approved by the Food and Drug Administration (FDA) in 2008 for the treatment of major depressive disorder (MDD). During episodes of depression, the serotonin norepinephrine system appears to be deregulated; therefore, this drug selectively inhibits the uptake of these neurotransmitters in the presynaptic membrane (3,4).

The salt form, desvenlafaxine succinate monohydrate (DVSM), is administered as extended release (ER) tablets. The recommended dose is 50 mg once daily, and this dosage regimen is well tolerated and effective in the treatment of MDD. DVSM presents good oral absorption with an absolute bioavailability of 80% (5). The chemical name and some physical and chemical characteristics of DVSM are described in Table I.

DVSM can be classified as a BCS class I drug, with high solubility and high permeability (6,8,9). The absorption of DVSM is mainly limited by the *in vivo* dissolution rate from the ER pharmaceutical form (8,10) Thus, the *in vitro* dissolution test of DVSM ER becomes an important tool not only in the development, production, and quality control but also in the prediction of the *in vivo* behavior of this drug. For this, the development of a discriminative method, which can reproduce *in vitro* dissolution closer to what occurs under physiological conditions, becomes necessary and allows the establishment of an IVIVC between the *in vitro* dissolution data and *in vivo* pharmacokinetic data (11–13). A level A IVIVC is the highest and most powerful level of correlation, as it represents a point-to-point relationship between *in vitro* and *in vivo* data. For ER formulations containing BCS class I drugs, level A is expected to be reached, which would allow the application of the correlation model to predict the *in vivo* behavior of the formulations (14,15).

[1] Department of Drugs and Pharmaceutics, Faculty of Pharmacy, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho, 373, CCS, Bss, sl15, Rio de Janeiro, RJ 21941-902, Brazil.
[2] Programa de Pós-graduação Stricto Sensu em Ciências da Saúde, Universidade São Francisco, Av. São Francisco de Assis, 214, Bragança Paulista, SP 12916-900, Brazil.
[3] Faculty of Pharmaceutical Sciences, University of Campinas – UNICAMP, Campinas, São Paulo, Brazil.
[4] To whom correspondence should be addressed. (e–mail: valeria@pharma.ufrj.br)

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Table I. Chemical Properties of Desvenlafaxine Succinate Monohydrate (6,7)

Structure	
Chemical name	*RS*-4-[2-dimethylamino-1-(1-hydroxycyclohexyl)ethyl]phenol succinate hydrate
Molecular formula	$C_{16}H_{25}NO_2$. $C_4H_6O_4$. H_2O
Molecular weight	399.48 g/mol
Melting point	131°C
pKa values	8.34 (dimethylamino group) and 10.11 (phenolic group)
Log *P*	0.21

Few *in vitro* dissolution methods for DVSM ER tablets can be found in the literature. The FDA method suggests the use of the USP apparatus 1 and the saline medium, while Franek et al. (2015) used the USP apparatus 3 and biorelevant media with different compositions and pH, simulating the fasting state, to investigate if *in vivo* dissolution and intestinal permeability rate limited the absorption of DV (10,16). However, no method that presents IVIVC for DVSM ER tablets has yet been described in the literature. Thus, the objective of this work was to establish a level A IVIVC for ER tablets containing 50 mg of DVSM from *in vitro* (dissolution) and *in vivo* (pharmacokinetics/bioequivalence) data and to evaluate the predictive capacity of this model.

MATERIALS AND METHODS

Reagents

Reference standard DVSM with purity of 99.8% was characterized by Thomson Laboratory (Campinas, Brazil) and raw material was obtained from Alembic Pharmaceuticals Ltd. (Mumbai, India). Monobasic ammonium phosphate and HPLC grade reagents acetonitrile and methanol were purchased from Merck (Merck Millipore, Darmstadt, Germany). Sodium chloride was obtained from J.T.Baker™ (J.T.Baker Chemical Co, NJ, USA). Analytical grade reagents hydrochloric acid, potassium phosphate monobasic, and sodium hydroxide were purchased from VWR (São Paulo, Brazil). Filtration procedures utilized 0.45 μm polyvinylidene (33 mm diameter) (Merck Millipore, Darmstadt, Germany).

Formulations

Test formulation DVSM 50 mg ER tablet was manufactured by a Brazilian pharmaceutical company, with the following composition: 75.8 mg DVSM (corresponding to 50 mg DV), microcrystalline cellulose, hypromellose, silicon dioxide, talc, magnesium stearate, polyvinyl alcohol, titanium dioxide, macrogol, red iron oxide. The reference product, Pristiq™ 50 mg ER tablet, was manufactured by Pfizer Ireland Pharmaceutical (imported and distributed by Pfizer SA Bogotá, Colombia), composed by 75.8 mg DVSM equivalent to 50 mg DV, hypromellose, microcrystalline cellulose, talc, magnesium stearate, Opadry® containing polyvinyl alcohol, titanium dioxide, macrogol, talc, red iron oxide, and yellow iron oxide.

The same batches of the reference and test products were applied in the *in vitro* and *in vivo* assays in order to establish the IVIVC model.

Determination of Sink Conditions

In order to ensure the sink conditions for dissolution profiles, an amount of drug has been added in each tested media for assessing the recovery of DVSM in each condition. Sink condition was considered as at least 3 times the volume necessary to solubilize the highest single therapeutic dose of DVSM (100 mg). The samples were prepared in triplicate and performed in an incubator with orbital shaking (Tecnal®, São Paulo, Brazil), adding 20 mg of DVSM in flasks containing 20 mL of dissolution media HCl pH 1.2, acetate buffer solution (ABS) pH 4.5, and phosphate buffer solution (PBS) pH 6.8. The solutions were kept under stirring at 150 rpm, at 37 ± 1°C. After 1 h of testing, the samples were removed and subjected to centrifugation at 3300 rpm for 2 min. The supernatant was filtered through a 0.45-μm PVDF membrane and analyzed by HPLC. The recovery of the drug was calculated by comparing the theoretical and experimental concentrations. The stability of DVSM in the dissolution media was achieved by comparing the areas of two injections at time 0 and 24 h. The solution was considered stable for 24 h with average recovery results within the range from 95 to 100%.

Development of Dissolution Test

The development of the dissolution test for IVIVC was carried out in the equipment UDT-814 (DCTech Laboratory Technologies, São Paulo, Brazil). The dissolution profiles of the DVSM ER 50 mg test tablets were evaluated in pH 6.8 medium with and without deaeration, to assess the need for this procedure, using a 900-mL medium volume, 100 rpm rotation, and USP apparatus 1.

The definition of the dissolution test parameters, such as apparatus, rotation speed, volume, and dissolution medium, was made by comparing the dissolution profiles of the test and reference formulations. For the evaluation of the apparatus, assays were carried out with the USP apparatus 1 (basket) at the rotational speeds of 75 and 100 rpm, and USP apparatus 2 (paddle) with sinker at the rotations of 50, 75, and 100 rpm (15), with 900 mL volume of PBS medium at pH 6.8. The dissolution medium volume was evaluated with the volumes of 500, 900, and 1000 mL of medium PBS pH 6.8. The selection of the dissolution medium for IVIVC was

carried out by comparing the dissolution profiles in the three media HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8.

All dissolution tests were performed at a temperature of 37 ± 1°C, for 24 h, with removal of 10 mL aliquots at times of 1, 4, 8, 16, and 24 h and analyzed by HPLC. The comparison between the dissolution profiles was performed using the independent model approach, using the difference (f_1) and similarity (f_2) factors. The curves were considered similar if the f_1 values are between 0 and 15 and f_2 values are between 50 and 100 (17).

Analytical Method

The samples for determination of sink conditions and dissolution tests were analyzed by high-performance liquid chromatography (HPLC) using an Elite LaChrom Hitachi chromatograph coupled to photodiode array detector (DAD/UV). Quantifications were performed using an Agilent Zorbax Eclipse XDB C_{18} column (150 × 4.6 mm, 5 μm, Agilent, CA, USA) at 25°C, mobile phase composed of buffer pH 3.2 and acetonitrile (85:15), flow of 0.9 mL/min, wavelength of 225 nm, and injection volume of 10 μL.

In Vivo Data

The bioequivalence study was carried out on male and female healthy volunteers (44 healthy volunteers, 22 female and 22 male, aged 18 to 50 years). The study was an open-label, randomized, crossover study with two treatments, a 2 × 2 crossover design and a 14-day washout period. The study was carried out in a Brazilian Contract Research Organization and had the opinion of the ethics committee approved under No. 572.456. Twenty-two blood samples (8.5 mL) were collected in each period, at the following intervals: 0 (predose), 1.0, 2.0, 3.0, 4.0, 5.0, 5.5, 6.0, 6.3, 6.7, 7.0, 7.3, 7.7, 8.0, 8.3, 8.7, 9.0, 10.0, 12.0, 24.0, 36.0, and 48.0 h after drug administration. The collections were carried out in tubes with anticoagulant heparin lithium. The samples were centrifuged at 3000 rpm for 10 min and in cryogenic tubes were stored at − 20°C.

The quantification of DVSM in human plasma was performed on HPLC coupled to MS/MS mass spectrometry, with electrospray ionization source (ESI) in positive mode, according to the methodology described and validated by Predrazzoli-Júnior et al. (2017) (18). Orphenadrine was used as an internal standard. The linearity of the method ranged from 1 to 250 ng/mL. Quantification was performed using the desvenlafaxine/orphenadrine peak area ratios, monitoring transitions from m/z 264.4 > 58.7 to desvenlafaxine and 270.2 > 181.1 to orphenadrine. The pharmacokinetic parameters were calculated in the program Phoenix WinNonlin™ (Pharsigh Corp., version 6.3).

In Vivo–In Vitro Correlation

IVIVC was established using the pharmacokinetic data of the reference product. The percentage of the *in vivo* absorbed fraction (Fa) was calculated using the deconvolution of the plasma concentration profile *versus* time, using the Wagner-Nelson mathematical equation (14,19):

$$F_t = \frac{C_t + k_{el}\int_0^t C\,dt}{k_{el}\int_0^\infty C\,dt}$$

where F_t is Fa *in vivo*, C_t is the plasma concentration in time t, and K_{el} is the constant elimination rate.

DVSM *in vitro* dissolution data were treated using the Weibull model, using the DD Solver 1.0 supplement in Microsoft Office Excel® 2019 (Microsoft Corporation, Redmond, WA, USA). The Weibull model was applied twice in the *in vitro* dissolution data. The first (Weibull 1) was relating the dissolution test times (1, 4, 8, and 24 h) with the percentage of drug dissolved in the medium, comparing the predicted values with those observed *in vitro*. The parameters α and β obtained in this first stage were used to calculate the $t_{in\ vitro}$ in the equation:

$$t_{invitro} = \sqrt[\beta]{\alpha(-1)\ln\frac{100-Fa}{100}}$$

where $t_{in\ vitro}$ is the time in which a certain amount of drug is dissolved *in vitro* at time t and Fa is the drug absorbed fraction *in vivo*.

Levy plot graph was constructed by correlating $t_{in\ vitro}$ with $t_{in\ vivo}$ (amount of drug absorbed *in vivo* at time t). Through the linear regression equation applied to these data, it was possible to determine the *in vivo* times corresponding to the *in vitro* dissolution times (1, 4, 8, and 24 h). Thus, the Weibull model (Weibull 2) by DDSolver was applied again, relating these calculated *in vivo* times to the percentage of drug dissolved in the medium, comparing the predicted values with those observed *in vitro*. The α and β parameters were applied to the Weibull equation and thus, the dissolved fraction (F_{diss}) of the drug was calculated (20–22):

$$F_{diss} = 100\left\{1-\exp\left[\left(-t_{invitro}^{\beta}\right)/\alpha\right]\right\}$$

where F_{diss} is the *in vitro* drug dissolved fraction, $t_{in\ vitro}$ is the time that a certain amount of drug is dissolved *in vitro* at time t, and α and β are Weibull function parameters. Level A correlation was investigated point by point comparing the amount of drug absorbed *in vivo* (Fa) with the dissolved fraction *in vitro* (13). IVIVC was evaluated through linear regression analysis using Microsoft Office Excel® 2019 (Microsoft Corporation, Redmond, WA, USA).

The internal validation of the IVIVC model was performed from the convolution of the predicted *in vivo* absorbed fraction and obtaining the predicted plasma concentration (Cp_{pred}) by the equation:

$$Cp_{pred} = \frac{\left(\frac{2\Delta F_{a\ pred} D}{V_d}\right) + Cp(2k_{el}\Delta t)}{(2 + k_{el}.\Delta t)}$$

where Cp_{pred} is the predicted plasma concentration, ΔF_{apred} is the difference of the absorbed fraction predicted

at the initial and final time, D is the drug dose, V_d is the distribution volume, Cp is the *in vivo* experimental plasma concentration, K_{el} is the constant of elimination, and Δt is the difference between the final and initial *in vivo* time. The percentage of prediction error (%PE) was calculated by comparing the C_{max} observed *in vivo* with the predicted one, from the equation:

$$\%PE = \left[\frac{(\text{observed value} - \text{predicted value})}{\text{Observed value}}\right] \times 100$$

The IVIVC model was considered predictive when the absolute mean PE% was less than or equal to 10% and the absolute PE percentage for the predicted parameter was less than 15% (13).

RESULTS AND DISCUSSION

Determination of Sink Conditions

The experimental concentrations (% recovery) of DVSM were 0.98 ± 0.74 mg/mL (99.9%), 0.96 ± 0.42 mg/mL (99.9%), and 0.99 ± 0.18 mg/mL (100.0%) in the media HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8, respectively. Then, the calculated volume to solubilize the highest single therapeutic dose (100 mg) was 101.3, 103.7, and 100.7 mL of the media HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8, respectively. Considering these data, the sink conditions (three times the volume necessary to solubilize the highest single therapeutic dose) would be achieved with 303.8, 311.2, and 302.0 mL of the media HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8, respectively. Therefore, the sink conditions were achieved in all dissolution profiles carried out. DVSM also proved to be stable for 24 h in the three tested media, with mass recovery of 100.7 ± 0.01%, 101.6 ± 0.1%, and 102.5 ± 0.02% for HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8.

Development of Dissolution Test

During the development of a discriminative dissolution method for the establishment of an IVIVC, it is necessary to evaluate some parameters, such as the choice of apparatus, volume, hydrodynamic conditions, and medium composition.

Initially, the need for deaeration of the PBS pH 6.8 medium was evaluated, before the dissolution assay (Fig. 1a). The similarity factor (f_2) of the dissolution profile of the test drug in the aerated and non-aerated media was 96.5, demonstrating that they are similar and discarding the need to perform such a procedure.

The use of apparatus USP 1 (basket) and 2 (paddle) is recommended by the FDA (13) for the dissolution assays of extended release pharmaceutical forms (23,24). Figure 1 b shows that changing apparatus basket, paddle, and rotational speed (USP 1, 75 rpm and 100 rpm; USP 2, 50, 75, and 100 rpm) has no significant impact on drug release, maintaining the overlapping dissolution curve profiles. Therefore, the USP 1 apparatus was chosen mainly because the pharmaceutical form fluctuates and its use is described in the dissolution method recommended by the FDA (16).

The rotation speed is another determining factor to the *in vitro* dissolution test, because it affects the drug release and can be less discriminative with the increase in speed (25). In Fig. 1c, it is possible to observe that there was no significant increase or reduction in the release of DVSM from the test and reference tablets, when using the USP apparatus 1 with 75 and 100 rpm speed. However, the chosen rotation speed was 75 rpm, because it had a lower similarity factor ($f_2 = 79.5$) between the two drugs when compared with the 100 rpm speed ($f_2 = 94.9$), which may be an indication of greater discriminative power of this condition.

Generally, the use of USP apparatus 1 in dissolution tests requires medium volumes ranging from 500 to 1000 mL to achieve sink condition (25). Thus, the test and reference tablets were dissolved in PBS buffer pH 6.8, USP apparatus 1, and rotational speed of 75 rpm in the medium volumes of 500 mL, 900 mL, and 1000 mL. Figure 1 d shows that, in the three different volumes of medium, DVSM release profiles were similar, with f_2 greater than 50. However, the 500 mL volume was chosen because it had a smaller similarity factor ($f_2 = 67.0$) than the others (900 mL, $f_2 = 94.9$; 1000 mL, $f_2 = 71.4$), indicating a greater discriminative power.

As observed in the assays for determination of sink conditions, both the DVSM standard solution and the sample solution in PBS pH 6.8 remained stable for 72 h at room temperature, with a mass recovery of 99.1% and 100.6%, respectively. Another observed factor was that the drug did not show retention in PVDF-type membranes (33 mm in diameter and 0.45 μm pore), discarding 1 or 3 mL of the dissolution test sample.

In ER pharmaceutical forms, different mechanisms are involved in drug release, which makes it difficult to establish standardized specifications and define the value of the amount of dissolved drug (Q). Therefore, it is recommended that the lower and upper limits at any point in the profile do not exceed a difference of ± 10% of the active substance labeled content in the formulation (total variability of 20%), unless a higher limit has been determined by bioequivalence studies. The specifications and the Q value can be established by at least three points: the first point, 1–2 h, with 20–30% drug release; the second point with approximately 50% release; and the last one, which represents complete or at least 80% drug dissolution (13,15,26). Based on this, the Q value was defined as follows: less than 20% in 1 h, 28 to 48% in 4 h; 48 to 68% in 8 h; 73 to 93% in 16 h; and a minimum of 80% in 24 h.

Often, the selection of a biorelevant medium can predict the *in vivo* dissolution conditions of the drug and reproduce a good IVIVC (25). The PBS pH 6.8 dissolution medium is shown to be the most representative of the *in vivo* tablet transit, since DVSM absorption from the ER tablet pharmaceutical form is expected to occur in the region of the duodenum to the colon (10), which has a pH range between 5.4 and 7.5 (27). However, after determining the dissolution method parameters, dissolution profiles were performed in the three physiological media with the USP apparatus 1, volume of 500 mL, at 37°C, and speed of 75 rpm. As seen in Fig. 2, the test and reference tablets showed f_1 values lower than 15 and f_2 values higher than 50, in all tested media, with RSD less than 20% in the 1- to 4-h collection times and less than 10% in other time points (8,

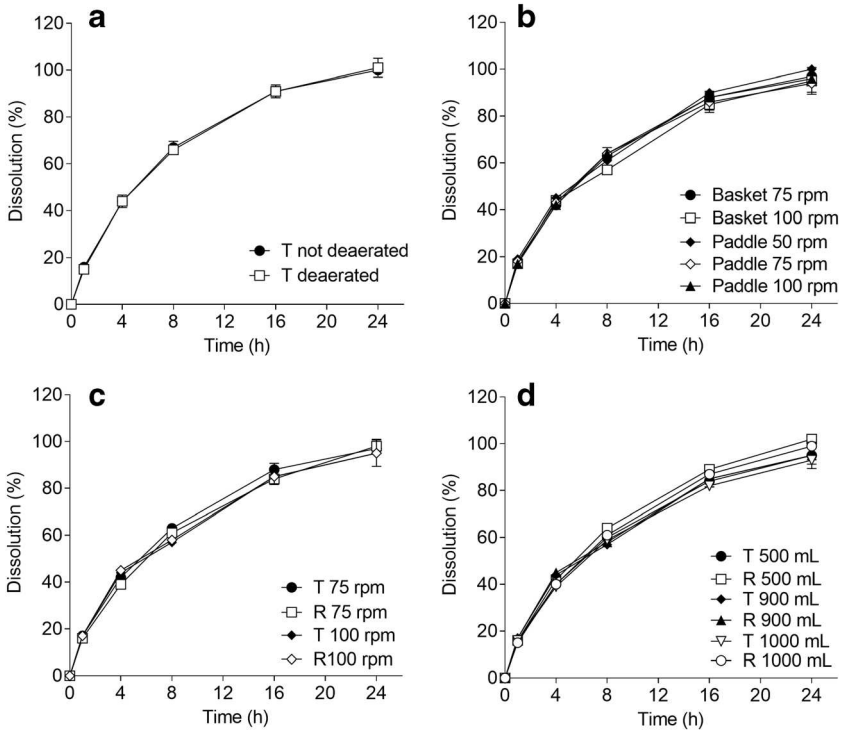

Fig. 1. Dissolution profiles of test in phosphate buffer pH 6.8 deaerated and not deaerated, using USP 1, in 900 mL of media at 37°C, under a rotational speed 100 rpm (**a**). Dissolution of test in phosphate buffer pH 6.8, using USP 1 and 2, in 900 mL of media at 37°C, under a rotational speed of 50, 75, and 100 rpm **(b)**. Dissolution of test and reference in PBS pH 6.8, using USP 1, in 900 mL of media at 37°C, under a rotational speed of 75 and 100 rpm (**c**). Dissolution of test and reference in PBS pH 6.8, using USP 1, in 500, 900, and 1000 mL of media at 37°C, under a rotational speed of 75 rpm (**d**)

16, and 24 h), as recommended for ER pharmaceutical forms (15). However, for the ABS pH 4.5 medium, f_1 and f_2 values were close to 15 and 50, respectively. Considering that the test and reference products were bioequivalent, IVIVC was established considering the dissolution data obtained in HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 media.

In Vivo Study

Pharmacokinetic data were obtained from the bioequivalence study. As seen in Table II, pharmacokinetic parameter values, such as $t_{1/2}$ and T_{max} of approximately 12 and 6 h, respectively, are in accordance with those described in the scientific literature (5,18,28), demonstrating that the bioequivalence study design was adequate to demonstrate the pharmacokinetic profile of DVSM in the administration of the test and reference products.

The C_{max} geometric mean ratio between the test and reference formulations was 94.1%, with a 90% confidence interval (90% CI) of 89.5 to 98.9%. For $AUC_{0\text{-}t}$, the ratio was 92.2% with a 90% CI of 86.5 to 98.2%. These results demonstrate that the two drugs are bioequivalent, because the extreme 90% CI values of the geometric mean ratio ($AUC_{0\text{-}t}$ test/$AUC_{0\text{-}t}$ reference and C_{max} test/C_{max} reference) are greater than 80% and less than 125% (26).

In Vitro–In Vivo Correlation

The test and reference formulations have the same drug release mechanism, since both contain hypromellosis, a matrix polymer responsible for controlled drug release (7,29). Based on this, IVIVC was performed with the pharmacokinetic data of the reference product. Therefore, Fa was obtained through deconvolution (Wagner-Nelson mathematical model) of the plasma concentration profile and pharmacokinetic data of the reference product, as shown in Fig. 3. The Wagner-Nelson model proved to be adequate, since the pharmacokinetics data modeled adequately in the single-compartment model.

The *in vitro* dissolved fraction data in both media was obtained through the application of the Weibull function, by estimating the time scale, as 4 collection points of the dissolution tests were used (1, 4, 8, and 24 h), and thus the correlation between *in vivo* and *in vitro* data (30). The values of Weibull 1 α and β parameters (Fig. 4a–f) were calculated from the collection times of the dissolution tests and used together with the Fa percentage for the prediction of the *in vitro* dissolution time and the construction of the Levy plot graph. In Fig. 5a, the correlation between the *in vivo* absorption time ($t_{in\ vivo}$) and the *in vitro* dissolution time ($t_{in\ vitro}$) is shown, demonstrating there is a linear relationship between *in vivo* and *in vitro* data, mainly between the first time

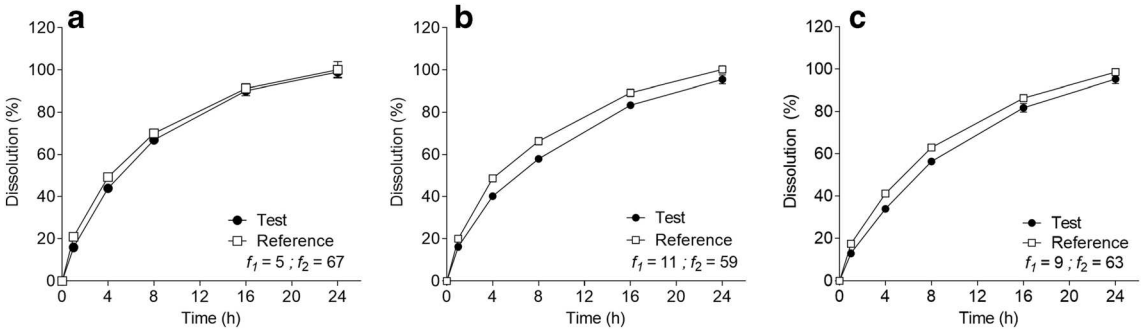

Fig. 2. Dissolution profiles of test and reference in HCl pH 1.2 (**a**), ABS pH 4.5 (**b**), and PBS pH 6.8 (**c**). USP apparatus 1, in 500 mL of media at 37°C, under a rotational speed of 75 rpm

points, for HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 dissolution media.

Weibull 2 α and β values, described in Fig. 4b–f, were obtained in relation to the *in vivo* time value ($t_{in\ vivo}$) calculated to obtain the *in vitro* dissolved fraction (F_{diss}). Figure 5 b shows the relationship between the reference product Fa and Fd, with correlation coefficients (r^2) of 0.9756, 0.9765, and 0.9741 for HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 media, respectively, showing a level A linear correlation (13).

Internal validation was performed to assess the accuracy of the mathematical model used for IVIVC. The predicted plasma concentration profile of DVSM was obtained from the convolution of *in vitro* pharmacokinetic and dissolution data. The predicted C_{max} was 71.3 ng/mL for HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 dissolution media, and the C_{max} observed *in vivo* in pharmacokinetic studies was 77.3 ng/mL. Figure 5 c shows that the predicted and observed plasma concentration curves are similar, especially after 6 h (T_{max}). The predicted error (PE) value was less than 10% (7.6%), when the predicted C_{max} values were compared with those observed *in vivo* (13,26), demonstrating that the model used in IVIVC is adequate and meets the criteria for internal validation in all media (HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8).

CONCLUSION

A level A IVIVC was established between Fd and Fa data obtained from the DVSM 50 mg ER tablets, using data from the test and reference products. The dissolution method proved to be discriminative using the USP apparatus 1 at 75 rpm, medium volume of 500 mL, at 37 ± 1°C, with 24-h duration.

The three dissolution media HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 showed high correlation coefficients ($r^2 > 0.95$). Considering the physiological pH and the drug absorption site (present in a modified release formulation), the PBS pH 6.8 medium, in addition to being able to be used in the quality control routine, also demonstrated biopredictive characteristics. When compared with the dissolution method suggested by the FDA (0.9% NaCl in water, USP 1, 100 rpm, 900 mL), the conditions proposed by

Table II. Pharmacokinetic Parameters After Oral Administration of Reference and Test Formulations in Healthy Volunteers ($n = 44$)

Pharmacokinetic parameters	Formulations	
	Reference	Test
C_{max} (ng/mL)	84.6 ± 22.9	79.2 ± 20.3
T_{max} (h)	6.3 ± 1.8	7.8 ± 4.0
$AUC_{0\text{-}t}$ (ng h/mL)	1872.4 ± 571.5	1729.9 ± 528.1
$AUC_{0\text{-inf}}$ (ng h/mL)	2088.2 ± 760.7	1910.2 ± 697.5
K_{el} (h^{-1})	0.071 ± 0.03	0.07 ± 0.03
$t_{½}$ (h)	12.0 ± 9.2	11.7 ± 6.2

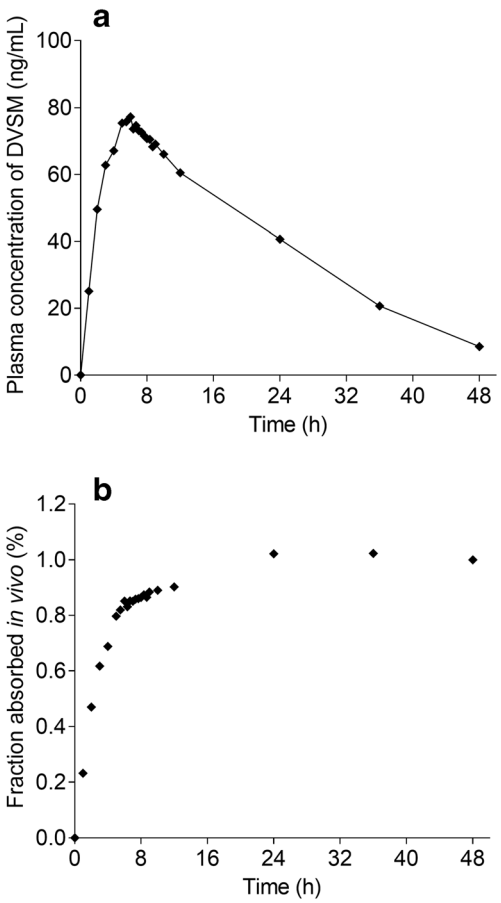

Fig. 3. Plasma concentration profiles *versus* time after fasting administration of the reference formulation (mean, $n = 44$) (**a**). DVSM fraction absorbed from reference formulation obtained by deconvolution using Wagner–Nelson model (**b**)

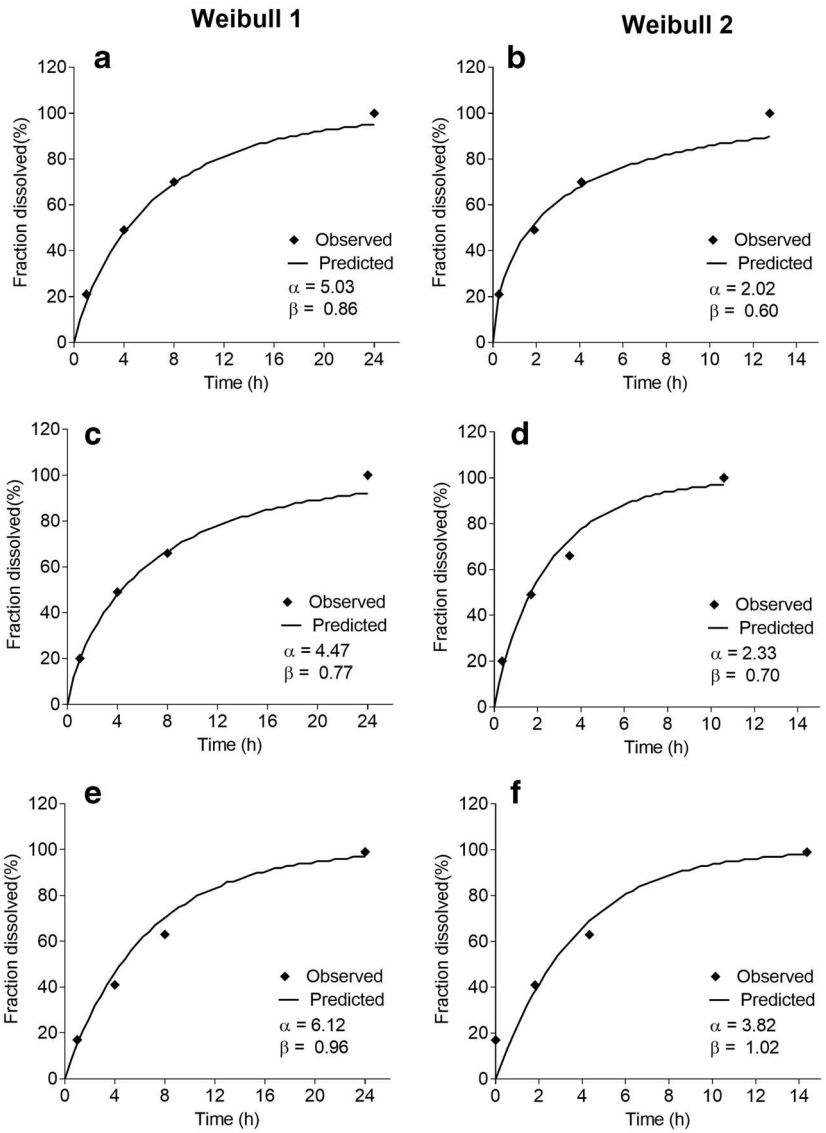

Fig. 4. *In vitro* dissolution data of HCl pH 1.2 fitted by Weibull 1 (**a**) and 2 (**b**). *In vitro* dissolution data of ABS pH 4.5 fitted by Weibull 1 (**c**) and 2 (**d**). *In vitro* dissolution data of PBS pH 6.8 fitted by Weibull 1 (**e**) and 2 (**f**)

this article are biorelevant, even if this medium is used for quality control of the finished product, since it uses a buffer with a pH similar to that of the small intestine, as well as rotation and medium volume compatible with the high solubility characteristic of the drug.

The mathematical model used to establish IVIVC was validated internally, confirming the good ability to predict *in vivo* absorption from the dissolution media evaluated. In addition, a deconvolution model by Wagner-Nelson was used, which does not require data on desvenlafaxine

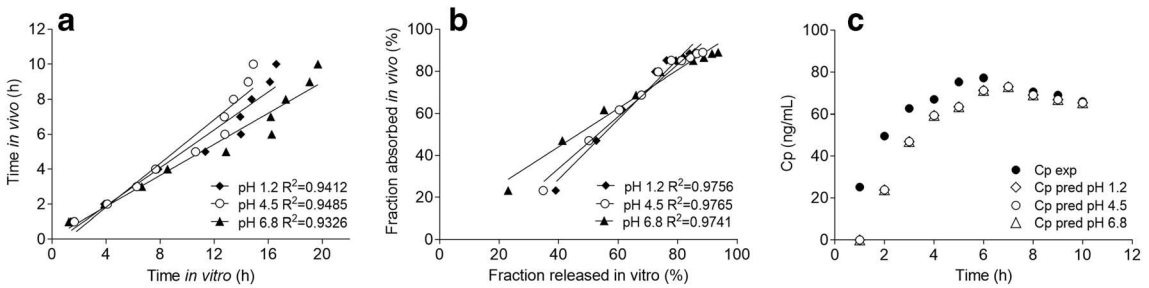

Fig. 5. Levy plot graph (**a**). *In vivo–in vitro* correlation analysis using fraction absorbed *in vivo* as a function of the *in vitro* fraction dissolved from reference in dissolution media pH 1.2, pH 4.5, and pH 6.8 (**b**). Comparisons of plasmatic concentrations observed *in vivo* and predict *in vitro* for HCl pH 1.2, ABS pH 4.5, and PBS pH 6.8 (**c**)

intravenous kinetic disposition. As per the package insert of the reference medicine (Pristiq™ 50 mg ER tablet), the kinetic disposition of the formulation does not change with food administration; we can infer that this dissolution medium could also be used to predict possible dose-dumping events for this formulation *in vivo*. Thus, the IVIVC established in this article can be used as an excellent tool to predict the *in vivo* behavior of DVSM ER formulations, thus contributing to the more assertive development of generic drugs, as well as predicting the *in vivo* impact of possible post-registration changes in these products.

COMPLIANCE WITH ETHICAL STANDARDS

The study was carried out in a Brazilian Contract Research Organization and had the opinion of the ethics committee approved under No. 572.456

Conflict of Interest The authors declare that they have no conflict of interest.

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3.3. CAPÍTULO III

BERMEJO, M., MEULMAN, J., DAVANÇO, M. G.; CARVALHO, P. O.; GONZALEZ-ALVAREZ, I.; CAMPOS, D. R. *In Vivo* Predictive Dissolution (IPD) for Carbamazepine Formulations: Additional Evidence Regarding a Biopredictive Dissolution Medium. **Pharmaceutics**, v. 12, n. 6, 558, 2020.

Este trabalho foi conduzido em parceria com pesquisadores da *Universidad Miguel Hernández de Elche* (Espanha) e Universidade Estadual de Campinas (Unicamp) e teve como objetivo proporcionar evidências adicionais a respeito de um meio de dissolução biopreditivo contendo 1% do tensoativo LSS para prever o comportamento *in vivo* de formulações teste e referência de carbamazepina 400 mg comprimido. Os dados de concentração plasmática foram obtidos de um estudo piloto de bioequivalência. As correlações de nível A foram estabelecidas utilizando as abordagens *one-step* e *two-steps*. Além disso, foi explorado a aplicação de *time-scaling* para avaliar as diferenças entre as taxas de dissolução *in vitro* versus *in vivo*. Os resultados mostraram que o meio de dissolução contendo 1% de LSS pode ser utilizado como uma ferramenta altamente relevante para predição do desempenho *in vivo* de formulações contendo carbamazepina. Ainda, ambas as abordagens aplicadas para construção do modelo (*one-step* e *two-steps*) foram consideradas adequadas para predição do desempenho *in vivo* a partir dos dados de dissolução.

Nota de correção:
Na página 7 de 21 deste artigo, onde se lê “Figure 2 represents the fractions absorbed….”, considerar “Figure 3 represents the fractions absorbed …”.

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Article

In Vivo Predictive Dissolution (IPD) for Carbamazepine Formulations: Additional Evidence Regarding a Biopredictive Dissolution Medium

Marival Bermejo [1,†], Jessica Meulman [2,†], Marcelo Gomes Davanço [3], Patricia de Oliveira Carvalho [3], Isabel Gonzalez-Alvarez [1,*] and Daniel Rossi Campos [3]

1 Department of Engineering, Pharmacy and Pharmaceutical Technology Area, Facultad de Farmacia, University Miguel Hernandez de Elche, San Juan de Alicante, 03550 Alicante, Spain; mbermejo@umh.es
2 Faculty of Pharmaceutical Sciences, University of Campinas—UNICAMP, Campinas, 13083-871 São Paulo, Brazil; jessicameulman@gmail.com
3 Postgraduate Program in Health Sciences, Universidade São Francisco, Bragança Paulista, 12916-900 São Paulo, Brazil; davanco.marcelo@gmail.com (M.G.D.); patricia.carvalho@usf.edu.br (P.d.O.C.); camposrossi@gmail.com (D.R.C.)
* Correspondence: isabel.gonzalez@umh.es
† Authors contributed equally.

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Abstract: The aim of the present study was to bring additional evidence regarding a biopredictive dissolution medium containing 1% sodium lauryl sulphate (SLS) to predict the in vivo behavior of carbamazepine (CBZ) products. Twelve healthy volunteers took one immediate release (IR) dose of either test and reference formulations in a bioequivalence study (BE). Dissolution profiles were carried-out using the medium. Level A in vitro–in vivo correlations (IVIVC) were established using both one-step and two-step approaches as well as exploring the time-scaling approach to account for the differences in dissolution rate in vitro versus in vivo. A detailed step by step calculation was provided to clearly illustrate all the procedures. The results show additional evidence that the medium containing 1% SLS can be classified as a universal biopredictive dissolution tool, and that both of the approaches used to develop the IVIVC (one and two-steps) provide good in vivo predictability. Therefore, this biopredictive medium could be a highly relevant tool in Latin-American countries to ensure and check the quality of their CBZ marketed products for which BE studies were not requested by their regulatory health authorities.

Keywords: carbamazepine; in vitro in vivo correlation; dissolution; biopredictive; bioequivalence; biowaiver

1. Introduction

Carbamazepine (CBZ) is a drug widely used in the treatment of epilepsy and trigeminal neuralgia. CBZ is a Biopharmaceutics Classification System (BCS) class II drug with a low aqueous solubility and high permeability [1]. Therefore, the drug is poorly soluble in aqueous media [2]. Besides its poor aqueous solubility, other attributes such as a narrow therapeutic index and relatively high variability have been recognized as obstacles for CBZ product development for bioequivalence proposals [3].

In vitro–in vivo correlations (IVIVC) are widely used tools in biopharmaceutic research in order to speed up the product development for quantifying the in vivo release, evaluating formulation-related effects on absorption and as a tool for setting in vitro dissolution specifications [4,5]. The FDA recognizes four levels of IVIVC: Level A, B, C, and multiple Level C. The most desired is the Level A category of IVIVC. It is defined as a point-to-point relationship between in vitro dissolution and

the in vivo response, such as plasma drug concentration or amount of drug absorbed [4]. Level A regression may be complicated due to differences in time scales as well as the lack of coincident times of similar release in vitro and in vivo [6,7]. Discrepancies between in vivo and in vitro times are observed by faster in vitro dissolution compared to in vivo release or by differences in shape between the two curves. In both cases, a direct relationship between in vitro and vivo data cannot be set up simply. The most used approach to determine time scaling is the so-called Levy plot. Times at which in vivo and in vitro the same percentage is absorbed and dissolved, respectively, are plotted in the Levy plot [7,8].

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. In classical two-stage approaches, fraction dissolved, obtained from in vitro dissolution profiles is typically used together with corresponding in vivo fraction absorbed obtained by deconvolution of observed plasma concentrations [6]. On the other hand, the one-stage approach uses the in vitro dissolution data and pharmacokinetic characteristics of the drug to obtain the adequate link function of the plasma drug concentrations, directly by convolution [9].

Some authors have already described a universal (for CBZ) biopredictive feature of dissolution medium containing 1% sodium lauryl sulphate (SLS), which is available in the USP Pharmacopeia [10,11]. This medium has been used for quality control proposal and additionally as a biorelevant dissolution medium since Kovaĉević, I. et al. [1] performed gastrointestinal simulations as well as established IVIVC for both immediate and modified release formulations of CBZ. Additionally, González-García, I. et al. [12] recently applied the IVIVC approach using the USP medium described above, showing the biopredictive feature of this medium, which causes the immediate release of formulations with conventional excipients, even if different batches and in vivo/in vitro studies are combined. The biopredictive dissolution method is defined by Suarez-Sharp et al. [13] as a set of testing conditions in which in vitro dissolution profiles can predict the pharmacokinetic profiles.

The aim of the present study was to perform an IVIVC for two CBZ immediate release formulations (used in a pilot BE study) using both a one-step and two-steps approaches as well as to explore the time-scaling approach to account for the differences in dissolution rate in vitro versus in vivo. A detailed step by step calculation was provided to clearly illustrate all the procedures. Moreover, this paper intends to bring additional evidence of using of IVIVC-based biowaiver for BCS class II drugs.

2. Materials and Methods

2.1. Formulations

CBZ 400-mg tablets (test formulation) and Tegretol® (reference formulation) were purchased in the local Brazilian market.

2.2. Bioanalytical Method

Plasma concentrations of CBZ were determined using liquid chromatography with tandem mass spectrometry assay—LC-MS/MS (Waters) with electrospray ionization source in positive mode. Carbamazepine-d8 was used as internal standard. The chromatographic separation was performed at 40 °C using a column X-Bridge C18 (4.6 × 50 mm, 3.5 µm) and flow rate of 0.50 mL/min. The quantification was performed by using multiple reaction monitoring (MRM) mode of the transitions at m/z 237.051 > 194.240 and m/z 244.600 > 202.300 for CBZ and carbamazepine-d8, respectively. The analytes were extracted from plasma using protein precipitation with methanol as solvent. The mobile phase used was 0.1% formic acid and methanol at a 35:65 ratio (v/v) and the injection volume was 5 µL and the total run time set as 4 min.

The bioanalytical method was validated in compliance with ANVISA guidance for bioanalytical method validation [14] and FDA Bioanalytical Method Validation Guidance for Industry [15].

2.3. Bioequivalence Study

The bioequivalence study was approved by the Research Ethics Committee (protocol number 3.085.454). All procedures were conducted in the Clinical Trials Center in accordance with the principles of Good Clinical Practice guidelines [16], the Declaration of Helsinki [17], and Resolution 466/2012 (Ministério da Saúde, Brazil) [18,19]. Written informed consent from all participants was obtained prior to the enrollment.

All participants were aged between 18 and 50 years with a body mass index between 18.5 and 29.9 kg/m^2. All subjects showed good health conditions or the absence of significant diseases after assessment of medical history, verification of vital signs, physical examination, electrocardiogram and routine laboratory tests. The study design was randomized, single dose, fasting, two-period, two-sequence crossover with a 14-days washout period. Twelve adult healthy subjects of both genders were enrolled in the study and eleven (6 women and 5 men) subjects completed the two study periods. The blood samples (7.5 mL each) were obtained at 0 h (pre-dose) and at 1.00, 2.00, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.0, 12.0, 24.0, 48.0 and 72.0 h post-dose during each period. Each blood sample was collected in EDTA tubes as anticoagulant at each time point. Collected blood samples were centrifuged immediately (3500 rpm for 10 min at 4 °C) and plasma was separated and stored frozen at −20 °C with appropriate labeling until sample analysis.

2.4. Pharmacokinetic and Fraction Absorbed Analysis

The pharmacokinetic parameters were obtained from the curves of plasma concentration versus time for CBZ and statistically compared for determination of bioequivalence, using Phoenix WinNonlin software version 8.0 (Bioequivalence Wizard module). The area under the curve from zero to the last quantifiable concentration (AUCt) was calculated by the trapezoidal method, and the area under the curve from zero to infinity (AUC∞) was calculated by the formula AUCt+ (Cn/kel), where Cn was the last quantifiable plasma concentration. Due to the long elimination half-life ($t_{1/2}$) of CBZ, it was considered area under the curve (AUC) truncated (72 h). The elimination rate constant (kel) was determined by the elimination phase of the graph of log plasma concentration versus time. The $t_{1/2}$ was defined using the equation $t_{1/2}$ = Ln(2)/kel. The maximum plasma drug concentration (Cmax) was obtained directly from the experimental data, as well as the time of the occurrence of Cmax (tmax).

Bioequivalence assessment was based on predefined acceptance criteria of 80.00–125.00% for the 90% confidence interval for the ratio of the test and reference products for the log-transformed data of AUC and Cmax, as recommended by (19), and FDA [20]. An ANOVA was performed for the primary parameters estimated (Cmax and AUC) to evaluate formulation, sequence, and period as fixed effects and to estimate the residual variance to construct the confidence intervals [21]. More detailed calculations are reported in Appendix A.

The drug absorption was estimated using numerical deconvolution method from Wagner–Nelson. This mass balance method, with First order elimination, was employed because it is the most suitable for one-compartment drugs and has been shown to adequately describe the pharmacokinetic absorption profile of CBZ:

$$Fabs = \frac{A_t}{A^{\infty}} = \frac{C_t + kel \times AUC_t}{kel \times AUC^{\infty}} \quad (1)$$

Equation (1) is the Wagner–Nelson equation that represents the fraction absorbed of the bioavailable dose at time t. $Fabs$ 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; kel is the elimination rate coefficient; AUC_t is the area under the curve from time zero to the last quantifiable concentration and AUC^{∞} is the area under the curve from zero to infinity.

2.5. In Vitro Dissolution Testing and Modelling

Dissolution study was performed in the PhEur/USP rotating paddle apparatus at 75 rpm using 900 mL of dissolution medium. The medium was a 1% sodium lauryl sulfate (SLS) aqueous solution.

Dissolution study was performed with the same batches used in the in vivo study. CBZ concentrations on the dissolution samples were analyzed by HPLC. Samples of 5mL were taken at 5, 10, 15, 30, 60 and 120 min. The experiment was performed with twelve tablets for each formulation.

First order and Weibull models were fitted to the data (fractions dissolved, F_{diss}) of each formulation.

Weibull equation

$$F_{diss} = 100 \cdot \left(1 - e^{\left(-\frac{(t^{\beta})}{\alpha}\right)}\right) \quad (2)$$

where F_{diss} are fractions dissolved and β and α are the Weibull parameters

First order equation

$$F_{diss} = 100 \cdot \left(1 - e^{(-kd \cdot t)}\right) \quad (3)$$

where kd is the dissolution rate constant. First order equation is a particular case of Weibull model when $\beta = 1$; then $kd = 1/\alpha$

The best model was selected based on the correlation coefficient of experimental versus predicted values, the Akaike's information criteria (AIC), and the residual variance comparison with Snedecor's F tests [22,23].

Fitting procedures were performed in Excel with DDsolver add-in [24].

With the Weibull parameters of each profile, it is possible to calculate the time needed for the dissolution of any desired fraction dissolved with Equation (4):

$$t_{vitro} = \sqrt[\beta]{\alpha * (-1) * ln \frac{100 - F_{abs}}{100}} \quad (4)$$

where β and α are the Weibull parameters (from Equation (2)) and F_{abs} the corresponding fraction absorbed (dissolved) in vivo.

2.6. IVIVC Two-Step Aproach

In the two-step approach fractions dissolved and absorbed at the same time points are correlated. The scheme of the calculations for the two-step approach is represented in Figure 1.

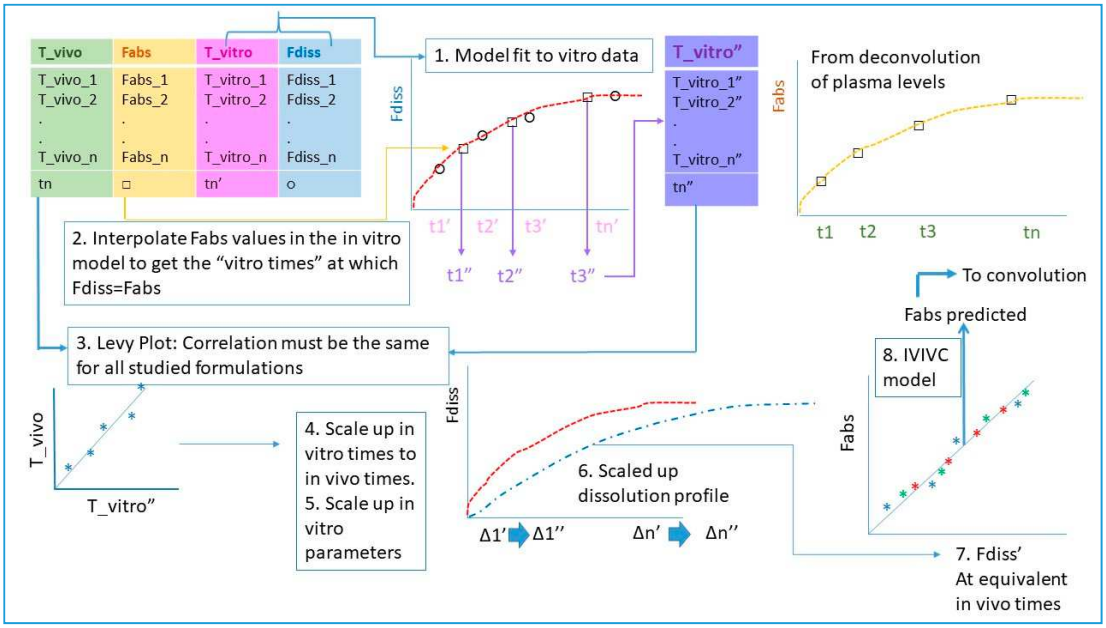

Figure 1. Steps describing the calculations to establish a two-step in vitro–in vivo correlations (IVIVC) when in vivo and in vitro dissolution processes take place at different rate and in consequence the time scale is different.

As the time scale was different on the in vitro and the in vivo assays, a Levy plot was necessary. To construct the Levy plot, a dissolution model is fitted to fraction dissolved data (step 1). Fraction-absorbed values were interpolated into the dissolution model and the equivalent in vitro times were obtained with Equation (4) and the Weibull parameters from each dissolution profile (step 2). In vitro and in vivo times were represented together and the Levy plot correlation parameters estimated (in the present paper, a linear one) (step 3). In vivo times were included up to 24 h for the test formulation and up to 12 h for the reference as later on the relationship was no longer univocal.

With the Levy equation, the in vitro sampling times were converted to their equivalent in vivo times (step 4). The objective of this procedure is to have the dissolution profile and the absorption profile in the same time-scale to check if they are directly superimposable.

As the dissolution in vitro profiles are scaled with the equivalent in vivo time, the Weibull model was fitted again to the data to obtain the scaled Weibull parameters (step 5).

The new Weibull parameters were used to estimate the fraction dissolved at the original in vivo times (step 7) so that, finally, fractions absorbed and dissolved at the same times could be plotted and the IVIVC linear relationship characterized (step 8).

To determine the predictability of the IVIVC correlation, predicted fractions absorbed (y) from the linear IVIVC (using the fractions dissolved (x)) were back-transformed into plasma concentrations using the following equation from Gohel et al. [25].

$$C_{t+1} = \frac{\left(\frac{2 \cdot \Delta F_{abs} \cdot D}{V_d}\right) + Ct \cdot (2 - Kel \cdot \Delta t)}{(2 + Kel \cdot \Delta t)} \quad (5)$$

where C_{t+1} is the plasma concentration at time (t + 1) and then C_t is the plasma concentration in the previous sampling time, t. Δt is the time interval between a sampling time and the next one and F_{abs} are the predicted fractions absorbed from the IVIVC correlation. D is the CBZ dose, kel the elimination rate constant and Vd the apparent distribution volume. This Vd values was estimated with the following equation.

$$Vd = \frac{D}{(AUC_0^{\infty} \cdot kel)} \quad (6)$$

kel (0.0159 h^{-1}) and AUC (area under the curve from time zero to infinity) (371,958.8 ng/mL*h) values were the average values from both formulations. The estimated or apparent Vd (Distribution volume divided by the bioavailability F (Vd/F)) was 67,698 mL.

2.7. IVIVC One-Step Approach

In the one-step approach, the fractions dissolved from test and reference were directly convoluted with the adequate scale factors with the pharmacokinetic parameters of CBZ (kel and Vd) to estimate plasma levels.

A system of differential equations was set up to simultaneously fit fractions dissolved (to a Weibull equation as described previously) and plasma levels.

Four differential equations were defined, two for each formulation, representing the fraction dissolved (dFdissx/dt) and the plasma levels (dCx/dt) where x is reference or test

$$\frac{dFdissTest}{dt} = \left(betaTest \times Fdissmax \times \left(t^{betaTest-1}\right)\right) \times e^{\left(\frac{(-(t^{betaTest}))}{alfaTest}\right)} / alfaTest \quad (7)$$

$$\frac{dFdissRef}{dt} = \left(betaRef \times Fdissmax \times \left(t^{betaRef-1}\right)\right) \times e^{\left(\frac{(-(t^{betaRef}))}{alfaRef}\right)} / alfaRef \quad (8)$$

where $Fdissmax$ is the maximum dissolved fraction which was fixed to 1 (100%); $betaTest$, $alfaTest$, $betaRef$ and $alfaRef$ were the parameters of the Weibull equation for the in vitro dissolution for both Test and

Reference formulations respectively. These two differential equations are simply the derivatives of Equation (2).

In order to estimate the plasma levels, the differential equation describing how drug amounts in the body change with time (dMass/dt) is designed taking into account the input rate and the elimination rate.

In a one-compartment pharmacokinetic model with First order elimination, the elimination rate in terms of drug mass is kel*Mass. The input rate into the system is limited by the drug dissolution (that is drug cannot be absorbed until dissolved), thus dissolution rate (defined by the two previous equations) corresponds to the input rate into the system (body). There are two modifications needed to estimate the in vivo input rate. In Equations (7) and (8), the dissolution rate is defined in terms of fractions dissolved, then to estimate the in vivo mass dissolved the previous equations are multiplied by the dose to get the input rate.

$$\frac{dMassTest}{dt} = \left(Dose \times betaTvivo \times \left(t^{betaTvivo-1}\right)\right) \times e^{\left(\frac{(-(t^{betaTvivo}))}{alfaTvivo}\right)} / alfaTvivo - kel \times MassTest \quad (9)$$

$$\frac{dMassRef}{dt} = \left(Dose \times betaRvivo \times \left(t^{betaRvivo-1}\right)\right) \times e^{\left(\frac{(-(t^{betaRvivo}))}{alfaRvivo}\right)} / alfaRvivo - kel \times MassRef \quad (10)$$

The Weibull parameters for the in vivo dissolution of the Test formulation were *betaTvivo* and *alfaTvivo*. *betaRvivo* and *alfaRvivo* were the Weibull parameters for the in vivo dissolution of the Reference formulation. *MassTest* and *MassRef* correspond to the amounts in plasma of CBZ from Test and Reference formulation and *kel* was the elimination rate constant.

Finally, amounts in plasma (Mass) are transformed in plasma levels dividing the amounts by the distribution volume (Vd) previously defined.

The link between in vitro and in vivo dissolution was established through a scaling factor (as in the two-step approach) of the Weibull parameters. The scaling factors (*scalfa* and *scbeta*) must be the same for both formulations.

$$alfaTvivo = scalfa \times alfaTest \quad (11)$$

$$alfaRvivo = scalfa \times alfaRef \quad (12)$$

$$betaTvivo = scbeta \times betaTest \quad (13)$$

$$betaRvivo = scbeta \times betaRef \quad (14)$$

Fitting procedures were carried out with Phoenix WinNonlin (version 8.0) and Berkeley Madonna 9.1.19 with similar results. Codes in both software are provided in the Appendix A.

3. Results and Discussion

Figure 1 shows the average plasma levels for both assayed formulations (test and reference). Figure A1 in the Appendix A represents the individual plasma levels, and Figure A2 includes the average levels with error bars.

When the Tmax variability across individuals is very high (due to, for instance, a highly variable lag time (Tlag)), it could be possible that the average plasma profile does not represent the individual behavior, which complicates the development of an IVIVC. Nevertheless, if the Tlag and Tmax of all subjects and of the mean curves are close together (as it is the case in the present data), the use of a mean curve will not dramatically modify the results [6]. Consequently, it was decided that average plasma levels for deconvolution would be used.

As there is no intravenous CBZ data available, we could not identify the pharmacokinetic compartmental model of the drug. Nevertheless, in the literature, CBZ oral profiles have been successfully described with a one-compartment model with reasonable accuracy [26–28].

For that reason, the Wagner–Nelson deconvolution method was selected to estimate bioavailable fractions.

Figure 2 represents the fractions absorbed (actually bioavailable fractions) obtained by the Wagner–Nelson method. The plot is restricted up to 40 h to clearly show the differences on the initial times.

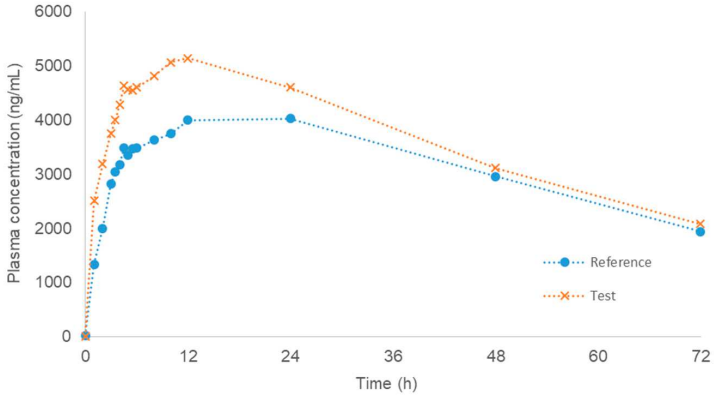

Figure 2. Reference and test average plasma levels (N = 11, pilot bioequivalence study).

In spite of the fact that the CBZ true compartmental pharmacokinetic model might be a two-compartment one, due to its lipophilicity ad long half-life, the Wagner–Nelson mass balance did not detect any relevant bias. As it can be observed in Figure 3, the fractions absorbed smoothly increase up to 100% without surpassing this value, as it is frequent when the procedure is applied to a two-compartment drug. This fact confirms the suitability of the one-compartment pharmacokinetic model to describe the CBZ absorption profile.

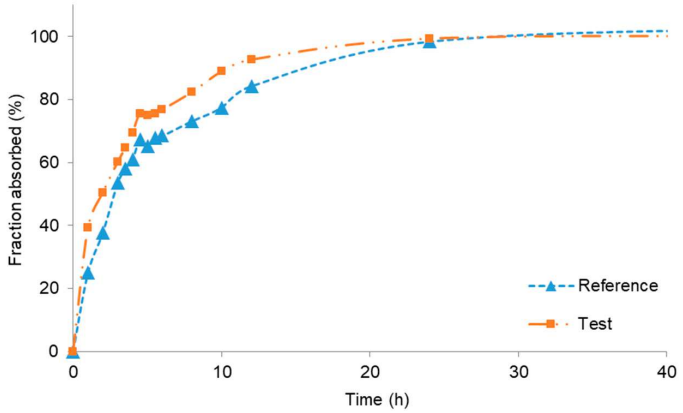

Figure 3. Bioavailable fractions (usually described as Fractions absorbed) obtained by Wagner–Nelson analysis from reference and test formulations.

3.1. Bioequivalence Results

Table 1 summarized the results of the pilot bioequivalence study (N = 11).

Statistical analysis of the bioequivalence study was performed with the adequate procedures for an average bioequivalence cross over design (two periods, two sequences, two formulations). The observed residual variability was low (less than 20%) thus the confidence interval calculation did not need any correction and the acceptance range was 0.8 to 1.25.

As it could be expected for a class II drug (low solubility, high permeability), the observed failure is on the pharmacokinetic parameter associated with the rate of absorption, Cmax. In a low solubility drug, the dissolution process can be the limiting factor for absorption, consequently formulation factors (as excipients) and drug factors (as particle size) affecting dissolution rate can influence Cmax. As long as the dissolution of both formulations is completed during transit time, the extent of absorption,

due to the high permeability, will be complete and similar (reflected in equivalent AUC values). In summary, Table 1 reflects the bioequivalence failure in absorption rate conditioned by the drug products dissimilar in vivo dissolution, leading to different input rates in the systemic circulation.

Table 1. Summary results of the pilot bioequivalence study (N = 11).

Parameter *	Geometric Mean Ratio %	90% CI	Power (1-Beta) %	CV_{ws} %
C_{max}	126.54	118.33–135.31	99.9	8.55
AUC_t	117.42	110.88–124.34	99.9	7.31
AUC_{inf}	111.60	104.34–119.37	99.9	8.59

* Parameters logarithmically Ln-transformed. Cmax, maximum plasma concentration; AUCt, area under the concentration-time curve from 0 to 96 h; AUCinf, area under the concentration–time curve extrapolated to infinity; CI, confidence interval; CVws, coefficient of variation within subject. Detailed equations for calculations of parameters (geometric mean ratio, CI and CV_{ws} are included in Appendix A).

3.2. Modeling Dissolution Data

First order and Weibull dissolution models were fitted to the in vitro dissolution data, but the best fit was obtained with the Weibull model. Table 2 summarizes the kinetic parameters and several indexes of goodness of fit. As it can be seen for both formulations, the Weibull model provided a statistically significant better fit.

Table 2. Fitted parameters of Weibull function and First order model to the in vitro dissolution data. Indexes of goodness of fit: R_obs_pred: correlation coefficient of experimental versus predicted values. AIC: Akaike's information criteria. SS: sum of squared residuals. Df: degrees of freedom estimated as number of data minus number of parameters. Ftab: tabulated F value for 0.05 probability. Fcal: calculated F value.

Parameter	Weibull		First Order	
	Test	Reference	Test	Reference
α	0.418	0.787	/	/
β	0.687	0.47	/	/
kd(h^{-1})	/	/	3.837	2.122
R_obs-pre	0.996	0.994	0.995	0.982
AIC	22.972	22.246	33.532	43.880
SS	23.618	20.926	191.575	1074.971
df	4	4	5	5
Ftab(0.05;1:4)	7.709			
Fcalc Test	28.445	Fcal > Ftab	Weibull model is the best	
Fcalc Ref	201.482	Fcal > Ftab	Weibull model is the best	

Correlation coefficients of the Weibull model were higher than the ones obtained with the First order model. AIC values were lower for the more complex model, indicating a better fit than the simple model. The sum of squared residuals was clearly smaller for the Weibull function. When residual variances (Sum of squared corrected by their degrees of freedom) were compared through F test, the comparison indicated a statistically significant improvement with the Weibull model versus the First order equation. As the best model was the same for both formulations, it also indicated a similar dissolution mechanism.

The dissolution media and method used in this study were selected based on previous reports, indicating its biopredictive ability and previously developed IVIVC. Taking into account the reported values for CBZ solubility (2.96 mg/mL in deionized water containing 1% of SLS [29] and

3.412 ± 0.13 mg/mL in the same media [30]) 900 mL of media does not provide sink conditions for a 400 mg tablet. The maintenance of sink conditions is, in general, required in quality control media, but for IVIVC, non-sink conditions can be of application. In actuality, non-sink conditions can be more reflective of the in vivo environment in which the available volume for dissolution is less than 500 mL [31]. Other authors have recently proposed a non-sink dissolution permeation method to discriminate among CBZ formulations with and IVIVC developed with mice data [32].

When in vitro fractions dissolved versus time plots and in vivo fractions absorbed versus time were compared, it is obvious that the time scales are different, i.e., dissolution is completed in less than 4 h while in vivo absorption took almost 20 h to be completed. In consequence, the direct correlation of fractions absorbed versus fractions dissolved is not possible without the time scaling procedure.

3.3. *In Vitro–In Vivo Data Modeling*

To establish the ivivc it is necessary to establish, first of all, a Levy-plot

Table 3 in columns 1 to 4 summarizes the calculations to obtain the in vitro times that can be used to construct the Levy plot represented in Figure 4.

Table 4 represents the in vitro original times scaled to in vivo times using the Levy plot. Once the in vitro times have been scaled up to in vivo times, then the scaled dissolution profile, represented in Figure 5, can be used to construct the Level A IVIVC.

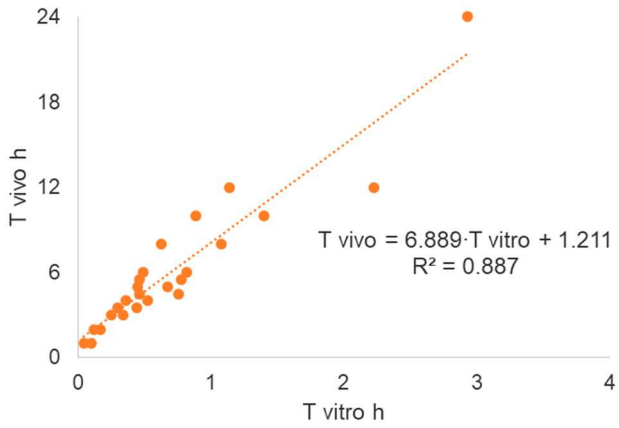

Figure 4. Levy plot constructed with data in Table 3. Times up to 24 h were used for test formulation and up to 12 h for reference formulation. Later points were excluded, as the relationship was no longer univocal.

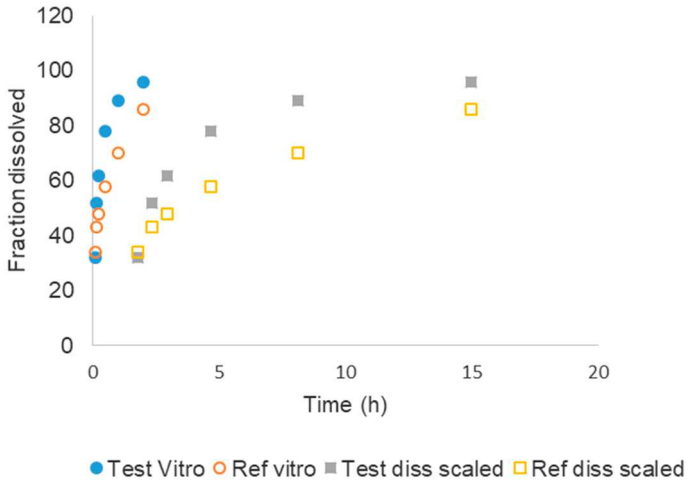

Figure 5. Dissolution profiles in their original in vitro time scale and scaled to equivalent in vivo times (see Table 4) with the Levy plot.

Table 3. Columns 1 to 4: Calculations for the Levy plot. Second and third column are the original in vivo data. Fourth column, "t vitro" are obtained from the Weibull function used to fit the in vitro profiles of each formulation using Equation (4) (see step 2 in Figure 1) and interpolation fraction absorbed values, i.e., in the in vitro dissolution profile it takes 0.1 h to get 39.23% dissolved. Column 5 fractions dissolved estimated at the original in vivo sampling times. Column 3 and 5 were used to represent the IVIVC. Columns 6 and 7 predicted fractions absorbed from the IVIVC linear relationship and the back-calculated predicted plasma concentrations.

1	2	3	4	5	6	7
Formulation	**T Vivo (h)**	**Fabs Vivo**	**T Vitro Equivalent to T Vivo (h)**	**Interpolated Fdiss**	**Fabs Predicted with IVIVC**	**Conc Predicted (ng/mL)**
Test	1	39.23	0.10	22.33	0.31	1829.6
Test	2	50.50	0.17	42.76	0.48	2813.7
Test	3	60.14	0.25	58.80	0.62	3564.5
Test	3.5	64.55	0.30	65.27	0.68	3858.3
Test	4	69.50	0.36	70.83	0.72	4104.3
Test	4.5	75.54	0.46	75.57	0.76	4307.9
Test	5	74.89	0.45	79.60	0.80	4474.5
Test	5.5	75.40	0.46	83.01	0.83	4608.8
Test	6	76.74	0.49	85.89	0.85	4715.3
Test	8	82.36	0.63	93.41	0.91	4938.1
Test	10	88.90	0.88	97.01	0.94	4960.6
Test	12	92.68	1.14	98.68	0.96	4887.2
Test	24	99.34	2.93	99.99	0.97	4096.8
Test				100.00	0.97	2785.3
Test				100.00	0.97	1893.4
Ref	1	24.90	0.04	25.96	0.34	2009.8
Ref	2	37.73	0.12	38.31	0.45	2590.2
Ref	3	53.43	0.34	47.14	0.52	2987.1
Ref	3.5	58.02	0.44	50.76	0.55	3143.6
Ref	4	60.96	0.53	53.99	0.58	3279.4
Ref	4.5	67.24	0.76	56.89	0.60	3397.9
Ref	5	65.22	0.67	59.52	0.63	3501.8
Ref	5.5	67.72	0.78	61.91	0.65	3593.2
Ref	6	68.49	0.82	64.10	0.67	3673.8
Ref	8	73.18	1.08	71.28	0.73	3911.7
Ref	10	77.45	1.40	76.62	0.77	4052.2
Ref	12	84.31	2.22	80.72	0.81	4127.4
Ref *	24	98.46	12.53	92.90	0.91	3964.7
				98.58	0.96	2933.2
				99.63	0.97	2038.4

* Indicated excluded value for the Levy plot.

Table 4. Vitro times scaled to vivo times using the Levy plot.

Formulation	Original t Vitro (hours)	Scaled t Vitro to t Vivo (Hours)
Test	0.083	1.785
Test	0.167	2.359
Test	0.250	2.933
Test	0.500	4.655
Test	1.000	8.099
Test	2.000	14.988
Ref	0.083	1.785
Ref	0.167	2.359
Ref	0.250	2.933
Ref	0.500	4.655
Ref	1.000	8.099
Ref	2.000	14.988

The scaled profiles were again used to fit the Weibull model and obtain the scaled Weibull parameters which are summarized in Table 5.

Table 5. Weibull parameters of the in vitro profile scaled up to the in vivo times.

Parameter	Test	Parameter	Reference
α	3.958	α	3.327
β	1.143	β	0.684

With the new Weibull parameters, it is possible to estimate the fraction dissolved at any time. Table 3 in column 5 shows the in vitro dissolved fractions estimated at the same equivalent in vivo times with the new scaled Weibull parameters.

The data in Figure 6 represent the final two-step Level A IVIVC.

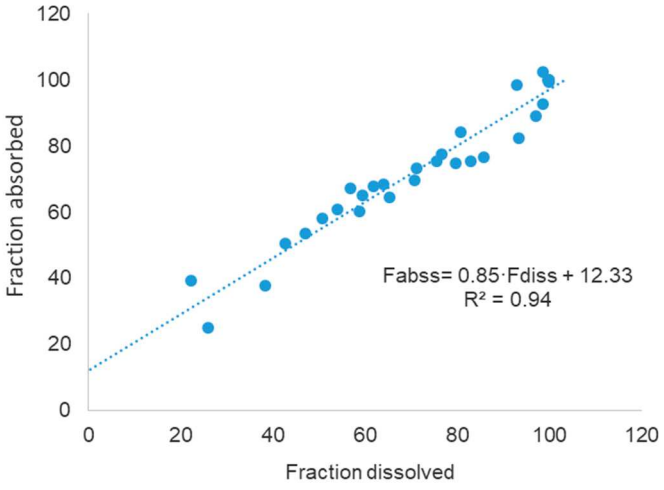

Figure 6. Two-step IVIVC model.

The linear correlation depicted in Figure 6 presents a good determination coefficient (R^2) and it is clearly a single relationship for both formulations. Both aspects indicate in the first place that

dissolution is the limiting step for the input of CBZ in the systemic circulation, and—in second place—that the in vitro method, despite its simplicity, reproduced the in vivo dissolution.

3.4. Two-Step IVIVC Predictability

In order to check the predictability of the correlation, the theoretical fractions absorbed were estimated from the fractions dissolved through the linear relationship (see Figure 6). The theoretical or predicted Fabs were back-transformed in plasma concentrations represented in Figure 7 and Table 3 (column 7), and the prediction error of Cmax and AUC was estimated.

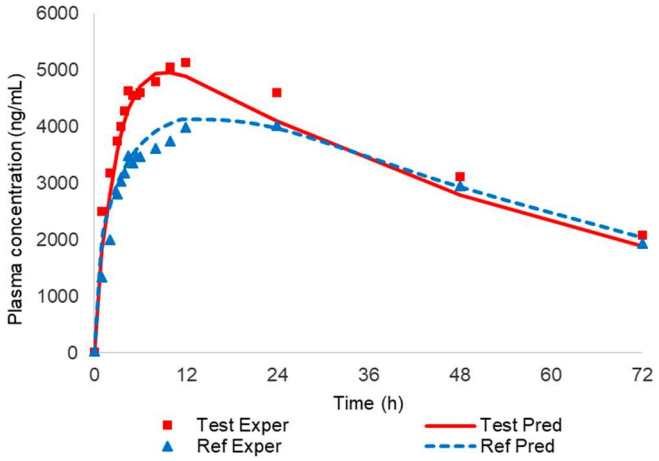

Figure 7. Experimental and predicted plasma levels of both carbamazepine (CBZ) formulations with the two-step IVIVC Level A approach.

Prediction errors of the two-step approach are summarized in Table 6.

Table 6. Predictability analysis of the two-step IVIVC approach.

Parameter	Experimental	Predicted	% Error
Cmax Test (ng/mL)	5135.86	4960.58	3.41
Cmax Ref (ng/mL)	3995.05	4127.41	−3.31
AUC_t Test (ng/mL∗h)	388,931.0	355,617.0	8.57
AUC_t Ref (ng/mL∗h)	354,986.6	364,858.3	−2.78

From a regulatory point of view, the prediction errors were within the accepted limits (up to 15% for each formulation with an average of 10% for all formulations) [4,5].

In conclusion, the in vitro dissolution profiles can be used to predict plasma levels of CBZ from its IR products.

3.5. IVIVC One-Step

In Figure 8 the experimental and predicted values in vitro and in vivo with the one-step approach are displayed.

The prediction errors with the one-step IVIVC approach are summarized in Table 7.

Parameters of the model are summarized in Table 8.

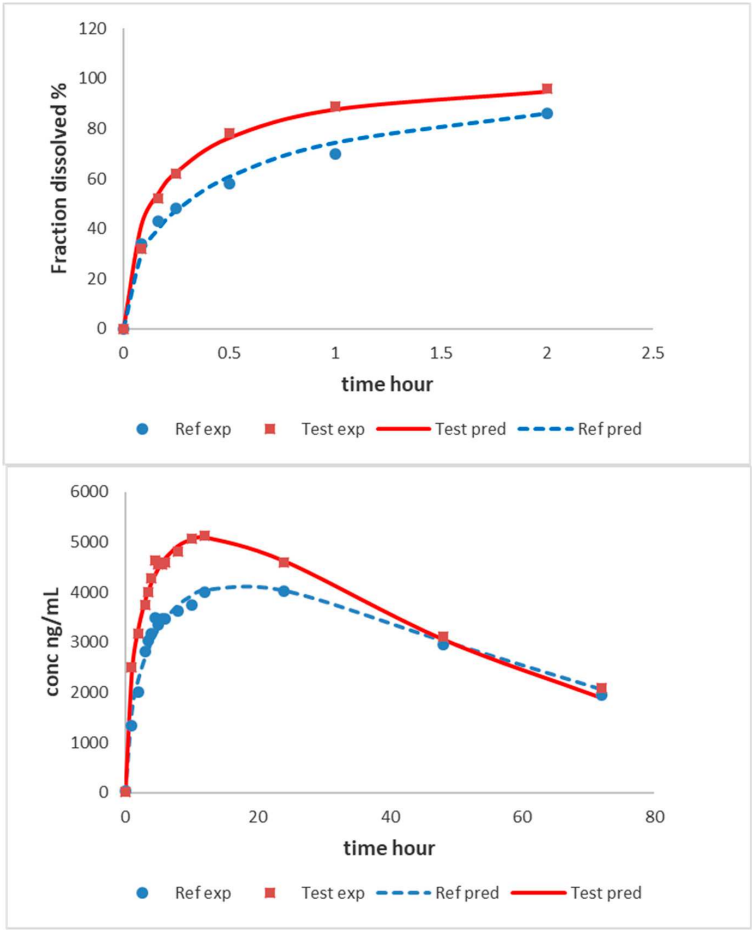

Figure 8. Experimental (symbols) and predicted (lines) in vitro fraction dissolved and plasma levels from reference and test formulations with the one-step IVIVC Level A approach.

Table 7. Predictability analysis of the one-step IVIVC approach.

Parameter	Experimental	Predicted	% Error
Cmax Test (ng/mL)	5135.86	5093.17	0.83
Cmax Ref (ng/mL)	3995.05	4053.67	−1.47
AUC_t Test (ng/mL*h)	388,931.0	374,575.0	3.69
AUC_t Ref (ng/mL*h)	354,986.6	368,207.0	−3.72

Table 8. Parameters of the model.

Parameter	Estimate	Standard Error
alfaT	0.455	0.722
betaT	0.555	1.269
scalfa	5.627	8.969
alfaR	0.717	1.138
betaR	0.542	1.240
scbeta	1.039	2.377
kel h^{-1}	0.022	0.003
Vd mL	51,686.649	6498.458

For the one-step procedure, the prediction errors were slightly lower for all the parameters, and it is interesting to observe that the plasma profiles are better captured in the one-step procedure compared with the two-step one (see predicted plasma profiles in Figure 7 versus Figure 8). Nevertheless, from the standpoint of prediction errors, both approaches are adequate and allowed for the prediction of the plasma levels from the dissolution profiles. In the EMA guidance [5], the recommended procedure is the one-step IVIVC using a mechanistic model. Nevertheless, it is also advised that constructing, as a first approach, the two-step calculations might give some insights into the mechanistic relationship between in vitro and in vivo dissolution such as, for instance, the need for a time-scaling factor detected with the Levy's plot. FDA guidance [4] requests the two-step procedure, which might not be possible to construct. For instance, for some complex relationships between in vitro and in vivo dissolution/absorption when a preferential dissolution segment or absorption windows exist, a single IVIVC linear two-step correlation might be challenging. In those complex situations, connecting the in vitro dissolution with in vivo plasma profiles requires a mechanistic model defined with differential equations [33,34].

3.6. Significance of the Results

CBZ is a non-ionizable molecule whose solubility does not change with pH [35]. Its permeability is high in human intestines compared to metoprolol [36] and passive diffusion is the main absorption mechanism due to its lipophilicity. In accordance with these characteristics, its dissolution process is not affected by the transit to one intestinal segment to the next, and the dissolved amounts are absorbed at a similar rate in the different intestinal segments. In consequence, the challenges for the classical two-step IVIVC approach are minimal as long as the in vitro dissolution method reflects the in vivo dissolution process [37]. In this work, similar predictive performance was achieved with both mathematical approaches. On the other hand, the mechanistic model used in the one-step approach only used a time scaling factor for the in vitro dissolution parameters, which can be directly convoluted with the disposition CBZ parameters without the need of any other kinetic feature.

The differences between formulations in vitro and in vivo may be explained by the difference in particle size distribution. As CBZ is a BCS class II drug, particle size as well as polymorphism are key topics to be investigated in order to ensure the bioequivalence between prototypes and reference products [38]. Both products have the same polymorphic form (form III) which was characterized by X-Ray Diffraction (in-house data). The work performed by Sehić S. et al. [39] demonstrated that commercial samples from the same polymorphic form III (anhydrous) presented particles with different morphology and size distribution. Those differences clearly impacted the kinetics of conversion from anhydrous to the dihydrate CBZ and the dissolution behavior of their formulations.

Moreover, in order to figure out the likely influence of excipients in the in vitro and in vivo behavior of both formulations, a dissolution profile was carried out with crushed tablets of both products in the media containing 1% of SLS (in-house data, not shown). The same difference with intact tablets was observed in the dissolution profiles with crushed tablets, and in consequence, differences in disintegration can be ruled out as a potential source of dissolution differences. Therefore, it is possible to infer that API characteristics may play a significant role in the in vitro and in vivo behavior of the CBZ formulations used in this study. On the other hand, it is not possible to completely rule out the role of excipients, as they might influence, for instance, particle wettability. A previous clinical study, using the same reference product and a test product manufactured with smaller particle size showed also statistically significant Cmax differences in epileptic patients. [40].

The assayed pharmaceutical products used in this work were formulated with common and non-problematic excipients (those affecting transit time or permeability) CBZ dissolution from these products is driven mainly by CBZ particle size. In combination with the previously discussed physicochemical characteristics, a simple unbuffered media (but with SLS at 1% to ease particle wetting and ensure complete dissolution) might reproduce the in vivo dissolution. Even if the in vivo dissolution environment is much more complex, most of the in vivo factors (as pH changes during

transit, bile secretions presence) do not affect the in vivo dissolution of CBZ. Consequently, these factors do not need to be incorporated in the in vitro dissolution model, which can be kept as simple as possible.

As this medium was able to distinguish between differences in API particle size, it could be a highly relevant tool in Latin-American countries to ensure and check the quality of their oral CBZ IR marketed products for which BE studies have not been requested by most health authorities [41].

In this work, it was shown that both approaches, two-step, recommended in FDA guidance, and one-step, recommended in EMA guidelines, provided good in vivo predictability for CBZ, a BCS class II drug.

This article reinforces the evidence that, for CBZ, the same dissolution medium can be used during the product development as well as for quality control purposes. Some authors have suggested approaches for establishing the link between the dissolution test and in vivo performance [42,43]. Therefore, this is a unique situation, as in general, quality control (QC) dissolution methods are of application only to a particular pharmaceutical product to check the consistence of the manufacturing process. QC methods do not have, in most cases, any biopredictive aim, while in the case of CBZ products, it would be possible with the QC method to correlate possible deviations in the manufacturing process (discriminatory power) and their in vivo impact (biopredictive relevance) for a narrow therapeutic index drug, such as CBZ.

4. Conclusions

As proposed by previous studies, the dissolution method in apparatus USP II at 75 rpm with 900 mL of aqueous media containing 1% of SLS was successfully used to develop a Level A IVIVC with two CBZ oral IR products which provides additional evidence that this medium can be classified as a biopredictive dissolution tool for CBZ oral IR products with conventional excipients. On the other hand, we confirmed the similar outcome of one-step versus two-step procedures for IVIVC in an uncomplicated drug (constant solubility, no absorption window, no carrier mediated absorption or saturated metabolic step).

Author Contributions: Methodology, Supervision, investigation, writing—review and editing I.G.-A.; Supervision, writing—review and editing, Conceptualization, project administration, funding acquisition, D.R.C.; Supervision, writing—review and editing, Conceptualization, Validation, Visualization, M.G.D. and P.d.O.C.; Methodology, software, Investigation, formal analysis, writing—original draft preparation, M.B. and J.M.; All authors have read and agreed to the published version of the manuscript.

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Appendix A

Berkeley Madonna Code

```
METHOD RK4

STARTTIME = 0.001
STOPTIME = 72
DT = 0.02
rename TIME = t

Init(QdissR) = 0; fraction dissolved Reference
Init(QcR) = 0; Plasma levels Reference

Init(QdissT) = 0; fraction dissolved Test
```

Init(QcT) = 0; Plasma levels Test

;initial estimations of parameters Reference
alfaR = 0.787
betaR = 0.470
FmaxR = 100; Fixed to 100
alfaResc = slope*alfaR
betaResc = slope2*betaR

;initial estimations of parameters Test
alfaT = 0.418
betaT = 0.687
FmaxT = 100; Fixed to 100
alfaTesc = slope*alfaR
betaTesc = slope2*betaT

; dose in ng; 400 mg
Dose = 400000000
;kel and Vc fixed
;kel h^{-1}
kel = 0.0159
;Vc in mL
Vc = 67,698.44

;initial estimations of scaling factor parameters
slope = 1
slope2 = 1

;Differential equations
QdissR′ = (betaR*FmaxR*(tˆ(betaR-1))*exp((-(tˆ(betaR)))/alfaR))/alfaR
QcR′ = ((Dose*betaResc*(FmaxR/100)*(tˆ(betaResc-1))*exp((-(tˆ(betaResc)))/alfaResc))/alfaResc)-(kel*QcR)

QdissT′ = (betaT*FmaxT*(tˆ(betaT-1))*exp((-(tˆ(betaT)))/alfaT))/alfaT
QcT′ = ((Dose*betaTesc*(FmaxT/100)*(tˆ(betaTesc-1))*exp((-(tˆ(betaTesc)))/alfaTesc))/alfaTesc)-(kel*QcT)

;integrated plasma levels
CpR = QcR/Vc
CpT = QcT/Vc

ASCII code Phoenix Winnonlin

Model 1
remark **********
remark Developer: Marival Bermejo
remark Model Date: 21-July-2019
remark Model Version: 1.0
remark **********

```
remark one-step ivivc for CBZ
remark-define model-specific commands
COMMANDS
NFUNCTIONS 4
NDERIVATIVES 4
NPARAMETERS 8
PNAMES 'alfaT','betaT','scalfa','alfaR','betaR','scbeta','kel','Vd'
Ncons 2
END
remark-define temporary variables
TEMPORARY
Dose = con(1)
Remark Dose in ng 400000000
Fmax = con(2)
Remark Fmax fixed at 100
alfaT = P(1)
betaT = P(2)
scalfa = P(3)
alfaR = P(4)
betaR = P(5)
scbeta = P(6)
kel = P(7)
Vd = P(8)
t = x
ti = t + 0.0001
Remark time shift to avoid floating point error
END
remark-define differential equations starting values
START
Z(1) = 0
Z(2) = 0
Z(3) = 0
Z(4) = 0
END
remark-define differential equations
DIFFERENTIAL
alfaTvivo = scalfa*alfaT
alfaRvivo = scalfa*alfaR
betaTvivo = scbeta*betaT
betaRvivo = scbeta*betaR
DZ(1) = (betaT*Fmax*(ti**(betaT-1))*exp((-(ti**(betaT)))/alfaT))/alfaT
DZ(2) = (betaR*Fmax*(ti**(betaR-1))*exp((-(ti**(betaR)))/alfaR))/alfaR
DZ(3) = ((Dose*betaTvivo*(ti**(betaTvivo-1))*exp((-(ti**(betaTvivo)))/alfaTvivo))/alfaTvivo)-kel*z(3)
DZ(4) = ((Dose*betaRvivo*(ti**(betaRvivo-1))*exp((-(ti**(betaRvivo)))/alfaRvivo))/alfaRvivo)-kel*z(4)
END
remark-define algebraic functions
FUNCTION 1
```

F = Z(1)
END
FUNCTION 2
F = Z(2)
END
FUNCTION 3
F = Z(3)/Vd
END
FUNCTION 4
F = Z(4)/Vd
END
remark-define any secondary parameters
remark-end of model
EOM

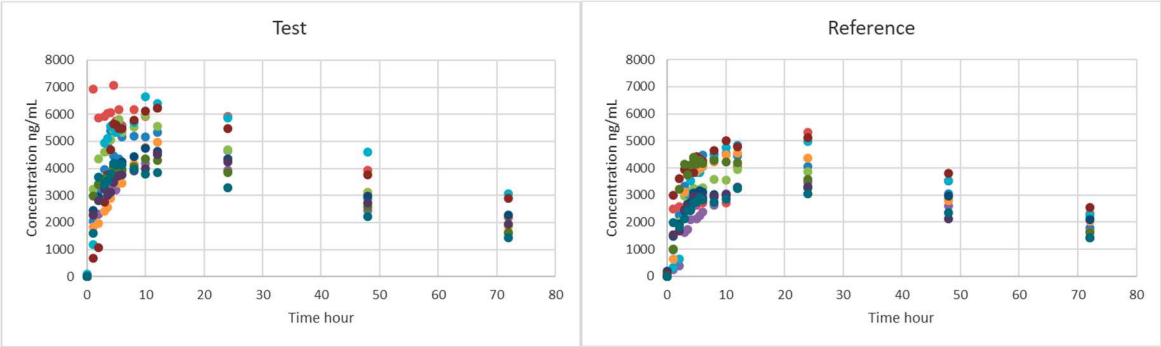

Figure A1. Individual CBZ plasma levels of the reference and test formulations.

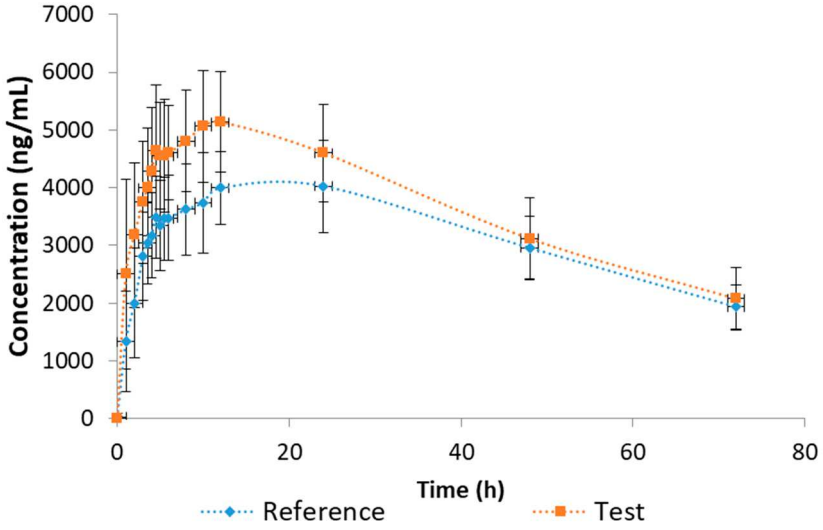

Figure A2. Average plasma levels of Reference (solid circles) and Test (solid squares) formulations and their standard deviation.

Bioequivalence calculations:

90% confidence intervals of the ratio of geometric means of the corresponding variable (Cmax or AUC) were estimated as

$$\left(\overline{LnY_{test}} - \overline{LnY_{reference}} \right) \pm t(alfa,\ N-2) * SE$$

In which LnY_{test} and $LnY_{reference}$ represents the average of the natural logarithms of individual AUC or Cmax values of test and reference respectively. A difference of arithmetic averages in the natural logarithm scale corresponds to the ratio of the geometric means in the numerical scale. Student *t* value for a probability of type I error (alfa) of 0.05 and N-2 degrees of freedom where *N* is the number of subjects in the BE trial. *SE* is the standard error, which is calculated from the ANOVA residual variance (also called within subject) as:

$$SE = \sqrt{2 * residual\ variance / N}$$

Within subject coefficient of variation (CV_{ws}) is then calculated (in the numerical scale) as

$$CV_{ws} = \sqrt{e^{residual\ variance} - 1}$$

The power of the test is calculated as 1-Beta, where Beta is the probability of a type II error (failing to reject the null hypothesis (Inequivalence or nonequivalence) when the null hypothesis is false. Note in BE analysis it is used a procedure called "reversed hypothesis" meaning the null hypothesis is nonequivalence and the alternative hypothesis is Bioequivalence. Power estimation is explained in detail in Chapter 5 of Chou SC and Liu J 2009 [44].

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4. CONCLUSÃO

Com base nos artigos avaliados na primeira etapa deste trabalho (*Capítulo I*) foi possível revisar e identificar as principais características dos estudos de CIVIV publicados nas últimas décadas para formulações orais, tais como: 1) os principais modelos matemáticos aplicados, 2) os principais aparatos utilizados nos ensaios de dissolução, 3) as principais classes biofarmacêuticas e formas farmacêuticas envolvidas, 4) as metodologias *in vivo* e *in vitro* adotadas, entre outros aspectos. Assim, por meio de uma robusta avaliação técnica destes dados foi possível pontuar os pontos fortes, bem como os que necessitam de aprimoramento por parte dos pesquisadores envolvidos nessa área. Além disso, um compilado das características dos estudos contemplando 45 substâncias diferentes foi construído e está disponível publicamente para consulta de pesquisadores que pretendem trabalhar em abordagens de CIVIV com formulações contendo esses fármacos. Assim, a primeira etapa deste trabalho possibilitou realizar uma importante "fotografia" do uso da CIVIV no desenvolvimento de formulações orais.

A segunda etapa deste trabalho (*Capítulos II* e *III*) envolveu a publicação de dois artigos científicos em periódicos internacionais. Primeiramente, uma CIVIV de nível A foi estabelecida entre os dados dissolução e fração absorvida de duas formulações de succinato de desvenlafaxina monoidratado 50 mg comprimido revestido de liberação prolongada. O modelo matemático utilizado para estabelecer CIVIV foi validado internamente, confirmando a capacidade de predição da performance *in vivo* da formulação através da metodologia de dissolução avaliada. Assim, este modelo poderá ser aplicado para o desenvolvimento mais assertivo de formulações de liberação modificada candidatas à medicamentos genéricos, bem como para prever o impacto *in vivo* de possíveis alterações pós-registro. Em um segundo momento, uma CIVIV nível A também foi estabelecida para uma formulação de carbamazepina 400 mg comprimido. Foi demonstrado que o meio de dissolução contendo 1% de LSS pode ser utilizado como uma ferramenta altamente relevante para predição do desempenho *in vivo* de formulações contendo este fármaco. Como essa metodologia está disponível para fins de controle de qualidade (farmacopeia americana), isso demonstra uma condição rara e altamente relevante que é a ocorrência de uma metodologia de dissolução apresentar ambas as propriedades, capacidade biopreditiva e discriminativa. Além disso, como se trata de um fármaco de estreita faixa terapêutica, tal condição se apresenta como uma poderosa

ferramenta para monitorar a qualidade dos lotes fabricados. Como exemplo da relevância desse trabalho, pode-se destacar a recente citação deste artigo pela monografia de bioisenção dessa molécula publicada pela *International Pharmaceutical Federation* (FIP) (GARCÍA et al., 2021)

Por fim, este trabalho foi concluído com a publicação de três artigos científicos em periódicos internacionais, os quais possibilitaram: 1) uma revisão sistemática do *status quo* da CIVIV para formulações orais, 2) a aplicação do modelo de CIVIV para uma formulação de liberação modificada e, por fim, 3) a aplicação do modelo de CIVIV para uma formulação de liberação imediata contendo um fármaco BCS classe 2. Tais dados científicos se apresentam como um conjunto de ferramentas que poderão ser utilizadas como importantes referências para pesquisadores interessados na aplicação da CIVIV no desenvolvimento de formulações candidatas a medicamentos a genéricos e similares, na definição da especificação dos limites de dissolução e, também, em possíveis bioisenções para alterações pós-registro. Essas frentes de trabalho poderão auxiliar na redução no número de ensaios de bioequivalência, ou seja, permitirão reduzir a exposição de indivíduos sadios aos procedimentos realizados nestes estudos, destacando a importância ética dessa linha de pesquisa.

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