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IVIVC approach based on carbamazepine bioequivalence studies combination

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

1. Introduction

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

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

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

Carbamazepine (CBZ) is an anticonvulsant and mood-stabilizing drug, classified as BCS Class II drug. Two different level A and a level C carbamazepine IVIVCs have been published (Lake et al. 1999; Veng-Pedersen et al. 2000; Kovacevic et al. 2009), using the same *in vitro* dissolution media and conditions. This level of correlation with the adequate internal and external validation is the warranty of similar drug absorption in terms of rate and extent and supports the claim of a biowaiver to the regulatory authority.

The aim of the present study was to explore the feasibility of obtaining an IVIVC by combination of data from two bioequivalence (BE) studies of CBZ in order to assess if the previously published dissolution media and conditions (Veng-Pedersen et al. 2000; Kovacevic et al. 2009) could be applicable to any other oral immediate release (IR) CBZ product with conventional excipients. Therefore, it would be possible to assess the usefulness of

Abbreviations

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

in vitro dissolution data from different laboratories to predict *in vivo* concentration profiles. The second objective was to evaluate the variability associated to the use of data obtained in different laboratories as well as data from different *in vivo* studies and the influence of the use of individual or average data on the establishment of an IVIVC.

2. Investigations and results

2.1. In vivo data

Figure 1 shows the average plasma profiles of CBZ test and reference formulations in both BE studies. Mean pharmacokinetic parameters of each *in vivo* BE study obtained from individual parameters are shown in Table II.

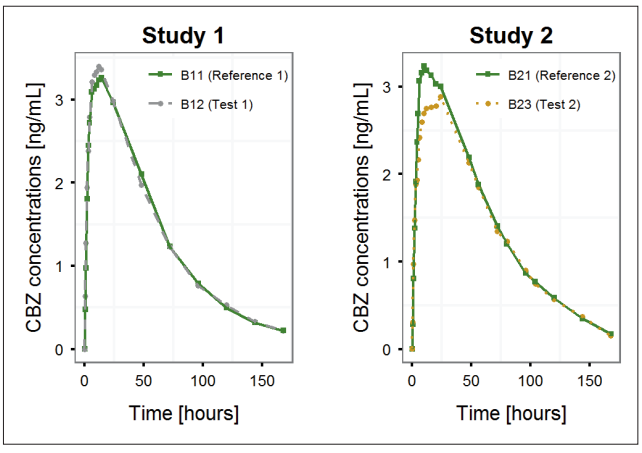

Fig. 1: Carbamazepine *in vivo* profiles. B = *in vivo* data; first digit study (1; 2); second digit product (1=reference, 2=test, 3=test).

2.2. In vitro data

Average dissolved fractions versus time from all the formulations obtained in two laboratories are presented in Fig. 2. Sampling

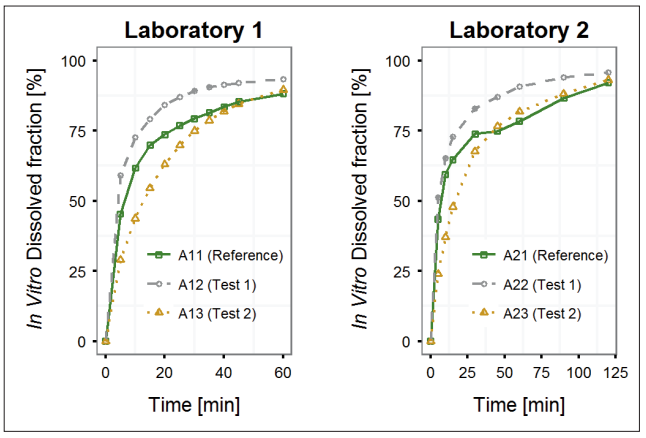

Fig. 2: Carbamazepine mean *in vitro* dissolution profiles from each laboratory. A = *in vitro* data; first digit study (1 = laboratory 1, 2 = laboratory 2); second digit product (1 = reference, 2 = test 1, 3 = test 2).

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

Table 1: *In vivo* parameters for both bioequivalence studies

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

Identification code B=*in vivo* data; first digit study (1;2); second digit product (1=reference, 2=test 1; 3=test 2). Formulations labeled with the asterisk are the normalized formulations. CV is expressed in %. Left values belongs to the mean parameters and right values were obtained from the averaged profiles.

Table 2: *In vitro* first order model parameters for both laboratories

	Identification code	AUC (%·min)		MDT (min)		t_{25} (min)		t_{50} (min)		t_{75} (min)		t_{80} (min)		f_2 (intra-laboratory)		f_2 (inter-laboratory)	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	EMA	FDA	EMA	FDA
Reference	A11	4462.2	2.1	10.2	5.3	1.2	12.9	4.3	9.0	11.8	6.3	14.6	6.6				
Test 1	A12	4896.3	1.0	7.8	12.5	0.8	19.0	8.3	8.5	8.3	8.5	10.4	7.8	51.06	51.57		
Test 2	A13	3997.6	1.0	15.4	1.1	4.0	0.5	9.9	0.5	20.4	1.2	23.9	2.7	44.93	47.30		
Reference	A21	9138.4	5.3	20.7	13.5	1.0	53.6	1.0	53.6	19.1	21.9	24.4	30.4			56.80	54.13
Test 1	A22	10158.9	3.5	13.9	10.7	0.9	37.2	4.0	17.9	13.9	6.6	18.7	10.4	54.57	51.62	57.71	58.75
Test 2	A23	8832.0	3.0	25.1	9.2	4.9	18.4	13.6	12.2	30.7	11.7	36.8	11.6	43.94	43.94	57.66	57.65

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

Table 3: Correlation coefficient values for IVIVC level C

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

Formulations labeled with the asterisk are the normalized formulations.

2.3. In vitro-in vivo correlations

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

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

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

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

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

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

The analysis of the extreme tablets (Table 4) i.e. those with the fastests and slowest dissolution rate showed that the fastest tablet of the fastest dissolving formulation (FTFF) fulfill the BE criteria, whereas the slowest tablet of the slowest dissolving formulation (STSF) did not satisfy the BE for C_{max}, being smaller than the lower limit of the 90% confidence interval (0.8) for the BE study.

Table 4: Results for the analysis of the extreme tablets based on the BE criteria

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

FTFF = fastest tablet of the fastest formulation; STSF = slowest table of the slowest formulation.

2.4. Internal validation

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

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

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

3. Discussion

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

3.1. In vivo data

In vivo parameters from both BE studies of CBZ were similar among studies and the coefficients of variation were low (7%). The double peak that appears in Study B profile may be due to CBZ

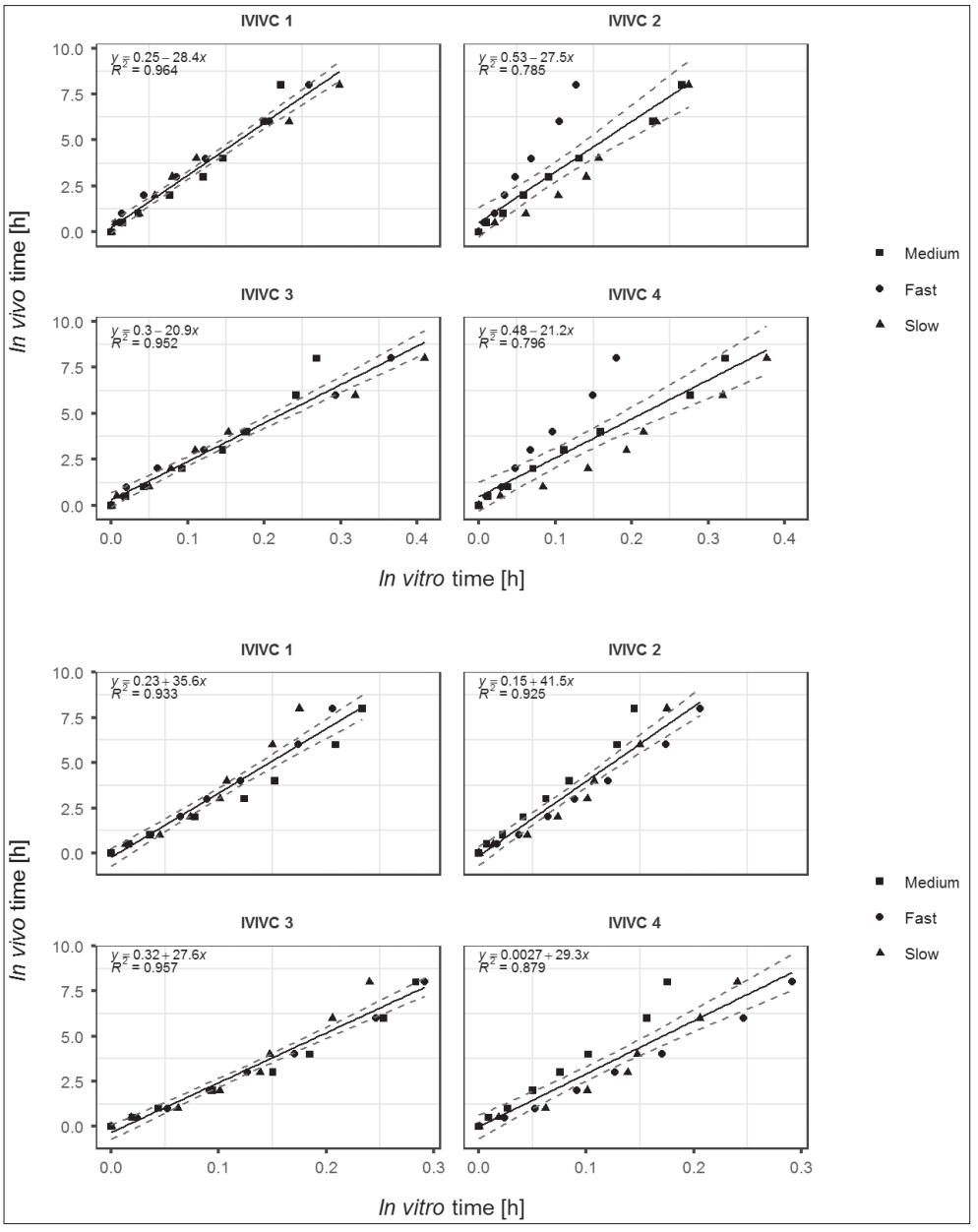

Fig. 3: Levy plots shows the correlation between *in vivo* absorbed time and *in vitro* dissolved time (top: average method, bottom: individual method). Linear regression is represented by the solid line and dashed lines represent the 95% prediction confidence interval.

entero-hepatic cycle (Fleischman and Chiang 2001). The absence of those peaks in profiles from Study A might be explained because of the absence of sampling in this time interval. This phenomenon does not strongly affect the estimation of pharmacokinetic parameters because only a slightly greater AUC value of Reference B was obtained due to the double-peak. The normalization procedure used with the reference (bioequivalent) products was done in order to reduce between-studies variability.

3.2. *In vitro data*

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

3.3. *IVIVC Level B*

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

3.4. *IVIVC Level C*

The best level C correlations were obtained for C_{max}. One of the goals of this work was to explore the influence of the *in vivo* and *in vitro* data sources in the quality of the IVIVC obtained. The differences associated with the use of different *in vitro* data sets are very small (Table 3). This is in accordance with other publications on IVIVC which conclude that variability between *in vitro* profiles is much lower than from *in vivo* data (Gaynor et al. 2009; Cardot and Davit 2012). Thus, the influence of laboratory/experimental conditions or inter-batch variability in the establishment of an IVIVC is much less evident, obtaining consistent IVIVC fittings among different laboratories, if experimental conditions are equally kept.

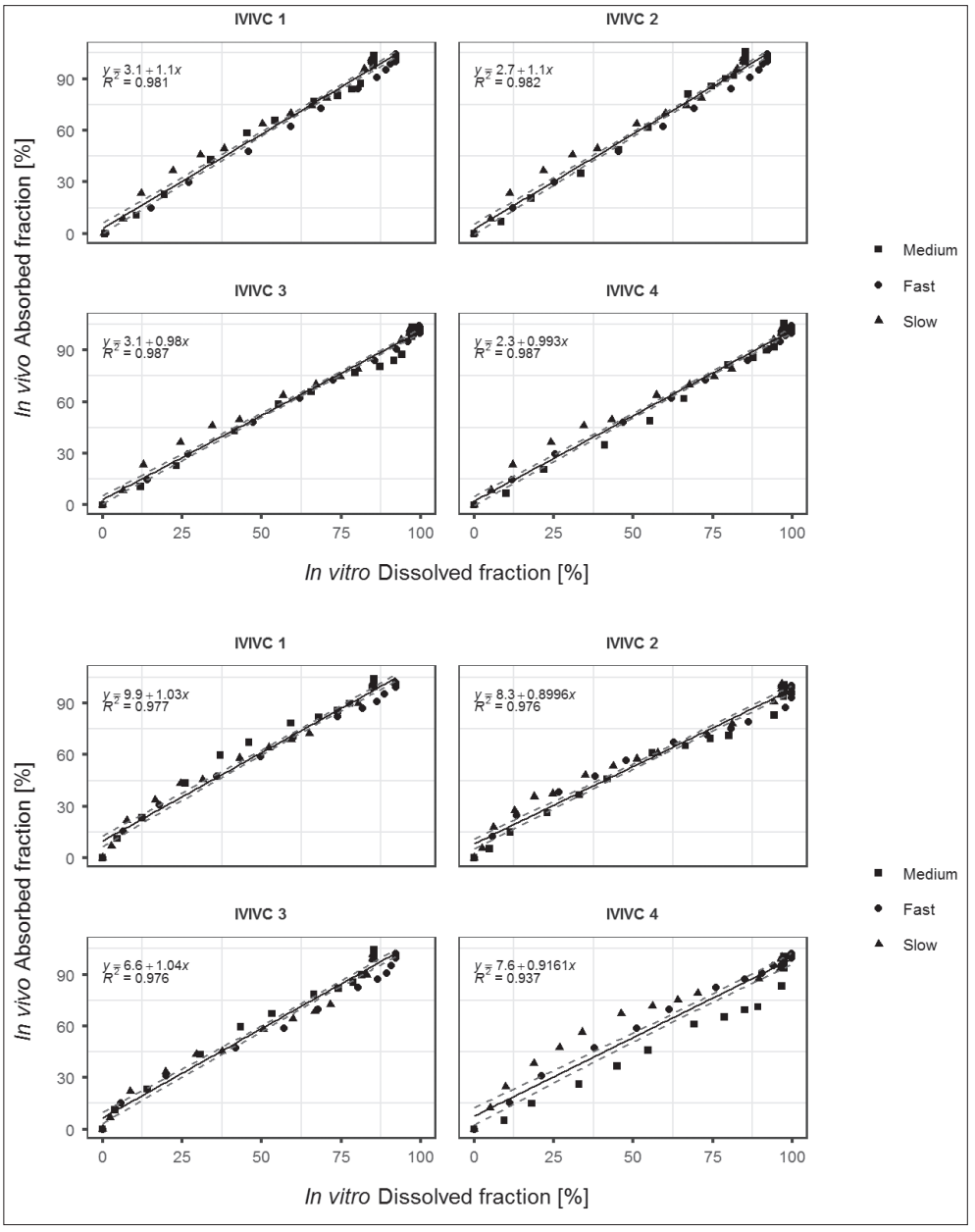

Fig. 4: IVIVC level A shows the correlation between *in vitro* dissolved fractions and *in vivo* absorbed fractions (top: average method, bottom: individual method). Linear regression is represented by the solid line and dashed lines represent the 95% prediction confidence interval.

3.5. IVIVC Level A

As it can be observed in the Levy plot (Figure 4), a good relationship has been achieved between *in vitro* dissolution times and *in vivo* absorption times. It demonstrates the slower *in vivo* dissolution rate compared to the *in vitro* drug dissolution which could be due to different agitation conditions and degree of sink conditions. Once *in vivo* fractions absorbed have been calculated based on the scaled *in vitro* times, good correlation coefficients (higher than 0.95) were obtained in both data analysis methods (mean or individual). As stated by Gaynor et al. (2009) and Cardot and Davit (2012), higher R^2 were observed based on individual data analysis, although it does not improve the internal validation predictions. Regarding the predicted profiles for the fastest and slowest tablets of test formulations using IVIVC1, the C_{max} predicted values are 1.09 (FTFF) and 0.72 (STSF). The first one (FTFF) is included on the 90%CI of the reference formulation for the bioequivalence study used to develop IVIVC1 but not the corresponding to the slowest tablet. Eventually that could mean that a tighter dissolution specification should be set to ensure bioequivalence of all the tested formulations.

3.6. Conclusion

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

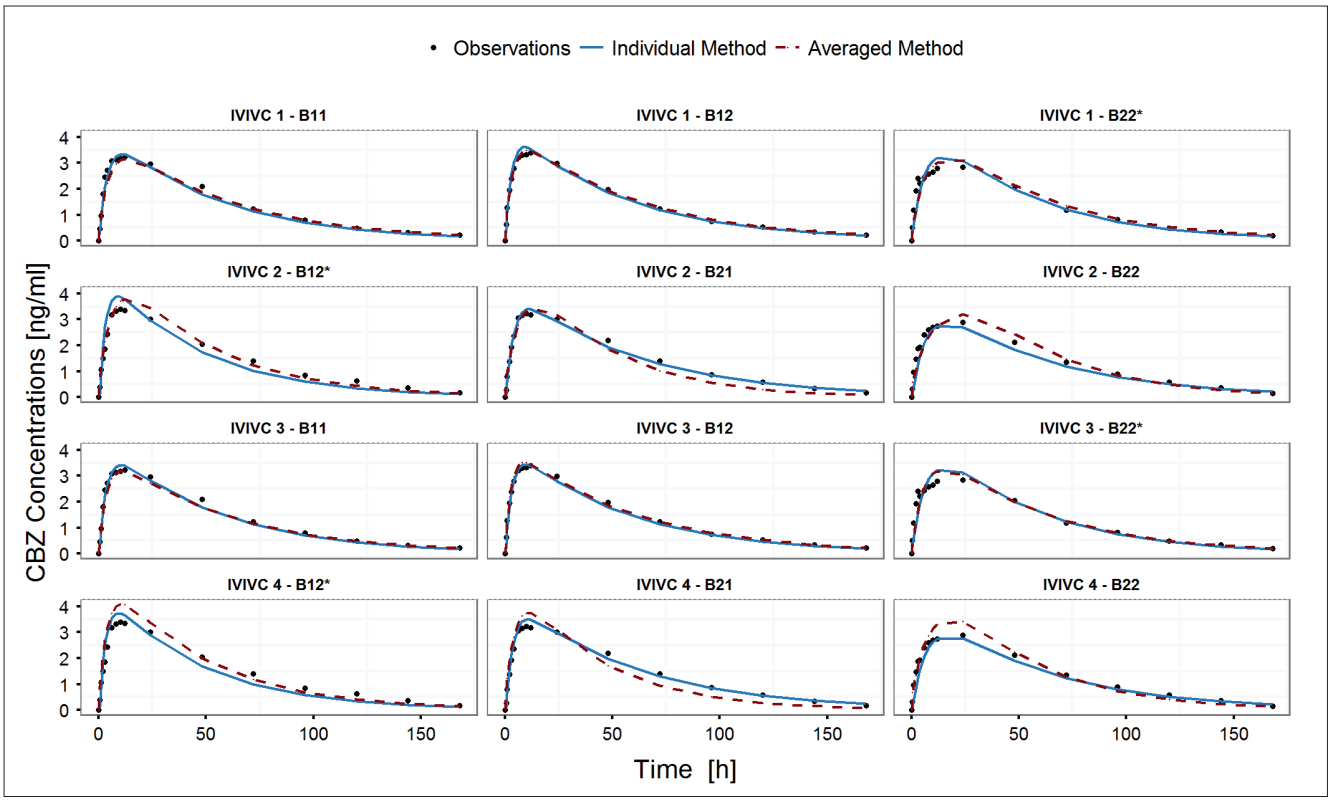

Fig. 5: Solid and dashed lines represent predicted concentrations; dots represent the observed concentrations for each formulation. Blue solid lines represent predictions of individual analysis and red dashed lines predictions of average analysis.

as a biopredictive dissolution medium of broader application to other immediate release formulations with conventional excipients, even if different batches and *in vivo/in vitro* studies were combined.

4. Experimental

4.1. In vivo studies

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

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

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

4.2. In vitro study

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

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

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

4.3. In vitro–in vivo correlation

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

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

Table 6: Codification for *in vitro* and *in vivo* formulations

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

Four different datasets combinations generated to develop level A, B and C IVIVC. Formulations labeled with the asterisk are the normalized formulations.

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

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

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

4.4. Internal validation

$F_{a,mean}$ and $F_{a,average}$ were convolved to obtain the predicted *in vivo* profiles using superposition principle to transform absorbed fractions into concentrations (Langenbucher 2003; Qureshi 2010). Those predicted profiles were utilized to obtain the predicted AUC and C_{max}. Internal predictability was calculated using Eq. (1). FDA (FDA 1997) and EMA (EMA 2014) guidelines validate the IVIVC when the mean prediction error (%PE) in AUC and C_{max} is less than 15% for each individual formulation, and 10% for the mean of all formulations.

$$Prediction\ errors\left(\%PE\right) = \frac{\left(Observed\ parameter - Predicted\ parameter\right)}{Observed\ parameter} \cdot 100 \quad (1)$$

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