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The Use of Saliva as a Biological Fluid in Relative Bioavailability Studies: Comparison and Correlation with Plasma Results

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ABSTRACT: The aim of the present study was to present new evidence supporting the use of saliva as a biological fluid in relative bioavailability studies. Carbamazepine was chosen as a model drug because of its suitability for salivary therapeutic drug monitoring and its well-documented plasma bioavailability. A relative bioavailability study of four different immediate release carbamazepine products was performed. Stimulated saliva samples were collected by chewing on parafilm wax and by the spitting method. *In vitro* dissolution testing of formulations, using 900 ml of 1% sodium lauryl sulphate in water, was also carried out. The *in vitro–in vivo* correlations obtained in this salivary study were consistent with previous correlations assessed using plasma. These results support the suitability of saliva as the biological fluid in relative bioavailability studies. Copyright © 2010 John Wiley & Sons, Ltd.

Key words: saliva; *in vitro–in vivo* correlations; relative bioavailability; dissolution profiles; carbamazepine

Introduction

Drug concentration monitoring in biological fluids is essential in many well-known pharmacokinetic applications, plasma being the most widely used biological fluid. Although not so extensively yet, many authors have used saliva in clinical research and in therapeutic drug monitoring (TDM). Good reviews can be found on this topic [1–5]. The use of salivary therapeutic drug monitoring (STDM) is broadly justified by the simplicity of obtaining samples, the ethical advantage of being a non-invasive method and the possibility of home monitoring: whole saliva can be collected by individuals with limited training and no special equipment is required to collect the fluid. However, further studies are needed before firm conclusions can be made about STDM.

The first step when considering a potential, particular application of STDM is to evaluate whether a certain drug allows this type of monitoring. Most authors state that a drug may be monitored through STDM when the drug has the general requirements for plasma drug monitoring [4] and, at the same time, some correlation between saliva and plasma or serum (as the fluids in contact with the site of action) concentrations can be proved [1,3].

The aim of the drug monitoring study should also be taken into consideration when analysing the suitability of STDM for a specific application. The use of saliva for clinical monitoring in patients requires the demonstration of a relationship

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between the saliva concentration and the pharmacological effect at least to the same extent as that existing between the plasma concentration and the pharmacological effect [6].

On the other hand, when saliva monitoring is considered for relative bioavailability (RBA) studies, the regulatory requirement states that pharmacokinetic parameters, such as maximum concentration (C_{max}) and area under concentration–time curve (*AUC*), obtained from salivary exposure should have the same meaning as those obtained from plasma exposure, in order to assess the systemic bioavailability of drug products. Assuming linear kinetics, the area under the curve from zero to infinity (AUC_{0-inf}) in plasma is proportional to the systemically available dose. Also, the AUC_{0-inf} in saliva is directly proportional to that dose. Hence, the relative bioavailability or extent of bioavailability of different drug product formulations given by the same route of administration could be determined from sampling saliva only [5].

Drug concentration in stimulated saliva samples meets both clinical and biopharmaceutical requirements, since salivary levels correlate better with free drug flowing throughout capillaries than the plasma levels currently determined in venous blood samples [5,6]. Because capillary drug concentrations, and stimulated saliva levels, are tightly related with arterial levels [7], the systemic bioavailability of a drug seems to be determined more appropriately than using the venous plasma levels.

The aim of the present study was to present new evidence supporting the use of saliva as a biological fluid in RBA studies, by exploring the same *in vitro–in vivo* correlation method used in already published bioavailability studies based on plasma concentrations. This is an original way to address the matter which avoids repeating human-volunteer based studies by using previously published data instead of new experimental results [8]. The applied methodology is intended to be a first approach to the study of saliva reliability as a surrogate of plasma as a matrix in RBA studies for a given drug. Although it is not strong enough to solve the matter by itself, it is a simple and fast way to obtain evidence that may support the use of saliva in this kind of study.

Carbamazepine (CBZ) was chosen as a model drug since it gathers the requirements to be used in STDM with acceptable variability [1,9,10] and, at the same time, many bibliographic data on its *in vitro* and *in vivo* behaviour can be found. It is also an interesting drug to use as a model since in many previous biopharmaceutical investigations authors have performed correlations between saliva and plasma CBZ concentrations both in patients [10–14] and healthy volunteers [9,15,16].

It is well known that there are clearly large economic advantages to the use of generic drug products. Free competition with generics based on price will lead to lower costs and greater availability of these important therapeutic options [17]. In most countries, it is required to carry out RBA studies in healthy volunteers during the premarketing phase of drug development [18,19]. The ease of use of saliva as a monitoring fluid in RBA studies facilitates obtaining healthy volunteers for RBA studies without the use of economic incentives (which imply an important bioethical issue: volunteers should not pursue an economic incentive and therefore RBA studies should be as comfortable as possible to promote participation). Thus, the use of saliva may indirectly facilitate generic product development and therefore should help pharmaceutical manufacturers to bring a new product onto the market, improving patients' access to drug therapy.

To accomplish the goal of this study, a relative bioavailability study of four immediate release CBZ 200 mg products was performed in saliva. Four previously published series of RBA studies for immediate release CBZ products using plasma as a biological matrix, involving 14 formulations, were analysed [20–23]. The use of the same commercial brand as the reference product and the same *in vitro* dissolution method, enabled us to assess whether saliva could be a surrogate of plasma, or furthermore, more useful for bioavailability/bioequivalence purposes.

Materials and Methods

Subjects and study design

A relative bioavailability study of four 200 mg immediate release CBZ products (A, B, C and D)

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was performed in a group of ten healthy volunteers, of both sexes (age range 27–33 years; body weight 50–90 kg) using Tegretol, Novartis (product A) as the reference product. None of the volunteers was currently receiving any medication, and they were all required to abstain from alcohol ingestion 48 h prior to the study. The study protocol was approved by the Ethical Committee of the Italian Hospital of La Plata, Argentina, and all the volunteers gave their written, informed consent. Only eight volunteers (three females and five males) completed the study, two volunteers withdrew for personal reasons not related to the products.

The administration of the drug products was accomplished in a four-way randomized cross-over design with washout periods of 2 weeks.

Each volunteer received a single dose of 200 mg of CBZ in the morning (at 08:00), after fasting for at least 10 h. Meals were programmed to be taken at 12:00, 16:00, 20:00 h during the first day of administration. Saliva samples were collected at the following intervals: predose, 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 10, 12, 24, 36, 48 and 84 h after dosing. After dose administration the mouth was exhaustively rinsed with water which was discarded.

Saliva was collected by chewing parafilm wax for 1 min before spitting into tubes. After centrifugation, the saliva samples were stored in a freezer (−20°C) before analysis.

Analytical method

The analytical method used in this study was as follows: 10 µl of a 25 µg/ml nitrazepam (NTZ, internal standard) methanol solution and 3 ml of chloroform was added to 1 ml of saliva and vortex mixed for 1 min. After centrifugation for 10 min at 3000 rpm, the organic layer was removed and evaporated to dryness under a stream of nitrogen at 40°C in a water bath. The residue was dissolved in 100 µl of methanol, centrifuged for 10 min at 13000 rpm and 20 µl was injected in a HPLC apparatus (Gilson SAS, Villiers-Le-Bel, France) with UV detection, with a LiChrospher RP Select B (250 × 4 mm, 5 µm) column and acetonitrile:methanol:KH_2PO_4 10 mM pH 7 (34:6:60) as the mobile phase. The flow rate was set at 1 ml/min and detection was at 220 nm.

Since the method is a modification of a previously published one [24], it was quantitatively evaluated in terms of linearity, limit of quantification, accuracy, precision, selectivity and sample stability.

Pharmacokinetic analysis

The areas under the concentration–time curves from zero to 84 h ($AUC_{0–84}$) and to infinity ($AUC_{0-\inf}$) were calculated with the linear trapezoidal rule. The maximum saliva drug concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were obtained directly from the saliva concentration versus time data. The elimination rate constant (K_e) was calculated by least-squares regression using the terminal points of each curve. In order to obtain $AUC_{0-inf,}$ extrapolation from 84 h to infinity was calculated as the last concentration measured divided by the elimination rate constant.

Dissolution study

Dissolution studies of the investigated CBZ tablets were performed in the USP [25] rotating paddle apparatus (Sotax AT 7, Switzerland) at 75 rpm using 900 ml of 1% sodium lauryl sulphate (SLS) aqueous solution at 37.0 ± 0.5°C, as dissolution media. Samples were withdrawn after 10, 20, 30, 45, 60, 90, 120, 150 and 180 min. The amount of dissolved CBZ was determined spectrophotometrically at 285 nm (Thermo spectrophotometer, Helios-beta model). Trials were performed with 12 tablets and the mean values reported.

Tablet assay

Assay and uniformity of dosage units of the four products were performed in order to assure that the differences observed in the dissolution study were due to different dissolution behaviour of the tablets instead of different drug content. The BP [26] analytical method and specifications were followed. Tablet content may be within the range 95–105% of the labelled amount. For the uniformity of dosage units, the content of each of the ten units must be within the range 85–115% of the labelled amount, with a relative standard deviation less than or equal to 6%. In all cases these acceptance criteria were fulfilled.

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Table 1. Summary of the scientific articles employed in this paper for comparison with salivary data

	Meyer *et al.* [20]	Jung *et al.* [21]	Kovacevic *et al.* [22]	Olling *et al.* [23]
Relative Bioavailability Study – *in vivo*				
Number of products	4	4	2	4
Administered dose	200	400	400	400
Number of volunteers	24	12	18	16
Age (years)	21–35	20–30	29–37	20–38
Weight (kg)	61–93	60–80	70–81	49–88
Dissolution Studies – *in vitro*				
Method	USP 31[a]	USP 31[a]	USP 31[a]	USP 31[a]
Number of tablets	6	12	6	9

[a]1% sodium lauryl sulphate, 900 ml, USP rotating paddle, 75 rpm, 37 ± 0.5°C [25].

Bibliographic data

After a careful bibliographic search, four articles including plasma RBA studies for CBZ immediate release tablets, as well as *in vitro* dissolution studies using 1% sodium lauryl sulphate as dissolution media, were selected. Table 1 summarizes the basic features of these studies.

Results

Analytical method

A linear response was observed in the concentration range of 0.02–3.0 μg/ml, with a coefficient of determination $r^2 = 0.99997$. The intercept (a) and the slope (b) with the respective 95% confidence interval were $a = -0.0091 \pm 0.0245$ and $b = 1.5981 \pm 0.0158$, respectively. Employing Student's test, a linear correlation was observed ($p > 0.05$), assuming that there is no correlation between X and Y as null hypothesis. The residuals' sum was -2.15×10^{-15}. The limit of quantification (LOQ) was 0.02 μg/ml according to the lowest standard concentration on the calibration curve, because the following conditions were met: the CBZ response at the LOQ was at least five times the response compared with the blank response and the CBZ peak was identifiable, discrete and reproducible with a precision of at least 20% and an accuracy of 80–120% [27].

The accuracy and precision of the method were established at three concentration levels (0.02, 0.875 and 3.0 μg/ml). The results expressed as a percentage of CBZ recovered (± relative standard deviation) were 96.8% (±3.2), 100.0% (±1.2) and 100.8% (±2.7) for the low, medium and highest concentration, respectively.

The method was specific for the biological matrix because no peaks were observed near CBZ or NTZ retention times.

Saliva samples inoculated with CBZ demonstrated their stability under three freeze–thaw cycles and 20 h at room temperature.

In vivo study

For all the four products assayed the mean values of saliva pharmacokinetic parameters are given in Table 2. Also, Table 2 summarizes plasma pharmacokinetic parameters recovered from bibliographic sources. Mean concentration versus time curves are shown in Figure 1.

In vitro study

Figure 2 shows the mean ($n = 12$) *in vitro* dissolution profiles for all four products assayed in 1% lauryl sulphate as dissolution media.

It is worth noting that the mean percentage dissolved at 30 min for the reference product (Tegretol in all cases) was very similar between the cited articles and the present work. All the five values were within the range of 68–72%, with a mean (±SD) of 70% (±1.2).

In vitro–in vivo correlations

In order to prove that STDM can be applied to RBA studies with comparable results to those of traditional plasma monitoring, correlations between a pharmacokinetic parameter and an *in vitro* parameter were established.

The main mean parameters (T_{max}, C_{max} and AUC_{0-t}) obtained in RBA studies using plasma as the monitoring fluid [20–23] and in the present salivary RBA study, were correlated with the percentage of CBZ dissolved *in vitro* at 30 min. Thirty minutes was chosen as the sampling time since it was the only time used in all the cited articles.

In order to make consistent all bibliographic plasma data and the salivary data obtained in this

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Table 2. Mean values of saliva (experimental) and plasma (bibliographic) pharmacokinetic parameters (±SD). Product denominations and decimal positions are the same as those used by the authors

	Product			
Saliva data	A	B	C	D
$AUC_{0-84\,hs}$ (mg.h/l)	27.61±6.66	28.49±5.24	27.26±5.91	20.80±7.77
AUC_{0-inf} (mg.h/l)	35.76±10.27	39.34±15.14	34.38±8.94	26.23±9.89
C_{max} (mg/l)	0.68±0.18	0.97±0.21	0.90±0.18	0.50±0.22
T_{max} (h)	6.8±2.8	4.1±1.8	5.7±1.9	9.3±6.8
K_e (h^{-1})	0.020±0.005	0.021±0.008	0.024±0.007	0.022±0.005
Plasma data				
Meyer *et al.* [20]	1 ($n = 24$)[b]	2 ($n = 24$)	3 ($n = 23$)	4 ($n = 23$)
$AUC_{0-169\,hs}$ (mg.h/l)	134.8±20.2	80.9±38.8	154.2±27.8	104.5±31.4
AUC_{0-inf} (mg.h/l)	143.5±21.5	86.5±40.6	162.2±32.4	111.7±32.4
C_{max} (mg/l)	1.89±0.38	1.15±0.71	2.69±0.48	1.40±0.55
T_{max} (h)	15.9±8.1	13.6±10.1	8.3±6.0	19.6±15.3
Jung *et al.* [21]	K ($n = 12$)[c]	C ($n = 12$)	J ($n = 12$)	A ($n = 12$)
$AUC_{0-120\,hs}$ (mg.h/l)	296.0±30.7	316.0±27.7	368.0±25.5	331.0±25.7
AUC_{0-inf} (mg.h/l)	NR	NR	NR	NR
C_{max} (mg/l)	4.24±0.92	4.81±1.04	5.98±0.57	5.98±0.83
T_{max} (h)	15.8±7.3	14.6±9.1	8.6±6.1	8.5±5.9
Kovacevic *et al.* [22]	Reference[a] ($n = 18$)	Test ($n = 18$)		
$AUC_{0-144\,hs}$ (mg.h/l)	211.37±52.97	220.42±55.94		
AUC_{0-inf} (mg.h/l)	259.15±63.97	259.03±69.02		
C_{max} (mg/l)	4.34±1.24	4.74±1.27		
T_{max} (h)	9.7±4.5	8.6±2.8		
Olling *et al.* [23]	D ($n = 16$)[c]	B ($n = 16$)	C ($n = 16$)	A ($n = 16$)
$AUC_{0-96\,hs}$ (mg.h/l)	249±61	253±73	253±53	198±48
AUC_{0-inf} (mg.h/l)	295±59	294±84	292±69	246±61
C_{max} (mg/l)	4.5±0.8	5.9±1.6	6.1±1.6	3.2±1.0
T_{max} (h)	11.0±5.2	5.6±5.9	3.6±4.1	16.0±6.5

NR, not reported in the original article.
[a]Reference product, Tegretol 200 mg, Novartis.
[b]Reference product, Tegretol 200 mg, Geigy Pharmaceuticals.
[c]Reference product, Tegretol 200 mg, Ciba Geigy.

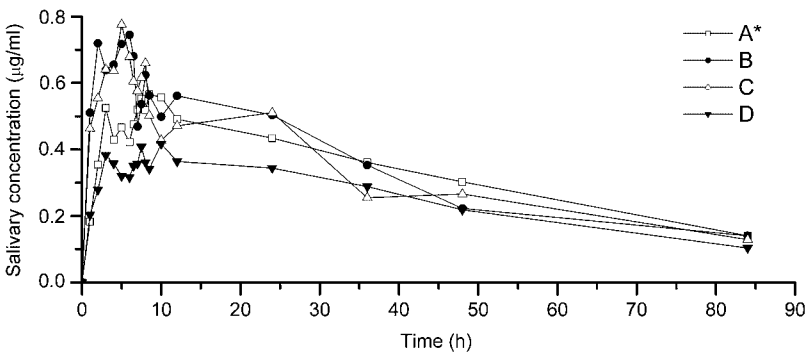

Figure 1. Mean salivary concentration vs time curves obtained after administration of 200 mg CBZ as four different immediate release products (the asterisk denotes the reference product)

work, C_{max} and AUC_{0-t} are reported here in relation to the value obtained for the reference product used in each trial, Tegretol (Test/Ref). Besides, normalization was performed in order to compare the results obtained in two fluids (plasma and saliva), where different C_{max} and AUC_{0-t}

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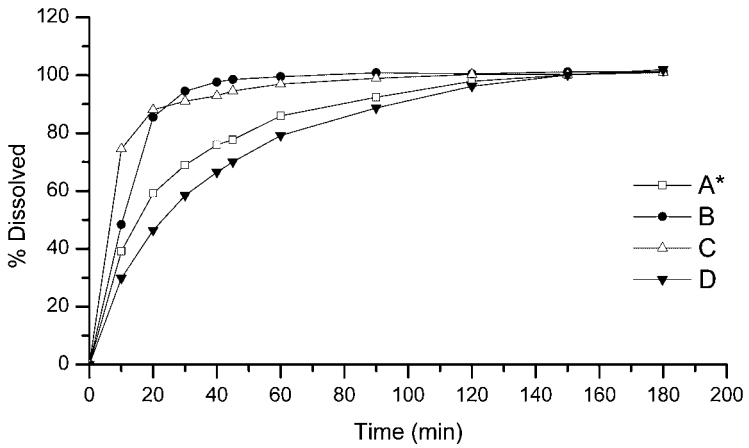

Figure 2. Mean *in vitro* dissolution profiles ($n = 12$) for all four CBZ 200 mg immediate release products in 1% lauryl sulphate (the asterisk denotes the reference product)

could be expected, since they are dependent on the clearance (individual and biological matrix related) and on the dose received in each experimental trial. AUC_{0-t} was used instead of AUC_{0-inf} since the former was reported in all the cited articles. In the case of T_{max} normalization was not considered to be appropriate because the time to reach C_{max} is dependent on the scheduled sampling time.

In all cases, the point corresponding to the reference product, with C_{max} Test/C_{max} Ref = 1 and AUC_{0-t} Test/AUC_{0-t} Ref = 1 as the y value, were included in the C_{max} and AUC_{0-t} normalized graphs, with the mean %Dissolved at 30 min obtained for all the reference products (70%) as the x value.

Correlations grouped taking into account plasma results or saliva results are shown in Table 3.

Once the three pairs of curves were obtained for each parameter, statistical testing for coincidence of two general straight lines [28] was performed for each pair, to prove whether or not the two sets of data (plasma and saliva) are associated with the same line. Under the null hypothesis (H_0) it is assumed that the two slopes and the two intercepts are equal. For the three parameters, H_0 could not be rejected ($p > 0.05$).

Consequently, three new correlations were calculated: (1) T_{max}; (2) C_{max} Test/C_{max} Ref; and (3) AUC_{0-t} Test/AUC_{0-t} Ref; vs % Dissolved at 30 min, but using both plasma and saliva data points. Figure 3 shows the obtained correlations considering plasma data exclusively and plasma plus saliva combined data.

Table 3. Linear relationships (T_{max}, C_{max} and AUC_{0-t} vs % Dissolved in 30 min), using data from four bibliographic sources [20–23] for plasma and our experimental data for saliva. The correlation for T_{max} was straightforward, while the remaining two parameters were normalized to the reference product, Tegretol (C_{max}, Test/C_{max}, Ref and AUC_{0-t}, Test/AUC_{0-t}, Ref)

	Plasma ($n = 14$ for Tmax, $n = 11$ for C_{max} and AUC)	Saliva ($n = 4$)
T_{max} vs % Dissolved in 30 min		
Slope (b) ±95% CI	−0.1420 ± 0.0633	−0.1187 ± 0.1253
Intercept (a) ±95% CI	21.50 ± 4.58	15.74 ± 9.98
Coefficient of determination (r^2)	0.6653	0.8925
C_{max} Test/C_{max} Ref vs % Dissolved in 30 min		
Slope (b) ±95% CI	0.0080 ± 0.0039	0.0179 ± 0.0069
Intercept (a) ±95% CI	0.5319 ± 0.2830	−0.2822 ± 0.5452
Coefficient of determination (r^2)	0.7079	0.9843
***AUC* Test/*AUC* Ref vs % Dissolved in 30 min**		
Slope (b) ±95% CI	0.0044 ± 0.0025	0.0058 ± 0.0139
Intercept (a) ±95% CI	0.6482 ± 0.1804	0.4901 ± 1.1064
Coefficient of determination (r^2)	0.6477	0.6167

Discussion

In order to establish the suitability of saliva as a biological fluid for RBA studies it is worth noting that the variance of the saliva data is similar to that of the plasma data. Table 4 shows the minimum and maximum variances observed

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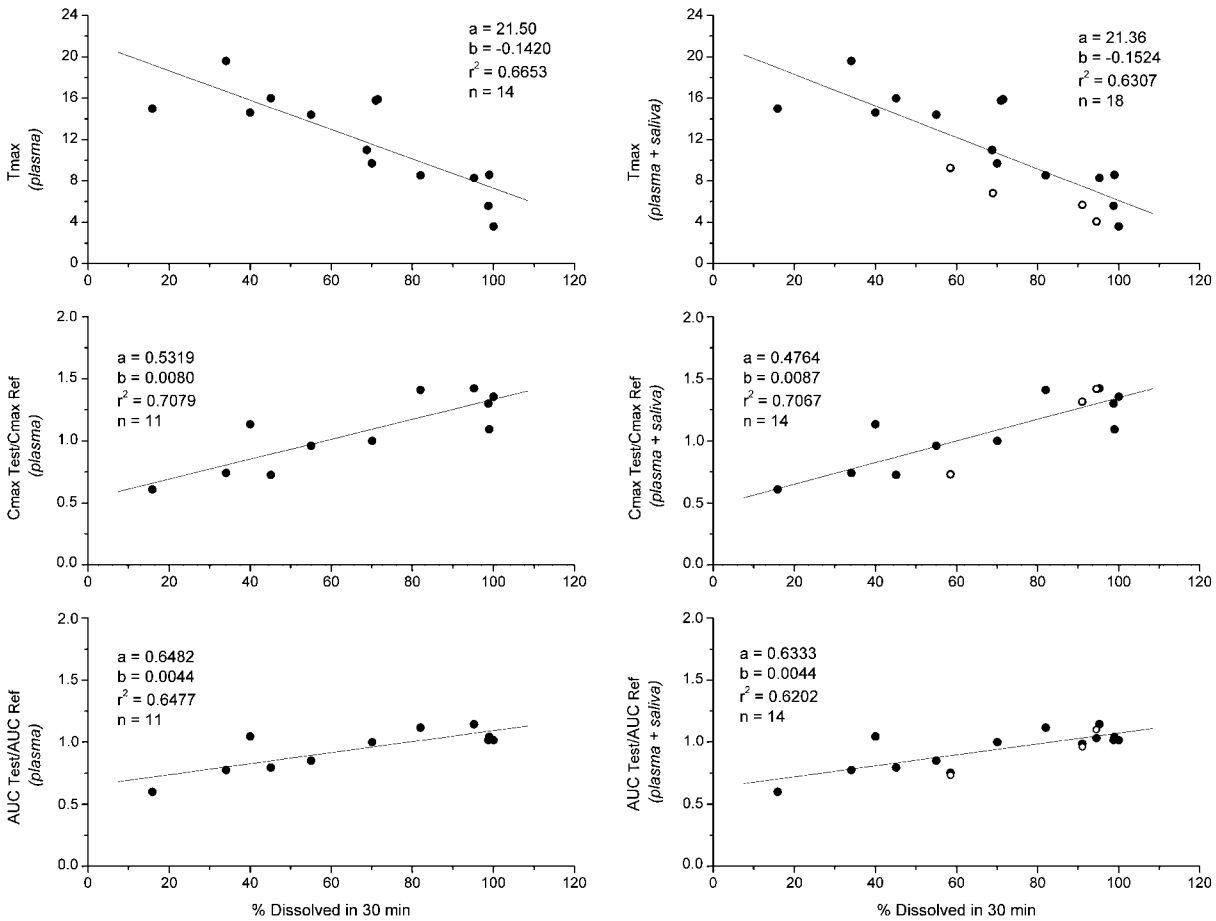

Figure 3. Correlations between pharmacokinetic parameters vs % Dissolved at 30 min. Plasma data are represented on left side graphics (full circles). Salivary data are included on the right side graphics (open circles represent salivary points)

Table 4. Ranges of relative standard deviations (% RSD) obtained for the different pharmacokinetic parameters in saliva and plasma

	Range of salivary % RSD obtained in the present work (4 products)	Range of plasma % RSD [20–23] (14 products)	Range of % RSD in saliva [24] (2 products)
AUC_{0-t} (mg.h/l)	18.4–37.4	6.9–48.0	22.2–33.4
AUC_{0-inf} (mg.h/l)	26.0–38.5	15.0–46.9[a]	NR
C_{max} (mg/l)	20.0–44.0	9.5–61.7	31.9–43.6
T_{max} (h)	33.3–73.1	32.6–113.9	55.8–90.0

NR, not reported in the original article.
[a]The range represents only 10 products because the parameter is not reported by one of the authors.

both for saliva and for plasma data, for each pharmacokinetic parameter, expressed as relative standard deviations (%RSD). The third column included in Table 4 presents the %RSD obtained in a previous work of RBA study using saliva as biological fluid, of two brands of 400 mg CBZ tablets in healthy volunteers [24].

Most drugs appear to enter saliva by simple passive diffusion. Working with healthy volunteers, for whom a normal physiology of the oral cavity may be assumed, the variables that influence this type of transport are pH and pKa, lipid solubility, charge, molecular weight and spatial configuration, free drug plasma level, dose and clearance of the drug, salivary flow rate, salivary binding proteins and

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concentration of salivary enzymes capable of metabolizing the drug [2,29,30].

Drugs which are not ionizable or are unionized within the salivary pH range (e.g. phenytoin, carbamazepine and theophylline) are candidates for STDM. Carbamazepine belongs to the group of neutral lipophilic drugs. Hence, its lipid solubility may be the determining factor in its ability to diffuse into saliva, the salivary pH being of minor importance [9,31]. Besides, changes in salivary pH have been shown to be wholly dependent on changes in flow rate [1], and it has been proved that carbamazepine is not so affected by salivary flow rate [10,32].

While the amount of protein in saliva is negligible and usually no significant binding of drugs to saliva contents is found [5], differences in plasma protein binding of a drug may increase both inter- and intra-individual variability of RBA studies. In the case of CBZ, the total plasma concentration is more sensitive to protein binding changes than free (and salivary) concentrations [6,13]. Nevertheless, the classic average bioequivalence statistical design (2×2) has the advantage of allowing each subject to be its own control, eliminating the inter-individual variability [33], which is larger than the intra-individual variability for most drugs. On the other hand, Miles and colleagues studied the intra-individual variability of salivary carbamazepine concentrations in healthy volunteers, concluding that it was not clinically significant [34].

From the correlations presented in Figure 3, it can be stated that saliva is comparable to plasma in RBA studies, since the saliva data obtained in this study and previously reported plasma data can be combined in a single linear correlation. With a significance level of 0.05, it has been demonstrated that both data groups cannot be considered different. In other words, T_{max}, C_{max} and AUC_{0-t} are equally correlated with *in vitro* data, whether they are derived from plasma or saliva samples, or from both of them combined.

In the case of C_{max} and AUC_{0-t}, saliva biases are eliminated when the data is standardized to the reference formulation, and a random distribution of the saliva data along the regression line is obtained. However, the case of T_{max} is different, because even though the variances of the saliva and plasma data statistically allow them to be grouped into a single group, all the points corresponding to saliva T_{max} fall below the resulting regression line. This confirms the anatomical–physiological model according to which salivary concentrations instantaneously reach equilibrium with arterial concentrations in blood capillaries, resulting in higher values than the venous plasma concentrations usually measured [6,7]. This explains the higher ratio between the salivary concentration and total plasma concentration (S/P_T ratio) generally found during the absorption phase as well as the lower T_{max} found in saliva.

Another interesting experimental fact that reflects what happens during the absorption phase are the higher values of the S/P_T ratio obtained when working with C_{max} than those obtained with AUC_{0-inf}. As shown in Table 5, using only the

Table 5. AUC_{0-inf} and C_{max} S/P_T ratio of the Reference Product (the value obtained for Tegretol in the present work divided between the value obtained for Tegretol in the cited work, for $AUC_{0\text{-}inf}$ or C_{max} respectively)

	S/P_T ratio of the Reference Product	
	For $AUC_{0\text{-}inf}$ (Tegretol AUC_{0-inf} obtained in the present work/Tegretol AUC_{0-inf} obtained in the cited work)	For C_{max} (Tegretol C_{max} obtained in the present work/Tegretol C_{max} obtained in the cited work)
Meyer *et al.* [20]	0.249	0.360
Jung *et al.* [21]	NR	0.321[a]
Kovacevic *et al.* [22]	0.276[a]	0.313[a]
Olling *et al.* [23]	0.242[a]	0.302[a]
Mean	**0.257**	**0.324**
SD	0.021	0.025

NR, not reported in the original article.
[a]Normalization to the dose was needed.

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data obtained for the reference formulation and computing the ratios between the saliva AUC_{0-inf} and the plasma AUC_{0-inf} (normalized to the dose if necessary), a mean $\pm$SD of 0.257 ± 0.021 is obtained, which represents a quite good estimation of the mean S/P_T ratio = 0.26 expected for CBZ [35]. However, if that calculation is repeated for C_{max}, the obtained value is 0.324 ± 0.025.

It also should be noted that in the graph of T_{max} vs % Dissolved at 30 min the point with the lowest % Dissolved at 30 min (15.9%) was extracted from Meyer *et al*. work [20]. The authors themselves have stated that this point may well be an outlier.

It is clear that the evidence presented here in favour of the use of saliva for RBA studies strongly depends on the results of the *in vitro* dissolution test, since the data of this test were used to establish the correlation between both fluids. Several studies support the validity of this approach, since good correlations between *in vitro* and *in vivo* data for CBZ have been established, both at a Level C [20,21,36] and at a Level A, point by point [22,37,38]. Moreover, Level A correlations were found using the same dissolution media that was used in the present study. Proposals to consider biowaiving of CBZ have arisen on the basis of the correlation between *in vitro* and *in vivo* data [22], despite the lack of agreement about the possibility of considering biowaivers for drugs assigned to BCS classes other than Class I (high solubility/high permeability), such as Class II CBZ [39].

Conclusion

From the discussed results, we believe that saliva is fairly comparable to plasma fluid in relative bioavailability studies of CBZ. The agreement between our results and those of others [9,15] supports the reliability of our methodology.

Though further studies are needed in order to establish the suitability of saliva as a widely accepted monitoring biological fluid for RBA studies, our results are a bioethically relevant first outcome in that direction: we obtained a fairly good correlation between CBZ saliva and CBZ plasma concentrations despite them having been obtained from the literature CBZ plasma data. When in-house, standardized plasma data are used, the correlation improved [9–13,15].

Therefore, we believe early studies using literature data are a fine approach to assess rough, preliminary results in a bioethical manner: if in the presence of many confounding factors due to the variability in plasma concentrations of the drug we still find a fair correlation, there are high probabilities of obtaining a better correlation when experimental data without confounding factors are used.

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