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Investigation on the need of multiple dose bioequivalence studies for prolonged-release generic products

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ABSTRACT

In the European Union multiple dose bioequivalence studies are required for the approval of generic prolonged-release products, but they are not required by the US-FDA. In order to investigate if the multiple dose bioequivalence studies are necessary, the bioequivalence studies assessed in the Spanish Agency for Medicines and Health Care Products in the last 10 years were searched to find all reasons for rejection and identify those cases where the multiple dose study had failed to show bioequivalence and the single dose study had shown bioequivalence. In these latter cases, the plasma concentration at the end of the dosing interval (C_τ) in the single dose study was assessed to investigate its sensitivity to predict non-bioequivalence in the steady state.

The search identified six cases where the non-equivalence in the multiple dose study was not detected by the corresponding single dose study. C_τ was not able to detect the difference in five cases and in general it was more variable than conventional metrics. In conclusion, the multiple dose bioequivalence study is necessary to ensure therapeutic equivalence and the use of C_τ would be counterproductive, increasing the sample size of the studies without enough sensitivity to detect differences in the steady state.

1. Introduction

The present CHMP Guideline on Modified Release Oral and Transdermal Dosage Forms (Committee for Proprietary Medicinal Products (CPMP), 1999) requires the investigation of bioequivalence of generic/hybrid prolonged release products in a single dose study in fasted state, a multiple dose study in fasted state and in presence of a high fat meal in those cases where the product is to be taken irrespective of the food intake, or only a single dose study in fed state and a multiple dose study in fed state in those products where the SPC of the reference products indicates intake only in fed conditions for safety/tolerability issues or for pharmacokinetic reasons.

After the announcement of the update of the present guideline (Committee for Medicinal Products for Human Use (CHMP), 2010a), EUFEPS sponsored recently a conference to address the pharmacokinetic requirements of this guideline (EUFEPS BABP Network Open Discussion Forum. Revision of BE Requirements for Modified Release Products). The need for multiple dose studies was one of the topics addressed (Becker, 2011). The literature was reviewed to identify cases where multiple dose studies were necessary, i.e. cases where the difference between products was detected more sensitively in a multiple dose study in steady state compared with a single dose study in fasted or fed state. No case was found and this was considered as demonstration that such a study is not needed to ensure bioequivalence. Unfortunately, the lack of published studies cannot be considered as scientific evidence of its insensitivity due to publication bias. In other words, absence of evidence is not evidence of absence. Furthermore, the theoretical ability of multiple studies to detect differences in the release profile of the prolonged release product at the end of the dosing interval was highlighted in this conference.

Multiple dose studies are not required for immediate release products because of its lower sensitivity to detect differences in release rate compared with single dose studies (Fernandez-Teruel et al., 2009a,b; Navarro-Fontestad et al., 2010; Jackson, 1987, 1989;

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el-Tahtawy et al., 1994, 1995, 1998). Therefore, multiple dose studies are only accepted for immediate-release products when a single dose study is not feasible for safety reasons and the product has to be administered in patients that require continuous treatment or when the plasma concentrations are measurable only after accumulation at steady state (Committee for Medicinal Products for Human Use (CHMP), 2010b).

For prolonged release products, the US-FDA does not require the investigation of bioequivalence at steady state after multiple doses because single-dose studies are considered more sensitive in addressing the primary question of BE (i.e. release of the drug substance from the drug product into the systemic circulation), even in instances where nonlinear kinetics are present (US Department of Health and Human Services, 2003). In Canada, demonstration of bioequivalence in a multiple dose study, in addition to single-dose studies, is required currently (Health Canada, 1996), but this requirement is under review and there is a proposal to eliminate it. Consequently, in Europe there are similar opinions that believe that this type of study does not provide any useful information.

On the contrary, from a theoretical point of view, the multiple dose study is necessary to assess the biopharmaceutical quality of prolonged release products since no other study is able to investigate the shape of the whole curve by means of $C_{max,ss}$ and $C_{min,ss}$ or the release rate of the product at the end of the dosing interval since the singled dose study only investigates AUC and C_{max}. In contrast to immediate release products, multiple dose studies are necessary in prolonged release products because the release rate determines the apparent elimination phase since the absorption rate is slower than the elimination rate. Therefore, differences in release at the end of the dosing interval cannot be detected with the current metrics of a single dose study, AUC_{0-t} or $AUC_{0-\infty}$ and C_{max}, since the difference could be masked in AUC by the similarity in the first part of the curve and C_{max} is observed in a wide range of sampling times, independently of the release rate, since the prolonged release profile is characterised by prolonged plateau levels and, therefore, the t_{max} can occur in a large range of values due to random variability. In other words, the shape of plasma profiles in a prolonged release product is much more dependent on the formulation than in an immediate release product. Consequently, after multiple doses the plasma concentrations at steady state would be different if at the end of the dosing interval the plasma concentration were notably different. Importantly, differences at the end of the plasma concentration–time curve may have a high clinical relevance if plasma concentration falls below the minimum effective concentration (e.g. analgesics).

The aim of the present study was to identify the reasons for rejection of prolonged release generic products in regulatory submissions to the Spanish Agency for Medicines and Health Care Products in the last 10 years, in order to investigate if the multiple dose bioequivalence studies are necessary based on the existence of cases where the multiple dose study had failed to show bioequivalence and the single dose study had shown bioequivalence. In these latter cases, the plasma concentration at the end of the dosing interval (C_τ) in the single dose study was assessed to investigate its sensitivity to predict non-bioequivalence in the steady state in order to include it as a requirement in the single dose study to ensure bioequivalence in steady state without performing such a study.

2. Materials and methods

2.1. Prolonged release products

All prolonged release generic products assessed in Spain by the Spanish Agency for Medicines and Health Care Products since 2000 approximately and whose assessment report is available in electronic format were investigated. These products may have been submitted by national, mutual recognition or decentralised procedure.

Table 1
Reasons for rejection of prolonged release generic products submitted to the Spanish Agency for Medicines and Health Care Products.

Reason for rejection	Number of cases
Inadequate reference product	3
Inadequate design	3
Inadequate characterisation	1
Insufficient number of studies	27
One or more failed studies	11

2.2. Identification of reasons for rejection

A search was performed in all assessment reports to identify files with the word “prolonged” (“prolongada” in Spanish) in any paragraph of the assessment report. This word is expected to be included in all assessment reports of prolonged release products since it is part of the standard term of the dosage form in the European Pharmacopoeia. Subsequently, the word “rejected” (“desfavorable” or “no conforme” in Spanish terminology) was searched in those previously identified files, since this is the legal term employed in the assessment report conclusions.

Finally, the assessment reports were collected and reviewed.

2.3. Investigation of an additional metric in single dose studies to avoid multiple dose studies

For those products where the multiple dose study failed to demonstrate bioequivalence and the single dose study demonstrated bioequivalence, the reason for the failure was investigated (i.e. differences between products or insufficient statistical power to conclude equivalence). In those cases where differences were detected in the multiple dose study the raw data of the single dose study was collected to calculate the 90% confidence interval of the ratio test/reference of plasma concentration at the end of the dosing interval (C_τ).

3. Results

The same development of a prolonged release generic product is frequently submitted to obtain a marketing authorisation of several generic (or hybrid) medicinal products. Therefore, several assessment reports contained the same bioequivalence studies. A total of 44 cases/developments with a negative opinion were identified. In one case the rejection was based on two reasons. Table 1 summarizes the 45 reasons for rejection. The products are not identified for reasons of confidentiality.

Only the 11 cases where the documentation was complete were considered for further investigation. Table 2 shows the results of these bioequivalence studies.

Table 3 lists the 90% CI for C_τ in the single dose study in those cases where bioequivalence was not concluded in the multiple dose study.

4. Discussion

Table 1 describes the reasons for a negative opinion, but it does not mean that all of the cases were finally rejected since, in a few of them, the deficiencies were solved afterwards.

Eleven cases were rejected because some of the required studies were not able to show bioequivalence. This is the dataset where we can investigate if the multiple dose study is necessary.

Table 2
Bioequivalence results of the complete cases with failed results (in bold).

Case	Single dose fasted			Multiple dose fasted				Single dose fed			Multiple dose fed			
	n	90% CI		n	90% CI			n	90% CI		n	90% CI		
		C_{max}	AUC_∞		$C_{max,ss}$	$C_{min,ss}$	AUC_t		C_{max}	AUC_∞		$C_{max,ss}$	$C_{min,ss}$	AUC_t
1	24	**67.63**–103.48	91.18–115.48	24	**64.82**–86.65	**158.37–276.59**	86.97–103.86	24	**64.63**–89.36	84.52–107.41				
2[a]	24	**64**–80	101–115	24	**133.54–159.62**	87.64–117.41	107.81–124.18	24	80–100	98–111				
3	24	118.66–**125.27**	108.13–118.82	22	107.00–116.64	82.70–**163.43**	105.08–124.14	24	112.06–**133.21**	107.56–**126.66**				
4	24	90.84–108.38	95.46–107.38					22	105.95–**133.65**	99.04–117.37	21	116.92–**136.40**	91.29–106.96	106.92–122.5
5	34	89.35–101.39	97.62–114.62					38	**75.63**–96.02	**79.20**–108.76	34	**78.42**–90.67	95.11–118.13	97.84–112.96
6	55	81.14–100.55	102.77–119.32	35	**74.56**–91.67	**125.48–170.59**	91.09–107.63	54	93.73–116.13	87.80–99.40				
7	34	97.21–114.70	94.50–112.95	34	88.08–106.58	**72.09**–97.56	80.87–99.45	34	90.20–122.36	92.98–114.01				
	34	97.86–124.11	95.55–109.94					35	89.00–110.89	86.44–99.87				
8	35	90.12–107.62	96.36–112.30											
				42	86.87–105.95	108.83–**133.03**	96.55–110.05							
9								24	94.18–123.27	95.36–115.82	24	**73.76**–95.99	**62.47**–103.42	86.47–100.34
10	28	82.98–109.38	91.83–124.95	59	104.77–119.19	107.25–**132.46**	107.89–122.70	57	93.44–108.30	101.86–114.33				
	24	92.41–99.47	93.93–103.22											
11	23	94.94–109.42	92.55–105.07					23	96.97–109.09	97.41–106.74	28	97.64–108.02	**71.27**–100.02	92.09–104.21

n, sample size; CI, confidence interval.
[a] The results were submitted without two decimal units.

The existence of a publication bias resulting from the publication of only positive studies is obvious. Therefore, this review includes products whose studies have not been published. However, this review is also affected by bias. There is also a submission bias, since most failed studies are not submitted. However, some failed studies are submitted for regulatory assessment because the Applicant also submits clinical studies to justify that the pharmacokinetic differences were clinically irrelevant or the Applicant believed that the 80–125% acceptance criteria was not required strictly (e.g. for C_{min}). This might explain why the incidence of failures in the multiple dose study was much higher than that observed in the other study designs.

According to Table 2, one case failed in all three studies because the formulations had a different release rate, which caused a different C_{max} in the single dose studies (fasted and fed) and a different $C_{max,ss}$ and $C_{min,ss}$ in the multiple dose study, but AUC was bioequivalent in all three studies. It is also evident that $C_{min,ss}$ is much more variable than $C_{max,ss}$ and C_{max}, but it also showed the most different point estimate, suggesting it seems to be the most sensitive to detect differences. Nevertheless, with such a large difference between products any type of study is able to detect the difference.

In the second case both studies in fasted state were able to detect a difference in absorption rate by means of C_{max} or $C_{max,ss}$, but the multiple dose study seems to be more sensitive to detect the difference. Or in other words, the detected difference is larger. In this case $C_{min,ss}$ was not able to detect the difference. Interestingly, in this case the fed study was unable to detect the difference. Therefore, we can conclude that the fed single dose study and the fasted single dose study are required for two different purposes. The fasted state study measure the quality of the release in optimal conditions and fed study investigates the formulation resistance to dose-dumping and the release in different/worse conditions.

In the third case the highest sensitivity was observed in the fed study. The single dose study in fasted state was almost bioequivalent and the multiple dose study failed due to the extremely high variability of $C_{min,ss}$ (67%), but the point estimate was only 116%. This study seems to indicate that the multiple dose study is not necessary.

In the fourth case the highest sensitivity was also detected in the fed state, both in the single dose study and the multiple dose study, but the difference was not detected in the single dose study in fasted state. Interestingly, when comparing the fed studies, there is more sensitivity to detect differences between formulation in the $C_{max,ss}$ than in C_{max}, and $C_{max,ss}$ exhibits lower variability.

In the fifth case the fed conditions were the most discriminative. Bioequivalence was shown in fasted state but not in fed state. The sensitivity of the multiple dose fed study based on point estimates seems to be slightly higher than that of the single dose fed study.

In the next 6 cases the differences between formulations were detected only in the multiple dose study in fasted state or fed state and the single dose study in fasted or fed state were not able to detect a difference. It is important to highlight that in five out of six cases the 90% confidence interval was not only out of the acceptance range but also it did not include the 100%. Therefore, statistically significant differences were detected with that confidence level (10% consumer risk). Only in one case the 100% was included, but it was marginally. Therefore, it is not possible to claim that these failures were due to an excessive variability, but it has to be admitted that differences between formulations really existed and they were detected only in the multiple dose study. Therefore, if in half of the identified rejected cases with complete documentation the most sensitive study is the multiple dose study, it does not seem reasonable to stop requiring this type of study, except if it is possible to identify an additional pharmacokinetic parameter in the single dose study able to provide the same information. To this end the 90% CI of the ratio of the concentration at the end of dosing interval

Table 3
90% CI for C_τ in the single dose study compared with the failed 90% CI of $C_{max,ss}$ or $C_{min,ss}$ in the multiple dose study.

Case	Single dose fasted/fed		Multiple dose fasted/fed	
	n	Point estimate (90% CI) CV% C_τ	*n*	Point estimate (90% CI) CV% $C_{max,ss}$ [a], $C_{min,ss}$ [b]
6			35	82.67 (74.56–91.67) 25.96%[a]
	55	145.57 (131.21–161.50) 33.05%	33	146.30 (125.48–170.59) 60.13%[b]
7	34	125.15 (103.99–150.62) 47.48%	34	83.86 (72.09–97.56) 38.11%[b]
8	33	106.67 (92.29–123.34) 35.85%		
	35	119.37 (100.15–142.27) 45.50%	42	120.32 (108.83–133.03) 27.84%[b]
9				84.12 (73.76–95.99) 27.07%[a]
	24	89.31 (70.79–112.67) 49.57%	24	80.38 (62.47–103.42) 54.32%[b]
10	28	112.28 (90.40–139.41) 50.33%	59	119.19 (107.25–132.46) 35.32%[b]
11	24	98.15 (88.94–108.33) 20.09%		
	23	92.08 (81.67–103.82) 23.96%	28	84.43 (71.27–100.02) 38.49%[a]

(C_τ) after a single dose between Test and Reference was calculated (see Table 3) for these 6 cases (cases 6–11).

In the sixth case the new parameter (C_τ) was able to detect the difference without an extremely large variability and the point estimate was similar to that of $C_{min,ss}$, but with half of the variability. Therefore, it seems to be an adequate solution to avoid the need of multiple doses studies.

In the seventh case C_τ also failed to show equivalence, which seems to indicate that it is able to predict the steady state bioequivalence conclusion, but, surprisingly, C_τ detected the difference in the opposite direction than $C_{min,ss}$, which seems to be illogical. In addition, C_τ was too variable and even more variable than $C_{min,ss}$. Therefore, this case raises some doubts concerning the validity of C_τ as predictor of the steady state.

In the eighth case two single dose studies are available because two proportional formulations were developed. In one of them, C_τ was equivalent and in the other one C_τ provides a point estimate similar to that of $C_{min,ss}$, but with a much higher variability. Therefore, C_τ does not seem to be consistent and the high variability is not desirable.

In the ninth case C_τ was not able to detect the difference detected in $C_{max,ss}$ and failed to show equivalence due to its high variability. In steady state $C_{min,ss}$ failed also due to variability, but the point estimate seems to be more discriminative (80.38% vs. 89.31%) and $C_{max,ss}$ did not fail due to variability but to a large difference (around 15%) in point estimate. Therefore, C_τ was not able to detect the problems detected in the multiple dose study.

In the tenth case C_τ was less sensitive and more variable than $C_{min,ss}$. Therefore, it does not seem to be an adequate parameter to be used instead of the multiple dose study due to insensitivity based on the point estimate and the larger sample size that it may require.

In the eleventh case two single dose studies are available because two proportional formulations were developed. In both cases C_τ was equivalent with a very similar point estimate (difference < 10%), therefore, it was not able to detect the 15% difference detected by $C_{max,ss}$.

In summary, these first five examples identified 2 cases where non-equivalence was concluded in all studies (cases 1 and 3). In one case (case 2) the fed state was the less sensitive and concluded equivalence, but in 3 out of 5 cases the fed studies were the most discriminative. In fact, in case 5 only the fed studies concluded non-equivalence. Therefore, although the fed study seems to be unnecessary based on case 2, it is obvious that the fed studies are essential to compare the *in vivo* performance of prolonged release products. Similarly, although case 3 seems to indicate that the multiple dose study is unnecessary, in 4 out of 5 studies the multiple dose study seems to be more discriminative than the single dose study. This is confirmed in the next six cases where the multiple dose study was the only design able to detect the differences and, therefore, it was essential when comparing the *in vivo* performance of prolonged release products.

Regarding the predictive value of C_τ, one case in Table 3 shows that it is predictive of the bioequivalence failure of $C_{min,ss}$, but in the other five cases, the results are not predictive or as sensitive as $C_{max,ss}$ or $C_{min,ss}$.

5. Conclusion

The reviewed data show that half of the rejected prolonged release generic products with complete documentation were rejected due to the multiple dose study. Even if variability in some of the failed PK parameters was high, the reason for failure was not due to the variability, but to the differences between formulations, since in almost all cases the differences was statistically significant with 90% CI that were not including the value of 100%. In most cases where the multiple dose study failed to show equivalence C_τ was not able to detect the difference observed in the multiple dose study. Only in one case it was less variable than, and as sensitive as, $C_{min,ss}$. Therefore, the existing evidence recommends that bioequivalence demonstration also in a multiple dose study must be required as a regulatory requirement to ensure bioequivalence in steady state and real chronic use, because for prolonged release products, in contrast to immediate release products, the single dose study is not able to ensure equivalence in steady state due to the complex shape of plasma concentration–time profiles.

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