Research Article

Theme: Facilitating Oral Product Development and Reducing Regulatory Burden through Novel Approaches to Assess Bioavailability/Bioequivalence
Guest Editors: James Polli, Jack Cook, Barbara Davit, and Paul Dickinson

Re-introduction of a Novel Approach to the Use of Stable Isotopes in Pharmacokinetic Studies

Alan Parr,^{1,5} Manish Gupta,² Timothy H Montague,³ and Frank Hoke⁴

Received 20 January 2012; accepted 10 May 2012; published online 9 June 2012

Abstract. The purpose of this investigation is to evaluate the scientific benefits of a novel approach in using stable isotopes to reduce the number of subjects needed to perform relative bioavailability and bioequivalence pharmacokinetic studies for formulations that are qualitatively and quantitatively the same and quality by design (QbD) pharmacokinetic studies. The stable isotope approach was investigated using simulations to determine the impact this approach would have on the estimation of variability and, subsequently, the sample size for a bioequivalence study. A biostudy was conducted in dogs in a two period crossover to explore the viability of the stable isotope approach. For a drug product with within-subject variability (CV_w) of 50% and assuming a correlation of 0.95 between the enriched and non-enriched pharmacokinetics (PK), simulations showed that the variability can be reduced by 70% and the required sample size can be reduced by 90% while maintaining 90% power to demonstrate bioequivalence. The dog study showed a strong correlation (R^2 , >0.99) between the enriched and non-enriched area under the curve and maximum observed concentration, and a significant reduction in the variability (reduction in % coefficient of variation from 79.9% to 6.3%). Utilization of a stable isotope approach can markedly improve the efficiency and accuracy of bioavailability and bioequivalence studies particularly for highly variable drugs in formulations that are qualitatively and quantitatively the same and for studies designed for QbD investigations.

KEY WORDS: bioequivalence; biopharmaceutics; quality by design; relative bioavailability; stable isotope.

INTRODUCTION

Establishing bioequivalence (BE) of a product has been a challenge to the development of many compounds over the years and therefore is a critical component in the drug development process. That importance has increased over time, especially with the desire to link *in vitro* data (e.g., dissolution) to *in vivo* data, as is the expectation with quality by design (QbD). While QbD clearly brings value to the development process and

the patient, it also brings challenges, particularly in the area of relative bioavailability and bioequivalence. The biggest challenge is how to develop this *in vitro/in vivo* link in the most efficient manner possible. Based on the product being developed and the number of Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) for the product, the size and number of studies needed to define the appropriate design space could be quite large. Additionally, if the compound/product exhibits a high *in vivo* variability, then the number of subjects needed for making a statistically significant conclusion could become quite high. Therefore, a method or study design that could deal with these issues could add significant value to the drug development process.

The application of stable isotopes may be one approach to overcome this challenge. This approach may also add value to studies that have very unique challenges such as studies that include patient populations that are difficult to recruit (e.g., oncology), or studies involving highly variable drugs, or a combination of both. The impact of these challenges can lead to long patient recruitment times and significant increases in patient numbers, leading to potentially slower delivery of medicines to patients. Additionally, this approach would work

¹ Product Development, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, North Carolina 27709, USA.

² Product Development, GlaxoSmithKline, Collegeville, Pennsylvania, USA.

³ Clinical Statistics, GlaxoSmithKline, King of Prussia, Pennsylvania, USA.

⁴ Clinical Pharmacology Modelling & Simulation, GlaxoSmithKline, Research Triangle Park, North Carolina, USA.

⁵ To whom correspondence should be addressed. (e-mail: alan.f.parr@gsk.com)

where bioavailability/bioequivalence (BE) studies need to be done in formulations that are qualitatively and quantitatively the same (*e.g.*, manufacturing site change).

Utilization of single- and dual-label stable isotopes (*e.g.*, ^{13}C , ^{15}N , ^{18}O) in pharmacokinetics (PK) studies has been in existence for many years (1–6). Utilization of a stable isotope provides the ability to measure plasma concentrations of an enriched and non-enriched drug substance from the same plasma sample (*i.e.*, subject). Thus, by co-administering an enriched and non-enriched drug substance, it has been demonstrated that the variability of the statistical test used to compare formulations can be reduced (1) and subsequently reducing the required number of subjects to be studied. Previous reports in the literature around the application of stable isotopes to PK studies took the position of incorporating the stable isotope into the formulation being studied. This has the limitation of requiring significant amounts of stable isotope to enable the manufacture of an appropriately size batch of drug product. The approach presented here is similar to that reported by Heck *et al.* (2), that the addition of a small dose of enriched compound, such as a solution or solid, is given along with the products being tested rather than incorporating them into the formulations. However, Heck *et al.* did not discuss the applicability of this approach to PK studies to support QbD and its application to BE studies for formulations that are qualitatively and quantitatively the same. Using this approach eliminates the need for large batches of enriched drug substance and the need to manufacture large-scale batches of drug product (containing enriched and non-enriched drug substance). By using the enriched PK measure as a “correction factor” in the analysis, the variability of the statistical test used to compare formulations is accurately determined. As such, the use of this approach may result in significant improvement in the efficiency of some bioavailability and bioequivalence studies.

To further understand the potential of this approach to improve the efficiency of these trials, we conducted both simulations and a dog PK study to evaluate the impact on both the estimated variability and the subsequent sample size needed for these studies. Based on the results of these two exercises, we discuss potential applications of the stable isotope approach in the context of product development and QbD.

MATERIALS AND METHODS

Simulations

Simulations were run to better understand the benefits of this approach in terms of reducing the variability of the statistical test used to compare formulations (in this example, the residual mean square error (RMSE) of the analysis of variance model) and subsequently the sample size. To this end, simulations were run to explore a range of within-subject variability ($\text{CV}_w=30$ –70%) and a range of correlation between the non-enriched and enriched PK measurements ($\rho=0.7, 0.8, 0.85, 0.90, 0.95$, and 0.99). Additionally, the cases of the within-subject variability of the non-enriched and enriched formulations being equal and unequal were explored.

For each simulated two-period crossover trial, individual non-enriched and enriched PK values were randomly generated for 16 subjects, assuming a bivariate log-normal distribution. The mean of the PK parameter (*e.g.*, area under the

curve (AUC) and maximum observed concentration (C_{max}) for the test (T) and reference (R) products and the enriched solution were 500, 500, and 50, respectively (this is 10% of the previous two due to the fact that the enriched isotope in solution was assumed to be given at 10% of the dose in the test and reference products).

For each combination of CV_w (equal and non-equal) and ρ , 1,000 trials were simulated. For each trial, the RMSE was estimated from both an analysis of variance model (ANOVA) and analysis of covariance model (ANCOVA) of the non-enriched PK. In both models, terms for subject, period, and regimen were fit and for the ANCOVA model, the enriched PK was fit as a covariate. For the simulations, subject was fit as a fixed effect. The average RMSE of the 1,000 simulations and the associated sample size required to provide 90% power to demonstrate bioequivalence were then calculated. The average RMSE is expressed as a coefficient of variation (CV_r). Sample size calculations were based on 2 one-sided testing procedures assuming a type I error rate of 5% and true ratio of T/R of unity. Simulations were carried out using SplusTM 7.0.

Dog Study

A PK study was conducted in six dogs (three males and three females) in a two-period crossover design to explore the viability of stable isotope approach using drug substance X. Dogs were randomized to receive 9 mg/kg dose of drug substance X as salt 1 (regimen A) and salt 2 (regimen B) in one of the two periods. A 1 mg/kg dose of ^{13}C salt 1 (total dose of drug substance per treatment was 10 mg/Kg) was co-administered in each of the two periods and serves as the internal control or covariant in this study. The study was conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Following log transformation, AUC and C_{max} of non-enriched substance X were analyzed using both an analysis of variance (ANOVA) and ANCOVA. In both models, subject, period, and regimen were fit as fixed effects and in the ANCOVA model the log-transformed AUC and C_{max} of the enriched control were fit as a covariate. Point estimates and 90% confidence intervals (CI) were calculated for the ratio of B/A, as was the within-subject coefficient of variation (CV_r). The AUC and C_{max} of the enriched form (eAUC and eC_{max}) were analyzed similarly using the ANOVA model. The residual mean square error was also calculated and is expressed as CV_r .

RESULTS

Simulations

In all cases, the average residual mean square error was reduced when adjusting for the enriched PK. Subsequently, the required sample size to demonstrate bioequivalence was also reduced. The reduction in the residual mean square error increased with increasing correlations between the non-enriched and enriched PK within a subject and period (ρ), with reductions in the residual mean square error being greater than 70% and sample size reductions of greater than 80% for correlations greater than 0.9. Figure 1 illustrates the estimated variability (CV_r) and sample size by correlation

Fig. 1. Simulation results: estimated variability (*solid bars*) and required sample size (*hashed bars*) for $CV_w=50\%$. The x -axis represents the correlation between the non-enriched and enriched PK measurements (ρ). The left y -axis provides the estimated residual mean square error from the analysis of covariance model expressed as a coefficient of variation (CVR; *solid bars*) and the right y -axis provides values for the reduced required sample size (*hashed bars*)

(ρ) for CV_w of 50%, Fig. 2 shows the corresponding percent reduction of variability and sample size. The percentage of reduction in both variability and sample size, as well as the relationship between the amount of reduction and ρ was independent of the assumed within-subject variability of the non-enriched PK (data not shown). The reduction in the residual mean square error was also independent of whether the variability of the non-enriched and enriched PK was equal or not (data not shown).

For example, a drug product with CV_w of 50% would require a sample size of 98 subjects to provide at least 90% power to demonstrate bioequivalence. If the stable isotope approach is used and the correlation between the enriched and non-enriched PK is assumed to be 0.95, the required sample size would be 10 subjects with a resulting variability (RMSE) being approximately 15%. This represents a reduction in sample size of 90% and a reduction in the residual mean square error of 70%.

Fig. 2. Simulation results: percent reduction in both variability (CVR) and sample size for $CV_w=50\%$. The x -axis represents the correlation between the non-enriched and enriched PK measurements (ρ). The y -axis provides values for the percent reduced in variability, CVr (*solid bars*) and the required sample size (*hashed bars*)

Dog Study

Table I provides the PK parameters (AUC and Cmax) for regimens A and B and corresponding PK parameters of the enriched internal control. As expected, the non-enriched and enriched AUCs and Cmax were highly correlated (Pearson correlation coefficient, >0.99, Fig. 3) and thus the residual mean square error was greatly reduced by an order of magnitude (when using the enriched PK as an internal standard, *i.e.*, ANCOVA analysis).

Of note, the point estimates of the adjusted analysis (ANCOVA) and unadjusted analysis (ANOVA) appear to be discordant with the point estimates for AUC being 1.05 and 0.80, respectively (Table II). Additionally, there appears to be concordance between the estimates from the unadjusted analysis (ANOVA) for the non-enriched and enriched AUCs, with ratios of 0.80 and 0.78, respectively. Similar trends were observed for Cmax.

This example highlights a potential pitfall of this approach which has not been previously discussed in the literature. It would be difficult to determine whether the adjusted analysis is biased or not, with the bias being attributed to the possibility of an interaction between the enriched drug substance and the non-enriched formulations. In other words, is there an excipient in one formulation that isn't in the other formulation and that excipient could affect the absorption of the enriched drug substance which then adds bias to the study results. In this example, the discordance observed between different analyses (ANCOVA and ANOVA) may be an indication that the two salts interact resulting in lower absorption of drug substance X. Or it might just be a reflection of the variability of substance X and sample size. By including the enriched AUC/Cmax as a covariate, the point estimate appears to be biased toward 1 and bioequivalence. It should be noted that following administration of regimen B, exposure of both non-enriched and enriched PK for dog #6 appear to be outliers having the lowest observed values. This dog was reported to have vomited following administration of regimen B and thus

Fig. 3. Scatter plot of non-enriched and enriched AUCs to show correlation

explains these low exposures. Repeating the analysis excluding all data from dog #6, the results from the ANCOVA and ANOVA analyses (Table III) appear to be more concordant, indicating that the discordance may be a function of this one outlier.

DISCUSSION

The simulations and dog study confirm what has been discussed in the literature: utilization of a stable isotope in some pharmacokinetic studies can result in significant reductions in the number of subjects required. However, the dog study raises the concern regarding potential for interactions between the non-enriched and enriched forms and potential bias of the adjusted point estimates. One way to eliminate this potential issue is to apply this approach when the composition of the formulations is both qualitatively and quantitatively identical.

Applying this approach to formulations that are qualitatively and quantitatively identical would permit its application to the evaluation of QbD design space and setting specifications for drug product, and for BE studies associated with a manufacturing site change.

Recently, the regulatory agencies have re-emphasized the need to relate the drug product and its manufacturing process

Table I. AUC and Cmax Values from Periods 1 and 2 of the Dog Study at a Dose of 10 mg/Kg (Ratio of Enriched to Non-Enriched of 1:9)

Regimen	Period	Subject	Sex	AUC inf (ng h/mL)	Cmax ng/mL	Relative F (AUC)	Enriched AUC inf (ng h/mL)	Cmax (ng/mL)	Relative F (AUC)
A	Period 1	1	Male	1,158	114.6		143	13.7	
A	Period 1	4	Male	2,857	369.6		314	42.9	
A	Period 2	5	Male	4,163	386.6		486	43.1	
A	Period 2	2	Female	7,023	759.9		783	82.3	
A	Period 2	3	Female	1,246	172.6		148	20.7	
A	Period 1	6	Female	8,058	854.2		845	95.0	
Mean				4,084	443		453	50.0	
Range				1,158–8,058	114.6–854.2		143–845	13.7–95.0	
B	Period 1	2	Female	7,988	795.5	114	869	88.3	111
B	Period 1	3	Female	3,006	456.8	241	319	50.0	215
B	Period 2	6	Female	968	91.9	12	125	10.3	15
B	Period 2	1	Male	1,434	124.8	124	168	12.4	118
B	Period 2	4	Male	2,566	308.2	90	279	33.3	89
B	Period 1	5	Male	2,970	285.1	71	296	25.4	61
Mean				3,155	344	109.0	343.0	36.6	102
Range				968–7,988	91.9–795.5	12–241	125–869	10.3–88.3	15–215

Table II. Point Estimates and 90% Confidence Intervals, and Estimated Coefficients of Variances (CV_r) for AUC Data

	Model	Point estimate B/A	90% CI	CV _r (%)
AUC	ANCOVA	1.048	0.958, 1.146	6.3
AUC	ANOVA	0.800	0.337, 1.900	79.9
eAUC ^a	ANOVA	0.780	0.353, 1.725	71.8

^a eAUC = enriched AUC; AUC = non enriched AUC

with patient safety and efficacy. Dissolution is a key drug product CQA that has the potential to serve as a surrogate marker for intended clinical performance. FDA (Christine Moore, Specifications for QbD containing applications, CMC Workshop, Translating science into successful regulatory submissions, Washington DC, Feb 2011 and Elaine Morefield, Performance based specifications, National AAPS Meeting, New Orleans, Nov 2010) has outlined the approaches for setting dissolution specification in different scenarios, where the absence of biodata linking *in vitro* dissolution to plasma concentrations may result in tighter dissolution specifications that may be overdiscriminating and lead to unnecessary batch failures. On the other hand, data establishing the range of dissolution characteristics that result in bioequivalence may provide wider dissolution specifications and increased regulatory flexibility for manufacturing changes. The prerequisite to the definition of a robust design space is the identification of CQAs and CPPs. In addition to considering the drug product formulation and its manufacturing process variables, the clinical relevance (*i.e.*, patient safety and efficacy) should be a key factor when developing the design space. This objective may be achieved by performing biostudies using the stable isotope approach using products with variations in CQAs (*e.g.*, particle size of drug substance) and variations in CPPs (*e.g.*, compression force to control tablet thickness/hardness). We are proposing the use of stable isotopes to reduce variability of the statistical test used to compare formulations and subsequently improve the efficiency of studies designed for relative bioavailability and bioequivalence. The enriched (stable isotope containing) compound would be co-administered with the non-enriched formulation. PK parameters would be determined for both the non-enriched and enriched compound. The analysis of the non-enriched PK parameters would be “corrected” by either analyzing the ratio of the non-enriched PK and the enriched PK or by fitting the enriched PK as a covariate. This correction results in a reduction in variability of the statistical test used to compare formulations used to assess bioequivalence hypothesis

Table III. Point Estimates and 90% Confidence Intervals, and Estimated Coefficients of Variance (CV_r) for AUC Data Excluding Dog #6

	Model	Point estimate B/A	90% CI	CV _r (%)
AUC	ANCOVA	1.069	0.973, 1.174	4.9
AUC	ANOVA	1.149	0.658, 2.005	38.0
eAUC ^a	ANOVA	1.076	0.610, 1.899	38.7

^a eAUC = enriched AUC; AUC = non enriched AUC

as including the enriched PK in the analysis accounts for any differences between periods within an individual subject. Since the non-enriched and enriched compounds are administered under the exact same conditions in a given subject and period, it is expected that their PK values will be highly correlated. For example, if the non-enriched AUC for subject 1 in period 1 is greater than the mean non-enriched AUC across all subjects, the enriched PK for that same subject in the same period is likely to be greater than the mean enriched AUC. Based on the literature (3) and in-house dog data (see “RESULTS” section), it is expected that the correlation will exceed 95% and possibly even 99%. The greater the correlation, the greater the reduction in the variability of the statistical test used to compare formulations.

Where applicable to demonstrate bioequivalence in humans, the preferred approach will be to use stable isotope approach using a crossover design consistent with the FDA Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations, March 2003. Subjects can be dosed with all the products (typically one reference and one test product for bioequivalence studies but may include three to four products with variations in CQAs and CPPs for QbD investigations). Additionally, each product will be co-dosed with drug substance labeled with a stable isotope (such as ¹³C) which will be given at no more than 10% of dose of the products.

One of the shortcomings of this approach is the difficulty in determining whether the “corrected” PK analysis is biased or not, with the bias being attributed to the possibility of an interaction between the enriched drug substance and the non-enriched formulations. Applying this stable isotope approach to products that are identical qualitatively and quantitatively will eliminate any issues associated with an interaction between the enriched drug substance and the non-enriched formulations. While this approach is limited to formulations that are qualitatively and quantitatively identical, this approach does allow the comparison of changes across input material properties and process parameters.

Some of the additional benefits and limitations/considerations for the use of stable isotope approach include the following,

Benefits

1. A stable isotope acts as a reference within each subject/period reducing the variability of the statistical test used to compare formulations and decreasing the number of subjects required to achieve the desired power thus providing an opportunity to set clinically relevant specifications and QbD design space using efficient biostudies.
2. Reduced time of the study offers advantages when there is a need to perform studies in difficult to recruit patient populations (compared to healthy volunteers).
3. Reduced number of dosing periods (when compared to the current replicate design approach for highly variable drugs)
4. No change in the number of PK samples that need to be analyzed (determine enriched as well as non-enriched API in the same plasma sample).

5. The approach of using stable isotopes does not use radioactivity.

Limitations/Additional Considerations

1. These studies require manufacturing of the stable isotope containing compound
2. Synthesizing two different stable isotopes if one is needed as an internal standard (typically with additional ^{13}C atoms) for analytical purposes
3. For low dose compounds the accuracy of administration of the stable isotope (at no more than 10% of actual dose) needs to be considered for the formulation
4. For poorly water-soluble drug substances, the use of solid state vs. solution state needs to be considered for the enriched formulation
5. Consideration of resources and cost for synthesis of these stable isotope containing compounds so that the labeled site is metabolically stable
6. Ideally, work in a dose range where linear PK is exhibited for the drug substance

To stay within compendia guidelines, the maximum amount of the enriched drug substance to be used is set to 10% or less of the target drug dose. There may be some concern around the impact of the enriched drug substance on the solubility and bioavailability of the non-enriched drug substance and *vice versa*. However, by using low doses of enriched drug substance and the fact that the subjects receive the same total dose at each dosing period that issue is addressed. The actual amount of enriched drug substance is driven by the analytical sensitivity of the assay. Our experience shows the average dose of enriched drug substance is between 3% and 5% of the target dose. The form (aqueous

suspension or solution) of the enriched drug substance used is dependent on a number of factors such as the solubility of the drug substance in the dosing solution and the amount of enriched drug substance to be administered. For compounds (e.g., BCS 1 and 3 compounds) where the dose of the enriched drug substance does not exceed the solubility of the drug in the volume of the dosing solution, then the enriched drug substance can be administered as an aqueous solution. In situations where the dose of the enriched drug substance (e.g., BCS 2 and 4 compounds) exceeds the solubility of the drug in the volume of the dosing solution, then the drug substance will need to be administered as an aqueous suspension. In the case of dosing a suspension, one needs to ensure that an accurate dose (*i.e.*, that the suspension is uniform) of the enriched drug substance is administered with each test dose. The ideal situation would be where the non-enriched and enriched drug substance is in the same form (*i.e.*, both are suspension or both are aqueous liquids) but this is not required. If one is a suspension and the other a liquid, this approach will still work as indicated by reports in the literature where solutions were compared to solid oral dosage forms (1). Simulations show a positive effect using this approach even with the correlation value as low as 0.8.

Example

A hypothetical case has been created to illustrate the proposed approach. For setting clinically relevant specifications, an example biostudy would include the following three tablet products (identical qualitative and quantitative formulation composition).

Fig. 4. Dissolution profile for products A, B, and C using a discriminating dissolution method

- 1 Reference product A, using micronized drug substance and at target compression (target porosity) manufacturing process
- 2 Test product B, using nonmicronized drug substance and at target compression (target porosity) manufacturing process
- 3 Test product C, using micronized drug substance and at high compression (low porosity) manufacturing process

The dissolution method would be designed to demonstrate discrimination between formulation/process variables and would ensure complete dissolution (>85%) from these products (although the rate of dissolution may vary). A hypothetical set of dissolution profiles for these products have been shown as Fig. 4.

A three period crossover study in healthy volunteer subjects (or patients as necessary) would be conducted to demonstrate that the two test products (B and C) are bioequivalent to the reference product (A). Subjects would receive each of the products in one period following one of six sequences (ABC, ACB, BAC, BCA, CAB, and CBA). In addition, in each period, the assigned product would be co-dosed with the enriched compound (such as ^{13}C) in a solution/solid dosage form. The dose of the enriched compound would be no more than 10% of dose of the product. The study would be appropriately powered to demonstrate bioequivalence assuming the reduced variability was achieved with the stable isotope approach.

Assuming that the CV_w is 50% and using a standard study design, a sample size of 98 subjects would be required to provide at least 90% power to demonstrate bioequivalence. However, if the stable isotope approach is used and the correlation between the enriched and non-enriched PK is assumed to be 0.95, the required sample size would be 10 subjects. Sample size calculations are based on a two-sided testing procedure, type I error rate of 5% and a true ratio of 1 and have been rounded up to the nearest multiple of six to ensure equal allocation to the six treatment sequences.

Following log transformation, AUC and C_{max} on the non-enriched products would be analyzed using analysis of covariance model fitting fixed effects for subject, period, and regimen and the AUC and C_{max} of the enriched solution/solid (following log transformation) as a covariate. Point estimates and associated 90% CI will be calculated for the ratios of B/A and C/A using the residual mean square error from the model. Bioequivalence will be demonstrated if the 90% CI are completely contained within the interval 0.80–1.25.

Availability of PK data allows setting clinically relevant specifications. If the above example showed bioequivalence across the three products, A, B, and C, these data will be used to justify data driven clinically relevant specifications for

dissolution, drug substance particle size, and compression (porosity), for example $Q=80\%$ at 45 min.

CONCLUSIONS

As per the simulations, for a drug product with CV_w of 50% and assuming a correlation of 0.95 between the enriched and non-enriched PK, variability of the statistical test used to compare formulations (ie. the residual mean square error of the analysis of covariance model) can be reduced by 70% and the required sample size can be reduced by 90%.

Using stable isotopes in a dog study allowed the “correction” for PK data to result in significant reduction in the variability of the statistical test used to compare formulations. The results of this dog study validated the results of the simulation performed and showed the applicability of this approach to bring value to preclinical studies as well.

Based on the work to date, it appears that with a minimum investment (synthesis of enriched drug substance) and slight modifications to PK study design, it is possible to reduce the number of subjects needed to perform certain bioequivalence studies. The higher the within-subject variability, the greater the benefits of using the proposed stable isotope approach.

ACKNOWLEDGMENTS

We thank GSK management and staff for their support of this project.

REFERENCES

1. Pieniaszek HJ, Mayersohn M, Adams MP, Reinhart RJ, Barrett JS. Morcizine bioavailability via simultaneous, dual, stable isotope administration: bioequivalence implications. *J Clin Pharmacol.* 1999;39:817–25.
2. Heck HA, Buttrill SE, Flynn NW, Dyer RL, Anbar M, Cairns T, Dighe S, Cabana BE. Bioavailability of imipramine tablets relative to a stable isotope-labelled internal standard: increasing the power of bioavailability tests. *J Pharmacokinet Biopharm.* 1979;7(3):233–48.
3. Browne TR. Stable isotope techniques in early drug development: an economic evaluation. *J Clin Pharmacol.* 1998;38:213–20.
4. Wolen RL. The application of stable isotopes to studies of drug bioavailability and bioequivalence. *J Clin Pharmacol.* 1986; 26:419–24.
5. Lappin G, Rowland M, Garner RC. The use of isotopes in the determination of absolute bioavailability of drugs in humans. *Expert Opin Drug Metab Toxicol.* 2006;2(3):419–27.
6. Schellekens RC, Stellaard F, Woerdenbag HJ, Frijlink HW, Kosterink JG. Applications of stable isotopes in clinical pharmacology. *Br J Clin Pharmacol.* 2011;72(6):879–97.