Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and *in vitro* immunoassay interference

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Aim: Biotin in human serum is a potential interfering factor for streptavidin-biotin-based assays. We aimed to evaluate the effective half-life of biotin and biotin metabolites, and establish a pharmacokinetic (PK) model to simulate the time taken for the biotin concentration to fall below a series of thresholds. **Materials & methods:** PK properties of biotin (5, 10 and 20 mg daily) were evaluated in healthy participants. Biotin serum concentrations were simulated for high-dose regimens (1 mg daily to 300 mg q.i.d.) using a population PK model. **Results:** Washout periods required for biotin concentrations to reach thresholds ranging from 10 to 100 ng/ml were successfully simulated. **Conclusion:** Our simulations provide valuable guidance on biotin washout periods necessary to avoid false assay results.

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Immunoassays that allow rapid measurement of analytes can be vital for the correct diagnosis of a broad range of diseases [1]. The interaction of streptavidin and biotin has been utilized for the development of robust and highly sensitive immunoassays by many manufacturers (Abbott, Beckman Coulter, Ortho Clinical Diagnostics, Roche Diagnostics, Siemens Healthcare Diagnostics and others). Biotin, a water-soluble vitamin, is a small and stable molecule that can be conjugated to many proteins without significantly affecting their biological activity; this interaction is the strongest known noncovalent binding between a protein and a ligand [2].

Exogenous biotin has the potential to interfere with streptavidin-biotin-based assay results. The impact of interference on test results can be the generation of falsely high values, obtained when using a competitive assay design, whereby an excess of biotin in the specimen competes with biotinylated analog for binding sites on streptavidin. Alternatively, when using a sandwich assay design, an excess of biotin in the specimen can displace biotinylated antibodies, which can generate falsely low values [3]. Reports of biotin interference leading to incorrect biochemical diagnoses in both adults and children have been published previously, along with warnings to clinicians and pathologists to interpret unexpected assay results with caution and consider the potential effect of biotin interference before making a diagnosis [4–7].

The normal serum concentration of biotin is very low; published average values range from below 0.1 to 0.8 ng/ml [8,9]. The adequate daily intake of biotin is 30 µg/day [10] and biotin deficiency is rare as the majority of diets contain enough biotin for this to be reached. However, biotin is increasingly being marketed as a lifestyle supplement which is claimed to strengthen hair and nails, despite no scientific confirmation of these benefits [11]. The unregulated, over-the-counter (OTC) product is available in doses ranging from 50 µg found in multivitamin

tablets, to as high as 10 mg in biotin-only products [12,13]. Consequently, the risk of biotin interference is increased for samples taken from patients choosing to take these supplements.

Guidance on how to mitigate the risk of biotin interference in streptavidin-biotin-based assays varies between manufacturers. The spectrum of guidance provided in package inserts ranges from no mention or a vague generic warning of biotin interference, which is insufficient as it does not create awareness of potential issues, to the more comprehensive specification of a serum biotin concentration below which assay interference is minimal, for example, $\leq 10\%$ change; these thresholds vary from 10 to 500 ng/ml, with the majority of thresholds being > 50 ng/ml. To our knowledge, the Elecsys assays (Roche Diagnostics) are the only assays to specify a recommended washout period that would ensure that biotin concentrations are below the interference threshold following the last biotin administration [14–19].

Due to increasing doses of OTC biotin products and the broader acceptance of biotin as a dietary supplement, there is a need for greater understanding of biotin pharmacokinetic (PK) properties and clearer guidance for clinicians and pathologists on the use of streptavidin-biotin-based assays in this setting. The overall aim of this study was therefore to characterize the PK properties of biotin following high-dose regimens and evaluate the time needed for biotin concentrations to fall below a range of interference thresholds.

Materials & methods

Trial design

This was an open-label, multiple-dose, single-center study. A total of 54 participants (male and female) provided a sufficient sample size for the collection of data for the derivation of PK model parameters.

Objectives

The objectives of the study were to characterize the PK properties of biotin using a population modeling approach, and to simulate biotin concentrations following different dosing regimens. Additionally, biotin PK properties were used to assess the time necessary for biotin concentrations to fall below a range of interference thresholds following last intake.

Participants

Apparently healthy participants (no known chronic or acute disease) aged ≥ 18 years were enrolled in the study. Participants were not eligible for enrollment if they were taking anticonvulsants, biotin or multivitamins, if they had a known intolerance to biotin or lactose or if they were participating in other trials during the active study phase. Pregnant women and breast-feeding mothers were also excluded from the study.

Standard protocol approvals, registration & patient consents

The clinical study protocol was approved by Ethik-Kommission der Bayerischen Landesärztekammer. The study was performed in accordance with ethical principles consistent with the Declaration of Helsinki and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use/Good Clinical Practice. Written informed consent was obtained from all participants before study initiation.

Interventions

Participants were divided into three different dosing groups (5, 10 or 20 mg). Each group was comprised of equal proportions of male and female participants. Participants were required to fast for 8 h prior to, and 1 h after, biotin intake. Biotin (5 mg tablet: $\times 1$, $\times 2$ or $\times 4$ depending on group) was administered orally, once daily, at the same time on 5 consecutive days. Blood samples were collected prior to biotin intake on Days 1, 2 and 7, and at 1, 3, 6, 8 and 12 h postdose on Days 3 and 7.

Biotin measurements

Samples were measured on the cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using an in-house competitive Elecsys research assay, which detects total serum biotin (free biotin, bound biotin/biotin and biotin metabolites). Samples were incubated with a streptavidin–ruthenium conjugate followed by the addition of a poly-biotinylated antibody that binds to still vacant biotin-binding sites on the streptavidin–ruthenium conjugate and to the streptavidin-coated microparticles. The reaction mixture was then aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were

removed with ProCell/ProCell M. Application of a voltage to the electrode induced chemiluminescent emission that was measured by a photomultiplier. Results were determined via a calibration curve which was generated prior to serum sample measurements using D-biotin standards. Those samples with a biotin concentration above the measuring range (0.1–0.75 ng/ml) were diluted, and results were subsequently multiplied by the dilution factor.

Assay quality was monitored using internal run controls and human serum samples spiked with a defined amount of D-biotin.

Pharmacokinetic model

The population PK model was developed using the first-order conditional estimation interaction method in NONMEM[®] version 7.3 (Icon Development Solutions, MD, USA). Simulations were also performed with NONMEM. The analysis and simulation datasets, as well as graphics, were created using SAS[®] System for Windows[®] version 9.4 TS Level 1M2. Validation checks were conducted by creating diagnostic plots using all PK data variables in order to identify possible errors or outliers.

A preliminary population PK model was developed using existing data (not shown) and used as a basis for this analysis. The model was a two-compartment disposition model, with first-order absorption and elimination. The best residual error model was a multiplicative error model. The PK model parameters were apparent clearance (CL/F), apparent volume of distribution of the central compartment (V₂/F), apparent intercompartmental clearance (Q/F), apparent volume of distribution of the peripheral compartment (V₃/F), first-order absorption rate constant (K_A) and lag time (TLAG). Graphical analyses were conducted to investigate any impact of the following baseline characteristics (i.e., covariates) on the PK of biotin: age, gender, race, weight, height, BMI and dose.

Final model selection criteria

Final model development and selection were based on various goodness-of-fit indicators. The final model was identified by using the following criteria: minimization obtained successfully in NONMEM; covariance step obtained successfully; at least three significant figures obtained for all θ estimates and final estimates of population PK parameters were not close to the initial estimate boundaries. It was required that there should be no trends in the following goodness-of-fit plots: population and individual predictions versus observations; conditional weighted residuals versus population predictions versus time; individual weighted residuals versus individual predictions versus time; individual plots comparing observed and individual predictions over time.

Effective half-life & accumulation ratio

The population PK parameters from the final biotin model were used to compute the biotin-effective half-life. An effective half-life ($t_{1/2, \text{eff}}$) was computed for the two-compartment PK model, taking into account the contribution of each phase of the PK profile to the elimination process. The accumulation ratio, based on the AUC, was also computed, taking into account the contribution of each phase of the PK profile.

Model predictive performance

The predictive performance of the population PK model was evaluated by conducting a visual predictive check (VPC). This approach aimed to verify the agreement between observed data from the biotin interference study and the simulated data using the parameters from the final population PK model. Since the simulations of different dosing regimens were conducted with the individual *post-hoc* PK parameters ($n = 54$), those parameters were used for the VPC and were randomly sampled and assigned to each dose group. For each individual, dosing information and sampling times were identical to those contained in the original dataset (used for model development). This process was then repeated 100 times for the entire dataset. For each simulation, median, 5th and 95th percentiles were calculated; the 90% confidence interval (using the 5th and 95th percentiles) was computed from results of the 100 simulation runs. Median, 5th and 95th percentiles derived from observed data were then superimposed on the same graph and compared with predicted 90% confidence intervals.

Simulations of biotin concentrations for different dosing regimens

Individual PK parameters (i.e., K_A, CL/F, V₂/F, Q/F and V₃/F) of the 54 participants (who received biotin 5, 10 or 20 mg once daily) included in the NONMEM analysis were used to perform the simulations with several very high biotin doses (1, 2.5, 5, 10, 20, 100 and 300 mg) and several dosing frequencies: single dose (s.d.); multiple

Table 1. Patient demographics.

	5-mg biotin (n = 18)	10-mg biotin (n = 18)	20-mg biotin (n = 18)	Total (n = 54)
Mean age, years (range)	43.9 (19–66)	48.2 (21–72)	35.9 (19–58)	42.7 (19–72)
Sex, n (%)				
– Female	9 (50)	9 (50)	9 (50)	27 (50)
– Male	9 (50)	9 (50)	9 (50)	27 (50)
Race, n (%)				
– Asian	1 (6)	0	0	1 (2)
– White	17 (94)	18 (100)	18 (100)	53 (98)
Mean BMI, kg/m ² (SD)	25.1 (4.8)	24.9 (2.5)	24.4 (4.0)	24.8 (3.8)

BMI: Body mass index; SD: Standard deviation.

doses up to steady state: once a day (q.d.); twice a day (b.i.d.): 8:00 am and 8:00 pm; three-times a day (t.i.d.): 8:00 am, 12:00 pm and 8:00 pm; four-times a day (q.i.d.): 8:00 am, 12:00 pm, 4:00 pm and 8:00 pm.

For each dosing regimen, biotin concentration-time profiles were simulated every 0.5 h from the time of last drug intake up to 24 h after the last drug intake and then every hour up to 250 h after the last drug intake.

Simulated biotin concentration-time profiles were summarized by dosing regimen using descriptive statistics. The times needed for biotin concentration percentiles to fall below different thresholds ranging from 10 to 100 ng/ml were computed.

Results

Patient characteristics

Between 22 September 2016 and 12 October 2016, 54 participants were enrolled in the study. Participant demographics are summarized in Table 1; the proportion of males and females, and mean BMI was consistent across the doses studied and 98% of the participants were White. The mean age of participants in the 20-mg group was slightly lower than that in the other two groups (Table 1). All 54 received treatment and completed the study. In total, 702 samples were collected for analysis. No serious adverse events were recorded during the study period. In total, nine adverse events occurred; all were deemed unlikely to be related to the study.

Biotin measurements & PK

The performance of the assay was assessed to ensure the accuracy of the results. The overall assay performance was good. Recoveries of internal run controls ranged from 94 to 106% and interassay CVs were $\leq 4\%$. No drift in signal within runs was detected (end measurement taken at ~ 6 –8 h). Recoveries of human serum control samples ranged from 87 to 113% and interassay CVs were $\leq 6\%$.

PK analysis showed that the biotin concentration was dose proportional within the studied range; Figure 1 shows the median biotin concentrations for all groups. The median (minimum–maximum) peak biotin concentration on Day 3 (1-h postdose) was 41 (10–73) ng/ml, 91 (53–141) ng/ml and 184 (80–355) ng/ml for the 5, 10 and 20 mg groups, respectively. Following 5 days of consecutive dosing (q.d.), accumulation was observed for each group, with the majority of values doubled 6 h postdose on Day 7 compared with the same time point on Day 3. PK analysis showed linear PK over the range of doses (5–20 mg) and time (first dose to steady state). Absorption was rapid, with a maximum serum concentration ($t_{\max}$) of less than 1 h.

Pharmacokinetic model

Final model selection

The PK of biotin was found to be appropriately described by the two-compartment disposition model, with first-order absorption and elimination. NONMEM population parameters are shown in Table 2. The population PK parameters were well estimated with a standard error of less than 10%. Interindividual variability ranged from 13.8 to 21.7%.

The covariates investigated (weight, height and age) had minimal impact on CL, V_2/F and F_1 , and thus were not further investigated in the population PK analysis. No trends were observed in the population or individual predictions versus observations, or in weighted residual plots. No effect of dose on the PK parameters was observed.

● Dosage group 1 (5 mpg daily):	0.1	0.1	40.5	16.2	6.6	4.9	3.6	4.9	46.4	19.2	11.2	9.1	7.0
● Dosage group 2 (10 mpg daily):	0.1	0.1	90.6	32.7	14.1	10.3	7.9	10.2	103.2	41.9	24.3	18.7	14.9
■ Dosage group 3 (20 mpg daily):	0.1	0.1	183.8	63.7	27.7	20.9	16.7	21.6	168.4	71.4	45.9	37.0	29.5

Figure 1. Median (minimum–maximum) biotin concentration in serum (ng/ml) for each dosing group (5, 10 and 20 mg).

D: Day; T0: Prebiotin intake; T1: 1-h postdose; T2: 3-h postdose; T3: 6-h postdose; T4: 8-h postdose; T5: 12-h postdose.

Table 2. NONMEM population parameter values for the final run with the biotin pharmacokinetic model.

Parameter	Estimate	Standard error (%)
CL/F	17.4 l/h	2.50
V2/F	73.2 l	3.50
V3/F	301 l	5.70
Q/F	24.1 l/h	3.50
KA	7.12 h	9.60
Lag time	0 h (fixed)	NA
F1	1 (fixed)	NA
CL	13.8%	47.8
VC	20.2%	32.4
F1	21.7%	24.3
σ (proportional)	14.4	6.8

σ : Standard deviation; CL: Clearance; CL/F: Apparent drug clearance; F1: Relative bioavailability; KA: Absorption rate constant; NA: Not applicable; PK: Pharmacokinetic; Q/F: Apparent intercompartmental clearance; VC: Volume of central compartment; V2/F: Apparent central volume of distribution; V3/F: Apparent peripheral volume of distribution.

Figure 2. Simulation of time required for biotin serum concentration to fall below 30 ng/ml following biotin intake in the 54 participants characterized in the pharmacokinetic study.

b.i.d.: Twice a day; q.d.: Once daily; q.i.d.: Four-times a day; s.d.: Single dose; t.i.d.: Three-times a day.

Effective half-life & accumulation ratio

The effective half-life was calculated to be 15 h, indicating that steady state would be reached in approximately 3 days. The AUC accumulation ratios were 1.4 and 1.8 for once daily and twice a day dosing, respectively.

Model predictive performance

A VPC of the predictive performance of the PK model was conducted for all dose groups at Day 3 and Day 7 to ensure accuracy of the model. The median, fifth and 95th percentiles of the observed PK profiles were contained in their respective CIs indicating that the set of 54 individual PK parameters captures both the central tendency and the interindividual variability of biotin pharmacokinetics.

Simulations of biotin concentrations for different dosing regimens

It is important to understand the time taken for the serum biotin concentration to fall below a specific threshold, to allow calculation of the washout period required before collection of serum samples for assessment using biotin-streptavidin based assays. Figure 2 shows the time needed for the biotin concentration to fall below 30 ng/ml following various dosing regimens in the 54 participants characterized in the PK study. The threshold of 30 ng/ml was chosen as a conservative approach to calculate the washout times, as for many assays, the biotin threshold is >50 ng/ml. Following doses of biotin at 5, 10 or 20 mg q.d. for 5 days, the 30 ng/ml interference threshold was reached within 3.5, 8 or 31 h, respectively. Supplementary Table 1 summarizes the time required for the biotin concentration to fall below thresholds ranging from 10 to 100 ng/ml, for each of the dose regimens simulated.

Discussion

This study aimed to characterize the PK properties of biotin and establish a PK model to simulate different high-dose regimens. These data are important as the use of OTC biotin supplements is increasing, and the strength of available doses is rising. Therefore clinicians and pathologists need to be confident that any recommendations for biotin interference thresholds or washout periods are sufficient to mitigate the risk for potential assay interference. To this end, validation or strengthening of data surrounding thresholds or washout periods stated on package inserts can help reassure clinicians and pathologists that accurate diagnoses can be made using these assays.

Results of the population PK analysis showed that biotin has linear PK over the range of doses studied (5–20 mg), is rapidly absorbed and has an effective serum half-life of 15 h. Following consecutive dosing over several days,

accumulation of biotin was observed within each dosing group; however, data analysis revealed that steady state is reached after 3 days of biotin intake.

The PK model showed very good predictive performance, indicating that simulations from the model can provide additional guidance on the use of washout times depending on assay-specific biotin interference thresholds. However, interindividual variability for clearance was moderate (13.8–21.7%) and the model was based on data from healthy participants. By including apparently healthy participants, the study more accurately reflects real-life situations, where renal function may be unknown and a spectrum of renal function is expected. The results should be interpreted with care; particularly in patients with impaired renal function where the half-life of biotin may be longer [4,8].

The results of the population PK analysis demonstrate that for biotin dosing regimens up to daily doses of 1 mg, as found in OTC supplements/multivitamin pills (i.e., more than 80-fold the adequate daily intake for biotin), serum biotin levels fell below an *in vitro* threshold of 10 ng/ml (one of the lowest recommended biotin interference thresholds [18]) after 2 h (Supplementary Table 1). For doses of biotin of up to 10 mg/day (e.g., 5 mg twice daily or 10 mg once daily; more than 300-fold the adequate daily intake of biotin), an *in vitro* serum biotin threshold of 30 ng/ml was consistently reached after 8 h.

Following extremely high doses of biotin (≥ 20 mg q.d.; more than 500-fold the adequate daily intake), the residual biotin serum concentration did not drop to 30 ng/ml until 31 h after last intake, and took 73 h to drop to 10 ng/ml. Therefore, in rare cases where extremely high doses of biotin are administered, extended washout periods would be needed for some immunoassays, particularly those with a low *in vitro* interference threshold (< 30 ng/ml).

A limitation of this study was that it was designed to relate *in vivo* biotin serum levels to *in vitro* interference thresholds. Consequently, the data do not directly provide information on the clinical relevance of biotin interference. Specifically, the present study does not give information on the size of any potential effect of biotin interference on test results or the proportion of laboratory values that are near a medical decision point and thus potentially vulnerable to interference. Future investigations could aim to address this limitation but *in vitro* thresholds will continue to be the accepted approach until robust methods of assessing biotin interference *in vivo* are published. Also, it could be argued that results of biotin measurements would be better presented in nmol/l rather than ng/ml since the MWs of the main metabolites of biotin are different. However, both of these units create uncertainty in relation to biotin metabolites, and the specific metabolites present and their concentrations are unknown.

This study used the cobas e 411 analyzer and an in-house research kit to detect total biotin in the serum samples. This was chosen over other methods, such as LC–MS as the research kit measures total serum biotin, including free biotin, bound biotin/biotin and biotin metabolites, all of which can interfere with streptavidin-biotin-based assays.

In a recent study investigating the extent of biotin interference with Roche Elecsys thyroid assays (free thyroxine, thyroid-stimulating hormone, total triiodothyronine and total thyroxine) using the Roche cobas e 601 immunoassay platform, the experimentally derived biotin interference thresholds corroborated the corresponding Roche package (free thyroxine: 61 ng/ml [package insert threshold: 25 ng/ml]; total thyroxine: 348 ng/ml [100 ng/ml]; total triiodothyronine: 19 ng/ml [10 ng/ml]; thyroid-stimulating hormone: 30 ng/ml [20 ng/ml]). Additionally, it was found that treating 0.45-ml biotin-spiked serum with 0.05-ml streptavidin agarose beads for 1 h effectively removed biotin and eliminated interference [20], highlighting that if biotin interference is suspected, additional steps can be taken by the laboratory in order to reduce the risk of a false result.

The biotin doses administered in this study were chosen to reflect the high doses of biotin that are available to take as a lifestyle supplement (50 μ g–10 mg), which can be purchased OTC, despite no available clinical evidence to support the claims that it leads to the desired improvements in health of hair and nails [11]. It should be noted that the doses examined were approximately 100- to 500-times higher than the recommended daily intake of 30 μ g/day [10]. Higher doses were also simulated; currently, very-high biotin doses are only being used in the clinical trial setting, as a potential treatment for multiple sclerosis [21,22]. A study in healthy volunteers ($n = 8$) found that, following a single 100 or 300 mg dose of biotin, median $t_{\max}$ occurred at 1.25 and 1.5 h, respectively. Elimination half-lives varied from 7.8 to 18.8 h, which are consistent with the elimination half-life observed in our study (15 h) [23]. The slight food effect observed by Peyro Saint Paul *et al.* [23] was avoided in our study by administration of biotin in the fasted state only. Also, biotin accumulation was observed in our study, which is in contrast to animal studies detailed by Peyro Saint Paul *et al.* [23] where no accumulation was reported.

The degree of biotin interference varies widely between assays and manufacturers of immunoassays are expected to disclose the identity of any substance that may interfere with their assay results. At the time of writing, few assays include statements on the serum biotin concentration thresholds required to avoid assay interference, or guidance on the necessary washout periods in their package inserts. Although our study used a washout period of 8 h as stated in the package insert of the specific Elecsys assay used, it is important to note that this should not be extrapolated to other assays where a washout period is not given in the package insert. However, the PK characteristics of biotin are independent of assay design; therefore, biotin half-life and plasma levels can still be used to relate to assay-specific thresholds and thus, to estimate corresponding washout periods for any assay. As some thresholds will be calculated using the total biotin concentration (i.e., including metabolites), and others will not measure biotin metabolites, cross-assay comparisons are difficult. Following a number of recent publications discussing case reports of biotin interference causing erroneous results [5–7], it is advised that clinicians review the supplements taken by their patients in addition to their prescribed medication. Laboratories and pathologists should also be aware of which assays are vulnerable to biotin interference, and communicate the specified washout periods to clinicians, when provided in the package insert of the specific assays used. Additionally, it is important for clinicians and pathologists to be aware of which immunoassays are streptavidin-biotin-based and therefore when an abnormal result may be the result of interference and require repeat analysis before a diagnosis is made [4].

Conclusion

In summary, our findings provide clear guidance on washout periods required for assays with interference thresholds ranging from 10 to 100 ng/ml, following biotin intake at dose regimens of 1 mg s.d. to 300 mg q.i.d. For assays with an *in vitro* interference threshold of ≥ 30 ng/ml, our simulations show that for patients taking biotin doses of up to 5 mg b.i.d. or 10 mg q.d., an 8-h washout period is sufficient to mitigate the risk of biotin interference. For a minority of assays with an *in vitro* interference threshold of < 30 ng/ml, or in the rare case of patients taking biotin at ≥ 10 mg daily doses, it is necessary to delay sample collection for a longer period (up to 73 h) after the last dose of biotin in order to avoid the risk of false assay results.

Future perspective

Healthcare is becoming increasingly personalized, as underlying mechanisms of disease continue to be elucidated and different patient subpopulations are described. Accordingly, companion diagnostics, including immunoassays, will continue to play a vital (and increasing) role in medicine. Streptavidin-biotin assays are commonplace, and their use is likely to continue. In parallel, the use of biotin supplements as lifestyle products of hair and nails, regardless of evidence, may continue to increase. Thus, for the foreseeable future, clear recommendations on the use of streptavidin-biotin assays in light of biotin supplement use will remain critical. The results presented here give clear guidance on washout periods needed for a range of biotin doses before the use of streptavidin-biotin immunoassays. These results will allow clinicians and pathologists to have confidence that the recommended washout periods are sufficient, even with patients who have taken high doses of biotin.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/full/10.4155/ipk-2017-0013

Summary points

- Biotin in human serum is a potential interfering factor for all streptavidin–biotin-based assay designs.
- Use of high doses of biotin as an over-the-counter lifestyle supplement is increasing, therefore the potential risk of erroneous immunoassay results due to biotin interference is growing.
- Biotin had linear pharmacokinetic over the range of doses studied (5–20 mg), was rapidly absorbed and had an effective serum half-life of 15 h (steady-state reached in 3 days).
- The time taken for biotin doses to drop below thresholds of 10–100 ng/ml was simulated for dosing regimens ranging from 1 mg s.d. to 300 mg q.i.d.
- For biotin regimens of ≤ 10 mg q.d. (10 mg is >300 -times the adequate daily intake), serum biotin levels were below an *in vitro* interference threshold of ≥ 30 ng/ml after 8 h.
- If the *in vitro* interference threshold of an immunoassay is <30 ng/ml, or in the extreme cases of patients taking a daily dose of >10 mg, extended washout periods are recommended.

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