Quantitative Analysis of Rabeprazole Sodium in Commercial Dosage Forms by Spectrophotometry

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The aim of this study was to develop and validate two spectrophotometric methods for the quantitative analysis of rabeprazole sodium in commercial dosage forms. Method A is based on the reaction of drug with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of ammonium cerium(IV) nitrate in acetic acid medium at room temperature to form red-brown product which absorbs maximally at 470 nm. Method B utilizes the reaction of rabeprazole sodium with 1-chloro-2,4-dinitrobenzene (CDNB) in dimethyl sulfoxide (DMSO) at 45±1°C to form yellow colored Meisenheimer complex. The colored complex has a characteristic band peaking at 420 nm. Under the optimized reaction conditions, proposed methods are validated as per ICH guidelines. Beer’s law is obeyed in the concentration ranges of 14—140 and 7.5—165 µg ml⁻¹ with linear regression equations of A=6.041×10⁻⁴+1.07×10⁻²C and A=1.020×10⁻³+5.0×10⁻³C for methods A and B, respectively. The limits of detection for methods A and B are 1.38 and 0.75 µg ml⁻¹, respectively. Both methods have been applied successfully for the estimation of rabeprazole sodium in commercial dosage forms. The results are compared with the reference UV spectrophotometric method.

Key words rabeprazole sodium; quantitative analysis; spectrophotometry; 3-methyl-2-benzothiazolinone hydrazone hydrochloride; 1-chloro-2,4-dinitrobenzene; validation

Rabeprazole sodium is chemically known as 2-((4-(3-methoxy propoxy)-3-methyl-2-pyridinyl)methyl)sulphyl)-1H-benimidazol-4-ium (C₁₈H₂₀N₃NaO₃S=381.4). Rabeprazole sodium represents the newest class of antisecretory reagents that are well known for their proton pump (H⁺/K⁺-ATPase) inhibitor activity, most profoundly diminishing gastric acid secretion and thus, lowering the luminal concentration of hydrogen ions. It has recently been demonstrated that rabeprazole sodium is the only proton pump inhibitor among tested (omeprazole, lansoprazole) that augments gastric mucin content. It has proven efficacy in healing, symptom relief and prevention of relapse peptic ulcers and gastroesophageal reflux disease. It is an important alternative to H₂ antagonists and an additional treatment option to other proton pump inhibitors in the management of acid related disorders.

The drug is officially listed in Martindale The Extra Pharmacopoeia. The assay of drug in bulk and formulations is not cited in the United States Pharmacopeia or British Pharmacopoeia. In view of the great importance and wide use of rabeprazole sodium, different analytical methods have been reported for its determination which include high performance liquid chromatography (HPLC), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), capillary electrophoresis (CE), derivative spectrophotometry, and UV-spectrophotometry. These reported methods such as HPLC, LC-MS/MS and CE are sensitive but expensive due to high cost. The main problem associated with these determinations is the laborious cleanup procedure required prior to analysis of drug. The preparation of the drug sample included liquid–liquid or solid–liquid extraction to isolate and preconcentrate the drug samples. Spectrophotometry is attractive because of speed, and simplicity. Extractive spectrophotometric methods have been utilized for the estimation of rabeprazole sodium in pharmaceutical formulations based on chloroform extractable ion pair complexes of the drug with bromothymol blue, bromocresol green, bromocresol purple, amido black and alizarin Red S in acidic medium at 424, 430, 422, 636 and 437 nm, respectively.

The aim of this study was to develop and validate two spectrophotometric methods for the determination of rabeprazole sodium in the presence of formulation excipients. Method A is based on the reaction of rabeprazole sodium with MBTH in the presence of ammonium cerium(IV) nitrate in acetic acid medium to form colored species which absorbs maximally at 470 nm. Method B utilizes the reaction of rabeprazole sodium with 1-chloro-2,4-dinitrobenzene in DMSO to form yellow σ or Meisenheimer complex peaking at 420 nm. The reaction conditions are optimized and validated as per ICH guidelines.

Experimental

Apparatus Shimadzu (UV-1240, Shimadzu Corporation, Kyoto, Japan) and Milton Roy Company (20D+, U.S.A.) spectrophotometers were used for absorbance measurements.

Reagents and Materials All chemicals used were of analytical or pharmaceutical grade. MBTH (s.d. finechem. Ltd., Mumbai, India) solution (1.7×10⁻³ M) was freshly prepared in distilled water. Ammonium cerium(IV) nitrate (Fluka Chemie AG) solution (2.0×10⁻² M) was prepared in 3.5×10⁻³ M acetic acid (Merck, India). CDNB (Fluka Chemie AG) solution (5.59×10⁻³ M) was prepared in DMSO (Merck, India).

Rabeprazole sodium reference standard drug was supplied by Hetero Drug Ltd., Hyderabad, India (Batch No.: RSO250305). Tablet formulations of rabeprazole sodium such as Rabepic–20 (Cipla, Mumbai, India), Rabetel-20 (Lupin, Mumbai, India), Rapeed-20 (Alkem, Mumbai, India) were purchased from local drug stores.

Test Solutions Rabeprazole sodium (1 mg ml⁻¹) solution was prepared in distilled water. Rabeprazole sodium (0.75 mg ml⁻¹) solution was prepared in DMSO.

Proposed Procedures for the Analysis of Rabeprazole Sodium. Method A Aliquots (0.14—1.4 ml) of standard rabeprazole sodium (1 mg ml⁻¹) solution corresponding to 140—1400 µg were pipette into a series of 10 ml volumetric flasks. To each flask, 1.7 ml of 2.0×10⁻⁷ M ammonium cerium(IV) nitrate and 1.9 ml of 1.71×10⁻³ M MBTH were added and diluted to volume with distilled water. The contents of the flask were mixed well and kept for 10 min at room temperature (25±1°C) to complete the reaction. The absorbance of each solution was measured at 470 nm against the reagent blank prepared simultaneously except drug within the stability time period of 6 h. The amount of the drug was calculated either
from the calibration graph or the corresponding regression equation.

**Method B** Into a series of boiling test tubes, different volumes (0.05—1.1 ml) of standard rabeprazole sodium (0.75 mg ml⁻¹) solution corresponding to 37.5—825 μg were pipetted. To each test tube, 2.5 ml of 5.59×10⁻⁵ M CDNB was added, mixed well and heated on water bath for 10 min at 45±1°C. After cooling at room temperature, the contents of the test tube were transferred to a 5 ml volumetric flask and the volume was completed with DMSO. The absorbance was measured at 420 nm against the reagent blank treated similarly within the stability period of 24 h.

**Procedure for the Analysis of Rabeprazole Sodium in Tablet Formulations** Five commercially available tablets of 20 mg strength of rabeprazole sodium were taken in distilled water and DMSO separately and kept for 10 min for complete dispersion of the drug. The distilled water and DMSO extracts were filtered through Whatmann No. 42 filter paper (Whatman International Limited, Kent, U.K.) in 100 ml volumetric flasks individually. The left residues were washed well with 5×10 ml portions of distilled water or DMSO, as the case may be, for complete recovery of the drug and diluted to volume with the corresponding solvent. The amount of drug in commercial tablets was assayed following the proposed procedures.

**Procedure for Reference Method** Aliquots (0.1—2.0 ml) of standard rabeprazole sodium (0.5 mg ml⁻¹) corresponding to 50—1000 μg were pipetted into a series of 10 ml volumetric flasks and diluted to volume with distilled water. The absorbance was recorded against the solvent blank at 290 nm. The amount of the drug in a given sample can be estimated either from the calibration graph or the corresponding regression equation.

**Validity Protocol: Specificity** The specificity of the proposed methods was ascertained by the analysis of placebo solution which was prepared with the excipients such as mannitol, magnesium oxide, low substituted hydrotrope and 2.57×10⁻⁵ M BMTH in 5.95×10⁻⁵ M Acetic Acid. (c) Sample Solution: Blank Solution + 100.0 μg ml⁻¹ Rabeprazole Sodium

**Equivalence Testing:** For pharmaceutical analysis, a bias of ±2.0% is acceptable[13] and can be calculated statistically[14] using the following quadratic equation:

\[ \theta^2 (\bar{x}_{1} - S_{\theta_{1}} t_{n/2})^2 + \theta( - 2 \bar{x}_{1} \bar{x}_{2} + \bar{x}_{2} - S_{\theta_{2}} t_{n/2}^2) = 0 \]  

where \( \bar{x}_{1} \) and \( \bar{x}_{2} \) are mean values based on \( n_{1} \) and \( n_{2} \) measurements, respectively. \( S_{\theta} \) is the pooled standard deviation and \( t_{n/2} \) is the tabulated one-sided t-value, with \( n_{1} + n_{2} - 2 \) degrees of freedom at 95% confidence level.

**Ruggedness and Robustness** For the evaluation of ruggedness of methods A and B, the contents of rabeprazole sodium (80 μg ml⁻¹) in 100 ml volumetric flasks individually. The left residues were washed well with 5×10 ml portions of distilled water or DMSO, as the case may be, for complete recovery of the drug and diluted to volume with the corresponding solvent. The amount of drug in commercial tablets was assayed following the proposed procedures.

**Results and Discussion**

**Method A. Mechanism** The literature citation revealed that MBTH on oxidation with cerium(IV) in acidic medium produces an active electrophilic intermediate[15] which further reacts with iminoheteroaromatic compounds such as indole, carbazole, phenothiazine and benzimidazole resulting in the formation of a colored azo cationic species.[16,17] Benzimidazole is the iminoheteroaromatic compound which undergoes electrophilic substitution in the benzene ring. The order of substitution is 7>6>5>4.[18] Rabeprazole sodium is a water soluble proton pump inhibitor having benzimidazole as the active group and hence undergoes similar electrophilic substitution at position 7 of the benzene ring with the electrophilic intermediate of MBTH in acetic acid medium resulting in the formation of azo cationic species, which absorbs maximally at 470 nm. The blank consisting of MBTH and Ce(IV) in acidic medium absorbed at 350 nm. The absorption spectra are shown in Fig. 1.

**Stoichiometry** The combining ratio was evaluated by limiting logarithmic method.[19] The plot of log absorbance vs. log [rabeprazole sodium] or [MBTH] or [Ce(IV)] gave values of the slopes of 1, 1 and 0.98, respectively (Fig. 2). Hence it is concluded that the reaction proceeds in the molar ratio of 1:1:1. The reaction sequence is shown in Chart 1.

**Method B. Mechanism** Polynitroaromatic and halo-polynitroaromatic compounds interact with a variety of Bronsted bases to give brightly colored species due to the activating effect of a nitro group with nucleophilic displace-
ment of an ortho substituent, especially halogen. Therefore, in general addition-elimination mechanism via an intermediate σ, or Meisenheimer complex is accepted.20) Halogen may be displaced by nitrogen bases (nucleophiles) such as imidazole, benzimidazole, 1,3,5-trimethyl pyrazole and 3,5-dimethyl pyrazole; and piperidine. 22) It was reported that piperidine is a nitrogen base interacted with 1,3,5-trinitrobenzene in DMSO to form colored species of 1,3,5-trinitrophenyl piperidine. In this reaction, 2 mol of nitrogen base were utilized with 1 mol of 1,3,5-trinitrobenzene. Rabeprazole sodium is a nitrogen base due to the presence of benzimidazole group which reacts with CDNB in DMSO at 45 ± 1 °C resulting in the formation of yellow σ or Meisenheimer complex which absorbs maximally at 420 nm. The blank consisting of CDNB in DMSO has a characteristic band at 353 nm (Fig. 3).

**Stoichiometry** The stoichiometry was established by mole ratio method. The results are shown in Fig. 4. It is apparent from the figure that the combining molar ratio between rabeprazole sodium and 1-chloro 2,4-dinitro benzene is 2 : 1. This stoichiometric ratio is comparable with the previous results showed by 1,3,5-trinitrophenyl piperidine complex. The reaction sequence is shown in Chart 2.

**Optimization of Variables** The optimization of variables for methods A and B was assessed by testing several parameters such as temperature, heating time, solvents, concentrations of ammonium cerium(IV) nitrate, MBTH and CDNB.
Method A. Effect of Reaction Time

The influence of the reaction time on the absorbance of the product was studied by taking 100 μg ml⁻¹ of rabeprazole sodium with 1.7 ml of 2×10⁻² M ammonium cerium(IV) nitrate and 1.9 ml of 1.7×10⁻³ M MBTH in 10 ml volumetric flask. It was found that the maximum absorbance was achieved at 8 min of reaction and remains constant up to 12 min (Fig. 5a). Therefore, a time of 10 min at room temperature was selected as an optimum reaction time.

Effect of the Concentration of Ammonium Cerium(IV) Nitrate

The influence of the concentration of ammonium cerium(IV) nitrate on the absorbance of the colored product was investigated at 100 μg ml⁻¹ rabeprazole sodium with 1.5 ml of 1.7×10⁻³ M MBTH in the range of 2.0×10⁻⁴—4.0×10⁻³ M ammonium cerium(IV) nitrate. It was observed that the maximum absorbance was attained with 3.0×10⁻³ M ammonium cerium(IV) nitrate (Fig. 6) and remained constant up to 4.0×10⁻³ M. Therefore, 3.4×10⁻³ M ammonium cerium(IV) nitrate was taken as the optimum concentration for the determination process.

Effect of the Concentration of MBTH

The effect of the concentration of MBTH on the absorbance of the colored product was studied at 100 μg ml⁻¹ rabeprazole sodium with 3.4×10⁻³ M ammonium cerium(IV) nitrate in the range of 1.71×10⁻⁵—3.25×10⁻⁴ M MBTH. The highest absorbance was obtained with 1.88×10⁻⁴ M MBTH, beyond this further increase in the concentration of MBTH up to 3.25×10⁻⁴ M, resulted in no change in the absorbance (Fig. 7). Thus, 3.25×10⁻⁴ M MBTH was adopted as an optimum concentration for the maximum absorbance in the determination procedure.

Method B. Effect of Temperature and Time

The effect of temperature on the reaction between rabeprazole sodium (150 μg ml⁻¹) and CDNB (2.84×10⁻² M) was studied at 35, 40, 45 and 50 °C. It was observed that the equilibrium was
attained at 18, 14, 8 and 8 min at temperature of 35, 40, 45 and 50 °C, respectively. To speed up the determination process and for the sake of good recovery results, optimum temperature of 45 °C was chosen for the estimation of rabeprazole sodium. It was also observed that the absorbance at 45 °C was constant in the range of 8—12 min (Fig. 5b). Therefore, the optimum time of heating for the maximum absorbance was chosen to be 10 min for determination procedure.

**Effect of the Concentration of CDNB** The influence of CDNB concentration on the absorbance of yellow colored complex was studied at 150 μg ml⁻¹ rabeprazole sodium in the concentration range of 1.18×10⁻³—3.32×10⁻² M CDNB at 45 °C. It was found that the maximum absorbance was obtained in the range of 2.37×10⁻²—3.32×10⁻² M CDNB (Fig. 8). Therefore, the optimum concentration of 2.84×10⁻² M CDNB was recommended for determination procedure.

**Validation Protocol. Specificity** The proposed spectrophotometric conditions were found to be specific and selective in the presence of tablet excipients. It was observed that common excipients present in tablet formulations did not cause any significant interference.

**Linearity** The calibration curves were constructed by plotting absorbance against concentration of rabeprazole sodium for the proposed methods. Beer’s law was obeyed over the concentration ranges 14—140 μg ml⁻¹ and 7.5—165 μg ml⁻¹ with molar absorptivity of 4.104×10¹¹ mol⁻¹ cm⁻¹ and 2.069×10¹⁰ mol⁻¹ cm⁻¹ for methods A and B, respectively. The calibration data were fitted to the equation, \( A=a+bC \), where \( A \) is the absorbance at relevant \( \lambda_{\text{max}} \); \( C \) is the concentration in μg ml⁻¹; \( b \) is the slope and \( a \) is the intercept of calibration. The regression parameters are summarized in Table 1. The high values of correlation coefficients (0.9999) for both methods indicated excellent linearity. In order to verify that the proposed methods are free from procedural error, the experimental intercept of the calibration lines were tested for significance of the deviation from the theoretical intercept as zero. For this justification, the values of \( r \)-calculated from the relation, \( r=a/S_{b} \) were found to be 0.246 and 1.596 for methods A and B, respectively, which did not exceed the theoretical \( r \)-value (2.365) at 95% confidence level. This indicated that the intercepts for methods A and B are not significantly different from zero.

**Precision** The intra day precision was evaluated by determining rabeprazole sodium at three concentration levels for five times within the same day (Table 2). As can be seen from Table 2 that the percent relative error and relative standard deviation (%) were in the ranges of 0.01—0.57; 0.04—0.47 and 0.04—0.14; 0.05—0.36 for methods A and B, respectively. Also, the inter day precision was evaluated over a period of 5 d and the percent relative error and relative standard deviation (%) were found to be 0.01—0.43; 0.07—0.56 and 0.05—0.27; 0.06—0.43 for methods A and B, respectively.

**Accuracy** The accuracy of the proposed methods A and B was ascertained by recovery studies using standard addition method. The results are summarized in Table 3. The mean recoveries and RSD for methods A and B were in the ranges 99.99±0.08—100.13±0.17% and 100.01±0.04—100.05±0.11%, respectively which can be considered to be very satisfactory.

**Ruggedness and Robustness** The ruggedness of methods A and B was evaluated by assaying the contents of rabeprazole sodium in tablet formulation using Spectronic 20D⁺ and Shimadzu UV 1240 spectrophotometers. The percent recoveries±RSD resulted from Spectronic 20D⁺ spectrophotometer (100.02±0.06 and 100.05±0.09 for methods A and B, respectively) and Shimadzu UV 1240 (100.05±0.06 and 100.04±0.06 for methods A and B, respectively) were compared. The results agreed well within the acceptable limits with permissible bias.

The robustness of the methods A and B relative to each operational parameter was challenged. The operational pa-

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**Table 1. Summary of Validation Data for the Determination of Rabeprazole Sodium**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>475</td>
<td>420</td>
<td>290</td>
</tr>
<tr>
<td>Beer’s law limit (μg ml⁻¹)</td>
<td>14—140</td>
<td>7.5—165</td>
<td>5.0—100</td>
</tr>
<tr>
<td>Molar absorptivity (mol⁻¹ cm⁻¹)</td>
<td>4.104×10⁰</td>
<td>2.069×10⁰</td>
<td>4.551×10⁰</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>( A=6.041×10^{-4}+1.07×10^{-4}C )</td>
<td>( A=1.020×10^{-3}+5.0×10^{-3}C )</td>
<td>( A=8.707×10^{-4}+1.2×10^{-4}C )</td>
</tr>
<tr>
<td>( \pm S_{a} )</td>
<td>6.825×10⁻⁴</td>
<td>1.774×10⁻³</td>
<td>3.026×10⁻³</td>
</tr>
<tr>
<td>( \pm S_{b} )</td>
<td>9.065×10⁻⁵</td>
<td>1.886×10⁻⁴</td>
<td>4.991×10⁻⁴</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Variance (S²) of calibration line</td>
<td>1.989×10⁻⁶</td>
<td>1.513×10⁻⁶</td>
<td>2.890×10⁻⁶</td>
</tr>
<tr>
<td>Detection limit (μg ml⁻¹)</td>
<td>1.378</td>
<td>0.750</td>
<td>0.471</td>
</tr>
<tr>
<td>Quantitation limit (μg ml⁻¹)</td>
<td>4.176</td>
<td>2.273</td>
<td>1.426</td>
</tr>
</tbody>
</table>
Table 2. Summary of Precision Results of the Proposed Methods Evaluated by Standard Addition Technique

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Amount (µg ml⁻¹)</th>
<th>Taken</th>
<th>Added</th>
<th>Found±S.D. (%)</th>
<th>Recovery±RSD (%)</th>
<th>SAE (%)</th>
<th>C.L. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabicip-20 (Cipla)</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>135.06±0.11</td>
<td>100.04±0.08</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Rablet-20 (Lupin)</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>135.05±0.12</td>
<td>100.04±0.09</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Rapeed-20 (Alkem)</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>134.99±0.14</td>
<td>99.99±0.14</td>
<td>0.06</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Method A

- room temperature, 25±1°C
- reaction time, 10±2 min
- volume of 1.7×10⁻³ M MBTH, 1.5±0.4 ml
- volume of 2.0×10⁻² M ammonium cerium(IV) nitrate, 1.7±0.3 ml

Method B

- heating temperature, 45±1°C
- reaction time, 10±2 min
- volume of 5.59×10⁻² M CDNB, 2.4±0.4 ml

The robustness of the proposed methods was assessed by analyzing active drug content in Rabicip-20. The quality control sample solution containing 80 µg ml⁻¹ of the drug was analyzed five times using methods A and B. The percent recoveries±RSD for methods A (100.02±0.09) and B (100.05±0.08) were found to be appreciable, thus indicated that the proposed methods are robust.

Equivalence Testing The proposed methods have been successfully applied to the analysis of rabeprazole sodium in commercial dosage forms. The results obtained (Methods A and B) were compared with those of reference method in terms of mean recovery, RSD, R.E., t- and F-values (Table 4). It is evident from Table 4 that the assay results showed good agreement between proposed methods and the UV reference spectrophotometric method as t- and F-values were less than the theoretical ones at 95% confidence level and θₚ and θₑ were less than ±2.0%. Therefore, it is concluded that the proposed methods A and B are applicable for routine quality control analysis of rabeprazole sodium in commercial dosage forms with acceptable recovery results less than ±2.0%.

Conclusion The proposed methods provide simple, accurate and reproducible quantitative analysis for the assay of rabeprazole sodium in commercial dosage forms. Both methods are specific and selective. In addition, the proposed methods have high molar absorptivity (4.1×10¹⁰ L mol⁻¹ cm⁻¹ for method A and 2.07×10¹⁰ L mol⁻¹ cm⁻¹ for method B) with broad linear dynamic range (14—140 µg ml⁻¹ for method A and 7.5—165 µg ml⁻¹ for method B) and high tolerance limit for ex-
cipients found in dosage forms. The molar absorptivity for method A is two times more than that for method B and hence method A is considered to be more superior to method B. Therefore the proposed methods are recommended for the routine quality control analysis of rabeprazole sodium in commercial dosage forms.

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References


<table>
<thead>
<tr>
<th>Formulations</th>
<th>Method A</th>
<th>Method B</th>
<th>Reference method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
<td>$t$, $F^{q}$</td>
</tr>
<tr>
<td>Rabicip-20</td>
<td>100.04</td>
<td>0.06</td>
<td>$t=0.05$</td>
</tr>
<tr>
<td>(Cipla)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rablet-20</td>
<td>100.02</td>
<td>0.10</td>
<td>$t=0.25$</td>
</tr>
<tr>
<td>(Lupin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapeed-20</td>
<td>100.05</td>
<td>0.06</td>
<td>$t=0.10$</td>
</tr>
<tr>
<td>(Alkem)</td>
<td></td>
<td></td>
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</table>

$^{a}$ Mean for five independent analyses. $^{b}$ Theoretical $t$-value ($v=8$) and $F$-value ($v=4,4$) at 95% confidence level are 2.306 and 6.39, respectively. $^{c}$ $\theta_{b}=0.98$ and $\theta_{c}=1.02$ are acceptable bias, based on recovery experiments and are within ±2%.