An approach was proposed for the estimation of measurement uncertainty for analytical methods based on one-point calibration. The proposed approach is similar to the popular multiple-point calibration approach. However, the standard deviation of calibration was estimated externally. The approach was applied to the estimation of measurement uncertainty for the quantitative determination of ketamine (K) and norketamine (NK) at a 100 ng/mL threshold concentration in urine. In addition to uncertainty due to calibration, sample analysis was the other major source of uncertainty. To include the variation due to matrix effect and temporal effect in sample analysis, different blank urines were spiked with K and NK and analyzed at equal time intervals within and between batches. The expanded uncertainties \( k = 2 \) were estimated to be 10 and 8 ng/mL for K and NK, respectively.

Introduction

Every measurement is subject to error. A measured value is unlikely to be equal to the true value because of the systematic and random errors present in any chemical test. Ideally, knowledge of systematic and random errors would provide the true value after correction. However, random error may vary in each measurement, and thus the true value is theoretically difficult to obtain. Although the true value cannot be known, one can characterize a parameter, the uncertainty of measurement, that describes the dispersion of the quantity values being attributed to a measurand \( (1) \). ISO/IEC 17025 \( (2) \) clearly specifies that an estimation of the measurement uncertainty (MU) is mandatory for all quantitative tests.

Two major approaches have been established to MU estimation. The bottom-up approach is described in the Guide to the Expression of Uncertainty of Measurement (GUM) \( (3) \), published by the International Organization for Standardization (ISO). In this approach, all sources of error are fundamentally considered, and the MU is estimated by statistical methods \( (\text{type A}) \) as well as by means other than statistical methods \( (\text{type B}) \). The guide provides a comprehensive understanding of all required analytical operations, which allows the analyst to focus efforts on improving the operations if required, to optimize the analytical method \( (4) \). The second, top-down approach, has been illustrated in the example A4 given in the EURACHEM/CITAC guide \( (5) \). This approach considers the MU as a whole. Uncertainties are evaluated using in-house validation data and intra-lab quality control (QC). The top-down approach has been suggested to be more suitable for complex chemical tests \( (4) \). For more complex test procedures, the bottom-up approach may not account for uncertainties that tend to cancel out \( (6) \).

Multiple-point calibration is the most popular method used in quantitative analysis. Because of the need of knowing the MU for any quantitative data, approaches for estimating the MU based on multiple-point calibration (often more than five points) have been reported \( (7–11) \). In general, these approaches are similar to the approach proposed in case A5 of the EURACHEM/CITAC guide of quantifying uncertainty in analytical measurement \( (5) \).

In areas that require a threshold value, such as in the detection of drugs of abuse, the MU at the threshold value is critical because of its significance in decision-making. One potential problem of using the MU based on multiple-point calibration is that the assumption of the same population standard deviation across the calibration range may not be correct. It is known that standard deviation is often increased with concentration. Therefore, the slope and standard deviation of calibration calculated from a non-weighted multiple-point calibration curve may not be the best estimates for the threshold concentration.

For drug-of-abuse testing laboratories in Taiwan, one-point calibration at the threshold value is the most commonly used quantitative approach. One-point calibration has shown similar bias and precision with multiple-point calibration \( (12) \). Because it shows similar performance and better efficiency with respect to time and work-load, one-point calibration has been applied to the study of drugs-of-abuse in whole blood \( (13) \), in the gas chromatography–mass spectrometry (GC–MS) drug confirmation guideline of the Clinical and Laboratory Standards Institute \( (14) \) and in the forensic drug laboratory accreditation program at the College of American Pathologists.

Although not in the field of drug-of-abuse testing, the MU of one-point calibration has been reported \( (15, 16) \). Attempts have been made to estimate the uncertainty of the peak area ratio of the calibration solution by standard deviations from repeated analysis \( (15) \). Uncertainty of the peak area, including repeatability, detector drift and peak integration, has also been reported \( (16) \).

None of the one-point calibration methods used the word “slope” to describe the equation \( (15, 16) \), and this could be the primary reason that the MU estimation for one-point calibration \( (15, 16) \) appeared to be dissimilar to multiple-point calibration \( (7–11) \). In practice, one-point calibration can be considered to be the simplest case of a multiple-point calibration curve without an intercept \( (17) \). This study proposes an MU approach for one-point calibration that is similar to multiple-point calibration.

The proposed approach was used in the estimation of MU for quantitative determination of ketamine and norketamine in urine at the cut-off concentration \( (100 \text{ ng/mL}) \). Ketamine is a...
general anesthetic with analgesic and hallucinogenic properties. It was originally used in veterinary medicine; however, its ability to produce intensely vivid psychedelic effects causes its frequent abuse. In Taiwan, it is at the top of the list for quantities seized by the law enforcement for the past six consecutive years. The procedures of the estimation of MU and the results of the estimation are reported and discussed.

**Experimental**

**Materials**

Different batches of ketamine (K), ketamine-d4 (K-d4), norketamine (NK) and norketamine-d4 (NK-d4) standards were purchased from Cerilliant Corporation (Round Rock, TX) for the preparation of calibration solution and spiked urine samples. QC samples (urine) for checking Cerilliant standards were purchased from UTAK Laboratories (Valencia, CA). All other chemicals and solvents were purchased from Merck (Darmstadt, Germany). A SPEC DAU solid-phase extraction column was purchased from Agilent Technologies (Santa Clara, CA).

**Preparation of standard solutions**

A schematic illustration of the experimental procedures is shown in Figure 1. One milliliter of K and NK reference solutions (1.0 mg/mL) were diluted to 10 mL with 50% methanol (methanol–water; v/v) to prepare the standard stock solution (0.1 mg/mL). To prepare the standard working solution at 1 μg/mL, 1 mL K/NK stock solution was diluted twice with 50% methanol to 10 mL. One milliliter blank urine was spiked with 100 μL K/NK standard working solution to prepare the 100 ng/mL K/NK standard calibration solution.

**Preparation of 100 ng/mL K/NK urine samples**

The urine samples of 100 ng/mL K/NK were prepared by spiking 100 μL K/NK standard working solution (prepared from a different batch of Cerilliant standard) into 1-mL drug-free urines that were collected from drug-free individuals.

**Preparation of spiked urine samples**

One milliliter of K/NK standard calibration solution and urine sample were added to 100 μL internal standard (IS, K-d4 and NK-d4, 1 μg/mL) and 1 mL phosphate buffer (pH 6.0) before the extraction procedure. The extraction was performed on a SPEC DAU column. Briefly, the extraction column was preconditioned with methanol (200 μL). Urine was loaded onto the extraction column and sequentially rinsed with acetic acid (500 μL) and methanol (500 μL) by applying 10–20 inHg (33.8–67.7 kPa) vacuum for 1 min. The eluting solvent (800 μL, 80 mL ethyl acetate mixed with 20 mL methanol and 2 mL ammonia added) was added to the column and gentle vacuum was applied [below 3 inHg (10.2 kPa)]. The eluate was collected. Acidic methanol (1%) with hydrochloric acid (50 μL) was added to the eluate and agitated for 30 s to prevent volatilizing K and NK. The eluate was evaporated to dryness at 55°C under a stream of nitrogen. After drying, the residue was reconstituted in 100 μL ethyl acetate for GC–MS analysis.

**Extraction procedure of urine**

One milliliter of K/NK standard calibration solution and urine sample were added to 100 μL internal standard (IS, K-d4 and NK-d4, 1 μg/mL) and 1 mL phosphate buffer (pH 6.0) before the extraction procedure. The extraction was performed on a SPEC DAU column. Briefly, the extraction column was preconditioned with methanol (200 μL). Urine was loaded onto the extraction column and sequentially rinsed with acetic acid (500 μL) and methanol (500 μL) by applying 10–20 inHg (33.8–67.7 kPa) vacuum for 1 min. The eluting solvent (800 μL, 80 mL ethyl acetate mixed with 20 mL methanol and 2 mL ammonia added) was added to the column and gentle vacuum was applied [below 3 inHg (10.2 kPa)]. The eluate was collected. Acidic methanol (1%) with hydrochloric acid (50 μL) was added to the eluate and agitated for 30 s to prevent volatilizing K and NK. The eluate was evaporated to dryness at 55°C under a stream of nitrogen. After drying, the residue was reconstituted in 100 μL ethyl acetate for GC–MS analysis.

Figure 1. A schematic illustration of the experimental procedures.
GC–MS analysis

GC–MS analysis was performed on an Agilent 6890 GC–5973 MSD system. A HP-5MS column was used for gas chromatographic separation. The oven temperature was initially set at 150°C for 1 min, ramped to 280°C at a ramping rate of 30°C/min and held at 280°C for 5 min. The injector and transfer line temperatures were set at 170 and 280°C, respectively. A constant flow mode was used, and the helium carrier gas was set at a flow rate of 0.8 mL/min. An aliquot of 1 μL was injected using the splitless mode. For MS analysis, selected ion monitoring (SIM) was chosen to monitor the following ions (m/z): K (180, 182, 209), K-d4 (184, 213), NK (166, 168, 195), NK-d4 (170, 199) (the quantification ions are in bold).

Methods and Results

Uncertainty estimations at the threshold concentration

To ensure that no possible contributions were missed, the bottom-up approach was adopted and all parameters in the mathematical model were identified. The following steps were taken to estimate the MU using the bottom-up approach described in GUM (3): (i) specifying the measurand; (ii) identifying sources of uncertainty; (iii) quantifying uncertainty; (iv) calculating the combined uncertainty.

Specifying the measurand

The sample concentration can be calculated using the following equation for one-point calibration:

$$C_x = \frac{Y}{b}$$  (1)

where $C_x$ is the concentration of K or NK in a urine sample; $Y$ is the ratio of the peak area of K or NK in the sample ($A_S$) to deuterated internal standard ($A_{IS}$) [Eq. (2)]; and $b$ is the slope of the one-point calibration curve [Eq. (3)]:

$$Y = \frac{A_S}{A_{IS}}$$  (2)

$$b = \frac{A_{Std}}{C_{Std}}$$  (3)

A single calibration solution ($C_{Std}$) with concentration equal to the threshold value of 100 ng/mL was analyzed to establish the slope, $b$. $A_{Std}$ is the peak area of $C_{Std}$.

Identifying sources of uncertainty

The critical step in the bottom-up approach is to identify all uncertainty sources. To facilitate the identification, a cause and effect diagram was sketched as shown in Figure 2. The uncertainty of the slope comprised the uncertainty of the calibrator’s concentration and the uncertainty owing to the calibrator’s signal variation. In terms of the uncertainty of $A_S/A_{IS}$ in sample analysis, sample preparation, matrix effect resulting from different urine sample compositions, and temporal effect (instrument stability) within the analysis batch were considered to be potential sources of uncertainty. Recovery was also added as a potential source of uncertainty.

The within-laboratory reproducibility was evaluated. The within-laboratory reproducibility included the uncertainty contributions from materials, analysts (two analysts), environmental
and measurement conditions and variations observations. The laboratory was not responsible for sampling and only one GC–MS was used; therefore, these two sources of uncertainty were not included.

Other potential uncertainty sources such as the finite resolution of balance, pipettes and flasks have been included in the precision study of volumetric apparatuses. Approximation in the method such as the purity of reference material has been included in the uncertainty of calibration solution. The traceability of reference material and equipment (balance, pipettes and volumetric flasks) has been included in the supplier’s certificate or reports of external calibration.

**Concentration of the calibrator**
The uncertainty in the calibrator’s concentration \( u(C_{\text{calib}}) \) was due to the reference materials and the volumetric apparatuses used for preparation of the standard solutions. The uncertainty of the reference material was provided by the reference material supplier. The volumetric apparatuses are subject to three sources of uncertainty: tolerance, precision and temperature variation. Table I summarizes tolerance and precision data for pipettes and flasks.

**Calibration**
In practice, the number of calibration points can be reduced to one, for a calibration curve that passes through the origin. However, to estimate the uncertainty due to calibration, the number of calibration points can be reduced to six standard deviations were collected and pooled using Eq. (4):

\[
s_p^2 = \frac{\sum_{i=1}^{n} (n_i - 1) s_i^2}{\sum_{i=1}^{n} n_i - 1}
\]

where the symbols \( s_p \) and \( s_i \) are the pooled standard deviation and individual standard deviations, respectively.

**Sample analysis**
For sample analysis, we considered that the possible sources of uncertainty would result from the urine sample preparation, matrix effect and temporal effect (GC–MS stability). To account for the matrix effect, spiked urine samples of 100 ng/ml K and NK were prepared using five different drug-free urines. To account for the temporal effect, spiked urine samples were analyzed at equal time intervals. The experiment was carried out in the following sequence (40 urine samples per batch):

CCCCCS1S2S3S4S5S6S7S8S9S10S11S12S13S14S15S16S17S18S19S20S21S22S23S24S25S26S27S28S29S30S31S32S33S34S35S36S37S38S39S40S41

C symbolizes the calibration, \( S_{1-40} \) represents the regular test urine samples and \( P_{n-1-5} \) represents the five spiked urine samples. Regular injections of QC samples and washing solvents were not shown in the preceding sequence. Data across six weeks were pooled to provide a more representative relative standard deviation.

**Quantifying uncertainty**

**Standard solutions**
According to the manufacturer’s certificate of analysis, the uncertainties were 0.006 and 0.031 mg/mL for K and NK, respectively. Standard uncertainties were obtained by dividing the uncertainties by the coverage factor \( k = 2 \).

For the volumetric pipette, the uncertainty of tolerance was obtained by dividing the uncertainty from the calibration report with the coverage factor \( k = 2 \). The tolerance of volumetric flask was based on the manufacturer’s certificate. The standard uncertainty is calculated (divided by \( \sqrt{6} \)) assuming a triangular distribution. The precision was estimated by the standard deviation based on 10 repetitive measurements using gravimetric determination.

The temperature in the laboratory was set at 20 ± 5°C. The expansion coefficient at 20°C was used with the assumption of a rectangular distribution. The thermal expansion coefficient of a water-methanol binary mixture was calculated using the equation \( \alpha = (1/V_0) (\partial V_0/\partial T)_P \), where \( V_0 \) is the molar volume and \( \partial V_0/\partial T \) is the variation of molar volume with temperature \( T \). The values of \( V_0 \) and \( \partial V_0/\partial T \) were obtained from the literature \( (19) \). The thermal expansion coefficients for 50% methanol and water at 20°C were \( 7.183 \times 10^{-4} \) (°C\(^{-1}\)) and \( 2.273 \times 10^{-4} \) (°C\(^{-1}\)), respectively.

Finally, all sources of uncertainty with respect to the preparation of standard solutions were combined. Table II summarizes these calculations.

**Calibration**
The calibrator (100 ng/mL) was analyzed five times (CCCCCC described previously) to calculate the standard deviation. To obtain a more representative estimate, standard deviations of six weeks were pooled. The standard deviations and pooled result are shown in Table III. To calculate uncertainty due to calibration, \( u(\text{Calibration}) \), at 100 ng/mL of the predicted concentration for K and NK, the externally estimated pooled standard deviation, the signal (\( Y \)) and the slope were substituted into Eq. (5) \( (17) \) for a calibration line through the origin:

\[
u(\text{Calibration}) = u\left(\frac{Y}{b}\right) = \frac{s}{b} \sqrt{1 + \left(\frac{Y}{b}\right)^2 / \sum x^2}
\]
Table II
Summary of the Calculations for Relative Uncertainty of $C_{\text{obs}}$, $\omega_{C_{\text{obs}}}$

Pipettes and Flasks:
Pipette-A (1 mL): Preparing 0.1 mg

Pipette-A (1 mL): Preparing 0.1 mg

Pipette (100 µL): Preparing 0.1 mg

Pipette (1 mL): Preparing 0.1 mg

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Expression</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega_{C_{\text{obs}}}$</td>
<td>$\frac{\sigma}{C_{\text{obs}}}$</td>
<td>0.006</td>
</tr>
<tr>
<td>$K : \omega_{C_{\text{obs}}}$</td>
<td>$\sigma_{C_{\text{obs}}} = \frac{\sqrt{\sum \sigma^2}}{1}$</td>
<td>0.033</td>
</tr>
<tr>
<td>$NK : \omega_{C_{\text{obs}}}$</td>
<td>$\sigma_{C_{\text{obs}}} = \frac{\sqrt{\sum \sigma^2}}{1}$</td>
<td>0.016</td>
</tr>
</tbody>
</table>

2. $\omega_{\text{pipette}(1 mL)}$

$\omega_{\text{B}} = \frac{0.00011}{2} = 0.000056$(ml)

$\omega_{\text{precision}} = 0.0018$(ml)

$\omega_{\text{temp.}} = \sqrt{\frac{\omega_{\text{pipette}(1 mL)}^2 \times \Delta T}{\sqrt{3}}} = \sqrt{\frac{(0.000056)^2 \times (5)}{\sqrt{3}}} = 0.021$(ml)

3. $\omega_{\text{flask}(10 mL)}$

$\omega_{\text{B}} = \frac{0.025}{\sqrt{6}} = 0.01$(ml)

$\omega_{\text{precision}} = 0.014$(ml)

$\omega_{\text{temp.}} = \sqrt{\frac{\omega_{\text{pipette}(1 mL)}^2 \times \Delta T}{\sqrt{3}}} = \sqrt{\frac{(0.000056)^2 \times (5)}{\sqrt{3}}} = 0.021$(ml)

$\omega_{\text{B}} = \frac{\sqrt{0.01^2 + 0.021^2 + 0.021^2}}{10} = 0.028$(ml)

4. $\omega_{\text{pipette}(100 µL)}$

$\omega_{\text{B}} = \frac{0.000096}{2} = 0.000048$(ml)

$\omega_{\text{precision}} = 0.00035$(ml)

$\omega_{\text{temp.}} = \sqrt{\frac{\omega_{\text{pipette}(1 mL)}^2 \times \Delta T}{\sqrt{3}}} = \sqrt{\frac{(0.000056)^2 \times (5)}{\sqrt{3}}} = 0.021$(ml)

$\omega_{\text{B}} = \sqrt{0.000048^2 + 0.00035^2 + 0.00021^2} = 0.004$(ml)

5. $\omega_{\text{pipette}(1 mL)}$

$\omega_{\text{B}} = \frac{0.000096}{1} = 0.000056$(ml)

$\omega_{\text{precision}} = 0.0018$(ml)

$\omega_{\text{temp.}} = \sqrt{\frac{\omega_{\text{pipette}(1 mL)}^2 \times \Delta T}{\sqrt{3}}} = \sqrt{\frac{(0.000056)^2 \times (5)}{\sqrt{3}}} = 0.021$(ml)

$\omega_{\text{B}} = \sqrt{0.000056^2 + 0.0018^2 + 0.00066^2} = 0.019$(ml)

6. $\omega_{C_{\text{obs}}}$

$K : \omega_{C_{\text{obs}}} = \sqrt{\omega_{C_{\text{obs}}}^2 + 3 \times (\omega_{\text{pipette}(1 mL)} - A)^2 + \omega_{\text{flask}(10 mL)}^2 + \omega_{\text{pipette}(100 µL)}^2 + \omega_{\text{pipette}(1 mL)} - B)^2}$

$\omega_{C_{\text{obs}}} = \sqrt{0.003^2 + 3 \times (0.0028^2 + 0.0027^2) + 0.004^2 + 0.0019^2}$

$\omega_{C_{\text{obs}}} = 0.0086$

$NK : \omega_{C_{\text{obs}}} = \sqrt{\omega_{C_{\text{obs}}}^2 + 3 \times (\omega_{\text{pipette}(1 mL)} - A)^2 + \omega_{\text{flask}(10 mL)}^2 + \omega_{\text{pipette}(100 µL)}^2 + \omega_{\text{pipette}(1 mL)} - B)^2}$

$\omega_{C_{\text{obs}}} = \sqrt{0.016^2 + 3 \times (0.0028^2 + 0.0027^2) + 0.004^2 + 0.0019^2}$

$\omega_{C_{\text{obs}}} = 0.018$
Eq. (5) provided the standard uncertainty for an unknown sample having a predicted concentration of $Y/b$. $S$ is the estimated standard deviation of one-point calibration. $\Sigma x^2$ is the sum of squares of all the concentrations used to establish the curve. One calibration point should produce a curve with a slope. As a result, 30 slopes were obtained over six weeks. The slopes obtained during the six-week period were 0.0118 to 0.0149 for K and 0.0117 to 0.0144 for NK. For a more conservative estimation, the largest $u(Calibration)$ was chosen for calculation. Among the 30 $A_y/A_S$ and 30 $b$, 1.22/0.0118 and 1.22/0.0117 provided the largest $u(Calibration)$ for K and NK, respectively.

$$u(Calibration)_K = \frac{0.033}{0.0118} \sqrt{1 + \left(\frac{1.22}{0.0118}\right)^2} / 100^2$$

$$= 4.1 \text{ ng/mL}$$

$$u(Calibration)_NK = \frac{0.024}{0.0117} \sqrt{1 + \left(\frac{1.22}{0.0117}\right)^2} / 100^2$$

$$= 3.0 \text{ ng/mL}$$

$A_y/A_S$

The precision of sample signals ($P_1$, $P_2$, $P_3$, $P_4$, $P_5$ described previously) is shown in Table IV. The uncertainty in $A_y/A_S$ was estimated by pooling the relative standard deviations of six weeks' data. Relative uncertainties of sample signals ($u_y(A_y/A_S)$) were estimated to be 2.8 and 2.4% for K and NK, respectively.

### Recovery study

The bias of the analytical procedure was investigated using the thirty spiked urine samples. They showed a mean recovery of 98.34 and 100.19% with a pooled standard deviation ($s$) of 2.8 and 2.4% for K and NK, respectively. The standard uncertainty of recovery was calculated as the standard deviation of the mean $u(R_{Rec}) = 0.028/\sqrt{30} = 0.0051$ for K and $u(R_{Rec}) = 0.024/\sqrt{30} = 0.0044$ for NK. A t-test was performed to determine whether the mean recovery is significantly different from 1. The $t_{calculated}$ was calculated using the following equations:

$$t_{calculated} = \frac{1 - \bar{R}_{Rec}}{u(R_{Rec})}$$

**Table III**

<table>
<thead>
<tr>
<th>Week</th>
<th>$SD$ (area ratio)</th>
<th>Week</th>
<th>$SD$ (area ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.026</td>
<td>1</td>
<td>0.010</td>
</tr>
<tr>
<td>2</td>
<td>0.029</td>
<td>2</td>
<td>0.022</td>
</tr>
<tr>
<td>3</td>
<td>0.041</td>
<td>3</td>
<td>0.048</td>
</tr>
<tr>
<td>4</td>
<td>0.024</td>
<td>4</td>
<td>0.008</td>
</tr>
<tr>
<td>5</td>
<td>0.035</td>
<td>5</td>
<td>0.012</td>
</tr>
<tr>
<td>6</td>
<td>0.041</td>
<td>6</td>
<td>0.017</td>
</tr>
<tr>
<td>pooled SD</td>
<td>0.033</td>
<td>pooled SD</td>
<td>0.024</td>
</tr>
</tbody>
</table>

### Calculating the combined uncertainty

The uncertainties in urine analysis for 100 ng/mL K and NK are shown in Table V. Four uncertainties were combined to calculate the combined uncertainty $u_y(y)$. For a calculation involving only quotients such as in Eq. (1), based on the law of propagation of uncertainty, the relative combined uncertainty is the positive square root of the total variance of all the uncertainty components expressed as relative standard deviations ($u_y(C_{Std})$, $u_y(Calibration)$, $u_y(A_y/A_S)$, $u_y(R_{Rec})$):

$$u_y(y) = \sqrt{u_y(C_{Std})^2 + u_y(Calibration)^2 + u_y(A_y/A_S)^2 + u_y(R_{Rec})^2}$$

$$= 100 \times \sqrt{0.0086^2 + \left(\frac{4.1}{1.22/0.0118}\right)^2 + 0.028^2 + \left(\frac{0.0051}{0.9834}\right)^2}$$

$$= 100 \times 0.05$$

$$= 5 \text{ ng/mL}$$

**Table IV**

<table>
<thead>
<tr>
<th>Week</th>
<th>RSD (%)</th>
<th>Week</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>3</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>6</td>
<td>1.8</td>
</tr>
<tr>
<td>$u_y(A_y/A_S)$</td>
<td>2.8</td>
<td>$u_y(A_y/A_S)$</td>
<td>2.4</td>
</tr>
</tbody>
</table>

These values were compared with $t_{table}$ for 24 degrees of freedom at 95% confidence. Because the $t_{table}$ value (2.06) is smaller than the $t_{calculated}$ value for K and larger than the $t_{calculated}$ value for NK, a recovery correction factor was needed for K (0.98), not for NK.

### Table V

<table>
<thead>
<tr>
<th>$x$ Description</th>
<th>$u_y(x)$ for K</th>
<th>$u_y(x)$ for NK</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{Std}$</td>
<td>0.0086</td>
<td>0.018</td>
</tr>
<tr>
<td>Calibration</td>
<td>0.040</td>
<td>0.029</td>
</tr>
<tr>
<td>$A_y/A_S$</td>
<td>0.029</td>
<td>0.024</td>
</tr>
<tr>
<td>$R_{Rec}$</td>
<td>0.0052</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

The expanded uncertainty $U$ provides an interval, within which the true value is believed to lie, with a high degree of confidence (5). $U$ is obtained by multiplying the combined uncertainty with a coverage factor $k$, which is dependent on

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the effective degrees of freedom and the level of confidence. The Welch-Satterthwaite formula was used to approximate the effective degrees of freedom of \( u_x(y) \):

\[
v_{\text{eff}} = \frac{\sum_i u_i^2(y)}{\sum_i u_i(y) / v_i}
\]

(6)

The effective degrees of freedom were calculated for K and NK as 52 and 62, respectively. Because of the high degree of freedom, the coverage factor \( k \) was assumed to be 2 with 95% confidence. The expanded uncertainties, \( U \), for K and NK were therefore 2 times the combined uncertainties for each:

\[
U(C_K) = 2 \times 5 = 10 \text{ ng/mL}
\]
\[
U(C_{NK}) = 2 \times 4 = 8 \text{ ng/mL}
\]

Discussion

In Taiwan, one-point calibration at the threshold concentration is commonly used by laboratories testing urine for drugs-of-abuse. Before analysis of samples, the instrument is calibrated using a blank urine sample, spiked with the threshold concentration of the target compound.

One-point (cut-off concentration) calibration curves were used for the analysis of K and NK in urine, primarily for the following reasons: (i) the multiple-point calibration curves for K and NK showed that the 95% confidence interval of the intercepts included zero (data not shown); (ii) for drug abuse law enforcement, the accuracy at cutoff concentration is more important than other concentrations. If the standard deviation is a function of concentration and the popular non-weighted multiple-point calibration curve is used for quantitation, the accuracy at cutoff concentration may not be better than that obtained from one-point calibration using the cutoff concentration as the calibrator; (iii) one-point calibration is more efficient with respect to time and work-load; (iv) multiple-point calibration is periodically performed to check one-point calibration. According to the rules of the Taiwan Food and Drug Administration (TFDA), a linearity study should be performed at six-month intervals.

For methods based on a multiple-point calibration curve, the uncertainty due to calibration can be easily calculated by assuming the same population standard deviation for all the calibration points. However, this strategy cannot be used for one-point calibration, because only a single calibration point is available. To estimate the uncertainty due to calibration, the standard deviation must be estimated externally. Other experiments have to be conducted to obtain the standard deviation of calibration. To make a differentiation between these two approaches, the term “external estimate of error standard deviation” has been used to refer to the calculation of standard deviation from other experiments (17).

In this study, to estimate the standard deviation due to calibration, the calibration solution was analyzed repeatedly and the standard deviation of the data was used as the estimated standard deviation of calibration. To obtain a more representative estimate, six standard deviations were obtained over a six-week period, the standard deviations were then pooled, and the equation of uncertainty, which is derived for a calibration curve without an intercept (17) was then used to calculate the uncertainty due to calibration.

The expanded uncertainties for K and NK are 10 and 8 ng/mL, respectively. If a sample is found with a concentration of K at 110 ng/mL (NK, 108 ng/mL), then there is 95% probability that the true concentration is above the threshold. Therefore, a positive decision can be made if the measured value is over 110 and 108 ng/mL, for K and NK, respectively.

Table V shows that the uncertainties due to calibration and sample signals were the major sources of MU. Because the uncertainties are propagated in the form of variance \( (u_x)^2 \), the uncertainty of the calibrator’s concentration (the preparation of standard solutions) made a minor contribution to MU.

Conclusions

A MU estimation approach for analytical methods based on one-point calibration was proposed. The strategy is similar to the popular multiple-point calibration. Generally, analysts are familiar with MU based on multiple-point calibration. This study provides an example for analysts with experiences in MU based on multiple-point calibration to do the MU for a method based on one-point calibration. The proposed approach was applied to the MU of ketamine and norketamine at the threshold (100 ng/mL) concentration. The MU for K and NK at 100 ng/mL were found to be 10 and 8 ng/mL, respectively. Uncertainties due to calibration and sample signals were found to be the major sources of MU. The uncertainty of the calibrator’s concentration (the preparation of standard solutions) made a minor contribution to MU.

Acknowledgments

The present work was funded by the Taiwan Food and Drug Administration.

References


