

Evaluation of Uncertainty in High-performance Liquid Chromatography

The concept of "uncertainty" was defined in the Guide to the expression of uncertainty in measurement⁽¹⁾ published by ISO in 1993. However, how uncertainty in measurement should be applied to chemical analysis is summarized in the CITAC (Cooperation on International Traceability in Analytical

Chemistry) guidelines⁽²⁾. This Application News introduces the method for calculating uncertainty in HPLC analysis using an analysis of aspartame in beverage as an example. Refer to the references below for more details.

■ Procedure for evaluating uncertainty

The procedure for the evaluation of uncertainty generally comprises five steps: investigation of the specifications, identification of uncertainty sources, quantity of uncertainty (calculation of the standard uncertainty for each source), calculation of the combined uncertainty and calculation of the expanded uncertainty.

Step 1 Investigation of specification

The aim is to quantify the aspartame in beverages by using high performance liquid chromatography according to the Food Hygiene Law⁽³⁾⁽⁴⁾.

Step 2 Identification of uncertainty sources

A 1000mg/L aspartame aqueous solution (in water) was prepared using 100mL volumetric flasks and a chemical balance. This was then diluted using 100mL volumetric flasks and 5mL whole pipettes to prepare the 50ppm standard solution. This solution was sequentially further diluted by a factor of 2 and 4, using 50mL volumetric flasks and 25mL whole pipettes, to prepare standard samples for the calibration curve.

As a sample preparation, the beverage was diluted by a factor of 10 using 50 mL volumetric flasks and 5 mL whole pipettes. As a HPLC quantitation of the sample, external standard method with the two points calibration curve was employed.

The mean results from ten times repeated analytical runs yielded 245.8ppm.

Step 3 Quantity of uncertainty (Calculation of the standard uncertainty for each source)

The related uncertainty sources for each operation are summarized in Table 1.

Step 4 Calculation of the combined uncertainty

The uncertainties determined at Step 3 are combined but the calculation is complicated. For simplicity, the Kragten spreadsheet method⁽⁵⁾ can be used. This calculation gave a combined standard uncertainty (uc) of 2.7420.

Step 5 Calculation of the expanded uncertainty

The combined standard uncertainty calculated at Step 4 multiplied by a coverage factor is called expanded uncertainty. A coverage factor of 2, for example, represents an approximately 95% confidence interval (in case of more than 6 degrees of freedom).

Multiplying the combined standard uncertainty (uc) of 2.7420 determined above by the coverage factor $k=2$ introduced an expanded uncertainty (U) of 5.4840, such that the result of this analysis can be expressed as $245.80 \pm 5.48 \text{ ppm } (\mu\text{g/mL})$.

■ References

- (1)Guide to the expression of uncertainty in measurement, ISO Geneva, Switzerland 1993 (ISBN 92-67-10188-9)
- (2)Eurachem-CITAC Second Edition, June 1999
- (3)Hamada, Takahashi, Fujita, Abstracts, The Japanese Society for Analytical Chemistry 48th

- Annual Conference, 276 (1999)
- (4)Hioki, Hamada, Takasaka, Kashiwadaira, Shimadzu Scientific Research Inc. Seminars, Abstracts "Accuracy Control and Uncertainty Evaluation in Analysis" (2000)
- (5)J. Kragten, Analyst 119, 2161-2166 (1994)

Table 4 Evaluation of uncertainty for aspartame analysis

Cause	Item	Standard uncertainty ⁽⁷⁾
① Balance accuracy ⁽¹⁾	$\sigma = 0.1\text{mg}$	$u(\text{SD})=0.1\text{mg}$
② Reagent purity ⁽²⁾	98% min.	$u(\text{SD})=100 \cdot 0.01/\sqrt{3}=0.5773\%$
③ 100mL volumetric flask ⁽³⁾	Tolerance ($\pm 0.08\text{mL}$)	$u(\text{SD})=0.08/\sqrt{3}=0.0462\text{mL}$
	Operator's skill ⁽⁴⁾	$u(\text{SD})=0.0199\text{mL}$
④ 5mL whole pipette	Tolerance ($\pm 0.01\text{mL}$)	$u(\text{SD})=0.01/\sqrt{3}=0.0058\text{mL}$
	Operator's skill	$u(\text{SD})=0.0090\text{mL}$
⑤ 50mL volumetric flask	Tolerance ($\pm 0.05\text{mL}$)	$u(\text{SD})=0.05/\sqrt{3}=0.0289\text{mL}$
	Operator's skill	$u(\text{SD})=0.0209\text{mL}$
⑥ 25mL whole pipette	Tolerance ($\pm 0.03\text{mL}$)	$u(\text{SD})=0.03/\sqrt{3}=0.0173\text{mL}$
	Operator's skill	$u(\text{SD})=0.0078\text{mL}$
⑦ Combined relative standard uncertainty for 1000ppm aspartame solution ⁽⁵⁾ (①+②+③) ⁽⁶⁾		$uc(\text{RSD})=\sqrt{(0.1/100)^2+(0.5773/100)^2+(0.0462/100)^2+(0.0199/100)^2}$ = 0.0059
⑧ Combined relative standard uncertainty for 50ppm standard solution (⑦+③+④)		$uc(\text{RSD})=\sqrt{0.0059^2+(0.0462/100)^2+(0.0058/5)^2+(0.0199/100)^2+(0.0090/5)^2}$ =0.0063
⑨ Combined relative standard uncertainty for 2x dilution (25ppm standard solution) (⑧+⑤+⑥)		$uc(\text{RSD})=\sqrt{0.0063^2+(0.0289/50)^2+(0.0209/50)^2+(0.0173/25)^2+(0.0078/25)^2}$ =0.0064
⑩ Combined relative standard uncertainty for 4x dilution (12.5ppm standard solution) (⑨+⑤+⑥)		$uc(\text{RSD})=\sqrt{0.0064^2+(0.0289/50)^2+(0.0209/50)^2+(0.0173/25)^2+(0.0078/25)^2}$ =0.0065
⑪ Combined relative standard uncertainty for 10x beverage test sample dilution (④+⑤)		$uc(\text{RSD})=\sqrt{(0.0289/50)^2+(0.0209/50)^2+(0.0058/5)^2+(0.0090/5)^2}$ =0.0023

(*1)Balance accuracy is indicated as $\sigma = 0.1\text{mg}$. This was used directly as the uncertainty.

(*2)Reagents for food additive analysis were used. A rectangular distribution function was adopted, allowing any value in the 98% to 100% purity range to be used.

(*3)A rectangular distribution was adopted.

(*4)SD determined from 10 measurements.

(*5)Combined after conversion of each cause to a relative standard uncertainty.

(*6)Indicates which items were included in each standard uncertainty calculation.

(*7)Standard uncertainty denoted as $u(\text{SD})$. Combined standard uncertainty denoted as $uc(\text{RSD})$.



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Printed in Japan 3295-12118-10A-IK