Evaluation of Uncertainty in Determination of Ethyl Maltol in Vegetable Oil by Ultra-high Performance Liquid Chromatograph-Mass Spectrometer (UPLC-MS)

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Abstract   [**Objectives**] This study was conducted to establish an uncertainty evaluation method for the determination of ethyl maltol by ultra-high performance liquid chromatograph-mass spectrometer (UPLC-MS). [**Methods**] A mathematical model of uncertainty was established by analyzing the method for determining ethyl maltol using UPLC-MS. The sources of uncertainty were analyzed, and the components of uncertainty were calculated to evaluate the expanded uncertainty of the method. [**Results**] When the content of ethyl maltol in edible vegetable oil was 1 657 µg/kg, the expanded uncertainty was 22.4 µg/kg ($K = 2$, $P = 95\%$). [**Conclusions**] The uncertainty in this evaluation model mainly came from standard solution preparation, sample weighing, dilution of sample to constant volume, standard curve fitting, and repeated measurement.

Key words   Ultra-high performance liquid chromatograph-mass spectrometer; Determination; Ethyl maltol; Uncertainty

Ethyl maltol is a kind of essence food additive, which is widely used in meat products, biscuits, beverages and other fields[1]. However, China's food safety standard[2] clearly stipulates that no essence shall be added to vegetable oil. According to reports[3], some edible oil companies in China have added ethyl maltol to inferior vegetable oil, which poses significant risks to consumers' health.

The method for the determination of ethyl maltol in vegetable oil by ultra-high performance liquid chromatograph-mass spectrometer (UPLC-MS) has the characteristics of low detection limit and good stability, and is widely used by a wide range of scientific research and food safety testing institutions[4-6]. In order to evaluate the reliability of the results of this method more accurately, it is necessary to evaluate the uncertainty of this method. Measurement uncertainty is a parameter associated with measurement results that characterizes the dispersion of values that are reasonably assigned to measurement. Its value reflects the quality of the measurement results, and is directly related to the qualification determination of inspection results[7]. In this study, the uncertainty of the method for the determination of ethyl maltol in vegetable oil by UPLC-MS was evaluated, hoping to provide a basis for quality control in testing laboratories.

Materials and Methods

Instruments, reagents, and materials

Ultra high performance liquid chromatography mass spectrometry: Shimadzu LC-MS-MS 8050; electronic balance: Mettler To-ledo XSE204 (resolution 0.000 1 g); pipettes: 100 − 1 000 µl; standard ethyl maltol reference material (LGC); 10 ml volumetric flask: allowable volume tolerance ± 0.020 ml; 50 ml volumetric flask: allowable volume tolerance ± 0.05 ml.

Standard preparation process

Purchased ethyl maltol standard substance was diluted to required concentrations using pipettes and 10 and 50 ml volumetric flasks.

Sample pretreatment process

A 10 g of the sample (accurate to 0.01 g) was accurately weighed and added into a 50 ml polypropylene centrifuge tube, and 10 ml of methanol was accurately added using a pipette. The obtained liquid was vortex-shaking for 2 min, and centrifuged at 9 000 r/min and 4 ℃ for 10 min. The supernatant was transferred into a 20 ml graduated tube with a stopper, and the underlying oil was extracted with 10 ml of methanol once again. The supernatants were combined and diluted with methanol to 20 ml of solution, which was filtered through a microporous filter membrane (0.22 µm, organic phase), and provided for liquid chromatography-tandem mass spectrometry analysis.

UPLC-MS conditions

Chromatographic conditions   Chromatographic column: Waters ACQUITY BEH C18 (100 mm × 2.1 mm, 1.7 µm); mobile phase A: 0.1% formic acid aqueous solution; mobile phase B: 0.1% formic acid methanol; flow rate: 0.3 ml/min; injection volume: 1.0 µl; column temperature: 40 ℃; elution method: gradient elution, with initial concentration of phase B at 50%.

The time sequence is shown in Table 1.

MS conditions   Ionization mode: ESI, positive ion mode simultaneous analysis; drying gas: nitrogen 10.0 L/min; heating gas: air 10.0 L/min; collision gas: argon 270 kPa; atomization gas: 3.0 L/min; interface temperature: 200 ℃; DL temperature:

250 ℃; dwell time: 150 ms; heating module temperature: 300 ℃; delay time: 3 ms; interface voltage: 1.0 KV; MRM parameters: Table 2; scanning mode: MRM; spray needle distance: +3 mm.

Establishment of mathematical model

$$X = \frac{c \times V \times 1\ 000}{m \times 1\ 000}$$

In the equation, X is the content of ethyl maltol, $\mu g/kg$; c is sample concentration, ng/ml; V is constant volume of test sample, ml; and m is sample weight.

Table 1 Gradient elution time procedure

Time//min	Module	Command	Value
0.50	Pumps	Pumps B Conc.	50
1.50	Pumps	Pumps B Conc.	85
2.00	Pumps	Pumps B Conc.	85
4.00	Pumps	Pumps B Conc.	95
5.00	Pumps	Pumps B Conc.	95
6.00	Pumps	Pumps B Conc.	50
7.00	Controller	Stop	

Table 2 MRM parameter

Name	CAS No.	Mode	Precursor ion	Product ion	Q1 Pre Bias//V	CE//V	Q3 Pre Bias//V
Ethyl Maltol	4940-11-8	ESI(+)	141.1	126.1	−17.0	−22.0	−22.0
		ESI(+)	141.1	71.0	−16.0	−32.0	−28.0

Results and Analysis

In this experiment, the sample weighing 10.0 g was tested twice. The detected content of ethyl maltol in the sample was 1 657 $\mu g/kg$.

Evaluation of uncertainty

Uncertainty brought by standard solutions

Uncertainty of reference material, from reference material certificate

Relative expanded uncertainty (%) ($k = 2$): 0.6

The standard uncertainty of standard solutions:

$$u_{rel}(STD) = 0.6\%/\sqrt{3} = 0.003\ 46$$

Uncertainty in weighing of reference material With a balance accuracy of 0.01 mg, 100 mg of ethyl maltol was weighed. According to rectangular distribution, $k = \sqrt{3}$, the relative standard uncertainty introduced by the weighing of reference material was:

$$u_{rel}(m_1) = \frac{0.01 \times \sqrt{2}/\sqrt{3}}{100} = 0.000\ 081\ 6$$

Uncertainty introduced in the process of diluting reference material to constant volume The standard material was diluted to 100 ml, and the allowable error of a 100 ml volumetric flask was 0.10 ml. According to rectangular distribution, $k = \sqrt{6}$, the uncertainty introduced by diluting reference material to constant weight was:

$$u_{rel}(V_1) = \frac{0.1}{100 \times \sqrt{6}} = 0.000\ 408$$

Uncertainty introduced in the preparation process of standard solution A 1 ml pipette (with an allowable error of 0.007 ml) and a 100 ml volumetric flask (with an allowable error of 0.10 ml) were used in this process. The uncertainty of the 1 ml pipette was $u_{11} = 0.007/\sqrt{3} = 0.040\ 4$, calculated based on uniform distribution, with $k = \sqrt{3}$; and the uncertainty of a 100 ml volumetric flask was $u_{12} = 0.010/\sqrt{6} \times 100 = 0.000\ 408$, calculated according to uniform distribution, with $k = \sqrt{6}$. The uncertainty introduced by the solvent methanol and temperature was negligible.

The uncertainty of this dilution process was:

$$u_1 = \sqrt{0.040\ 4^2 + 0.000\ 408^2} = 0.004\ 06$$

Synthesis of standard uncertainty in the dilution process of standard solution

$$u_{crel}(STD) = \sqrt{0.003\ 46^2 + 0.000\ 081\ 6^2 + 0.000\ 408^2 + 0.004\ 06^2}$$
$$= 0.00\ 535$$

Uncertainty introduced by sample weighing The uncertainty introduced in sample weighing mainly comes from the calibration uncertainty of electronic balances. The calibration certificate of the balance gave a maximum allowable error of ±0.01 g. Assuming a rectangular distribution, the uncertainty was converted to: $0.01 \times \sqrt{2}/\sqrt{3} = 0.008\ 16$. The relative standard uncertainty caused by weighing a sample of 10 g was:

$$u_{rel}(m) = \frac{0.008\ 16}{10} = 0.000\ 816$$

Uncertainty introduced in the process of diluting sample to constant volume The sample was extracted with methanol and diluted to constant volume in a 20 ml volumetric flask. The volume tolerance was ±0.03 ml based on evaluation of Class A. Assuming a rectangular distribution, the uncertainty was: $0.03/\sqrt{6} = 0.01\ 225$ ml

$$u_{rel}(V) = \frac{0.012\ 25}{20} = 0.000\ 612\ 5$$

Uncertainty caused by standard curve fitting The least square method was adopted to fit the standard solution mass concentration-peak area curve, and ethyl maltol standard solutions with five mass concentrations of 12.5, 25, 50, 250 and 500 ng/ml were measured, three times for each mass concentration, to obtain the corresponding peak areas A. The measurement results are shown Table 3.

The equation for the fit calibration curve was $A_i = 14\ 667\rho_i + 10\ 663$, wherein A_i is chromatographic peak area, B_0 is the intercept of the fit calibration curve, B_1 is the slope of the fit calibration curve, and ρ_i is the mass concentration of ethyl maltol in the calibration standard solution.

Table 3   Fitting results of standard solution mass concentration-peak area by least squares method

A Peak area // Au · s	ρ_i mass concentration // ng/ml				
	12.5	25	50	250	500
Y_1	181 087	366 717	767 473	3 679 525	7 330 880
Y_2	178 545	365 240	771 984	3 661 555	7 389 983
Y_3	176 902	375 132	780 035	3 681 603	7 328 208

The standard deviation $S(A)$ of peak area residuals of the standard solutions was calculated from the following formula:

$$S(A) = \sqrt{\frac{\sum_{i=1}^{n}\left[A_i - B_0 - B_1\rho_i\right]^2}{n-2}}$$

The content of ethyl maltol in the sample extract measured from the above standard curve was c = 416 ng/ml, and the standard deviation $S(A)$ of peak area residuals of the standard solutions was 22 030.

The standard uncertainty when calculating the concentration from the standard curve was calculated by following equation:

$$u(C_0) = \frac{S(A)}{B1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{\sum_{i=1}^{n}(c_i - \bar{c})^2}} \quad (1)$$

In equation (1), p is sample measurement times, and it was equal to 5 in this study; n is the measurement times of standard solutions, and it was equal to 15 in this study; C_0 is the average concentration of sample; $\bar{C}$ is the average concentration of standard solutions; and $S(A)$ is the sum of squares of the concentration residuals of standard solutions.

The relative uncertainty was calculated by following equation:

$$u_{rel}(C_0) = \frac{u(C_0)}{C_0} \times 100\% \quad (2)$$

The results calculated from equations (1) and (2) were as follows: $u(C_0) = 1.18127$ ng/ml, and $u_{rel}(C_0) = 1.18127/416 = 0.0028396$.

Standard uncertainty of repeated measurement results   The results of the six repeated determinations of the sample are shown in Table 4.

Table 4   Repeated determination data

No.	1	2	3	4	5	6	Mean
Content // μg/kg	1 658	1 663	1 653	1 651	1 661	1 659	1 657

$$u(x) = \sqrt{\frac{\sum_{i=1}^{n}(x - x_i)^2}{n-1}} = 4.605$$

Relative uncertainty of repeated measurement results:

$$u_{rel}(\bar{X}) = \frac{u(x)}{x} = 0.002779$$

Combined relative standard uncertainty

According to mathematical model $X = \frac{\rho \times V}{m \times 1\,000}$, the relative uncertainty in the determination of ethyl maltol content in edible vegetable oil by UPLC-MS was synthesized from $u_{rel}(\text{STD})$, $u_{rel}(\text{V})$, $u_{rel}(\text{x})$ and $u_{rel}(\text{m})$.

$$u_{crel}(X) = \sqrt{u_{rel}(STD)^2 + u_{rel}(V)^2 + u_{rel}(\bar{X})^2 + u_{rel}(m)^2 + u_{rel}(C_0)^2}$$
$$= \sqrt{0.00535^2 + 0.000816^2 + 0.0028396^2 + 0.0006125^2 + 0.002779^2}$$
$$= 0.0067$$
$$U(c) = 0.0067 \times 1\,657 = 11.1$$

Expanded uncertainty

A 95% confidence probability was taken with factor $k = 2$. The expanded uncertainty of ethyl maltol content was: $U = k \times u_{rel}(X) \times X = 22.2$, and the determination result could be expressed as $(1\,657 \pm 22)$ μg/kg, $(K = 2, P = 95\%)$.

Discussion

In this study, the uncertainty of the determination result of ethyl maltol in vegetable oil by UPLC-MS was evaluated. The results showed that when the content of ethyl maltol in vegetable oil was 1 657 μg/kg, the determination result was expressed as $(1\,657 \pm 22)$ μg/kg, $(k = 2, P = 95\%)$. Specifically, the main source with the greatest contribution to the uncertainty was standard solutions, and the fitting of standard curve took the second place. Therefore, in the future testing process, special attention should be paid to the preparation of standard solutions and the maintenance of instruments to improve accuracy.

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