

Statement of measurement



4005

Malachite Green Oxalate {4-[(4-dimethylaminophenyl)phenylmethylidene]cyclohexa-2,5-dien-1-ylidene}dimethylazanium oxalate Reference Material LGC1706

Assessed Value

	Assessed value (mass %)	Uncertainty ¹ (mass %)	Malachite green cation fraction ² (mass %)	Weight ³ (g)
Purity	94.2	1.4	67.0	0.05

Notes:

1. The uncertainty quoted is the half-width of the expanded uncertainty interval calculated using a coverage factor of 2.46, which gives a level of confidence of approximately 95 %
2. Fraction of malachite green cation in the reference material LGC1706
3. Weight of sample taken for the homogeneity assessment

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Signed: _____
Gill Holcombe (Mrs)
for the Government Chemist



Setting standards
in analytical science

Indicative values

The following figures have been provided for information purposes and should be regarded as indicative values. The four main impurities were identified and their levels are shown in Table 1.

Table 1

Constituent	mass %
Monode-malachite green	1.62
4-(Dimethylamino)benzophenone	0.78
Malachite green carbinol (MG-carbinol)	0.19
Leucomalachite green (LMG)	1.16
Unidentified	0.39

Material Preparation

A batch of malachite green oxalate was obtained and purified by supercritical fluid extraction (SFE) at QinetiQ (Fort Halstead, Sevenoaks, Kent). The purified material was homogenised by tumbling and rolling on a laboratory mill. The material was sub-sampled into a number of fractions. The sub-fractions were dispensed as 0.25 g portions into amber glass vials with screw-caps. The final material was stored at 5 °C ± 4 °C.

Homogeneity Assessment

The material was considered to be homogeneous on the basis of High Performance Liquid Chromatography with UV detection (HPLC-UV) measurements on 3 randomly selected 50 mg portions, which showed no significant variation in purity value.

Stability Assessment

The material has been monitored over a period of 5 months at ambient temperature (20 °C ± 5 °C). No significant change in purity of the material was observed over this time period. LGC1706 will be monitored as part of the LGC reference material stability testing programme, and customers will be notified of any changes that affect the assessed value.

Characterisation

The identity of the SFE purified material was confirmed by 500 MHz 1H-NMR spectroscopy. The purity assessment of the material was carried out by LGC using only one chromatographic method (i.e. HPLC-UV) because malachite green oxalate is thermally unstable and could not be analysed by gas chromatography (GC) and differential scanning calorimetry (DSC). Reversed phase HPLC was used with the experimental conditions described in Table 2. The buffer was composed of 75 x 10⁻³ mol/L ammonium acetate and 25 x 10⁻³ mol/L pentanesulfonic acid sodium salt monohydrate titrated to pH 4 using acetic acid.

Table 2

HPLC system	Waters 2695 separation module		
Detector	Waters 996 Photodiode Array detector		
Processing software	Empower 2		
Analytical column	Phenomenex, Luna 5 μm , C18(2), 100A, 150 x 2.0 mm		
Flow	0.2 mL/min		
Injection volume	10 μL		
Analyte concentration	0.005 % m/m		
Gradient	Time (min)	Buffer (%)	Acetonitrile (%)
	0	50	50
	10	25	75
	15	25	75
	25	50	50
	30	50	50
λ_{max}	265 nm (λ_{max} for LMG)		
Auto sampler temperature	8 °C		
Run time	40 minutes		

The purity assessment of LGC1706 was carried out at 265 nm because most of the impurities exhibit a λ_{max} at that wavelength and are not detectable at the λ_{max} of malachite green (MG) (620 nm). Because monode-malachite green exhibits very similar spectral properties and is very similar structurally to MG, it was quantified at the λ_{max} of MG (620 nm) assuming an equal response from both compounds. However, calibration standards were used to quantify the other identified impurities, due to their difference in molar extinction coefficients at 265 nm ($\epsilon_{(265 \text{ nm})}$) and consequently their different response factors compared to MG. The spectral properties of the unidentified impurities were very similar to those exhibited by LMG and MG-carbinol. Molar extinction coefficients were determined experimentally for those two impurities and used to estimate the molar extinction coefficient of the unidentified impurities.

Using equation (1), it is possible to derive concentration from absorbance (peak height).

$$\epsilon_{(265 \text{ nm})} = \frac{A}{c \cdot l} \quad (1)$$

Where,

$\epsilon_{(265 \text{ nm})}$ is the molar extinction coefficient; A is the absorbance; c is the concentration (mol/L) and l the path length (1 cm).

To calculate the concentration of the sum of the unknown impurities present in purified MG, the absorbance values were summed and the concentration derived using an appropriate $\epsilon_{(265 \text{ nm})}$ value.

This approach relies on two assumptions:

- $\epsilon_{(265 \text{ nm})}$ values of the unknown impurities are similar to $\epsilon_{(265 \text{ nm})}$ values of MG-carbinol or LMG. The effect of the variation of molecular weight (Mw) and $\epsilon_{(265 \text{ nm})}$ on the concentration of the impurities was assessed and an impurity concentration uncertainty was calculated.
- Mw of the impurities are similar to that of LMG (330.47). The effect of a Mw range from 230 to 430 and an $\epsilon_{(265 \text{ nm})}$ range from 2.09×10^9 ($\epsilon_{(265 \text{ nm})}$ MG-carbinol) to 2.66×10^9 ($\epsilon_{(265 \text{ nm})}$ LMG) $\text{L mol}^{-1} \text{cm}^{-1}$ were assessed and shown in Table 3.

Table 3

Total unidentified impurities mass %	Mw	$\epsilon_{(265 \text{ nm})}$ $\text{mol}^{-1} \text{cm}^{-1}$	L
0.23 (minimum)	430	2.09×10^9	
0.54 (maximum)	230	2.66×10^9	

From the calculated minimum and maximum levels of unidentified impurities in Table 3, the mean level was calculated as 0.39 mass % with a range of 0.31 mass %. The range was converted to a standard uncertainty assuming a rectangular distribution and was found to be 0.09 mass %, which was combined with the standard uncertainty associated with the measurement of the absorbance of the sum of the impurities (0.033 mass %). Therefore, the expanded uncertainty was estimated to be 0.2 mass % at the 95 % confidence interval.

Moisture

Moisture was determined by coulometric oven Karl Fischer titration and the value subtracted from the purity determined by HPLC-UV, which does not measure moisture. A Merck Apura 1 % oven standard (Lot F1334054511.188054.0005) (nominal water content: 1.00 ± 0.03 mass %) was used as the QC standard to check instrument performance.

Inorganic Content

The percentage of inorganic material was assessed by ashing. About 2 g of sample was weighed into a crucible and heated from room temperature to 600 °C over 90 minutes. The inorganic content (mass %) was calculated using the weight of residue after completion of the ashing and the initial sample weight.

The value obtained was subtracted from the purity value determined by HPLC-UV.

Uncertainties

Standard uncertainties for the MG purity assessment analytical techniques (u_{meth}) were either taken from historical method data, or calculated using equation (2).

$$u_{\text{meth}} = \frac{\sigma}{\sqrt{n}} \quad (2)$$

Where:

σ = Standard deviation of the results.

n = Number of independent analyses performed.

Historical data on a range of organic compounds analysed at LGC by 2 or 3 independent methods (HPLC, GC, DSC) shows that the between-method standard deviation only once exceeded 0.5 % (actual value 0.53 %). As the assessment for this material is based on only one method, the value of 0.5 % is included as the maximum expected between-method standard deviation for that compound.

$$u_{\text{MG}} = \sqrt{u_{\text{imp}}^2 + u_{\text{moisture}}^2 + u_{\text{ashing}}^2 + u_{\text{non-homogeneity}}^2 + u_{\text{single method}}^2} \quad (3)$$

Where u_{imp} includes the uncertainty contribution for the estimation of all the impurities and the uncertainty contribution arising from the purity of the calibration standards of the identified impurities.

The number of effective degree of freedom (dof_{eff}) used for the purity determination of MG was calculated using the Welch-Satterthwaite [1] equation (4).

$$dof_{\text{eff}} = \frac{(u_{\text{Tot}})^4}{\left(\frac{(u_{\text{imp}})^4}{n_{\text{imp}} - 1}\right) + \left(\frac{(u_{\text{moisture}})^4}{n_{\text{moisture}} - 1}\right) + \left(\frac{(u_{\text{ashing}})^4}{n_{\text{ashing}} - 1}\right) + \left(\frac{(u_{\text{non-homogeneity}})^4}{n_{\text{non-homogeneity}} - 1}\right)} \quad (4)$$

The t-value for the effective degrees of freedom was estimated using a linear interpolation between the two known t-values for integer degrees of freedom that encompass the effective degrees of freedom.

Table 4

Purity (mass %)	mean	s.e.m ²
Purity ¹ (100 % - sum impurities)	94.2	
Total standard uncertainty for purity (including moisture, possible inhomogeneity and ash)		0.19
Uncertainty contribution from possible instability		0.13
Uncertainty contribution (single method)		0.5
Total standard uncertainty		0.55
Effective degrees of freedom (dof _{eff})		5.86
t for 5.86 dof and 95 % confidence		2.464
Expanded uncertainty (k = 2.46) at 95 % confidence		1.4
1) Corrected mean is the mean minus, where appropriate, the mean moisture content (1.09 mass %) and the mean inorganic content (0.55 mass %)		
2) s.e.m = standard error (uncertainty) of corrected mean		

In October 2007, an error was found in the statement for this material. The IUPAC name quoted for malachite green oxalate was incorrect. Page 1 of the statement was revised to include the correct IUPAC name.

Intended Use

The material is intended for the use as an analytical standard for the determination of malachite green oxalate in foodstuff, especially fish.

Instructions for Use

A detailed protocol for the preparation of the standard, including the stability of prepared solutions, is available separately. (LGC Guide to the Preparation of Malachite Green Oxalate Standards using LGC1706)

Storage

The reference material should be stored in the unopened vial at a temperature of 5 °C ± 4 °C in the dark.

Shelf Life

If stored under the recommended conditions, the assessed value of the unopened material will remain valid for 12 months from the date of shipment.

References

1. Guide to the Expression of Uncertainty in Measurement. ISO, Geneva, Switzerland, 1995 (ISBN 92-67-10188-9).

Unit Number:

Date of Shipment:

Legal Notice

(revised November 2008)

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