

# Statement of measurement



4005

## Allergen reference material - Walnut powder – partially defatted

Reference Material LGC7426

### Assessed Values

Property	Content g/100 g	Uncertainty <sup>3</sup> g/100 g	$k^4$
Nitrogen <sup>1</sup>	6.15	0.19	2.00
Water <sup>2</sup>	6.11	0.65	2.00

#### Notes:

1. Determined by an automated Dumas procedure. See later section for metrological traceability.
2. Determined by oven Karl Fischer titration. See later section for metrological traceability.
3. The quoted uncertainty is the half-width of the expanded uncertainty interval calculated using a coverage factor <sup>4</sup>, which gives a level of confidence of approximately 95 %.
4. Coverage factor.

Date of Issue: December 2019  
Latest document revision June 2022

Signed: \_\_\_\_\_  
Gill Holcombe (Mrs)  
for the Government Chemist



## Material sourcing and preparation

The raw material was sourced by the University of Manchester from a reputable supplier to minimise the risk of contamination, and was described as 'Origin: Italy. *Juglans regia* cultivar Lara. Lipid content 27 g/100g'.

The walnut powder was prepared by grinding using a centrifugal mill to pass a 0.5 mm sieve. The sieved material was combined and mixed before weighing in  $(1.1 \pm 0.1)$  g portions into amber glass vials. The vials were closed under argon with a rubber stopper and a crimp cap. Each unit was sealed inside a metallised sachet to minimise changes in water content. 550 units were prepared and stored at  $(5 \pm 4)$  °C.

## Homogeneity Assessment

Homogeneity was assessed for nitrogen and water content. The procedure used is outlined in the section entitled 'Analytical Methods Used'. For both nitrogen and water, fifteen units were analysed in triplicate over three runs using a randomised block design. For both analytes, the homogeneity of the material was considered fit for purpose.

## Stability

Deterioration is not expected over the lifetime of the material when stored under the recommended conditions; however LGC7426 will be subjected to testing under LGC's stability monitoring programme. Purchasers will be informed of any changes to the assessed values.

## Characterisation

This reference material has been prepared by LGC and characterised using the analytical methods described below.

## Analytical Methods Used

**Nitrogen** was determined at LGC using an automated Dumas procedure using a rapid N cube nitrogen analyser (Elementar Analysensysteme GmbH) which is within LGC's scope of accreditation to ISO/IEC 17025 for testing. The instrument was calibrated using an EDTA Organic Analytical Standard which has a certified value for nitrogen traceable to NIST SRM 143d.

**Water** was determined by oven Karl Fischer (KF) coulometry using a Metrohm 774 oven sample processor and 831 KF coulometer. This method is within LGC's scope of accreditation to ISO/IEC 17025 for calibration.

LGC7426 was also characterised at the University of Manchester by a combination of advanced proteomic techniques including gel electrophoresis, immune-based analysis and mass spectrometry, confirming the presence of the relevant allergen protein molecules.

## DNA sequencing of LGC7426 (Walnut)

Purified DNA that had been extracted from the walnut candidate reference material sample was subject to testing for species identity using a Sanger DNA sequencing-based approach.

Pairs of species-specific oligonucleotides were used to direct the PCR amplification of polymorphic regions for the plastid *matK* and *rbcL* loci. Sanger DNA sequencing was performed on the amplified products in both forward and reverse directions.

The resulting DNA sequence information, consisting of sequence read lengths between 644bp – 951bp, were evaluated for sequence identity by comparison to annotated DNA sequence data using the BLASTn (v. 2.9.0) sequence alignment software accessible at the National Centre For Biotechnology Information, Washington. USA (NCBI).

All of the analysed sequences exhibited at least 99.36 % sequence identity with DNA sequences for walnut (*Juglans regia*) accessible at the NCBI GenBank database (accessed 06/09/2019). Corresponding E-values (probability that a score as high as the ones observed between two DNA sequences arising by chance alone) were estimated as being equal to or less than  $10^{-155}$ .

## Intended Use

The material is intended for use in (a) method development: e.g. in the generation of allergen kit calibrator extract solutions, (b) method validation: e.g. in the generation of external check calibrator extract solutions for allergen measurements (c) recovery estimates: to spike food matrices either by way of an extract, but preferably by addition of the raw material itself to assess allergen recovery in real life situations for which no other RMs are available.

LGC7426 can also be used in the quality control of methods for the determination of nitrogen and water in food ingredients and processed food products.

## Metrological Traceability

The values for nitrogen and water are traceable to the SI.

For nitrogen, the instrument was calibrated using an EDTA Organic Analytical Standard which has a certified value for nitrogen traceable to NIST SRM 143d (Gaithersburg, USA), which in turn is traceable to the SI.

The value for water content by coulometric Karl Fischer titration is traceable to the SI via (a) the use of coulometric titration equipment certified for performance using electrical test equipment calibrated and traceable to national and/or international standards (Certificate of Performance no. 1831001014106), (b) the use of certified reference materials for performance verification and quality control, and (c) the use of calibrated balances for mass determination.

## Accreditation

All values are within LGC's scope of accreditation to ISO 17034.

## Instructions for Use

The sample should be brought to room temperature before opening. Mix the contents of the vial using a clean spatula, being careful to avoid contamination. The sample portion should be removed and used rapidly to prevent changes in the water content. Open vials should not be stored for reuse.

The minimum recommended sample size is based on the amount of material taken for the homogeneity assessment and is shown below:

- 0.25 g for nitrogen
- 0.01 g for water

Use in allergen analysis should follow published guidance.

## Storage Conditions

The material should be stored unopened at  $(5 \pm 4) ^\circ\text{C}$ .

## Shelf Life

This statement is valid for 6 months from the date of shipment provided the kit is stored unopened under the recommended conditions.

## Acknowledgements

The University of Manchester School of Biological Sciences, Division of Infection, Immunity and Respiratory Medicine, is thanked for providing the walnut powder used to prepare this reference material and for carrying out proteomic characterisation.

## Additional information

This material is also available as a component in the LGC reference material kit 'LGC746-KT Allergen kit – Milk, Egg, Almond, Hazelnut and Walnut'.

## Document Revision

In September 2020, this document was updated to confirm the material is within LGC's scope of accreditation to ISO 17034 as a reference material producer.

The document was revised in June 2022 to reflect an update to the UKAS symbol.

SPECIMEN

Unit Number

Date of Shipment

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