Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

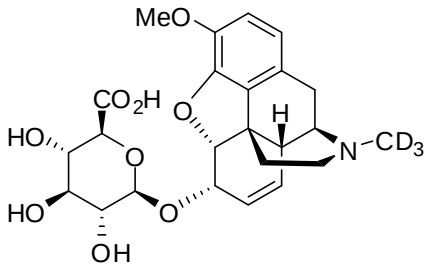
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D695: d3-Codeine glucuronide

Report ID: D695.2021.03 (Ampouled 140807)

Chemical Formula: $C_{24}H_{26}D_3NO_9$

Molecular Weight: 478.5 g/mol (base)



Property value

Batch No.	CAS No.	Mass per ampoule
01-D-04	**219533-59-2**	**71 ± 3µg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: d_3-(5α,6α)-3-Methoxy-17-methyl-7,8-didehydro-4,5-epoxymorphinan-6-yl β-D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 18 November 2031, i.e. ten years from the date of recertification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The shelf life does not apply to ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D695. The material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 71 µg of anhydrous codeine glucuronide (d_3, d_2, d_1 and d_0). The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

Recommended storage: When not in use, this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

Stability: This material has demonstrated stability over a minimum period of ten years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials.
The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on seven randomly selected ampoules of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
14 September 2022.

This report supersedes any issued prior to 14 September 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

HPLC:	Instrument:	Waters alliance 2695 or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4 mm × 150 mm)
	Mobile Phase:	Methanol/NH_4OAc (20 mM, pH 5.4) (15:85)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 or 2998 operating at 215 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 96.5%, s = 0.1% (7 ampoules in duplicate, August 2014)
	Re- analysis:	Mean = 96.5%, s = 0.3% (5 ampoules in duplicate, July 2017)
	Re- analysis:	Mean = 96.5%, s = 0.2% (5 ampoules in duplicate, November 2021)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^1H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by qualitative elemental microanalysis.

Moisture and sodium acetate are associated with the glucuronide from the purification process cannot be readily removed.

The main component of this material is d_3-codeine glucuronide. d_2-, d_1- and d_0- codeine glucuronide are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_3, d_2 and d_1) and d_0- codeine glucuronide in the material.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity:	d3 ≈ 99% [= d3/(d3 +d2 +d1 +d0) x 100]	
	d0 < 0.5% [= d0/(d3 +d2 +d1 +d0) x 100]	
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4 mm × 150 mm)
	Mobile Phase:	Methanol/NH_4OAc (20 mM, pH 5.4) (20:80)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at 215 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 94.7%, (10 sub samples in duplicate, October 2003)
	Re-analysis:	Mean = 94.5%, s = 0.1% (5 sub samples in duplicate, April 2007)
Karl Fischer analysis:		Moisture content 11.7% mass fraction (May 2007) Moisture content 16.6% mass fraction (July 2014)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH_4OAc, pH 7.5: MeOH (1:1)
	Major ions:	523 (8), 502 (14, MNa^+), 479 (100, MH^+), 462 (8) m/z
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH4OAc, pH 7.5: MeOH (1:1)
	Major ion:	477 ($[M^-H]^-$, 100) *m/z*
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3400 (br), 1607, 1507, 1056, 792 cm^{-1}
^1H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Key spectral data:	δ 3.81 (3H, s), 4.60 (1H, d), 6.60 & 6.72 (2 x 1H, d) ppm
	Absorbance attributed to sodium acetate was observed at δ 1.9 ppm. The mass fraction of sodium acetate present was estimated as 12.7% from integration of relative peak areas.	
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz
	Solvent:	MeOH-d_4 (49.0 ppm)
	Spectral data:	δ 20.6, 33.8, 39.0, 42.3, 46.0, 55.5, 59.2, 72.1, 72.2, 73.7, 75.0, 76.1, 88.8, 101.3, 113.3, 119.3, 125.7, 126.7, 129.9, 131.4, 142.2, 146.8, 175.4 ppm