Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

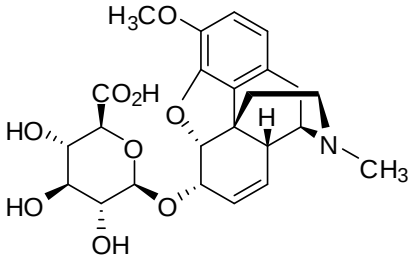
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D694: Codeine glucuronide

Report ID: D694.2020.03 (Ampouled 130430)

Chemical Formula: $C_{24}H_{29}NO_9$

Molecular Weight: 475.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
01-D-03	N/A	76 ± 6 µg

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

Expiration of certification: The property values are valid till 15 May 2025, i.e. five years from the date of re-certification 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 expiry date/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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D694. This material was prepared by synthesis and certified for identity and purity by NMIA.

Intended use: This certified reference material may be used for instrument calibration.

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 76 ± 6 µg of codeine glucuronide. 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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% coverage 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 homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence interval from that observed on repeat analysis of the same sample.

Safety: Treat as 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:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified 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 ^{1}H 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.

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

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler or 2695 Separation module
	Column:	Alltech or Grace Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	20 mM Ammonium Acetate buffer pH= 5.4 /Methanol A = Ammonium Acetate; B = Methanol 0-8 min 15% B; 8-9 min 15-90% B; 9-14 min 90% B; 14-15 min 90-15% B; 15-22 min 15% B Or B: A (20:80) [2013] and B: A (15:85) [2014]
	Flow rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating 217 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 91.1%, s = 0.1% (7 ampoules in duplicate, May 2013)
	Re-analysis:	Mean = 90.9%, s = 0.1% (5 ampoules in duplicate, April 2014)
	Re-analysis:	Mean = 90.9%, s = 0.3% (5 ampoules in duplicate, May 2015)
	Re-analysis:	Mean = 91.6%, s = 0.1% (5 ampoules in duplicate, May 2016)
	Re-analysis:	Mean = 91.7%, s = 0.04% (5 ampoules in duplicate, May 2020)

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

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltech Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	MeOH/MilliQ water (20:80) The aqueous phase was buffered at pH 5.4 using 20 mM NH4OAc and AcOH
	Flow rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating 217 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 90.9 %, s = 0.05 % (7 sub samples in duplicate, May 2013)
Karl Fischer analysis:		Moisture content ca. 12% mass fraction (August 2006) Moisture content ca. 17.8% mass fraction (April 2013)
Inorganic analysis:		Sodium ion content 1.4% mass fraction (August 2006)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Finnigan MAT TSQ 700		
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH4OAc, pH 7.5: MeOH (1:1)		
	Major ions:	498 (10, MNa^+), 476 (100, MH^+) *m/z*		
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH4OAc, pH 7.5: MeOH (1:1)		
	Peak:	474 ($[M^-H]^-$, 100) *m/z*		
LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro		
	Column:	X-Bridge C-18, 100 mm × 2.1 mm I.D. × 3.5 μm		
	Column temp:	40 °C		
	Solvent system:	15% Acetonitrile/85% MilliQ water with formic acid		
	Flow rate:	1 mL/min		
	Sample prep:	1000 μg/g in MeOH/MilliQ water (20:80)		
	Injection volume:	10 μL		
	Ionisation mode:	Electrospray positive ion		
	Capillary voltage:	3.5 kV		
	Cone voltage:	30 V		
	Source temp:	130 ºC	Desolvation gas temperature:	350 ºC
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate:	753 L/hr
	The retention time of Codeine glucuronide is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.			
	Major ion:	476.3 (M^-H^+) *m/z*		
IR:	Instrument:	FT-IR, Biorad WIN FTS40		
	Range:	4000-400cm^{-1}, KBr pellet		
	Peaks:	3400 (br), 1607, 1507, 1056, 792 cm^{-1}		
^{1}H NMR:	Instrument:	Bruker DMX-500		
	Field strength:	500 MHz		
	Solvent:	MeOH-d_4 (3.3 ppm)		
	Key spectral data:	δ 2.75 (3H, s), 3.81 (3H, s), 4.60 (1H, d), 6.50 & 6.64 (2 x 1H, d) ppm		
	Sodium acetate estimated at 6.4% mass fraction was observed in the ^{1}H NMR			
^{13}C NMR:	Instrument:	Bruker DMX-500		
	Field strength:	125 MHz		
	Solvent:	MeOH-d_4 (49 ppm)		
	Spectral data:	δ 21.1, 32.8, 38.1, 40.2, 41.9, 46.3, 55.7, 59.9, 71.8, 72.3, 73.6, 75.3, 76.2, 88.3, 101.1, 114.0, 119.5, 124.2, 125.6, 129.2, 132.0, 142.5, 147.1, 175.6 ppm		
Microanalysis:	Found:	C = 50.3%; H = 6.0%; N = 2.2% (August, 2006)		
	Calculated:	C = 60.6%; H = 6.2%; N = 2.9% (Calculated for $C_{24}H_{29}NO_9$)		