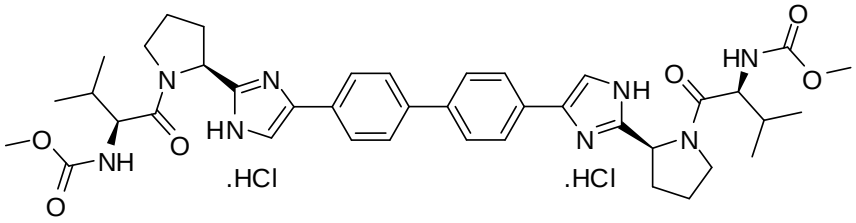
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: GP2U3.2015.01

Compound Name: **Daclatasvir dihydrochloride**
Collection Number: GP2U_3
Chemical Formula: $C_{40}H_{50}N_8O_6.2HCl$
CAS Number: 1009119-64-5
Description: White powder
Batch Number: 15-GP-03
Molecular Weight: 811.8 (HCl), 738.9 (base)
Release date: 7th October 2015

Structure:



Synonym: Methyl [(2*S*)-1-{(2*S*)-2-[4-(4'-{2-[(2*S*)-1-{(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-2-pyrrolidinyl]-1*H*-imidazol-4-yl}-4-biphenylyl)-1*H*-imidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo-2-butanyl]carbamate
N,*N'*-[[1,1'-biphenyl]-4,4'-diylbis[1*H*-imidazole-5,2-diyl-(2S)-2,1-pyrrolidinediyl[(1S)-1-(1-methylethyl)-2-oxo-2,1-ethanediyl]]]bis-carbamic acid, *C*,*C'*-dimethyl ester

Purity (mass fraction): 98.1 ± 0.5% (95% coverage interval)

Note: The assigned stereochemistry of this sample of daclatasvir has not been confirmed.

The purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, and Karl Fischer analysis. The purity value by qNMR was obtained using the four methyl doublets at 0.6-1.0 ppm measured against a certified internal standard of potassium hydrogen maleate. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent and elemental microanalysis.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water buffered at pH 10 with NH_4^+ ^-OAc; B = MeCN
	Gradient	0 min 35% B; 0-15 min 35% B; 15-18 min 35-75% B; 18-23 min 75% B.
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 310 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.2%, s = 0.01% (10 sub samples in duplicate, September 2015)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (September 2015). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis.
Karl Fischer analysis:		Moisture content 0.6% mass fraction (August 2015)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz Solvent: DMSO-d_6 (2.50 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis:	Mean (0.86 ppm) = 98.2%, s = 0.2% (6 sub samples, September 2015)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Thermo Scientific Dionex UltiMate 3000 Degasser,	
	Column:	ZORBAX RRHD SB-C8, 2.1 x 50 mm, 1.8 µm (Agilent, 857700-906)	
	Column temp:	30.0 °C	
	Solvent system:	Mobile phase A: 10 mM ammonium formate, 0.01% (v/v) formic acid in Milli-Q® water. Mobile phase B: 0.01% (v/v) formic acid in acetonitrile. Gradient from 90% A to 100% B	
	Flow rate:	0.25 mL/min	
	Sample prep:	2 mg/mL in MeOH with trace of formic acid	
	Injection volume:	10 µL	
	Ionisation mode:	Electrospray positive ion	
	Capillary voltage:	4.5 kV	
	Capillary temp:	360ºC	Desolvation gas temperature: 300 ºC
	Cone gas flow rate:	10 (arbitrary unit)	Desolvation gas flow rate: 70 (arbitrary unit)
	The retention time of daclatasvir is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	9.98 min:	739.39545 ($M+H^+$) m/z	
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888	
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 µm	
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)	
	Injector:	150 °C	Transfer line temp: 280 °C
	Carrier:	Helium, 1.2 mL/min	Split ratio: 50/1
	Solvents detected:	Ethyl acetate	
TLC:	Conditions:	Kieselgel $60F_{254}$. Ethyl acetate : methanol (95/5) Single spot observed, R_f = 0.18. Visualisation with UV at 254 nm The TLC was performed on the liberated free base.	
IR:	Instrument:	Bruker Alpha FT-IR	
	Range:	4000-400 cm^{-1}, neat	
	Peaks:	1723, 1697, 1643, 1523, 1439, 1235, 1099, 1024 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	500 MHz	Solvent: DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.77 (6H, d, *J* = 6.7 Hz), 0.83 (6H, d, *J* = 6.7 Hz), 2.01 (2H, m), 2.07 (2H, m), 2.12-2.27 (4H, m), 2.38 (2H, m), 3.54 (6H, s), 3.84 (2H, m), 3.97 (2H, m), 4.12 (2H, t, *J* = 7.7 Hz), 5.18 (2H, t, *J* = 7.0 Hz), 7.31 (2 N-H, d, *J* = 8.5 Hz), 7.94 (4H, d, *J* = 8.4 Hz), 7.99 (4H, d, *J* = 8.4 Hz), 8.16 (2H, s) ppm Ethyl acetate estimated at 0.6% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	126 MHz	Solvent: DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 17.8, 19.6, 25.0, 29.0, 31.2, 47.3, 51.6, 52.9, 58.0, 115.1, 125.9, 126.6, 127.3, 131.8, 139.2, 149.4, 157.0, 171.1 ppm	
Melting point:		> 250 °C	
Microanalysis:		Found: C = 59.0%; H = 6.5%; N = 13.7% (August 2015) Calc: C = 59.2%; H = 6.5%; N = 13.8% (Calculated for $C_{40}H_{50}N_8O_6.2HCl$)	

Expiration of certification
The long-term stability of the compound in both solid form and in solution has not been examined.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by HPLC with UV detection on ten randomly selected 1-2 mg sub samples 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.

Metrological Traceability
The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. The purity was derived by subtraction of the mass of impurities from the mass of the reference material. Organic purity is traceable to the SI-derived coherent unit one through chromatographic separation and response factor determination of individual components. Volatile and non-volatile residue content is directly traceable to mass through use of Karl Fischer and thermogravimetric analysis. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

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.

Intended Use
For ***in vitro*** laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

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Dr Stephen R. Davies,
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Dated: 7 October, 2015.