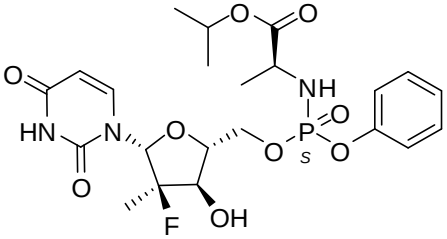
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: GP2U1.2015.01

Compound Name: **Sofosbuvir**
Collection Number: GP2U1
Chemical Formula: $C_{22}H_{29}FN_3O_9P$
CAS Number: 1190307-88-0
Structure:



Description: White powder
Batch Number: 15-GP-01
Molecular Weight: 529.5
Release date: 7th October 2015

Synonyms: Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate
N-[[*P*(S),2'R]-2'-deoxy-2'-fluoro-2'-methyl-*P*-phenyl-5'-uridylyl]-L-alanine-1-methylethyl ester

Purity (mass fraction): 97.9 ± 0.9% (95% coverage interval)

The purity estimate was obtained from ^{31}P quantitative nuclear magnetic resonance (qNMR). Supporting evidence is provided by HPLC analysis with UV detection at 262 nm (a λ_{MAX} of sofosbuvir), thermogravimetric analysis, Karl Fischer analysis, headspace GC-MS analysis of occluded solvents elemental microanalysis and ^{1}H qNMR. The purity value by ^{1}H qNMR was obtained using a combination of all four methyl protons (the six-proton doublet at 1.14 ppm, the three-proton doublet at 1.22 ppm and the three-proton doublet at 1.23 ppm) and the five phenyl protons with one uracil olefinic proton (the one-proton triplet at 7.18 ppm, the two-proton doublet at 7.22 ppm, the two-proton triplet at 7.37 ppm and the one-proton doublet at 7.56 ppm) measured against a certified internal standard of dimethyl sulfone. The total purity estimate by ^{1}H qNMR is corrected for by the organic purity estimate using HPLC with UV detection.

HPLC:	Instrument:	Thermo Scientific Ultimate 3000 RS
	Column:	Alltima C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water; B = MeOH; C = 2% formic acid in Milli-Q water.
	Gradient	0 min 10% B, 10% C; 0-10 min 10-55% B, 10% C; 10-20 min 55% B, 10% C; 20-25 min 55-90% B, 10% C; 25-30 min 90% B, 10% C.
	Flow rate:	1.0 mL/min
	Detector:	RS DAD operating at 262 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.08% (10 sub samples in duplicate, September 2015)
Thermogravimetric analysis:		Volatile content < 0.1% mass fraction (September 2015). The non-volatile residue (e.g. inorganic salts) could not be determined by thermogravimetric analysis.
Karl Fischer analysis:		Moisture content 0.1% mass fraction (August 2015)
^{1}H QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz Solvent: MeCN-d_3 (1.94 ppm)
	Internal standard:	Dimethyl sulfone (100% mass fraction)
	Initial analysis:	Mean (methyls) = 99.3%, s = 0.1% (5 sub samples, September 2015)
	Initial analysis:	Mean (aromatics) = 99.3%, s = 0.1% (5 sub samples, September 2015)
^{31}P QNMR:	Instrument:	Bruker Avance-III-600
	Field strength:	243 MHz Solvent: DMSO-d_6
	Internal standard:	Triphenylphosphine oxide (100% mass fraction)
	Initial analysis:	Mean = 97.9%, s = 1.9% (5 sub samples, October 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 sofosbuvir is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.		
	9.51 min:	530.17145 ($M+H^+$) 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:	Propan-2-ol and *tert*-butyl methyl ether	
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate Single spot observed, R_f = 0.45. Visualisation with UV at 254 nm	
IR:	Instrument:	Bruker Alpha FT-IR	
	Range:	4000-400 cm^{-1}, neat	
	Peaks:	1744, 1713, 1688, 1454, 1378, 1208, 1082, 1012, 940 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	500 MHz	Solvent: DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 1.14 (6H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.7 Hz), 1.25 (3H, d, J_{H-F} = 22.3 Hz), 3.80 (1H, m), 3.86 (1H, bs), 4.00 (1H, m), 4.23 (1H, m), 4.36 (1H, m), 4.84 (1H, septet, J = 6.3 Hz), 5.54 (1H, d, J = 7.9 Hz), 5.87 (1H, bs), 6.00 (1H, bs), 6.06 (1H, dd, J = 10.4, 12.7 Hz), 7.18 (1H, t, J = 7.5 Hz), 7.22 (1H, d, J = 8.3 Hz), 7.37 (1H, t, J = 7.8 Hz), 7.55 (1H, bd, J = 7.9 Hz), 11.5 (1H, s) ppm The ^{1}H NMR matches that for a sample of sofosbuvir extracted from tablets of Twinvir™, and the spectrum reported in J. Med. Chem., 2010, 53, 7202-7218.	
^{13}C NMR:	Instrument:	Bruker Avance III 500	
	Field strength:	126 MHz	Solvent: DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 16.6 (d, J_{C-F} = 25 Hz), 19.8 (d, J_{C-P} = 6.4 Hz), 21.39, 21.43, 49.8, 64.8 (br), 68.0, 71.5 (br), 79.5 (br), 88.7 (br), 100.3 (J_{C-F} = 181.3 Hz), 102.3, 120.1 (J_{C-P} = 4.9 Hz), 124.6, 129.7, 139.5 (br), 150.5, 150.7 (J_{C-P} = 6.3 Hz), 162.8, 172.6 (J_{C-P} = 5.1 Hz) ppm	
Microanalysis:		Found: C = 50.1%; H = 5.5%; N = 8.0%; F = 3.6%; P = 5.9% (August 2015) Calc: C = 49.9%; H = 5.5%; N = 7.9%; F = 3.8%; P = 5.9% (Calculated for $C_{22}H_{29}FN_3O_9P$)	

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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Dated: 7 October, 2015.