

BDG SYNTHESIS

Certificate of Analysis

BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

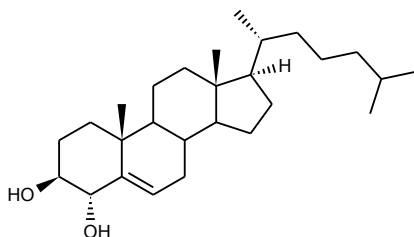
Barry Dent

Barry R. Dent, PhD, Director
14 June 2012

Name: 4 α -Hydroxycholesterol

CAS Number: 34310-86-6

Structure:



Molecular Weight: C₂₇H₄₆O₂ = 402.65

Lot Number: BDG 12527.2

Appearance: White, crystalline solid

Purity By HPLC: 97.4 %

Re-test Date: 14 June 2013

Storage and Handling:

Temperature:	refrigerate for prolonged storage; may be handled and shipped at ambient temperature.
Humidity:	not believed to be hygroscopic; may be handled in normal laboratory atmosphere.
Light:	protect from strong sunlight.
Caution:	only experienced laboratory personnel should handle the material.

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Residual Solvents: no residual solvents are observed.

Impurities: no significant impurities are evident in the spectrum.

Carbon-13 NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

High-resolution Mass Spectrum (ESI+)

Found m/z 425.3392. $C_{27}H_{46}O_2$ $[M+Na]^+$ requires m/z 425.3396. The deviation of 0.9 ppm is within normally accepted limits for the establishment of identity by HRMS.

HPLC

A sharp, symmetrical peak is observed (97.4 %). Note: in the absence of reference materials for preparing calibration curves, it is assumed that all peaks have the same detector response. Where possible, the conditions of analysis follow a pharmacopeial or literature method, or have been adapted from same.

Elemental Analysis

	Found:	C 80.44, H 11.58 %
$C_{27}H_{46}O_2$	Requires:	C 80.54, H 11.51 %

The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

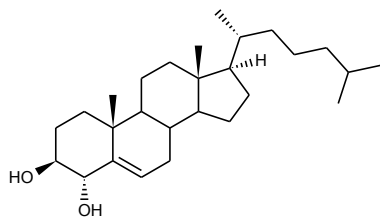
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials often contain higher levels of residual solvents and/or water, and we urge you to use the corrected purity where needed rather than the raw HPLC purity. This compound is intended for use as an analytical reference material and it is not for human administration. Structures are shown with relative stereochemistry unless otherwise specified.

The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.

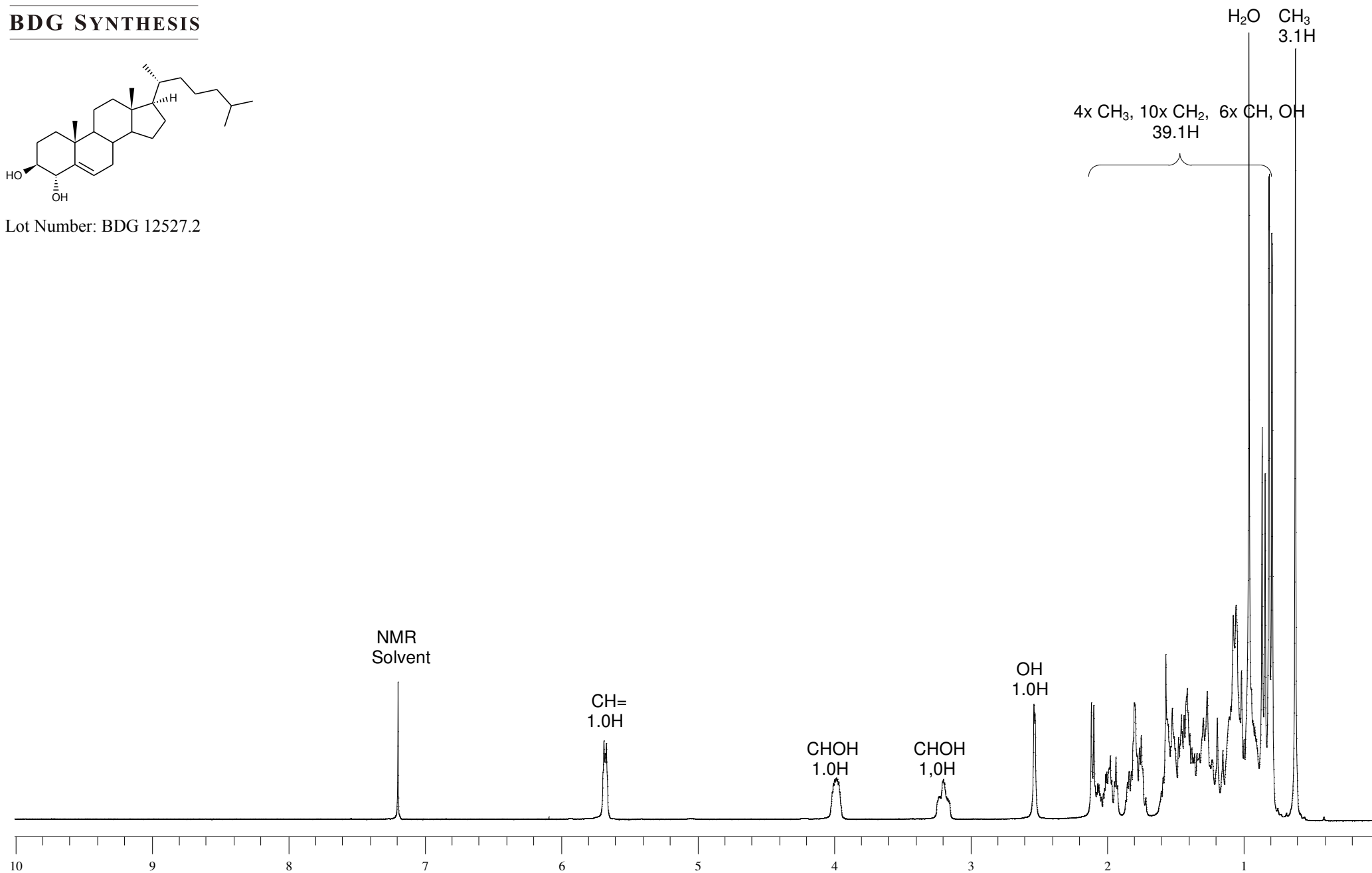


Proton NMR Spectrum of 4 α -Hydroxycholesterol in CDCl₃

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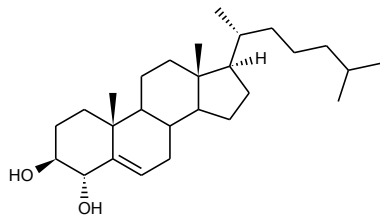
Lot Number: BDG 12527.2



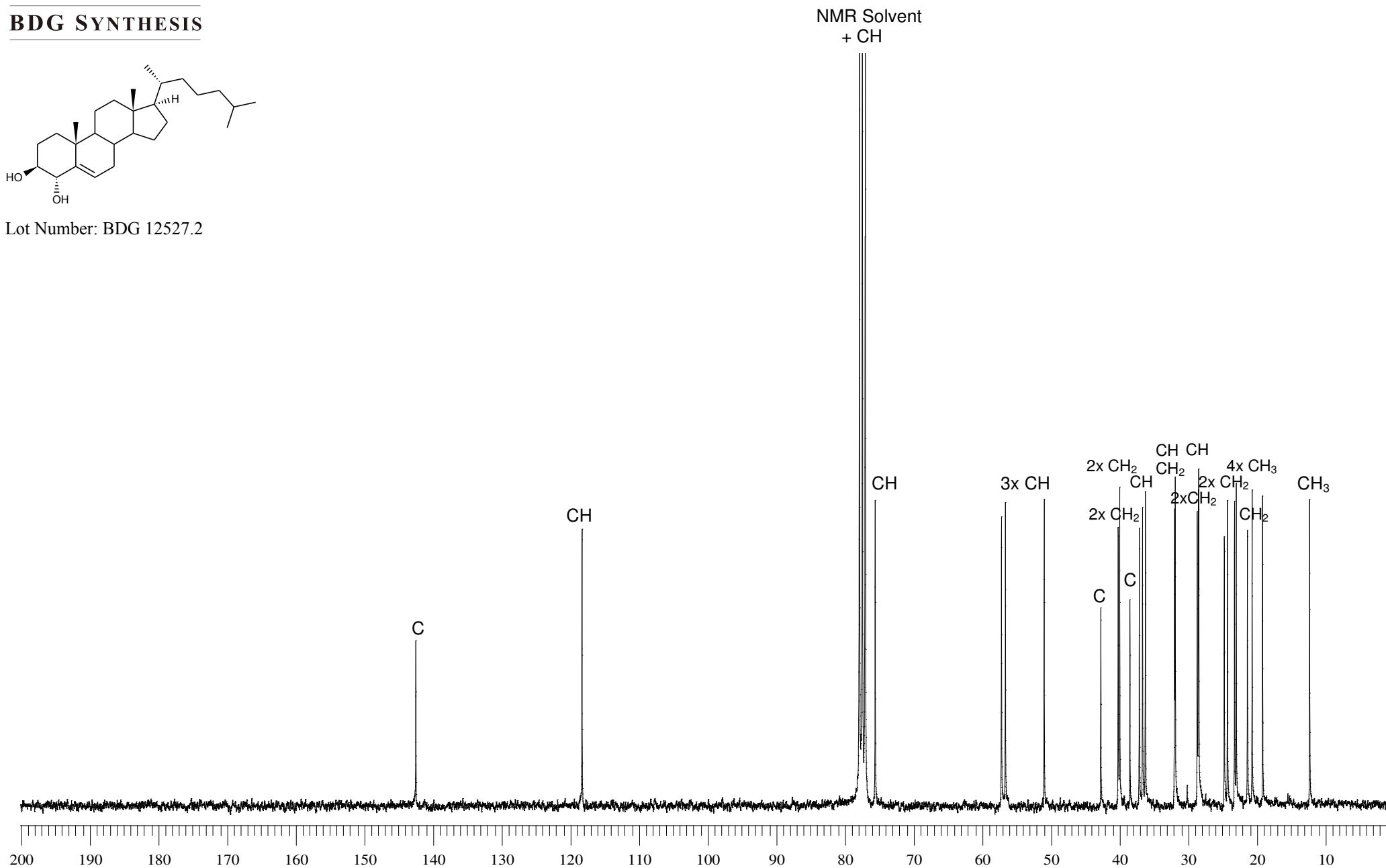


Carbon-13 NMR Spectrum of 4 α -Hydroxycholesterol in CDCl₃

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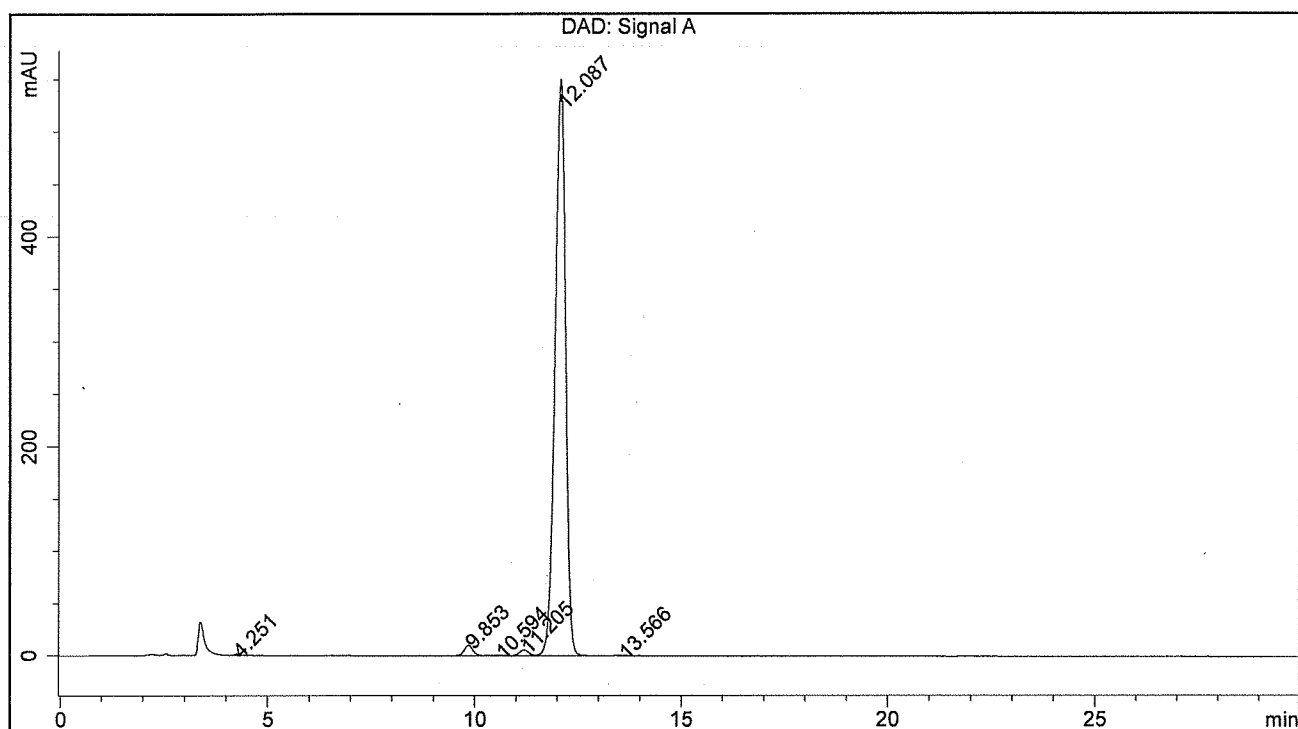
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BDG - Analysis of 4-alpha-Hydroxycholesterol

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
 Mobile Phase : 97 : 1.5 : 1.5 Methanol : Acetonitrile : Water
 Flow Rate : 1.0 mL/min Column Temperature : 20C
 Sample Solvent : Mobile Phase
 Injection Volume : 25 uL Detection : UV at 210 nm

Sample Name	BDG 12527.2	Instrument	AnalyticalLC01
Acquisition	14/06/2012, 10:17:47	Method (rev.)	LC10520e (5)
Sequence	BDG_14Jun2012a	Vial Position	3
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	4.25 min	1.2764	10.2872	0.1200 min	0.103 %
2	9.85 min	9.8451	138.9408	0.2186 min	1.398 %
3	10.59 min	0.6513	10.1191	0.2173 min	0.102 %
4	11.20 min	5.3781	84.2804	0.2448 min	0.848 %
5	12.09 min	549.8118	9685.9327	0.2735 min	97.447 %
6	13.57 min	0.5186	10.1504	0.2443 min	0.102 %