

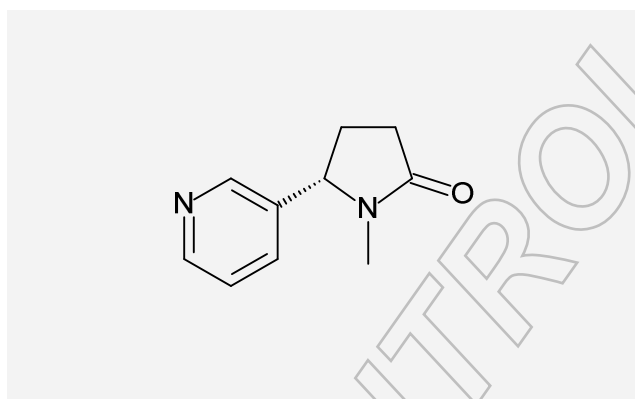
Certificate of Analysis

Reference Substance

Cotinine

Catalogue Number: LGCFOR0517.03
Lot Number: 47162
Molecular Formula: C₁₀H₁₂N₂O
Molecular Weight: 176.22
CAS Number: [486-56-6]

Long-term Storage: 2 to 8 °C, dark
Appearance: white solid
very hygroscopic
Assay 'as is': 98.4 %



Date of shipment: **2016-May-20**

This certificate is valid for two years from the date of shipment provided the substance is stored under the recommended conditions.

Release Date: 2014-02-13

LGC GmbH



Dr. Sabine Schröder
Product Release

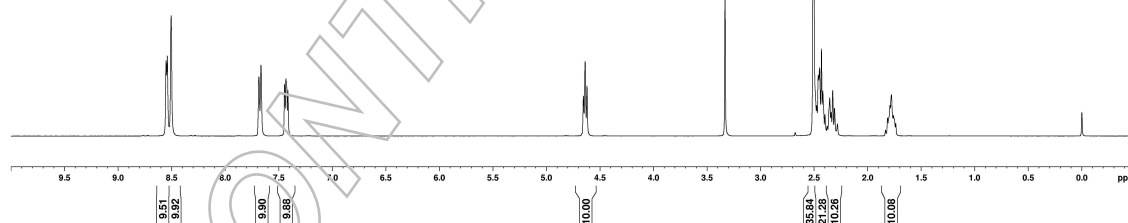
I. Identity

The identity of the reference substance was established by following analyses.

Ia. ¹H-NMR Spectrum

Conditions: 400 MHz, DMSO-d₆

The structure is confirmed with the signals of the spectrum and their interpretation.



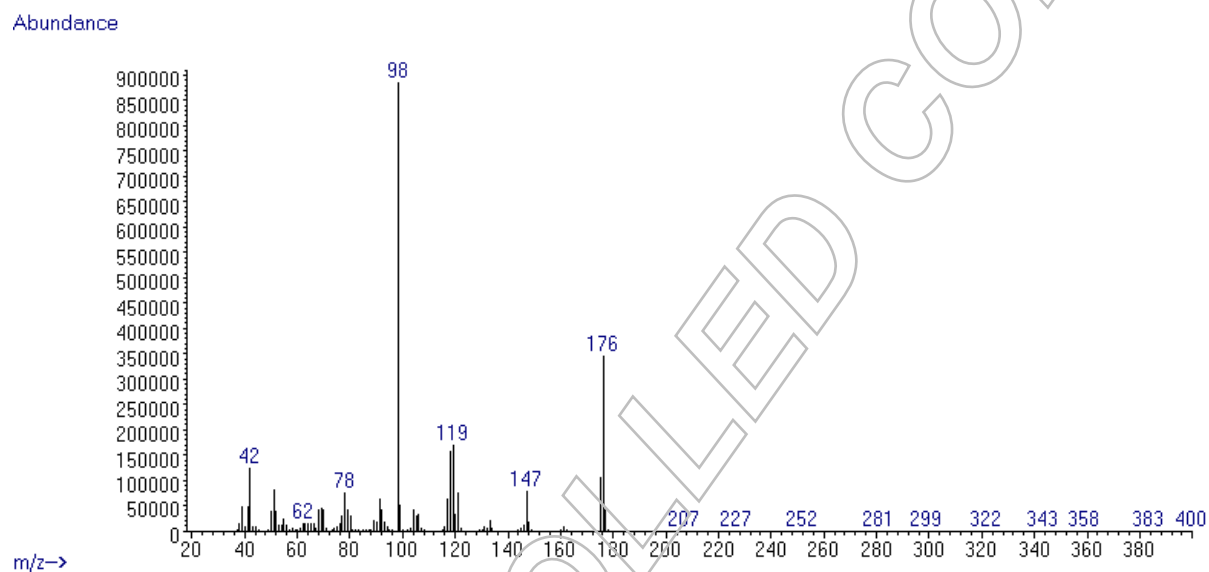
LGCFOR0517.03 Lot Number 47162

LGC GmbH, Im Biotechnologiepark, TGZ II, D-14943 Luckenwalde, Germany

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1b. Mass Spectrum

Method: EI, 70eV, detector temperature: 280 °C

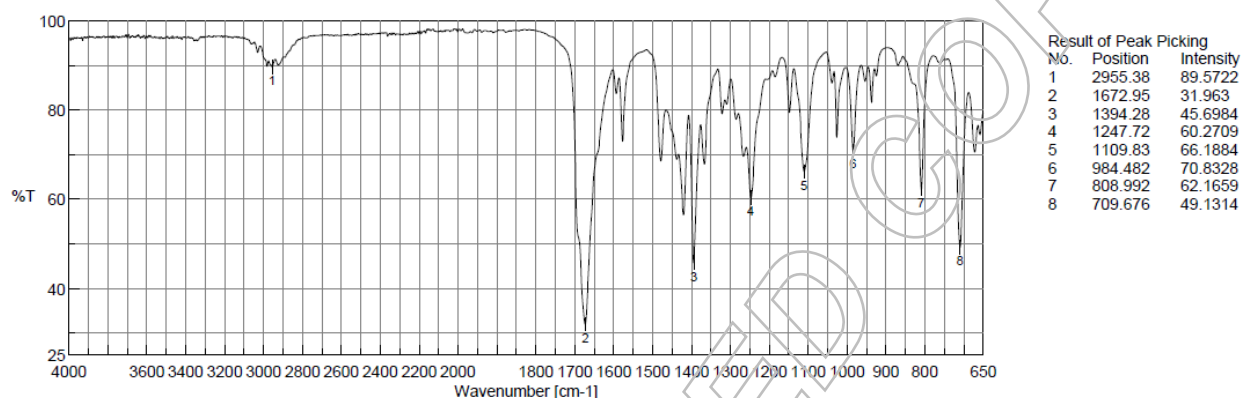


m/z	fragments
176	[M]
147	[M – NCH ₃]
119	[C ₈ H ₉ N]
98	[C ₅ H ₈ NO]

The signals of the mass spectrum and their interpretation are consistent with the structural formula.

Ic. IR Spectrum

Method: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy



The signals of the IR spectrum and their interpretation are consistent with the structural formula.

II. Purity

The purity of the reference substance was analysed by high performance liquid chromatography (HPLC).

HPLC Conditions:

Column:

BetaBasic-8
5 µm, 100 x 4.6 mm

Conditions:

1.0 ml/min, 40 °C

mob. Phase A: Water/Acetonitrile 95/5 (v/v)

mob. Phase B: Water/Acetonitrile 50/50 (v/v)

0 – 4 min	A/B	100/0
4 – 7 min	A/B	to 0/100
7 – 9 min	A/B	to 100/0
9 – 14 min	A/B	100/0 (v/v);

0.1 % H₃PO₄ +

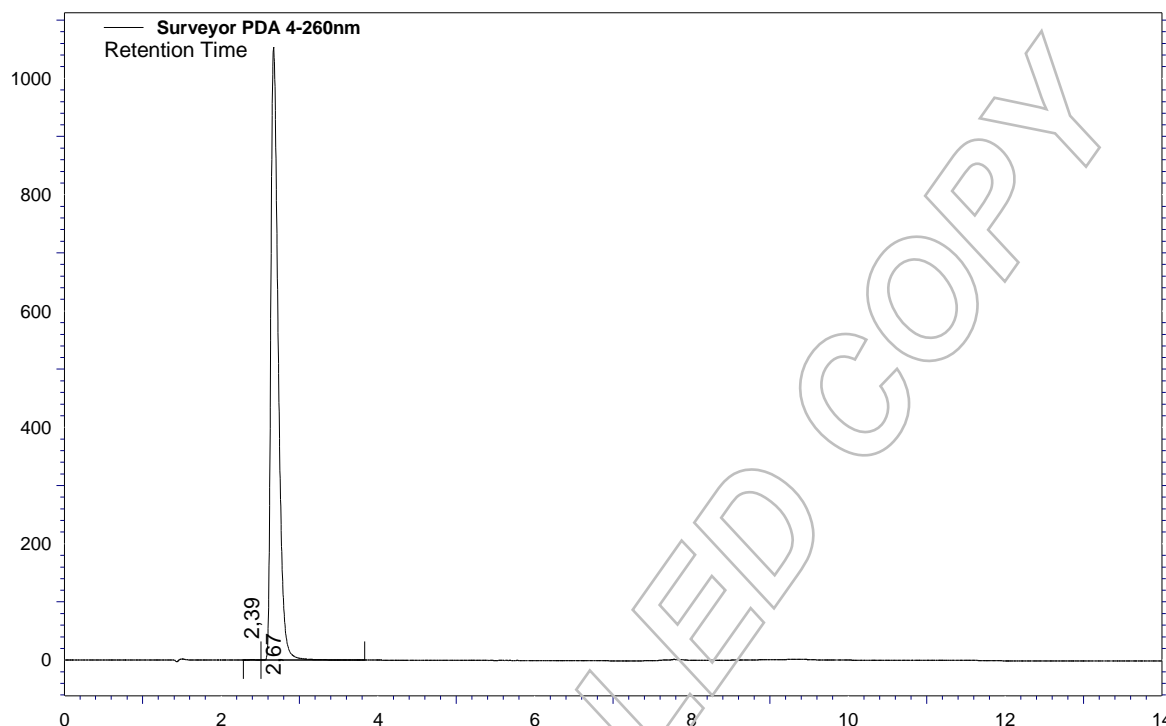
0.025 % Sodium hexanesulphonate

Detector:

DAD
260 nm

Injector:

Auto
6 µl; 0.1728 mg/ml in
Water/Acetonitrile
50/50 (v/v)



Area Percent Report - Sorted by Signal

Pk #	Retention Time	Area	Area %
1	2.39	4780	0.07
2	2.67	6902738	99.93
Totals		6907518	100.00

For the calculation the system peaks were ignored. The content of the analyte was determined as ratio of the peak area of the analyte and the cumulative areas of the purities, added up to 100 %.

Results:

Average 99.93 %
Number of results n=3
Standard deviation < 0.01 %

III. Water Content

Method: Karl Fischer titration

Results:

Average	1.57 %
Number of results	n=4
Standard deviation	0.74 %

IV. Residual Solvents

Method: ¹H-NMR

No significant amounts of residual solvents were detected (< 0.05 %).

V. Final Result

Total impurities (HPLC)	0.07 %
Water content	1.57 %
Residual solvents	n. d. (not detected)
Assay (100 % method) ¹	98.36 %

The assay is assessed to be 98.4 % 'as is'

The assay 'as is' is equivalent to the assay based on the not anhydrous and not dried substance respectively.

¹ The calculation of the 100 % method follows the formula:

$$\text{Assay (\%)} = (100 \% - \text{KF} - \text{RES}) \times \frac{\text{Purity HPLC (\%)}}{100 \%}$$

Water (KF) and Residual solvents (RES) are considered as absolute contributions, HPLC purity is considered as relative contribution.

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