



Certificate of Analysis

Product Name: Caffeine

Grade Working standard

Product Number: C0003 **Lot Number:** -

Brand:HamyLabCAS Number:58-08-2Formula: $C_8H_{10}N_4O_2$ Formula Weight:194.19 g/molLong term storage:2-30 °CQuality Release Date:00-???-2023

PubChem Preview ID: -

HO OH OH

Application Note: For peak identification. It is freely soluble in ethanol, DMSO or dimethyl formamide to approximately 25 mg/mL, and has a pKa = 3.57.

Test	Specification	Test Result
Appearance	prismatic crystals	prismatic crystals
Color	White	White
Solubility	Water	Water
Purity, (HPLC)	$\geq 99.2 \pm 0.4\%$	$\geq 99.2 \pm 0.4\%$
Identification (UV spectrum)	Conform	Conform
Identification (IR-spectroscopy)	Conform	Conform
Identification (1H-NMR-spectroscopy)	Conform	Conform
Peak purity, (HPLC)	Conform	Conform
Residual solvents, (headspace-GC)		
Water content, (micro determination, coulometric titration),		
Melting point		

The identity of the caffeine has been substantiated by at least two independent analytical methods: IR, NMR, UV and HPLC analysis. A mass balance approach, which takes chromatographic purity into account, as well as the contents of water, residual solvents, inorganic impurities, and the counter ion (if the reference standard is present as a salt) is applied in the calculation of the absolute purity as given in this COA

The absolute purity value (and not just the chromatographic purity result obtained by means of HPLC or GC) must be used in all quantitative calculations as the chromatographic techniques do not yet account for water, residual solvents and inorganic impurities.

Identity:

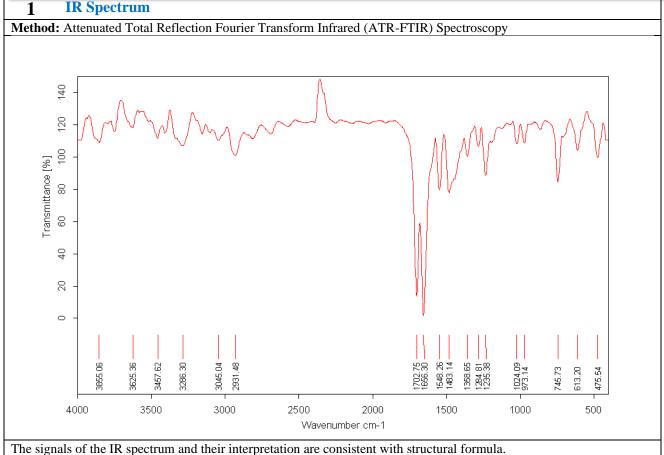
The identity of the reference substance was established by following analyses.

No. 219, Science and Technology Park, Incubator of Shahid Beheshti University, Shahid Beheshti University, Daneshjoo Square, Velenjak, Tehran, Iran.,

Website: www.hamylabs.ir Email: hamylabs@gmail.com & hamylabs@gmail.com







2 HPLC

Method: The purity of the reference substance was analysed by high performance liquid chromatography

(HPLC). Analytical HPLC analysis was carried out using reverse-phase chromatography under the

following conditions:

Column: Ultisil XB-C18, 5 µm, 4.6 mm X 250 mm, 1/pk

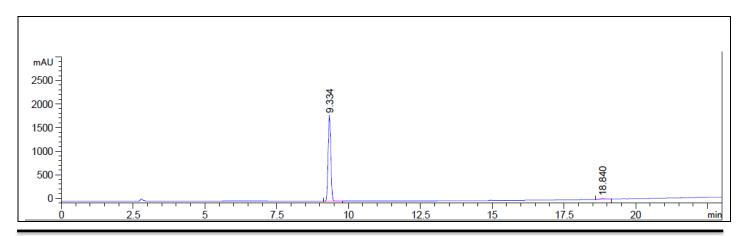
Detector: Photodiode-Array Detection (PDA)

Flow:

Injector: Manually

mob. Phase A: Buffer phosphate (Ph=2.7)

mob. Phase B: Methanol



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Area Percent Report - Sorted by Signal						
No	Retention Time	Area	Width min	Height mAU	Area mAU*min	Real area %
1	9.334	0.1007	333.72778	62.9402	BB	
2	18.842	0.2296	196.50201	37.0598	BV R	
Total:						100%
For the calculation the system peaks were ignored. The content of the analyte was determined as the ratio of the peak area of the analyte and the cumulative areas of the purities, added up to 100 %. Results:						
Average:						
Number of results:		n=6				
Standard deviation:		< 0.01 %				

3	¹ H-NMR Spectrum	
Conditions: 400 MHz, DMSO-d ₆		
The structure is confirmed by the signals of the spectrum and their interpretation.		

4 Water Conte	nt	
Method: Karl Fischer titration		
Results:		
Average	0.55 %	
Number of results	n=3	
Standard deviation	0.08 %	

5 Residual Solvents		
Residual Solvent	Average	Method
Dioxane	0.08 %	¹H-NMR

Final Result:

Chromatographic purity (HPLC) 99.42 %

Water content 0.55 %

Residual solvents 0.08 %

Assay (100 % method)¹ 98.79 %

The assay is assessed to be 98.8 % 'as is'

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

Maryam Yousefi /PhD/PhD, Supervisor Quality Assurance

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¹The calculation of the 100% method follows the formula: Assay (%)= (100%-volatile contents) * Purity (%)/100% Volatile contents are considered as absolute contributions, purity is considered as relative contribution

Disclaimer:

HamyLab warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Hamylabs.com. For further inquiries, please contact technical service (hamylabs@gmail.com). Purchaser must determine the suitability of the product for its particular use. HamyLab company makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by HamyLab company. We do not guarantee that the product can be used for a special application.