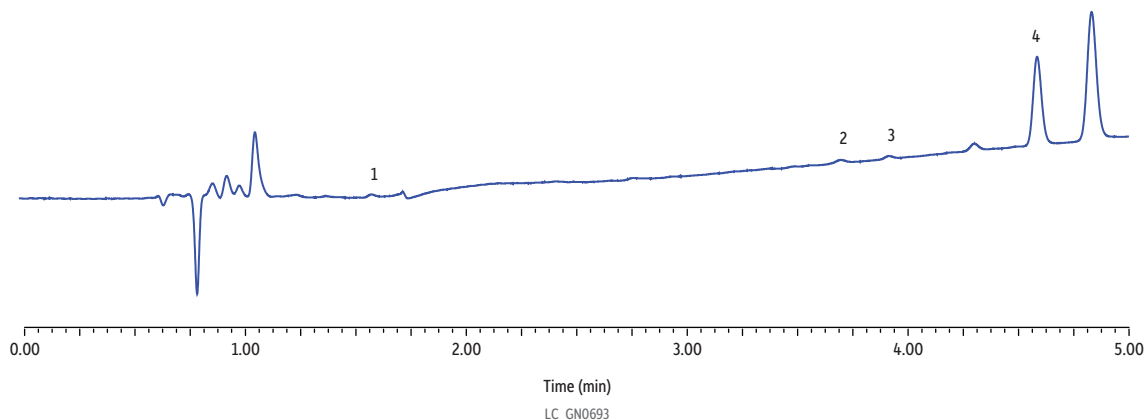


Analysis of Psychoactive Alkaloids in Infused Chocolate by HPLC-UV



Peaks	tr (min)
1. N,N-desmethyl Psilocybin (Norbaeocystin)	1.585
2. Psilocybin	3.358
3. 4-hydroxy-N-Methyltryptamine (Norpsilocin)	3.904
4. 4-hydroxy-N,N-Dimethyltryptamine (Psilocin)	4.583

Column Force Biphenyl (cat.# 962931E)
Dimensions: 100 mm x 3.0 mm ID
Particle Size: 3.0 µm
Pore Size: 100 Å
Guard Column: Force Biphenyl EXP Guard Cartridge 5.0 mm, 3.0 mm ID (cat.# 962950253)
Temp.: 50 °C

Standard/Sample

Diluent: Water
Inj. Vol.: 3 µL

Mobile Phase

A: Water, 10 mM ammonium formate, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.8	95	5
4.00	0.8	65	35
4.01	0.8	5	95
5.00	0.8	5	95
5.01	1.0	95	5
7.50	1.0	95	5

Detector UV/Vis @ 222 nm
Instrument Agilent 1100

Sample Preparation 1 g of freeze-dried, homogenized chocolate was weighed into a 50 mL Environmental Express tube (6.0 µm) followed by the addition of 2 mL of isopropanol and 1 min of vortexing. 18 mL of water with 1% acetic acid was added, tube capped, and vortexed for 5 min at 2500 RPM. The sample was then filtered with a plunger. An aliquot was obtained using a 3 mL syringe (cat.#22773) equipped with a 0.22 µm PTFE syringe filter (cat.# 23984). The sample was then diluted 5x with HPLC grade water into a 2.0 mL amber, short-cap vial (cat.# 21142) and capped with a 9 mm short cap (cat.#24497). The guard cartridge was equipped with an UltraShield precolumn filter, 0.22 µm (cat.# 25809).

Notes

System and column were passivated using LC Passivation Solution (cat.# 32475). The outlined method conditions were used to perform five injections of passivation solution (5 µL) on column, diverting to waste. Five warm-up injections were completed prior to analysis.

Storage Conditions: Flush column with acetonitrile, 0.1% formic acid for 5 min.

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