RESTEK Pure Chromatography

The HPLC-UV Analysis of Psilocin, Psilocybin and Others Found in Psychedelic Mushroom and Infused Products

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Abstract

Psilocin and psilocybin are tryptamine alkaloids found in some plants and fungi. Often found in certain species of mushrooms, these compounds are used for their psychotropic effects, but microdosing has shown promise for the treatment of anxiety, depression, and other mental health disorders. With recent decriminalization and legalization in certain areas, of both naturally derived and synthetic psychedelic compounds, labs are seeking to begin potency testing of psychedelic compound containing mushrooms and infused products. Providing accurate and reliable potency values is crucial for labs to ensure the safety of the consumer when receiving dosages. A set of efficient methods were developed for the testing of two psychedelic compound containing mushroom species. Sample preparation and extraction processes were established for infused chocolates, gummies, and extracts, as well as freshly ground mushrooms. Due to the robust nature, an HPLC-UV system was used to collect all data.

Sample Preparation

Whole Dried Mushroom	Infused Chocolates	Gummies	Extracts	Gummies (Amanita)
Homogenize dried mushroom, by using a grinder and pulsing until homogenized*	Homogenize chocolate, by using dry ice or liquid nitrogen and a mortar and pestle	Homogenize gummies, by using a mortar and pestle with dry ice or liquid nitrogen	Dilute with HPLC grade water for injection	Homogenize gummies by using a mortar and pestle with dry ice or liquid nitrogen
Weigh 500 mg ground mushroom into Environmental Express tube	Weigh 1.0 g of freeze-dried chocolate into Environmental Express tube	Weigh 1.0 g gummy into Environmental Express tube		Weigh 1.0 g gummy into Environmental Express Tube
Add 20 mL methanol, 0.5% acetic acid (v/v)	Add 2 mL of isopropanol and cap, vortex for 1 minute	Add 20 mL HPLC Grade Water, 0.5% acetic acid (v/v)		Add 20 mL of 50:50 Water: Methanol
Using shaker table, shake 5 min	Add 18 mL methanol, 0.5% acetic acid (v/v)	Using shaker table, shake 5 min @ 2500 RPM		Using shaker table, vortex 10 min
Insert plunger and pushdown to filter	Using shaker table, shake 5 min at 2500 RPM	Insert plunger and pushdown to filter		Insert plunger and push down to filter
Filter using a 3 mL syringe with 0.22µm filter into 4 mL vial	Insert plunger and pushdown to filter	Filter using a 3 mL syringe with 0.22µm filter into 4 mL vial		Aliquot into vial for injection
Dilute with HPLC Grade water for injection	Filter using a 3 mL syringe with 0.22µm filter into 4 mL vial	Dilute with HPLC Grade water for injection		
	Dilute with HPLC Grade water for injection			

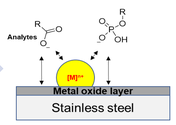
*Care should be taken to not over grind. Over grinding can cause dust and particulates as well as clogging of filters, frits or columns. If there are large stems remaining (typical) remove stems pour homogenized plant into appropriately sized container and add remaining stems back to grinder. Pulse until homogenized.

Figure I: Sample Preparation Techniques for Matrix

Considerations

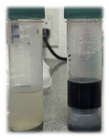
Non-specific Binding

- Any binding or adsorption that was NOT intended
- Molecules tend to adhere to exposed surface
- Sources
  - Hydrophilic/hydrophobic/Ionic interactions



Stability

- Temperature
- Photosensitivity
- Enzymatic reactions



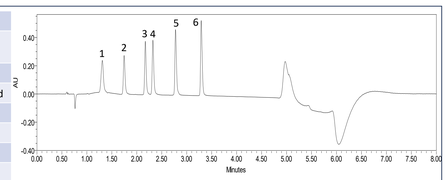
Other

- Filtration to prevent column and system clogging
- Column overload
- Column Storage

Methods

Column:	Force Biphenyl 100 x 3 mm, 3µm (cat.# 962931E)
Guard:	Force Biphenyl EXP Guard Column Cartridge (cat.# 962950253)
Pre-column Filter:	UtraShield UHPLC PreColumn Filter (cat.#25809)
Mobile Phase A:	Water, 10 mM ammonium formate, 0.1% formic acid
Mobile Phase B:	Methanol, 0.1% formic acid
Temperature:	50 °C
Injection Volume:	2 µL
Diluent:	Water
Flow Rate:	0.8 mL/min
Detector	UV-vis
Wavelength:	222 nm

Conditions: Time (min)	%B
0.00	5
4.00	35
4.01	95
5.00	95
5.01	5
8.00	5

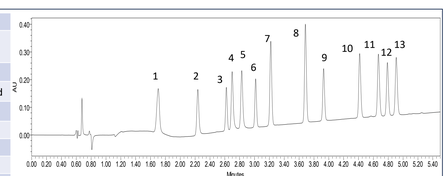


Peak #	Compound
1	Norbaeocystin
2	Baeocystin
3	Aeruginascin
4	Psilocybin
5	Norpsilocin
6	Psilocin

Figure III: Method, peak list, and chromatogram of 50 ppm standard for six endogenous analytes found in psychedelic mushrooms.

Column:	Force Biphenyl 100 x 3 mm, 3µm (cat# 962931E)
Guard:	Force Biphenyl EXP Guard Column Cartridge (cat.# 962950253)
Pre-column Filter:	UtraShield UHPLC PreColumn Filter (cat.#25809)
Mobile Phase A:	Water, 10 mM ammonium formate, 0.1% formic acid
Mobile Phase B:	Methanol, 0.1% formic acid
Temperature:	60 °C
Injection Volume:	1 µL
Diluent:	Water
Flow Rate:	0.8 mL/min
Detector:	UV/vis
Wavelength:	222 nm

Conditions: Time (min)	%B
0.00	0
5.00	45
5.01	95
6.00	95
6.01	0
9.00	0

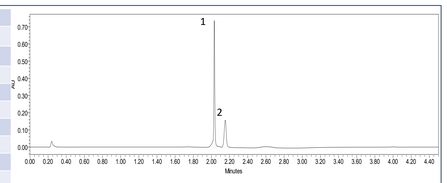


Peak #	Compound	Peak #	Compound
1	Norbaeocystin	8	Psilocin
2	Baeocystin	9	4-hydroxy-TMT
3	5-hydroxy-NMT	10	5-methoxy-NMT
4	Aeruginascin	11	5-methoxy-AMT
5	Psilocybin	12	Psilacetin
6	Bufotenine	13	N,N-DMT N-Oxide
7	Norpsilocin		

Figure IV: Method, peak list, and chromatogram of 50 ppm standard for expanded panel of 13 analytes.

Column:	Ultra IBD 30 x 3.0 mm, 3µm (cat.# 917533E)
Mobile Phase A:	Water, 0.05% H_3PO_4
Mobile Phase B:	Acetonitrile
Temperature:	40 °C
Injection Volume:	1 µL
Diluent:	50:50 MeOH: H_2O
Flow Rate:	0.8 mL/min
Detector:	UV/vis
Wavelength:	210 nm

Conditions Time (min)	%B
0.00	98
1.00	98
2.00	70
2.50	70
2.51	98
7.00	98



Peak #	Compound
1	Muscimol
2	Ibotenic Acid

Figure V: Method, peak list, and chromatogram of 100 ppm standards compounds endogenous to amanita muscaria.

Results

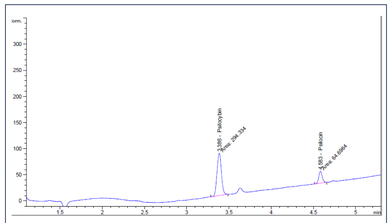


Figure VI: Chromatogram of ground mushroom matrix using method found in Figure III.

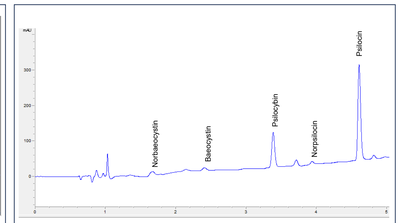


Figure VII: Chromatogram of extract matrix using method found in Figure III.

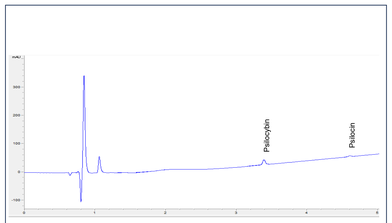


Figure VIII: Chromatogram of gummy matrix using method found in Figure III.

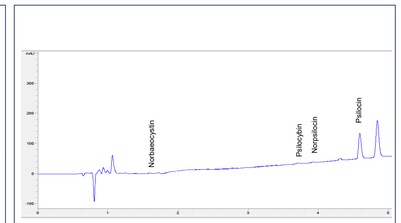


Figure IX: Chromatogram of chocolate matrix using method found in Figure III.

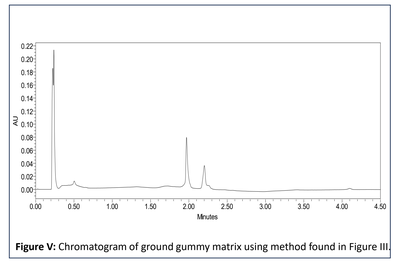


Figure V: Chromatogram of ground gummy matrix using method found in Figure III.

Conclusions

For psilocybin compounds, the introduction of matrix required minor adjustments to the methods method. This included on column passivation, and the addition of both a guard and pre-column filter to prevent clogging. Prior to analysis, on column passivation was utilized. Using method conditioned outlined in figure III, a total of five injections were completed using 5 µL of passivation solution (cat.#32475).

Four full work-flow solutions were developed for psilocybin mushrooms and infused matrix. One workflow was developed for Amanita mushroom infused gummies.

Future Work

- Optimize extraction for Amanita Muscaria matrix
- Replace on column passivation for chelating analytes with inert solution
- Continue to add analytes to the expanded panel

References

1. Kuypers KP, Ng L, Erritzoe D, et al. Microdosing psychedelics: More questions than answers? An overview and suggestions for future research. Journal of Psychopharmacology. 2019;33(9):1039-1057. doi:10.1177/0269881119857204
2. Lopez, D. A. & Bischof, J. Methods for the Passivation of HPLC Instruments and Columns. *LCGC ADVANCES IN LC COLUMN TECHNOLOGY* (2023). Available at: www.chromatographyonline.com.