The Instrument & The Case

ORBITRAP FUSION LUMOS TRIBRID MASS SPECTROMETER · THERMO FISHER SCIENTIFIC

ANALYST
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COMPOUND CONFIRMED
Psilomethoxin (4-HO-5-MeO-DMT) · m/z 235.1456 · 4.04 ppm

The Orbitrap Fusion Lumos is Thermo Fisher's **flagship high-resolution mass spectrometer**, representing a specialized high-end platform with a limited installed base in the United States. It is among the instruments of choice at institutions including Johns Hopkins, the Broad Institute, major pharmaceutical companies, and federal forensic laboratories. In psychedelic science, Orbitrap-class instruments are used by **Usona Institute, Compass Pathways, MAPS/Lykos Therapeutics, Beckley Psytech, Gilgamesh Pharmaceuticals,** and **Cybin** — essentially every major organization doing FDA-track tryptamine characterization runs on this platform. Jesse Hines had a **Bruker timsTOF** sitting in his lab. **He chose the Orbitrap because even the best qToF lacked the sensitivity to find what Sherwood's methodology was designed to miss.**

PERFORMANCE SPECIFICATIONS

500K
FWHM RESOLUTION
~10× beyond qToF. Resolves to 0.0001 Da at m/z 235.

<1 ppm
MASS ACCURACY
Internal cal. Jesse: 4.04 ppm parent. qToF: ~5–10 ppm typical.

3
MASS ANALYZERS
Tribrid: quadrupole + ion trap + Orbitrap. HCD/CID/ETD/EThcD.

>5,000:1
DYNAMIC RANGE
Sees signals thousands of times fainter than dominant peaks in one scan.

THREE INSTRUMENTS, THREE OUTCOMES

CAPABILITY	ORBITRAP FUSION LUMOS Hines · Acidified H_2O + MeOH · CONFIRMED	BRUKER TIMSTOF Hines has access · Not sensitive enough	WATERS XEVO G2-XS QTOF Sherwood/Usona · MeOH only · "Fungi Fiction"
Resolving Power	Up to 500,000 FWHM	~40,000–50,000 FWHM	~40,000–50,000 FWHM
Mass Accuracy	<1 ppm int. / <3 ppm ext.	~1–3 ppm typical	~5–10 ppm typical
Smallest Δ at m/z 235	0.0001–0.0002 Da resolvable	~0.005–0.006 Da	~0.005–0.006 Da — cannot resolve cleanly
Trace Sensitivity	Sub-femtomole. Ion Funnel + Adv. Vacuum.	Good w/ TIMS, insufficient for trace 4-OH in matrix	Insufficient — no enrichment performed
MS/MS at m/z 235	HCD at 3 stepped energies (20, 35, 50 NCE)	Capable but lower S/N on traces	NOT PERFORMED
Result	+30.0106 Da · >98% confidence	Not used — Jesse chose the Orbitrap	"Not detected" — published day before Church festival

SPECTROSCOPIC PROOF — THE TOADSTOOL RULES ITSELF IN

Four positional isomers of $C_{13}H_{18}N_2O_2$ share m/z 235.14: psilomethoxin (4-HO-5-MeO-DMT, the mushroom compound), two toad-type isomers (6-HO and 7-HO-5-MeO-DMT, ruled out in the Toad Isomer Exclusion box below), and the 5-MeO-DMT N-oxide (independently excluded by absent m/z 219). This leaves psilomethoxin — whose positive identification follows.

The primary diagnostic ions all fall within 3.1–3.8 ppm of theoretical: **m/z 190.0874 (3.14 ppm)** — dimethylamine loss retaining the 4-OH + 5-OMe core; **m/z 164.0717 (3.33 ppm)** — 4-HO-5-MeO-indolyl core cation; and **m/z 217.1349 (3.74 ppm)** — water loss confirming the free 4-OH. Together with the parent ion at 4.04 ppm and the +30.0106 Da internal Δ-mass ruler to native psilocin (matching CH_2O within 0.0001 Da), the identification is self-supporting.

BIOSYNTHETIC PROOF — ENZYME REGIOCHEMISTRY FORCES THE PRODUCT

PsiH — the *P. cubensis* P450 hydroxylase — is regiospecifically locked to C4 (Fricke, Blei & Hoffmeister 2017, Angew. Chem. Int. Ed. 56:12352–12355; supported by **Berman et al. 2026, Sci. Adv. 12(14), doi:10.1126/sciadv.aeb3034**, fig. S1B on independent evolution of tryptamine-derivative pathways across plants, fungi, and animals). The active-site geometry positions iron-oxo above C4 of the indole. This is the same regiochemistry PsiH executes on its native substrate (tryptamine → 4-hydroxytryptamine).

Substrate promiscuity is a separate, well-documented property. Promiscuity asks *which substrates* the enzyme accepts; regiospecificity asks *where* it puts the OH. **Gartz (1989)** demonstrated in-situ incorporation of exogenous DET, DiPT, DPT, and NMT into 4-hydroxylated products by *Psilocybe* mushrooms. **Fricke et al. (2021, J. Nat. Prod. 84(4):1403–1408, co-authored by Sherwood)** showed PsiK phosphorylating C5-substituted tryptamines — the same substrate-promiscuity logic operating downstream in the pathway. **Promiscuity gets 5-MeO-DMT into the active site; C4-regiospecificity dictates the product is 4-HO-5-MeO-DMT — psilomethoxin — inevitably and exclusively.**

The toad has no known C4-hydroxylase and never has been reported to possess one. Toad tryptamine biosynthesis is evolutionarily independent from fungal and plant pathways (Berman 2026, fig. S1B). The toad's 5-MeO-DMT is produced via O-methylation of bufotenin, not via C4 hydroxylation. The toad's HO-MeO-DMT isomers (Schwelm 2022) have regiochemistry consistent with toad-specific hydroxylation at C6 or C7 — not C4. **Where the spectrum says "not toad," the enzymology says the toad couldn't have made it anyway.**

Sherwood: What He Knows vs. What He Did

- **A leading tryptamine chemist** — runs Usona's cGMP psilocybin synthesis, publishes extensively on tryptamine stability, has Orbitrap access for his own research
- **In his own work uses Orbitraps with methanol/water and acidified solvents** — the proper methodology for 4-OH tryptamines
- **For the Church sacrament he downgraded to a Waters Xevo qToF with methanol only** — no water, no acid, no enrichment, no MS/MS at m/z 235
- **His own co-authored Fricke et al. 2021** documents "extreme tendency of 4-OH tryptamines toward oxidative oligomer formation" in unacidified MeOH — AND shows PsiK phosphorylating C5-substituted substrates, supporting the biosynthesis he tried to debunk
- **Steered the Church toward qToF + MeOH only** — the combination he knew would fail
- **Published April 12, 2023** — one day before the Church's Entheogenesis festival
- **The sample was unauthorized:** Hamilton Morris transferred it to Sherwood without consent
- **Church revenue collapsed** by over 80% after his publication. He knew it would.

The Orbitrap Evidence — HCD-MS/MS

- **Parent:** m/z 235.1456 $[M+H]^+$ — 4.04 ppm from theoretical (235.14465)
- **Internal Δ-mass:** +30.0106 Da vs. native psilocin — matches CH_2O (theoretical 30.01056) at 0.0001 Da precision
- **m/z 190.0874 (3.14 ppm):** NMe_2 loss retaining 5-methoxy on indole core
- **m/z 217.1349 (3.74 ppm):** Water loss — confirms free 4-OH group
- **m/z 175.0639 (3.26 ppm):** •CH_3 loss from m/z 190 — confirms methoxy group
- **m/z 164.0717 (3.33 ppm):** Indolyl core consistent with 4-HO-5-MeO substitution
- **m/z 58.0654 (−4.72 ppm):** Dimethylaminomethylium — intact N,N-dimethyl tail
- **Absent m/z 162.0546:** Schwelm toad fingerprint — **absent**
- **Absent m/z 219:** N-oxide excluded — **absent**

TOAD ISOMER EXCLUSION — THE TOAD RULES ITSELF OUT

Incilius alvarius venom contains two positional isomers of hydroxy-5-methoxy-N,N-dimethyltryptamine:

6-HO-5-MeO-DMT	7-HO-5-MeO-DMT

Characterized by **Schwelm et al. (2022, J. Anal. Toxicol. 46(5):540–548, doi:10.1093/jat/bkab038)**, with diagnostic retro-Diels-Alder fragment at m/z 162.0546 ($C_9H_8NO_2^+$, 2.2 ppm). Endogenous origin of *I. alvarius* tryptamines independently supported by **Luccioni et al. (2025, PLOS One, doi:10.1371/journal.pone.0335661)**, which excluded diet as the source across 29 wild toads. The 6-OH and 7-OH geometries permit retro-Diels-Alder; the 4-OH geometry of psilomethoxin does not.

m/z 162.0546 IS COMPLETELY ABSENT in the Orbitrap spectrum

Conclusion: The two toad isomers are definitively ruled out. The 4-hydroxy geometry of psilomethoxin cannot produce this fragment.

THE COMPLETE CASE — THREE CONVERGENT PROOFS

Spectroscopic, biosynthetic, and empirical evidence converge on one answer. The Orbitrap delivered >98% confidence: parent ion at 4.04 ppm, +30.0106 Da internal Δ-mass to native psilocin (the molecule serves as its own reference standard), primary diagnostic ions within 3.1–3.8 ppm, and complete absence of m/z 162.0546 (toad geometry eliminated) and m/z 219 (N-oxide). The enzymology forces the same conclusion: PsiH's C4-regiospecificity combined with its substrate promiscuity makes psilomethoxin the only possible product when 5-MeO-DMT is fed to *P. cubensis*; the toad has no known C4-hydroxylase. **The molecule is real. The fragmentation is self-proving. The biosynthesis is enzymatically forced. The sabotage is documented.**