ISSUE 2

Cayman NPS Metabolism Monograph

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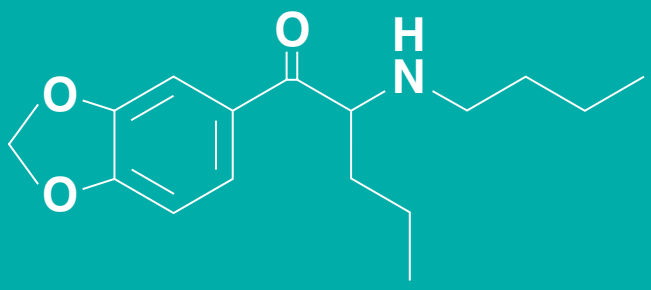
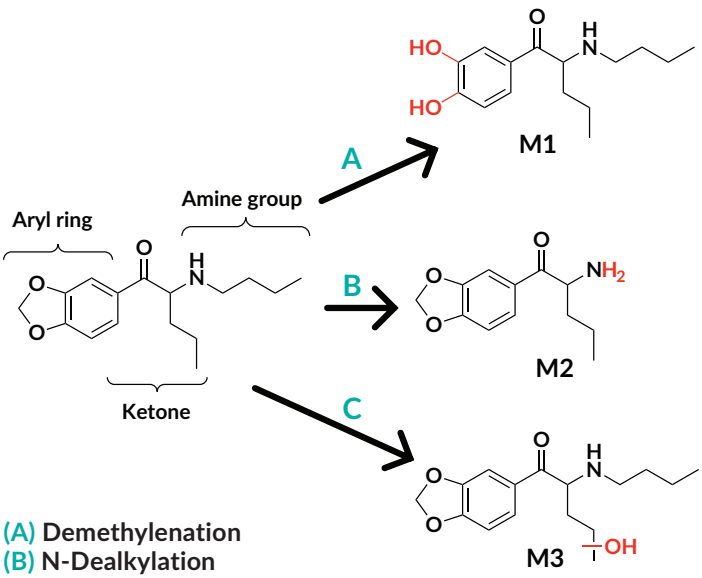
N-butyl Pentylone

Item No. 26701

Synthetic Cathinone



N-butyl Pentylone is a synthetic cathinone first reported in 1960s German patent literature, emerging on the recreational drug market in late 2018.[1] Cathinone analogs have seen widespread abuse since the mid-2000s and are derived from the alkaloid cathinone found in khat.[2] N-butyl Pentylone has been identified on illicit research chemical websites and in seized drug samples. While several similar cathinone analogs are Schedule I controlled substances, N-butyl pentylone remains uncontrolled as of mid-2020. The goal of this monograph is to identify its probable phase I metabolites using HLM assays. This study can provide insights into the metabolic pathway of N-butyl pentylone, and by analysis with high-resolution Orbitrap mass spectrometry, we aim to provide forensic toxicologists with the key ions/fragments that can help identify this novel synthetic cathinone in biological samples.



Observed phase I metabolites of N-butyl pentylone detected using high mass accuracy fragments generated by LC-MS/MS.

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Mass Spectrum and Fragments of N-butyl Pentylone

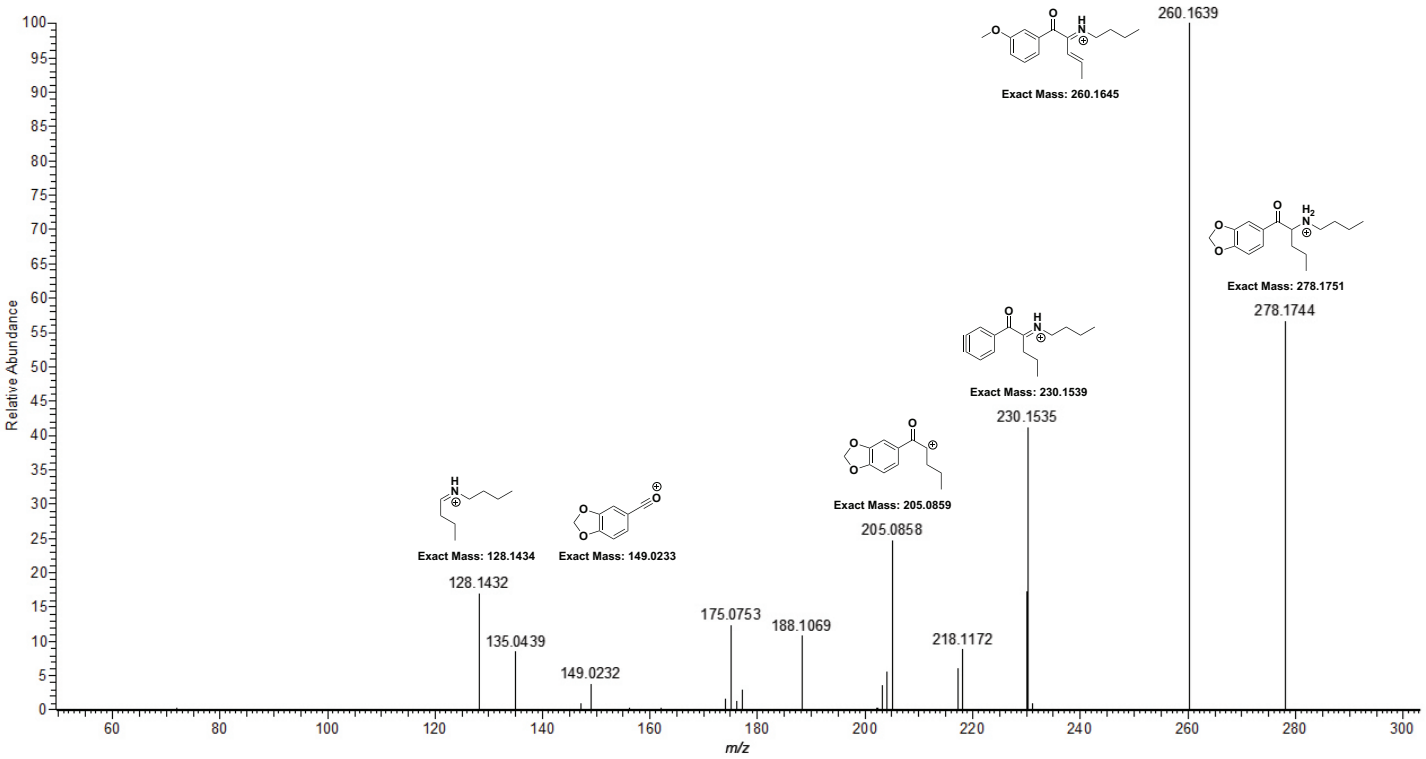

Figure 1. High-resolution mass spectrum of the parent compound N-butyl pentylone with proposed fragment structures.

N-butyl Pentylone Metabolite Formation and Detection

The parent compound N-butyl pentylone was incubated with human liver microsomes (HLMs) to form phase I metabolites. An aliquot from the incubation was quenched with acetonitrile, and the mixture was centrifuged at 13,200 rpm for ten minutes. The obtained supernatant was injected onto a Dionex Ultra-High Performance Liquid Chromatography (UHPLC) system for metabolite separation. The eluted metabolites were detected using a high-resolution Orbitrap mass spectrometer (Thermo Scientific). For comparison, a control sample was prepared by incubating N-butyl pentylone with deactivated HLMs. The total ion chromatogram (TIC) along with extracted ion chromatograms (EICs) of the incubation compared with those of the control were used to identify metabolites formed from the HLM assay with N-butyl pentylone. The TIC shows potential metabolites at 4.75 (**M1**), 4.80 (**M2**), and 5.09 (**M3**) min retention times (RT), as well as the parent, N-butyl pentylone, at 5.70 min. The EICs show the three main phase I metabolites by their exact masses, formed from our HLM incubation. All three metabolites gave masses consistent with the known ways 3,4-methylenedioxycathinones are metabolized: demethylenation, N-dealkylation, and oxidation (hydroxylation).[3] In our study, we did not observe the possible hydrogenation metabolite that results from reduction of the β-ketone to the corresponding alcohol that can occur with some cathinones. This result agrees with the literature on the metabolism of 3,4-methylenedioxy-substituted cathinones.[3] Interestingly, we observed two peaks at 5.38 min and 5.70 min in the metabolite incubation that correspond with the MS/MS spectrum of the parent compound. However, we only observed a single peak of the parent compound at 5.72 min in the control sample. The earlier 5.38 min peak displays no evidence of a mass shift, indicating that it results from a change in the parent N-butyl pentylone because of incubation and not as the result of metabolism.

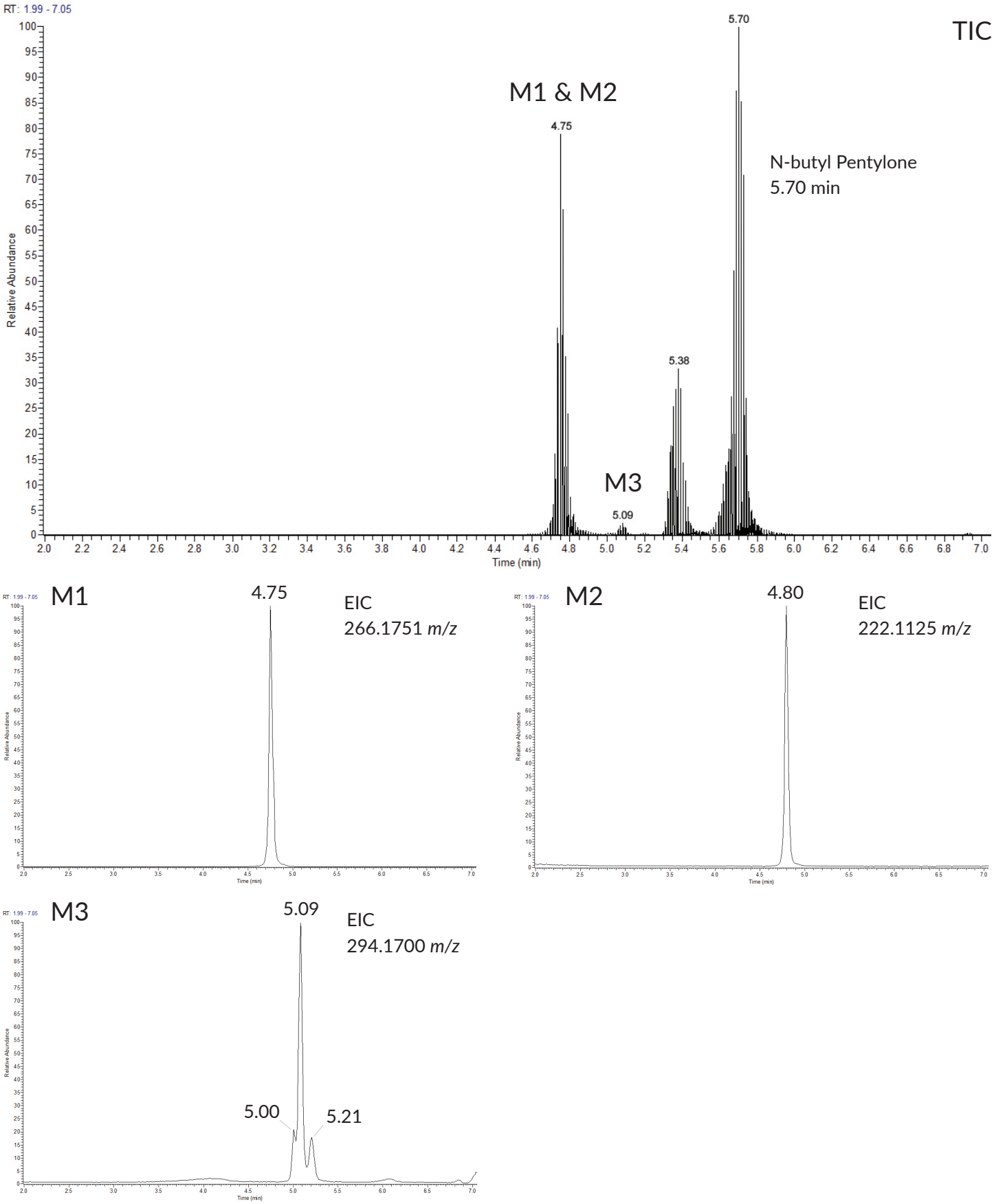

Figure 2. TIC (top) and EICs (bottom) of N-butyl pentylone post-incubation. EICs and corresponding exact masses for metabolites M1, M2, and M3 represent demethylenation, N-dealkylation, and hydroxylation, respectively.

Metabolite 1 (M1)

The first eluting metabolite (4.75 min) yielded the MS/MS spectrum below with proposed major fragments. This demethylenation metabolite is consistent with literature metabolism data on structurally similar analog N-ethyl pentylone, which includes authentic human biological samples.[4] Two key ions generated from fragmentation of this metabolite are 266.1743 *m/z* and 193.0857 *m/z*, both of which show the loss of the methylenedioxy methylene (CH_2) group.

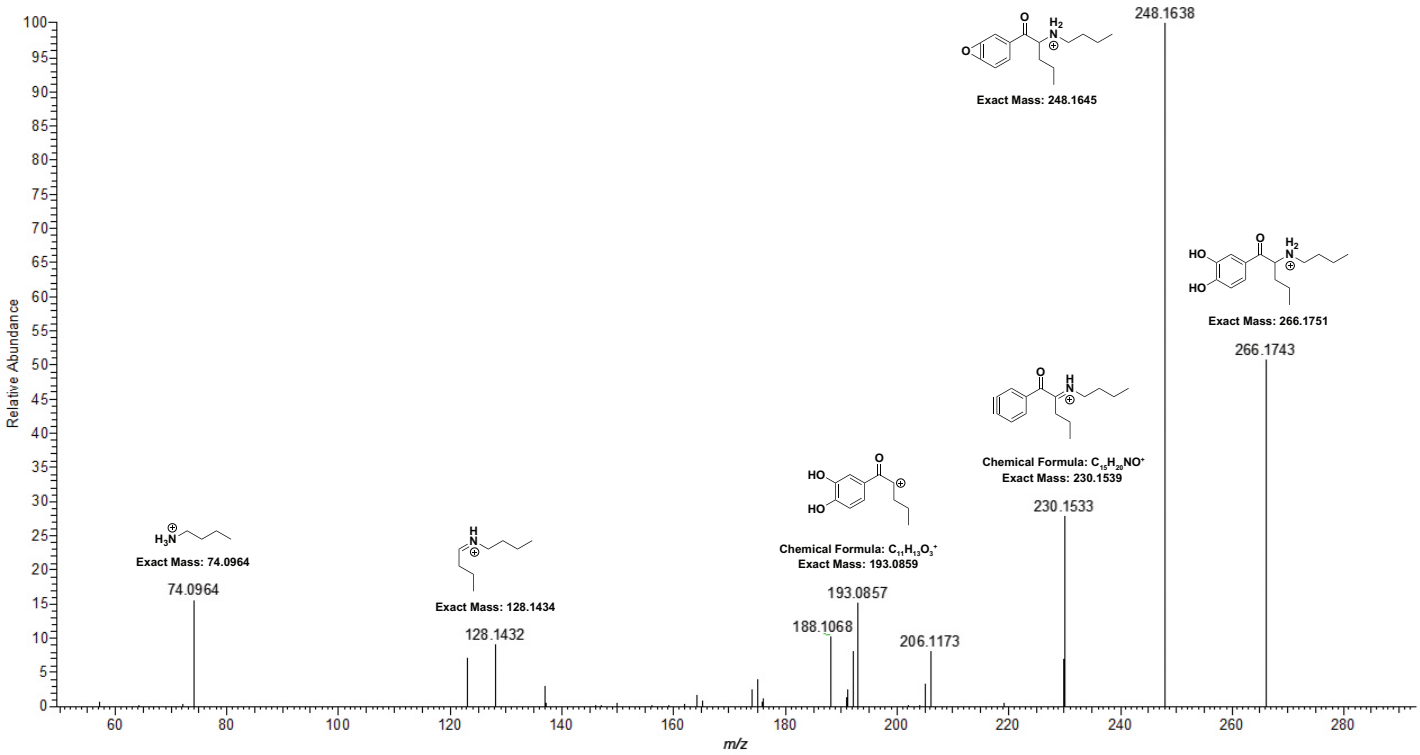

Figure 3. High-resolution MS/MS spectrum of demethylenation metabolite M1 and proposed fragment structures.

Metabolite 2 (M2)

The second eluting metabolite had a retention time of 4.80 min and yielded the MS/MS spectrum and fragments below. The key fragments that indicate N-dealkylation include 222.1120 *m/z*, 204.1016 *m/z*, and 174.0912 *m/z*. These fragments have proposed structures based on the fragmentation of similar cathinones.

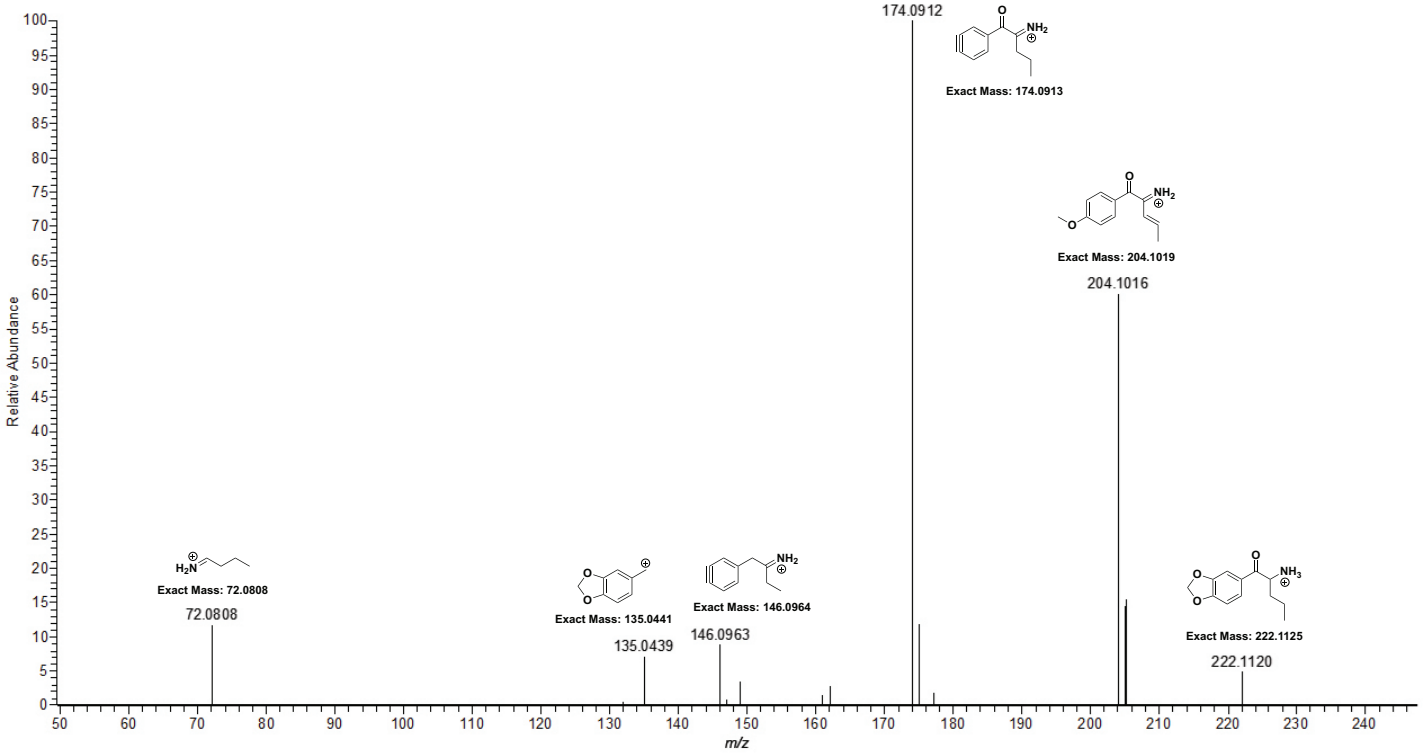

Figure 4. High-resolution MS/MS spectrum of N-dealkylation metabolite M2 and proposed fragment structures.

Metabolite 3 (M3)

The latest eluting metabolites had retention times of 5.00 min/5.09 min/5.21 min and yielded the averaged mass spectrum and proposed fragments below. This spectrum represents the various possible mono-hydroxylation metabolites. All three metabolites yielded the two key fragments, with 294.1693 *m/z* and 276.1587 *m/z* representing the hydroxylation product and elimination fragment. However, each peak showed different abundances of the ions 258.1482 *m/z*, 234.1119 *m/z*, and 216.1014 *m/z*, indicating possible differences in the position of hydroxylation. As reported in the literature, the hydroxylation of 3,4-methylenedioxy-substituted cathinones occurs predominately on the ketone acyl chain and not on the N-alkyl group.[5]

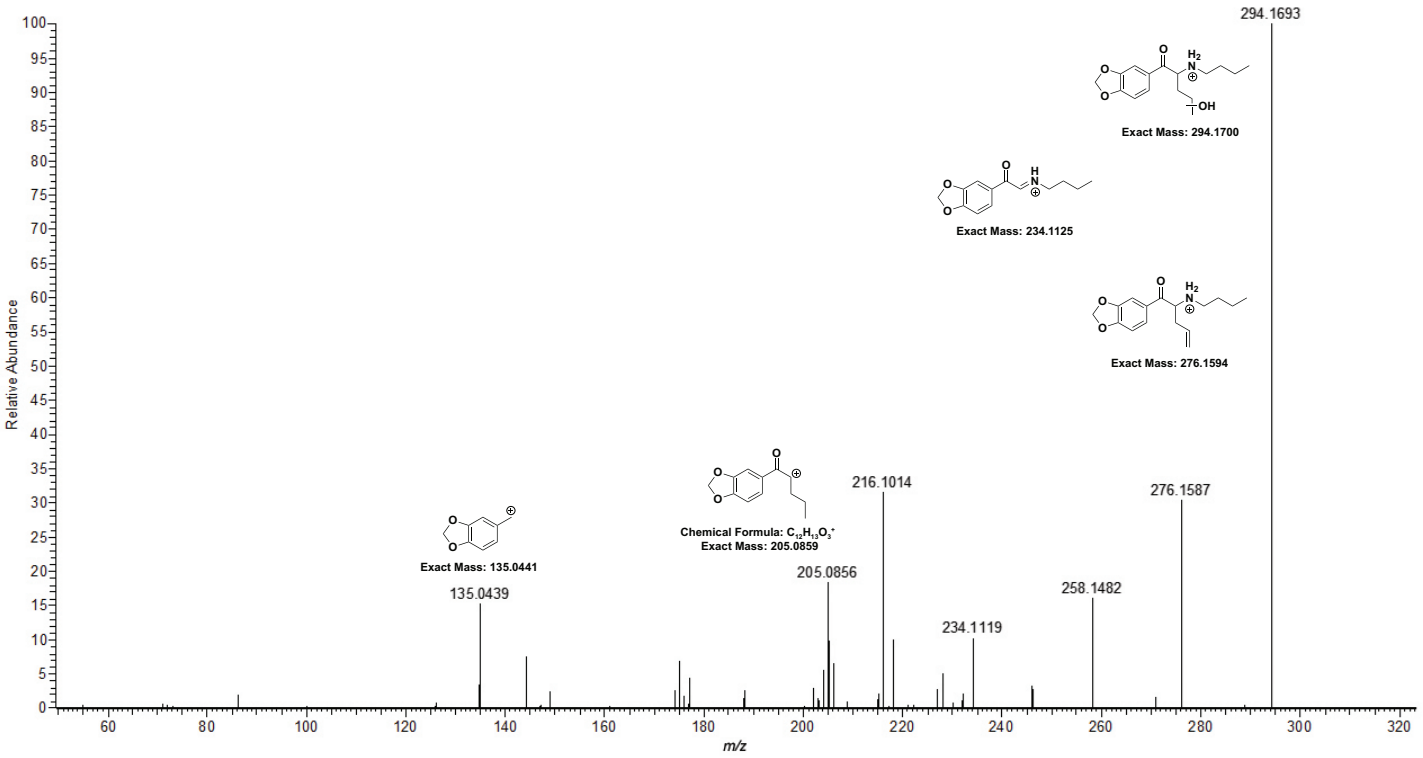

Figure 5. High-resolution MS/MS spectrum of hydroxylation metabolites M3 and proposed fragment structures.

Conclusion

Incubation of N-butyl pentylone with HLMs resulted in three different phase I metabolites (**M1**, **M2**, **M3**). Based on MS/MS fragmentation data, we can conclude that the first of the observed metabolites is the result of demethylenation of the 3,4-methylenedioxy moiety. The second observed metabolite occurs as the result of N-dealkylation of the butylamine group. The third set of metabolites arises from hydroxylation at possible sites along the pentanone chain. These metabolites can all serve as biomarkers for cathinone abuse, and our results indicate that the N-dealkylation and demethylenation metabolites are the most abundant in our HLM study. The parent compound is also a valuable indicator in the case of 3,4-methylenedioxy-substituted cathinones because of their greater stability.[6]

Methodology and Instrumentation

In vitro metabolism studies

The compound N-butyl pentylone was diluted to the necessary concentration by preparing a 50 mM stock DMSO solution and further diluting with phosphate buffer (pH 7.4). The compound solution was then incubated for two hours at 37°C with HLMs and the added cofactor NADPH. At two hours, the reaction was quenched with acetonitrile and spun down on a centrifuge. An aliquot of the mixture was injected onto the UHPLC. The eluates were detected using an Orbitrap mass spectrometer.

- Microsomes: 50-pool mixed-gender microsomes, 20 mg/ml protein conc. (Sekisui XenoTech, H0610)
- Analyte: N-butyl pentylone (hydrochloride) (Cayman Chemical, Item No. 26701)
- Incubation time of two hours
- Sample prepared in pH 7.4 phosphate buffer, 50 mM stock DMSO solution
- Instrumentation and analysis LC-MS/MS:
  - Dionex™ UltiMate™ 3000 UHPLC (Thermo Scientific™)
  - Acquity UPLC® BEH C8 column, 1.7 µm, length 2.1 x 100 mm (Waters™)
  - Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Scientific™)
  - Xcalibur™ software v. 4.0 (Thermo Scientific™)

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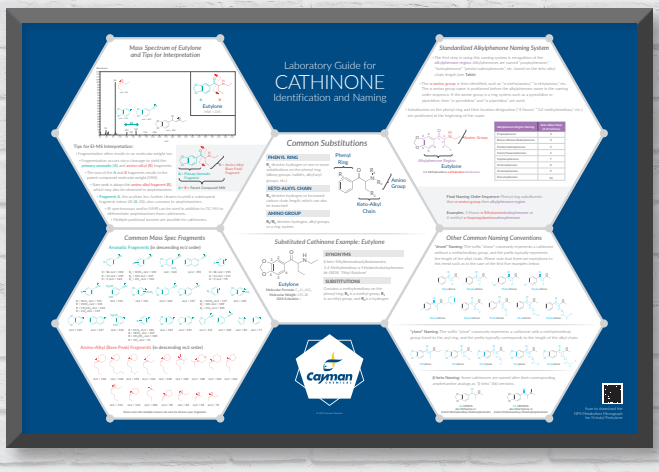
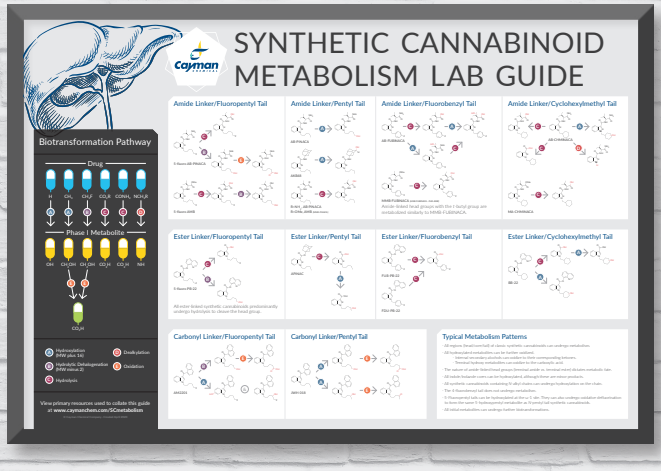
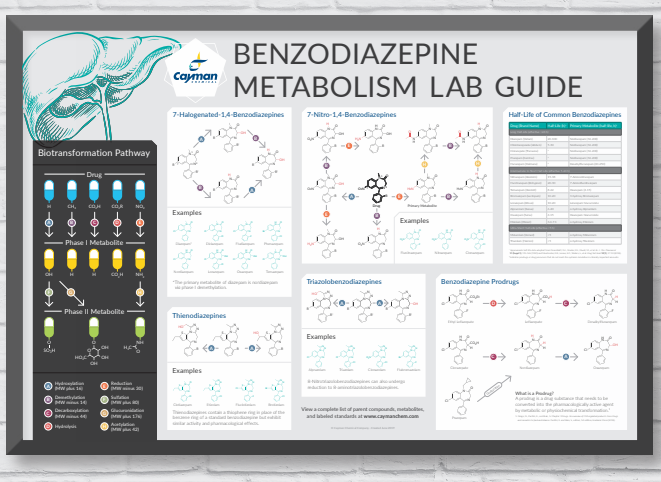


Cathinone Identification and Naming Lab Guide



Synthetic Cannabinoid Metabolism Lab Guide



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