Supplementary information

Fatal intoxication involving amphetamine, clonazafone, a benzodiazepine prodrug, and fluoro-etonitazene, a new synthetic opioid

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1. Isolation of fluoro-etonitazene

Due to the lack of commercially available reference material, fluoro-etonitazene was extracted and purified from a vaping liquid. The vaping liquid was purified using an Agilent 1260 Infinity II HPLC preparative system equipped with an XBridge® Prep Shield RP 18.5 µm OBDTM 19 x 150 mm column. The flow rate was 17.1 mL/min, and detection wavelengths 210 and 254 nm. The purity of the dried eluate was evaluated using an Agilent 1260 Infinity II HPLC-DAD system equipped with an XBridge® Shield RP18 5µm XB-C18 100 Å 150 x 4.6 mm column. The flow rate was 1.00 mL/min. Eluent A was 0.05% TFA, and eluent B was acetonitrile containing 0.05% TFA. The gradient profile was as follows: Starting at 10% eluent B, at 4 min, the concentration of B was increased linearly to 100% B over 15 min. Next, eluent B was reduced to 90% over 0.5 min, followed by another decrease to 10% B. The chromatogram can be found in the supplementary information (SI) in *Figure S1*.

2. Supplementary Figures

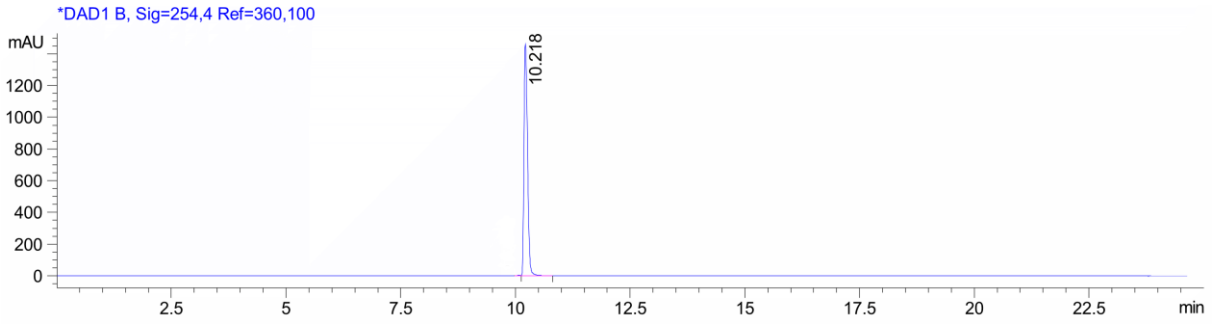

Figure S1: Chromatogram of preparative chromatography for the purification of fluoro-etonitazene (retention time 10.2) using high-performance liquid chromatography with diode-array detection (HPLC-DAD) at a wavelength of 254

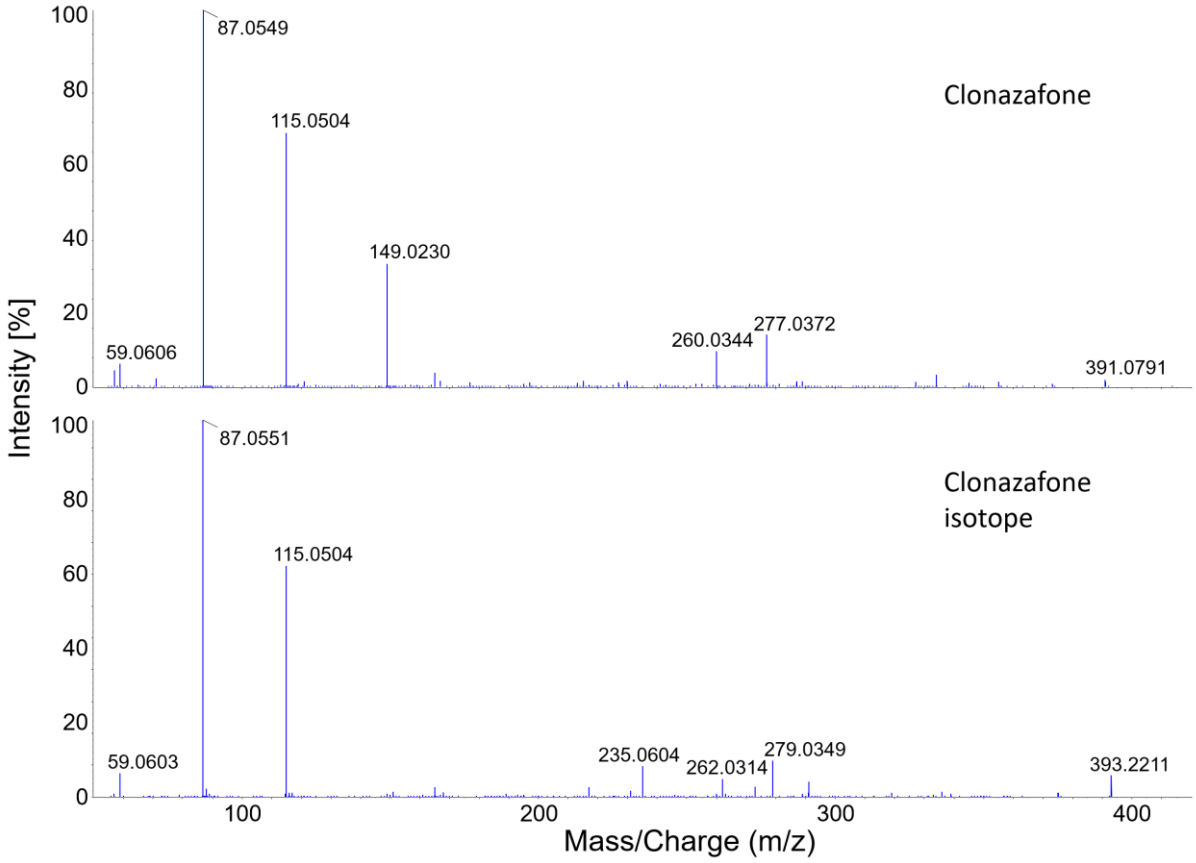

Figure S2: Spectra of clonazafone and its isotope included in the typical chlorine cluster - therefore, it can be concluded that the fragments m/z 260, 277, and 391 contain chlorine

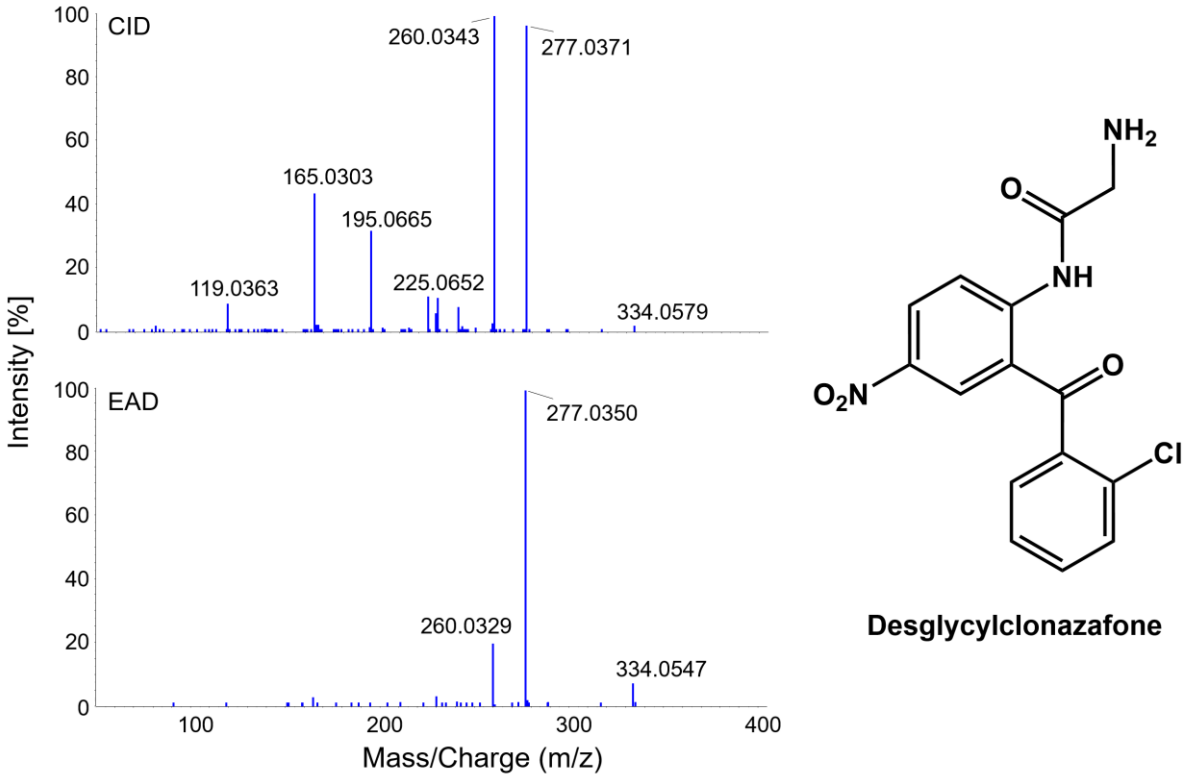

Figure S3: Spectra of desglycylclonazafone (C1) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)

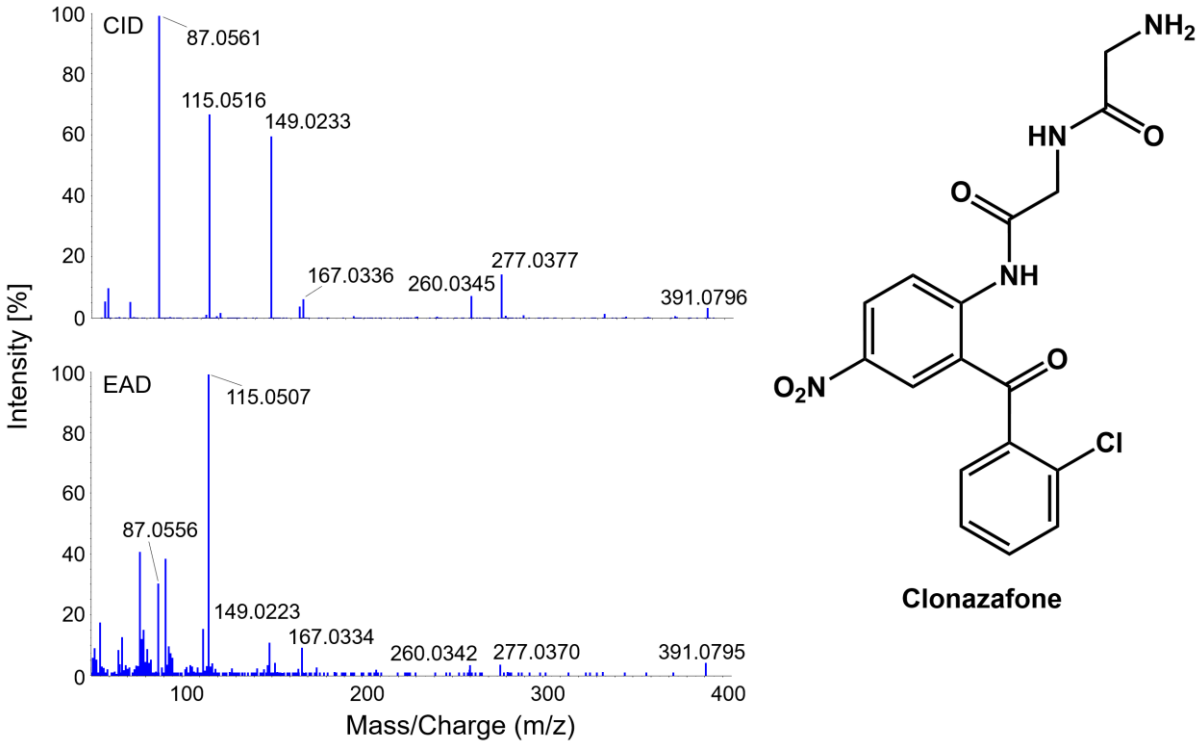

Figure S4: Spectra of clonazafone (C2) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)

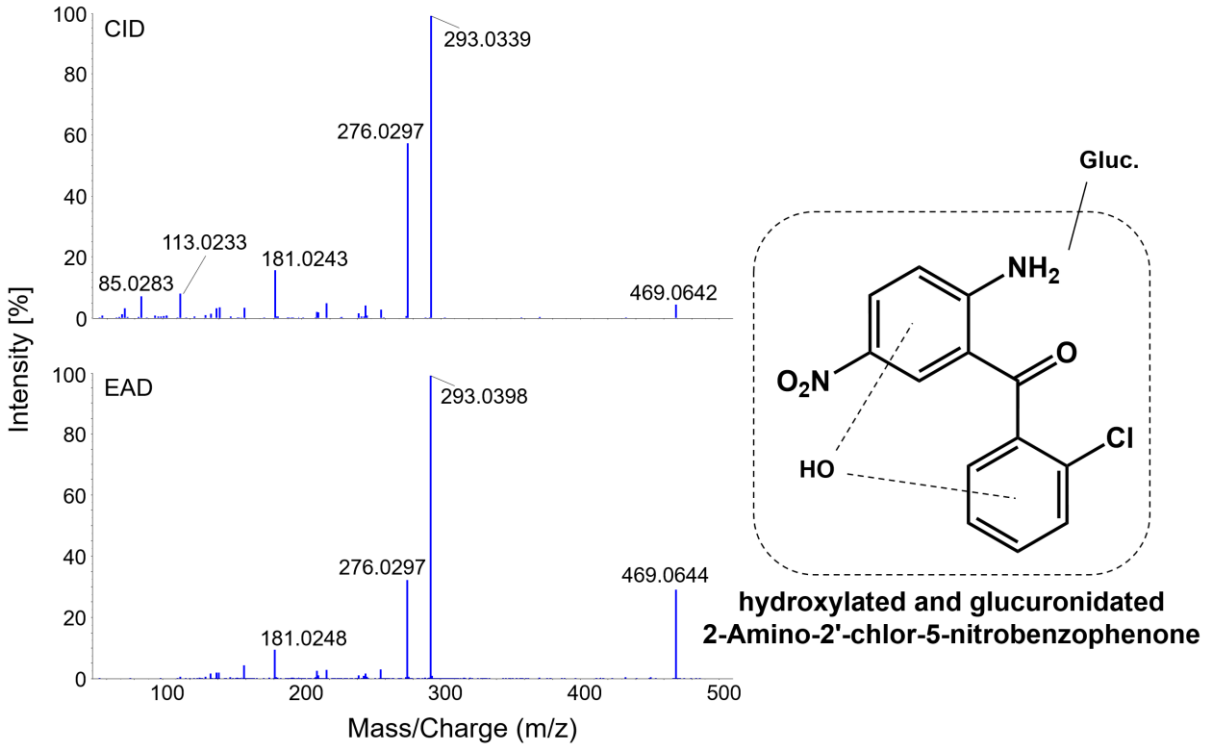

Figure S5: Spectra of hydroxylated and glucuronidated 2-Amino-2'-chlor-5-nitrobenzophenon (C3) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)

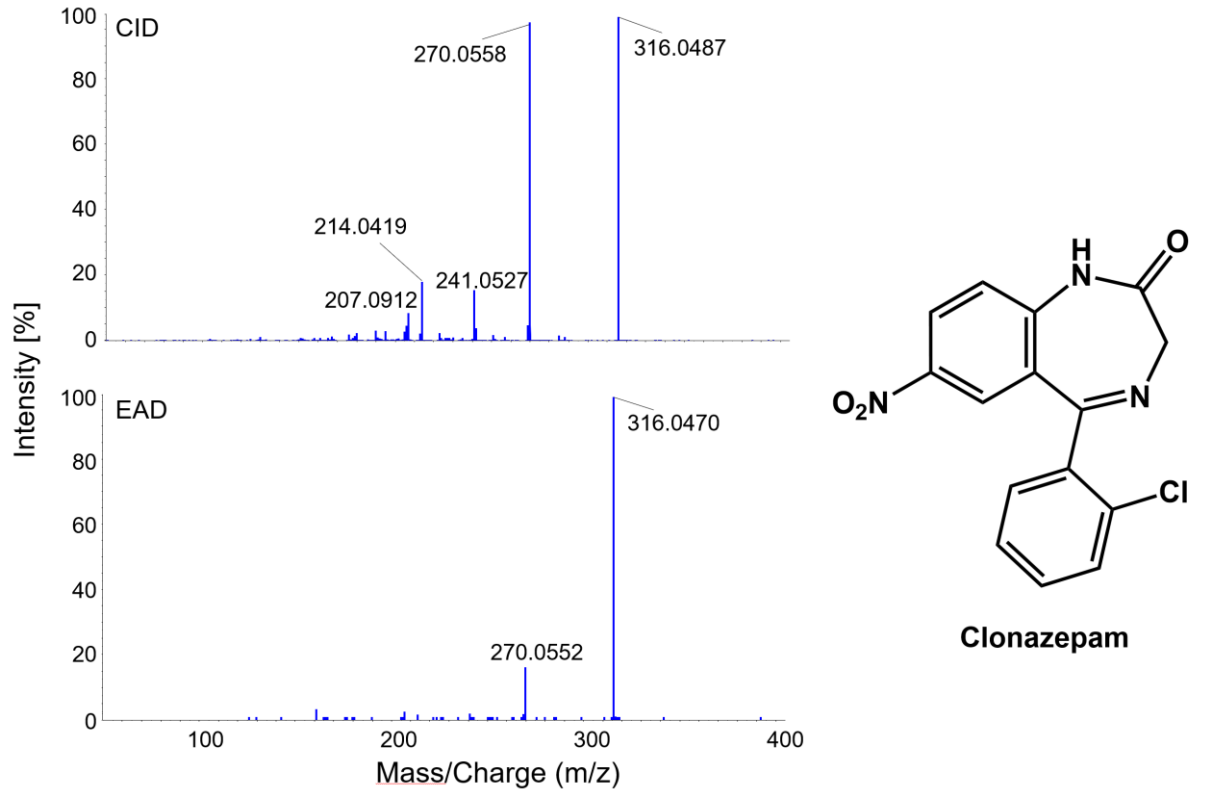

Figure S6: Spectra of clonazepam (C4) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)

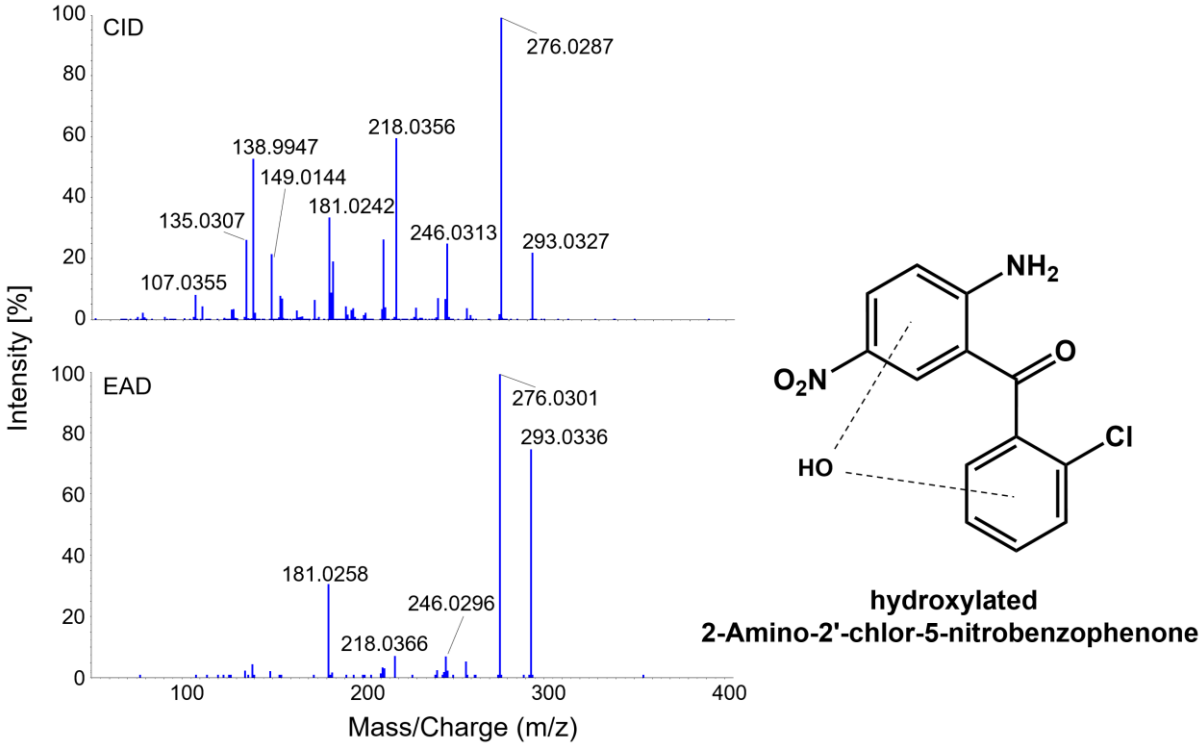

Figure S7: Spectra of hydroxylated 2-Amino-2'-chlor-5-nitrobenzophenon (C5) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)

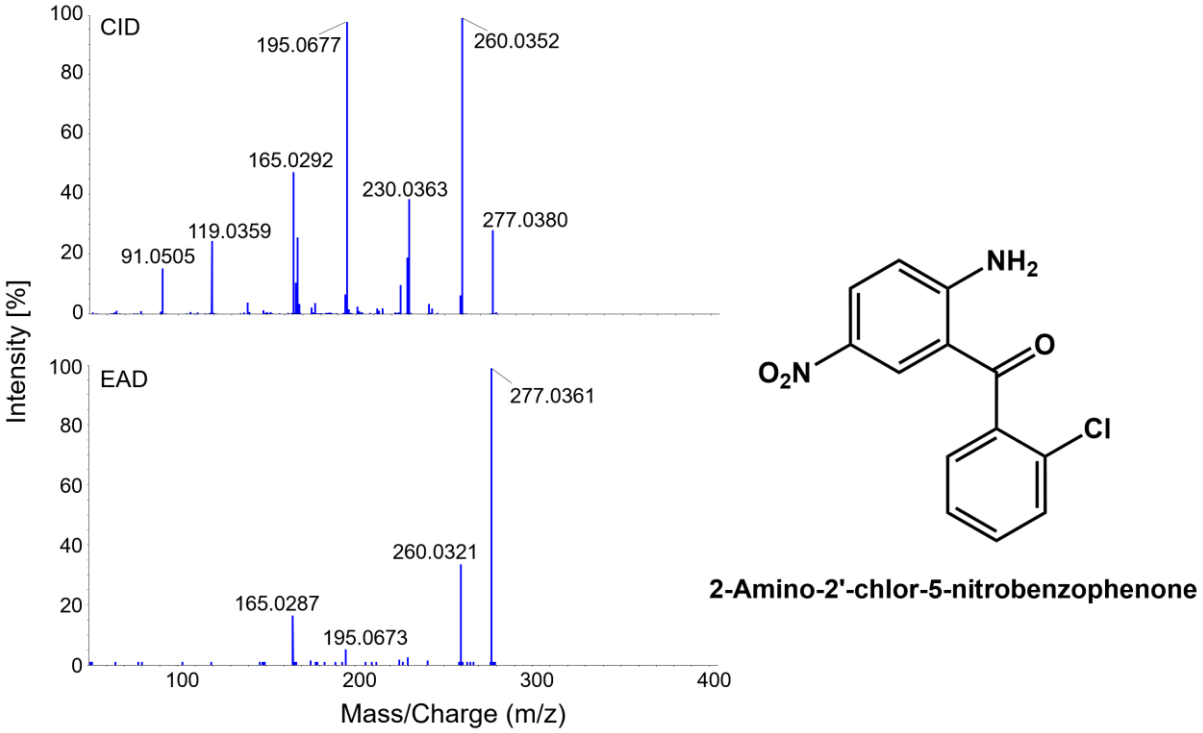

Figure S8: Spectra of 2-Amino-2'-chlor-5-nitrobenzophenon (C6) generated with collision-induced dissociation (CID) in comparison to electron-activated dissociation (EAD)