

Chemical Attribution of Fentanyl: The Effect of Human Metabolism

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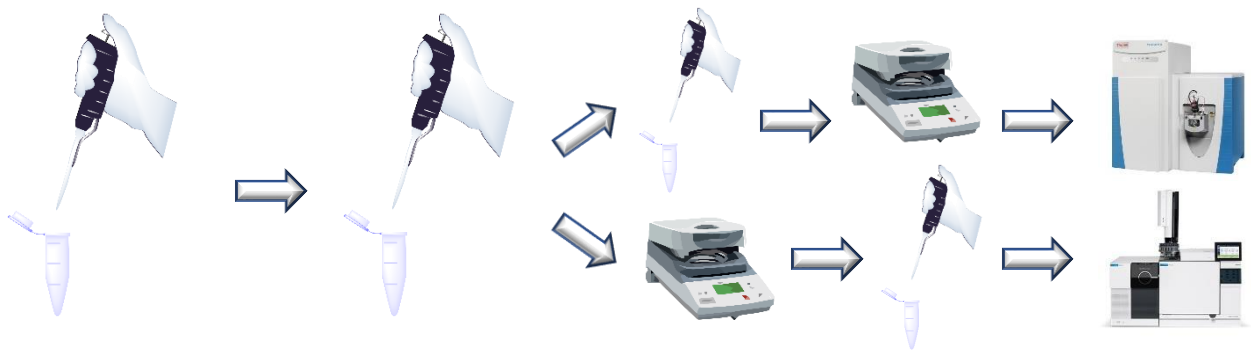
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Supplementary material

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PRE-INCUBATION

- 500 μL Buffer: 0.1 M K_3PO_4 , 2.5 mM MgCl_2 (pH = 7.4)
 - 100 μL of 1 mg/mL fentanyl
 - 200 μL of 2.5 mg/mL human liver microsomes
- 3 min., 300 rpm @37 °C

INCUBATION

- 200 μL NADPH-regenerating system:
 - 1 mM NADP+
 - 5 mM glucose-6-phosphate
 - 1 U/mL glucose-6-phosphate dehydrogenase
 - 2 mM UDPGA
- 72h, 300 rpm @37 °C

SAMPLE PREPARATION

LC

- Collect 500 μL sample
 - Add 500 μL acetonitrile
- Vortex and centrifuging: 10 min., 14,000 rpm
- Collect supernatant (proteins precipitate)
 - Add 100 μL benzylfentanyl of 1 $\mu\text{g}/\text{mL}$
 - Add 400 μL MilliQ
 - Analyse by LC

GC

- Collect 500 μL sample
- Centrifuging: 10 min., 14,000 rpm
- Collect supernatant (proteins precipitate)
 - Add 400 μL DCM and vortex
 - Collect bottom layer
 - Add 100 μL d_5 -norfentanyl of 100 $\mu\text{g}/\text{mL}$
 - Analyse by GC

Figure 1. Method for microsomal incubation and sample preparation. LC-Orbitrap-MS © Thermo Fisher Scientific (Bremen). Printed with OTRS permission. GC-MS © Agilent Technologies, Inc. Reproduced with Permission.

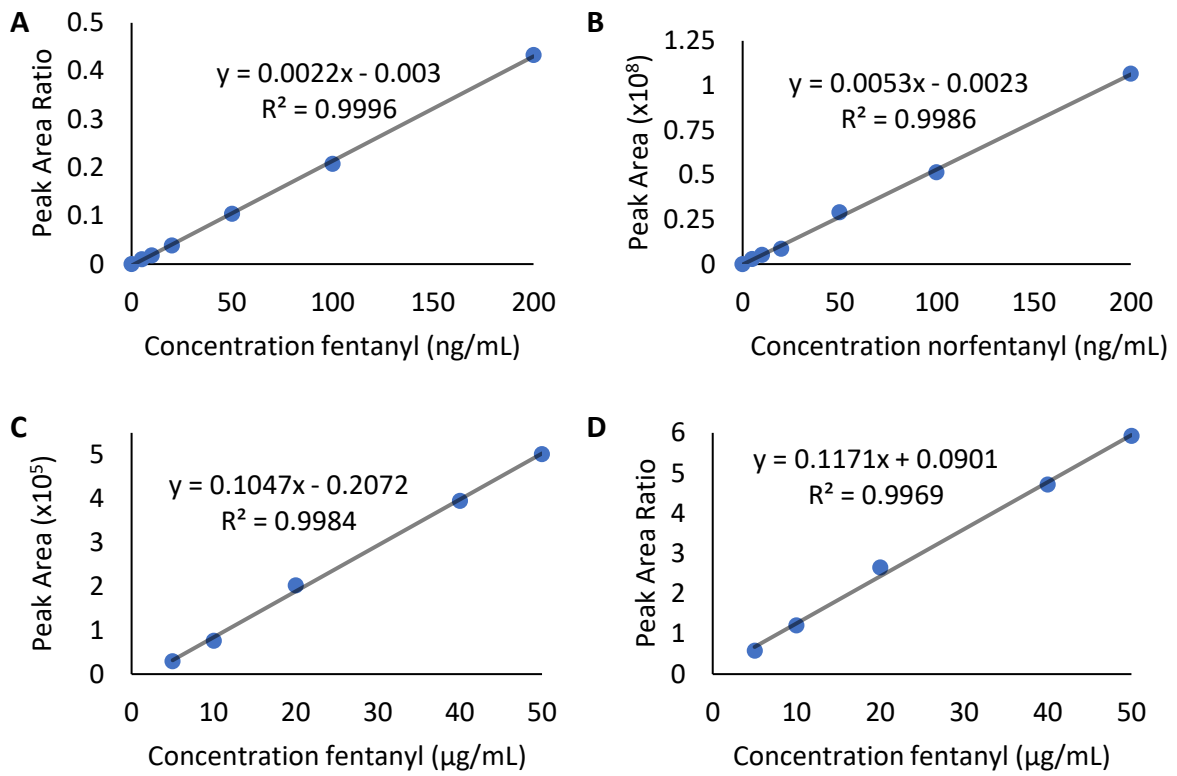
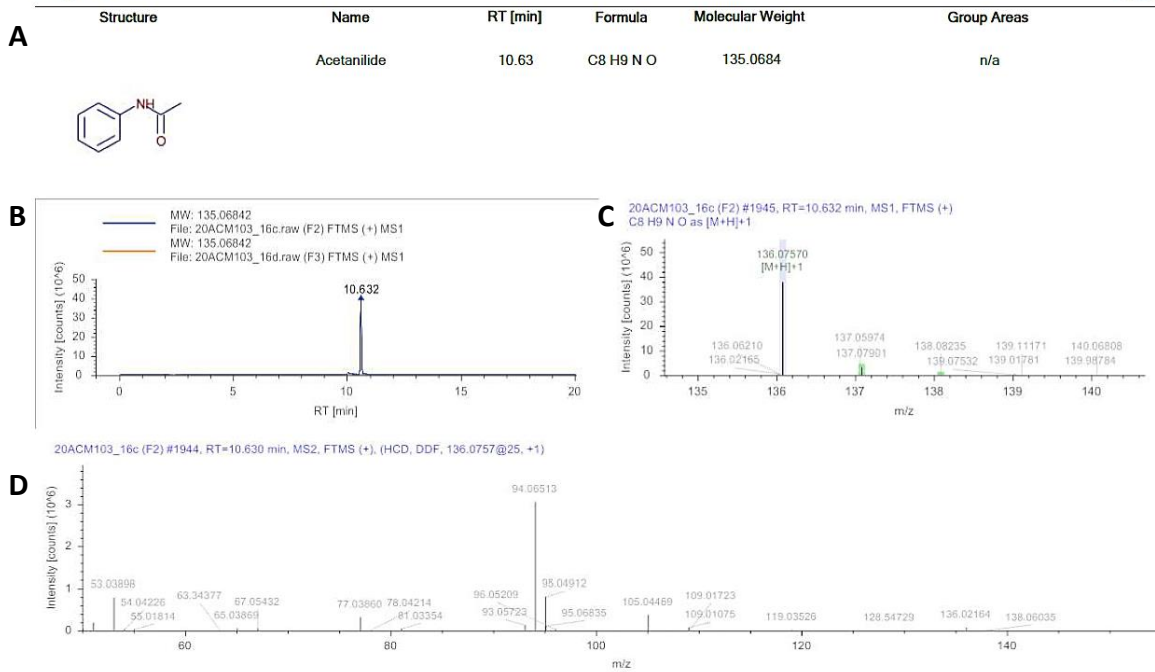


Figure 2. Calibration curves of A) Fentanyl with internal standard benzylfentanyl detected by LC-MS/MS. B) Norfentanyl detected by LC-MS/MS. C) Fentanyl detected by GC-MS. D) Norfentanyl with internal standard d_5 -norfentanyl detected by GC-MS.



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Figure 3. Typical identification of a tentative structure by LC-Orbitrap-MS/MS reported by Compound Discover 2.1. The impurity identified as N-phenylacetamide (J) is presented. A) Summary of results. B) Chromatogram. C) Mass spectrum. D) MS/MS spectrum.

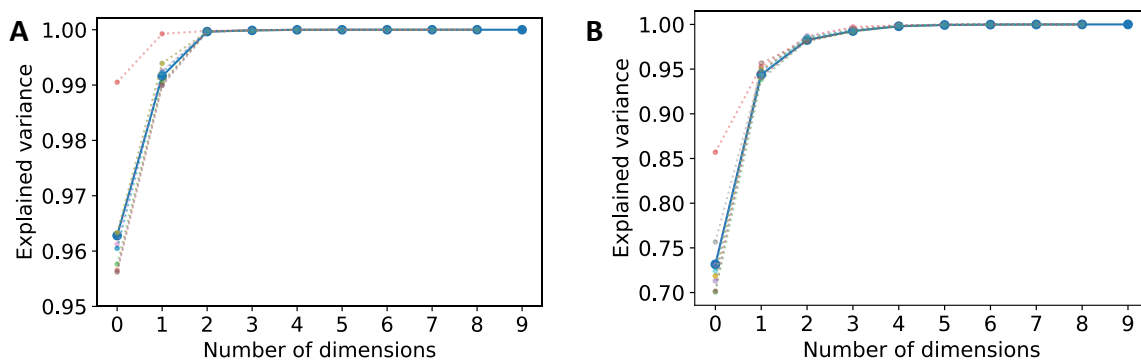


Figure 4. Effect of leave-one-out validation on PCA robustness. Blue line: PCA including all samples; dashed lines: PCA with one sample left out. For each dashed line another measurement is left out. The PCA model showed good robustness, since leaving out one sample resulted in similar explained variance. A) Pre-metabolism measured by LC-Orbitrap-MS. B) Post-metabolism measured by LC-Orbitrap-MS.