Electronic Supplementary Material for:

Stability of Cocaine and its Metabolites in Municipal Wastewater – the Case for Using Metabolite Consolidation to Monitor Cocaine Utilization

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Description: The supplementary material contains a reaction schematic (Figure S1) and rate equations used in modeling the hydrolysis of cocaine and its metabolites, as well as a table of model-fitted rate constants (Table S1). It also contains results from the variance/covariance analysis of cocaine and its metabolites in independent urine samples (Table S2).
Rate Equations Used to Model Cocaine Transformations

The equations used to model the stability of cocaine and its metabolites in untreated municipal wastewater are provided below. Reactions and corresponding rate constants for the hydrolysis of cocaine and its metabolites are also displayed (with abbreviations) in Figure S1.

Hydrolysis of Cocaine, Cocaethylene, and Their Metabolites:

\[
\begin{align*}
\frac{dCOC}{dt} &= -k_1COC - k_2COC \\
\frac{dCE}{dt} &= -k_3CE - k_4CE \\
\frac{dBEC}{dt} &= k_1COC + k_3CE - k_5BE \\
\frac{dEME}{dt} &= k_2COC - k_6EME \\
\frac{dEEE}{dt} &= k_4CE - k_7EEE \\
\frac{dmOHBE}{dt} &= -k_8mOHBE \\
\frac{dpOHBE}{dt} &= -k_9pOHBE \\
\frac{dEC}{dt} &= k_5BE + k_6EME + k_7EEE + k_8mOHBE + k_9pOHBE
\end{align*}
\]

Mass Balance = COC + CE + BE + EME + EEE + mOHBE + pOHBE + EC

Hydrolysis of the Pyrolized Cocaine Metabolites:

\[
\begin{align*}
\frac{dAEME}{dt} &= -k_{10}AEME \\
\frac{dAEC}{dt} &= k_{10}AEME
\end{align*}
\]

Mass Balance = AEME + AEC
Hydrolysis of the Norcocainic Cocaine Metabolites:

\[
\frac{dNC}{dt} = -k_{11}NC - k_{12}NC \\
\frac{dNBE}{dt} = k_{11}NC - k_{13}NBE
\]

As verifiable standards for norecgonine methyl ester and norecgonine could not be located, the hydrolysis of the norcocaine metabolites could not be fully modeled. Hydrolysis of norcocaine to form norbenzoylecgonine (rate constant \(k_{11}\)) was fit against experimental data. The remaining norcocaine that degraded was assumed to form norecgonine methyl ester (NEME; rate constant \(k_{12}\)). The rate constant for the hydrolysis of norbenzoylecgonine to norecgonine (\(k_{13}\)) was forced to zero because model-fit hydrolysis rate constants corresponding to the loss of the benzoyl group (or substituted benzoyl group) were indistinguishable from zero for benzoylecgonine, \(m\)-hydroxybenzoylecgonine, and \(p\)-hydroxybenzoylecgonine at all temperatures investigated. Degradation of NEME to norecgonine (\(k_{14}\) in Figure S1) was not included in the model, as no analytical standards exist for either compound.

Normalization Procedure Used in Modeling Cocaine Hydrolysis:

The concentration data for all cocaine species were normalized to initial concentrations in order to prevent biases in model fitting. These biases arise from the large differences in initial spiking concentrations of the various species, which results in large differences in residual errors from model fitting. The Scientist models were then provided these normalized data and equations. An example normalized rate equation for benzoylecgonine is:

\[
\frac{dBE_N}{dt} = k_1CO_CN_{BE_0} + k_3CE_N_{BE_0} - k_5BE_N; \text{ In this equation, } BE_N = \frac{BE}{BE_0}
\]
Figure S1. Schematic for the hydrolysis of cocaine and its major metabolites. Solid lines represent hydrolysis reactions that are likely to occur in aqueous environments. Dashed lines represent transformation pathways that occur during metabolism (“ox” = oxidation; “EtOH” represents transesterification) or administration (e.g., pyrolysis when administered as crack cocaine).
Table S1. Model-fit rate constants (d^{-1}; ± 95% confidence intervals) for the hydrolysis of cocaine and its metabolites in untreated municipal wastewater at different temperatures.\(^a\)

<table>
<thead>
<tr>
<th>Transformation(^b)</th>
<th>Temperature</th>
<th>31 °C</th>
<th>23 °C</th>
<th>9 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cocaine and Cocaethylene</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_1) COC (\rightarrow) BE</td>
<td></td>
<td>2.27 ± 0.37</td>
<td>1.16 ± 0.18</td>
<td>0.21 ± 0.11</td>
</tr>
<tr>
<td>(k_2) COC (\rightarrow) EME</td>
<td></td>
<td>1.07 ± 0.37</td>
<td>0.27 ± 0.19</td>
<td>0.03 ± 0.11</td>
</tr>
<tr>
<td>(k_3) CE (\rightarrow) BE</td>
<td></td>
<td>1.29 ± 0.10</td>
<td>0.54 ± 0.06</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>(k_4) CE (\rightarrow) EEE</td>
<td></td>
<td>0.00 ± 0.64</td>
<td>0.00 ± 0.37</td>
<td>0.00 ± 0.29</td>
</tr>
<tr>
<td>(k_5) BE (\rightarrow) EC</td>
<td></td>
<td>0.00 ± 0.52</td>
<td>0.00 ± 0.28</td>
<td>0.00 ± 0.04</td>
</tr>
<tr>
<td>(k_6) EME (\rightarrow) EC</td>
<td></td>
<td>4.99 ± 0.81</td>
<td>1.65 ± 0.40</td>
<td>0.23 ± 0.26</td>
</tr>
<tr>
<td>(k_7) EEE (\rightarrow) EC</td>
<td></td>
<td>1.77 ± 0.13</td>
<td>0.59 ± 0.06</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>(k_8) mOHBE (\rightarrow) EC</td>
<td></td>
<td>0.00 ± 0.07</td>
<td>0.00 ± 0.11</td>
<td>0.00 ± 0.04</td>
</tr>
<tr>
<td>(k_9) pOHBE (\rightarrow) EC</td>
<td></td>
<td>0.00 ± 0.08</td>
<td>0.00 ± 0.09</td>
<td>0.00 ± 0.07</td>
</tr>
<tr>
<td><strong>Pyrolysis Metabolites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_{10}) AEME (\rightarrow) AEC</td>
<td></td>
<td>0.16 ± 0.09</td>
<td>0.00 ± 0.09</td>
<td>0.00 ± 0.19</td>
</tr>
<tr>
<td><strong>Norcocainic Metabolites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_{11}) NC (\rightarrow) NBE</td>
<td></td>
<td>0.62 ± 0.20</td>
<td>0.45 ± 0.12</td>
<td>0.33 ± 0.13</td>
</tr>
<tr>
<td>(k_{12}) NC (\rightarrow) NEME(^c)</td>
<td></td>
<td>0.93 ± 0.23</td>
<td>0.28 ± 0.14</td>
<td>0.00 ± 0.29</td>
</tr>
<tr>
<td>(k_{13}) NBE (\rightarrow) NEC</td>
<td></td>
<td>NA(^d)</td>
<td>NA(^d)</td>
<td>NA(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Refer to Figure S1 for a description of the hydrolysis model. \(^b\) Refer to Figure S1 for abbreviations. \(^c\) Analyses for noregonine methyl ester (NEME) could not be conducted; rate coefficient determined from model fits of NC and NBE. \(^d\) Rate coefficient could not be determined because analysis for NEC could not be conducted; value assumed equal to zero.
**Variance/Covariance Analysis of Cocaine and its Metabolites in Urine Samples**

The variance of a linear combination of variables is the sum of the variances of each variable, plus two times the sum of the covariance among variables (Tabachnick and Fidell 1989):

\[
\text{Var}(X + Y) = \text{Var}(X) + \text{Var}(Y) + 2\text{Cov}(X,Y)
\]

For normally-distributed variables:

\[
\text{Var}(X) = \frac{1}{n-1} \sum_{i=1}^{n} (X_i - \bar{X})^2 \quad \text{and} \quad \text{Cov}(X,Y) = \frac{1}{n-1} \sum_{i=1}^{n} [(X_i - \bar{X})(Y_i - \bar{Y})]
\]

For the samples from Paul et al. 2005: EC\textsubscript{hyd} = COC + CE + EME + EEE + BE + mOHBE + EC

\[
\text{Var}(\text{EC\textsubscript{hyd}}) = \text{Var}(\text{COC} + \text{CE} + \text{EME} + \text{EEE} + \text{BE} + \text{mOHBE} + \text{EC})
\]

\[
= \text{Var}(\text{COC}) + \text{Var}(\text{CE}) + \text{Var}(\text{EME}) + \text{Var}(\text{EEE}) + \text{Var}(\text{BE}) + \text{Var}(\text{mOHBE}) + \text{Var}(\text{EC}) + 2\left[\text{Cov}(\text{COC,CE}) + \text{Cov}(\text{COC,EME}) + \text{Cov}(\text{COC,EEE}) + \text{Cov}(\text{COC,BE}) + \text{Cov}(\text{COC,mOHBE}) + \text{Cov}(\text{COC,EC}) + \text{Cov}(\text{CE,EME}) + \text{Cov}(\text{CE,EEE}) + \text{Cov}(\text{CE,BE}) + \text{Cov}(\text{CE,mOHBE}) + \text{Cov}(\text{CE,EC}) + \text{Cov}(\text{EME,EEE}) + \text{Cov}(\text{EME,BE}) + \text{Cov}(\text{EME,mOHBE}) + \text{Cov}(\text{EME,EC}) + \text{Cov}(\text{EEE,BE}) + \text{Cov}(\text{EEE,mOHBE}) + \text{Cov}(\text{EEE,EC}) + \text{Cov}(\text{BE,mOHBE}) + \text{Cov}(\text{BE,EC}) + \text{Cov}(\text{mOHBE,EC})\right]
\]

Sum of individual variances (diagonal of covariance matrix in Table S2) = 950

Sum of covariances (minor cells of covariance matrix in Table S2) = −421.5

\[
\text{Var}(\text{EC\textsubscript{hyd}}) = \text{sum of individual variances} + 2(\text{sum of covariances})
\]

\[
\text{Var}(\text{EC\textsubscript{hyd}}) = 950 + 2(-421.5) = 107 \quad \text{SD (EC\textsubscript{hyd})} = \sqrt{107} = 10.4
\]

<table>
<thead>
<tr>
<th></th>
<th>COC</th>
<th>CE</th>
<th>EME</th>
<th>EEE</th>
<th>BE</th>
<th>mOHBE</th>
<th>EC</th>
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</thead>
<tbody>
<tr>
<td>COC</td>
<td>15.5</td>
<td>4.4</td>
<td>24.7</td>
<td>7.3</td>
<td>-16.9</td>
<td>-0.3</td>
<td>-32.6</td>
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<tr>
<td>CE</td>
<td></td>
<td>3.1</td>
<td>9.4</td>
<td>8.4</td>
<td>-10.3</td>
<td>-0.2</td>
<td>-11.6</td>
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<tr>
<td>EME</td>
<td></td>
<td></td>
<td>133.2</td>
<td>28.7</td>
<td>-43.8</td>
<td>-0.3</td>
<td>-138.1</td>
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<td>EEE</td>
<td></td>
<td></td>
<td></td>
<td>31.8</td>
<td>-32.8</td>
<td>-0.5</td>
<td>-35.0</td>
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<td>BE</td>
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<td>341.7</td>
<td>0.1</td>
<td>-182.7</td>
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<tr>
<td>mOHBE</td>
<td></td>
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<td>0.6</td>
<td>0.7</td>
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<tr>
<td>EC</td>
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<td></td>
<td>424.2</td>
</tr>
</tbody>
</table>

\(^a\) Data from (Paul et al. 2005); reported as concentration (ng/mL) in urine and presented as mass fraction of COC measured in sample. \(^b\) See Figure S1 for abbreviations.
References
