Assessing Cocaine Use Patterns in the Brazilian Capital by Wastewater-Based Epidemiology

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# SUPPLEMENTAL MATERIAL

# Linearity and limits of detection and quantification

Linear regression parameters of the analytical curves constructed for quantification of cocaine (COC), benzoylecgonine (BE) and cocaethylene (COE) in wastewater samples by liquid chromatography coupled to high-resolution hybrid quadrupole-time-of-flight mass spectrometry (LC-QTOF) are presented in Table S1.

Table S1. Parameters of the analytical curves and limits of determination and quantification for the investigated analytes determined by LC-QTOF.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | IS | Working Range  | Analytical Curve | R2 | LOD*i*  | LOQ*i*  | LOD*m*\* | LOQ*m*\* |
|  |  | *µg L-1* | *y* = a + b *x* |  | *µg L-1* | *µg L-1* | *ng L-1* | *ng L-1* |
| COC | COC-d3 | 90 − 700 | *y* = 0.045 + 0.005 *x* | 0.996 | 27 | 90 | 86 | 288 |
| BE | BE-d3 | 70 − 1000 | *y* = -0.046 + 0.007 *x* | 0.999 | 21 | 70 | 65 | 215 |
| COE | COE-d3 | 15 − 100 | *y* = 0.005 + 0.051 *x* | 0.991 | 5 | 15 | 14 | 42 |

COC: cocaine, BE: benzoylecgonine, COE: cocaethylene, IS: internal standard, a: linear coefficient, b: angular coefficient, LOD*i*: limit of detection of the instrument, LOQi: limit of quantification of the instrument, LOD*m*: method limit of detection, LOQm: method limit of quantification. \*Limits calculated considering the efficiency of the method ($Ef$) and a concentration factor of 250.

Analytical curves using internal standards were homoscedastic. Instrumental limits of quantification (LOQ*i*) were expressed by the lower standard concentration of the analytical curves, while instrumental limits of detection (LOD*i*) were calculated using LOQ*i* divided by the factor of 3.3. Limits of detection and quantification of the method, LOD*m,* and LOQ*m*, respectively, were obtained using LOD*i* and LOQ*i* multiplied by the efficiency of the method ($Ef$) and divided by the pre-concentration factor of 250.

# *Analytes quantification in Brazil*

The Carnival Day sample, as well as the samples from the stability test, were analyzed in Brazil using liquid chromatograph (Agilent 1200 Series) coupled to triple-quadrupole (QqQ) mass spectrometer (QTRAP 3200, Sciex) with electrospray ionization (ESI) interface operating at 550 °C and 4500 V, using nitrogen as curtain gas at 15 psi and as auxiliary nebulizing gas (GS1 and GS2) at 45 psi.

Separation was performed using an Eclipse XDB-C18 column (4,6×150 mm, particle size of 5 μm, Agilent) with gradient elution (0.3 mL min-1) water and in methanol as mobile phases containing 0.1% (v/v) of formic acid. The gradient was achieved by maintaining for 4 min a relative methanol concentration of 10%, followed by the increase to 100% in 6 min, and held constant for another 1 min. After readjusting to the initial conditions, the system was re-equilibrated for 7 min. The injection volume was 2.0 μL.

Mass spectrometric analyses were carried out using the multiple reaction monitoring (MRM) mode in order to identify and quantify the target analytes by measuring the fragmentation products of each protonated molecular ions [M+H]+. Table S2 shows the MRM transitions used alongside with other instrumental parameters.

Table S2. LC-MS/MS parameters used during the analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Analyte | DP (V) | EP (V) | CEP (V) | MRM transitions (m/z) | CE (V) | CXP (V) |
| COC | 51 | 1 | 26 | 304,1$⟶$182,0a304,1$⟶$105,1 | 3945 | 1024 |
| BE | 51 | 5 | 20 | 290,1$⟶$168,1a290,1$⟶$105,0 | 2541 | 44 |
| COE | 26 | 4.5 | 14 | 318,1$⟶$196,2a318,1$⟶$105,3 | 2355 | 44 |
| COC-d3 | 16 | 5 | 22 | 307,1$⟶$185,1a | 25 | 4 |
| BE-d3 | 16 | 4.5 | 98 | 293,1$⟶$171,1a | 25 | 4 |
| COE-d3 | 11 | 5 | 28 | 321,1$⟶$199,1a | 27 | 4 |

aTransition used for quantification. DP: declustering potential, EP: entrance potential, CEP: collision cell entrance potential, CE: collision energy, CXP: collision cell exit potential, RT: retention time.

The preparation of the samples analyzed in Brazil was identical to those sent to Spain, except for the pre-concentration step. After elution of the analytes with 6 mL of methanol and the addition of internal standards, the eluates were then evaporated under vacuum in a parallel evaporator Syncore Analyst (Buchi) to a final volume of 1.5 mL. Table S3 shows the parameters of the analytical curves constructed for quantification of the selected analytes by liquid chromatography coupled to triple-quadrupole mass spectrometry (LC-QqQ), as well as limits of detection and quantification.

Table S3. Parameters of the analytical curves and limits of determination and quantification for the investigated analytes determined by LC-QqQ.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | IS | Working Range  | Analytical Curve | R2 | LOD*i*  | LOQ*i*  | LOD*m*\* | LOQ*m*\* |
|  |  | *µg L-1* | *y* = a + b *x* |  | *µg L-1* | *µg L-1* | *ng L-1* | *ng L-1* |
| COC | COC-d3 | 10 − 500 | *y* = 0.001 + 0.006 *x* | 0.98 | 3 | 10 | 73 | 242 |
| BE | BE-d3 | 10 − 500 | *y* = 0.077 + 0.723 *x* | 0.99 | 3 | 10 | 71 | 233 |
| COE | COE-d3 | 10 − 500 | *y* = 0.012 + 0.092 *x* | 0.99 | 3 | 10 | 65 | 215 |

COC: cocaine, BE: benzoylecgonine, COE: cocaethylene, IS: internal standard, a: linear coefficient, b: angular coefficient, LOD*i*: limit of detection of the instrument, LOQi: limit of quantification of the instrument, LOD*m*: method limit of detection, LOQm: method limit of quantification. \*Limits calculated considering the efficiency of the method ($Ef$) and a concentration factor of 33.