Overview

- First validated method for regulated analysis of cocaine and metabolites benzoylecgonine (BE) and ecgonine methyl ester (EME) in human plasma.
- Cocaine is rapidly metabolized in vivo and in vitro. Benzoylecgonine and ecgonine methyl ester are two of the metabolites formed by ester hydolysis of cocaine.
- Sodium fluoride and potassium oxalate (NaF/KOx) are used as plasma anticoagulants to slow the hydrolysis of cocaine esters.
- All analytes are highly polar.
- Two minute injections allow for rapid analysis of up to nearly 500 samples/day.

Method

- Validated range: 1.00-1000 ng/mL for cocaine and BE and 0.500-500 ng/mL for EME.
- Conventional reverse phase chromatography was unable to retain EME.
- HLIC phase chromatography provided excellent retention and separation of each analyte.
- Isocratic LC program eliminates need for column re-equilibration.
- Triple quadrupole MS with positive electrospray ionization provides excellent sensitivity.
- Declustering potential was applied to reduce response drift during a run (~96 injections).
- A secondary pump delivered unbuffered 50:50 water/methanol to flush buffer salts from the HESI probe between injections.
- Utilizes 50 µL of plasma per analysis.
- Plasma samples are handled on wet ice, which slows ester hydrolysis more than 10-fold compared to room temperature.
- Deuterated internal standards are used for each analyte (Cocaine-D3, BE-D8, EME-D3).
- Analytes were extracted using acetonitrile protein precipitation. The organic supernatant can be directly injected without the need for evaporation or concentration.

Validation Results Overview

- 7 of 7 validation runs met acceptance CS/QC criteria for all three analytes.
- Recovery >90% for each analyte and ISTD.
- Benchtop stability established through 24 hours at refrigerated temperatures.
- Five freeze/thaw cycles established at -20°C and -80°C on wet ice.
- Long term stability for plasma QC’s was established through 279 days at both -20°C and -80°C.
- Dual plate run (up to 192 injections) was successfully validated.

Conclusions

A sensitive and specific LC-MS/MS assay to measure cocaine and metabolites benzoylecgonine and ecgonine methyl ester in NaF/KOx human plasma has been developed and validated for regulated use. A simple sample processing procedure combined with a robust and efficient LC-MS/MS analysis method delivers reliable results with high sample throughput. This is the first known method validated for analysis of all three analytes together in plasma. Despite known stability issues for cocaine, over 700 clinical samples were successfully analyzed, including successful incurred sample reproducibility (ISR) testing for 10% of samples.

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