

K20 Hydroxycocaine Concentrations in Head and Body Hair as Determined by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Neil Stowe, PhD*, Psychomedics Corporation, Culver City, CA 90230; Ryan B. Paulsen, PhD, Psychomedics Corporation, Culver City, CA 90230; Virginia Hill, BS, Psychomedics Corporation, Culver City, CA 90230; Michael I. Schaffer, PhD, Psychomedics Corporation, Culver City, CA 90230

Learning Overview: After attending this presentation, attendees will have viewed an investigation of hydroxycocaine concentrations, as determined by LC/MS/MS, in head and body hair samples after an extended aqueous wash.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by imparting knowledge regarding methods useful for determining hydroxycocaine concentrations in hair samples after an extended aqueous wash. Interpretation of hydroxycocaine concentrations regarding cocaine ingestion is presented.

Statement of Hypothesis: It was hypothesized that hydroxycocaine isomers could be detected in head and body hair samples by LC/MS/MS. As part of this investigation, concentrations of hydroxycocaine isomers, effects of hair color, and stability of the hydroxycocaine isomers in hair were examined.

Statement of Methods: Head and body hair samples containing cocaine were also analyzed for Benzoylcegonine (BE), Cocaethylene (CE), norcocaine (Ncoc), para-Hydroxycocaine (p-OHCoc), meta-Hydroxycocaine (m-OHCoc), and ortho-Hydroxycocaine (o-OHCoc) by LC/MS/MS. The confirmation process consisted of a hair aliquot that was first washed using an extended aqueous method, followed by solid phase extraction and quantitation using either an AB SCIEX™ API 3200 LC/MS/MS for cocaine, BE, CE, and NCoc or an AB SCIEX™ 6500+ for p-OHCoc, m-OHCoc, and o-OHCoc. The confirmation used primary and secondary ions for each analyte in positive Multiple Reaction Mode (MRM). The cocaine LC/MS/MS method was linear from 25 to 15,000pg/mg hair for cocaine, BE, CE, and NCoc; the hydroxycocaine LC/MS/MS method was linear from 0.4 to 300pg/mg for o-, m-, and p-OHCoc.

Results: A total of 2,902 head and body hair samples were analyzed in this study. The percentage of samples having hydroxycocaine isomers in head hair below the Lower **Limit Of Quantification** (LLOQ) for p-OHCoc, m-OHCoc, and o-OHCoc was 4%, 2%, and 9.6%, respectively, and in body hair was 9%, 9%, and 4.3%. In this sample population, the median concentrations of p-OHCoc, m-OHCoc, and o-OHCoc were 1.6, 2.1, and 3.6 times greater, respectively, in body hair than head hair. The average concentrations of p-OHCoc, m-OHCoc, and o-OHCoc, respectively, as a percentage of cocaine in head hair were 1.143, 1.079, and 0.108%, and 0.788, 0.943, and 0.193% in body hair. The lower concentration of p-OHCoc and m-OHCoc as a percentage of cocaine in head hair versus body hair was significant (Z-test), with the higher concentration of o-OHCoc as a percentage of cocaine in body hair also significant (Z-test). With head hair, 5.0% of samples failed to be $\geq 0.05\%$ of cocaine for one or both of m-OHCoc or p-OHCoc. In body hair, 10% of samples failed to equal $\geq 0.05\%$ cocaine for one or both isomers. Comparison of % cocaine for black and brown head hair found a higher percentage of p-OHCoc in brown hair (Z-test), with no difference in % cocaine observed for p- and m-OHCoc in body hair (Z-test). Concentration ratios of m-OHCoc/o-OHCoc, and p-OHCoc/o-OHCoc were examined, with a ratio of 2 required for a head hair sample to be positive for cocaine ingestion, and body hair evaluated at ratios of 1.5 and 2. The effects of CE on hydroxycocaine concentrations were examined, with higher concentrations of p-OHCoc and m-OHCoc apparent in concentrations up to 3,000pg/mg CE in hair. Samples were analyzed after one year in storage at ambient temperature, with hydroxycocaine isomers proving stable over this time. Treatment of cocaine-positive samples with peroxide showed a large increase in o-OHCoc as percentage of cocaine as compared with smaller changes in p-OHCoc and m-OHCoc before sample washing. After an extended wash, only o-OHCoc showed an increase as percentage of cocaine.

Conclusion: Hydroxycocaines were analyzed for concentrations in hair, their concentrations in hair relative to cocaine and cocaethylene, and hair color effects. The hydroxycocaines were stable over one year storage at ambient temperature, with p-OH and m-OHCoc also stable to hair peroxide treatment. The p- and m-OHCoc isomers represent useful metabolites that can be used as biomarkers for evidence of cocaine ingestion.

Hair, Hydroxycocaine, LC/MS/MS