## B41 Formation of a Glutathione Adduct With a Cocaine Pyrolyis Product - Anhydroecgonine Methyl Ester

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The goal of this presentation is to develop an in vitro model for the formation of adducts of a pyrolysis product of cocaine with cellular nucleophiles, such as glutathione.

The smoking of crack cocaine is a major drug abuse concern. Anhydroecgonine methyl ester (AEME) is a known pyrolysis product of cocaine derived from heating crack cocaine. AEME contains an alphabeta unsaturated carbonyl functional group that can undergo a Michael addition reaction with nucleophiles. Glutathione is a tripeptide found in most mammalian tissues. The nucleophilic thiol group within the cysteine residue of glutathione functions biologically as a free radical scavenger. The formation of glutathione adducts with xenobiotics, such as AEME, may serve as markers of toxicity for new or established compounds. Glutathione conjugation products are further metabolized via enzyme mediated pathways to mercapturic acid derivatives that are commonly excreted in mammalian urine. The glutathione and mercapturate adducts of AEME may provide markers that could facilitate forensic identification of crack cocaine smokers and serve as a basis for a better understanding of the toxicology of smoked cocaine.

AEME (1 mg/mL in acetonitrile, Cerilliant, Austin, TX) was combined with reduced glutathione (10 mg/mL in water, Sigma, St. Louis, MO) and was stirred in a sealed tube under a nitrogen atmosphere. Aliquots (50 µL) were taken at 0, 30, and 60 min, diluted with HPLC-grade methanol, and analyzed by direct infusion into the electrospray ionization source of a ThermoQuest Model LCQ/DECA 1000 ion trap mass spectrometer.

Glutathione-AEME adduct (*m/z* 489), and reactants, AEME (*m/z* 182) and glutathione (*m/z* 308), were detected within 5 min following mixing. Further mass spectral analysis of the parent adduct ion (*m/z* 489) by $MS^2$ showed daughter ions consistent with the masses of AEME and glutathione. Further fragmentation ($MS^{3)}$ of the daughter ion at *m/z* 182 provided the same spectrum as that produced by the $MS^2$ fragmentation of authentic AEME. In addition, fragmentation ($MS^3$) of the daughter ion at *m/z* 308 yielded the same spectrum as that produced by the $MS^2$ fragmentation of authentic glutathione.

An adduct between AEME and glutathione was detected and its structure elucidated by mass spectrometry. Identification of this adduct suggests that AEME can covalently link with cellular constituents which may explain in part toxic responses to smoked cocaine. Further studies on the stability of the adduct will be conducted to establish the applicability of AEME-glutathione adduct as a standard material for forensic analysis.

**AEME, Glutathione, Adduct**

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