URINE COCAINE AND BENZOYLECGONINE
by Flame-Ionization Gas Chromatography

Principle:

Cocaine and benzoylecgonine are extracted from urine using a chloroform/ethanol solvent mixture. One aliquot is analyzed directly by flame-ionization gas chromatography for cocaine, while another is subjected to methylation to convert benzoylecgonine to cocaine prior to chromatography. The difference between the two results represents the benzylecgonine concentration.

Reagents:

Stock solutions -
1 mg/ml cocaine in methanol
1 mg/ml benzylecgonine (Applied Science) in methanol

Urine standards -
1, 2, 5 and 10 mg/l for cocaine and
10, 20, 50 and 100 mg/l for benzoylecgonine

Quality control urine -
5 mg/l cocaine and 50 mg/l benzoylecgonine in urine containing 0.5% NaF and adjusted to pH6 (stable for 1 month at 4° C.)

Internal standard -
50 mg/l butyl anthraquinone (Aldrich) in chloroform

Extracting solvent -
chloroform/ethanol, 80/20 by volume

Methylating reagent -
add 1 volume conc. H_2SO_4 slowly to 2 volumes of Methanol
Ether
Sodium Bicarbonate -
solid $NaHCO_3$

Instrumental Conditions:

Gas chromatograph with flame-ionization detector
1 m X 2 mm i.d. glass column containing 3% OV-17 on 80/100 mesh Supelcoport
Injector, 290° C; column, 220° C; detector, 290° C
Nitrogen flow rate, 30 ml/min.

Procedure:

1. Transfer 5 ml of urine to a 50 ml glass-stoppered centrifuge tube and add 25 ml extraction solvent. Shake for 2 min. and centrifuge to separate layers.

2. Discard the upper aqueous layer and transfer 10 ml aliquots of the solvent to two separate 15 ml conical centrifuge tubes. Evaporate both to dryness at 55° C under a stream of air.

3. Cocaine determination: add 200 ul of the internal standard to one tube, rinsing the walls of the vessel to dissolve the residue. Inject 5 ul into the gas chromatograph.

4. Total cocaine/benzoylecgonine determination: add 0.6 ml methylating reagent to the second tube and vortex. Incubate at 85° C for 10 min.

5. Cool and wash the solution twice with 10 ml portions of ether. Centrifuge each time and discard ether layers. Evaporate remaining traces of ether under a stream of air at 55° C.

6. Add 1 ml water and sufficient solid $NaHCO_3$ to neutralize the solution. Add 200 ul internal standard and vortex for 2 min.

7. Centrifuge to separate layers and inject 5 ul of the lower organic layer into the gas chromatograph.

	Retention Time (min.)
Cocaine	3.7
Internal Standard	4.5

Calculation:

Calculation is based on a response factor derived from a standard curve. The benzoylecgonine concentration is obtained by subtracting the peak height ratio obtained in step 3 from that in step 7. A quality control specimen prepared as described is analyzed daily.

Evaluation:

Sensitivity: 0.2 mg/l
Linearity: 0.25-20 mg/l for cocaine, 0.5-100 mg/l for benzoylecgonine
C.V.: 5% within-run
Relative recovery: not established

Interferences:

Drug-free urine specimen yielded apparent cocaine concentrations averaging 0.14 mg/l.
Propoxyphene and amitriptyline were found not to interfere in the procedure.

Reference:

J.E. Wallace, H.E. Hamilton, D.E. King et al. Gas-liquid chromatographic determination of cocaine and benzoylecgonine in urine. Anal. Chem. 48: 34-38, 1976.