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# Screening and Identification of Cocaine

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## Introduction

Several studies have demonstrated that cocaine (CO) is extensively metabolized in man. The main metabolites in urine are benzoylecgonine (BE) and ecgonine methyl ester (EME) which are excreted mainly free in urine. BE and EME are formed from CO by the hydrolysis of either alkyl ester or phenyl ester bond. In urine parent CO, if detectable at all, is present in extremely low concentrations.

BE has been suggested to be the most sensitive indicator of cocaine use. Due to its chemical nature (amphoteric, very polar and hydrophilic), BE is very often screened separately from other drugs. Methods commonly used include immunoassay, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC) with or without mass spectrometry (MS). In laboratories involved in doping control an increasing number of separate screening procedures adds to the workload and expenses and thus should be avoided.

The aim of this work was to investigate whether it is possible to detect the use of cocaine by the routine analytical procedures in use in doping control laboratories. The applicability of the screening procedures for stimulants, anabolic steroids and beta-blockers to detect BE, EME and CO was studied. An excretion study after oral administration of cocaine was also carried out. For comparison, the urine samples were analyzed also by means of a commercial immunoassay.

## Experimental

*Excretion study.*
A 22 mg oral dose of cocaine hydrochloride was given to a volunteer. Urine specimens were collected up to 48 hours after the drug ingestion. The samples were stored frozen until analysis.

*Screening procedure I (stimulants).*
5 ml of urine was adjusted to pH 9.6 and extracted with 2 ml of diethyl ether using salting out. Diphenylamine was used as an internal standard. Samples were analyzed on GC (a Hewlett-Packard HP 5890A GC equipped with a nitrogen-phosphorous detector). The fused-silica capillary column was Ultra-2 (Hewlett-Packard), 12 m x 0.2 mm with a 0.33 $\mu$m film thickness. Carrier gas was helium (1.1 ml/min at 100 °C). The oven was programmed from 100 to 310 °C at 20 °C per minute. Split injection (1 $\mu$l, 1:17) was done at 280 °C.

*Screening procedure II (beta-blockers).*
5 ml of urine was hydrolyzed enzymatically (Helix Pomatia) at pH 5.2 for 1.5 hours at 55 °C. After washing with 5 ml of diethyl ether the aqueous phase was extracted with 1 ml of t-butanol and 5 ml of diethyl ether at pH 9.6 using salting out. After evaporation the dry residue was derivatized with 100 $\mu$l of MSTFA (5 min at 60 °C) and 30 $\mu$l of MBTFA (5 min at 60 °C). Dextromethorphan was used as an internal standard. Samples were analyzed on GC/MS (HP 5890A GC combined with HP 5970B mass-selective detector). The column was Ultra-2 with dimensions as described above. Carrier gas was helium (1.1 ml/min at 100 °C). The oven was programmed from 120 to 310 °C at 15 C per minute. Split injection (3 $\mu$l, 1:16) was done at 280 °C. MS was operated in SIM-mode (ions 82, 182 m/z for CO and 82, 240 m/z for O-TMS-derivative of BE).

*Screening procedure IVa (anabolic steroids/free fraction).*
The extraction procedure was as in screening I. Stanozolol was used as an internal standard. After extraction, samples were derivatized with 50 $\mu$l of MSHFBA/TMSCl/TSIM (100:5:3) for 5 min at 60 °C and 100 $\mu$l of MBHFBA/acetonitrile (1:9) at 80 °C for 20 min. Samples were analyzed on GC/MS (HP 5995A). The column was Ultra-1 with dimensions as described above. Carrier gas was helium (1.1 ml/min at 150 °C). After 2 min at 170 °C the oven was programmed to 310 °C at 25 °C per minute. Splitless injection (3 $\mu$l) was done at 280 °C. MS was operated in SIM-mode (monitored ions were the same as described above).

*Screening procedure IVb (anabolic steroids/combined fraction).*
2.5 ml of urine was introduced into Sep-Pak C18 cartridge. After washing with water (3 ml), the sample was eluted with 3 ml of methanol and evaporated to dryness. The residue was dissolved in 1 ml of phosphate buffer of pH 7 and hydrolyzed enzymatically (beta-glucuronidase from Escherichia coli) for 1 hour at 60 °C. After adjusting pH to 11, the sample was extracted with 5 ml of diethyl ether using salting out. After evaporation the dry residue was derivatized with 50 $\mu$l of MSTFA/TMSI/dithioertyhritol (1000:2:4) for 15 min at 60 °C. Methyltestosterone was used as an internal standard. Samples were analyzed on GC/MS (the same instrument as in screening IVa). Oven was first programmed from 160 to 200 °C at 12 °C per min and then to 226 °C at 2 °C/min and finally to 300 °C at 10 °C/min. Split injection (3 $\mu$l, 1:15) was done at 280 °C. MS was operated as described in screening IVa.

*Immunoassay of benzoylecgonine.*
Fluorescent polarization immunoassay (FPIA) was performed using TDx-system from Abbot according to the manufacture's instructions.

*Quantitation.*
CO and BE were quantitated using screening procedure II. Spiked urine samples (0.1- 5 $\mu$g/ml of CO and BE) were used as standards.
EME was semiquantitated using screening procedure I. Because EME was not available as a pure substance the concentration of EME was estimated using urine samples spiked with CO (0.3 - 5 $\mu$g/ml) as standards.

**Results**

EME can be screened using procedure I for stimulants (figure 1). The relative retention to diphenylamine was 0.83. It elutes very near to phendimetrazine. The concentration down to 0.3 $\mu$g/ml can be detected but 1 $\mu$g/ml can be regarded as a reasonable limit for screening. The mass spectrum of EME is presented in figure 2.

Both BE and CO can be screened by screening II for beta-blockers (figure 3). CO is analyzed underivatized and BE as its O-TMS -derivative. Concentrations down to 0.05 - 0.1 $\mu$g/ml can be detected. The relative retention times to dextromethorphan were 1.11 and 1.06 for BE and CO, respectively. They both elute very near to the O-TMS,N-TFA -derivative of parent propranolol. The mass spectra are presented in figure 2.

Concentrations of BE gave a good correlation with the results measured by fluorescent polarization immunoassay (figure 4).

BE can also be screened using screening procedures IVa and IVb for anabolic steroids. In screening IVa for free steroids the relative retention time to stanozolol was 0.87. In screening IVb for conjugated steroids the relative retention time to methyltestosterone was 0.40 which means that it elutes very near to the O-TMS -derivative of parent probenecid.

The urine specimens collected after oral administration of cocaine were found to contain cocaine and/or metabolites. The concentration of EME was highest (about 3.7 $\mu$g/ml) in the specimen collected 2-5 hours after ingestion. EME was still detectable at 30-36 hours the concentration, however, being quite low. CO was detectable only for a few hours after ingestion in very low concentrations (0.1-0.2 $\mu$g/ml). On the other hand, BE which appeared to reach a peak level at 8-14 hours (6.8 $\mu$g/ml) was still detectable 48 hours after administration. The results of the excretion study are summarized in figure 5.

## Conclusions

It is shown that the screening procedures generally used in doping control laboratories are very suitable for the detection of cocaine use as well. Both BE and EME, the most important urinary metabolites of cocaine, can be measured by these methods. The sensitivity obtained for BE is at least as good as with most immunoassays. Commercial immunoassays do not detect EME at all.

The excretion study confirmed that CO is rapidly and extensively metabolized to BE and EME. Both metabolites were detectable in urine for a long time after cocaine ingestion. The urinary concentrations of both were about at same level. CO itself was present in urine only for a few hours and in low quantities.

To detect the use of cocaine, screening of both EME and BE is recommended. EME can easily be measured using screening I for stimulants. Screening of BE can be included in the screening procedures for either beta-blockers or anabolic steroids. For mass spectrometric confirmation the same extraction and chromatographic conditions as in screening procedures can be used.

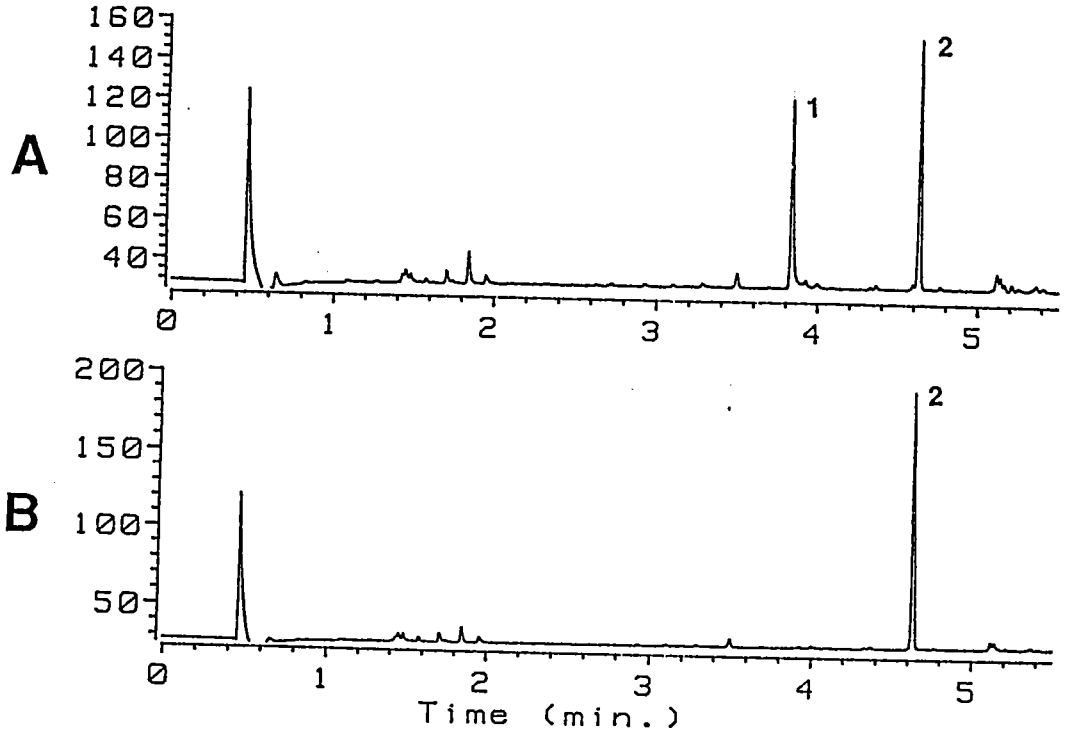


Figure 1.
Screening of ecgonine methyl ester (EME) by screening procedure I. Chromatograms of (A) urine collected 2 - 5 hours after oral administration of 22 mg of cocaine hydrochloride and (B) blank urine. The peaks are (1) EME and (2) diphenylamine (internal standard).

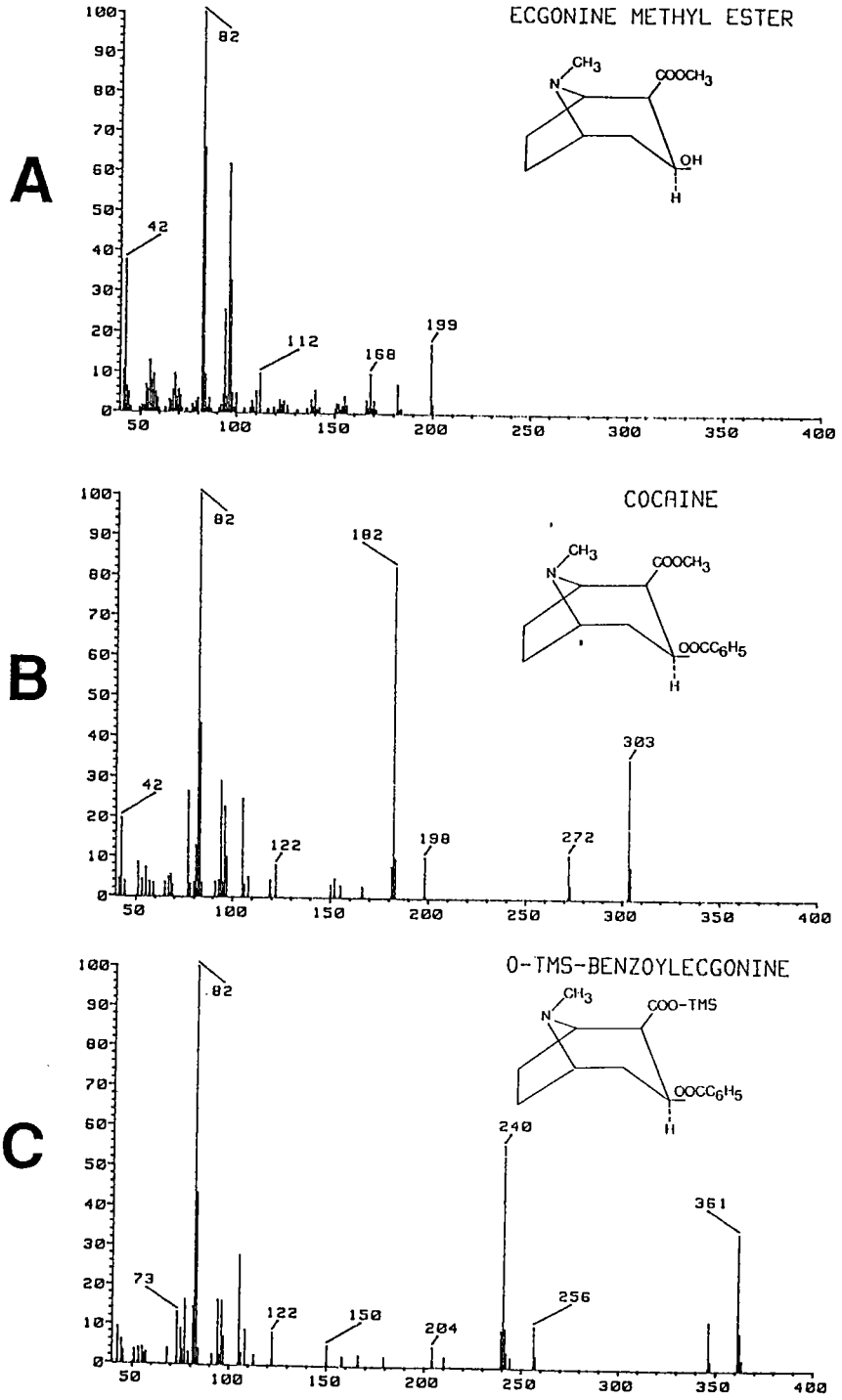


Figure 2.
Mass spectra of (A) ecgonine methyl ester, (B) cocaine and (C) O-TMS -derivative of benzoylecgonine.

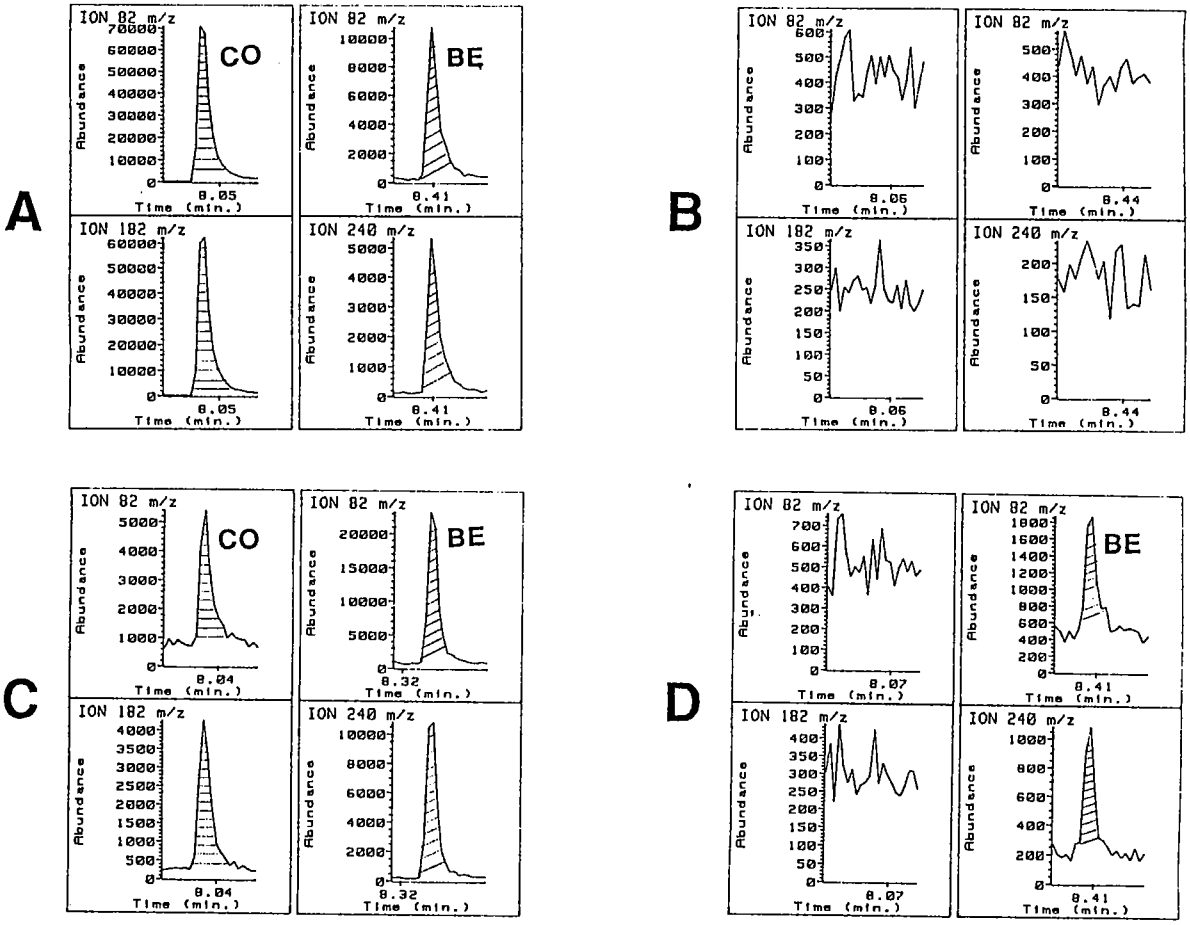


Figure 3.
Screening of cocaine (CO) and benzoylecgonine (BE) with screening procedure II. Selected ion chromatograms with ions (m/z) 82, 182 for CO and 82, 240 for BE. (A) spiked urine containing 5 $\mu$g/ml of CO and 2 $\mu$g/ml of BE, (B) blank urine, (C) urine collected 2 - 5 hours after oral administration of 22 mg of cocaine hydrochloride and (D) urine collected 45 - 48 hours after administration of cocaine.

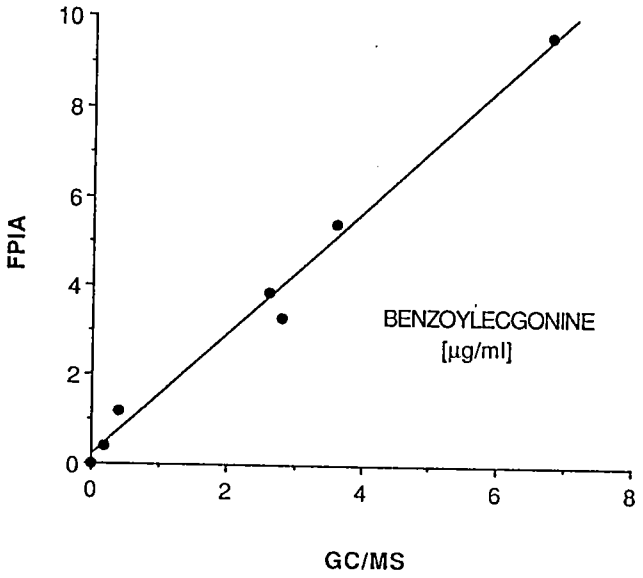


Figure 4.
Correlation of urinary benzoylecgonine as measured by GC/MS (screening procedure II) and fluorescent polarization immunoassay (FPIA). Urine samples originated from the excretion study were analyzed.

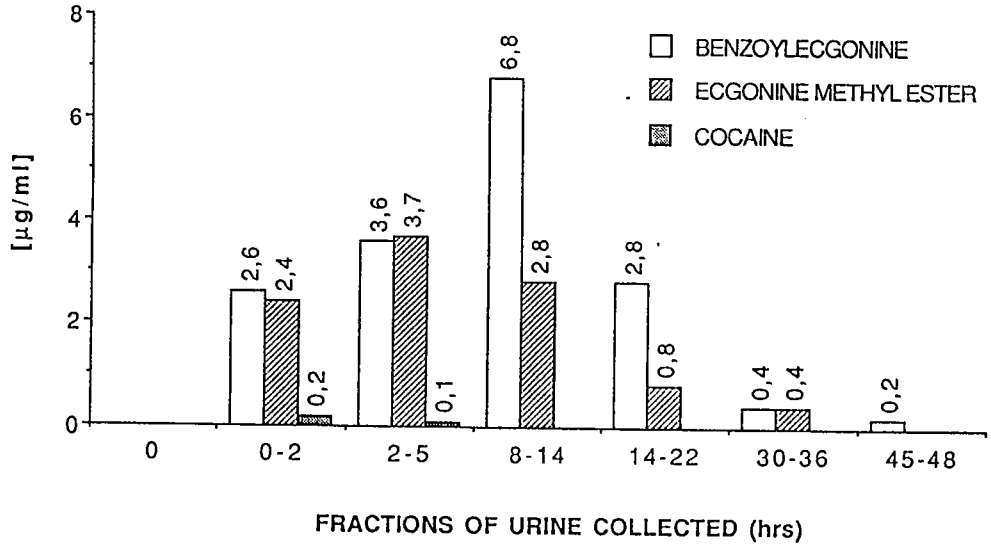


Figure 5.
Excretion of cocaine and its main metabolites in urine after oral administration of 22 mg of cocaine hydrochloride. Concentrations of cocaine and benzoylecgonine were determined by GC/MS in screening procedure II. Ecgonine methyl ester was semiquantitated by GC in screening procedure I.