Laboratory Testing for Cocaine

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Laboratory testing for benzoylecgonine, the principle metabolite of cocaine, is becoming increasingly common. While this article deals specifically with analysis of benzoylecgonine, the general principles of chain of custody, security seals, and consent for testing, apply to all drugs of abuse testing.

Increasingly, America is becoming a litigious society. Its marquee is "If you don't like it, sue the SOB." Nowhere is this more apparent than in the area of consumer business law. The following guidelines have resulted from an industry concern for maximizing the accuracy of sample identification and laboratory results while minimizing its exposure to litigation.

The analysis of a sample for the presence of a foreign substance may be broken down into three major phases. These three are:

1. correct identification of applicant
2. sample collection and chain of custody
3. laboratory testing and reporting

While 1 and 2 do not deal directly with the laboratory analysis per se, all three must be correctly completed to assure accuracy of analysis.

At the time of collection, it is imperative that the applicant be correctly identified. This is accomplished by the paramed or examiner obtaining a photo identification of the applicant; normally this is a driver's license.

After identification of the applicant, the sample should be collected in a fashion that does not compromise the privacy of the applicant and yet assures the paramed that a sample was voided. The paramed can accomplish this in two ways. By positioning oneself adjacent to the restroom, the examiner can assure himself that a sample was voided. Likewise, the examiner should immediately take the temperature of the sample and record this on the consent form and observe the sample for color and foam. By following these simple steps, the paramed is reassured of the sample's integrity.

Following the collection, the applicant is asked to initial a security tape and to then place it over the capped urine container. The tamper evident seal is to assure the applicant and the testing laboratory that no one has opened the container prior to its analysis. The security seal and modified chain of custody was initiated in June of 1988. As of March of 1989 approximately 83% of urine samples have a security seal affixed. Each month the percentage of samples with the security seal continues to increase.

The sample along with the required completed paperwork is placed in a shipping container, sealed, initialed at the top of the chain of custody bag by the applicant, signed and dated by the applicant and paramed. The signatures provide the required legal chain of custody documentation. The chain of custody document and the security seal assure the laboratory that the sample that is analyzed in the laboratory was correct and not tampered with after collection. The sample is then sent to the laboratory for analysis.

Upon arrival in the laboratory the sample is signed in. The following three things are documented: whether a security seal was present; if present, the integrity of the seal; and the date and initials of the laboratory person who opened the chain of custody bag and shipping container. The sample identification is verified to correspond with the consent form. A unique barcode number is affixed to the sample and the consent form. The sample is then ready for analysis. Laboratory analysis is segregated into two major operations; screening and confirmation.

Screening for benzoylecgonine is accomplished by reaction of a small aliquot of sample with an antibody that is reactive with cocaine and its metabolites. A number of different commercial products currently use the competitive antibody approach to identify initially reactive samples. These include, but are not limited to Roche RIA, Syva EMIT, Abbott TDX or ADX and various solid phase enzyme linked immunosorbent assays (ELISA). All have inherent limitations due to serological cross-reactivity with drugs other than cocaine. As a direct consequence, all initially reactive samples require confirmation by a technique that has the ability to differentiate benzoylecgonine from all other chemicals.

When a sample has screened presumptive positive for benzoylecgonine, the medical technologist initials the results and initials the log book entry for the sample. This provides the necessary legal documentation for chain of custody and also documents that a certified medical technologist has performed the assay.

Prior to GC/MS confirmation testing of the urine, an internal standard of 500 ng/ml of benzoylecgonine-d_3 (3-benzoyloxy-8 [methyl-d_3] – 8-azabicyclo [3.2.1] octane-2-carboxylic acid, Sigma Chemical Company, St. Louis, MO) is added to each

sample. This internal standard is the same chemical structure as benzoylecgonine with one exception. The hydrogen atoms on the 8-methyl group have been replaced with the deuterium isotope of hydrogen. This gives the internal standard a mass that is three atom units greater than non-enriched benzoylecgonine. The d_3 internal standard and the benzoylecgonine share identical chemical properties as far as solid phase extraction, reactivity with derivatization reagent and migration on the gas chromatograph are concerned.[1,2]

A presumptive positive sample is prepared for gas chromatography/mass fragmentation spectroscopy by solid phase extraction. Solid phase extraction offers a number of advantages over aqueous/organic (liquid/liquid) extraction. The advantages include automation of extraction, better selectivity for the drug of interest and, subsequently, less contamination of the mass fragmentation detector. Cocaine and its metabolites are isolated from urine by solid phase extraction on a bonded silica particle column. These columns are specially prepared and tested for extraction of benzoylecgonine and cocaine (Bond Elut Certify, Analytichem International, Harbor City, CA).

Following the solid phase extraction of the urine sample, the column eluate is concentrated by evaporation under nitrogen gas. The dried sample is then derivatized with pentafluoropropionic anhydride in pentafluoropropanol by heating at 60°C for 15 minutes.[3] The sample is again dried under nitrogen. Following evaporation of the solvent the residue is dissolved in ethyl acetate and the solution transferred to an autosampler vial.

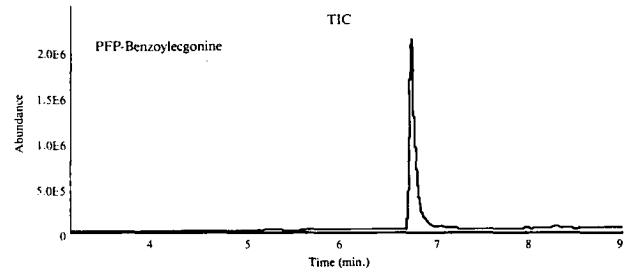
A quick word about derivatization. An organic molecule that contains hydrophilic groups such as carboxylic acids, amines and or alcohols does not resolve or separate well during gas chromatography (GC). In order to optimize the separation on GC, the hydrophilic groups are modified with a chemical or chemicals. The modification is designed to reduce the hydrophilicity of the molecule, increase the chemical volatility, reduce the chemical's interaction with the solid phase of the GC column increase its partitioning into the carrier gas and/or provide reporter groups for detection. These effects by themselves or combined provide for increased resolution in shorter elution times on the GC.

The Analysis

Gas chromatography/mass fragmentation spectroscopy is based on two technologies. Gas chromatography provides a technique for the separation of a drug of interest away from all or most other chemicals that are present. Separation of the drug is extremely important where very complex mixtures are being analyzed. This is certainly the case for urine and blood samples. Separation of a complex mixture is accomplished by adjusting initial and final temperature gradients to achieve the desired resolution of benzoylecgonine. A number of different strategies have been developed that successfully resolve benzoylecgonine. The one presented in this paper was initially reported by Mulé and Casella (1988).[4] The particulars for the temperature gradient and column selection are reported in Figure 1.

Figure 1

Gas Chromatography of Pentafluoropropyl derivative of Benzoylecgonine.



500 ng of Benzoylecgonine was reacted with 40 µl pentafluoropropionic anhydride and 20 µl pentafluoropropanol at 60° for 15 minutes. The solvent was evaporated with a stream of dry nitrogen. The residue was dissolved in 50 µl of ethyl acetate. A one (1) microliter sample was injected.

The GC column was 5% Phenyl Silicone Gum Phase, 530 microns by 12.5 meters. The column was developed with a linear temperature gradient of 60° to 280°C over ten minutes. The injector temperature was 220°C. The GC to MS transfer line was set at 280°C.

These data were acquired as a total ion spectra.

The Mass Fragmentation Spectra

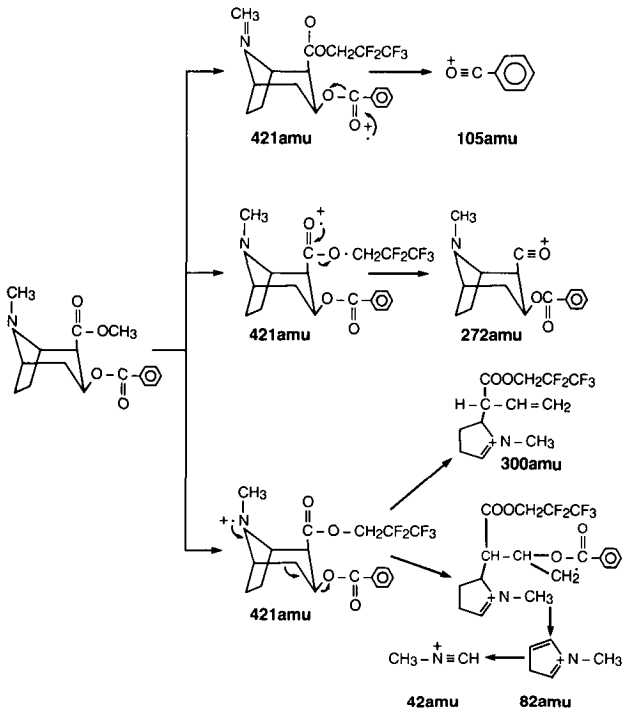
The mass fragmentation spectra reported in this article are produced on a single focusing magnetic sector mass spectrometer with electron ion source. Quite a mouthful; here is what that means. The Hewlett Packard Mass Selection Detector (HP MSD) electrically charges chemicals to be analyzed by subjecting them to a beam of electrons. Following ionization, the chemical is accelerated into a single focusing quadrupole magnet where it is focused depending on its mass to charge ratio. This allows the mass detector to print a molecular picture of the chemical and its fragmentation parts.

The pattern of fragmentation for a chemical is the result of four factors; (i) the strengths of the bonds which are broken, (ii) stability of the fragments, (iii) the internal energy of the fragmenting ions and (iv) time interval between ion formation and detection. These factors produce unique molecular fingerprints for a particular chemical. By comparing both elution times and the mass fragmentation patterns of an unknown and the appropriate standards, it is possible to provide unambiguous identification of all drugs of abuse. As an example the fragmentation of pentafluoropropylbenzoylecgonine is presented in Figure 2.

The proposed fragmentation pattern should produce ions with mass to charge ratios of 421, 316, 300, 272 and 82. A benzoylecgonine positive urine sample was extracted and derivatized as described in this article. A total ion mass spectra was prepared on an HP MSD 5970. A representative spectra is presented in Figure 3. The lower scan is the total

Figure 2

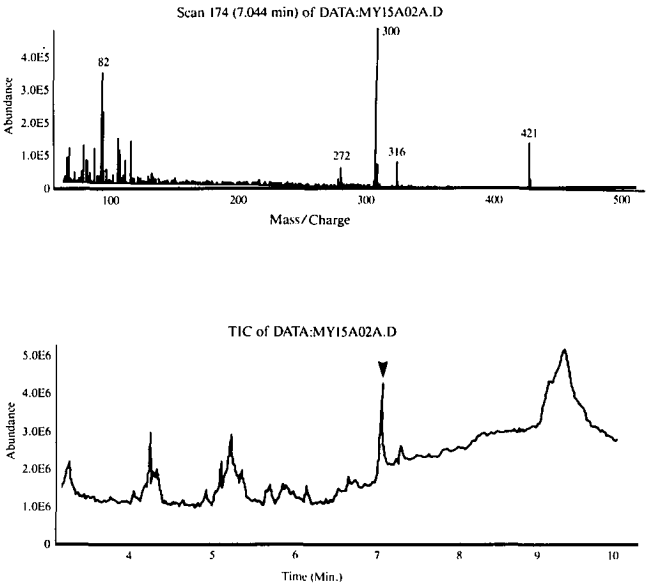
Major Electron Impact Fragmentation Pattern of Pentafluoropropyl-benzoylecgonine.



*The methyl group attached to the ring nitrogen group is the site of replacement of the 3 hydrogen atoms by 3 deuterium atoms for the internal standard.

Figure 3

GC/MS of a Benzoylecgonine Positive Urine.



The lower window is the total ion chromatogram of a benzoylecgonine positive urine sample. The ions present in the chemical that eluted from the GC at 7.044 minutes are presented in the upper window. The elution time and the mass/charge ion pattern is identical to PFA-benzoylecgonine standard. This provides unambiguous identification of benzoylecgonine.

spectra as a function of time. The cursor (arrow) is set at the elution time for PFP-benzoylecgonine. The spectra for the compound eluting at 7.04 minutes is shown in the upper window. As predicted, all of the major ions for benzoylecgonine are present. This procedure provides the scientist with unambiguous identification, but how is quantitation accomplished on the GC/MS?

Quantitation of benzoylecgonine is accomplished by comparing the integrated peak at 300 Au with the integrated peak at 303 Au. The peak at 303 is the instrument response to the 500 ng/ml d3 labeled benzoylecgonine. If the peak at 300 Au (the unknown) is twice as large as the 303 Au peak then the concentration of benzoylecgonine is 1000 ng/ml. Likewise, if the peak was half the size of the 303 Au, the concentration of the unknown would be 250 ng/ml. This strategy provides the most reliable method for determination of the concentration of an unknown. The effect of differences in peak intensities is shown in Figure 4. The upper spectra is for a positive unknown sample; the peak is about 200,000 counts. The 500 ng internal standard should have a peak of about 2,000,000 and that is what was found. The concentration of this unknown is calculated to be 50 ng/ml.

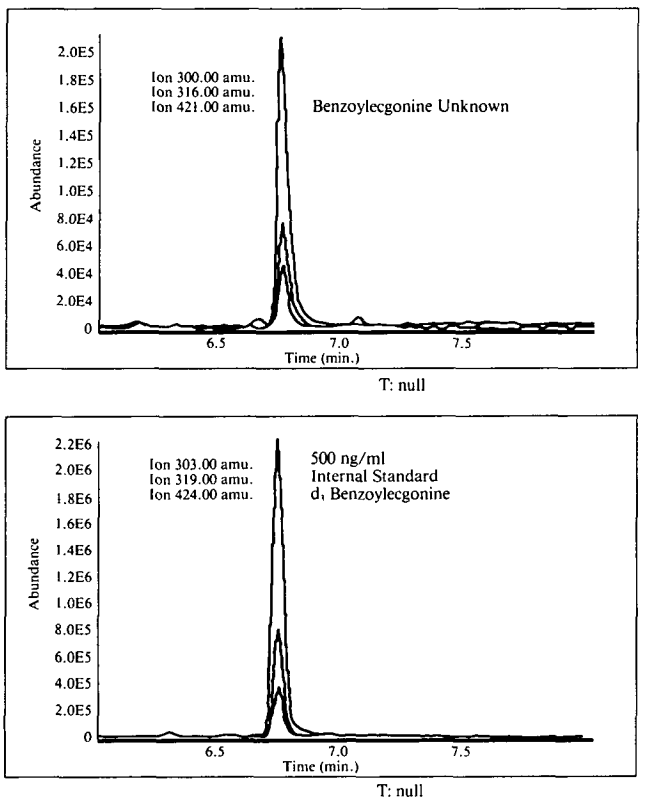
The procedures summarized in this article follow the recommendations of the National Institute of Drug Abuse (NIDA). These techniques provide a method for the definitive identification of benzoylecgonine. The Federal Court System has recognized the GC/MS confirmation as the "Gold Standard" for definitive, unambiguous identification of cocaine and its metabolites in urine. The GC/MS has proven to be the single most powerful tool for analysis of organic compounds. It is the method of choice in forensic toxicology.

Figure 4

Selected Ion Monitoring for Benzoylecgonine and d_3-Benzoylecgonine.

The difference in molecular mass for each of the ion pairs (300-303, 316-319, 421-424) is due to the change in mass associated with the substitution of 3 deuterium isotopes $^2H_3$ for 3 hydrogen atoms $^1H_3$ on methylene carbon C_8.

Selected ion monitoring for d_3 benzoylecgonine and benzoylecgonine are presented. Ions for benzoylecgonine are 300, 316, and 421 while d_3 benzoylecgonine is monitored at 303, 319, and 424 mass/charge units. Ions for both drugs show an identical elution time. Derivatization and chromatography are the same as Figure 1.



The calculated concentration of the unknown is

$$\frac{200{,}000}{2{,}000{,}000} \times 500 \text{ ng/ml} = 50 \text{ ng/ml}$$

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