Quantitation of Cocaine by Gas Chromatography-Flame Ionization Detection Utilizing Isopropylcocaine as a Structurally Related Internal Standard

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ABSTRACT: The quantitation of cocaine by gas chromatography-flame ionization detection using isopropylcocaine as a structurally related internal standard is presented. The selectivity, precision, and accuracy of the method are detailed. The facile, multi-gram synthesis of isopropylcocaine standard from cocaine (via two different routes) is described.

KEYWORDS: Isopropylcocaine, Synthesis, Gas Chromatography, Flame Ionization Detection, Cocaine Quantitation, Internal Standard, Forensic Chemistry

Introduction

The analysis of cocaine exhibits has been a major task in forensic, crime, and toxicological laboratories over the past 20 - 25 years. Federal Sentencing Guidelines (1), as well as some state criminal statutes, require quantitative analysis of cocaine exhibits. In addition, the accurate assay of cocaine is also a critical element for laboratories that are conducting cocaine signature analyses (2-3). In the DEA's Cocaine Signature Program (CSP), target alkaloids are quantitated relative to the amount of cocaine present, not to the total sample weight. Therefore, even samples that are cut, either with an adulterant or a diluent, can still be analyzed for signature purposes as though they were uncut. However, this technique requires highly accurate quantitations of all of the target alkaloids.

Several gas chromatographic methods have been developed and validated for cocaine quantitation (4-8). These methods utilize an internal standard (ISTD) to improve the precision of the quantitative analysis; however, the ISTDs utilized in these studies (tetraphenylethylene, morphine HCl, cyclobenzapine HCl, methylpalmitate, or eicosane) are not structurally related to cocaine, and in fact in most instances have dissimilar chemical and physical properties. Thus, the presence of impurities, possible acid hydrolysis of cocaine (9), dirty injection ports, and the formation of artifacts (10), can decrease the accuracy of the assay (11). The use of a structurally related internal standard (SR-ISTD) minimize the factors that affect the resulting analyte signal (in this case cocaine), since the SR-ISTD will have virtually the same response to the detector (3). The gas chromatographic method presented herein employs isopropyl cocaine as the SR-ISTD. Isopropylcocaine is not commercially available; however, also as described herein it can be synthesized from cocaine and commercially available reagents (see Figure 1, next page).

Experimental

Materials: Pharmaceutical cocaine base and hydrochloride were obtained from Merck Chemical (Rahway, NJ). Chloroform was a distilled-in-glass product of Burdick and Jackson Laboratories (Muskegon, MI). All other

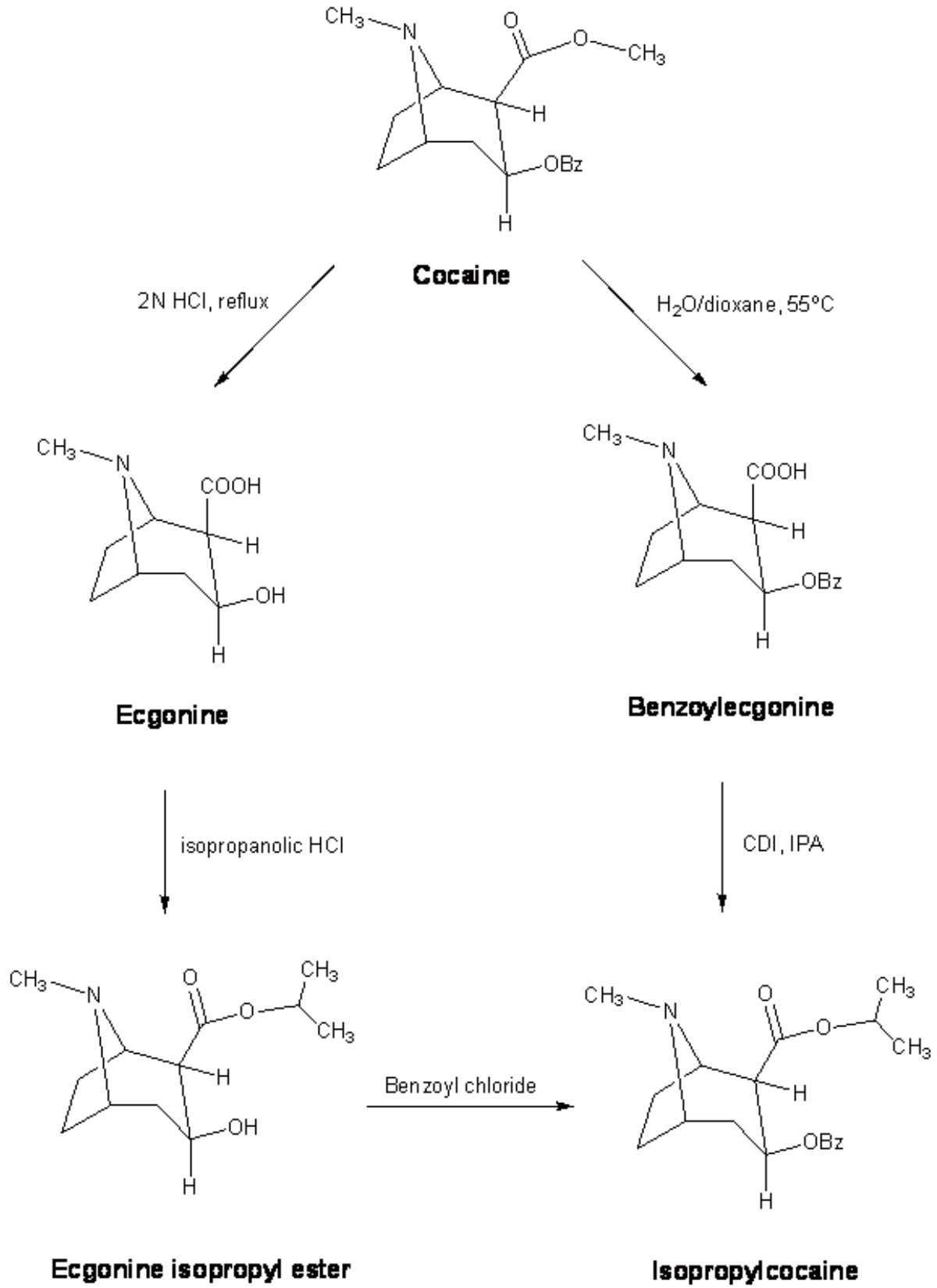

Figure 1. Synthetic Routes to Isopropylcocaine.

reagents and chemicals were reagent-grade quality products of the Sigma-Aldrich Chemical Company (Milwaukee, WI). Illicit refined cocaine HCl was acquired from the reference collection of this laboratory.

Syntheses (Acid Hydrolysis Route):
Ecgonine HCl: Refined illicit cocaine HCl (250 grams, 0.736 mol) was combined with water (500 mL) and concentrated hydrochloric acid (25.0 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed, with stirring, for 6 days. Benzoic acid precipitated from the solution upon cooling. The reaction mixture was extracted with chloroform (5 x 500 mL) to remove benzoic acid and methyl benzoate. The aqueous phase was then added slowly, with stirring, to acetone (7.2 liters) to precipitate ecgonine HCl. The precipitate was captured via suction filtration, washed with additional acetone (1.5 liters), then dried to provide ecgonine HCl as a white powder (107 grams, 65 percent yield).

Ecgonine Isopropyl Ester HCl: Ecgonine HCl (40.0 grams, 0.18 mol) was combined with isopropanolic HCl (2.0 liters, 0.14 grams/mL) in a 5-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed, with stirring, for 3 days. The isopropanol was evaporated *in vacuo* to an oil. The oil was dissolved in water (500 mL), adjusted to pH 10 with concentrated NaOH, and extracted with methylene chloride (3 x 200 mL). The combined extracts were washed with water (3 x 400 mL) and brine (200 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to a clear oil (42.0 grams). The oil was dissolved in anhydrous diethyl ether (500 mL), and isopropyl ecgonine HCl was precipitated by adding ethereal HCl (0.05 grams/mL) until a pH of 4 was achieved. The ether was decanted from the crystalline product, and acetone (400 mL) added with stirring. The product was captured via suction filtration, washed with additional acetone (400 mL) and diethyl ether (400 mL), then dried to provide ecgonine isopropyl ester HCl as a white powder (32.0 grams, 67 percent yield).

Isopropylcocaine: Ecgonine isopropyl ester HCl (31.1 grams, 0.118 mol) was combined with pyridine (200 mL) and benzoyl chloride (19.8 grams, 0.142 mol) in a 1-liter round-bottom flask fitted with a drying tube. After stirring for 1 hour, acetone (400 mL) was added to precipitate isopropylcocaine HCl. The product was captured via suction filtration, washed with additional acetone (2 x 200 mL) and diethyl ether (200 mL), then dried to provide isopropylcocaine HCl as a white powder containing a small amount of pyridine HCl. The product was dissolved in water (100 mL), adjusted to pH 9 with solid sodium carbonate, then extracted with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to provide isopropylcocaine HCl as a white crystalline powder (31.6 grams, 81 percent yield, 99+ percent purity).

Syntheses (Base Hydrolysis Route):
Benzoylecgonine: Pharmaceutical cocaine base (70.6 grams, 0.233 mol) was combined with water (250 mL) and dioxane (350 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was heated at 55 ^{O}C for 9 days. The reaction mixture was evaporated *in vacuo* to provide crude benzoylecgonine tetrahydrate as a white powder. The powder was washed with diethyl ether (2 x 400 mL) to remove any remaining cocaine base, then dried to give 67.5 grams of benzoylecgonine tetrahydrate. The product was dissolved in boiling acetone (750 mL), cooled to room temperature, diluted with diethyl ether (2.25 liters), and allowed to stand overnight at 5 ^{O}C. The resulting crystalline product was captured via suction filtration, washed with additional diethyl ether (600 mL), then dried to provide anhydrous benzoylecgonine as a white powder (53.5 grams, 79 percent yield).

Isopropylcocaine: Anhydrous benzoylecgonine (30.7 grams, 0.106 mol) was combined with methylene chloride (500 mL) and 1',1'-carbonyldiimidizole (17.2 grams, 0.106 mol) in a 1-liter round-bottom flask fitted with a drying tube, and stirred overnight. Isopropanol (26.8 grams, 0.447 mol) was added, and the solution was stirred for 6 days. The reaction was extracted with 3 N HCl (2 x 200 mL). The combined aqueous extracts were washed with methylene chloride (200 mL), adjusted to pH 9 with concentrated ammonium hydroxide, and extracted with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give a clear oil (30.0 grams). The oil was dissolved in petroleum

ether (20 - 40 ^{O}C boiling range, 300 mL) and allowed to stand overnight, resulting in precipitation of the imidizole by-product. The solution was suction filtered to remove this byproduct, and the filtrate evaporated *in vacuo* to give a clear oil which crystallized upon standing. This was recrystallized from petroleum ether, then dried to provide isopropylcocaine base as a white powder (23.3 grams, 66 percent yield, 99+ percent purity).

Gas Chromatography - Flame Ionization Detection (GC-FID): Analyses were performed with an Agilent (Palo Alto, CA) Model 6890N gas chromatograph. One mL of the prepared solutions was placed into an autosampler vial for analysis under the following conditions: A 30 m x 0.25 mm ID fused-silica column coated with 0.25 µm HP-1 (Agilent) was used. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/minute. The injection port and flame ionization detector were maintained at 280 ^{O}C. Samples (2 µL) were injected in the split mode (25:1) by an Agilent 7683 Series Auto Injector. The oven temperature was programmed isothermally at 250 ^{O}C for 7.00 minutes. Nitrogen was used as the auxiliary make-up gas for the detector.

Structurally Related Internal Standard Stock Solution: Isopropylcocaine base was dissolved into chloroform at a concentration of 0.9 mg/mL (equivalent to 1.0 mg/mL isopropylcocaine hydrochloride). The solution was stored at 4 ^{O}C when not in use. Solutions can be stored for one year at 4 ^{O}C without detectable degradation. The solution should be warmed to room temperature before use.

Standard Solutions for Linearity Study and Calibration: Individual solutions containing 0.038, 0.087, 0.23, 0.44, 0.63, 0.83, 1.00, 1.53, and 2.03 mg/mL of cocaine base in chloroform were prepared. Each also contained 0.18 mg/mL of the SR-ISTD.

Standard and Sample Preparation: About 18 to 20 mg of cocaine hydrochloride (or 16 to 18 mg for cocaine base) was accurately weighed (to the nearest 0.01 mg) into a 50 mL Erlenmeyer flask, and 5.0 mL of the SR-ISTD stock solution and 20 mL of chloroform containing 50 µL of diethylamine were added. The solutions were allowed to sit for 5 minutes. Aliquots of standard and sample solutions were transferred to autosampler vials for analyses.

Results and Discussion

The synthesis of isopropylcocaine is relatively simple, and can be performed on a large scale with common glassware and reagents. The mass spectrum of isopropylcocaine is illustrated in Figure 2. Isopropylcocaine was selected as the SR-ISTD for several reasons. Its close structural similarity to cocaine means it will have a similar FID response. Second, it has excellent chromatographic properties, and does not interfere with any other coca alkaloids or commonly encountered diluents and adulterants (see Figure 3 for chromatographic profiles of illicit cocaine base and illicit cocaine HCl). Third, only a small amount (about 5 mg) is needed for each analysis. Fourth and finally, it was found to be very stable. A stock solution stored for up to one year at 4 ^{O}C in chloroform yielded no detectable hydrolysis or degradation products, and produced the same number of integrated area counts over that entire time frame.

The linearity of the method was confirmed over the concentration range listed in the Experimental Section, and linear regression analysis determined the correlation coefficient (R^2) as 0.9999 (Figure 4). The average error difference between the known concentrations and the predicted concentrations was +/- 0.75 percent between 0.44 mg/mL and 1.53 mg/mL. For routine analyses, a single point calibration of approximately 0.75 mg/mL was used. Method selectivity was excellent; the identities and retention times of some common adulterants and diluents using the presented methodology are shown in Table 1 (reported retention times are relative to cocaine). Other coca alkaloids and common cutting agents do not interfere with cocaine or isopropylcocaine. The precision of the method was determined using the nine linearity concentrations, with five replicate injections per concentration. The resulting calculated Relative Standard Deviation (RSD) for each concentration was less than 0.21 percent, and in some instances was as low as 0.02 percent. The accuracy of the method was tested over a 14 month period by having eleven different chemists quantitate a secondary cocaine standard (having a known cocaine

concentration of 84.6 percent) against the pharmaceutical cocaine standard during routine casework. Over that time period, 188 quantitative observations for the secondary standard were recorded. The average value obtained was 84.7 percent, with a range of 83.4 - 86.0 percent. The RSD for all 188 observations was found to be 0.58 percent. The overall absolute error of the assay was determined to be less than 1 percent.

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[Table 1 and Figures 2 - 4 Follow.]

Table 1. Relative Retention Times (RRT) of Some Common Adulterants/Diluents and Coca Alkaloids.

Compound	RRT (min)
Ecgonine methyl ester	0.48
Benzocaine	0.49
Acetaminophen	0.52
Caffeine	0.61
Lidocaine	0.65
Procaine	0.76
Cocaine	1.00
Isopropylcocaine	**1.14**
cis-Cinnamoylcocaine	1.36
trans-Cinnamoylcocaine	1.78
Benzoylecgonine	1.89

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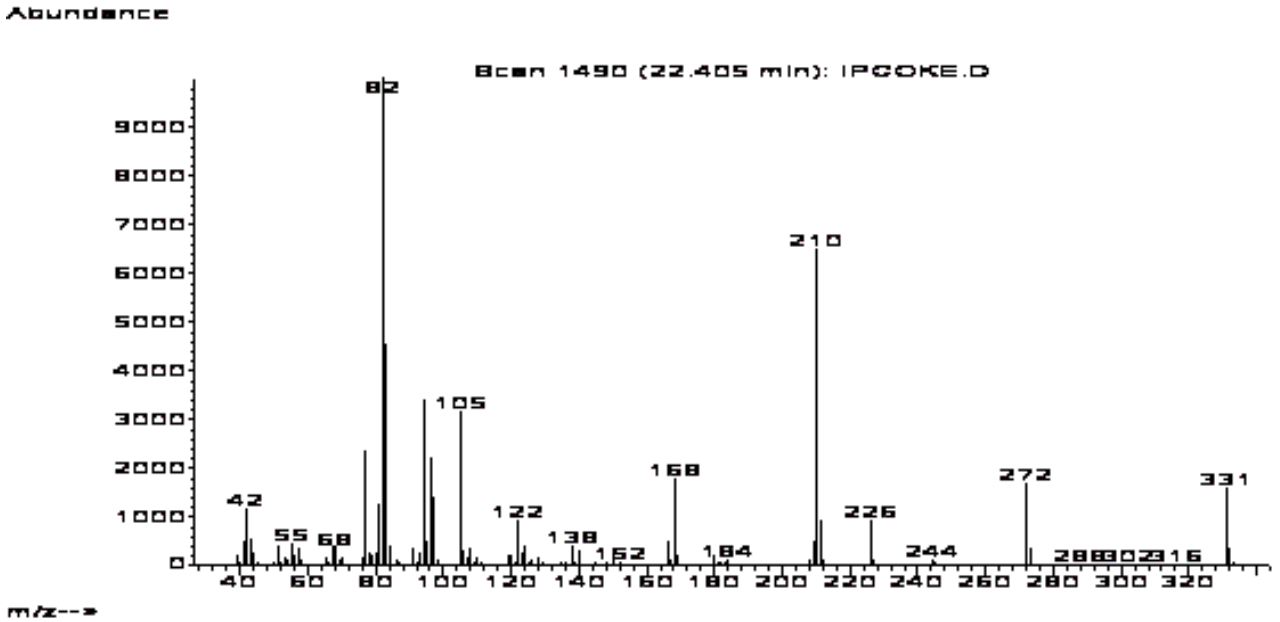

Figure 2. Electron Ionization Mass Spectrum of Isopropylcocaine.

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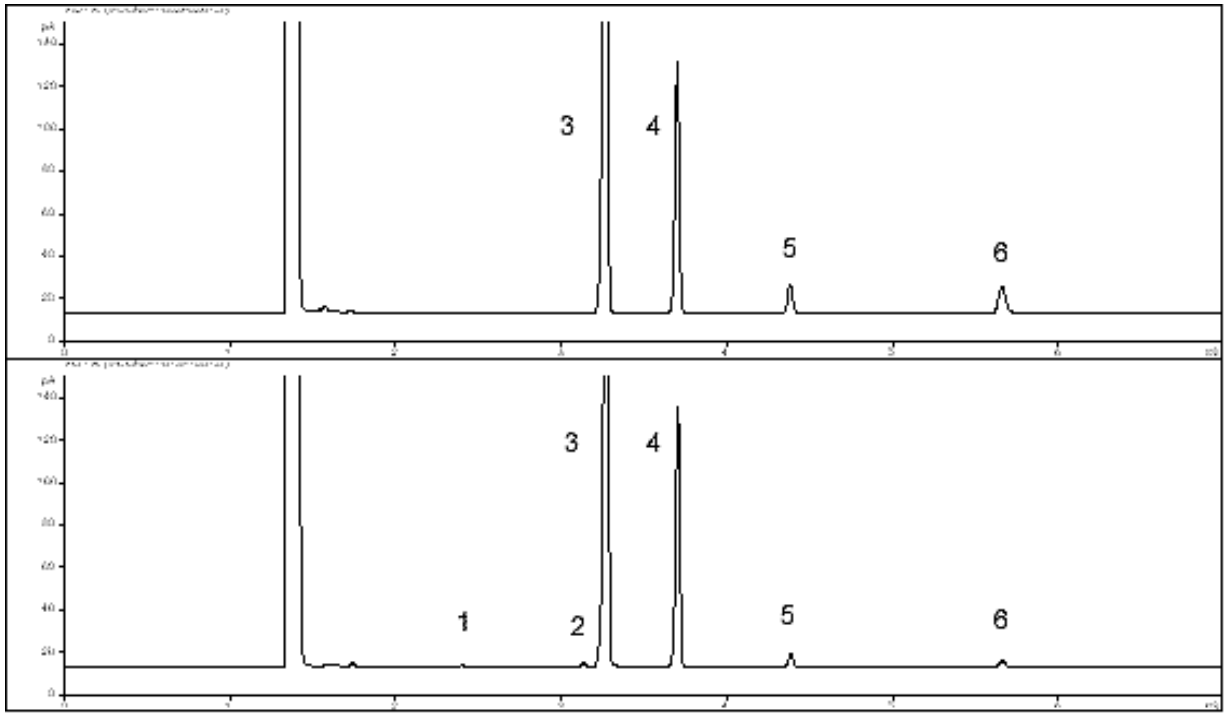

Figure 3. Capillary Gas Chromatographic Profiles of (Upper) 86.1 Percent Illicit Cocaine Base Exhibit and (Lower) 86.2 Percent Illicit Cocaine HCl Exhibit. Peak Identification: 1 = Tropacocaine, 2 = Norcocaine, 3 = Cocaine, 4 = Isopropylcocaine (SR-ISTD), 5 = *cis*-Cinnamoylcocaine, and 6 = *trans*-Cinamoylcocaine.

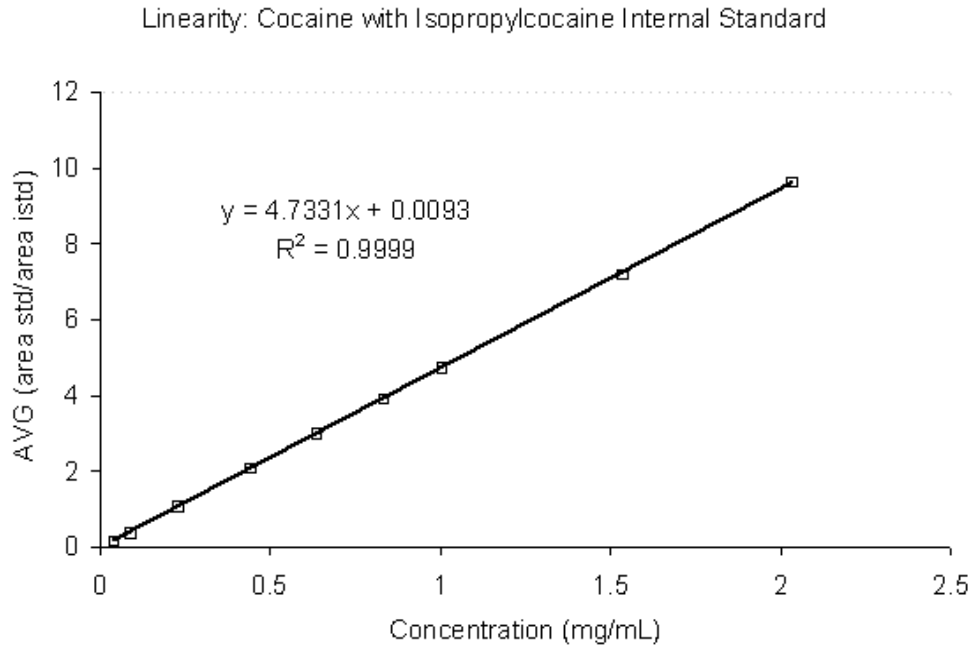

Figure 4. Calibration Curve for the Isopropylcocaine Internal Standard Methodology.