The Use of Dipropionylmorphine as a Structurally-Related Internal Standard for Gas Chromatographic Quantitation of Heroin

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ABSTRACT: Dipropionylmorphine is utilized as a structurally similar internal standard for quantitation of illicit heroin via gas chromatography with flame ionization detection. The described method has excellent selectivity, precision, and accuracy, with a relative standard deviation of less than 0.2 percent and a correlation coefficient of 0.99999. The quantitative results were in excellent agreement with other quantitative methods. The synthesis of high-purity dipropionylmorphine from morphine is detailed.

KEYWORDS: Dipropionylmorphine, Synthesis, Gas Chromatography, Flame Ionization Detection, Heroin, Quantitation, Internal Standard, Forensic Chemistry.

Introduction

The quantitative determination of illicit heroin (Figure 1) and related opium alkaloids is important for forensic, toxicological, and judicial purposes. Many methods have been published for this purpose, including using, for example, high pressure liquid chromatography (HPLC) [1], capillary electrophoresis (CE) [2], nuclear magnetic resonance (NMR) [3], and gas chromatography (GC) [4-6].

Internal standards are routinely incorporated in quantitative methods. Structurally similar internal standards are preferred because they improve method accuracy and precision. However, most analytical methods that employ internal standards use non-structurally related hydrocarbons such as *n*-tetracosane [4], *n*-octacosane [4], and *n*-triacontane [5] because of their wide availability, high purity, high stability, and relative ease in handling. In contrast, methods using structurally related internal standards are uncommon.

Specifically looking at gas chromatography analysis with flame ionization detection (GC/FID), an ideal internal standard should have similar chemical and physical properties as well as comparable FID responses to the analyte of interest. As such, the structurally related internal standard would maximize precision and accuracy [6] and minimize issues related to reactivity, absorption, solubility, and inlet/on-column degradation [4,7]. Diacetylnalorphine has been previously utilized as a structurally similar internal standard for heroin quantitation [8], and satisfies the above criteria. However, it is not an ideal compound for routine use because of the relatively high cost of its precursor (nalorphine), and its moderate instability in solution (three months at 4^{O}C).

To date, only diacetylnalorphine has been reported for use as a structurally similar internal standard for heroin quantitation. However, dipropionylmorphine (Figure 1) was previously utilized as a target compound for quantitation of morphine in toxicological samples [9] (in this study, propionic anhydride was utilized as a derivatization reagent). *A priori*, dipropionylmorphine would be an ideal internal standard for heroin quantitation by GC/FID, providing it is non-coincident with any other opium alkaloids or typical adulterants and diluents. Herein we report the facile synthesis and successful use of dipropionylmorphine as a structurally similar internal standard for GC/FID analysis of heroin.

Experimental

Reagents: All solvents were obtained from Burdick and Jackson Laboratories (Muskegon, MI). Diethylamine, propionylchloride, ammonium hydroxide, alumina, and activated carbon were obtained from Sigma Aldrich Inc. (St. Louis, MO). Heroin hydrochloride and morphine hydrochloride standards were obtained from this laboratory's reference collection.

Synthesis of Dipropionylmorphine: Morphine hydrochloride monohydrate (40.0 g, 0.124 mol) was combined with 800 mL of acetonitrile and propionyl chloride (51.1 g, 0.552 mol) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was refluxed gently, with stirring, for 22.5 hours. Upon cooling, the reaction mixture was split into four 200 mL aliquots. Each portion was added slowly with stirring to a mixture of isooctane (1.0 liter) and diethyl ether (800 mL), causing crude dipropionylmorphine hydrochloride to precipitate from solution. The crystals were captured via suction filtration and washed with anhydrous diethyl ether (200 mL). All four crops of crude material were then dissolved into 400 mL of water and filtered. The filtrate was washed with isooctane (500 mL), then with anhydrous diethyl ether (400 mL). The solution was adjusted to pH 9 with concentrated ammonium hydroxide and extracted with methylene chloride (2 x 300 mL). The extracts were combined, dried over anhydrous sodium sulfate, filtered, and treated with activated carbon (2 g). The carbon was removed by filtering through a celite pad. The filtrate was evaporated *in vacuo* to provide approximately 40 grams of 96 percent dipropionylmorphine base. The material was chromatographed on a basic alumina column (600 g containing 4 percent H_2O) using methylene chloride. The first 800 mL of eluate was collected and evaporated *in vacuo* to a clear oil. The oil was dissolved into a minimal volume of anhydrous diethyl ether in a flask, and the flask was scratched to crystallize dipropionylmorphine base as a chromatographically pure (99+ percent) white powder (34.2 g, 70 percent yield).

Gas Chromatography - Flame Ionization Detection (GC/FID): An Agilent 6890N GC with a DB-5 column (30 m x 0.25 mm I.D., 0.25 μm film thickness) was utilized. The oven temperature program began at 205^{O}C (1 minute hold), ramped to 240^{O}C at 12^{O}C/minute (5 minute hold), ramped at 4^{O}C/minute to 275^{O}C (1 minute hold), and then ramped at 15^{O}C/minute to 285^{O}C (2.33 minute hold). The carrier gas was hydrogen (99.999 percent UHP) at a flow rate of 0.9 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280^{O}C.

Gas Chromatography - Mass Spectrometry (GC/MS): An Agilent 6890N GC/MSD with a DB-1 column (30 m x 0.25 mm I.D., 0.25 μm film thickness) was utilized. The oven temperature program began at 90^{O}C (2 minute hold), ramped to 300^{O}C at 14.0^{O}C /minute (10.0 minute hold). The carrier gas was ultra high purity Helium at a flow of 1.0 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280^{O}C. The mass spectrum of dipropionylmorphine is presented in Figure 2.

Internal Standard Stock Solution: A stock solution of dipropionylmorphine was prepared at 1 mg/mL in chloroform. The solution was used at room temperature and can be stored at 4^{O}C for up to two years without detectable degradation.

Standard and Sample Preparation: Approximately 18 - 20 mg of heroin hydrochloride standard was accurately weighed into a 50 mL Erlenmeyer flask. 5.00 mL of the internal standard stock solution and 20 mL of chloroform (containing 50 μL of diethylamine) were added to the Erlenmeyer flask and the solution was allowed to sit for 5 minutes. Samples were prepared in the same manner with slight modifications in sample weight to maintain sample concentration within the linear range of the method (see below). Aliquots of both standard and sample were transferred to separate autosampler vials for analysis.

Linearity and Precision: Nine individual concentrations of heroin hydrochloride were prepared (at 0.106, 0.207, 0.418, 0.611, 0.757, 0.807, 1.011, 1.632, and 2.039 mg/mL) with the internal standard, as described above. All nine concentrations were utilized to calculate the method linearity and precision.

Results and Discussion

High purity dipropionylmorphine is easily prepared from morphine hydrochloride. As expected, heroin and dipropionylmorphine give highly similar FID responses. The selectivity of dipropionylmorphine on a DB-5 column was excellent, with no interferences with any of the opium alkaloids, adulterants, and diluents typically present in illicit heroin (see Figure 3 and Table 1). The stock solution was stable for over 2 years at $4^{O}C$ (no detectable degradation or hydrolysis, and consistent FID peak area and height counts (see Figure 4)).

The method linearity was determined over the concentration range stated in the Experimental section. The calculated correlation coefficient (R^2) was 0.99999 (see Figure 5). The method precision was determined using all nine of the solutions listed in the Experimental section, with seven replicate injections per solution. The Relative Standard Deviations (RSDs) ranged from 0.04 to 0.17 percent. The method accuracy was determined by quantitating 11 illicit samples that had previously been analyzed in this laboratory via proton nuclear magnetic resonance (^{1}H-NMR) and capillary electrophoresis (CE). The average difference for the three methods was determined to be 2.6 percent absolute (see Table 2).

Finally, the GC/FIDs of four different types of heroin are presented in Figure 6. Highly refined samples, such as Southeast Asian (SEA/4) and South American (SA) heroin, as well as crudely refined samples, such as Southwest Asian (SWA/A) and Mexican black tar (MEX) heroin, can all be routinely quantitated utilizing this method.

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

Compound	RRT (minute)
Acetaminophen	0.16
Phenacetin	0.17
Caffeine	0.22
Diphenhydramine	0.23
Theophylline	0.27
Procaine	0.30
Cocaine	0.39
Codeine	0.51
Morphine	0.54
Acetylcodeine	0.61
O6-Monoacetylmorphine	0.62
Heroin	0.75
Quinine	0.97
Dipropionylmorphine	**1.00**
Papaverine	1.03
Noscapine	1.53

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Table 2. Comparison of 11 Samples Quantitated by Different Methods.

Sample	% by CE	% by NMR	% by GC/FID using Dipropionylmorphine
1	11.7	N/A	10.9
2	83.6	N/A	81.2
3	74.2	72.2	71.8
4	86.3	N/A	86.3
5	93.3	93.2	89.4
6	86.0	N/A	86.1
7	80.9	78.0	77.8
8	47.6	N/A	45.8
9	56.5	58.0	55.2
10	11.2	10.9	10.7
11	10.2	11.9	9.7

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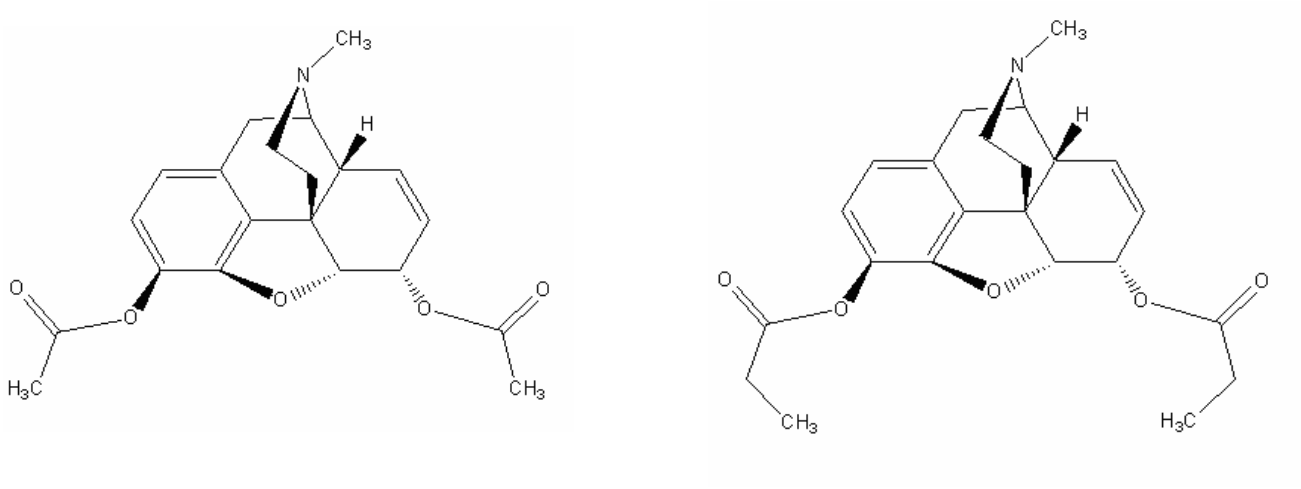

Figure 1. Structures of Heroin (Left) and Dipropionylmorphine (Right).

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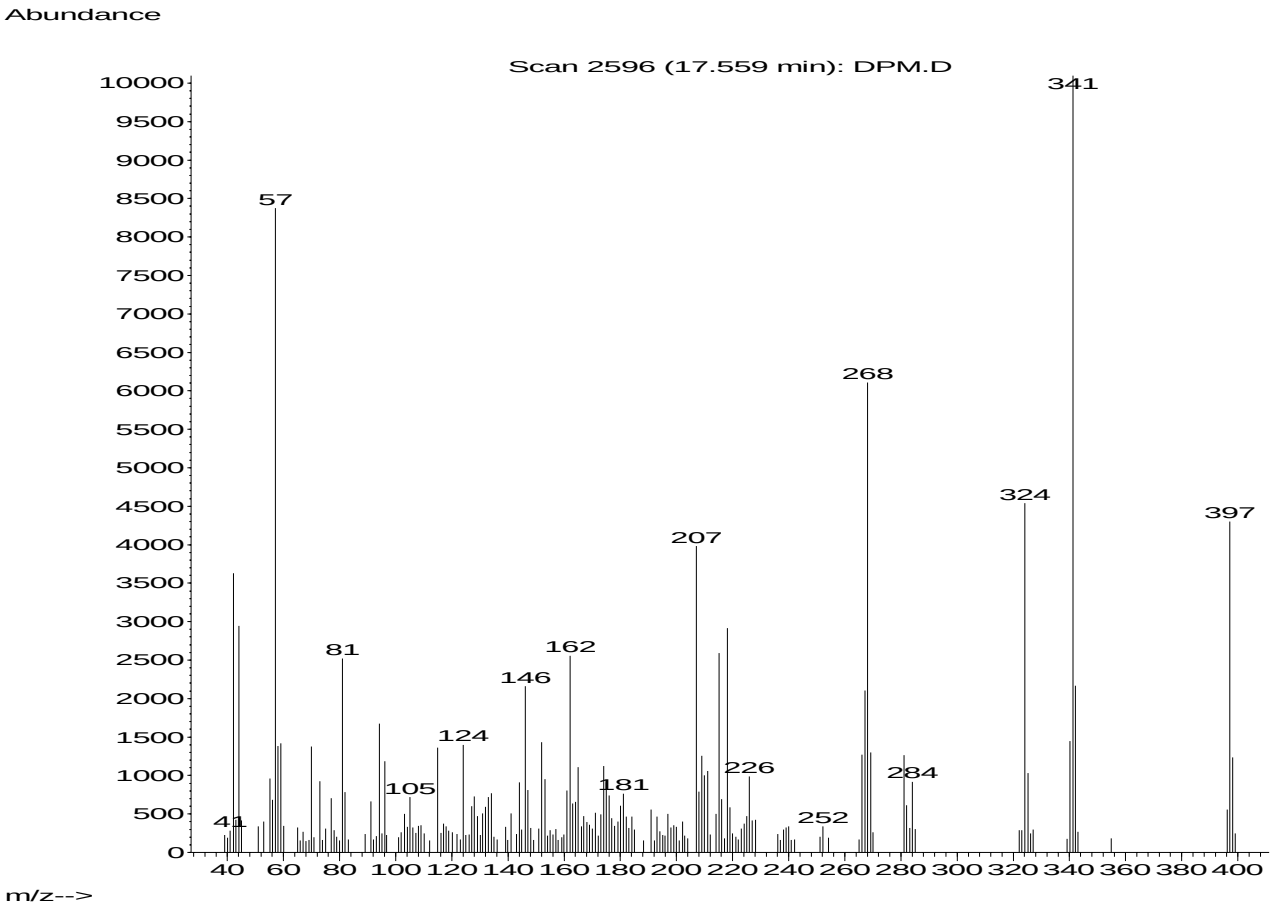

Figure 2. Mass Spectrum of Dipropionylmorphine.

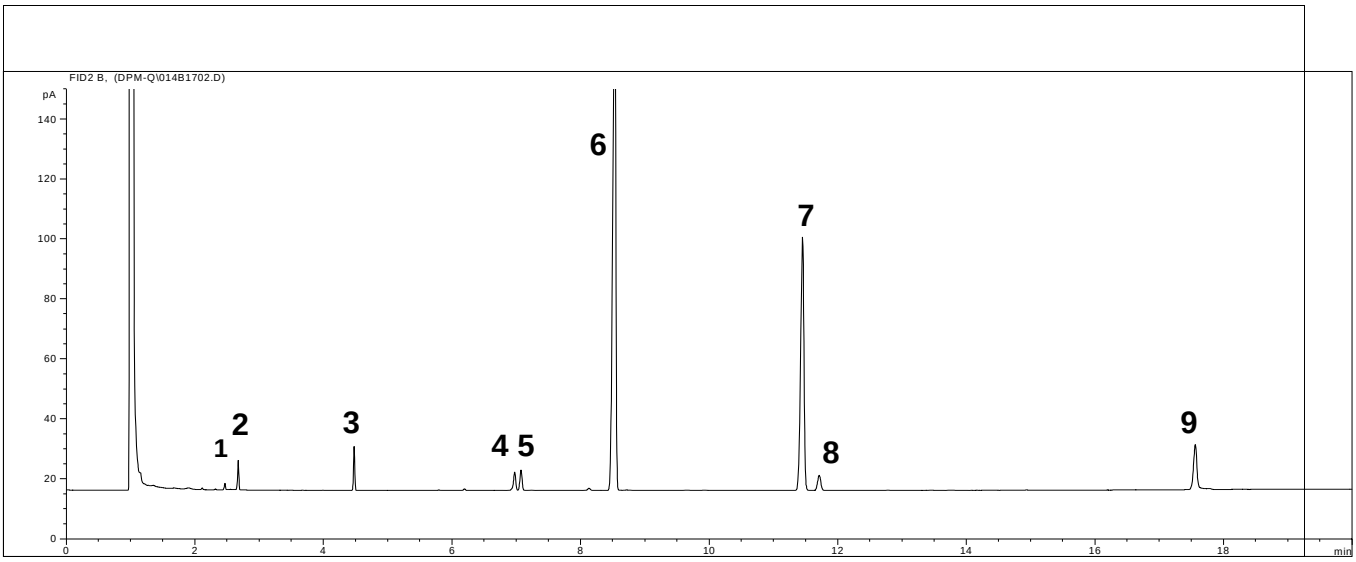

Figure 3. Capillary GC/FID Profile of a Typical Street Heroin Sample Containing Several Adulterants. Peak Identification: 1 = Caffeine, 2 = Lidocaine, 3 = Cocaine, 4 = Acetylcodeine, 5 = O6-Monoacetylmorphine, 6 = Heroin, 7 = Internal Standard, 8 = Papaverine, 9 = Noscapine.

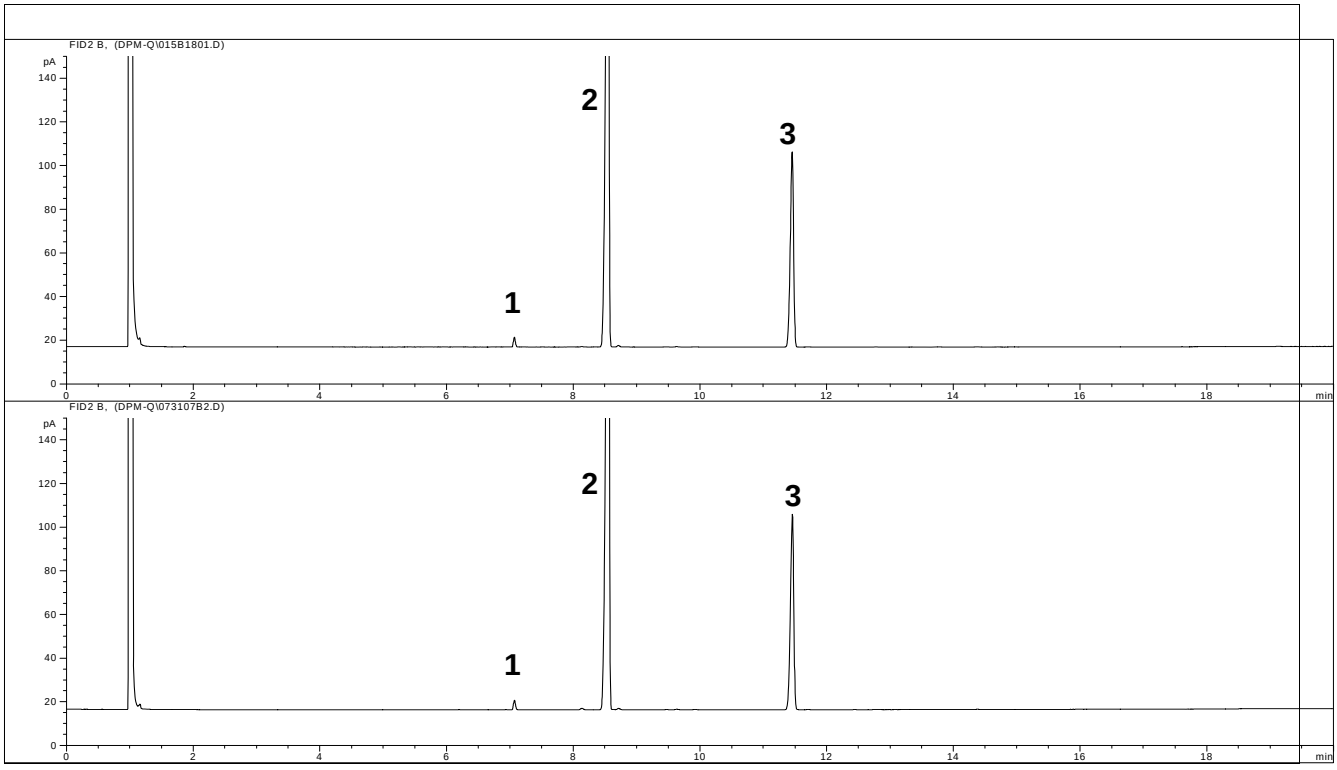

Figure 4. GC/FID Comparison of Internal Standard Prepared on May 14, 2004 (Upper) and Internal Standard Prepared on November 6, 2007 (Lower); No Loss in Peak Area Detected.
Peak Identification: 1 = O6-Monoacetylmorphine, 2 = Heroin Standard (contains a small amount of O6-Monoacetylmorphine), 3 = Dipropionylmorphine Internal Standard.

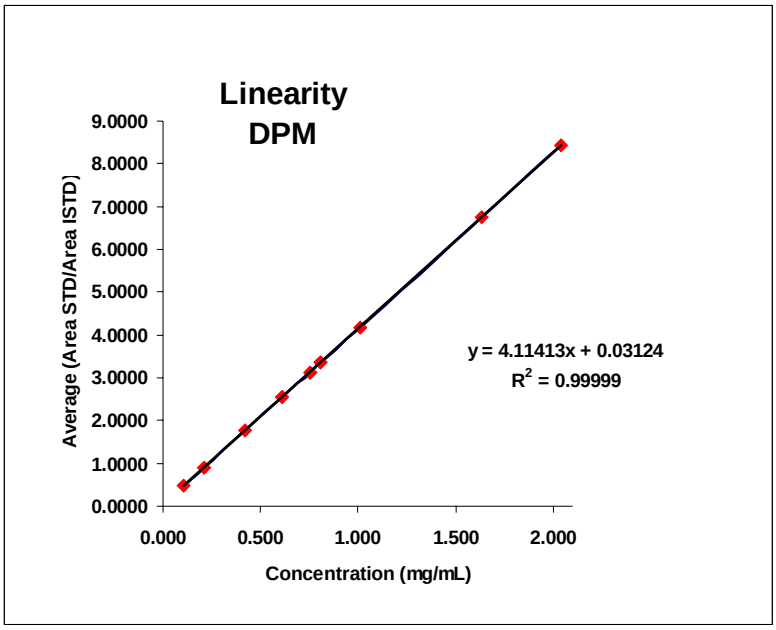

Figure 5. Linearity for Heroin with Dipropionylmorphine Internal Standard.

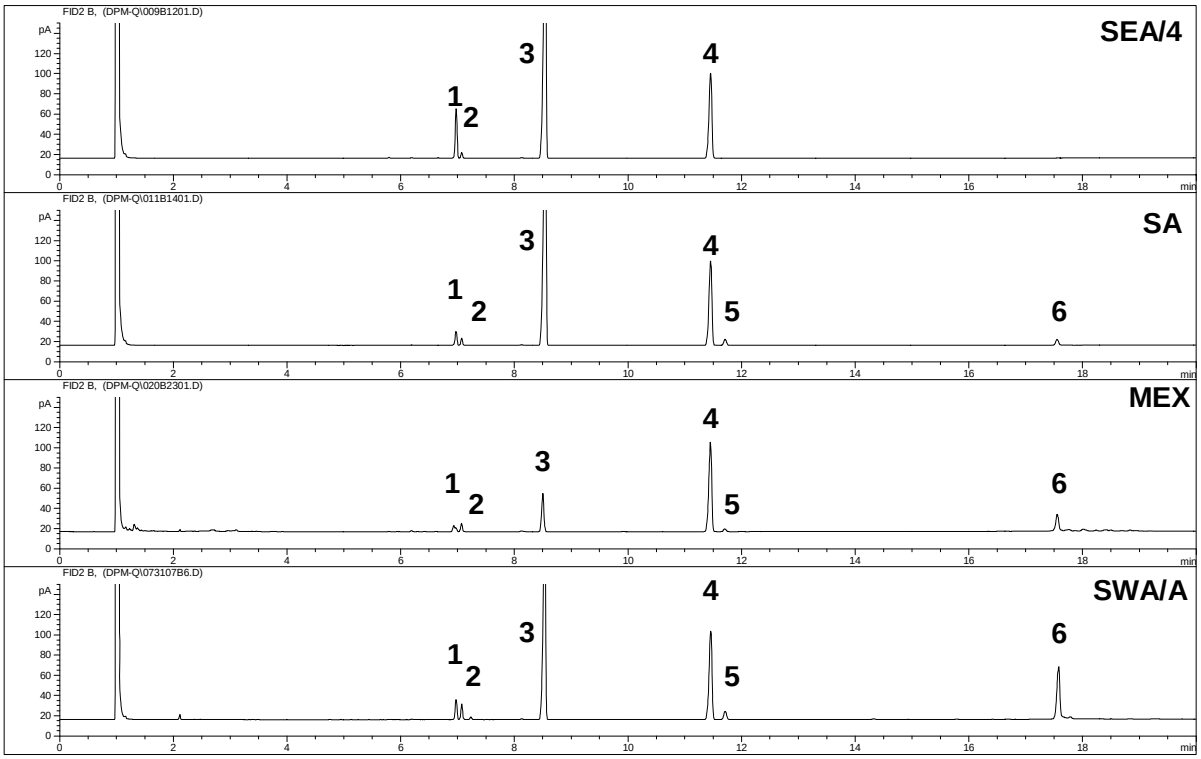

Figure 6. GC/FID Comparison of Four Types of Heroin. Southeast Asian (SEA/4) 86.3 % Heroin Hydrochloride, South American (SA) 86.1 % Heroin Hydrochloride, Mexican (MEX) 9.8 % Heroin as Hydrochloride, and Southwest Asian (SWA/A) 57.6 % Heroin Base. Peak Identification: 1 = Acetylcodeine, 2 = O6-Monoacetylmorphine, 3 = Heroin, 4 = Dipropionylmorphine Internal Standard, 5 = Papaverine, 6 = Noscapine.