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Comparison Of Two Methods For Differentiating Negative From Inconclusive GC-MS Test Results Using An Isotopic Analog Of The Analyte As The Internal Standard - 11-Nor-Δ9-Tetrahydrocannabinol-9-Carboxylic Acid Example

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COMPARISON OF TWO METHODS FOR DIFFERENTIATING NEGATIVE FROM INCONCLUSIVE GC-MS TEST RESULTS USING AN ISOTOPIC ANALOG OF THE ANALYTE AS THE INTERNAL STANDARD — 11-NOR-Δ9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID EXAMPLE

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COMPARISON OF TWO METHODS FOR DIFFERENTIATING NEGATIVE FROM INCONCLUSIVE GC-MS TEST RESULTS USING AN ISOTOPIC ANALOG OF THE ANALYTE AS THE INTERNAL STANDARD — 11-NOR-Δ9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID EXAMPLE

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FORENSIC SCIENCE

ABSTRACT

In the testing of urine samples for illicit drugs by gas chromatography-mass spectrometry (GC-MS), a drop in internal standard response is an indicator of possible sample adulteration. In 2007, Dennis V. Canfield (Liu et al. 2007) developed a formula to calculate factor A, an empirically determined value for the lowest acceptable internal standard (IS) signal-to-noise (S/N) response. Using this method, Liu et al. were able to distinguish true negatives from samples showing interference by ibuprofen and hydrogen peroxide (H_2O_2) in the detection of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine samples.

To determine if Liu's method can be universally applied, a set of samples containing THC-COOH, deuterated IS (THC-COOH-d_3), and ibuprofen were prepared at concentrations similar to Liu et al., but tested under a different set of laboratory conditions, and therefore a new value for factor A. Two types of ratios were used to evaluate the IS response of the samples. The first approach was to divide the IS signal by the noise. Two sets of data were produced, from ratios calculated using corrected and uncorrected values, respectively. The second approach was to divide the uncorrected IS intensity by the uncorrected THC-COOH intensity.

While the uncorrected IS S/N ratio and the uncorrected IS intensity to THC-COOH intensity methods provided some indication of ibuprofen interference, the

corrected IS S/N ratio method proved the most effective at identifying negative, positive, and inconclusive samples. Further research is needed to more closely examine the transition from no, to partial, and to full interference by ibuprofen in such samples. It is recommended that samples which produce an IS S/N ratio very close to the cutoff value of factor A also be considered for follow-up testing, as they may be exhibiting partial interference. The factor A method could also be useful in identifying other types of chemical interference encountered in drug testing, in addition to that of ibuprofen or H_2O_2 on THC-COOH.

DEDICATION

To my family, for their unconditional love and support.

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INTRODUCTION

In cases where forensic biological samples may have been chemically altered, from either environmental processes or deliberate manipulation, the drug of interest may be present, but undetectable. In such cases, the analyst has the critical task of distinguishing a true negative result from a negative caused by the presence of interfering substances. One indication of interference is a drop in internal standard response. An equation developed by Dennis V. Canfield (Liu et al. 2007) for factor A, an empirically determined cutoff value for the lowest acceptable internal standard signal-to-noise (S/N) response, was used to identify urine samples showing interference by ibuprofen in the detection of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH). The objective of my research is to apply the equation for factor A to a set of samples similar in composition to Liu et al. 2007, but which were tested under a different set of laboratory conditions, and therefore a new value of factor A, to determine if Liu's method can be universally applied.

Methods of Urine Sample Adulteration

As noted by Scholer (2004) in his review of urine sample adulteration techniques, the majority of samples tested for illegal drugs in the U.S. originate from workplace drug screening programs. Many possible methods of altering a urine sample drug test result exist; however, they can be broadly classified as either deliberate or accidental means of

sample manipulation. The first category includes substitution or dilution of the urine, as well as efforts to mask or destroy drugs present in the sample through the use of chemical additives. In the second type of sample adulteration, the ingestion of certain foods or medications by the subject can lead to false positive or negative drug results.

Both the preliminary immunoassay screening and the confirmatory testing by gas chromatography-mass spectrometry (GC-MS) are susceptible to deliberate attempts at sample manipulation. The addition of household chemicals such as baking soda or ammonia to urine samples can cause false negatives during immunoassay screening, because changes in sample pH can modify protein structures and therefore hinder the antigen-antibody binding necessary for drug detection (Scholer 2004). Other types of adulterants can chemically alter a given drug to prevent its detection. Oxidizing agents such as hypochlorite bleach and hydrogen peroxide have been shown to decrease the recovery of the marijuana metabolite 11-nor-9-carboxy-Δ^9-THC (THC-COOH) in urine samples subjected to GC-MS analysis (Baiker et al. 1994, Paul 2004).

Medications taken by the subject also have the potential to affect drug test results. Examples include antipsychotics, antidepressants, vitamins, and nonsteroidal anti-inflammatory drugs (NSAIDs) (Scholer 2004). One commonly used NSAID, ibuprofen, has been observed to interfere with the detection of THC-COOH by GC-MS (Brunk 1988). A related study of ibuprofen interference on THC-COOH by Liu et al. (2007) compared the response of a deuterated internal standard (IS) to an empirically calculated factor A, and identified false negatives in adulterated samples which had suspiciously low IS responses.

The purpose of my research is to test the general applicability of the factor A method using samples containing deuterated THC-COOH, THC-COOH, and ibuprofen in concentrations similar to those of Liu et al., but with a new set of laboratory conditions, and therefore a different value of factor A.

Analysis of THC-COOH in Urine by GC-MS Using a Deuterated IS

The primary psychoactive component found in marijuana is Δ^9-tetrahydrocannabinol (Δ^9-THC) (Clarke's Drugs & Poisons 2004). This substance is oxidized in the body by the hepatic P450 cytochrome enzyme system to numerous metabolites; of these, 11-nor-9-carboxy-Δ^9-THC (THC-COOH) is found at the greatest concentration in urine samples of THC users (Fig. 1). The THC-COOH metabolite is generally excreted as a conjugate with glucuronic acid, and so hydrolysis of urine samples is required to obtain the total amount of free THC-COOH present.

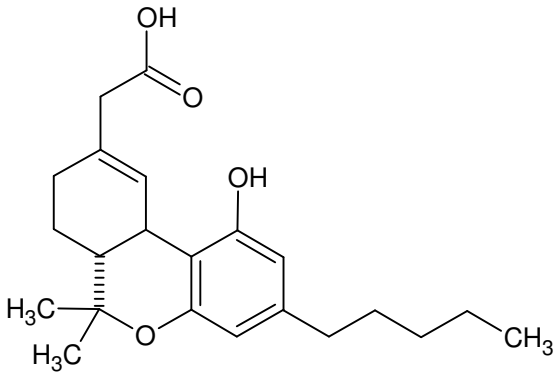

Figure 1. 11-nor-9-Carboxy-Δ^9-THC (THC-COOH).

Gas chromatography-mass spectrometry can detect very small concentrations of THC-COOH in urine, but it requires sample derivatization. Chemical conversion of the

analyte can increase the volatility or stability of the resulting compound, and can also enhance the GC separation and target the MS fragmentation (Liu & Gadzala 1997). Derivatization of compounds with carboxylic acid groups, such as THC-COOH, is of particular importance in that it reduces the formation of hydrogen bonds between the analyte and the siloxane component of the GC-MS column, preventing loss of sample to the column.

Several types of GC-MS derivatizing methods, including those adding trimethylsilyl- (TMS) groups to the analyte, have been applied to the testing of THC-COOH in urine. The approach most often used by investigators, however, has been methylation (Tindall et al. 2005). The resulting derivatization product has methyl groups attached to the carboxylic acid and phenolic oxygen atoms (Fig. 2). The MS fragmentation of methylated THC-COOH includes a base peak at m/z=313 and a molecular ion (M^+) at m/z=372. The major ions, with relative intensities in parentheses, are as follows: 313 (100), 357 (79), and 372 (52) (Pfleger 1992). Other minor ions are present, with relative intensities of less than 10 (Fig. 3).

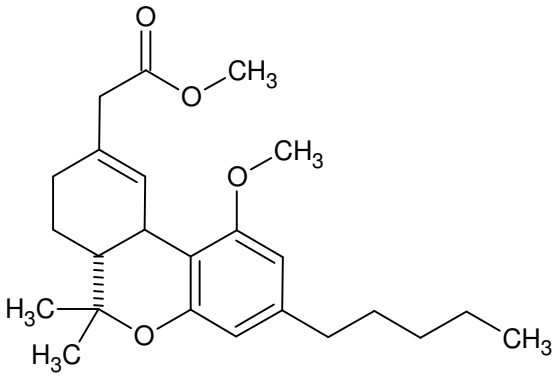

Figure 2. Methylated 11-nor-9-Carboxy-Δ^9-THC (THC-COOH).

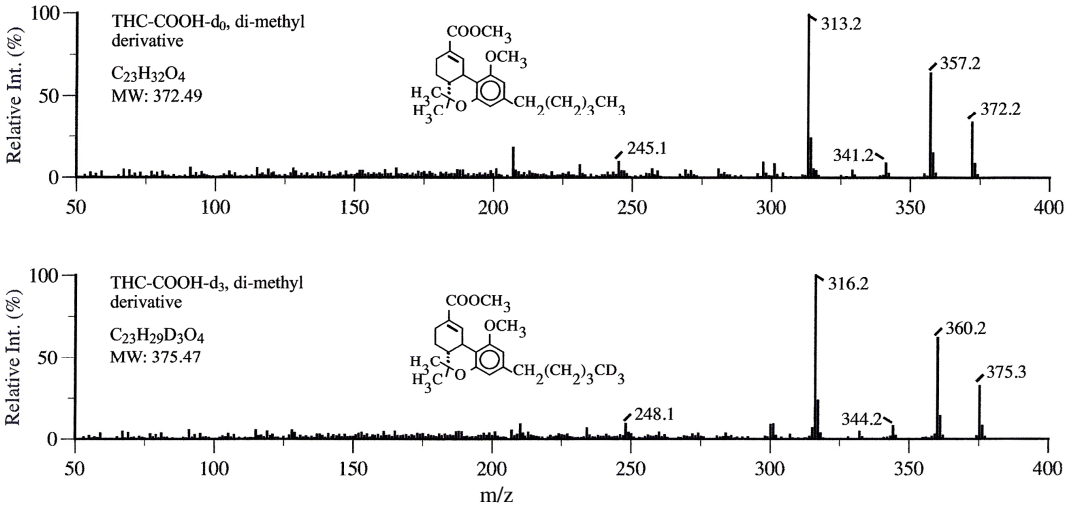

Figure 3. Mass spectra* of methylated 11-nor-9-Carboxy-Δ^9-THC (THC-COOH) and its deuterated analog.

*Full-scan mass spectrometric data were stored as digital files that were then converted into mass spectra of a more desirable format for systematic presentation. This conversion was carried out using the DeltaGraph software (DeltaPoint: Seattle, WA, US) on an Apple iMac G5 computer (Cupertino, CA, US)

In addition to sample derivatization, quantitative analysis of THC-COOH by GC-MS also requires selection of an appropriate internal standard (IS). The chosen IS may be either a chemically similar, but distinct compound (analog) or it may be identical to the IS, except for the substitution of multiple deuterium atoms for the corresponding hydrogen atoms in the analyte (deuterated). While the use of an analog rather than deuterated IS may have been adopted in older studies on THC-COOH, due to the relatively low cost (Brunk 1988), the deuterated form of THC-COOH has since become the more commonly employed IS (THC-COOH-d_3) (Yinon 1995).

The addition of an IS to the sample can help ensure the reliability of a given analytical method. Either type of IS compensates for loss of the analyte during sample preparation, as well as for variations in the GC-MS system during sample runs (Liu & Gadzala 1997). However, a deuterated IS, with its near-identical chemical structure,

provides a more specific marker for interfering substances than an analog IS. A low response from the deuterated IS is an indication of chemical interference, and samples that initially test negative for the analyte can be checked for possible adulterants.

Chemical and Pharmacological Properties of Ibuprofen

Ibuprofen (Fig. 4) is a popular medication for the relief of pain and inflammation, including that from chronic conditions such as rheumatoid arthritis (Furst & Ulrich 2007). As an NSAID, it reduces the cellular production of prostaglandins, substances which mediate inflammation and various other physiological processes, through inhibition of the cyclooxygenase (COX) enzyme system. While lower doses are sufficient for general pain relief, the suggested oral dose of ibuprofen for adequate anti-inflammatory effects is approximately 2400 mg daily.

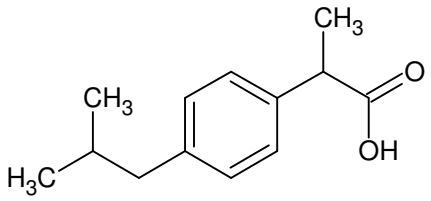

Figure 4. Ibuprofen.

Oral formulations of the drug are well absorbed, and over 60% of a dose is excreted in the urine as the conjugated and non-conjugated forms of both its 2-hydroxy and 2-carboxy metabolites. Less than 10% is excreted as unchanged ibuprofen (Clarke's Drugs & Poisons 2004). Although free ibuprofen is present in urine at relatively low concentrations compared to those of its metabolites, it can serve as an interferent for

THC-COOH analysis due its carboxylic acid functional group. Competition between ibuprofen and THC-COOH for methylating reagent, with a corresponding decrease in methylated THC intensity and appearance of a GC-MS peak for methylated ibuprofen (Fig. 5), was observed in a 1988 study by Brunk, as described below.

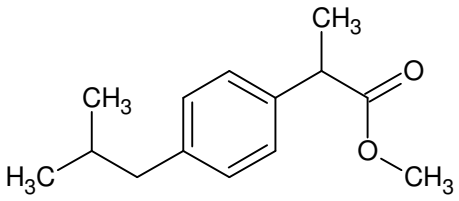

Figure 5. Methylated ibuprofen.

Interfering Effects of Ibuprofen on the Detection of Methylated THC-COOH in GC-MS Analysis

Interference by ibuprofen on the detection of methylated THC-COOH in urine samples undergoing GC-MS analysis was demonstrated by Brunk (1988). In this study, a urine sample tested positive for cannabinoids when immunoassay and thin layer chromatography (TLC) methods were used, but was negative for THC-COOH when analyzed by GC-MS. The subject of the urine sample had disclosed the use of a drug containing ibuprofen on a preemployment drug screening test form. Sample derivatization using iodomethane was unique to the GC-MS method, and it was hypothesized that ibuprofen was interfering with the methylation of THC-COOH, reducing or eliminating the GC-MS peak corresponding to methylated THC-COOH.

Tests of samples with a THC-COOH concentration of 50 ng/mL and increasing amounts of ibuprofen resulted in a disappearance of the THC-COOH peak at ibuprofen

concentrations of 250–500 μg/mL (Brunk 1988). Within this ibuprofen concentration range, GC chromatogram peaks for methylated ibuprofen and free ibuprofen were present; these compounds had a common base peak of 161, and molecular ions of 220 and 206, respectively. The proportion of methylated to free ibuprofen decreased as the ibuprofen concentration increased. Retesting of the subject's urine with double (10 μL) the volume of iodomethane used in the standard protocol resulted in a detectable THC-COOH peak, thus indicating that an increase in the amount of methylating reagent is one possible means of countering the interference effect.

In Brunk's study, the internal standard was 1-pyrenebutyric acid, a carboxylic acid compound which is an analog rather than a deuterated version of THC-COOH. It was acknowledged by the author that the use of a deuterated internal standard could have revealed the ibuprofen interference more readily, because the deuterated internal standard peak would also have been reduced or eliminated.

Factor A as a Cutoff Value for the IS Signal-to-Noise

In 2007, Canfield (Liu et al. 2007) developed a method for distinguishing negative from inconclusive THC-COOH results, based on the equation $A = (R \times I \times S)/L$, where A is a cutoff value for the internal standard (IS) signal-to-noise (S/N) ratio of the sample. The equation variables were defined as the following: (1) R, the relative response of the analyte and the IS when they are of equal concentration in the test sample; (2) I, the IS concentration; (3) S, the lowest acceptable value of the S/N ratio; and (4) L, the limit of detection for the analyte under the given experimental conditions.

An increase in the value of R, which is calculated as a ratio of IS intensity to analyte intensity, reflects an increase in the relative intensity of the IS signal and will result in a higher value of A. Raising the value of I also increases the IS response and leads to a larger A value. A greater value of S will require a higher relative IS signal and will also increase A. A higher limit of detection (L), however, indicates a decrease in instrumentation sensitivity and produces a lower acceptable value for the IS response.

If the *m/z* = 313 peak of THC-COOH was present in the mass spectrum at greater than three times the noise, the sample was considered "positive". If the THC-COOH intensity of a sample was less than three times the noise, and its IS signal-to-noise ratio was greater than the calculated value of A, the sample was considered "negative"; otherwise, it was classified as "inconclusive". Within a group of samples testing negative for THC-COOH, those samples with unusually low IS signal-to-noise ratios (below the cutoff value of A), could be isolated from those with sufficient IS response (greater than the cutoff value) and subjected to further analysis.

The 2007 study by Liu et al. examined the interfering effects of ibuprofen, in the range of 350–450 μg/mL, and 35% hydrogen peroxide (H_2O_2), in the range of 75–200 μL, in urine specimens containing 0–5 ng/mL of THC-COOH. The internal standard concentration (I) used was 15 ng/mL, and the lowest acceptable S/N ratio (S) was set at 3, reflective of common drug testing laboratory parameters. The values of R, or the relative response of the analyte (THC-COOH, *m/z* 313) to the internal standard (THC-COOH-d_3, *m/z* 316) when they are of equal concentration in a sample, and L, the limit of detection for the analyte (THC-COOH), were experimentally determined and were found to be 0.85

and 3 ng/mL, respectively. The resulting value for A = (R×I×S)/L was therefore (0.85×15×3) / 3 = 13 rounded up to the nearest integer.

The data obtained by Liu et al. indicate that the A = (R×I×S)/L equation is effective in identifying potentially adulterated samples. Within the critical concentration ranges of the ibuprofen or H_2O_2, several samples at 3 ng/mL or higher THC-COOH were classified as inconclusive by the equation, when they would have been considered negative due to the disappearance of the THC-COOH peak. In addition, all of the negative and positive designations made using the equation were correct.

Application of Factor A to Ibuprofen Using Different Experimental Parameters

The objective of my research is to apply the equation A = (R×I×S)/L to samples which contained an analyte, THC-COOH, a deuterated internal standard, THC-COOH-d_3, and ibuprofen as the adulterant, in concentrations similar to those used by Liu et al., but which were tested under different laboratory conditions. Additional goals include a comparison of different methods used to calculate the IS signal-to-noise ratios of the samples, and an assessment of the ratio of IS signal to analyte signal as an alternative value for comparison with factor A. It is proposed that use of the A = (R×I×S)/L equation in drug testing research could be extended to evaluate other types of chemical interference, in addition to that of ibuprofen or H_2O_2 on THC-COOH.

EXPERIMENTAL

Reagents and Chemicals

Standard (±)-11-nor-9-carboxy-Δ^9-THC (THC-COOH, 100 μg/mL in methanol) and deuterated internal standard (±)-11-nor-9-carboxy-Δ^9-THC-d_3 (THC-COOH-d_3, 100 μg/mL in methanol) were purchased from Cerilliant Corporation (Round Rock, TX). The interference reagent, ibuprofen, was obtained from Sigma (St. Louis, MO). A.C.S. reagent grade water from Sigma-Aldrich (St. Louis, MO) was used to dilute samples prior to extraction. Hydrolysis and extraction were performed using potassium hydroxide and glacial acetic acid from Fisher Scientific (Fair Lawn, NJ) and n-hexane and ethyl acetate from Baxter Healthcare Corporation (Muskegon, MI). Sample derivatization was achieved using tetramethylammonium hydroxide (TMAH), dimethyl sulfoxide (DMSO), and iodomethane from Aldrich, and reagent-grade hydrochloric acid.

The sample processing methods used in a previous study (Liu et al. 2007) were adopted for this analysis, with the following modifications. In place of the 1 mL of drug-free urine, 1 mL of A.C.S. reagent grade water was used. Samples were derivatized with 30 μL undiluted iodomethane, rather than 100 μL diluted iodomethane (1:50, v/v, in DMSO). In addition, reconstitution of samples for GC/MS analysis was performed using ethyl acetate, instead of the cyclohexane used in the prior study.

Sample Preparation and Extraction

Prior to sample hydrolysis and extraction, the following working solutions were prepared: 1 μg/mL THC-COOH, 1 μg/mL IS, and 10 μg/mL ibuprofen. For each experimental sample, 1 mL of distilled water was pipetted into a conical-bottom borosilicate glass centrifuge tube (Kimble: Vineland, NJ). The specified amounts of THC-COOH and ibuprofen were then added. The volume of IS added was 15 μL for all samples. Following the addition of 200 μL of 10 N KOH, the samples were incubated in a 60˚C oven for 20 min. Upon cooling to room temperature, the samples were treated with 2 mL glacial acetic acid and 2 mL of n-hexane/ethyl acetate (9:1 v/v). The samples were vortex-mixed and centrifuged at 2500 rpm for 5 min. The organic phase was removed and evaporated to dryness under nitrogen at 50˚C.

Derivatization

The dried residue was suspended in 100 μL of TMAH:DMSO (1:20) and vortexed for 2 min. Derivatization was accomplished by the addition of 30 μL undiluted iodomethane, followed by incubation at room temperature for 5 min. The samples were acidified with 200 μL of 0.1 N HCl, and 1 mL n-hexane was then added. After centrifugation at 2500 rpm for 5 min, the organic phase was removed and evaporated to dryness under nitrogen at 50˚C. The dried residue was reconstituted in 100 μL ethyl acetate.

GC/MS Analysis

The GC/MS analysis was performed on an Agilent 6890N GC interfaced to an Agilent 5975 MS (Agilent: Palo Alto, CA, US) equipped with a 30-m HP-5MS

((5% Phenyl) - methylpolysiloxane) column (250 μm ID, 0.25 μm film thickness). The carrier gas was helium at a flow rate of 1.2 mL/min. The temperatures of the injector and GC-MS interface were 250°C and 280°C, respectively. Each sample was injected at a volume of 1 μL in the splitless mode. The initial oven temperature of 150°C was held for 1 min, and then increased to 270°C at 30°C/min, followed by a hold of 7 min. The final temperature was 300°C, and was held for 4 min to clear excess sample from the column before the next injection. The following ions were selected for monitoring in SIM mode: *m/z*, 313, 357, and 372 for methylated THC-COOH; and 316, 360, and 375 for methylated THC-COOH-d_3. Quantitative analysis was performed using the first ion listed for each compound.

The GC-MS parameters used here differ in a few ways from those of the previous study (Liu et al. 2007), which utilized a column of shorter length (12 m) and slightly different internal dimensions (200 μm ID, 0.33 μm film thickness), a helium flow rate of 1.0 mL/min, an injector temperature of 260°C, and a hold time of 5 min upon reaching 270°C during the sample run.

RESULTS

Calculation of Factor A

The equation A = (R×I×S)/L, where A is the cutoff value for an acceptable internal standard (IS) signal-to-noise (S/N) ratio, requires a determination of the following variables: (1) R, the relative response of the analyte and the IS when they are of equal concentration in the test sample; (2) I, the IS concentration; (3) S, the lowest acceptable value of the S/N ratio; and (4) L, the limit of detection for the analyte under the given experimental conditions. R was calculated (data not shown) using samples containing 15 ng/mL of THC-COOH and 15 ng/mL of IS (THC-COOH-d_3), and was found to be 0.77. The values for I and S were identical to those used by Liu et al., and were 15 ng/mL and 3, respectively.

The limit of detection, L, was determined from the first set of sample data (lsw38 through lsw42), and was defined as the lowest concentration at which the THC-COOH intensity was greater than three times the noise. In the GC-MS analysis, each sample was run in triplicate, and the THC-COOH intensity and noise values were obtained by averaging the corresponding values over three sample runs. The THC-COOH signal was the corrected *m/z*=313 peak height, found by subtracting the average noise value of a region (9.60 to 10.15 min) immediately prior to the THC-COOH peak from the intensity of the THC-COOH peak. For samples lacking a visible THC-COOH peak, the *m/z*=313 intensity at 10.492 min, the retention time of THC-COOH, was used as the THC-COOH intensity. Noise was defined as the peak-to-peak noise, or the difference between the

maximum and minimum noise values of the same noise region described above. The resulting value for L was 2 ng/mL.

Under these experimental conditions, the value of factor A was therefore A = (0.77×15×3)/2 = 17 rounded to the nearest integer.

Calculation of the Signal-to-Noise (S/N) Ratio of the IS

The S/N ratio of the IS was calculated from the corrected IS peak height (*m/z*=316) and the peak-to-peak noise. Corrected peak height and peak-to-peak noise were calculated as described above, including the averaging of values over three sample runs. For samples lacking a detectable IS peak, the *m/z*=316 intensity at 10.444 min, the retention time of the IS, was used as the IS intensity. The IS S/N ratio for each sample was therefore calculated by dividing the average corrected IS intensity by the average peak-to-peak noise.

Preliminary Identification of Samples as Negative or Positive Using the THC-COOH S/N Ratio

The corrected THC-COOH intensity and noise values for each sample were calculated as described above, and were used to initially classify samples as negative or positive for the analyte. If the corrected THC-COOH intensity was less than three times the noise, the sample was considered negative (N); otherwise, it was considered positive (P).

Table 1 shows the initial N and P designations for five data sets, each containing samples with 0, 1, 2, 3, and 5 ng/mL THC-COOH, at the following ibuprofen concentrations (μg/mL): 0 (lsw38-42, lsw44-48), 350 (lsw49-lsw53), 400 (lsw67-lsw71), and 450 (lsw72-lsw76). Included are the corrected THC-COOH intensity and noise values; also

shown is the product of three times the corrected noise, a value which can be directly compared with the corrected THC-COOH signal. All samples contained 15 ng/mL IS. It was noted that the N assignment of sample lsw46 is not consistent with the experimental limit of detection (L) value of 2 ng/mL; this result will be addressed in the Discussion section.

Classification of Samples as Negative, Positive, or Inconclusive Using Factor A

For samples which tested positive (P) according to their THC-COOH S/N ratios, an evaluation using the IS S/N and factor A was not performed. A positive result according to the THC-COOH S/N was considered a sufficient requirement for identifying the sample as positive, regardless of the IS S/N response.

If a sample was found to be negative according to its THC-COOH S/N ratio, it was then subjected to evaluation using factor A. If the IS S/N ratio of the sample was greater than A, the sample was considered negative (N). If the IS S/N ratio was less than A, the sample was designated as inconclusive (I). In either of these two cases, final classification of the sample was dependent on the IS S/N response.

Factor A and IS S/N Ratios Calculated From Corrected IS Intensity and Noise Values

Table 2 contains the N, P, and I designations made by comparing the factor A value of 17 to ratios of corrected IS intensity to corrected (peak-to-peak) noise for samples lsw38 through lsw76. The IS S/N ratios labeled "N/A" were not calculated because the corresponding samples were positive for THC-COOH. As expected, the samples with 0 μg/mL ibuprofen (lsw38-42, lsw44-48) had calculated IS S/N ratios

above the value of factor A. In the first data set (lsw38-42), the 2, 3, and 5 ng/mL THC-COOH samples were positive, while the 0 and 1 ng/mL THC-COOH samples were negative. The results of the second data set (lsw44-48) were similar, except that the 1 ng/mL THC-COOH sample was positive and the 2 ng/mL THC-COOH sample (lsw45) was negative (see Discussion).

Of the samples containing 350 μg/mL ibuprofen (lsw49-lsw53), the 0 ng/mL THC-COOH was negative, and the 1, 2, 3, and 5 ng/mL THC-COOH samples were positive. The lack of interference at this concentration of ibuprofen is consistent with the findings of Liu et al. (2007). The 350 μg/mL ibuprofen samples tested by Liu et al. were positive when the level of THC-COOH was at or above the experimental limit of detection (3 and 5 ng/mL), and were negative at lower THC-COOH concentrations (0, 1, and 2, ng/mL).

Interference, as demonstrated by corrected IS S/N ratios below the value of factor A, could be observed at the 400 and 450 μg/mL levels of ibuprofen. In Table 2, the samples with the three lowest THC-COOH concentrations at the 400 μg/mL ibuprofen level, and all of the 450 μg/mL samples, were classified as inconclusive by the $A = (R \times I \times S)/L$ equation. At 400 μg/mL ibuprofen, the 5 ng/mL THC-COOH sample (lsw71) tested positive; however, the 3 ng/mL THC-COOH sample (lsw70) tested negative, due to a low THC-COOH S/N ratio (Table 1) and an IS S/N ratio which was just above the value of factor A (see Discussion).

In the Liu et al. study, all of the samples containing 450 μg/mL ibuprofen were designated inconclusive. At a slightly lower ibuprofen concentration (420 μg/mL), the 3 and 5 ng/mL THC-COOH samples were found to be positive, and the 0, 1, and 2 ng/mL

THC-COOH samples were inconclusive. The data of Liu et al. include two sets of samples with 400 μg/mL ibuprofen. In the first set, all of the samples (0, 1, 2, 3, and 5 ng/mL THC-COOH) tested inconclusive; however, in the second set (0, 1, 3, and 5 ng/mL THC-COOH), the 0 and 1 ng/mL samples were negative, while the 3 and 5 ng/mL samples tested positive. The inconsistent results of Liu et al. at 400 μg/mL ibuprofen, as well as the data obtained for samples containing 400 μg/mL ibuprofen in this study (Table 2), are intriguing and warrant further investigation (see Discussion).

Factor A and IS S/N Ratios Calculated From Uncorrected IS Intensity and Noise Values

To more closely compare the results of this research to those of Liu et al., N, P, and I assignments were made to the samples using uncorrected IS intensity and noise values to calculate the IS S/N ratios (Table 3). The uncorrected IS signal was the intensity of the *m/z*=316 peak, while the uncorrected noise was defined as the average of the maximum and minimum noise values within the region of 9.60 to 10.15 min.

Due to the higher absolute values of the uncorrected average noise, as compared to the corrected values shown in Table 2, the corresponding limit of detection for THC-COOH was higher than the previous value of 2 ng/mL. Using the uncorrected THC-COOH S/N ratios of lsw38 through lsw42, as well as those of samples containing 10 ng and 15 ng THC-COOH and 15 ng IS (data not shown), the limit of detection of THC-COOH was estimated as 8 ng/mL. The corresponding value of A for the uncorrected IS S/N method was therefore (0.77×15×3)/8 = 4.33.

As shown in Table 3, the values of the uncorrected IS S/N ratios of the samples are lower, and narrower in range, than those calculated from the corrected IS S/N method

(Table 2). Despite the changes in these values, the results from the uncorrected IS S/N method are similar to those of the corrected method for samples in the 400-450 μg/mL ibuprofen range. A comparison of the data in Tables 2 and 3 shows that either method produces designations of inconclusive for all samples at 450 μg/mL ibuprofen, and for the three lowest THC-COOH concentrations for the samples at 400 μg/mL ibuprofen. The negative assignments given to all of the remaining samples are reasonable, given that the highest THC-COOH concentration (5 ng/mL) was below the limit of detection for this approach (8 ng/mL).

Factor A and Ratios Calculated From Uncorrected IS Intensity and THC Intensity Values

An alternative means of evaluation, made by comparing factor A with ratios of uncorrected IS intensity to THC intensity, is shown in Table 4. The uncorrected IS intensity was identical to that used in the previous method, and the uncorrected THC-COOH signal was the intensity of the *m/z*=313 peak.

As indicated by the data in Table 4, the ratios of uncorrected IS intensity to THC-COOH intensity are lower than both the corrected and uncorrected IS S/N ratios (Tables 2 and 3). All of the samples at 400 and 450 μg/mL ibuprofen tested inconclusive; therefore, except for the 3 and 5 ng/mL THC-COOH samples with 400 μg/mL ibuprofen (lsw70 and lsw71), the results are identical to those of the previous methods at this level of ibuprofen.

The two negative designations made were for samples containing 0 ng/mL THC-COOH and 0 and 350 μg/mL ibuprofen (lsw38 and lsw49), and are reasonable given the lack of interference at these ibuprofen levels. All of the other samples at 0 and 350

μg/mL ibuprofen, however, were inconclusive. These assignments are problematic in that they suggest a lowering of IS and THC-COOH response at ibuprofen concentrations where no significant interference is expected to occur.

Tables 5 and 6 contain the raw data obtained from samples lsw38 through lsw76 at *m/z*=316 and *m/z*=313, respectively.

DISCUSSION

Factor A and IS S/N Ratios Calculated From Corrected IS Intensity and Noise Values

Comparison of factor A to the corrected IS S/N ratios of the samples produced results which agreed well with those of Liu et al (2007). At ibuprofen concentrations of 0 or 350 μg/mL ibuprofen, the samples were generally positive at or above the limit of THC-COOH detection (2 ng/mL), and negative at lower levels of THC-COOH (Table 2). Two samples with 1 ng/mL THC-COOH (lsw45 and lsw50) tested positive, while one sample at 2 ng/mL THC-COOH (lsw46) was negative. The latter finding is problematic as it occurs at the limit of THC-COOH detection, a concentration level which, in the absence of chemical interference, should reliably produce a positive result. However, given that none of the other samples with 2 ng/mL or higher THC-COOH at the 0 or 350 μg/mL ibuprofen concentrations tested negative using the corrected IS S/N method, and that the corrected THC-COOH intensity of sample lsw46 was only slightly below three times the noise (Table 1), the negative assignment for this sample was considered an outlying result.

As shown in Table 2, the lowest ibuprofen concentration at which interference occurred was 400 μg/mL. In this set of samples (lsw67-71), those with the three lowest THC-COOH concentrations were classified as inconclusive, the 3 ng/mL sample tested negative, and the 5 ng/mL sample was positive. The 3 ng/mL sample (lsw70) was initially classified as negative due to its low THC-COOH signal (Table 1), and its IS S/N ratio of 17.4 was slightly higher than the cutoff value of 17; therefore, it did not qualify

as inconclusive. Considering the concentration of the IS (15 ng/mL), it is reasonable to assume that interference by ibuprofen could have reduced the THC-COOH peak below a detectable level, while leaving the IS peak intensity reduced, but high enough to produce an IS S/N ratio just above the cutoff value for designating a sample as inconclusive.

It is noted that, in the absence of rounding, the difference between the corrected IS S/N ratio of sample lsw70 and factor A is only (17.388–17.325) = 0.063, or 0.36% of the value of factor A. Rounding of the factor A value of 17.4 to the next highest whole number, 18, instead of rounding down to 17, would have caused this sample to be judged inconclusive rather than negative. In Liu et al. the value of factor A used was $A = (R \times I \times S)/L = (0.85 \times 15 \times 3) / 3 = 12.75 = 13$ rounded up to the nearest integer. Such a conservative approach to rounding in the determination of factor A would appear to reduce, although probably not eliminate, the number of false negative designations.

The results for the 450 μg/mL ibuprofen samples (lsw72-lsw76) were identical to those of Liu et al. in that all samples at this ibuprofen concentration were inconclusive. The findings at 400 μg/mL ibuprofen, however, differ from either of the two sets of corresponding samples evaluated by Liu et al. In that study, all of the samples in the first set tested inconclusive, while in the second set the 0 and 1 ng/mL samples were negative, and the 3 and 5 ng/mL samples were positive. Interestingly, the designations of a set of 420 μg/mL ibuprofen samples tested by Liu et al. were inconclusive at 0, 1, and 2 ng/mL THC-COOH, and positive at 3 and 5 ng/mL THC-COOH.

The data obtained in Liu et al. and in this study indicate that samples containing ibuprofen in the 400–420 μg/mL range can produce inconsistent results. Further testing of sample sets with ibuprofen concentrations between 400 and 450 μg/mL could help

identify where the expected crossovers from no interference (negative or positive assignments) to partial (some inconclusive and some negative/positive assignments) and full (all inconclusives) interference occur. In addition, the detection and quantitation of peaks corresponding to methylated and free ibuprofen, as performed by Brunk (see Introduction), in such samples is another potential means of examining the interference process.

Factor A and IS S/N Ratios Calculated From Uncorrected IS Intensity and Noise Values

The uncorrected IS S/N method produces results which are roughly comparable to those of the corrected method for samples in the 400-450 μg/mL ibuprofen range (Tables 2 and 3). The samples with three lowest THC-COOH concentrations at 400 μg/mL ibuprofen, and all of the 450 μg/mL ibuprofen samples were inconclusive. While the uncorrected IS S/N ratios were smaller in magnitude and range than those of the corrected method, both approaches showed a decrease in ratio values at 400 and 450 μg/mL ibuprofen. The negative assignments given to all of the 0 and 350 μg/mL ibuprofen samples, as well as to the two highest THC-COOH concentration samples at 400 μg/mL may reflect the increased limit of THC-COOH detection for this method (8 ng/mL) and the resulting decrease in the value of factor A to 4.33.

Additional samples with THC-COOH concentrations at and above the estimated limit of detection, and containing ibuprofen at the levels used in this experiment, could be tested to determine if the pattern of negative and positive assignments seen in the corrected method could be observed. By requiring the adoption of a higher limit of detection, however, the uncorrected IS S/N method could prove impractical when samples

showing small but clearly discernable THC-COOH peaks are nevertheless classified as negative due to the high absolute value of the noise.

Factor A and Ratios Calculated From Uncorrected IS Intensity and THC Intensity Values

As the data in Table 4 indicate, the calculated ratios of uncorrected IS intensity to THC-COOH intensity values are lower than those of either the corrected or uncorrected IS S/N methods. At ibuprofen concentrations of 0 or 350 μg/mL ibuprofen, the ratios generally decreased with increasing THC-COOH concentration, as would be predicted for dividing a relatively constant value (the intensity of the IS) by an increasing value (the intensity of the THC-COOH). The ratios of the uncorrected IS to THC-COOH intensities decreased as the ibuprofen levels increased to 400 and 450 μg/mL, but the drop in ratio values was not as large as that observed with the corrected and uncorrected IS S/N methods (Tables 2 and 3).

When compared with the uncorrected factor A value of 4.33, the ratios of all of the samples except two (lsw38 and lsw49) tested inconclusive. As a result, this approach was not able to discriminate between samples with interfering amounts of ibuprofen and those for which interference was absent.

Concluding Remarks

Of the three types of data analysis used to evaluate the $A = (R \times I \times S)/L$ equation, the corrected IS S/N ratio method proved the most effective at identifying negative, positive, and inconclusive samples. While the uncorrected IS S/N ratio and the uncorrected IS intensity to THC-COOH intensity methods can provide some information about the

interfering effects of ibuprofen, each showed significant drawbacks which limited their usefulness in predicting potentially adulterated samples.

Future research could include the testing of more samples in the 400–450 μg/mL range of ibuprofen, in order to more closely examine the transition from no, to partial, and to full interference. In particular, it is recommended that samples which produce an IS S/N ratio very close to the cutoff value of factor A also be considered for follow-up testing, as they may be showing signs of partial interference. As noted previously (see Introduction), the $A = (R \times I \times S)/L$ equation identifies experimental factors which could apply to other types of chemical interference on an analyte, and research could be carried out in this area as well.

TABLE 1—*Signal-to-noise calculations from corrected THC-COOH intensity and noise values.*

	Sample	THC-COOH conc. (ng/mL)	Ibuprofen conc. (µg/mL)	Corrected THC intensity	Corrected Noise x 3	Corrected Noise	(N/P)†
*	lsw38	0	0	-7.07	590	197	N
	lsw39	1	0	514	615	205	N
	lsw40	2	0	624	555	185	P
	lsw41	3	0	1414	597	199	P
	lsw42	5	0	1464	617	206	P
*	lsw44	0	0	-15	438	146	N
	lsw45	1	0	772	535	178	P
	lsw46	2	0	441	491	164	N
	lsw47	3	0	1308	603	201	P
	lsw48	5	0	1740	603	201	P
*	lsw49	0	350	-19	613	204	N
	lsw50	1	350	652	542	181	P
	lsw51	2	350	1153	566	189	P
	lsw52	3	350	1698	543	181	P
	lsw53	5	350	3973	541	180	P
*	lsw67	0	400	-37	854	285	N
*	lsw68	1	400	134	867	289	N
*	lsw69	2	400	-11	934	311	N
	lsw70	3	400	774	927	309	N
	lsw71	5	400	1298	901	300	P
*	lsw72	0	450	-54	1084	361	N
*	lsw73	1	450	-126	1057	352	N
*	lsw74	2	450	-61	1069	356	N
*	lsw75	3	450	-79	932	311	N
*	lsw76	5	450	-91	1018	339	N

† = N = negative; P = positive

* = The sample contained no detectable m/z=313 peak; the THC-COOH value listed is the m/z=313 intensity at 10.492 min.

N = Negative. The corrected THC-COOH intensity is less than 3 times the corrected noise.

P = Positive. The corrected THC-COOH intensity is greater than 3 times the corrected noise.

TABLE 2—*Factor A ratios calculated from corrected IS intensity and noise values.*

	Sample	THC-COOH conc. (ng/mL)	Ibuprofen conc. (µg/mL)	Corrected IS intensity	Corrected Noise	Ratio	(N/P/I)†
	lsw38	0	0	4407	121	36.5	N
	lsw39	1	0	3806	119	32.1	N
	lsw40	2	0	3340	117	N/A‡	P
	lsw41	3	0	3865	108	N/A‡	P
	lsw42	5	0	3174	106	N/A‡	P
	lsw44	0	0	2325	108	21.5	N
	lsw45	1	0	3602	114	N/A‡	P
	lsw46	2	0	3616	133	27.3	N
	lsw47	3	0	4211	109	N/A‡	P
	lsw48	5	0	4717	142	N/A‡	P
	lsw49	0	350	5247	116	45.2	N
	lsw50	1	350	5439	125	N/A‡	P
	lsw51	2	350	6268	129	N/A‡	P
	lsw52	3	350	5465	96	N/A‡	P
	lsw53	5	350	6125	103	N/A‡	P
*	lsw67	0	400	-36	138	-0.3	I
	lsw68	1	400	1573	124	12.6	I
	lsw69	2	400	1107	130	8.5	I
	lsw70	3	400	2643	152	17.4	N
	lsw71	5	400	2976	151	N/A‡	P
*	lsw72	0	450	-42	128	-0.3	I
*	lsw73	1	450	-59	160	-0.4	I
*	lsw74	2	450	-50	130	-0.4	I
*	lsw75	3	450	-49	130	-0.4	I
*	lsw76	5	450	-39	147	-0.3	I

† N = negative; P = positive; I = inconclusive

* The sample contained no detectable m/z=316 peak; the IS value listed is the m/z=316 intensity at 10.444 min.

‡ The sample met the THC-COOH S/N criteria for a positive sample.

N Negative. The 313 S/N ratios were < 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.
P Positive. The 313 S/N ratios were > 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.
I Inconclusive; the ratio was less than the cutoff value of 17.

TABLE 3—*Factor A ratios calculated from uncorrected IS intensity and noise values.*

	Sample	THC-COOH conc. (ng/mL)	Ibuprofen conc. (µg/mL)	Uncorrected IS intensity	Uncorrected Noise	Ratio	(N/P/I)†
	lsw38	0	0	5001	594	8.4	N
	lsw39	1	0	4406	596	7.4	N
	lsw40	2	0	3935	602	6.5	N
	lsw41	3	0	4454	588	7.6	N
	lsw42	5	0	3790	618	6.1	N
	lsw44	0	0	2804	485	5.8	N
	lsw45	1	0	4134	534	7.7	N
	lsw46	2	0	4190	583	7.2	N
	lsw47	3	0	4753	546	8.7	N
	lsw48	5	0	5238	533	9.8	N
	lsw49	0	350	5787	547	10.6	N
	lsw50	1	350	5959	521	11.4	N
	lsw51	2	350	6837	575	11.9	N
	lsw52	3	350	5992	529	11.3	N
	lsw53	5	350	6662	539	12.4	N
*	lsw67	0	400	553	580	1.0	I
	lsw68	1	400	2129	562	3.8	I
	lsw69	2	400	1678	572	2.9	I
	lsw70	3	400	3195	554	5.8	N
	lsw71	5	400	3547	576	6.2	N
*	lsw72	0	450	523	561	0.9	I
*	lsw73	1	450	517	596	0.9	I
*	lsw74	2	450	507	556	0.9	I
*	lsw75	3	450	509	556	0.9	I
*	lsw76	5	450	500	560	0.9	I

† = N = negative; P = positive; I = inconclusive

* = The sample contained no detectable m/z=316 peak; the IS value listed is the m/z=316 intensity at 10.444 min.

N = Negative. The *m/z*=313 S/N ratios were < 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.

P = Positive. The 313 S/N ratios were > 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.

I = Inconclusive; the ratio was less than the cutoff value of 4.33.

TABLE 4—*Factor A ratios calculated from uncorrected IS intensity and THC intensity values.*

Sample	THC-COOH conc. (ng/mL)	Ibuprofen conc. (µg/mL)	Uncorrected IS intensity	Uncorrected THC intensity	Ratio	(N/P/I)†
lsw38	0	0	5001	1163	4.3	N
lsw39	1	0	4406	1704	2.6	I
lsw40	2	0	3935	1791	2.2	I
lsw41	3	0	4454	2688	1.7	I
lsw42	5	0	3790	2673	1.4	I
lsw44	0	0	2804	946	3.0	I
lsw45	1	0	4134	1788	2.3	I
lsw46	2	0	4190	1541	2.7	I
lsw47	3	0	4753	2363	2.0	I
lsw48	5	0	5238	2800	1.9	I
lsw49	0	350	5787	1036	5.6	N
lsw50	1	350	5959	1698	3.5	I
lsw51	2	350	6837	2232	3.1	I
lsw52	3	350	5992	2722	2.2	I
lsw53	5	350	6662	4998	1.3	I
lsw67	0	400	553	1035	0.5	I
lsw68	1	400	2129	1149	1.9	I
lsw69	2	400	1678	1029	1.6	I
lsw70	3	400	3195	1782	1.8	I
lsw71	5	400	3547	2319	1.5	I
lsw72	0	450	523	1058	0.5	I
lsw73	1	450	517	1018	0.5	I
lsw74	2	450	507	1034	0.5	I
lsw75	3	450	509	946	0.5	I
lsw76	5	450	500	917	0.5	I

† = N = negative; P = positive; I = inconclusive
* = The sample contained no detectable m/z=316 peak; the IS value listed is the m/z=316 intensity at 10.444 min.
N = Negative. The *m/z*=313 S/N ratios were < 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.
P = Positive. The 313 S/N ratios were > 3 for all 3 sample runs over the 9.60 - 10.15 min. noise range.
I = Inconclusive; the ratio was less than the cutoff value of 4.33.

TABLE 5—*Absolute IS (m/z=316) intensity and noise values of all samples.*

Sample	THC* (ng)	IS (ng)	IBU† (ug)	Absolute IS Intensity			Max Noise			Min Noise			Average Noise		
				Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
lsw38	0	15	0	4931	5209	4862	660	682	622	543	555	504	603.2	613.4	564
lsw39	1	15	0	4069	4694	4454	631	680	654	513	572	524	581.3	622.1	596.6
lsw40	2	15	0	3971	4244	3591	699	674	609	570	580	481	621.5	624.5	540.2
lsw41	3	15	0	4235	4660	4466	619	670	635	523	553	525	577.1	608.1	580.5
lsw42	5	15	0	3905	3639	3825	688	687	638	564	581	549	625.9	623.6	596.5
lsw44	0	15	0	2492	2909	3012	532	530	554	405	435	451	453.8	485.9	498.5
lsw45	1	15	0	3908	4244	4251	585	588	599	474	473	483	523.6	533.1	539.7
lsw46	2	15	0	3873	4318	4379	615	666	666	500	517	532	552.4	584.6	585.2
lsw47	3	15	0	4853	4747	4660	600	612	590	502	484	488	547.6	539.3	541.4
lsw48	5	15	0	4531	5548	5636	564	620	626	443	473	469	492.8	530.3	542.3
lsw49	0	15	350	5863	5726	5771	627	576	613	491	493	484	542.4	535.9	541.9
lsw50	1	15	350	5974	6162	5742	563	591	596	431	473	471	505.8	524.2	531.2
lsw51	2	15	350	6469	6798	7245	626	664	629	507	500	526	563.1	570.6	575.4
lsw52	3	15	350	5926	5703	6346	546	589	595	468	486	488	510.9	530.3	539.3
lsw53	5	15	350	6540	7026	6421	600	584	588	487	487	489	534	538.4	538.2
lsw67	0	15	400	534	583	543	631	648	667	493	518	522	580.9	591.6	594.6
lsw68	1	15	400	2021	2095	2271	589	646	636	482	490	526	540.6	555.2	573.3
lsw69	2	15	400	1734	1533	1766	648	618	644	506	515	498	570	570.8	572.6
lsw70	3	15	400	3090	3328	3168	621	646	622	469	474	490	535.1	564.3	559
lsw71	5	15	400	3468	3661	3511	658	632	665	510	492	501	564.5	575.6	571.3
lsw72	0	15	450	489	529	551	603	637	635	462	532	496	548.6	572.6	573
lsw73	1	15	450	527	490	535	682	648	698	510	529	510	569.8	583.2	577.2
lsw74	2	15	450	506	513	502	608	617	639	468	506	500	539.5	557.1	574.1
lsw75	3	15	450	499	521	508	635	615	613	502	495	476	557.9	559.6	558
lsw76	5	15	450	473	502	526	628	607	665	472	476	510	522.2	539.5	556.4

* THC-COOH
† Ibuprofen

TABLE 6— *Absolute THC-COOH (m/z=313) intensity and noise values of all samples.*

Sample	THC* (ng)	IS (ng)	IBU† (ug)	Absolute THC Intensity			Max Noise			Min Noise			Average Noise		
				Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
lsw38	0	15	0	1231	1186	1073	1301	1269	1235	1101	1084	1030	1209.8	1182.9	1118.5
lsw39	1	15	0	1682	1760	1670	1291	1299	1292	1074	1102	1091	1174.3	1222	1172.5
lsw40	2	15	0	1902	1846	1624	1333	1335	1133	1147	1133	966	1232.2	1228.6	1040.4
lsw41	3	15	0	2593	2804	2668	1275	1351	1270	1080	1154	1065	1423	1243.2	1157.7
lsw42	5	15	0	2757	2629	2632	1362	1335	1278	1140	1136	1082	1233.6	1226.9	1165.7
lsw44	0	15	0	943	930	965	1012	1001	1086	865	860	936	948.9	929.1	1004.8
lsw45	1	15	0	1803	1749	1813	1107	1093	1143	926	921	961	994	1010.1	1044.5
lsw46	2	15	0	1457	1544	1622	1108	1190	1250	965	1039	1053	1043.1	1117.5	1139.7
lsw47	3	15	0	2426	2340	2323	1159	1108	1202	942	947	977	1078.1	1014.4	1071.1
lsw48	5	15	0	2493	2917	2991	1134	1181	1207	938	998	983	1016.1	1064.6	1099.7
lsw49	0	15	350	1082	983	1043	1187	1074	1225	988	908	977	1082.1	1002.8	1080.3
lsw50	1	15	350	1714	1670	1711	1148	1120	1130	989	907	960	1071.7	1031.4	1036.8
lsw51	2	15	350	2128	2236	2332	1150	1136	1274	983	967	1044	1071.3	1039.5	1127.5
lsw52	3	15	350	2729	2642	2795	1104	1114	1126	897	943	961	1009.8	1016.4	1044.6
lsw53	5	15	350	4878	5277	4839	1159	1073	1118	977	899	933	1062.2	984.7	1029.4
lsw67	0	15	400	1113	1064	929	1307	1254	1199	1012	950	944	1110.8	1059.1	1047.5
lsw68	1	15	400	1081	1151	1214	1194	1213	1198	878	930	930	987.2	1021.9	1036.2
lsw69	2	15	400	1071	959	1058	1268	1221	1281	957	928	951	1043.3	1024.4	1052.5
lsw70	3	15	400	1695	1825	1827	1201	1201	1204	862	924	893	975.9	1018.1	1029.8
lsw71	5	15	400	2232	2378	2347	1196	1286	1204	928	938	919	1018.4	1030.8	1014.6
lsw72	0	15	450	1045	1034	1095	1325	1358	1367	971	986	1009	1083.7	1137.8	1114.7
lsw73	1	15	450	976	1042	1035	1356	1441	1345	1002	1055	1028	1125.4	1168.4	1138
lsw74	2	15	450	1008	999	1094	1265	1338	1393	944	977	1006	1058.8	1102.5	1123.3
lsw75	3	15	450	974	899	964	1240	1190	1232	906	910	914	1034	1012.1	1027.5
lsw76	5	15	450	919	926	905	1197	1227	1254	834	899	927	971.1	1012.8	1038.6

* THC-COOH
† Ibuprofen

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