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Increasing Gas Chromatography-Flame Ionization Detection Sensitivity for Carfentanyl Detection: An Examination of Procaine at Different Concentrations and Split Ratios

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Abstract

An opioid abuse crisis in recent years has contributed to many overdoses and overdose-related deaths in the United States. In 2017, fentanyl and fentanyl analogs contributed to more than half of NYC's overdose deaths in 2017. Carfentanyl – an extremely dangerous fentanyl analogue 100 times more potent than fentanyl – is very difficult to detect in drug samples because it is typically present in concentrations lower than analytical instruments can detect at standard settings. In this work, I hypothesize that gas chromatography-flame ionization detection (GC-FID), when optimized for the detection of carfentanyl by adjusting the split ratio, is more sensitive than standard GC-FID settings. Procaine in methanol solutions of 10 different concentrations ranging from 0.01mg/mL to 0.10mg/mL were prepared by carrying out serial dilutions and run using GC-FID. The 0.05mg/mL solution was then run at various split ratios ranging from 10:1 to 200:1. I found that the size of the procaine peak increases as the procaine concentration increases. Both an increase in concentration and a decrease in split ratio individually result in an increase in the size of the procaine peak, supporting my hypothesis. Based on the properties of procaine in this analysis, GC-FID can be optimized for the detection of small quantities of carfentanyl in non-biological samples by increasing the split ratio to increase the sensitivity of the instrument. Being able to detect carfentanyl that may be present in drug samples would assist in the tracking and regulation of carfentanyl and prevent related overdose deaths, as well as those related to fentanyl and other fentanyl analogues.

Keywords: opioid, fentanyl, carfentanyl, procaine, split ratio, gas chromatography-flame ionization detection, GC-FID

1. Introduction

Opioids are a class of drugs derived from *Papaver somniferum*, the opium poppy. Heroin, morphine, codeine, oxycodone, hydrocodone, and fentanyl are some examples of natural and synthetic opioids. Opioids are prescribed for pain and are commonly misused and abused [1]. In recent years, there has been an opioid abuse crisis, which has led to many overdoses and deaths. Opioids can be natural, extracted directly from the opium poppy plant, or synthetic, produced in laboratories. Fentanyl (*see Figure 1*) is an example of a synthetic opioid. It is an analgesic drug, used for pain relief, as well as an anesthetic drug.

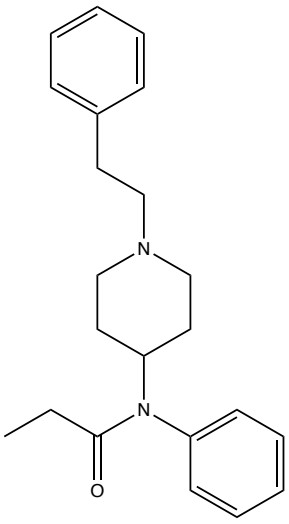


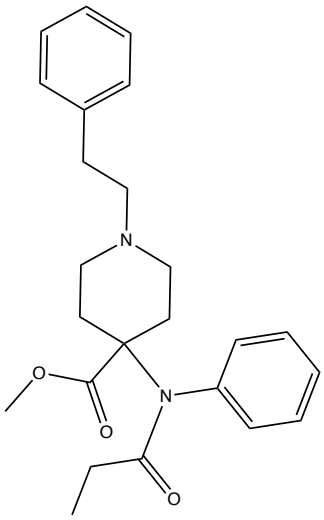
FIGURE 1: Structure of Fentanyl

Fentanyl is extremely potent; it is up to 100 times as potent as morphine [2]. This makes fentanyl very dangerous and lethal in small quantities. According to the Drug Enforcement Administration (DEA), the lethal dose of fentanyl is approximately 2 milligrams [3]. Illicitly manufactured fentanyl is commonly mixed into other drugs, including opioids, methamphetamine,

benzodiazepines, xylazine, and synthetic cannabinoids [4]. Drug users are often unaware that their drugs have been laced with fentanyl. The United States has experienced three major fentanyl-related epidemics within the last four decades [5].

Based on data from the NYC Office of the Chief Medical Examiner, fentanyl-related overdose deaths in NYC increased at a rate of almost 3,000% from 2014 to 2017, with a steep increase beginning in 2015 [6]. Colon-Berezin and colleagues (2019) explain that overdose deaths involving fentanyl – which accounted for approximately 2% of all overdose deaths in NYC from 2000 to 2014 (246 of 10,673 total deaths over the 14 years) – had soared to 57% in 2017 (842 of 1,487 total deaths in 2017 alone). This increase in fentanyl-related deaths resulted in an 81% rise in all of NYC's overdose deaths between 2014 to 2017. The detection of fentanyl and its analogues, substances that are similar to fentanyl chemically and behaviorally, is important because detecting fentanyl is the first step towards regulating fentanyl to prevent more fentanyl-related deaths.

Fentanyl has a variety of analogues, including 4-fluorobutyrylfentanyl, acetylfentanyl, butyrylfentanyl, furanylfentanyl, ocfentanyl, and carfentanyl [2]. My research focuses on carfentanyl – which may alternatively be spelled "carfentanil." For the purpose of this paper, I will be using the former spelling. Carfentanyl (*see Figure 2*), which is not approved for human use, is used as a veterinary tranquilizer [6] and is far more potent than fentanyl. Whereas fentanyl is 100 times as potent as morphine, carfentanyl is 10,000 times as potent as morphine [2]. Therefore, carfentanyl is 100 times as potent as fentanyl and is lethal in much lower doses [3]. Because carfentanyl is so potent, it is typically present in quantities even smaller than fentanyl, often in the ng/mL (nanograms per milliliter) range. These low concentrations make carfentanyl difficult, and sometimes even impossible if the concentration falls below the analytical instrument's limit of detection, to detect in other drug samples using analytical instruments.



Carfentanyl

FIGURE 2: Structure of Carfentanyl

Different instrumental methods can be employed for carfentanyl detection. Casale and colleagues (2017) discuss the detection of carfentanyl in case exhibits using a variety of analytical instruments [5]. This study detects carfentanyl using gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance spectroscopy (NMR), quantitative determination via gas chromatography-flame ionization detection (GC-FID), infrared spectroscopy (IR), and isotope ratio mass spectrometry (IRMS). Rab and colleagues (2019) discuss the detection of fentanyl and fentanyl analogues (including carfentanyl) in biological samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS) [2]. Gozdzialski and colleagues (2021) discuss how a portable GC-MS instrument can be used to detect carfentanyl in samples of opioids [4]. My research focuses on studying the properties of a noncontrolled substance using GC-FID to determine how GC-FID settings can be altered to detect carfentanyl in non-biological samples of purchased and seized drugs.

Chromatography is a method for separating the components of a mixture. Gas chromatography (GC) is one type of chromatography that vaporizes samples for analysis. It separates mixtures by utilizing an inert carrier gas mobile phase like hydrogen, nitrogen, or helium to carry the vaporized sample (the eluent) and a liquid or solid stationary phase that coats the inside of the column. Interactions between the eluent and the stationary phase cause the eluent to separate into its components. Because like dissolves like, some analytes have a higher affinity for the stationary phase than others. This causes some of the analytes to move faster or slower than others, depending on how much they interact with the stationary phase, and results in each analyte having a different retention time. The different retention times can be used to identify the different analytes.

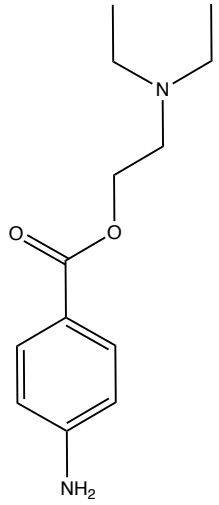
Gas chromatographs contain an injector (syringe), an inert carrier gas mobile phase, a GC column (inside of which the stationary phase coats), a GC oven, and a detector. First, a volatile sample is heated and vaporized in the injection port. This vaporized sample is called the eluent and is injected by the syringe into the GC column. The GC column into which the vaporized sample is injected is coated with the stationary phase. The stationary phases have an optimal temperature at which they can be used. The GC column within the GC oven heats the column to an appropriate temperature. After running through the column, the sample enters the detector. There are many types of detectors, but the most common GC detectors are Mass Spectrometers (MS) and Flame Ionization Detectors (FID). An FID was used for my research. In an FID, an H_2-generated flame is used to pyrolyze organic compounds eluted, producing electrons and ions. A collector electrode gathers these electrons and ions. This generates a current, which is measured to produce a signal representing the analyte mass based on how many carbon atoms there are. FID is appropriate for

both quantitative and qualitative analysis and has a wide linear response range, high sensitivity, and low noise. However, it is a destructive method of analysis.

GC-FID is a very sensitive method ideal for carfentanyl detection. GC-FID and liquid chromatography with triple quadrupole mass spectrometry (LC-MS-MS) are the most commonly used methods for detecting carfentanyl, as these methods both have very low limits of detection and can detect samples with concentrations in the ng/mL range [7,8]. GC-MS is another method commonly used for drug analysis. It is also a sensitive instrumental method but with limits of detection typically in the mg/mL (milligrams per milliliter) range, it is not quite as sensitive as GC-FID and LC-MS-MS [9,10]. Therefore, GC-MS is not an ideal method for the analysis of carfentanyl, a low level drug. GC-FID was selected over LC-MS-MS, as the former is more commonly used for drug analysis in DEA laboratories than the latter. For GC-FID analysis of most drug samples, including fentanyl, a small portion of the drug sample can be dissolved in an organic solvent, such as methanol (MeOH). Most controlled substances are organic compounds, so an organic solvent must be used, as like dissolves like. The mixture can then be directly injected into the gas chromatograph for processing.

GC can use either split or splitless modes of injection. Splitless mode means that all of the vaporized sample is injected into the GC column. In split mode, only portions of the eluent make it into the column; the rest of the gaseous sample is discarded as waste. The split ratio controls how much of an injected sample enters the GC column. Split ratio is written in a format resembling 20:1, 50:1, 100:1, etc. This ratio describes how much of the sample is discarded and how much goes into the GC column. If the split ratio is 20:1, for example, 1 unit of the eluent enters the GC column for every 20 units of eluent that are discarded as waste before entering the column. My research expands upon prior research by determining how GC-FID sensitivity could be optimized

for carfentanyl detection based on the chromatographic properties of procaine, a similar non-controlled substance. Procaine (*see Figure 3*) is an analgesic and anesthetic drug. It is a non-controlled substance. Procaine was selected for my research because it was the most similar non-controlled substance (in terms of structure and behavior) to fentanyl available. My research explores the following hypothesis: gas chromatography-flame ionization detection (GC-FID), when optimized for the detection of carfentanyl by adjusting the split ratio, is more sensitive than standard GC-FID settings.



Procaine

FIGURE 3: Structure of Procaine

2. Methods

2.1 Preparation of procaine in methanol solutions

A ~0.10mg/mL (0.103mg/mL) stock solution of procaine in MeOH was prepared by measuring 10.3mg of procaine into a 100mL volumetric flask and adding methanol up to the fill line.

Solutions of varying concentrations were then prepared by carrying out serial dilutions. 9mL of stock solution was measured into a 10mL volumetric flask using a 9mL Pyrex volumetric pipet to produce a 0.09mg/mL procaine in MeOH solution. This was repeated eight more times using decreasing volumes of stock solution to produce procaine in MeOH solutions of decreasing concentrations. See Table 1 for the volumes of stock solution used for each dilution. This resulted in a total of ten 10mL procaine in MeOH solutions with concentrations of approximately 0.10mg/mL, 0.09mg/mL, 0.08mg/mL, 0.07mg/mL, 0.06mg/mL, 0.05mg/mL, 0.04mg/mL, 0.03mg/mL, 0.02mg/mL, and 0.01mg/mL respectively.

Table 1

Preparing Procaine in MeOH Solutions of Varying Concentrations by Serial Dilutions

Procaine in MeOH Solution #	**Volume of Stock (mL)**	**Final Volume (mL)**	**Concentration (mg/mL)**
1	10	10	0.103
2	9	10	0.093
3	8	10	0.082
4	7	10	0.072
5	6	10	0.062
6	5	10	0.052
7	4	10	0.041
8	3	10	0.031
9	2	10	0.021
10	1	10	0.010

2.2 *Instrumentation*

Chromatographic analysis was performed on an Agilent Technologies 8890 Gas Chromatography (GC) system with a flame ionization detector. The GC system was equipped with an Agilent Technologies 7693A Autosampler. The injector was operated in split mode with the split ratio set to 50:1. 1.0uL of each solution was injected into the GC-FID system with the injector at 280°C. Separation was carried out on an Agilent Technologies HP-5 30m x 0.320mm x 0.25um capillary column. Hydrogen (H_2) was used as the carrier gas with a flow rate of 1.0mL/min for the first 2 minutes, followed by a gradual increase from 0.5mL/min to 3.0mL/min for the remainder of the 7.4333 minute run time. The column was set to 170°C for the first 0.5 minutes, increased at 30°C/min to 300°C, and finally increased to 20°C/min to 320°C for the final 1.6 minutes. The flame ionization detector was set to 280°C.

2.3 Adjusting split ratio

After running all ten solutions at a split ratio of 50:1, the 0.05mg/mL solution was re-run at split ratios of 10:1, 25:1, 100:1, and 200:1 to understand how increasing and decreasing the sensitivity of the instrument would affect the procaine peak. Aside from the split ratio, all GC-FID parameters described in the Instrumentation subsection remained the same.

3. Results

After running all of the prepared solutions, the procaine peak sizes of each spectrum were examined. The peak heights and peak areas, in particular were compared. According to the results procaine eluted at retention times ranging from 3.555 minutes to 3.560 minutes. As the concentration of the solution increased, the size of the peak also increased, both in height and area. As the concentration of the solution decreased, the height and area of the peak also decreased. At the lowest concentration (0.010 mg/mL), the smallest peak height of 4.476 and the smallest peak area of 2.181 were achieved. At the highest concentration (0.103 mg/mL), the greatest peak height of 45.902 and the greatest peak area of 21.092 were achieved. See Table 2 for the peak heights and peak areas of the procaine peaks of all 10 solutions at a split ratio of 50:1.

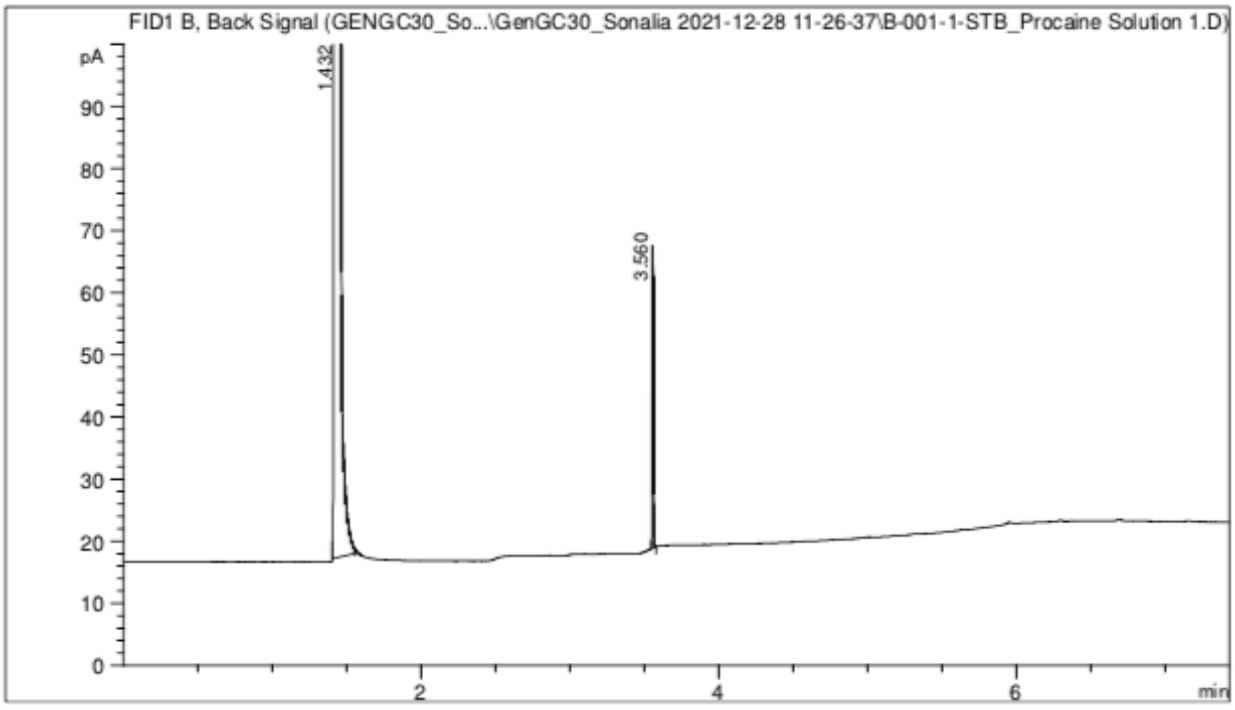


FIGURE 4: Procaine in MeOH Solution #1, Concentration – 0.103 mg/mL; Split ratio – 50:1

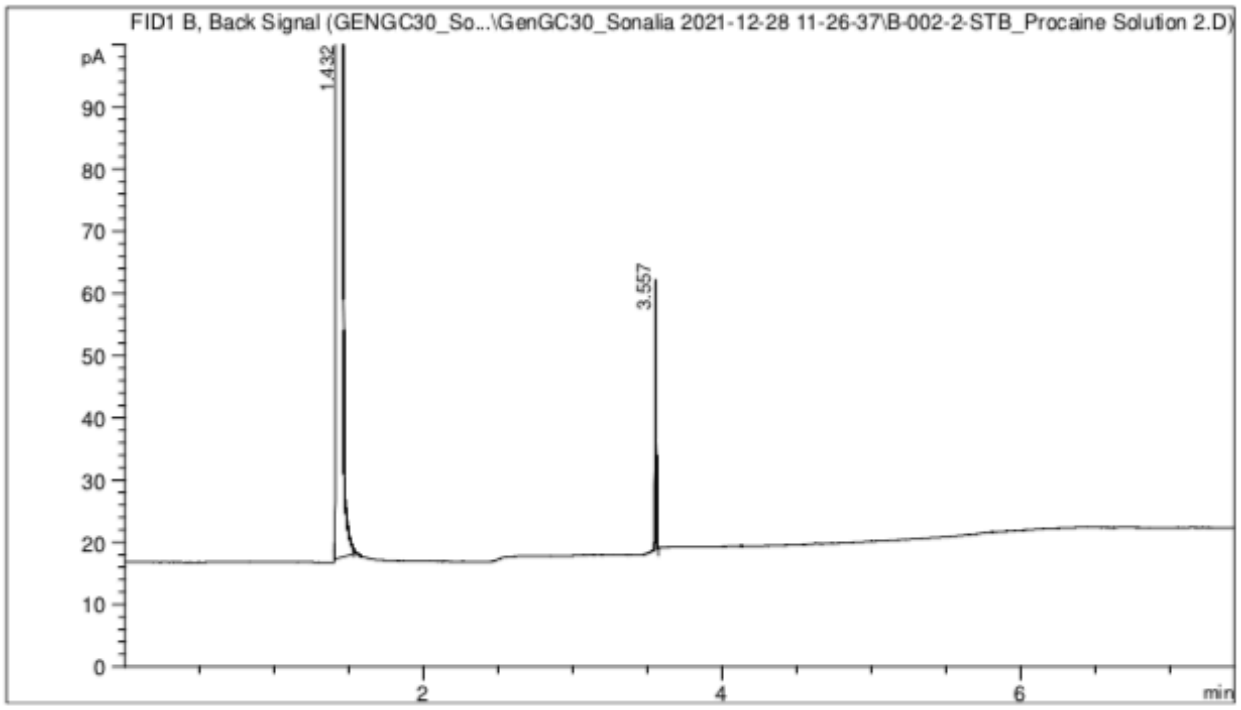


FIGURE 5: Procaine in MeOH Solution #2, Concentration – 0.093 mg/mL; Split ratio – 50:1

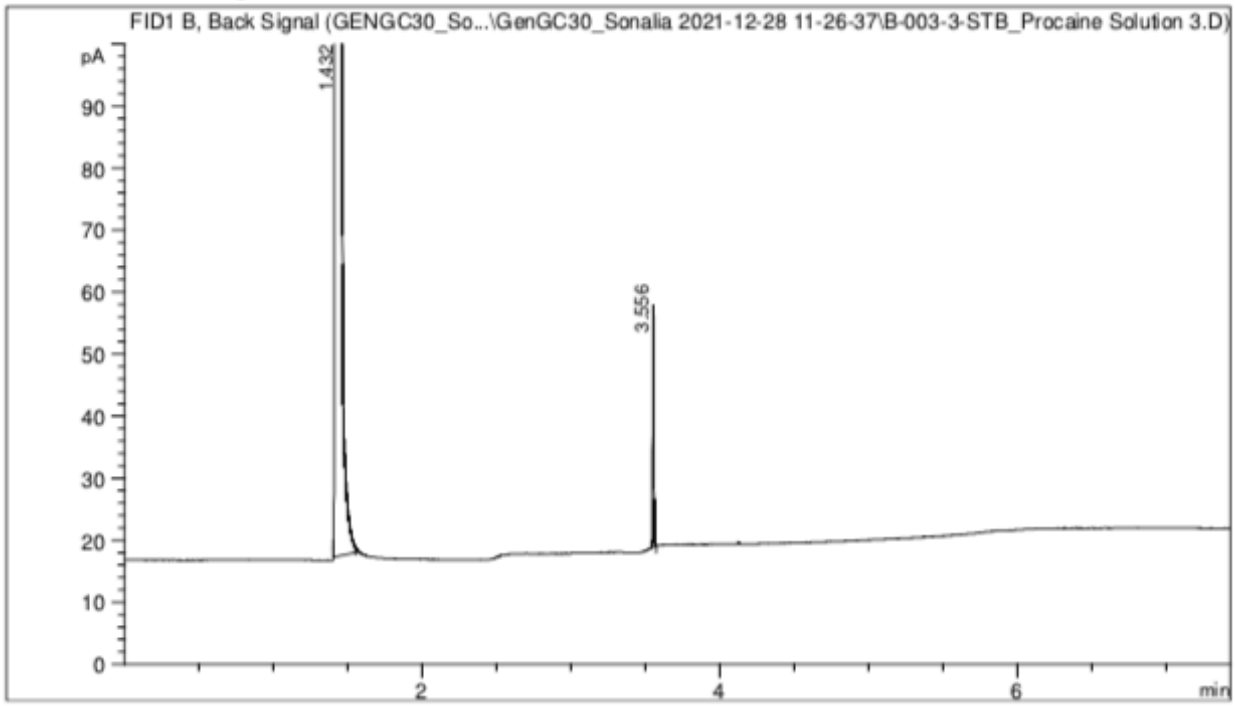


FIGURE 6: Procaine in MeOH Solution #3, Concentration – 0.082 mg/mL; Split ratio – 50:1

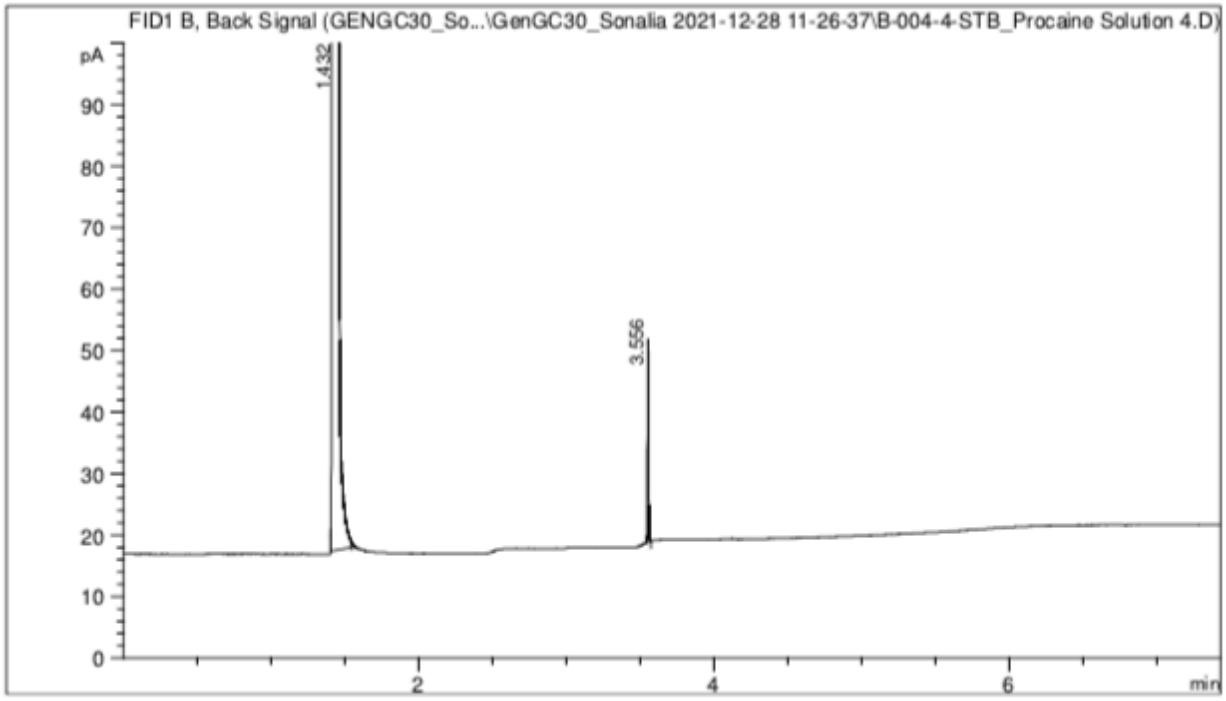


FIGURE 7: Procaine in MeOH Solution #4, Concentration – 0.072 mg/mL; Split ratio – 50:1

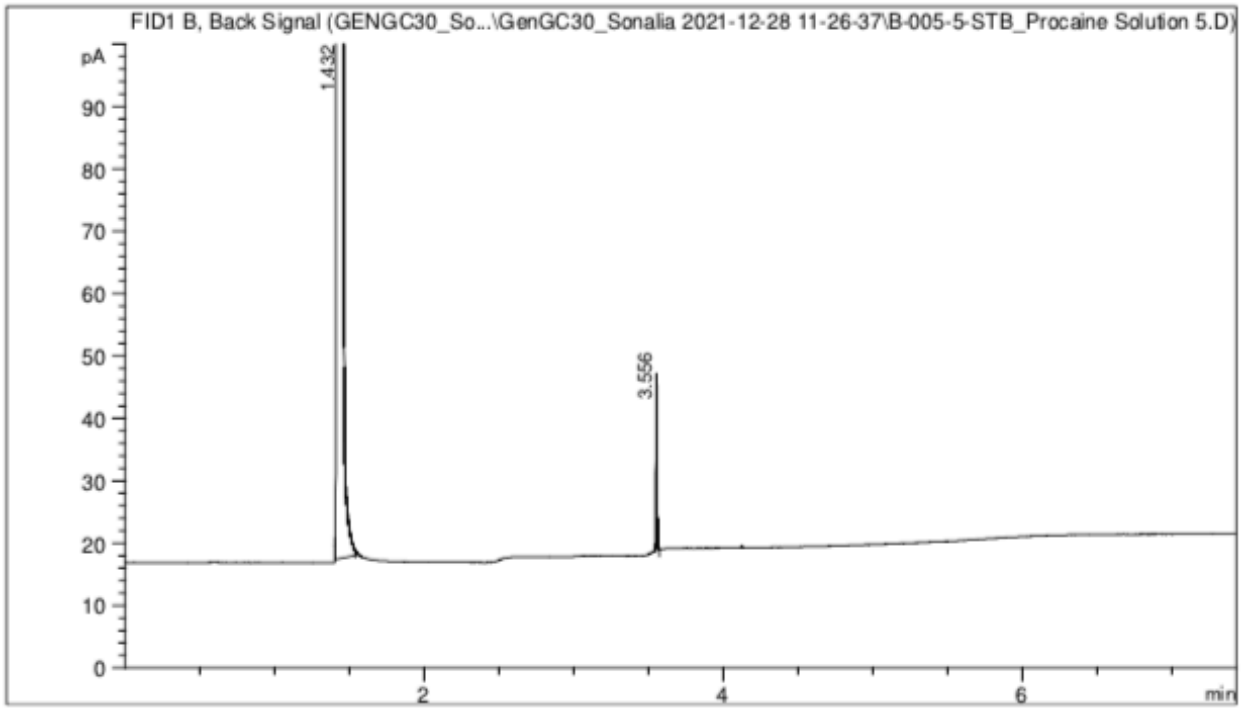


FIGURE 8: Procaine in MeOH Solution #5, Concentration – 0.062 mg/mL; Split ratio – 50:1

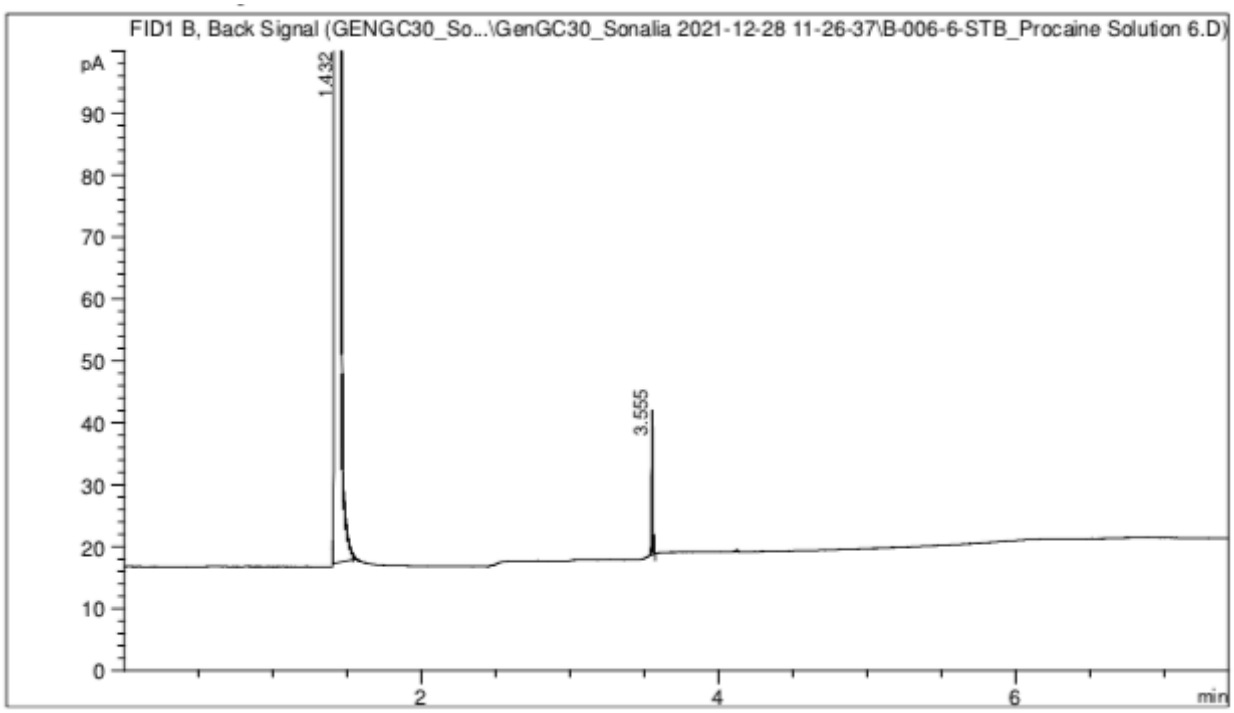


FIGURE 9: Procaine in MeOH Solution #6, Concentration – 0.052 mg/mL; Split ratio – 50:1

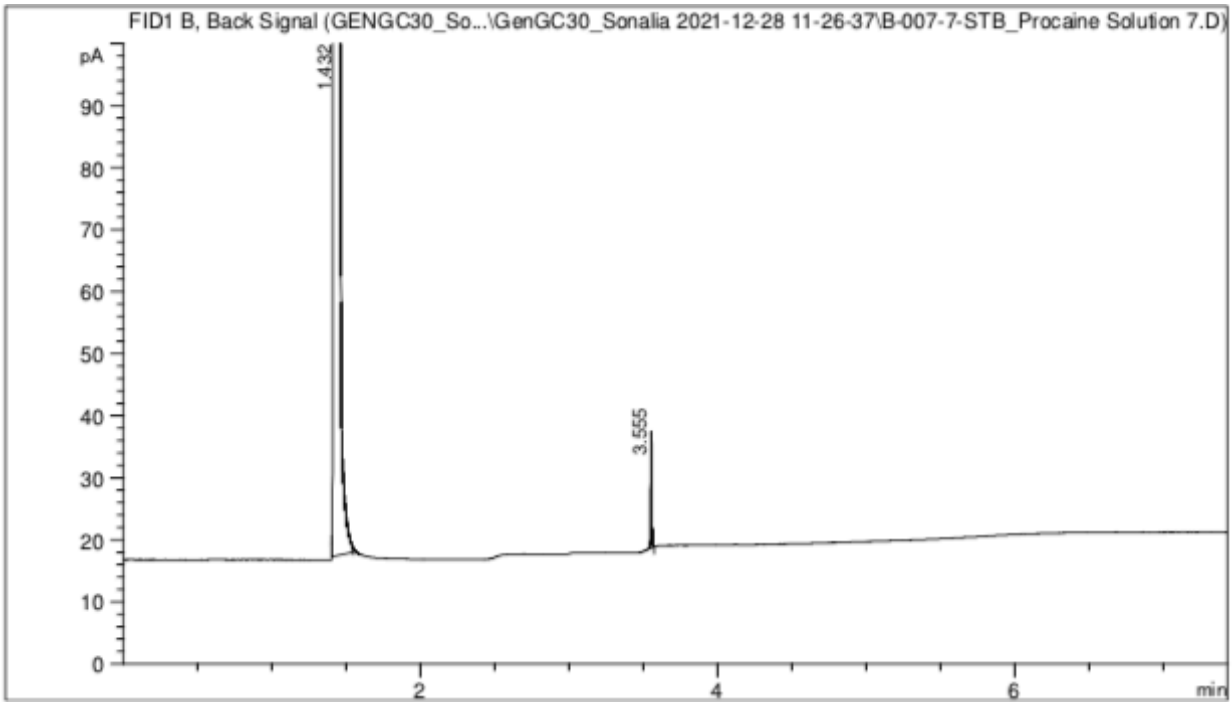


FIGURE 10: Procaine in MeOH Solution #7, Concentration – 0.041 mg/mL; Split ratio – 50:1

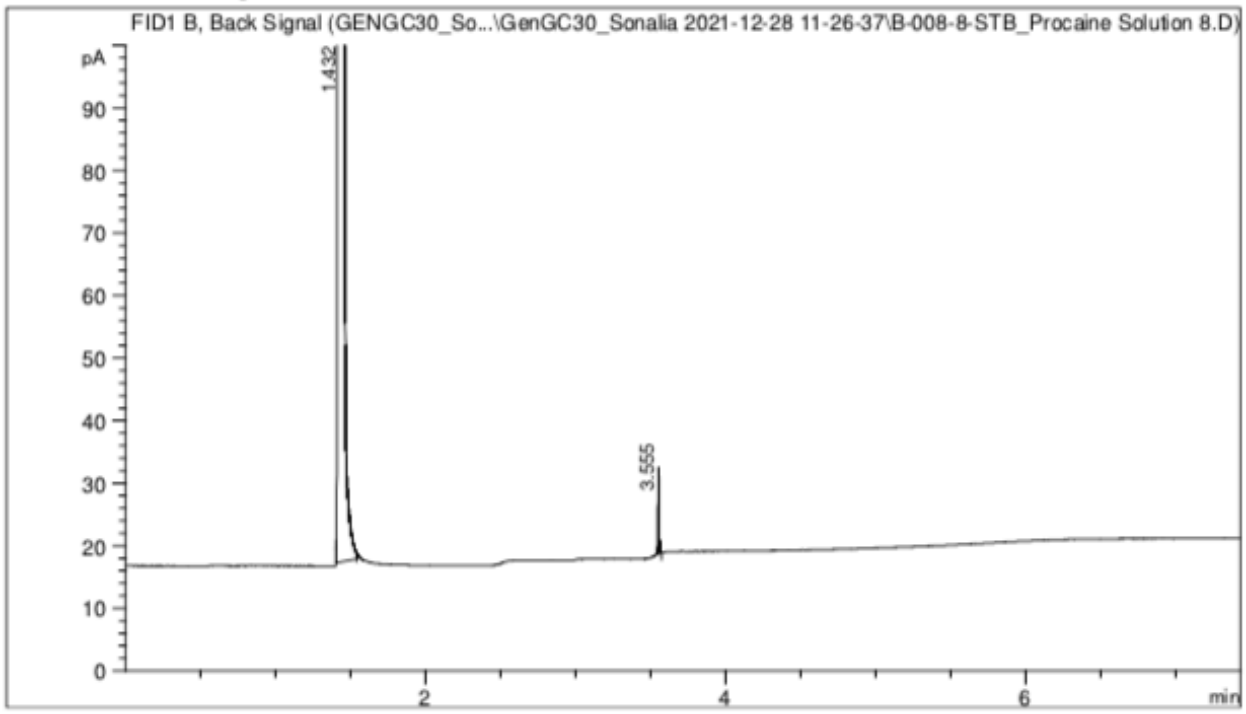


FIGURE 11: Procaine in MeOH Solution #8, Concentration – 0.031 mg/mL; Split ratio – 50:1

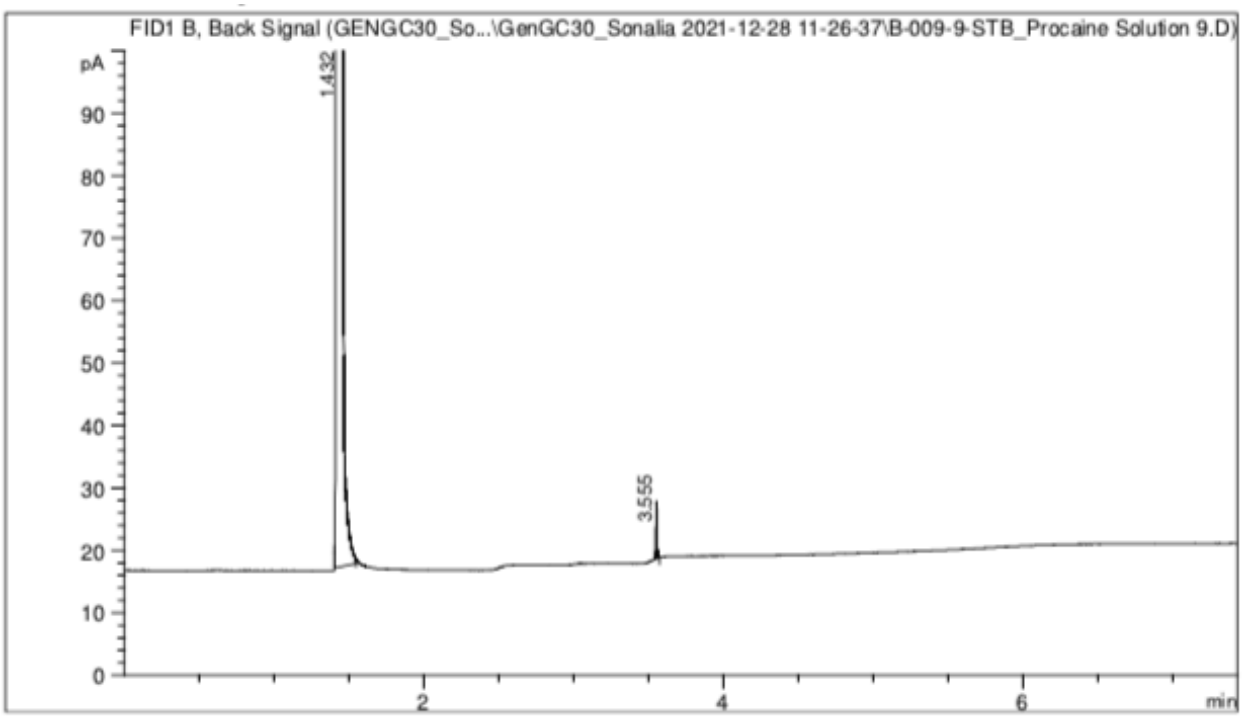


FIGURE 12: Procaine in MeOH Solution #9, Concentration – 0.021 mg/mL; Split ratio – 50:1

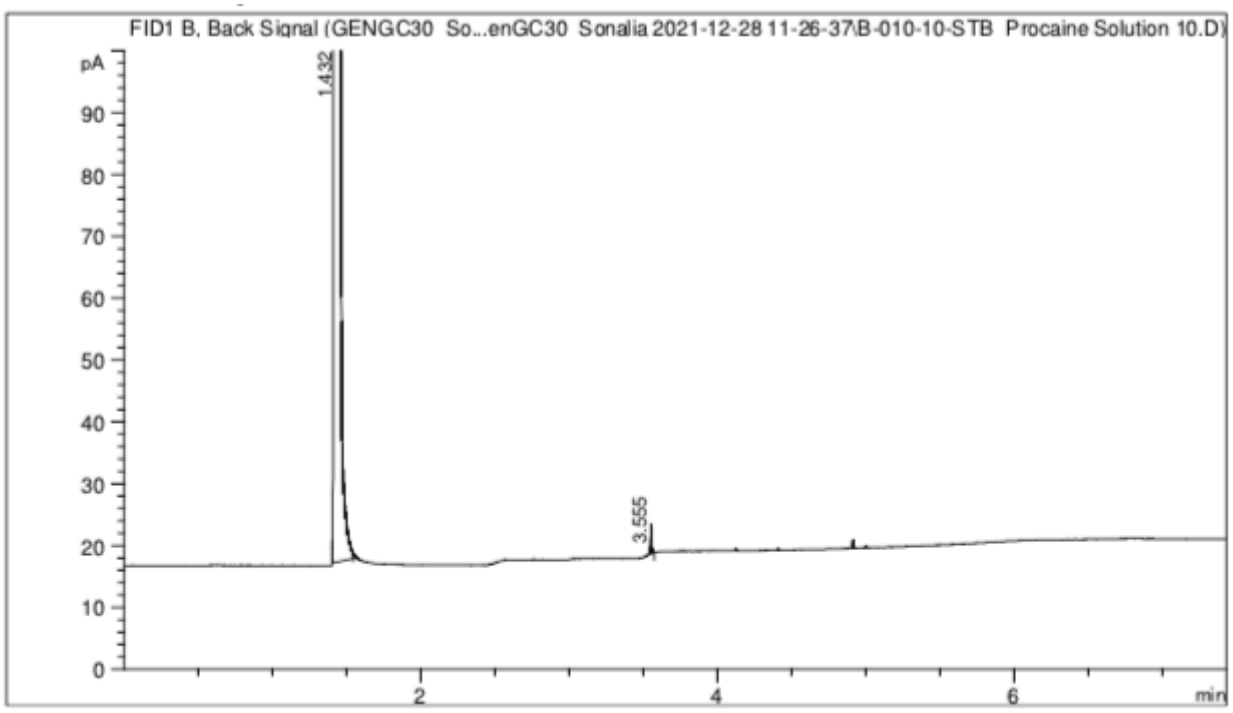


FIGURE 13: Procaine in MeOH Solution #10, Concentration – 0.010 mg/mL; Split ratio – 50:1

Table 2

GC-FID Procaine Peak Properties for Procaine in MeOH Solutions of Concentrations Ranging from 0.01mg/mL to 0.10mg/mL at a 50:1 Split Ratio

Procaine in MeOH Solution #	Concentration (mg/mL)	Split Ratio	Procaine Retention Time	Procaine Peak Area	Procaine Peak Height
1	0.103	50:1	3.560	21.092	45.902
2	0.093	50:1	3.557	18.915	41.328
3	0.082	50:1	3.556	16.819	37.507
4	0.072	50:1	3.556	14.438	31.659
5	0.062	50:1	3.556	12.318	27.154
6	0.052	50:1	3.555	10.266	21.957
7	0.041	50:1	3.555	8.244	17.77
8	0.031	50:1	3.555	6.182	13.042
9	0.021	50:1	3.555	4.085	8.626
10	0.010	50:1	3.555	2.181	4.476

As the split ratio was increased for solution #6, decreasing the sensitivity of the instrument, the peak height and area both decreased. However, as the split ratio was decreased for solution #6, increasing the sensitivity of the instrument, the peak height and area both increased. At the lowest split ratio (10:1), the greatest peak height of 95.788 and the greatest peak area of 44.148 were achieved. At the highest split ratio (200:1), the smallest peak height of 6.146 and the smallest peak

area of 2.869 were achieved. See Table 3 for the peak heights and peak areas of the procaine peaks of solution #6 at varying split ratios. The hypothesis that optimal carfentanyl detection could be achieved by altering the split ratio to increase GC-FID sensitivity was supported by these findings.

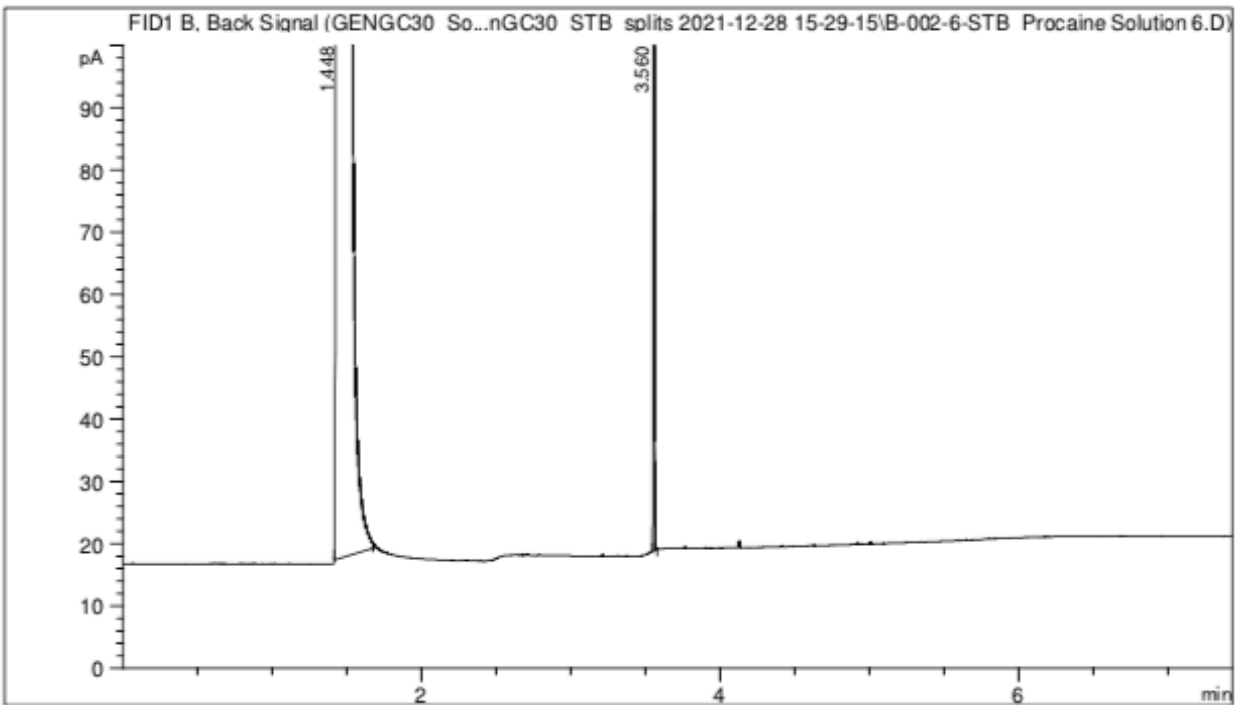


FIGURE 14: Procaine in MeOH Solution #6, Concentration – 0.052 mg/mL; Split ratio – 10:1

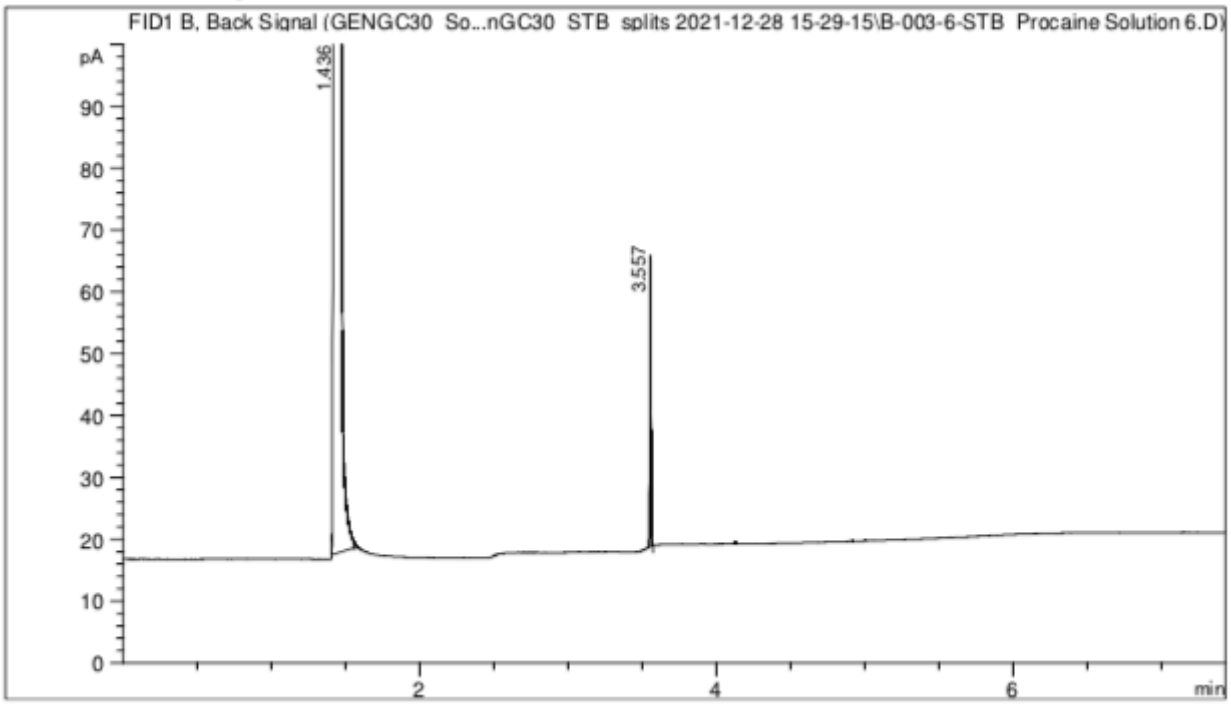


FIGURE 15: Procaine in MeOH Solution #6, Concentration – 0.052 mg/mL; Split ratio – 25:1

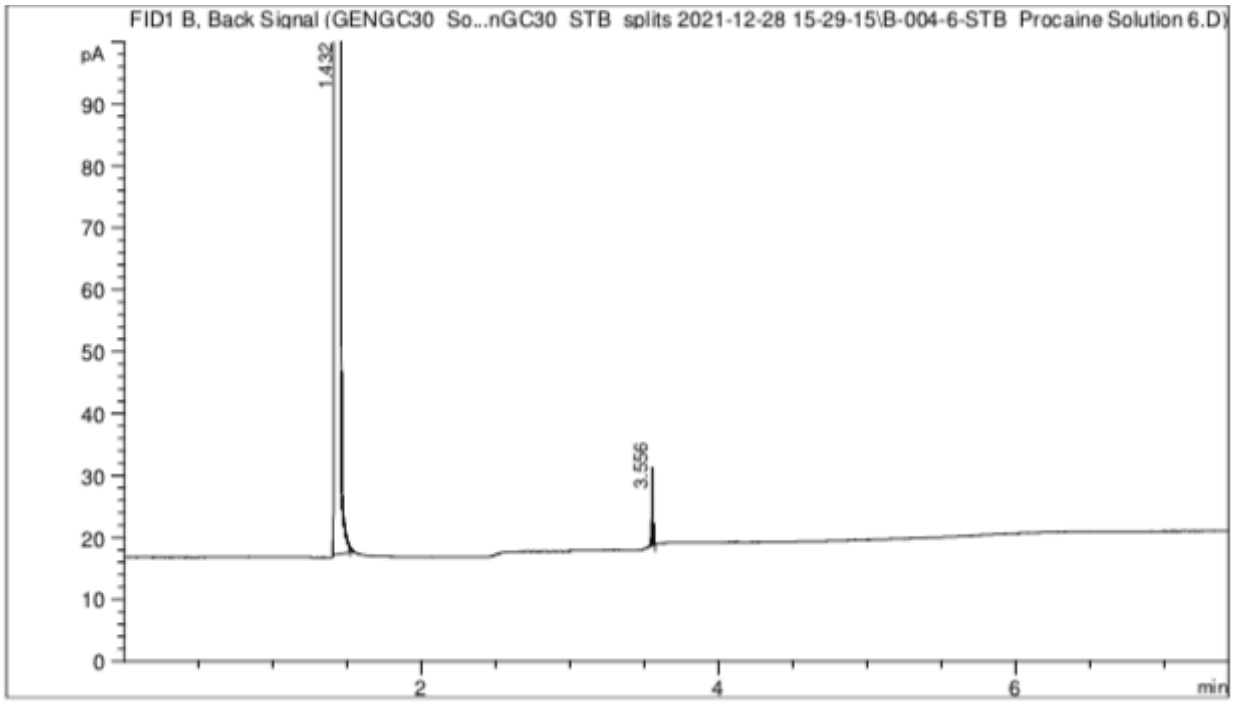


FIGURE 16: Procaine in MeOH Solution #6, Concentration – 0.052 mg/mL; Split ratio – 100:1

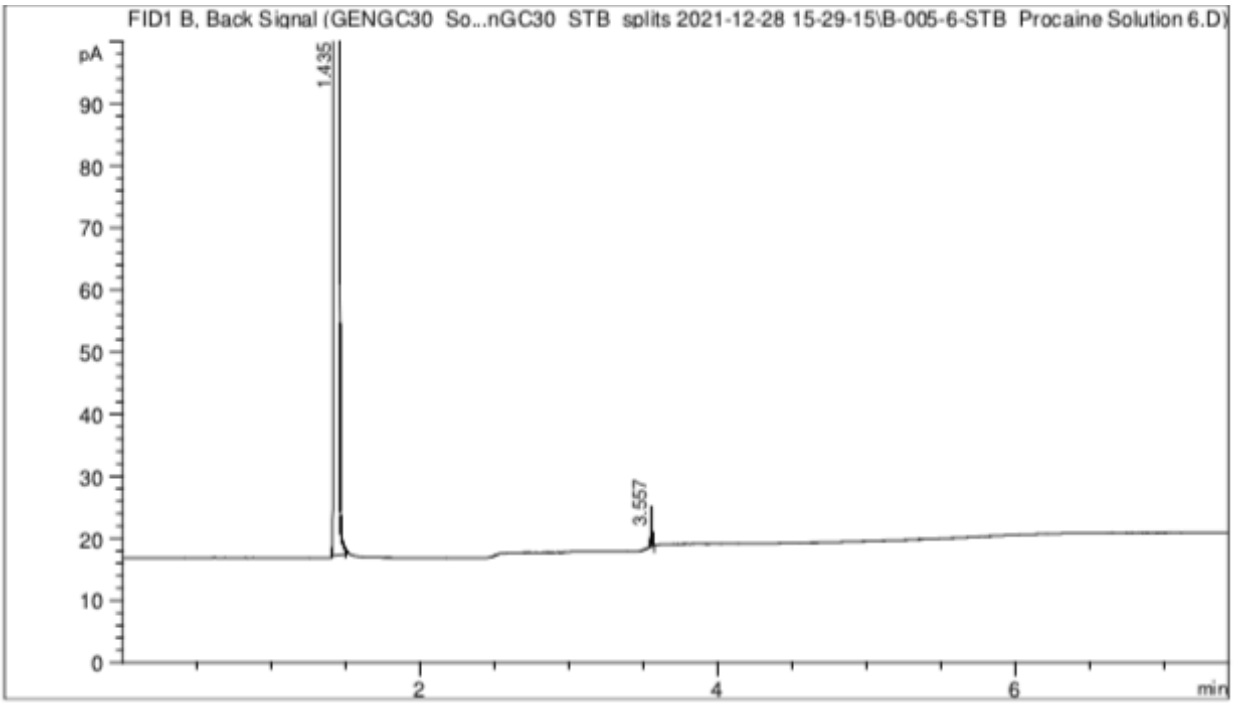


FIGURE 17: Procaine in MeOH Solution #6, Concentration – 0.052 mg/mL; Split ratio – 200:1

Table 3

GC-FID Procaine Peak Properties for a 0.052mg/mL Procaine in MeOH Solution at Varying Split Ratios

Procaine in MeOH Solution #	Concentration (mg/mL)	Split Ratio	Procaine Retention Time	Procaine Peak Area	Procaine Peak Height
6	0.052	10:1	3.560	44.148	95.788
6	0.052	25:1	3.557	20.148	43.817
6	0.052	50:1	3.555	10.266	21.957
6	0.052	100:1	3.556	5.431	11.986
6	0.052	200:1	3.557	2.869	6.146

4. Discussion

I hypothesized that optimizing gas chromatography-flame ionization detection for the detection of carfentanyl involves adjusting the split ratio to make the instrument settings more sensitive than standard GC-FID settings. This is reliant on the properties of procaine in different concentrations and at different split ratios using GC-FID. The results demonstrate the properties of procaine when analyzed by GC-FID at varying concentrations. The data in Table 2 indicate that an increase in the concentration of the procaine solutions with a constant split ratio correlates to an increase in the size of the procaine peak, both in height and area. Therefore, the greater the amount of procaine present, the larger the procaine peak will be. This is the result of the instrument detecting more sample as the concentration increases. So, the more sample that is present, the easier it will be to detect and identify that sample above the level of detection.

Showing support for my hypothesis, the sensitivity of the instrument was increased by adjusting the split ratio to increase the analyte peak size. The purpose of this was to allow for the detection of low concentrations of analyte by replicating the results of increasing the procaine concentration. The results demonstrate the properties of procaine when analyzed by GC-FID at varying split ratios. The data in Table 3 indicate that an increase in the split ratio with a constant concentration of procaine in the various methanol solutions correlates to a decrease in the size of the procaine peak, both in height and area. As the split ratio increases, the sensitivity of the GC-FID instrument decreases by limiting the amount of procaine entering the instrument. As the split ratio decreases, the sensitivity of the GC-FID instrument increases by increasing the amount of procaine entering the instrument. For comparison, one part of the injected sample enters the GC column for every ten parts of injected sample that are discarded as waste at the low split ratio of

10:1; however, at the high split ratio of 200:1, one part of the injected sample enters the GC column for every 200 parts of injected sample that are discarded as waste. Therefore, lower split ratios are able to detect lower concentrations of procaine than higher split ratios can. If a sample has a very low concentration and cannot be detected at a high split ratio, lowering the split ratio may make it possible to detect the sample.

The challenge that inspired this work is the detection of trace quantities of carfentanyl. While I was not able to directly test carfentanyl in this assay, what we have learned about the properties of procaine in GC-FID may be applied to carfentanyl to understand how low-concentration samples of carfentanyl could be detected using GC-FID. Carfentanyl was not accessible for carrying out research, so procaine (a similarly-structured noncontrolled substance) was used instead to determine how adjusting the split ratio affects the size of the analyte peak. Drug samples – especially those with a higher carfentanyl contamination risk, such as opioids – can be screened using GC-FID at lower split ratios to attempt to detect low concentrations of carfentanyl, which may not be possible to detect at higher concentrations. Increasing the sensitivity by lowering the split ratio has the same effect on the analyte peak as increasing the analyte concentration while using a constant split ratio. Based on my results, samples of smaller concentrations could be more easily detected by decreasing the split ratio to increase the sensitivity of the GD-FID system. Therefore, analysts may be able to optimize GC-FID for carfentanyl detection by decreasing the split ratio and achieving a more sensitive instrument setting.

The number of opioid-related deaths is on the rise as the United States faces an opioid crisis. The majority of these deaths are related to fentanyl and fentanyl analogues, including carfentanyl. Illicit drugs – including but not limited to opioids – are commonly laced with fentanyl and its analogues unbeknownst to users and even some distributers. Fentanyl is extremely potent,

and carfentanyl is about 100 times more potent than fentanyl [3]. Carfentanyl is deadly in far lower concentrations than fentanyl and typically found in smaller quantities, making it much more difficult to detect than fentanyl. The ability to more regularly detect carfentanyl in non-biological samples of purchased and seized drugs will allow for better detection, surveillance, and regulation of carfentanyl. Detecting carfentanyl would, therefore, help to reduce the number of opioid overdose victims by keeping carfentanyl, along with fentanyl and other fentanyl analogues, off the streets.

5. Conclusions

Testing serially-diluted procaine in methanol solutions in order of increasing concentration resulted in an increase in size of the procaine peak in the GC-FID spectra. Decreasing the split ratio without altering the procaine concentration mimicked these results. Based on the GC-FID properties of procaine, it was determined that GC-FID could be optimized for carfentanyl detection by decreasing the split ratio to increase the instrument's sensitivity and detect samples of lower concentrations.

Acknowledgements

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