Method Development and Validation for the Identification and Separation of Acetyl Fentanyl, Fentanyl, and Heroin

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Abstract

The identification of fentanyl and fentanyl analogs in heroin samples has become of growing importance in the United States, as the number of samples submitted to forensic laboratories containing these substances has increased. The use of GC/MS and GC-FID for the identification of acetyl fentanyl has previously been proven difficult due to acetyl fentanyl, fentanyl and heroin having similar retention times. The objective of this research was the development of a method for the separation and identification of fentanyl and acetyl fentanyl in heroin samples by GC/MS and GC-FID. To determine the success of the method developed the peak separation and resolution were calculated. The results from this research demonstrate that the method developed could adequately separate acetyl fentanyl, fentanyl and heroin.

Introduction

Heroin is a schedule I narcotic that is a semi-synthetic form of morphine, and has been considered one of the most addictive controlled substances (1). Heroin has been a commonly analyzed substance in drug laboratories for years. Until recently, this analysis mainly consisted of positively identifying heroin in samples submitted to forensic laboratories. The presence of the synthetic opioid fentanyl and its analogs (primarily acetyl fentanyl) in heroin samples analyzed at the Kentucky State Police (KSP) Eastern Laboratory has become an increasing problem. Fentanyl is a schedule II narcotic substance. Many fentanyl analogs including alfentanyl, acetyl fentanyl, and sufentanyl were also synthesized in addition to fentanyl itself. Much of the concern surrounding fentanyl analogs is due to their potential for dependence and misuse, their high potency and their associated risk of fatal overdose (2).

Compared to other fentanyl analogs, acetyl fentanyl is most commonly identified in forensic drug laboratories. Drug users are often unknowingly sold heroin that contains fentanyl or one of its analogs. This can lead to accidental overdose or death (3).The significant risk to public health that acetyl fentanyl presents has led to its emergency scheduling into schedule I of the controlled substances act in May of 2015 (4).

The large increase in the number of cases where acetyl fentanyl has been present in heroin samples has resulted in the need for gas chromatography mass spectrometry (GC/MS) and gas chromatography with a flame ionization detection (GC-FID) methods for the identification and separation of acetyl fentanyl, fentanyl, and heroin. Previously, acetyl fentanyl and heroin have been difficult to separate and identify due to similar retention times and many overlapping ions making it difficult to positively identify both in a sample. The goal of the following research was to develop a GC/MS method that could readily be applied in forensic drug laboratories to differentiate between heroin, fentanyl, and acetyl fentanyl. Once the new acetyl fentanyl/heroin method was developed through this project, it was compared to the current method used by KSP for drug identification and found to be an improvement with an increase in the amount of separation between drugs and a decrease in the amount of overlapping ions.

Methods and Materials

Certified reference standards of acetyl fentanyl (1 mg/mL), fentanyl (1 µg/mL), and heroin (1 mg/mL) were purchased from Cerilliant (Round Rock, TX) for use in analysis. In order to determine the degree of separation of acetyl fentanyl and heroin in samples in which they are both present, solutions containing various ratios of acetyl fentanyl, fentanyl, and heroin were prepared. Samples of unknown concentration and combinations of the same drugs were also analyzed.

Methods and Materials

An Agilent Technologies 7890B gas chromatograph with a 5977A mass spectral detector (MSD) and flame ionization detector (FID) was used for analysis throughout this project. The GC/MS contained a DB-5MS Ultra Inert capillary column with a length of 15 meters, a diameter of 0.250 millimeters, and a film thickness of 0.25 micrometers.

Data was first collected using the standard method for drug analysis used by the KSP Eastern Laboratory. The parameters for that method are available in Table 1.

Table 1: Parameters for KSP GC/MS Method of Separation

Oven Temperature			Pressure			Injector	
		Hold Time			Hold Time	Mode	Split
Initial	100°C	0.50 min	Mode	Ramped		Split Ratio	50:1
Ramp	20°C /min	8.5 min	Initial	5 psi	0.5 min	Carrier Gas	Helium
Final	315°C	0 min	Ramp	150 psi/min to 40 psi		Injection Volume	1.00 µL

The oven temperature program, pressure program, and split ratio were adjusted throughout the project in order to achieve optimal separation of acetyl fentanyl, fentanyl and heroin in samples. The parameters for the final method demonstrating the greatest separation, and therefore the best identification of acetyl fentanyl and heroin are listed in Table 2.

Table 2: Parameters for Optimal GC/MS Separation of Acetyl Fentanyl, Fentanyl, and Heroin

Oven Temperature			Pressure			Injector	
		Hold Time			Hold Time	Mode	Split
Initial	230°C	13.5 min	Mode	Ramped		Split Ratio	25:1
Isothermal Temperature Program			Initial	5 psi	0.5 min	Carrier Gas	Helium
			Ramp	150 psi/min to 10 psi		Injection Volume	1.00 µL

The retention times obtained throughout the research were used to determine the peak resolution and peak separation for each method. The average retention time for each drug was calculated by averaging all the retention times obtained for each drug throughout the research. This was completed for each method. After the retention times for each sample were determined the separation between the different components of each sample solution and the peak resolution between the components were calculated.

Results

The GC-FID method currently in place in the KSP Eastern Laboratory was determined to adequately separate acetyl fentanyl, fentanyl, and heroin, allowing the focus to be on developing a new GC/MS method for identification and separation of acetyl fentanyl and heroin.

The results of GC/MS portion of this research showed that an improved method for the identification and the separation of acetyl fentanyl, fentanyl and heroin could be developed. The overall average retention times can be found in Table 3. The average retention times were used to calculate the amount of separation between the peaks. The overall average peak separation can be found in Table 4.

Results

Table 3: Overall Average Retention Times (in minutes)

	Heroin	Acetyl Fentanyl	Fentanyl
KSP Method	8.145	8.302	8.520
New Method	6.370	7.125	8.257

Table 4: Overall Average Peak Separation

	Acetyl Fentanyl/Heroin	Fentanyl/Acetyl Fentanyl	Fentanyl/Heroin
KSP Method	0.156	0.221	0.375
New Method	0.746	1.143	1.893
Increase in Separation	**0.590**	**0.922**	**1.518**

The peak resolution was also calculated for the two methods. The peak resolution for each of the sample solutions increased when they were analyzed using the new method. The peak resolution for all of the samples showed at least a two-fold increase in peak resolution. The average peak resolution for each method can be found in Table 5.

Table 5: Overall Average Peak Resolution

	Acetyl Fentanyl/Heroin	Acetyl Fentanyl/Fentanyl	Fentanyl/Heroin
KSP Method	4.397	5.003	10.304
New Method	10.136	12.897	23.163
Increase in Resolution	**5.739**	**7.894**	**12.859**

The amount of separation between acetyl fentanyl, fentanyl, and heroin using the KSP method did not allow for their individual identification in samples because heroin ions carried over into the acetyl fentanyl mass spectrum. With the method developed in this study several of the smaller ions present in heroin carried over into acetyl fentanyl as well as larger ones such as 268, 327, and 369. However, with the increased separation obtained using the new method, both acetyl fentanyl and heroin could be identified when present in the same samples. The amount of carryover from heroin in the mass spectra of acetyl fentanyl and fentanyl greatly decreased. Only a few of the smaller heroin ions and the 369 ion carried over into the spectra for the other substances. The difference in the amount of carryover ions in the spectra was determined by a visual comparison of Figures 1 and 2.

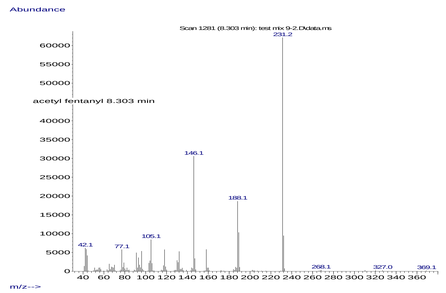

Figure 1: Acetyl Fentanyl Mass Spectrum from KSP Method

Results

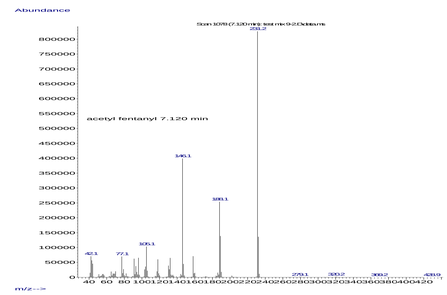

Figure 2: Acetyl Fentanyl Mass Spectrum from New Method

Conclusions

Developing a method for the identification and separation of acetyl fentanyl and heroin is of growing importance due to the increasing number of reports where acetyl fentanyl is being found in heroin. The development of this method increased in importance for drug analysts in Kentucky when acetyl fentanyl became a schedule I narcotic in early 2015 (5). Even more recently, acetyl fentanyl was given temporary placement as a schedule I controlled substance by the Drug Enforcement Administration. With the individual scheduling of acetyl fentanyl, it would be a good assumption that other fentanyl analogs will also be individually scheduled. The objective of this research project was to develop a method that could successfully separate acetyl fentanyl, fentanyl, and heroin so that both could be positively identified in samples together.

This research project was successful in developing and validating a new method for the separation of acetyl fentanyl and fentanyl in heroin, however, more research is needed to further improve and expand the method. Future studies, should include elimination of the few remaining heroin ions, that carry over into the acetyl fentanyl and fentanyl spectra. The source of the unexplained ions present in the mass spectra of acetyl fentanyl and fentanyl, should also be determined. Additionally, it would be beneficial to determine if the method developed in this research project could be applied to other drugs with similar retention times to aid in their identification.

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