Time Optimization of Gas Chromatography-Mass Spectrometry Method for Controlled Substances Analysis

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Abstract *— An optimization of the gas chromatography- mass spectrometry (GC-MS) method was conducted by establishing a temperature ramp of 150°C, held by 3 min and raising at 12°C/min until reaching 300°C, which from 25.00 min was reduced to 15.50 min the analysis time and identified qualitatively the Caffeine, Cocaine, Delta 9 THC, Oxycodone and Heroin. A validation of the results was performed to determine the precision, instrument detection limit (IDL) and selectivity from six replicates analysis. For the controlled substances identification a 200 ppm QC-Standard Drug mix solution was prepared. A low variability was observed in the retention time of each compound. Instead, the peaks area demonstrates a high variability from the acceptance criteria of RSD, especially the Oxycodone affecting the IDL results. All the compounds showed a good resolution with acceptance criteria of R<2, except Oxycodone. Further measurements will be necessary to acquire more tight values to the acceptances criteria*.

Key Terms *— Controlled substances, GC-MS, Temperature, Validation*

INTRODUCTION

This project aims for a time optimization for the analysis of controlled substances in a gas chromatography- mass spectrometry (GC-MS). Unlike a previous method that was set to 25.00 minutes, by doing modifications in the oven temperature settings a shorter time analysis of 15.50min can be acquired. For that reason, an improvement of the method was conducted by establishing a different oven temperature ramp, which save time and identified qualitatively compounds of interest. A validation of the results was performed to determine the precision, instrument detection limit (IDL) and selectivity from the six replicate analysis as part of a system suitability.

The majorities of the research articles suggest changing multiple supplies and parameters at the same time to achieve faster GC-MS analysis. That's why is proposed to start with changes of parameters that can accelerate the process of analysis, but in the other hand not require additional costs. Also this project contribute to the literature for the purpose that other entities could adopt the method to obtain results in less time from controlled substance analysis by just doing modifications in temperature parameters and has guide to verify the system suitability of the equipment.

LITERATURE REVIEW

In analytical chemistry GC-MS is one of the equipment of major relevance, where is capable to identify qualitatively the characteristic of chemicals in a sample by the identification of the molecules that is compose. The GC is in charge of separating the molecules of the analyte in the chromatography column due to their properties differences. In other way, MS detector split components into ionized species and segregated them in their mass to charge relation. For the qualitatively detection gas chromatography makes the first step of separation and the mass spectrometry do the second one. During the samples screening is preferable the GC-MS, as it's designed for the use a gaseous phase for the component separation in the column which give faster results. In fact, the GC-MS is considered a predominant forensic technique that can be

presented as evidence in law court, because generates unbiased results that prove certainly what types of substance were present.

A number of analysis applicability could be attributed to the GC-MS, such as in medicine, environmental monitoring and clean-up, food, beverage and perfumes, security, criminal forensics and the law enforcement for the identification of controlled substances; as related to this project. [4] Actually, comparative analysis is what normally is presented as evidence, in which the acquired spectrum result is compared to a spectrum library to match characteristic that are present in the sample. Otherwise always a quantitatively analysis is needed to support the evidence to determine the current amount of the substance. [2][4][5]

When substances have to be screened and identified for example in a case of law enforcement, generally the analytical results are required in short time and be cost effective. In the development of a fast GC-MS method multiple parameters and additional features could be integrated to reduce time of the chromatographic run. [3][6] Some of the factors that are possible to modify in a GC-MS for a rapid analysis may be a faster splitless injection, liberty to choose a suitable column, ultra-fast ion response time, compatibility with the scanning speed of quadrupole MS and improved compatibility with low thermal mass ultra fast GC. But have to be put on perspective that when analysis methods are implemented, they are modified to optimize the performance of the equipment according with the supplies that are available, due to acquisition limitations of the same apparatuses as other laboratories. Usually rapid GC-MS is accomplished based on the exchange of GC resolution for speed of analysis and the separation is remunerated with enhanced separation of the MS detector. [3] Additionally, technology is in constant development into the latest instruments, where sensitivity and specificity had been improved. For that reason MS detectors has acquired popularity because, are more affordable and can detect multiple analytes. [1]

According to literature the oven program is one of the parameters in a GC-MS that can be modified to obtain shorter run time and better resolution between multiple analytes from a standard. [8] The changes that more effect has in the mentioned factors are in the initial column temperature, initial temperature hold time and column temperature column rate. [7][8] Therefore, the focus of this project is to modify does parameters from a previous method used for qualitative identification of controlled substances. In view of the fact that limitations were confronted to change other features that as reviewed can make faster GC-MS analysis, is a cost effective alternative that have to be considerate for development.

METHODOLOGY

The analysis was performed on April 24, 2014 at the Customs and Border Protection Laboratory facilities in San Juan. For the procedure development, some of the main gas chromatography-mass spectrometry default parameters were established as follows:

- GC-MS model: GC Agilent 7890 coupled quadrupole MS detector Agilent 5975
- Column of J&W HP-5 (5% Phenyl Methyl Siloxan) with the dimensions of 30m X 250 µm X 0.25 µm, which holds a temperature until the 325°C
- Carrier gas: Helium Ultra High Purity at a flow rate of 1.2 mL/min
- Front SS Inlet He split mode, heater 250°C, pressure 9.1312 psi and total flow 64.2 mL/min
- Purge flow 3 mL/min
- Split radio 50:1 and split flow 60 mL/min
- The full scan acquisition mass range was 30.0-550.0

A 200 ppm QC-Standard solution was prepared for the identification of controlled substances by mixing each of the next certified high purity compounds in 250 mL of (4:1) Chloroform/Methanol: Cocaine (0.0511 g), Heroin (0.0515 g), Oxycodone (0.0516 g), Delta 9-Tetrahydrocannabinol (0.0500 g) and Caffeine

(0.0489 g). In addition a standard solution of 1000 ppm D-Amphetamine Sulfate was spiked to an aliquot of 2 mL containing the drug mix solution.

From that aliquot 1 µL was directly injected in the GC-MS and analyzed by each adjusted range of temperatures in the oven settings. After those trials, it was determined that chromatogram peaks were identified in a period time of 15.50 min at a temperature ramp of 150°C, held by 3 min and raising at 12°C/min until reaching 300°C. By using that temperature six replicate injections were performed for the acquisition of the method validation statistical data.

For qualitative methods validations it is suggested that at least selectivity and the LOD (Limit of Detection) have to be evaluated and the precision determination would be of added value. [1] [6] In this case, the method was validated with those three metrics. In which resolution is the metric for the determination of selectivity, as an approach to LOD was evaluated with IDL (Instrument Detection Limit) and the standard deviation with the RSD for precision.

- **Precision:** Injection reproducibility was stated as RSD (relative standard deviation) to know how GC-MS performed in the moment that samples were analyzed. RSD should be ≤ 1% for n≥5 samples [10]. The equations are:

$$STD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}} \quad (1)$$

$$RSD = \frac{STD}{\bar{X}} \cdot 100 \quad (2)$$

Where X_i is the obtained value from peaks area ratio, n is the number of determinate samples and $\bar{X}$ is the mean of values.

- **IDL:** The IDL (Instrumental Detection Limit) is the minimum amount of substance that is statistically greater than zero within a specified probability. IDL is linked to the standard deviation (STD) of the measured area resulting from replicate injections and a statistical correction factor t_α as of the following equation [9]:

$$IDL = (t_\alpha)\,(RSD)\,(\text{amount standard})/100\% \quad (3)$$

Since the number of consecutively measurements is of n=6 (n<30), the one sided Student t-distribution was used to determine the confidence factor t_α with n-1 degrees of freedom at 99% confidence level. [10] [11]

- **Selectivity:** The selectivity was applied to determine whether a particular compound in the matrix will interfere or interact with the substances of interest. The targets analytes should have a baseline chromatographic resolution of R>2 from all other components in the sample. Resolution is calculated with the subsequent equation [9]:

$$R = [2(t2-t1)] / (w2+w1) \quad (4)$$

Also a comparison analysis was performed with the GC-MS library matching the detecting substances, where the minimum quality acceptance is 70%. [12]

DISCUSSION OF RESULTS

The developed method could simultaneously identify the analytes of interest in which, all the compounds were successfully identified in the comparison analysis with above the minimum quality acceptance of 70% [12]. One thing that has to be established is that the spiked sample of D-Amphetamine Sulfate in the 2 mL aliquot data, was took only into consideration for the comparison analysis and the selectivity calculations. Due to it was not determined the volume added to the aliquot, the compound final concentration is unknown. Therefore can't be used for the determination of precision and IDL.

The precision determination is summarized in Table 1. By using the average of chromatogram peaks area from six replicates, could be determined the standard deviation and relative standard deviation for each substance. The compounds relative standard deviations were of 3.04% to 60.07%, which are over the acceptance criterion (RSD ≤1%). These percents can be associated to the peaks abundance as shown in Figure 1, where

Caffeine (at a retention time of 8.617 min) is the peak with greater abundance and have the lower RSD percent. Instead, Oxycodone is peak with lower abundance and higher RSD percent.

Table 1
Determination of Relative Standard Deviation of Chromatogram Peaks Area

Analyte	Peak Average	STD	RSD (%)
Caffeine	6048383.667	183730.5167	3.04
Cocaine	1072660.167	47420.74597	4.42
Delta 9 THC	242010.3333	21596.72202	8.92
Oxycodone	157575.7821	94661.85716	60.07
Heroin	245584.5	21316.59914	8.68

*In this table is presented the relative standard deviation (RSD) of each compound chromatogram peaks area from six consecutive injections.

In Table 2, the precision of the retention times was evaluated calculating an average for the chromatogram peaks that correspond to each substance from six replicates. For all substances the relative standard deviations were within the acceptance criterion (RSD ≤1%) in a range of 0.003% to 0.032%.

Table 2
Determination of Relative Standard Deviation of Retention Time

Analyte	Rt Average	STD	RSD (%)
Caffeine	8.616	0.000516398	0.006
Cocaine	11.854	0.000408248	0.003
Delta 9 THC	13.757	0.000752773	0.005
Oxycodone	14.292	0.004578937	0.032
Heroin	14.920	0.000983192	0.007

*This table shows the relative standard deviation of each compound retention time.

This represents a very low variability in retention times for each substance. But Oxycodone has the higher variability in comparison with the other compounds. It might be attributed to the peak tailing, in which the software integrator confronts difficulties in the determination of where or when the peaks end [10]. It can be appreciated in Figure 1, that the Oxycodone peak tailing is longer than other peaks.

For the instrument detection limits (IDL) evaluation, was used the equation (3) for each substance. It was determinate that for six replicates (n=6) at 99% of confidence level the correction factor t_α for the equation is of 3.365 [11]. In Table 3 are collected the values used in the equation such as RSD from peaks area and the amount in ppm of each substance and also the instrument detection amount limits results. The IDL results go from 20 ppm to 417 ppm, being Oxycodone the bigger amount of the detectable limit. Oxycodone IDL result could have been inflated by the low abundance of the substance that reflects the chromatogram (Refer to Figure 1).

Table 3
Determination of Instrument Detection Limit (IDL)

Analyte	RSD	Amount (ppm)	IDL (ppm)
Caffeine	3.04	196	20
Cocaine	4.42	204	30
Delta 9 THC	8.92	200	60
Oxycodone	60.07	206	417
Heroin	8.68	206	60

*This table summarizes the data that was used to obtain the IDL results at a confidence level of 99% with a t_α of 3.365.

The selectivity results are presented in Table 4 as resolution values for each substance by replicates of six injections in the GC-MS. All compounds fulfilled the acceptance criterion of R>2, except for Oxycodone that his resolution values during the consecutive replicates fluctuated in a range of 1.47 to 1.89. The lower resolution results of Oxycodone are due to the proximity of an impurity peak that appears at a of retention time 14.195. (Refer to Figure 1)

CONCLUSION

The method for qualitative identification of controlled substance was optimized and validated. A reduction from 25.00 to 15.50 minutes of run time analysis was successfully achieved with

Table 4
Determination of Resolution

Resolution	Run #1	Run #2	Run #3	Run #4	Run #5	Run #6
Caffeine	152.26	124.76	155.39	155.39	152.26	152.26
Cocaine	64.47	66.73	67.94	66.75	66.75	64.47
Delta 9 THC	39.23	28.65	41.41	42.19	41.43	39.27
Oxycodone	1.51	1.51	1.43	1.89	1.83	1.47
Heroin	10.59	11.07	10.30	12.74	12.33	9.96

*The table summarizes the resolution results for each analyte in six consecutive injections.

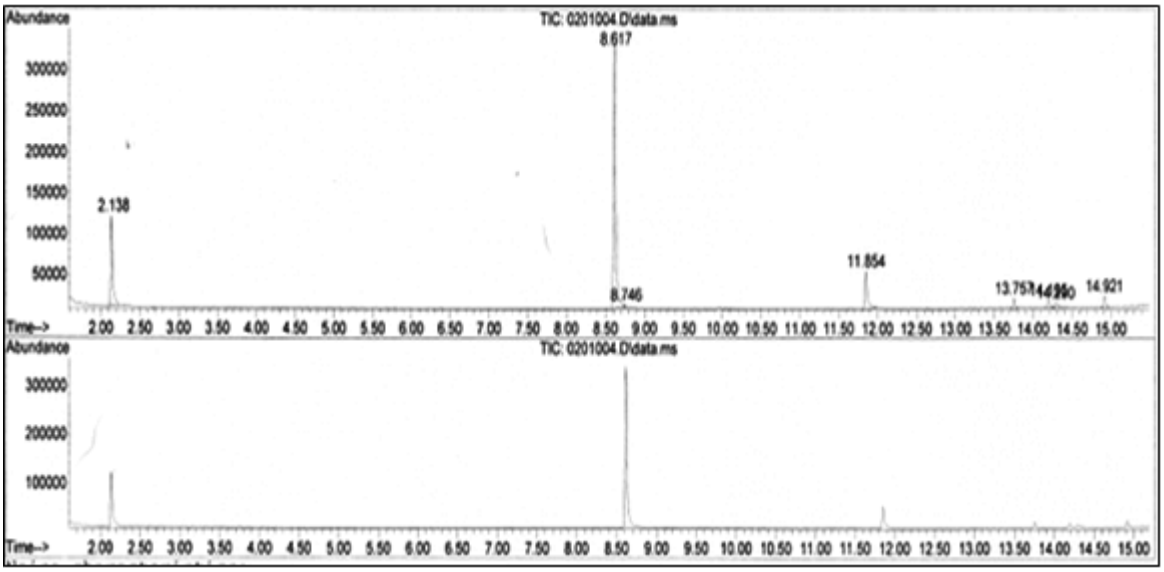

Figure 1
GC-MS Chromatogram at 15.50 Minutes (D-Amphetamine Sulfate, Caffeine, Cocaine, Delta 9 THC, Oxycodone and Heroin).

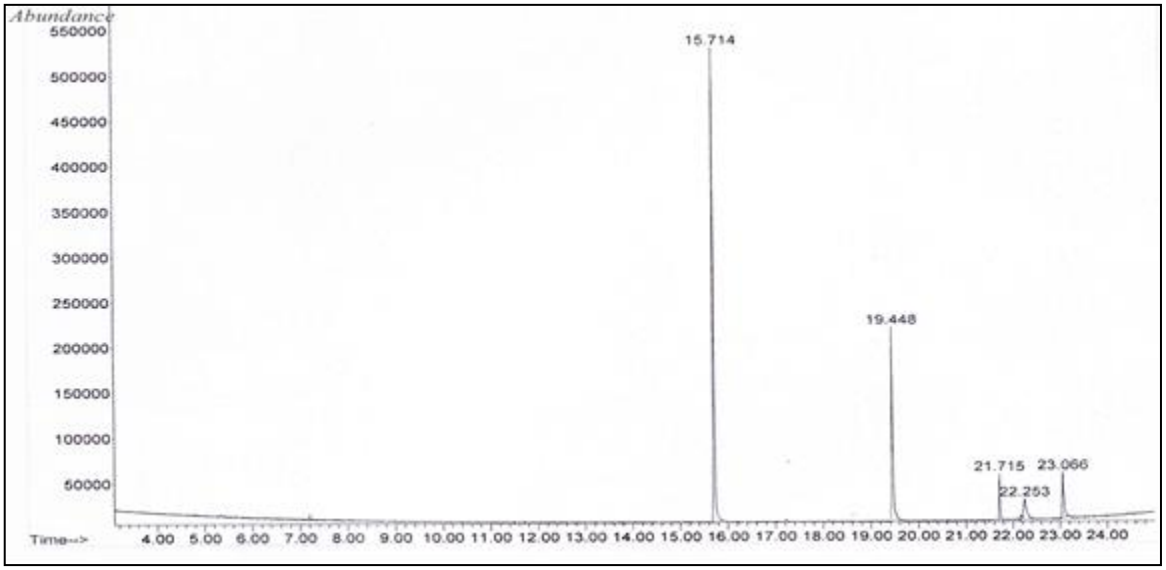

Figure 2
GC-MS Chromatogram at 25.00 Minutes (Caffeine, Cocaine, Delta 9 THC, Oxycodone and Heroin).

the settled oven temperature as it could be observed by comparing Figure 1 and 2. During the system suitability validation was determined the precision, instrument detection limit and selectivity using the collected data as result of six replicate injections in the GC-MS.

From precision results (refer Table 1), the peaks area of each substance demonstrates a high variability with respect to the RSD acceptance criterion, especially Oxycodone with a RSD of 60.07%. But if Figure 1 is compared with the RSD results, can be conclude that the relation between peaks abundance and variability is inversely proportional. In contrast, a low variability was observed in the retention time RSD for all substances in Table 2.

Besides of the precision data, in Table 3 Oxycodone showed a higher value in the instrumental detection limits results than other compounds. As of that the RSD for Oxycodone was of 60.07%, an inflated statistical value was obtained causing that the detectable amount of substance was greater than the amount analyzed. Unlike of Oxycodone, the other compounds expressed lower IDL values which are in accordance with expected values.

In Table 4 selectivity was evaluated by the calculation of chromatogram peaks for every compound in six consecutive injections. All substances showed a good resolution above the acceptance criterion (R<2) except Oxycodone, where his poor resolution is associated to the proximity of an impurity peak that appears at a of retention time 14.195. (Refer Figure1)

Finally can be concluded that the new adjustment of temperature is suitable for the identification of the controlled substances of interest, but further measurements have to take place to acquire more tight values to the acceptances criteria.

RECOMMENDATIONS

The variability observed from RSD results may be reduced using higher concentrations of the substances to reach upper peak abundance as the Caffeine, who was the peak with greater abundance and lower variability.

Although greater variations can be acceptable for low detectable levels would be helpful for a future evaluation a bigger number of replicates as another feature to reduce variability percent.

In the selectivity evaluation, a better resolution might be obtained by adding a greater concentration of the compound.

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