Reproducibility and Repeatability in Calibration for Gas Chromatography in Fusel and Esters Analysis

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Abstract — *Gas chromatography is a very sensitive method of analysis that requires understanding what will be analyzed in order to choose the correct parts and analysis parameters. This project seeks to find the ideal conditions to achieve reproducibility and reliable values of samples with high and low levels of fusel and esters. Chromatography of two different liners was evaluated and the calibration levels were created separately to maintain the individual conditions of analysis. The result of this study helped to obtain better reproducibility in the reference samples. On the other hand, maintaining two calibration conditions created flexibility in the use of GC instruments, which could speed up the customer's response to important decisions.*

Key Terms — *AGT ("aguardiente"), Calibration, Ester, Fusel, Gas Chromatography, Liner, Method Parameters, Standard.*

PROBLEM STATEMENT

The reproducibility of the reference samples, as well as in samples high in fusel oils has been a recurrent problem in the Quality Control Laboratory. This situation leads to the necessity of instrument calibration several times during a week, repetition of samples preparation and/or injections, thus causing delays and accuracy of analysis results to our customers Distillery and Process (Figure 1). This situation also causes the analysts assigned to the area, to fully work in 'Troubleshooting' without being able to attend other areas.

This project aims to ensure repeatability and reproducibility in calibration in a gas chromatography equipment for the analysis of fusel and esters. During a calibration, the equipment is expected to remain within a set range. Standard addition calibration is useful for compensate for the effect that other substances can produce on the signal measured by the equipment. For the quality laboratory to which this project applies, a weekly calibration was established to ensure optimal conditions and only a daily verification to corroborate compliance. Two Gas Chromatography (GC) equipment were dedicated for this analysis.
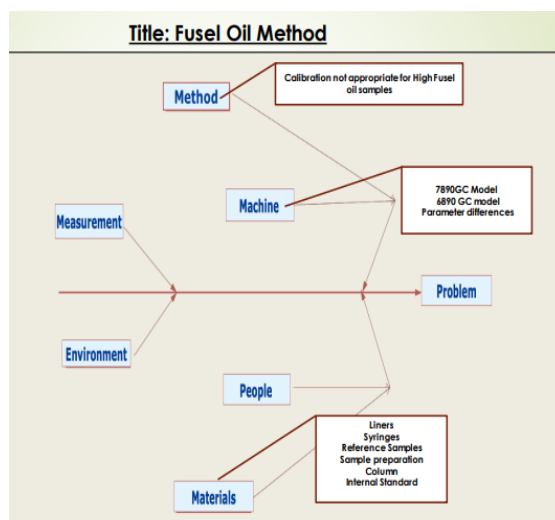

Figure 1
Method Analysis

One of the goals that the quality laboratory have is continuous improvement. It is important to identify the elements that affect the compliance of the GC calibration in order to keep availability and to be able to trust the results obtained.

RESEARCH DESCRIPTION

This research work aims to improve procedure to establish calibration conditions and standard values for the analysis of fusel and esters faster and accurate. This procedure directly affects the quantitative analysis of samples that require only fusel and ester results.

RESEARCH OBJECTIVES

It is important to establish an efficient and robust calibration protocol prior to the analysis of a sample. Figure 2 shows daily fault causes from August to September 2018. As it can be seen, calibration is the main fault cause. The requirements of this project are:

- Minimize recalibrations due to lack of reproducibility results in high solids products and high fusel (> 100 mg/100ml) product
- Achieve 75% of compliance with the daily reference samples criteria. (Actual: 45%).
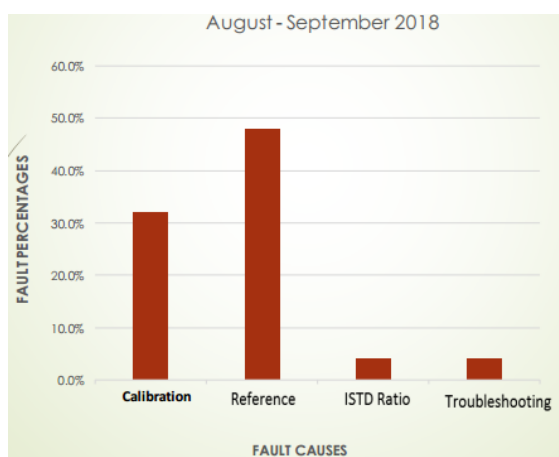
Figure 2
Daily Start-Up Fault Causes

This work started with a previously established protocol that requires improvements since the calibration conditions are not reproducible and repeatable. There´s a need to determine the causes that affect compliance in the calibration of the GC of the Agilent 6890 Technology. Variations in the methodology, the environment, the qualification of the personnel, the matrix, the equipment and the material were analyzed. The previously validated calibration method will be use, using two standards with specific value ranges. This project will determine what elements affect the repeatability of calibration results.

Research Contributions

This procedure will help the analyst to perform the correct troubleshooting at the time of having atypical calibration results so that it returns to the expected values. In this way, it is guaranteed that the equipment is always in good condition and produces the best results. This project will contribute to avoid rework of calibration and reference samples, further improvement in delivery of results on time and optimize the use of human resources. This will save time and cost in solving problems. One of the most important contributions of this project will address the requirements established in the quality standards and finally, guarantee reliability and traceability.

Literature Review

Definitions:

- **Total Fusel Oils**: Is the sum of the following components: n Propanol, Sec-butanol, Iso-butanol, N-butanol, Iso-amyl, Act-amyl, N-Amyl.
- **Reproducibility**: The measurement can be obtained with stated precision by a different team, a different measuring system, in a different location on multiple trials.
- **Gas Chromatography (GC):** Is a technique for separating chemical substances that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the components in a mixture [1]. This technique is use for determinate fusel and esters Oils. Figure 3 shows major GC instrument components.
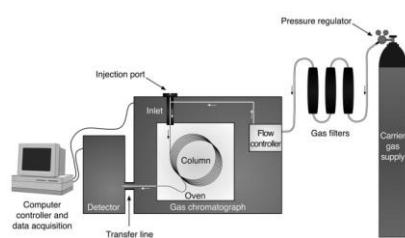
Figure 3
Overview Gas Chromatography Instrument

In a reliable measuring system, precision, linearity and stability are considered to ensure an acceptable pattern. The rum industry needs to measure the amount of fusel and ester present in the manufacturing base, since these components

determine the odor of the product already manufactured [2]. For a laboratory that analyzes large amounts of samples daily, it is necessary to add repeatability and reproducibility as part of the calibration analysis.

In this project, repeatability is in relation to the amount of variability in the measurement system that is caused by the measuring device. In contrast, reproducibility will have to do with how much of the variability in the measurement system is caused by the differences between the analyst (operator). In this case, it is applied to gas chromatography for the analysis of esters and fusel, specifically to the GC model Agilent Technology 6890 (Figure 4) using Chemstation as software [3].

A standard of 14 components supplied by Sigma-Aldrich will be used as calibration standard for fusel and ester ranges. Table 1 is use as a standard 1 compound concentration range and Table 2 for standard 2 compound concentration range. As Figure 5 and 6 shows, calibration chromatography for standard 1 and 2 respectively.

Figure 4
Gas Chromatography Instrument

The validated method for the determination of this study is based on the statistical evaluation of the dispersion of the results in the form of minimum and maximum range:

- The calibration ranges acceptable for standard 1 are:
  Fusel: 29mg/100mL to 31mg/100mL
  Esters 20mg/100mL to 21mg/100mL

- The calibration ranges acceptable for standard 2 are:
  Fusel: 117mg/100mL at 122mg/100mL.
  Esters: 82mg/100mL at 85mg/100mL

Table 1
**Fusel and Ester Oils Concentrations Standard 1 using Custom Mix**

Fusel Oils Concentration Standard using Fusel Oils Custom Mix								
Composed	Amount in Custom Mix STD (ug/mL)	Purity	Amount in Custom Mix STD (mg/mL) Purity Corr.	Amount in WK STD, (.5mL/100mL)	Amount in WK std (mg/100mL)	-2%	Amount in Wk STD (mg/100mL) STD 1	2%
Methanol	4000	1	4.0000	0.0200000	2.00000	1.960	2.000	2.040
Acetaldehyde	4000	0.999	3.9960	0.0199800	1.99800	1.958	1.998	2.038
Acetone	8000	1	8.0000	0.0400000	4.00000	3.920	4.000	4.08
Methyl Acetate	16000	0.999	15.9840	0.0799200	7.99200	7.832	7.992	8.152
N-Propanol	8000	1	8.0000	0.0400000	4.00000	3.920	4.000	4.08
sec-Butanol	8000	0.998	7.9840	0.0399200	3.99200	3.912	3.992	4.072
Ethyl acetate	8000	0.999	7.9920	0.0399600	3.99600	3.916	3.996	4.076
iso-Butanol	8000	0.997	7.9760	3.9880000	3.98800	3.908	3.988	4.068
n-Butanol	8000	0.999	7.9920	3.9960000	3.99600	3.916	3.996	4.076
Iso Amyl alcohol	16000	0.999	15.9840	7.9920000	7.99200	7.832	7.992	8.152
Act. Amyl (2-Methyl-1-butanol)	4000	0.998	3.9920	1.9960000	1.99600	1.956	1.996	2.036
N. Amyl (Amyl alcohol)	8000	0.999	7.9920	3.9960000	3.99600	3.916	3.996	4.076
Total Esters						**20**	**20.900**	**21**
Total Fusel						**29**	**30.000**	**31**
Ethyl Formate Concentrate STD								
Composed	Amount in Ethyl formate STD (g/100mL)	Purity	Amount in Ethyl formate STD (mg/mL) Purity Corr.	Amount in WK STD (2mL/100mL)		-2%	Amount in Wk STD (mg/100mL) STD 1	2%
Ethyl formate	1.8294	0.9700	17.7452	8.8730		8.6950	8.8730	9.0500

Table 2
Fusel and Ester Oils Concentrations Standard 2 using Custom Mix

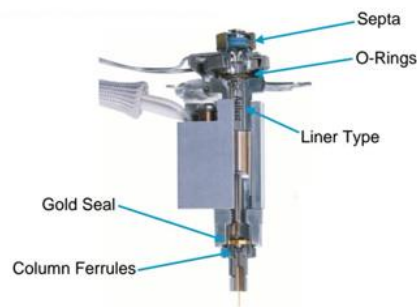| Fusel Oils Concentration Standard using Fusel Oils Custom Mix								
Composed	Amount in Custom Mix STD (ug/mL)	Purity	Amount in Custom Mix STD (mg/mL) Purity Corr.	Amount in WK STD, (2mL/100mL)	Amount in WK std (mg/100mL)	-2%	Amount in Wk STD (mg/100mL) STD 2	2%
Methanol	4000	1	4.0000	0.0800000	8.00000	7.840	8.000	8.160
Acetaldehyde	4000	0.999	3.9960	0.0799200	7.99200	7.832	7.992	8.152
Acetone	8000	1	8.0000	0.1600000	16.00000	15.680	16.000	16.32
Methyl Acetate	16000	0.999	15.9840	0.3196800	31.96800	31.329	31.968	32.607
N-Propanol	8000	1	8.0000	0.1600000	16.00000	15.680	16.000	16.32
sec-Butanol	8000	0.998	7.9840	15.9680000	15.96800	15.649	15.968	16.287
Ethyl acetate	8000	0.999	7.9920	15.9840000	15.98400	15.664	15.984	16.304
iso-Butanol	8000	0.997	7.9760	15.9520000	15.95200	15.633	15.952	16.271
n-Butanol	8000	0.999	7.9920	15.9840000	15.98400	15.664	15.984	16.304
Iso Amyl alcohol	16000	0.999	15.9840	31.9680000	31.96800	31.329	31.968	32.607
Act. Amyl (2-Methyl-1-butanol)	4000	0.998	3.9920	7.9840000	7.98400	7.824	7.984	8.144
N. Amyl (Amyl alcohol)	8000	0.999	7.9920	15.9840000	15.98400	15.664	15.984	16.304
Total Esters						**82**	**83.400**	**85**
Total Fusel						**117**	**119.800**	**122**
Ethyl Formate Concentrate STD								
Composed	Amount in Ethyl formate STD (g/100mL)	Purity	Amount in Ethyl formate STD (mg/mL) Purity Corr.	Amount in WK STD (2mL/100mL)		-2%	Amount in Wk STD (mg/100mL) STD 2	2%
Ethyl formate	1.8294	0.9700	17.7452	35.4900		34.7810	35.4900	36.2000
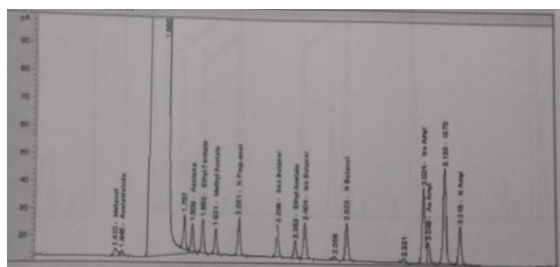
Figure 5
Standard 1 Chromatography
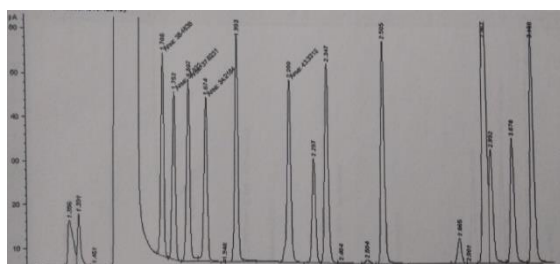
Figure 6
Standard 2 Chromatography

METHODOLOGY

As part of the research process, executed trials were done, and presented below.

Resolve & Execute – Trials

Trial 1 - Comparison of 2 liners: Liners are made of glass that helps limit the degradation of the sample and improve vaporization [4]. Figure 7 shows where the liner was inside the injection port. This is where the liquid sample is vaporized and transported to the column by the carrier gas.
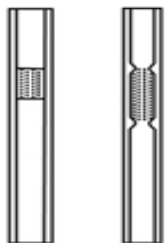

Figure 7
Injection Port

This trial aim to seek better reproducibility using standard trials on different liner design. Figure 8 is an example of precision liner (deactivated glass wool) and Cycle Splitter used for those trials.
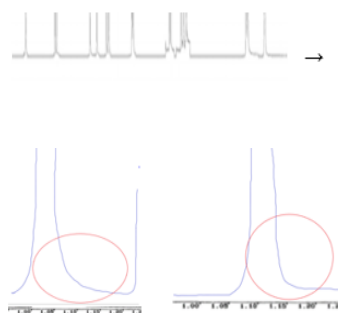
Figure 8
Precision Liner and Cycle Liner

Precision liner resulted in a deformation of the baseline in the chromatography (Figure 9). This shows that there is less resolution. On the other hand, the cycle liner, after several injections, showed improvement of resolution in the peaks (Figure 10).
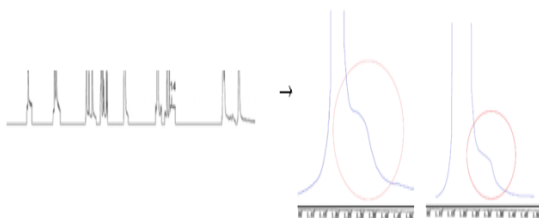
Figure 9
A Peak Deformation Due to Precision Liner

Figure 10
Baseline with Cycle Split Liner

Trial 2 - Validation of values for "Aguardiente" calibration standard: It is important to determine the real value of the sample standard in order to have reproducibility. For this reason it was used an external laboratory for certification purpose. The trials consist of a certain number of injections in the gas chromatography equipment to determine average values. Table 3 shows trials that reflect averages standard values. This average value will be the actual value used for the calibration that applies. These trials were carried out to challenge method, pieces of equipment as well as the preparation of solution, equipment measures and effects on environmental conditions [5]. Two GCs used. Five readings were made for each standard and at the end; its real value was determined by averaging.

Table 3
**Accepted Criteria for "Aguardiente" Sample Standard (AGT)**

	Agte	
	Esters	Fusel
GC#9	17.51925	193.13064
	17.55522	195.46489
	16.58967	194.84769
	16.65961	195.49564
	16.78087	194.76464
GC#12	16.53654	189.09627
	16.79686	190.88187
	17.01085	189.54324
	17.79474	192.20447
	17.65552	190.58204
Interlab Results	17.66	192.1
Average	17.14	192.56

Accepted Criteria		
	Agte	
Range	Esters	Fusel
Minimum	16	190
Maximum	18	196

Injection Reproducibility will be tested with trial samples and ethanol as blank- seek when the injector gets dirty.

Trial 3 - Calibration Results Using Separate Method Parameters: Individual calibration parameters were created for standard 1 (level 1) and standard 2 (level 2). Figure 11 shows calibration behavior as established (both standards under same method parameter).

Figure 12 aim to collect the results of fusel and esters in Standards 1 and 2 but with individual methods. That is, for calibrating the standard 1 a

method called Fusel Ester Level 1 was created and for calibration with standard 2 apart a method called Fusel Ester Level 2. With this, separate calibration conditions were created. Figure 12 shows calibration results for Level 1 and Level 2 calibration.
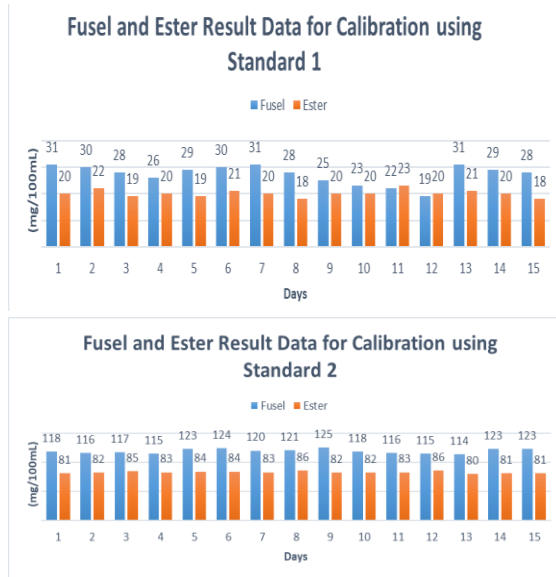
Figure 11
Fusel and Ester Result Data for Calibration using Standard 1 and Standard 2 / Same Method Parameters
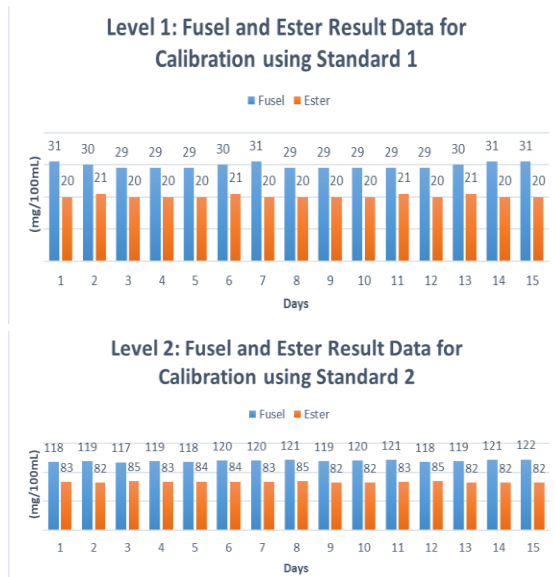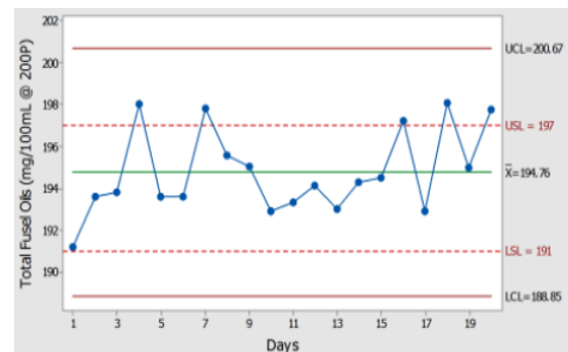

Figure 12
Level 1 and Level 2 / Fusel and Ester Result Data for Calibration using Separate Method Parameters

RESULTS AND DISCUSSION

In trial 1 was able to obtain better resolution in the chromatography baseline using a split liner cycle. After several injections, it could be noticed that the liner had residuals of samples that leads to conclude that the change of the same is necessary. The frequency of the liner change could not be specified. An ocular inspection or atypical chromatography would be the criterion to change liner. The function of the liner is to form a container in which the sample can be injected and heated. The cycle split liner plays an important role by allowing a sample that is injected into the liquid phase to pass into the gas phase and into the GC column giving better resolution and therefore reproducibility.

The standard of AGT maintains a specific range of the type of sample that is analyzed. The theoretical value of an external laboratory was compared with a live exercise and the range of esters and fusel was determined for calibration of samples of AGT for both GC equipment: Esters 16 mg/100mL to 18 mg/100mL and fusel 190 mg/100mL to 196 mg/100mL.

Level 1 calibration improvement and reproducibility was obtained in standard one results for samples with low concentrations of fusel. This meant that the amount of re-calibration decreased and the reproducibility increased (see Figure 13). It was also show that for level 2 of calibration, reproducible results could be achieved for standard 2 and for samples with high concentration of fusel.
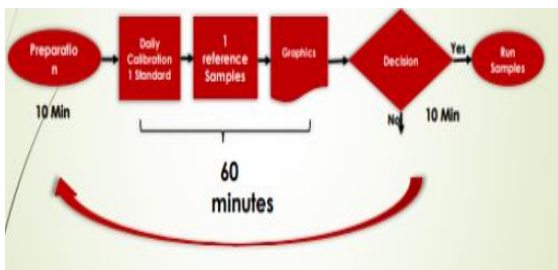

Figure 13
Chart of Reference Samples Result

Before the improvement of the project, two levels of calibration in the same programmed method consumed 130 minutes of working time (see Figure 14). After the analysis of results and implementation of two separate calibration levels this time was reduced to 60 minutes (see Figure 15).
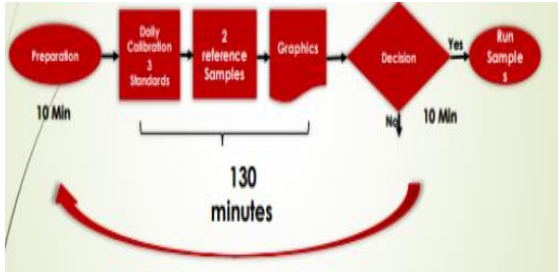
Figure 14
Fusel Oil Method: Two Level Calibration

Figure 15
Fusel Oil Method: Separated Level Calibration

In a period of 19 days of compilation of AGT standard readings, we were able to obtain 20 readings, of which 16 were within the range. The carry-over due to high fusel oil sample was minimized. In this case references with low fusel oil concentration was analyzed before high fusel oil concentration samples and a breakout programs between samples was created.

CONCLUSIONS

According to the results previously discussed, significant conclusions are presented below.

Important Findings

- Comparison of results between different levels of calibration demonstrated a significant difference.

- Significant difference in results was observed when using 2 different liners
- Samples with Low Fusel Oil results should be analyzed with STD 1 20/30 mg/100 ml
- Samples with High Fusel Oil results should be analyze with STD 2 (80/120 mg/100 ml)
- All samples should be analyzed at 80P × Using a one-point Calibration results for our control samples improved a 75% in reproducibility compared to the 45 % obtained using a two-point calibration
- Using a one-point calibration was observed more stable results in the reference samples.

Benefits

Calibration:

- Advantage of a one level calibration provides a better reproducibility in the references samples.
- Greater flexibility in the use of GC instruments. One instrument could be dedicated to run high fusel oils samples and one instrument for low fusel oils samples.
- Rapid response to clients for important decision-making.

Lessons Learned

Expertise:

- The availability of an expert to help Quality Laboratory during the Fusel oil project to accelerate the investigation.

Samples:

- Complexity of the samples led to some difficulties during the investigation to help determine the proper standard concentration and preparation of samples.

Project Benefits

- Business Benefits
- Compliance System Improvement
- Waste Reduction
- Defects
- Waiting
- Intellect
- Over-processing

Next steps

- Fusel Method needs to be re-validated due to new products with a High Fusel Oils and Solids (columns, liners, temperature ramps, Internal Standard, etc.).
- One representative control sample should be considered to ensure daily calibration instead of having a Control Sample for each product.
- Further study with high solids and fusel needs to be performed to understand the caramel and sugar content effect on the columns, liners, etc.

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