# REPRODUCIBILITY AND REPETIBILITY 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.

## Introduction

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. This situation also causes the analysts assigned to the area, to fully work in 'Troubleshooting' without being able to attend other areas.

## Background

Previously validated calibration method will be use, using two standards with specific value ranges according to Sigma-Aldrich custom mix. 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. Individual calibration parameters were created for standard 1 (level 1) and standard 2 (level 2).

As part of the trials it was determined to use two types of liners such as precision and cycle split liner. Both will help the resolution of the peaks and it is expected that it will therefore improve the accuracy of the areas.

## Problem

It is important to establish an efficient and robust calibration protocol prior to the analysis of a sample. Calibration is the main fault cause. The requirements of this project are:

1) Minimize recalibrations due to lack of reproducibility results in high solids products and high fusel (> 100 mg/100ml) product

2) Achieve 75% of compliance with the daily reference samples criteria. (Actual: 45%)

## Methodology

- Trial 1: Comparison of 2 liners.

Precision liner (deactivated glass wool) and Cycle Splitter Liners are made of glass that helps limit the degradation of the sample and improve vaporization. This trial aim to seek better reproducibility using standard trials on different liner design (figure 1).

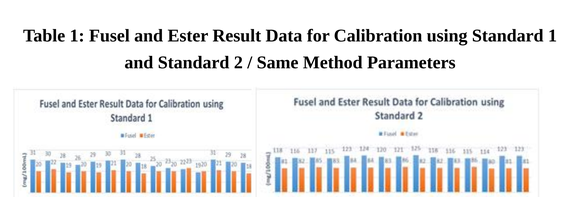

**Figure 1: A peak deformation due to precision liner vs Baseline with cycle split liner**

- Trial 2: Validation of values for Aguardiente calibration standard.

The trials consist of a certain number of injections in the gas chromatography equipment to determine average 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. 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.

For calibrating 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 was created. Table 2 aim to collect the results of fusel and esters in Standards 1 and 2 but with individual methods. Table 1 shows calibration behavior as established (both standards under same method parameter).

**Table 1: Fusel and Ester Result Data for Calibration using Standard 1 and Standard 2 / Same Method Parameters**




**Table 2: Level 1 and Level 2 / Fusel and Ester Result Data for Calibration using Separate Method Parameters**


## Results and Discussion

In trial 1 we were able to obtain better resolution in the chromatography baseline using a splitter liner cycle. After several injections, we could notice that the liner had residuals of samples that leads us 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 aguardiente (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.

Before the improvement of the project, two levels of calibration in the same programmed method consumed 130 minutes of working time. After the analysis of results and implementation of two separate calibration levels this time was reduced to 60 minutes.

In a period of 19 days of compilation of aguardiente 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

**Important Findings:**

- Significant difference in results was observed when using 2 different liners
- Samples with Low Fusel Oil results should be analyze 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 analyze 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 we observed more stable results in our reference samples

**Benefits:**

- Calibration:
- Advantage of a one level calibration provides a better reproducibility in our 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 our samples led to some difficulties during the investigation to help determine the proper standard concentration and preparation of samples.

## Future Work

**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 our high solids and fusel need to performed to understand the caramel and sugar content effect on the columns, liners , etc.

## References

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