



Routine Analytical Chemistry Sub-Group

Technical Report on 2007 Joint Experiment to Update Repeatability and Reproducibility Statistics for:

- **CORESTA Recommended Method 60, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by Gas Chromatography**
- **CORESTA Recommended Method 61, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by High Performance Liquid Chromatography**

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1. BACKGROUND

The CORESTA Recommended Methods (CRMs) for the determination of 1,2-propylene glycol (PG) and glycerol (GLY) in tobacco and tobacco products were published in February 2005. These resulted from a series of joint experiments which were carried out between 1993 and 1999 to evaluate sample preparation, extraction and analysis parameters when analysing PG and GLY in tobacco.

During 2007, the CORESTA Scientific Commission requested the Routine Analytical Chemistry (RAC) Sub Group carry out a collaborative study to investigate whether the repeatability (r) & reproducibility (R) values quoted in the methods were still relevant or could be improved.

The CRMs in question are listed below:

CRM 60

Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by Gas Chromatography

CRM 61

Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by High Performance Liquid Chromatography

The r and R statistics published in CRMs 60 and 61 were relatively large. Sample non-homogeneity was suspected to be a key contributor to the observed methods' variability. R.J. Reynolds Tobacco Company (RJRT) volunteered to prepare a series of appropriate test samples and carry out an initial sample assessment. Data were presented at the April 2007 meeting of the RAC Sub Group as shown below in Tables 1 and 2, and displayed graphically in Figures 1 and 2.

Table 1: Loose Cut Tobacco Analysis for % 1,2-Propylene Glycol

| Sample ID | A | B | C | D |
|-----------------|--------------|--------------|--------------|--------------|
| Point 1 | 0.43 | 1.21 | 2.03 | 3.30 |
| Point 2 | 0.42 | 1.23 | 2.07 | 3.26 |
| Point 3 | 0.41 | 1.21 | 2.05 | 3.33 |
| Average | 0.42 | 1.22 | 2.05 | 3.33 |
| Std.Dev. | 0.010 | 0.012 | 0.020 | 0.035 |

Table 2: Loose Cut Tobacco Analysis for % Glycerol

| Sample ID | A | B | C | D |
|-----------------|--------------|--------------|--------------|--------------|
| Point 1 | 3.33 | 2.14 | 1.29 | 0.56 |
| Point 2 | 3.36 | 2.23 | 1.39 | 0.54 |
| Point 3 | 3.33 | 2.15 | 1.34 | 0.54 |
| Average | 3.34 | 2.17 | 1.34 | 0.55 |
| Std.Dev. | 0.017 | 0.049 | 0.050 | 0.012 |

Figure 1: Loose Cut Tobacco Analysis for % 1,2-Propylene Glycol

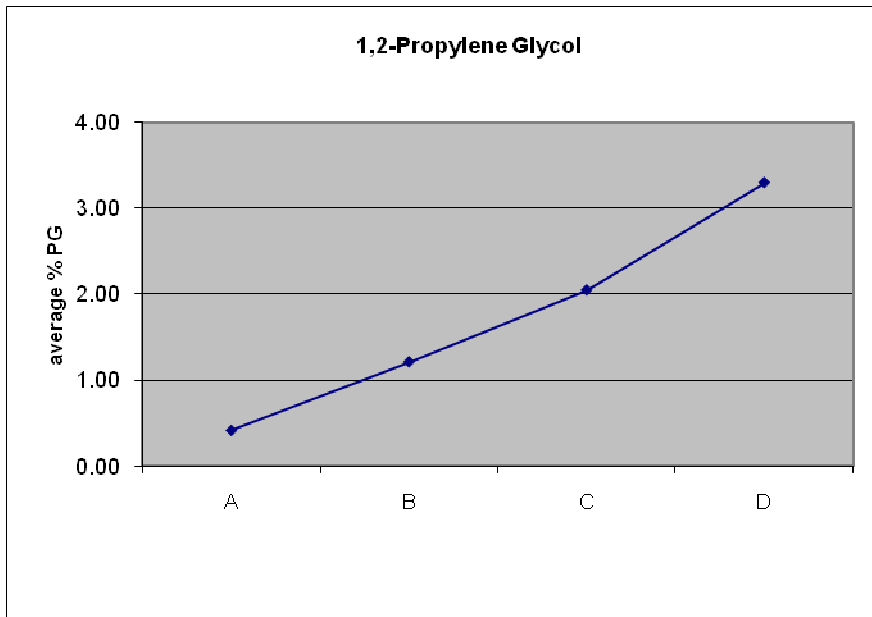


Figure 2: Loose Cut Tobacco Analysis for % Glycerol



*

The RAC agreed that the samples covered an appropriate range and therefore were acceptable for the study.

2. SUMMARY

Eight tobacco samples were despatched to 19 laboratories. These samples consisted of four pouches of loose cut tobacco and four pouches of finely ground (ground to pass through a 1mm sieve) and homogenized tobacco from the same population as the loose cut tobacco samples.

The list of participating laboratories is in APPENDIX A.

The protocol was also sent to the participating laboratories, a copy of which is in APPENDIX B.

Duplicate preparations of each sample pouch were made with two injections per sample preparation, consistent with the original studies. Results were reported on an 'as received' basis.

All results were received within the deadline.

Analysis of the data showed that the larger *r* and *R* values found in the previous study were due to the lack of sample homogeneity. Therefore, for the purpose of this study every effort was made to prepare the samples for humectants analysis as homogeneously as possible.

Another outcome of this study was to update CRM 60 and 61 to include the *r* and *R* statistics found in this piece of work. The following table compares the values from both studies.

Table 3: CORESTA Collaborative Study of 1,2-Propylene Glycol and Glycerol Measurements – Comparison of Previous and Current Study Performance Measures

| % Propylene Glycol in Cut Loose Tobacco - GC Method | | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|--|
| Previous Study | | | | | 2007 Study | | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | |
| Mean | 0.597 | 1.567 | 2.873 | 4.693 | 0.416 | 1.227 | 2.049 | 3.362 | |
| r | 0.046 | 0.079 | 0.120 | 0.244 | 0.016 | 0.057 | 0.067 | 0.135 | |
| R | 0.146 | 0.434 | 0.360 | 0.544 | 0.089 | 0.227 | 0.248 | 0.429 | |

| % Propylene Glycol in Cut Loose Tobacco - HPLC Method | | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|--|
| Previous Study | | | | | 2007 Study | | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | |
| Mean | 0.585 | 1.568 | 2.724 | 4.723 | 0.439 | 1.251 | 2.103 | 3.573 | |
| r | 0.120 | 0.062 | 0.208 | 0.153 | 0.077 | 0.071 | 0.090 | 0.174 | |
| R | 0.530 | 0.315 | 0.825 | 0.48 | 0.358 | 0.408 | 0.503 | 0.307 | |

| % Glycerol in Cut Loose Tobacco - GC Method | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.260 | 1.291 | 2.444 | 4.462 | 0.585 | 1.387 | 2.202 | 3.386 |
| r | 0.117 | 0.173 | 0.156 | 0.698 | 0.059 | 0.060 | 0.132 | 0.184 |
| R | 0.325 | 0.401 | 0.522 | 2.668 | 0.163 | 0.236 | 0.313 | 0.418 |

| % Glycerol in Cut Loose Tobacco - HPLC Method | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.223 | 1.244 | 2.442 | 4.396 | 0.560 | 1.413 | 2.324 | 3.707 |
| r | 0.048 | 0.053 | 0.106 | 0.181 | 0.035 | 0.051 | 0.064 | 0.208 |
| R | 0.448 | 0.311 | 0.862 | 1.369 | 0.129 | 0.196 | 0.217 | 0.450 |

| % Propylene Glycol in Ground Tobacco - GC Method | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.591 | 1.549 | 2.803 | 4.580 | 0.404 | 1.181 | 2.011 | 3.303 |
| r | 0.033 | 0.056 | 0.110 | 0.179 | 0.019 | 0.029 | 0.028 | 0.073 |
| R | 0.090 | 0.170 | 0.334 | 0.514 | 0.058 | 0.204 | 0.210 | 0.360 |

| % Propylene Glycol in Ground Tobacco - HPLC Method | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.574 | 1.596 | 2.669 | 4.554 | 0.424 | 1.236 | 2.072 | 3.435 |
| r | 0.054 | 0.067 | 0.102 | 0.096 | 0.050 | 0.054 | 0.093 | 0.075 |
| R | 0.555 | 0.276 | 0.666 | 0.450 | 0.230 | 0.390 | 0.469 | 0.204 |

| % Glycerol in Ground Tobacco - GC Method | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.259 | 1.311 | 2.439 | 4.389 | 0.584 | 1.365 | 2.159 | 3.361 |
| r | 0.056 | 0.100 | 0.143 | 0.173 | 0.032 | 0.048 | 0.094 | 0.145 |
| R | 0.289 | 0.286 | 0.620 | 0.910 | 0.178 | 0.183 | 0.276 | 0.444 |

| % Glycerol in Ground Tobacco - HPLC Method | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| Previous Study | | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.167 | 1.211 | 2.367 | 4.281 | 0.558 | 1.425 | 2.307 | 3.555 |
| r | 0.039 | 0.090 | 0.095 | 0.108 | 0.037 | 0.059 | 0.023 | 0.070 |
| R | 0.328 | 0.214 | 0.810 | 0.572 | 0.131 | 0.155 | 0.245 | 0.369 |

3. INTRODUCTION

In spring 2007, eight samples of tobacco were despatched by RJRT to 19 laboratories (APPENDIX A). These samples consisted of four pouches of loose cut tobacco that had been carefully processed in a V Blender* to improve homogeneity and four pouches of finely ground (ground to pass through a 1mm sieve) and homogenized tobacco. The protocol, designed by RJRT, was also sent to the participating laboratories (APPENDIX B).

*Note: V-Blenders are often used for the intimate dry blending of free flowing solids. Materials being blended are constantly being split and intermixed as the shell rotates.

Duplicate preparations of each sample pouch were requested with two injections per sample preparation - consistent with the original studies. Results to be reported on an 'as received' basis with a deadline set as September 4, 2007.

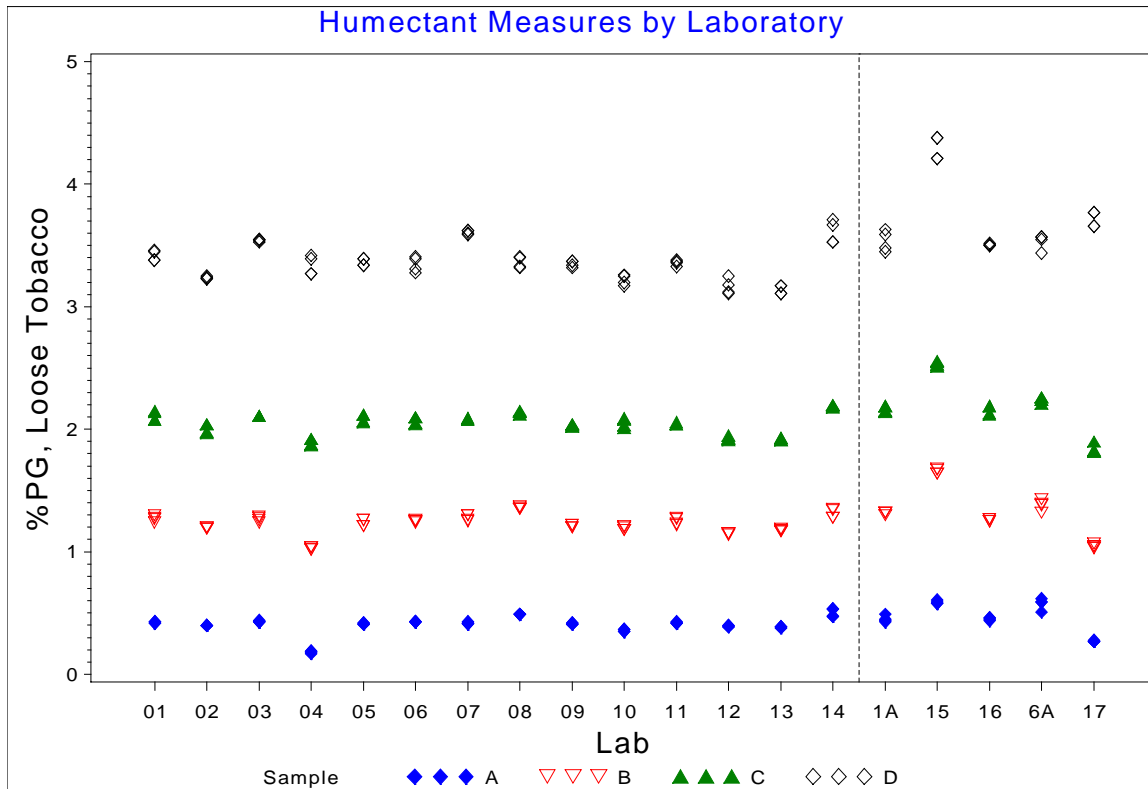
19 sets of results were received by the coordinating company (RJRT) within the deadline. A preview of the study data was presented at the April 2008 meeting of the CORESTA RAC Sub Group.

4. DATA TREATMENT

4.1. RAW DATA

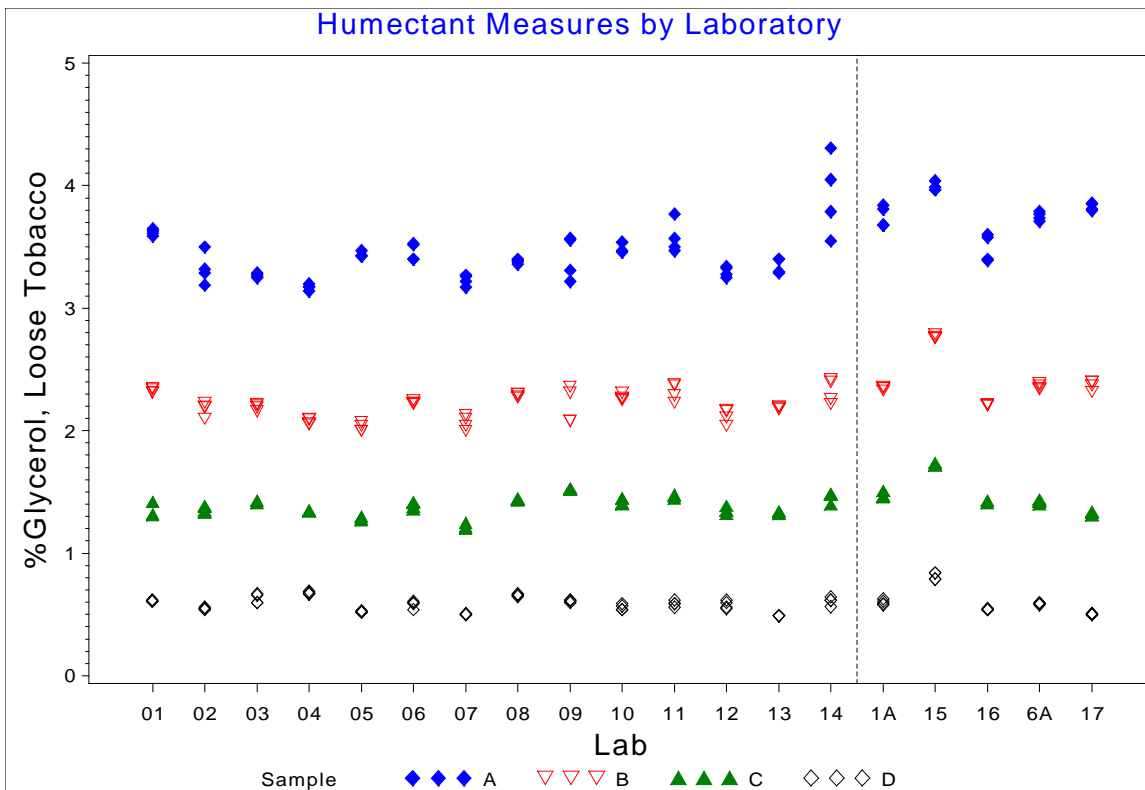
Graphical representations of the data are shown in Figures 3 – 14. These depict the individuals, mean and SDs from each lab plotted for each sample.

Figure 3: PG Cut Loose Tobacco Measurements by Laboratory



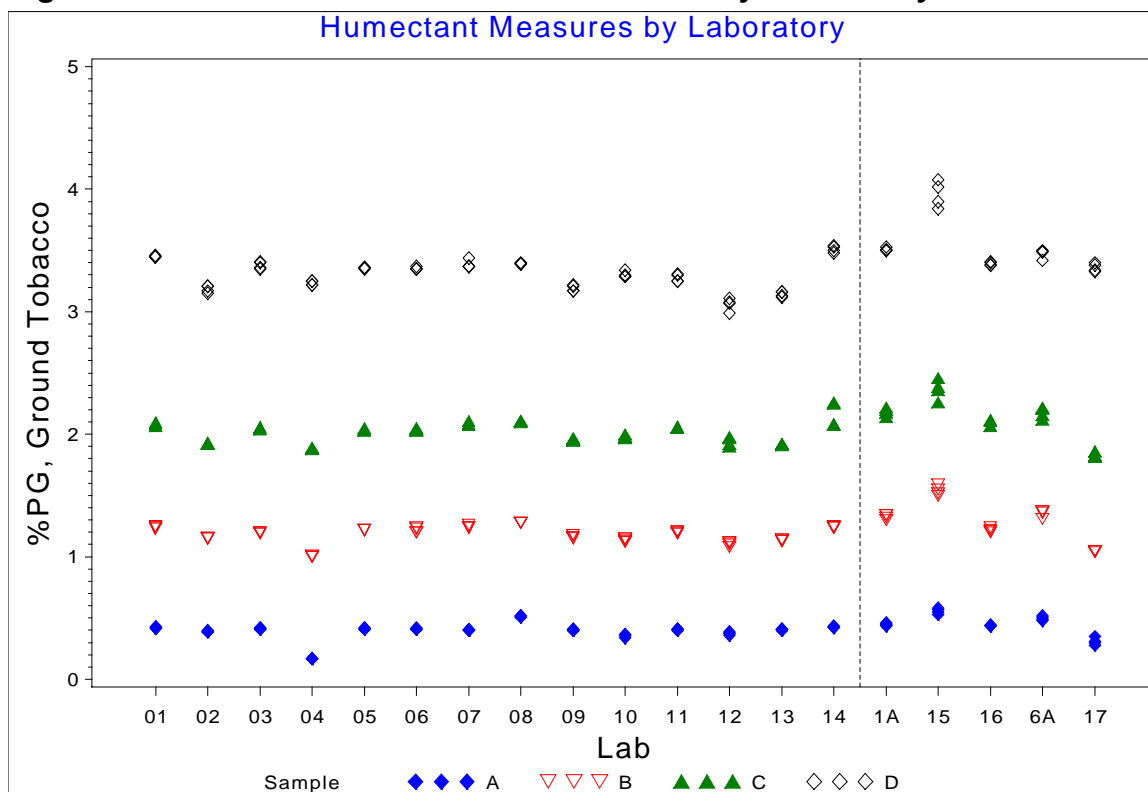
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 4: Glycerol Cut Loose Tobacco Measurements by Laboratory



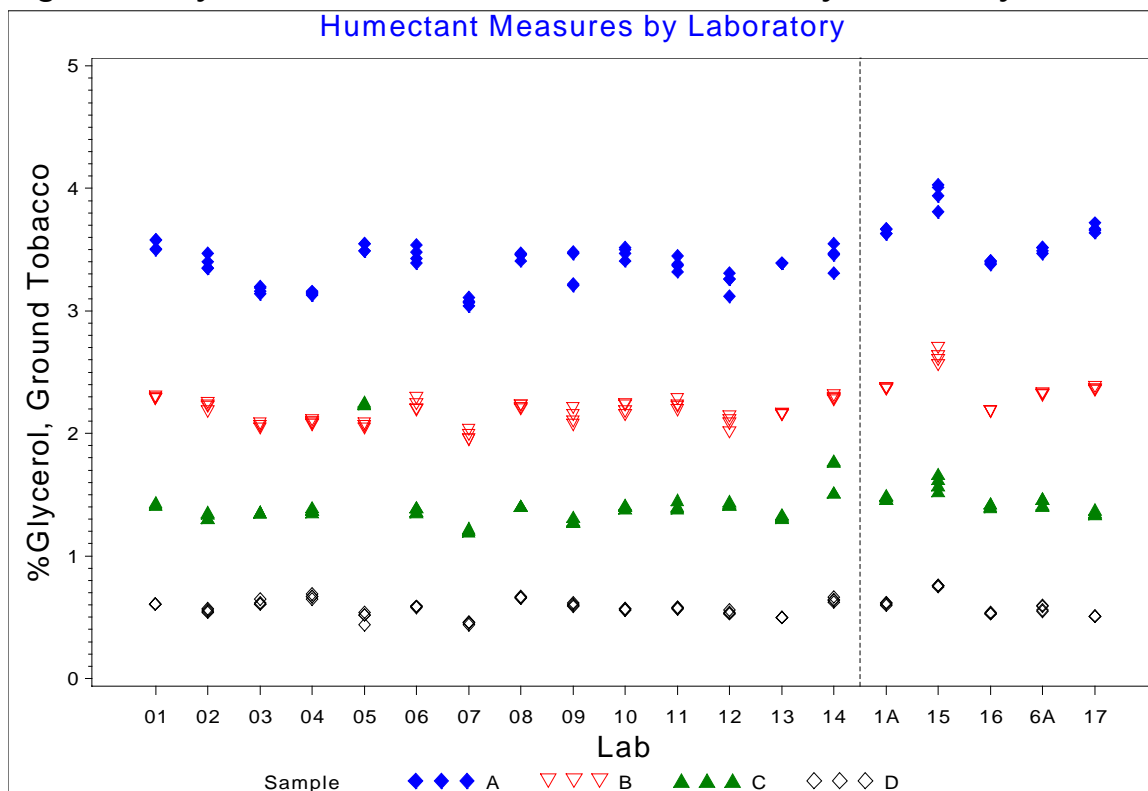
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 5: PG Ground Tobacco Measurements by Laboratory



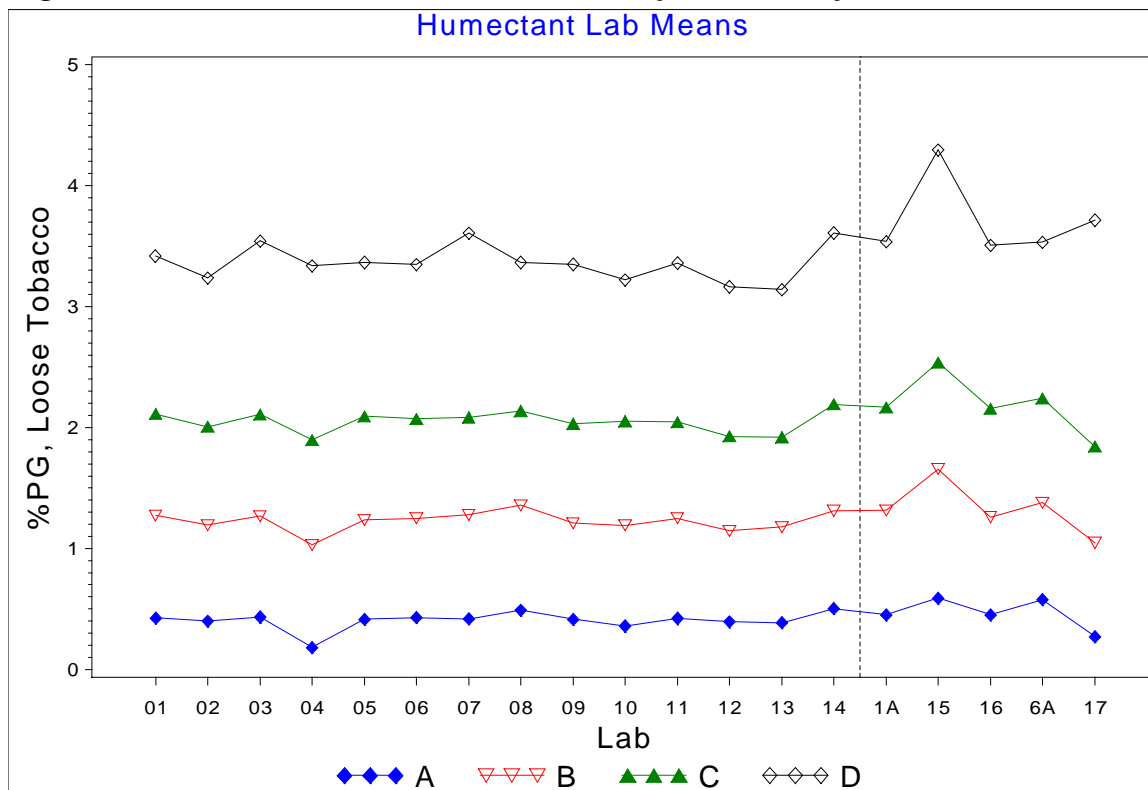
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 6: Glycerol Ground Tobacco Measurements by Laboratory



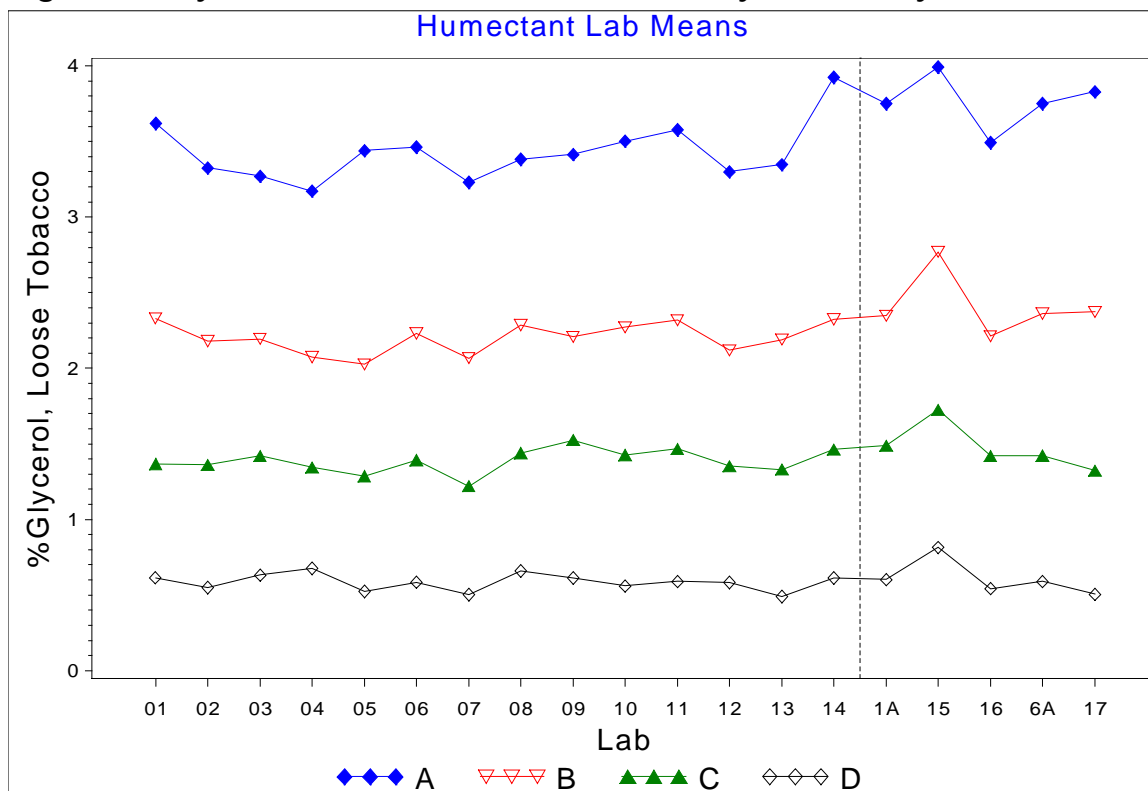
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 7: PG Cut Loose Tobacco Means by Laboratory



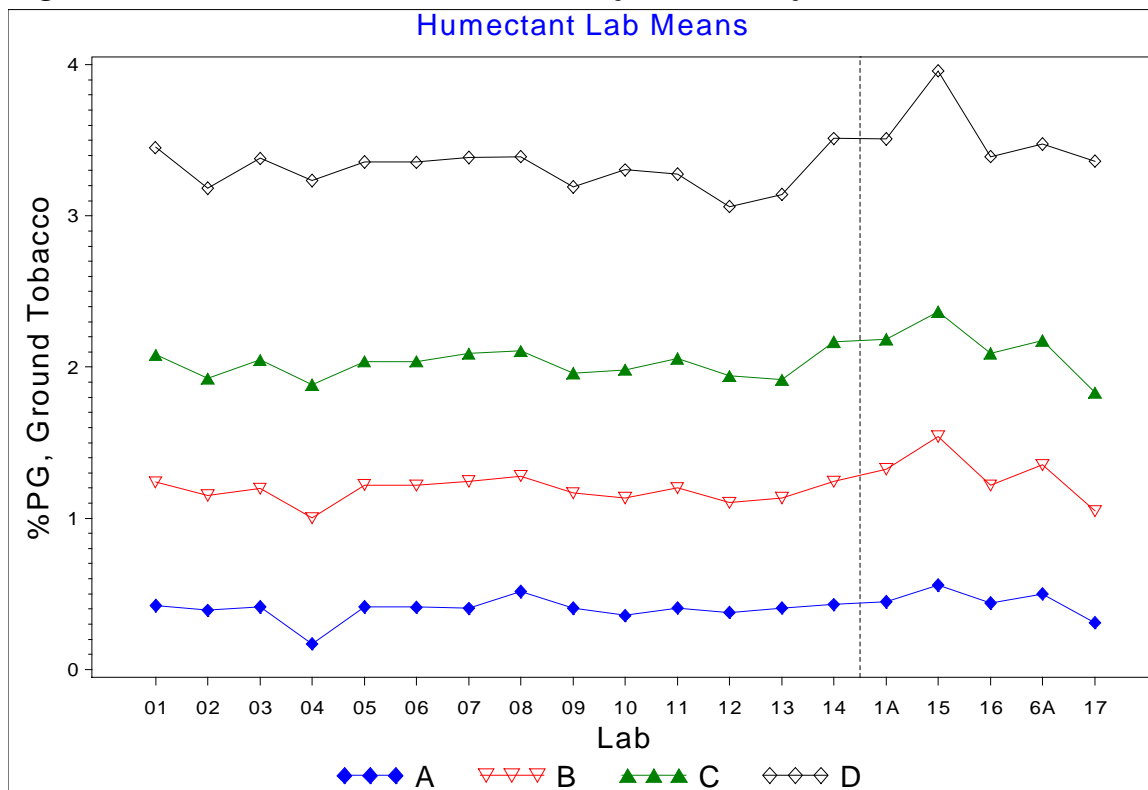
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 8: Glycerol Cut Loose Tobacco Means by Laboratory



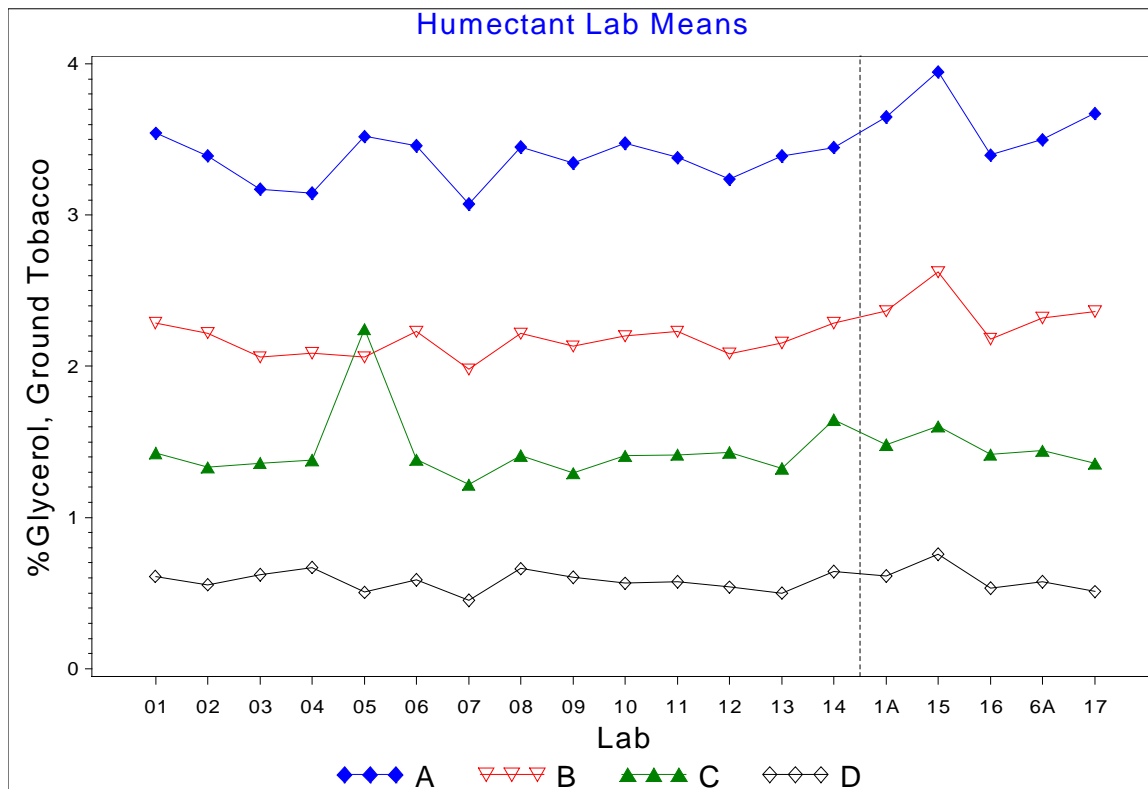
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 9: PG Ground Tobacco Means by Laboratory



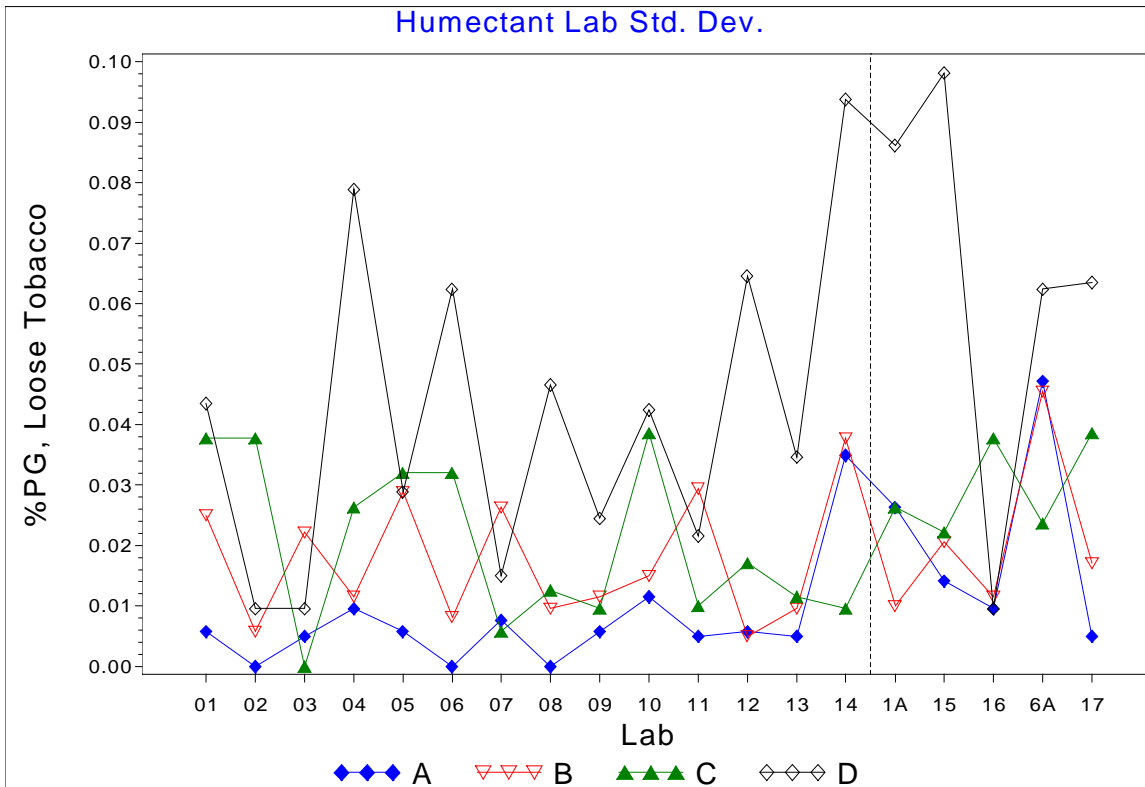
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 10: Glycerol Ground Tobacco Means by Laboratory



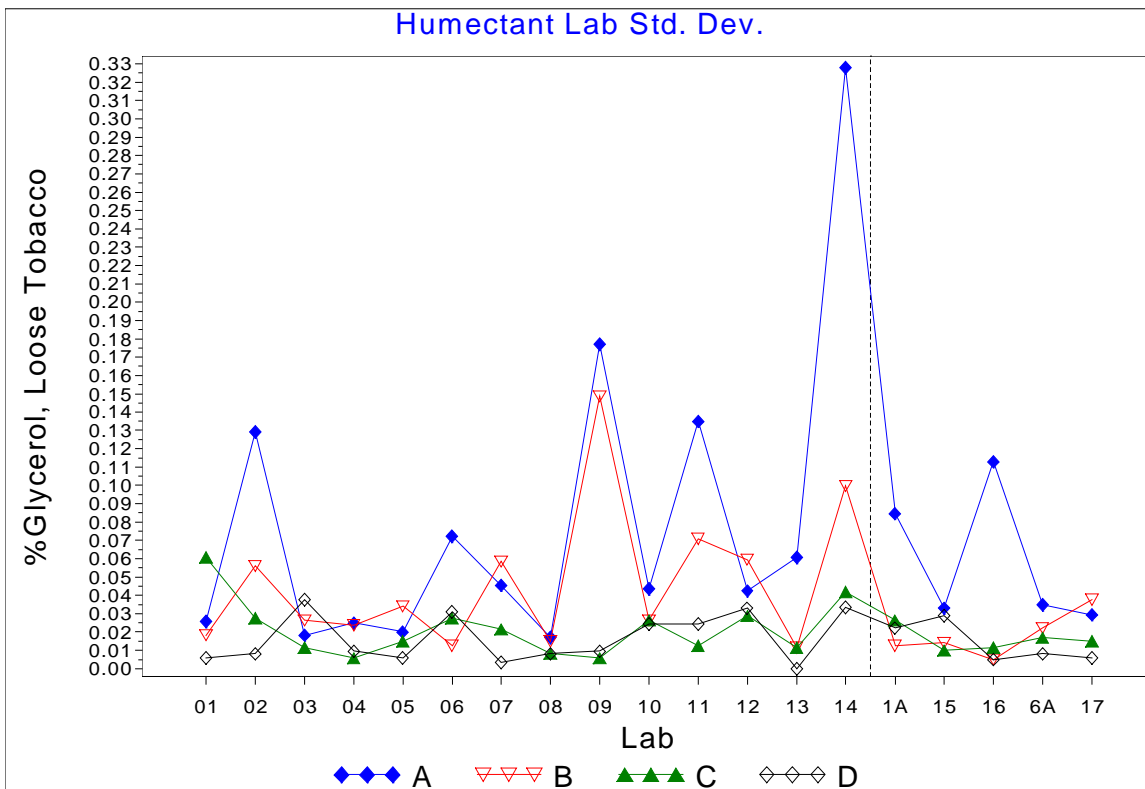
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 11: PG Cut Loose Tobacco SDs by Laboratory



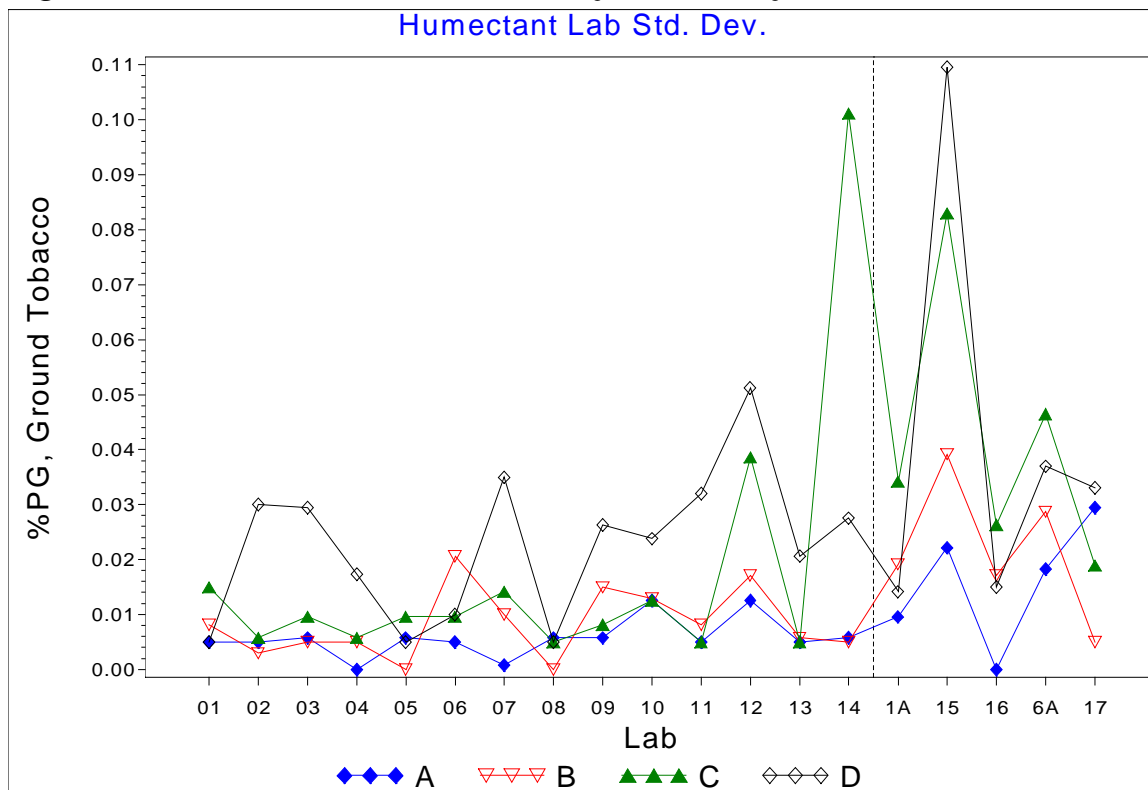
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 12: Glycerol Loose Tobacco SDs by Laboratory



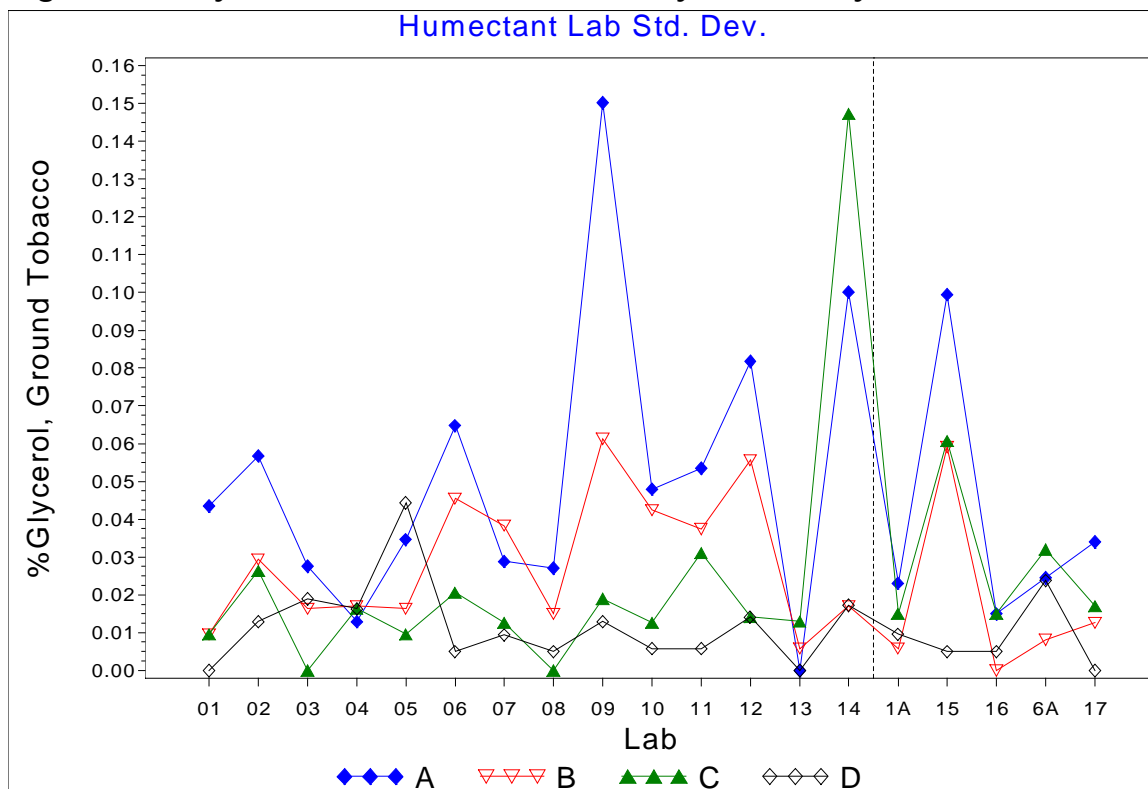
Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 13: PG Ground Tobacco SDs by Laboratory



Note: Vertical dotted line depicts separation of GC and HPLC results

Figure 14: Glycerol Ground Tobacco SDs by Laboratory



Note: Vertical dotted line depicts separation of GC and HPLC results

4.2. MANDEL's *h* and *k*

The data were then checked for the presence of outliers using MANDEL's *h* and *k* graphical consistency techniques. The results from this are shown in Figures 15 – 22a. Laboratory 15 was identified as an outlier in the statistical analysis and therefore excluded from the subsequent *r* and *R* calculations.

Figure 15: Propylene Glycol in Cut Loose Tobacco (GC), Mandel *h* Chart for Lab Means

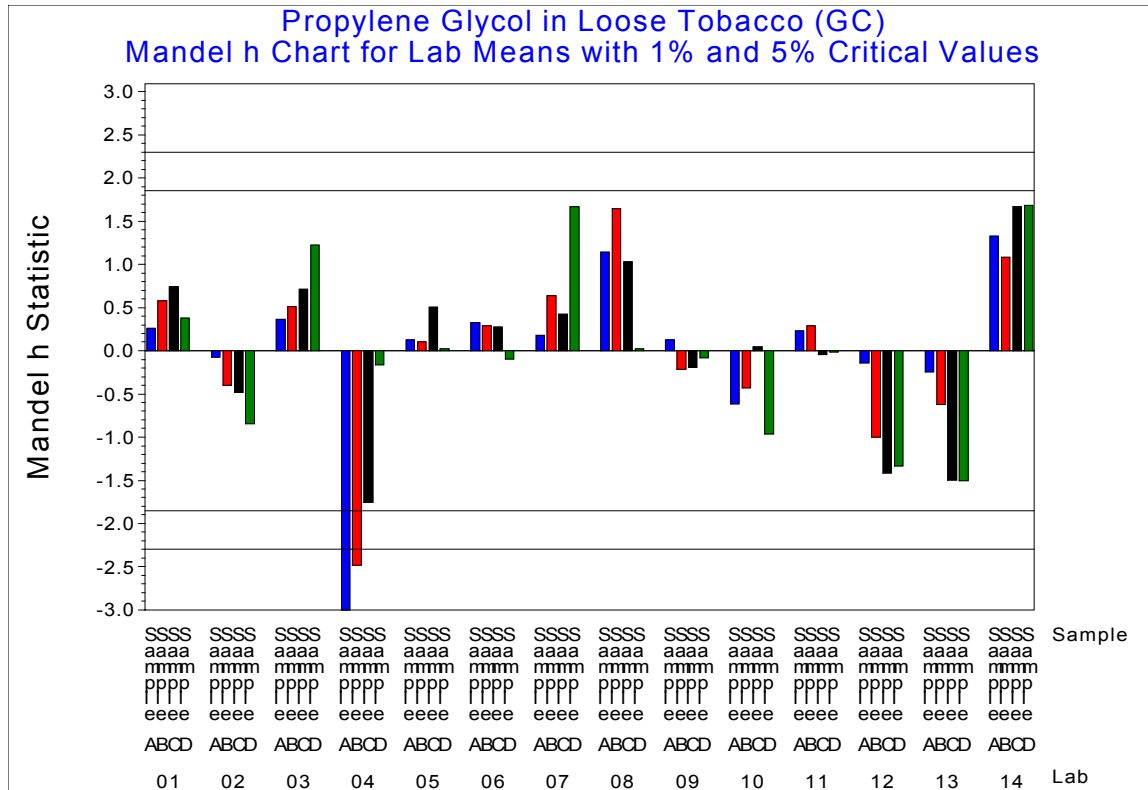


Figure 15a: Propylene Glycol in Cut Loose Tobacco (GC), Mandel k Chart for Lab SDs

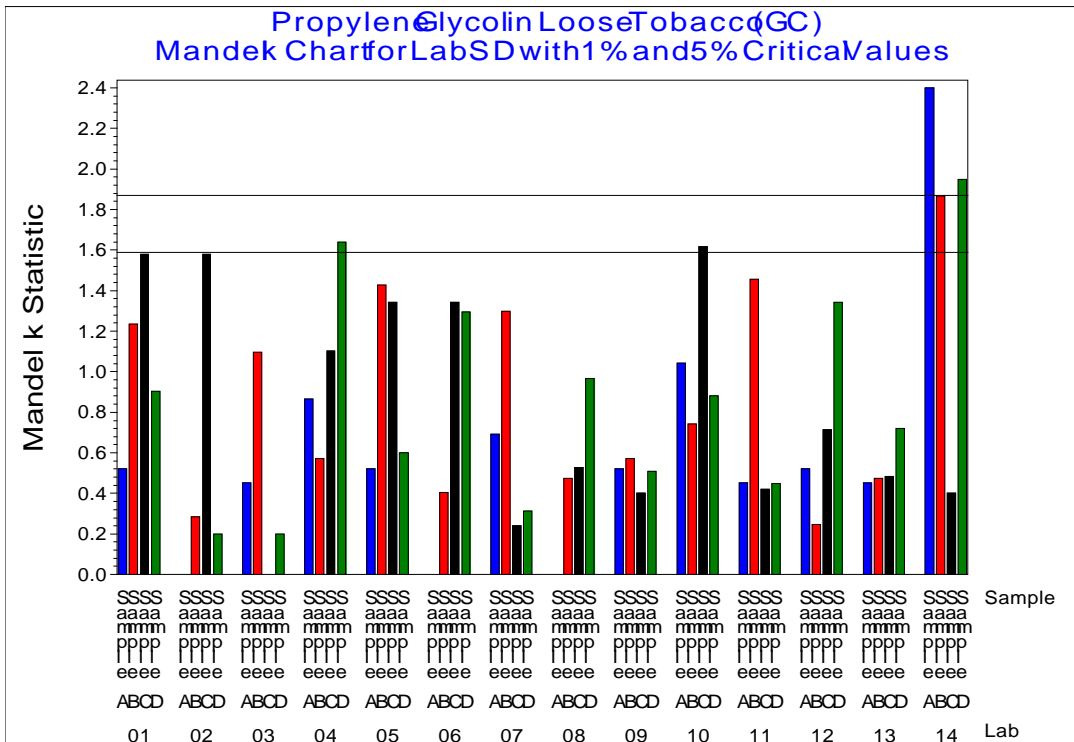


Figure 16: Glycerol in Cut Loose Tobacco (GC), Mandel h Chart for Lab Means

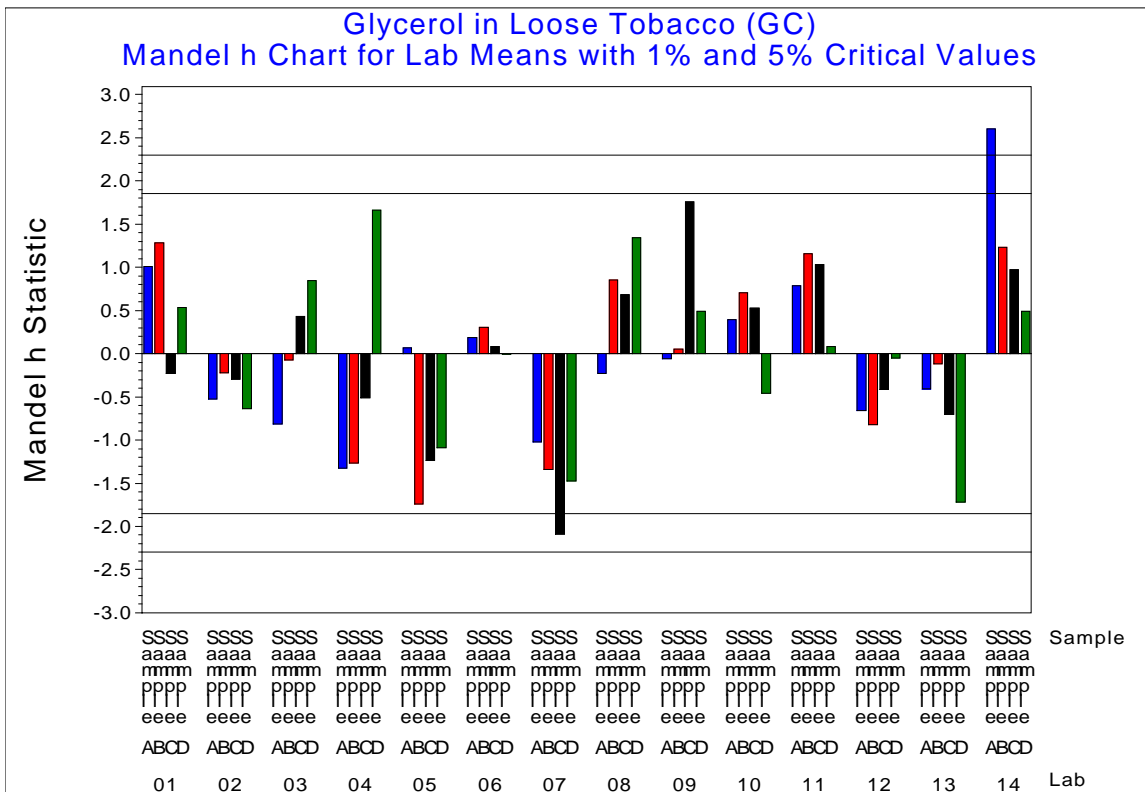


Figure 16a: Glycerol in Cut Loose Tobacco (GC), Mandel k Chart for Lab SDs

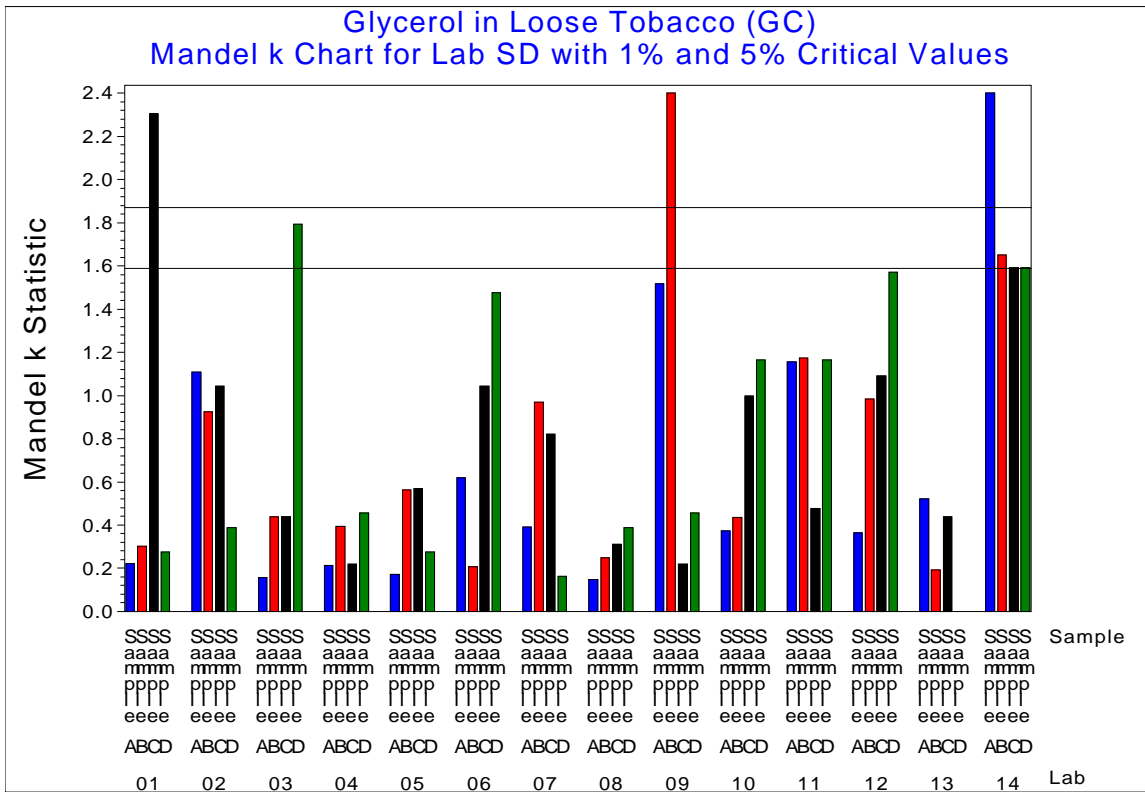


Figure 17: Propylene Glycol in Ground Tobacco (GC), Mandel h Chart for Lab Means

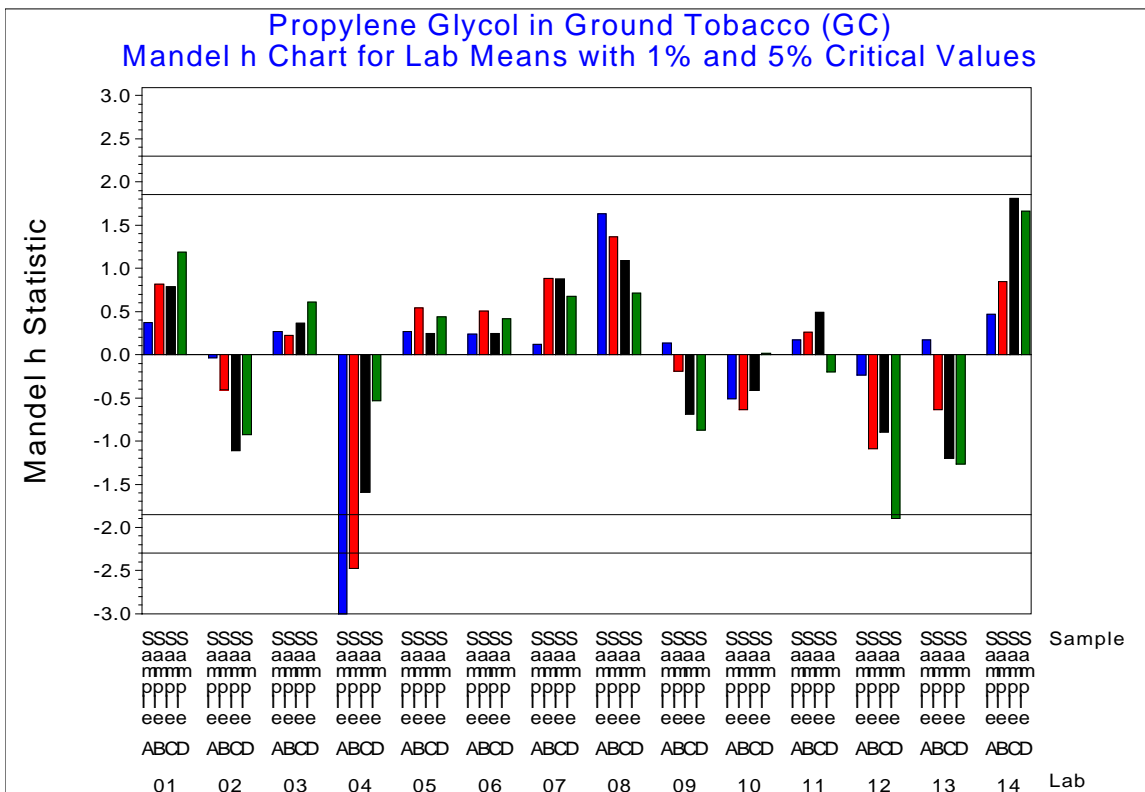


Figure 17a: Propylene Glycol in Ground Tobacco (GC), Mandel k Chart for Lab SDs

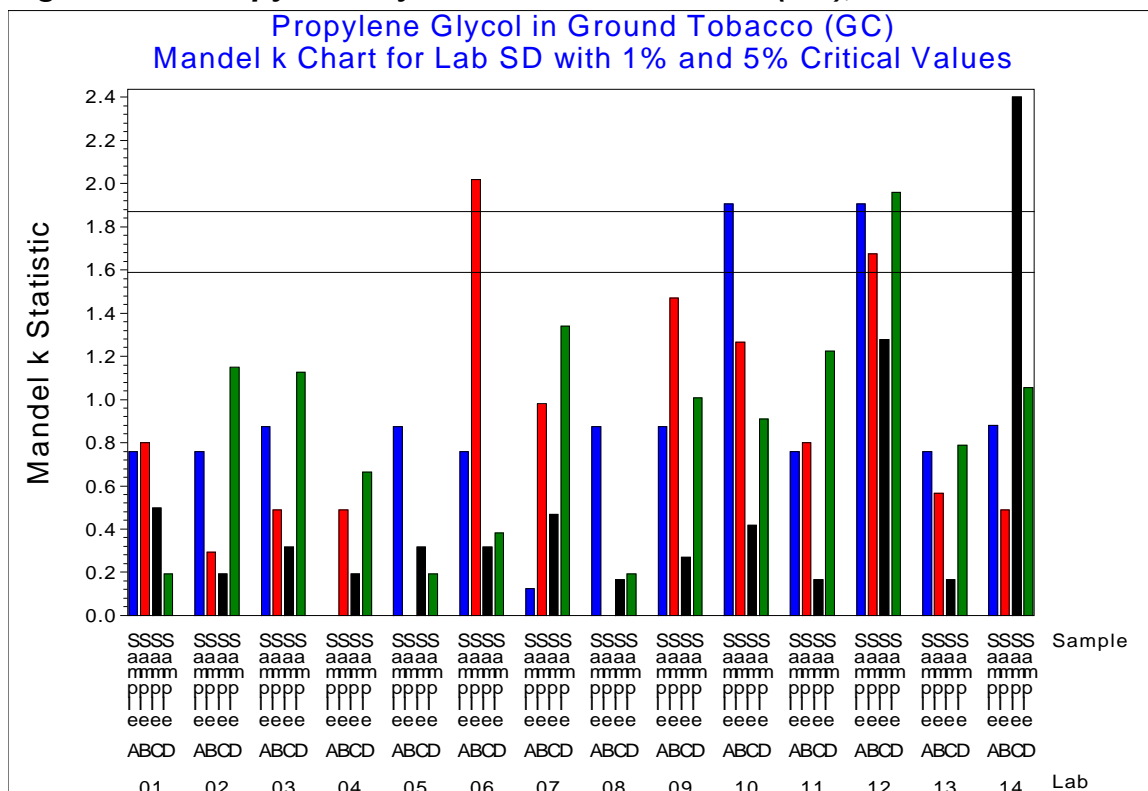


Figure 18: Glycerol in Ground Tobacco (GC), Mandel h Chart for Lab Means

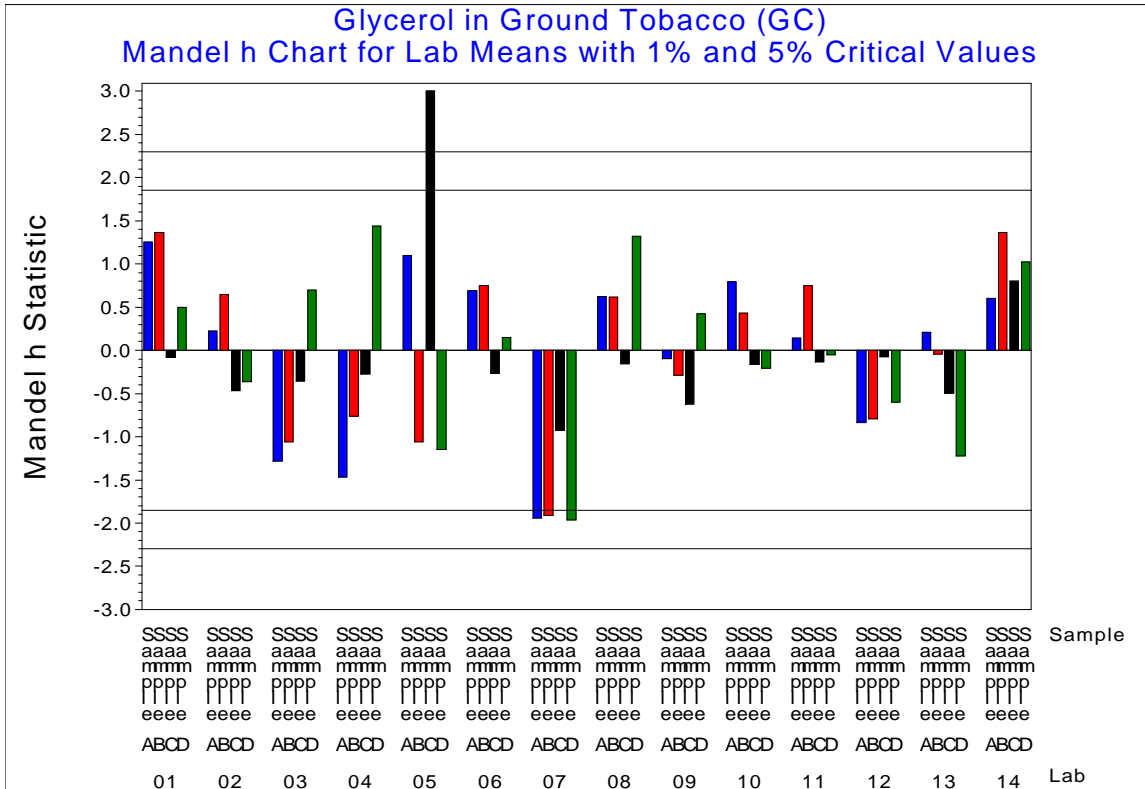


Figure 18a: Glycerol in Ground Tobacco (GC), Mandel k Chart for Lab SDs

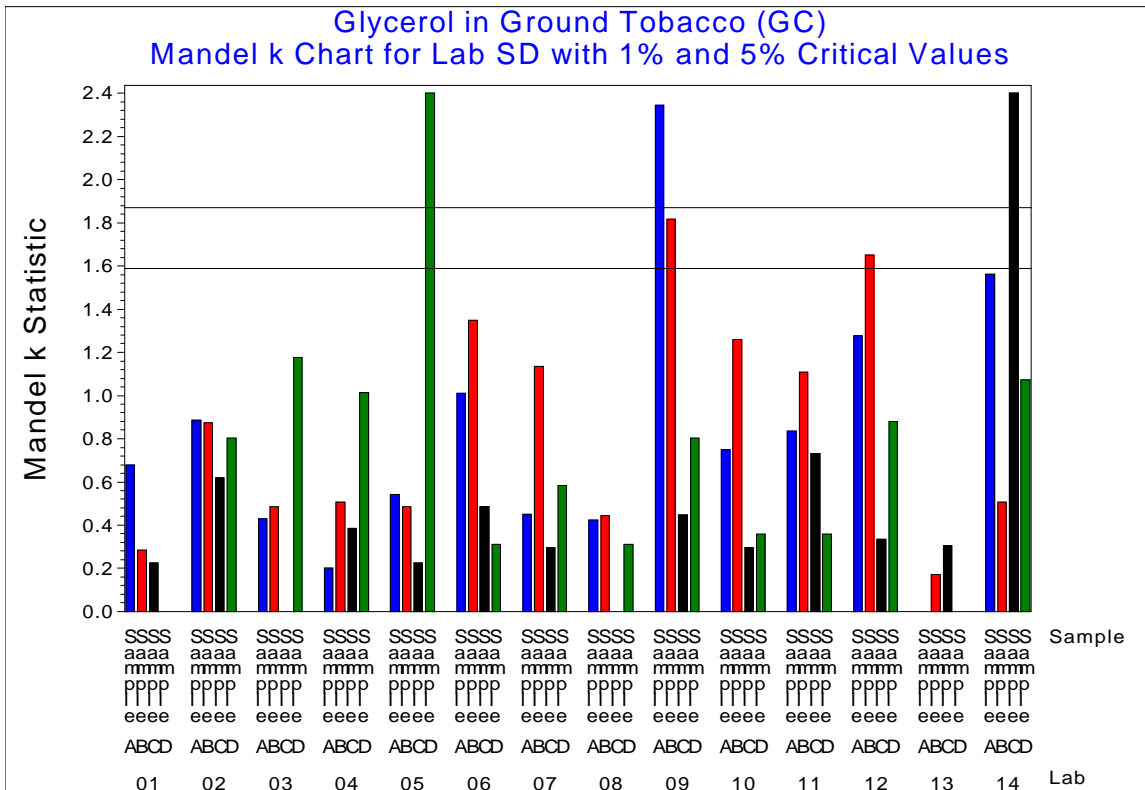


Figure 19: Propylene Glycol in Cut Loose Tobacco (HPLC), Mandel h Chart for Lab Means

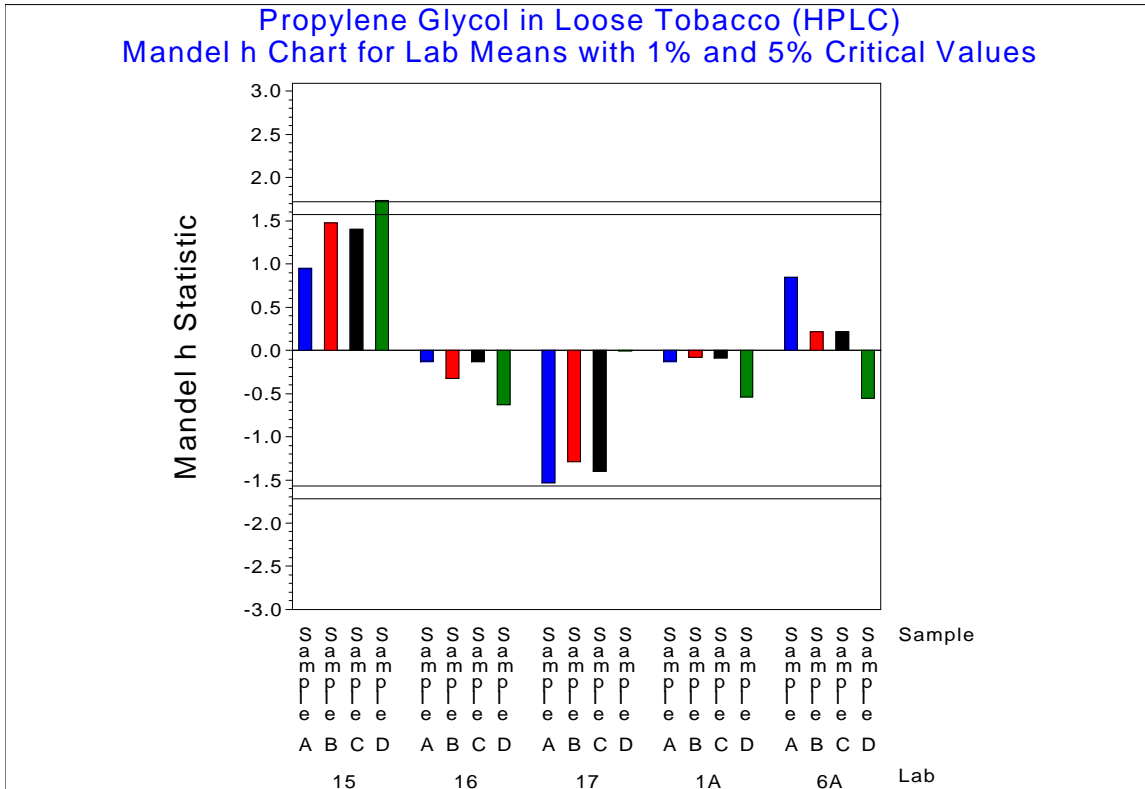


Figure 19: Propylene Glycol in Cut Loose Tobacco (HPLC), Mandel k Chart for Lab SDs

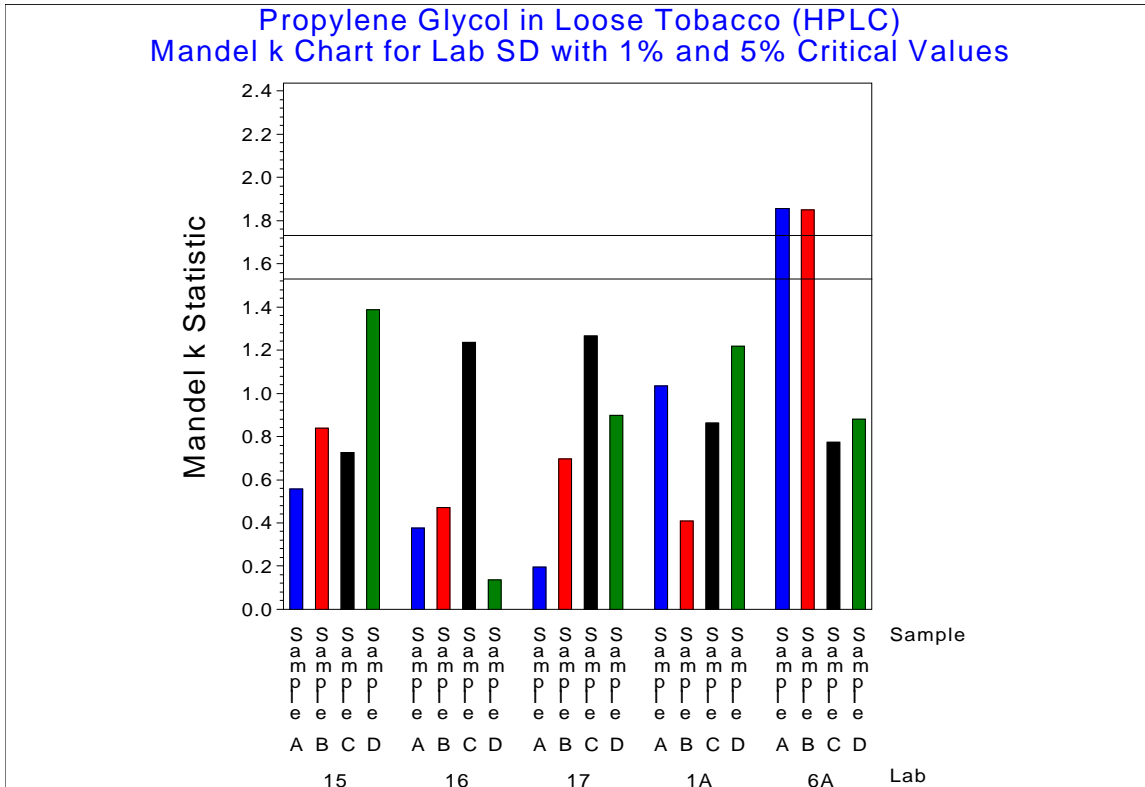


Figure 20: Glycerol in Cut Loose Tobacco (HPLC), Mandel h Chart for Lab Means

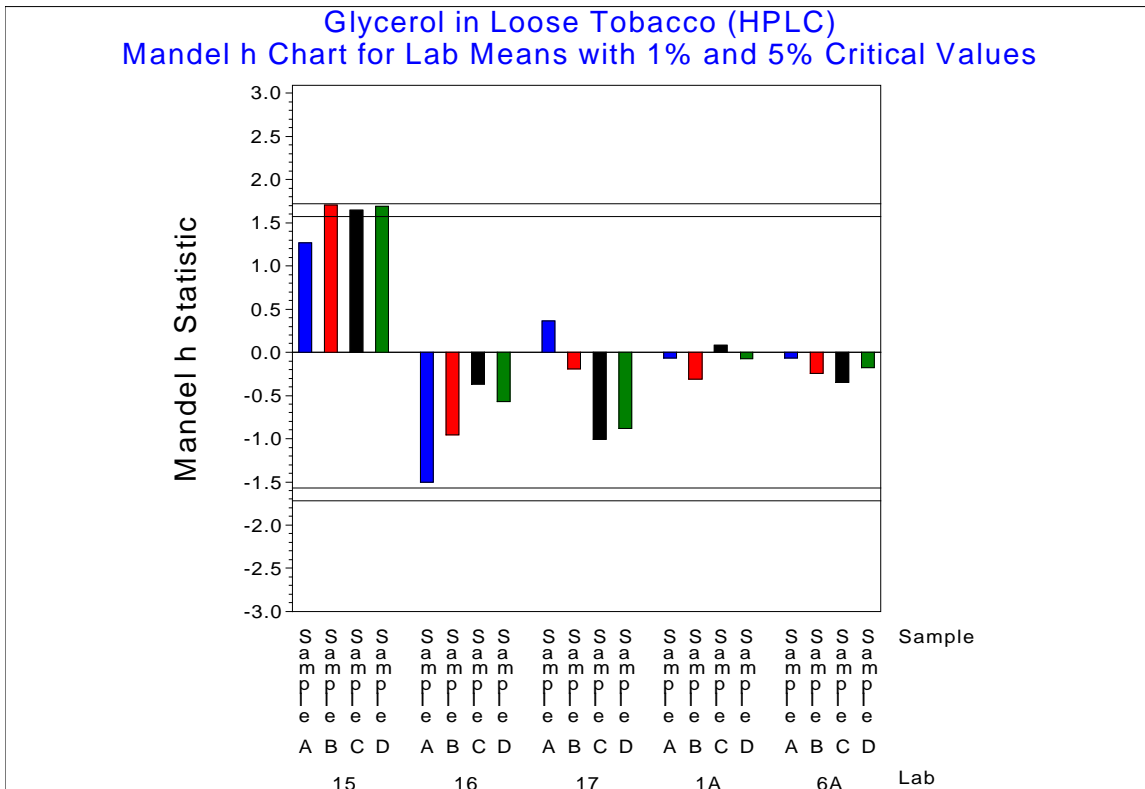


Figure 20a: Glycerol in Cut Loose Tobacco (HPLC), Mandel k Chart for Lab SDs

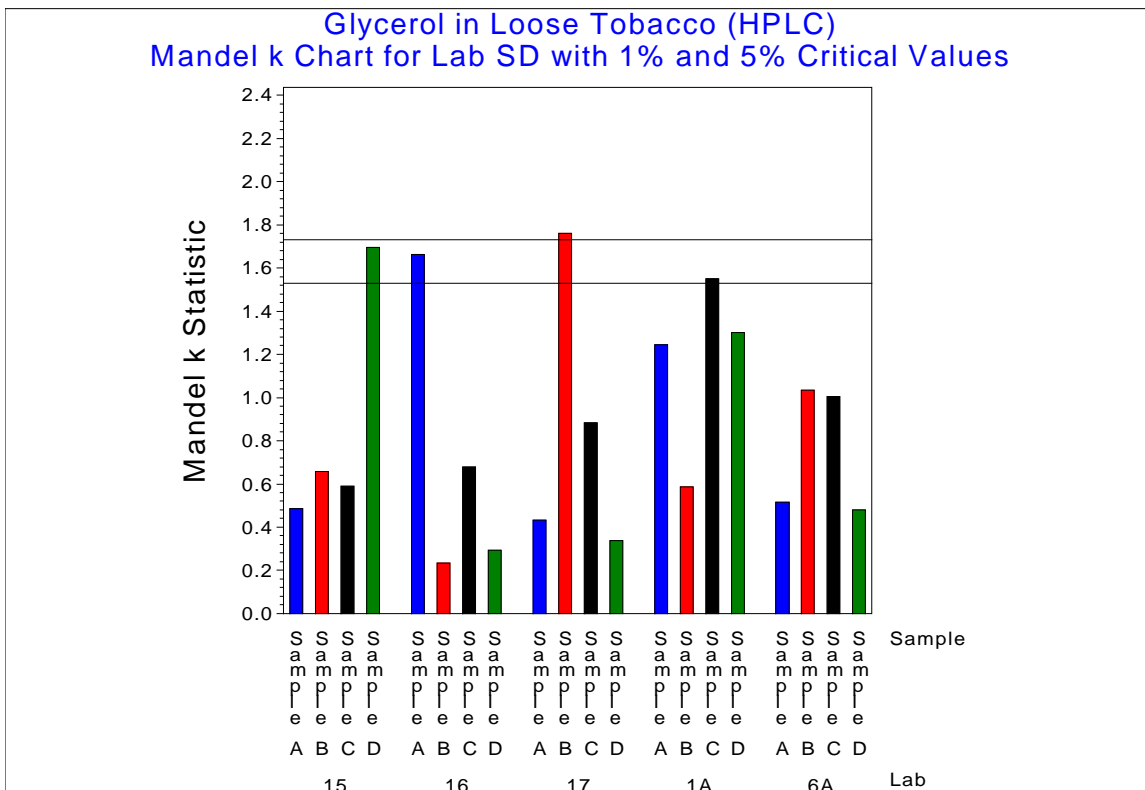


Figure 21: Propylene Glycol in Ground Tobacco (HPLC), Mandel h Chart for Lab Means

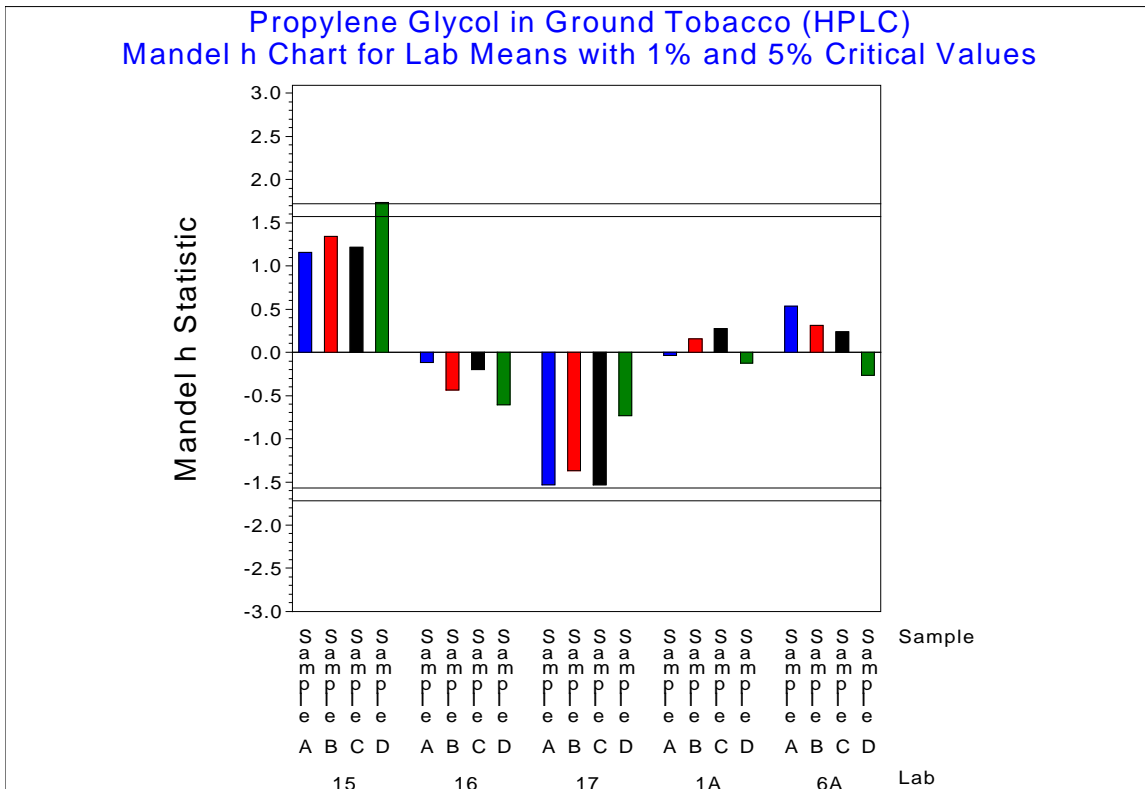


Figure 21a: Propylene Glycol in Ground Tobacco (HPLC), Mandel k Chart for Lab SDs

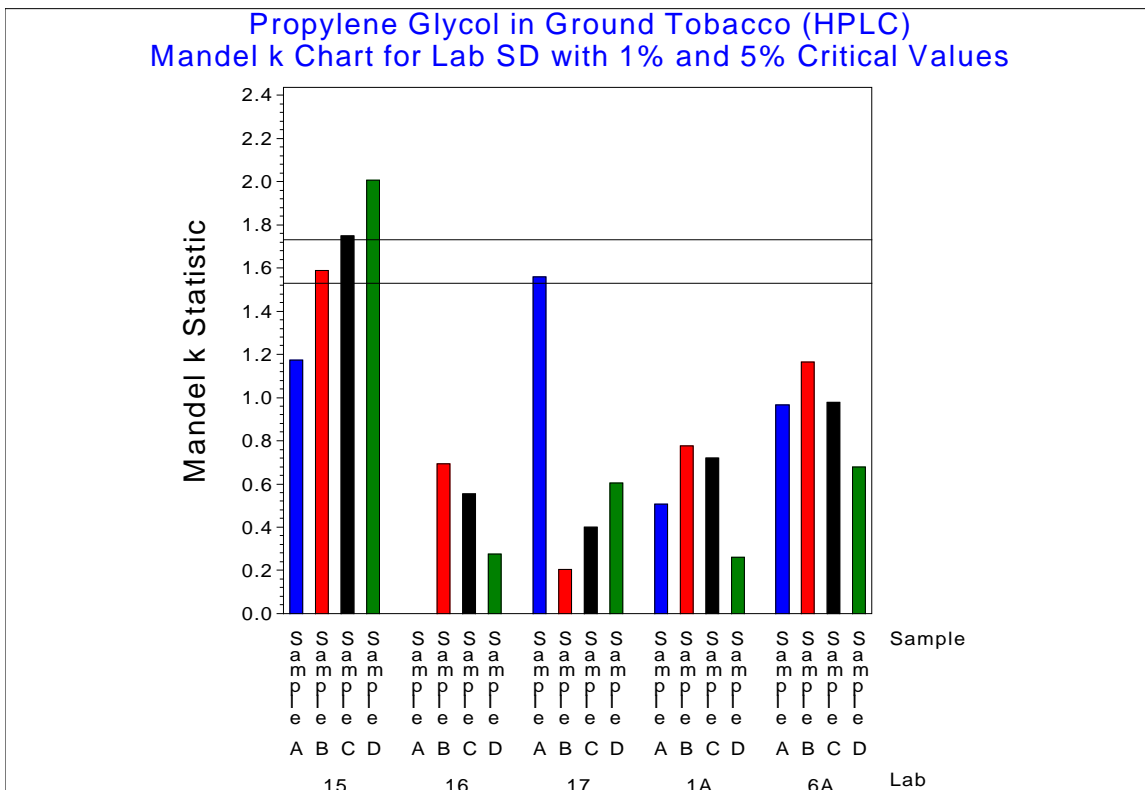


Figure 22: Glycerol in Ground Tobacco (HPLC), Mandel h Chart for Lab Means

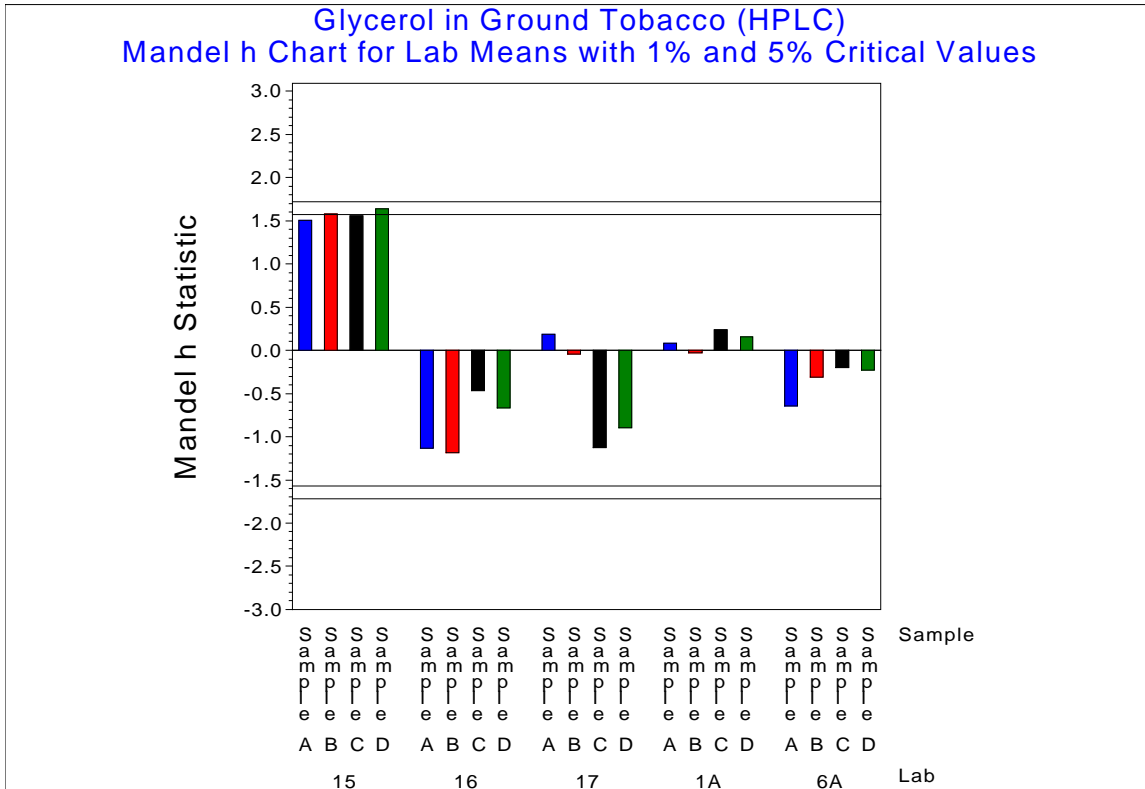
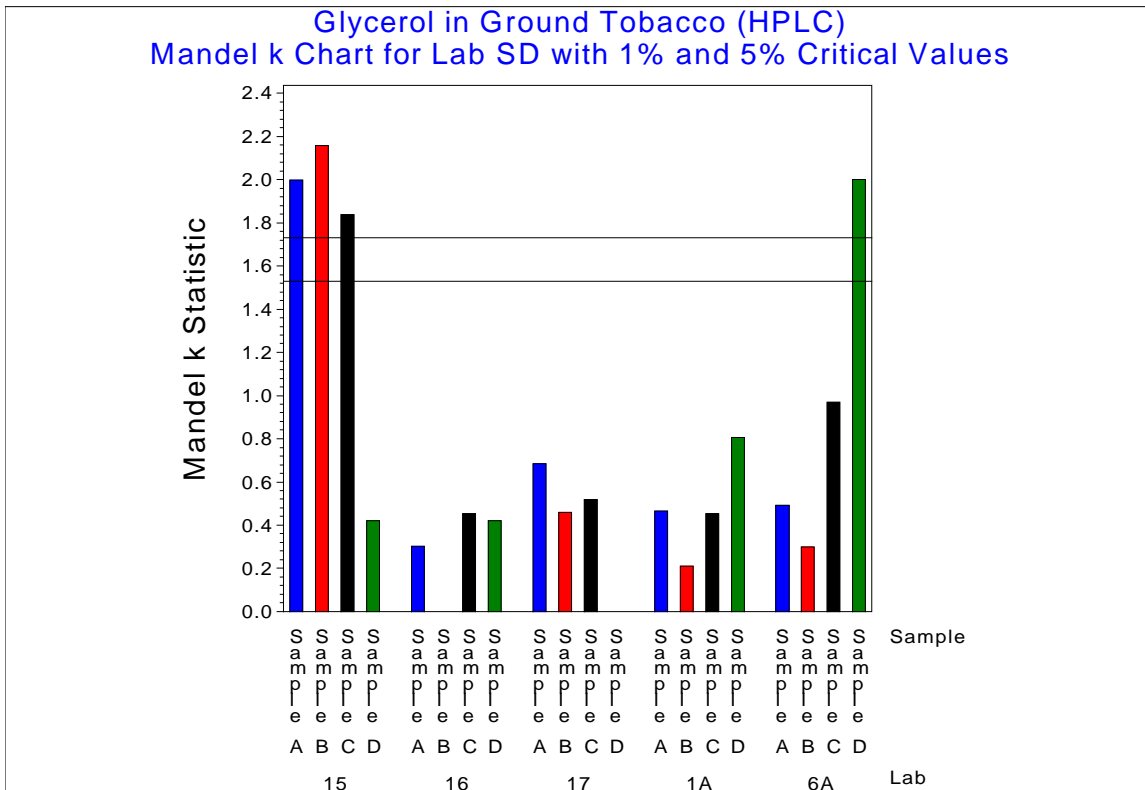


Figure 22a: Glycerol in Ground Tobacco (HPLC), Mandel k Chart for Lab SDs



5. CONCLUSION

The following figures 23 – 26 and Table 4 compare the current mean, r and R results to those results published in the 2005 CRMs. Lab 15 data is excluded from these graphs as it was an outlier.

As can be seen, the care taken during sample preparation of the test portions to ensure homogeneity significantly improved method precision.

APPENDIX C contains a summary table of deviations from the CRMs, as all laboratories were not able to strictly follow the protocol.

Figure 23: Propylene Glycol in Cut Loose Tobacco Method Precision – Exclude HPLC Lab 15

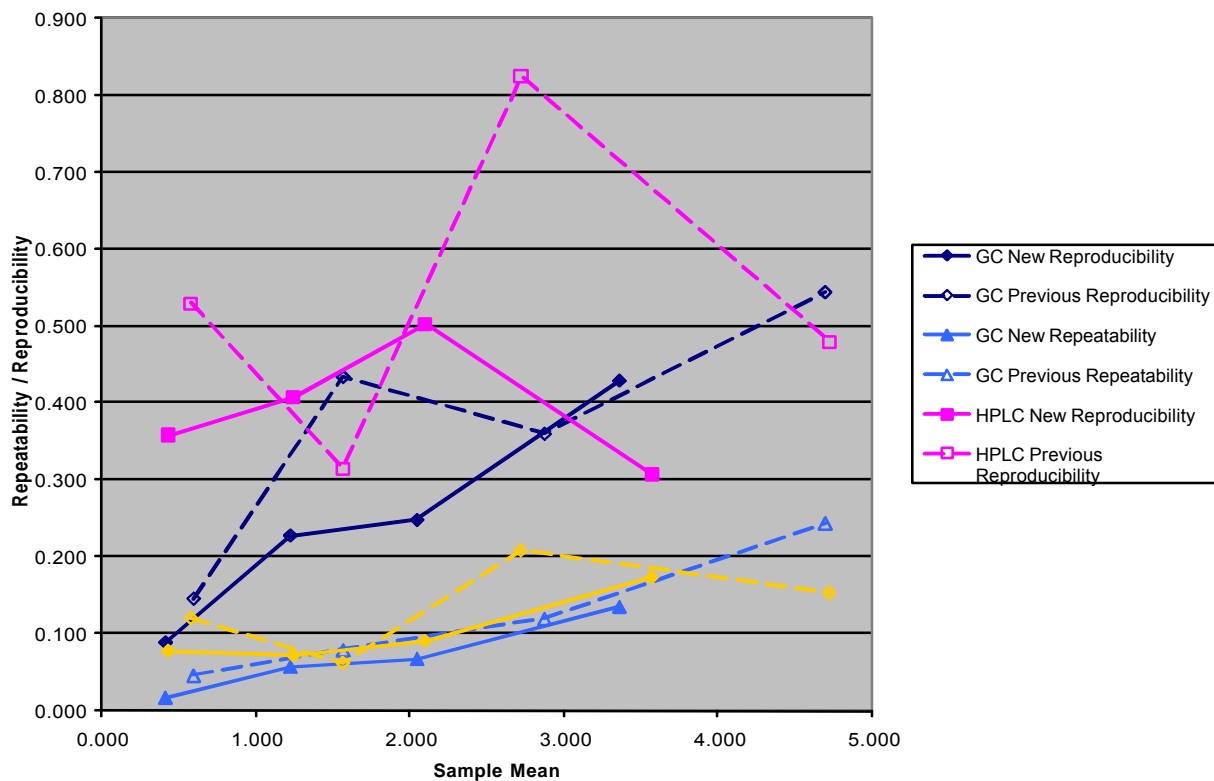


Figure 24: Glycerol in Cut Loose Tobacco Method Precision – Exclude HPLC Lab 15

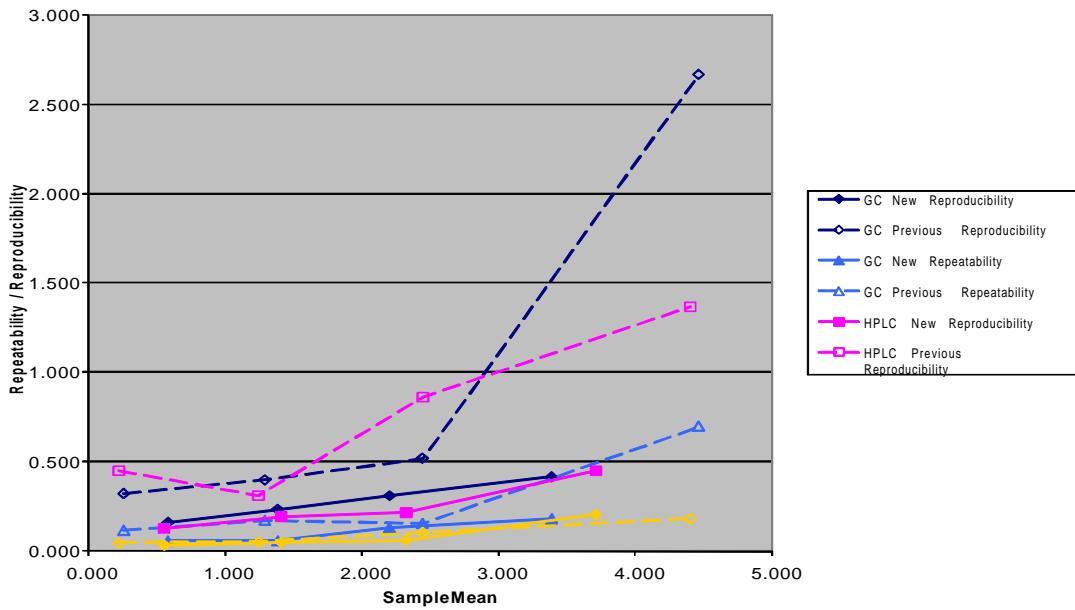


Figure 25: Propylene Glycol in Ground Tobacco Method Precision – Exclude HPLC Lab 15

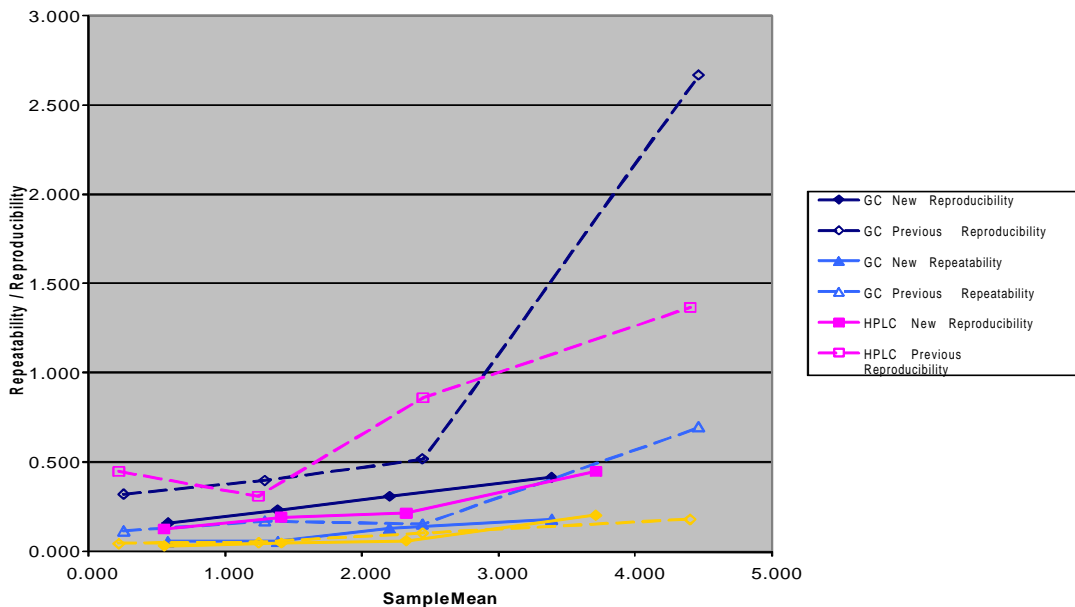


Figure 26: Glycerol in Ground Tobacco Method Precision – Exclude HPLC Lab 15

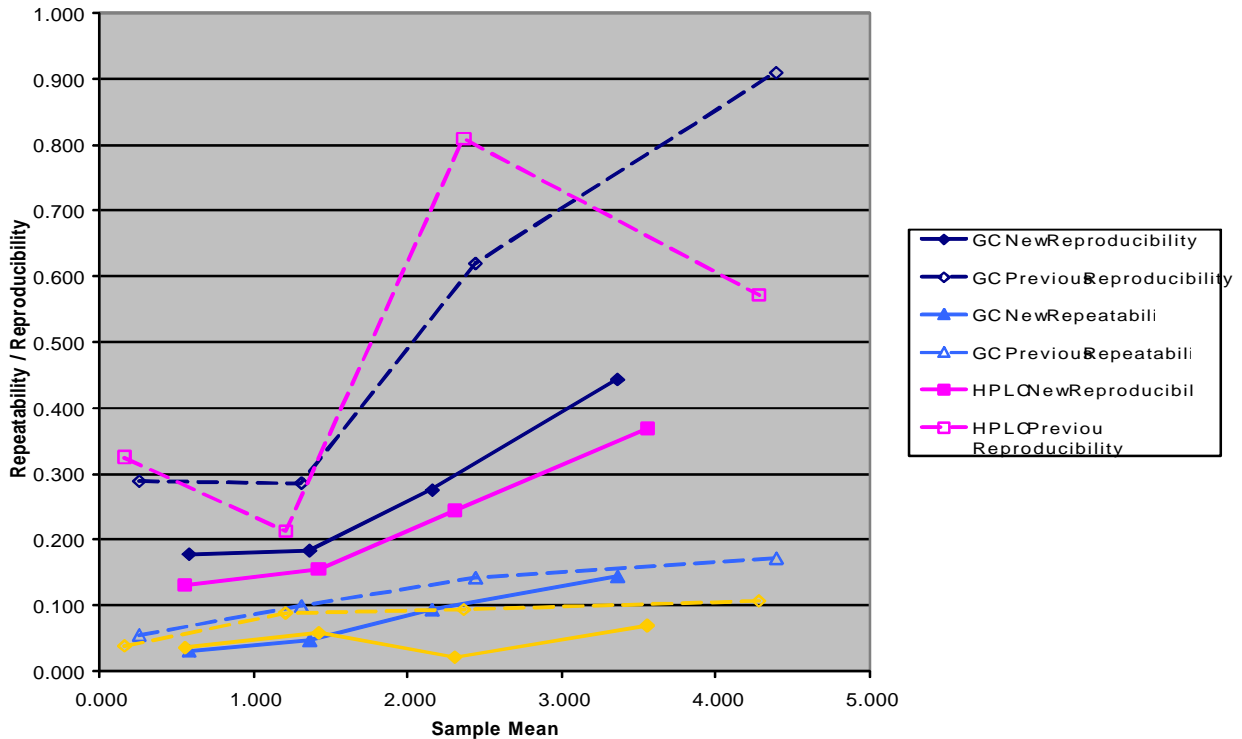


Table 4: CORESTA Collaborative Study of 1,2-Propylene Glycol and Glycerol Measurements – Comparison of Previous and Current Study Performance Measures (Excluding HPLC Lab 15)

| | Propylene Glycol in Cut Loose Tobacco - GC Method | | | | | | | |
|-------------|---|----------|----------|----------|------------|----------|----------|----------|
| | Previous Study | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.597 | 1.567 | 2.873 | 4.693 | 0.416 | 1.227 | 2.049 | 3.362 |
| r | 0.046 | 0.079 | 0.120 | 0.244 | 0.016 | 0.057 | 0.067 | 0.135 |
| R | 0.146 | 0.434 | 0.360 | 0.544 | 0.089 | 0.227 | 0.248 | 0.429 |

| | Propylene Glycol in Cut Loose Tobacco - HPLC Method* | | | | | | | |
|-------------|--|----------|----------|----------|------------|----------|----------|----------|
| | Previous Study | | | | 2007 Study | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.585 | 1.568 | 2.724 | 4.723 | 0.439 | 1.251 | 2.103 | 3.573 |
| r | 0.120 | 0.062 | 0.208 | 0.153 | 0.077 | 0.071 | 0.090 | 0.174 |
| R | 0.530 | 0.315 | 0.825 | 0.48 | 0.358 | 0.408 | 0.503 | 0.307 |

| Glycerol in Cut Loose Tobacco - GC Method | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.260 | 1.291 | 2.444 | 4.462 | 0.585 | 1.387 | 2.202 | 3.386 |
| r | 0.117 | 0.173 | 0.156 | 0.698 | 0.059 | 0.060 | 0.132 | 0.184 |
| R | 0.325 | 0.401 | 0.522 | 2.668 | 0.163 | 0.236 | 0.313 | 0.418 |

| Glycerol in Cut Loose Tobacco - HPLC Method* | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.223 | 1.244 | 2.442 | 4.396 | 0.560 | 1.413 | 2.324 | 3.707 |
| r | 0.048 | 0.053 | 0.106 | 0.181 | 0.035 | 0.051 | 0.064 | 0.208 |
| R | 0.448 | 0.311 | 0.862 | 1.369 | 0.129 | 0.196 | 0.217 | 0.450 |

| Propylene Glycol in Ground Tobacco - GC Method | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.591 | 1.549 | 2.803 | 4.580 | 0.404 | 1.181 | 2.011 | 3.303 |
| r | 0.033 | 0.056 | 0.110 | 0.179 | 0.019 | 0.029 | 0.028 | 0.073 |
| R | 0.090 | 0.170 | 0.334 | 0.514 | 0.058 | 0.204 | 0.210 | 0.360 |

| Propylene Glycol in Ground Tobacco - HPLC Method* | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.574 | 1.596 | 2.669 | 4.554 | 0.424 | 1.236 | 2.072 | 3.435 |
| r | 0.054 | 0.067 | 0.102 | 0.096 | 0.050 | 0.054 | 0.093 | 0.075 |
| R | 0.555 | 0.276 | 0.666 | 0.450 | 0.230 | 0.390 | 0.469 | 0.204 |

| Glycerol in Ground Tobacco - GC Method | | | | | | | | |
|--|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.259 | 1.311 | 2.439 | 4.389 | 0.584 | 1.365 | 2.159 | 3.361 |
| r | 0.056 | 0.100 | 0.143 | 0.173 | 0.032 | 0.048 | 0.094 | 0.145 |
| R | 0.289 | 0.286 | 0.620 | 0.910 | 0.178 | 0.183 | 0.276 | 0.444 |

| Glycerol in Ground Tobacco - HPLC Method* | | | | | | | | |
|---|----------|----------|----------|----------|-------------------|----------|----------|----------|
| <i>Previous Study</i> | | | | | <i>2007 Study</i> | | | |
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| Mean | 0.167 | 1.211 | 2.367 | 4.281 | 0.558 | 1.425 | 2.307 | 3.555 |
| r | 0.039 | 0.090 | 0.095 | 0.108 | 0.037 | 0.059 | 0.023 | 0.070 |
| R | 0.328 | 0.214 | 0.810 | 0.572 | 0.131 | 0.155 | 0.245 | 0.369 |

***Note: r and R statistics for the HPLC methods are based on a very small number of laboratories, eight in the original study and four in this study with Lab 15 excluded.**

4. Recommendations

Analysis of the data showed that the larger r and R values found in the previous study were due to the lack of sample homogeneity. Therefore one recommendation is to prepare samples for humectants analysis as homogenously as possible.

Another outcome of this study is to update CRM 60 and 61 to include the r and R statistics found in this study.

APPENDIX A: List of Participating Laboratories

| |
|--|
| BAT RPC, GERMANY |
| BAT GR&D, U.K. |
| Imperial Tobacco, GERMANY |
| LTR Industries, FRANCE |
| ARISTA Laboratories U.S, U.S.A. |
| House of Prince, DENMARK |
| ALTADIS, FRANCE |
| JAPAN Tobacco Inc, JAPAN |
| JTI GALLAHER. U.K. |
| Rothmans B&H Inc., CANADA |
| BAT PPS, Africa Middle East, SOUTH AFRICA |
| PMI, SWITZERLAND |
| PTHM Sampoerna Tbk, INDONESIA |
| Landewyck Group Sarl, LUXEMBOURG |
| R.J. Reynolds Tobacco Company, U.S.A. |
| Labstat International, CANADA |
| KT&G, KOREA |
| Scandinavian Tobacco S.A., POLAND |
| Swedish Match, SWEDEN |

APPENDIX B: Study Protocol

CORESTA SUB-GROUP ROUTINE ANALYTICAL CHEMISTRY

JOINT EXPERIMENT 2007

TEST PROTOCOL

1. Analytical Methods:

- a. CORESTA Recommended Method 60, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by Gas Chromatography
- b. CORESTA Recommended Method 61, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by High Performance Liquid Chromatography

Report any deviations from the methods listed above, as well as any observations you deem useful to share on attached Data Sheet 1.

If a methodology other than those specified for propylene glycol and glycerol are used, please document deviations carefully on the attached Data Sheet 1.

2. SAMPLES

Upon receipt of samples store the sealed bags in an air conditioned room in order to ensure moderate ambient temperature (20-25 °C). Samples consist of four pouches of loose cut tobacco that have been carefully processed in a V Blender to improve homogeneity and four pouches of finely ground and homogenized tobacco. Samples contain nominally .4% - 3.3% Propylene Glycol and 0.5% - 3.3% Glycerol.

3. TESTS

Samples should be analyzed “**as received**” and reported on Data Sheet 2 with **no Oven Volatiles Correction**. Perform each extraction in **duplicate and inject each extract twice**.

Note: Please retain samples (well sealed and stored at moderate ambient temperature) until results for the Joint Experiment are finalized.

4. REPORTING

Report results to Linda Crumpler by **September 4, 2007** at the latest for inclusion in the data analysis to be presented at the October meeting of the Routine Analytical Chemistry Sub-Group.

Results should be sent electronically on the attached data sheets to e-mail address crumpll@rjrt.com.

APPENDIX C: Table Summarizing the Methodologies used by the Participating Laboratories.

(*Indicates insignificant deviations from protocol)

| Deviations from Protocol | | | | | | | | | |
|--------------------------|--------------|-----|-------------------|------------------------------------|--|-------------------------------|------------------------|---|------------------------------|
| Lab ID | Followed CRM | CRM | Internal Standard | Column | GC Conditions | Sample Amount | Extraction Vessel Size | Extraction Time | Filtration |
| 1 | Yes | 60 | | DB-Wax 15m x 0.53mm i.d., 1µm film | Change in Temp. profile | | 200 mL | | 0.45 µm PTFE membrane filter |
| 1A | Yes | 61 | | Varian Metcarb 67C @ 95°C | | | 200 mL | | 0.45 µm PTFE membrane |
| 2 | Yes | 60 | 1,3-butanediol | DB-Wax 15m x 0.53mm i.d., 1µm film | Change in Temp. profile | 4g | | | |
| 3 | Yes | 60 | | ZB 5 30m x 0.53mm i.d., 5µm film | Adjusted GC conditions & temp. profile | | | | |
| 4 | Yes | 60 | | | | | | | |
| 5 | Yes | 60 | | | | | | | |
| 6 | Yes | 60 | | | | | | | |
| 6A | Yes | 61 | | | | | | | |
| 7 | Yes | 60 | | | | 2 g/25 mL extraction solution | | 60 minutes shaking & 30 minute stand time | |

| | | | | | | | | | |
|----|-----|----|----------------|---|--|--------------------------------|--|--------------------|---|
| 8 | Yes | 60 | 1,3-butanediol | AT 35 ex Alltech Associates | | | | | |
| 9 | Yes | 60 | | | | | | | |
| 10 | Yes | 60 | n-undecane | | | 2 g/25 mL extraction solution | | | |
| 11 | Yes | 60 | | | | | | | |
| 12 | Yes | 60 | 1,3-butanediol | J&W 5m x 0.2mm i.d., 0.4µm film DBWAX ETR | Adjusted GC conditions & temp. profile | 8g/100 mL extraction solution | | 3 hours shake time | |
| 13 | Yes | 61 | | | | 2g/20 mL extraction solution | | | 0.45 µm PTFE membrane |
| 14 | *No | 60 | 1,3-butanediol | Rtx-Wax 30m x 0.32mm i.d., 0.1µm film | Adjusted GC conditions & temp. profile | ASE 200 Solvent Extractor used | | | 13mm PTFE syringe filter |
| 15 | *No | 61 | | Monosaccharide specific column (Ca ²⁺ based) | | 1g/50 mL H ₂ O | | 3 hours shake time | Nylon filter - SAX SPE tube combination |
| 16 | *No | 61 | | Varian Metacarb H Plus 300 x 7.8mm | 0.005M H ₂ SO ₄ mobile phase | different | | | 0.45 µm CA-membrane, SAX SPE |
| 17 | Yes | 61 | | | | 2g/50 mL H ₂ O | | | |