COMPARING 3 BRANDS OF ASPIRIN PILLS

Analysis by: Gavin Gunawardena

Using dataset from: LC-MS/MS in the Clinical Laboratory – Where to From Here?

by Stefan KG Greve and Ravinder J Singh and which can be found here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3052391/

Intro

Background and Dataset

- Machine generated dataset analyzing the chemical make-up and intensities of three brands of aspirin pills
- Dataset consists of:
  - 3 pills from 3 different brands of aspirin (Bayer, PV, and Walgreens)
  - 63 tsv files grouping the pills by brand, peak of intensity, replicant, and aliquot
  - 3 aliquots for each replicant, 3 replicants for each peak, and 7 peaks for each brand in the dataset
    - Peak: time when the intensity of each pill is at its highest, usually when a chemical is released from the pill and at artificially timed intervals
    - Replicant: replicas of the same pill; 3 replicas of 3 different brands have been tested
    - Aliquots: Series of measurements within a timeframe of less than a second

Intro

Objective and Assumptions

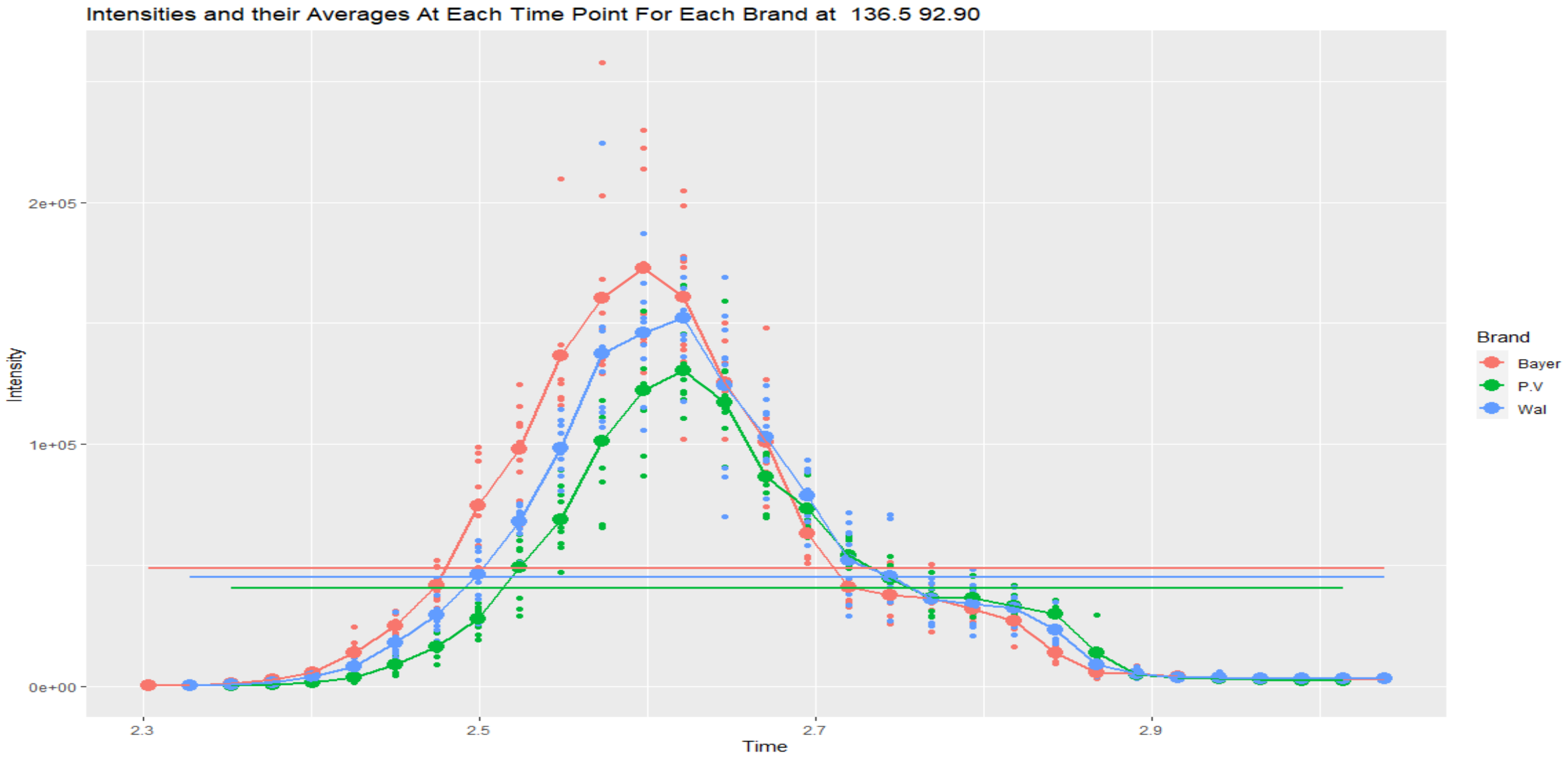
- Objective is to test the null hypothesis that the 3 brands of aspirin are the same with a 95% confidence interval
  - Plan is to measure the Area Under a Peak (AUP) for each aliquot of each replicant
  - Next, run various ANOVA analyses to compare the AUPs between brands for each peak to confirm or deny the null hypothesis
- Assumptions:
  - Data was gathered using a standardized process, since it was machine generated
  - There is still some possibility for error within the process utilized to attain the data
    - Thus, missing data will be removed, and statistical tests will be run with and without outliers.

Exploratory Analysis

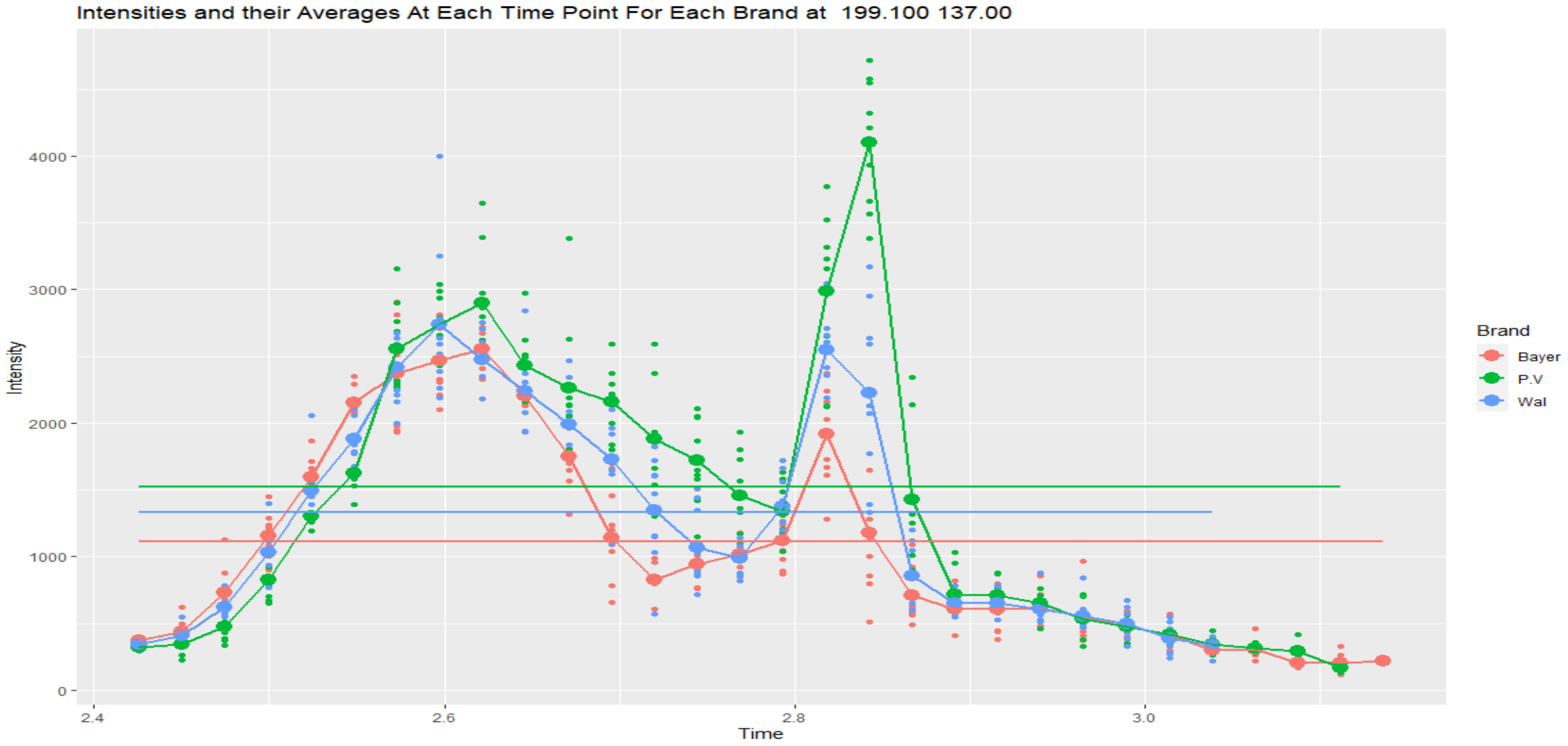
Dataset Summary

```
##     Brand                Peak              Replicant       Aliquot
##  Length:4798        Length:4798        Min.   :1.00   Min.   :1.000
##  Class :character   Class :character   1st Qu.:1.00   1st Qu.:1.000
##  Mode  :character   Mode  :character   Median :2.00   Median :2.000
##                                        Mean   :1.99   Mean   :2.003
##                                        3rd Qu.:3.00   3rd Qu.:3.000
##                                        Max.   :3.00   Max.   :3.000
##       Time          Intensity
##  Min.   :2.303   Min.   :    10
##  1st Qu.:2.548   1st Qu.:   270
##  Median :2.720   Median :   785
##  Mean   :2.717   Mean   : 10071
##  3rd Qu.:2.867   3rd Qu.:  2590
##  Max.   :3.161   Max.   :257870
```

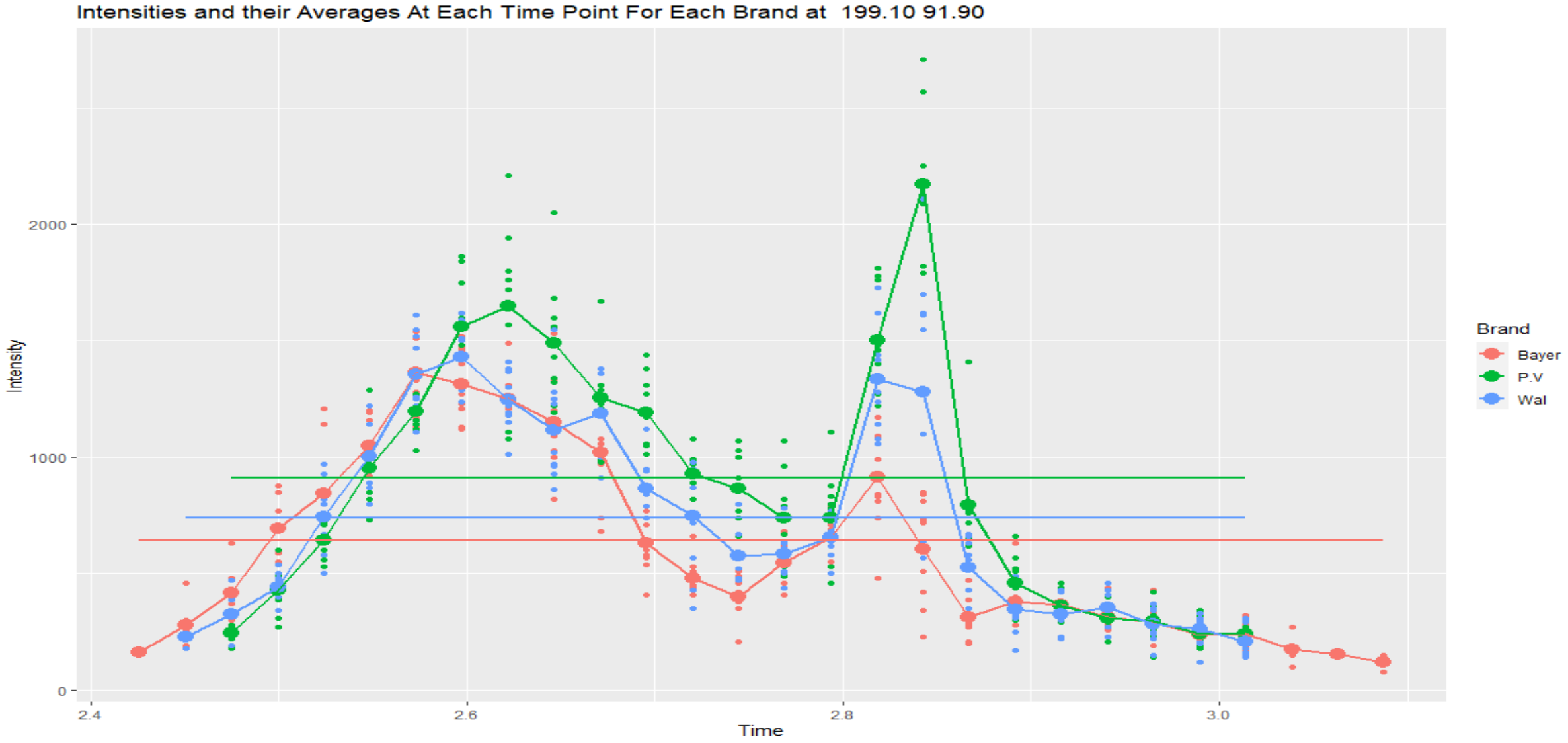
Exploratory Analysis



Exploratory Analysis



Exploratory Analysis



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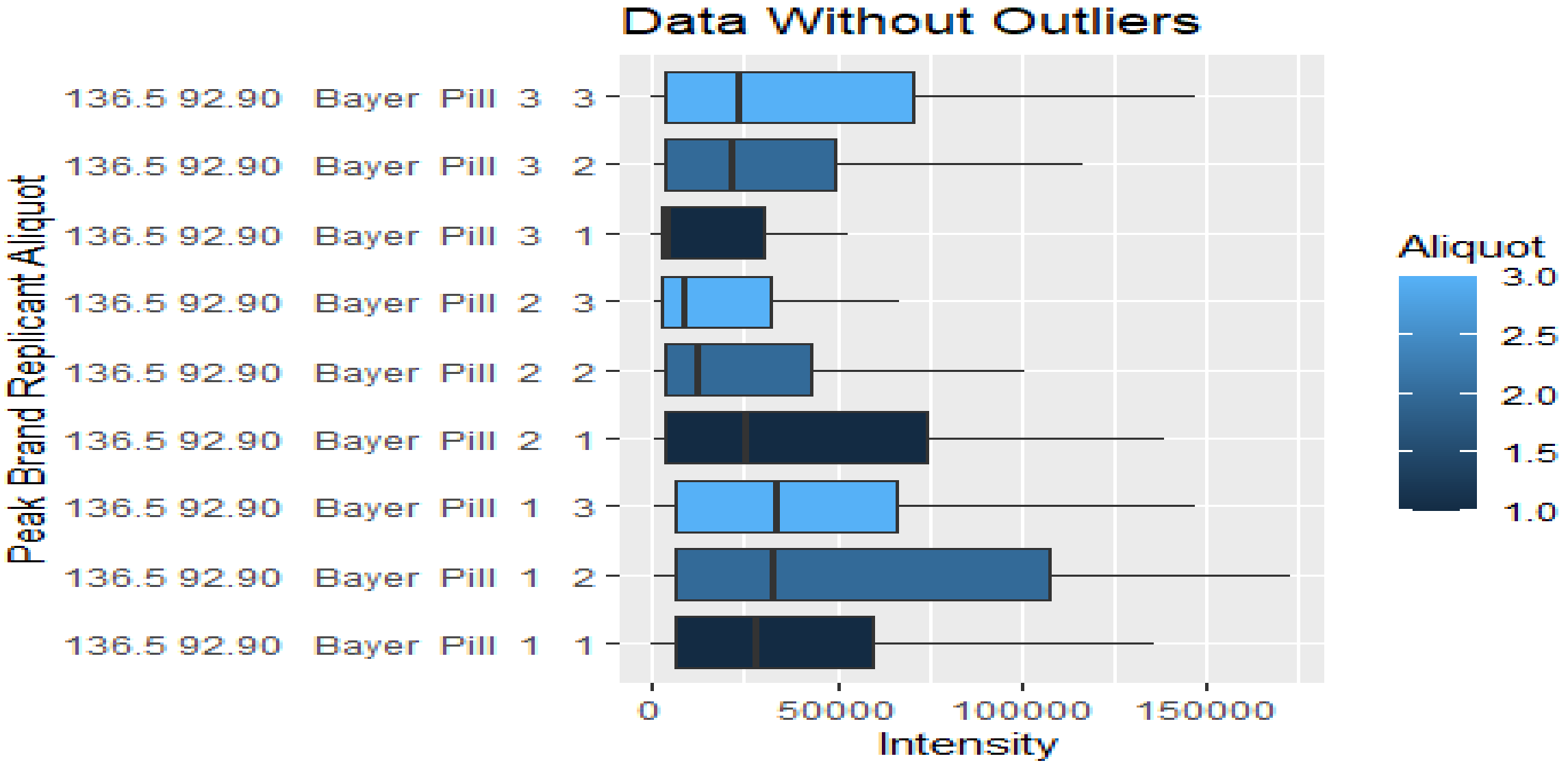
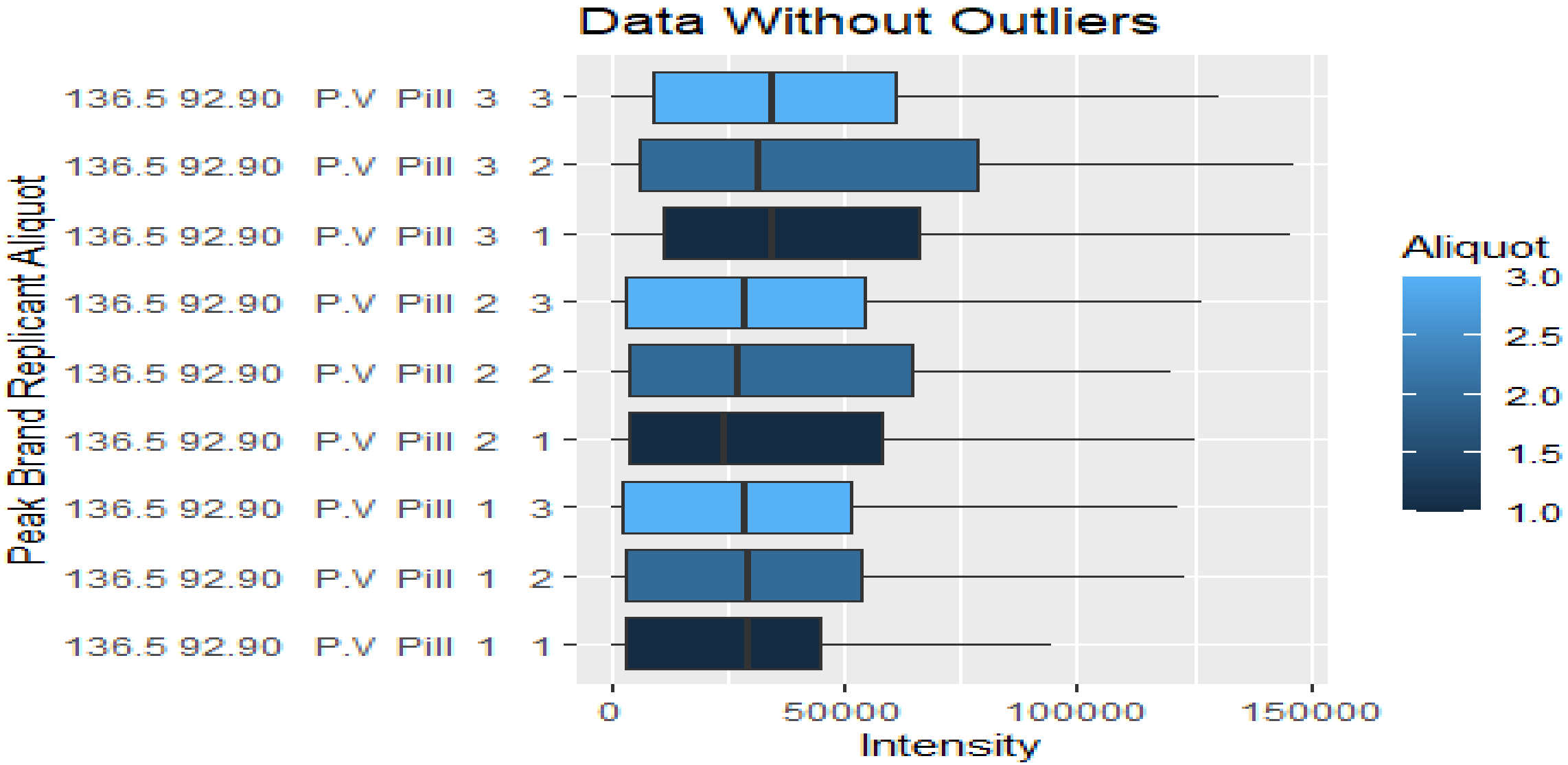
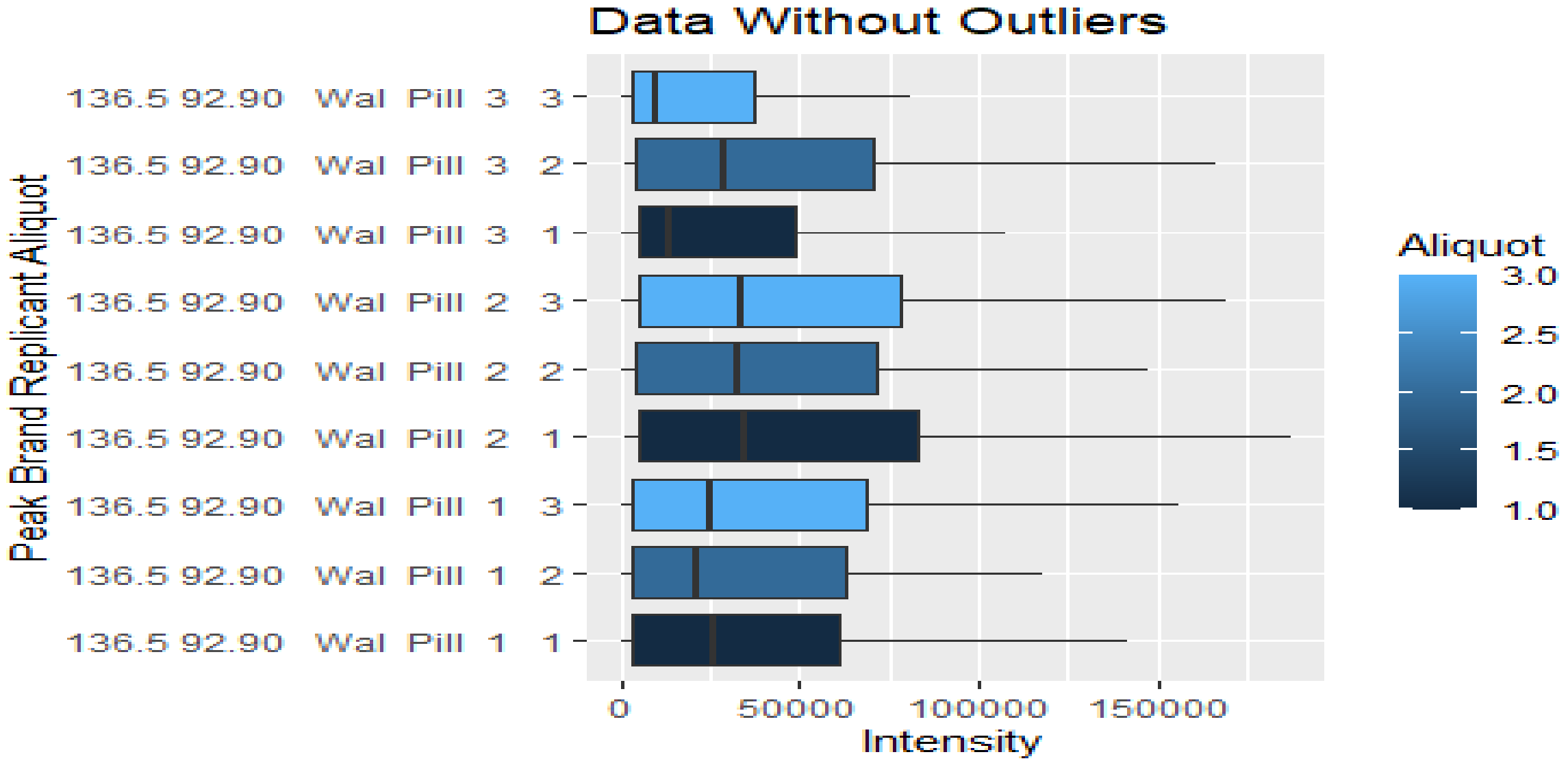


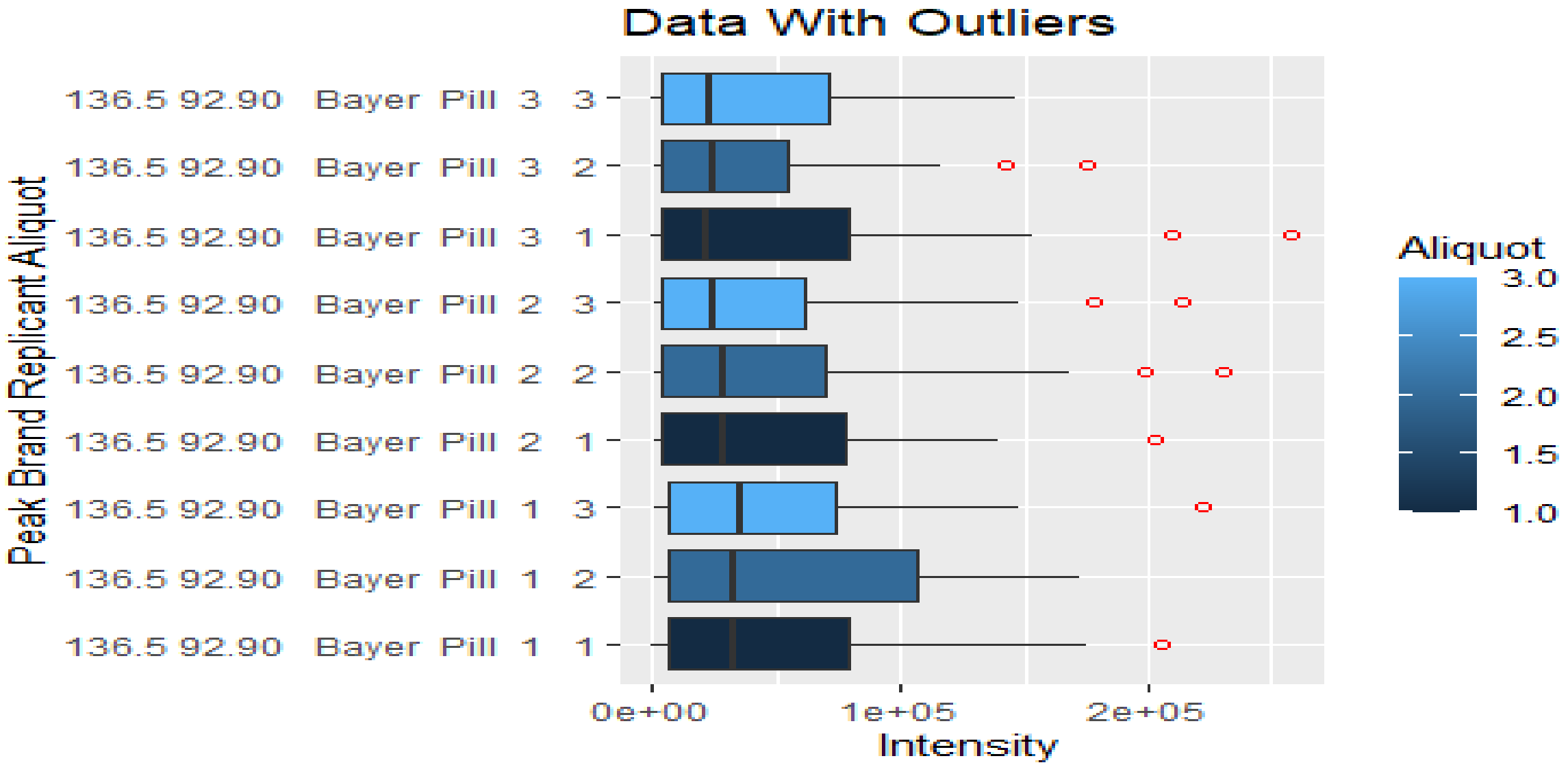
Exploratory Analysis



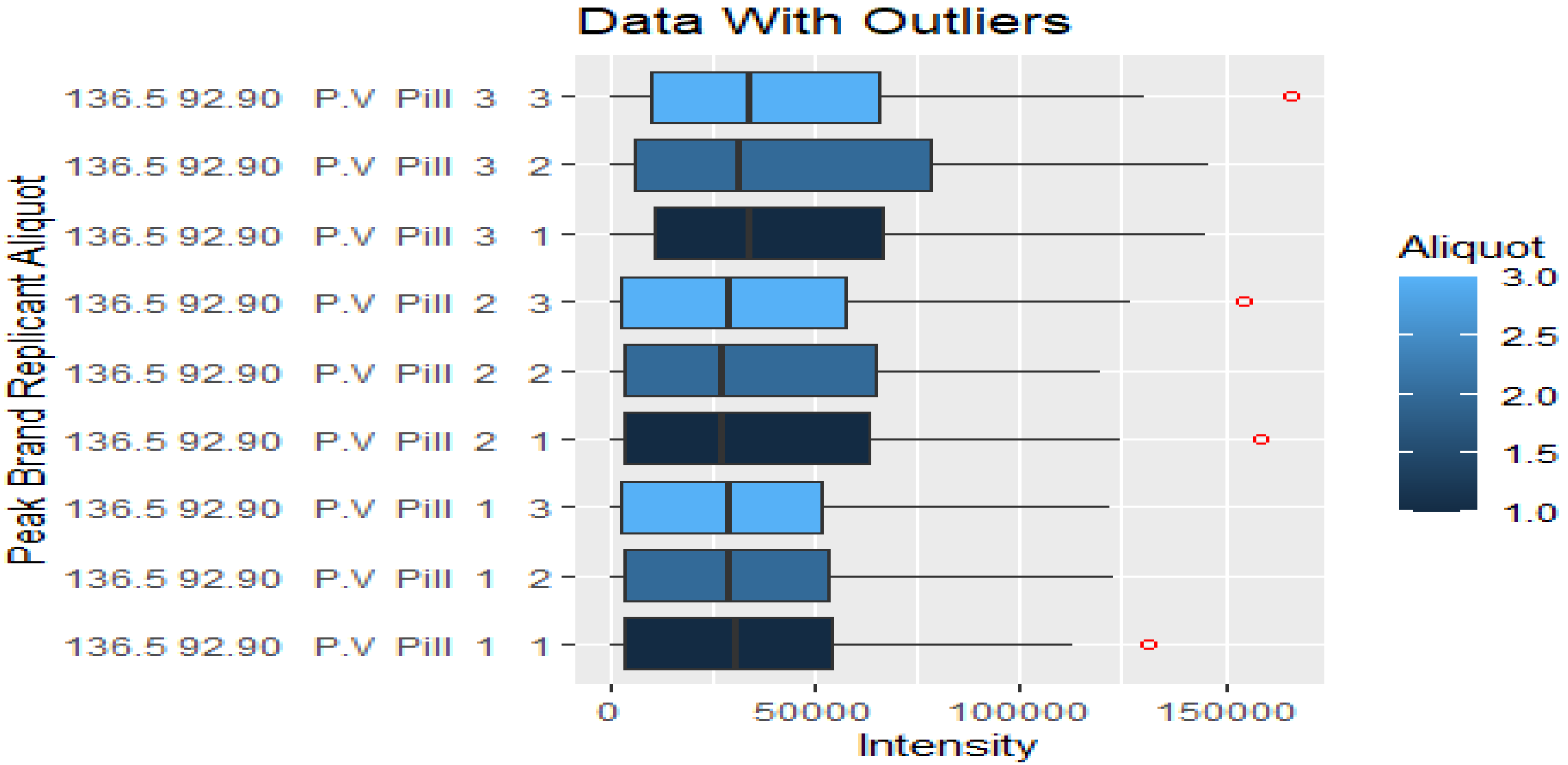
Exploratory Analysis



Exploratory Analysis



Exploratory Analysis



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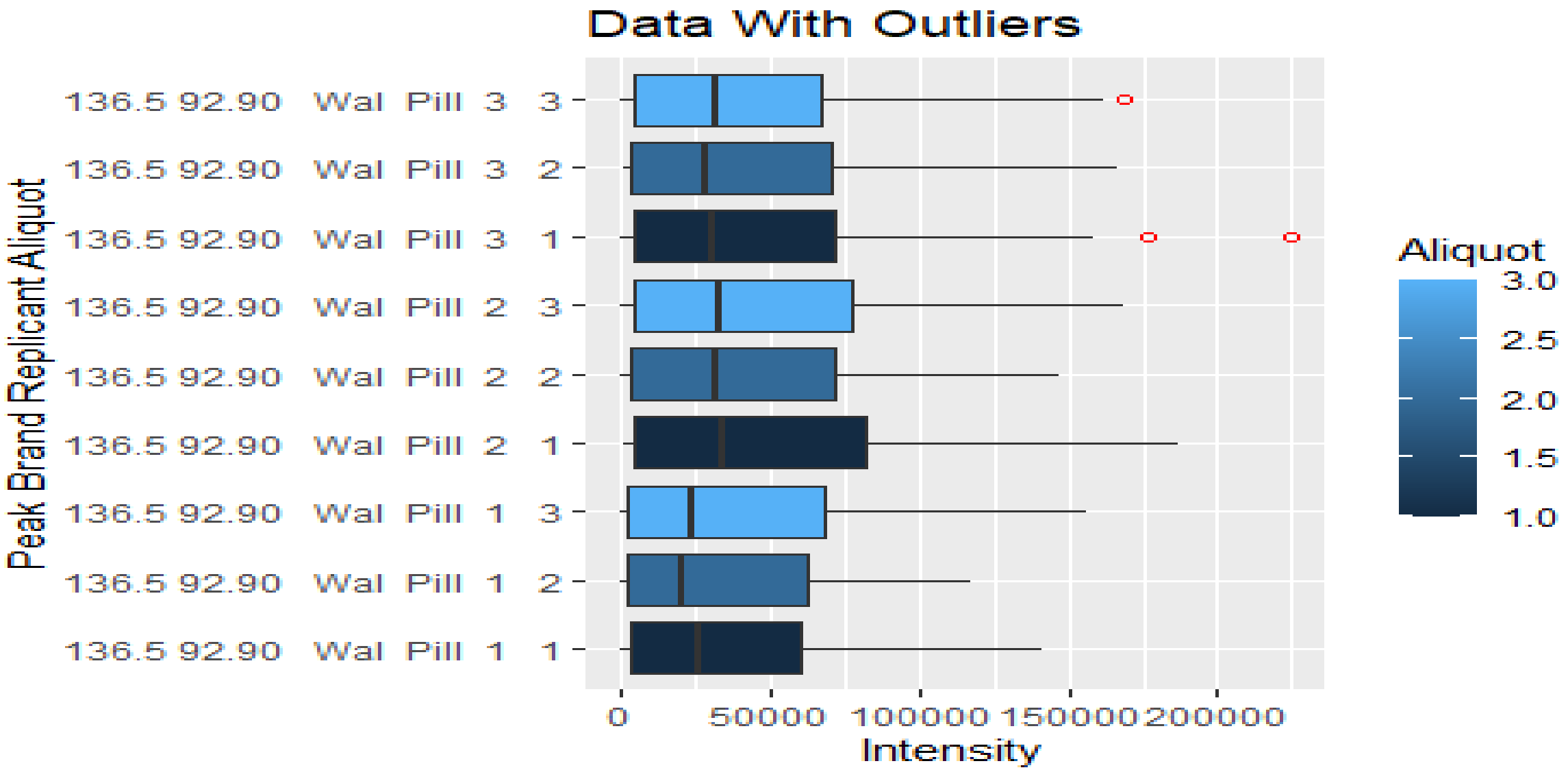

Analysis

One Way ANOVA and Pairwise comparisons of AUPs for each Brand

- Complete with an alpha of .5 to get a 95% confidence interval statistical significance comparison
- All 3 at once and pairwise comparisons using Student's t-distribution tests

Brand	AoV_P_Value	Pairwise_T_Test_P_Value
Brand - With Outliers	0.973	
Brand - w/o Outliers	0.729	
P.V. vs Bayer - With Outliers		0.85
Wal vs Bayer - With Outliers		0.98
Wal vs P.V. - With Outliers		0.83
P.V. vs Bayer - w/o Outliers		0.53
Wal vs Bayer - w/o Outliers		0.46
Wal vs P.V - w/o Outliers		0.91

Analysis

Ad-Hoc Pairwise comparisons

- Tukey-Kramer comparison which has an error correction that assists when comparing pairs within a group with sample sizes that aren't exactly the same.
- Bonferroni comparison which has an error correction that, similarly to the Tukey-Kramer test, also assists when comparing pairs within a group with sample sizes that aren't exactly the same.

Brand	Tukey_Kramer_P_Values	Bonferroni_P_Values
P.V. vs Bayer - With Outliers	0.489	0.818
Wal vs Bayer - With Outliers	0.984	0.887
Wal vs P.V. - With Outliers	0.389	0.118
P.V. vs Bayer - w/o Outliers	0.078	0.140
Wal vs Bayer - w/o Outliers	0.030	0.132
Wal vs P.V - w/o Oultiers	0.924	0.624

Conclusions

Null Hypothesis that the Aspirin Pills Are the Same

- Failed to reject null hypothesis that the aspirin pills are the same
  - Initial ANOVA test revealed that at a 95% confidence interval, there is no difference between the brands with or without the outliers being removed
  - This result was confirmed when running pairwise t-tests between the 3 brands
- Ad-Hoc Analysis
  - When outliers are not removed and when comparing the individual brands between each other using either Tukey-Kramer or Bonferroni analyses, the null hypothesis is not rejected at a 95% confidence interval
  - When outliers are removed and the Tukey-Kramer analysis but not the Bonferroni analysis is used, the Walgreens and Bayers brand aspirin pills are found to be different at a 95% confidence interval, rejecting the null hypothesis
- Standard One-Way ANOVA is being prioritized and thus the null hypothesis fails to be rejected

Sources

- https://www.vedantu.com/formula/anova-formula
- https://www.educba.com/t-test-formula/
- https://www.statology.org/tukey-vs-bonferroni-vs-scheffe/
- https://aaronschlegel.me/tukeys-test-post-hoc-analysis.html
- https://www.itl.nist.gov/div898/handbook/prc/section4/prc473.htm
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3052391/