Validation of Bioequivalence Test Performed by BE R package

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Contents

1 Introduction

To assess bioequivalence, the 90% confidence interval for the difference in the means of the log-transformed data should be calculated using appropriate methods to the study design. The antilogs (exponents) of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the T and R products. (Center for Drug Evaluation and Research (CDER), Food and Drug Administration, U.S. Department of Health and Human Services 2001; Chow and Liu 2008; Hauschke, Steinijans, and Pigeot 2007) To establish bioequivalence, the calculated confidence interval should fall within a bioequivalence limit, usually 80-125% for the ratio of the product averages. For nonreplicated crossover designs, general linear model procedures available in PROC GLM in SAS are preferred, although linear mixed-effects model procedures can also be indicated for analysis. (Center for Drug Evaluation and Research (CDER), Food and Drug Administration, U.S. Department of Health and Human Services 2001)

`BE` R package (Bae 2018) can analyze bioequivalence study data with industrial strength. The current version of `BE` performs bioequivalency tests for several pharmacokinetic parameters of the conventional two-treatment, two-period, two-sequence (2x2) randomized crossover design. The statistical model includes factors accounting for the following sources of variation: sequence (SEQ), subjects nested in sequences (SUBJ(SEQ)), period (PRD), and treatment (TRT).

In this document, the author performed validation of bioequivalence tests performed by `BE` R package as compared to bioequivalence tests performed by PROC GLM or PROC MIXED in SAS.

2 Methods

2.1 Dataset

A simulated dataset of the conventional 2×2 crossover study for this analysis, `BE::NCAResult4BE` is shown in Appendix A. The number of subjects in the sequence 'RT' is 17 and that in the sequence 'TR' is 16. (total N=33) The 4 variables, SUBJ (subject), GRP (group or sequence), PRD (period), and TRT (treatment), and the 3 pharmacokinetic parameters, AUC_{last}, C_{max}, and T_{max} are presented and there is no missing values. (total 66 observations with 7 variables)

2.2 Bioequivalence tests in R

The required R packages are following.

```
library(BE)          # install.packages("BE", repos="http://r.acr.kr")
library(dplyr)       # install.packages("dplyr")
library(readr)       # install.packages("readr")
```

`tab_r_be_results` function uses `BE::test2x2()` and returns the 90% confidence interval.

```
tab_r_be_results <- function(parameter){
  BE::test2x2(BE::NCAResult4BE, parameter)[[4]] %>%
  as.data.frame() %>%
  mutate(Analysis = 'R: BE package') %>%
  select(Analysis, `Lower Limit`, `Point Estimate`, `Upper Limit`)
}
```

2.3 Bioequivalence tests in SAS

To run BE analysis, PROC GLM and PROC MIXED in SAS version 9.4 were used. The SAS program statements include the variables, SEQ (sequence), TRT (treatment), SUBJ (subject), and PRD (period). LNAUCL (log-transformed AUC_{last}) or LNCMAX (log-transformed C_{max}) denotes the response measure. A part of SAS scripts are shown below and the full SAS scripts are appended in Appendix B.

```
PROC GLM DATA=BE OUTSTAT=STATRES; /* GLM use only complete subjects. */
CLASS SEQ PRD TRT SUBJ;
MODEL LNAUCL = SEQ SUBJ(SEQ) PRD TRT;
RANDOM SUBJ(SEQ)/TEST;
LSMEANS TRT /PDIFF=CONTROL('R') CL ALPHA=0.1 COV OUT=LSOUT;
```

```
PROC MIXED DATA=BE; /* MIXED uses all data. */
CLASS SEQ TRT SUBJ PRD;
MODEL LNAUCL = SEQ PRD TRT;
RANDOM SUBJ(SEQ);
ESTIMATE 'T VS R' TRT -1 1 /CL ALPHA=0.1;
ODS OUTPUT ESTIMATES=ESTIM COVPARMS=COVPAR;
```

A function, `tab_sas_proc_results()` reads SAS analysis results exported to Microsoft Excel files (`.xls`) and converted to comma separated version file (`.csv`). It returns a data frame of 90% confidence interval calculated either `PROC GLM` or `PROC MIXED` in SAS.

```
tab_sas_proc_results <- function(filename, skip_no, analysis_name){
  read_lines(filename, skip = skip_no, n_max = 2) %>%
  paste(collapse='\n') %>% read_csv() %>%
  mutate(Analysis = analysis_name) %>%
  select(Analysis, `Lower Limit` = LL, `Point Estimate` = PE, `Upper Limit` = UL)
}
```

3 Results

3.1 AUC_{last}

Comparison of 90% confidence interval for the ratio of the geometric means of AUC_{last} between the T and R products is shown in Table 1.

```
AUClast_R_BE <- tab_r_be_results("AUClast")

AUClast_proc_glm <- tab_sas_proc_results('sas/SAS_results_AUClast.csv',
                                         skip = 206, 'SAS: PROC GLM')
AUClast_proc_mixed <- tab_sas_proc_results('sas/SAS_results_AUClast.csv',
                                           skip = 278, 'SAS: PROC MIXED')

# Combine all analyses of AUClast
AUClast_all_analyses <- bind_rows(AUClast_R_BE, AUClast_proc_glm, AUClast_proc_mixed)
```

Table 1: Comparison of 90% confidence interval for the ratio of the geometric means of AUClast

Analysis	Lower Limit	Point Estimate	Upper Limit
R: BE package	0.88944	0.95408	1.02341
SAS: PROC GLM	0.88944	0.95408	1.02341
SAS: PROC MIXED	0.88944	0.95408	1.02341

3.2 C_{max}

Comparison of 90% confidence interval for the ratio of the geometric means of AUC_{last} between the T and R products is shown in Table 2.

```
Cmax_R_BE <- tab_r_be_results("Cmax")

Cmax_proc_glm <- tab_sas_proc_results('sas/SAS_results_Cmax.csv',
                                      skip = 206, 'SAS: PROC GLM')
Cmax_proc_mixed <- tab_sas_proc_results('sas/SAS_results_Cmax.csv',
                                        skip = 278, 'SAS: PROC MIXED')

# Combine all analyses of Cmax
Cmax_all_analyses <- bind_rows(Cmax_R_BE, Cmax_proc_glm, Cmax_proc_mixed)
```

Table 2: Comparison of 90% confidence interval for the ratio of the geometric means of Cmax

Analysis	Lower Limit	Point Estimate	Upper Limit
R: BE package	0.90136	0.97984	1.06515
SAS: PROC GLM	0.90136	0.97984	1.06515
SAS: PROC MIXED	0.90136	0.97984	1.06515

4 Conclusion

There is no discrepancy between bioequivalence tests performed by `BE` R package and those performed by PROC GLM or PROC MIXED in SAS. We also performed multiple analyses with the actual clinical trial datasets and have found no differences (data not shown: confidential).

Bioequivalence tests performed by the open-source `BE` R package for the conventional two-treatment, two-period, two-sequence (2x2) randomized crossover design can be **qualified and validated** enough to acquire the identical results of the commercial statistical software, SAS.

Please report issues regarding validation of the R package to https://github.com/asancpt/BEreport/issues.

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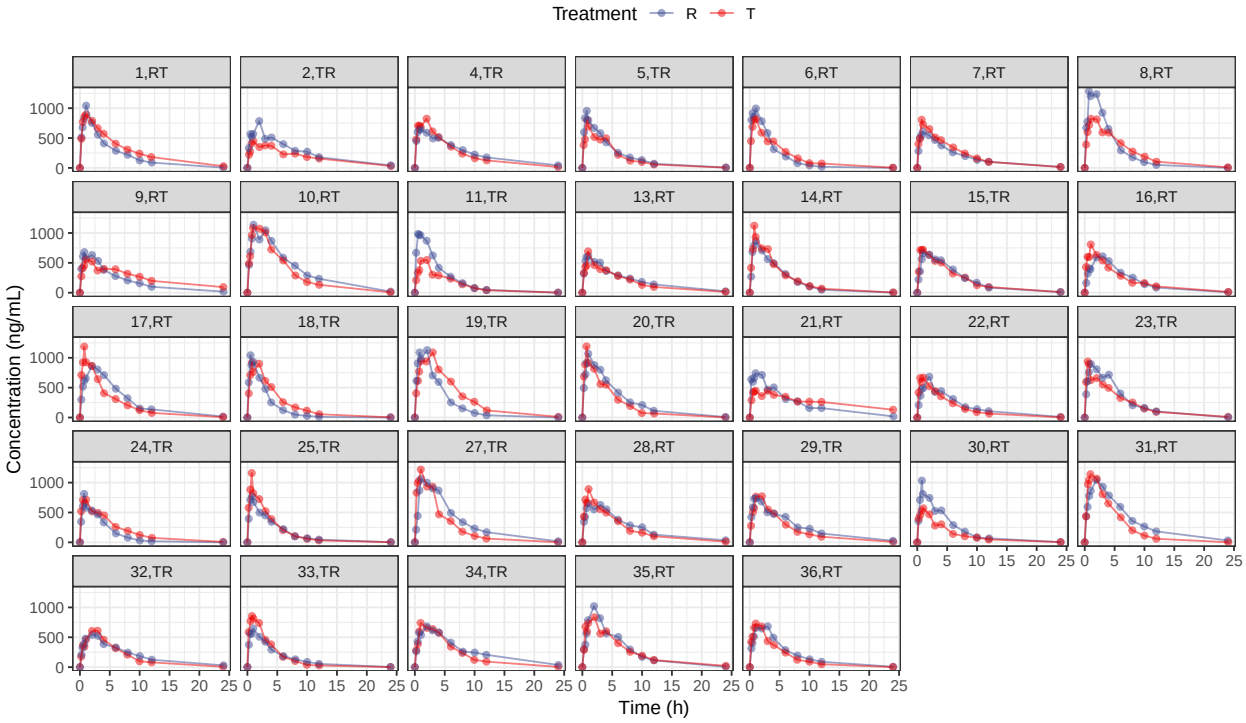

Figure 1: Concentration-time curves

A Raw data

The concentration-time curves are ploted in Figure 1 and the result of noncomparmental analysis is presented in Table 3.

Table 3: The raw data used for analysis in R and SAS

SUBJ	GRP	PRD	TRT	AUClast	Cmax	Tmax
1	RT	1	R	5018.927	1043.13	1.04
1	RT	2	T	6737.507	894.21	1.03
2	TR	1	T	4373.970	447.26	1.01
2	TR	2	R	6164.276	783.92	1.98
4	TR	1	T	5592.993	824.42	1.97
4	TR	2	R	5958.160	646.31	0.97
5	TR	1	T	3902.590	803.70	0.80
5	TR	2	R	4620.156	955.30	0.74
6	RT	1	R	3735.274	995.34	1.02
6	RT	2	T	4257.802	816.33	1.00
7	RT	1	R	4314.993	608.99	0.95
7	RT	2	T	5030.372	806.57	0.74
8	RT	1	R	6053.098	1283.67	0.72
8	RT	2	T	5790.067	822.95	1.03
9	RT	1	R	4602.582	679.39	0.74
9	RT	2	T	6042.462	556.55	0.98
10	RT	1	R	8848.988	1136.91	1.03
10	RT	2	T	7349.822	1082.79	0.97

11	TR	1	T	3054.096	547.73	2.02
11	TR	2	R	4719.175	984.69	0.54
13	RT	1	R	4828.682	615.17	1.00
13	RT	2	T	4175.434	692.26	0.97
14	RT	1	R	4566.275	864.56	1.03
14	RT	2	T	5042.649	1122.75	0.75
15	TR	1	T	4950.980	719.40	0.97
15	TR	2	R	4959.554	660.17	0.96
16	RT	1	R	4577.432	609.64	3.01
16	RT	2	T	4773.723	807.65	1.01
17	RT	1	R	6462.652	861.56	2.02
17	RT	2	T	5246.032	1187.75	0.73
18	TR	1	T	4754.625	919.87	0.77
18	TR	2	R	3214.809	1042.84	0.53
19	TR	1	T	7619.304	1089.84	3.00
19	TR	2	R	5210.569	1127.94	2.04
20	TR	1	T	5063.471	1191.46	0.71
20	TR	2	R	6406.634	1069.19	1.00
21	RT	1	R	5580.289	742.67	0.97
21	RT	2	T	6304.119	447.85	0.99
22	RT	1	R	4398.887	682.73	2.02
22	RT	2	T	3760.359	669.01	1.04
23	TR	1	T	5141.165	937.02	0.51
23	TR	2	R	5835.275	894.72	1.04
24	TR	1	T	4343.439	713.57	1.03
24	TR	2	R	2848.448	811.83	0.71
25	TR	1	T	3983.260	1160.32	0.73
25	TR	2	R	3476.389	769.63	0.78
27	TR	1	T	5772.972	1219.56	0.99
27	TR	2	R	7673.260	1063.29	1.03
28	RT	1	R	5679.039	650.24	1.00
28	RT	2	T	5160.875	891.63	1.05
29	TR	1	T	4800.455	770.63	2.02
29	TR	2	R	5772.925	738.17	1.04
30	RT	1	R	4722.324	1034.11	0.77
30	RT	2	T	2896.939	569.22	1.03
31	RT	1	R	8032.393	1043.82	1.98
31	RT	2	T	6076.359	1141.43	0.96
32	TR	1	T	4245.372	608.93	2.97
32	TR	2	R	4745.770	539.66	2.04
33	TR	1	T	3648.195	856.18	0.76
33	TR	2	R	3356.777	647.95	0.98
34	TR	1	T	5015.499	739.42	0.96
34	TR	2	R	6325.746	682.41	1.99
35	RT	1	R	6259.347	1020.55	1.96
35	RT	2	T	5802.468	835.87	2.04
36	RT	1	R	4669.384	682.87	3.01
36	RT	2	T	3783.584	729.63	1.00

B SAS Scripts and results

To run these scripts, the dataset `BE::NCAResult4BE` should be exported from R by `write.csv()`.

B.1 AUC_{last}

```
DATA BE;
  INFILE 'c:\Users\mdlhs\asancpt\BEreport\sas\NCAResult4BE.csv' FIRSTOBS=2 DLM=",";
  INPUT SUBJ $ SEQ $ PRD $ TRT $ AUClast Cmax Tmax;
  IF CMAX =< 0 THEN DELETE;
  LNCMAX = LOG(Cmax);
  LNAUCL = LOG(AUClast);

PROC PRINT; RUN;

PROC GLM DATA=BE OUTSTAT=STATRES; /* GLM use only complete subjects. */
  CLASS SEQ PRD TRT SUBJ;
  MODEL LNAUCL = SEQ SUBJ(SEQ) PRD TRT;
  RANDOM SUBJ(SEQ)/TEST;
  LSMEANS TRT /PDIFF=CONTROL('R') CL ALPHA=0.1 COV OUT=LSOUT;
RUN;

PROC PRINT DATA=STATRES; RUN;
PROC PRINT DATA=LSOUT; RUN;

DATA STATRES;
  SET STATRES;
  IF _TYPE_='ERROR' THEN CALL SYMPUT('DF', DF);

DATA LSOUT;
  SET LSOUT;
  IF TRT='R' THEN CALL SYMPUT('GMR_R', LSMEAN);
  IF TRT='T' THEN CALL SYMPUT('GMR_T', LSMEAN);
  IF TRT='R' THEN CALL SYMPUT('V_R', COV1);
  IF TRT='T' THEN CALL SYMPUT('V_T', COV2);
  IF TRT='T' THEN CALL SYMPUT('COV', COV1);

DATA LSOUT2;
  LNPE = &GMR_T - &GMR_R;
  DF = &DF;
  SE = SQRT(&V_R + &V_T - 2*&COV);
  LNLM = TINV(0.95, DF)*SE;
  LNLL = LNPE - LNLM ;
  LNUL = LNPE + LNLM;
  PE = EXP(LNPE);
  LL = EXP(LNLL);
  UL = EXP(LNUL);
  WD = UL - LL;

PROC PRINT DATA=LSOUT2; RUN;

PROC MIXED DATA=BE; /* MIXED  uses all data. */
  CLASS SEQ TRT SUBJ PRD;
  MODEL LNAUCL = SEQ PRD TRT;
```

```
  RANDOM SUBJ(SEQ);
  ESTIMATE 'T VS R' TRT -1 1 /CL ALPHA=0.1;
  ODS OUTPUT ESTIMATES=ESTIM COVPARMS=COVPAR;
RUN;

DATA COVPAR;
  SET COVPAR;
  IF CovParm = 'Residual' THEN CALL SYMPUT('MSE', Estimate);

DATA ESTIM;
  SET ESTIM;
  MSE = &MSE;
  LNLM = (Upper - Lower)/2;
  PE = EXP(Estimate);
  LL = EXP(Lower);
  UL = EXP(Upper);
  WD = UL - LL;

PROC PRINT Data=ESTIM; RUN;
```

Table 4: Table of analysis of variance for log-transformed AUClast (PROC GLM)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SUBJ(SEQ)	31	2.7730360	0.0894530	3.17	0.0010
PRD	1	0.0000303	0.0000303	0.00	0.9741
TRT	1	0.0364350	0.0364350	1.29	0.2646
Error: MS(Error)	31	0.8749020	0.0282230		

B.2 C_{max}

```
DATA BE;
  INFILE 'c:\Users\mdlhs\asancpt\BEreport\sas\NCAResult4BE.csv' FIRSTOBS=2 DLM=",";
  INPUT SUBJ $ SEQ $ PRD $ TRT $ AUClast Cmax Tmax;
  IF CMAX =< 0 THEN DELETE;
  LNCMAX = LOG(Cmax);
  LNAUCL = LOG(AUClast);

PROC PRINT; RUN;

PROC GLM DATA=BE OUTSTAT=STATRES; /* GLM use only complete subjects. */
  CLASS SEQ PRD TRT SUBJ;
  MODEL LNCMAX = SEQ SUBJ(SEQ) PRD TRT;
  RANDOM SUBJ(SEQ)/TEST;
  LSMEANS TRT /PDIFF=CONTROL('R') CL ALPHA=0.1 COV OUT=LSOUT;
RUN;

PROC PRINT DATA=STATRES; RUN;
PROC PRINT DATA=LSOUT; RUN;

DATA STATRES;
  SET STATRES;
```

```
  IF _TYPE_='ERROR' THEN CALL SYMPUT('DF', DF);

DATA LSOUT;
  SET LSOUT;
  IF TRT='R' THEN CALL SYMPUT('GMR_R', LSMEAN);
  IF TRT='T' THEN CALL SYMPUT('GMR_T', LSMEAN);
  IF TRT='R' THEN CALL SYMPUT('V_R', COV1);
  IF TRT='T' THEN CALL SYMPUT('V_T', COV2);
  IF TRT='T' THEN CALL SYMPUT('COV', COV1);

DATA LSOUT2;
  LNPE = &GMR_T - &GMR_R;
  DF = &DF;
  SE = SQRT(&V_R + &V_T - 2*&COV);
  LNLM = TINV(0.95, DF)*SE;
  LNLL = LNPE - LNLM ;
  LNUL = LNPE + LNLM;
  PE = EXP(LNPE);
  LL = EXP(LNLL);
  UL = EXP(LNUL);
  WD = UL - LL;

PROC PRINT DATA=LSOUT2; RUN;

PROC MIXED DATA=BE; /* MIXED  uses all data. */
  CLASS SEQ TRT SUBJ PRD;
  MODEL LNCMAX = SEQ PRD TRT;
  RANDOM SUBJ(SEQ);
  ESTIMATE 'T VS R' TRT -1 1 /CL ALPHA=0.1;
  ODS OUTPUT ESTIMATES=ESTIM COVPARMS=COVPAR;
RUN;

DATA COVPAR;
  SET COVPAR;
  IF CovParm = 'Residual' THEN CALL SYMPUT('MSE', Estimate);

DATA ESTIM;
  SET ESTIM;
  MSE = &MSE;
  LNLM = (Upper - Lower)/2;
  PE = EXP(Estimate);
  LL = EXP(Lower);
  UL = EXP(Upper);
  WD = UL - LL;

PROC PRINT Data=ESTIM; RUN;
```

Table 5: Table of analysis of variance for log-transformed Cmax (PROC GLM)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SUBJ(SEQ)	31	2.861394	0.092303	2.31	0.0113
PRD	1	0.004717	0.004717	0.12	0.7335

TRT	1	0.006838	0.006838	0.17	0.6820
Error: MS(Error)	31	1.238856	0.039963		

C Session Information

```
devtools::session_info()
```

```
## - Session info ---------------------------------------------------------
##  setting  value
##  version  R version 3.5.1 (2018-07-02)
##  os       Windows 7 x64 SP 1
##  system   x86_64, mingw32
##  ui       RTerm
##  language (EN)
##  collate  Korean_Korea.949
##  ctype    Korean_Korea.949
##  tz       Asia/Seoul
##  date     2018-10-24
##
## - Packages -------------------------------------------------------------
##  package     * version date       lib source
##  assertthat    0.2.0   2017-04-11 [1] CRAN (R 3.5.0)
##  backports     1.1.2   2017-12-13 [1] CRAN (R 3.5.0)
##  base64enc     0.1-3   2015-07-28 [1] CRAN (R 3.5.0)
##  BE          * 0.1.1   2018-07-19 [1] CRAN (R 3.5.1)
##  bindr         0.1.1   2018-03-13 [1] CRAN (R 3.5.0)
##  bindrcpp      0.2.2   2018-03-29 [1] CRAN (R 3.5.0)
##  bookdown      0.7     2018-02-18 [1] CRAN (R 3.5.0)
##  callr         3.0.0   2018-08-24 [1] CRAN (R 3.5.1)
##  cli           1.0.1   2018-09-25 [1] CRAN (R 3.5.1)
##  crayon        1.3.4   2018-06-08 [1] Github (gaborcsardi/crayon@3e751fb)
##  debugme       1.1.0   2017-10-22 [1] CRAN (R 3.5.0)
##  desc          1.2.0   2018-05-01 [1] CRAN (R 3.5.0)
##  devtools      2.0.0   2018-10-19 [1] CRAN (R 3.5.1)
##  digest        0.6.18  2018-10-10 [1] CRAN (R 3.5.1)
##  dplyr       * 0.7.7   2018-10-16 [1] CRAN (R 3.5.1)
##  evaluate      0.12    2018-10-09 [1] CRAN (R 3.5.1)
##  fs            1.2.6   2018-08-23 [1] CRAN (R 3.5.1)
##  glue          1.3.0   2018-07-17 [1] CRAN (R 3.5.1)
##  highr         0.7     2018-06-09 [1] CRAN (R 3.5.0)
##  hms           0.4.2   2018-03-10 [1] CRAN (R 3.5.0)
##  htmltools     0.3.6   2017-04-28 [1] CRAN (R 3.5.0)
##  knitr       * 1.20    2018-02-20 [1] CRAN (R 3.5.0)
##  magrittr      1.5     2014-11-22 [1] CRAN (R 3.5.0)
##  memoise       1.1.0   2017-04-21 [1] CRAN (R 3.5.0)
##  pillar        1.3.0   2018-07-14 [1] CRAN (R 3.5.1)
##  pkgbuild      1.0.2   2018-10-16 [1] CRAN (R 3.5.1)
##  pkgconfig     2.0.2   2018-08-16 [1] CRAN (R 3.5.1)
##  pkgload       1.0.1   2018-10-11 [1] CRAN (R 3.5.1)
##  prettyunits   1.0.2   2015-07-13 [1] CRAN (R 3.5.0)
##  processx      3.2.0   2018-08-16 [1] CRAN (R 3.5.1)
##  ps            1.2.0   2018-10-16 [1] CRAN (R 3.5.1)
##  purrr         0.2.5   2018-05-29 [1] CRAN (R 3.5.0)
```

```
##  R.methodsS3   1.7.1   2016-02-16 [1] CRAN (R 3.5.0)
##  R.oo          1.22.0  2018-04-22 [1] CRAN (R 3.5.0)
##  R6            2.3.0   2018-10-04 [1] CRAN (R 3.5.1)
##  Rcpp          0.12.19 2018-10-01 [1] CRAN (R 3.5.1)
##  readr       * 1.1.1   2017-05-16 [1] CRAN (R 3.5.0)
##  remotes       2.0.1   2018-10-19 [1] CRAN (R 3.5.1)
##  rlang         0.3.0   2018-10-22 [1] CRAN (R 3.5.1)
##  rmarkdown     1.10    2018-06-11 [1] CRAN (R 3.5.0)
##  rprojroot     1.3-2   2018-01-03 [1] CRAN (R 3.5.0)
##  rtf         * 0.4-13  2018-05-17 [1] CRAN (R 3.5.1)
##  sessioninfo   1.1.0   2018-09-25 [1] CRAN (R 3.5.1)
##  stringi       1.2.4   2018-07-20 [1] CRAN (R 3.5.1)
##  stringr       1.3.1   2018-05-10 [1] CRAN (R 3.5.0)
##  testthat      2.0.1   2018-10-13 [1] CRAN (R 3.5.1)
##  tibble        1.4.2   2018-01-22 [1] CRAN (R 3.5.0)
##  tidyselect    0.2.5   2018-10-11 [1] CRAN (R 3.5.1)
##  usethis       1.4.0   2018-08-14 [1] CRAN (R 3.5.1)
##  withr         2.1.2   2018-03-15 [1] CRAN (R 3.5.0)
##  xfun          0.4     2018-10-23 [1] CRAN (R 3.5.1)
##  yaml          2.2.0   2018-07-25 [1] CRAN (R 3.5.1)
##
## [1] C:/Users/mdlhs/Rlib
## [2] C:/Program Files/R/R-3.5.1/library
```

References

Bae, Kyun-Seop. 2018. *BE: Bioequivalence Study Data Analysis*. https://CRAN.R-project.org/package=BE.

Center for Drug Evaluation and Research (CDER), Food and Drug Administration, U.S. Department of Health and Human Services. 2001. *Guidance for Industry Statistical Approaches to Establishing Bioequivalence*. https://www.fda.gov/downloads/drugs/guidances/ucm070244.pdf.

Chow, Shein-Chung, and Jen-pei Liu. 2008. *Design and Analysis of Bioavailability and Bioequivalence Studies (Chapman & Hall/Crc Biostatistics Series)*. Chapman; Hall/CRC.

Hauschke, Dieter, Volker Steinijans, and Iris Pigeot. 2007. *Bioequivalence Studies in Drug Development: Methods and Applications*. Wiley.