

RI Correction Extra

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March 10, 2021

Abstract

This vignette explains how to transform retention time (RT) to retention index (RI) when the RI markers are not spiked in the samples. The same principle can be applied to other uncommon scenarios.

Package

TargetSearch 1.46.3

Report issues on <https://github.com/acinostroza/TargetSearch/issues>

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1 Motivation

The *TargetSearch* package assumes that the retention index markers (RIM), such as n-alkanes or FAMEs (fatty acid methyl esters), are injected together with the biological samples. A different approach often used is to inject the RIMs separately, alternating between RIMs and biological samples. For example, a GC-MS run may look like the list shown in Table 1.

Measurement Order	Sample Type
1	Alkanes
2	Biological
3	Biological
4	Biological
5	Biological
6	Alkanes
7	Biological
8	Biological
9	Biological
10	Biological
11	Alkanes
12	Biological
13	Biological
14	Biological
15	Biological

Table 1: An example of a GC-MS run
Alkanes samples are retention index markers (RIMs).

In the example (Table 1), samples 1, 6, 11 are RIMs and the rest are biological samples. The assumption is that the retention time shifts between consecutive runs are not significant, so sample #1 is used to correct samples #2-5, sample #6 corrects samples #7-10, and so on. This document shows how to perform RI correction in such case with *TargetSearch*, using the available chromatograms of the *TargetSearchData* package.

2 Retention Index correction

First, we load the required packages

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```
library(TargetSearch)
## Loading required package: ncdf4
library(TargetSearchData)
```

Specify the directory where the CDF files are. In this example we will use the CDF files of [TargetSearchData](#) package.

```
cdfPath <- file.path(find.package("TargetSearchData"), "gc-ms-data")
dir(cdfPath, pattern="cdf$")
## [1] "7235eg04.cdf" "7235eg06.cdf" "7235eg07.cdf" "7235eg08.cdf"
## [5] "7235eg09.cdf" "7235eg11.cdf" "7235eg12.cdf" "7235eg15.cdf"
## [9] "7235eg20.cdf" "7235eg21.cdf" "7235eg22.cdf" "7235eg25.cdf"
## [13] "7235eg26.cdf" "7235eg30.cdf" "7235eg32.cdf"
```

Create a `tsSample` object using all chromatograms from the previous directory. Also set the RI path to the current directory. Use the R commands `setwd()` and `getwd()` to set/get the working directory.

```
samples.all <- ImportSamplesFromDir(cdfPath)
RIpath(samples.all) <- "."
```

Import retention index marker times limits. We will use the ones defined in [TargetSearchData](#).

```
rimLimits <- ImportFameSettings(file.path(cdfPath, "rimLimits.txt"))
rimLimits
## An object of class "tsRim"
## Slot "limits":
##           LowerLimit UpperLimit
## RI.Marker 1         230         280
## RI.Marker 2         290         340
## RI.Marker 3         350         400
##
## Slot "standard":
## [1] 262320 323120 381020
##
## Slot "mass":
## [1] 87
```

Run the retention index correction methods. Here we use an intensity threshold of 50, the peak detection method is "ppc", and the time window width is $2 * 15 + 1 = 31$ scan points (equal to 1.5 seconds in this example).

Note: We skip conversion to CDF-4 files for illustration purposes. In a normal workflow you would set `writeCDF4path=TRUE` (the default).

```
RImatrix <- RIMcorrect(samples.all, rimLimits, writeCDF4path=FALSE,
                      Window=15, pp.method="ppc", IntThreshold=50)
RImatrix
##           7235eg04 7235eg06 7235eg07 7235eg08 7235eg09 7235eg11 7235eg12
## RI.Marker 1   252.11  252.009  251.578   252.46   252.11   252.275  251.871
```

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```
## RI.Marker 2 311.16 311.059 310.978 311.36 311.26 311.325 311.171
## RI.Marker 3 369.36 368.409 368.528 369.61 368.81 369.575 368.671
##          7235eg15 7235eg20 7235eg21 7235eg22 7235eg25 7235eg26 7235eg30
## RI.Marker 1 252.025 252.091 252.006 252.391 251.675 251.76 252.341
## RI.Marker 2 311.125 311.141 311.106 311.291 311.025 310.96 311.241
## RI.Marker 3 368.525 368.641 368.556 368.741 368.525 369.41 369.291
##          7235eg32
## RI.Marker 1 252.31
## RI.Marker 2 311.11
## RI.Marker 3 368.96
```

Now you should make sure that the retention times (RT) of the RIMs (not the biological samples!!) are correct by using a chromatogram visualization tool such as LECO Pegasus, AMDIS, etc. Because there are no RIMs injected in the biological samples, the RTs of the RIMs found in `RImatrix` will make no sense, so you don't need to check them.

Up to now we have run the usual [TargetSearch](#) workflow which you can find in the main vignette. To correct the RI of the biological samples, the following procedure can be used.

First, we need a logical vector indicating which samples are RIMs and which biological. For example, you could create a vector like this.

```
isRIMarker <- c(T, F, F, F, F, T, F, F, F, F, T, F, F, F, F)
```

where `isRIMarker` is `TRUE` if the respective sample is a RIM and `FALSE` otherwise. Note that here we have set components 1, 6, 11 to `TRUE` just like the example in Table 1.

Then we have to copy the RIM columns of `RImatrix` to their respective biological sample columns. In other words, copy column 1 to columns 2, 3, 4, 5; column 6 to columns 7, 8, 9, 10; and so on. There are many ways to achieve that, here I show two examples.

```
RImatrix2 <- RImatrix
RImatrix2[, 2:5] <- RImatrix[,1]
RImatrix2[, 7:10] <- RImatrix[,6]
RImatrix2[, 12:15] <- RImatrix[,11]
```

This code snippet is a straight forward method, but has the disadvantage that the column indexes must be filled manually. A more general approach could be.

```
RImatrix2 <- RImatrix
z <- cumsum(as.numeric(isRIMarker))
for(i in unique(z))
  RImatrix2[, z==i] <- RImatrix[, z==i][,1]
RImatrix2

##          7235eg04 7235eg06 7235eg07 7235eg08 7235eg09 7235eg11 7235eg12
## RI.Marker 1 252.11 252.11 252.11 252.11 252.11 252.275 252.275
## RI.Marker 2 311.16 311.16 311.16 311.16 311.16 311.325 311.325
## RI.Marker 3 369.36 369.36 369.36 369.36 369.36 369.575 369.575
##          7235eg15 7235eg20 7235eg21 7235eg22 7235eg25 7235eg26 7235eg30
## RI.Marker 1 252.275 252.275 252.275 252.391 252.391 252.391 252.391
## RI.Marker 2 311.325 311.325 311.325 311.291 311.291 311.291 311.291
## RI.Marker 3 369.575 369.575 369.575 368.741 368.741 368.741 368.741
##          7235eg32
```

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```
## RI.Marker 1 252.391
## RI.Marker 2 311.291
## RI.Marker 3 368.741
```

After the `RImatrix2` object is corrected, the RI files of the biological samples must be fixed as well.

```
fixRI(samples.all, rimLimits, RImatrix2, which(!isRIMarker))
```

Finally, we remove the standards, since we don't need them anymore.

```
samples <- samples.all[!isRIMarker]
RImatrix <- RImatrix2[, !isRIMarker]
```

After that, we can continue with the normal [TargetSearch](#) workflow. If you prefer to use the GUI, run `TargetSearchGUI()` from this point and import the RI files by selecting the option *Apex Data* (do not import the standard files).

A script with all the commands used in this document is located in the 'doc' directory of the [TargetSearch](#) package.

3 Session info

Output of `sessionInfo()` on the system on which this document was compiled.

- R version 4.0.4 (2021-02-15), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Running under: Ubuntu 18.04.5 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.12-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.12-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: TargetSearch 1.46.3, TargetSearchData 1.28.0, ncd4 1.17
- Loaded via a namespace (and not attached): BiocManager 1.30.10, BiocStyle 2.18.1, assertthat 0.2.1, compiler 4.0.4, digest 0.6.27, evaluate 0.14, highr 0.8, htmltools 0.5.1.1, knitr 1.31, magrittr 2.0.1, rlang 0.4.10, rmarkdown 2.7, stringi 1.5.3, stringr 1.4.0, tools 4.0.4, xfun 0.21, yaml 2.2.1