

RI correction when standards are not co-injected with biological samples

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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

```
> library(TargetSearch)
> 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" "7235eg09.cdf"
[6] "7235eg11.cdf" "7235eg12.cdf" "7235eg15.cdf" "7235eg20.cdf" "7235eg21.cdf"
[11] "7235eg22.cdf" "7235eg25.cdf" "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 a mass scan range of 85-320 m/z , 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).

```
> RImatrix <- RIcorrect(samples.all, rimLimits, massRange=c(85,320),
+                       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
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						
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.

```
> fixRIcorrection(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 we 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" (don't import the standard files).

You could find a copy of all the commands used in this document in the `doc` directory of the *TargetSearch* package.