Filtering of chromatographic peaks in an XCMS object

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```
#> Warning in fun(libname, pkgname): mzR has been built against a different Rcpp version (1.0.6)
#> than is installed on your system (1.0.7). This might lead to errors
#> when loading mzR. If you encounter such issues, please send a report,
#> including the output of sessionInfo() to the Bioc support forum at
#> https://support.bioconductor.org/. For details see also
#> https://github.com/sneumann/mzR/wiki/mzR-Rcpp-compiler-linker-issue.
```

Introduction

This tutorial describes the CPC package functionality on an example data set included with the package. We will load the example dataset, perform peak picking using XCMS and apply the CPC algorithm to filter the peaks detected. Furthermore, we will showcase the data extraction methods and visualize the results.

The data originates from 4 replicate injections of protein precipitated plasma on a HILIC platform. The example data is heavily filtered in order to keep package size and processing times to a minimum. The filtering is made in two steps: (1) The full data set was processed using XCMS and CPC in the same way described below. A subset of 100 peaks was randomly selected from those that were retained in the filtering step, based on the calculated signal-to-noise of the peaks (25 from each quartile). Similarily 100 peaks were randomly selected from those removed in the filtering step (33-34 each from those removed due to not being

detected, having too low signal to noise, or having too few data points along the peak range). (2) The data set was then filtered to (i) only include scan indices 1-1200, (ii) only include mass peaks with intensity > 2.225*median intensity (removes a lot of noise peaks), and (iii) include only mass peaks whose m/z value coincide with one of the selected peaks in step (1) within a ppm value of 50 and a retention window of +/- 120 seconds.

Setup environment

```
library(xcms)
library(cpc)
library(ggvenn)
```

XCMS processing

The first step in this tutorial is to use XCMS to process the reduced data set. This step normally takes a long time to run on a full dataset, however, thanks to the significant filtering made, it should run very fast on an average desktop PC. For more information on how to use XCMS for processing LC/MS data, see XCMS documentation. Only the peak picking will be performed as the retention alignment will not work properly with this heavily filtered dataset, and is not important to illustrate the workflow.

```
# get example raw data file paths from package
fp_raw <- list.files(system.file("extdata", package = "cpc"), full.names = T)

# setup multi-core processing for XCMS
register(bpstart(SnowParam()))

# setup metadata
(pd <- data.frame(sample_name = paste0("HILIC_", seq(1,4,1)),
                  sample_group = rep("HILIC_POS", 4),
                  stringsAsFactors = F))
#>   sample_name sample_group
#> 1     HILIC_1    HILIC_POS
#> 2     HILIC_2    HILIC_POS
#> 3     HILIC_3    HILIC_POS
#> 4     HILIC_4    HILIC_POS

# Create raw data object
(msraw <- MSnbase::readMSData(files = fp_raw,
                              pdata = new("NAnnotatedDataFrame", pd),
                              mode = "onDisk", msLevel. = 1, centroided. = T))
#> MSn experiment data ("OnDiskMSnExp")
#> Object size in memory: 1.54 Mb
#> - - - Spectra data - - -
#>  MS level(s): 1
#>  Number of spectra: 5200
#>  MSn retention times: 0:02 - 11:18 minutes
#> - - - Processing information - - -
#> Data loaded [Wed Jan 12 14:52:05 2022]
#> Filter: select MS level(s) 1. [Wed Jan 12 14:52:05 2022]
```

```
#>  MSnbase version: 2.18.0
#> - - - Meta data  - - -
#> phenoData
#>   rowNames: 1 2 3 4
#>   varLabels: sample_name sample_group
#>   varMetadata: labelDescription
#> Loaded from:
#>   [1] hilic015.mzML...  [4] hilic021.mzML
#>   Use 'fileNames(.)' to see all files.
#> protocolData: none
#> featureData
#>   featureNames: F1.S0001 F1.S0002 ... F4.S1300 (5200 total)
#>   fvarLabels: fileIdx spIdx ... spectrum (35 total)
#>   fvarMetadata: labelDescription
#> experimentData: use 'experimentData(object)'

# --- PEAK DETECTION ---

# run peak detection
xd <- xcms::findChromPeaks(msraw,
                           param = xcms::CentWaveParam(ppm = 60,
                                                       peakwidth = c(5, 50),
                                                       fitgauss = T,
                                                       noise = 200,
                                                       integrate = 2,
                                                       prefilter = c(5, 1000),
                                                       verboseColumns = T,
                                                       mzdiff = 0.01),
                           msLevel = 1L)
#>
#> Attaching package: 'BiocGenerics'
#>
#> The following objects are masked from 'package:parallel':
#>
#>     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
#>     clusterExport, clusterMap, parApply, parCapply, parLapply,
#>     parLapplyLB, parRapply, parSapply, parSapplyLB
#>
#> The following objects are masked from 'package:stats':
#>
#>     IQR, mad, sd, var, xtabs
#>
#> The following objects are masked from 'package:base':
#>
#>     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
#>     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
#>     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
#>     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
#>     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
#>     union, unique, unsplit, which.max, which.min
#>
#> Welcome to Bioconductor
#>
```

```
#>     Vignettes contain introductory material; view with
#>     'browseVignettes()'. To cite Bioconductor, see
#>     'citation("Biobase")', and for packages 'citation("pkgname")'.
#>
#>
#> Attaching package: 'S4Vectors'
#>
#> The following objects are masked from 'package:base':
#>
#>     expand.grid, I, unname
#>
#>
#> Attaching package: 'ProtGenerics'
#>
#> The following object is masked from 'package:stats':
#>
#>     smooth
#>
#>
#> This is MSnbase version 2.18.0
#>   Visit https://lgatto.github.io/MSnbase/ to get started.
#>
#>
#> Attaching package: 'MSnbase'
#>
#> The following object is masked from 'package:base':
#>
#>     trimws
#>
#>
#> This is xcms version 3.14.1
#>
#>
#> Attaching package: 'xcms'
#>
#> The following object is masked from 'package:stats':
#>
#>     sigma
#>
#> Detecting mass traces at 60 ppm ... OK
#> Detecting chromatographic peaks in 1837 regions of interest ... OK: 268 found.
#>
#> Attaching package: 'BiocGenerics'
#>
#> The following objects are masked from 'package:parallel':
#>
#>     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
#>     clusterExport, clusterMap, parApply, parCapply, parLapply,
#>     parLapplyLB, parRapply, parSapply, parSapplyLB
#>
#> The following objects are masked from 'package:stats':
#>
#>     IQR, mad, sd, var, xtabs
```

```
#>
#> The following objects are masked from 'package:base':
#>
#>     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
#>     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
#>     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
#>     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
#>     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
#>     union, unique, unsplit, which.max, which.min
#>
#> Welcome to Bioconductor
#>
#>     Vignettes contain introductory material; view with
#>     'browseVignettes()'. To cite Bioconductor, see
#>     'citation("Biobase")', and for packages 'citation("pkgname")'.
#>
#>
#> Attaching package: 'S4Vectors'
#>
#> The following objects are masked from 'package:base':
#>
#>     expand.grid, I, unname
#>
#>
#> Attaching package: 'ProtGenerics'
#>
#> The following object is masked from 'package:stats':
#>
#>     smooth
#>
#>
#> This is MSnbase version 2.18.0
#>   Visit https://lgatto.github.io/MSnbase/ to get started.
#>
#>
#> Attaching package: 'MSnbase'
#>
#> The following object is masked from 'package:base':
#>
#>     trimws
#>
#>
#> This is xcms version 3.14.1
#>
#>
#> Attaching package: 'xcms'
#>
#> The following object is masked from 'package:stats':
#>
#>     sigma
#>
#> Detecting mass traces at 60 ppm ... OK
#> Detecting chromatographic peaks in 1782 regions of interest ... OK: 264 found.
```

```
#>
#> Attaching package: 'BiocGenerics'
#>
#> The following objects are masked from 'package:parallel':
#>
#>     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
#>     clusterExport, clusterMap, parApply, parCapply, parLapply,
#>     parLapplyLB, parRapply, parSapply, parSapplyLB
#>
#> The following objects are masked from 'package:stats':
#>
#>     IQR, mad, sd, var, xtabs
#>
#> The following objects are masked from 'package:base':
#>
#>     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
#>     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
#>     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
#>     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
#>     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
#>     union, unique, unsplit, which.max, which.min
#>
#> Welcome to Bioconductor
#>
#>     Vignettes contain introductory material; view with
#>     'browseVignettes()'. To cite Bioconductor, see
#>     'citation("Biobase")', and for packages 'citation("pkgname")'.
#>
#>
#> Attaching package: 'S4Vectors'
#>
#> The following objects are masked from 'package:base':
#>
#>     expand.grid, I, unname
#>
#>
#> Attaching package: 'ProtGenerics'
#>
#> The following object is masked from 'package:stats':
#>
#>     smooth
#>
#>
#> This is MSnbase version 2.18.0
#>   Visit https://lgatto.github.io/MSnbase/ to get started.
#>
#>
#> Attaching package: 'MSnbase'
#>
#> The following object is masked from 'package:base':
#>
#>     trimws
#>
```

```
#>
#> This is xcms version 3.14.1
#>
#>
#> Attaching package: 'xcms'
#>
#> The following object is masked from 'package:stats':
#>
#>     sigma
#>
#> Detecting mass traces at 60 ppm ... OK
#> Detecting chromatographic peaks in 1774 regions of interest ... OK: 296 found.
#>
#> Attaching package: 'BiocGenerics'
#>
#> The following objects are masked from 'package:parallel':
#>
#>     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
#>     clusterExport, clusterMap, parApply, parCapply, parLapply,
#>     parLapplyLB, parRapply, parSapply, parSapplyLB
#>
#> The following objects are masked from 'package:stats':
#>
#>     IQR, mad, sd, var, xtabs
#>
#> The following objects are masked from 'package:base':
#>
#>     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
#>     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
#>     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
#>     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
#>     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
#>     union, unique, unsplit, which.max, which.min
#>
#> Welcome to Bioconductor
#>
#>     Vignettes contain introductory material; view with
#>     'browseVignettes()'. To cite Bioconductor, see
#>     'citation("Biobase")', and for packages 'citation("pkgname")'.
#>
#>
#> Attaching package: 'S4Vectors'
#>
#> The following objects are masked from 'package:base':
#>
#>     expand.grid, I, unname
#>
#>
#> Attaching package: 'ProtGenerics'
#>
#> The following object is masked from 'package:stats':
#>
#>     smooth
```

```
#>
#>
#> This is MSnbase version 2.18.0
#>   Visit https://lgatto.github.io/MSnbase/ to get started.
#>
#>
#> Attaching package: 'MSnbase'
#>
#> The following object is masked from 'package:base':
#>
#>     trimws
#>
#>
#> This is xcms version 3.14.1
#>
#>
#> Attaching package: 'xcms'
#>
#> The following object is masked from 'package:stats':
#>
#>     sigma
#>
#> Detecting mass traces at 60 ppm ... OK
#> Detecting chromatographic peaks in 1768 regions of interest ... OK: 282 found.

print(xd)
#> MSn experiment data ("XCMSnExp")
#> Object size in memory: 1.54 Mb
#> - - - Spectra data - - -
#>  MS level(s): 1
#>  Number of spectra: 5200
#>  MSn retention times: 0:02 - 11:18 minutes
#> - - - Processing information - - -
#> Data loaded [Wed Jan 12 14:52:05 2022]
#> Filter: select MS level(s) 1. [Wed Jan 12 14:52:05 2022]
#>  MSnbase version: 2.18.0
#> - - - Meta data  - - -
#> phenoData
#>   rowNames: 1 2 3 4
#>   varLabels: sample_name sample_group
#>   varMetadata: labelDescription
#> Loaded from:
#>   [1] hilic015.mzML...  [4] hilic021.mzML
#>   Use 'fileNames(.)' to see all files.
#> protocolData: none
#> featureData
#>   featureNames: F1.S0001 F1.S0002 ... F4.S1300 (5200 total)
#>   fvarLabels: fileIdx spIdx ... spectrum (35 total)
#>   fvarMetadata: labelDescription
#> experimentData: use 'experimentData(object)'
#> - - - xcms preprocessing - - -
#> Chromatographic peak detection:
#>  method: centWave
```

```
#>  1110 peaks identified in 4 samples.
#>  On average 278 chromatographic peaks per sample.
```

CPC peak filtering

The way in which the CPC package is applied to the workflow is via two wrapper functions, *characterize_peaklist()* and *filter_xcms_peaklist()*. The first one will only characterize the peaks detected by XCMS and returns a *cpc* object, whereas the second will call *characterize_peaklist()* as well as run the filtering method and can return either a *cpc* object containing all the information, including the filtered XCMSnExp object, or only the filtered XCMSnExp object. For incorporating the package into an XCMS workflow, it is recommended to use the function *filter_xcms_peaklist()* with *return_type = "xcms"* or use the *getFilteredXCMS()* method on the *cpc* object returned by *filter_xcms_peaklist()* as both cases will give the same result. The latter case will be used in this tutorial as we want to keep the information contained in the *cpc* object.

The *cpcProcParam* object holds processing parameters for both peak characterization as well as peak filtering.

```
cpc <- cpc::filter_xcms_peaklist(xd = xd, return_type = "cpc",
                                 param = cpc::cpcProcParam(min_sn = 10,
                                                           min_pts = 10,
                                                           min_intensity = 2000))
#> Processing file: C:\R\library\cpc\extdata\hilic015.mzML
#> Found 268 peak(s).
#> % complete:
#> 0
#> 10
#> 20
#> 30
#> 40
#> 50
#> 60
#> 70
#> 80
#> 90
#> 100
#> Done!
#> Finished in 5.353 secs.
#> Processing file: C:\R\library\cpc\extdata\hilic017.mzML
#> Found 296 peak(s).
#> % complete:
#> 0
#> 10
#> 20
#> 30
#> 40
#> 50
#> 60
#> 70
#> 80
#> 90
#> 100
#> Done!
#> Finished in 4.843 secs.
```

```
#> Processing file: C:\R\library\cpc\extdata\hilic019.mzML
#> Found 264 peak(s).
#> % complete:
#> 0
#> 10
#> 20
#> 30
#> 40
#> 50
#> 60
#> 70
#> 80
#> 90
#> 100
#> Done!
#> Finished in 6.12 secs.
#> Processing file: C:\R\library\cpc\extdata\hilic021.mzML
#> Found 282 peak(s).
#> % complete:
#> 0
#> 10
#> 20
#> 30
#> 40
#> 50
#> 60
#> 70
#> 80
#> 90
#> 100
#> Done!
#> Finished in 4.271 secs.
#> Keeping 923 of 1110 (83.15%) peaks.
```

Processing times will depend heavily on the computer hardware used. With this data set the processing times are very fast (<10 seconds per file), but that is due to the heavy filtering that has been done. More realistic processing times, when working with full LC/MS data sets, range from a 2-10 minutes per file, scaling linearly with the number of peaks detected by XCMS.

After processing we can get the original peak table from the XCMS object and the characterized peak table from the CPC object using the methods *cpt* and *getPeaklist*.

```
cpcPeaktable <- cpc::cpt(cpc)
```

The peak table that you get from the CPC package has a number of determined peak characteristics as well as calculated peak characteristics.

Column	Description
id	The row number of the peak in the original peak table from XCMS.
rt	Peak retention time in seconds.
rtmin	Peak front bound in seconds.

Column	Description
rtmax	Peak tail bound in seconds.
rtf1b	Peak front bound at 1% peak height.
rtt1b	Peak tail bound at 1% peak height.
rtf5b	Peak front bound at 5% peak height.
rtt5b	Peak tail bound at 5% peak height.
rtf10b	Peak front bound at 10% peak height.
rtt10b	Peak tail bound at 10% peak height.
rtf50b	Peak front bound at 50% peak height.
rtt50b	Peak tail bound at 50% peak height.
apex	The most intense scan point in the peak.
finf	The interpolated front inflection point of the peak where the second derivative crosses zero.
tinf	The interpolated tail inflection point of the peak where the second derivative crosses zero.
fblb	The determined front baseline boundary scan.
tblb	The determined tail baseline boundary scan.
fpkb	The determined front peak bound.
tpkb	The determined tail peak bound.
fcode	The front peak bound type (B = baseline, V = valley, S = shoulder, R = rounded peak).
tcode	The tail peak bound type (B = baseline, V = valley, S = shoulder, R = rounded peak).
blslp	The slope of the baseline calculated between fblb and tblb.
emu	The mu value of the fitted exponentially modified gaussian (EMG) function. This is only calculated if EMG deconvolution is turned on.
esigma	The sigma value of the fitted EMG function. This is only calculated if EMG deconvolution is turned on.
elambda	The lambda value of the fitted EMG function. This is only calculated if EMG deconvolution is turned on.
earea	The area modifier of the fitted EMG function. This is only calculated if EMG deconvolution is turned on.
econv	An indicator if the Nelder-Mead minimizer converged when performing EMG deconvolution.
note	A character statement describing the result of the peak processing. This can be “detected”, “not_detected”, “too_narrow”, “low_sn”, “too_small”.
height	The peak height calculated between the apex scan point and the interpolated baseline value at that point.
fh1b	Front bound at 1% peak height.
th1b	Tail bound at 1% peak height.
fh5b	Front bound at 5% peak height.
th5b	Tail bound at 5% peak height.
fh50b	Front bound at 50% peak height.
th50b	Tail bound at 50% peak height.
wb	Base width of the peak calculated as th5b-fh5b.
fwhm	Full width at half maxima of the peak calculated as th50b-fh50b.
area	The area of the peak calculated using a trapezoid integrator between fh1b and th1b.
a	*apex* - *fpkb*. Used to calculate the tailing factor.
b	*tpkb* - *apex*. Used to calculate the tailing factor.

Column	Description
tf	The tailing factor calculated as $tf = b/a$.
sn	The calculated signal-to-noise ratio calculated as $sn =$ 2**height*/noise.
file	Which sample file the peak was detected in.
exectime	The execution time for the peak in seconds.

Visualizing the results

First lets determine which peaks are kept and which are filtered. In the *cpc* object, there are two *XCMSnExp* objects, *xd* and *xdFilt*. *xdFilt*, as the name implies, is the filtered *XCMSnExp* object. It can be extracted using the *filteredObject()* method.

```
# get the original xcms object using getOriginalXCMS()
xdOrig <- cpc::getOriginalXCMS(cpc)

xdOrig
#> MSn experiment data ("XCMSnExp")
#> Object size in memory: 1.54 Mb
#> - - - Spectra data - - -
#>  MS level(s): 1
#>  Number of spectra: 5200
#>  MSn retention times: 0:02 - 11:18 minutes
#> - - - Processing information - - -
#> Data loaded [Wed Jan 12 14:52:05 2022]
#> Filter: select MS level(s) 1. [Wed Jan 12 14:52:05 2022]
#>  MSnbase version: 2.18.0
#> - - - Meta data  - - -
#> phenoData
#>   rowNames: 1 2 3 4
#>   varLabels: sample_name sample_group
#>   varMetadata: labelDescription
#> Loaded from:
#>   [1] hilic015.mzML...  [4] hilic021.mzML
#>   Use 'fileNames(.)' to see all files.
#> protocolData: none
#> featureData
#>   featureNames: F1.S0001 F1.S0002 ... F4.S1300 (5200 total)
#>   fvarLabels: fileIdx spIdx ... spectrum (35 total)
#>   fvarMetadata: labelDescription
#> experimentData: use 'experimentData(object)'
#> - - - xcms preprocessing - - -
#> Chromatographic peak detection:
#>  method: centWave
#>  1110 peaks identified in 4 samples.
#>  On average 278 chromatographic peaks per sample.

# get the original peak table
xcmsPeaktableOrig <- data.frame(xcms::chromPeaks(xdOrig))

# get the filtered xcms object using getFilteredXCMS()
```

```
xdFilt <- cpc::getFilteredXCMS(cpc)

xdFilt
#> MSn experiment data ("XCMSnExp")
#> Object size in memory: 1.54 Mb
#> - - - Spectra data - - -
#>  MS level(s): 1
#>  Number of spectra: 5200
#>  MSn retention times: 0:02 - 11:18 minutes
#> - - - Processing information - - -
#> Data loaded [Wed Jan 12 14:52:05 2022]
#> Filter: select MS level(s) 1. [Wed Jan 12 14:52:05 2022]
#>  MSnbase version: 2.18.0
#> - - - Meta data  - - -
#> phenoData
#>   rowNames: 1 2 3 4
#>   varLabels: sample_name sample_group
#>   varMetadata: labelDescription
#> Loaded from:
#>   [1] hilic015.mzML...  [4] hilic021.mzML
#>   Use 'fileNames(.)' to see all files.
#> protocolData: none
#> featureData
#>   featureNames: F1.S0001 F1.S0002 ... F4.S1300 (5200 total)
#>   fvarLabels: fileIdx spIdx ... spectrum (35 total)
#>   fvarMetadata: labelDescription
#> experimentData: use 'experimentData(object)'
#> - - - xcms preprocessing - - -
#> Chromatographic peak detection:
#>  method: centWave
#>  923 peaks identified in 4 samples.
#>  On average 231 chromatographic peaks per sample.

# get the filtered peak table
xcmsPeaktableFilt <- data.frame(xcms::chromPeaks(xdFilt))

# get the filtered cpc peak table
cpcPeaktableFilt <- cpcPeaktable[row.names(xcmsPeaktableFilt), ]
```

In the original XCMS object there were 1110 peaks identified across 4 files. After filtering using our processing, 913 peaks remain.

To see how many peaks were removed in a file-by-file basis you can use the table function on the chromatographic peak table from the xcms object.

```
rbind(Original = table(xcmsPeaktableOrig[, "sample"]),
      Filtered = table(xcmsPeaktableFilt[, "sample"]),
      PercentKept = round(100*table(xcms::chromPeaks(xdFilt)[, "sample"]) /
        table(xcms::chromPeaks(xdOrig)[, "sample"]), 1))
#>                 1     2     3     4
#> Original    268.0 296.0 264.0 282.0
#> Filtered    226.0 238.0 221.0 238.0
#> PercentKept  84.3  80.4  83.7  84.4
```

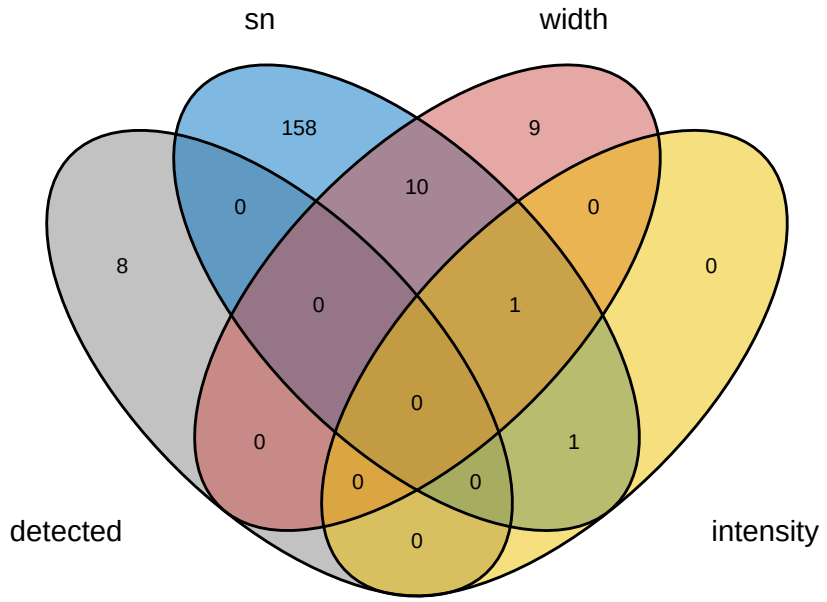
The filtering outcomes can be extracted from the object using the *getFilterOutcomes()* method. This returns a data.frame indicating if a peak passed the filter criteria or not for the different filter characteristics.

```
outcomes <- cpc::getFilterOutcomes(cpc)

head(outcomes)
#>        detected    sn width fwhm   tf intensity
#> CP0001     TRUE FALSE FALSE TRUE TRUE      TRUE
#> CP0002     TRUE  TRUE  TRUE TRUE TRUE      TRUE
#> CP0003     TRUE  TRUE  TRUE TRUE TRUE      TRUE
#> CP0004     TRUE  TRUE  TRUE TRUE TRUE      TRUE
#> CP0005     TRUE  TRUE  TRUE TRUE TRUE      TRUE
#> CP0006     TRUE  TRUE  TRUE TRUE TRUE      TRUE
```

In the example presented here we have the peak width, peak area, and signal-to-noise ratio filters active. For that reason the other filters just show TRUE for all peaks as they were not filtered based on this.

To check which peaks are retained and which are removed, the outcomes data.frame can be used. A character vector of removed and retained peak IDs can also be obtained using the *getRemovedPeaks()* and *getRetainedPeaks()* methods, respectively. We can visualize the filtering outcomes using a Venn diagram.

```
# setup a list for the Venn diagram
outcomesList <- lapply(outcomes, function(x) which(!x))

# output a Venn diagram for the used filters
ggvenn::ggvenn(data = outcomesList[c(1, 2, 3, 6)], show_percentage = FALSE,
               stroke_size = 0.5, set_name_size = 4, text_size = 2.8,
               fill_color = c("#868686FF", "#0073C2FF", "#CD534CFF",
                              "#EFC000FF", "#073b4cFF"))
#> Warning in sprintf("%d", n, 100 * n/sum(n)): one argument not used by format
#> '%d'
```



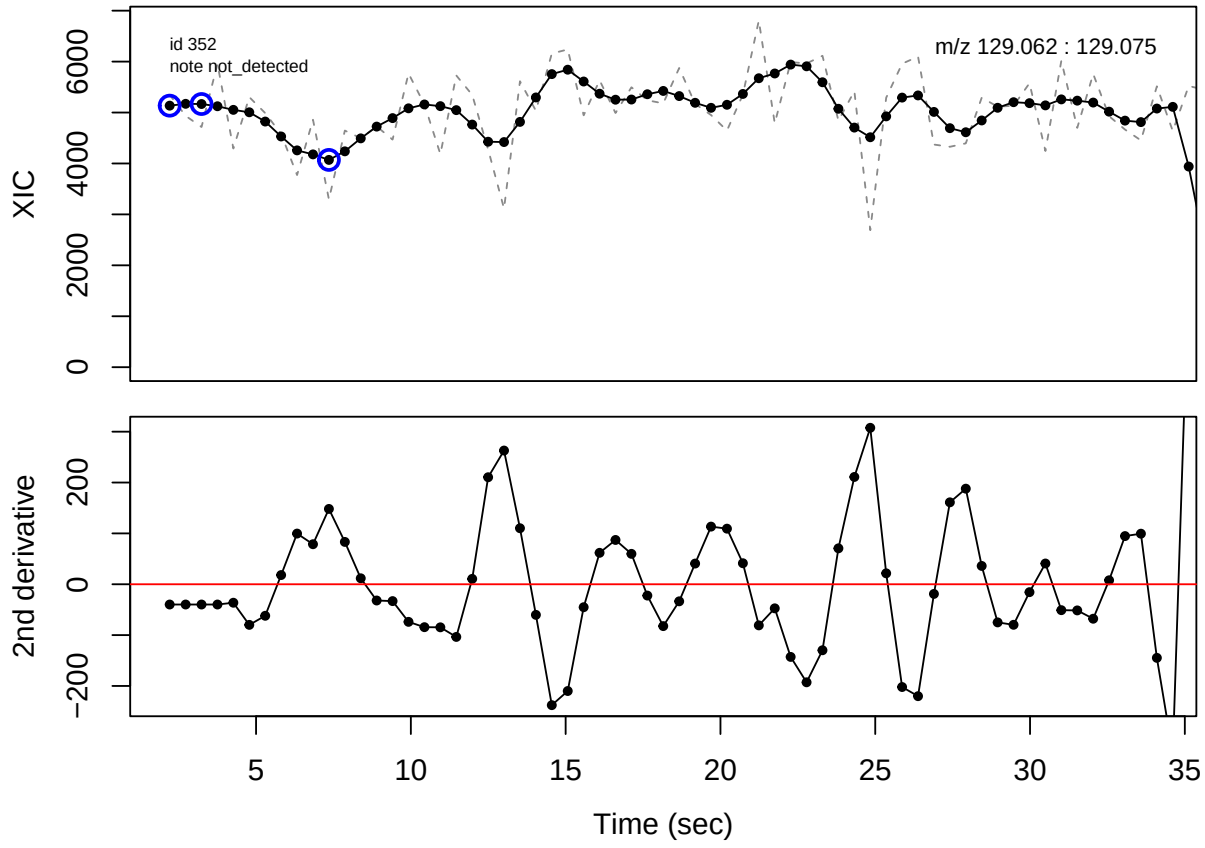
We can see that 8 peaks were not detected at all, ie. did not exhibit the characteristic pattern in the second derivative. Apart from those, the majority of peaks were removed due to their signal-to-noise being too low (SN<10).

```
# set a seed for sample() to make it reproducible
seed <- 12345

# as an example we will select 2 peaks from each filter outcome
# (1) Not detected as a peak,
# (2) Too low signal-to-noise,
# (3) Too low intensity (only 1 peak here)
# (4) Too narrow
set.seed(seed); randomRemoved <- list(
  nd = sample(which(apply(outcomes, 1, function(x) { # (1)
    return(!x[1] &
             x[2] &
             x[3] &
             x[4] &
             x[5] &
             x[6])
  })), 2),
  lowsn = sample(which(apply(outcomes, 1, function(x) { # (2)
    return(x[1] &
             !x[2] &
             x[3] &
             x[4] &
```

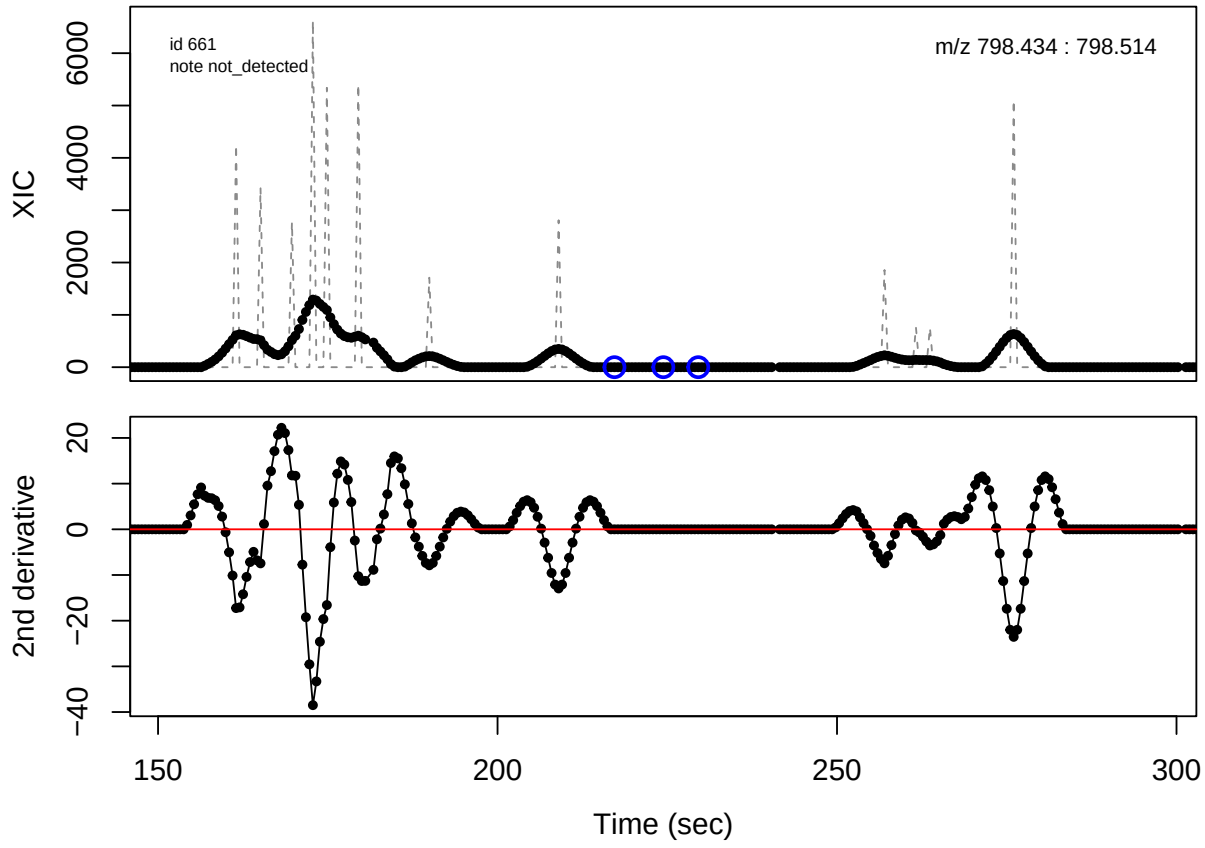
```
            x[5] &
            x[6])
  })), 2),
  lowint = which(apply(outcomes, 1, function(x) { # (3)
    return(x[1] &
            !x[2] &
            x[3] &
            x[4] &
            x[5] &
            !x[6])
  })),
  toonarrow = sample(which(apply(outcomes, 1, function(x) { # (4)
    return(x[1] &
            x[2] &
            !x[3] &
            x[4] &
            x[5] &
            x[6])
  })), 2)
)

randomRemoved
#> $nd
#> CP0661 CP0352
#>    661    352
#>
#> $lowsn
#> CP1033 CP0359
#>   1033    359
#>
#> $lowint
#> CP0245
#>    245
#>
#> $toonarrow
#> CP0906 CP0549
#>    906    549
```

And then we can plot these peaks using the *plotPeaks()* function. This function takes a *cpc* object as well as a list of peak IDs as argument. The peak IDs can be supplied as indices in the full peak list (ie. not the filtered peak list) or as a character vector with peak IDs (row names in the peak tables). In the present example they are the same and we can use either the names of randomRemoved or the values.

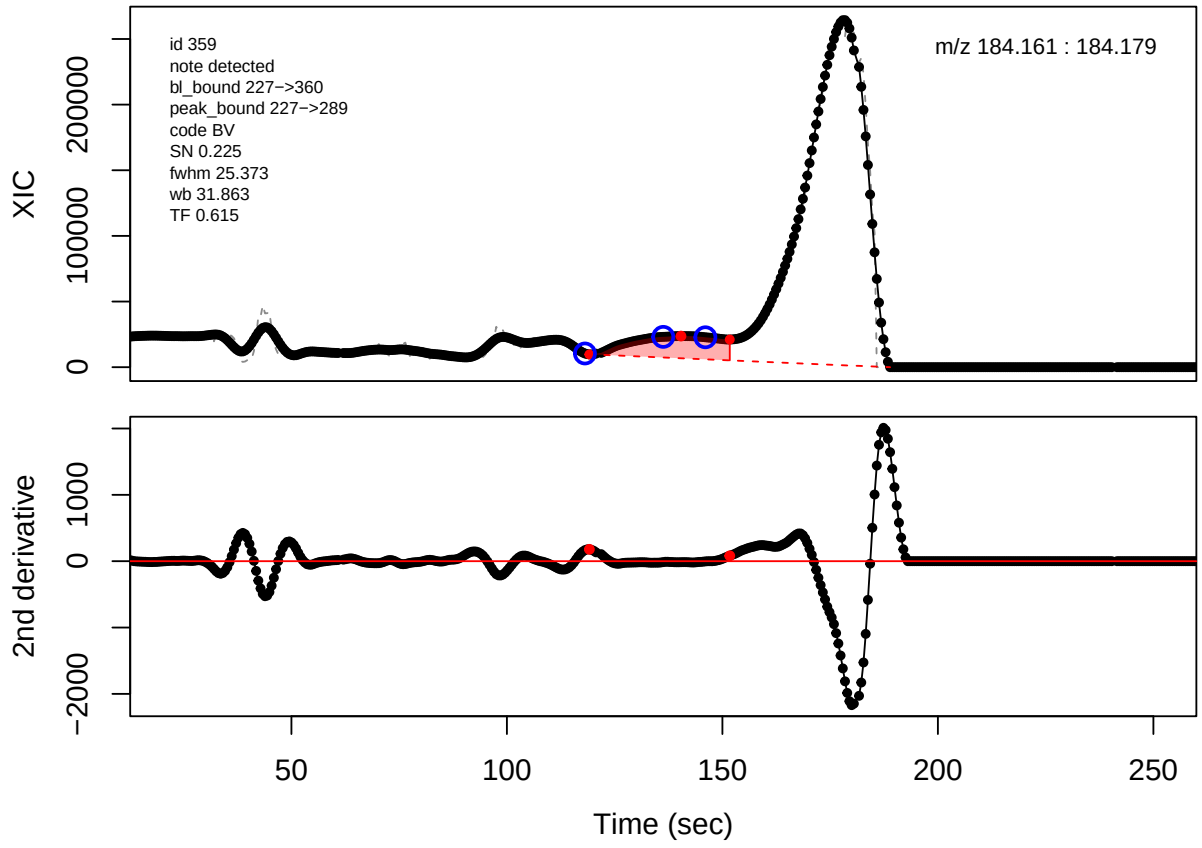
```
cpc::plotPeaks(cpc, peakIdx = randomRemoved[[1]])
#> Opening file: C:\R\library\cpc\extdata\hilic017.mzML
```



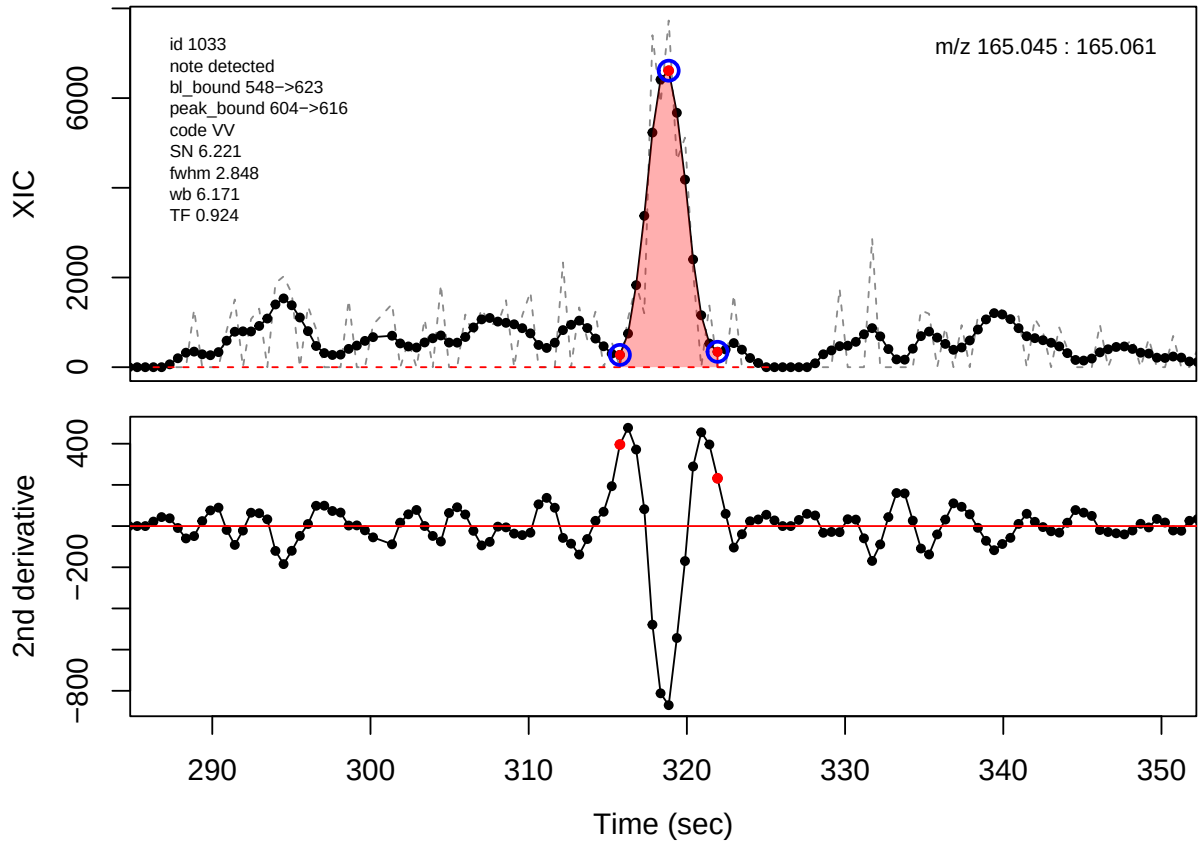

```
#> Opening file: C:\R\library\cpc\extdata\hilic019.mzML
```



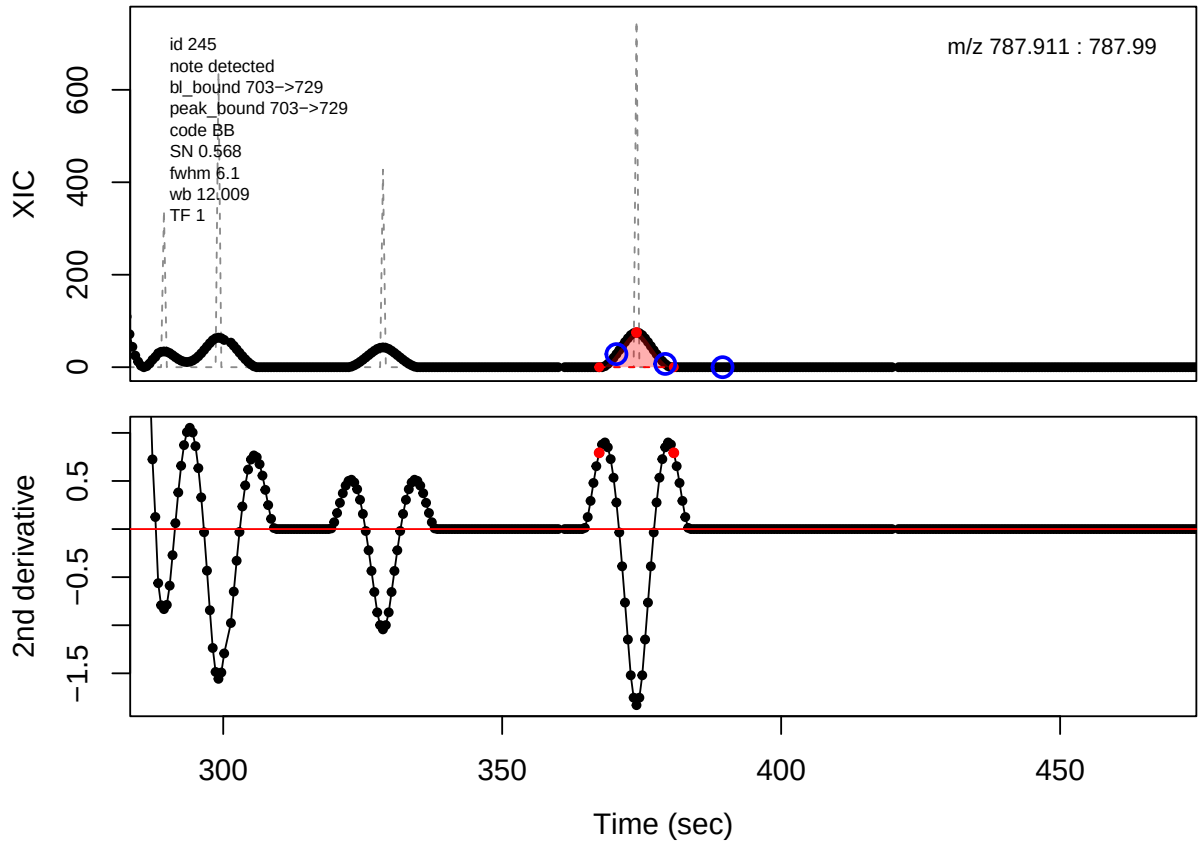

```
cpc::plotPeaks(cpc, peakIdx = randomRemoved[[2]])
#> Opening file: C:\R\library\cpc\extdata\hilic017.mzML
```



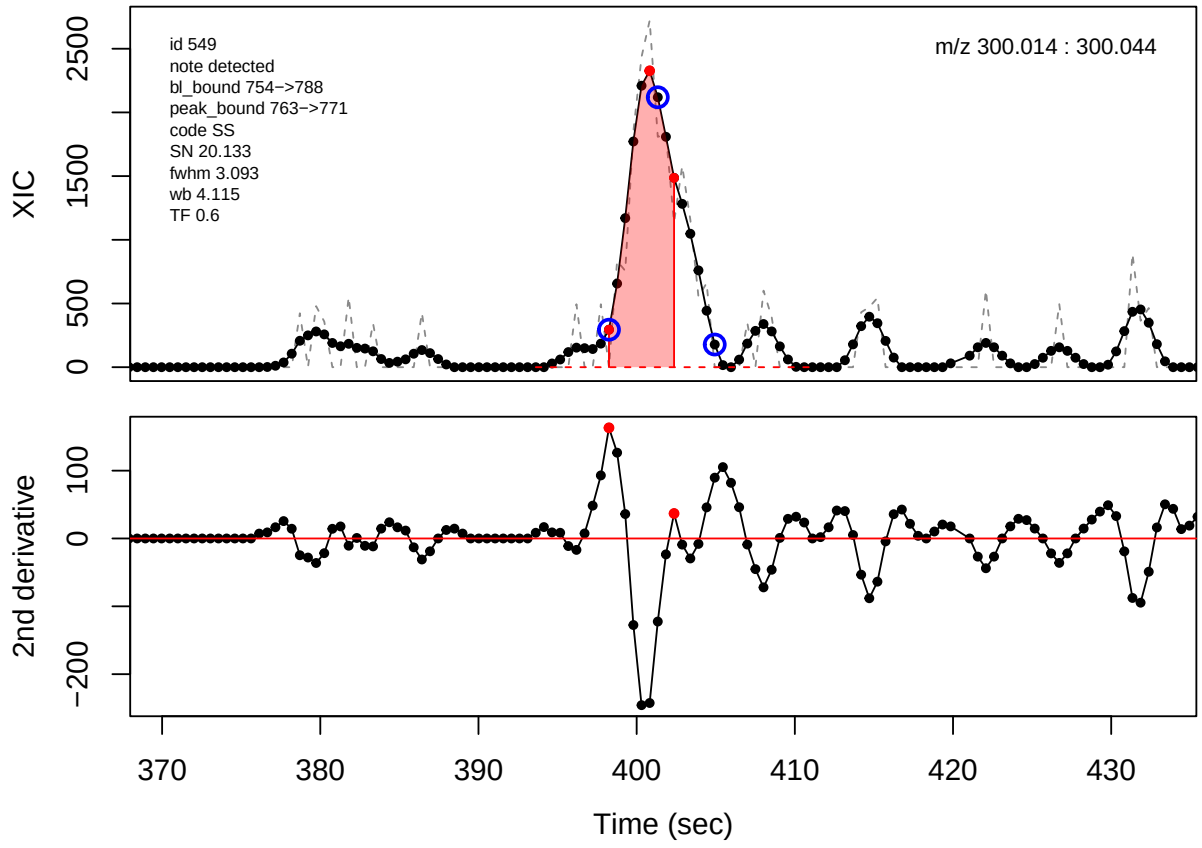

```
#> Opening file: C:\R\library\cpc\extdata\hilic021.mzML
```



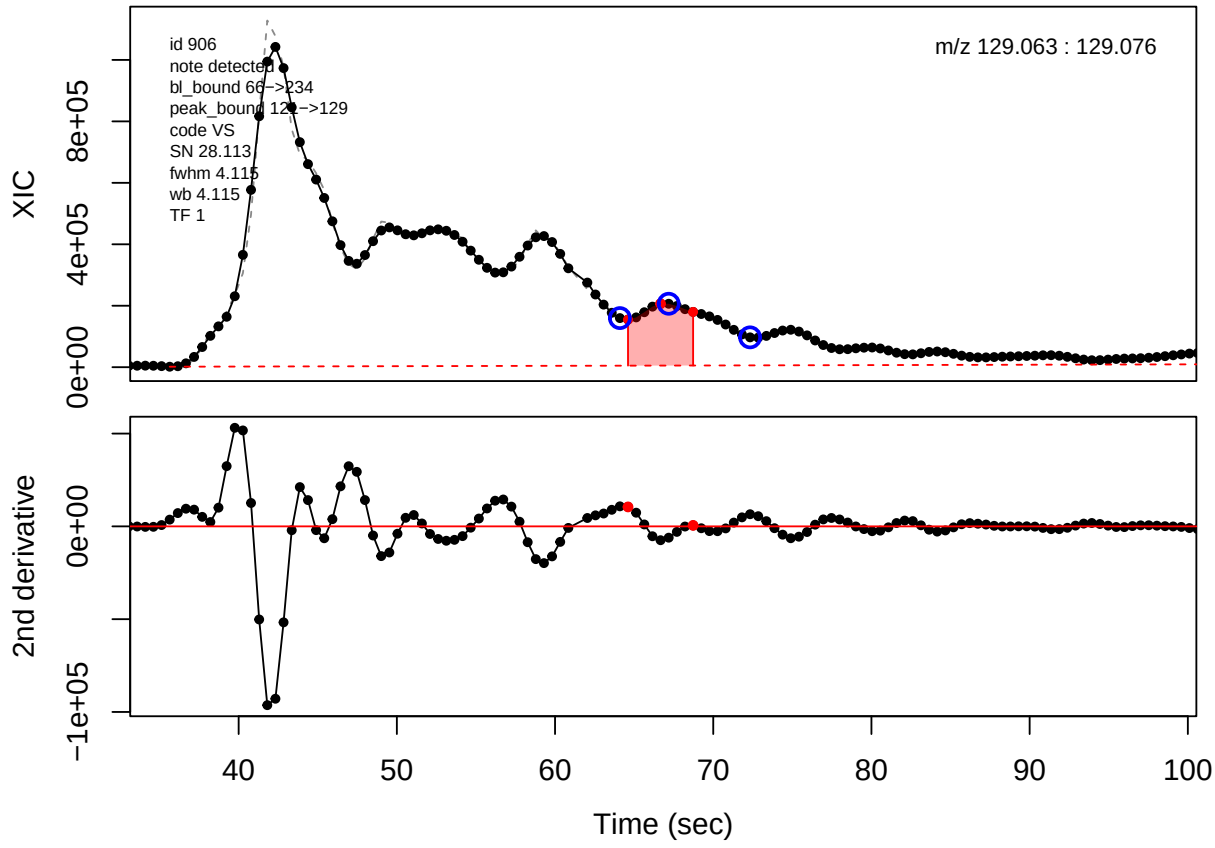

```
cpc::plotPeaks(cpc, peakIdx = randomRemoved[[3]])
#> Opening file: C:\R\library\cpc\extdata\hilic015.mzML
```




```
cpc::plotPeaks(cpc, peakIdx = randomRemoved[[4]])
#> Opening file: C:\R\library\cpc\extdata\hilic017.mzML
```




```
#> Opening file: C:\R\library\cpc\extdata\hilic021.mzML
```



In the above plots the original XIC trace is plotted as a gray dashed line with the smoothed chromatogram overlaid on top in black. The black dots are the actual scan points. The blue circles represent the front boundary, apex location, and tail boundary, as determined by XCMS. The red fill indicates the peak boundaries determined by CPC with the red dot representing the apex point. The red dashed line is the baseline of the peak, as estimated by CPC.

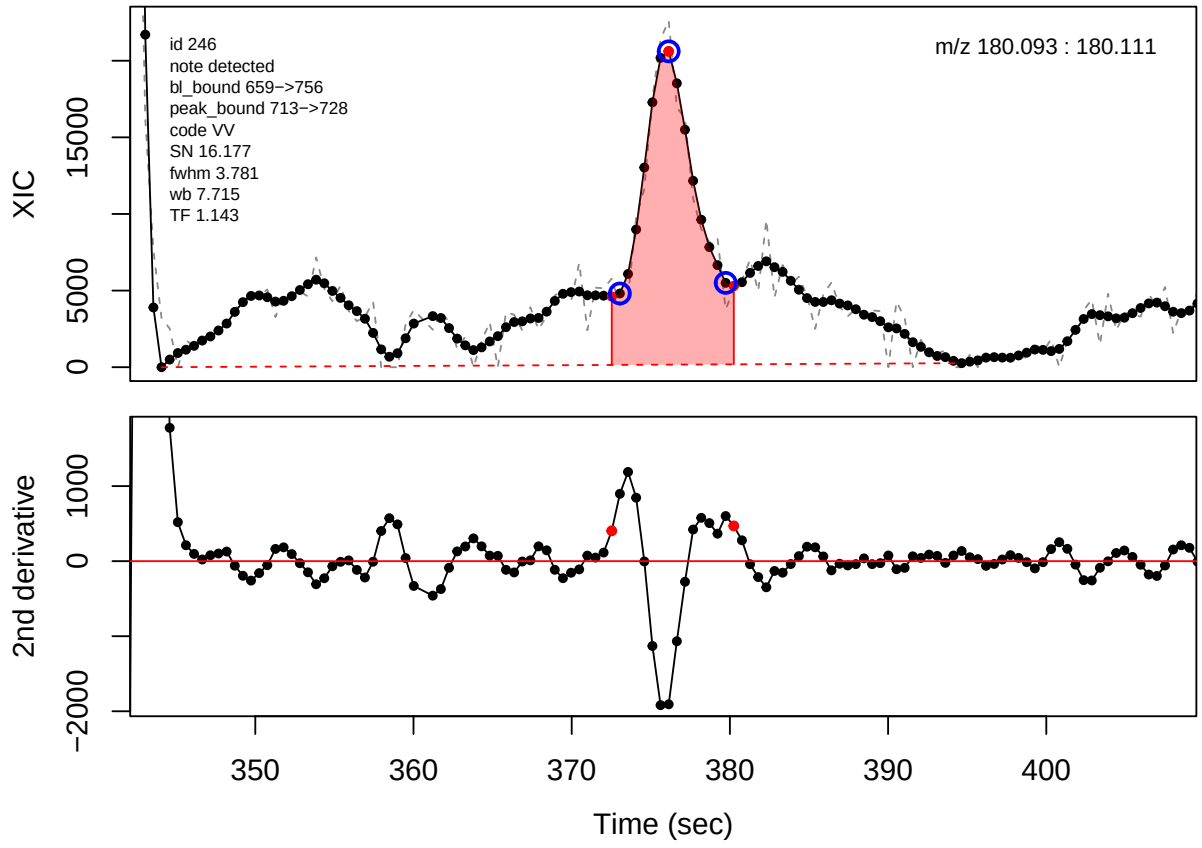
We can also plot some of the retained peaks in the same way. Here we will randomly select one peak from each quartile of the signal-to-noise range.

```
# get a vector of all detected peaks
allRetainedPeaks <- cpc::getRetainedPeaks(cpc)
allRetainedPeakIdx <- match(allRetainedPeaks, row.names(cpcPeaktable))

# determine the quartile break points
quarts <- quantile(cpcPeaktable$sn[allRetainedPeakIdx])
quarts[1] <- 0 # set the first boundary to be 0

quarts
#>        0%       25%       50%       75%      100%
#>   0.00000  28.49219  64.91197 161.98079       Inf

# randomly select peaks in each quartile
noPeakFromEachQuartile <- 2

# randomly select the specified number of peaks from each quartile
```

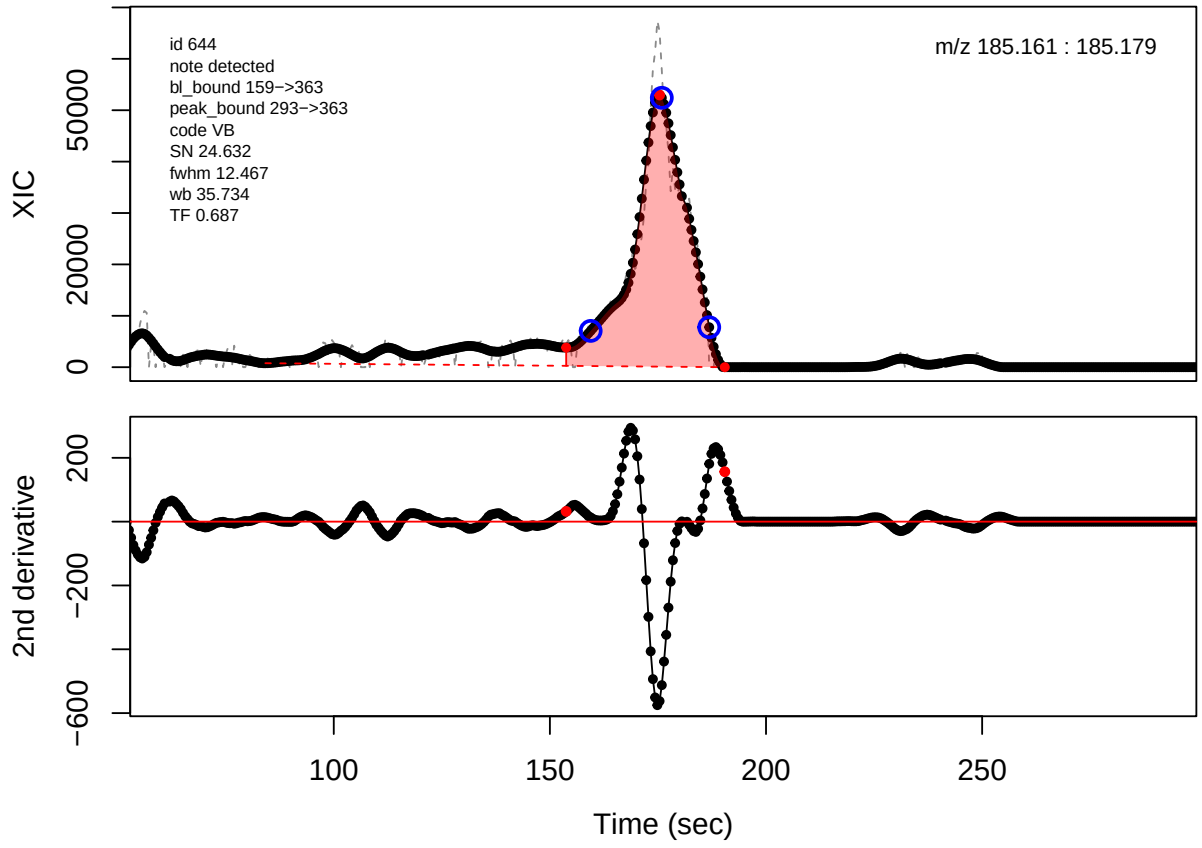
```
set.seed(seed); randomRetained <- list(
  low <- sample(allRetainedPeaks[
    cpcPeaktable$sn[allRetainedPeakIdx] >= quarts[1] &
      cpcPeaktable$sn[allRetainedPeakIdx] < quarts[2]
  ], noPeakFromEachQuartile, FALSE), # 0% <= SN < 25%
  lowmed <- sample(allRetainedPeaks[
    cpcPeaktable$sn[allRetainedPeakIdx] >= quarts[2] &
      cpcPeaktable$sn[allRetainedPeakIdx] < quarts[3]
  ], noPeakFromEachQuartile, FALSE), # 25% <= SN < 50%
  medhigh <- sample(allRetainedPeaks[
    cpcPeaktable$sn[allRetainedPeakIdx] >= quarts[3] &
      cpcPeaktable$sn[allRetainedPeakIdx] < quarts[4]
  ], noPeakFromEachQuartile, FALSE), # 50% <= SN < 75%
  high <- sample(allRetainedPeaks[
    cpcPeaktable$sn[allRetainedPeakIdx] >= quarts[4] &
      cpcPeaktable$sn[allRetainedPeakIdx] < quarts[5]
  ], noPeakFromEachQuartile, FALSE)) # 75% <= SN < 100%

randomRetained
#> [[1]]
#> [1] "CP0644" "CP0246"
#>
#> [[2]]
#> [1] "CP1018" "CP1072"
#>
#> [[3]]
#> [1] "CP1048" "CP0709"
#>
#> [[4]]
#> [1] "CP0253" "CP0470"
```

And then we can plot all these peaks in the same way as with the removed peaks.

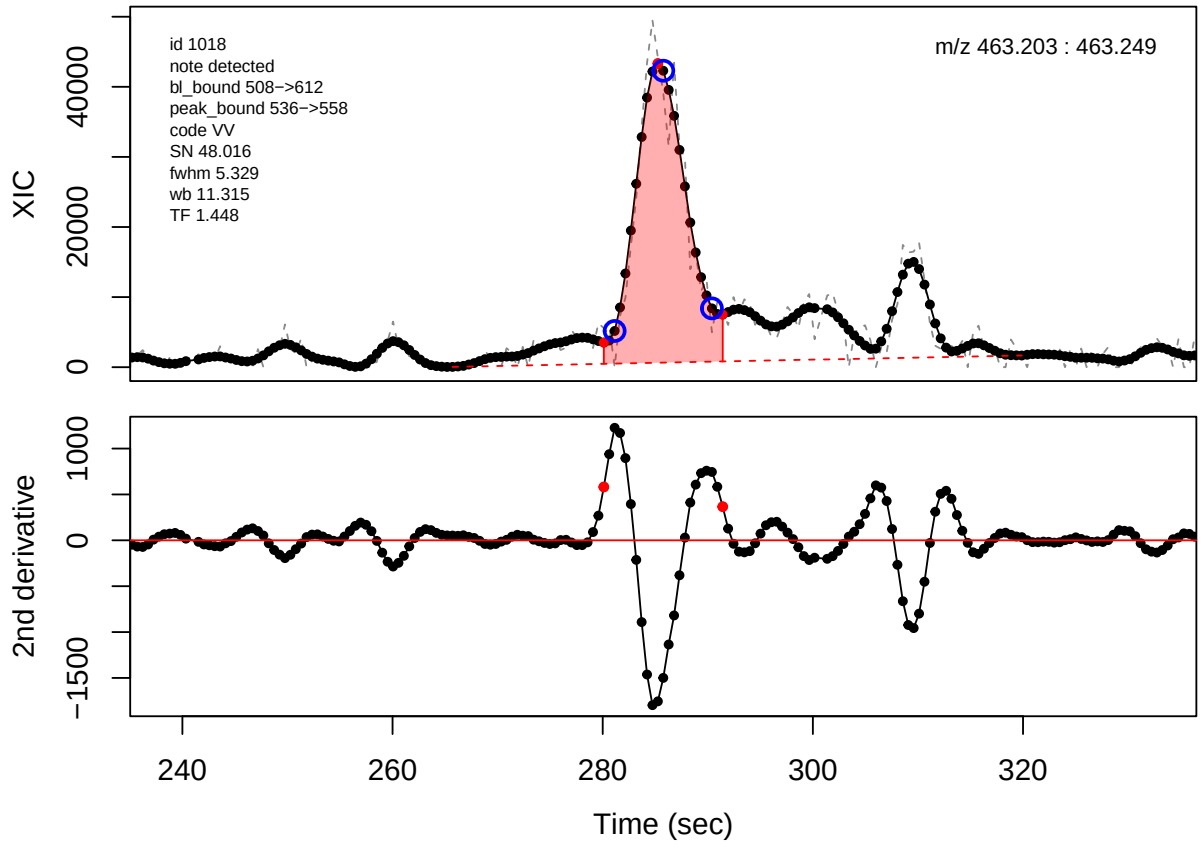
```
cpc::plotPeaks(cpc, peakIdx = randomRetained[[1]])
#> Opening file: C:\R\library\cpc\extdata\hilic015.mzML
```

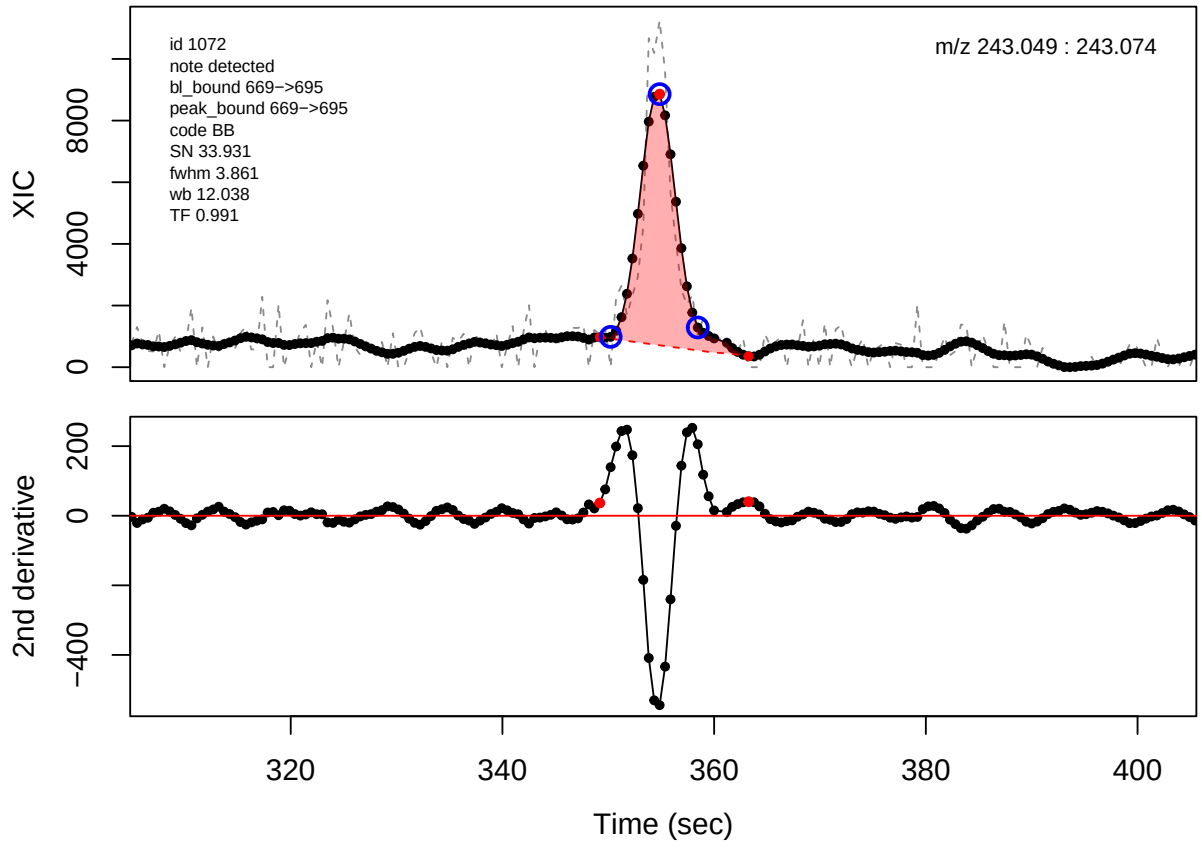



```
#> Opening file: C:\R\library\cpc\extdata\hilic019.mzML
```

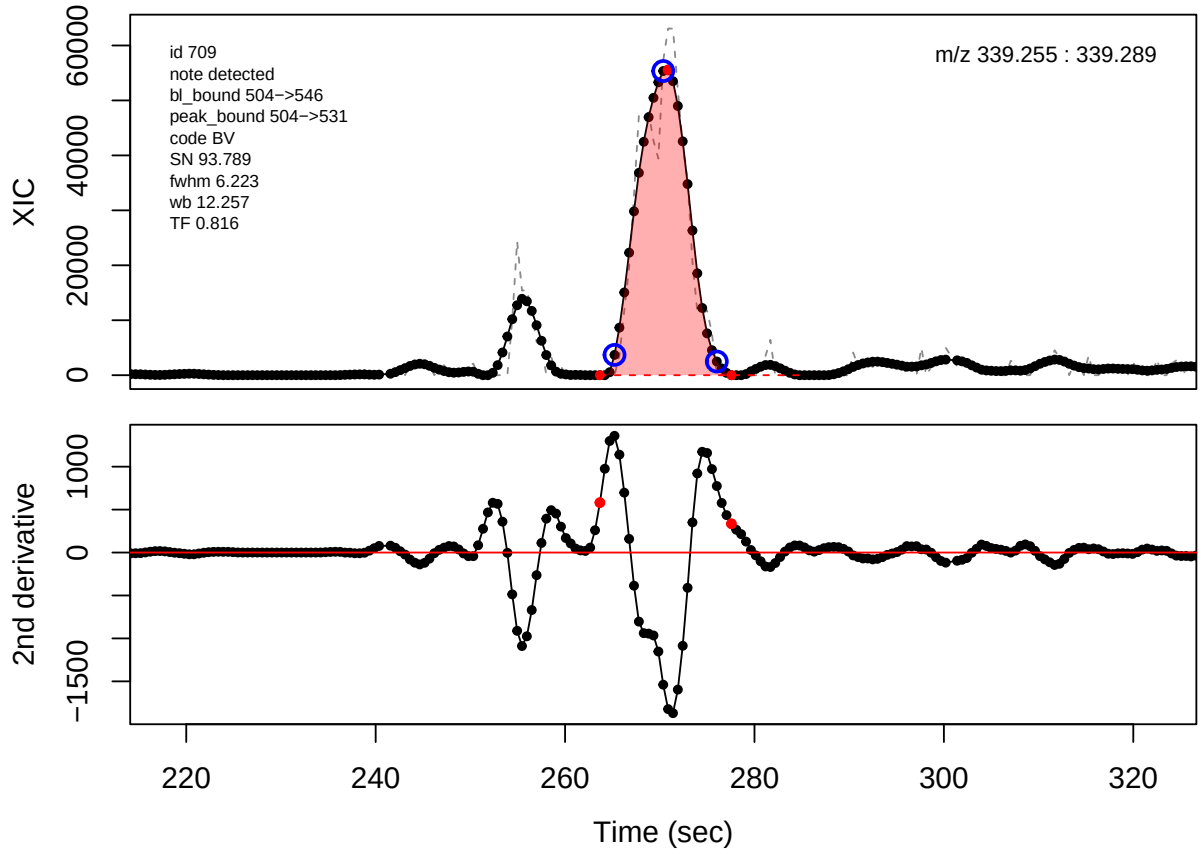



```
cpc::plotPeaks(cpc, peakIdx = randomRetained[[2]])
#> Opening file: C:\R\library\cpc\extdata\hilic021.mzML
```

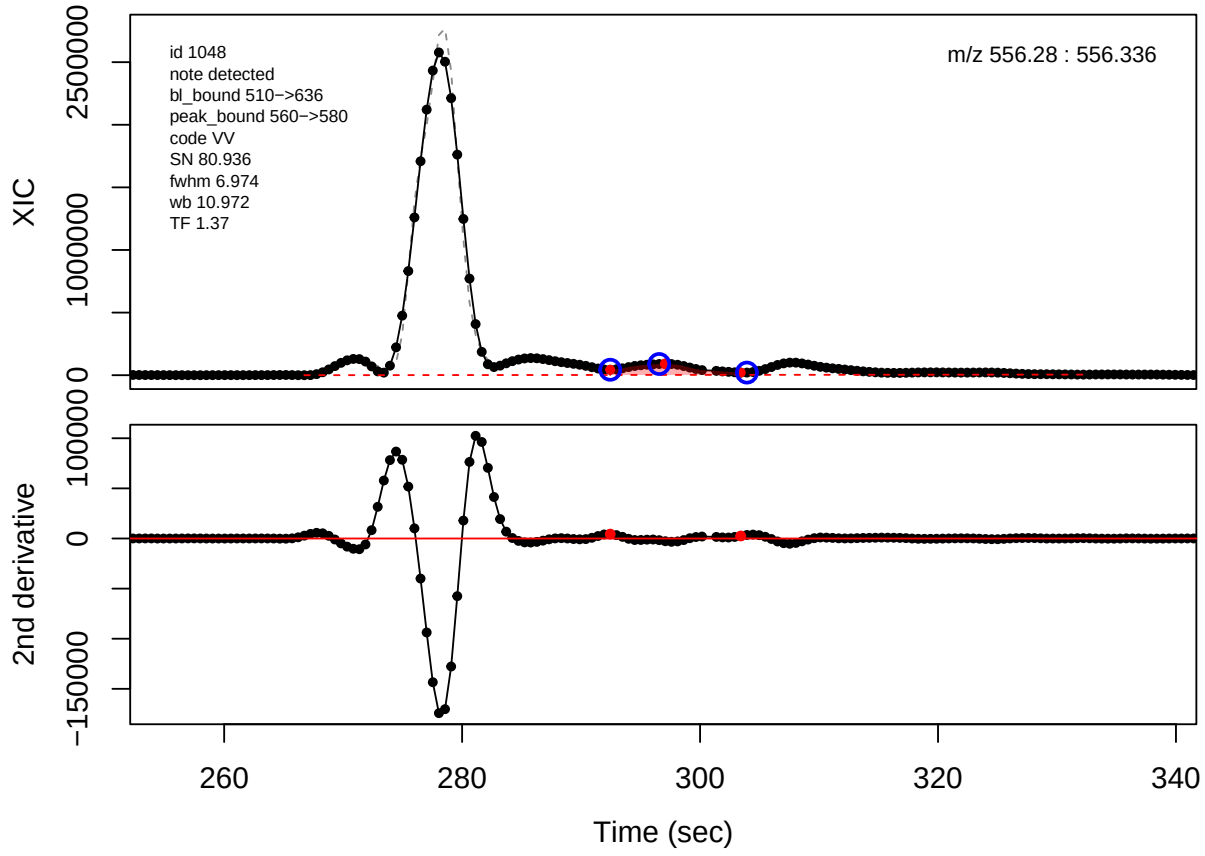

id 1018
note detected
bl_bound 508−>612
peak_bound 536−>558
code VV
SN 48.016
fwhm 5.329
wb 11.315
TF 1.448
m/z 463.203 : 463.249
XIC
0
20000
40000
2nd derivative
−1500
0
1000
240
260
280
300
320
Time (sec)



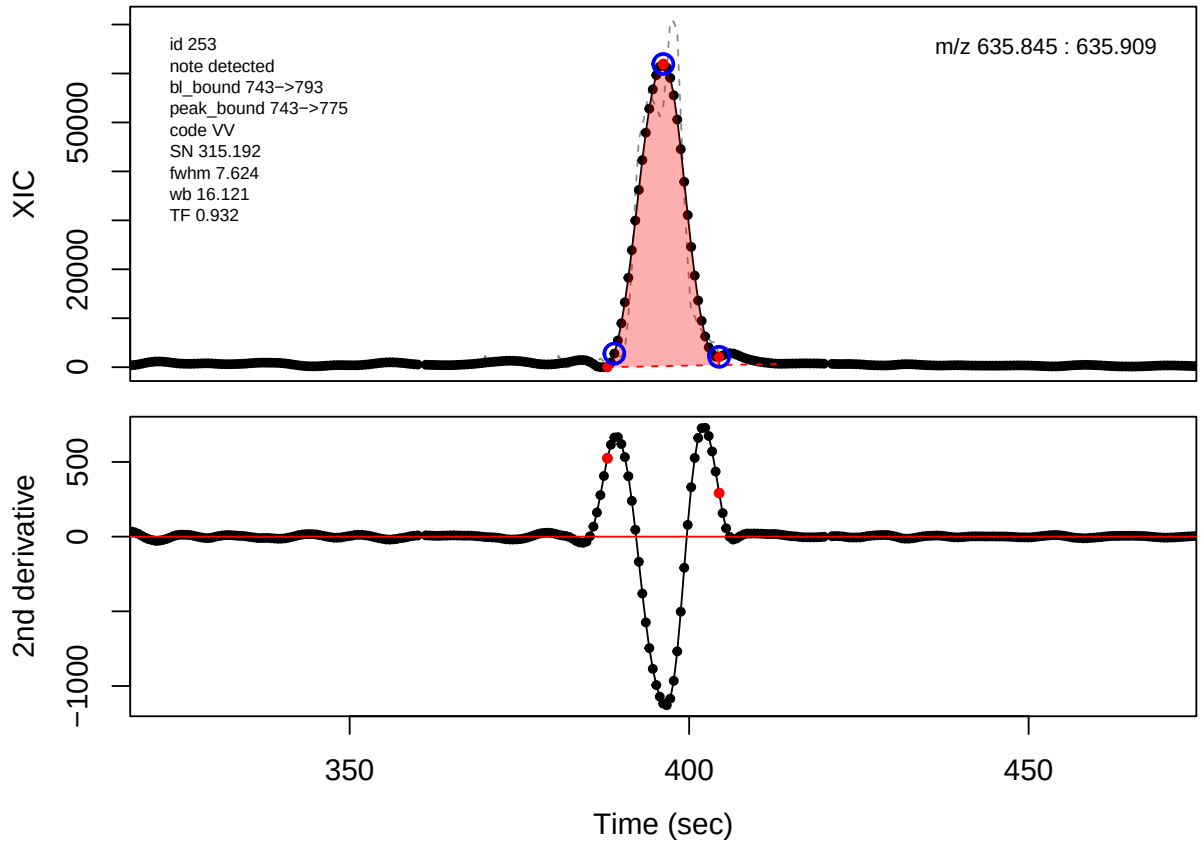

```
cpc::plotPeaks(cpc, peakIdx = randomRetained[[3]])
#> Opening file: C:\R\library\cpc\extdata\hilic019.mzML
```



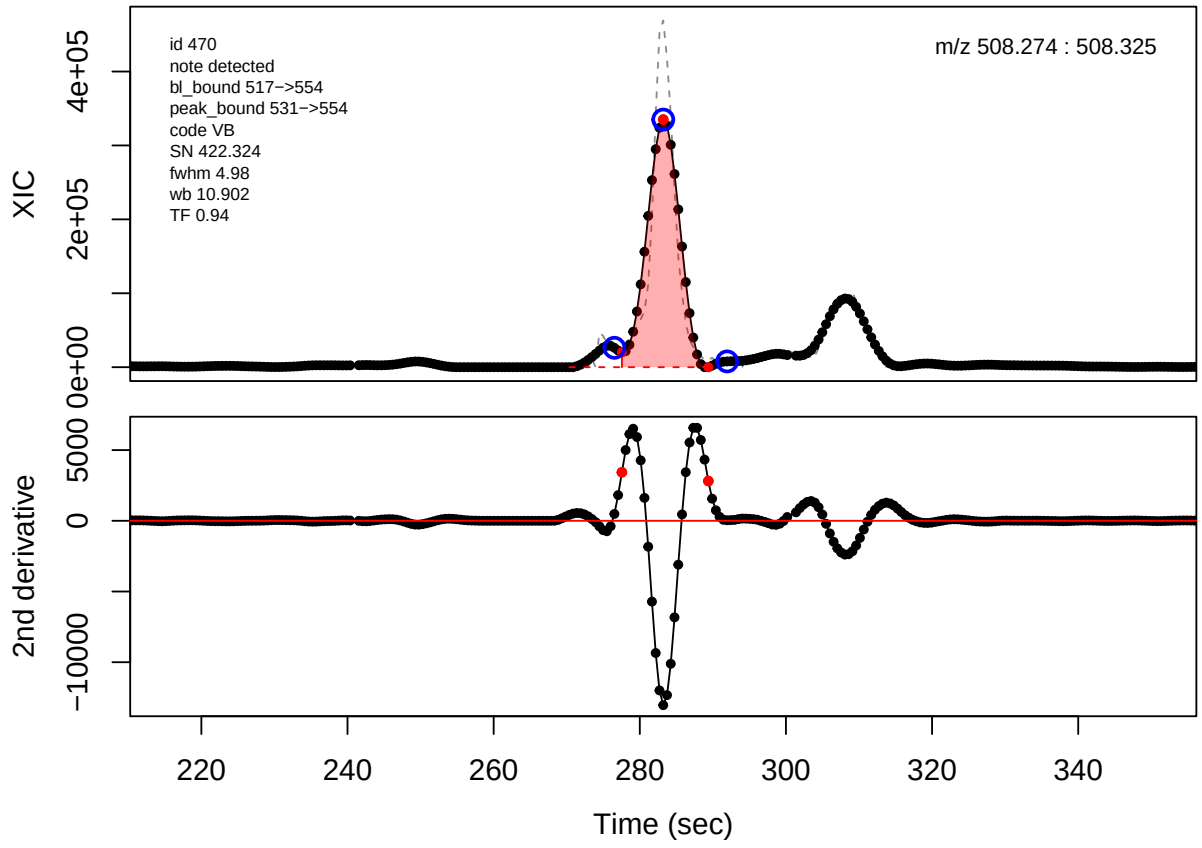

```
#> Opening file: C:\R\library\cpc\extdata\hilic021.mzML
```




```
cpc::plotPeaks(cpc, peakIdx = randomRetained[[4]])
#> Opening file: C:\R\library\cpc\extdata\hilic015.mzML
```

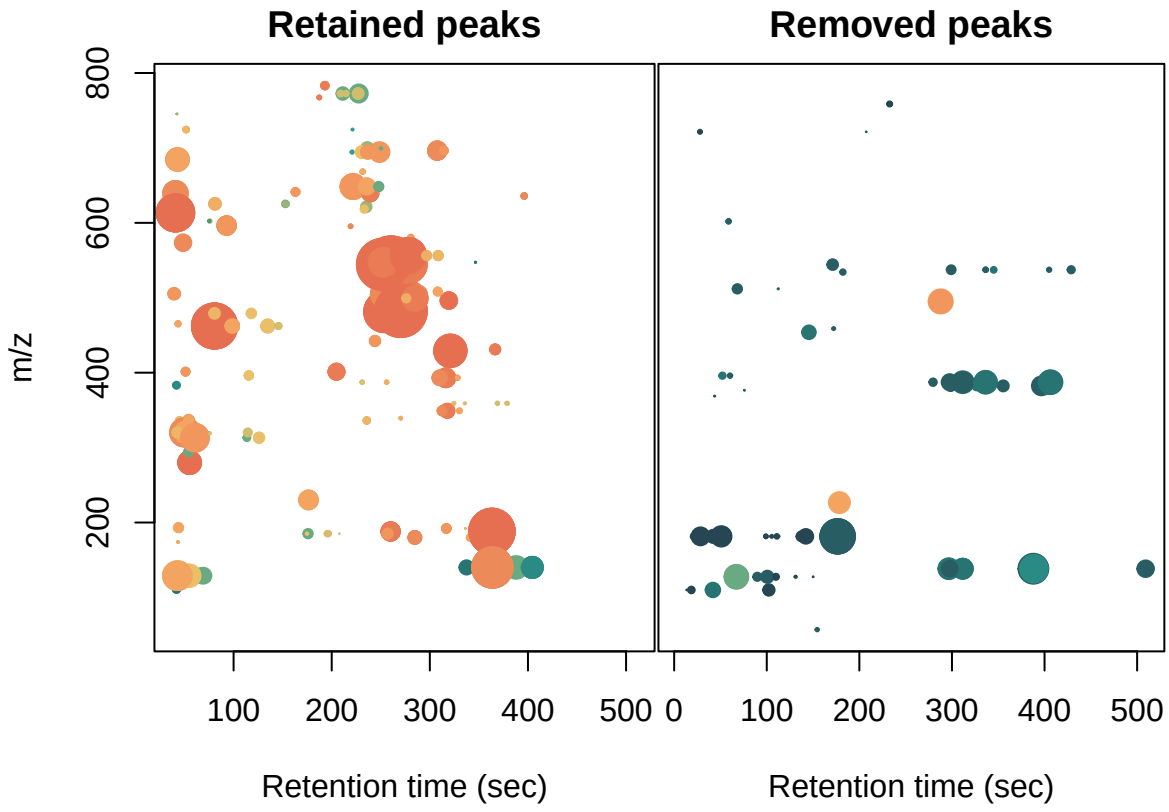



```
#> Opening file: C:\R\library\cpc\extdata\hilic017.mzML
```



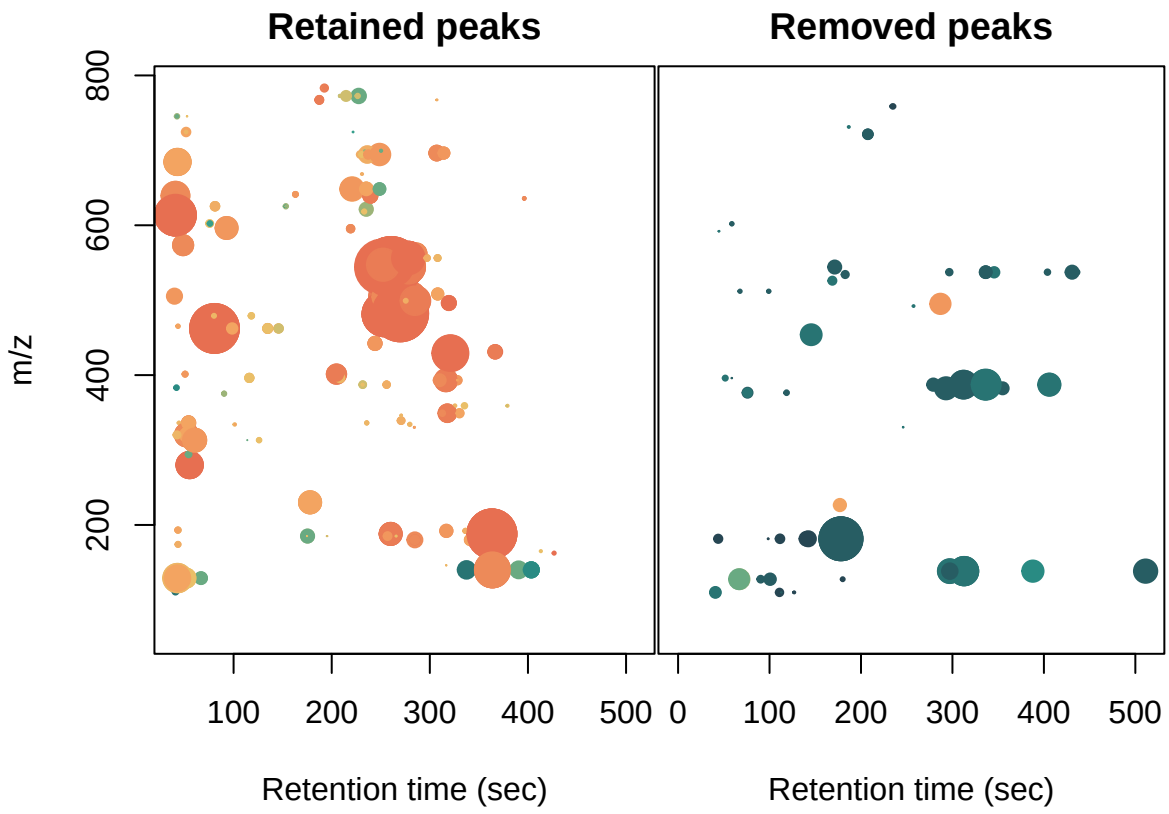

```
# get a vector of all removed peak numeric IDs
removedPeaksIdx <- match(cpc::getRemovedPeaks(cpc), row.names(cpcPeaktable))

snVals <- cpcPeaktable$sn
snVals[which(snVals == Inf)] <- max(snVals[which(snVals != Inf)])*2
quanBreaks <- quantile(snVals, probs = seq(0,1,length.out = 17))
quanBreaks[1] <- 0

colPalette <- colorRampPalette(colors = c("#264653", "#2A9D8F", "#E9C46A",
                                          "#F4A261", "#E76F51"))(16)

colVec <- colPalette[as.integer(sapply(snVals, FUN = function(x) {
  which(quanBreaks[1:(length(quanBreaks)-1)] < x &
          quanBreaks[2:length(quanBreaks)] >= x)
}))]

# m/z ~ rt (xcms data) with separate panels for retained and removed peaks
layout(mat = matrix(c(1,2), nrow = 1), widths = c(0.53,0.47))
par(mar = c(5.1,4.1,2.1,0.1))
plot(x = xcmsPeaktableOrig$rt[-removedPeaksIdx],
     y = xcmsPeaktableOrig$mz[-removedPeaksIdx], pch = 20,
     col = colVec[-removedPeaksIdx],
     cex = log(pmax(0, xcmsPeaktableOrig$into[-removedPeaksIdx]) /
                 median(pmax(0, xcmsPeaktableOrig$into[-removedPeaksIdx]))),
```

```
     xlab = "Retention time (sec)", ylab = "m/z", main = "Retained peaks")

par(mar = c(5.1,0,2.1,2.1))
plot(x = xcmsPeaktableOrig$rt[removedPeaksIdx],
     y = xcmsPeaktableOrig$mz[removedPeaksIdx], pch = 20,
     col = colVec[removedPeaksIdx],
     cex = log(pmax(0, xcmsPeaktableOrig$into[removedPeaksIdx]) /
                 median(pmax(0, xcmsPeaktableOrig$into[removedPeaksIdx]))),
     xlab = "Retention time (sec)", yaxt = "n", ylab = "", main = "Removed peaks")
```




```
# m/z ~ rt (cpc data) with separate panels for retained and removed peaks
layout(mat = matrix(c(1,2), nrow = 1), widths = c(0.53,0.47))
par(mar = c(5.1,4.1,2.1,0.1))
plot(x = cpcPeaktable$rt[-removedPeaksIdx],
     y = xcmsPeaktableOrig$mz[-removedPeaksIdx], pch = 20,
     col = colVec[-removedPeaksIdx],
     cex = log(pmax(0, cpcPeaktable$area[-removedPeaksIdx]) /
                 median(pmax(0, cpcPeaktable$area[-removedPeaksIdx]))),
     xlab = "Retention time (sec)", ylab = "m/z", main = "Retained peaks")

par(mar = c(5.1,0,2.1,2.1))
plot(x = cpcPeaktable$rt[removedPeaksIdx],
     y = xcmsPeaktableOrig$mz[removedPeaksIdx], pch = 20,
     col = colVec[removedPeaksIdx],
     cex = log(pmax(0, cpcPeaktable$area[removedPeaksIdx]) /
```

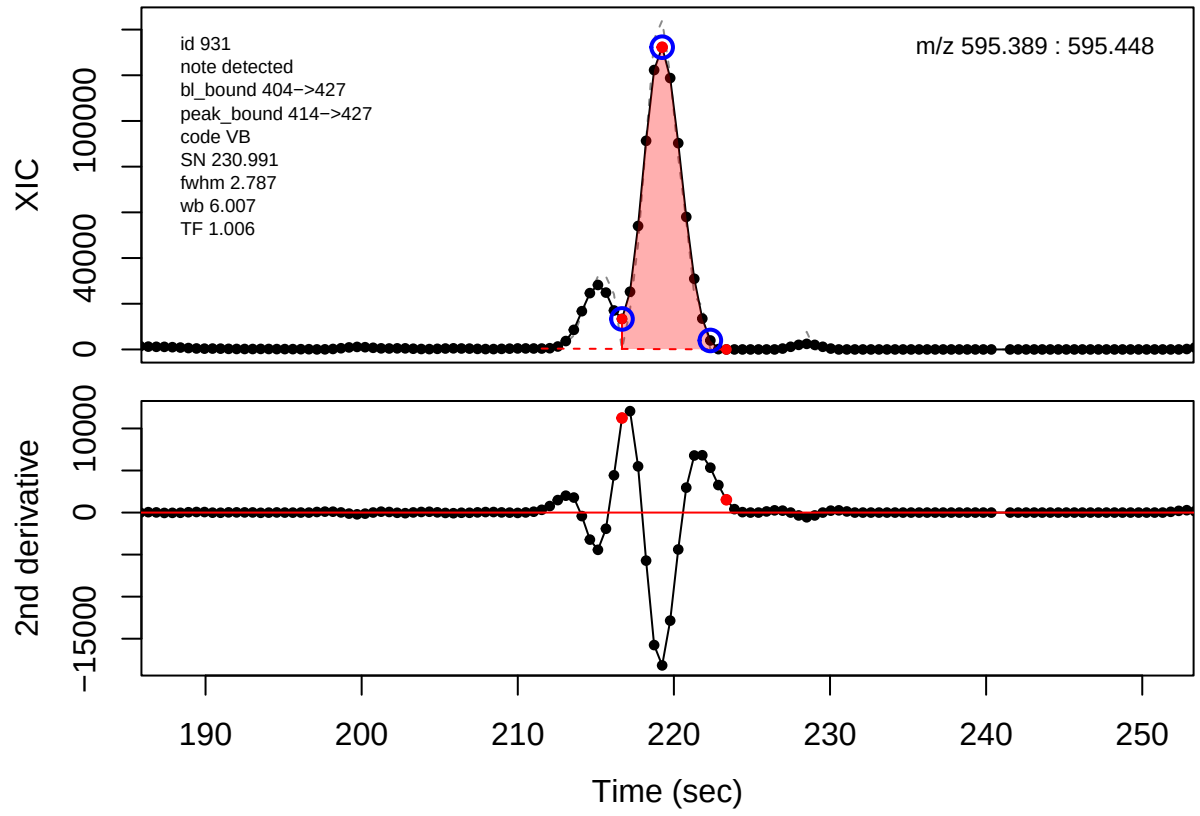
```
                median(pmax(0, cpcPeaktable$area[removedPeaksIdx]))),
    xlab = "Retention time (sec)", yaxt = "n", ylab = "", main = "Removed peaks")
```



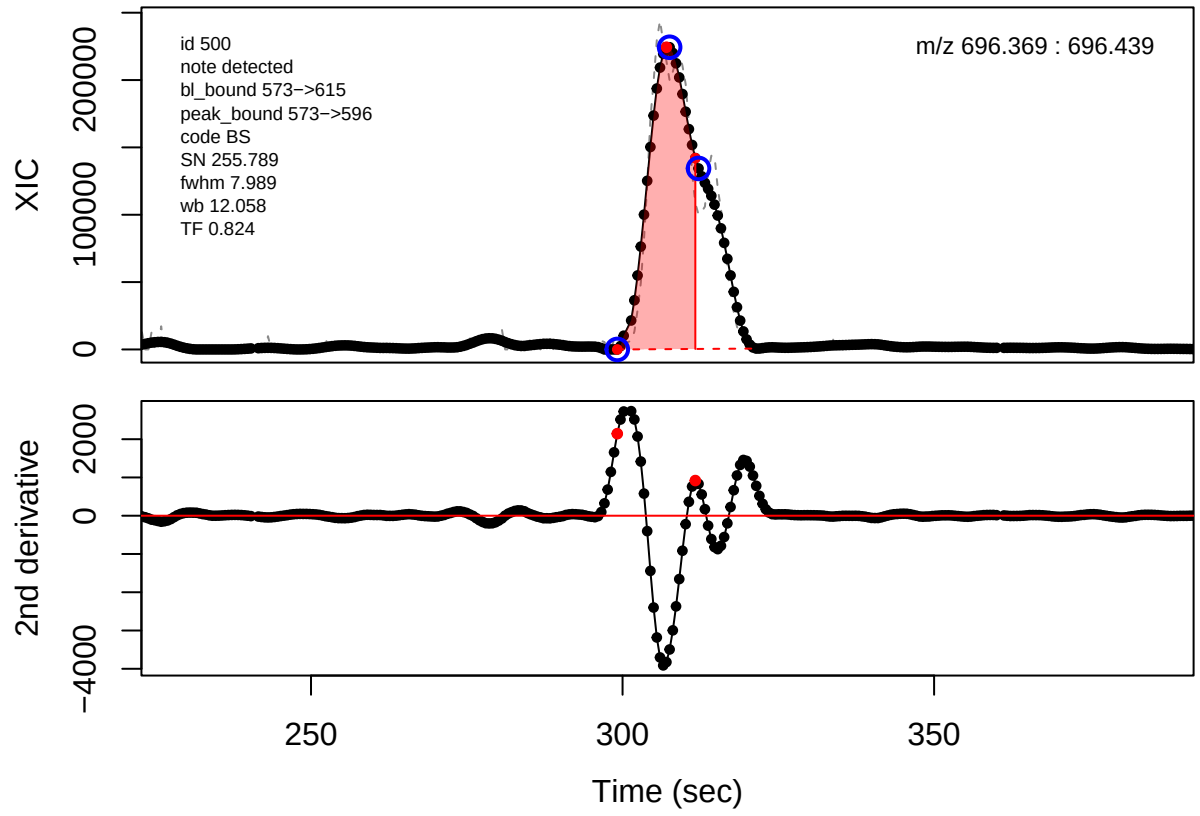
Peak cluster boundaries

Overlapping peak clusters can be detected by the algorithm as their baseline boundaries after expansion will overlap. Consequently the boundaries between peaks in clusters need to be determined and updated. This is handled differently depending on the degree of overlap. Valley boundaries between peaks are set to the lowest point between the peak apices along the ion trace. Shoulder boundaries are set to the maxima between the peak apices along the second derivative of the ion trace. When two similarily sized peaks overlap sufficiently much, it can lead to a rounded peak shape. This is detected as two peak apices that share inflection points along the second derivative which a negative valued maxima in between the apice points. The boundary is then set to the negative maxima point between the apices.

```
# Valley boundary example
cpc::plotPeaks(cpc, 931)
#> Opening file: C:\R\library\cpc\extdata\hilic021.mzML
```




```
# Shoulder boundary example
cpc::plotPeaks(cpc, 500)
#> Opening file: C:\R\library\cpc\extdata\hilic017.mzML
```

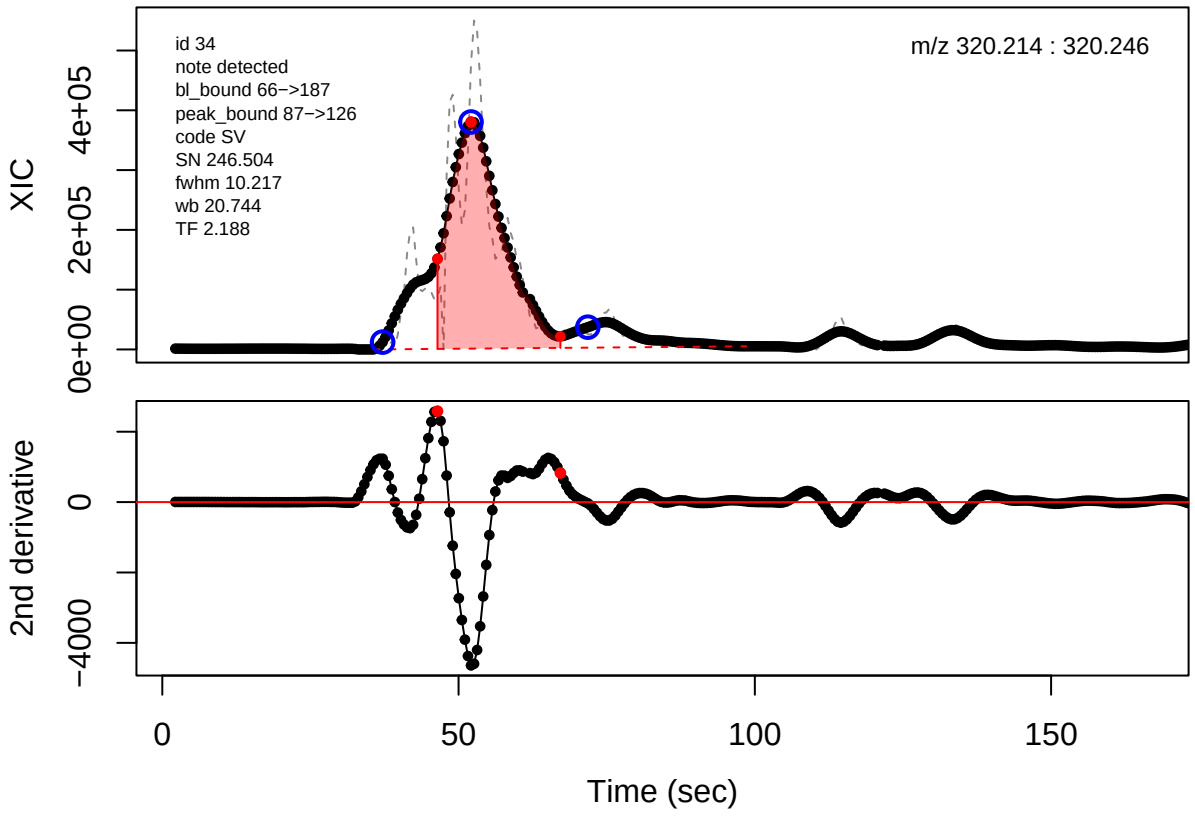


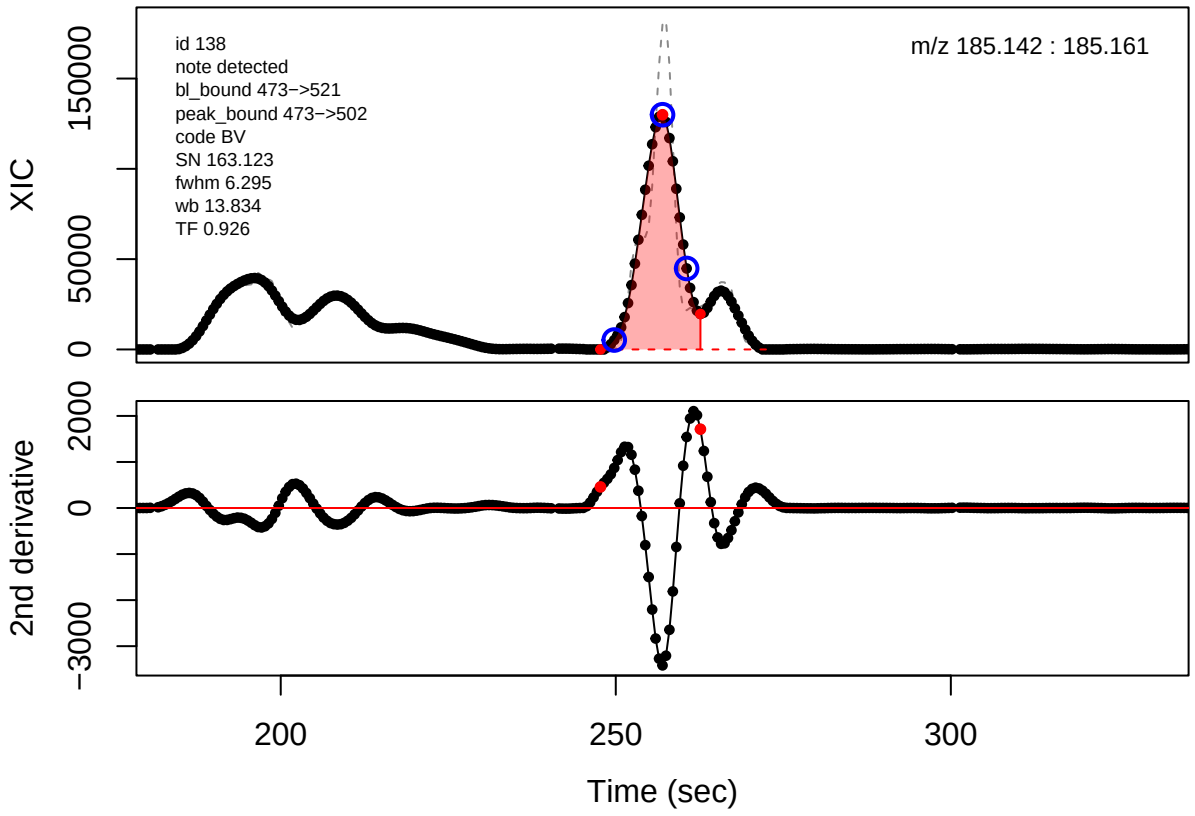
Example of EMG deconvolution

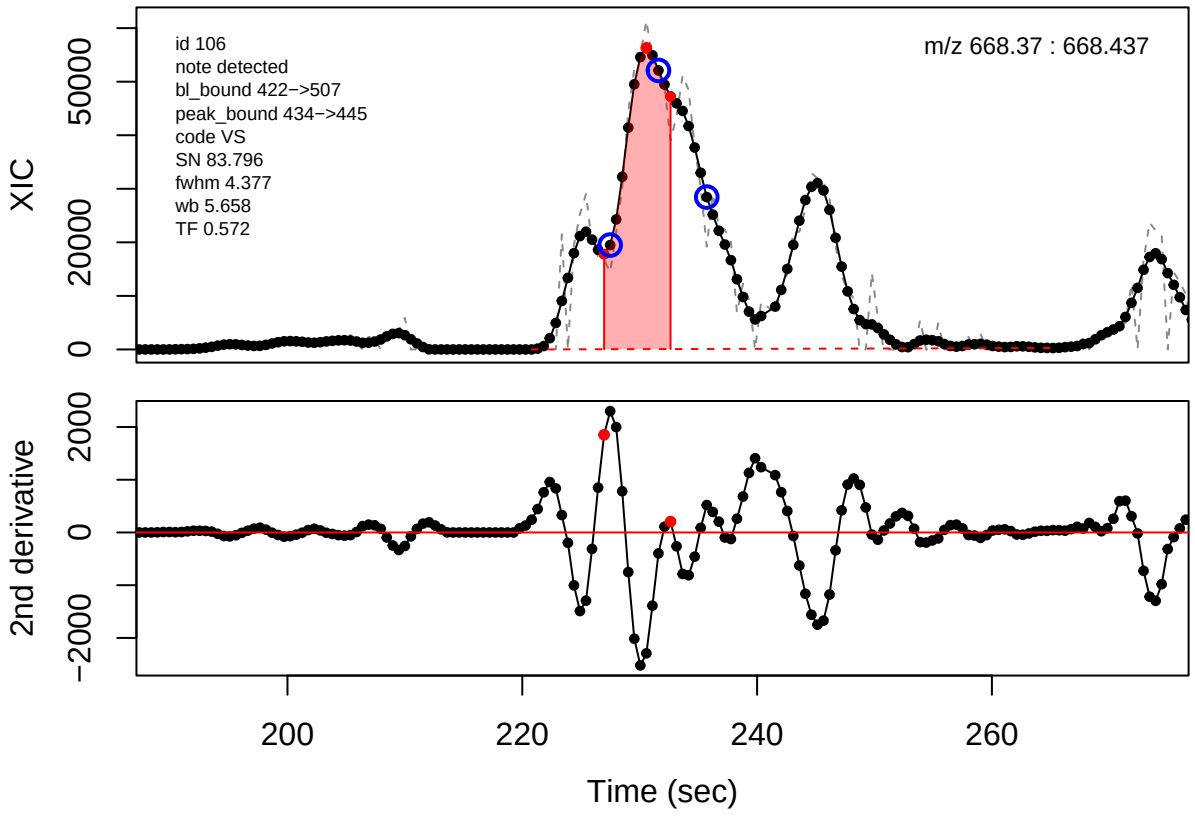
The processing engine used in the CPC package includes EMG deconvolution of peak clusters to achieve better estimates of the peak boundaries. This can be included in the processing by setting the argument *fit_emg = TRUE* in the *cpcProcParam* object.

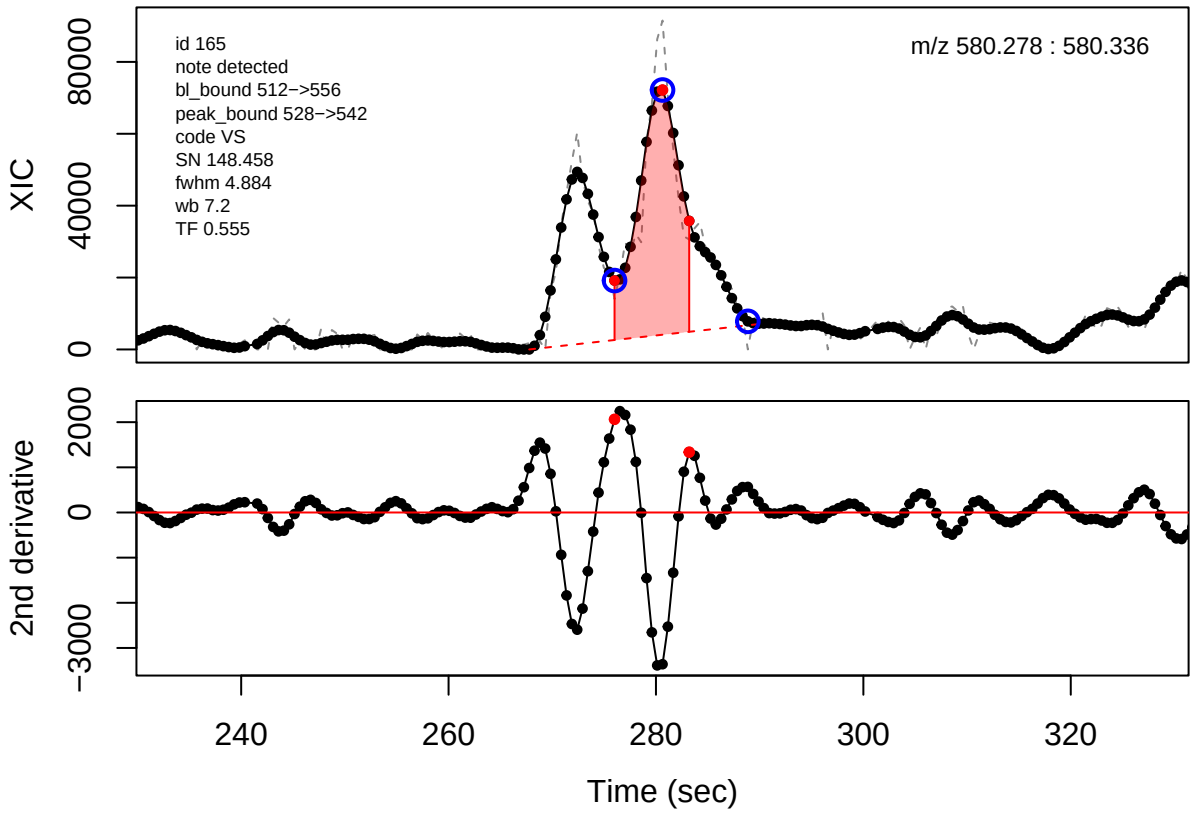
To illustrate this, we first need to find a few peaks to perform EMG deconvolution on.

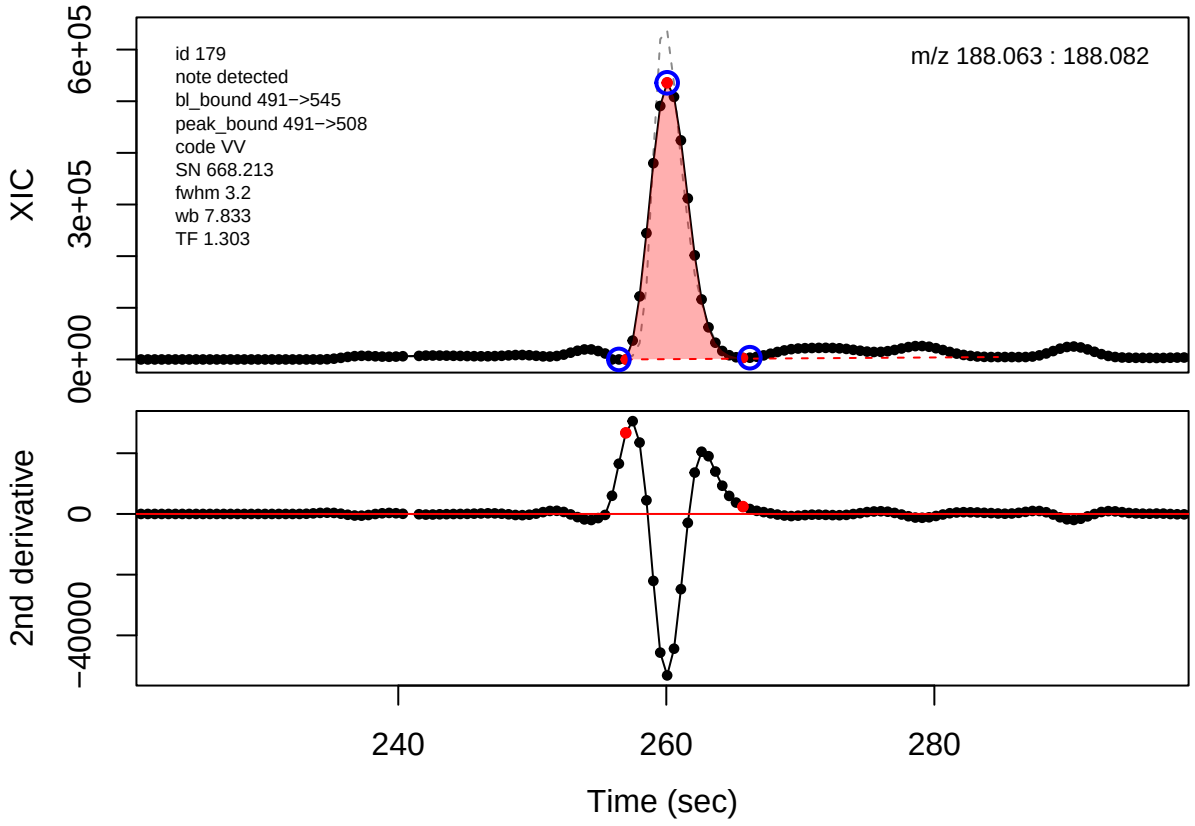
```
(selection <- c(34, 138, 106, 165, 179, 217))
#> [1]  34 138 106 165 179 217

(peakIdx <- row.names(cpcPeaktable[selection, ]))
#> [1] "CP0034" "CP0138" "CP0106" "CP0165" "CP0179" "CP0217"

(selection <- cpcPeaktable$id[selection])
#> [1]  34 138 106 165 179 217

cpc::plotPeaks(cpc, peakIdx = selection)
#> Opening file: C:\R\library\cpc\extdata\hilic015.mzML
```

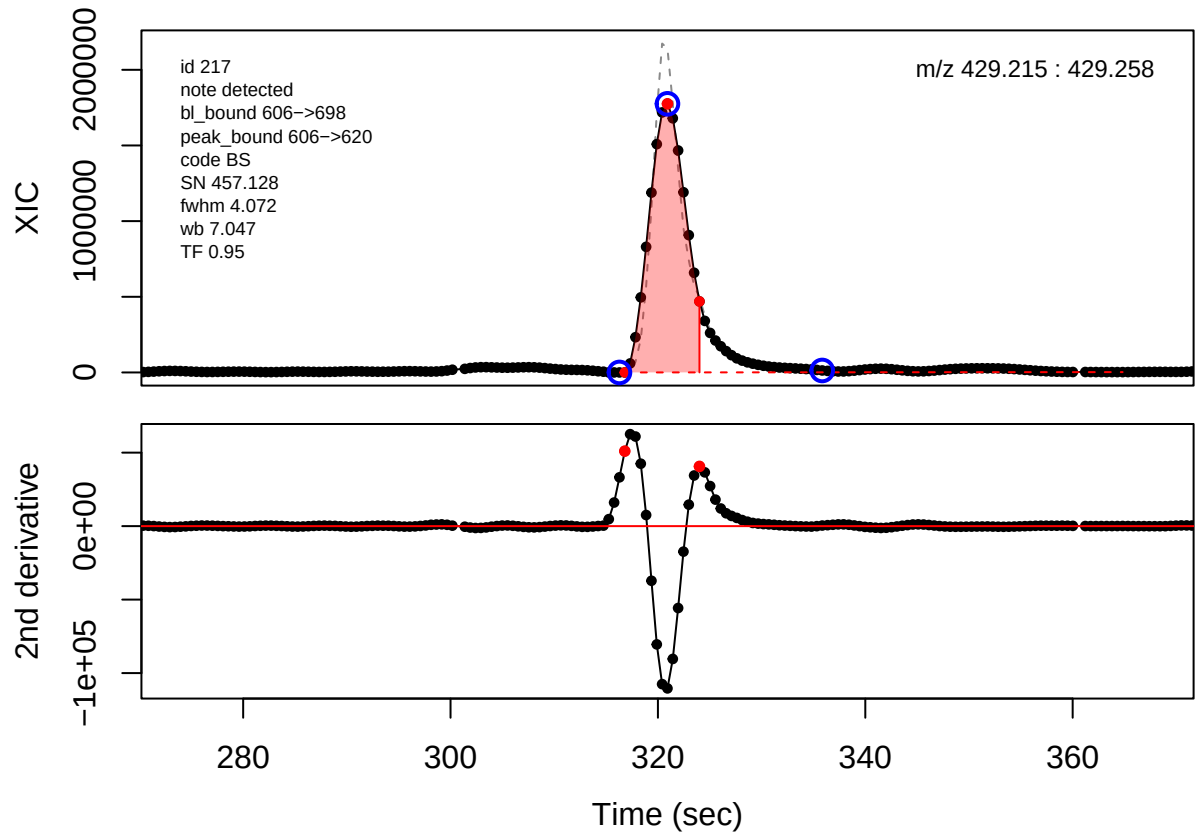

id 34
note detected
bl_bound 66−>187
peak_bound 87−>126
code SV
SN 246.504
fwhm 10.217
wb 20.744
TF 2.188
m/z 320.214 : 320.246
XIC
0e+00
2e+05
4e+05
2nd derivative
−4000
0
0
50
100
150
Time (sec)


id 138
note detected
bl_bound 473−>521
peak_bound 473−>502
code BV
SN 163.123
fwhm 6.295
wb 13.834
TF 0.926
m/z 185.142 : 185.161
XIC
150000
50000
0
2nd derivative
2000
0
−3000
200
250
300
Time (sec)


id 106
note detected
bl_bound 422–>507
peak_bound 434–>445
code VS
SN 83.796
fwhm 4.377
wb 5.658
TF 0.572
m/z 668.37 : 668.437
XIC
0
20000
50000
2nd derivative
−2000
0
2000
200
220
240
260
Time (sec)

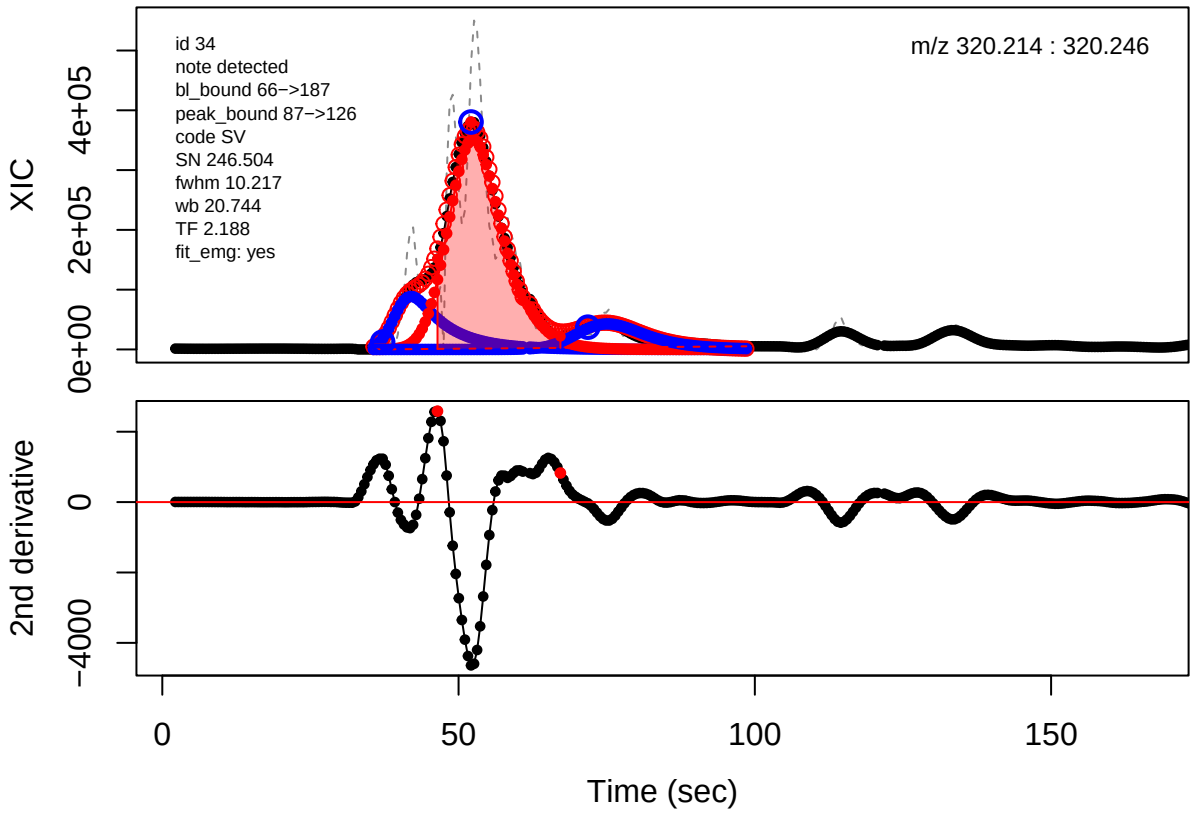

id 165
note detected
bl_bound 512−>556
peak_bound 528−>542
code VS
SN 148.458
fwhm 4.884
wb 7.2
TF 0.555
m/z 580.278 : 580.336
XIC
0
40000
80000
2nd derivative
−3000
0
2000
240
260
280
300
320
Time (sec)

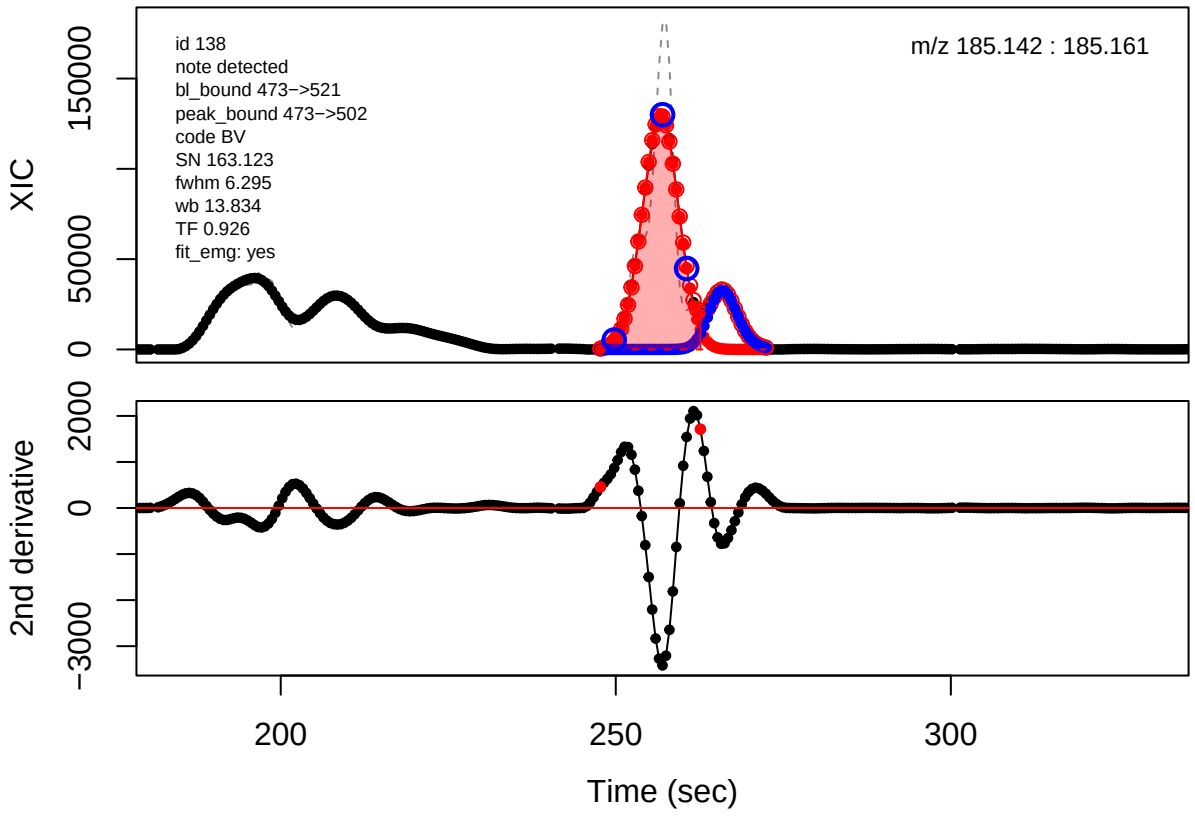

id 179
note detected
bl_bound 491−>545
peak_bound 491−>508
code VV
SN 668.213
fwhm 3.2
wb 7.833
TF 1.303
m/z 188.063 : 188.082
XIC
0e+00
3e+05
6e+05
2nd derivative
−40000
0
240
260
280
Time (sec)

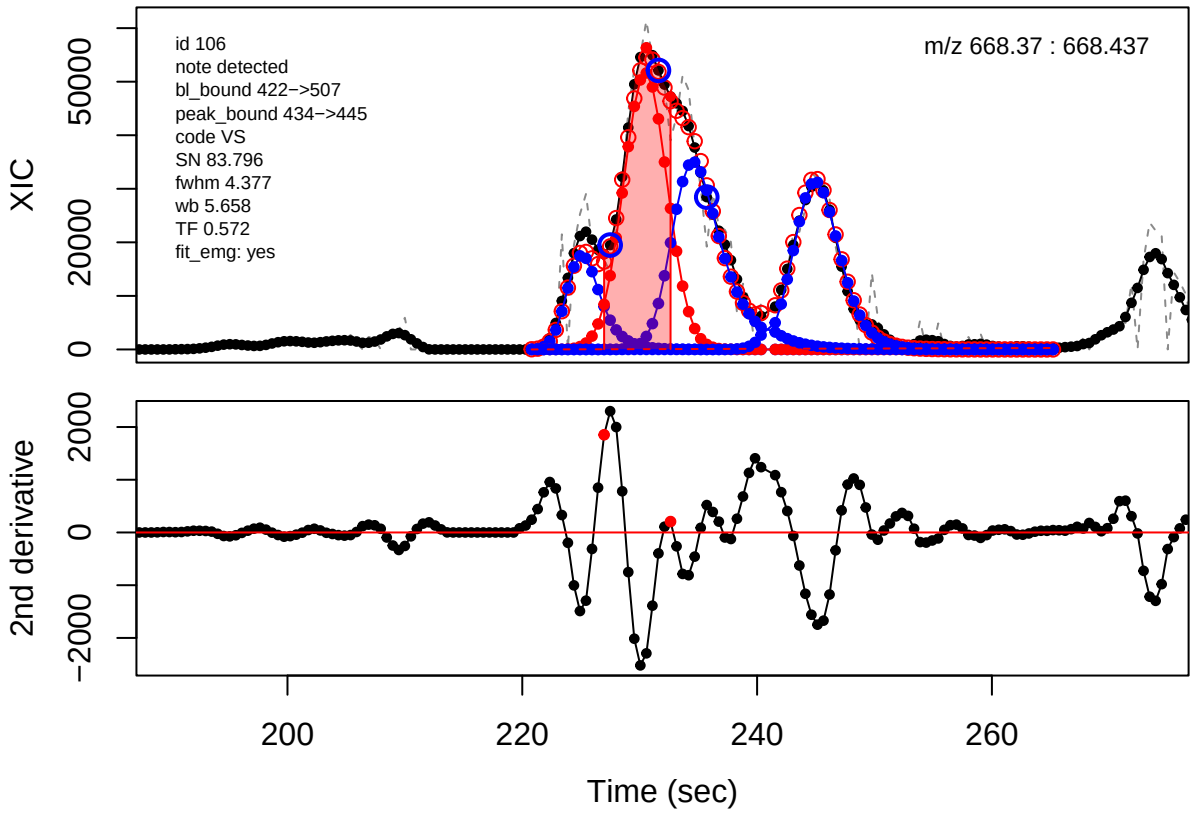


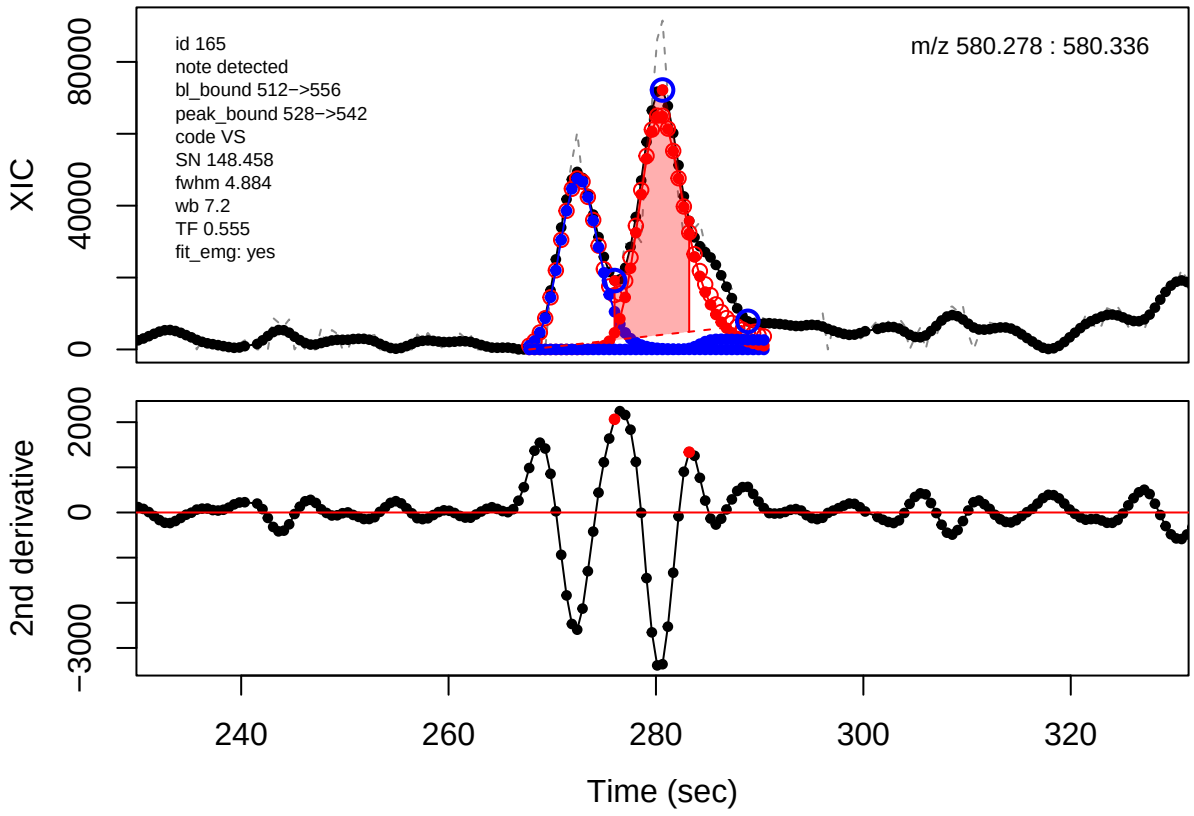

```
cpcParam <- cpc::cpcProcParam(fit_emg = TRUE, sel_peaks = selection, plot = F,
                              save_all = TRUE)
cpcDeconv <- cpc::characterize_xcms_peaklist(xd = xd, param = cpcParam)
#> Processing file: C:\R\library\cpc\extdata\hilic015.mzML
#> Found 6 peak(s).
#> % complete:
#> 10
#> 30
#> 50
#> 60
#> 80
#> 100
#> Done!
#> Finished in 0.788 secs.
```

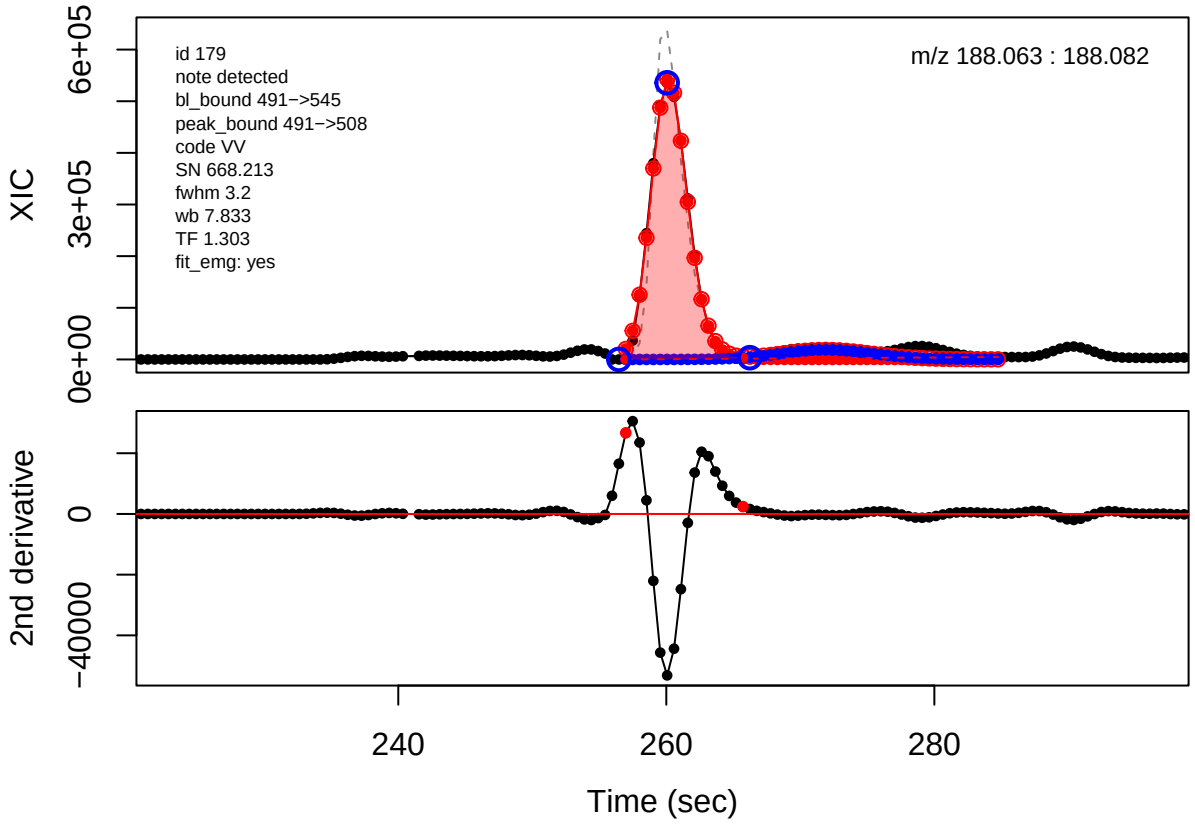
```
cpc::plotPeaks(cpc = cpcDeconv, peakIdx = peakIdx, plotEMG = T)
#> Opening file: C:\R\library\cpc\extdata\hilic015.mzML
```

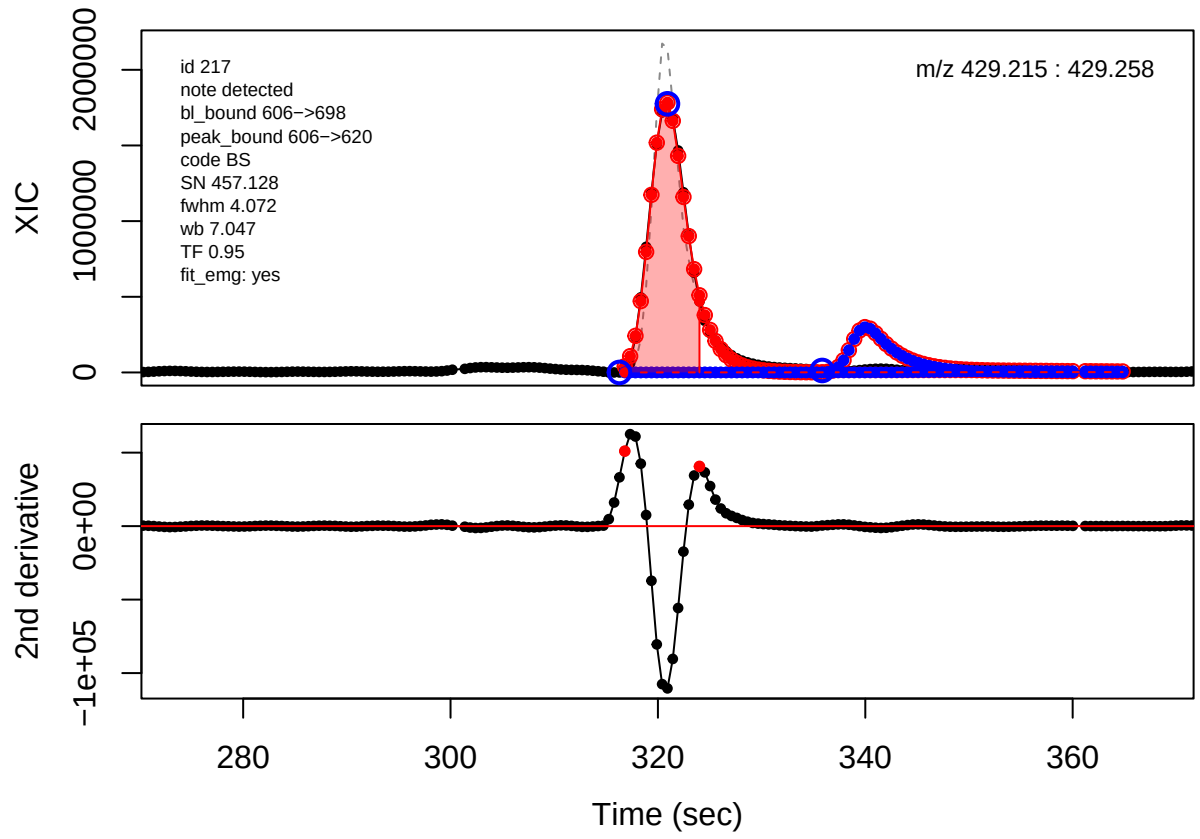

id 34
note detected
bl_bound 66−>187
peak_bound 87−>126
code SV
SN 246.504
fwhm 10.217
wb 20.744
TF 2.188
fit_emg: yes
m/z 320.214 : 320.246
XIC
0e+00
2e+05
4e+05
2nd derivative
−4000
0
0
50
100
150
Time (sec)


id 138
note detected
bl_bound 473−>521
peak_bound 473−>502
code BV
SN 163.123
fwhm 6.295
wb 13.834
TF 0.926
fit_emg: yes
m/z 185.142 : 185.161
XIC
150000
50000
0
2nd derivative
2000
0
−3000
200
250
300
Time (sec)


id 106
note detected
bl_bound 422–>507
peak_bound 434–>445
code VS
SN 83.796
fwhm 4.377
wb 5.658
TF 0.572
fit_emg: yes
m/z 668.37 : 668.437
XIC
0
20000
50000
2nd derivative
−2000
0
2000
200
220
240
260
Time (sec)


id 165
note detected
bl_bound 512−>556
peak_bound 528−>542
code VS
SN 148.458
fwhm 4.884
wb 7.2
TF 0.555
fit_emg: yes
m/z 580.278 : 580.336
XIC
0
40000
80000
2nd derivative
−3000
0
2000
240
260
280
300
320
Time (sec)


id 179
note detected
bl_bound 491−>545
peak_bound 491−>508
code VV
SN 668.213
fwhm 3.2
wb 7.833
TF 1.303
fit_emg: yes
m/z 188.063 : 188.082
XIC
0e+00
3e+05
6e+05
2nd derivative
−40000
0
240
260
280
Time (sec)



Session info

```
sessionInfo()
#> R version 4.1.1 (2021-08-10)
#> Platform: x86_64-w64-mingw32/x64 (64-bit)
#> Running under: Windows 10 x64 (build 17134)
#>
#> Matrix products: default
#>
#> locale:
#> [1] LC_COLLATE=Swedish_Sweden.1252  LC_CTYPE=Swedish_Sweden.1252
#> [3] LC_MONETARY=Swedish_Sweden.1252 LC_NUMERIC=C
#> [5] LC_TIME=Swedish_Sweden.1252
#>
#> attached base packages:
#>  [1] grid      stats4    parallel  stats     graphics  grDevices utils
#>  [8] datasets  methods   base
#>
#> other attached packages:
#>  [1] ggvenn_0.1.9        ggplot2_3.3.5       dplyr_1.0.7
#>  [4] cpc_0.1.0           signal_0.7-7        xcms_3.14.1
#>  [7] MSnbase_2.18.0      ProtGenerics_1.24.0 S4Vectors_0.30.2
#> [10] mzR_2.26.1          Rcpp_1.0.7          Biobase_2.52.0
```

```
#> [13] BiocGenerics_0.38.0 BiocParallel_1.26.2 pander_0.6.4
#>
#> loaded via a namespace (and not attached):
#>  [1] bitops_1.0-7                matrixStats_0.61.0
#>  [3] doParallel_1.0.16           RColorBrewer_1.1-2
#>  [5] GenomeInfoDb_1.28.4         tools_4.1.1
#>  [7] utf8_1.2.2                  R6_2.5.1
#>  [9] affyio_1.62.0               DBI_1.1.1
#> [11] colorspace_2.0-2            withr_2.4.2
#> [13] tidyselect_1.1.1            compiler_4.1.1
#> [15] MassSpecWavelet_1.58.0      preprocessCore_1.54.0
#> [17] DelayedArray_0.18.0         labeling_0.4.2
#> [19] scales_1.1.1                DEoptimR_1.0-9
#> [21] robustbase_0.93-9           affy_1.70.0
#> [23] stringr_1.4.0               digest_0.6.28
#> [25] rmarkdown_2.11              XVector_0.32.0
#> [27] pkgconfig_2.0.3             htmltools_0.5.2
#> [29] MatrixGenerics_1.4.3        highr_0.9
#> [31] fastmap_1.1.0               limma_3.48.3
#> [33] rlang_0.4.12                impute_1.66.0
#> [35] farver_2.1.0                generics_0.1.1
#> [37] mzID_1.30.0                 RCurl_1.98-1.5
#> [39] magrittr_2.0.1              GenomeInfoDbData_1.2.6
#> [41] MALDIquant_1.20             Matrix_1.3-4
#> [43] munsell_0.5.0               fansi_0.5.0
#> [45] MsCoreUtils_1.4.0           lifecycle_1.0.1
#> [47] vsn_3.60.0                  stringi_1.7.5
#> [49] yaml_2.2.1                  MASS_7.3-54
#> [51] SummarizedExperiment_1.22.0 zlibbioc_1.38.0
#> [53] plyr_1.8.6                  crayon_1.4.1
#> [55] lattice_0.20-45             knitr_1.36
#> [57] pillar_1.6.4                GenomicRanges_1.44.0
#> [59] codetools_0.2-18            XML_3.99-0.8
#> [61] glue_1.4.2                  evaluate_0.14
#> [63] pcaMethods_1.84.0           BiocManager_1.30.16
#> [65] vctrs_0.3.8                 foreach_1.5.1
#> [67] gtable_0.3.0                RANN_2.6.1
#> [69] purrr_0.3.4                 clue_0.3-60
#> [71] assertthat_0.2.1            xfun_0.27
#> [73] ncdf4_1.17                  tibble_3.1.5
#> [75] snow_0.4-3                  iterators_1.0.13
#> [77] IRanges_2.26.0              cluster_2.1.2
#> [79] ellipsis_0.3.2
```