

HelmholtzZentrum münchen

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Working with MS² data in XCMS3

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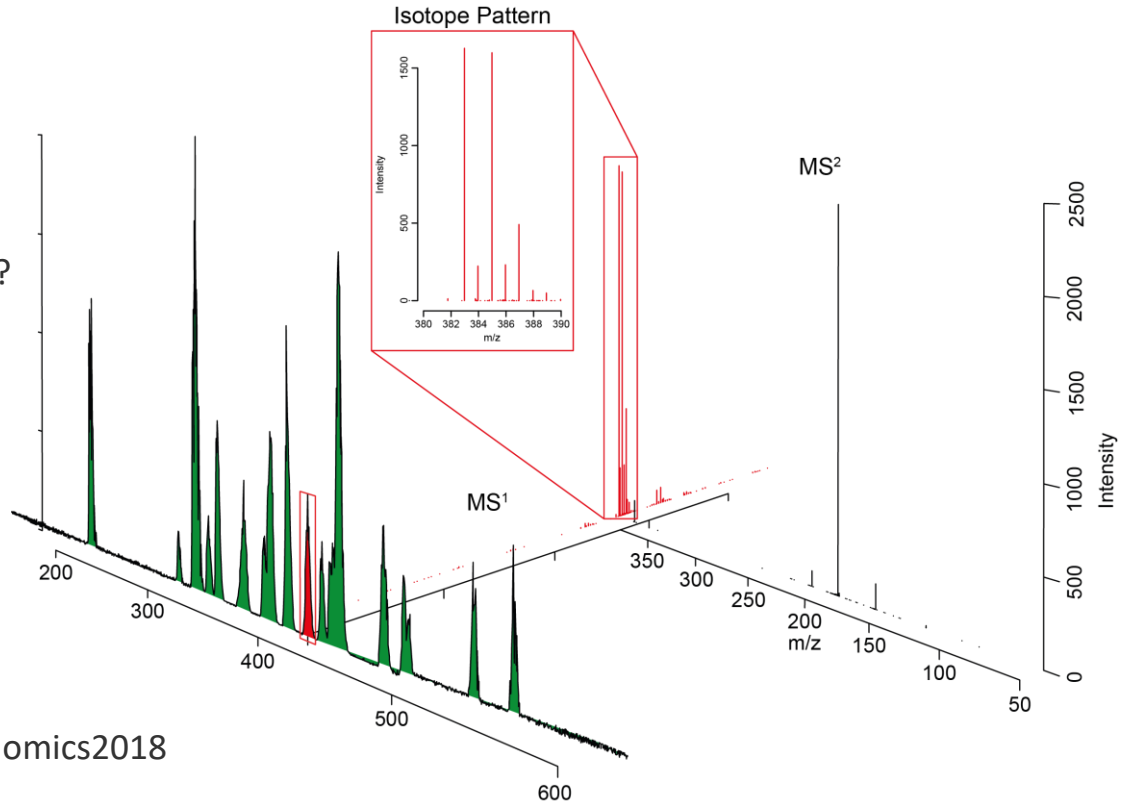
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GROSSE HERAUSFORDERUNGEN

Accessing MS² data in XCMS3

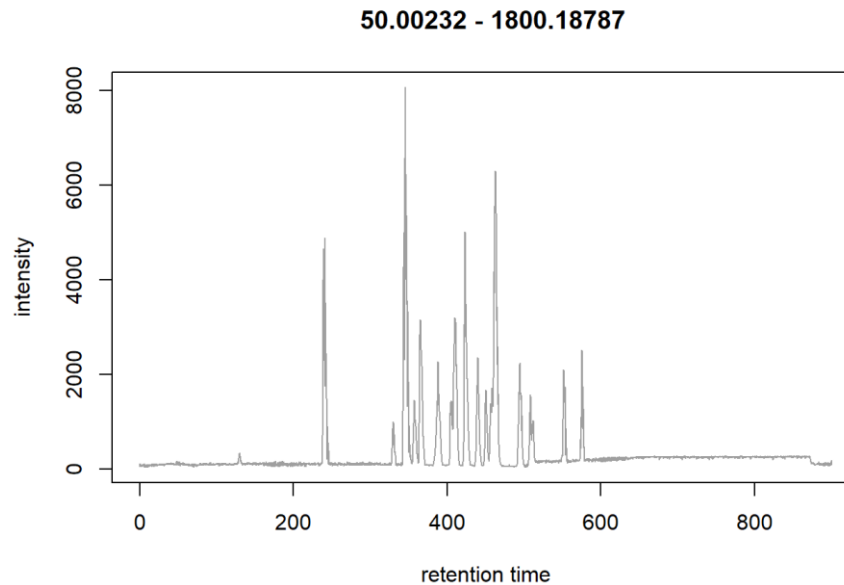
- Important Questions:

- How to access the MS² data?
- What is noise? What is a real MS² spectrum?
- How to further analyze?
- Data available under:
<https://github.com/michaelwitting/metabolomics2018>



Step 1: Reading the raw data

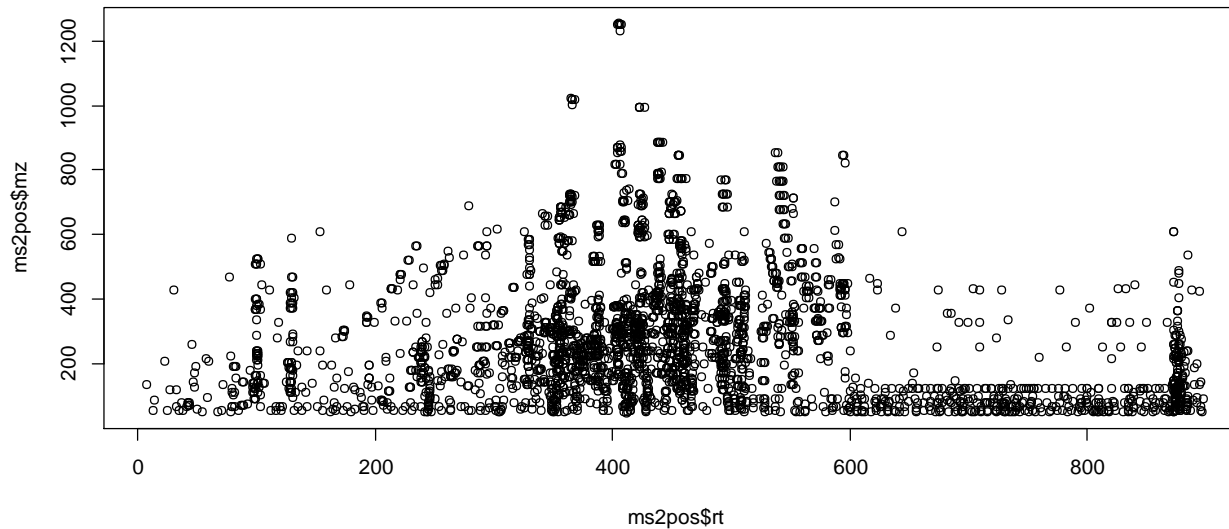
```
## MS1 and MS2 data and file has to be read only once.  
xrms <- readMSData("data\\PestMix1_DDA.mzML", mode = "onDisk", centroided = TRUE)  
  
#plot BPC  
bpcis <- chromatogram(xrms, aggregationFun = "max") plot(bpcis)
```



Step 2: Isolate MS² spectra

```
#get all MS2 spectra from DDA experiment
ms2spectra <- spectra(filterMsLevel(xrms, msLevel = 2))

#plot position of all acquired MS2 spectra
ms2pos <- data.frame(rt = unlist(lapply(ms2spectra, function(x) {return(x@rt)})),
                    mz = unlist(lapply(ms2spectra, function(x) {return(x@precursorMz)})))
plot(ms2pos$rt, ms2pos$mz)
```

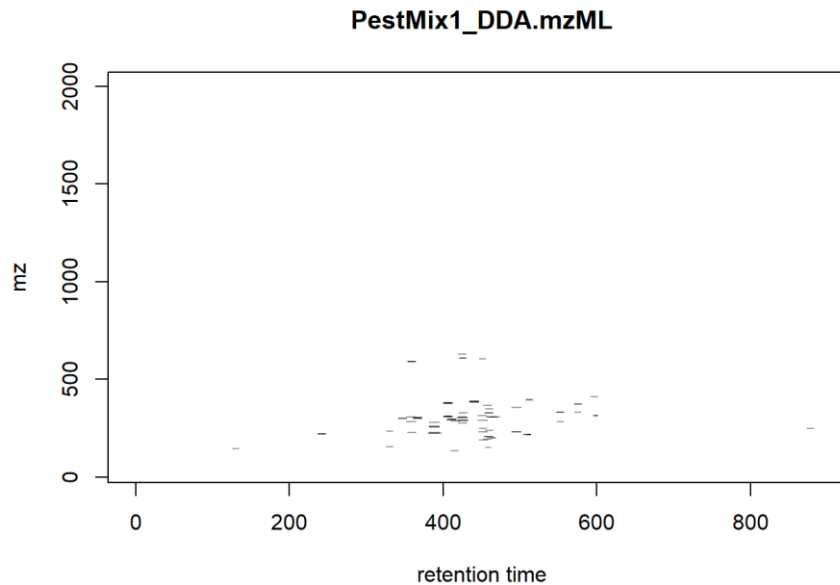


Step 3: Detect chromatographic peaks

```
#set parameters and find chromatographic peaks
mslcwp <- CentWaveParam(snthresh = 5, noise = 100, ppm = 10, peakwidth = c(3,30))
msldata <- findChromPeaks(xrms, param = mslcwp, msLevel = 1)

#get all peaks
chromPeaks <- chromPeaks(msldata)

#check detected peaks
plotChromPeaks(msldata)
```



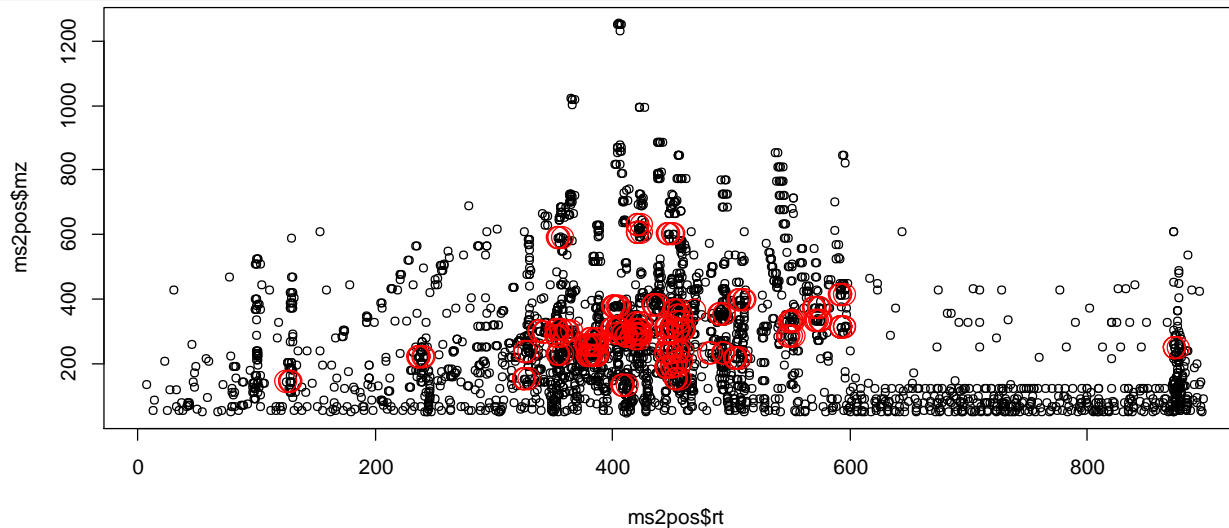
Step 4: Filter MS² spectra

```
#create empty list
filteredMs2spectra <- list()

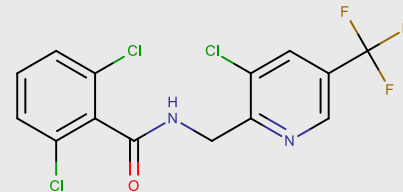
# iterate over all chromatographic peaks and get only spectra in range of peaks
for(i in 1:nrow(chromPeaks)) {
  chromPeak <- chromPeaks[i,]
  filteredMs2spectra_clipboard <- getDdaMS2Scans(chromPeak, ms2spectra)
  filteredMs2spectra <- c(filteredMs2spectra, filteredMs2spectra_clipboard)
}

#plot the filtered spectra as red circles
ms2pos_filtered <- data.frame(rt = unlist(lapply(filteredMs2spectra, function(x) {return(x@rt)})),
                             mz = unlist(lapply(filteredMs2spectra, function(x) {return(x@precursorMz)})))

points(ms2pos_filtered$rt, ms2pos_filtered$mz, col = "red", cex = 2.5)
```



DDA example: Fluopicolide

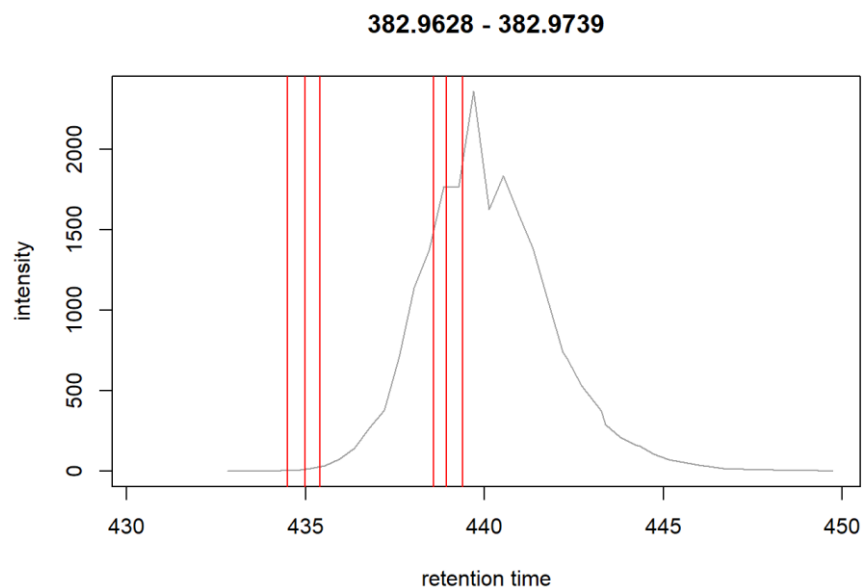


```
#isolate Fluopicolide
chromPeak <- chromPeaks[57,]

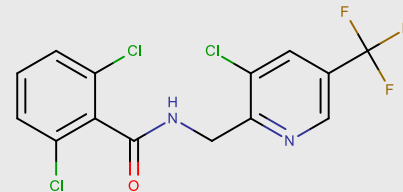
#Fluopicolide, exact mass = 381.965430576, [M+H]+ = 382.972706
eic <- chromatogram(xrms, aggregationFun = "max", mz = c(382.96, 382.98), rt = c(430,450))
plot(eic)

#filter out fitting spectra
filteredMs2spectra <- getDdaMS2Scans(chromPeak, ms2spectra)

#mark position in EIC
abline(v = unlist(lapply(filteredMs2spectra, function(x) {return(x@rt)})), col = "red")
```



DDA example: Fluopicolide

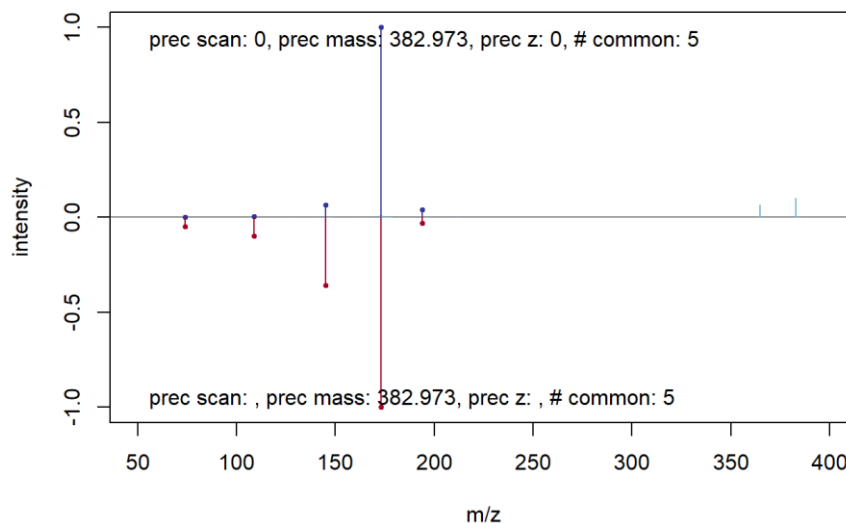


```
#example spectrum (taken from Metlin MID 72270, ESI-Q-ToF)
librarySpectrum <- new("Spectrum2",
  precursorMz = 382.9727,
  mz = c(193.9949, 172.9555, 144.9603, 108.9841, 74.0161),
  intensity = c(3, 100, 36, 10, 5), centroided = TRUE)

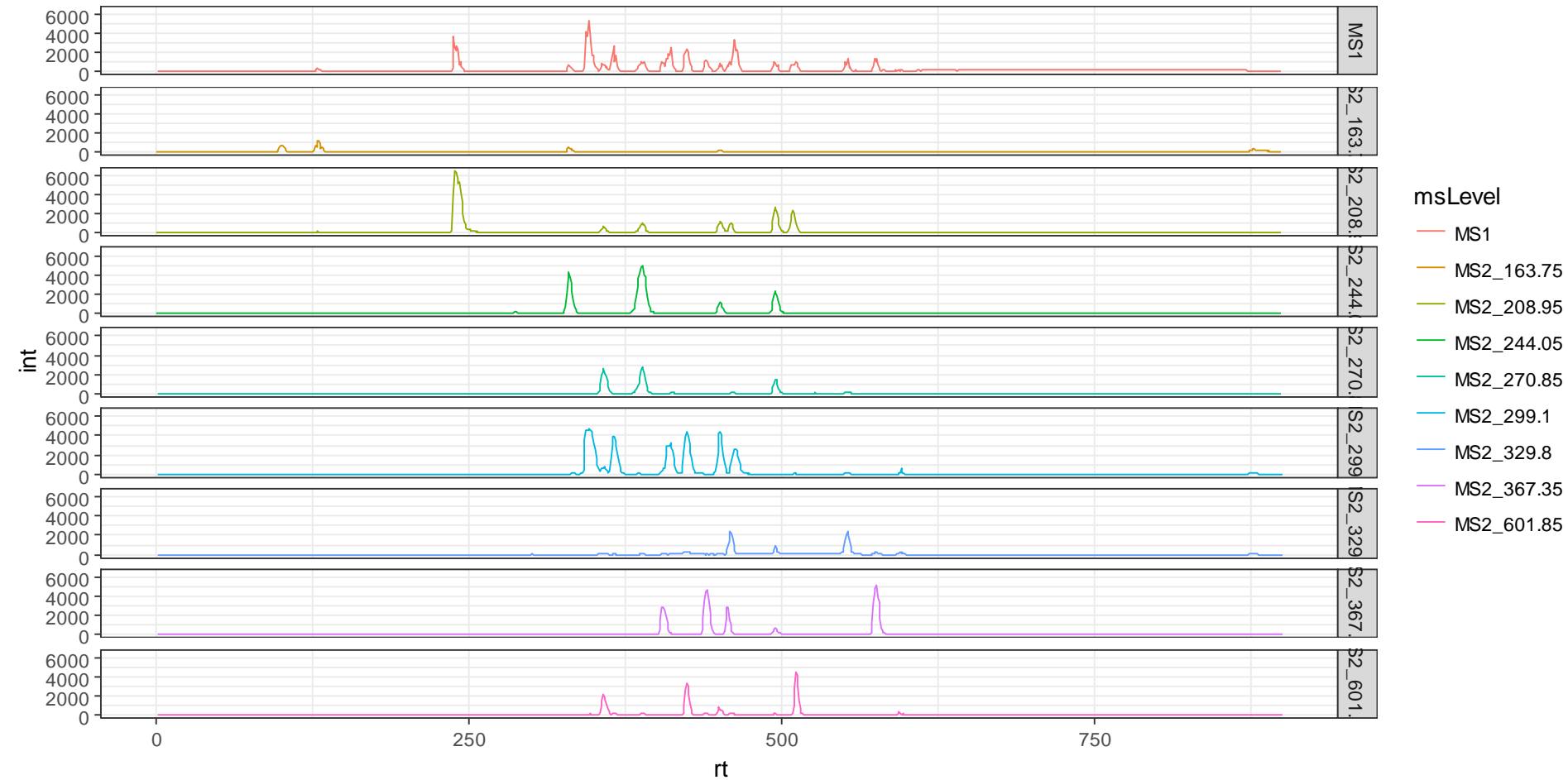
# make mirror plot of two spectra
plot(filteredMs2spectra[[4]], librarySpectrum)

# calculate dot product
compareSpectra(filteredMs2spectra[[4]], librarySpectrum, binSize = 0.01, fun = "dotproduct")

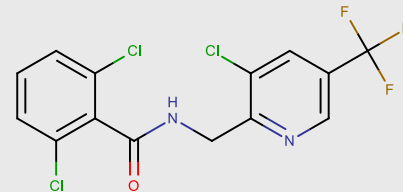
## [1] 0.9488386
```



Preview: DIA data in XCMS

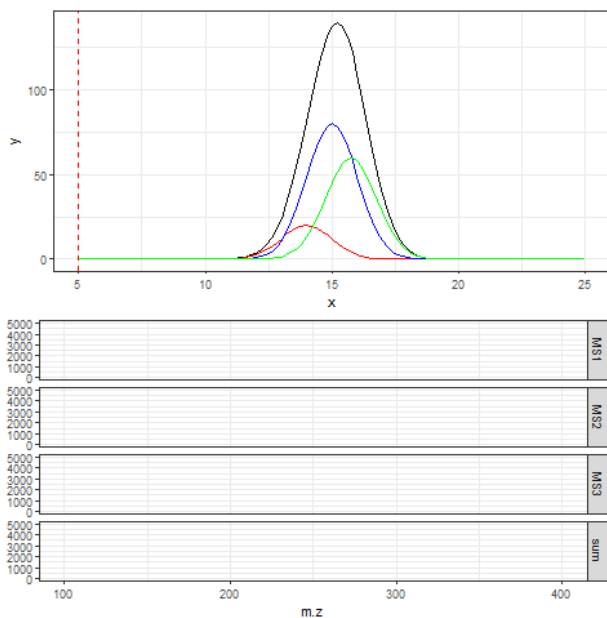


DIA example: Fluopicolide – MS2



- **Reconstruction of MS² spectra from DIA**

- Step 1: Splitting of data into MS¹ and MS² data sets
- Step 2: Splitting of MS² data into defined SWATH pockets
- Step 3: Chromatographic peak detection
- Step 4: Deconvolution of MS² data using EICs correlation
- Step 5: Reconstruction of MS² spectrum



MSMS_383@440.pdf

