Package ‘FAMetA’

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Description Fatty Acid Metabolic Analysis aimed to the estimation of FA import (I), de novo synthesis (S), fractional contribution of the 13C-tracers (D0, D1, D2), elongation (E) and desaturation (Des) based on mass isotopologue data.
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Add missing FA annotations

Usage

```r
addFA(msbatch, dmz = 5, faid, adducts = "M-H", mz, from, to)
```

Arguments

- `msbatch`: annotated msbatch.
- `dmz`: mz tolerance in ppm.
- `faid`: character vector specifying FA names (i.e. "FA(16:1)").
- `adducts`: character vector specifying adducts.
- `mz`: numeric vector specifying FA mz.
- `from`: numeric vector specifying the peak start.
- `to`: numeric vector specifying the peak end.

Value

annotated msbatch.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>
**annotateFA**  
*FA annotation*

**Description**  
FA annotation

**Usage**  
```
annotateFA(msbatch, dmz = 5, rt, adducts = c("M-H"), db)
```

**Arguments**  
- `msbatch`: msbatch obtained from LipidMS package.
- `dmz`: mz tolerance in ppm.
- `rt`: Optional. Numeric vector of length two specifying the rt range to search for FA.
- `adducts`: character vector specifying adducts.
- `db`: FA database. Data frame with three columns: formula, total (number of carbons and double bounds, i.e. "18:1") and Mass.

**Value**  
annotated msbatch.

**Author(s)**  
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

**Examples**
```
## Not run:
# from the msbatch obtained using LipidMS R package
msbatch <- annotateFA(msbatch, dmz = 5)

## End(Not run)
```
blankSubstraction  
*substract blank samples.*

**Description**
substract blank samples.

**Usage**
```r
blankSubstraction(fadata, blankgroup = "blank", verbose = TRUE)
```

**Arguments**
- `fadata`: fadata.
- `blankgroup`: name used to define blank samples group.
- `verbose`: print information messages.

**Value**
blank substracted fadata.

**Author(s)**
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

---

changeFArt  
*Modify rt peak limits of annotated FAs*

**Description**
Modify rt peak limits of annotated FAs

**Usage**
```r
changeFArt(msbatch, id, from, to)
```

**Arguments**
- `msbatch`: annotated msbatch.
- `id`: integer vector specifying FA ids to be modified.
- `from`: numeric vector specifying the peak start.
- `to`: numeric vector specifying the peak end.
correctNatAb13C

Value
annotated msbatch.

Author(s)
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

correctNatAb13C correct data for natural abundance of 13C using accucor algorithm.

Description
correct data for natural abundance of 13C using accucor algorithm.

Usage
correctNatAb13C(fadata, resolution = 140000, purity = 0.99)

Arguments
fadata fadata.
resolution resolution of the mass spectrometer.
purity purity of the tracer employed.

Value
corrected fadata.

Author(s)
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

References
curateFAannotations  Modify FA annotations

Description

after FA annotation using annotateFA, the resulting data frame can be modified to remove rows with unwanted annotation, iniRT and endRT can be changed to redefine peak limits and extra rows may be written to add new annotations. FAid should also be modified to contain unique names such as "FA(16:1)n7" and "FA(16:1)n10" instead of generic "FA(16:1)". For unknown fatty acids use FA(16:1)nx (nx, ny and nz are available for all FA).

Internal standards can also be added to normalize data later. Leave ID and Adducts columns empty, write "IS" at the FAid column and add mz, RT, iniRT and endRT information.

Usage

curateFAannotations(msbatch, faid, dmz = 10, verbose = TRUE)

Arguments

msbatch  annotated msbatch.
faid   data frame with 7 columns (ID, FAid, Adducts, mz, RT, iniRT and endRT) containing curated FAs.
dmz   mz tolerance in ppm.
verbose print information messages.

Details

Modify FA annotations

Value

annotated msbatch.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

## Not run:
# from the msbatch obtained using LipidMS R package
msbatch <- annotateFA(msbatch, dmz = 5)
plots <- plotFA(msbatch, dmz = 10)

pdf("FAs.pdf")
for (p in 1:length(plots)){
```r

print(plots[[p]])
}
dev.off()

write.csv(msbatch$fas, file="faid.csv", row.names=FALSE)

faid <- read.csv("faid_curated.csv", sep="", dec=".")

msbatch <- curateFAannotations(msbatch, faid)

## End(Not run)
```

**dataCorrection**

*Data correction for natural abundance of 13C and data normalization using internal standards followed by blank subtraction.*

**Description**

Data correction for natural abundance of 13C and data normalization using internal standards followed by blank substraction.

**Usage**

```r
dataCorrection(
  fadata,
  correct13C = TRUE,
  blankgroup = "blank",
  externalnormalization = c(),
  resolution = 140000,
  purity13C = 0.99,
  verbose = TRUE
)
```

**Arguments**

- `fadata` : fadata list.
- `correct13C` : logical. If TRUE, data is corrected for natural abundance of 13C. Set to FALSE if data has been already been corrected.
- `blankgroup` : name used to define blank samples group.
- `externalnormalization` : column name at the metadata data frame of any additional measure that must be used to normalize data (i.e. protein).
- `resolution` : resolution of the mass spectrometer.
- `purity13C` : purity of the tracer employed.
- `verbose` : print information messages.
**Value**

corrected fadata.

**Author(s)**

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

**References**


**Examples**

```r
ssdata <- dataCorrection(ssexamplefadata, blankgroup = "Blank")
```

---

`desaturationAnalysis`  
*Desaturation analysis of fatty acids.*

**Description**

Desaturation analysis of fatty acids.

**Usage**

```r
desaturationAnalysis(
  fadata,
  desaturationsdb = FAMeTA::desaturationsdb,
  SEThr = 0.05
)
```

**Arguments**

- `fadata`: fadata containing synthesis and elongation results.
- `desaturationsdb`: desaturation reactions considered. It can be modified to change them or to add new reactions.
- `SEThr`: minimum S or E value allowed to perform estimate desaturations.
Details

Once synthesis and elongation parameters have been estimated, these results can be used to calculate the FA fraction that comes from desaturation in unsaturated FA. For a given unsaturated FA (e.g. FA(18:1n9) we can conceptually consider a one-step elongation-desaturation reaction (in this example directly from FA(16:0) to FA(18:1n9) (E1') or a two-step elongation followed by desaturation process (in this example FA(16:0) is elongated to FA(18:0) (E1) and then desaturated to FA(18:1n9) (Des). Therefore, desaturation can be estimated based on the fraction of E1', which is E1 from FA(18:1n9), and E1, which is E1 from FA(18:0). This same model can be used for all known desaturation steps (see FAMeta::desaturationsdb) as long as precursor and product FA isomers have been correctly and uniquely identified and stationary state has been reached.

Value

fadata list. Desaturation analysis results will be saved at the desaturation element of the fa list.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

```r
ssdata <- dataCorrection(ssexamplefadata, blankgroup = "Blank")
ssdata <- synthesisAnalysis(ssdata, R2Thr = 0.95, maxiter = 1e3, maxconvergence = 100, startpoints = 5)
ssdata <- elongationAnalysis(ssdata, R2Thr = 0.95, maxiter = 1e3, maxconvergence=100, startpoints = 5, D2Thr = 0.1)
ssdata <- desaturationAnalysis(ssdata, SEThr = 0.05)
```

```r
## Not run:
fadata <- dataCorrection(examplefadata, blankgroup = "Blank")
fadata <- synthesisAnalysis(fadata, R2Thr = 0.95, maxiter = 1e3, maxconvergence = 100, startpoints = 5)
fadata <- elongationAnalysis(fadata, R2Thr = 0.95, maxiter = 1e4, maxconvergence = 100, startpoints = 5, D2Thr = 0.1)
fadata <- desaturationAnalysis(fadata, SEThr = 0.05)
```

```r
## End(Not run)
```

---

**desaturationsdb**

Desaturation reactions database.

**Description**

Desaturation reactions database.
Usage

data("desaturationsdb")

Format

A data frame with 12 observations on the following 3 variables.

precursor character vector.
product character vector.
parameter parameter required to estimate desaturation.

Examples

data(desaturationsdb)

elongationAnalysis

Elongation analysis of fatty acids longer than 16 carbons.

Description

Elongation analysis of fatty acids longer than 16 carbons.

Usage

elongationAnalysis(
  fadata,
  R2Thr = 0.98,
  maxiter = 10000,
  maxconvergence = 100,
  startpoints = 5,
  D2Thr = 0.1,
  parameters = FAMetA::parameters,
  verbose = TRUE
)

Arguments

fadata fadata containing synthesis results.
R2Thr positive numeric between 0 and 1 specifying the minimum R2 allowed for fits.
maxiter parameter passed to nls.control. Positive integer specifying the maximum number of iterations allowed.
maxconvergence positive integer specifying the maximum number of successes before choosing the winning model.
startpoints positive integer specifying the number of starting points for each parameter to be estimated.
**elangationAnalysis**

D2Thr  
minimum D2 value allowed to perform the elongation analysis.

parameters  
parameters to be estimated for each fatty acid. It can be modified to change them or to add new fatty acids (adding new rows).

verbose  
print information messages.

**Details**

Main route of de novo synthesis plus elongation starts at 16 carbons and then adds blocks of 2 carbons. Therefore, isotopologue distributions for FA longer than 16 carbons will be modeled taking into account de novo synthesis until FA(16:0), followed by single and independent elongation steps (E1, E2, ..., En). Parameters D0, D1 and D2 are imported from FA(16:0) or FA(14:0) and thus, the only relevant parameters to be estimated in the elongation analysis are Ei and I. For n6 and n3 series, elongation is expected from FA(18:2)n6 and FA(18:3)n3 so that synthesis (S16:0) and first elongation step (E1) are set to 0.

**Value**

fadata list. Elongation analysis results will be saved at the elongation element of the fa list.

**Author(s)**

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

**Examples**

```r
ssdata <- dataCorrection(ssexamplefadata, blankgroup = "Blank")
ssdata <- synthesisAnalysis(ssdata, R2Thr = 0.95, 
maxiter = 1e3, maxconvergence = 100, startpoints = 5)
ssdata <- elongationAnalysis(ssdata, R2Thr = 0.95, maxiter = 1e3, 
maxconvergence=100, startpoints = 5, D2Thr = 0.1)

## Not run:
fadata <- dataCorrection(examplefadata, blankgroup = "Blank")
fadata <- synthesisAnalysis(fadata, R2Thr = 0.95, maxiter = 1e3, 
maxconvergence = 100, startpoints = 5)
fadata <- elongationAnalysis(fadata, R2Thr = 0.95, maxiter = 1e4, 
maxconvergence=100, startpoints = 5, D2Thr = 0.1)

## End(Not run)
```
Description
Example fadata list.

Usage
\begin{verbatim}
data("examplefadata")
\end{verbatim}

Format
A list with 4 elements.
- metadata: data frame with metadata information for samples.
- fattyacids: data frame with compound name and label for each isotopologue (intensities df).
- IS: data frame with IS intensities for each sample.
- intensities: data frame with isotopologue intensities for each sample.

Examples
\begin{verbatim}
data(examplefadata)
\end{verbatim}

Description
External normalization using additional measures (i.e. protein levels).

Usage
\begin{verbatim}
externalNormalization(fadata, externalnormalization, verbose = TRUE)
\end{verbatim}

Arguments
\begin{verbatim}
fadata: fadata list.
externalnormalization: column names of metadata data frame used to define external measures.
verbose: print information messages.
\end{verbatim}

Value
normalised fadata by external measures.
Author(s)
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iisafe.es>

Description
Fatty Acids database.

Usage
data("fattyacidsdb")

Format
A data frame with 35 observations on the following 3 variables.

- `formula` a character vector.
- `total` a character vector. Number of carbons and double bounds.
- `Mass` a numeric vector.

Examples
data(fattyacidsdb)

normalizeIS
Data normalization using internal standards.

Description
Data normalization using internal standards.

Usage
normalizeIS(fadata, verbose = TRUE)

Arguments
- `fadata` fadata list.
- `verbose` print information messages.

Value
normalised fadata by IS.
Author(s)
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

parameters

Parameters for FA metabolic analysis.

Description
Parameters for FA metabolic analysis.

Usage
data("parameters")

Format
A data frame with 167 observations on the following 8 variables.

- **FattyAcid** a character vector.
- **M** integer vector. Number of carbons.
- **S16** De novo synthesis. If equal to 1 it is estimated.
- **E1** a numeric vector. If equal to 1 it is estimated.
- **E2** a numeric vector. If equal to 1 it is estimated.
- **E3** a numeric vector. If equal to 1 it is estimated.
- **E4** a numeric vector. If equal to 1 it is estimated.
- **E5** a numeric vector. If equal to 1 it is estimated.

Examples
data(parameters)

plotFA

Plot FA EICs

Description
Plot FA EICs

Usage
plotFA(msbatch, dmz, verbose = TRUE)
Arguments

- `msbatch`: annotated msbatch.
- `dmz`: mz tolerance in ppm for EIC extraction.
- `verbose`: print information messages.

Value

annotated msbatch with saved plots.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

```r
## Not run:
# from the msbatch obtained using LipidMS R package
msbatch <- annotateFA(msbatch, dmz = 5)

plots <- plotFA(msbatch, dmz = 10)

pdf("FAs.pdf")
for (p in 1:length(plots)){
  print(plots[[p]])
}
dev.off()

## End(Not run)
```

readfadatafile

read FA data from a csv file.

Description

First rows must contain metadata information such as sample groups (row named samptype) and any other extra information like protein levels for external normalization. Then, the following row must contain a Compound and Label columns followed by all sample names. FA names must be unique and omega series must be indicated (i.e. FA(20:4)n3, FA(24:1)n9, FA(16:0)). Unknown FA series can be named as nx, ny, nz to differentiate between isomers. Labels must be specified with integer numbers for 0 to maximum number of carbons.

Usage

```r
readfadatafile(file, sep = ",", dec = ".")
```
removeFA

Arguments

file csv file name.
sep column delimiter.
dec character used for decimal points.

Details

read FA data from a csv file.

Value

fadata.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

## Not run:
```r
defad <- readfadatafile("externafadata.csv", sep="", dec=" .")
## End(Not run)
```

---

removeFA Remove incorrect FA annotations

Description

Remove incorrect FA annotations

Usage

```r
removeFA(msbatch, ids)
```

Arguments

msbatch annotated msbatch.
ids integer vector specifying FA ids to be removed.

Value

annotated msbatch.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>
searchFAisotopes  

Search FA isotopes  

Description  
Search FA isotopes  

Usage  
```r  
searchFAisotopes(msbatch, dmz = 5, coelCutoff = 0.7)  
```  

Arguments  
- `msbatch` annotated msbatch.  
- `dmz` mz tolerance in ppm.  
- `coelCutoff` coelution score threshold between parent and isotope peaks.  

Value  
fadata list.  

Author(s)  
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>  

Examples  
```r  
## Not run:  
# from the msbatch obtained using LipidMS R package and annotated with  
# annotateFA()  
fadata <- searchFAisotopes(msbatch, dmz = 10, coelCutoff = 0.4)  
## End(Not run)  
```  

searchIS  

Search internal standards.  

Description  
Search internal standards.  

Usage  
```r  
searchIS(msbatch, mz, rt, minRT, maxRT, dmz = 10)  
```
ssexamplefadata

Arguments
msbatch annotated msbatch.
mz numeric vector specifying IS mz.
rt numeric vector specifying IS rt.
minRT numeric vector specifying lower limits for IS rt.
maxRT numeric vector specifying upper limits for IS rt.
dmz mz tolerance in ppm.

Value
annotated msbatch.

Author(s)
M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Description
Example fadata list.

Usage
data("ssexamplefadata")

Format
A list with 4 elements.
metadata data frame with metadata information for samples.
fattyacids data frame with compound name and label for each isotopologue (intensities df).
IS data frame with IS intensities for each sample.
intensities data frame with isotopologue intensities for each sample.

Examples
data(ssexamplefadata)
summarizeResults

Obtain result tables and heatmaps that help interpreting your results.

Description

Obtain result tables and heatmaps that help interpreting your results.

Usage

summarizeResults(fadata, controlgroup = NA, parameters = FAMetA::parameters)

Arguments

- fadata: fadata containing synthesis, elongation and desaturation results.
- controlgroup: name of the control group to compare the results.
- parameters: parameters to be estimated for each fatty acid. It can be modified to change them or to add new fatty acids.

Value

fadata list with a results element which contains: results data frame (results for the main parameters for each fatty acid and sample), summary data frame (mean and sd by sample groups for each parameter and fatty acids from the results table), different heatmaps representing pool size and results (values represented are also saved in data frames) and tables summarizing all parameters values for synthesis and elongation (S16, E1, E2, E3, E4 and E5).

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

```r
ssdata <- dataCorrection(ssexamplefadata, blankgroup = "Blank")
ssdata <- synthesisAnalysis(ssdata, R2Thr = 0.95, 
maxiter = 1e3, maxconvergence = 100, startpoints = 5)
ssdata <- elongationAnalysis(ssdata, R2Thr = 0.95, maxiter = 1e3, 
maxconvergence = 100, startpoints = 5, D2Thr = 0.1)
ssdata <- desaturationAnalysis(ssdata, SEThr = 0.05)
ssdata <- summarizeResults(ssdata, controlgroup = "Control13Cglc")
```

```r
## Not run:
fadata <- dataCorrection(examplefadata, blankgroup = "Blank")
fadata <- synthesisAnalysis(fadata, R2Thr = 0.95, maxiter = 1e3, 
maxconvergence = 100, startpoints = 5)
fadata <- elongationAnalysis(fadata, R2Thr = 0.95, maxiter = 1e4, 
maxconvergence = 100, startpoints = 5, D2Thr = 0.1)
```
synthesisAnalysis <- desaturationAnalysis(fadata, SEThr = 0.05)
fadata <- summarizeResults(fadata, controlgroup = "Control13Cglc")

## End(Not run)

synthesisAnalysis

*De novo synthesis analysis of fatty acids until 16 carbons.*

### Description

De novo synthesis analysis of fatty acids until 16 carbons.

### Usage

```r
synthesisAnalysis(
  fadata,
  R2Thr = 0.98,
  maxiter = 1000,
  maxconvergence = 100,
  D1 = NA,
  D2 = NA,
  P = NA,
  startpoints = 5,
  parameters = FAMetA::parameters,
  propagateD = TRUE,
  verbose = TRUE
)
```

### Arguments

- **fadata**: fadata obtained from the msbatch with `searchFAisotopes` function or read from csv file with `readfadatafile` function.
- **R2Thr**: positive numeric between 0 and 1 specifying the minimum R2 allowed for fits.
- **maxiter**: parameter passed to `nls.control`. Positive integer specifying the maximum number of iterations allowed.
- **maxconvergence**: positive integer specifying the maximum number of successes before choosing the winning model.
- **D1**: positive numeric between 0 and 1 specifying the contribution of acetate M+1. If NA it is estimated.
- **D2**: positive numeric between 0 and 1 specifying the contribution of acetate M+2. If NA it is estimated.
- **P**: overdispersion parameter. If NA it is estimated (quasi-multinomial distribution). If set to 0, no overdispersion is assumed (multinomial distribution).
- **startpoints**: positive integer specifying the number of starting points for each parameter to be estimated.
parameters parameters to be estimated for each fatty acid. It can be modified to change them or to add new fatty acids.

propagateD logical. If TRUE, unsaturated fatty acids use estimated D0, D1, D2 and P values for saturated fatty acids (14:0 for FA shorter than 16C and 16:0 for FA with 16C).

verbose print information messages.

Details

Synthesis analysis will model FA data for FA up to 16 carbons to estimate 13C-tracer contribution to the acetyl-CoA pool for FA synthesis (D) and the FA fraction that has been synthesized de novo. D0, D1 and D2 represent the contribution of M+0, M+1 and M+2 acetate, respectively, and P (phi) is the overdispersion parameter of the quasi-multinominal distribution. D0, D1, D2 can also be fixed if they are known. This is particularly useful in case inhibitors have been used as they could reduce S below the confidence interval and thus, S and D parameters could be misestimated.

Value

fadata list. Synthesis analysis results will be saved at the synthesis element of the fa list.

Author(s)

M Isabel Alcoriza-Balaguer <maribel_alcoriza@iislafe.es>

Examples

```r
ssdata <- dataCorrection(ssexamplefadata, blankgroup = "Blank")
ssdata <- synthesisAnalysis(ssdata, R2Thr = 0.95, maxiter = 1e3, maxconvergence = 100, startpoints = 5)

# Not run:
فادata <- dataCorrection(examplefadata, blankgroup = "Blank")
فادата <- synthesisAnalysis(فادата, R2Thr = 0.95, maxiter = 1e3, maxconvergence = 100, startpoints = 5)
# If inhibitors have been used, make sure D2 has not been underestimated. If so, D2 could be set as the one calculated for 13-Glc Control samples to improve the results:
# D2 <- فادата$analysis$results$D2[examplefadata$analysis$results$FA == "FA(16:0)"]
# فاداته$analysis$results$Group[examplefadata$analysis$results$FA == "FA(16:0)"]
# D2[4:12] <- rep(mean(D2[1:3]))
# relaunch synthesis analysis fixing D2
# فاداة <- synthesisAnalysis(فاداة, R2Thr = 0.95, maxiter = 1e3, #
# maxconvergence = 100, startpoints = 5, D2 = D2)

# End(Not run)
```
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