BART User Manual

Version 1.1, May 30, 2023

1. Prerequisite

BART is a program developed in Java to predict gradient retention times of bile acids on different LC-MS instruments based on isocratic retention modeling. The Java run time environment (JRE 15) required by BART has been bundled with the program. Currently the database only supports the following LC condition:

Column: Waters BEH C18 (2.1 mm × 100 mm, 1.7 µm)
Mobile phase A: 7.5 mM ammonium acetate (adjusted to pH 4.9 using acetic acid) in water
Mobile phase B: ACN
Flow rate: 0.45 mL/min
Column temperature: 45 ℃

CRITICAL STEP

1.It is very important that mobile phase A is prepared accurately, and the chemicals used are of high quality and fresh. We recommend preparing it by weighing using the following protocol:

Reagents
ammonium acetate (Sigma-Aldrich, 73594, LiChropur™, eluent additive for LC-MS)
acetic acid (Sigma-Aldrich, 695092, glacial, ACS reagent, ≥99.7%)
acetonitrile (Fisher Chemical, A955-4, Optima LC/MS grade)

Procedure

a. Measure 996.7 g water in a 1 L clean, dry bottle (mobile phase A).
b. Measure 578.1 mg ammonium acetate in a 1.5 mL eppendorf tube.
c. Transfer 1 mL water from the 1 L bottle to the 1.5 mL tube containing the ammonium acetate and vortex. Pour the solution back into the 1 L bottle.
d. Repeat step c three more times.
e. Measure 297.6 mg acetic acid in another 1.5 mL tube rapidly.
f. Pour the acetic acid into the 1 L bottle and wash the tube with mobile phase A.
g. Mix the contents of the 1 L bottle thoroughly.

2. If the LC system has an active preheater (e.g. Waters UPLC), it needs to be uninstalled and replaced by an appropriate tubing (preferred) or disabled to be consistent with the condition of the database.

3. Run a series of bile acid reference standards under the above LC condition with customized gradient and record their retention times. These experimental retention times are used to correct for the differences of extra-column volume and dwell volume between instruments, as well as small imperfection of actual gradient. The following 21 bile acids are recommended as calibration compounds:

LCA	CDCA	DCA	UDCA	CA	
TLCA	TCDCA	TDCA	TUDCA	TCA	Tα-MCA
LCA-3S	CDCA-3S	DCA-3S	UDCA-3S	CA-3S	
TLCA-3S	TCDCA-3S	TDCA-3S	TUDCA-3S	TCA-3S	

NOTE
1. CDCA. DCA and UDCA are isomers. Prepare them in separate mixes if necessary.
2. If the above bile acid standards are not available, slight modifications are acceptable, such as replacing Tα-MCA with Tβ-MCA, or replacing TBA and TBA-3S with GBA and GBA-3S. Removing CDCA and UDCA series may increases the prediction errors slightly. It is not recommended to remove Tα-MCA/Tβ-MCA.

2. Prepare the input files

Three input files were required by BART. They are all text file (.txt) separated by tab and could be prepared easily using Excel and then copied to a text editor such as Notepad.

1.Isocratic retention model library

The retention library for 272 bile acids under default LC condition is provided with BART. Users can also edit the library or make their own library as long as the following format is kept:

name class m/z c_0 c_1 c_2

c_0, c_1 and c_2 are the adjustable parameters of quadratic solvent strength model (QSSM):

$$\boldsymbol{lnk = c_0 - c_1\varphi + c_2\varphi^2}$$

2. gradient profile

The gradient file contains two columns: 1) time in minute; 2) the fraction of mobile phase B. Below is an example:

0.5	0.2
13.5	0.4
15.5	0.6
18	0.7

3. experimental gradient retention times of compounds

The experimental retention time file contains three columns, 1) name of compound, which needs to be exactly same as the name in the isocratic library; 2) experimental retention time in minute; 3) a label for the calibration compound (cal). If the compound is not labeled, it will not be used as the calibration compound, but the predicted retention time and error will still be calculated. Below is an example:

LCA;BA	16.4	cal
LCA;BA-3S	12.19	cal
LCA;GBA	12.2	
LCA;GBA-3S	8.18	
LCA;TBA	12.28	cal
LCA;TBA-3S	7.96	cal

3. To start

BART can be started from batch file on 64-bit Windows system. Support for Mac OS is coming soon.
To create the batch file, open a text editor such as Notepad and put in the following text. Save it as a .bat file and double click to run it.

BART_win.exe lib=argument1 gradient=argument2 ref=argument3 output=argument4
(optional) noSrefAll=TRUE t0=argument5 tD=argument6

Explanation:
BART_win.exe: the full pathway and name of the exe file
argument1: the full pathway and name of the retention library
argument2: the full pathway and name of the gradient file
argument3: the full pathway and name of the experimental retention time file
argument4: the full pathway and name of the output file
Below are optional parameters:
noSrefAll=TRUE: to use all calibration standards to correct for none-sulfated bile acids, only useful when Tα-MCA/Tβ-MCA is not available.
argument5: starting point of the optimization of holdup time in minute. Default value is 0.58 min.
argument6: starting point of the optimization of dwell time in minute. Default value is 0.48 min.

Example:
D:\BART_win64\BART_win.exe lib=D:\Data\lib.txt gradient=D:\Data\gradient.txt ref=D:\Data\caliRT.txt output=D:\Data\predictedRT.txt

NOTE
1.The arguments were separated by space. Therefore, no space is allowed in the pathway and file name.
2. If JDK 15 was installed on the computer, java -jar BART.jar also works.

4. Output Files

The output is a tab-separated text file that could be opened by Excel by drag-and-drop. It is composed of a brief summary of the prediction results and the list of predicted retention times and errors (if the experimental retention time is available) for all compounds in the isocratic retention database. By default, two sets of results are reported, one with all compounds calculated together, the other with sulfated and none-sulfated compounds calculated separately.

Below is an example of result summary:

optimized t0: 0.61
optimized tD: 0.34
optimized time: [0.5, 13.5, 15.4, 18.1]
optimized perB: [0.2, 0.4, 0.58, 0.72]
average/RMS error of calibration compounds after optimization: 0.01/0.023
average/RMS error of all compounds after optimization: 0.018/0.034

The predicted retention times could be formatted and copied to a separate .txt file to be used for compound identification with BAFinder. Please see the user manual for BAFinder for more details.

5. Contact us

To report bugs, ask questions and offer suggestions, please contact us by email: mayan@nibs.ac.cn.

Thanks for using BART!