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Operation Manual of GUI-based T-SEN

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Hardware Requirements for TSEN.exe

- OS: Windows XP, Vista, 7 (English Edition)
- This version is only for 64 bit OS.
- More than 10 GB RAM is recommended for large data.
- Installation of Statistical software “R” is required. Freely downloadable from the site below.
- <http://cran.md.tsukuba.ac.jp/bin/windows/base/>
- netCDF data from Agilent, Waters, JEOL (GC and LC) have been confirmed to work.
- Don't touch the computer keyboard during first minute, because this tool automatically types the required information instead of you. Please save and close important files before starting the tool (Don't place the important files at “My documents”, for safety.).
- Data for demonstration is available via the following link (Japanese site).
- <http://www.nies.go.jp/analysis/member.html#downloads>

How to Operate 1

The screenshot shows the T-SEN exe special edition (64 bit version) window. It has a blue title bar and a light beige background. The window is divided into several sections. On the left side, there are five numbered steps in white boxes with blue borders: 1, 2, 3, 4, and 5. The main area of the window contains the following sections:

- Choose files -**
 - Step 1: Select .cdf file for T-SEN processing. Below this is a button labeled "File...".
 - Step 2: Select database .csv file for T-SEN processing. Below this is a button labeled "File...".
- Select save files position -**
 - Step 3: Select save folder and input file name. Two chromat pictures (.jpg) and a result file (.csv) are created. Below this is a button labeled "File...".
- T-SEN Package installation -**
 - Step 4: ☐ Install Packages. Check that you are online!
☒ Package T-SEN and ncdf are installed
- Check your R version -**
 - Step 5: R-2.14.2 Input your R version for using.
☐ Place of R folder is [Program files]. Check off if C drive directly
☐ R is 32 bit version. T-SEN with spetial edition is needed.
- Separation parameter** (selected) / **Mass parameter**
 - A dropdown menu shows "Two-dimensional" with a downward arrow. To its right is the text "Select dimension."
 - Below this are several input fields with labels:
 - 70 BaseIS.search: Search range of base IS for RT correction in entire chromatogram (\pm sec).
 - 0.1 Range.correct: The degree of peak range correction after peak finding (\pm sec).
 - 8 search.range.x-axis: Search range in first GC (\pm sec). Rounded to MPereod each in 2D.
 - 0.5 Search.range.y-axis: Search range of a peak in second GC (\pm sec). 2D only
 - 4 MPeriod: The modulation time period.
 - 1.3 Phase.shift: The time of phase shift.
- At the bottom right is a button labeled "T-SEN run".

① Choose the data that you want to analyze (.cdf).

② Choose the database file that includes your target compounds (.csv).

③ Set the output folder and file name.

④ In the first time, installation of program packages are needed. Choose the upper one.

⑤ Set file path for calling "R". You need to recognize where and which version of "R" is installed on your computer. Located at C drive directly or "Program Files"? Is this a 64 bit OS? Is the version number right?

How to Operate 2

☐ It is 32 bit version. T-SEN with special edition is needed.

Separation parameter Mass parameter

 Select dimension.

 BaseIS.search: Search range of base IS for RT correction in entire chromatogram (\pm sec).

 Range.correct: The degree of peak range correction after peak finding (\pm sec).

 search.range.x-axis: Search range in first GC (\pm sec). Rounded to MPeriod each in 2D.

 Search.range.y-axis: Search range of a peak in second GC (\pm sec). 2D only

 MPeriod: The modulation time period.


 Phase.shift: The time of phase shift.

- Set the parameter of separation in your data. 1D or 2D data?
- In case of 1D, "Search.range.y-axis", "Mperiod" and "Phase.shift" are not used, so you don't need to change the default settings.
- If you apply LC data, the values of "search.range.x-axis" and "Range.correct" should be set to larger values. Be sure that the default setting is for GCxGC.

How to Operate 3

✓ R is 32 bit version. T-SEN with special edition is needed.

Separation parameter **Mass parameter**

Accurate MS  Select MS type.


 CountMS: The number of MS counting for searching/assignment of target compounds.

Tolerance (m/z) = HRwide.order × HRwide.order.fluc

 HRwide.order: The decimal point position in accurate mass.

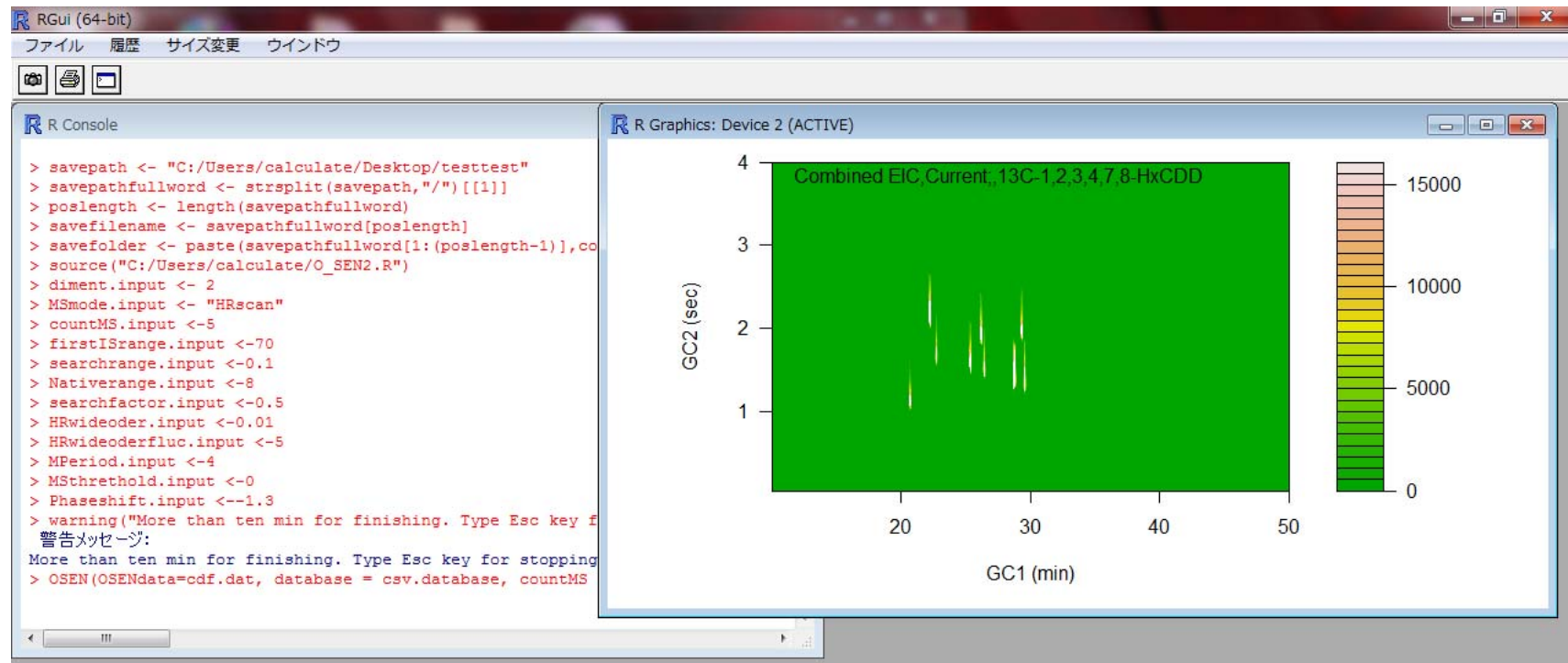
 HRwide.order.fluc: The degree of fluctuation.

 MSthreshold: The minimum threshold of ion intensity.



- Set the parameter of Mass in your data.
- Accurate or Nominal MS?
- If you chose “Accurate MS”, information on mass accuracy (Tolerance) is needed. Input the parameter according to the screen displays.
- In the case of nominal MS, “HRwide.order” and “Hrwide.order.fluc” is not used, so you don’t need to change the default setting.
- After setting all the parameters, click the “T-SEN run” button.

Screenshot of the Tool Execution



- If all the parameters are correctly set, “R” will boot up and your setting will be loaded. Don’t touch to keyboard during this process.
- If all the code is loaded in “R”, target search will be started.
- Generally, it takes 4 seconds for 1 compound search in GCxGC data. If you have 100 compound list, it takes around 10 minutes for all the calculation.
- After the calculation, you can find the output files, such as XXXTIC.jpeg, XXXCombinedEIC.jpeg and XXX.csv (the file names depends on your setting), in the folder you set.

Output (.csv) File

Sum of Ion intensity
(see *Analytica Chimica Acta*, 778, (17), 54–62, 2013. for details)

The positions (RT1, RT2 and pixel)
where the peaks were detected

Intensity ratios of native to IS.

	A	B	C	D	E	F	G	H	I	J	K	L	M		
1		Compound	Combined	SN	Noise	canc	Noise	num	RT1 (min)	RT2 (sec)	peak top	ID of IS/Native	ID of IS	ISratio	ISratio_fitting
2	1	13C-2,3,7,8-P	300909.6	165.5138	8	71	22.21	2.02	18534	1	0	0	0		
3	2	13C-1,3,6,8-P	372666	2145.417	5	59	20.68	1.19	16190	1	1	0	0		
4	3	13C-2,3,7,8-P	551239.8	1226.493	3	57	22.21	1.98	18533	1	2	0	0		
5	4	13C-2,3,7,8-P	398560.1	4836.689	1	59	22.75	1.47	19328	1	3	0	0		
6	5	13C-1,2,3,7-P	456085.9	Inf	0	57	25.35	1.07	23257	1	4	0	0		
7	6	13C-2,3,4,7-P	496599.3	1372.298	4	55	26.15	1.35	24476	1	5	0	0		
8	7	13C-1,2,3,7-P	344504.9	721.0077	8	60	26.41	0.87	24868	1	6	0	0		
9	8	13C-1,2,3,7-P	719750.6	336.2663	3	74	28.75	0.52	28394	1	7	0	0		
10	9	13C-1,2,3,7-P	861053.5	337.5983	5	74	28.75	0.52	28394	1	8	0	0		
11	10	13C-2,3,4,7-P	418019.4	1365.074	4	73	29.28	1.03	29215	1	9	0	0		
12	11	13C-1,2,3,7-P	622425.3	Inf	0	71	29.55	0.36	29602	1	10	0	0		
13	12	13C-1,2,3,7-P	537917.3	129.2953	11	61	29.55	0.36	29602	1	11	0	0		
14	13	13C-1,2,3,7-P	351636.3	723.8645	8	61	29.75	0.63	29912	1	12	0	0		
15	14	13C-1,2,3,7-P	413853.1	1156.906	4	56	30.01	0.79	30320	1	13	0	0		
16	15	13C-1,2,3,7-P	369360.7	721.2165	6	66	31.35	0.2	32325	1	14	0	0		
17	16	13C-1,2,3,7-P	255620.4	3623.173	1	58	32.35	0.16	33839	1	15	0	0		
18	17	13C-1,2,3,7-P	369778.1	504.0314	1	70	32.81	0.24	34548	1	16	0	0		
19	18	13C-OCDF	407048.6	3542.264	1	84	34.88	0.16	37677	1	17	0	0		
20	19	13C-OCDF	443746.7	344.8065	6	61	35.01	0.2	37880	1	18	0	0		
21	20	2,3,7,8-TC	2665337	5567.459	4	61	22.21	2.02	18534	0	2	4.835168	0		
22	21	2,3,7,8-TC	2095995	1425.677	12	70	22.75	1.47	19328	0	3	5.258918	0		
23	22	1,2,3,7,8-P	2480828	1882.569	6	67	25.35	1.07	23257	0	4	5.439389	0		
24	23	2,3,4,7,8-P	2506915	5026.745	5	60	26.15	1.35	24476	0	5	5.048164	0		
25	24	1,2,3,7,8-P	1571282	4949.105	2	65	26.41	0.87	24868	0	6	4.560985	0		

- The results of compound assignment and its ion quantified value is reported (Sum of ion intensity is calculated and adjusted by several ions which you set, and finally the intensity of the first ion in the database is returned.). The intensity ratio of native to IS whose combination is set in database is also calculated.

About the Database

Compound ID

IDs below 0 are not used for quantification. They are just used for RT correction.

RT correction

1: RT is corrected based on this compound peak.

0: RT is not corrected.

IS (1) or native (0)?

Please always start from 1.

* Put the number of compound ID of IS corresponding to the native (IS and native combination is determined from this setting). In the case of IS, just use the IS number.

MS fragment

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
	BlobID	Compound Abreviation	Group Name	Rtcorrect	Internal Standard	Peak I	Peak II	IS	Native ref	IS	Native	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9	MS10	MS11	MS12
1	-2	13C-2,3,7,8-TCDF	PCDD	1		22.24	0.834			0		317.95283	315.94825	319.93246	318.92842	316.93021	321.92677						
2												15.265677	12.887926	9.9872899	4.4107563	2.5250874	2.3886481	1					
3	1	13C-1,3,6,8-TCDD	PCDD	0		20.63	0.001			1		333.9487	331.94479	335.93219	267.97001	269.96701	204.00319	3					
4												12.049672	10.114774	8.0782124	6.1127712	5.8802501	2.8517746	2					
5	2	13C-2,3,7,8-TCDF	PCDD	4		22.24	0.834			2		317.95283	315.94825	319.93246	251.9708	253.96762	182.03453	3					
6												15.265677	12.887926	9.9872899	4.4107563	2.5250874	2.3886481	1					
7												305.89596	303.89921	307.89436	240.9406	242.93661	171.00252	3					
40	19	2,3,7,8-TCDF	PCDD	0		22.24	0.834			2		22.536902	17.63751	10.841941	4.0036323	3.9958859	3.8846943						
41												321.89055	319.88321	323.88787	256.93117	258.92932	193.97054	3					

Native

- Calculation starts from the top of this list. It start from IS, then the same group of native is calculated. After that, ISs of next group are started again. The same process is repeated over other chemical list in this order.
- Information of one compound is stored in two rows. First row is for m/z. Second row is for relative intensity (total should be 100%).
- Cells from "I" to "P" is available for remarks, in case you want to use them.
- In the calculation, IS or native is judged by the information in the cell "E" (IS=1, Native=0).
- Corresponding ID of IS should be put in the cell "H" for its Native. Intensity ratio is calculated from this combination. In the case of IS, you don't need to enter a value.

Precautions

-About the source file

- Keep the position of source file “T_SEN.R” with the same position of TSEN.exe file.

-If the software freeze, exit from it by clicking icon in bottom light.

-License: Artistic License 2.0

-Disclaimer: Any hard- or software damages, data losses and false inferences caused by using this software is not guaranteed.

-Please cite this tool as *Zushi et al., Anal. Chim. Acta (2013) 778, 54-62.*