Part VI: Creating and Using Retention Indices in NIST Software

12/3/2022

James Little
tvasailor@gmail.com
https://littlemsandsailing.wordpress.com/
"Little Mass Spec and Sailing" Website
Kingsport, TN

- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services.*

Eastman Chemical Company, Main Site, Kingsport, TN
50 Manufacturing Sites Worldwide, ~14,500 Employees

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company

NIST Retention Indices (RI) Resources

- RI's used in combination with mass spectrometry to provide nearly unambiguous identification of isomers
- Very difficult task to accomplish with EI mass data alone
- NIST offers extensive resources including:
  - RI's for >139K compounds
  - RI and MS data >114K compounds
  - GC methods and RI citations from >400K literature references
- AMDIS to determine RI's for compounds in GCMS analyses
- Results sent to NIST search for evaluation

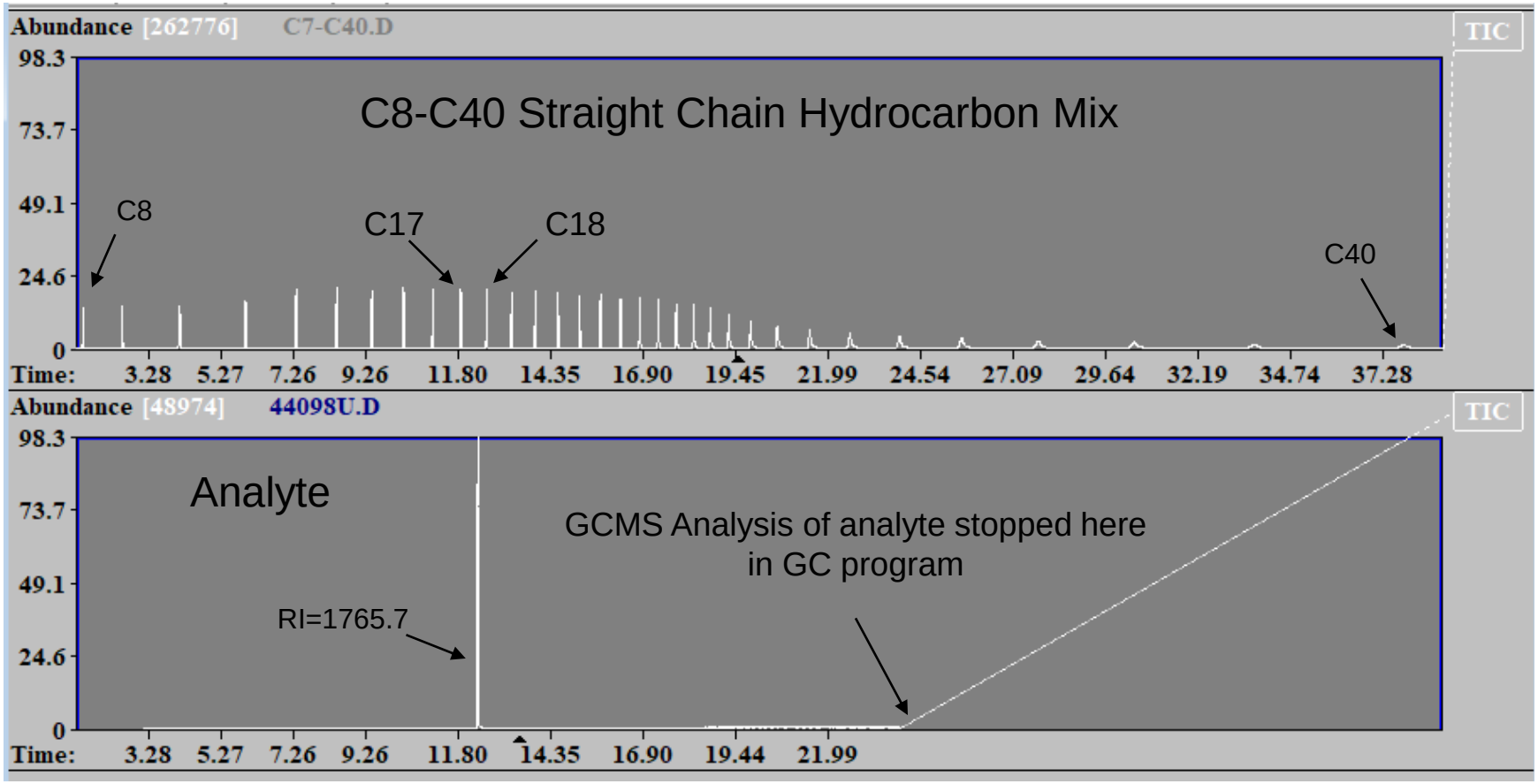
Series of Other Courses on NIST Mass Spec Software

This tutorial ***assumes*** user is familiar with content in ***Parts I and III***

Part I: Spectral Searches with NIST MS Search
Part II: Structure Searches with NIST MS Search and Using MS Interpreter
Part III: AMDIS(NIST) for Processing EI Mass Spectral Data Files
Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra
Part V: Creating and Sharing User EI and MS/MS Libraries

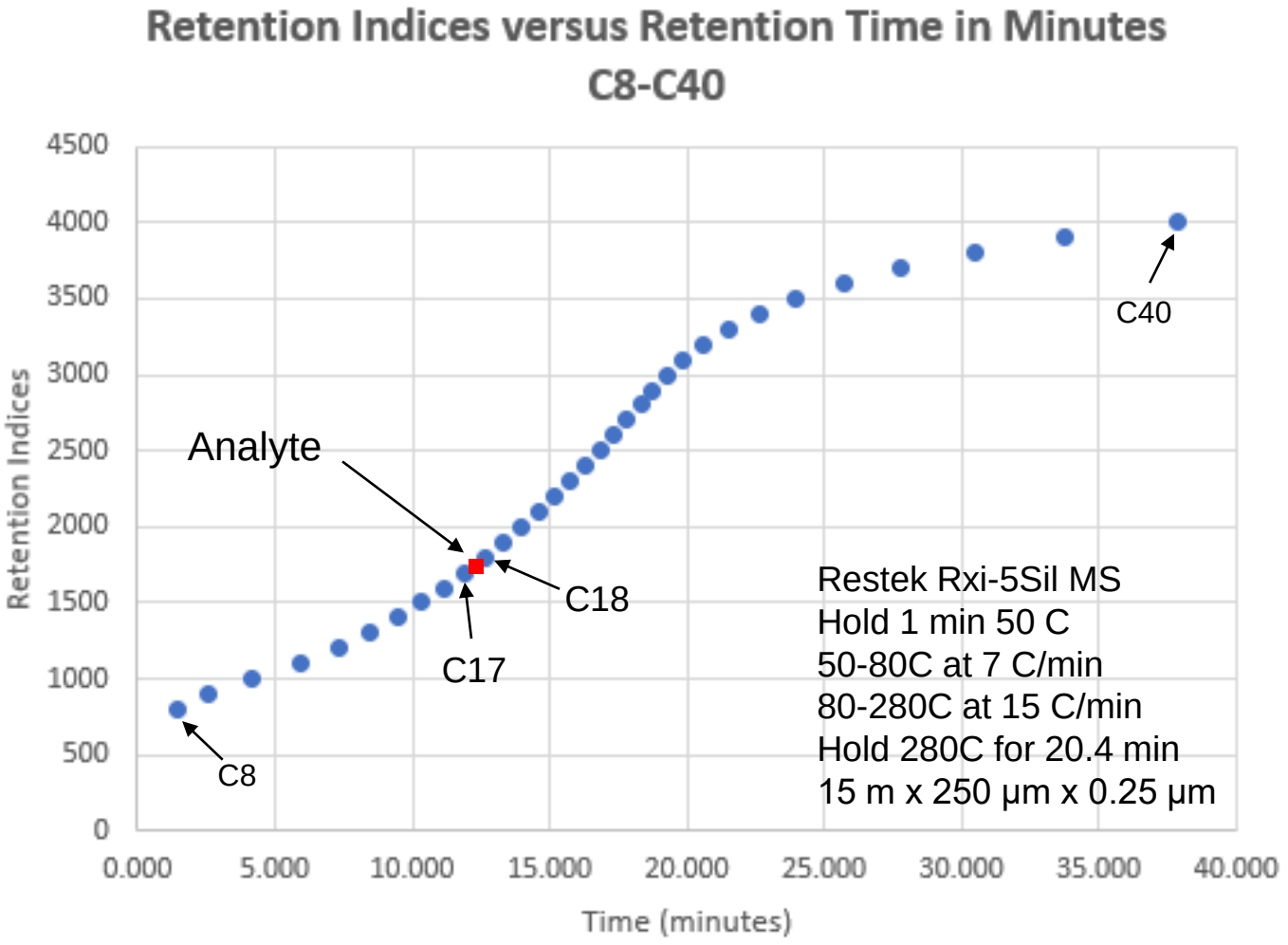
Determining Retention Index (RI) of Compound

- Run sample and C8-C40 straight chain standard mix using ***same*** GC/MS conditions
- AMDIS ***interpolates*** between standards to determine analyte's RI
- The RI of a straight chain hydrocarbon is defined as its carbon number x 100



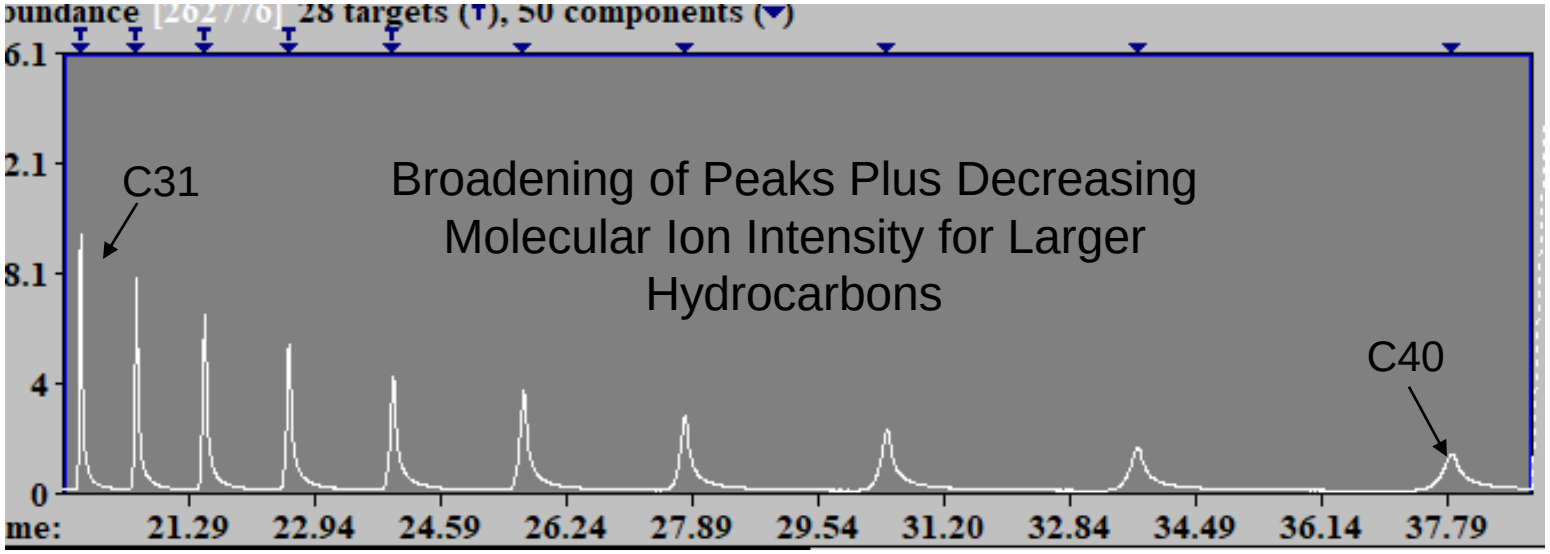
Plot of Retention Index Versus GCMS Retention Time

- Slope of curve ***changes*** during column temperature program
- Important to ***bracket*** analyte between two standard peaks for AMDIS to interpolate



Creation of AMDIS Calibration File (*.cal)

- AMDIS uses a *.csl file for standard C8-C35 to automatically create calibration file (*.cal)
- *.csl file contains an EI spectrum of each hydrocarbon to locate position of peaks
- Higher molecular weight hydrocarbon peaks tend to ***broaden*** at the end of GCMS analysis
- Lack of reliable signals for molecular ion intensity for C36-C40 ***hinders*** their automatic detection and calibration
- ***Thus***, easier to add C36-C40 RI's and associated retention times ***manually*** to calibration file



Example of Typical *.csl File

- CSL files can be viewed and edited with notepad Windows utility
- Supplied c7-c35.csl and c8-c35.csl files in a resource.zip file on my website
- Thus, no need for user to create their own
- Example for ***1st*** record of 27 records in c8-c35.csl file below

C8 standard record

Defined value 8 x 100

Value of 1 indicates used in calibration

Requires *m/z* 114 be found at >0.1%

***m/z* versus intensity table**

c8-c35.csl - Notepad

File Edit View

```
NAME:c8
COMMENT: 1.4871 min C7-C40|RI:800.00
RI:800.00
CASNO:C7-C40-N1002
CALIB: 1
RT:1.487
PEAK: 114 0.1
SOURCE:C:\NIST20\AMDIS32\TUTORIAL\c8-c40.csl
NUM PEAKS:  44
( 15    1) ( 26    5) ( 27   90) ( 28   20) ( 29  141)
( 30    3) ( 37    1) ( 38    4) ( 39  108) ( 40   20)
( 41  406) ( 42  143) ( 43 1000) ( 44   34) ( 50    3)
( 51    9) ( 52    3) ( 53   26) ( 54   11) ( 55  150)
( 56  266) ( 57  481) ( 58   20) ( 62    1) ( 63    2)
( 65    3) ( 66    1) ( 67    4) ( 68    1) ( 69   18)
( 70  137) ( 71  242) ( 72   13) ( 77    1) ( 79    1)
( 83    2) ( 84  101) ( 85  459) ( 86   30) ( 87    1)
( 98    1) ( 99    1) (114   82) (115    7)
```

Example of Typical *.cal File *After* AMDIS *Automatic* Calibration

- ***Only*** values in first two columns important, GCMS retention time and RI for hydrocarbon
- 27 entries for C8-C35

C8 retention time and defined RI

c8-c35.CAL - Notepad

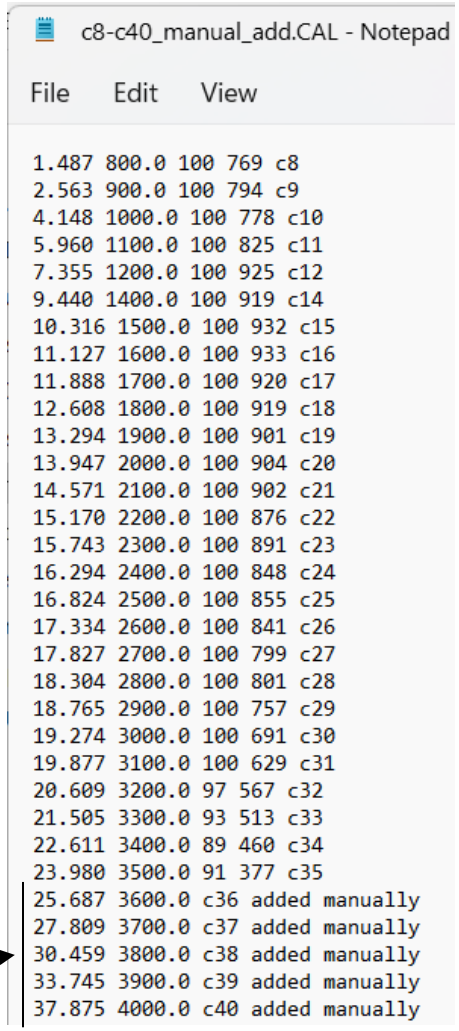
File Edit View

```
1.487 800.0 100 769 c8
2.563 900.0 100 794 c9
4.148 1000.0 100 778 c10
5.960 1100.0 100 825 c11
7.355 1200.0 100 925 c12
9.440 1400.0 100 919 c14
10.316 1500.0 100 932 c15
11.127 1600.0 100 933 c16
11.888 1700.0 100 920 c17
12.608 1800.0 100 919 c18
13.294 1900.0 100 901 c19
13.947 2000.0 100 904 c20
14.571 2100.0 100 902 c21
15.170 2200.0 100 876 c22
15.743 2300.0 100 891 c23
16.294 2400.0 100 848 c24
16.824 2500.0 100 855 c25
17.334 2600.0 100 841 c26
17.827 2700.0 100 799 c27
18.304 2800.0 100 801 c28
18.765 2900.0 100 757 c29
19.274 3000.0 100 691 c30
19.877 3100.0 100 629 c31
20.609 3200.0 97 567 c32
21.505 3300.0 93 513 c33
22.611 3400.0 89 460 c34
23.980 3500.0 91 377 c35
```

C35 retention time and defined RI

Example of Typical *.cal File with C36-C38 *Added Manually*

- Addition of higher C36-C40 RI's and associated retention times manually
- Added user comments to the left of retention time and RI



User added values for C36-C40

Demo of Software

- Comparison of C8-C40 to Analyte File
- Automatic creation of a *.cal file with a *.csl file for C6-C35 retention indices
- Manual addition of higher hydrocarbons such as C8-C40 to *.cal file
- Checking that calibration file works using original C8-C40 calibration mixture
- Determining RI for typical analyte
- Sending analyte with its RI to NIST search for processing

Experimental

- All the example files on my website in zip file named ***resources.zip***
- Experimental for C8-C40 found in C7-C40.D, acqmeth.txt file
- C7-C40 Standard available from Sigma Aldrich, **49452-U**

Additional Information

- ***After*** acknowledgements, will demonstrate formation of user CSL library
- However, as stated before, just use mine
- In resources.zip file included a CSL file for C8-C35 and C7-C35
- Didn’t use C7-C35 in example, C7 not resolved from solvents in GCMS of reference mix

Useful Links on My Website
Little Mass Spec and Sailing
https://littlemsandsailing.wordpress.com/

- Parts I-V of the NIST Search Software Course
- AMDIS Manual, start reading page 29, Section 2.9
- NIST Manual, search for retention index or retention indices in document

Acknowledgements

NIST Technical Advice:

- Gary Mallard
- Stephen Stein
- Sandy Markey
- Weihua Ji
- Nirina Rabe Andriamaharavo
- Nino G. Todua

Creation of CSL File Library
Demo of Software