Retention Index Made Easy for GC/MS Unknown Compound ID

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GC/MS Search Limitations

- Library search “Hit Lists” are not always definitive
- Co-eluting chromatographic peaks or high background can lead to poor and/or misleading library matches
- Compounds may not be in the library
- Good analytical practice requires additional metrics to confirm matches with confidence

Which is the correct compound?

Conventional Library results are not definitive!

Hit	NIST	MW	Formula	Name
1	968	95.953	C2H2Cl2	Ethylene, 1,2-dichloro-, (Z)-
2	932	95.953	C2H2Cl2	1,2-Dichloroethylene
3	932	95.953	C2H2Cl2	Ethylene, 1,2-dichloro-, (E)-
4	867	95.953	C2H2Cl2	Ethene, 1,1-dichloro-
5	787	153.959	C4H4Cl2O2	Dihydro-4,5-dichloro-2(3H)furanone
6	661	233.885	C4H5BrCl2O2	Methyl 3-bromo-2,2-dichloro-propanoate
7	642	95.98	C2H5ClS	Chloromethylmethyl sulfide
8	599	189.936	C4H5Cl3O2	Methyl 2,3,3-trichloropropanoate
9	493	189.936	C4H5Cl3O2	Propanoic acid, 2,2,3-trichloro-, methyl ester
10	487	123.985	C4H6Cl2	Cyclobutane, 1,1-dichloro-
11	479	93.938	C2Cl2	Ethyne, dichloro-

What's the right answer?

Wrong! It's this one

Are you sure?

How to Improve Search Confidence?

Provide complimentary and/or orthogonal metrics

Classic “unknown” identification uses multiple measurements such as MS, IR, NMR, elemental analysis, etc. **However these techniques do not align well with GC/MS with it’s high sensitivity and direct chromatography interface.**

What’s left?

Additional metrics must be derived from the GC or from the MS, which leaves

✓***Retention time index (RI) (truly orthogonal)***

- Accurate mass formula ID (molecular ion + fragment ions)

What is Retention Index

Kovats retention index (Kovats index, retention index (RI)) is used to convert retention times (RT) into a system-independent constant.

- RI is a powerful tool for identifying unknown compounds that is very complementary to library search.
- RI is column chemistry dependent
- GC must be calibrated for RI

Retention Index (RI)

- Readily available RI Databases
  - NIST20-23 has nearly 100% of compounds assigned accurate RI values – GAME CHANGER!
- Excellent orthogonal metric!
  - Good for distinguishing isomers (MS is not!)
- The GC can be calibrated with commercially available standards
- <u>Downside</u> – The extra work in running a separate RI standard

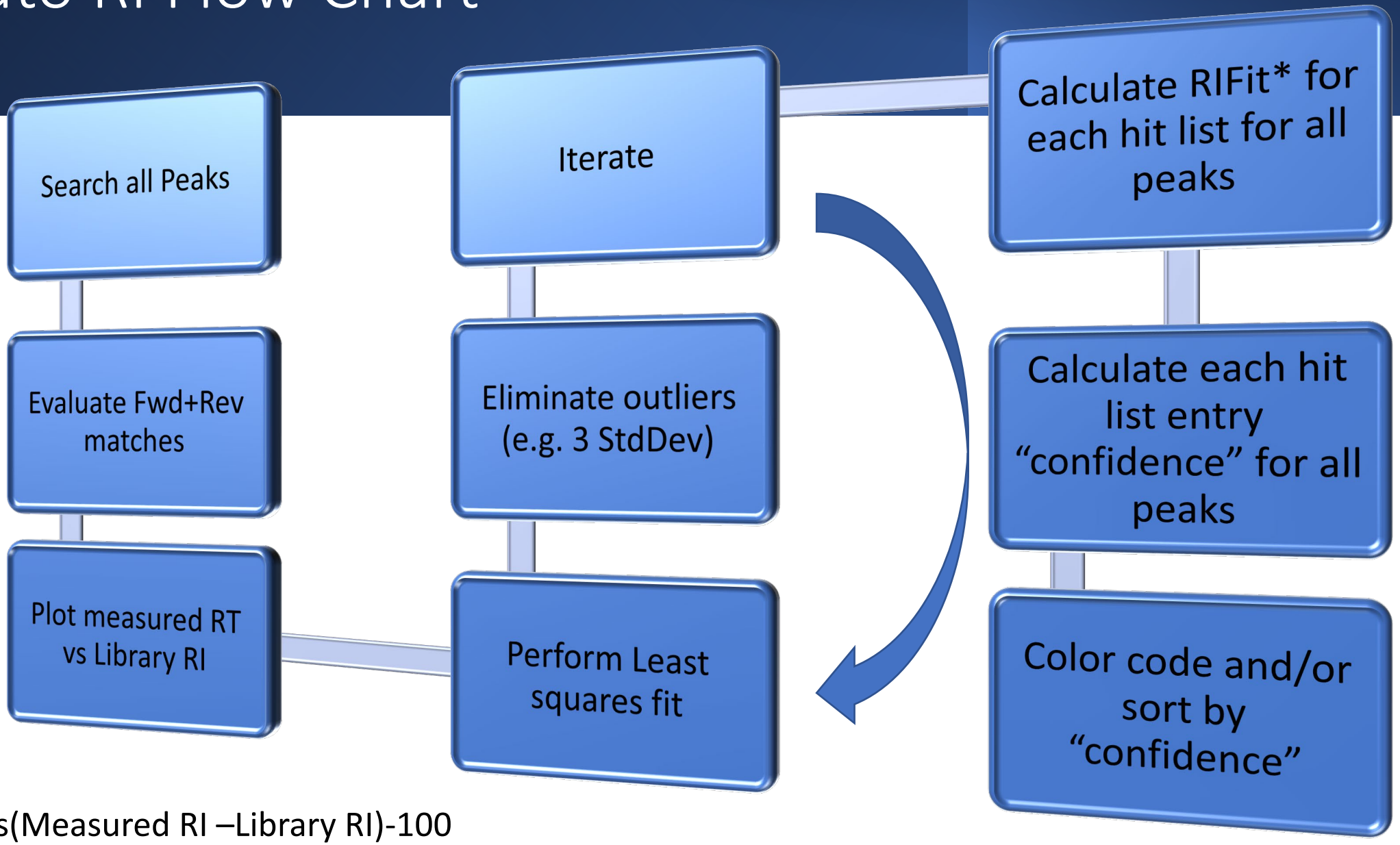
How to Calibrate the GC for RI

- Run an n-alkane ladder in a separate run (e.g. C5-C30)
  - 👍 Builds the calibration against the accepted “standards”
  - 👎 Requires a separate GC run
- Use an internal standard, possible one already in use
  - 👍 No additional run
  - 👎 May not have suitable RT coverage to produce accurate RI
  - 👎 Complicates the run
  - 👎 Complicates the sample preparation

A New Approach: Auto RI

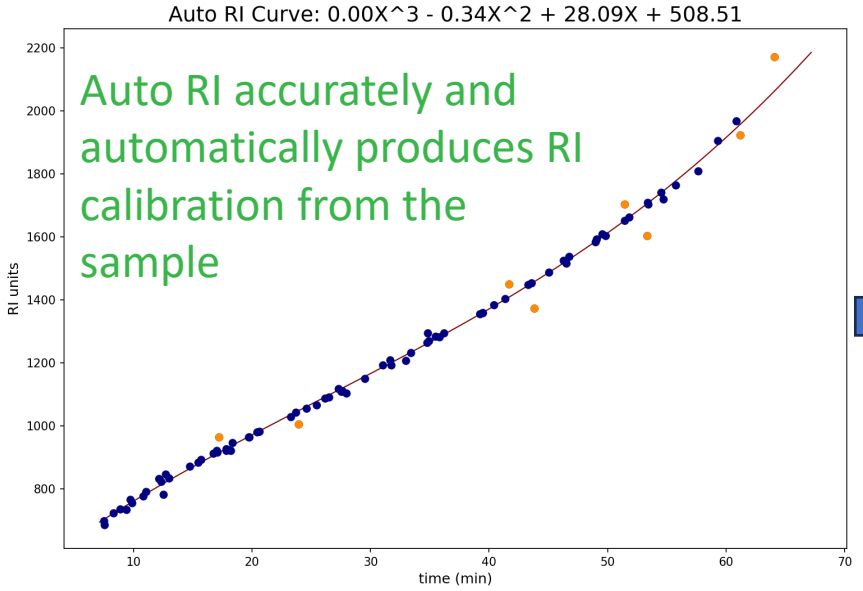
- **“Auto RI” requires <u>NO STANDARDS,</u> allowing you to get RI information on almost any run, new or old!**
  - Uses the sample data to calibrate RI
  - Leverages “high confidence” search matches that accurately match the “RI trend line”
  - Works with most samples

Auto RI Flow Chart



*RIFit =Abs(Measured RI –Library RI)-100

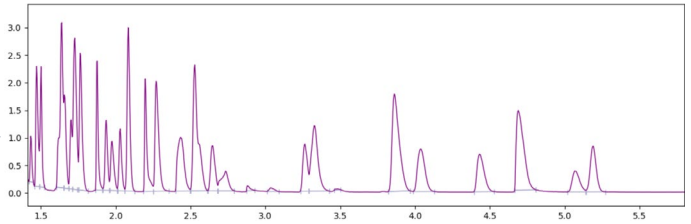
GC Retention Index Calibraton

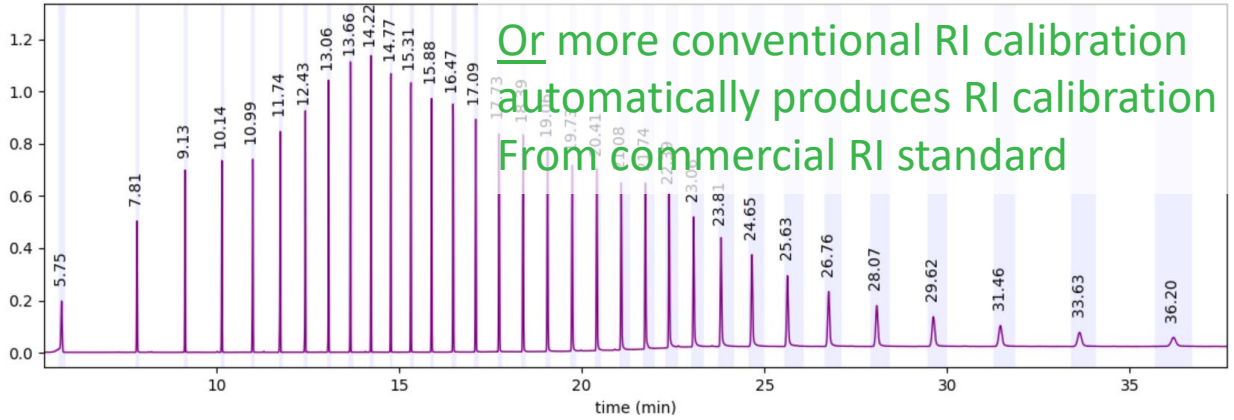


Auto RI accurately and automatically produces RI calibration from the sample

Calibrated GC

Fully RI calibrated chromatogram





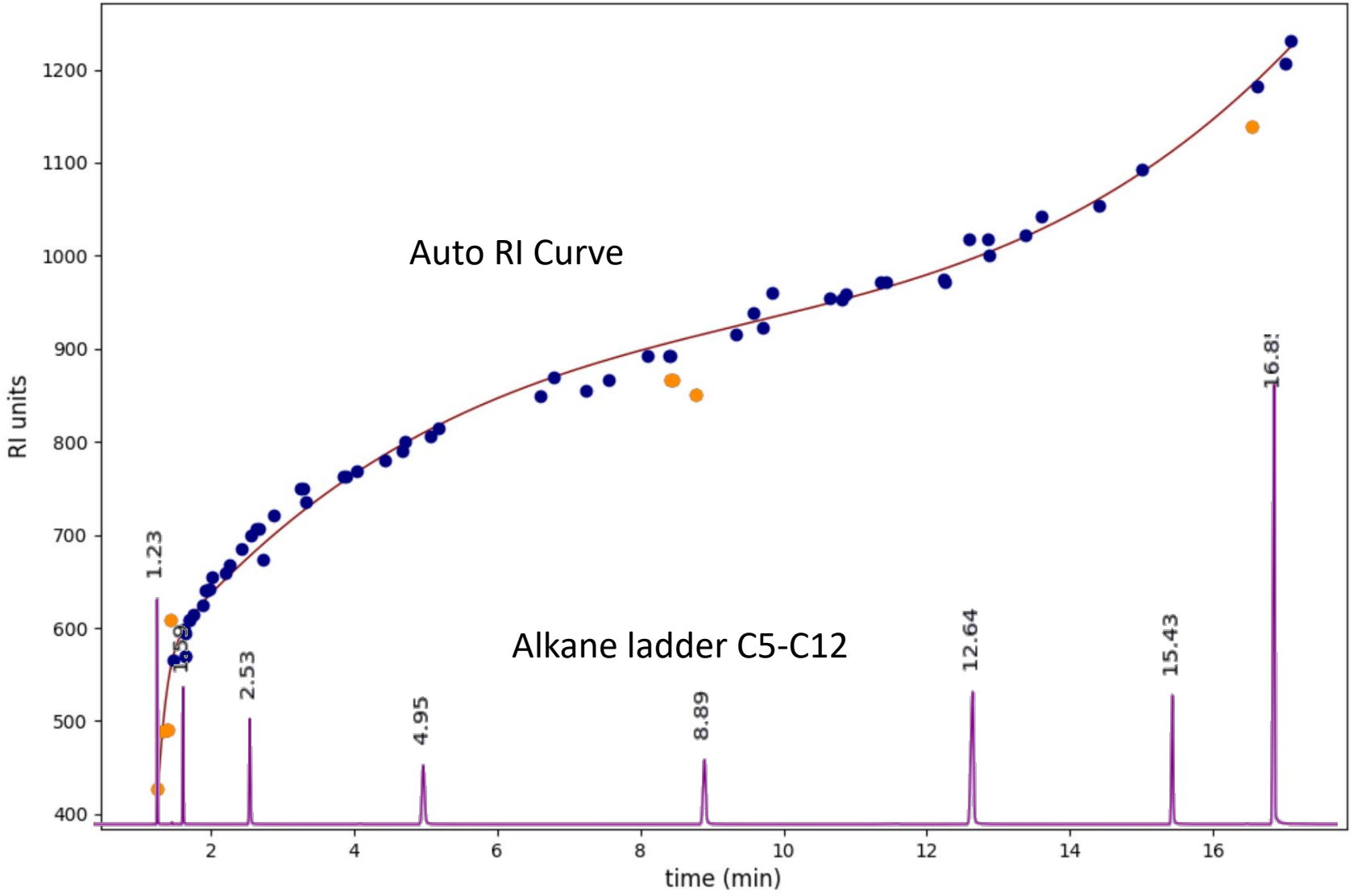
Or more conventional RI calibration automatically produces RI calibration From commercial RI standard

New NIST database with “full” RI coverage

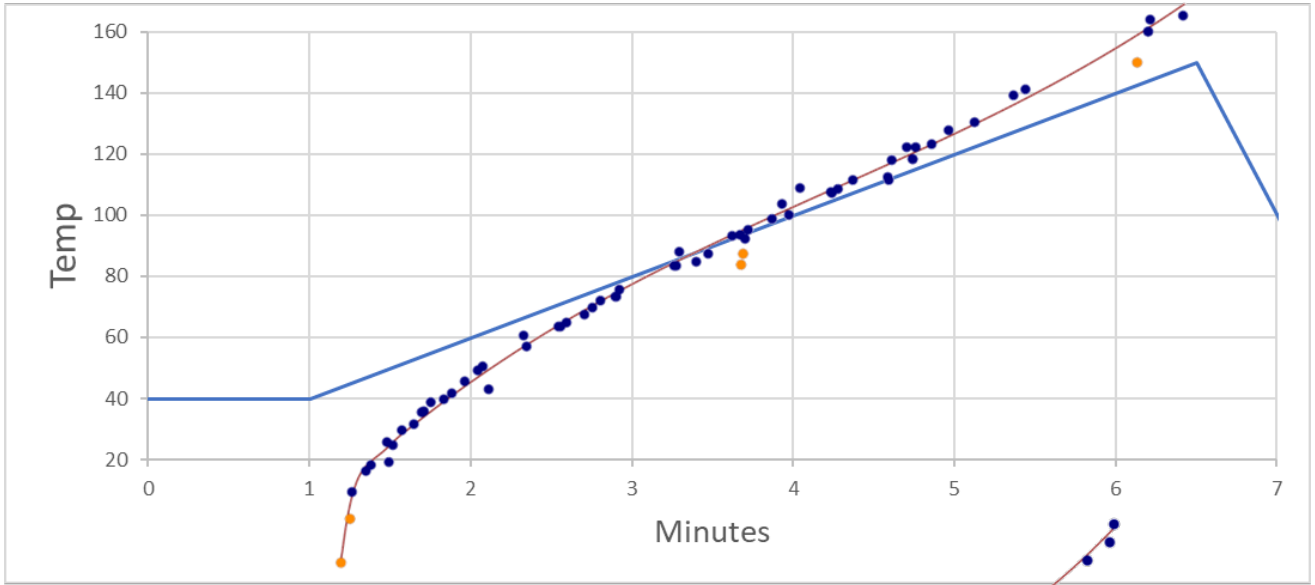
RI match for every compound for superior identification

Hit	Match	NIST	RIFit	Name
1	96.96	932	94.62	Ethylene, 1,2-dichloro-, (E)-
2	92.88	932	69.38	1,2-Dichloroethylene
3	88.36	968	54.38	Ethylene, 1,2-dichloro-, (Z)-
4	86.96	867	57.62	Ethene, 1,1-dichloro-

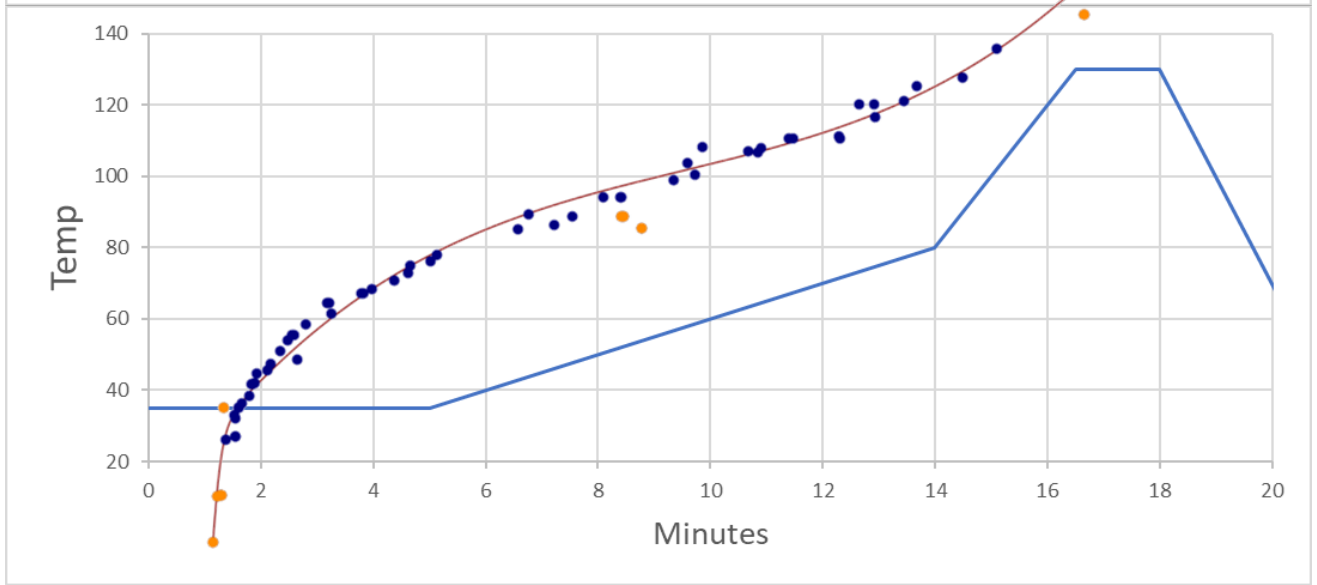
Sampling Frequency: Auto RI vs Alkane Ladder



RI Curves as a Function of Temperature Programming

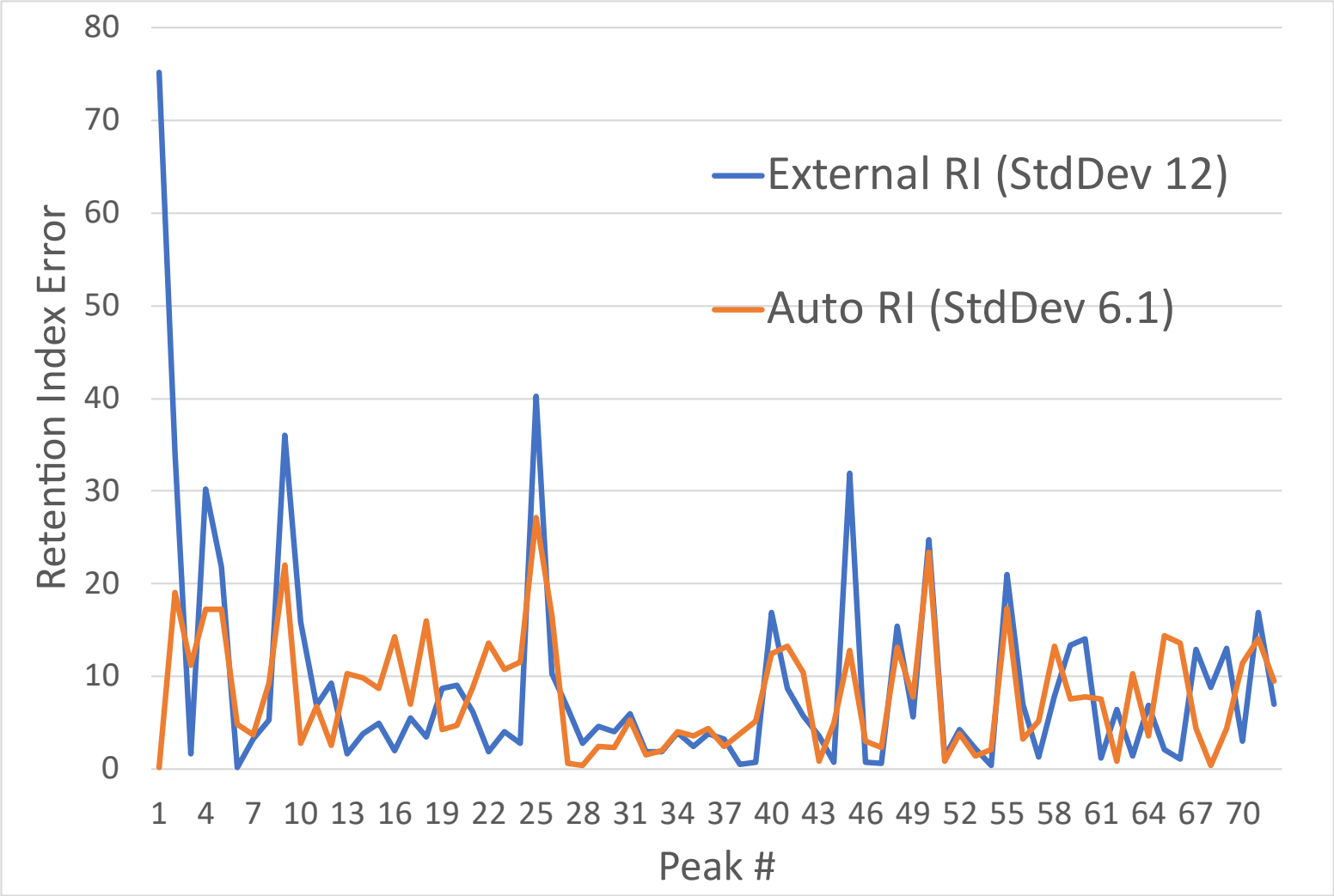


"Fast" VOC run with single fast ramp temperature program



"Slow" VOC run with multiple ramp temperature program

External Ri vs Auto RI Calibration



Which is the correct compound?

But GC/ID knows!

Because it weighs *MW, Match, NIST, RevS, SA-M, SA-F, RIFit, Formula, Name, Retention Index*

Conventional Library results are not definitive!

Composite Scoring

Hit	Match	NIST	RevS	SA-M	SA-F	RIFit	MW	Formula	Name
1	96.96	932	932	99.28	99.18	94.62	95.953	C2H2Cl2	Ethene, 1,2-dichloro-, (E)-
2	92.88	932	932	99.28	99.18	69.38	95.953	C2H2Cl2	1,2-Dichloroethylene
3	88.36	968	968	99.28	99.18	54.38	95.953	C2H2Cl2	Ethene, 1,2-dichloro-, (Z)-
4	86.96	867	876	99.28	99.18	57.62	95.953	C2H2Cl2	Ethene, 1,1-dichloro-
5	76.6	422	423	98.45	99.18	94.62	97.969	C2H4Cl2	Ethane, 1,1-dichloro-
6	64.5	642	644	97.24	99.18	0	95.98	C2H5ClS	Chloromethylmethyl sulfide
7	50.68	787	806	26.28	99.18	0	153.959	C4H4Cl2O2	Dihydro-4,5-dichloro-2(3H)-fu
8	41.97	479	512	14.48	85.65	82.38	93.938	C2Cl2	Ethyne, dichloro-
9	37.42	661	662	-29.09	99.18	0	233.885	C4H5BrCl2O2	Methyl 3-bromo-2,2-dichloro
10	37.09	487	777	-25.77	99.18	0	123.985	C4H6Cl2	Cyclobutane, 1,1-dichloro-
11	34.67	599	600	-26.37	99.18	0	189.936	C4H5Cl3O2	Methyl 2,3,3-trichloropropan

What's the right answer?

Wrong! It's this one

Are you sure?

Summary

- Search alone cannot confidently ID many samples
- RI can significantly improve ID confidence but
  - Requires either external or internal calibrations which each have significant drawbacks
- Auto RI can provide confident RI GC calibration without standards for most samples
  - Can allow improved confidence for old or new runs by simple re-analysis

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