Welcome to our E-Seminar:

Pesticide Analysis using Retention Time Locking and RTL Databases

A History of Leadership in GC and GC/MS

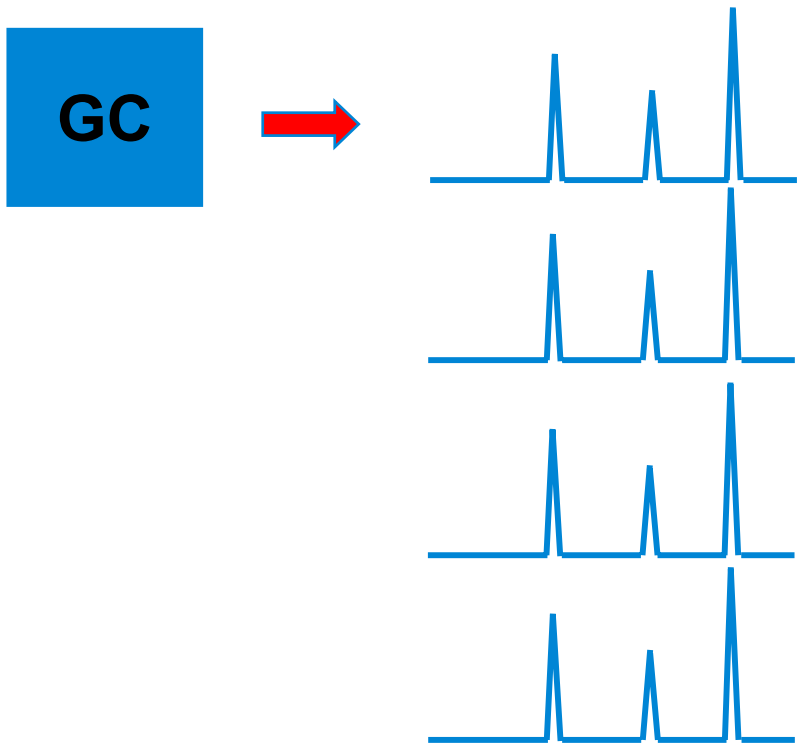
- 1959 – First linear temperature programmed GC
- 1963 – First GC with automatic temperature programming & oven cooling
- 1968 – Introduces digital integrator
- 1969 – First automatic sampler for GC
- 1974 – First microprocessor-controlled GC
- 1974 – First digital GC integrator
- 1976 – First digital bench top GC/MS
- 1979 – Invents fused silica capillary column
- 1982 – First Mass Selective Detector for GC
- 1984 – First fast-injection autosampler for GC
- 1989 – First electronic pressure programming for GC
- 1995 – First electronic pneumatic control of all flows and pressures
- **And recently…** **Retention Time Locking (RTL)**
  RTL database screening

Outline

- What is Retention Time Locking?
- Locking GC Methods
- Locking GC/MSD Methods
- Pesticide Analysis using Agilent's RTL Pesticide Databases

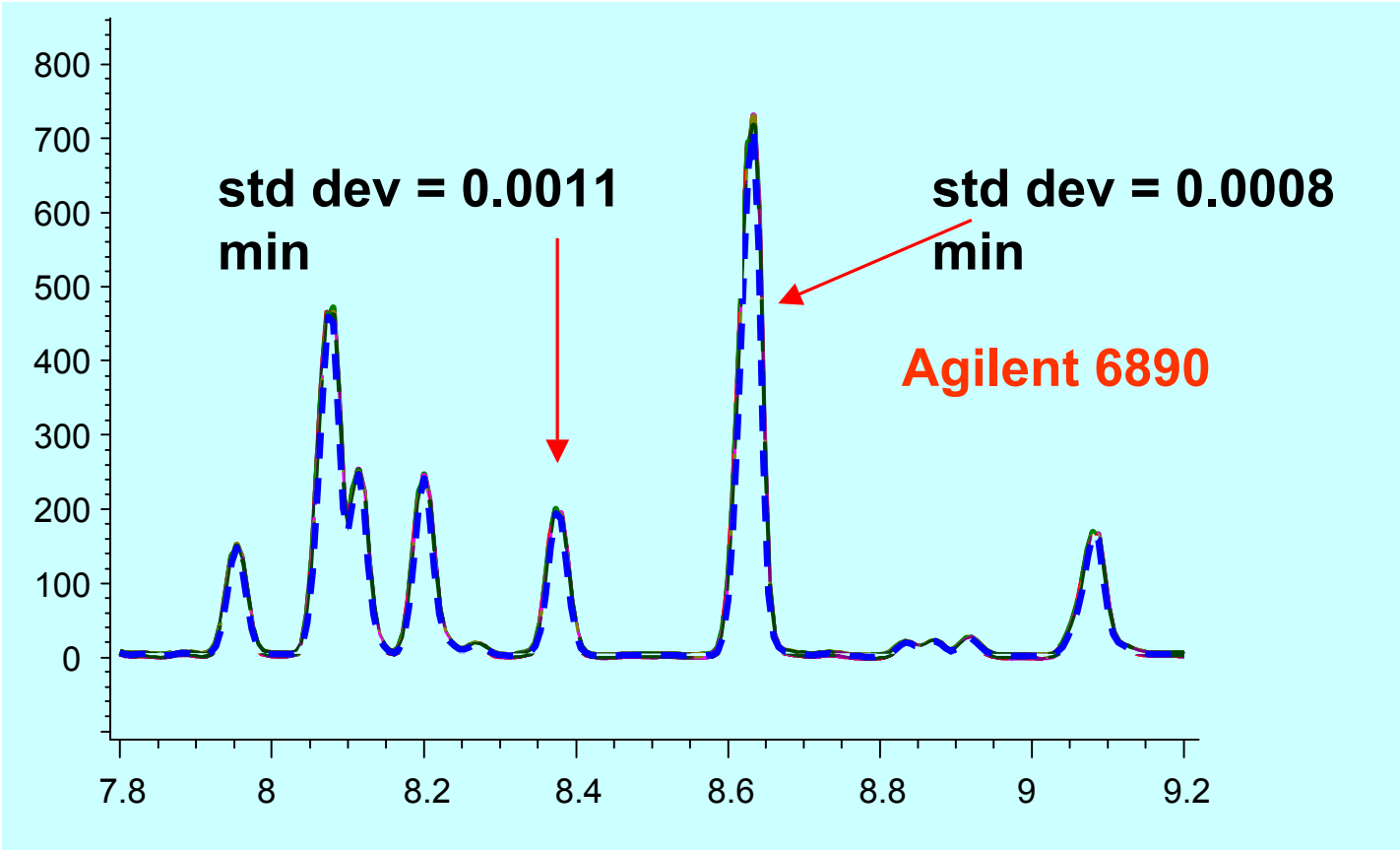
What's the Problem?

Retention times are well matched in a single instrument to 0.010 min or better...



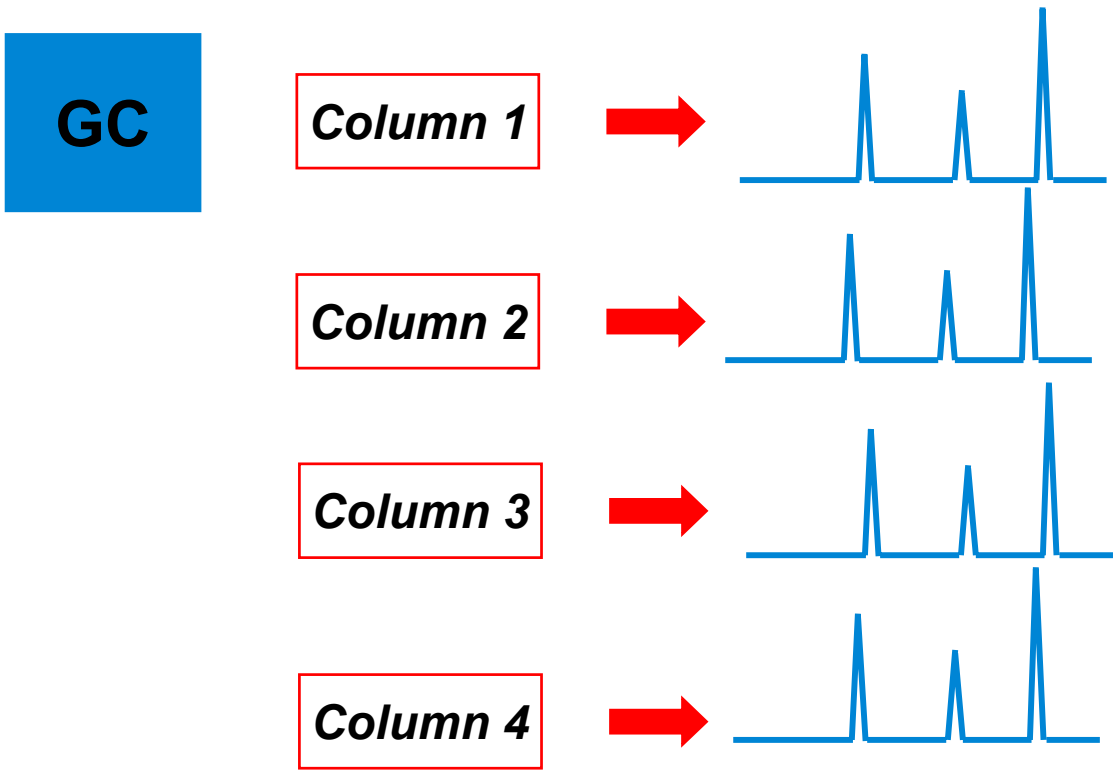
Retention Time Precision, 6890

Gasoline, 15 Runs Over 5 Days



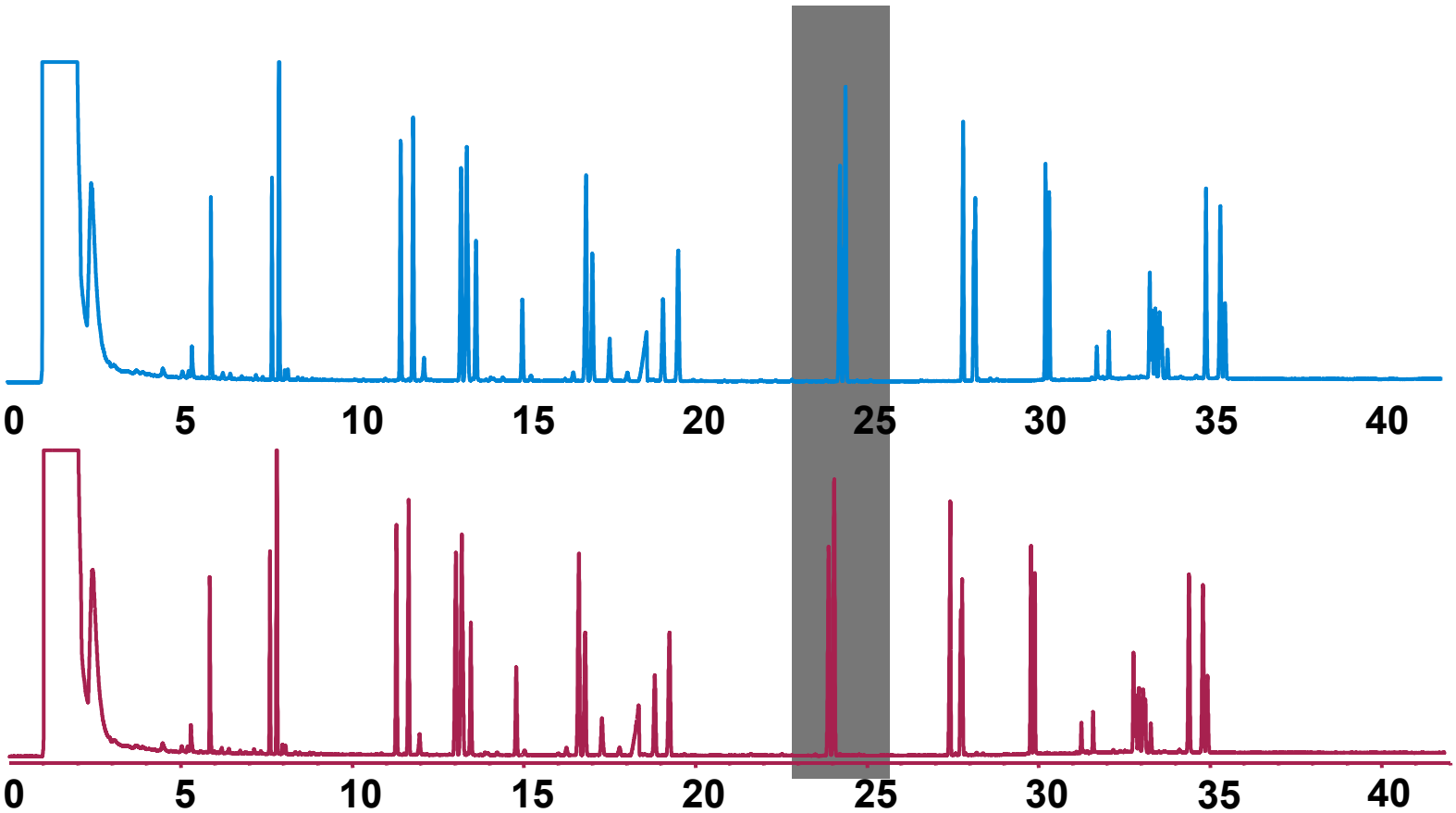
Different Columns, Same GC

Retention times vary from column-to-column by as much as 0.5 min. Column maintenance increases variation.

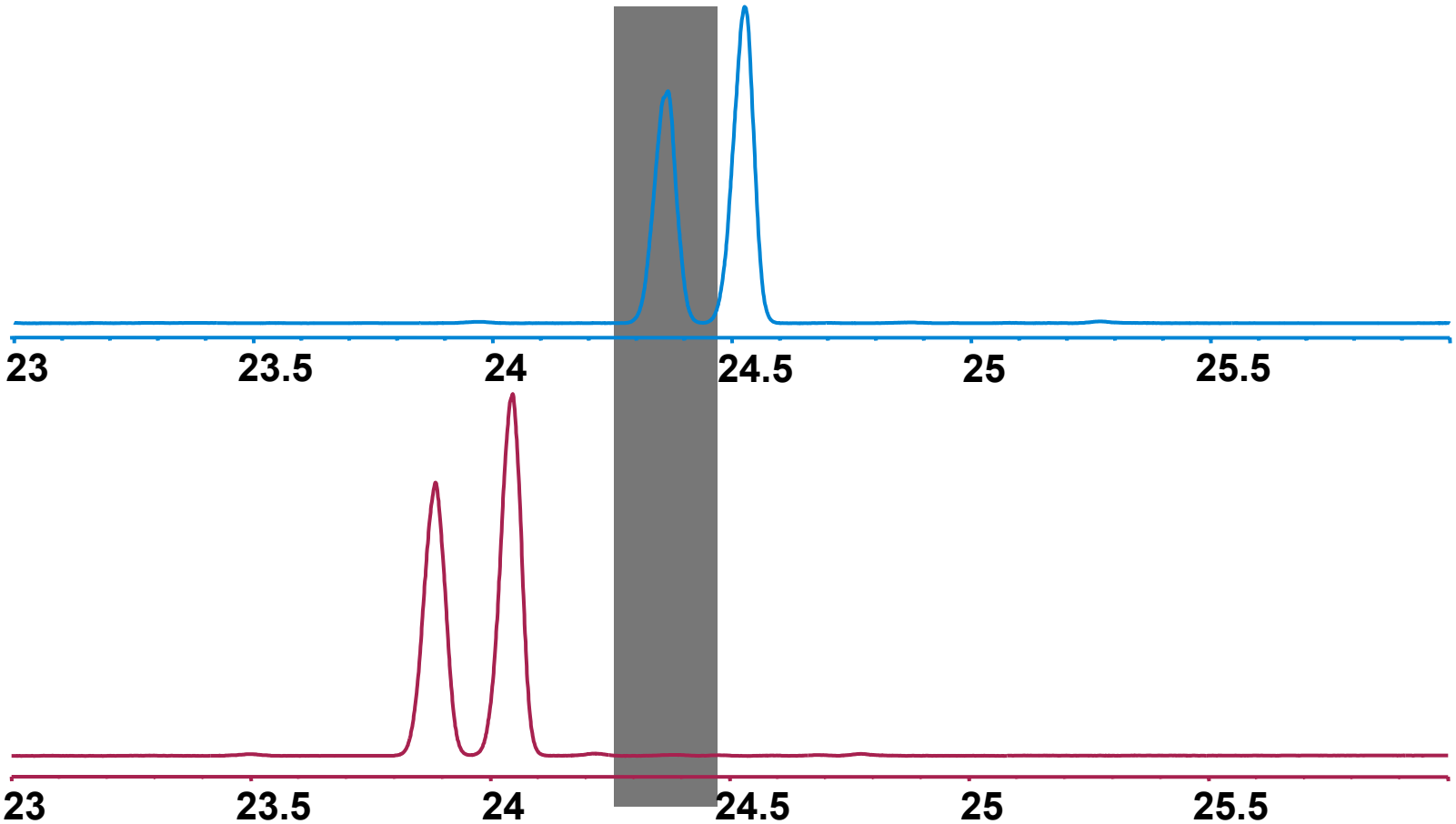


Pesticides on Two Different GCs

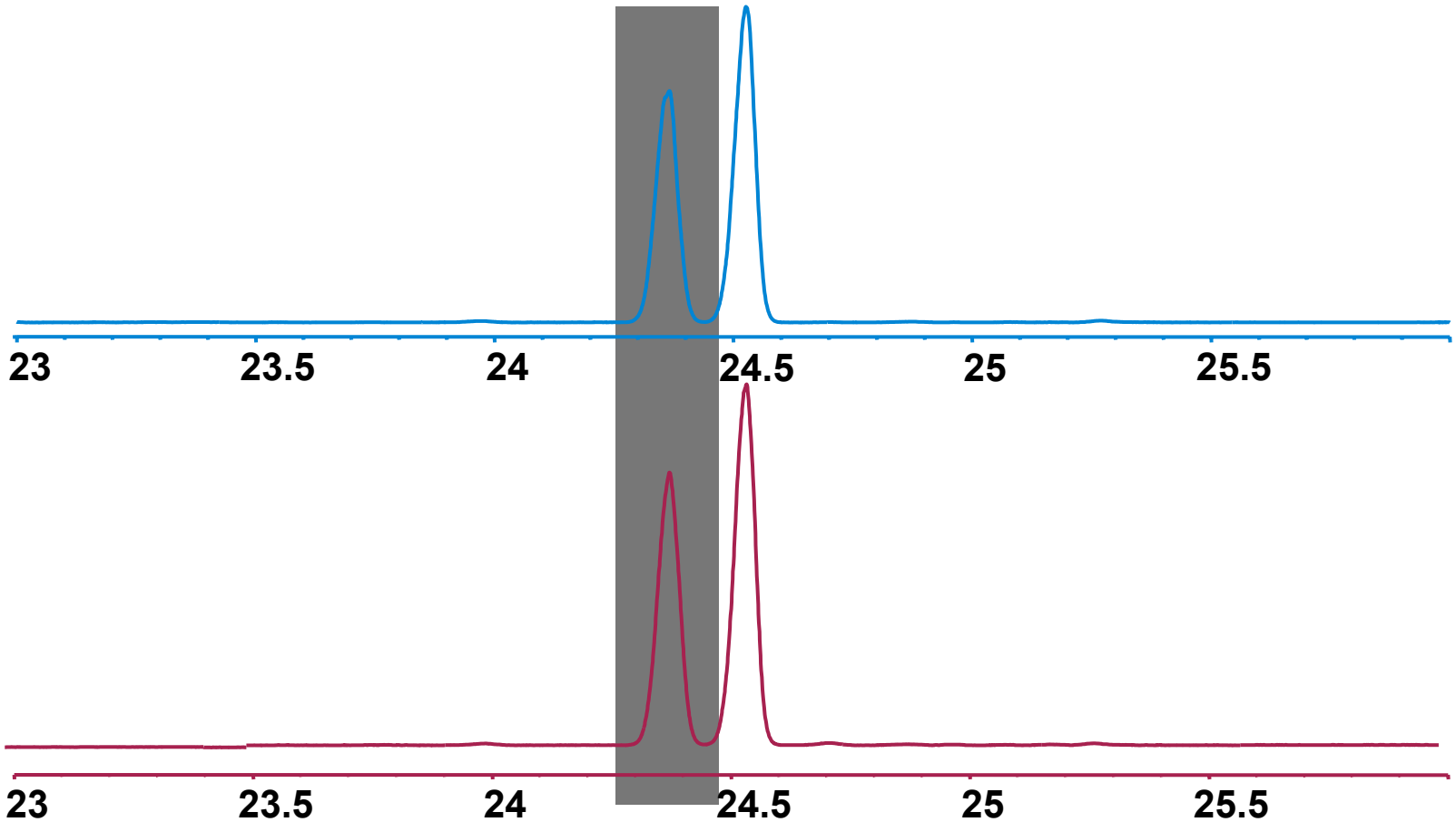
Retention times vary from column-to- column and instrument-to-instrument



Pesticides on Two Different GCs



Pesticides on Two Different GCs After Retention Time Locking



Retention Time Locking

What is Retention Time Locking (RTL)?

The ability to precisely match chromatographic retention times in any systems to those in another chromatographic system with the same nominal method and column.

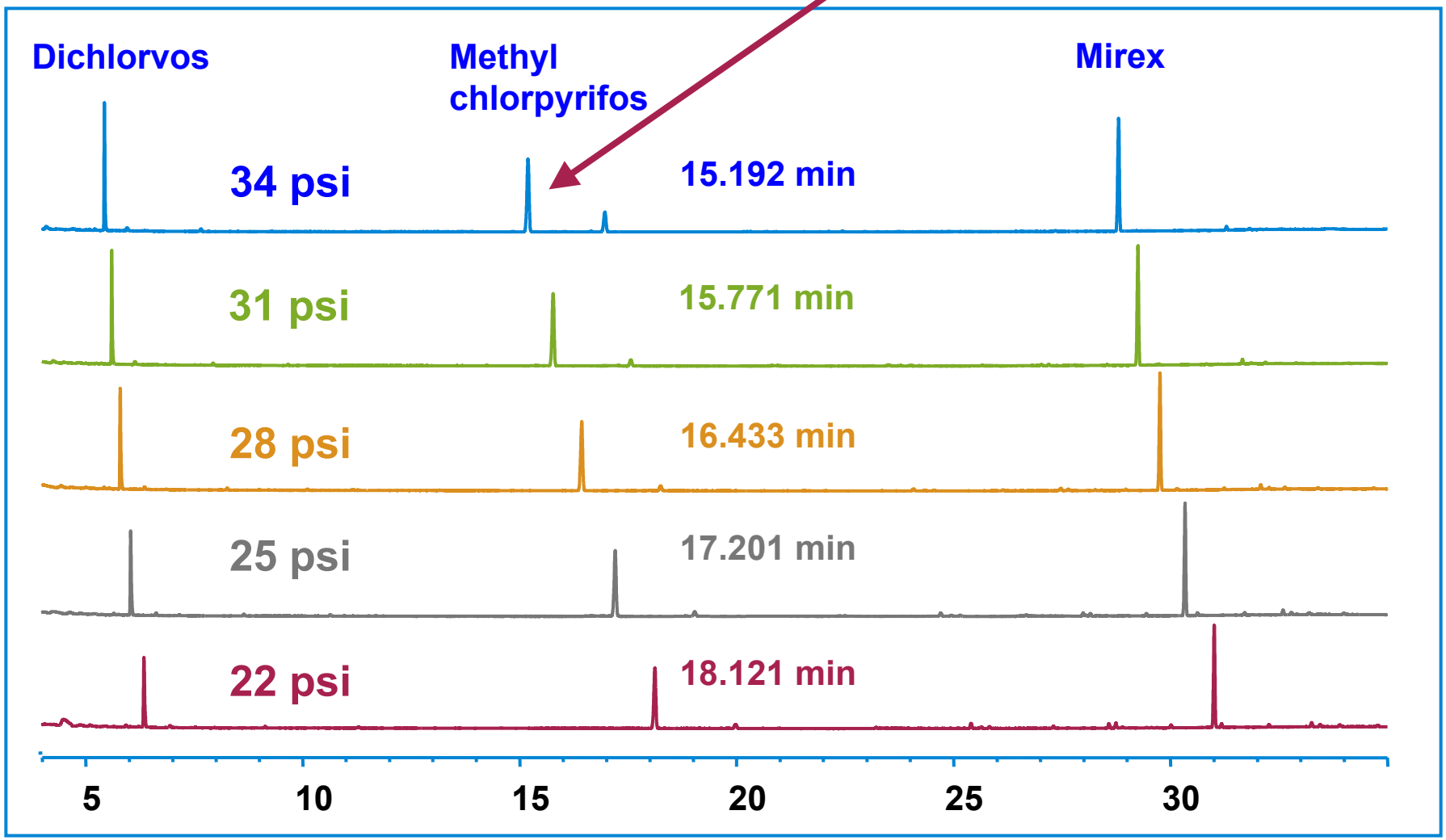
How is Retention Time Locking Done?

By adjusting column head pressure via EPC using interactive HP ChemStation software.

Outline

- What is Retention Time Locking?
- Locking GC Methods
- Locking GC/MSD Methods
- Pesticide Analysis using Agilent's RTL Pesticide Databases

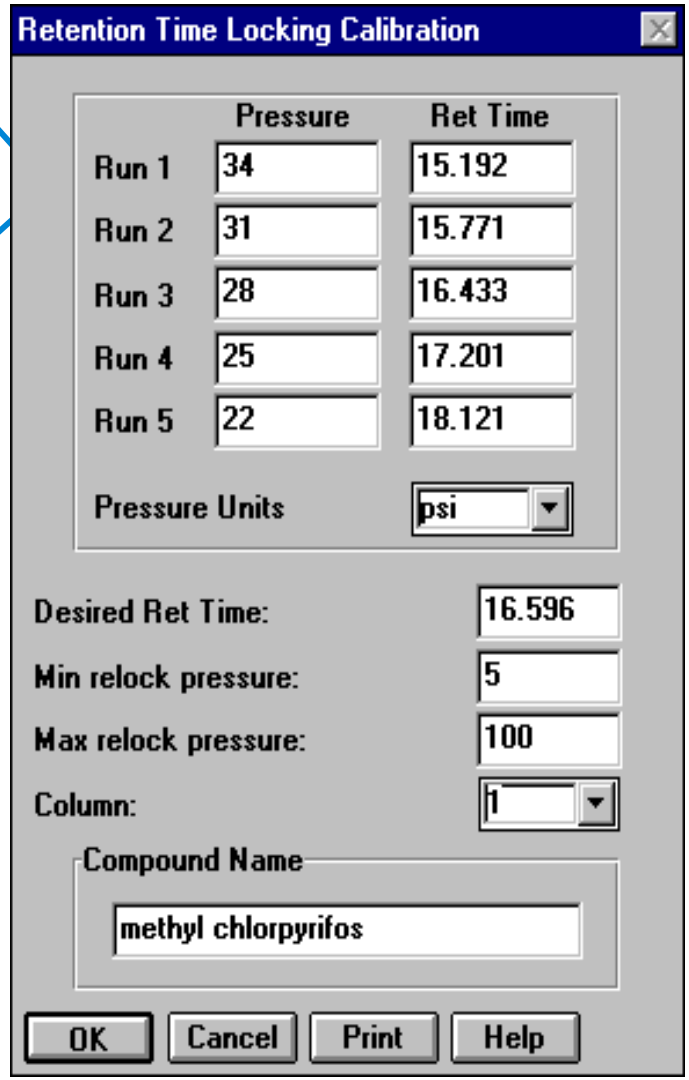
RTL Calibration Runs



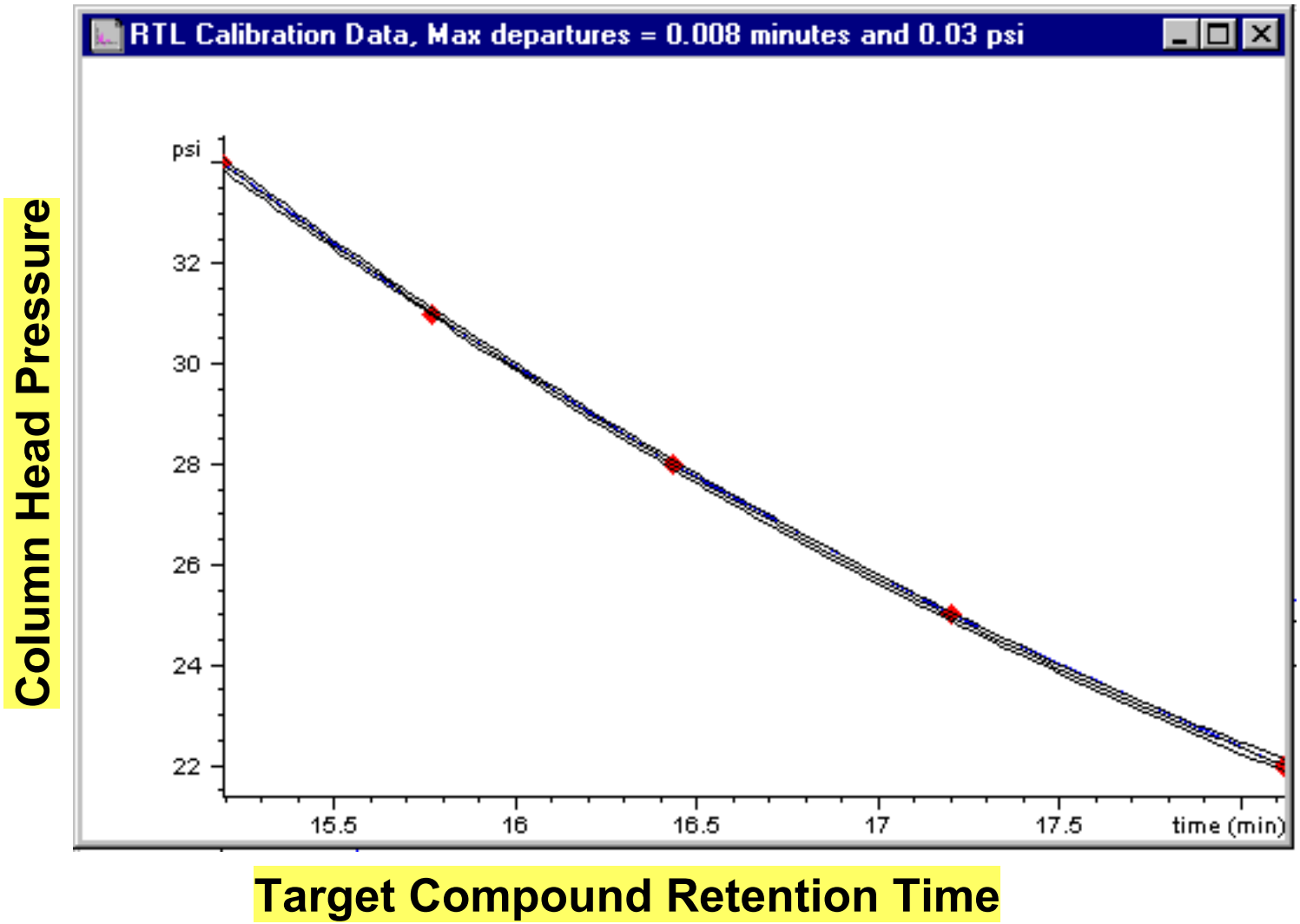
RTL Calibration - Done Once for a Given Method

- Make 5 runs at 5 different inlet pressures:
  - target -20%
  - target -10%
  - target
  - target +10%
  - target +20%
- Determine RT of your target compound in
- Enter values into the RTL Software

This process is automated!

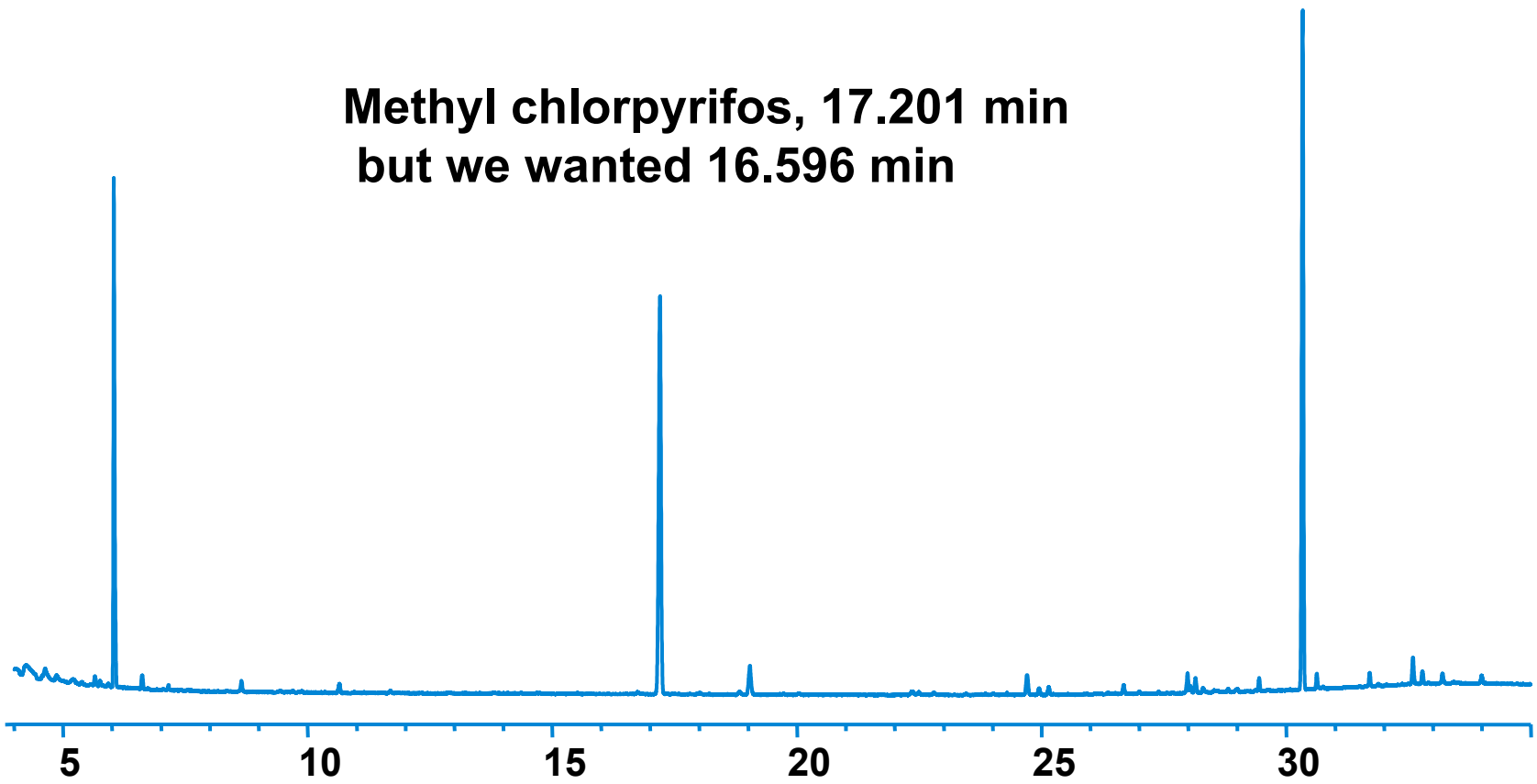


RTL Calibration Curve



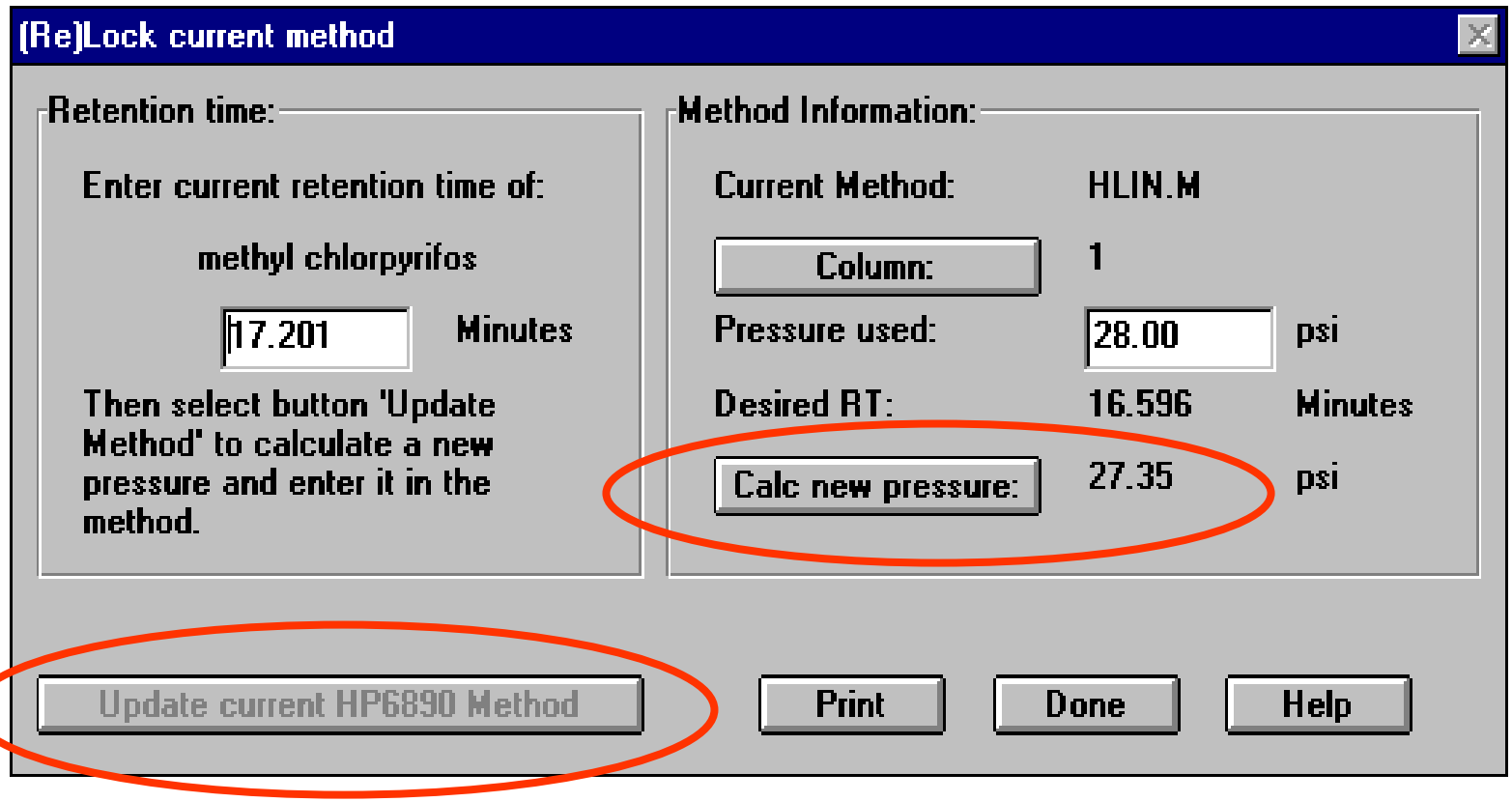
Locking A New System or Column

Run locking standard at nominal pressure (28 psi) and determine RT of target peak.



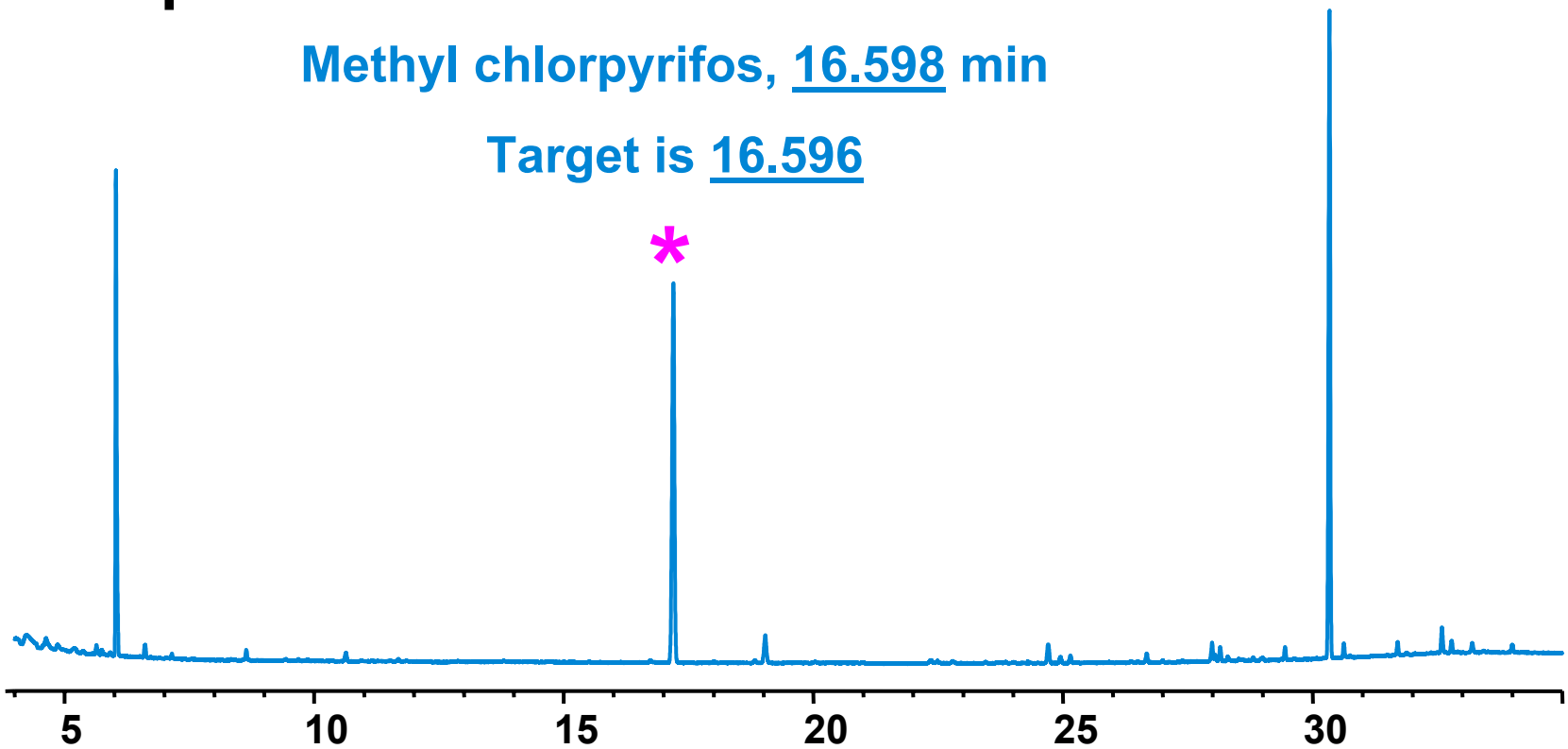
Locking a GC System

Type in pressure used and RT of target compound to calculate locking pressure (27.35 psi)



Confirming RT Lock

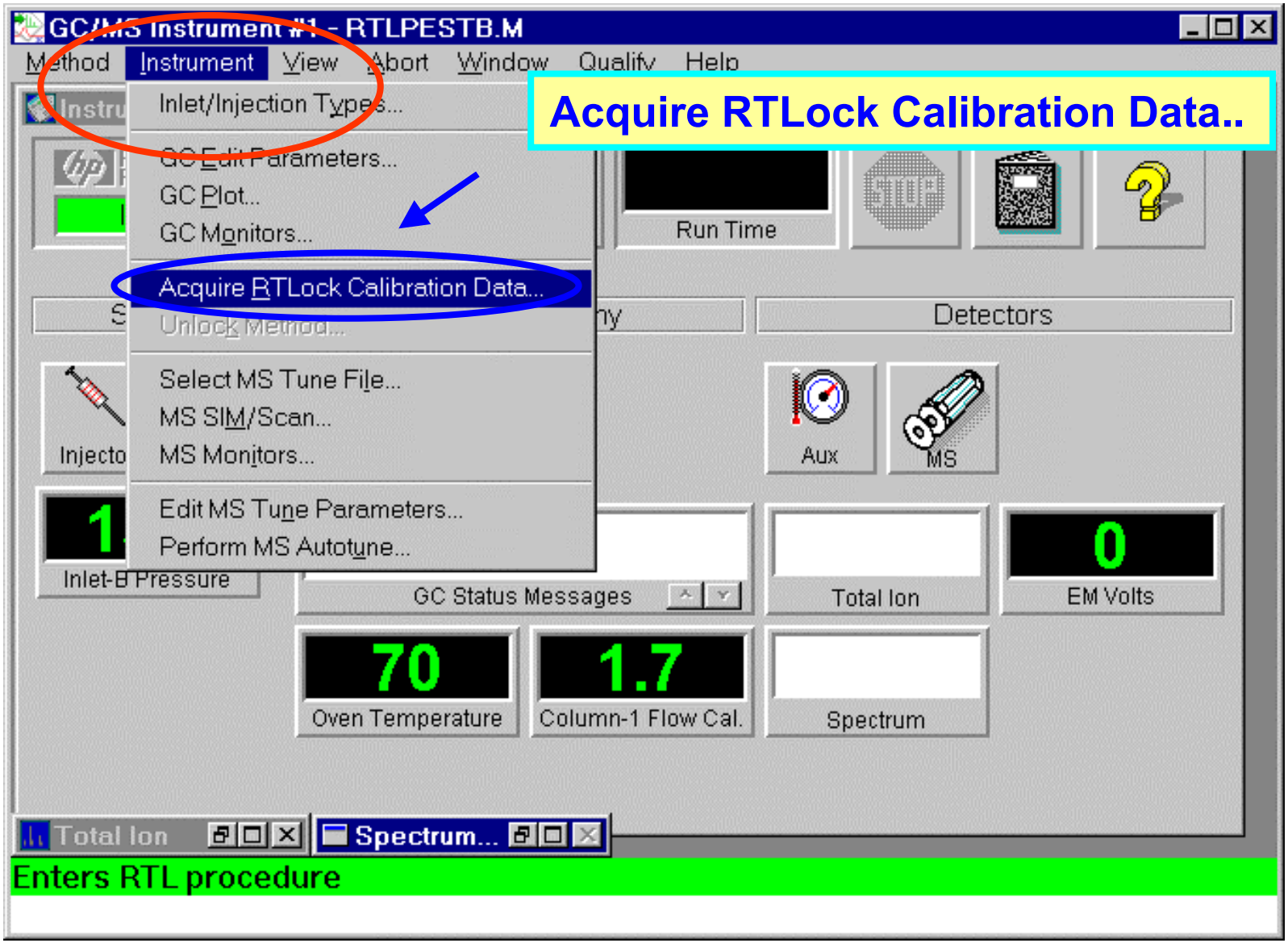
Set inlet pressure to that calculated by software, 27.35 psi. Confirmation run...



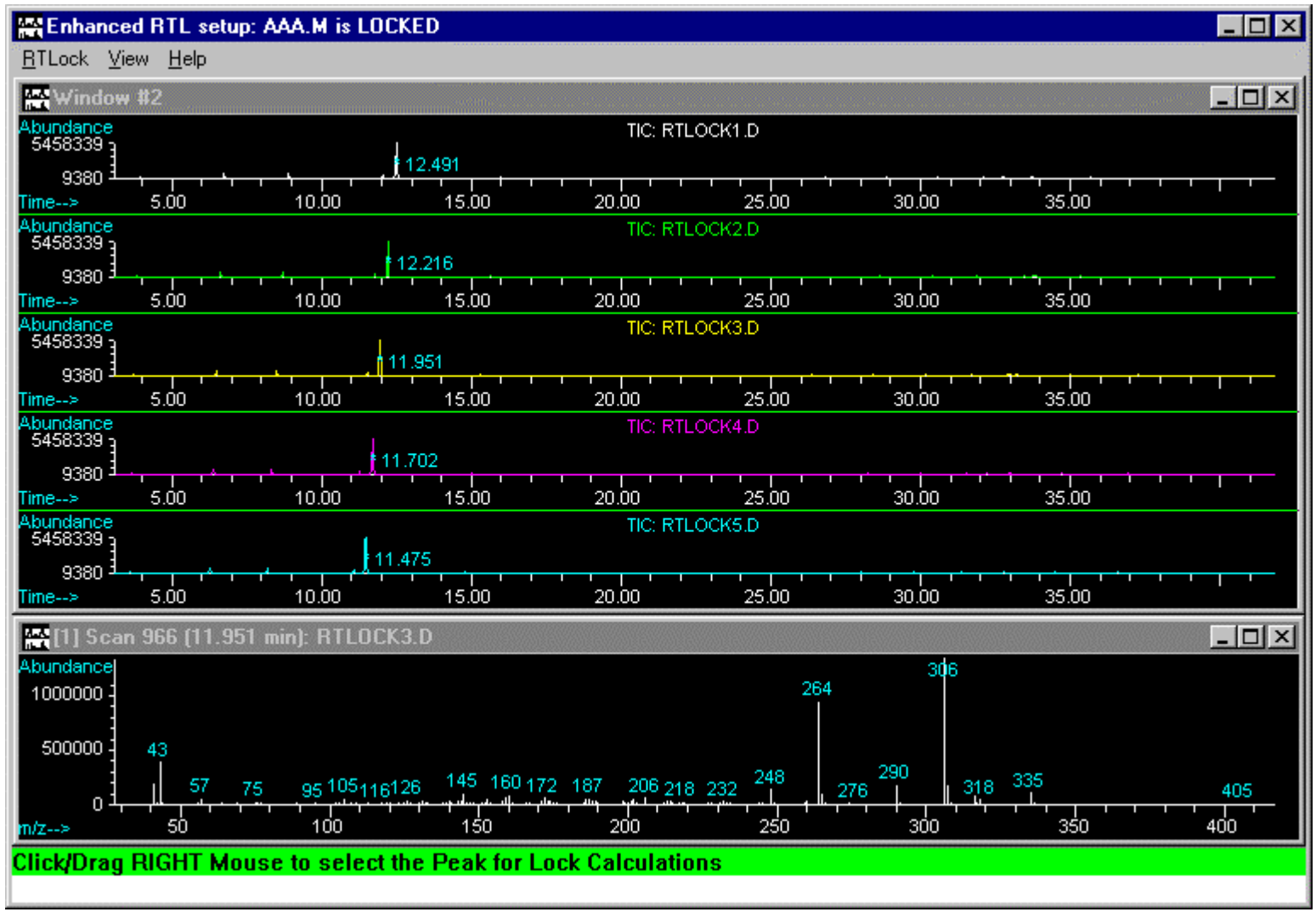
Outline

- What is Retention Time Locking?
- Locking GC Methods
- Locking GC/MSD Methods
- Pesticide Analysis using Agilent's RTL Pesticide Databases

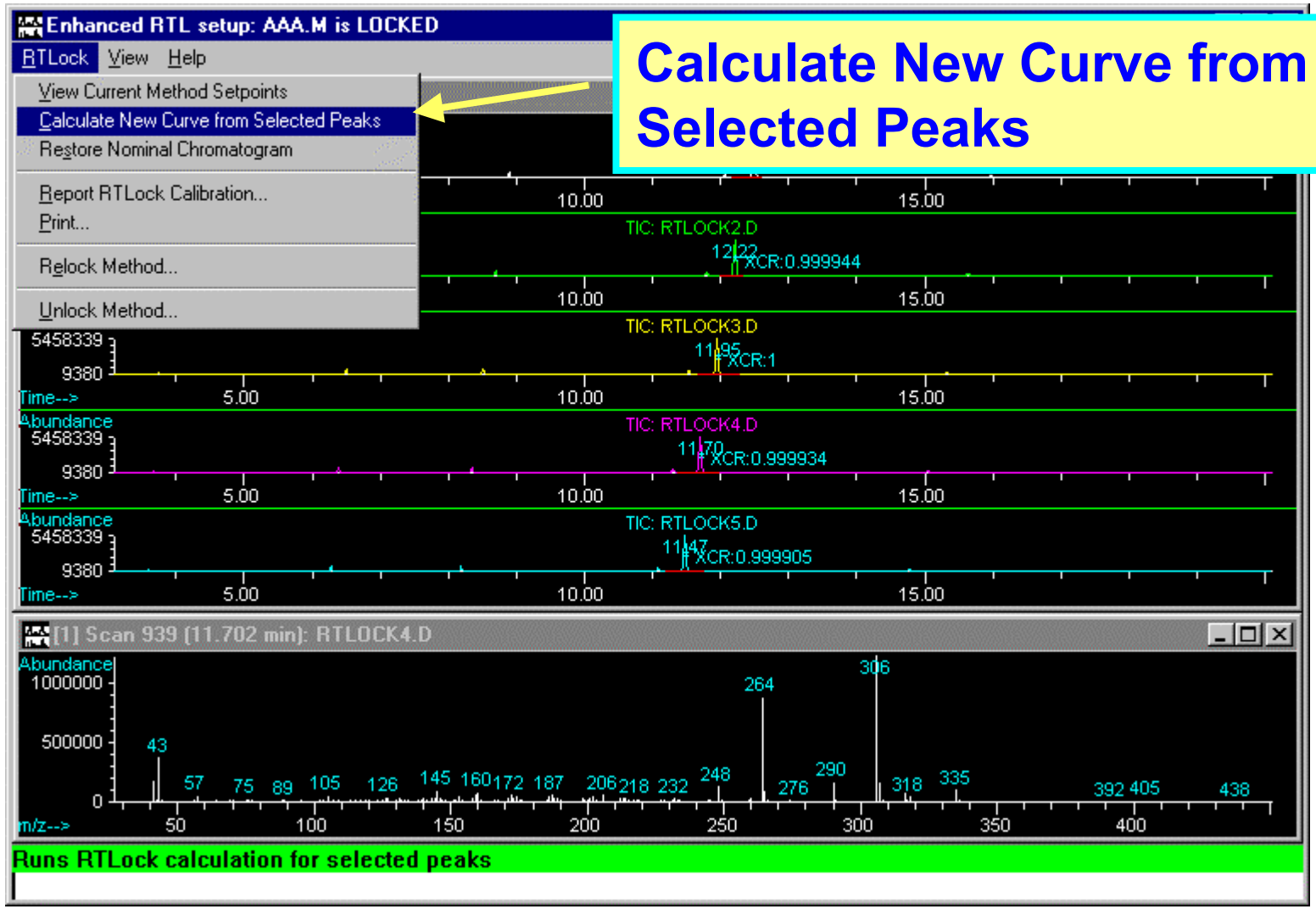
Acquiring RTLock Calibration Data..



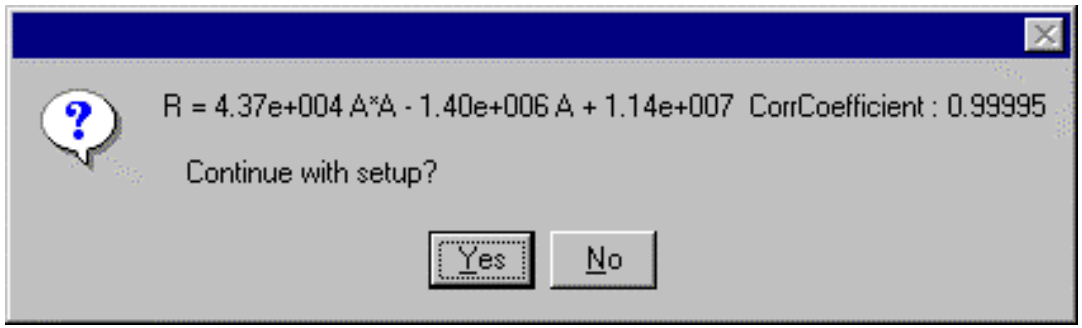
RT Lock Data Files Are Automatically Collected

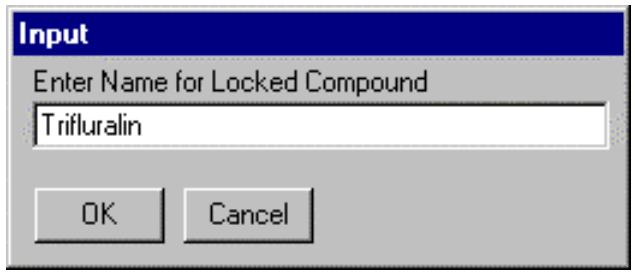


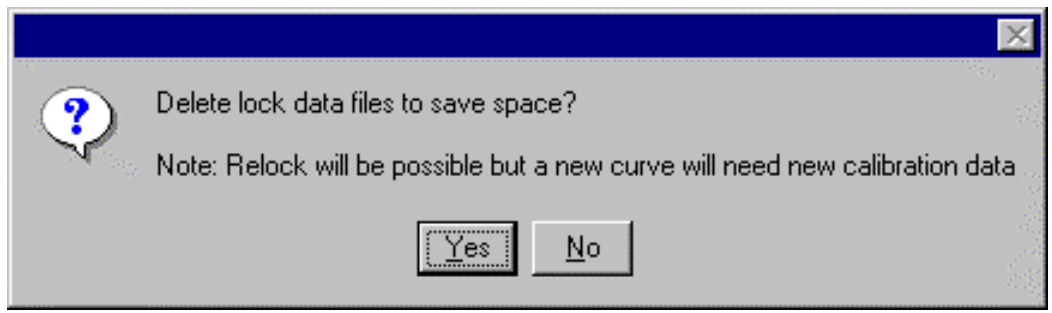
Generate RT vs Head Pressure Equation



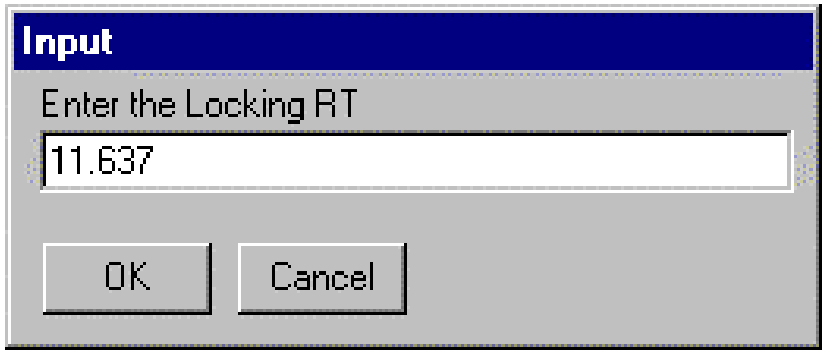
Working with the software

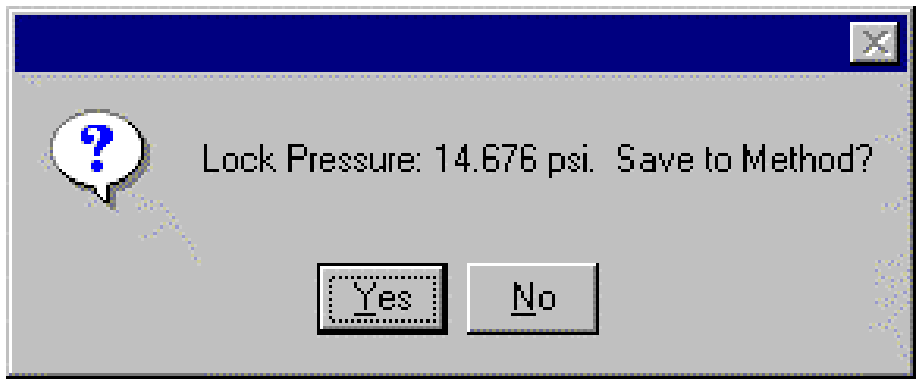






Working with the software





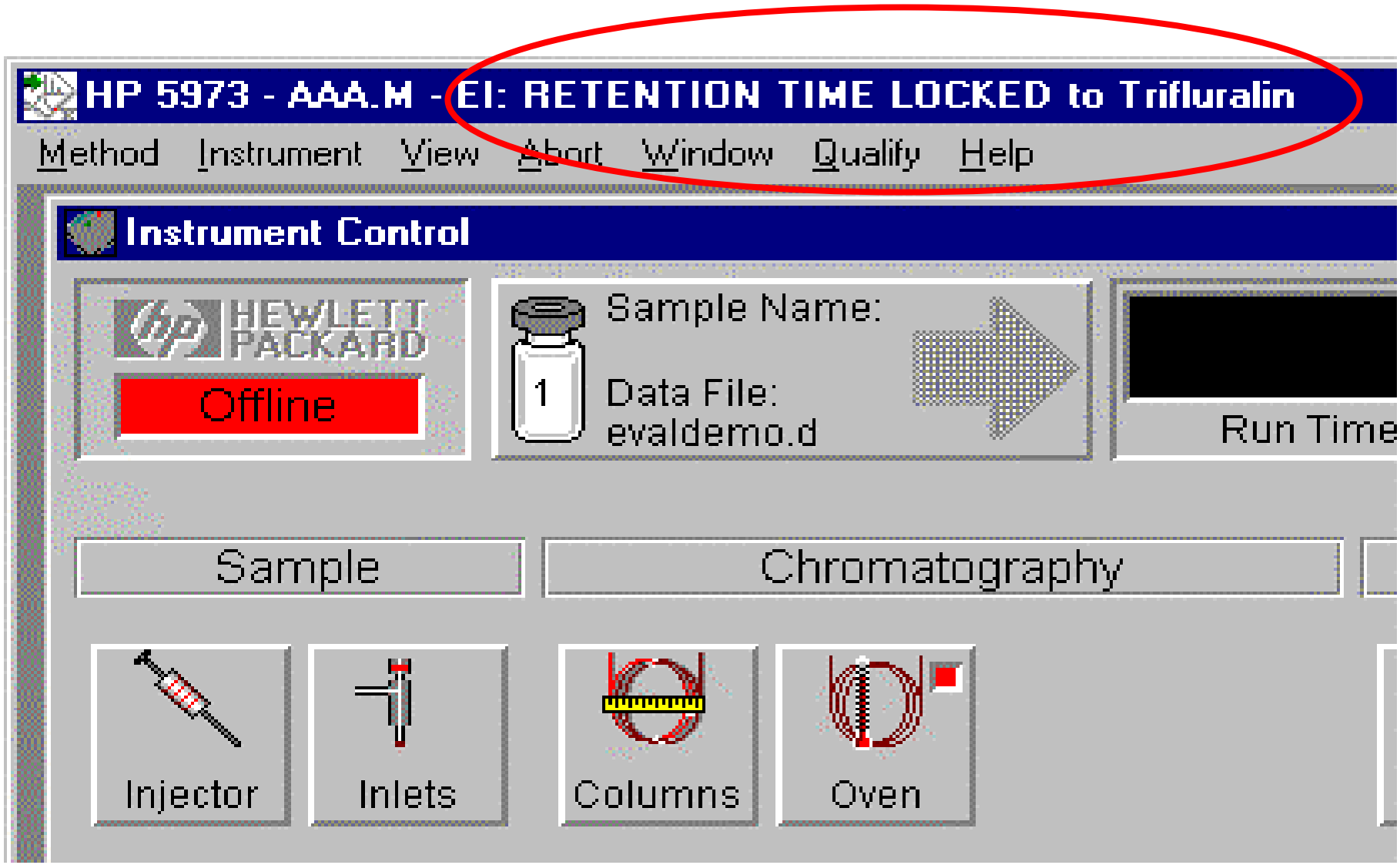
RTL Method Calibration Report

MultiVu - [C:\MSDCHEM\1\METHODS\PEST_RTL.M\rtlrep.txt]

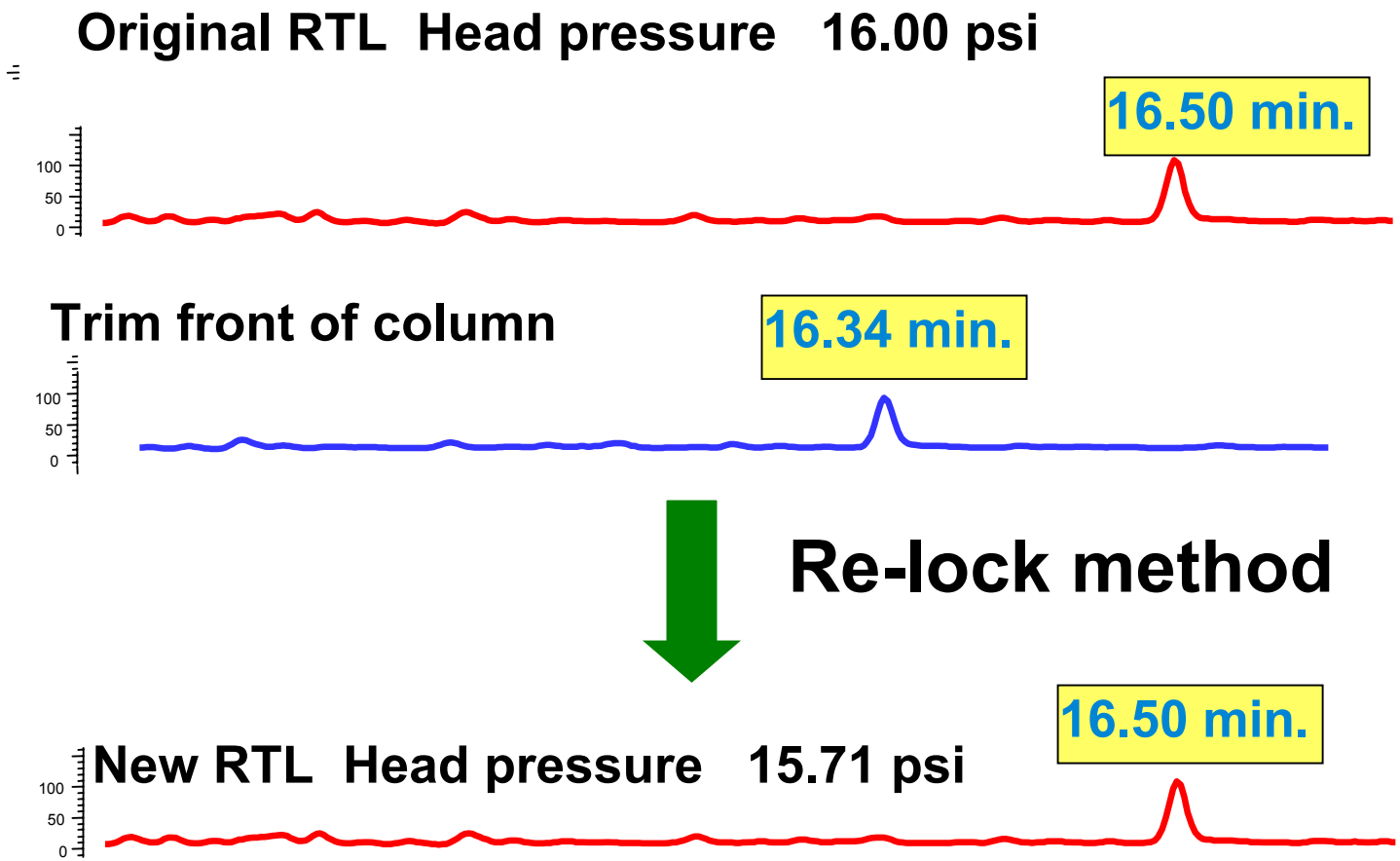
File Edit Search Window

```
              Retention Time Locking Data Report

  Retention Locked Method: C:\MSDCHEM\1\METHODS\PEST_RTL.M
Retention Locked Cal Date: 21 Jan 2000    1:26 pm
                Instrument: 5973N
                  Operator: Chris Sandy

Method Lock is currently On

Compound: Trifluralin

Retention Time Calibration:

                 psi         Time        Spec    Deviation
    File      Pressure       min.        Xcor     Seconds

  RTLOCK1.D     12.80      12.373     0.99536      35.616
  RTLOCK2.D     14.40      12.065     0.99763      17.130
  RTLOCK3.D     16.00      11.779     1.00000       0.000
  RTLOCK4.D     17.60      11.516     0.99749     -15.762
  RTLOCK5.D     19.20      11.277     0.99631     -30.138

   Maximum Deviation: 35.616 seconds
           RTL Curve: R = 6.10e+004 A*A - 1.84e+006 A + 1.44e+007

   Terms of Curve Fit:
       Constant = 1.43685e+007
         Linear = -1.845e+006
      Quadratic = 61025.7
    Coefficient = 0.999981 ** Good Fit **

Locked Retention Time information:

    Retention Locked File: C:\MSDCHEM\1\DATA\IRISH_SL\TRIFLOCK.D
                 Acq Date: 6 Jul 2000    8:40 am
               Instrument: 5973N
                 Operator: Chris Sandy

  Measured Retention Time: 11.384  Pressure: 16.86 (psi)
    Locked Retention Time: 11.637  Pressure: 15.24 (psi)
   ** Locked RT and Pressure Within Calibrated Limits **

   Lock run spectrum XCor: 0.9434


 Report created: Thu Feb 07 10:27:53 2002
```

The Method is Now Locked

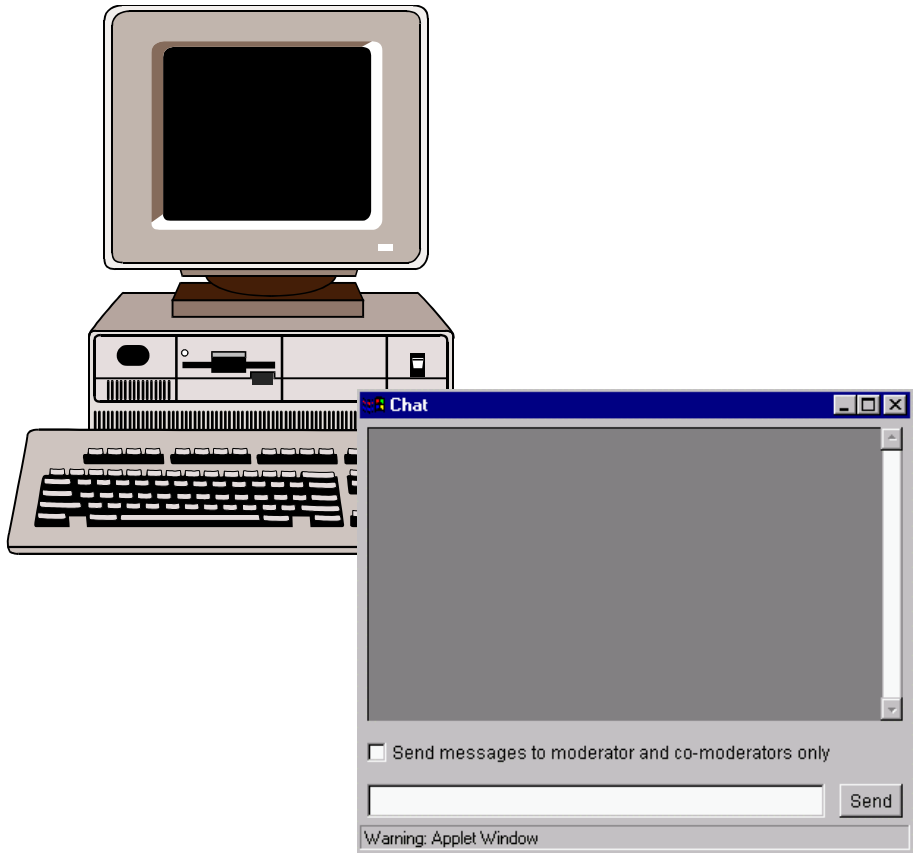


Example: RTL Re-Locking Procedure for Trimmed Column



Break Number 1

Please type your question into the Chat Box at any time during the presentation.



Outline

- What is Retention Time Locking?
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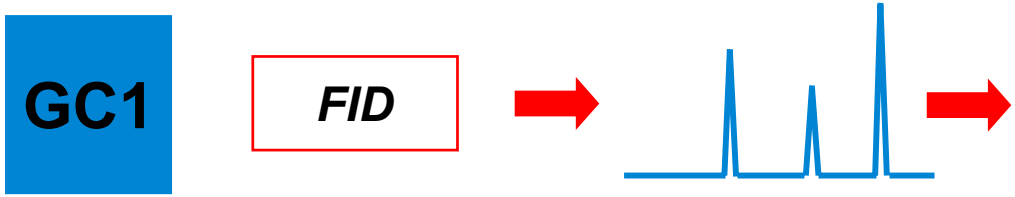
RTL Database Screening: Build a Retention Time Table

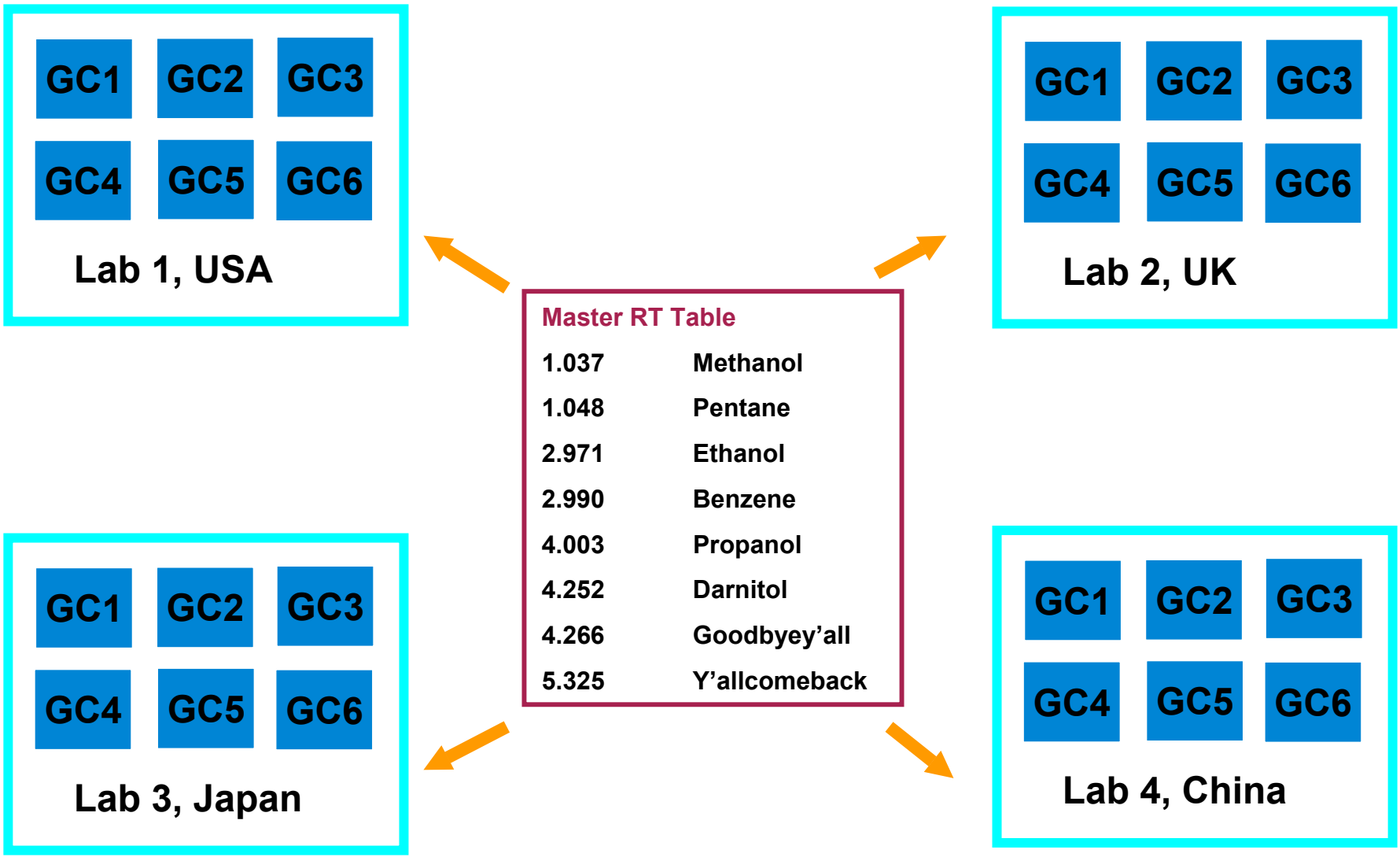
Record retention times under RT Locked conditions



Master RT Table

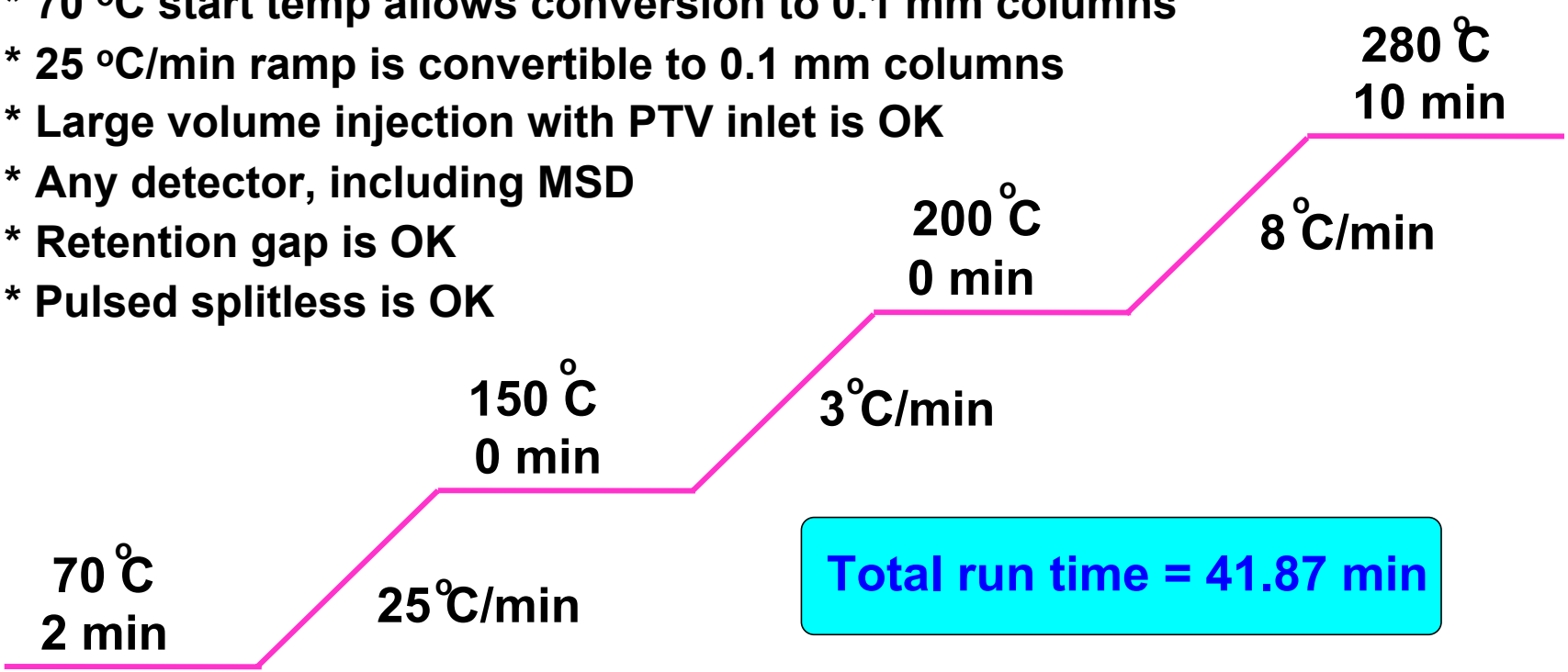
1.037	Methanol
1.048	Pentane
2.971	Ethanol
2.990	Benzene
4.003	Propanol
4.252	Darnitol
4.266	Goodbye y'all
5.325	Y'all comeback

Use Locked RT Table Worldwide



Universal Pesticide Method

* Designed for 30 m X 0.25 mm X 0.25 um HP-5 MS & HP-35 columns
* Excellent separation in reasonable time
* 70 °C start temp allows conversion to 0.1 mm columns
* 25 °C/min ramp is convertible to 0.1 mm columns
* Large volume injection with PTV inlet is OK
* Any detector, including MSD
* Retention gap is OK
* Pulsed splitless is OK



Pesticide & Endocrine Disrupter Library: GC Version

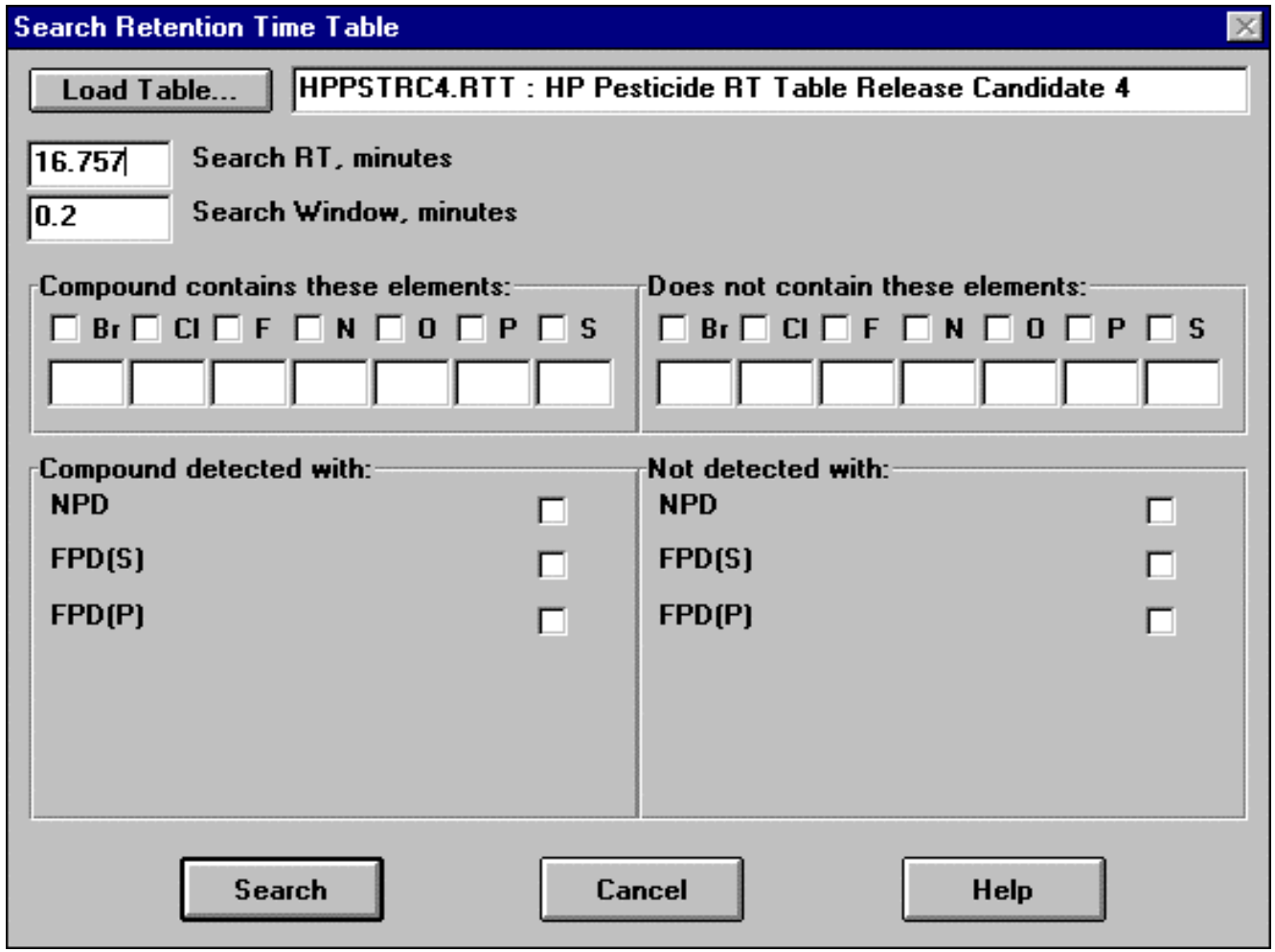
1 of 4 User-defined columns

FID RT	Name	CAS#	Mol. Formula	MW	MS RT	
16.542	Acetochlor	34256-82-1	C:14,H:20,Cl:1,N:1,O:2,	269.77	16.542	HP2364
16.549	Fuberidazole	3878-19-1	C:12,H:8,N:2,O:1,	196.21	16.549	HP2377
16.583	Methyl parathion	298-00-0	C:8,H:10,N:1,O:5,P:1,S:1,	263.20	16.594	HP2068
16.596	Chlorpyrifos Methyl	5598-13-0	C:7,H:7,Cl:3,N:1,O:3,P:1,S:1,	322.53	16.596	HP2142
16.637	Vinclozolin	50471-44-8	C:12,H:9,Cl:2,N:1,O:3,	286.11	16.630	HP2280
16.650	Plifenat	21757-82-4	C:10,H:7,Cl:5,O:2,	336.43	16.641	HP2540
16.689	Terbucarb	001918-11-2	C:17,H:27,N:1,O:2,	277.41	16.686	HP2638

Contains 567 compounds

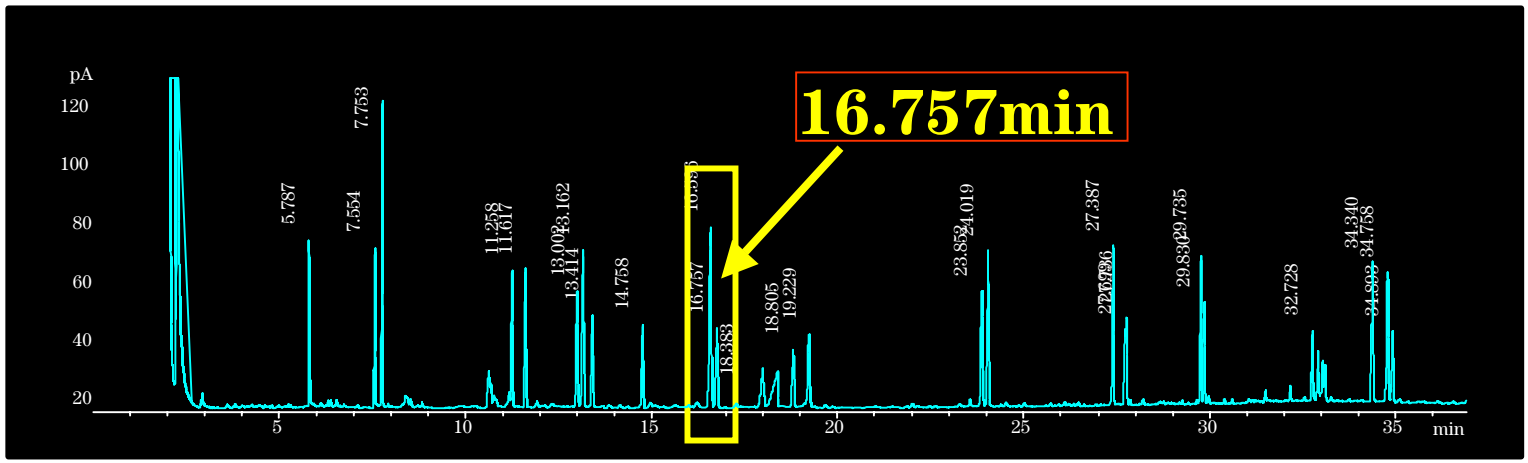
Searching a Retention Time Database

Entering RT and Element qualifiers



Pesticide Database Search

Using an unknown's Retention Time only yields 5 possibilities



RT Table Search Results

Start New Report | Add Results to Current Report | Help...

View Current Report | Close Window

FID_RT	Name	CAS No.	Mol_Formula	MW
16.730	Chloranocryl	9/2/64	C:10,H:9,Cl:2,N:1,O:1,	230.09
16.773	Heptachlor	76-44-8	C:10,H:5,Cl:7,	373.32
16.800	Carbaryl	63-25-2	C:12,H:11,N:1,O:2,	201.22
16.827	Simetryn	1014-70-6	C:8,H:15,N:5,S:1,	213.30
16.850	Silvex	93-72-1	C:10,H:9,Cl:3,O:3,	283.54

Searching a Retention Time Database

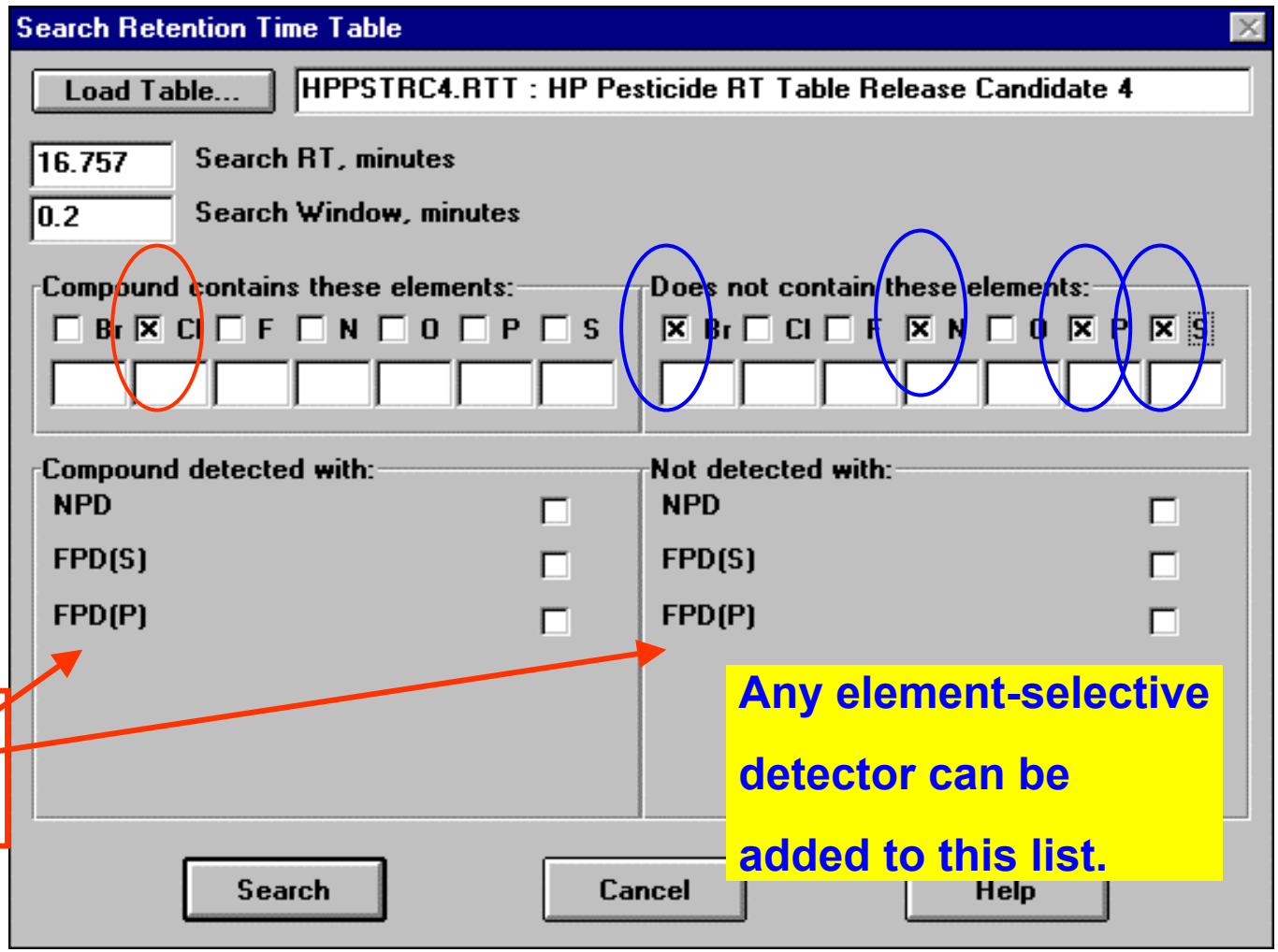
Entering RT and Element Qualifiers

Choose elements

Unknown has Cl

Unknown does not have Br, N, P or S

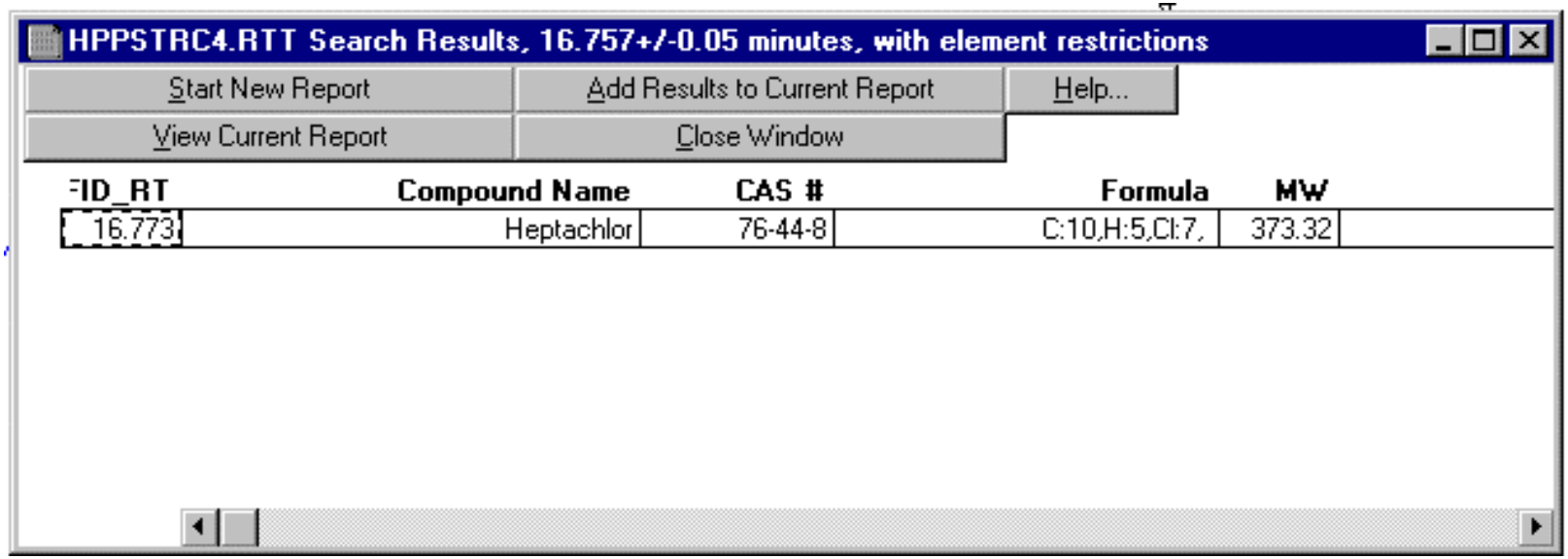
Or… choose detector response



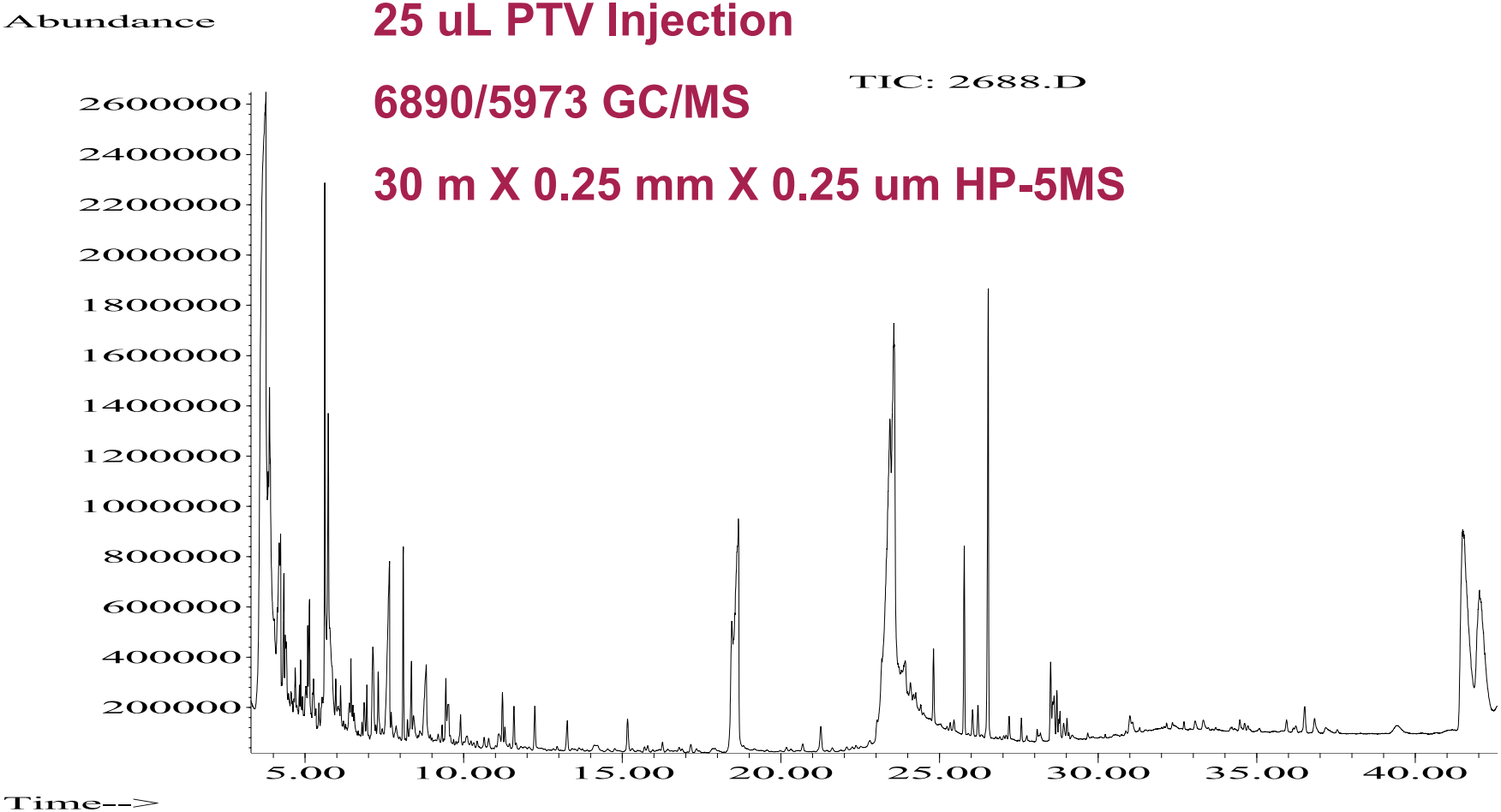
Any element-selective detector can be added to this list.

Pesticide Database Search

Using unknown's element information yields just 1 possibility



Strawberry Extract

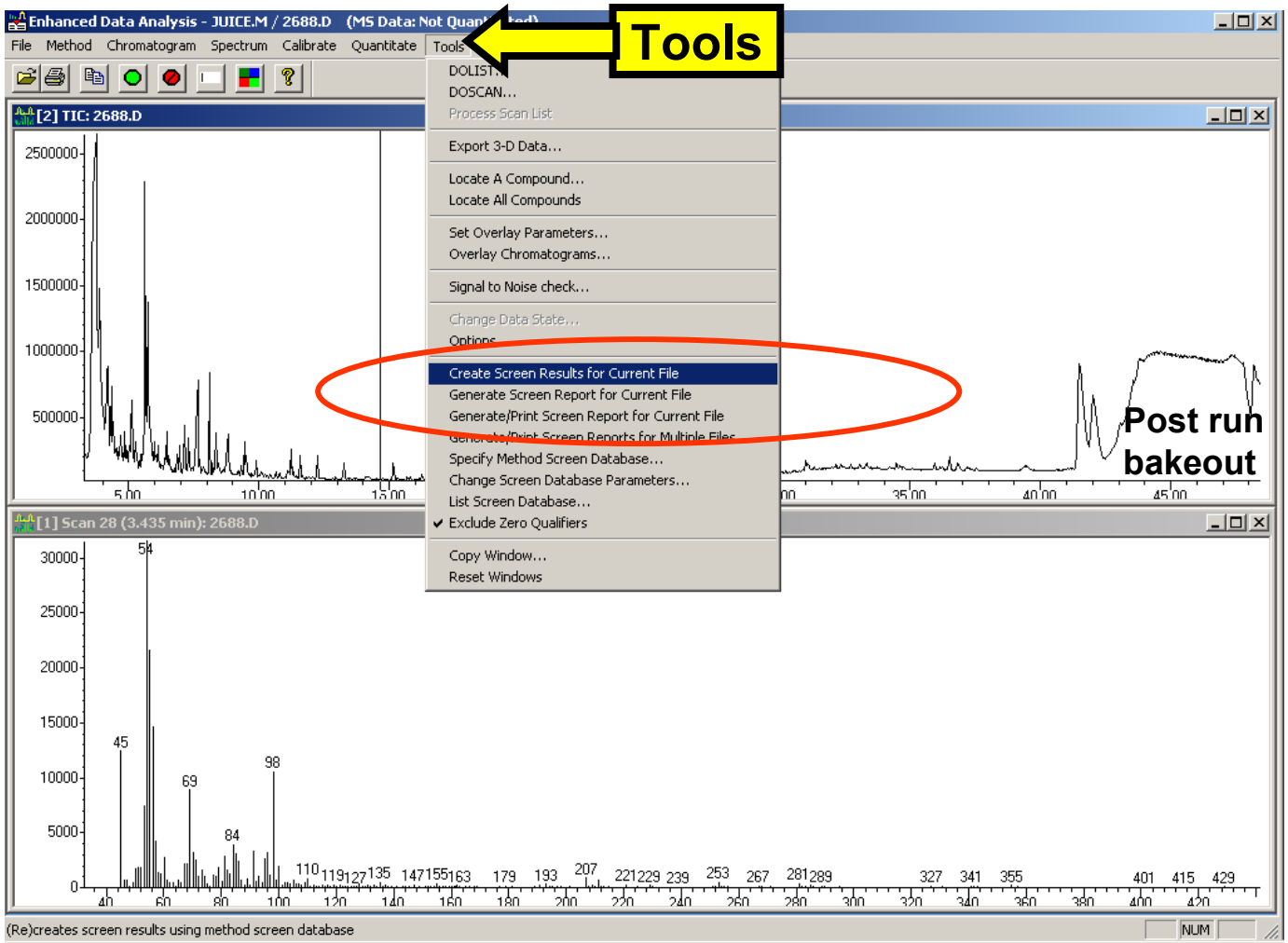


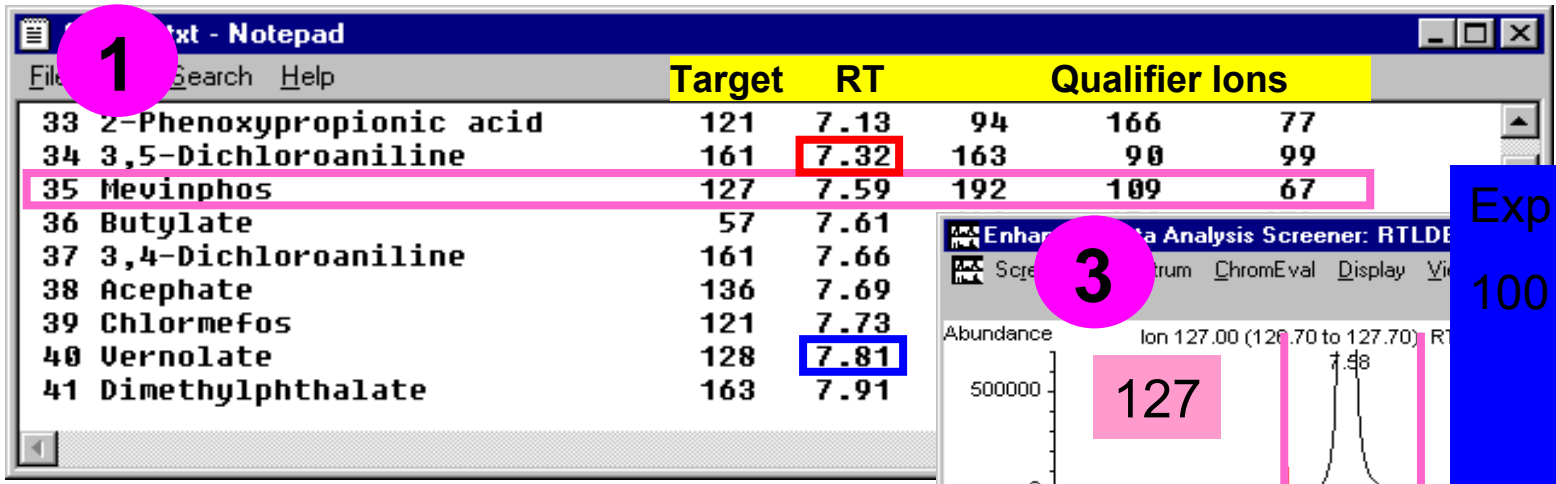
25 uL PTV Injection

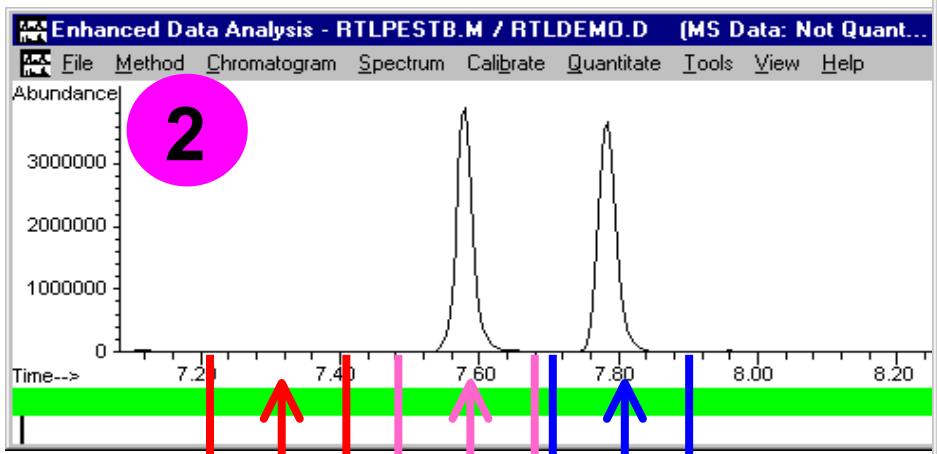
6890/5973 GC/MS

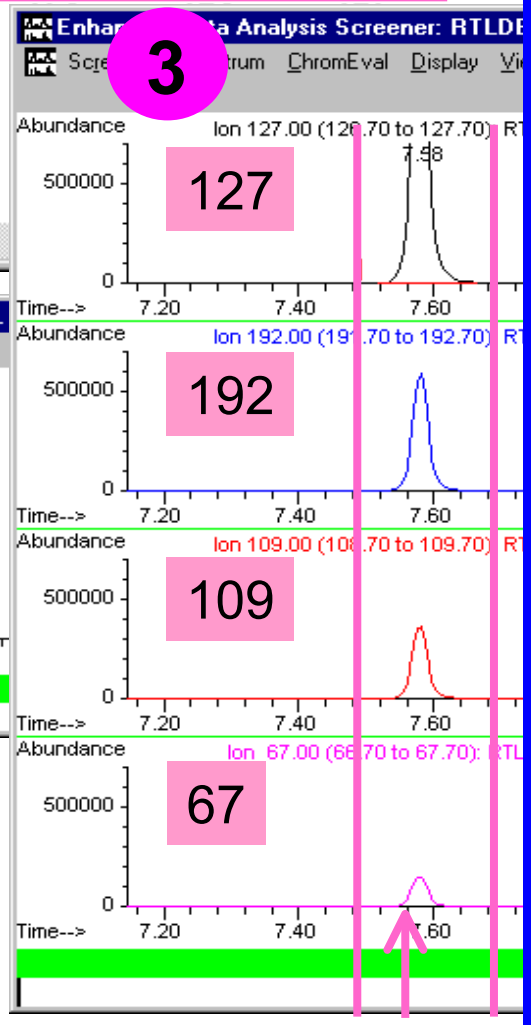
30 m X 0.25 mm X 0.25 um HP-5MS

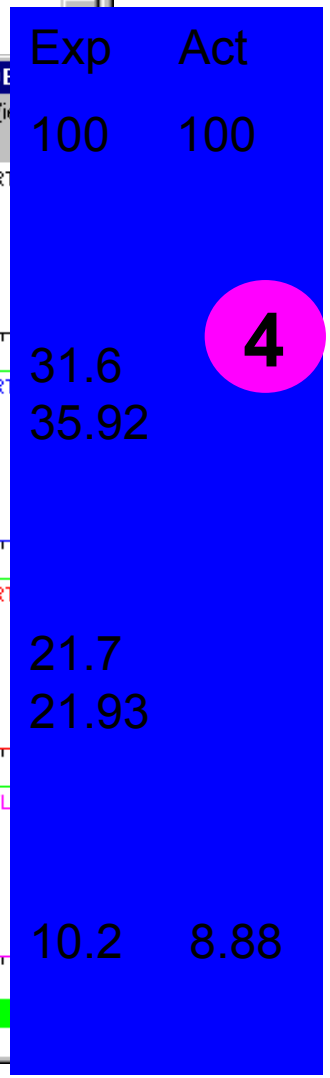
Under Tools, Choose: *Create Screen Results for Current File* then *Generate Screen Report for Current File*







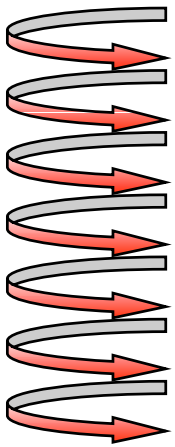




7.32 ± 0.1 | 7.59 ± 0.1 | 7.81 ± 0.1

After a sample is run, ion currents are automatically extracted and integrated for each database entry in sequential time windows.

GC/MSD RTL Screener Software Searches for All Compounds in the Database in a Few Seconds



Scdlist.txt - Notepad

File Edit Search Help

33	2-Phenoxypropionic acid	121	7.13	94	166	77
34	3,5-Dichloroaniline	161	7.32	163	90	99
35	Mevinphos	127	7.59	192	109	67
36	Butylate	57	7.61	146	156	174
37	3,4-Dichloroaniline	161	7.66	163	99	90
38	Acephate	136	7.69	94	42	95
39	Chlormefos	121	7.73	97	154	234
40	Vernolate	128	7.81	43	86	146
41	Dimethylphthalate	163	7.91	77	164	194

Initial Screener Report for Strawberry Extract

MultiVu - [C:\MSDCHEM\1\DATA\CDFA SAMPLES FROM KAI AND MIKE\2688.D\scrntemp.txt]

File Edit Search Window

Screen Report (Not Reviewed)

Data File : C:\MSDCHEM\1\DATA\CDFA SAMPLES FROM KAI AND MIKE\2688.D Vial: 8
Acq On : 7 Apr 2003 19:03 Operator: mikeski
Sample : 25 ul PTV 300 RTLpest 1X Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: RTEINT.P

Screen File: rtlpest2.RES
Screen Database: rtlpest2.SCD

Extraction Window: +/- 0.100 min
Qualifier Mode : Absolute
Qualifier % : 20
Zero qualifiers : Excluded
Subtraction Mode : Sub Average Start/Stop

567 Compounds Screened

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
3	p-Dichlorobenzene	?	3.882	+0.004	146	2459	148,111,75	0.04
8	4-Methylphenol	?	4.415	+0.052	107	2066	108,77	0.00
31	Biphenyl	x	7.104	+0.064	154	4420		0.00
36	Butylate	?	7.607	-0.036	57	2520	146,156,174	0.43
50	Chloroneb	?	8.675	-0.042	191	2719	193,206,208	0.44
72	Diethyl phthalate	x	9.958	+0.036	149	7891		0.99
84	Benzophenone	?	10.667	-0.019	105	3179	77,51	0.37
95	Dibrom (naled)	?	11.221	+0.003	109	3041	185,145,79	0.02
263	Di-n-butylphthalate	x	18.415	+0.033	149	466128		0.99
330	Captan	?	21.227	+0.037	79	69148	151	0.89
429	Flamprop-isopropyl	x	25.821	-0.040	105	2649		0.00
459	Butyl benzyl phthalate	x	27.007	+0.020	149	6538		0.95
505	Fenpropathrin	x	28.990	+0.026	97	26411		0.45
513	Bis(2-ethylhexyl)phthalat	x	29.648	+0.017	149	12285		0.96

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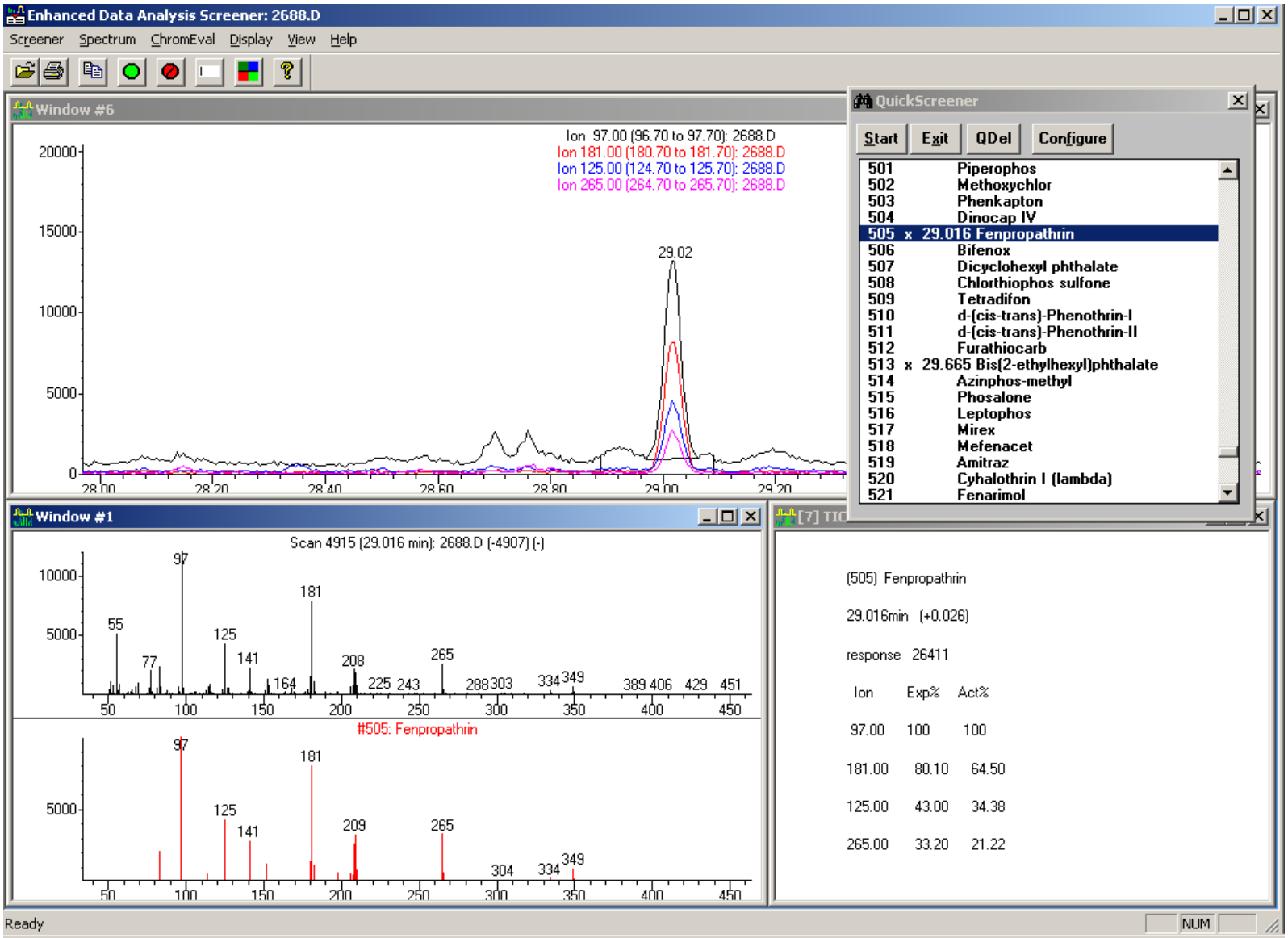
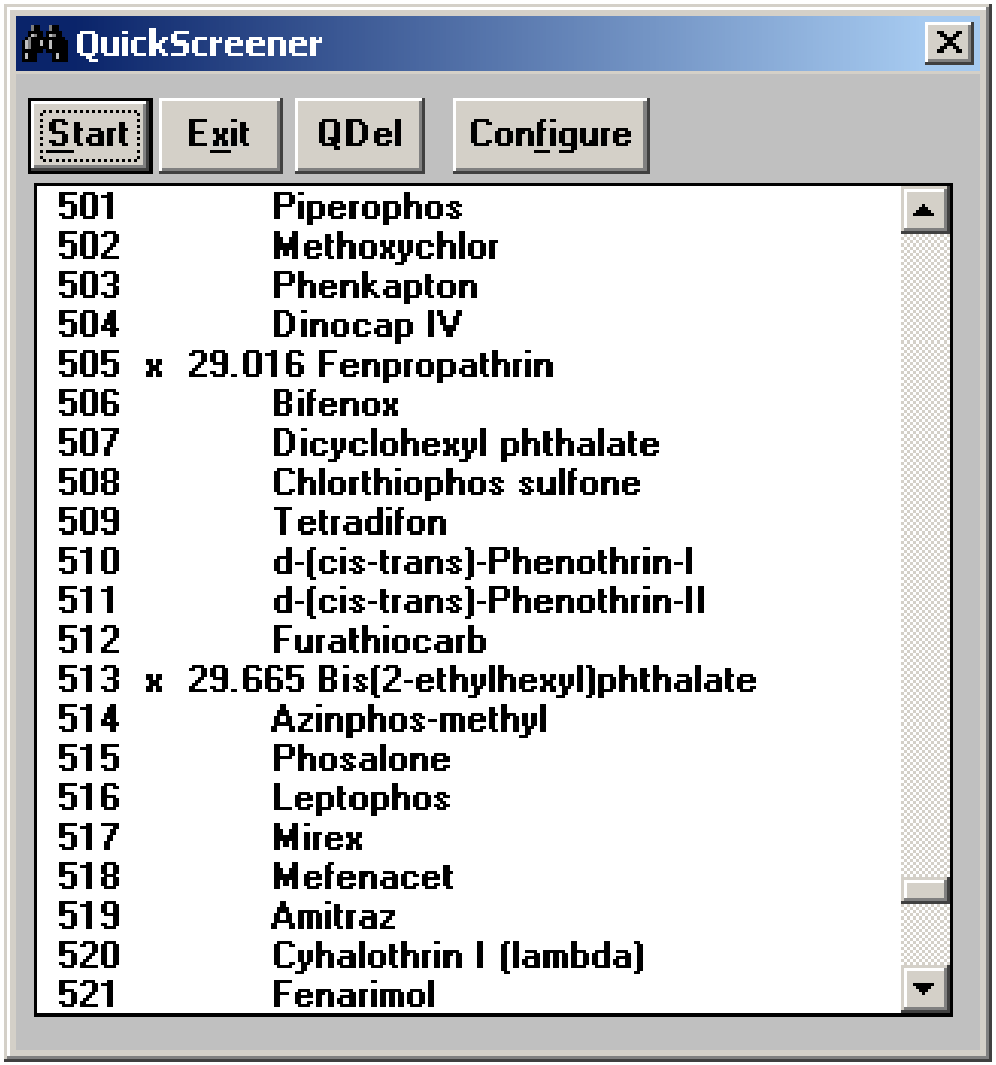
14 possible 'hits'

Results Screener Software for Verification

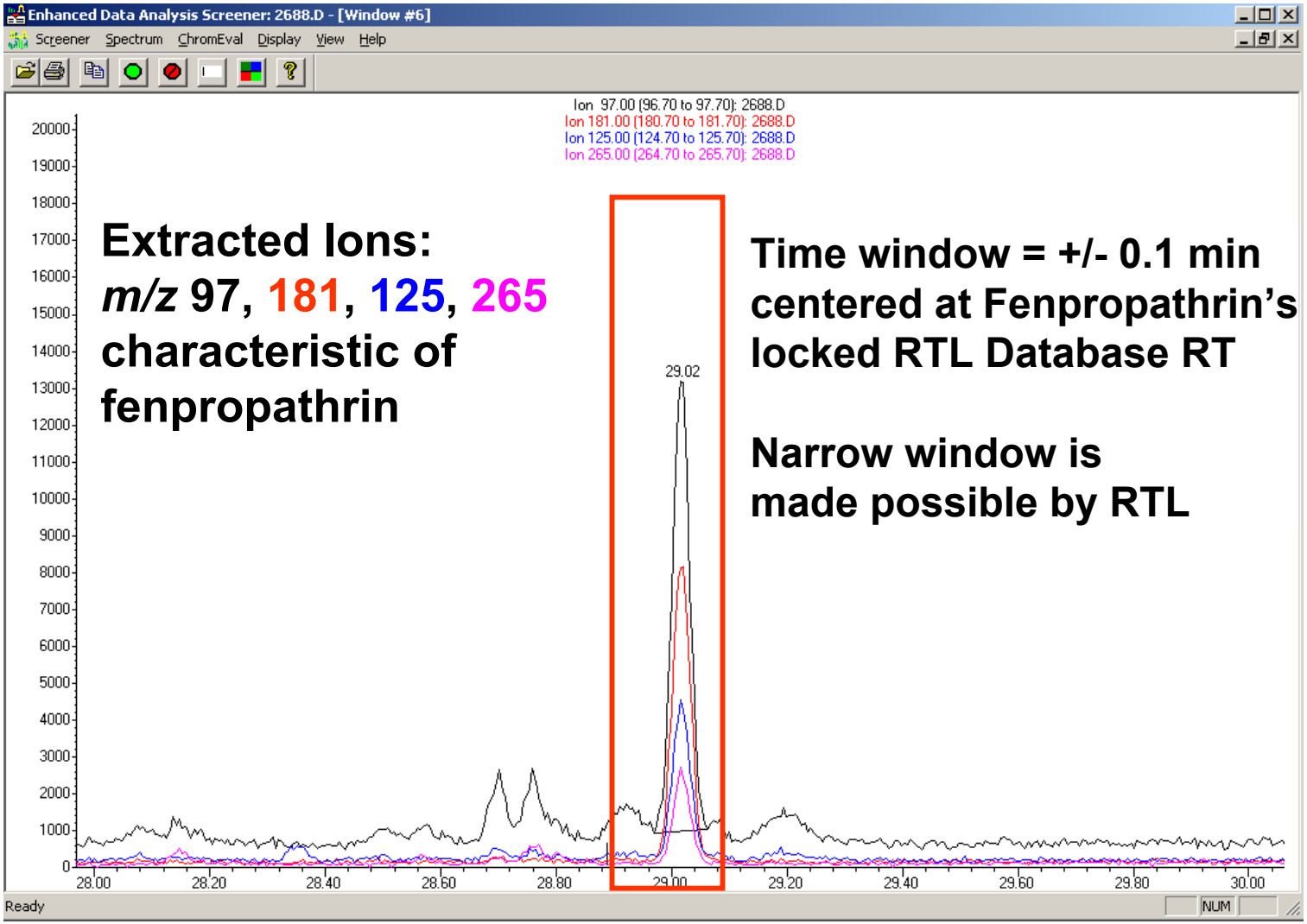


Results Screener: Compound List with 'Hits' Marked with ? or X

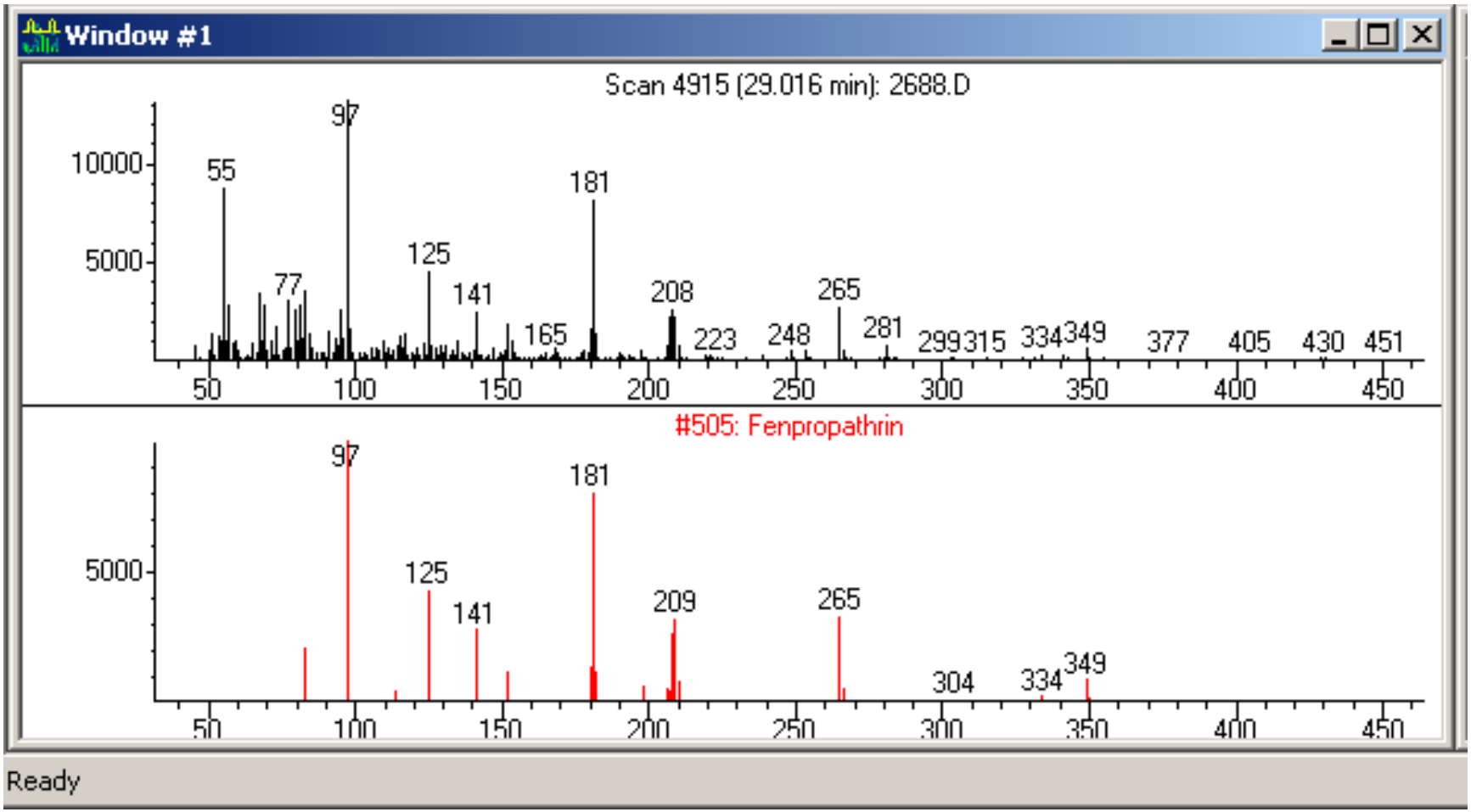


Fenpropathrin and Bis(2-ethylhexyl)phthalate are marked with an X because all four target ions are present in the correct ratios inside the retention time window (0.2 min)

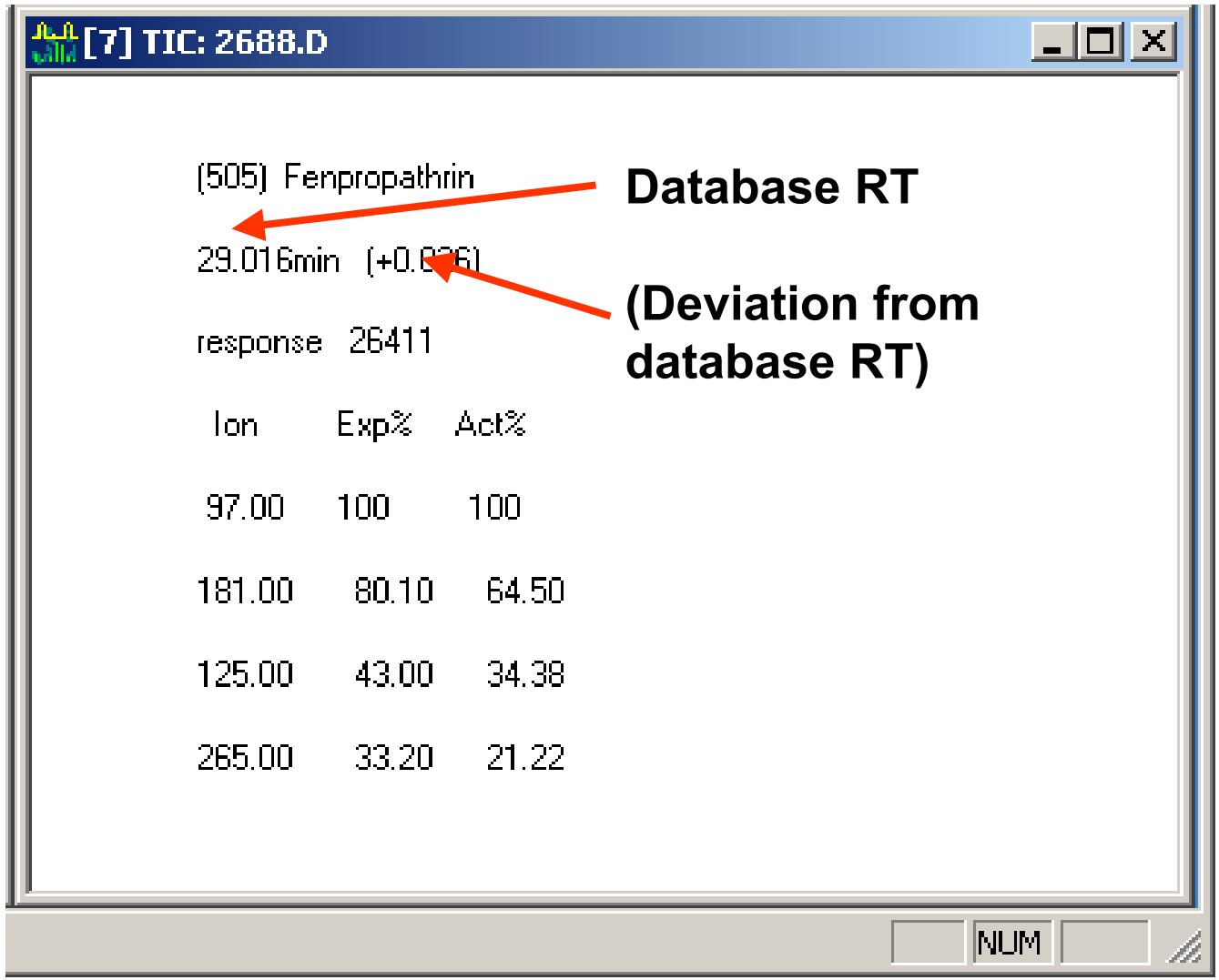
Results Screener: 4 Ions of Fenpropathrin Extracted - peaks found inside time window



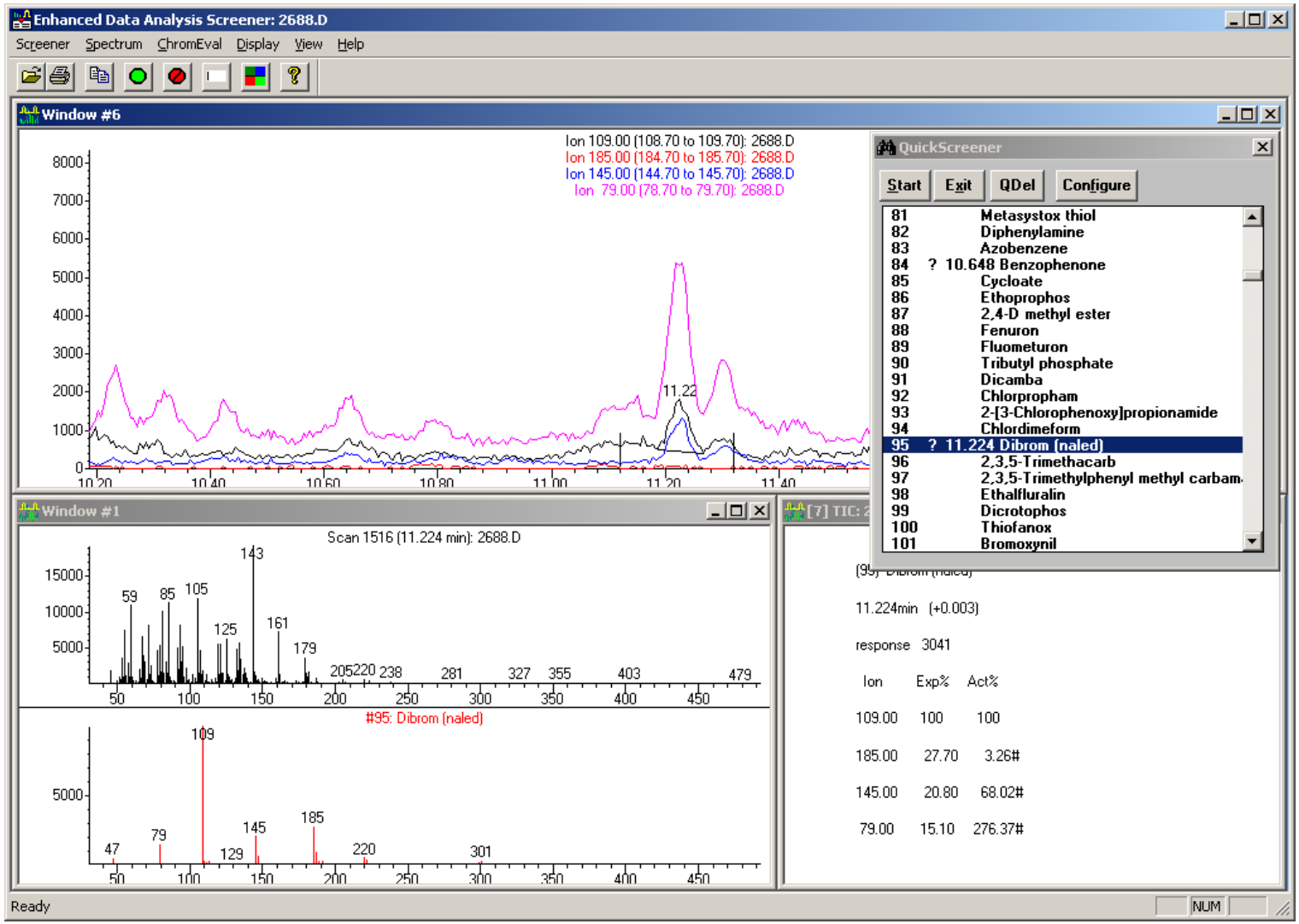
Results Screener: Apex Spectrum Compared to RTL Pesticide Library Spectrum



Results Screener: Expected and Actual Ion Ratios and Retention Time Deviation



Dibrom: Originally Marked with a '?' Discarded During Review



Strawberry After Data Review Using the Results Screener: Captan & Fenpropathrin Identified

MultiVu - [C:\MSDCHEM\1\DATA\CDFA SAMPLES FROM KAI AND MIKE\2688.D\scrntemp.txt]

File Edit Search Window

Screen Report (Screen Reviewed)

Data File : C:\MSDCHEM\1\DATA\CDFA SAMPLES FROM KAI AND MIKE\2688.D Vial: 8
Acq On : 7 Apr 2003 19:03 Operator: mikeski
Sample : 25 ul PTV 300 RTLpest 1X Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: RTEINT.P

Screen File: rtlpest2.RES
Screen Database: rtlpest2.SCD

Extraction Window: +/- 0.100 min
Qualifier Mode : Absolute
Qualifier % : 20
Zero qualifiers : Excluded
Subtraction Mode : Sub Average Start/Stop

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
72	Diethyl phthalate	x	9.958	+0.036	149	7891		0.99
263	Di-n-butylphthalate	x	18.415	+0.033	149	466128		0.99
330	Captan	x	21.227	+0.037	79	88406	151	0.88
459	Butyl benzyl phthalate	x	27.007	+0.020	149	6538		0.95
505	Fenpropathrin	x	28.990	+0.026	97	26411		0.45
513	Bis(2-ethylhexyl)phthalat	x	29.648	+0.017	149	12285		0.96

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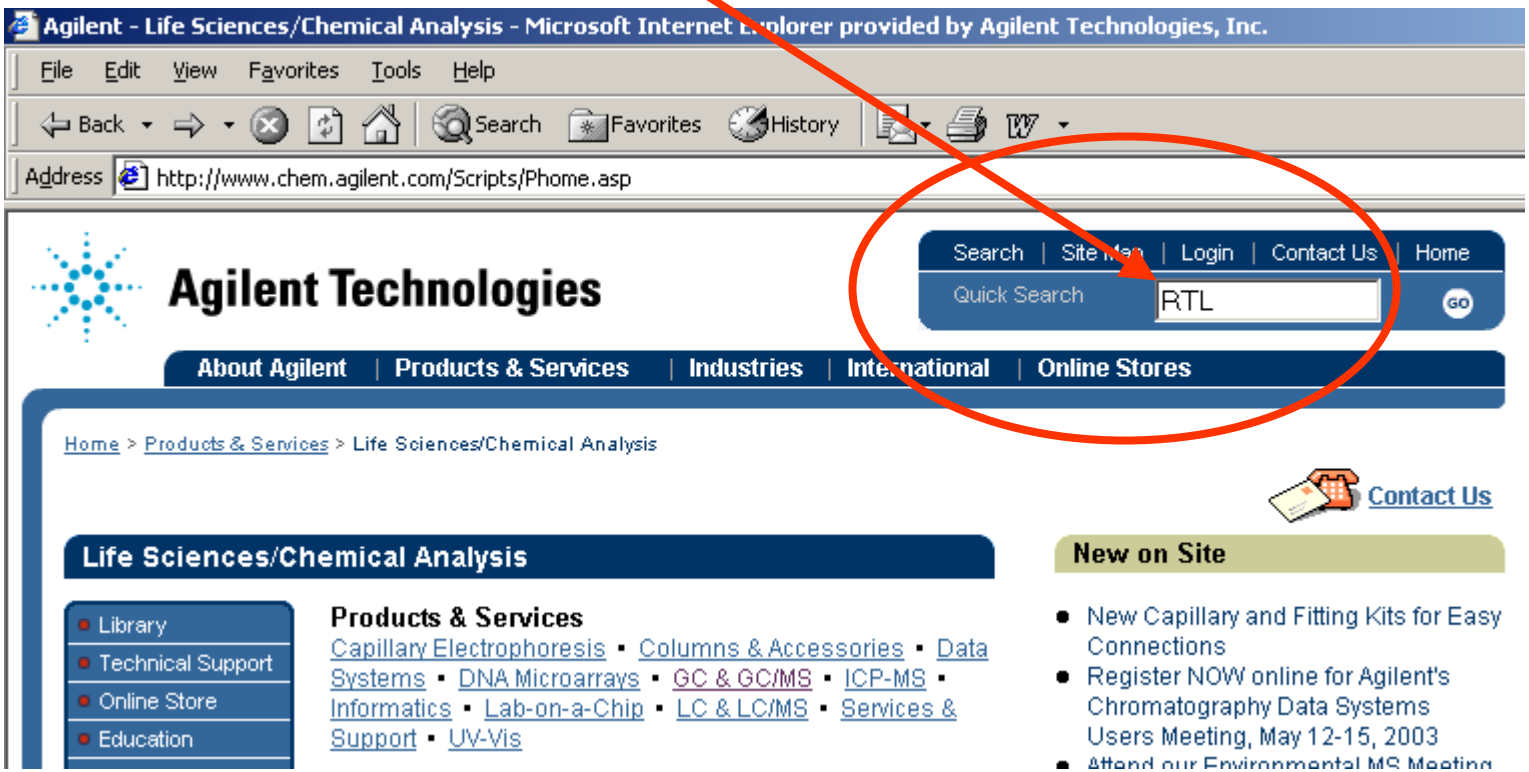
Where to get More RTL & Pesticide Analysis Information

At www.Agilent.com, choose Life Sciences/Chemical

Type RTL or Pesticide into the quick search window



Ordering RTL Libraries or RTL Software for GC

(Note: RTL Software is built into the GC/MS ChemStation)

- G1049A RTL Mass Spectral Pesticide Library
- G2081AA RTL Pesticide Library for GC
- G2080AA Retention Time Locking software for GC

Why Use RTL with Agilent's Pesticide Databases?

- Retention times are critical for pesticide identification
- The databases are comprehensive
  - USA, European, & Japanese pesticides
  - Many, many others including endocrine disrupters
- Never update quant or SIM table RTs
- Identify non-target pesticides
- Fewer false positives & false negatives
- Portable methods
- Compare results among instruments and labs
- GC and GC/MS retention times are the same