Welcome to our E-Seminar:

Pesticide Analysis using Retention Time Locking and RTL Databases

A History of Leadership in GC and GC/MS

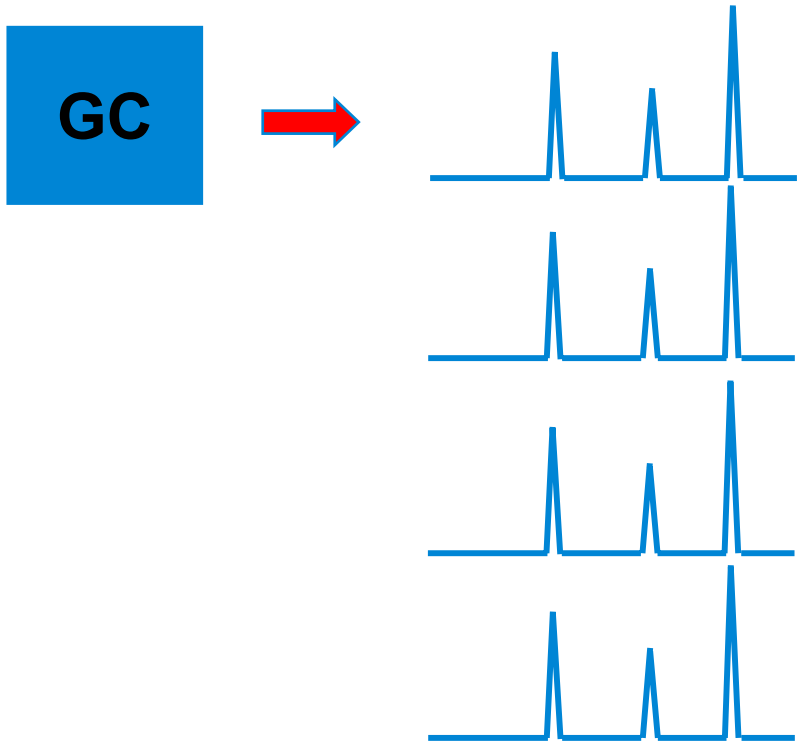
- 1959 – First linear temperature programmed GC
- 1963 – First GC with automatic temperature programming & oven cooling
- 1968 – Introduces digital integrator
- 1969 – First automatic sampler for GC
- 1974 – First microprocessor-controlled GC
- 1974 – First digital GC integrator
- 1976 – First digital 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

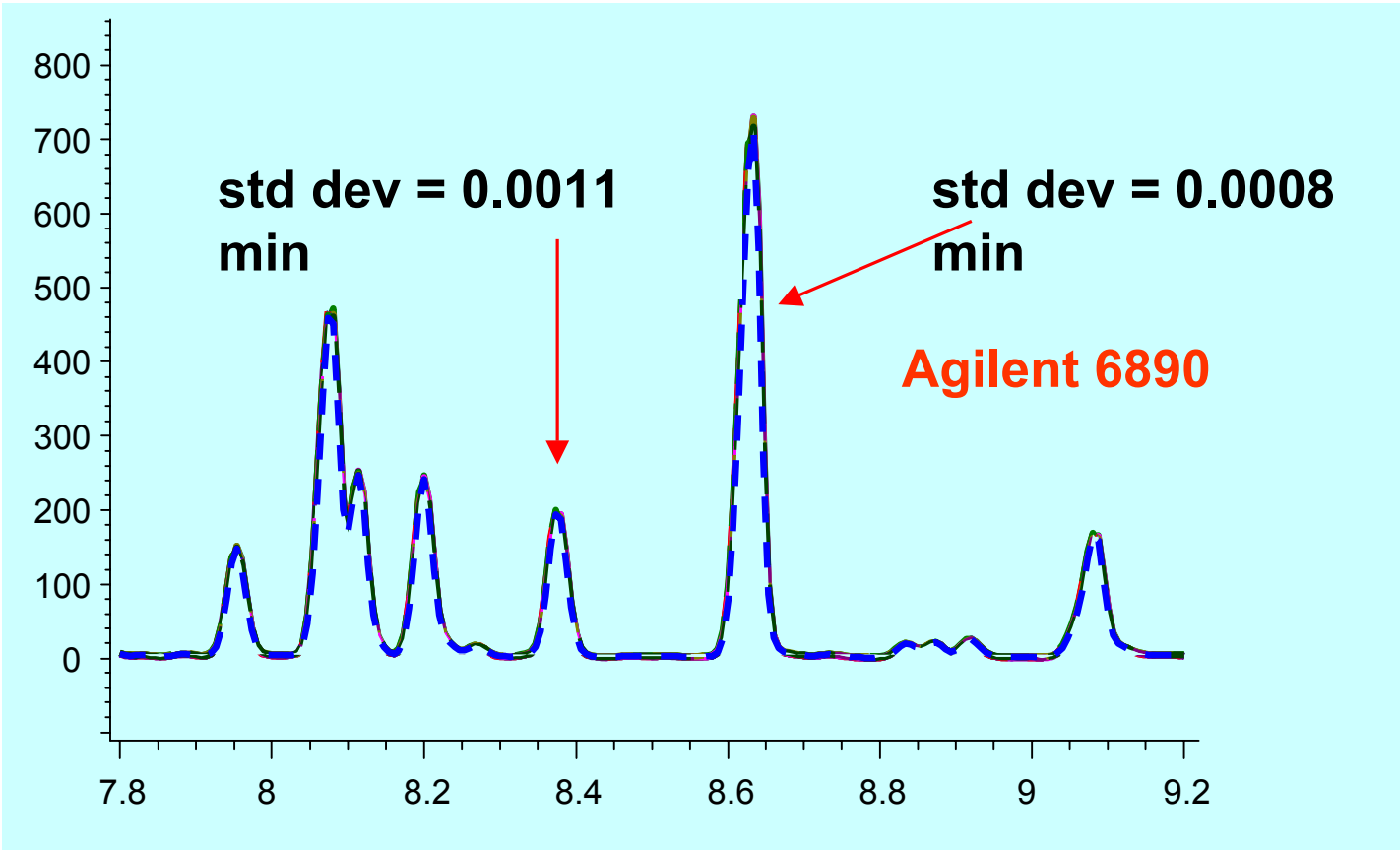
What's the Problem?

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



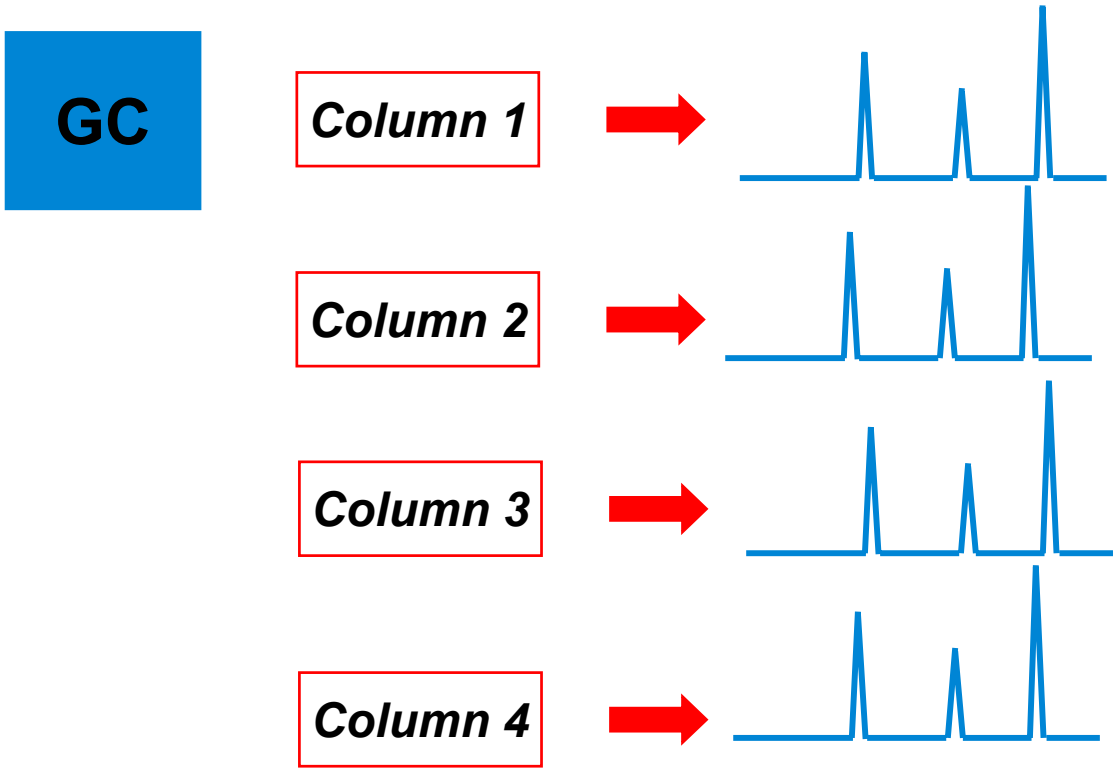
Retention Time Precision, 6890

Gasoline, 15 Runs Over 5 Days



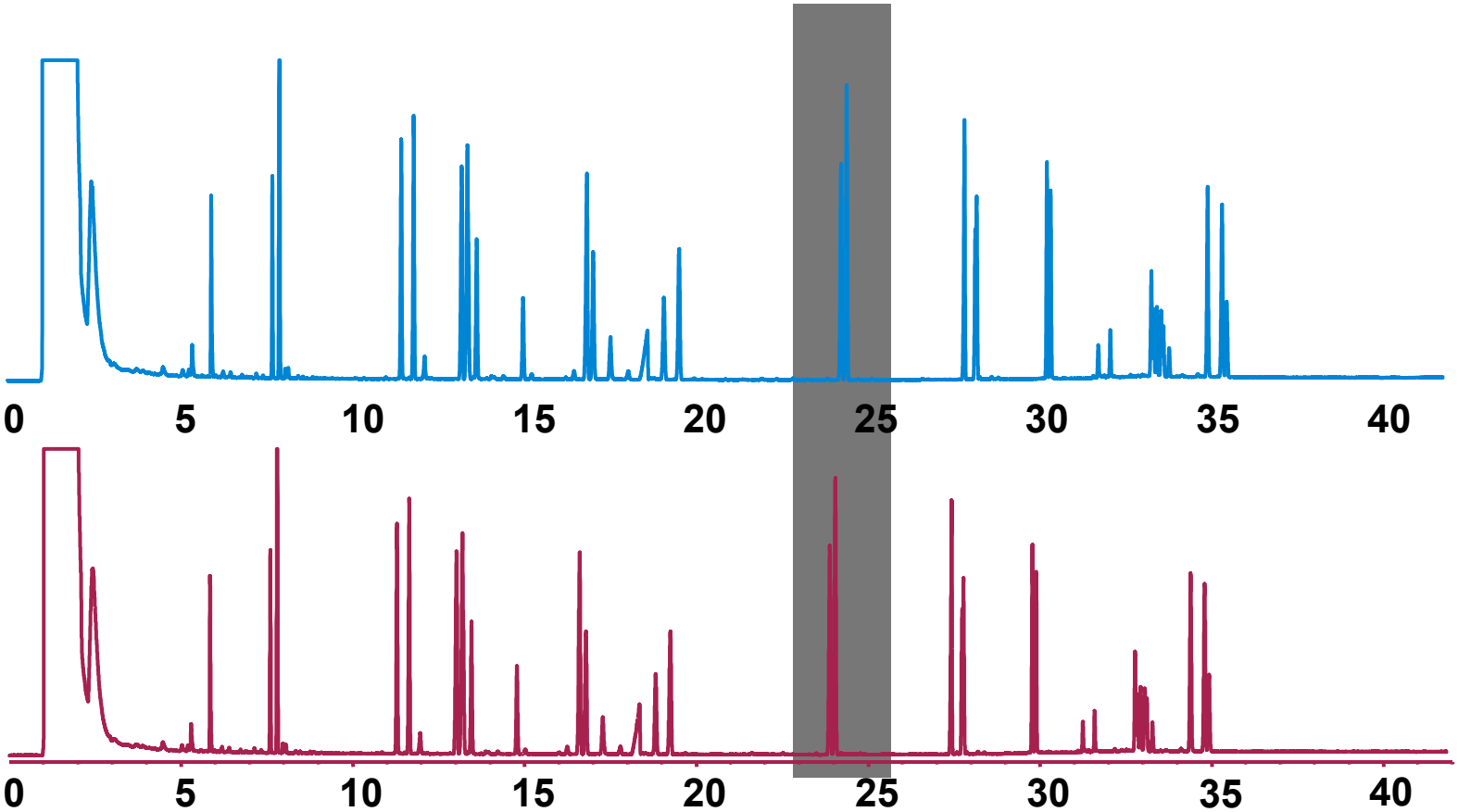
Different Columns, Same GC

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

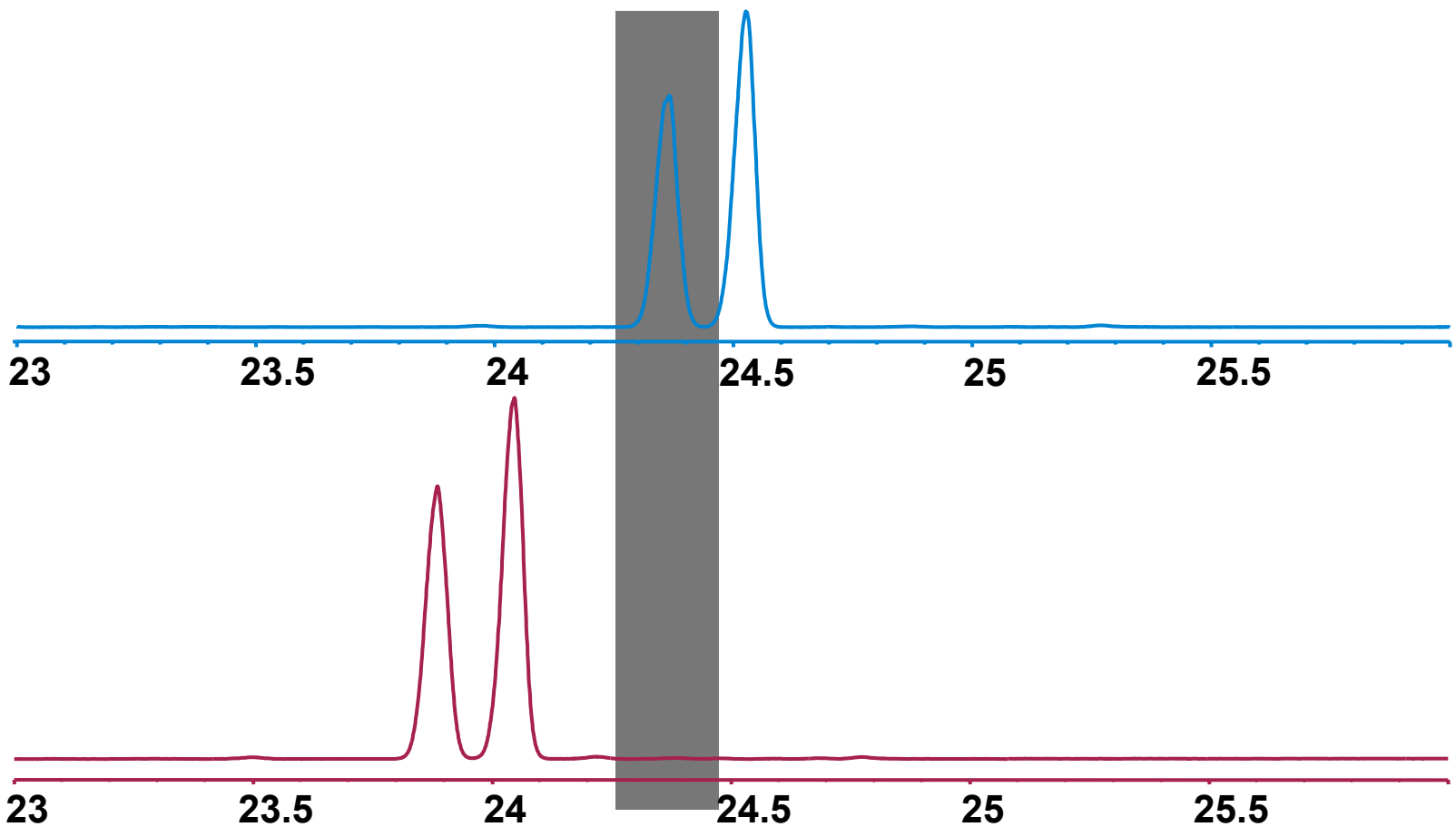


Pesticides on Two Different GCs

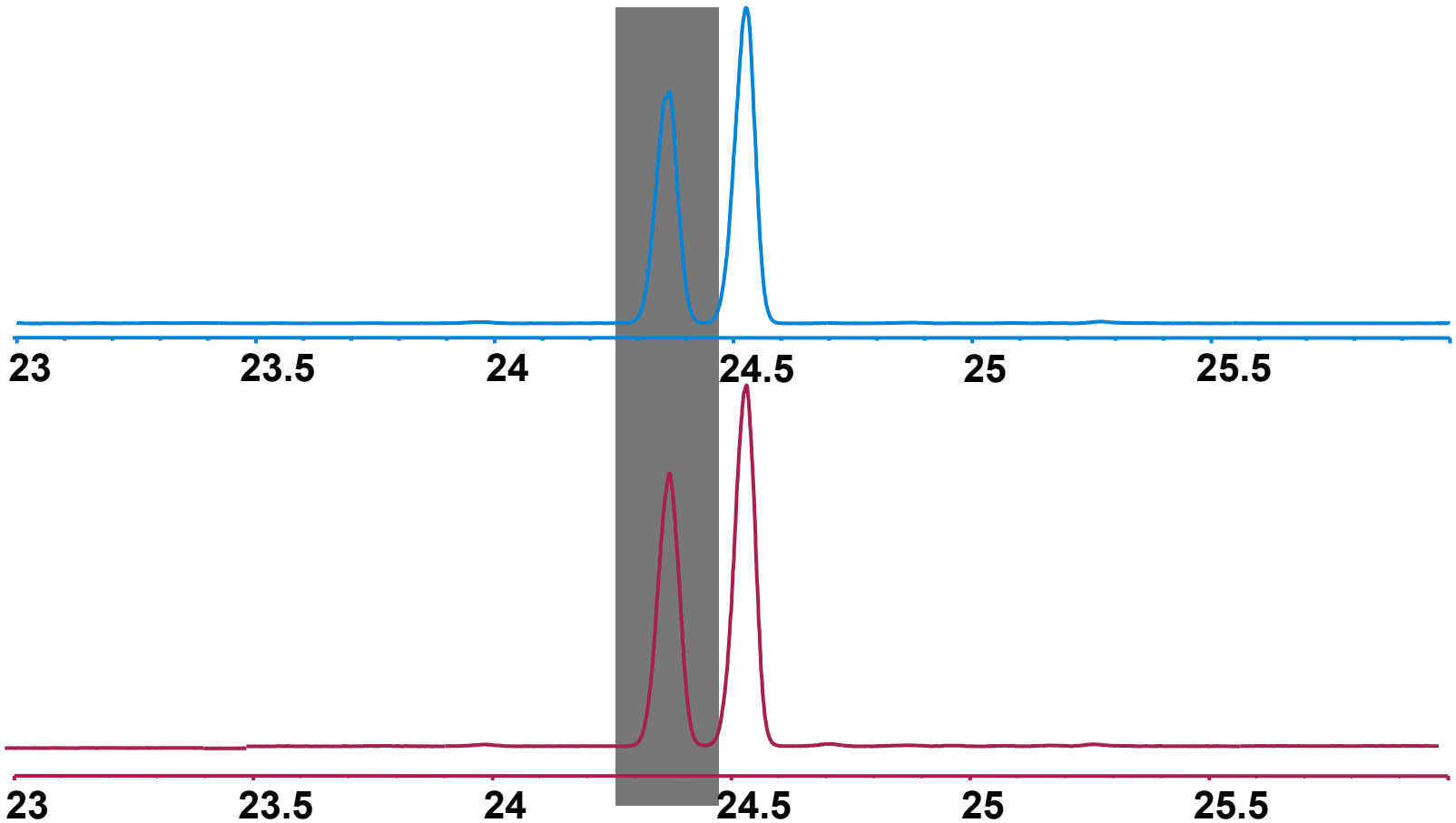
Retention times vary from column-to- column and instrument-to-instrument



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.

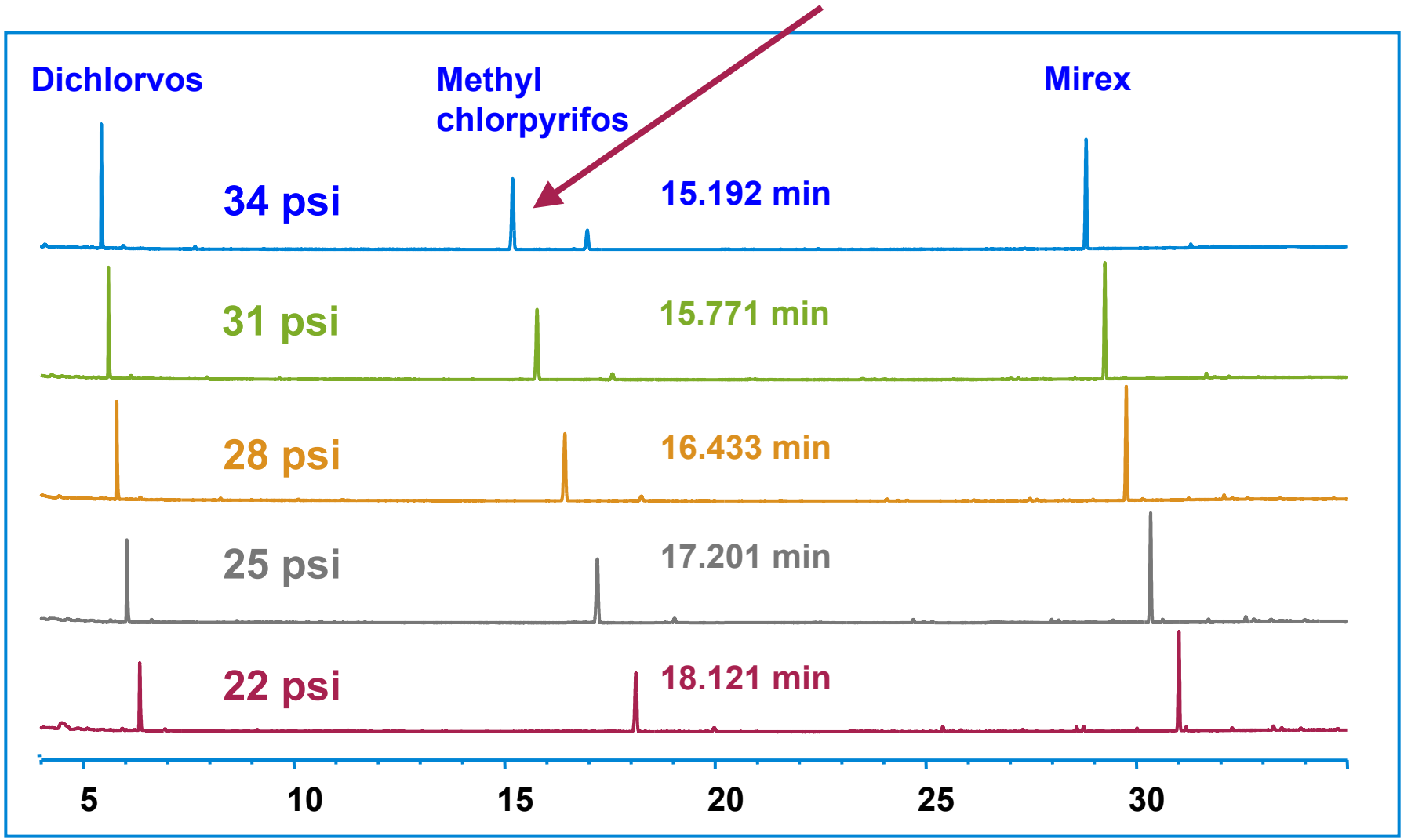
How is Retention Time Locking Done?

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

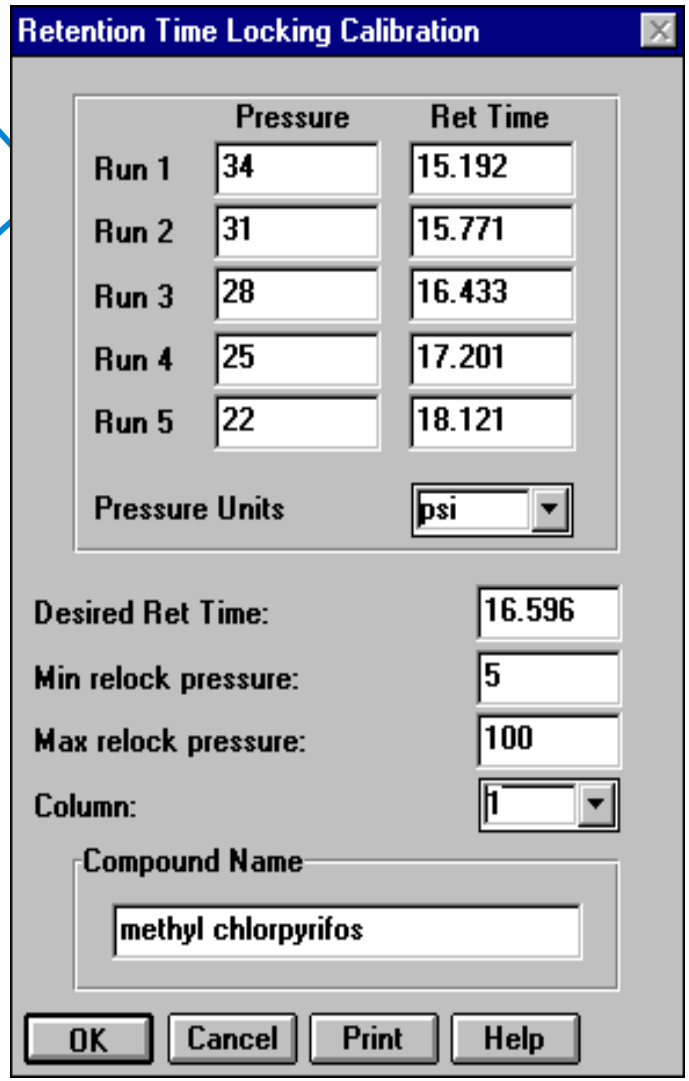
RTL Calibration Runs



RTL Calibration - Done Once for a Given Method

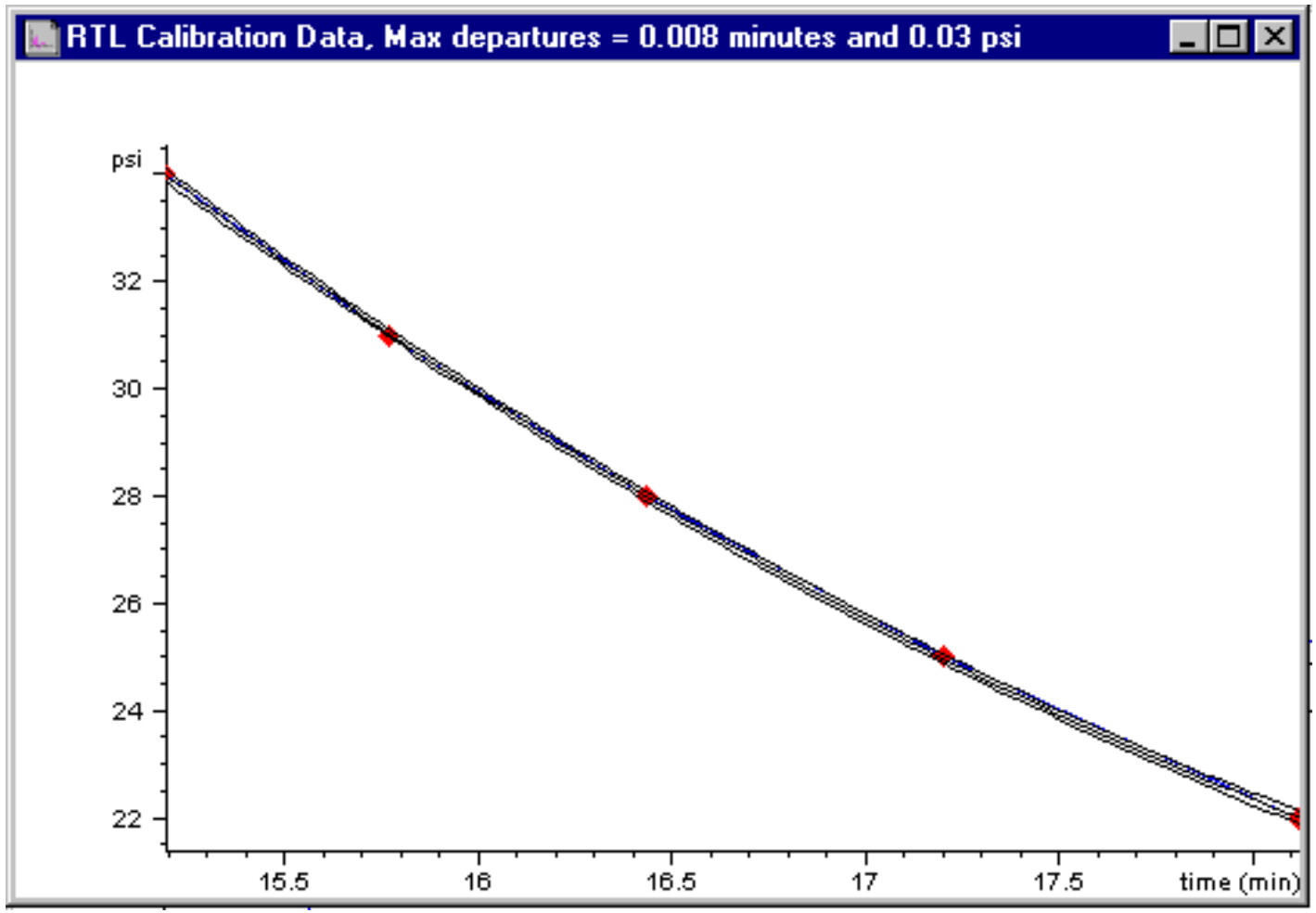
- Make 5 runs at 5 different inlet pressures:
  - target -20%
  - target -10%
  - target
  - target +10%
  - target +20%
- Determine RT of your target compound in
- Enter values into the RTL Software

This process is automated!



RTL Calibration Curve

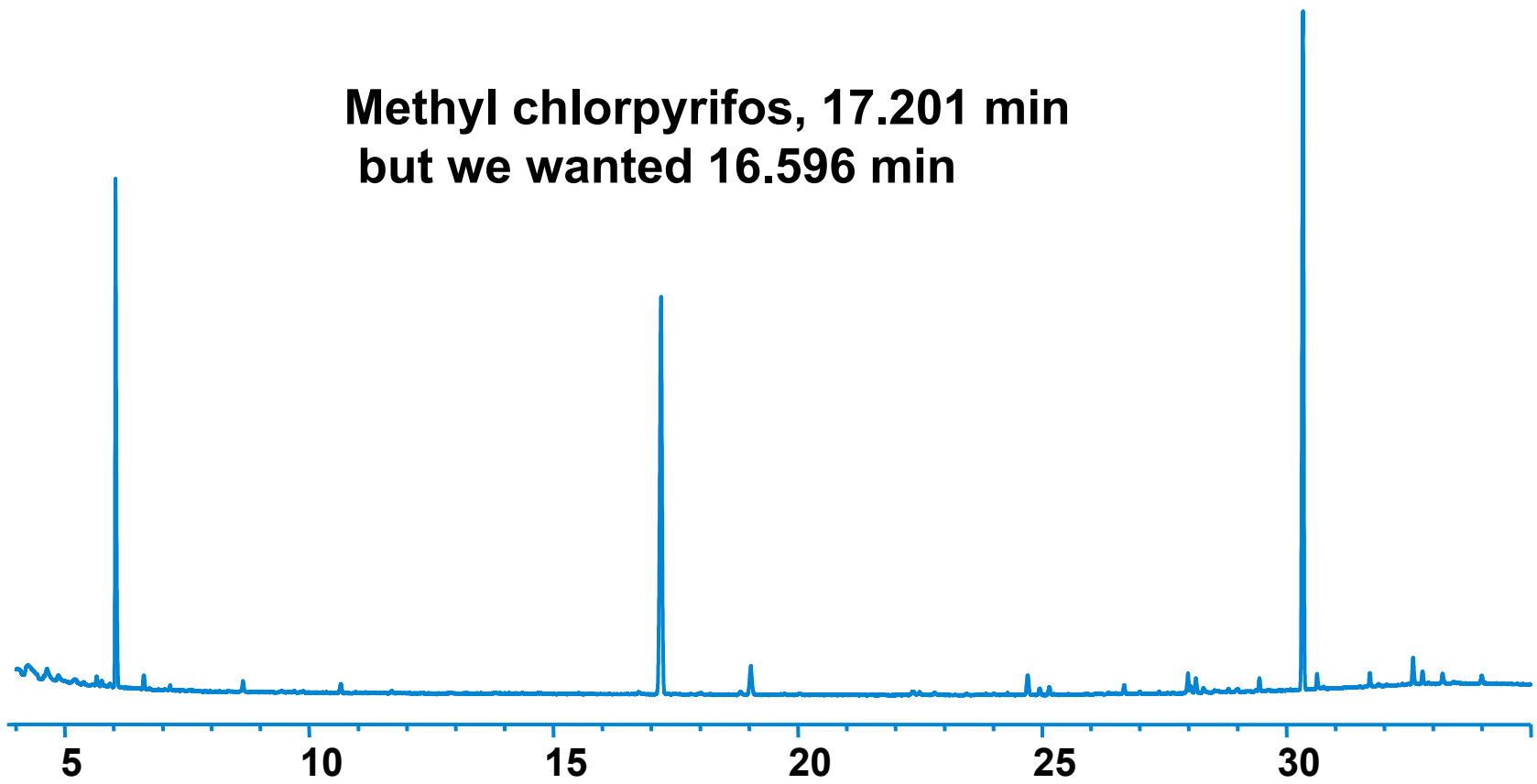
Column Head Pressure



Target Compound Retention Time

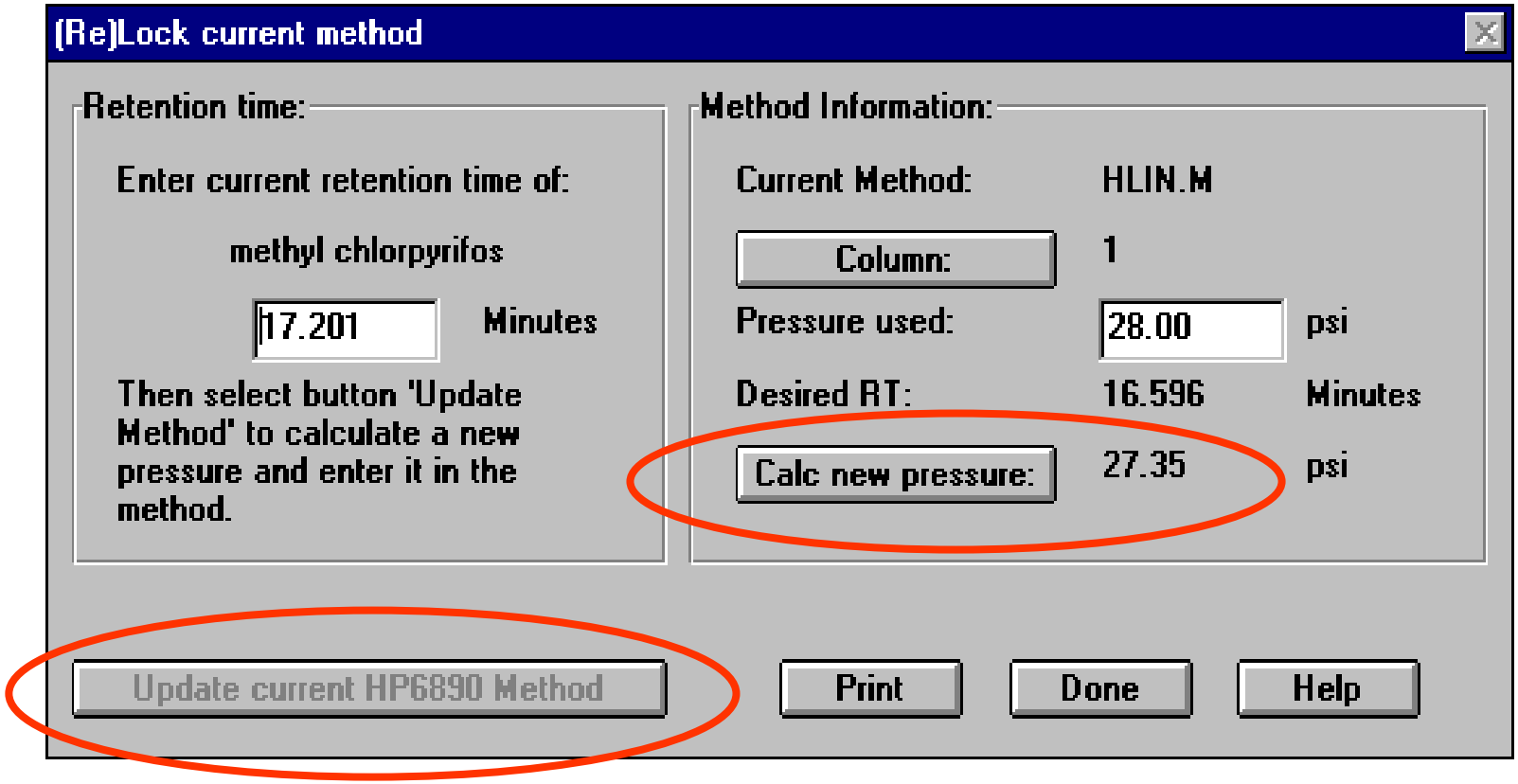
Locking A New System or Column

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



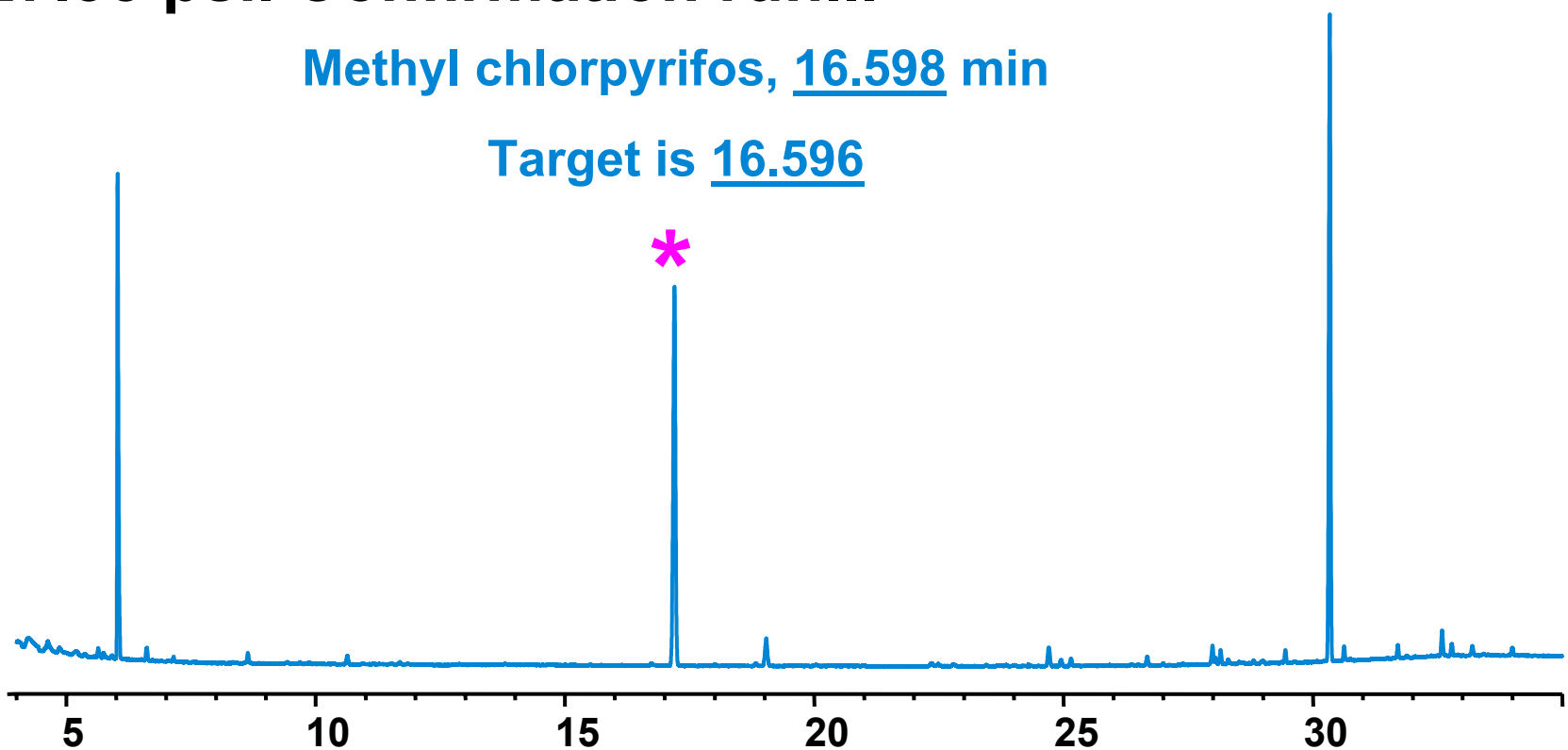
Locking a GC System

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



Confirming RT Lock

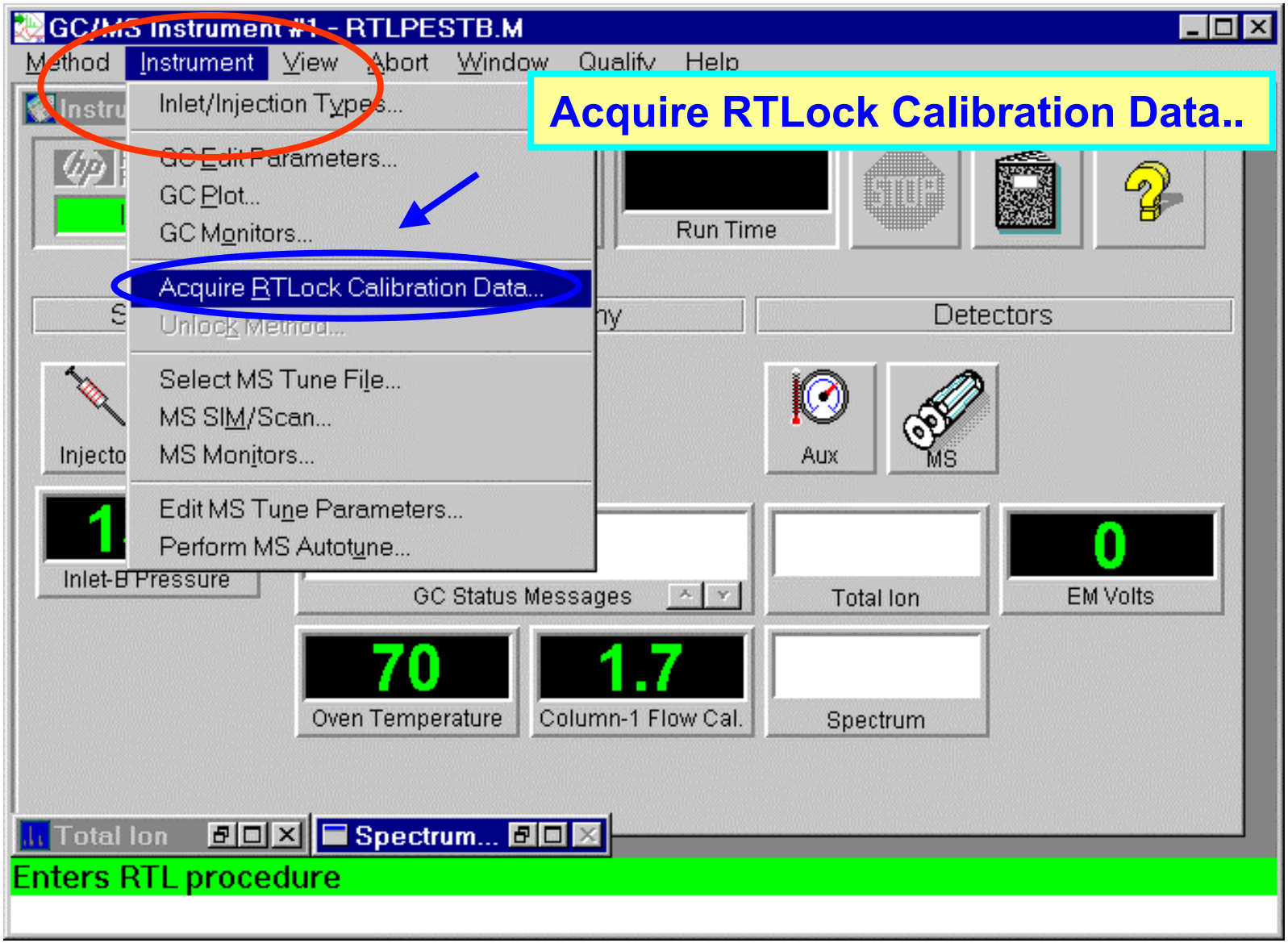
Set inlet pressure to that calculated by software, 27.35 psi. Confirmation run...



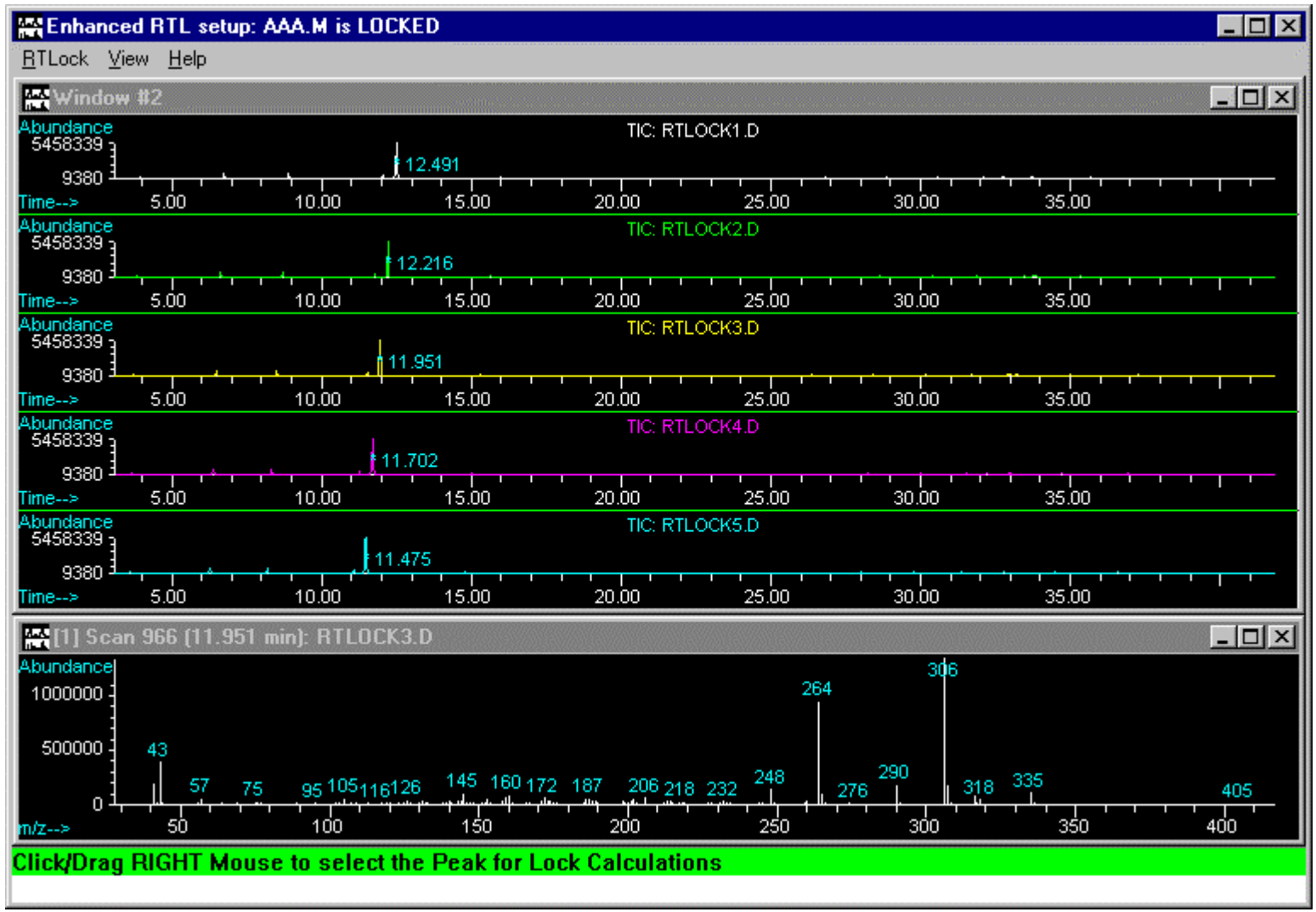
Outline

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

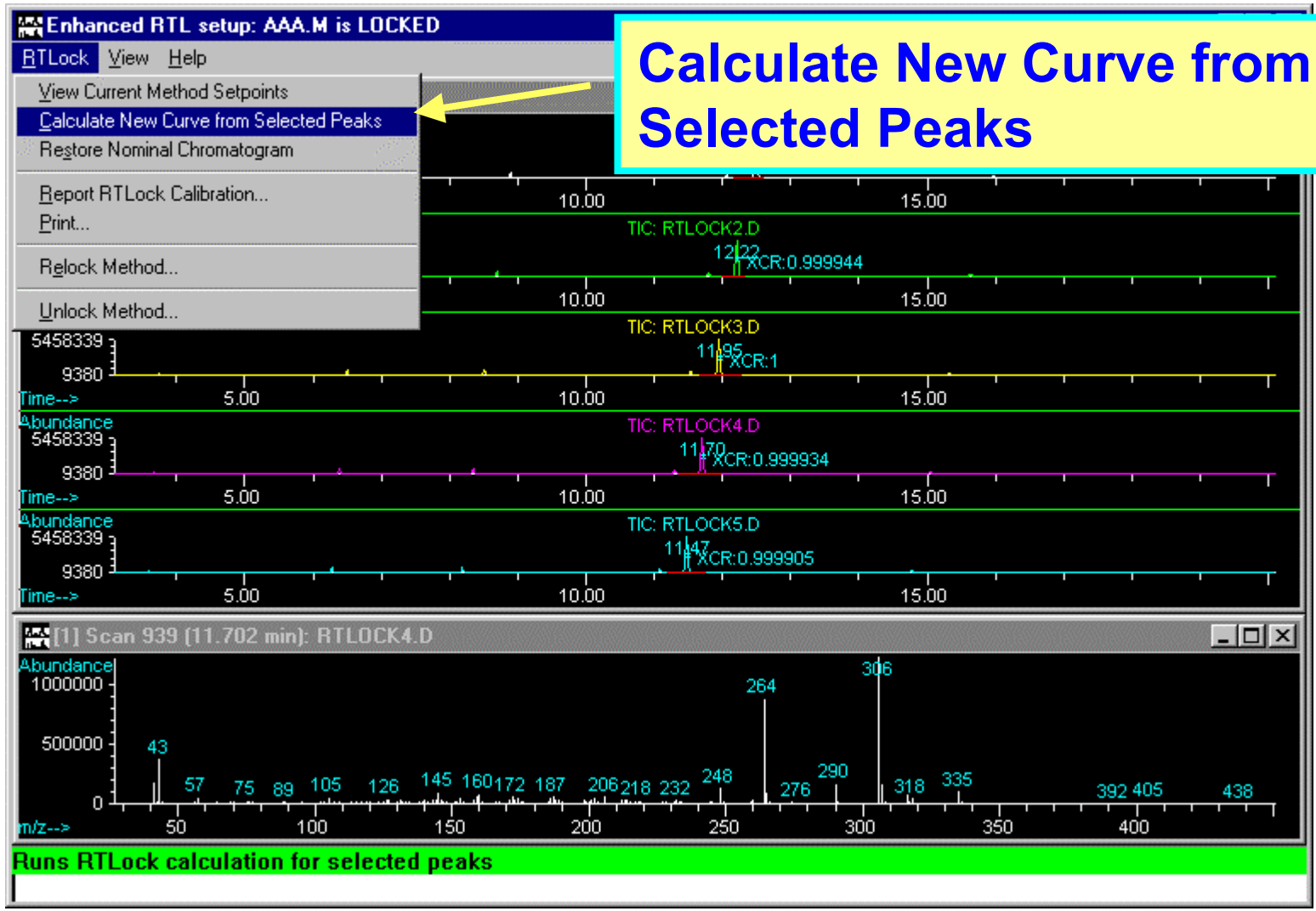
Acquiring RTLock Calibration Data..



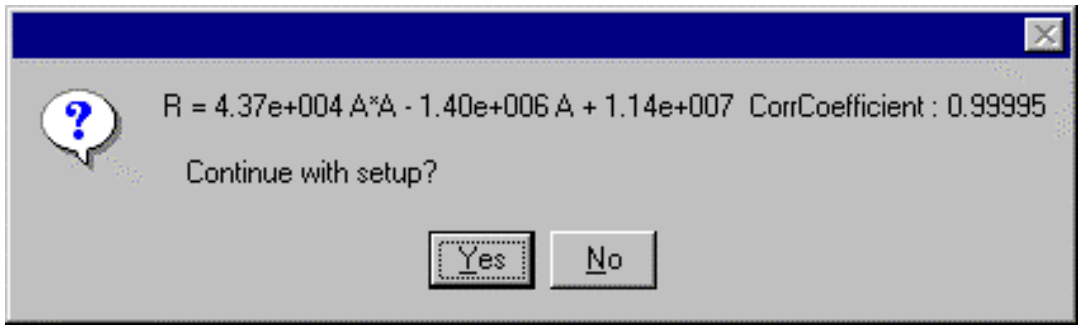
RT Lock Data Files Are Automatically Collected

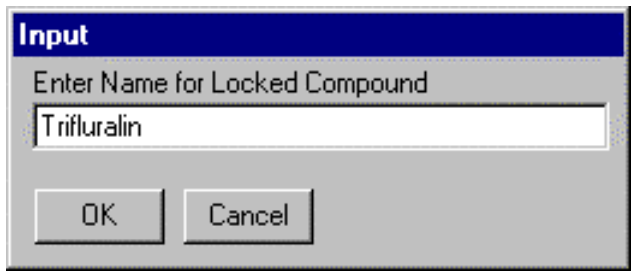


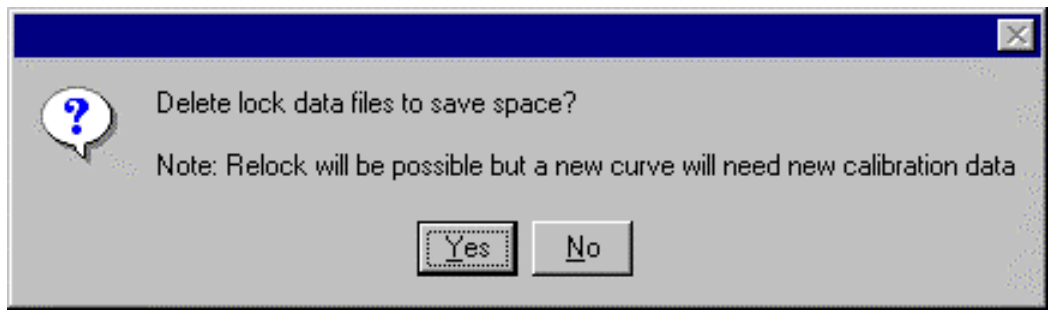
Generate RT vs Head Pressure Equation



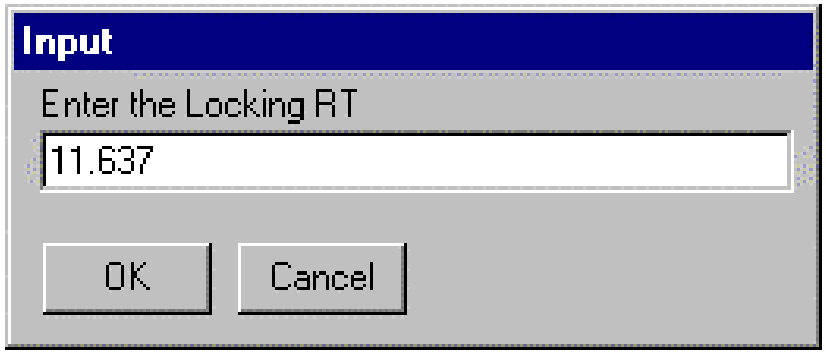
Working with the software

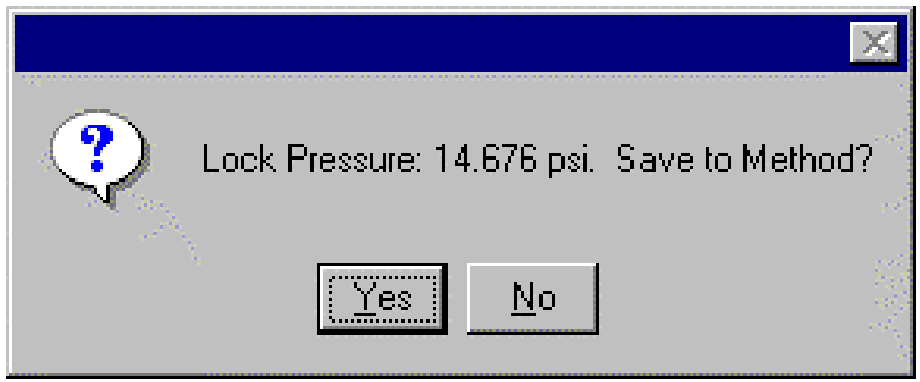






Working with the software





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

HP 5973 - AAA.M - EI: RETENTION TIME LOCKED to Trifluralin

Method Instrument View Abort Window Qualify Help

Instrument Control

HEWLETT PACKARD

Offline

1

Sample Name:

Data File:
evaldemo.d

Run Time

Sample

Chromatography

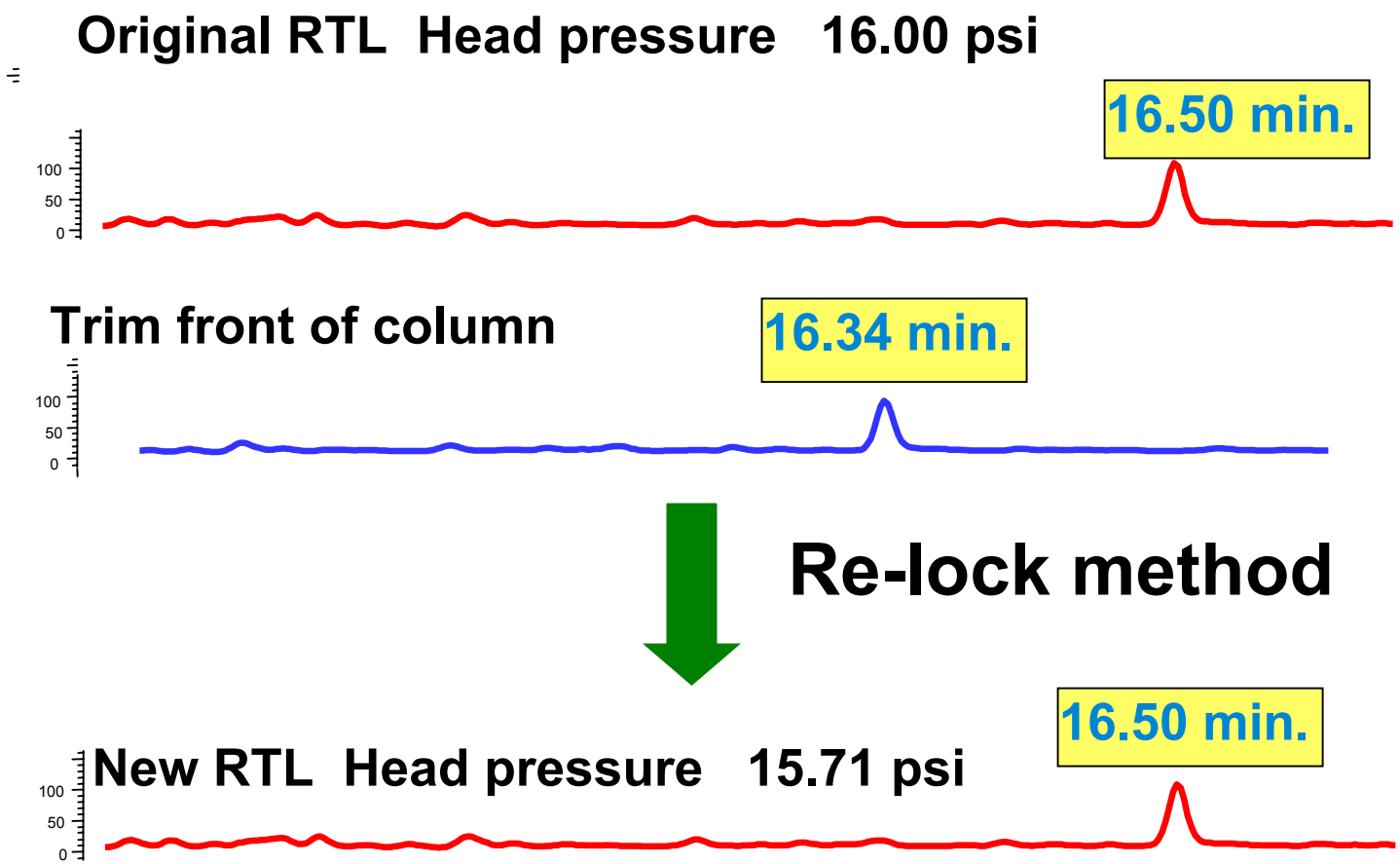
Injector

Inlets

Columns

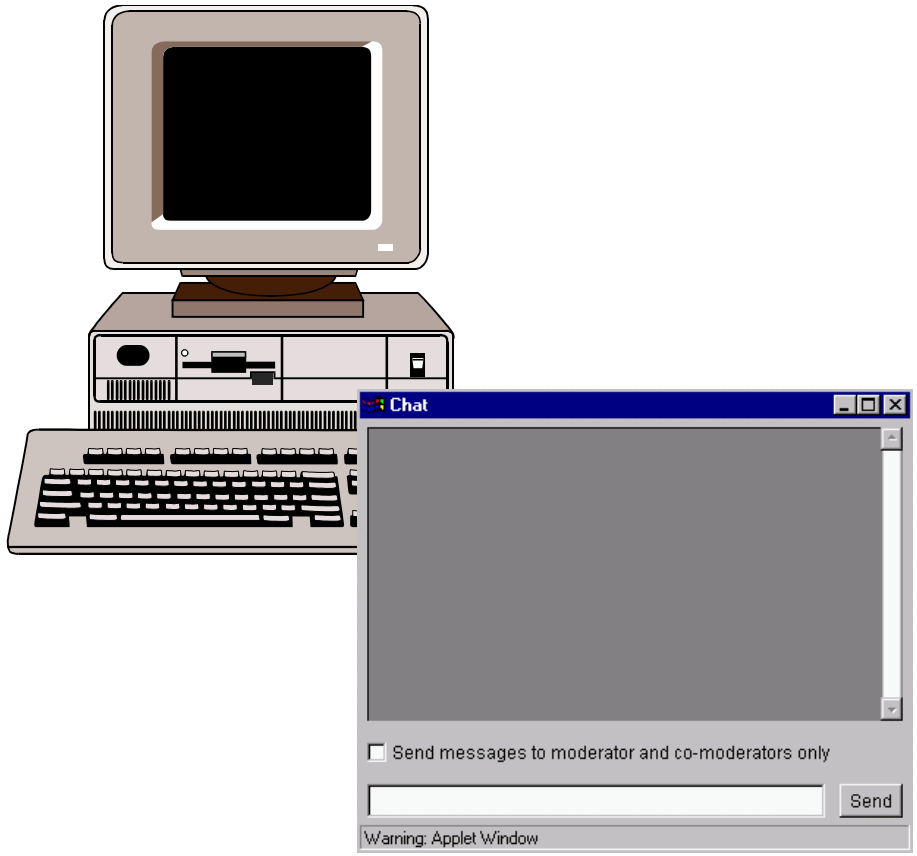
Oven

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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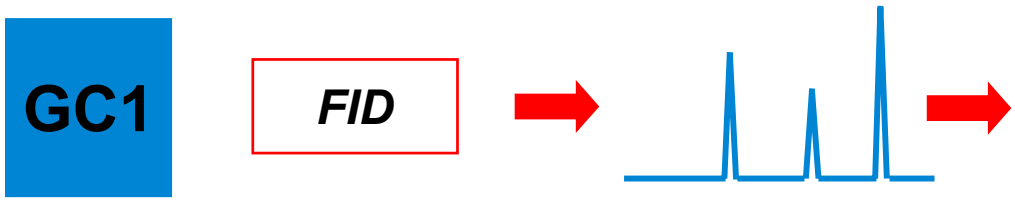
RTL Database Screening: Build a Retention Time Table

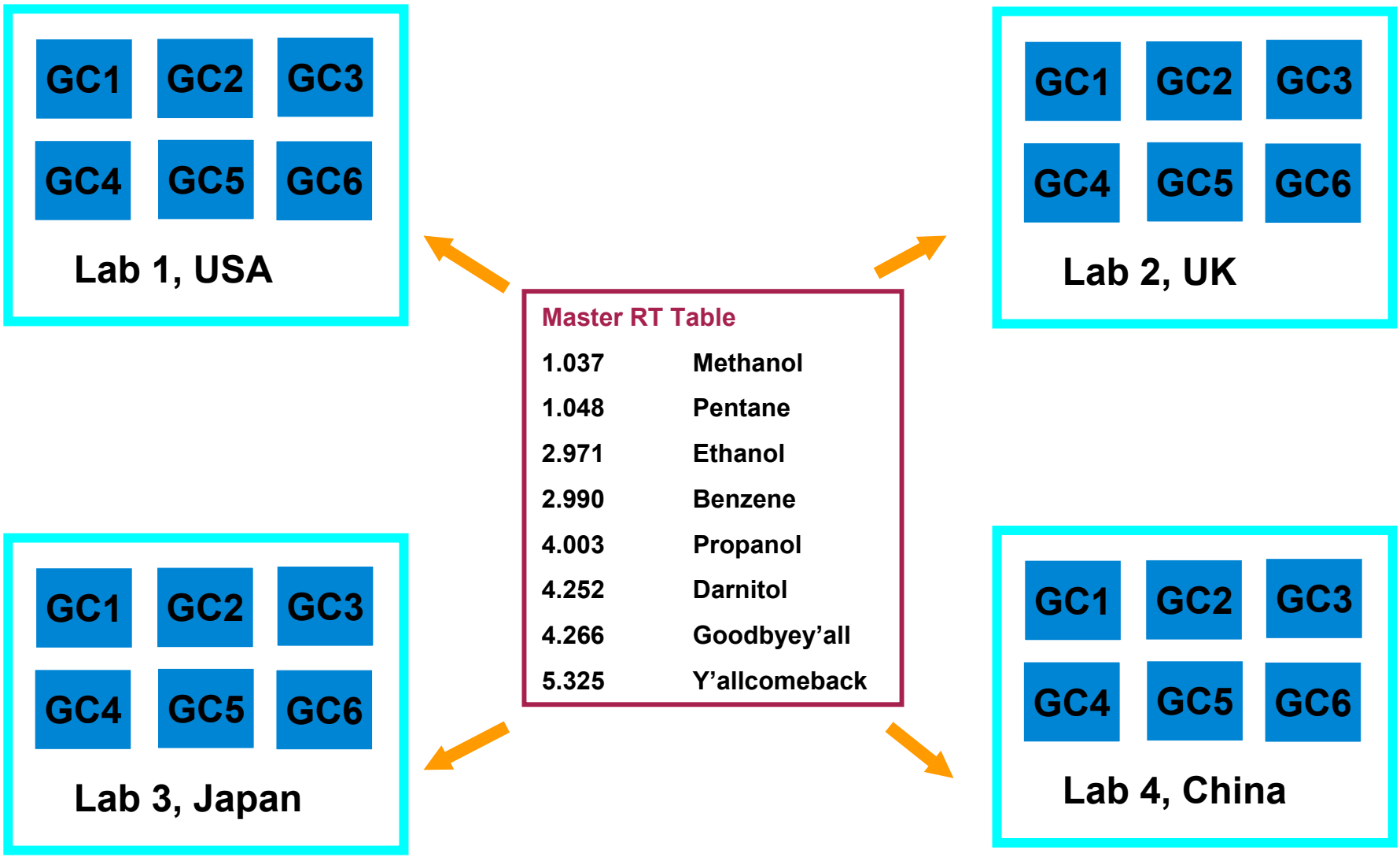
Record retention times under RT Locked conditions



Master RT Table

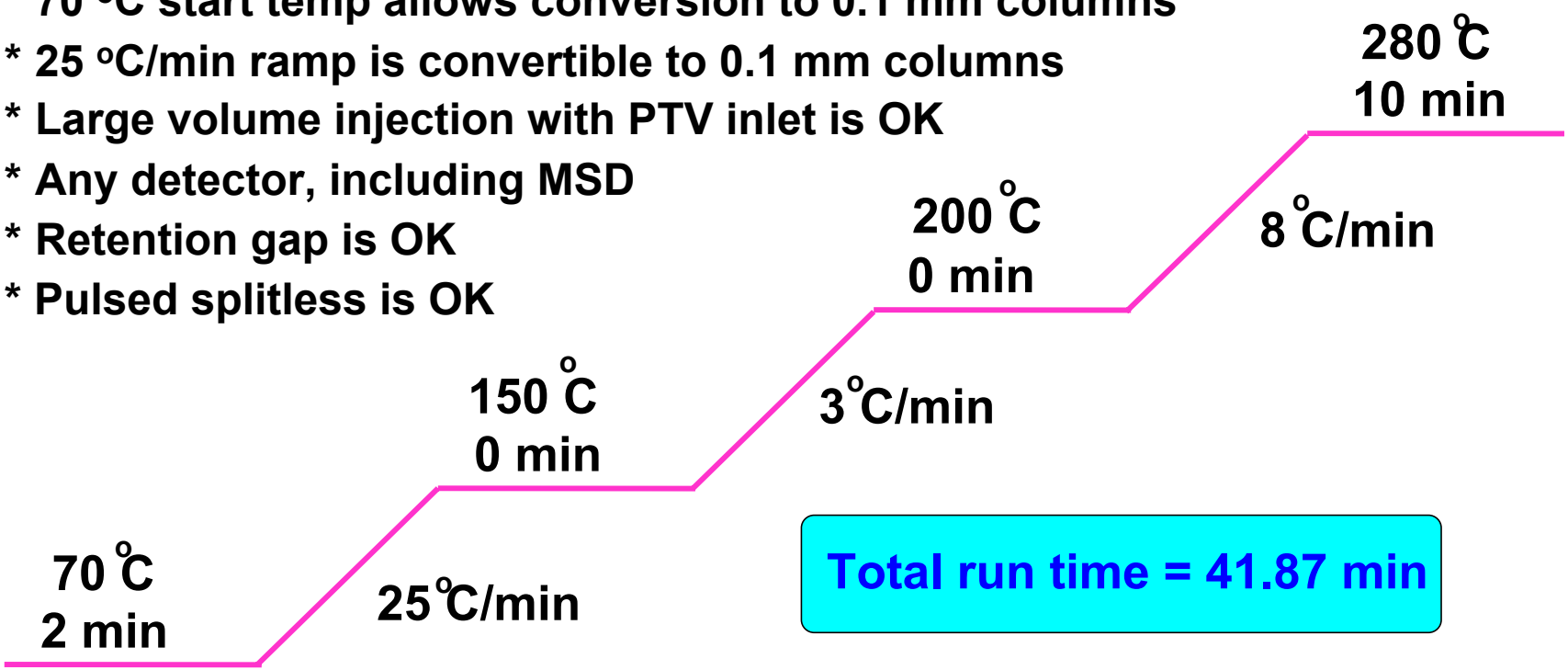
1.037	Methanol
1.048	Pentane
2.971	Ethanol
2.990	Benzene
4.003	Propanol
4.252	Darnitol
4.266	Goodbye y'all
5.325	Y'all comeback

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



Total run time = 41.87 min

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

Search Retention Time Table

Load Table... | HPPSTRC4.RTT : HP Pesticide RT Table Release Candidate 4

16.757 Search RT, minutes

0.2 Search Window, minutes

Compound contains these elements:

☐ Br ☐ Cl ☐ F ☐ N ☐ O ☐ P ☐ S

Does not contain these elements:

☐ Br ☐ Cl ☐ F ☐ N ☐ O ☐ P ☐ S

Compound detected with:

NPD ☐

FPD(S) ☐

FPD(P) ☐

Not detected with:

NPD ☐

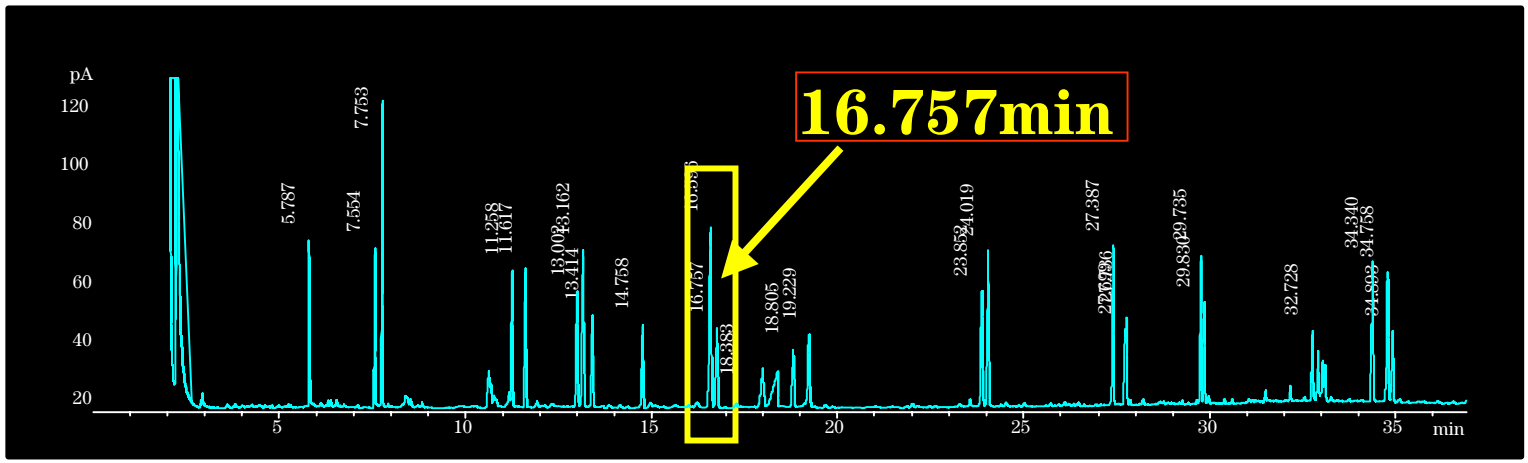
FPD(S) ☐

FPD(P) ☐

Search | Cancel | Help

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

Search Retention Time Table

Load Table... | HPPSTRC4.RTT : HP Pesticide RT Table Release Candidate 4

16.757 Search RT, minutes

0.2 Search Window, minutes

Compound contains these elements: ☐ Br ☒ Cl ☐ F ☐ N ☐ O ☐ P ☐ S

Does not contain these elements: ☒ Br ☐ Cl ☐ F ☒ N ☐ O ☒ P ☒ S

Compound detected with: NPD ☐ FPD(S) ☐ FPD(P) ☐

Not detected with: NPD ☐ FPD(S) ☐ FPD(P) ☐

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

Search | Cancel | Help

Pesticide Database Search

Using unknown's element information yields just 1 possibility

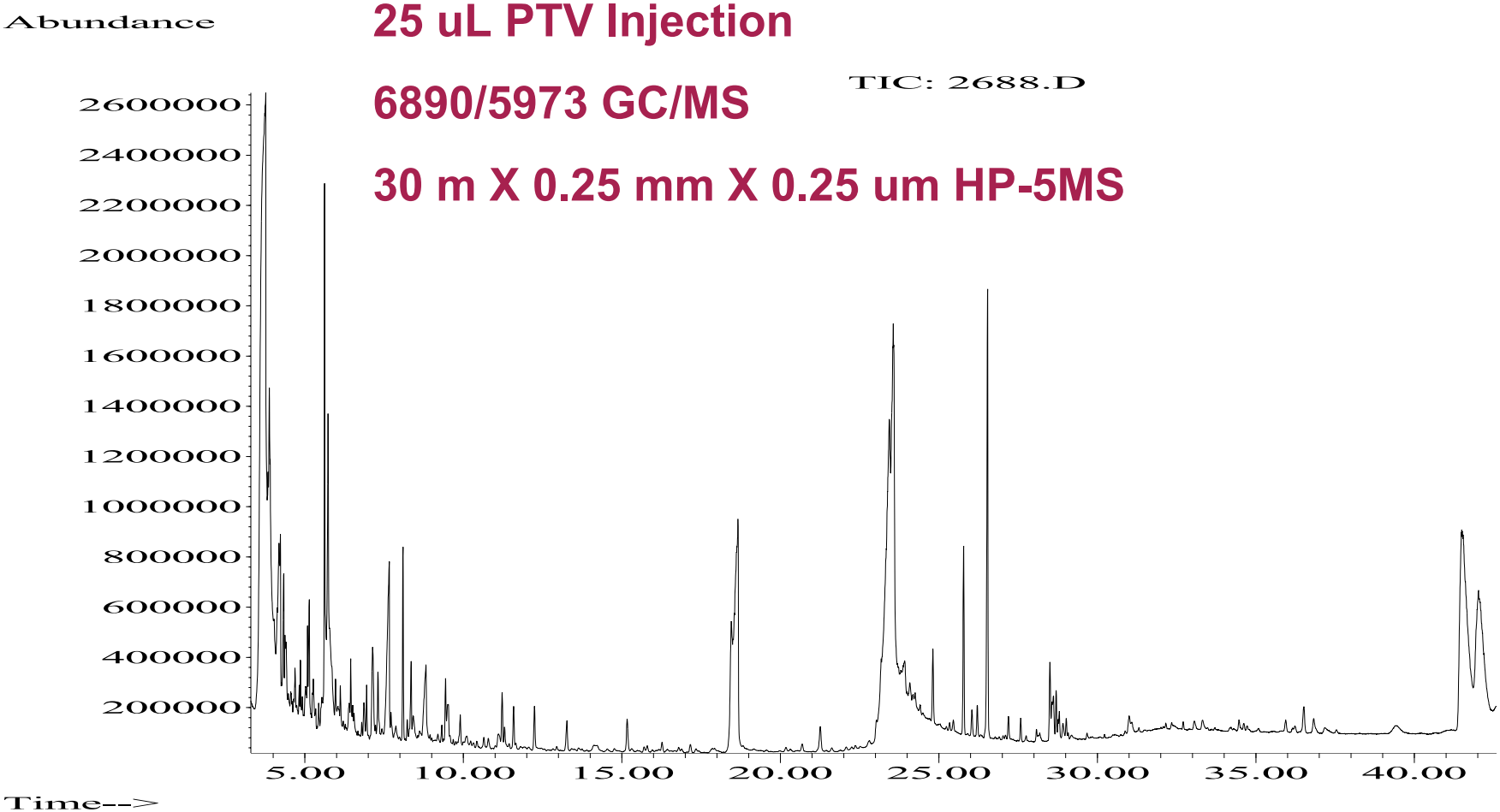
HPPSTRC4.RTT Search Results, 16.757+/-0.05 minutes, with element restrictions

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

View Current Report | Close Window

FID_RT	Compound Name	CAS #	Formula	MW
16.773	Heptachlor	76-44-8	C:10,H:5,Cl:7,	373.32

Strawberry Extract

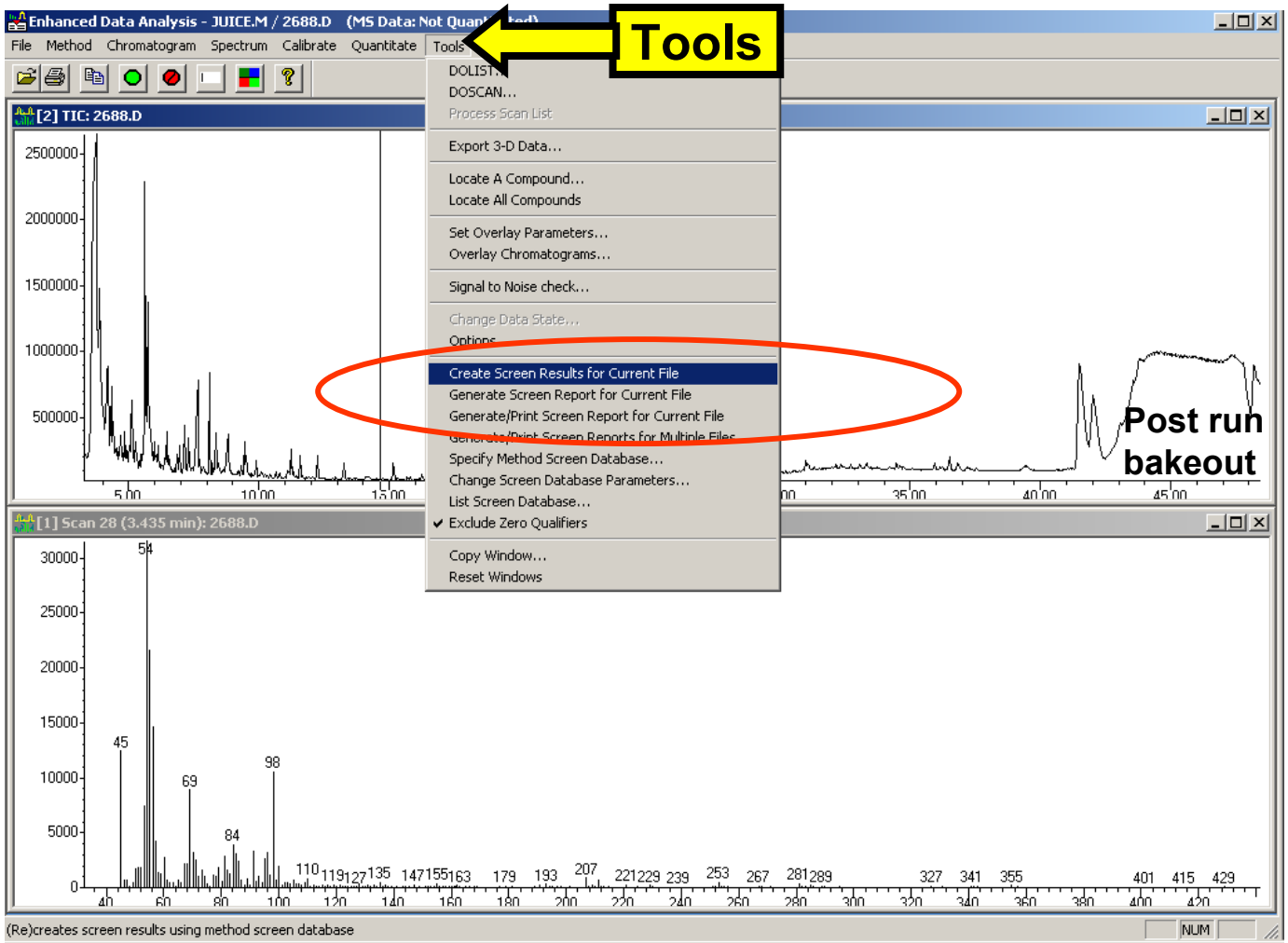


25 uL PTV Injection

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*



txt - Notepad

File Search Help

		Target	RT	Qualifier Ions		
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			
37	3,4-Dichloroaniline	161	7.66			
38	Acephate	136	7.69			
39	Chlormefos	121	7.73			
40	Vernolate	128	7.81			
41	Dimethylphthalate	163	7.91			

1

Enhanced Data Analysis - RTLPESTB.M / RTLDEMO.D (MS Data: Not Quant...

File Method Chromatogram Spectrum Calibrate Quantitate Tools View Help

2

Abundance 3000000 2000000 1000000 0

Time--> 7.20 7.40 7.60 7.80 8.00 8.20

7.32 ± 0.1 | 7.59 ± 0.1 | 7.81 ± 0.1

Enhanced Data Analysis Screener: RTLDE

Screen Spectrum ChromEval Display View

3

Abundance Ion 127.00 (126.70 to 127.70) RT 7.58 500000 0 127

Time--> 7.20 7.40 7.60

Abundance Ion 192.00 (191.70 to 192.70) RT 500000 0 192

Time--> 7.20 7.40 7.60

Abundance Ion 109.00 (108.70 to 109.70) RT 500000 0 109

Time--> 7.20 7.40 7.60

Abundance Ion 67.00 (66.70 to 67.70): RTL 500000 0 67

Time--> 7.20 7.40 7.60

Exp	Act
100	100
31.6	
35.92	
21.7	
21.93	
10.2	8.88

4

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              Extraction Window: +/- 0.100 min
Screen Database: rtlpest2.SCD          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

14 possible 'hits'

Screen Report Wed Apr 16 15:04:43 2003

Results Screener Software for Verification

Enhanced Data Analysis Screener: 2688.D

Screener Spectrum ChromEval Display View Help

Window #6

Ion 97.00 (96.70 to 97.70): 2688.D
Ion 181.00 (180.70 to 181.70): 2688.D
Ion 125.00 (124.70 to 125.70): 2688.D
Ion 265.00 (264.70 to 265.70): 2688.D

29.02

QuickScreener

Start Exit QDel Configure

501		Piperophos
502		Methoxychlor
503		Phenkapton
504		Dinocap IV
505	x	29.016 Fenpropathrin
506		Bifenox
507		Dicyclohexyl phthalate
508		Chlorthiophos sulfone
509		Tetradifon
510		d-(cis-trans)-Phenothrin-I
511		d-(cis-trans)-Phenothrin-II
512		Furathiocarb
513	x	29.665 Bis(2-ethylhexyl)phthalate
514		Azinphos-methyl
515		Phosalone
516		Leptophos
517		Mirex
518		Mefenacet
519		Amitraz
520		Cyhalothrin I (lambda)
521		Fenarimol

Window #1

Scan 4915 (29.016 min): 2688.D (-4907) (-)

#505: Fenpropathrin

[7] TIC

(505) Fenpropathrin

29.016min (+0.026)

response 26411

Ion	Exp%	Act%
97.00	100	100
181.00	80.10	64.50
125.00	43.00	34.38
265.00	33.20	21.22

Ready NUM

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

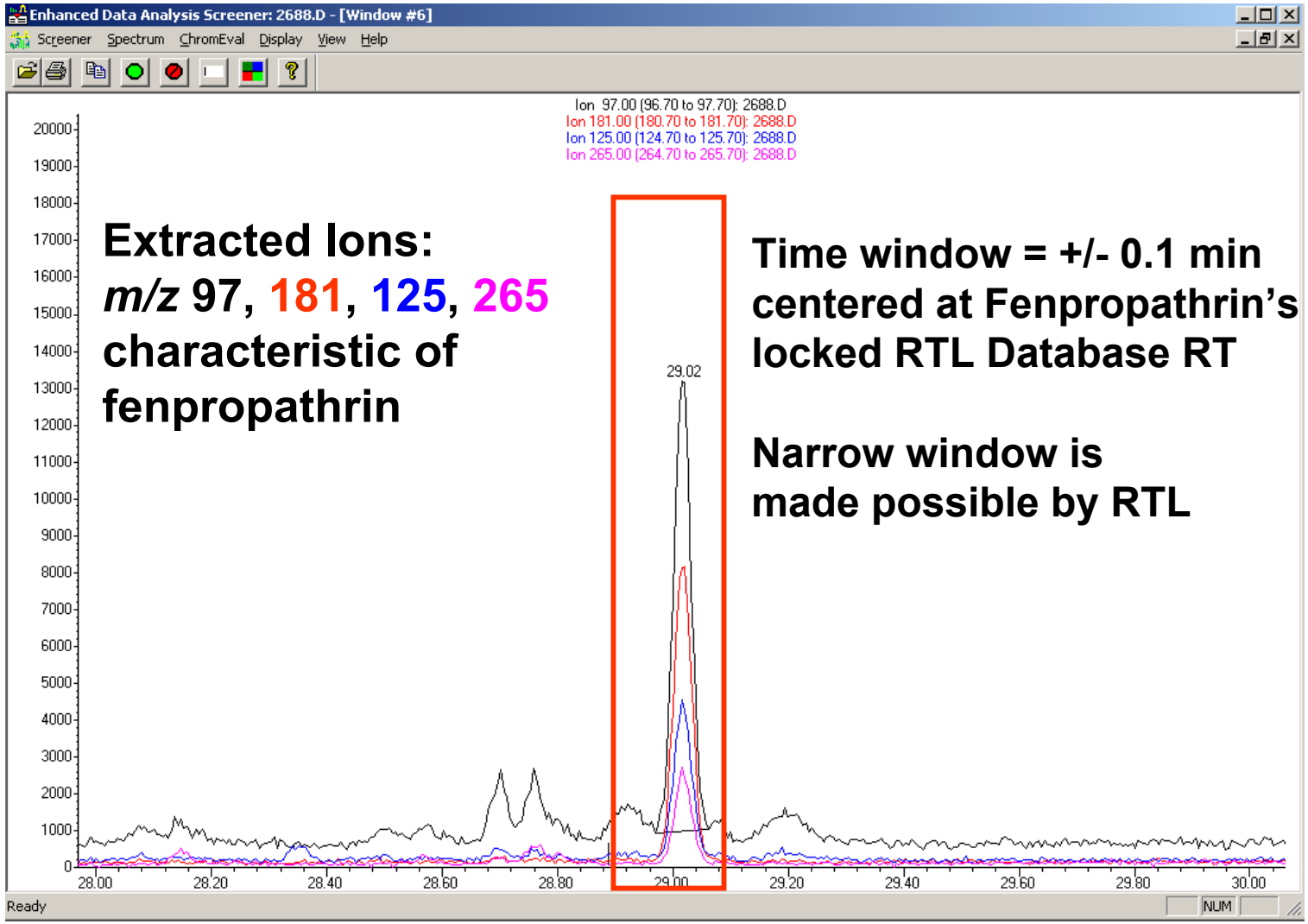
QuickScreener

Start | Exit | QDel | Configure

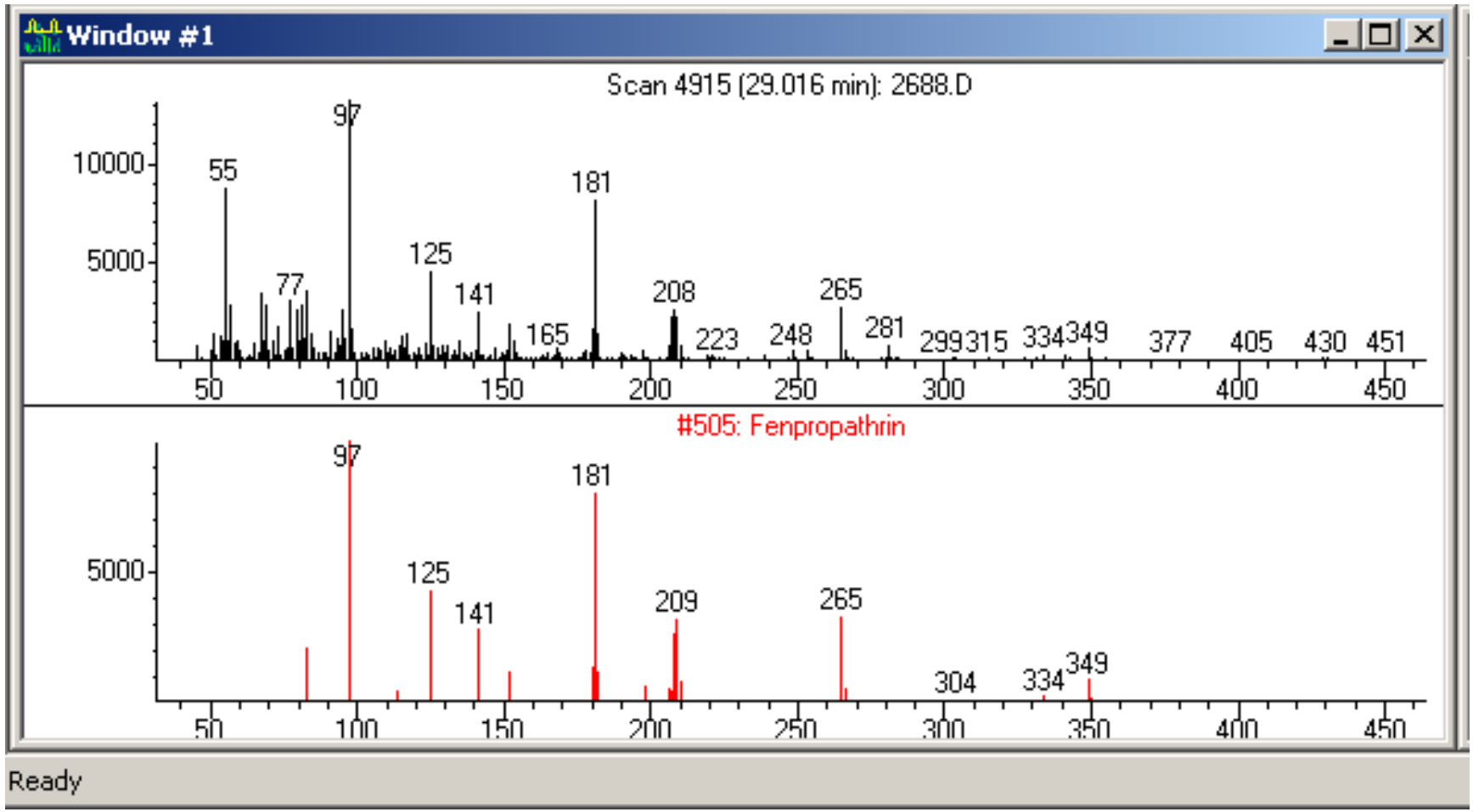
501			Piperophos
502			Methoxychlor
503			Phenkapton
504			Dinocap IV
505	x	29.016	Fenpropathrin
506			Bifenox
507			Dicyclohexyl phthalate
508			Chlorthiophos sulfone
509			Tetradifon
510			d-(cis-trans)-Phenothrin-I
511			d-(cis-trans)-Phenothrin-II
512			Furathiocarb
513	x	29.665	Bis(2-ethylhexyl)phthalate
514			Azinphos-methyl
515			Phosalone
516			Leptophos
517			Mirex
518			Mefenacet
519			Amitraz
520			Cyhalothrin I (lambda)
521			Fenarimol

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

[7] TIC: 2688.D

(505) Fenpropathrin

29.016min (+0.026)

response 26411

Ion	Exp%	Act%
97.00	100	100
181.00	80.10	64.50
125.00	43.00	34.38
265.00	33.20	21.22

Database RT

(Deviation from database RT)

NUM

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

Enhanced Data Analysis Screener: 2688.D

Screener Spectrum ChromEval Display View Help

Window #6

Ion 109.00 (108.70 to 109.70): 2688.D
Ion 185.00 (184.70 to 185.70): 2688.D
Ion 145.00 (144.70 to 145.70): 2688.D
Ion 79.00 (78.70 to 79.70): 2688.D

QuickScreener

Start Exit QDel Configure

81		Metasystox thiol
82		Diphenylamine
83		Azobenzene
84	? 10.648	Benzophenone
85		Cycloate
86		Ethoprophos
87		2,4-D methyl ester
88		Fenuron
89		Fluometuron
90		Tributyl phosphate
91		Dicamba
92		Chlorpropham
93		2-[3-Chlorophenoxy]propionamide
94		Chlordimeform
95	? 11.224	Dibrom (naled)
96		2,3,5-Trimethacarb
97		2,3,5-Trimethylphenyl methyl carbam
98		Ethalfluralin
99		Dicrotophos
100		Thiofanox
101		Bromoxynil

Window #1

Scan 1516 (11.224 min): 2688.D

#95: Dibrom (naled)

[7] TIC:

(95) Dibrom (naled)

11.224min (+0.003)

response 3041

Ion	Exp%	Act%
109.00	100	100
185.00	27.70	3.26#
145.00	20.80	68.02#
79.00	15.10	276.37#

Ready NUM

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                Extraction Window: +/- 0.100 min
Screen Database: rtlpest2.SCD            Qualifier Mode   : Absolute
                                         Qualifier %      : 20
                                         Zero qualifiers  : Excluded
                                         Subtraction Mode : Sub Average Start/Stop


                                                Target      Qualifiers
   Compound                 Status ExpRT  Delta m/z   Resp. 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

Screen Report Wed Apr 16 16:25:14 2003
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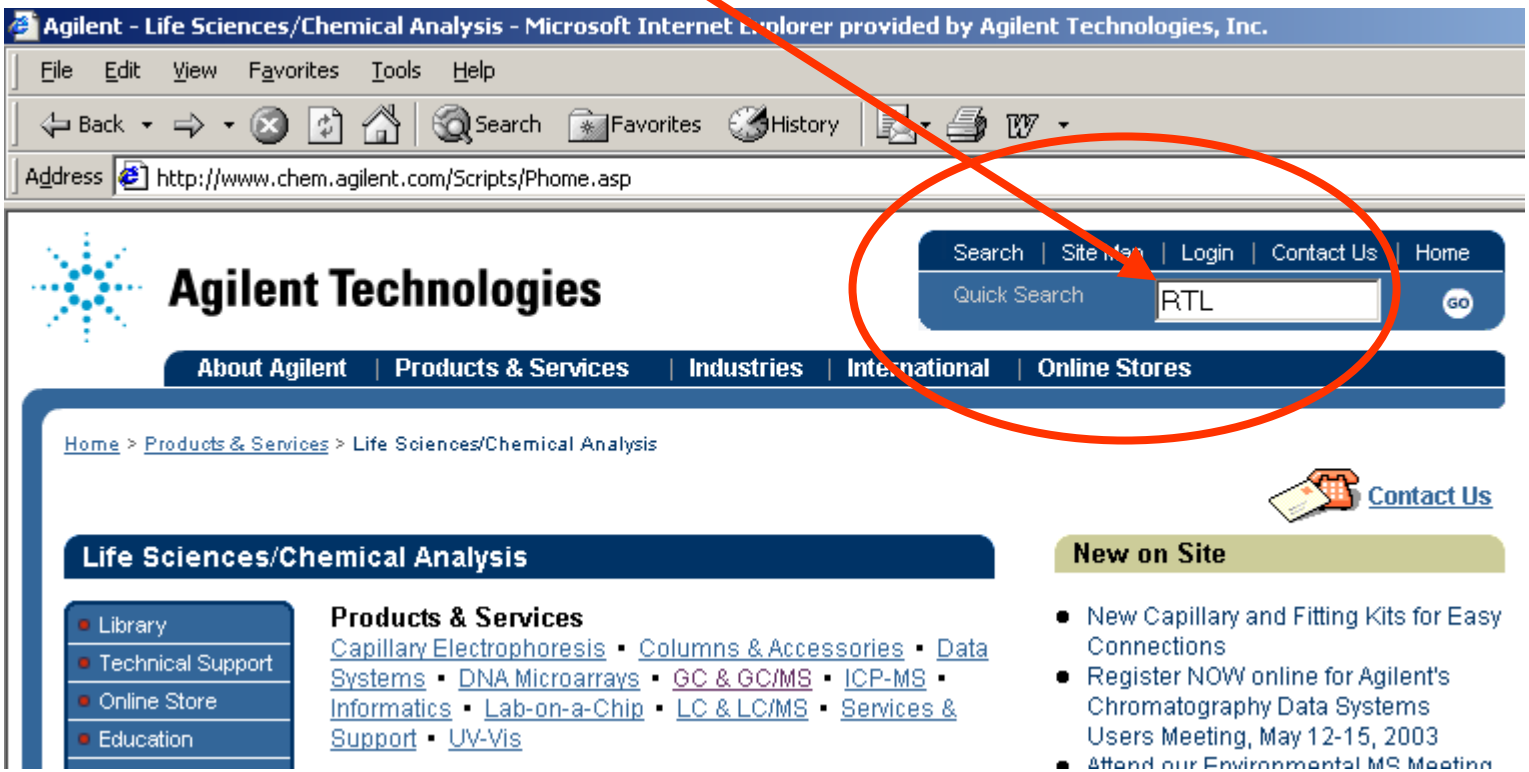
Where to get More RTL & Pesticide Analysis Information

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