

**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**

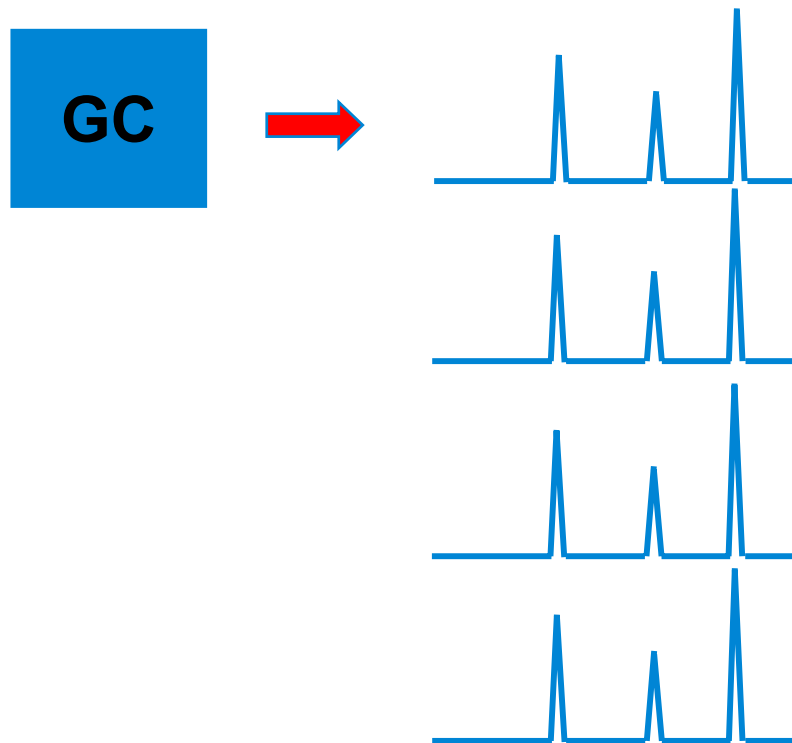
# Outline

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



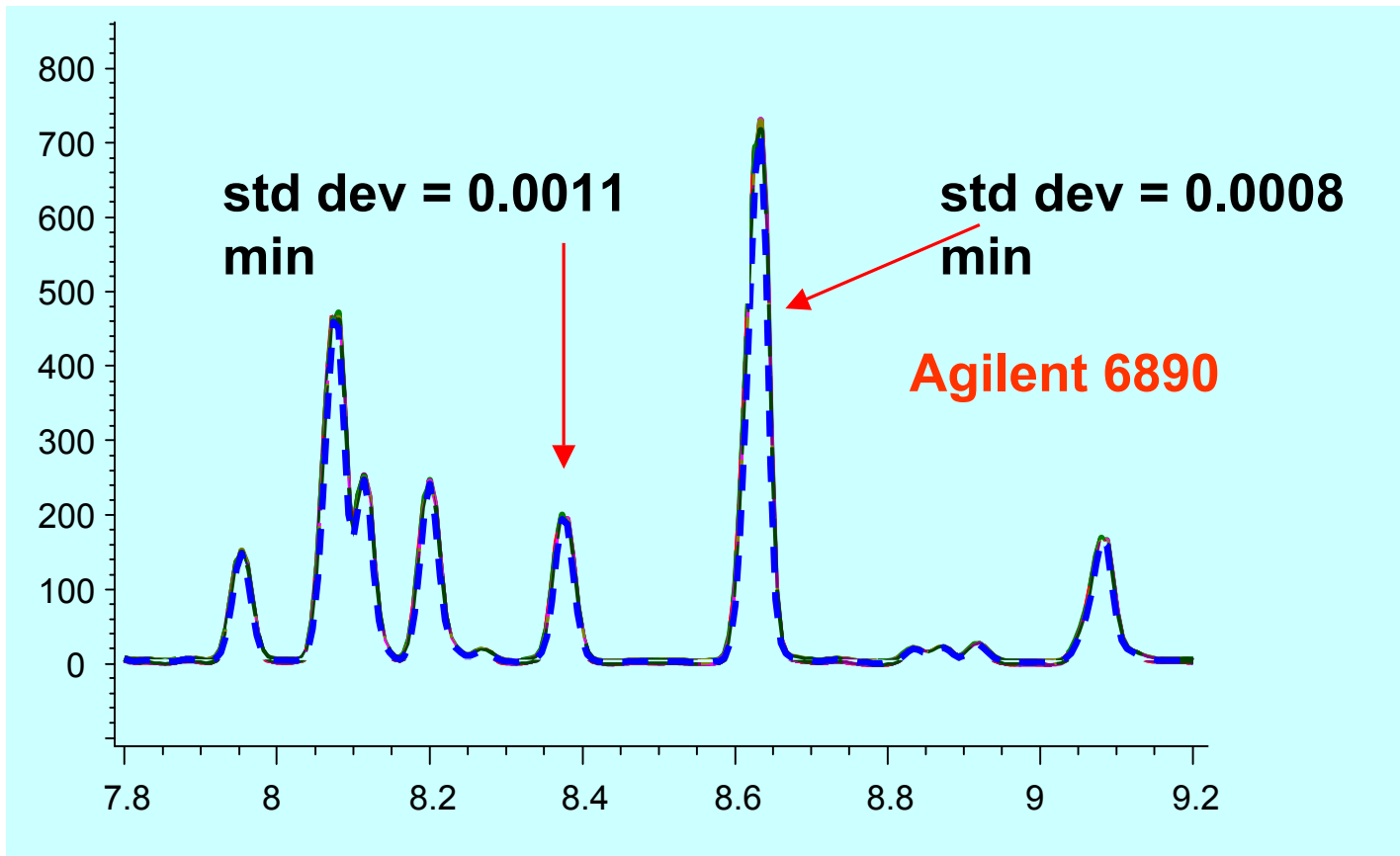
# What's the Problem?

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



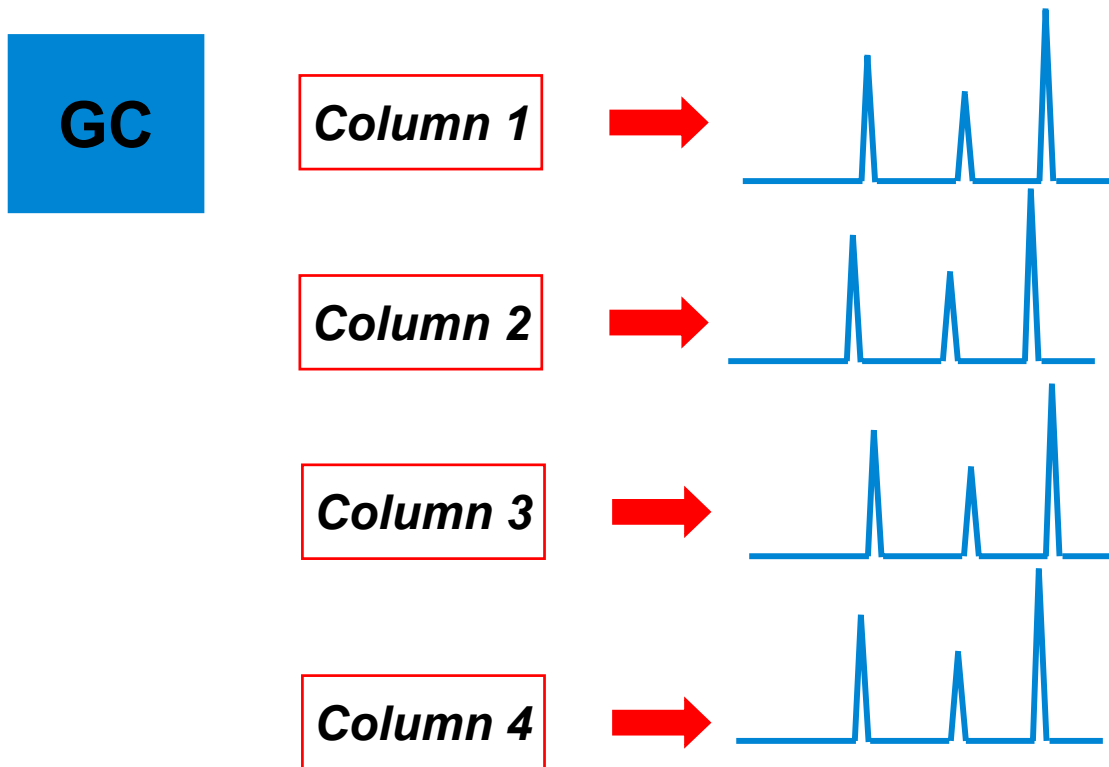
# Retention Time Precision, 6890

## Gasoline, 15 Runs Over 5 Days



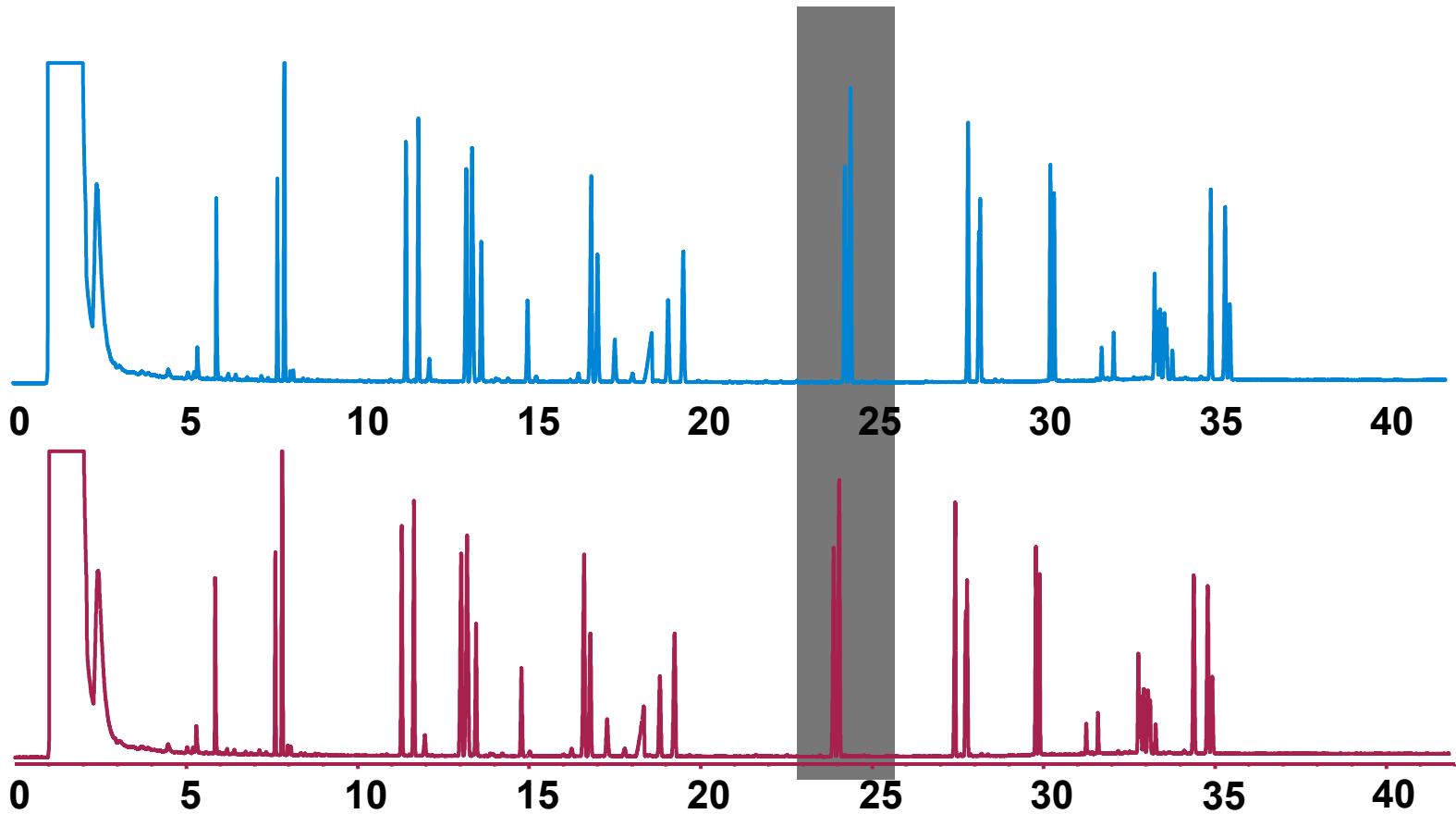
# Different Columns, Same GC

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

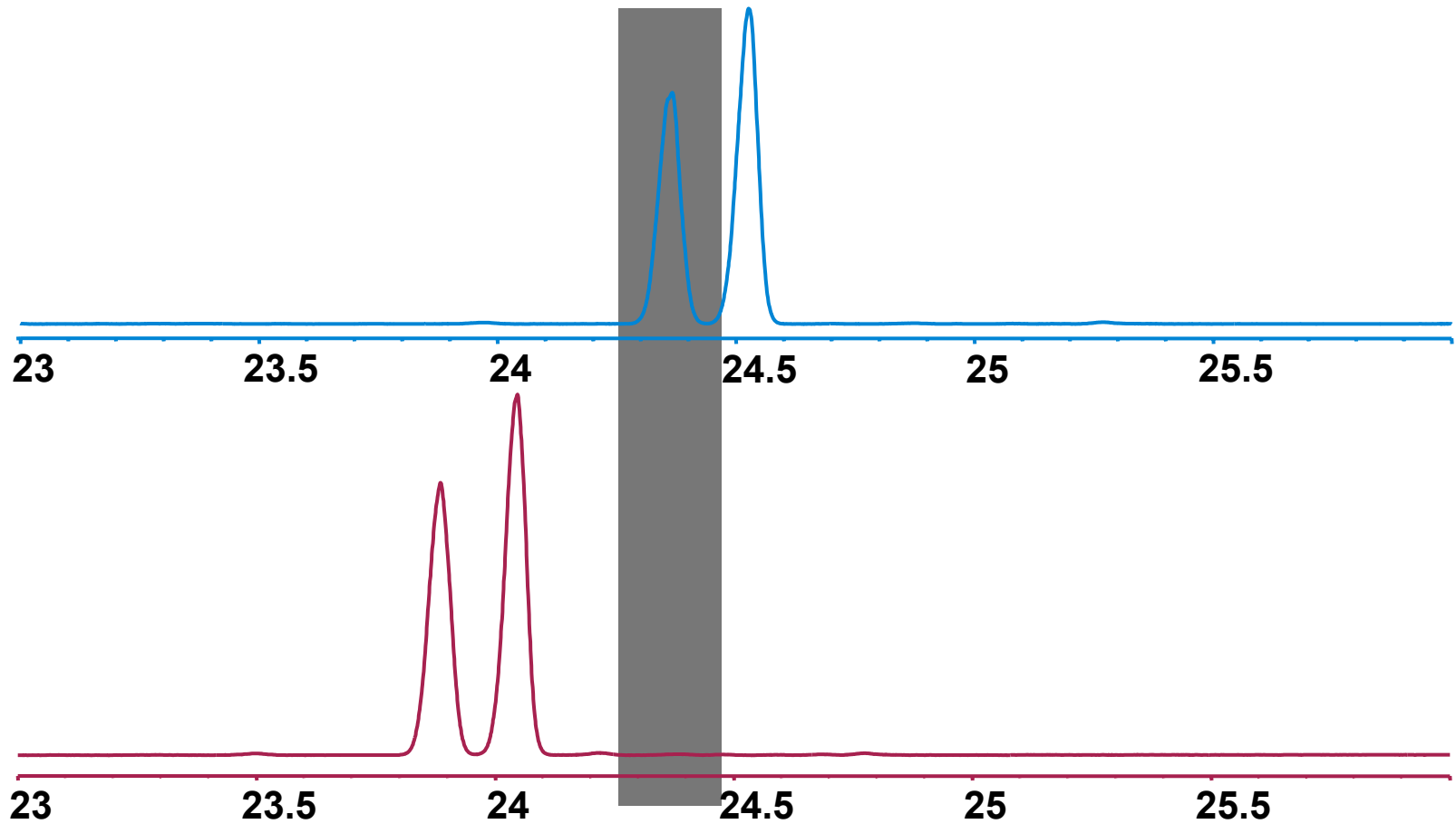


# Pesticides on Two Different GCs

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

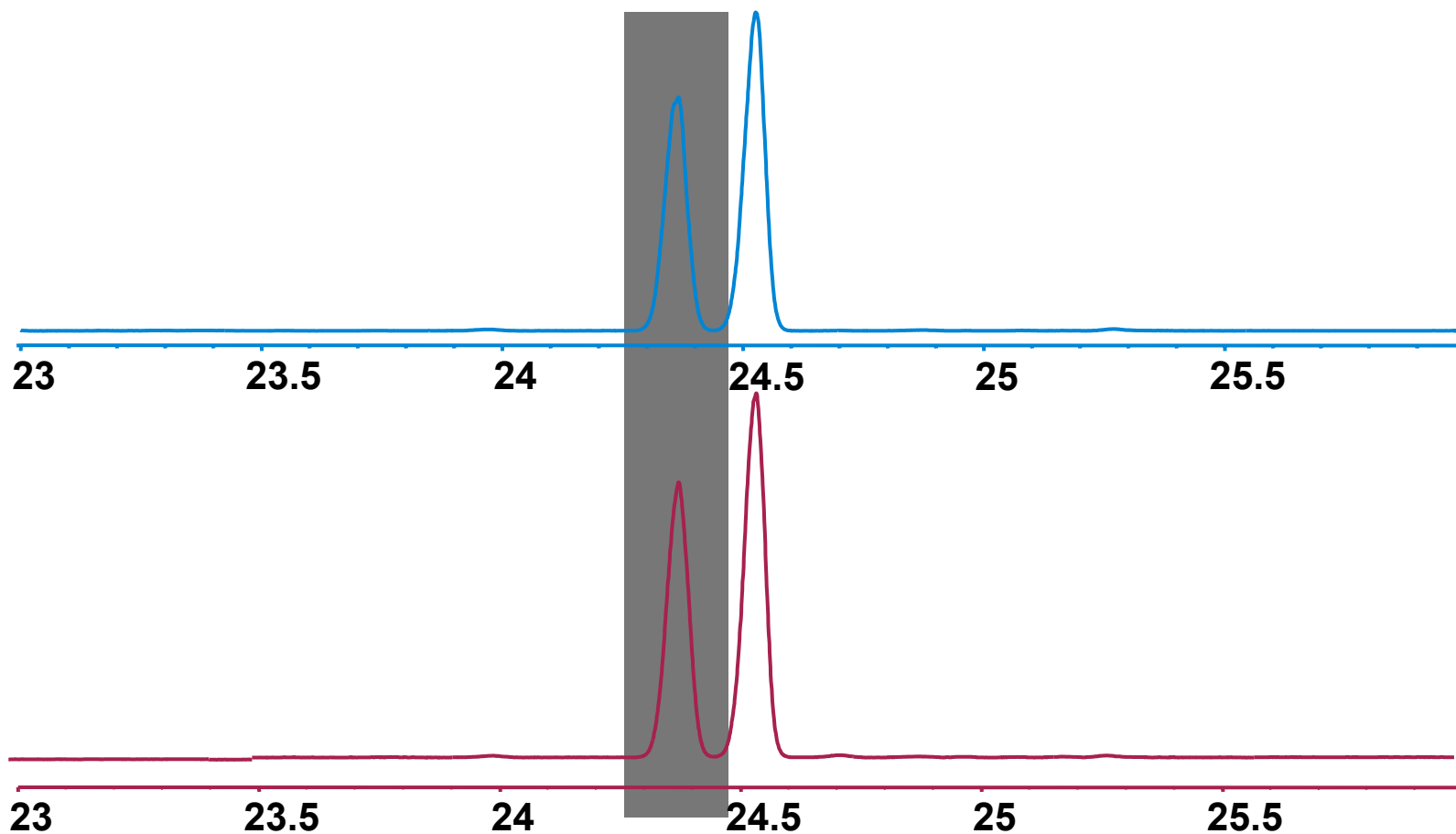


# Pesticides on Two Different GCs





# 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.

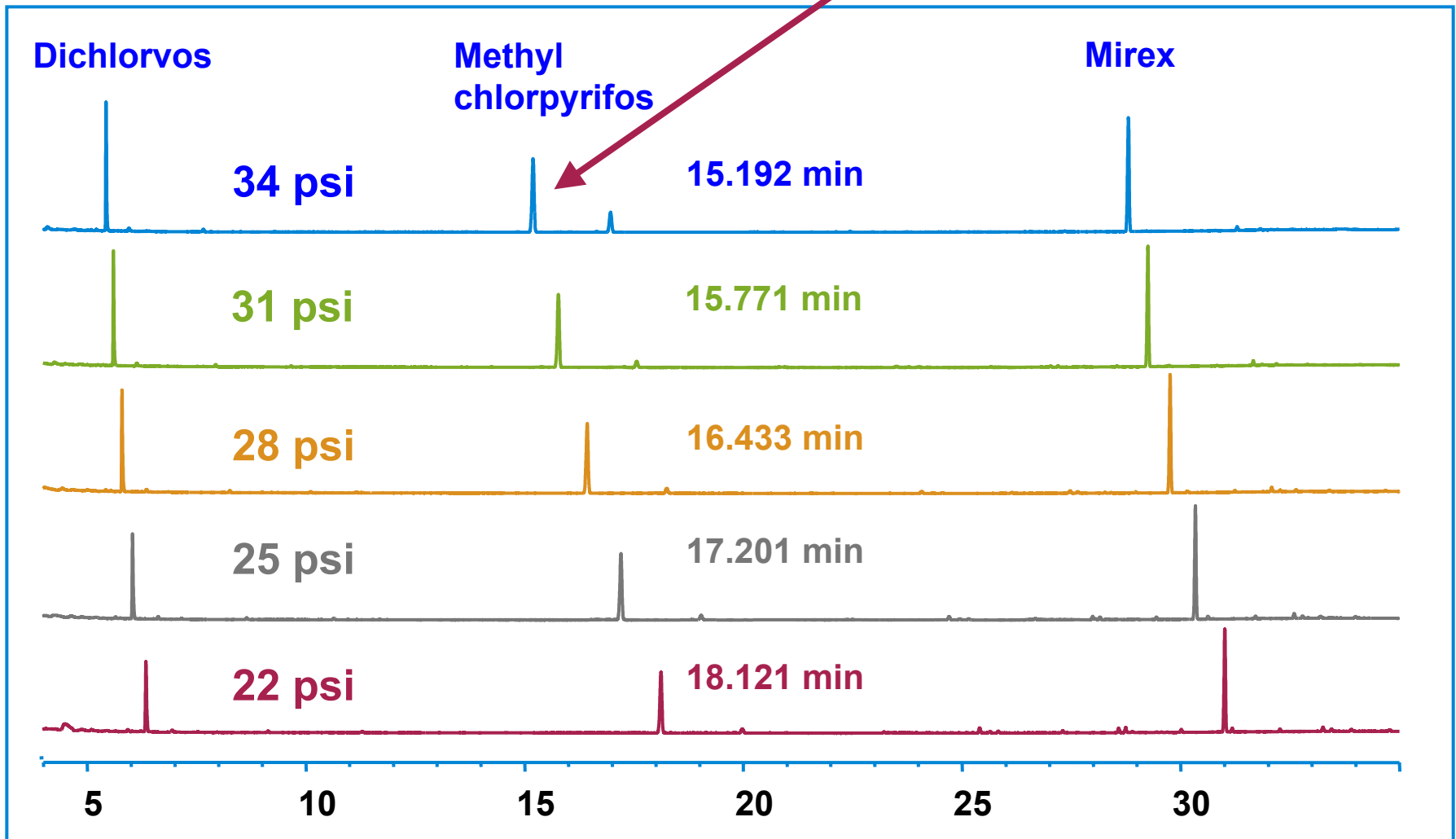
# Outline

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



# RTL Calibration Runs

Target Compound for RTL



# 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!**

The screenshot shows the 'Retention Time Locking Calibration' dialog box. It contains a table with 5 runs, each with a pressure value and a retention time. Below the table are input fields for 'Desired Ret Time', 'Min relock pressure', 'Max relock pressure', 'Column', and 'Compound Name'. The 'Compound Name' field contains 'methyl chlorpyrifos'. At the bottom are buttons for 'OK', 'Cancel', 'Print', and 'Help'.

	Pressure	Ret Time
Run 1	34	15.192
Run 2	31	15.771
Run 3	28	16.433
Run 4	25	17.201
Run 5	22	18.121

Pressure Units:

Desired Ret Time:

Min relock pressure:

Max relock pressure:

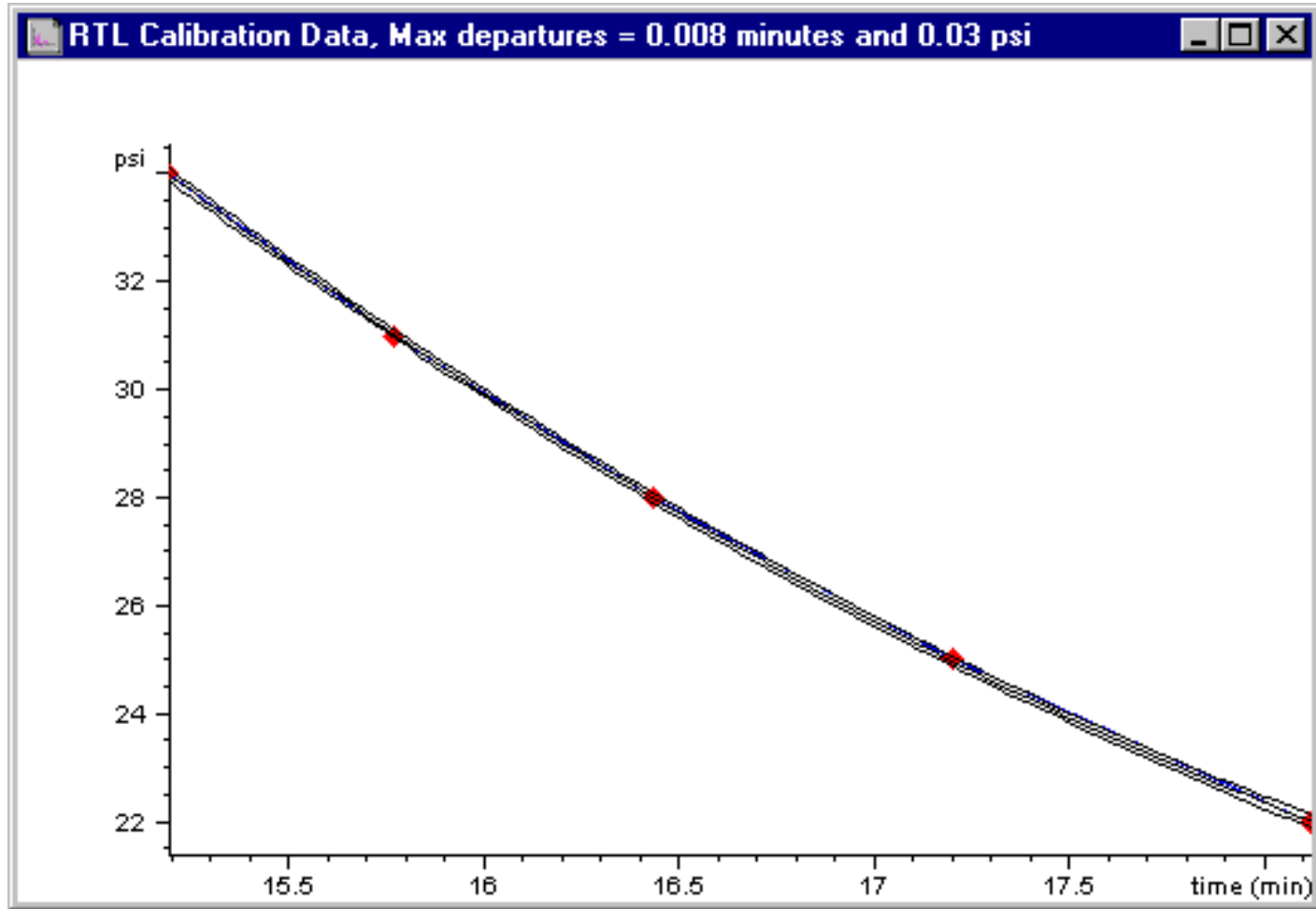
Column:

Compound Name:

Buttons: OK, Cancel, Print, Help

# RTL Calibration Curve

Column Head Pressure

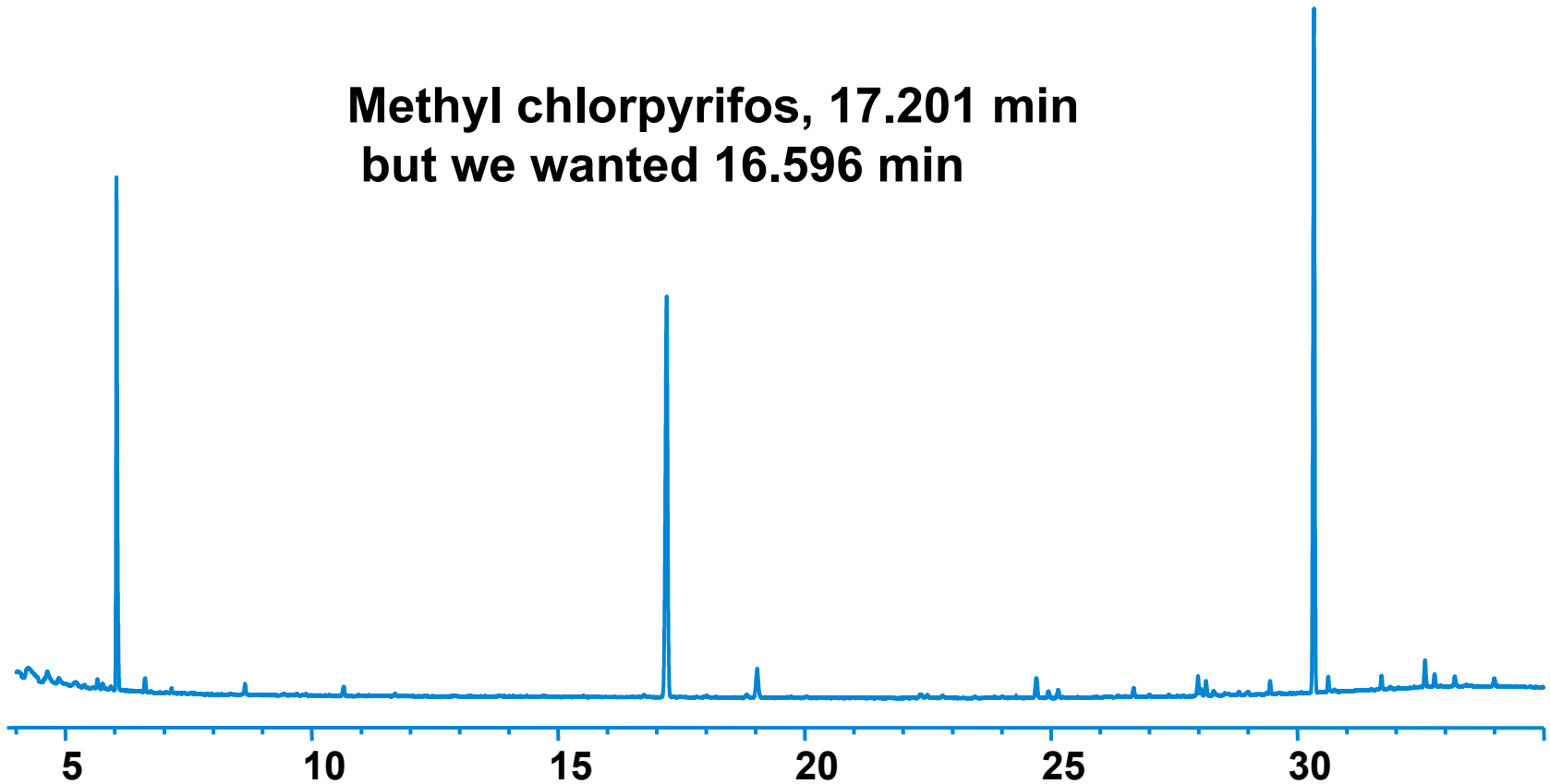


Target Compound Retention Time

# Locking A New System or Column

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

Methyl chlorpyrifos, 17.201 min  
but we wanted 16.596 min



# Locking a GC System

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

**[Re]Lock current method**

**Retention time:**

Enter current retention time of:  
methyl chlorpyrifos  
17.201 Minutes

Then select button 'Update Method' to calculate a new pressure and enter it in the method.

**Method Information:**

Current Method:	HLIN.M
Column:	1
Pressure used:	28.00 psi
Desired RT:	16.596 Minutes
Calc new pressure:	27.35 psi

Update current HP6890 Method    Print    Done    Help

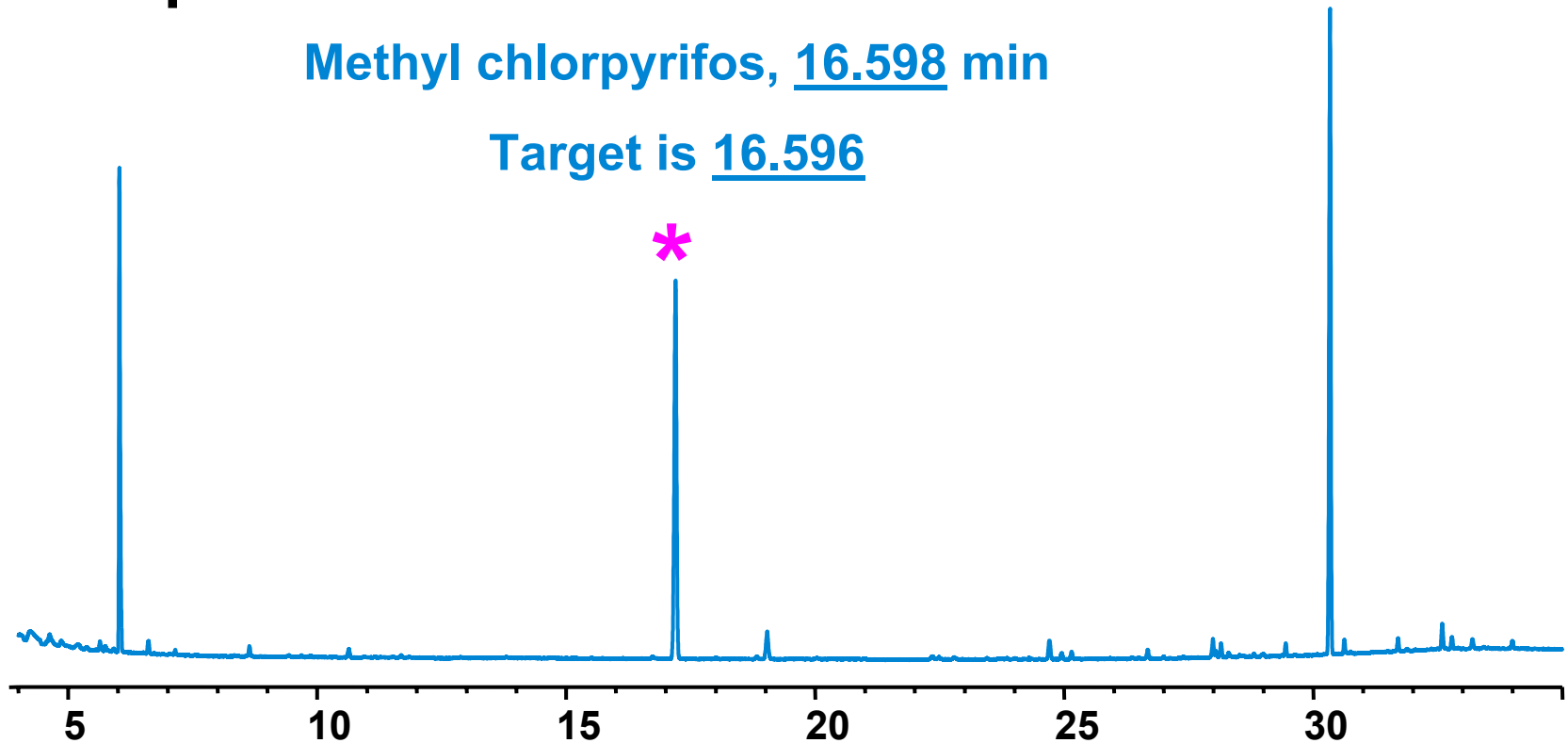


# Confirming RT Lock

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

Methyl chlorpyrifos, 16.598 min

Target is 16.596



# Outline

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

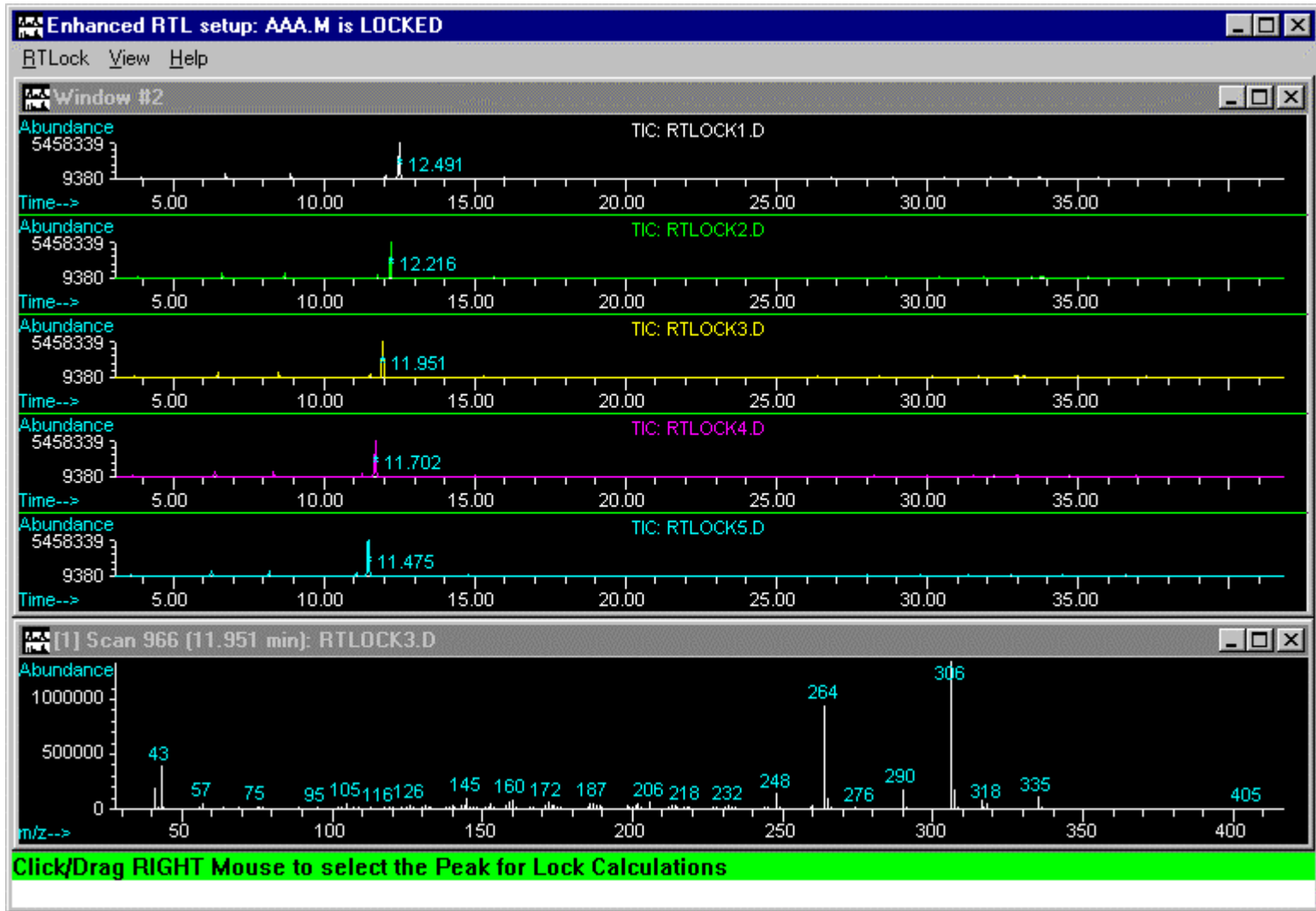


# Acquiring RTLock Calibration Data..

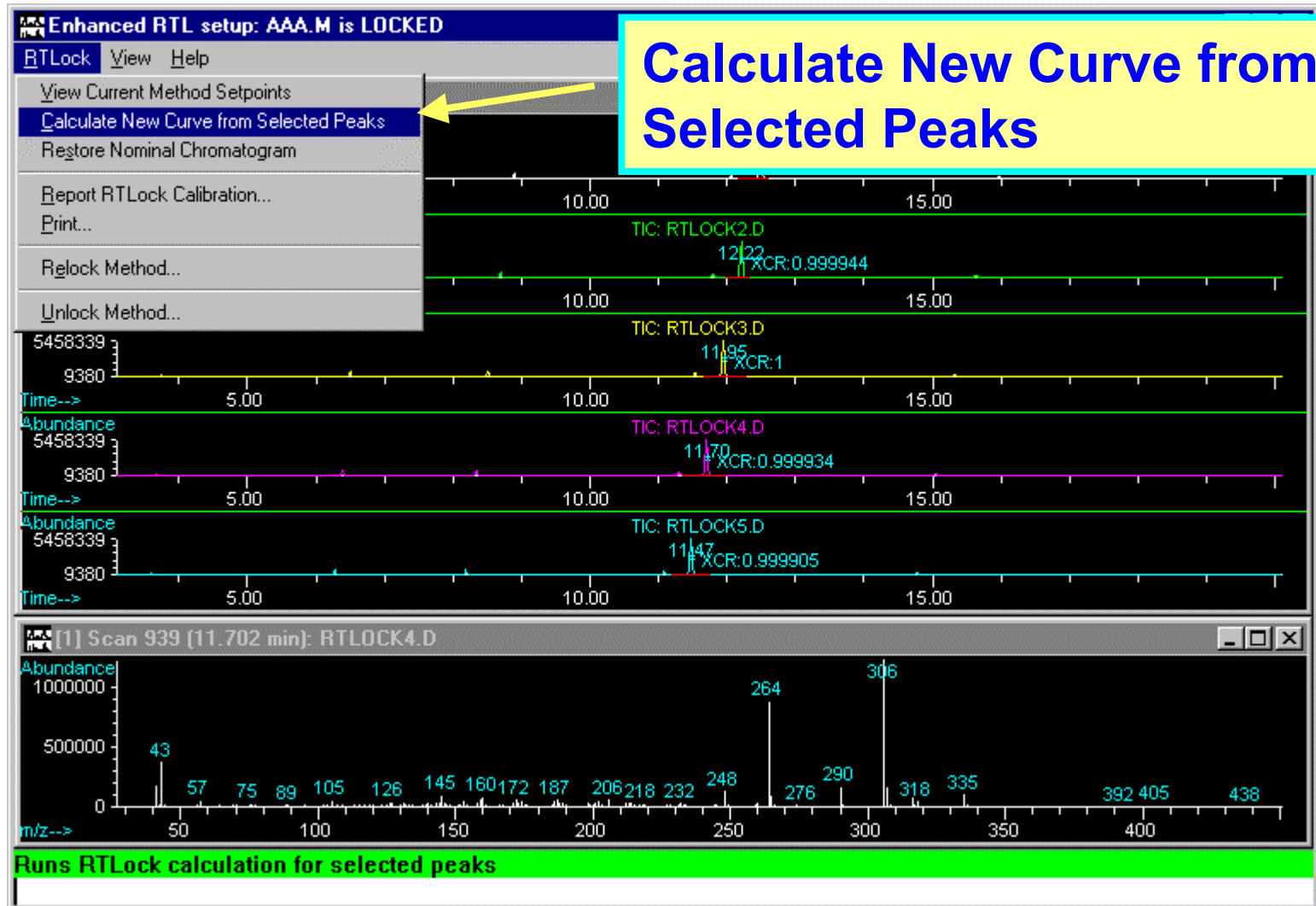
The screenshot displays the software interface for a GC/MS instrument. The title bar reads "GC/MS Instrument #1 - RTLPESTB.M". The menu bar includes "Method", "Instrument", "View", "Abort", "Window", "Qualify", and "Help". The "Instrument" menu is open, showing options such as "Inlet/Injection Types...", "GC Edit Parameters...", "GC Plot...", "GC Monitors...", "Acquire RTLock Calibration Data..." (highlighted with a blue oval and a blue arrow), "Unlock Method...", "Select MS Tune File...", "MS SIM/Scan...", "MS Monitors...", "Edit MS Tune Parameters...", and "Perform MS Autotune...". A yellow callout box with the text "Acquire RTLock Calibration Data.." points to the highlighted menu item. The interface also features a "Run Time" display, "Detectors" section with "Aux" and "MS" indicators, "Total Ion" and "EM Volts" displays, and "Oven Temperature" (70) and "Column-1 Flow Cal." (1.7) displays. A green bar at the bottom of the interface contains the text "Enters RTL procedure".



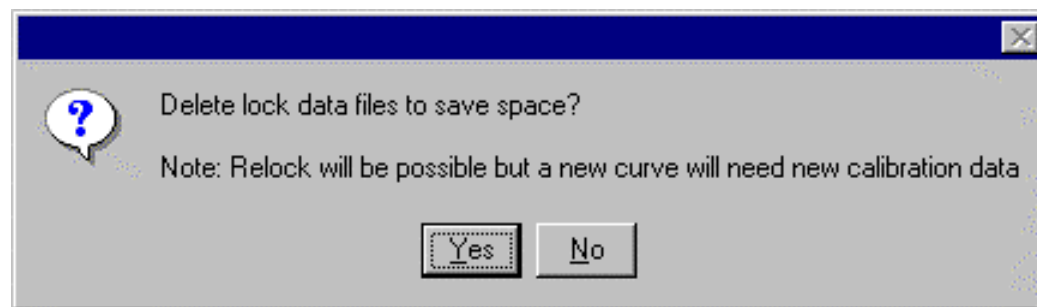
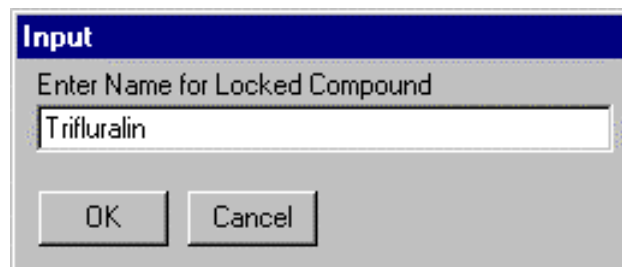
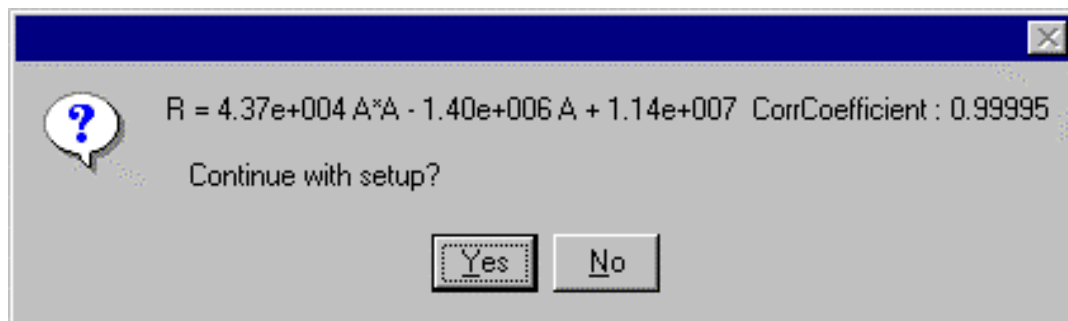
# RT Lock Data Files Are Automatically Collected



# Generate RT vs Head Pressure Equation



# Working with the software




# Working with the software

**Input**


Enter the Locking RT

11.637

OK Cancel

 Lock Pressure: 14.676 psi. Save to Method?

Yes No



RT	Pressure (psi)
12.491	10.40
12.216	11.70
11.951	13.00
11.702	14.30
11.475	15.60

OK

# 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:

  File      psi      Time      Spec      Deviation
  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
```

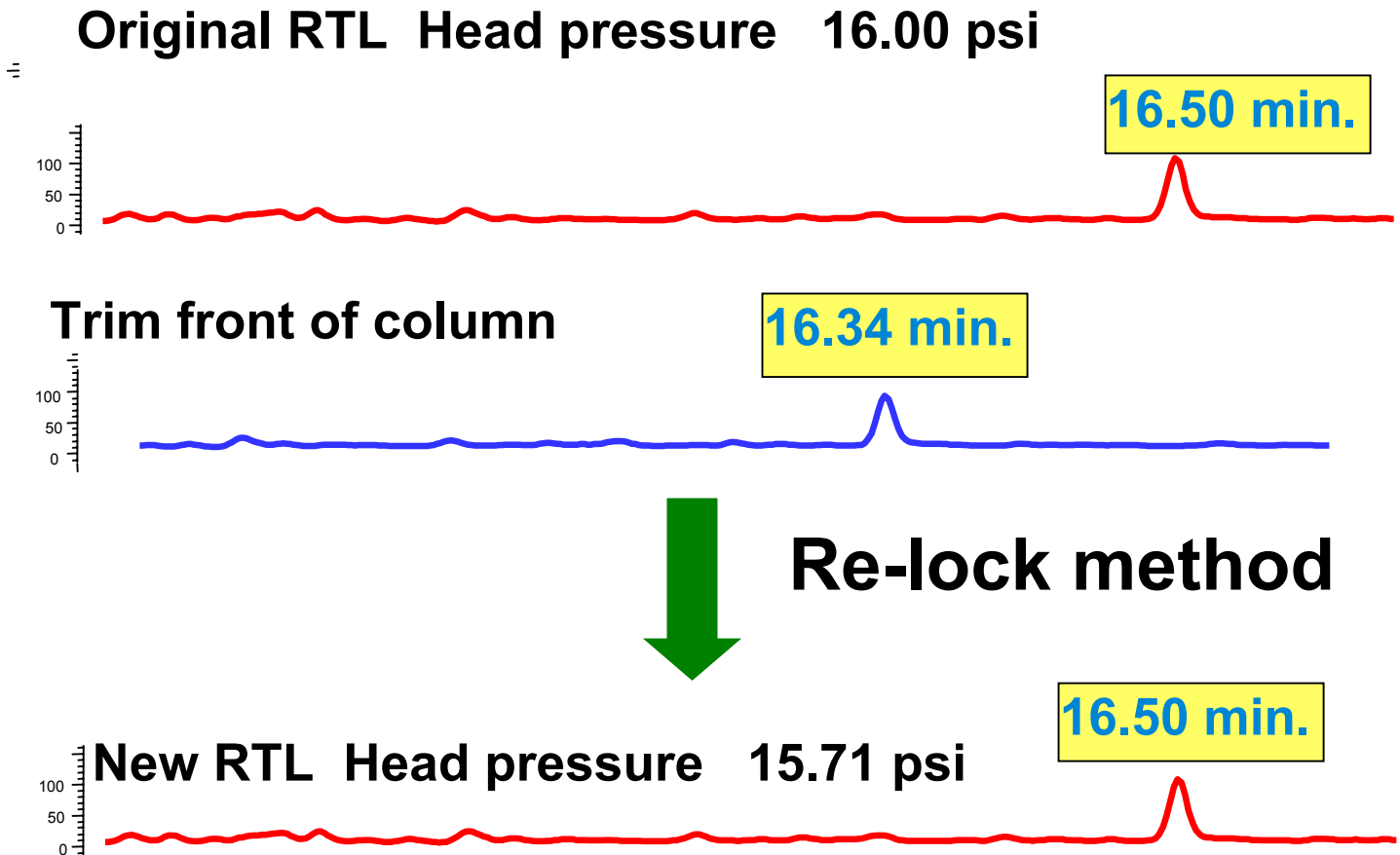




# The Method is Now Locked

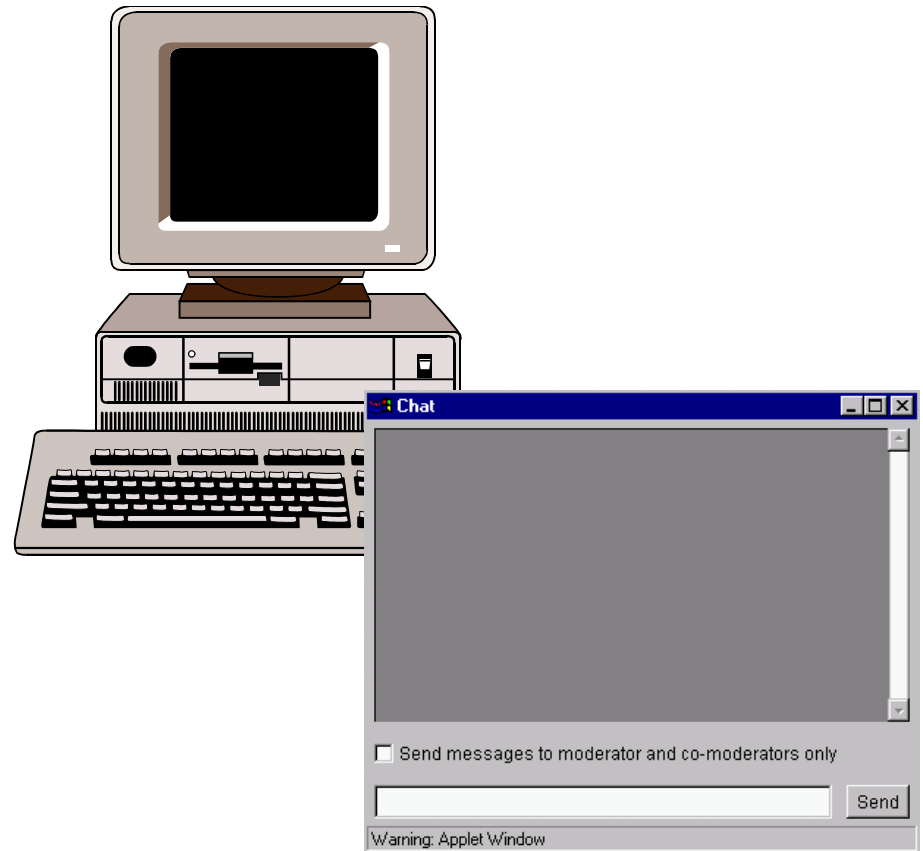
The screenshot shows the HP 5973 software interface. The title bar at the top reads "HP 5973 - AAA.M - EI: RETENTION TIME LOCKED to Trifluralin", with the latter part circled in red. Below the title bar is a menu bar with options: Method, Instrument, View, Abort, Window, Quality, Help. The main window is titled "Instrument Control" and contains several panels. On the left, there is a Hewlett-Packard logo and a red "Offline" button. In the center, there is a sample vial icon labeled "1" and a "Sample Name:" field. Below the vial is a "Data File:" field containing "evaldemo.d". To the right of the sample name and data file fields is a large grey arrow pointing right. Further right, there is a black box labeled "Run Time". Below these panels are two tabs: "Sample" and "Chromatography". Under the "Chromatography" tab, there are four icons representing different components: "Injector" (a syringe), "Inlets" (a T-junction), "Columns" (a coiled tube with a yellow bar), and "Oven" (a coiled tube with a red square).

# 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?
- Locking GC Methods
- Locking GC/MSD Methods
- Pesticide Analysis using Agilent's RTL Pesticide Databases

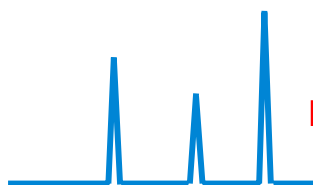


# RTL Database Screening: Build a Retention Time Table

Record retention times under  
RT Locked conditions

**GC1**

*FID*



## Master RT Table

1.037 Methanol

1.048 Pentane

2.971 Ethanol

2.990 Benzene

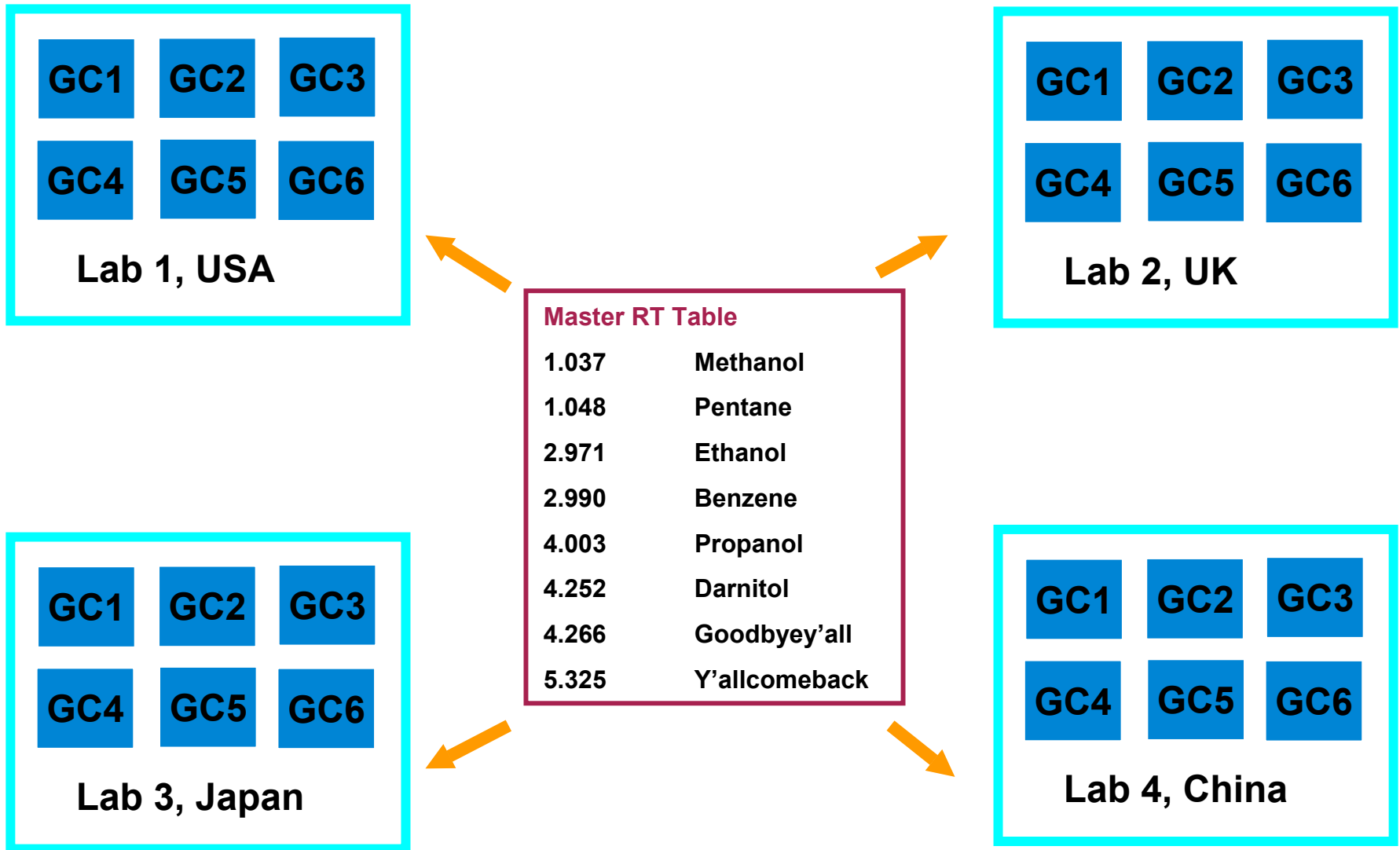
4.003 Propanol

4.252 Darnitol

4.266 Goodbye y'all

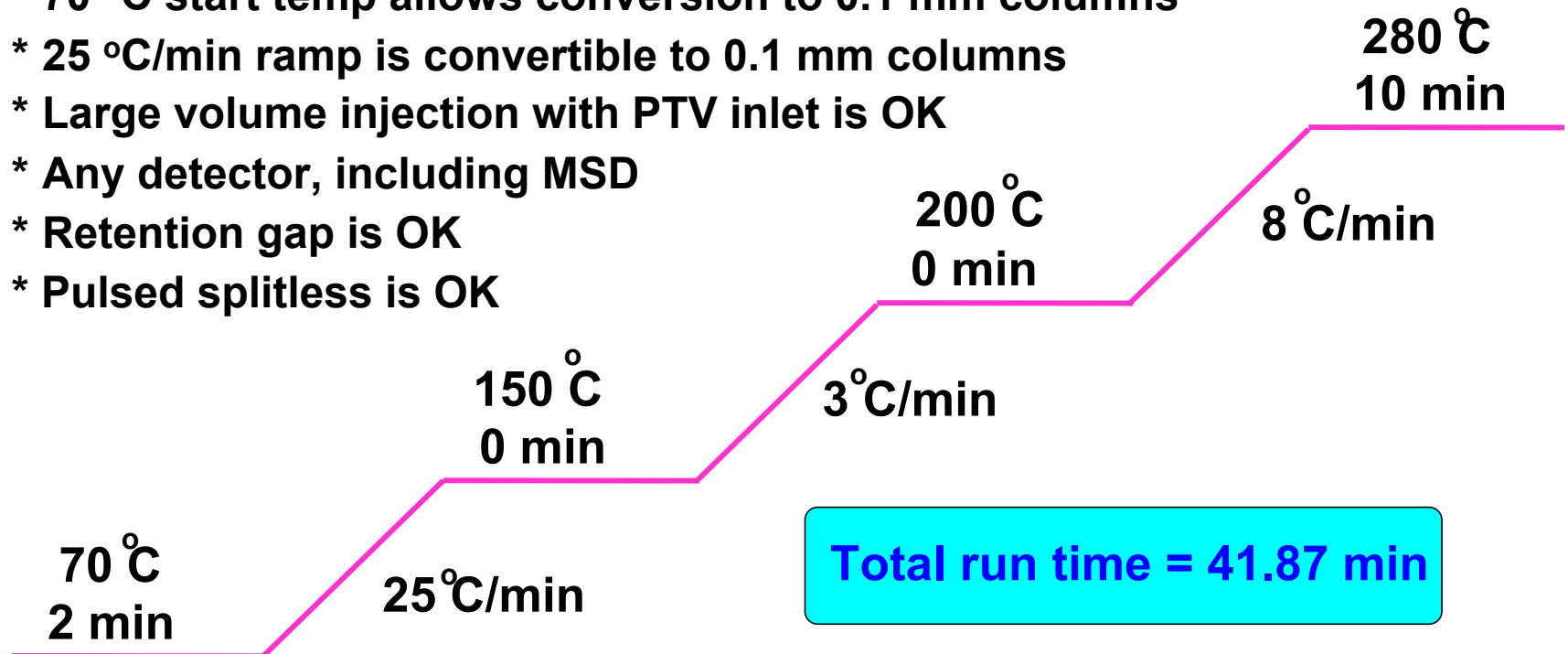
5.325 Y'all comeback

# Use Locked RT Table Worldwide



# Universal Pesticide Method

- \* Designed for 30 m X 0.25 mm X 0.25  $\mu$ m 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	HP
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

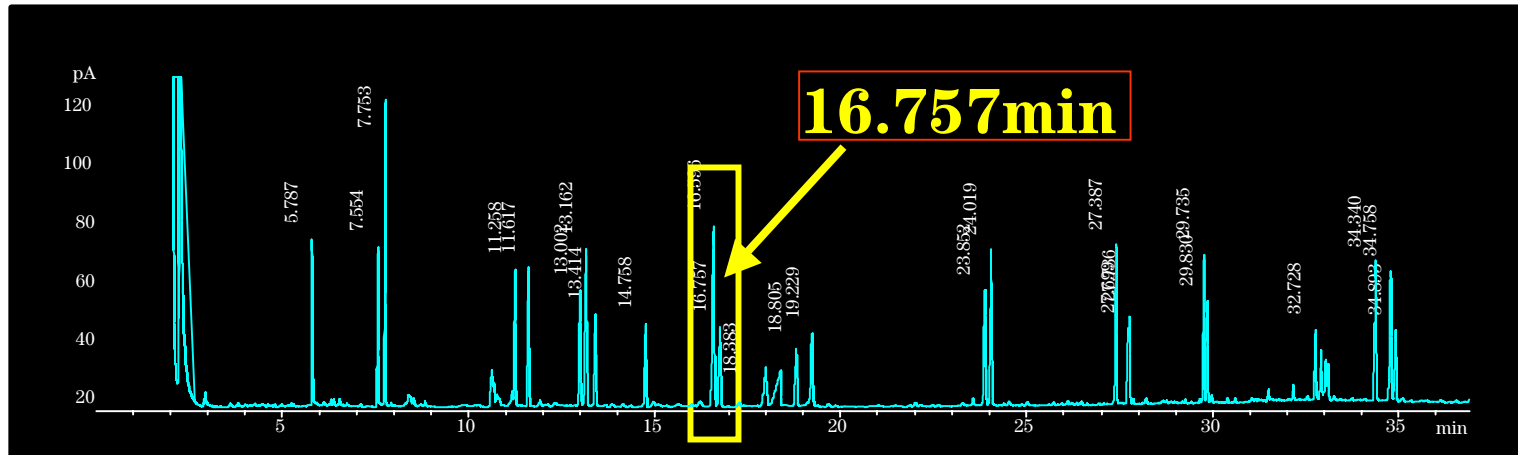
FPD(S)

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

ID_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

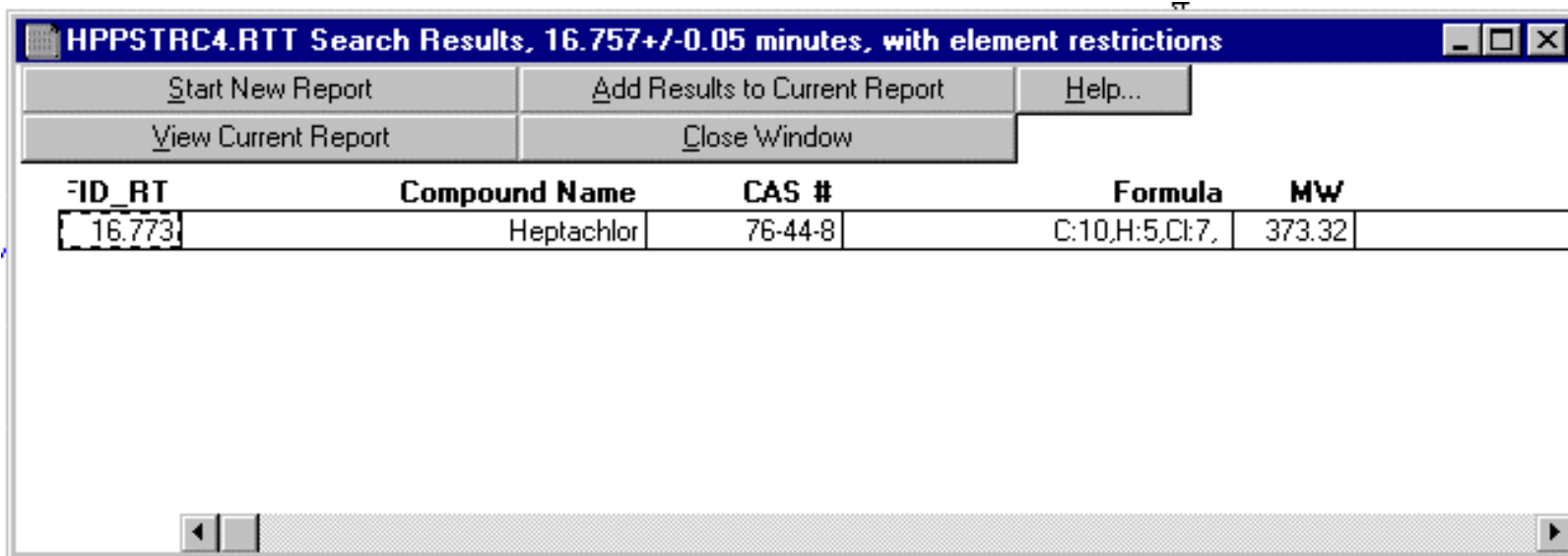
FPD(S)

FPD(P)

Search Cancel Help

# Pesticide Database Search

*Using unknown's element information yields just 1 possibility*



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

# Strawberry Extract

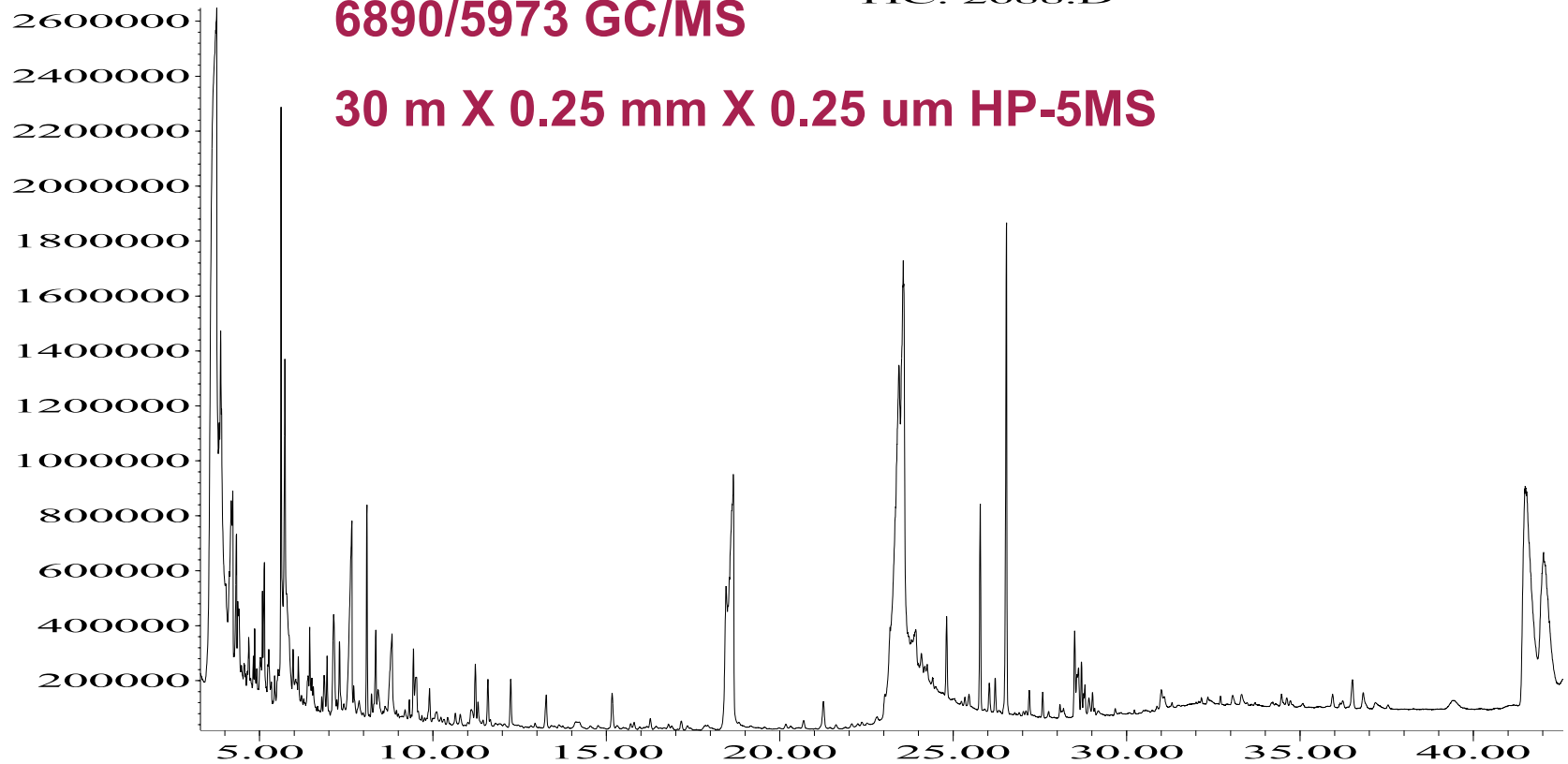
25 uL PTV Injection

6890/5973 GC/MS

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

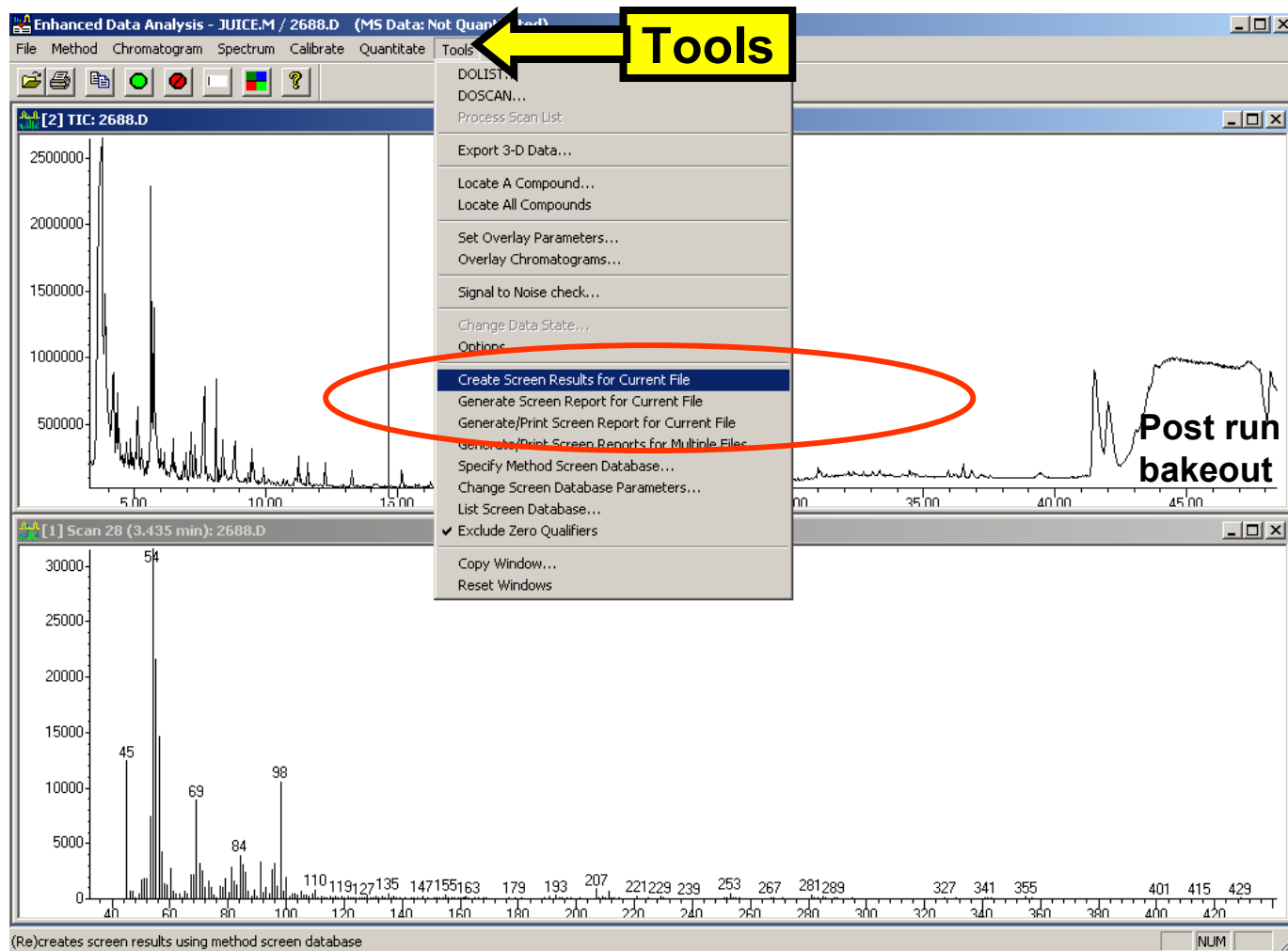
TIC: 2688.D

Abundance



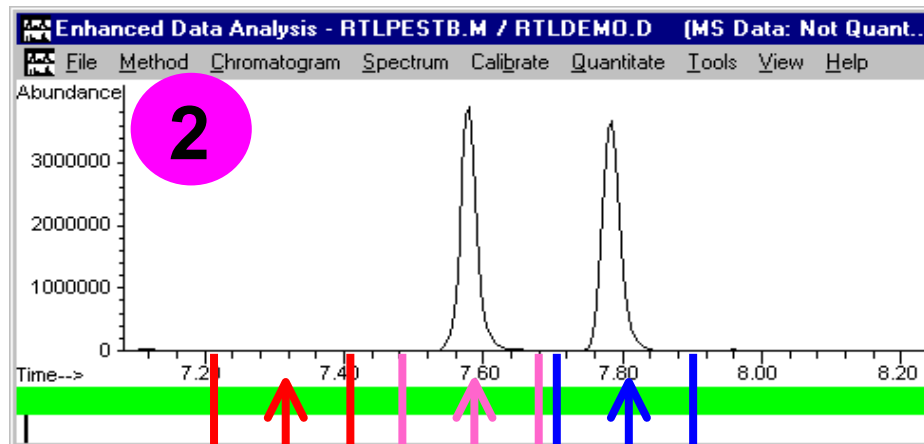
Time-->

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



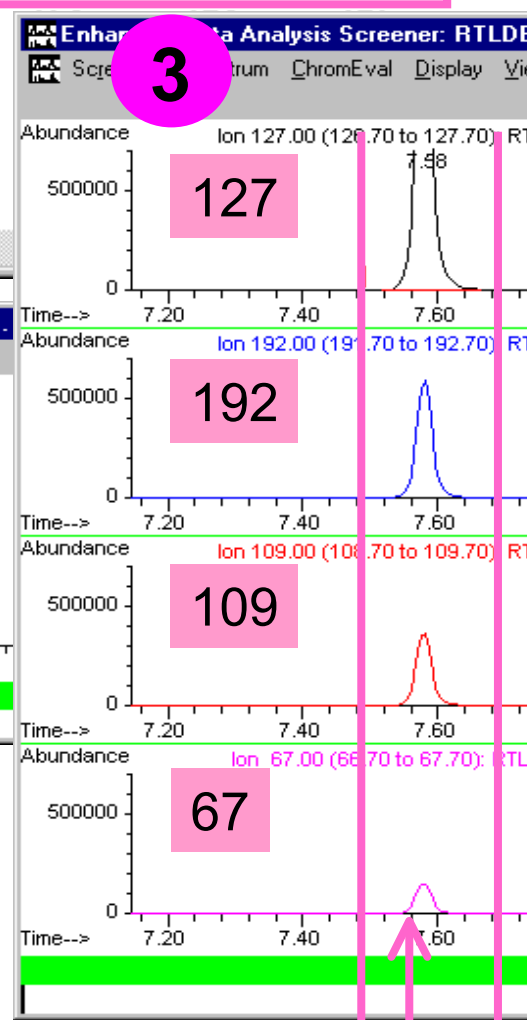
1

	Target	RT	Qualifier Ions			
33	2-Phenoxypropionic acid	121	7.13	94	166	77
34	3,5-Dichloroaniline	161	7.32	163	98	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			



7.32 ± 0.1  
7.59 ± 0.1  
7.81 ± 0.1

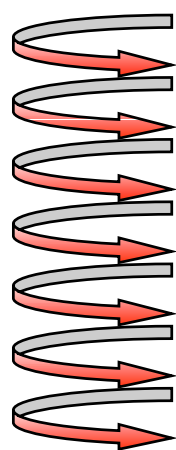
After a sample is run, ion currents are automatically extracted and integrated for each database entry in sequential time windows.



4

Exp	Act
100	100
31.6	
35.92	
21.7	
21.93	
10.2	8.88

# 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

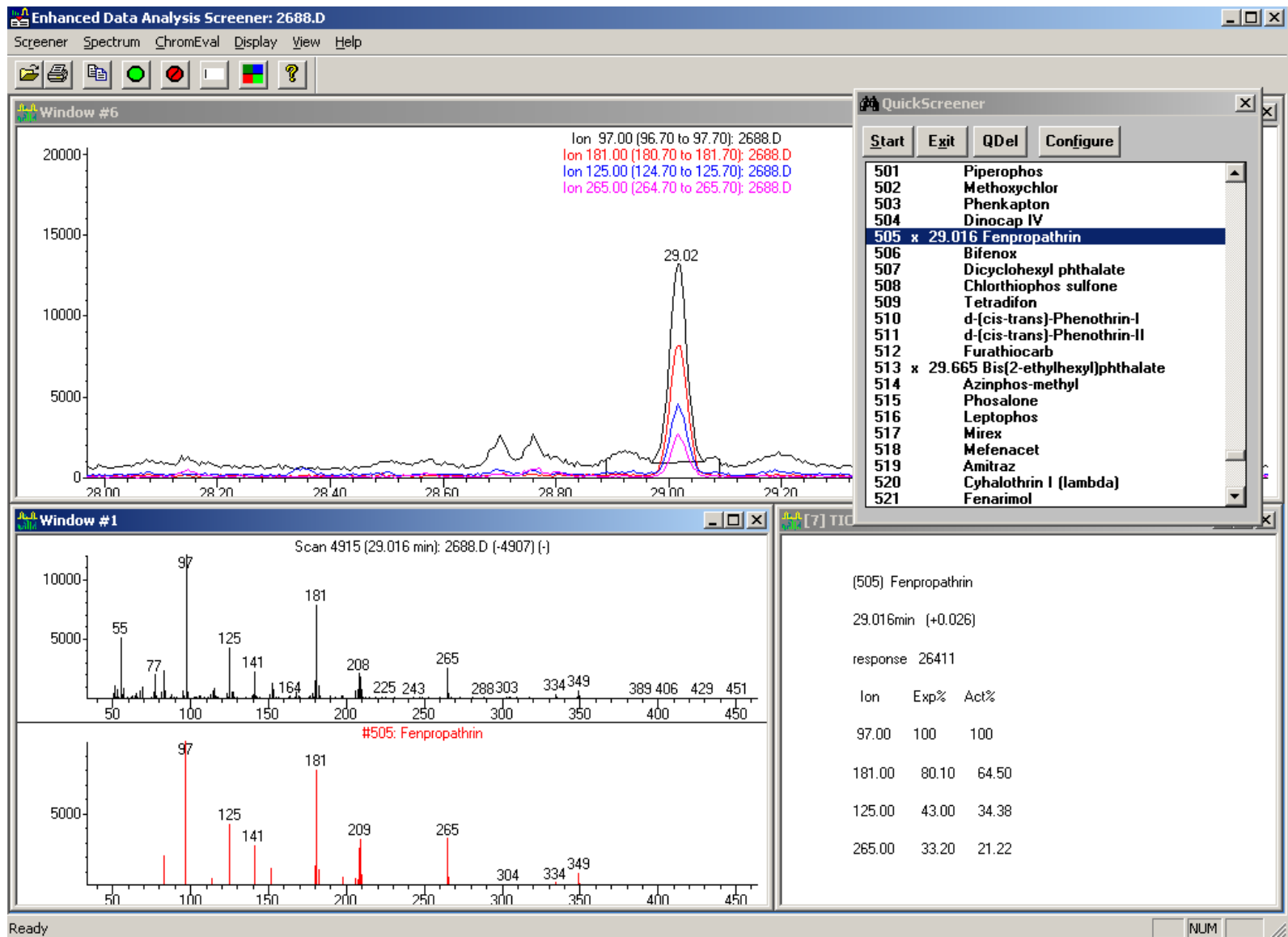
**567 Compounds Screened**

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
<b>263 Di-n-butylphthalate</b>	<b>x</b>	<b>18.415</b>	<b>+0.033</b>	<b>149</b>	<b>466128</b>		<b>0.99</b>
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

Screen Report Wed Apr 16 15:04:43 2003

**14 possible 'hits'**

# Results Screener Software for Verification

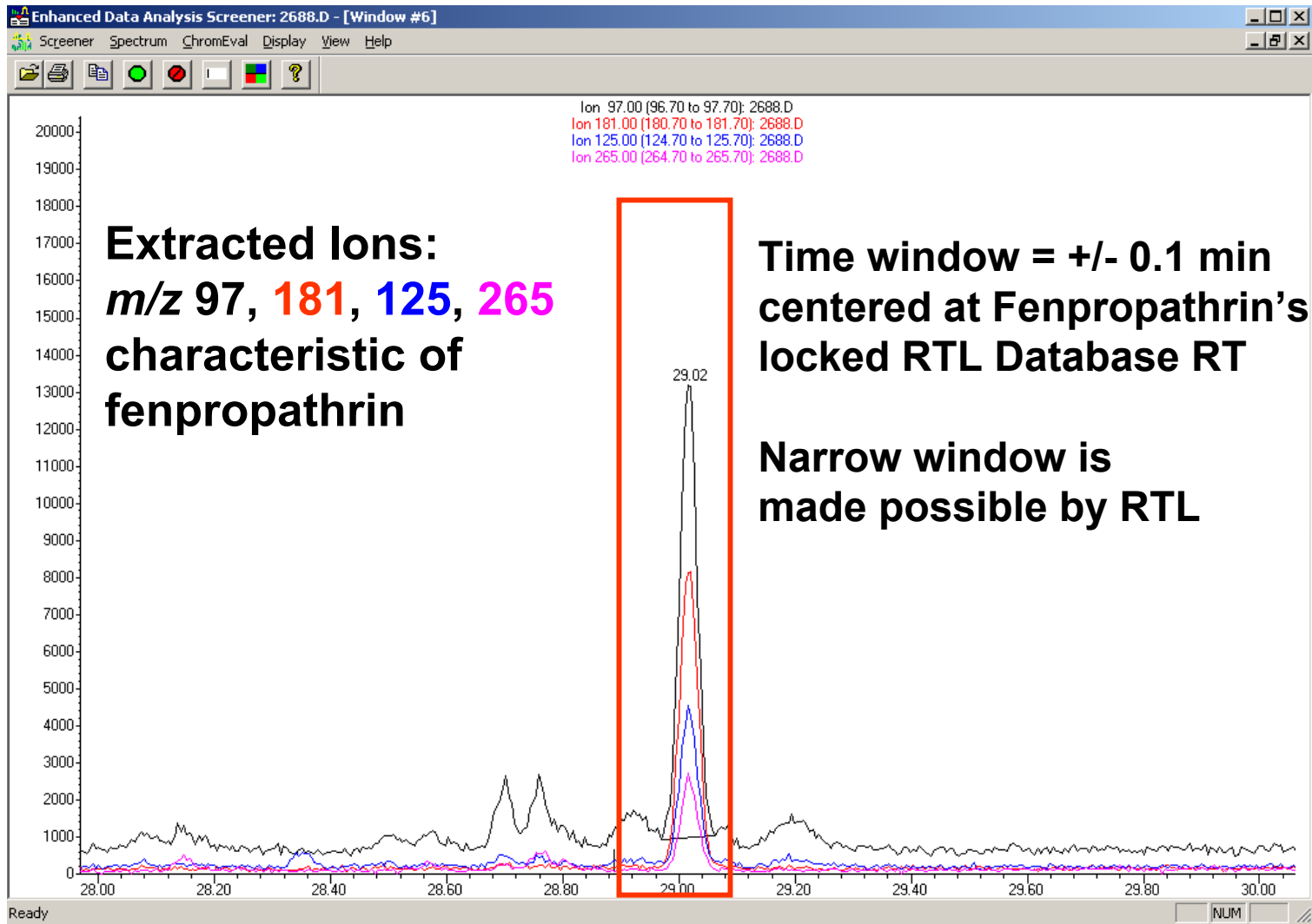


# Results Screener: Compound List with 'Hits' Marked with ? or X

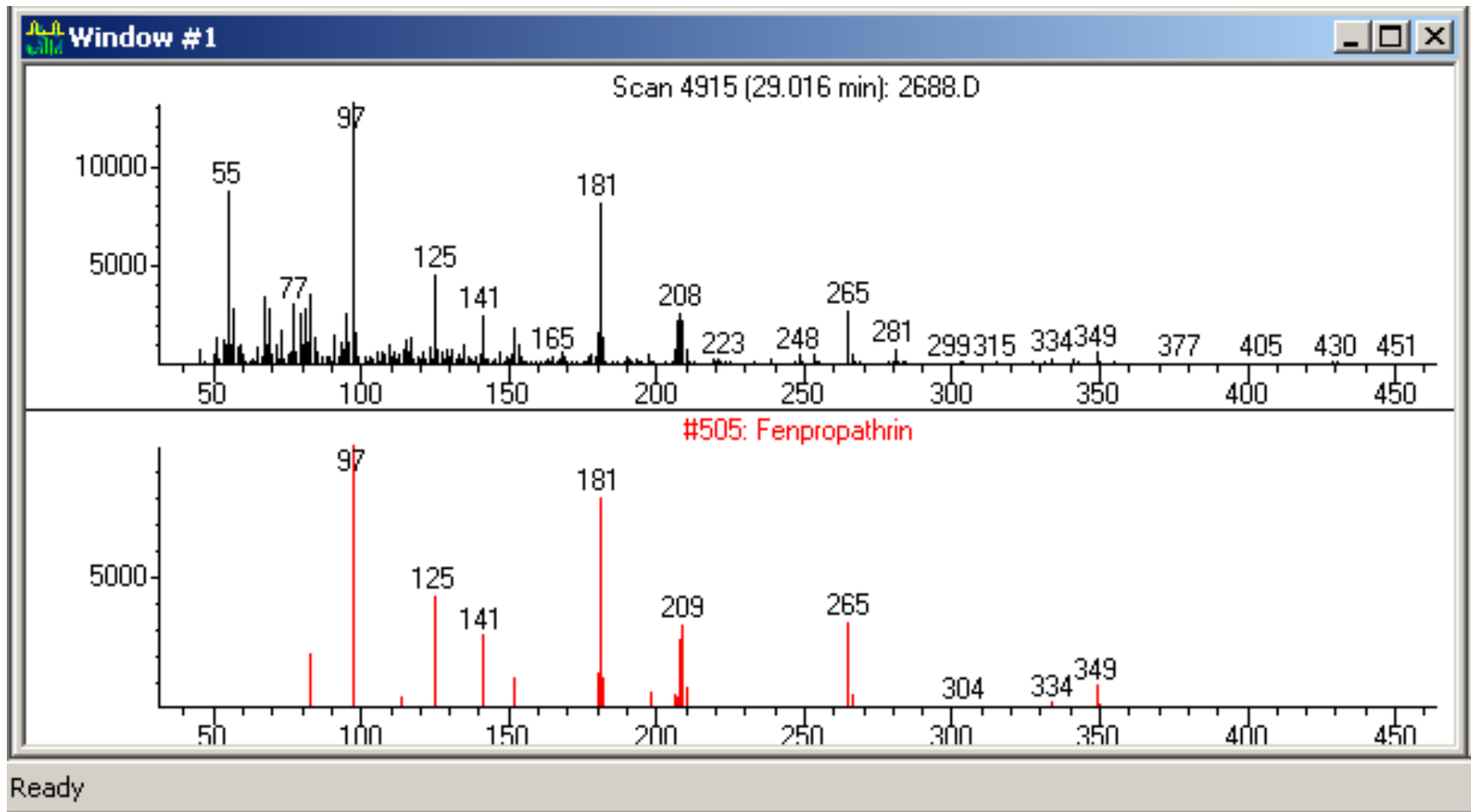
ID	Retention Time	Compound Name
501		Piperophos
502		Methoxychlor
503		Phenkapton
504		Dinocap IV
505	x 29.016	Fenpropathrin
506		Bifenox
507		Dicyclohexyl phthalate
508		Chlorthiophos sulfone
509		Tetradifon
510		d-(cis-trans)-Phenothrin-I
511		d-(cis-trans)-Phenothrin-II
512		Furathiocarb
513	x 29.665	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)**

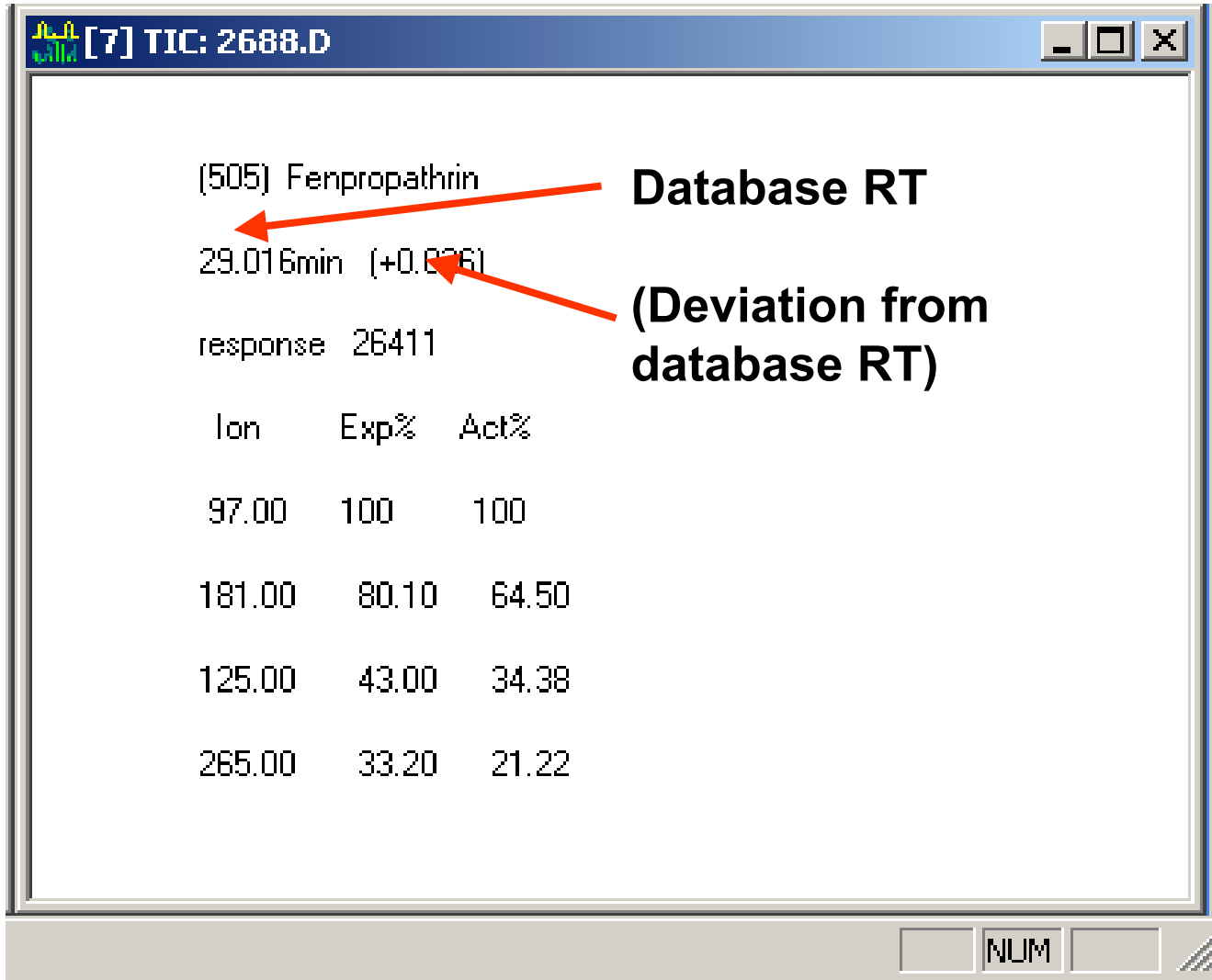
# Results Screener: 4 Ions of Fenpropathrin Extracted - peaks found inside time window



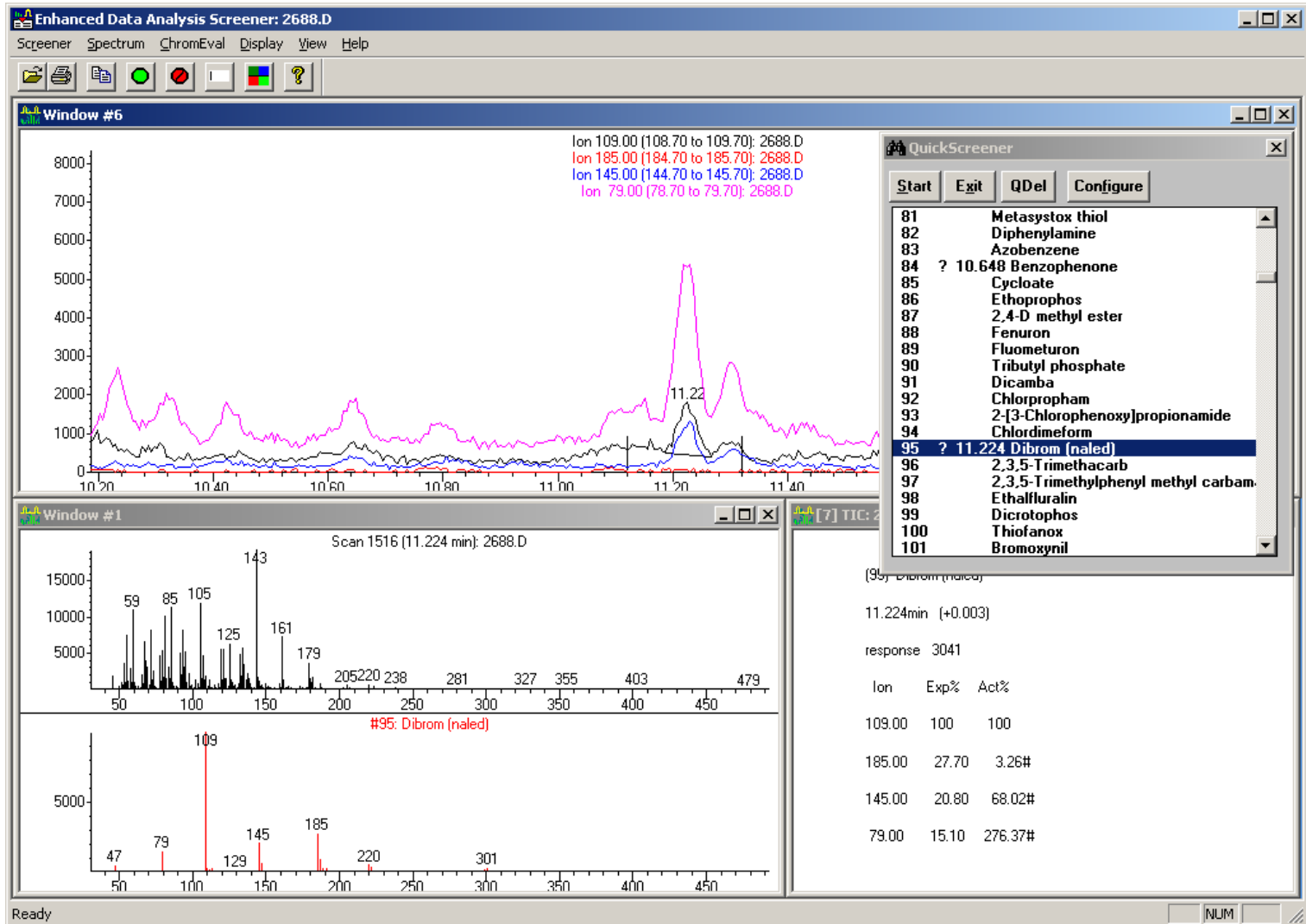
# Results Screener: Apex Spectrum Compared to RTL Pesticide Library Spectrum



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



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

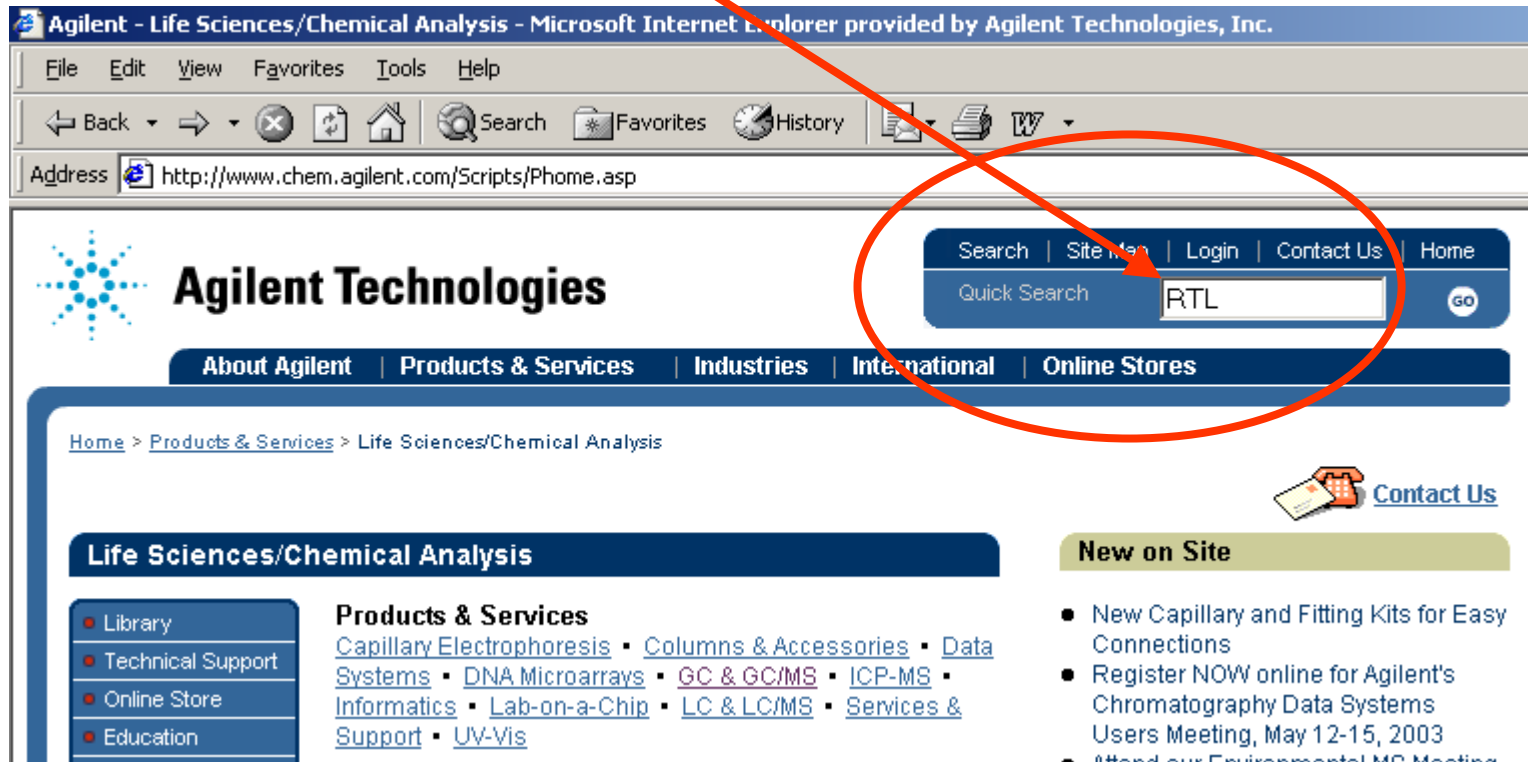
Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
72 Diethyl phthalate	x	9.958	+0.036	149	7891		0.99
263 Di-n-butylphthalate	x	18.415	+0.033	149	466128		0.99
330 Captan	x	21.227	+0.037	79	88406	151	0.88
459 Butyl benzyl phthalate	x	27.007	+0.020	149	6538		0.95
505 Fenpropathrin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

Screen Report Wed Apr 16 16:25:14 2003



# Where to get More RTL & Pesticide Analysis Information

At [www.Agilent.com](http://www.Agilent.com), choose Life Sciences/Chemical  
Type **RTL** or **Pesticide** into the quick search window



The screenshot shows a Microsoft Internet Explorer browser window displaying the Agilent website. The address bar shows <http://www.chem.agilent.com/Scripts/Phome.asp>. The website header includes the Agilent logo and navigation links: Search, Site Map, Login, Contact Us, and Home. A "Quick Search" box is highlighted with a red circle, containing the text "RTL" and a "GO" button. Below the header is a navigation bar with links: About Agilent, Products & Services, Industries, International, and Online Stores. The main content area features a breadcrumb trail: Home > Products & Services > Life Sciences/Chemical Analysis. On the left, there is a "Life Sciences/Chemical Analysis" section with links to Library, Technical Support, Online Store, and Education. In the center, the "Products & Services" section lists various analytical techniques and equipment. On the right, there is a "New on Site" section with a "Contact Us" button and a list of recent news items.

# 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

