Innovations in... Large Volume Injection

Applications of a Chromatographic Zone as Inlet System for GC/MS

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Introduction

In typical GC/MS analyses,
only a fraction of the injected components is of analytical interest;
the remainders are interferences or uninteresting
(e.g., sample solvent, matrix related, etc…).

Eliminating these components provides substantial improvements;
increased analytical integrity, less frequent maintenance, etc…

These concerns become amplified in

Large Volume Injection (LVI)!

ProSep™ Function

The ProSep™ system consist of 4 components, the pre-column module which is the inlet port, two control modules and a glass or silica precolumn which fits inside the Precolumn Module similar to a split/splitless glass liner. The "*ProSep™ Function*" is diagrammed in Figure 1.

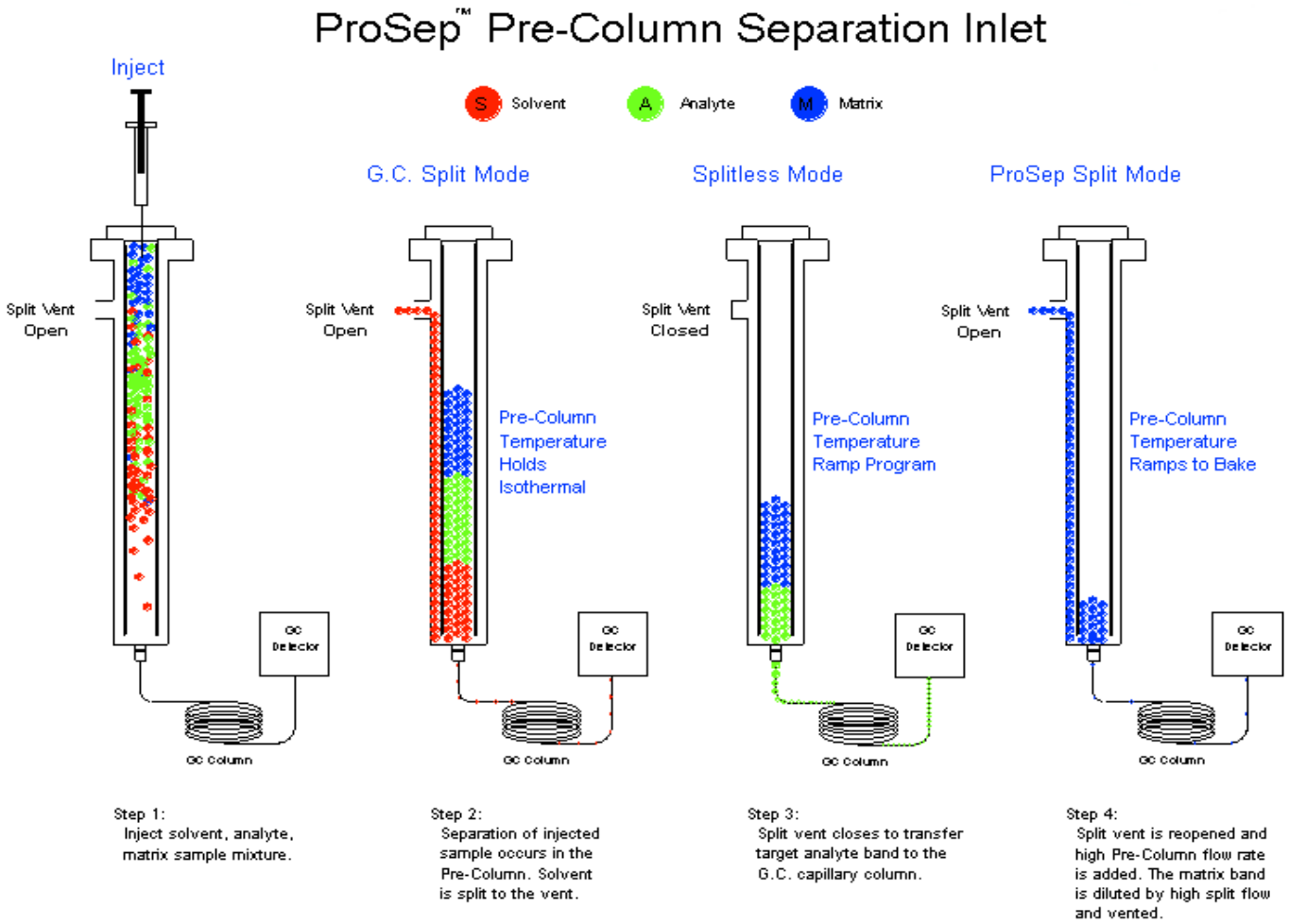
At injection, ProSep™ sets the GC in split mode. Because ProSep™ provides some separation, injected components are organized in the pre-column according to boiling point; here solvent, analyte and matrix are ordered in the port and the low boiling solvent rapidly vented (Step 2).

After solvent elimination, the Pre-separation Column Module temperature is ramped and the split vent closed transferring analytes into the analytical column (Step 3).

After transfer of the last analyte of interest, the split vent is again opened and the Pre-separation Column Module temperature ramped to bake off uninteresting matrix components (Step 4).

Software controls all aspects of the pre-separation module condition.

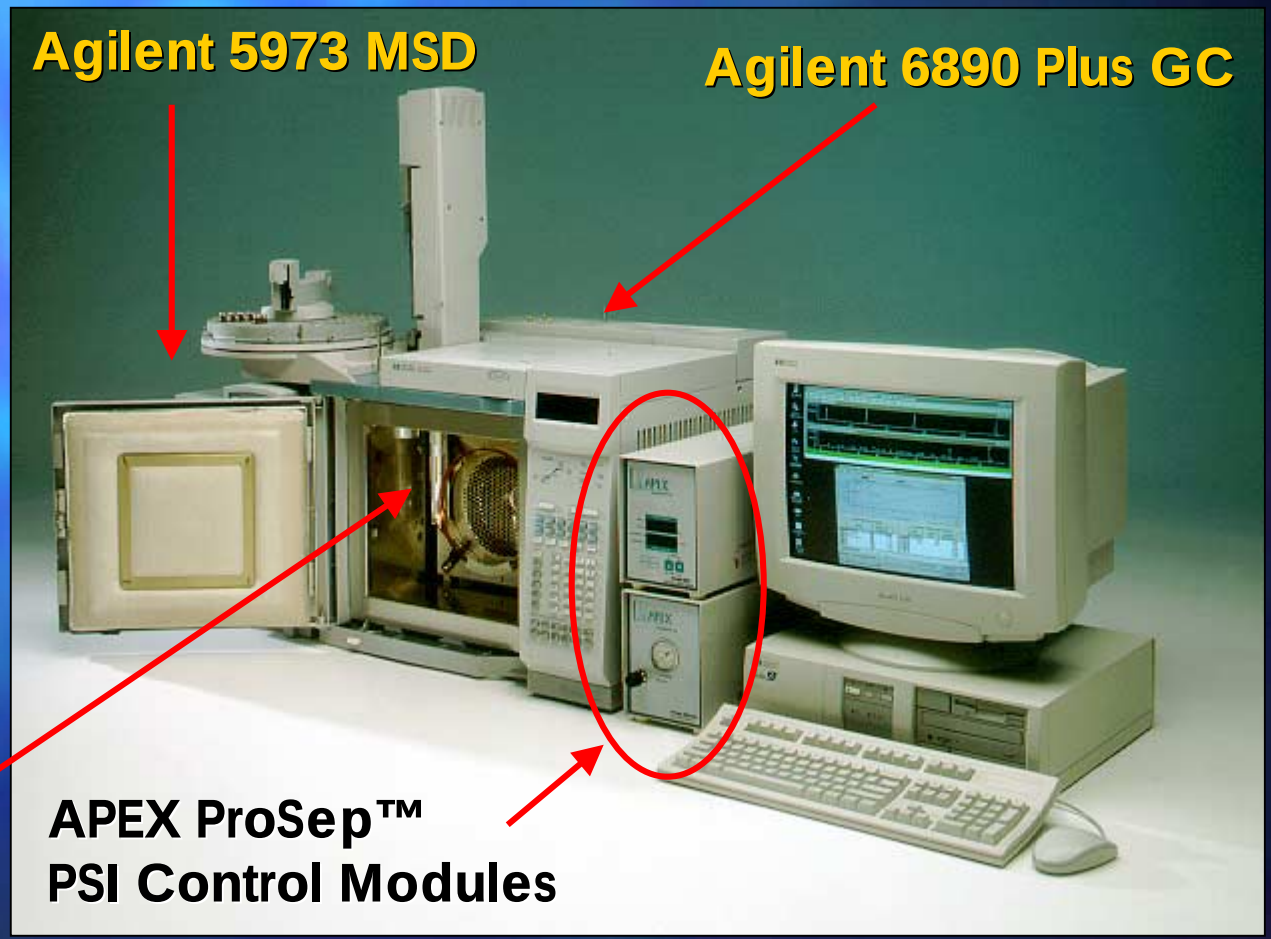
ProSep™ Function



Experimental Instrument Configuration

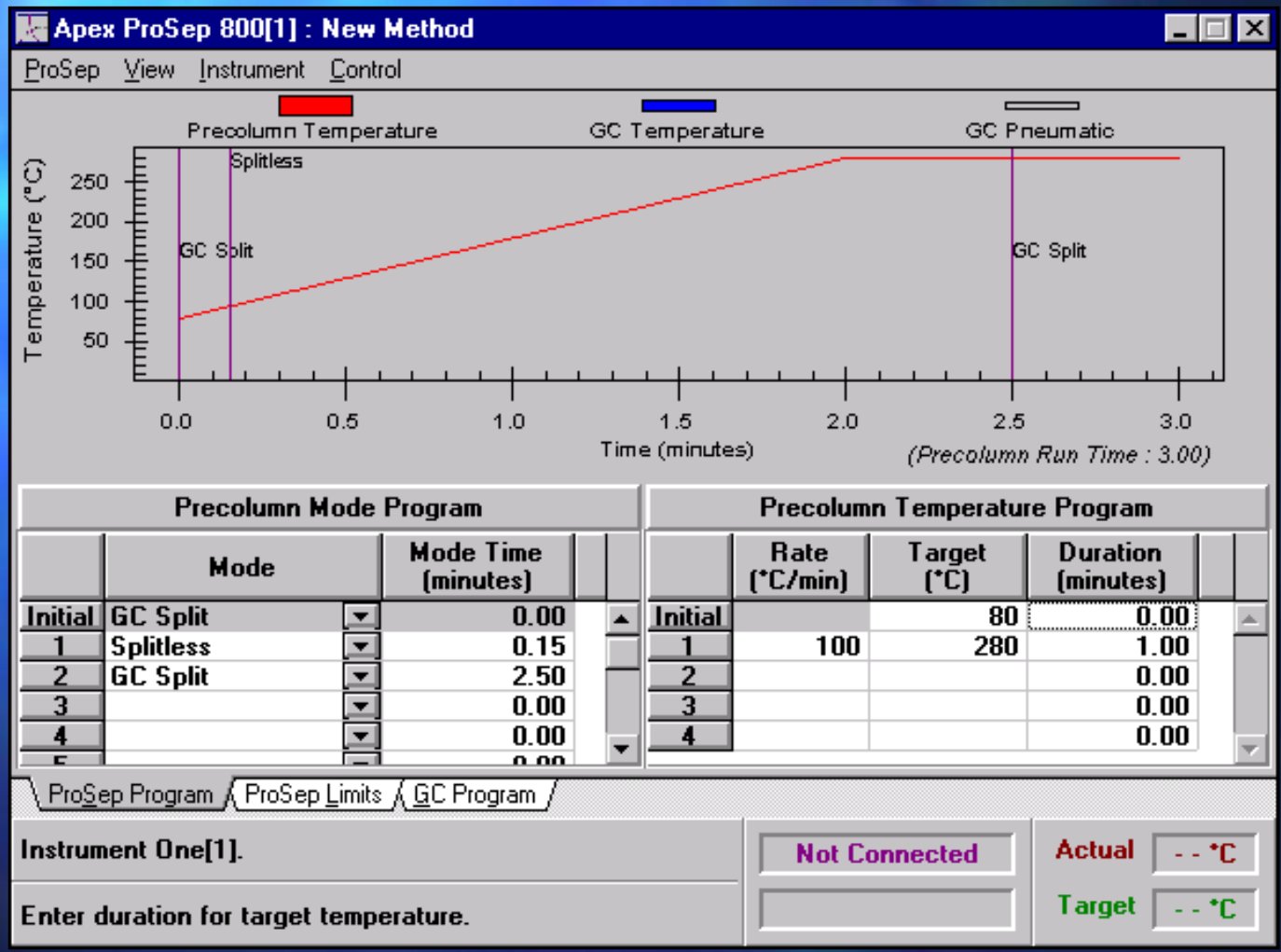
Agilent Technologies 6890 GC - 5973 Mass Selective Detector with APEX ProSep™ 800 XT Plus Precolumn Separation Inlet (PSI) and controller modules

Pre-separation Column Module



Control of ProSep™ Parameters

Navigator Software



Experimental

A 30 m, 0.25 mm OD, 0.25 um film Agilent Technologies-5MS was used as the analytical column. The Agilent Technologies 5973 MSD was operated in full scan (56-280 amu) with the only change between small volume (SV) and large volume injections being an increase in threshold (500-5000) to suppress peaks from impurities.

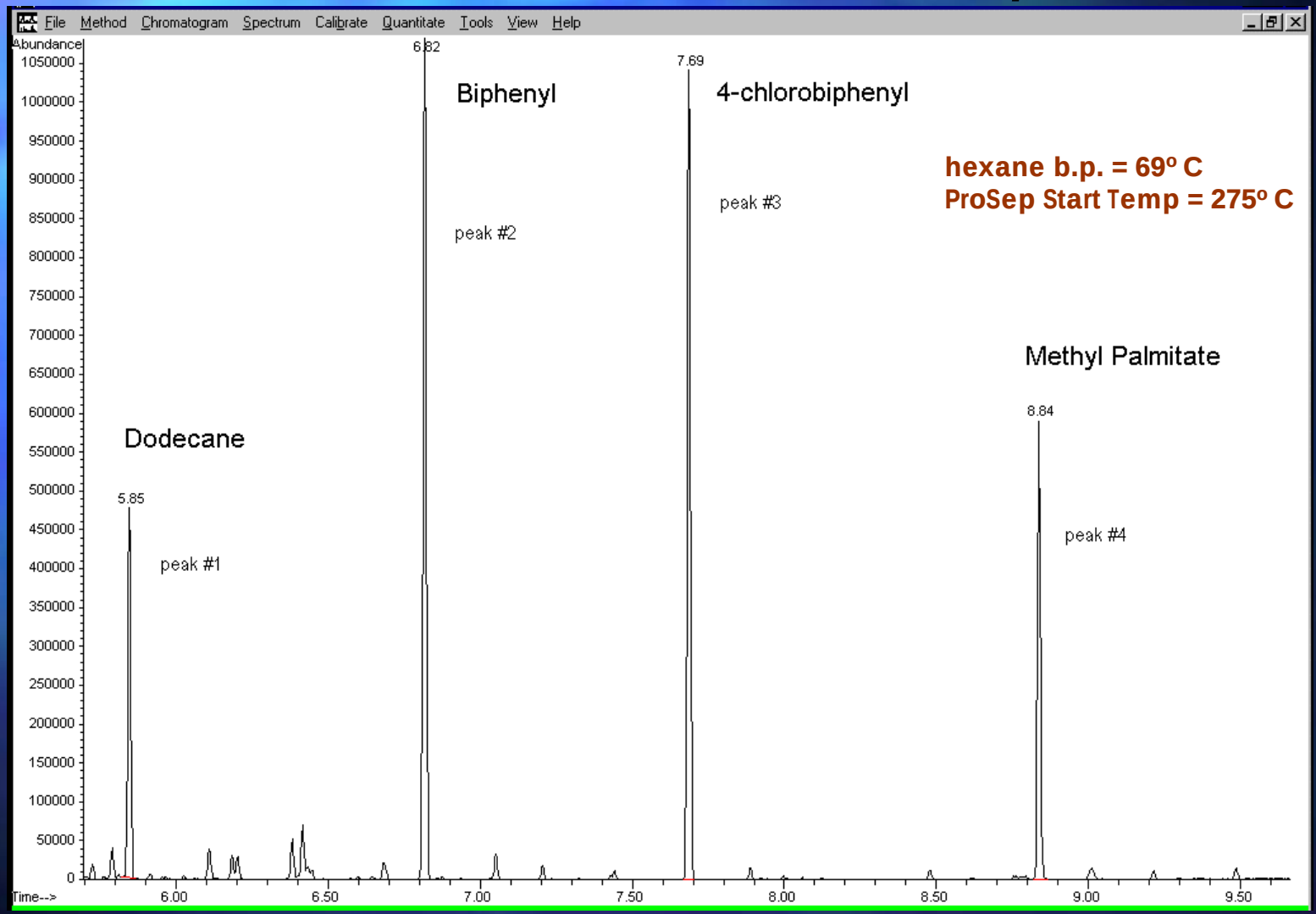
The ProSep™ precolumn was phase coated with HT-5, contained HT-5 coated fibers and a plug of silica wool in the upper 4-7 cm of the column.

Before applying LVI, a SV 1 ul injection of the 4 component mixture in hexane solvent was made to establish relative abundances of the compounds, Figure 3. The ProSep™ precolumn was held isothermal at 275° C to elute all components and maintained in the splitless mode to transfer all analytes to the GC column.

1µl injection of the test mixture

Figure 3

Note relative abundances and composition



LVI Experiments

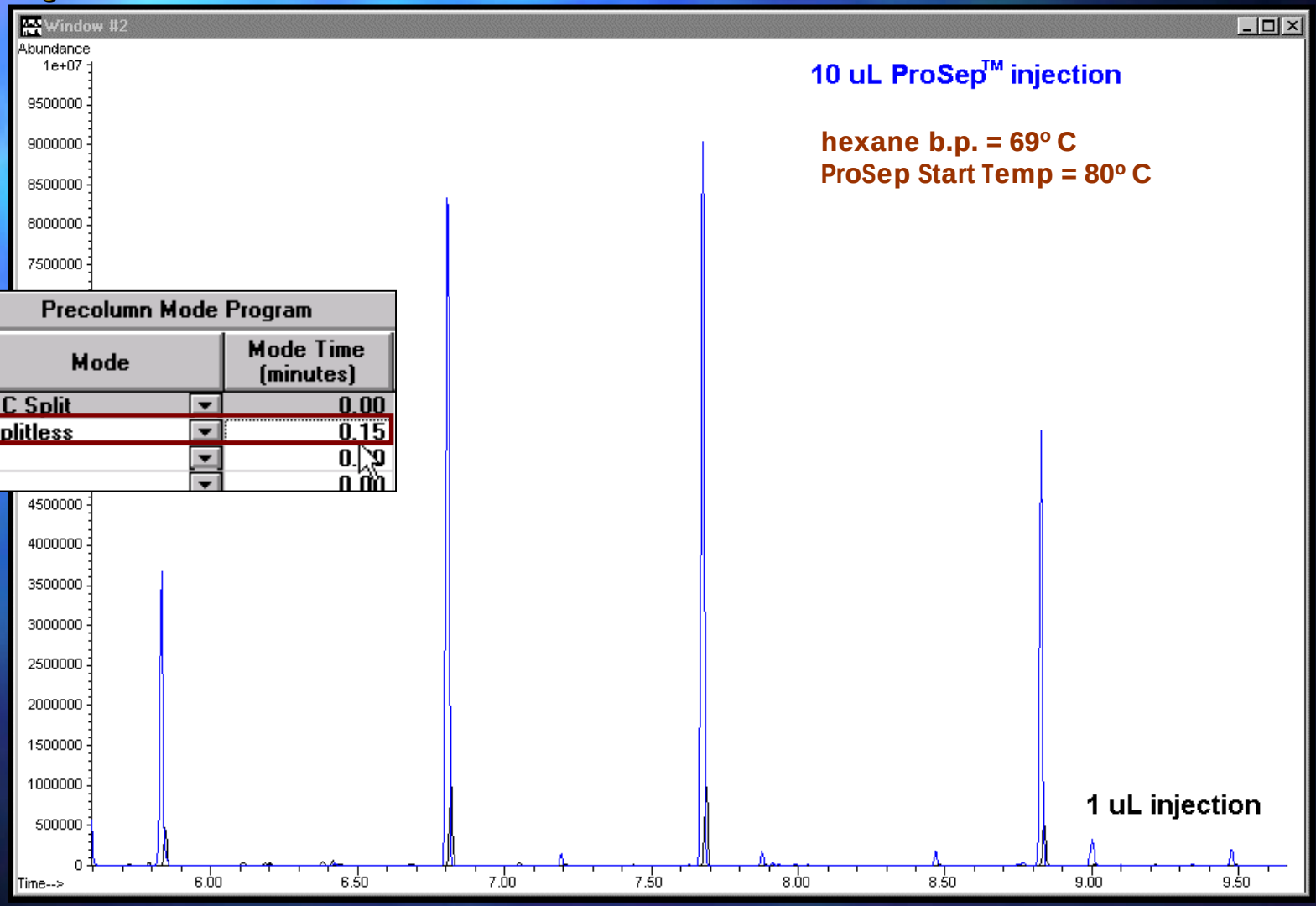
Next, the ProSep™ was used to scale from 1uL to 10 ul injections. The precolumn temperature is ***above*** the boiling point of the hexane (69° C) at injection (80° C) and the split mode is held for about 6 seconds to vent the hexane. By optimizing these ProSep™ parameters, a nearly ten-fold increase in signal is obtained, Figure 4 (The high value for the MS threshold holds the gain just under a factor of ten).

Consider the dodecane component (peak #1) as an uninteresting solvent impurity. By delaying the transition to splitless mode, ProSep™ is capable of selectively attenuating just this first peak, Figure 5. While this is still a 10 ul injection, dodecane has been attenuated by a factor of 20 fold; less than in the initial 1 ul injection.

Selectively removing dodecane implies separating dodecane (boiling point 216 °C) from biphenyl (boiling point 255 °C) in the precolumn.

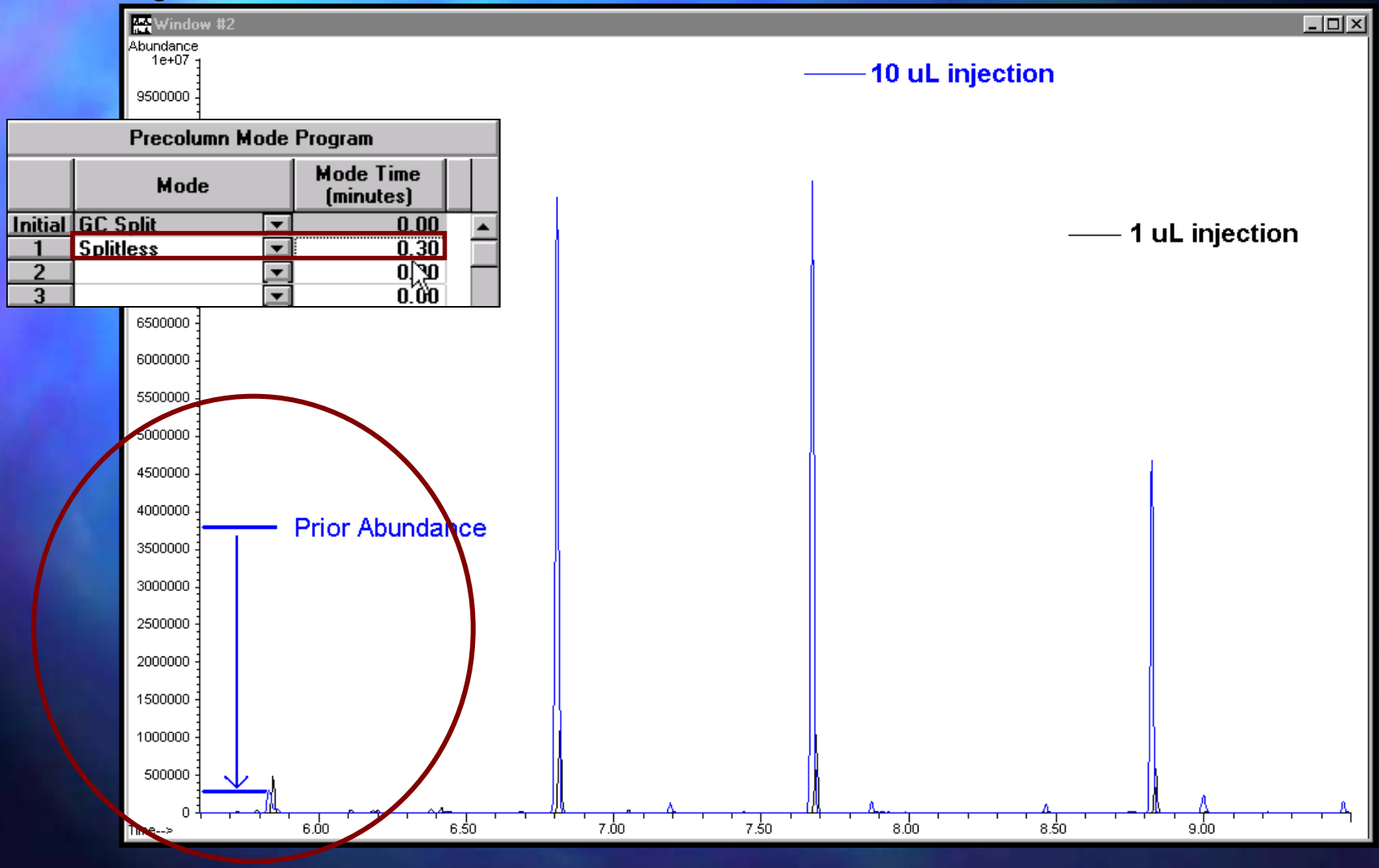
10 µl ProSep™ LVI

Figure 4



Reducing *just* the first component

Figure 5



LVI Experiments: *continued*

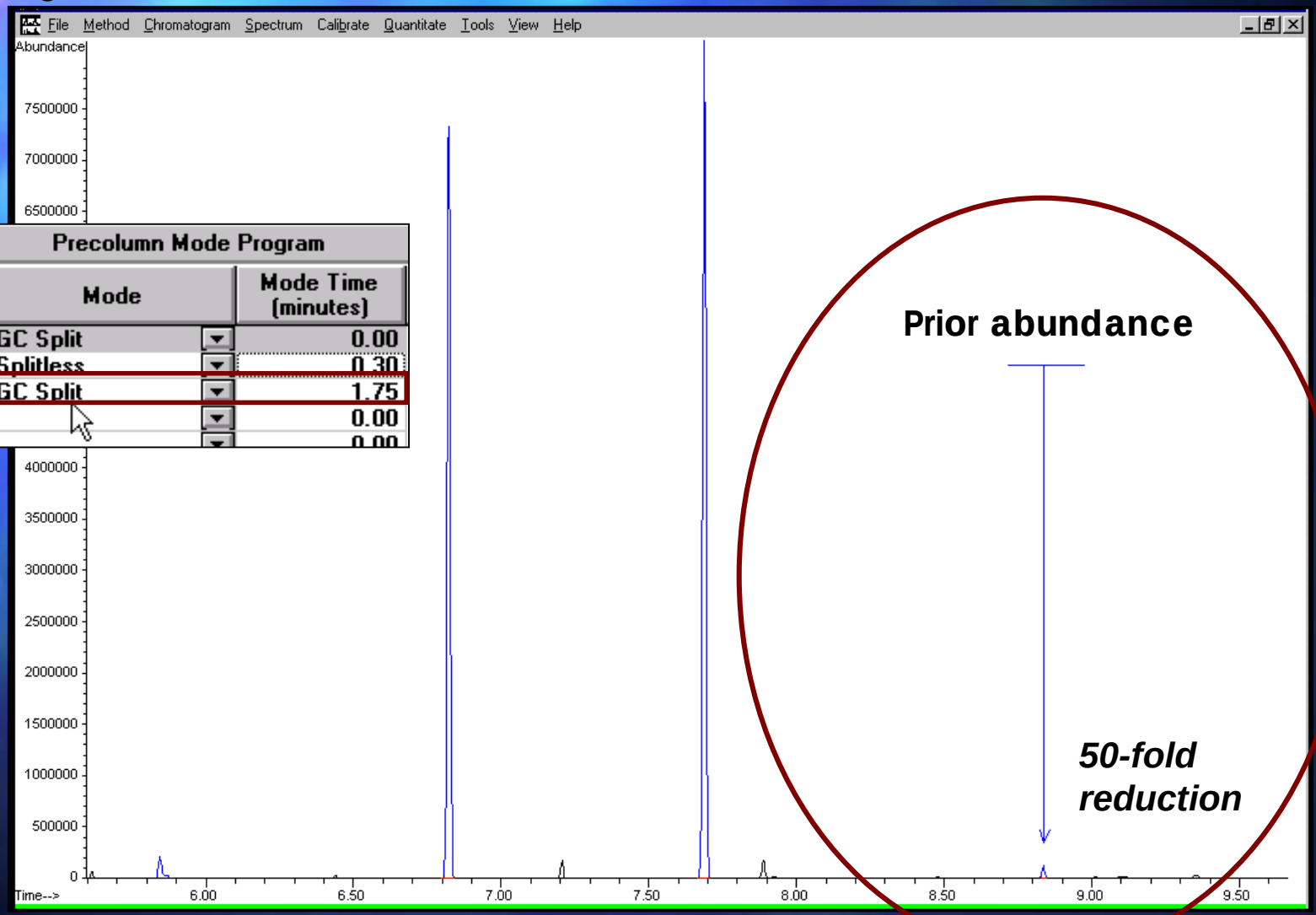
Imagine the last eluting peak, methyl palmitate (peak #4), to be a high boiling, matrix-related contaminant that can adversely effect detector performance or degrade the column.

By returning to split mode later in the precolumn program, ProSep™ can reduce introduction of this component. Figure 6 shows a reduction in this component by approximately 50 fold; on the order of the GC split ratio.

ProSep™ is also capable of applying additional purge gas to the pre-separation column in a mode known as "*ProSep™ Split*". Essentially this implements very high the split ratios, e.g., ratios >1:250, and very high reductions of matrix contaminants are possible. Applying "*ProSep™ Split*" mode to the methyl palmitate reduces its response below the threshold, Fig. 7. Combining this mode with a high Pre-separation Column Module temperature flushes matrix contaminants from the precolumn and prevents matrix accumulation during LVI.

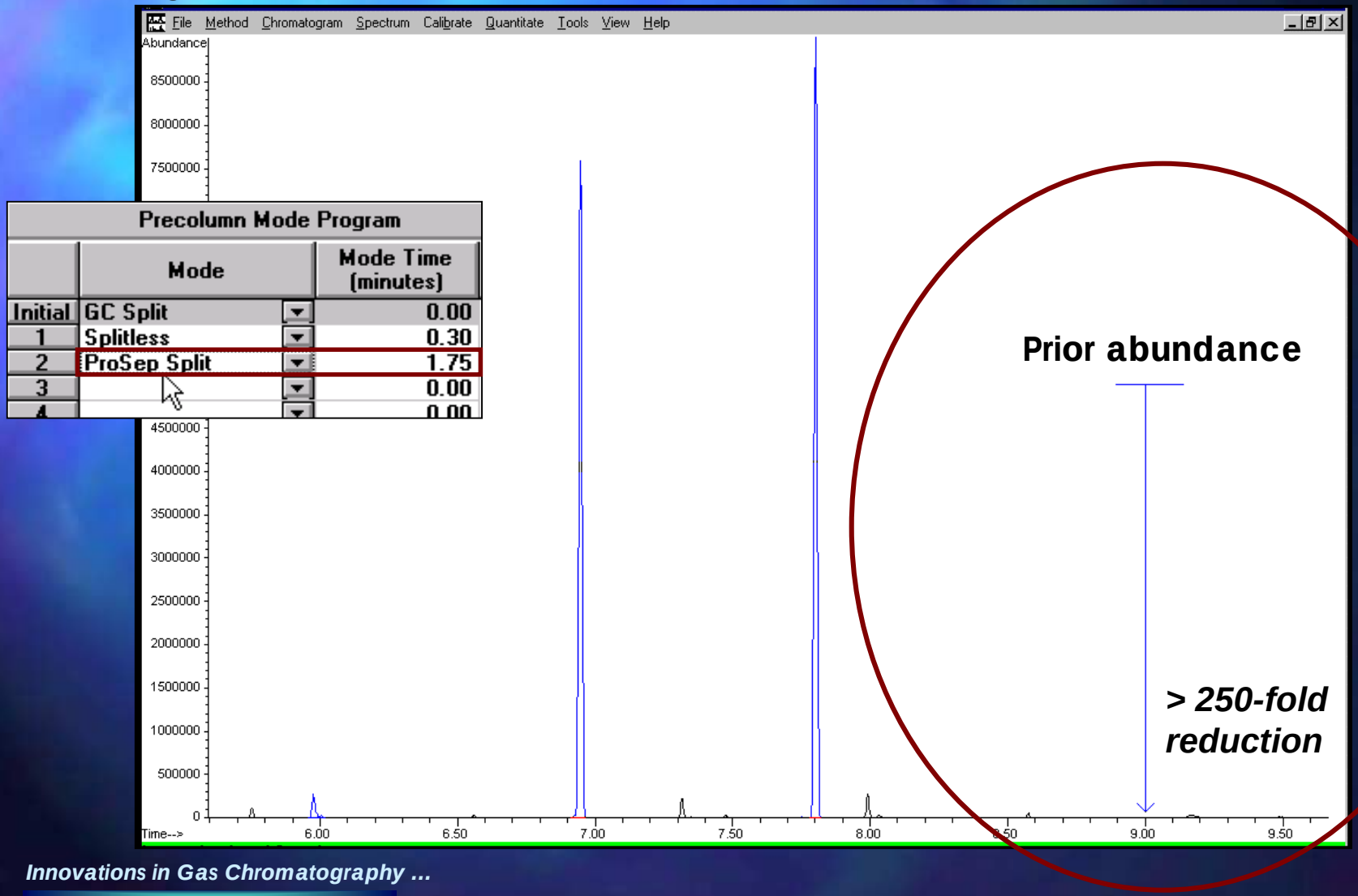
Attenuating *just* the first and last peak

Figure 6

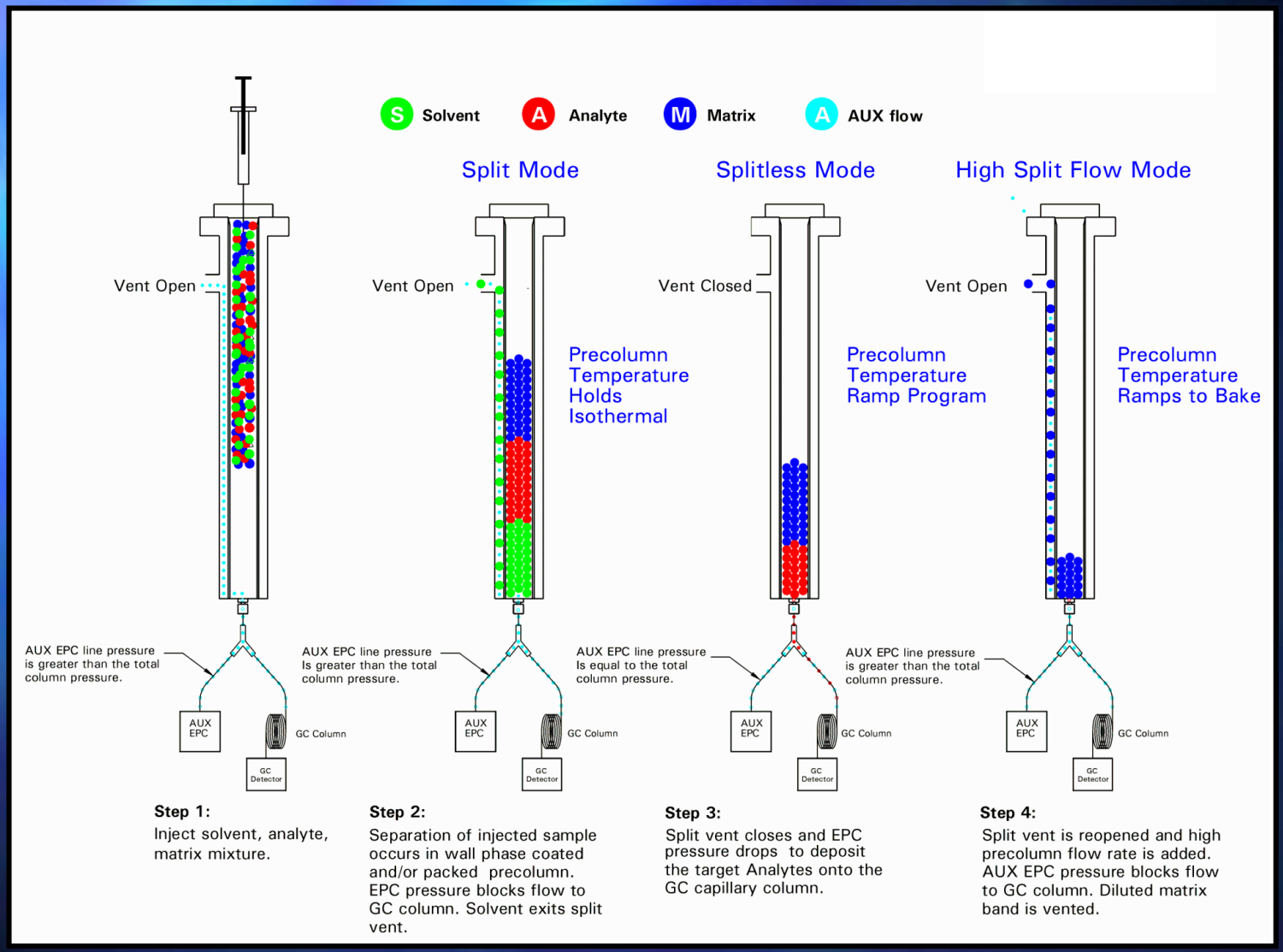


"Eliminating" the last peak: "ProSep™ Split" Mode

Figure 7

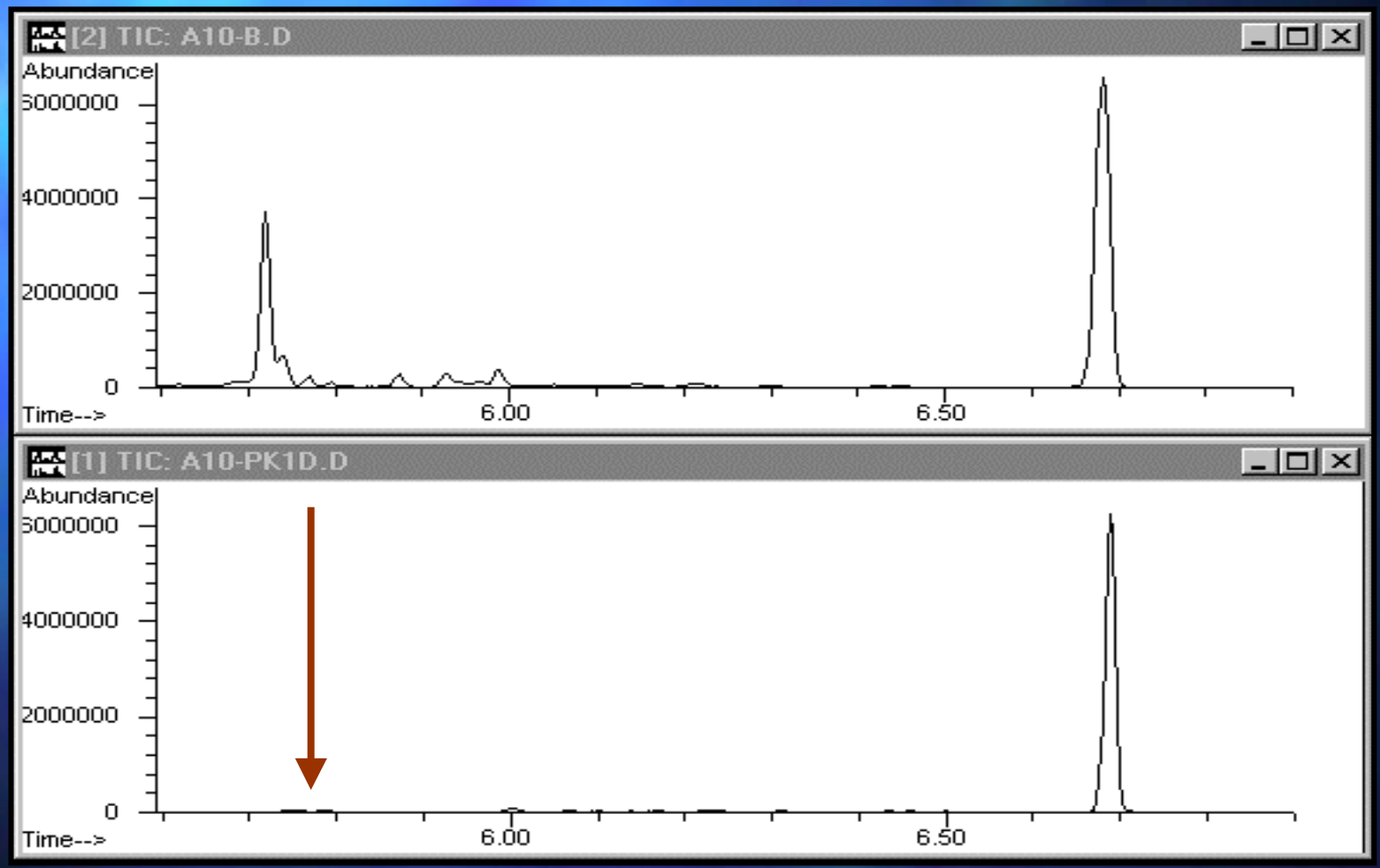


Pressure Switch Configuration



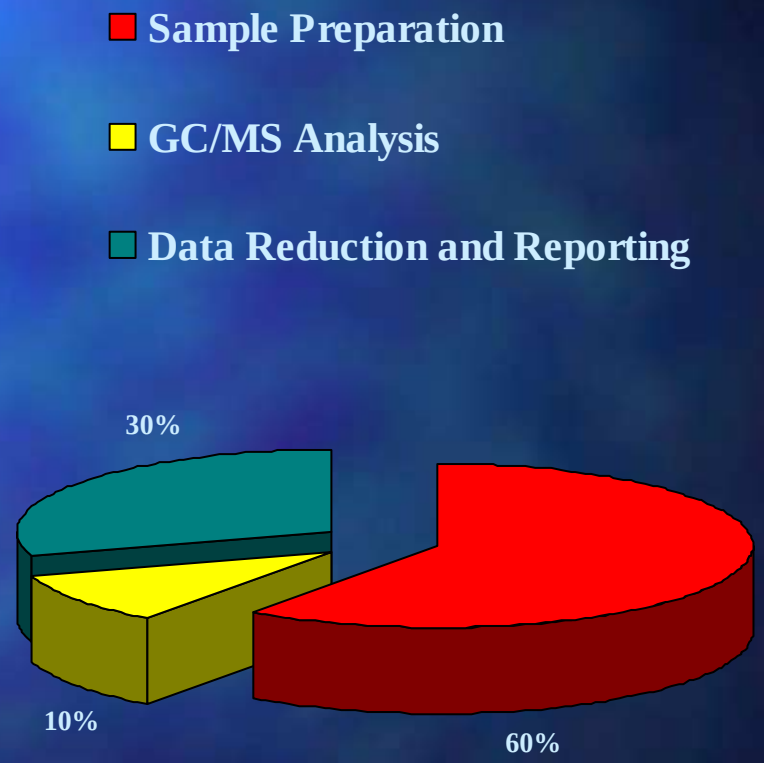
Pressure Switch Configuration:

Total Elimination of dodecane



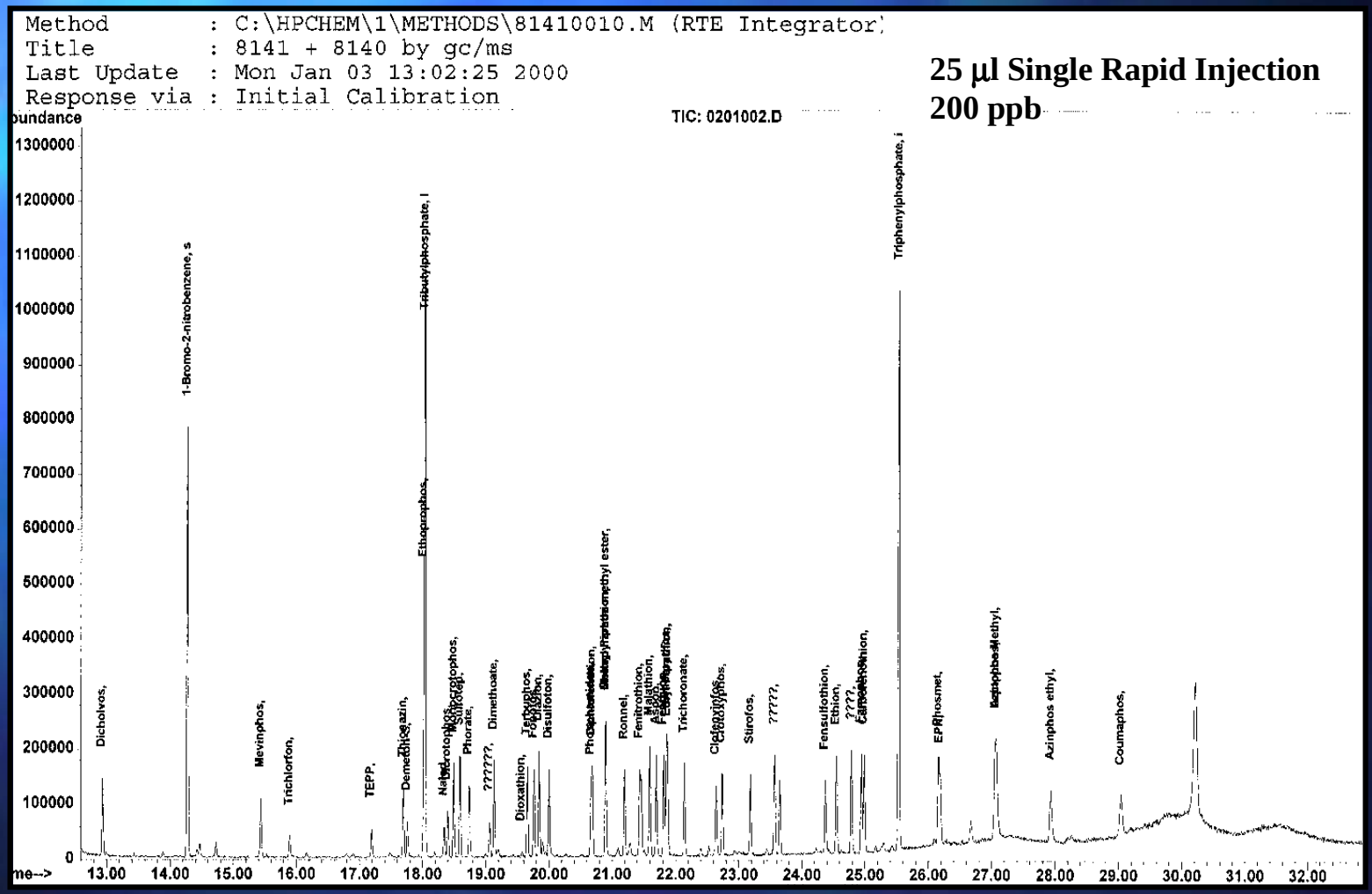
Cost and Time Distribution in Sample Analysis

- Solvent Types
- Solvent Acquisition
- Solvent Extraction
- Preparative Media
- Disposable Glassware
- Solvent Disposal
- Hourly Cost of Laboratory Operation



Combined EPA Methods 8140 and 8141 Pesticides and Herbicides by ProSep™ GC/MSD

Normally run by GC/NPD, this configuration combines two methods into a single run and provides GC/MS spectral confirmation at low levels.



Innovations in Gas Chromatography ...

Combined EPA Methods 8140 and 8141 Pesticides and Herbicides by ProSep™ GC/MSD

Initial Calibration Curve – 200 ppb to 2 ppm.

Response Factor Report GC/MS Ins

Method : C:\HPCHEM\1\METHODS\81410010.M (RTE Integrator)
Title : 8141 + 8140 by gc/ms
Last Update : Mon Jan 03 13:02:25 2000
Response via : Initial Calibration

Calibration Files
0.6 =0301003.D 0.2 =0201002.D 1 =0401004.D
1.4 =0501005.D 2 =0601006.D

		Compound	0.6	0.2	1	1.4	2	Avg	%RSD
1)	I	Tributylphosphate	ISTD						
2)		Dicholvos	0.530	0.545	0.499	0.507	0.490	0.514	4.41
3)	s	1-Bromo-2-nitrobenzen	0.499	0.574	0.449	0.445	0.394	0.472	14.38
4)		Mevinphos	0.415	0.407	0.400	0.372	0.372	0.393	5.18
5)		Trichlorfon	0.061	0.056	0.063	0.065	0.069	0.063	7.76
6)		TEPP	0.093	0.075	0.099	0.101	0.109	0.095	13.37
7)		Thionazin	0.120	0.121	0.119	0.119	0.124	0.120	1.66
8)		Demeton-S	0.139	0.135	0.146	0.151	0.157	0.145	6.25
9)		Ethoprophos	0.137	0.142	0.142	0.145	0.147	0.142	2.48
10)		Naled	0.186	0.173	0.190	0.197	0.205	0.190	6.29
11)		Dicrotophos	0.322	0.306	0.340	0.342	0.344	0.331	4.90
12)		Monocrotophos	0.595	0.557	0.610	0.610	0.628	0.600	4.44
13)		Sulfotep	0.117	0.112	0.115	0.116	0.121	0.116	2.70
14)		Phorate	0.341	0.348	0.360	0.358	0.370	0.355	3.20
15)		??????	0.154	0.143	0.164	0.165	0.172	0.160	7.15
16)		Dimethoate	0.352	0.352	0.357	0.368	0.370	0.360	2.42
17)		Dioxathion	0.021	0.024	0.022	0.022	0.026	0.023	8.79
18)		Terbuphos	0.194	0.193	0.203	0.209	0.215	0.203	4.59
19)		Fonofos	0.449	0.440	0.469	0.471	0.496	0.465	4.69
20)		Diazion	0.158	0.165	0.164	0.169	0.171	0.165	3.02
21)		Disulfoton	0.348	0.339	0.370	0.374	0.398	0.366	6.32
22)		Phosphamidon	0.207	0.197	0.222	0.219	0.225	0.214	5.53
23)		Dichlofenthion	0.155	0.155	0.159	0.164	0.170	0.161	4.11
24)		Methyl Parathion	0.271	0.239	0.297	0.312	0.320	0.288	11.42
25)		Chlorpyriphos methyl	0.198	0.192	0.210	0.211	0.216	0.206	4.83
26)		Ronnel	0.249	0.252	0.262	0.268	0.276	0.261	4.19
27)		Fenitrothion	0.198	0.176	0.209	0.215	0.217	0.203	8.30
28)		Malathion	0.242	0.234	0.242	0.244	0.252	0.243	2.63
29)		Aspon	0.301	0.301	0.311	0.310	0.315	0.307	2.06
30)		Fenthion	0.305	0.302	0.314	0.312	0.321	0.311	2.47
31)		Chlorpyrifos	0.053	0.058	0.056	0.057	0.056	0.056	3.99
32)		Ethyl Parathion	0.096	0.081	0.107	0.109	0.112	0.101	12.38
33)		Trichoronate	0.116	0.120	0.120	0.124	0.125	0.121	3.00
34)	i	Triphenylphosphate	ISTD						
35)		Clofenvinfos	0.631	0.601	0.653	0.658	0.678	0.644	4.56
36)		Crotoxyphos	1.693	1.665	1.829	1.830	1.948	1.793	6.42
37)		Stirofos	0.653	0.662	0.677	0.687	0.718	0.680	3.73
38)		?????	0.449	0.484	0.483	0.477	0.515	0.482	4.89
39)		Fensulfothion	0.559	0.407	0.591	0.601	0.655	0.563	16.65
40)		Ethion	1.186	1.216	1.243	1.254	1.287	1.237	3.10
41)		????	0.805	0.854	0.853	0.834	0.892	0.848	3.74
42)		Famophos	2.129	2.213	2.212	2.200	2.294	2.209	2.65

(#) = Out of Range
81410010.M Thu Feb 03 15:45:16 2000 Page 1

Response Factor Report GC/MS Ins

Method : C:\HPCHEM\1\METHODS\81410010.M (RTE Integrator)
Title : 8141 + 8140 by gc/ms
Last Update : Mon Jan 03 13:02:25 2000
Response via : Initial Calibration

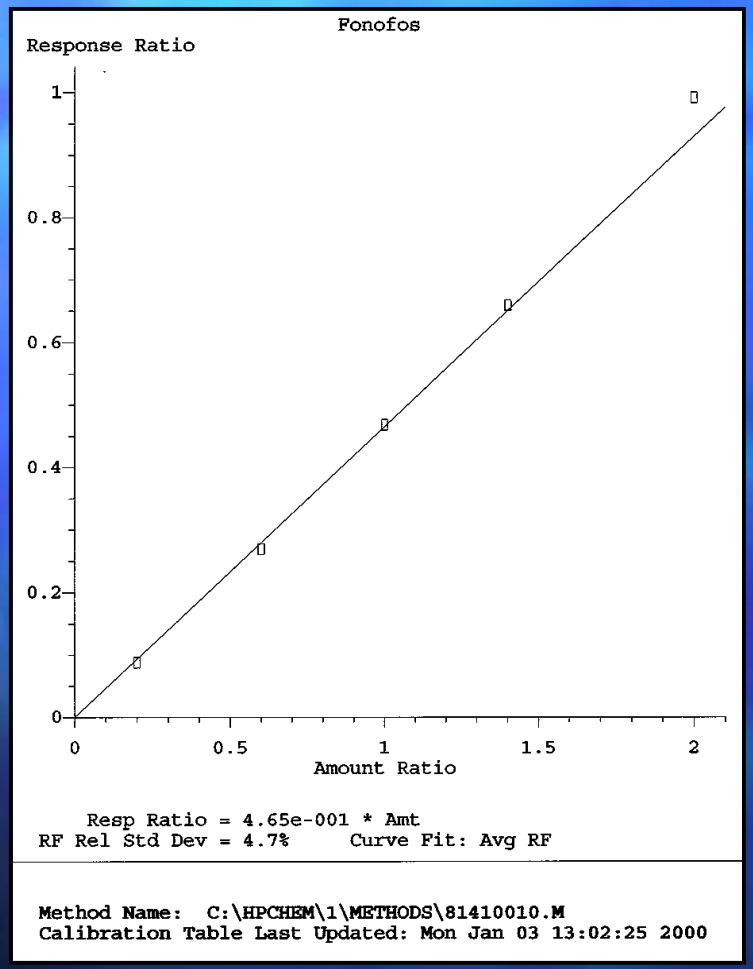
Calibration Files
0.6 =0301003.D 0.2 =0201002.D 1 =0401004.D
1.4 =0501005.D 2 =0601006.D

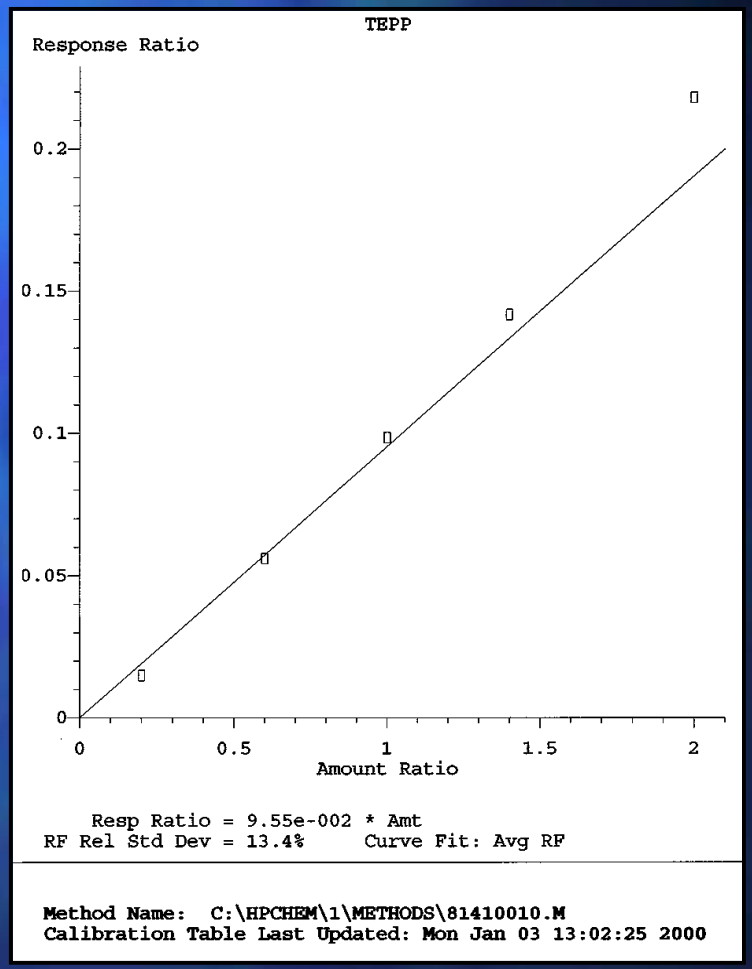
	Compound	0.6	0.2	1	1.4	2	Avg	%RSD
43)	Carbofenothion	1.062	1.062	1.117	1.093	1.149	1.097	3.41
44)	Phosmet	3.924	3.914	4.118	4.093	4.309	4.072	3.99
45)	EPN	1.508	1.231	1.663	1.698	1.884	1.597	15.30
46)	Leptophos	1.146	1.407	1.134	1.133	1.158	1.196	9.91
47)	Azinphos Methyl	1.312	1.455	1.358	1.363	1.448	1.387	4.45
48)	Azinphos ethyl	1.159	1.192	1.222	1.234	1.295	1.220	4.15
49)	Coumaphos	0.377	0.399	0.390	0.387	0.403	0.391	2.68

(#) = Out of Range
81410010.M Thu Feb 03 15:45:21 2000 Page 2

Combined EPA Methods 8140 and 8141 Pesticides and Herbicides by ProSep™ GC/MSD

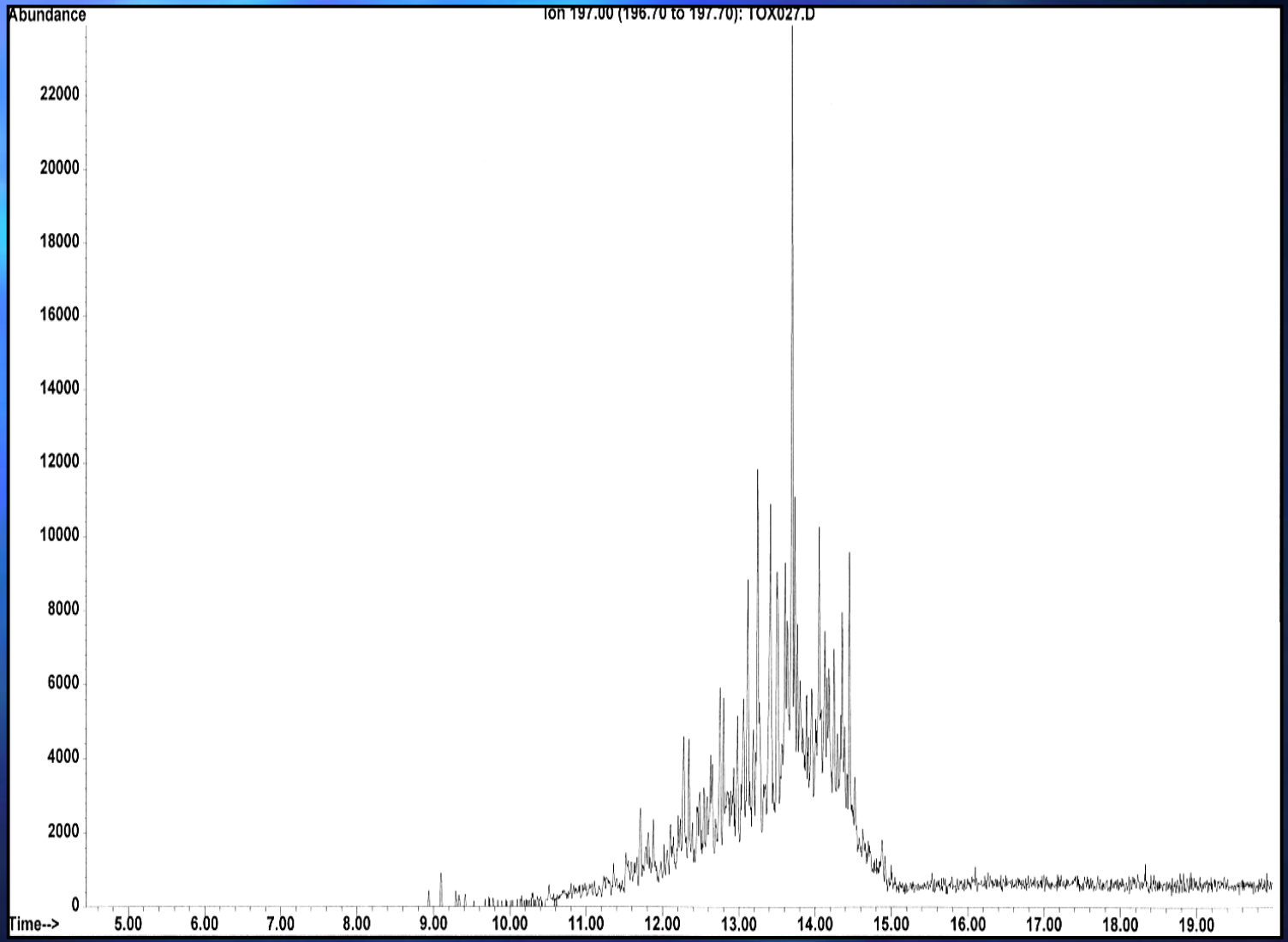
Initial Calibration Curves for Fonofos and TEPP - 200 ppb to 2





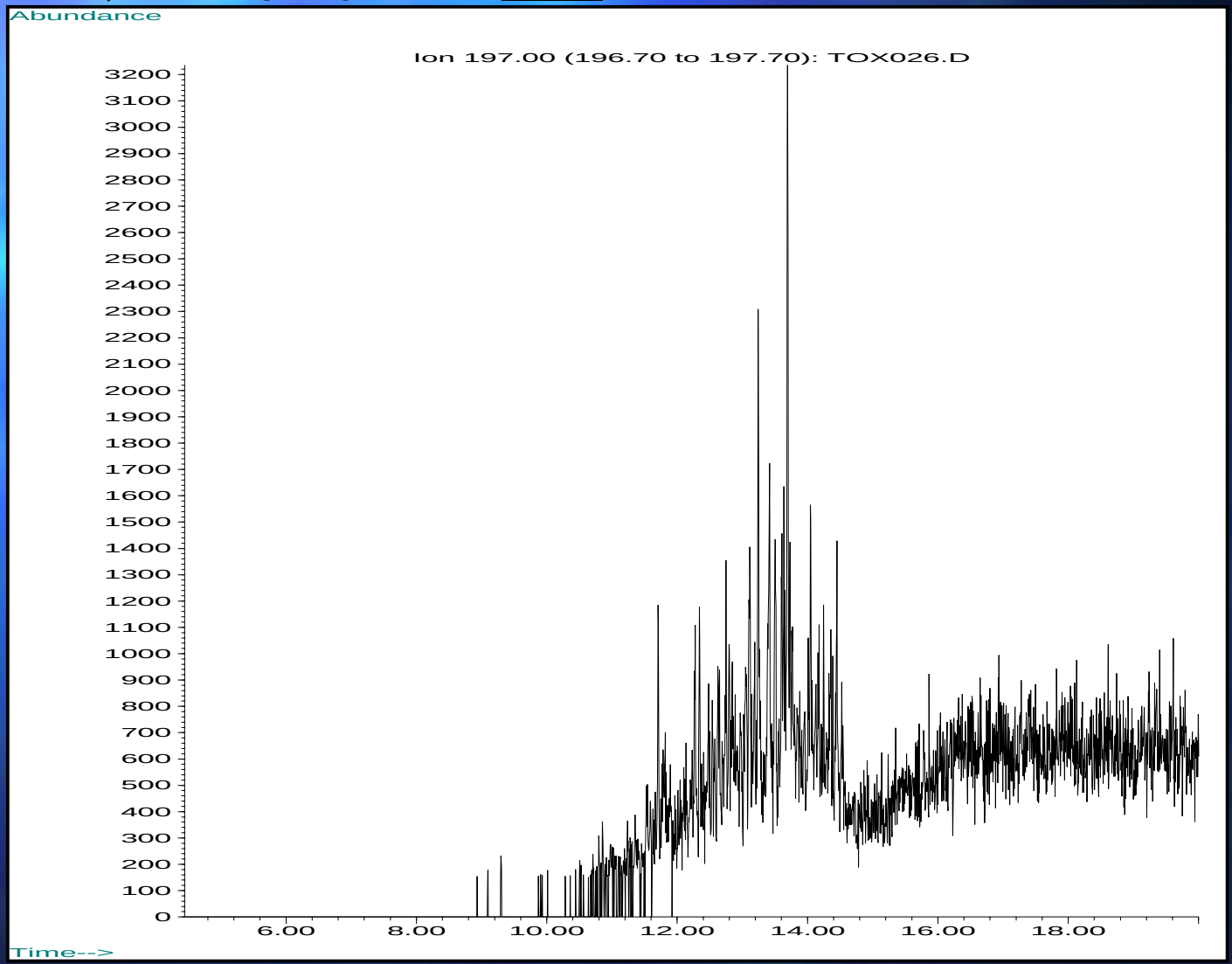
Low-Level Detection of Toxaphene by ProSep™ GC/MSD

100 µl Fast Single Injection at 5 ppm.



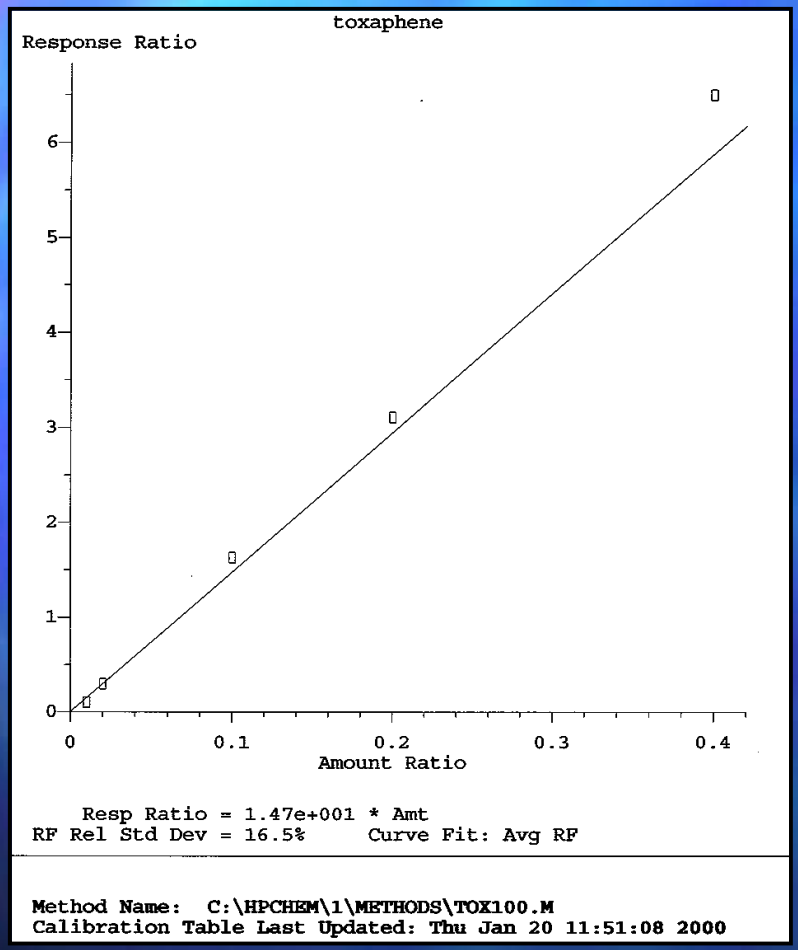
Low-Level Detection of Toxaphene by ProSep™ GC/MSD

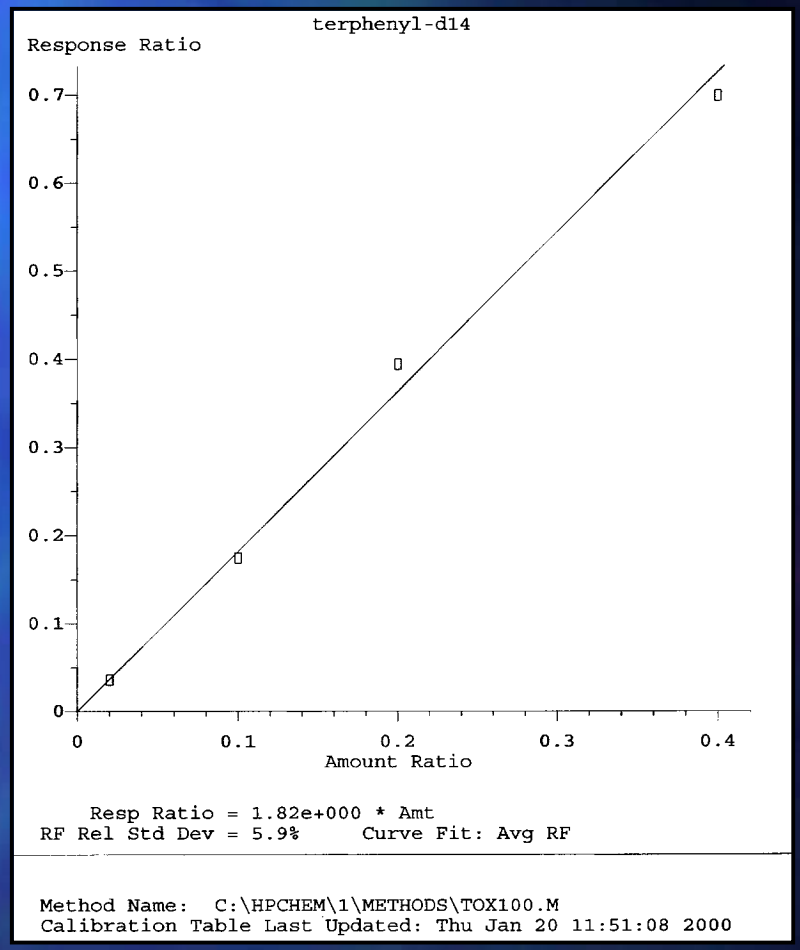
100 µl Fast Single Injection at 1 ppm.



Low-Level Detection of Toxaphene by ProSep™ GC/MSD

Initial Calibration Curves for toxaphene and terphenyl – d_{14}.





N-nitrosodimethylamine

PROBLEM:

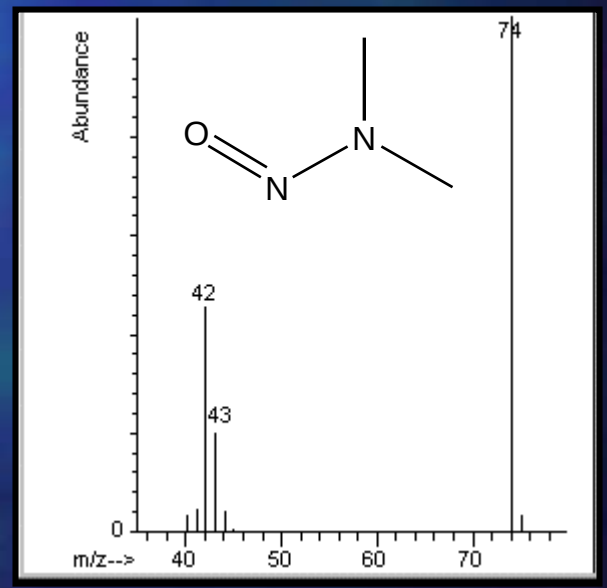
NDMA is a suspected human carcinogen and teratogen.

Based on risk assessments NDMA, has been regulated in drinking water sources at the 0.7 ng/l (ppt) level by the USEPA and at 2 ng/l (ppt) by California Dept. of Health Services.

Existing methods have *quantitation* limits in the 7- 100 ng/l range and *detection* limits at best 1-3 ng/l; higher than the regulated limits.

Losses increase with sample evaporation using either SPE or Liquid/Liquid extraction.

The electron impact ionization spectrum for NDMA is not very favorable.



NDMA acquired using Positive Chemical Ionization with Selected Ion Monitoring using ProSep™ PSI

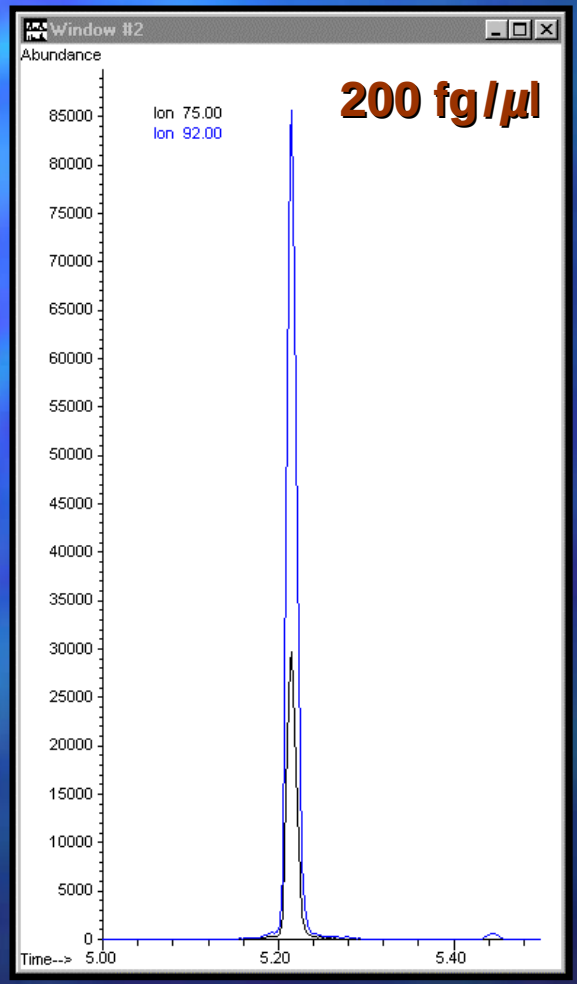
Using NH_3 as PCI reagent gas, NDMA produces $[NDMA+H]^+$ and $[NDMA+NH_4]^+$ ions corresponding to m/z = 75 and 92.

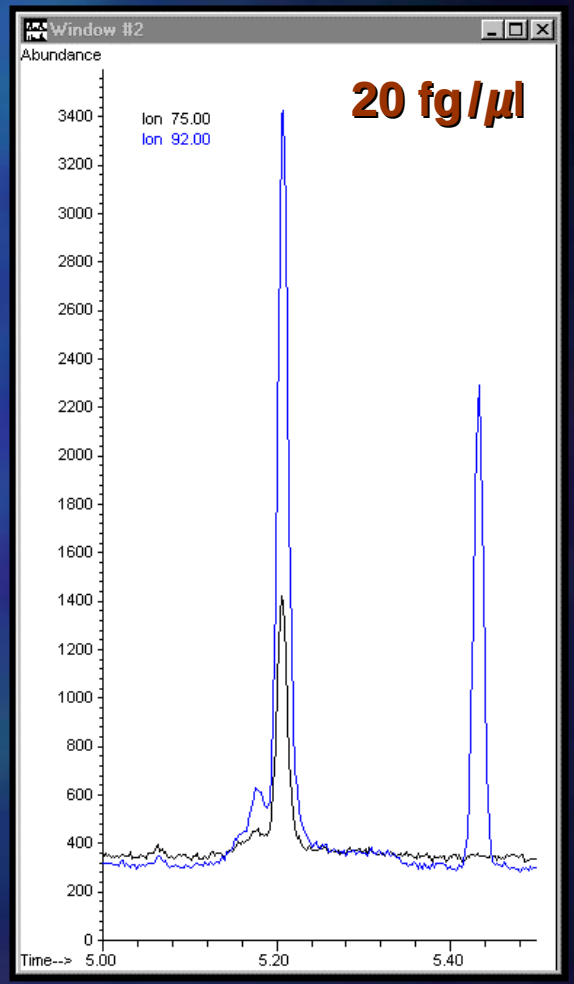
PCI-NH_3-SIM chromatograms of 50 ul injections of NDMA standards in CH_2Cl_2 .

Applying a concentration & recovery factor of 500…

200 fg/µl => 0.4ng/l (0.4 ppt)

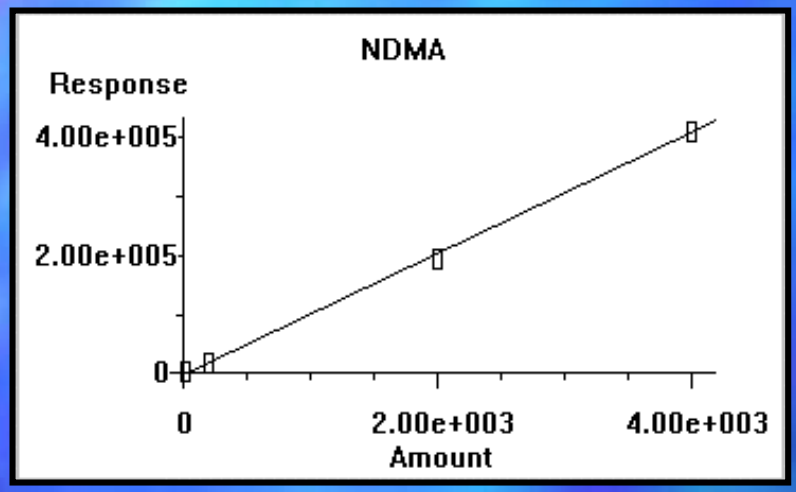
20 fg/µl => 0.04ng/l (40 ppq)





NDMA analysis

ProSep LVI and PCI linearity and reproducibility



NDMA quantitation curve from 20 fg/µl to 4000 fg/µl
r^2 =0.999

Reproducibility in 75/92 m/z ratio and response (n=5) for concentrations of NDMA from 20 to 4000 fg/µl

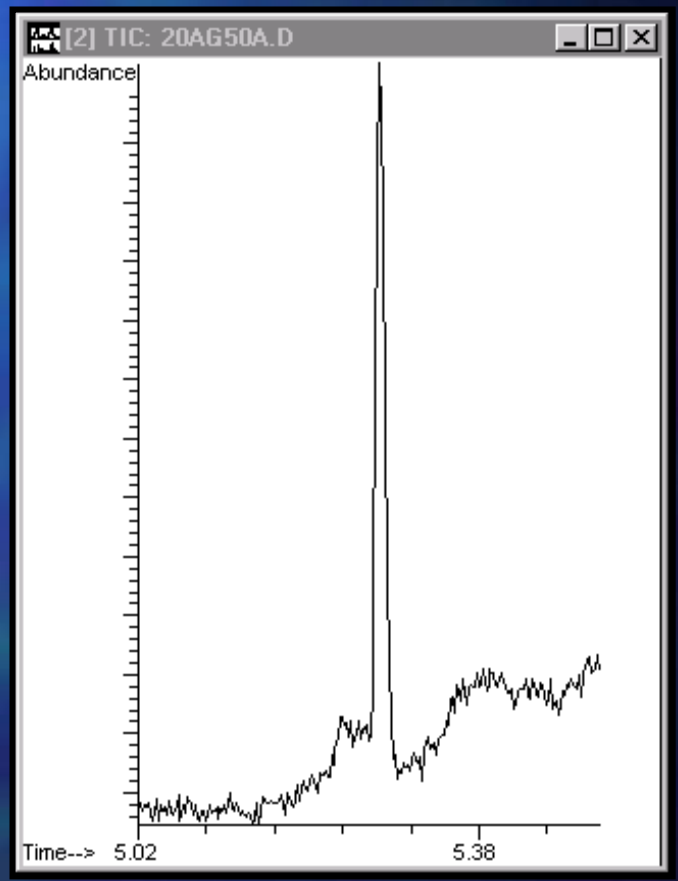
Concentration	RSD Ratio 75/92	RSD Response
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

ProSep LVI with ECNI/MS

- **Increased selectivity and sensitivity**

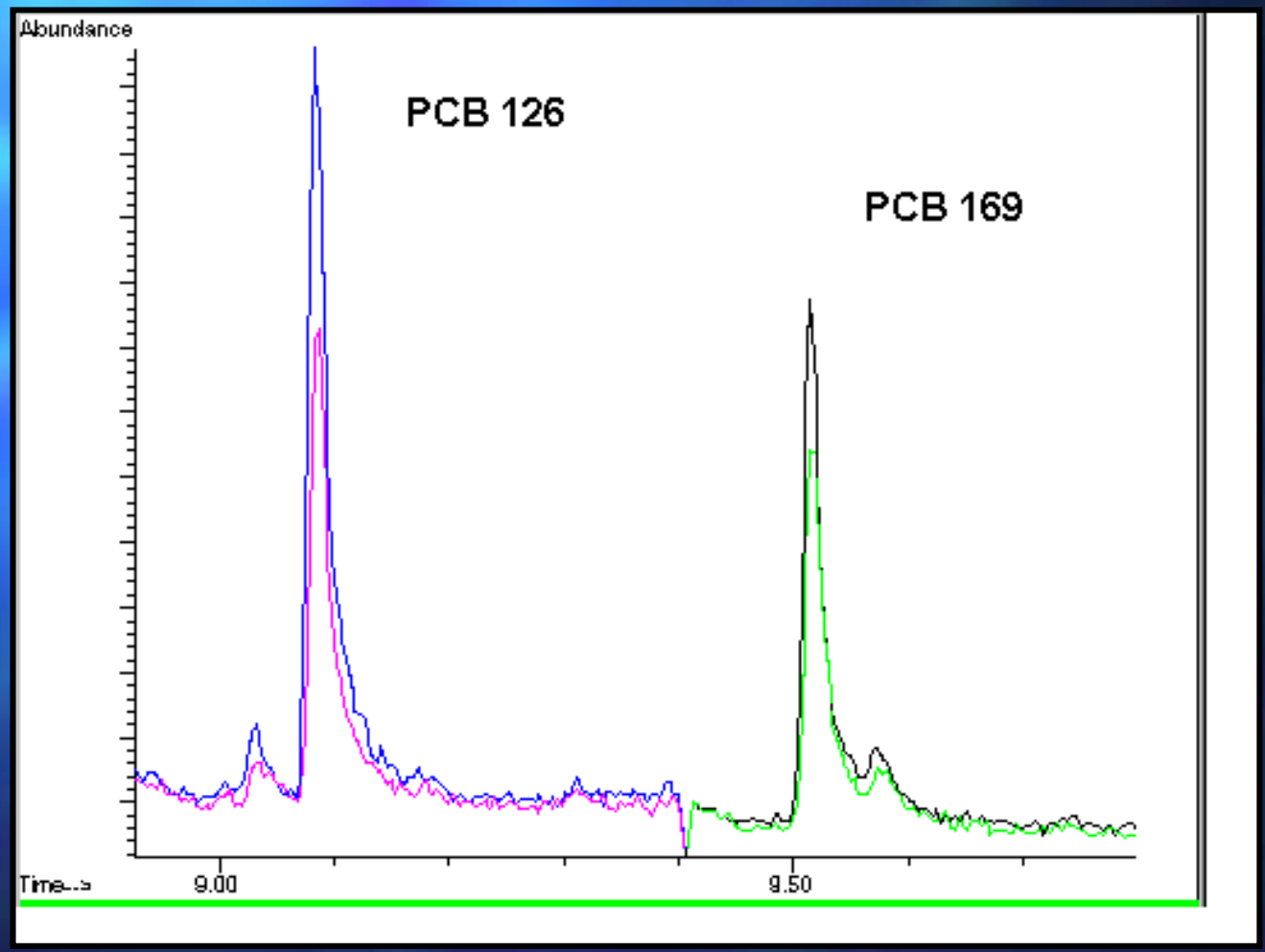
20 attograms/µl of Octafluoronaphthalene acquired using LVI with ECNI – MS on the Agilent 5973 MSD

(44,000 molecules/µl)!



ProSep LVI with ECNI/MS

1 fg/µl of PCB 126 & PCB 169 using ECNI/MS-SIM



Conclusions

- Behaves as a Chromatographic Zone Inlet System
- Performs separations in SVI or LVI modes
- Increased analytical integrity, lowered column and detector

  maintenance, etc…
- Work with authentic samples and a wide variety of matrices has shown the ProSep™ to be very reproducible and robust.
- Injection (1 to 125 ul) is rapid so sample throughput is high.

For additional information…

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