



Improving the Analysis of Trace Level of Active Compounds in High Matrix Samples

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The Challenge for GC(MS) - Our World is Changing



Increased concerns about safety of food supply and environment



Need for Lower Method Detection Limits from pg to fg



Focus on productivity lower costs and less skilled operators

Is leading to

Use of QuEChERS sample extraction methods with minimal cleanup

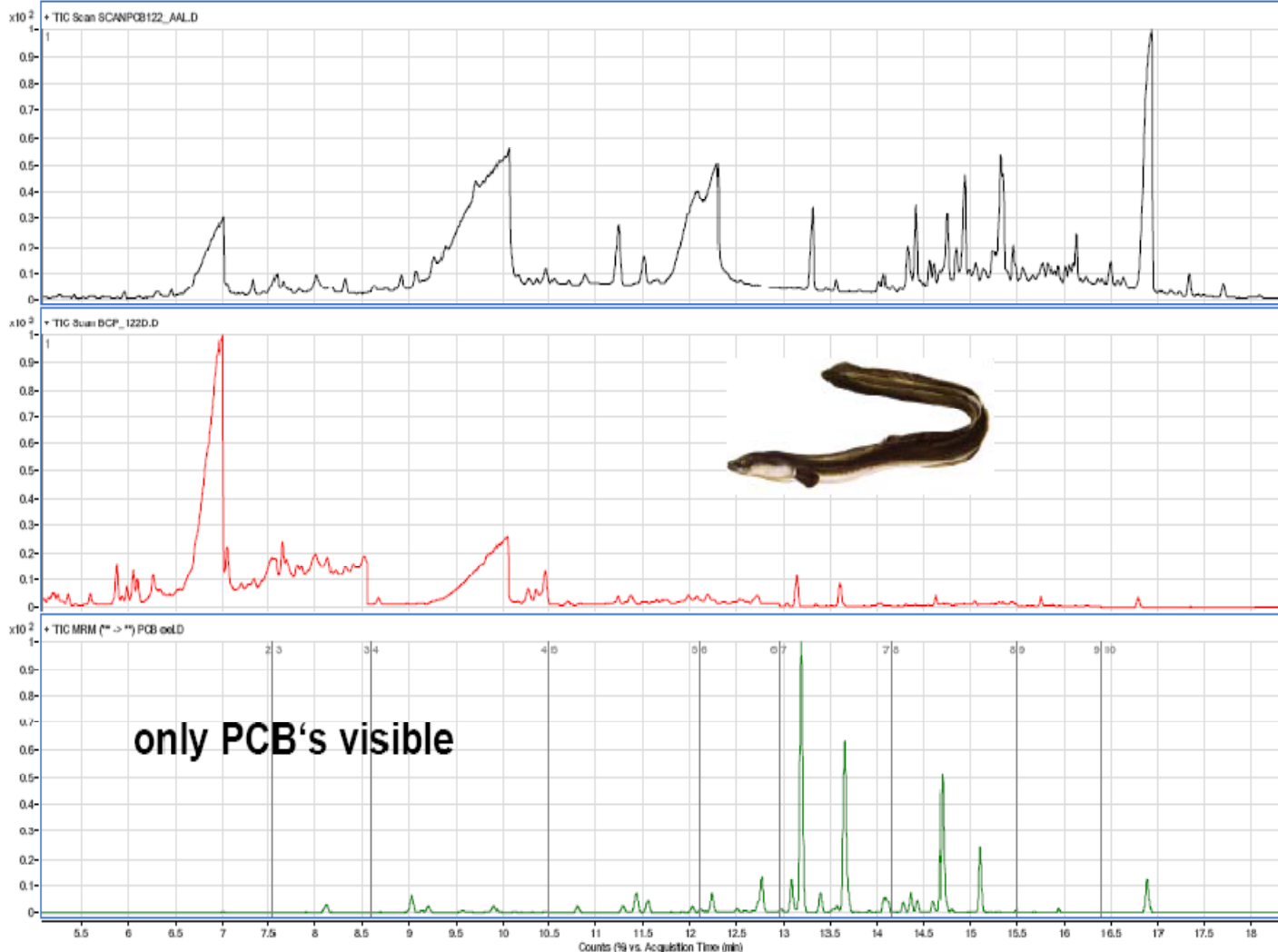
Use of high sensitivity, selectivity GC/MS/MS

Need for easy to use solutions which can accept high matrix samples

Requiring us to

Develop and promote new HW, SW and consumables which allow customers to maximize productivity when analyzing trace levels of toxic organic compounds in samples with high matrix like Food and Environmental

Selectivity of the GC/MS/MS for high matrix samples, PCB's in Fish Oil



SCAN Q

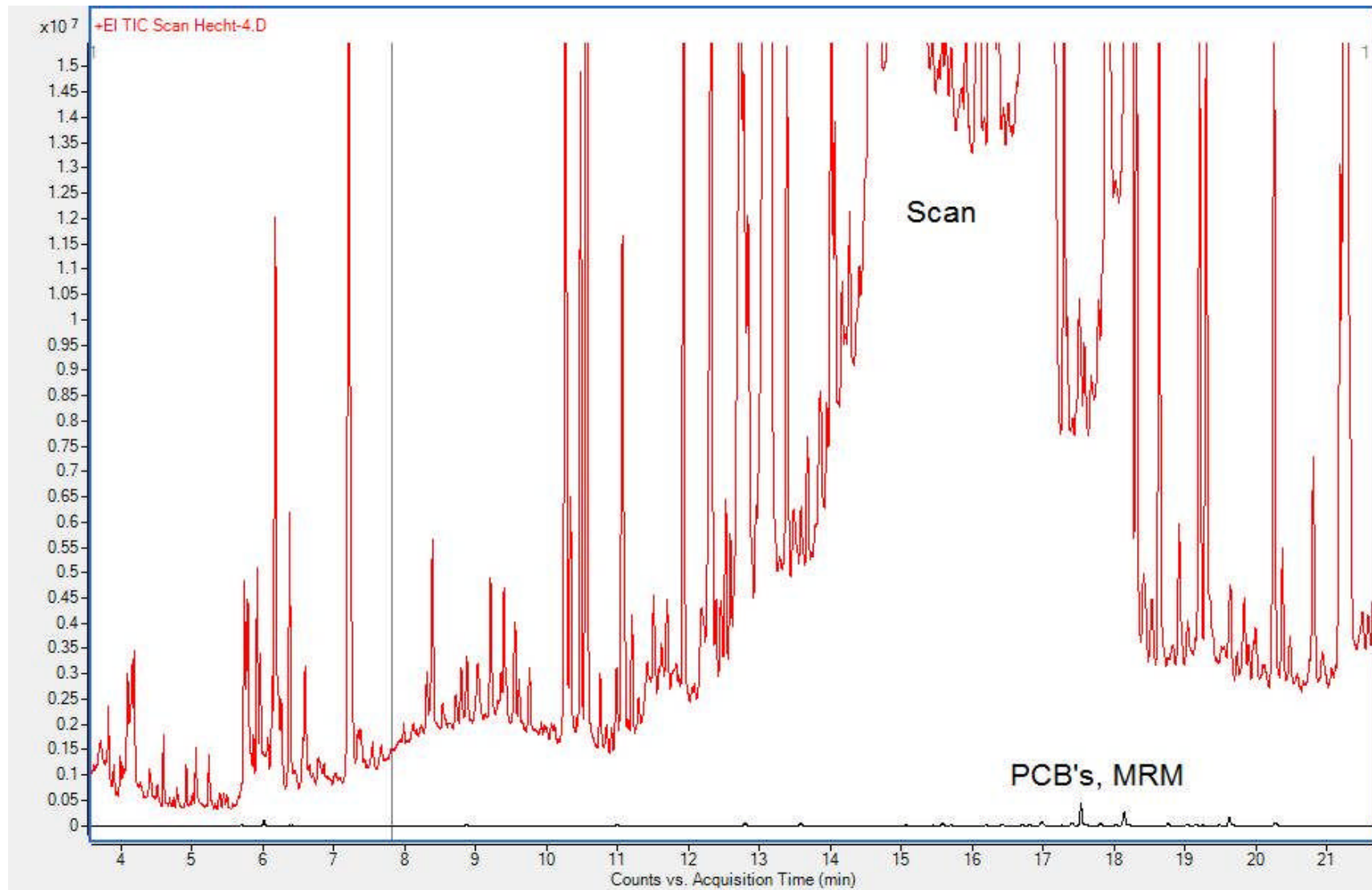
SIM Q

MRM QQQ

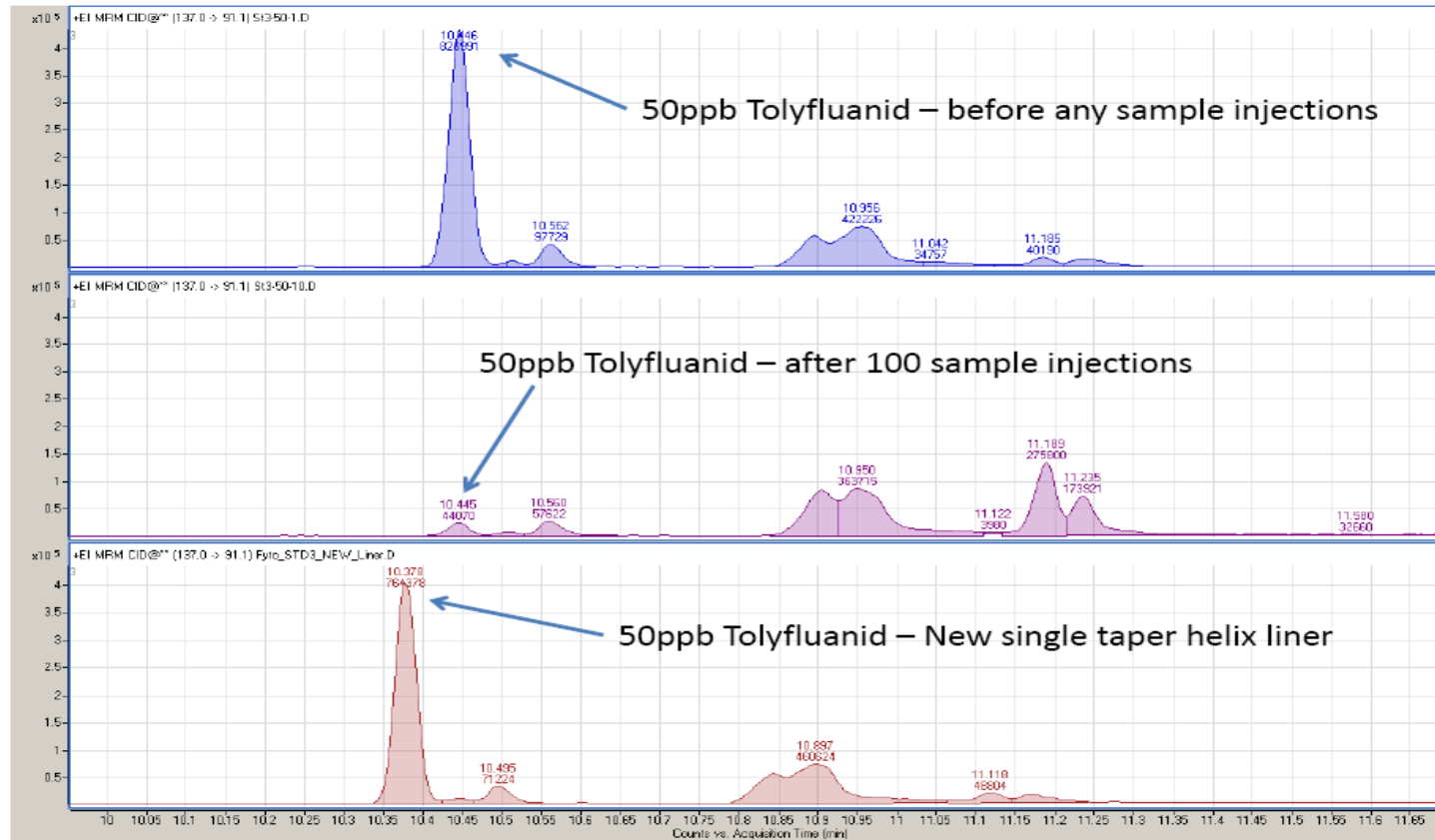
The problem with trace analysis of high matrix samples



Pike Extract, QQQ Scan and MRM Analysis



Loss of Response with Injection of High Matrix Samples



What is QuEChERS (pronounced “Catchers”)

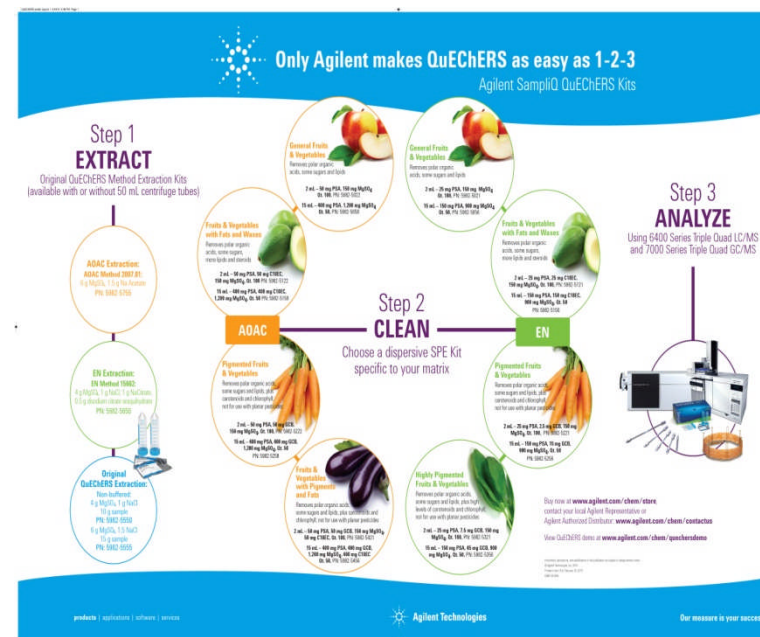
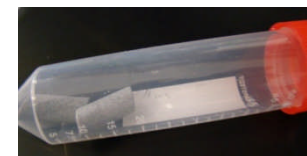
- Quick, Easy, Cheap, Effective, Robust and Safe
- Sample Preparation method developed jointly by US FDA and EU Food Regulatory Agencies
- Methodology for simplified extraction of a large number of pesticide residues in fruits and vegetables
- QuEChERS is a fast sample preparation method for screening for analytes of interest, not for concentrating samples
- No automated solution for QuEChERS exists in the market today
- QuEChERS is still in it's “Infancy”; being adopted worldwide

	Luke method or traditional SPE	QuEChERS	Bottom Line
Estimated Time to process 6 samples (min)	120	30	4x faster
Solvent Used (mL)	90 mL	10mL	9 x less solvent
Chlorinated Waste (mL)	30 mL	none	safer, greener, less costly
Glassware/ specialized equipment	Funnel, water bath, 200mL containers, evaporator, etc.	None	No additional supplies needed

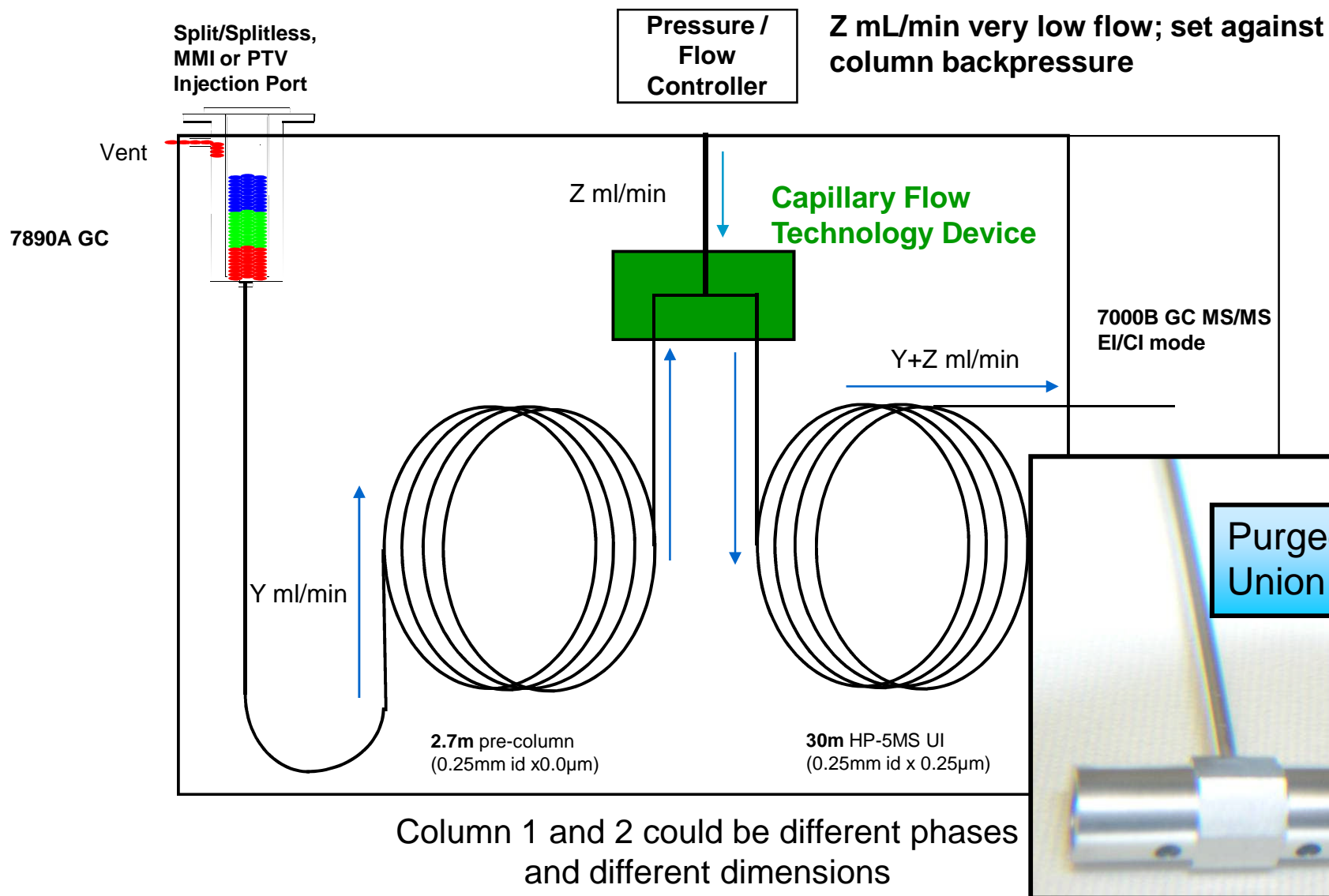
QuEChERS Brochure: literature #5990-3562EN

The Value of Agilent SampliQ QuEChERS Kits

- **Reduced time and labor: New Ceramic Homogenizers**
 - With addition of ceramic homognizers in tubes, shaking step is minimized to only 20 seconds
 - Saves you time, money and improves results
 - Proof of performance included in QuEChERS brochure (5990-3562EN)
- **Improved Recoveries: Individually packaged extraction salts**
 - Vacuum-sealed anhydrous foil packs ensure salt integrity
 - avoid exothermic reactions by adding salts *after* solvent
 - Exothermic reactions cause samples to degrade
 - No need for additional centrifuge tubes
- **Easy Product Selection: QuEChERS Kits Identified by method and matrix**
 - Select an extraction kit based on methodology (AOAC or EN)
 - Select a dispersive kit based on food type (meat, fruits, vegetables, etc)
 - Extraction packets available without tubes
- **Technical support:** Over 14 application notes on QuEChERS:
 - All available on our website: www.agilent.com/sampliQ
 - QuEChERS Food Application “Notebooks”: Lit number: 5990-4977EN
 - Customer Seminars and hands-on training available worldwide
 - Contact Joni Stevens or Melanie Rothermich for information



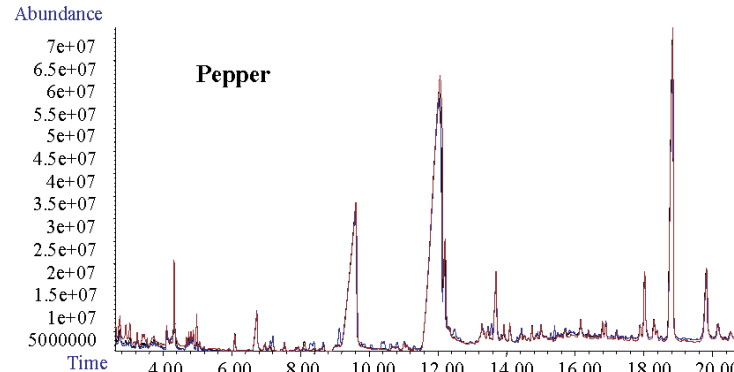
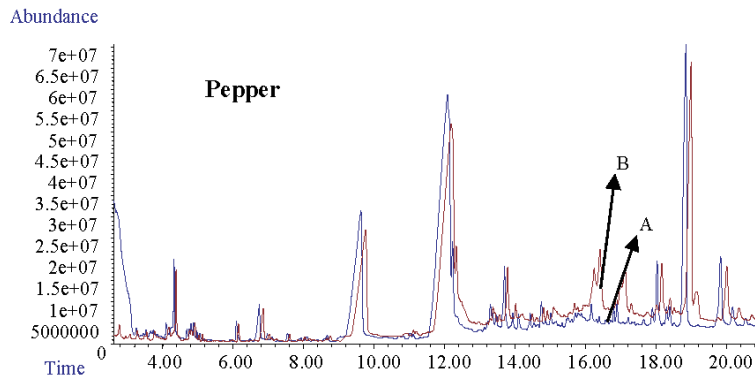
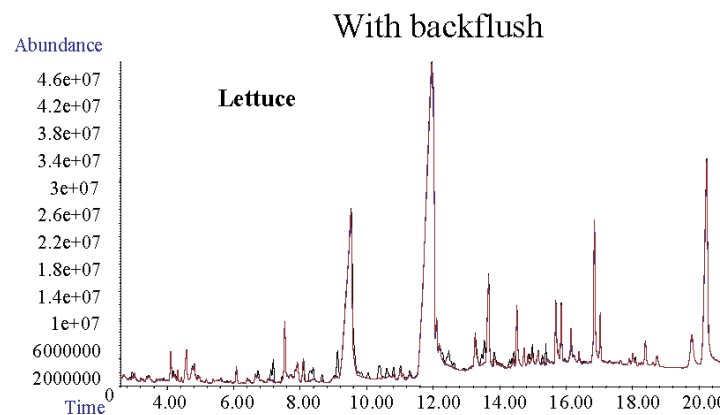
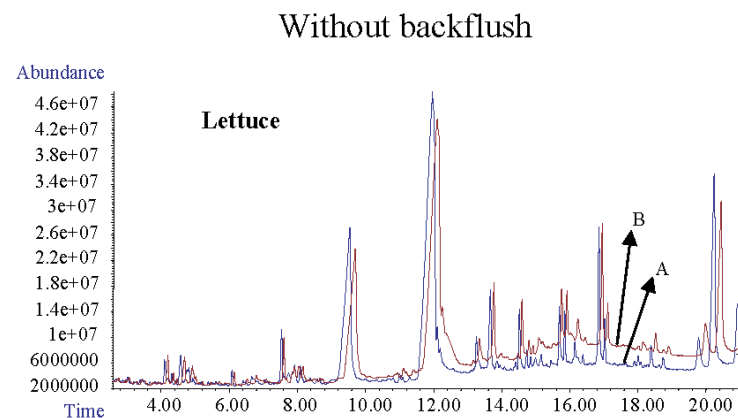
Agilent Capillary Flow Technology - Backflush



If Backflush is So Beneficial Why Aren't More Customers Using It?

- CFT devices are “delivered” but not “applied”, so customers often do not use them
- Even though backflushing could benefit many customers in many ways, adding backflush to a current method is not straightforward and easy for most users
- Backflush of Pre-column and/or Mid-column Backflush currently requires training and expertise
- Competitors (especially Shimadzu and Perkin-Elmer) are starting to copy the gas-phase microfluidics message

Backflush Minimizes Carryover, Matrix Interference and RT Shifts and Decreases Maintenance



Extracts of lettuce and pepper injected 3 times with a 2 min backflush and 3 times without backflush. The first and third injections are shown in each case. Without backflushing, the third injection (B) shows carryover, and retention time shifts compared to the first injection (A).

MEZCUA ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 92, NO. 6, 2009

Why Should You Implement Capillary Column Backflushing?

- More samples/day/instrument
- It's "green" + has lower operating costs (gases + power)
- Less frequent and faster GC & MSD maintenance
- Longer column life
- Better data quality
 - less chemical background (better detection limits)
 - cleaner mass spectra (better ion ratios)
 - better peak integration (less intervention)
- More rugged multidimensional methods
 - more stable peak retention times
 - less background



Coming Soon (End 2010) - New Backflush Software and Utilities will be Added to the 7890A GC Driver

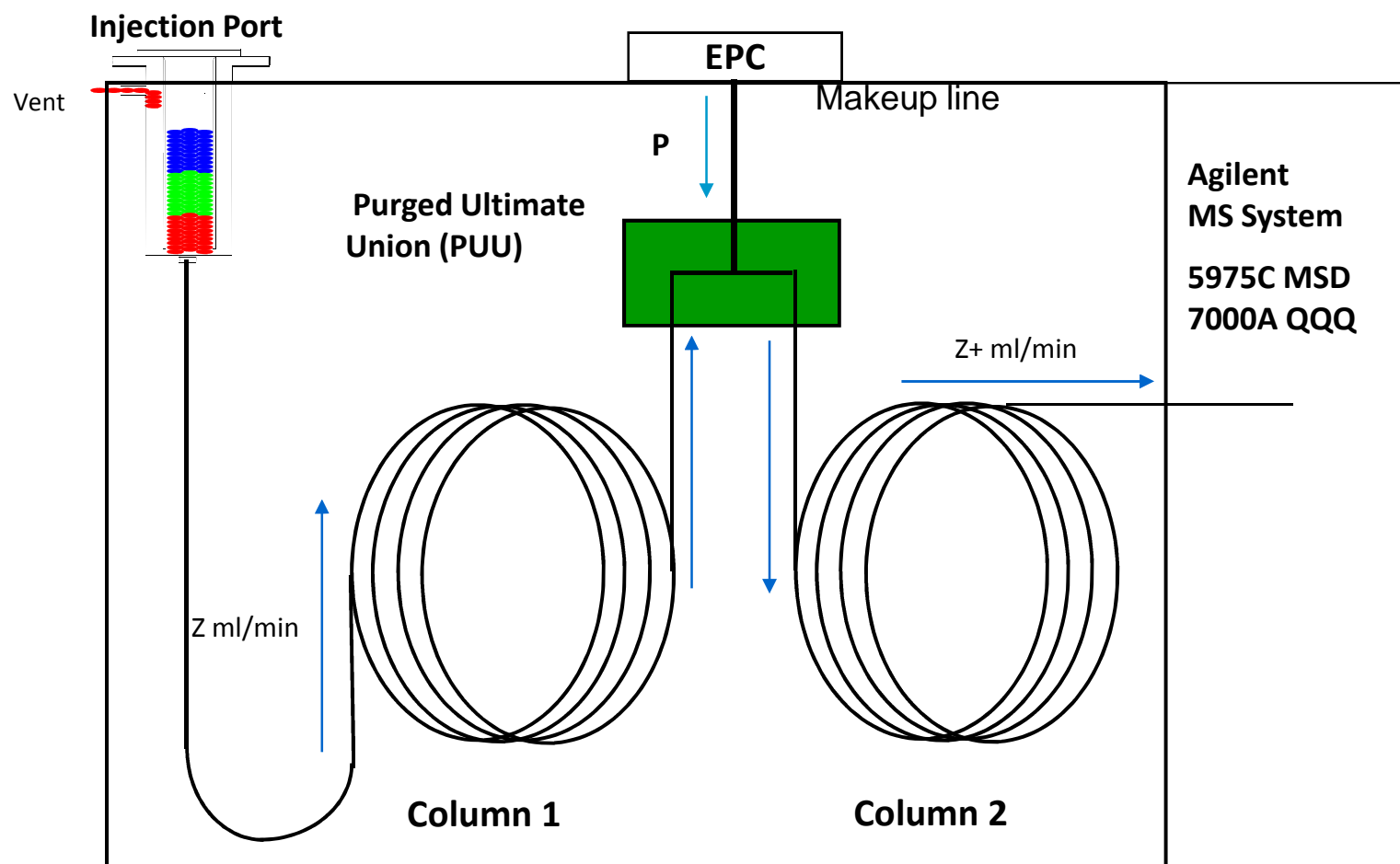
- Facilitate addition of backflush to a current method
- Help selecting which of three backflush modes to use
- Guidance during installation of backflush hardware
- Automation of critical system update and health checking procedures
- Easy to use tool to assist in selection and implementation of backflush
 - Graphical UI, customer picks last peak of interest, tool recommends appropriate backflush timing and conditions
- Context relevant Help and supporting documentation (theory, application notes, definitions, troubleshooting, etc.)

Three Configurations and Two Modes of BF

1. Post-Column
Post-run backflush
2. Uncoated Pre-Column
Concurrent backflush
3. Coated Pre-Column (split column)
Post-run
Concurrent



New GC/MS Backflushing Configurations



Agilent 7890A GC

Vocabulary: Backflush Modes Post Run & Concurrent

- Post Run Backflush:
 - ≡ begins after the last analyte has eluted and the MS is OFF !

- Concurrent Backflush
 - ≡ begins before the last analyte has eluted and the MS is ON !

Easy to Use - User Interface

Convert to Backflush - My Third Favorite GC Method

Backflush Time | Backflush Conditions | Method Overview | Setpoints Check | Trial Run | Blank Run

Specify which compounds should be backflushed

Chromatographic Attributes data: My Favorite Method CA Run1

Data File Location: C:\Chem32\1\data\NEW_CONFIG.D\newdata.csv

Both .asc and .csv files are accepted. Mass spec data must be a TIC exported to .csv

Backflush start time: 8.4 minutes

Select Last Peak of Interest

#	Time	Area	Height	Width	Area%	Symmetry
5	6.456	405.4	130.2	0.0268	0.047	0.662
6	6.521	19.4	29.7	0.0213	0.000	0.81
7	7.310	11.9	20.1	0.0193	0.014	1.039
8	7.423	108.2	70.4	0.0196	0.013	1.068
9	7.477	106.7	70.4	0.0195	0.012	0.98
10	8.698	205.4	130.2	0.019	0.012	1.109
11	8.897	105.9	75.6	0.0205	0.024	1.206

Backflush Kit Preparation

Configuration | PID Update | Zero Pressure Sensors | Gas Check | Frit Check | Setpoint Attainability | Run Verification

Select modules to update with kit PIDs

Coated Pre-Column w/ Retention Gap

Front: Split-Splitless Inlet

Composite Column 1: Retention Gap to Coated Pre-Column

Column 2: Analytical Column

Back: Flame Ionization Detector

Update with Kit PIDs

Or Launch PID Tool

Live Setpoint Check

Time: 0.4 min

GC Ready State: No: ready
Front Inlet: Pressure not ready
Front Inlet: Flow not ready

view Status

Reference Chromatogram and a Selected Last Peak of Interest

Select sample and signal source from {0}

Standard Data File

C:\ccviews\cachance_view7\Blast\parts\BF5setupSt

MSD Data

Comparison Data File

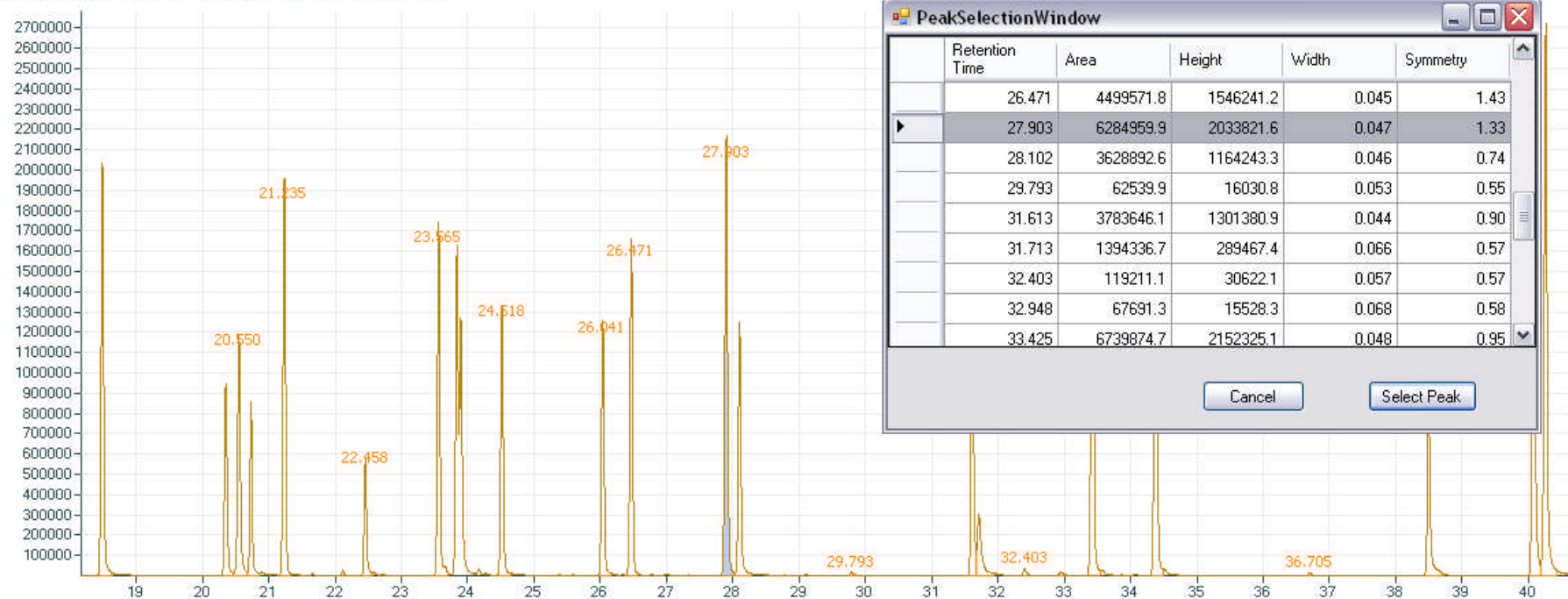
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MSD Data

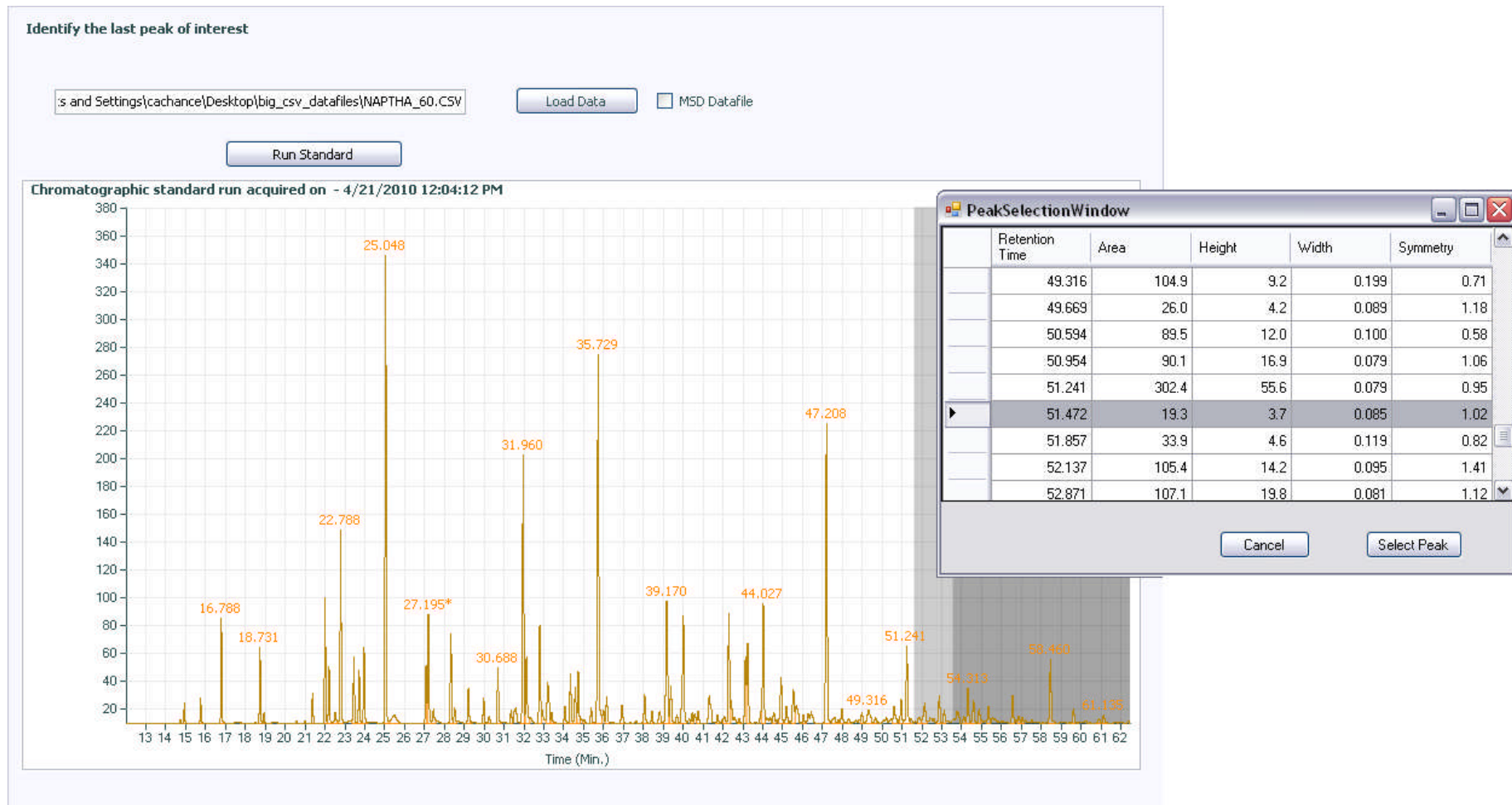
Compare Data Files

Only the plot

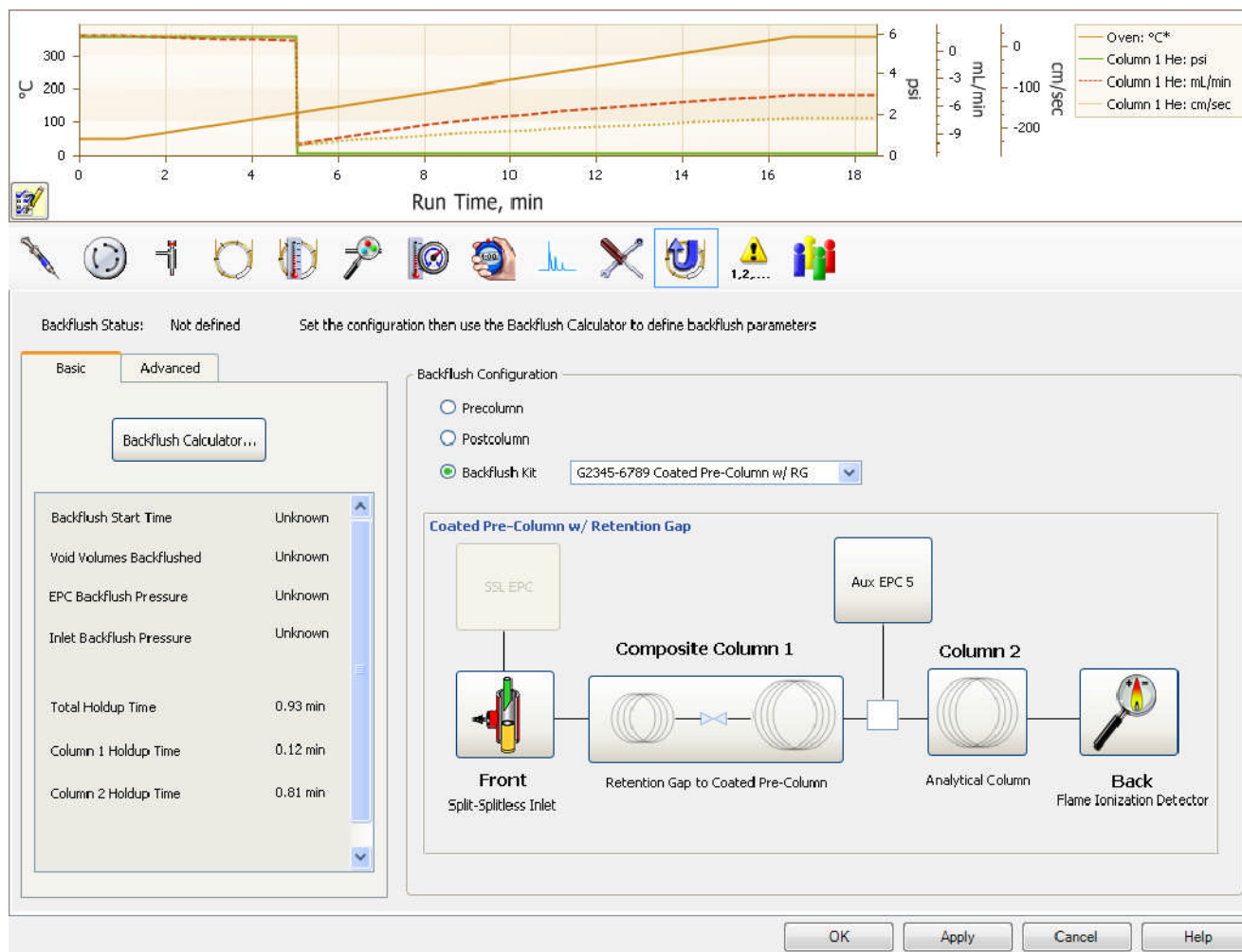
Chromatographic standard run "" acquired on - 4/21/2010 1:04:24 PM



Selecting Last Peak of Interest in Reference Chromatogram (from which optimal backflush conditions are automatically predicted)



Methods are Automatically Updated with Backflush Conditions



Comparing Backflushed and Reference Chromatograms

Select sample and signal source from {0}

Standard Data File

C:\ccviews\cachance_view7\Blast\parts\BFSetupSt



Comparison Data File

C:\ccviews\cachance_view7\Blast\parts\BFSetupSt



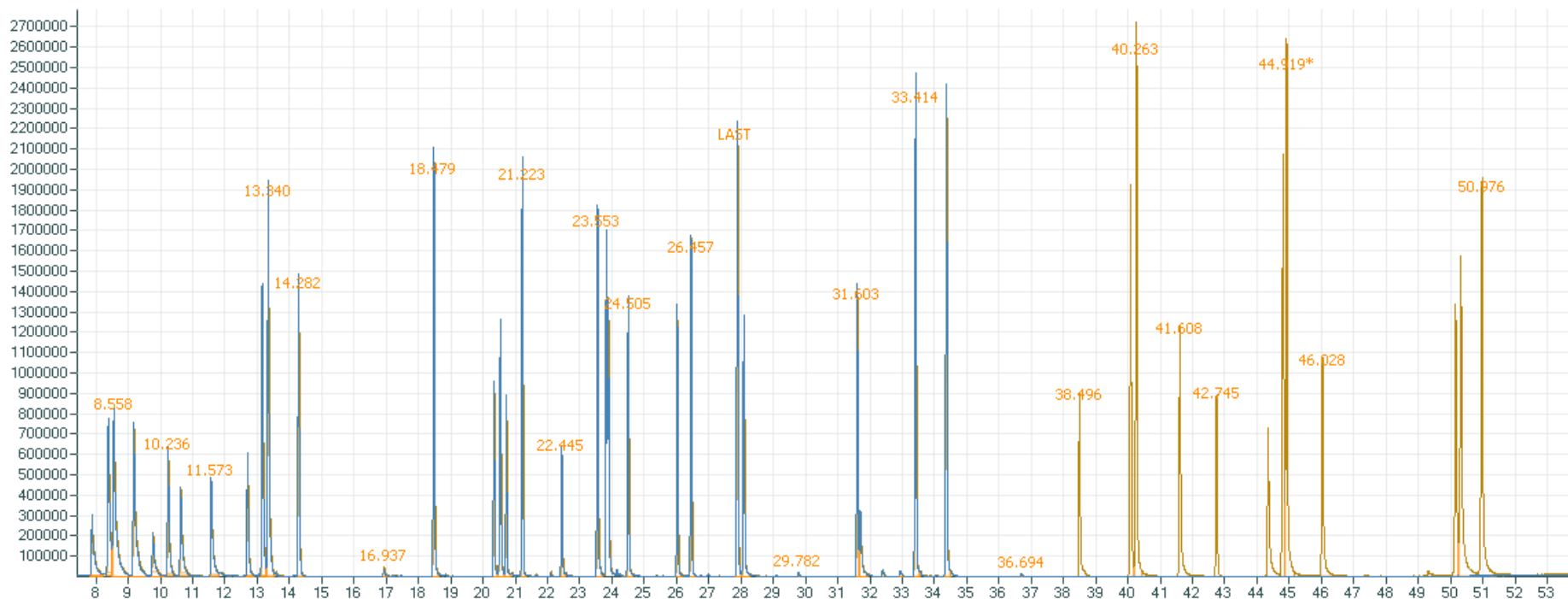
MSD Data

MSD Data

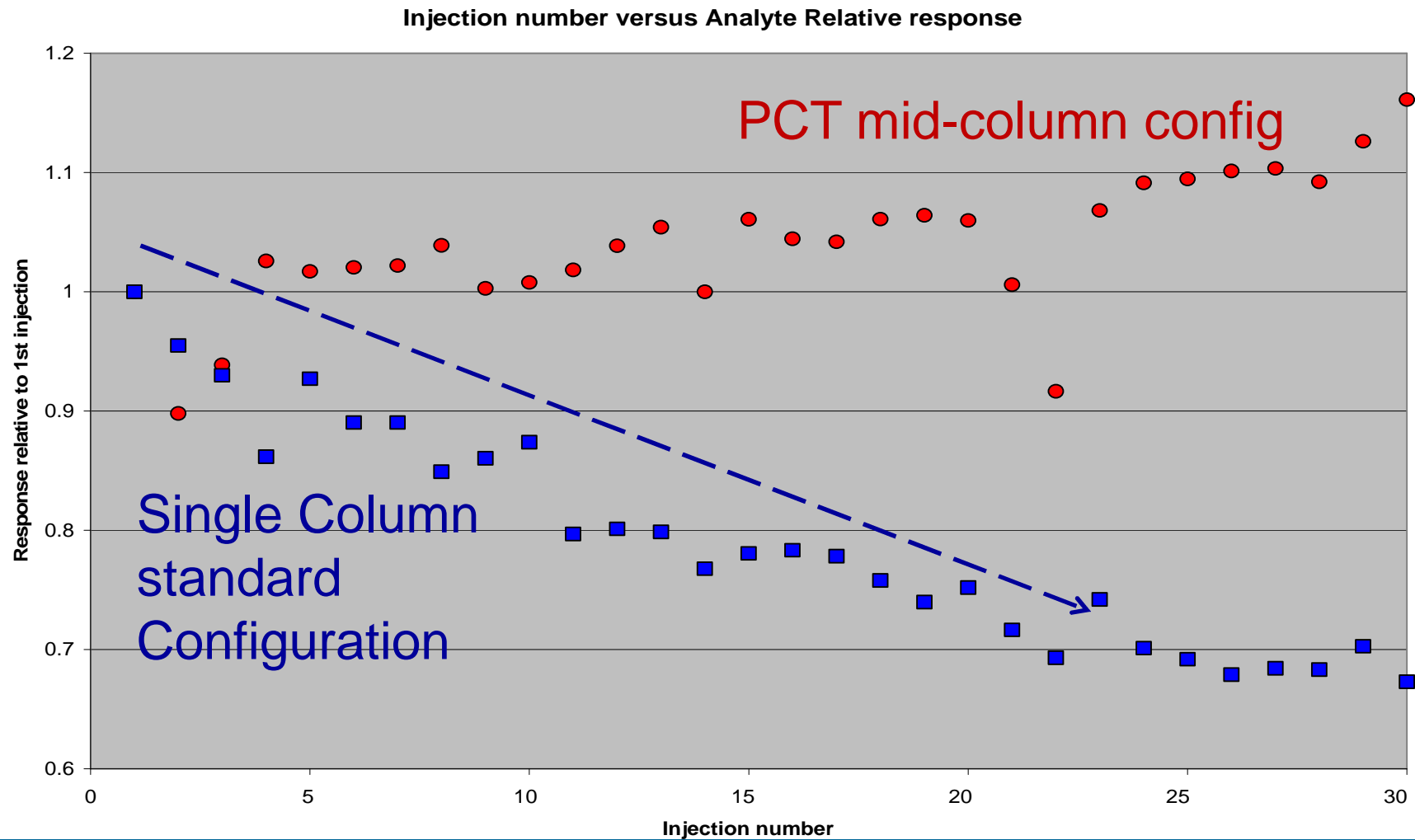
Compare Data Files

Only the plot

Results of comparison of <AreaRatio> between peaks 0 and 0 against standard ""

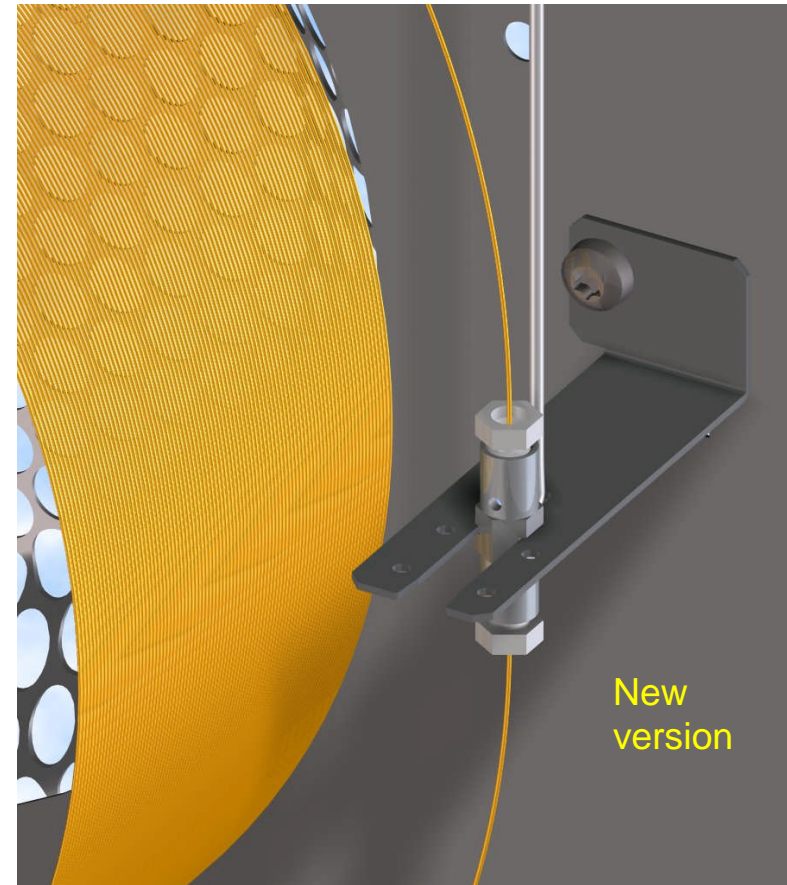


Backflushing Extends Column Life and Minimizes Maintenance due to Matrix Effects



What makes it all work?

7890 EPC control, the G1472A kit and ...
the Purged Ultimate Union



MMI Programmable Temperature Vaporizing Inlet – injection modes

- Hot split/splitless (also pulsed)
- similar to the S/SL inlet using the same liners
- Cold split/splitless (also pulsed)
- Significantly more inert than hot splitless
- Can inject 3-5 uL with no solvent venting
- Better sensitivity than hot splitless because large vapor cloud is not formed which travels outside the liner and portions are lost
- LVI-Solvent Vent
- An extension of cold splitless
- Large volume injection for maximum sensitivity

MMI Design Features

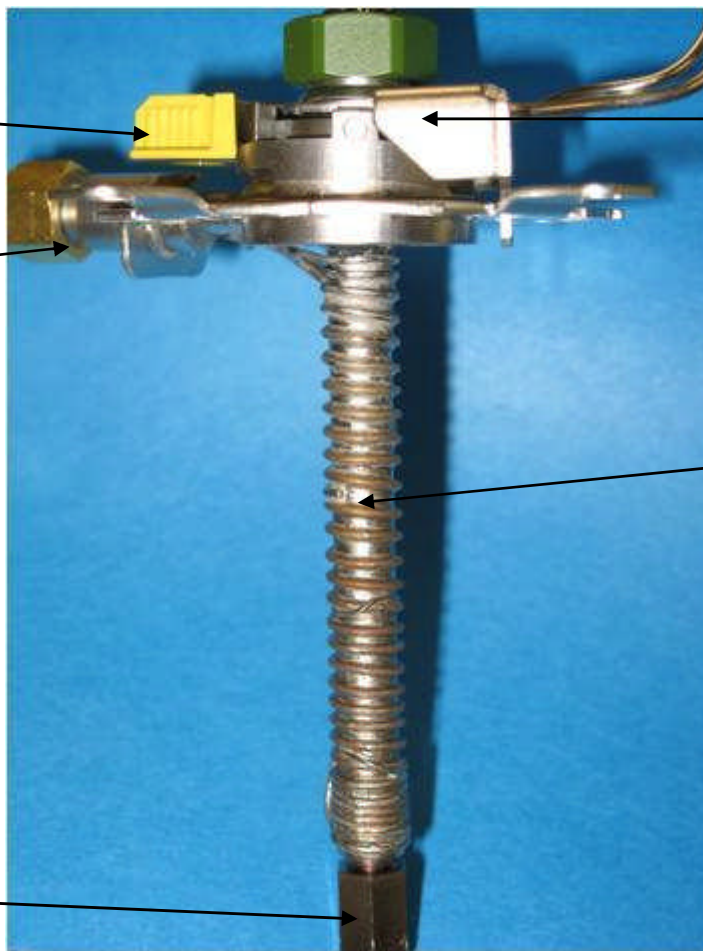
7890 turn top

7890 standard
pneumatics

S/SI standard
liners, o-rings,
and septa

Thermocouple
sensing

Graphite standard
column connection



Improved Performance with Cold Splitless - MMI

1 Hot Splitless

2 Pulsed Hot Splitless

3 Pulsed Hot Splitless with SPC

4 Hot Splitless with SPC

6,7

5 Cold Splitless

6 Pulsed Cold Splitless

7 Pulsed Cold Splitless with SPC

8 Cold Splitless with SPC

3

2

4

1

8

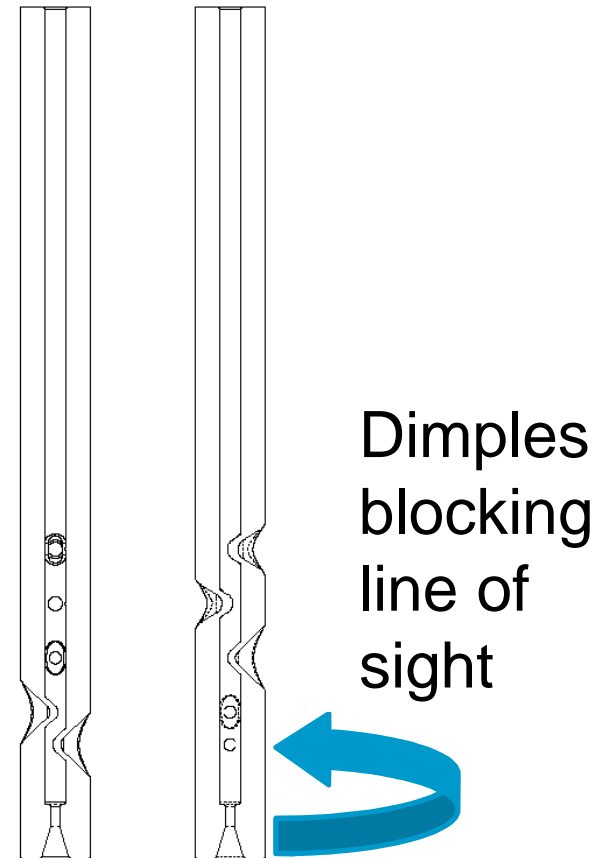
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An active compound where cold splitless is better than the best hot splitless



New Splitless Liner for dirty matrix samples

- Liner, 2 mm dimpled splitless with single taper
- Agilent proprietary deactivation
- Blocked line of sight to gold seal
- Recommended for heavy matrix samples
 - Pesticides in food extracts
 - Multi Mode Inlet in cold splitless mode
- Part Number: 5190-2296



2 mm Splitless Liner with Single Taper and Dimples

Competitive Pricing	US list	€ list
<i>NEW</i> Dimpled 2MM Splitless	\$ 47	45 €
Splitless Single taper w/ wool	\$32.75	32 €
Helix - Siltek	\$ 109	103 €
Cyclosplitter (Restek)	\$ 76	85 €
Cyclosplitter -Siltek (Restek)	\$ 81	91 €

How can Agilent's' GC/MS/MS with QuEChERS, MMI Inlet and Backflush better meet customer needs and win the order from the competition?

Top 3 Customer-Business Values	Customer Needs/ Problems Solved	Solution Feature/Spec
System must be rugged and easy to use with proven methodology	New backflush software quickly optimizes methods, MMI with “turntop” makes liner changing easy without MS venting	New liner design for MMI maximizes number of high matrix injections at a reasonable cost, QuEChERS provides recovery with minimal prep
For lower MDLs use MMI in Cold Splitless with solvent vent	Customers need more sensitivity to meet future regulations and this system provides lower MDLs	Combining MMI and Backflush with the Agilent GC/MS/MS allows customers achieve MDL requirements
Analyze high matrix samples and with less maintenance and lower costs	QuEChERS, Cold Splitless and Backflush keeps the column and source cleaner, GC/MS has sens & selectivity	Minimizing sample prep and maintenance permits analysis of more samples with less down time, increasing productivity

Analysis of High Matrix Samples - Summary and Conclusions

- Agilent now has an **improved solution for Cold Splitless injection (MMI)** using **a new liner (2 mm dimpled liner)** optimized for the analysis of high-matrix samples.
- **Backflush extends column lifetime and time between source cleanings** but does not change the impact of sample matrix on the liner.
- **Easy Backflush software** will make backflush more reliable and easy to use for our customers.
- Using **QuEChERS with dispersive SPE cleanup** will extend the number of injections between liner changes.

Questions ?