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OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

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LC-UHPLC Hybrid 2D Platform for LC/MS analysis of Biological Samples: A New Paradigm

Eduard Rogatsky

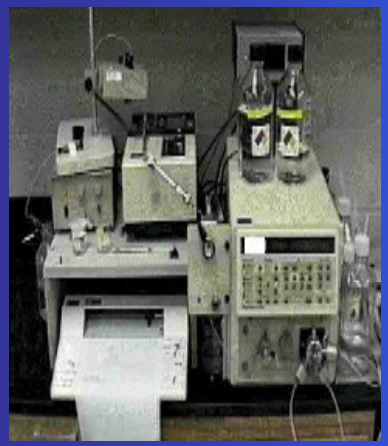
Albert Einstein College of Medicine

Bronx, NY

Critical Review on Recent Developments in Analytical & Bioanalytical Techniques

Liquid Chromatography

HPLC Instrument Development Milestones



1960s-1980s HPLC



1980s-2000s
“Conventional”
HPLC



2004 - Present
Acquity UPLC (2004)
UHPLC (2009)
UFLC (2009)
H-Class UPLC (2010)

?

HPLC Instrumentation Development

1992, MS-DOS



1998, Windows 95



2001, Windows XP



UHPLC was introduced 10 years ago. This new technology, while having great promise for improved efficiency and productivity, has not to date displaced HPLC. This transition will be slow process. Currently, both HPLC and UHPLC can be working in the same lab.

UHPLC vs HPLC

UHPLC it is not only a system that's capable of operating at higher pressures. UHPLC is optimized to work with sub 2 micron ultra – high resolution columns to produce very narrow sharp chromatographic peaks.

UHPLC –in contrast to conventional HPLC, is a low dwell volume system designed for fast runs at high pressure.

Current trends in chromatography

- Transition from HPLC to UHPLC
- Evolution of conventional HPLC for full support of Fused-Core chromatographic columns (delay volume reduction)
- Evolution of Liquid Chromatography with Mass Spectrometry analysis.

National Automobile Museum

The Harrah Collection, Reno, NV



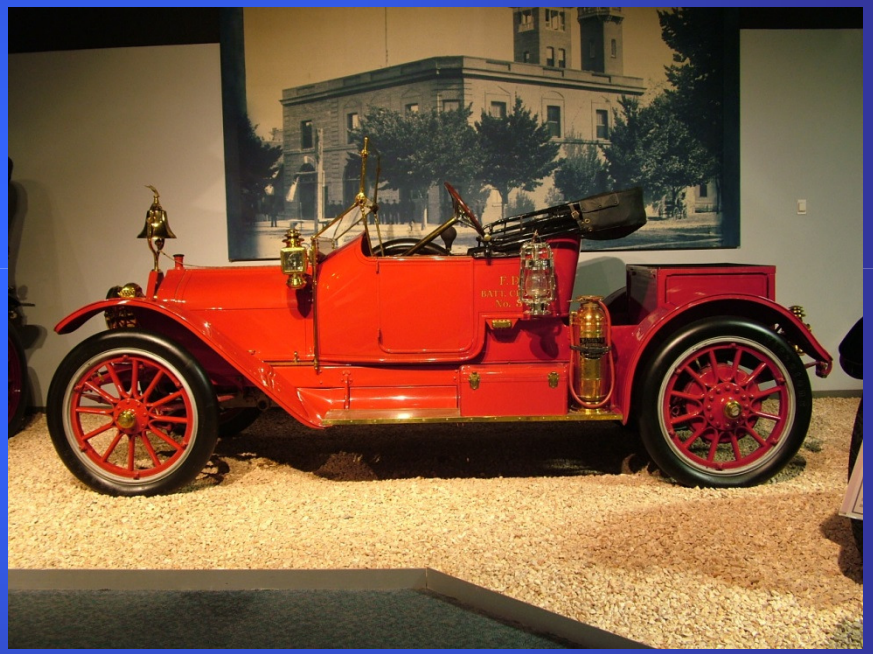


1912 BAKER - electric



1913 STANLEY -steam

In July 1886 the newspapers reported on the first public outing of the three-wheeled Benz Patent Motor Car, model no. 1.



1912 SELDEN - gasoline



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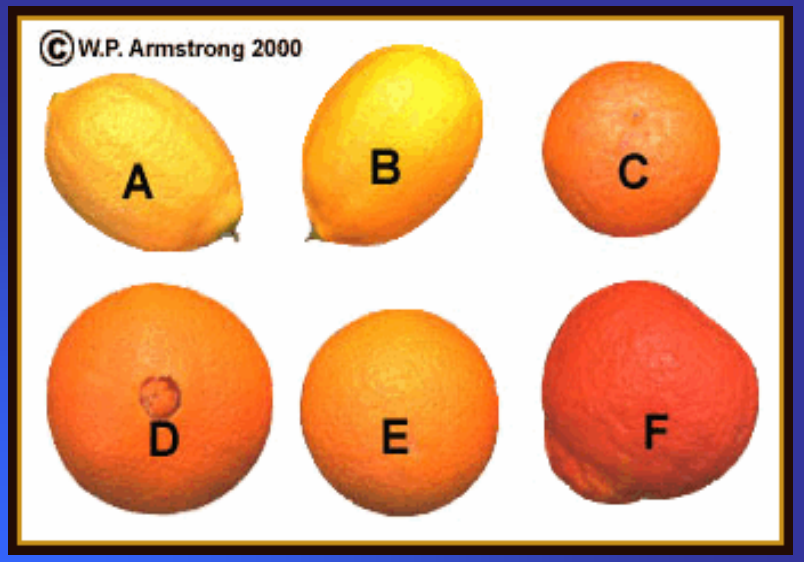
Hybrid

Something that is produced or formed by combining two [or more] things of different kinds.

Or: that has two different types of components that performing essentially the same function.

Or: mixture of different things or styles.





Hybrid Citrus Fruits: A. and B. Eureka and Meyer's Lemons (**Citrus limon**); C. Tangerine or Mandarin Orange (**C. reticulata** = **C. nobilis**); D. and E. Navel and Valencia Oranges (**C. sinensis**); F. Tangelo (**C. x tangelo**). The tangelo is a hybrid produced by crossing a Tangerine (**C. reticulata)**with a Grapefruit (**C. x paradisi**). The grapefruit (**C. x paradisi**) is another hybrid between the Shaddock or Pomelo (**C. maxima**) and the Sweet Orange (**C. sinensis**).





HPLC is also hybrid

- HPLC is an apparatus- hybrid machine - composed of autosampler, pump, column thermostat and detector…regardless – they are in the same box or in separate modules.
- HPLC is a symbiosis and not one unit [an inlet].



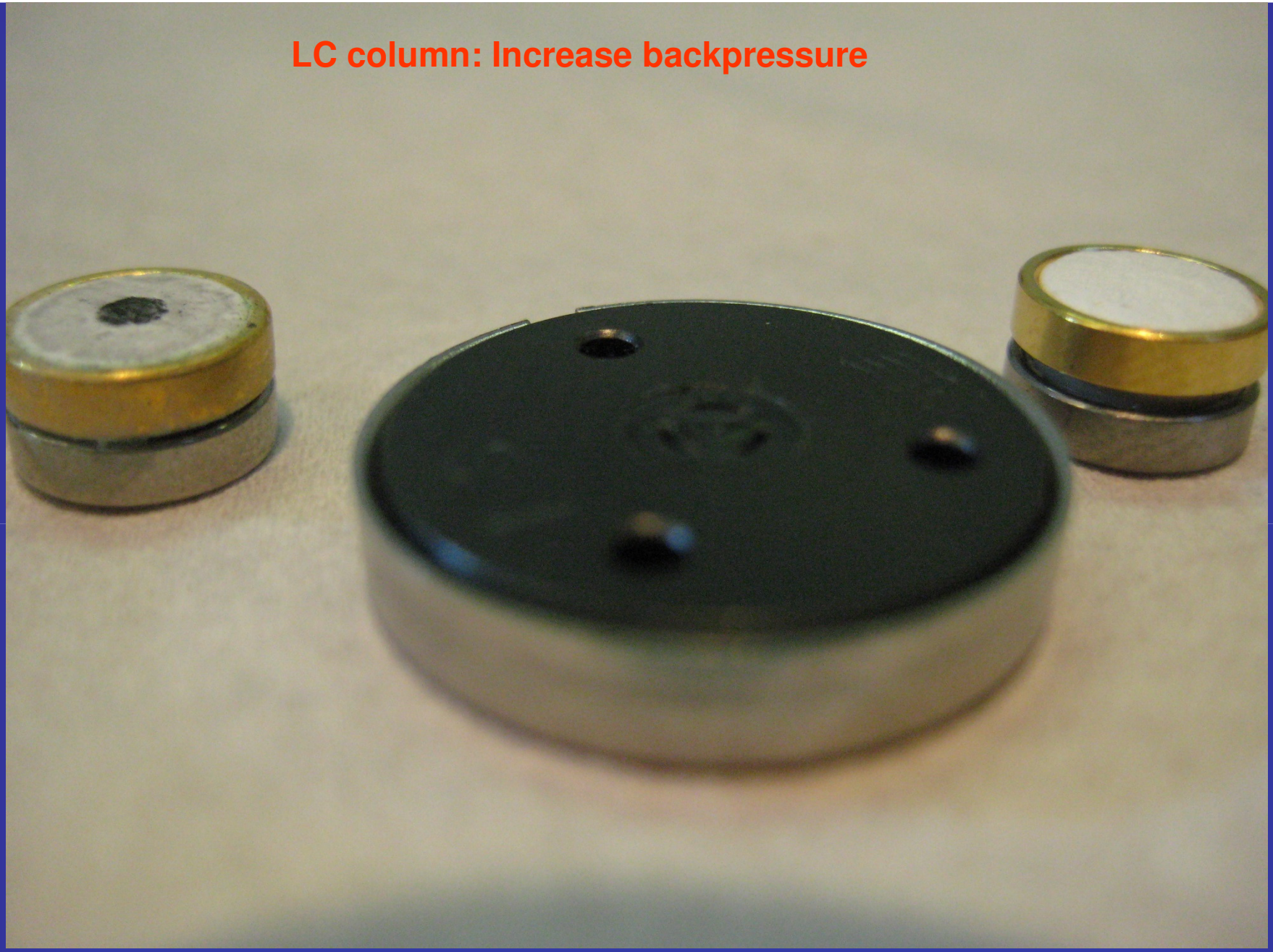
HPLC performance

- **HPLC performance is mostly based upon correct choice of flow rate, operating pressure, dwell/post-column volume and chromatographic column.**
- **Choice of these parameters is not module-based. Correct choice is system-based.**

HPLC is also hybrid:
pressure and flow rate impact

LC devices	Critical components and parameters
• Autosampler	• Injector valve, wash port, sample loop size
• Pump	• Purge valve, mixer
• Column thermostat	• Heat exchanger • Switching valve
• Detector	• Flow-cell design, pressure and volume

LC column: Increase backpressure

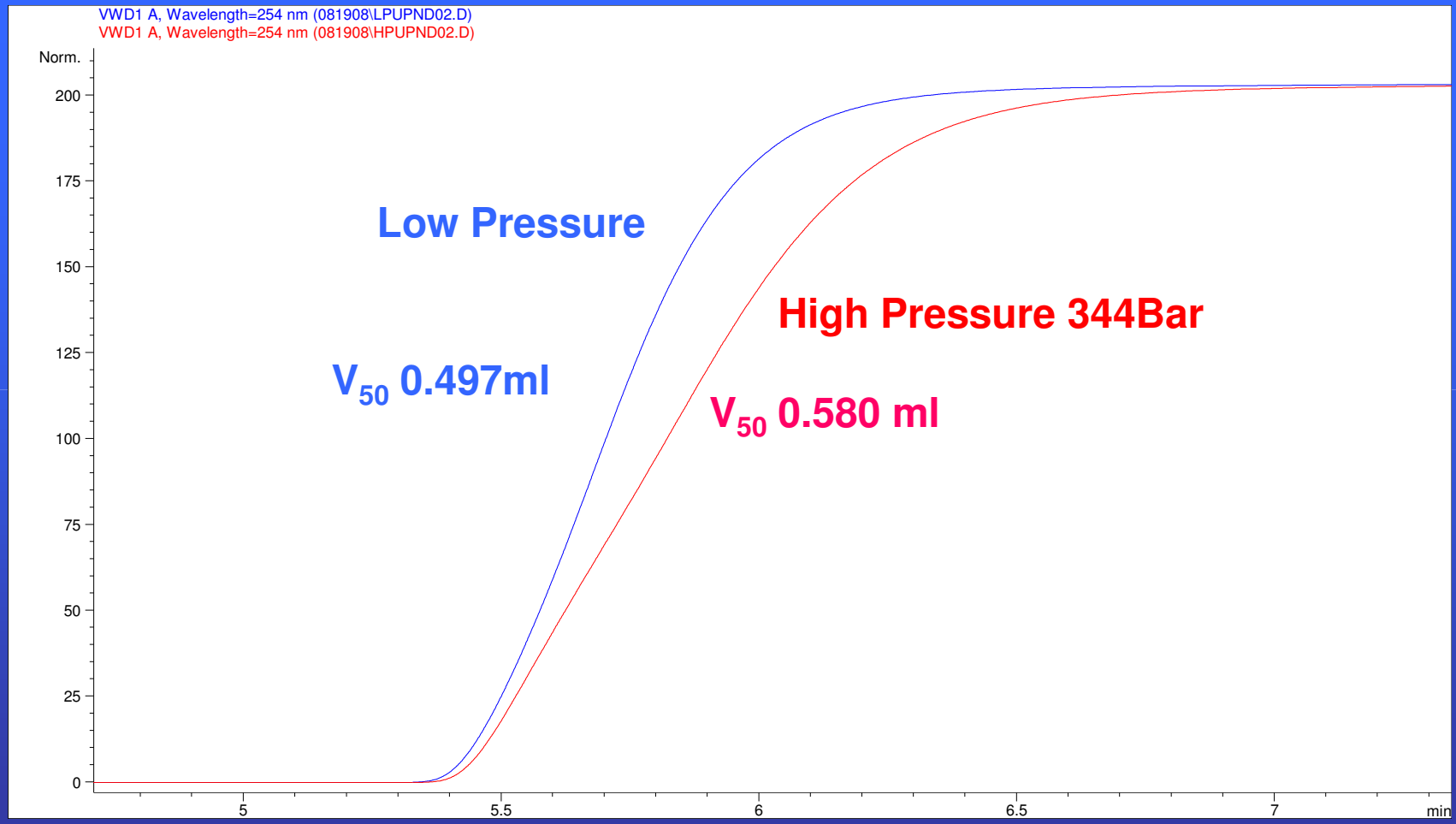


Column frit: contamination by rotor seal wear debris

Rotor seal dust

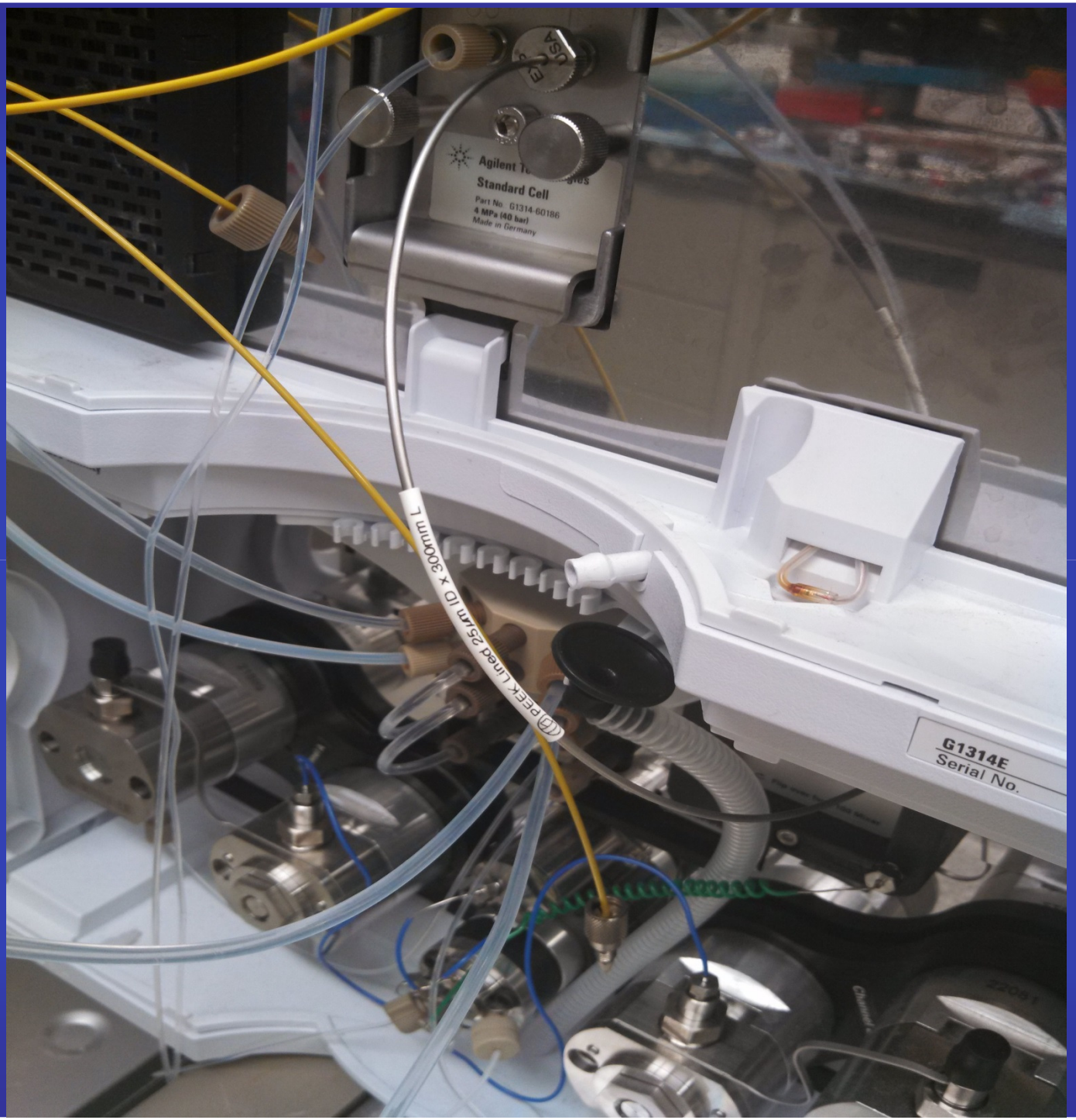


Delay volume-pressure impact: 15% drop of mixing efficiency

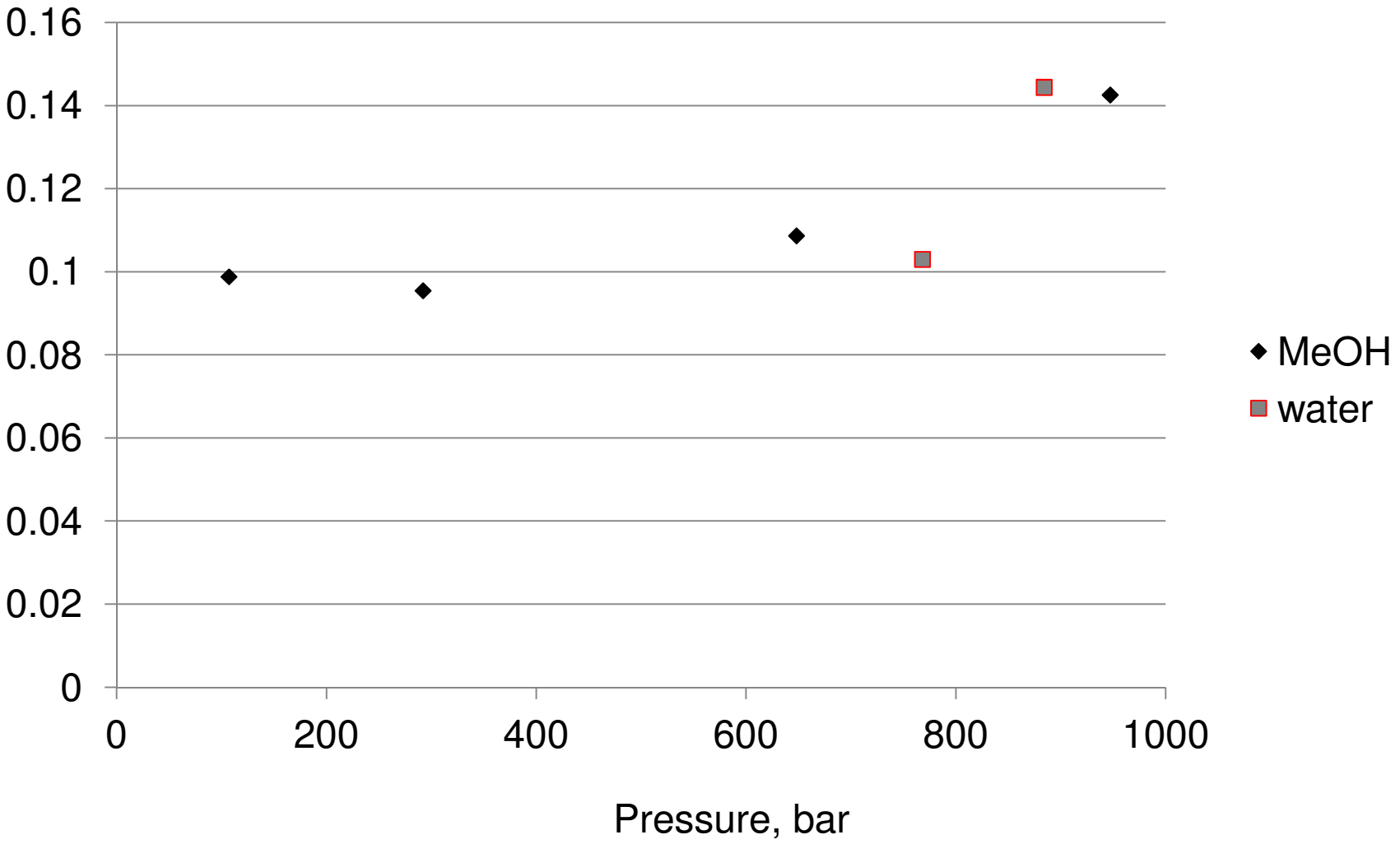


Upchurch filter in the pump, no damper, valve, restrictor, Flow rate 0.7 ml/min
Blue: Low Pressure V_0 0.318 ml; V_{50} 0.497ml
Red: High Pressure 344Bar, V_0 0.305 ml; V_{50} 0.580 ml
The more shallow slope results in a larger V_{50}, [poor mixing] as well as smaller V_0

IDEX PEEK Lined SS



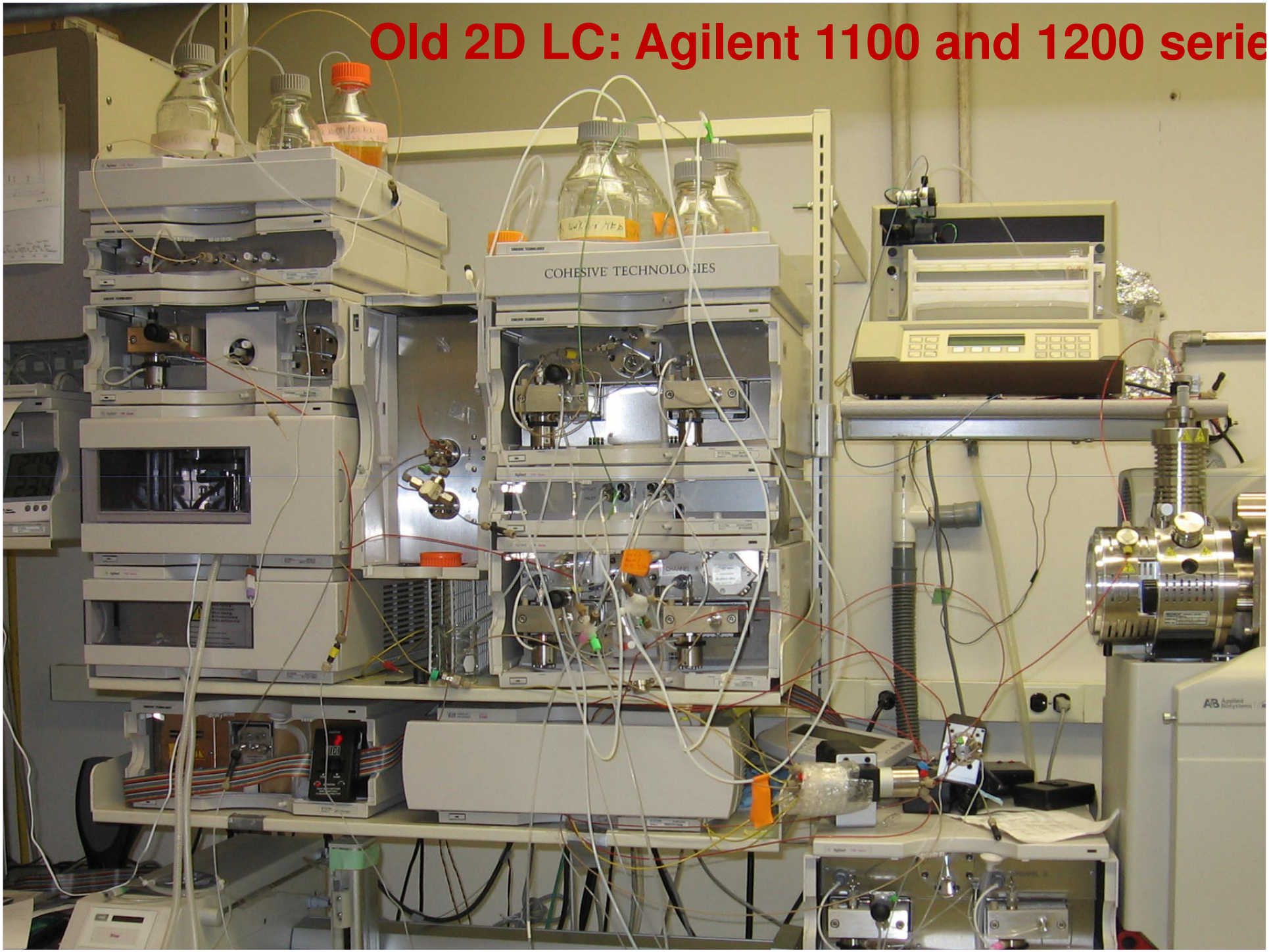
V50 increase with pressure



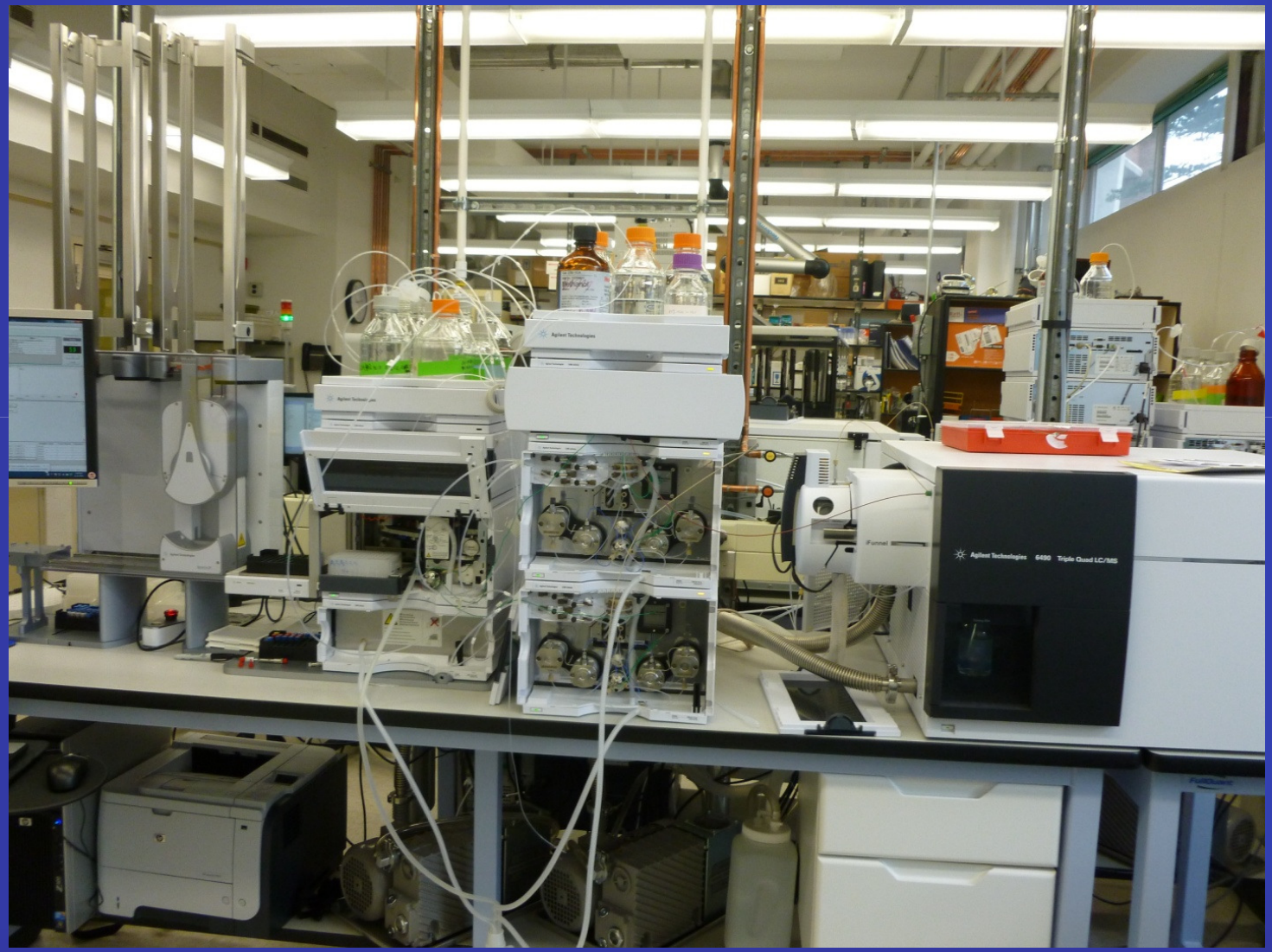
Summary

- **New experience: moving parts and mixers are less rugged and efficient in high pressure applications.**

Old 2D LC: Agilent 1100 and 1200 serie



UHPLC 2D/MS Agilent 1290 series



2D and LCMS

2D chromatography – efficient way to increase peak capacity. It is important for UV applications, but in general, not for LC/MS applications

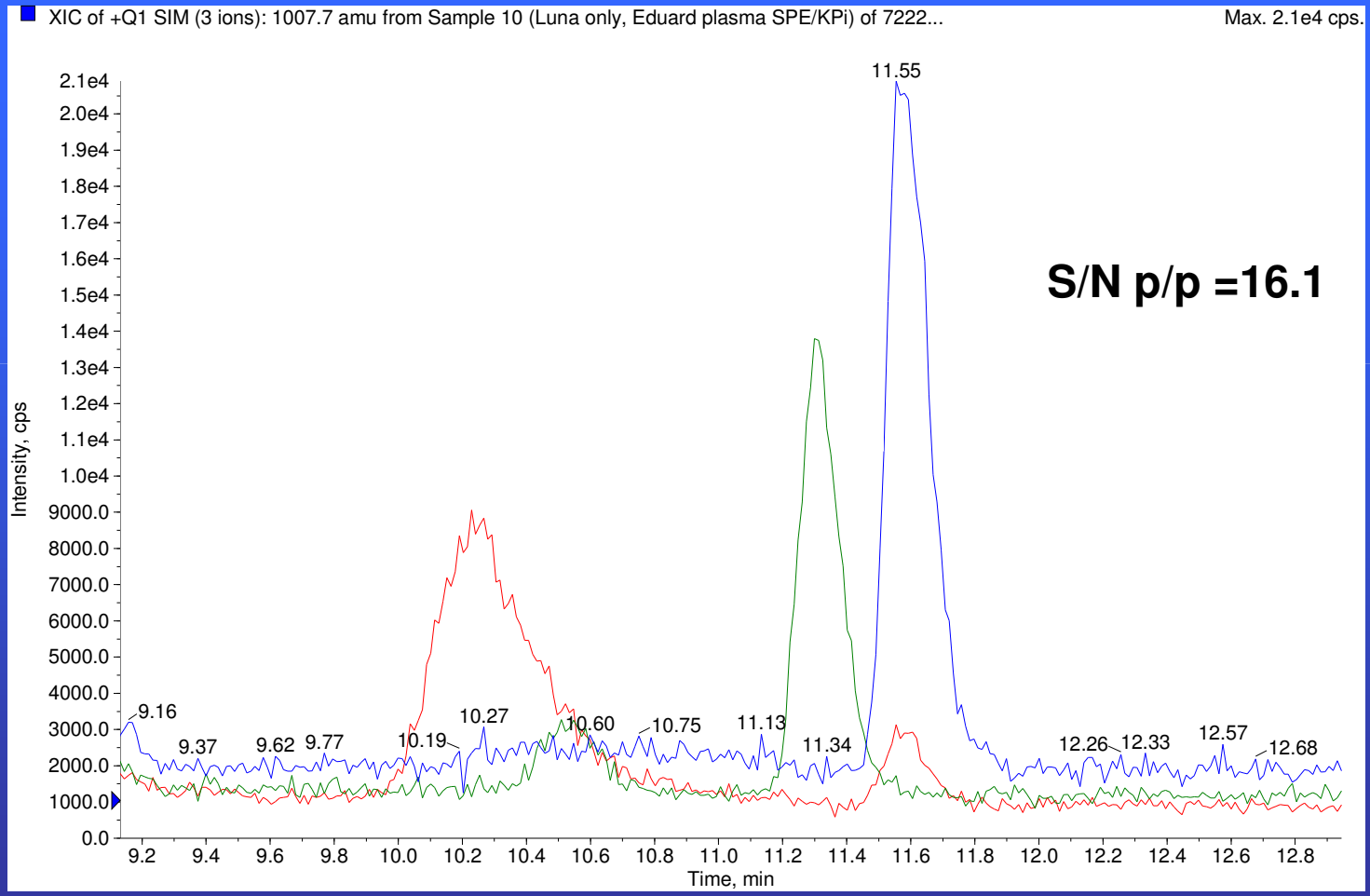
MS detection utilizes ions resolution by m/z and chromatographic peaks overlay is often in LC/MS analysis.

Benefits of 2D in MS:

Minimum of signal suppression
Better background
Better peak shape
More clean instrument

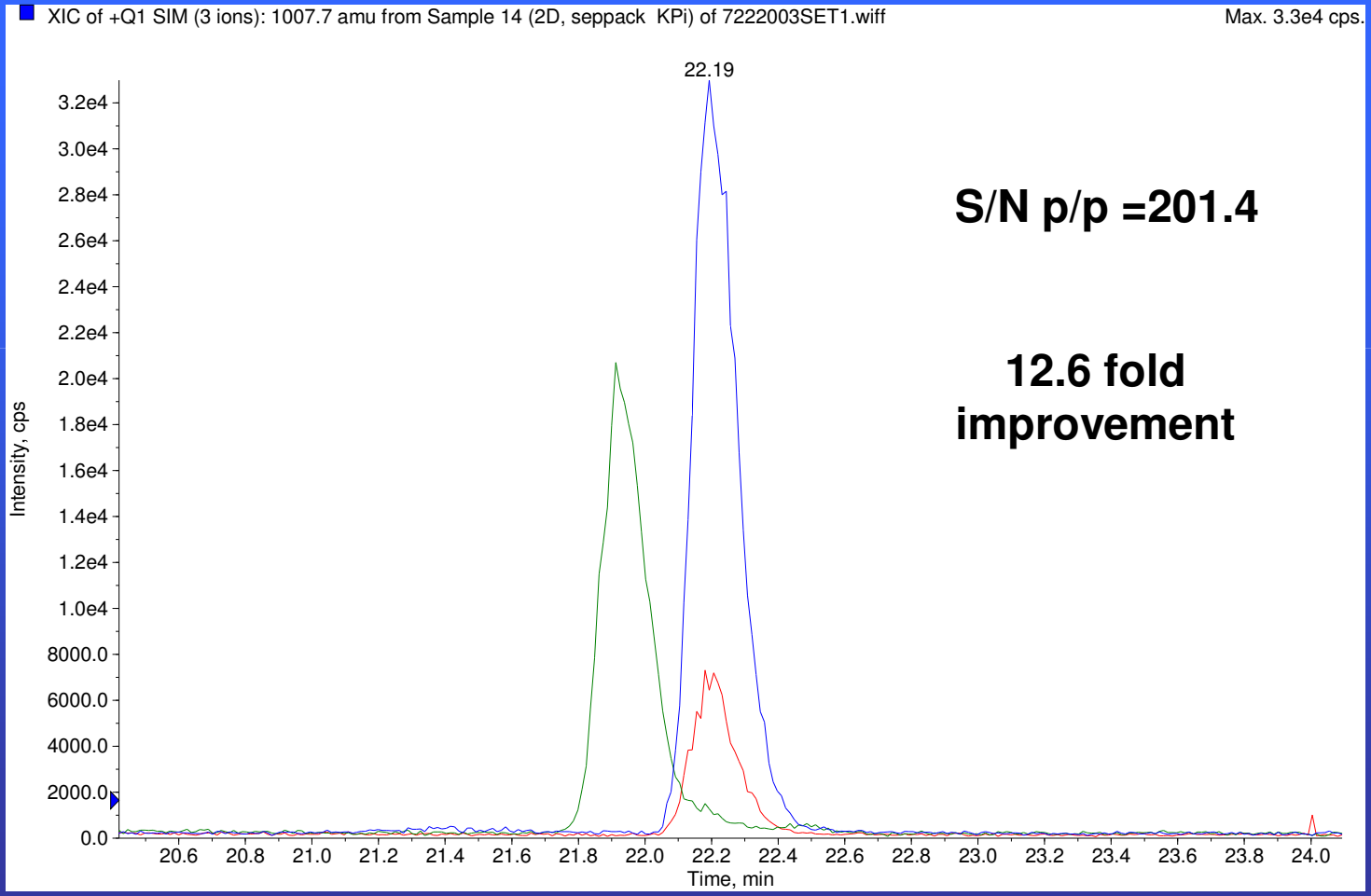
Matrix effect reduction - One column method

SPE AcN/Phospate sample



Matrix effect reduction - Two column method

SPE AcN/Phospate sample



S/N p/p =201.4

12.6 fold improvement

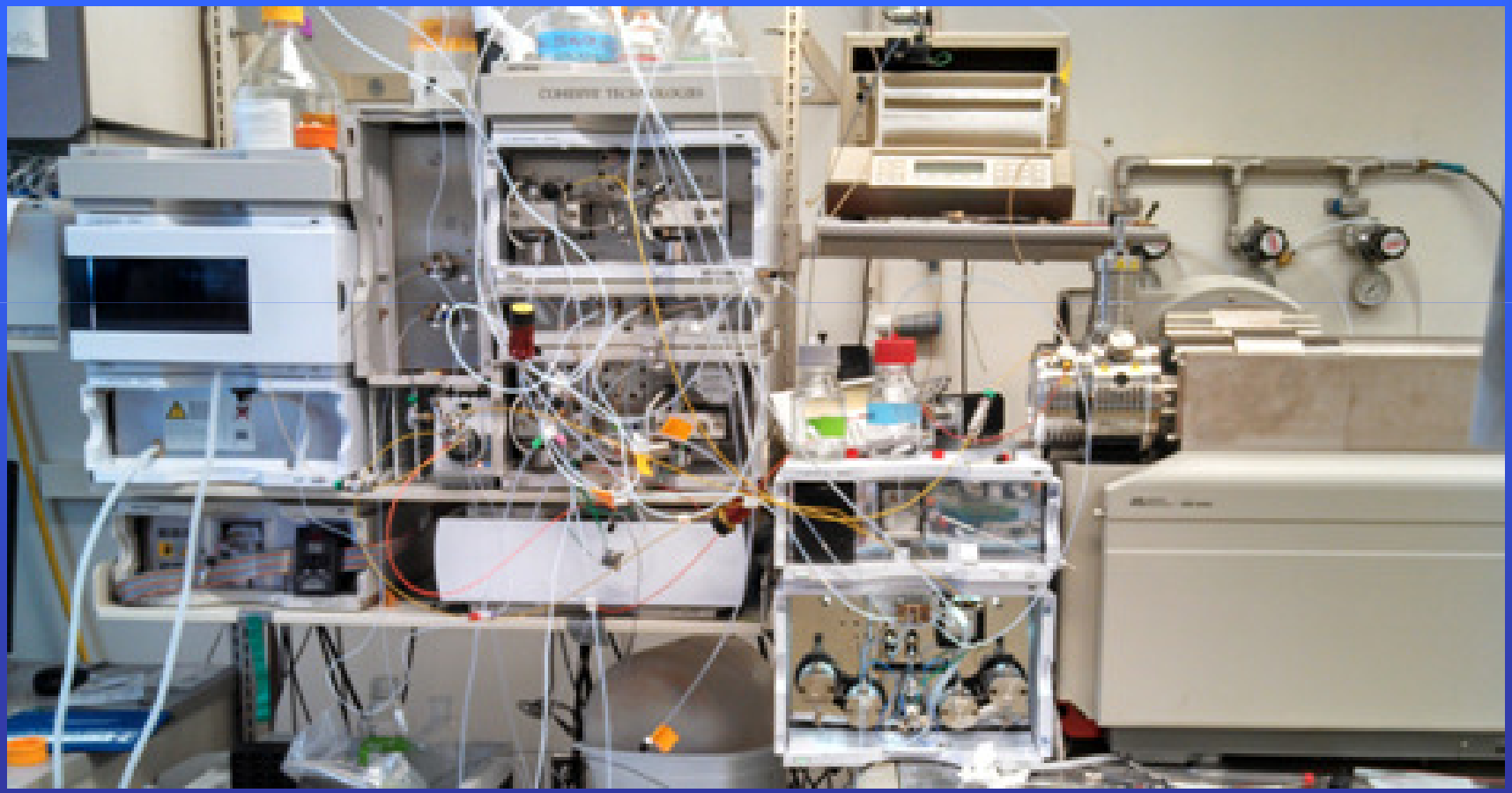
Chromatography myth

- Single column chromatography is always more robust, compared to column switching applications. That's true – if we are comparing standard pressure ranges.
- High pressure operations are less robust and more expensive - compare to standard LC
- New paradigm: low pressure injection by standard LC and UHPLC chromatography.

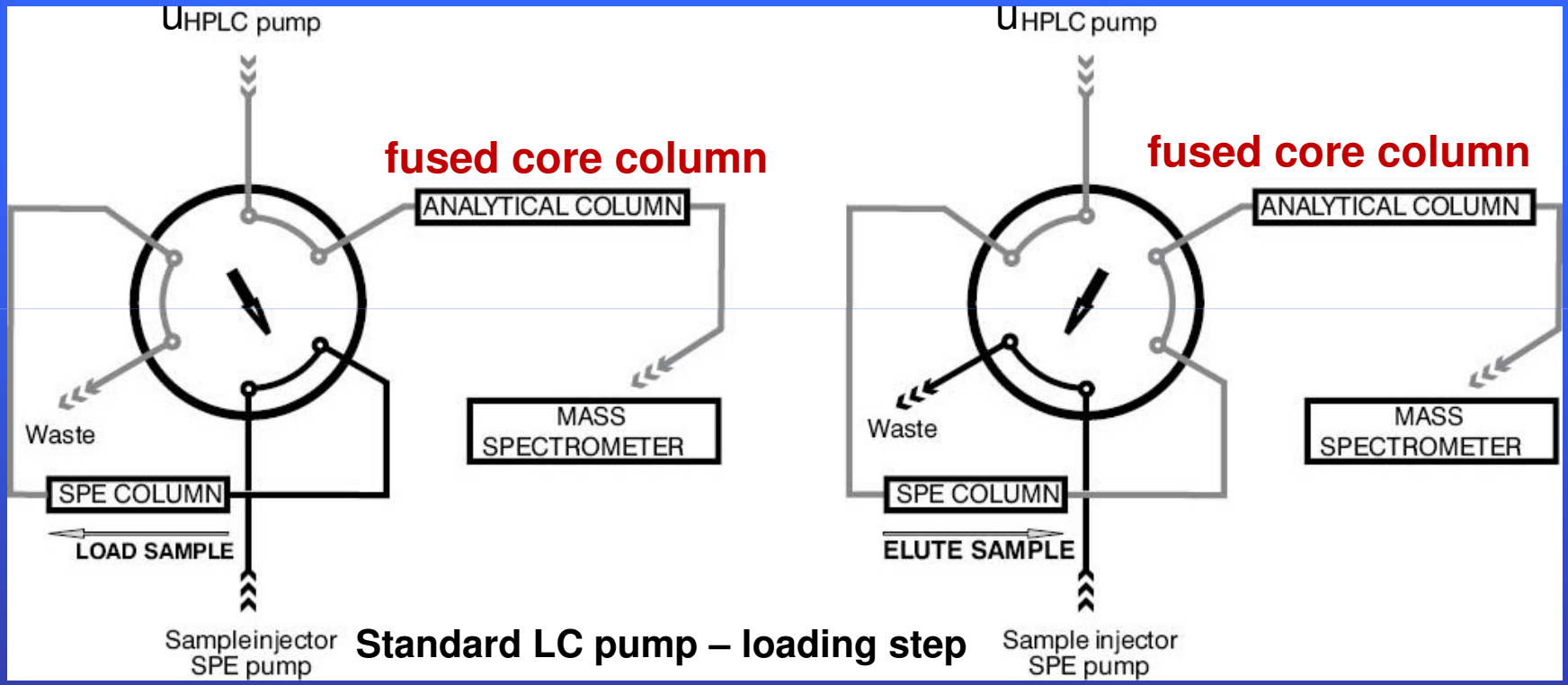
Outline

The sample was injected by a standard (not UHPLC) autosampler into a pre-analytical column, where it was desalted and partially purified. A standard pressure range binary HPLC pump was used. The fraction containing analytes of interest was transferred through a UHPLC valve to a fused core column. A fast gradient was performed using a UHPLC 1290 pump.

LCxUHPLC



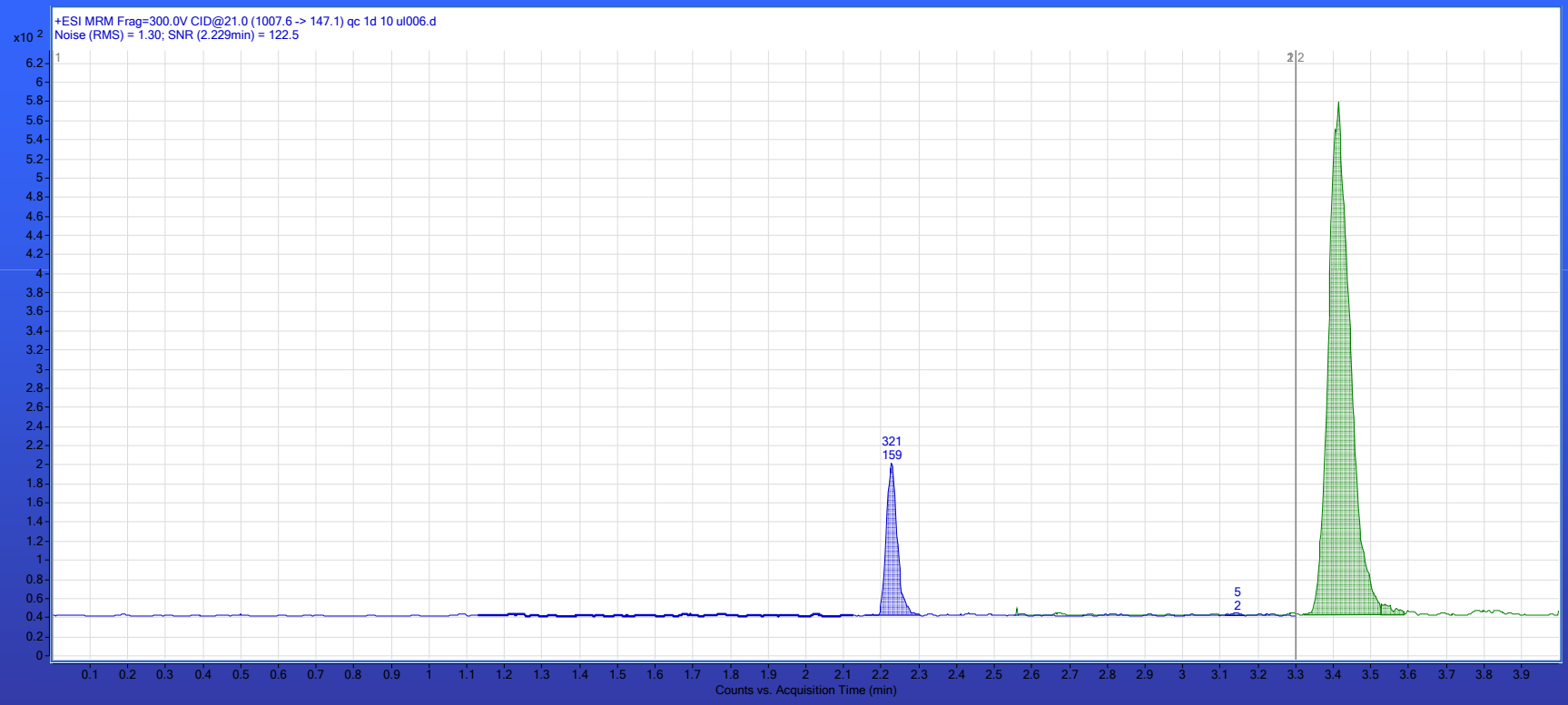
Online SPE design



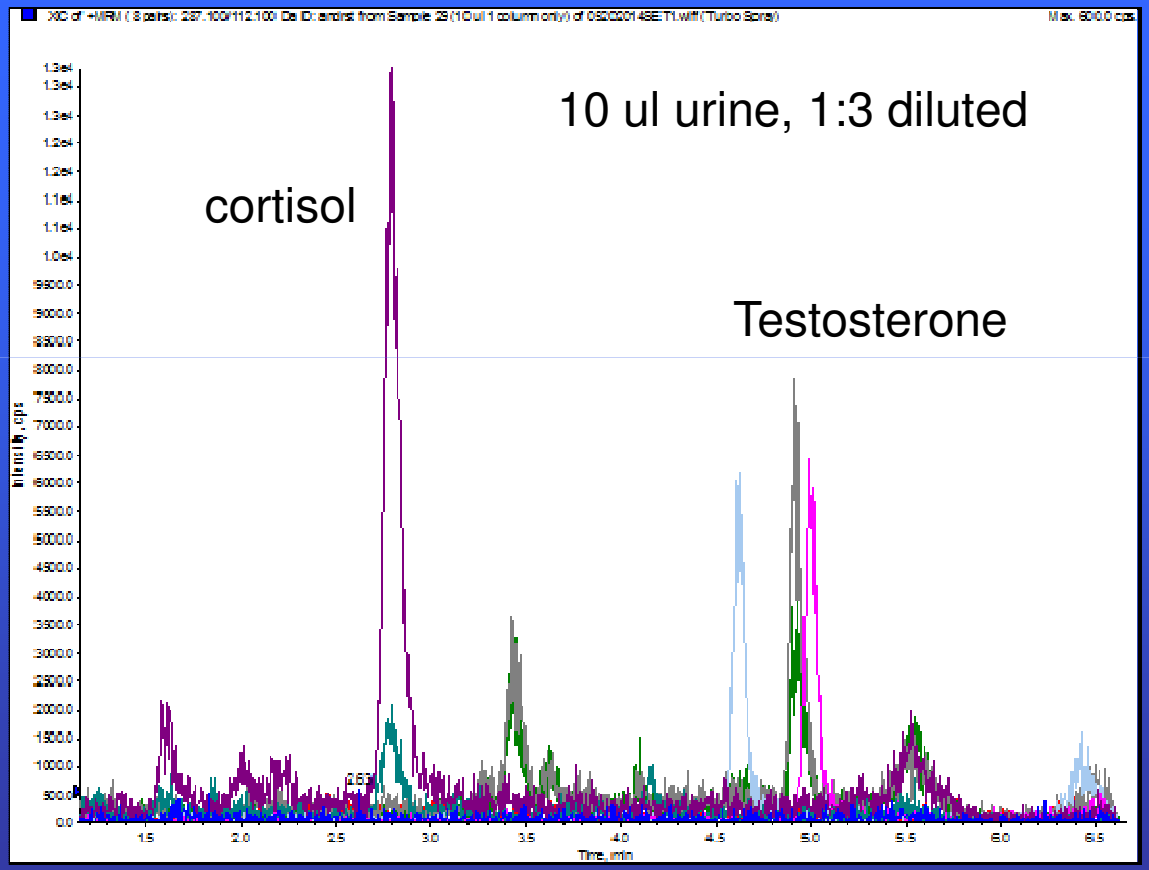
Standard LC pump – loading step

LCxUHPLC design based on HPLC loading pump and UHPLC LC/MS pump

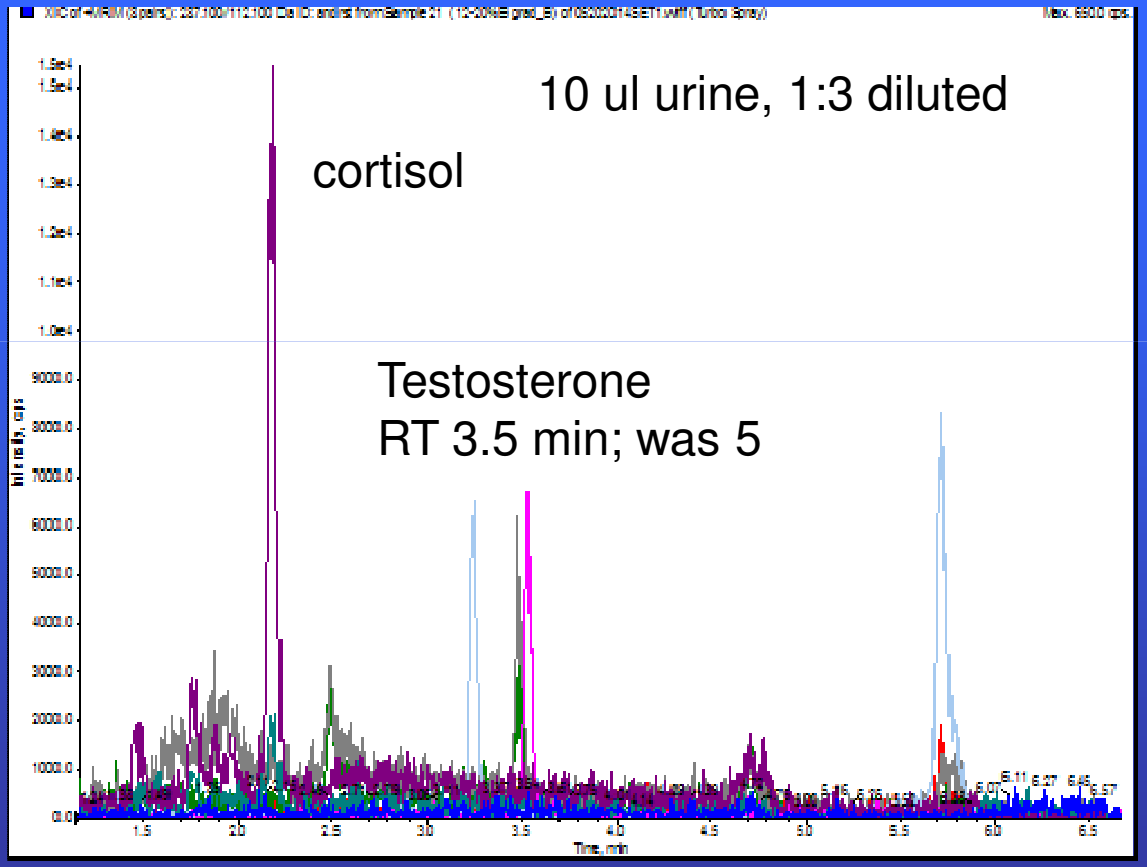
Human C-peptide Plasma SPE



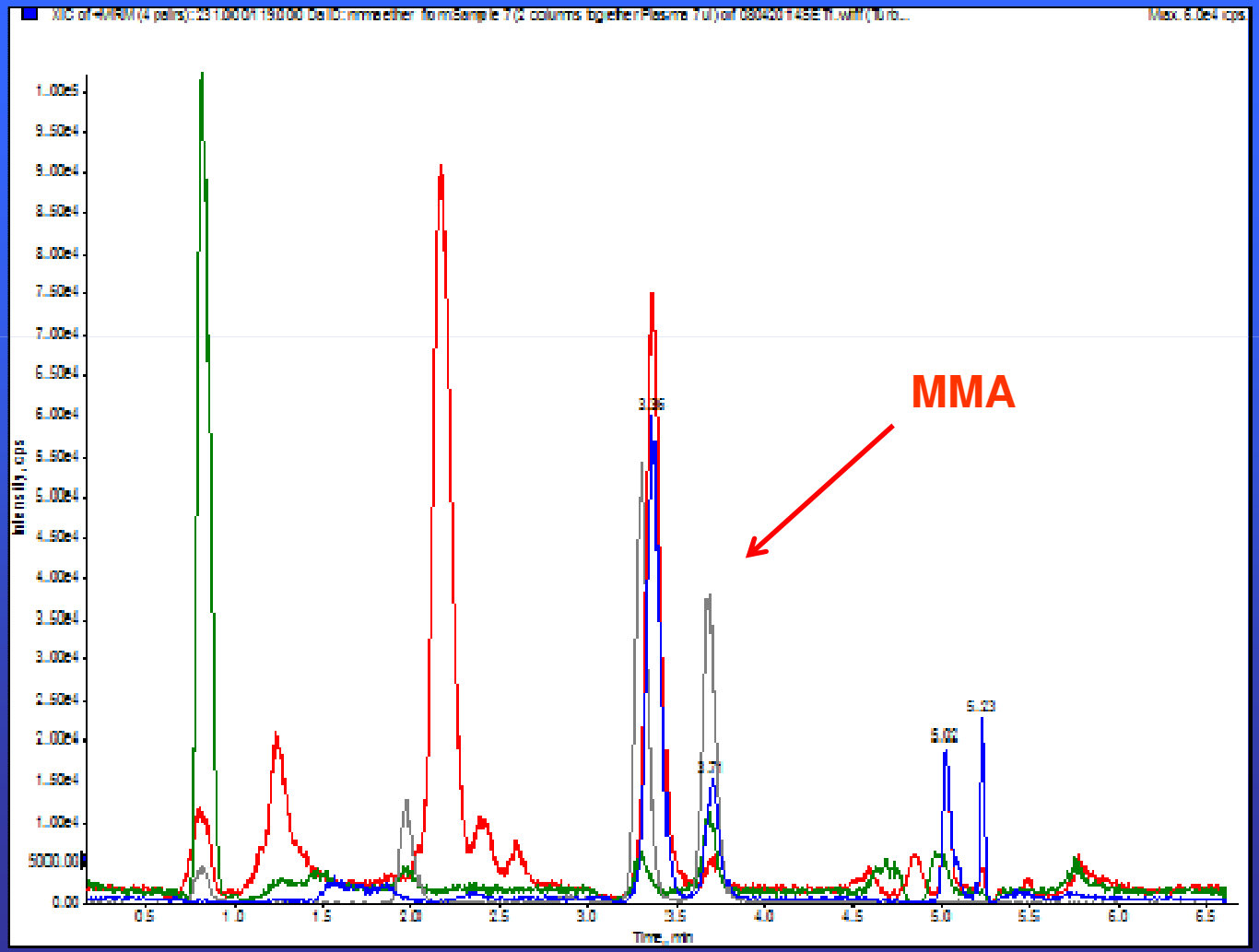
Urinary cortisol and testosterone –single column



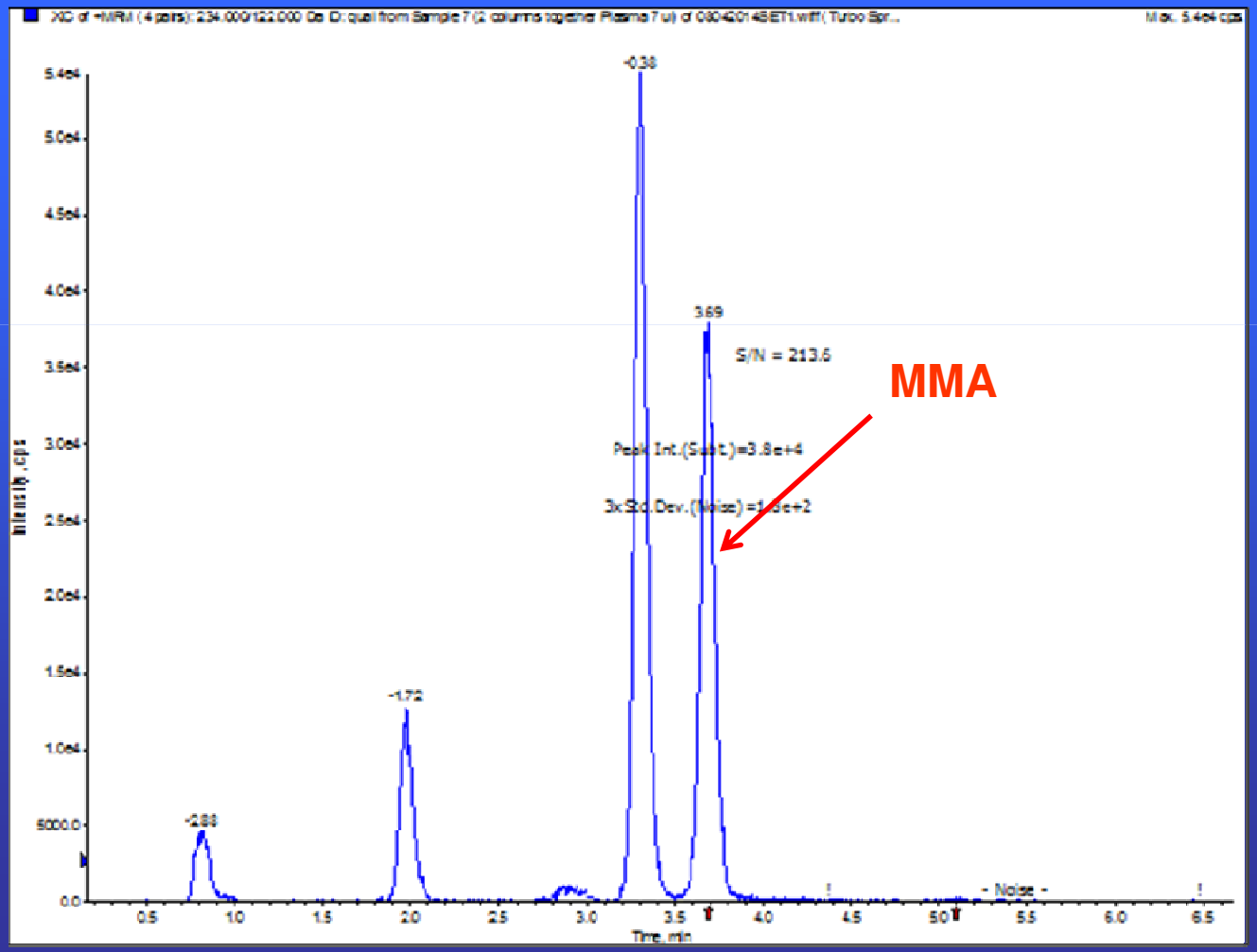
Urinary cortisol and testosterone -trap



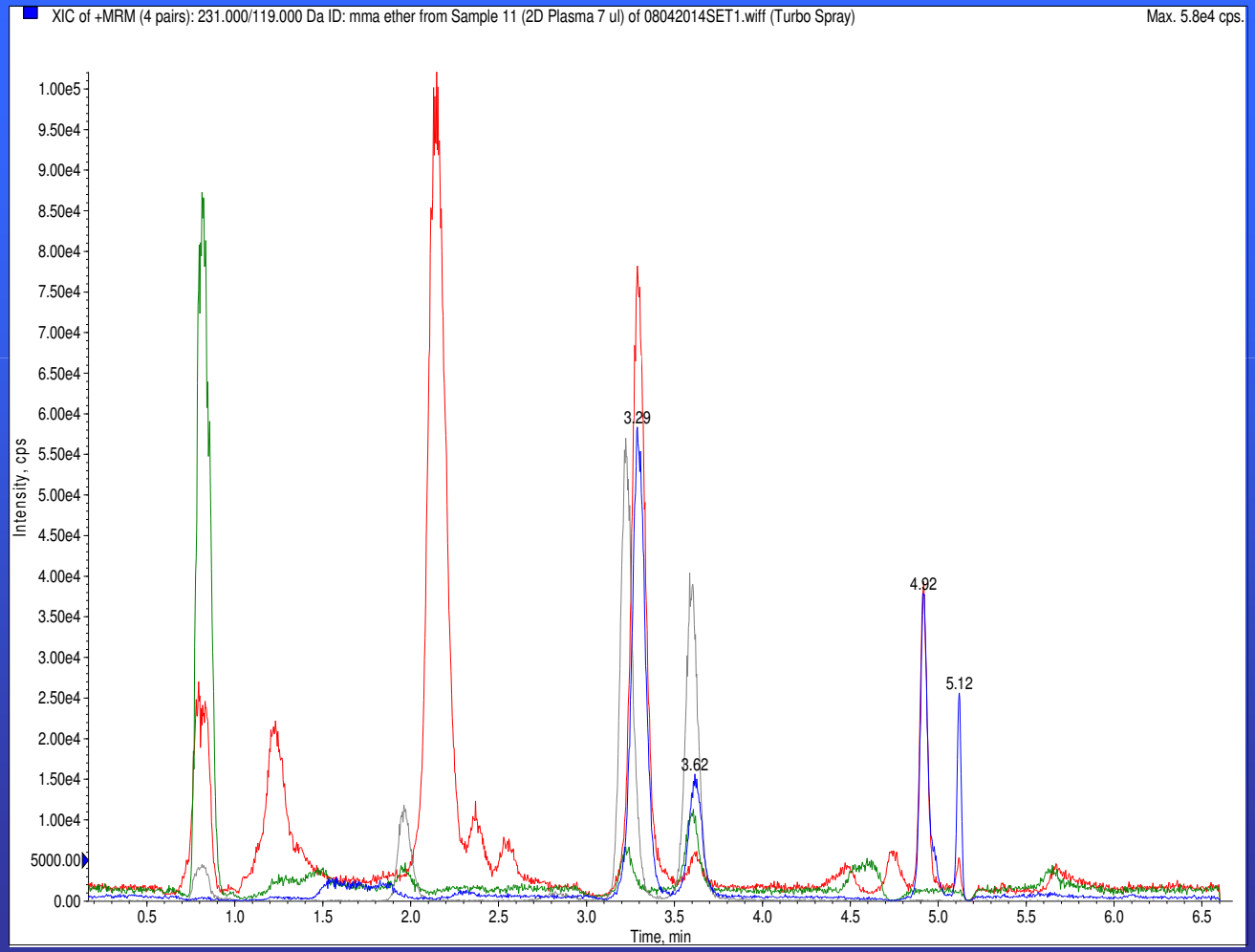
MMA from plasma
1D: trap +column



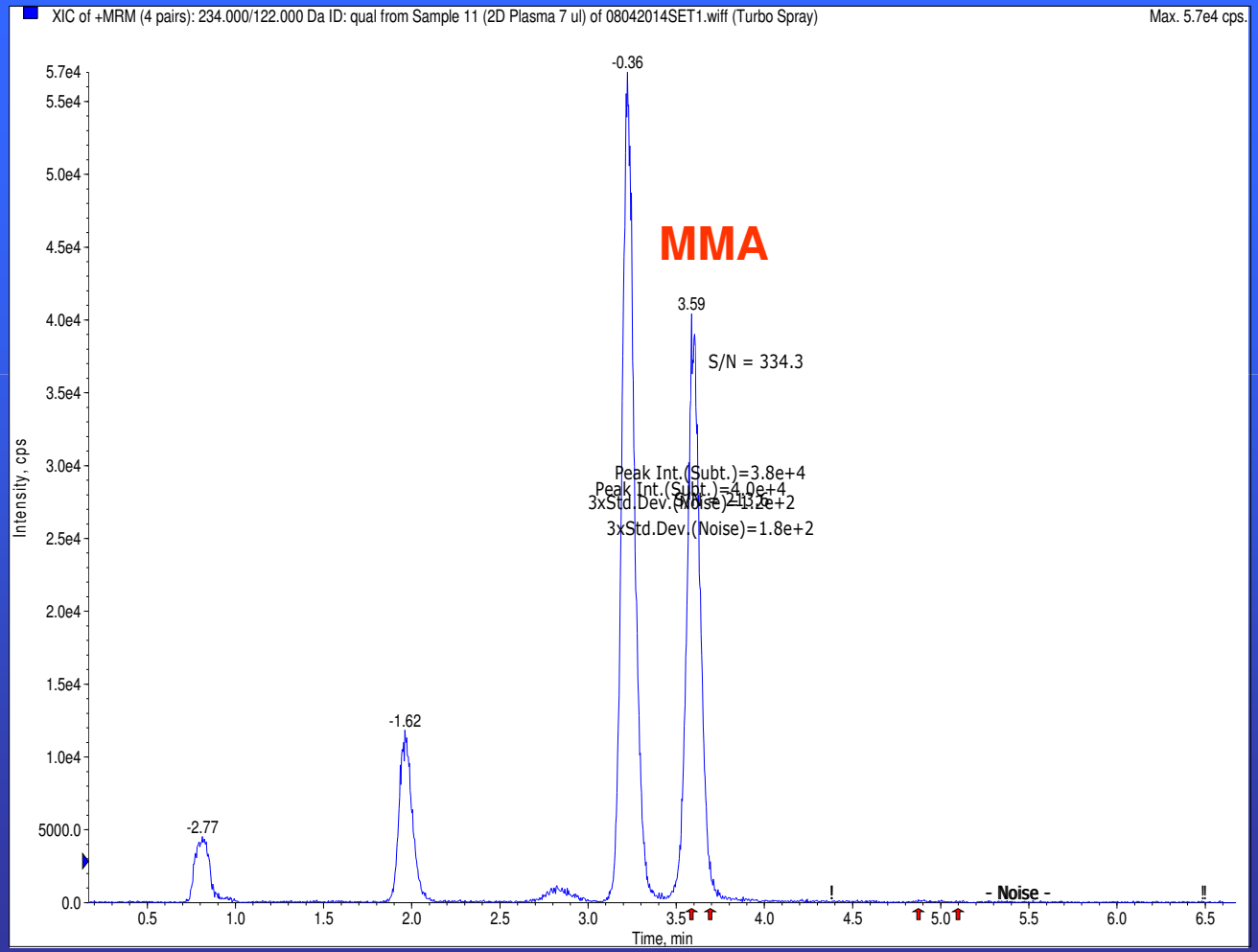
MMA from plasma
1D: trap +column



MMA from plasma 2D: trap loading +column switching



MMA from plasma 2D: trap loading +column switching



Column switching improves S/N from 214 to 334; aprox. 40% gain

Summary 1

- Hybrid LCxUHPLC is more rugged column switching system, compare to 2D UHPLC
- UHPLC sample loading is limited
- Sample loading to trap, using standard LC pump and autosampler is more convenient and cost efficient
- Loading and wash step using trap greatly improve ruggedness of mass spectrometer operations

Expert • Independent • Nonprofit
ConsumerReports.org®

Hybrids typically have:
More functionality
Higher cost, compare to its components,
Only basic control and features
Less ruggedness
Limited support

Hybrid LCxUHPLC is free from these disadvantages

Take Home Message

We successfully used LCxUHPLC platform for LC/MS analysis. Instead of retiring an entire functioning Agilent 1100 LC system, we just added one UHPLC pump to achieve much greater overall performance, functionality and lower cost, compared to a single pump UHPLC system purchase.

Summary 2

- The addition of a UHPLC valve and pump to a standard 1100/1200 series LC system (autosampler, pump and column compartment) greatly extends operational flexibility including column selection, while standard LC – which is already available in the lab, performs the initial steps of sample loading and clean-up

Traps and guards: choice of selectivity, performance and cost



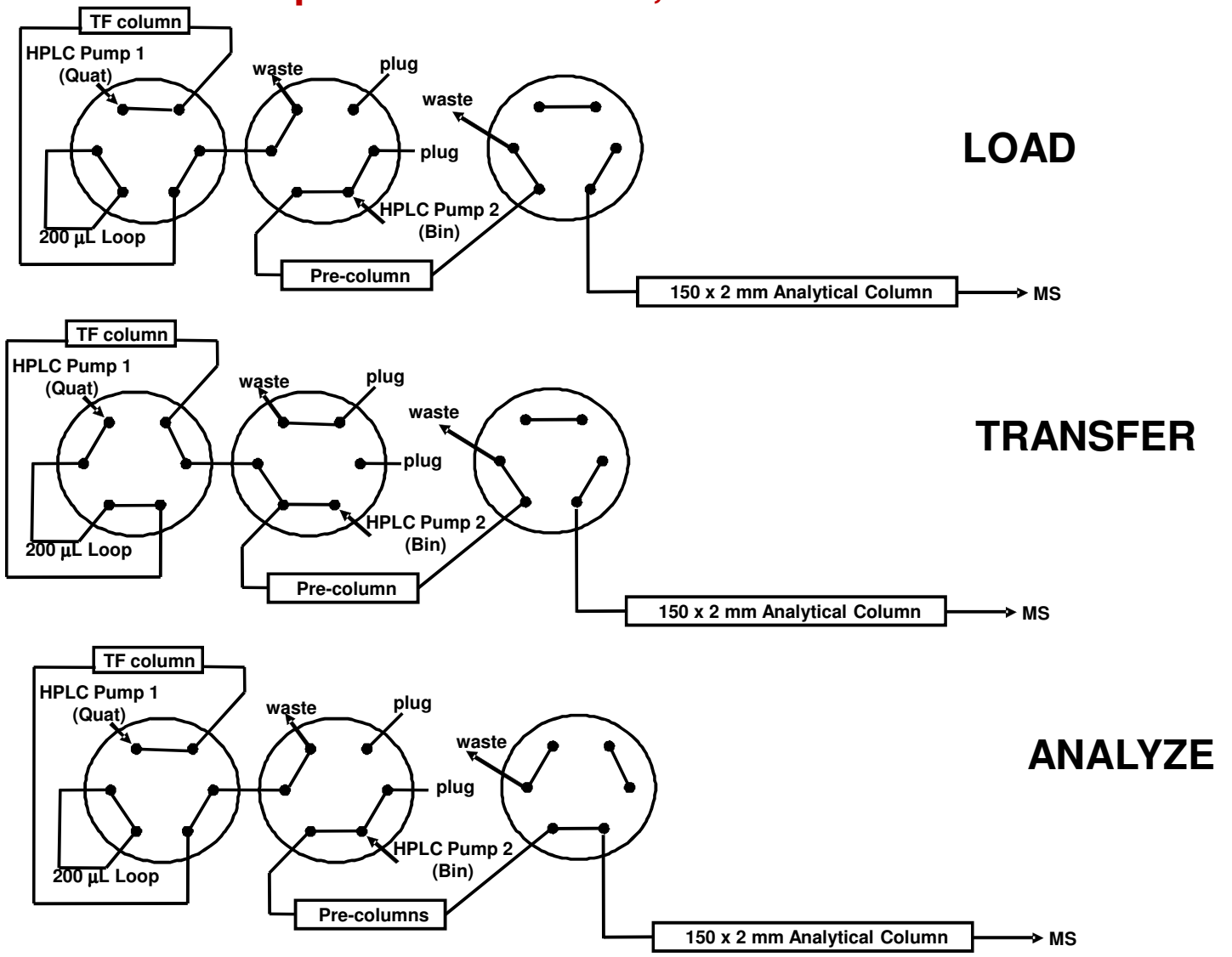
Trap column selection

Commonly accepted “rule” of HPLC method development suggest:

Guard column made from the same material as the main analytical column

That’s true, if there is no valve between guard and column

Choice of trap becomes critical; it is a NEW CENTER of LC configuration

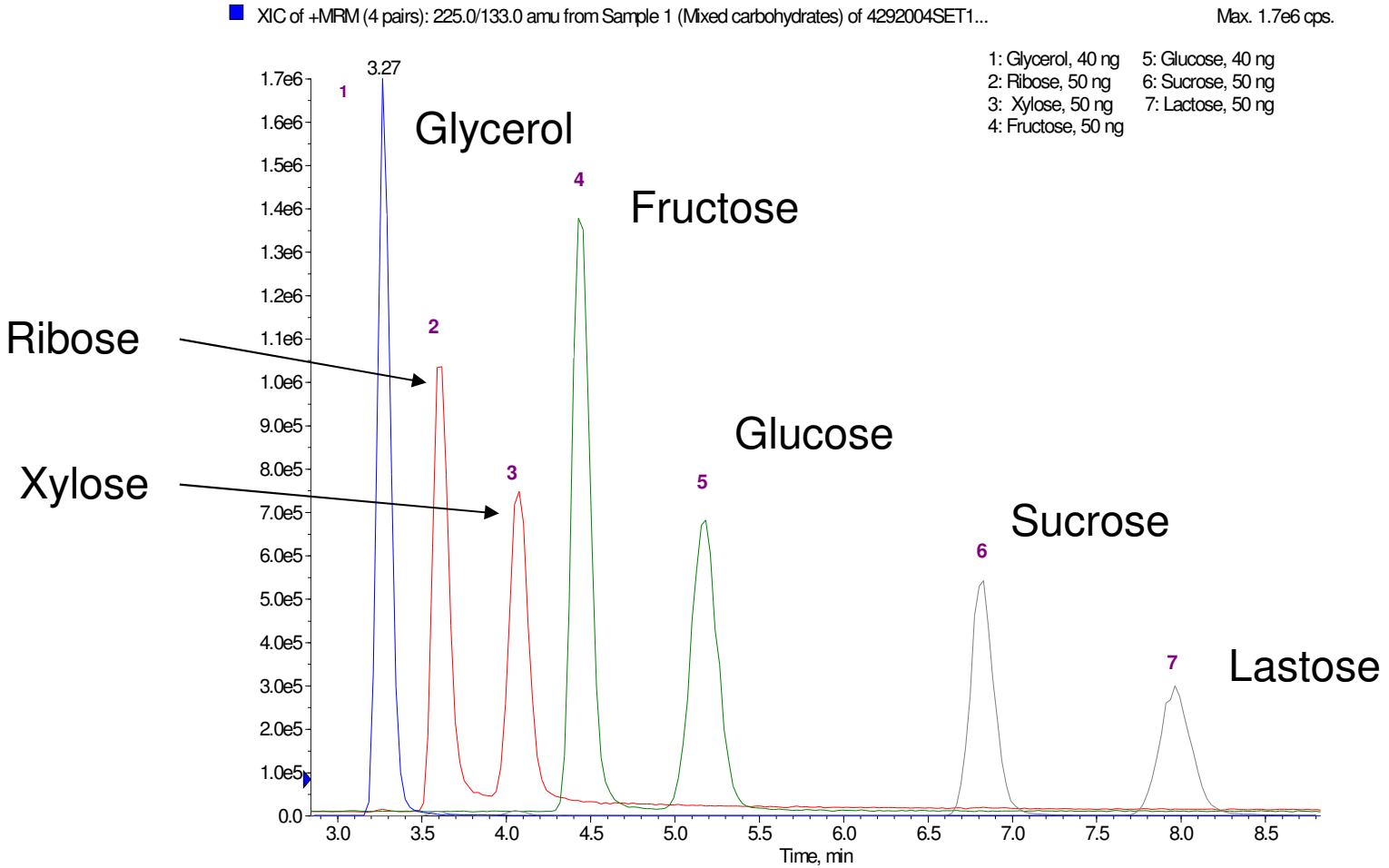


J.L. Herman. The Use of Turbulent Flow Chromatography and the Isocratic Focusing Effect to Achieve On-line Cleanup and Concentration of Neat Biological Sample for Low-level Metabolite Analysis. RCM 2005, 19, 696-700.

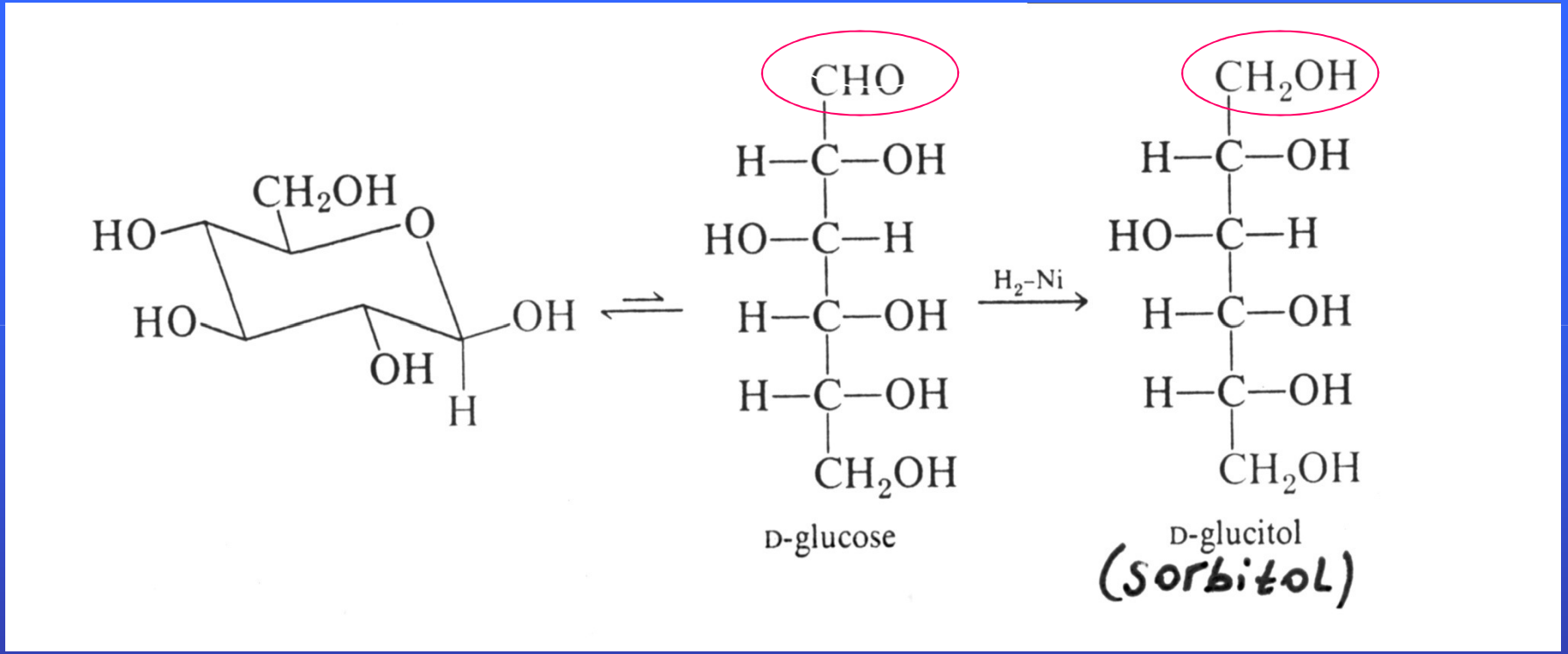
Sorbitol analysis from biological samples

by liquid chromatography - mass spectrometry

Carbohydrate detection by Cs^+ attachment



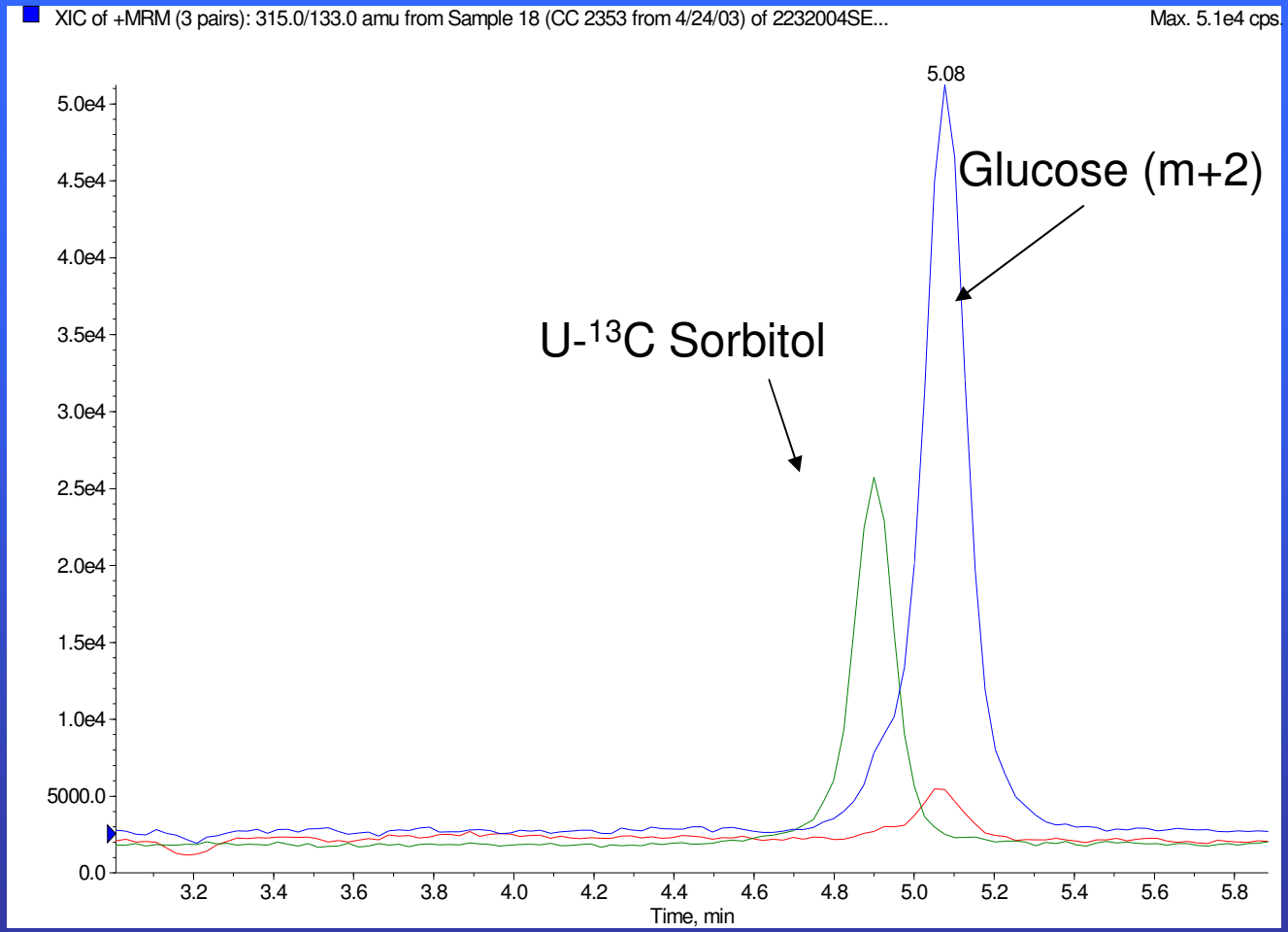
Glucose (MW 180) and sorbitol (MW 182)



Sorbitol LC analysis from plasma: Implications of glucose presence

- Sorbitol concentration in plasma < 1µM
- Glucose concentration is ~ 5 mM (> 5000 fold concentration difference).
- Incomplete resolution of these analytes results in overlap of sorbitol (M=182) with m+2 glucose (M=182) (APE 1.45%)
- Potential 70 fold signal contamination!

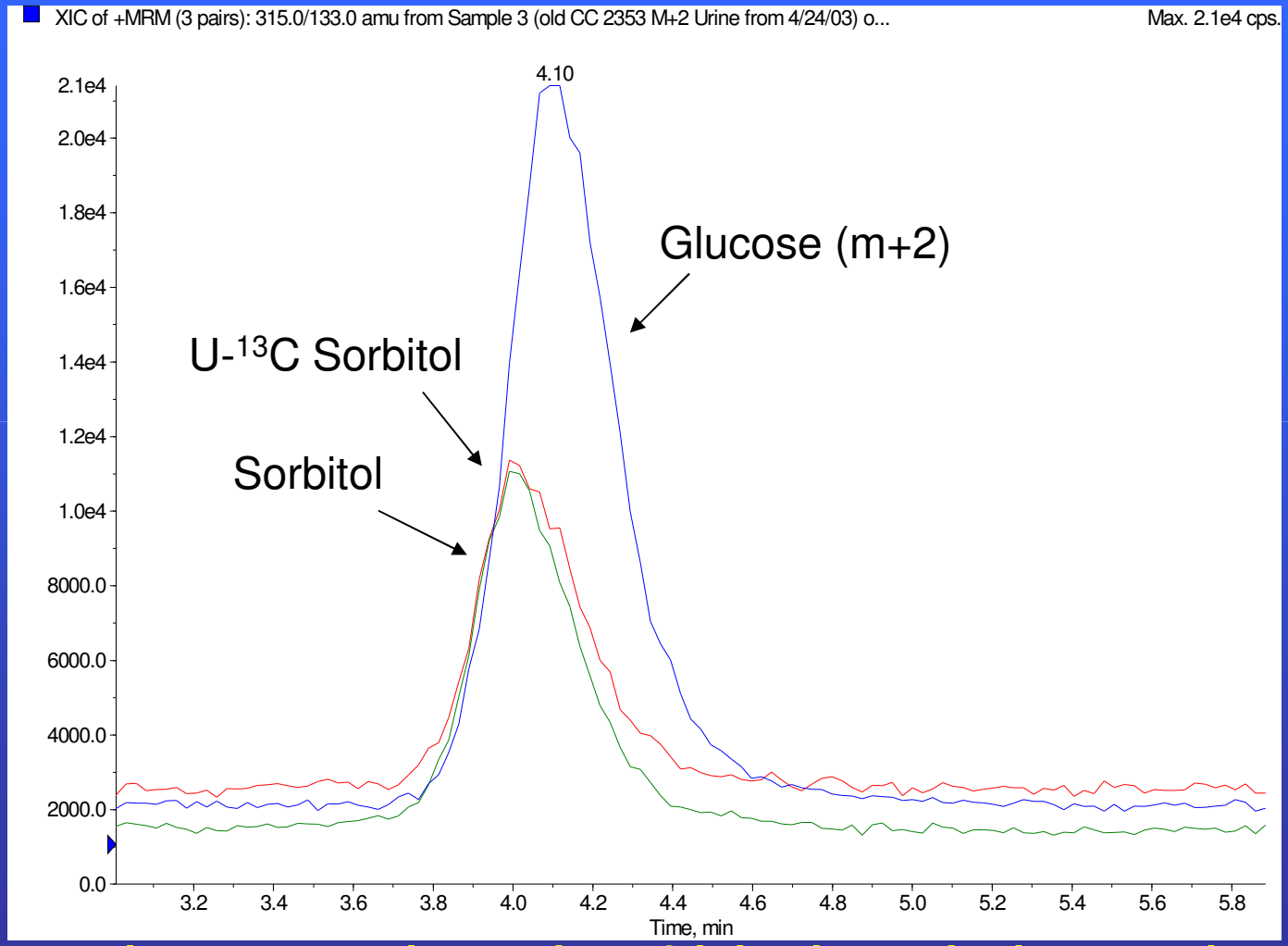
Brand new 3µ **Luna Amino column**, 2 x 150 mm, 2.5µl/inject



New Amino column; Sample –Urine, 1/20 in Acetone

-- U-C13 Sorbitol;

Amino column: degradation of resolution



Same sample to same column after 50 injections of urine samples.

-- U-C13 Sorbitol; --D2 Sorbitol;

Typical column lifetime

Clean samples: 1000 -2000 injections;
column cost per analysis is about $0.20,
Representing only about 1% of total cost per analysis

More complex samples: 200 -500 injections;
column cost per analysis is about $1,
Representing about 4% of total cost per analysis

Biological samples: 50 -200 injections;
column cost per analysis is about $3,
Representing about 10% of total cost per analysis

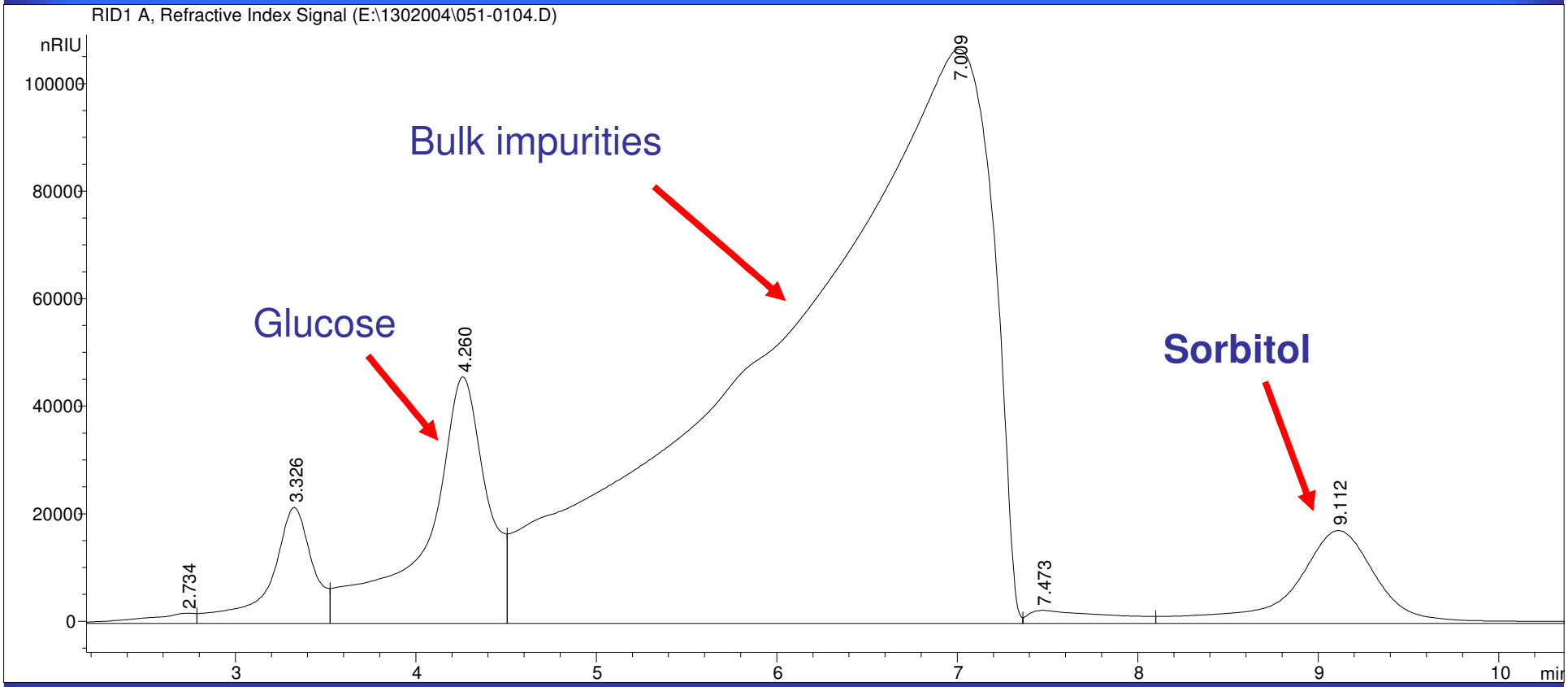
Practical HPLC method development, Second edition.
L. R. Snyder, J. L. Kirkland, J.L. Glajch. p 211.

Part 3. Current strategy and solution to the problem of glucose and sorbitol separation

Since the simultaneous separation of sorbitol from glucose, together with MS detection on one column is impossible, we are have decided to perform off-line separation of sorbitol from glucose.

Our new analytical solution is based on 2D LC pre-purification of sorbitol from glucose and other impurities in plasma samples. We will show that this scheme has significant advantages for MS detection versus "classic" single column methods.

Pre-purification of sorbitol from plasma extract



Purification was performed on 2 guard cartridges of REZEX Lead (Phenomenex) 7.8 x 50 mm

Off-line sorbitol purification: method development challenges

- **Sorbitol can be separated from glucose on Ion Exchangers, charged by Calcium, Sodium, Silver or Lead. These columns widely used in food analysis for carbohydrate analysis.**

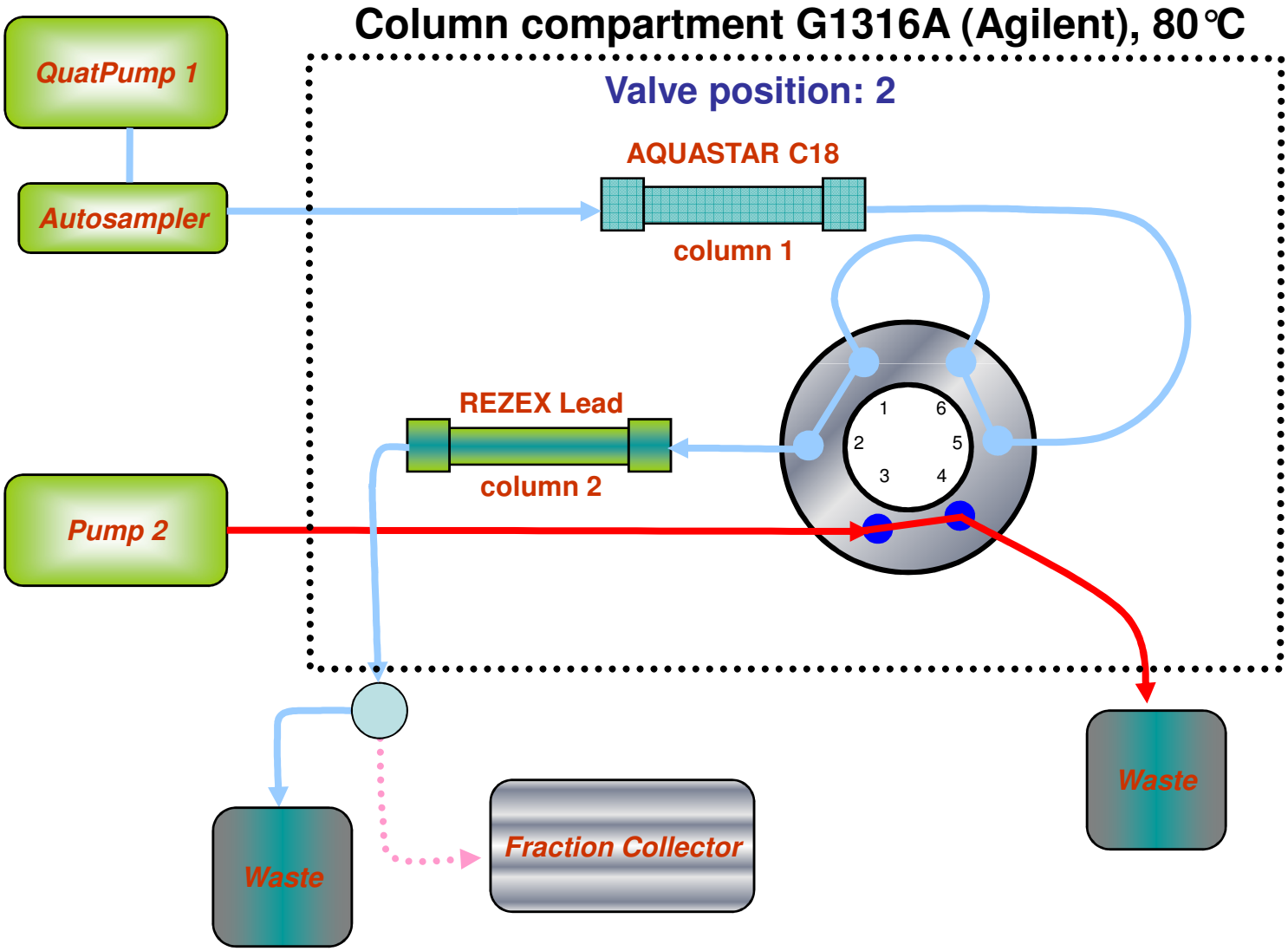
- **Plasma extract contains significant amount of lipids which bind tightly (poisoning) an anion exchanger. Regeneration from lipid contamination requires column wash by organic solvent.**

- **Typical carbohydrate IEX resin does not tolerate organic solvents. The problem: routine analysis of plasma samples is not robust, since the column cannot be regenerated after plasma lipid contamination.**

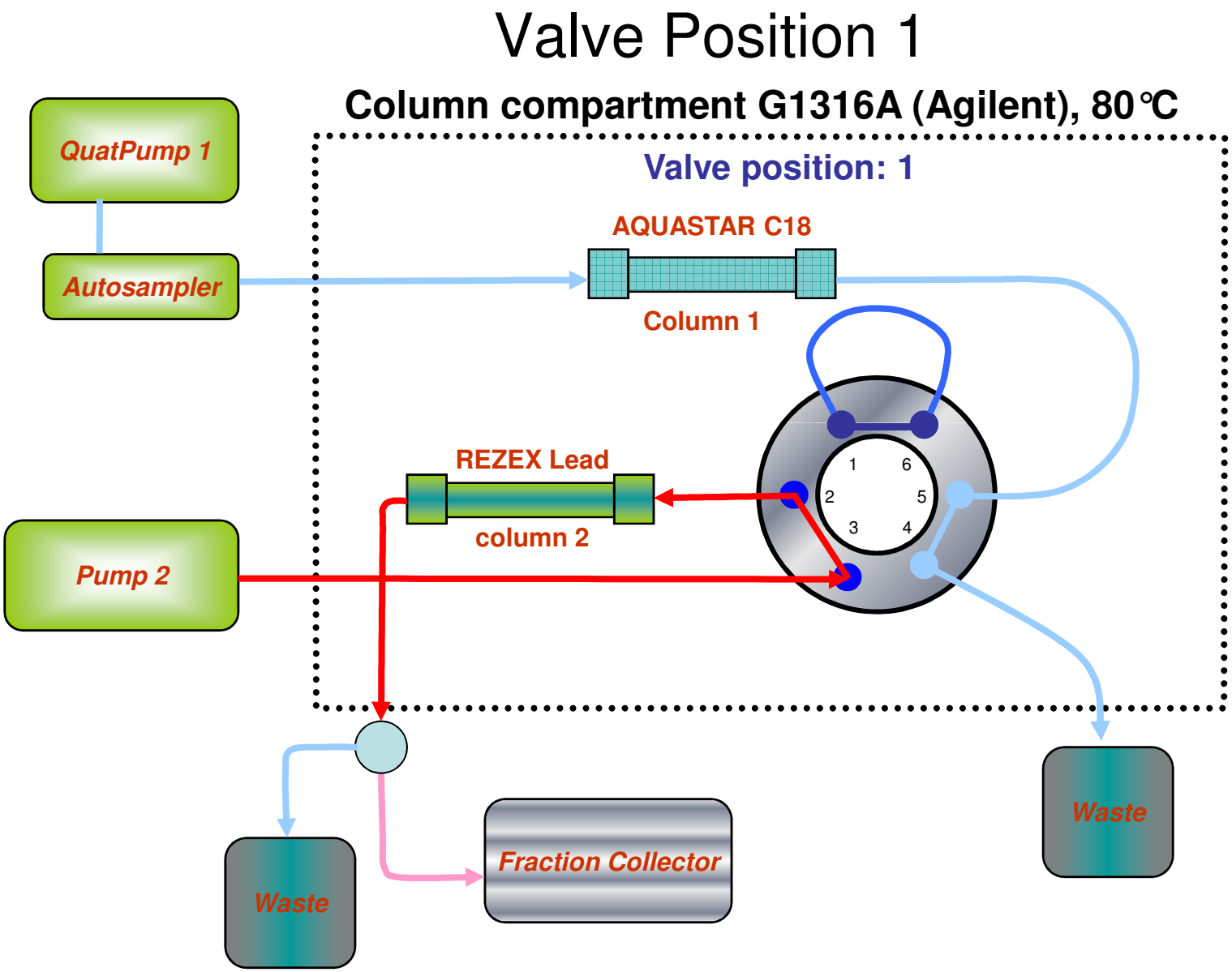
This problem was resolved by using
2D LC technique

Offline 2D pre-purification of sorbitol:

Valve Position 2



Offline 2D pre-purification of sorbitol:



2D LC method development summary

- Commonly accepted “rules” of HPLC method development suggest:
- 1. Guard column made from the same matrix as the main analytical column.
- 2. Most 2D LC applications use IEX in a first step, and RP – in the second.
- 3. The primary purpose of 2D technique development is a sensitivity enhancement of on-line analysis (by UV or MS) and as a rule, not used for fraction collection.
- In our method, all these 3 rules were inverted 180°. We demonstrate how opposing “common sense” can resolve difficult problems specific to bioanalytical mass spectrometry.

Conclusions: MS

- **Efficient chromatography plays a greater role in optimizing mass spectrometry sensitivity than advanced MS tuning.**

- **For LC/MS analysis from complex matrices - LC it is not just an "inlet" – it is the key first step toward increased mass spectrometry sensitivity.**

Acknowledgements

Funding:

This research was supported in part by a Clinical and Translational Science Award (CTSA) from the National Institutes of Health (UL11RR025740) awarded to the Albert Einstein College of Medicine of Yeshiva University.

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Agilent Technologies
IDEX corporation/Upchurch scientific
Optimize Technologies


Good seal;
no excess volume