Making LC/MS Analyses Accessible to Non Specialists

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Topics for Today

Workflows

Enabling Technologies

- Small particle LC columns
- Versatile LC systems to take advantage of LC columns
- Simple robust LC/MS instruments
  - Single Quadrupole
  - Time of Flight
  - (QQQ, QTOF, Ion Trap)
- The Multi-mode Source and Agilent JetStream Technology

What should an end user need to know?

- Reporting MS data or “just give me the answer”?

Enabling Software

The Target User

- Users who need a relatively simple answer from an LC/MS instrument with minimal interaction.
- Users who primarily don't have time to manage the LC/MS instrument. They want the instrument to wake up, run samples and go to sleep with miminal consumable waste. They want the instrument to maintain itself in a state of readiness.
- Environments where there are limited LC/MS experts, but large numbers of users!

Workflows – What's the question?

Did I make what I thought I made?

Synthetic Chemists

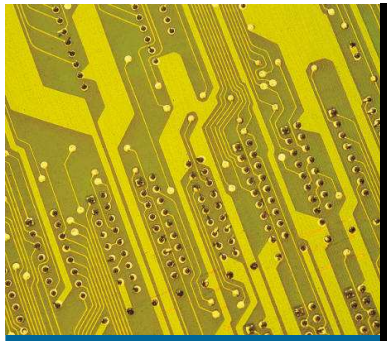
Medicinal Chemists

What's in this stuff?

What is it contaminated with?

Environmental and Toxicological Screening

Raw materials monitoring

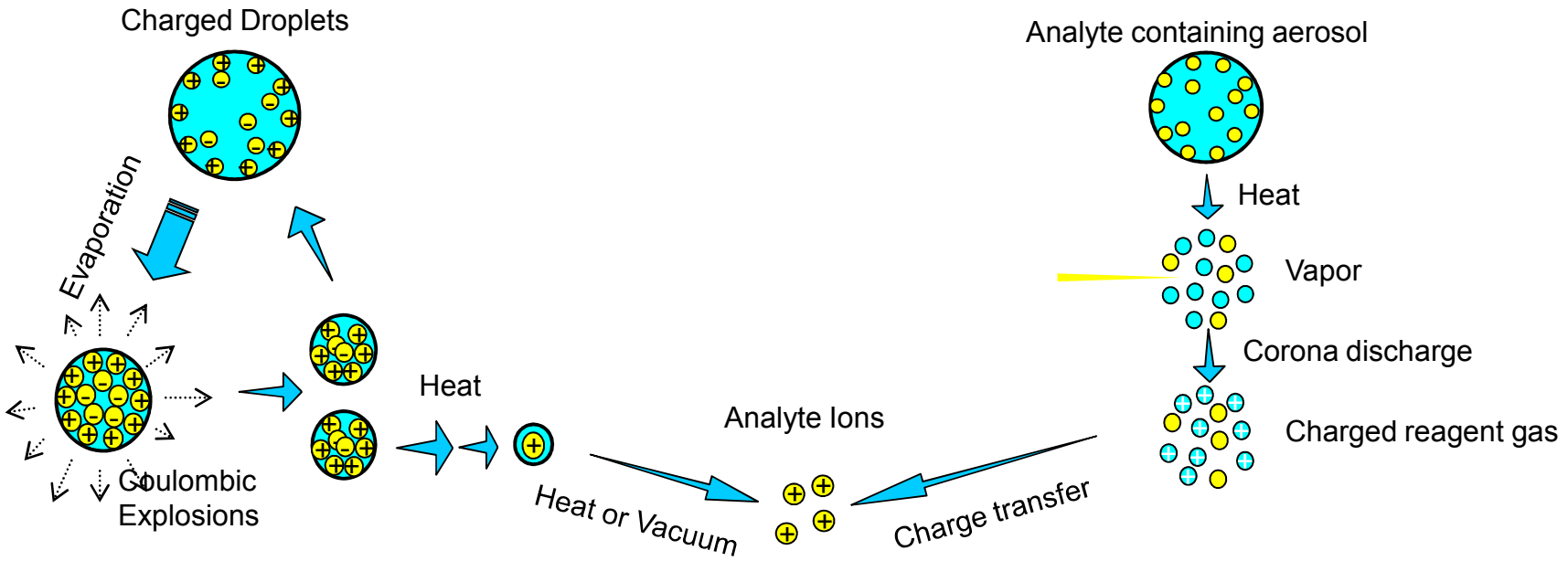




The Theory of Common LC/MS Ionization Modes

Atmospheric Pressure
Electrospray Ionization (ESI)

Atmospheric Pressure
Chemical Ionization (APCI)



- Spray into a charged environment
- Droplets evaporate and explode
- Single ion droplets evaporate

- Spray into a heated environment
- Vaporize into the gas phase
- Corona discharge creates ions
- Charge transfer generates analyte ions

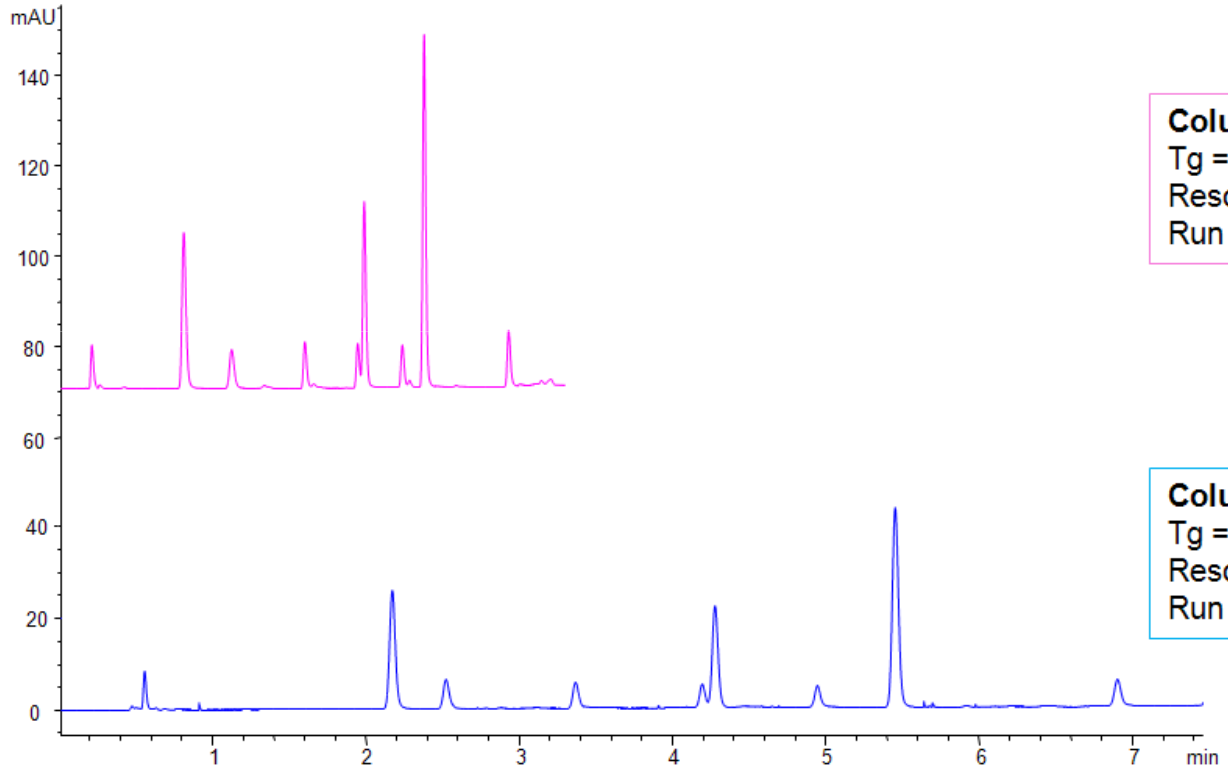
Enabling Technologies

From HPLC to RRLC on the 1220 Infinity LC

Varying Pore Size and Column Length

From HPLC to RRLC

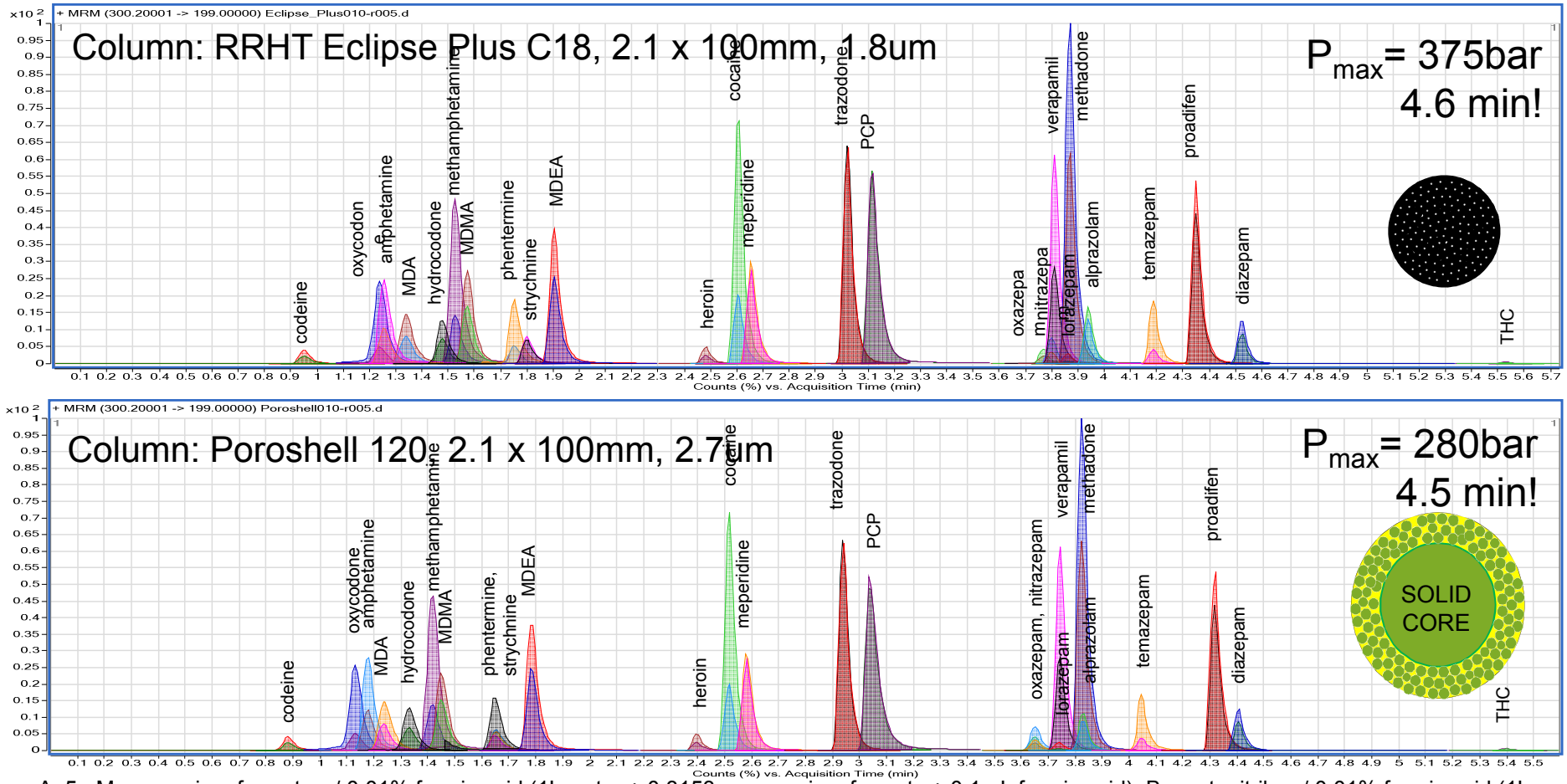
Flow rate: 1.2 mL/min at 40°C



Column: 3 x 50, 1.8 µm
Tg = 3 min
Resolution peak 6 = 1.09
Run time = 3.2 min

Column: 3 x 150, 5 µm
Tg = 9 min
Resolution peak 6 = 1.23
Run time = 7.5 min

Phenomenal Column Technologies: Identical Interchangeable Chemistries Totally Porous 1.8µm particles and Superficially Porous 2.7µm particles



A: 5mM ammonium formate w/ 0.01% formic acid (1L water + 0.3153g ammonium formate + 0.1mL formic acid), B: acetonitrile w/ 0.01% formic acid (1L acetonitrile + 0.1mL formic acid); 0.5mL/min; 10%B at t_0, ramp to 15%B in 0.5min, ramp to 50%B in 2.5min, ramp to 95%B in 1min, hold 95%B for 2min; stop time 6min, post run 2min; Sample: injector program: draw 5µL water, draw 1µL LC/MS Toxicology Test Mixture (PN 5190-0470), inject; TCC=60°C; MS Source: electrospray AP-ESI, drying gas temperature and flow: 350°C, 12L/min, nebulizer gas pressure: 30psi, capillary voltage: 2000V; MS Acquisition: dynamic MRM (+ve ionization polarity)

Agilent LC/MS Toxicology Test Mixture with **25 components** analyzed on an Agilent 1200 RRLC + Agilent 6410 QQQ.

Matching Needs to Technical Capabilities

1290 – Adds very fast gradients at up to 1200 bar

- 1200 bar pressure rating
- High sensitivity Diode Array Detector @ 160Hz
- Highest precision, lowest carryover

1260 - Adds fast gradients at up to 600 bar

- Total versatility….. All 1260 modules
- Add high precision low carryover autosampler options
- Both high and low pressure pump mixing options
- X 10 UV sensitivity
- Available in Bio-Inert model BIO inert
- Available as SFC (liquid CO_2) system

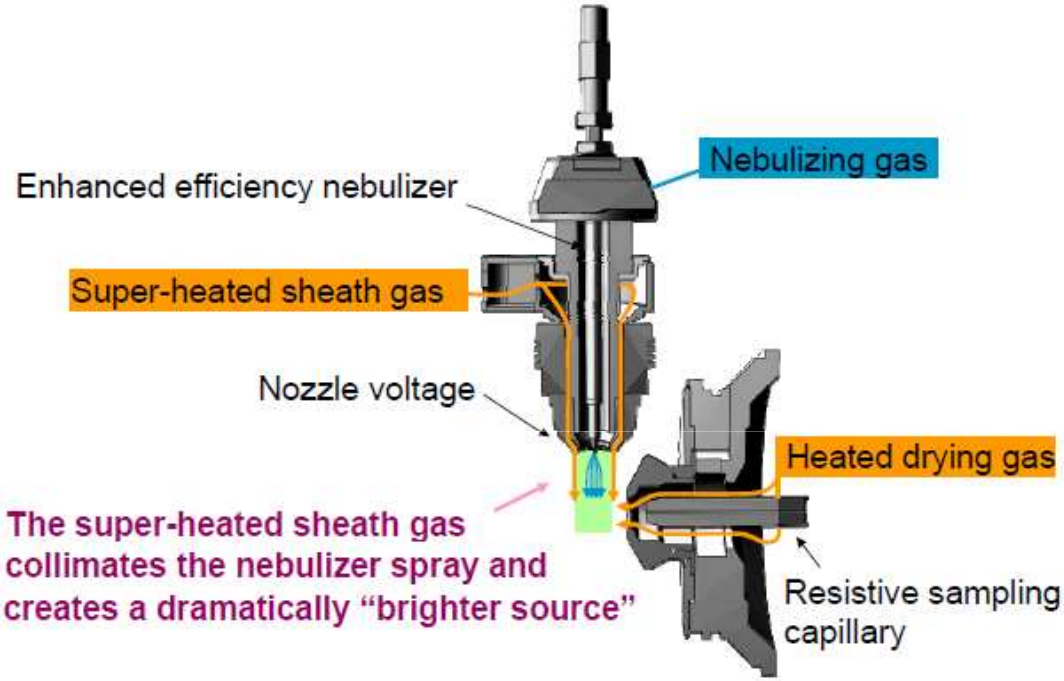
1220 – Integrated Simplicity

- Injection from vials or manual injection
- Single wavelength UV detection
- 600 bar pressure rating
- Isocratic or Low pressure mixing versions

RRLC – Rapid Resolution LC
The ability of a LC-system to make use of sub-two micron particle size columns to achieve faster - **more rapid** - and **higher resolving** analysis. This is also called UHPLC and has many other synonyms.

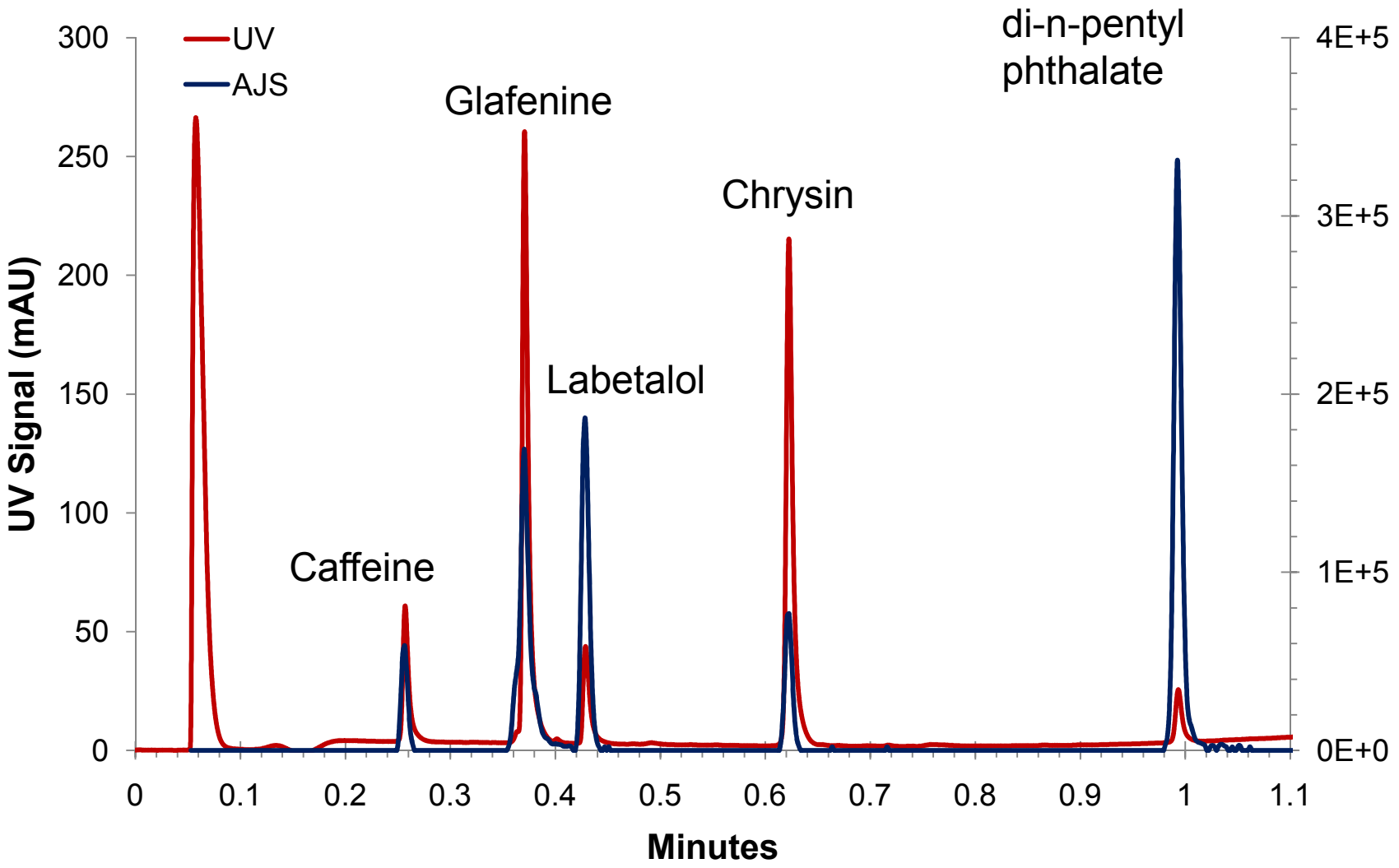
Agilent Jet Stream Technology

The sample is nebulized into a charged environment and inside a sheath of superheated nitrogen gas.



- ✓ Narrow chromatographic peak widths
- ✓ Very high signal to noise
- ✓ Ionization by multiple mechanisms
- ✓ Matched to the Agilent 1290 Infinity LC
- ✓ Integrated in the 6150 SQ and 6230 TOF

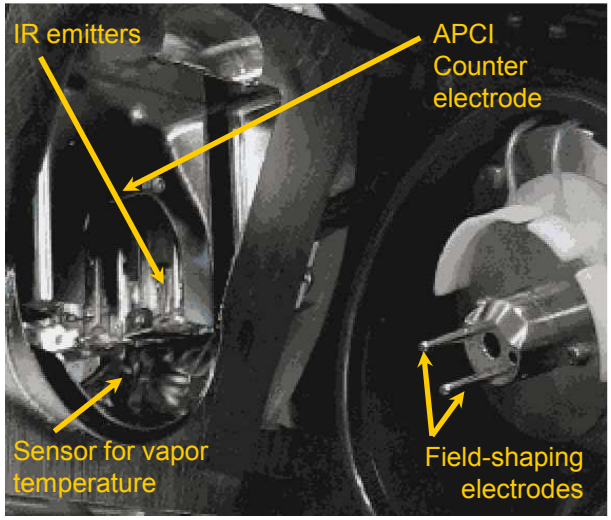
UV vs MS Peak Width Comparison

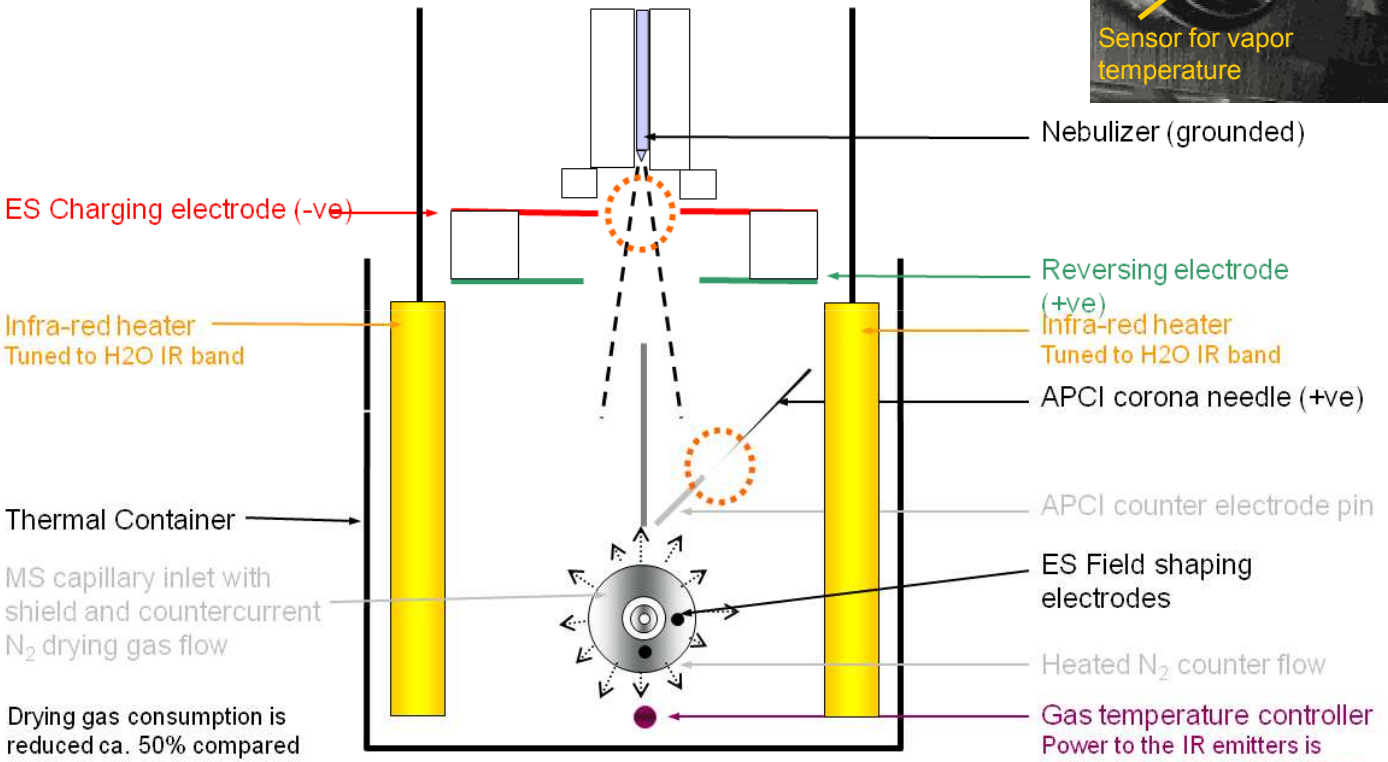


1290/6150 system, 1 min Gradient (water/**acetonitrile** with 0.05% TFA), 2.1x30mm Eclipse Plus C18, 1.8um

The Agilent Multi-Mode Source

- ✓ *Simultaneous* ESI and APCI
- ✓ Ionizes more than ESI or APCI alone
- ✓ Time saving



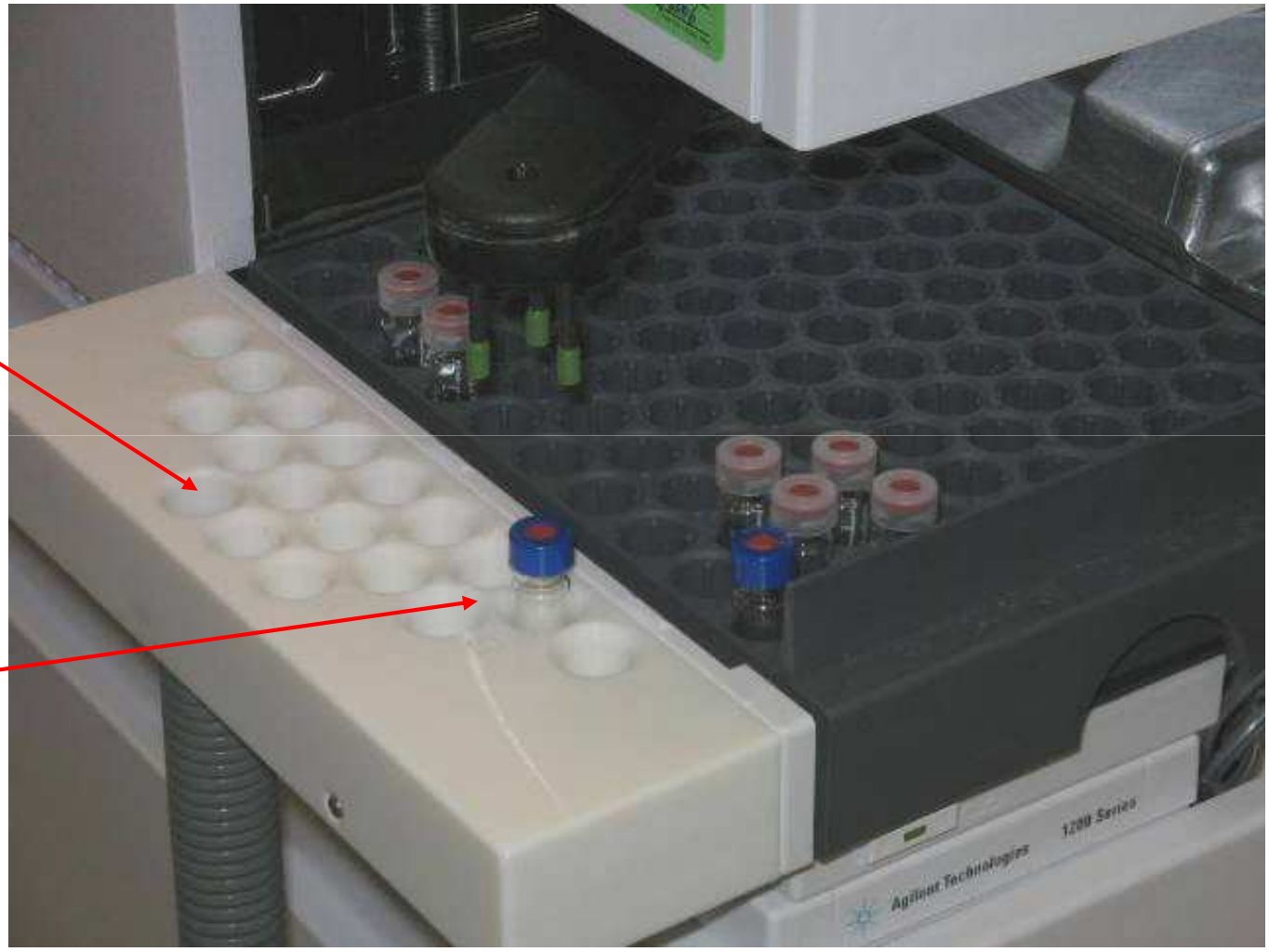


Building the Right Solution

External Tray for Standard Autosampler

Vials discarded here on completion (with optional delay)

Up to 17 vials in external tray Sampler arm moves them inside



LC and MS options: SQ and TOF instruments

1290
1200 bar
Highest precision
Highest speed

6150 SQ with Agilent JetStream Technology

Mass range	2-1350m/z
Max scan speed	**10000u/S**
50 x more sensitive than 6120B	
+/- switch time	20msec

1260
600 bar
Versatility

6130B SQ adds Versatility

Mass range	2-3000m/z
Max scan speed	5250u/S
10x more sensitive than 6120B	
+/- switch time	50msec

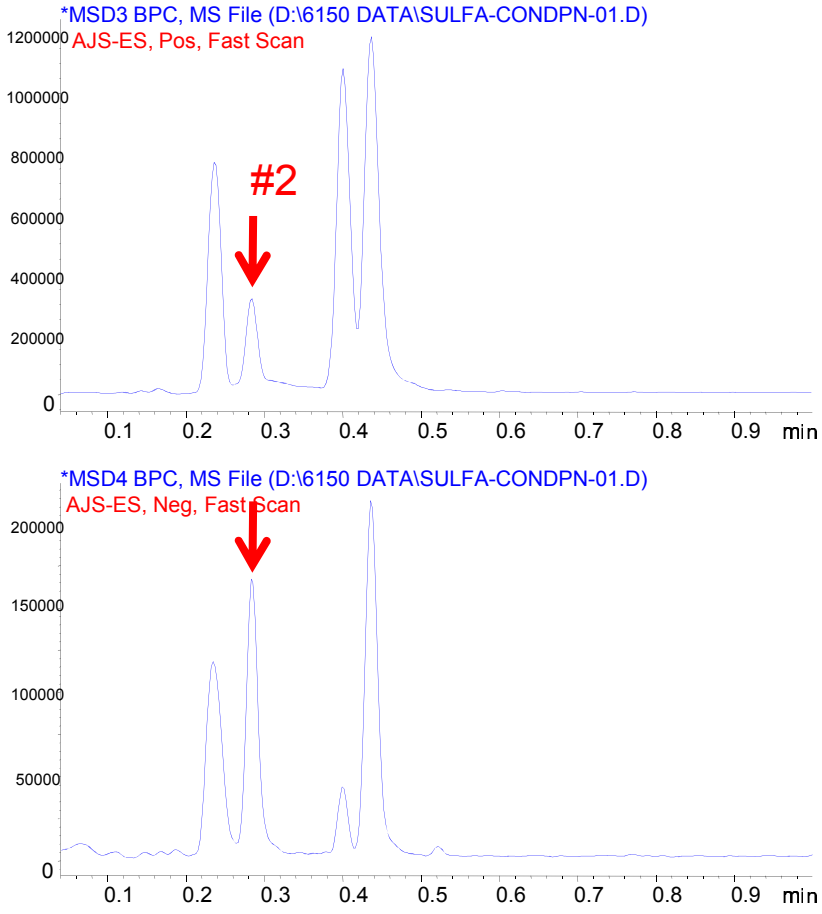
1220
600 bar
Simplicity
Lowest cost

6120B SQ Simple, robust

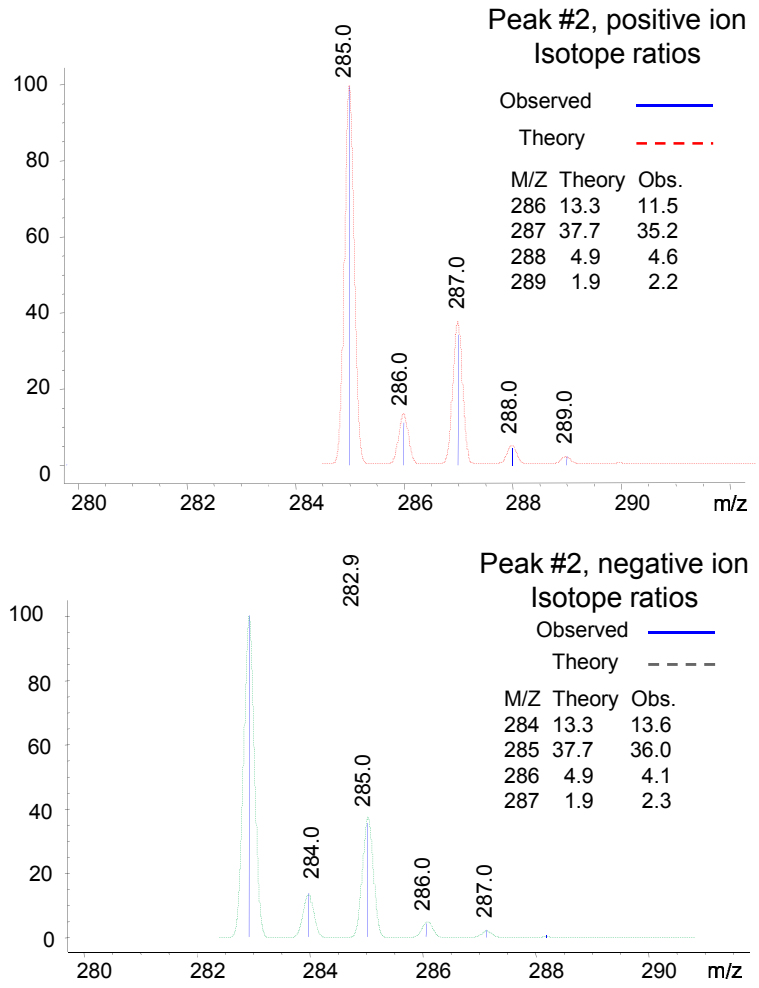
Mass range	2-2000m/z
Max scan speed	2500u/S
+/- switch time	300msec

Fast Scan POS/NEG Switching at 10,000 Da / sec and 20 msec

Gives more confident compound identifications



Base Peak Chromatogram



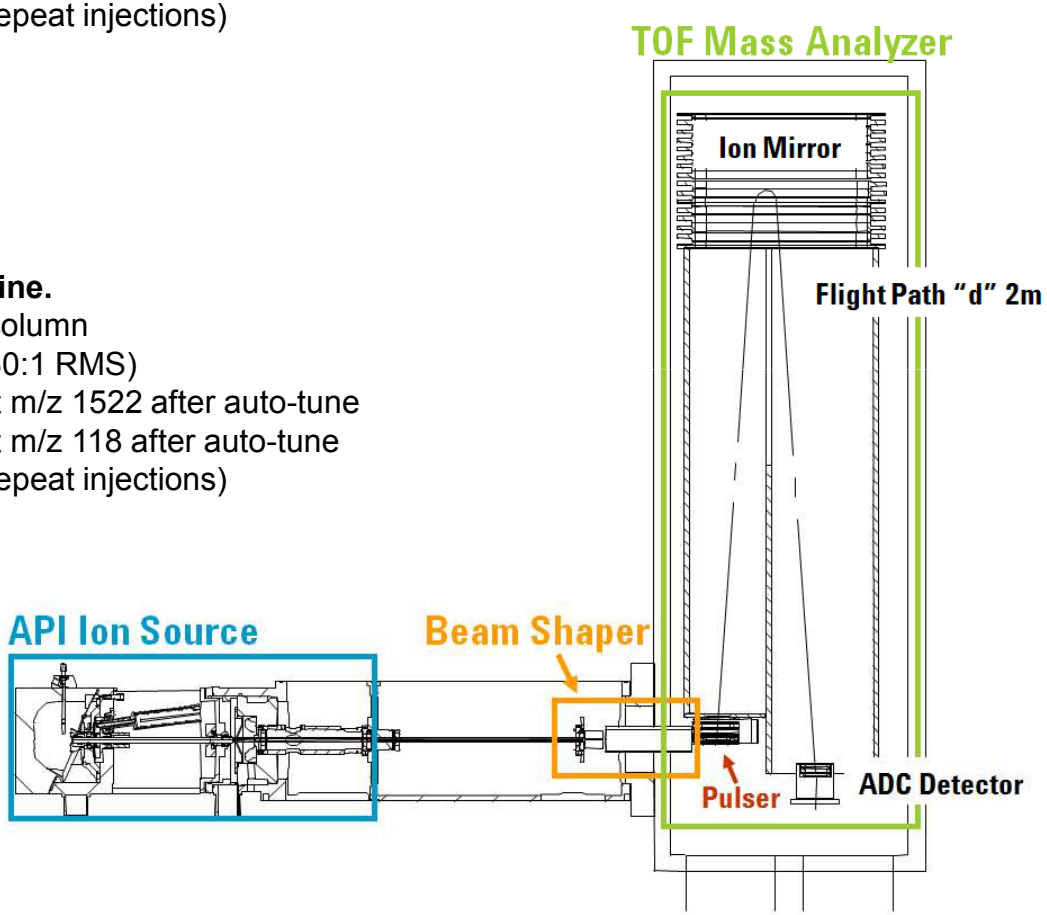
Accurate isotope ratios

Agilent 6230 TOF with Agilent JetStream Technology

Sensitivity	**2 pg: 20:1 reserpine.** MS mode, ESI,on-column 10:1 RMS (typical 30:1 RMS)
Mass resolution	> **20,000 FWHM** at m/z 1522 after auto-tune > 10,000 FWHM at m/z 118 after auto-tune
Mass accuracy	< 2 ppm RMS (10 repeat injections)
Mass range	20000 m/z
Acquisition speed	40 scans/sec

Agilent 6224 TOF

Sensitivity	**10 pg: 20:1 reserpine.** MS mode, ESI,on-column 10:1 RMS (typical 30:1 RMS)
Mass resolution	> **20,000 FWHM** at m/z 1522 after auto-tune > 10,000 FWHM at m/z 118 after auto-tune
Mass accuracy	< 2 ppm RMS (10 repeat injections)
Mass range	20000 m/z
Acquisition speed	40 scans/sec



How Much Accuracy is Needed?

Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066

Single quad reports mass to +/- 0.1 = 165 ppm

Number of possible formulas using only C, H, O & N:

- 165 ppm 209
- 10 ppm 13
- 5 ppm 7
- 3 ppm 4
- 2 ppm 2

Accurate mass reduces number of candidates and risk of investing effort with the wrong molecule

LC and MS options: SQ and TOF instruments

1290
1200 bar
Highest precision
Highest speed

6150 SQ
Adds Agilent JetStream Technology

6230 TOF
Adds Agilent JetStream Technology,
5x Sensitivity + greater peak capacity

1260
600 bar
Versatility

6130B SQ
Adds Versatility

6224 TOF
Simple, Stable
Sub 1-2ppm mass accuracy

1220
600 bar
Simplicity
Lowest cost

6120B SQ
Simple, robust

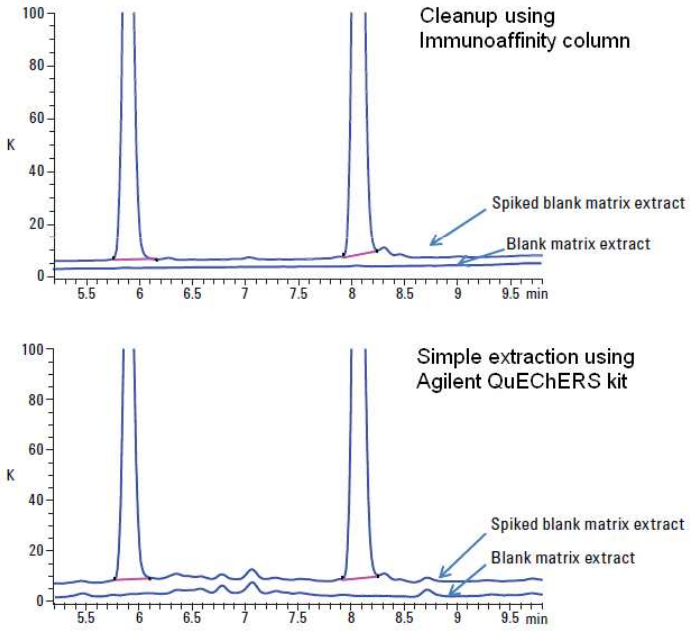
Implementing Solutions

Analysis of fumonisin, FB1 & FB2 Mycotoxins, in Corn

Ref. Syed Salman Lateef, Agilent publication number: 5990-6560EN

Fumonisins are fungal metabolites, which can cause mycotoxicosis when ingested. The FB1, FB2 and FB3 types can contaminate corn samples. Amounts in food and feed are regulated.

"The major challenge … is that different extraction procedures may be required for different food matrices."



Overlay of blank matrix peaks and the standard spiked in blank matrix peak.

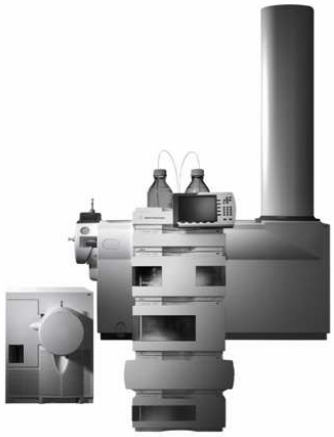
Sample Name	FB1 (µg/Kg)	FB2 (µg/Kg)	Total Fumonisin (µg/Kg)
Dried Corn - IAC	797	204	1001
Dried Corn – EM	735	189	924
Popcorn – IAC	380	139	519
Popcorn – EM	319	123	442
Corn sour/turtle food/rice grain - IAC	ND	ND	ND
Corn sour/turtle food/rice grain - EM	ND	ND	ND

Fumonisin analysis on food and feed samples extracted by IAC and EM methods.

LC/MS: Agilent 1220 Compact LC plus Agilent 6120 Single Quadrupole LC/MS

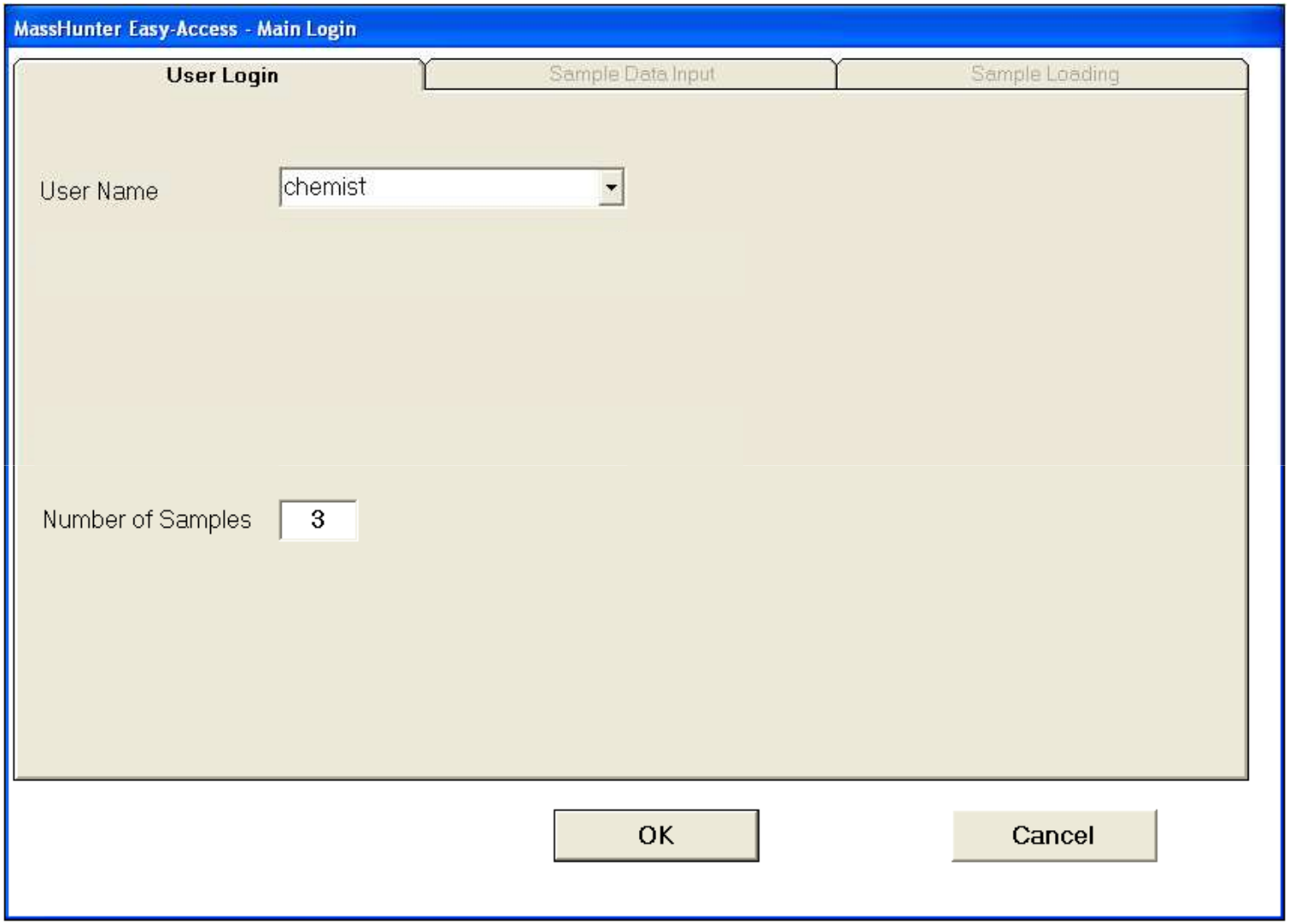
Agilent MassHunter Easy-Access Software

Walk-up LC and LC/MS

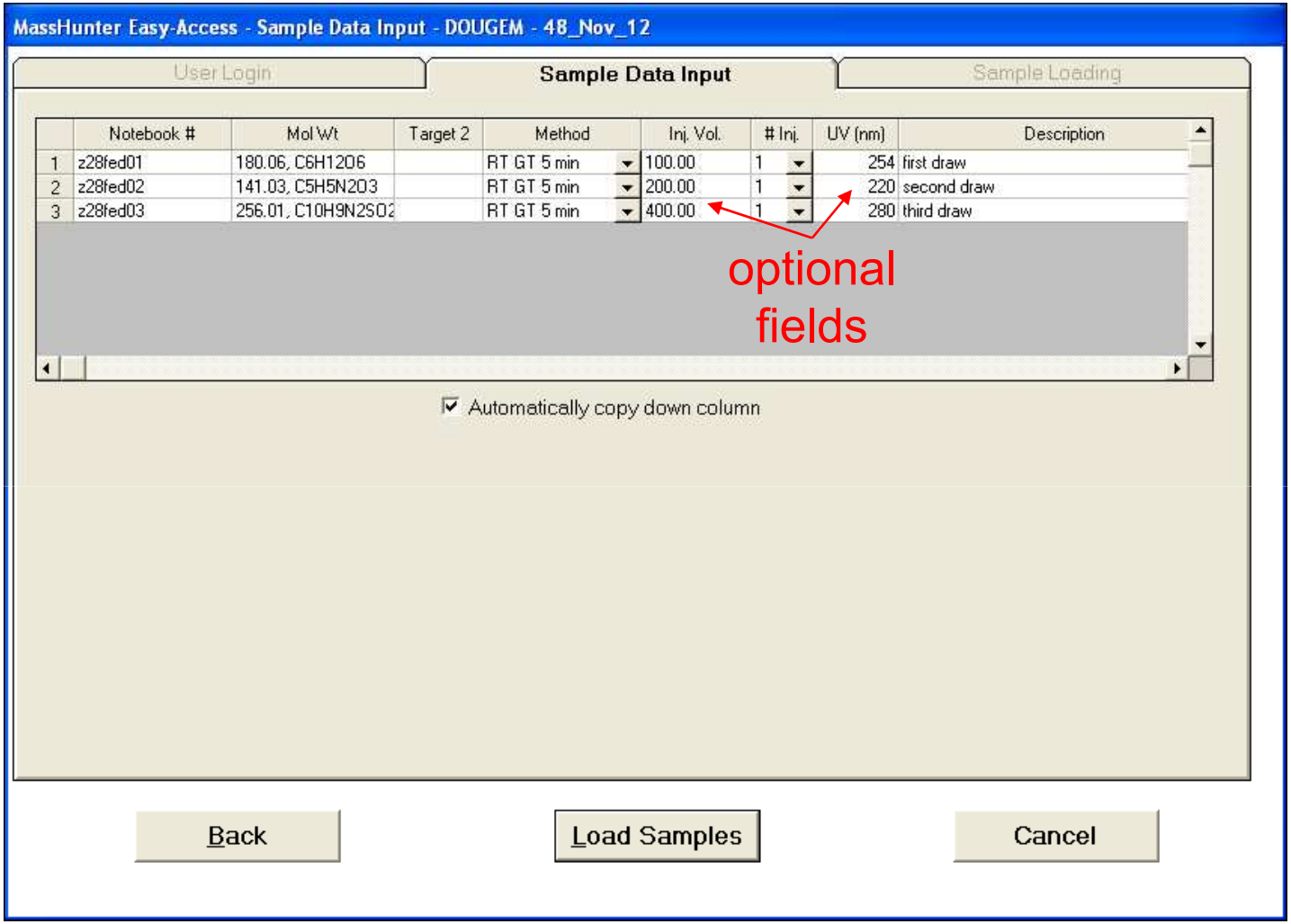


- ✓ Simplified user interface for anyone using LC, LC/MS single quad, or LC/MS TOF systems
- ✓ Expandable to large numbers of users and multiple instruments in different locations
- ✓ Configurable to match laboratory SOPs, specific terminology, usage rules, etc.
- ✓ One skilled user required for setup and administration
- ✓ One semi-skilled user can manage multiple systems!

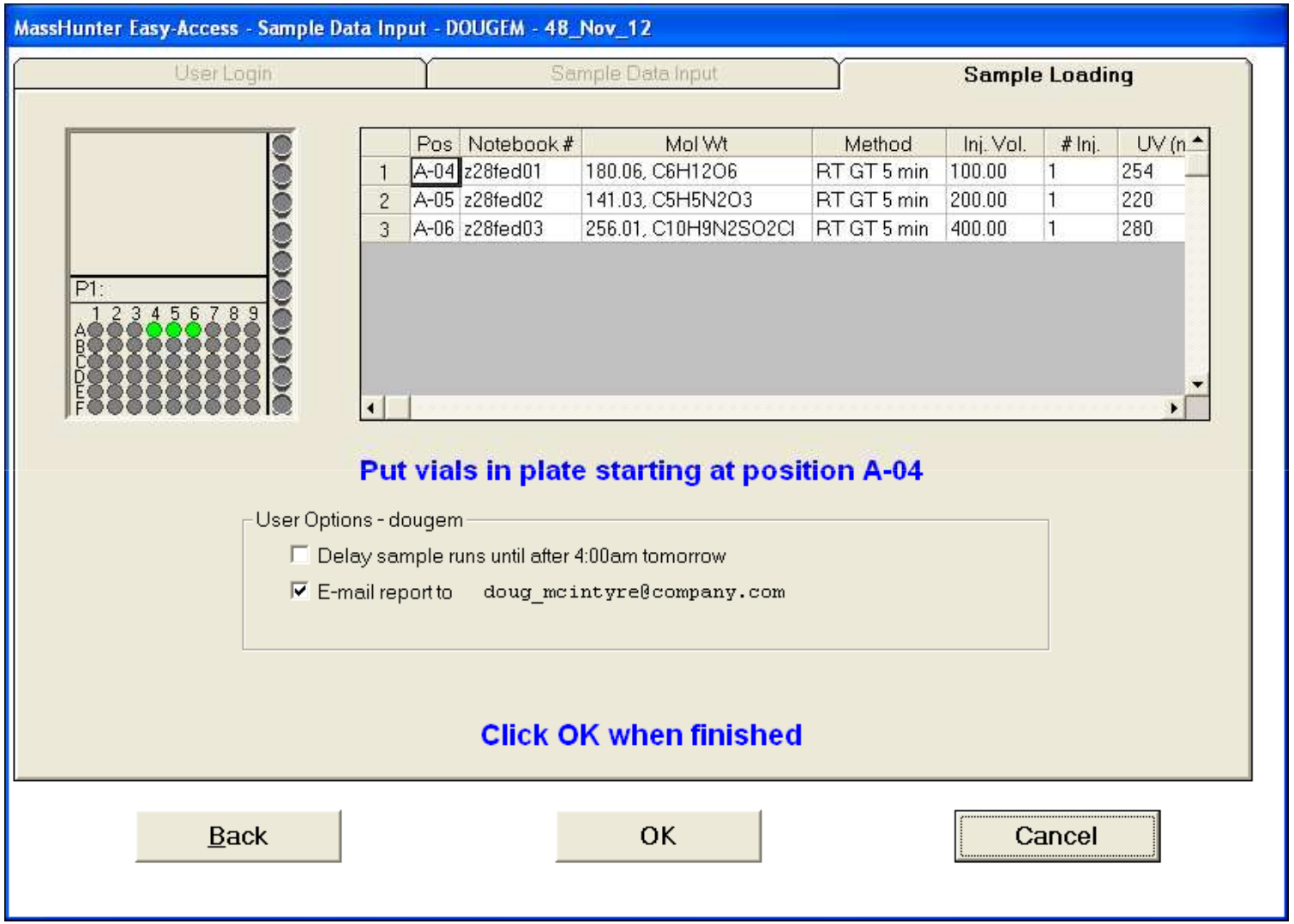
Sample Login – Step 1 of 3 – Who and How Many?



Sample Login – Step 2 of 3 – Sample Details

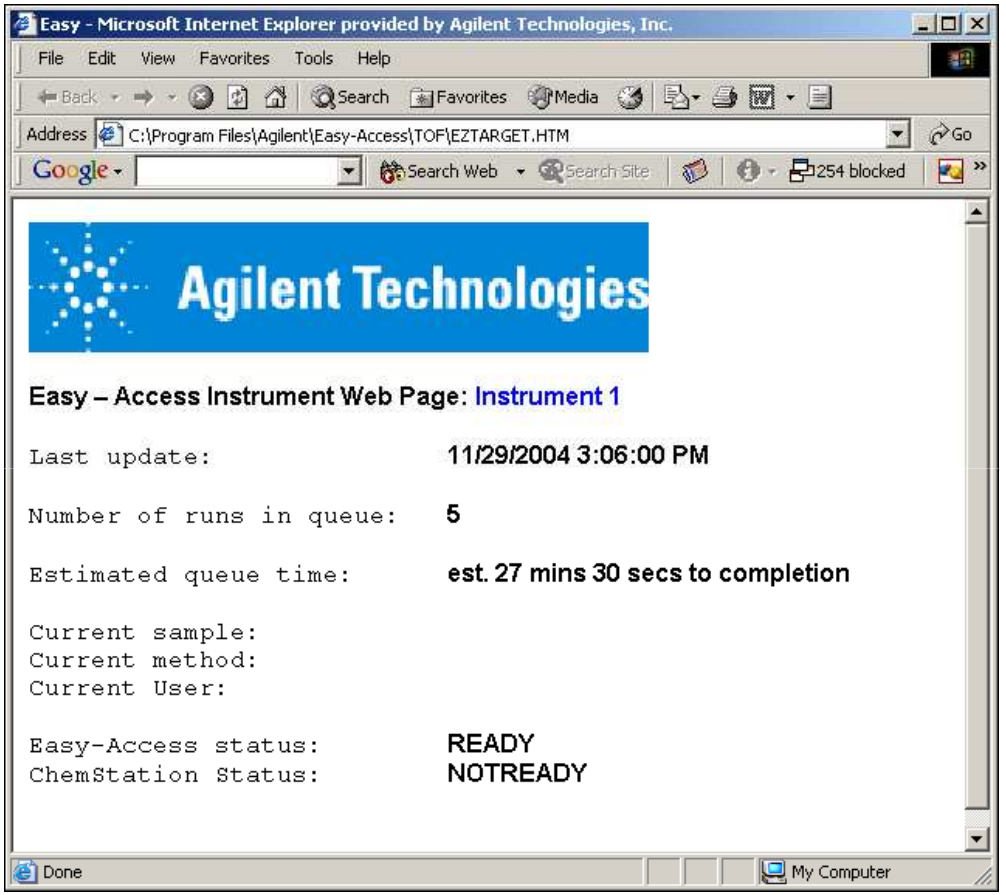


Sample Login – Step 3 – Confirm and Commit

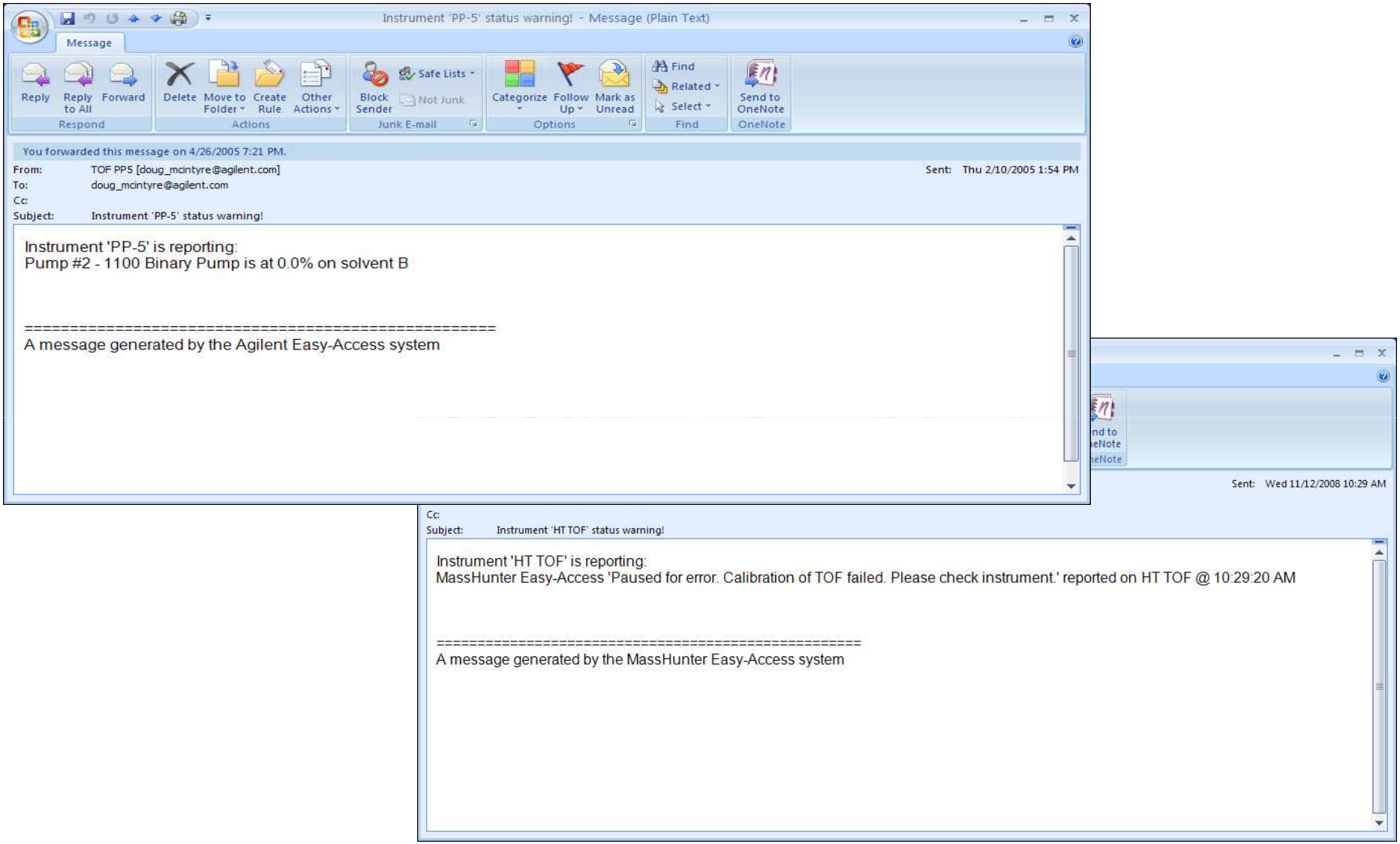


Web Access to Instrument Status with Easy Access

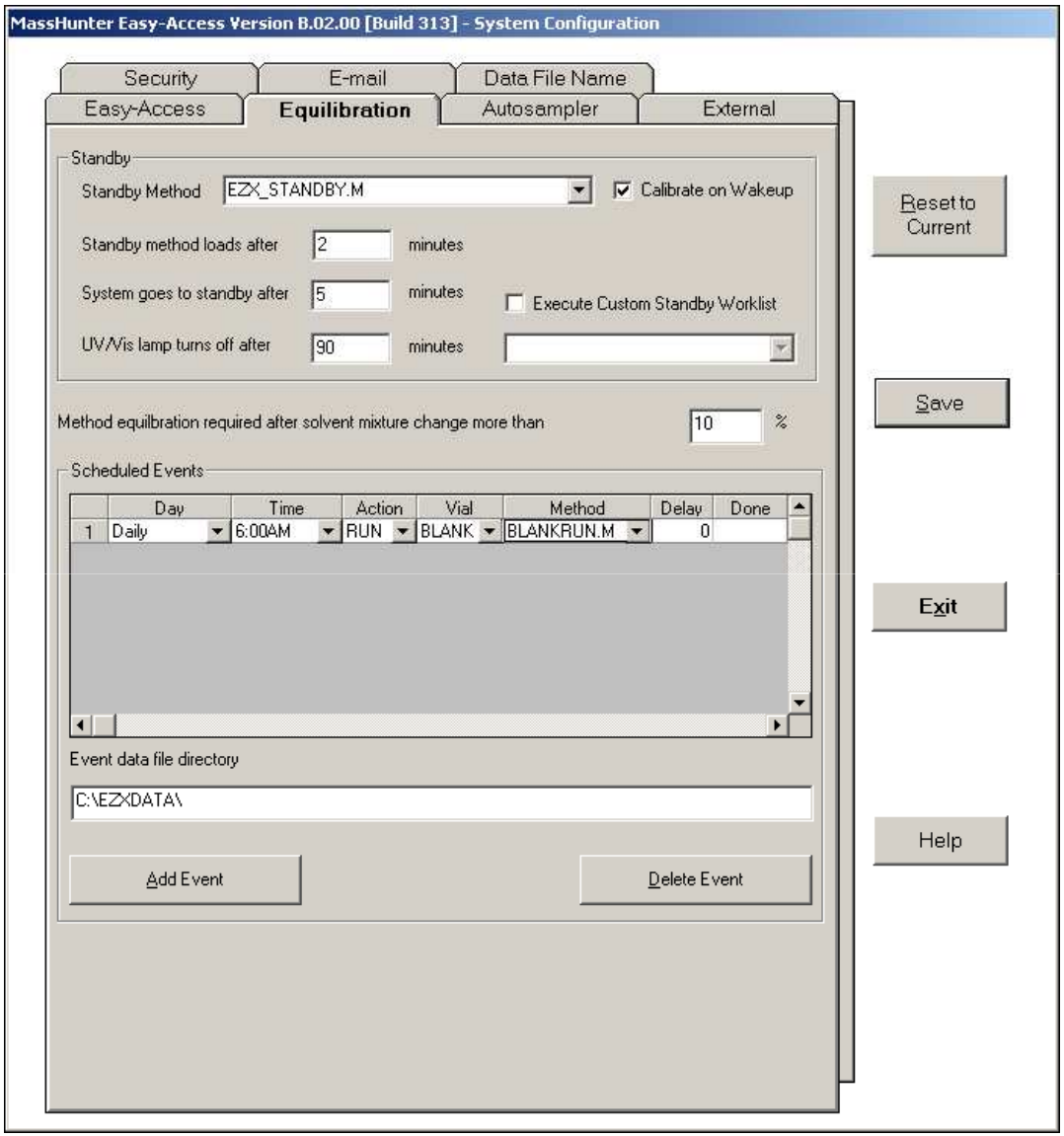
Instrument status can be viewed over the internet with a customizable template



E-mail Notification of System Problems



Administration – Events and Shutdown Protocol

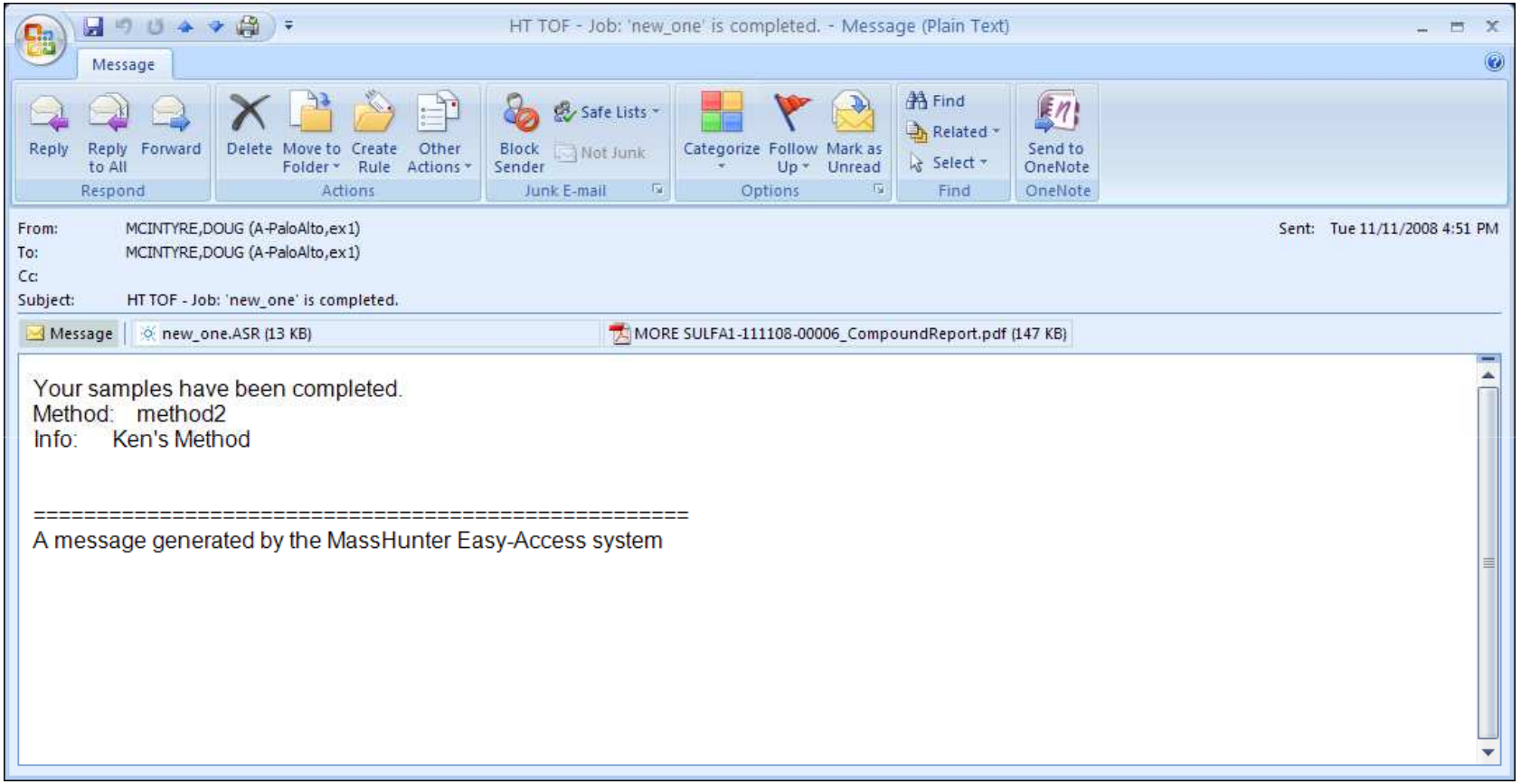


Automatic calibration of TOF on Wakeup or through Events Scheduler

Automatic calibration of TOF on Wakeup or through Events Scheduler

Events can be scheduled to run at a specific time, every ‘n’ samples or on Wakeup or Startup

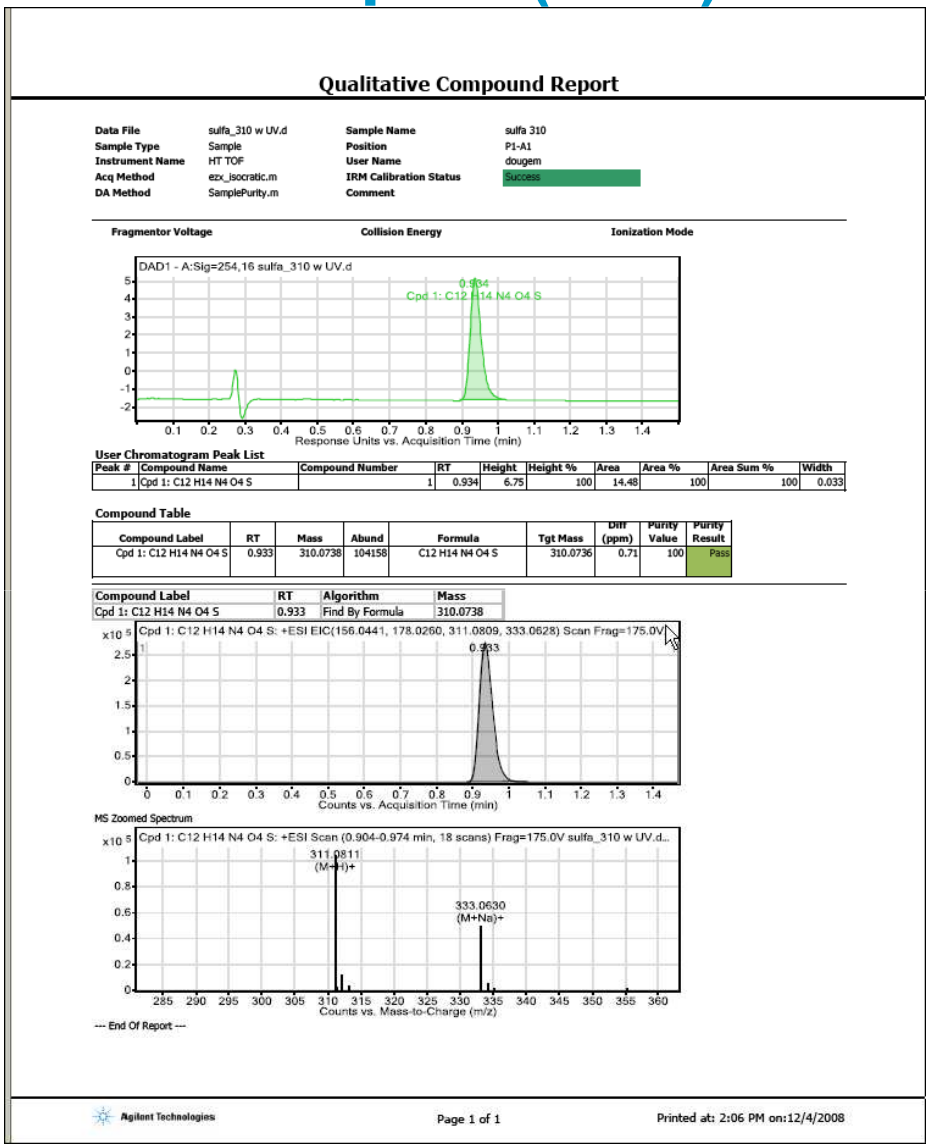
E-mail Notification of Sample Results



Will e-mail ASR files, reports, and/or raw data

Empirical Formula Confirmation Report (TOF)

- Formula input by submitter and system calculates expected monoisotopic mass
- Extracted ion chromatogram covering specified adducts
- Zoomed spectrum covering adduct range
- Calculated mass error results
- Calculated purity results based on MS, UV or other detector data



Qualitative Compound Report

Data File	sulfa_310 w UV.d	Sample Name	sulfa 310
Sample Type	Sample	Position	P1-A1
Instrument Name	HT TOF	User Name	dougem
Acq Method	ezx_isocratic.m	IRM Calibration Status	Success
DA Method	SamplePurity.m	Comment	

Fragmentor Voltage | Collision Energy | Ionization Mode

DAD1 - A:Sig=254,16 sulfa_310 w UV.d

0.934
Cpd 1: C12 H14 N4 O4 S

Response Units vs. Acquisition Time (min)

User Chromatogram Peak List

Peak #	Compound Name	Compound Number	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	Cpd 1: C12 H14 N4 O4 S	1	0.934	6.75	100	14.48	100	100	0.033

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Purity Value	Purity Result
Cpd 1: C12 H14 N4 O4 S	0.933	310.0738	104158	C12 H14 N4 O4 S	310.0736	0.71	100	Pass

Compound Label	RT	Algorithm	Mass
Cpd 1: C12 H14 N4 O4 S	0.933	Find By Formula	310.0738

Cpd 1: C12 H14 N4 O4 S: +ESI EIC(156.0441, 178.0260, 311.0809, 333.0628) Scan Frag=175.0V

0.933

Counts vs. Acquisition Time (min)

MS Zoomed Spectrum

Cpd 1: C12 H14 N4 O4 S: +ESI Scan (0.904-0.974 min, 18 scans) Frag=175.0V sulfa_310 w UV.d...

311.0811
(M+H)+

333.0630
(M+Na)+

Counts vs. Mass-to-Charge (m/z)

--- End Of Report ---

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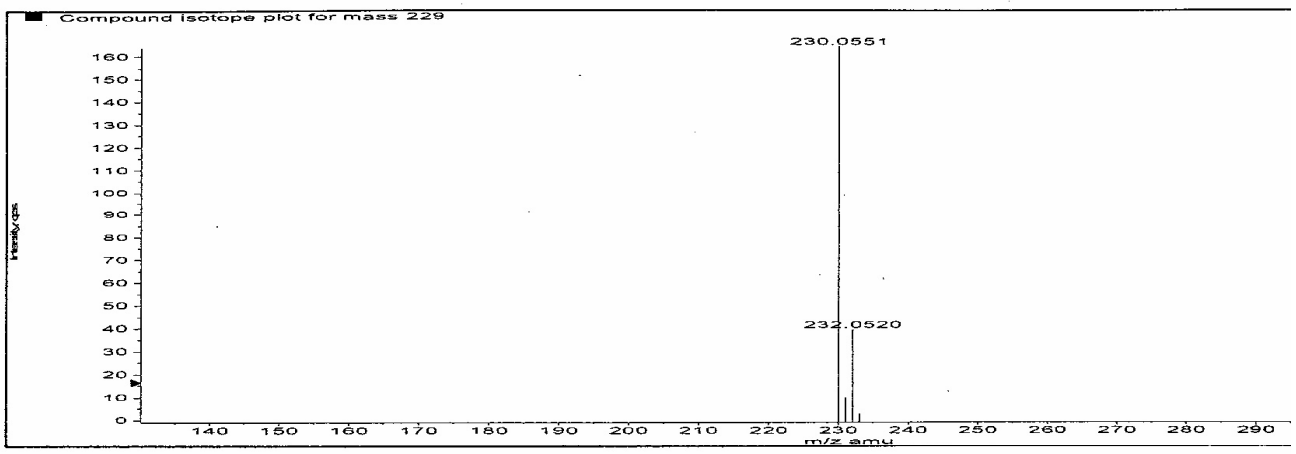
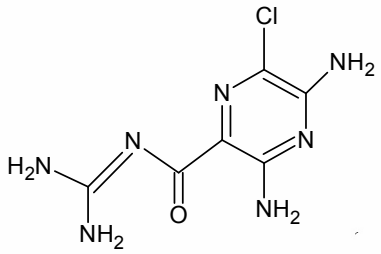
MassHunter Data Analysis
MFE and MFG – Fundamental Algorithms

- **Both algorithms are crucial for Agilent's MassHunter SW**
  - Used in several software applications for accurate mass LC/MS data
  - Very competitive and unique compared to other vendor's offerings
- **Molecular Feature Extractor (MFE)**
  - Allows finding molecular features (= compounds) in an LC/MS run in the MS data domain (identify co-eluting and related mass signals)
  - Works on accurate mass MS data from TOF and Q-TOF.
- **Molecular Formula Generator (MFG)**
  - Allows determination of molecular formulas of unknown compounds using accurate mass TOF MS and Q-TOF MS/MS data
  - If multiple adducts or charge states selected, get cross score
  - Unique scoring algorithm

Automated Molecular Feature Extraction - TOF



Experiment# : 1 Compound# : 5 Mass : 229.04786



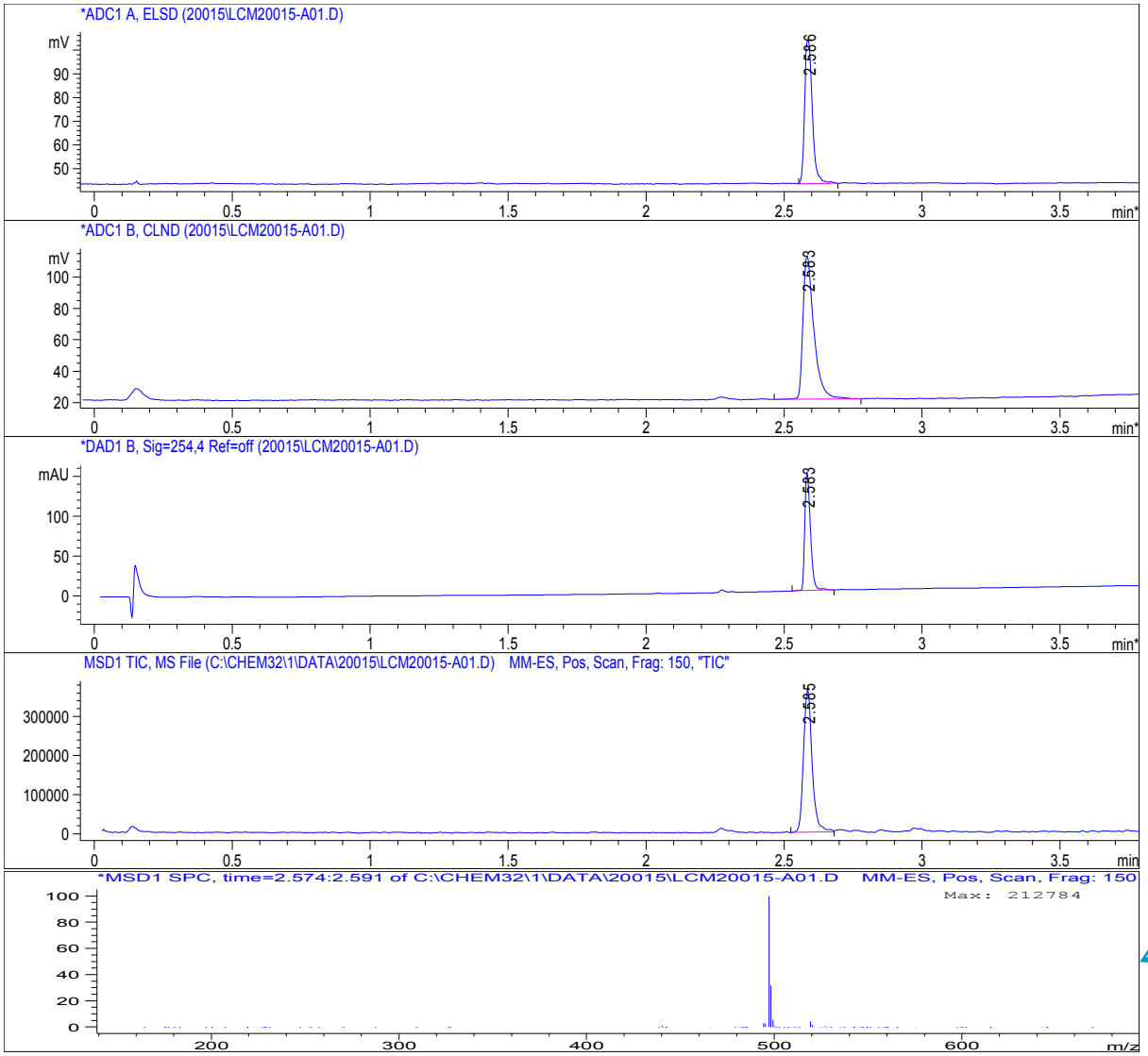
Mass Value = 229.04786				
#	Ion	Retention Time	Mass	Volume
1	M+H	2.06	230.05514	164.15
2	M+H+1	2.06	231.05628	10.11
3	M+H+2	2.06	232.05195	39.39
4	M+H+3	2.06	233.0538	2.78

Mass Value = 229.04786							
#	Formula	Compound	Mass	Error (mDa)	* Error (ppm)	Ret. Time Error	Description
1	C6H8N7OCl	Amiloride	229.04789	-0.02	-0.1	--	Diuretikum

Report of identified pharmaceutical compounds - Identification of diuretic amiloride with 0.1 ppm.

Compound Characterization

A good sample



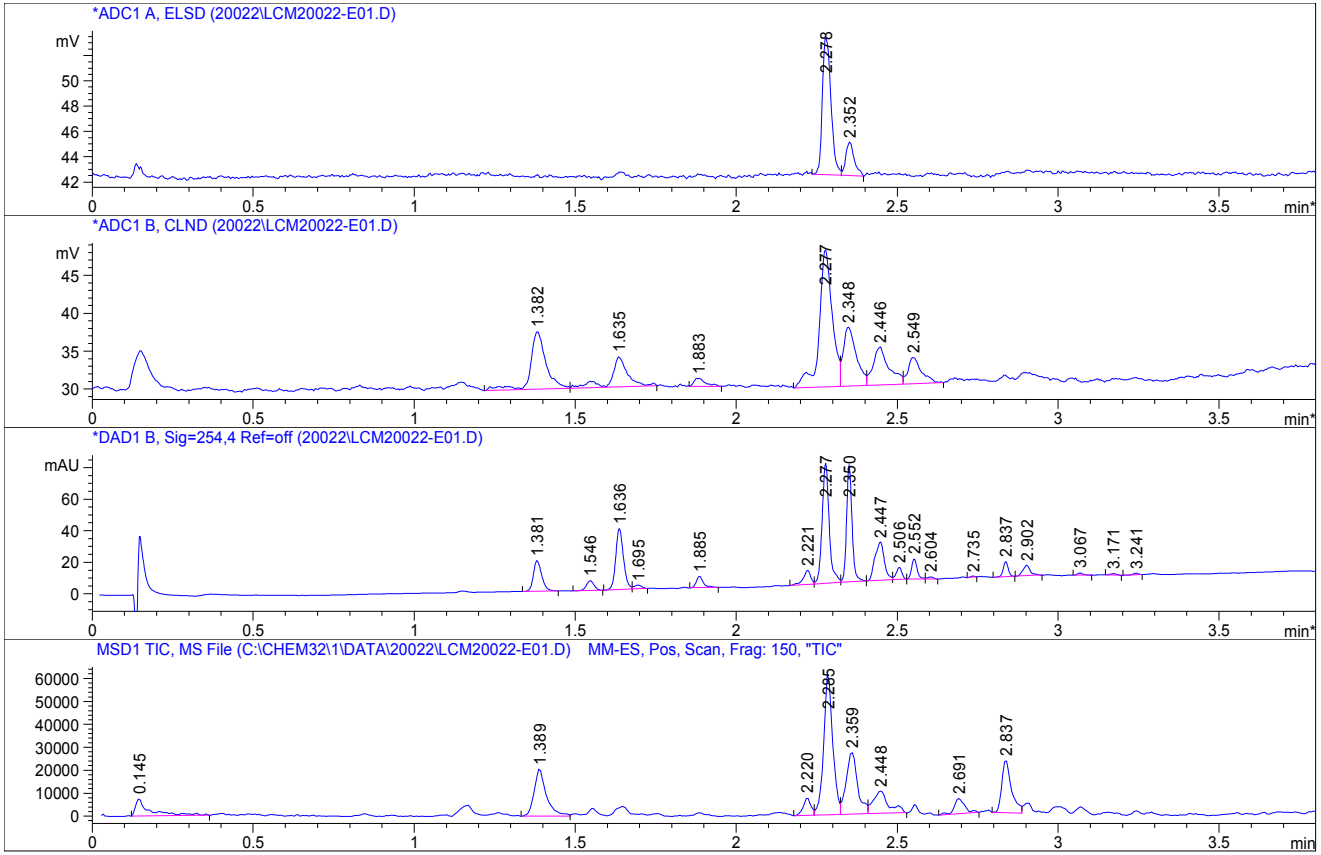
Single peak identified for compound

Utilize similar setup of LC-UV/ELSD/CLND/MS for full evaluation

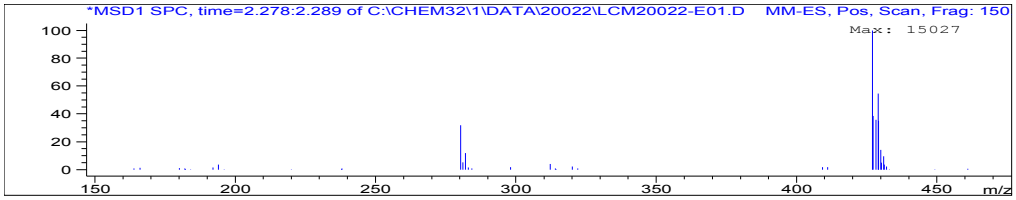
Single MW identified with spectra matching desired compound

Compound Characterization

A poor sample

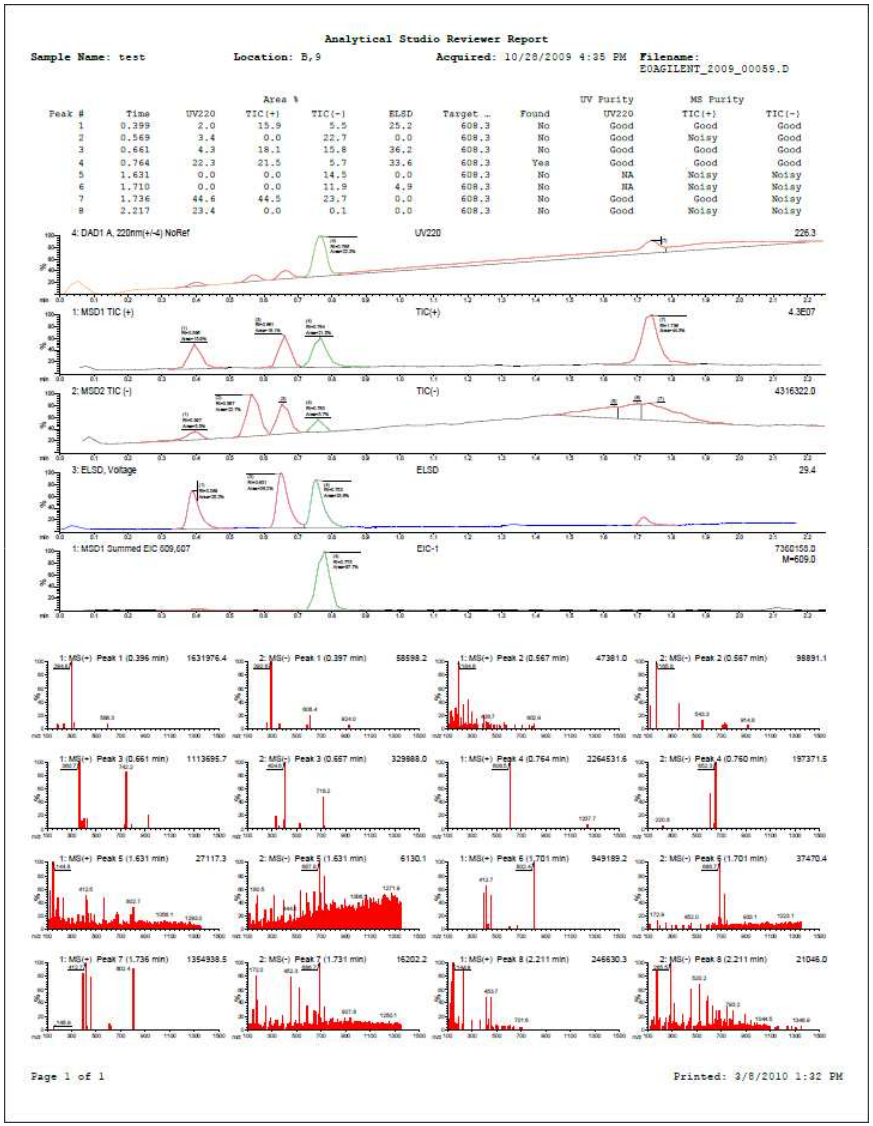


Clearly several other peaks in system



No clean single ion recognizable as the M+H ion from the target mass or formula

Simple Brief Clear Reports (Single Sample)



Analytical Studio Reviewer Report

Sample Name: test Location: B,9 Acquired: 10/28/2009 4:35 PM Filename: E0AGILENT_2009_00059.D

Peak #	Time	UV220	Area % TIC(+)	Area % TIC(-)	ELSD	Target …	Found	UV Purity UV220	MS Purity TIC(+)	MS Purity TIC(-)
1	0.399	2.0	15.9	5.5	25.2	608.3	No	Good	Good	Good
2	0.569	3.4	0.0	22.7	0.0	608.3	No	Good	Noisy	Good
3	0.661	4.3	18.1	15.8	36.2	608.3	No	Good	Good	Good
4	0.764	22.3	21.5	5.7	33.6	608.3	Yes	Good	Good	Good
5	1.631	0.0	0.0	14.5	0.0	608.3	No	NA	Noisy	Noisy
6	1.710	0.0	0.0	11.9	4.9	608.3	No	NA	Noisy	Noisy
7	1.736	44.6	44.5	23.7	0.0	608.3	No	Good	Good	Noisy
8	2.217	23.4	0.0	0.1	0.0	608.3	No	Good	Noisy	Noisy

4: DAD1 A, 220nm(+/-4) NoRef UV220 226.3

1: MSD1 TIC (+) TIC(+) 4.3E07

2: MSD2 TIC (-) TIC(-) 4316322.0

3: ELSD, Voltage ELSD 29.4

1: MSD1 Summed EIC 609,607 EIC-1 7360156.0 M=609.0

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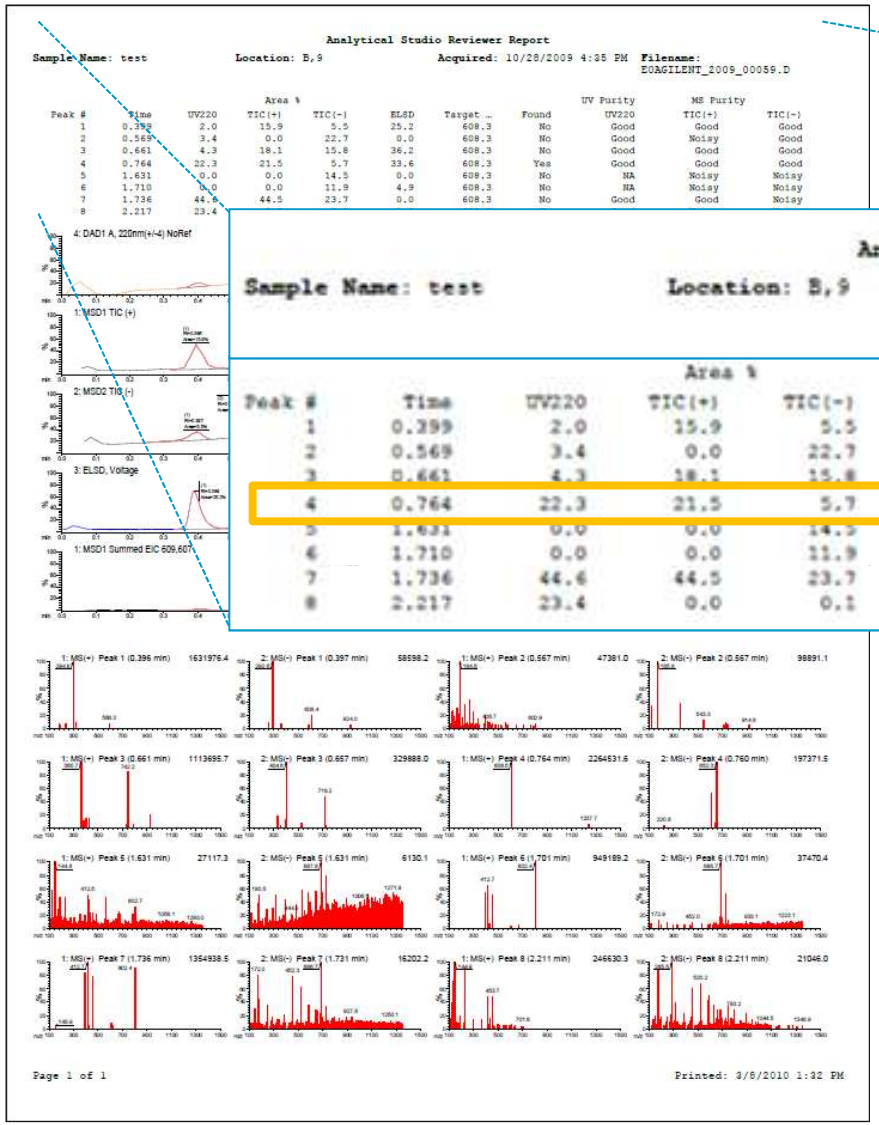
- ✓ YES, this really is a single page report from an Agilent SQ LC/MS.
- ✓ Simple clean reports with full options to customize to workflow.
- ✓ Automated e-mailing of PDF reports with Easy-Access
- ✓ Includes UV and MS peak purity
- ✓ Reports sample purity for target masses

The Concise Clean ASR report



Analytical Studio Reviewer Report

Sample Name: test Location: B,9 Acquired: 10/28/2009 4:35 PM Filename: E0AGILENT_2009_00059.D

Peak #	Time	UV220	Area % TIC(+)	Area % TIC(-)	ELSD	Target …	Found	UV Purity UV220	MS Purity TIC(+)	MS Purity TIC(-)
1	0.399	2.0	15.9	5.5	25.2	608.3	No	Good	Good	Good
2	0.569	3.4	0.0	22.7	0.0	608.3	No	Good	Noisy	Good
3	0.661	4.3	18.1	15.8	36.2	608.3	No	Good	Good	Good
4	0.764	22.3	21.5	5.7	33.6	608.3	Yes	Good	Good	Good
5	1.631	0.0	0.0	14.5	0.0	608.3	No	NA	Noisy	Noisy
6	1.710	0.0	0.0	11.9	4.9	608.3	No	NA	Noisy	Noisy
7	1.736	44.6	44.5	23.7	0.0	608.3	No	Good	Good	Noisy
8	2.217	23.4	0.0	0.1	0.0	608.3	No	Good	Noisy	Noisy

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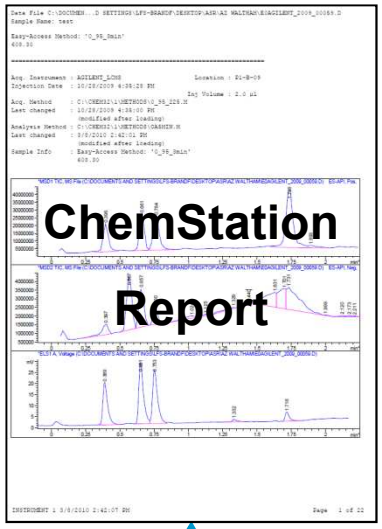
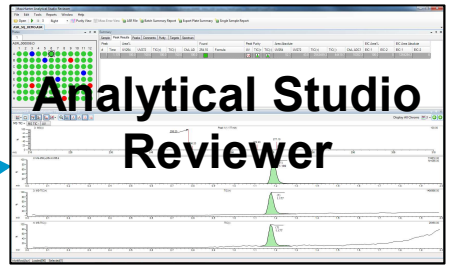
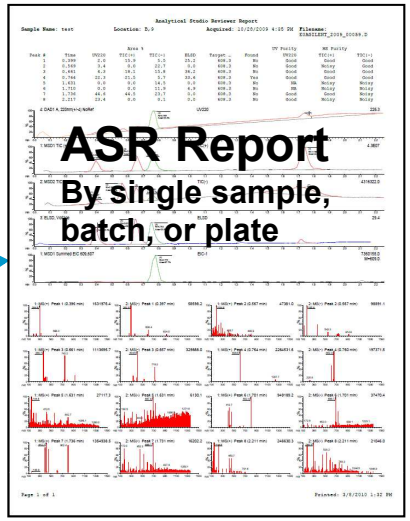
From Data to Information.. Agilent MSD solutions

Flexible automatable reporting and fast data review at desktop when needed



ASR File
A text file containing summary data on samples, chromatograms, peaks and targets data





ChemStation

ChemStation Raw Data Folder

CS-2-MH translator

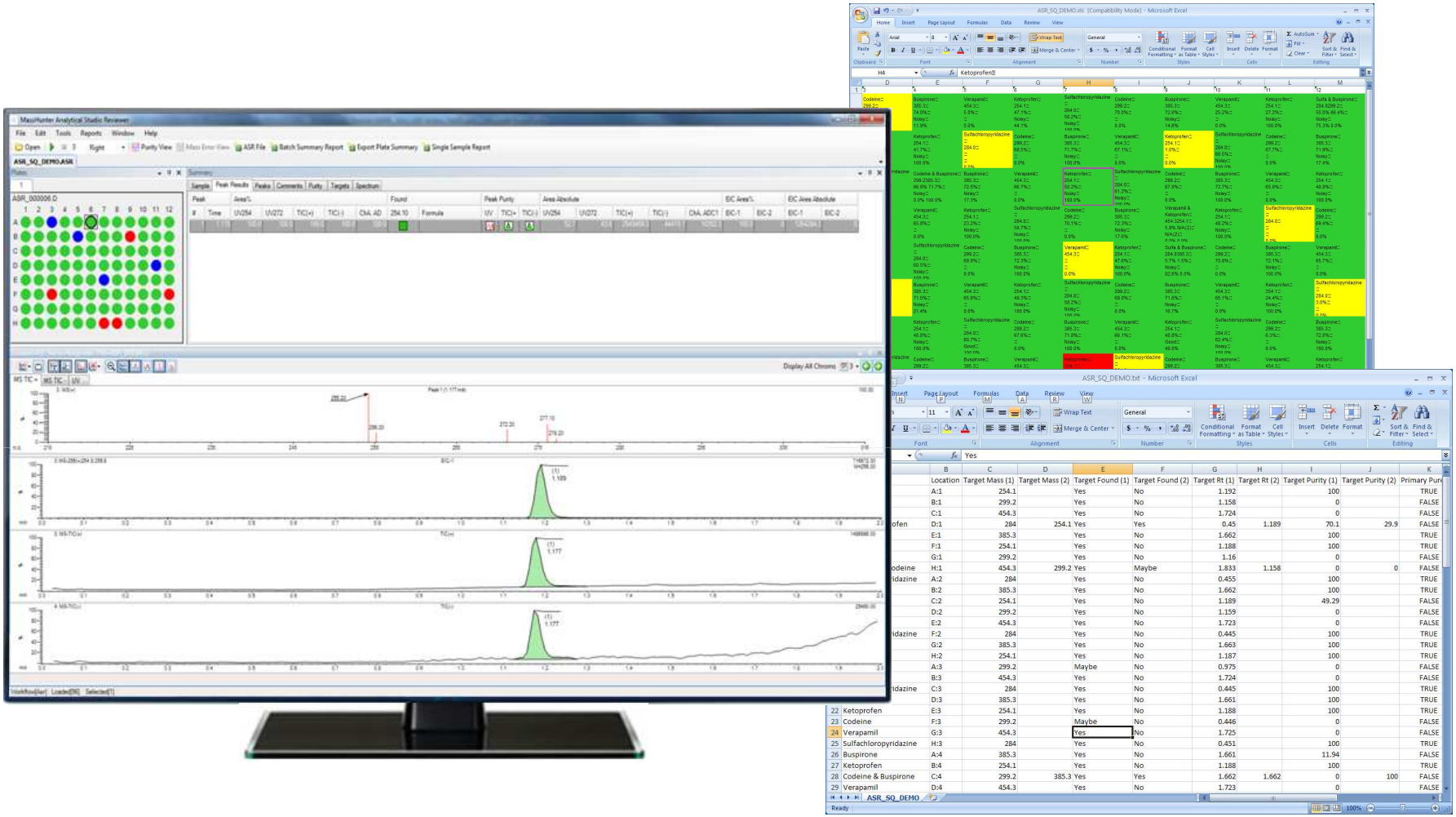
MassHunter Raw Data

MassHunter Qual

MassHunter Quant

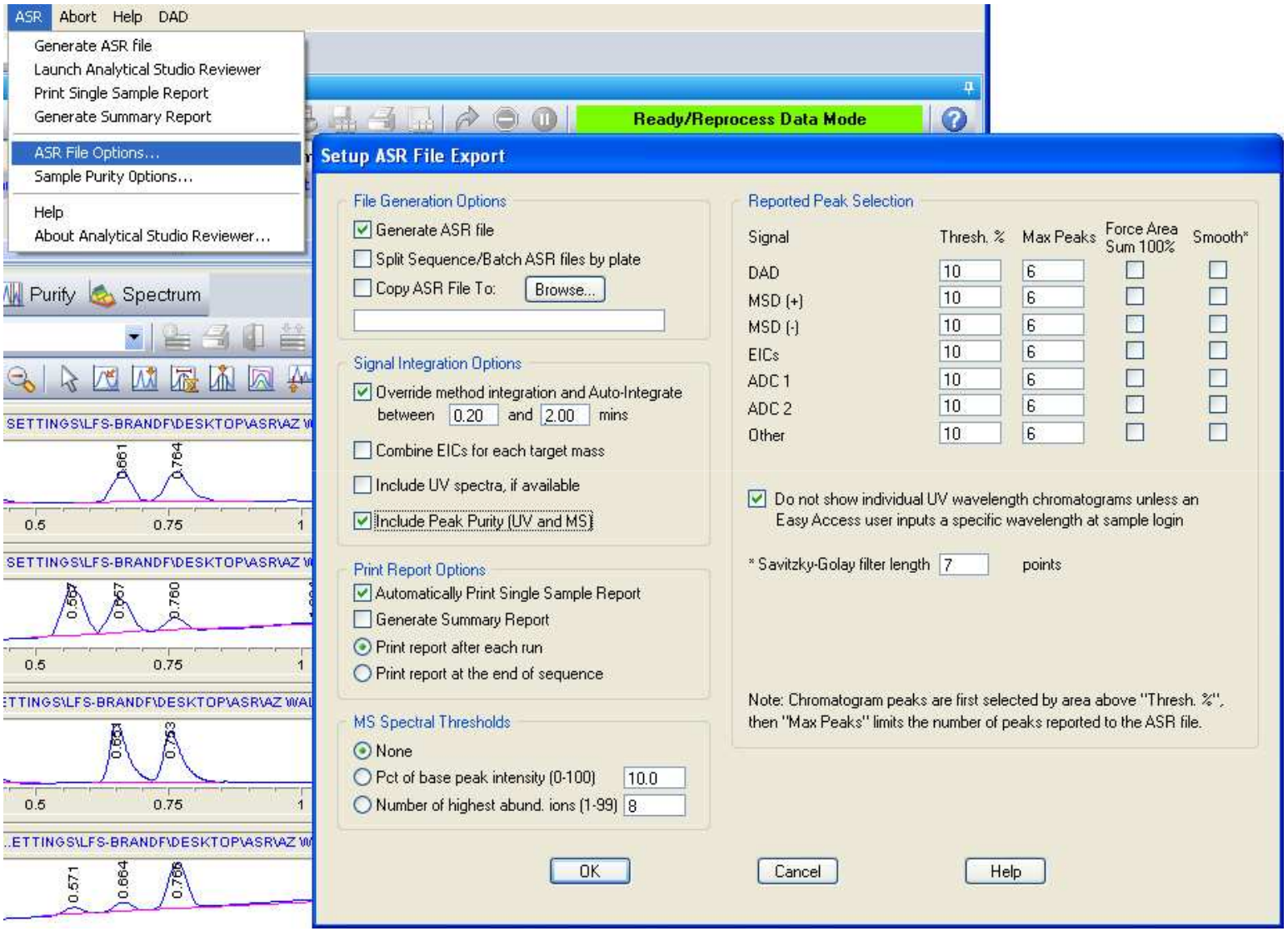
MassHunter Reporting Engine

MassHunter Analytical Studio Reviewer (ASR)



A simple data reviewer and reporting tool for LC/MS data

ASR File Creation Setup: A Single Panel



Purity – What is it really? The eternal question

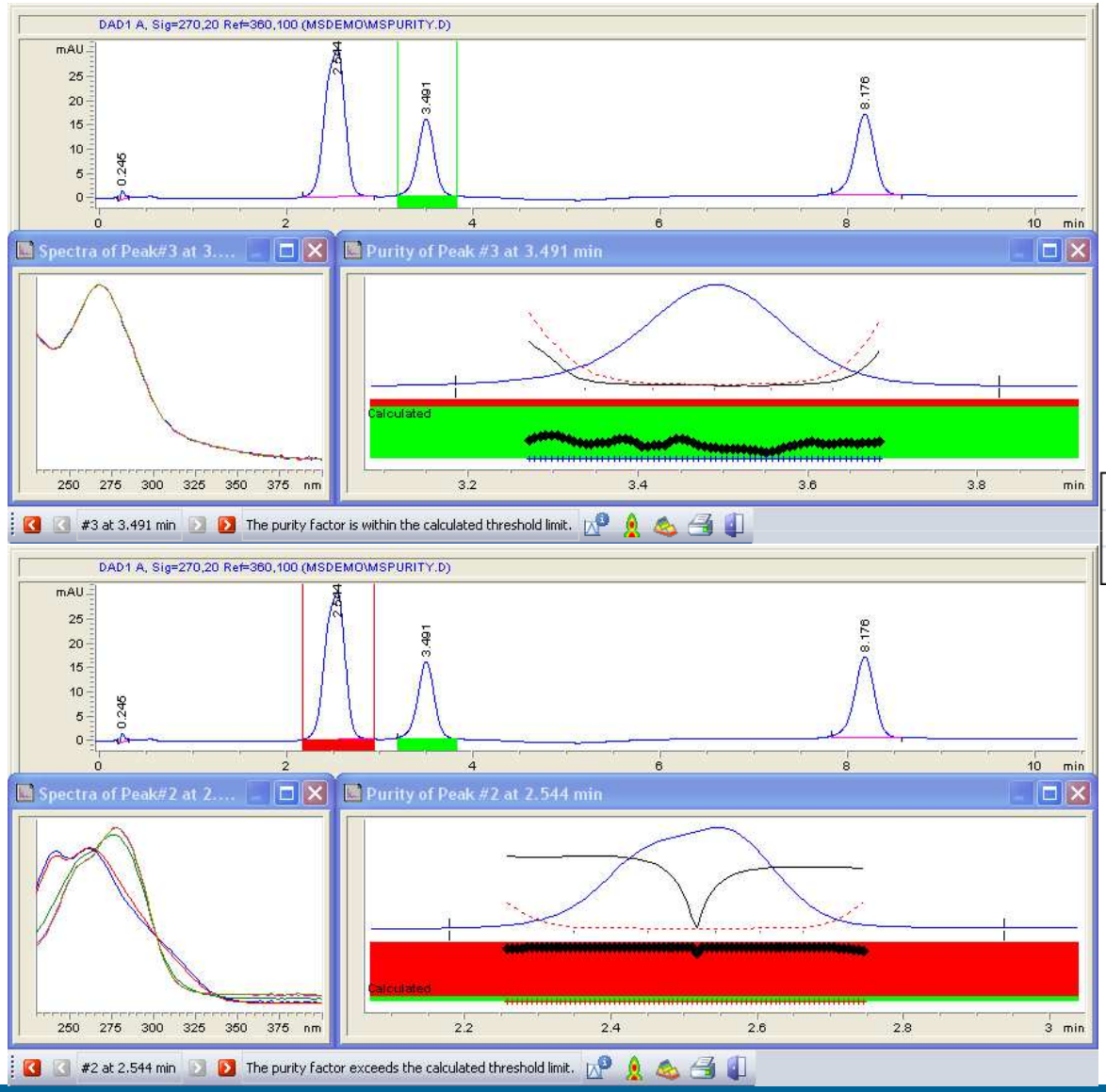
Sample purity

The percent area, usually in a UV chromatogram, e.g. 254nm, of a peak determined, usually by mass spec., to contain the target compound.

Peak purity

An evaluation of the spectra, either DAD UV or MS, to look for co-elution. *NOT DEFINITIVE, but worthwhile!*

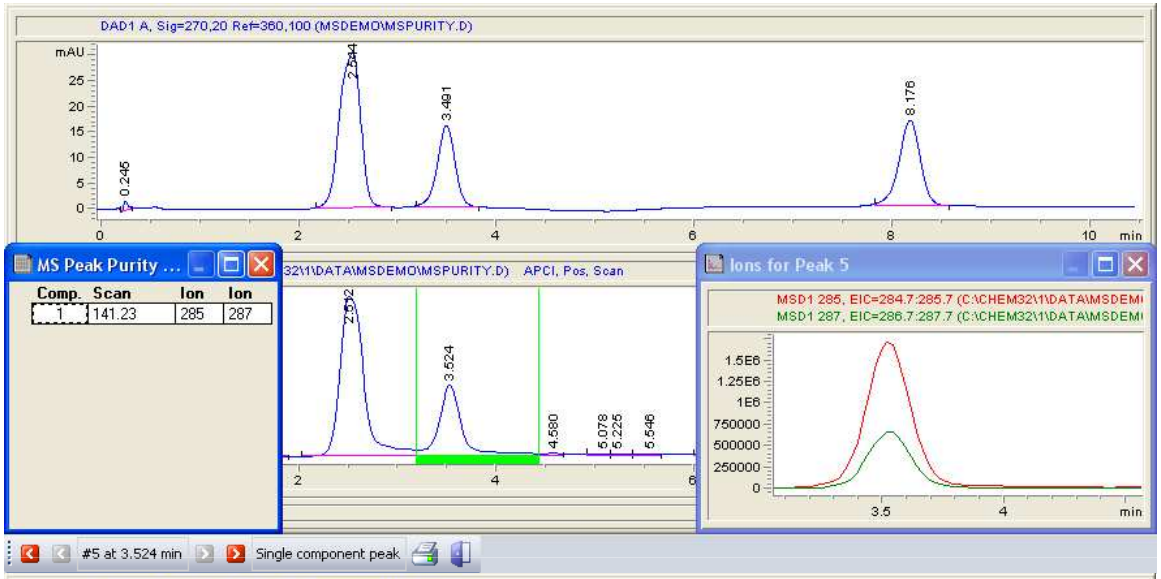
Peak Purity – Diode Array Detector

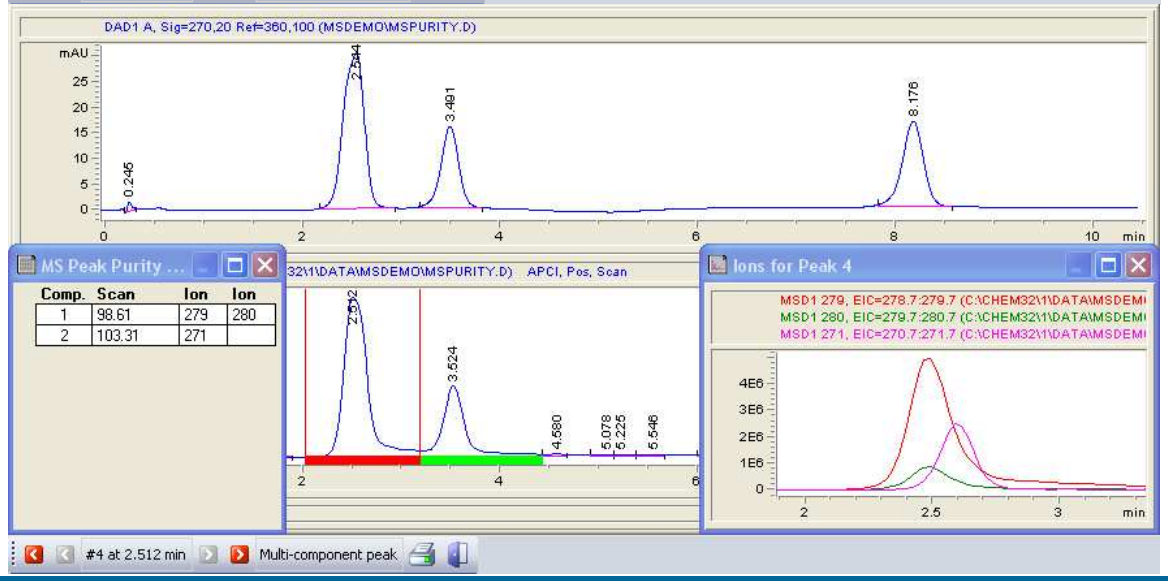


In ASR, this peak purity data has been distilled down to a single icon or word in a table.

Graphic	Report	Comment
	Good	
	Noisy	

Peak Purity – MS

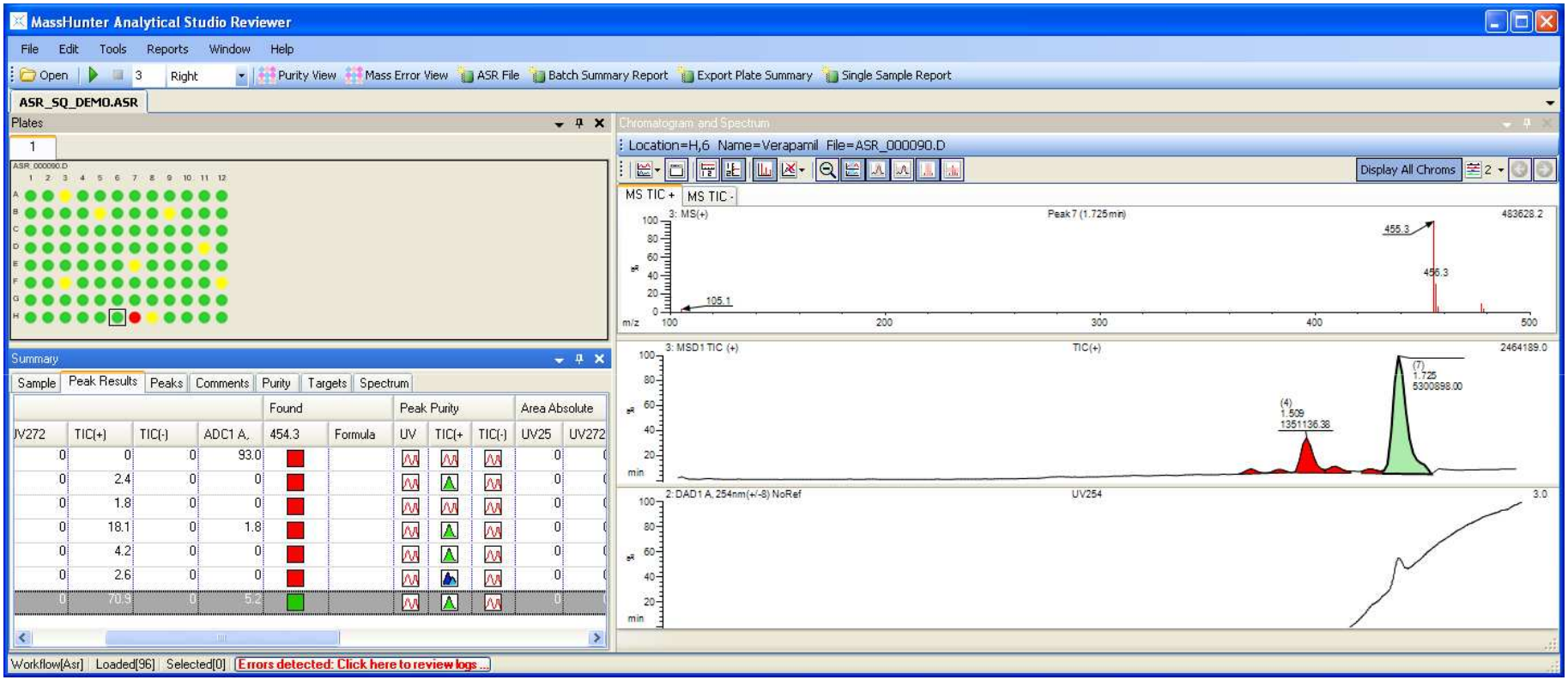




Graphic	Report	Comment
	Good	
	Noisy	
	Mix	MS only, multiple ion distributions

ASR Reporting of Peak Purity

Graphic	Report	Comment
	Good	
	Noisy	
	Mix	MS only, multiple ion distributions



Conclusions

- Easy-Access manages the LC/MS system to save resources (both people and consumables).
- Easy-Access makes sample submission simple.
- Sub-2 micron particle columns and Poroshell 120 columns make UHPLC practical on low end LC systems.
- SQ or TOF: what information do you need?
- Analytical Studio Reviewer makes simple elegant LC/MS reporting a reality.
- The rich environments for configuration and customization within the ChemStation and MassHunter platforms offer a unique opportunity to **encapsulate LC/MS systems** into a simple solution.

Thanks for listening