

# Opiates, Opioids and Benzodiazepines, Amphetamines & Illicit Drug Forensic Analysis by LC/MS

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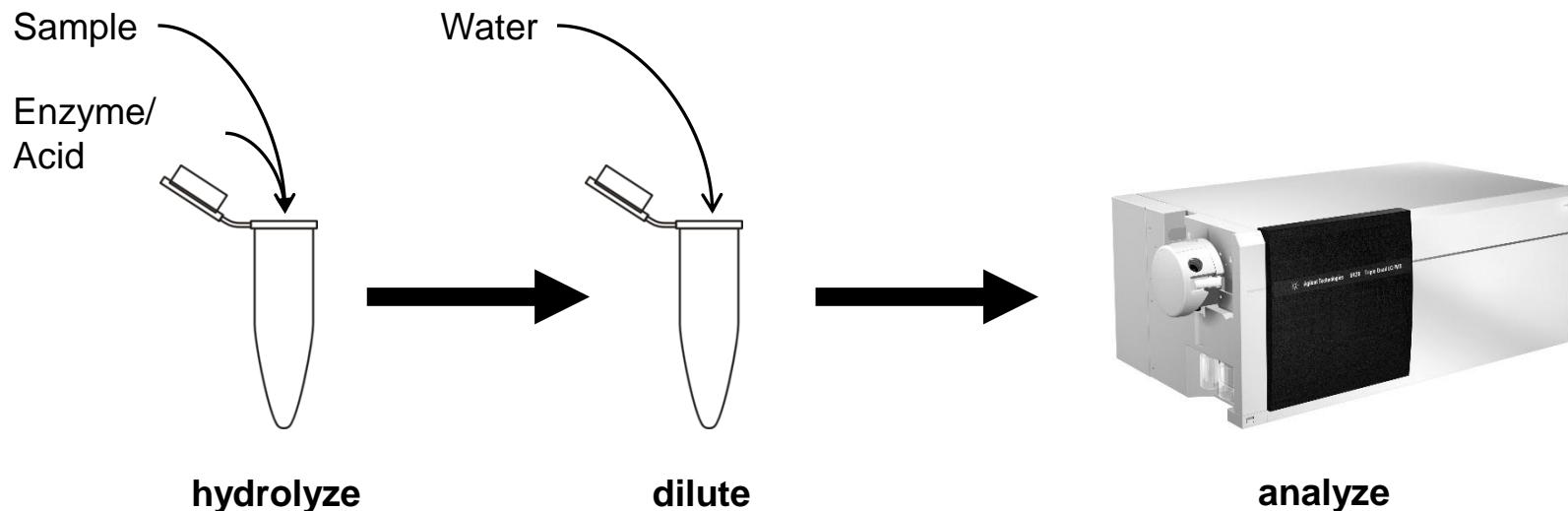
# Agenda

- A method for the rapid analysis of over 65 analytes in a single LC/MS analysis run
- Simplified method development through the use of Dynamic MRM (dMRM) and databases
- Qualitative and Quantitative data analysis
- Customized Reporting



# Targeted Analysis of Over 65 Analytes for Toxicology

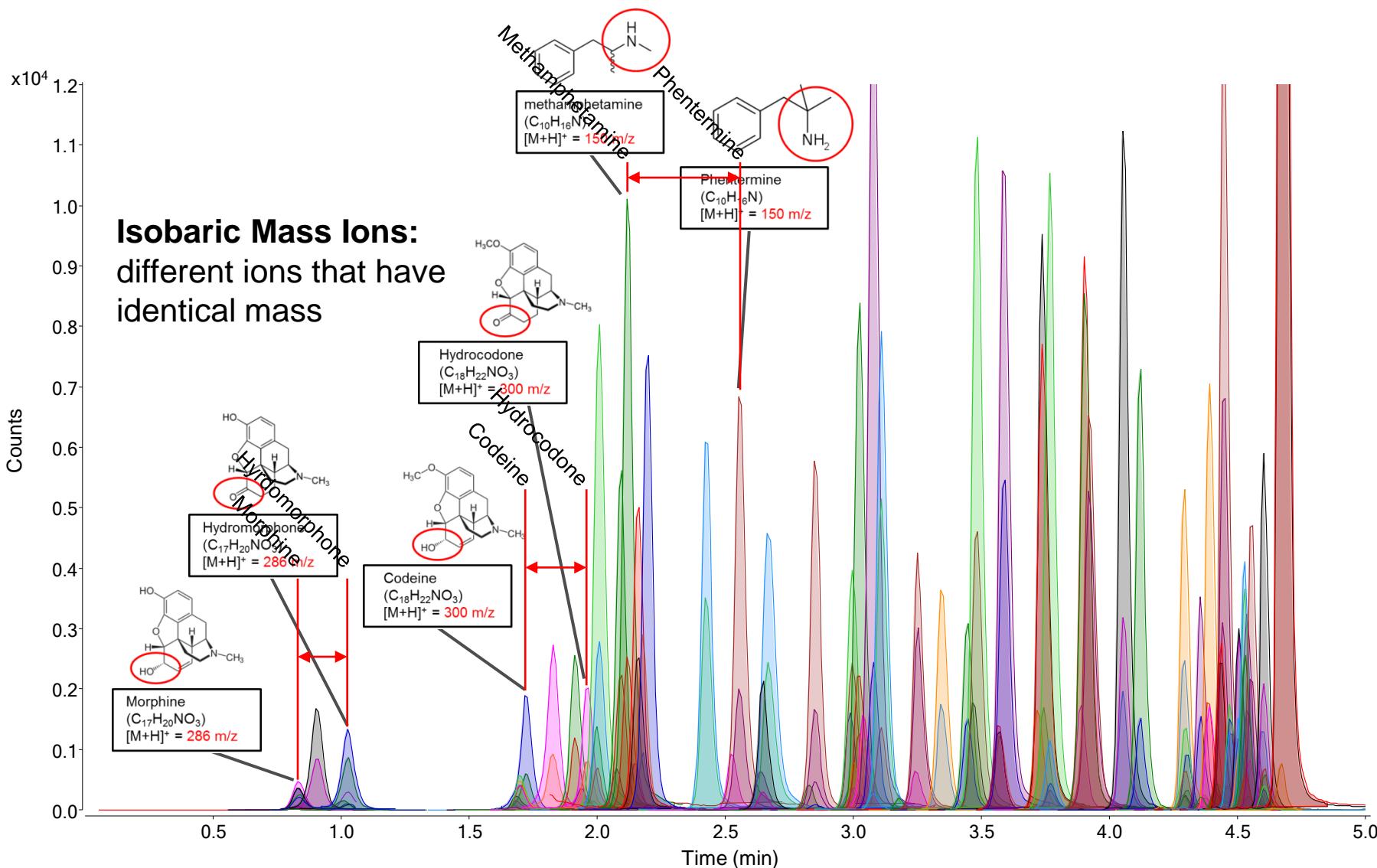
- An extensive screen and quantification in 5 to 6 minutes
- Internal standard corrected quantification
- Multi-point calibration curve covering a wide dynamic range
- Secondary qualifier ion for each analyte
- Simple sample preparation:



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# Chromatographic Separation of Over 65 Analytes



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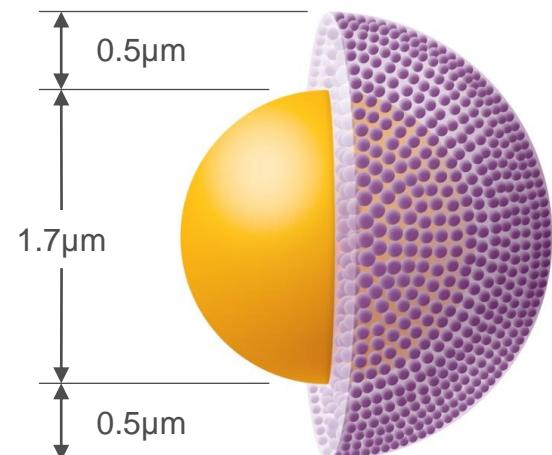
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# Poroshell 120 Columns for HPLC and UHPLC

Poroshell 120 is a **high efficiency, high resolution column choice** for enhancing productivity in LC and LC/MS

## Poroshell 120 Columns have:

- 80-90% efficiency of sub-2 $\mu$ m columns
- ~40-50% lower pressure
- 2x efficiency of 3.5 $\mu$ m (totally porous)
- A 2 $\mu$ m frit to reduce clogging
- A 600 bar pressure limit for HPLC or UHPLC
- The superficially porous particle is 2.7 $\mu$ m with a solid core (1.7 $\mu$ m) and porous outer layer with a 0.5 $\mu$ m diffusion path



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# Poroshell 120 Performance After 3000 Injections

- Dilute and shoot sample preparation
- Analytes covering a wide range of retention times show excellent reproducibility

Analyte	%RSD (RT)	Analyte	%RSD (RT)	Analyte	%RSD (RT)
Morphine	0.7	Meperidine	0.4	Triazolam	0
Codeine	0.4	zolpidem	0.3	Naltrexone	0.1
hydrocodone	0.4	Fentanyl	0.1	chlordiazepoxide	0.1
MDMA	0.3	EDDP	0.1	Desmethyl diazepam	0.1
norFentanyl	0.2	Nitrazepam	0.1	Buprenorphine	0.3
Heroin	0.2	Propoxephine	0.1	Cocaethylene	0.2
Methyl Phenidate	0.2	Buprenorphine	0.3	11-nor-9-carboxy-delta9-thc	0

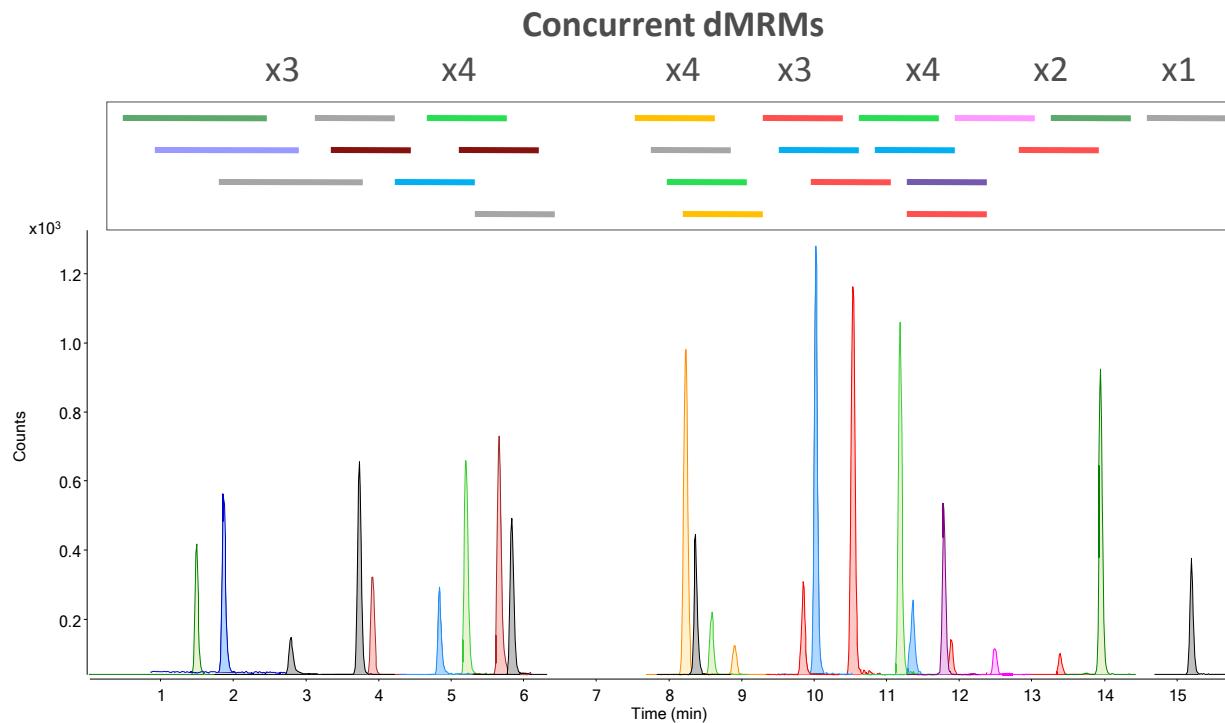


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# The Need for Dynamic MRM (dMRM)

- Nearly 200 MRM transitions for analytes, qualifier ions, and internal standards
- Monitoring all MRM transitions over the entire run results in poor quantification due to short dwell times and long cycle times
- dMRM only monitors each transition during the appropriate retention time window



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# Easy LC/MS Method Creation and Customization

## Agilent Analyzers and Application Kits

# Triple Quadrupole LC/MS Forensic Toxicology Application Kit



A Database  
containing over  
2500x compounds  
each with multiple  
MRM transitions  
ensures fast  
method creation for  
development

Quickly establish screening methods for complex matrices using these leading-edge technologies



**A Dynamic MRM database with more than 200 compounds, plus Agilent MassHunter Data Acquisition and Analysis software, let you quickly generate acquisition and analysis methods, which can be modified to meet your future needs.**



**The Agilent 1200 Series SL Rapid Resolution LC, interfaced with Agilent's 6400 Series Triple Quadrupole LC/MS System delivers fast, high-resolution LC/MS/MS analysis.**



## Agilent's Jet Stream Electrospray lowers detection levels of analytes in complex matrices.



**Dynamic MRM** maximizes the Quadrupole's detection capability – without sacrificing sensitivity – when a large number of compounds are being analyzed at femtomole concentrations.

# Qualitative Screening

## Find By MRM



Agilent MassHunter Qualitative Analysis B.04.00 - Pete\_02.m

File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help

Data Navigator

Sort by Data File

- Compounds
  - Cpd 1: morphine-d6
  - Cpd 2: nor-Codene
  - Cpd 3: Morphine
  - Cpd 4: Dymorphine
  - Cpd 5: hydromorphone-d6
  - Cpd 6: Hydromorphone
  - Cpd 7: codeine-d6
  - Cpd 8: dihydrocodeine
  - Cpd 9: naloxone
  - Cpd 10: Codeine
  - Cpd 11: Oxycodone
  - Cpd 12: naltrexone
  - Cpd 13: hydrocodone-d6
  - Cpd 14: 6-acetylmorphine-d6
  - Cpd 15: Hydrocodone
  - Cpd 16: 6-monoacetyl morphine
  - Cpd 17: mda-d5
  - Cpd 18: dAmphetamine
  - Cpd 19: mdma-d5
  - Cpd 20: MDA
  - Cpd 21: methamphetamine
  - Cpd 22: m-hydroxybenzoylegonine
  - Cpd 23: MDMA**
  - Cpd 24: o-desmethyltramadol
  - Cpd 25: MDEA
  - Cpd 26: phenetermine-d5
  - Cpd 27: phenetermine
  - Cpd 28: benzoylegonine-d8
  - Cpd 29: Benzoylegonine
  - Cpd 30: italinic acid
  - Cpd 31: norfentanyl-d5
  - Cpd 32: nor-Fentanyl
  - Cpd 33: zanclizone-d4

Compound List

Show/Hide	Cpd	Label	Name	Diff (DB, ppm)	RT	Formula	Score (DB)	Hits (DB)
✓	15	Cpd 15: Hydrocodone	Hydrocodone		1.946			
✓	16	Cpd 16: 6-monoacetyl m...	6-monoacetyl morphine		1.982			
✓	17	Cpd 17: mda-d5	mda-d5		2.057			
✓	18	Cpd 18: dAmphetamine	d-Amphetamine		1.991			
✓	19	Cpd 19: mdma-d5	mdma-d5		2.134			
✓	20	Cpd 20: MDA	MDA		2.077			
✓	21	Cpd 21: methamphetamine	methamphetamine		2.107			
✓	22	Cpd 22: m-hydroxybenz...	m-hydroxybenzoylegonine		2.16			
✓	23	Cpd 23: MDMA	MDMA		2.144			
✓	24	Cpd 24: o-desmethyltra...	o-desmethyltramadol		2.181			

Chromatogram Results

x10<sup>4</sup> Cpd 23: MDMA: +ESI MRM Freq=97.0V CID@9.0 (194.1000 > 163.0000) Spiked Urine\_L08:003.d

Method Explorer: Pete\_02.m

Reports

Find Compounds

Find by Auto MS/MS

Find by Targeted MS/MS

Find by Molecular Feature

**Find by MRM**

Find Compounds by Formula

Compound Identification Results: Cpd 23: MDMA Chromatogram Results

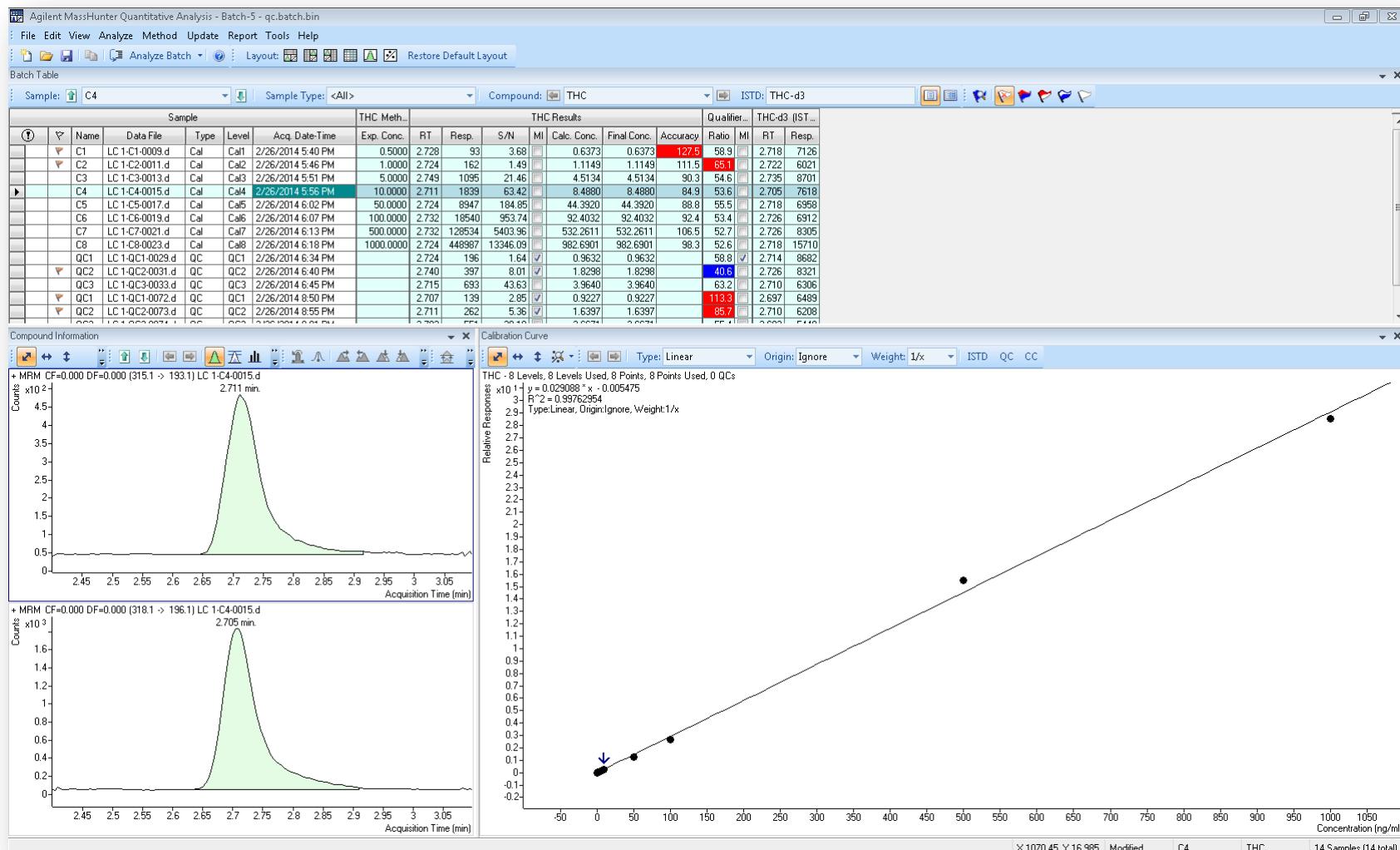
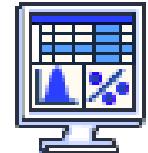


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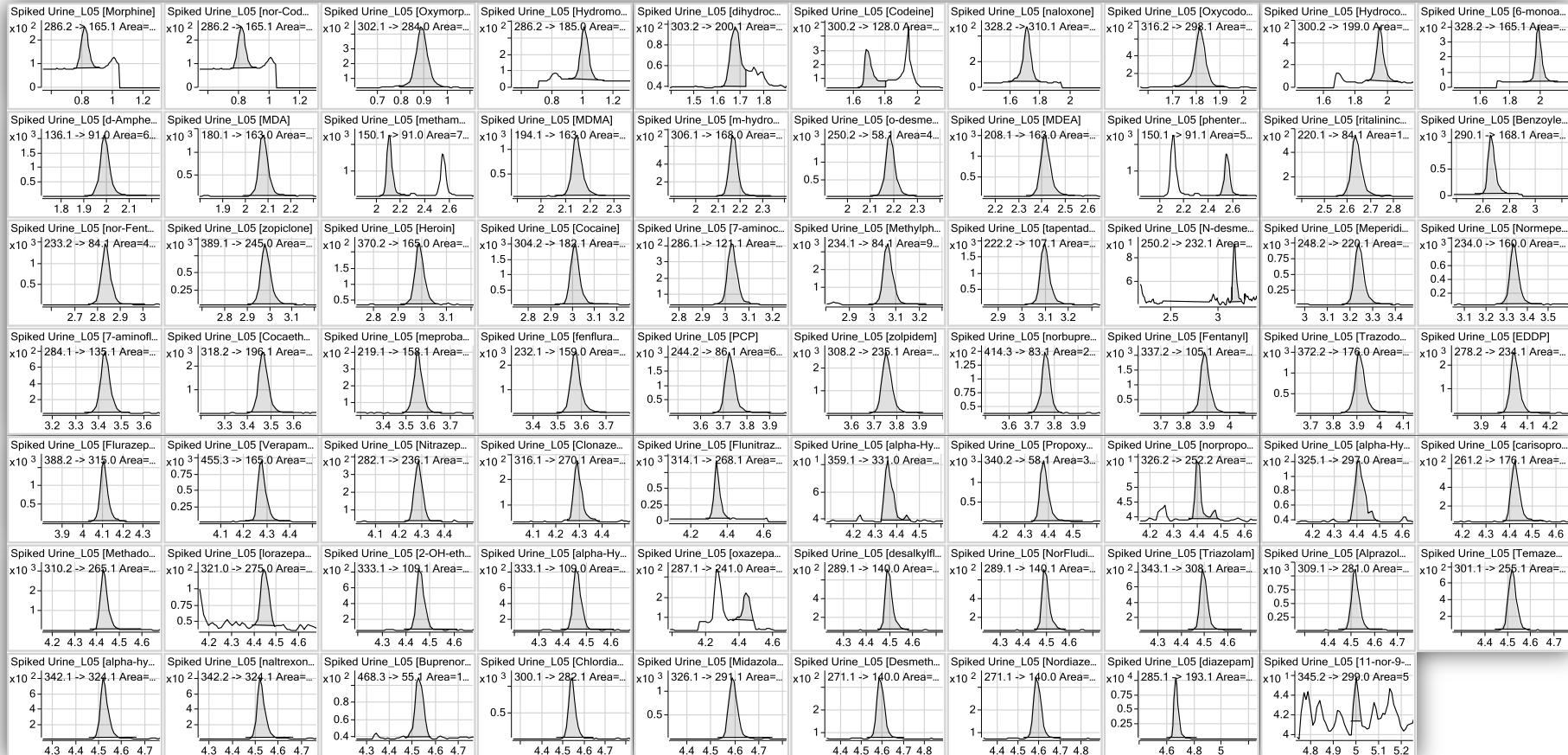
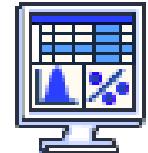
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# Quantitative Analysis

## Batch at a Glance



# Data Review with Compounds at a Glance

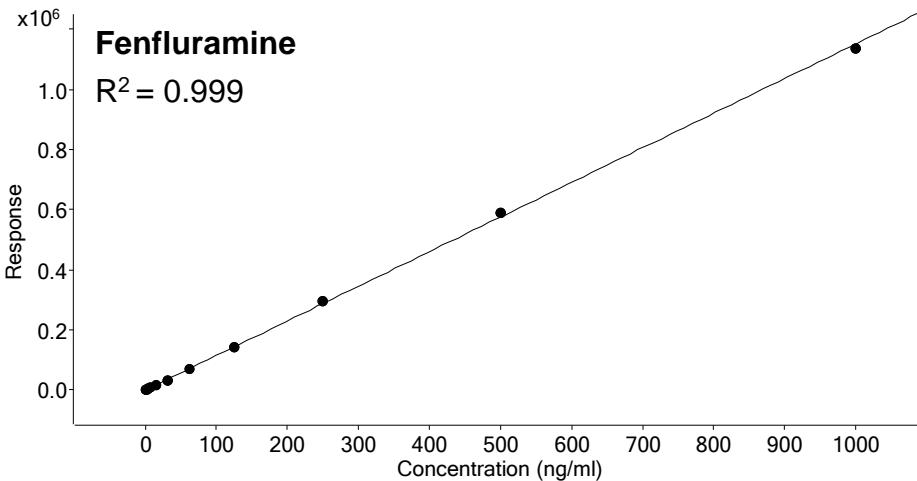
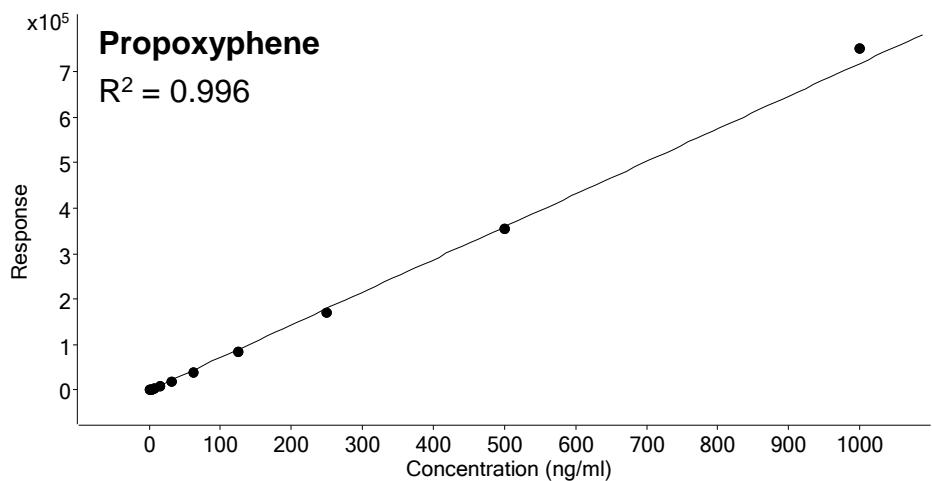
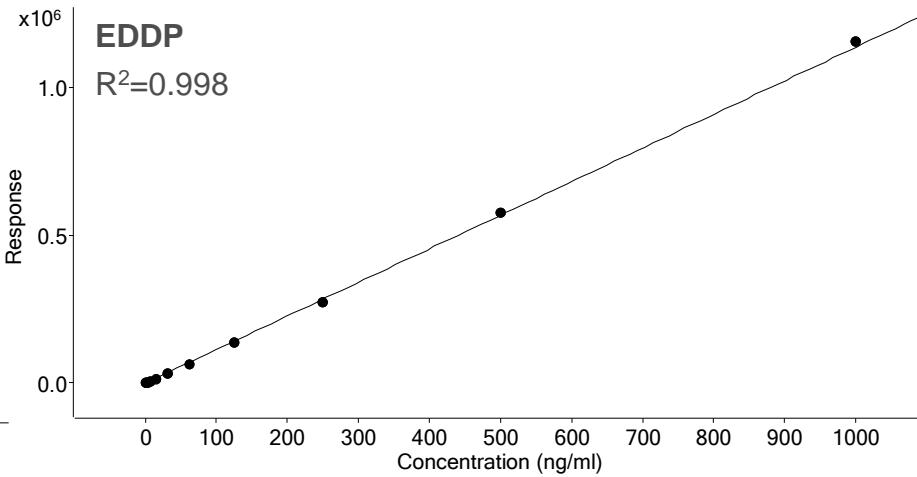
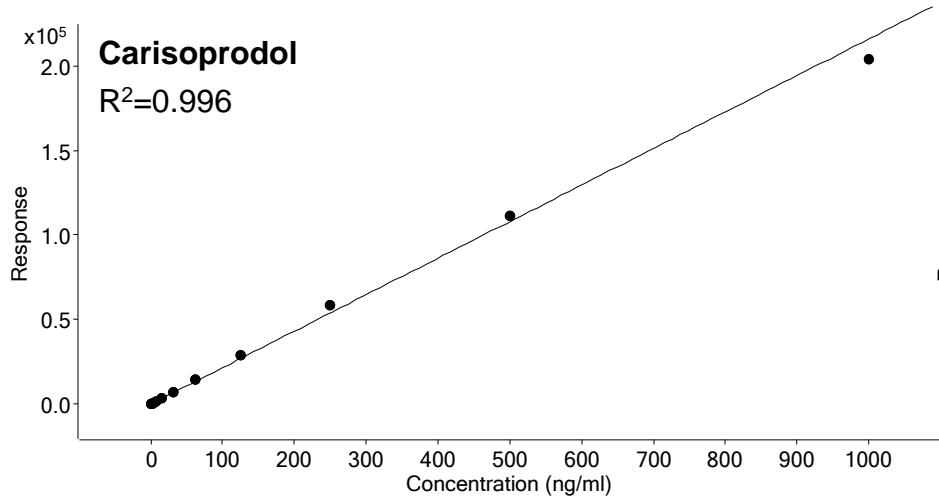


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# Quantitative Analysis

## Calibration Curves



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# Customizable Reporting

**Quantitative Analysis Sample Report**

**Batch Info**

Batch Data Path	C:\Demo Data
Analysis Time	#####
Report Time	#####
Last Calib Update	#####

**Instrument** 6430    **Sample Name** PM\_Mix\_50ppb  
**Operator** Calibration    **Data File** PMSeqA\_056.d  
**Sample Type** Calibration    **Acq Method File** Optimized\_dNRM\_ESI\_Methanol\_01.m  
**Dilution** 1    **Acq Time** 2010-10-09 00:24  
**Position** P2-E9    **Sample Info**  
**Injector Vol.** 5 Comment

**Compound** **Test Outcome** **Measured Result** **Cutoff** **RT** **Area**

Morphine	Positive	50.3	40.0 ng/ml	0.863	15955
Oxymorphone	Positive	51.4	40.0 ng/ml	0.948	68076
Hydromorphone	Positive	49.4	40.0 ng/ml	1.090	37464
Codeine	Negative	50.4	60.0 ng/ml	1.817	22263
Naloxone	Positive	51.6	40.0 ng/ml	1.881	71272
Oxycodone	Positive	50.8	40.0 ng/ml	1.959	144027
Naltrexone	Positive	50.9	40.0 ng/ml	2.039	73330
Hydrocodone	Positive	49.8	35.0 ng/ml	2.085	85546
6-monooacetyl morphine	Positive	52.6	40.0 ng/ml	2.133	35081
d-Amphetamine	Positive	50.8	40.0 ng/ml	2.230	128541
methamphetamine	Negative	50.1	80.0 ng/ml	2.318	184766
MDMA	Positive	49.8	40.0 ng/ml	2.332	123486
Strychnine	Negative	49.3	80.0 ng/ml	2.492	26033
MDEA	Negative	50.1	80.0 ng/ml	2.587	138131
nor-Fentanyl	Positive	51.1	8.0 ng/ml	2.976	152310
Heroin	Positive	51.4	25.0 ng/ml	3.097	2098
Tramadol	Negative	49.3	75.0 ng/ml	3.122	252341
Cocaine	Positive	50.0	50.0 ng/ml	3.136	117743
7-Aminodionazepam	Positive	50.0	40.0 ng/ml	3.154	38927
Mepеридин	Negative	50.4	60.0 ng/ml	3.364	251258
Normeperидин	Positive	50.0	40.0 ng/ml	3.451	97713
Meprobamate	Positive	50.1	40.0 ng/ml	3.747	18383
PCP	Positive	50.0	40.0 ng/ml	3.779	210618
Norbuprenorphine	Positive	51.0	40.0 ng/ml	3.831	2172
MDA	Negative	7.6	40.0 ng/ml	3.890	81000
Fentanyl	Positive	4.9	2.0 ng/ml	3.975	20177
Trazodone	Positive	49.6	40.0 ng/ml	4.067	136292
EDDP	Positive	48.5	25.0 ng/ml	4.075	312209
Verapamil	Positive	46.6	40.0 ng/ml	4.310	61548
Nitazepam	Positive	50.1	40.0 ng/ml	4.391	21352
Propoxyphene	Positive	48.2	40.0 ng/ml	4.425	160040
Norpropoxyphene	Negative	29.6	40.0 ng/ml	4.442	898
Methadone	Positive	49.7	40.0 ng/ml	4.454	595919
alpha-Hydroxylalprazolam	Negative	49.5	50.0 ng/ml	4.485	4798
Lorazepam	Positive	49.2	40.0 ng/ml	4.538	2106
Oxazepam	Negative	48.4	55.0 ng/ml	4.545	3204
Alprazolam	Negative	49.9	50.0 ng/ml	4.566	78443
Temazepam	Positive	49.4	40.0 ng/ml	4.610	31380
Buprenorphine	Positive	50.2	50.0 ng/ml	4.638	9526
Nordiazepam	Negative	51.2	65.0 ng/ml	4.692	8293
Prouufen	Positive	48.3	40.0 ng/ml	4.688	109420
Diazepam	Positive	50.1	35.0 ng/ml	4.756	48262

PM\_Mix\_50ppb    Page 1 of 4    Printed at: 1:07 PM on: 10/27/2011

**Quantitative Analysis Sample Report**

**Batch Info**

Batch Data Path	C:\Demo Data\ACME_Toxicology_Inc
Analysis Time	#####
Report Time	2/15/2014 5:43 PM
Last Calib Update	#####

**Instrument** 6430    **Sample Name** Mix\_50ppb  
**Operator** P. John Doe    **Data File** SeqA\_056.d  
**Sample Type** Calibration    **Acq Method File** Optimized\_dNRM\_ESI\_Methanol\_01.m  
**Dilution** 1    **Acq Time** 10/9/2013 0:24  
**Position** P2-E9    **Sample Info**  
**Injector Vol.** 5 Comment

**Sample Chromatogram**

+ TIC MRM (\*\* -> \*) PMSeqA\_056.d

Counts x10<sup>5</sup>

0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8

Acquisition Time (min)

**Compound** **Test Outcome** **Measured Result** **Cutoff** **RT** **Area**

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PM\_Mix\_50ppb    Page 1 of 4    Printed at: 11:48 AM on: 2/15/2014



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# Conclusions

- Agilent makes it easy to create custom analytical panels for the measurement of Opiates, Opioids, Benzodiazepines, Amphetamines & Illicit
- Panels can be comprehensive or focused to suit your needs
- Dynamic MRM functionality, automatic optimization and MRM transition Database help make method development straightforward
- Results can be achieved with a simple Dilute & Shoot sample preparation
- Agilent's MassHunter software is optimized for your data review and reporting workflow

