



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

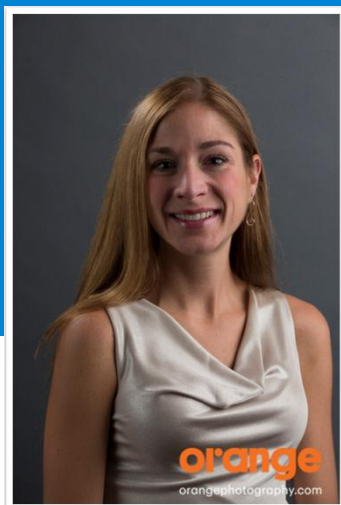
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Julie Cichelli, PhD

Agilent Technologies

Application Engineer

April 29, 2014



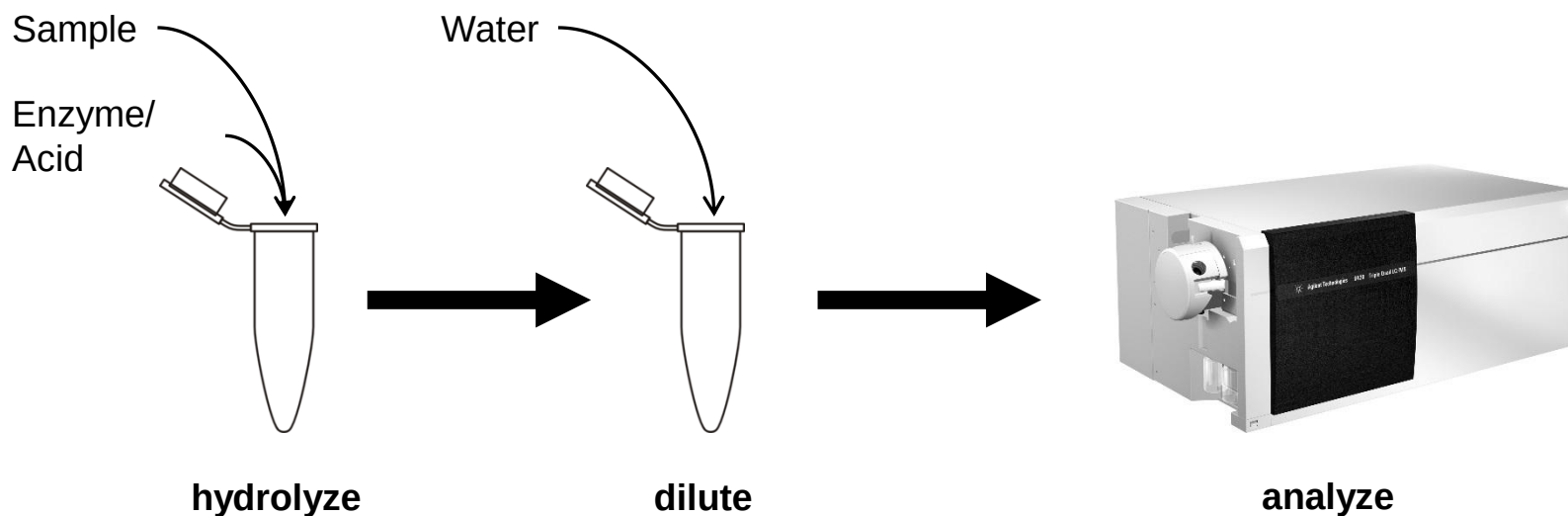
# Agenda

- 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

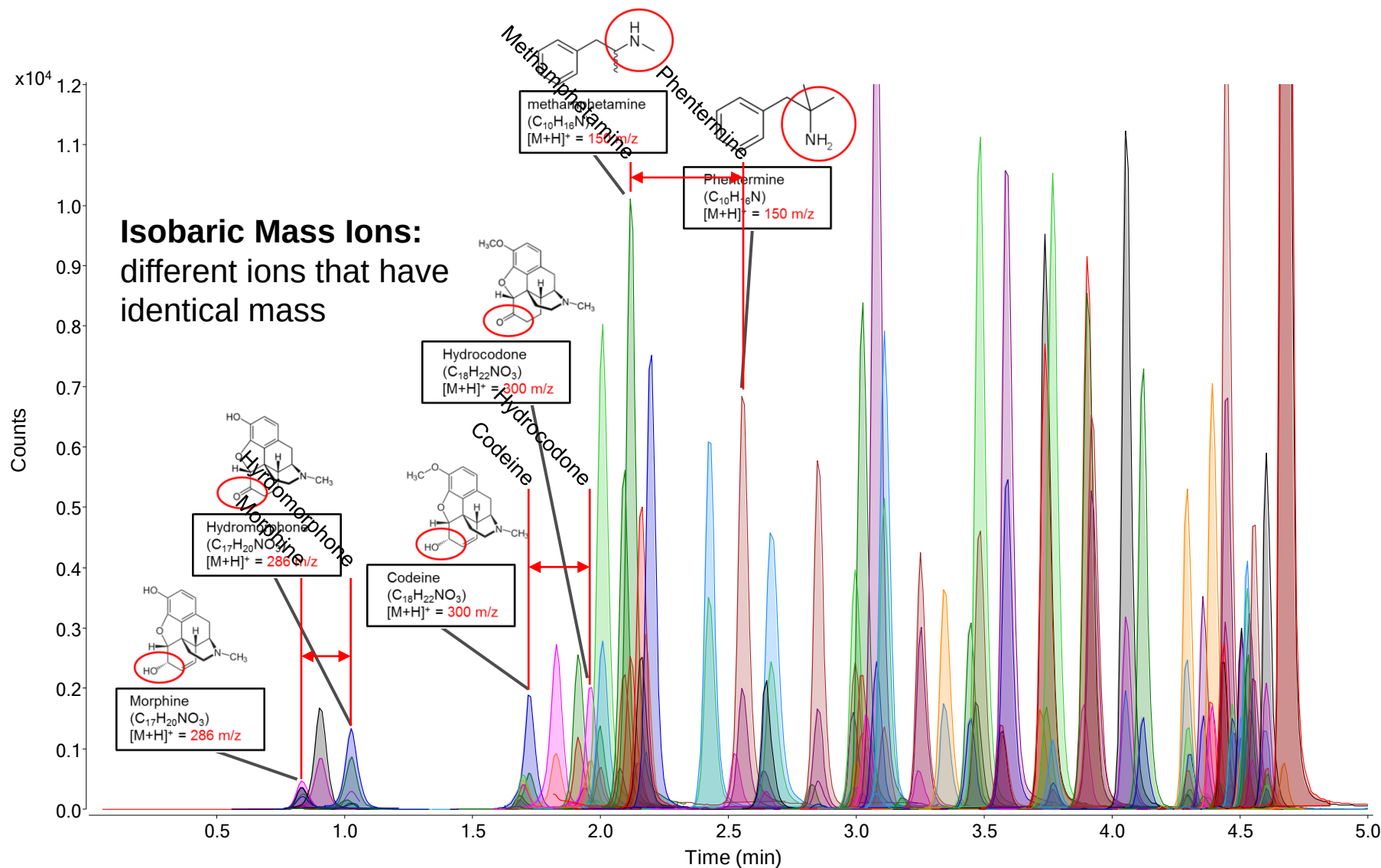


# Targeted Analysis of Over 65 Analytes for Toxicology

- 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:



# Chromatographic Separation of Over 65 Analytes

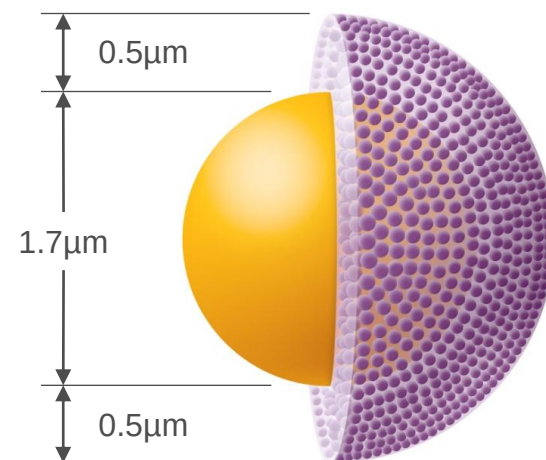


# Poroshell 120 Columns for HPLC and UHPLC

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



# Poroshell 120 Performance After 3000 Injections

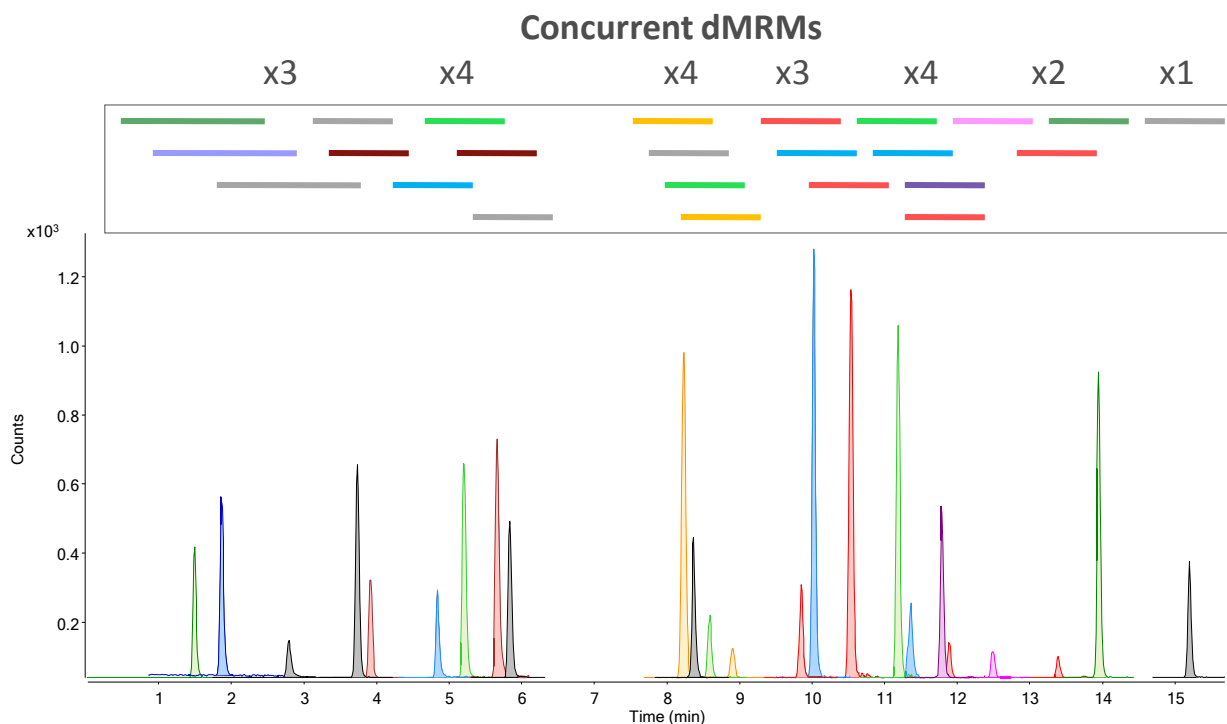
- Dilute and shoot sample preparation
- Analytes covering a wide range of retention times show excellent reproducibility

| 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         |



# 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





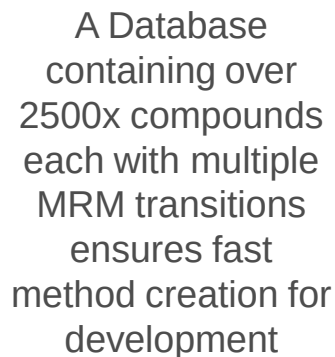
Agilent Analyzers and Application Kits

# Triple Quadrupole LC/MS Forensic Toxicology Application Kit

**Quickly and efficiently implement target screening methods**

Quickly and efficiently implement target screening methods





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

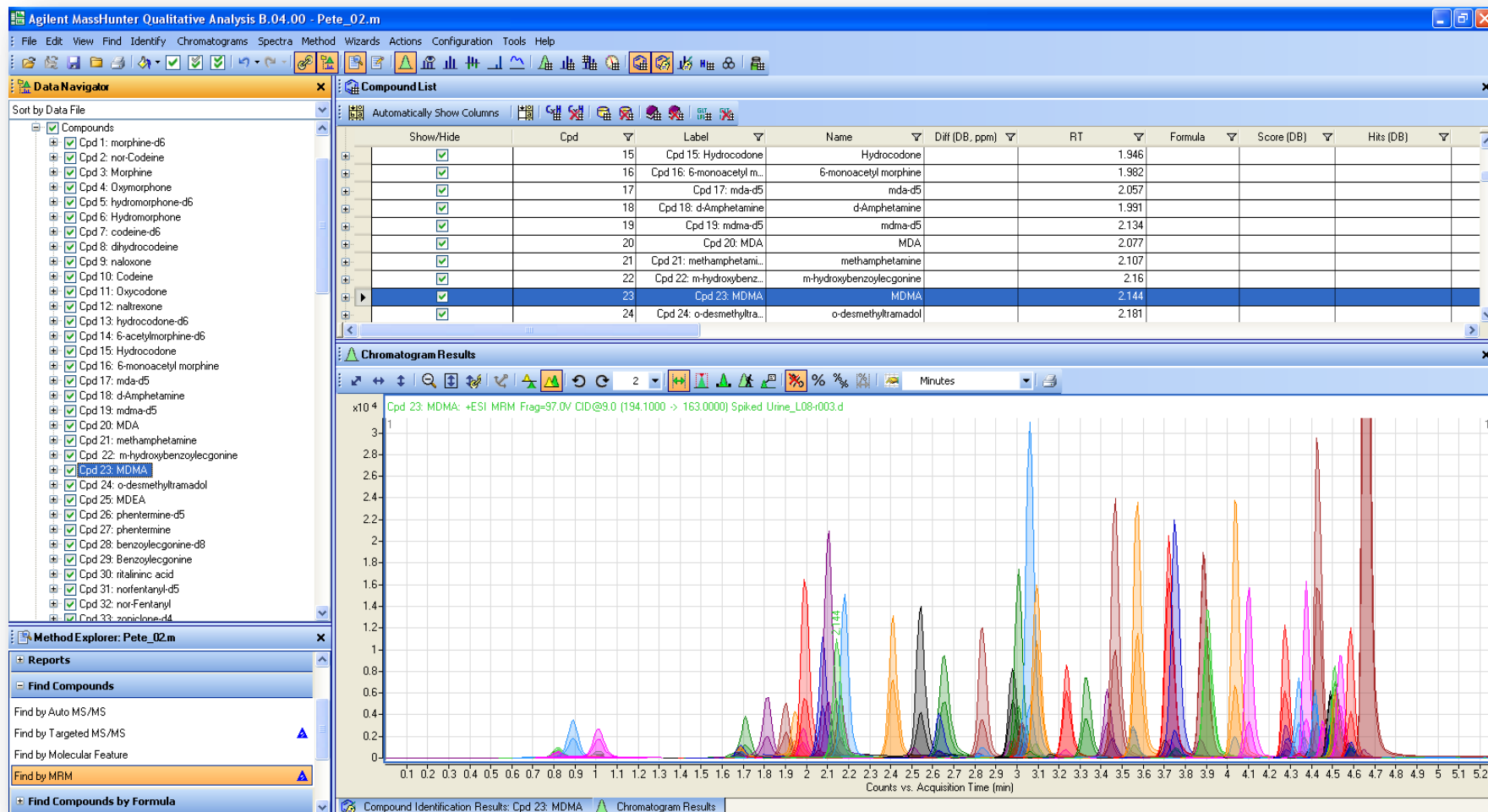


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# Qualitative Screening

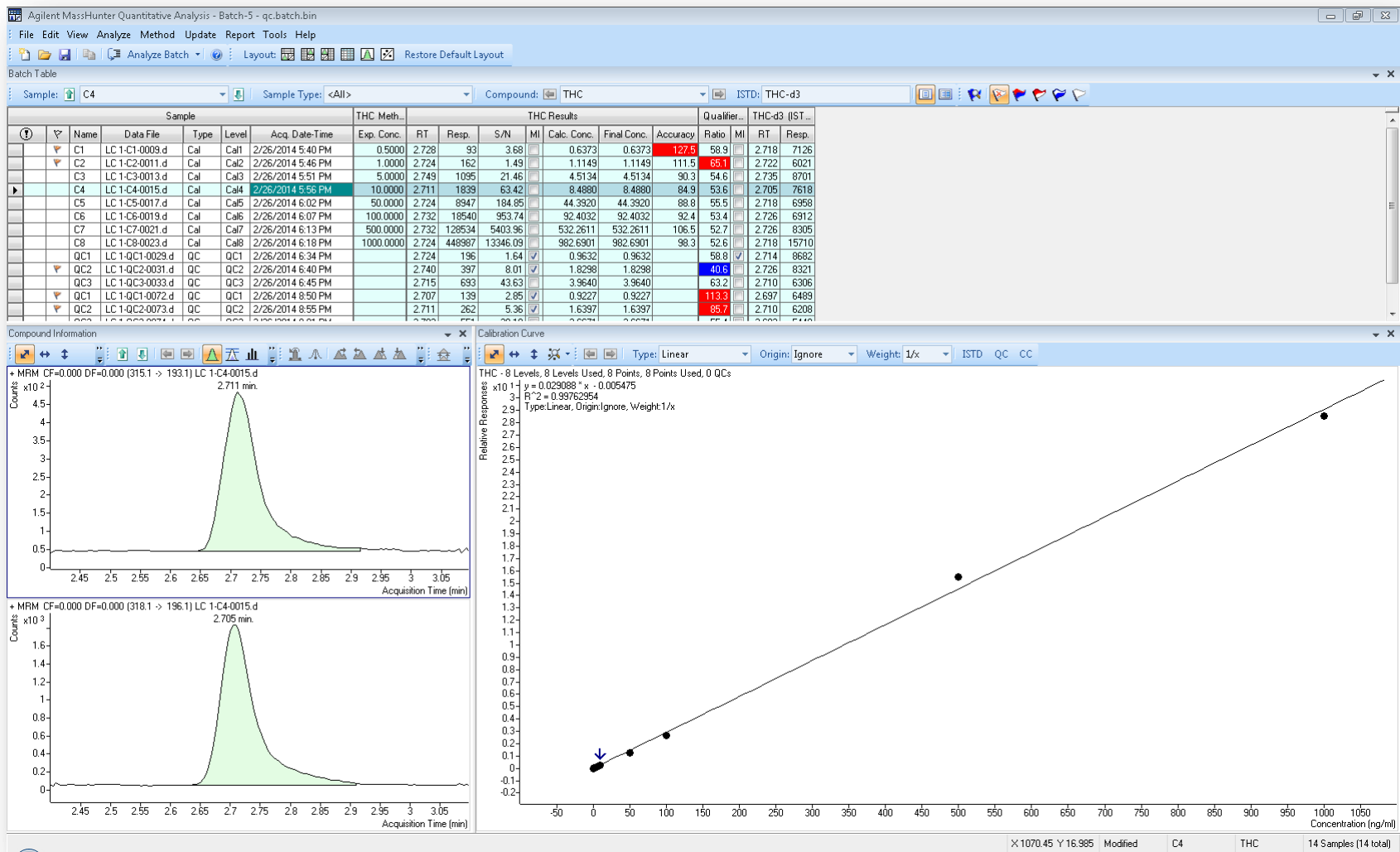
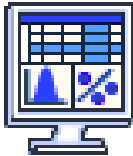
## Find By MRM



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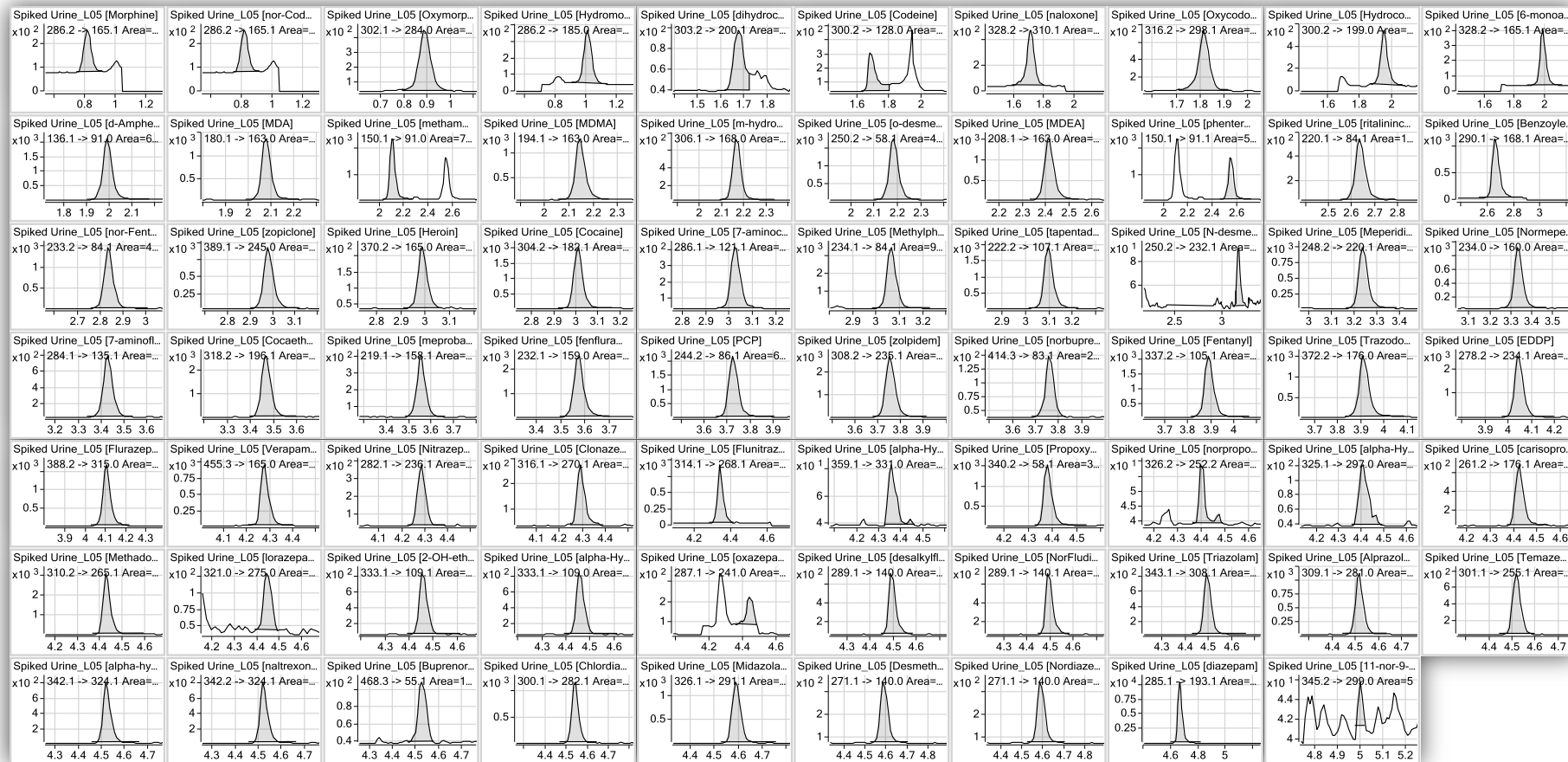
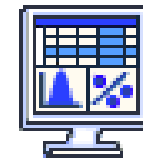
# Quantitative Analysis Batch at a Glance



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# Data Review with Compounds at a Glance

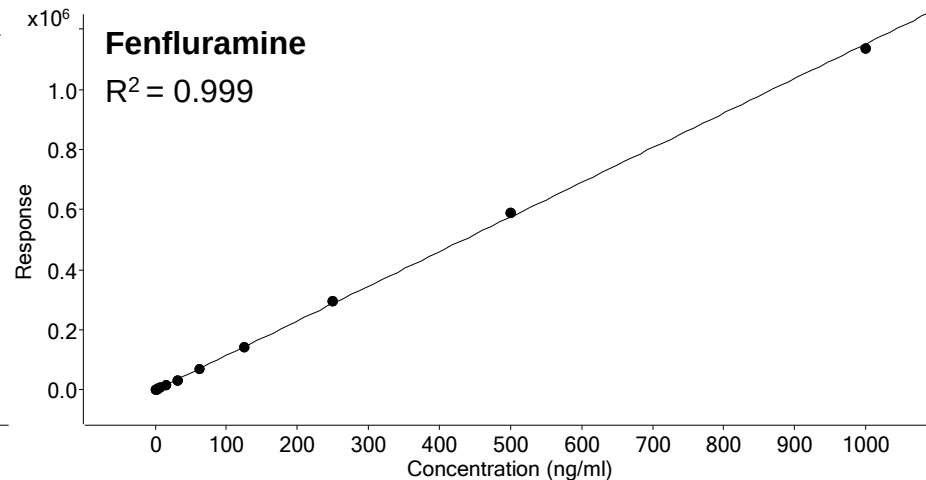
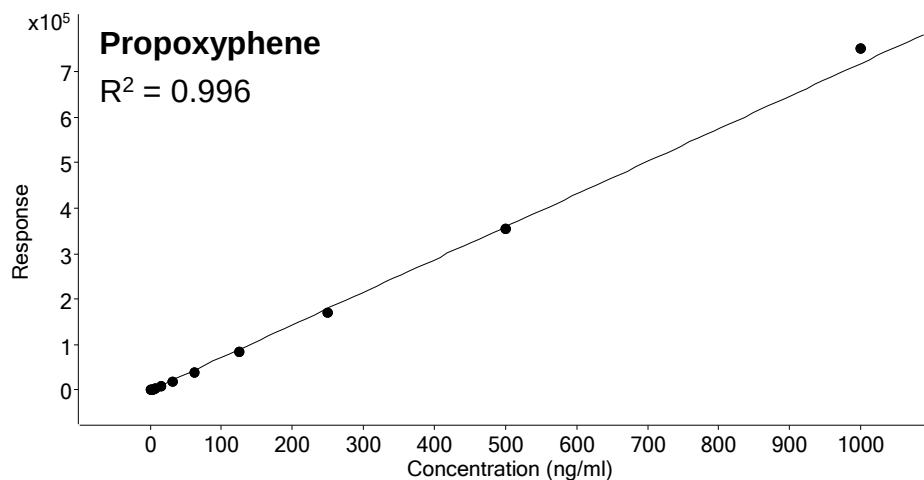
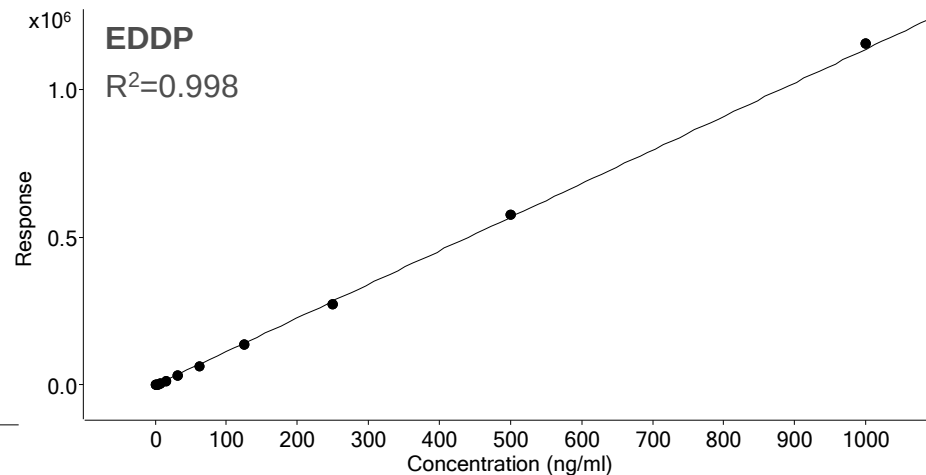
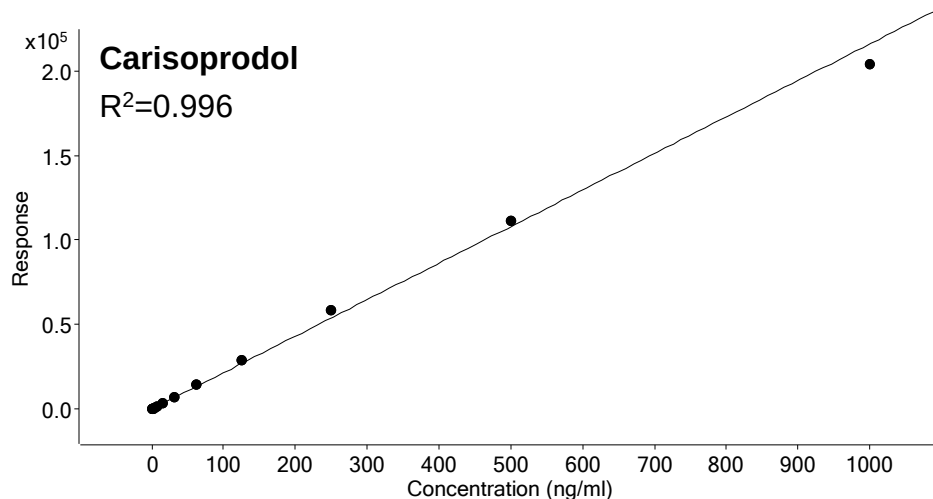
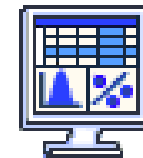


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# Quantative Analysis

## Calibration Curves



# 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 PMSegA\_056.d  
Sample Type Calibration Acq Method File Optimized\_dMRM\_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  |
| Oxycodone              | Positive     | 51.4            | 40.0 ng/ml | 0.948 | 68076  |
| Hydrocodone            | 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-monoacetyl morphine  | Positive     | 52.6            | 40.0 ng/ml | 2.133 | 35081  |
| d-Amphetamine          | Positive     | 50.8            | 40.0 ng/ml | 2.230 | 128641 |
| 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-Aminoclonazepam      | Positive     | 50.0            | 40.0 ng/ml | 3.154 | 38927  |
| Meperidine             | Negative     | 50.4            | 60.0 ng/ml | 3.364 | 252158 |
| Normeperidine          | 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  |
| Triazodone             | Positive     | 49.6            | 40.0 ng/ml | 4.067 | 136292 |
| EDDP                   | Positive     | 48.5            | 25.0 ng/ml | 4.075 | 312209 |
| Verapamil              | Positive     | 46.5            | 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-Hydroxylprazosin | 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   |
| Proclifen              | Positive     | 48.3            | 40.0 ng/ml | 4.688 | 109420 |
| Diazepam               | Positive     | 50.1            | 35.0 ng/ml | 4.756 | 48262  |

PM\_Mix\_50ppb

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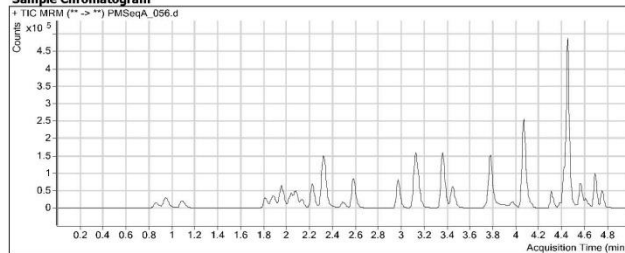
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## Quantitative Analysis Sample Report

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\_dMRM\_ESI\_Methanol\_01.m  
Dilution 1 Acq Time 10/9/2013 0:24  
Position P2-E9 Sample Info  
Injector Vol. 5 Comment

### Sample Chromatogram



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| PCP                   | Positive     | 50.0            | 40.0 ng/ml | 3.779 | 210618 |
| Norbuprenorphine      | Positive     | 51.0            | 40.0 ng/ml | 3.831 | 2172   |

PM\_Mix\_50ppb

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# Conclusions

- Agilent makes it easy to create custom analytical panels for the measurement of Opiates, Opioids, Benzodiazepines, Amphetamines & Illicits
- 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

