

# A UPLC-MS/MS Method for Analyzing Eight Drugs: A Sixty Percent Reduction in Runtime vs. HPLC-MS/MS

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## Abstract:

**Objective:** To improve the efficiency and quality of one of our routinely performed tests, an HPLC-MS/MS method for eight drugs was converted to a UPLC-MS/MS method. Using the new method, we saw improvements in the chromatographic properties and a decrease in the overall run time. The method we selected for comparison quantified Cocaine, Benzoylcegonine, Coca-ethylene, Diphenhydramine, Methadone, EDDP (Methadone metabolite), Chlorpheniramine, and Dextromethorphan in white blood, serum, and plasma.

**Relevance:** We describe a simple and rapid analytical method for the determination of eight commonly used and abused drugs. Additionally, we have converted a method from traditional HPLC to UPLC (Ultra Performance Liquid Chromatography), a faster chromatographic technique which results in turnaround time improvements. This became increasingly important as the use of tandem mass spectrometry grows in clinical laboratory medicine.

**Methodology & Validation:** Sample preparation was the same for both methods. A 200 µL aliquot of specimen was precipitated with 1.0 mL of Acetonitrile containing deuterated internal standards. Specimens were vortexed, centrifuged, and 200 µL of the supernatant was transferred to a new tube. The extract was diluted with water, placed in an autosampler vial, and 10 µL was injected. Methods A and B were validated for both HPLC-MS/MS and UPLC-MS/MS. UPLC-MS/MS analysis was performed on a Waters TQD tandem quadrupole mass spectrometer. Separation was performed at 30°C on a Waters Acquity UPLC HSS T3, 2.1 x 100 mm, 1.8 µm particle size column. Mobile phases consisted of Solvent A: 0.1% Formic Acid in water, and Solvent B: 0.1% Formic Acid in Acetonitrile. A gradient elution from 75%A/25%B to 50%A/50%B provided adequate separation. HPLC-MS/MS analysis was performed on a Waters Quattro Micro mass spectrometer coupled with a Waters 2695 HPLC. The gradient proportioning was similar to that of the UPLC method. The mass spectrometer method was divided into 4 functions to allow for collection of an adequate number of data points.

**Results and Conclusions:** A simple protein precipitation method was developed to analyze eight drugs in blood, serum, and plasma. Specimens were run on both HPLC-MS/MS and UPLC-MS/MS, and the results were comparable. Overall run time was reduced from 10 minutes with HPLC to 4 minutes using UPLC. Both methods had adequate performance characteristics. Within-run precision was less than 12% CV for each analyte. The lower limit of quantitation was 25 ng/mL for Cocaine, Coca-ethylene, Benzoylcegonine, Methadone, Diphenhydramine, and EDDP (group A). The lower limit of quantitation for Dextromethorphan and Chlorpheniramine (group B) was 10 ng/mL. The linear range for group A analytes was 25 to 2000 ng/mL, and the linear range for group B analytes was 10 to 800 ng/mL. A simple, rapid UPLC-MS/MS method for the analysis of Cocaine, Coca-ethylene, Benzoylcegonine, Dextromethorphan, Chlorpheniramine, Methadone, EDDP, and Diphenhydramine was developed. The run time was significantly reduced without any negative effect on quality. A batch of 30 sample is now performed in approximately 2.5 hours by UPLC versus 6.5 hours by traditional HPLC.

## Introduction:

The objective was to improve the efficiency and quality of an HPLC-MS/MS method by converting it to a UPLC-MS/MS method.

- HPLC-MS/MS Method: Waters 2695 HPLC coupled with Waters Quattro Micro
- UPLC-MS/MS: Waters Acquity UPLC coupled with Waters TQD detector

•Simple protein precipitation extraction used to remove analytes from biological specimen

•Analytes were extracted from variety of blood or serum specimens

- Post-mortem Specimen:
  - To determine cause of death: overdose (accidental or intentional), drug interactions, impairment of user
- Clinical/Forensic:
  - Useful in legal proceedings: impairment of user, child custody
  - Drug Compliance Testing

- Protein precipitation extraction used to remove analytes from biological specimen
- The following analytes were included in this method:

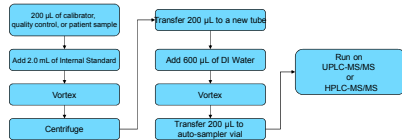
- Cocaine
- Benzoylcegonine
- Coca-Ethylene
- Diphenhydramine
- Methadone
- EDDP
- Chlorpheniramine
- Dextromethorphan

•The Analytes included in this method have different medicinal uses:

- Cocaine - CNS stimulant
- Benzoylcegonine - Cocaine metabolite
- Coca-Ethylene - metabolite of Cocaine when Ethanol is present.
- Diphenhydramine - anti-histamine
- Methadone - analgesic, anti-nausea, to treat opiate addiction
- EDDP - Methadone metabolite
- Chlorpheniramine - anti-histamine
- Dextromethorphan - anti-tussive

## Methodology:

### Extraction:



### HPLC-MS/MS Method:

**Initial Method:**  
 •Column: Atlantis dC18, 3.0 x 100 mm, 3 µm  
 •Guard Column: Atlantis dC18, 3.9 x 20 mm, 3µm  
 •20°C column temperature  
 •Gradient Analysis

Time (min)	DI Water (%)	10mM NH <sub>4</sub> HCO <sub>3</sub> (pH 4.5) (%)	Acetonitrile (%)	Flow Rate (mL/min)
Initial	55.0	10.0	35.0	0.600
1.00	55.0	10.0	35.0	0.600
3.50	20.0	10.0	70.0	0.600
7.00	20.0	10.0	70.0	0.600
7.10	55.0	10.0	35.0	0.600

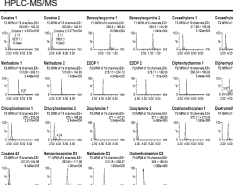
### MS Method:

Analyte	Retention Time (min)	Dwell Time (Secs)	Ion(s)
Cocaine	2.25	0.100	303.85 > 182.03 304.14 > 149.91
Benzoylcegonine	1.15	0.100	290.90 > 168.94 290.90 > 104.86
Cocacethylene	2.80	0.100	317.99 > 196.08 317.99 > 149.90
Methadone	4.90	0.100	310.08 > 265.10 310.08 > 104.88
EDDP	4.50	0.100	278.17 > 234.12 278.17 > 186.06
Diphenhydramine	3.85	0.100	256.04 > 166.93 256.04 > 151.88
Chlorpheniramine	2.75	0.100	275.00 > 230.04 275.00 > 166.94
Dextromethorphan	3.65	0.100	272.11 > 170.96 272.11 > 213.02

### Sample Chromatograms:

200 ng/mL Standard in Blood

#### HPLC-MS/MS



### UPLC-MS/MS Method:

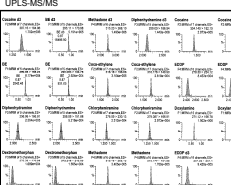
**Initial Method:**  
 •Acquity UPLC HSS T3 2.1 x 100 mm, 1.8 µm column  
 •30°C column temperature  
 •Gradient Analysis

Time (min)	DI Water (%)	0.1 % Formic Acid in DI Water (%)	0.1 % Formic Acid in ACN (%)	Flow Rate (mL/min)
Initial	75.0	25.0	0.500	
0.50	75.0	25.0	0.500	
3.00	50.0	50.0	0.600	
3.01	75.0	25.0	0.600	

### MS Method:

Analyte	Retention Time (min)	Dwell Time (Secs)	Ion(s)
Cocaine	1.38	0.010	304.14 > 182.10 304.14 > 150.16
Benzoylcegonine	0.85	0.010	290.90 > 168.24 290.90 > 104.78
Cocacethylene	1.76	0.010	318.18 > 196.08 318.18 > 149.90
Methadone	2.74	0.010	310.18 > 265.10 310.18 > 104.88
EDDP	2.40	0.010	278.30 > 234.12 278.30 > 186.06
Diphenhydramine	2.09	0.010	256.05 > 166.91 256.05 > 151.88
Chlorpheniramine	1.37	0.025	275.09 > 230.13 275.09 > 166.91
Dextromethorphan	2.02	0.025	272.25 > 170.88 272.25 > 213.09

#### UPLS-MS/MS



## Validation & Results:

The following parameters were used to validate the method:

- Limit of Quantitation (LOQ)
- Within-Run Precision
- Overall Precision
- Accuracy
- Ion Suppression Study

•Calibrators and controls were prepared by spiking drug free whole blood with reference standard

•The linear range was determined by analyzing seven sets of calibrators

•Observed versus expected values were evaluated by quadratic regression.

•Total Precision was estimated using 40 consecutive measurements of each quality control material. The values were pulled from "routine" production batches.

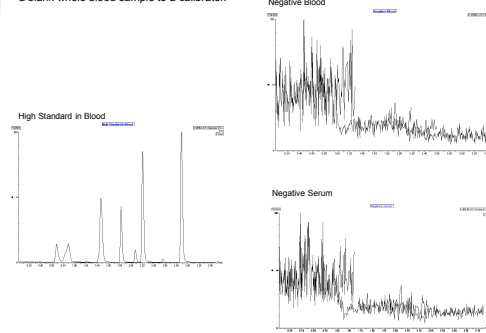
•Within run precision was estimated by measuring five replicates of the two quality control levels in one analytical batch.

•Accuracy was determined by comparing the actual results of the quality control samples to the target value of the quality control.

•Ion Suppression was performed by comparing a blank serum sample and a blank whole blood sample to a calibrator.

### Ion Suppression Study:

•Ion Suppression was performed by comparing a blank serum sample and a blank whole blood sample to a calibrator.



### Linearity:

Analyte	LOQ (ng/ml)	Linear Range (ng/ml)
Cocaine	25	25 – 2000
Benzoylcegonine	25	25 – 2000
Coca-ethylene	25	25 – 2000
Methadone	25	25 – 2000
EDDP	25	25 – 2000
Diphenhydramine	25	25 – 2000
Chlorpheniramine	10	10 – 800
Dextromethorphan	10	10 – 800

### Analyte

Analyte	QC Values (ng/mL)
Cocaine, Benzoylcegonine, Coca-ethylene, Methadone, EDDP, Diphenhydramine	200 & 800
Chlorpheniramine, Dextromethorphan	50 & 200

### Accuracy:

Analyte	Lower Level (%)	Higher Level (%)
Cocaine	97.9	101.7
Benzoylcegonine	93.5	97.6
Coca-Ethylene	99.6	105.6
Methadone	94.2	97.7
EDDP	96.7	101.5
Diphenhydramine	92.8	99.0
Chlorpheniramine	96.0	101.3
Dextromethorphan	98.2	105.3

### Overall Precision:

Analyte	Low Level (%CV)	High Level (%CV)
Cocaine	5.8	5.0
Benzoylcegonine	4.1	4.2
Coca-Ethylene	6.6	6.1
Methadone	5.3	6.1
EDDP	8.4	8.5
Diphenhydramine	8.6	7.8
Chlorpheniramine	7.7	6.4
Dextromethorphan	9.1	9.2

### Within-Run Precision:

Analyte	Low Level (%CV)	High Level (%CV)
Cocaine	4.9	3.8
Benzoylcegonine	4.0	2.8
Coca-Ethylene	2.1	5.6
Methadone	2.8	1.8
EDDP	4.7	1.7
Diphenhydramine	5.3	9.3
Chlorpheniramine	8.0	4.0
Dextromethorphan	8.5	5.4

## Conclusions:

Benefits of the UPLC-MS/MS method:

- Shorter run time which allows for a savings of 4 hours per batch of 30 samples.
- Better separation of components especially in forensic samples.
- Less matrix effects which improves the accuracy of results as compared with HPLC-MS/MS.
- Shorter dwell time in the UPLC-MS/MS method allows for more points across the peaks of the chromatograms ensuring better chromatographic results

## Acknowledgements:

The authors would like to thank Dr. Michael Evans for his continual support.