# Ultrafast, Quantitative Analysis of Buprenorphine, Methadone and Their Metabolites in Human Urine Using Online SPE/MS/MS

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## Introduction

New high throughput methods are desired to overcome the increasing number of samples requiring analysis in clinical research and forensic toxicology laboratories. Ultrafast SPE/MS/MS systems are capable of analyzing many analytes in biological matrices at a rate of 11-15 seconds per sample. In the present study, we evaluated the capabilities of the Agilent RapidFire High-throughput Mass Spectrometry, an ultrafast SPE/MS/MS system, to quantitatively measure multiple drug panels in human urine; methadone (and its metabolite), and buprenorphine (and its metabolite). The results were then compared to traditional LC/MS/MS analysis.

## Experimental

Online SPE methods for each analyte were optimized using a RapidFire High-throughput Mass Spectrometry system coupled to an Agilent 6460 triple quadrupole mass spectrometer. In case of bup/norbup, after the enzymatic hydrolysis, samples were extracted by an offline solid phase extraction procedure using 96 well SPE plates, dried down and reconstituted in 5% methanol in water. Analysis of all samples was performed at a rate of <13 seconds per sample covering the range of 2.5-400 ng/mL. For the MDN/EDDP a simple dilute and shoot method was used over a linear range of 10-5000 ng/mL.

### Sample Preparation

For the bup/norbup panel, after enzymatic hydrolysis, samples were centrifuged at 3,000 rpm for 5 minutes. Then a solid phase extraction protocol was applied on the supernatant using Plexa PCX plates. The eluate from the SPE was dried down under nitrogen, reconstituted with 5% methanol and injected on the RapidFire/MS/MS.

For the MDN/EDDP; 10 µL of each urine sample, blank, calibrators and QC were combined with 1 mL of 50% ultrapure water: 50% HPLC grade methanol containing internal standards. The plate was then sealed and shaken for 30 seconds prior to RapidFire/MS/MS analysis.

## Experimental

The following MRM transitions were monitored for both panels using Agilent 6460 triple quadrupole mass spectrometer.

| Analyte | Q1 | Q3 | Fragmentor | CE |
|---|---|---|---|---|
| Buprenorphine-d4 | 472.3 | 59.1 | 200 | 62 |
| Buprenorphine Quant | 468.3 | 55.1 | 200 | 62 |
| Buprenorphine Qual | 468.3 | 396.2 | 200 | 45 |
| NorBuprenorphine-d3 | 417.3 | 83.1 | 188 | 60 |
| Norbuprenorphine Quant | 414.3 | 83.1 | 188 | 60 |
| Norbuprenorphine Qual | 414.3 | 101.1 | 188 | 50 |

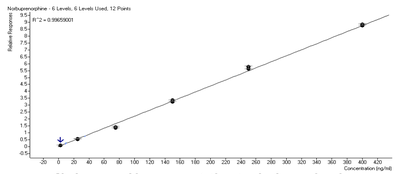
| Analyte | Q1 | Q3 | Fragmentor | CE |
|---|---|---|---|---|
| Methadone-d3 | 313.2 | 268.3 | 85 | 13 |
| Methadone Quant | 310.2 | 265.0 | 95 | 13 |
| Methadone Qual | 310.2 | 105.1 | 95 | 29 |
| EDDP-d3 | 281.2 | 234.1 | 100 | 33 |
| EDDP Quant | 278.2 | 234.1 | 150 | 29 |
| EDDP Qual | 278.2 | 249.1 | 150 | 25 |

| Ionization mode | ESI + Agilent 6460 | |
|---|---|---|
| Panels | Bup/Norbup | MDN/EDDP |
| Drying gas temp. | 350 °C | 300 °C |
| Drying gas flow | 10 L/min | 10 L/min |
| Sheath gas temp. | 350 °C | 350 °C |
| Sheath gas flow | 12 L/min | 12 L/min |
| Nebulizer pressure | 35 psi | 40 psi |
| Nozzle voltage | 0 V | 0 V |
| Capillary voltage | 2800 V | 2800 V |

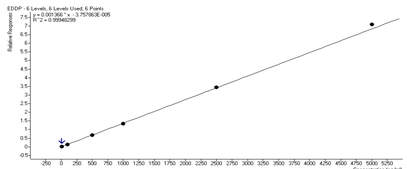
| RapidFire Method | MDN/EDDP | Bup/Norbup |
|---|---|---|
| Solvent A | $H_2O$ + 0.1% FA | $H_2O$ + 5 mM Ammonium Acetate + 0.01%TFA + 0.1% FA |
| Solvent B | 50% MEOH + 25% IPA + 25% ACN + 0.1% Formic acid | 50% MeOH + 25% IPA + 25% ACN + 0.1% Formic acid + 0.01% TFA |
| Solvent C | 50% MEOH + 25% IPA + 25% ACN + 0.1% Formic acid | 50% MeOH + 25% IPA + 25% ACN + 0.1% Formic acid + 0.01% TFA |
| SPE Cartridge | C18 | C18 |
| RF State 1: Aspirate | 600 ms | 1000 ms |
| RF State 2: Load/Wash | 2000 ms | 3500 ms |
| RF State 3: Extra Wash | 0 ms | 0 ms |
| RF State 4: Elute | 4000 ms | 6000 ms |
| RF State 5: Re-equilibrate | 800 ms | 2000 ms |

## Results

**Linearity:** The analytes in both panels had excellent linearity within the measured ranges with $R^2$ values greater than 0.995.




**Norbuprenorphine representative standard curve in urine**

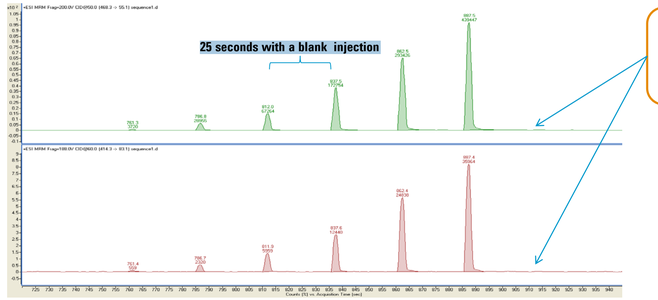



**EDDP representative standard curve in urine**

Bup/Norbup standard curves in urine had excellent linearity within the measured range of 2.5-400 ng/mL. The LOQ was determined to be 2.5 ng/mL for both analytes.

MDN/EDDP standard curves in urine had excellent linearity within the measured range of 10-5000 ng/mL. The LOQ was determined to be 5 ng/mL for both analytes.

**Timing and Carryover:** Example of carryover assessment for Bup/Norbup




**Precision:**

Inter and intra-day accuracies determined were within 10% and coefficient of variation values were all less than 10% for concentrations within the measured range for both panels.

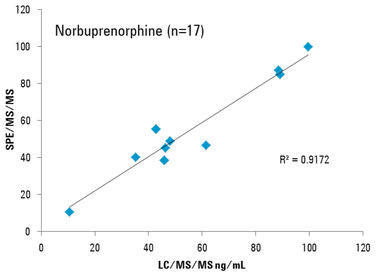
| Norbuprenorphine (ng/mL) | Interday % Accuracy (n=6) | Interday % Precision (n=6) | Intraday % Accuracy (n=6) | Intraday % Precision (n=6) |
|---|---|---|---|---|
| 10 QC | 96.3 | 0.8 | 98.3 | 1.1 |
| 200 QC | 101.6 | 3.1 | 107.6 | 4.7 |
| 50 G QC | 105.0 | 2.2 | 95.2 | 2.8 |
| 330 G QC | 108.5 | 4.9 | 100.6 | 4.9 |

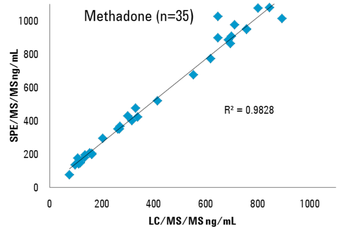
| EDDP (ng/mL) | Interday % Accuracy (n=6) | Interday % Precision (n=6) | Intraday % Accuracy (n=6) | Intraday % Precision (n=6) |
|---|---|---|---|---|
| 150 QC | 96.1 | 1.4 | 99.4 | 3.3 |
| 750 QC | 97.8 | 1.2 | 100.3 | 2.3 |
| 2000 QC | 101.7 | 1.2 | 103.5 | 2.5 |

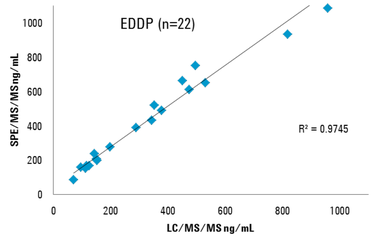
## Results

**Method Comparison with Blinded Human Samples:**

Two sets of 50 and 282 blinded human samples of Bup/Norbup and MDN/EDDP respectively were analyzed by SPE/MS/MS and LC/MS/MS. The results had 100% concordance for positives and negatives and correlated well within the linear range shared between the two methods.




## Discussion

- The drug Buprenorphine and its metabolite Norbuprenorphine were accurately and precisely quantified using an Agilent RapidFire High-throughput Mass Spectrometry System. Samples containing analytes were simultaneously analyzed at 12 seconds per sample, using a high-throughput method of quantitation for these analytes that is capable of analyzing more than 270 samples per hour. This SPE/MS/MS methodology provides comparable results to LC/MS/MS, but at >10x the speed and efficiency of typical LC/MS/MS methods.

- The drug methadone and its metabolite EDDP were rapidly, accurately and precisely measured in urine using a simple dilute and shoot procedure and the Agilent RapidFire/MS/MS System. This method covered a broad linear range of 10 to 5000 ng/mL for each analyte. Samples were analyzed at 11 seconds per sample, providing a high-throughput method of analysis for these analytes. This methodology is capable of throughputs greater than 300 samples per hour. The Agilent RapidFire/MS/MS system may be useful for fast and efficient detection of similar small molecule analytes in urine.

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