The following MRM transitions were monitored for both panels using Agilent 6440 triple quadrupole mass spectrometer:

**Analyte** | **Q1** | **Q3** | **Fragmentor** | **CE** | **Range** | **LOD**
--- | --- | --- | --- | --- | --- | ---

**Buprenorphine Qual** | 468.3 | 310.2 | 200 | 45 | 10-5000 ng/mL | 0.1 ng/mL
**Buprenorphine Quant** | 472.3 | 278.2 | 200 | 62 | 10-5000 ng/mL | 0.1 ng/mL

**Norbuprenorphine Qual** | 414.3 | 234.1 | 188 | 50 | 10-5000 ng/mL | 0.1 ng/mL
**Norbuprenorphine Quant** | 468.3 | 234.1 | 200 | 62 | 10-5000 ng/mL | 0.1 ng/mL

**Methadone Qual** | 278.2 | 220.1 | 120 | 25 | 10-5000 ng/mL | 0.1 ng/mL
**Methadone Quant** | 278.2 | 220.1 | 120 | 25 | 10-5000 ng/mL | 0.1 ng/mL

**EDDP Qual** | 278.2 | 220.1 | 120 | 25 | 10-5000 ng/mL | 0.1 ng/mL
**EDDP Quant** | 278.2 | 220.1 | 120 | 25 | 10-5000 ng/mL | 0.1 ng/mL

**MDN/EDDP**

**RF State 1:** Aspirate
**RF State 2:** 600 ms 1000 ms
**RF State 3:** 600 ms 1000 ms
**RF State 4:** 2500 ms 2900 ms
**RF State 5:** Re-equilibrate

**Flow:**
- **Solvent A:** 50% MEOH + 25% IPA + 25% ACN + 0.1% Formic acid + 0.01% TFA
- **Solvent B:** 100% H2O + 0.1% FA
- **Solvent C:** 100% H2O + 5 mM Ammonium acid + 0.01% TFA

**Injection volume:** 5 μL
**Drying gas:** 350 °C
**Drying gas flow:** 10 L/min
**Sheath gas:** 350 °C
**Sheath gas flow:** 12 L/min
**Nebulizer pressure:** 2800 V
**Capillary voltage:** 2800 V

**Precise:** 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.

**Precision:**
- **Intraday:**
- **Interday:**

**Linearity:** The linearity of the standard curves was excellent within the measured range of 2.5-400 ng/mL. The LOD was determined to be 2.5 ng/mL for both analytes.

**Results and Discussion**

• The drug Buprenorphine and its metabolite Norbuprenorphine were accurately and precisely quantified using an Agilent RapidFire High-throughput Mass Spectrometer System. Samples containing analytes were simultaneously analyzed at 12 seconds per sample, using a high-throughput method of quantification for these analytes. The precision was 100% concordance for positives and negatives and correlated well within the linear range shared between the two 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 50000 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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