# SENSITIVE AND RAPID HOMOGENEOUS IMMUNOASSAY FOR THE DETECTION OF BUPRENORPHINE AND ITS MAJOR METABOLITES IN URINE

#B-296

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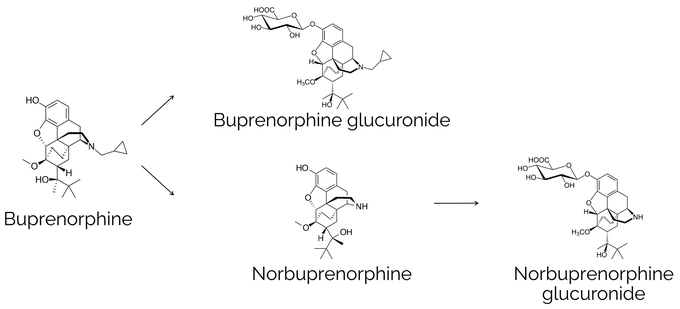
## BACKGROUND

Buprenorphine is a synthetic opioid derived from thebaine. It is structurally and pharmacologically similar to morphine but is 20-30 times more potent. It is a partial agonist receptor modulator and has a longer duration of action relative to morphine due to its unusual slow rate of dissociation from its receptor. Buprenorphine produces a variety of symptoms including, but not limited to addiction, life-threatening respiratory depression, neonatal opioid withdrawal syndrome, severe hypotension. After administration in humans, buprenorphine is primarily metabolized through N-dealkylation to form norbuprenorphine. Both buprenorphine and norbuprenorphine undergo further metabolism via conjugation with D-glucuronic acid to form buprenorphine-glucuronide and norbuprenorphine-glucuronide. Pharmaceutical buprenorphine is a medication used to treat opioid use disorders (OUDs) and manage severe pain that necessitates an opioid analgesic when other treatments are insufficient. Furthermore, it has occasionally been used off-label (i.e., for purposes not approved by the FDA) via injection, including applications in perineural anesthesia and managing withdrawal in hospitalized patients dependent on heroin. In 2002, buprenorphine, including its salts, isomers and salts of isomers, became a Schedule III narcotic substance under the Controlled Substances Act for its potential for abuse and risk of dependence. ARK Diagnostics has developed the ARK Buprenorphine Assay to detect buprenorphine and its metabolites at a cutoff concentration of 5 ng/mL of buprenorphine with high cross-reactivity to its metabolites, norbuprenorphine, buprenorphine-glucuronide, and norbuprenorphine-glucuronide without additional treatment of glucuronidase.




## METHODS

The ARK Buprenorphine Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, with a cutoff concentration of 5.0 ng/mL and semi-quantitative range up to 100 ng/mL. The performance of this assay was evaluated on the Beckman Coulter AU680 Automated Clinical Chemistry Analyzer. Precision, analytical recovery, specificity, Histogram Overlap Analysis of ± 40% controls and the cutoff, and method comparison with LC-MS/MS were evaluated.

## RESULTS

### PRECISION

Pooled human urine was spiked with buprenorphine to achieve concentrations at 0 to 10.0 ng/mL for 5.0 ng/mL Cutoff. Each concentration level was tested in quadruplicate, twice daily, over a 20-day period (N = 160). Semi-quantitative mode of analysis was evaluated according to the Clinical Laboratory Standards Institute (CLSI) Protocol EP05-A3. The qualitative mode of analysis was also assessed.

#### Qualitative Precision

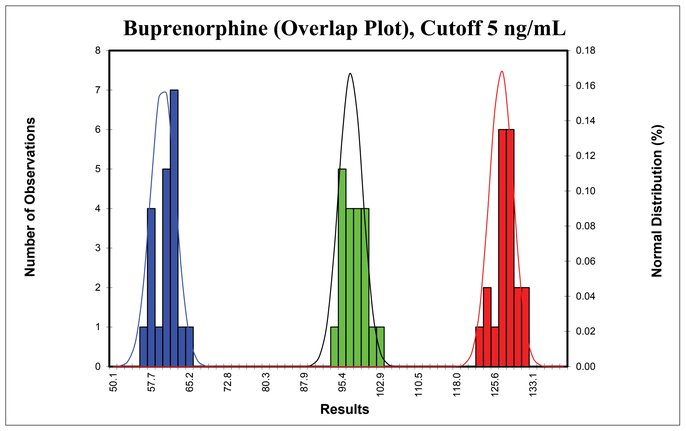
| Buprenorphine (ng/mL) | Relative % Cutoff | # of Results | Results |
|---|---|---|---|
| 0.0 | -100 | 160 | 160 Negative |
| 1.25 | -75 | 160 | 160 Negative |
| 2.50 | -50 | 160 | 160 Negative |
| 3.75 | -25 | 160 | 160 Negative |
| 5.0 | Cutoff | 160 | 108 Negative / 52 Positive |
| 6.25 | +25 | 160 | 160 Positive |
| 7.50 | +50 | 160 | 160 Positive |
| 8.75 | +75 | 160 | 160 Positive |
| 10.0 | +100 | 160 | 160 Positive |

#### Semi-quantitative Precision

| Buprenorphine (ng/mL) | Relative % Cutoff | # of Results | Mean (ng/mL) | SD | CV (%) |
|---|---|---|---|---|---|
| 0.0 | -100 | 160 | 0.0 | 0.07 | N/A |
| 1.25 | -75 | 160 | 1.19 | 0.17 | 14.6% |
| 2.50 | -50 | 160 | 2.40 | 0.16 | 6.9% |
| 3.75 | -25 | 160 | 3.50 | 0.22 | 6.3% |
| 5.0 | Cutoff | 160 | 4.89 | 0.27 | 5.5% |
| 6.25 | +25 | 160 | 6.20 | 0.31 | 4.9% |
| 7.50 | +50 | 160 | 7.41 | 0.39 | 5.3% |
| 8.75 | +75 | 160 | 8.48 | 0.39 | 4.7% |
| 10.0 | +100 | 160 | 9.83 | 0.45 | 4.6% |

### HISTOGRAM OVERLAP ANALYSIS (QUALITATIVE ANALYSIS)

Frequency of distribution of buprenorphine values for each sample is shown by histogram analysis. Twenty replicates each of Negative Control (3.0 ng/mL), Cutoff Calibrator (5.0 ng/mL), and Positive Control (7.0 /mL) were assayed together in a single run. The distributions of measurements did not overlap.




Histogram analysis, Blue (Low 3.0 ng/mL), Green (Cutoff 5.0 ng/mL), Red (High 7.0 ng/mL)

### ANALYTICAL RECOVERY/LINEARITY

Recovery across the assay range was evaluated using in-house prepared samples. Proportional dilutions of a 125 ng/mL buprenorphine stock solution were prepared using pooled negative human urine to yield concentrations of 2.0 ng/mL (LOQ), 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, and 100 ng/mL. Two separately calibrated runs with three replicates of each sample per run were assayed (N=6) in semi-quantitative mode. The percent recoveries ranged from 92.5 to 100.1%.

| Samples (ng/mL) | Mean (ng/mL) | SD | CV (%) | %Nominal | N |
|---|---|---|---|---|---|
| 2.0 | 1.85 | 0.0548 | 3.0 | 92.5 | 6 |
| 5.0 | 4.63 | 0.207 | 4.5 | 92.7 | 6 |
| 10.0 | 9.55 | 0.472 | 4.9 | 95.5 | 6 |
| 20.0 | 19.62 | 0.763 | 3.9 | 98.1 | 6 |
| 30.0 | 29.97 | 1.323 | 4.4 | 99.9 | 6 |
| 40.0 | 38.87 | 1.426 | 3.7 | 97.2 | 6 |
| 50.0 | 49.67 | 1.773 | 3.6 | 99.3 | 6 |
| 60.0 | 59.10 | 4.568 | 7.7 | 98.5 | 6 |
| 70.0 | 68.43 | 3.049 | 4.5 | 97.8 | 6 |
| 80.0 | 79.25 | 2.581 | 3.3 | 99.1 | 6 |
| 90.0 | 90.07 | 3.276 | 3.6 | 100.1 | 6 |
| 100.0 | 98.12 | 5.703 | 5.8 | 98.1 | 6 |

### SPECIFICITY

#### Desirable Structurally Related Compounds

Cross-reactivity of the ARK Buprenorphine Assay to buprenorphine metabolites—norbuprenorphine, buprenorphine glucuronide, and non-buprenorphine glucuronide—was evaluated in semi-quantitative mode to determine the concentration of each compound that produced a response equivalent to the 5.0 ng/mL buprenorphine cutoff.

| Compound | Concentration Approximately Equivalent to to the Cutoff (ng/mL) | Percent Cross-reactivity (%) |
|---|---|---|
| Norbuprenorphine | 9.8 ng/mL | 51.3 |
| Buprenorphine glucuronide | 4.8 ng/mL | 104.6 |
| Norbuprenorphine glucuronide | 7.6 ng/mL | 66.2 |

#### Undesirable structurally related or unrelated opiate compounds

The following 46 structurally related or unrelated opiate compounds were tested in drug-free negative human urine. The ARK Buprenorphine Assay showed no cross reactivity to them.

| Compound | Concentration Tested (ng/mL) | Compound | Concentration Tested (ng/mL) |
|---|---|---|---|
| 6-Acetylcodeine | 100,000 | Morphine-6-Glucuronide | 100,000 |
| 6-Acetylmorphine | 100,000 | Nalbuphine | 250,000 |
| Codeine | 100,000 | Nalorphine | 100,000 |
| Codeine-6-β-Glucuronide | 100,000 | Naloxegol | 100,000 |
| Dextromethorphan | 100,000 | Naloxone* | 100,000 |
| Dextrorphan | 100,000 | Naltrexone | 100,000 |
| Diacetylmorphine (heroin) | 100,000 | N-desmethyl tramadol | 100,000 |
| Dihydrocodeine | 100,000 | Norcodeine | 100,000 |
| Dihydrocodeine-6-glucuronide | 100,000 | Norhydrocodone | 100,000 |
| Dihydromorphine | 100,000 | Normorphine | 100,000 |
| EDDP | 100,000 | Noroxycodone | 100,000 |
| EMDP | 100,000 | Noroxymorphone | 100,000 |
| Ethyl morphine | 100,000 | Norpropoxyphene | 100,000 |
| Fentanyl | 100,000 | O-desmethyl tramadol | 100,000 |
| Hydrocodone | 100,000 | Oxycodone | 100,000 |
| Hydromorphone | 100,000 | Oxymorphone | 100,000 |
| Hydromorphone Glucuronide | 100,000 | Oxymorphone-3-β-Glucuronide | 100,000 |
| Levallorphan | 100,000 | Pentazocine (Talwin) | 100,000 |
| Levorphanol | 100,000 | Propoxyphene | 100,000 |
| Meperidine | 100,000 | Tapentadol | 100,000 |
| Methadone | 320,000 | Thebaine | 100,000 |
| Morphine | 120,000 | Tilidine | 100,000 |
| Morphine-3-Glucuronide | 520,000 | Tramadol | 150,000 |

#### Undesirable Structurally Unrelated Compounds

No interference was observed by testing the following 196 structurally unrelated compounds at tested concentrations.

**1. No interference was observed by testing the following structurally unrelated compounds at tested concentrations.**

Up to 500,000 ng/mL: Butalbital
Up to 250,000 ng/mL: Quetiapine (Seroquel), Sulfamethoxazole
Up to 200,000 ng/mL: Sulpiride
Up to 125,000 ng/mL: Trimethoprim
Up to 100,000 ng/mL:
(+)-MDA, 11-hydroxy-delta-9-THC, 4-Bromo-2,5-Dimethoxyphenethylamine, 4-Fluoromethcathinone (4-FMC), 6-Naltrexol, 7-Aminoclonazepam, 7-Aminoflurnitrazepam, 7-Aminonitrazepam, Alprazolam, Amobarbital, Aripiprazole (Abilify), Atenolol (Tenormin), Benzylpiperazine, Bromazepam, Budesonide (Pulmicort), Bupropion, Buspirone (Buspar), Butabarbital Cannabidiol, Cannabinol, Carbamazepine-10,11-epoxide, Carisoprodol, Clobazam, Clonazepam, Cocaine, Cyanocobalamin (Vitamin B12), Delta-9-THC, Demoxepam, Deoxymethoxetamine, Desalkylflurazepam, Doxylamine, Duloxetine (Cymbalta), Ecgonine, Ecgonine Methyl Ester, Ethyl-D glucuronide, Famotidine (Pepcid), Fenfluramine (-), Fenfluramine (+), Flunitrazepam, Flurazepam, Fluticasone Furoate (Trelegy Ellipta), Formoterol (Foradil), Hexobarbital, Lamotrigine, Lorazepam, Losartan (Cozaar), Lurasidone (Latuda), MDEA, MDPV (Methylenedioxypyrovalerone), Meprobamate, Metformin (Glucophage), Methoxisopropamine, Methylone (3,4-Methylenedioxy-N-methylcathinone), Methylphenidate, Midazolam, Mirtazepine (Remeron), N-desmethyltapentadol, N-desmethyl venlafaxine, Nicotine, Nitrazepam, Norpseudoephedrine, Norsertraline, O-desmethyl venlafaxine, Olodaterol (Striverdi Respimat), Paliperidone (Invega), Paraxanthine, Pentobarbital, Phentermine, Phenylephedrine, Phenylpropanolamine, PMA, Prazepam, Propranolol, Quinine, S (+) MHD (10-monohydroxy derivative), Steviol, Steviol Glucuronide, Temazepam, Testosterone, Theophylline, Tianeptine (Stablon, Tatinol, and Coaxil), Tiotropium (Spiriva), trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine, Triazolam, Trifluoromethylphenylpiperazine, Valacyclovir (Valtrex)

Up to 50,000 ng/mL: Lorazepam Glucuronide, Sufentanil Citrate

**2. No interference was observed by testing the following structurally unrelated compounds at 100,000 ng/mL in the presence of buprenorphine at 3 ng/mL and 7 ng/mL.**

10,11-Dihydrocarbamazepine, 7-hydroxymitragynine, Acetaminophen, Acetylsalicylic acid, Albuterol or Salbutamol (Ventolin), Amisulpride, Amitriptyline, Amoxicillin, Amphetamine, Atorvastatin (Lipitor), AZT (Zidovudine), Benzoylecgonine, Brompheniramine, Caffeine, Captopril, Carbamazepine, Chlordiazepoxide, Chloroquine, Chlorpromazine, Cimetidine, Ciprofloxacin, Citalopram, Clomipramine, Clonidine, Cyclobenzaprine, d-Amphetamine, Desipramine, Desmethyl ofloxacin, Diazepam, Diclofenac, Digoxin, Diphenhydramine, d-Methamphetamine, Doxepin, Enalapril, Ephedrine (1R, 2S), Ephedrine (1S, 2R), Fluoxetine, Fluphenazine, Gabapentin, Haloperidol, Hydroxychloroquine, Hydroxyzine (dihydrochloride), Ibuprofen, Imipramine, Ipratropium (Atrovent), Ketamine, Ketorolac Tromethamine, L-Cotinine, Levofloxacin, Levothyroxine (L-Thyroxine), Lidocaine, Loratadine (Claritin), Lormetazepam, LSD, Maprotiline, MDMA (Ecstasy), Methaqualone, Methoxetamine, Mitragynine, Montelukast (Singulair), Naproxen, Nicotinic Acid, Nifedipine, Nordiazepam, Norfentanyl, Norketamine, Nortriptyline, Ofloxacin, Omeprazole (Prilosec and Losec), Oxazepam, Oxcarbazepine (Trileptal), Paroxetine, Perphenazine, Phencyclidine, Phenelzine (sulfate), Phenobarbital, Phenytoin, Prazosin (Minipress), Procainamide (HCl), Procyclidine (HCl), Promethazine (HCl), Protriptyline (HCl), Pseudoephedrine (R,R), Pseudoephedrine (S,S), Quinacrine (HCl), R (-) MHD (10-monohydroxy derivative), Ranitidine, Ritalin, S (+) MHD (10-monohydroxy derivative), Salicylic Acid, Scopolamine, Secobarbital, Sertraline, Sulpiride, THC (11-nor-Δ9-THC-9-COOH), Thioridazine, Trazodone, Trimethoprim, Trimipramine, Tyramine, Venlafaxine, Verapamil, Xylazine, Zolpidem

### METHOD COMPARISON

A total of three-hundred and eighty (380) unaltered, un-pretreated with glucuronidase, clinical human urine specimens that are not individually identifiable were analyzed for buprenorphine and its metabolites with the ARK Buprenorphine Assay in semi-quantitative mode and the results were compared to LC-MS/MS. Results are summarized in the tables below.

| | LC-MS/MS Results (ng/mL) | | | |
|---|---|---|---|---|
| ARK Buprenorphine Assay Result | Low Negative Less than 50% below the Cutoff (< 2.5 ng/mL) | Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (2.5 - 4.9 ng/mL) | Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (5.0 – 7.5 ng/mL) | High Positive Greater than 50% above the Cutoff (> 7.5 ng/mL) |
| Negative (< 5 ng/mL) | 275 | 0 | 0 | 0 |
| Positive (≥ 5 ng/mL) | 0 | 0 | 0 | 105 |

## CONCLUSIONS

The ARK Buprenorphine Assay measures buprenorphine and its major metabolites, norbuprenorphine, buprenorphine glucuronide, and Norbuprenorphine glucuronide in human urine with good analytical performance. The assay has superior specificity as other opiates and opioids do not cross react. The assay is sensitive, rapid, and applicable to a wide range of clinical chemistry analyzers.

## REFERENCES

1. Drug Enforcement Administration, Office of Diversion Control, Drug & Chemical Evaluation Section. 2022. Buprenorphine.
2. Belsey SL, Couchman L, Flanagan RJ. 2014. Buprenorphine detection in urine using liquid chromatography-high-resolution mass spectrometry: comparison with cloned enzyme donor immunoassay (ThermoFisher) and homogeneous enzyme immunoassay (immunalysis). J Anal Toxicol. 38(7):438-43.
3. Kumar R, Viswanath O, Saadabadi A. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): June 08, 2024. Buprenorphine.

## REGULATORY STATUS

Product under development. Not FDA cleared for sale in the U.S.