SENSITIVE HOMOGENEOUS ENZYME IMMUNOASSAY FOR CARISOPRODOL METABOLITE (MEPROBAMATE)

#B-312

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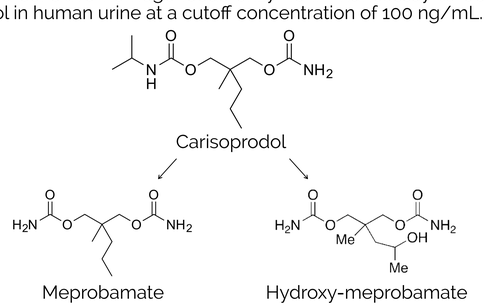
BACKGROUND

Carisoprodol is a carbamate derivative medication used for musculoskeletal pain. Therapeutic use of carisoprodol produces common side effects such as headache, dizziness, and sleepiness, but more serious side effects include allergic reactions and seizures. Isolated case reports suggest that carisoprodol may have the potential for abuse. Carisoprodol was approved for medical use in the United States in 1959. Its approval in the European Union was withdrawn in 2008 due to the risk of abuse or addiction. In the United States, it is a Schedule IV controlled substance. In humans, carisoprodol primarily metabolizes to meprobamate, an active metabolite, via the polymorphic CYP2C19 enzyme. Meprobamate is also a schedule IV-controlled substance used to treat anxiety and has a noted potential for abuse. ARK Diagnostics has developed a sensitive homogeneous enzyme immunoassay to detect meprobamate and carisoprodol in human urine at a cutoff concentration of 100 ng/mL.



METHODS

The ARK Carisoprodol Metabolite (Meprobamate) Assay is a liquid-stable homogenous enzyme immunoassay consisting of two reagents. The assay has a 100 ng/mL cutoff. The performance characteristics of this assay, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU680 automated clinical analyzer.

RESULTS

PRECISION

Drug-free, negative human urine was supplemented with Meprobamate (0.0 to 200 ng/mL for 100 ng/mL Cutoff). Each level was assayed in quadruplicate twice a day for 20 days (N=160) and evaluated qualitatively and semi-quantitatively. Results are summarized in the tables below.

Qualitative Precision

Human Urine (ng/mL)	Relative % Cutoff	# of Results	Results
0.0	-100	160	160 Negative
25.0	-75	160	160 Negative
50.0	-50	160	160 Negative
75.0	-25	160	160 Negative
100.0	Cutoff	160	48 Negative / 112 Positive
125.0	+25	160	160 Positive
150.0	+50	160	160 Positive
175.0	+75	160	160 Positive
200.0	+100	160	160 Positive

Semi-quantitative Precision

Meprobamate (ng/mL)	Relative % Cutoff	# of Results	Mean (ng/mL)	Results
0.0	-100	160	0.2	160 Negative
25.0	-75	160	21.4	160 Negative
50.0	-50	160	49.9	160 Negative
75.0	-25	160	75.3	160 Negative
100.0	Cutoff	160	103.6	29 Negative / 131 Positive
125.0	+25	160	129.7	160 Positive
150.0	+50	160	155.8	160 Positive
175.0	+75	160	184.8	160 Positive
200.0	+100	160	209.0	160 Positive

LOWER LIMIT OF QUANTIFICATION

The LLOQ of the ARK Meprobamate Assay is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed. A set of samples were prepared by spiking Meprobamate into negative urine to give a theoretical concentration of 10, 20, and 30 ng/mL. Eight replicates of the test samples were assayed from one calibration curve. This was repeated 5 times to yield 40 replicates of the test samples. ARK Meprobamate Assay met the criteria of LLOQ at 20.0 ng/mL.

HISTOGRAM OVERLAP ANALYSIS (QUALITATIVE ANALYSIS)

Frequency of distribution of meprobamate values for each sample is shown by histogram analysis. Twenty replicates each of Negative Control (75 ng/mL), Cutoff Calibrator (100 ng/mL), and Positive Control (125 ng/mL) were assayed together in a single run. The distributions of measurements did not overlap.

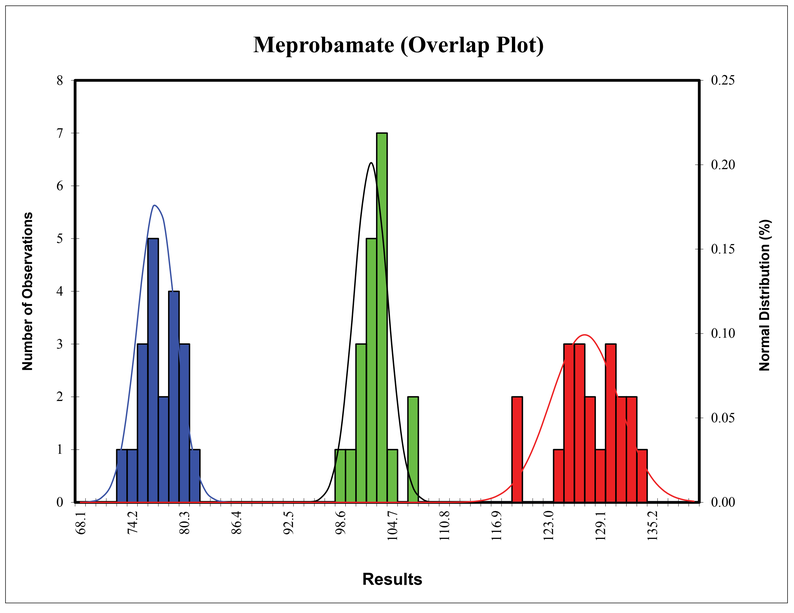


Figure 1. Histogram analysis, Blue (Low 75.0 ng/mL), Green (Cutoff 100.0 ng/mL), Red (High 125.0 ng/mL)

ANALYTICAL RECOVERY/LINEARITY

Recovery across the assay range was assessed using in-house prepared samples. Proportional dilutions of 625 ng/mL Meprobamate were made with pooled negative human urine to give concentrations of 20 (LOQ), 30, 50, 100, 200, 300, 400, and 500 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 88.3% to 101.5% from 20.0 to 500.0 ng/mL.

Samples (ng/mL)	Mean (ng/mL)	SD	CV (%)	%Nominal	N
20.0	17.67	0.942	5.3	88.3	6
30.0	27.42	1.107	4.0	91.4	6
50.0	50.50	1.740	3.4	101.0	6
100.0	103.03	2.022	2.0	103.0	6
200.0	201.03	8.039	4.0	100.5	6
300.0	294.53	14.562	4.9	98.2	6
400.0	402.88	13.410	3.3	100.7	6
500.0	507.50	35.466	7.0	101.5	6

SPECIFICITY

Carisoprodol and Hydroxy-meprobamate

The cross-reactivity to Carisoprodol and Hydroxy-meprobamate compounds with the ARK Carisoprodol Metabolite (Meprobamate) Assay was tested in semi-quantitative mode to obtain the concentration of each compound equivalent to the 100 ng/mL cutoff.

Compound	Concentration Approximately Equivalent to 100 ng/mL Cutoff (ng/mL)	Percent Cross-reactivity (%)
Carisoprodol	100	106.7
Hydroxy-meprobamate	15,000	0.75

Structurally Related Compounds

No interference was observed by testing the following 29 structurally related compounds.

Compound	Highest concentration negative to 100 ng/mL Cutoff (ng/mL)	Compound	Highest concentration negative to 100 ng/mL Cutoff (ng/mL)
Buprenorphine	100,000	Naltrexone	100,000
Codeine	100,000	Neostigmine	200,000
Darunavir	200,000	Norbuprenorphine	100,000
Dihydrocodeine	100,000	Norcodeine	100,000
Efavirenz	200,000	Normorphine	100,000
Felbamate	45,000	Oxycodone	100,000
Hydrocodone	100,000	Oxymorphone	100,000
Hydromorphone	100,000	Propoxyphene	100,000
Meperidine	100,000	Retigabine	200,000
Methocarbamol	200,000	Ritonavir	200,000
Mitomycin C	200,000	Rivastigmine	200,000
Morphine	100,000	Trazodone	100,000
Morphine-3-glucuronide	100,000	Venlafaxine	100,000
Morphine-6-glucuronide	100,000	Zafirlukast	200,000
Naloxone	100,000		

Structurally Unrelated Compounds

The following structurally unrelated compounds were tested in negative urine and meprobamate concentrations of 75 ng/mL and 125 ng/mL (the negative and positive control concentrations, respectively). No interference or cross-reactivity was observed by the addition of compounds up to the indicated concentrations.

Up to 500,000 ng/mL:
Acetaminophen, Acetylsalicylic Acid, Caffeine, Diphenhydramine, Ibuprofen, Methadone

Up to 100,000 ng/mL:
6-Acetylcodeine, 6-Acetylmorphine, Albuterol (Salbutamol), Alprazolam, 7-Aminoclonazepam, 7-Aminoflunitrazepam, 7-Aminonitrazepam, Amitriptyline, Amobarbital, d-Amphetamine, S-(+) Amphetamine, Aprobarbital, Barbital, Benzoylecgonine, Benzylpiperazine, Bromazepam, 4-Bromo-2,5-Dimethoxyphenethylamine, Bupropion, Butabarbital, Butalbital, Cannabidiol, Carbamazepine, Cetirizine, Chlordiazepoxide, Chlorpheniramine, Chlorpromazine, Clobazam, Clomipramine, Clonazepam, Clozapine, Cocaine, Cotinine, Cyclobenzaprine, Dehydronorketamine, Demoxepam, Desakylflurazepam, Desipramine, N-desmethyl Tapentadol, O-desmethyl Tramadol, N-desmethyl Venlafaxine, Dextromethorphan, Diazepam, Digoxin, Doxylamine, Ecgonine, Ecgonine methyl ester, EDDP, EMDP, 1R,2S(-)-Ephedrine, 1S,2R(+)-Ephedrine, Ethyl glucuronide, Ethylmorphine, Fenfluramine, Fentanyl, Flunitrazepam, Fluoxetine, Fluphenazine, Flurazepam, Haloperidol, Heroin, Hexobarbital, Imipramine, Ketamine, Lamotrigine, Levorphanol Tartrate, Lidocaine, Loratadine, Lorazepam glucuronide, Lormetazepam, LSD, Maprotiline, MDA (3,4-Methylenedioxyamphetamine), MDEA, MDMA (3,4-Methylenedioxymethamphetamine), d-Methamphetamine, S(+)-Methamphetamine, Methapyrilene, Methaqualone, Methoxetamine, Methylone, Methylphenidate, Metronidazole, Midazolam, Nalorphine, Naloxone, Naproxen, Nicotine, Nitrazepam, Nordiazepam, Norketamine, Norpropoxyphene, Norpseudoephedrine, Nortriptyline, Olanzapine, Oxazepam, PCP (Phencyclidine), Pentazocine, Pentobarbital, Phenazepam, Phenobarbital, Phentermine, r-(-)-Phenylephrine, Phenylpropanolamine, Phenytoin, PMA, Prazepam, d-Propoxyphene, Propranolol, Protriptyline, R,R(-)- Pseudoephedrine, S,S(+)-Pseudoephedrine, Ranitidine, Ritalinic Acid, Salicylic Acid, Secobarbital, Sertraline, Tapentadol, Temazepam, THC-OH (11-hydroxy-delta-9-THC), THC-COOH (11-nor-9-carboxy-THC), Theophylline, Thioridazine, Tramadol (cis-Tramadol), Triazolam, Trifluoromethylphenyl-piperazine, Trimipramine, Valproic Acid, Venlafaxine, Verapamil, Zolpidem Tartrate

Up to 80,000 ng/mL: Alphenal

Up to 50,000 ng/mL: Sufentanil citrate

METHOD COMPARISON

A total of two hundred and ninety-eight (298) unaltered, clinical human urine specimens that are not individually identifiable were analyzed for carisoprodol and meprobamate with the ARK Carisoprodol Metabolite (Meprobamate) Assay in semi-quantitative mode and the results were compared to LC-MS/MS. ARK Carisoprodol Metabolite (Meprobamate) Assay showed 95.7 % specificity and 97.7 % sensitivity.

	LC-MS/MS Results (Meprobamate + Carisoprodol) (ng/mL)			
ARK Result	Low Negative Less than 50% below the Cutoff (< 50 ng/mL)	Near Cutoff Negative Between 50% below the Cutoff and the Cutoff (50-99.9 ng/mL)	Near Cutoff Positive Between the Cutoff and 50% above the Cutoff (100-149.9 ng/mL)	High Positive Greater than 50% above the Cutoff (> 150 ng/mL)
Negative (< 100 ng/mL)	200	3	1	1*
Positive (≥ 100 ng/mL)	1**	8	20	64

* 89.4 ng/mL (ARK Assay) vs 172.24 (LC-MS/MS)
** 105 ng/mL (ARK Assay) vs 49.77 (LC-MS/MS)

CONCLUSIONS

The ARK Carisoprodol Metabolite (Meprobamate) Assay enables the sensitive, rapid, and reliable measurement of meprobamate and carisoprodol in human urine. The ARK Carisoprodol Metabolite (Meprobamate) Assay is applicable to a wide range of clinical chemistry analyzers.

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REGULATORY STATUS

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