ARK™ OXCARBAZEPINE METABOLITE ASSAY FOR THE BECKMAN COULTER AU480® AUTOMATED CLINICAL CHEMISTRY ANALYZER

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Introduction

ABSTRACT

Rationale

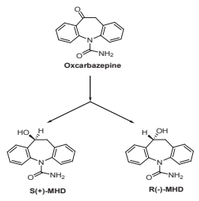
Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Oxcarbazepine (OXC) and eslicarbazepine acetate are second and third generation antiepileptic drugs (AED) respectively. These prodrugs are metabolized to 10-monohydroxy derivative (MHD), the active agent. Serum levels of MHD usually range 3 µg/mL to 35 µg/mL. Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of MHD is described. Both the S and R enantiomers of the active metabolite are detected.

Methods

The ARK™ Oxcarbazepine Metabolite Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.0, 2.0, 5.0, 12.0, 25.0 and 50.0 µg/mL) and 3 controls (3.0, 10.0 and 30.0 µg/mL). The performance of the ARK assay was evaluated on the Beckman Coulter AU480® analyzer. Precision, limit of quantitation (LOQ), recovery, specificity and method comparison were studied.

Results

Total precision ranged 5.5% to 6.5%CV and within-run precision ranged 3.8% to 4.8%CV in a 20-day study using quality controls and spiked serum samples. Acceptable quantitation and recovery was observed from 1.0 µg/mL (LOQ) to 35.0 µg/mL. The assay crossreacted with structurally similar oxcarbazepine (22.2%), eslicarbazepine acetate (22.1%), and carbamazepine (20.4%) and its metabolites (cis-10, 11-dihydroxy carbamazepine, dihydro-carbamazepine, and carbamazepine epoxide). The assay did not crossreact with other AEDs tested (gabapentin, lamotrigine, levetiracetam, topiramate, and zonisamide). The assay was not interfered by endogenous substances or other potentially coadministered drugs tested. One hundred specimens (1.7 to 34.3 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to LC/MS/MS values: ARK = 1.01 LC/MS/MS - 0.38 (r^2=0.96).



Structures of Oxcarbazepine and Metabolites

Precision

Precision was determined as described in CLSI Protocol EP5-A3. Tri-level controls and sera containing oxcarbazepine metabolite were assayed in quadruplicate twice a day for 20 days. Mean determinations of oxcarbazepine metabolite, standard deviation (SD) for within-run, between-day, and total coefficients of variation (% CVs) were calculated.

			Within Run		Between Day		Total	
Sample	N	Mean (µg/mL)	SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Control								
LOW	160	3.0	0.14	4.5	0.10	3.3	0.19	6.2
MID	160	10.0	0.38	3.8	0.41	4.1	0.57	5.7
HIGH	160	30.6	1.48	4.8	1.25	4.1	2.00	6.5
Human Serum								
LOW	160	3.0	0.13	4.5	0.10	3.4	0.17	5.8
MID	160	9.9	0.45	4.6	0.32	3.3	0.55	5.6
HIGH	160	29.6	1.28	4.3	1.00	3.4	1.64	5.5

Lower Limit of Quantitation

Limit of quantitation was evaluated according to CLSI EP17-A2. Pooled human serum was supplemented with known amounts of oxcarbazepine metabolite and assayed 40 times. The LLOQ of the ARK Oxcarbazepine Metabolite Assay is defined as the lowest concentration that meets both acceptable inter-assay precision (≤20% CV) and ±15% recovery. The criteria of LLOQ were met at 1.0 µg/mL.

Nominal (µg/mL)	Mean Recovery (µg/mL)	Percent Recovery (%)	CV (%)	N
0.5	0.39	78.0	10.9	40
1.0	0.90	90.0	7.7	40
1.5	1.38	92.0	8.3	40

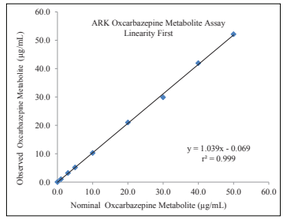
Analytical Recovery

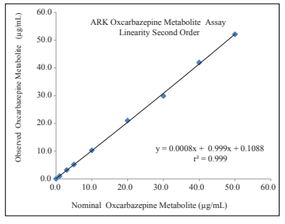
Analytical recovery throughout the measurement range was assessed by supplementing negative human serum with enantiomers of oxcarbazepine metabolite. The S:R ratio of each enantiomer was varied. The mean of six (6) replicate measurements of oxcarbazepine metabolite was tabulated as a function of the enantiomer ratio.

Mean Recovered Concentration (µg/mL)				
Theoretical Concentration (µg/mL)	S:R 1:1	S:R 4:1	S:R 9:1	S:R 19:1
1.0	0.77	0.93	0.98	0.95
4.0	3.78	3.92	3.94	3.86
8.0	7.47	8.18	8.16	7.82
15.0	14.10	15.80	14.91	15.42
20.0	19.03	21.69	19.81	21.02
35.0	33.74	34.71	33.52	36.16
45.0	42.89	46.88	44.63	49.46

Linearity

Linearity studies were performed as suggested in CLSI Protocol EP6-A. A 60.0 µg/mL serum sample was prepared and dilutions were made proportionally with human serum negative for oxcarbazepine metabolite. Oxcarbazepine metabolite concentrations ranged from 1.0 to 50.0 µg/mL. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values or ≤ 0.20 µg/mL below 2.0 µg/mL. A linear relationship was demonstrated between 1.0 and 50.0 µg/mL.





Specificity - Coadministered Drugs

Specificity studies were conducted using CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry.

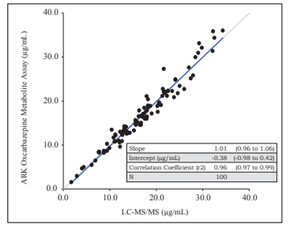
Potentially co-administered medications, other anti-epileptic drugs and antibiotic drugs were tested to determine whether these compounds affect the quantitation of oxcarbazepine metabolite concentrations using the ARK™ Oxcarbazepine Metabolite Assay. The compounds were prepared with 3.0 µg/mL and 30.0 µg/mL of oxcarbazepine metabolite present.

The compounds listed in Table below did not interfere (≤ 10% interference) at the levels tested in the ARK Oxcarbazepine Metabolite Assay.

Compound	Tested (µg/mL)	Compound	Tested (µg/mL)
Acetaminophen	200	Lincomycin	1000
Acetazolamide	100	Mephenytoin	100
Acetylsalicylic acid	1000	Mesoridazine	10
Amikacin	100	Methicillin	250
Amitriptyline	10	Naproxen	600
Amoxapine	10	Neomycin	1000
Amphotericin B	100	Niacin	100
Ampicillin	100	Nitrazepam	20
Ascorbic acid	100	Nortriptyline	10
Baclofen	100	Olanzapine	10
Bupropion	10	Paroxetine	10
Caffeine	100	2-phenyl-2-ethyl-malonamide (PEMA)	1000
Chloramphenicol	250	Penicillin V	100
Chlorpromazine	10	Perphenazine	50
Citalopram	10	Phenobarbital	200
Clobazam	100	Phenytoin	200
Clonazepam	10	Pregabalin	200
Cyclosporin A	40	Primidone	100
Diazepam	20	Procainamide	100
Digoxin	10	Prochlorperazine	10
Doxepin	10	Ranitidine	100
Erythromycin	200	Rifampin	100
Ethanol	4000 (0.4%)	Risperidone	10
Ethotoin	100	Sertraline	100
Ethosuximide	250	Spectinomycin	100
Felbamate	250	Stiripentol	100
Fluoxetine	20	Sulfamethoxazole	400
Furosemide	100	Theophylline	200
Gentamicin	100	Thioridazine	10
Haloperidol	10	Tobramycin	100
Heparin	200 U/mL	Tiagabine	200
Ibuprofen	500	Topiramate	250
Imipramine	10	Trimethoprim	40
Kanamycin A	200	Valproic Acid	600
Gabapentin	200	Vancomycin	250
Lamotrigine	400	Vigabatrin	150
Levetiracetam	400	Zonisamide	400
Lidocaine	100		

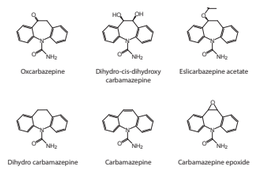
Method Comparison

Correlation studies were performed using CLSI Protocol EP9-A3. Results from the ARK Oxcarbazepine Metabolite Assay were compared with results from LC-MS/MS. Oxcarbazepine metabolite concentrations ranged from 1.7 µg/mL to 34.3 µg/mL. Results of the Passing-Bablok regression analysis for the study are shown below (with 95% confidence limits).



Specificity - Structurally Similar Compounds

Parent drugs (oxcarbazepine and eslicarbazepine acetate) and carbamazepine and its available metabolites were tested for crossreactivity at the concentrations listed in the presence of 20.0 µg/mL oxcarbazepine metabolite. Means measurements (6 replicates) were compared to a serum control to calculate the percentage crossreactivity.



Structures of Oxcarbazepine and Structurally Similar Compounds

Compound	Nominal (µg/mL)	Result (µg/mL)	Serum Control (µg/mL)	Crossreactivity %
Carbamazepine	20.0	23.8	19.7	20.4
Carbamazepine-epoxide	10.0	23.2	21.9	13.6
Dihydro –Carbamazepine	5.0	21.0	21.9	6.0
Dihydro-cis-10, 11-dihydroxy Carbamazepine	5.0	21.6	21.9	-11.3
Eslicarbazepine acetate	20.0	24.1	19.7	22.1
Oxcarbazepine	20.0	24.9	20.5	22.2

Endogenous Interfering Substances

Interference studies were conducted using CLSI Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of oxcarbazepine metabolite (approximately 3.0 and 30.0 µg/mL) were evaluated. Each sample was assayed using the ARK Oxcarbazepine Metabolite Assay, along with a serum control of oxcarbazepine metabolite. Measurement of oxcarbazepine metabolite resulted in ≤ 10% error in the presence of interfering substances at the levels tested.

		Percentage Recovery	
Interfering Substance	Interferent Concentration	3.0 µg/mL Oxcarbazepine Metabolite	30.0 µg/mL Oxcarbazepine Metabolite
Human Albumin	12 g/dL	102.2	95.1
Bilirubin - conjugated	70 mg/dL	108.6	100.2
Bilirubin - unconjugated	70 mg/dL	102.7	92.4
Cholesterol	400 mg/dL	101.4	99.4
Human IgG	12 g/dL	93.1	93.1
Hemoglobin	1000 mg/dL	105.7	100.7
Rheumatoid Factor	1000 IU/mL	101.0	103.9
Triglycerides	1000 mg/dL	96.6	94.3
Uric Acid	30 mg/dL	107.5	95.5

Conclusions

The ARK™ Oxcarbazepine Metabolite Assay measures oxcarbazepine MHD in human serum with excellent precision, recovery and clinical accuracy versus LC-MS/MS. Care should be taken regarding interpretation when coadministration of carbamazepine occurs. Ability to measure trough levels of oxcarbazepine MHD with high accuracy and fast turn-around time makes this method clinically useful for TDM.

PROPOSED INTENDED USE

The ARK Oxcarbazepine Metabolite Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of oxcarbazepine metabolite in human serum on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of oxcarbazepine metabolite to help ensure appropriate therapy.

REGULATORY STATUS

The assay has not been cleared by the U.S. FDA for in vitro diagnostic use.
Europe – CE Marked
Canada – Medical device license