ARK™ TOPIRAMATE ASSAY ON ROCHE/HITACHI 917 SYSTEM

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Introduction

BACKGROUND

Chemically, topiramate (2,3:4,5-Di-O-isopropylidene-β-D-fructopyranose sulfamate) is a sulfamate-substituted monosaccharide, related to fructose. It is used as both adjunctive and monotherapy therapy for patients with partial onset or primary generalized tonic-clonic seizures as well as Lennox-Gastaut syndrome. Topiramate has also been approved by the FDA for the prophylaxis of migraines. Pharmacokinetics varies widely, particularly with co-medications, age, and/or compromised renal function. For example, topiramate is eliminated at a faster rate in children, the magnitude of the increase in clearance compared with adult's ranges in different studies from 25% to 170%. Also, the use of enzyme-inducing antiepileptic drugs (AED's) such as phenytoin and carbamazepine gives rise to an increased metabolism of topiramate. The additional metabolism is capable of producing a 50% decrease in plasma concentration compared with patients receiving non-enzyme inducing AED's. The faster elimination rate in children and effect of coadministered AED's on the plasma concentration of topiramate make monitoring even more important due to the variability of seizure control with plasma concentrations. The proposed therapeutic range for seizure control is 2-25 µg/mL.

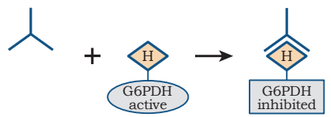
OBJECTIVE

To evaluate the performance of the new ARK Topiramate Assay on the Roche/Hitachi 917 automated clinical chemistry analyzer.

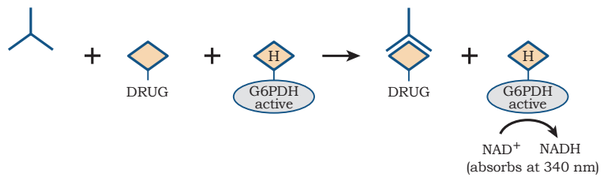
METHODS

The ARK Topiramate Assay is a homogenous enzyme immunoassay for quantifying topiramate in human serum or plasma. The assay was evaluated on the Roche/Hitachi 917. The assay was calibrated using a six point calibration curve (0 to 60µg/mL) where increasing reaction rate correlates to increasing topiramate concentration. Tri-level (2.5, 10.0 and 40.0 µg/mL) quality controls were run. Performance of the assay assessed precision, sensitivity, linearity, accuracy, specificity and method comparison to the INNOFLUOR® FPIA Assay System for topiramate (Seradyn Diagnostics/Thermo Scientific, Indianapolis, IN).

A) Absence of drug



B) Presence of drug



RESULTS

Using a 20-day protocol (2 runs per day, quadruplicate measurements) total (within-lab) precision for tri-level controls was 4.3% CV (2.4 µg/mL), 2.7% CV (10.2 µg/mL), and 3.2% CV (40.2 µg/mL). The within-run precision component was 3.5% CV, 2.4% CV and 2.9% CV, respectively. Analytical sensitivity or Limit of Quantitation (LOQ) was 1.0 µg/mL. The assay was linear from 0.6 to 60.0 µg/mL, determined with samples prepared by serial dilution. Accuracy as analytical recovery was 95.6% to 107.1% for nominal values 1.5 to 55.0 µg/mL. Endogenous substances and commonly co-administered drugs did not interfere with measurement of topiramate at the levels tested. Using Passing Bablok regression analysis for method comparison, ARK = 0.99 FPIA -0.17 (r2 = 0.98, n=116, range 1.0 to 59.1 µg/mL).

Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. Tri-level controls containing topiramate were assayed in quadruplicate twice a day for 20 days. Mean determinations of topiramate, 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 (%)
QC Low (2.5 µg/mL)	160	2.4	0.08	3.5	0.05	2.0	0.10	4.3
QC Mid (10.0 µg/mL)	160	10.2	0.24	2.4	0.14	1.4	0.28	2.7
QC High (40.0 µg/mL)	160	40.2	1.19	2.9	0.64	1.6	1.29	3.2

Lower Limit of Quantitation

Limit of quantitation was evaluated according to CLSI/NCCLS EP17-A. Pooled human serum was supplemented with known amounts of topiramate and assayed 40 times. The LLOQ of the ARK Topiramate Assay is defined as the lowest concentration for which acceptable inter-assay precision (≤20% CV) and recovery (±15%) is observed. The criteria of LLOQ were met at 1.0 µg/mL; the precision was 5.3% CV and the recovery was 96.0%.

Conc. Tested (µg/mL)	Mean (µg/mL)	RMS SD	CV (%)	Recovery (%)	N
0.5	0.3	0.03	8.8	62.0	40
1.0	1.0	0.05	5.3	96.0	40
1.5	1.4	0.05	3.7	94.2	40

Analytical Recovery

Accuracy (analytical recovery) was performed by adding concentrated topiramate drug (USP) into human serum negative for topiramate. Test sample concentrations were 1.5, 2.5, 4.0, 5.0, 6.0, 10.0, 15.0, 30.0, 45.0, and 55.0 µg/mL. Two analytical runs of three replicates of each sample were assayed. The results of the six replicates were averaged and compared to the theoretical target concentration and the percentage recovery was calculated. The amount of topiramate recovered from nominal ranged from 95.6% to 107.1%.

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
1.5	1.4	0.10	7.2	95.6
2.5	2.7	0.05	1.9	106.7
4.0	4.2	0.15	3.6	104.2
5.0	5.3	0.22	4.1	106.0
6.0	6.4	0.25	4.0	106.7
10.0	10.4	0.35	3.4	103.8
15.0	15.5	0.28	1.8	103.4
30.0	30.8	0.89	2.9	102.6
45.0	47.3	1.22	2.6	105.0
55.0	58.9	1.77	3.0	107.1

Specificity

A high concentration of each compound was spiked into normal human serum with known levels of topiramate (approximately 5.0 and 20.0 µg/mL) and assayed along with a serum control of topiramate. Measurement of topiramate resulted in ≤10% error in the presence of drug compounds at the levels tested. The following compounds did not interfere with the measurement of topiramate at the levels tested.

Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)	Compound	Conc. (µg/mL)
Acetominophen	50	Diazepam	50	Lamotrigine	100	Phenytoin	50
Acetazolamide	50	Dichlorphenamide	40	Levetiracetam	200	Primidone	100
Alprazolam	20	Ethosuximide	500	Methysergide	100	Protriptyline	20
Amitriptyline	10	Famotidine	50	Metoprolol	100	Salicylic Acid	750
Acetylsaliyclic Acid	100	Felbamate	500	Nadolol	150	Sulfanilamide	2000
Atenolol	50	Flurazepam	20	Naproxen	600	Tiagabine	200
Caffeine	100	Furosemide	10	Nimodipine	100	Tolbutamide	750
Carbamazepine	100	Gapapentin	100	Notriptyline	10	Valproic Acid	200
Chlorthalidone	100	Hydrochlorthiazide	60	Oxcarbazepine	50	Verapamil	100
Clonazepam	50	Ibuprofen	500	Phenelzine	15	Viagabatrin	150
Clorazepate	20	9 OH Topiramate*	40	Phenobarbitol	40	Zonisamide	200

* The metabolite 9-Hydroxytopiramate did not crossreact (≤ 1.6%), within error of the method.

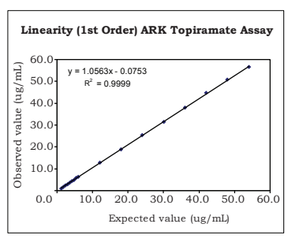
Endogenous Interference

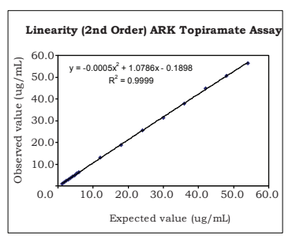
Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of topiramate (approximately 5.0 and 20.0 µg/mL) were evaluated. Each sample was assayed using the ARK Topiramate Assay, along with a serum control of topiramate. Measurement of topiramate resulted in ≤10% error in the presence of interfering substances at the concentrations tested.

Substance	Concentration
Albumin	12 g/dL
Bilirubin	60 mg/dL
Cholesterol	301 mg/dL
Gamma-Globulin	10 g/dL
Hemoglobin	100 mg/dL
Intralipid®	1500 mg/dL
Rheumatoid Factor	1000 IU/mL
Triglycerides	1105 mg/dL
Uric Acid	30 mg/dL
Heparin	200 units/mL

Linearity

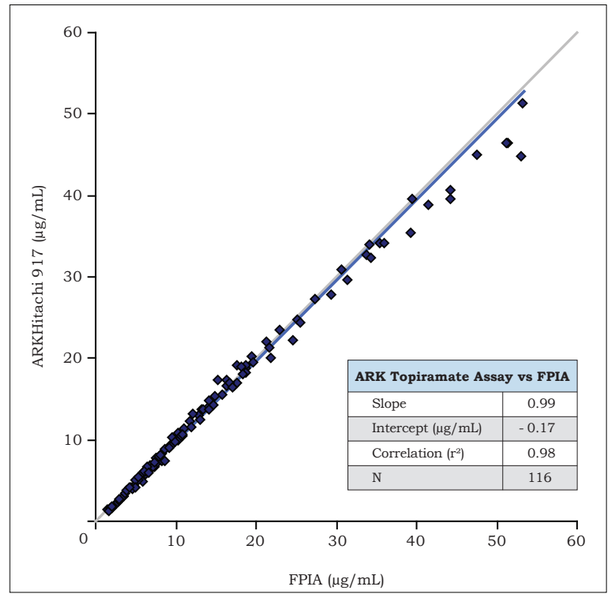
Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. Negative pooled human serum was supplemented with topiramate to give 60.0 µg/mL and then diluted proportionally. The assay was considered linear when the percentage difference between the predicted 1st order and 2nd order polynomial was within 10%. The assay was linear for individual dilution segments (0.6 to 6.0 µg/mL and 6.0 to 60.0 µg/mL). Regression plots of observed versus expected concentrations are shown for the entire linear range determined on the Roche/Hitachi 917, 1.2 to 54.0 µg/mL.





Method Comparison

Clinical specimens from patients treated with topiramate were analyzed using the ARK Topiramate Assay on the Roche/Hitachi 917 chemistry analyzer and FPIA. Comparison by Passing-Bablock regression of the results is shown in the figure below for the range 1.0 to 59.1 µg/mL, including 19 specimens supplemented with topiramate to evaluate the upper range of the assay. The highest neat concentration tested was 51.4 µg/mL.



Conclusions

The ARK™ Topiramate Assay System is FDA-Cleared, Licensed in Canada, and CE Marked.

Performance was demonstrated on the Roche/Hitachi 917 System. The assay has an extended calibration range (0 to 60 µg/mL). Performance of the assay showed good precision, accuracy, specificity and linearity with excellent correlation to FPIA. The ARK Topiramate reagents, calibrators and controls are provided in liquid form ready-to-use.

INTENDED USE

The ARK Topiramate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers. The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.

ASSAY RANGE

The range of the assay is 1.5 to 54.0 µg/mL. Report results below this range as <1.5 µg/mL or below the analyzer-specific lower LOQ established in your laboratory. Report results above this range as >54.0 µg/mL or above the analyzer-specific upper LOQ established in your laboratory.