

# Validation of a New Immunoassay for Quantification of Topiramate

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## Abstract

Topiramate (Topamax<sup>®</sup>) is an important anticonvulsant drug for which therapeutic drug monitoring (TDM) can be clinically indicated and would be improved through commercialization of additional automated assays performed using common open channel analyzers. OBJECTIVE: Immunoassay reagents developed by ARK Diagnostics, Inc. (Sunnyvale, CA) for quantification of topiramate were evaluated and results compared to those generated by FPIA. METHODS: The ARK<sup>™</sup> Topiramate Assay was performed with an Olympus AU400 (Olympus Diagnostics, Center Valley, PA). The INNOFLUOR<sup>®</sup> FPIA Assay System for topiramate (Seradyn Diagnostics/Thermo Scientific, Indianapolis, IN) was performed using an Abbott TDx<sup>®</sup> Analyzer. Both assays were performed according to manufacturer instructions and all measurements were performed in triplicate (or more). The ARK assay was evaluated for accuracy (recovery) by analyzing two batches of samples spiked at ten concentrations between 1.5 and 55 µg/mL. Linearity (assay range) was determined by analyzing two batches of spiked samples over two concentration ranges (0.6 to 6.0 µg/mL, and 6.0 to 60.0 µg/mL) that were prepared by serial dilution. Precision was determined by analyzing quadruplicate control samples at three concentrations for five days. Carryover was evaluated by a series of samples containing 80.0 µg/mL, followed by a sample containing 2.0 µg/mL, in five replicates. Analytical interference was evaluated with residual clinical samples containing various concentrations of potentially interfering substances with known concentrations of topiramate, along with a topiramate control. Three replicates of each sample and their respective controls were analyzed. The ARK assay was compared to the FPIA assay with residual patient specimens. All residual patient samples were de-identified in compliance with an IRB-approved protocol. Twenty specimens were fortified with topiramate to achieve a concentration of 30 to 60 µg/mL to evaluate the upper range of the ARK assay, because the FPIA assay had a reportable range of 0.6 to 32.0 µg/mL. These 20 specimens were diluted with zero calibrator for analysis by FPIA. Nine discordant specimens whose ARK results differed by more than 20% from the FPIA results were retested twice by both methods. RESULTS: The LLOQ for the ARK assay was 1.0 µg/mL. The ULOQ was 60.0 µg/mL. Percent recovery ranged from 94.5 to 107.5%. A first order regression fit of the linearity data was  $y = 1.1620x - 0.1111$  for sample concentrations 0.6 to 6.0 µg/mL and  $y = 1.0156x + 1.2407$  for sample concentrations 6.0 to 60 µg/mL. All precision data (within run, between day and total) demonstrated  $\leq 6.8\%$  CV. The linear regression equation for the patient specimens tested by both methods was  $y = 0.93x - 0.06$ ,  $r^2 = 0.98$  ( $n=116$ , range 1.0 to 59.1 µg/mL). CONCLUSION: Good correlation was observed between patient specimens analyzed using the ARK assay and the FPIA assay. The ARK topiramate assay performed comparably to the FPIA assay but offers a wider reportable range (1.0 to 60.0 µg/mL vs 1.0 to 32.0 µg/mL) while maintaining excellent precision. Commercialization of the ARK reagents may improve accessibility of topiramate TDM.

## Introduction

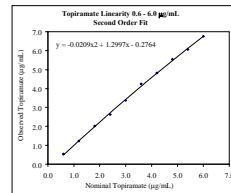
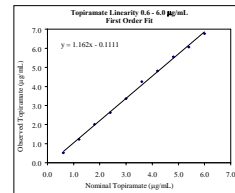
Topiramate (Topamax<sup>®</sup>) is an important anticonvulsant medication, and since 2004 has also been widely used for the treatment of migraines. Indeed, of the top 200 brand name drugs ranked by number of prescriptions in 2008, Topamax was ranked number 33, with 7.9 million prescriptions written (1), and estimated sales of \$2.2 billion (USD). Generic formulations of topiramate for the treatment of seizures gained FDA approval in April, 2009, and several manufacturers have been licensed to begin producing topiramate. This could lead to broader use of topiramate as an anticonvulsant, and increase the need for topiramate analysis for therapeutic drug monitoring. Additional automated assays for topiramate could increase laboratory throughput and improve accessibility of results for physicians. Results of a new automated immunoassay (ARK Diagnostics, Inc. Sunnyvale, CA) for quantification of topiramate was evaluated and compared to those generated by FPIA (INNOFLUOR<sup>®</sup> Seradyn Diagnostics/Thermo Scientific, Indianapolis, IN).

## Linearity

Linearity was evaluated by analyzing two batches of samples spiked at 18 concentrations in triplicate. Results are shown for concentrations from 0.6 to 6.0 µg/mL and 6.0 to 60.0 µg/mL ( $n = 6$  for each concentration). The analytical measurement range was 1.0 to 32.0 µg/mL for the FPIA assay and 1.0 to 60.0 µg/mL for the ARK assay.

### Linearity 0.6 to 6.0 µg/mL

Estimated Value	Dilution Factor	Results (µg/mL)	1st order Predicted Results	2nd Order Predicted Results	Percent Difference (Acceptance Criteria: ±10%)
0.6	10%	0.5	0.6	0.5	-15.4
1.2	20%	1.2	1.3	1.3	-2.3
1.8	30%	2	2	2	0.7
2.4	40%	2.6	2.7	2.7	1.7
3	50%	3.4	3.4	3.4	1.8
3.6	60%	4.3	4.1	4.1	1.5
4.2	70%	4.8	4.8	4.8	0.9
4.8	80%	5.6	5.5	5.5	0.3
5.4	90%	6.1	6.2	6.1	-0.5
6	100%	6.8	6.9	6.8	-1.3



## Precision

Precision was determined by analyzing two batches of spiked samples prepared in quadruplicate at three concentrations for five days ( $n = 40$  for each concentration).

Target (µg/mL)	N	Mean (µg/mL)	Within Run SD	Within Run CV (%)	Between Day SD	Between Day CV (%)	Total SD	Total CV (%)
2.0	40	2.4	0.11	4.5	0.12	5.3	0.16	6.8
10.0	40	10.2	0.26	2.6	0.17	1.7	0.44	4.3
40.0	40	40.3	1.58	3.9	0.9	2.2	1.73	4.3

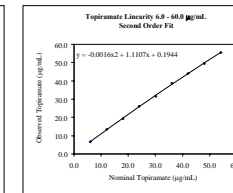
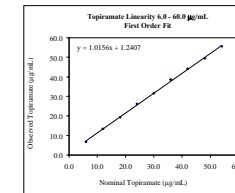
## Total Recovery

Recovery was evaluated by analyzing two batches of samples spiked at 10 concentrations in triplicate ( $n = 6$  for each concentration). The percent recovery ranged from 94.5% to 107.5%.

Target (µg/mL)	Mean (µg/mL)	SD	%CV	Percent Recovery	N
1.5	1.6	0.08	5.2	106.7	6
2.5	2.4	0.17	7.2	96	6
4	4.3	0.38	8.7	107.5	6
5	5.1	0.35	6.9	102	6
6	6.4	0.35	5.4	106.7	6
10	10.6	0.94	8.9	106	6
15	15	0.02	6.8	100	6
30	28.5	1.61	5.7	95	6
45	44	1.13	2.6	97.8	6
55	52	1.99	3.8	94.5	6

### Linearity 6.0 to 60 µg/mL

Estimated Value	Dilution Factor	Results (µg/mL)	1st order Predicted Results	2nd Order Predicted Results	Percent Difference (Acceptance Criteria: ±10%)
6	10%	6.8	7.3	6.8	-7.3
12	20%	13.4	13.4	13.3	-1
18	30%	19.4	19.5	19.7	0.8
24	40%	26.2	25.6	25.9	1.2
30	50%	31.6	31.7	32.1	1.2
36	60%	38.7	37.8	38.1	0.8
42	70%	44.1	43.9	44	0.3
48	80%	49.6	50	49.8	-0.3
54	90%	55.6	56.1	55.5	-1



## Determination of LOQ

The LOQ was determined by analyzing five batches of eight replicates spiked at 0.5, 1.0 and 1.5 µg/mL ( $n = 40$  for each concentration). The LOQ was determined to be 1.0 µg/mL.

Target (µg/mL)	Mean (µg/mL)	RMS SD	%CV	Percent Recovery	N
0.5	0.3	0.05	15.4	61	40
1.0	1.2	0.06	5.4	116	40
1.5	1.6	0.09	5.7	106.3	40

## Carryover and Interfering Substances

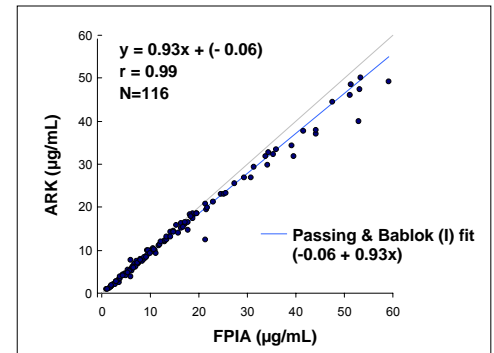
Carryover was evaluated by a series of samples containing 80.0 µg/mL, followed by a sample containing 2.0 µg/mL, in five replicates. No carryover was observed. Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering substances in serum with known levels of topiramate (approximately 5 and 20 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  $\leq 10\%$  error in the presence of interfering substances at the levels tested.

Interfering Substance	Interferent Concentration
Albumin	12 g/dL
Bilirubin	70 mg/dL
Cholesterol	301 mg/dL
Gamma-Globulin	12 g/dL
Hemoglobin	1000 mg/dL
Heparin	200 units/mL
Rheumatoid Factor	1000 IU/mL
Triglycerides	1105 mg/dL
Uric Acid	30 mg/dL

## Method Comparison

The ARK assay was compared to the FPIA assay by analyzing 116 patient specimens and spiked samples. Twenty specimens were fortified with topiramate to achieve a concentration of 30 to 60 µg/mL to evaluate the upper range of the ARK assay, because the FPIA assay had a reportable range of 1.0 to 32.0 µg/mL. These 20 specimens were diluted with zero calibrator for analysis by FPIA.

### First order linear regression fit for patient specimens and spiked samples



## Conclusions

- ◆ Results of the ARK assay were comparable to the FPIA assay.
- ◆ The analytical measurement range of the ARK assay was greater than the FPIA assay.
- ◆ Commercialization of the ARK assay may improve accessibility to topiramate TDM.

## Acknowledgements

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## References

1. Drug Topics Magazine, May 26, 2009.