

Thermo Scientific QMS® Topiramate Immunoassay on HITACHI® 917 System

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Abstract

Topiramate (2,3:4,5-Di-O-isopropylidene-β-D-fructopyranose sulfamate; Topamax®) is a sulfamate substituted monosaccharide derived from D-fructose and used as both adjunctive and monotherapy for patients with partial onset or primary generalized tonic-clonic seizures as well as Lennox-Gastaut syndrome. More recently topiramate has been approved by the FDA for the prophylaxis of migraines.¹ 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 of topiramate as compared to that of the patients receiving non-enzyme inducing AED's.² The substantial effect of these AED's on the plasma concentration of topiramate makes monitoring even more important due to the individual variability of seizure control with topiramate plasma concentrations. Clear improvements are seen in the serum concentration range of 2 to 25 µg/mL. Serum concentrations above approximately 25 µg/mL lead to decreased seizure control.³ Thermo Fisher Scientific has developed a homogenous turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology for analyzing topiramate concentrations in human serum or plasma. A six point calibration curve (0, 2, 4, 8, 16 and 32 µg/mL) is generated in which high rates of agglutination are observed with low topiramate concentrations, while low rates are observed with high topiramate concentrations. The Thermo Scientific QMS Topiramate Immunoassay was developed on the HITACHI 917 platform. Performance of the immunoassay was determined by assessing precision, sensitivity, accuracy and method comparison. Within-run precision on tri-level, serum based controls was found to be 3.02% CV (2.87 µg/mL), 1.6% CV (10.29 µg/mL) and 3.5% CV (26.60 µg/mL). The 5-day precision performed on the same controls is as follows: 3.91% total CV (2.84 µg/mL), 2.77% total CV (10.16 µg/mL) and 3.76% total CV (26.05 µg/mL). Sensitivity was determined by assessing the Limit of Quantitation (LOQ) and Lowest Detectable Dose (LDD) using protocols in NCCLS EP17-A.⁴ LOQ was found to be 1.12 µg/mL and LDD to be 0.15 µg/mL. Accuracy by dilution was determined to be 0.58 to 37.57 µg/mL. An abbreviated method comparison conducted on 24 patient samples spanning 1.29 to 25.89 µg/mL was conducted using the Innofluor® Topiramate immunoassay on the TDX® analyzer as a reference method. The correlation coefficient was 0.983, slope of 0.922 and y-intercept of 0.383 µg/mL using Passing-Bablok⁵ regression analysis.

Introduction

Thermo Fisher Scientific has developed the first homogenous immunoassay that utilizes the QMS technology for analyzing topiramate in human serum and plasma. Topiramate (Figure 1a) is a sulfamate substituted monosaccharide derived from D-fructose and used as both adjunctive and monotherapy for patients with partial onset or primary generalized tonic-clonic seizures as well as Lennox-Gastaut syndrome. More recently topiramate has been approved by the FDA for the prophylaxis of migraines.¹ 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 of topiramate as compared to that of the patients receiving non-enzyme inducing AED's.² The substantial effect of these AED's on the plasma concentration of topiramate makes monitoring even more important due to the individual variability of seizure control with topiramate plasma concentrations. Clear improvements are seen in the serum concentration range of 2 to 25 µg/mL. Serum concentrations above approximately 25 µg/mL lead to decreased seizure control.³ Monitoring of topiramate serum or plasma concentration allows physicians to aid in their patients' anticonvulsant drug therapy. The therapeutic range for topiramate has been reported in literature as 2 to 25 µg/mL.³ Topiramate has an elimination half life of 20 hours and an 80% bioavailability.¹ Topiramate drug concentrations should not be the only means of therapeutic drug management. Clinicians should carefully monitor patients during therapy initiation and dosage adjustments. It may be necessary to obtain multiple samples to determine expected variation of optimal concentrations for individual patients.

Methods & Materials

The immunoassay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the topiramate antibody reagent (Figure 1b). The topiramate-coated microparticle reagent is rapidly agglutinated in the presence of the anti-topiramate antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of agglutination of the particles. When a sample containing topiramate is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with the highest rate of agglutination at the lowest topiramate concentration and the lowest agglutination rate at the highest topiramate concentration.

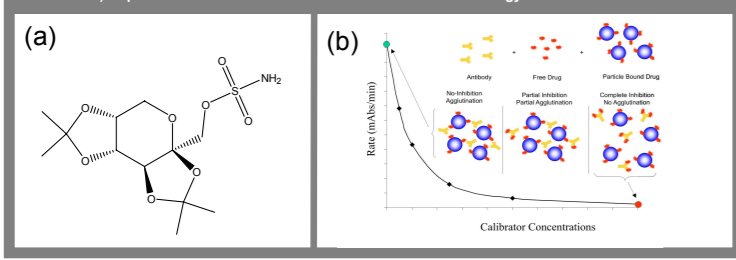
Reagents: R1 = Anti-Topiramate Polyclonal Antibody (Sheep), R2 = Topiramate-Coated Microparticles

Calibrators: QMS Topiramate Calibrators A-F: 1x 1mL each

Controls: QMS Topiramate Controls 1-3: 1x 2mL each

FIGURE 1. a) Structure of Topiramate

b) Representation of Calibration Curve via the QMS Technology



Results

Specificity: Cross-reactivity and interfering substance studies were conducted using NCCLS protocol EP7-A2 as a guideline.⁶ Studies were conducted to examine the cross-reactivity of topiramate metabolite, 9-Hydroxytopiramate, was tested at varying concentrations (Table 1). Commonly co-administered drugs were tested (Table 2) at 3 times their high therapeutic values with low and high topiramate concentrations in serum. Both the testing conditions resulted in less than 10% cross reactivity, except for Ibuprofen, Phenytoin and Tiagabine, which displayed a cross-reactivity of 11.38%, 28.14% and 29.60% respectively.

TABLE 1. Cross-Reactivity with 9-Hydroxytopiramate

Metabolite	Cross Reactivity		
	Conc of Cross-reactant spiked (µg/mL)	Low TPM Conc	High TPM Conc
9-Hydroxy topiramate	4	19.75%	14.50%
	8	22.63%	12.50%
	32	15.56%	18.25%

TABLE 3. Endogenous Substances Tested

Substance	Concentration
Albumin	12 g/dL
Bilirubin	70 mg/dL
Cholesterol	250 mg/dL
Hemoglobin	1000 mg/dL
IgG	12 g/dL
HAMA Type-1	-
HAMA Type-2	-
Heparin	185.5 units/mL
Rheumatoid Factor	500 IU/mL
Uric Acid	30 mg/dL
HAMA = Human Anti Mouse Antibody	

TABLE 2. Cross-Reactivity of Common & Co-administered Drugs.

Acetaminophen	Lamotrigine
Acetazolamide	Levetiracetam
Alprazolam	Methysergide
Amitriptyline	Metoprolol
Acetylsalicylic acid	Nadolol
Atenolol	Naproxen
Caffeine	Nimodipine
Carbamazepine	Nortriptyline
Chlorthalidone	Phenelzine
Clonazepam	Phenobarbital
Clorazepate	Phenytoin
Diazepam	Primidone
Dichlorphenamide	Protriptyline
Ethosuxamide	Salicylic Acid
Famotidine	Sulfanilamide
Felbamate	Tiagabine
Flurazepam	Tolbutamide
Furosemide	Valproic Acid
Gabapentin	Verapamil
Hydrochlorothiazide	Vigabatrin
Ibuprofen	Zonisamide

Precision: Precision (Table 4) was conducted according to NCCLS protocol EP5-A2.⁷ A tri-level human serum based commercial control containing topiramate was immunoassayed on the HITACHI 917. Measurements were taken in duplicate, twice a day, with a minimum of 2 hours difference for 20 non-consecutive days, giving 80 measurements per control level. Additional variability was captured by collecting data on multiple HITACHI 917's and multiple calibrations.

TABLE 4: Precision Summary

	N	Mean	Within Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV
Low Control	80	2.94	0.08	2.77	0.0617	2.1	0.1238	4.22
Mid Control	80	10.14	0.19	1.83	0.24	2.34	0.34	3.37
High Control	80	25.69	0.83	3.23	0.74	2.87	1.14	4.44
Acceptance Criteria: < 10% total CV								

Linearity & Accuracy: Linearity (Figure 2, Table 5) by dilution was determined by a study based on the NCCLS guideline EP6-A.⁸ A high topiramate patient pool was made by combining three patient samples of high therapeutic levels. The patient pool topiramate concentration was not sufficiently high, it was supplemented (less than 10% total volume) with a topiramate stock solution in order to attain a concentration 20 to 30% above the desired reportable range (approximately 40 µg/mL). The pool was then diluted with QMS Topiramate Calibrator A (blank calibrator, negative for topiramate) to achieve concentrations across the immunoassay range. Each level was analyzed in triplicate. The dilution factor was calculated for each topiramate level. The calculated dilution factor was plotted versus the mean recovered concentration. Regression equations of the first and second order polynomial deviation were used to calculate the predicted first and second order values. Less than 10% difference was observed between the predicted first and second order values.

Accuracy by recovery (Table 6) was determined by diluting the high calibrator to twelve concentrations across the immunoassay range. The samples were analyzed in triplicate and its mean percent recovery were compared with the original value.

FIGURE 2. Regression Analysis for Linearity Determination

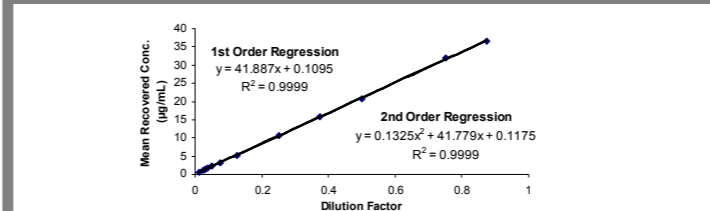


TABLE 5. Linearity

Estimated Value	Dilution Factor	Result	1 st Order Predicted	2 nd Order Predicted	Percent Difference
40	1	36.57	NA	NA	NA
35	0.875	36.57	36.76	36.78	-0.04%
30	0.75	31.87	31.52	31.53	0.00%
20	0.5	20.86	21.05	21.04	0.06%
15	0.375	15.89	15.82	15.8	0.09%
10	0.25	10.54	10.58	10.57	0.10%
5	0.125	5.28	5.35	5.34	0.06%
3	0.075	3.11	3.25	3.25	-0.02%
2	0.05	2.22	2.20	2.21	-0.13%
1.5	0.0375	1.68	1.68	1.68	-0.25%
1.2	0.03	1.43	1.37	1.37	-0.36%
1	0.025	1.22	1.16	1.16	-0.47%
0.5	0.0125	0.71	0.63	0.64	-1.05%

Acceptance Criteria: ±10% recovery between 1st and 2nd order predicted regressed values.

TABLE 6. Accuracy

Theoretical Conc. (µg/mL)	Rep 1	Rep 2	Rep 3	Mean Result	% Recovery
32.00	33.57	32.63	31.24	32.48	102%
24.00	24.26	24.41	24.83	24.50	102%
16.00	16.88	16.57	16.78	16.74	104%
8.00	8.27	8.30	8.48	8.35	104%
6.40	6.60	6.59	6.63	6.61	103%
3.20	3.45	3.41	3.54	3.47	108%
2.56	2.60	2.70	2.70	2.67	104%
1.92	2.16	2.03	2.15	2.11	110%
1.60	1.59	1.71	1.66	1.65	103%
1.28	1.30	1.33	1.36	1.33	104%

Acceptance Criteria: ±10% recovery

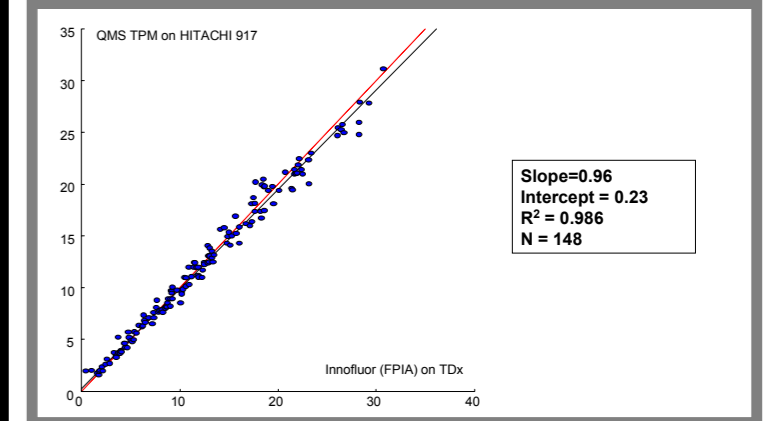
Sensitivity:

Least Detectable Dose (LDD): The LDD for QMS Topiramate Immunoassay is 0.12µg/mL. LDD, or analytical sensitivity, of the immunoassay is defined as the lowest drug concentration that can be distinguished from zero with 95% confidence. The LDD was determined by analyzing the lowest level calibrator n = 20.

Limit of Quantitation (LOQ): The LOQ for QMS Topiramate Immunoassay is 1.5µg/mL. The LOQ, or functional sensitivity, of the immunoassay is defined as the lowest drug concentration for which acceptable intra-immunoassay precision is observed (often considered less than or equal to 20% CV) and recovery is between 85 to 115% of theoretical value. The LOQ was determined by analyzing diluted samples twice (n=2) over five days with the QMS Topiramate Immunoassay.

Method Comparison: A study was conducted according to NCCLS Guideline EP9-A2⁹ to compare accuracy of recovery of topiramate by the QMS Topiramate Immunoassay to that of the predicate Innofluor TDX/TDXFLx Topiramate immunoassay. Patient samples ranged from 1.56 to 30.72 µg/mL of topiramate were tested using both the Innofluor TDX Topiramate immunoassay and the QMS Topiramate Immunoassay on HITACHI 917.

FIGURE 3. Method Comparison of QMS Topiramate on HITACHI 917 vs Innofluor (FPIA) Topiramate on TDX



Stability

- QMS Topiramate reagents show superb reagent stability (based on accelerated stability testing at higher temperatures).
- On-Board reagent stability on a HITACHI platform was 100 days when uncapped.
- Calibration curve is stable for 3 weeks on HITACHI platforms.

Conclusions

- The performance results of QMS Topiramate Immunoassay on the HITACHI 917 show that the immunoassay provides precise and reliable topiramate concentrations within the therapeutic range to aid in optimal patient care.
- The QMS Topiramate reagents and calibrators are liquid stable and are easy to use.

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