ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 3, 2013

ISSN - 0974-2441

Research Article

ESTIMATION OF TOPIRAMATE IN HUMAN PLASMA USING LC-MS/MS METHOD

DAS GANESH KUMAR

Shri Jagdishprasad Jhabarmal Tibrewala University, Vidyanagari, Churu Jhunjhunu Road, Chudela, District-Jhunjhunu, Rajasthan India
Email: gnsh.das@gmail.com

Received: 15 June 2013, Revised and Accepted: 5 July 2013

ABSTRACT

Objective: The objective of this research was to develop and validate a simple, sensitive and specific liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for quantification of Topiramate in human plasma.
Method: The analytical method consists of solid phase extraction of plasma sample followed by the determination of Topiramate by a LC–MS/MS. The analyte was separated on Advance Hypurity C18 (50 x 4.6 mm, 5µ) column with an isocratic mobile phase of Acetonitrile and 2mM ammonium acetate buffer in the volume ratio of 80:20 v/v at a flow rate of 0.8 mL/min. Results: Protonated ions formed by a turbo ion spray in a negative mode were used to detect analyte and internal standard (IS). The MS/MS detection was made by monitoring the fragmentation of m/z 338.00→77.50 for Topiramate and m/z 350.40→90.10 for internal standard on a mass spectrometer. The method was validated over the concentration range of 20.721 ng/mL to 605.656 ng/mL for Topiramate in human plasma with correlation coefficient greater than 0.99.
Conclusion: The developed method was successfully applied for analyzing Topiramate in plasma samples for a bioequivalence study with healthy volunteers.

Keywords: Topiramate; Validation; Analysis; Bioavailability; LC-MS/MS.

INTRODUCTION

Topiramate (TPM; RWJ- 17021-000, McN-4853) was originally synthesized as part of a research project to discover structural analogs of fructose- 1, 6-diphosphate capable of inhibiting the enzyme fructose 1, 6- bisphosphatase, thereby blocking gluconeogenesis [1]. Topiramate is a newer AED with a broad pharmacological profile, thereby providing the rationale for use as broad-spectrum anti-epileptic monotherapy. It is a sulfamate-substituted monosaccharide, with multiple mechanisms of action that include blockade of voltage-sensitive sodium channels, potentiation of $GABA_A$-evoked chloride flux, blockade of kainate/AMPA type of glutamate receptors, and reduction of type L-calcium channels activity. It also inhibits carbonic anhydrase [2]. Also, the clinical efficacy and safety of topiramate has been thoroughly evaluated in adults. It has been shown to be effective as an adjunctive therapy for multiple seizure types including localization-related and primary generalized seizures [3].

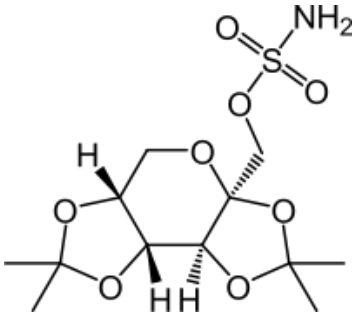

Fig. 1: Chemical Structure of Topiramate

Several analytical methods have been reported for the determination of Topiramate such as HPLC [4-8], Capillary electrophoresis [9-10], Gas chromatography [11] and mass spectrometry [12-17]. The purpose of the present study was to develop and validate an LC-MS-MS method as per USFDA Bioanalytical Method Validation Guideline [18], with simple sample preparation technique to determine Topiramate concentration in human plasma and to apply it to a bioequivalence study of Topiramate tablet. This assay method demonstrated acceptable sensitivity (LLOQ: 20.614 ng/mL), precision, accuracy, selectivity, recovery and stability, and less absolute and relative matrix effect. In addition, most of the reported method for Topiramate utilizes non deuterated/non stable isotope as internal standard. For control of extraction of analyte and internal, HPLC injection and ionization variability, it is recommended to use a deuterated/ stable isotope of analyte. The present study utilizes deuterated Topiramate-D12 as an internal standard which has advantage over the other reported method.

MATERIAL AND METHODS

Instrumentation

Shimadzu Prominence HPLC System and AB Sciex MS/MS API-4000.

Reagents / Materials

Methanol (HPLC Grade), Acetonitrile (HPLC Grade), Water (HPLC Grade), Acetic Acid (AR Grade), Formic acid (AR Grade), Ammonium acetate (AR grade) Topiramate USP Working Standard and Topiramate-D12 internal standard.

Stock Solutions

Topiramate stock solutions and Topiramate-D12 stock solutions were prepared in acetonitrile.

Biological Matrix

Human plasma containing Sodium Heparin as anticoagulant was used as a biological matrix

during method validation. Selectivity and sensitivity tests were performed before bulk spiking.

Calibration Curve (CC) Standards and Quality Control (QC) Sample Concentrations

The Calibration Curve standards (CC) were prepared at ranges from 20.721ng/mL to 605.656 ng/mL concentrations for Topiramate. The quality control samples for Topiramate were prepared at concentrations of 20.614 ng/mL (LLOQ QC), 57.261 ng/mL (LQC), 357.883 ng/mL (MQC) and 577.230 ng/mL (HQC).

Buffer (2 mM Ammonium acetate)

Weigh accurately around 77.08 mg of Ammonium acetate and transfer it to 500 mL volumetric flask. Make up the volume up to the mark with Milli-Q water.

Mobile Phase

Take Acetonitrile and 2 mM Ammonium acetate Buffer in the ratio 80:20 v/v and adjust the pH to 6.5±0.1 with acetic acid. Mix well, sonicate and degas in an ultrasonicator.

Diluent

Take Acetonitrile and Milli-Q Water in a ratio of 80:20 v/v. Mix well, sonicate and degas in an ultrasonicator

Bioanalytical Conditions

A summary of the chromatographic conditions is given in Table 1:

Table 1: Summary of Bioanalytical Chromatographic Conditions

Parameters	Conditions
System	LC-MS/MS API 4000
Detector	Mass Spectrometry
Software	Analyst software 1.5
Column	Advance Hypurity C18 50 x 4.6 mm, 5 µ
Mobile Phase	Acetonitrile **:** 2mM ammonium acetate Buffer (80:20 v/v) (pH 6.5±0.1 with acetic acid)
Flow Rate	0.8 mL / min.
m/z	Topiramate **-** 338.00/77.50 Topiramate-D12-350.40/90.10
Injection Volume	5 µL
Column Oven Temperature	40° C
Diluent	Acetonitrile: Water (80:20v/v)
Auto Sampler Temp	5° C

Sample Preparation

The thawed plasma samples were vortexed to ensure complete mixing of the contents. 200 µL of the sample was pipetted into prelabelled RIA tubes and then 50µL of Topiramate-D12 dilution (400.000ng/mL) was added to it as an internal standard (IS) except in blank sample wherein 50µL of Diluent was added, and vortexed for 30 seconds followed by addition of 0.1 mL of 0.1% formic acid and vortexed for 30 seconds. Conditioned SPE cartridge with 1mL of methanol and equilibrated with 1mL of water. Then load the above sample on SPE cartridge, washed twice with 1.0mL of Milli-Q grade water. Elution was carried out with 1 mL of Mobile Phase. Transferred the above solution into HPLC vials and Load the sample in the auto sampler and inject 5µL of volume.

Data Processing

The chromatograms were acquired and the data was processed by peak area ratio method using Analyst 1.5 software. The concentration of the unknown was calculated from the following equation using regression analysis of spiked calibration standard with the reciprocal of the drug concentration ratio as a weighting factor (1/x* x).

$$y = mx + c$$

Where, y = peak area ratio of Topiramate to internal standard
m = slope of the calibration curve
x = concentration ratio of Topiramate ng/mL
c = y-axis intercept of the calibration curve

RESULTS AND DISCUSSION

LC-MS/MS method for the estimation of Topiramate in human plasma was developed and validated using Topiramate-D12 as an internal standard (IS). Sample preparation was accomplished by solid phase extraction method. The processed samples were chromatographed on Advance Hypurity C18 50 x 4.6 mm, 5 µ columns using a mobile phase consisting a mixture of Acetonitrile and 2mM ammonium acetate buffer in the volume ratio of 80:20 v/v at a flow rate of 0.8 mL/min. The method was validated over a concentration range of 20.721ng/mL to 605.656 ng/mL concentrations for Topiramate. During validation selectivity, sensitivity and recovery exercise was carried out. Precision and accuracy exercise was carried out by processing four precision and accuracy batches. Results of various stabilities, reinjection reproducibility and ruggedness were carried out.

Selectivity

No significant interference from endogenous components was observed at the retention times of Topiramate and internal standard in all the batches screened.

Sensitivity

The lowest limit of reliable quantification for Topiramate was set at the concentration of the LLOQQC 20.614ng/mL. The precision and accuracy for Topiramate at this concentration was found to be 3.05% and 98.86% respectively for plasma containing Sodium Heparin as anticoagulant.

Matrix Effect

Extracted four blank samples from each of six batches of matrix. Prepared LQC, MQC & HQC spiking dilution and spiked in the above extracted blank samples. Prepared aqueous sample at LQC, MQC & HQC and injected six replicates of the same and spiked samples. For Topiramate the matrix effect ratio is between 1.01 to 1.59 at all three QC levels. Precision of area response of replicate injection of aqueous samples is 1.48% to 5.09% and precision of area response of post extracted samples is 0.25% to 1.48% for Topiramate. So there is no matrix effect.

Carryover Test

For carryover test two samples of upper limit of quantification (ULOQ) and 4 samples of blank plasma were processed. These samples were injected in the following sequence.

a} 2 blank samples b) 2 ULOQ samples c) 2 blank samples

The step (b) and (c) were repeated 2 times.

The results demonstrate that there was no interference from the previous injection.

Linearity

A regression equation with a weighting factor 1/x*x of ratio of drug to IS concentration was judged to produce the best fit for the concentration response relationship for Topiramate in human plasma. Correlation coefficients (r) were greater than 0.99 in the concentration range of 20.721 ng/mL to 605.656 ng/mL for Topiramate.

Precision and Accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentration range of LLOQ QC, LQC, MQC and HQC samples respectively during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the low, middle and high quality control samples to their respective nominal values, expressed in percentage.

Within-Batch Precision and Accuracy

For LLOQ QC within-batch precision ranged from 2.96% to 7.03% and the within batch accuracy ranged from 91.45% to 98.86% for Topiramate. For LQC, MQC & HQC within-batch precision ranged from 1.25% to 7.68% and the within batch accuracy ranged from 92.23% to 106.91%for Topiramate.

Intra-day Precision and Accuracy

For LLOQ QC, the intra-day precision was 5.27% and the intra-day accuracy was 97.71% for Topiramate. For LQC, MQC & HQC, the

intra-day precision ranged from 1.39% to 9.15% and the intra-day accuracy ranged from 96.15% to 100.53% for Topiramate.

Inter-run Precision and Accuracy

For LLOQ QC, the inter-day precision was 0.05% and the inter-day accuracy was 95.87% for Topiramate. For LQC, MQC & HQC, the inter-day precision ranged from 0.08% to 4.00% and inter- day accuracy ranged from 94.44% to 98.36% for Topiramate.

Stabilities

Standard Stock Solution & Intermediate stock solution Stability Room Temperature Stock Solution Stability for 48 Hrs

Room temperature stock solution stability was carried out at 48.0 Hours by injecting six replicates of stock dilutions of both stability standard stock solution and comparison (fresh) standard stock solution of Topiramate and Topiramate-D12. The response of stability sample was corrected by multiplying with correction factor. The 48.0 Hours stock solution stability of Topiramate was found to be 101.32%. The 48.0 Hours stock solution stability of Topiramate-D12 was found to be 99.31%. The precision of area response of replicates injections for Topiramate stability and comparison stock solution was found to be 0.78% and 0.97% respectively. The precision of area response of replicates injections for Topiramate-D12 stability and comparison stock solution was found to be 0.79% and 0.93% respectively.

Room Temperature Intermediate stock solution and Spiking solution of IS Stability for 48 Hrs

Room temperature Intermediate stock solution stability and Spiking solution IS stability was carried out at 48 Hours by injecting six replicates of stock dilutions of both stability Intermediate standard solution and comparison (fresh) Intermediate standard stock solution of Topiramate and Topiramate-D12 . The response of stability sample was corrected by multiplying with correction factor. The 48 Hours Intermediate stock solution stability of Topiramate was found to be 103.46%. The 48 Hours stock solution stability of Topiramate-D12 was found to be 99.68%. The precision of area response of replicates injections for Topiramate stability and comparison Intermediate stock solution was found to be 2.16% and 2.18% respectively. The precision of area response of replicates injections for Topiramate-D12 stability and comparison Intermediate stock solution was found to be 2.26% and 3.73% respectively.

Refrigerated Stock Solution Stability at 2 to 8°C for 9 days

Refrigerated stock solution stability was carried out by injecting six replicates of stock dilutions of both stability standard stock solution and comparison (fresh) standard stock solution of Topiramate and Topiramate-D12. The response of stability sample was corrected by multiplying with correction factor. The nine days stock stability of Topiramate was found to be 100.02%. The nine days stock stability of Topiramate-D12 was found to be 104.44%. The precision of area response of replicates injections for Topiramate stability and comparison stock solution was found to be 2.44% and 1.21% respectively. The precision of area response of replicates injections for Topiramate-D12 stability and comparison stock solution was found to be 2.05% and 4.35% respectively.

Refrigerated Intermediate Stock Solution Stability at 2 to 8°C for 9 days

Refrigerated Intermediate stock solution stability was carried out by injecting six replicates of stock dilutions of both stability standard stock solution and comparison (fresh) standard stock solution of Topiramate and Topiramate-D12. The response of stability sample was corrected by multiplying with correction factor. The nine days Intermediate stock stability of Topiramate was found to be 100.70%. The nine days stock stability of Topiramate-D12 was found to be 93.14%. The precision of area response of replicates injections for Topiramate stability and comparison Intermediate stock solution was found to be 2.15% and 1.74% respectively. The precision of area response of replicates injections for Topiramate-D12 stability and comparison Intermediate stock solution was found to be 1.85% and 0.40% respectively.

Bench Top Stability in Human Plasma for 40 Hrs

Bench top stability, using six sets each of LQC, MQC and HQC, was determined at 40 hours. The quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for calculation of precision and accuracy. Topiramate was found to be stable up to 40 hours as per the acceptance criteria. For Topiramate the percent nominal ranged from 96.71% – 98.64% and the precision ranged from 1.61% - 2.08%

Auto sampler Stability 55 Hrs

In assessing the auto sampler stability, six sets of QC samples (LQC, MQC and HQC) were processed and placed in the auto sampler. These samples were injected after a period of 55 hours and were quantified against freshly spiked calibration curve standards of concentration range equivalent to that used for calculation of precision and accuracy. The results demonstrate that the processed samples were stable for 55 Hours. The percent nominal at 55 Hours for Topiramate ranged from 90.56% to 98.27% and precision at 55 Hours for Topiramate ranged from 0.38% - 4.50%

Freeze-Thaw Stability 5th Cycle

The stability in human plasma was determined for five freeze-thaw cycles. Six replicates of LQC, MQC and HQC were analyzed on five freeze-thaw cycles. The freeze-thaw quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for the calculation of precision and accuracy. The percent nominal for Topiramate ranged from 93.92% to 98.96% for five freeze-thaw cycles and precision ranged from 0.90% to 2.47% for five freeze thaw cycles.

At -20°C Stability 5 days

The stability of Topiramate in case of temporary storage of plasma samples at -20°C deep freezer for five days was carried out by quantifying six sets each of LQC, MQC, MQC1 and HQC against the freshly spiked calibration curve standards of concentration range equivalent to those used for the calculation of precision and accuracy. Topiramate was found to be stable up to 5 days at -20°C deep freezer as per the acceptance criteria. The mean stability ranged from 97.59% to 102.88% and the precision ranged from 1.61% to 8.51%, for 5 days stability samples.

At - 20°C Stability in human plasma for 9 days

The stability of Topiramate in case of temporary storage of plasma samples at -20°C deep freezer for nine days was carried out by quantifying six sets each of LQC, MQC and HQC against the freshly spiked calibration curve standards of concentration range equivalent to those used for the calculation of precision and accuracy. Topiramate was found to be stable up to 9 days at -20°C deep freezer as per the acceptance criteria. The mean stability ranged from 99.57% - 101.60% and the precision ranged from 0.22% - 1.75% for 9 days stability samples.

At - 70°C Stability in human plasma for 9 days

The stability of Topiramate in plasma samples below -70°C deep freezer for nine days was carried out by quantifying six sets each of LQC, MQC and HQC against the freshly spiked calibration curve standards of concentration range equivalent to those used for the calculation of precision and accuracy. Topiramate was found to be stable up to 9 days at -70°C deep freezer as per the acceptance criteria. The mean stability ranged from 98.47% - 100.11% and the precision ranged from 0.14% -2.46% for 9 days stability samples.

Reinjection Reproducibility for 24 hrs

Reinjection reproducibility was carried out by reinjecting six sets of LQC, MQC and HQC samples after 24 Hours and was back calculated concentration against initial calibration curve standards injected at zero hours. After 24 Hours precision and accuracy ranged from 0.28% - 0.93% and 96.80% to 99.33% respectively for Topiramate.

Recovery

Prepared six sets of recovery comparison samples by spiking 10 µL of mixture of spiking dilution of quality control samples (LQC, MQC and HQC) Topiramate of in 1000 µL Eluent of extracted blank. For recovery comparison sample of IS, 50 µL of internal standard dilution 400.000 ng/mL was spiked in 1000 µL Eluent of extracted blank. The recovery comparison samples of Topiramate were compared against extracted samples of LQC, MQC and HQC, and recovery comparison samples of Topiramate were compared against extracted samples LQC, MQC and HQC which were within the acceptance criteria. The mean overall recovery of Topiramate was 93.22% with a precision of 2.59% & mean recovery of internal standard (Topiramate-D12) was 98.80%.

Dilution Integrity

Dilution integrity samples were prepared by spiking approximately 1.6 times highest standard concentration. Six sets of dilution integrity samples were processed by diluting them twice and four times. These quality control samples were analyzed along with a processed calibration curve standards. The quality control sample concentrations were calculated using appropriate dilution factor. Results demonstrate acceptable dilution integrity for two times and four times dilution. For Topiramate the precision and accuracy, for a dilution factor of 2 was1.62% and 94.55% respectively and the precision and accuracy, for a dilution factor of 4 was 8.22% and 100.51% respectively. Dilution integrity for two times and four times dilution is acceptable as per acceptance criteria.

Ruggedness

Ruggedness was carried out by another analyst. Ruggedness exercise was carried out by processing one precision and accuracy batch. A regression equation with a weighting factor $1/x^{*}x$ of ratio of drug to IS concentration was judged to produce the best fit for the concentration-detector response relationship for Topiramate in human plasma. Correlation coefficients (r) were greater than 0.99 in the concentration range of 20.721 ng/mL to 605.656 ng/mL for Topiramate. For LLOQ QC within-batch precision was 4.46 % and the within batch accuracy ranged was 97.99% for Topiramate. For LQC, MQC & HQC within-batch precision ranged from 0.88% to 2.32% and the within batch accuracy ranged from 98.82 to 100.33% for Topiramate.

CONCLUSION

The LC–MS/MS validated method has proved to be very simple, sensitive and reliable and successfully applied for the pharmacokinetic study in human plasma. The assay method is specific due to the inherent selectivity of tandem mass spectrometry. The major advantage of this method is the use of deuterated Topiramate-D12 as an internal standard. The run time is within 2 min and only 0.2 mL of plasma was required for each determination of Topiramate, and thus the stress to volunteers or patients in clinical studies was greatly reduced. This method is very suitable and convenient for pharmacokinetics and bioavailability study of the drug Topiramate.

REFERENCES

1. Shank RP, Gardocki JF, Streeter AJ, Maryanoff BE. An Overview of the Preclinical Aspects of Topiramate: Pharmacology, Pharmacokinetics, and Mechanism of Action. Epilepsia 2000; 41(1):3.
2. Guerrini R, Carpay J. Topiramate monotherapy as broad-spectrum antiepileptic drug in a naturalistic clinical setting. Seizure 2005; 14:371.
3. Ajlouni SA, Shorman A, Daoud AS. The efficacy and side effects of topiramate on refractory epilepsy in infants and young children: A multi-center clinical trial. Seizure 2005; 14:459.
4. Styslo-Zalasik M, Weiyong Li. Determination of topiramate and its degradation product in liquid oral solutions by high performance liquid chromatography with a chemiluminescent nitrogen detector. J. Pharm. Biomed. Anal. 2005; 37(3):529.
5. Bahrami G, Mohammadi B. A novel high sensitivity HPLC assay for topiramate, using 4-chloro-7-nitrobenzofurazan as pre-column fluorescence derivatizing agent. J. Chromatogr. B 2007; 850:400.
6. Gh. Bahrami, Sh. Mirzaeei, Kiani A. Sensitive analytical method for Topiramate in human serum by HPLC with pre-column fluorescent derivatization and its application in human pharmacokinetic studies. J. Chromatogr. B 2004; 813:175.
7. Bahrami B, Mirzaeei S, Mohammadi B, Kiani A. High performance liquid chromatographic determination of topiramate in human serum using UV detection. J. Chromatogr. B 2005; 822:322.
8. Mercolini L, Mandrioli R, Amore M, Raggi MA. Simultaneous HPLC-F analysis of three recent antiepileptic drugs in human plasma. J. Pharm. Biomed. Anal. 2010; 53:62.
9. Mandrioli R, Musenga A, Kenndler E, Donno M, Amore M, Raggi MA. Determination of topiramate in human plasma by capillary electrophoresis with indirect UV detection. J. Pharm. Biomed. Anal. 2010; 53:1319.
10. Klockow-Beck A, Nick A, Schaufelberger D. Determination of the inorganic degradation products sulfate and sulfamate in the antiepileptic drug topiramate by capillary electrophoresis. J. Chromagr. B: Biome. Sci. Appl. 1998; 720:141.
11. Carl E. Wolf, C. Richard Crooks, Poklis A. Rapid Gas Chromatographic Procedure for the Determination of Topiramate in Serum. J. Anal. Toxicol. 2000; 24:661.
12. Matar KM, Therapeutic drug monitoring of topiramate by liquid chromatography-tandem mass spectrometry. Clin. Chim. Acta 2010; 411 (9-10):729.
13. Pineyro-Lopez A, Pineyro-Garza E, Gomez-Silva M. Bioequivalence of single 100-mg doses of two oral formulations of topiramate: An open-label, randomized-sequence, two-period crossover study in healthy adult male Mexican volunteers. Clin. Therap 2009; 31:411.
14. Contin M, Riva R, Albani F, Baruzzi A. Simple and rapid liquid chromatographic–turbo ion spray mass spectrometric determination of topiramate in human plasma. J. Chromatogr. B: Biomed. Sci. Appl. 2001; 761:133.
15. Goswami D, Kumar A, Khuroo AH, Monif T, Rab S. Bioanalytical LC-MS/MS method validation for plasma determination of topiramate in healthy Indian volunteers. Biomed. Chromatogr. 2009: 23:1227.
16. Park JH, Park YS. Determination of plasma topiramate concentration using LC-MS/MS for pharmacokinetic and bioequivalence studies in healthy Korean volunteers. Biomed. Chromatogr. 2008, 22:822.
17. Malka B, Stefan S, Nina I. Analysis of Topiramate and Its Metabolites in Plasma and Urine of Healthy Subjects and Patients with Epilepsy by Use of a Novel Liquid Chromatography-Mass Spectrometry Assay. Therapeu. Drug Monit, 2003; 25:314.
18. Guidance for Industry, Bioanalytical Method validation, CDER, May 2001.