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A Sensitive Capillary GC-MS Method for Analysis of Topiramate from Plasma Obtained from Single Dose Studies

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Abstract

Topiramate (Topamax®) is an antiepileptic medication used as adjunctive and monotherapy in patients with epilepsy and for migraine prophylaxis. A GC-MS assay was developed that was capable of detecting topiramate plasma concentrations following a single rectal or oral dose administration. Topiramate plasma samples were prepared by solid phase extraction and were quantified by GC-MS analysis. The topiramate standard curves were split from 0.1–4 µg/mL and 4–40 µg/mL in order to give a more accurate determination of the topiramate concentration. The accuracy of the standards ranged from 94.6% to 107.3% and the precision (% CV) ranged from 1.0 % to 5.3% for both curves at all concentrations. The % CV for quality controls was <7.6%. The assay is both accurate and precise and will be used to complete future pharmacokinetic studies.

Keywords

topiramate; mass spectrometry; gas chromatography; humans; cyheptamide

Introduction

Topiramate (Topamax®, Ortho-McNeil, [2,3:4,5-Di-O-isopropylidene- -D-fructopyranose sulfamate]) is an antiepileptic drug (AED) indicated for adjuvant treatment of partial onset, generalized tonic-clonic, and Lennox-Gastaut seizures in children (ages 2 to 16) and adults. It is also approved for monotherapy treatment of partial and generalized onset seizures in patients 10 years of age or older and for prophylaxis of migraine headaches in adults. Topiramate is a weak carbonic anhydrase inhibitor and its anticonvulsant activity is mediated via multiple mechanisms of action including a specific effect on GluR5 kainate receptors and an enhancement of GABA release (Braga 2009).

Previous analytical methods for the quantification of topiramate in plasma, serum, or saliva have been described, including fluorescent polarization immunoassay (Berry and Patsalos 2000; Tang 2000; Christensen 2002) and HPLC after fluorescence derivatization (Bahrami and Mohammadi 2007). Several HPLC-MS assays have also been developed with a variety

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of ionization methods such as negative ion electrospray (Chen and Carvey 2001), turbo ion spray (Contin 2001), thermospray (Masucci 1998), electrospray with MS/MS detection (Christensen 2002), atmospheric pressure chemical ionization and negative ion LC-MS (Subramanian 2008). For example, Britzi et al. developed a LC-MS method for the analysis of topiramate in plasma and several metabolites in urine with $^2\text{H}_{12}$ -topiramate as an internal standard (Britzi 2003). The lower limit of quantification for this method in plasma was $0.625\mu\text{g/mL}$. Several assays are available that use liquid-liquid extraction and measurement by gas chromatography methods with either flame ionization detection (FID) or nitrogen-phosphorus detection (NPD) (Holland 1988; Riffitts 1999; Tang 2000; Wolf 2000). Gidal and Lensmeyer developed a plasma and whole blood method by solid phase extraction and analysis by GC-NPD with a limit of detection of $0.42\mu\text{g/mL}$ (Gidal and Lensmeyer 1999).

GC-MS is now used less commonly than LC-MS and HPLC hyphenated MS methods for therapeutic drug monitoring and pharmacokinetic studies of drugs. However, analysis by GC-MS has many attractive features including high selectivity due to the greater number of theoretical plates and requirement of volatile compounds. It also has high sensitivity, lower instrumentation cost, and obviates the needs to make mobile phases and buffers reducing organic solvent waste.

In order to study intensive pharmacokinetic profiles in humans following a single dose of topiramate administered either orally or rectally, a GC-MS method with solid phase extraction was developed. The samples were extracted by a modified method originally developed by Gidal and Lensmeyer (Gidal and Lensmeyer 1999) and detection by GC-MS. Cyheptamide, a commercially available carbamazepine analog that is commonly used as an internal standard for simultaneous assays of anticonvulsants, was used as the internal standard. The goal of this project was to develop a rapid and precise analytical method for pharmacokinetic studies of topiramate in humans to support a post-marketing, single dose, bioavailability clinical study.

Experimental

Chemicals and Materials

Topiramate powder was generously supplied by R.W. Johnson Pharmaceutical Research Institute, Spring House, PA. The internal standard was cyheptamide (Sigma, St. Louis, MO). Working standards were prepared from a stock standard solution of 1 mg/mL topiramate in 50% water and 50% methanol. Methanol (Fischer Scientific, Fair Lawn, NJ), hexane (Fischer Scientific, Fair Lawn, NJ), toluene (Mallinckrodt, Paris, KT) and ethyl acetate (Mallinckrodt, Paris, KT) were all HPLC grade. 25% saturated ammonium acetate solution was prepared with ammonium acetate (ACS grade, Fischer Scientific, Fair Lawn, NJ). All standards and quality control samples were prepared in human plasma. All lots of human plasma were verified to not contain interfering substances by inspection of chromatograms after preparation as blank samples prior to use in all assays.

Instrumentation

The GC-MS system was comprised of a HP5890 series II gas chromatograph connected to a HP5971 mass selective detector. The system was controlled by Chemstation software provided by Hewlett Packard. The GC column was a DB-5MS $30\text{ mm} \times 0.25\text{ mm}$ i.d. capillary of 0.5 micron film thickness (J&W Scientific, Folsom, CA). The temperature program was set at 230°C for 0.5 min and then raised to 290°C at 10° per min . The final temperature of 290°C was held for 1 min . The carrier gas was 99.95% high purity helium with a head pressure of 1.5 kg/cm^2 and a split ratio of 30:1. Electron multiplier voltage was set to 70 eV . The transfer line temperature was set to 280°C , and the injection port and the detector temperatures were 280°C and 300°C , respectively. Two microliters of the sample

were injected onto the column in the split injection mode with a 1:30 split ratio. Typical retention times were 4.68 (topiramate) and 5.75 min (cyheptamide). Ions monitored were m/z 324.1 (topiramate) and m/z 193.1 (cyheptamide).

Sample Preparation and Extraction procedure

Blood samples (10 mL) were drawn from human subjects at each time point into heparin-containing Vacutainer® tubes. From this sample, approximately 4 mL of plasma were procured by centrifugation at 2000 g for 5 min. Plasma samples (0.5 mL) were extracted by a modified method developed by Gidal and Lensmeyer (Gidal and Lensmeyer 1999). The standard curve was prepared by combining 500 μ L of human plasma, 50 μ L of cyheptamide internal standard, 50 μ L of topiramate standard, and 500 μ L of 25% saturated ammonium acetate. The internal standard solution was prepared by placing 8.8 mg of cyheptamide into a 10 mL volumetric flask and diluted to volume with methanol. An aliquot (125 μ L) of this stock solution was transferred into a 10 mL volumetric flask and diluted to volume with methanol (working stock final concentration 1.1 mg/mL). Fifty microliters were added to each sample and standard (1.1 μ g/mL cyheptamide final concentration in plasma). Samples were mixed on a Vortex® mixer and transferred to an Empore™ (3M, St. Paul, MN) C18 solid-phase extraction disk cartridge. One mL of methanol and then 1 mL of water were drawn through the disk by a vacuum manifold prior to the addition of sample. The sample was loaded onto the disk by low-speed centrifugation (120g) for 5 min. The disk was washed with 500 μ L of water, then 500 μ L hexane by vacuum manifold, and finally 1 mL of ethyl acetate was added and topiramate and cyheptamide were eluted from the disk by centrifugation (120 g) for 5 min. The eluate was evaporated to dryness under nitrogen and the final samples were reconstituted in 50 μ L of toluene.

Method validation

Peak-area ratios (topiramate/internal standard) were used to calculate topiramate concentrations from extracted plasma standards and samples. Standard curves were prepared at the following concentrations: 0.1, 0.2, 0.4, 1, 4, 10, 20, and 40 μ g/mL in blank human plasma. The standard curves were prepared in triplicate for each day on five different days. Quality control samples were prepared in blank human plasma at 0.6, 6, and 15 μ g/mL and were run in triplicate along with the unknowns.

The estimated with-in run variability of the topiramate assay for a single plasma sample on different days was determined by one-way analysis of variance (ANOVA) from calculated concentrations of multiple triplicate weighted ($1/x$) standard curves or quality control samples run in triplicate. The square root of the MS_w (mean square, within run) statistic (S_{wr}) represents the average coefficient of variation (C.V.) from five triplicate standard curve or quality control samples. The between-run variability was determined from the standard deviation (S_b) of all of the standard curve samples ($n=15$). Accuracy was calculated by dividing the calculated mean concentration by the true values (known concentration) multiplied by 100. Accuracy was improved when the standard curve was split, with the lower curve containing the 0.1, 0.2, 0.4, 1 and 4 μ g/mL standards, and the upper curve consisting of the 4, 10, 20, and 40 μ g/mL standards. The percentage C.V. was calculated by dividing the S_{wr} and S_b by the mean of the calculated concentration values multiplied by 100 (Rodbard 1974, Rosing 2000).

Results and Discussion

Gas chromatographic mass spectrometry

GC-electron impact MS in the scan mode was used to identify the appropriate ions to monitor topiramate and cyheptamide. Figures 1 and 2 shows the fragmentation patterns and

structures for topiramate and the internal standard (cyheptamide). For cyheptamide (MW 237.1), the major fragment ions were $m/z = 193.1$ [$M-CONH_2$, base peak] and at m/z 179, 165, and 115. The base peak for topiramate (MW 339.2) was at $m/z = 324.1$ [$M-NH$], along with several other fragment ions (see Fig. 1). Select ion monitoring for topiramate and cyheptamide were done at 324.1 and 193.1, respectively. Under the described chromatographic conditions, the retention times were 4.68 min (topiramate) and 5.75 min (cyheptamide). Figures 3 and 4 show a human plasma blank, the lower level of quantification (LOQ), a mid level standard, and a patient sample. This GC-MS assay is both accurate and precise with an LOQ of 0.1 $\mu\text{g/mL}$.

Derivatization was commonly used to increase volatility and prevent toiling observed with older packed glass GC columns; however, modern inlet systems and capillary columns have few exposed reactive sites. Many compounds including topiramate can be analyzed without derivatization. Therefore typical extraction schemes involving liquid-liquid extraction or solid phase extraction are no more complicated than those used for HPLC.

The split injection technique improves reproducibility and the high column oven temperatures (250°C to 290°C) allow for rapid cycling times and short retention time (~8 minutes between injections). However, split-less injection is more sensitive due to cold-trapping/concentrating at the head of the column. The LOQ was 0.1 $\mu\text{g/mL}$, but could be improved by split-less injection.

The stability of topiramate in stored plasma has been previously studied. Riffitts et. al., reported that quality control urine and plasma samples were stable for up to 6 months at -20°C (Riffitts 1999). Matar et. al, recently reported that topiramate was stable during five freeze thaw cycles and was stable in plasma at -80°C for at least 1 month (Matar 2010).

Extraction recovery

The extraction recovery for topiramate at four different concentrations is shown in Table 1. The extraction recovery for the internal standard, cyheptamide, was similar to topiramate and was $65.8 \pm 8.6\%$ at the concentration used in the assay (1.1 $\mu\text{g/mL}$). The extraction ratio appeared to decline modestly as the topiramate concentration increased, but the ratio of topiramate/internal standard was constant across the entire concentration ratio with a mean ratio of 0.658 ± 0.036 . Solid phase extraction on C18 Empore™ disk cartridges was used with a hexane wash (to remove non-polar lipids) followed by elution by ethyl acetate. This method was based on a prior topiramate GC method (Wolf 2000). Other investigators have employed liquid-liquid extraction with good success. The extraction recovery for this solid phase extraction method resulted in a highly reproducible assay.

Assay validation

Validation consisted of five standard curves performed in triplicate on five different days. The analytical method for quantification of topiramate with cyheptamide was both accurate and precise. Table 2 shows the accuracy, within-day and between-day components for variation and total coefficient of variation for topiramate from the compiled standard curve data. All CV values were less than or equal to 5.3% based on results from five triplicate standard curves. Mean accuracy was less than 7.2% of the true value with a precision of less than 6%. The LOQ was 0.1 $\mu\text{g/mL}$. Table 3 shows the statistical data for the quality control samples. The average plasma level in human subjects after a single oral dose of 200 mg was 6 $\mu\text{g/mL}$. This assay was sensitive enough to detect topiramate in human plasma up to 4 to 5 half-lives after a single dose. A pharmacokinetic profile in a patient that received an oral dose is shown in Figure 5.

Conclusions

GC-MS was selected because of the high specificity and sensitivity of the technique. Since no derivatization is required for topiramate for GC analysis and sufficient sensitivity was obtained with a split injection method, a rapid separation was achieved with a shallow temperature gradient from 230°C to 290°C. The cycle time between injections was <10 min. Gas chromatography with a simple mass selective detector (MSD-electron impact ionization) has several advantages over HPLC-MS methods. Capillary GC columns have excellent peak resolution and the sensitivity of the MSD is comparable to published LC-MS methods and is sufficient for pharmacokinetic studies of topiramate. GC-MS instrumentation is simpler and less expensive than LC-MS to purchase and maintain, mobile phase preparation is not required, and column life is very good (>2000 injections) if the split liner is cleaned or changed after each run and the first 6 cm are removed from the column after every 200 injections. Other AEDs are not expected to interfere because of the high specificity and sensitivity of capillary GC-MS. The analytical method has sufficient sensitivity for pharmacokinetic studies and the precision is also very good (<5%) for therapeutic drug monitoring in patients taking maintenance doses of topiramate for epilepsy or migraine headaches.

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Mass Spectrum of Topiramate

Figure 1.
MS fragmentation and structure of topiramate

Figure 2.
MS fragmentation and structure of cyheptamide (internal standard)

Figure 3a.

Figure 3b.

Figure 3c:

Figure 3.

Sample chromatograms from a typical assay preparation

a) Blank plasma chromatogram b) Limit of quantification chromatogram (0.1 $\mu\text{g/ml}$)

* The peak at 4.86 minutes corresponds to an analogue of topiramate that was used as an exploratory internal standard. Due to the lack of commercial availability we did not use this internal standard.

c) Extracted ion chromatogram for a mid level standard (2 $\mu\text{g/ml}$). Top panel: Selected ion chromatogram ($m/z=193.1$) for cyheptamide (internal standard) at 5.75 minutes. Bottom panel: Selected ion chromatogram ($m/z=324.1$) for topiramate at 4.68 minutes.

Figure 4. Extracted ion chromatogram for a plasma patient sample. Top panel: Selected ion chromatogram ($m/z=193.1$) for cyheptamide (internal standard) at 5.75 minutes. Bottom panel: Selected ion chromatogram ($m/z=324.1$) for topiramate at 4.68 minutes.

Figure 5. Ninety-six hour plasma profile of topiramate in a single human subject given a single, 200 mg oral dose of topiramate.

Table 1

Extraction Recovery of topiramate from Human Plasma

Concentration of TPM ($\mu\text{g/mL}$)	Extraction recovery (mean $\pm$ SD)	C.V. (%)
1	69.3 $\pm$ 7.4	10.7
4	58.0 $\pm$ 13.9	24.1
10	56.7 $\pm$ 4.2	7.5
20	52.1 $\pm$ 2.6	4.9

TPM = topiramate

Table 2
Accuracy, Between-Run, and Within-Run Variation for topiramate in Standard Curve Samples

Concentration of TPM (µg/mL)	N	Mean measured concentration (µg/mL)	Accuracy (%)	S _b ^a (µg/mL)	C.V. _b ^b (%)	S _{wr} ^a (µg/mL)	C.V. _{wr} ^b (%)
40	15	40.1	100.3	1.84	4.6	2.13	5.3
20	15	20.0	100.1	0.77	3.8	0.80	4.0
10	15	9.77	97.7	0.26	2.7	0.25	2.6
4	15	4.07	101.8	0.086	2.1	0.04	1.0
1	15	0.97	97.0	0.034	3.5	0.031	3.2
0.4	15	0.38	94.6	0.012	3.3	0.014	3.7
0.2	15	0.20	99.9	0.005	2.5	0.006	2.9
0.1	15	0.107	107.3	0.004	3.8	0.004	3.4

^aStandard deviation: S_b = between run, S_{wr} = within-run

^bCoefficient of variation: C.V._b = between-run, C.V._{wr} = within-run

Table 3
Accuracy, Between-Run, and Within-Run Variability for topiramate Quality Control Samples

Concentration of TPM QC (µg/mL)	N	Mean Measured Concentration (µg/mL)	Accuracy (%)	S _b ^a (µg/mL)	C.V. _b ^b (%)	S _{wr} ^a (µg/mL)	C.V. _{wr} ^b (%)
0.6	15	0.563	93.9	0.038	6.8	0.0428	7.6
2	15	2	100	0.124	6.2	0.1184	5.9
15	15	14.3	95.3	0.691	4.8	0.6039	4.2