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Note

Automated capillary gas chromatographic assay using flame ionization detection for the determination of topiramate in plasma

MARY L. HOLLAND*, JANET A. UETZ and KUNG T. NG

Department of Drug Metabolism, McNeil Pharmaceutical, Spring House, PA 19477-0776 (U S A)

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Topiramate, 2,3:4,5-bis-O-(1-methylethylidene)-β-D-fructopyranose sulfamate (Fig. 1), is an anticonvulsant drug currently under clinical evaluation for the treatment of epilepsy. Topiramate has been evaluated extensively in animal studies both at McNeil and at the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). It has been shown to be an effective anticonvulsant with a good safety profile after oral administration in animals [1,2].

The present study reports the development of a reproducible and specific capillary gas chromatographic (GC) assay with flame ionization detection (FID) for the determination of topiramate in plasma. Solid-phase cartridges (cyano moiety) are used in the isolation of the drug from plasma. The assay has been used successfully in preclinical and clinical pharmacokinetic studies of topiramate.

EXPERIMENTAL

Instrumentation

A Hewlett-Packard 5880A capillary gas chromatograph (Avondale, PA, U.S.A.) equipped with a Hewlett-Packard 7672A autosampler and a flame ionization detector was used. Compounds were separated on a DB-5 fused-silica capillary column (25 m×0.32 mm I.D., 0.25 μm film thickness, J & W Scientific, Rancho Cordova, CA, U.S.A.) using temperature programming from 165 to 205°C at 9°C/min. Helium carrier gas was used at a flow-rate of 3 ml/min at 165°C. Injector and detector temperatures were 250 and 300°C, respectively. Splitless injection with a purge at 0.3 min was used in conjunction with the autosampler.

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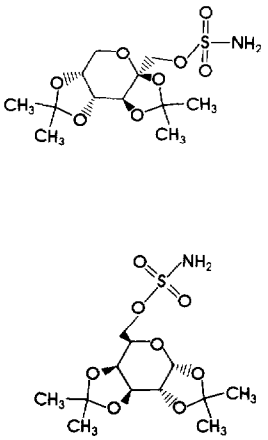

Fig. 1. Chemical structures of topiramate (top) and the internal standard (bottom).

A Hewlett-Packard 3354 laboratory automation system was used for automatic data acquisition and processing.

Reagents and supplies

Nanograde methanol, chloroform, ethyl acetate, hexane and toluene were obtained from Mallinckrodt (Paris, KY, U.S.A.) and used without further purification. HPLC-grade hexane and isopropanol were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Triply purified distilled water was obtained from Ephrata Mountain Water (Manheim, PA, U.S.A.). Absolute ethanol was obtained from Pharmco (Dayton, NJ, U.S.A.).

Potassium phosphate monobasic and sodium phosphate dibasic were purchased from Mallinckrodt and Fisher Scientific, respectively. These reagents are used to prepare a 0.05 *M* phosphate buffer (pH 7.4).

Cyano (CN) Bond-Elut® cartridges, 500 mg capacity, and the Vac-Elut® manifold were purchased form Analytichem International (Harbor City, CA, U.S.A.).

Topiramate and the internal standard, a positional isomer of topiramate (Fig. 1) were obtained in-house (McNeil Pharmaceutical, Spring House, PA, U.S.A.).

Extraction procedure

The isolation procedure consists of an initial wash of the plasma with hexane–isopropanol (95:5), extraction of topiramate and the internal standard into ethyl acetate, evaporation of the ethyl acetate and further sample clean-up on a CN Bond-Elut cartridge. The procedure is described in detail in the following paragraphs.

To a 0.5-ml plasma sample, 50 μl of methanol containing 2.5 μg internal standard, 1 ml distilled water, 200 μl pH 7.4 phosphate buffer and 5 ml hexane–isopropanol (95:5) are added. The sample is shaken on a linear shaker, centrifuged for 5 min at 681 *g*, and the organic layer aspirated and discarded. Ethyl acetate (5 ml) is added and the sample is again shaken and centrifuged for 5 min at 681 *g*. After freezing the aqueous layer using dry ice, the ethyl aceate is decanted into a clean tube and evaporated to dryness.

The sample is reconstituted by first adding 500 μl of chloroform, vortex-mixing and then adding 500 μl of hexane and mixing again. The chloroform–hexane solution is applied to a CN solid-phase extraction cartridge that was conditioned by rinsing with 3 ml of ethanol. After washing the cartridge twice with 3 ml of chloroform–hexane (50:50), topiramate and the internal standard are eluted with four 500-μl aliquots of ethanol. The ethanol is evaporated and the sample is reconstituted with toluene–methanol (90:10, v/v). Samples with expected concentrations of 5 μg/ml or less and unknown samples are reconstituted with 100 μl of solvent and those with higher expected concentrations are reconstituted with 500 μl to avoid overloading the capillary column (unknown samples may be further diluted to 500 μl and reinjected if the initially measured concentrations exceed 5 μg/ml). The reconstituted samples are then transferred to autoinjector vials for injection into the capillary gas chromatograph.

Standard curves

To establish a calibration curve, a series of topiramate standard solutions (1–500 μg/ml) containing 50 μg/ml internal standard were prepared in methanol. A 50-μl volume of these solutions (instead of the internal standard solution) was added to 0.5 ml of plasma and the samples were extracted according to the procedure above. Duplicate standard curves were run on each analysis day. The peak-height ratios of topiramate/internal standard were weighted by 1/variance and plotted against the topiramate concentrations. Linear regression analysis gave a calibration line which was used to calculate topiramate concentrations in frozen seeded controls and unknown samples.

As an additional control, spiked plasma pools were prepared at two concentrations (2.0 and 20 μg/ml), separated into 1-ml aliquots and stored frozen. Two samples from each pool were analyzed with each calibration curve to assess the precision of the assay procedure.

Application of the assay

In order to demonstrate the utility of the assay for pharmacokinetic studies, plasma samples from beagle dogs which had received topiramate (10 mg/kg intravenously or 300 mg/kg orally) were analyzed. Blood samples were taken from 5 min (intravenous dose) or 15 min (oral dose) to 24 h following dose administration. Plasma was obtained from the blood samples and appropriate sample aliquots (50 μl to 0.5 ml) were analyzed.

RESULTS AND DISCUSSION

Gas chromatography

Topiramate is a weakly acidic drug which degrades in the presence of strong acid. At neutral pH, the drug is not extracted into non-polar solvents such as hexane which would provide a clean sample for analysis. The use of a three-step liquid–liquid extraction clean-up was therefore not feasible for the isolation of topiramate from plasma. Additionally, the drug has no significant UV absorption, so high-performance liquid chromatographic analysis was not practical. Finally,

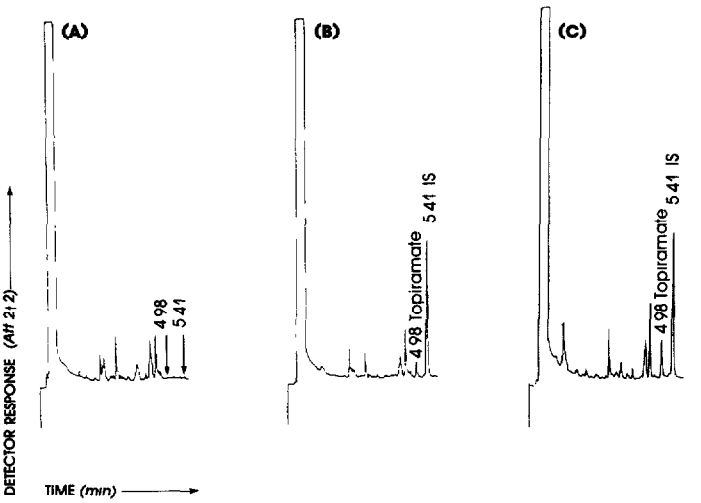

Fig. 2. Capillary GC–FID profiles of (A) blank plasma, (B) plasma spiked with 0.5 μg/ml topiramate and 5 μg/ml internal standard (IS) and (C) a clinical plasma sample containing 2.48 μg/ml topiramate (0.5 ml was analyzed).

topiramate is not detectable using a thermionic GC detector. Thus, the use of the CN cartridge in a normal-phase mode permits the isolation of topiramate and provides a sample that is clean enough for analysis by capillary GC with FID.

Chromatograms of plasma with and without topiramate and the internal standard are shown in Fig. 2. The retention times for topiramate and the internal standard are 4.98 and 5.41 min, respectively. No significant interfering peaks appear in the chromatogram of blank plasma and the two compounds are well separated. Since anticonvulsants are often administered concomitantly, the potential interference of several commonly used anticonvulsants was evaluated. None of the anticonvulstants evaluated, including carbamazepine, phenytoin, phenobarbital, diazepam and valproic acid, were found to interfere with the quantification of topiramate.

Standard curves, precision and accuracy

Duplicate calibration curves run on three consecutive days were linear over the concentration range studied here (0.1–50 μg/ml) (Table I). Regression analysis of the peak-height ratios gave the following equation for the composite calibration curve: $y = 0.237x + 0.003$ where y = drug/internal standard peak-height ratio and x = topiramate concentration. The correlation coefficient for the three-day composite curve was 0.998. The precision of the assay, as measured by the relative standard deviations at each concentration, was within 9%. The mean of the calculated concentration was within 4% of the actual value at all concentrations except at the highest concentration (10.3%).

The average measured values of the frozen seeded control samples were within 5% of their seeded concentrations with relative standard deviations of $< 7\%$.

TABLE I

SUMMARY OF CALIBRATION CURVE DATA FOR THE ANALYSIS OF TOPIRAMATE IN PLASMA

Duplicate calibration curves analyzed on three separate days.

Actual concentration (μg/ml)	*n*	Mean concentration measured (μg/ml)	Relative standard deviation (%)	Deviation of mean from actual concentration (%)
0.10	5	0.096	8.3	−3.5
0.25	6	0.26	7.2	+3.9
0.50	6	0.51	6.9	+1.6
1.0	6	1.0	5.0	0.0
2.5	6	2.6	7.1	+3.4
5.0	6	4.8	5.2	−3.1
10.0	6	10.3	4.6	+2.7
25.0	6	25.3	2.1	+1.1
50.0	6	44.9	5.4	−10.3

Day	Linear regression analysis		
	Slope	*y*-Intercept	Correlation coefficient
1	0.241	0.008	0.999
2	0.238	0.005	0.999
3	0.216	0.002	1.00
Three-day composite	0.237 ± 0.018	0.003 ± 0.001	0.998

Recovery and sample stability

The extraction efficiencies for topiramate and the internal standard were estimated by comparing the peak heights obtained by extracting six standards at two concentrations (1 and 10 μg/ml for topiramate; 5 μg/ml for the internal standard) with those obtained by seeding extracted blank plasma samples at the same concentrations. Using this method, the recoveries of both topiramate and the internal standard were estimated to be greater than 90%.

Analysis of frozen seeded control samples up to five months after their initial preparation gave measured concentrations within 10% of the originally measured values. Extracted and reconstituted samples can be stored refrigerated for at least three days without significant alteration in measured concentrations of topiramate.

Application of the assay

Topiramate plasma concentrations were determined in each of the dog samples using plasma aliquots as small as 50 μl (diluted to 0.5 ml with blank plasma) when plasma concentrations exceeded the linear range of the assay. Plasma concentrations ranged form 1.2 to 201 μg/ml. Plasma concentration versus time pro-

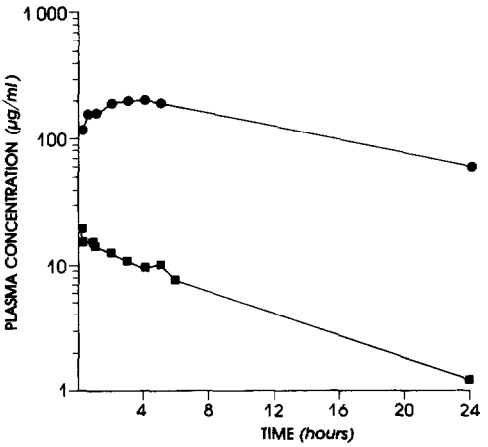

Fig. 3. Plasma concentration–time profiles obtained from two dogs following the administration of (●) a single oral dose (300 mg/kg) or (■) a single intravenous dose (10 mg/kg) of topiramate.

files are given for an intravenously and an orally dosed dog (Fig. 3), demonstrating the utility of this assay for pharmacokinetic studies. The assay has been used successfully in preclinical studies in mice, rats and dogs as well as in several clinical trials.

In summary, a capillary GC–FID assay for topiramate in plasma has been developed that is capable of measuring from 0.1–50 μg/ml topiramate in plasma using a 0.5-ml sample. The drug isolation procedure permits the analysis of topiramate without interference from other anticonvulsants. Sample aliquots as small as 50 μl have been analyzed successfully; this has been an important feature of the assay for pharmacokinetic studies in rodents where sample size is limited.

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REFERENCES

1 B.E. Maryanoff, S O. Nortey, J.F. Gardocki, R.P. Shank and S.P Dodgson, J. Med. Chem., 30 (1987) 880–887.
2 J.F. Gardocki, L.S. Labinsky, G.L. Brown and B.E. Maryanoff, Epilepsia, 27 (1986) 648–649.