

# Development and validation of a new method for determination of topiramate in bulk and pharmaceutical formulation using high performance liquid chromatography-UV detection after pre-column derivatization

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## ABSTRACT

In published high performance liquid chromatographic (HPLC) methods for analysis of topiramate (TPM) in pharmaceutical dosage forms and raw materials Refractive Index Detector (RID) has been used which is not available in many laboratories and has low sensitivity. We described a new, sensitive and simple HPLC method for determination of topiramate in pharmaceutical forms and In-virto dissolution studies which avoids the use of RID detector. The method is based on derivatization of topiramate and an internal standard by reaction with 4-chloro-7-nitrobenzofurazan (NBD-CL), and reverse-phase chromatography using phenyl column and spectrophotometer detection at 264 nm. A mixture of phosphate buffer (0.05 M) containing triethylamine (0.1% V/V; pH=2.3) and methanol (28:72, V/V) at a flow rate of 2.2 ml/min was used as mobile phase. The analysis performance was studied and the method was shown to be selective and linear for determination of topiramate in pharmaceutical forms and dissolution studies.

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## Introduction

Topiramate (2,3:4,5-bis-*O*-(-1-methyl)[beta]-D-fructopyranose sulfamate), a sulfamate-substituted derivative of the monosaccharide D-fructose that has been approved for the treatment of epileptic disorder [1]. The action mechanism of TPM is unknown but appear its blocked voltage-sensitive sodium channels and increase activity of gamma-amino butyrate (GABA) in central nerve system [2,3]. The oral bioavailability of the drug was reported to be 80% with time to peak plasma concentration of 2-4h [4]. The analysis of topiramate is complicated, because the molecule has no ultraviolet, visible or fluorescence absorption. Topiramate has been measured with several methods such as gas chromatography (GC) [5,6] liquid chromatography-tandem mass [7-9], capillary electrophoresis [10] and HPLC [11-13]. In previous studies we have reported the development of a sensitive and reliable HPLC method for quantifying of topiramate in human serum after its derivatization by 9-fluorenylmethylchloroformate (FMOC-Cl) and NBD-CL (using fluorescence detection) [11,12]. However, there is noticeable shortage of methods described in the literature for determination of the drug in pharmaceutical dosage forms and in in-vitro dissolution studies. Quantification method has been reported for analysis of the drug in bulk production, raw materials using HPLC equipped with RID detector [14], but this detection device is not generally available in routine pharmaceutical analysis laboratory. Moreover the sensitivity of RID detector for quantitation of topiramate in in-vitro studies such as dissolution test is low. In the previously reported method for analysis of topiramate in formulation [14], the drug has poorly been detected with retention time of about 28 min. Thus more simple and sensitive methods are needed for in-vitro studies of topiramate. Present paper avoids the use of RID detector and introduces a new, simple and sensitive HPLC method for quantification of topiramate in pharmaceutical dosage forms and in-vitro dissolution studies using UV detection.

## Materials and Methods

### Chemicals

Topiramate was from (Johnson- Cilag) and kindly donated by Arya pharmaceutical company (Tehran, Iran). Methanol (HPLC grade), boric acid, potassium chloride, potassium hydroxide, potassium dihydrogen phosphate, phosphoric acid and glycine were purchased from Merck (Darmstadt, Germany). NBD-CL and amantadine (I.S.) were obtained from Sigma (St.Louis, MO, USA). All reagents used were of analytical grade except methanol which was HPLC grade. Water was glass-double distilled and further purified for HPLC with a Maxima purification system (USF ELGA, England).

### Preparation of standard solutions

Stock solutions of topiramate and the I.S. were prepared by dissolving the compounds in distilled water at concentration of 500 and 40 µg/ml, respectively. Topiramate stock solution was further diluted with the same medium to obtain the different working solutions ranging from 1 to 300 µg/ml. A borate buffer was prepared by dissolving 0.625 g of boric acid and 0.750 g of potassium chloride in 100 ml water and adjusting the pH to 7.7 with 0.2 M potassium hydroxide solution. A 500 µg/ml solution of NBD-CL was prepared in acetonitrile. Stock solution of glycine (4mg/ml) was prepared in water. All solutions were stored at 4°C and were stable at least for 30 days.

### Absorption spectra

For measurement of absorption curve of derivatized topiramate a solution of 200 µg/ml the drug in acetonitrile was used. A 25µl of this solution was subjected to the derivatization and analysis with HPLC using fluorescence detection which has previously been described [12]. A fraction covering the retention time range (3.2-4.5 min) where derivatized topiramate elutes ( but not excess of NBD-Cl and endogenous peaks) was collected and subjected to absorption spectra measurement using the mobile phase as the blank with a Uvikon 933 double beam UV VIS

spectrophotometer (Italy). The compound shows sufficient absorption in the UV region, with maximum absorption at around 264 nm.

### **Chromatography**

The HPLC system used consisted of two pumps of Shimadzu LC-10A solvent delivery system, a system controller (SCL 10AD), a variable wavelength UV-VIS spectrophotometric detector (SPD-10A), a injection valve with a 20 µl filling loop, a column oven (CTO-10A) set at 62 °C, a degasser (DGU-3A) and a data processor (C-R4A) all from Shimadzu, Kyoto, Japan. Analysis was performed on a Shim-pack G-phenyl precolumn (4.0 mm i.d. ×1cm) and a reverse phase phenyl column (150 ×6 mm ID) which was packed with 5 µm particles (Shim-pack-CLC-Phenyl). A mobile phase which was comprised of mixture of phosphate buffer (0.05 M) containing triethylamine (1ml/L V/V) adjusted to pH 2.3 with o-phosphoric acid and methanol (28:72, V/V) was used. The elution was filtered through a 50 µm filter (Milipore, Bedford, MA, USA) and degassed before use. A flow rate of 2.2 ml/min at a pressure of 140 kg/fcm<sup>2</sup> was used. The detection was performed at the wavelength of 264 nm.

### **Optimization of the derivatization conditions**

samples prepared with the drug working solutions to make low (0.02 µg/mL), medium (10 µg/mL) and high (200 µg/mL) concentrations of TPM were used to optimize derivatization of the drug with NBD-CL. Concentrations of the NBD-CL solutions were optimized in the range of 100–1000 µg/mL. pH and concentrations of the buffer solutions ranging from 2.2 to 8 and 0.01 to 0.5 M respectively, were tested to obtain optimal conditions. The polarity of the reaction solution was tested using various organic solvents–water proportions, ranging from 1:1 to 10:1; the reaction was allowed to proceed in a water bath at different temperatures ranging from 40 to 80 °C and times between the ranges of 5 and 25 min.

### **Sample preparation and derivatization**

For assay test the number of 20 tablets (25, 100 or 200mg), were weighted and finely powdered. The combined contents were mixed and quantity of the powder equivalent to one tablet was suspended in distilled water and vigorously stirred for 10 min. A 500 µl from this assay test medium was sampled. For dissolution test each topiramate tablet was suspended in 900 ml of distilled water (Erveka Dt 60 apparatus, 50 rpm) and sampling was performed (500 µl) at different times up to 45 min. All the samples were transferred to a disposable glass tube containing 500 µl amantadine (40µl/ml). After brief vortex mixing, 50 µl of these solutions are sampled and subjected to derivatization. To each 50 µl of the sample 300 µl NBD-Cl (500 µg/ml in acetonitrile) and 25 µl of borate buffer (pH 7.7) were added and after brief mixing for 10 s the samples were kept at 50°C for 15 min. The reaction was stopped by adding 20 ul glycine (0.1 M) and, after 1 min, a volume (20 µl) of the reaction mixture was injected in to the chromatograph.

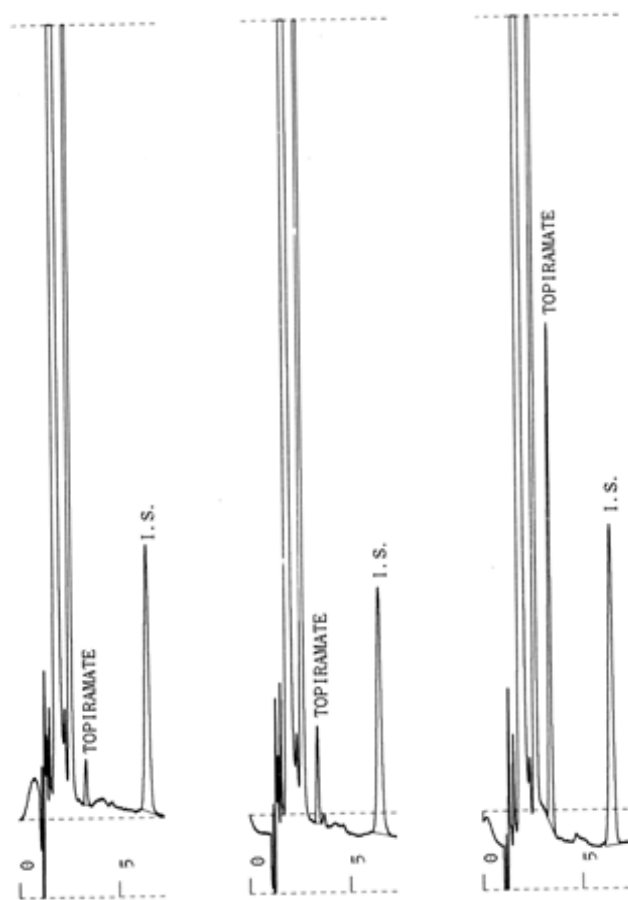
### **Method validation**

Calibration curves were prepared by the analysis of 50 µl from different working standard solutions ranging from of 1-300 µg/ml. The samples were subjected to the derivatization and chromatographic analysis described above. Calibration curves were obtained by linear least-squares regression analysis plotting of peak-area ratios (topiramate /I.S.) versus the topiramate concentrations. Intra and inter day variations were determined by repeated analysis(n=6) of different topiramate concentrations within the range of calibration curve in a single analytical run and in ten analytical run performed on 10 different days, respectively. The limit of detection (LOD) was defined as the concentration of drug giving a signal to noise ratio of 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of topiramate quantified with a coefficient of variation of less than 20% [15].

## Results and Discussion

### Specificity and selectivity

Representative chromatograms of topiramate (3, 7.2 and 28  $\mu\text{g/ml}$ , respectively) and the I.S. in different times of dissolution test are shown in fig 1. chromatograms show the excellent chromatographic specificity without evidence of interfering of either topiramate degradation products or additive substances with drug analysis during the study.



**Fig. 1.** Chromatograms of topiramate (25 mg; Johnson-Cilag) in dissolution test after 5, 10 and 30 min sampling, corresponding to 11, 26 and 101% dissolution of the drug (3, 7.2 and 28  $\mu\text{g/ml}$ , respectively).

### Sensitivity and linearity

The standard calibration curve was linear over the concentration ranges of 1-300  $\mu\text{g/ml}$ . The correlation coefficients for calibration curves were equal to or better than 0.9975. Intra-assay reproducibility was determined for calibration curves prepared the same day in replicate ( $n=6$ ). The intra-day average slope of the fitted straight lines was  $0.9198 \pm 0.042 \text{ ng/ml}$  (C.V. = 2.48%) and the mean intercept of the calibration curves was  $5.9823 \pm 0.2241$  (C.V. = 2.59%). The corresponding mean ( $\pm$  SD.) coefficient of the linear regression analysis was  $0.9987 \pm 0.005$  (C.V. = 0.105%). For calibration curves prepared on different days ( $n=10$ ), the mean  $\pm$  SD. of results were as follows: slope =  $0.9226 \pm 0.033 \text{ ng/ml}$  (C.V. = 3.54), coefficient of the linear regression analysis =  $0.9985 \pm 0.010$  (C.V. = 0.121%) and intercept =  $6.1214 \pm 0.1218$ . The LOD was approximately 500 ng/ml at a signal to noise ratio of 3:1 and LOQ corresponding with a coefficient of variation of less than 20% was 1 ng/ml.

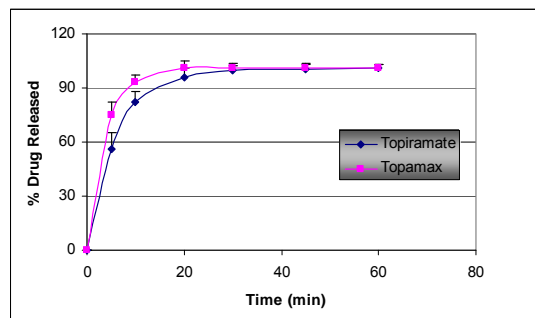
### Precision, accuracy and stability

Intraday precision and accuracy were studied for different concentrations in the range of calibration curve. For intra-day variation an accuracy ranged from -4.2 to +2.8% and precision was <13.5 % and for inter-day variation an accuracy ranged from -4.8 to +0.6% and precision were <14.1 %. The results of precision and accuracy of the method are given in table 1. Stock solutions of topiramate and amantadine were stable for at least 60 days when stored at 4°C and the derivatized solutions were found to be stable (>95%) for 24h.

### Conclusion

In conclusion present method obviates the need of RID detector and describes a new, simple and more sensitive method for quantification of topiramate in pharmaceutical dosage form and in-vitro dissolution studies. This method has been used in comparative in vitro study of two different topiramate preparations and proved to be suitable for assay of the drug in in-vitro bioequivalence

studies of topiramate. The dissolution profiles two different topiramate preparations have been shown in Fig 2.



**Fig. 2.** Drug release profile of two different topiramate preparations (n=6) manufactured by Johnson- Cilag (Topamax) or Arya (Topiramate) pharmaceutical companies.

### Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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