

## Development of a Stability-Indicating High Performance Liquid Chromatographic Method for the Analysis of Topiramate and Dissolution Rate Testing in Topiramate Tablets

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A stability-indicating high performance liquid chromatographic (HPLC) method was developed and validated for the quantitation and dissolution determination of topiramate in tablet dosage forms. An isocratic separation was achieved using a phenyl column with a flow rate of 1 mL/min using UV detection at 264 nm. Topiramate has low UV absorbivity and was subjected to derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl). The mobile phase for the separation consisted of acetonitrile: 50 mM sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) containing 3 % v/v triethylamine (pH 2.8) in a 48:52 v/v ratio. Topiramate was subjected to oxidation, hydrolysis, photolysis and heat for the purposes of stress testing. Separation was achieved for the parent compound and all the degradation products in an overall analytical run time of approximately 15 min with the parent compound topiramate eluting at approximately 9.2 min. The method was linear over the concentration range of 1-100  $\mu\text{g/mL}$  ( $r = 0.9996$ ) with limits of quantitation and detection of 1 and 0.3  $\mu\text{g/mL}$ , respectively.

**Key Words: Topiramate, Stability-indicating, UV-High performance liquid chromatographic (UV-HPLC), Dissolution testing.**

### INTRODUCTION

Topiramate is chemically 2,3:4,5-bis-O-(1-methylethylidene)- $\beta$ -D-fructopyranose sulfamate (Fig. 1), a sugar derivative intended for use as an antiepileptic drug. Pharmacologically the compound is characterized by a complex mechanism of action and has the ability to affect most currently known mechanisms by which seizures are induced<sup>1</sup>. Several HPLC<sup>2-10</sup>, gas chromatographic<sup>11-13</sup> and fluorescence polarization immunoassay<sup>14,15</sup> methods for the quantitative determination of topiramate in biological fluids are reported. A capillary electrophoresis method<sup>16</sup> with indirect UV detection and an ion chromatographic method<sup>17</sup> have also been reported for

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monitoring inorganic sulfate and sulfamate degradation products in topiramate dosage forms and active pharmaceutical ingredient (API). The published methods are not directly applicable to the analysis of dissolution samples collected during dissolution testing of topiramate tablets and require further investigation into method development and subsequent validation.

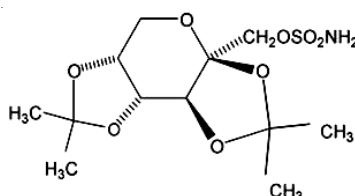


Fig. 1. Structural formulae for topiramate (MW = 339.4)

Due to the lack of an intrinsic chromophore, topiramate is not readily monitored by UV detection without appropriate pretreatment. The determination of topiramate is feasible by derivatization with chromophoric agents<sup>2</sup> and subsequent analysis by HPLC. 9-Fluorenylmethyl chloroformate (FMOC-Cl) reacts with primary and secondary amines to form apolar UV-absorbing products which can be detected by UV absorbance<sup>18</sup>. The aim of this study is to develop an HPLC-UV analytical procedure for use in the routine analysis and dissolution testing of topiramate in tablets by derivatization with FMOC-Cl. The US<sup>19</sup> and British pharmacopoeias<sup>20</sup> have yet to include an official method for the quantitation of topiramate in dosage forms or for use in dissolution testing. Furthermore the methods reported previously focused primarily on the analysis of topiramate in biological fluids. Therefore the development of an analytical method that is applicable for routine quality control of the active pharmaceutical ingredients is beneficial. The method was validated in terms of linearity, precision, accuracy, selectivity, limits of quantitation (LOQ) and detection (LOD) and was applied to the quantitation and dissolution testing of topiramate in tablets.

## EXPERIMENTAL

Topiramate standard powder (100.5 %) was kindly supplied by Janssen-Cilag (Berehem, Belgium) and was used without further purification. Topiramate tablets containing 100 mg topiramate were purchased from a local pharmacy. Topamax<sup>®</sup> (Janssen-Cilag, Berehem, Belgium) tablets were used as the reference formulation. HPLC grade acetonitrile, triethylamine, NaH<sub>2</sub>PO<sub>4</sub>, boric acid, glycine, NaOH, HCl and H<sub>2</sub>O<sub>2</sub> were obtained from Merck (Darmstadt, Germany). 9-Fluorenylmethyl chloroformate (FMOC-Cl) ( $\geq 99.0$  %) was purchased from Fluka (Buchs, Switzerland). All chemicals were at least of analytical grade and were used as received. Purified HPLC grade water was prepared by reverse osmosis and filtration through a Milli-Q<sup>®</sup> system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

**HPLC instrumentation and conditions:** The HPLC system consisted of a Waters® 600 solvent delivery with a solvent degasser, a Waters® 717 plus autosampler, a Waters® 486 tunable absorbance detector. Data was recorded, monitored and evaluated using a Millennium® chromatographic data system that was coupled to the detector via a SAT/IN Module (Waters Chromatography Division, Milford, MA, USA). Chromatographic separation was achieved using a Perfectsil® target phenyl, 250 mm × 4.6 mm i.d., 5 µm column (Mainz, Germany) and a mobile phase consisting of acetonitrile: 50 mM NaH<sub>2</sub>PO<sub>4</sub> containing 3 % v/v triethylamine (pH 2.8) in a ratio of 48: 52 v/v. Samples were injected at a constant flow rate of 1 mL/min and the analytical wavelength was set at 264 nm. The column was maintained at 52 °C and 50 µL of samples were injected onto the column. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter (Apple Valley, MN, USA) prior to use.

**Preparation of stock and standard solutions:** A stock solution of topiramate (1 mg/mL) was prepared by accurately weighing approximately 20 mg of topiramate into a 20 mL A-grade volumetric flask and making up to volume with HPLC grade acetonitrile. The stock solution was protected from light using aluminium foil and stored for 1 week at 4 °C. The stock solution was stable during this period. Aliquots of the standard stock solution were transferred using A-grade bulb pipettes into 10 mL volumetric flasks and the solutions were made up to volume with borate buffer to produce final concentrations for the analytical standards of 1, 2, 5, 20, 50 and 100 µg/mL. The borate buffer was prepared by dissolving 6.25 g of boric acid and 7.50 g of potassium chloride in 1000 mL of HPLC grade water and adjusting to a pH of 7.8 using a 1 M potassium hydroxide solution. A stock solution of glycine (10 mg/mL) was prepared by dissolving glycine in HPLC grade water. The FMOC-Cl solution (5 mg/mL) was prepared in acetonitrile and stored at 4 °C until required. Topiramate standard solutions were then subjected to derivatization with FMOC-Cl as described in the procedure described in the sample derivatization section of this manuscript.

**Preparation of tablets for analysis:** Twenty tablets were weighed, crushed and mixed in a mortar and pestle. A portion of the powder equivalent to the weight of one tablet was accurately weighed into each of six 50 mL A-grade volumetric flasks and 25 mL of acetonitrile was added to each flask. The volumetric flasks were sonicated for 15 min to effect complete dissolution of the topiramate and the solutions were then made up to volume with purified HPLC grade water. Suitable aliquots of solution were filtered through a 0.45 µm nylon filter (Chrom Tech, Apple Valley, MN, USA) and 0.5 mL of the filtered solution was quantitatively transferred to a 20 mL volumetric flask and made up to volume with borate buffer, prepared as previously described, to yield a concentration of topiramate in the linear range of the analytical method. The solution was then subjected to derivatization with FMOC-Cl as described in the procedure described in the sample derivatization section of this manuscript.

**Forced degradation studies of active pharmaceutical ingredient (API) and topiramate tablets:** In order to determine whether the analytical method and assay

were stability-indicating, topiramate tablets and topiramate API powder were stressed under various conditions to conduct forced degradation studies. Regulatory guidance provided in ICH<sup>21,22</sup> require the development and validation of stability-indicating procedures for the analysis of drugs and drug products. Unfortunately these guidance documents do not indicate the detailed conditions required to conduct stress testing studies. The experimental conditions required to conduct such stress tests are described in a general manner in the documents and the exact conditions to be applied are not explicitly stated. Furthermore the extent to which degradation should continue is also not described. Some guidelines suggest that the experimental conditions required for stress testing are dependent on the nature of the drug substance and the drug product being tested. Typically the necessary stress conditions are determined on the basis of the experience of the analyst conducting the studies or by trial and error. The conditions used to effect degradation including the concentration of stress agent used and the time of stress application was found to be effective and degradation of not less than 10 % but without complete destruction of the topiramate was achieved.

**Oxidation, acid degradation, alkali degradation and neutral degradation studies:** Solutions for oxidation, acid degradation, alkali degradation and neutral degradation studies were prepared in acetonitrile and 3 % H<sub>2</sub>O<sub>2</sub> (50:50, v/v), acetonitrile and 0.1 M HCl (50:50, v/v), acetonitrile and 0.1 M NaOH (50:50, v/v) and acetonitrile and water (50:50, /v), respectively. The resultant solutions were heated for 1 h in a water bath at 70 °C in order to accelerate the degradation of the compound. The solutions were diluted with borate buffer to yield starting concentrations of topiramate of 100 µg/mL and were then subjected to derivatization with FMOC-Cl using the conditions described in the sample derivatization section of this manuscript.

**Temperature studies:** In order to conduct temperature stress studies, 20 tablets and API powder were exposed to dry heat (90 °C) in an oven for 3 days. The tablets and API powder were removed from the oven and the tablets were weighed, crushed and mixed in a mortar and pestle. An aliquot of powder equivalent to the weight of one tablet and API powder were then prepared for analysis using the same conditions and procedures described in the preparation of tablets for analysis.

**Photostability studies:** Topiramate API, tablet powder and solutions of topiramate were prepared and exposed to light to determine the effects of light irradiation on the stability of topiramate in solution and in the solid state. Approximately 50 mg of topiramate API powder was spread on a glass dish in a layer that was less than 2 mm thick. A 1 mg/mL solution of topiramate powder was prepared in acetonitrile and water (50:50, v/v). Tablets were prepared for analysis in a similar manner to that previously described. All samples for photostability testing were placed in a Suntest CPS/CPS<sup>+</sup> light cabinet (Atlas Material Testing Technology, Linsengericht, Germany) and exposed to light for 40 h resulting in an overall illumination of  $\geq 210$  w h/m<sup>2</sup> at 25 °C with UV radiation of between 320-400 nm. Control samples that had been protected with aluminium foil were also placed in the light

cabinet and exposed at the same time as the test samples. Following exposure for the desired period, samples were removed from the light cabinet and prepared for analysis as described in the procedure in the sample derivatization.

**Dissolution test conditions:** Dissolution testing was performed using an Erweka® (Heusenstamm, Germany) Model DT-700 USP Apparatus II (paddle). Dissolution testing was undertaken by dropping a tablet ( $n = 12$ ) into a dissolution vessel containing a 900 mL dissolution medium (borate buffer, pH 7.8). The temperature of the dissolution fluid was maintained at  $37 \pm 1$  °C and the paddle was rotated at a speed of 50 rpm for 50 min. Five milliliters of the receptor medium were removed at 5, 10, 20, 30 and 45 min following the commencement of the dissolution test. Five milliliters of the fresh dissolution medium was replaced into each vessel after removal of the test sample. The samples were filtered through Whatman No. 1 filter paper and analyzed using the proposed method as described in the procedure for sample derivatization.

**Procedure of sample derivatization:** In order to derivatize the compound of interest 50  $\mu$ L Fmoc-Cl solution was added to 100  $\mu$ L of each sample and the solutions were mixed for 20 s and then incubated at 50 °C for 15 min in a water bath. In order to terminate the reaction, 10  $\mu$ L of glycine solution was added to the solution and mixture was vortexed for 10 s. The solution was allowed to stand for 1 min prior to injection, of a 50  $\mu$ L aliquot of the resultant solution, onto the chromatographic system.

## RESULTS AND DISCUSSION

**Derivatization, HPLC method development and optimization:** A derivatization procedure in which Fmoc-Cl was selected as the derivatizing agent was adapted<sup>2</sup> for the analysis of topiramate in raw material and dosage forms. The optimum conditions for the derivatization procedure included the use of a borate buffer and a reaction temperature of 50 °C with a reaction time of 15 min. It is important to note that neither the ionic strength of the reacting medium nor the buffer concentration affect the derivatization reaction<sup>18</sup>. The composition, pH and the flow rate of the mobile phase in addition to the column temperature were altered in a systematic manner in order to optimize the separation conditions using unstressed and stressed test samples. Several preliminary investigatory chromatographic runs were evaluated and optimal chromatographic separation was achieved using a Perfectsil® target phenyl, 250 mm  $\times$  4.6 mm i.d., 5  $\mu$ m column maintained at 52 °C temperature with a mobile phase mixture of acetonitrile: 50 mM NaH<sub>2</sub>PO<sub>4</sub> containing 3 % v/v triethylamine (pH 2.8) in a ratio of 48:52 v/v at a flow rate of 1 mL/min. The eluant was monitored using UV detection at a wavelength of 264 nm. The peak shape of topiramate was symmetrical under the experimental conditions described.

**Validation:** The method was validated with respect to linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and recovery<sup>23-28</sup>.

**Linearity:** The linearity of the method was evaluated by least squares linear regression analysis of three calibration curves constructed for topiramate over the concentration range of 1-100 µg/mL. The peak area of topiramate was plotted *versus* concentration and linear regression analysis performed on the resultant curve. The resultant curves were found to be linear with correlation coefficients of  $R = 0.9999$ ,  $R = 0.9995$  and  $R = 0.9996$  for the three curves and with % RSD values ranging from 0.22-3.25 % across the concentration range studied. A typical regression equation for a calibration curve was found to be  $y = 0.495X + 4.85$ .

**LOQ and LOD:** The LOQ and LOD of the analytical method were determined based on signal to noise (S/N) criteria. The respective values were found to be 1 µg/mL (S/N = 10) and 0.3 (S/N = 3) for the LOQ and LOD, respectively.

**Precision:** Repeatability and reproducibility were evaluated to determine the precision of the analytical method. Repeatability was investigated by injecting 10 replicate samples of each of the 1, 10 and 100 µg/mL standards where the mean concentrations were found to be 1.02, 9.85 and 100.75 µg/mL with associated % RSD, values of 1.85, 2.20 and 0.35 %, respectively. Inter-day precision was assessed by injecting the same three concentrations over three consecutive days, resulting in mean concentrations of topiramate of 1.03, 10.13 and 101.25 µg/mL and associated % RSD values of 3.23, 2.95 and 1.7 %, respectively.

The ruggedness of the analytical method was assessed by comparison of the intra- and inter-day assay results for topiramate following analyses undertaken by two independent analysts. The % RSD values for intra - and inter- day determinations of topiramate performed in the same laboratory by the two analysts did not exceed 3.5 %, thus indicating the ruggedness of the method. The mean retention time of topiramate was 9.2 min with a % RSD of 0.12 %.

**Accuracy:** The accuracy of the assay procedure was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards (1, 10 and 100 µg/mL) from a calibration curve prepared as previously described. In each case, the per cent relevant error and accuracy was calculated. The resultant concentrations were  $1.032 \pm 0.030$  mg/mL (mean  $\pm$  SD),  $10.152 \pm 0.22$  and  $99.60 \pm 0.35$  mg/mL with per cent relevant errors of 3.25, 1.55 and 0.43 %, respectively.

**Selectivity:** The results of stress testing studies indicate a high degree of selectivity of this method for topiramate. The degradation of topiramate was found to be similar for both the tablets and API powder. Typical chromatograms obtained following the assay of pure bulk sample and stressed samples are shown in Fig. 2.

**Recovery:** A known amount of topiramate standard powder was added to samples of tablet powder and the powders were then mixed, extracted and subsequently diluted to yield a starting concentration of 75 µg/mL and the solution was analyzed as previously described. The recovery determination was repeated (n = 9) over 3 consecutive days to obtain intermediate precision data. The observed concentration of topiramate was found to be  $74.95 \pm 1.25$  µg/mL (mean  $\pm$  SD) and the resultant % RSD value for these studies was found to be 1.67 % with a corresponding percentage recovery of 99.93 %.

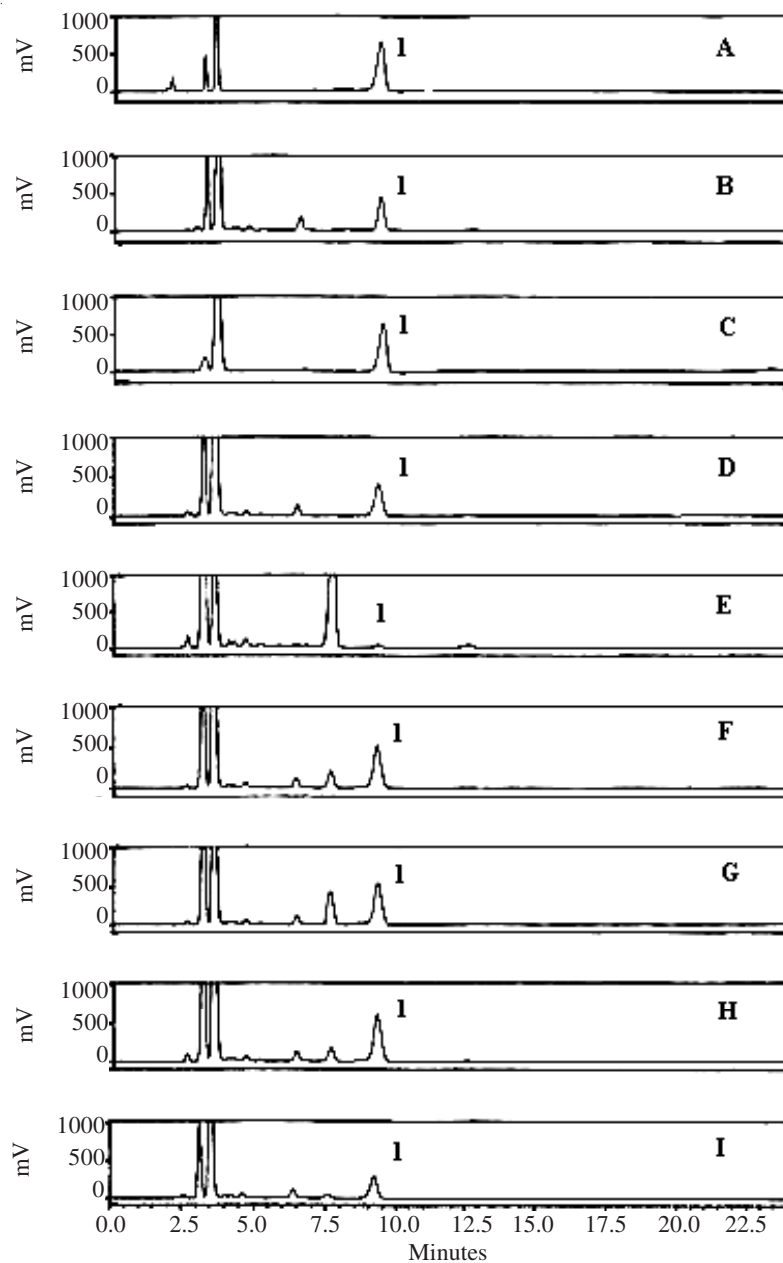


Fig. 2. Typical HPLC chromatograms of: (A) standard solution of active pharmaceutical ingredient (API) showing topiramate (1); (B) neutral-hydrolysis degraded API (1 h in a water bath at 70 °C); (C) untreated API in light cabinet(control); (D) acid hydrolysis-degraded API; (E) dry heat-degraded API; (F) neutral-hydrolysis degraded API (90 °C in an oven for 3 days); (G) photodegraded API; (H) oxidative degraded API; (I) base hydrolysis-degraded API

**Stability studies:** In the solid state and under normal storage conditions topiramate is stable. However it degrades at elevated temperatures and humidity to produce degradation and insoluble polymeric products in addition to the inorganic anions sulfate and sulfamate<sup>16,17</sup>. In these studies the drug was subjected to oxidative, hydrolytic, photolytic and heating conditions to apply stress to the molecule. All stressed samples in both the solid and solution state remained colourless following exposure to the different conditions. Topiramate was found to be unstable under dry heat conditions and more than 95 % of the drug decomposed after exposure to dry heat (90 °C) for 3 days. In spite of alkaline conditions, the drug was found to be more stable in acidic conditions and the concentration decreased by 55 % on heating for 1 h in a water bath at 70 °C. The drug was found to be more stable in light and under oxidative stress conditions. The stability of the stock solution was determined by quantitation of topiramate in the stock solution and then comparing the response to that obtained from a freshly prepared standard solution. No significant change (< 2 %) was observed in the stock solution response, relative to a freshly prepared standard solution.

**Application of the analytical method:** The dissolution profiles generated following dissolution testing under described conditions are shown in Fig. 3. It is clearly evident that more than 90 % of the drug is released after 20 min. The percentage of drug dissolved over time and the associated % RSD values are summarized in Table-1. The difference ( $f_1$ ) and similarity factors ( $f_2$ ) were found to be 2.48 and 77.94, respectively, clearly indicating the curves are similar<sup>28</sup>.

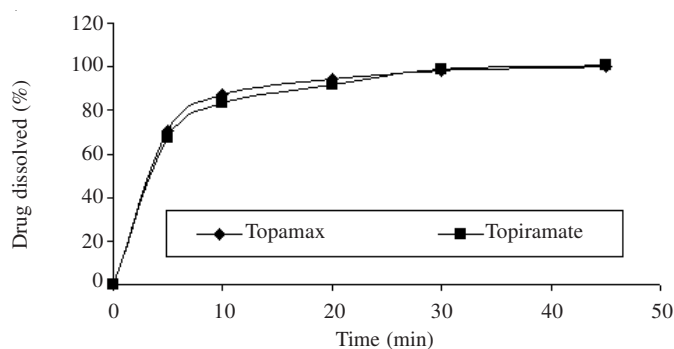


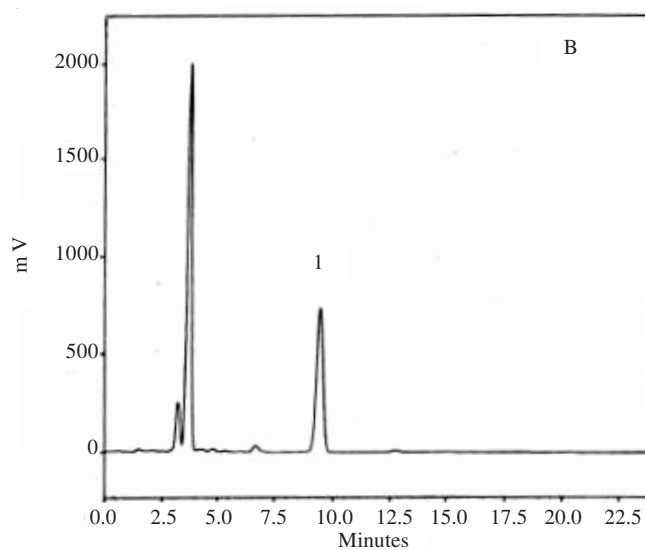
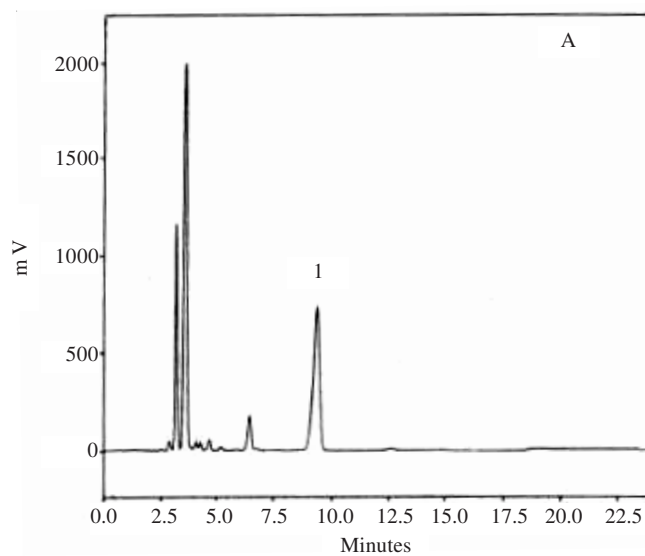
Fig. 3. Dissolution profiles of topiramate (multisource) and Topamax<sup>®</sup> (reference) tablets

The proposed method was also applied to the determination of topiramate in a multisource (generic) dosage form of topiramate and Topamax<sup>®</sup> tablets. A typical chromatogram obtained following the assay of Topamax<sup>®</sup> tablets is depicted in Fig. 4. The result of these analyses yielded 99.01 % (% RSD = 1.350 %) and 100.25 % (% RSD = 1.40 %) of label claim for the generic product and Topamax<sup>®</sup> tablets, respectively. The results of the assay and dissolution test indicate that the method is selective for the analysis of topiramate without interference from the excipients that are used in the production of these tablets.



TABLE-1  
PERCENTAGE OF TOPIRAMATE DISSOLVED IN THE DISSOLUTION TEST (n = 12),  
USING BORATE BUFFER pH 7.8 MEDIUM AT  $37 \pm 1$  °C AND SPEED OF 50 rpm

Time (min)	Drug dissolved (%), RSD (%)	
	Topiramate	Topamax®
5	70.50, 2.54	67.51, 3.15
10	87.08, 1.10	83.20, 1.45
20	94.30, 0.65	91.50, 0.53
30	98.50, 0.25	99.00, 0.17
45	100, 0.30	101, 0.43



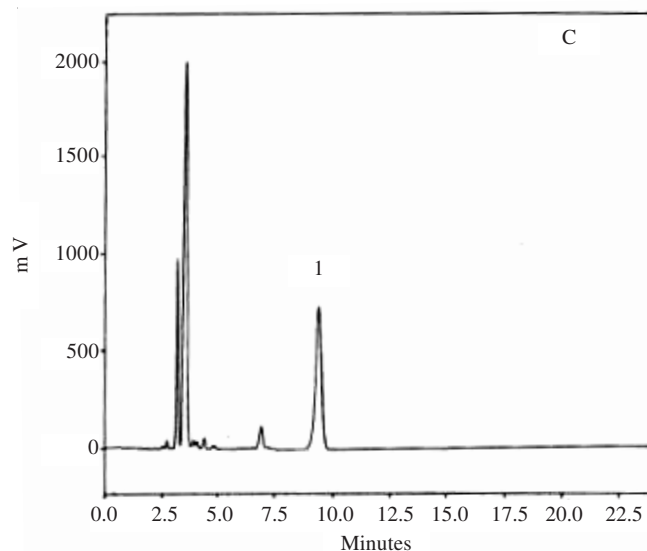


Fig. 4. Resultant HPLC chromatograms obtained following the analysis of topiramate tablets (A), topiramate standard solution (100  $\mu\text{g}/\text{mL}$ ) (B) and Topamax® tablets (C) showing topiramate (1)

### Conclusion

An accurate, precise, selective stability-indicating HPLC analytical method has been developed and validated for the determination of topiramate in API raw material, tablet dosage forms and samples collected during dissolution testing. A dissolution test procedure for application to topiramate tablets was developed using 900 mL of borate buffer (pH 7.8) and USP Apparatus II (paddle). The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The simplicity of this HPLC-UV method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS<sup>6-10</sup> and that require complicated and costly methods to successfully analyze test samples.

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### REFERENCES

1. H. Stefan and T.J. Feuerstein, *Pharmacol. Ther.*, **113**, 165 (2007).
2. Gh. Bahrami, Sh. Mirzaeei and A. Kiani, *J. Chromatogr. B*, **813**, 175 (2004).
3. Gh. Bahrami, Sh. Mirzaeei and A. Kiani, *J. Chromatogr. B*, **822**, 322 (2005).
4. Gh. Bahrami and B. Mohammadi, *J. Chromatogr. B*, **837**, 24 (2006).
5. Gh. Bahrami and B. Mohammadi, *J. Chromatogr. B*, **850**, 400 (2007).
6. S. Chen and P.M. Carvey, *Rapid Commun. Mass Spectrom.*, **13**, 1980 (1999).

7. J.A. Masucci, M.E. Ortegón, W.J. Jones, R.P. Shank and G.W. Caldwell, *J. Mass Spectrom.*, **33**, 85 (1998).
8. S. Chen and P. Carvey, *Rapid Commun. Mass Spectrom.*, **15**, 159 (2001).
9. M. Contin, R. Riva, F. Albani and A. Baruzzi, *J. Chromatogr. B*, **761**, 133 (2001).
10. M. Britzi, S. Soback, N. Isoherranen, R. Levy, E. Perucca, D. Doose, B. Maryanoff and M. Bialer, *Therap. Drug Monit.*, **25**, 314 (2003).
11. M.L. Holland, J.A. Uetz and K.T. Ng, *J. Chromatogr.*, **433**, 276 (1988).
12. J.M. Riffitts, L.G. Gisclon, R.J. Stubbs and M.E. Palmer, *J. Pharm. Biomed. Anal.*, **19**, 363 (1999).
13. P.H. Tang, M.V. Miles, T.A. Glauser, L. Coletta, N. Doughman, D. Doose, M. Frey and A. DeGrauw, *Therap. Drug Monit.*, **22**, 195 (2000).
14. D.J. Berry and P.N. Patsalos, *Therap. Drug Monit.*, **22**, 460 (2000).
15. J. Christensen, C.S. Hojskov and J.H. Poulsen, *Therap. Drug Monit.*, **24**, 658 (2002).
16. A. Klockow-Beck, A. Nick, St. Geissshuesler and D. Schaufelberger, *J. Chromatogr. B*, **720**, 141 (1998).
17. A.P. Micheel, C.Y. Ko and H.Y. Guh, *J. Chromatogr. B*, **709**, 166 (1998).
18. A. A. Descombes, J. L. Veuthey and W. Haerdi, *Fresenius J. Anal. Chem.*, **339**, 480 (1991).
19. The United States Pharmacopeia 31, Incorporating the National Formulary 26, United States Pharmacopeial Convention, Inc. Rockville, Maryland, United States of America (2007).
20. British Pharmacopoeia, The Stationery Office, London, United Kingdom (2007).
21. ICH, Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonization, IFPMA, Geneva (2005).
22. ICH, Impurities in New Drug Products (Q3B(R2)). International Conference on Harmonization, IFPMA, Geneva (2006).
23. ICH, Stability Testing of New Drug Substances and Products (Q1AR2). International Conference on Harmonization, IFPMA, Geneva (2003).
24. ICH Draft Guidelines on Validation of Analytical procedures: Definitions and Terminology, Federal Register, Vol. 60, IFPMA, Switzerland (1995).
25. A. Mohammadi, A. Mehramizi, F.A. Moghaddam, L.E. Jabarian, M. Pourfarzib and H.N. Kashani, *J. Chromatogr. B*, **854**, 152 (2007).
26. A. Mohammadi, I. Haririan, N. Rezanour, L. Ghiasi and R.B. Walker, *J. Chromatogr. A*, **1116**, 153 (2006).
27. A. Mohammadi, N. Rezanour, M.A. Dogaheh, F.G. Bidkorbeh, M. Hashem and R.B. Walker, *J. Chromatogr. B*, **846**, 215 (2007).
28. J. Moore and H. Flanner, *Pharm. Tech.*, **20**, 64 (1996).