Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF PHENTERMINE AND TOPIRAMATE IN PURE AND ITS PHARMACEUTICAL DOSAGE FORMS

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Article Received on: 30/05/14 Revised on: 21/06/14 Approved for publication: 12/07/14

DOI: 10.7897/2230-8407.0507125

ABSTRACT

Developing a single analytical method for estimation of individual drug from a multidrug composition is a very challenging task. A simple, rapid, precise, and reliable simultaneous stability indicating RP - HPLC method was developed for the separation and estimation of two drugs phentermine and topiramate in bulk drug mix and pharmaceutical dosage forms. An isocratic separation was achieved using a phenyl column with a flow rate of 1 mL/min using UV detection at 263 nm. Phentermine dose not has and topiramate has low UV absorbtivity and was subjected to derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl). The mobile phase for the separation consisted of acetonitrile: 50 mM sodium dihydrogen phosphate (NaH₂PO₄) containing 3 % v/v triethylamine (pH 2.8) in a 55:45 v/v ratio at a flow rate of 1.0 mL/min using phenyl column, and UV detection was performed at 263 nm. Total run time was 10 min; Phentermine and topiramate were eluted with retention times of 2.72 min and 6.13 min, respectively. The method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation revealed that the method is specific, rapid, accurate, precise, reliable, and reproducible. The high recovery and low coefficients of variation confirmed the suitability of the method for simultaneous analysis of the two drugs in tablet dosage form.

KEYWORDS: Phentermine, Topiramate, Analytical method development, UV-HPLC, Dosage form, Validation.

INTRODUCTION

Phentermine is chemically 2-methyl-1-phenylpropan-2-amine (Figure 1) is a psycho stimulant drug of the phenethylamine class, with pharmacology similar to amphetamine. It is used medically as an appetite suppressant. It is approved as an appetite suppressant to help reduce weight in obese patients when used short-term and combined with exercise, diet, and behavioural modification. It is typically prescribed for individuals who are at increased medical risk because of their weight and works by helping to release certain chemicals in the brain that control appetite.¹ Topiramate is chemically 2,3,4,5-bis-O-(1-methylethylidene)-d-fructopyranosesulfamate (Figure 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.¹ Several HPLC²⁻¹⁰, gas chromatographic¹¹⁻¹³ and fluorescence polarization immunoassay^{14,15} methods for the quantitative determination of topiramate in biological fluids are reported. A capillary electrophoresis method¹⁶ with indirect UV detection and an ion chromatographic method¹⁷ have also been reported for monitoring inorganic sulfate and sulfate 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. Due to the lack of an intrinsic chromophore, topiramate is not readily monitored by UV detection without appropriate pre-treatment. The determination of topiramate is feasible by derivatization with chromophoric agents² and subsequent analysis by HPLC. 9-Fluorenylmethyl chloroformate (FMOC-Cl) reacts with primary and secondary amines to form a polar UV-absorbing products which can be detected by UV absorbance¹⁸. 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 US¹⁹ and British pharmacopoeias²⁰ 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 of phentermine and topiramate in tablets.

Topiramate

Figure 1: Chemical structure

In the present work, an analytical method based on LC using detection was developed and validated for assay determination of phentermine and topiramate in formulation. The analytical conditions were selected, keeping in mind the different chemical nature of phentermine and topiramate. The development trials were taken by using the degraded sample of each component and keeping them in various extreme conditions. The column selection has been done on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between phentermine and topiramate peak. After evaluating all these factors, Pinnacle DB Phenyl (250mm x 4.6 mm i.d., 5 μ m particle size) column was found to be giving satisfactory results. The selection of buffer was based on chemical structure of both the drugs. The acidic pH

range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components.

Validation of method

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity. Accuracy was studied by measurement of recovery at five different levels 50, 75, 100, 125 and 150% of the amount expected in the formulation, in accordance with ICH guidelines²¹⁻²⁴. Precision was measured both intra-day and inter-day. In the intra-day study the concentrations of both the drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the concentrations of both the drugs were measured on three different days. The selectivity and specificity of the method were validated by injecting solutions containing both the drugs; two sharp peaks were obtained for both drugs. The limits of detection and quantitation of the method were studied to detect the lowest amount of analyte and quantitative determination of analyte in a sample, respectively.

RESULTS AND DISCUSSION

Derivatization, HPLC method development and optimization

A derivatization procedure in which FMOC-Cl was selected as the derivatizing agent was adapted for the analysis of phentermine and 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 reaction¹⁸. 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. Several preliminary investigatory chromatographic runs were evaluated and optimal chromatographic separation was achieved using a Perfectsil® target phenyl, 250 mm × 4.6 mm i.d., 5 µm column maintained at 52 °C temperature with a mobile phase mixture of acetonitrile: 50 mM NaH₂PO₄ containing 3 % v/v triethylamine (pH 2.8) in a ratio of 55:45 v/v at a flow rate of 1 ml/min. The eluant was monitored using UV detection at a wavelength of 263 nm. Under the optimum chromatographic conditions, the retention times obtained for phentermine and topiramate were 2.72 and 6.13 min, respectively (Figure 2). The peak shape of phentermine and topiramate were symmetrical under the experimental conditions described.

Figure 2: Typical Chromatogram of phentermine (2.1mins) and topiramate (6.2)

Validation

The method was validated with respect to linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and recovery.

System Suitability

The suitability of the system was studied by performing the experiment and looking for changes in separation, retention times, and asymmetry of the peaks. Five injections of the standard and two injections of the sample were injected for this purpose. The resolution, areas, retention time, theoretical plates values and peak asymmetry were calculated for standard and sample solutions. Results obtained are given in Table 1.

Table 1: Result from System Suitability Study

Parameters	Phentermine	Topiramate
Retention time	2.72	6.13
Tailing factor	1.20	1.16
Capacity factor	0.30	0.05
No of theoretical plates	8859	17719
Resolution	-	7.03

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of both the drugs. It was observed that two sharp peaks for phentermine and topiramate were obtained at retention times 2.72 and 6.13 min, respectively; these peaks were not obtained from placebo solution.

The specificity of the method was assessed by comparing chromatograms obtained from drug standards with that obtained from tablet solutions. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets.

Linearity

The linearity of the method were evaluated by least squares linear regression analysis of calibration curves constructed for phentermine and topiramate over the concentration range of 72-288 µg/mL and 12-48 µg/mL respectively. The peak area of phentermine and topiramate were plotted versus concentration and linear regression analysis performed on the resultant curve. The linear regression equations for phentermine and topiramate were: Phentermine $y = 32071x + 7785.6$ ($n = 5$; $r^2 = 0.9993$) and Topiramate $y = 16430x + 117782$ ($n = 5$; $r^2 = 0.9995$) where y is response (peak area) and x the concentration. The correlation coefficient (r^2) was calculated, and it was between 0.98 to 1.00 which is well within the acceptance criteria. The results are shown in Table 2. The concentration was found to be proportional to the area, and the response of the detector was determined to be linear over the range of 0.2 to 0.6 mg/ml.

Table 2: Linearity results

Component	Linearity range	Correlation coefficient
Phentermine	50–150%	0.997
Topiramate	50–150%	0.995

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 50, 75, 100, 125 and 150% of those expected, in accordance with ICH guidelines, by replicate analysis (n=6). Standard drug solutions were added to a pre-analyzed sample solution and percentage drug

content was measured. The results from study of accuracy are reported in Table 3. From these results it was clear that the method enables very accurate quantitative estimation of phentermine and topiramate in tablet dosage form, because all the results were within acceptable limits, that is, COV < 2.0% and S.D. < 1.0.

Table 3: Results for accuracy of the method

Drug	Amount taken mg/ml	Amount added %	Recovery (% ± SD)	COV %
Phentermine	3.75	50	100.01 ± 0.019	0.0030
		75	100.02 ± 0.018	0.0030
		100	99.97 ± 0.034	0.0021
		125	99.98 ± 0.032	0.0020
		150	100.07 ± 0.020	0.0024
Topiramate	23	50	100.02 ± 0.018	0.0030
		75	99.99 ± 0.030	0.0027
		100	99.95 ± 0.036	0.0025
		125	99.91 ± 0.037	0.0047
		150	100.02 ± 0.018	0.0030

Table 4: Results from Determination of Intra-Day and Inter-Day Precision

Component	Intra day precision (COV %)	Inter day precision (COV %)
Phentermine	0.06	0.05
Topiramate	0.343	0.321

Table 5: Results from Determination of LOD and LOQ

Component	LOD($\mu\text{g/ml}$)	LOQ($\mu\text{g/ml}$)
Phentermine	0.11	0.31
Topiramate	0.52	1.59

Table 6: % RSD at different flow rates

Flow rate	Component	Retention time RSD%	Area RSD%	Sample area RSD%
0.7ml/min	Phentermine	0.05	0.14	0.4
	Topiramate	0.04	0.09	0.22
1. ml/min	Phentermine	0.04	0.71	0.12
	Topiramate	0.06	0.57	0.03
1.5ml/min	Phentermine	0.07	0.69	0.14
	Topiramate	0.03	0.05	0.01

Table 7: RSD of the drugs on different days and different analysts

Day	Analyst	Component	Retention time % RSD	Area % RSD	Sample area % RSD
1	1	Phentermine	0.03	0.01	0.04
		Topiramate	0.08	0.02	0.02
2	2	Phentermine	0.04	0.04	0.05
		Topiramate	0.06	0.02	0.06

Table 8: Estimation of the drugs in commercial samples

Qsymia	Component	Label claim	Acquired data	Assay%
	Phentermine	3.75 mg/tab	3.71mg/tab	98.93%
	Topiramate	23 mg/tab	23.06mg/tab	100.2%

Method Precision

Precision was studied both intra-day and inter-day. Six replicate samplesolutions were prepared from the stock solution. For study of intra-dayprecision the concentrations of the two drugs were measured three timeson the same day at intervals of 1 hr. In the inter-day study the drug concentrationswere measured on three different days (Table 4).

Limits of Detection (LOD) and Limits of Quantification (LOQ)

The limits of detection and quantitation, LOD and LOQ, were calculatedby use of the equations $\text{LOD}=3.3\sigma/s$ and $\text{LOQ}=10\sigma/s$, where σ is the standard deviation of the blank and s is the slope of the calibration plot. The results are reported in Table 5.

Robustness

Due to deliberate change in the method, no changes were found in the chromatogram, the method developed is robust. The results are shown in Table 6.

Ruggedness

Data acquired and compared, % RSD of area and RT has been calculated and tabulated in Table 7. Based on the data, it is evident that the method is Rugged.

Performance of the Drug/Batch Analysis

One markete sample have been analysed to see the performance of the method. Capsules taken was Qsymia which contains Low strength 3.75 mg phentermine and 23 mg topiramate. Results obtained have been summarized in the Table 8.

CONCLUSION

The optimized chromatographic parameters of the RP-HPLC method prove to be simple, rapid and sensitive. The total run time was about 10mins with in which two drugs were separated. Linear relationship between concentration and corresponding response has been shown by proposed methods over a wide concentration range. The results of assay of drugs in formulation obtained by proposed method are quite concurrent and reproducible indicating precise and ruggedness of the method. The recoveries of the drugs were about 100% indicating accuracy of the method and non-interference of excipient states method specificity, hence the developed method can be used for routine assay of drug. From the studies it can be conclude that RP-HPLC can be successfully used for estimation of topiramate and phentermine in their combined dosage form.

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Cite this article as:

Kavitha K.Y, Geetha G, Hariprasad R, Venkatnarayana R and Subramanian G. Development and validation of RP-HPLC analytical method for simultaneous estimation of phentermine and topiramate in pure and its pharmaceutical dosage forms. *Int. Res. J. Pharm.* 2014; 5(7):613-618
<http://dx.doi.org/10.7897/2230-8407.0507125>

Source of support: Nil, Conflict of interest: None Declared