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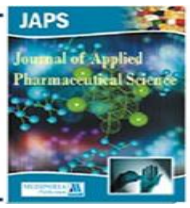

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Development and Validation of RP-HPLC-PDA Method for Simultaneous Estimation of Baclofen and Tizanidine in Bulk and Dosage Forms

Buchi N. Nalluri, K. Sushmitha, B. Sunandana and D. Prasad Babu

ABSTRACT

A simple, specific, and accurate reverse phase liquid chromatographic method was developed for the estimation of Tizanidine (TZN) and Baclofen (BCF) in combination. A Phenomenex -C_{18} (150×4.60 mm Dimensions, 5µm Particle size) column with mobile phase containing methanol: water (53:47) was used at isocratic mode and eluents were monitored at 228 nm. The retention times of TZN and BCF were 2.03 and 4.1min respectively and both the drugs showed good linearity in the concentration range of 10-50 µg/mL with a correlation coefficient (R) of 0.9992 and 0.9993 respectively. The proposed method was validated as per ICH guidelines and method showed good precision with percent relative standard deviation less than 2%. The percentage assay values of TZN and BCF were found to be 99.72 and 98.56 respectively and recovery values are within the limits of 98-102% indicating the proposed method was accurate and precise for the simultaneous estimation of TZN and BCF in bulk and pharmaceutical dosage forms.

Keywords: Simultaneous estimation, Reverse phase liquid chromatography, Validation.

Buchi N. Nalluri, K. Sushmitha, B. Sunandana and D. Prasad Babu
KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, India.

For Correspondence
Buchi N. Nalluri
Director for PG Studies and Research, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada – 520 010, AP, India.
Tel: +91-866-2493346

INTRODUCTION

TZN is centrally acting α2- adrenergic agonist, and its chemical name is 5-chloro-4-(2-imidazoline-2-ylamino)-2,1,3-benzothiodiazole hydrochloride (Seal, 1999). It acts by reducing spasticity and by increasing presynaptic inhibition of motor neurons. It is used as a central muscle relaxant (Carter, 2009). BCF is chemically (*RS*)-4-amino-3-(4-chlorophenyl) butanoic acid (Seal, 1999) a derivative of gamma-amino butyric acid (GABA)**,** and acts as a synthetic, antispastic agent (muscle relaxant). It is primarily used to treat spasticity as it decreases the frequency and amplitude of muscle spasms that arise in response to muscle stretching (Carter, 2009) and is under investigation for the treatment of alcoholism. Clinical study suggests that TZN is likely to be used in combination with other antispastic agents with different mechanism of action, such as BCF (Kent, 1998), hence we developed HPLC-PDA method for the simultaneous estimation of both drugs in bulk and dosage forms.

Some HPLC, Spectrophotometric (Sanjay Kumar, 2006) and HPTLC methods (Mei-Ling, 2003; Kaul, 2005) were reported for the estimation of TZN either alone or in combination with other drugs in biological and formulation samples and few analytical methods were reported for the analysis of BCF by LC-TMS (Goda, 2004) and HPLC (Abu, 1989; Sedat, 1985; Alain, 1996). However, there was no validated HPLC-UV/PDA method published so far for the simultaneous estimation of TZN and BCF in bulk and dosage forms. Hence, the present investigation was aimed at developing a fully validated HPLC-PDA method for the simultaneous estimation of TZN and BCF in bulk and dosage forms.

EXPERIMENTAL

Materials and Methods

BCF and TZN were supplied by Sun Pharma Laboratories, Mumbai, as gift samples. All the reagents used were of HPLC grade.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex-C_{18} column (150 × 4.6mm, 5µ).

Chromatographic Conditions

Mobile phase consisting of methanol: water (53:47) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 10µL. PDA detection was performed at 228 nm and the separation was achieved at ambient temperature.

Preparation of standard stock solution

Accurately weighed quantities (10mg each) of TZN and BCF were dissolved separately in sufficient quantity of 0.1N HCl in a 10mL volumetric flask. The volume was adjusted up to the mark with 0.1N HCl to obtain a stock solution of mg/mL each of TZN and BCF.

VALIDATION

Linearity

The linearity responses in the concentration range of 10-50µg/mL for both TZN and BCF were determined and the data was given in Table-1.

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at concentrations of 30µg/mL for both TZN and BCF. The data was given in Table 1 and shown in Fig 2.

Accuracy

Accuracy of the method was ascertained by performing recovery studies. Recovery studies were carried out by addition of standard drug solution to pre-analysed tablet sample solution at three different concentrations levels (80%, 100% and 120%) within the range of linearity. Results of recovery studies were shown in Table-1.

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with blank (diluents) and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by analyzing the purity index data. The data was shown in Figures 3&4.

System suitability

System suitability was carried out by injecting 30 µg/mL of TZN and BCF at different injection volumes in the range of 10-50 µg/mL. The data was given in Table-3, with increment of injection volumes, the %RSD for tailing factor and theoretical plate number was less than 1% and is satisfactory.

LOD and LOQ

The LOD and LOQ values were determined by the formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves) and the results were given in Table 1.

Table. 1: Linearity, Precision and Accuracy data.

	Validation data of TZN and BCF	
	TZN	**BCF**
Linearity (n=3)	Range 10-50 µg/mL y =4272x - 20522 R=0.999 R^2=0.999 LOD=0.358µg/mL LOQ=1.087µg/mL	Range 10-50 µg/mL y =14018x -56174 R=0.999 R^2=0.999 LOD=0.330µg/mL LOQ=1.001µg/mL
	Average peak area of the standard sample (%RSD)	
Precision (n=6)	337093 (0.443)	986239 (0.241)
Accuracy (n=3) Level of addition	**Mean Percent Recovery (%RSD)**	
80%	100.12 (0.83)	99.89 (1.24)
100%	99.04 (1.01)	99.45(1.15)
120%	99.51 (1.01)	99.46(0.80)

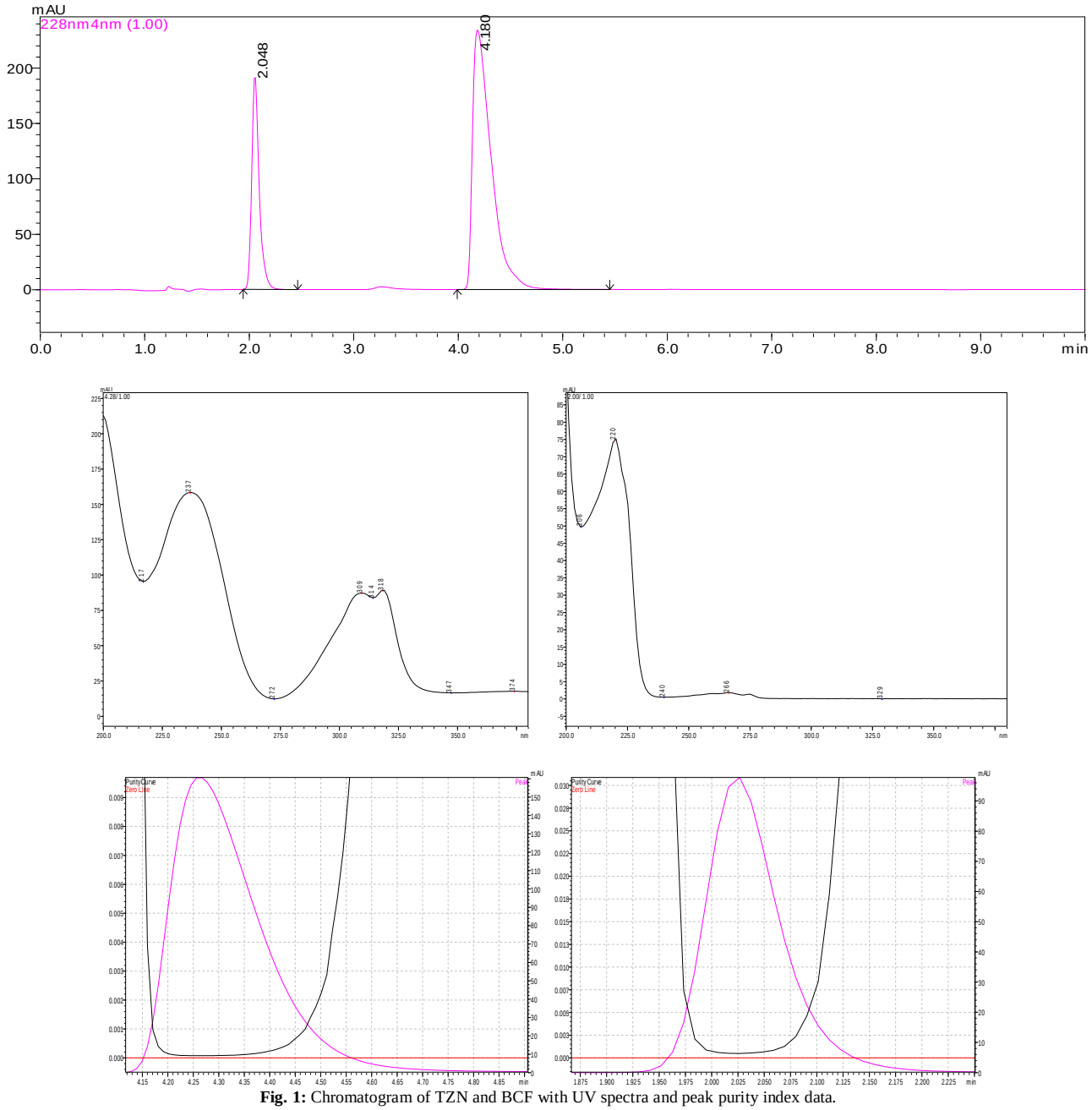

Fig. 1: Chromatogram of TZN and BCF with UV spectra and peak purity index data.

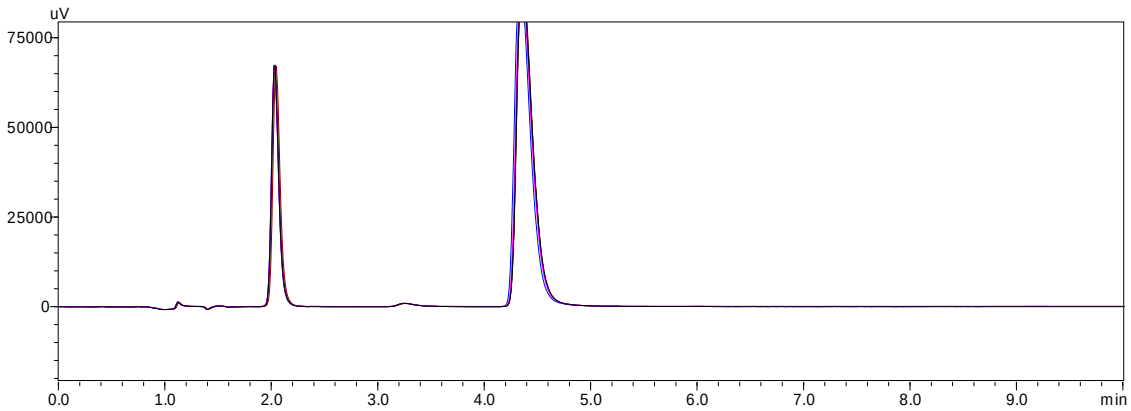

Fig. 2: Overlay chromatograms of precision data.

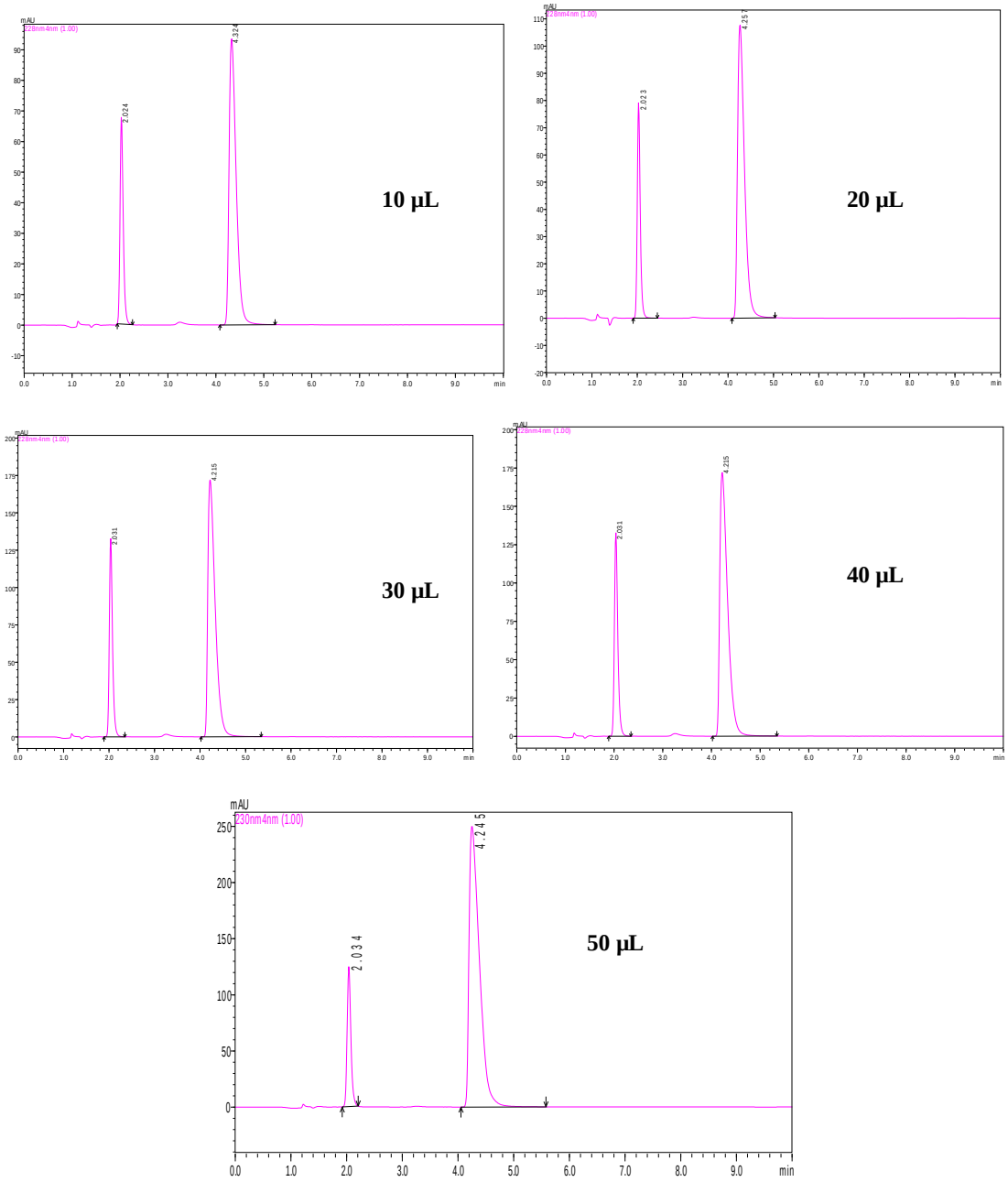

Fig. 3: Chromatograms of TIZ and BCF with increment injection volumes.

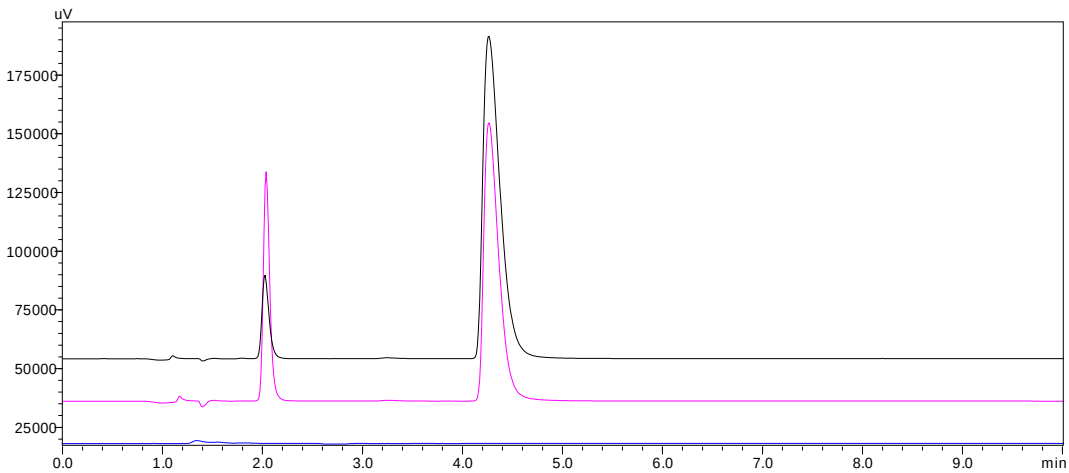

Fig. 4: Overlay of the chromatograms of A-placebo; B-standard chromatogram; C-sample chromatograms.

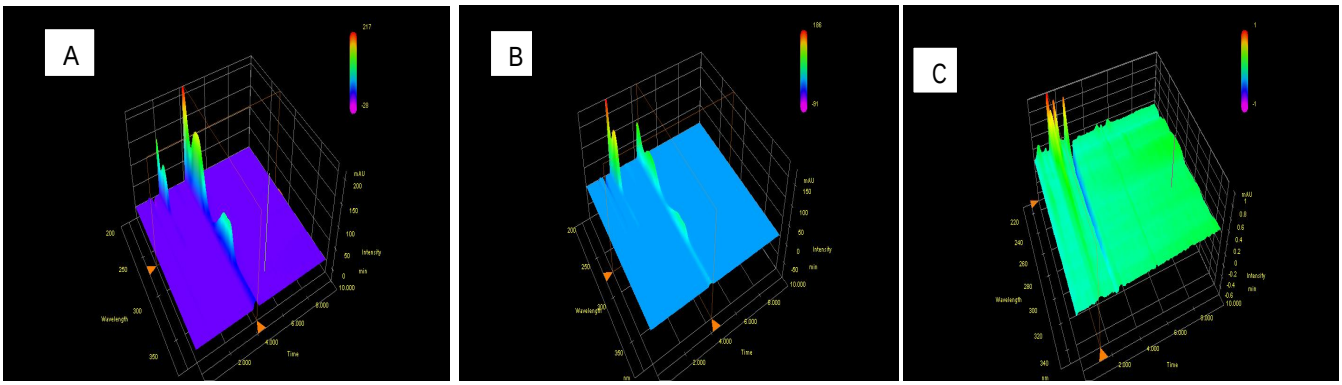

Fig. 5: 3D plots of the chromatograms of standard (A), sample (B) and placebo (C).

Assay

Twenty tablets of in house made tablets containing TZN (2mg) and BCF (10mg) were taken and crushed to fine powder. Then powder equivalent to 10mg of BCF was taken in 10mL volumetric flask and dissolved in 0.1N HCl and vortexed for 5-10min. Solution was filtered through 0.45µ nylon disc filter and the 100µL of filtrate was diluted with methanol to get a solution containing 10µg/mL of BCF and 2µg/mL of TZN. The solution was injected three times into the column. The amount present in the each tablet was calculated by comparing the areas of standards with the test samples.

RESULTS AND DISCUSSION

Method Development

Several HPLC - UV analytical methods were published for the estimation of TZN and BCF alone or in combination with other drugs in bulk and pharmaceutical dosage forms and there were no methods reported on TZN and BCF combination. Also the published methods were not economical and used higher percentages of organic solvents and retention times were longer. The aim of the present work was to develop and validate a simple, efficient, sensitive and selective method for the simultaneous estimation of TZN and BCF in bulk and dosage form. In the present investigation, initial trials were made to develop LC conditions for the separation of TZN and BCF using 0.02%v/v Formic acid (pH 2.5) as aqueous phase and methanol as organic modifier (50:50v/v) at a flow rate of 1.0 mL/min, With C_{18} Phenomenex column (250 x 4.6 mm, 5µ) at a flow rate of 1.0 mL/min and the peak eluted was broad in shape. In another trial with above mobile phase composition using Inertsil ODS- C_{18} (250 x 4.6 mm, 5µ) column the peaks were eluted before solvent front. Whereas, with the same column with change in mobile phase to Water: Acetonitrile (70:30v/v) at a flow rate of 1.0 mL/min the TZN was eluted before solvent front and BCF was at 3.25min with broad peak shape. Further trials were carried out with Methanol: Water (30:70 v/v) at 1mL/min flow rate and the drug peaks were eluted before the solvent front and resolution was not good. In another trail, C_{18} Phenomenex column (150 x 4.6 mm, 5µ) and a mobile phase composition of Methanol: Water (50:50 v/v) the resolution between peaks is not good and shape is broad for both drugs. In another trail with same column with change in mobile phase composition of (51:49 v/v), the TZN and BCF peaks were eluted at 2min and 4 min respectively. However, the peak shape of BCF is broad with peak tailing. Then by changing mobile phase composition of (53:47 v/v), a good resolution with sharp peaks were obtained (Figure 1), and these conditions were finalized for simultaneous estimation of TZN and BCF in bulk and pharmaceutical dosage forms. For quantitative analytical purpose wavelength was set at 228 nm, which provided better reproducibility with minimum or no interference.

Method validation

The method described above has been validated as per the ICH guidelines (ICH–Guidelines Q2B, Switzerland, 1996) and the results were summarized below.

Linearity

A linear relationship was evaluated across the range (10-50 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient (R^2) and correlation coefficient (R) for both the drugs were found to be >0.99 and indicating good linearity. The linearity data was given in Table 1.

Precision

Precision studies were carried out in terms of repeatability and reproducibility. Six replicate determinations were carried out and percent relative standard deviation for both the drugs was less than 2%, indicating the high degree of precision and results were given in Table 1 and shown in Figure 2.

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown and for drug substance the analyte peak is evaluated by 3D plot of the chromatogram in order to confirm the existence of single components at 2.01 and 4.02 min for TZN and BCF respectively as the impurities are not available.

The obtained recovery results were given in Table-1. The recovery of the added standard to the drug product sample was calculated and it was found to be 99.04-100.12 % for TIZ and 99.45-99.89% for BCF and the % RSD was less than 2 for both the drugs which indicates a good accuracy of the method to that of the label claim. The 3 D plots for standard chromatogram were shown in Figure 5. From the 3 D plot it is clear that the peaks eluted at 2.01 and 4.021 mins were of one component and free from impurities as confirmed by peak purity indices (Figure-5).

System suitability

System suitability was carried out by injecting 30 µg/mL of TZN and BCF at different injection volumes and the resultant chromatograms were shown in Figure 3. With increment of injection volumes, the %RSD for tailing factor and theoretical plate number was less than 1% and is satisfactory.

Specificity

Diluent, placebo, standard and sample (formulation) solutions were run individually as per the method to examine any interference. From the base shifted overlay chromatograms as shown in (Figure 4) and the 3D plots of placebo and formulation in (Figure-5), it can be inferred that there were no co eluting or interfering peaks at the retention times of TZN and BCF. This shows that the peak of analyte was pure and excipients in the formulation did not interfere with the analysis and the peak purity indices of the standard and sample peaks were greater than 0.999 and confirms the specificity of the method (Figure 5).

LOD and LOQ

LOD and LOQ were calculated from the average slope and standard deviation of y-intercepts of the calibration curve. LOD was found to be 0.0895µg/mL and 0.2505 µg/mL respectively for TZN and BCF, LOQ was found to be 0.2714µg/mL and 0.7593µg/mL respectively for TZN and BCF indicating high sensitivity of the method.

Assay

Assay of in house made TZN and BCF tablets was performed by the proposed method and the % assay of the both drugs were calculated as an average of 3 determinations, which was about 99.72 ± 0.057 and 98.5 ± 0.25 for TZN and BCF respectively. These results indicate that the present HPLC method can be successfully used for the simultaneous assay of TZN and BCF in bulk and dosage forms.

CONCLUSION

The proposed RP-HPLC - PDA method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of TZN and BCF using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of TZN and BCF without interference from diluent and placebo. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Therefore, this method can be employed in quality control to estimate the amount of TZN and BCF in bulk and in combined dosage forms.

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REFERENCES

Abu MR. Simple and rapid RP- HPLC determination of Baclofen in human plasma with UV detection: Application to a pharmacokinetic study. J Chromatogr B Biomed Sci Appl. 1989; 487; 107-115.

Alain M, Laurette M, Murielle B, Virginie G. High-Performance liquid Chromatographic determination of Baclofen in Human Plasma. J. Chromatogr. A. 1996; 729(1-2); 309-314.

Carter LP. Koek W, France CP. "Behavioural analyses of GHB: receptor mechanisms". Pharmacol Ther. 2009; 121 (1); 100-14.

Kent SM, Lesley G, Jaymin S, and Gary DN. A controlled pharmacokinetic evaluation of Tizanidine and Baclofen at steady state by American society for pharmacology and experimental therapeutics. 1998; 27(2); 201-204.

Mei-ling Qi, Peng W, Lei W. Validated Liquid chromatography method for assay of Tizanidine in drug substance & formulated products**.** Anal. Chem. Acta. 2003; 478; 171-177.

Neeraj K, Dhaneshwar SR, Himani A, Abhijit K, Bharat P. Application of HPLC and HPTLC for the simultaneous determination of Tizanidine and Rofecoxib in Pharmaceutical Dosage form. J. Pharmaceut. Biomed. Anal. 2005; 37; 27-38.

Sanjay KR, Karthikeyan C, Moorthy N, Trivedi P. New Spectrophotometric methods applied to the simultaneous determination of Diclofenac potassium and Tizanidine. Indian J Pharm Sci. 2006; 68(3); 317-322.

Goda R, Murayama N, Fujimaki Y, Sudo K Simple and sensitive LC-TMS method for the determination of S (+) - &R (-) - enantiomers of Baclofen in human plasma & CSF. J. Chromatogr. B. 2004**;** 801 (2); 257-264.

Seal SC. Martindale-The Complete drug reference, 33rd edition, Pharmaceutical press; London, (1999) 643.

Sedat T, Lale E. Determination of Baclofen in human plasma & Urine by HPLC with Fluorescence detection. Analyst. 1995; 120; 373-375.