INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received; accepted

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF DICLOFENAC POTASSIUM AND TIZANIDINE HYDROCHLORIDE IN BULK AND SOLID DOSAGE FORMS

Amit Kumar[1] , M.S. Uma Shankar[1] , Prabhsimran Singh[2]*, and Gagan Shah[3]

1. Department of Pharmaceutical Sciences, Lovely Professional University, Chaheru, Phagwara, Punjab-144402, India
2. S.B.S College Of Pharmacy, Patti, Taran- Tarn 143416 Punjab, India.
3. B.I.S College Of Pharmacy, Moga, Punjab. India.

Keywords:
Diclofenac Potassium, Tizanidine Hydrochloride, UV Spectrophotometric, Dosage forms.

For Correspondence:

Prabhsimran Singh

S.B.S College Of Pharmacy, Patti, Taran-Tarn 143416 Punjab, India

E-mail:

prabh.7750@gmail.com

ABSTRACT

A simple, accurate, precise, and sensitive and a highly selective ultra violet spectrophotometric method have been developed for the simultaneous estimation of diclofenac potassium and tizanidine hydrochloride in bulk and solid dosage form. The estimation of diclofenac potassium was carried out at 274 nm while tizanidine hydrochloride was estimated at 319.6 nm. The developed method was validated for linearity, range, precision, recovery studies and interference study for mixture. All these parameters showed the adaptability of the method for the quality control analysis of the drug in bulk and in combination formulations.

INTRODUCTION

Chemically diclofenac potassium is 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid, monopotassium salt as shown in Figure-(1)[1]. Therapeutically diclofenac potassium is indicated for treatment of primary dysmenorrhea, relief of mild to moderate pain, relief of the signs and symptoms of osteoarthritis and for relief of the signs and symptoms of rheumatoid arthritis. Diclofenac potassium is a nonsteroidal anti-inflammatory drug (NSAID) that shows preferential inhibition of the cyclooxygenase-2 (COX-2) enzyme [2].

Tizanidine hydrochloride (6-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-7-amine) (fig. 2) is an alpha-2 adrenergic receptor agonist that may reduce spasticity by increasing presynaptic inhibition of motor neurons [3-6].

The combination of both the drugs would be beneficial for the treatment of lower back pain. However the combination therapy of two drugs if they are administered in the form of a single formulation, a simultaneous estimation by simplest method of analysis like UV spectrophotometric method would be required for the simultaneous estimation of these two drugs. So it is worthwhile to pursue the present work. The quantitative estimation is the method to determine how much of each constituent is in the sample. Estimation of a given drug or medicine in the dosage forms needs the quantitative analysis of that drug or medicinal in it [7-8].

In this simultaneous estimation is carried out with the spectrophotometric analysis, in which a source of radiation is used that extends into the ultraviolet region of the spectrum. From this, definite wavelengths of radiation are chosen possessing a bandwidth of less than 1 nm. This process necessitates the use of a more complicated and consequently more expensive instrument. All atoms and molecules are capable of absorbing energy in accordance with certain restrictions; these limitations depend upon the structure of the substance. Energy may be furnished in the form of electromagnetic radiation (light). The kind and amount of radiation absorbed by a molecule depend upon the structure of the molecule, the amount of radiation absorbed also depend upon the number of molecules interacting with the radiation.

MATERIALS & METHODS

MATERIAL USED

Instrumentation

A Systronic UV-Visible Spectrophotometer 2203, with 1 cm matched quartz cell was used for the absorbance measurement over the range of 270-330 nm.

Reagents and Chemicals

Samples of Diclofenac potassium and Tizanidine hydrochloride used in this study were gifted by Aarti drugs pvt. ltd, India and Ipzah pharmaceutical pvt. ltd., India respectively. All the reagents and chemicals used were of analytical grade.

METHODS

Determination of wavelength and calibration graph

Standard stock solution was prepared by dissolving diclofenac potassium in methanol to make final concentration of 1000 µg/ml. Different aliquots were taken from stock solution and diluted with pH 6.8 phosphate buffer to prepare the series of concentration from 2-34 µg/ml. The same procedure was used for tizanidine hydrochloride vice versa but drug was dissolved in water and dilutions from stock solution were prepared from 2-20 µg/ml using pH 6.8 phosphate buffer. The absorption λmax for both the drugs were measured and the calibration curves were prepared by plotting absorbance versus concentration of the drugs.

Effect of solvent on wavelength of both the drugs

The effect of solvent on λmax of both the drugs was carried out by dissolving tizanidine hydrochloride and diclofenac potassium in 100 ml mixture of methanol and water (1:1).

Validation of analytical method

The absorbances of sample solutions of diclofenac potassium and tizanidine hydrochloride were measured at 274.6 nm and 319.6 nm respectively. The results were calculated by the following formula using Vierodt's method [9-10].

$A1 = ax1\ Cx + ax2\ Cy$ at 274.6 nm

$A2 = ay1\ Cx + ay2\ Cy$ at 319.6 nm

Where, A1 and A2 are absorbance of diluted mixture of drugs at 274.6 nm and 319.6 nm respectively, Cx and Cy are the concentration of diclofenac potassium and tizanidine hydrochloride respectively (µg/ml), ax1 and ax2 are absorptivities of diclofenac potassium at 274.6 nm and 319.6 nm respectively, ay1 and ay2 are absorptivities of tizanidine hydrochloride at 274.6 nm and 319.6 nm respectively.

Linearity and range

The linearity of an analytical procedure is its ability to obtain test results and directly proportional to the concentration of analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample. It has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Accuracy and Precision

Accuracy and precision (repeatability and intermediate precision) were investigated by analyzing three concentrations of diclofenac potassium and tizanidine hydrochloride mixtures in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). Intra-day and Inter-day relative standard deviation were calculated[11].

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were evaluated from the calibration curves plotted in concentration range of 2-34 µg/mL for diclofenac potassium and 2-20 µg/mL for tizanidine hydrochloride, with formula LOD = 3.3 S.D/S and LOQ = 10 S.D/S (where S.D = Standard Deviation and S= slope of the calibration curve). The LOD and LOQ for each drug were thus obtained[11].

Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by the standard addition technique of drug with excipients. A known amount of tizanidine hydrochloride and diclofenac potassium was added to the preanalyzed sample solution of drug with excipients in the three different concentrations. Percentage recoveries of three concentrations were calculated[11].

RESULTS AND DISCUSSION

Statistical evaluation of analysis was carried out. The data obtained from the proposed method showed suitability of method. The values of relative standard deviation were satisfactorily low (≤ 2).

Determination of wavelength and calibration graph

The λmax of diclofenac potassium was found to be 274.6 nm nm in pH 6.8 phosphate buffer (fig.4).The absorbance was measured at 274.6nm against pH 6.8 phosphate buffer as a blank. The calibration curve was prepared by plotting absorbance versus concentration of drug (fig. 5).

The λmax of tizanidine hydrochloride was found to be 319.6 nm in pH 6.8 phosphate buffer (fig.3).The absorbance was measured at 319.6 nm against pH 6.8 phosphate buffer as a blank and the calibration curve was prepared (fig. 6).

Effect of solvent on wavelength of both the drugs

No shift in λmax of both the drugs was observed (Fig. 7 and 8)

Results for validation of analytical method

The method was validated with respect to linearity and range, accuracy and precision, limit of detection and limit of quantitation, and recovery studies.

Linearity and Range

The prepared aliquots of diclofenac potassium (2-34 µg/ml) were scanned for absorbance at 274.6 nm. The absorbance range was found to be 0.067-1.030. These aliquots obeyed Beer-Lambert's law with correlation coefficient (R^2) of 0.9996 and for tizanidine hydrochloride the aliquots prepared (2-20 µg/ml) were scanned at 319.6 nm. Absorbance range was found to be 0.112-0.985 with correlation coefficient of 0.9996 (Fig 6). The optical characteristics are shown in Table 1.

Accuracy and precision

The low RSD values obtained for repeatability and intermediate precision indicated good precision of the method. The data evaluated has been summarized in Table 2 and Table 3.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) calculated for diclofenac potassium was found to be 0.4234 and 1.2828 respectively and for tizanidine hydrochloride 0.3085 and 0.9349 respectively.

Recovery Studies

The percentage recoveries of three concentrations (10, 14 and 18 µg/ml) were found within the limit, indicative of high accuracy. The high percent recoveries indicate no interference from ingredients and excipients that might be present in formulation. Data of recovery studies are summarized in Table 4 and Table 5.

CONCLUSION

The developed spectrophotometric method was validated for simultaneous estimation of diclofenac potassium and tizanidine hydrochloride using linearity and range, accuracy and precision and recovery studies. The relative standard deviations for all parameters were found to be less than two, indicated the validity of method. The assay results obtained by this method were fair agreement. So the developed method can be used for routine quantitative simultaneous estimation of diclofenac potassium and tizanidine hydrochloride in various dosage forms.

ACKNOWLEDGEMENTS

Authors of the present study are thankful to Aarti Drugs Pvt. Ltd., India and Ipzah Pharmaceutical Pvt. Ltd., India for providing gift sample of Diclofenac Potassium and Tizanidine Hydrochloride respectively.

Table 1: Optical characteristics of Diclofenac Potassium and Tizanidine Hydrochloride

S.No.	Parameters	Diclofenac Potassium	Tizanidine Hydrochloride
1	Wavelength (λmax)	274.6	319.6
2	Beer's limit (µg/ml)	2-34	2-20
3	Correlation coefficient (R^2)	0.9996	0.9996
4	Slope	0.0304	0.0492

Table-2: Results of Repeatability of Diclofenac Potassium and Tizanidine Hydrochloride

S.No.	Conc. of DP taken (µg/ml)	Conc. of DP observed (µg/ml) ± S.D	% recovery of DP	Conc. of TH taken (µg/ml)	Conc. of TH observed (µg/ml) ± S.D	% recovery of TH
1	10	9.94±0.080	99.43	10	9.95±0.077	99.53
2	14	13.95±0.070	99.67	14	13.99±0.045	99.95
3	18	17.94±0.061	99.68	18	17.97±0.075	99.83

Each value is average of three determinations

R.S.D of diclofenac potassium = 0.55

R.S.D of tizanidine hydrochloride = 0.5

Table-3: Results of Intermediate Precision of Diclofenac Potassium and Tizanidine Hydrochloride

S.No.	Drug	Conc. of drug taken (µg/ml)	Average conc. found in Intra days studies (µg/ml) ± S.D	Average conc. found in Inter days studies (µg/ml) ± S.D
1	Diclofeanc Potassium	10	10.45±0.05	10.39±0.035
2	Tizanidine HCl	10	9.71±0.055	9.79±0.015

Each value is average of three determinations

R.S.D. of diclofenac potassium = 0.4815 (Intra days) and 0.3378 (Inter days)

R.S.D. of tizanidine hydrochloride = 0.5670 (Intra days) and 0.1559 (Inter days)

Table-4: Results of recovery studies for Diclofenac Potassium

S.No.	Excipients added	Conc. of DP taken (µg/ml)	Conc. of TH added (µg/ml)	Conc. observed (µg/ml) ± S.D
1	HPMC (50cps) 35 mg	10	10	10.44±0.0305
2	EC 100 mg	10	14	9.77±0.0351
3	Sod. CMC 35 mg	10	18	9.74±0.0152

Each value is average of three determinations

R.S.D = 0.2695

Table-5: Results of recovery studies for Tizanidine Hydrochloride

S.No.	Excipients added	Conc. of TH taken (µg/ml)	Conc. of DP added (µg/ml)	Conc. observed (µg/ml) ± S.D
1	HPMC (50cps) 35 mg	10	10	9.68±0.020
2	EC 100 mg	10	14	9.77±0.036
3	Sod. CMC 35 mg	10	18	10.03±0.0251

Each value is average of three determinations

R.S.D = 0.0278

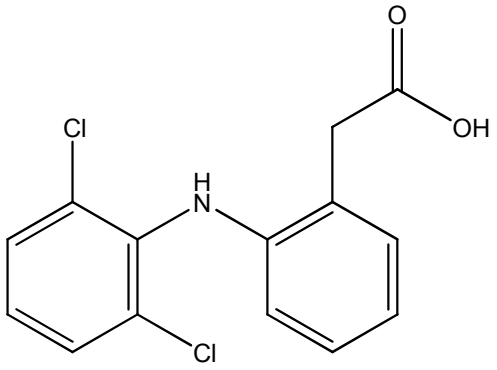

Fig.-1: Chemical structure of Diclofenac Potassium

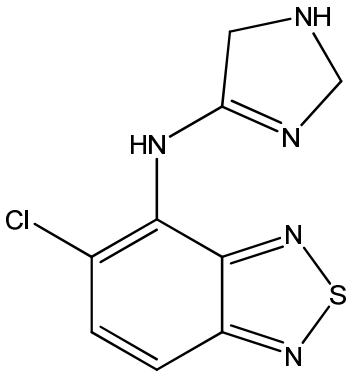

Fig.-2: Chemical structure of Tizanidine Hydrochloride

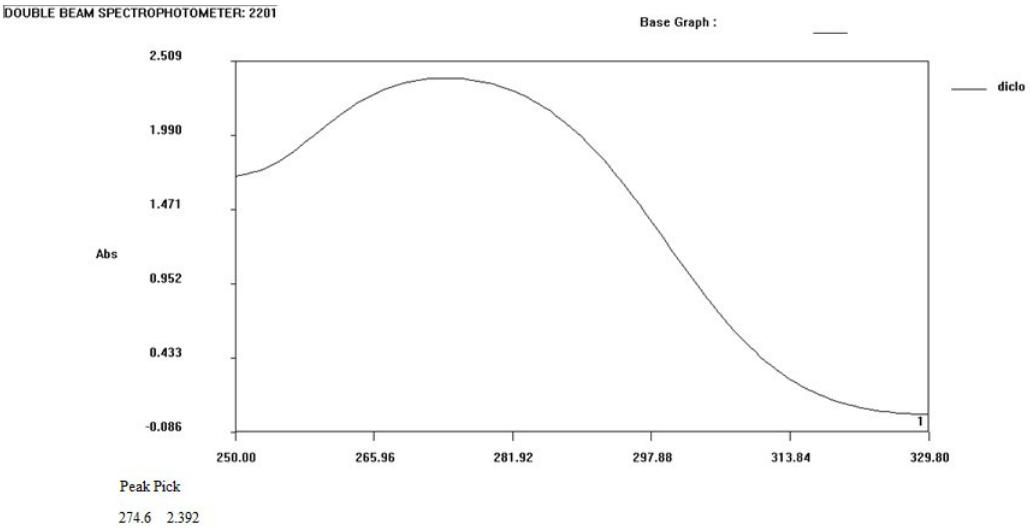

Fig.-3: UV Absorption Spectra of Diclofenac Potassium

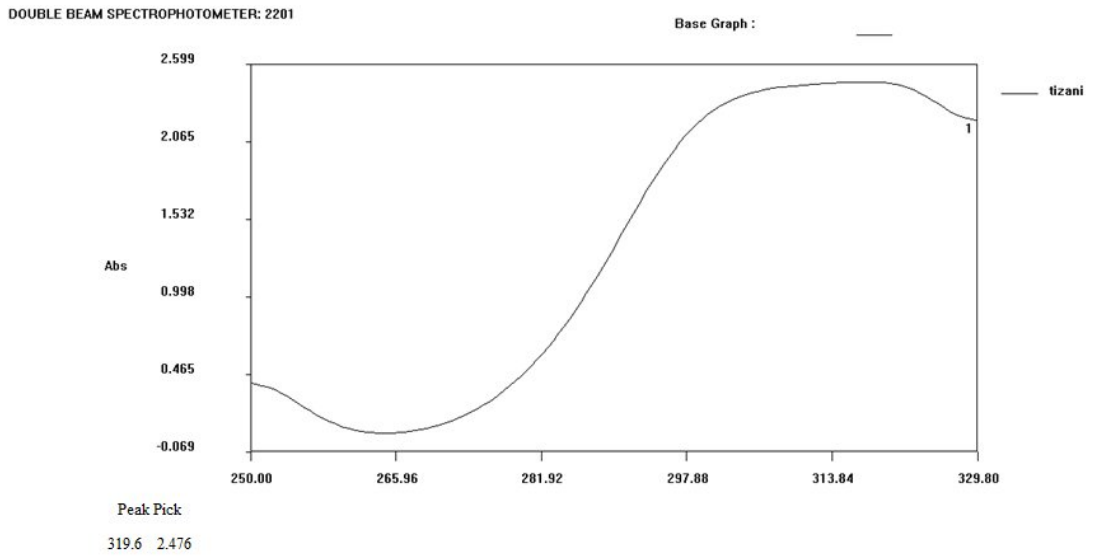

Fig.-4: UV Absorption Spectra of Tizanidine Hydrochloride

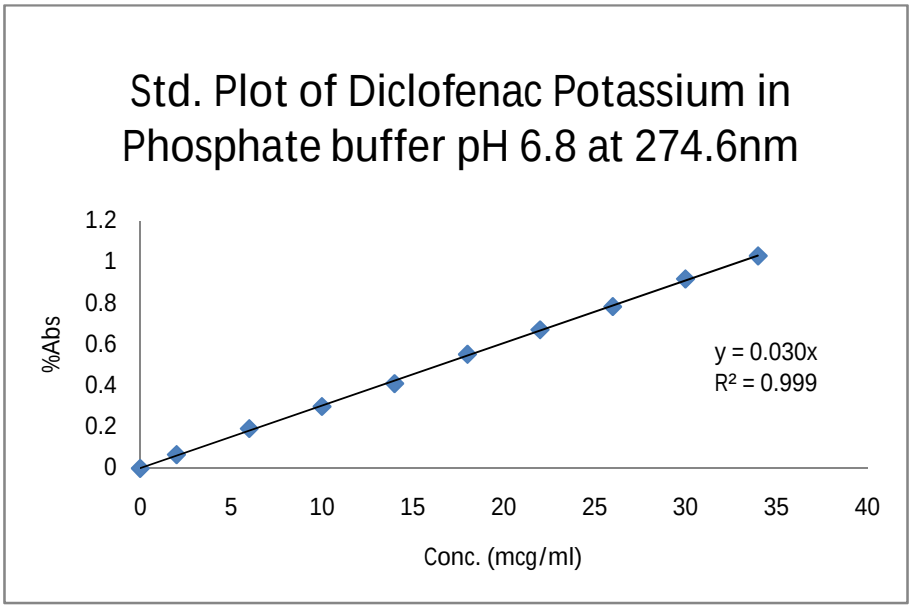

Fig.-5: Calibration graph of Diclofenac Potassium at 274.6 nm

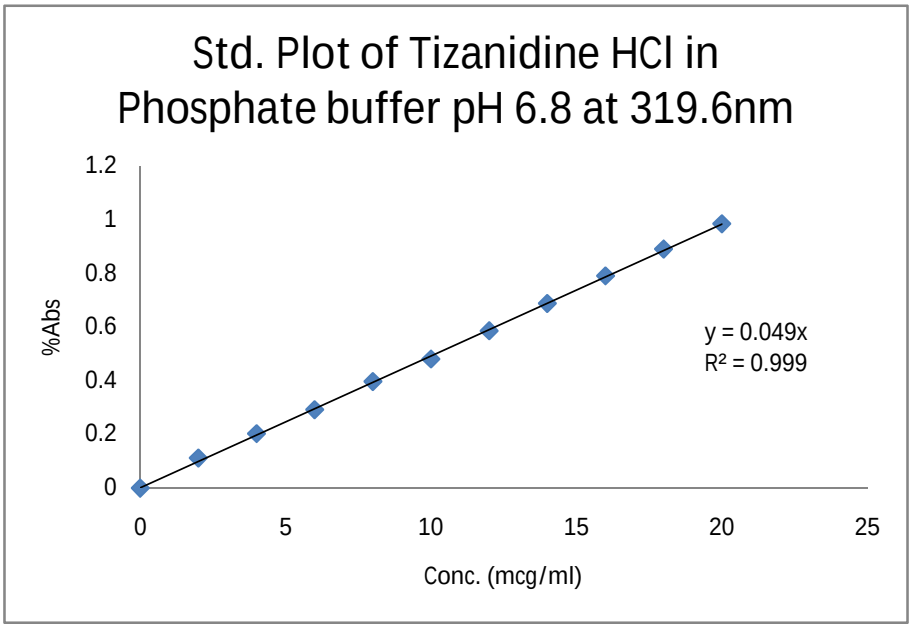

Fig.-6: Calibration graph of Tizanidine Hydrochloride

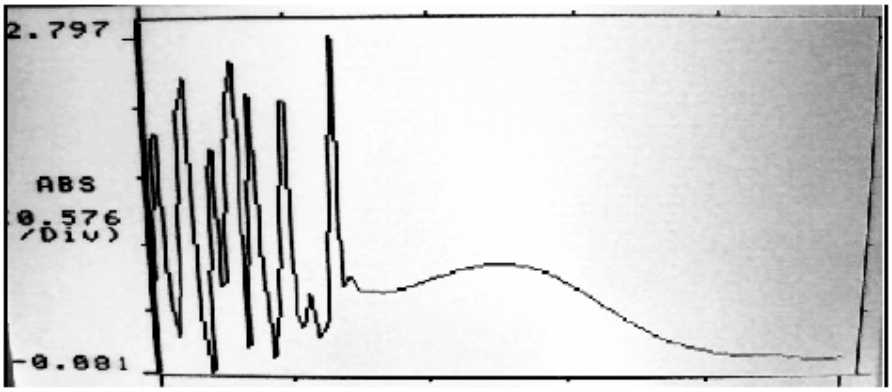

Fig.-7: Effect of solvent on λmax of Diclofenac Potassium

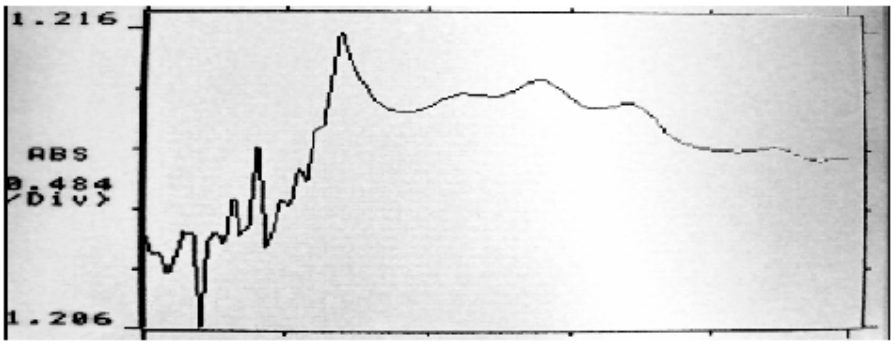

Fig.-8: Effect of solvent on λmax of Tizanidine Hydrochloride

REFERENCES

1. Hinz B, Rau T, Auge D, et al. Aceclofenac spares cyclooxygenase 1 as a result of limited but sustained biotransformation to diclofenac. Clin Pharmacol Ther. 2003;74:222–35.

2. D. E. Furst and S. H. Dromgoole, in *Drugs for Rheumatic Disease*, ed. H. E. Paulus, D. E. Furst and S. H. Dromgoole, Churchill Livingstone, New York, 1987, ch. 20.

3. Nance PW, Bugaresti J, Shellenberger K, the North American Tizanidine Study Group. Efficacy and safety of tizanidine in the treatment of spasticity in patients with spinal cord injury. Neurology 1994;44:S44–51.

4. United Kingdom Tizanidine Trial Group. A double blind, placebo-controlled trial of tizanidine in the treatment of spasticity caused by multiple sclerosis. Neurology 1994;44:S70–8.

5. Wagstaff AJ, Bryson HM. Tizanidine: a review of its pharmacology, clinical efficacy, and tolerability in the management of spasticity associated with cerebral and spinal disorders. Drugs 1997;53:435–52.

6. Acorda Therapeutics, Inc.. Zanaflex® (tizanidine hydrochloride) tablets and capsules prescribing information. Hawthorne: Acorda Therapeutics; 2006.

7. S.Sayed, A.Thomas, Journal Of Pharmacy Research, 2009(2) 1485.

8. R.S.Kumar, C.Karthikeyan, N.S.H.N.Moorthy, P.Trivedi, *Indian Journal of Pharmaceutical Sciences*, 2006,68, 317.

9. ICH Q2A, Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines (1994).

10.Vasudevan, R. and Mathai, I. M., Ind. J. Chem**.,** 1972, 10, 175.

11.Vasudevan, R., Subramanian, P. S. and Mathai, I. M., J. Ind. Chem. Soc**.,** 1984, 61, 395.