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Validated RP-HPLC method for the simultaneous determination of diclofenac potassium and tizanidine hydrochloride in tablet dosage form

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ABSTRACT

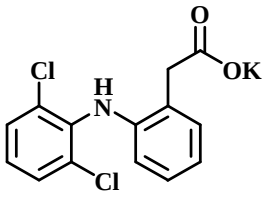
A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Diclofenac Potassium and Tizanidine Hydrochloride in tablet dosage forms. A hypersil C_{18} Octa Decyl Silane (ODS) column 250mm×4.6mm i.d, 5µm particle size in isocratic mode, with mobile phase Acetonitril and Phosphate Buffer in the ratio of 55:45 v/v at pH -7 were used. The flow rate was 1.5 ml/min and effluents were monitored at 235 nm. The Retention time of Diclofenac Potassium and Tizanidine HCl were 4.75 and 8.78 respectively. The Linearity for Diclofenac Potassium and Tizanidine HCl were in the range of 10-50µg/ml and 0.2-1.0µg/ml respectively. Results of the analytical method were validated statically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

KEYWORDS

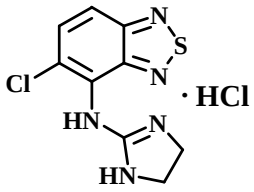
Diclofenac potassium;
Tizanidine HCl;
RP-HPLC.

INTRODUCTION

Diclofenac Potassium and Tizanidine HCl combination is used clinically for its NSAID properties. Diclofenac K is chemically 2-[(2,6-dichlorophenyl) amino] benzeneaceticacid, monopotassium salt and Tizanidine HCl is 5-chloro-4-(2-imidazoline-2-ylamino)-2,1,3-benzothiodiazole hydrochloride.



Diclofenac potassium



Tizanidine HCl

The mechanism of action of Diclofenac Potassium is not completely under-stood but may be related to prostaglandin synthetase inhibition. Tizanidine is an agonist at α2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. On detailed literature survey, it was found that these drugs have been estimated individually and in combinations by various methods[1-11]. Besides, UV method for simultaneous estimation of this combination was reported[1] In this communication we report a new RP-HPLC method for simultaneous estimation of Diclofenac Potassium and Tizanidine HCl from Tablet dosage form, which is simple, rapid and precise.

EXPERIMENTAL

Instrumentation

A Shimadzu LC system comprising pump (model:

SPD-10 ATVP) with Hamilton 100 naduz schweiz Rheodyne valve with injector 20µl fixed loop comprising with PDA detector was used for the analysis. The column used was C18 column 250×4.6mm, the mobile phase was pumped at a flow rate of 1.5 ml/min and the detection was performed at 235 nm. The separation was carried in isocratic mode at ambient temperature.

Chemicals and reagents

- Acetonitrile HPLC grade
- Phosphate buffer
- Diclofenac Potassium working standard
- Tizanidine HCl working standard

Preparation of mobile phase

Acetonitrile HPLC grade was mixed with buffer solution in the ratio of 55:45 v/v and the pH was adjusted to 7.0, which was then filtered through membrane filter of 0.45µ millipore membrane filter before use and degassed in an ultrasonic bath.

Standard stock solution

About 100 mg of Diclofenac Potassium and 20mg of Tizanidine HCl were transferred to a 100ml volumetric flask, dissolved with Acetonitrile and buffer solution and made up to 100ml with Acetonitrile and buffer solution. From this 5ml is pipetted out into a 25ml volumetric flask, and made up the volume to give a concentration of 200mcg/ml.

Working standard solution

2.5, 5.0, 7.5, 10.0, 12.5ml of Diclofenac Potassium was taken from the above solution and made up to 50ml with mobile phase to give 10, 20, 30, 40, 50µg/ml concentrations of Diclofenac Potassium. 0.5 ml of Tizanidine HCl was taken and made up to 10ml with mobile phase to give 10µg/ml of Tizanidine HCl. From this 0.2, 0.4, 0.6, 0.8, 1.0ml of Tizanidine HCl is taken and made up to 10 ml with mobile phase to give 0.2, 0.4, 0.6, 0.8, 1.0µg/ml of Tizanidine HCl.

Sample stock solution

Twenty tablets (Tizaran) were finely powdered and powder equivalent to 100 mg was accurately weighed and transferred to a 100ml volumetric flask .The Acetonitrile and Buffer solution were added and sonicated for 15 minutes, made up to 100ml with solvent. This solution was filtered through a membrane filter, first 10ml of the solution is discarded. Then 5ml of the solution is pipetted out into a 25ml volumetric flask, and made up to 25ml with Acetonitrile and Buffer solution.

RESULTS AND DISCUSSION

The method was chosen after several trails with Acetonitril and Buffer solution in various proportions and at different pH values. A mobile phase consisting of Acetonitrile and Ortho-phosphoric acid in the ratio 55:45 v/v was selected to achieve maximum separation and sensitivity. The effects of flow rates in range 0.5 to 2.0 ml/min were examined where the flow rate1.5 ml/min gave an optimal signal to noise ratio with reasonable separation time. The overlain spectra of Diclofenac Potassium and Tizanidine HCl in mobile phase showed isoabsorptive at 235nm. Hence the detection was selected at 235nm. Using reverse phase C_{18} column, the retention time of Diclofenac Potassium and Tizanidine HCl were found to be 4.75 min and 8.78 min respectively. The total time of analysis was less than 10minutes.

Estimation method

The chromatogram of samples extracted from tablets were observed where the peaks showed retention times at 4.76 min for diclofenac potassium and 8.78 min for tizanidine HCl.

Experimental results of the amount of drugs in marketed tablets (TIZARAN) expressed as % of label claim were in good agreement with label claims, there by suggesting that there is no interference from any excipients which are normally present in tablets. The average drug content was found to be 50.29 mg for Diclofenac Potassium and 2.024 mg for Tizanidine HCl which also depicts datas for statistical validation such as percentage relative standard deviation.

Validation parameters

Linearity

Linearity of detector response shows the linear relationship between the concentration and the detector response. The coefficient of correlation of Diclofenac Potassium and Tizanidine HCl was found to be 0.9999 and 0.9974.

Precision

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The obtained precision values obtained by performing with two different analysts were found to be present with in the specified limits. The values were expressed in terms of % RSD which were found below 2%.

Accuracy

The accuracy of the method was established by using the sample addition method. The recovery of the added samples was found at five different concentrations levels for each drug. i.e. 50mcg/ml to 150mcg/ml of Diclofenac Potassium and Tizanidine HCl. The average recovery for Diclofenac potassium was found to be 100.20% and 100.12% for Tizanidine HCl.

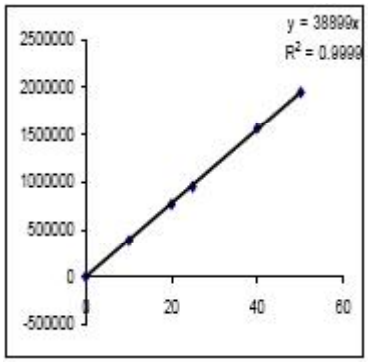
Specificity

No peaks were obtained while running the solution containing placebo ingredients which proves that the method is specific.

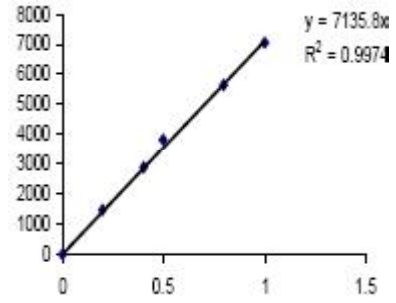
System suitability parameters

- Retention time for Diclofenac Potassium and Tizanidine HCl are 2.71 and 4.7 respectively.
- Tailing factor for Diclofenac Potassium and Tizanidine HCl are 1.12 and 2.08 respectively.
- Number of theoretical Plates for Diclofenac Potassium and Tizanidine are 13312 and 6659 respectively.
- RSD for Diclofenac Potassium and Tizanidine HCl are 0.0667 and 0.0564 respectively. The peak is reasonably symmetrical. High numbers of theoretical plates indicate efficient performance of the column. These parameters represent specificity of the method.

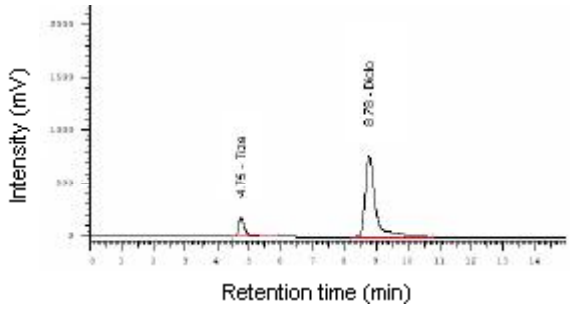
Parameters	Diclofenac potassium	Tizanidine HCl
Linearity	10-50 µg/ml	0.2-1.0µg/ml
STD DEV of precision	0.1909	0.3256
Mean % recovery	100.20%	100.12%
Retention time	8.77 min	4.75 min
Tailing factor	1.12	2.08
RSD	0.0667	0.0564
No of theoretical plates	13312	6659
Resolution	3.23	-



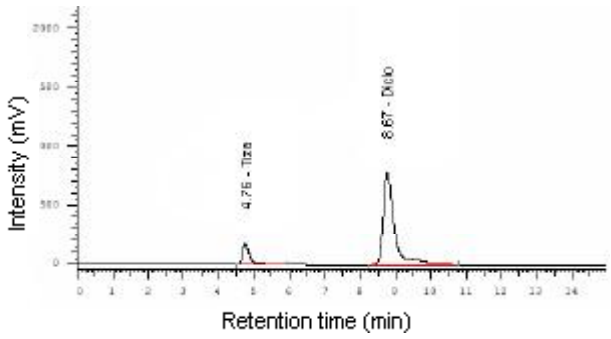
Linearity of diclofenac potassium



Linearity of Tizanidine HCl



Chromatogram of Diclofenc Potassium and Tizanidine HCl at 235 nm



Chromatogram of Diclofenac potassium and Tizanidine HCl for Recovery studies

CONCLUSION

In this present study an attempt has been made to develop an analytical method for the simultaneous estimation of Diclofenac Potassium and Tizanidine Hydrochloride in combined tablet dosage form.

The present combination of Diclofenac Potassium and Tizanidine Hydrochloride was marketed as one formulation.

Diclofenac Potassium - 50mg /tab; Tizanidine Hydrochloride - 2mg/tab

The fixed dose combination tablet of Diclofenac Potassium and Tizanidine Hydrochloride was subjected to simultaneous estimation by RP-HPLC method.

The proposed HPLC method was validated by evaluation of the validation parameters. The LOD, LOQ values, relative standard deviation of slope, correlation coefficient, with in and between day reproducibility, resolution and tailing factors for this techniques were obtained. Assay was performed with in a short analysis time. Assay parameters used in this study reduced tailing for all peaks and improved the resolution.

Highly reliable and cost efficient HPLC method was developed for the quantitative estimation of Diclofenac Potassium and Tizanidine Hydrochloride in combined tablet dosage form.

The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained.

From the forgoing it is concluded that the method developed is simple, rapid, selective and precise hence suitable for application in routine analysis of pharmaceutical preparations.

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