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Simultaneous RP HPLC determination of aceclofenac and tizanidine in pharmaceutical preparations

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ABSTRACT

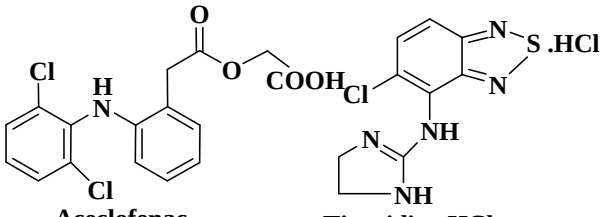
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of aceclofenac and tizanidine using paracetamol as an internal standard. Chromatographic separation of the three drugs were performed on a hypersil C_{18} column (250mm × 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of mix phosphate buffer pH 7.0: acetonitrile (40:60 v/v), at a flow rate of 0.7mL min^{-1} and UV detection at 230nm. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 100-300 - µg mL^{-1} for aceclofenac and 2-6 - µg mL^{-1} for tizanidine hcl equivalent to tizanidine. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Aceclofenac;
Tizanidine HCl.

INTRODUCTION

Aceclofenac {[2-(2', 6'-dichlorophenyl) amino] phenyl acetoxyacetic acid} is a new phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties and improved gastric tolerance[1]. Tizanidine HCl is 5-chloro-4-[2-imidazolin-2-yl-amino]-2, 1,3-benzothiadiazole. It is used as skeletal muscle relaxant[2]. The structures of these two drugs are shown in figure 1. One such combination contains 100mg of aceclofenac and 2 mg of tizanidine hcl equivalent to tizanidine. It is widely used as Anti-inflammatory- Analgesic. The literature revealed no method was available for simultaneous determination of these two drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed for determination of aceclofenac and tizanidine from their combined dosage form[5-10]. The method described is simple, fast, precise and accurate for simultaneous determination of aceclofenac and tizanidine from pharmaceutical preparation using paracetamol as an internal standard.



Aceclofenac ($C_{16}H_{13}Cl_2NO_4$) **Tizanidine HCl** ($C_9H_8ClN_5S$. HCl)

Figure 1: Structures of aceclofenac and tizanidineHCl

Chemicals and reagents

Standards were supplied from J.B. Chemicals and pharmaceutical Ltd., Mumbai, India. Starmoto-TZ tablets manufactured by Minova life sciences, India was

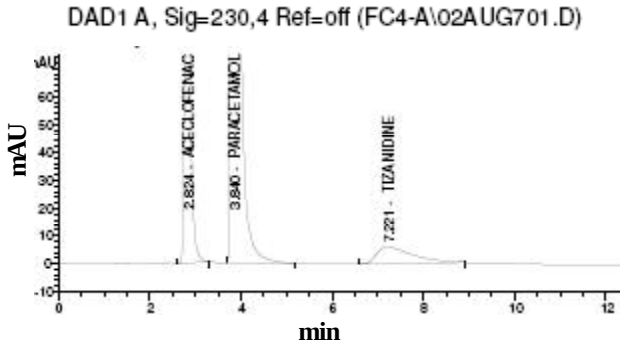


Figure 2: Chromatogram of aceclofenac, paracetamol and tizanidine in standard preparation

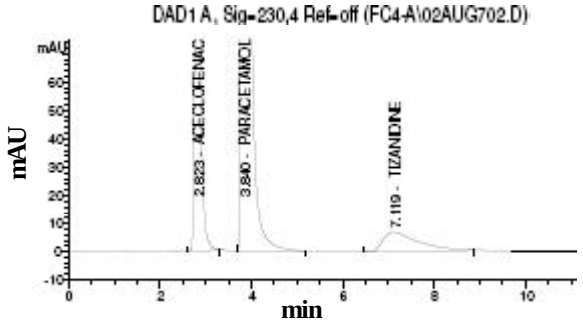


Figure 3: Chromatogram of aceclofenac paracetamol and tizanidine in sample preparation

procured from the market. Acetonitrile, potassium dihydrogen orthophosphate and sodium dihydrogen orthophosphate were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of Aceclofenac and tizanidine using paracetamol as an internal standard in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Agilent 1100 series High performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of all the three drugs were scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs were checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. A hypersil C_{18} column (250mm × 4.6 mm, 5mm particle) was used for the analysis. The mobile phase comprising of Mix phosphate buffer pH 7.0: acetonitrile in the ratio (40:60) v/v. The system was run at a flow rate of 0.7mL min^{-1}, 5µL of sample was injected in the chromatographic system and detection wavelength was set at 230nm for simultaneous determination of these two drugs. A typical HPLC chromatogram for simultaneous determination of aceclofenac, paracetamol and tizanidine from pharmaceutical formulation is shown in figures 2 and 3.

Preparation of standard stock solutions

The stock solution of aceclofenac (4000µg mL^{-1}) was prepared by dissolving 99.11 mg of aceclofenac (99.9 %) in water:acetonitrile (50:50) in a standard 25mL volumetric flask (solution A). The stock solution of tizanidine hcl equivalent to tizanidine (4µg mL^{-1}) was prepared by dissolving 11.45 mg of tizanidine hcl (99.8 %) in water:acetonitrile (50:50) in a standard 25mL volumetric flask (solution B). The internal standard stock solution of paracetamol (4000µg mL^{-1}) was prepared by dissolving 99.21 mg of paracetamol (99.8 %) in water:acetonitrile (50:50) in a standard 25mL volumetric flask (solution C).

Working standard solution

Transferred 5.0 mL of stock solution A, 1.0mL of stock solution B and 5.0mL of stock solution C to a 100 mL volumetric flask and diluted up to the mark with water:acetonitrile (50:50).

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and a quantity equivalent to one tablet was transferred in a 500mL volumetric flask, dissolved in water:acetonitrile (50:50), and filtered through Whatman no. 41 filter paper.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the

reproducibility of the equipment is adequate for the analysis to be carried out[3-4]. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 5µL standard solutions of aceclofenac, tizanidine and paracetamol of strengths 200µg mL^{-1}, 4µg mL^{-1} and 200µg mL^{-1} respectively. This was repeated five times. The RSD values of aceclofenac and tizanidine were 0.09 and 0.77 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 31. Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1: Result of system suitability

Parameters	Aceclofenac	Paracetamol	Tizanidine
Resolution	-	5.490	4.209
Tailing factor	1.359	1.637	2.480
Theoretical plates	3291	7841	1129

TABLE 2: Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Aceclofenac	14.13	0.86	0.9998
Tizanidine	3.20	1.66	0.9998

TABLE 3: Results of assay experiment

	Aceclofenac	Tizanidine HCl equivalent to tizanidine
Drug found in mg/tablet (mean)	99.86	1.99
Mean %	99.91	100.63
RSD	0.31	0.16

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Linearity

Linearity was evaluated by analysis of working standard solutions of aceclofenac, tizanidine of seven different concentrations[3-4]. The range of linearity was from 100 - 300 µg mL^{-1} for aceclofenac and 2- 6µg mL^{-1} for tizanidine hcl equivalent to tizanidine. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained are represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[3-4]. The LOD and LOQ of aceclofenac and tizanidine were experimentally determined by six injections of each drug. The LOD of aceclofenac and tizanidine were found to be 0.3µg mL^{-1} and 0.03µg mL^{-1} respectively. The LOQ of aceclofenac and tizanidine were found to be 1.0µg mL^{-1} and 0.1µg mL^{-1} respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3-4]. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of aceclofenac and tizanidine in the product. The %RSD were 0.31 and 0.16 respectively. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of each drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of each drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[3-4]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drugs from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of these drugs.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between aceclofenac, paracetamol and tizanidine were observed using the mobile phase mentioned in chromatographic conditions.

The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and can be used for simultaneous determination of aceclofenac and tizanidine using paracetamol as an internal standard from pharmaceutical preparations.

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