Simultaneous RP HPLC Determination of Aceclofenac, Paracetamol and Tizanidine in Pharmaceutical Preparations

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Abstract: A simple, fast and precise reverse phase high performance liquid chromatographic method is developed for the simultaneous determination of aceclofenac, paracetamol and tizanidine. Chromatographic separation of the three drugs were performed on a hypersil C₁₈ column (250 mm x 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 0.7 mL min⁻¹ and UV detection at 230 nm. 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, 500-1500 µg mL⁻¹ for paracetamol and 2-6 µg mL⁻¹ for tizanidine HCl equivalent to tizanidine. It can be conveniently adopted for routine quality control analysis.

Keywords: Validation, Pharmaceutical preparations, Aceclofenac, Paracetamol, Tizanidine HCl.

Introduction

Aceclofenac {[2-(2', 6'-dichlorophenyl) amino] phenyl acetoxycetic acid} is a new phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties and improved gastric tolerance¹. Paracetamol is chemically 4-hydroxy acetanilide, a centrally and peripherally acting analgesic and antipyretic agent². Tizanidine HCl (TIZ) is 5-chloro-4-[2-imidazolin-2-yl-amino]-2,1,3-benzothiadiazole. It is used as skeletal muscle relaxant³. The structures of these three drugs are shown in Figure 1. One such combination contains 100 mg of aceclofenac, 500 mg of paracetamol and 2 mg of tizanidine HCl equivalent to tizanidine.

It is widely used as anti-inflammatory analgesic. The literature revealed that, no method was available for simultaneous determination of these three drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed for determination of aceclofenac, paracetamol and tizanidine from their combined dosage form⁶⁻¹¹. The method described is simple, fast, precise and accurate for simultaneous determination of aceclofenac, paracetamol and tizanidine from pharmaceutical preparation.

Figure 1. Structures of aceclofenac.

Figure 2. Structures of tizanidine hydrochloride.

Figure 3. Structures of paracetamol.

Chemicals and reagents

Standards were supplied from J.B. Chemicals & Pharmaceuticals Ltd., Mumbai, India. Zerodol-MR tablets manufactured by Ipca laboratories, India was 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

To develop a suitable LC method for the analysis of aceclofenac, paracetamol and tizanidine 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 spectra of all the three drugs were scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs was checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. A hypersil C₁₈ column (250 mm x 4.6 mm, 5 µm 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 0.7 mL min⁻¹, 5 µL of sample was injected in the chromatographic system and detection wavelength was set at 230 nm for simultaneous determination of these three drugs. A typical HPLC chromatogram for simultaneous determination of aceclofenac, paracetamol and tizanidine from pharmaceutical formulation is shown in Figure 4 and Figure 5.

Preparation of standard stock solutions

The stock solution of aceclofenac (4000 µg mL⁻¹) was prepared by dissolving 99.11 mg of aceclofenac (99.9%) in water:acetonitrile (50:50) in a standard 25 mL volumetric flask (solution A). The stock solution of paracetamol (10000 µg mL⁻¹) was prepared by dissolving 250.21 mg of paracetamol (99.8%) in water:acetonitrile (50:50) in a standard 25 mL volumetric flask (solution B). The stock solution of tizanidine HCl equivalent to tizanidine (4 µg mL⁻¹) was prepared by dissolving 11.45 mg of tizanidine HCl (99.8%) in water:acetonitrile (50:50) in a standard 25 mL volumetric flask (solution C).

Figure 4. Chromatogram of aceclofenac, paracetamol and tizanidine HCl in standard preparation.

Figure 5. Chromatogram of aceclofenac paracetamol and tizanidine HCl in sample preparation.

Working standard solution

Transferred 5.0 mL of stock solution A, 10.0 mL of stock solution B and 1.0 mL 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 500 mL 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 out⁴⁻⁵. 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 5- μ L standard solutions of aceclofenac, paracetamol and tizanidine of strengths 200 μ g mL⁻¹, 1000 μ g mL⁻¹ and 4 μ g mL⁻¹ respectively. This was repeated five times. The RSD values of aceclofenac, paracetamol and tizanidine were 0.09, 0.52 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

Linearity

Linearity was evaluated by analysis of working standard solutions of aceclofenac, paracetamol and tizanidine of seven different concentrations^{4,5}. The range of linearity was from 100-300 $\mu\text{g mL}^{-1}$ for aceclofenac, 500-1500 $\mu\text{g mL}^{-1}$ for paracetamol and 2-6 $\mu\text{g mL}^{-1}$ 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 within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

Table 2. Results of linearity.

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Aceclofenac	14.13	0.86	0.9998
Paracetamol	89.30	-0.63	0.9999
Tizanidine	3.20	1.66	0.9998

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^{4,5}. The LOD and LOQ of aceclofenac, paracetamol and tizanidine were experimentally determined by six injections of each drug. The LOD of aceclofenac, paracetamol and tizanidine were found to be 0.3 $\mu\text{g mL}^{-1}$, 0.3 $\mu\text{g mL}^{-1}$ and 0.03 $\mu\text{g mL}^{-1}$ respectively. The LOQ of aceclofenac, paracetamol and tizanidine were found to be 1.0 $\mu\text{g mL}^{-1}$, 1.0 $\mu\text{g mL}^{-1}$ and 0.1 $\mu\text{g mL}^{-1}$ 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^{4,5}. 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, paracetamol and tizanidine in the product. The %RSD were 0.31, 0.09 and 0.16 respectively (Table 3).

Table 3. Results of assay experiment

	Aceclofenac	Paracetamol	Tizanidine HCl equivalent to Tizanidine
Drug found in mg/tablet(mean)	99.86	501.2	1.99
Mean %	99.91	100.21	100.63
RSD	0.31	0.09	0.16

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^{4,5}. 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 the three drugs.

Table 4. Accuracy of the method.

Analyte	Initial conc., mg	Conc. added, mg	Total conc., mg	Conc. found, mg	RSD, % n= 3	Recovery, %
Aceclofenac	100	0	100	99.86	0.11	99.86
	100	10	110	109.94	0.16	99.95
	100	20	120	120.56	0.09	100.47
	100	30	130	130.11	0.07	100.08
	500	0	500	501.2	0.16	100.24
Paracetamol	500	50	550	555.23	0.34	100.95
	500	100	600	599.10	0.09	99.85
	500	150	650	649.94	0.23	99.99
Tizanidine HCl equivalent to Tizanidine	2	0	2	1.99	0.14	99.50
	2	0.2	2.2	2.19	0.05	99.55
	2	0.4	2.4	2.40	0.15	100.00
	2	0.6	2.6	2.59	0.10	99.62

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 was 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, paracetamol and tizanidine from pharmaceutical preparations.

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