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Simultaneous RP-HPLC determination of aceclofenac, paracetamol and tramadol HCl in pharmaceutical preparations

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ABSTRACT

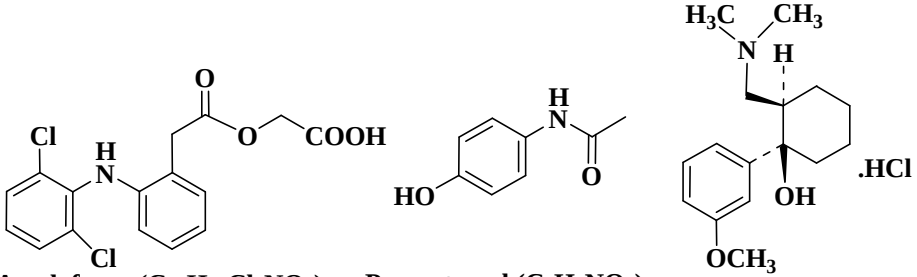
A simple, fast and precise RP-HPLC method is developed for the simultaneous determination of aceclofenac, paracetamol and tramadol HCl using methyl paraben as an internal standard. Chromatographic separation of these drugs were performed on a inertsil C_{18} column (250mm × 4.6 mm, 5µm) as stationary phase with a mobile phase comprising of 0.02M potassium dihydrogen orthophosphate pH adjusted to 3.0 with orthophosphoric acid : acetonitrile (40:60 v/v), at a flow rate of 1.5mL min^{-1} and UV detection at 270nm. 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, 325-975 -µg mL^{-1} for paracetamol and 37.5- 112.5 -µg mL^{-1} for tramadol HCl. The retention time were found 1.40 min for tramadol HCl, 1.88 min for paracetamol, 2.77 min for methyl paraben (internal standard) and 7.38 min for aceclofenac. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Aceclofenac;
Paracetamol;
Tramadol 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]. Paracetamol is chemically 4-hydroxy acetanilide, a centrally and peripherally acting analgesic and antipyretic agent[2]. Tramadol HCl (TIZ) is(1RS, 2RS)-[(dimethylamino) methyl]-1-(3-methyoxyphenyl)cyclohexanol hydrochloride. It is used as an analgesic[3]. The structures of these three drugs are shown in figure 1. One such combina-



Aceclofenac ($C_{16}H_{13}Cl_2NO_4$) **Paracetamol ($C_8H_9NO_2$)** **Tramadol HCl ($C_{16}H_{26}ClNO_2$. HCl)**

Figure 1: Structures of aceclofenac, paracetamol and tramadol HCl

tion contains 100mg of aceclofenac, 375mg of para cetamol and 37.5 mg of tramadol HCl. It is widely used as Anti-inflammatory- Analgesic. The literature revealed 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 tramadol HCl from their combined dosage form [6-12]. The method described is simple, fast, precise and accurate for simultaneous determination of aceclofenac, paracetamol and tramadol HCl from pharmaceutical preparation.

Chemicals and reagents

Standards were supplied from J.B.Chemicals and pharmaceutical Ltd., Mumbai, India. Zerodol-PT tablets manufactured by Ipca laboratories, India were procured from the market. Acetonitrile, potassium dihydrogen orthophosphate and ortho-phosphoric acid 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, paracetamol and tramadol HCl in their combined dosage form using methyl paraben as an internal standard, different mobile phase 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 Shimadzu LC-2010 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 Class-VP software. A inertsil C_{18} column (250mm × 4.6 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.02M potassium dihydrogen ortho phosphate buffer pH 3.0: acetonitrile in the ratio (40:60) v/v. The system was run at a flow rate of 1.5 mL min^{-1}, 20µL of sample was injected in the chromatographic system and detection wavelength was set at 270nm for simultaneous determination of these three drugs. A typical HPLC chromatogram for simultaneous determination of aceclofenac, paracetamol and tramadol HCl from pharmaceutical formulation is shown in figures 2 and 3.

Preparation of standard stock solutions

The stock solution of aceclofenac (2000µg mL^{-1}) was prepared by dissolving 99.11 mg of aceclofenac (99.9 %) in water:acetonitrile (50:50) in a standard 50 mL volumetric flask (solution A). The stock solution of paracetamol (6500µg mL^{-1}) was prepared by dissolving 325.05 mg of paracetamol (99.8 %) in water: acetonitrile (50:50) in a standard 50 mL volumetric flask (solution B). The stock solution of tramadol HCl (750µg mL^{-1}) was prepared by dissolving 37.25 mg of tramadol HCl (99.8 %) in water:acetonitrile (50:50) in a standard 50 mL volumetric flask (solution C). The stock solution of methyl paraben (5000µg mL^{-1}) was prepared by dissolving 250.04 mg of methyl paraben (99.9

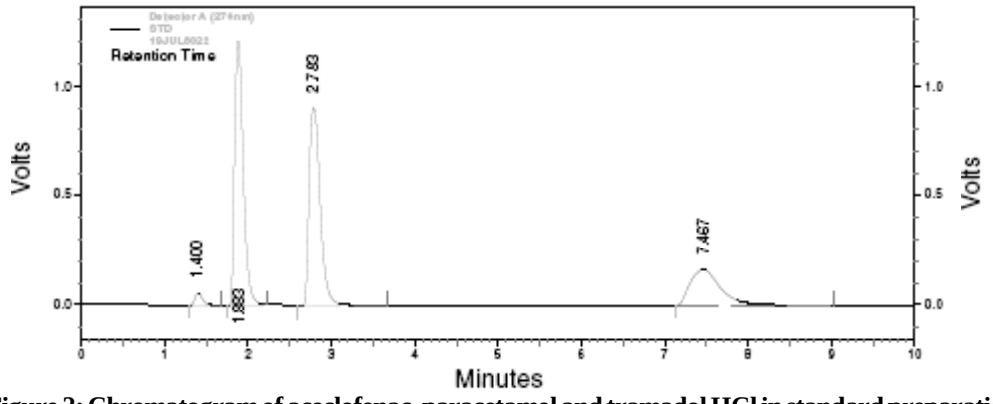


Figure 2: Chromatogram of aceclofenac, paracetamol and tramadol HCl in standard preparation

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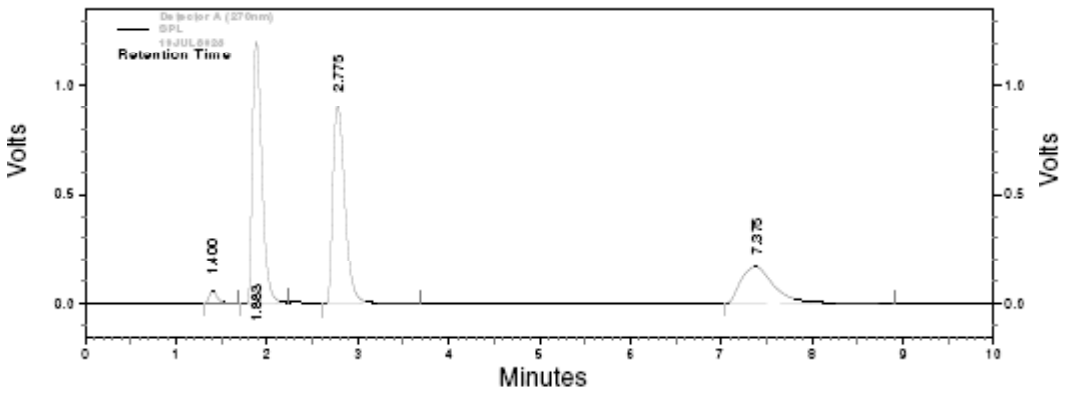

Figure 3: Chromatogram of aceclofenac paracetamol and tramadol HCl in sample preparation

%) in water:acetonitrile (50:50) in a standard 50 mL volumetric flask (solution D).

Working standard solution

Transferred 10.0 mL each of stock solution A, stock solution B, stock solution C and 5.0 mL of stock solution D 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 containing 25.0mL of stock solution D, 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[4-5]. 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 20µL standard solutions of aceclofenac, paracetamol and tramadol HCl of strengths 200µg mL^{-1}, 650µg mL^{-1} and 75µg mL^{-1} respectively. This was repeated five times. The RSD values of aceclofenac, paracetamol and tramadol HCl were 0.07, 0.16 and 0.14 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	Tramadol HCl	Paracetamol	Methyl Paraben	Aceclofenac
Resolution	-	2.72	4.24	10.67
Tailing factor	1.44	1.48	1.57	1.57
Theoretical plates	1108	1619	2267	2201

TABLE 2: Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Tramadol HCl	3.48	-3.48	0.9996
Paracetamol	87.57	-66.43	0.9999
Aceclofenac	41.66	-28.71	0.9999

Linearity

Linearity was evaluated by analysis of working standard solutions of aceclofenac, paracetamol and tramadol HCl of seven different concentrations using methyl paraben as an internal standard[4-5]. The range of linearity was from 100 - 300µg mL^{-1} for aceclofenac, 325-975µg mL^{-1} for paracetamol and 37.5- 112.5µg mL^{-1} for tramadol HCl. 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 fetection 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 tramadol HCl were experimentally determined by six injections of each drug.

TABLE 3: Results of assay experiment

	Aceclofenac	Paracetamol	Tramadol HCl
Drug found in mg/tablet (mean)	99.96	324.88	37.32
Mean %	99.96	99.96	99.53
RSD	0.08	0.01	0.26

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	99.99	0.10	99.99
	100	10	110	109.99	0.06	99.99
	100	20	120	120.12	0.20	100.10
	100	30	130	130.46	0.49	100.36
Paracetamol	325	0.0	325	324.88	0.01	99.96
	325	32.5	357.5	357.57	0.06	100.02
	325	65.0	390	390.01	0.24	100.00
	325	97.5	422.5	422.40	0.10	99.98
Tramadol HCl	37.5	0	0	37.34	0.24	99.56
	37.5	3.75	41.25	41.23	0.06	99.95
	37.5	7.5	45	45.30	0.58	100.67
	37.5	11.25	48.75	48.61	0.18	99.71

The LOD of aceclofenac, paracetamol and tramadol HCl were found to be 0.4μg mL^{-1}, 0.2μg mL^{-1} and 0.8μg mL^{-1} respectively. The LOQ of aceclofenac, paracetamol and tramadol HCl were found to be 0.8μg mL^{-1}, 0.7μg mL^{-1} and 1.5μg 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 five independent determinations at 100% of the test concentrations of aceclofenac, paracetamol and tramadol HCl in the product. The % RSD were 0.31, 0.09 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[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.

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 tramadol HCl 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, paracetamol and tramadol HCl from pharmaceutical preparations.

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