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Validated High Performance Liquid Chromatography Method for Simultaneous Estimation of Paracetamol and Aceclofenac in Tablet Dosage Forms

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A simple, specific, precise and rapid HPLC method has been developed for estimation of paracetamol and aceclofenac simultaneously in tablet dosage form. In this method standard solution and sample solution of paracetamol and aceclofenac were applied on C_{18} reversed phase column with a mobile phase consisting of methanol, buffer, water and acetonitrile (85:15:25:5 v/v/v/v) at a flow rate of 1 mL/min and spectrophotometric detection was carried out at 274 nm. This HPLC system was quantitatively evaluated in terms of linearity, accuracy, precision, repeatability and specificity proving the utility in estimation of drug content in tablet dosage forms.

Key Words: HPLC, Paracetamol, Aceclofenac.

INTRODUCTION

Paracetamol is one of the most popular among the other counter analgesic and antipyretic drugs. It is chemically 4-hydroxy acetanilide. Aceclofenac is a non-steroidal analgesic, antipyretic and antiinflammatory drug. It is chemically 2-[[2-[2(2,6-dichlorophenyl)amino phenyl] acetyl]oxy]acetic acid. The fixed combination of paracetamol (500 mg) and aceclofenac (100 mg) is available in the market as tablet and is used for the treatment of painful skeletal muscle spasm associated with musculoskeletal disorder, low back pain and joint stiffness.

Numerous methods have been reported for the analysis of paracetamol and its combinations in pharmaceuticals or in biological fluids. Paracetamol has been determined individually and in combination with other drugs by using UV-spectrophotometry[1-3], high performance liquid chromatography[3-8] in pharmaceutical preparations. Aceclofenac has been determined by titrimetric[9], spectrophotometric, spectroflourimery and high performance

liquid chromatographic methods[10-13] in formulations and in biological fluids. In the present investigation an attempt was made to develop a simple and economical validated RP-HPLC with greater precision, accuracy and sensitivity for the simultaneous estimation of paracetamol and aceclofenac in pure and tablet dosage forms.

EXPERIMENTAL

Paracetamol and aceclofenac in pure and powder forms were obtained as gift sample from Aeon Therapeutics Ltd., Chennai. Hypersil C_{18} ODS column (5 µ, 25 cm × 4.6 mm) was used as stationary phase. Zenodol-p and X-trap (paracetamol 500 mg and aceclofenac 100 mg) were purchased from local pharmacy. Methanol and acetonitrile of HPLC-grade purity were procured from E.Merck Ltd., Mumbai. Phosphoric acid and sodium dihydrogen phosphate were obtained from S.D fine Chemicals Ltd., Mumbai.

Standard and sample preparation: The standard stock solution of paracetamol and aceclofenac were prepared by dissolving 500 mg of paracetamol and 100 mg of aceclofenac accurately weighed in 100 mL of methanol. Working standard solutions were obtained by dilution of standard stock solution with mobile phase to get the final concentration of 100, 200, 300, 400 and 500 µg/mL of paracetamol and 20, 40, 60, 80 and 100 µg/mL of aceclofenac. 20 Tablets of marketed samples were separately weighed and ground to a fine powder. A weight equivalent to 500 mg of paracetamol and 100 mg of aceclofenac were transferred to a conical flask and extracted with methanol. The extract was filtered through Wattman filter paper No.1 and the residue was washed with sufficient amount of methanol. The extract and washings were pooled, transferred to a 100 mL volumetric flask and the final volume was made up to 100 mL with methanol. Further 5 mL was diluted to 50 mL with mobile phase to obtain the sample solution, which contains 500 µg/mL of paracetamol and 100 µg/mL of aceclofenac.

HPLC method and chromatographic conditions: HPLC analysis was performed by isocratic elution with a flow rate of 1 mL/min. The mobile phase composition was methanol-buffer-water-acetonitrile (85:15:25:5). All solvents were filtered through a Millipore filter before use and degassed in an ultrasonic bath. 20 µL of standard solutions and sample solutions were injected into the column and the chromatogram is developed. Quantification was effected by measuring at 274 nm. The chromatographic run time was 8 min.

Method validation: The method was validated as per ICH guidelines in terms of linearity, accuracy, inter-day and intra-day precision, reproducibility of measurement of peak area, reproducibility of sample application

and the specificity. The accuracy of the analysis was evaluated by carrying out a recovery study. For that purpose known concentration of standard drug was added to a pre-analysis tablet sample at three different levels namely 80, 100 and 120 % and average recovery was calculated. The intra-day precision was determined by analyzing standard drug solution in the concentration range of 500 to 1000 ng per injection for 3 times on the same day, while inter-day precision was determined by analyzing corresponding standards daily for a period of 1 week. Repeatability of measurement of peak area was determined by injecting 20 µL of standard drug solution and elutes the peaks. Injecting a sample solution of paracetamol and aceclofenac and developing the chromatogram and checked the specificity of the proposed method. Purity was also checked by overlain spectra of standard paracetamol and aceclofenac solution with spectra of sample.

RESULTS AND DISCUSSION

Various methods have been reported for the analysis of paracetamol and aceclofenac individually in pharmaceutical preparations. Most of them are colorimetric, HPLC, UV-spectrophotometric methods, but there is no simultaneous determination of these drugs. The developed method may turn out to be easy and cost effective for the routine analysis purposes. This is a versatile speedy and cost effective technique. The solvent system having a combination of methanol, buffer, water and acetonitrile (85:15:25:5) offered maximum resolution for the two drugs with R_t values of 2.92 and 5.31 min for paracetamol and aceclofenac, respectively. Since these drugs are freely soluble in methanol, the tablet powder was extracted with methanol. The amount of drug in tablet formulation was calculated on applying suitable dilution factor and comparing the peak area of the standard and sample solutions. The assay of paracetamol and aceclofenac in tablet formulation calculated as per peak area was found to be 100.13 ± 0.10 and 100.80 ± 0.22 %, respectively (Table-1).

TABLE-1
ESTIMATION OF PARACETAMOL AND ACECLOFENAC

Drug	Label Claim (mg/tab)	Amount found ± SD (mg)*	Assay ± SD* (%)	RSD (%)	SE
Paracetamol	500	500.69 ± 0.49	100.13 ± 0.10	0.096	0.222
Aceclofenac	100	100.80 ± 0.22	100.80 ± 0.22	0.218	0.098

*Mean and ± Standard deviation for five determinations
RSD = Relative standard deviation, SE = Standard error.

The good average recovery values obtained in recovery studies indicate that proposed method is accurate for estimation of drug in tablets (Table-2). The intra-day and inter-day relative standard deviation for these two drugs was found to be in the range of 0.09 to 1.370 % and 0.314 to 2.268 %, respectively. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. It was observed that excipients present in formulation did not interfere with peaks of paracetamol (R_t = 2.92) aceclofenac (R_t = 5.31). Different validation parameters for the proposed HPLC method for determination of paracetamol and aceclofenac have been summarized (Table-3). The specificity of the HPLC method is illustrated by complete separation of paracetamol and aceclofenac was noticed in presence of tablet excipients. The average retention time and standard deviation for paracetamol and aceclofenac were found to be 2.92 ± 0.05 and 5.33 ± 0.07 min, respectively, for four replicates. The peaks obtained were sharp and have clear baseline separation.

TABLE-2
RECOVERY OF PARACETAMOL AND ACECLOFENAC

Drug	Label claim (mg/tablet)	Amount added (mg)	Amount Recovered* (mg)	Recovery* (%)	RSD (%)	Average recovery (%)
Paracetamol	500	80	80.81 ± 0.25	101.03	0.277	100.97
		100	100.9 ± 0.31	100.90	0.307	
		120	121.18 ± 0.31	100.98	0.257	
Aceclofenac	100	80	81.68 ± 0.55	102.10	0.657	101.27
		100	101.02 ± 0.56	101.02	0.554	
		120	120.84 ± 1.15	100.71	0.953	

*Average value ± standard deviation of 3 determinations.

TABLE-3
METHOD VALIDATION PARAMETERS

Parameter	Results	
	Paracetamol	Aceclofenac
Linearity range (µg/mL)	100-500	20-100
Correlation coefficient (r)	0.997	0.999
Accuracy	100.97	101.27
Precision (RSD %) repeatability		
Intra-day (n = 3)	0.090-0.264	0.392-1.370
Inter-day (n = 3)	0.314-0.989	1.161-2.268
Specificity	2.920 ± 0.050	5.33 ± 0.07

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