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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC SODIUM AND PARACETAMOL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Diclofenac sodium and paracetamol in tablet dosage form. Phenomenex C18 column (250 mm X 4.6 mm i.d., 5 µm particle size) in Isocratic mode with mobile phases containing phosphate buffer (pH 5.5) and Acetonitrile (70:30 %v/v) was used. The flow rate was 1.0mL/min and effluents were monitored at 280 nm.) The retention time of Diclofenac sodium and paracetamol was 2.90min and 5.27min respectively. The concentration curves of Diclofenac sodium and paracetamol were linear in the concentration range of 2-10 µg/mL and 10-50 µg/mL respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD, LOQ, robustness. The developed method can be successfully applied in routine quality control analysis.

KEYWORDS: Paracetamol, Diclofenac sodium RP-HPLC method, simultaneous estimation.

INTRODUCTION

Diclofenac Sodium is chemically Sodium salt of 2-[{2, 6-dichlorophenyl} amino] benzene acetic acid. It is having anti-inflammatory and analgesic properties. Many methods have been reported in literature for determination of Diclofenac sodium and paracetamol with other drugs. The present work describes a validated new reverse phase HPLC method by changing the mobile phase, for simultaneous determination of these drugs in combined tablet dosage form.

MATERIALS AND METHODS

Instrumentation

A Youngling's HPLC (Acme-9000), UV Detector, Manual injector of 20-µl loop, Column - Phenomenex C_{18} column (250 mm X 4.6 mm i.d., 5 µm particle size) Software – Auto chrome 3000, Digital pH meter (Ecoscan), Corning volumetric flasks (10mL, 50mL and 100 mL), A CP224S analytical balance (Sartorius), Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai), Vacuum pump, Pipettes – 1mL, 5mL, 10mL, beakers, measuring cylinder.

Chemicals and Reagents

Authentic samples of Diclofenac sodium and paracetamol were supplied from Camper Healthcare,Mehsana, India as gift samples. Phosphate buffer and Acetonitrile (HPLC grade, S.D. Fine Chemicals Ltd.,Mumbai), 0.22 µm filter (Millipore). The tablet formulations were procured from a local pharmacy.

Chromatographic conditions

Phenomenex C_{18} column (250 mm X 4.6 mm i.d., 5 µm particle size), Mobile phase - Phosphate buffer and Acetonitrile (70:30 %v/v). Flow rate: 1.0 mL/min, Filter: Nylon 0.22 µm membrane filter, Mobile Phase was degassed before use, Detection wavelength: 280nm, the injection volume: 20 µl, and Temperature: 30 ±2^{o} C.

Preparation of mobile phase

Phosphate buffer and Acetonitrile in the ratio of 70:30 v/v, respectively (pH 5.5) was mixed. The mobile phase was filtered through nylon 0.22 µm membrane filter and was degassed before use.

Preparation of Solutions

Standard stock solution (100 µg/mL)

Accurately weighed 100 mg of Diclofenac sodium and 100 mg of Paracetamol were transferred to two separate 100 mL volumetric flasks. 50 mL Water was added to the flask. The drug was dissolved with sanitations and the final volume was adjusted with Water up to the mark to prepare 100 µg/mL stock solutions of both drugs.

Preparation of working standard solution

Accurately measured working standard solutions of Diclofenac Sodium(0.2, 0.4, 0.6, 0.8, and 10 mL) and Paracetamol(1, 2, 3, 4 and 5 mL) were transferred to a series of 10 mL

volumetric flasksand the volume in each flask was adjusted to 10 mL with Water.

Preparation of Sample solution

Twenty tablets were weighed and powdered. Quantity of the powder equivalent to about 50 mg of Diclofenac sodium and 100mg of paracetamol was transferred into 100 mL volumetric flask contain 50 mL of mobile phase and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with Water. The filtrate and washings were combined in a 100 mL volumetric flask and diluted to the mark with Methanol to get a final concentration 50 µg/mL of Diclofenac sodium and 100 µg/mL of paracetamol. From this, 0.6 mL of solution was transferred to the 10 mL volumetric flask and diluted to mark with methanol to get final Concentration 6 µg/mL of Diclofenac sodium and 30 µg/mL of Paracetamol.

Determination of Maximum wavelength

The standard solution of Diclofenac sodium and Paracetamol were scanned in the range of 200-400 nm against mobile phase as a blank. Diclofenac sodium and Paracetamol showed maximum absorbance at 280 nm. So the wavelength selected for the determination of Diclofenac sodium and Paracetamol was 280 nm.

Chromatographic method

Pre-treatment of column

Phenomenex C_{18} was properly washed with of Acetonitrile (HPLC grade previously filtered with Nylon 0.22 µm membrane filter and degassed properly) for 30 min at 1.0 mL/min of flow rate.

Chromatographic separation

With the help of micro liter syringe and loop, 20 mL of each working standard solutions or sample solution was injected into the column through loop at 1.0 mL/min flow rate. The Peaks of Diclofenac sodium and Paracetamol were detected at 280 nm and retention times were found to be 2.90 and 5.27 minutes respectively.

Calibration curve of standard Diclofenac sodium and Paracetamol

A calibration curves were plotted over a concentration range of 2- 10µg/mL for Diclofenac sodium and 10- 50µg/mL for Paracetamol. Accurately measured standard stock solutions of Diclofenac sodium (0.2, 0.4, 0.6, 0.8, and 1.0 mL) and Paracetamol (1, 2, 3, 4 and 5 mL)

were transferred to a series of 10 mL corning volumetric flasks and the volume in each flask was adjusted to 10 mL with mobile phase. The resulting solution was injected into the column and the peak area obtained at retention time 2.90 and 5.27 minutes and flow rate 1.0 mL/min were measured at 280 nm for Diclofenac sodium and paracetamol respectively. Calibration curves were constructed for Diclofenac sodium and Paracetamol by plotting peak area versus concentration at 280 nm.

Quantization of Diclofenac sodium and Paracetamol in formulation

Test solution from tablets which contain Diclofenac sodium (6.0 µg/mL) and Paracetamol (30 µg/mL) were prepared from the sample solution and solutions were injected into HPLC system and area was measured.

METHOD VALIDATION

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The linear response of Diclofenac sodium and Paracetamol were determined by analyzing five independent levels of the calibration curve in the range of 2- 10µg/mL for Diclofenac sodium and 10-50µg/mL for Paracetamol.

Precision

It provides an indication of random error. The precision of an analytical method is usually expressed as thestandard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of Diclofenac sodium (6µg/mL) and Paracetamol (30µg/mL) for six times and analyzed as per the proposed method.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of Diclofenac sodium and Paracetamol in triplicate at three different

concentrations. Inter-day precision was evaluated by using freshly prepared solutions of Diclofenac sodium and Paracetamol in triplicates at three different days.

RESULT AND DISCUSSION

The proposed method can determine Diclofenac sodium and Paracetamol in combined dosage form and the validity of this method was confirmed in accordance with the ICH guidelines. In proposed method retention times were recorded at 2.90 min and 5.27 min at 1.0 mL/min for Diclofenac sodium and Paracetamol respectively. The calibration graphs for Diclofenac sodium and Paracetamol were constructed by plotting the area versus their corresponding concentrations, over the range 2-10 µg/mL for Diclofenac sodium and 10 - 50 µg/mL for Paracetamol. The proposed method has been applied to the assay of Diclofenac sodium and Paracetamol in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate that the additives present do not interfere with analysis of the studied mixtures (Table 1). The regression characteristics and validation parameters are reported in (Table 4). Results of application of proposed method to the pharmaceutical dosage form are shown in (Table 5)

Table 1: Percentage recovery of Diclofenac sodium and Paracetamol.

Drug	Spiked level(%)	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL) ± S.D	% Recovery ± S.D (n=3)
Diclofenac sodium	80	6	4.7	4.705 ± 0.005	100.112 ± 0.111
	100	6	6	6.002 ± 0.009	100.047 ± 0.146
	120	6	7.3	7.313 ± 0.005	100.184 ± 0.074
Paracetamol	80	3	2.3	2.302 ± 0.001	100.036 ± 0.053
	100	3	3	3.002 ± 0.001	100.073 ± 0.044
	120	3	3.7	3.703 ± 0.002	100.064 ± 0.035

Table 2: Repeatability data for analysis of Diclofenac sodium.

Concentration	Area (mV*sec)	Amount found	% Amount found
6 µg/mL	653.15	6.004	100.162
	650.17	6.001	100.025
	651.93	5.988	99.954
	650.89	5.987	99.961
	652.52	6.012	100.250
	651.45	6.003	100.038
Mean	651.685	5.999	100.065
SD (n = 6)	0.7268	0.0078	0.1135
%RSD	0.1115	0.1147	0.1133

Table 3: Repeatability data for analysis of Paracetamol.

Concentration	Area (mV*sec)	Amount found	% Amount found
30 µg/mL	1285.70	30.001	100.149
	1290.45	31.008	100.287
	1287.65	32.006	100.221
	1284.64	29.004	100.144
	1286.71	31.006	100.228
	1288.49	30.003	100.134
Mean	1287.3	30.504	100.193
SD (n = 6)	0.7608	0.0015	0.0598
%RSD	0.0590	0.0594	0.0596

Table 4: Intermediate precision data for Diclofenac sodium at 280 nm (Intraday & Inter day Precision).

Concentration (µg/mL)	Intraday Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
2	231.58 ± 0.644, 0.276	232.66 ± 0.724, 0.312
4	433.93 ± 0.438, 0.101	430.62 ± 0.612, 0.141
6	643.82 ± 0.664, 0.103	642.45 ± 0.856, 0.132
8	868.45 ± 0.823, 0.094	867.63 ± 0.967, 0.112
10	1079.83 ± 0.585, 0.054	1077.86 ± 0.770, 0.071

Table 5: Intermediate precision data for Paracetamol at 280 nm (Intraday & Inter day Precision).

Concentration (µg/mL)	Intraday Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
1	455.46±0.810,0.178	452.58±0.998,0.220
2	885.57±0.777,0.087	887.40±0.873,0.098
3	1287.58±0.744,0.056	1289.64 ±0.791,0.061
4	1755.66±0.693,0.039	1744.7±0.723,0.041
5	2187.53±0.669,0.031	2186.55±0.699,0.0321

Table 6: LOD and LOQ data of Diclofenac sodium and Paracetamol.

Parameter	Diclofenac sodium	Paracetamol
LOD (µg/mL)	0.023	0.014
LOQ (µg/mL)	0.066	0.033

Table 7: System suitability parameters.

Parameter	Diclofenac sodium	Paracetamol
Retention time (Minutes)	2.90	5.27
Theoretical plates	3247.55	2145.7
Tailing factor (Tf)	1.27	1.54

Table 8: Method validation parameters for Diclofenac sodium and Paracetamol.

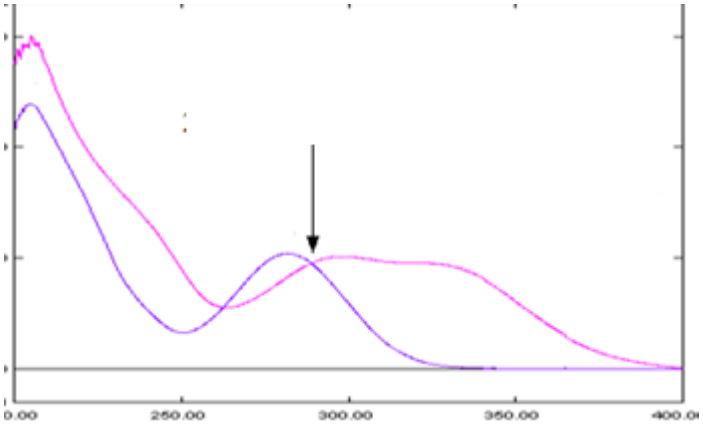
Parameters		Diclofenac sodium	Paracetamol
Calibration range		2-10 µg/mL	10-50 µg/mL
Slope		106.32	432.44
Intercept		13.32	16.47
Correlation coefficient		0.9996	0.9996
Precision (%RSD)	Intra-day	0.054 – 0.276	0.031 – 0.178
	Inter-day	0.071 – 0.312	0.032 – 0.220
LOD(µg/mL)		0.023	0.014
LOQ(µg/mL)		0.066	0.033

Table 9: Assay results of Diclofenac sodium and Paracetamol.

Formulation	Label Claim (mg/tablet)		Assay (Content inmg)		% Assay (Mean* ± S.D, n=6)	
	Diclo	Para	Diclo	Para	Diclo	Para
DPSYL	50	500	100.04	50.06	100.05 ± 0.17	100.06 ± 0.17

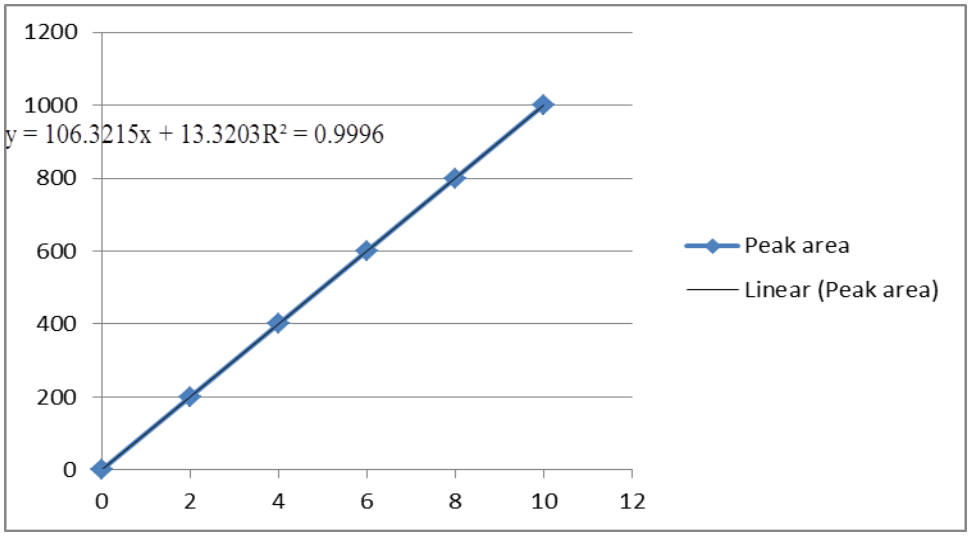
Table 10: Solution Stability Studies of Diclofenac sodium and Paracetamol.

Time (hr.)	Peak Area of Diclofenac sodium		Peak Area of Paracetamol	
	Standard	Test	Standard	Test
0	651.16	650.75	1287.43	1287.86
2	651.34	651.09	1286.91	1286.28
4	650.85	649.38	1288.78	1287.76
6	651.20	649.95	1287.84	1287.54
24	650.75	650.32	1287.22	1286.84
Average	651.06	650.30	1287.64	1287.26
SD	0.2297	0.5767	0.7910	0.7259
% RSD	0.0354	0.0885	0.0616	0.0565



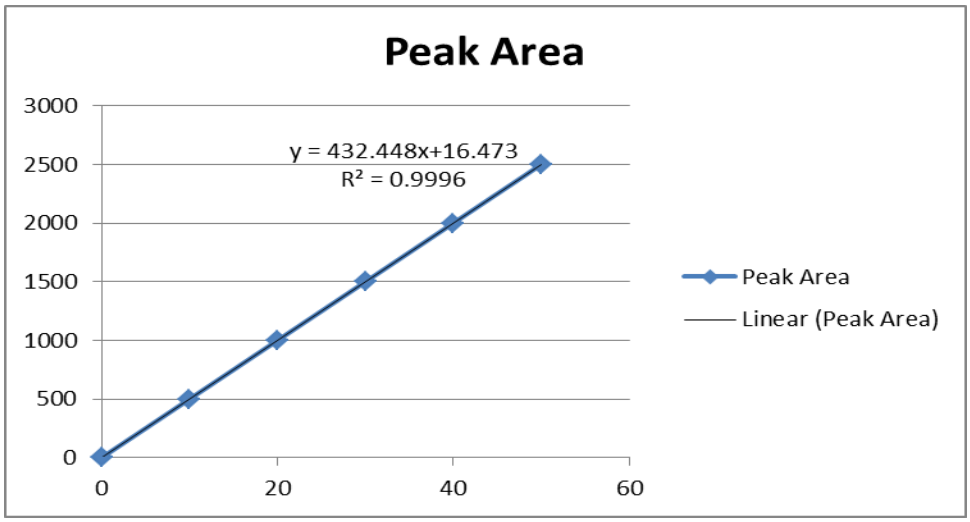
280nm

Calibration curve of Diclofenac sodium at 280nm

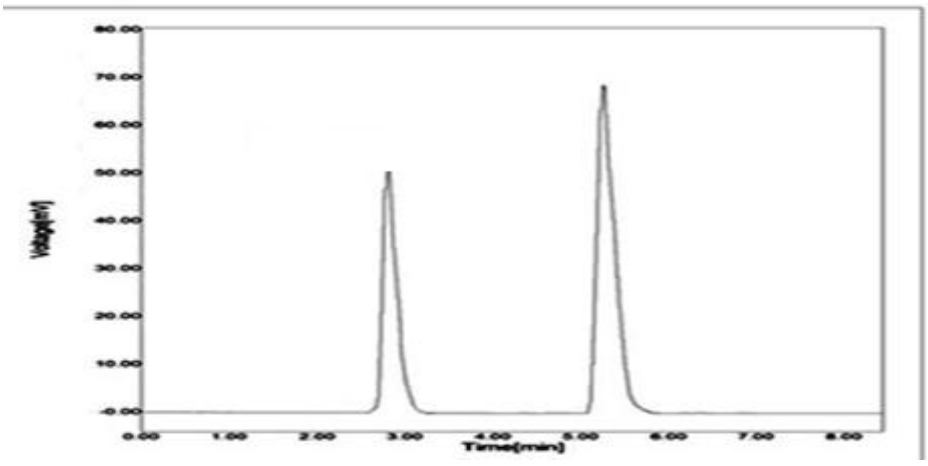


Concentration

Calibration curve of Paracetamol at 280 nm



Concentration



Diclofenac sodium **Paracetamol**

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