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A New Modified Reverse Phase High Performance Liquid Chromatography Method for Estimation and Validation of Paracetamol and Diclofenac Sodium in Combined Dosage Pharmaceutical Formulation

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A new modified simple, selective, rapid, precise and economical reversed phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous estimation of paracetamol and diclofenac sodium from solid oral dosage form. This combination is used as an analgesic and antipyretic disorders. The method was carried out on a LIChroCART® RP-18 (125 mm × i.d - 4 mm, pore- 5 µm) column with a mobile phase consisting of methanol:sodium acetate buffer (0.808 % solution of anhydrous sodium acetate in water, pH: 8.0) in the ratio 70:30 (v/v). The flow rate was 0.5 mL/min and the effluent was monitored for paracetamol at 257 nm and for diclofenac sodium at 254 nm (Waters 2487 dual absorbance detector). The validation of the proposed method was also carried out in terms of linearity, accuracy, precision, symmetry factor, plate count, regression and recovery. The retention time of paracetamol and diclofenac sodium was 2.12 and 4.08 min, respectively. Due to its simplicity, accuracy and economic value, the proposed method can be used for routine quality control analysis of these drugs in combined dosage form.

Key Words: RP-HPLC, Paracetamol, Diclofenac sodium.

INTRODUCTION

Paracetamol (acetaminophen) is chemically N- (4-hydroxyphenyl) acetamide[1] and it is used as analgesic and antipyretic[2] drug (Fig. 1) and one of the most popular over-the-counter analgesic and antipyretic drug. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias[3,4].

Diclofenac sodium or sodium [*o*-(2, 6- dichlorophenyl)-amino-phenyl]acetate (Fig. 2) is a non-steroidal antiinflammatory analgesic with potent cycloxygenase inhibition activity[5-8]. This drug is commonly used for pain control and treatment of rheumatic diseases[9,10]. Diclofenac is well absorbed after oral administration with

extensive hepatic metabolism[11,12]. This compound exhibits a terminal half-life of 1-2 h, volume of distribution of 0.17 L/kg and 99 % protein binding[13-15] and enters the synovial fluid[16]. The drug is well absorbed orally and dissolves in the intestinal fluid[17].

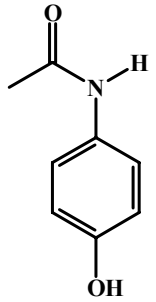

Fig. 1. Structure of pracetamol

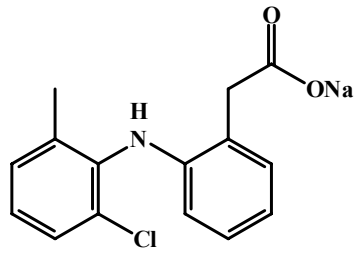

Fig. 2. Structure of diclofenac sodium

Fixed dose combination of paracetamol 500 mg and diclofenac sodium 50 mg is available in the tablet form in the market. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of paracetamol and diclofenac sodium in combined dosage form.

EXPERIMENTAL

Methanol (HPLC grade) was procured from Thomas Baker (Chemicals) Pvt. Ltd., Mumbai. Anhydrous sodium acetate (AR grade) and acetic acid (HPLC grade) were purchased from Merck Ltd., Mumbai, India. Water (HPLC grade) was obtained from aurium® 611UV water purification system of Sartorius, Germany. Working standard of paracetamol and diclofenac sodium was obtained from Granules India Ltd. and Umedica Laboratories Pvt. Ltd. respectively as a gift sample.

Chromatograpic conditions: A Waters® HPLC (515 pumps) system was used for analysis. The method was carried out on LIChroCART® RP-18 (125 mm × i.d-4 mm, pore-5 µm) column as a stationary phase and mobile phase consisting of methanol:sodium acetate buffer (0.808 % solution of anhydrous sodium acetate in water, pH: 8.0) in the ratio 70:30 (v/v). The mobile phase was filtered through 0.45 µm membrane and degassed. The flow rate was 0.5 mL/min. Rheodyne injector and 10 µL loop was used for the injection of samples. Detection was done for paracetamol at 257 nm and for dichlofenac sodium at 254 nm (Waters 2487 dual detector). Empower2 software provided by Waters was used throughout this experiment.

Preparation of standard solution

For assay: 0.1002 g working standard of paracetamol (99.87 %) and 0.0122 g working standard of diclofenac sodium (99.61 %) were taken in a 25 mL of volumetric flask and volume was made up to 25 mL by the mobile phase (solution A). 1 mL of aliquot was taken in a 25 mL volumetric flask and volume was made up to the mark by the mobile phase (100 %) (solution B).

For linearity: 0.8 mL of solution A was taken in a 25 mL of volumetric flask and volume was made up to 25 mL with mobile phase (80 %) (solution C). To another 25 mL of volumetric flask 1.2 mL of solution A was taken and volume was made up to the mark by the mobile phase (120 %) (solution D).

Preparation of sample solution:

For assay: Twenty tablets were taken and finely powered. 0.174 g of powder was accurately weighed equivalent to 5.25 mg of pracetamol and 0.47 mg of diclofenac sodium, respectively in a 25 mL volumetric flask and the volume was made up to 25 mL by the mobile phase (solution E). 1 mL of aliquot was taken in a 25 mL volumetric flask and made up to the mark with mobile phase (solution F).

For recovery and accuracy: 1 mL of solution E was separately taken in a three volumetric flask of 25 mL. 0.1 mL of solution A was added in the first volumetric flask (solution G), 0.2 mL of solution A was added in the second volumetric flask (solution H) and 0.3 mL of solution A was added in the third volumetric flask (solution I) as spike. The volume of all three volumetric flasks was made up to 25 mL by the mobile phase.

Assay method: With the optimized chromatographic conditions (room temperature 25 ºC), a steady baseline was recorded. In the first phase standard paracetamol solution was injected 6 times. This procedure was repeated for the sample solution obtained from the formulation. Retention time of paracetamol was 2.128. The peak area of the standard and sample solution was obtained from the chromatogram.

In the second phase, 6 replicate standard diclofenac sodium solutions were injected. This procedure was repeated for the sample solution obtained from the formulation. Retention time of diclofenac sodium was 4.077. The peak area of the standard and sample solution was obtained from the software. The concentrations of the drugs were calculated using following formula:

$$\text{Concentration of drugs (mg/tablet)} = \frac{\text{Sample area} \times \text{standard concentration}}{\text{Standard area} \times \text{sample concentration}} \times \text{Dilution factor}$$

Method validation: Accuracy of the method was studied by recovery experiments. To the powdered tablets formulation (500 mg of paracetamol and 50 mg of diclofenac sodium) and working standard drugs were added at the level of 7.62, 15.24 and 22.86 % of the actual assay value. The extraction of drugs was followed using sample preparation procedure and were analyzed. The method validation result sheet of paracetamol and diclofenac sodium was given in Table-1. The percentage recovery of spiked sample was calculated and presented in Tables 2-4. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for 6 times and passing them through the assay procedure.

TABLE-1
THE METHOD VALIDATION RESULT SHEET OF
PARACETAMOL AND DICLOFENAC SODIUM

No.	Parameters	Experiment	Result	Limit	Ref.
1.	Plate Count	w.r.t. Paracetamol peak	3040.89	>2000	Manufacturer
		w.r.t. Diclofenac sodium peak	4159.21	>2000	Manufacturer
2.	Symmetry factor	w.r.t. Paracetamol peak	1.06	0.8-1.5	B.P 2007
		w.r.t. Diclofenac sodium peak	1.04	0.8-1.5	B.P 2007
3.	Resolution	w.r.t. Paracetamol and Diclofenac sodium peak	8.99	>1.5	B.P 2007
4.	% RSD (Precision)	Paracetamol peak area	0.20	Not more than 2.0	B.P 2007
		Paracetamol peak R.T	0.20		
		Paracetamol in amount	0.20		
		Diclofenac sodium peak area	0.20	Not more than 2.0	B.P 2007
		Diclofenac sodium peak R.T	0.20		
		Diclofenac sodium in amount.	0.20		
5.	Regression (R^2)	For Paracetamol	1.00	Not more than 1.0	Statistics
		For Diclofenac sodium	0.99		
6.	Recovery difference in solution G (%)	For Paracetamol	0.23	-	-
		For Diclofenac sodium	0.14		
7.	Recovery difference in solution H (%)	For Paracetamol	1.06	-	-
		For Diclofenac sodium	0.05		
8.	Recovery difference in solution I (%)	For Paracetamol	0.86	-	-
		For Diclofenac sodium	2.34		
9.	Accuracy for solution G (%)	For Paracetamol	99.76	-	-
		For Diclofenac sodium	99.85		
10.	Accuracy for solution H (%)	For Paracetamol	98.94	-	-
		For Diclofenac sodium	99.94		
11.	Accuracy for solution I (%)	For Paracetamol	99.13	-	-
		For Diclofenac sodium	97.65		
12.	Accuracy (%RSD)	For Paracetamol	0.43	Not more than 2.0	Statistics
		For Diclofenac sodium	1.30		

Linearity and range of the method was determined by analyzing mixed standard containing 0.1002 g of paracetamol and 0.0122 g of diclofenac sodium respectively. The calibration curve was plotted using peak area *vs.* concentration of standard solution; the values are presented in Tables 2-5. The limit of detection (LOD) and limit of quantification (LOQ) of the method was determined by injecting progressively low concentration of the standard solutions with the optimized chromatographic conditions.

Limit of detection (LOD) and Limit of quantitation (LOQ): The LOD and LOQ were separately determined based on the standard calibration curve. The residual standard deviation (RSD) of the regression line or the standard deviation of Y- intercept of regression lines may be used to calculate LOD and LOQ. LOD = 3.3 × D/S and LOQ = 10 × D/S, where D is the standard deviation of the Y intercepts of regression line and S is the slope of the calibration curve.

TABLE-2
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Name	Actual concentration in sample / mg MX332 (PS)	Concentration in solution G (mg)	Excess amount (%)	Concentration in solution H (mg)	Excess amount (%)	Concentration in solution I (mg)	Excess amount (%)
Paracetamol	5.25	5.65	7.61	6.05	15.24	6.45	22.85
Diclofenac sodium	0.47	0.51	10.33	0.56	20.67	0.62	31.02

TABLE-3
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Name	Average assay in sample MX332 (PS) (mg)	Average assay from Solution G (mg)	Excess Amount %	Average assay from Solution H (mg)	Excess Amount %	Average assay from Solution I (mg)	Excess Amount %
Paracetamol	508.02	545.51	7.38	580.05	14.18	619.74	21.99
Diclofenac sodium	45.46	50.10	10.19	54.84	20.62	60.63	33.36

TABLE-4
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Name	Recovery difference in Solution G (%)	Accuracy for Solution G (%)	Recovery difference in Solution H (%)	Accuracy for Solution H (%)	Recovery difference in Solution I (%)	Accuracy for Solution I (%)	Accuracy % RSD
Paracetamol	0.24	99.76	1.06	98.94	0.87	99.13	0.43
Diclofenac sodium	0.14	99.86	0.06	99.94	2.34	97.66	1.30

Maxrel® (Dey's Medical Stores (Mfg.) Ltd., 62 Bondel Road, Kolkata-700 019) each tablet contains paracetamol 500 mg and diclofenac sodium 50 mg.

TABLE-5
LINEARITY AND RANGE

Paracetamol			Diclofenac sodium		
Solution	Concentration (mg)	Peak area	Solution	Concentration (mg)	Peak area
C	80.49	11699205	C	9.90	310230
	80.07	11637763		9.97	312403
	80.26	11665364		9.29	307733
B	100.07	14544886	B	12.15	380628
	99.95	14526866		12.14	380099
	99.48	14458713		12.15	380658
D	116.78	16974086	D	14.54	455288
	118.65	17246092		14.56	456059
	118.42	17211433		14.50	454108

RESULTS AND DISCUSSION

The chromatograms of mixed sample solutions are presented in Figs. 3 and 4. The accuracy of the method was determined by recovery studies and the percentage of recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate. The precision procedure was satisfactory. The concentration range from 5.6539-6.4545 mg/mL of paracetamol and 0.5188-0.6161 mg/mL of diclofenac sodium were examined by the assay procedure and the calibration curves were plotted (Figs. 5 and 6). The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve shows linearity, which justifies the use of single point calibration and the proximity of maximum points to the calibration line demonstrated that the method has accurate linearity to the concentration to the analyte. The retention time of paracetamol and diclofenac sodium was found to be 2.12 and 4.08 min, respectively. The asymmetry factors of all the peaks were lesser than 2.0 and it showed that all peaks were symmetrical in shape. The precision of the proposed method was lesser than 2 % for both the drugs. The limit of detection (LOD) for paracetamol and diclofenac sodium was found to be 6 and 7 ng/mL, respectively (Table-4). The limit of quantification (LOQ) for paracetamol and diclofenac sodium was found to be 18 and 21 µg/mL, respectively (Table-4). The ruggedness of the method was determined by carrying out the experiment of different instruments like Waters HPLC 600 pumps, Merck Hitachi HPLC Lachrom pump-L-7100 with Merck Hitachi UV Lachrom detector L-7400 *etc*. by different operators using different columns of similar type like X-terra® (15 cm, i.d 4.6 mm, particle size 5 µm) of Waters. The recovery studies are showed in the Table-2. The mean % recovery was found to be 99.76 % in paracetamol and 99.86 % for diclofenac sodium. Assay of the combination in tables

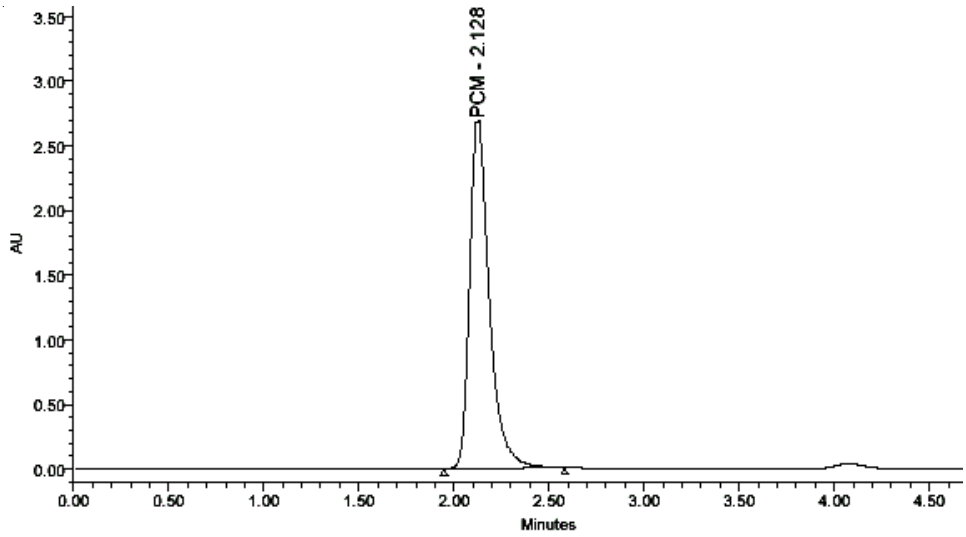

Fig. 3. A typical HPLC chromatogram of paracetamol in mixed sample at 257 nm

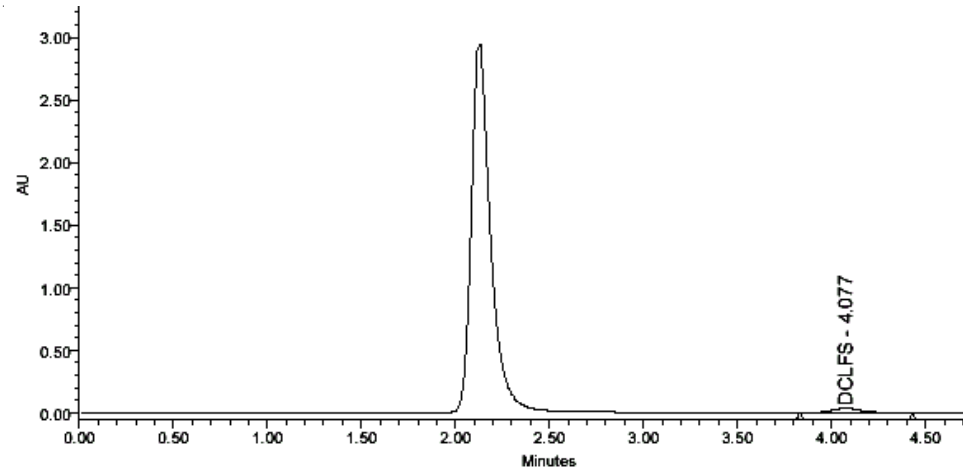

Fig. 4. A typical HPLC chromatogram of diclofenac sodium in mixed sample at 254 nm

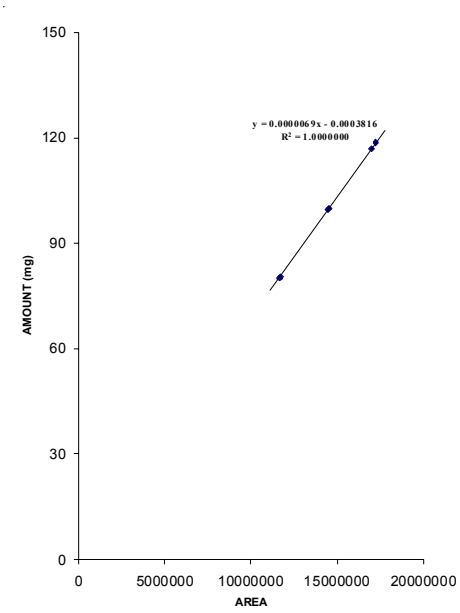

Fig. 5. Linearity curve for paracetamol

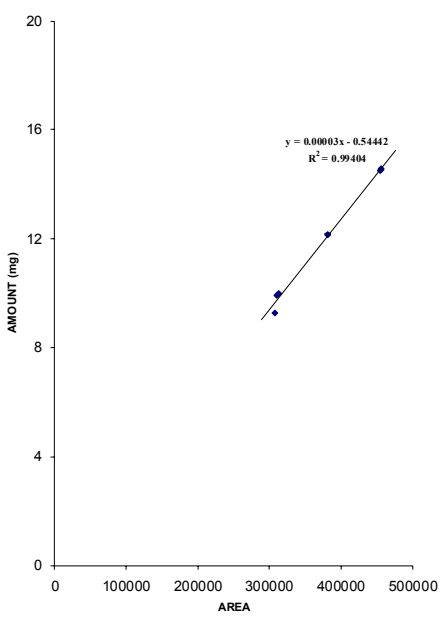

Fig. 6. Linearity curve for diclofenac sodium

dosage form was found to be for paracetamol 58.02 % and for diclofenac sodium 46.46 %. The estimated amount was within the acceptable limits of the labeled claim of the formulation. The total run time of the proposed method was 5 min. Robustness of the method was determined by making slight changes in the chromatographic conditions. After that there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table-6).

TABLE-6
SYSTEM SUITABILITY STUDIES

Parameters	Paracetamol	Diclofenac sodium
Theoretical plate count 5 sigma method	3040.89	4159.21
Resolution factor	8.99	8.99
Symmetry factor	1.06	1.04
LOD (ng/mL)	6.00	7.00
LOQ (µg/mL)	18.00	21.00

Conclusion

In conclusion the proposed RP-HPLC method was simple and precise because of the commonly used buffer, easier extraction procedures and shorter run time. The low retention time and requirement of minimum amount of solvent is the main focus in this experiment in comparison to other methods. The proposed method is highly accurate which showed good recovery of the drug sample. The analysis was less time consuming and more economical value. LOC and LOQ minimum than the other methods. The method gives a good resolution between paracetamol and diclofenac sodium within a short analysis time (< 6 min). Hence, the proposed method is very useful in routine quality control of combined dosage from containing paracetamol and diclofenac sodium in tablets.

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