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Simultaneous Estimation of Paracetamol and Aceclofenac in Combined Dosage Forms by RP-HPLC Method

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A simple, selective, rapid, precise and economical reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of paracetamol and aceclofenac from tablets. The method was carried out on a Hichrom C_{18} (25 cm × 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: 20 mM phosphate buffer (adjusted to pH 5.0 using ortho-phosphoric acid) (60 : 40 v/v) at a flow rate of 0.8 mL/min. Detection was carried out at 265 nm. Etoricoxib was used as internal standard. The retention time of paracetamol, aceclofenac and etoricoxib were 4.75, 6.44 and 8.83 min, respectively. The validation of the proposed method was also carried out according to ICH guidelines. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key Words: RP-HPLC, Paracetamol, Aceclofenac, Etoricoxib.

INTRODUCTION

Paracetamol is chemically *N*-(4-hydroxyphenyl)acetamide[1] and it is used as analgesic and antipyretic[2]. Aceclofenac is chemically designated as [[2-(2',6'-dichlorophenyl) amino]phenylacetoxyacetic acid] is a phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties and an improved gastro-intestinal tolerance[3]. Many methods have been described in the literature for the determination of paracetamol and aceclofenac individually and combination with other drugs[4-14]. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination of paracetamol 500 mg and aceclofenac 20 mg is available in tablets form in the market. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of paracetamol and aceclofenac in combined dosage forms. The method was validated according to procedures and acceptance criteria based on FDA guidelines[15] and recommendations of ICH[16].

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EXPERIMENTAL

Acetonitrile (HPLC grade) was procured from E. Merck (India) Ltd., Mumbai. Disodium hydrogen orthophosphate and orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water (HPLC grade) was obtained from a Milli-QRO water purification system. Reference standards of paracetamol and aceclofenac were procured from Aristo pharmaceuticals, Mumbai and etoricoxib was procured from Cadila Pharmaceuticals Ltd., Ahmedabad.

Chromatographic conditions: A Shimadzu® HPLC (LC-10AT *VP*) system was used for the analysis. The method was carried out on Hichrom C_{18} (25 cm × 4.6 mm i.d., 5µ) column as a stationary phase and acetonitrile: 20 mM phosphate buffer (adjusted to pH 5.0 using orthophosphoric acid), (60:40 v/v) as the mobile phase at flow rate of 0.8 mL/min. Rheodyne 7725*i* injector with 50 µL loop was used for the injection of samples. Detection was done at 265 nm. The mobile phase was filtered through 0.2 µ membrane filter and degassed.

Preparation of solutions: Standard stock solutions of 1 mg/mL of paracetamol, aceclofenac and etoricoxib were prepared separately using a solvent mixture of water and acetonitrile (1:1 v/v). From this standard stock solution, the mixed standard solution was prepared to contain 50 µg/mL of paracetamol, 2 µg/mL of aceclofenac and 100µg/mL of etoricoxib as internal standard.

Twenty tablets, each tablet containing 500 mg of paracetamol and 20 mg of aceclofenac were finely powdered and a quantity of powder equivalent to 50 mg of paracetamol and 2 mg of aceclofenac were weighed and transferred to a sintered glass crucible. To this 10 mL of 1 mg/mL of etoricoxib was added and the drugs were extracted with three quantities, each of 20 mL of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 mL with mobile phase and further dilution were made to get a concentration of 50 µg/mL of paracetamol and 2 µg/mL of aceclofenac (theoretical value) and 100 µg/mL of etoricoxib as internal standard and this solution was used for the estimation.

Assay method: With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of paracetamol, aceclofenac and etoricoxib were 4.75, 6.44 and 8.83 min, respectively. This procedure was repeated for the sample solution obtained from the formula-

tion. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated (Table-3). The concentrations of the drugs were calculated (Table-1) using following formula.

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

Method validation: Accuracy of the method was studied by recovery experiments. To the powdered tablet formulation (50 mg of paracetamol and 2 mg of aceclofenac), 10 mL of 1 mg/mL of etoricoxib solution and reference standard drugs were added at the level of 25, 50 and 100 % of the label claim. The extraction of drugs was followed using sample preparation procedure and those were analyzed. The percentage recovery was calculated and presented in Table-1. The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factors of drug peak and percentage RSD were calculated. In the interday variation studies, six repeated injection of standard and sample solutions were made for three consecutive days and the response factors of drug peak and percentage RSD were calculated and presented in Table-2.

TABLE-1
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Amount mg/tab		Label	Recovery*
	Label claim	Found ± SD*	claim* (%)	(%)
Paracetamol	500	499.07 ± 0.147	99.81	98.89
Aceclofenac	20	19.01 ±0.037	95.05	95.01

*Average of six determinations
OLFENAC-P (Olcare pharmaceuticals) each tablets containing 500 mg of paracetamol and 20 mg of aceclofenac.

Linearity and range of the method was determined by analyzing mixed standard containing 20-80 µg/mL of paracetamol and 0.5-3.5 µg/mL of aceclofenac (50-150 % of targeted level of the assay concentration) containing 100 µg/mL of etoricoxib as internal standard, respectively. The calibration curve was plotted using response factor *vs.* concentration of standard solution; the values are presented in Table-3. The limit of detection (LOD) and limit of quantification (LOQ) of the method was determined by injecting progressively low concentration of the standards solutions with the optimized chromatographic conditions; the values are presented in Table-4.

TABLE-2
INTRADAY AND INTERDAY PRECISION STUDIES

Intraday Studies				Interday Studies				
R_f Paracetamol	Mean (CV)	R_f Aceclofenac	Mean (CV)	Day	R_f Paracetamol	Mean (CV)	R_f Aceclofenac	Mean (CV)
				1	0.3610	0.3611 (0.0324)	0.0522	0.0522 (0.1974)
					0.3611		0.0521	
					0.3612		0.0523	
					0.3613		0.0520	
					0.3611		0.0522	
					0.3610		0.0522	
0.3612	0.3612 (0.0286)	0.0521	0.0521 (0.1981)	2	0.3609	0.3611 (0.0407)	0.0520	0.0521 (0.2017)
0.3613		0.0522			0.3610		0.0521	
0.3611		0.0521			0.3613		0.0522	
0.3610		0.0523			0.3611		0.0520	
0.3612		0.0520			0.3610		0.0521	
0.3612		0.0521			0.3612		0.0519	
				3	0.3611	0.3610 (0.0407)	0.0521	0.0521 (0.2017)
					0.3608		0.0520	
					0.3611		0.0519	
					0.3612		0.0521	
					0.3610		0.0522	
					0.3609		0.0520	

TABLE-3
LINEARITY AND RANGE

Internal standard peak area (100 µg/mL etoricoxib)	Paracetamol			Aceclofenac		
	Conc. (µg/mL)	Peak area	Response factor	Conc. (µg/mL)	Peak area	Response factor
11979960	20	1718492	0.143	0.5	151147	0.013
	30	2577835	0.215	1.0	302534	0.025
	40	3437240	0.287	1.5	455216	0.038
	50	4315895	0.360	2.0	624543	0.052
	60	5205470	0.435	2.5	755658	0.063
	70	6114715	0.510	3.0	906413	0.076
	80	6873960	0.574	3.5	1054874	0.088

TABLE-4
SYSTEM SUITABILITY STUDIES

Parameters	Paracetamol	Aceclofenac
Theoretical plates/meter	25478	29784
Resolution factor	1.32	
Asymmetry factor	0.91	1.02
LOD (ng/mL)	5	10
LOQ (ng/mL)	15	30

RESULTS AND DISCUSSION

The chromatograms of mixed sample solutions are presented in Fig. 1. The overlain UV spectrum of paracetamol, aceclofenac and internal standard are shown in Fig. 2. 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 20-80 µg/mL of paracetamol and 0.5-3.5 µg/mL of aceclofenac were examined by the assay procedure and the calibration curves were plotted (Fig. 3). The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve passes through the origin, which justifies the use of single point calibration and the proximity of all points to the calibration line demonstrated that the method has adequate linearity to the concentration of the analyte.

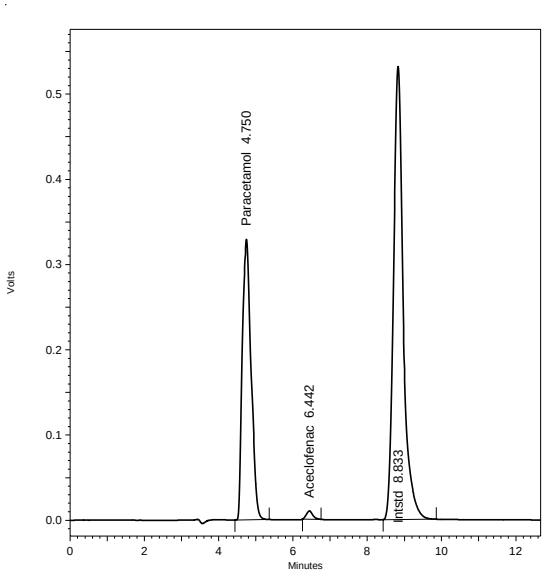

Fig. 1. Typical chromatogram of sample solution

The limit of detections (LOD) for paracetamol and aceclofenac were found to be 5 ng/mL and 10 ng/mL, respectively (Table-4). The limit of quantifications (LOQ) for paracetamol and aceclofenac were 15 ng/mL and 30 ng/mL, respectively (Table-4). The ruggedness of the method was determined by carrying out the experiment of different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil, Phenomenex LUNA, and Hichrom. Robustness of the method was determined by making slight changes in the chromatographic conditions.

Further there is no interference due to excipient. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table-4). The proposed HPLC method is simple, selective, precise, rugged, robust, linear and rapid. Hence this method can be applied for the quality control of raw materials, formulations and dissolution studies.

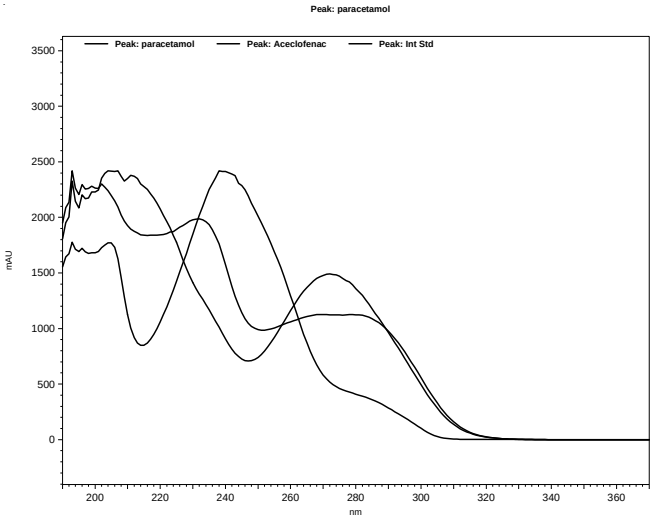

Fig. 2 Overlain UV spectrum of paracetamol, aceclofenac and internal standard

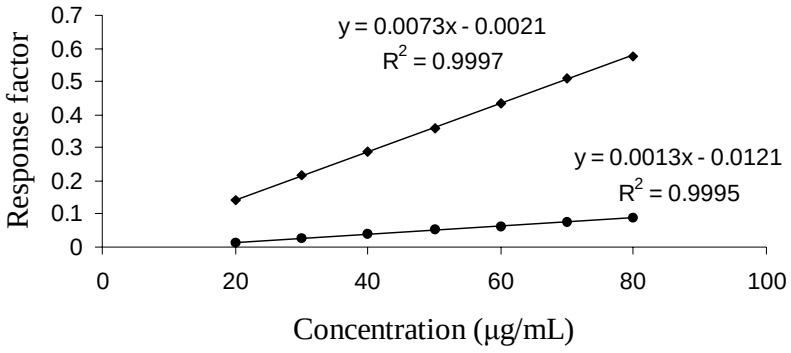

Fig. 3. Calibration curve of paracetamol and aceclofenac

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