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Simultaneous Estimation of Paracetamol and Valdecoxib 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 valdecoxib from tablets. The method was carried out on a Phenomenex Gemini C_{18} (25 cm × 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: 20 mM octane sulphonic acid (adjusted to pH 3.0 using orthophosphoric acid) (50:50 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 250 nm. Bromhexine was used as an internal standard. The retention time of paracetamol, bromhexine and valdecoxib were 2.97, 5.85 and 8.05 min, respectively. The validation of the proposed method was also carried out according to ICH guidelines.

Key Words: RP-HPLC, Paracetamol, Valdecoxib, Bromhexine.

INTRODUCTION

Paracetamol is chemically *N*-(4-hydroxyphenyl) acetamide[1] and it is used as analgesic and antipyretic[2]. Valdecoxib is chemically designated as 4-(5-methyl-3-phenylisoxazol-4-yl) benzene sulfonamide, diaryl substituted isoxazole and also a novel cox-2 inhibitor[3] with a lower incidence of ulcer complication. It has been found to be effective analgesic in postoperative pain[4]. Many methods have been described in the literature for the determination of valdecoxib and paracetamol individually and combination with other drugs[5-14]. 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 valdecoxib 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 valdecoxib 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. Octane sulphonic acid and orthophosphoric acid (AR grade) were procured from Ranbaxy fine chemicals, New Delhi. Water (HPLC grade) was obtained from a Milli-QRO water purification system. Reference standards of paracetamol and valdecoxib were procured from Unichem Pharmaceuticals Ltd., Mumbai and bromhexine 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 Phenomenex Gemini C_{18} (25 cm × 4.6 mm i.d., 5 µ) column as a stationary phase and acetonitrile: 20 mM octane sulphonic acid (adjusted to pH 3.0 using orthophosphoric acid), (50:50 v/v) as the mobile phase at flow rate of 1.0 mL/min. Rheodyne 7725*i* injector with 20 µL loop was used for the injection of samples. Detection was done at 250 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 and valdecoxib 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 valdecoxib, 300 µg/mL of bromhexine as internal standard.

Twenty tablets, each tablet containing 500 mg of paracetamol and 20 mg of valdecoxib were finely powdered, and a quantity of powder equivalent to 50 mg of paracetamol and 2 mg of valdecoxib were weighed and transferred to a sintered glass crucible. To this 30 mL of 1 mg/mL of bromhexine 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 valdecoxib (theoretical value) and 300 µg/mL of bromhexine 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, bromhexine and valdecoxib were 2.97, 5.85 and 8.05 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. 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-2). The concentration 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 valdecoxib), 30 mL of 1 mg/mL of bromhexine 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. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for 10 times and passing them through the assay procedure.

TABLE-1
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Amount mg/tab		Label claim* (%)	Recovery* (%)
	Label claim	Found ± SD*		
Paracetamol	500	499.07 ± 0.012	99.99	99.95
Valdecoxib	20	19.94 ±0.043	99.70	99.89

*Average of six determinations
Alcox plus (Unichem Pharmaceuticals, Mumbai) each tablets containing 500 mg of paracetamol and 20 mg of valdecoxib

TABLE-2
LINEARITY AND RANGE

Internal standard peak area (300 µg/mL bromhexine)	Paracetamol			Valdecoxib		
	Conc. (µg/mL)	Peak area	Response factor	Conc. (µg/mL)	Peak area	Response factor
15988845	20	2991062	0.157	0.5	51997	0.003
	30	4489587	0.281	1.0	103978	0.007
	40	5982131	0.374	1.5	155961	0.010
	50	7478557	0.468	2.0	208935	0.013
	60	8973279	0.561	2.5	259818	0.016
	70	10478729	0.655	3.0	303788	0.019
	80	11964263	0.748	3.5	351755	0.022

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 valdecoxib (50-150 % of targeted level of the assay concentration) containing 300 µg/mL of bromhexine as internal standard, respectively. The calibration curve was plotted using response factor *vs.* concentration of standard solution; the values are presented in Table-2. 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.

RESULTS AND DISCUSSION

The chromatograms of mixed sample solutions are presented in Fig. 1. The overlain UV spectrum of paracetamol, valdecoxib and internal standard are shown in Fig. 2. The accuracy of the method was determined by recovery studies were carried out 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 valdecoxib were examined by the assay procedure and the calibration curves were plotted (Fig. 3 and 4). 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 maximum points to the calibration line demonstrated that the method has adequate linearity to the concentration of the analyte.

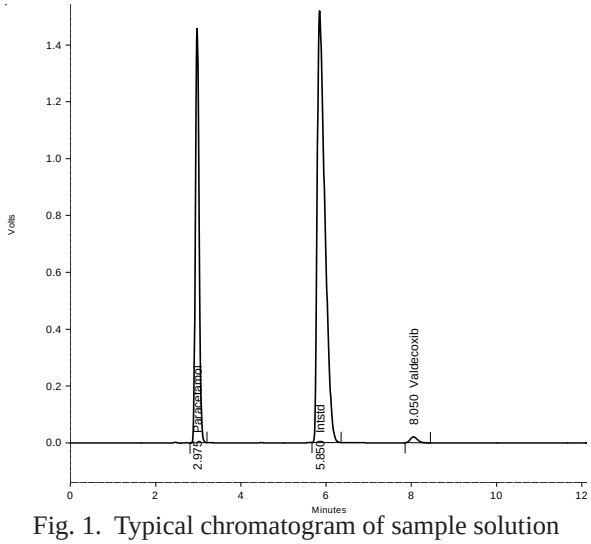

Fig. 1. Typical chromatogram of sample solution

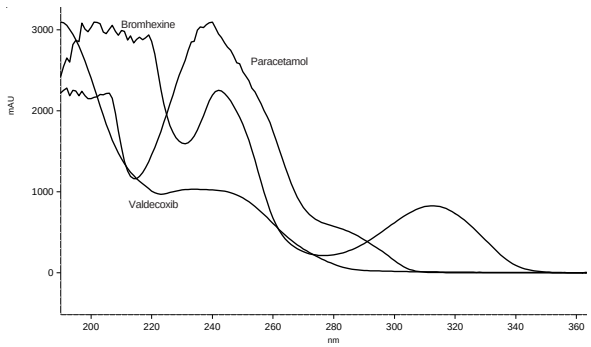

Fig. 2. Overlain UV spectrum of paracetamol, bromhexine and valdecoxib

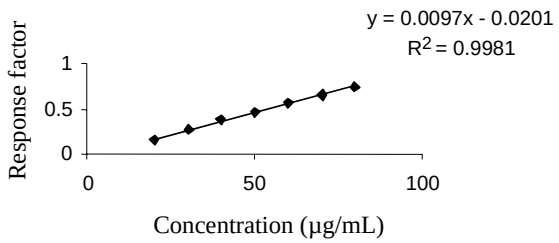

Fig. 3. Calibration curve for paracetamol

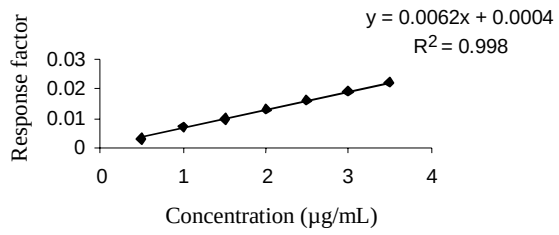

Fig. 4. Calibration curve for valdecoxib

The limit of detections (LOD) for paracetamol and valdecoxib were found to be 5 ng/mL and 10 ng/mL, respectively (Table-3). The limit of quantifications (LOQ) for paracetamol and valdecoxib were 15 ng/mL and 30 ng/mL (Table-3). 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-3). 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.

TABLE-3
SYSTEM SUITABILITY STUDIES

Parameters	Paracetamol	Valdecoxib
Theoretical plates/meter	27894	312478
Resolution factor	1.35	
Asymmetry factor	0.98	1.01
LOD (ng/mL)	5	10
LOQ (ng/mL)	15	30

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