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A Reverse Phase High Performance Liquid Chromatography Method For Simultaneous Estimation Of Paracetamol And Valdecoxib In Pharmaceutical Formulation

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase 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 x 4.6 mm i.d., 5μ) column with a mobile phase consisting of acetonitrile: 20 mM octane sulphonic acid buffer (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 240 nm. Bromhexine was used as an internal standard. The retention time of paracetamol, bromhexine and valdecoxib was 2.97, 5.85 and 8.05 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

KEYWORDS

RP-HPLC;
Paracetamol;
Valdecoxib.

INTRODUCTION

Paracetamol is chemically *N*-(4-hydroxyphenyl) acetamide. It is used as an analgesic and antipyretic. Valdecoxib is chemically designated as 4-(5-methyl-3-phenyl-4-isox-azolyl) benzene sulfonamide and is a diaryl substituted isoxazole. Valdecoxib is a non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic and antipyretic properties. Many methods have been described in the literature for the determination of valdecoxib and paracetamol individually and combination with other drugs[1-11]. However, there is no HPLC method reported for the simultaneous estimation of these

drugs in combined dosage forms. Fixed dose combination containing paracetamol 500 mg and valdecoxib 20 mg is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of paracetamol and aceclofenac in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines[12,13].

EXPERIMENTAL

Reagents and chemicals

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 standard of valdecoxib and paracetamol are procured from Unichem pharmaceuticals, Mumbai and bromhexine was procured from Cadila Pharmaceuticals Ltd, Ahmedabad.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 50µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Phenomenex Gemini C_{18} column (25cm x 4.6mm i.d., 5µ) was used for the separation, mobile phase of a mixture of acetonitrile and 20 mM octane sulphonic acid buffer (adjusted to pH 3.0 using orthophosphoric acid), (50:50 v/v) was delivered at a flow rate of 1.0 ml/min with detection at 240 nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 50µl; analysis was performed at ambient temperature.

Preparation of standard solutions

Standard stock solutions of 1mg/ml of paracetamol and valdecoxib were prepared separately using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solution, mixed standard solution was prepared to contain 50µg/ml of paracetamol, 2µg/ml of valdecoxib and 300µg/ml of bromhexine as internal standard.

Preparation of sample solutions

Twenty tablets, each containing 500mg of paracetamol and 20 mg of valdecoxib were weighed and finely powdered; a quantity of powder equivalent to 50 mg of paracetamol and 2 mg of valdecoxib was weighed and transferred to a sintered glass crucible. To this 30ml of 1mg/ml solution 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 dilutions were made to get a concentration of 50µg/ml of paracetamol, 2µg/ml of valdecoxib (theoretical value) and 300µg/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 was found to be 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

TABLE 1: Results of analysis of formulation and recovery studies

Drug	Amount mg/ tab		%	%
	Labeled	Found*	Label claim*	Recovery*
Paracetamol	500	499.98 ± 0.012	99.99 ± 1.041	99.95 ± 0.871
Valdecoxib	20	19.94 ± 0.043	99.70 ± 1.096	99.89 ± 0.681

***Average of 6 determinations, mean ± standard deviation**
VALCOX PLUS (Unichem pharmaceuticals, Mumbai) each tablet containing 500 mg of Paracetamol and 20 mg of Valdecoxib

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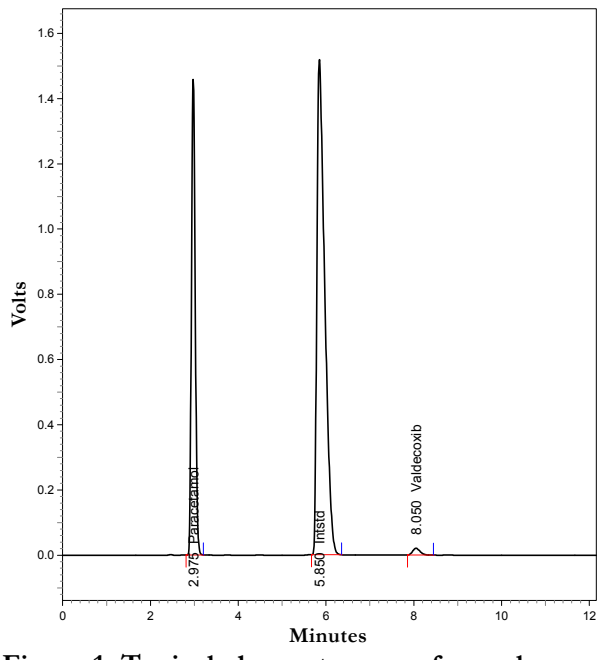

Figure 1: Typical chromatogram of sample solution

calculated. The concentration of the drugs were calculated (TABLE 1) using following formula

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

RESULTS AND DISCUSSION

Estimation of paracetamol and valdecoxib in dosage forms

Estimation of paracetamol and valdecoxib in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The typical chromatogram of sample solution is given in figure 1. Detection was done at 240 nm. The overlaid UV spectrum of paracetamol and valdecoxib is shown in figure 2. The

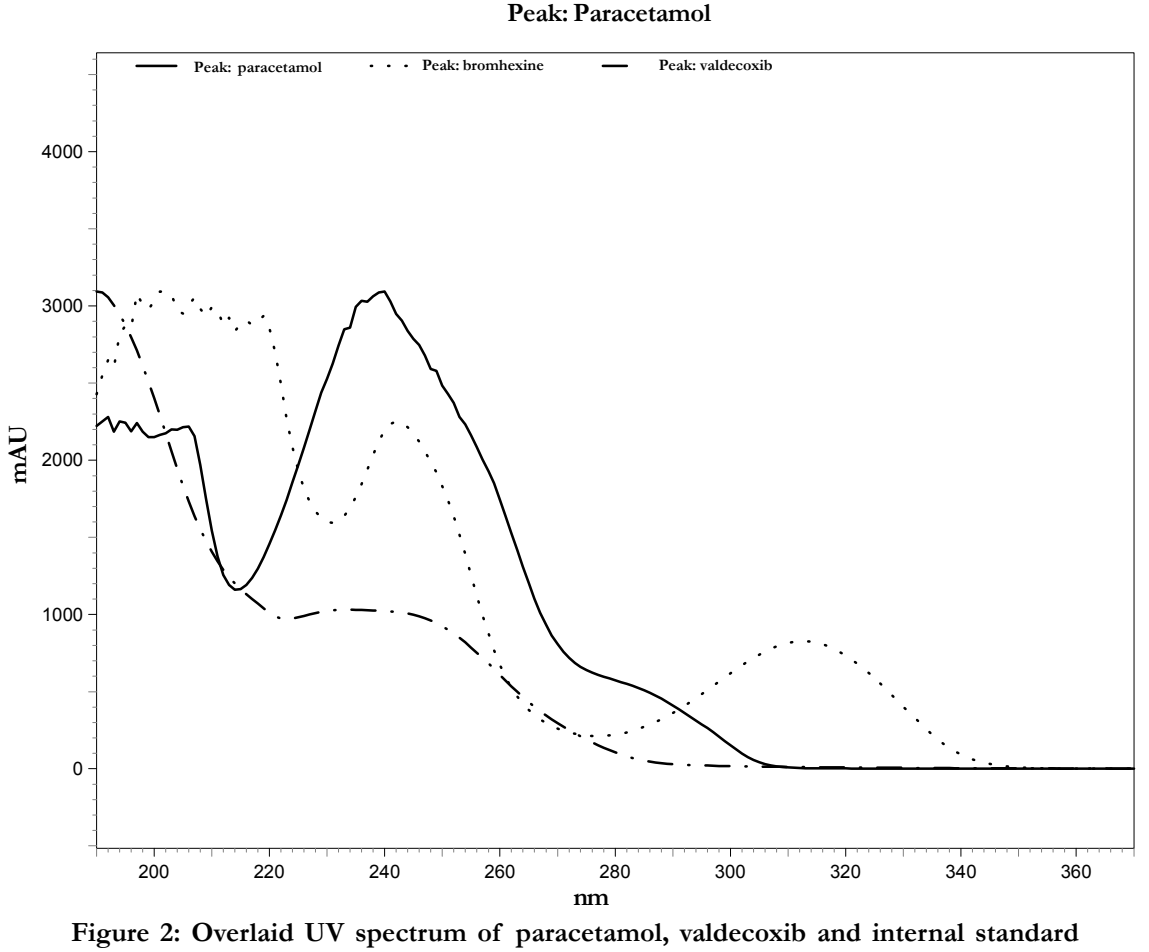

Figure 2: Overlaid UV spectrum of paracetamol, valdecoxib and internal standard

peak area ratio of standard and sample solutions were calculated. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in TABLE 1. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

Method validation

1. Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drugs peaks and percentage RSD were calculated and presented in TABLE 2. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated and presented in TABLE 2. From the data obtained, the developed RP-HPLC method was found to be precise.

2. Linearity and range

The linearity of the method was determined at seven concentration levels ranging from 20.0 to 80.0 µg/ml for paracetamol and 0.5 to 3.5µg/ml for valdecoxib (TABLE 3). The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y=0.0094x-0.0016 (R^2=0.9998) for paracetamol and y=0.006 x + 7E-05 (R^2=0.9998) for valdecoxib. The results show that an excellent correlation exists between response factor and concentration of drugs within the concen-

TABLE 2: Intraday and interday precision studies

Intraday studies				Interday studies				
RF* of Paracetamol	Mean (%RSD*)	RF of Valdecoxib	Mean (%RSD)	Day	RF of Paracetamol	Mean (%RSD)	RF of Valdecoxib	Mean (%RSD)
0.3656		0.0571			0.3677		0.0534	
0.3574		0.0536			0.3663		0.0552	
0.3663	0.3632	0.0541		Day 1	0.3674	0.3672	0.0534	0.0527
0.3677	(0.0741)	0.0522			0.3653	(0.4121)	0.0551	(0.2143)
0.3588		0.0534	0.0548		0.3652		0.0531	
0.3634		0.0548	(0.1981)		0.3672		0.0527	
					0.3619		0.0525	
					0.3653		0.0535	
				Day 2	0.3647	0.3644	0.0529	0.0526
					0.3653	(0.4712)	0.0533	(0.2412)
					0.3662		0.0541	
					0.3644		0.0526	
					0.3639		0.0544	
					0.3621		0.0542	
					0.3671		0.0541	
				Day 3	0.3633	0.3627	0.0543	0.0545
					0.3622	(0.5413)	0.0548	(0.2217)
					0.3627		0.0545	

*** RF-Response Factor, % R.S.D - Relative standard deviation**

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TABLE 3: Linearity and range

Internal standard peak area (300µg/ml Bromhexine)	Paracetamol			Valdecoxib		
	Concentration (µg/ml)	Peak area	Response factor	Concentration (µg/ml)	Peak area	Response factor
15988845	20	2991062	0.187	0.5	51997	0.0030
	30	4489587	0.281	1.0	103978	0.0061
	40	5982131	0.364	1.5	155961	0.0091
	50	7478557	0.468	2.0	208935	0.0123
	60	8973279	0.561	2.5	259818	0.0151
	70	10478729	0.655	3.0	311813	0.0180
	80	11964263	0.748	3.5	364886	0.0210

tration range indicated above. The calibration curves are shown in figure 3 and 4.

3. Limit of detection and limit of quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for paracetamol and valdecoxib was found to be 5 ng/ml and 10 ng/ml, respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 15 ng/ml and 30 ng/ml for paracetamol and valdecoxib, respectively (TABLE 4).

TABLE 4: System suitability studies

S. No.	Parameters	Paracetamol	Valdecoxib
1	Theoretical plate/meter	27894	312478
2	Resolution factor	1.35	
3	Asymmetric factor	0.98	1.01
4	LOD (ng/ml)	5	10
5	LOQ (ng/ml)	15	30

4. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different opera-

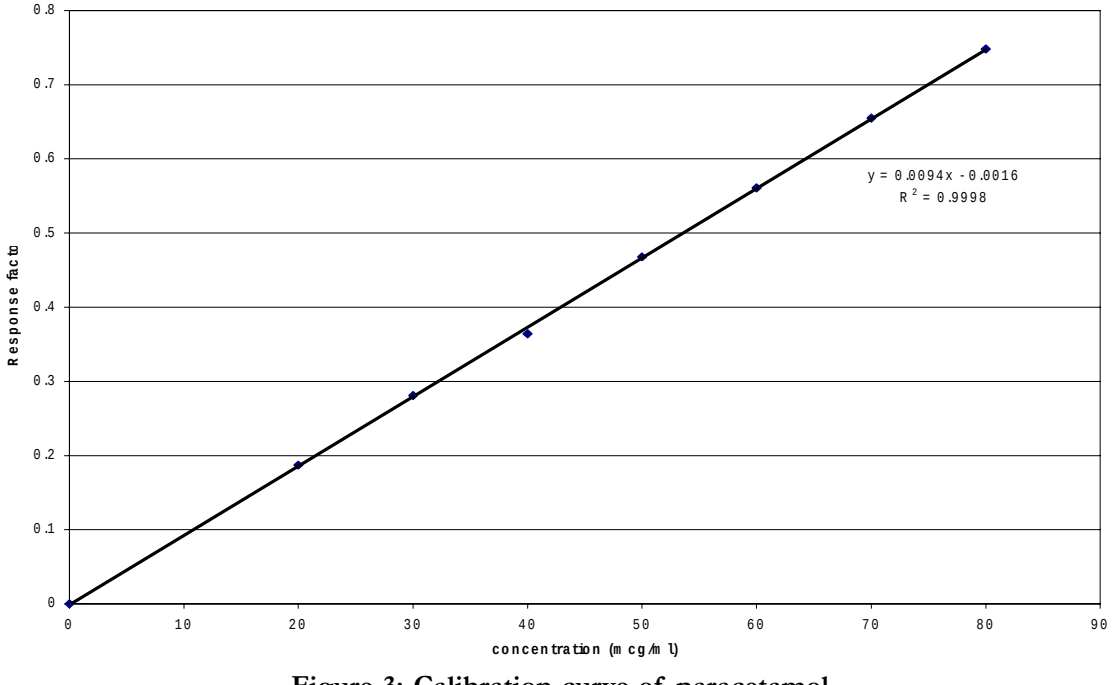

Figure 3: Calibration curve of paracetamol

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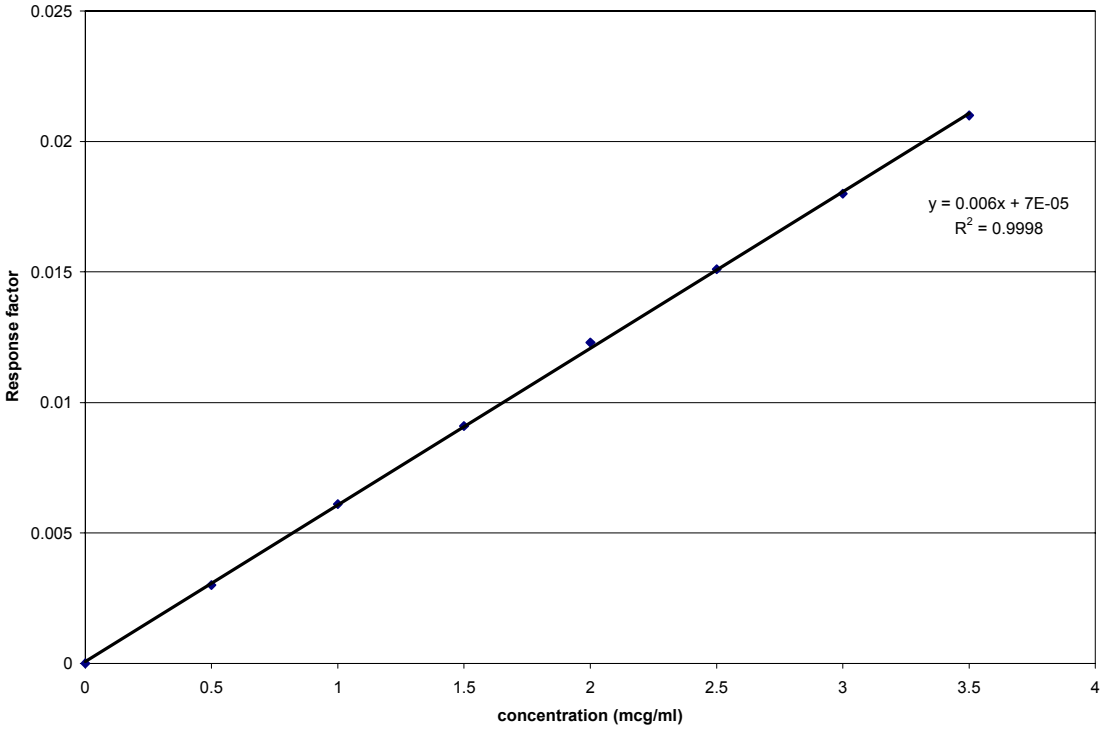

Figure 4: Calibration curve of valdecoxib

tors using different columns of similar type like Hypersil C_{18}, Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrate that the RP-HPLC methods have developed are rugged and robusted.

5 Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of paraceta mol and valdecoxib remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

6. System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (TABLE 4). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of paracetamol and valdecoxib in combined dosage forms are accurate, precise, linear, rugged, robusted, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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