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Research Article

Analytical Method Development and validation for simultaneous Estimation of Paracetamol and Propyphenazone in their Combined Pharmaceutical dosage form by RP-HPLC Method

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Paracetamol and Propyphenazone in their combined pharmaceutical dosage form. The method was carried out on a Inertsil ODS 3V (250 x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile: water in the ratio of 70:30 v/v at a flow rate of 1.0 mL/min. Detection was carried out at 238 nm.
The retention times of Paracetamol and Propyphenazone were 5.87 and 13.63min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of Quantitation. The proposed method can be used for the estimation of these drugs in routine quality control.

Keywords: Paracetamol, Propyphenazone, RP-HPLC method

INTRODUCTION

Paracetamol is chemically as *N*-(4-hydroxyphenyl) acetamide. It is used as an analgesic and antipyretic [1, 2,6]. Propyphenazone is chemically 4-Isopropyl-2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one, a dipyrazolone derivatives exhibits anti-inflammatory and analgesic properties[3,4,5]. Many methods have been described in the literature for the determination of Paracetamol and no single method has been available for estimating the Propyphenazone individually and No single method was reported for the estimation in combined dosage form. Fixed dose combination containing Paracetamol 100mg and Propyphenazone 100mg is available in the tablet form in the market. The present work describes the development of a validated RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated following the ICH guidelines [7,8]. The chemical structures of Paracetamol and Propyphenazone are shown in Figure 1 (A), (B).[1, 4]
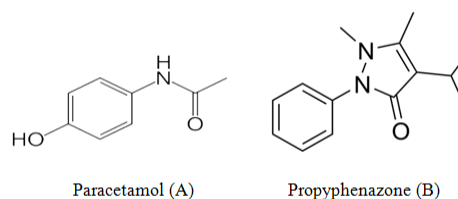

Figure-1: Chemical structure of (A) Paracetamol and (B) Propyphenazone

EXPERIMENTAL WORK

Reagents and chemicals:

Acetonitrile (HPLC grade) and water (HPLC grade) was procured from HD fine chemicals (India) Ltd, Ahmedabad. Reference standards of Paracetamol and Propyphenazone were procured from Meghmani pharmaceuticals, Ahmedabad and Vani Pharma Pvt. Ltd, Hyderabad.

Apparatus and chromatographic conditions:

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-2010 CHT with, UV-detector of 2010 CHT, auto injector loop system.LC-solution was applied for data collecting and processing (Shimadzu, Japan). A Inertsil ODS 3V C_{18} column (250 x 4.6 mm i.d., 5μ) was used for the separation, mobile phase of a mixture of Acetonitrile and water in the ratio of 70:30 v/v was delivered at a flow rate of 1.0 mL/min with detection at 238nm. Analysis was performed at ambient temperature.

Preparation of standard solutions:

Standard stock solutions of 1.0 mg/mL Paracetamol and Propyphenazone 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 10.0 μg/Ml of Paracetamol, and 10.0 μg/mL of Propyphenazone.

Preparation of sample solution:

Twenty Tablets, each containing 100mg of Paracetamol and 100mg of Propyphenazone were weighed and finely powdered; a quantity of powder equivalent to 100mg of Paracetamol and 100mg of Propyphenazone was weighed and transferred to a 100ml volumetric flask. From this withdraw 10ml of the resulting solution to another volumetric flask of 100ml.make up the volume using the mixture of acetonitrile and water (1:1 v/v). The necessary dilution were made with mobile phase to get a concentration of 10.0 μg/mL of Paracetamol, and 10.0 μg/mL of Propyphenazone 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 times of Paracetamol, and Propyphenazone were found to be 5.87 and 13.63min, respectively. This procedure was repeated for the sample solution for five times obtained from the formulation.

RESULTS AND DISCUSSION

Estimation of Paracetamol and Propyphenazone in dosage forms:

The HPLC procedure was optimized with a view to develop precise and stable assay method. Both the pure drugs Paracetamol and Propyphenazone were run in different mobile phase compositions with Inertsil ODS 3V C_{18} column. The flow rate was also varied from 0.5 mL to 1.4 mL/min. Finally Inertsil ODS 3V C_{18} column (250 x 4.6 mm i.d., 5μ), with a mobile phase of a mixture of acetonitrile: water (70:30 v/v) at a flow rate of 1.0 mL/min with detection at 238nm gave sharp and symmetrical peaks with retention time 5.87 and 13.63 for Paracetamol and Propyphenazone respectively. The typical chromatogram of sample solution linearity is shown in Fig.2. The assay procedures were repeated for five times and mean peak area and mean weight of standard drugs was calculated. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

METHOD VALIDATION

Accuracy and precision:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out three times for each sample of standard edition (80%, 100%, and 120%) and the percentage recovery and standard deviation of the percentage recovery were calculated. 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 intraday studies, three repeated injections of sample solutions of three different concentrations were made. In the inter-day variation studies, three repeated injections of sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range:
The linearity of the method was determined at five concentration levels ranging from 10μg/mL to 50μg/mL for Paracetamol and 10 to 50μg/mL for Propyphenazone. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 44226x+1017.657 (R^2=0.998) for Paracetamol and y=66836x+10000 (R^2=0.998) for Propyphenazone. The results shows that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above. The calibration curves are shown in Fig. 3, 4.

Limit of Detection and Limit of Quantification:
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by auto-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. The LOD for Paracetamol and Propyphenazone was found to be 0.0759ng/mL and 0.49374ng/mL, respectively.

The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ was 0.23010ng/mL and 1.4961ng/mL for Paracetamol and Propyphenazone, respectively (Table II).

Ruggedness and Robustness:
The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010cHT), by different operators using different columns of similar type like Hypersil C_{18}, Inersil C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

System suitability studies:
The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table II). 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 Propyphenazone in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations.

Table – 1: Results of Analysis of formulation and Recovery Studies

Drug	Amount mg/ tab		% Label claim*	% Recovery*
	labelled	found		
Paracetamol	100mg	98.104 ±1.6551	99.92 ± 1.010	98.110
Propyphenazone	100mg	100.58 ± 1.4078	99.87 ± 1.210	100.010

*Average of five determinations of sample, mean ± standard deviation cipladon (cipla pharmaceuticals) each tablet containing 100 mg of Paracetamol and 100 mg of Propyphenazone.

Table 2: System Suitability Studies

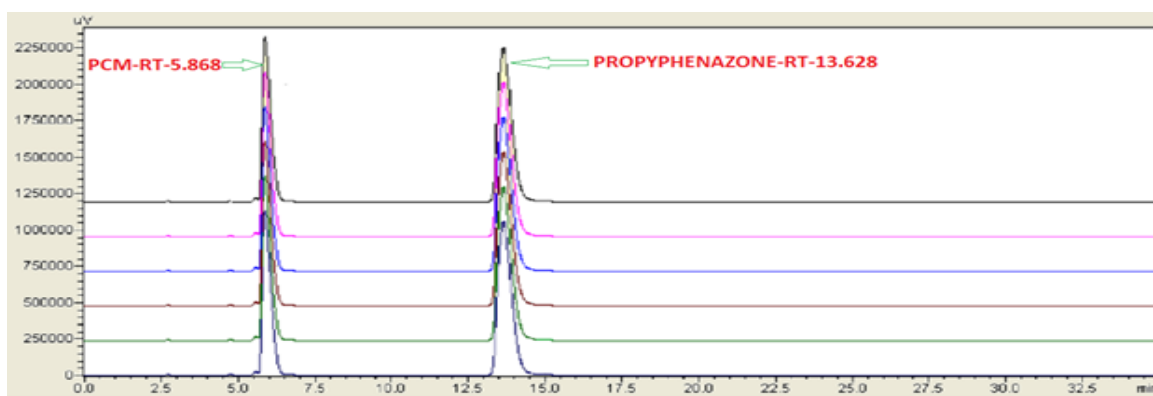| S.No.	Parameters	Paracetamol	Propyphenazone
1	Linearity range	10-50µg/mL	10-50µg/mL
2	Regression equation Y = mx + c*	44226x+10705	66836x+30691
3	Correlation coefficient	0.998	0.998
4	Theoretical plate/meter	1855.398	4054.551
5	Resolution factor	2.23	11.0
6	Asymmetric factor	1.89	1.46
7	LOD (ng/mL)	0.0759ng/mL	0.4937ng/mL
8	LOQ (ng/mL)	0.23010ng/mL	1.4937ng/mL

Fig 2. Typical Chromatogram of Sample Solution for linearity (10-50µg/Ml) and Propyphenazone (10-50µg/mL) Baseline mode overlapping
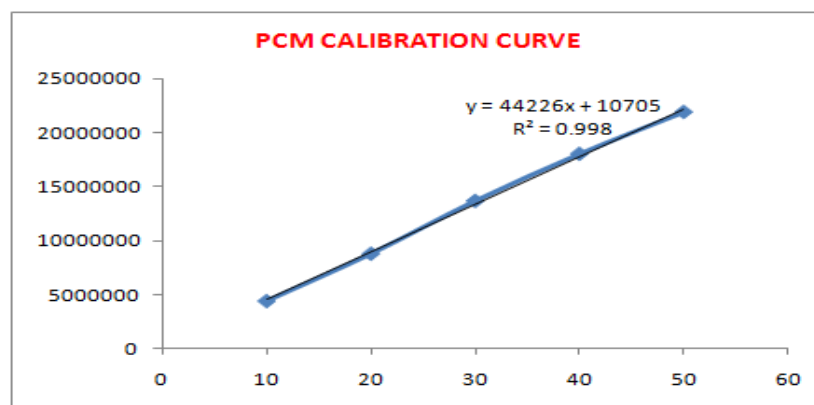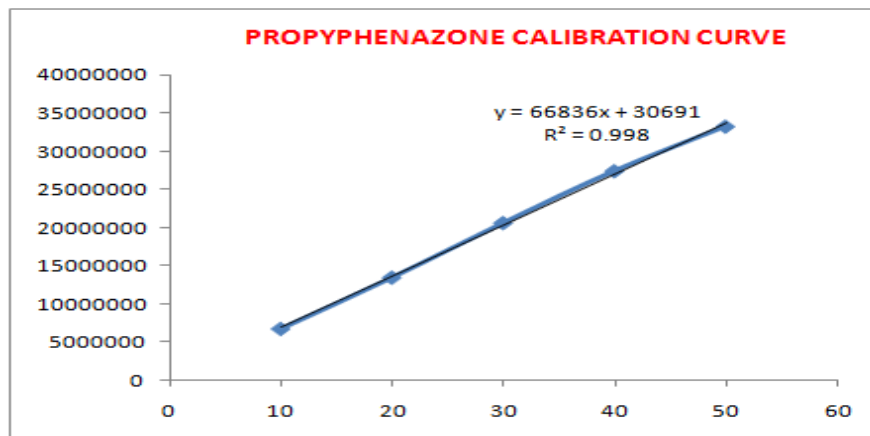

Fig 3. Calibration Curve of Paracetamol

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Fig 4. Calibration Curve of Propyphenazone

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