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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICLOFENAC POTASSIUM IN PHARMACEUTICAL FORMULATION

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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 diclofenac potassium from pharmaceutical formulation. The method was carried out on a Phenomenex LUNA C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: sodium dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid) in the ratio of 70:30 v/v at a flow rate of 1.0 mL/min. Detection was carried out at 278 nm. Aceclofenac was used as an internal standard. The retention times of paracetamol, diclofenac potassium and aceclofenac were 5.9, 9.4 and 3.12 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.
KEY WORDS: RP-HPLC, Paracetamol, Diclofenac potassium and Acelofenac.

INTRODUCTION

Paracetamol is chemically as *N*-(4-hydroxyphenyl) acetamide. It is used as an analgesic and antipyretic. Diclofenac potassium is chemically potassium (o-(2, 6-dichloroanilino) phenyl) acetate, a non steroidal anti-inflammatory drug (NSAID) exhibits anti-inflammatory and analgesic properties. Many methods have been described in the literature for the determination of paracetamol and diclofenac potassium individually and in combination with other drugs[1-6]. No single method was reported for the estimation in combined dosage form. Fixed dose combination containing paracetamol 500mg and diclofenac potassium 50mg 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].

EXPERIMENTAL

Reagents and chemicals:

Acetonitrile (HPLC grade) was procured from E.Merck (India) Ltd, Mumbai. Sodium dihydrogen ortho phosphate 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 diclofenac potassium were procured from Unichem pharmaceuticals, Mumbai and aceclofenac was procured from Divi`s laboratories Ltd, Hyderabad.

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 50 µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Phenomenex LUNA C_{18} column (25 cm x 4.6 mm i.d., 5µ) was used for the separation, mobile phase of a mixture of acetonitrile and sodium dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid in the ratio of 70:30 v/v was delivered at a flow rate of 1.0 mL/min with detection at 278 nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 50 µL; Analysis was performed at ambient temperature.

Preparation of standard solutions:
Standard stock solutions of 1.0 mg/mL paracetamol, diclofenac potassium and aceclofenac 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 50.0 µg/mL of paracetamol, 5.0 µg/mL of diclofenac potassium and 50.0 µg/mL of aceclofenac as internal standard.

Preparation of sample solution:
Twenty Tablets, each containing 500mg of paracetamol and 50mg of diclofenac potassium were weighed and finely powdered; a quantity of powder equivalent to 50mg of paracetamol and 5mg of diclofenac potassium was weighed and transferred to a sintered glass crucible. To this 5.0 mL of 1.0 mg/mL solution of aceclofenac 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 50.0 µg/mL of paracetamol, 5.0 µg/mL of diclofenac sodium (theoretical value) and 50.0 µg/mL of aceclofenac 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 times of paracetamol, diclofenac potassium and aceclofenac were found to be 5.9, 9.4 and 3.12 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. The concentration of the drugs were calculated (Table I) 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 diclofenac potassium in dosage forms:
The HPLC procedure was optimized with a view to develop precise and stable assay method. Both the pure drugs paracetamol and diclofenac potassium were run in different mobile phase compositions with different C_{18} columns (Kromacil 25 cm x 4.6 mm i.d., 5µ), Phenomenex Luna C_{18} column (25cm x 4.6mm i.d., 5µ) . The flow rate was also varied from 0.5 mL to 1.2 mL/min .Finally Phenomenex C_{18} column (25 cm x 4.6 mm i.d., 5µ), with a mobile phase of a mixture of acetonitrile: sodium dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid) (70:30 v/v) at a flow rate of 1.0 mL/min with a detection at 278nm gave sharp and symmetrical peaks with retention time 5.9 and 9.4 for paracetamol and diclofenac potassium respectively. The typical chromatogram of sample solution is shown in Fig.1. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in Table I. 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 six times 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 intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. 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. 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 100.0 to 600.0 µg/mL for paracetamol and 10 to 60.0µg/mL for diclofenac potassium. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0015x-0.0217 (R^2=0.9982) for paracetamol and y=0.0017x-0.001 (R^2=0.9803) for diclofenac potassium. 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. 2.

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 diclofenac potassium was found to be 10 ng/mL and 5.0 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 30 ng/mL and 15 ng/mL for paracetamol and diclofenac potassium, 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-2010A_{HT}), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex Gemini C_{18} and Hichrom 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.

Solution stability:
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for both solutions, the retention time and peak area of paracetamol and diclofenac potassium 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 hours, which was sufficient to complete the whole analytical process.

CONCLUSION
The proposed RP-HPLC method for the simultaneous estimation of paracetamol and diclofenac potassium 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 and dissolution studies.

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

Drug	Amount mg/ tab		% Label claim*	% Recovery*
	Labelled	Found *		
Paracetamol	500.0	499.07 ± 1.047	99.81 ± 1.02	98.89 ± 0.81
Diclofenac potassium	50.0	48.59 ± 1.132	97.18 ± 1.41	95.01 ± 0.57

*Average of six determinations, mean ± standard deviation
DIAPRASE (Arigo pharmaceuticals, Chennai) each tablet containing 500 mg of paracetamol and 50 mg of diclofenac Potassium

Table 2: System Suitability Studies

S. No.	Parameters	Paracetamol	Diclofenac
1	Linearity range	100-600 µg/mL	10-60 µg/mL
2	Regression equation Y = mx + c*	0.0015x-0.0217	0.0017x-0.001
3	Correlation coefficient	0.9982	0.9803
4	Theoretical plate/meter	2085.64	3015.46
5	Resolution factor	2.23	2.56
6	Asymmetric factor	0	2.15
8	LOD (ng/mL)	10	5
9	LOQ (ng/mL)	30	15

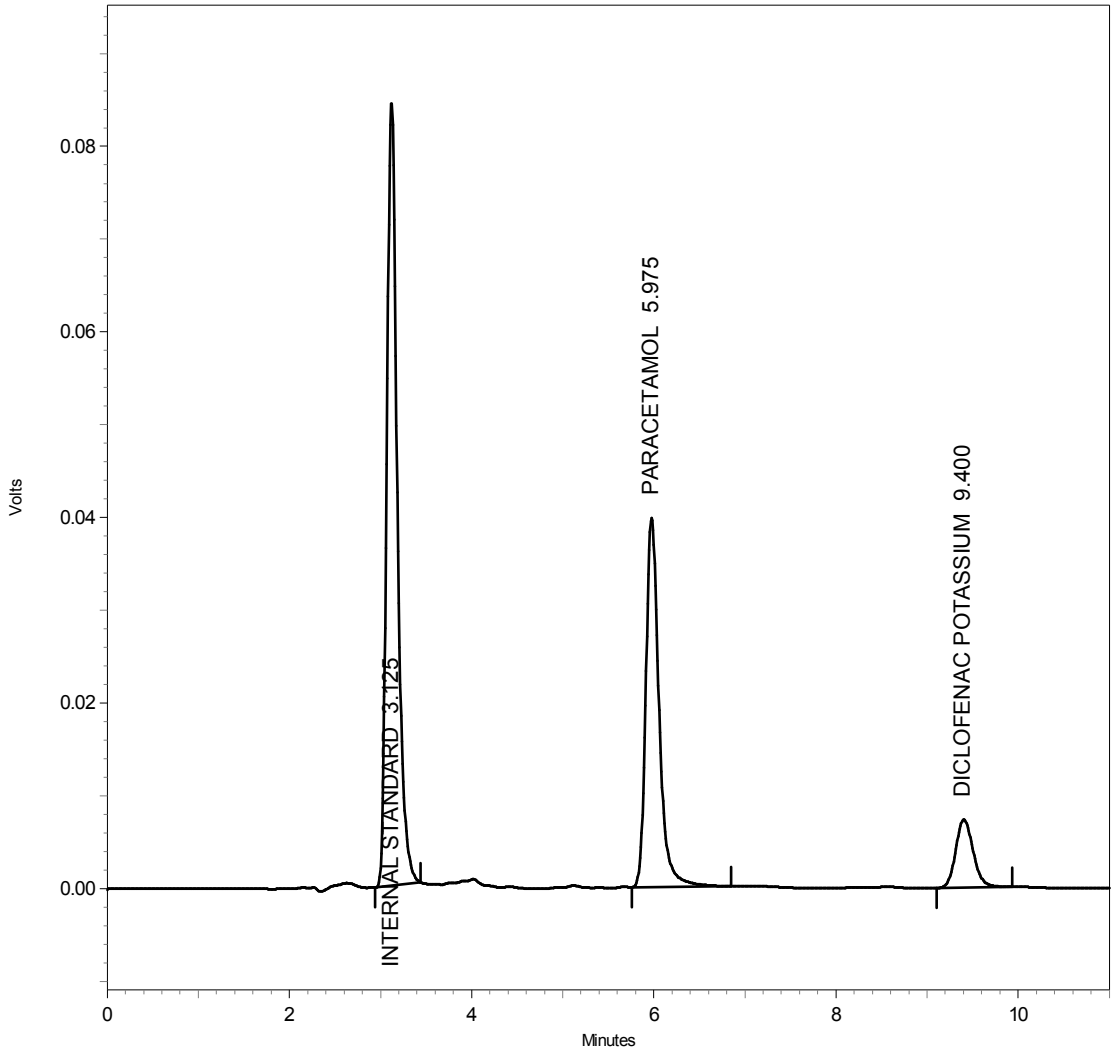

Fig 1. Typical Chromatogram of Sample Solution

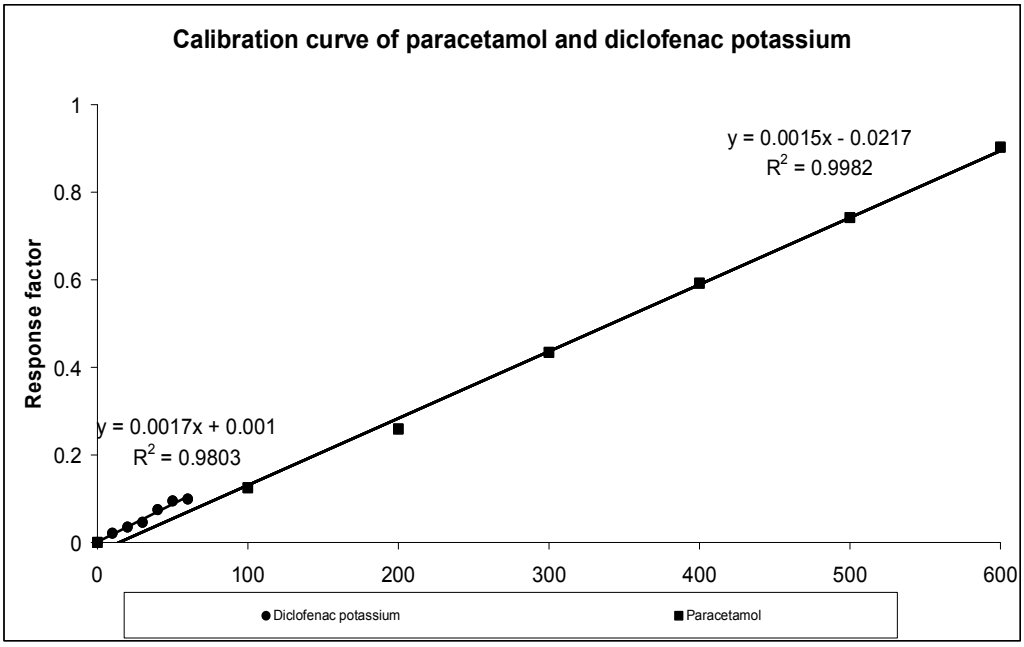

Fig 2. Calibration Curve of Paracetamol and Diclofenac potassium

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