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Research Article

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Development and validation of a RP-HPLC method for the simultaneous determination of paracetamol and diclofenac potassium on stainless steel surface of pharmaceutical manufacturing equipments

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ABSTRACT

In Pharmaceutical industry, the important step in the cleaning of equipment involves the removal of possible drug residues from the equipment surface and surrounding areas. The cleaning procedure must be validated and the methods to determine trace amounts of drugs have, therefore, to be considered with special attention. 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 on stainless steel equipments in order to control cleaning procedure. Cotton swab, moistened with water: methanol(50:50) is used to remove any residue from surface. The drug analysis was carried out on a Hibar C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: potassium dihydrogenortho phosphate (adjusted to pH 3 using orthophosphoric acid) in the ratio of 70:30 v/v at a flow rate of 0.8 ml/min. Detection was carried out at 275 nm. The retention times of paracetamol and diclofenac potassium were 3.1 and 7.4 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The developed method was also successfully applied to evaluate the residue and drugs on the surface of the stainless steel equipments used for manufacturing solid tablet dosage form.

Key Words: RP-HPLC, Paracetamol, Diclofenac potassium, Cleaning Validation.

INTRODUCTION

An important step in the manufacturing of pharmaceutical products is the cleaning of equipment and surfaces for the possible removal of residual potent drugs used earlier. The cleaning procedures for the equipment must be validated according to good manufacturing practices (GMP) rules and guidelines. The main objective of cleaning validation is to avoid contamination between different productions or cross-contamination. This cleaning is verified by determining the amount of residues on surfaces involved in the manufacturing process. The acceptance limit for residue in equipment is not established in the current regulations. According to FDA, the limit should be based on logical criteria, involving the risks associated with residues of determined product. The calculation of an acceptable residual limit, the maximum allowable carryover of active products in production equipment should be based on therapeutic doses, the toxicological index and a general limit(10ppm). Several mathematical formulates were proposed to establish the acceptable residual limit[1-4].

Paracetamol is chemically 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) exhibiting 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[5-7].The aim of the present work is to develop a validated RP-HPLC method, which can quantify the traces of paracetamol and diclofenac potassium in the production areaand equipments. The developed RP-HPLC method was validated following the ICH guidelines [8,9].

EXPERIMENTAL SECTION

Chemicals and Reagents

Acetonitrile (HPLC grade) and Methanol (HPLC grade) was procured from E Merck (India) Ltd, Mumbai. Potassium dihydrogenortho 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 Crescent Therapeutics Limited, Baddi, Himachal Pradesh.

Equipment 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 collection and processing (Shimadzu, Japan). A Hibar C_{18} column (25 cm x 4.6 mm i.d., 5µ) was used for the separation. The mobile phase consisting of a mixture of acetonitrile and potassiumdihydrogenortho phosphate (adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 70:30 v/v was delivered at a flow rate of 0.8 ml/min with detection at 275 nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed prior to analysis.

Standard solution preparation

The stock solution of standards Paracetamol and Diclofenac Potassium were prepared by accurately weighing 10mg of each drug separately in methanol. The further dilutions were made with the mobile phase such that the final standard solution to be injected consists of the 50µg/ml and 5µg/ml of paracetamol and diclofenac potassium respectively.

Sample solution preparation

The selected surface (10cm × 10cm) of stainless steel,was previously cleaned and dried. The surface was wiped in one direction with the swab wetted with the methanol. The swab was placed in the swab tube containing the mobile phase. The tube was placed in the ultrasonicator for 10 min.The drug if present in the swab is dissolved in the mobile phase. The solution from the tube is injected into the HPLC and the chromatogram was recorded. The area of the peaks from the chromatogram was noted and the areas were plotted into the calibration data to find out the concentration of the drugs in the sample.

RESULTS AND DISCUSSION

Acceptance criteria for the samples on the stainless steel surface[3]

In pharmaceutical manufacturing process careful examination of the trace residues is vital which can contaminate subsequent products. The maximum allowable carryover (MACO) is the acceptable transferred amount of drug from the previous to the following product. The MACO is determined based on the therapeutic dose, toxicity and generally 10ppm criterion. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the residue limit in terms of the contamination level of active ingredient per surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculation. The limit per surface area was calculated from the equipment surface area and the most stringent maximum allowable carryover. The 0.1% dose limit criterion is justified by the principle that an active pharmaceutical ingredient (API) at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects on human health.

Swab samples from different locations within the manufacturing equipment train have been analysed to determine the residues of paracetamol and diclofenac potassium. The samples were analysed by the proposed method. (Fig: 1)

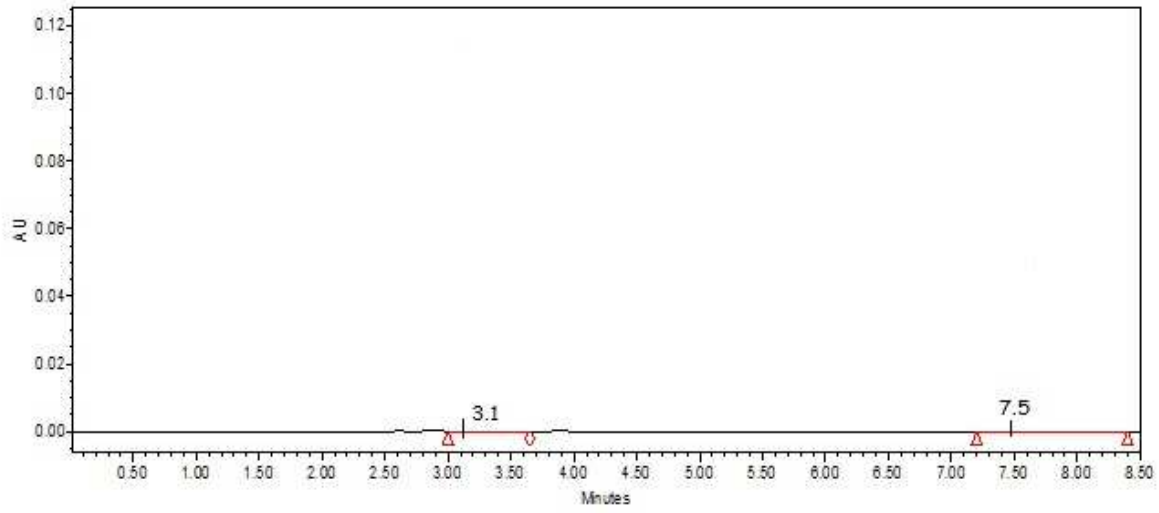

Fig1: Typical Chromatogram of swab sample after cleaning

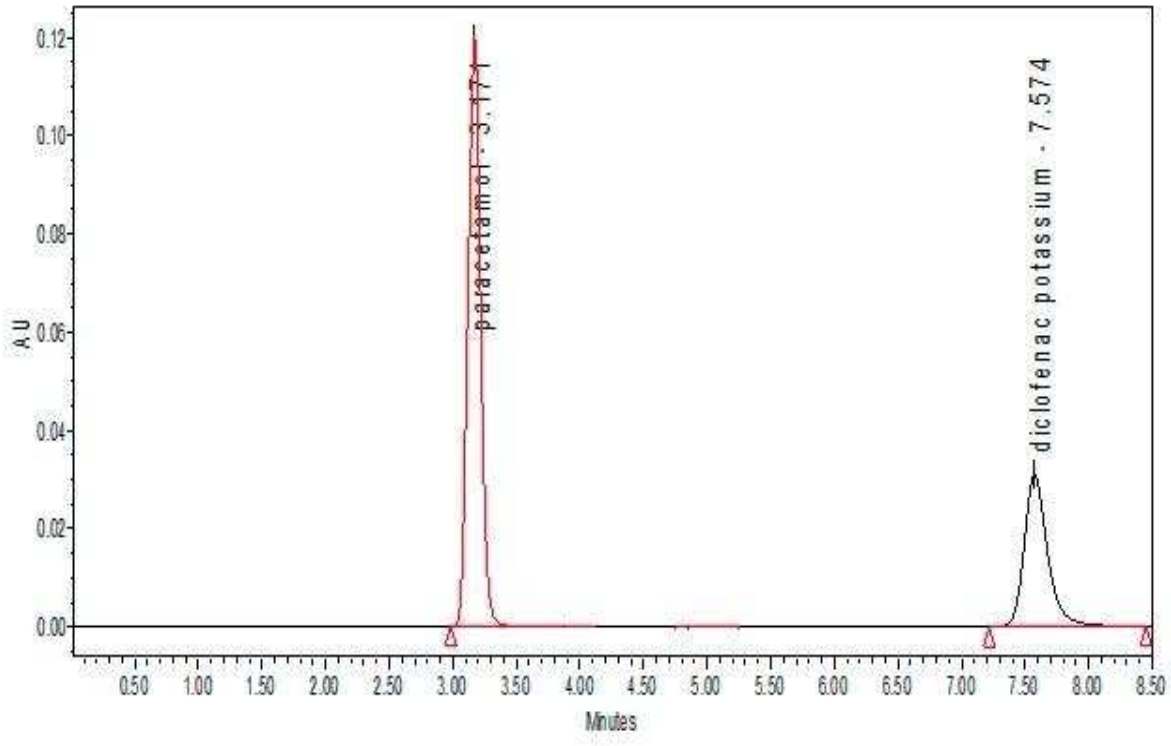

Fig:2 Standard chromatogram of Paracetamol and Diclofenac Potassium

Chromatographic conditions

The HPLC procedure was optimized with a view to develop precise and stable method to determine the traces of the drugs. 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), Hibar 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 Hibar C_{18} column (25 cm x 4.6 mm i.d., 5µ), with a mobile phase of a mixture containing acetonitrile: potassium dihydrogenortho phosphate (adjusted to pH 3 using orthophosphoric acid) (70:30v/v) at a flow rate of 0.8 ml/min

with a detection at 275nm gave sharp and symmetrical peaks with retention time 3.1 and 7.5 for paracetamol and diclofenac potassium respectively. The typical chromatogram of standard solution is shown in Fig 2. The peak area ratio of standard and sample solutions was calculated.

Method Validation

Accuracy and precision

The accuracy of the method was determined byrecovery 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 intraday 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.

Table 1: precision studies

Parameters	Intraday		Interday					
	Paracetamol	Diclofenac	Paracetamol			Diclofenac		
			Day 1	Day2	Day3	Day1	Day2	Day3
Average	1233301	208925	1229912	1232972	1224810	209216	210049	210266
SD	11891.87	1831.2	6792.85	7891.5	8602.09	1241.6	1444.4	894.64
%RSD	0.964	0.876	0.552	0.640	0.702	0.593	0.687	0.425

Linearity and Range:

The linearity of the method was determined at five concentration levels ranging from 10.0 to 60.0 µg/ml for paracetamol and 1 to 6.0µg/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=27764X-76768(R^2=0.995) for paracetamol and Y=48807X-32582(R^2=0.9963) for diclofenac potassium. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The calibration curves are shown in Fig. 3 and Fig 4 respectively.

Table 2: Linearity and range of paracetamol

Concentration (µg/ml)	**Peak Area**
10	235076
20	491274
30	698914
40	1014321
50	1299867
60	1630287
Correlation Coefficient	**0.995**

Table 3: Linearity and range of Diclofenac potassium

Concentration (µg/ml)	**Peak Area**
1	21981
2	60473
3	107901
4	168544
5	206947
6	263620
Correlation Coefficient	**0.996**

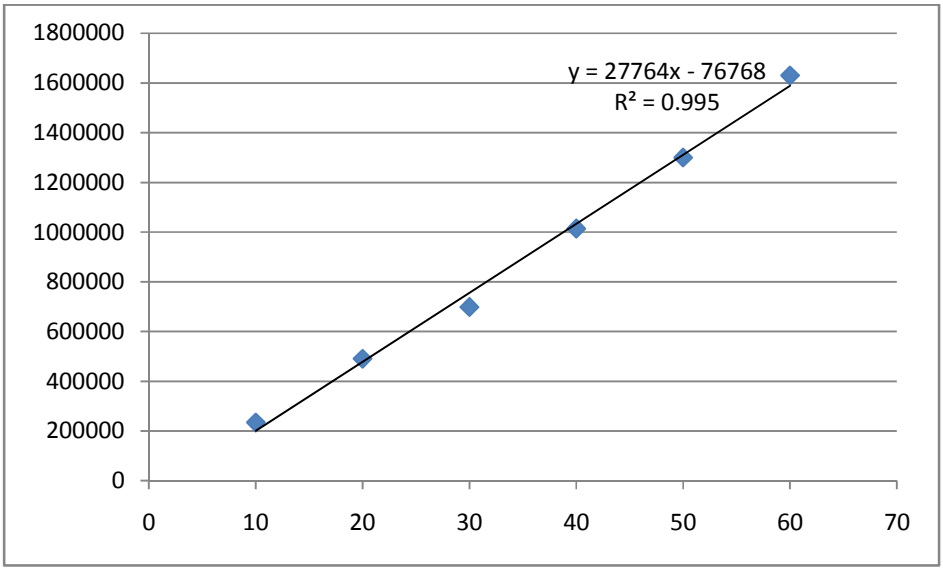

Fig 3: Linearity graph of Paracetamol

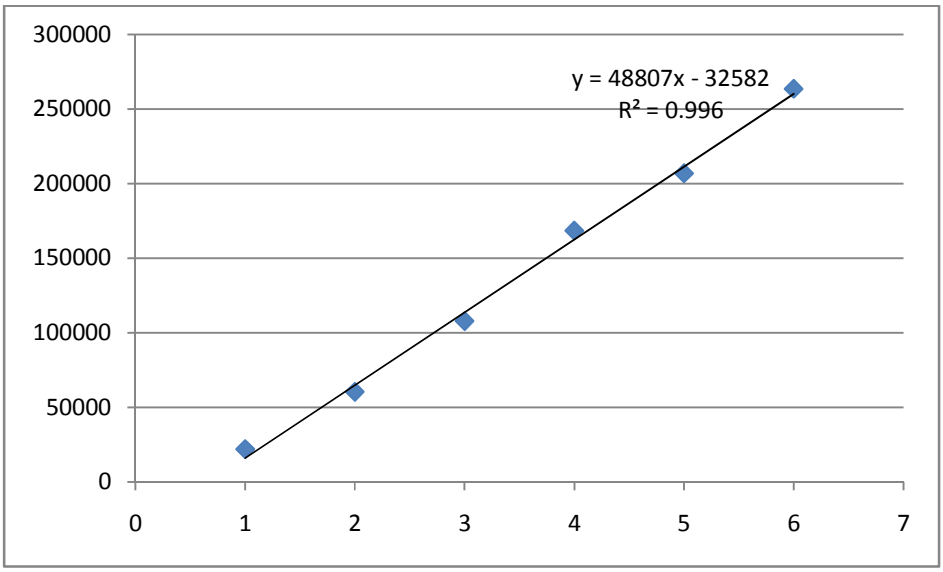

Fig 4: Linearity graph of Diclofenac Potassium

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 0.1 ng/ml and 1.0 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 4 ng/ml and 10 ng/ml for paracetamol and diclofenac potassium, respectively.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010AHT), and Water's Breeze HPLC by different operators using different columns of

similar type like Hypersil C_{18}, Phenomenex Gemini C_{18} and Hibar 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 RPHPLC method developed, are rugged and robust.

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.

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 degradationwithin the indicated period, thus indicated that both solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

Table 4: System suitability parameters of Paracetamol and Diclofenac Potassium

Parameters	Paracetamol	Diclofenac Potassium
Linearity	10-60µg/ml	1-6µg/ml
CorrelationCoeffificent	0.995	0.996
Regression equation	Y=27764X-76768	Y=48807X-32582
LOQ	4ng/ml	10ng/ml
LOD	0.1ng/ml	1ng/ml
Theoretical plates	1537600	4042775
Tailing factor	1.04	1.06
Asymmetric factor	1.02	1.05

CONCLUSION

A simple, accurate HPLC method developedis used to quantify residues of the API of paracetamol and diclofenac potassium on swabs, in support of cleaning validation of pharmaceutical manufacturing equipment.

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