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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF FIVE DIFFERENT DRUG SUBSTANCE IN DRUG PRODUCT USING SUB TWO MICRON COLUMN.

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ABSTRACT

A novel selective and fast stability indicating High performance liquid chromatography method has been developed for the simultaneous assay of Paracetemol, Nimesulide, Aceclofenac, Diclofenac, Ibuprofen by using sub 2 micron column. In reverse phase chromatography efficient separation was achieved between all five compounds. This method was validated as per International Conference on Harmonization guidelines (Q2R1). The proposed RP-HPLC method utilizes a Reprosil Gold C18, 50 x 4.6 mm 1.8µ column and mobile phase consisting of Ammonium acetate buffer pH-3.5 with formic acid and Methanol using gradient program. UV detection at 220 nm for Ibuprofen, Paracetemol and 280nm for Nimesulide, Aceclofenac and Diclofenac Sodium was employed. The described method is accurate & linear in the given range. The observed mean recoveries are in the range of 99.91 to 100.53%.

KEYWORDS: Paracetemol, Nimesulide, Aceclofenac, Diclofenac, Ibuprofen, Assay, Reverse phase and sub 2 microncolumn, Fast High performance liquid chromatography.

INTRODUCTION

Paracetemol is classified as a mild analgesic and is commonly used for the relief of headaches, minor aches and pains. Paracetemol is a major ingredient in numerous cold and flu remedies in combination with analgesics.[1] Nimesulide is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties.[2,6] Aceclofenac and Diclofenac

are analog of each other. These are a non-steroidal anti-inflammatory drugs. They are used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and spondylitis.[3,4] Ibuprofen is a Non - Steroidal anti-inflammatory drug used for relieving pain, helping with reducing fever and inflammation.[1] These are the drugs formulated in combination and require different method of analysis for different molecules[3,4], which intern consumes additional time and cost to change over from one combination of drug to other combination for analysis in quality control department and subsequent release of these products in the market. Method of analysis for Assay for all these compounds is different and takes minimum time of 30-40 minutes. Therefore a study was carried out to develop fast High- performance liquid chromatography method to test Assay of different compounds in a single method to save time and cost of analysis.

OBJECTIVES OF THE STUDY

The objective of the study was to reduce the cost and time of the analysis significantly at laboratory by developing a single fast and selective HPLC method for multi components which can be used to analyze many products. Also to demonstrate that the procedure when correctly applied produce results that are fit for purpose.

PROCEDURE AND PROCESS

Materials and Reagent

Paracetemol, Nimesulide, Aceclofenac, Dicyclofenac and Ibuprofen standards were obtained from Indoco Remedies Ltd, Navi Mumbai, India where as Ammonium Acetate, Methanol and Formic acid were procured from Merck.

Instrumentation

High performance liquid chromatography system consists of UV/PDA detector 2998 (Waters) and Empower software for data handling. All pH measurements were performed on a pH meter (Thermo).

Chromatographic conditions

The mobile phase is 10 mM Ammonium acetate in water pH 3.5 adjusted with Formic acid and methanol in gradient proportion and column used is Reprosil Gold C18 50 mm x 4.6 mm, 1.8µm.

Table No. 01: Gradient Program

Time in Min	% Buffer	% Methanol
0	80	20
1	80	20
3	50	50
5	20	80
7	80	20
10	80	20

Solvent mixture: Buffer: Methanol (50:50).

Standard solution

Weigh accurately 200 mg of Paracetemol, 250 mg Ibuprofen, 60 mg Nimesulide, 40 mg Aceclofenac, 20 mg Diclofenac Standard into a 250 mL volumetric flask. Dissolve in 10 mL solvent mixture and make up to volume with same. Further diluted to 12.5 ml above solution in to the 50ml volumetric flask.

Test solution for

a) Paracetamol+Aceclofenac Tablet: Average wt: 760 mg/Tab) Transfer about 70.2 mg accurately weighed of crushed tablet in to a 250 mL volumetric flask, dissolve in 100 mL solvent mixture, sonicate and make up to volume with solvent mixture.

b) Paracetamol+Nimesulide Tablet: Average wt: 548 mg/Tab) Transfer about 84 mg accurately weighed of crushed tablet in to a 250 mL volumetric flask, dissolve in 100 mL solvent mixture, sonicate and make up to volume with solvent mixture.

c) Paracetamol+Diclofenac Tablet: Average wt: 894 mg/Tab) Transfer about 87 mg accurately weighed of crushed tablet in to a 250 mL volumetric flask, dissolve in 100 mL solvent mixture, sonicate and make up to volume with solvent mixture.

d) Paracetamol+Ibuprofen Tablet: Average wt: 894 mg/Tab) Transfer about 140 mg accurately weighed of crushed tablet in to a 250 mL volumetric flask, dissolve in 100 mL solvent mixture, sonicate and make up to volume with solvent mixture.

Analytical method Development

To establish a selective and sensitive method; the primary concern during development was to achieve separation of each component, peak symmetry of the peaks of all five molecules and resolution between these compounds. Different types of buffer at different pH were

studied in combination with methanol. The chromatographic data with number of theoratatical plates (N) was recorded during this study. Solutions of all these compounds were prepared in diluent at different concentration and UV-visible spectra were acquired by PDA detector. A UV absorption maximum of Paracetemol and Ibuprofen is at 220nm while Aceclofenac, Dicyclofenac and Nimesulide are 280 nm. Selection of the column being very critical to achieve short run time with selectively and efficiently. Reprosil Gold C18 1.8µ, 50 x 4.6 mm was selected as stationary phase. pH of the buffer is also critical especially dealing with more than one component. The pH of the buffer should be selected in such a way so that at least in the range of ±1.0 from the pKa value of each component and also it gives the correct separation and resolution between all the components. For this different experiments were carried out and finely pH of the 3.5 was selected as an optimum pH of the mobile phase.

Method Validation

The validation work was conducted according to the ICH (International Conference on Harmonization) guidelines method validation parameters include Specificity, Linearity, Accuracy and Precision.[2,6]

1) Specificity

The specificity of the HPLC method was determined by injecting, individually samples of all five components under study all these peak were well resolved without any interference from each other. The specificity of the method was also proven by passing peak purity criteria of all the peaks.

2) Accuracy

The accuracy of the method was determined by determining recovery of each component after spiking the known amount of each component at different level from 80 to 120% of target concentration.

3) Linearity

The Linearity of method was established by observing the response of each component against concentration of individual component for the concentration range of 50% to 150%. of the targest concentration. Linear regression graph was plotted and Correlation coefficient was reported.

4) Precision

Precision of the method was evaluated in terms of degree of repeatability. The Repeatability is determined by calculating the relative standard deviation (% RSD) of six replicate determinations of standard solution containing all five components under study.

Table No. 03: Method validation parameter

Parameter	Paracetamol	Ibuprofen	Aceclofenac	Nimesulide	Diclofenac
Linearity Range (%)	50% to 150%	50% to 150%	50% to 150%	50% to 150%	50% to 150%
Correlation coefficient	0.999	0.999	0.999	0.999	0.999
Repeatability (% RSD)	0.66	0.64	0.40	1.03	0.52
% Recovery					
80 %	98.61	97.22	98.69	98.18	98.18
100 %	98.15	98.36	98.92	98.74	99.20
120 %	98.11	97.32	99.16	98.18	98. 48

RESULT AND DISCUSSION

A novel fast HPLC method has been developed and validated for the simultaneous quantitative determination of Paracetemol, Nimesulide, Aceclofenac, Diclofenac and Ibuprofen. The develop method is specific and selective for the given purpose. Since the method was sub two micron stationary phase. It is a fast HPLC method which consumes very less time and reagent as compare to conventional HPLC. The selection of volatile buffer enables suitability of this method for LC-MS determination also. The validation data proves repeatability and reproducibility of the method in terms of linearity, precision and accuracy. Since method is short can be used for cleaning validation. Method is suitable for five drugs product so analyst can switch over form one combination drug to another combination of drug hassle free and it leads to lot of time saving of laboratories one more advantage is that conman analytical error get minimized since method is same for any combination of drug.

Table No. 02 Specificity

Name of Drug	Retention time (min)	Purity Angle	Purity Threshold
Paracetemol	0.930	20.57	49.25
Ibuprofen	5.589	1.41	4.31
Nimesulide	3.558	0.21	0.32
Aceclofenac	4.282	0.09	0.46
Dclofenac Na	4.861	0.29	1.23

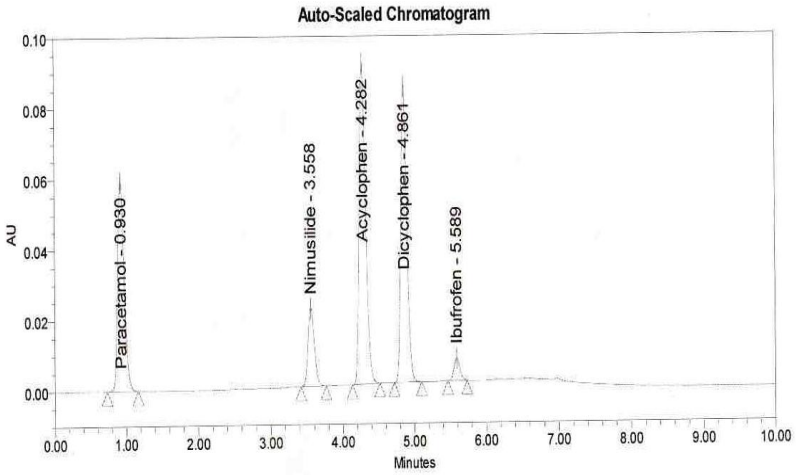

Fig. 1

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