

Hplc method for piroxicam analysis



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ABSTRACT

A simple, precise method using HPLC method was developed for the determination of piroxicam in gel. A reversed phase HPLC system was used consisting of C18 column with the dimension size of 150mm x 4.6mm. 55 volumes of methanol and 45 volumes of phosphate buffer (0.05M, pH 7) are used as the mobile phase. The flow rate was 1ml/min and the effluent was monitored at 254nm. The retention time was found to be around 6.0 minutes. The stock solution of piroxicam was prepared and the standard solutions ranging from 5 to 20 µg/ml were prepared with phosphate buffer x methanol (60 x 40, v/v). This was injected into HPLC and the chromatograms were obtained. Test solution was prepared from the marketed product Feldene gel and injected into HPLC. From the regression equation, which was obtained from the standard concentrations, the concentration of Feldene gel was determined. Validation methods are performed to demonstrate the accuracy of the method, precision of the method and the linearity. This method has shown linearity and the correlation coefficient was found to be 0.999, the accuracy of the method was good in the range of 91% – 99.03%, and the precision which is expressed as %RSD was found to be 4.44%, the method was not precise to repeat. The proposed method was successfully applied to the quantitative determination of piroxicam in topical dosage regimen gel.

Key words: Piroxicam, HPLC, Feldene gel

INTRODUCTION:

Piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-H-1, 2-benzothiazine - 3 - carboxamide-1, 1-di-oxide] is a non-steroidal anti-inflammatory and

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analgesic agent. It exhibits a weakly acidic 4-hydroxy proton and a weakly basic pyridyl nitrogen. Its molecular formula is C₁₅H₁₃N₃O₄S. Molecular weight was 331.35 [25].

Piroxicam is a non-steroidal anti-inflammatory agent from the oxicam class [14]. NSAIDs are the drugs with analgesic and antipyretic effects which have in higher concentration anti-inflammatory effects [26]. Piroxicam is used in the treatment Rheumatoid Arthritis, Osteoarthritis, ankylosing spondylitis and also used in acute pain in musculoskeletal disorders and in acute gout [6]. Piroxicam acts by reducing the hormones that cause inflammation and pain in the body. The anti-inflammatory effect of piroxicam may result from the reversible inhibition of cyclooxygenase, causing the peripheral inhibition of prostaglandin synthesis. The prostaglandins are produced by Cox-1 enzyme, resulting in the disruption of production of prostaglandins. It also inhibits the migration of leukocytes into sites of inflammation and prevents the formation of thromboxane A₂, aggregating agent, by the platelets [14]. Piroxicam has a prolonged duration of action that is one dose can provide pain relief throughout the day [25]. Piroxicam is very slightly soluble in water and slightly soluble in alcohol. The other names for Piroxicam are Candy, Cycladol, Feldene, Novo-pirocam, and Piroflam. Piroxicam is a white crystalline powder [12].

Piroxicam is well absorbed by oral route. When piroxicam is administered through the oral route, the drug plasma concentrations peak within three to four hours. The prolonged half life of 50hrs, has steady state plasma concentrations throughout the day on one dose. With the food there is a minor delay in the rate of absorption but not the extent of absorption after

oral administration. The simultaneous administration of antacids with the piroxicam has no effect on plasma drug concentration when administered orally. The apparent volume of distribution of piroxicam is 0.14L/kg, 99% of the plasma piroxicam was bound to plasma proteins. Metabolism of the piroxicam drug occurs by the hydroxylation at the fifth position of the pyridyl side of the chain and a sequence of reactions occurs which involve hydrolysis of the amide linkage, decarboxylation, N- demethylation and the ring contraction. And the drug gets excreted in urine and the in the feces. [25].

Commercially Piroxicam is available in the form of capsules, tablets, injectables, gel. Piroxicam is well absorbed by oral administration but has been associated with a number of side effects on stomach, nausea, dyspepsia, diarrhoea, constipation and some renal ones. And also it can cause gastric mucosal damage which may result in ulceration or bleeding [1]. In order to reduce these adverse effects a topical formulation has been prepared [4]. It also has the additional advantage of avoiding hepatic first-pass metabolism and providing the controlled delivery of the drug for an extended period [21]. Several analytical procedures for the detection and also quantification of piroxicam in pharmaceutical preparations and in biological fluids have been used [23]. These involve Quantitative determination of piroxicam by thin layer chromatography-matrix-assisted laser desorption (MALDI) TOF mass spectrometry [3]. UV spectrophotometric method UV detection is most commonly employed, as it is sufficiently sensitive to detect levels associated with common oral dosage regimens [7], spectrofluorimetric method [5], Chemiluminescence method [15], Piroxicam has been quantified in capsules, suppositories, injection solutions, tablets,

suspensions by capillary zone electrophoresis and micellar electro kinetic capillary chromatography [10], A colorimetric determination of piroxicam in capsules has been used [8], Liquid chromatography has also been reported [13], HPLC with amperometric detection of piroxicam in human plasma and tissues was reported [2], there are many methods for the determination of piroxicam in plasma by HPLC by using different mobile phases [23], And there is an HPLC method for the determination of piroxicam in pharmaceutical products like capsules, tablets, ointments, suppositories, Ophthalmic suspensions [9]. And there was also an hplc method for the quantitative determination of piroxicam in a new formulation piroxicam- β -cyclodextrin [7].

There are so many HPLC methods for quantitative determination of piroxicam in all pharmaceutical preparations [23], but there was a less literature about quantitative determination of piroxicam in gel, there was a literature about stability study of piroxicam gel by using HPMC and ACUPEC HV-505 bases [21], So a simple, rapid and sensitive method has to be developed for topical dosage regimen (gel). When Feldene gel is applied to the skin where the inflammation is there it gets absorbed through the skin into the underlying tissues [27]. Feldene gel is used to relieve pain and inflammation in the applied area.

In general, high-performance liquid chromatography has been the most employed method to measure piroxicam [1]. The HPLC has the major improvement over column chromatography regarding the detection methods which are used [19]. These methods are highly automated and extremely sensitive, It is used to analyze, identify, purify & quantify the compounds <https://assignbuster.com/hplc-method-for-piroxicam-analysis/>

[19]. There are two variants in use in HPLC depending on the relative polarity of the solvent and the stationary phase, they are normal phase and the reversed phase HPLC. High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres which makes it much faster [19]. The schematic diagram of an HPLC was given below, this shows how the HPLC works.

HPLC requires a pump to move the liquid mobile phase through the system, a column to perform the separation, a detector and data collector to record a chromatogram, and a fraction collector to collect the output. When sample is injected into stream of mobile phase the motion of analytes in the sample depends on the physical and chemical attractions with the stationary phase in the column and passes through the detector and this sends signals to the processing unit and also to the display [19].

The time taken for a particular analyte to pass through the column to the detector is known as its retention time. The amount of analyte can be related to the area under the peak obtained [19].

If the peak is smaller then the concentration will be smaller and vice versa. If there are two different substances in the mixture (X and Y) then it is difficult to observe the concentration from the area under the peak, because one sample absorbs UV light at one wave length and the other at different wave length. Frequently a series of tests are performed on the analyte and a

number of trial runs may be processed in order to find the HPLC method which gives the best peaks [19].

In this paper an HPLC method has been used for the quantitative determination of Piroxicam in gel. In the previous papers for the quantitative determination of piroxicam in pharmaceutical preparation different mobile phases used like acetate buffer, methanol water etc [23], the mobile phase selected in this proposed method was methanol: Phosphate buffer (0.05M pH 7) 55: 45 v/v, the effluent was monitored at 254nm. A mobile phase has to be buffered to ionize the analyte, because when an analyte gets ionized it becomes less hydrophobic, therefore retention time will get decreased [16]. And this method was performed in a purely isocratic mode. Isocratic methods are used when a single analyte has been widely considered and the compound is being run to confirm its identity [19]. There are two methods of HPLC normal and reversed phase [19], the one which is used in this study was reversed phase hplc. In this the solvent used will be polar solvent example methanol and a non polar stationary phase is used like silica, to make it non polar long hydrocarbon chains was attached to its surface with either 8 or 18 carbon atoms in them and the column size used in this will be same like normal phase, a typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm [19]. The flow rate of mobile phase was set to be at 1ml/min. And the injection volume was 20 μ l.

Before this method, one method has been tried but this does not give good results. In the previous method the retention time has been changed for inter day runs, it has been changed from 7 to 8 and then to 13. So slight

changes has been done to the previous method, the mobile phase used was the same but the proportions used are different 40 volumes of methanol and 60 volumes of phosphate buffer 0.05M are used, in the present method methanol composition has been changed to decrease the retention time, and the wave length used was 240nm, this has been changed to 254nm because in many journals different wave lengths are used so this wave length was used for a trial run, it gave good results so it has been used for further studies. The retention time was found to be around 6 minutes after the method has been changed and it was constant for inter day runs. The validation of any new method has to be carried out to check whether the proposed method is giving good results or not [20]. So validation has to be carried out for the hplc method for accuracy, precision, Limit of detection, Limit of quantification, linearity, specificity, robustness, linearity, range [20].

OBJECTIVES: To develop a new method for the quantitative determination of piroxicam in gel which have less literature.

MATERIALS AND METHOD:

Apparatus:

Analysis was performed by using analytical balance, pH meter, the HPLC used is equipped with a series 200 binary pump with variable single wave length UV/VIS detector set at 254nm, Column used is of SNIXF13, C18 column and the dimensions of the column is 150mm x 4.6mm with a flow rate of 1ml/min(Isocratic). The mobile phase consists pH 7, 0.05M phosphate buffer: methanol (60: 40 v/v), the injection volume was 20 μ l and the sample injection was manual.

Materials:

Piroxicam manufactured by sigma Aldrich, UK. Phosphate buffer (pH 7.0, 0.05M) this is prepared by dissolving 7.8g of sodium dihydrogen phosphate (analytical grade, purchased from sigma Aldrich, UK) in 1000ml of water and pH adjusted with 1.0 N NaOH (analytical grade purchased from sigma Aldrich, UK). Methanol HPLC grade was manufactured by Fischer UK Ltd. Company. Feldene gel was manufactured by Pfizer laboratory and the percent purity of piroxicam in gel was 0.5%w/w in 60g of gel. Bi distilled water.

Standard preparation:

Piroxicam (50mg) was weighed out accurately into a 50ml volumetric flask, dissolved in mobile phase (0.05M phosphate buffer ×f methanol, 60×f40, v/v), Standard solutions ranging from 5 to 20 $\hat{1}\frac{1}{4}$ g/ml were prepared, diluted to 10ml with mobile phase (phosphate buffer×f methanol, 60×f40, v/v).

These standard solutions were injected into HPLC which are used to construct calibration graph. This calibration graph was obtained as absorbance peak area versus concentration. A couple of runs are performed on the standard solutions to get the better peaks and the average of both peak areas are taken for the calibration graph, calibration graph was constructed by taking peak area on X-axis and the concentration on Y-axis.

Sample solution:

An accurately weighed 500mg of Feldene gel was transferred to a 50.0 ml volumetric flask, dissolved with methanol, volume was then adjusted to the mark with methanol. The solution was sonicated in an ultrasonic sonicator for 20 min, from this 4ml of solution was transferred to 10ml volumetric flask,

diluted to 10 ml with mobile phase (phosphate buffer: methanol, 60: 40(v/v)) prior to analysis. And for calculating accuracy different concentrations were taken, after the analysis of 4ml sample 5ml, 6ml were taken from the sample solution and diluted to 10ml with the mobile phase 60: 40 v/v,

Frequently a series of tests are performed on the analyte and a number of trial runs should be carried out in order to get best peaks [19], the mean of the both peak areas were taken for accuracy and precision. By using calibration curve, quantitative determination of piroxicam in gel was determined.

Validation procedure:

Validation is required for any new method to ensure that the proposed method is capable of giving good reliable and reproducible results when performed by different operators in the same laboratory and with the same equipment. A validation of the assay consisting of linearity, accuracy, precision of the method was performed for this proposed method with the same equipment in the same laboratory [20].

Precision:

The precision of the method was studied by the analysis of the sample for the multiple runs. The precision was expressed as the %RSD. The precision value should not be more than 2% [14]. If this method exceeds that then the method was not precise to repeat again or to follow in the regular laboratory analysis.

$\%RSD = \text{standard deviation} \times 100$

Mean

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Accuracy:

Accuracy for this method can be performed by applying the method in triplicate samples. The sample with different concentrations were taken and injected into the HPLC and the chromatograms were recorded from which the peak area can be noted down and the concentration can be calculated by submitting the peak area value in the place Y in the regression equation obtained by the calibration graph of the standard solutions ranging from 5 $\frac{1}{4}$ g/ml to 25 $\frac{1}{4}$ g/ml. The obtained value is considered as the amount found value and it is compared with the true value to calculate the percentage recovery. Therefore it can be defined as the measurement of degree of closeness of measurements of the quantity of its true value. This was expressed as the %recovery [11].

$$\% \text{ Recovery} = \text{amount found} \times 100$$

True value

Linearity and range:

The linearity is the method's ability to get the results which are proportional to the concentration of the analyte within a given range [20]. Linearity is determined by calculating regression line using peak area versus analyte concentration [14]. The linearity of the responses of the detector can be established by the graph constructed as peak versus concentration of the standard solutions and also by determining the correlation coefficient [20]. A series of standard solutions of piroxicam ranging from 5 $\frac{1}{4}$ g/ml, 10 $\frac{1}{4}$ g/ml, 15 $\frac{1}{4}$ g/ml, 20 $\frac{1}{4}$ g/ml, and 25 $\frac{1}{4}$ g/ml were injected into HPLC and the peak areas were noted. These standard solutions are injected into HPLC for a couple of times to get the best peaks and the average of the peak areas

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were considered to construct the calibration graph to find out the linearity and also range of the responses.

RESULTS AND DISCUSSION:

Several methods are reported for the quantitative determination of piroxicam in pharmaceutical preparations [9], but there was a less literature about quantitative determination of piroxicam in gel so, this has been chosen, two methods have been tried for the determination of piroxicam, one method has shown good results so it has been considered for further studies. Piroxicam standard solutions concentration ranging from 5 $\frac{1}{4}$ g/ml – 25 $\frac{1}{4}$ g/ml and the sample were injected into HPLC system, the piroxicam peak in the sample was identified by comparing with the piroxicam standard solutions and also identified by the retention time which was found to be around 6.0 minutes. The estimation of piroxicam was carried out by HPLC using mobile phase having a composition of 55 volumes of methanol and 45 volumes of 0.05M phosphate buffer(55: 45v/v). The pH of the phosphate buffer was 7. The column used was c18 (150mm x 4mm dimension). Flow rate of mobile phase was 1ml/min; the effluent was monitored at 254nm.

The Chromatograms obtained for the standard solutions and sample solutions when injected into HPLC were shown below.

The standard solutions of piroxicam concentrations ranging from 5 $\frac{1}{4}$ g/ml to 25 $\frac{1}{4}$ g/ml were injected into HPLC a couple of times in order to determine the better peak and the average of the peaks were taken to construct the calibration graph.

The Validation of the hplc method has been carried out. To report the precision of the method the sample has to be run for 10 times in the HPLC. The selected method shown good linearity and accuracy but the method was not precise because the method exceeded the normal limits for the precision, it has shown the %RSD value of 4.44, the normal value is 2%. For accuracy different concentrations of sample solutions were taken and these are injected into hplc for triplicate and the average of these values are considered to calculate the accuracy, the accuracy was good with the value of 91.9%- 99.03% recovery of the drug.

Linearity: Under the optimum conditions described above, the linearity of the piroxicam in the standard solutions having concentrations 5 $\frac{1}{4}$ g/ml to 25 $\frac{1}{4}$ g/ml were injected into hplc for couple of times, the average values of the peak areas were taken to plot the graph. Graph was plotted by taking peak area on y-axis and the concentration on x-axis. This is shown in figure 5. There is a linearity between 5 $\frac{1}{4}$ g/ml, 10 $\frac{1}{4}$ g/ml, 20 $\frac{1}{4}$ g/ml, 25 $\frac{1}{4}$ g/ml, the correlation coefficient (r^2) value was found to be 0.993 and the regression equation was $y = 37623x + 61207$ where y is the peak area of piroxicam test solution and x is concentration of piroxicam in the test solution. There might be some problem with the injections with the 15 $\frac{1}{4}$ g/ml concentration of standard solution. The value of 15 $\frac{1}{4}$ g/ml has been slightly varied from the linearity so the point has been left out to get the linearity. If that point has been left out the correlation coefficient value was found to be 0.999, and the regression equation was $y = 37623x + 71625$, the values after the point has been left out and before that has been shown in the table 5, the graph with the point left out was shown figure 6.

Statistical analysis: To test the linearity of the standard concentrations a statistical simple linear regression test has been performed on the standard concentrations. The output was attached in the appendix.

In this the 't' value was found to be 67.604 and the p value was found to be 0.000 which states that there is a linear relationship between peak area and the concentration that is if there is a slight change in the concentration there will be change in the peak area as the concentration increases peak area increases.

In this the F value was found to be 4570.291 and the value of P value was found to be 0.000 which is less than 0.001 this states that peak area linearly depends on the concentration that is as the concentration increases the peak area will increase.

Precision: The method precision is evaluated by inter and intraday repeatability [20]. In this method intraday repeatability was performed. For the intraday repeatability the sample with same concentration has to analyse for many times (10 times) in the same day, in this method the sample has been analysed for 8 times. The precision is expressed as % RSD and the %RSD for the samples was 4.44%, it exceeds the normal limit of the precision that is 2% this indicates that the method may not be precise to repeat again. The precision was satisfactory. Need to be performed again to check whether the method was not satisfactory or if there are any problems with the injections made earlier.

Accuracy: The accuracy of this method was checked at three volumes containing three different concentrations. The accuracy is expressed as

%recovery, and the %recovery is the range of 91.5- 99.03%. The validation of this method shows that the accuracy is within the limits, which shows that this method is capable of showing good accuracy.

Table 6: Percent Recovery of the test solution.

Volume of sample taken

True value

Amount found

%recovery

4ml

0.5%

0.474%

94.8%

5ml

0.5%

0.459%

91.9%

6ml

0.5%

0.495%

99.03%

The validation methods tested in this method were accuracy, precision and the linearity. Accuracy and linearity were good but the precision was satisfactory. May be that was not performed correctly or may be due to the machine reproducibility problems. If any further study has to be carried out, the method for the precision has to be performed again because as this method gives good results for accuracy and linearity, the precision has to be checked again. In linearity, 15¼g/ml concentration of standard solution has to be analysed again because it was slightly varied from the calibration curve. This may due to some problems in the injections or may be due to the impurities in the volumetric flask of that concentration or may be in the pipette tip.

There are so many methods reported for quantitative determination of piroxicam in pharmaceutical preparations [9, 23], but there was less literature about the determination of piroxicam in topical dosage forms, there was a literature about hplc method to determine the quantity of piroxicam in ointments but there is no literature for gel. Hence Gel has been used for the quantitative determination of piroxicam. Commonly HPLC method was used to determine the quantity of piroxicam in the pharmaceutical preparations, therefore HPLC was selected as the method to analyse the quantity of piroxicam in the topical dosage form gel. So the method has been tried for the quantitative determination of the marketed product Feldene gel. Different mobile phases are used to quantify the

piroxicam in pharmaceutical dosage forms, the mostly used mobile phase was methanol and the phosphate buffer, in this the mobile phase has to be buffered to reduce the retention time that is when an analyte gets ionised the analyte becomes less hydrophobic, if the analyte is less hydrophobic the retention time will be decreased. This method was performed in isocratic mode because when a single analyte has to be analysed then this mode was used. There the parameters used were given in the below table.

Table 7: Optimised conditions for chromatograph

Parameters

Optimised conditions

Mobile phase

Methanol : 0.05M pH 7 phosphate buffer(40: 60 v/v)

Column size

250mm x4.6 mm dimension

Detection

240nm

Flow rate

0.8ml/min

With the use of these parameters the results were not good; the retention time was changed for day to day, it was changed from 7minutes to 9minutes

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and then to 13minutes and the chromatograms was also not good. And there was no linearity in the standard concentrations. When the calibration graph has been plotted there is no linearity a v- shaped calibration curve was obtained. This method was repeated for so many times but the results were same. So the method has been changed, the method changed was mentioned in the table below.

Table 8: Optimised chromatographic conditions:

Parameters

Optimised conditions

Mobile phase

Methanol: 0. 05M pH7 Phosphate buffer 55: 45 v/v

Column size

150mm x 4. 6mm dimension

Detection

254nm

Flow rate

1ml/min

After the changes in the method has been done, there are changes in the retention time, it was found to be around 6. 0 minutes, if the methanol proportions increases the retention time will be decreased so it the

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proportion has been increased and the results found were good that is the retention time has been changed from 13 minutes to 6 minutes and this was constant which was not constant earlier. And the chromatograms obtained were good. When the calibration graph was plotted the graph was linear with the results obtained from the changed method. With the changed method the results were good and the validation methods were performed on the method to know its accuracy and precision, the results for precision was satisfactory and the accuracy was good and the linearity was good.

In the previously reported methods quantitative determination of piroxicam in a new formulation piroxicam - β - cyclodextrin the method was accurate and precise. And the several other methods are reported many other pharmaceutical formulations they were accurate and precise, the method proposed here was accurate but the precision it has to be repeated again, as there was no time to repeat the method it was not done if any future work has to be carried out on this method, then firstly the precision has to be performed for inter and intraday. This proposed method was the new method for the determination of piroxicam of piroxicam in gel. If this method was repeated again for precision, and if the precision was good after the repetition of the method then it said to be a development of new method with good accuracy and precision for the quantitative determination of the piroxicam in the pharmaceutical preparation gel.

CONCLUSION:

High performance liquid chromatography is one of the most sophisticated tools of analysis at present. In this the estimation of piroxicam is done by reversed phase HPLC. In this method the mobile phase used was 45 volumes

of 0.05M pH7 phosphate buffer and 55 volumes of methanol. The detection is carried out using UV/VIS detector at 254nm. The retention time for piroxicam was found to be around 6.0 minutes. Linearity range for piroxicam was found to be 5 $\frac{1}{4}$ g/ml, 10 $\frac{1}{4}$ g/ml, 20 $\frac{1}{4}$ g/ml, and 25 $\frac{1}{4}$ g/ml. The quantitative estimation of piroxicam in Feldene gel was carried out by Reversed phase HPLC. A statistical test, simple linear regression model, was performed on the standard concentrations ranging from 5 $\frac{1}{4}$ g/ml to 25 $\frac{1}{4}$ g/ml. The validation of the assay consisting of linearity, accuracy and precision were carried out. The values of Relative standard deviation value was found to be 4.4% which was more than 2% indicating that the precision was satisfactory for this method. The percentage recovery was found to be 91.5% to 99.03% for piroxicam in the topical dosage form (Feldene gel).

The results obtained on the validation procedure for the precision does not meet the requirements. The accuracy and the linearity were good. It says that the method was accurate, and the linearity was good. The method was found to have satisfactory application to use in the routine laboratory analysis with a high degree of accuracy. A further work has to be carried out to repeat the precision again.

FURTHER WORK: The validation method precision has to be carried out again, to check whether the proposed method was not satisfactory or there are any problems during the injections of the test solution to the HPLC. If precision was satisfactory after the repetition then the developed method for the quantitative determination of piroxicam in the topical dosage regimen, Feldene gel (0.5%w/w of piroxicam which was present in 60g of gel) can be used in the routine laboratory analysis with a high