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Stability indicating HPTLC determination of piroxicam☆

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Abstract

A rapid and sensitive HPTLC method was developed and validated for the estimation of Piroxicam (PM). Spectrodensitometric scanning-integration was performed at an absorbance wavelength of 360 nm. To justify the suitability, accuracy and precision of the proposed method, recovery studies were performed at three concentration levels. One of the degradation products of PM is 2-aminopyridine (2AP). It becomes imperative to separate this compound as it is a precursor during synthesis of the drug. A TLC aluminium plate precoated with silica gel 60F-254 was used as the stationary phase. The solvent system toluene–acetic acid (8:2 v/v) gave a dense and compact spot of PM with a Rf value of 0.58 ± 0.01 which was well separated from 2AP (Rf 0.23 ± 0.01). The polynomial regression data for the calibration plots exhibited good linear relationship (coefficient of correlation $r = 0.9982$) over a concentration range of 400–800 ng. Statistical analysis proves that the proposed method is accurate and reproducible. The method is stability indicating and being economical can be employed for the routine analysis in bulk drug as well as pharmaceutical formulations.

Keywords: Piroxicam; HPTLC; Stability indicating; 2-aminopyridine

1. Introduction

Piroxicam (PM), a non-steroidal antiinflammatory agent from the Oxicam class is used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and acute pain in musculoskeletal disorders and acute gout. In addition, this drug has been in clinical practice as an analgesic in cases of dental, post operative and post partum pain. Commercially PM is available in the form of capsules, tablets, injectables and gel.

The drug can be determined with a variety of methods viz. TLC [1,2], LC [3–14], spectrophotometry [15], derivative spectrophotometry [16,17], spectrofluorometry [18], solid-phase extraction coupled with UV [19] and 2-D gel electrophoresis [20]. However, the chromatographic techniques reported are tedious and time consuming. In addition, not much attention has been paid to the determination of PM and it's degradation products in pharmaceuticals [21]. As a result a reliable and rapid determination method was developed which could also be used to obtain the optimum

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separation of the degradation component and from the parent compound. The proposed technique utilises a two component solvent system. This method is advantageous in that a large number of samples can be simultaneously subjected to analysis and comparatively less amounts of the solvents are required. The proposed method is highly sensitive. The time for analysis is considerably reduced as compared to the other documented methods. In addition, the method can be used as a qualitative tool as well.

The primary goal was to develop and validate a HPTLC method for the rapid quantitation of the drug. A second objective was to develop a stability indicating technique which could be employed for the routine quantitation of PM in the presence of 2-aminopyridine (2AP), one of the main degradation products of PM. In pharmaceutical dosage forms, the maximum allowable limit of 2AP is 2%. Moreover, 2AP is a precursor in the synthesis of PM.

2. Experimental

2.1. Materials

Piroxicam (purity: 99.98%) was a gift from IPCA Laboratories Ltd, India. Toluene (Ranbaxy Chemicals, India), acetic acid (Ranbaxy Chemicals Ltd, India) and 2-aminopyridine (Loba Chemie Pvt. Ltd, India) were of analytical grade.

2.2. Instrumentation

Piroxicam was spotted as a narrow band of 3 mm width at a constant application rate of 15 s/μl using a Camag microlitre syringe on precoated silica gel aluminium plate 60 F-254, (format: 10 × 10 cm having a thickness of 250 μm; E. Merck, Germany) using a Camag Linomat IV (Switzerland). A solvent system consisting of toluene-acetic acid (8:2, v/v) was selected. A linear ascending technique was employed for the development of the chromatogram in a Camag twin trough glass chamber. The chamber saturation time for the mobile phase was optimized to 15 min to ensure a concentrated zone of the compound and hence better resolution. The length of chromatogram run was set at 9 cm. After development, the TLC plates were dried in a current of air using an air blower.

The densitometric evaluation was performed on a Camag TLC scanner II (Switzerland) at 360 nm. Densitograms were obtained by integration performed using a Perkin Elmer integrator system LCI-100 (Switzerland).

2.3. Calibration plots

A stock solution of PM (10 μg/ml) in methanol was prepared. A series of standard curves were constructed over a concentration range of 400 to 800 ng following the same procedure described in Section 2.2. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated in triplicate.

Both PM and 2-AP are stable moieties. The solution state stability of the two compounds were assessed. Accurately weighed amounts of the pure drug and 2-AP were dissolved in acetone to get a final concentration of 10 μg/ml. The solutions were subjected to HPTLC analysis immediately and after a period of 6, 12, 24, 48 and 72 h.

2.4. Analysis of marketed formulation

Marketed tablet formulation (20 tablets) were weighed and powdered. Accurately weighed amounts of the tablet triturate corresponding to the label claim of the drug was dissolved in acetone and subjected to sonication for 15 min. The volume was made up to 25 ml and filtered. The filtrate after suitable dilution was spotted onto the plate followed by development and scanning as discussed in Section 2.2. The analysis was repeated in triplicate.

The tablet composition included dicalcium phosphate, lactose, starch, magnesium stearate, talc and aerosil in addition to the active ingredient. Placebo tablets prepared according to the same tableting technology were subjected to extraction as discussed above and spotted. This experiment was undertaken to find any possibility of interference from the excipients in the analysis of the drug.

2.5. Method validation

To justify the suitability, accuracy and precision of the proposed method, reproducibility and recovery studies were performed. A concentration within the linearity range (500 ng) was selected and analysed six times. This assay was repeated six times. In order to determine the recovery, known quantities of a previously analysed reference standard corresponding to 50, 100 and 150 percent of the label claim were added during the extraction procedure. The extraction solvent employed was methanol. Samples were analysed in the same way as described above. Each level was repeated in triplicate. The within day and day-to-day variation was studied and the percent recovery was computed using the following formula,

$$\% \text{ recovery} = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \times 100$$

Where X is the amount of standard drug added, Y is the amount of drug found by the proposed method and N is the total number of observations.

In order to obtain an estimate of the limit of detection and limit of quantitation, blank acetone was spotted six times following the same procedure explained in Section 2.2. The noise level was determined. While the limit of detection was calculated to be three times the standard deviation, the limit of quantitation was ten times the standard deviation value. Ruggedness of the proposed method was studied using reagents from different lots and different manufacturers.

Table 1
Marketed product: piroxicam content analysis[a]

Label claim	Experimental content	Recovery (%) (average ± s.d.)
640 mg	Within day, 20 mg	99.5 ± 1.1
	Day-to-day, 20 mg	99.3 ± 1.4

[a] $n = 6$, Not statistically significant (ANOVA; $P > 0.05$).

2.6. Stability indicating method

Pure PM and 2AP were spotted and the chromatogram was developed as described in Section 2.2. The degradation compound could be effectively separated from the pure drug by the eluent system used in the study.

3. Results and discussion

3.1. Optimization of chromatogram

In developing a suitable solvent system for PM, toluene alone gave a spot that exhibited a tailing phenomena. Among the various other components tried such as ethyl acetate, isopropyl alcohol and acetic acid, the acid was able to give a compact spot with the desired Rf value. A solvent combination of toluene and acetic acid (8:2, v/v) gave a dense and compact spot with a Rf value of 0.58 ± 0.01 ($n = 6$). It was observed that well defined spots of the drug under study were obtained when the chamber was equilibrated with the mobile phase for a period of 15 min.

3.2. Calibration plots

A representative standard curve of PM was constructed by plotting the peak area versus drug concentration. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation $r = 0.9982 \pm 0.012$; slope $= 11.449 \pm 0.137$ and intercept $= 6488.17 \pm 118.733$ ($n = 6$) over the concentration range studied. The range of reliable quantification was set at 400–800 ng as no significant difference was observed in the slopes of the standard curves in this range (ANOVA; $P > 0.05$). The RSD for within day and day-to-day analysis was found to be less than 2% in all the cases.

The drug was found to be stable in solution state during the HPTLC analysis. The assay content of PM after 72 h was found to be $99.98 \pm 0.89\%$.

Table 2
Recovery studies of marketed tablet formulation of piroxicam[a]

Experimental level	Theoretical content	Recovery (%) (average ± s.d.)
Level 1	Within day 15 mg	99.58 ± 1.07
	Inter day 15 mg	99.82 ± 1.30
Level 2	Within day 20 mg	100.14 ± 1.18
	Inter day 20 mg	99.42 ± 1.02
Level 3	Within day 25 mg	100.41 ± 1.16
	Inter day 25 mg	99.70 ± 1.10

[a] $n = 6$, Not statistically significant (ANOVA; $P > 0.05$).

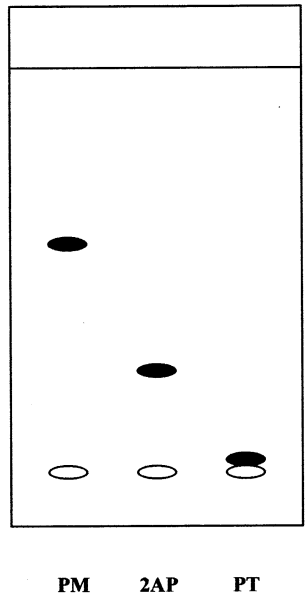

Fig. 1. A schematic representation of separation of piroxicam from it's degradation product (2-aminopyridine) and tablet excipients on TLC plate (after development). Key: Piroxicam (PM), 2-aminopyridine (2AP) and placebo tablet (PT).

3.3. Marketed product analysis

The results of the content analysis of marketed tablet formulations is shown in Table 1. The coefficient of variation for the studies is 1.11% (within day) and 1.42% (day-to-day). The tablet components gave a diffuse spot near to the point of spotting. This spot was well separated from the that of the drug. Thus, excipient interference was not observed.

3.4. Validation

The system precision results reveal that the proposed method was precise with an RSD as low as 0.93%. The data on recovery studies are shown in Table 2. In both, the intra day and inter day analysis the RSD was found to be less than 2%. The limit of detection was found to be 40 ng where the drug could be detected without any noise. The limit of quantitation was 150 ng. The method was found to be rugged with respect to the solvents employed form different manufacturers, with the RSD for system precision and recovery studies was found to be 1.32% and 1.58% (for different lots of reagents) and 1.08% and 1.68% (for different manufacturers) respectively.

3.5. Stability indicating

Degradation component 2AP gave an Rf value of 0.23 ± 0.01 ($n = 6$). The separation of both the compounds is given in Fig. 1. Thus, the method proves to be a stability indicating one.

4. Conclusion

The proposed HPTLC method of analysis is selective. Statistical data analysis (ANOVA; $P > 0.05$) proves that the method is precise and reproducible. The system being economical can be employed for the routine estimation of the drug in pharmaceutical formulations as well as in bulk drug analysis.

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