Spectrophotometric method for the simultaneous determination of piroxicam and 2-aminopyridine

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A rapid and simple spectrophotometric method is proposed for the simultaneous determination of piroxicam (Pi) and 2-aminopyridine (2-Ap). Piroxicam is stable under basic hydrolysis, but yields 2-Ap as one of the degradation products under acid hydrolysis. The method is based on the measurement of absorbances of 2-Ap and Pi at 300 and 360 nm, respectively, and the calculations are based on the binary method. The absorbances of both compounds obey Beer-Lambert's law over the concentration range of 5-25 μg mL⁻¹ with good linearity (r² > 0.99). The recoveries are within 100.8-106.4% for Pi and are within 98.4-98.9% for 2-Ap. Precision is good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The method has been applied for the determination of Pi and 2-Ap in piroxicam capsules. The average content of two different brands of piroxicam is 97.4 and 98.5% (n = 3), which complies with the USP 26 (92.5-107.5%). Under the stress condition (refluxing with 0.1 N HCl), the percentages of piroxicam decrease from 100% (0 h) to 18.9% (21 h) and 2-Ap increase from 0% (0 h) to 63.6% (21 h).

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Piroxicam (Pi), 4-hydroxy-2-methy-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a prototype of a non-steroidal anti-inflammatory drug (NSAID). The drug is an enolic acid, which exists in two tautomeric forms¹, with a pKa of 6.3 in dioxane:water (2:1)¹². Piroxicam is insoluble in water with a partition coefficient of 1.8 between octanol and aqueous buffer (pH 7.4)³.

The synthetic pathways of piroxicam are already known and the final step in the synthetic route involves the treatment of compound 5 with 2-aminopyridine (2-Ap) in refluxing xylene³. 2-Ap is not only used as a reagent in the synthesis, but it is also one of the degradation products of piroxicam. The presence of the 2-Ap residue in piroxicam raw material or as the degraded compound in piroxicam formulations is undesirable. 2-Ap can cause the irritation of eyes, nose, throat, headache, dizziness, excitement, nausea, high blood pressure, respiratory distress, weakness, convulsion and stupor⁴. Toxicity data in human show that after 5 h exposure to 5 μg ml⁻¹ of 2-Ap causes severe headache, increased blood pressure, flushing of the extremities and nausea⁵.

Several analytical methods have been reported for the analysis of piroxicam in formulations such as spectrophotometry⁶, spectrophotometry⁷, spectrophotometry⁸, high-performance liquid chromatography (HPLC)⁹-¹¹ and capillary electrophoresis (CE)¹². Determination of the drug in human plasma and urine has been demonstrated mainly by HPLC¹³-¹⁵. Stability indicating assays of piroxicam have been compared by HPLC, CE and high-performance thin layer chromatography (HPTLC)¹⁶-¹⁷.

A rapid and simple spectrophotometric method for the analysis of piroxicam and 2-Ap and studies on the stability of piroxicam under acid and basic hydrolysis are reported here. The remaining piroxicam and the generated 2-Ap are calculated using the binary method¹³. The method is valuable where a mixture shows overlapped ultra-violet (UV) spectra, i.e., the absorbance of compound X is interfered by that of compound Y and vice versa. Thus, the absorbance of each compound is the sum of the absorbance of both compounds present in the mixture as shown in Eqs (1) and (2). The amount of the individual compound can be derived by Eqs (3) and (4), which are obtained by the substitution of Eqs (1) and (2) with Beer-Lambert's law (A = abc).

\[ A_{X} = A_{X}\alpha + A_{Y}\beta \]  
\[ A_{Y} = A_{X}\alpha + A_{Y}\beta \]  
\[ A_{X} = \alpha \lambda_{X}bc + \alpha \lambda_{Y}bc \]  
\[ A_{Y} = \alpha \lambda_{X}bc + \alpha \lambda_{Y}bc \]

where, \( A_{X} \) is the absorbance of the mixture at the maximum wavelength of compound X; \( A_{Y} \) the absorbance of the mixture at the maximum wavelength of compound Y; \( \lambda_{X}, \lambda_{Y} \) the maximum wavelength of compound X and Y, respectively; \( \alpha, \beta \) absorbivity (L g⁻¹ cm⁻¹); \( b \) pathlength (cm); and, \( c \) the concentration (g L⁻¹).

Experimental

Analytical grade reagents and solvents were used here. Piroxicam and 2-aminopyridine were obtained
from Sigma (St. Louis, MO, USA). Piroxicam capsules were from SeaPharm Manufacturing (Ayutthaya, Thailand). Hydrochloric acid was obtained from BHD Lab (Poole, England) and sodium hydroxide was from LabGuard (Xalostoc, Mexico). Double distilled water was used.

Stock standard solutions of piroxicam and 2-Ap (10 mg L\(^{-1}\)) was prepared by separately dissolving appropriate amount of each compound in 0.1 N HCl. Working standard solutions were obtained by diluting the stock standard solution with water to obtain the concentration of 1 mg L\(^{-1}\) and adjusted pH to 7.0 with 0.1 N NaOH. The standard mixture solutions were prepared by mixing the working standard solutions to obtain the concentration of piroxicam and 2-Ap at ratios of 10:0, 7:3, 5:5, 3:7 and 0:10 µg L\(^{-1}\). Solutions for calibration data were obtained from diluting the working standard solution to the final concentration of 5-25 µg L\(^{-1}\).

pH measurements were carried out with a Consort C830 pH meter equipped with a glass combined electrode (Turnhout, Belgium). UV absorbance and spectra were obtained from a UV-160A Shidmadzu.

Assay of piroxicam in capsules by spectrophotometry

Solvents and sonication time were optimized using standard piroxicam. Ten mg of piroxicam was dissolved in a mixture of water and methanol at various ratios (100:0, 75:25, 50:50, 25:75 and 0:100 v/v) and sonicated in an ultrasonic bath for different times (0, 10, 15, 20 and 25 min). The optimum condition was employed for establishment of a calibration curve in a range of 5-25 µg L\(^{-1}\) and UV absorbance was obtained by measurement at the maximum wavelength scanning in a range of 200-600 nm.

Powder from twenty capsules of piroxicam capsules was mixed and finely grounded. An amount equivalent to 10 mg of the drug was weighed accurately, transferred into a 100 mL volumetric flask and diluted with the appropriate solvent. The solution was sonicated, filtered through a Whatman paper No. 1 and diluted to obtain the final concentration of 10 µg L\(^{-1}\). The solution was analyzed as described earlier.

Stability study of piroxicam under acid and basic hydrolysis

A preliminary study on the stability of piroxicam under acid and basic hydrolysis of piroxicam was performed by refluxing 10 mg of the drug in 100 mL of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide at 100°C. Aliquots were taken after 0, 3 and 6 h, divided into two portions and each was diluted to obtain the concentration of 10 µg L\(^{-1}\)of the original concentration. One portion was neutralized to pH 7.0 with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide and the others were kept un-neutralized at pH 1 or pH 13, respectively. UV spectra of all solutions were scanned in a range of 200-600 nm. The conditions, which gave 2-Ap as the degradation product was further employed for the stability study of piroxicam capsules.

Analytical performance characteristics

Analytical performance characteristics including linearity, precision, accuracy, limit of detection and limit of quantitation for the analyses of 2-Ap and the remaining piroxicam and by spectrophotometry using the binary method calculation were evaluated. Calibration curves were obtained by measurement the UV absorbance of the standard solutions of piroxicam and 2-Ap in a range of 5-25 µg L\(^{-1}\). Linear regression and correlation coefficient (r\(^2\)) were calculated using Microsoft Excel\(^{b}\) program. Precision of the method was determined by repetitive measurements (n = 5) of the UV absorbance of the pure piroxicam and 2-Ap solutions and percent relative standard deviations (%RSD) were calculated. Recoveries (%R) of the method were determined by preparing synthetic mixtures containing piroxicam and 2-Ap at ratios of 7:3, 5:5 and 3:7 µg L\(^{-1}\) and %R was calculated by the ratios of the amount added and amount found. Limits of detection (LOD) and quantitation (LOQ) were calculated from 3.3 and 10 times of the ratios of the standard deviation and the slope of the calibration curve, respectively.

Results and discussion

Assay of piroxicam in capsules by spectrophotometry

Assay of piroxicam in capsules was performed to check whether the piroxicam content in the investigated capsules complied with the USP 26 requirement\(^7\). The optimal solvent for dissolving piroxicam was in 50% v/v methanol and the appropriate sonication time was 15 min. The maximum UV absorbance of piroxicam was obtained at the wavelength of 360 nm and the linear equation was y = 0.0522x + 0.0112 (r\(^2\) = 0.9997). The average content of two different brands of piroxicam was 97.4 and 98.5% (n = 3), which complies with the USP 26 (92.5-107.5%).

Stability study of piroxicam under acid and basic hydrolysis

Results from preliminary study on the stability of piroxicam under acid and basic hydrolysis show that
UV absorbance of piroxicam at 360 nm remained unchanged after refluxing in basic condition for 3 or 6 h, but in acid condition the value dramatically decreased. Percentages of 2-Ap and the remaining piroxicam were calculated by the binary method. The percentages of piroxicam were unaffected under basic hydrolysis, which were 100% and 99.0% after 0 and 6 h, respectively. However, the percentages of piroxicam decreased from 100% (0 h) to 43.6% (6 h) under acid hydrolysis. We reasoned that acid hydrolysis of piroxicam easily occurred at the higher rate than basic hydrolysis due to the protonation of the amine group of the pyridine ring. The hypochromic shift (blue shift) of piroxicam from 360 to 332 nm occurred when the pH of the solution changed from pH 7 (neutralized solution) to pH 1 (non-neutralized solution), respectively. Additionally, the UV spectrum from acid hydrolysis of the neutralized solution (pH=7) shows the second peak of the degraded product at about 300 nm, which was sharper than that obtained from the non-neutralized solution (pH=1). The identity of the degradation product, 2-Ap, was confirmed by comparison of its UV spectrum with that of the standard 2-Ap, which provided the same peak shape and the same maximum absorbance at the wavelength of 300 nm. It was concluded that piroxicam was stable under basic hydrolysis, whereas it degraded under acid hydrolysis and yielded 2-Ap as one of the degradation products.

Further study focused on the acid hydrolysis of piroxicam from capsules by refluxing the drug for a longer period. In an attempt to obtain the complete acid hydrolysis, the refluxing time was increased from 0-21 h. Aliquots were prepared as described earlier, the UV absorbance was measured at 300 and 360 nm and percentages of the remained piroxicam and 2-Ap were calculated by the binary method. Fig. 1 shows that, after refluxing in 0.1 N hydrochloric acid, the percentages of piroxicam decreased from 100% (0 h) to 18.9% (21 h) and 2-Ap increased from 0% (0 h) to 63.6% (21 h). In comparison, the same lot of piroxicam capsules, which were not refluxed with hydrochloric acid, and the 2-Ap spiked samples were analyzed. These samples were dissolved in 0.1 N HCl, sonicated for 30 min, filtered and diluted to the desirable concentrations. This condition did not cause any degradation of piroxicam. UV absorbance of all samples were measured as described earlier. Data showed that the samples did not contain any significant amount of 2-Ap (LOD = 4.7 μg L⁻¹).

### Analytical performance characteristics

The validity of the methods for the analyses of piroxicam and 2-Ap was examined using the proposed procedures. Summary of optical and analytical performance characteristics is shown in Table 1. The calibration curves and linear equations of piroxicam at the wavelengths of 300 and 360 nm were $y = 0.0221x - 0.0099 (r^2 = 0.9999)$ and $y = 0.036x + 0.0737 (r^2 = 0.9996)$, respectively. For 2-Ap, the linear equation at the wavelengths of 300 was $y = 0.0643x + 0.0989 (r^2 = 0.9991)$. At the wavelength of 360 nm, 2-Ap did not show significant absorption. From the absorptivity values (Table 1), and calibration curves, it is evident that piroxicam strongly interferes the UV absorbance of 2-Ap at the wavelength of 300 nm.
Whereas, 2-Ap did not interfere the UV absorbance of piroxicam at the wavelength of 360 nm. This is a situation when the binary method plays an important role for calculations. Precision of the method, calculated from %RSDs of the absorbance of piroxicam and 2-AP at the wavelengths of 300 and 360 nm, were less than 5.23% for all cases (n = 5) and recoveries of piroxicam were within 100.8-106.4% (%RSD = 0.04%) and of 2-AP were within 96.4-98.9% (%RSD = 0.03). The LOD and LOQ values for both compounds were within 6.3 and 19.0 μg ml\(^{-1}\), respectively (Table 1).

**Conclusion**

Unlike other researchers, which use advanced techniques (e.g. HPLC, HPTLC and CE), we describe the utilization of spectrophotometric method for the determination of piroxicam and its degradation product, 2-Ap. Additionally, the direct UV measurement is rapid, convenient and in-expensive.

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**References**