Fast HPLC Method for the Determination of Piroxicam and its Application to Stability Study

ALINA DIANA PANAINTE, MADALINA VIERIU*, GLADIOLA TANTARU, MIHAI APOSTU, NELA BIBIRE
Grigore T. Popa University of Medicine and Pharmacy, Faculty of Pharmacy, 15th University Str., 700115, Iasi, Romania

A fast and robust RP-HPLC isocratic method was developed for determination of piroxicam in bulk materials and pharmaceutical formulations. Optimum separation of piroxicam and stress induced degradation a product was achieved using a SB-C_{18} Eclipse column (150x4.6; 5µm). The mobile phase was a mixture of water: acetonitrile (50:50) with a flow rate of 0.5mL/min. The UV detection was performed at 360nm. The method was validated in accordance with the current ICH guidelines in terms of linearity, limit of detection, limit of quantification, precision, accuracy, recovery and system suitability. The retention time for piroxicam was 2.55 min. The calibration graph was linear in the concentration range 5-90µg/mL. The assay proved to be sensitive, specific and reproducible. The method was applied for the determination of piroxicam in tablets.

Keywords: piroxicam, pharmaceutical formulations, HPLC, UV

Rheumatoid arthritis is an autoimmune disease that may cause chronic inflammation of the joints and other areas of the body. It is not life-threatening, but it creates a major discomfort and many people are suffering because of it. The treatment of rheumatoid arthritis optimally involves a combination of patient education, rest and exercise, joint protection, medications, and occasionally surgery.

The medications used in the treatment of rheumatoid arthritis includes nonsteroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs, immunosuppressants, and steroids. One of most important nonsteroidal anti-inflammatory drugs is piroxicam.

Piroxicam is a nonsteroidal anti-inflammatory drug from the oxicam class, which shows chemopreventive and chemosuppressive effects against various cancer cell lines in animal models. It is used to treat various painful conditions such as injuries and dental inflammation [1-3].

Piroxicam works by reducing hormones that cause inflammation and pain in the body. The anti-inflammatory effect of piroxicam result from the reversible inhibition of cyclooxygenase, causing the peripheral inhibition of prostaglandin synthesis [4, 5].

The chemical name of piroxicam shown in figure 1 is 4-hydroxy-2-methyl-1,1-dioxo-N-pyridin-2-yl-1ë6,2-benzothiazine-3-carboxamide. Piroxicam is official in European Pharmacopoeia [6], British Pharmacopoeia [7], United States Pharmacopeia [8], and Indian Pharmacopoeia [9].

Piroxicam may be quantitatively determined from tablets and biological fluids using various analytical methods such as high performance liquid chromatography (HPLC) [10-12], UV-Vis spectrophotometry [13, 14], electrophoresis [15, 16].

Because today's pharmaceutical industry and biomedical analysis are looking for new ways to cut costs and shorten time for the development of drugs, the main objective of this work was to develop and validate a simple, rapid, accurate and economical method for the determination of piroxicam using a high performance liquid chromatographic method with ultraviolet detection (HPLC-UV) from various pharmaceutical products.

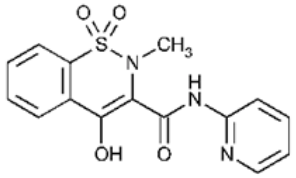

Fig. 1. Structural formula of piroxicam

Experimental part

Reagents

Solvents and other materials used were HPLC grade as provided by Fluka. Ultrapure water was obtained with a Milli-Q Ultrapure equipment. Piroxicam and tenoxicam reference substance were provided by Antibiotice Iasi Company, Romania. Pharmaceutical formulations with piroxicam were acquired from local pharmacies.

Apparatus

An Agilent Technologies 1100 High Performance Liquid Chromatograph equipped with Diode Array Detector, a Kern 770 analytical balance and a Cole Parmer ultrasonic bath were used.

Chromatographic Conditions

The chromatographic separation of piroxicam was achieved using isocratic elution on a SB-C_{18} Eclipse (150x4.6; 5µm) reverse phase stainless steel column. The temperature of the column and autosampler was maintained constant at 25°C. A 50µL sample loop injector was used. The detection was done spectrophotometrically 360nm. The mobile phase was a mixture of acetonitrile and water (50:50) used at a flow rate of 0.50mL/min.

Preparation of calibration standard solutions

100µg/mL piroxicam stock standard solution was diluted with mobile phase to appropriate concentrations for the calibration standard solutions. The solutions were deposited into 2mL vials in order to prevent evaporation and contamination. The stock solution of the internal standard (tenoxicam) was prepared in methanol, and added to the analyzed solutions.

* *email: vieriu_madalina@yahoo.com*

Preparation of sample solutions

The developed method for the determination of piroxicam was applied to the quality control of pharmaceutical products from various manufacturers.

Two types of piroxicam tablets produced by two different manufacturers were analyzed. For each pharmaceutical product, ten tablets were pulverized and a precisely weighed amount of powder equivalent to 5mg piroxicam was dissolved in 25 mL chloroform using an ultrasonic bath for 15 min. That solution was diluted with mobile phase to obtain a 50µg/mL solution of piroxicam, then it was filtered through a 0.45µm Millipore membrane. Three such sample solutions for each pharmaceutical product were analyzed.

The injectable solution from 1mL vials containing 20mg piroxicam was successively diluted with mobile phase to obtain a 50µg/mL sample solution. Three such sample solution were prepared for each of the three vials that were analyzed.

Method validation

Validation of the method was carried out following the norms of The International Conference on Harmonization (ICH) guidelines for selectivity, linearity, detection limit, quantification limit, system suitability, precision, accuracy, recovery, stability and robustness [17].

The linearity was investigated in the 2.5-120µg/mL concentration range and the calibration curve was obtained by plotting the peak area values against piroxicam concentration using linear regression.

The sensitivity of the analytical method was evaluated by determining the detection limit (LOD) and quantitation limit (LOQ) using the signal-to-noise ratio method (3:1 for LOD and 10:1 for LOQ).

The precision and accuracy were assessed by determining the active compound concentration at three concentration levels in the same day and in different days. The precision of the method was evaluated through standard deviation (SD) and covariance (CV) (%). The accuracy was evaluated through recovery.

The recovery of tablet extraction for three piroxicam concentrations was determined. Known amounts of standard piroxicam were added to sample solutions obtained from tablets. After the chromatographic analysis, the peak areas were compared to those obtained for standard solutions of piroxicam of the same concentration.

To evaluate the robustness of the developed RP-HPLC method, some small deliberate variations in the optimized method parameters were made. The effects of column temperature and modifying flow rate on the peak area were studied.

The stability was assessed by comparing the area of the standard solution that was maintained for 8 h at room temperature with that of a freshly prepared standard solution.

Stability tests were achieved in stressed conditions such as hydrolytic (acidic and basic), oxidation, and thermal conditions [18].

Results and discussions

Optimization of chromatographic conditions

Several parameters were examined for the optimization of HPLC analysis of piroxicam. The first step was establishing the composition of the mobile phase and the retention time (fig. 2). According to the results it was established that the optimum mobile phase was a mixture of acetonitrile:water and the retention time for piroxicam was 2.55min.

Various proportions of acetonitrile:water were tested when establishing the optimum mobile phase: 20:80, 30:70, 40:60, 50:50, 60:40, 70:30 and 80:20. The most appropriate chromatographic peaks were obtained when a 50:50 (v/v) mobile phase was used. Then the influence of the mobile phase flow rate on peak normalization was studied.

The optimum flow rate was 0.5mL/min. The detection was performed at a wavelength of 360nm as it was determined from the absorption spectra of piroxicam (fig. 3).

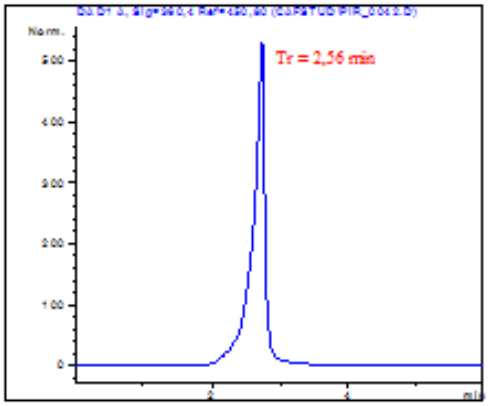

Fig. 2. Retention time of piroxicam

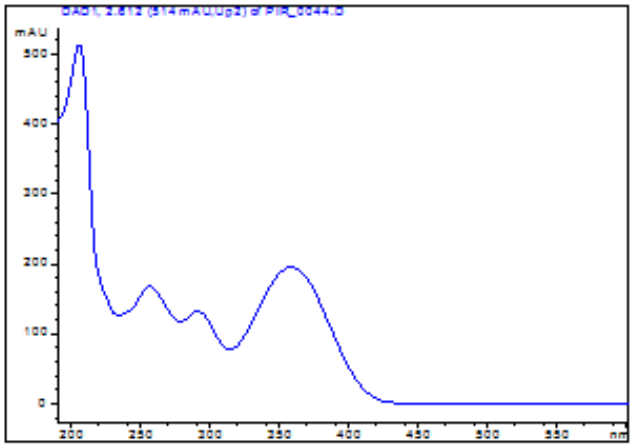

Fig. 3. The absorption spectra of piroxicam

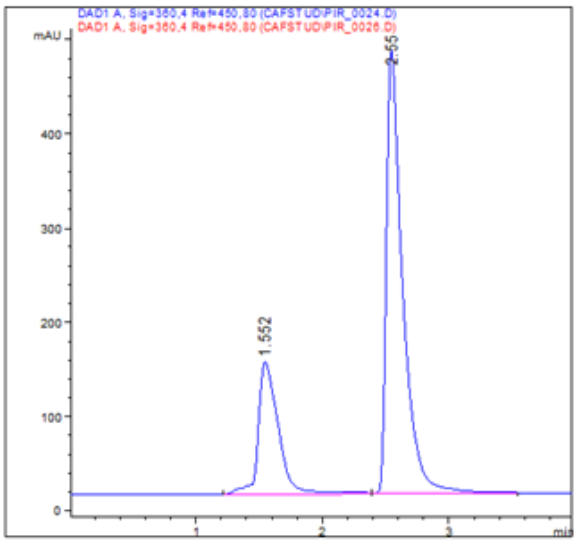

Fig. 4. Chromatogram of mixture with tenoxicam and piroxicam

Method validation

The specificity was assessed by using tenoxicam as internal standard (fig. 4).

That chromatogram showed that the method was selective because it had the ability to separate the signal corresponding to piroxicam from that of tenoxicam.

For the linearity study a calibration graph (fig. 5) that plotted the concentration against piroxicam peak area was used; that graph showed a good linearity in the 0.50-90.0µg/mL concentration range. The statistical parameters are summarized in table 1.

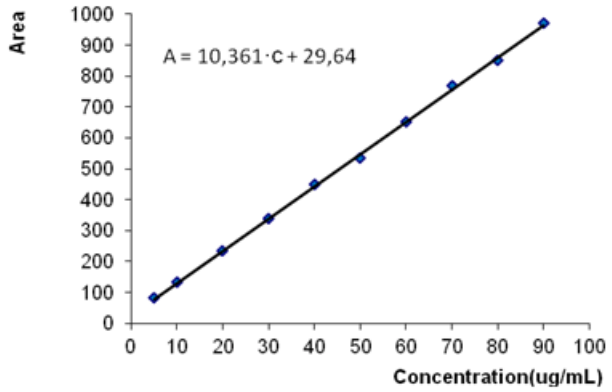

Fig. 5. Calibration curve

Table 1
STATISTICAL PARAMETERS

Regression coefficient	0.9993
Correlation coefficient	0.9996
Standard error	8.33
Intercept	29.64
Slope	10.36

The LOD and LOQ of piroxicam were 1.04 and 1.15µg/mL, respectively. Those values were lower than those obtained for other reported methods.

The system suitability was evaluated using piroxicam standard solution. Because $RSD_{area} \leq 2\%$ and $RSD_{retention\ time} \leq 1.5\%$, we concluded that the chromatographic system was robust (table 2).

The intra- and inter-day precision of measurements (table 3) for three different concentration levels (36, 45, and 54µg/mL) were lower than the accepted criteria (RSD ≤ 2%).

The proposed method was accurate because the maximum value of RSD(%) was lower than 2% and mean recovery yield was 1000.83 with values in between 99.08% and 101.98% (table 4).

The robustness of the method was evaluated through deliberate changes of flow rate (±0.2 mL) and of temperature of chromatographic column (±5°C). That study revealed that there were no significant variations in % assay (table 5).

The influence of interferers such as formulation excipients was evaluated before applying the method to the quantitative analysis of piroxicam in pharmaceutical products. A 40 µg/mL piroxicam standard solution was spiked with magnesium stearate, lactose monohydrate, starch, talcum, and gelatin at levels of 0.1% and then the solution was filtered through 0.45 µm membrane. The recovery values proved that none of the excipients had influenced the results of the analysis (table 6).

The experimental results of HPLC determination of piroxicam from 20mg tablets and from 20mg injectable solution are summarized in tables 8 and 9. Figure 6 presents the chromatogram of piroxicam analyzed from tablets and injectable solution.

The results of analysis proved that the amount of piroxicam was in good agreement with the label claim of the formulation, and the mean recovery of piroxicam as

Table 2
SYSTEM SUITABILITY

No.	Pick area	Statistical Data	Retention time (min)	Statistical Data
1	488.56	Mean = 487.64 SD = 7.72 RSD(%) = 1.58	2.55	Mean = 2.57 SD = 0.03 RSD(%) = 1.06
2	476.98		2.52	
3	496.10		2.59	
4	487.25		2.58	
5	491.74		2.56	
6	498.56		2.55	
7	479.23		2.54	
8	477.56		2.59	
9	496.86		2.57	
10	483.58		2.54	

Table 3
METHOD PRECISION

Repeatability			**Intermediate precision**		
Theoretical concentration (µg/mL)	Concentration found		**Theoretical concentration (µg/mL)**	Concentration found	
	µg/mL ± SD	RSD(%)		µg/mL ± SD	RSD(%)
35	34.95 ± 0.9	0.91	35	35.01 ± 0.55	0.58
45	45.01 ± 1.1	1.2	45	44.98 ± 0.88	0.9
54	53.98 ± 0.85	0.87	54	53.88 ± 1.1	1.2

Table 4
ACCURACY

No	Theoretical concentration (µg/mL)	Pick area	Found concentration (µg/mL)	Recovery (%)
1	36	408.17	36.53	101.48
2		402.83	36.01	100.05
3		410.05	36.71	101.98
4	45	497.68	45.17	100.38
5		503.41	45.72	101.61
6		494.23	44.84	99.64
7	54	583.96	53.50	99.08
8		598.82	54.93	101.73
9		597.95	54.85	101.58
Statistical data			Mean recovery	100.83
			Minimum	101.98
			Maximum	99.08
			RSD(%)	1.06

No.	Concentration (µg/mL)	Flow rate (mL/min)	Column temperature (°C)	Average area (n = 3)	Recovery (%)
1	36	0.45	20	405.01	100.63
2		0.50	25	403.35	100.19
3		0.55	30	399.87	99.25
4	45	0.45	20	491.21	99.00
5		0.50	25	498.23	100.50
6		0.55	30	502.02	101.32
7	54	0.45	20	589.13	99.99
8		0.50	25	596.03	101.23
9		0.55	30	599.99	101.940

Table 5
ROBUSTNESS OF CHROMATOGRAPHIC PARAMETERS

Excipient	Concentration (µg/mL)	Recovery ± RSD (%) for n = 3
Magnesium Stearate	25	98.91 ± 0.97
Lactose monohydrate		100.58 ± 0.52
Starch		100.62 ± 0.53
Talcum		101.01 ± 0.84
Gelatin		99.61 ± 0.41

Table 6
RECOVERY OF PIROXICAM IN THE PRESENCE OF EXCIPIENTS

No.	Label claim (mg/tablet)	Concentration found (mg/tablet) for n = 3	Recovery (%)	RSD (%)
1.	20	19.96	99.80	0.77
2.	20	19.93	99.65	0.62

Table 7
PIROXICAM ANALYSIS RESULTS FROM TABLETS

No.	Label claim (mg/vial)	Concentration found piroxicam/vial (mg) for n = 3	Recovery (%)	RSD (%)
1.	20	19.98	99.90	0.98
2.	20	20.03	100.15	0.68
3.	20	19.88	99.40	0.89

Table 8
PIROXICAM ANALYSIS RESULTS FROM INJECTABLE SOLUTION

mg/tablet and mg/vial were in agreement with the requirements of the 10th Romanian Pharmacopoeia (it admits a percentage deviation of ± 7.5% from the declared value for the active substance content).

In order to evaluate the piroxicam stability in solution, 8 replicate 40µg/mL sample solutions were analyzed and compared with 8 replicate 40µg/mL standard solutions. All those solutions were kept at room temperature for 8 h in the dark. The obtained results are summarized in table 9 and they showed that piroxicam was found to be stable in solution for at least 8 h at room temperature (CV% ≤15%).

Intentional degradation was carried out by exposing each sample to 5mL of 2M hydrochloric acid at 40°C for five hours and 5mL 2M sodium hydroxide solution at 40°C for ten hours on a water bath.

Oxidative degradation studies were performed at room temperature by adding 1mL 3% hydrogen peroxide to the

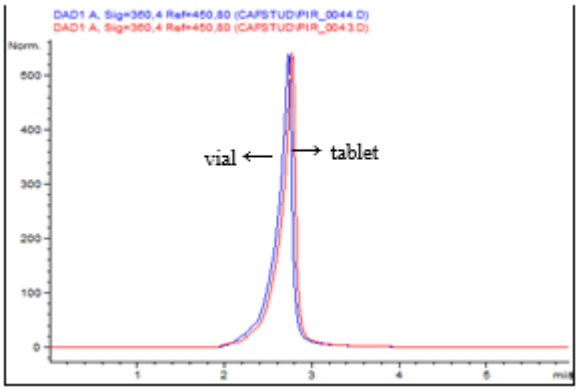

Fig. 6. The chromatograms of piroxicam from tablets and injectable solution

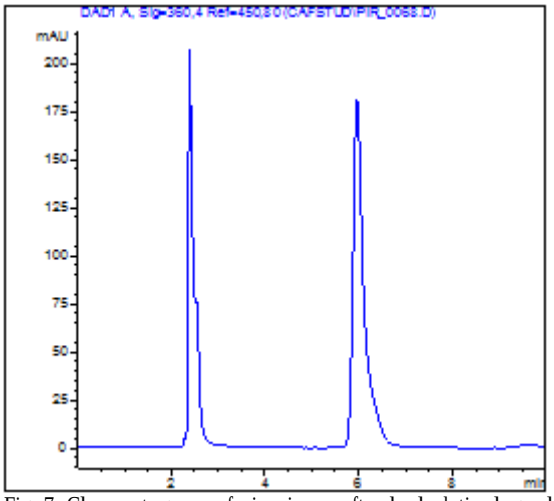

Fig. 7. Chromatogram of piroxicam after hydrolytic degradation in acidic medium

Analyte		piroxicam
Solvent		mobile phase
Storage conditions		8 hours at room temperature
Comparison Samples		stability samples
40µg/mL		40µg/mL
Analyte response		
451.31		455.05
439.45		439.87
439.21		439.13
396.68		491.23
456.41		439.02
454.23		453.41
447.68		434.23
454.52		453.65
Statistics		
Mean	451.18	449.44
SD	7.57	7.85
CV(%)	1.67	1.74

Table 9
ANALYTE STABILITY IN SOLUTION AT ROOM TEMPERATURE

Nature of stress	Storage conditions	Time (hours)	Remaining piroxicam (%)
2M HCl	40°C	5.00	95.56
2M NaOH	40°C	10.0	90.25
3% H_2O_2	25°C	0.30	80.25
Photolytic	sunlight	0.30	91.25
UV	UV lamp	0.30	79.56
Thermal	105°C	2.00	60.25

Table 10
STRESS TESTING RESULTS

standard solutions. After 30 min the standard solution was analyzed.

The photolytic degradation studies was performed on dry powder of piroxicam standard substance. After 30 min the powder was dissolved in mobile phase and then it was analyzed.

For UV degradation studies standard stock solution was kept under UV light for 30 min.

For the thermal stress study, piroxicam standard substance was heated in the oven at 105°C for 2 h. Afterwards the powder was dissolved in mobile phase and then it was analyzed.

The typical chromatogram of piroxicam degraded in acidic medium is shown in figure 7.

Piroxicam was found to degrade under acidic conditions. The degradation process was more intense and quicker in alkaline conditions. The highest degradation of piroxicam occurred under UV and thermal stress conditions (table 10).

Also, numerous degradation products (impurities) were detected after piroxicam was exposed to stress conditions.

Conclusions

The present study described the development and validation of a novel fast and accurate HPLC method with UV detection for the quantitative determination of piroxicam in bulk and pharmaceutical formulations. In the study, the recovery percentage of piroxicam was higher

than that obtained using other methods. Also, the newly developed method was much faster. It was a simple method that eliminated the need for mass spectrometry and large volumes of organic solvents. The proposed method was used to for the analysis of piroxicam in pharmaceutical formulations used for the treatment of arthritis. Those studies also provided information about the degradation pathways and degradation products formed during storage.

References

1.VIJAY-KUMAR, R., MADHUKAR, A., SANJEEVA, Y., SAMEER, G.N., UMA-MAHESH, K., Der Pharm. Lett., **2**, no. 2, 2010, p. 217.
2.COX, S., YARBROUGH, J., VEIGA-PARGA, T., GREENACRE, C., Chromatogr. Res. Int., 2014, Article ID 521697.
3.TITA, B., STEFANESCU, M., TITA, D., Rev. Chim. (Bucharest), **62**, no. 10, 2011, p. 1002.
4.NICOLESCU, L.C., TITA, B., JURCA, T., MARIAN, E., TITA, D., Rev. Chim. (Bucharest), **66**, no. 11, 2015, p. 1802.
5.ROY, S., BANERJEE, R., SARKAN, M., J. Inorg. Biochem., **100**, 2006, p. 1320.
6.***European Pharmacopoeia, 3rd Edition, Council of Europe, France, 1997, p 55.
7.*** British Pharmacopoeia, Her Majesty's Stationery Office, London, 2006, p 1508.
8.*** United States Pharmacopeia/ The National Formulary, (USP28/ NF 23) United States Pharmacopeial Convention, Rockville, 2005, p. 16, 1569.
9.*** Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Delhi, 1996, p. 554.
10.SAVAS, A., KARATAS, A., OZKAN, Y., YUKSEL, N., OZKAN, S.A., BAYKARA, T., Chromatograph., **59,** no. 9/10, 2004, p. 555.
11.PASCUAL-REGUERA, M.I., AYORA-CANADA, M.J., RUIZ, M.S.C., Eur. J. Pharm. Sci. **15**, no. 9, 2002, p. 179.
12.BURCEA DRAGOMIROIU, G.T.A., CIMPOIESU, A., GINGHINA, O., BALOESCU, C., BARCA, M., POPA, D.E., CIOBANU, A.M., ANUTA, V., Farmacia, **63**, no. 1, 2015, p. 123.
13.BONAZZI, D., ANDRISANO, V., GATTI, R., CAVRINI, V., J. Pharm. Biomed. Anal., **13**, no. 11, 1995, p. 1321.
14.EL-RIES, M.A., MOHAMED, G., KHALIL, S., EL-SHALL, M., Chem. Pharm. Bulletin., **51, no.** 1, 2003, p. 6.
15.GUL DAL, A., OKTAYER, Z., DOLRUKOL-AK, D., J. Anal. Methods Chem., **2014,** Article ID 352698.
16.CHEN, Y.L., WU, S.M., Anal. Bioanal. Chem., **381**, no. 4, 2005, p. 907.
17.*** ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1), in: Proceedings of International Conference on Harmonization, 2005.
18.*** International Conference on Harmonization (ICH) Q1A(R2) Stability of new drug substances and products, 2003, CPMP/ICH/2736/ 99

Manuscript received: 19.12.2016