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A NEW RP-HPLC METHOD FOR ANALYSIS OF MELOXICAM IN TABLETS

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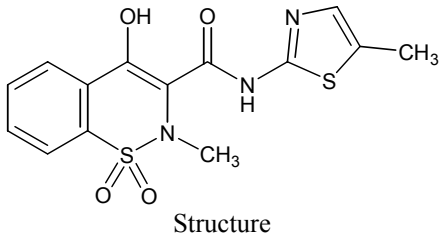
A simple and rapid high-performance liquid chromatographic (HPLC) method for the determination and quantification of meloxicam has been developed. The chromatographic system consisted of a Shimadzu LC-10 AT VP pump, SPD-10 AV VP UV/ visible detector, and a CBM-102 Bus Module integrator. Separation was achieved on the μ Bondapak 125 A C18 10 μm column at room temperature. The sample was introduced through an injector valve with a 10 μl sample loop. Methanol:water (70:30 v/v) was used as mobile phase, with flow rate 2 ml/minutes. pH was adjusted to 2.6 with phosphoric acid. U.V detection was performed at 230 nm. The results obtained showed a good agreement with the declared content. Recovery values of meloxicam in tablets (in Melfax 15 mg tablets) were from 99.27 % to 100.06 %. Piroxicam was used as an internal standard. The proposed method is rapid, accurate and selective; it may be used for the quantitative analysis of meloxicam from raw materials, in bulk drugs and other dosage formulations.

Keywords: Meloxicam, Piroxicam, HPLC determination.

INTRODUCTION

Meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-1,3-thiazol-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide having molecular formula $C_{14}H_{13}N_3O_4S_2$, molecular weight 351.4, melting point 254ºd (structure). It is practically insoluble in water, slightly soluble in acetone, soluble in dimethylformamide, very slightly soluble in ethanol (96 %), and methanol. Meloxicam is a non steroidal antiinflammatory oxicam derivative, which selectively inhibits cyclooxygenase-2 (Cox-2) and has analgesic and antipyretic activities (The Merck index. 2001; Martindale 1996; United State Pharmacopoeia 2004; British Pharmacopoeia 2003). The usual dosage of meloxicam is 7.5 mg once in a day, which may be doubled to 15 mg per day in acute painful conditions with severe pain like rheumatoid arthritis.



Structure

There are number of methods reported in the literature to determine meloxicam. Velpandian *et al* (2000) reported HPLC method for the determination of meloxicam in biological samples, while a pulse polarographic method for the determination of meloxicam is also available (Altiokka 2001). Methods for LC determination of meloxicam in plasma were developed by Dasandi *et al* (Dasandi *et al* 2002) and Baeyens *et al* (Baeyens *et al* 2003) while LC-tanden mass spectrometry method for the determination of meloxicam in human plasma was reported by Wiesner *et al* (Wiesner *et al* 2003). A liquid chromatographic method was also reported to determine meloxicam in bulk drug and pharmaceutical formulation by Zawilla *et al* (Zawilla *et al* 2003).

The present work describes a simple reverse phase HPLC method for the determination of meloxicam from reference materials, bulk raw materials and dosage formulations. The determination was carried out on a Hypersil, ODS, C-18 (150x4.6 mm, 5 micron) column using a mobile phase of methanol/water (70:30) and pH was adjusted to 2.6 with phosphoric acid. The flow rate was adjusted to 2 ml/minutes and retention time was 3.3 minutes. The eluent was monitored at 230 nm. The method was found to be reproducible, with good resolution between meloxicam and piroxicam. The detector response was found to be linear in the concentration range of 5 ng to 30 ng of drug. The validity of method was determined over a wide range of period for any possible intra-day and inter-day variations.

EXPERIMENTAL

Material and reagents

Samples of piroxicam, meloxicam and melfax 15 mg tablets were a kind gift from AGP (Private) Limited, which were used without further purification. HPLC grade methanol and phosphoric acid were obtained from Merck Germany. The mobile phase and solution were prepared in deionized water, which was freshly prepared in the laboratory before use. Stock solutions of the compounds were prepared in methanol. Fresh working solutions were prepared daily. All solutions were filtered (0.45 μm) and degassed by sonicator.

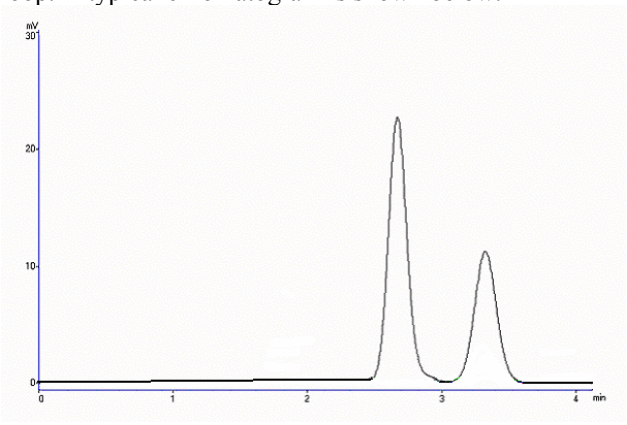
Instrument

The HPLC systems used in both Labs were identical and consisted of LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector, a μ-Bondapak 125 A C18 10

cm column (particle size 10 µm). The chromatographic data was integrated using a CBM-102 communication Bus Module Shimadzu and was recorded on an IBM PC.

Chromatographic conditions

The mobile phase used was methanol/water (70:30). The pH of this mobile phase was adjusted to 2.6 with phosphoric acid (85 %). Prior to delivering into the system it was filtered through 0.45 µm filter and degassed using a sonicator. The analysis was carried out under isocratic conditions using a flow rate 2.0 ml /minutes at room temperature. Chromatograms were recorded at 230 nm using a detector SPD-10AV VP Shimadzu UV visible. The samples were introduced by injector with a 10-µliter sample loop. A typical chromatogram is shown below.



A typical chromatogram showing separation runs of meloxicam from its internal standard.

Retention time = Piroxicam (Internal standard) = 2.3 minutes
Meloxicam = 3.3 minutes

Analytical procedure

10 mg piroxicam and 10 mg of meloxicam were dissolved in methanol in 100 ml volumetric flask and made up the volume with the same solvent (stock solution of 100 µg/ml). Aliquots were appropriately diluted. Twenty tablets of melfax 15 mg were weighed to obtain the average tablets weight, (320 mg) and were then powdered; 213.3 mg of the powdered tablets were mixed with 100 ml methanol. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug (stock solution). This stock solution was filtered and this clear filtrate was diluted to desired concentrations. 10-µliter volume of each sample was injected and chromatographed under above conditions.

RESULTS AND DISCUSSION

The use of HPLC methods for the quantitation of drugs has received considerable attention in the recent past and its importance in the quality control of drugs and drug products is unquestioned. The goal of this study was to develop a rapid, more accurate, precise reliable least time consuming HPLC method for the analysis of meloxicam in the form of raw materials, bulk drug samples and its tablets or formulations using the most commonly employed C-18 column with UV detector. During these studies piroxicam was used as an internal standard.

The newly developed method has been validated and holds well for the determination of drug in raw materials and other dosage formulation. The method has been successful in determining the meloxicam in concentrations, as low as 5 ng, with retention time of only 3.3 minutes. The internal standard, piroxicam was separated earlier at 2.3 minutes.

Chemical structure and chemical properties are the most important facts that predict chromatographic behavior. In the present investigation the best separation of meloxicam from its internal standard was achieved using a µ-Bondapak 125 A C18 (10 µm) column. Using other type of column under similar experimental condition, the separation lasted about 28 minutes. For the determination of meloxicam and its separation from piroxicam, the internal standard, best results were obtained using mobile phase methanol/water (70:30 v/v). The lower percentage of methanol in mobile phase results in peak tailing of both components and long analysis duration while higher percentage of methanol in mobile phase results very little analysis duration. Optimal retention times (piroxicam — 2.3 minutes and meloxicam — 3.3 minutes) were achieved when the pH of mobile phase was adjusted to 2.6 with 85 % phosphoric acid. Small changes in pH of the mobile phase had a great influence to the chromatographic behavior of these substances. The higher pH of the mobile phase also results in peak tailing and at a lower pH retention time of piroxicam and meloxicam was extremely long.

Accuracy and precision

The accuracy of the method was evaluated by analyzing independently prepared solutions of meloxicam. The recovery data is expressed in tables 2, 3 and 5. These results show that the method is accurate for determination of meloxicam in nano-gram levels.

The precision of the method was investigated with respect to repeatability. For intra-day precision, six concentration of each compound were analyzed in the same day. Each concentration of sample was injected four times. Table 1 summarizes the standard deviation and relative standard deviation (RSD). Generally acceptable repeatability of the results with in one day and day-to-day was observed. Data of the relative retention times obtained in a series of four consecutive injections also showed acceptable repeatability when analyzed not only on the same day but also on three consecutive days. Table 4 showed relative response factor of estimation of meloxicam, standard deviation and coefficient of variation of response factor.

Table 1
Intra and inter day variations in the analysis of meloxicam

Drug injected	Time	Day 1	Day 2	Day 3	Day 4	(±)S.D	RSD	Mean
5 ng	8:00	6108	6962	6409	5978	437.48	0.07	6364.25
	11:00	6161	6970	6420	5996	426.20	0.07	6386.75
	02:00	6126	6964	6400	5942	445.68	0.07	6358.00
	05:00	6142	6968	6411	5966	437.60	0.07	6371.75
	Mean	6134.25	6966.00	6410.00	5970.50			
	S.D.	22.60	3.65	8.21	22.65			
	RSD	0.0037	0.0005	0.0013	0.0038			
10 ng	8:00	12149	14046	12903	11892	966.15	0.08	12747.50
	11:00	12206	13992	12846	11920	919.47	0.07	12741.00
	02:00	12186	14009	12800	11909	932.85	0.07	12726.00
	05:00	12192	13949	12882	11806	939.90	0.07	12707.25
	Mean	12183.25	13999.00	12857.75	11881.75			
	S.D.	24.32	40.24	45.12	51.80			
	RSD.	0.0020	0.0029	0.0035	0.0044			
15 ng	8:00	18346	20899	19344	18016	1294.73	0.07	19151.25
	11:00	18442	20942	19326	17946	1315.59	0.07	19164.00
	14:00	18412	20920	19280	17909	1320.73	0.07	19130.25
	17:00	18386	20907	19249	17929	1312.41	0.07	19117.75
	Mean	18396.50	20917.00	19299.75	17950.00			
	S.D.	40.71	18.78	43.25	46.53			
	RSD.	0.0022	0.0009	0.0022	0.0026			
20 ng	8:00	24496	27906	25832	23796	1808.36	0.07	25507.50
	11:00	24523	27942	25740	23849	1798.20	0.07	25513.50
	14:00	24504	27876	25659	23890	1756.28	0.07	25482.25
	17:00	24384	27893	25682	23747	1830.89	0.07	25426.50
	Mean	24476.75	27904.25	25728.25	23820.50			
	S.D.	62.86	28.00	77.11	62.30			
	RSD.	0.0026	0.0010	0.0030	0.0026			
25 ng	8:00	30593	34799	32322	29749	2229.55	0.07	31865.75
	11:00	30692	34856	32296	29836	2207.00	0.07	31920.00
	14:00	30647	34802	32443	29809	2210.29	0.07	31925.25
	17:00	30620	34780	32420	29966	2155.22	0.07	31946.50
	Mean	30638.00	34809.25	32370.25	29840.00			
	S.D.	42.21	32.65	72.13	91.53			
	RSD.	0.0014	0.0009	0.0022	0.0031			
30 ng	8:00	36692	41705	38146	35916	2565.77	0.07	38114.75
	11:00	36776	42039	38209	35940	2699.79	0.07	38241.00
	14:00	36749	41696	38188	35892	2558.35	0.07	38131.25
	17:00	36780	41833	38320	35959	2598.04	0.07	38223.00
	Mean	36749.25	41818.25	38215.75	35926.75			
	S.D.	40.57	159.92	74.27	29.09			
	RSD.	0.0011	0.0038	0.0019	0.0008			

System suitability and specificity

System suitability of the method was evaluated by analyzing the symmetry of the meloxicam peaks (symmetry factor), theoretical plates of the column, resolution between the peaks of meloxicam and internal standard, mass distribution ratio (capacity factor) and relative retention. The specificity of the method was evaluated to ensure separation of meloxicam and was demonstrated by assaying a sample of meloxicam and piroxicam. The method demonstrated resolution between both the drugs.

Quantification limit

The limit of quantitation of developed method was found 500 ng/ml.

Table 2
Recovery of meloxicam in reference drug and dosage form

Conc.(ppm) Injected	<——— Reference drug ———>			<——— Dosage form ———>		
	Peak area	Found	% Recovery	Peak area	Found	% Recovery
0.5	6134.25	0.500	100.15	5970.5	0.498	99.77
1.0	12183.25	0.994	99.45	11881.7	0.992	99.27
1.5	18396.50	1.501	100.11	17950.0	1.499	99.98
2.0	24476.75	2.998	99.90	23820.5	2.990	99.51
2.5	30638.00	2.501	100.04	29840.0	2.493	99.73
3.0	36749.25	3.000	100.00	35926.7	3.001	100.06

Table 3
Recovery of meloxicam in dosage forms (tablets) by the proposed method

Dosage formulation	Labeled amount	Observed amount	% Recovery	% Error
Melfax (AGP) tablet	15 mg	14.95	99.66	0.33
		15.009	100.06	0.06

Table 4
Relative response factor for the estimation of meloxicam by the proposed method

Concentration μg ml^{-1}	Day 1	Day 2	Day 3	Day 4	(±)S.D	C.V. (n=16)
0.5	1.00	1.00	1.00	1.00	0.000	0.0000
1.0	1.99	2.01	2.01	1.99	0.012	0.0019
1.5	3.00	3.00	3.01	3.01	0.005	0.0006
2.0	3.99	4.01	4.01	3.99	0.012	0.0010
2.5	4.99	5.00	5.05	5.00	0.027	0.0018
3.0	6.00	6.00	5.96	6.02	0.024	0.0013

Table 5
Recovery and regression characteristics of the proposed method

Concentration	<——— Recovered concentration ———>			
(μg^{-ml}) injected	Day1	Day 2	Day 3	Day 4
0.50	0.50	0.50	0.50	0.50
1.00	0.99	1.00	1.01	0.99
1.50	1.50	1.50	1.52	1.50
2.00	2.00	2.00	2.02	1.99
2.50	2.50	2.50	2.54	2.49
3.00	3.00	3.00	3.00	3.00
Correlation coefficient (R)	0.9999	0.9999	0.9997	0.9999
Standard error of estimate	0.0029	0.0023	0.0158	0.0051
Intercept	-0.0018	0.0024	0.0063	-0.0045
P value	0.0000	0.0000	0.0000	0.0000

Ruggedness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Department of Chemistry, Faculty of Science, University of Karachi, while Lab 2 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi. In both the labs instruments of the same model and make were used.

CONCLUSION

A rapid, precise, accurate, low cost and least time consuming RP-HPLC method for the qualitative and quantitative analysis, determination and quantification of meloxicam in raw materials as well as dosages formulation has been successfully developed.

Table 6
Precision of analysis of meloxicam and piroxicam by proposed method

Concentration (ppm) meloxicam	Day 1		Day 2		Day 3		Day 4	
	S.D	RSD	S.D	RSD	S.D	RSD	S.D	RSD
0.5	22.6	0.0037	3.65	0.0005	8.21	0.0013	22.65	0.0038
1	24.32	0.002	40.24	0.0029	45.12	0.0035	51.8	0.0044
1.5	40.71	0.0022	18.78	0.0009	43.25	0.0022	46.53	0.0026
2	62.86	0.0026	28	0.001	77.11	0.003	62.3	0.0026
2.5	42.21	0.0014	32.65	0.0009	72.13	0.0022	91.53	0.0031
3	40.57	0.0011	159.92	0.0038	74.27	0.0019	29.09	0.0008

The proposed RP-HPLC method enables the determination of meloxicam because of good separation and resolution of the chromatographic peaks from internal standard. The obtained results are in a good agreement with the declared contents of dosage formulations. Results are accurate and precise and are confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this HPLC method give it advantage over to the other reported HPLC methods for determination of meloxicam.

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HYPOGYLCEMIC ACTIVITY OF AQUEOUS EXTRACT OF SOME INDIGENOUS PLANTS

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Pakistan is rich in medicinally important plants and has an ancient herbal treatment methods. Our work is based on the study of some indigenous plants which show inhibitory effect of glucose utilization, and are in use as hypoglycemic agent in traditional system of medicine. Gymnema sylvestre, Momordica charantia and Eugenia jumbolana have been shown to possess hypoglycemic activity of varying degree. The results in three different media revealed that, hypoglycemic activity is more prominent in neutral and basic media as compared to acidic medium.

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Table 6
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