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Short Communication

LC determination and pharmacokinetics of meloxicam

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Abstract

A simple and rapid HPLC assay method for the estimation of meloxicam in plasma was developed. The method totally eliminated the solvent extraction procedure. The plasma proteins were precipitated using perchloric acid (70%) and acetonitrile mixture (1:1 v/v) and the supernatant was directly injected to the HPLC system. The separation was achieved on a Lichrospher C_{18} 5μ (125 × 4.0 mm) analytical column with a mobile phase of sodium acetate buffer (pH 3.3, 170 mmol):acetonitrile (62:38 v/v) mixture. Detection was by UV detector at 355 nm. The retention time observed for meloxicam and piroxicam (internal standard) were at 6.0 and 4.0 min, respectively. The response was linear over a range of 50–1500 ng ml^{-1} in human plasma. The method was simple, specific, precise and accurate. The method was also used for the bioequivalence study of meloxicam formulation in healthy, human, Indian, male volunteers.

Keywords: Meloxicam; Piroxicam; One step sample preparation; ODS column; UV detection; Pharmacokinetics; Indian male volunteers

1. Introduction

Meloxicam is a new NSAID of the enol carbamide class. Animal studies have shown that in addition to high anti-inflammatory efficacy meloxicam appears to have low ulcerogenic potency [1] and exhibits less gastric irritation and local tissue irritation in comparison to other NSAIDs [2]. This good tolerability profile may be explained by the ability of meloxicam to preferentially inhibit the inducible cyclo oxygenase present in inflamed tissue (COX-2) over COX-1 that has a house keeping function in prostaglandin formulation [3].

Previous single dose pharmacokinetic studies [4] in healthy fasting volunteers have shown that meloxicam has prolonged absorption after oral administration. This avoids high initial drug concentration and is suitable for once daily dosage [5]. The plasma protein binding of meloxicam is more than 99.5% [6]. Few LC assay methods for meloxicam quantitation in biological fluids have been reported. The method reported by Busch et al. involves long extraction procedures and requires sophisticated equipments like column switching [7]. The method reported by Velpandian

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et al. [8] is simple, but involves the conventional extraction procedure. The method reported here bypasses the whole process of extraction, separation and evaporation. The plasma is treated with perchloric acid and acetonitrile mixture and the supernatant was directly injected to HPLC. Another method reported by Schmed et al. [9] also involves a complicated process of precolumn enrichment of the sample with ammonium formate and gradient elution procedure.

This paper describes a sensitive, specific and simple method involving one-step rapid sample preparation technique. Human plasma samples containing meloxicam and internal standard were precipitated using perchloric acid (70%): acetonitrile (1:1 v/v) with more than 85% recovery. Determination of meloxicam was performed on reversed phase C_{18} column.

The developed method was applied to bioequivalence study of two oral dosage forms of meloxicam (test and reference). The open randomized, cross over study performed on a group of 12 healthy, Indian male volunteers confirmed the bioequivalence of both the formulations.

2. Experimental

2.1. Materials

Meloxicam and piroxicam were obtained from Cadila Pharma (Ahmedabad, India) as gift sample. Sodium acetate, glacial acetic acid, perchloric acid (70% v/v) of analytical grade and acetonitrile and water of HPLC grade were obtained from Merck, India (Mumbai).

2.2. Apparatus and conditions

Analysis was performed using HPLC system consisting of a pump (L-7110, Merck Hitachi), UV–visible Detector (L-7400, Merck Hitachi) and auto sampler (L-7200, Merck Hitachi). The system was connected with help of D-7000 interface to HSM software in a computer system for data collection and processing.

The analytical column used was Lichrospher C_{18} (5μ, 4.0×125 mm, Merck, Germany). The protein precipitating solution was a mixture of acetonitrile and perchloric acid (70%) in the ratio of 1:1 v/v. The mobile phase contained 62% of buffer and 38% of acetonitrile and delivered at a rate of 1 ml min^{-1}. The buffer was 170 mmol of sodium acetate in water with pH adjusted to 3.3 with glacial acetic acid. The eluent was monitored at 355 nm. Under these conditions the retention times observed for meloxicam and piroxicam were 6.0 and 4.0 min, respectively.

2.3. Pharmacokinetics

Two formulations of meloxicam (test and reference) were administered to 12 healthy, Indian male volunteers in a double blind, randomized, cross over design. The washout period was 7 days. The volunteers were selected on a pre set inclusion–exclusion criteria. The volunteers were screened for vital signs, blood and urine analysis before enrolment. Oral dose of 30 mg meloxicam was administered with 240 ml of water. Blood samples were withdrawn at 0 h and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 h post dose. The samples were stored at -20 °C pending analysis and analyzed by the above method. A concentration time curve was plotted and AUC calculated by trapezoidal rule (AUC_{0-72}). $AUC_{0-\infty}$ was also calculated. Time to achieve the maximum concentration (C_{max}) t_{max} was obtained directly from the concentration time curve without interpolation. All the pharmacokinetic data are calculated using 'QUICKCALC', in house software.

2.4. Preparation of standard curve

One hundred microlitres of meloxicam solution prepared using drug free plasma, of appropriate concentration and 100 μl of piroxicam of (5 μg ml^{-1}) were added to 900 μl of drug free plasma contained in a clean borosilicate glass tube and vortexed for 10 s. To this 100 μl of protein precipitating reagent was added and vortexed for 1 min. After centrifugation at 3000 rpm for 20 min, 100 μl of the supernatant was injected to the HPLC system.

3. Results and discussions

3.1. Specificity

Representative chromatograms of processed human blank plasma, LQC and HQC were presented in Figs. 1–3, respectively. The chromatograms shown are the true scale, unmodified ones. Though the peaks look slightly broad, they are quiet reproducible and did not effect the

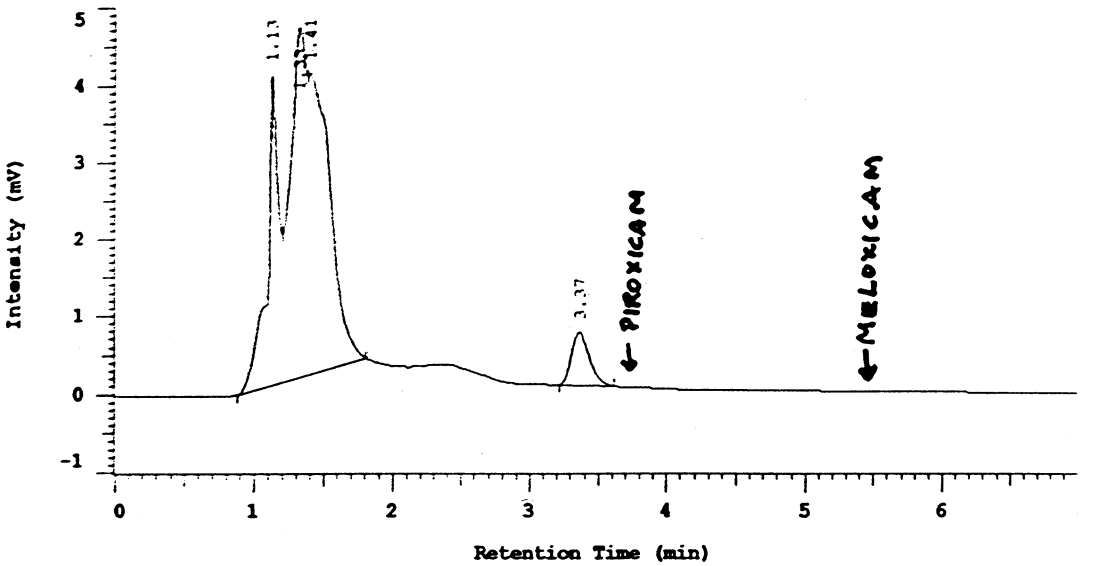

Fig. 1. Representative chromatogram of processed blank human plasma.

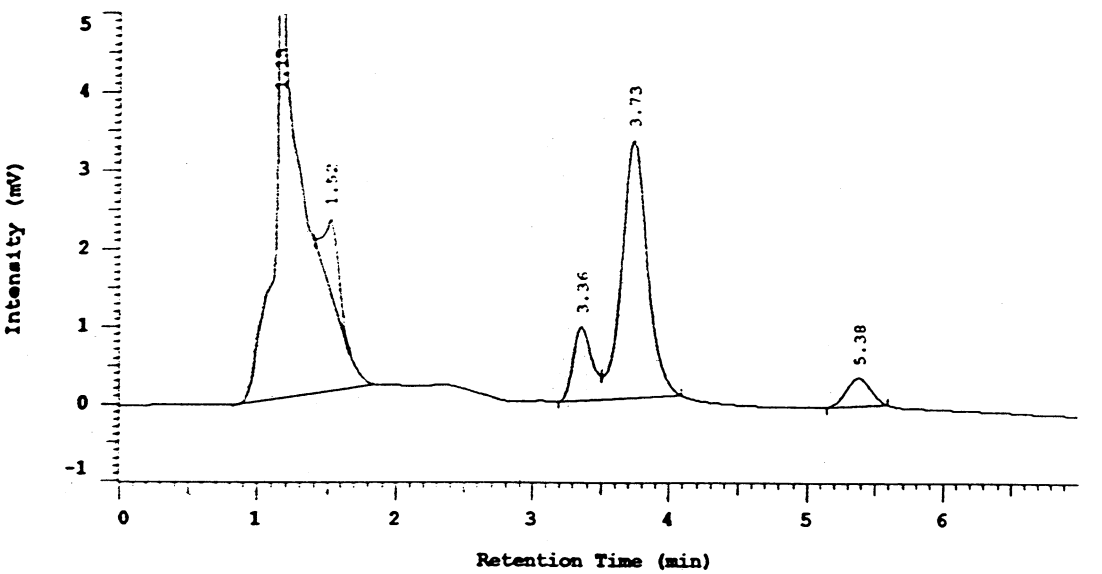

Fig. 2. Representative chromatogram of LQC.

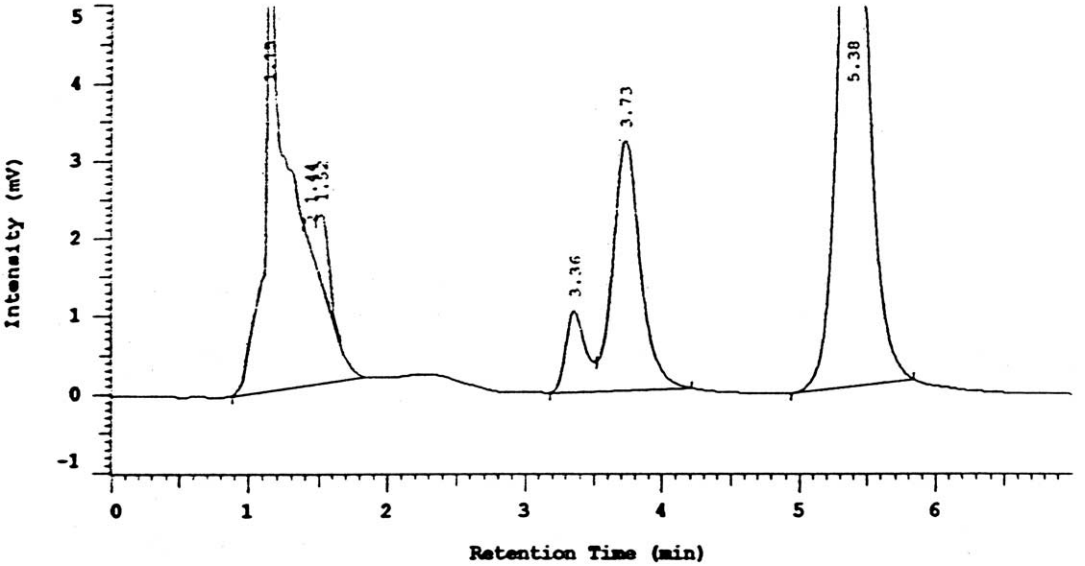

Fig. 3. Representative chromatogram of HQC.

results in any manner. No interfering peaks were observed in blank at retention time corresponding to the drug and internal standard. This shows that the assay procedure is specific to meloxicam. All human plasma samples used for calibration standards and QC were free from interfering peaks.

3.2. Linearity of calibration curves

A standard curve of seven points was plotted ($n = 5$). A straight-line equation with weightage factor of $1/X^2$ was derived for the observed response. Calibration curve data and calibration curve parameters for meloxicam and piroxicam (internal standard) in human plasma demonstrate that calibration curves were linear in the concentration range from 50 to 1500 ng ml^{-1}. The correlation coefficient was found to be 0.9991 ± 0.0005 (Table 1).

3.3. Precision and accuracy

The limit of detection (LOD) for this assay was 10 ng ml^{-1}. The limit of quantitation (LOQ) of the method is 50 ng ml^{-1}. The interday and intraday accuracy and precision was assessed by replicate analysis of three QC samples. The mean RSD values for intraday and interday assay reproducibility ($n = 5$) were 7.23 and 6.41%, respectively (Tables 2 and 3, respectively). Data presented in the above tables are the coefficient of variation (%CV) for each sample processed.

3.4. Recovery

Absolute recoveries were determined by comparing the ratio of peak height of meloxicam to

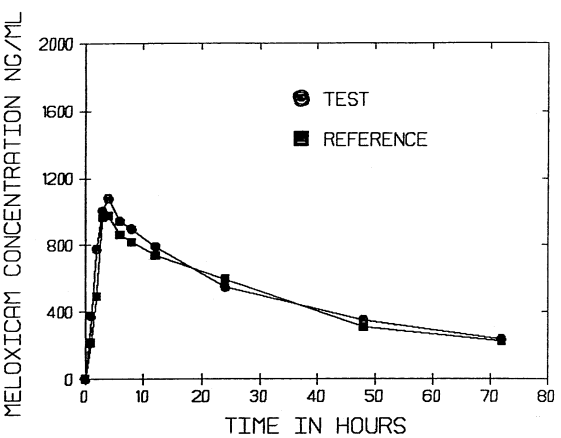

Fig. 4. Concentration vs. time graph of meloxicam for test and reference formulation.

Table 1
Calibration curve data of meloxicam in human plasma

Day	Slope	Intercept	r^2
1	0.001247	−0.009165	0.9995
2	0.002564	−0.03727	0.9982
3	0.002341	−0.009043	0.9995
4	0.002051	−0.009120	0.9991
5	0.001996	−0.009060	0.9994
Mean ± S.D.	0.002039 ± 0.0005	NA	0.9991 ± 0.00055
R.S.D. (%)	24.46	NA	0.055

internal standard for standard preparations against those of the same preparations in processed samples. Recoveries were performed at three QCs were greater than 85%. Evaluation of short-term storage of extracted plasma samples on the standard curve characteristics and chromatographic behavior of meloxicam and piroxicam (internal standard) were also performed. Stability data of the extracted sample inside the autosampler at 20 °C is given in Table 4. These samples were stable even after 8 h inside the autosampler. Regression analysis of the standard curve data gave correlation coefficients and values for the slope and y-intercept within the same order of magnitude following storage of samples in the auto sampler. Freeze thaw stability results are given in Table 5.

3.5. Pharmacokinetics

Overlay graph of mean concentration vs. time of the two formulations (Test and Reference) is shown in Fig. 4. The area under the curve from 0 to 72 h was determined with the help of linear trapezoidal rule. The extrapolation to infinity that

Table 2
Interday variability of the assay of quality control samples

Concentration added (ng ml^{-1})	Concentration analyzed (ng ml^{-1}) (mean ± S.D.)	CV (%)	Bias (%)
50	50.63 ± 3.83	7.57	1.19
600	563.37 ± 33.84	6.01	−6.66
1000	863.08 ± 48.88	5.66	−14.21

Table 3
Intraday variability of the assay of quality control samples

Concentration added (ng ml^{-1})	Concentration analyzed (ng ml^{-1}) (mean ± S.D.)	CV (%)	Bias (%)
50	46.23 ± 4.11	8.88	−7.59
600	573.06 ± 57.36	10.01	−5.06
1000	897.01 ± 25.26	2.82	−10.83

Table 4
Auto injector stability

Concentration (ng ml^{-1})	Concentration determined after			Mean ± S.D.	CV (%)
	2 h	4 h	8 h		
50.0	52.921	48.521	49.586	586 ± 2.95	5.95
600.0	575.513	545.090	568.724	563.109 ± 15.97	2.84
1000.0	876.985	910.411	845.920	877.772 ± 32.25	3.67

Table 5
Freeze thaw cycle

Concentration (ng ml^{-1})	Recovery (%)			Mean ± S.D.	CV (%)
	1	2	3		
50.0	80.02	76.26	78.34	78.21 ± 1.88	2.41
600.0	82.24	86.08	88.66	83.66 ± 2.11	2.52
1000.0	80.78	88.23	84.54	84.54 ± 3.77	4.45

Table 6
Pharmacokinetic data of meloxicam

Parameter	Test	Reference	Ratio	90% confidence interval
AUC_{0-72} (ng h^{-1} ml^{-1})	35027.58	33580.3	1.169	98.11–103.11
$AUC_{0-\infty}$ (ng h^{-1} ml^{-1})	46051.08	42155.20	1.209	99.46–102.53
C_{max} (ng ml^{-1})	1126.75	1111.70	1.051	83.15–117.18

is necessary for $AUC_{0-\infty}$ evaluation was calculated using a linear regression model from the last three data points in the elimination phase that has been log transformed. Maximum concentration achieved (C_{max}) was obtained directly from the measured concentration without interpolation.

Assuming the multiplicative models expected medians of these parameters of the test and reference formulations were computed and presented in Table 6 as their ratios. The confidence intervals, suitable for bioequivalence testing were found well within the bioequivalence range of 0.8–1.25 adopted in a recent cGMP guidelines on bioequivalence studies [10].

4. Conclusions

The HPLC assay described here is simple, selective, precise and accurate for quantitation of meloxicam in human plasma. The sensitivity, simplicity and the rapidity of the method were the main advantages that can be applied to routine therapeutic monitoring of the drug and have proved useful in evaluating the pharmacokinetic in human volunteers.

References

[1] G. Engelhardt, D. Homma, K. Schlegel, C.H.R. Schnitzler, Inflamm. Res. 44 (1995) 423.
[2] P. Stei, B. Kruss, J. Wiegleb, V. Trach, Boehringer Ingelheim, 1993, Data on File.
[3] G. Engelhardt, Presented at Ninth International Conference on 'Prostaglandins and Related Compounds' June 6–10, 1994, Florence, Italy.
[4] D. Turck, U. Busch, G. Heinzel, H. Narjes, G. Nehmiz, Clin. Drug Invest. 9 (1995) 270.
[5] U. Busch, G. Heinzel, G. Bozler, Abstracts of XVIIth ILAR Conference of Rheumatology, Brazilian Society of Rheumatology, Campinas, Brazil, 1989, p. 190.
[6] D. Turck, U. Busch, G. Heinzel, H. Narjes, Eur. J. Rheumatol. Inflamm. 15 (1995) 23.
[7] U. Busch, Boerhinger Ingelheim, 1989, Data on file.
[8] T. Velpandian, J. Jaiswal, R.K. Bhardwaj, S.K. Gupta, J. Chromatogr. B. Biomed. Sci. Applic. 738 (2) (2000) 431.
[9] J. Schmud, W. Roth, in: D.J. Benford, J.W. Bridges, G.G. Gibson (Eds.), Drug Metabolism: From Molecule to Man, Taylor and Frances, London, 1987.
[10] M. Gibaldi, Biopharmaceutics and Clinical Pharmacokinetics, fourth ed., Lea and Febiger, Philadelphia, 1991.