Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 1, 2015

Original Article

DEVELOPMENT AND VALIDATION OF HPLC/UV METHOD FOR DETERMINATION OF MELOXICAM IN HUMAN PLASMA AND APPLICATION IN PHARMACOKINETIC STUDIES

JAAFAR J. I. AL-TAMIMI

Department of Pharmaceutics, College of Pharmacy, Baghdad University, Baghdad, Iraq.
Email: drJaafarJaber@yahoo.com

Received: 13 Jul 2014 Revised and Accepted: 25 Aug 2014

ABSTRACT

Objective: To develop and validate a modified isocratic reversed-phase high performance liquid chromatographic/ultraviolet (RP-HPLC/UV) method for determination of Meloxicam in human plasma to be used for pharmacokinetic studies.

Methods: The drug was extracted from plasma samples by direct protein precipitation technique using perchloric acid: acetonitrile (1:1). Piroxicam was used as internal standard (IS). Samples were analyzed on phenomenex C_{18} column(150 x 4.6 mm, 5 µm), applying sodium acetate buffer (0.17M): acetonitrile, at a ratio of 62:38 v/v in isocratic mode as a mobile phase at a flow rate of 1 ml/min to attain adequate resolution. Using a spectra autosampler, separations were performed at room temperature and monitored at a wavelength of 353 nm after injection a 100µl samples into the HPLC system. The analytical method was validated according to FDA bioanalytical method validation guidance. The method was applied for pharmacokinetic study of Meloxicam tablets (Mobic®,Boehringer Ingelheim, Germany). Mobic®15mg tablets were administered as a single dose to 12 healthy male adult volunteers under fasting condition. Twenty blood samples were withdrawn from each volunteer over 72 hours periods. From the plasma concentration-time data of each individual, the pharmacokinetic parameters; C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, β and $t_{0.5}$ were calculated.

Results: A peak area was obtained for piroxicam and Meloxicam at 6.1 and 10.3 min retention time, respectively. Linearity was established at a concentration range of 50– 1500ng/ml with R^2 = 0.999 as the regression coefficient. The lower limit of quantitation (LLOQ) was identifiable and reproducible at 50ng/ml with a precision of 1.012%. The coefficients of variation(% CV) of the intra-day and inter-day precision at 150, 750 and 1200ng /ml levels were found to be 2.906%, 1.139%, 2.938%; and 4.347%, 4.985%, 3.556%, respectively, which are lower than the accepted criteria limits (15-20 %). The relative recovery (%) of Meloxicam at 150, 750, and 1200ng/ml was found to be 100.706%, 102.638% and 100.292%, respectively. Stability at different conditions and in autosampler was also established. The mean pharmacokinetic parameters; C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, β and $t_{0.5}$ were; 1262.2 ng/ml, 4.8 hr, 40905.2 ng. hr/ml, 45460.5 ng. hr/ml, 0.029 hr^{-1}, 0.035 hr^{-1}, 19.9 hr, respectively.

Conclusion: The present analytical method was found to be specific, sensitive, accurate and precise for quantification of Meloxicam in human plasma. It can be successively applied for pharmacokinetics, bioavailability and bioequivalence studies.

Keywords: Meloxicam, HPLC/UV, Human plasma, Pharmacokinetics.

INTRODUCTION

Meloxicam, an oxicam derivative, is a member of non-steroidal anti-inflammatory drugs (NSAIDs). Chemically, it is described as 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1, 1-dioxide. Its empirical formula is: $C_{14}H_{13}N_3O_4S_2$; the molecular weight is 351.4. The structural formula is shown in fig. 1[1, 2].

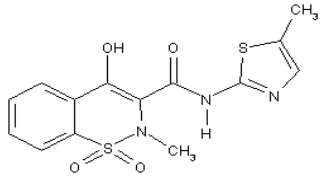

Fig. 1: It shows chemical structure of Meloxicam

Meloxicam is reported to be a selective inhibitor of cyclo-oxygenase-2 (COX-2). The drug is used in the management of rheumatoid arthritis, for the short-term symptomatic treatment of acute exacerbations of osteoarthritis, and for the symptomatic treatment of ankylosing spondylitis [2, 3]. In the treatment of rheumatoid arthritis and ankylosing spondylitis, Meloxicam is given in a usual oral dose of 15mg daily as a single dose. A dose of 7.5mg daily is recommended for long-term treatment in the elderly [2]. Meloxicam is well absorbed after oral or rectal doses and peak plasma concentration occur within 6 hours. It is 99% bound to plasma proteins. Meloxicam has elimination half-life of about 20 hours. It is extensively metabolized, mainly by oxidation to its major metabolite, 5'-carboxyMeloxicam. Meloxicam, in the form of metabolites, is excreted in similar amounts in the urine and in the feces; less than 5% of a dose is excreted unchanged [2, 3].

To date several methods have been developed for the quantification of Meloxicam either alone or in combination with other drugs in different matrices [4-6]. HPLC methods have been reported for the determination of Meloxicam alone in human plasma [7-9] and in pharmaceutical formulations [9-12]. The simultaneous quantification of Meloxicam with diclofenac, with paracetamol, and with pridinol mesylate in oral formulations was reported [13-15]. Nevertheless, there is still an increasing interest for development of more specific, accurate, precise and rapid method for determination of Meloxicam especially in human plasma. The aim of this study was to develop and validate HPLC/UV analytical method for determination of Meloxicam in human plasma in order to be utilized for studying the pharmacokinetics of Meloxicam after a single oral dose of Mobic® 15mg tablet.

MATERIALS AND METHODS

Bioanalytical part

Materials and reagents

Meloxicam 15mg tablets (Mobic®, Boehringer Ingelheim, Germany). Meloxicam standard powder. Piroxicam standard powder. Acetonitrile HPLC grade (Acros, Belgium). Glacial acetic acid (Scharlau, Spain). Milli-Q HPLC water (Millipore, France). N, N-Dimethylformamide (Frutarom, England). Perchloric acid (Panreac, Spain). Sodium acetate (Scharlau, Spain).

Instruments

Waters 2690 alliance HPLC system (USA). Waters 2487U detector (USA). Millennium 3.2 ® software (USA). Stuart scientific vortex shaker (England).

Preparation of standard solutions

Stock solution of Meloxicam (1mg/ml) was prepared in n,n-dimethylformamide using Meloxicam standard powder. The internal standard stock solution of piroxicam (1mg/ml) was prepared in methanol using piroxicam standard powder. All stock solutions were prepared every week.

Working standard solutions of Meloxicam and piroxicam were prepared by dilution of their respective stock solution with a mixture of acetonitrile: water (1:1) to produce a final concentration of 100µg/ml for each.

Standard calibration curve preparation

Standard solutions of Meloxicam were prepared by serial dilution of working solution (100µg/ml) with a mixture of acetonitrile: water (1:1) to attain a concentration of 0.5, 1.0, 2.0, 5.0, 10.0 and 15.0µg/ml, keeping internal standard piroxicam at a concentration of 7.5µg/ml in each one. All solutions were prepared daily.

Plasma sample preparation for calibration curve

Human blood samples transferred to heparinized tubes and then immediately centrifuged at 4000rpm for 10 min. Plasma was separated by polypropylene disposable tips and transferred to eppendorf tubes and then immediately stored at −20°C ± 2 in the deep freezer until analysis.

The calibration curve of Meloxicam in plasma was constructed by spiking 900 µl of plasma samples (which was first thawed at room temperature) with 100 µl of the previously prepared standard solutions (0.5, 1.0, 2.0, 5.0, 10.0 and 15.0µg/ml, keeping internal standard piroxicam at a concentration of 7.5µg/ml in each one). Accordingly, the plasma samples contain a final concentration of Meloxicam equivalent to 50, 100, 200, 500, 1000 and 1500ng/ml, respectively; and 750ng/ml of piroxicam as internal standard in each. While blank plasma samples were spiked with 100µlof a mixture of acetonitrile: water (1:1). The samples were vortex to mix for30 seconds to be applied to the extraction and analytical procedure.

Quality Control (QC) samples preparation

Standard solutions of Meloxicam were prepared by serial dilution of working solution (100µg/ml) with mixture of acetonitrile: water(1:1)to attain a concentration of 1.5, 7.5 and 12.0µg/ml for Meloxicam QC samples preparation, keeping piroxicam internal standard at a concentration of 7.5µg/ml in each. All solutions were prepared daily. Quality Control plasma samples were prepared by spiking 900 µl of plasma samples (which was thawed at room temperature) with 100 µl of the freshly prepared standard solutions (1.5, 7.5, and 12.0µg/ml of Meloxicam; with7.5µg/ml of piroxicam). Accordingly, plasma samples contain a final concentration of Meloxicam equivalent to 150, 750, and 1200ng/ml, respectively; and 750ng/ml of piroxicam as internal standard.

Sample preparation for HPLC injection

Drug was extracted from plasma samples using direct protein precipitation technique. Mixture of 100 µl of perchloric acid: acetonitrile (1:1) was added to 900 µl of spiked plasma sample. The samples were shaken and then centrifuged at 4000rpm for 20 minutes. Finally, 100 µl of the clear supernatant were injected into the HPLC column.

Chromatographic conditions

Previously published HPLC methods using UV detection and piroxicam as internal standard [7-9] were modified for the determination of Meloxicam in human plasma. The different HPLC experimental parameters were optimized. The optimized chromatographic conditions were; column: phenomenex C_{18} 5 µm (150 x 4.6 mm), mobile phase: 0.17M sodium acetate buffer: acetonitrile (62:38, v/v), detection: UV detector set at a wavelength λ of 353 nm, flow rate: 1.0 ml/min, injection volume: 100 µl, auto sampler temperature: ambient.

The mobile phase was always degassed and clarified by filtration through porous membranes with 0.45 µm pore size. A mobile phase degasser was connected on line during the analysis runtime, and then pumped at a flow rate of 1 ml/min, in isocratic mode on the column. The sample (100µl) was injected into HPLC system and the data was acquired employing Millennium 3.2 ®Software.

Method modification and development

The combination of samples extraction and HPLC were modified and developed to provide a rapid assay and a valid analytical method for the determination of Meloxicam, free from interfering with endogenous plasma components and to obtain adequate resolution. Separations were performed at room temperature.

Method validation

The analytical method was validated according to standard guidelines [16 – 18] with respect to the following parameters:

Calibration and linearity

The linearity of the method was established from the standard calibration curve constructed at several concentration levels (50 – 1500ng/ml of Meloxicam with constant concentration (750ng/ml) of the internal standard piroxicam for six consecutive days. Calibration curve were constructed for Meloxicam in the spiked plasma samples by plotting the relative peak area (ratio of peak area of drug to peak area of internal standard) against their respective concentrations using a linear least squares regression analysis. In addition, a blank and a zero sample were prepared to confirm the absence of interferences.

Specificity/selectivity

The specificity**/s**electivity of the analytical method was investigated by confirming the complete separation and resolution of the required peak area of Meloxicam from the internal standard (piroxicam) in human plasma samples spiked with appropriate concentration of these compounds.

The method's specificity was determined by screening six different batches of healthy human plasma. The tests were accomplished to ensure absence of interfering from endogenous plasma components.

Accuracy and precision

Intra-day accuracy and precision

The intra-day precision and accuracy of the assay were measured by analyzing five spiked samples of Meloxicam at three different concentrations (50, 750 and 1200ng/ml); the concentrations were calculated by applying the regression equation of the calibration curve. The deviation of the mean from the true value serves as the measure of accuracy. The precision and accuracy deviation values should be less than 15% of the actual values except at lower limit of quantitation (LLOQ) where it shouldn't deviate by more than 20%. The statistical evaluation includes mean, standard deviation (SD), coefficient of variation (%CV), accuracy, and relative error (%RE).

Inter-day accuracy and precision

The inter-day precision was done at three different concentrations (50, 750 and 1200 ng/ml) over three days, the concentrations were measured by analyzing forty five samples (five determinations from each concentration per day) and were calculated applying the regression equation of the calibration curve. The statistical evaluation includes mean, SD, %CV, accuracy and %RE.

Accuracy and precision for Quality Control (QC) samples

The accuracy and precision for QC samples were demonstrated by analyzing over two days duplicates of QC sample at three concentration levels representing the entire range of the standard calibration curve. The low QC samples (150ng/ml) were designed to

be three times the LLOQ (50ng/ml), while the mid QC samples were taken at the center (750ng/ml) and the high QC samples were taken near the upper limit of quantitation (ULOQ) which is (1200ng/ml).

Recovery

The absolute peak area (detector response) obtained from the injections of the prepared plasma standards was compared to the absolute peak area (detector response) of an equivalent pure authentic standard, which was prepared to contain a drug concentration assuming 100% recovery. The absolute recoveries were calculated for both Meloxicam and internal standard by comparing peak areas of the extracted samples with the un-extracted pure authentic standard solutions peak areas, while the relative recovery was determined for Meloxicam comparing the calculated concentrations of extracted samples to their respective nominal values. Both absolute and relative recoveries of Meloxicam were measured at three concentration levels (150, 750 and 1200ng/ml).

Sensitivity

The lowest concentration in the calibration curve was considered as the LLOQ and should meet the following criteria [16]; LLOQ response is five times the response of the blank, LLOQ response is identifiable, discrete and reproducible with precision of 20% and accuracy of 80-120%.

The peak was identifiable, precise and accurate at this concentration. The LLOQ of Meloxicam in plasma was considered to be50ng/ml.

Stability

Sufficient aliquots of human plasma were spiked with Meloxicam to reach final concentrations of 150, 750 and 1200ng/ml. Five determinations were assigned soon after aliquot preparation for initial concentration determination for each aliquot. Samples were extracted, analyzed and their concentrations were determined. The following allocation for each of spiked aliquots was applied:

Short-term stability

Five samples from each of the stored plasma aliquots were thawed and kept at room temperature for a period of time exceeded that expected to be encountered during routine sample preparation (around 6 hrs). Samples were extracted and then analyzed.

Post- preparative stability

The autosampler stability was conducted by preparing 10 determinations from each of the stored plasma aliquots after thawing and extraction. The processed samples were pooled and 5 measurements were initially done. The remaining pooled processed samples were kept under the auto sampler conditions (ambient) for a period of time more than that expected during the analysis run time (24 hrs) and then analyzed.

Freeze and thaw stability

Testing for freeze and thaw stability was determined through three freeze and thaw cycles. Five samples from each of the stored plasma aliquot were thawed completely unassisted at room temperature and re frozen at the same conditions (−20°C). This cycle was repeated two more times. Samples were then extracted and analyzed.

Long- term stability

Samples from each of the aforementioned plasma aliquots were stored to perform long-term stability analysis at −20°C. The total storage period exceeded the time between the date of volunteers first sample collection and date of last sample analysis. After samples analysis, the concentrations of all stability samples were compared to the mean of that calculated initially at the first day of long-term stability.

Stock solutions stability

All stock solutions were prepared weekly. The stability of Meloxicam stock solution and internal standard piroxicam stock solution was evaluated using triplicate injections of each. Twenty µl of each stock solution were diluted with acetonitrile: water (1:1) up to 1 ml (20:1000). Ten µl of each solution was injected into the HPLC column soon after preparation. The stock solutions of Meloxicam and piroxicam were kept at room temperature for 6 hours and then re-injected into the HPLC after being diluted with acetonitrile: water (1:1) up to 1 ml (20:1000). Then they were kept at room temperature for 7 days and re-injected into the HPLC after being diluted with the same solvent (20:1000). Mean peak areas of the late injected stock solutions of Meloxicam and piroxicam were compared to those injected freshly. So that, the stability of Meloxicam and piroxicam stock solutions was evaluated by testing their validity for 6 hours and for7 days at room temperature. Stability of stock solutions was expressed as % recovery.

Clinical part

Study design, drug administration, blood sampling and drug determination

Twelve healthy male adult volunteers were recruited for this study with age between 25 – 40 years. The volunteers were considered healthy on the basis of medical history, physical and clinical examinations, and routine laboratory tests. The drug was administered as a single oral dose of 15mg Mobic tablet with 240 ml of water after an overnight fasting of 12 hrs. Blood samples were withdrawn from the volunteers up to 72 hours post dosing. Blood samples (7 ml) were withdrawn via an Indwelling Cannula placed in the forearm anticubital vein. The blood was sampled at zero time (30 minutes before dosing), and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10, 12.0, 16.0, 24.0, 36.0, 48.0, 60.0 and 72.0 hours post dosing. A total of 20 blood samples were collected from each volunteer. The blood samples were transferred to heparinized tubes and then immediately centrifuged for 10 min at 4000 rpm. The plasma was separated and then stored at −20°C ± 2 in the deep freezer until analysis for determination of Meloxicam concentrations.

Before extraction, plasma samples were spiked with the internal standard only. For this purpose, 90 µl of solution containing 7.5µg/ml of an internal standard were added to 810 µl of each plasma sample. Plasma samples of each volunteer were analyzed together with their own calibration curve and QC samples (low, medium & high) as one batch in a single run. A standard curve including blank matrix was generated for each analytical run and was used to determine Meloxicam concentrations in the unknown authentic samples.

Pharmacokinetic (PK) parameters calculations of Meloxicam [19-21]

The PK parameters C_{max}, t_{max}, AUC_{0-t}, $AUC_{t-\infty}$, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, β and $t_{0.5}$were calculated for each subject applying non-compartmental analysis. The % extrapolated AUC was calculated as $(AUC_{t-\infty}/AUC_{0-\infty}) \times 100$. The value of (% extrapolated AUC) should not exceed 20% of the total AUC value ($AUC_{0-\infty}$). The terminal elimination rate constant (β) was estimated for each subject via linear regression of the last points (at least three points) of the terminal phase of the log-concentration versus time curve. The values of C_{max} and t_{max} were obtained directly from the concentration versus time curves of each individual.

The mean drug concentrations in plasma + SD versus time plot is presented.

Definitions of the PK parameters [19-21]

C_{max} = maximum concentration of drug in plasma. t_{max} = time to attain C_{max}.

AUC_{0-t} = area under the plasma concentration-time curve from time zero to t_{last} calculated by Trapezoidal rule. $AUC_{t-\infty}$ = extrapolated area ($AUC_{Extrapolated}$ or called $AUC_{Residual}$) which is the area under the plasma concentration-time curve from t_{last} to infinity, calculated as C_{last}/β. $AUC_{0-\infty}$ = total area under the plasma concentration-time curve from time zero to infinity, calculated from the sum of AUC_{0-t} + $AUC_{t-\infty}$. β = first order terminal elimination rate constant. $t_{0.5}$ = first

order terminal elimination half-life which is equal to 0.693/$_{\beta}$.

RESULTS AND DISCUSSION

Linearity

The linearity of the method was evaluated from the calibration curve of spiked plasma samples at several concentration levels of Meloxicam (constructed for six consecutive days). The mean area (ratio of peak area of the drug to the peak area of the internal standard) yielded a linear correlation over a concentration range of 50–1500ng/ml. The method exhibited excellent linearity for this range. A typical calibration curve of spiked plasma samples with the regression equation and their respective correlation coefficient (R^2) of Meloxicam is shown in fig. 2.

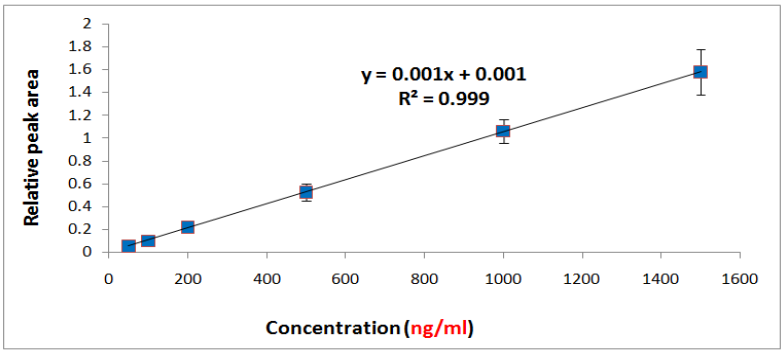

Fig. 2: It shows calibration curve of Meloxicam with piroxicam as internal standard in human plasma

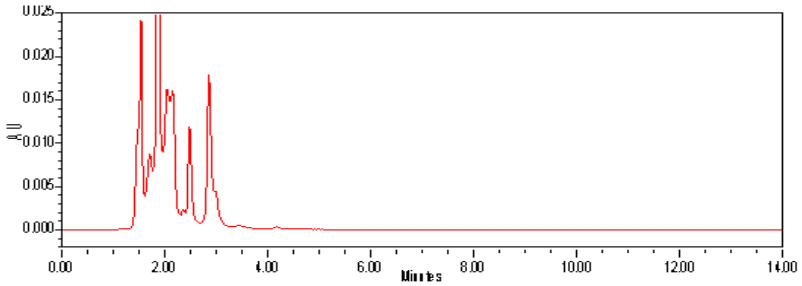

Fig. 3: It shows HPLC chromatogram of a blank human plasma sample

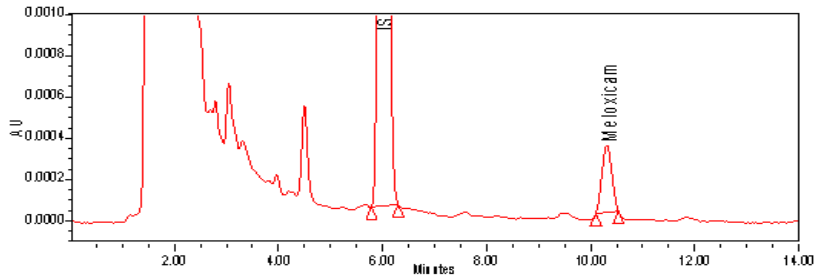

Fig. 4: It shows HPLC chromatogram representing complete resolution of the internal standard (IS) piroxicam peak (750ng/ml) from Meloxicam peak (50ng/ml) at a retention time 6.1 and 10.3 minutes, respectively

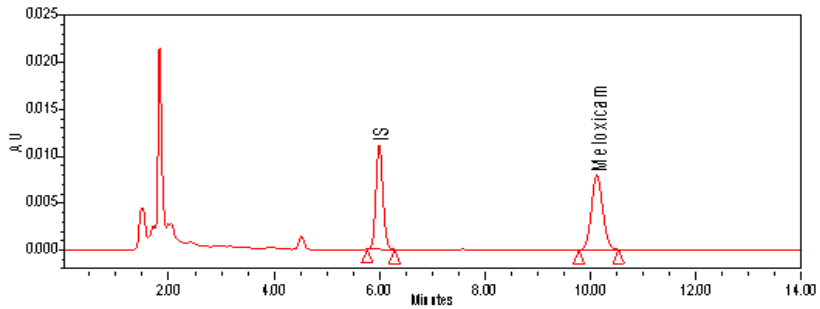

Fig. 5: It shows HPLC chromatogram representing complete resolution of the internal standard (IS) piroxicam peak (750ng/ml) from Meloxicam peak (1200ng/ml) at a retention time 6.1 and 10.3 minutes, respectively

Table 1: It shows intra-day precision, accuracy and relative error (RE) for Meloxicam in spiked human plasma samples

	Meloxicam concentration in human plasma		
	150ng/ml	750ng/ml	1200ng/ml
Mean	153.938	758.14	1223.507
±SD	4.475	8.641	35.947
Precisions as CV%	2.906	1.139	2.938
Accuracy%	102.626	101.085	101.959
RE%	2.626	1.085	1.958

Specificity/selectivity

Representative chromatogram of blank plasma confirmed the presence of very little interference from the endogenous component as shown in fig. 3.

Chromatograms of spiked plasma samples of Meloxicam at concentration ranging from 50–1500ng/ml with the internal standard piroxicam at a constant concentration (750ng/ml) confirming that Meloxicam and piroxicam were well resolved and completely separated at retention times of 10.3 and 6.1 min, respectively as shown in fig. 4 and 5.

Accuracy and precision

Intra-day accuracy and precision

Intra-day accuracy of the method for Meloxicam ranged from 101.09% to 102.63%, while the intra-day precision ranged from 1.14% to 2.94% at the concentrations of 150, 750 and 1200ng/ml. The results are presented in table 1.

Inter-day accuracy and precision

Inter-day precision of the method for Meloxicam ranged from 3.56% to 4.99% at the concentrations of 150, 750 and 1200ng/ml. The results are presented in table 2.

Accuracy and precision for Quality Control (QC) samples

Quality control samples were analyzed for Meloxicam at the three levels150, 750 and 1200ng/ml. The results are shown in table 3.

Recovery

The absolute and relative recovery determined for Meloxicam shown to be consistent, precise and reproducible at the three levels 150, 750 and 1200ng/ml. The data is depicted in table 4. While, the absolute recovery of piroxicam (IS) was found to be 69.572%.

Table 2: It shows inter-day precision, accuracy and relative error (ER) for Meloxicam in spiked human plasma samples

	Meloxicam concentration in human plasma		
	150ng/ml	750ng/ml	1200ng/ml
Mean	157.319	764.635	1227.857
±SD	6.839	38.117	43.666
Precisions as CV%	4.347	4.985	3.556
Accuracy%	104.879	101.951	102.321
RE%	4.879	1.951	2.321

The coefficient of variation (CV%) for all levels of Meloxicam plasma samples was found to be within acceptable limit indicating a reasonable intermediate precision (intra and inter- day) of the method.

Table 3: It shows accuracy and precision for Meloxicam Quality Control samples

	Meloxicam concentration in human plasma		
	QC Low (150ng/ml)	QC Mid (750ng/ml)	QC High (1200ng/ml)
Mean	154.288	769.864	1266.58
±SD	1.811	29.622	55.106
Precisions as CV%	1.174	3.848	4.351
Accuracy%	102.859	102.649	105.548
RE%	2.859	2.649	5.548

Table 4: It shows absolute and relative recovery of Meloxicam

Concentration (ng/ml)	Absolute recovery %	Relative recovery %
150	58.031	100.706
750	63.677	102.638
1200	58.899	100.292

Table 5: It shows data for the Lower Limit of Quantitation (LLOQ)

Concentration (ng/ml)	Actual concentration (ng/ml)	Accuracy %	RE%	Mean (ng/ml)	CV%
50	53.959	107.917	7.917	53.946	1.012
	53.619	107.238	7.238		
	54.054	108.108	8.108		
	54.776	109.551	9.551		
	53.325	106.651	6.651		

Table 6: It shows short-term stability of Meloxicam in human plasma for five run at different concentration levels

	Short-term stability					
	Low level (150ng/ml)		Mid level (750ng/ml)		High level (1200ng/ml)	
	Initial conc. (ng/ml)	After 6 hrs, conc. (ng/ml)	Initial conc. (ng/ml)	After 6 hrs, conc. (ng/ml)	Initial conc. (ng/ml)	After 6 hrs, conc. (ng/ml)
Mean (ng/ml)	154.1	152.818	755.904	760.669	1221.652	1242.367
± SD	1.424	2.5435	2.617	3.9	14.238	7.543
CV%	0.924	1.659	0.346	0.513	1.165	0.607
Recovery%		99.167		100.632		101.702

Table 7: It shows post-preparative stability of Meloxicam in human plasma at different concentration levels

	Post-term stability					
	Low level (150ng/ml)		Mid level (750ng/ml)		High level (1200ng/ml)	
	Initial conc. (ng/ml)	After 24 hrs, conc. (ng/ml)	Initial conc. (ng/ml)	After 24 hrs, conc. (ng/ml)	Initial conc. (ng/ml)	After 24 hrs, conc. (ng/ml)
Mean (ng/ml)	152.059	149.306	761.802	759.507	1225.34	1218.816
± SD	1.295	1.465	2.967	2.444	5.137	4.632
CV%	0.851	0.98	0.389	0.322	0.419	0.38
Recovery%		98.198		99.69		99.463

Sensitivity

The sensitivity of the method was established at 50ng/ml (LLOQ), with a precision of 1.012%. The data for LLOQ is presented in table 5. The chromatogram of an extracted plasma sample spiked with 50ng/ml of Meloxicam is shown in fig. 4.

Stability

Short-term stability

The stability of Meloxicam plasma samples was tested. The calculated data indicated reliable stability behavior under the experimental conditions of the regular run. The results are given in table 6.

Post-preparative stability

Stability of samples in the autosampler was assessed. The data is shown in table 7.

Freeze and thaw stability

The data that represents the stability of Meloxicam plasma samples over the cycles of freeze (at -20°C) and thawing (at room temperature) is given in table 8.

Long-term stability

The stability data of Meloxicam in plasma samples stored for a period of six weeks (beyond that expected for finalizing the authentic samples analysis) at –20°C is summarize in table 9.

The stability study of Meloxicam in human plasma showed reliable stability behavior, thus suggesting that storage of volunteer's plasma at - 20°C is adequate, and no stability-related problems would be expected during the samples routine analysis for pharmacokinetic studies.

Stock solutions stability

The stability of Meloxicam and the internal standard (IS) piroxicam stock solutions was tested and established at room temperature after 6 hours and then after 7 days. Stability of stock solutions was expressed as relative recovery % as shown in table 10.

The results revealed acceptable stability for the prepared stock solutions through-out the period intended for both their daily and 7 day uses. Working solutions and serial dilution of standard solutions were prepared freshly just before spiking of plasma for both the calibration curve and the QC samples.

Table 8: It shows freeze and thaw stability of Meloxicam in human plasma at different concentration levels

	Freeze and thaw stability					
	Low level (150ng/ml)		Mid level (750ng/ml)		High level (1200ng/ml)	
	Initial conc. (ng/ml)	After 3 freeze and thaw cycles, conc. (ng/ml)	Initial conc. (ng/ml)	After 3 freeze and thaw cycles, conc. (ng/ml)	Initial conc. (ng/ml)	After 3 freeze and thaw cycles, conc. (ng/ml)
Mean (ng/ml)	154.694	156.569	753.639	772.017	1224.059	1251.7
± SD	1.425	1.871	4.106	9.785	16.4	27.912
CV%	0.921	1.195	0.545	1.267	1.339	2.229
Recovery%		101.223		102.441		102.289

Application of the present validated method of analysis for pharmacokinetic study of Meloxicam in human

The method was applied for studying the pharmacokinetics of the drug after administration of Mobic® 15 mg tablets (Boehringer Ingelheim, Germany) to 12 healthy male adult subjects.

The mean concentrations versus time data obtained from the subjects are presented in table 11 and depicted in fig. 6. The mean pharmacokinetic parameters calculated from the individual pharmacokinetic analysis of the concentration-time data of each subject are summarized in Table 12.

Thirty minutes before dosing (zero time) of Mobic® tablets, Meloxicam was not detected in plasma of all volunteers (Table 11 and fig. 6). This confirms the absence of carry-over effect. The drug was detected in plasma samples of 9 volunteers after 0.5hr of Mobic® tablets administration, while the drug was detected in all the 12 volunteers after 1.0hr of drug administration (Table 11 and fig. 6). The rapid absorption of Meloxicam tablet was also confirmed by

the ratio $C_{max}/AUC_{0-\infty}$ (Table 11) which was calculated as an estimate for the rate of drug absorption [22-24]. This indicates rapid absorption of Meloxicam from tablets. The mean t_{max} found in the present investigation was about 5 hrs (range 3-12 hrs). Similar values were reported in other literature [2, 25, 26]. A first peak in Meloxicam concentration observed around 5 hrs post dosing. However, a second peak in Meloxicam concentration was also noticed 12 to 14 hours post dosing (Table 11 and fig. 6) suggesting gastrointestinal recirculation resulting in prolonged absorption and stable effective concentration for a longer period.

Table 9: It shows long-term stability of Meloxicam in human plasma at different concentration levels

	Long-term stability					
	Low level (150ng/ml)		Mid level (750ng/ml)		High level (1200ng/ml)	
	Initial conc. (ng/ml)	After 6 weeks storage, conc. (ng/ml)	Initial conc. (ng/ml)	After 6 weeks storage, conc. (ng/ml)	Initial conc. (ng/ml)	After 6 weeks storage, conc. (ng/ml)
Mean ng/ml)	153.506	158.11	756.659	771.94	1217.037	1254.49
± SD	1.746	1.22	3.861	7.977	23.593	25.196
CV%	1.137	0.772	0.510	1.033	1.939	2.01
Recovery%		103.012		102.019		103.097

Table 10: It shows Meloxicam and piroxicam (IS) stock solutions stability

Stock solution	Recovery %	
	After 6 hrs	After 7 days
Meloxicam	98.87	99.676
Piroxicam	99.28	102.35

Table 11: It shows mean plasma concentrations of Meloxicam versus time obtained after a single dose of Mobic® 15 mg tablet administered to 12 healthy male adult subjects

Time (hr)	Mean (ng/ml)	± SD	%CV	Minimum (ng/ml)	Maximum (ng/ml)
0.5	213.384	169.305	79.343	55.497	594.695
1.0	461.127	331.299	71.845	71.841	1088.618
1.5	612.336	358.102	58.481	121.047	1225.465
2.0	772.996	375.027	48.516	145.136	1263.157
2.5	944.98	393.098	41.598	171.888	1304.117
3.0	1007.031	360.386	35.787	260.117	1377.826
3.5	1115.06	303.152	27.187	385.485	1412.969
4.0	1149.124	253.436	22.055	519.368	1461.183
5.0	1210.666	211.798	17.494	714.546	1439.07
6.0	1044.218	187.909	17.995	580.336	1230.687
8.0	992.007	193.495	19.505	549.231	1318.548
10.0	1011.775	208.781	20.635	622.884	1435.585
12.0	1027.692	180.722	17.585	629.762	1258.984
16.0	917.402	247.242	26.95	472.611	1345.83
24.0	727.782	197.055	27.076	412.008	1018.953
36.0	536.262	177.719	33.14	279.231	816.851
48.0	382.533	184.844	48.321	168.861	816.168
60.0	243.409	101.117	41.542	119.289	380.45
72.0	151.711	67.928	44.775	66.357	245.195

Table 12: It shows mean pharmacokinetic parameters of Meloxicam obtained after a single dose of Mobic® 15 mg tablet administered to 12 healthy male adult subjects

Pharmacokinetic parameters	Mean	± SD	%CV	Minimum	Maximum
C_{max} (ng/ml)	1262.2	203.7	16.1	714.5	1461.2
t_{max} (hr)	4.8	2.4	49.6	3.0	12.0
AUC_{0-t} (ng. hr/ml)	40905.2	10966.5	26.8	20956.9	56471.8
$AUC_{t-\infty}$ (ng. hr/ml)	4555.3	2516.4	55.2	1673.2	8795.2
$AUC_{0-\infty}$ (ng. hr/ml)	45460.5	13203.7	29.0	22781.0	63026.0
$\%AUC_{extra}$	9.4	3.1	33.3	5.5	14.4
$C_{max}/AUC_{0-\infty}$ (hr^{-1})	0.029	0.006	19.4	0.023	0.040
β (hr^{-1})	0.035	0.0045	12.7	0.028	0.042
$t_{0.5}$ (hr)	19.9	2.6	13.3	16.3	24.9

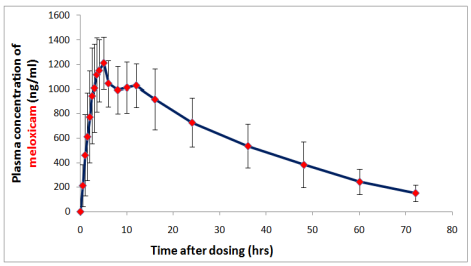

Fig. 6: It shows mean plasma concentrations (± SD)of Meloxicam versus time profile obtained after a single dose of Mobic® 15 mg tablet administered to 12 healthy male adult subjects

The First Order terminal elimination rate constant (β) for Meloxicam was measured from not less than three points in the terminal phase of the log-concentration vs. time data (Tables 11). This procedure is assumed to give the reliable estimation of the β value and consequently the values of pharmacokinetics parameters calculated from β value; namely $AUC_{extrapolated}$ and $t_{0.5}$ [19-21]. The reported value of the mean terminal elimination half-life of Meloxicam in plasma following administration of Meloxicam tablets was about 20 hours[2, 25, 26,]. Similar value is found in the present investigation (Table 12). Therefore, blood sampling for 72 hours following Meloxicam administration was considered adequate enough for pharmacokinetic, bioavailability and bioequivalence studies [19-21].

The mean $C_{max}$and $AUC_{0-\infty}$values obtained in the present study (Table 12) are comparable to that reported in other studies [25-29]. Meloxicam exhibited great individual variation in the pharmacokinetic parameters C_{max}, t_{max} and $AUC_{0-\infty}$ (Table 12). Beside, population differences in the clinical pharmacokinetics of Meloxicam were observed [27, 30]. Therefore, caution is required in the selection of dose. The mean value of the % $AUC_{extrapolated}$ ($AUC_{t-\infty}/AUC_{0-\infty}$ × 100) had small contribution to the total AUC ($AUC_{0-\infty}$) since the maximum % $AUC_{extrapolated}$ did not exceed 20 % (Table 12). Thus, indicating an adequate enough sampling program used in the study [19-21].

CONCLUSION

The present study introduced the pharmacokinetic characteristics of Meloxicam tablet administered to human. Beside, the current investigation provides a specific, sensitive, precise, accurate and rapid assay for Meloxicam in human plasma. This method would be efficient in analyzing large number of plasma samples for routine analysis of drug concentrations for pharmacokinetics, bioavailability and bioequivalence studies of Meloxicam tablets.

ACKNOWLEDGMENT

The author would like to appreciate the clinical staff and the analytical staff for their kind help and expertise in conducting this study. Beside, sincere thanks to college of pharmacy, Baghdad university, Baghdad, Iraq for their support.

CONFLICT OF INTERESTS

Declared None.

REFERENCES

1. The Merck index, an encyclopedia of chemicals, drugs, and biological. 14th ed. Maryadele JO Neil, Editor, Merck & Co. INC, USA; 2006. p. 5823.
2. Martindale. The Complete Drug Reference, 37th ed. Sweetanon SC, arm BP. Pharms FR. London. Chicago, Ph. Press; 2011. p. 84–5.
3. Remington. The science and practice of pharmacy 21th ed. Lippincott Williams and Wilkins; 2006. p. 1538.
4. Shaji J, Varkey D. Development of a validated stability–indicating HPTLC method for determination of Meloxicam in bulk and pharmaceutical formulations: pertinence to ICH guidelines. Int J Pharm Pharm Sci 2012;4(Suppl 1):160–9.
5. Aejaz A, Jafar M, Dehgan MHG, Shareef AS. Meloxicam-PVP-SLS Ternary dispersion systems: *in-vitro* and *in-vivo* evaluation. Int J Pharm Pharm Sci 2010;2(Issue 1):182–90.
6. Wiesner JL, de Jager AD, Sutherland FCW, Hundt HKL, Swart KJ, Hundt AF. Sensitive and rapid liquid chromatography–tandem mass spectrometry method for the determination of Meloxicam in human plasma. J Chromatogr B 2003;785(Issue 1):115-21.
7. Bae JW, Kim MJ, Jang CG, Lee SY. Determination of Meloxicam in human plasma using a HPLC method with UV detection and its application to a pharmacokinetic study. J Chromatogr B 2007;859(1):69-73.
8. Dasandi B, Shivaprakash, Saroj H, Bhat KM. LC determination and pharmacokinetics of Meloxicam. J Pharm Biomed Anal 2002;28(5):999-1004.
9. Jessy S, Dhanila V. Development and validation of a reverse phase–HPLC method for determination of Meloxicam in pharmaceutical dosage forms and human plasma. Int J Pharm Sci Rev Res 2012;12(Issue1):153–60.
10. Shaji J, Jain V. Reverse phase HPLC method development and validation for determination of Meloxicam in bulk and pharmaceutical formulation. Int J Pharm Sci Res (IJPSR) 2011;2(1):107-15.
11. Wali AF, Masoodi MH, Akbar M, Mushtaq A. Method development and validation of a stability-indicating RP-HPLC method for analysis of Meloxicam using DAD detector. J Pharm Res Dev 2013;1(5):33–9.
12. Bandarkar FS, Vavia PR. A stability indicating HPLC method for the determination of Meloxicam in bulk and commercial formulations. Trop J Pharm Res 2009;8(3):257-64.
13. Madani A, Ahmad M, Usman M, Zubair MM, Qamar UZM. New high performance liquid chromatographic method for simultaneous determination of diclofenac and Meloxicam in oral formulation of liposomes and human plasma. J Chem Soc Pak 2010;32(5):654–61.
14. Induri M, Mantripragada BR, Yejella RP, Kunda PR, Meechel AM. Simultaneous quantification of paracetamol and Meloxicam in tablets by high performance liquid chromatography. Trop J Pharm Res 2011;10(4):475-81.
15. Vignaduzzo SE, Castellano PM, Kaufman TS. Method development and validation for the simultaneous determination of Meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations. J Pharm Biomed Anal 2008;46(2):219–25.
16. Guidance for Industry. Bioanalytical Method Validation for human studies. U. S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2001. p. 1–22.
17. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis, understanding the differences and similarities between validation requirements of the US food and drug administration, the US pharmacopeia and the international conference on harmonization. J Chromatogr A 2003;987:57–66.
18. Pathuri R, Muthukumaran M, Krishnamoorthy B, Nishat AA. Review on analytical method development and validation of pharmaceutical technology. Curr Pharm Res 2013;3(2):855-70.
19. ShargelL, Wu-Pong S, YUA. Applied Biopharmaceutics & Pharmacokinetics. International edition, 6th ed. The McGraw-Hill Companies, Inc. New York; 2012.
20. Design and Analysis of Bioavailability and Bioequivalence Studies. 2nd ed. Revised and Expanded. Shein-Chung Chow & Jen-Pei Liu; 2000.
21. Guidance for Industry. Bioavailability and Bioequivalence Studies for Orally Administered Drug Products–General Considerations. U. S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER); 2003.
22. Lacey LF, Keene ON, Duquesony C, Bye A. Evaluation of different indirect measures of rate of drug absorption in comparative pharmacokinetic studies. J Pharm Sci 1994;83:(2):212-5.
23. Endrenyi L, Tothfalusi L. Secondary metrics for the assessment of bioequivalence. J Pharm Sci 1997;86(3):401-2.

24. Duquesony C, Lacey LF, Keene ON, Bye A. Evaluation of different partial AUCs as indirect measures of rate of drug absorption in comparative pharmacokinetic studies. Eur J Pharm Sci 1998;6(Issue 4):259-63.
25. Turck D, Roth W, Busch U. A review of the clinical pharmacokinetics of Meloxicam. Br J Rheumatol 1996;35 Suppl 1:13-6.
26. Türck D, Busch U, Heinzel G, Narjes H. Clinical pharmacokinetics of Meloxicam. Arzneim-Forsch Drug Res 1997;47(3):253-8.
27. Rani S, Guttikar S, Rathod R, Cherian B, Nivsarkar M, Harish P. Determination of oral Meloxicam pharmacokinetic parameters in Asian Indians: comparison with a German population. Saudi Pharm J 2004;12(4):144-9.
28. Xu HY, Zhong DF, Zhao LM, Zhang YF, Zhang BJ. Pharmacokinetics of Meloxicam in healthy Chinese volunteers. Yao Xue Xue Bao 2001;36(1):71-3.
29. Türck D, Schwarz A, Höffler D, Narjes H, Nehmiz G. Pharmacokinetics of Meloxicam in patients with end-stage renal failure on haemodialysis: a comparison with healthy volunteers. Eur J Clin Pharmacol 1996;51(3-4):309-13.
30. Hasan SMF, Shoaib MH, Hassan F, Jabeen S, Siddiqui AA. Determination of the pharmacokinetic parameters of Meloxicam in healthy Pakistani volunteers using non-compartmental analysis. Pakistan J Pharmacol 2008;25(1):25-8.