DEVELOPMENT OF A VALIDATED STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF MELOXICAM IN BULK AND PHARMACEUTICAL FORMULATIONS: PERTINENCE TO ICH GUIDELINES

JESSY SHAJI*, DHANILA VARKEY

Department of Pharmaceutics, Prin. K.M. Kundani College of Pharmacy, Plot No. 23, Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade, Mumbai-400005, India. Email: jshaji@rediffmail.com

ABSTRACT

A rapid, precise, selective and sensitive, stability-indicating HPTLC method for quantitative estimation of Meloxicam (MLX) has been developed and validated. TLC aluminium plates (Merck) precoated with silica gel 60F$_{254}$ was used as the stationary phase. The solvent system comprising of toluene: ethyl acetate: methanol: glacial acetic acid in the ratio of 4:4:1.6:0.4 (v/v/v/v) gave a dense compact spot with an R$_f$ value of 0.61± 0.02, n=6. Densitometric analysis was carried out in reflectance-absorbance mode at 358 nm. The method was validated in compliance with ICH Harmonized Tripartite Guideline Q2 (R1) for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, specificity, accuracy, repeatability and robustness. The drug response with respect to peak area was linear over the concentration range 200-700 ng/spot (n=6). The mean (±SD) values of the slope, intercept and correlation coefficient were 8.192 (±0.089), 1640.1 (±24.516) and 0.9965 (±0.00083), respectively. The LOD and LOQ were 23.20 and 70.30 ng/spot respectively. Statistical evaluation proved that the established method was accurate, specific, precise, repeatable and robust for the estimation of MLX. The degradation products were well resolved from the pure drug with significantly different R$_f$ values and thus can be used to monitor stability. Being simple and economical, the method can be employed for the routine quality control analysis of MLX in bulk and pharmaceutical dosage forms.

Keywords: HPTLC; Meloxicam; Stability-indicating; Degradation; Validation; Bulk

INTRODUCTION

Meloxicam, [MLX; 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl) - 2H-benzothiazine-3-carboxamide-1,1-dioxide)] is a highly effective non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class of oxicam derivatives[1]. It has the molecular weight of 351.4 Dalton[2] and has two pKa values (pKa$_1$ = 1.09, pKa$_2$ = 4.18)[3]. Its chemical structure is shown in Fig.1.
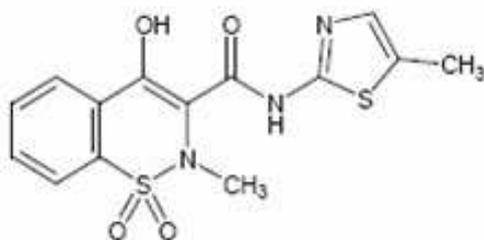
Fig. 1: Chemical Structure of MLX

MLX demonstrated a high potency in animal tests for anti-arthritic action, and has a wider spectrum of anti-inflammatory activity, combined with less gastric and local tissue irritation than NSAIDs available prior to its discovery. It is recurrently used to treat rheumatoid arthritis, osteoarthritis, pauciarticular and polyarticular course juvenile rheumatoid arthritis. Besides its main therapeutic application as an anti-inflammatory and analgesic agent, it is also rising as a promising drug for the treatment of Alzheimer's disease and cancer (mainly colorectal and adenocarcinoma)[4,5]. Clinical trials data on MLX showed that it has a superior gastrointestinal tolerability, as would be predicted from its more selective inhibition of COX-2 relative to COX-1[6]. MLX is slowly but almost completely absorbed after oral administration with an absolute bioavailability of 89%. Like many other NSAIDs, MLX is practically insoluble in water. MLX can be graded in Class II, of the Biopharmaceutical Classification System (BCS), which means low aqueous solubility and rapid absorption (high permeability) through the gastrointestinal tract[7]. MLX is strongly bound to plasma proteins (99.5%) and displays linear pharmacokinetics. Peak plasma concentrations are reached 5-6 h after oral dosing when taken concomitantly with a light meal, as generally recommended. It has a favorable tolerability profile and a higher therapeutic index than that of other NSAIDs, including piroxicam, diclofenac and indomethacin[8]. MLX undergoes extensive metabolism, primarily by cytochrome P450 CYP2C9 and to a minor extent by CYP3A4, forming four major inactive metabolites. The pharmacokinetics of MLX is linear over the dose range 7.5-30 mg and remains unchanged from single to multiple dosing. Total MLX clearance is 7-8 ml min^{-1} and the terminal elimination half-life is approximately 20 h, making MLX suitable for once daily dosing. Steady-state drug plasma concentrations are reached within 3-5 days[9].

Although MLX is one of the most widely used anti-inflammatory drugs, no reference procedure exists for its determination in pharmaceutical formulations in International Pharmacopoeias. Effectively, there is only one monograph for MLX in the British Pharmacopoeia, based on a non aqueous titration, which is not applicable to tablets due to interference from excipients used in pharmaceutical formulations[10]. Few methods have been reported for the determination of MLX including non aqueous titration[11], spectrophotometric methods[12,13], high performance liquid chromatography (HPLC) methods[14-17], fluorimetric methods[18,19], turbidimetric method[20], densitometric method[21], electrochemical method[22], voltammetric method[23], chemiluminometric[24] and electrophoretic method[25]. However most of these analytical methods have some limitations for routine analysis such as tedious and time consuming sample preparation, constant dependency on operator, long sample analysis time and use of expensive solvents and apparatus.

In view of this, high performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase, thereby reducing the time and cost of analysis[26]. HPTLC can be used for the analysis of large as well as small samples[21]. Furthermore for such methods extraction procedure is not always required and could be used for analyzing drug without any interference from excipients.

The parent drug stability guidelines [Q1A (R), 2000][27] issued by ICH suggest that stress studies should be conducted on a drug to establish its inherent stability characteristics. Stress degradation studies are necessary to develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. They

can be functional in the investigation of the chemical and physical stability of crystal forms, stereochemical stability and differentiating drug substance-related degradation products in formulations, thereby supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be fully validated and the assays should be stability-indicating[28]. Accordingly, the objective of this work was to subject MLX to variety of stress test conditions to establish intrinsic stability of the drug and to develop a validated stability method. The present study reports development of an accurate, specific, repeatable and stability indicating method for determination of MLX in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its pharmaceutical dosage form.

MATERIALS AND METHODS

Materials

Pharmaceutical grade of MLX was obtained as a gift sample from Zest Pharma, Indore, India (Batch No. ALC/MLX/090302). It was certified to contain 99.78% w/w (on dried basis) and was used without further purification. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, Mumbai, India. Commercial pharmaceutical preparation Muvera 15 (Sun Pharma, Sikkim) containing MLX 15mg was purchased from the local pharmacy. All dilutions were performed in calibrated volumetric flasks.

Instrumentation

Linomat V sample applicator (Camag, Switzerland) with 100 μl sample syringe (Hamilton, Bonaduz, Switzerland), twin trough glass chamber (20cm x 10cm, Camag, Switzerland), TLC plate heater (Camag, 25-250°C), Camag TLC scanner (4) with winCATS software (version 1.4.5), TLC visualizer (Camag), UV inspection cabinet (Camag), Mettler analytical balance AB 104-S/FACT (Mettler Toledo) (accuracy ±0.01mg) and ultrasonic bath sonicator (PCI Analytics, Mumbai).

Chromatographic HPTLC conditions

Experimental analysis was performed on silica gel 60F$_{254}$ HPTLC plates (20cm x 10cm with 250 μm thickness; E Merck, Darmstadt, Germany, Batch-HX011551) using mobile phase consisting of toluene: ethyl acetate: methanol: glacial acetic acid in the ratio of 4:4:1.6:0.4 (v/v/v/v). Prior to chromatographic analysis the plates were washed in methanol, dried in a current of dry air and activated at 110°C for 5 min. Samples were spotted in the form of bands of width 8mm with a Camag microlitre syringe. A constant application rate of 150nl/s was used and the space between two bands were 5mm. Monochromator band width was set at 20nm, each track was scanned six times and baseline correction was used. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (26±2°C) and relative humidity (50±5%). No neckless effect was seen during the development and it took approximately 20 min for the complete development of the TLC plate. The length of each chromatogram run was 80mm. Consequent to development, the plates were dried in current of air by use of an air dryer. Densitometric scanning was performed in the reflectance-absorbance mode at 358nm, operated by Camag winCATS software (V 1.4.5). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400nm. The slit dimension was kept at 6mm x 0.30mm and the scanning speed was 20mm/s. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. Scanned peak areas were recorded for each sample at each concentration level. The average peak areas and variations in peak area obtained were expressed as percent relative standard deviation (% RSD).

Calibration plot of MLX

A stock solution of MLX (1mg/ml) was prepared in chloroform. 0.5 ml of stock solution was further diluted to 10 ml with chloroform to get standard solution of 0.05mg/ml. Appropriate volumes of standard solution were spotted to obtain MLX in the concentration range of 200-700ng (n=6). The plate was developed and scanned as described above. Well resolved compact band of drug was scanned at 358nm. The linearity plot was obtained by plotting average peak area at each concentration against corresponding band concentrations of MLX (ng/spot). Linear regression analysis was employed to calculate the regression equations and the correlation coefficients.

Method Validation

The proposed method was validated in compliance with ICH Guidelines. The method was validated for linearity and range, limit of detection (LOD), limit of quantitation (LOQ), precision, specificity, accuracy, repeatability and robustness.

Limit of detection and limit of quantitation

Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

LOD= 3.3 σ/S

Where σ = the standard deviation of the response

S = the slope of the calibration curve

LOQ = 10 σ/S

Where σ = the standard deviation of the response

S = the slope of the calibration curve

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Precision

Precision is a measure of the reproducibility of the analytical method under normal operating conditions. Precision is expressed as relative percent standard deviation (% RSD). The intra- and inter-day precisions were carried out at three different concentration levels, 300, 500 and 700 ng/spot (n=6) respectively. Repeatability of measurement of peak area and % content can be defined as the precision of the method when repeated under same operating conditions (same equipments, reagents, settings and laboratory) over a short interval of time. Repeatability was assessed by chromatography of six replicates of 100% of the test concentration (500ng/spot) and expressed in terms of coefficient of variation (CV).

Selectivity and specificity

Selectivity of an analytical method is its capability to determine precisely and specifically the analyte in the presence of components that may be expected to be present in the sample matrix like matrix components, impurities or degradation products. UV spectrum of standard drug and sample at selected wavelength was compared. Specificity ensures that the signal measured comes from the analyte of interest and that there is no interference from excipients, impurities or degradation products. Specificity was determined by analyzing standard drug and test sample. The spot for MLX in the sample was confirmed by comparing the R$_f$ and spectrum of the spot with that of the standard. The peak purity of MLX was judged by comparing the spectrum at three different regions of the spot i.e. peak start, peak apex, and peak end positions of the spot.

Accuracy (Recovery studies)

Accuracy of an analytical method is the closeness of test results to true value. It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in preanalyzed sample solutions. The amount of drug recovered in accuracy study was in the range, which indicated that the method is accurate. Accuracy was studied at different levels i.e. 0%, 80%, 100% and 120% by addition of pure drug to previously analyzed test samples. The mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate and percent recovery was calculated.

Robustness

The robustness of an analytical method may be defined as the measure of its ability to remain unchanged by small, but purposeful variations in the method parameters and provides an indication of its dependability during normal usage. The robustness of the method was determined by variations in mobile phase composition (±0.2ml), volume of mobile phase (±2ml), chamber saturation period (±5min), development distance (±0.5cm), and time from development to scanning (0, 10, 20 and 30 min). One factor at a time was changed at a concentration level of 400ng/band (n=6) of MLX, to study the effect on peak areas of the drug.

Analysis of marketed formulation

Marketed tablet formulations (20 tablets, label claim 15mg/tablet) were weighed and powdered. Tablet triturate equivalent to the label claim of the drug was dissolved in chloroform and subjected to sonication for 10 min. The volume was made up to 100 ml and filtered through Whatman filter paper. The filtrate obtained was suitably diluted and spotted onto the plate followed by development and scanning as described in section "chromatographic HPTLC conditions". The analysis was repeated in triplicate. Any possibility of excipients interference was also studied.

Forced degradation studies

A stock solution containing 10 mg/ml MLX was prepared. This solution was used for stress degradation to indicate specificity of the proposed analytical method and its stability indicating property. In all the degradation studies the average peak area of three replicates was obtained.

Acid and base induced degradation studies

Decomposition studies were carried out by refluxing 5ml of drug solution with 5ml each of 1N HCl and 1N NaOH. The mixtures were refluxed for 6h respectively at 60°C. The forced degradation in acidic and alkaline media was carried out in dark to exclude the possible degradation effect of light. The solutions (0.5ml) were taken and neutralized and subsequently diluted to 10 ml with chloroform. The resulting solutions were applied to TLC plate in such a way that the final concentration attained was 5000ng/spot for both acid and base degradation products and the chromatograms were run as described in previous section.

Dry heat and wet heat degradation

The standard drug was stored in oven at 50°C for 72h to study dry heat degradation and for wet degradation drug was stored at 50°C and 75% relative humidity for 3 months.

Photo degradation

The photochemical stability of the drug was studied by exposing the stock solution to direct sunlight for 7 days. After suitable dilution, concentration of 5000ng/spot was applied and the chromatograms were run as described in previous section.

Oxidative degradation (Hydrogen peroxide induced)

Studies were performed in 3% (v/v) and 30% (v/v) hydrogen peroxide at room temperature for 48h respectively. The mixture was stored in dark to exclude the possible effect of light. The solution

was then heated in boiling water bath for 10 min to completely remove excess of hydrogen peroxide. The resultant solutions were applied to TLC plate to achieve a final concentration of 5000ng/spot and the chromatograms were run as described in previous section.

Detection of the related impurities

Related impurities were determined by spotting higher concentrations of the drug with an aim to detect and quantify them. MLX (50 mg) was dissolved in 5ml of chloroform to attain a concentration of 10mg/ml and this solution was termed as sample solution. 0.5 ml of the sample solution was diluted to 100ml with chloroform to get a standard solution (0.05mg/ml). 1 μl of sample solution (10,000ng/spot) and 10μl of standard (500ng/spot) were applied on the TLC plate and the chromatograms were run as described in previous section.

RESULTS AND DISCUSSION

Development of optimum mobile phase

The HPTLC method was optimized with an aim to develop a stability-indicating assay method. During mobile phase optimization various mobile phase compositions including toluene: methanol (8:2), toluene: methanol: glacial acetic acid (8:1.5:0.5), toluene: ethyl acetate: methanol (4:4:2) and different proportions of toluene: ethyl acetate: methanol: glacial acetic acid were tried. Both pure drug as well as forced degradation samples were spotted onto precoated TLC plates and tried with the afore mentioned mobile phase systems. Higher concentrations of toluene resulted in low R_f values of 0.32. Increasing the solvent strength (ε^o) of the mobile phase decreases retention and increases the R_f value. As a result ethyl acetate was used in equal proportions as toluene along with methanol to have considerable ε^o. The spot obtained was diffused with an R_f value of 0.34. Glacial acetic acid was used in the mobile phase as a modifier to reduce the diffusion of the spots. Amongst the combinations tried toluene: ethyl acetate: methanol: glacial acetic acid (4:4:1.6:0.4, v/v/v/v) was finalized as the mobile phase.

The spots developed were dense and compact with an R_f value of 0.61± 0.02. Typical peak of MLX is shown in Fig.2. The peak obtained was sharp and symmetrical in nature. The spectrum obtained after densitometric scanning showed peak at 358 nm and is shown in Fig 3.

Validation paradigms

Linearity

The linearity of an analytical method is its ability, within a given range, to obtain test results which are either directly or through mathematical transformation, proportional to concentration of the analyte in the sample. The method was found to be linear ($r^2 = 0.9965 \pm 0.00083$) in the concentration range of 200-700 ng/spot (n=6), with respect to peak area. Peak area and concentration was subjected to linear regression analysis to determine calibration equation and correlation coefficients. No significant difference was observed in the slopes of standard curves (ANOVA, P< 0.0001 (P value, one sample t test). The three dimensional overlay of densitograms of the calibration spots of MLX at 358nm is displayed in Fig.4. The regression data is reported in Table 1.

Table 1: Linear regression data for the calibration curves[a]

Parameters	Value
Detection Wavelength (nm)	358
Beer's Law Limit (ng/band)	200-700
Correlation Coefficient ($r^2 \pm$ SD)	0.9965 ± 0.00083
Intercept (c) ± SD	1640.1± 24.51
Confidence limit of intercept[b]	1385.5-1804.1
Slope (m) ± SD	8.192 ± 0.08
Confidence limit of slope[b]	7.901-8.771
SD of residuals from line (Sy.x)	58.608

[a]n=6; [b]Confidence interval: 95%
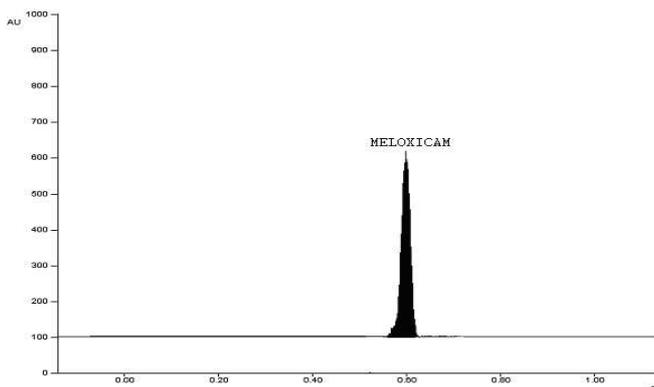
Fig. 2: Densitogram of MLX (R$_f$: 0.61±0.02), Wavelength: 358nm, Mobile phase: toluene: ethyl acetate: methanol: glacial acetic acid (4:4:1.6:0.4, v/v/v/v)
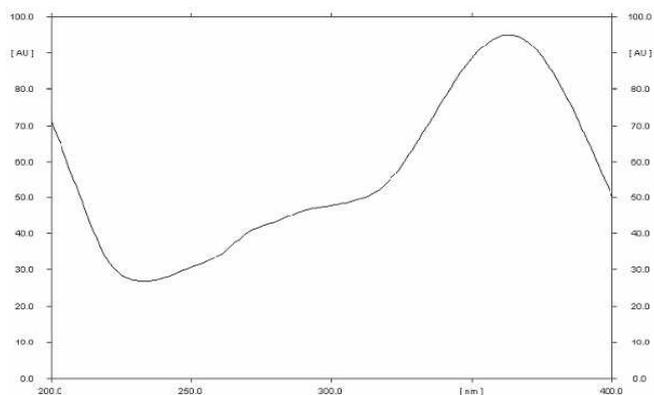
Fig. 3: Spectrum of MLX measured from 190-400nm
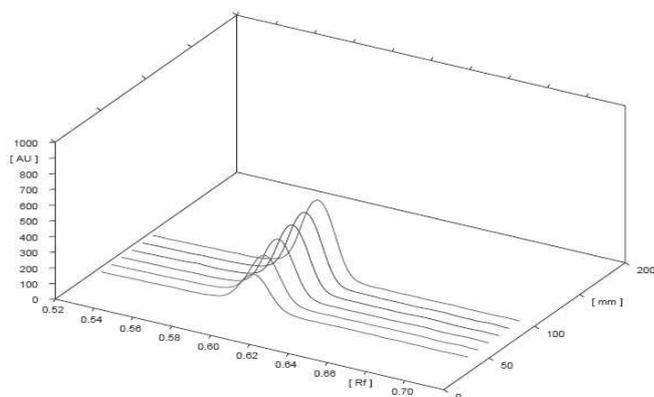
Fig. 4: Three dimensional overlay of densitograms of the calibration spots of MLX at 358nm

Table 2: Statistical evaluation of precision (repeatability) of developed method

Drug: MLX Repeatability[a]				
Conc. (ng/spot)	Area ± SD	% Content ± SD	Coefficient of Variation	
			Peak Area	% Content
500	5803.09± 68.44	101.88± 1.65	0.011	0.016

[a]n=5

Table 3: Summary of Intra-day and Inter-day method precision[a]

Amount (ng/spot)	Intra-day precision by peak area			Inter-day precision by peak area		
	Mean ± SD	% Content ± SD	%RSD	Mean ± SD	% Content ± SD	%RSD
300	4126±3.04	101.37±0.10	0.073	4134.06 ± 7.96	101.53±0.16	0.192
500	5787±0.02	101.34 ±0.001	0.003	5786.73 ±0.27	101.33 ±0.01	0.004
700	7285.3±1.93	98.47 ± 0.02	0.026	7286±0.70	98.48 ±0.015	0.009

[a]n=5

LOD and LOQ

The LOD and LOQ were found to be 23.20 and 70.30 ng respectively.

Precision

The repeatability of measurement of peak area and % content was expressed as CV and found to be 0.011 and 0.016 for MLX (Table 2). Results for intra- and inter-day variation studied at three levels 300, 500 and 700 ng/spot is shown in Table 3. The % RSD was found to be <2% in all cases and thus indicate that the method is highly precise.

Specificity and selectivity

The method was selective and specific for the determination of MLX. Good correlation (r=0.9999) was obtained between the standard and sample spectra of MLX.

The overlay spectrum of standard and sample is shown in Fig. 5. A single spot with an R_f value of 0.61 was observed in MLX standard and samples from marketed MLX tablet. In degradation studies the spots of degraded products were well resolved from the drug spot.
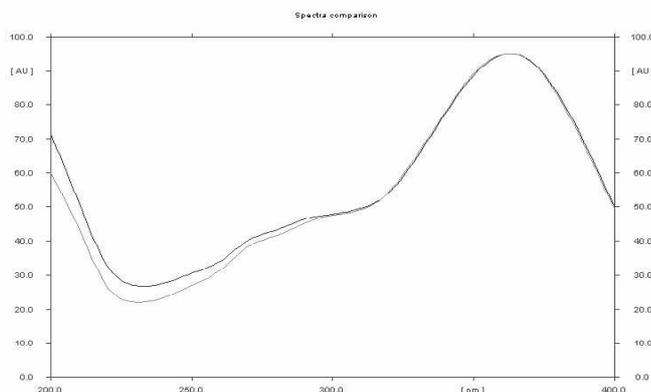
Fig. 5: Spectral overlay of standard and sample (formulation)

Accuracy (Recovery studies)

The proposed method for extraction and estimation of MLX from pharmaceutical dosage form after spiking with 80%, 100% and 120% showed good recoveries in the range of 101.45-101.97% as listed in Table 4.

Robustness

Robustness was studied by determining the effect of small variations in the mobile phase composition, mobile phase volume, chamber saturation time, migration distance and time from chromatography to scanning. The % RSD of peak areas was calculated for each variable and was found to be less than 2%. The low values of % RSD as listed in Table 5 indicate that the method is robust.

Solution stability

Solution of 100% test concentration (500ng/spot) was stored at room temperature for 6, 12, 24, 48 and 72h, respectively. The chromatographic analysis was performed on the same TLC plate. After development, the chromatogram was studied for additional spots if any. The peak area and % RSD was found to be 5849.78 ±

106.31 and 1.81 respectively. No indication of instability of the analyte was found in the sample solution.

Analysis of marketed formulation

A single and compact spot at R_f value of 0.61 was observed in the chromatogram of the samples extracted from tablets. No interference from the excipients was found. The drug content was found to be 100.74% ± 1.15, with 1.14 % RSD. MLX was stable in the marketed formulation analyzed by this method and no degradation had occurred. Acceptable limit of % RSD value indicated the suitability of the proposed method for routine analysis of MLX in pharmaceutical dosage form.

Forced Degradation (Stability indicating property)

Acid and base induced degradation product

The rate of degradation in acid was slower as compared with alkali. The chromatogram of the acid degraded sample showed peak at R_f value of 0.16 (Fig.6). For base degraded sample, peak at R_f value of 0.16 and 0.69 were observed (Fig.7). The results indicate that MLX undergoes degradation under acidic and basic conditions.

Table 4: Recovery studies of MLX[a]

Recovery Level	Initial amount (ng)	MLX added (ng)	Total amount (ng)	MLX found (ng) ± SD	% Recovery ± SD
0	300	0	300	305.12 ± 0.75	101.70 ± 0.25
80	300	100	400	407.85 ± 11.52	101.96 ± 2.88
100	300	150	450	456.53 ± 11.59	101.45 ± 2.58
120	300	200	500	509.85 ± 16.46	101.97 ± 3.29

[a]n=3

Table 5: Results of Robustness studies[a]

Parameter	Mean R_f	%RSD of peak area
Mobile phase composition (±0.2ml)	0.59 ± 0.007	1.15
Mobile phase volume (±2ml)	0.60 ± 0.009	1.19
Chamber saturation time (±5 min)	0.61 ± 0.009	1.42
Migration distance (±0.5 cm)	0.60 ± 0.003	1.18
Time from chromatography to scanning (±10 min)	0.59 ± 0.002	0.59

[a]n=6
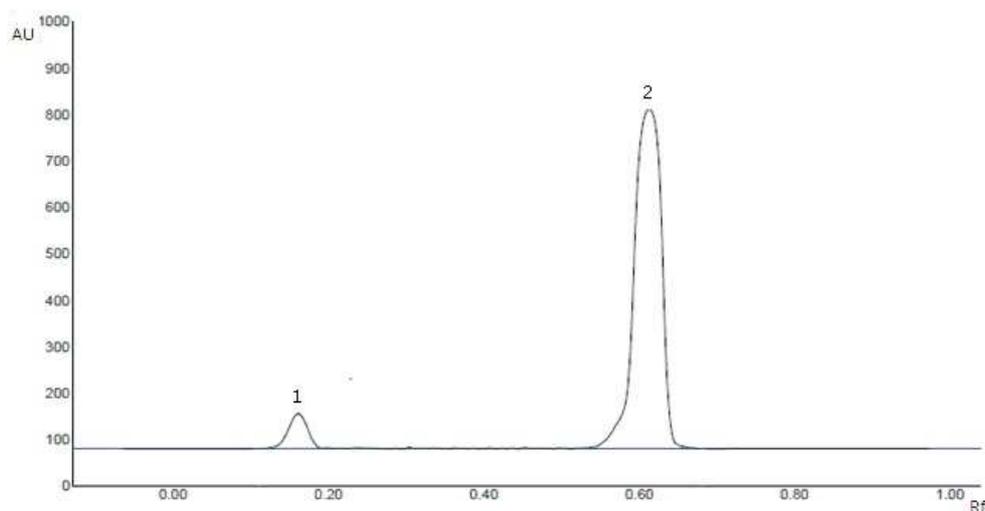
Fig. 6: Chromatogram of acid induced degradation; peak 1(degradation product) (R_f: 0.16), peak 2 (MLX) (R_f: 0.61)
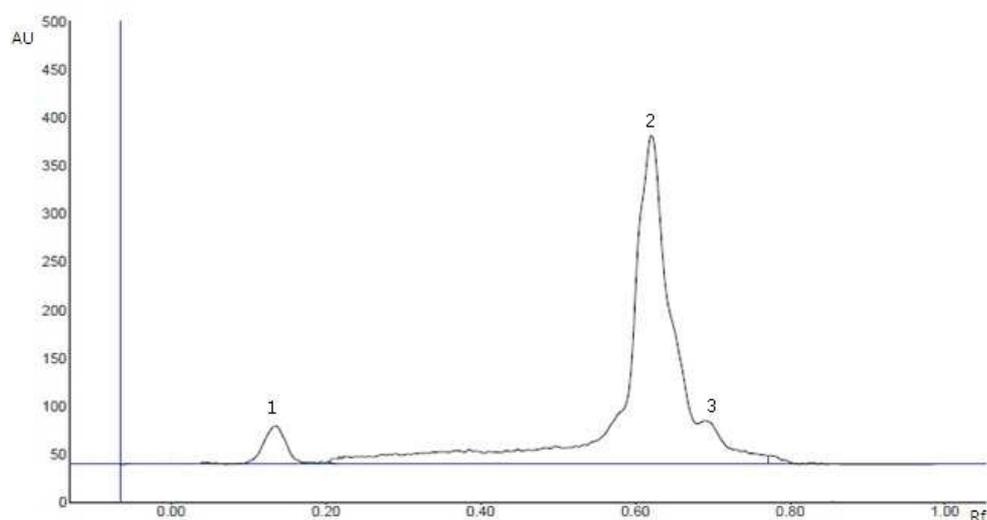
Fig. 7: Chromatogram of base induced degradation; peak 1, 3 (degradation product) (R_f: 0.18, 0.69), peak 2 (MLX) (R_f: 0.62)

Hydrogen peroxide induced degradation product

The sample showed an additional peak with 3% (w/v) (Fig.8) and 30% (w/v) at R_f value of 0.16 (Fig.9). The drug was considerably stable at room temperature.
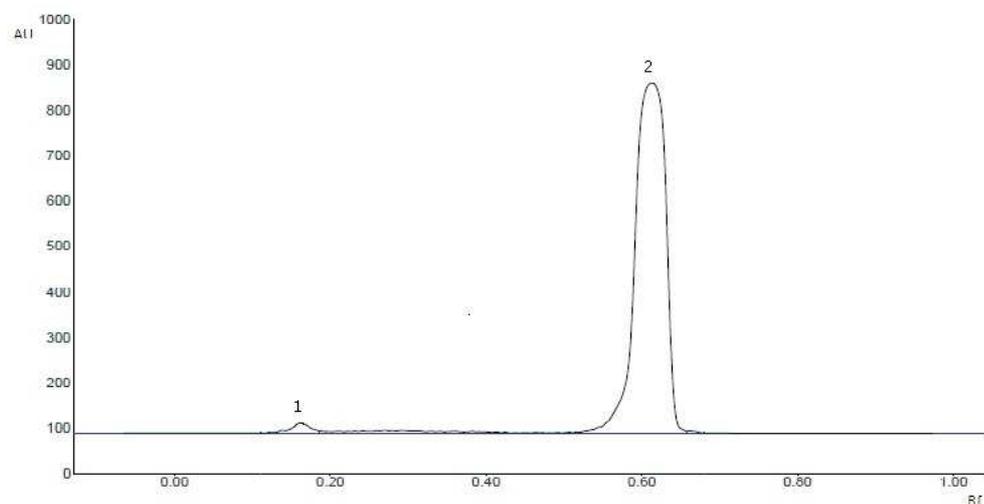
Fig. 8: Chromatogram of hydrogen peroxide (3%) induced degradation; peak 1(degradation product) (R_f: 0.16), peak 2(MLX) (R_f: 0.61)
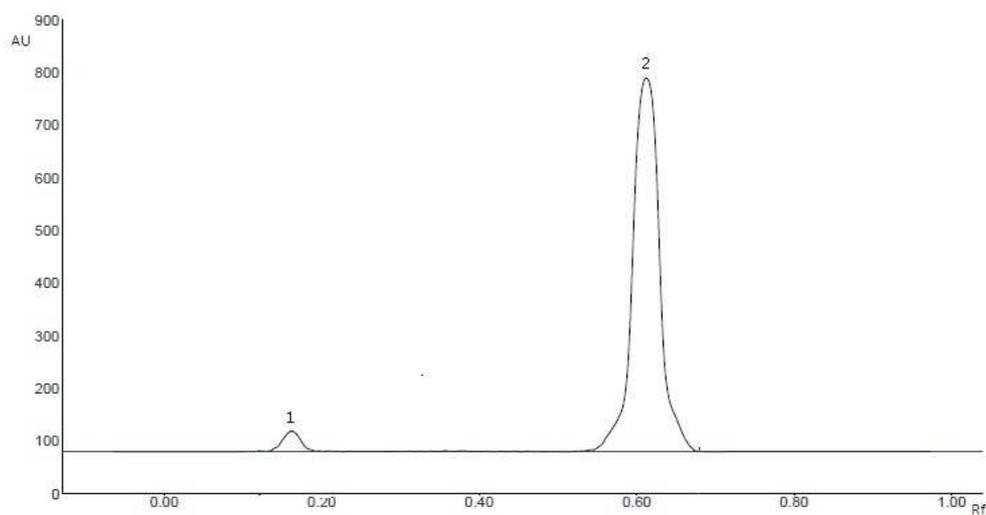
Fig. 9: Chromatogram of hydrogen peroxide (30%) induced degradation; peak 1(degradation product) (R$_f$: 0.16), peak 2(MLX) (R$_f$: 0.61)

Dry and wet degradation product

No additional peaks were observed (Fig.10, 11). MLX was stable under these conditions showing negligible degradation to dry and wet heat degradation.
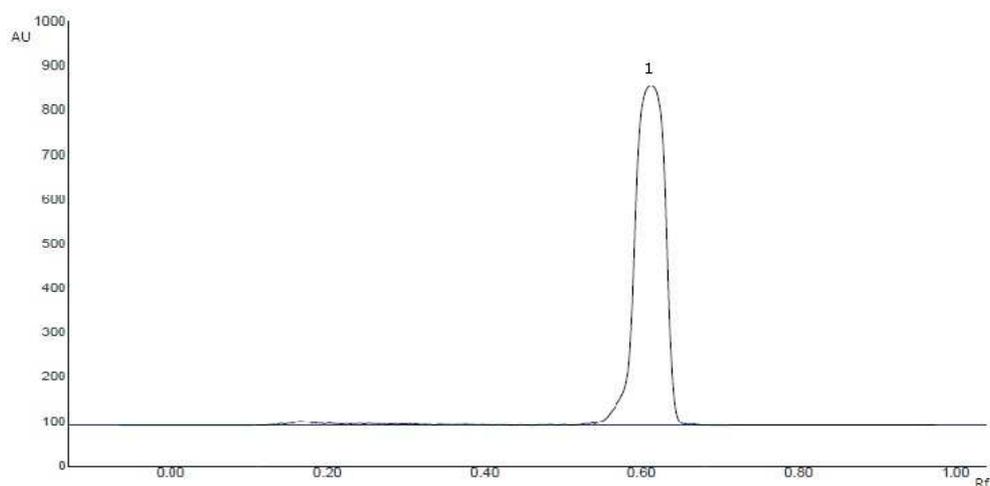
Fig. 10: Chromatogram of dry heat induced degradation; peak 1 (MLX) (R$_f$: 0.61).
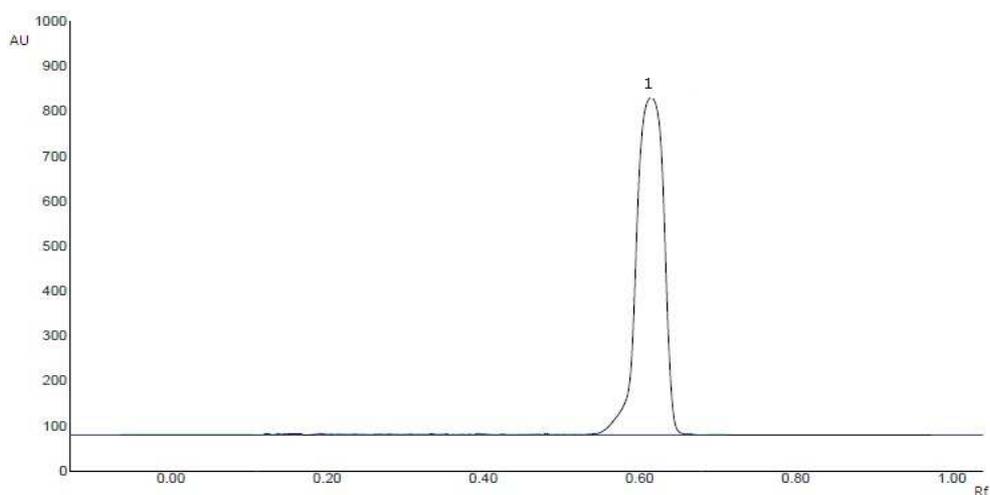
Fig. 11: Chromatogram of wet heat degradation; peak 1 (MLX) (R$_f$: 0.61)

Photolytic degradation product

An additional peak was observed at R_f value of 0.17 (Fig.12), however % recovery of MLX showed negligible degradation.

The results for forced degradation studies are listed in Table 6.
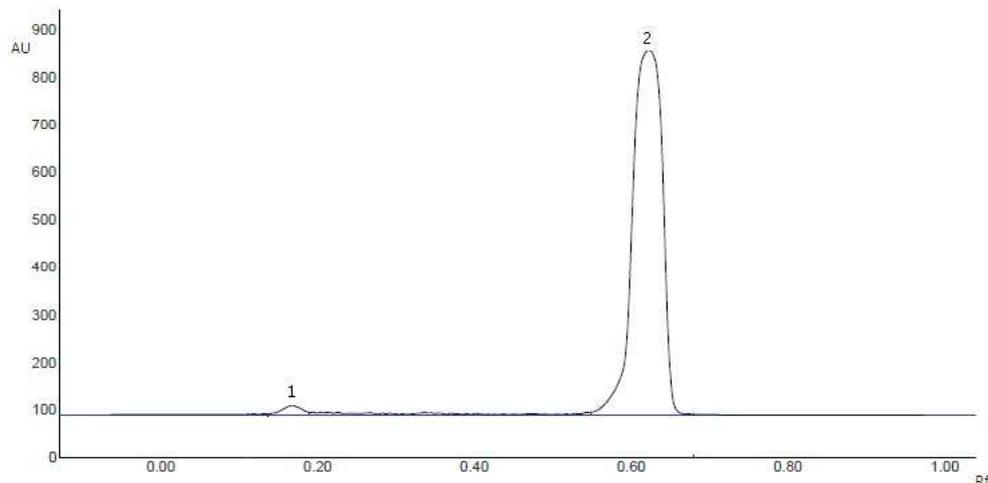
Fig. 12: Chromatogram of photo degraded MLX peak 1(degradation product) (R_f: 0.17); peak 2 (MLX) (R_f: 0.61)

Table 6: Forced Degradation of MLX

Degradation Condition	% Recovery	R_f value of degradation products
Acid 1N HCl[a]	93.12	0.16
Base 1N NaOH[a]	84.09	0.18, 0.69
Oxidative 3% H_2O_2	98.29	0.16
Oxidative 30% H_2O_2	96.29	0.16
Dry heat (50ºC,72h)	100	-
Wet heat (50ºC, 75% RH)	100	-
Photolytic (sunlight, 7days)	98.47	0.17

[a]Refluxed

Detection of related impurities

The chromatographic analysis revealed that the sample showed one additional spot at R_f value of 0.16 (Fig.13). The area of the additional spot (1702.8) was found to be much less as compared to the main spot (23048.7) from the standard solution. From the degradation results it can be observed that the R_f value of acid, base, oxidative and photo degradation components equal with the impurity present in the drug. However base induced degradation showed an additional degradation component eluted at R_f value of 0.69, which was not detected in the present determination. The spectra recorded for both sample and impurity is shown in Fig.14.

The data of summary of validation parameters are listed in Table 7.
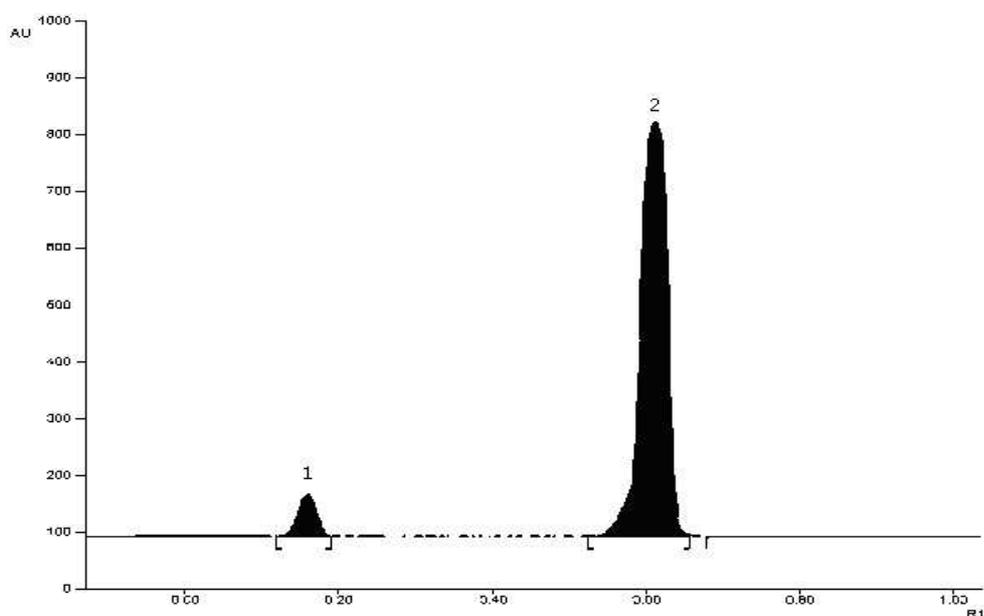
Fig. 13: Chromatogram of MLX and its impurity; peak 1 impurity, R_f: 0.16: peak 2 MLX, R_f: 0.61
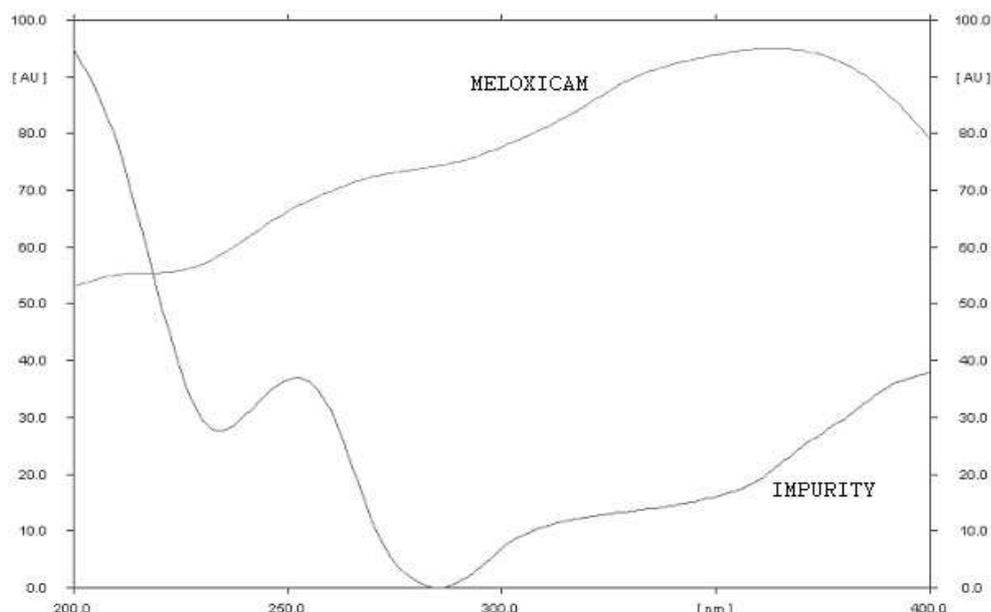
Fig. 14: Spectra Comparison: MLX and impurity

Table 7: Summary of validation parameters

Parameter	Data
Linearity range	200-700
Correlation coefficient	0.9965 ± 0.00083
Regression equation	Y= 8.192x + 1640.1
Limit of detection	23.20 ng/spot
Limit of quantification	70.30 ng/spot
Accuracy	101.70 ± 0.25
Precision	
Repeatability of application (n=5)	CV: 0.011 and 0.016 for peak area and % content respectively
Inter-day (n=3)	0.068
Intra-day (n=3)	0.034
Robustness	Robust
Specificity	Specific

CONCLUSION

Statistical analysis of the data establishes that the developed HPTLC method is specific, accurate, precise and stability-indicating. The validated method is suitable for analysis of MLX in both bulk and pharmaceutical dosage form without any interference from the excipients. The method can be employed to determine the purity of the drug by detecting any related impurities present. The method is efficient in separating the degradation components from the main analyte MLX, and hence can be considered as stability-indicating. This analytical method may be extended for determination of MLX in plasma and other biological fluids and warrant further studies.

ACKNOWLEDGEMENT

REFERENCES

1. Ah YC, Choi JK, Choi YK, Ki HM, Bae JH. A novel transdermal patch incorporating meloxicam: i*n vitro* and i*n vivo* characterization. Int. J. Pharm. 2010; 385: 12-19.
2. Mahmood KT, Khan B, Ashraf M, Haq IU. Specific and simple HPLC assay of ecofriendly meloxicam in pharmaceutical formulations. Journal of Pharmaceutical Sciences and Research 2010; 2(12): 878-883.
3. Luger P, Daneck K, Engel W, Trummlitz G, Wagner K. Structure and physicochemical properties of meloxicam, a new NSAID. Eur. J. Pharm. Sci. 1996; 4: 175-187.
4. Ambrus R, Kocbek P, Kristl J, Sibanc R, Rajko R, Revesz PS. Investigation of preparation parameters to improve the dissolution of poorly water-soluble meloxicam. Int. J. Pharm. 2009; 381:153-159.
5. Bandarkar FS, Vavia PR. A stability indicating HPLC method for the determination of meloxicam in bulk and commercial formulations. Tropical Journal of Pharmaceutical Research 2009; 8(3): 257-264.
6. Martin RM, Biswas P, Mann RD. The incidence of adverse events and risk factors for upper gastrointestinal disorders associated with meloxicam use amongst 19 087 patients in general practice in England: cohort study. Br. J. Clin. Pharmacol. 2000; 50: 35-42.
7. Vignaduzzo SE, Castellano PM, Kaufman TS. Development and validation of a dissolution test for meloxicam and pridinol mesylate from combined tablet formulation. Indian J. Pharm. Sci. 2010, 72(2): 197-203.
8. Mahmood KT, Ashraf M. Absolute bioavailability of oral meloxicam in healthy dogs. The Journal of Animal & Plant Sciences 2010; 20(3): 193-196.
9. Meineke I, Turck D. Population pharmacokinetic analysis of meloxicam in rheumatoid arthritis patients. Br. J. Clin. Pharmacol. 2002; 55: 32-38.
10. Vasiliki V, Pinto PCAG, Lucia M, Saraiva MFS, Lima JLFC. Sequential injection determination of meloxicam in pharmaceutical formulations with spectrophotometric detection. Can. J. Anal. Sci. Spectros. 2007; 52(6): 351-358.
11. Zawilla NH, Mohammad MAA, Kousy NME, Aly SMEM. Determination of meloxicam in bulk and pharmaceutical formulations. J. Pharm. Biomed. Anal. 2003; 32: 1135-1144.

12. Khan F, Lohiya RT, Umekar MJ. Development of UV spectrophotometric method for the simultaneous estimation of meloxicam and paracetamol in tablet by simultaneous equation, absorbance ratio and absorbance correction method. International Journal of ChemTech Research 2010; 2(3): 1586-1591.
13. Pomykalski A, Hopkala H. Comparison of classic and derivative UV spectrophotometric methods for quantification of meloxicam and mefenamic acid in pharmaceutical preparations. Acta Pol. Pharm. 2011; 68(3): 317-23.
14. Eroglu H, Bozkurt NB, Uma S, Oner L. Validation of the analytical method for *in-vivo* determination of meloxicam and bioequivalence study from meloxicam containing microparticle formulations in rabbits. Hacettepe University Journal of the Faculty of Pharmacy 2009; 29 (2): 115-130.
15. Zhang H, Choi HK. Analysis of meloxicam by high-performance liquid chromatography with cloud-point extraction. Anal. Bioanal. Chem. 2008; 392: 947-953.
16. Velpandian T, Jaiswal J, Bhardwaj RK, Gupta SK. Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples. J. Chromatogr. B. 2000; 738: 431-436.
17. Dasandi B, Saroj SH, Bhat KM. LC determination and pharmacokinetics of meloxicam. J. Pharm. Biomed. Anal. 2002; 28: 999-1004.
18. Taha EA, Salama NN, Fattah LESA. Spectrofluorimetric and spectrophotometric stability-indicating methods for determination of some oxicams using 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl). Chem. Pharm. Bull. 2006; 54(5): 653-658.
19. Hassan EM. Spectrophotometric and fluorimetric methods for the determination of meloxicam in dosage forms. J. Pharm. Biomed. Anal. 2002; 27: 771–777.
20. Murarasu AE, Mandrescu M, Spac AF, Dorneanu V. A method for the turbidity assay of meloxicam using molybdophosphoric acid. Farmacia 2010; 58(3): 315-321.
21. Desai N, Amin P. Stability Indicating HPTLC determination of meloxicam. Indian J. Pharm. Sci. 2008; 70(5): 644-647.
22. Beltagi AM, Ghoneim MM, Radi A. Electrochemical reduction of meloxicam at mercury electrode and its determination in tablets dosage form. J. Pharm. Biomed. Anal. 2002; 27(5): 795-809.
23. Altinoz S, Nemutlu E, Kir S. Polarographic behaviour of meloxicam and its determination in tablet preparations and spiked plasma. Farmaco 2002; 57(6): 463-468.
24. Ye H, Qiu B, Chen J, Lin J, Chen G. Flow-injection analysis for meloxicam based on tris(2,2'-bipyridine) ruthenium(II)-Ce(IV) chemiluminescent system. Luminescence 2009; 24(4): 260-265.
25. Nemutlu E and Kir S. Method development and validation for the analysis of meloxicam in tablets by CZE. J. Pharm. Biomed. Anal. 2003; 31(2): 393-396.
26. Patel RB, Patel MR, Bhatt KK, Patel BG. HPTLC method development and validation: quantification of paliperidone in formulations and *in vitro* release study. Anal. Methods 2010; 2: 525-531.
27. FDA, "International Conference on Harmonization: Draft Revised Guidance on Q1A(R) Stability Testing of New Drug Substances and Products," 2000. Federal Register 65 (78), 21446.
28. Mahadik MV, Dhaneshwar SR. Application of a stability-indicating HPTLC method for the quantitative determination of ezetimibe in pharmaceutical dosage forms. Asian J. of Pharm. Sci. 2007; 2(5): 182-190.