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# Specific and Simple HPLC Assay of Ecofriendly Meloxicam in Pharmaceutical Formulations

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## Abstract

A simple, precise, specific ,robust and rapid HPLC assay for the of measurement meloxicam in pharmaceutical formulations was developed and validated at Department of Pharmacy and Central Quality Assurance Laboratory, LCWU, Lahore. The solution of meloxicam standard was prepared in HPLC grade acetonitrile (5 µg.mL$^{-1}$) and ultra sonicated for 15 minutes. One mL sample mixed well with one mL of HPLC grade water and filtered through 0.22 µm filter. Ten micro-litter sample was injected into HPLC system through an injector valve with a ten micro-litter sample loop. The mobile phase comprising of phosphate buffer(0.2 Normal) and acetonitrile (38:62, v/v) was pumped into Water 1525 Binary HPLC Pump 1525 at the rate 0.5mL/min Separation was achieved by using a reversed phase C18 column (Phenomenex, particle size 5 µm;4.6 mm ×150 mm) at retention time of 7.4 minutes. Oven temperature was set at 25 °C. The meloxicam was distinctly and specifically detected at 352nm by using Water 2487 dual absorbance detectors. The method was validated prior to the analysis of samples. The limit of detection and limit of quantification were .0.06 and 6 micrograms respectively. The sample from commercial pharmaceutical formulation were prepared in HPLC grade methanol in concentration of 5 µg.mL$^{-1}$. The developed and validated method was successfully applied for assay of meloxicam in these formulations including injections, tablets and suspension.

**Keywords :***Meloxicam; HPLC; C1.8 and Pharmaceutical Formulations*

## INTRODUCTION

Meloxicam is non-steroidal anti-inflammatory drug (NSAID), registered as an anti-inflammatory and analgesic agent for management of pain arising from different conditions such as rheumatoid arthritis and osteoarthritis in human as well as animal, in many countries including USA It is available in the dosage form of tablets, injection and liquid oral suspension.[1,2,3,4].

Meloxicam is chemically designated as 4-hydroxy-2-methyl- N -(5-methyl-2-thiazalyl)-2 H -1,2-benzothiazine-3-carboxamide-1,1-dioxide and belongs to oxicam class of NSAIDs. It has the molecular weight of 351.4 dalton and its formula is shown in fig 1.[5]

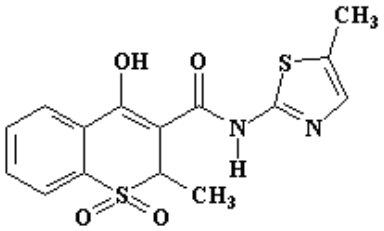


**Figure 1** Chemical formula for Meloxicam ($C_{14}H_{13}N_3O_4S_2$)

The therapeutic index of meloxicam is higher when compared with other NSAIDs like piroxicam, diclofenac, and indomethacin. This more favorable risk: benefit profile is due to capability of meloxicam for preferential inhibition of COX-2 over COX-1. [6] . Meloxicam undergoes fast elimination, leading to a shorter $t_{1/2}$ in comparison with piroxicam and tenoxicam. It has no capability for nephrotoxicity [7,8,and 9]

Diclofenac was banned for veterinary use in Pakistan, India and Nepal during 2005-06, following evidence of its role in the decline of vulture [10,11,and 12]. Meloxicam has been reported as a safe and ecofriendly alternate of diclofenac in veterinary practice [13,14] .7.5mg and 15mg tablets are registered for human use whereas injection meloxicam for veterinary use have been registered after ban on veterinary formulation in Pakistan.[15]

Some HPLC methods for determination of meloxicam in pharmaceutical formulations and plasma have been reported. [16,17,18,19].

The present paper describes a specific, precise accurate, reproducible, robust, efficient, economical and quicker HPLC method for measurement of meloxicam in different formulation. The work was done at Department of Pharmacology & Toxicology

,University of Veterinary and Animal Sciences (UVAS); Lahore and Department of Pharmacy/Central Quality Assurance Laboratory, LCWU, Lahore.

## MATERIALS AND METHODS

### Experimental Drugs / Chemicals:

Meloxicam standard of company Sigma was purchased for use as reference external standard .HPLC grade acetonitrile and phosphoric acid (E. Merck Germany) were purchased. All other chemicals of reagent grade were used..The purified water prepared by using a Milli-Q system was used for the preparation of buffer and other aqueous solutions .Injections of meloxicam 5mg/mL manufactured by INTAS Pharmaceutical Limited Matoda 382210 Dist. Ahmadabad, India, Metacam oral suspension 5mg/mL Boehringer UK ,Mobic oral suspension 7.5mg/mL and tablets 7.5mg of Boehringer were procured. Meloxicam Tablets 7.5mg manufactured by Mega, Lahore ,Pakistan were received as gift.

### Statistical analysis

The software SPSS (Statistical Package for the Social Sciences) 13.0 was used for statistical analysis. The values in the data were expressed as range, mean, SEM (standard error of means); median and standard deviation.

### Preparation of mobile phase

1. The buffer was prepared by use of 85% phosphoric acid. The pH 4.0 was adjusted. Buffer was filtered through 0.22 µm filter
2. 380mL of the buffer were added to 400mL acetonitrile in flask of one litter capacity. The volume was made up to one litter by adding HPLC grade acetonitrile.. High speed vortex mixing was done for 30minutes followed by 30ultrasonication for 15minutes.Mobile phase was filtered through 0.22 µm filter

### Solutions of standard and samples

#### i. Stock solution

Stock solution of standard meloxicam was prepared in acetonitrile at concentration of 1mg.mL$^{-1}$. Mixing, ultrasonication and filtration was done in the same way as for mobile phase.

#### ii. Solutions of standard

The solution at concentration of 5µgmL$^{-1}$ was prepared with external standard meloxicam by diluting with mobile phase.

The samples at concentration of 10.0, 9.0,8.0,6.0,4.0.2.0,1.5,1.0,0.5, 0.25 and 0.12 µg.mL$^{-1}$ were also prepared by diluting stock solution of external standard meloxicam in mobile phase for constructing standard curve. All these samples were mixed well, ultrasonicated for 30 minutes and filtered through 0.22 µm

#### iii. Solutions of Samples

1. Sample A. 20 tablets meloxicam 7.5mg were weighed and powdered. Powder equivalent to 5mg meloxicam was transferred to a 50 mL volumetric flask, followed by addition of 30 mL methanol. The contents in the flask mixed well and ultrasonicated for 30 minutes to complete dissolution and diluted to the mark with methanol and then filtered through 0.22 µL filter. 0.5 mL sample solution was diluted to 10mL with mobile phase to get concentration of 5µg/mL.
2. Sample B Mobic suspension equivalent to 5mg meloxicam was transferred to a 50 mL volumetric flask. The sample prepared in the similar fashion as sample A .
3. Sample C Metacam suspension equivalent to 5mg meloxicam was transferred to a 50 mL volumetric flask. The sample prepared in the similar fashion as sample A .
4. Sample D. one mL of injection equivalent to 5mg meloxicam was transferred to a 50 mL volumetric flask. The sample prepared in the similar fashion as sample A .

### High performance liquid chromatography

The maximum absorbance $\lambda_{max}$ for meloxicam external standard was determined by scanning in UV-Visible range of wave length ($\lambda_{max}$ = 352nm).

Method validation is vital issue in any drug analysis and is also legal requirement USP_NF (2009). The validation ascertain suitably and reliability of a method for its

intended use. The developed HPLC method was validated with respect to stability, linearity, accuracy, precision, sensitivity, and robustness

The stability studies on stock solution of meloxicam(1 $mg.mL^{-1}$) were carried out for six weeks at 4 ºC and 25ºC. The stability studies on meloxicam 5 $\mu g.mL^{-1}$ in mobile phase were carried out for 24h at 25ºC.

The AUC of samples of meloxicam at concentration of 10.0,9.0,8.0,6.0, 4.0. 2.0, 1.5, 1.0,0.5, 0.25 and 0.12 $\mu g.mL^{-1}$ were determined for making calibration curve.

The intraday and assays were carried out by testing three samples meloxicam at the concentration 1, 2.5 and 5.0 $\mu g.mL^{-1}$.Six reading were taken for each samples.

The assays were performed by different analysts to explore robustness in the method. 6 samples at the concentration of 5$\mu g.mL^{-1}$ were analyzed. The result was compared statistically (Wilcoxon paired test).

The samples prepared from all the formulations were analyzed by use of above method. The concentrations of meloxicam and other conditions were same for samples and standard, The meloxicam was determined by the following formula.

**Meloxicam in dosage form** = A mg

$$= \frac{AUC_{sample} \times \text{Strength per unit dosage form}}{AUC_{Standard}}$$

**% Meloxicam in dosage form**

$$= \frac{\text{A mg (Determined meloxicam)} \times 100}{\text{Stated meloxicam in dosage form}}$$

## RESULTS AND DISCUSSION

The maximum absorbance $\lambda_{max}$ for meloxicam external standard was determined 352nm

The distinct peak was visible in chromatograms of meloxicam external standard at retention time of 7.4 minutes when UV detector was set 352nm.There was no interference peak around this time. The representative chromatogram is shown in figure 2. There was no peak when placebo mobile phase was run as sample.

The calibration curve shown in figure 3 was prepared by plotting AUC versus concentration of meloxicam.

**Table 1:**HPLC method for determination of meloxicam Percent yield/recovery in standard tablets 7.5mg

| | |
|---|---|
| *Mean concentration meloxicam recovered from tablets 7.5mg (mean ±/-S.D.)* | *7.49±0.16* |
| % concentration meloxicam recovered from tablets 7.5mg | 99.87% |
| C.V% (RSD) | 0.5% |

C.V = coefficient of variation, RSD = Relative standard deviation

**Table.2:** Stability studies on stock solution of meloxicam at concentration of 1mg/mL

| ***Temp.*** | ***Mean (%) concentration of meloxicam recovered (mean ±SD)*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Day1*** | ***Day 2*** | ***Day4*** | ***Day 8*** | ***Day 16*** | ***Day24*** | ***Day42*** |
| 4 ºC | 100.09± 0.044% | 100.08± 0.073% | 99.99± 0.054% | 100.00± 0.06% | 99.81± 0.55% | 99.82± 0.046% | 99.80± 0.04% |
| 25 ºC | 100.03± 0.046% | 99.91± 0.055% | 100.00± 0.073% | 100.00.± 0.04% | 99.88± 0.06% | 99.82± 0.043% | 99.73± 0.05% |

**Table 3:**Stability of meloxicam in mobile phase at concentration of 5µg/ mL

| ***Mean ± SD concentration of meloxicam in mobile phase at 25 ºC (n=6)*** | | | | | | |
|---|---|---|---|---|---|---|
| ***At 0h*** | ***At 2h*** | ***At 6h*** | ***At 8h*** | ***At 12h*** | ***At 18h*** | ***At 24h*** |
| 100.00.± 0.019% | 99.91± 0.015% | 100..00± 0.019% | 100.09± 0.037% | 99.88± 0.055% | 99.7± 0.064% | 99.73± 0.037% |

**Figure 2:**Representative chromatogram of meloxicam external standard (5 µg / mL)

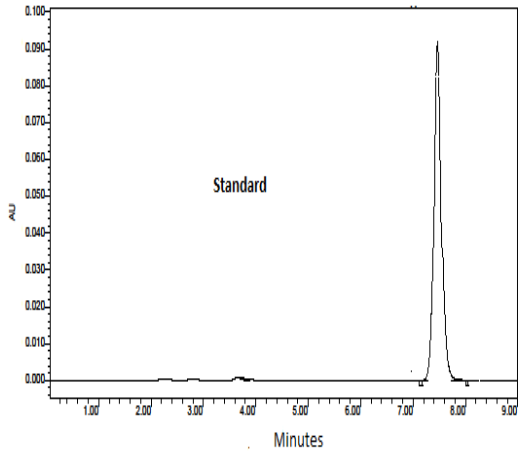


**Figure.3** Standard calibration curve for HPLC method for determination of meloxicam in different formulations.

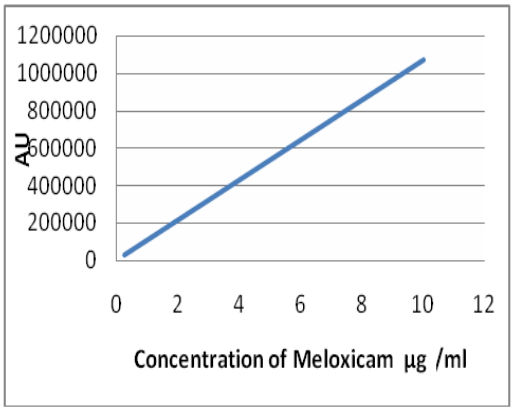


**Figure.4** Chromatogram of meloxicam tablets 7.5mg

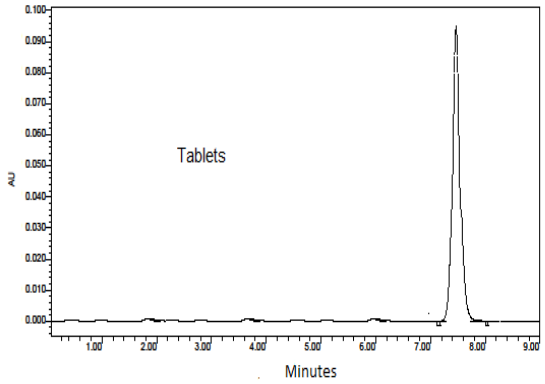


**Figure.5** Chromatogram of Mobic Suspension 7.5mg/5mL

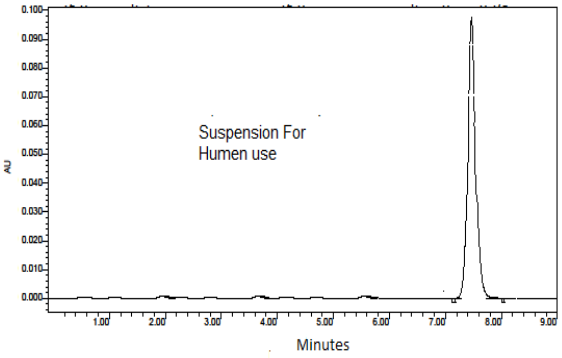


**Figure.6** Chromatogram of Metacam Suspension 5mg/mL.

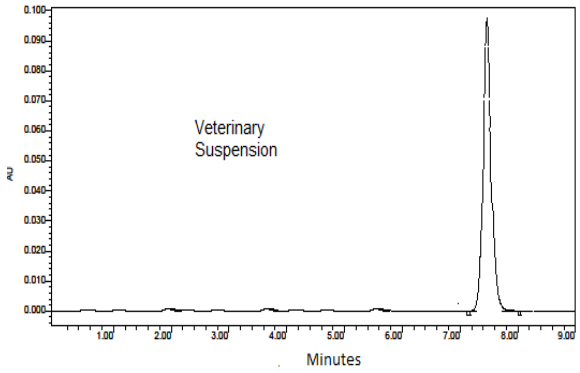


**Figure.7.** Chromatogram of Injection meloxicam 5mg/mL

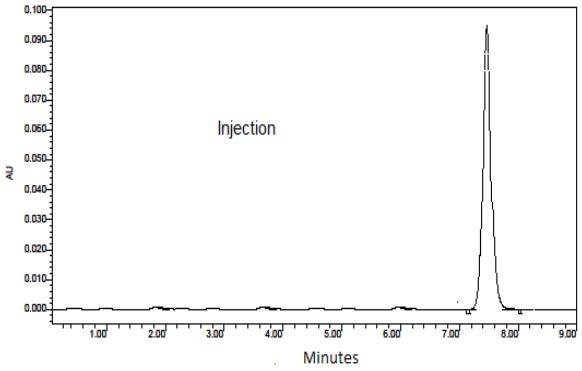


**Table.4:**Intraday and intraday variability in HPLC method for determination of Meloxicam

| ***Meloxicam added*** | ***intraday variability***(n=6) | | | ***Interday variability***(n=6) | | |
|---|---|---|---|---|---|---|
| | ***1 µg/ mL*** | ***2.5µg/ mL*** | ***5µg/ mL*** | ***1 µg/ mL*** | ***2.5µg/ mL*** | ***5µg/ mL*** |
| Mean% meloxicam recovered ±S.D. | 99.28±1.413 | 99.65±1.177 | 99.85±0.872 | 99.89±0.82 | 99.87±0.91 | 99.96±1.25 |
| C.V%(RSD) | 1.420 | 1.181 | 0.873 | 0.821 | 0.911 | 1.251 |

**Table 5:** HPLC determination of meloxicam in 5 µg/ mL solution done by two different analysts.

| *Analysts* | *Mean % concentration meloxicam ±S.D. (n=6)* |
|---|---|
| Analyst.1 | 99.01%±0.027 |
| Analyst.2 | 100.04%±0.037 |

The results regarding recovery/ percentage yield of meloxicam in the patent tablets 7.5 mg are presented in table 1. The mean recovery of 99.84%% with C.V% (RSD) 0.5% had shown that HPLC method was accurate.

The method was cross checked by two different analysts. Results are presented in table 5.The statistical comparison (Wilcoxon paired test) had shown that there was no difference between results (p=0.217, p=0.050). Therefore, the method was found to be robust.

The representative chromatogram of tablets meloxicam 7.5mg shown in figure 4.

The representative chromatogram s of Mobic suspension 7.5mg shown in figure 5

The representative chromatogram s Metacam suspension 5mg shown in figure 6

The representative chromatogram s of Injection meloxicam 5.0mg shown in figure 7

The peaks of meloxicam in the samples of all the above dosage were matched with the peak of standard. These were similar and distinct at same retention time 7.4 minutes, without any interference peak. Thus showing that method was specific.

## CONCLUSION

This method for measurement of meloxicam was specific, precise, accurate, reproducible, robust, efficient, economical and quicker. The HPLC method described above was different and better due to high speed vortex mixing of the samples as well as better mobile phase, filtration through 0.22 µm ultrafilter and better ultrasonication. It was successfully used for measurement of meloxicam in pharmaceutical formulations .It is an economical method for routine analysis of meloxicam in different commercial preparations including tablets, suspension and injections..

After ban on diclofenac and registration of meloxicam as its alternate in veterinary practice, this convenient method will be helpful for the routine assay of veterinary formulations of meloxicam.

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