METHOD DEVELOPMENT FOR 4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide BY REVERSE PHASE HPLC: NEW ANALYTICAL TECHNIQUE

Husnul Maab[1], Hammad Yousaf*[2], Syed Saeed ul Hassan[3], Umar Farooq[2]

[1]Faculty of Pharmacy, Punjab University, Lahore, Pakistan

[2]Rashid Latif College of Pharmacy, Lahore Pakistan

[3]Faculty of Pharmacy, The University of Lahore.

*Corresponding

Hammad Yousaf
Assistant Professor, Rashid Latif College of Pharmacy, Lahore
h_y_84@hotmail.com

Authors' contributions

This work was carried out in collaboration between all authors. Author SH has supervised the designed research work and evaluated the results critically. Author HM conducted the research activities in collaboration with co-authors and wrote the first draft of the manuscript. Author HY evaluated the results and guided about protocols of thesis writing and help out in manuscript writing. Author HY and UF gathered the literature review data and arranged the data and references according to guidelines of thesis. All authors read and approved the final manuscript.

ABSTRACT

Purpose: In this study, development of a new analytical method for the evaluation of 4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (Meloxicam) by reverse phase HPLC was carried out. The basic aim of this research was to develop and validate a simple, precise, accurate and sensitive method for qualitative and quantitative analysis of Meloxicam in pharmaceutical raw material and its dosage forms. The existing reported method (BP) for the analysis of Meloxicam is potentiometric method which is an old, lengthy and tedious method.

Method: In the new method of reverse phase HPLC, C18 column was used while the mobile phase was acetonitrile and methanol (70:30). The flow rate of mobile phase was 0.6ml/min and retention time was found to be 1.5min. Separately equal volume of standard solution and sample solutions in HPLC vials were injected in auto sampler compartment of HPLC in six replicates. Chromatogram and peak areas of Meloxicam in standard and sample solutions of different concentrations were recorded.

Results: This method was later validated in different ways by which the calibration curve proved to be linear with linearity coefficient of 0.999 over the range of 100 to 600ppm. The precision was equivalent to 0.0003%. The LOD and LOQ were 0.0003ug/ml and 0.001ug/ml respectively. The system also showed accuracy over the range of 95 to 99%.

Conclusion: Hence, this method proved to be an alternative to the existing reported method of potentiometric titration because the new method showed accuracy, reproducibility and sensitivity.

Key words 4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide (Meloxicam), acetonitrile, methanol, HPLC.

INTRODUCTION

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID). It is an oxicam derivative with general formula of $C_{14}H_{13}N_3O_4S_2$ and falls in the category of enolic acid group of NSAIDs (1). It was developed by Boehriger Ingelhemin in 1996 and since then it is used to relieve symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and pain. Meloxicam mainly acts by inhibiting cyclo-oxygenase that plays an important role in the formation of prostaglandins (2). The basic aim of this study was to develop and validate a simple, precise, accurate and sensitive method for qualitative and quantitative analysis of Meloxicam in pharmaceutical raw material and its dosage forms.

The reported method for the analysis of Meloxicam is potentiometric titration (BP 2013). Potentiometric titration is a slow and tedious method. It involves the proper calibration of the instrument since this affects the final results. This method is based on acid-base reaction and pH change at the equivalence point. A lot of care is required for the correct preparation of solvents involved, as the slightest change in acid-base composition may lead to faulty results (3). In addition to this potentiometric titration, the method requires plotting of titration curve for analysis of the compound which is a time consuming process. Reactivity of the elements to be titrated with acids and bases should be researched, since this may affect the end point. Addition of titrant should be very carefully narrowed down as the titration approaches equivalence point; for all these tasks, great care and expertise are required (4). Importantly, potentiometric titration is highly temperature dependent method (5). For these reasons, a newer method of reverse phase HPLC was developed for the analysis of Meloxicam in raw material as well as pharmaceutical dosage forms.

HPLC is a highly advanced automated process that takes only a few minutes to give results. It is a powerful and adaptable method with increased productivity by managing all the areas of analysis from sample to instrumentation and from separation to reporting of results. It is an easy method that has resolution, reproducibility and speed of analysis. A Small amount of sample is required and columns can

be reused without repacking. HPLC shows greater reproducibility due to close control of the parameters affecting the efficiency of the method. The results produced are of high resolution and easy to read and the tests are easily reproduced by automated process (6).

MATERIALS AND METHODS

Apparatus

Agilent 1200 HPLC system, Bandelinsonoplus DT2200, Sarstedt 0.45 micro membrane filter, Sartorius laboratory L420S analytical balance.

Chemicals

Meloxicam working standard was donated by Pharmacare Laboratories (Pvt) Ltd. Acetonitrile, methanol, hexane, acetone, hydrochloric acid, sodium hydroxide, distilled water, acetic acid, perchloric acid and sulphuric acid were of analytical grade and purchased from Asif Chemicals (Pvt) Ltd. A Pharmaceutical preparation of Meloxicam was purchased from local pharmacy of Lahore Pakistan.

Solubility studies

Initially, the solubility of Meloxicam in various solvents was studied visually as shown in **Table 1**. Later various combinations of solvents were made and absorbance spectrum of Meloxicam in these combinations was studied in UV. The combination of Meloxicam in acetonitrile and methanol showed a clear peak in UV as shown in **Figure 1**. Thus, it was selected as a suitable solvent system for Meloxicam. This solvent was applied to reverse phase HPLC.

HPLC ANALYSIS

Preparation of the mobile phase

60 ml Acetonitrile and 40 ml methanol were mixed to form mobile phase. The mobile phase was filtered through 0.45 micro membrane filter by vacuum filtration unit and degassed in ultrasonic bath.

Preparation of standard solution

100mg of meloxicam was weighed accurately on an analytical balance. 80ml of mobile phase was taken in volumetric flask and weighed amount of meloxicam was dissolved in it. The volume was made up to 100ml by mobile phase. This solution was filtered by micromembrane filter and degassed by an ultrasonic

bath. The concentration of this solution was found to be 1mg/ml. The Solution of different concentration were prepared from it and their standard curve was studied.

Preparation of sample solution

5 tablets were weighed accurately in an analytical balance and powdered in pestle and mortar. 100mg of powdered meloxicam was dissolved in 100ml of the mobile phase. This solution was filtered in micro filters and degassed. The Concentration of this solution was 1mg/ml. Sample solution of different concentration were prepared from this solution and the calibration curve was plotted.

Procedure

Mobile phase - acetonitrile:methanol

60 : 40

Solvent - acetonitrile:methanol

60 : 40

Column: C 18 silicone 250*50mm

Flow rate: 0.6ml/min

Lambda max: 355nm

Individually, an equal volume of standard preparation and sample preparations in HPLC vials were kept in an auto-sampler compartment in six replicates. Chromatogram and peak areas of meloxicam in standard and sample solutions of different concentrations were recorded and studied.

RESULTS

In this research a new method was developed to study the purity and authenticity of Meloxicam other than the method given in official pharmacopeia (BP 2013). The new method was developed and later validated and authenticated by various analytical methods.

The official method is potentiometric titration which is a slow, tedious and complicated method. This method is more time consuming and difficult method due to the use of buffers and maintenance of pH. A slight change in composition of the solutions may lead to faulty results. The non-aqueous titration method

was also used for analysis of raw material as well as tablet dosage form, although this method gave the results within official limit but this method was found to be slow with involvement of the preparation of standard solution of perchloric acid and its standardization using non aqueous medium. Therefore our aim was to look into fast and sensitive method for analysis of Meloxicam raw material as well as its determination in solid dosage form.

Initially, the solubility of Meloxicam in various solvents such as water, polyethylene glycol, 1N NaOH, 1N HCl, Methanol, ethanol, chloroform, hexane, acetonitrile, were studied visually. Acetonitrile is most commonly used in the study of Meloxicam in combination with different solvents as shown in the work of Bae (2), Bandarkar (3), El-Ries (4), Joseph (6), Mahmood (7) on Meloxicam. This was due to its good solubility and inert nature in the presence of Meloxicam.The interaction between drug and various solvent systems was also well understood by the study of Babu (1). Thus, different combinations of solvents were made with acetonitrile and methanol, and absorbance of Meloxicam was studied. From the results, it was concluded that combination of methanol and acetonitrile in ratio of 30:70 with Meloxicam, gave very clear peak without reactivity both in UV and HPLC method as shown in Figure 1 (a & b).

Table 1: Solubility profile of various solvents

SOLVENTS	SOLUBILITY
water	Insoluble
Polyethylene glycol	Insoluble
0.1M NaOH	Soluble
0.1M HCl	Insoluble
methanol	Soluble
ethanol	Soluble
chloroform	Freely soluble
hexane	Insoluble
acetonitrile	Soluble
acetone	Soluble

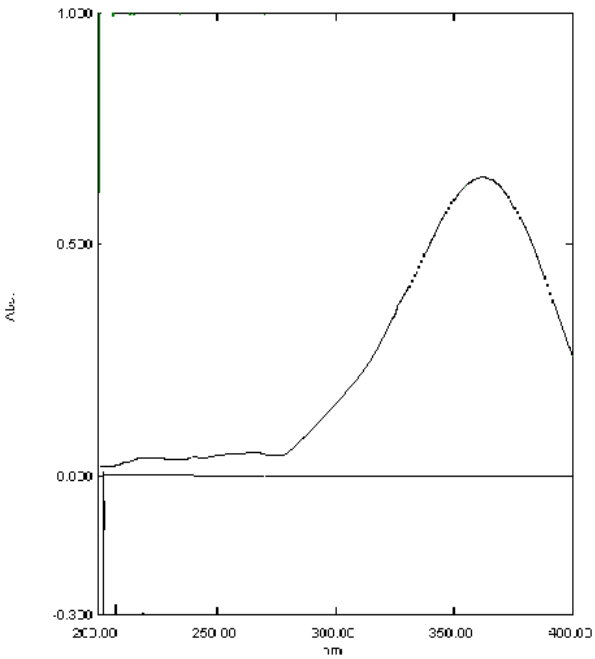


Figure1a: Standard peak of meloxicam in acetonitrile and methanol mixture at 355nm by UV

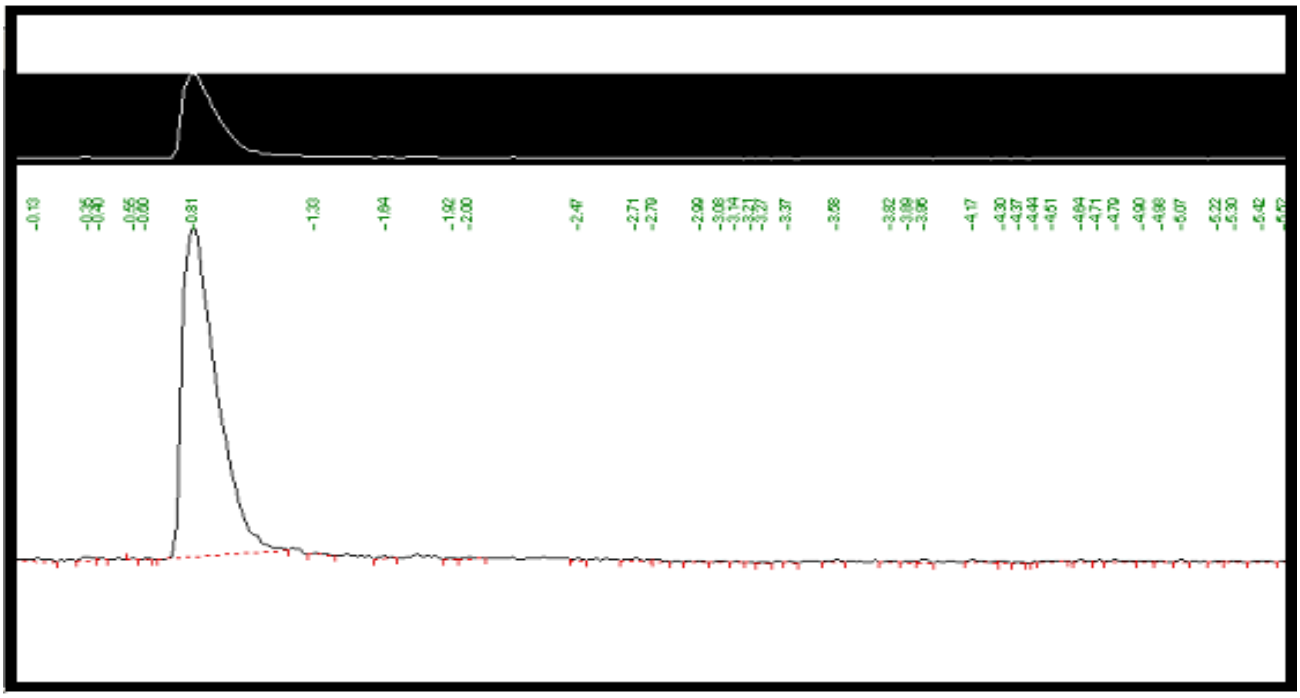


Figure1b: Chromatogram of meloxicam in acetonitrile and methanol by HPLC.

Thus by trial and error method a good solvent system was developed to study the characteristics of Meloxicam. After the development of solvent system for Meloxicam, its application in different instrumental techniques for Meloxicam analysis was studied.

Extensive research of Nageswara *et al*. (7) showed that UV and HPLC proved to be the most readily used method for analysis of drugs. From the study of Meloxicam in methanol: acetonitrile (30:70) solvent system by UV visible spectroscopy, it was observed that the method proved to be linear with good precision (0.25%), appropriated accuracy and very minimum value of limit of detection (0.006ug/ml) and limit of quantification(0.02ug/ml). Hence this system can be used for qualitative and quantitative study of Meloxicam by UV method.

The method developed was later applied and validated on High performance liquid chromatography. The mobile phase was acetonitrile and methanol (30:70), column used was C18. The flow rate was kept at 0.6ml/min and the retention time of the system was approximately 1.5 minutes.

Firstly, the precision of six similar concentrations of raw material was determined by injecting them on the column. The six peak areas were obtained whose standard deviation and relative standard deviation was found to be 2.8 and 0.00003%. The same procedure was repeated for six similar concentrations of tablets of Meloxicam and later their standard deviation and relative standard deviation were found to be 2.67 and 0.000035% respectively. The results obtained for both raw material and tablet samples showed that this HPLC system developed for Meloxicam showed very good precision and degree of reproducibility and hence can generate very close and appropriate results as shown in Table 2.

Table 2: Precision of meloxicam raw material by HPLC method

S. No.	Concentration ppm	Peak area mV	mean	Standard deviation	RSD
1	300ppm	8353568.1	8353565.1	2.8	0.000033%
2	300ppm	8353562.3			
3	300ppm	8353563.9			
4	300ppm	8353568.5			
5	300ppm	8353562.2			
6	300ppm	8353566.1			

*ppm parts per million

*mV millivolt

Later six different concentrations of Meloxicam raw material were made. These were injected into the column and their respective peak areas were noted given in Table 3. Graph was plotted between the concentration and peak areas given as shown in Figure 2. From the graph it was observed that Meloxicam raw material in methanol, acetonitrile solvent system showed good level of linearity in HPLC method over the range of 100 to 600ppm. The linearity coefficient and calibration equation were calculated to be 0.999 and y+27986x+92440. The similar procedure was repeated for Meloxicam tablet.

Again the obtained results showed sufficient level of linearity with linearity coefficient of 0.999 and calibration equation of y=30227x+41454 respectively. Hence, the developed method has the ability to show good degree of linearity over the range of 100 to 600ppm.

Table 3: Analysis of raw material of meloxicam by HPLC method

S. No.	Concentration ppm	Peak area mV
1	100	2956981.52
2	200	5636878.31
3	300	8455317.46
4	400	11273756.07
5	500	14092195.08
6	600	16910400.27

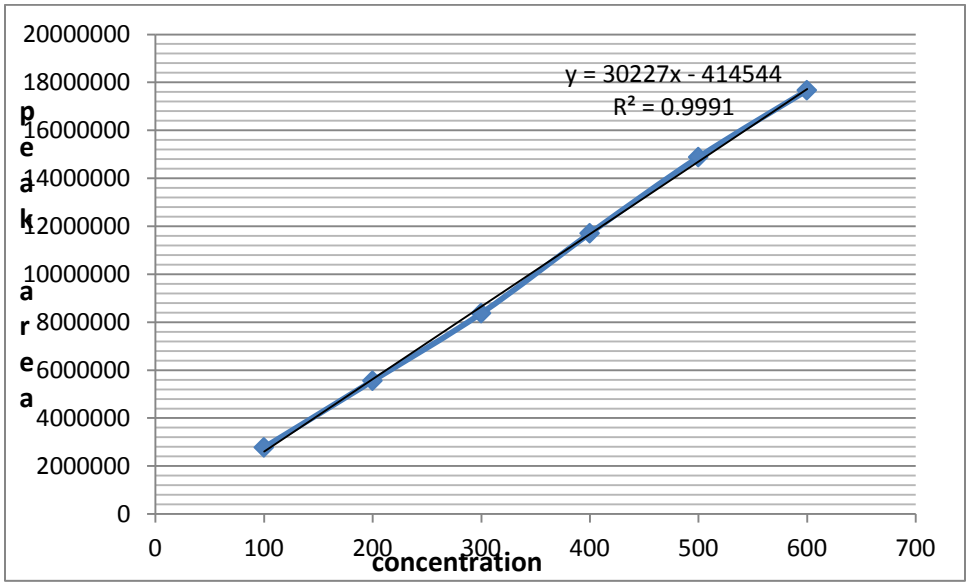


Figure 2: Calibration curve between concentration and peak area of meloxicam raw material

Limit of detection and limit of quantification was calculated from the graph and were found to be 0.000344ug/ml and 0.001ug/ml for raw material and 0.00031ug/ml and 0.001ug/ml for dosage form. From these results, it was observed that the system has the ability to detect and quantify up to 1ppm of Meloxicam in solvents by HPLC (Table 4).

Table 4:LOD and LOQ Raw material by HPLC:

S. No.	FACTOR	FORMULA	RESULT
1	Limit of detection	3.3*SD/slope	0.000344ug/ml
2	Limit of quantification	10*SD/slope	0.00104ug/ml

*SD standard deviation

Finally, the accuracy of the method was calculated from the concentration taken and concentration found. The adopted system showed good level of accuracy over the range of 95 to 100% as shown in Table 5.The method developed was later applied on various brands which showed accurate results as shown in Table 6, figure 3.

Table 5: Determination of percentage accuracy of meloxicam by HPLC

Amount of standard solution taken	Amount of sample solution added	Percentage composition	Peak area mV	Practical con	Actual con *X=y-b/m	%accuracy=actual concentration/practical concentration *100
10ml	8ml	80%	579494	18mg/ml	17.82mg/ml	99%
10ml	10ml	100%	615767	20mg/ml	19.0mg/ml	95%
10ml	12ml	120%	685289	22mg/ml	21.3mg/ml	96%

X=actual concentration, y=peak area, b=slope,

DISCUSSION

Thus, from this HPLC method it has been concluded that this system has good level of precision (0.00003%), linearity (R^2=0.999), accuracy, LOD (0.0003ug/ml) and LOQ (0.001ug/ml) for both raw material and dosage form of Meloxicam by HPLC method and hence can be used for analysis of Meloxicam. In this study the new method was also applied to different brands for their analysis as shown in Table 4.

Hence, from the study conducted above, it is proved that by solvent system developed (methanol: acetonitrile) for Meloxicam and new method developed and validated on HPLC can be successfully used for the analysis of Meloxicam. Quantitative analysis of different brands of meloxicam by HPLC is presented in Figure 3.

This method can be used against acid base and potentiometric titration for the qualitative and quantitative analysis of Meloxicam. The reported methods for Meloxicam analysis showed retention time greater than 1.5, as shown in the work of Bae (11.6min) (2), Bandarkar (6.8min) (3), Bao-xiu (6.2min) (5), Mahmood (7.3min) (8), and hence these systems prove to be slow as compared to our developed method, which is more fast and accurate due to its less retention time of 1.5 minutes. Similarly many reported methods require involvement of buffer system which makes the process more time consuming and complicated (9). In such systems pH maintenance is a major requirement for development of appropriate results. Our method is also advantageous on the basis of less number of solvent systems involved. In many researches more than two solvents are involved which make the system more complicated. In our research method, there is no requirement of pH maintenance and accurate results can be generated by the use of simple and readily available solvents that is acetonitrile and methanol. Hence, a new method has been developed for the analysis of Meloxicam in raw material and pharmaceutical dosage form.

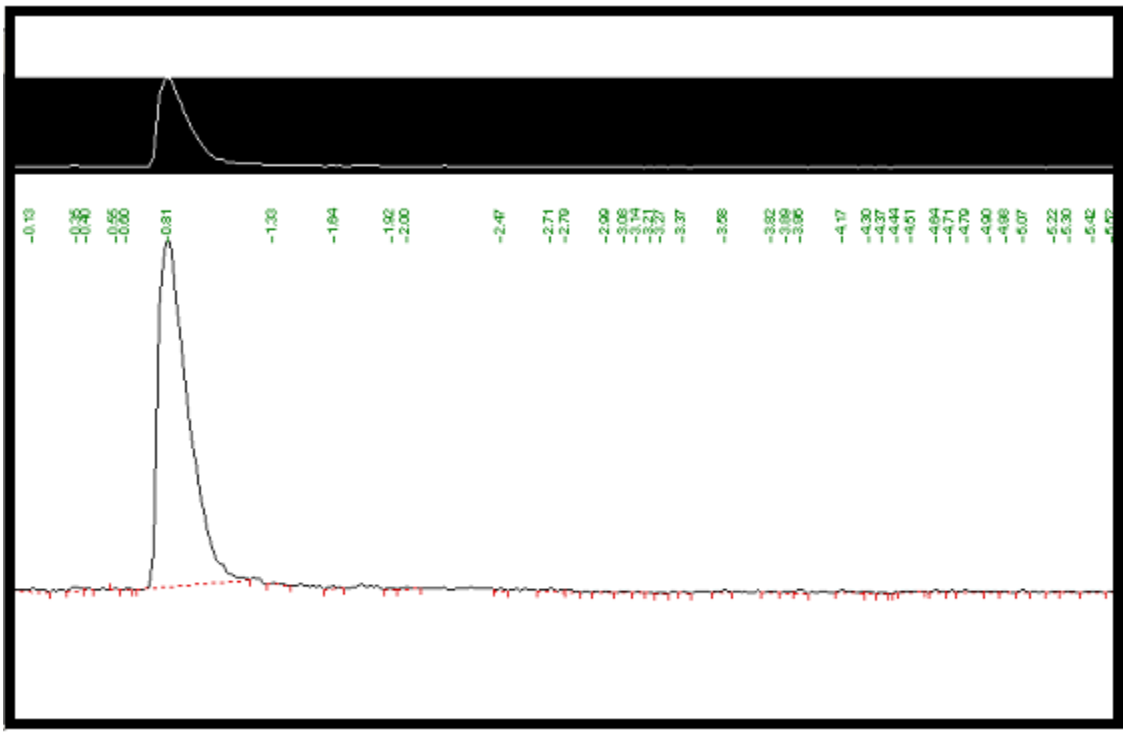
Table 6: Quantitative analysis of different brands of meloxicam by HPLC

Name of brand	Retention time of standard (min)	Area of standard (mV)	Retention time of sample (min)	Area of sample (mV)	Percentage purity= area of sample/area of standard*100
melfax	1.5min	8455317.46	1.48min	8064292.02	95.3%
melor	1.5min	8455317.46	1.44min	8353568.10	98.7%
mits	1.5min	8455317.46	1.48min	8221186.50	97.2%
mocam	1.5min	8455317.46	1.50min	7922236.90	93.6%
Talgesic 15	1.5min	8455317.46	1.50min	7897989.36	93.4%
Xobix	1.5min	8455317.46	1.49min	8307192.61	98.2%

Min= minutes

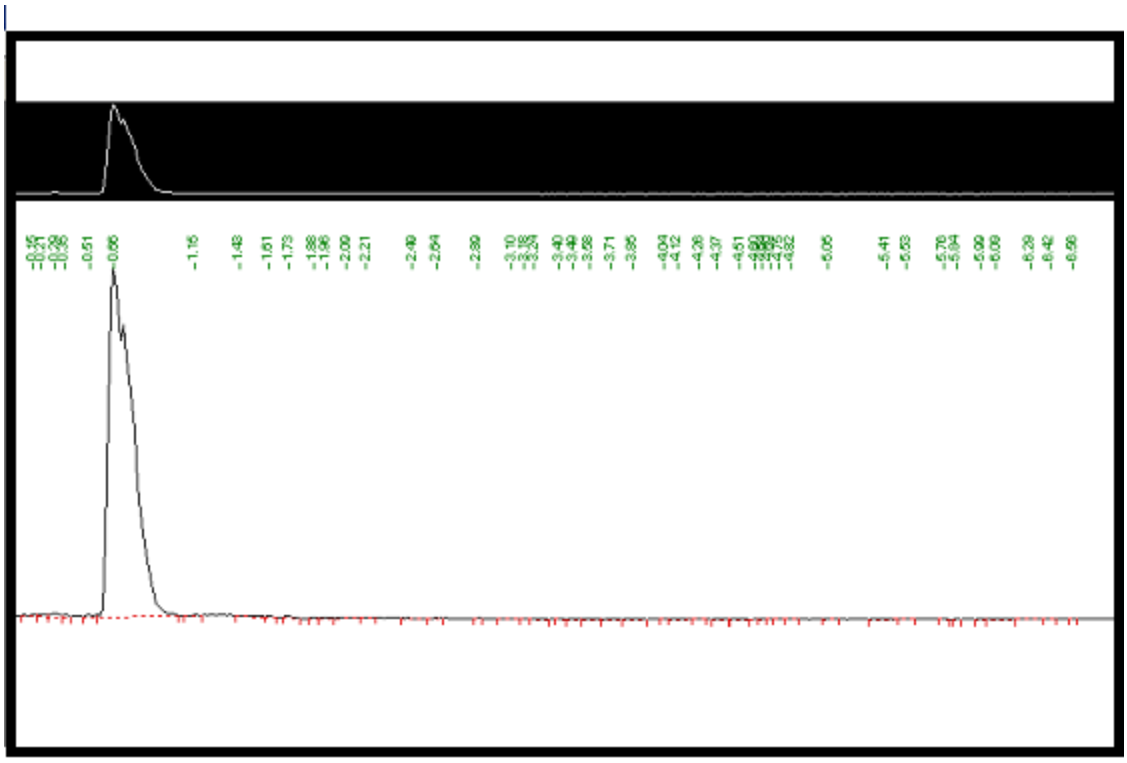
Figure3: Quantitative analysis of different brands of meloxicam by HPLC

CHROMATOGRAM OF MITS:



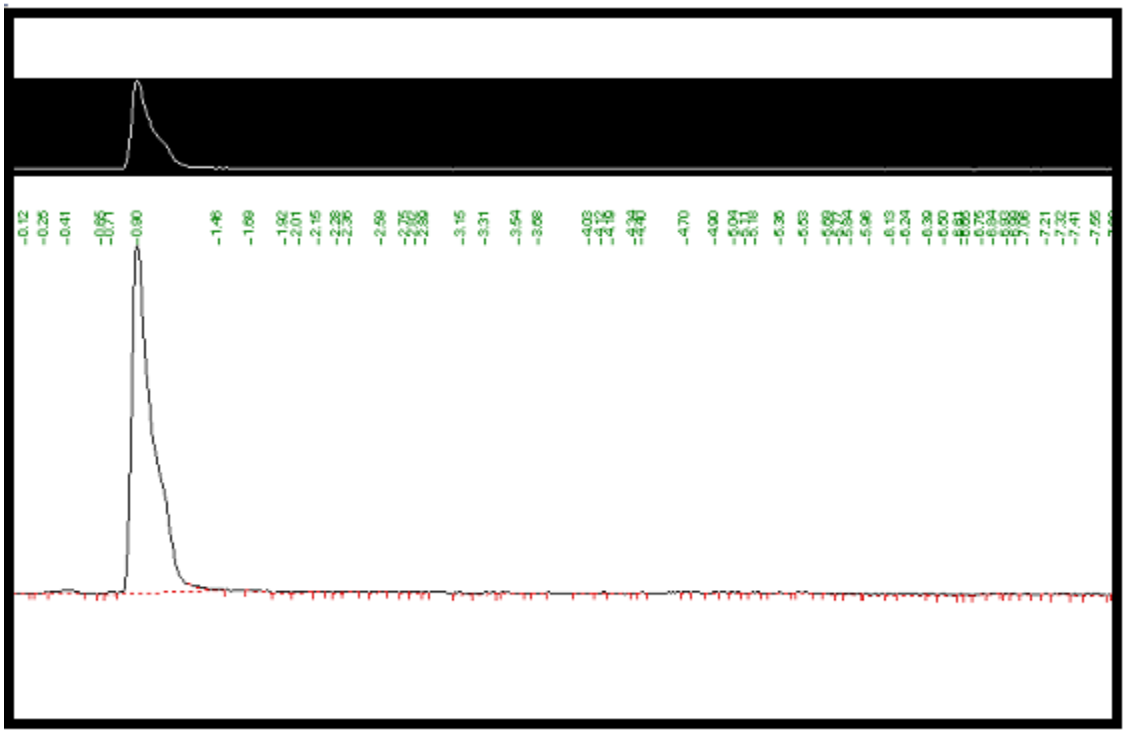
Retention time	*Peak area*	*Height*	*pts*
1.48	*8353568.10*	*711.95*	*117*

CHROMATOGRAM OF MOCAM:



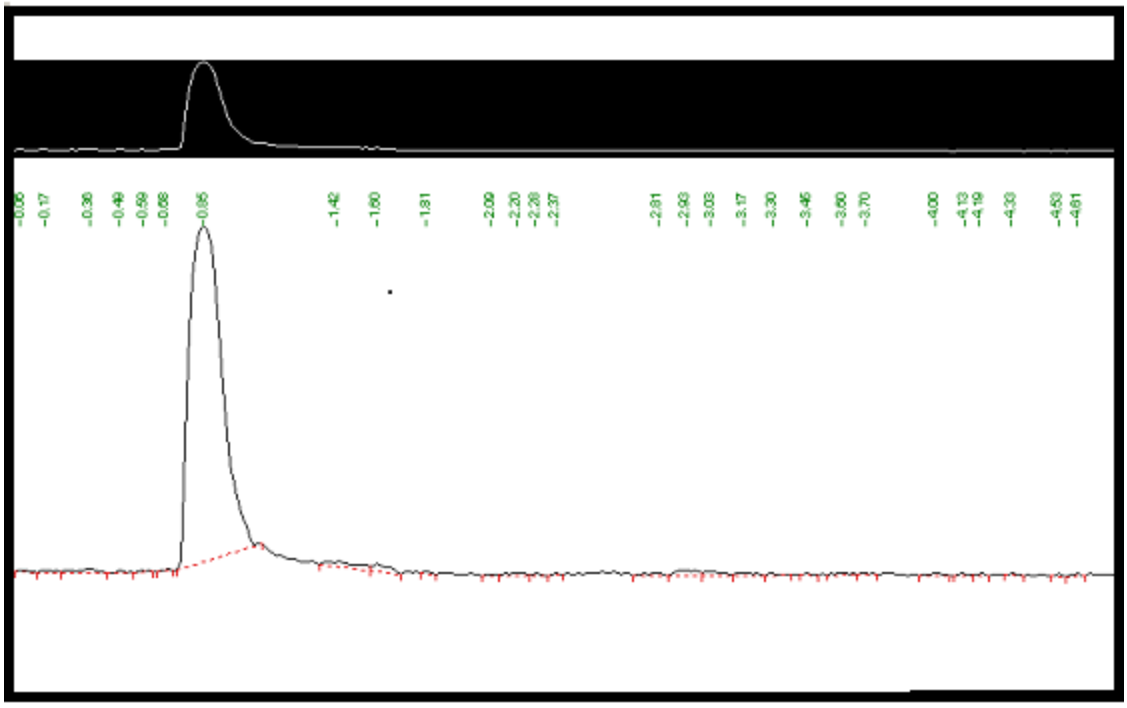
Retention time	*Peak area*	*Height*	*pts*
1.48	*8221186.50*	*970.72*	*127*

CHROMATOGRAM OF TALGESIC 15:



Retention time	*Peak area*	*Height*	*pts*
1.48	*7922236.90*	*907.28*	*121*

CHROMATOGRAM OF XOBIX:



Retention time	*Peak area*	*Height*	*pts*
1.48	*7897989.36*	*971.01*	*125*

LIST OF SYMBOLS

NSAID Nonsteroidal anti-inflammatory drug

HPLC High pressure liquid chromatography

UV ultra violet

LOD limit of detection

LOQ limit of quantification

BP British pharmacopeia

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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