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Comparison of normal and reverse phase HPLC study of meloxicam

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ABSTRACT

In this study, work was done for the development of new precise, accurate and sensitive analytical methods for the evaluation of Meloxicam by reverse and normal phase HPLC. After development of two separate methods they are compared to determine the more authentic method of the two. In reverse phase HPLC, C18 column was used and mobile phase was acetonitrile and methanol. Similarly chloroform was used as mobile phase with silicon column for normal phase HPLC. Separately equal volume of standard solution and sample solutions in HPLC vials were injected in auto sampler compartment of HPLC. Chromatogram peak areas of Meloxicam in standard and sample solutions of different concentrations were recorded and compared. These methods are later validated in different ways. The calibration curve proved to have linearity coefficient of 0.999 for reverse and 0.994 for normal phase HPLC. The precision was equivalent to 0.0003%for reverse and 0.003%for normal phase. The LOD and LOQ were 0.0003ug/ml and 0.001ug/ml respectively for reverse and 0.002 and 0.006ug/ml for normal phase. The system also showed accuracy over the range of 95 to 99% for reverse and 91 to 97% for normal phase. These methods showed accuracy, reproducibility and sensitivity and reverse phase proved to be better than normal phase but both can be used alternatively.

Key words: Meloxicam, acetonitrile, methanol, HPLC.

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INTRODUCTION

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) used to relieve the symptoms of arthritis, dysmenorrhea, fever; and also used as an analgesic and anti-inflammatory component. Chemically Meloxicam is an organic heterocyclic benzothiazine with general formula $C_{14}H_{13}N_3O_4S_2$. It is a pastel yellow solid, practically insoluble in water, with higher solubility observed in strong acids and bases. It also shows good solubility in DMF and DMSO. meloxicam crystallized in four different prototrophic forms; the anion, the acidic enol, the zwitterion and the cation forms which is responsible for the variable solubility character of meloxicam.

This moiety was discovered in 1996. Although a lot of work has been done on it since then but a lot more aspects have yet to be discovered. Meloxicam has been evaluated by chromatography, electrophoresis, polarography, potententiometric methods, calorimetric methods and spectrofluorimetric methods. The newer and more advanced techniques of HPLC and UV spectrophotometry are now used for Meloxicam analysis.

Extensive research of Nageswara et al.[1] showed that UV and HPLC are among the most readily used methods for analysis of drugs. This has been proved by reviewing a number of studies using these techniques for analysis of meloxicam [2,3,4,5,6,7,8,9,10].

In this study HPLC method of meloxicam analysis is analyzed. High performance liquid chromatography is one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid. HPLC can be applied to any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

The main components of HPLC are the stationary phase and the mobile phase. The sample along with mobile phase is allowed to pass through the stationary phase column. The compounds contained in the sample distribute, or partition differently between the moving solvent and the particles of stationary phase. This causes each compound to move at a different speed, thus creating a separation of the compounds. Thus the compounds that were more strongly attracted to the particles slow down, while other compounds more strongly attracted to the solvent move faster. Separation methods are classified into the four modes: adsorption**,** partition**,** ion exchange**,** and size exclusion or hydrophilic interaction. HPLC is of two types, normal and reverse phase HPLC. Polar stationary phase and nonpolar solvent makes up normal phase whereas non polar stationary phase and polar solvent makes up reverse phase HPLC.

Meloxicam shows solubility both in polar as well as nonpolar solvents. In this study the normal and reverse phase method of HPLC are compared to find which of the method is more authentic and accurate for meloxicam analysis.

MATERIAL AND METHOD:

Apparatus

Agilent 1200 HPLC system, Bandelin sonoplus DT2200, Sarstedt 0.45 micro membrane filter Sartorius laboratory L420S analytical balance supplied by Punjab university.

Chemicals

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

HPLC Analysis

Initially, the solubility of Meloxicam in various solvents was studied visually as shown in Table 1.

Table-1: Solubility of meloxicam in different solvents

SOLVENTS	SOLUBILITY
Water	insoluble
Polyethylene glycol	insoluble
0.1 M NaOH	soluble
0.1 M HCl	insoluble
Methanol	soluble
Ethanol	soluble
Chloroform	Freely soluble
Hexane	insoluble
Acetonitrile	Freely soluble
Acetone	Soluble

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 clear peak in UV at 355nm. Thus, it was selected as a suitable solvent system for Meloxicam. This solvent system was polar in nature and hence applied to reverse phase HPLC study of meloxicam.

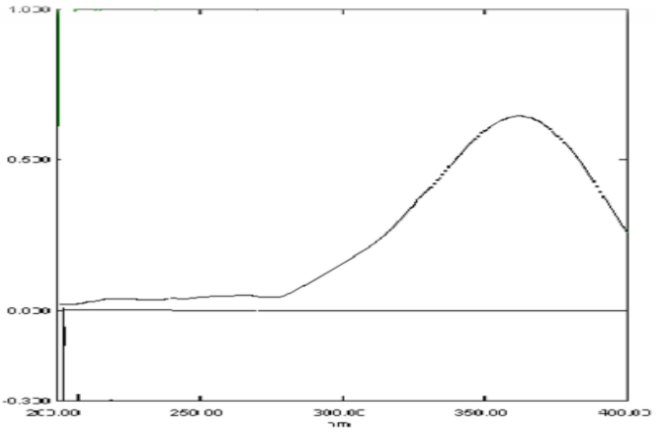


FIGURE 1: Absorption peak of Meloxicam in acetonitrile and methanol mixture in UV at 355nm

Similarly meloxicam was also studied by UV in chloroform and clear peak was obtained at 363nm. This solvent was applied for normal phase HPLC study of Meloxicam because chloroform is nonpolar in nature. These solvents where not previously used.

Preparation of mobile phase

60ml Acetonitrile and 40ml methanol were mixed to form reverse phase mobile phase and 100ml chloroform for normal phase study. These mobile phases were 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 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 micro membrane filter and degassed by ultrasonic bath. The concentration of this solution was found to be 1mg/ml. 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 mobile phase. This solution was filtered in micro filters and degassed. Concentration of this solution was 1mg/ml. Sample solution of different concentration were prepared from this solution and calibration curve was plotted.

PROCEDURE

Reverse phase:

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

Normal phase:

Mobile phase: chloroform (100ml)

Solvent: chloroform (100ml)

Column: silicone 250*50mm

Flow rate: 0.6ml/min

Lambda max: 363nm

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

Method Validation Tests:

Precision:

Precision of a method is the degree of agreement among individual test results, when the procedure is applied repeatedly to multiple samplings. Precision is determined statistically. Standard deviation and percentage relative deviation are the considered as important tools for determination of precision.

The precision and reproducibility were analyzed on both raw material and dosage form with the help of R software - an open source software environment for statistical computing and graphics.

Linearity:

The amount of error change throughout an instrument's measurement range is known as linearity. The linear response of the method was studied by measuring absorbance of different concentrations and plotting different concentrations from 0.1,0.2,0.3,0.4,0.5,0.6 ug/ml verses their absorbance.

Sensitivity:

Sensitivity refers to the response obtained for a given amount of analyte and is often denoted by two analytical factors, the limit of detection (LOD) and the limit of quantification (LOQ).The lowest concentration of the analyte that can be detected is called as limit of detection (LOD) and has been calculated by using the formula, 3.3*SD/slope, where SD shows standard deviation. Limit of quantification (LOQ) is determination of the lowest concentration of the analyte with

good precision & accuracy in the sample and has been determined by using the formula, 10*SD/slope, where SD shows standard deviation.

Accuracy:

Accuracy of a method is the degree of agreement between an individual test result generated by the method and the true value.

By applying this tool, we determined the wavelength and its best accuracy of dosage form while comparing with standards. Calibration curves were constructed at all the wavelengths to check the accuracy of the spectrum, percentage purity was determined by taking ratio of sample absorption to standard absorption and multiplying the result by 100, and results were calculated.

RESULTS AND DISCUSSION

HPLC is among the most prevailing and latest method of evaluation. This method has been used for the analysis of Meloxicam in this study. The official method is potentiometric titration, which is a slow, tedious and lengthy method involving the formation of buffer and management of pH . HPLC has been selected because it is more accurate, latest and short method involving few solvents and no pH maintenance is required. The authenticity of this method is validated by studying following parameters.

Precision:

This experimental work revealed that standard deviation and relative standard deviation of meloxicam raw material were 2.8 and 0.000033 %, respectively. Similarly the standard deviation and relative standard deviation of meloxicam tablet was found to be 2.67 and 0.000035 %, respectively for reverse phase HPLC. These statistical results show a good degree of agreement among test results and hence proved to be an applicable method in terms of repeatability and reproduction of results.

Similarly for normal phase HPLC standard deviation and relative standard deviation of meloxicam raw material were 25.5 and 0.00030%, respectively. Similarly the standard deviation and relative standard deviation of meloxicam tablet was found to be 24.1 and 0.00029 %, respectively for reverse phase HPLC. These statistical results show a moderate degree of agreement among test results and hence proved to be an applicable method in terms of repeatability and reproduction of results.

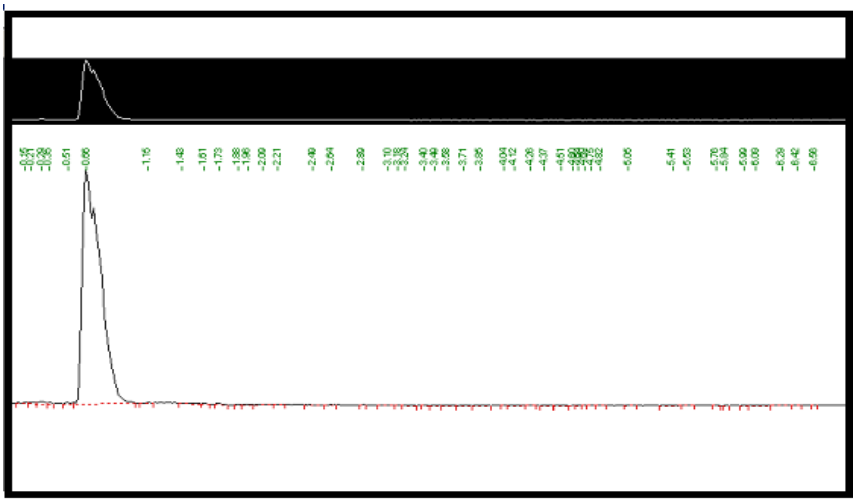
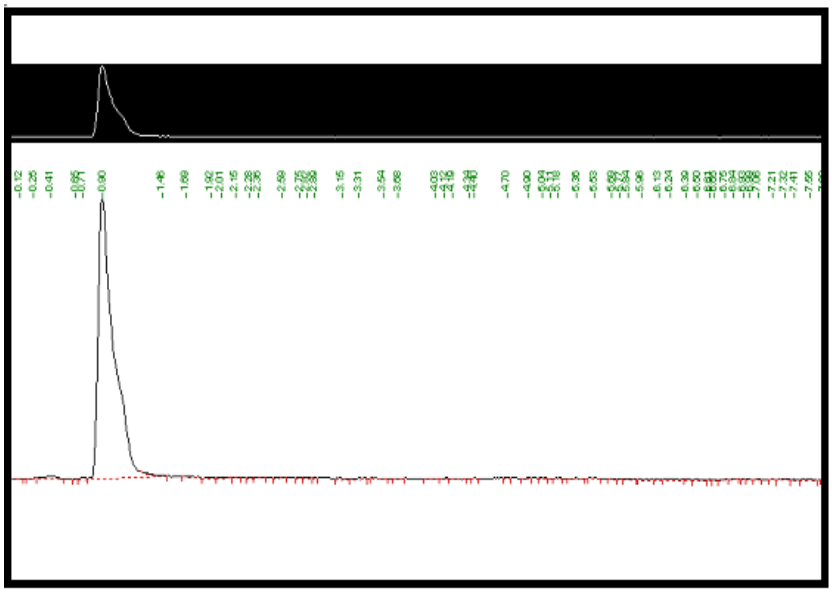


Figure 2. Chromatogram of meloxicam raw material(acetonitrile:methanol)in reverse phase HPLC.



Fifure 3. Chromatogram of meloxicam talgesic15 in (acetonitrial:methanol) by reverse phase HPLC.

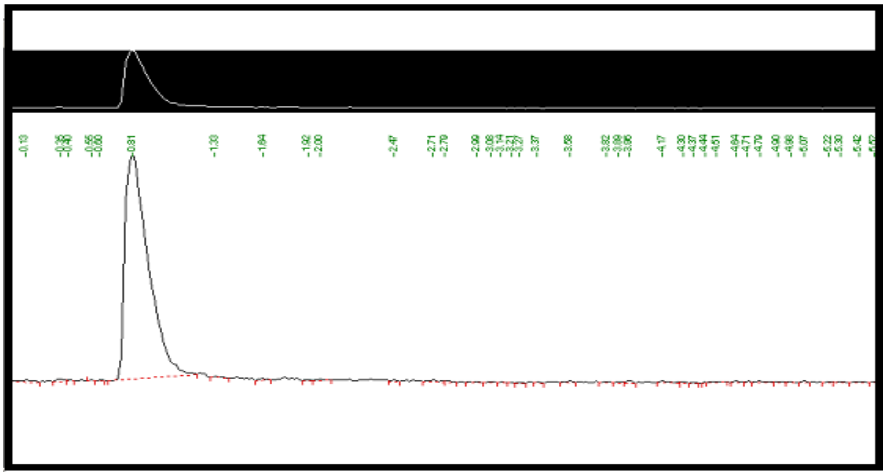


Figure 4. Chromatogram of meloxicam raw material in chloroform by normal phase HPLC.

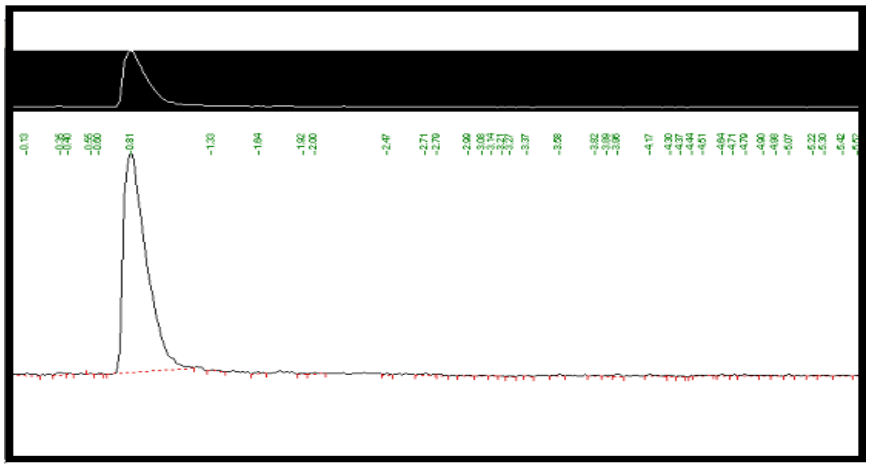


Figure 5. Chromatogram of meloxicam Talgesic15 in chloroform by normal phase HPLC.

Linearity:

A good determination coefficient of 0.999 was obtained and calibration equation was found to be y+27986x+92440 for raw material and y=30227x+41454 for dosage form. Value of coefficient of determination (R^2) is clearly showing that the regression line in our research perfectly fits the data.

Similarly in normal phase HPLC a moderate determination coefficient of 0.9947 was obtained and calibration equation was found to be y = 38357x - 31006 for raw material and y = 38093x – 32306 for dosage form. the coefficient of determination shows that regression line does not fits the data completely.

Sensitivity

The lowest concentration of analyte that can be detected by this method was found to be 0.000344ug/ml for raw material and 0.00031 ug/ml for tablet dosage form. This minute quantity shows that the system is capable of analyzing even very small amount of meloxicam in the solution. Similarly, LOQ of this method was calculated to be 0.001 ug/ml for both meloxicam raw material and dosage form.

For normal phase LOD of raw material and dosage form was found to be 0.002ug/ml and LOQ for both raw and sample material was found to be 0.006ug/ml. this amount shows that normal phase analysis can be used to analyze small quantity also.

Accuracy:

Accuracy was found to be within the range of 99.5 to 100 % for reverse phase and 91 to 97for normal phase HPLC.

F TEST:

From raw material analysis of HPLC normal and reverse phase the F=0.0012, the result shows

that the two tailed probability shows two set of values are not quite different. Similarly from dosage form analysis of HPLC normal and reverse phase values F=0.000309, the two tailed probability that the variances obtained from normal and reverse phase are not significantly different.

Table-2:comparison of Different validation parameters and their results

Validation Parameter	Validation Result	
	Reverse phase HPLC	Normal phase HPLC
Standard deviation and Relative standard deviation of meloxicam raw material	2.8 and 0.000033 %, respectively	25.5 and 0.0003%
Standard deviation and Relative standard deviation of meloxicam tablet	2.67 and 0.000035 %, respectively	24 and 0.00029%
Determination coefficient	0.999	0.994
Lowest concentration of analyte for raw material that can be detected by this method	0.000344 ug/ml	0.002 ug/ml
Lowest concentration of analyte for tablet dosage form that can be detected by this method	0.00035 ug/ml	0.002 ug/ml
LOQ for meloxicam, raw material, and dosage form	0.001 ug/ml	0.006 ug/ml
Accuracy of method	99.5 % - 100 %	91 % - 97 %

In this research firstly two new solvent systems were developed for both normal and reverse phase HPLC study of meloxicam. Meloxicam exists in four different forms in different solvents such as polar solvents eg methanol, nonpolar solvents such as chloroform as well as acids and basis. Thus it has the ability to show solubility in different solvents which has been studied visually table 1. In precision study it has been clearly revealed that reverse phase is much more precise and accurate, having the ability to generate closely resembling test results on repeated sampling. In comparison to this normal phase also has been proved to be precise yet not as close resemblance as in reverse phase.

In studying the linear response of the two methods it was revealed that reverse phase gave a very good differentiation coefficient of 0.999 as compared to normal phase of 0.994. Similarly the sensitivity of response for reverse phase in terms of LOD and LOQ normal phase also proved to be smaller than that of normal phase. The accuracy range for normal phase was calculated to be smaller than reverse phase.

Thus it was concluded that the reverse phase was much more authentic as compared to normal phase. Although chloroform shows good solubility, the main reason of drawback this is that chloroform is a highly volatile and unstable as compared to methanol and acetonitrile.

Chloroform is also affected by heat and light hence solution composition varies on keeping solution for some time for inter and intraday effect and sensitivity study of meloxicam.

By f test study it is also revealed that although the two methods varies in terms of sensitivity, accuracy, precision and linearity yet its reading values shows close resemblance and are not significantly different. Hence reverse phase being superior in terms of validation characteristics yet it can be replaced with normal phase study of Meloxicam in case of unavailability of reverse phase setup.

CONCLUSION

This study not only provides us with the comparison of two types of HPLC methods that is normal and reverse phase HPLC, but it gives authenticity of HPLC study of Meloxicam. It shows that HPLC is also a fast method for Meloxicam study conducted in just 15minutes. The simple solvents selected for the study shows that we can perform rapid study by selecting solvents that does not require buffer formulation and pH maintenance. Hence it is concluded that the stated two methods can be used for Meloxicam analysis but reverse phase method is superior.

list of Abbreviation：

NSAID Nonsteroidal anti-inflammatory drug, HPLC High pressure liquid chromatography, UV ultra violet, LOD limit of detection, LOQ limit of quantification, BP British pharmacopeia

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