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Development and Validation of RP-HPLC method for the Simultaneous estimation of Meloxicam and Paracetamol

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Abstract:
A novel, rapid, sensitive, specific RP-HPLC method was developed and validated for the simultaneous estimation of meloxicam and paracetamol in bulk and its dosage form. Efficient chromatographic separation was achieved on a Hypersil ODS C18 (150mm * 4.6mm, 5.0µm) column containing UV-Visible detector with methanol and water as mobile phase in the ratio of 70:30 at a flow rate of 0.6ml/min and the eluent was monitored at 240nm. Retention times were found to be 3.5min and 5.0min for meloxicam and paracetamol respectively. Results of the analysis were validated statistically and by recovery studies. The developed method has shown to be linear (r^2 = 0.939 for Mlx and 0.996 for Pct), precise (R.S.D < 2), accurate (recovery of 99.26% for Mlx and 94.96% for Pct) and specific with limit of detection (0.102 for Pct and 0.591 for Mlx) and limit of quantitation (0.310 for Pct and 1.79 for Mlx) as per ICH guidelines.

Keywords: Meloxicam (Mlx), Paracetamol (Pct), RP-HPLC, Validation.

Introduction

Meloxicam is chemically 4-hydroxy, 2-methyl-N(5-methyl,1,3-thiazol-2-yl),2H-1,2-Benzothiazin,3-Carboxamide, 1,1-dioxide[1] . It is used to relieve pain, tenderness, swelling and stiffness caused by ***Osteoarthritis*** (arthritis caused by a breakdown of lining of joints); ***Rheumatoid arthritis*** (arthritis caused by swelling of lining of joints); ***Juvenile rheumatoid arthritis*** (a type of arthritis that affects children); ***Ankylosing spondylitis*** (arthritis that mainly affects spine) [2]. The mechanism of action of meloxicam, like that of other NSAIDs, may be related to prostaglandin synthetase (cyclo-oxygenase) inhibition which is involved in the initial steps of the arachidonic acid cascade, resulting in the reduced formation of Prostaglandins, thromboxanes and prostacylin[3].

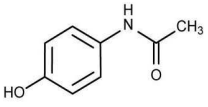


Fig-1: Structure of paracetamol

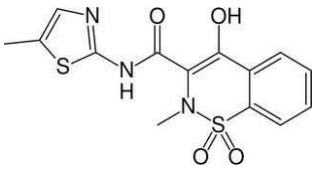


Fig-2: Structure of meloxicam

Paracetamol is chemically N-(4-hydroxy phenyl) acetamide [4]. It is used as an analgesic and anti-pyretic [5]. The mechanism of action of paracetamol is related to the selective inhibition of COX-2 so that it cannot suppress the inflammation of rheumatoid arthritis. The combination of pct and Mlx works effectively in relieving pain and inflammation related to arthritis. Literature survey revealed that few HPLC [7-9], spectrophotometric [10, 11] and LC-MS-MS [12] methods have been reported for the determination of paracetamol in combination with other drugs. Several analytical methods are also reported for the determination of meloxicam by flourimetry [13], capillary electrophoresis [14],

HPLC [15, 16], LC/MS [17] and spectrophotometry [18].Only one RP-HPLC [19] method has been reported for the simultaneous estimation of meloxicam and paracetamol in bulk and its dosage forms. The aim of the present work was to develop and validate a rapid, specific, sensitive RP-HPLC method for the simultaneous estimation of meloxicam and paracetamol.

Experimental:

Chemicals and materials-

Meloxicam was obtained as a gift sample. Paracetamol was purchased from GSC (G.S.chemical testing lab, Mumbai). Tablets containing 7.5mg of mlx and 325mg of Pct (Melodol®- mfg by: Aristo pharmaceuticals Pvt. Ltd) were purchased from local pharmacies. Methanol and water (HPLC grade) were purchased from MERCK laboratories, New Delhi.

Instrumental and Analytical conditions:

The HPLC analysis was carried out on shimadzu system (LC solutions software) with UV detector, Hypersil ODS C18 (150mm * 4.6mm, 5.0µm) column. UV detection was performed at 240 nm. The injection volume of sample was 20 µL. An isocratic mobile phase containing methanol and water (70:30) was pumped with a flow rate of 0.6ml/min.

Mobile phase preparation:

Prior to use, the mobile phase (70 parts of methanol and 30 parts of water) was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.2µ high vacuum filter.

Preparation of standard solutions:

About 100mg of Mlx and Pct were accurately weighed and transferred to separate 100ml volumetric flasks, dissolved and volume was adjusted with mobile phase. The solutions were filtered through 0.2µ membrane filter. Further dilutions were made to get the concentration of 100µg/ml and from that 10-50µg/ml solutions were prepared.

Preparation of sample:

Tablet powder equivalent to 100mg of Pct was accurately weighed, dissolved in mobile phase, sonicated for 20min and the volume was adjusted by using mobile phase. This solution was filtered through 0.2µ filter. Further dilutions were made until the concentration within the standard range is obtained.

Injection of solutions:

20µl volumes of standard and sample solutions were injected into the chromatographic system, under optimized chromatographic conditions. The peak areas were measured at 240nm and concentration in samples were determined by interpolation from calibration plots of each drug previously obtained.

Validation [20, 21]:

Linearity:

Standard solutions containing 1mg/ml of Mlx and Pct were prepared individually. Aliquots of these solutions were diluted in mobile phase to five different concentrations (10-50µg/ml) of both the drugs. The calibration curves for concentration Vs peak area were plotted for both the drugs; obtained data were subjected to regression analysis using the least squares method with a weighing factor of 1/x.

Precision:

The precision is the degree to which repeated measurements under unchanged conditions show the same results. It was evaluated by analyzing six sample solutions at the concentration (30µg/ml) of both the drugs. Similarly, the inter day precision was evaluated. The concentrations of Mlx and Pct were determined, relative standard deviation (RSD) was calculated.

Accuracy:

Accuracy is the degree of closeness of measurement of a quantity to the quantity's true value. Accuracy of the method was demonstrated at three different concentration levels (20-40µg/ml) by spiking a known quantity of standard drugs of Mlx and Pct three times each; the analysis being done in replicate.

Specificity:

Specificity demonstrates that the response due to analyte of interest in the sample is not affected by potential differences which may also be present in sample as well as due to solvents used. The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample).

Detection and quantitation limits:

Limit of detection LOD (signal-to- noise ratio of 3) and limit of quantification LOQ (signal-to- noise ratio of 10) were measured based on the signal-to noise ratio. Signal-to-noise is determined by comparing measured signals samples with known low concentration of analyte with those of blank samples establishing the minimum concentration at which the analyte can be reliably detected and quantified.

Results and discussion:

The chromatographic parameters were evaluated using a Hypersil ODS C18 (150mm * 4.6mm, 5.0µm) column and a mobile phase composed of methanol and water (70:30). After the evaluation of Mlx and Pct UV spectrum in the range of 200-400 nm, the wavelength of 240 nm was selected for detection.

Validation:

System suitability parameters:

To ensure the validity of the system and analytical method, system suitability test was performed. (RSD) Relative standard deviations of Mlx and Pct from the five consecutive injections of the standard solutions were 0.42 and 0.47 respectively. The tailing factor for Mlx and Pct peaks were 0.804 and 1.690 respectively, thus reflecting good peak symmetry. The resolution (Rs) between Pct and Mlx was 4.347 indicating good separation of both analytes from each other.

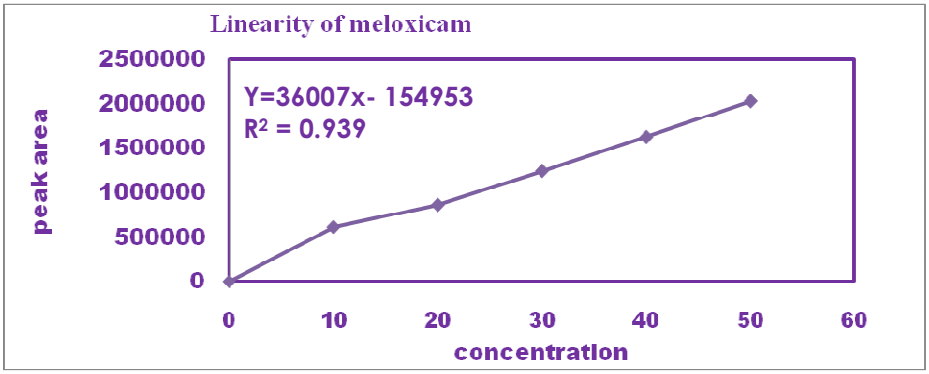
Specificity:

There was no interference of impurities and also no change in the retention times; therefore the method was found to be specific and also confirmed with the results of analysis of formulation.

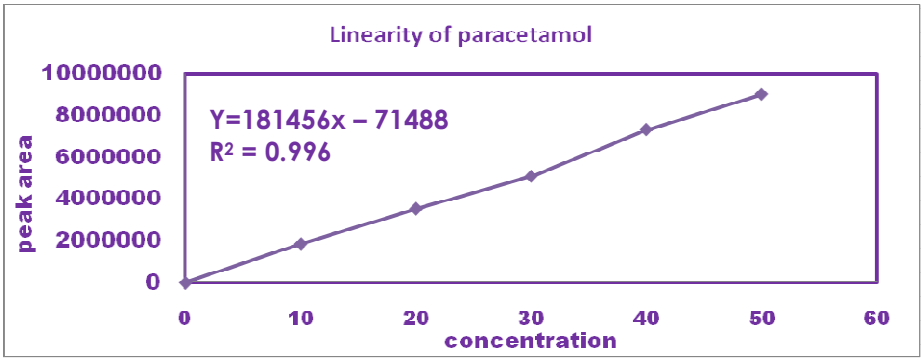
Linearity:

A linear correlation was found between the peak areas and the concentrations of Mlx and Pct in the assayed range. The regression coefficients (r2) obtained were 0.939 and 0.996 for Mlx and Pct respectively (graph 1&2), which indicates the linearity of the method.

Graph-1: Linearity of Meloxicam



Graph-2: Linearity of Paracetamol



Accuracy:

Accuracy was confirmed by studying the mean recovery at three different concentrations and the results (99.26% for Mlx and 94.96% for Pct) in all cases were within the acceptable limits as tabulated.

Drug	Theoretical content	% recovery	Mean recovery
Mlx	20	104.5%	102.5%
	20	102%	
	20	101%	
Mlx	30	98%	97.7%
	30	95%	
	30	100.2%	
Mlx	40	95.25%	97.58%
	40	96.25%	
	40	101.25%	

Drug	Theoretical content	% recovery	Mean recovery
Pct	20	97%	96.4%
	20	97.35%	
	20	95.10%	
Pct	30	94.33%	94.29%
	30	93.4%	
	30	95.11%	
Pct	40	89.98%	94.19%
	40	97%	
	40	95.6%	

Table1: Accuracy (% recovery) of meloxicam and paracetamol

Precision:

Interday and Intraday precision was performed. The RSD values for inter day precision for Mlx and Pct were 0.029 and 0.031 respectively and for intraday were 0.023 and 0.045 respectively which was less than 2.

Detection and quantitaion limits:

According to the determined signal -to –noise ratio, Meloxicam and Paracetamol presented limits of detection (0.102 for Pct and 0.591 for Mlx) and limit of quantitation (0.310 for Pct and 1.79 for Mlx).

Fig 3: chromatogram of blank showing specificity

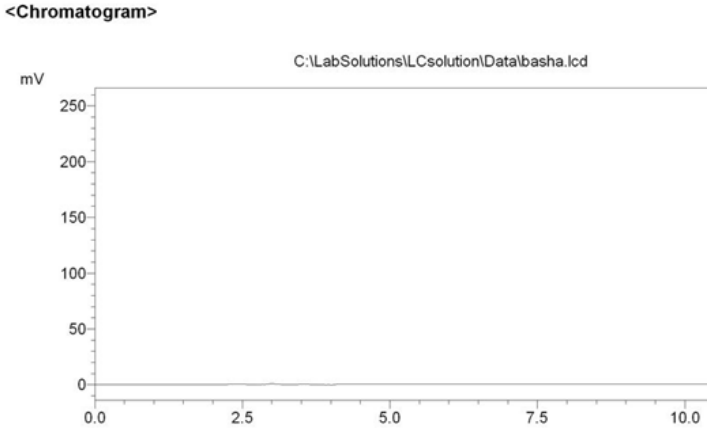
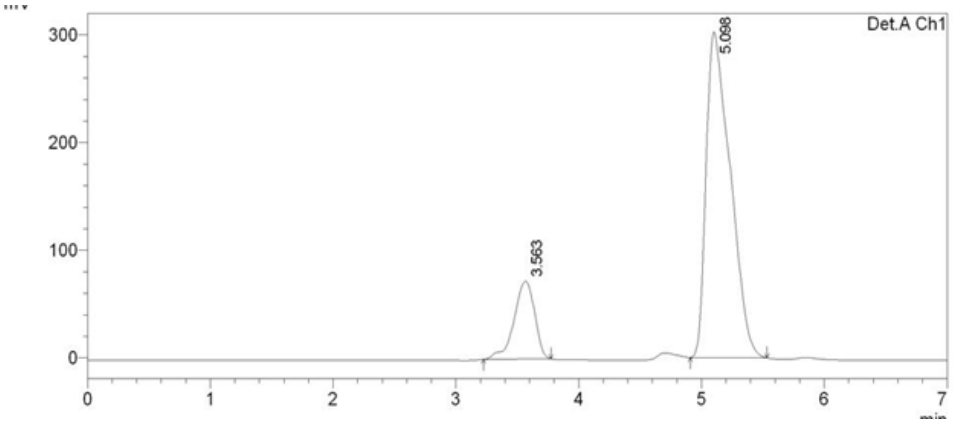


Fig 4: Typical chromatogram of meloxicam and paracetamol



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Conclusion:

The validated HPLC method was found to be rapid, precise and accurate and can be readily utilized for analysis of Meloxicam and Paracetamol in bulk and in pharmaceutical formulations.

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