METHOD DEVELOPMENT AND COMPARATIVE STATISTICAL EVALUATION OF HPLC & HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL (PCM)) AND MELOXICAM (MLX).

Ankita Jain[1*], Tamanna Narsinghani[2], Rajesh Sharma[3]

Abstract

Rapid, precise, accurate, specific, and sensitive high performance liquid chromatographic method and high performance thin layer chromatographic methods have been developed for simultaneous determination of paracetamol and meloxicam in their tablet formulation. The HPLC method was standardised using Phenomenex Luna reversed-phase C18 analytical column (25cm X 4.6mm, 5μm) with mobile phase constituted of Acetonitrile: Buffer, pH adjusted to 7 using orthophosphoric acid delivered at the flow rate of 1.0 ml min-1 and detection was performed at 300nm. For HPTLC analysis separation was carried out on precoated TLC plates, coated with silica gel 60F-254 and using mobile phase dichloromethane: isopropanol:glacial acetic acid (10.5:1.5:0.1 v/v/v). Scanned at 300nm with CAMAG TLC scanner controlled by Cats Software. Different analytical performance parameters such as linearity, accuracy, precision, repeatability, robustness LOD and LOQ were determined according to International conference of Harmonization ICH Q2B guidelines. As a result HPLC method was found to be more precise and robust whereas HPTLC method was found to be more sensitive. As number of sample per analysis, different samples per shift, mobile phase cost, system cleanup cost, method development and speed of analysis is far much less in HPTLC as compared to HPLC.

Keywords: HPLC, HPTLC, Paracetamol, Meloxicam, Linearity, Accuracy.

[1*,2,3]School of Pharmacy, Takshashila campus, Eastern Ring Rd, Devi Ahilya VishwaVidyalaya, Indore (M.P)

*Corresponding author: Ankita Jain

*School of Pharmacy, Takshashila campus, Eastern Ring Rd, Devi Ahilya VishwaVidyalaya, Indore (M.P)
Email: Aajainusa@gmail.com

Introduction

Method development and comparative statistical evaluation of HPLC and HPTLC methods for the simultaneous estimation of Paracetamol (PCM) and Meloxicam (MLX) represent a critical aspect of pharmaceutical analysis. The process begins with method development, which entails the systematic design and optimization of analytical techniques to accurately quantify the concentrations of both drugs within a given sample.

In method development, various parameters are considered, including the selection of appropriate solvent systems, stationary phases, mobile phase compositions, and detection methods for both HPLC and HPTLC. Each parameter must be carefully optimized to ensure optimal separation, resolution, and sensitivity for PCM and MLX. Additionally, factors such as sample preparation techniques and instrument parameters are also optimized to enhance the performance of the methods.

Following method development, a comparative statistical evaluation is conducted to assess the performance of the developed HPLC and HPTLC methods. This evaluation involves the systematic comparison of various analytical parameters, including linearity, accuracy, precision, sensitivity, and specificity. Statistical tests such as correlation coefficient, regression analysis, t-tests, and ANOVA may be employed to compare the results obtained from both methods.

The simultaneous estimation of PCM and MLX is of particular importance in pharmaceutical analysis, as many formulations contain multiple active ingredients. Therefore, the developed methods should be capable of accurately quantifying the concentrations of both drugs within complex matrices.

High-Performance Liquid Chromatography (HPLC) is a widely used technique for pharmaceutical analysis due to its high sensitivity, selectivity, and reproducibility. Conversely, High-Performance Thin-Layer Chromatography (HPTLC) offers advantages such as rapid analysis, low cost, and minimal sample preparation requirements.

By conducting a comparative evaluation of HPLC and HPTLC methods for the simultaneous estimation of PCM and MLX, researchers can determine the most suitable technique based on factors such as analytical performance, cost-effectiveness, and practicality. This comprehensive approach ensures the development of robust analytical methods that meet the stringent requirements of pharmaceutical analysis. HPLC and HPTLC methods are simple, sensitive, reproducible and rapid. They were designed to be suitable for the quality assessment of these compounds in their pharmaceutical preparation. It is also specific, linear, accurate and rugged method. To provide a quality control procedure for PCM and MLX in their pharmaceutical preparation relaying on a compelling quantitative approach, alternative and competitive with HPLC, in terms of rapidity of execution, high throughput and routine amenability, HPTLC-densitometry procedure was also adopted. The present work aims to develop and validate RP-HPLC and HPTLC methods for the simultaneous determination of PCM and MLX in tablet dosage form and statistically compare the two developed methods.

Material and Methods
HPLC Method

Mobile phase consisting of Ammonium Acetate Buffer (pH-7; 0.5M) - Acetonitirile (20:80v/v), pH 7 adjusted with orthophosphoric acid offered a good separation at a flow rate of 1.0 ml/min and a runtime of 10 min. PCM elutes first and then MLX gets eluted as shown in the chromatogram (Fig. 1), which illustrate the separation of both active ingredients in this system. The detection wavelength of 300 nm was chosen in order to achieve a good sensitivity for quantitative determination of PCM and MLX in tablet dosage form. The isocratic program throughout HPLC method was adopted to analyze both components in a single run.

HPTLC Method

It is well-established fact that chromatographic techniques are more specific than other analytical methods. The R_f value of PCM was found to be in the range of 0.35-0.37 and that of MLX is 0.76-0.78. Spectrum of all tracks was recorded between 200-400nm wavelengths using deuterium lamp. A typical spectrum of PCM and MLX are depicted in Calibration curve was constructed by plotting area of respective drug against concentration in ng/ml. A linear relationship was observed for PCM and MLX in concentration range of 400-900 ng/ml and 300-700 µg/ml respectively. Mobile phase consisting of Dichloromethane: isopropanol: glacial acetic acid (10.5:1.5:0.1 v/v/v), offered a good separation at a 20 ml of mobile phase at runtime of 10 min.

Results and Discussion
HPLC method

The plot of peak area response against concentration is linear over the concentration range of 520-790μg/ml and2-20 g/ml for PCM and MLX respectively. The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. Intraday (n=3) and interday (n=5) precision was carried out to and % RSD was found <2 -3 ensuring repeatability of procedure (Table 1).

Table 1: Intra- and inter-day precision of PCM (a) and MLX (b)

HPLC				HPTLC			
Intra-day precision		**Inter-day precision**		**Intra-day precision**		**Inter-day precision**	
S.D of areas	%R.S.D	S.D of areas	%R.S.D	S.D of areas	%R.S.D	S.D of areas	%R.S.D
PCM(n=4)				PCM(n=4)			
0.0231	0.0381	0.0134	0.0730	8.51	0.59	98.96	3.088
MLX(n=5)				MLX(n=5)			
0.0155	0.1206	0.0165	0.0914	34.29	2.431	96.84	3.007

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels (80%, 100% and 120%). The results of recovery studies were presented in Table 2. Robustness of the method was determined by small deliberate changes in, mobile phase ratio and pH. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in Table 3. During assay study, there was no change in the content of drug due to presence excipient, which reveals that the method is specific.

Table 2: Standard addition technique for determination of PCM (a) and MLX (b) by TLC densitometry and HPLC (*n*=3)

HPLC				HPTLC			
PCM							
Excess drug added to the analyte (%)	Theoretical content (μl)	Recovery (%)	R.S.D %	Excess drug added to the analyte (%)	Theoretical content (μl)	Recovery (%)	R.S.D %
80	520	100.00	0.0083	80	4.8	93.4	0.65
100	585	100.01	0.0065	100	6	92.6	0.41
120	650	99.95	0.0031	120	7.2	90.4	0.40
MLX							
Excess drug added to the analyte (%)	Theoretical content (μl)	Recovery (%)	R.S.D %	Excess drug added to the analyte (%)	Theoretical content (μl)	Recovery (%)	R.S.D %
80	12.0047	100.0392	0.191	80	3.2	87.1	0.73
100	13.5055	100.0407	0.767	100	4	97.5	1.3
120	15.0129	100.0862	0.763	120	4.8	91.4	0.82

Table no 3: Robustness of developed HPLC and HPTLC Methods

HPLC(%RSD)(n=3)					HPTLC(%RSD)(n=3)	
DRUGS	**pH (7)**		**Ratio of mobile phase (20:80)**		**Time (spotting to (development)**	**Time (development to scanning)**
	+0.1	-0.1	+1%	-1%	+30 min	+30 min
PCM	1	0.9	0.75	0.9	0.43	0.36
MLX	1.08	0.8	0.71	0.8	0.51	0.46

n =No of times analysis repeated

HPTLC method

The low value of standard deviation showed that the method was precise (**Table 22**). Intraday (n=3) and interday (n=3) precision was carried out and % RSD was found <2, ensuring repeatability of procedure (Table 1). The result of recovery analysis for PCM and MLX are tabulated in Table 2. From the result it is revealed that there is good correlation between amount of standard added and amount of drug found at all concentration level.
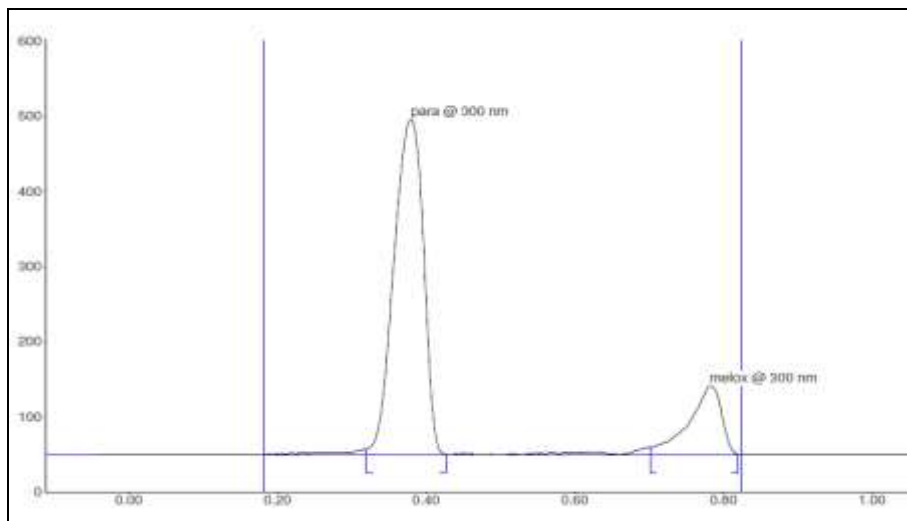
Fig 1: Resolution Study for Paracetamol and Meloxicam

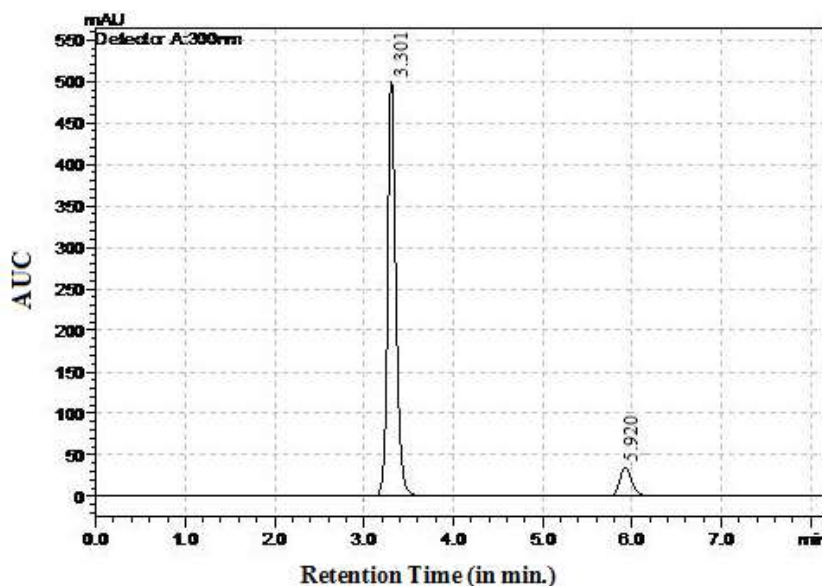**[R$_f$ value of Paracetamol: 0.35-0.37min. and that of Meloxicam: 0.76-0.78 min.]**

Fig 2: Peaks for Paracetamol and Meloxicam

(R$_t$ value of Paracetamol: 3.301 and that of Meloxicam: 5.920)

Table no 4: Result of Parameters used for validation of PCM and MLX

Parameters	HPLC		HPTLC	
S.No	PCM	MLX	PCM	MLX
Linearity (r^2)	0.9993	0.9992	0.9992	0.9991
% RSD (n=6) (Indicates Precision)	<2%	<2%	<2%	<2%
Mean % Recovery	99.95	99.5	97.5	93.4
Limit of Detection	0.299 µg/ml	0.019µ g/ml	3.19 ng/ml	2.035 ng/ml
Limit of Quantitation	0.908µg/ml	0.061µg/ml	6.980 ng/ml	6.175 ng/ml
Range	520-800	2-20	400-800	200-800
Assay(n=5)	99.9998	100.0773	99.75	99.67
Robustness study	Robust	Robust	Robust	Robust
Specificity Study	Specific	Specific	Specific	Specific

CONCLUTION:

A thin-layer chromatography–UV scanning densitometric technique was used for the simultaneous determination of Paracetamol and Meloxicam. A favourable advantage of TLC–UV densitometry over HPLC is its ability to separate

the contents of the analyzed samples, thus eliminating the possibility of interference between active ingredients or due to additives, excipients or impurities. In addition, the method is amenable to the simultaneous analysis of six samples on the same TLC plate with precision and accuracy comparable with alternate chromatographic. Other advantages of the TLC–UV method are its fast scanning speed, its low limit of detection and its broad linear ranges and recovery. On the other hand, HPLC methods are simple, rapid and sensitive and therefore suitable for the routine analysis of PCM and MLX presents in multidrug pharmaceutical preparations. HPLC is found to be more accurate, precise, specific and robust as compare to HPTLC. In case of HPTLC determination of medicaments in formulation is not so complicated, time-consuming pre-treatment and a complicated elution system for separating of medicaments are not required.

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