Research Article

Validated HPTLC Method for Simultaneous Estimation of Meloxicam and Paracetamol in their Formulation

Savita Yadav[1]*, Vividha Dhapte[2]

1. Department of Pharmaceutical Chemistry, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune-411 038, India.
2. Department of Pharmaceutics, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Erandwane, Pune-411 038, India.

***Corresponding author's E-mail:** savitang2729@yahoo.com

Received: 12-06-2019; **Revised:** 20-07-2019; **Accepted:** 28-07-2019.

ABSTRACT

An accurate, simple, rapid and sensitive high performance thin layer chromatographic (HPTLC) method for the determination of meloxicam and paracetamol was developed and validated in the bulk drug and tablet dosage form. Aluminum foil precoated Silica gel G 60F254 plates were used as stationary phase and toluene: ethyl acetate: methanol: formic acid 8: 2: 0.5: 0.5 (v/v/v/v) as mobile phase. Wavelength selected for analysis was 287 nm. The two drugs were satisfactorily resolved with Rf 0.19 ± 0.02 for Paracetamol and Rf 0.54 ± 0.05 for Meloxicam. The method was validated according to ICH guidelines. Linearity was found to be in the concentration range of 75–450 ng/band and 325-1950 ng/band for Meloxicam and Paracetamol, respectively. The mean recovery of Meloxicam and Paracetamol was found to be 99.22 and 99.72, respectively. Developed HPTLC method was found to be accurate, precise, selective and rapid for simultaneous estimation of meloxicam and paracetamol. Thus, it can be used for routine quality control of paracetamol and meloxicam in various dosage forms.

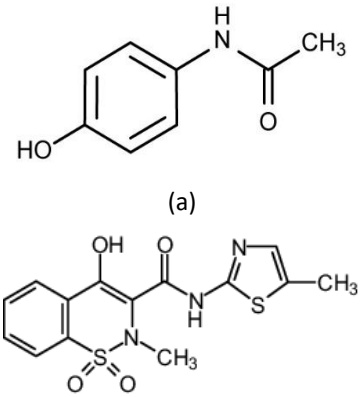
Keywords: HPTLC, validation, Meloxicam and Paracetamol.

INTRODUCTION

Meloxicam (MX) is a nonsteroidal anti-inflammatory drug (NSAID) the oxicam class, used to relieve the symptoms of arthritis, primary dysmenorrhea, fever; and as an analgesic. Chemically, it is (3E)-3-[hydroxy- [(5-methyl-1, 3-thiazol-2-yl) amino] methylidene]-2-methyl-1, 1-dioxobenzo [e] thiazin-4-one. Meloxicam inhibits cyclooxygenase (COX), the enzyme responsible for converting arachidonic acid into prostaglandin H_2—the first step in the synthesis of prostaglandins, which are mediators of inflammation[1]. Paracetamol (PARA) is an analgesic, antipyretic derivative of acetanilide. It has weak anti-inflammatory properties and is used as a common analgesic, but it may cause liver, blood cell, and kidney damage. Paracetamol chemically termed as N-(4-hydroxyphenyl) acetamide[2], is official in various pharmacopoeias [3-5].

Several methods have been reported for the analysis of MX and PARA, independently as well as its combinations in pharmaceuticals or in biological sample. These include spectrophotometric and fluorimetric [6-11], HPLC[12-24], LC-MS[25-27], capillary zone electrophoresis[29] and HPTLC [29-31].

In accordance with ICH guidelines, the objective of this study was to develop and validate a specific, accurate, precise and quality control HPTLC method for meloxicam and paracetamol in their formulations. The structures of drugs are shown in Figure 1a and 1b.



(a)

(b)

Figure 1: Chemical structures of Paracetamol (a) and Meloxicam (b)

MATERIALS AND METHODS

Meloxicam (MX) and Paracetamol (PARA) working standards were kindly supplied as gift samples by Lupin Laboratory Ltd. (Maharashtra, India), Torrent Pharmaceuticals, Ahmedabad (Gujarat, India) and Aristo Pharmaceutical Pvt. Ltd. India, respectively. All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, Mumbai, India. Melodol tablets containing 7.5 mg of MX and 325 mg of PARA were purchase from local chemist market.

Chromatographic conditions

Analysis was performed on 20 cm × 20 cm aluminum plates precoated with silica gel G $60F_{254}$. The solutions of MX and PARA were spotted in the form of bands of width 6 mm at

6 mm interval with Hamilton 100 µL syringe under a stream of nitrogen by means of a Camag Linomat IV. The mobile phase used was toluene: ethyl acetate: methanol: formic acid (8: 2: 0.5: 0.5 v/v/v/v), chamber and plate saturation time of 20 min, migration distance allowed was 80 mm. Ascending development was carried out in a saturated twin-trough TLC chamber. Densitometric scanning was performed in absorbance/reflectance mode at 287 nm using 5×0.45 mm slit dimensions. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum.

Preparation of standard solution

Accurately weighed quantity of 7.5 mg of MX and 325 mg of PARA were dissolved and volume was adjusted to 10 mL with methanol. Further dilutions were made up to concentration of 75–450 ng/band and 325-1950 ng/band for MX and PARA, respectively.

Preparation of Sample solution

Twenty tablets (Melodol) were selected, each containing 7.5 mg of MX and 325 mg of PARA, weighed and finely powdered. Powder equivalent to 7.5 mg of MX and 325 mg of PARA was transferred into a volumetric flask and extracted with methanol. The solution was centrifuged for 15 min at 600 rpm. The extract was filtered through Whatmann filter paper no. 41 and residue was washed with methanol. Required further dilutions were made with methanol.

Method validation[32-34]

According to ICH guidelines the developed method was validated for various analytical performance parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

RESULTS AND DISCUSSION

Method optimization

The method was optimized with a vision to develop a simultaneous assay method for MX and PARA. The HPTLC method was optimized through the assessment of number of solvent mixtures. Initially, ethyl acetate, toluene and methanol were tried in different ratios. Finally, the mobile phase of toluene: ethyl acetate: methanol: formic acid (8: 2: 0.5: 0.5 v/v/v/v) resulted in sharp, well-defined peaks with good resolution.

Specificity

The specificity of the method was evaluated by analyzing standard drugs and samples extracted from formulations. The spots for MX and PARA were confirmed by comparing the R_f of the samples with those of the standards. The method was specific for MX and PARA, since it resolved the peak of PARA (R_f = 0.19 ± 0.02) and MX (R_f= 0.54 ± 0.05) in presence of other excipients in the formulation (Figure 2).

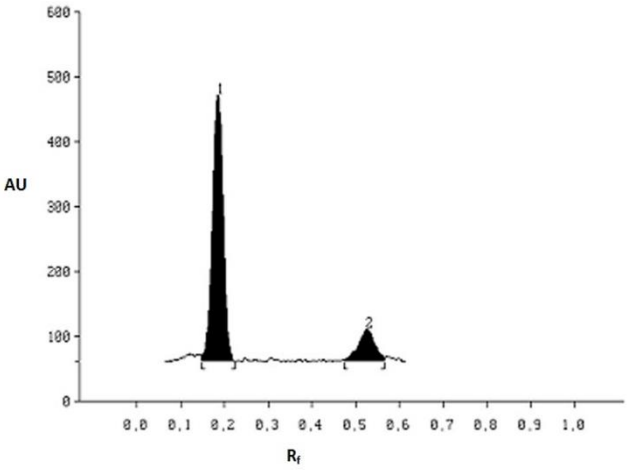


Figure 2: Typical densitogram of PARA (peak 1) and MX (peak 2)

Method validation

Optimized HPTLC method was validated with respect to the following parameters as per ICH guidelines.

Linearity and range

From the standard solution, six different concentrations, 0.1 to 0.6 µL were spotted on the TLC plate. Each concentration was applied in replicates on the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. A good linear correlation coefficient (R^2 = 0.9992 for MX and 0.9993 for PARA) was obtained over the concentration range of 75-450 ng/spot for MX and 325-1950 ng/spot for PARA, respectively as shown in Figures 3a and 3b.

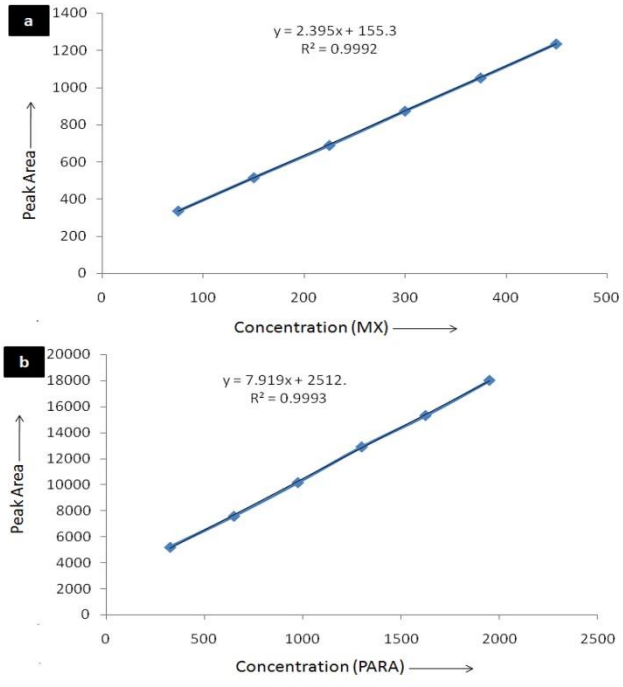


Figure 3: Calibration curves for (a) MX and (b) PARA

Limit of detection (LOD) and limit of quantification (LOQ)

From the slope (s) of the calibration plot and the standard deviation (SD) of the response, limits of detection (LOD) and quantification (LOQ) were calculated i.e. signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ were found to be 22.5 ng/band and 60 ng/band for MX and 32.5 ng/band and 162.5 ng/band for PARA respectively.

Precision

The precision of the method was verified by intraday and interday precision studies. Intraday studies were performed by analysis of three different concentrations (150, 300 and 450 ng/band for MX and 650, 1300, 1950 ng/band for PARA) of the drugs six times on the same day. The interday precision of the method was checked by repeating studies on three different days. The developed method was found to be precise as the relative standard deviation RSD values for intraday and interday precision studies were < 2%. The results of the intraday and interday precision are shown in Table 1.

Robustness of the method

Making small deliberate changes in chromatographic conditions, robustness of the method was examined. The following changes were made in the method such as mobile phase composition, development distance, duration of saturation time of chamber and the time from application to chromatography. In the robustness study, when small changes were made to the method conditions there were no marked changes in chromatographic behaviors and the % RSD were found to be less than 2%, indicating the method is robust as shown in Table 2.

Accuracy

A known concentration of the standard drug was added to a preanalysed tablet sample at three levels namely 80%, 100% and 120%. Dilutions were made and accuracy studies were performed. From the data obtained, good recoveries of MX and PARA were obtained as shown in Table 3.

Table 1: Precision studies of MX and PARA

Conc. (ng/band)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured Conc.	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery (%)
MX						
150	149.62	1.12	99.75	148.64	1.02	99.09
300	297.66	1.06	99.22	298.26	1.15	99.42
450	446.85	1.17	99.30	446.27	1.08	99.17
PARA						
650	643.63	1.10	99.02	643.89	1.20	99.06
1300	1291.42	1.03	99.34	1292.85	1.09	99.45
1950	1933.62	1.08	99.16	1935.18	1.14	99.24

Table 2: Robustness evaluation of MX and PARA (n=3)

Parameter	MX		PARA	
	SD of area	% RSD	SD of area	% RSD
A: Composition of toluene (± 0.1 mL)				
Toluene: ethyl acetate: methanol: formic acid 8.1: 2: 0.5: 0.5 (v/v/v/v)	17.50	1.23	15.20	1.10
Toluene: ethyl acetate: methanol: formic acid 8: 2: 0.5: 0.5 (v/v/v/v)	16.32	1.11	19.21	1.07
Toluene: ethyl acetate: methanol: formic acid 7.9: 2: 0.5: 0.5 (v/v/v/v)	20.12	1.15	17.41	1.27
B: Development distance (± 0.5 cm)				
7.5 cm	11.39	1.34	10.69	1.10
8 cm	9.45	1.13	10.14	1.12
8.5 cm	11.49	1.21	10.56	1.18
C: Duration of saturation (± 5 min.)				
25 min	10.67	1.12	12.28	1.07
20 min.	10.21	1.26	11.09	1.15
30 min	12.08	1.16	13.98	1.18
Time from application to chromatography (+10 min)	18.23	1.18	12.83	1.03

Table 3: Accuracy study of MX and PARA (n=3)

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)	Mean (%) Recovery (± SD)
MX (7.5)	80 100 120	13.5 15 16.5	13.42 14.93 16.29	99.40 99.53 98.73	99.22 ± 0.429
PARA (325)	80 100 120	585 650 715	582.30 648.15 714.20	99.54 99.72 99.89	99.72 ± 0.175

Assay of the marketed formulation

The % assay of drugs in the tablet dosage form was found to be 99.40 % for MX and 99.71 % for PARA, respectively.

CONCLUSION

The developed HPTLC method for simultaneous assessment of meloxicam and paracetamol is simple, rapid, economic and less time consuming. It offers more flexibility than HPLC method. Method validation proved that this method is precise, accurate, robust and specific for simultaneous analysis of meloxicam and paracetamol as bulk drugs and in tablet formulations without any interference from the excipients and thus, it can be explored for routine quantification and quality control procedures.

Acknowledgements: The authors are grateful to Lupin laboratory Ltd. (Maharashtra, India), Aristo Pharmaceutical Pvt. Ltd. India and Torrent Pharmaceuticals, Ahmedabad (Gujarat, India) for providing samples of meloxicam and paracetamol, respectively, as gifts. The authors are also grateful to Dr K R Mahadik, Principal, Bharati Vidyapeeth (Deemed To Be University), Poona College of Pharmacy, Pune, India for providing excellent facilities to carry out the work.

REFERENCES

1. https://www.drugs.com/meloxicam.html
2. https://pubchem.ncbi.nlm.nih.gov/compound/Acetaminophen
3. The Indian Pharmacopoeia, 1996 edition, Vol.II, 554.
4. The British Pharmacopoeia, 2007 edition, Vol. II, 1575.
5. USP-NF Asian edition 2007 volume 2, 1269.
6. Ramesh S, Rupali J, Deepali K, Varsha S. Development and validation of spectrophotometric methods for simultaneous estimation of paracetamol and meloxicam in pure and tablet dosage form, Der Pharm Lett, 2(2), 2010, 471-478.
7. Khan F, Lohiya R T, Umekar M J. Development of UV spectrophotometric method for the simultaneous estimation of meloxicam and paracetamol in tablet by simultaneous equation, absorbance ratio and absorbance correction method, International Journal of ChemTech Research, 2 (3), 2010, 1586-1591.
8. Abbas A, Nahid S, Ali RZ, Spectrophotometric determination of salicylamide and paracetamol in biological samples and pharmaceutical formulations by a differential kinetic method, Acta Chim Slov, 53, 2006, 357-362.
9. Sawant R, Joshi R, Kawade D, Sarode V. Development and validation of spectrophotometric methods for simultaneous estimation of paracetamol and meloxicam in pure and tablet dosage form, Der Pharmacia Lettre, 2(2), 2010, 471-478.
10. Master S M, Mehta R S, Bhatt K K, Panchal H S. First order derivative spectroscopic method for simultaneous estimation of meloxicam and paracetamol in their combined dosage form, International Journal of Research in Pharmacy and Chemistry, 1(2), 2011, 193-198.
11. Hassan EM. Spectrophotometric and fluorimetric methods for the determination of meloxicam in dosage forms, J Pharm Biomed Anal, 27(5), 2002, 771-777.
12. Kamberi M, Riley CM, Ma Sharon X, Huang C W, A validated, sensitive HPLC method for the determination of trace impurities in acetaminophen drug substance, J Pharm Biomed Anal, 34 (1), 2004, 123-128.
13. Shaikh K A, Devkhile A.B, Simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone by RP-HPLC in pharmaceutical dosage form, J Chromatogr Sci, 46(7), 2008, 649-652.
14. Uttam D P, Abhijit V N, Aruna V S, Tirumal A D, Kiran V M, Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by HPLC in tablet dosage form, E-J Chem, 6(1), 2009, 289-294.
15. Sivasubramanian L, Lakshmi K S, Reverse phase-high performance liquid chromatographic method for the analysis of paracetamol, cetirizine and pseudoephedrine from tablets, Der Pharma Chemica, 1(1), 2009, 37-46.

16. Gopinath R, Rajan S, Meyyanathan SN, Krishaveni N, Suresh B, A RP-HPLC method for simultaneous estimation of paracetamol and aceclofenac in tablets, India J Pharm Sci, 69(1), 2007, 137-140.

17. Induri M, Mantripragada B R, Yejella R P, Kunda P R, Arugula M, Boddu R, Simultaneous quantification of paracetamol and meloxicam in tablets by high performance liquid chromatography. Tropical Journal of Pharmaceutical Research. 10(4), 2011, 475-481.

18. Arayne M S, Sultana N, Siddiqui F A, A new RP-HPLC method for analysis of meloxicam in tablets, Pak J Pharm Sci, 18(1), 2005, 58-62.

19. Farzana S B, Pradeep R V, A stability indicating HPLC method for the determination of meloxicam in bulk and commercial formulations, Trop J Pharm Res, 8 (3), 2009, 257-264.

20. Mahmood K T, Khan B, Ashraf M, Haq I U, Specific and simple HPLC assay of ecofriendly meloxicam in pharmaceutical formulations, Journal of Pharmaceutical Sciences and Research, 2(12), 2010, 878-883.

21. Zawilla N H, Abdul-Azim Mohammad M, El kousy N M, El-Moghazy Aly SM, Determination of meloxicam in bulk and pharmaceutical formulations, J Pharm Biomed Anal, 32(6), 2003, 1135-44.

22. Velpandian T, Jaiswal J, Bhardwaj R K, Gupta S. K, Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples, J. Chromatogr B Biomed Sci. Appl, 738(2), 2000, 431-436.

23. Vignaduzzo S E, Castellano P M, Kaufman T S, Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations, J Pharm Biomed Anal, 46(2), 2008, 219-25.

24. Madhusudhanareddy Induri, Bhagavan R Mantripragada, Rajendra P Yejella, Pavankumar R Kunda, Meechel Arugula and Rajkumar Boddu, Simultaneous Quantification of Paracetamol and Meloxicam in Tablets by High Performance Liquid Chromatography, Tropical Journal of Pharmaceutical Research 10(4), 2011, 475-481.

25. Hao Z, Shouhong G, Wansheng C, Yanqiang Z, Xuetao J, Yuanying P, Simultaneous quantification of paracetamol, pseudoephedrine and chlorpheniramine in dog plasma by LC-MS-MS, Chromatographia, 68(3-4): 2008, 251-257.

26. Wiesner J L, De Jager A D, Sutherland FCW, Hundt HKL, Swart K J, Hundt AF, Els J, Sensitive and rapid liquid chromatography-tandem mass spectrometry method for the determination of meloxicam in human plasma, J Chromatogr B Analyt Technol Biomed Life Sci, 785 (1), 2003, 115-21.

27. Rigato H M, Mendes GD, Borges N C, Moreno R A, Meloxicam determination in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) in Brazilian bioequivalence studies, Int J Clin Pharmacol Ther, 44 (10), 2006, 489-98.

28. Nemutlu E, Kir S. Method development and validation for the analysis of meloxicam in tablets by CZE, J Pharm Biomed Anal, 31(2), 2003, 393-405.

29. Shaji J, Varkey D, Development of a Validated Stability-Indicating HPTLC Method for Determination of Meloxicam in Bulk and Pharmaceutical Formulations: Pertinence to ICH Guidelines, International Journal of Pharmacy and Pharmaceutical Sciences, 4 (l), 2012, 160-169.

30. Farid N F, Abdelaleem E A, HPTLC Method for the Determination of Paracetamol, Pseudoephedrine and Loratidine in Tablets and Human Plasma, J Chromatogr Sci, 54(4), 2016, 647–652.

31. Patil A, Mulla S, Development and Validation of HPTLC Method for the Simultaneous Estimation of Paracetamol and Acelofenac in Combined Dosage Form, International Journal of Pharmacy and Pharmaceutical Sciences, 6 (1), 2014, 641-643.

32. Gorantla N, Dodlapati J , Jadi S, A New Validated RP-HPLC Method for Simultaneous Estimation of Lumacaftor and Ivacaftor in Pharmaceutical Dosage Form, Int. J. Pharm. Sci. Rev. Res., 56(1), 2019, 30-37.

33. Joshi H, Khristi A, Simultaneous Equation Method Development and Validation for the Simultaneous Estimation of Teneligliptine hydrobromide hydrate (TEN) and Metformin hydrochloride (MET) in Tablet Dosage Form, Int. J. Pharm. Sci. Rev. Res., 49(2), 2018, 9-15.

34. ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology.

Source of Support: Nil, **Conflict of Interest:** None.