IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2022 Vol.:25, Issue:4

High Performance Thin Layer Chromatography for Simultaneous Estimation of Miconazole Nitrate and Betamethasone Valerate in Bulk and Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Varsha Kamble*[1], Bharti Fegade[2], V. H. Bhaskar[3]

[1]*M. Pharmacy Student, Department of Pharmaceutical Quality Assurance, Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai, Maharashtra, India*

[2]*Assistant Professor, Department of Pharmaceutical Quality Assurance, Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai, Maharashtra, India*

[3]*Professor and Principle of Gahlot Institute of Pharmacy, Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai, Maharashtra, India*

Submitted: 25 October 2022
Accepted: 31 October 2022
Published: 30 November 2022

Keywords: Betamethasone Valerate, Miconazole Nitrate, Toluene, Ammonia, Acetone, HPTLC.

ABSTRACT

A simple, precise, accurate, and reliable HPTLC method has been developed and validated for the analysis of BETA-Betamethasone Valerate and MCN- Miconazole Nitrate in their combined dosage form. Separation and analysis were performed on precoated silica gel 60 F254 plate having thickness of layer 0.2 mm, which were then eluted with Toluene:Acetone:Ammonia 25% (8.0:2.0:0.1). Calibration plots were established showing the dependence of response (peak area) on the amount chromatographed. The validated calibration ranges were 100-900 ng/spot for BETA and MCN with correlation coefficient (R2) 0.997 and 0.994, respectively. Average % recovery was between 85-102% for BETA and MCN, respectively and gave Rf 0.82 and 0.05 For BETA and MCN respectively. The spots were scanned at 245 nm for BETA and the Ninhydrin (post derivatizing reagent) reagent sprayed on plate for visualization of MCN and plate scan at 224 nm. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of BETA and MCN in their combined semisolid dosage form.

HUMAN JOURNALS
www.ijppr.humanjournals.com

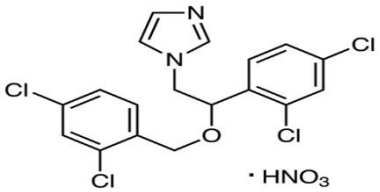
INTRODUCTION:

Science and technology have never been so promising nor have delivered so many opportunities to improve health and extend lives, but continued investments are being invested in both the public and private sector, in spite of the current economic climate.[1,2] Increasing pharmaceutical industry success rates and delivering more medicines are very challenging, but very few predictive scientific and analytical tools are available. Research on drugs involves production control of bulk drug and final product, toxicological analysis of side effects of the drug or its possible impurities, and determination of the fate of a drug and its metabolites in an organism by the monitoring of body fluids.[3,4] Common criteria for drug evaluation include the quality and therapeutic value of the bulk drug and pharmaceutical product, identification studies, purity, content, uniformity, chemical and physical stability, and biological availability.[5,6] High-performance thin-layer chromatography (HPTLC) is an enhanced form of thin-layer chromatography (TLC). A number of enhancements can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements.[7]

Miconazole nitrate (MCZ), or **imidazole**: Miconazole nitrate is an antibacterial of the class of imidazole. Miconazole Nitrate is the nitrate salt form of miconazole, an antifungal synthetic derivative of imidazole and used in the treatment of candida skin infections, Miconazole selectively affects the integrity of fungal cell membranes, high in ergosterol content and different in composition from mammalian cells membranes.[8,9,10]

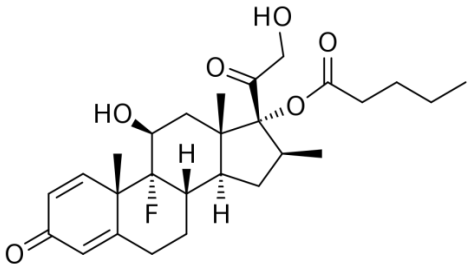
MOLECULAR FORMULA: $C_{18}H_{15}Cl_4N_3O_4$

MOLECULAR WEIGHT: 479.135 g/mol



Betamethasone valerate:

Betamethasone valerate is a synthetic glucocorticoid corticosteroid and a corticosteroid ester. It is the 17-acetate ester of betamethasone.[11,12]



MOLECULAR FORMULA: $C_{27}H_{37}FO_6$

MOLECULAR WEIGHT: 476.585g/mol

METHOD DEVELOPMENT:

- Method selection and information of samples
- Selection of initial method conditions
- Checking the analytical method in aqueous standards
- Development and optimization of sample processing method

1) Apparatus and instruments:

1. Pre- coated silica gel aluminum plate 60F-254 (10 x 10 cm, 20x 20cm with 250µm thickness)
2. Desaga - 25µl dosing syringe (Hamilton)
3. Desaga Applicator, AS30win
4. Desaga – Twin through chamber (200 x 100) with stainless steel lid
5. Desaga TLC scanner
6. Desaga photo chamber, provide with canon power shot G5 digital camera
7. UV cabinet with dual wavelength UV lamp (254 nm and 366 nm)
8. Balance, model ALC210.4 (Acculab)
9. Ultra sonicator (fast clean Ultrasonic cleaner)

2) Reagents and materials:

1. Miconazole nitrate (yarrow chem Pvt Ltd, Mumbai)
2. Betamethasone valerate (yarrow chem Pvt Ltd, Mumbai)

3. AR grade Toluene, Acetone, Ammonia 25% (Finar Chemicals Pvt Ltd, Ahmedabad, India)

4. Silica gel 60 GF254 plates were obtained from Merck, Mumbai, India.

5. Whatman filter paper no. 41

3) Formulation:

BETNOVATE- M (Betamethasone valerate and Miconazole nitrate 0.12%/ 2%/5gm)

4) Chromatographic condition:

1. Stationary phase- 10 x 10 cm precoated silica gel 60 F254 plate
2. Mobile phase- Toluene: Acetone: Ammonia25% (8.0: 2.0: 0.1)
3. Detection wavelength- 225 nm
4. Chamber saturation time- 5 min
5. Band width- 8mm
6. Distance between spots- 20mm
7. Rate of spotting- 5s/µl
8. Spotting volume- 10µl
9. Distance run- 70mm
10. Lamp – Deuterium

5) Preparation of the mobile phase:

The mobile phase was prepared by mixing 8.0 ml Toluene, 2.0 ml Acetone, 0.1 ml Ammonia. The mobile phase was transferred into a twin- trough chamber covered with lid and allowed to stand for 5 min before use.

6) Preparation of standard solution:

A. Preparation of standard stock solution:

Accurately weighed betamethasone valerate (10mg) and miconazole nitrate (10mg) were transferred to two separate 10 ml volumetric flasks, dissolved in 5 ml of methanol and sonicate it for 10 min. make up the volume with methanol to obtain standard stock solution (1000µg/ml) of each.

B. Preparation of combined standard stock solution:

Add 5 ml of betamethasone valerate and 5 ml of miconazole nitrate from above stock solution in 10 ml of volumetric flask to obtain a solution of 0.5µg/ml. i.e., 500 ng/spot.

C. Preparation of test solution:

A quantity equivalent to 6mg of betamethasone valerate and 100mg of miconazole nitrate (1gm) were transferred to 50 ml of volumetric flask, diluted with methanol to volume, mixed well with ultra- sonication, and filtered through Whatman Filter Paper no. 41 to obtain 0.12µg/ml (6µg/µl) of betamethasone valerate and 2.0µg/ml (100µg/µl) of miconazole nitrate.

METHOD VALIDATION:

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.[13,14]

Method validation helps to validate the analytical method for a range of concentrations so that the change in formulation or concentration do not require additional validation.[15]

As per ICH guideline Q2R1, the method validation parameters studied were specificity, linearity, accuracy (% recovery), precision, limit of detection and limit of quantitation.[16]

1. Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of components that may be expected to be present in the sample matrix. Chromatograms of standard and sample solution of betamethasone valerate and miconazole nitrate were compared, and peak purity spectra at three different levels i.e., peak start (S), peak apex (M) and peak end (E) of a spot were recorded in recorded in order to provide an indication of specificity of the method.

2. Linearity and Range

The linearity was determined by using working standard solutions between 100-900 µg/spot. The spectra of these solutions were recorded at wavelength 246 nm. Calibration curve of peak absorbance v/s Concentration was plotted after suitable calculation and simple linear regression was performed. Regression equation and correlation coefficient were obtained.

The range of solutions has been decided according to statistical parameters of the generated equation. Linearity and Range of Betamethasone Valerate and Miconazole nitrate.

Acceptance criteria: Correlation coefficient "R^2" should be not less than 0.99.

3. Calibration curve

In analytical chemistry a calibration curve, also known as a standard curve, is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.[17] Calibration curves were plotted by taking the absorbance of respective concentration of samples.

4. Precision

The repeatability was checked by repeatedly (n=6) injecting standard solutions of betamethasone valerate and miconazole nitrate. Area of each curve of these solutions was measured at 225 nm. The intra- day and inter- day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of same concentration of ng/spot of both drugs.

Repeatability: The precision of the method was checked by repeatedly injecting (n= 10) standard solutions of betamethasone valerate (500 µg/spot) and miconazole nitrate (500 µg/spot). Absorption of these solutions was measured at 245 nm and 224 nm. Relative standard deviation (% RSD) was calculated in the following table.

Reproducibility: The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of the same concentrations of 500 µg/spot of betamethasone valerate and miconazole nitrate (500 µg/spot). The results were reported in terms of percentage relative standard deviation (%RSD). The results have been tabulated in the below table.

Acceptance criteria- The results were calculated in terms of percentage relative standard deviation (% RSD). The RSD values were found to be below 2% which indicates that proposed methods are precise.

5. Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of betamethasone valerate and miconazole nitrate by the standard addition method. Known amount of standard solution of betamethasone valerate and miconazole nitrate (1200, 1500, 1800 ng/ spot) were

added to a pre- quantified sample solution. Each solution was applied to a plate in triplicate as 80%, 100% and 120%.

Acceptance criteria- Accuracy should be between 85% - 102% and RSD should not be more than 2.0. The overall average % Recovery should be between 85% - 102% with % RSD not more than 2.0.

6. Limit of detection and Limit of quantification

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Nine sets of known concentrations (100-900 µg/spot) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation (Table 5) and following formulae as; LOD = 3.3 SD/S LOQ = 10 SD/S Where, SD is standard deviation of y-intercept of the calibration curves S is mean slope of five calibration curves.

Formula for Limit of Detection and Limit of Quantification is LOD= 3.3XN/S and LOQ= 10X N/S

Acceptance criteria: The acceptance criterion is that the **LOD** has to be less than the **LOQ**.

***Analysis of formulation: (BETNOVATE-M, 5gm)**

Label claim- Betamethasone valerate (0.12%), Miconazole nitrate (2%)

Preparation:

5 gm of cream was emptied in the beaker and 20 ml of methanol was added. This mixture was ultra-sonicated for 15 min and was transferred to 50ml of volumetric flask. Volume was made up to 50 ml with methanol. Above mixture was centrifuged for 10 min at 2000 rpm. The cloudy upper supernatant was used for analysis. A quantity equivalent to 6mg of betamethasone valerate and 100mg of miconazole nitrate (1gm) were transferred to 50 ml of volumetric flask, diluted with methanol to volume, mixed well with ultra- sonication, and filtered through Whatman Filter Paper no.41 to obtain 0.12µg/ml (6µg/µl) of betamethasone valerate and 2.0µg/ml (100µg/µl) of miconazole nitrate.

Drug	**Label Claim(mg)**	**% Assay**
BETA	0.12%	98.93%
MCN	2%	99.59%

RESULTS AND DISCUSSION:

1. Specificity

Table No. 1: Concentration and Absorbance of Miconazole Nitrate

Concentration µg/spot	Absorbance
100	0.0016
200	0.0020
300	0.0034
400	0.0045
500	0.0059
600	0.0067
700	0.0072
800	0.0083
900	0.0095

Table No. 2: Concentration and Absorbance of Betamethasone Valerate

Concentration µg/spot	Absorbance
100	0.0014
200	0.0025
300	0.0036
400	0.0047
500	0.0057
600	0.0066
700	0.0073
800	0.0083
900	0.0094

2. Linearity and Range:

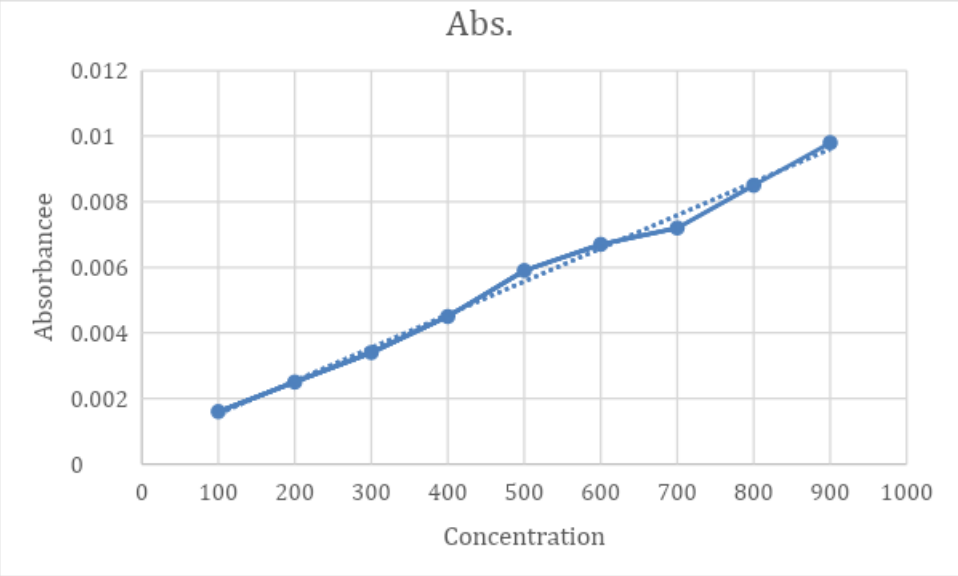
This table shows sequence of linearity and range:

Parameter	Betamethasone valerate	Miconazole nitrate
Linearity range ng/spot	100-900	100-900
R^2	0.997	0.994
Slope	0.00001	0.00001
Intercept	0.0006	0.0005

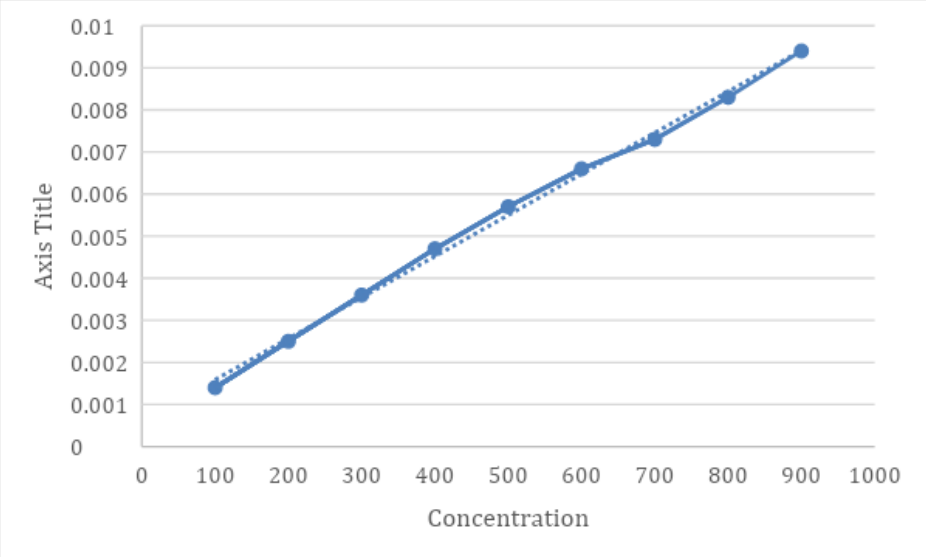
3. Calibration curve:

A. Calibration curve of Miconazole Nitrate.

B. Calibration curve of Betamethasone Valerate.



A



B

4. Precision

Concentration (µg/spot)	**Absorbance**	**Mean absorbance**	**%RSD**
500	0.0039	0.00427	1.940
500	0.0042		
500	0.0042		
500	0.0042		
500	0.0041		
500	0.0041		
500	0.0042		
500	0.0045		
500	0.0045		
500	0.0048		

A. Table of Miconazole Nitrate

Concentration (µg/spot)	Absorbance	Mean absorbance	%RSD
500	0.0038	0.00426	1.938
500	0.0042		
500	0.0041		
500	0.0040		
500	0.0042		
500	0.0041		
500	0.0042		
500	0.0045		
500	0.0046		
500	0.0049		

B. Table of Betamethasone Valerate

Reproducibility:

Concentration (µg/spot) (Miconazole Nitrate)	%RSD	
	Intraday	Interday
500	1.51	1.61
500	1.17	1.88
500	1.55	1.66

***n=3**

Concentration (µg/spot) (Betamethasone Valerate)	%RSD	
	Intraday	Interday
500	1.52	1.60
500	1.16	1.87
500	1.53	1.67

***n=3**

5. Accuracy (% Recovery)

Following table shows sequence of accuracy:

Concentration taken in µg/spot(A)	Standard addition in µg/spot(B)	Total drug concentration (µg/spot) (A+B)	Area	Average	% Recovery
200	160	360	5356 5560 5475	5463.666	97.02
200	200	400	5659 5486 5523	5556	85.04
200	240	440	6189 6236 6145	6190	83.22

A. Accuracy table of Miconazole nitrate

Concentration taken in µg/spot(A)	Standard addition in µg/spot(B)	Total drug concentration (µg/spot) (A+B)	Area	Average	% Recovery
200	160	360	5257 5530 5380	5372.667	96.06
200	200	400	5759 5583 5524	5622	85.89
200	240	440	6290 6337 6244	6290.333	84.23

B. Accuracy table of Betamethasone Valerate

6. Limit of detection and Limit of quantification

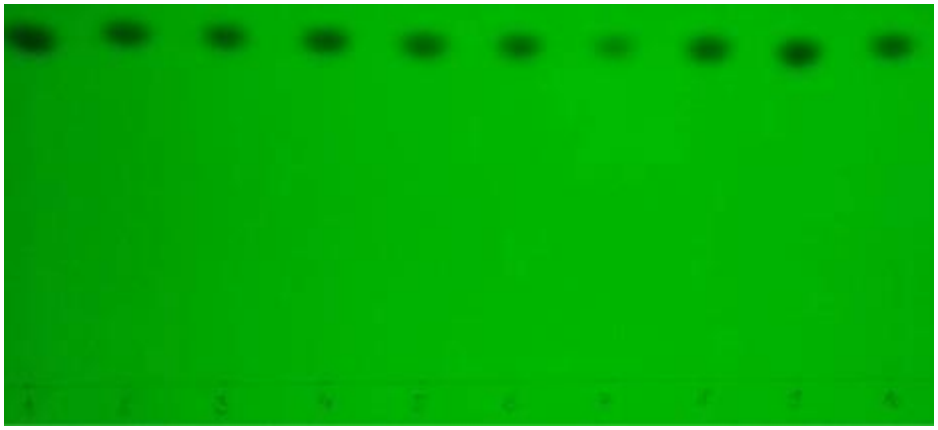
Drug	LOD	LOQ
Betamethasone Valerate	0.97	2.96
Miconazole Nitrate	0.98	2.97

7. Analytical validation parameters of proposed HPTLC method for simultaneous estimation of Betamethasone valerate and Miconazole nitrate:

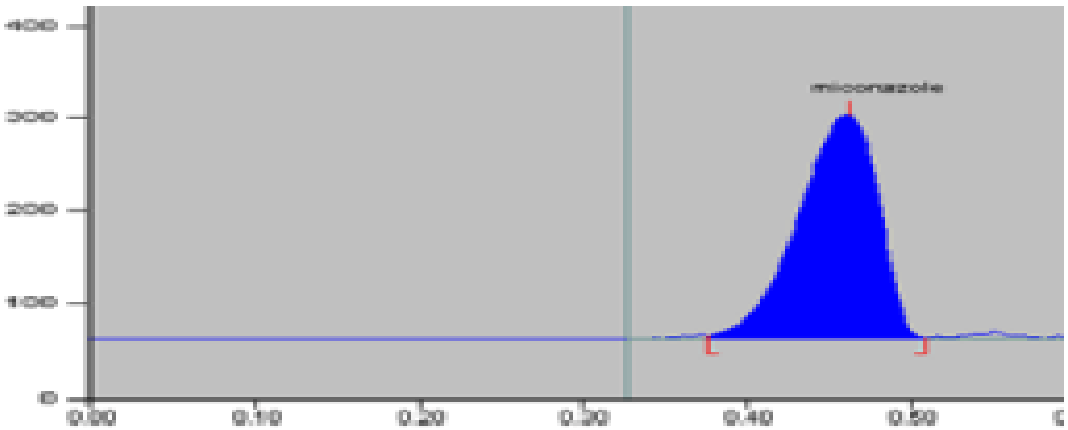
Parameter	BETA	MZN
Linearity Range(ng/spot)	100-900	100-900
Correlation Coefficient	0.997	0.994
Limit of Detection (LOD)	0.97	0.98
Limit of Quantification (LOQ)	2.96	2.97
%Recovery(n=3) 360% 400% 440%	96.06 85.89 84.23	97.02 85.04 83.22
Precision(n=3), %RSD Interday Intraday	1.938 1.71 1.40	1.940 1.71 1.41

8. UV image and HPTLC chromatogram of Betamethasone valerate and Miconazole nitrate:

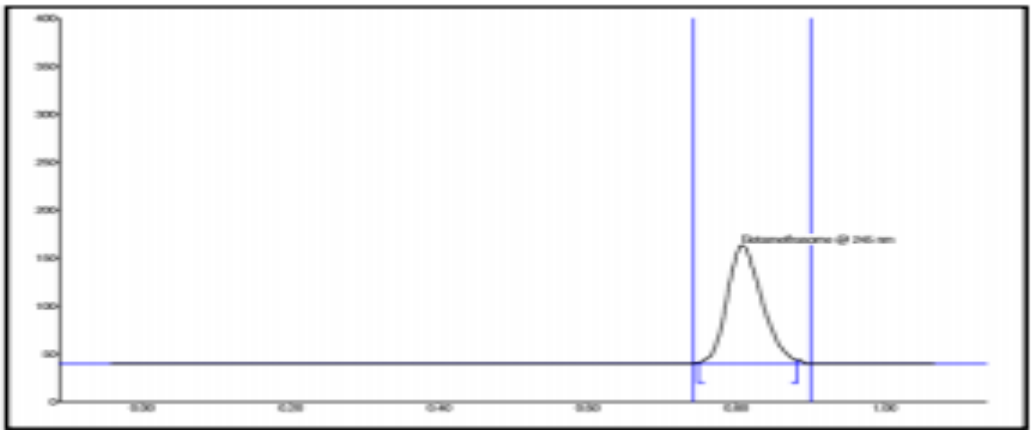
A. UV image of developed chromatogram of Betamethasone Valerate



B. UV image of developed chromatogram of Miconazole Nitrate



C. HPTLC Chromatogram of Miconazole Nitrate scan at 224nm



D. HPTLC Chromatogram of Betamethasone Valerate scan at 245 nm

CONCLUSION:

Thus, the objective of project work was development and comparison of analytical methods of BETA and MCN in their combined dosage form. The developed and validated HPTLC method for BETA and MCN was found to be simple, specific, and cost effective and can be routinely applied for analysis of BETA and MCN in their combined dosage form. We can say that the HPTLC method is more sensitive giving precise results (interday, intraday) for both drugs, and also the HPTLC method is more sensitive in terms of LOD and LOQ. It also requires least solvents for analysis. The proposed method has the advantages of simplicity and convenience for the separation and quantitation of BETA and MCN in combination and can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedures. The additives usually present in the pharmaceutical formulations of the assayed analytes did not interfere with determination of BETA and MCN. The method can be used for the routine simultaneous analysis of BETA and MCN in pharmaceutical preparation.

ACKNOWLEDGEMENT:

The authors would like to thank the Gahlot Institute of Pharmacy for the constant support and encouragement to carry out work.

REFERENCES:

1. Zlatkis A, Kaiser RE. HPTLC-high performance thin-layer chromatography. Elsevier; 2011 Aug 26.
2. Sethi PD. HPTLC: high performance thin-layer chromatography; quantitative analysis of pharmaceutical formulations. CBS publishers & distributors; 1996.
3. Arup U, Ekman S, Lindblom L, Mattsson JE. High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. The Lichenologist. 1993 Jan;25(1):61-71.
4. Neumann C, Margot P. New perspectives in the use of ink evidence in forensic science: Part I. Development of a quality assurance process for forensic ink analysis by HPTLC. Forensic science international. 2009 Mar 10;185(1-3):29-37.
5. Sherma J. Review of HPTLC in drug analysis: 1996-2009. Journal of AOAC international. 2010 May 1;93(3):754-64.
6. Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. Natural product reports. 2008;25(3):475-516.
7. Srivastava M, editor. High-performance thin-layer chromatography (HPTLC). Springer Science & Business Media; 2010 Nov 15.
8. Zlatkis A, Kaiser RE. HPTLC-high performance thin-layer chromatography. Elsevier; 2011 Aug 26.
9. Shewiyo DH, Kaale EA, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of pharmaceutical and biomedical analysis. 2012 Jul 1; 66:11-23.
10. Patel KG, Shah PM, Shah PA, Gandhi TR. Validated high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate cream using fractional factorial design. journal of food and drug analysis. 2016 Jul 1;24(3):610-9.

11. Franz TJ, Parsell DA, Halualani RM, Hannigan JF, Kalbach JP, Harkonen WS. Betamethasone valerate foam 0.12%: a novel vehicle with enhanced delivery and efficacy. International journal of dermatology. 1999 Aug;38(8):628-32.
12. Meshram DB, Bagade SB, Tajne MR. Simultaneous determination of metronidazole and miconazole nitrate in gel by HPTLC. Pakistan Journal of Pharmaceutical Sciences. 2009 Jul 1;22(3).
13. Zanwar AS, Sen DB, Maheshwari RA, Chandrakar VR, Seth AK, Sen AK. Simultaneous analysis of mometasone furoate, miconazole nitrate, and nadifloxacin in cream formulation by HPTLC. Journal of Applied Pharmaceutical Science. 2020 Jul 4;10(7):108-15.
14. Wulandari L, Indrayanto G. HPTLC determination of betamethasone in tablets and its validation. Journal of liquid chromatography & related technologies. 2003 Oct 1;26(16):2709-17.
15. Sinnarkar NS, Phoujdar MS. Development and validation of HPTLC method for determination of betamethasone valerate in API and pharmaceutical dosage form. World Journal of Pharmaceutical Sciences. 2017 Apr 27:186-90.
16. Fereja TH, Hymete A, Bekhit AA. Stability indicating HPTLC method development for determination of betamethasone and dexchlorpheniramine maleate in a tablet dosage form. AASCIT Journal of Chemistry. 2015;2(2):32-42.
17. Indrayanto G, Widjaja S, Sutiono S. Simultaneous densitometric determination of betamethasone valerate and miconazole nitrate in cream, and its validation. Journal of liquid chromatography & related technologies. 1999 Jan 1;22(1):143-52.