ISSN 2515-8260 Volume 10, Issue 01, 2023

A Novel HPTLC Method for the Estimation of Triamcinolone Acetonide, Budesonide, Beclomethasone, Mometasone Furoate and Fluticasone Propionate in Various Nasal Sprays Using Common Mobile Phase

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Abstract - A novel, simple, precise, sensitive, rapid and specific high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of Triamcinolone acetonide, Budesonide, Beclomethasone, Mometasone furoate and Fluticasone propionate in various nasal sprays using common mobile phase. The stationary phase used was precoated silica gel 60F_{254} plate. The mobile phase used was a mixture of Toluene: Acetonitrile: Triethyl amine [6.5: 3.5: 0.2, v/v/v]. The detection of spots was carried out densitometrically using a UV detector at 240 nm in absorbance mode. This system was found to give compact spots for Triamcinolone acetonide, Budesonide, Beclomethasone, Mometasone furoate and Fluticasone propionate with Rf values of 0.30, 0.46, 0.55, 0.71 and 0.82, respectively. The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity. The calibration curve was found to be linear between 100-600 ng/spot for all drugs with significantly high value of correlation coefficient [r^2 > 0.99]. The limits of detection and quantitation values reveals sensitive determination of all drugs in various formulations using common mobile phase. The low value of percent relative standard deviation reflects the repeatable and precise nature of the developed method. The average percent recovery for all drugs between 98.00 – 102.0% with low value of percent relative standard deviation indicates the accuracy of the method. The comparison of associated spectra at peak points of start, apex and end of the band or spot for all drugs with very good correlation or peak purity [r > 0.99] indicates specificity of the method. The assay results are found in good agreement with the label claim indicates quantification of all drugs without interferences of sample matrix. The proposed method can be used in the quality control of bulk drugs and pharmaceutical dosage forms with very fast, accurate, precise and specific results.

Index Terms - Quality control, Assay, HPTLC, Nasal spray

I. INTRODUCTION

Respiratory disease, which includes conditions of the trachea, bronchi, bronchioles, alveoli, pleura and pleural cavity, as well as the nerves and muscles of breathing, the common cold, which is mild and self-limiting while life-threatening illnesses such as pneumonia, pulmonary embolism, severe asthma, and lung cancer are also examples of respiratory ailments. [1]. A bacterial infection caused by streptococci, staphylococci, pneumococci, or other bacteria can often worsen viral rhinitis [2]. COPD includes two entities; one chronic bronchitis and another emphysema. A productive cough that lasts at least three months each year for two years is still referred to as "chronic bronchitis." [3]

Asthma is a chronic inflammatory condition of lung airways characterized by variable, reversible and episodic airflow blockage due to bronchospasm. Wheezing, coughing, chest tightness and shortness of breath are common symptoms of Asthma. [4]

Triamcinolone acetonide [TRI] is a synthetic gluco-corticosteroid, is chemically 1S,2S,4R,8S,9S,11S,12R,13S)-12-fluoro-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo [10.8.0.02,9.04,8.013,18] icosa-14,17-dien- 16-one. Budesonide [BUD] is gluco-corticoid with immunomodulating and anti-inflammatory properties, is chemically 11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo [10.8.0.02,9.04,8.013,18] icosa-14,17-dien-16-one. Beclomethasone [BEC] is a gluco-corticoid with immunomodulating and anti-inflammatory properties, is chemically 9-chloro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16- trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one. Mometasone furoate [MOM] is a topical glucocorticoid receptor [GR]

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agonist with dermatological properties, is chemically [(8S,9R,10S,11S,13S,14S,16R,17R)-9-chloro-17-(2-chloroacetyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] furan-2-carboxylate. Fluticasone propionate [FLU] is trifluorinated gluco-corticoid receptor with anti-allergic and anti-inflammatory effect, is chemically [(6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-17-(fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] propanoate [5].

TRI, BUD, BEC, MOM and FLU are official in Indian Pharmacopoeia [IP] 2018, British Pharmacopoeia [BP] 2020, United States Pharmacopoeia [USP] 2020, and European Pharmacopoeia [EP] 2020. The pharmacopoeia describes liquid chromatography and spectroscopic method for estimation of these drugs. Literature review reveals chromatographic, voltammetry, and spectroscopic methods for estimation of these drugs in either single or combination with other drugs from API, dosage forms or biological fluids [6]-[14]. In view of this, high-performance thin layer chromatography [HPTLC] based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. The present paper describes the development and validation of simple, sensitive, fast, economic and specific HPTLC method for routine estimation of TRI, BUD, BEC, MOM and FLU from bulk and pharmaceutical dosage forms such as nasal spray.

II. MATERIAL AND METHODS

Apparatus

The HPTLC system [Camag, Muttenz, Switzerland] consisted of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber [10 × 10 cm], a derivatization chamber, and a plate heater. Precoated silica gel 60 F_{254} TLC plates [10 × 10 cm], layer thickness 0.2 mm [E. Merck KGaA, Darmstadt, Germany] was used as stationary phase. TLC plates were prewashed twice with 10 ml of methanol and activated at 110^0C for 5 min prior to sample application. Densitometric analysis was carried out using a TLC scanner 3 with winCATS software.

Reagents and Materials

TRI, BUD, BEC, MOM and FLU pure powder was obtained as gift sample from Maharshi Pharma [India] and Vadish Pharma [India], respectively with 99.9% purity. Nasal spray formulations were procured from the local pharmacy. Toluene, acetonitrile, triethyl amine, methanol [AR grade, S.D. Fine Chemical Ltd., Mumbai, India] and Whatman filter paper no. 41 [Whatman International Ltd., England] were used in the study.

Chromatographic Conditions

The chromatographic separation was carried out by Silica Gel G60F254 HPTLC plates that had been treated with methanol and kept for 5 minutes at 110ºC. Both substances were spotted to the stationary phase [10×10 cm] using automated spotter with a 100 µl Hamilton syringe and sets of 6 mm wide band, 10 mm distance from the border and bottom of the plate. Using the win-CATS software, the distance between the bands were automatically set. The stationary phase was built in chamber that had been kept for 30 minutes saturation with the mobile phase Toluene: Acetonitrile: Triethyl amine [6.5: 3.5: 0.2, v/v/v] to run almost 85 mm distance at a room temperature [25± 3ºC]. A TLC scanner at 240 nm with absorbance mode having slit width of 6 × 0.45 mm and speed of scanning 1 mm/second was used for densitometric scanning. Win-CATS software was used to examine the scanned data.

Standard Stock Solution

TRI, BUD, BEC, MOM and FLU standard materials were carefully weighed [100 mg] and placed to volumetric flasks of 100 ml, where they were solubilized in methanol. The flask was stirred, and the volume was raised to the marked height with methanol to produce a solution with concentrations of 1 mg/ml.

Mixed Working Solution

Placing 10 ml of stock solution into a volumetric flask of 100 ml yielded the working solutions. Methanol was used to adjust the volume to the desired level, and the flask was stirred.

Sample Solution

To estimate the amount of TRI, BUD BEC, MOM, and FLU in various spray solutions; 6 ml, 5 ml, 2 ml, 4 ml, and 6 ml spray solutions of TRI, BUD, BEC, MOM, and FLU, consecutively, were carefully placed to volumetric flask of 100 ml, shaken with methanol [25 ml], and kept 30 minutes for sonication. To accomplish the desired solution, the solvent was added up to the marked height.

Preparation of Mobile Phase

Solvents with an AR grade, the mobile phase composition of 6.5: 3.5: 0.2, v/v/v mixture of Toluene, Acetonitrile and Triethyl amine. Solvents were stirred well and placed into the TLC chamber, which was held at 25 ± 3ºC for 30 minutes for saturation.

Determination of Analytical Wavelength

The working solutions of TRI, BUD BEC, MOM and FLU were scanned in spectral range of 200-400 nm against methanol as a baseline. Considerable response for all the drugs was noted at 240 nm. So, the wavelength picked for the quantification was 240 nm.

Method Validation

Validation of the developed HPTLC method was carried out as per the International Council for Harmonization [ICH] guidelines Q2 [R1] for below mentioned parameters [15].

Linearity

On a 10×10 cm stationary phase, the analysis was carried out. A calibration chart was graphed across a range of 100-600 ng/band. For the calibration chart, a Linomat 5 semiautomatic spotter was used to spot precisely measured mixed working solutions [1, 2, 3, 4, 5, and 6 µl]

ISSN 2515-8260 Volume 10, Issue 01, 2023

of TRI, BUD BEC, MOM and FLU on TLC plates. As mentioned in above section, the stationary phase was kept in the air to dry, developed in mobile phase chamber, and scanned. The linear regression was calculated by mapping peak areas vs concentrations for each spot to plot the calibration charts.

Precision

Method Precision

The repeatability of the spectrophotometer was determined by continuous observing and noting the response of solutions [n = 6] for TRI, BUD, BEC, MOM and FLU without modifying the parameter of the designed approach were used to verify the instrument's precision. The findings were expressed as % RSD.

Intermediate Precision

The designed approach's intra-day and inter-day variability were assessed by evaluating the relevant findings for varying concentrations of TRI, BUD, BEC, MOM and FLU three times on the same day and on different days. The outcomes are expressed as % RSD.

LOD and LOQ

The proposed method's LOD and LOQ were obtained by solving the below formulas.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the S.D. of the responses, S= Slope of calibration chart

Accuracy

The accuracy was determined by calculating TRI, BUD, BEC, MOM and FLU recoveries independently using the standard addition method. Known amounts of TRI, BUD, BEC, MOM and FLU standard solution was transferred to prequantified sample solutions at 50 %, 100 %, and 150 % accordingly. The recovered amount of standard powder of TRI, BUD, BEC, MOM and FLU was estimated by applying these values to the regression equation of the calibration curve.

Specificity

The method's specificity was determined by examining a standard substance and a sample. By studying the comparison of the Rf and spectra of bands to those of the standard, the presence of substance in samples was confirmed. By studying the comparison of at three different level of the spectra, namely at start [S], apex [M], and end [E] of peak positions of the band, the peak purity of substance was determined.

Solution Stability

The standard and sample solutions were kept in firmly closed container at working temperature to test the solution's stability. In 24 hours of storage, stability was assessed in time interval. Methanol was used to make both solutions. The observed results were compared to a freshly made solution. By matching peak area spot, the solution's stability was assessed.

Formulation Analysis

The prepared sample solution [10 µl] was spotted to the stationary phase and developed as stated above. The quantity of substances was calculated by integrating the area response into the regression equation.

III. RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

So far, there hasn't been a way for estimating TRI, BUD, BEC, MOM and FLU using HPTLC. The goal of this method was to establish an HPTLC approach for analysis of TRI, BUD, BEC, MOM and FLU in different formulations at the same time. To achieve improved separation and peak symmetry of TRI, BUD, BEC, MOM and FLU, many mobile phases were explored. Initially, plain solvents such as benzene, acetonitrile, toluene, n-hexane, chloroform, acetone and alcohol were examined as mobile phases based on existing TRI, BUD, BEC, MOM and FLU research articles with different drug combinations. When Triethylamine was added to the mobile phase, better separation and peak symmetry were attained. Better separation was achieved using the optimized mobile phase Toluene: Acetonitrile: Triethyl amine [6.5:3.5: 0.2, v/v/v] and stationary phase 10 x 10 cm Silica Gel G60F254 HPTLC plates, with Rf values of 0.30 for TRI, 0.46 for BUD, 0.55 for BEC, 0.71 for MOM and 0.82 for FLU. Methanol was used to pre-treat the TLC plates and activated at 110ºC for 5 minutes, both peaks seemed to be more symmetrical and more compact. At room temperature mobile phase was kept for 30 minutes to saturate in chamber, well-defined spots were obtained. For the quantification of both drugs, a wavelength of 240 nm was employed. Fig. 1 and 2, respectively show a typical densitogram and 3-D chromatogram exhibiting TRI, BUD, BEC, MOM and FLU peaks in various concentrations at 240 nm.

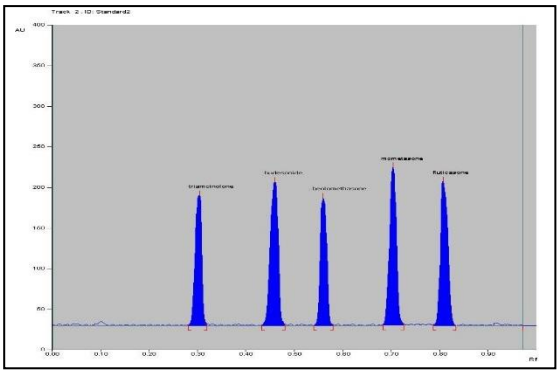

Fig. 1. Densitogram of band for TRI, BUD, BEC, MOM, and FLU [200 ng/band] at 240 nm

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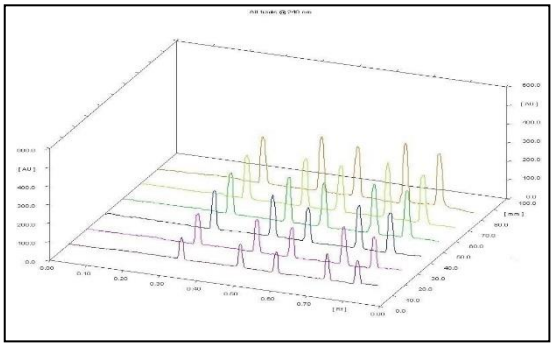

Fig. 2. 3-D chromatogram of TRI, BUD, BEC, MOM and FLU at 240 nm

Validation Parameters

The designed HPTLC approach was validated as per ICH Q2R1 factors like linearity & range, precision, recovery study, limit of detection and quantification. Validation was performed in compliance with ICH standards.

Linearity

The method's linearity was tested at different concentration standards, in 100-600 ng/band range for all drug. Over this concentration range, the calibration plot was linear, and the drug followed Beer's law of linearity. The high correlation coefficient [r^2=0.9995, 0.9984, 0.9996, 0.9997 and 0.9992] for TRI, BUD, BEC, MOM and FLU throughout this concentration range verified the calibration chart's linearity. Table I contains a list of all regression parameters.

Table I. Calibration data and regression parameters

PARAMETERS	TRI	BUD	BEC	MOM	FLU
Wavelength [nm]	240	240	240	240	240
Linear range [ng/band]	100-600	100-600	100-600	100-600	100-600
Regression Equation y = mX + C	y = 6.071x + 123.46	y = 6.8583x + 81.44	y = 5.5401x + 148.38	y = 8.0841x + 134.55	y = 6.5923x + 122.66
Intercept [C]	123.46	81.44	148.38	134.55	122.66
Slop [m]	6.071	6.8583	5.5401	8.0841	6.5923
Correlation coefficient [r^2]	0.9995	0.9984	0.9996	0.9997	0.9992

Precision

Method Precision

The precision of the approach was determined by spotting and recurrent studying of standard TRI, BUD, BEC, MOM and FLU having concentration 200 ng/band. The designed approach was proven to be precise, with low % RSD values.

Intermediate Precision

A study of intra-day variations and inter-day variations demonstrated intermediate precision. The % RSD was computed using replicate observations of three working standard concentrations [200, 300 and 400 ng/band] for all drug. The results shows %RSD values less than 2% indicates the high level of repeatability and precision.

LOD and LOQ

LOD and LOQ for TRI, BUD, BEC, MOM and FLU are found to be 1.92, 1.79, 3.06, 2.26, 7.33 ng/band and 5.83, 5.43, 9.29, 6.85, 22.23 ng/band, respectively indicates sensitivity of the method.

Accuracy

A recovery study was used to determine the method's accuracy. An accuracy study was repeated three times, with the % recovery between 98 – 102% and % RSD values less than 2% reveals the accuracy of the developed method.

Formulation Analysis

In order to quantify TRI, BUD, BEC, MOM and FLU in formulation using same mobile phase. The findings obtained for assay were closer to labelled content reveling that this approach is applicable for the quantification of all drugs in various nasal formulations using common mobile phase. [Table II].

Table II. Formulation Analysis

Drug	Label claim (µg/spray)	Qty. found (µg/spray) (n = 6)	% Label claim ± S. D (%)
TRI	55	53.79	98.24 ± 0.92
BUD	32	31.42	98.21 ± 1.04
BEC	50	49.44	98.88 ± 0.83
MOM	50	49.32	98.64 ± 1.07
FLU	50	50.07	100.1 ± 1.17

CONCLUSION

A HPTLC approach for estimating TRI, BUD, BEC, MOM and FLU in different formulations using common mobile phase has been designed and verified according to ICH standards. Based on the findings of the combined mixture analysis utilizing the provided approach, it can be stated that this technique has a linear response for 100-600 ng/band for all drug. The analytical findings demonstrate that the proposed approach could be used to quantify TRI, BUD, BEC, MOM and FLU in different formulations using common mobile phase.

The HPTLC wavelength approach described is simple, cost-effective, accurate, precise, and sensitive. The development of a less time-consuming, simple technique with improved sensitivity was prioritized. The key significance was given to design time- saving, economic, less usage of mobile phase and stationary phase and simple method with greater sensitivity and accuracy. As a result, the suggested technique can be adopted for routine analysis of TRI, BUD, BEC, MOM and FLU in different formulations using common mobile phase.

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REFERENCES

[1] Jeffrey A, and Berkowitz, "Decrements in vigilance and cognitive functioning associated with ragweed-induced allergic rhinitis," Annals of Allergy Asthma & Immunology, vol. 89, pp. 372–380, October 2002.

[2] D. M. Quillen, and D. B. Feller, "Diagnosing rhinitis: Allergic vs. Nonallergic," American Family Physician, vol. 73, pp. 1583–1590, May 2006.

[3] Custovic A, and Simpson A, "The role of inhalant allergens in allergic airways disease," J. Invest Allergy and Clin Immunology, vol. 22, pp. 393–401, October 2012.

[4] Phillips, and William R, "Screening for chronic obstructive pulmonary disease," JAMA, vol. 315, pp. 1372-1377, April 2016.

[5] M. J. O'Neil, "The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals," vol. ED-14, pp. 169-1650, October 2006.

[6] S. K. Gupta, B. Kumar, and P. K. Sharma, "Development and validation of a spectrophotometric method for estimation of Triamcinolone in solid dosage form," Asian J. Pharm. Ana., vol. 3, no. 2, pp. 42-43, June 2013.

[7] C Vedhi, R. Eswar, H. G. Prabu, and P. Manisankar, "Determination of Triamcinolone acetonide steroid on glassy carbon electrode by stripping voltametric methods," Int. J. Electro. Chem. Sci. vol. 3, no. 4, pp. 509-518, October 2008.

[8] R. B. Gudimitla, L. R. Atmakuri, and V. R. Jangala, "A novel method for the estimation of Budesonide in human plasma by using LC-MS/MS," Der Pharma Chemica. vol. 10, no. 4, pp. 181-185, April 2018.

[9] D. D. Sanap, A. M. Sisodia, S. H. Patil, and M. V. Janjale, "Novel and validated spectrophotometric determination of Budesonide from bulk and tablets using mixed hydrotropic solubilization technique," Int. J. Pharm. Sci. Res. vol. 2, no. 9, pp. 2419-23, September 2011.

[10] A. Singh, and M. Hinge, "Spectrophotometric determination of Beclomethasone Dipropionate in its pharm dosage form using quality by design approach," J. Pharm. Sci. Biosci. Res. vol. 10, no. 1, pp. 113-119, May 2020.

[11] A. S. Zanwar, D. B. Sen, and A. K. Seth, "Simultaneous estimation of Mometasone Furoate and Formoterol Fumarate by HPLC method in rotacaps," Int. J. Pharm. Pharm. Sci. vol. 11, no. 2, pp. 12-16, February 2019.

[12] V. S. Vichare, V. P. Choudhari and M. V. Reddy, "Simultaneous estimation of Mometasone Furoate and Salicylic Acid in topical formulation by UV-Visible spectrophotometry," Int. J. Chem. Sci. vol. 15, no. 2, pp. 129-136, May 2017.

[13] R. K. Godge, S. S. Satpute, and M. M. Sagar, "Development and validation of analytical method for simultaneous estimation of Formoterol Fumarate Dihydrate and Fluticasone Propionate from bulk and dry powder inhaler formulation," J. Drug. Delivery and Therapeutics. vol. 9, no. 3, pp. 212-222, June 2019.

[14] R. R. Kulkarni, D. G. Phadtare, and R. B. Saudagar, "UV spectrophotometric method development and validation of Fluticasone Propionate," Asian J. Res. Pharm. Sci. vol. 6, no. 2, pp. 135-138, June 2016.

[15] ICH Guideline Q2 (R1), "Validation of analytical procedures: text and methodology," November 2005.