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Research Article

A Sensitive High-Performance Thin Layer Chromatography Method for Simultaneous Determination of Salbutamol Sulphate and Beclomethasone Dipropionate from Inhalation Product

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ABSTRACT

Background: A sensitive high-performance thin layer chromatographic (HPTLC) method was developed for simultaneous determination of beclomethasone dipropionate and salbutamol sulphate from inhalation product.

Methods: Chromatographic separation was achieved on aluminium plates pre-coated with silica gel G60 F254 as the stationary phase and methanol:ethyl acetate:toluene:ammonia (3:1:3:0.15) as the mobile phase. The densitometric evaluation was carried out at 232 nm. The developed method was validated as per the ICH Q2 (R1) guidelines. Robustness of the proposed method was assessed using experimental design approach, and results were analyzed graphically. The results of sample analysis by proposed HPTLC method and reported HPLC method were statistically compared.

Results: The R_f value of salbutamol sulphate and beclomethasone dipropionate was found to be 0.38±0.02 and 0.72±0.02, correspondingly. The response in terms of peak area was linear over the concentration range of 100-500 ng/spot and 200-1000 ng/spot for beclomethasone dipropionate and salbutamol sulphate, individually, with the regression coefficient values greater than 0.99 for both the drugs. The limit of detection and limit of quantification for beclomethasone dipropionate were found to be 27 ng/spot and 84 ng/spot, respectively and for salbutamol sulphate were 40 ng/spot and 112 ng/spot, respectively. The proposed method was found to be statistically insignificant when compared to the reported method.

Conclusion: The proposed method is robust, sensitive, accurate and precise. The method can be applied for the simultaneous estimation of beclomethasone dipropionate and salbutamol sulphate from inhalation product.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the presence of chronic bronchitis that may lead to the development of airways obstruction.[1] The major symptom of chronic bronchitis is a daily cough and mucus production, at least three months a year for two consecutive years. COPD symptoms often do not appear until significant lung damage has occurred, and they usually worsen over time. The most important risk factor for COPD is cigarette smoking. Other risk factors may include air pollution, childhood infections, heredity, advanced age, airway hyperresponsiveness, and occupational exposures.[2] COPD remains a major public health problem. It is projected to rank third-leading cause of death in 2030 worldwide, according to a study published by the World Bank/World Health Organization.[3] Inhalation products containing bronchodilators, steroids, phosphodiesterase-4 (PDE4) inhibitors and combinations thereof are used in the treatment of COPD symptoms.[4]

Salbutamol sulphate (SAL) belongs to a class of drugs known as bronchodilators and used in the treatment of asthma and COPD symptoms.[5] Chemically, it is (RS)-4-[2-(tert-Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol (Figure 1). It is a short-acting β_2-adrenergic receptor agonist and highly selective to the receptors in bronchial muscle, resulting in bronchodilation. Beclomethasone dipropionate (BD) is a synthetic gluco-corticoid (Figure 1) with a potent anti-inflammatory activity and weak mineralocorticoid activity.[6] The combination of SAL and BD is used for the treatment of obstructive airways disease such as COPD.[7] Spectrophotometric,[8,9] square wave voltametric,[10] capillary electrophoresis with contactless conductivity detection[11] and RP-HPLC[12,13] methods are reported in the literature for determination of SAL in pharmaceutical formulation.

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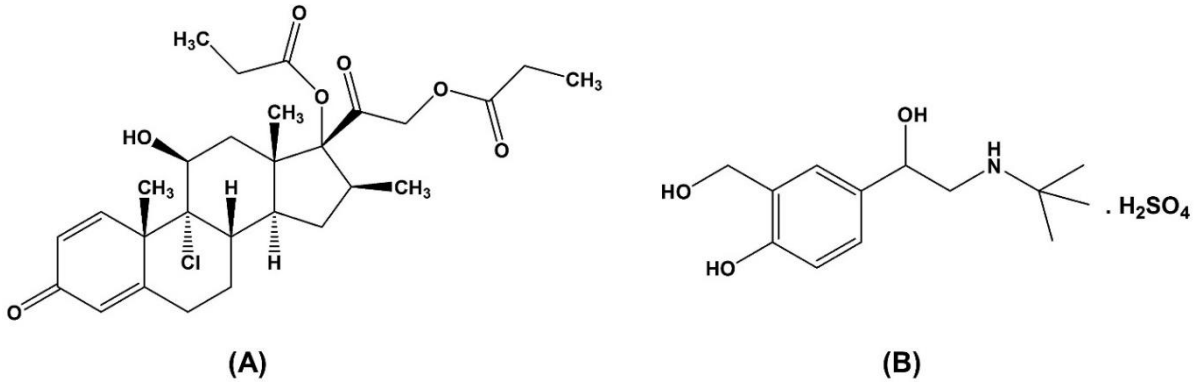

Figure 1. Chemical structure of (A) BD and (B) SAL.

Several methods are reported for simultaneous determination of components of fixed-dose combination products containing SAL and other drug substance in the form of pulmonary product.[14-16] The HPLC methods with fluorescent[17] and electrochemical[18,19] detection and LC-MS/MS method[20] are reported for determination of SAL in biological fluids such as plasma and urine. The HPLC methods are reported for assay of BD in bulk drug in Indian Pharmacopoeia,[21] British Pharmacopoeia[22] and European Pharmacopoeia.[23] The HPTLC,[24] HPLC[24] and UV spectrophotometric[25] methods are reported for determination of BD from combined inhalation products. Chemometric-assisted UV spectrophotometric,[26] UV/Visible spectrophotometric,[27] TLC-spectrodensitometric,[27] isocratic RP-HPLC[28] methods were reported for simultaneous determination of BD and SAL in inhalation preparations. The reported methods are less sensitive for assay and content uniformity testing of inhalation product containing BD and SAL. Therefore, the aim of the present work was to develop rapid and sensitive HPTLC method for simultaneous determination of BD and SAL in inhalation product. The developed HPTLC method was validated and found to be simple, rapid, sensitive and robust. The robustness testing is generally performed using one factor at a time (OFAT) approach, which requires a large number of experiments. The present paper describes the use of experimental design to predict the possible interactions between the factors with the limited number of experiments. Further, the design of experiment (DoE) approach supports the application of quality by design (QbD) to find factors negatively affecting the method. It was successfully applied for assay and content uniformity testing of the pharmaceutical formulation containing BD and SAL.

Materials and Methods

Instrumentation

The HPTLC system (Camag Sonnenmattstr, Mutenz, Switzerland) consisting of a Linomat V semi-automatic spotting device connected to a nitrogen cylinder, a glass twin-trough TLC chamber (20×10 cm), a TLC scanner-IV, a data station with winCATS (V 1.4.7) software and an HPTLC syringe (100 µL capacity; Hamilton Company, NV, USA) was used for thin layer chromatographic studies.

Chemicals

SAL (99.08 %w/w) was procured from Montage Laboratories, Himmatnagar, India. BD (99.20 %w/w) was supplied as gratis sample from Tripda Biotech Private Limited, Ahmedabad, India. Methanol, ethyl acetate, toluene and ammonia were purchased from Loba Chemicals, Mumbai, India. Analytical reagent grade solvents were used for HPTLC analysis. Marketed inhalation formulation Rotacaps containing BD and SAL (Label claim: BD 100 µg, SAL 200 µg in each Rotacap) was purchased from local pharmacy.

Chromatographic conditions

Separation was performed on pre-coated silica gel G60 F_{254} aluminum plates (20×10 cm) with 0.2 mm thickness (E. Merck, Darmstadt, Germany). Samples were spotted on the TLC plate in the form of band leaving 10 mm from the bottom edge using Linomat V semi-automatic spotter and analyzed using following parameters; bandwidth, 4 mm; track distance, 10 mm; migration distance, 40 mm; spraying rate, 150 nL/s; volume of mobile phase, 7.15 mL; temperature, 27±2 ˚C; chamber saturation time, 15 min; migration distance, 40 mm; slit dimension, 3.00×0.30 mm; scanning speed, 20 mm/s; detection wavelength, 232 nm. Mobile phase consisted of methanol: ethyl acetate: toluene: ammonia (3: 1: 3: 0.15).

Stock solutions

Accurately weighed SAL (20 mg) and BD (10 mg) were transferred to 10 mL volumetric flask, dissolved and diluted up to the mark with methanol to get the solution containing 2 mg/mL of SAL and 1 mg/mL of BD. Aliquot (1 mL) of stock solution is diluted up to 10 mL with methanol to prepare working standard solution containing 200 µg/mL of SAL and 100 µg/mL of BD.

Calibration curves

Aliquots (1, 2, 3, 4, and 5 µL) of working standard solution (corresponding to 100, 200, 300, 400, 500 ng/spot and 200, 400, 600, 800, 1000 ng/spot for BD and SAL, respectively) were spotted on a TLC plate and analyzed. Calibration curves were prepared by plotting peak area of BD and SAL against their respective concentrations.

Sample preparation

Assay

Twenty capsules were weighed and emptied. The capsule powder equivalent to 100 µg of BD and 200 µg of SAL was transferred to 10 mL volumetric flask. Five milliliters of methanol was added. The capsule shells were rinsed twice with methanol (3 mL), dried and weighed. The solution was sonicated for 15 min, diluted up to the mark with methanol and filtered using 0.45 µm filters (Millipore, Milford, MA, USA) to get the sample solution containing 10 µg/mL of BD and 20 µg/mL of SAL.

Content uniformity

Ten capsules were taken for content uniformity test. Each capsule was weighed and emptied. The content of each capsule was transferred to a series of 10 mL volumetric flasks. The capsule shells were rinsed with methanol (3 mL) and the rinsing solution was transferred to respective volumetric flask. The volume was made up to the mark with methanol. The mixtures were sonicated for 15 min. The resulting solution was filtered using 0.45 mm filters (Millipore, Milford, MA, USA) to get the sample solution containing 10 µg/mL of BD and 20 µg/mL of SAL.

Validation of the proposed method

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample solutions. The spot for BD and SAL in sample solution prepared from marketed formulation was confirmed by comparing the absorbance/reflectance spectrum with that of standard BD and SAL. The peak purity of BD and SAL was assessed by correlating the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) position of the spot.

Linearity

The linearity of BD and SAL was determined in the range of 100-500 ng/spot and 200-1000 ng/spot respectively. Five sets of such solutions were prepared and analyzed by plotting a calibration curve of mean peak area versus concentration. Standard deviation (SD), slope, intercept and correlation coefficient (r) of the calibration curves were calculated to ascertain linearity of the method.

Precision

The precision is measured by the degree of reproducibility and repeatability of analytical method. The precision of analytical method is expressed as a %RSD.

Repeatability of measurement of peak area was carried out by repeated scan of the same spot (300 ng/spot of BD and 600 ng/spot of SAL) seven times without changing the plate position. The % RSD for peak area was calculated. Repeatability of sample application is based on seven-time application of combined standard solution. The % RSD for peak area was computed. Variations of results within day (intra-day precision) and between days (inter-day precision) are called as reproducibility. The intra-day precision (% RSD) was determined by analyzing standard solution of BD and SAL for three times on the same day. The inter-day precision (% RSD) was determined by analyzing standard solution of BD and SAL for three days. The intra- and inter-day variation for determination of BD and SAL were carried out at three different concentration levels 200, 300, 400 ng/spot of BD and 400, 600, 800 ng/spot of SAL.

Accuracy

The accuracy studies were carried out through the recovery experiments at three levels of BD and SAL concentration in sample solution. Each level was determined in triplicate and % recoveries of BD and SAL were calculated. The average recoveries after the analysis were calculated along with SD.

Robustness

Robustness study was carried out using experimental design approach. Plackett-Burman design was applied on eight selected factors namely, (A) change in wavelength, nm (B) change in migration distance, cm (C) change in volume of toluene, mL (D) change in volume of ethyl acetate, mL (E) change in volume of methanol, mL (F) change in saturation time, min (G) change in slit dimension (H) change in band length. Each factor was studied at two levels (Table 1). The selection of factors and their levels were based on observations during method development and own experience. Experiments were designed using Design expert 9 software. Experiment was carried out according to design generated in the software. Standard solution containing BD (100 ng/spot) and SAL (200 ng/spot) was analyzed at each design experiment. The responses (R_f and peak area) were calculated for BD and SAL at each experiment designed. The experiment was repeated three times.

Limit of detection and limit of quantification

The measurement of signal to noise ratio approach was used for determination of LOD and LOQ.

Table 1. Eight factors and their levels for Placket-Burman experimental design for HPTLC.

Factors	Levels		
	(-)	Nominal (0)	(+)
(A) Change of wavelength (nm)	230	232	234
(B) Change in migration distance (cm)	3.5	4	4.5
(C) Change in volume of toluene (mL)	2.8	3	3.2
(D) Change in volume of ethyl acetate (mL)	0.8	1	1.2
(E) Change in volume of methanol (mL)	2.8	3	3.2
(F) Change in saturation time (min)	10	15	20
(G) Change in slit dimension	2.0×0.30	3.0×0.30	4.0×0.30
(H) Change in band length (mm)	2	4	6

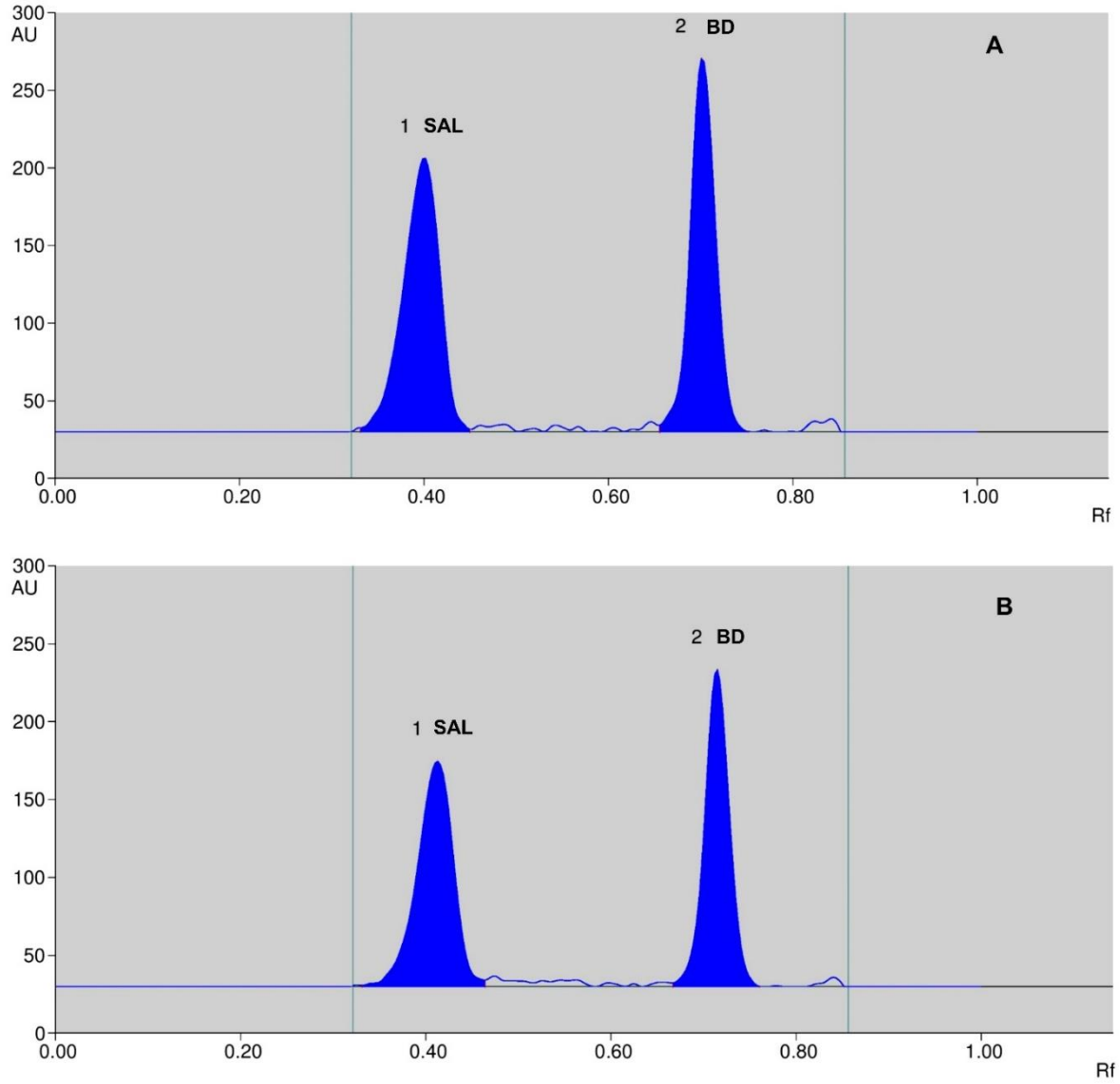

Figure 2. Densitogram of (A) Standard SAL; peak 1 (Rf: 0.38 ± 0.02) and BD; peak 2 (Rf: 0.72 ± 0.02). (B) Sample SAL; peak 1 (Rf: 0.38 ± 0.02) and BD; peak 2 (Rf: 0.72 ±0.02) measured at 232 nm, mobile phase methanol: ethyl acetate: toluene: ammonia (3: 1: 3: 0.15).

Signal to noise ratio of 3:1 and 10:1 were considered acceptable for estimating the detection limit and quantification limit respectively. Determination of the signal to noise ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank sample and the minimum concentration at which the analyte could be reliably detected.

Analysis of marketed formulations

Assay

Sample solution (10 µL) was spotted on the TLC plate and analyzed. The experiment was repeated 3 times. The peak areas of the spots were measured. The % content was calculated using straight line equation derived from calibration curves for BD and SAL.

Content Uniformity

Sample solution (10 µL) was spotted from each flask on TLC plate and analyzed. The peak areas of the spots were measured. The concentrations of BD and SAL from sample solutions were determined using linear regression equation.

Statistical comparison

Assay sample solutions were analyzed simultaneously by proposed HPTLC and reported HPLC[28] method. The results of both methods were compared by Student's *t*-test.

Results

Method development and optimization

To optimize the chromatographic conditions for the separation of BD and SAL, mobile phase composition, the effect of saturation time, and detection wavelength were investigated. Initially, trials for mobile phase optimization were carried out using experimental conditions: stationary phase, pre-coated silica gel G60 F_{254} aluminium sheets;

the standard solution, BD 200 ng/spot and SAL 400 ng/spot; detection wavelength, 254 nm; saturation time, 30 min. The solvent system consisting of methanol:ethyl acetate:toluene:ammonia (3:1:3:0.15) resulted in the separation of BD and SAL spots at R_f values of 0.38±0.02 and 0.72±0.02, respectively (Figure 2A). The chromatographic plate was developed up to 40 mm migration distance. Pre-saturation of TLC chamber with mobile phase for 15 min produced good reproducibility and peak shape. The photometric evaluation was performed at 232 nm. Quantitative determinations of BD and SAL were made by considering the peak areas from chromatograms and regression line equation using optimized conditions.

Method validation

Specificity

Comparison of chromatograms of standard solution and sample solution from formulation showed identical R_f values, i.e. 0.38±0.02 for SAL and 0.72±0.02 for BD (Figure 2). Comparison of the spectra scanned at peak start (S), middle (M) and end (E) showed a high degree of correlation (above 0.990) (Figure 3). This confirmed the purity of the corresponding spots. Furthermore, the spectrum of individual drug was compared to the spectrum of standard BD and SAL. The correlation obtained was 0.9991 for BD and 0.9997 for SAL; this confirmed the identity of spots. The excipients and other components present in the tablet did not interfere in the resolution of BD and SAL.

Linearity

The calibration curves for BD and SAL were found to be linear in the concentration range of 100-500 ng/spot for BD and 200-1000 ng/spot of SAL with correlation coefficients greater than 0.99. The linear regression equations were found to be y = 5.76x+1077.3 for BD and y = 2.38x+444.96 for SAL, where, y – peak area and x – concentration in ng/spot.

Precision

The repeatability (% RSD) of sample application was found to be 1.54 and 1.97 for BD and SAL, respectively. The scanner precision (% RSD) for measurement of peak area was found to be 0.30 and 0.58 for BD and SAL, respectively. The repeatability studies ensured precision of scanner and spotting devices. The % RSD for intra-day precision was found to be 0.12-0.31 and 0.28-0.45 for BD and SAL, respectively (Table 2). The % RSD for inter-day precision was found to be 0.86-1.75 and 0.81-3.18 for BD and SAL, respectively (Table 2).

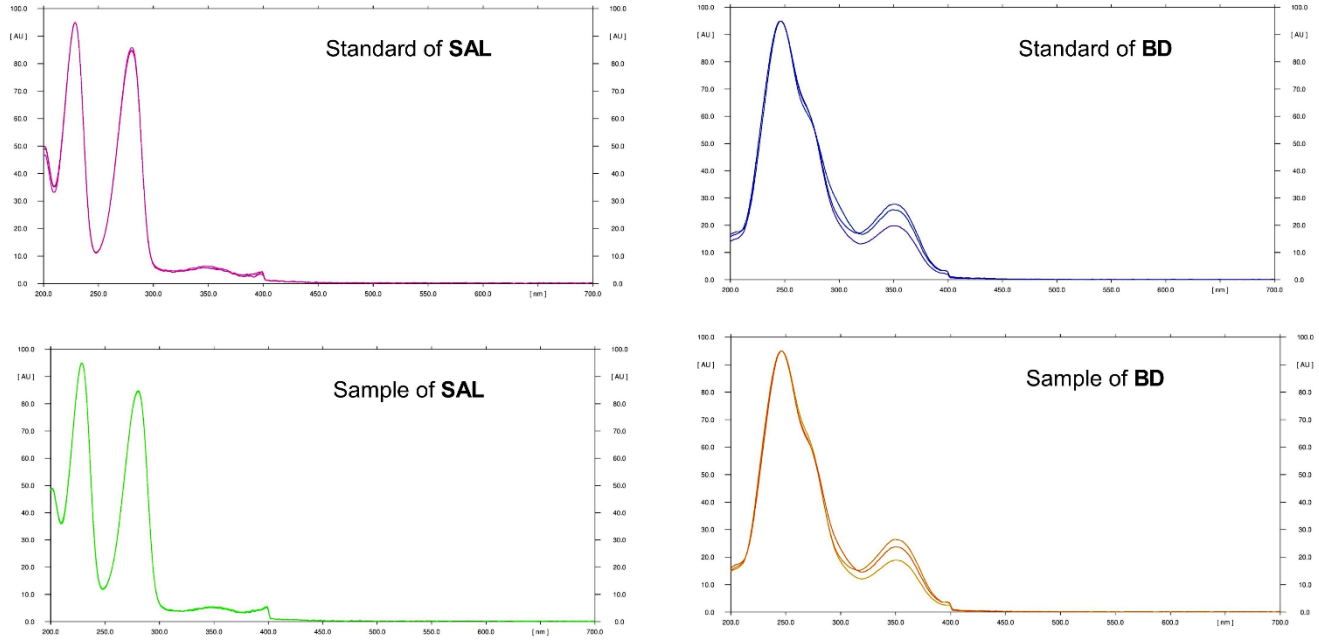

Figure 3. Peak purity spectrum of standard and sample BD and SAL.

Table 2. Intra- and Inter-day Precisions of BD and SAL (*n*=3).

Drug	Amount taken (ng/spot)	Intra-day precision		Inter-day precision	
		Amount Found[a] ±SD	% RSD	Amount Found[a] ±SD	% RSD
BD	200	200.91±0.25	0.12	202.15±2.59	1.28
	300	301.57±0.46	0.15	301.57±0.46	1.75
	400	398.98±1.23	0.31	398.98±1.23	0.86
SAL	400	399.56±1.13	0.28	400.21±3.26	0.81
	600	601.05±2.06	0.34	602.35±18.61	3.09
	800	802.39±3.64	0.45	804.09±25.54	3.18

[a] Average of three determinations

Table 3. Recovery study for proposed HPTLC method (*n*=3).

Content of drug in sample (ng/spot)		Drug spiked (ng/spot)		Theoretical content (ng/spot)		% Recovery		% RSD	
BD	SAL	BD	SAL	BD	SAL	BD	SAL	BD	SAL
150	300	50	100	200	400	99.50	102.00	2.65	1.61
150	300	150	300	300	600	101.56	102.66	1.17	2.84
150	300	250	500	400	800	100.42	99.54	1.51	2.65

Accuracy

Accuracy of the developed method was calculated by performing recovery studies. Proposed method was employed for estimation of amount of BD and SAL from pre-analyzed sample solutions spiked at three different levels of standard. Results of recovery studies are shown in Table 3. The % recoveries were found out to be 99.50–101.56 % for BD and 99.54–102.66 % for SAL.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LODs and LOQs were found to be 27 ng/spot and 84 ng/spot for BD and 40 ng/spot, 112 ng/spot for SAL, respectively.

Robustness

Plackett-Burman design was utilized in order to test robustness of developed HPTLC method. In the present study, eight factors were tested for twelve experiments. The total 12 experimental plans of PB design and the corresponding responses are summarized in Table 4. The obtained Pareto charts are presented in Figure 4.

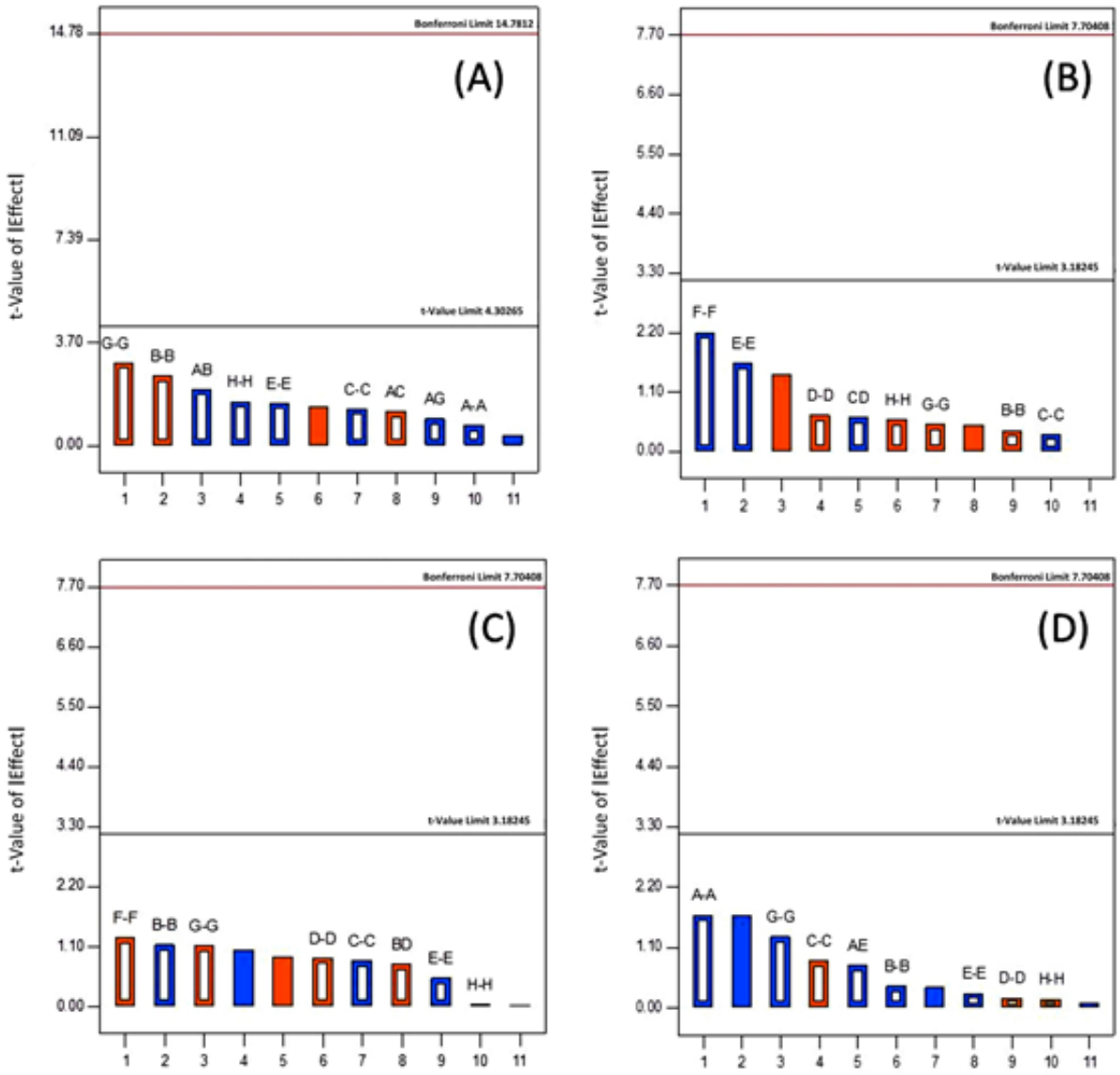

Figure 4. Pareto chart showing effect of various HPTLC parameters on (A) Rf of BD, (B) Rf of SAL, (C) peak area of BD and (D) peak area of SAL.

Table 4. Twelve Experiment Plackett-Burman (PB) design to examine the eight factors (A-H).

Experiments	Factors								Responses			
									Peak Area		R_f value	
	A	B	C	D	E	F	G	H	BD	SAL	BD	SAL
1	-1	+1	-1	+1	+1	-1	+1	+1	1783	789	0.78	0.50
2	+1	+1	+1	-1	-1	-1	+1	-1	1723	755	0.69	0.46
3	+1	-1	+1	+1	+1	-1	-1	-1	1704	764	0.70	0.35
4	-1	-1	+1	-1	+1	+1	+1	-1	1811	763	0.75	0.34
5	+1	-1	-1	-1	+1	-1	+1	+1	1733	655	0.70	0.36
6	-1	+1	+1	-1	+1	+1	+1	-1	1633	729	0.68	0.35
7	-1	-1	-1	+1	-1	+1	+1	-1	1799	690	0.69	0.39
8	+1	+1	-1	-1	-1	+1	-1	+1	1728	713	0.76	0.38
9	+1	+1	-1	+1	+1	+1	-1	-1	1775	678	0.67	0.35
10	+1	-1	+1	+1	-1	+1	+1	+1	1830	731	0.73	0.40
11	-1	+1	+1	+1	-1	-1	-1	+1	1642	796	0.72	0.39
12	-1	-1	-1	-1	-1	-1	-1	-1	1733	809	0.69	0.37

Analysis of marketed formulation

The spots at R_f value 0.38 (for BD) and 0.72 (for SAL) was observed in the densitogram of the drug samples extracted from capsule. Amounts of BD and SAL were calculated using linear regression equation derived. The % assay was found to be 98.44 % for BD and 99.98 % for SAL (Table 5). Content uniformity testing for ten capsules was found in the range of 94–104 % for BD and 90–99 % for SAL. The acceptable value (AV) was calculated for each of the capsules and was found to be within the acceptable range (LI). The results of statistical comparison between proposed HPTLC method and reported HPLC method[28] are shown in Table 5. The *t* calculated values were lower than *t* tabulated values obtained from Student's distribution table for a risk factor of 5%.

Discussion

Method development and optimization

Various solvent systems composed of toluene, methanol, ethyl acetate, hexane, acetone or mixtures thereof were tried for optimization of mobile phase for HPTLC separation of BD and SAL. Both, BD and SAL, did not separate in the mixture of polar solvents such as methanol, acetone or water. Solvent mixture like ethyl acetate:hexane (5:4 v/v), methanol: toluene (6:3 v/v) showed R_f value less than 0.2 for SAL whereas mobile phases, acetone:ethyl acetate:hexane (8:0.5:1.5), methanol:ethyl acetate:toluene:ammonia (1:1.5:3:1) resulted in R_f value more than 0.85 for BD. Different ratio of methanol, ethyl acetate and toluene were tired and the mixture of methanol:ethyl acetate:toluene (3:1:3) was optimized for better separation of BD and SAL spots with the addition of 0.15 mL of ammonia for improvement of peak shape of both the drugs.

The densitometric scanning at 232 nm, the iso-absorptive point determined from overlain absorbance/reflectance spectra of BD and SAL (Figure 5), resulted in increased sensitivity of the method. The optimization of chamber saturation time at 15 min and migration distance at 40 mm resulted in rapid development of densitogram and quick analysis of samples. Thus, the proposed method allows determination of several samples on a TLC plate at the same time.

Method Validation

The goal of the study was to develop sensitive and rapid chromatographic method for simultaneous determination of BD and SAL in quality control of the pharmaceutical formulation containing BD and SAL. The proposed method was validated according to the ICH guidelines.[29,30]

The identical R_f values and absorbance/reflectance spectra for BD and SAL between standard and sample track proved the specificity of the method. Further, the peak purity analysis confirmed the non-interference of excipients in the analysis of BD and SAL using the proposed method. The high value of correlation coefficient (>0.99) and the SD for intercept value (<2%) of regression line ascertained the linearity of calibration graphs for BD and SAL. The repeatability studies ensured precision of sample application device and densitometric scanner of HPTLC system. The method was found to be precise based on % RSD values for intra-day and inter-day precision studies. The proposed method showed acceptable % recoveries when used for extraction and subsequent estimation of BD and SAL from inhalation product after spiking with the additional drug. The proposed method was tested for robustness using experimental design approach.

Table 5. Statistical comparison of the results obtained by application of proposed HPTLC method and the reported HPLC method for assay of BD and SAL from marketed formulation.

Parameter	BD		SAL	
	Proposed HPTLC method	Reported HPLC method (28)	Proposed HPTLC method	Reported HPLC method (28)
Label claim (µg)	100	100	200	200
Drug content (%) ±SD	98.44±1.57	99.12±0.89	99.98±2.51	99.49±1.01
d.f.	4		4	
***t* calculated**	-1.42		0.77	
***t* tabulated**	2.13			

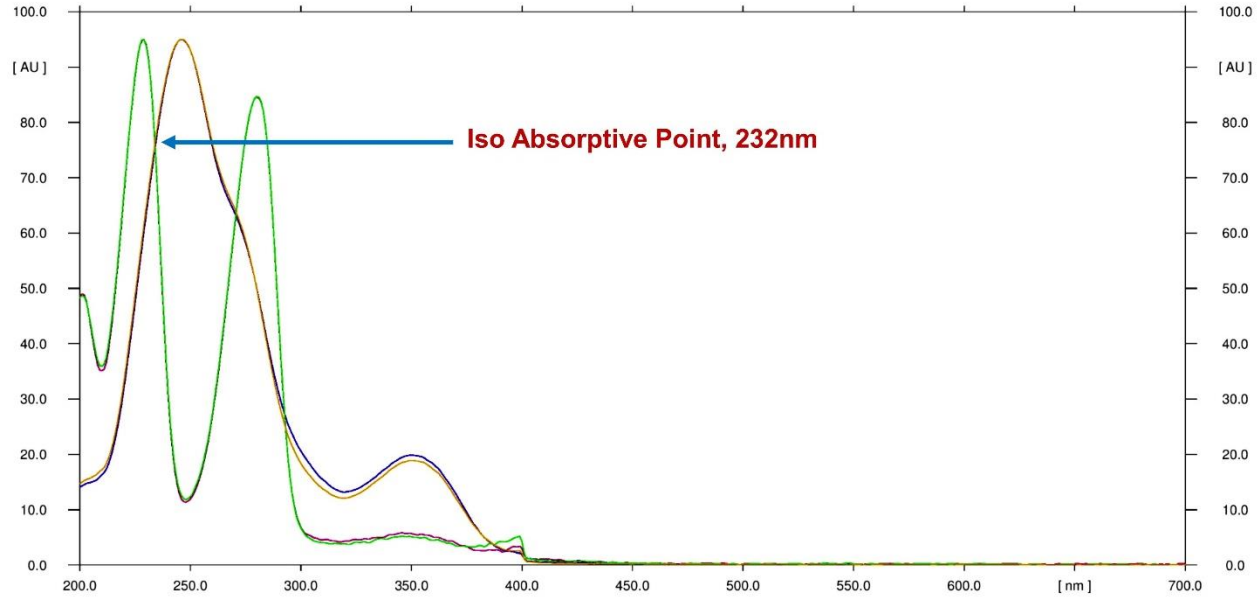

Figure 5. Overlain absorbance/reflectance spectra of BD and SAL.

Table 6. Comparison of method validation parameters of proposed method and reported method.

Parameter	Proposed HPTLC method		Reported TLC-densitometric method[27]	
	BD	SAL	BD	SAL
Range	100-500 ng/spot	200-1000 ng/spot	2-7 µg/spot	2-9 µg/spot
Intra-day precision (%RSD)	0.18	0.38	1.134	0.667
Inter-day precision (%RSD)	1.31	2.00	0.955	1.212
% Recovery	100.49	99.54	101.35	99.07
LOD	27 ng/spot	40 ng/spot	0.277 µg/spot	0.382 µg/spot
LOQ	84 ng/spot	112 ng/spot	0.992 µg/spot	1.272 µg/spot

The results of robustness testing were evaluated using Pareto Charts. It may be more comfortable viewing the Pareto Chart that has the significant effects selected. The Pareto graphs consist of bars with a length proportional to the absolute value of the estimated effect, divided by the pseudo-standard error defined by Lenth (Lenth's PSE). All main effects and interaction terms are found to be statistically as absolute values of main effects are below the critical t-value which indicates that the small deliberate changes to the method do not affect the results. Thus, it proves the method is robust.

The figures of merit such as linearity range, inter-day precision, intra-day precision, recovery, limit of detection and limit of quantitation of the proposed method are compared with those of the reported method[27] (Table 6). The proposed method is found to be linear over wider dynamic range, sensitive, accurate and precise as compared to reported method.

Analysis of marketed formulation

Both the drugs have good solubility in methanol; therefore, methanol was selected for the extraction of drugs from the formulation. The formulation powder was sonicated with methanol to ensure complete dissolution of drugs. No interfering peaks were found in the chromatograms of sample solutions. The method was found to be suitable for content uniformity testing. The results of statistical comparison between HPTLC and HPLC methods showed that there was no statistically significant difference between two analytical methods.

Conclusion

The proposed HPTLC method provides precise, accurate and reproducible quantitative analysis for the simultaneous determination of BD and SAL in inhalation product. The method was validated as per the ICH guidelines. The method was found to be linear in the range of 100-500 ng/spot for BD and 200-1000 ng/spot for SAL. The robustness of the proposed methods was studied using DoE and found to be robust to deliberate changes made in experimental conditions. It can be concluded that the developed method is simple, accurate, sensitive and precise. The HPTLC method is also applicable for the content uniformity test of marketed formulation. The method is suitable for routine analysis of BD and SAL in their commercial dosage form.

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Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Snider GL. Chronic obstructive pulmonary disease: risk factors, pathophysiology and pathogenesis. Annu Rev Med. 1989;40(1):411-29. doi:10.1146/annurev.me.40.020189.002211
2. Edelman NH, Kaplan RM, Buist AS, Cohen AB, Hoffman LA, Kleinhenz ME, et al. Chronic Obstructive Pulmonary Disease. Chest. 1992;102:243S-56S.
3. WHO (2008) World health statistics 2008. World Health Organization.
4. Singh D. Pharmacological treatment for COPD; GOLD 2017 changes direction. Br J Clin Pharmacol. 2017;83(5):935-7. doi:10.1111/bcp.13212
5. Aliverti A, Rodger K, Dellacà RL, Stevenson N, Mauro AL, Pedotti A, et al. Effect of salbutamol on lung function and chest wall volumes at rest and during exercise in COPD. Thorax, 2005;60(11):916-24. doi:10.1136/thx.2004.037937
6. Dompeling E, Van Schayck CP, Molema J, Folgering H, Van Grunsven PM, Van Weel C. Inhaled beclomethasone improves the course of asthma and COPD. Eur Respir J. 1992;5(8):945-52.
7. Angus R, Reagon R, Cheesbrough A. Angus R, Reagon R, Cheesbrough A. Short-acting β2-agonist and oral corticosteroid use in asthma patients prescribed either concurrent beclomethasone and long-acting β2-agonist or salmeterol/fluticasone propionate combination. Int J Clin Pract. 2005;59(2):156-62. doi:10.1111/j.1742-1241.2005.00455.x
8. Sadler NP, Jacobs H. Application of the Folin-Ciocalteau reagent to the determination of salbutamol in pharmaceutical preparations. Talanta. 1995;42(10):1385-88. doi:10.1016/0039-9140(95)01534-i
9. Mishra AK, Kumar M, Mishra A, Verma A, Chattopadhyay P. Validated UV spectroscopic method for estimation of salbutamol from tablet formulations. Arch Appl Sci Res. 2010;2(3):207-11.
10. Edelman NH, Kaplan RM, Buist AS, Cohen AB, Hoffman LA, Kleinhenz ME, et al. Chronic Obstructive Pulmonary Disease. Chest. 1992;102(3):243S-56S. doi: 10.1378/chest.102.3_Supplement.243S
11. Felix FS, Quintino MS, Carvalho AZ, Coelho LH, do Lago CL, Angnes L. Determination of salbutamol in syrups by capillary electrophoresis with contactless conductivity detection (CE-C 4 D). J Pharm Biomed Anal. 2006;40(5):1288-92. doi:10.1016/j.jpba.2005.09.027
12. Ray S, Bandopadhyay A. Reversed phase high performance liquid chromatographic determination of salbutamol sulphate in pharmaceutical formulations. Indian Drugs. 1990;27:313-16.
13. Beaulieu N, Cyr TD, Lovering EG. Liquid chromatographic methods for the determination of albuterol (salbutamol), albuterol sulphate and related compounds in drug raw materials, tablets and inhalers. J Pharm Biomed Anal. 1990;8(7):583-9. doi:10.1016/0731-7085(90)80084-3
14. Chitlange SS, Chaturvedi KK, Wankhede SB. Development and validation of spectrophotometric and HPLC method for the simultaneous estimation of salbutamol sulphate and prednisolone in tablet dosage form. J Anal Bioanal Tech. 2011;2(1):1-5.
15. Blewett AJ, Varma D, Gilles T, Butcher R, Jacob J, Amazan J, et al. Development and validation of a stability-indicating high-performance liquid chromatography method for the simultaneous determination of albuterol, budesonide, and ipratropium bromide in compounded nebulizer solutions. J AOAC Int. 2011;94(1):110-7.
16. Kasawar GB, Farooqui M. Development and validation of a stability indicating RP-HPLC method for the simultaneous determination of related substances of albuterol sulfate and ipratropium bromide in nasal solution. J Pharm Biomed Anal. 2010;52(1):19-29. doi:10.1016/j.jpba.2009.11.026
17. Hutchings MJ, Paull JD, Morgan DJ. Determination of salbutamol in plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl. 1983;277:423-6. doi:10.1016/s0378-4347(00)84870-7
18. Kurosawa N, Morishima S, Owada E, Ito K. Reversed-phase high-performance liquid chromatographic determination of salbutamol in rabbit plasma. J Chromatogr B Biomed Sci Appl. 1984;305:485-8. doi:10.1016/s0378-4347(00)83366-6
19. Tan YK, Soldin SJ. Determination of salbutamol in human serum by reversed-phase high-performance liquid chromatography with amperometric detection. J Chromatogr B Biomed Sci Appl. 1984;311:311-7. doi:10.1016/s0378-4347(00)84724-6
20. Chan SH, Lee W, Asmawi MZ, Tan SC. Chiral liquid chromatography–mass spectrometry (LC–MS/MS) method development for the detection of salbutamol in urine samples. J Chromatogr B. 2016;1025:83-91. doi:10.1016/j.jchromb.2016.05.015
21. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Vol 2, 6th ed. Ministry of Health and Family Welfare, Government of India. Ghaziabad; 2010. pp. 873-1386.
22. British Pharmacopoeia Commission. British Pharmacopoeia, Secretariat of the Medicines and Healthcare Products Regulatory Agency, London;2011. pp. 219-963.
23. Council of Europe's European Directorate for the Quality of Medicines and Healthcare (EDQM) and European Medicines Agency joint meeting on raw materials used for the production of cell-based and gene-therapy products, Council of Europe: European Directorate for the Quality of Medicines (EDQM). European Pharmacopoeia. Vol 2. Strasbourg; 2011. pp. 1449-2067.
24. Parmar VK, Patel HN, Patel BK. Sensitive and Robust Methods for Simultaneous determination of beclomethasone dipropionate and formoterol fumarate

dihydrate in rotacaps. J Chromatogr Sci. 2014;52(10):1255-66. doi:10.1093/chromsci/bmt208
25. Blanco M, Serrano D, Bernal JL. UV-spectrophotometric determination of beclomethasone dipropionate and phenylethyl alcohol in a nasal spray by inverse least-squares regression. Talanta. 1999;50(3):527-32. doi:10.1016/s0039-9140(99)00141-1
26. Gandhi SV, Pahade AR, Sutar AS, Kuchekar BS, Tapale SR. Simultaneous estimation of beclomethasone dipropionate and salbutamol sulphate in capsules by chemometric assisted UV-spectrophotometric method. International Journal of Pharmaceutical Chemistry and Analysis. 2016;3(2):84-9. doi:10.5958/2394-2797.2016.00013.7
27. Samir A, Lotfy HM, Salem H, Abdelkawy M. Development and validation of simultaneous spectrophotometric and TLC-spectrodensitometric methods for determination of beclomethasone dipropionate and salbutamol in combined dosage form. Spectrochim Acta A Mol Biomol Spectrosc. 2014;128:127-36. doi:10.1016/j.saa.2014.02.044
28. Martis EA, Gangrade DM. Reverse phase isocratic HPLC method for simultaneous estimation of salbutamol sulphate and beclomethasone dipropionate in rotacaps formulation dosage forms. Int J Pharm Pharm Sci. 2011;3(1):64-7.
29. ICH, Q2A; Harmonised tripartite guideline, text on validation of analytical procedures, IFPMA. In Proceedings of the International Conference on Harmonization, Geneva; 1994. pp. 1-5.
30. ICH, Q2B; Harmonised tripartite guideline, validation of analytical procedure: methodology, IFPMA In Proceedings of the International Conference on Harmonization, Geneva; 1996. pp. 1-8