Research Paper

Development and Validation of HPTLC Method for Simultaneous Estimation of Budesonide and Levalbuterol Hydrochloride in their Combined Pharmaceutical Dosage Forms

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ABSTRACT

An accurate, specific and precise HPTLC method has been developed for the simultaneous estimation of Budesonide and Levalbuterol hydrochloride in their combined pharmaceutical dosage form. The chromatographic separation of Budesonide and Levalbuterol Hydrochloride was performed using aluminium plate precoated with silica gel 60 F_{254} as stationary phase and toluene: methanol : triethylamine (7 : 3 : 0.2, v/v/v) as mobile phase. The quantification was carried out at 275 nm. The Rf values were found to be 0.38 ± 0.02 and 0.61 ± 0.02 for Levalbuterol Hydrochloride and Budesonide respectively. The linearity was observed in range of 200-1000 ng/spot for Budesonide and 500-2500 ng/spot for Levalbuterol Hydrochloride. The correlation coefficient (R^2) was found to be0.9930 and 0.9960 for Budesonide and Levalbuterol Hydrochloride respectively. The method was validated for precision, accuracy, LOD and LOQ as per ICH guideline. The method was applied for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride in their combined pharmaceutical dosage form. The assay results were found to be 99.12%± 0.36 for Budesonide and 98.99%±0.47 for Levalbuterol Hydrochloride of percentage label claim of their combined pharmaceutical dosage form.

Keywords: High Performance Thin Layer Chromatography, Budesonide, Levalbuterol Hydrochloride.

1. INTRODUCTION:

Budesonide is designated chemically as (RS)-11-β, 16-α, 17, 21-tetrahydroxypregna-l, 4-diene-3, 20-dionecyclic 16, 17-acetal butyraldehyde.[1]Chemical structure of Budesonide is shown in Figure 1a. Budesonide is a second generation glucocorticoid, exhibits high affinity to the corticosteroid receptors with a high ratio of topical to systemic anti-inflammatory activity. Glucocorticoids have effects such as gluconeogenesis, proteolysis, lipolysis, suppression of inflammation and immune responses. It has poor systemic availability due to extensive first pass metabolism in the liver. Therefore, Budesonide is used for the treatment of asthma by inhalation administration to reduce inflammation of airways smooth muscle.[2,3]

Levalbuterol Hydrochloride (also known as Levosalbutamol Hydrochloride) is the R - enantiomer of short acting β_2-adrenergic receptor agonist of Salbutamol (Albuterol). Chemically it is designated as (R)-α1-[[(1,1-

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dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol hydrochloride.[1]Chemical structure of Levalbuterol Hydrochloride is shown in Figure 1b. Levalbuterol leads to activation of β_2-adrenergic receptors on airways smooth muscle resulting in muscle relaxation and bronchodilatation. Levalbuterol relaxes the smooth muscle of all airways, from the trachea to the terminal bronchioles. Levalbuterol Hydrochloride is a highly selective β_2 agonist; cardiac side effects are less prominent. Selectivity is further increased by inhaling the drugs. Inhaled Levalbuterol Hydrochloride is used to abort and terminate attacks of asthma. [2,3]

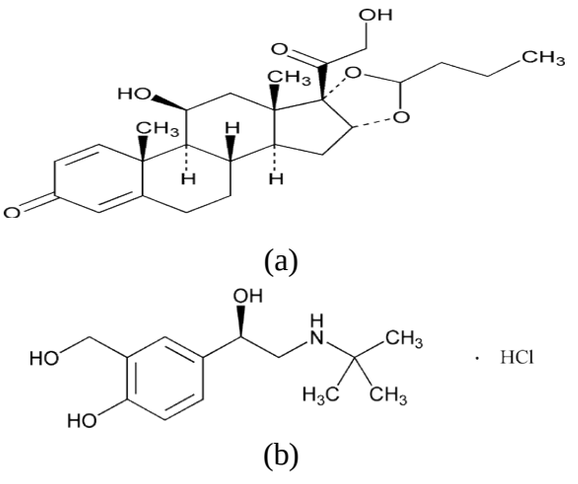

Figure 1: Chemical structure of a) Budesonide b) Levalbuterol Hydrochloride

The literature review described HPLC,UV-Visible spectrophotometric, LC-MS method for determination of Budesonide individually and combination with other drugs in their pharmaceutical dosage form.[4-22]The literature review described HPLC and UV-Visible spectrophotometric method for determination of Levalbuterol Hydrochloride individually and combination with other drugs in their pharmaceutical dosage form.[23-32] The literature review also described stability indicating HPLC methods for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride in combined dosage form.[34]. But there was no reported HPTLC method for simultaneous estimation of Budesonide and Levalbuterol hydrochloride in their combined dosage forms. So, it was thought of interest to develop and validate HPTLC method for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride in their combined dosage form. The present work deals with simultaneous estimation of these drugs in combined dosage form by HPTLC method.

2. MATERIALS AND METHOD:

2.1. Instrumentation:

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

2.2. Chemicals and reagents:

Budesonide was kindly supplied as a gift sample by Sun Pharma Ltd, Ahmednagar, Maharashtra, India and Levalbuterol Hydrochloride was kindly supplied by Cure Life Science, Surat, Gujarat, India. All chemicals and reagents used were of LR grade and purchased from s.d. Fine-Chem Limited, Mumbai, India. Budesal respule (2 ml, Manufacturer – Cipla Ltd.), containing Budesonide 0.5 mg and Levalbuterol Hydrochloride 1.25 mg was procured from local pharmacy.

2.3. Chromatographic conditions:

Chromatographic separation was performed on 10 × 10 cm aluminium plates pre coated with 250 µm layer of silica gel 60 F_{254} (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 60 °C for 5 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 100 nl/s. The TLC plate was developed in twin through chamber using toluene : methanol : triethylamine (7 : 3 : 0.2, v/v/v) as

mobile phase at temperature, 27 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 275 nm.

2.4. Preparation of solutions:

2.4.1. Preparation of stock solution of Budesonide:

Accurately weighed 10mg of standard Budesonide was transferred into 10 ml volumetric flask, dissolved in methanol and diluted upto mark with methanol to get standard stock solution having strength of 1000 µg/ml of Budesonide.

2.4.2. Preparation of stock solution of Levalbuterol Hydrochloride:

Accurately weighed 10 mg standard Levalbuterol Hydrochloride was transferred into 10 ml volumetric flask, dissolved and diluted upto mark with methanol to get standard stock solution having strength of 1000 µg/ml of Levalbuterol Hydrochloride.

2.4.3. Preparation of combined working standard solution:

The mixed working standard solution was prepared by mixing of 0.4 ml of Budesonide standard stock solution and 1 ml of Levalbuterol Hydrochloride standard stock solution into 10 ml volumetric flask and diluted up to mark with methanol to get a solution having strength of 40 µg/ml of Budesonide and 100 µg/ml of Levalbuterol Hydrochloride.

2.4.4. Procedure for calibration curve:

From combined working standard solution, 5, 10, 15, 20 and 25 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Calibration curve was obtained by plotting peak area against respective concentration of both drugs.

2.5. Method Validation:

2.5.1. Specificity:

From combined working standard solution of Budesonide and combined marketed formulation, 15 µl were spotted on same TLC plate. The TLC plate was developed and dried as described under chromatographic conditions [Section 2.3]. The spot of Budesonide and Levalbuterol Hydrochloride from standard and marketed formulation were scanned in range of 200 nm to 700 nm to obtain in situ UV spectrum of all spots. The spot of Budesonide and Levalbuterol Hydrochloride from marketed formulation were confirmed by comparing its R_f values and reflectance-absorbance spectrum with that of standard Budesonide and Levalbuterol Hydrochloride. The peak purity of Budesonide and Levalbuterol Hydrochloride was determined by correlating the spectra of Budesonide and Levalbuterol Hydrochloride scanned at peak start, peak apex and peak end position of the spot.

2.5.2. Linearity:

From combined working standard solution 5, 10, 15, 20 and 25 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Linearity of proposed method was evaluated by repeating same procedure for five times. Calibration curve for Budesonide and Levalbuterol Hydrochloride was obtained by plotting graph of mean peak area of five determinations vs. respective concentration of both drugs. The correlation coefficient and regression line equation was calculated.

2.5.3. Precision:

2.5.3.1. Repeatability of sample application:

From combined working standard solution, 15 µl was spotted seven times on a same TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The peak area of seven spots was measured and the %RSD of peak area was calculated for both standard drugs.

2.5.3.2. Repeatability of measurement of peak area:

From combined working standard solution, 15 µl was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The spots were scanned for seven times without changing plate position and %RSD for measurement of peak area was calculated for both standard drugs.

2.5.3.3. Intraday precision:

From combined working standard solution, 5, 10, 15, 20 and 25 µl were spotted on TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Same procedure was carried out three times on same day and % RSD of peak area was calculated for both standard drugs.

2.5.3.4. Interday Precision:

From combined working standard solution, 5, 10, 15, 20 and 25 µl were spotted on TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Same procedure was carried out on three consecutive days and % RSD of peak area was calculated for both standard drugs.

2.5.4. Accuracy:

The accuracy was determined by standard addition method. The proposed method was applied for estimation of Budesonide and Levalbuterol Hydrochloride in their combined dosage forms. The recovery experiment was carried out in triplicate by spiking previously analysed sample i.e. 300 ng/spot of Budesonide and 750 ng/spot of Levalbuterol Hydrochloride with different concentration of both standard drugs at 80%, 100% and 120%.The percentage recovery of Budesonide and Levalbuterol Hydrochloride were calculated at each level.

2.5.5. Limit of detection and limit of quantification:

LOD and the LOQ of the method were calculated using the following equations as per ICH guideline Q2 (R1).

$$LOD = 3.3\ N/S \qquad LOQ = 10\ N/S$$

Where, N = Standard deviation of intercepts of five calibration curves

S = Mean slope of five calibration curves

Table 1: Summary of validation parameters

Sr. No	Parameters	Results	
		BUD	LEVA
1	Linearity Range (ng/spot)	200-1000	500-2500
2	Correlation coefficient (R^2)	0.9930	0.9960
3	Precision (%RSD)		
	Repeatability of measurement of peak area (n=7)	0.96	1.05
	Repeatability of sample application (n=7)	1.02	1.13
	Intraday (n=3)	0.93 – 1.27	1.03 – 1.41
	Interday (n=3)	1.57 – 1.78	1.56 – 1.83
4	Accuracy (% Recovery)	98.47 – 99.08	98.42 – 99.87
5	Limit of Detection (ng/spot)	15.88	33.12
6	Limit of Quantitation (ng/spot)	48.12	100.38
7	Specificity	Specific	Specific

2.6. Procedure for assay of marketed formulation:

Twenty respules were taken and solution of respules was collected, weighed and mixed. From combined repsule solution, aliquot equivalent to 0.5 mg of Budesonide was accurately weighed and transferred in 10 ml volumetric flask, mixed with 5 ml of methanol and diluted up to mark with the same. From resulting solution, 12 µl was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Amount of Budesonide and Levalbuterol Hydrochloride from marketed formulation was calculated using calibration curve data.

3. RESULTS AND DISCUSSION:

3.1. Selection of wavelength:

Standard Budesonide and Levalbuterol Hydrochloride were spotted on TLC plate. The TLC plate was developed and dried as described under chromatographic conditions [Section 2.3]. Both spots were scanned from 200 nm to 700 nm to obtain in situ UV spectrum. The overlain UV spectra of Budesonide and Levalbuterol Hydrochloride (figure 2) indicate that both drugs were showed reasonable absorbance at 275 nm wavelength. So, 275 nm was selected as wavelength for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride.

3.2. Mobile phase optimization:

Different solvent systems have been tried for separation of Budesonide and Levalbuterol Hydrochloride but good separation was found to be in toluene :methanol : triethylamine (7 : 3 : 0.2, v/v/v). The R_f values were found to be 0.38 ± 0.02 and 0.61 ± 0.02 for Levalbuterol Hydrochloride and Budesonide respectively.Chromatogram of Levalbuterol Hydrochloride (R_f: 0.38 ± 0.02) and Budesonide (R_f: 0.61 ± 0.02) is shown in Figure 3.

3.3. Method validation:

3.3.1. Calibration curve and Linearity:

A good linear relationship over the concentration range 200 to 1000 ng/spot for Budesonide and concentration range 500 to 2500 ng/spot for Levalbuterol Hydrochloride was observed. The correlation of coefficient was found to be 0.9930 for Budesonide and 0.9960 for Levalbuterol Hydrochloride. The regression line equation for Budesonide and Levalbuterol hydrochloride was found to be y = 2.948 x + 1903 and y = 1.644 x + 2253 respectively. The 3D chromatogram of calibration curve for Budesonide and Levalbuterol Hydrochloride is shown in figure 4.

3.3.2. Specificity:

To confirm specificity of proposed method 15 µl of combined working standard solution of Budesonide and Levalbuterol Hydrochloride and 15 µl from sample solution of combined marketed formulation of Budesonide and Levalbuterol Hydrochloride were spotted on same TLC plate. The spotted plate was developed, dried and scanned as described under chromatographic conditions [Section 2.3]. Both the track showed only two spots having same R_f values 0.38 ± 0.02 and 0.61 ± 0.02 for Levalbuterol Hydrochloride and Budesonide respectively. The In situ UV spectra of both tracks were recorded.

Peak purity check of both drug from marketed formulation showed high degree of correlation between spectra scanned at peak start, peak apex and peak end position [r (s,m) = 0.9992 and r (m, e) = 0.9996]. The good correlation (r = 0.9994) between absorbance reflectance spectrum of both standard drugs and sample drugs from combined marketed formulation confirms the purity of both drugs.

3.3.3. Precision:

The %RSD for repeatability of sample application was found to be 1.02 and 1.13 for Budesonide and Levalbuterol Hydrochloride respectively. The %RSD for repeatability of measurement of peak area was found to be 0.96 and 1.05 for Budesonide and Levalbuterol Hydrochloride respectively. The %RSD for intraday precision was found to be 0.93 - 1.27 for Budesonide and 1.03 - 1.41 for Levalbuterol Hydrochloride respectively. The %RSD for interday precision was found to be 1.57 - 1.78 for Budesonide and 1.56 - 1.83 for Levalbuterol Hydrochloride respectively.

3.3.4. Accuracy:

Accuracy was determined by standard addition method. The proposed method was applied for estimation of Budesonide and Levalbuterol Hydrochloride in their combined pharmaceutical dosage forms. The recovery experiment was carried out in triplicate by spiking previously analysed sample i.e. 300 ng/spot of Budesonide and 750 ng/spot of Levalbuterol Hydrochloride with different concentration of both standard drugs at 80 %, 100% and 120%.

Table 2: Assay data for marketed formulation (Budesal respules)

Respule Formulation	Label Claim (mg)		Amount found (mg) (n=3)		Assay (% Label Claim)	
	BUD	LEVA	BUD	LEVA	BUD	LEVA
1	0.5	1.25	0.499	1.236	99.88	98.91
2	0.5	1.25	0.497	1.228	99.37	98.24
3	0.5	1.25	0.491	1.248	98.12	99.83
Mean					99.12	98.99
Standard Deviation (n=3)					0.36	0.47
% RSD					0.36	0.48

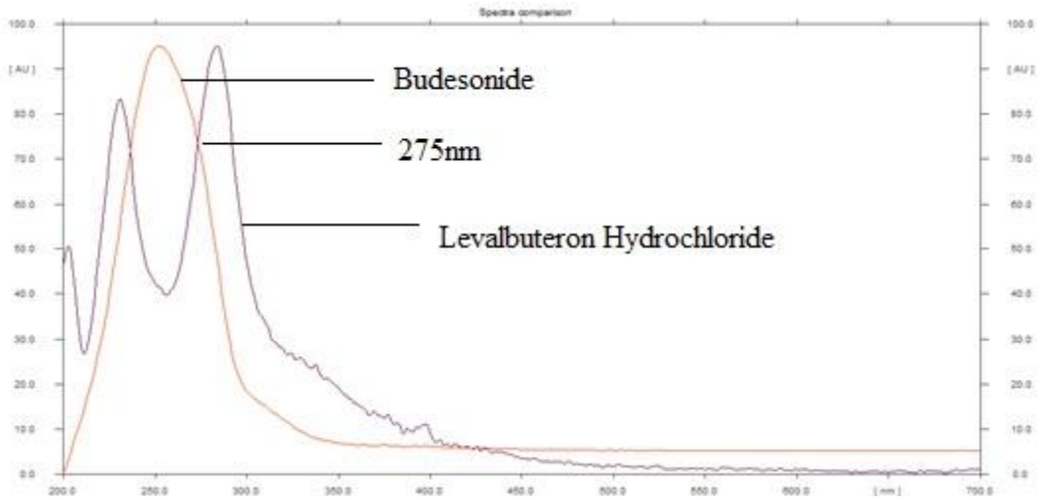

Figure 2: Overlain UV spectra of Budesonide and Levalbuterol Hydrochloride

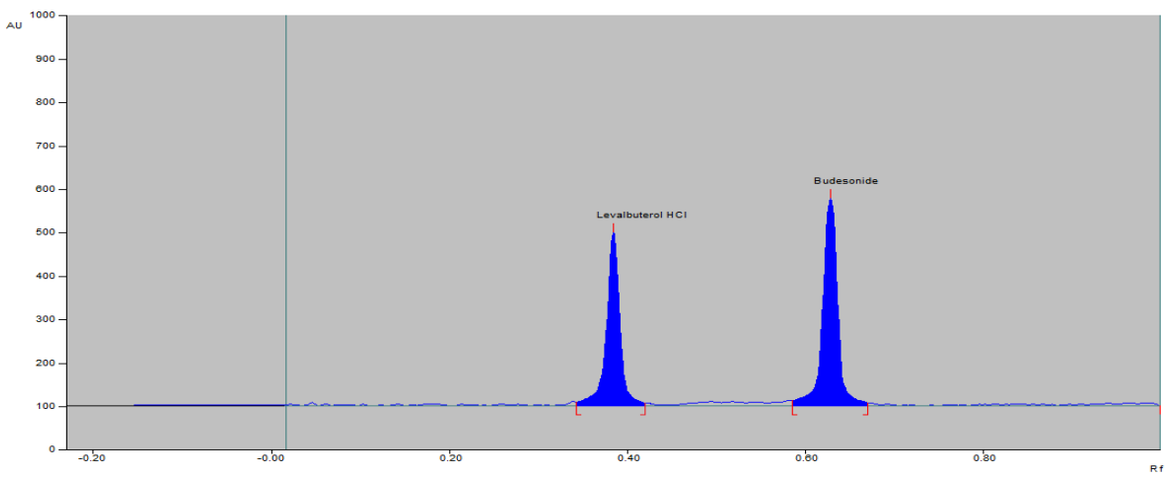

Figure 3: Chromatogram of standard Levalbuterol Hydrochloride (1500 ng/spot); peak 1 (R_f: 0.38 ± 0.02) and Budesonide (600 ng/spot); peak 2 (R_f: 0.61 ± 0.02), measured at 275 nm

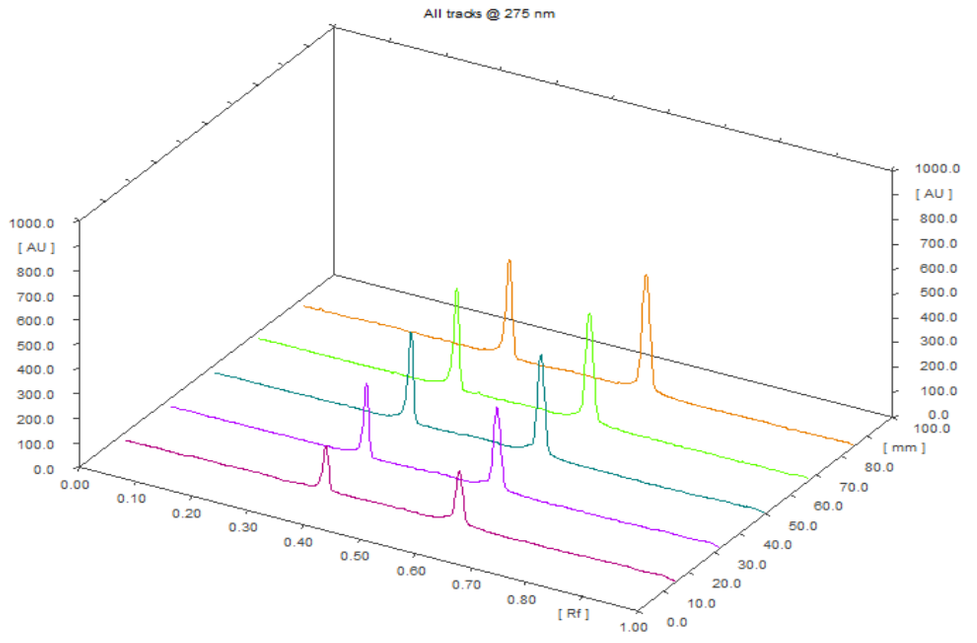

Figure 4: 3D chromatogram of calibration curve for Levalbuterol Hydrochloride (R_f: 0.38 ± 0.02) and Budesonide (R_f: 0.61 ± 0.02) at 275 nm

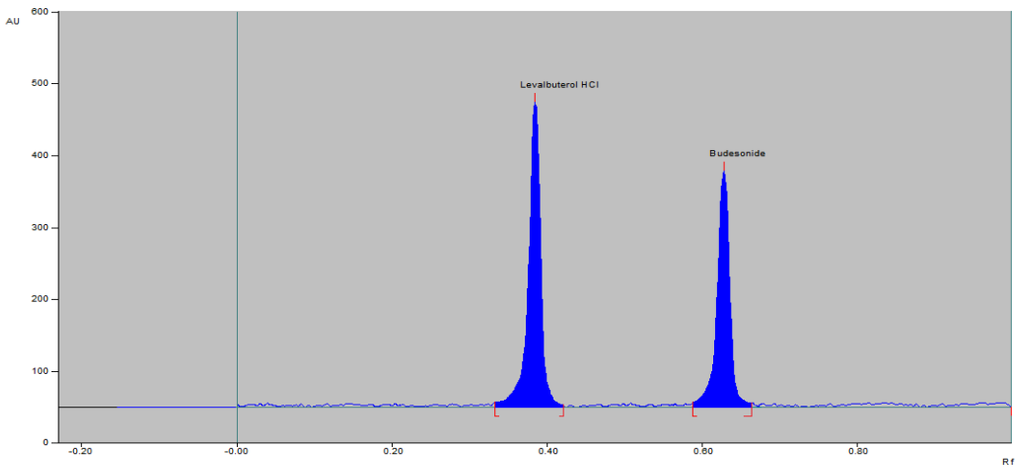

Figure 5: Chromatogram of separation of and LEVA; peak 1 (R_f: 0.38 ± 0.02) and BUD; peak 2 (R_f: 0.61 ± 0.02) from marketed formulation, measured at 275 nm

The percentage recovery was found to be 98.47% - 99.08% for Budesonide and 98.42% - 99.87% for Levalbuterol Hydrochloride.

3.3.5. LOD and LOQ:

The LOD was found to be 15.88 ng/spot for Budesonide and 33.12 ng/spot for Levalbuterol Hydrochloride. The LOQ was found to be 48.12 ng/spot for Budesonide and 100.38 ng/spot for Levalbuterol Hydrochloride. The summary of validation parameters is shown in Table 1.

3.4. Assay of marketed formulation:

The spots at R_f 0.38 (for Levalbuterol Hydrochloride) and 0.61 (for Budesonide) were observed in the chromatogram of the drug sample

from marketed formulation in Figure 5. The drug content was found to be 99.12% ± 0.36 (%RSD = 0.36) and 98.99% ± 0.47 (%RSD = 0.48) for Budesonide and Levalbuterol Hydrochloride of label claim of their combined dosage form respectively. There was no additional peak observed in the chromatogram of combined marketed formulation except Budesonide and Levalbuterol Hydrochloride that indicated no interference of excipients in estimation of Budesonide and Levalbuterol Hydrochloride in their pharmaceutical dosage form. The result for assay of marketed formulation is shown in table 2.

4. CONCLUSION:

The proposed HPTLC method was developed for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride in their combined pharmaceutical dosage form. The %RSD of precision was found to be less than 2% and percentage recovery was found to be in range of 98-102% proves that the developed method is precise and accurate for simultaneous estimation of Budesonide and Levalbuterol Hydrochloride. The proposed method was applied for assay of combined pharmaceutical dosage form of Budesonide and Levalbuterol Hydrochloride. The assay results for Budesonide and Levalbuterol Hydrochloride were in good agreement with label claim of their combined pharmaceutical dosage form.

5. ACKNOWLEDGEMENT:

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