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Stability–indicating High Performance Thin Layer Chromatography /densitometry estimation of Formoterol fumarate dihydrate in bulk and capsules

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Abstract

Novel, simple, rapid and reliable High-Performance Thin-Layer Chromatographic (HPTLC) methods were developed and validated for the analysis of Formoterol Fumarate Dihydrate (FFD) in bulk and in in-house capsules formulation. HPTLC quantitation of FFD was done at UV detection of 281 nm and analysis was performed on (10 cm × 10 cm) aluminium sheets precoated with silica gel 60-F_{254} (E. Merck) as stationary phase and ethyl acetate: methanol: triethylamine (3.2:1.5:0.3 *v/v*) as mobile phase. Quantitation by HPTLC method was performed over the concentration range of 400 - 900 ng/band. The HPTLC method resulted into compact and well resolved band for FFD at retention factor (R_f) of 0.51 ± 0.3. Linear regression analysis data for calibration of HPTLC method represented good linear relationship with regression coefficient; r^2 = 0.999. The developed methods were validated for precision, robustness, ruggedness, accuracy, sensitivity as per guidelines laid by International Conference on Harmonisation (ICH). FFD was subjected to acid and alkali hydrolysis, oxidation, neutral, photo and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation, and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different R_f value. Statistical analysis proved that the developed methods were precise, robust, sensitive and accurate and can be used effectively for the analysis of FFD in bulk and pharmaceutical formulations.

Keywords: Formoterol fumarate dihydrate, FFD, Formoterol, HPTLC, Stability indicating HPTLC.

1. Introduction

Formoterol Fumarate Dihydrate (FFD), N-[2-hydroxy-5-(1-hydroxy-2-{[1-(4-methoxyphenyl) propan-2-yl] amino} ethyl) phenyl]formamide [1] [Figure 1], is a long-acting β_2-selective adrenoceptor agonist, used as Bronchodilator and also used in Chronic Obstructive Pulmonary Disease (COPD). [2].

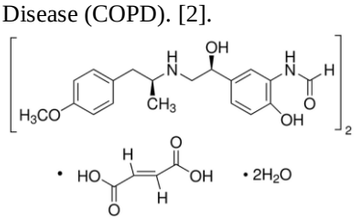

Figure 1: Chemical Structure of FFD

In literature survey few analytical methods includes LC-MS [3] method for quantitation of FFD inhuman urine, Assay of Formoterol by HPLC [4] and spectrophotomertic method by formation of colour chromogens of FFD has also studied for the estimation of FFD in pharmaceutical formulation.

Many analytical methods such as RP-HPLC [5-7], UV- spectrophotometry [8,9] have been reported for simultaneous determination of FFD in combination with Budossunide and Memontasone Furoate in Pharmaceutical formulation.

Analysis of pharmaceuticals is an imperative and chief aspect of the complete drug development process. Therefore, simple and rapid methods for quality control of commercial formulations are needed. High – Performance thin – layer chromatography (HPTLC) is an important separation technique since many samples can be chromatographed simultaneously with small amount of solvent [10].

Forced degradation experiments are used to relieve the development of analytical methodology, to achieve better insightful of the stability of the active pharmaceutical ingredient (API) and the drug product, and to provide information about degradation pathways and degradation products [11]. However, no literature is available for which deals with the stress degradation profile of LTD in accordance with ICH guidelines using any of the above analytical techniques.

The objective of this work was to develop a stability-indicating High-Performance Thin-Layer Chromatography (HPTLC) / Densitometry method for analysis FFD. Since there are no reports of analysis of FFD alone in formulations, the method has also been used for analysis of FFD in capsules. This paper describes an accurate, specific, repeatable, and stability-indicating method for analysis of FFD in the presence of its degradation products. The method was validated in accordance with the guidelines of the US Pharmacopoeia [12] and the International Conference on Harmonization (ICH) [13].

2. Materials and Methods

Formoterol fumarate dihydrate standard was supplied as a gift sample from a well reputed pharmaceutical industry. Ethyl acetate and Methanol (HPLC Grade) and Triethylamine used during study were procured from (E.Merck, Mumbai, India). Capsules containing 12 mcg of

2.1 Equipments and Experimental conditions

A Camag TLC system (Muttenz, Switzerland) comprising of Camang Linomate 5 automatic sample applicator, Hamilton syringe (100 µL), Camang TLC scanner 3, Camang winCATS software (version 1.3.0), Camang twin trough chamber (10 cm x 10 cm) and ultrasonicator; ENERTECH Electronics Pvt. Ltd., India was used during the study.

Chromatographic study was carried out on aluminium-backed precoated silica gel 60-F_{254} (10 cm × 10 cm) HPTLC plates having thickness 200 µm (E.Merck, Mumbai, India). Prior to use; the HPTLC plates were pre-washed and dried in oven at 105 ^{O}C. Densitometric detection was performed with a Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) installed with winCATS software (version 1.3.0). Drug standards and samples were applied on the HPTLC plates using Linomat 5 (Camag) applicator. Ten microliter samples were spotted 6 mm from the edge of the plates. The plates were developed in twin trough glass chamber (20 cm × 10 cm) (Camag, Muttenz, Switzerland). The volume of mobile phase was 10 ml. Mobile phase components were mixed prior to use and the

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development chamber was left for saturation with mobile phase vapours for 20 min before each run at room temperature. Development of the plate was carried out by the ascending technique to a migration distance of 8 cm followed by drying with the help of an air drier. Densitometric scanning was done in absorbance-reflectance mode at 281 nm using a deuterium lamp emitting a continuous UV-spectrum between 190 nm - 400 nm.

2.2 Preparation of Stock Standard Solution and Study of Calibration Curve

A stock standard solution was prepared by dissolving 10 mg FFD in a 100 ml volumetric flask containing 25 ml of methanol and sonicated for 10 min using ultrasonicator and volume was made up to the mark to get a stock standard solution of 0.1 mg/ml of FFD. From the standard solution, appropriate volumes of 4 - 9 ml were transferred with the help of previously calibrated pipette (10.0 ml) into series of 10 ml volumetric flasks. A fixed volume 10 µL was applied on the HPTLC plates to obtain concentration 400, 500, 600, 700, 800 and 900 ng/band of FFD respectively. Each concentration was applied six times to the plates and plates were developed as described above. Peak areas were plotted against corresponding concentrations to obtain the calibration plot.

2.3 Validation of Analytical Method

2.3.1 Precision

The repeatability of sample application and measurement of peak area were measured using six replicates of the same band (600 ng/band of FFD). Intra-day and inter-day precision for analysis of FFD was measured at three different concentrations 600, 700, and 800 ng/band.

2.3.2 Accuracy

The accuracy of the experiment was established by over spotting drug standard solution to the pre-analyzed sample solution. The recovery study was performed at three different levels i.e. 80, 100, and 120%. The experiment was demonstrated in triplicate.

2.3.3 Ruggedness and Robustness

The ruggedness of the method was performed by two different analysts using same operational and environmental conditions.

For robustness study, few parameters like mobile phase composition (±0.5ml), mobile phase volume (±2ml) development distance (±0.5cm) and duration of saturation (±5min.), were changed deliberately and the effects on the results were examined. The robustness and ruggedness of the method was assessed at concentration (600 ng/band) for six times.

2.3.4 Limits of Detection (LOD) and Quantification (LOQ)

The LOQ and LOD were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B where 'N' is the standard deviation of the peak areas of the drug (n = 3), taken as a measure of the noise, and 'B' is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal- to-noise ratio was 3:1 and LOQ as the amount for which the signal-to-noise ratio was 10:1. The LOD and LOQ estimated at concentration range 400 – 500 ng/band.

2.3.5 Specificity

The specificity of the method was ascertained by analyzing standard FFD and FFD extracted from capsule formulation. The band for FFD in sample was confirmed by comparing the R_f and spectra of the band with those obtained from standard. The peak purity of FFD was assessed by comparing spectra acquired at three different positions on the band, i.e. peak-start (S), peak-apex (M), and peak-end (E).

2.4 Analysis of marketed formulation

Twenty capsules were weighed and powdered. An amount of powder equivalent of 10 mg of FFD was weighed accurately and extracted with 100 ml methanol in 100 ml flask using reciprocating shaker for 10 min (the optimal extraction time) and transferred to a 100 ml volumetric flask. After filtration through a Whatman filter paper, extract (6µl; 600 ng/band) was applied to a HPTLC plate followed by development and scanning as described above. The analysis was repeated in six times.

2.5 Forced Degradation Studies

Acid and base-induced degradation was attempted by separately adding 10 mg of FFD in 10 ml each of 0.1 N HCl and 0.1 N NaOH solutions separately. These solutions were kept for 8 h in the dark in order to exclude the possible degradative effect of light. The solutions (1 ml) were neutralized and diluted to 10 ml with methanol. The 8 µL of the resulting solutions (800ng/band) were applied to HPTLC plate and chromatograms were obtained as described above.

For oxidative degradation, 10 mg of FFD added in 10 ml of 3% (*v/v*) hydrogen peroxide solution and kept in dark for 8 h. The solution (1 ml) was diluted to 10 ml with methanol and treated as described for acid and base-induced degradation.

For dry heat degradation, FFD powder was placed in an oven at 70°C for 8 h. A solution of 10 mg FFD in 10 ml methanol was prepared from the dry heat treated sample. The solution (1 ml) was diluted to 10 ml with methanol and treated as described for acid and base-induced degradation.

To assess the neutral degradation, a 10 mg of FFD added in 10 ml water and photochemical degradation was studied by exposing 1 mg/ml solution in methanol to sun light for 72 h. The resulting solutions (1 ml) were diluted to 10 ml with methanol and analyzed using process described above.

3. Results and Discussion

3.1 Selection of the Optimum Mobile Phase

The HPTLC procedure was optimized to develop a stability-indicating assay method to quantify FFD in bulk and capsules formulation. The drug standard was applied on HPTLC plates and chromatographed with different composition of mobile phases. The high resolution and reproducible peak was achieved using ethyl acetate: methanol: triethylamine (3.2:1.5:0.3 *v/v*) as mobile phase and chamber saturation period was for 20 min. In this mobile phase FFD showed compact spot with R_f = 0.51 when the scanning was performed at 281 nm [Figure 2].

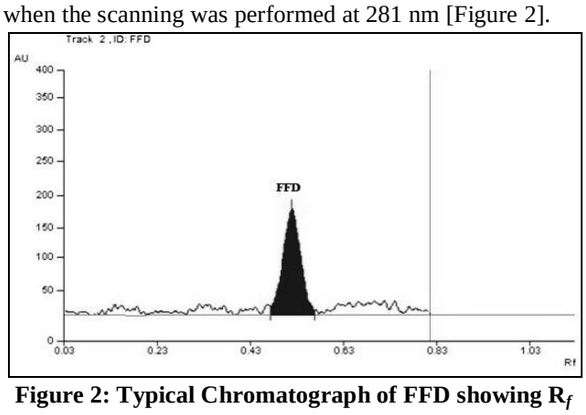

Figure 2: Typical Chromatograph of FFD showing R_f 0.51

3.2 Calibration Plots

Calibration curves were constructed by plotting peak area against drug quantity per band. A good linearity was obeyed in the concentration range of 400 – 900 ng/band; calibration plot is y = 8.400x - 1.228 and correlation coefficient (r^2 > 0.99) as shown in [Figure 3] All measurements were repeated six times.

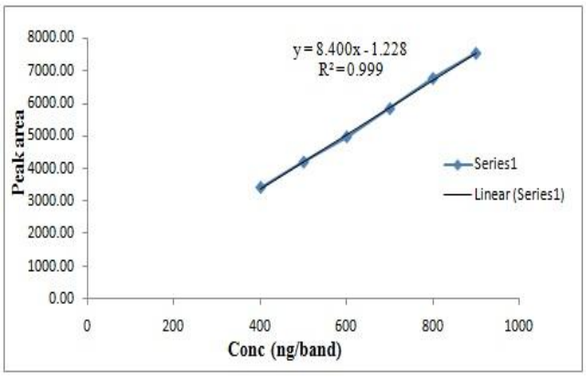

Figure 3: Calibration Curve of FFD

3.3 Validation of developed stability indicating method

3.3.1 Precision and % Recovery study

The precision of the method was studied as repeatability, intra-day and inter-day variations. The results from study of precision are shown in Table 1. The developed method was found to be précised as the RSD values for repeatability and intra-day and inter-day precision studies were < 2%, respectively as recommended by ICH guideline.

Table 1: Intra-day and inter-day precision [*n* = 6]

Precision	Amount [ng per band]	% Amount found	±SD	RSD [%]
Repeatability (n=6)	600	600.04	5.89	0.98
Intra-day (n=3)	600	592.70	01.66	0.28
	700	693.22	04.27	0.62
	800	800.17	01.01	0.13
Inter-day (n=3)	600	589.77	06.33	1.07
	700	691.16	13.10	1.90
	800	796.93	08.32	1.04

When the method was used for extraction and subsequent analysis of FFD in capsule dosage forms after spiking with 80, 100, or 120% excess drug the recovery was found 99.06 – 99.59 %, as listed in Table 2.

Table 2: Results from recovery studies [*n* = 3]

Amount of drug Added to capsule formulation [%]	Theoretical content [ng per band]	Recovery [%]	RSD [%]
80	720	99.42	0.49
100	800	99.06	0.65
120	880	99.59	0.32

3.3.2 Ruggedness and Robustness

The standard deviation of peak areas was calculated for each condition and % RSD was less than 2 %. The low values of % RSD are indicative of the robustness and ruggedness of the method. The results from study of robustness are shown in Table 3.

Table 3: Results from robustness testing [*n* = 6]

Conditions	RSD [%]
Mobile phase composition (± 0.5 ml)	1.42
Mobile phase volume (± 2 ml)	1.59
Development distance (± 0.5 cm)	1.58
Plate saturation time (± 5 min)	1.60
Time from application to chromatography (±10 min)	1.05
Time from chromatography to scanning (±10 min)	0.78

2.3.3 Limits of Detection (LOD) and Quantification (LOQ)

Detection limit and quantitation limit for signal-to-noise ratios of 3:1 and 10:1 were 17.06 ng and 53.35 ng, respectively, which indicate adequate sensitivity of the method.

3.3.4 Specificity

Peak purity for FFD was assessed by comparing spectra acquired at the peak -start (S), peak -apex (A) and at the peak -end (E) positions of the band, r^2(S,M) = 0.996 and r^2(M,E) = 0.998. Good correlation (r^2 = 0.999) was also obtained between FFD standard and FFD extracted from capsules FFD [Figure 4].

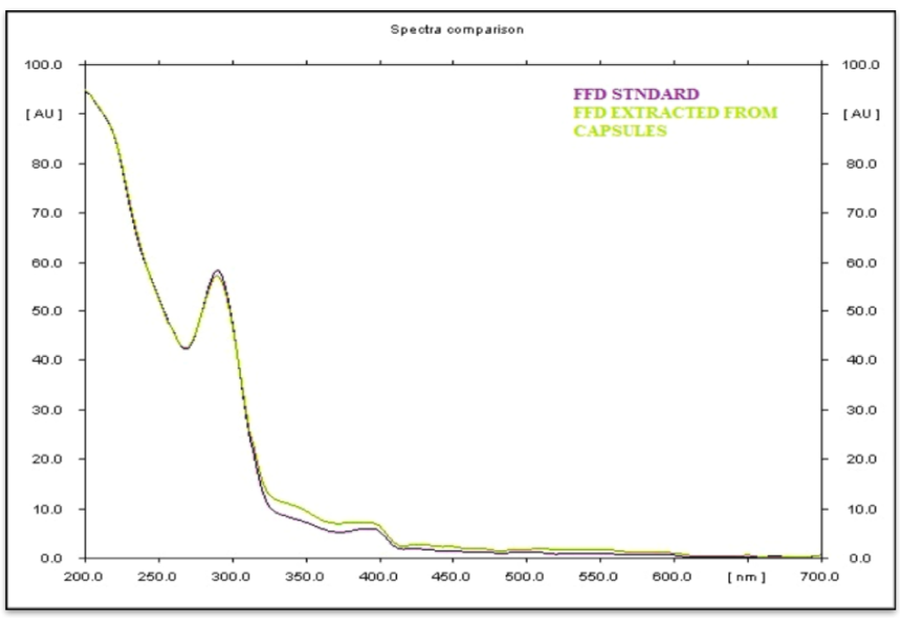

Figure 4: Overlain Peak - purity spectra of FFD standard and FFD extracted from capsules at peak start, peak apex and peak end position in HPTLC

These correlation values indicate the ability of the method to separate and specifically detect FFD from sample solutions.

The validation data are summarized in Table 4.

Table 4: Summary of validation data

Parameter	Results
Linear range [ng per band] [n = 6]	400 – 900
Correlation coefficient	0.999
Limit of detection [ng]	17.60
Limit of quantification [ng]	53.35
% Recovery [n = 9]	99.06 – 99.59
Precision [%RSD]	
Repeatability [n = 6]	0.98
Intra-day [n = 3]	0.13 – 0.62
Inter-day [n = 3]	1.04 – 1.90
Ruggedness [% RSD]	
Analyst – I [n = 6]	1.07
Analyst – II [n = 6]	1.10
Robustness	Robust
Specificity	Specific

3.4 Analysis of marketed formulation

The results obtained for the amount of FFD in capsules as against the label claims were in good agreement. The amount of drug estimated using this method was found to be 98.72 ± 0.96, demonstrated that there was no interference from the excipients usually present in capsule formulation. It may therefore be inferred that degradation of FFD had not occurred in the formulations analyzed by the method described above. The good performance of the method indicated the suitability of this method for routine analysis of FFD in capsules dosage forms.

3.5 Forced Degradation studies

The chromatograms obtained from samples exposure to acidic, alkaline, oxidative and dry heat degradation depicted well separated bands of pure FFD and some additional peaks at different R_f values. No major degradation product was seen when sample were subjected to neutral and photochemical degradation. The % of degradation products with their R_f values is listed in Table 5. Peak purity of these FFD peaks during stability studies was also examined by comparing the respective spectra obtained from RP-HPTLC/ densitometry at the peak start (S), peak apex (M), and peak end (E). The values of r (S, M) and r (M, E) were all > 0.99 with standard spectra FFD peak, indicating the purity of peaks of the remaining FFD. The result of forced degradation shown in [Figure 5].

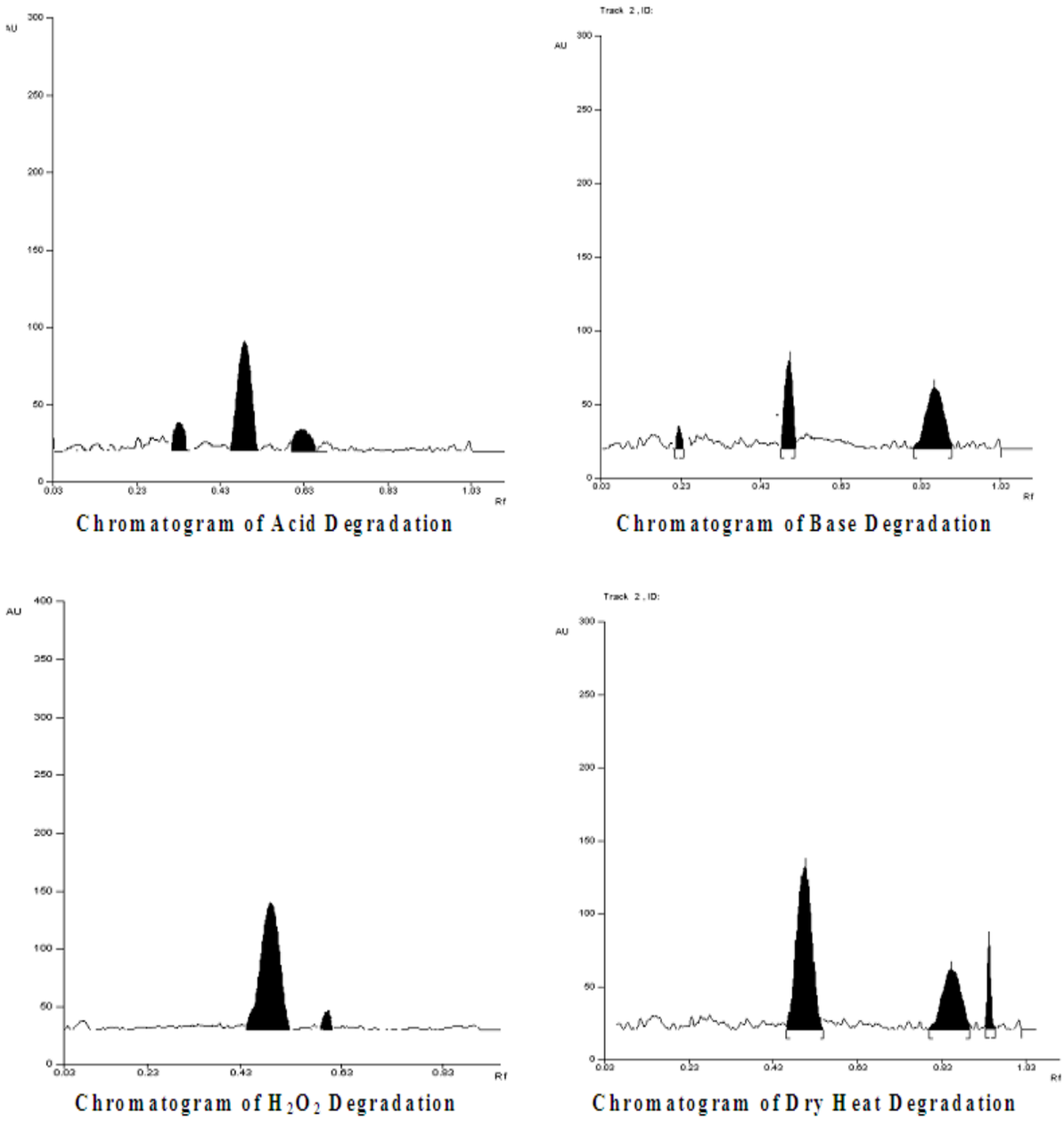

Figure 5: Degradation Study of FFD

Table 5: Results from degradation studies

Condition	Time (h)	R*f* of degradation product	% of degradation product
Acid, 0.1 N HCl, RT	8	0.32 and 0.63	12.75 and 16.71%
Base, 0.1 N NaOH, RT	8	0.23 and 0.77	9.22 and 49.97%
H_2O_2, 3% (w/v), RT	8	0.61	6.25 %
Dry heat (70 ± 2°C)	8	0.84 and 0.97	35.24 and 17.53%
Neutral degradation	8	Not detected	
Photo degradation	72	Not detected	

4. Conclusions

This densitometric HPTLC method is quite simple, accurate, highly precise, reproducible, rapid, sensitive and specific. It was successfully used to study the stability of FFD under different stress degradation conditions. Statistical analysis proves that the method is repeatable and selective for the analysis of FFD as the bulk drug and in pharmaceutical formulations, without interference from excipients. It can also be utilized successfully for determination of the chemical stability and shelf life of FFD in capsules formulations, because it has stability-indicating properties.

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References

[1] Maryadele J. O'Neil, The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 14th Ed., Merck Research Laboratories, New Jersey, USA, 2006. p. 728.

[2] Indian Pharmacopoeia Commission. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Government of India, Ghaziabad, Vol. 2, 6th ed. India, 2007. P. 1386.

[3] Youxuan Xu *et al*, An Improved Liquid Chromatography – Tandem Mass Spectrometric Method to Quantify Formoterol in Human Urine, *J Chromatogr Sci.*, 2013, 12:1-4.

[4] Appala. Raju *et al*, A High Performance Liquid Chomatographic Assay for Formoterol in Bulk Drug and its Pharmaceutical Dosage Forms, *Int. J Biomed Adv. Res*, 2010; 2(1):18-24.

[5] Pai N. and Patil S.S, Development and validation of RP-HPLC method for estimation of Formoterol fumarate and budesonide in pressurised meter dose inhaler form, *Der Pharmacia Sinica*, 2013; 4(4):15-25

[6] Ahmed S., Jayakar B. and Aleem M.A., Development of Reverse Phase High Performance Liquid Chromatography Method and its Validation for Estimation of Formoterol Fumarate Rota Caps, *Int. J Pharm. Sci. Res.*, 2011; 2(2): 319-24.

[7] Srinivasarao K., Gorule V., Venkata R.C., and Venkata K.A., Validated Method Development for Estimation of Formoterol Fumarate and Mometasone Furoate in Metered Dose Inhalation Form by High Performance Liquid Chromatography, *J Anal Bioanal. Tech.* 2012, 3(7):1-4.

[8] Gurjar *et al*, Development of First Derivative Spectroscopy Method for Estimation of Budesonide and Formeterol in Combined Dosage Form, *Int. J Pharm. Sci*., 2012; 3(1):82-92.

[9] Prasad A.V.S.S *et al*, Simultaneous Spectrophotometric Determination of Formoterol Fumarate and Budesonide in their Combined Dosage Form *Indian Journal of Chemical Technology*, 13:81-83.

[10] Sethi P.D., HPTLC - Quantitative Analysis of Pharmaceutical Formulations, 1st Edition, CBS Publishers and Distributors, New Delhi, 1996, 1-4.

[11] Alsante K., Ando A., Brown R., Ensing J., Hatajik T., Kong W., Tsuda Y., *Adv. Drug Del. Rev*. 2007, 59:29–37.

[12] United States Pharmacopeia, United States Pharmacopeial Convention, Rockville, 2004.

[13] ICH-Q2B Guidline for Industry, Validation of Analytical procedures: Methodology. International Conference on Harmonization IFPMA Geneva, 2005.