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ORIGINAL ARTICLE

Development of Stability Indicating and Effective Analytical Method of Budesonide and Formoterol by RP-HPLC

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ABSTRACT
A simple, accurate, precise and rapid High Performance Liquid Chromatographic Method for the analysis of Budesonide (BN) and Formoterol (FT) in its formulations was developed and validated in the present study. The peak for Budesonide and Formoterol was well resolved from the peaks of degradation products, using a Agilent (100 mm × 4.6 mm, 2.5 µm) column and mobile phase consist a mixture of Methanol : 0.1% OPA (pH adjusted to 4.5 with triethyl amine) mixture are used for the separation and quantification. The flow rate was 1.0 ml/min and the eluents were detected by UV detector at 231 nm. The retention times of Budesonide and Formoterol were found to be 3.811 mins and 6.342 mins. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. All the result were found to be within the specification limit.
KEYWORDS: *Budesonide, Formoterol, RP-HPLC, Stability study.*

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INTRODUCTION

It is designated chemically as (RS)-11-beta, 16-alpha,17,21-tetrahydroxypregna-l,4-diene-3,20-dionecyclic 16, 17- acetal with butyraldehyde. Due to the introduction of the alkyl chain at the C22 atom, budesonide is a mixture of two epimers (22R and 22S) as shown in figure 1. Both epimers appear to have similar pharmacological effects; however *in vitro* studies suggested that the R-epimer was two to three times more potent with respect to its anti inflammatory effects [1-2].

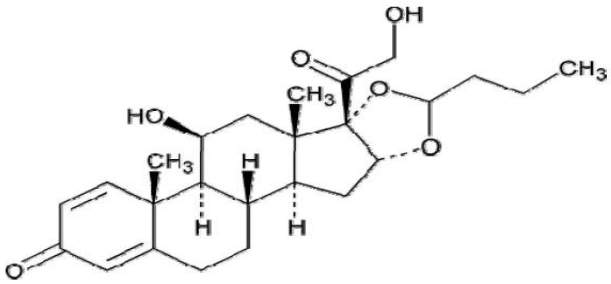

Figure 1. Chemical structure of Budesonide

The empirical formula of budesonide is C25H34O6 and its molecular weight is 430.5. Budesonide is a white to off-white, tasteless, odourless powder that is practically insoluble in water and heptane, sparingly soluble in ethanol,and freely soluble in chloroform. [3, 4].

Formoterol Fumarate is a dihydrate salt of fumaric acid with (RS)-2′-hydroxy-5′-[(RS)-1-hydroxy-2-[[(RS)-p-methoxy-α-methylphenethyl] amino] ethyl] formanilide. The structure of Formoterol Fumarate is represented below in Figure 2. Formoterol is long acting selective beta-2-adrenergic receptor agonist used as bronchodilator in treatment of asthma [12, 5].

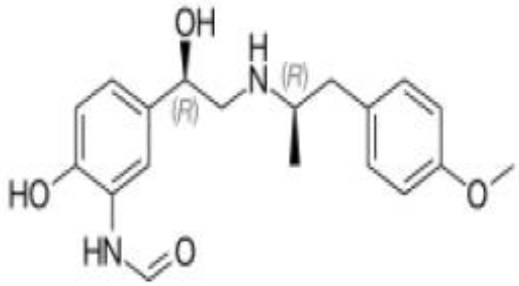

Figure 2. Chemical structure of Formoterol

its empirical formula is (C19H24N2O4) Formoterol is a white to yellowish crystalline powder, which is freely soluble in glacial acetic acid, soluble in methanol, sparingly soluble in ethanol and isopropanol, slightly soluble in water and practically insoluble in acetone, ethyl acetate, and diethyl ether. [6-9].

In literature, many analytical methods have been reported for estimations of these drugs individually from biological fluids. These drugs are official in BP and USP. Therefore, an attempt has been made to develop simple, accurate, precise and rapid UV–Spectrophotometric and HPLC methods for determination of Budesonide and Formoterol.

MATERIAL AND METHODS

Materials

Analytical pure samples of Budesonide and Formoterol were obtained as gift samples from Swapnroop Drug ang Pharmaceuticals LTD, Aurangabad, India. Methanol, Ortho-phosphoric acid, triethyl amine Acetonitrile, Sodium hydroxide, Hydrogen peroxide (H_2O_2), and Hydrochloric acid were used in the study. double distilled water were used in analysis.

Equipment

Agilent (1100series) with Auto sampler and DAD detector (G13148 S.NO. DE71365875) with Chemstation software were used.

Chromatographic Conditions

The Chromatographic system was performed using a Agilent (100 mm x 4.6 mm), 2.5µm column. Separation was achieved using a mobile phase consisting of mixture of Methanol : 0.1% OPA (pH adjusted to 4.5 with Triethyl amine). The flow rate of the mobile phase was 1.0 ml/min with a short run time (15 min). The eluent was monitored using UV detection at 231 nm. The column temperature was maintained at Ambient and the injection volume 20µl was used. The mobile phase was filtered through a 0.45µm micron filter prior to use [10, 12].

Preparation of Standard Stock Solution

100 mg of Budesonide was weighed accurately as a working standard and 6 mg of Formoterol working standard, was transferred into a 20 ml volumetric flask, followed by addition of 15 ml diluent, and sonicated it to dissolve the drug completely, The volume was made up to the mark with diluents (concentration of Budesonide was 5000 mcg/ml and concentration of Formoterol was 300 mcg/ml). From the prepared solution 0.1-0. 5 ml was pipette out in 10 ml volumetric flask and diluted it up to the mark with diluents (concentration of Budesonide 50-250 mcg/ml and concentration of Formoterol is 3-15 mcg/ml) [10].

RESULTS AND DISCUSSION

Selection of λ max for Budesonide and Formoterol

Solution of Budesonide and Formoterol (10 µg/ml) was prepared separately in methanol (as it has complete solubility in methanol). The λ_{max} was then determined on UV-Visible Spectrophotometer in the range of 400-200nm **(Figure. 3)**. The wavelength of maximum absorbance was noted.

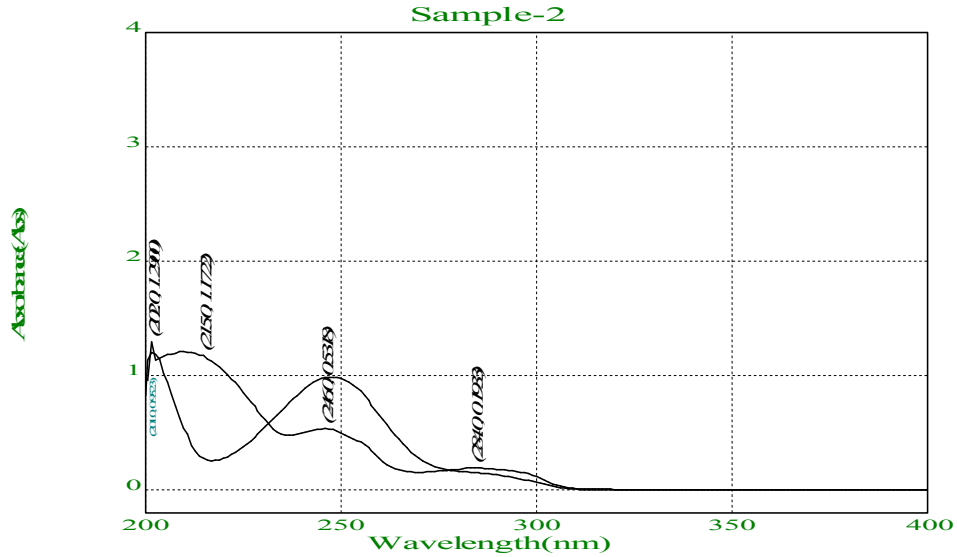

Figure 3. Overlaid spectrum UV

System Suitability Test

System suitability was Pharmacopoeia necessity and is utilized to check, regardless of whether the determination and reproducibility of chromatographic framework are sufficient for examination to be finished. The test was performed by gathering information from six replicate injection infusion of standard drug solution. System suitability information is shown in (Table 1, Figure 4)

Acceptance Criteria:

1. RSD should Not be more than 1.0% for five replicate injection of standard.
2. USP Tailing Factor is Not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

Table 1. System Suitability Test

Sr. No.	Parameter	Budesonide	Formoterol
1	RT	3.873 min	6.389 min
2	% RSD	0.90	1.07
3	USP Tailing	0.67	0.62
4	Theoretical Plate Count	5614	7723
5	Peak Area	5456.5737	1263.1075

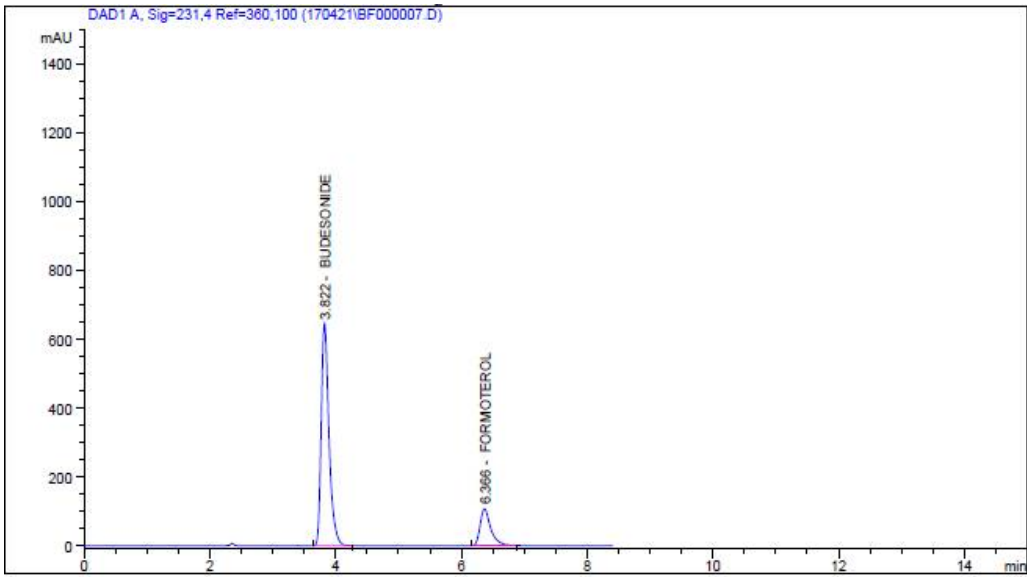

Figure 4. Chromatogram of optimized method.

METHOD VALIDATION

Linearity

Linearity of a diagnostic technique is its capacity to inspire test comes about that are specifically or by a very much characterized numerical change, relative to the convergence of analyte in tests inside a given range. From the above stock solution five different concentration of analyte having concentration range of 50-250 mcg/ml for BN and 3-15 mcg/ml for FT. Linearity arrangements were infused in duplicate. The calibration graph was acquired by plotting peak area against the concentration of drug. The calibration graph was observed to be straight in the previously mentioned focuses with correlation coefficient 0.999 and 0.999 (Figure 5 & 6 and Table 2).

Acceptance Criteria:

1. Correlation coefficient should not be less than 0.999.

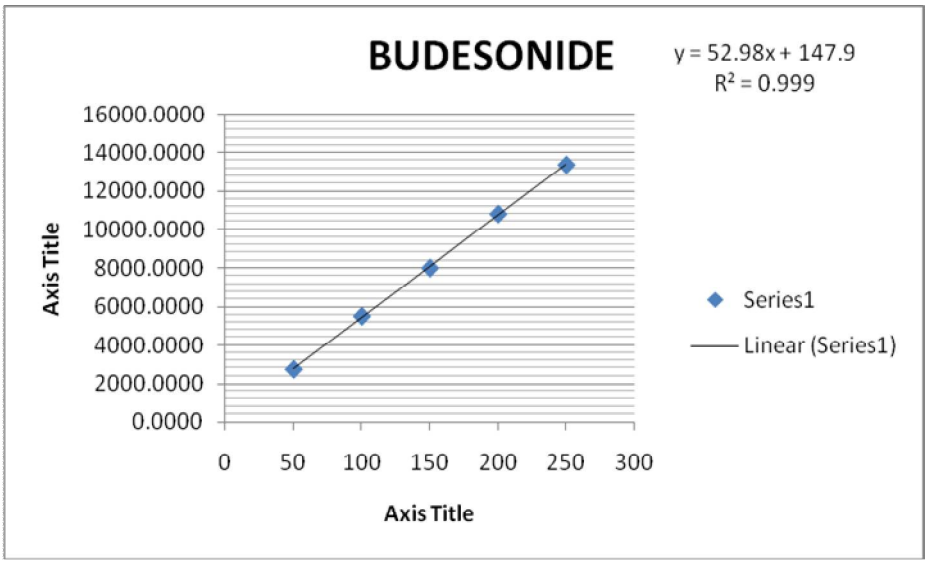

Fig 5: Linearity Graph for Budesonide

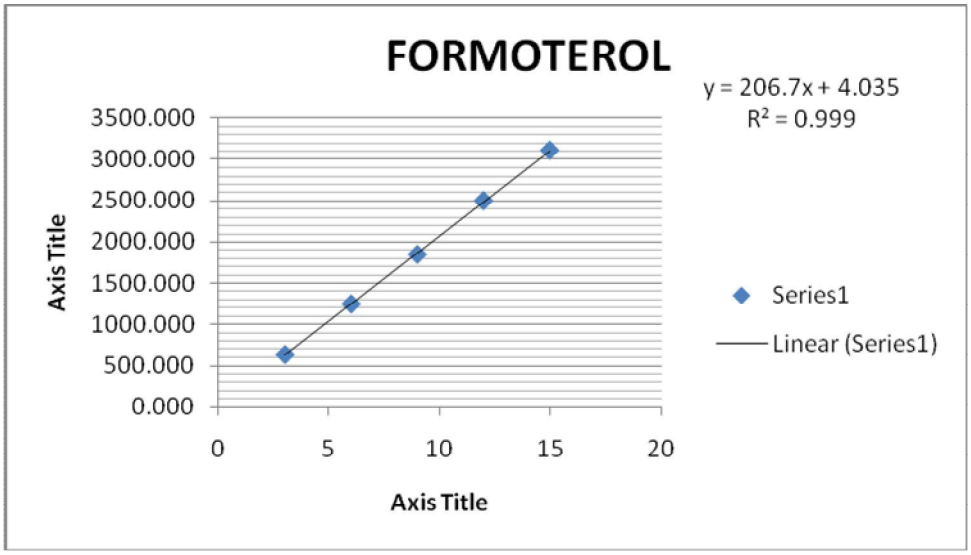

Fig 6: Linearity Graph for Formoterol

Table 2. Linearity and Correlation coefficient

Parameter	Budesonide	Formoterol
Regression equation	52.98x+147.9	206.7x- 4.035
Linearity (µg/ml)	50-250 µg/ml	3-15 µg/ml
Correlation coefficient	0.999	0.999

Accuracy (recovery)

Accuracy of the method was studied by estimation experiments. The estimation experiments were performed by adding known amounts of the drugs in the placebo. The estimation was performed at three levels, 80%, 100% and 120% of the standard working concentration of drug. The estimation samples were prepared as per different concentration of solution. Three samples were prepared for each estimation level. The solutions were then analysed and the percentage estimation were calculated. The estimation values for Budesonide and Formoterol for all determinations were in range of 100.31 to 100.93% and 99.90 to 101.07 respectively. The average estimation of three levels was 100.69% and 100.31% respectively (Table 3 & 4).

Acceptance criteria:

1. RSD of the six replicate injections is NMT 2.0%.
2. % Assay should be in between 95-105%.

Table 3. Results of accuracy study (Budesonide)

Level (%) n=3	Amount of drug use(mg)	Amount estimated (mg)	% Estimation (n=2)
80	40	40.33	100.84
100	50	50.15	100.31
120	60	60.56	100.93
Mean			100.69
RSD			0.12

Table 4. Results of accuracy study (Formoterol)

Level (%) n=3	Amount of drug use(mg)	Amount estimated (mg)	% Estimation (n=3)
80	2.4	2.40	99.90
100	3	3.0	99.98
120	3.6	3.64	101.07
Mean			100.31
RSD			0.373

Precision

To check the reproducibility of the method, suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1h and on three different days for intra and inter day study respectively. The standard deviation and Relative Standard Deviation were calculated (RSD) were calculated **(Table 5).**

Acceptance Criteria:

1. RSD should not be more than 2.0%.

Table 5. Precision studies

Drug	Intraday Precision n=6 %RSD	Interday Precision n=6 %RSD
Budesonide	0.40	0.20
Formoterol	0.11	1.25

Robustness

The robustness of an analytical method were determined by analysis of aliquots from homogenous lots by varying physical parameters that may differ but are still inside the predetermined parameters of the measure. For instance change in physical parameters like flow rate ,wavelength and mobile phase.

Standard preparation, placebo preparation and sample preparation in duplicates were prepared. The sample alongside standard and placebo were injected under various chromatographic conditions as shown below **(Table 6).**

- Changes in flow rate. (±0.1ml/min)
- Changes in wavelength. (±1nm)
- Changes in mobile phase. (±1%).

Acceptance criteria:

1. % RSD of the nine replicate injections is NMT 2.0%.

2. % Assay should be in between 98-102%.

Table 6. Results of Robustness study

Sr. No.	Parameter	Variation	Budesonide		Formoterol	
			% RSD	SD	% RSD	SD
1	Flow rate (± 0.1ml/min)	0.9 ml/min	0.05	2.94	0.72	8.67
		1.1 ml/min	0.05	2.60	0.17	1.92
2	Change in wavelength (± 1nm)	230nm	0.01	0.62	0.15	1.96
		232nm	0.03	1.68	0.12	1.47
3	Change in of mobile phase (±1%)	29+71 %	0.04	2.07	0.13	1.64
		31+69%	0.07	4.11	0.23	2.87

Analysis of BN and FT from marketed tablets

The percentage assay of tablet formulation was found to be 98.71 and 101.80% for BN and FT respectively. **(Table 7).**

Acceptance criteria:

1. % Assay should be in between 98-102%.
2. The percentage label claim of all samples calculated against the standard preparation The mean assay value of the filtered samples correlated with the mean assay value of centrifuged solution.

Table 7. Marketed tablets For Budesonide and Formoterol

Sr.No.	Sample Identity	Area	Amount found	%LC
1	BN	10776.45	200.61	100.31
2	FT	2481.64	11.99	99.89

Specificity

Stability study observation for Budesonide and Formoterol are shown in (Table 8, Figure 7 & 11).

Stability Study.

The stability of the drug solutions was observed for 2 h. In degradation studies, the drug was exposed to various stress conditions. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of BN and FT. Optimum degradation was observed in the presence of acid and alkali. Substantial degradation was observed in the presence of water, light, and peroxide. minor degradation was observed in the presence of peroxide and thermal for BN and thermal for FT. The results of the percentage of degradation are presented in Table 8 and Fig. 7-11. Hence, the method was found to be specific.

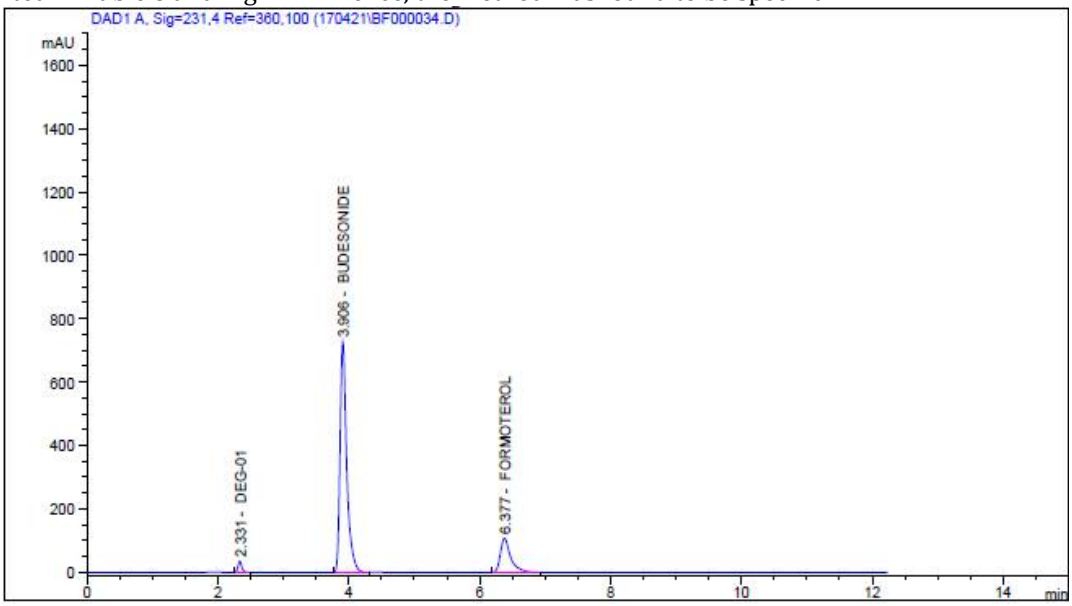

Fig.7: Chromatogram of BN and FT degraded with acid hydrolysis

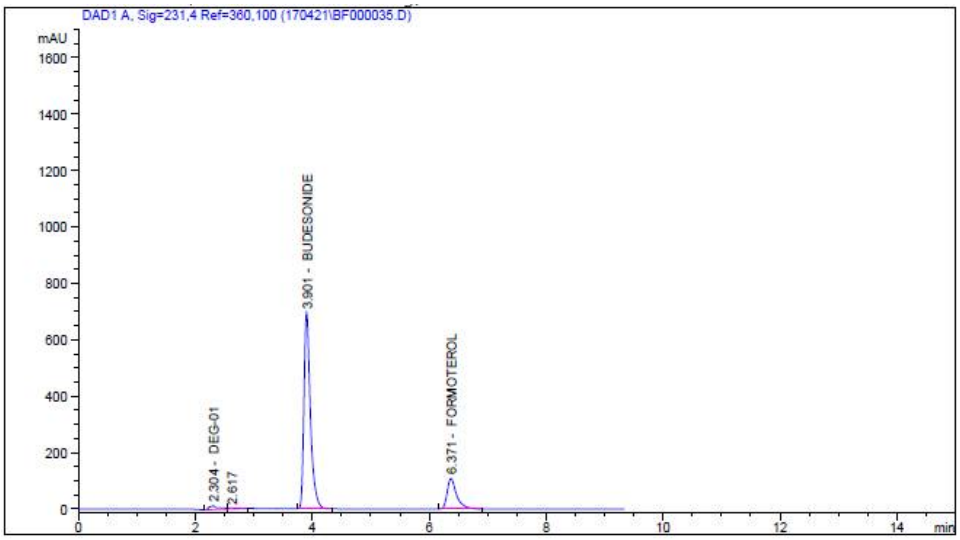

Fig.8: Chromatogram of BN and FT degraded with alkali hydrolysis

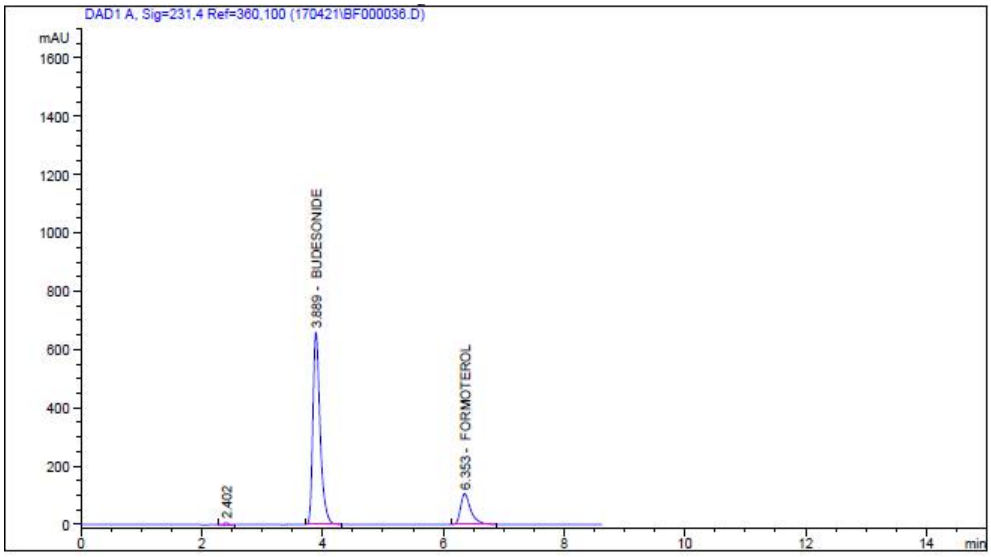

Fig. 9: Chromatogram of mixture of BN and FT degraded with neutral hydrolysis

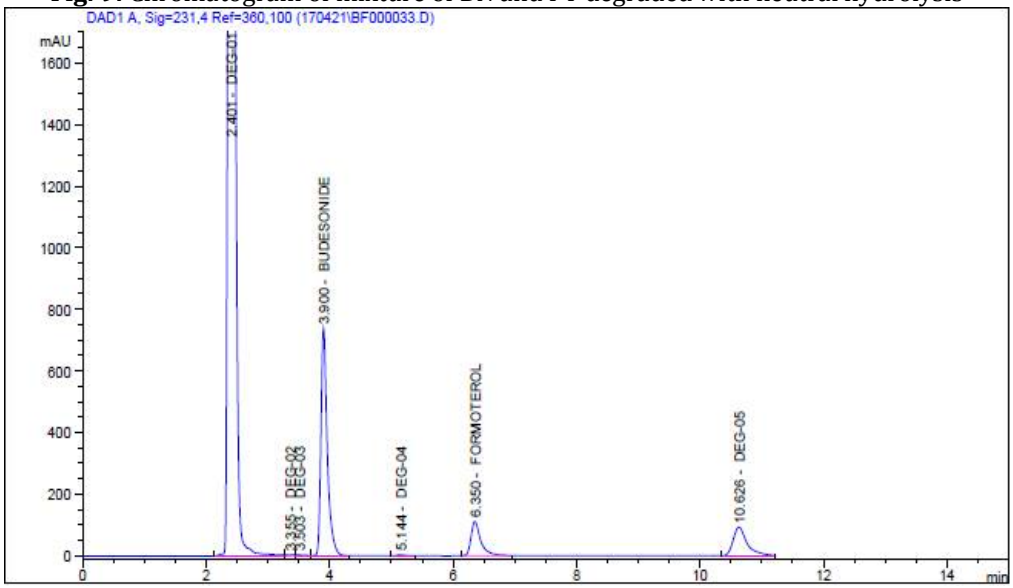

Fig.10: Chromatogram of BN and FT degraded with oxidative hydrolysis

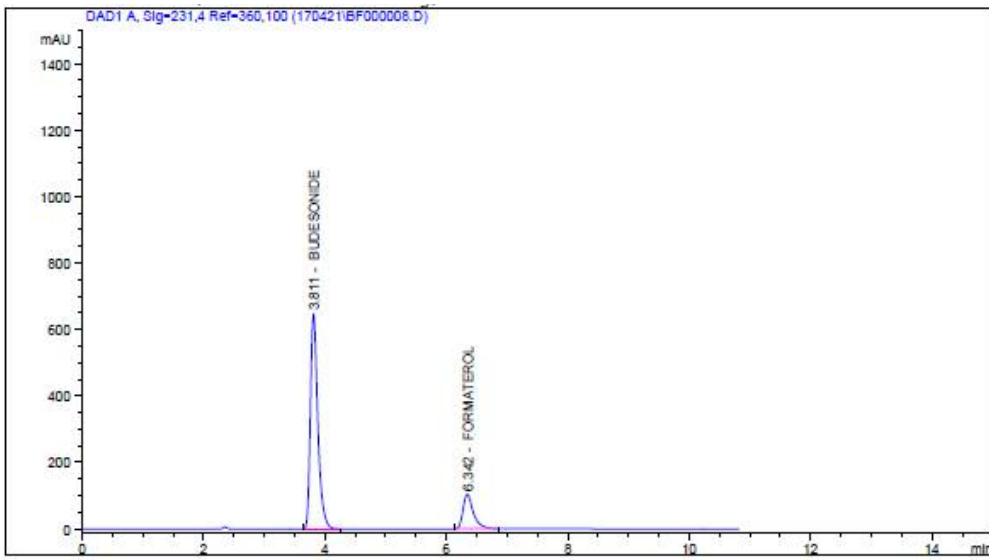

Fig.11: Chromatogram of BN and FT degraded with exposed to direct sunlight

Table 8: Stability-indicating method data for BN and FT

Parameter	BN (% degradation)	FT(% degradation)
Acidic(0.1N HCL for 2 hr)	83.13	25.15
Alkaline (0.1N NaH for 2 hr)	7.32	3.36
Hydrolytic(HPLC waters for 2hr)	0.39	0.41
Oxidative(3% H2O2 for 2 hr)	4.86	2.41
Photo(sun light for 24 hr)	0.13	0.19

HPLC: High Performance Liquid Chromatography

CONCLUSION

The Validated RP-HPLC method employed here proved to be simple, fast, accurate, precise, and robust, thus can be used for routine analysis of Budesonide and Formoterol in combined tablet dosage form.

REFERENCES

1. Pimpale, A., & Kakde, R. (2020). Development and Validation of Stability-Indicating Assay Method by RP-HPLC for Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Pharmaceutical Dosage Form. *Journal of Drug Delivery and Therapeutics*, *10*(4), 79-86.
2. Guideline, I. H. T. (2003). Stability testing of new drug substances and products. *Q1A (R2), current step*, *4*, 1-24.
3. Singh, P. S., & Shah, G. (2011). Analytical method development and validation. *Journal of Pharmacy Research*, *4*(7), 2330-2332..
4. ICH Expert Working Group. (1995). Q2 (R1): validation of analytical procedures: text and methodology. *Federal Register*, *60*, 11260.
5. Pharmacopoeia, I. (2007). Government of India Ministry of Health & Family Welfare, Published By the Indian Pharmacopoeia Commission, Ghaziabad. *Volume 1st*, *477*(478), 177-183.
6. Pai, N., & Patil, S. S. (2013). Development and validation of RP-HPLC method for estimation of formoterol fumarate and budesonide in pressurised meter dose inhaler form. *Der Pharmacia Sinica*, *4*(4), 15-25.
7. El-Bagary, R. I., Fouad, M. A., Manal, A., & Tolba, E. H. (2016). Forced degradation of mometasone furoate and development of two RP-HPLC methods for its determination with formoterol fumarate or salicylic acid. *Arabian Journal of Chemistry*, *9*(3), 493-505.
8. Bharti, P., Sachan, N., Chandra, P., Gahlo, K., Gouda, M. M., Shabaraya, A. R., ... & Kumar, P. R. (2011). Development and validation of selective UV spectrophotometric analytical method for budesonide pure sample.
9. Alkhateeb, F. L., Wilson, I., Maziarz, M., & Rainville, P. (2021). Ultra high-performance liquid chromatography method development for separation of formoterol, budesonide, and related substances using an analytical quality by design approach. *Journal of Pharmaceutical and Biomedical Analysis*, *193*, 113729.
10. Kumar, A. (2018). Development and validation of RP-HPLC method for Simultaneous Estimation of Formoterol Fumarate and Budesonide in their Combined Dosage Form (Doctoral dissertation, Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam).
11. Elkady, E. F., Tammam, M. H., & Elmaaty, A. A. (2017). Development and validation of RP-HPLC method for simultaneous estimation of tiotropium bromide, formoterol fumarate, and olodaterol HCL in bulk and metered

dose aerosols: Application to olodaterol HCL forced degradation study and degradation kinetics. *Chromatographia*, *80*(12), 1749-1760.

12. Taşkın, D., ErEnSoy, G., & SunGur, S. (2016). A validated spectrophotometric method for determination of formoterol fumarate dihydrate in bulk and dosage form using methyl orange as ion pair reagent. *Marmara Pharmaceutical Journal*, *20*(3).19-24