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A New Validated Stability indicating RP-HPLC Method for Simultaneous Quantification of Impurities of Fluticasone Propionate and Salmeterol Xenafoate in Metered Dose Inhalation Aerosol

SURYA PRAKASH MAMILLAPALLI[1,*], SHIRISHA KOYYA[1], B. VENKATA SUBBAIAH[1] and N. ANNAPURNA[2]

[1]Analytical Research and Development, IPDO, Dr Reddys Laboratories Ltd, Hyderabad-502325, India
[2]Department of Engineering Chemistry, Andhra University, College of Engineering, Visakhapatnam-530003, India

*Corresponding author: E-mail: suryapm@drreddys.com; suryanfg@gmail.com

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A simple, specific, precise, accurate and stability indicating reversed phase HPLC method for simultaneous quantification of total 12 impurities of fluticasone propionate and salmeterol xenafoate in metered dose inhalation aerosol has been developed in the present work. Chromatographic separation between impurities of both compounds were achieved on Altima C18 250 × 4.6 mm, 5 µ column using a step-gradient elution at a flow rate of 1.4 mL/min, 0.1% v/v orthophosphoric acid as buffer and acetonitrile as mobile phase constituents. Forced degradation studies for drug product were performed and revealed that Salmeterol is acid sensitive (about 21.3%), degrades to IMP-D and fluticasone is alkali sensitive (about 7.6%) and degrades to IMP-A. All degradant and process related impurities of both compounds were monitored at 214 nm and spectral purity along with % mass balance is assessed using PDA detector, which proved stability indicating capability of the method. The developed method is fully validated as per current ICH guidelines, where precision is achieved at % RSD of < 5, Correlation of < 0.999 for linearity, LOD-LOQ at < 0.02% and < 0.05%, along with satisfactory system suitability results under robustness conditions.

Keywords: Salmeterol xenafoate, Fluticasone propionate, Metered dose inhalers, Forced degradation, Photo diode array.

INTRODUCTION

Salmeterol xinafoate (SAL) is a long-acting β_2 adrenergic receptor agonist (LABA) used in the maintenance and prevention of asthma symptoms and chronic obstructive pulmonary disease (COPD). The chemical name of SAL is (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(4-phenylbutoxy)-hexyl]amino]ethanol-1-hydroxynaphthalene-2-carboxylate. Fluticasone propionate (FLC) is a highly selective agonist at the glucocorticoid receptor with negligible activity at androgen, estrogen or mineralocorticoid receptors, thereby producing anti-inflammatory and vasoconstriction effects. The chemical name of FLC is (6α,11β,16α,17α)-6,9-difloro-11-hydroxyl-16-methyl-3-oxo-17-(1-oxopropoxy)androsta-1,4-diene-17-carbothioic acid, S-fluoro methyl ester [1,2]. The orally administered MDI aerosol drug product is available in pressurized multidose canister containing FLC and SAL 250/25 µg, 125/25 µg and 50/25 µg per actuation and HFA134a as a propellant [1,2].

To evaluate quality and quantity of drugs in metered dose inhaler (MDI), till date a very few stability indicating analytical methods are available in public domain as well as pharmacopial forums. Few researchers [3-6] simultaneous HPLC methods for the estimation of impurities as well as assay and content uniformity of actives (salmeterol xenafoate and fluticasone propionate) in dry powder inhalers. However, these works lacks studies related to prove stability indicating strength of the method through forced degradation, mass balance and even placebo interference which is a mandate requirement for industrial applications. Nikam *et al.* [7] has demonstrated a gradient RP-UPLC method for simultaneous assay of salmeterol xenafoate and fluticasone propionate from DISKUS® inhalers. Although claimed as QbD based, this work doesn't provided any clarity and data related to QbD parameters and carry a disadvantage of high run time of 14 min which is inappropriate for UPLC-an equipment intended to shorten runtimes.

Ahmed *et al.* [8] reported a spectrophotometric method for simultaneous determination of salmeterol xinafoate and

fluticasone propionate in bulk powder and provided the comparision study with HPLC in resolving the bands and peaks of both compounds by UV-isobestic practices. Apart from simultaneous estimation, few methods were also reported with individual assays of salmeterol xinafoate and fluticasone propionate by HPLC, MEKC and HPTLC from bulk, dry powders, nasal sprays and inhalation particles on several matrices [9-15].

Based on literature survey, none of the references listed above supports data related to stress testing and in turn proving specificity and stability indicating capability of salmeterol xinafoate and fluticasone propionate. Considering this significance and novelty, we have chosen and developed a new stability indicating RP-HPLC method for simultaneous quantification of impurities of fluticasone propionate (FLC) and salmeterol xenafoate (SAL) delivered through metered dose inhalers (MDIs). The proposed method is stability indicating and thoroughly validated as per ICH [16] and system suitability parameters within compendial guidelines [17]. Challenges related to reduction in run time, adequate separation, forced degradation studies to demonstrate the method's stability indicating power, identification of process and degradation impurities were fully addressed.

EXPERIMENTAL

Qualified working standards and respective impurities (> 98%) for fluticasone propionate and salmeterol xenafoate were procured from Dr. Reddys Labs, Hyderabad, India. Orthophosphoric acid 88%, HPLC-Gradient grade acetonitrile and methanol were procured from Merck, Germany for mobile phase preparation and diluent preparation. 0.45µ filtered deionized water is attained from Milli-Q system, Millipore, USA.

HPLC system (Model: Alliance 2695, Make: Waters, Milford, USA) was used consists of a quaternary pump, auto sampler and a photo-diode array detector (PDA 2998). The output signal was monitored and processed using Empower-3 software. Sonicator (Power sonic 420), Centrifuge (Thermo electron GmbH, Germany) was used during preparation of solutions. Photo stability studies were carried out in a photo stability chamber (SUNTEST XLS+, Atlas USA) and thermal stability studies were performed in a dry air oven (Thermolab, India).

Chromatographic conditions: The method is developed using Altima C18 250 × 4.6 mm, 5 µ column (Alltech®). Buffer was prepared by dissolving 1 mL of orthophosphoric acid to 1000 mL of Milli-Q water and filtered through 0.45µ membrane filter. Mobile phase-A was prepared by mixing buffer and acetonitrile in the ratio of 900:100 v/v respectively and mobile phase-B was prepared by mixing buffer and acetonitrile in the ratio of 100:900 v/v, respectively. The gradient program (time in min/%B) is set as 0.0/20, 2.0/20, 45.0/50, 68.0/80, 70.0/100, 82.0/100, 85.0/20, 90.0/20 with flow rate of 1.4 mL/min. The column oven temperature was maintained at 60 ºC and sample cooler at 5 ºC. Blank as diluent (0.1% orthophosphoric and acetonitrile in the ratio of 60/40), Impure mixture, standard and samples were analyzed using HPLC system with 50 µL injection volume. Fluticasone propionate, salmeterol xenafoate and their related impurities were monitored at wavelength of 214 nm. Impurities shown in Figs. 1 and 2 are considered from USP and EP pharmacopoeial monographs. Total 12 impurities along with analyte peaks were targeted to separate in single chromato-graphic method for quantification (Fig. 3).

Standard solutions: Weighed and diluted salmeterol xenafoate and fluticasone propionate working standards in diluent to make a concentration of 1.2 µg/mL and 2 µg/mL, respectively.

Impurity stock solutions: Each individual impurities of salmeterol xenafoate (A, B, C, D, E, F, G) and fluticasone propionate (A, C, D, F, H) were weighed and dissolved in 10 mL of acetonitrile separately to attain a concentration of about 100 µg/mL.

Spiked impurity solutions: Pipetted 0.1 mL of each impurity into a 10 mL volumetric flask and diluted to volume with standard stock solution and mixed well.

Procedure: Metered dose inhaler (MDI) is available in 3 strengths, 250/25 µg per actuation, 125/25 µg per actuation and 50/25 µg per actuation. As a worst case scenario, considering drug to placebo ratio, 250/25 µg per actuation of fluticasone propionate and salmeterol xenafoate was chosen for method validation studies in comparision to other available strengths. Placed MDI canisters in a freezer at -40 ºC for 2 h. After 2 h retrieved the canisters from freezer, carefully cut at top with cutter and allowed to attain room temperature. Transferred the content into 200 mL volumetric flask and rinsed the canister with 10 mL diluent and transferred rinsed content to the same flask. Repeated rinsing for atleaset 5 times with diluent and transferred rinsed content to the same flask, further added 50 mL diluent and sonicated for 15 min. Transferred the solution to HPLC vials using a polypropylene dropper and subjected for analysis.

Method validation: As a part of current ICH guidelines [16], method validation is performed for all listed specified impurities of both the compounds, evaluating specificity, placebo interference and forced degradation, mass balance, LOD, LOQ establishment, precision, accuracy, linearity and range, Ruggedness and robustness. System suitability is monitored throughout the parameters of validation and verified resolution, tailing and % RSD for all impurity peaks.

RESULTS AND DISCUSSION

Method development: Fluticasone propionate (FLC) and salmeterol xenafoate (SAL) have pK_a value of about 13.56 and 10.12 [18,19]. Both active compounds have a strong UV absor-bance around 210 to 220 nm and upon screening individual impurity UV λ_{max} of both compounds, a common wavelength of 214 nm was selected for simultaneous estimation of both actives along with respective impurities. Development trials were initiated to optimize chromatographic conditions comp-rized of simple buffers and solvents with least UV cut-off, where 0.1% orthophosphoric acid (pH about 2.2), acetonitrile were chosen to achieve better separations and elution pattern with negligible baseline noise. Based on bonding chemistry, carbon loading, end capping efficiency, Hyperclone 150 × 4.6, 5 µ ODS (C18), ascentis express C18 150 × 4.6, 2.7µ, Altima C18, 250 × 4.6, 5µ, Inertsil ODS-3, 150 × 4.6, 3 µ columns were initially screened to attain

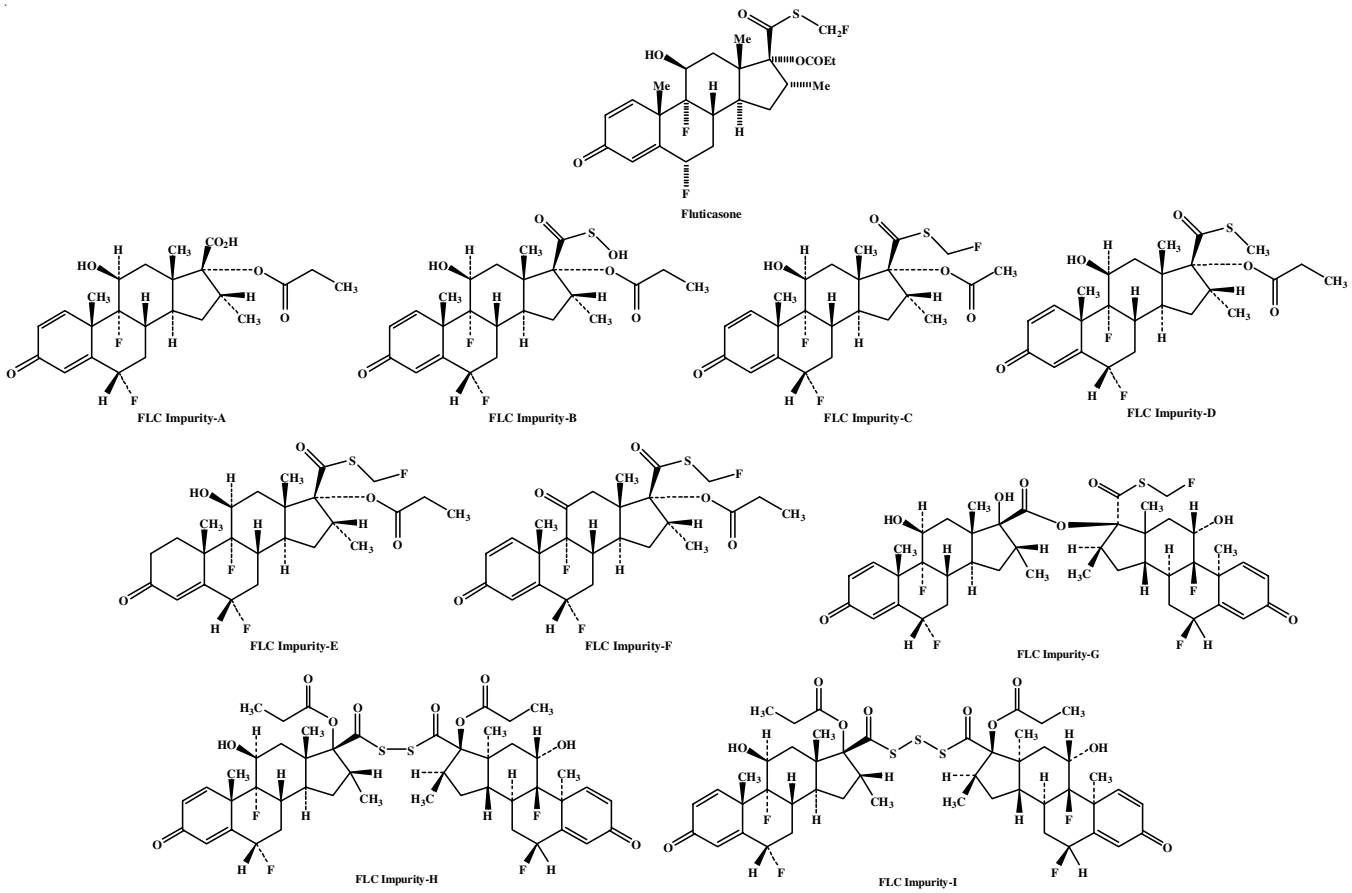

Fig. 1. Structure of fluticasone and its impurities

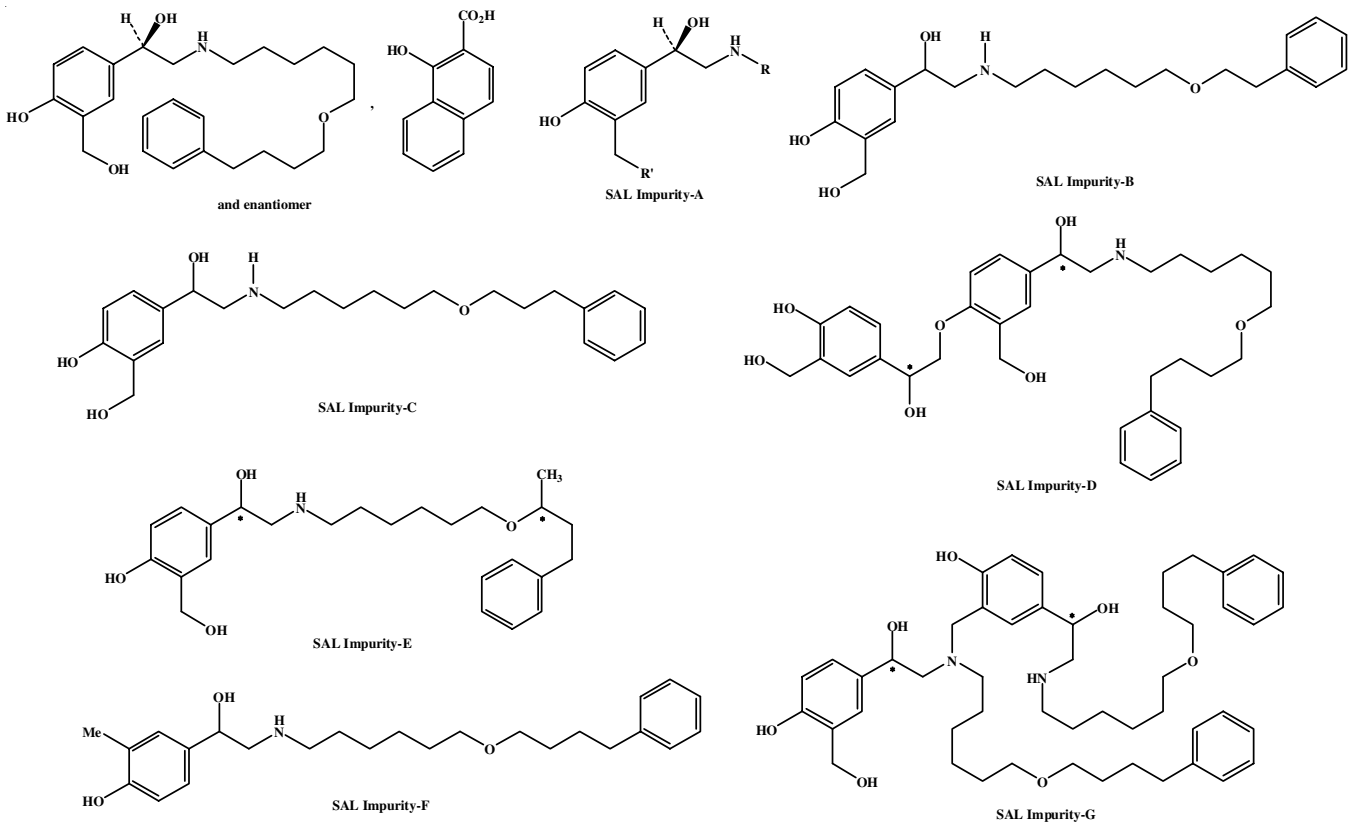

Fig. 2. Structure of salmeterol and its impurities

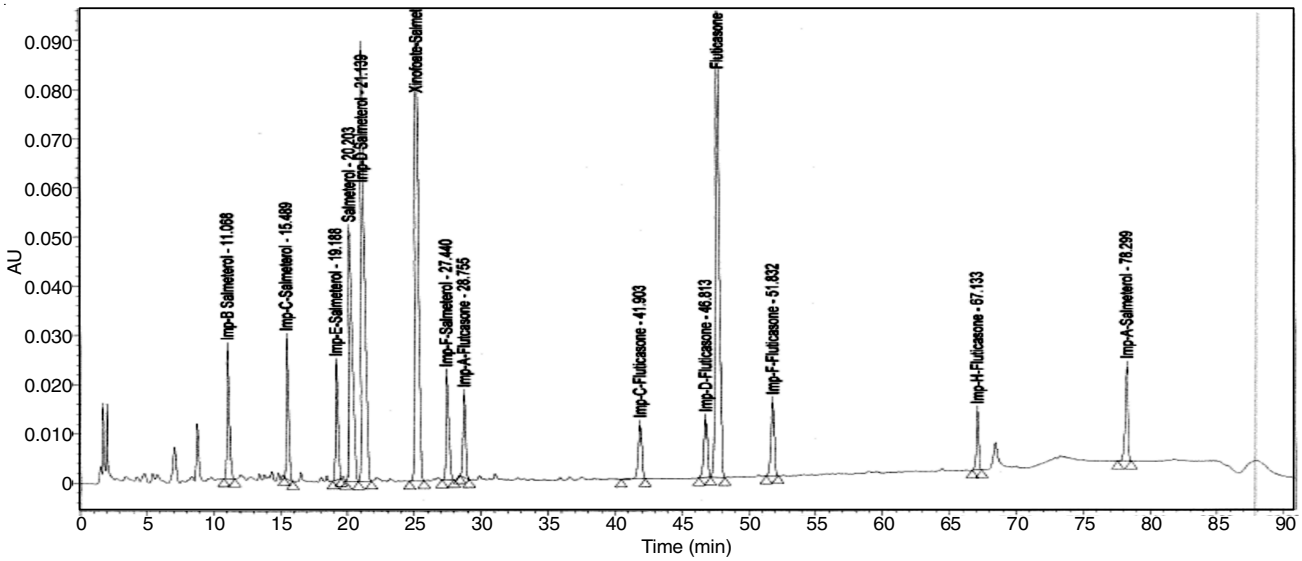

Fig. 3. Typical chromatogram of impurity spiked sample solution

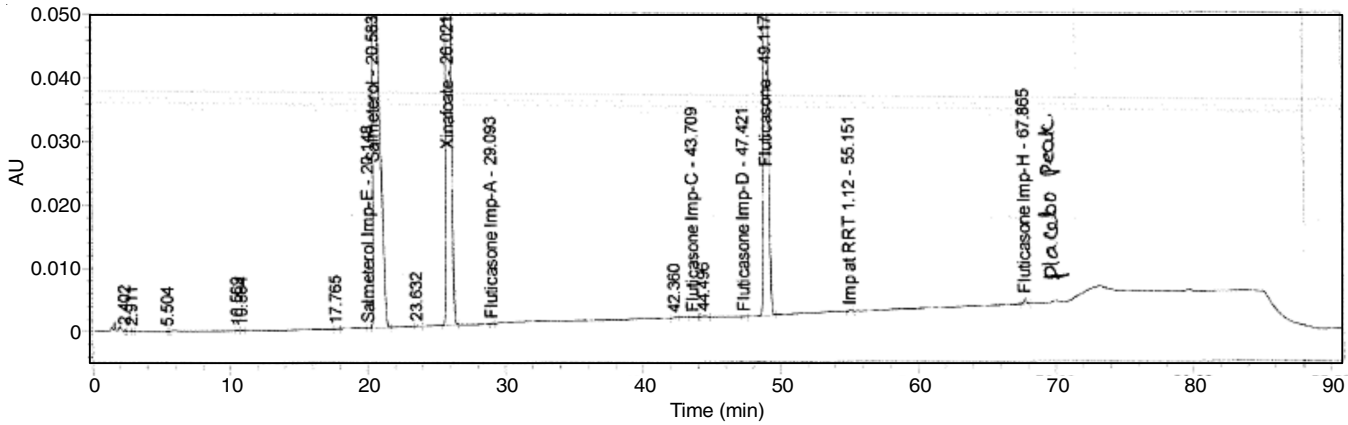

Fig. 4. Typical chromatogram of control test preparation

separation and optimal system suita-bility between analyte peaks (USP Resolution NLT 1.5, Tailing NMT 2.0).

Trials were initiated with isocratic mode to understand elution pattern of impurities and later trials were performed by selecting linear gradients, where chromatograms were run with higher aqueous mobile phase (A) to high organic phase (B) (Figs. 4 and 5). Although extraneous gradient peaks with noisy baseline were observed, smooth and consistent baseline has been attained by selecting buffer and acetonitrile mixtures 900:100 v/v as mobile phase-A and 100:900 v/v buffer and acetonitrile as mobile phase-B. Upon deliberate trials, Altima C18, 250 × 4.6, 5 µ column with step-gradient (time in min/%B) is set as 0.0/20, 2.0/20, 45.0/50, 68.0/80, 70.0/100, 82.0/100, 85.0/20, 90.0/20 with flow rate of 1.4 mL/min at column oven temperature at 60 ºC gave superior impurity peak shape and separation between impurities with satisfactory system suitability.

Specificity and forced degradation: Specificity is the ability of the method to measure the analyte response in the presence of inactive matrix (placebo), forced degradants peaks and other related impurities. To evaluate the stability indicating capability, separation and quantification of all possible degradants, forced degradation studies were performed on drug

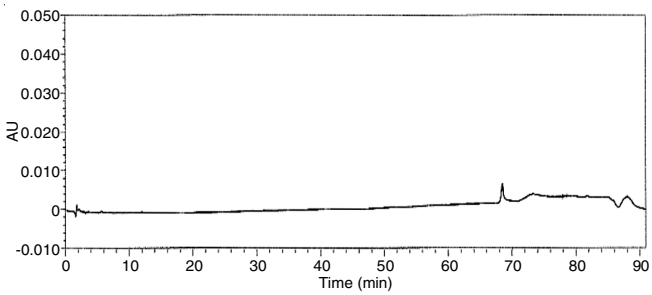

Fig. 5. Typical chromatogram of placebo solution

product as well as placebo. Exposed the test and placebo samples to different physico-chemical stress conditions and analyzed stress samples using photodiode array detector.

Salmeterol xenafoate was observed to be sensitive to acid and oxidation stress and degrades to impurity D as a major degradant. In other conditions, no significant degradation was observed for salmeterol xenafoate (Table-1). Fluticasone propionate was observed as slightly sensitive to acid stress (< 2%) but highly sensitive to alkali and oxidation conditions (Table-1). When exposed to alkaline stress, fluticasone propionate degrades to impurity A (7.6%) as a major degradant. Oxidative

TABLE-1
STRESS RESULTS OF SALMETEROL XENAFOATE (SAL) AND FLUTICASONE PROPIONATE (FLC)

Sample name	Assay of active (%)		Net degradation (%)		Mass balance		**Purity angle		**Purity threshold		**Purity flag	
	SAL	FLC	SAL	FLC	SAL	FLC	SAL	FLC	SAL	FLC	SAL	FLC
Unstressed	99.03	98.12	0.3	0.4298	100.3	100.39	0.135	0.080	0.313	0.254	No	No
Acid stress (0.1 N HCl at 85°C for 2 h)	80.76	97.91	21.4	0.5562	103.1	101.54	0.134	0.198	0.354	0.295	No	No
Base stress (0.1 N NaOH for 3 h)	97.87	87.89	1.8	7.6796	100.7	97.39	0.146	0.088	0.327	0.264	No	No
Oxidation stress (1% H_2O_2 for 3 h)	99.32	97.02	1.8	0.4310	102.1	99.32	0.272	0.112	0.336	0.254	No	No
Water stress (at 75°C for 11 h)	98.05	97.48	2.3	0.4633	101.4	99.77	0.146	0.092	0.396	0.305	No	No
Heat stress (at 70°C for 12 h)	100.09	95.69	0.2	0.4359	100.2	100.79	0.158	0.080	0.359	0.266	No	No
Photo stress (1.2 million Lux/h)	96.21	97.58	2.8	0.3973	100.0	99.81	0.131	0.071	0.357	0.257	No	No

**As per Empower software: Purity angle should be less than purity threshold with no flag.

stress resulted in numerous peaks (< 0.04%), however no significant degradants were noticed atleaset above 2%. These major degradants were confirmed by comparing relative retention times (RRT) of individual impurities spiked against degradation samples along with their UV spectra by PDA. Hence, further studies with LC-MS were not conducted to identify the degradants.

Accuracy and precision: Accuracy or recovery of impurities in presence of active and inactive matrix plays a critical role to assess the level and extent of recovery and extraction efficiency of the method. Inadequacy of recovery leads to non-reproducible and unreliable results, which in turn impacts the quality of product. Prepared six replicates of impurity stock spiked test samples at each 100% and 120% levels and calculated the precision (% impurity, % RSD) and recovery (%found/%added). As a part of ruggedness, intra-day precision analysis was performed with different HPLC system (Make: Agilent 1200 series, Germany), different column and different analyst.

LOQ, LOD and linearity: Considering the LOQ value, where the s/n ratio was about 10 and for LOD about 3, specified levels were spiked on placebo and attained chromatograms were processed using empower software by signal-to-noise method. After achieving desired LOQs, six replicates of specified concentrations were spiked on placebo and drug product and precision, recoveries for all impurities along with active peaks were evaluated (Table-2). From stocks of impurities and active components, serial dilutions were carried to attain solutions ranging from LOQ to 120% of specification level. Linearity of detector response is assessed by reporting slope, y-intercept and correlation coefficient (< 0.990).

Stability: Established stability of solution by spiking all impurities on test sample till 5 days on bench top and refrigerator. Stability of mobile phase and standard are established by injecting samples storing at refrigerator for 5 days. Established on day-1, day-2 and day-5, observed that the solutions standard, sample are stable for 2 days at refrigerator condition *i.e.* 2-8 ºC. No extra peaks and compatibility issue found with samples filtered through PVDF and nylon membrane filters and results were found satisfactory.

Robustness: Established robustness by allowing deliberate changes to existing method conditions, *i.e.*, flow rate (± 10%), column oven temperature (± 5 ºC), organic component variation in mobile phase-A and mobile phase-B (± 10%). System suitability and resolution between critical pairs were monitored along with relative retention times (RRT). Elution pattern were found comparable to actual chromatogram and system suitability found within acceptance limits of USP requirements. This proves the method is robust and can sustain variability in chromatographic conditions within established range (Tables 3 and 4).

Conclusion

The proposed method for the simultaneous quantification of total 12 impurities of fluticasone propionate and salmeterol

TABLE-2
SUMMARY OF RESULTS FOR LOD, LOQ, LINEARITY, RECOVERY AND PRECISION

Name of Impurity	LOQ (%)	LOD (%)	Correl	Recovery			%RSD from precision at 100% level
				LOQ (%)	100% level	120% level	
Fluticasone propionate	0.03	0.01	0.999	95.40	101.10	102.20	3.90
Impurity-A	0.04	0.02	0.999	97.50	96.40	101.40	6.70
Impurity-C	0.03	0.01	0.999	104.20	101.10	98.30	5.40
Impurity-D	0.03	0.01	0.999	101.60	103.40	97.40	7.60
Impurity-F	0.04	0.02	0.999	104.70	94.50	98.70	8.70
Impurity-H	0.04	0.02	0.999	96.30	96.70	99.20	5.90
Salmeterol xenafoate	0.03	0.01	0.999	98.20	97.80	104.30	6.50
Impurity-A	0.04	0.02	0.998	101.10	103.40	105.20	7.30
Impurity-B	0.03	0.01	0.998	106.20	104.80	103.20	6.20
Impurity-C	0.03	0.01	0.999	98.90	106.50	104.20	8.20
Impurity-D	0.04	0.02	0.998	95.40	101.30	100.20	3.90
Impurity-E	0.04	0.02	0.997	94.90	98.30	97.30	8.10
Impurity-F	0.03	0.01	0.998	96.20	99.20	96.50	7.80
Impurity-G	0.04	0.02	0.998	97.30	101.30	95.60	5.50

TABLE-3
RELATIVE RETENTION TIMES (RRT) OF IMPURITIES OBSERVED IN DIFFERENT ROBUSTNESS CONDITIONS

Name of the peak	Control	Flow rate (mL/min)		Temperature		Mobile phase-A		Mobile phase-B	
		1.2	1.6	40 °C	50 °C	90%	110%	90%	110%
Salmeterol xenafoate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Impurity-B	0.54	0.56	0.52	0.55	0.54	0.55	0.53	0.54	0.54
Impurity-C	0.78	0.80	0.77	0.79	0.78	0.79	0.77	0.78	0.78
Impurity-E	0.98	0.98	0.98	0.97	0.98	0.98	0.97	0.98	0.98
Impurity-D	1.10	1.10	1.12	1.11	1.11	1.10	1.10	1.11	1.11
Impurity-F	1.50	1.47	1.53	1.50	1.51	1.47	1.53	1.49	1.50
Impurity-G	2.06	1.97	2.18	2.04	2.11	1.99	2.14	2.08	2.08
Impurity-A	4.02	4.12	3.92	4.05	3.95	4.04	3.98	4.03	4.01
Fluticasone propionate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Impurity-A	0.56	0.57	0.55	0.56	0.55	0.57	0.54	0.56	0.56
Impurity-C	0.87	0.88	0.87	0.88	0.87	0.88	0.87	0.87	0.87
Impurity-D	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Impurity-F	1.08	1.07	1.09	1.07	1.09	1.07	1.09	1.08	1.09
Impurity-H	1.31	1.28	1.36	1.29	1.33	1.29	1.34	1.31	1.32

TABLE-4
ROBUSTNESS-RESOLUTION BETWEEN CRITICAL PAIRS

Parameter	Control	Temperature		Flow rate (mL/min)		Mobile phase-A		Mobile phase-B	
		55 °C	65 °C	1.2	1.6	90%	110%	90%	110%
SAL Imp-E and SAL	2.2	2.4	2.1	2.2	2.3	2.2	2.2	2.2	2.1
SAL and SAL Imp-D	1.6	1.8	1.7	1.5	1.9	1.8	1.7	2.1	2.0
SAL Imp-F and FLC Imp-A	3.3	3.5	3.0	3.7	2.8	3.2	3.2	3.1	3.2
FLC Imp-D and FLC	1.8	2.1	1.6	1.6	1.8	1.7	1.7	1.8	1.7
Placebo Peak and FLC Imp-H	1.8	2.3	1.5	1.6	2.1	1.8	1.8	1.7	1.8

xenafoate in metered dose inhaler found to be precise, specific, accurate, linear, robust and rugged. Trials related to selection of best chromatographic conditions to achieve consistent gradient and robust method were discussed. Stress studies revealed the degradation behaviour of drug product and all degradants were well separated from main peaks. Data related to the intentional changes to chromatographic conditions confirmed the method is robust enough to sustain routine laboratory variations at quality control level. The method can be confirmed as stability indicating and can be used for development laboratory scale batches as well as for stability screening of drug product.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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