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A New Validated Stability Indicating RP-HPLC Method for Simultaneous Quantification of Formoterol Fumarate Dihydrate and Mometasone Furoate in Metered Dose Inhalation Aerosol

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Stability indicating reversed phase-HPLC method for simultaneous estimation of mometasone furoate (MAF) and formoterol fumarate (FFD) in metered dose inhalation aerosol (MDI) dosage formulation has been developed and discussed in the present work. The chromatographic separation was achieved using Hypersil ODS column (250 mm × 4.6 mm, 3 µm) using an isocratic separation mode at a flow rate of 1.2 mL/min, column temperature of 50 ºC. The system operates with a mobile phase comprising of solution-A (buffer): Solution-B (acetonitrile) mixed in the ratio of 70:30 %v/v at a UV detection wavelength of 214 nm. Retention times of mometasone furoate and formoterol fumarate found to be about 3 min and 7 min, respectively. All possible degradation products of both compounds were monitored at 214 nm and spectral purity along with % mass balance is assessed using PDA detector. Both analyte were subjected to force degradation studies, found all degradants were resolved from analyte peaks and also other process-related impurities. The proposed method is validated for specificity, linearity, accuracy, precision and robustness as per ICH guidelines and found to be adequate. Method stood to be robust with variation in column temperature, flow rate, pH of buffer and organic content in mobile phase.

Keywords: Formoterol fumarate dihydrate, Mometasone furoate, Metered dose inhalers, Forced degradation, Photodiode array.

INTRODUCTION

Metered dose inhaler dosage forms (MDIs) were developed and targeted mainly for COPD (chronic obstructive pulmonary disease) patients, as well as for disease conditions which were not adequately controlled by a long-term asthma medication such as an inhaled corticosteroid (ICS). MDIs have distinct advantage over other conventional dosage forms in delivering fixed therapeutic dose directly to the lungs, surpassing first pass metabolism, with enhanced efficacy and ease of administration [1]. Subsequently MDIs are portable, easy to use, provides immediate relief, prefilled multidose delivery actuations and low risk of bacterial contamination. Since a decade, demand for MDIs is increasing due to rise in pollution across the world and climatic changes collectively leading to COPD, bronchial and other related respiratory issues. To meet the global demand, pharmaceutical companies have to equip a distinct portfolio of inhalations, starting from research and development, clinical studies, chemistry, manufacturing and controls throughout the lifecycle which were driven by stringent regulatory guidelines. To estimate purity and efficacy of active components in metered dose inhalers, stability indicating analytical methods are not available in industry, open province and pharmacopial forums.

Several researchers have published simultaneous HPLC methods for estimation of assay in meter dose inhalers [2-5]. Nonetheless available mechanism lacks revisions associated to demonstrate stability indicating power of the method through stress studies which is a mandatory requirement for industrial applications. Other researchers [6-8] also developed method and performed forced degradation studies but not demonstrated separation of possible degradants which strengthens specificity of methodology. Based on systematic works review, not any of the orientations listed above supports data related to degradation studies and to demonstrate specificity and stability indicating capability of mometasone furoate and formoterol fumarate. Bearing in mind this consequence and uniqueness, we have chosen and established a new stability indicating

reversed phase HPLC method for instantaneous quantification of formoterol fumarate dihydrate (FFD) and mometasone furoate (MAF) delivered through MDIs. This process is skilful to distinct most of possible impurities through different synthetic route of active manufacturing with reduced run time.

Formoterol fumarate dihydrate (FFD) is a β_2-agonist by pharmacological action and very effective bronchodilating agent [9]. Chemically named as (±)-2′-hydroxy-5′-[(R*)-1-hydroxy-2-[[(R*)-*p*-methoxy-α-methylphenethyl]amino]ethyl]-formanilide fumarate (2:1) (salt), dihydrate (Fig. 1a) and is a white crystalline powder, soluble in ethanol and methanol, slightly soluble in water, practically insoluble in acetonitrile. Mometasone furoate is a topical corticosteroid having anti-pruritic, anti-inflammatory and vasoconstrictive properties [10]. Chemically named as 11β,16α-9, 21-dichloro-11β-hydroxy-16-methyl-3, 20-dioxopregna-1,4-dien-17-yl 2-furoate (Fig. 1b) and is a white crystalline powder, soluble in acetone and dichloromethane and slightly soluble in ethanol, practically insoluble in water. The combination of these two drugs are effective in the management of asthma in patients of 12yrs and older by acting on the lungs locally by bronchodilating and relaxing the airway muscles for improved breathing [11]. The orally administered MDI aerosol drug product is available in pressurized multidose canister containing formoterol fumarate dihydrate and mometasone furoate as 5/100 µg and 5/200 µg per actuation and HFA 227ea as a propellant [12].

EXPERIMENTAL

Tested and certified secondary standards having purity > 98% and individual impurities for formoterol fumarate dihydrate (FFD) and mometasone furoate (MAF) were procured from different API manufacturers and qualified by Dr. Reddys Labs. Orthophosphoric acid 88%, hydrogen peroxide HPLC-Gradient grade acetonitrile and methanol were procured from Rankem, India for mobile phase preparation and diluent preparation. A 0.45 µ filtered deionized water was attained from Milli-Q system, Millipore, USA.

HPLC system (Model: 1200 series, Make: Agilent) equipped with quaternary pump (G1311A), UV-visible detector (G1314B), injector (G1328B) with (100 µL) injector loop and degasser (G1322A). The output signal was monitored and processed using Empower-3 software. The Heraeus Megafuge 8 centrifuge (Thermo-Fisher Scientific) and Sonicator was used during the preparation of solutions. Photo stability studies were carried out in a photo stability chamber (SUNTEST XLS+, Atlas USA) while the thermal stability studies were performed in a dry air oven (Thermolab, India).

Chromatographic conditions: Chromatographic separation and quantitation was achieved on Hypersil ODS C18 column (250 mm × 4.6 mm, 3 µm). The mobile phase comprising of solution-A (10 mM of sodium dodecyl sulphate in 25 mM of phosphate buffer pH 3.0 and acetonitrile (70:30 %v/v)): Solution-B (acetonitrile) mixed in the ratio of 70:30 %v/v. The chromatographic system was operated at a flow rate of 1.2 mL/min, column temperature of 50 ºC and a detection wavelength of 214 nm. Impurity mixture, standard and samples were analyzed using HPLC system with 50 µL injection volume. Formoterol fumarate dihydrate (FFD) and mometasone furoate (MF) were monitored at wavelength of 214 nm. Impurities shown in Fig. 2 are considered from USP, EP pharmacopoeial monographs and diverse API vendors. Total 14 impurities along with two analyte peaks were targeted to separate in single isocratic chromatographic method. Diluent was prepared by mixing water and acetonitrile in the ratio of 35:65 v/v, respectively.

Standard solution preparation: Weighed and diluted formoterol fumarate dihydrate (FFD) and mometasone furoate (MAF) working standards in diluent to make a concentration of 4 µg/mL and 86 µg/mL, respectively.

Impurity stock preparation: All the individual impurities of formoterol fumarate dihydrate (A, B, C, D, E, F, G and H) and mometasone furoate (β-epoxide, Imp-7, Imp-G, Imp-D, Imp-H and icomethasone) were weighed and liquefied in 10 mL of acetonitrile independently to achieve a attention of about 100 µg/mL.

Spiked impurity solution preparation: Pipette out 0.1 mL of individual impurity stock into a 10 mL volumetric flask, diluted to volume with standard stock solution and mixed well.

Test preparation and process: Metered dose inhaler dosage forms (MDIs) of the drug product is available in two different strengths, 100/5 µg per actuation and 200/5 µg per actuation. Considering drug to placebo ratio, 200/5 µg per actuation of FFD and MAF was chosen for method validation studies in comparision to other available strengths. Located the canisters in a freezer at -30 ºC for 2 h. Post exposure for 2 h, recovered the canisters from freezer, cautiously cut the canister and allowed to reach room temperature. Collected the content

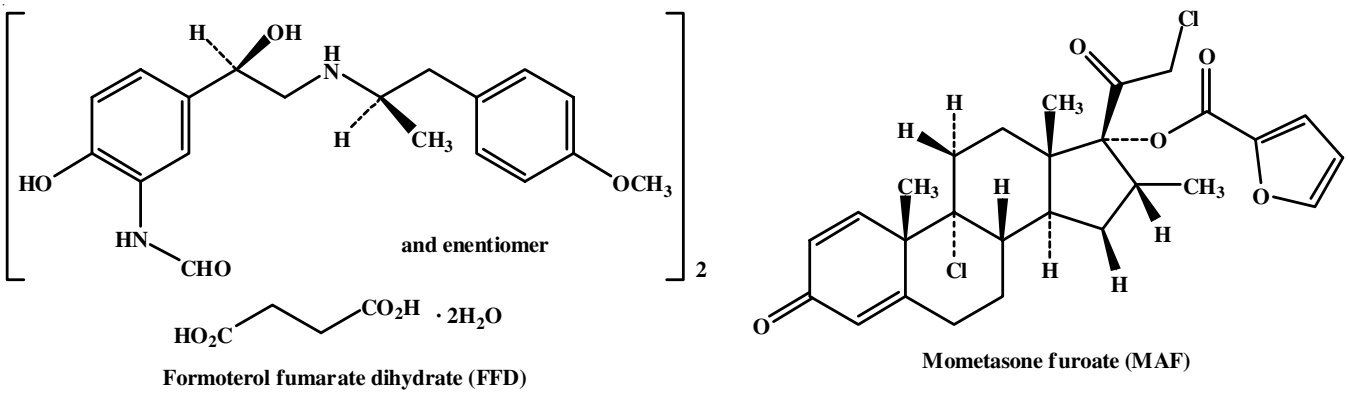

Fig. 1. Structure of formoterol fumarate dihydrate and mometasone furoate

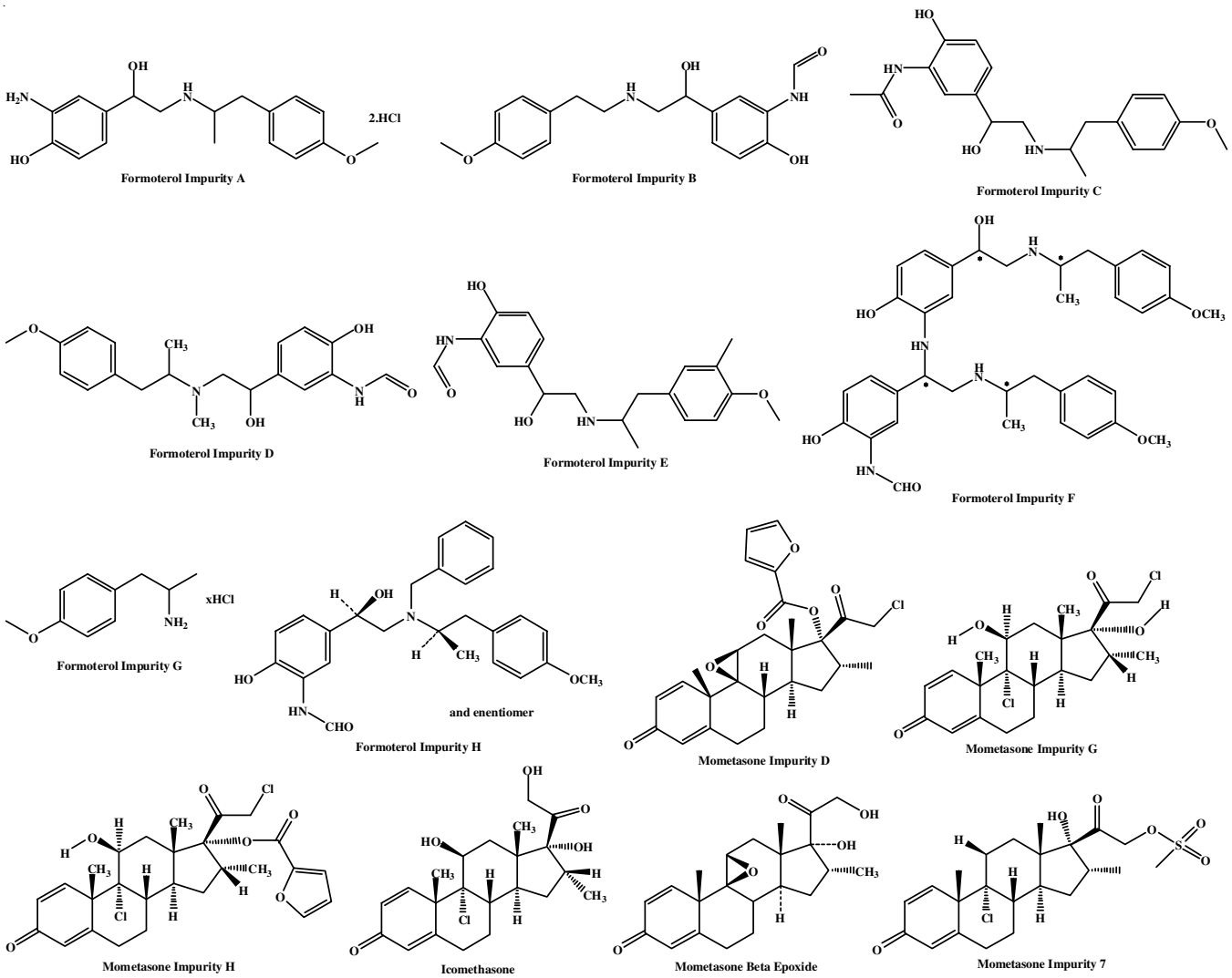

Fig. 2. Structure of impurities

into 250 mL volumetric flask and recovered the total content by rinsing with diluent. Added 100 mL diluent and sonicated for 15 min, made up the volume with diluent. Used the solution as test preparation and injected to HPLC.

Method validation: Method validation was performed for both active compounds by demonstrating specificity that is Impurity separation, interference from excipients, stress study, method precision, accuracy, linearity and range, ruggedness and robustness as per current ICH guidelines.

RESULTS AND DISCUSSION

Method development: Formoterol fumarate dihydrate (FFD) has pK_a of about 7.9 and 9.2, while mometasone furoate (MAF) has pK_a about 13.02 and 3.59. Individually, FFD and MAF has UV absorbance around 220 nm, a common wavelength of 214 nm was selected for synchronized assessment of both active compounds. Method optimization experiments were executed to optimize chromatographic parameters comprised of simple buffers and solvents to accommodate UV cut-off range, where phosphate buffer (pH 3), acetonitrile were selected to attain better resolutions and elution pattern with insignificant baseline disturbance. After evaluating column chemistry for silica generation, particle size and end capping efficiency, YMC Triart C18 75 × 3.0, 3µ, Hypersil ODS C18 250 × 4.6, 3µ, Hypersil BDS C18 250 mm × 4.6 mm, 3µ and Inertsil ODS-3V, 150 mm × 4.6 mm, 3µ columns were initially screened to accomplish resolution and system suitability between analyte peaks. Factors like Resolution of NLT 1.5, Tailing NMT 2.0 were chosen to select the right column and chromatography.

Initially, experiments were executed with isocratic mode and understood elution pattern of active compounds and impurities. Later trials were performed by introducing ion pair reagent (sodium octane suphonic acid) to attain better separation between impurities and active components. After performing a series of developmental trails, consistent baseline has been achieved by selecting buffer and acetonitrile mixtures 700:300 v/v as mobile phase-A and acetonitrile as mobile phase-B. Upon deliberate trials, Hypersil ODS C18 250 mm × 4.6 mm, 3µ column with isocratic elution was chosen in the ratio of mobile phase A and mobile phase B in the ratio of 70:30 v/v, respectively.

Flow rate of 1.2 mL/min at column oven temperature at 50 ºC provides desired separation, peak shape with acceptable resolution and system suitability.

Specificity and forced degradation: Specificity refers to the competency of a method to estimate peaks from forced degradation studies, the response of analytes in presence of an inactive ingredient (placebo) and other possible impurities. To determine stability for obtaining resolution and method nature and quantifying possible degradants, stress investigations were conducted on drugs and placebo. The placebo and test samples were treated under various physico-chemical stress conditions. The stress samples were studied using a photo diode array detector.

Formoterol fumarate dihydrate (FFD) degraded into impurity A, the key degradant and was sensitive to oxidation stress. Under other stress conditions, FFD did not degraded considerably. Mometasone furoate degraded into impurity D, the major degradant and was highly sensitive to base stress. At > 2%, no other major degradants were obtained. The main degradants were determined using PDA by comparing the degraded samples with their UV spectra against the relative retention times (RRTs) of specified spiked impurities (Tables 1 and 2). Hence, mass spectroscopic studies were not conducted to identify degradants. Each chromatogram was processed. The % assay was calculated for MAF and FFD. Spectral analysis (threshold; peak purity angle) was conducted. Placebo solutions were subjected to similar stress conditions. The results evidenced that at MAF and FFD retention time, no interference was obtained from excipients (Fig. 3).

Accuracy and precision: Accuracy or recovery of active components in presence of active and inactive placebo acting a critical role to absolutely assess the level and extent of recovery and extraction competence of the procedure. Insufficiency of recovery results to non-reproducible and defective results, which may in turn effects superiority of product for the intended use. Prepared three replicates of test sample at 50%, 100% and 150% levels and calculated the precision (% assay, % RSD) and recovery (Tables 3 and 4). As a part of ruggedness, intra-day precision analysis was performed with different HPLC system (Agilent 1200 series, Germany), altered column and altered analyst.

Linearity: Linearity was established by plotting a graph between concentrations *versus* peak area of formoterol fumarate dihydrate (FFD) and mometasone furoate (MAF), determined the correlation coefficient. A series of solutions of mometasone furoate and formoterol fumarate were prepared in concentration ranges of 43 ppm to 131 ppm for MAF and 2.1 ppm to 6.3 ppm for FFD, analyzed as per test method. A graph was plotted to concentration in ppm on *x*-axis *versus* peak area on *y*-axis. The correlation coefficient and biased value at 100% response were within limits. Intercept, slope value and residual sum of squares were calculated and the results are shown in Table-5 for results.

Stability: Performed stability of standard and test solution for a period of 5 days at room temperature and at cool temperature about 2-8 ºC. Stability of mobile phase also determined by storage at room temperature for 5 days. Determined stability of solutions on day-1, day-2 and day-5, observed that the solutions standard and test solutions are stable for 5 days at room temperature. No additional or extraneous peaks and compatibility issue found with samples filtered through 0.45 micron PVDF and Nylon membrane filters (Merck) and results are found satisfactory (Table-6).

TABLE-1
STRESS STUDY RESULTS FOR FORMOTEROL FUMARATE DIHYDRATE (FFD) AND MOMETASONE FUROATE (MAF)

	%Assay of active		**Purity angle		**Purity threshold		**Purity flag	
	FFD	MAF	FFD	MAF	FFD	MAF	FFD	MAF
Unstressed FFD/MF	99.7	96.6	0.167	0.144	1.165	1.031	No	No
Acid stress – 0.5 N HCl at room temperature for 30 min	101.6	85.8	0.223	0.111	0.249	0.233	No	No
Base stress – 0.05 N NaOH at room temperature for 10 min	102.7	81.4	0.204	0.126	0.307	0.235	No	No
Oxidation stress- 3% peroxide for 15 min	92.9	96.8	0.192	0.173	0.323	0.262	No	No
Humidity- >90% RH for 7 days	103.2	98.6	0.273	0.033	1.180	1.012	No	No
Hydrolytic - at 60 °C for 120 min	98.8	96.8	1.141	0.157	1.142	1.029	No	No
Heat stress - at 105 °C for 11 h	98.2	97.6	0.261	0.173	1.131	1.032	No	No
Photo stress-1.2 million Lux/h	103.3	98.6	0.422	0.084	1.079	1.005	No	No

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

TABLE-2
SPECIFICITY DATA OF THE RETENTION TIME OF IMPURITIES OF
FORMOTEROL FUMARATE DIHYDRATE (FFD) AND MOMETASONE FUROATE (MAF)

Name of the component	Retention time (min)	Name of the component	Retention time (min)
Formoterol fumarate dihydrate	3.15	Mometasone furoate	7.7
Formoterol Imp-A	3.76	Mometasone-Beta epoxide	2.19
Formoterol Imp-B	2.87	Mometasone Imp-7	3.36, 4.17
Formoterol Imp-C	3.39	Mometasone Imp-G	4.18
Formoterol Imp-D	3.31	Mometasone Imp-D	8.85
Formoterol Imp-E	4.09	Mometasone Imp-H	3.44
Formoterol Imp-F	3.31, 3.82 and 4.09	Mometasone Imp-Icomethasone	2.35
Formoterol Imp-G	3.53		
Formoterol Imp-H	5.78		

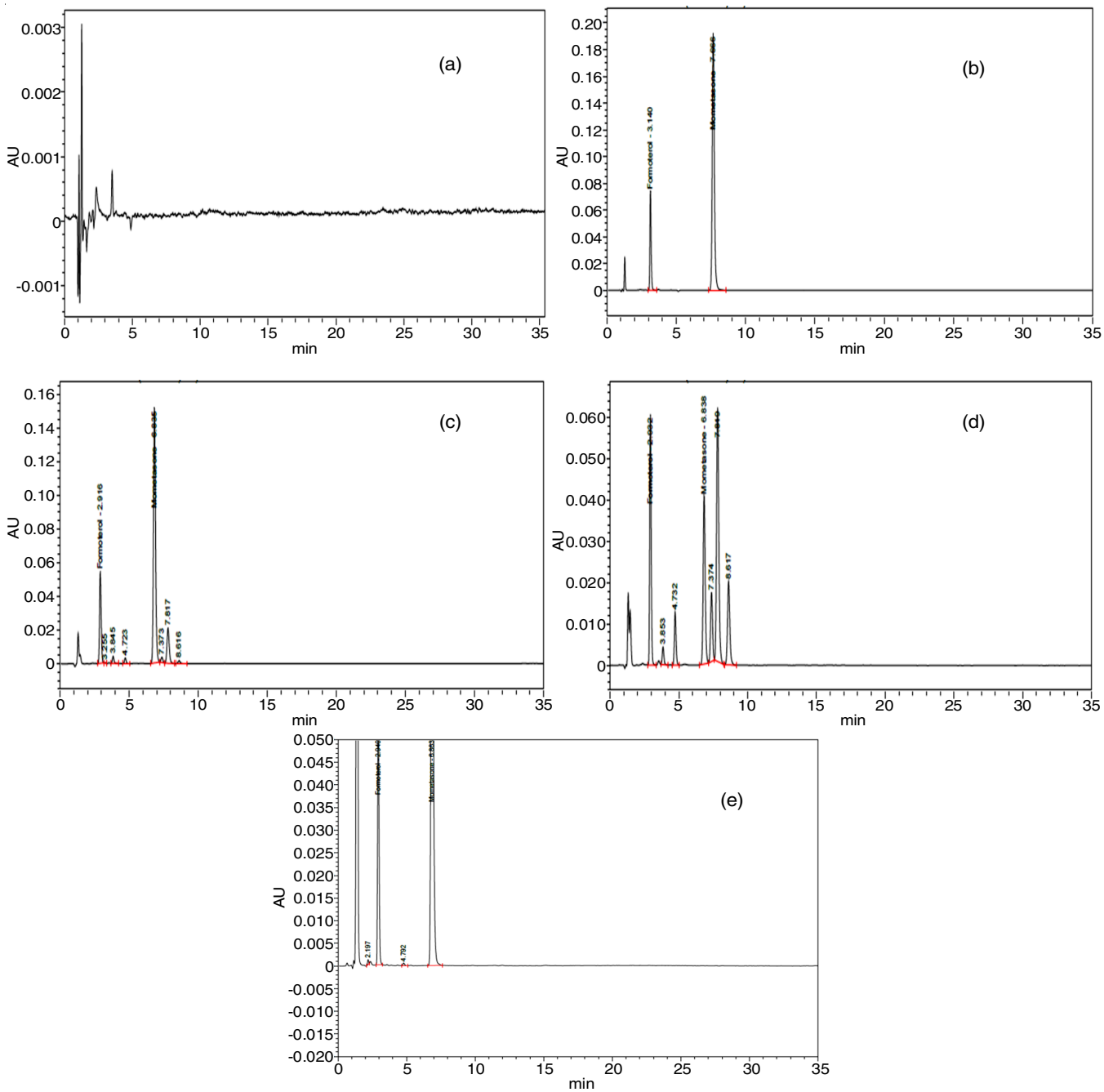

Fig. 3. Typical chromatogram of (a) placebo, (b) unstressed sample, (c) acid stress, (d) base stress and (e) oxidative stress

TABLE-3
ACCURACY RESULTS OF FORMOTEROL FUMARATE DIHYDRATE (FFD) AND MOMETASONE FUROATE (MAF)

Preparation	Formoterol fumarate dihydrate (FFD)			Mometasone furoate (MAF)		
	%Recovery at 50% level	Recovery at 100% level	Recovery at 120% level	%Recovery at 50% level	Recovery at 100% level	Recovery at 120% level
Prep-1	100.9	101.6	101.8	100.0	101.8	101.1
Prep-2	101.5	101.4	101.9	100.9	101.6	101.0
Prep-3	101.7	101.3	101.5	101.1	101.3	101.0
Mean recovery	101.4	101.4	101.7	100.7	101.6	101.1

Robustness and ruggedness: Determined robustness by permitting measured deviations to existing method conditions, *i.e.* flow rate (± 0.2 mL), column oven temperature (± 5 ºC), pH of mobile phase buffer (± 0.2), organic component variation in mobile phase (± 10%). The chromatographic elution pattern were found similar to actual/control chromatogram and system suitability found within acceptance limits of USP requirements. The results obtained demonstrates the method is robust and

TABLE-4
RESULTS FROM METHOD PRECISION AND INTERMEDIATE PRECISION

Sample No	Assay (%)			
	Mometasone furoate (MAF)		Formoterol fumarate dihydrate (FFD)	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2
Test preparation-1	96.7	96.6	98.4	97.9
Test preparation-2	97.7	95.9	99.9	98.3
Test preparation-3	97.8	95.4	99.9	97.1
Test preparation-4	96.8	96.3	98.8	98.5
Test preparation-5	97.2	95.9	99.4	98.0
Test preparation-6	96.8	95.7	99.3	98.1
Average	97.2	96.0	99.3	98.0
%RSD	0.5	0.5	0.6	0.5
95% Confidence interval	96.8 & 97.6	95.6 & 96.3	98.8 & 99.8	97.6 & 98.4
Cumulative %RSD	0.8		0.9	

TABLE-5
LINEARITY RESULTS OF FORMOTEROL FUMARATE DIHYDRATE (FFD) AND MOMETASONE FUROATE (MAF)

Name of component	Slope	Intercept	Correlation coefficient	Bias at 100% response	Residual sum of squares
FFD	111219.90929	434.7667	0.9999	0.09	2994955.8211
MAF	25103.98201	-877.4821	0.9999	-0.04	310632981.2189

TABLE-6
RESULTS OF SYSTEM SUITABILITY FOR FFD AND MAF FROM ROBUSTNESS

	Control		Flow (mL/min)				Column oven temp. (°C)				pH buffer of mobile phase				Variation of acetonitrile (%)			
	FFD	MAF	FFD		MAF		FFD		MAF		FFD		MAF		FFD		MAF	
			1.0	1.4	1.0	1.4	45	55	45	55	2.8	3.2	2.8	3.2	90	110	90	110
Tailing factor	1.2	1.1	1.3	1.2	1.1	1.1	1.2	1.3	1.1	1.1	1.4	1.3	1.1	1.1	1.1	1.1	1.0	1.1
%RSD for replicate std.	0.1	0.1	0.03	0.1	0.01	0.02	0.1	0.1	0.1	0.04	0.1	0.1	0.1	0.04	0.6	0.1	0.04	0.1

can sustain variability in chromatographic conditions within established range (Tables 4 and 6). Performed intermediate precision on different day, different instrument and different analyst and observed that the data is comparable with analyte-1.

Conclusion

According to the aforementioned results, the proposed method is specific, precise, linear, accurate, rugged and robust. This study discussed experiments related to chromatographic condition selection for achieving a robust and reliable method. The target analyte peaks for forced degradation studies indicated that the degradation behaviour of degradants and drug product was well resolved. The results of chromatographic condition change confirmed the robustness of the method and that at the quality control level, the method withstands frequent lab discrepancies. This method can be employed for lab-scale batch development and drug product stability screening.

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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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