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Quantitative Estimation of 10 Known Impurities from Indacaterol Acetate, Glycopyrronium, and Mometasone Furoate Dry Powder Inhalation Product

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Abstract

Indacaterol, glycopyrronium, and mometasone furoate triple combination inhalable fixed-dose medicines are effectively used to treat asthma and various chronic pulmonary disorders. The study aimed to develop and validate a simple single-run RP-HPLC impurity quantitation method. The chromatographic separation was accomplished using gradient elution mode of mobile phase A (potassium dihydrogen phosphate buffer pH 2.2) and mobile phase B (mixture of acetonitrile and methanol), with a flow rate of 0.8 mL/min using YMC Triart, C_{18} (250 × 4.6 mm, 5 µm) HPLC column at 45 °C and the detection wavelength of 210 nm (for indacaterol, glycopyrronium and their impurities) and 248 nm (for mometasone furoate and its impurities). Water and methanol (20:80) were used as a diluent. Quantitation of 10 known and several unknown impurities was successfully performed with the determination of relative response factors for all the known impurities. The developed method was validated as per the ICH Q2(R1) guidelines. The stability indicating the nature of the method was proved by performing stress study on the sample and placebo. The linearity and range of the method were proved by calculating the r^2 values (> 0.998). The overall precision was found to be within 1.82 – 7.76% RSD. The recovery for all the actives and known impurities were within 90 – 115% with 0.4 – 12% RSD. The sample solution was stable for 2 days at room temperature. The developed method can be successfully used for the impurity analysis of routine, stability, and commercial samples in a quality control laboratory of the pharmaceutical industry.

Keywords RP-HPLC–PDA · Impurity profiling · Method development · Indacaterol · Glycopyrronium · Mometasone furoate · Relative response factor

Introduction

Worldwide majority of the population is badly affected by asthma and various chronic obstructive pulmonary disorders (COPD) due to an increase in air pollution. Lung-related disorders are the third leading cause of death in the world today. Indacaterol acetate (INA) (5-[(1R)-2-[(5,6-diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one) is ultra-long acting b2-agonist, glycopyrronium (GLC) (3-[2-Cyclopentyl(hydroxy) phenylacetoxy]-1,1-dimethylpyrrolidinium) is a long-acting muscarinic antagonist, and mometasone furoate (MOF)

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(11_,16_)-9,21-dichloro-11-hydroxy-16-methyl-3,20-dioxo-pregna-1,4-dien-17-yl-2-furoate) is corticosteroid drug. This combination product is used in the maintenance treatment of adult asthma patients [1].

Two possible toxic degradants of INA, are INA impurity A (INA-A) 5-[(1R)-2-[5-Ethyl-2,3-dihydro-1H-inden-2-yl) amino]-1-hydroxyethyl]-8-hydroxy-2(1H)-quinolinone and INA impurity B (INA-B) (R)-8-(Benzyloxy)-5-[2-(5-,6-diethyllindan-2-ylamino)-1-hydroxyethyl]-1H-quinolin-2-one-melate. As per the United States Pharmacopeia (USP), three known major possible degradants of GLC active pharmaceutical ingredient (API), are USP-glycopyrrolate-related compound A (GLC-A) 5-Nitrobenzene-1/3-dicarboxylic acid, USP-glycopyrrolate related compound B (GLC-B) 1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetatem and USP glycopyrrolate-related compound C (GLC-C) 2-Cyclopentyl-2-hydroxy-2-phenylacetic. As per European Pharmacopoeia (EP) MOF API has five major possible toxic degradants namely MOF impurity C (MOF-C) 21-Chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate, MOF impurity D (MOF-D) 21-Chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate, MOF impurity J (MOF-J) 9,21-Dichloro-11β-hydroxy-6α,16α-dimethyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate, MOF impurity L (MOF-L) 9,11β-Epoxy-17,21-dihydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione and MOF impurity Q (MOF-Q) 21-Chloro-9,11β-epoxy-17-hydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione. The molecular structures of actives and known impurities are depicted in Fig. 1.

For the quality, safety, and efficacy of drug products, it is necessary to maintain the impurities within acceptable limits till the expiry of drug products as per the stringent regulatory guidelines [2]. Hence, an accurate estimation of such impurities is very critical for any pharmaceutical industry during the registration of pharmaceuticals in different regulated/non-regulated markets [3, 4]. GLC and MOF API are official in the USP and British Pharmacopoeia (BP). An extensive literature search revealed that several methods for the assay tests of INA, GLC, and MOF are available either individually or in combination with other drugs [5–23]. Few reported methods are also available for the related substances/impurity testing of these individual drugs or in combination with one more drug [24–29]. As of today, we have not found any reported methodology for a triple combination product for assay and impurity testing. Hence, an attempt has been made to develop and validate a simple yet robust stability-indicating impurity quantitation method using the RP-HPLC technique. This developed new method simultaneously quantitates the ten known and several unknown impurities of these three active drugs in a single HPLC run. The relative response factor (RRF) is established for the first time for all the ten known impurities to make the method more simple, accurate, and economical. The validation of the developed method was successfully executed as per the "International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use" (ICH) guidelines [30]. Post validation, the method was successfully used in the testing of both in-house and marketed dry powder inhalation (DPI) products.

Materials and Methods

Standards, Impurity Standards, and Samples

INA standard (purity 99.79% Chemicea, India) INA-A (purity 97.39%) and INA-B (Purity 99.43% Asvinau Pharma Pvt Ltd, India), Glycopyrrolate reference standard (purity 100%), GLC-A (purity 100%), GLC-B (purity 97.92%), GLC-C (purity 100%), MOF-D (purity 96.58%), MOF-Q (purity 98.79%) were purchased from Synzeal Research Pvt. Ltd., India. MOF-C (purity 99.5%), MOF-J (purity 95.4%), and MOF-L (purity 99.4%) were purchased from Pharmaffiliates Analytics and Synthetics (P) Ltd, India. MOF EP reference standard (purity 100%) was used. The in-house DPI capsules product containing INA 0.15 µg, GLC 0.05 µg, and MOF 0.16 µg per 25 mg was used along with the placebo sample (without INA, GLC, MOF drugs, and with all excipients).

Chemicals, Solvents, and Materials

Potassium dihydrogen phosphate (AR grade), acetonitrile (HPLC grade), methanol (HPLC grade), orthophosphoric acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide (AR grade, Rankem, India) were used. 0.45 µm nylon filters (mdi membrane technologies, India) were used for the filtration of all the samples.

Instrumentation

The resolutions were achieved on YMC triart, C_{18}, 250 mm × 4.6 mm, 5 µm—HPLC column (YMC, USA). Shimadzu, LC-2010C_{HT} HPLC system (Shimadzu, Japan) equipped with photodiode array (PDA) detector (SPD-MZOA). The peak integration was performed using Chromeleon (c) version 7.2.5991 network software (Dionex, USA). The analytical balance (Sansui vibra, model- HTR-220E) and microbalance (Mettler Toledo, model-Xp6, and XP26) were used for weighing the standards, samples, chemicals, etc. The calibrated pH meter (Mettler Toledo, model—Seven Compact) was used for pH adjustment of buffer and forced degradation study samples. Suntest XLS + apparatus (Atlas, Germany), and stability chambers (Thermo lab, India) were

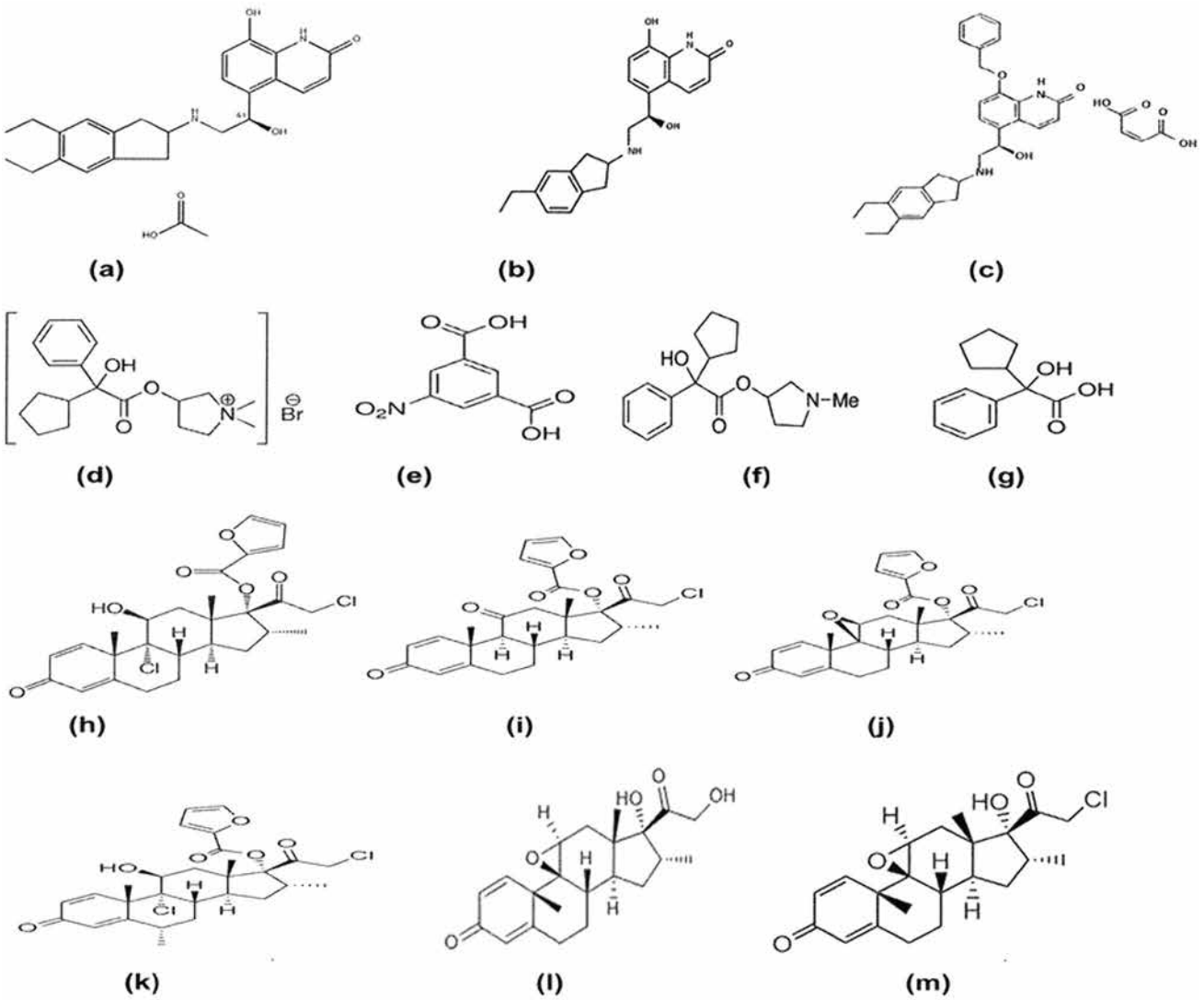

Fig. 1 Molecular structures **a** indacaterol acetate, **b** indacaterol acetate impurity A, **c** indacaterol acetate impurity B, **d** Glycopyrronium, **e** glycopyrrolate-related compound A, **f** glycopyrrolate-related compound B, **g** glycopyrrolate-related compound C, **h** mometasone furoate, **i** mometasone furoate impurity C, **j** mometasone furoate impurity D, **k** mometasone furoate impurity J, **l** mometasone furoate impurity L, and **m** mometasone furoate impurity Q

used to carry out the photostability and temperature/humidity studies, respectively. Milli-Q water type 1 (Merck Life Science Pvt Ltd, Germany) was used for all the sample and buffer preparations.

Methodology

Mobile phase A: 2.72 g of potassium dihydrogen phosphate (PDP) in 1000 mL of water, adjusted to pH 2.2 with dilute orthophosphoric acid (10% v/v in water), filtered and degassed through 0.45 µm filter and used.

Mobile phase B: Acetonitrile and methanol 75:25%v/v.

HPLC Column: YMC Triart, C_{18}, 250 × 4.6 mm, 5 µm.

Diluent: Water and methanol 20:80%v/v.

Column temperature: 45ºC.

Flow rate: 0.8 mL/min.

Injection volume: 25µL.

Detector wavelength: for INA, GLC, and their impurities at 210 nm and for MOF and its impurities at 248 nm.

Elution mode: gradient.

HPLC gradient program: time in min/%B was set as 0.01/25, 25/28, 45/30, 70/70, 90/70, 90.1/25, and 100/25.

Retention times: GLC eluted at about 32 min, GLC-A 14 min, GLC-B 35 min, GLC-C 64.5 min, INA 53 min, INA-A 22.4 min, INA-B 67.8 min, MOF 76.1 min, MOF-L 59.5 min, MOF-Q 73.3 min, MOF-C 75.2 min, MOF-D 77.2 min and MOF-J 77.6 min.

Preparation of Mixed-Standard Solutions

The mixture of 1.5 µg/mL of INA (equivalent to 1% IND concentration in sample), 2 µg/mL of GLC (equivalent to 4% GLC concentration in sample), and 1.6 µg/mL of MOF (equivalent to 1% MOF concentration in sample) was prepared and used.

Preparation of Resolution/Peak Identification Solution µg/mL of each INA, INA-A, INA-B 2 µg/mL of GLC, GLC-A, GLC-B, GLY-C, and 1.6 µg/mL of MOF, MOF-C, MOF-D, MOF-J, MOF-L, MOF-Q was prepared in diluent and used

Preparation of Test Solution

Transferred sample powder of about 250 mg (equivalent to 1.5 mg of INA, 0.5 mg of GLC and 1.6 mg of MOF) into a 10 mL volumetric flask, about 7 mL of diluent was added, vortexed for 5 min, and sonicated under controlled room temperature (25 ºC) with intermittent shaking for 15 min. The volume was made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 µ nylon filter by discarding the first 5 mL of filtrate and used.

Preparation of Placebo Solution

About 250 mg of placebo powder was weighed and transferred into a 10 mL volumetric flask and processed as per the method described for test solution preparation.

Preparation of Impurity Spiked Test Solution

About 3.0 µg/mL of each INA-A, INA-B about 4.0 µg/mL of GLY-A, GLY-B, GLY-C, and about 3.2 µg/mL of MOF, MOF-C, MOF-D, MOF-J, MOF-L, MOF-Q impurity stock solution was spiked into 10 mL volumetric flask containing 250 mg test sample and processed as per the test solution preparation method.

Preparation of Stress Study (Specificity) Test Solutions

Thermal stress study was conducted at 60ºC temperature for 2 days (48 h), for the photolytic study, samples were exposed to visible and UV light with an exposure of 1.2 million lux-hours and 200 W h/m2 respectively. A humidity study was performed at 40ºC/75%RH for 7 days. Owing to the sensitive nature of active drugs towards the acid and base mild stress conditions were used (1 mL 0.1N HCL at room temperature for 10 min and 1 mL of 0.01N NaOH at room temperature for 15 min). The oxidation study was carried out using 1 mL of 3% H_2O_2 at room temperature for 2 h. The stressed acid/base test samples were neutralized and further processed as per the test solution preparation method and injected into the HPLC system.

System Suitability Test (SST)

Resolution between the adjacent peaks should be not less than (NLT) 1.6; the signal-to-noise (s/n) ratio should be NLT 10 for the resolution solution. The similarity factor (between standard -1 and standard -2) should be within 95%-105%, Theoretical plates NLT 2000 and tailing factor not more than 2 for INA, GLC, and MOF in standard solution.

Known Impurities Relative Retention Time (RRT)

GLC known impurities RRT: at 210 nm, regarding to GLC (retention time about 32.4 min)—GLC-A about 0.431; GLC-B about 1.081; GLC-C about 1.986.

INA known impurities RRT: at 210 nm, regarding to INA (retention time about 53 min)—INA-A about 0.422 and INA-B about 1.277.

MOF known impurities RRT: at 248 nm, regarding to MOF (retention time about 76.1 min)—MOF-L about 0.781; MOF-Q about 0.949; MOF-C about 0.987; MOF-D about 1.014 and MOF-J about 1.045.

% Impurity Calculations

The peaks from the diluent and placebo were disregarded from the sample chromatogram at both wavelengths.

Impurities at 210 nm: The GLC known impurities were calculated against the GLC standard peak area from the standard solution. The INA known and all unknown impurities (up to impurity RRT about 1.3 against INA peak) were calculated against the INA peak area from the standard solution.

Impurities at 248 nm: The MOF known impurities were calculated against the MOF peak area from the standard solution. All unknown impurities peaks (after RRT about 0.95 against MOF peak) were calculated against MOF peak from standard solution.

The impurity calculation was performed using the following Eq. (1):

$$\frac{(Aimp \times RRF)}{Astd} \times \frac{StdC\ (\mu g/mL)}{SplC\ (\mu g/mL)} \times \frac{AvgCW\ (mg)}{LabelClaim\ (mcg)} \times 100 \times 1000 \quad (1)$$

where *Aimp* area of respective impurity, *RRF* relative response factor of known impurity, *Astd* area of respective standard, *StdC* concentration of respective standard, *SplC* concentration of sample, *AvgCW* net content weight of sample, *Label Claim* Label claim of the respective drug.

Table 1 Relative response factor and linearity study results

Components	RRF	Linearity equation	Coefficient of determination (r^2)
INA	NA	y = 212,607.0225x + 973.3938	0.999
INA-A	1.25	y = 170,677.8583x + 9,819.9905	0.998
INA-B	1.55	y = 137,505.4847x + 944.8005	1.000
GLY	NA	y = 41,738.8664x + 879.3875	0.999
GLY-A	0.25	y = 168,505.8102x + 1,807.6301	1.000
GLY-B	1.14	y = 36,538.8163x + 320.2287	1.000
GLY-C	0.68	y = 60,992.8623x—569.5055	0.999
MOF	NA	y = 83,812.1901x + 3,300.9162	1.000
MOF-C	0.88	y = 94,928.6417x + 1,253.4673	1.000
MOF-D	0.85	y = 98,140.8277x + 552.3242	1.000
MOF-J	1.04	y = 80,421.0935x + 2,354.8125	1.000
MOF-L	1.18	y = 70,848.2113x + 743.6469	0.999
MOF-Q	1.21	y = 69,113.4447x + 947.1707	0.999

The known impurities RRF values are reported in Table 1. The RRF factor 1.00 was used for the calculation of all unknown impurities. Total impurities were calculated as a sum of all known and unknown impurities.

Results

Method Development and Optimization

The prime objective of the study was to develop a simple yet robust and accurate method for the quantitation of all 10 known and several unknown potential degradants of INA, GLC, and MOF present in this triple combination DPI product for routine use in QC laboratories. The physicochemical properties of INA, GLC, and MOF were assessed before the finalization of the diluent. Based on our experimental observations, these drugs are sensitive to both acid and alkali solvents resulting in the formation of significant degradants. We observed that the active drugs and their impurities are more soluble and stable in water – methanol (20:80%v/v) as a diluent. For the selective wavelength selection, the UV spectrum of each individual standard and impurities (1 µg/mL) was recorded at 200 – 400 nm. Based on the maximum absorption, the wavelength of 210 nm was selected for INA, GLC, and their known impurities and 248 nm was selected for MOF and its impurities. The method development trials were initiated with reverse phase stationary phases like C_8, and C_{18} of inertsil ODS, BDS, and YMC Triart makes HPLC columns with water, and organic solvents (acetonitrile, methanol) in isocratic and gradient elution modes but it fails to elute all components and gaussian peak shapes. Hence the various buffers like PDP, sodium dihydrogen phosphate (SDP), etc. at different concentrations (mobile phase A) with organic modifiers (mobile phase B) in both isocratic and gradient elution modes were tried. Due to the close structural similarity between actives and their impurities, optimum resolution between critical pairs and their peak shape was a real challenge. The trials using PDP buffer shows the well separated and gaussian peak shapes as comparison with the SDP buffer. The further method developmental trials are carried using PDP buffer with various concentrations and pH. Simultaneously, optimization of the gradient program, column temperature, and mobile phase flow rate was also performed. The trials with pH 2.2 PDP buffer in a gradient elution mode on YMC Triart, C_{18}, 250 × 4.6 mm, 5 µm HPLC column showed significant improvement in resolution between critical pairs and gaussian peak shapes of components (refer Fig. 2). For overlay standard solution chromatogram refers supplementary Fig. S1 as file ESM_1.pdf.

RRF Determination

RRF is used to correct the differences in the detector response of impurities present in the sample and the respective API. According to ICH guidelines, RRF is used to estimate the impurities accurately and consistently. Quantification of impurity by using external standards is an ideal method but due to the unavailability or lack of sufficient quantities of impurities due to challenges in the impurity isolation, and synthesis, it is difficult to inject the impurity standards during every analysis. Once the RRF is estimated, there is no need to inject freshly prepared impurity standards at every analysis for calculation purposes. RRF helps in saving both the cost and time of the analysis. RRF is calculated by slope method by injecting linear range solutions of drugs and their impurities at different linearity concentration levels targeting to the specification levels of impurities under

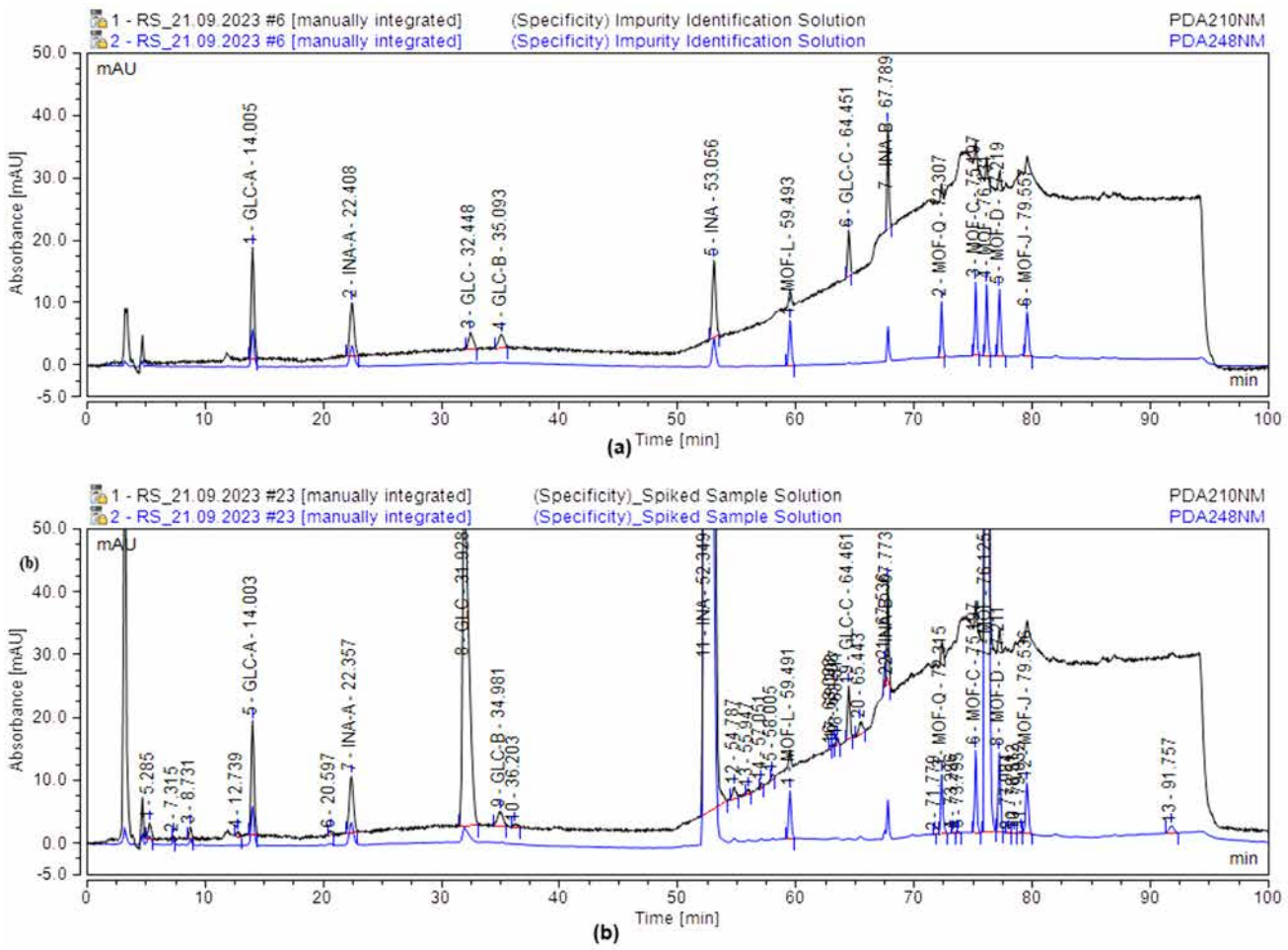

Fig. 2 HPLC overlay chromatograms at 210 nm and 248 nm **a** resolution solution and **b** impurity spiked test sample

identical conditions and calculated by the ratio of the slope of the area of respective active ingredient peak and its impurity peak. RRF estimation study was performed by preparing the mixtures of equal concentrations levels of solutions of impurities and their respective actives drugs from 5 to 150% of targeted impurity specification values. Calculated RRF factors for known impurities are summarized in Table 1.

Method Validation Study

The developed RP-HPLC method was validated concerning the critical method validation parameters recommended by ICH Q2(R1) analytical method validation guidelines (30).

Selectivity

Using a PDA detector selectivity study was carried out, the chromatograms of the diluent, placebo, standards, individual selectivity solutions, and test solutions (as such and impurity spiked) were evaluated for any interference from diluent and placebo peaks at the retention times of impurities and active ingredient peaks. The retention times of all standards/impurities were evaluated by injecting individual selectivity solutions of active drugs and known impurities. The resolution between the active ingredient and its adjacent impurity peaks and between two impurities peaks was found to be more than 1.6. The peak purity index for each impurity peak was found to be > 990. The selectivity study chromatograms are illustrated in Fig. 3.

Stress Study (Specificity)

Stability-indicating nature and specificity of the method were demonstrated by performing the stress study on a test sample and placebo as per ICH guidelines Q2(R1) (30). About 250 mg of test sample and placebo were subjected to each stress condition. The peak purity index for standards and all known impurity peaks were determined using a PDA detector and found > 990. The mass balance was calculated (%assay of the active ingredient and %degradants) for all

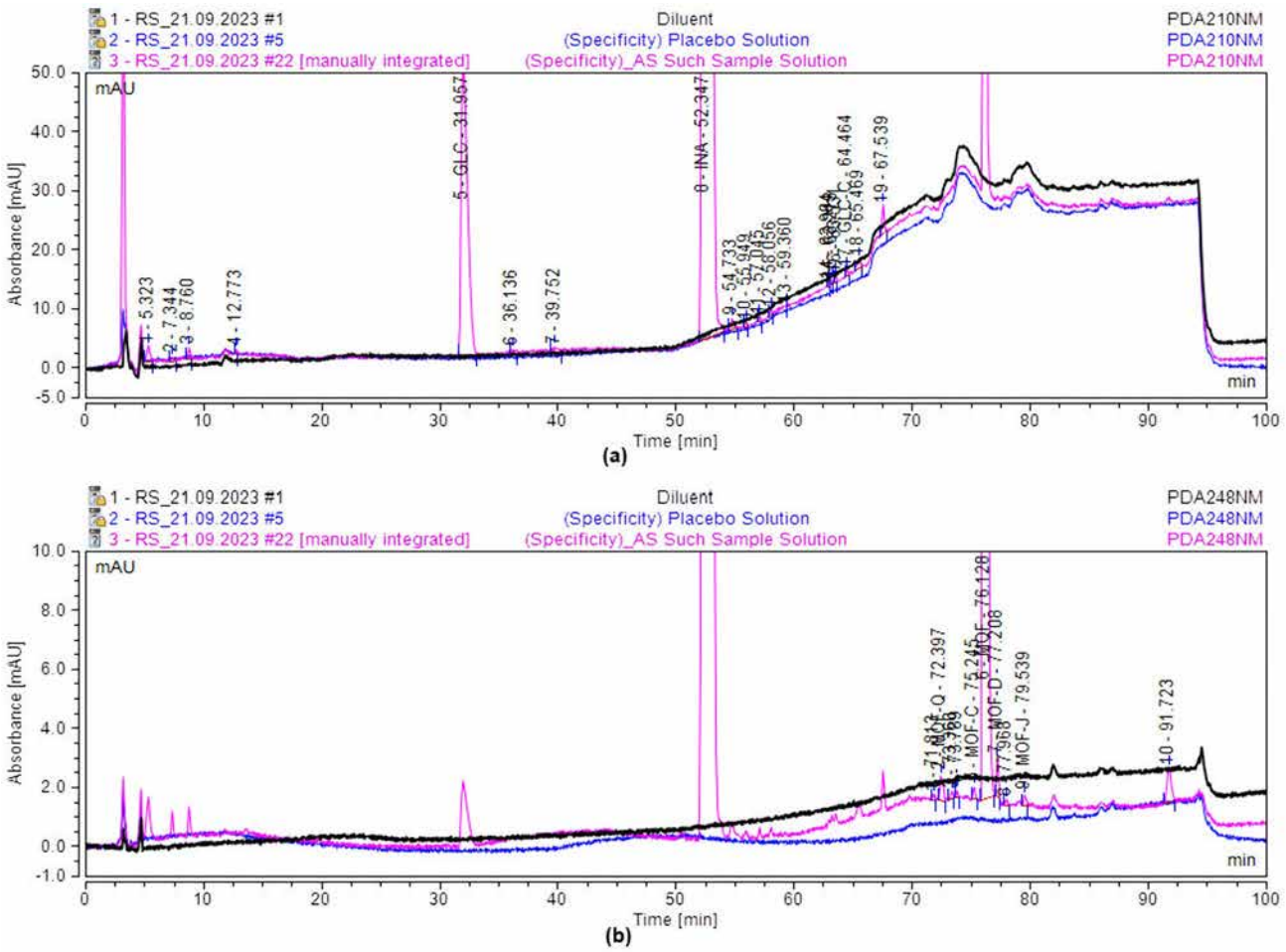

Fig. 3 Selectivity study overlay chromatograms for diluent, placebo, and test solution **a** at 210 nm and **b** at 248 nm

stressed test samples results (refer to supplementary Table 1 as file ESM_2.pdf). In majority of stress conditions, the mass balance for all the actives is achieved and found more than 90%. The mass balance of INA after acid treatment is 83.8%, GLC after humidity treatment is 85.3, and MOF after the acid treatment is 76.0%. The low mass balance observed may be due to the generation of non-chromophoric impurities or the low response of the generated impurities at the specified wavelength. The forced degradation study overlay chromatograms at 210 nm and 248 nm for test samples using various stress conditions are depicted in Figs. 4, 5 and 6.

LOD and LOQ (Sensitivity)

A series of known low concentrations of mix solutions of each active ingredient along with each known impurity standard from 50 to 5% of impurities specification levels were prepared and injected. LOD/LOQ values were calculated using the standard deviation of the y-intercept and mean slope (n = 5). LOD/LOQ values (µg/mL) for active drugs and their impurities along with the established LOQ values during the LOQ precision study with s/n ratio are reported in the supplementary Table S2 as file ESM_3.pdf. For LOQ study chromatograms refer to supplementary Fig. S2 as file ESM_4.pdf.

Linearity and Range

Linearity studies of active drugs and known impurities were established by preparing mixed linearity solutions at various concentration levels ranging from LOQ to 150% of each impurity specification level. The calibration graph was plotted between the average area and the concentration of the respective component. For the linearity equation and coefficient of determination of each active and known impurity refer to Table 1.

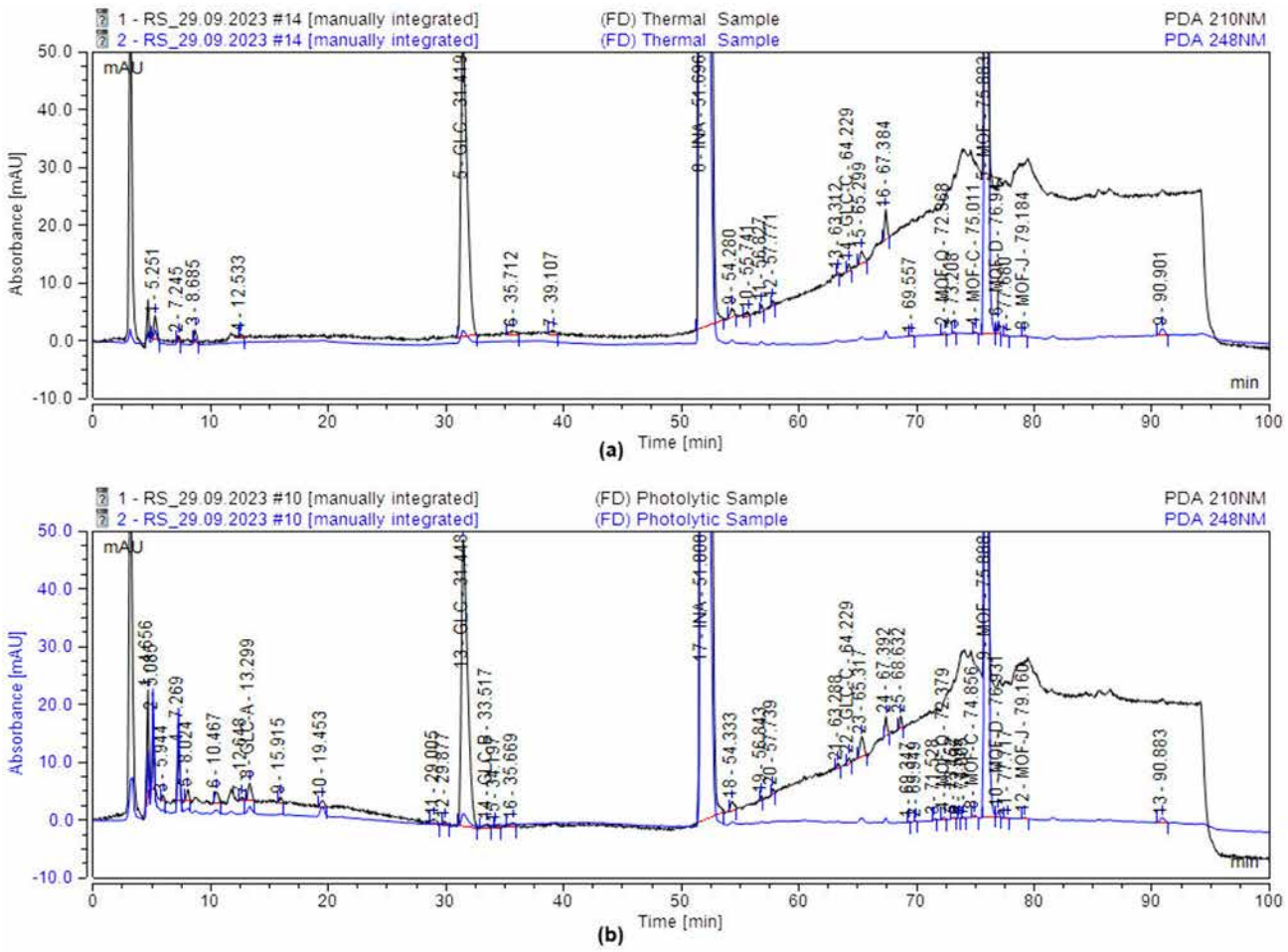

Fig. 4 Forced degradation study overlay chromatograms of the test sample **a** thermal stress sample and **b** photolytic stress sample

Precision Study

The overall precision of the method was evaluated on a set of 12 test samples (six method and six intermediate precision study). The intermediate precision study was evaluated by repeating the same experiment on a different day by different analysts using different HPLC systems and columns. The known, unknown and total impurities present in the test samples were calculated. The results are summarized in Table 2. For detailed results refer supplementary Table S3 as file ESM_5.pdf.

Accuracy

The accuracy of the method was measured as a %recovery of impurities at four concentration levels (LOQ, 50%, 100%, and 150%) of each impurity specification level. The accuracy study was performed in two ways for known impurities and unknown impurities. For known impurities, a mixed solution of each known impurities was spiked in the test sample, and for unknown impurities accuracy study, mixed INA, GLC, and MOF standards were spiked in the placebo. Accuracy study findings are summarized in Table 2.

Accuracy study % recovery was calculated using Eq. (2):

$$Accuracy\%(\text{Recovery}) = \frac{Amountfound}{Amountadded} \times 100 \quad (2)$$

Solution Stability

The study was carried out on a mix standard solution and test sample solution stored for up to 3 days (72 h.) at room temperature. The results were compared against freshly prepared mix standard at each interval period. Solutions were found stable up to 48 h. The % assay for INA, GLC, and MOF standards were 98.4, 101.1, and 102.3

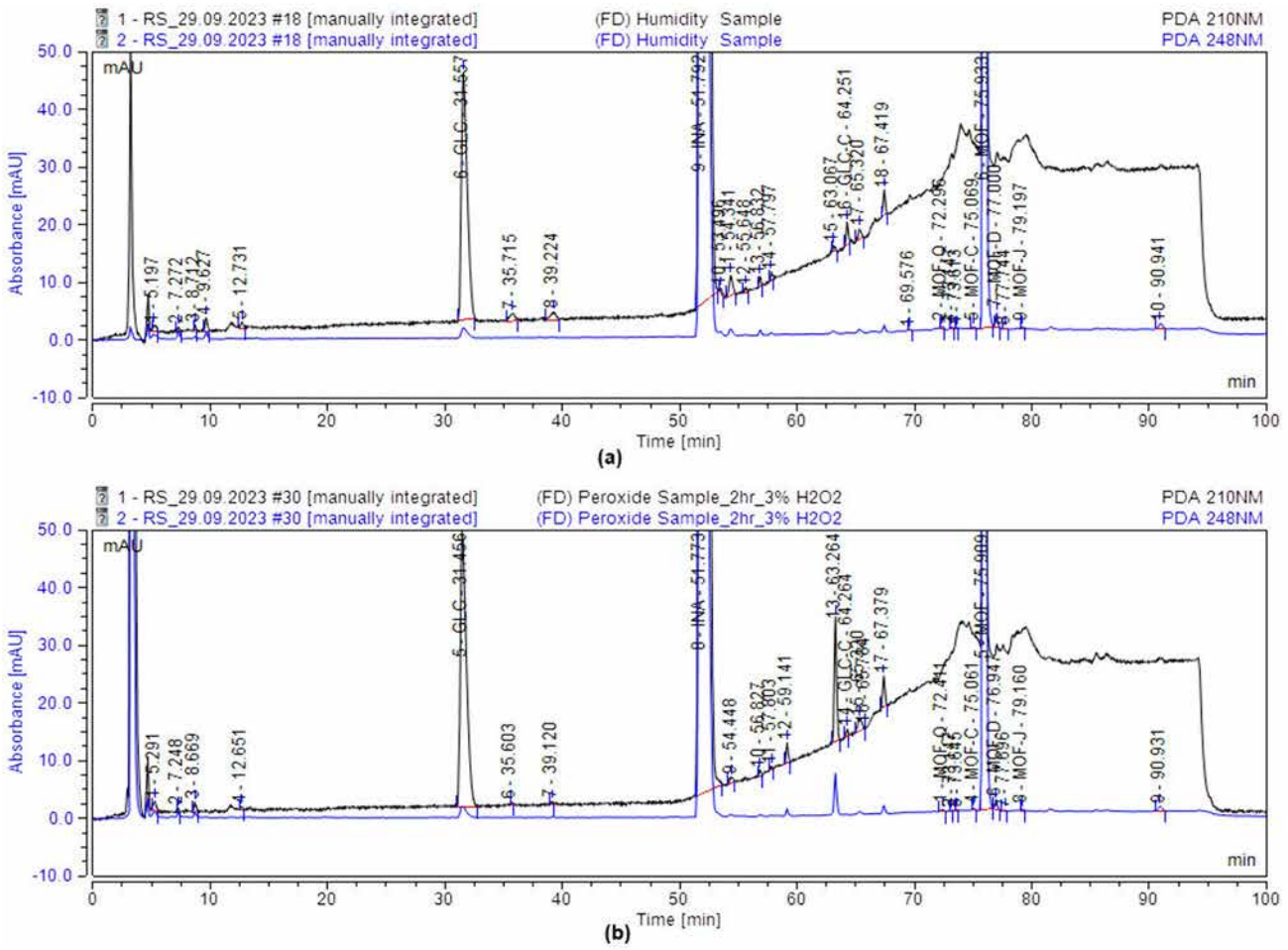

Fig. 5 Forced degradation study overlay chromatograms of the test sample **a** humidity stress sample and **b** peroxide stress sample

respectively within the predefined criteria of 95—105%, similarly, the %RSD for impurity results are found to be less than 11%, indicating the stability of solutions in the selected diluent up to the 2 days (48 h.) at room temperature.

Filter Study

Filter interference study was evaluated by comparing the chromatographic pattern and areas of 0, 2, and 5 mL discarded filtered test sample solutions (through 0.45 µm nylon membrane filter) against the centrifuged (at 5000 rpm for 10 min) test sample solution. No significant differences in the area of centrifuged and filtered test sample solutions were observed.

Robustness Study

The critical method parameters like flow rate (0.8 ± 0.1 mL/min), mobile phase buffer pH (2.2 ± 0.2), mobile phase gradient change (mobile phase B ± 5%), column oven temperature (45 ± 5ºC) and wavelength (at 210 and 248 nm ± 3 nm) were studied by intentionally modifying to assess their impact. Resolution between the peaks, peak area, and similarity factor between two standards, theoretical plates, and tailing factors in each injection was recorded (refer to supplementary Table 4 in file ESM_6.pdf.), and found that all these parameters complied with predefined SST criteria.

Testing of Commercial/Marketed Samples

The commercial sample Difizma (Lupin Ltd, India) was successfully analyzed for the known and unknown

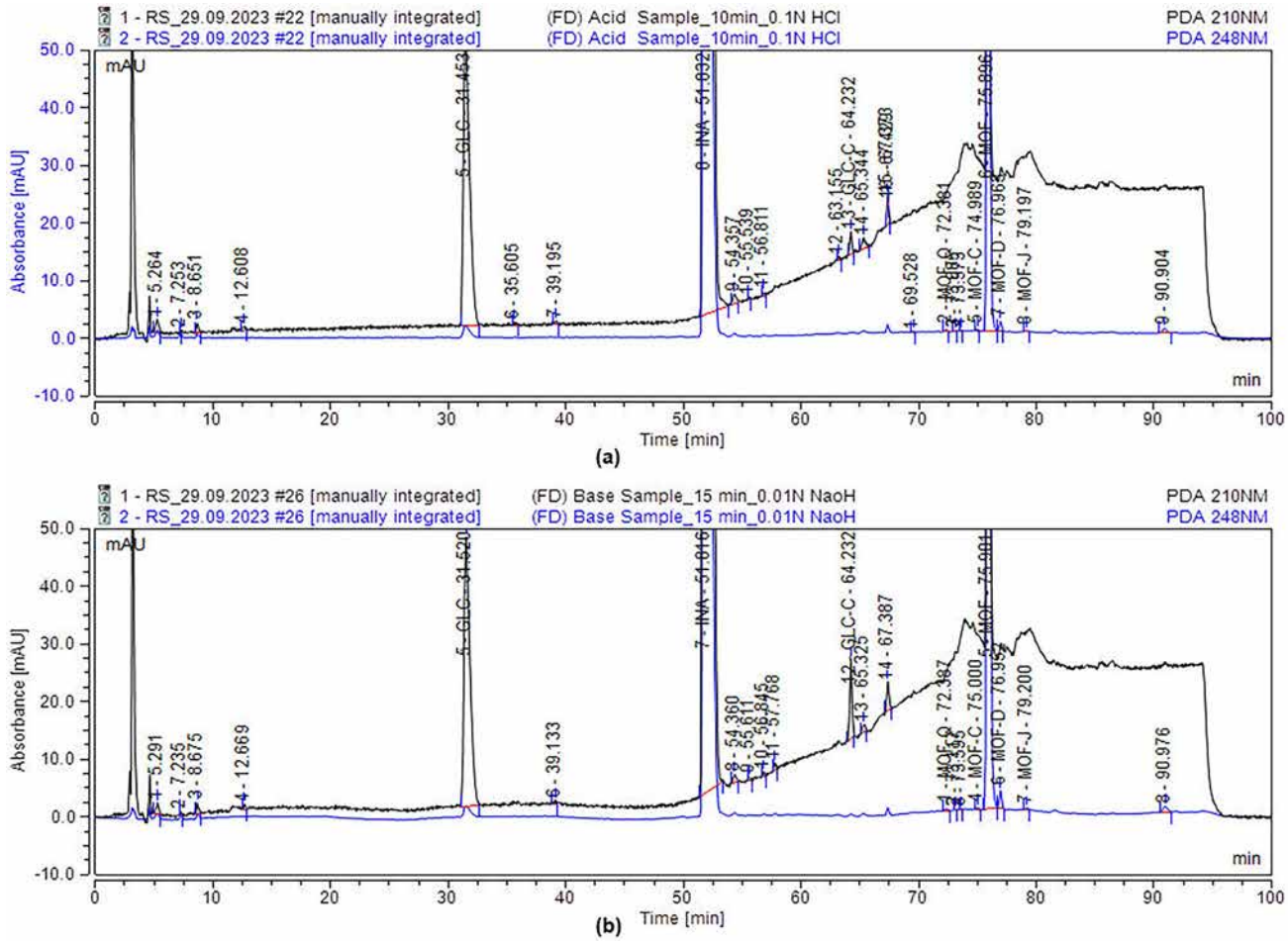

Fig. 6 Forced degradation study overlay chromatograms of the test sample **a** acid stress sample and **b** base stress sample

impurities, and the method was found specific, selective, linear, accurate, and robust.

Discussion

The INA, GLC, and MOF drug combination in DPI dosage form is very commonly prescribed worldwide for Asthma and COPD disorders. This product is not official in any of the pharmacopeia. Table 3 summarizes the sensitivity of the developed method against the reported impurity methods [24–29]. The reported data shows that, to date, no method is available for the estimation of impurities for this combination product in a single HPLC run. The reported methods are either using HPLC [24, 28, 29], tandem mass spectroscopy [25], HPTLC [26], and capillary electrophoresis [27] techniques. Mostly, the published methods are for individual drugs [27, 29] or drug formulations with [24–26, 28] GLC or MOF. No reported method is available for the estimation of INA impurities. Further, no reported methods have established the RRF values for the known impurities. The established RRF data for all the 10 known impurities make the method simpler yet accurate. The LOD, LOQ, and linearity range of the developed method is comparatively more sensitive and selective than the reported methods.

Conclusion

The impurity profiling methods are receiving critical attention from various regulatory authorities like the US FDA, UK MHRA, etc., from the product safety point of view. Hence, the development of a simple, selective, stability-indicating, and accurate single-run RP-HPLC method for the simultaneous quantitation of all known and unknown degradants of INA, GLC, and MOF from a combination drug product was the need of the hour. The developed RP-HPLC method quantifies ten known (two INA impurities A, B, three GLC impurities A, B, C, and five MOF impurities C, D, J, L, Q) and several unknown impurities at a very low concentration. Moreover, the established RRF values for all the known impurities make the method more

Table 2 Precision and recovery study results in summary expressed in mean values and %RSD

Precision study results												
Parameter	INA-A	INA-B	GLC-A	GLC-B	GLC-C	MOF-C	MOF-D	MOF-J	MOF-L	MOF-Q	[@]Unk Imp	[@]Tot. Imp
Mean[MP]	[*]ND	0.249	ND	ND	0.660	0.037	0.146	0.033	ND	0.099	0.233	1.905
%RSD[MP]	[#]NA	8.14	NA	NA	7.26	2.82	4.73	7.24	NA	6.18	5.25	6.70
Mean[IP]	ND	0.265	ND	ND	0.856	0.046	0.139	0.035	ND	0.084	0.244	2.162
%RSD[IP]	NA	7.53	NA	NA	5.00	1.79	1.87	2.56	NA	6.13	2.24	5.91
Mean[OP]	ND	0.257	ND	ND	0.758	0.041	0.142	0.034	ND	0.091	0.238	2.033
%RSD[OP]	NA	7.76	NA	NA	5.65	1.98	1.82	2.62	NA	5.62	2.29	6.29
Accuracy study results												
Parameter	INA-A	INA-B	GLC-A	GLC-B	GLC-C	MOF-C	MOF-D	MOF-J	MOF-L	MOF-Q	INA	GLC	MOF
Mean[LOQ]	107.5	106.1	114.1	103.1	104.9	105.6	112.5	112.3	108.1	114.4	103.4	93.7	110.3
%RSD[LOQ]	5.20	1.97	3.20	11.89	4.94	2.04	2.70	3.50	5.89	0.36	6.66	5.05	1.71
Mean[50%]	104.2	112.8	105.4	96.4	93.2	98.3	97.6	98.4	104.7	93.6	103.2	100.8	101.6
%RSD[50%]	1.35	1.31	1.48	2.08	3.72	3.64	0.41	0.71	2.00	1.07	3.53	1.50	3.69
Mean[100%]	106.1	102.7	105.1	102.8	93.2	100.5	99.4	98.6	103	98.4	103.4	102.0	101.8
%RSD[100%]	1.64	1.26	1.04	1.44	1.91	1.61	1.01	0.97	0.85	0.67	1.30	0.91	0.60
Mean[150%]	106.2	101.3	107.5	106.3	98.7	102.2	102.9	101	105.4	103.8	102.4	101.7	100.8
%RSD[150%]	2.79	2.52	2.76	3.13	4.21	2.74	2.98	2.83	2.35	2.61	0.79	0.71	0.89

[MP] Method precision (repeatability) results of six samples; [IP] Intermediate precision (ruggedness) results of six samples; [OP] Overall precision results of twelve samples; [LOQ] Recovery at LOQ level; [50%] Recovery at 50% accuracy level; [100%] Recovery at 100% accuracy level; [150%] Recovery at 150% accuracy level; *ND Not detected; [#]NA Not applicable; [@]Unk Imp Unknown impurity; [@]Tot. Imp Total impurities

Table 3 Comparison of sensitivity of a developed method with the reported methods

Sr. no	Test—technique	Analytes	LOD/LOQ (µg/mL)	Linearity (µg/mL)	Components stability	Filter study	References
1	RS—RP-HPLC	MOF impurities	[*]NR	[*]NR	[*]NR	[*]NR	Musmade BD [24]
2	RS—Tandem mass spectroscopy	GLC 1,1-Dimethyl-3-HydroxyPyrrolidinium bromide impurity	0.017/0.051	0.05–2.000	2 days	[*]NR	Chawla RK [25]
3	RS—HPTLC	GLC Imp	0.05/0.2 µg/spot	0.2–4 µg/spot	[*]NR	[*]NR	Soliman SM [26]
4	RS—Capillary electrophoresis	GLC Imp	[*]NR	[*]NR	[*]NR	[*]NR	Zuo L [27]
5	RS—RP-HPLC	MOF unknown Impurities	[*]NR	[*]NR	[*]NR	[*]NR	Sharma N [28]
6	RS—HPLC	GLC impurities	0.0015–0.0035/ 0.0045–0.01	[*]NR	[*]NR	[*]NR	Nebiu D [29]
7	RS—HPLC	INA, GLC, MOF and their ten impurities	0.03–0.10/0.07–0.10	INA–0.04–2.22 GLC–0.01–3.07 MOF—0.08—2.46	2 days (48 h)	0.45 µm nylon	Present study

Sr. no Serial numbers, *RS* related substances, *MOF* mometasone furoate, *GLC* glycopyrronium bromide, *INA* indacaterol acetate, **NR* not reported

simple, accurate, and economical. The developed method is successfully validated as per the ICH Q2(R1) guidelines and can be used for routine use, such as testing of commercial batches, and stability studies of combination drug products in the QC laboratories of pharmaceutical industries worldwide.

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Declarations

Competing interests Authors are declaring no financial or non-financial interests to declare.

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