

METHOD DEVELOPMENT, VALIDATION AND ESTIMATION OF RELATIVE RESPONSE FACTOR FOR THE QUANTITATION OF KNOWN IMPURITIES IN MOMETASONE FUROATE NASAL SPRAY DOSAGE FORM BY RP-HPLC WITH UV/PDA DETECTOR

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Mometasone furoate is a corticosteroid drug used in various pharmaceutical dosage forms, mainly nasal spray, nasal implant, inhalation powder, topical cream, lotion, ointment etc. for wide therapeutic applications. From literature survey, it was found that no any HPLC method was reported for the quantitation of four known toxic impurities of mometasone furoate. In the present work, an attempt was made to develop an accurate, precise and robust analytical method for the quantitation of these known toxic impurities in mometasone furoate API and its nasal spray dosage form by using a simple RP-HPLC technique with PDA detector. The known impurities of mometasone furoate are either not available easily or rather costly to purchase. Hence, we have determined the relative response factor of these known impurities for accurate quantitation. A mixture of octanesulfonic acid sodium salt and phosphate buffer at pH 3.0 was used as mobile phase A and pure acetonitrile as mobile phase B. Linear isocratic elution was set at 45:55 v/v ratio of mobile phases A and B, respectively, and 0.8 mL/min flow rate. Required resolution between the peaks of mometasone furoate and all the known and unknown impurities was achieved using Bakerbond C18, 250 × 4.6 mm, 5 μm column. The column was operated at 50°C with 254 nm as a UV/PDA detector wavelength. The developed method was validated as per ICH guidelines. The forced degradation study was carried out by using acid, alkali, thermal, photolytic and oxidative stress conditions for the evaluation of stability indicating nature of the method. The maximum degradation (12.4%) was observed in 0.1 N NaOH. The developed method has been found accurate, linear, precise, specific and robust and can be routinely used for the quantitation of four known toxic impurities in drug quality control laboratories.

Keywords: mometasone furoate; impurities; relative response factor, RP-HPLC, method validation.

1. INTRODUCTION

Impurity analysis is a very important and critical test factor for active pharmaceutical ingredient (API) and finished pharmaceutical drug products. It gives assurance for the stability, safety and efficacy of API and drug product. Impurities are classified as organic impurities, inorganic impurities, and residual solvents depending on the drug origin [1]. In addition, impurities are specified as per regulatory requirements as specified impurities, individual unspecified impurities, and total degradants. Impurities generated during

the synthesis of a drug substance are called process impurities. Generally, such impurities are controlled during manufacturing of a drug substance. Hence, these impurities are not included in the calculation of total impurities contained in the drug product [2]. During the analytical method development for impurities in drug substances or drug products, the goal is to resolve all the individual impurities from each other as well as from principle analyte peaks without any interference with placebo or diluent solution. For precise and robust method development, we need to have a minimum resolution of 1.5 between the peaks of impurities and principle analyte [3].

The IUPAC name of mometasone furoate is (11β,16α)-9,21-dichloro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl-2-furoate (Fig. 1). It is a topical corticosteroid drug possessing anti-inflammatory, antipretic, and vasocon-

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strictive properties [4], which is used in the treatment of dermatitis and allergic rhinitis [5]. The P_{ka} value of mometasone furoate is 13.84 at an aqueous solubility of 0.0108 mg/mL. Thus, it is almost insoluble in water, soluble in acetone and methylene chloride, and slightly soluble in 96% ethanol [6].

During the literature review, it was found that most of the proposed analytical methods were intended for the assay of either individual mometasone furoate or that in combination with other drug substances and preservatives in various dosage forms, viz. topical [4], cream [5, 7–9], ointment [10], nasal spray [11–13] and inhaler [14]. In one of the reported methods a forced degradation study was carried out for a combination dosage form [15]. The official monograph of mometasone furoate aqueous nasal spray in British Pharmacopoeia describes the related method of analysis by thin layer chromatography (TLC) technique [16]. For the quantitation of toxic impurities, TLC is less precise and accurate in comparison to the HPLC technique. According to the European Pharmacopoeia (EP), the DMCF impurity (Impurity D) has acute toxicity, causes serious skin and eye irritation and exhibits reproductive toxicity [17]. Globally harmonized System of Classification and Labeling of Chemicals indicated this impurity as a dangerous chemical. Hence, proper separation and quantitation of these impurities from various pharmaceutical dosage forms with specified limits is an urgent need of the pharmaceutical industry. Unfortunately, no method is available for complete impurity profiling in this respect by calculating the relative response factors (RRFs) for mometasone furoate in various pharmaceutical dosage forms.

In the present work, we have developed the stability indicating, robust and accurate HPLC method for the determination of known and unknown related substances along with the determination of the RRFs for all the known impurities in mometasone furoate contained in nasal spray dosage form. The complete validation of developed method was carried out as per ICH guidelines [18]. The developed method was successfully applied to the analysis of marketed sample (Nasonex) and in-house nasal spray product.

2. EXPERIMENTAL

2.1. Sample Materials, Chemicals, and Equipment

The mometasone furoate working standard (99.3%) was used for method development, validation and analysis purposes. The mometasone furoate EP impurity G (99.9%), DMCF (EP impurity D) (96.6%), DMC impurity (98.8%), 8-DM impurity (EP impurity L) (99.4%), and impurity J (99.5%) were used during the entire method development and validation procedure and for the calculation of relative response factors. Analytical method development and validation were performed for in-house R&D material batches. The validated method was further used for routine analysis of the marketed sample (Nasonex) manufactured by Merck Inc.

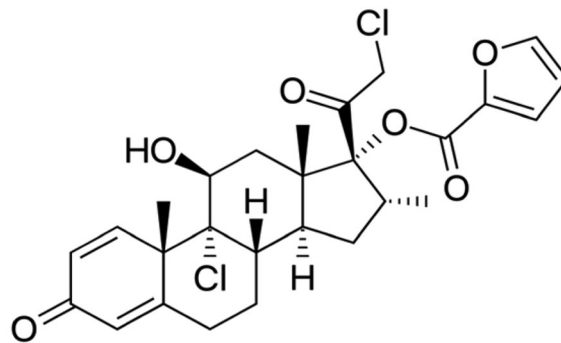


Fig. 1. Chemical structure of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

The complete development and validation was carried out on liquid chromatography system (Make-Shimadzu, Japan) LC-2010 C_{HT} with VU/Vis detector. Intermediate precision and selectivity study was performed on Waters e2695 system with PDA detector (Waters Model 2998). The analytical balance (Make SANSUI, VIBRA, Model-HTR-220E) and microbalance (Make- Mettler Toledo, Model-Xp6 and XP26) were used for sample weighing. HPLC grade methanol and acetonitrile (Make-Rankem) were used as mobile phase solvents. The AR grade potassium dihydrogen phosphate (Make-Rankem), octanesulfonic acid sodium salt (Make-Finar) and orthophosphoric acid (Make-Rankem) were used for buffer preparation. The calibrated pH meter (Make-Mettler Toledo, Model-Seven Compact) was used for pH adjustment. Separation of all impurities was achieved on Bakerbond (Make-Avantor) C18, 250×4.6 mm, $5\mu m$ column.

2.2. Chromatography Conditions

About 4.08 g potassium dihydrogen phosphate and 1 g of 1-octanesulfonic acid sodium salt were transferred in 1000 mL of water, the mixture was sonicated to dissolve it completely, and pH was adjusted to 3.0 ± 0.1 with dilute orthophosphoric acid. The buffer was filtered through $0.45\mu m$ filter, degassed and used as mobile phase A, while acetonitrile was used as mobile phase B. The mixture of methanol, water, and orthophosphoric acid in a ratio of 80:20:0.5% v/v/v was used as diluent. For better resolution between all the known impurities, unknown impurities, and principle peaks, the method was optimized by using Bakerbond column operated at $50^\circ C$. The mobile phases A and B were separately pumped at 0.8 mL/min flow rate with keeping the composition at 45:55% v/v, respectively. All solutions were injected in $50\mu L$ volume and the chromatogram was run for 60 min and detected at 254 nm. Mometasone furoate along with the known impurities was injected as a system suitability test solution.

2.3. Preparation of Solutions

System suitability testing solutions. About 1 mg of EP Impurity 8DM, Impurity G, Impurity DMC and Impurity DMCF along with mometasone furoate working standard were weighed and carefully transferred into 100 mL volumetric flask, dissolved, diluted up to the mark with diluent, and mixed well. The solution was further diluted to make 0.3 ppm of each impurity and mometasone furoate.

Standard solutions. Accurately weighed 20 mg of mometasone furoate working standard was transferred in to a 200 mL volumetric flask containing about 150 mL diluent. The flask was sonicated at controlled temperature to dissolve the substance completely and further diluted up to the mark with diluent. The solution was further diluted to make 0.3 ppm of mometasone furoate.

Sample solutions. About 5 g of accurately weighed Nasal spray sample equivalent to 2.5 mg of mometasone furoate was transferred to a 25 mL volumetric flask, about 15 mL diluent was added, vortexed and sonicated for 10 min with intermittent shaking. The volume was made up to the mark with diluent and mixed well (100 ppm). The sample was further centrifuged at 5000 rpm for 10 min and the supernatant liquid was filtered through 0.45 μm filter paper and used for HPLC analysis.

Placebo solutions. About 5 g placebo (formulation without API) was weighed and transferred into a 25 mL volumetric flask, 15 mL of diluent was added, and the mixture was further processed as per sample preparation method.

2.4. System Suitability Criteria

The resolution between all the known impurities and principle analyte was kept at NLT 2.0 while the % relative standard deviation of five replicate standard injections was set at NMT 5.0%. The limit for theoretical plates and peak tailing factor for mometasone furoate peak was NLT 2000 and NMT 2.0, respectively.

2.5. Estimation of Relative Response Factor

The relative response factor (RRF) of all the known impurities was calculated for quantitation purposes. The RRF value is used to correct for differences in the detector response of impurities against the principle analyte. The RRF determination helps us avoid the purchase of known impurities during routine testing especially when the impurities are not easily available, costly and critical to synthesize. Equal concentration of mometasone furoate working standard and all the known impurities were spiked at 10%, 20%, 30%, 50%, and 80% 100%, 120% and 150% of the specification level (0.3 ppm). All the solutions were injected in triplicate to determine the slope, intercept and correlation coefficient. The RRF values were calculated by dividing the slope of each impurity by the slope of mometasone furoate. The obtained values are listed in Table 1.

3. METHOD VALIDATION

The developed method was validated as per ICH guideline by using different quality parameters including specificity, precision, LOD, LOQ, linearity and range, accuracy, robustness, and stability of analytical solutions.

3.1. Specificity

Specificity study was carried out to prove the ability of a method to assess unequivocally the analytes in the presence of components which may be expected to be present in sample. To prove specificity of the developed method, the diluent as a blank solution, placebo solution, solutions of EP impurities G, DMCF, DMC, 8-DM, and J, mometasone furoate standard solution, sample solutions, and impurity spiked sample solutions at specification level (0.3 ppm) were prepared and injected. The response of the individual analyte and peak purity was recorded by RP-HPLC with photodiode array (PDA) detector.

3.2. Forced Drug Degradation Study

Forced degradation study was carried out to prove the selectivity of the method and to evaluate the stability indicating nature of the method. Sample and placebo was exposed under relevant stress factor conditions including heat, light, humidity, acid or base, and oxidation. The stressed samples were analyzed for percentage degradation and generated impurities. Acid and alkali degradation study was carried out by using 0.1 N hydrochloric acid and 0.1 N sodium hydroxide, respectively, for 30 minutes at room temperature. Similarly, the thermal degradation study (60°C for 2 days), oxidative stress study (3% hydrogen peroxide for 10 min) and photolytic study (1.2 million lux-hour at 200 watt hour/square meter UV energy) were performed.

3.3. LOD and LOQ Determination

The LOD and LOQ determination was performed by preparing series of standard and known impurity solutions over a range starting from 1% to 50 % of working concentration of known impurities. The linearity graph was plotted for the average area at each level against the concentration in PPM to determine the correlation coefficient, slope and intercept of analytes. The LOD & LOQ values were derived from linearity study by slope method.

3.4. Precision at LOQ Level

The six solutions were prepared by spiking the mometasone furoate working standard and all the known impurities at LOQ level in the placebo. The solutions were injected and % relative standard deviation of all the impurities and mometasone furoate was calculated.

3.5. Linearity and Range

A series of mixed impurity solution and mometasone furoate working standard solutions were prepared from

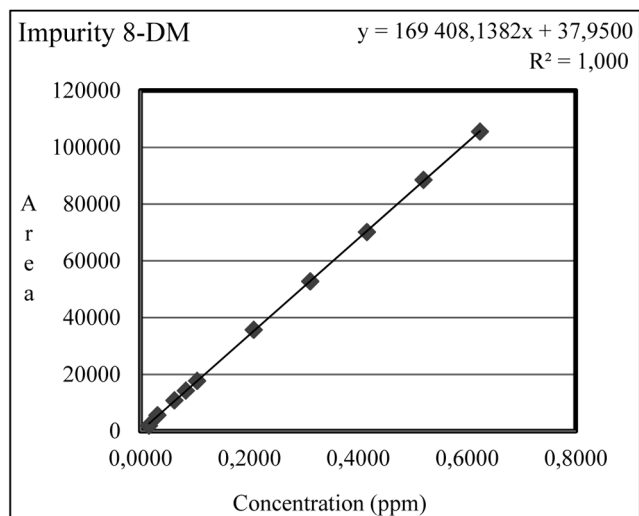


Fig. 2a. Linearity of Impurity 8-DM determination.

150% to LOQ level (150%, 120%, 100%, 80%, 50%, 20% and LOQ level). The solutions were injected in triplicate in the HPLC system and the linearity graph was plotted for the area of individual analyte peak against the concentration in PPM. The correlation coefficient (R^2) was then calculated for each impurity.

3.6. System Precision, Method Precision and Intermediate Precision

Mometasone furoate working standard solution was prepared and injected in six replicate into the HPLC system. The % relative standard deviation (RSD) was calculated and monitored as per system suitability criteria. Six sample solutions were prepared by spiking the known impurities at working concentration and injected into the HPLC system by same analyst in the same HPLC column. The % RSD for six samples was calculated for known impurities, individual unspecified impurities and total impurities. Intermediate precision study was carried out by changing the analyst, HPLC column, and HPLC system on different days. The six samples were prepared as per method precision and injected in the sequence. The % known impurities, individual impurities and total impurities was calculated. The % RSD of results obtained from six samples were determined.

3.7. Accuracy

Accuracy study was carried out by spiking all the known impurities and mometasone furoate working standard from the stock solutions at LOQ, 50%, 100%, and 150 % level in the placebo preparation. Six preparations at LOQ Level and three preparations each at 50%, 100% and 150% level were prepared and injected. The % recovered amount was calculated for each known impurity and mometasone furoate. The limit of recovery was set at 80% to 120% for LOQ level and 85% to 115 % for 50% to 150% levels in accordance with the

TABLE 2a. Linearity of Impurity 8-DM Determination

Conc. (ppm)	Average area
0.0153	3161
0.0613	11082
0.1532	26607
0.2451	41994
0.3064	52454
0.3677	63589
0.4596	79128
Slope	170861.1284
Intercept	452.5413
Correlation Coefficient	1.000

ICH guidelines. The percentage standard deviation for sample recovery at the LOQ level was set at maximum 15%.

3.8. Robustness

To prove the ability of the analytical method to remain unaffected by small but deliberate changes in method parameters, the study was conducted by changing the critical method parameters like, flow rate (± 0.1 mL) pH of mobile phase (± 0.2), column temperature ($\pm 5^\circ\text{C}$), detector wavelength (± 2 nm), and changes in gradient composition. The diluent, standard solution, sample solution (as such) and sample solutions spiked with known impurities at a specification level (0.3 ppm) were injected to check for robustness of the analytical method.

3.9. Stability of Analytical Solutions

The mometasone furoate working standard solution and sample solutions were prepared by spiking each impurity at a specification level (0.3 ppm) as per ICH. The solutions were stored at room temperature and evaluated against freshly prepared standard solution on day 0, day 1, day 2 and day 3. The % of known impurities and total impurities was calculated for the stored samples at particular time points and obtained results were compared against the freshly prepared sample.

TABLE 1. System Suitability Parameters

Analyte	RT (min)	RRT	RRF
Mometasone furoate Impurity 8DM	5.5	0.32	1.16
Mometasone furoate Impurity G	9.9	0.56	2.02
Mometasone furoate Impurity DMC	12.4	0.72	1.34
Mometasone furoate	17.5	1.0	1.0
Mometasone furoate Impurity DMCF	20.95	1.19	0.88

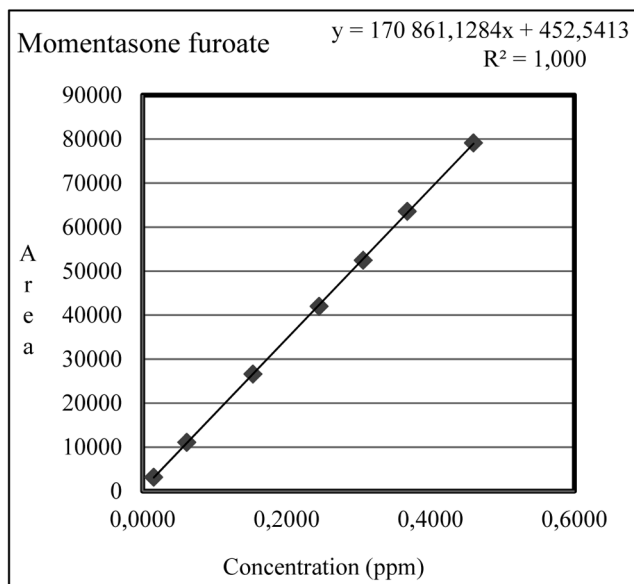


Fig. 2b. Linearity of mometasone furoate determination.

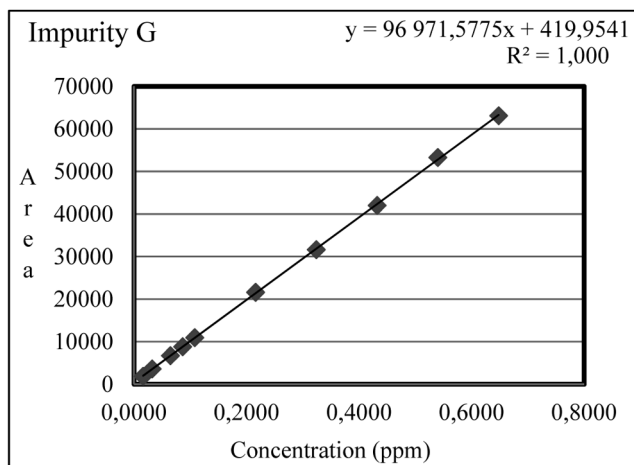


Fig. 2c. Linearity of impurity G determination.

TABLE 2b. Linearity of Mometasone Furoate Determination

Conc. (ppm)	Average area
0.0156	1924
0.0312	5571
0.0624	10828
0.0832	14282
0.1040	17679
0.2080	35706
0.3120	52745
0.4160	70138
0.5200	88542
0.6240	105546
Slope	169408.1382
Intercept	37.95
Correlation coefficient	1.000

TABLE 2c. Linearity of Impurity G Determination

Conc. (ppm)	Average area
0.0162	1871
0.0324	3595
0.0648	6721
0.0864	8810
0.1080	10933
0.2160	21601
0.3240	31628
0.4320	41986
0.5400	53285
0.6480	63076
Slope	96971.5775
Intercept	419.9541
Correlation coefficient	1.000

4. RESULTS AND DISCUSSION

4.1. System Specificity, Selectivity and Suitability

Specificity. No any interfering peaks were observed from blank and placebo solutions at the retention times of known and/or unknown impurities and mometasone furoate.

Forced drug degradation study. Generally, the force degradation testing is carried out to understand the degradation pathway of principle analyte and to assess the stability indicating nature of the method. During this study we found 2.47% and 12.4 % degradation of mometasone furoate in acid and alkali. The degradation observed in oxidative stress (0.152 %), thermal (0.185%) and photolytic condition (0.168%) was very small.

Suitability. The developed method was validated as per ICH guidelines. Resolution between peaks of all the known and unknown impurities was above 2.0. The retention time (RT), relative retention time (RRT) and relative response factor (RRF) for all impurities are listed in Table 1.

4.2. System Precision, Method Precision and Intermediate Precision

During the system precision testing, the % RSD of six replicate injections was 0.31%. For the method precision, the values of % RSD of Impurities 8DM, G, DMC, and DMCF were found to be 0.37%, 0.55%, 0.32%, and 0.45%, respectively. During the intermediate precision study, the % RSD of

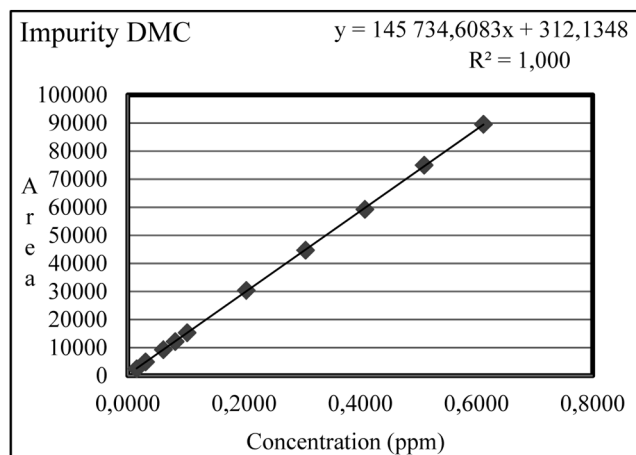


Fig. 2d. Linearity of impurity DMC determination.

TABLE 2d. Linearity of Impurity DMC Determination

Conc. (ppm)	Average area
0.0153	2425
0.0306	4868
0.0612	9263
0.0816	12144
0.1020	15271
0.2040	30341
0.3060	44669
0.4080	59246
0.5100	75011
0.6120	89547
Slope	145734.6083
Intercept	312.1348
Correlation coefficient	1.000

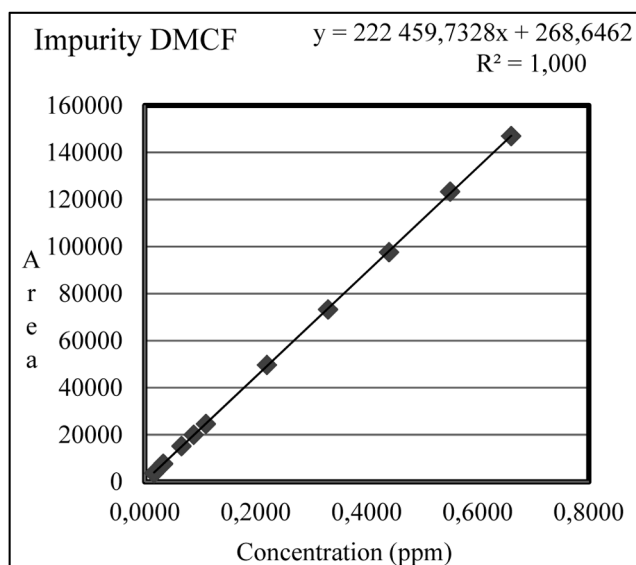


Fig. 2e. Linearity of impurity DMCF determination.

TABLE 2e. Linearity of Impurity DMCF Determination

Conc. (ppm)	Average area
0.0165	3638
0.0330	7659
0.0660	15170
0.0880	19982
0.1100	24613
0.2200	49701
0.3300	73253
0.4400	97567
0.5500	123325
0.6600	146931
Slope	222459.7328
Intercept	268.6462
Correlation Coefficient	1.000

impurities 8DM, G, DMC, and DMCF were found to be 0.51%, 1.73%, 1.11%, and 1.81%, respectively.

4.3. LOD and LOQ

The LOD values for mometasone furoate and impurities 8DM, G, DMC and DMCF were found to be 0.005, 0.019, 0.002, 0.003, 0.004 $\mu\text{g/mL}$ and their LOQ values were 0.014, 0.058, 0.007, 0.009, 0.011 $\mu\text{g/mL}$ respectively. During the precision study at LOQ level, we observed the % RSD of NMT 15% for any of the impurities, showing good method precision at the LOQ level.

4.4. Linearity

The linearity data is reported in Tables 2a to 2e and Figs. 2a to 2e. The correlation coefficient observed for all the known impurities was 1.000.

4.5. Accuracy

During the accuracy study, the % recovery was calculated against the spiked amount in ppm. The results are sum-

TABLE 3. Accuracy Study

Accuracy level.	Impurity 8DM	Impurity G	Impurity DMC	Impurity DMCF	Mometasone furoate
LOQ	101.6	104.2	96.4	104	95.5
50%	99.2	108.9	89.9	93.2	103.5
100%	97.5	102.6	89.0	96.0	96.6
150%	98.6	100.4	89.5	98.2	96.1

TABLE 4. Robustness Study

Robustness condition	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	%RSD
Flow rate 0.7 mL/min.	61182	61011	61126	60710	61328	61146	0.34
Flow rate 0.9 mL/min.	47567	47483	47529	47150	47185	47036	0.48
Low column temp. 45°C	48240	48425	47455	48582	46667	47663	1.51
High column temp. 55°C	48313	50291	48342	49499	48669	47983	1.79
Low pH 2.8	48652	49394	49311	48304	49539	49761	1.14
High pH 3.2	47960	48672	48546	49645	49529	49194	1.32
Low wave-length 251 nm	50499	50384	50282	49671	51738	49679	1.50
High wave-length 257 nm.	47422	47559	48859	46792	48176	46088	2.06

marized in Table 3. The recovery at precision level was found to be between 80 – 120% for all the known impurities meeting the specification limit as per ICH guidelines.

4.6. Robustness

The results of system suitability parameters evaluation during the robustness study for each variable parameter are compiled in Table 4.

5. CONCLUSION

From analysis of the available literature we concluded that there was an urgent need for simple analytical method for the accurate determination of all toxic impurities in mometasone furoate drug preparations widely used in various therapeutic indications worldwide. A simple yet robust analytical method was developed and validated as per the ICH guidelines for the quantification of all these known impurities from mometasone furoate API and nasal spray product. The forced degradation study was successfully performed to investigate the stability indicating nature of the method. Maximum degradation was observed under alkaline conditions (12.4%) followed by acidic conditions (2.47%), with no significant degradation under oxidative stress, thermal and photolytic conditions. During the accuracy study, we observed recovery between 89 % to 108% for all the known impurities from LOQ level to 150%. During the robustness study, the system suitability parameters were well within the acceptance criteria indicating good robustness of the method. The adopted HPLC procedure effectively resolved all the peaks of known and unknown impurities from mometasone furoate peak. Hence, this method can be routinely used in the

quality control laboratory for the release and stability study of commercial batches of mometasone furoate API and nasal spray product.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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