RJC

RASĀYAN *J. Chem.*
Vol. 13 | No. 3 |1522-1530| July - September | 2020
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SIMULTANEOUS DETERMINATION OF MOMETASONE FUROATE AND BENZALKONIUM CHLORIDE-A STABILITY INDICATING METHOD

Pankaj N. Kulkarni[1], Alaknanda M. Dodake-Supekar[2] and Charansingh H. Gill[1,*]

[1] Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India 431004

[2] Department of Chemistry, S.B.E.S.College of Science, Aurangpura, Aurangabad, Maharashtra, India 431001

*E-mail: chgill16@gmail.com

ABSTRACT

Stability indicating reverse phase HPLC method for simultaneous determination of Mometasone Furoate and Benzalkonium Chloride in nasal spray formulations has been developed. The analysis performed with the optimized chromatographic condition of mobile phase Phosphate buffer (pH 3.0): Acetonitrile: Methanol in the ratio of 30:50:20 v/v/v, in an isocratic mode at 1.2 mL/min flow rate, over Waters X-Bridge C18, 4.6*150mm, 3.5µm column by maintaining column oven temperature at 45°C and recorded chromatograms at 210 nm. The developed method has been validated and qualified in all parameters recommended by the International Conference on Harmonization in case of system suitability and specificity, precision within the limit of 2.0%.Accuracy observed within the limit of 98% to 102%, the superb linear response observed with correlation coefficient (R^2) for Mometasone 0.99996 and BKC 0.99994. Forced degradation study also has been performed to demonstrate the stability of drugs in various stress conditions. A novel, simple and accurate method for simultaneous Determination of Mometasone Furoate and Benzalkonium Chloride has been developed and validated successfully.

Keywords: Mometasone Furoate (MOM), Benzalkonium Chloride (BKC), HPLC, ICH, Forced Degradation, Validation

INTRODUCTION

Asthma is an inflammatory disease; it makes breathing difficult and can make some physical activities difficult or even impossible. Inflammation causes the airways to puff up and mucus to be formed. Inflammation makes the airways more receptive to asthma patients. Anti-inflammatory medicines help to end this process and prevent asthma attacks. There are three main types of drugs available for anti-inflammatory effects are antihistamines, decongestants and corticosteroids.[1,2] Steroids are power full drugs that can be dangerous when not taken as prescribed. The existing medical investigation shows that when corticosteroids consumed as per prescription, they are safe and well-tolerated. Corticosteroids are the most effective medications for asthma treatment.[3]

Mometasone furoate is also known as Mometasone, is a kind of corticosteroid drug, it is used to alleviate inflammation, this diminishes the symptoms such as hay fever and other allergies, including nasal congestion, pruritus, discharge and sneezing.[4]

Benzalkonium Chloride (BKC), is an organic salt, is a type of cationic surfactant. It classified as a quaternary ammonium compound. Especially for its antimicrobial activity, it is commonly used as a pharmaceutical preservative since the 1940s. Benzalkonium chlorides are fast-acting biocidal agents with a quite long extent of action. They are active against viruses, bacteria, protozoa and fungi. BKC can be safe and sound at concentrations ranges from 0.002% to 0.02% but it can be up to 0.2% depending on a variety of factors in the drug products.[5] Higher concentrations might be caustic and it may cause permanent damage.

http://dx.doi.org/10.31788/ RJC.2020.1335783

CrossMark

In the United States, nasal steroid formulations are without benzalkonium chloride. BKC classed Category III by the USFDA. Drugs are categorized as Category III once "available data are inadequate to classify as safe and effectual and additional testing is required". Benzalkonium chloride is excluded from over-the-counter drug products.[6]

Concerning the safety concerns of human beings, it is outmost necessary to quantify the concentration of Mometasone and BKC from a finished product. Several analytical methods have been reported with or without combination for the estimation of Mometasone and BKC in literature search.[7-9] However, in the current scenario, there is no official method or literature available showing a stability-indicating method for the quantitation of Mometasone and BKC in Mometasone Furoate Nasal Spray formulations. Our significance of work was to develop a simple, suitable and sensitive HPLC technique required for estimation and characterization of Mometasone and BKC from nasal spray formulations.

EXPERIMENTAL

Chemical and Reagents

The working standards Mometasone Furoate, Benzalkonium chloride and Common Placebo were a kind gift of Red Cross Formulations, Aurangabad, Maharashtra, India. Test samples were purchased from the market place medical shop. HPLC grade Methanol, Acetonitrile, Potassium dihydrogen phosphate (KH2PO4), HPLC Water and Triethylamine were procured from Ranbaxy Chemicals, Mumbai, India.

HPLC System and Chromatographic Conditions

A high-performance liquid chromatographic system of Waters 2695 equipped with 2998 Photodiode Array detector was used for all the experiments in this analysis. The data were recorded using the latest Empower 3 system software. Analytical method development and validation performed on X Bridge C18, 4.6*150mm, 3.5µm stationary phase. The analysis was performed at a column temperature 45° C over the isocratic condition. The flow rate of the mobile phase was 1.20 mL/minute for 10 min exactly. The injection volume was 30 µL for blank, placebo, standard and sample solution. Before analysis, every standard and sample was filtered through a 0.45 µm Nylon syringe filter. The complete analysis was monitored at a wavelength of 210 nm.

Preparation of Mobile Phase and Diluent

Weighed and dissolved about 6.80 gm of Potassium dihydrogen phosphate in 1000 ml of water then added 4 mL of Triethylamine and allowed sonication to dissolved (pH observed 6.67), adjusted to pH 3.00 with diluted Orthophosphoric acid solution. Buffer filtered through a 0.45µm PVDF membrane filter and to degas sonicated. Prepared a mixture of Buffer (pH 3.0): Acetonitrile: Methanol (30:50:20), v/v/v, degas by sonication. The mobile phase is used as a diluent.

Preparation of Standard Solution

Exactly weighed and transferred 100.23 mg of Mometasone Furoate and 80.46 mg of Benzalkonium chloride working standard into 200 mL volumetric flask, added about 30 mL of Methanol and sonicated to dissolve, wait to cool and diluted to volume with diluent. Transferred 5 mL of this solution into 50 mL volumetric and diluted up to volume with diluent and mixed well.

Preparation of Sample Solution

Brand Name: Mometasone Furoate Monohydrate Nasal Spray (German Remedies)

Carefully transferred 2.0 mL of Sample solution into 20 mL volumetric flask, added about 15 mL of mobile phase and sonicated to dissolved, diluted to volume with mobile phase and mixed well. Samples were filtered through a 0.45 µm Nylon syringe filter used and performed analysis using the proposed analytical methods.

Preparation of Placebo Solution

Accurately weighed and transferred 2.0 mL of Placebo solution in 20 mL volumetric flask, added about 15 mL of diluent and sonicated to dissolved, wait to cool and diluted to volume with diluent.

Forced Degradation

According to ICH guidelines to determine the content of the new drug substances a specific and stability-indicating procedure should be included.[10] The stability of the drug product is a very important matter as it impacts the effectiveness and safety of pharmaceutical finished products.[11,12] The following stress conditions applied for degradation study, Acid treated (2 mL 1.0 N HCL), Alkali treated (1 mL 0.1N NaOH) and H2O2 treated (2 mL of 5% H2O2 treated).[13] Each sample was analyzed with the proposed HPLC method, the peak purity of every stressed sample solution was monitored with the help of a photodiode array detector in the wavelength range of 200 nm to 400 nm.

Method Validation

Method validation is the procedure used to prove that the analytical method employed for a specific test is suitable for its intentional use.[14] Several trials performed with a different combination of stationary phase and mobile phase to optimize the appropriate chromatographic condition, it was extremely challenging to optimizing the run time and symmetry of all three peaks concerning system suitability. This proposed method has been developed and validated according to ICH guidelines ICH Q2 (R1).[15] Typical parameters verified are as below, system suitability, specificity, precision, accuracy, linearity and Robustness.

System Suitability

System suitability is an essential parameter of any good analytical method development and ensures the quality of the method for accuracy of the results.[16] It is required to perform before every sample analysis. To determine system suitability, the standard solution was prepared and injected under the mentioned chromatographic condition for six times consecutively into the HPLC system. The mean, Standard Deviation and % Relative Standard Deviation for peak areas of Mometasone and Benzalkonium chloride was calculated.

Specificity

To determine the specificity of the proposed analytical method blank, placebo and sample solution were injected and confirmed that there is no interference observed at the retention time of the main analyte peaks from blank and placebo solution.

Method Precision

Method Precision is the measure of agreement with individual test results when an analytical technique used repeatedly to multiple samplings from a homogeneous sample. The precision of the assay method was evaluated by concerning repeatability and reproducibility. In method precision single uniform batch used to prepare sample six times and analyzed as per the proposed method, % assay of Mometasone and Benzalkonium chloride calculated. Method precision is expressed as the relative standard deviation of a series of measurements.

Accuracy

The accuracy of the method has been demonstrated over its range. Accuracy can be reported as percent recovery by the assay of the known added amount of analyte in the placebo solution of test concentration. In this experiment, triplicate analysis for three different concentrations of standard solution50%, 100% and 150% was injected into the HPLC system to determine the accuracy of the method.

Linearity

Linearity is the ability of the method to provide the results that are directly proportional to injected concentration.[17] Linearity experiment has been performed on different standard concentrations of 50 %, 75%, 100%, 125% and 150%. The linearity of this analytical method was evaluated by a calibration curve to calculate the coefficient of correlation, slope, and intercept.

Robustness

Robustness experiment is a measure of the dependability of an analytical method during its regular usage. The particular method can remain unaffected by small but deliberate modifications in method parameters.

The effects of the following deliberate modifications in chromatographic conditions were recorded: Detector wavelength±2 nm, Flow rate±10% and Temperature±2 °C.

Solution Stability

This experiment provides information that how the quality of a drug and finished pharmaceutical product varies with time by the influence of various environmental factors such as light, humidity and temperature. Solution stability has been performed and evaluated with standard and test sample solution.

Forced Degradation

Forced degradation also is known as stress degradation. Forced degradation study is performed to determine the stability of the drug in the finished product and to resolve stability related issues. Acid, Alkali and Oxidation stress degradation performed on the blank solution and test sample solution.

RESULTS AND DISCUSSION

Method Development and Optimization

Analytical method development is a continuous process. To maintain the quality of pharmaceutical finished product it is very important and most essential. A very simple stability-indicating reverse phase HPLC method has been developed for the simultaneous determination of Mometasone Furoate and Benzalkonium Chloride from Mometasone Furoate Nasal Spray Formulation finished product. Critical parameters studied while performing method development such as solubility of the drug product, the effect of pH in buffer solution, column oven temperature and particle size effect of stationary phase. The minimum particle size of the stationary phase and application of Triethylamine in mobile phase preparation significantly reflects in results for better resolution and improved peak shape. The analysis method optimized by several no trials, in buffer 6.80 gm of Potassium dihydrogen phosphate and 4.0 mL of Triethylamine for 1000 mL water with pH 3.00 has been optimized. To maintain the resolution decided to use the premixed mobile phase of composition Buffer (pH 3.0): Acetonitrile: Methanol (30:50:20). To minimize the runtime column oven temperature was adjusted at 45° C and hybrid stationary phase used of 150 mm in length with an advanced 3.5 µm particle size. Detector used at wavelength 210 nm.

Method Validation

This developed analytical method has been validated according to the analytical procedures provided in the ICH guidelines.

Specificity

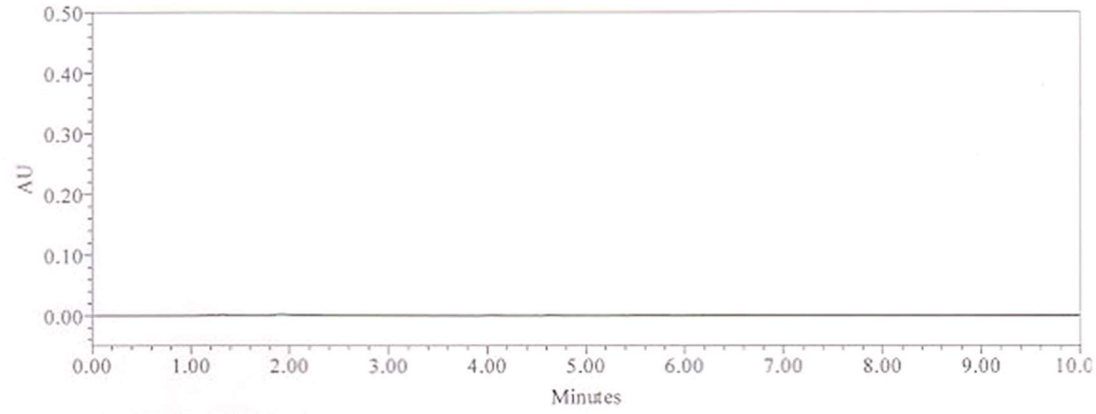
The specificity performed is as follows, injected blank, placebo and sample solution and monitor the interference from the main peak, no interference observed from the blank and placebo solution to the main peak of Mometasone, BKC peak 1 and BKC peak 2. Chromatograms of Blank, Placebo and Sample injections represented in Fig.-1.

System Suitability

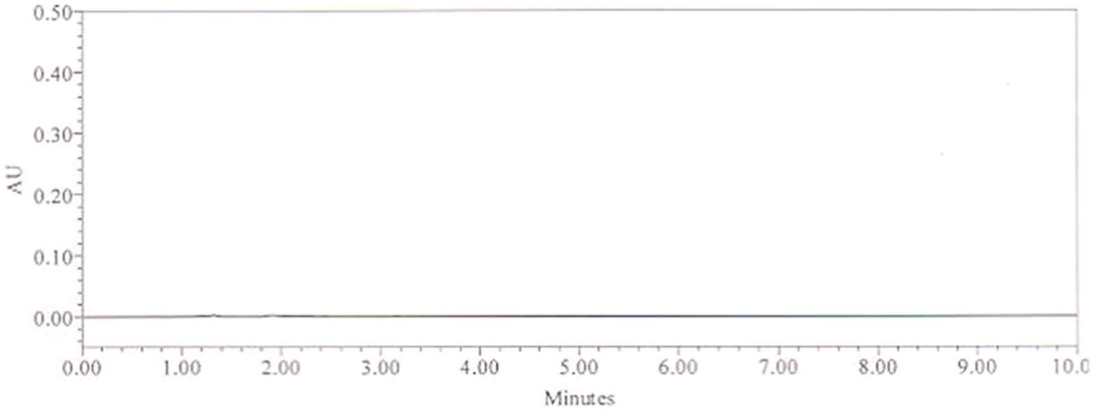
To evaluate System suitability freshly prepared standard solution injected in six replicates. Standard peak area, tailing factor and theoretical plates were monitored and tabulated in Table-1. The satisfactory results observed % RSD for peak area of the standard is within 2.0%, tailing factor for all the peaks is not less than 0.8 and not more than 2.0 and theoretical plates observed more than 2000. Method precision evaluated by injecting six different sample preparations from a single batch of the finished product in duplicate, calculate % assay of Mometasone and BKC. Assay values observed satisfactory i.e. between the limit range of 98.0% to 102.0% and tabulated in Table-2.

Accuracy

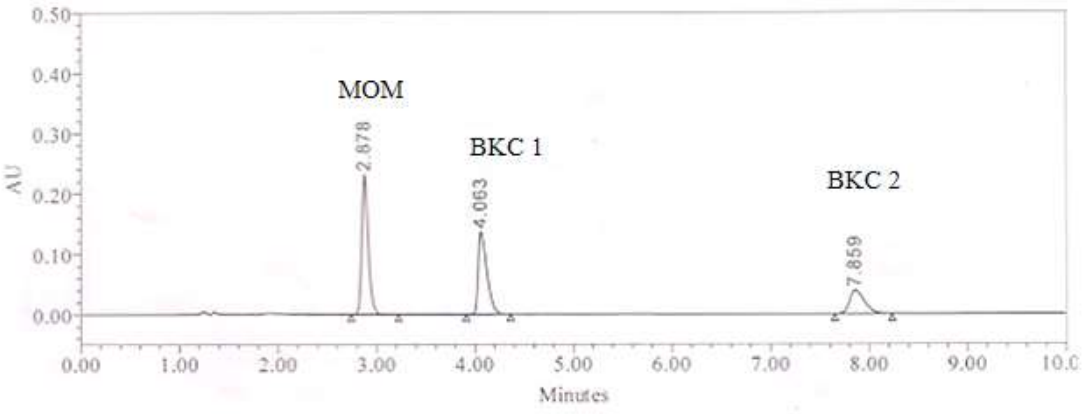
In the Accuracy experiment, the Known amount of Mometasone Furoate and BKC was spiked in placebo at about 50%, 100% and 150% of test concentration. The recovery of Mometasone and BKC was calculated as per the developed method. The % recovery was calculated from the amount found and the actual amount added. The accuracy results were tabulated in Table-3 and Table-4. The overall recovery of Mometasone Furoate and BKC in the samples was between 98.0 to 102.0% (RSD<2%).



(A)



(B)



(C)

Fig.-1: Representative Chromatograms of (A) Blank Injection, (B) Placebo injection and (C) Sample Injection

Table-1: Results From System Precision

Injection	Area of MOM	Tailing Factor	Plate Counts	Area of BKC*	Resolution MOM and BKC Peak 1	Tailing Factor	Plate Counts
1	1069161	1.3	9185	1252025	8.0	1.7	9412
2	1070300	1.3	9211	1250288	8.0	1.7	9527
3	1071094	1.3	9243	1252879	8.1	1.7	9569
4	1076007	1.3	9209	1261184	8.1	1.7	9557
5	1073561	1.3	9221	1258042	8.1	1.7	9434
6	1075530	1.3	9259	1260896	8.1	1.7	9562

Mean	1072609			1255886			
SD	2846.06			4756.14			
%RSD	0.27			0.38			

* Area of BKC=Sum of peak area counts of BKC Peak 1 and Peak 2

Table-2: Results From Method Precision

Sample No.	% Assay of MOM	% Assay of BK
1	99.2	99.5
2	99.6	99.7
3	99.6	99.9
4	99.4	99.6
5	100.2	100.2
6	99.5	99.9
Mean	99.6	99.8
SD	0.34	0.27
%RSD	0.35	0.27

Table-3: Accuracy of Mometasone Furoate

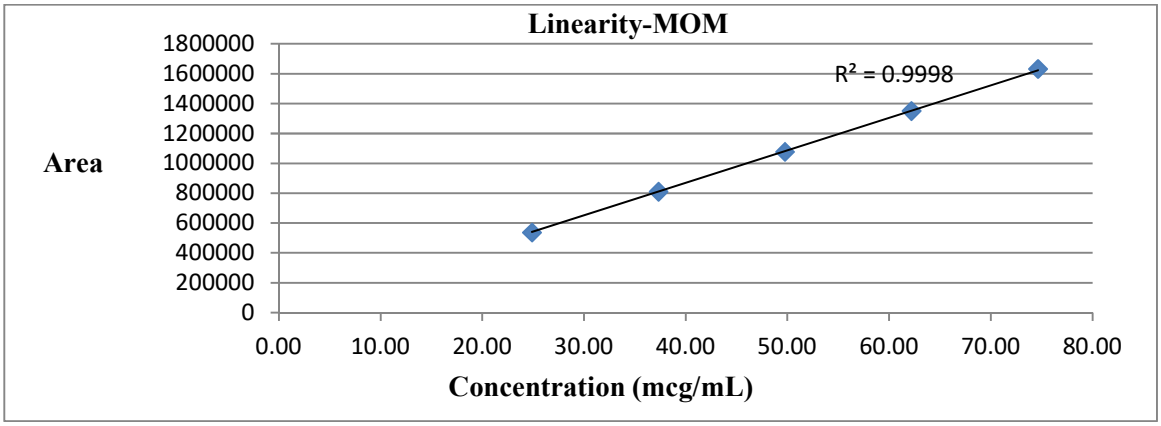
Accuracy (%) / Sample No.	Amount of API (MOM) Added (mg)	Amount of MOM Found (mg)	% Recovery	Mean	SD	%RSD
50% Sample-1	0.5039	0.4982	98.9	98.9	0.12	0.12
50% Sample-2	0.5039	0.4988	99.0			
50% Sample-3	0.5039	0.4976	98.7			
100% Sample-1	1.0079	0.9993	99.1	99.1	0.30	0.30
100% Sample-2	1.0079	0.9950	98.7			
100% Sample-3	1.0079	1.0008	99.3			
150% Sample-1	1.5118	1.5244	100.8	100.8	0.09	0.08
150% Sample-2	1.5118	1.5239	100.8			
150% Sample-3	1.5118	1.5220	100.7			
			Overall Mean	99.6		
			Overall SD	0.92		
			Overall % RSD	0.93		

Linearity

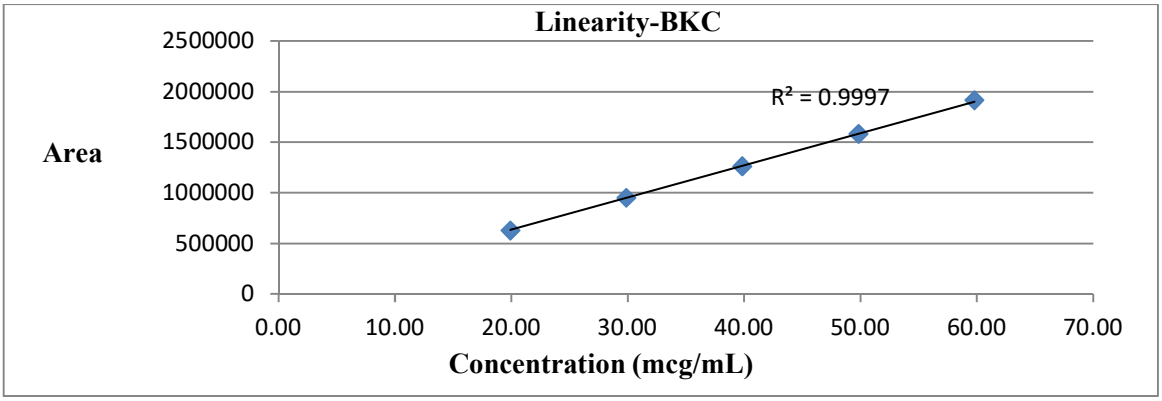
Five different linearity levels prepared by using the standard stock solution. All the linearity solutions were injected in duplicate. The Correlation coefficient value was calculated and observed within acceptance criteria i.e. correlation coefficient (R^2) =0.995. The results are tabulated in Table-5 and Linearity plots represented in Fig.-2.

Table-4: Accuracy of Benzalkonium Chloride

Accuracy (%) / Sample No.	Amount of API (BKC)Added(mg)	Amount of BKC Found (mg)	% Recovery	Mean	SD	%RSD
50% Sample-1	0.4025	0.3995	99.2	99.0	0.18	0.19
50% Sample-2	0.4025	0.3983	99.0			
50% Sample-3	0.4025	0.3981	98.9			
100% Sample-1	0.8051	0.8023	99.7	99.4	0.29	0.30
100% Sample-2	0.8051	0.7979	99.1			
100% Sample-3	0.8051	0.8016	99.6			
150% Sample-1	1.2076	1.2240	101.4	101.3	0.08	0.08
150% Sample-2	1.2076	1.2242	101.4			
150% Sample-3	1.2076	1.2225	101.2			
			Overall Mean	99.9		
			Overall SD	1.07		
			Overall % RSD	1.07		



(A)



(B)

Fig.-2: Linearity Plot for (A) Mometasone Furoate and (B) Benzalkonium Chloride

Table-5: Results of Linearity Experiment

	MOM		BKC	
Linearity level %	Concentration (mcg/mL)	Average Area (N=2)	Concentration (mcg/mL)	Average Area (N=2)
50	24.88	535778	19.93	625752
75	37.32	809422	29.90	947879
100	49.76	1075854	39.87	1258430
125	62.21	1350774	49.83	1580934
150	74.65	1632284	59.80	1912952
Slope		21978.57		32365.85
Y-Intercept		-12923.8		-17793.4
Correlation Coefficient ®		0.99996		0.99994

Robustness

Robustness of the method monitored and evaluated by injecting Blank solution and Standard solution of test concentration. The robustness of the method was verified by applying the following chromatographic conditions as shown in Table -6 and Table-7. All the applied variations observed satisfactory.

Table-6: Results From Robustness Experiment for Mometasone Furoate

S. No.	Robustness Parameter	Retention Time (min)	Tailing Factor	Theoretical Plate Counts	% RSD of Standard Solution
1	Wavelength 208 nm	2.88	1.3	9188	0.25
2	Wavelength 212 nm	2.88	1.3	9298	0.31

3	Flow rate (1.08 mL/min)	3.18	1.3	9543	0.42
4	Flow rate (1.32 mL/min)	2.62	1.3	9329	0.57
5	Column Temp 43° C	2.93	1.3	9204	0.10
6	Column Temp 47° C	2.82	1.3	9150	0.63

Table-7: Results From Robustness Experiment for Benzalkonium Chloride

S. No.	Robustness Parameter	Retention Time (min)	Resolution	Tailing Factor	Theoretical Plate Counts	% RSD of Standard Solution
1	Wavelength 208 nm	4.07	8.0	1.7	9363	0.29
2	Wavelength 212 nm	4.07	8.0	1.7	9477	0.37
3	Flow Rate (1.08 mL/min)	4.48	8.1	1.8	9584	0.54
4	Flow Rate (1.32 mL/min)	3.7	8.0	1.7	12462	0.52
5	Column Temp 43° C	4.18	8.2	1.7	9528	0.21
6	Column Temp 47° C	3.94	7.8	1.7	9496	0.65

Solution Stability in Analytical Solution

In this experiment, solution stability monitored for standard and sample solution up to 12 hrs and 11 Hrs respectively, at 25°C and found within the acceptable limit i.e. % deviation concerning initial should not more than 2.0% represented in Table-8 and Table-9.

Table-8: Results of Solution Stability of Standard Solution at 25°C

	Standard (MOM)		Standard (BKC)	
Time (Hr)	Area	% Deviation w.r.t. Initial	Area	% Deviation w.r.t. Initial
Initial	1069161	-	1252025	-
8	1078416	0.87	1266918	1.19
12	1080651	1.07	1271365	1.54

Forced Degradation

Forced degradation experiment of Mometasone Furoate and Benzalkonium chloride carried out on Blank solution and Test sample solution of the finished product. All solutions were prepared for test concentration and applied specific stress conditions. Prepared solutions injected in a single, monitored peak purity angle and peak purity threshold from each tested stressed condition and calculate % assay of Mometasone and BKC peak.[18] The practical approach used for forced degradation to produce the desire amount of degradation i.e. 5-20% and the acceptable degradation has been observed.[19] Based on recorded chromatograms from forced degradation experiments; the degradation at each specific condition is represented in Table-10.

The degradation products of Mometasone Furoate and Benzalkonium chloride were efficiently resolved by the developed method. Hence, this developed method is the most specific and selective for its intended applications.

Table-9: Results of Solution Stability of Sample Solution at 25°C

	Sample (MOM)		Sample (BKC)	
Time (Hr)	Area	% Deviation w.r.t. Initial	Area	% Deviation w.r.t. Initial
Initial	1068919	-	1249710	-
1	1072495	0.31	1253868	0.15
11	1074153	0.47	1254369	0.19

Table-10: Results From Forced Degradation

Sample Condition	Purity Factor (MOM and BKC Peak)	Assay % of Label Claim		% Degradation with respect to Control Sample	
		MOM	BKC	MOM	BKC
Control Sample(Sample as such)	Peak purity passes	99.2	99.5	NA	NA
Acid treated(2 mL, 1.0 N HCL, 30 min)	Peak purity passes	89.8	98.8	9.4	0.7

Alkali treated(1 mL 0.1N NaOH, 10min)	Peak purity passes	98.3	99.8	0.9	0.4
H2O2 treated (2 mL of 5% H2O2, 30min)	Peak purity passes	99.8	100.0	0.6	0.5

CONCLUSION

Successfully developed and validated, a novel, accurate and most suitable reversed-phase HPLC method for the sample analysis of Mometasone Furoate and Benzalkonium chloride in Mometasone Furoate Nasal Spray Formulation finished product. By applying advanced column chemistry, this method having a very short run time i.e. of 10 min only which is cost-effective, economic and user friendly. It has been also proved that the method is stability-indicating and can be used efficiently for quantitative as well as qualitative analysis purposes.

ACKNOWLEDGMENT

We sincerely thanks Mrs. Vijeta Korade, Red Cross Formulations, Research and Development Laboratory, Waluj MIDC, Aurangabad, Maharashtra, India for providing required laboratory facilities and Mr. Yashwant Kor, Senior Research Scientist, Wockhardt Research center, Aurangabad, Maharashtra, India for sharing zest to carry out this research work.

REFERENCES

1. https://emedicine.medscape.com/article/296301-overview
2. C.H. Gill, P.N. Kulkarni, A.S. Nipte, C.K. Jadhav, A.V. Chate, A.M. Dodake-Supekar, *Journal of Drug Delivery and Therapeutics*, **8(6-s)**, 236(2018), **DOI:** 10.22270/jddt.v8i6-s.2222
3. https://acaai.org/asthma/asthma-treatment
4. https://en.wikipedia.org/wiki/Mometasone
5. J. Liu, G.W. Lu, M. Sandoval, Y. Ciringh, G. Xue, D. Jaeger, *American Association of Pharmaceutical Scientists*, **10(4)**, 1216(2009), **DOI:** 10.1208/s12249-009-9314-2
6. https://en.wikipedia.org/wiki/Bezalkonium_chloride
7. A. Kabeer, A.T. Patil, *Journal of Trace Analysis in Food and Drugs*, **1**, 14(2013), **DOI:** 10.7726/jtafd.2013.1002
8. P. K. Gupta, V. Chaturvedi, *Asian Journal of Pharmaceutical and Clinical Research,* **10(11)**, 374(2017), **DOI:** 10.22159/ajpcr.2017.v10i11.21701
9. D. A. Kostic , S.S. Mitic, D.Č. Naskovic, A.R. Zarubica, M.N. Mitic, *E-Journal of Chemistry*, **9(3)**, 1599(2012)
10. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-test-procedures-cceptance-criteria-new-drug-substances-new-drug-products-chemical_en.pdf
11. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-3-b-r2-impurities-new-drug-products-step-5_en.pdf
12. https://www.fda.gov/files/drugs/published/ANDAs--Impurities-in-Drug-Products.pdf
13. R. Maheshwaran, *Pharmaceutical Technology*, **36**, 73(2012)
14. P.N. Kulkarni, A.M. Dodake-Supekar, A.S. Nipte, C.K. Jadhav, C.H. Gill, *Journal of Drug Delivery and Therapeutics*, **9(4-A)**, 505(2019), **DOI:** 10.22270/jddt.v9i4-A.3483
15. https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf
16. V. Dasari, D. Swarnalatha, G.V. Subbareddy, *Rasayan Journal of Chemistry*, **11(3)**, 1300(2018), **DOI:** 10.31788/RJC.2018.1133085
17. P.G. Swaroopa, K.R.S. Prasad, K.S. Babu, J.V.S Kumar, *Rasayan Journal of Chemistry*, **12(4)**, 2338(2019), **DOI:** 10.31788/RJC.2019.1245438
18. S.S. Prasad, G.V.K. Mohan, A.N. Babu, *Rasayan Journal of Chemistry*, **12(3)**, 1434(2019), **DOI:** 10.31788/RJC.2019.1235281
19. M.N. Blessy, R.D. Patel, P.N. Prajapati, Y.K. Agrawal, *Journal of Pharmaceutical Analysis*, **4(3)**, 159(2014), **DOI:** 10.1016/j.jpha.2013.09.003

[RJC-5783/2020]