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Research Article

Stability-Indicating HPLC Method for the Determination of Mometasone Furoate, Oxymetazoline, Phenyl Ethanol and Benzalkonium Chloride in Nasal Spray Solution

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Abstract

A sensitive stability-indicating reversed-phase high performance liquid chromatographic method was developed for the simultaneous determination of mometasone furoate (MON), oxymetazoline (OXY), phenyl ethanol (PEL) and benzalkonium chloride analogs (BKC1and BKC2) in nasal spray solution. The chromatographic separation was achieved on Cosmosil CN 150 mm x 4.6mm, 5 um column Using a mobile phase consisting of 50 mM KH_2PO_4 and Acetonitrile in the ratio of 60:40 (v/v), at a flow rate of 2.0 ml/min. *The column compartment temperature was set at 40°C.* The typical HPLC chromatograms were extracted at 215 nm using a photodiode-array detector (PDA). The inter-day and intra-day precision values are found within 2% of relative standard deviation. The described method gives LOQ values of 21 ng/mL for MON, 23 ng/mL for OXY, 25 ng/mL for PEL, 10 ng/mL for BKC1 and 9 ng/mL for BKC2. The method shows accuracy of 99.6% for MON, 99.8% for OXY, 99.8% for PEL, 99.0% for BKC1 and 99.5% for BKC2. The described method shows excellent linearity over a range of LOQ to 150% of test concentration. The correlation coefficient for MON, OXY, PEL, BKC1 and BKC2 was 0.9998, 0.9999, 0.9998, 0.9999 and 0.9996 respectively.

Keywords: Mometasone furoate; Oxymetazoline; Phenyl ethanol; Benzalkonium chloride; Nasal spray solution; HPLC

1. Introduction

Mometasone Furoate (USP, 2007) (Fig.1) is (11β,16α)-9,21-dichloro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl 2-furoate. Mometasone Furoate nasal inhalation is used for the treatment and prevention of nasal symptoms of seasonal and year-round allergies, including runny nose, sneezing and itchy nose. Mometasone Furoate nasal inhalation is in a class of medications called topical steroids. Mometasone Furoate works by reducing inflammation in the nasal passages. Oxymetazoline (USP, 2010) (Fig.1) is 3-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2,4-dimethyl-6-tert-

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butyl-phenol. Oxymetazoline is an adrenergic used as the hydrochloride salt as a vasoconstrictor to reduce nasal or conjunctival congestion. Phenyl ethanol (Fig.1) is 2-Phenyl Ethanol used as a preservative in the nasal spray solution. Benzalkonium Chloride (Fig.1) is a mixture of alkylbenzyl dimethyl ammonium chloride salts used as a bacteriocidal agent due to its ability to disrupt bacterial cell membrane function. Mometasone Furoate in combination with Oxymetazoline provides more relief to patients from nasal congestion in comparison with separate use of Mometasone Furoate and Oxymetazoline (Rael et al., 2011). Hence, it is necessary to develop an HPLC method for the determination of these two drugs in combination with the preservatives.

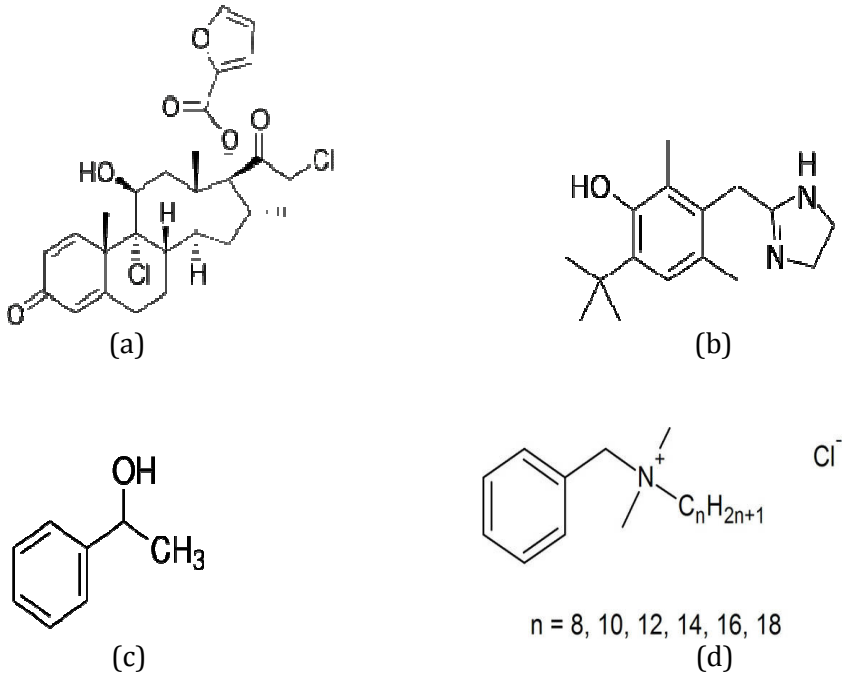

Fig 1. Structure of (a) mometasone furoate, (b) oxymetazoline, (c) phenyl ethanol and (d) benzalkonium chloride

A thorough literature survey revealed some quantification methods are available, such as a quantitative method for determination of oxymetazoline in combination triamcinolone acetonide in nasal spray solution (Sirimas et al., 2006), which is less sensitive and does not propose stability-indicating capability, as compared with the proposed method; determination of the stability of oxymetazoline hydrochloride in aqueous solution by HPLC(Stanisz et al., 2002); simultaneous determination of mometasone furoate along with chlorocresol(Shaikh et al., 2009) and with nadifloxacin(Kulkarniet al., 2010); determination of degradation and metabolism of mometasone furoate in humans by HPLC(Neal et al., 2004); determination of mometasone in human plasma (Teng et al., 2001), which is also less sensitive, as compared with the proposed method; a supercritical fluid chromatography method for the determination of impurities in mometasone furoate(Wang et al., 2011); HPLC analysis of benzalkonium chloride in different formulations (Prince et al., 1999; Dudkiewicz et al., 2004; Blanco et al., 1999) and a UV spectrophotometric method for the determination of phenyl ethanol(Parhizkari et al., 2011).

There are many HPLC methods available for the analysis of single components as mometasone furoate and oxymetazoline or in combination with different analytes. To the best of our knowledge, a stability-indicating RP-HPLC method for the simultaneous determination of MON, OXY, PEL and BKC in nasal spray is not available in any pharmacopoeia. It was felt essential to develop and validate a sensitive, accurate, and stability-indicating RP-HPLC method for the simultaneous determination of MON, OXY, PEL and BKC in nasal spray solution. The proposed method is sensitive, stability-indicating and time-saving. The proposed method is cost-effective, as all the four analytes are able to be analyzed within a single run. Hence, the method is useful for routine use in quality-control analysis.

2. Material and Methods

2.1 Chemicals and Reagents

MON, OXY, PEL and BKC working standards were obtained from Sir Sayyed College, Aurangabad, India. Anhydrous potassium dihydrogen orthophosphate, orthophospheric acid, triethylamine, HPLC-grade acetonitrile, sodium hydroxide, hydrochloric acid and hydrogen peroxide were obtained from Merck Ltd., Mumbai, India. A combination product of mometasone furoate (0.05%W/V) and oxymetazoline (0.05%W/V) nasal spray solution was prepared using preservatives benzalkonium chloride (0.02%W/V) and phenyl ethanol (0.25% W/V). Double-distilled water was used throughout the experiment.

2.2 Chromatographic Conditions

An Agilent HPLC system (1100 series) comprising online degasser, quaternary pump, auto injector, column compartment and photodiode-array detector was used and controlled through Empower Software. A mobile phase consisting of 50 mM KH_2PO_4 and acetonitrile in the ratio of 60:40 (v/v) was used at a flow rate of 2.0ml/min. The column compartment temperature was set at 40°C and the injection volume was 20µL. The UV spectrum of all components exhibits a relative absorption maximum at 215 nm. Typical HPLC chromatograms are extracted at this wavelength, and system suitability results are listed in Table 1.

Table 1 System Suitability Parameters

Component (n=6)	USP Tailing	USP Plate counts	RSD (%)[a]
MON	1.1	7770	0.15
OXY	1.2	9091	0.20
PEL	1.0	6770	0.32
BKC 1	1.1	9709	0.48
BKC 2	1.0	8906	0.86

[a]Mean ± % RSD for six determinations

2.3 Solution Preparation

2.3.1 Preparation of Standard Solution

A solution containing 50 µgmL^{-1} of MON and OXY, 250 µg mL^{-1} of PEL and 20 µg mL^{-1} of BKC was prepared in mobile phase.

2.3.2 Preparation of Sample Solution

5g of sample, equivalent to 2.5 mg of MON, was accurately weighed and transferred to a 50mL volumetric flask, to which 20mL of mobile phase was added and sonicated for about 20 min to dissolve, and the volume was made up to 50 mL with mobile phase. A typical HPLC chromatogram of the sample solution is shown in Fig.2. Benzalkonium chloride used for the nasal spray solution preparation was a mixture of two homologs, n-C_{12} and n-C_{14}. The concentration of these two homologs in the sample and standard is determined using the following formulae:

$$C_{C_{12}} = \frac{AC_{12}}{A_{C_{12}} + A_{C_{14}}} \times C \quad (1)$$

$$C_{C_{14}} = \frac{AC_{24}}{A_{C_{12}} + A_{C_{14}}} \times C \quad (2)$$

where A_{C12} and A_{C14} represent peak areas of homologs n-C_{12} and n-C_{14} respectively, C_{C12} and $C_{C_{14}}$ represent concentrations of homologs n-C_{12} and n-C_{14} respectively, and C is the concentration of benzalkonium chloride.

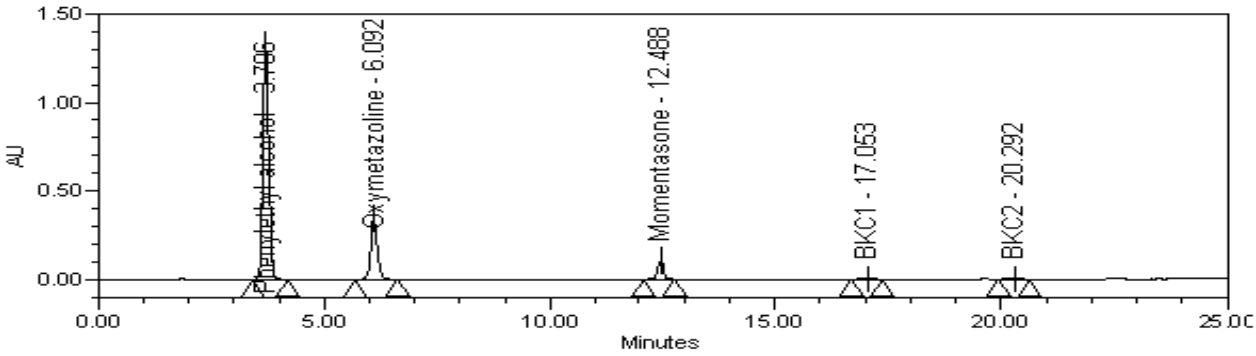

Fig 2. Typical chromatogram of Sample preparation.

3. Experimental

3.1 Method Validation

The developed method was validated according to ICH guidelines with respect to specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, solution stability, and robustness.

3.2 Specificity

The specificity of the method was checked by injecting a solution containing excipient without drug substances, and no chromatographic interference was observed from excipients on the retention time of the analyte peaks. Peak purity was verified by confirming homogeneous spectral data for MON, OXY, PEL and BKC homolog. The specificity of the method was also checked by performing the stressed study and no interference from degradation products on the retention time of MON, OXY, PEL and BKC homolog was found.

3.3 Linearity

Linearity of the method was tested from LOQ to 150% of the targeted level of the assay concentration (MON, OXY 50 μg mL^{-1}, PEL 250 μg mL^{-1} and 10 μg mL^{-1} for BKC1 and BKC2) for all analytes. The calibration graphs were obtained by plotting peak area ratios against the concentration of the drugs and the results are presented in Table 2.

3.4 Limit of Quantification (LOQ) and Detection (LOD)

The limit of detection (LOD) was estimated as three times the signal to noise ratio and the limit of quantification (LOQ) was estimated as ten times the signal to noise ratio. LOD and LOQ were achieved by injecting a series of dilute solutions of MON, OXY, PEL and BKC. The precision of the developed method for MON, OXY, PEL and BKC was checked by analyzing six test solutions prepared at LOQ and LOD level and determining the percentage relative standard deviation of peak area. The LOD and LOQ values are as shown in Table 2.

Table 2 Regression Statistics

S. No.	Statistics	MON	OXY	PEL	BKC 1	BKC 2
1	Linearity range (μg mL^{-1})	25 to 75	25 to 75	125 to 375	5 to 15	5 to 15
2	Correlation coefficient	0.9998	0.9999	0.9998	0.9999	0.9996
3	Slope	11218	12342	13469	7123	6123
4	Intercept	+1348	+1246	-1446	+342	-471
5	LOQ (ng/mL)	21	23	25	10	9
6	LOD (ng/mL)	7	8	9	3	3

3.5 Precision

The repeatability of the analytical method was evaluated by analyzing six test sample solutions of MON, OXY, PEL and BKC nasal spray solution, during the same day (intra-day), under the same experimental conditions. Intermediate precision (inter-day) was evaluated by analyzing six test solutions on different days. The percentage assay for each component was determined and compared. Precision was expressed as percentage relative standard deviation of percentage assay, which was found to be well within the limit (<2). Hence, the method was found to be precise. The inter-day and intra-day precision values are as shown in Table 3.

Table 3 Method precision results for assay of individual compound

Parameters	MON	OXY	PEL	BKC1	BKC2
Intra-day precision [a]	100.1	99.7	100.5	100.2	99.8
Intra-day precision (% R.S.D.)	0.23	0.12	0.54	0.34	0.54
Inter-day precision[a]	100.0	99.9	100.1	99.6	99.8
Inter-day precision(% R.S.D.)	0.34	0.56	0.42	0.43	0.60

[a]Denotes mean of six determinations.

3.6 Accuracy

Accuracy was evaluated by the simultaneous determination of analytes in solution prepared by standard addition method.

Table 4 Recovery study of the method (using the standard addition method)

Name of analyte	50% Accuracy			100% Accuracy			150% Accuracy		
	ug mL^{-1} added	ug mL^{-1} found	% Accuracy	ug mL^{-1} added	ug mL^{-1} found	% Accuracy	ug mL^{-1} added	ug mL^{-1} found	% Accuracy
MON	25.00	24.90	99.6	50.00	49.80	99.6	150.00	149.80	99.9
OXY	25.00	24.80	99.2	50.00	49.90	99.8	150.00	149.50	99.7
PEL	125.00	124.60	99.7	250.00	249.60	99.8	375.00	374.10	99.8
BKC1	5.00	4.90	98.0	10.00	9.90	99.0	15.00	14.80	98.7
BKC2	5.00	4.95	99.0	10.00	9.95	99.5	15.00	14.85	99.0

The experiment was carried out by adding a known amount of each component corresponding to three concentration levels of 50%, 100%, and 150% of target analyte concentration in placebo solution. The samples were prepared in triplicate at each level. The solutions were then analyzed as per the proposed method and the quantification of added analyte was carried out using an external standard of corresponding main drug prepared at the analytical concentration. The recovery values for MON, OXY, PEL and BKC are as shown in Table 4**.**

3.7 Stability in Analytical Solution

Standard solution and sample solution were found to be stable for about two days at room temperature. Similarity factor between the freshly prepared standard and the two day standard solution was found to be 1. Percentage assay was checked at initial sample preparation and on the second day at room temperature against the freshly prepared standard; the percentage difference between the second day sample and initial sample assay was less than 1%.

3.8 Robustness

The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were deliberately altered and the percentage assay was checked for MON, OXY, PEL and BKC. The altered parameters were change in flow rate ($\pm$0.2mLmin^{-1}), column compartment temperature ($\pm$5°C), and absolute organic composition ($\pm$10%). The percentage assay for both the components was found to be well within the limits.

3.9 Forced Degradation Study of Drug Product

5g of sample, equivalent to 2.5 mg of MON, was weighed and transferred into a 50mL volumetric flask, to which 20mL mobile phase was added, and sonicated for about 20 min to dissolve, then treated with acid, alkali, and peroxide, as described in Table 5. The stress studies were performed as per International Conference on Harmonisation (ICH) recommendation. Results of stressed studies are summarized in Table 5.

Table 5 Summary of forced degradation result

Conditions	Degradedness	%Degradation				
		MON	OXY	PEL	BKC 1	BKC 2
Acid	0.1N HCl/60°C/15 min	5.1	1.0	2.5	1.2	1.5
Alkali	0.1N NaOH/60°C/15 min	2.1	1.4	1.0	0.5	0.7
Oxide	30% H_2O_2/ambient/45 min	stable	5.2	stable	6.1	4.0

4. Results and Discussion

4.1 Method Development and Optimization

The main objective of development of an HPLC method for determination of MON, OXY, PEL and BKC homolog in nasal spray solution was that the method should be able to determine assays of both drugs along with preservatives within a single run. As dose strength is at mcg level, the method should be more sensitive, stability indicating, free of interference from degradation products, and straight forward enough for routine use in the quality-control laboratory. During optimization of chromatographic conditions, different mobile phase compositions, HPLC columns, organic modifiers, such as acetonitrile and methanol, and flow rates were tried to achieve acceptable system suitability parameters, as well as good separation between MON, OXY, PEL and BKC homolog. The optimum wavelength selected was 215nm because of higher sensitivity to all analytes at this wavelength.

4.2 Effect of the Organic Modifier

50 mM potassium dihydrogen orthophosphate was used as the buffer for the mobile phase preparation. Different combinations of buffer and acetonitrile within the range of 10:90–90:10, as well as buffer and methanol within the range of 10:90–90:10 were tested. It was observed that methanol in combination with a buffer leads to more retention of both BKC homolog peaks and asymmetric peak shape. An increase in the organic modifier volume in the mobile phase produces a reduced retention time of BKC homologs. The mobile phase combination of buffer : acetonitrile (60:40) gives a symmetric peak shape, an acceptable tailing factor.

4.3 Effect of Stationary Phase

Reversed-phase columns are silica-based bonded phases, and C18-type bonded phase is most frequently used. On an Inertsil ODS 3V 150mm x 4.6 mm, 5 um column PEL was eluted with a solvent front peak. On Cosmosil CN 150 mm X 4.6mm, 5 um column PEL was retained to get expected retention time and symmetrical peak shape for all analytes. The flow rate of the mobile phase was changed to obtain the expected retention time of the analyte. An increase in the flow rate led to reduced retention time of the analyte.

5. Conclusion

The proposed HPLC method is specific, accurate, and precise for simultaneous determination of MON, OXY, PEL and BKC in nasal spray solution. The method was validated as per ICH guidelines and the results show the stability-indicating capability of the method. The method is suitable for routine analysis and quality-control analysis of nasal spray solution containing MON, OXY, PEL and BKC as active pharmaceutical ingredients.

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