A Rapid, Stability-Indicating RP-UPLC Method for the Simultaneous Determination of Fluticasone Furoate and Benzalkonium Chloride in a Pulmonary Drug Product

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Abstract: A stability-indicating reversed-phase ultra performance liquid chromatography (RP-UPLC) method was developed for the simultaneous determination of fluticasone furoate (FF) and benzalkonium chloride (BKC) in a pulmonary drug product. The desired chromatographic separation was achieved on the BEH C18, 1.7 μm (50x2.1 mm) column, using isocratic elution at 215 nm detector wavelength. The optimized mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 45:55% v/v. The developed method separated FF and BKC within 5 minutes. The stability-indicating capability was established by forced degradation experiments. The developed RP-UPLC method was validated according to the International Conference on Harmonization (ICH) guidelines. This validated method was applied for the simultaneous estimation of FF and BKC in commercially available Furamist® (Nasal spray formulation) sample. Furthermore, this method can be extended for individual estimation of FF and BKC in various commercially available pulmonary drug products.

Introduction

Fluticasone furoate, the active component of Furamist® Nasal Spray, is a synthetic fluorinated corticosteroid having the chemical name (6α,11β,16α,17α)-6,9-difluoro-17 {[(fluoro-methyl) thio]carbonyl}-11-hydroxy-16-methyl-3-oxoandrosta-1,4-dien-17-yl 2furancarboxylate and the empirical[1] formula is $C_{27}H_{29}F_3O_6S$. Chemical structure of fluticasone furoate (FF) and benzalkonium chloride (BKC) are presented in Figure 1.

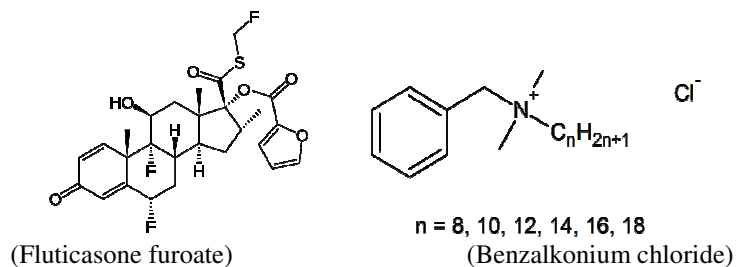(Fluticasone furoate)   (Benzalkonium chloride)

Figure 1. Chemical structure of FF and BKC

Fluticasone furoate is a white powder with a molecular weight of 538.6. It is practically insoluble in water. Furamist® Nasal Spray is an aqueous suspension of micronized fluticasone furoate for topical administration to the nasal mucosa by means of a metering (50 microliters), atomizing spray pump. After initial priming, each actuation delivers 27.5 mcg of fluticasone furoate in a volume of 50 microliters of nasal spray suspension. Furamist® Nasal Spray also contains 0.015% w/w benzalkonium chloride as a preservative.

The advantages of the therapeutic profile of fluticasone furoate have led to increasing use in the clinical practice, which encourages the development of new analytical method to provide driving force in today's pharmaceutical industry. Higher sample throughput with more information per sample may decrease the time to market, an important driving force in today's pharmaceutical industry. UPLC is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 μm particles for stationary phase[2]. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceuticals and biomedical analysis[3-4]. In the present work, this technology has been applied to the method development and validation study of simultaneous assay determination of FF and BKC in nasal spray formulation.

Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients[2]. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the product formulation[5]. The finished product release specifications should include an identification test and a content determination test with acceptance criteria and limits for each antimicrobial preservative present in the formulation[5]. The finished product self-life specification should also include an identification test and limits for the antimicrobial preservatives present[5]. BKC is a mixture of alkyls, including all or some of the group beginning with n-C_8H_{17} and extending through higher homologs, with n-$C_{12}H_{25}$ and n-$C_{14}H_{29}$ comprising the major portion.

A detailed literature survey for fluticasone revealed that few analytical methods are available using Spectrophotometry[6], HPTLC[7], LC-MS/MS[8], HPLC[9] and visible spectroscopy[10] are reported for its determination from pharmaceutical formulation and biological fluids. Literature survey revealed that several HPLC methods[11], LC-MS method[12], electrophoresis[13] have been reported for determination of BKC as individual and in a combination with other drug. HPLC method for the simultaneous estimation of fluticasone propionate and BKC content are reported as a long run time and high solvent consumption for determination of compounds[14]. Therefore the aim of this work is the development and validation of rapid, simple and specific method for the simultaneous determination of FF and BKC in nasal spray formulation.

Experimental

Drug product, placebo solution, working standards and reference standards were provided by Dr. Reddy's laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile was obtained from J.T.Baker (NJ., USA). GR grade potassium dihydrogen phosphate, was obtained from Merck Ltd. (Mumbai, India). 0.22 μm nylon membrane filter and nylon syringe filters were purchased from Pall life science limited (India). 0.22 μm PVDF syringe filter was purchased from Millipore (India). High purity water was generated by using Milli-Q Plus water purification system (Millipore®, Milford, MA, USA).

Equipments

Cintex digital water bath was used for specificity study. Photo stability studies were carried out in a photo-stability chamber (SUNTEST XLS+, ATLAS, Germany). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India).

Chromatographic conditions

Analyses were performed on Acquity UPLC^{TM} system (Waters, Milford, USA), consisting of a binary solvent manager, sample manager and PDA (photo diode array) detector. System control, data collection and data processing were accomplished using Waters Empower^{TM-2} chromatography data software. The chromatographic condition was optimized using Waters BEH C18, 1.7 μm (50 mm x 2.1 mm) column. Mixture of 0.05 M phosphate buffer (KH_2PO_4) and acetonitrile in the ration of 45:55% v/v was used as mobile phase. Mobile phase was filtered through 0.22 μm nylon membrane filter and degassed under vacuum prior to use. The separation of FF and BKC was achieved by isocratic elution. Mixture of water and acetonitrile in the ratio of 50:50 (v/v) respectively was used as a diluent. The finally selected and optimized conditions were as follows: injection volume 5 μL, isocratic elution, at a flow rate of 0.5 mL/min at 45 °C (column oven) temperature, detection wavelength 215 nm. The stress degraded samples and the solution stability samples were analyzed using a PDA detector covering the range of 200-400 nm.

Standard solution preparation

An accurately weighed 55.0 mg of FF and 30 mg of BKC (50% solution) standard substances were taken into the 50 mL volumetric flask. Then added 10 mL of acetonitrile in the same volumetric flask and sonicated in an ultrasonic bath for 5 min. Then added 10 mL of water in the same volumetric flask and sonicated in an ultrasonic bath for 5 min. This solution was then diluted up to the mark with diluent and mixed well. 0.5 mL of this solution was then diluted to 20 mL with diluent and mixed well.

Sample solution preparation

An accurately weighed 500 mg of sample solution was taken into the 10 mL volumetric flask. About 3 mL of acetonitrile was added and sonicated in an ultrasonic bath for 5 min. Then added 3 mL of water in the same volumetric flask and sonicated in an ultrasonic bath for 5 min. This solution was then diluted up to the mark with diluent and mixed well. It was then filtered through 0.22 μm PVDF syringe filter and the filtrate was collected after discarding first few milliliters.

Placebo (other substances without FF and BKC) solution preparation

An accurately weighed 500 mg of placebo solution was taken into the 10 mL volumetric flask. About 3 mL of acetonitrile was added and sonicated in an ultrasonic bath for 5 min.

Then added 3 mL of water in the same volumetric flask and sonicated in an ultrasonic bath for 5 min. This solution was then diluted up to the mark with diluent and mixed well. It was then filtered through 0.22 µm PVDF syringe filter and the filtrate was collected after discarding first few milliliters.

Results and Discussion

Method development and optimization

The main objective of the RP-UPLC method development was to rapid and simultaneous determination of FF and BKC in nasal spray formulation were: the method should be able to determine assay of FF and BKC compounds in single run and should be accurate, reproducible, robust, stability indicating, filter compatible, linear, free of interference and straightforward enough for routine use in quality control laboratory.

The spiked solution of FF (27.5 µg/mL) and BKC (7.5 µg/mL) was subjected to separation by RP-UPLC. Initially the separation of all compounds was studied using water as a mobile phase-A and acetonitrile as a mobile phase -B on UPLC column BEH C18, 1.7 µm (50 mm x 2.1 mm) and Waters (UPLC) system with the linear gradient program. The flow rate of 0.5 mL/min was selected with regards to the backpressure and analysis time as well. During this study column oven temperature was capped at 50 °C. When study performed with above condition we observed broad peak of all the compounds. Various types of buffer solution and solvents were studied to optimize the method. Based on buffer and solvent selection study optimized UPLC parameters were; flow rate 0.5 mL/min; column oven temperature 45 °C; isocratic program; mixture of 0.05M phosphate buffer (KH_2PO_4) and acetonitrile in the ration of 45:55% v/v as mobile phase. Based on compounds UV spectrums 215 nm was found more appropriate for the simultaneous determination. FF and BKC are well resolved with each other in reasonable time of 5.0 minutes which is presented in Figure 2. There was no any chromatography interference due to blank (diluent) and excipients (placebo) at the retention time of FF and BKC which was presented in Figure 2.
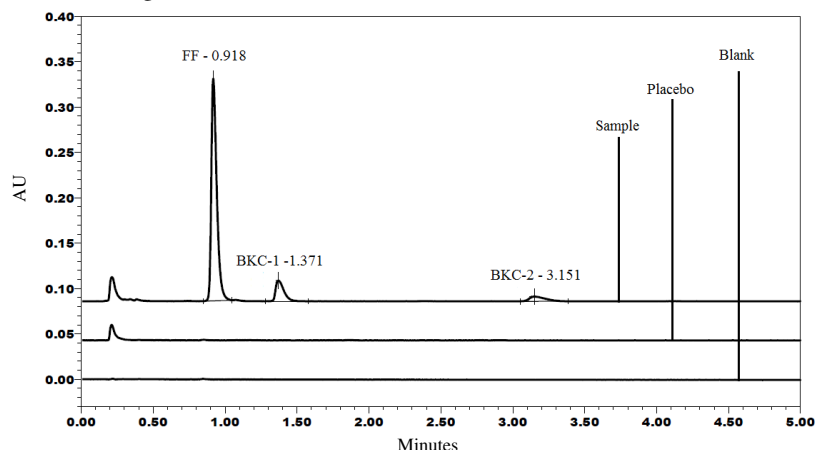

Figure 2. Overlaid specimen chromatograms of blank, placebo and sample

Analytical parameters and validation

After satisfactory development of method it was subjected to method validation as per ICH guideline[15]. The method was validated to demonstrate that it is suitable for its intended purpose

by the standard procedure to evaluate adequate validation characteristics (accuracy, precision, linearity, robustness, solution stability, filter compatibility and stability indicating capability).

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[15]. Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed RP-UPLC method. Figure 2 is shows that there is no any interferences at the RT (retention time) of FF and BKC due to blank, placebo and degradation products. Peaks due to FF and BKC were investigated for spectral purity in the chromatogram of all exposed samples and found spectrally pure.

Precision

Method precision: (Repeatability)

The precision of the assay method was evaluated by carrying out six independent determinations of FF and BKC (27.5 μg/mL of FF and 7.5 μg/mL of BKC) test samples against qualified working standard. The method precision study shows the repeatability of the results obtained by the testing method. The %RSD (n=6) was 0.6% for FF and 0.5% for BKC, which are well within the acceptable limit of 2.0%. It was confirmed from results that the method is precise for the intended purpose (Table 1).

Intermediate precision: (Reproducibility)

The purpose of this study is to demonstrate the reliability of the test results with variations. The reproducibility was checked by analyzing the samples by different analyst using different chromatographic system and column on different day. The analysis was conducted in the same manner as the method precision and the %RSD of all six sets of sample preparations was determined (Table 1). The %RSD was 0.5% for FF and 0.7% for BKC, which are well within the acceptance criteria of 2.0%, so this study proved that the method to be rugged enough for day to day use.

Table 1. Precision (n=6) and intermediate precision (n=6) results

Substance	Precision at 100%		Intermediate precision	
	Mean % assay[#]	% RSD*	Mean % assay[#]	% RSD*
FF	99.4	0.6	99.0	0.5
BKC	98.3[$]	0.5	98.6[$]	0.7

[#]*Average of six determinations;* *Determined on six values;* [$]*Results are mean of total area of BKC*

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique. The accuracy of the method was carried out by adding known amounts of each drug corresponding to three concentration levels; 50, 100 and 150% of the label claim (Table 2) along with the excipients in triplicate. The samples were given the same treatment as described in sample preparation. The percentage recoveries of FF and BKC at each level and each replicate were determined. The mean of percentage recoveries (n=3) and the relative standard deviation was calculated. The amount recovered was within ±1.5% of amount added, which indicates that there is no interference due to excipients present in nasal spray formulation. It was confirmed from results that the method is highly accurate (Table 2).

Table 2. Accuracy results

Substance	At 50% (n=3)		At 100% (n=3)		At 150% (n=3)	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
FF	100.3	0.5	99.8	0.4	99.3	0.7
BKC	99.2	0.6	100.2	0.5	100.5	0.4

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. The nominal concentrations of standard and test solutions for FF and BKC were 27.5 and 7.5 μg/mL, respectively. The response function was determined by preparing standard solutions at seven different concentration levels ranging from 13.75-55.0 μg/mL for FF and 3.75-15.0 μg/mL for BKC (50 to 200% of analyte concentration). The response was found linear from 50% to 200% of standard concentration. For all compounds the correlation coefficient was greater than 0.999. The regression statistics are shown in Table 3.

Table 3. Linearity results

Compound	Linearity Range, μg/mL	Correlation coefficient, R^2	Linearity (equation)	Y- intercept bias in %
FF	13.75 to 55.0	0.9998	Y=11572.5904(x)+1289.2981	0.4050
BKC	3.75 to 15.0	0.9990	Y=9590.7336(x)+113.2630	0.1575

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The effect of change in flow rate (±0.05 mL/min) and column oven temperature (±5 °C) on the retention time, resolution (between FF and BKC), theoretical plates and tailing factor were studied. During study other chromatographic conditions were kept same as per the experimental section. No significant differences are observed, when above changes are made.

Stability of sample in diluent

Drug stability in pharmaceutical formulations is a function of storage conditions and chemical properties of the drug, preservative and its impurities. Condition used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data is required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling. Stability of sample solution was established by storage of sample solution at ambient temperature (25 °C) for 24 h. Sample solution was re-analyzed after 12 and 24 h time intervals and assay were determined for the compounds (FF and BKC) and compared against fresh sample. Sample solution did not show any appreciable change in assay value when stored at ambient temperature up to 24 h, which are presented in Table 4. The results from solution stability experiments confirmed that sample solution was stable for up to 24 h during assay determination.

Table 4. Solution stability results

Time intervals	FF	BKC
% Assay Initial	99.5	98.3
% Assay after 12 h	99.3	98.0
% Assay after 24 h	99.5	98.1

Filter compatibility

Filter compatibility was performed for nylon 0.22 μm syringe filter (Pall Life sciences) and PVDF 0.22 μm syringe filter (Millipore). To confirm the filter compatibility in proposed analytical method, filtration recovery experiment was carried out by sample filtration technique. Sample was filtered through both syringe filters and percentage assay was determined and compared against centrifuged sample. Sample solution was not showing any significant changes in assay percentage with respect to centrifuged sample. Percentage assay results are presented in Table 5. In displayed result difference in % assay was not observed more than ±0.5, which indicates that both syringe filters having a good compatibility with sample solution.

Table 5. Filter compatibility results (Assay % w/w)

Compound	Centrifuged	PVDF filter 0.22 μm (Millipore)	Nylon filter 0.22 μm (Pall Life Sciences)
FF	99.6	99.5	99.5
BKC	98.2	98.0	98.1

Application of the method to dosage forms

The present method was applied for the estimation of drugs and preservatives in the commercially available Furamist® (Cipla Ltd.) nasal spray. The results obtained are 100.5% for FF and 98.7% for BKC. Based on obtained results developed method is suitable for the various marketed dosage form also.

Conclusion

An isocratic RP-UPLC method was successfully developed for the simultaneous estimation of FF and BKC in liquid pharmaceutical formulation. The developed method is selective, precise, accurate, linear, filter compatible and robust. Forced degradation data proved that the method is specific for the FF and BKC. The run time (5.0 min) enables for rapid determination of drugs and preservatives. Moreover, it may be applied for individual and simultaneous determination of FF and BKC compound in pulmonary drug product and substance. Also it can be utilized for assay determination of bulk and finished product (for FF and BKC) and spray content uniformity of nasal spray (finished product), where sample load is higher and high throughput is essential for faster delivery of results.

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