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A RAPID AND STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TIOTROPIUM, FORMOTEROL AND CICLESONIDE IN A DRY POWDER INHALER

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ABSTRACT

A stability indicating reversed phase high performance liquid chromatographic method has been developed and validated for simultaneous assay of Tiotropium, Formoterol and Ciclesonide from a dry powder inhalation formulation. Separation was achieved on a C18, 5µm x 250 mm column at 40°C temperature using Acetonitrile as organic modifier; Disodium hydrogen phosphate anhydrous as buffer and Decane sulfonic acid sodium salt as the ion pairing agent at pH 3.5 and UV detection at 230 nm. Forced degradation studies was performed and the method could effectively separate the interferences from placebo, primary packaging material, impurities and degradants, hence the method is specific and stability indicating method. The method was validated in line with the ICH guidelines in terms of linearity, precision, accuracy, specificity, robustness and solution stability.

Keywords: Dry powder inhaler, Tiotropium Bromide monohydrate, Formoterol Fumarate dihyrdate, Ciclesonide, HPLC, Assay and Validation.

INTRODUCTION

Nearly 235 million people are globally suffering from Asthma and Chronic obstructive pulmonary disease (COPD) [17]. Metered dose inhalers (MDI) and Dry powder inhalers (DPI) constitute main-stream therapy regime for both Asthma & COPD. Advantages of inhalation therapy include drug delivery directly to the site of action avoiding drug metabolism in the gastrointestinal tract. Inhaled β_2 agonists both short acting (SABA) and Long acting (LABA),

Anticholinergic agents and Corticosteroids are the cornerstone in the management of Asthma and COPD. These medications can be given as a liquid inhalation formulation in a metered dose inhaler or as a dry powder inhaler. They are available as rescue medication for relief of bronchoconstriction and as preventers for long term therapy. Combination drugs product are available ensuring dose compliance and ease of use for the patient as against taking multiple individual drug products. One such product is Triohale Rotacaps manufactured by Cipla Ltd. Triohale Rotacaps contains three active ingredients; A Long-acting anticholinergic agent Tiotropium (TIO) 18 mcg as Tiotropium Bromide monohydrate *Fig 1*, It acts mainly on M_3 muscarinic receptors located on smooth muscle cells and submucosal glands leading to a reduction in smooth muscle contraction and mucus secretion, thus producing a bronchodilatory effect. A long acting β_2 agonist (LABA) Formoterol Fumarate dihydrate (FFD) 12 mcg *Fig. 2* which causes bronchodilation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. And a non-halogenated glucocortico steroid Ciclesonide (CIC) 400 mcg *Fig 3* which is enzymatically hydrolyzed to a pharmacologically active metabolite, C21-desisobutyryl-ciclesonide (des-ciclesonide or RM1) following oral inhalation. Des-ciclesonide has anti-inflammatory activity with affinity for gluco corticoid receptors. The three active ingredients are mixed and blended with lactose which act as a carrier for the drug. This blend is partially filled in a capsule. This capsule is used with a dry powder inhaler device for drug delivery via oral inhalation.

Chemical name and structure of the three drugs

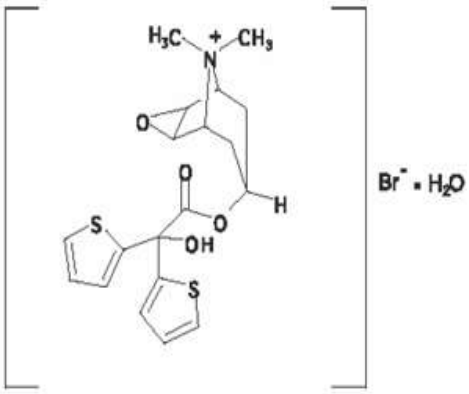


Fig. 1: Tiotropium bromide monohydrate It is chemically described as (1α, 2β, 4 β, 5α,7β)-7-[(Hydroxydi-2-thienylacetyl)oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo [3.3.1.02,4]nonane bromide monohydrate.

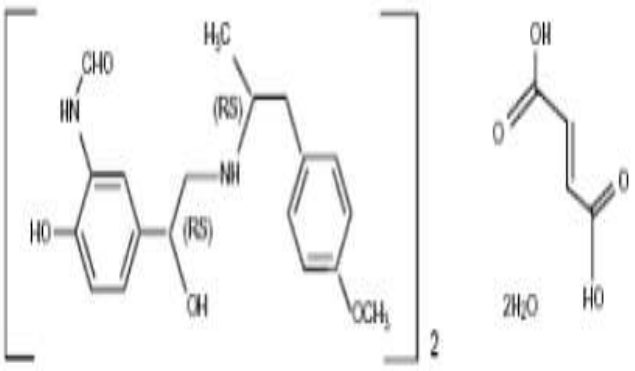


Fig. 2: Formoterol fumarate dihydrate, its chemical name is (±)-2-hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(4-methoxyphenyl)-1methylethyl]-amino]ethyl]formanilide fumarate dehydrate.

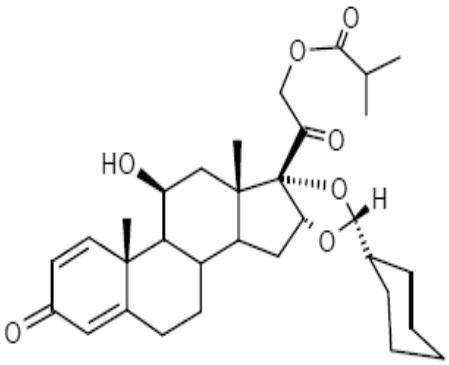


Fig. 3: I Cicleconide its chemical name is pregna-1,4-diene-3,20-dione, 16,17-[[(R)cyclohexylmethylene] bis(oxy)]-11-hydroxy-21-(2-methyl-1-oxopropoxy),(11β, 16α).

At present no official monograph is available for testing this triple combination drug product. There have been limited reports on HPLC methods available for the assay of the triple drug combination in a Metered dose inhaler which contains primarily drug and volatile propellant but none available for the Dry powder inhaler which contains drug and lactose in a capsule. Several analytical methods for the mono and dual components have been reported for determination of TIO, FFD, and CIC pulmonary drug product formulations, by HPLC and other techniques [3–10].

There are two reported methods for assay of the triple drug combination product in a metered dose inhaler Ravi Pratap *et al*. (2) it describe simultaneous estimation of TIO, FFD and CIC in pharmaceutical metered-dose inhalers which was not validated as per ICH guidance(for specificity and forced degradation study) and Rakshit Trivedi et al (3) which describes a HPLC method in which the known impurities are not identified and the forced degradation

study is not suitable for a DPI as it does not take into account the interaction between the drug and excipient and drug and primary packaging - the capsule. The excipient Lactose monohydrate (5-6 % moisture) and capsule(~ 5 - 18% moisture) may interact with the drug to give degradation products. In addition, the capsule color and residual lubricant in the capsule can leach into the powder blend over a period of time giving rise to unknown degradants.

The published methods are not satisfactory for the combination dry powder inhaler hence there is a need to develop a method for assay of the triple drug combination dry powder Inhaler. With the advances in analytical chemistry it is now generally expected that the assay methods are not only Linear, Accurate, Precise and Robust but also stability indicating. Hence a stability indicating HPLC method was developed and validated in line with the ICH Guidelines (12).

MATERIALS AND METHODS

Chemicals and reagents

Working standards and known impurities of Tiotropium bromide, Formoterol Fumarate dehydrate and Ciclesonide were obtained as generous gifts from various pharmaceutical firms and was used as such without further purification. HPLC grade Acetonitrile and Methanol, Buffer Disodium hydrogen phosphate anhydrous and Ortho-phosphoric acid manufactured by Merck were used. Decane sulfonic acid sodium salt anhydrous make: Rankem and purified water were employed throughout the work and quantitative determination.

Instrumentation and Chromatographic conditions

The separations were performed using Quaternary Gradient system – LC 100 Cyber Lab, LC-UV100 UV Detector, AS 100 Autosampler and data acquisition was done using WS 100 work station.

The mobile phase consisted of Solvent A - Buffer (0.01 M Disodium hydrogen phosphate anhydrous + 0.05 M decane sulfonic acid sodium salt solution , pH adjusted to 3.5 with OPA) and Solvent B - Acetonitrile. The chromatographic column used was C18, 5 micron (25 cms X 4.6 mm) column. The UV detection wavelength was 230 nm. Analysis was done at 40° C at a flow rate of 1.0 ml / minute and an injection volume of 50 µl. The method runtime was maintained at 22 mins. Diluent used was Acidified Water : ACN : Methanol (40:30:30). *(Acidified water - 0.5 ml, ortho phosphoric acid in 1000 ml purified water.)* The gradient programme followed was as per Table 1 given below :

Table 1: Gradient for test of assay

Time (min)	Mobile phase A (Buffer)	Mobile phase B (Acetonitrile)
0	50	50
7	50	50
10	10	90
18	10	90
20	50	50
22	50	50

Preparation of standard solution.

A standard solution containing Tiotropium (1.8 ppm), Formoterol Fumarate dihyrdate (1.2 ppm) and Ciclesonide (40 ppm) was prepared in the diluent.

Preparation of Sample solution.

Transfer contents equivalent to 10 capsules in a 100 ml volumetric flask add ~ 70 ml diluent, sonicate and dilute to 100 ml with diluent.

Forced degradation studies

The Drug product blend and placebo were subjected to stress testing as per ICH recommended test conditions and analyzed as per proposed method. The acid hydrolysis was performed using 0.5 N HCl and base hydrolysis by using 0.1 N Sodium Hydroxide. Oxidation degradation was done using 30 % Hydrogen peroxide, Thermal degradation was achieved by exposing drug product at controlled temperature of 60°C and Photolytic degradation was done by exposing the drug product blend in a photolytic chamber.

The objective of the forced degradation study was to generate the degradation products under various stress condition and confirm that the degradation product peaks are resolved from the main peaks of interest. Peak purity of Tiotropium, Formoterol and Ciclesonide was checked at every condition and was found to be greater than 950 at all conditions ensuring that there are no co-eluting peaks.

Method validation

The method was validated for specificity, linearity, accuracy, precision and robustness and solution stability in line with ICH guidelines.

System suitability

The system suitability was assessed by six replicate injections of 1.8 ppm, 1.2 ppm and 40 ppm solution of TIO, FFD and CIC. The Tailing Factor, number of theoretical plates, the percentage relative standard deviation of peak area and peak retention time of standard solution were calculated as represented in Table 2.

Table 2: System Suitability

Parameter	TIO	FFD	CIC	LIMIT
Area - % RSD	0.45	0.13	0.12	NMT 2.0%
RT - % RSD	0.26	0.27	0.27	NMT 1.0%
Tailing Factor	1.30	1.26	0.97	0.8 - 2.0
Theoretical Plates	12308	12396	178568	NLT 4000

Specificity

Specificity was demonstrated as shown in *Figure 4*, TIO , FFD CIC are very well resolved of interferences from Tiotropium impurity A, E and F, Formoterol impurities A, C and G, Ciclesonide Imp 1 and Imp 2 (process impurities), degradants impurities, placebo lactose monohydrate and capsule shell, confirming specificity of the method.

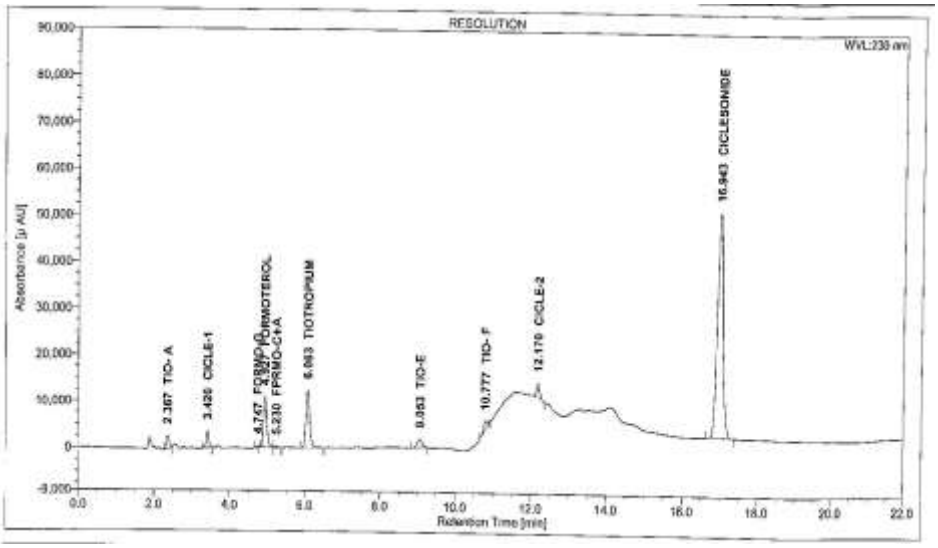
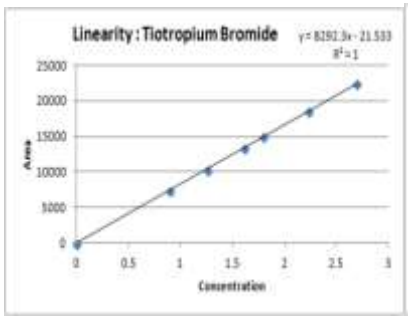


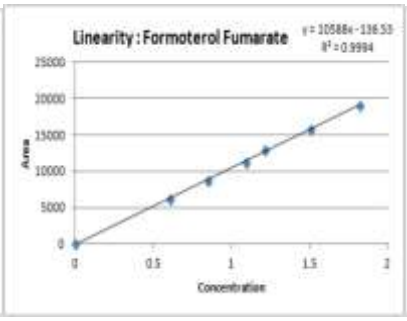
Fig. 4: Specificity

Linearity

Linearity of the method was determined by injecting six calibration standards covering 50% to150 % range of the target concentrations. The linearity of the method was demonstrated by plotting peak area versus concentrations and finding out the co-eff of correlation Table 3. It

can be seen that the plot is linear over the 50-150 % range for all three active ingredients. *Fig 5,6 and 7* TIO, FFD and CIC resp.





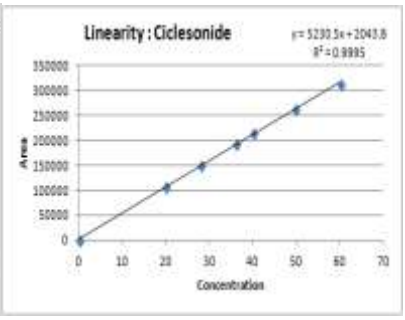


Fig 5: Linearity Tiotropium Fig 6: Linearity Formoterol Fig 7: Linearity Ciclesonide

Table 3 : **Linearity Results**		
	Linearity Range(n=5)	**Correlation Coefficient (r^2)**
Tiotropium	50 to 100 %	1.0000
Formoterol	50 to 100 %	0.9996
Ciclesonide	50 to 100 %	0.9996

Precision

System precision was determined from the % RSD of six replicate standards of TIO, FFD and CIC. System suitability criteria of % RSD, Tailing factor and Theoretical plates were studied; they meet the acceptance criteria established for the method, the results are tabulated in Table 2.

Method precision was carried out by analyzing six independent assays of the sample and finding out the % RSD, observations are tabulated in Table 4. The results indicate the % RSD is well within the limit of NMT 2.0 % confirming precision of method.

Table 4 : Method precision

Assay(%)	**TIO**	**FFD**	**CIC**
Average	99.4	101.5	101.1
%RSD	0.66	1.06	1.43

Intermediate precision was carried out by analyzing samples at different days and by different analyst Table 5. The purpose of this study was to demonstrate the reliability of the test results with variations arising out of regular QC release scenario. There is no significant difference

in the % RSD and recovery result data of analyst 1 and analyst 2. Thus the method is suitability precise and reproducible.

Table 5: Intermediate Precision

Assay (%)	TIO		FFD		CIC	
Sr.no	Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	99.4	98.1	102.2	98.9	101.4	99.6
2	99.6	99.6	103.3	102.4	103.6	99.9
3	99.6	101.6	101.0	100.6	99.8	102.2
4	98.5	100.8	100.2	101.2	101.4	101.8
5	99.0	101.4	101.1	101.8	99.6	102.6
6	100.4	102.1	101.3	100.9	101.0	103.2
Mean	100.0		101.2		101.3	
% RSD	1.26		1.12		1.40	

Accuracy

The accuracy of the method was assessed from the recovery studies of the three drugs TIO, FFD and CIC at three different levels 80%, 100% and 120% of the target concentration. The experiment was performed in triplicate and the recovery % and RSD (%) were calculated as shown in Table 6. Excellent recovery and the % RSD value confirm that the method is accurate for assay determination of the dry powder inhaler Table 6.

Table 6: Accuracy – Recovery data

Parameter	TIO			FFD			CIC		
Level (%)	80	100	120	80	100	120	80	100	120
% Recovery	99.0	99.8	101.4	99.7	100.1	100.02	100.4	99.4	100.36
% RSD	0.16	0.1	0.33	0.25	0.27	0.24	0.20	0.32	0.15

Robustness

In method robustness studies the influence of small deliberate variations of the analytical parameters are examined to understand and establish suitability of the method under variability condition arising out of routine analysis. The following two factors were selected for change the column temperature (40° ± 2C) and pH of the buffer 3.5 ±0.1, Table 7 and 8 resp. One factor at a time was changed to study the effect. Each condition was tested in three replicates at 3 levels and the % RSD calculated for each condition indicates that the method is robust for these conditions.

Table 7: Results for Robustness; Variable Temperature

Parameter	TIO			FFD			CIC		
Temperature (°C)	38	40	42	38	40	42	38	40	42
Assay (%)	100.3	100.8	99.6	101.7	101.9	101.8	102.4	101.2	101.5
% RSD	1.1	1.5	0.9	1.1	1.4	1.1	1.4	1.8	0.8

Table 8: Results for Robustness; Variable pH

Parameter	TIO			FFD			CIC		
pH	3.4	3.5	3.6	3.4	3.5	3.6	3.4	3.5	3.6
Assay (%)	100.7	100.8	99.5	101.6	101.9	102.2	102.8	101.2	101.0
% RSD	1.6	1.5	1.1	1.7	1.4	0.2	1.7	1.8	1.3

Solution stability

The stability of standard and sample solution was examined by analyzing the solutions stored at room temperature in the Autosampler for 48 hrs. Both the solutions did not show significant change in the area after the storage period Table 9. The % RSD for each of the analyte peak area was calculated and was found to be below 2.0% confirming solution stability for a time period of 48 hrs.

Table 9: Solution stability

	Standard Solution - Area			Sample Solution - Area		
Time	TIO	FFD	CIC	TIO	FFD	CIC
O Hrs	14871.2	11729.0	219249.8	15472.8	12775.1	236233.2
24 Hrs	15050.7	11927.2	219887.2	15172.4	12857.4	236699.6
48 Hrs	15036.3	11780.2	221421.2	15165.5	12849.4	233829.9
Mean	14986.1	11812.1	220186.1	15270.2	12827.3	235587.6
% RSD	0.7	0.9	0.5	1.1	0.4	0.7

RESULTS AND DISCUSSION

Triohale Rotacap contains three active ingredients Tiotropium Bromide monohydrate, Formoterol Fumarate Dihydrate and Ciclesonide, each of them in different concentration ranging from 12 mcg to 400 mcg; each having different solubility and different uv maxima. Detection wavelength was selected by reviewing the UV spectra of each of the drugs, TBM exhibits maxima at 238 nm, Formoterol at 211 nm and Ciclesonide at 240 nm the detection wavelength of 230 nm was chosen in order to achieve good sensitivity for all the three active ingredients. To achieve separation between TIO, FFD, CIC, known impurities, degradants,

impurities, excipient and capsule shell different Buffers, ion pairing agent and different proportions of organic modifiers were evaluated. C8 and C18 columns of different makes and geometry were evaluated. The differences in solubility of the three ingredients, lactose and capsule led to development of a gradient HPLC method. The best separation was achieved with Disodium hydrogen orthophosphate as buffer and Decane sulphonic acid as the ion paring agent. The forced degradation study data and the specificity data demonstrate that the method is stability indicating. To ensure solution stability of Tiotropium the pH of the diluent was kept mildly acidic. Under these conditions and using a flow rate of 1.0 ml/min and a run time of 22 mins. Formoterol elutes at 5.0 mins. Tiotropium elutes at 6.8 mins and Ciclesonide elutes at 15 mins. Linearity over the range of 50 to 150 % of the target concentration was found to be excellent with coeff. of correlation between 0.9996 and 1.0. The method precision (%RSD<2) and the Accuracy (% Recovery between 99-102% and % RSD < 1) indicate that the method is precise and accurate. Further, the results from the robustness study confirm the applicability of the method for routine Quality control. The solution stability of 48 hrs confirms that this method can not only be used for the test of assay but also delivered dose uniformity and aerodynamic assessment of fine particles which are tests specific to inhalation formulations and require multiple runs per test resulting in samples being analyzed over a period of time.

CONCLUSION

A stability indicating gradient HPLC method has been developed and validated for simultaneous Assay estimation of Tiotropium, Formoterol and Ciclesonide from a dry powder inhalation formulation. The validated method is specific, linear, precise, accurate and robust and can be used for day to day routine QC analysis.. The developed method can also be used for assay of single and dual drug combination of Tiotropium Bromide Monohydrate, Formoterol fumarate dihydrate and Ciclesonide. In addition, the method is also suitable for testing metered dose inhaler formulation having similar active ingredient combinations as the metered dose inhaler formulation generally contains only drug and volatile propellants.

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