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Research Article

ASSESSMENT OF FINE PARTICLE FRACTION OF INHALER DRUG USING CASCADE ANDERSON IMPACTOR AND HPLC

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A B S T R A C T

Asthma is a chronic respiratory disease characterized by constriction of smooth muscles of lung. Deposition of inhaled drug in respiratory system is the key parameter to treat these obstructive air diseases. Aerodynamic particle size of all inhaled products directly influencing regional deposition of APIs in the lungs and respiratory tract. Its measurement is critical during the product development cycle and for quality control. Cascade impactors are preferable for inhaler characterization because they provide the required degree of resolution in the particle size range of greatest interest between 0.5-5µm for the inhalation products. The aim of this study is to develop the successful method for estimation of triple combination metered dose inhaler Formoterol, Tiotropium and Ciclesonide by HPLC as well as analyze the deposition of the fine particle fraction of active pharmaceutical ingredients of inhaler drug into the in-vitro respiratory tract model (Andersen cascade impactor) with the help of HPLC. Improved APSD of inhalers will help to reduce the dosage and frequency of dosing to patient. HPLC method was developed and validated for measuring particle size deposition of MDI.

INTRODUCTION

Aerosolised medicine is used for treating asthma, chronic obstructive pulmonary disease (COPD) and other respiratory diseases (Stephen W *et al*., 2013). The concept of classical bioequivalence and bioavailability is usually not applicable for oral inhalation aerosols (Irwin M *et al*., 2018).The dose administered is typically so small that blood or serum concentrations are generally undetectable by routine analytical methods. Approximately 10-15 percent of the dose reaches the biological target, the remainder of the dose, trapped in the mouth and pharynx, is swallowed and absorbed through the gastrointestinal (GI) tract. Together with the delivered dose the aerodynamic particle size distribution (APSD) is widely recognized as a critical quality attribute in the in-vitro characteristics of inhalers (Shelton *et al*.,) It is generally accepted that to be therapeutically effective the particles should be in the range of 1-5µ.Since particles >5µ will generally impact in the oropharynx and be swallowed whereas <1µ the possibility exists that the particles will remain entrained in the air stream and be exhaled (Copley, 2015).CITDAS calculates fine particle dose using statistical analysis.

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Two devices working on this principle have been included in the British Pharmacopoeia, Apparatus A and Apparatus B(British Pharmacopoeia,2001). Apparatus A is Twin Stage Liquid Impinger (TSLI). It comprised two stage reservoirs [Fig.1]

Stage I representing the amount of dose deposited in the oropharyngeal region.

Stage II (also termed as respirable fraction), representing the amount of drug deposited in lungs The "respirable fraction" of the MDI (i.e., the fraction with size <6.4 microns) can be measured in the lower stage. TSLI is operated on a flow rate of 60lit/minute. Major advantage is that it is manufactured solely from glass so it is not prone to corrosion.

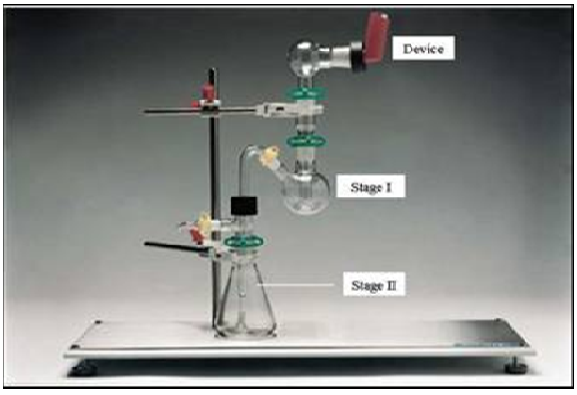

Fig 1 Twin Stage Liquid Impinger

Apparatus B is Anderson Cascade Impactor (ACI) [Fig-2] the preferred choice for measuring APSD of inhaled products. ACI consists of total eight stages. The inhaler is connected to the induction port by means of the mouthpiece adapter which provides the airtight seal between induction port and the medical device under test. Once discharged from the inhaler, the aerosol cloud is drawn through the impactor by means of a vacuum pump connected to the outlet of the impactor using a vacuum pump.

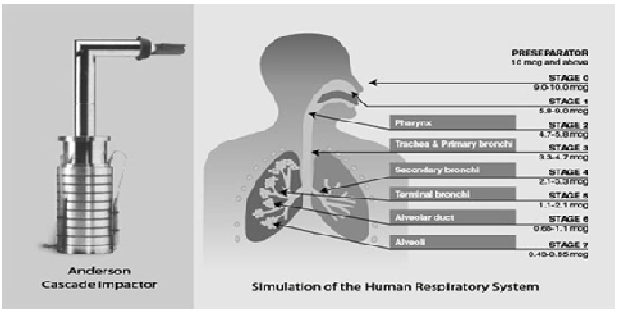

Fig 2 Diagram of Anderson cascade apparatus and simulation of the human respiratory system

Tiotropium

Tiotropium [Fig.3] is anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease. It is a muscarinic receptor antagonist. It is abbreviated as X in further studies.

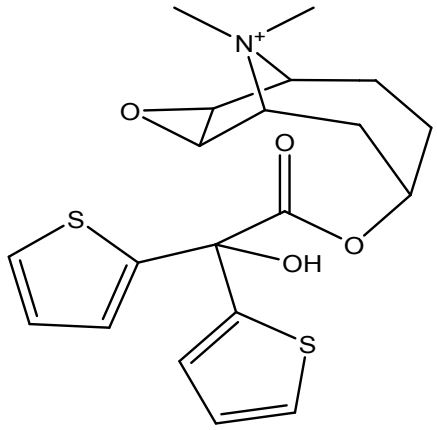

Fig 3 Chemical Structure of Tiotropium

Formoterol

Formoterol [Fig.4] is a highly selective β_2 adrenergic agonist. Formoterol cause bronchodilatation through relaxation of smooth muscle. It is abbreviated as Y in further studies.

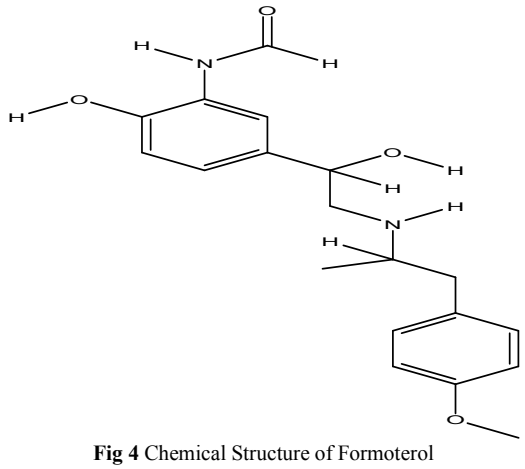

Fig 4 Chemical Structure of Formoterol

Ciclesonide

Ciclesonide [Fig.5] is a new inhaled corticosteroid used inhaler for the treatment of persistent asthma in adults and older. It is a prodrug enzymatically hydrolyzed in the lungs to a pharmacologically active metabolite which has anti-inflammatory activity with an affinity for Glucocorticoid receptors. It is abbreviated as Z in further studies.

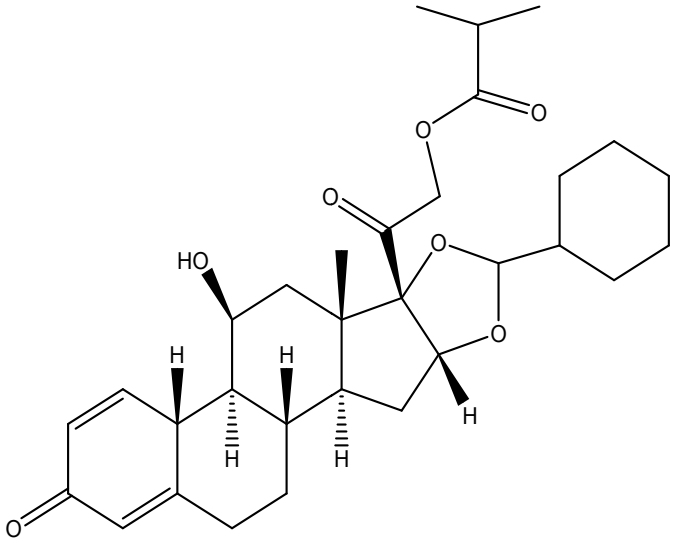

Fig 5 Chemical Structure of Ciclesonide

MATERIALS AND METHOD

Chemicals and Reagents

API were procured from different sources as the study involves simultaneous estimation of three drugs. Formoterol was procured from Sai Life Sciences, tiotropium from Vamsi Laboratories and ciclesonide from Arch Pharma. All other chemicals and reagents were of HPLC analytical grade. Copley anderson apparatus was used to prepare sample and estimation was done using waters HPLC equipped with UV detector. Data analysis was done using Copley Scientific Testing Data Analysis Software (CITDAS).

Chromatographic conditions

Methanol and pH6.5 potassium dihydrogen orthophosphate buffer solution was used as mobile phase in a gradient mode [Table.1].Buffer solution was prepared by dissolving 3.4 gm KH_2PO_4in 1000 ml water and pH was adjusted to 6.5±0.05 using 10%w/v KOH solution. Prior to use mobile phase was filtered through 0.45 µm membrane filter and degassed by sonication for 10 mins. Analysis was carried out on waters HPLC system fitted with Kromasil C18 (150×4.6mm), 5µ.The operating temperature of column was set at 30ºC and of sample tray at 5ºC.Injection volume was 100 µl and flow rate of mobile phase was maintained at 1.5 ml/min. Run time was 25 mins and effluents were detected at 230 nm.

Table 1 Mobile Phase Gradient

Time (min)	Buffer	Methanol
0	70	30
10	10	90
16	10	90
18	70	30
25	70	30

Preparation of stock solution

As study involved simultaneous estimation of three drugs, two different diluent systems were used to prepare stock standard solution. Water: methanol: acetonitrile (50:40:10) was labelled as diluent-1 and water: methanol (50:40) was labelled as diluent-2. All three standard stock solutions were prepared separately which were mixed later to prepare standard solution.

Stock standard solution of tiotropium

6 mg of tiotropium fumarate working standard was weighed and transferred to a 100 ml volumetric flask.50 ml of diluent-1 was added, sonicated to dissolve and volume was made-up with the same. Further 10ml was diluted to 100ml with diluent-1. It was labelled as solution A.

Stock standard solution of formoterol

11 mg of formoterol bromide working standard equivalent was transferred to a 100 mL volumetric flask. 50 ml of diluent-1 was added and dissolved and volume was made up with the same. Further 10ml solution was diluted to 100ml with diluent-1.It was labelled as solution B.

Stock standard solution of ciclesonide

About 40 mg of ciclesonide working standard was transferred to a dry 100mL volumetric flask. 50 ml of methanol was added dissolved and volume was made up with methanol. Further 10ml of solution was diluted to 50ml with diluent-1.It was labelled as solution C.

Preparation of standard solution

To prepare standard solution 10ml of solution A, 10ml of solution B and 20 ml of solution C was transferred to a 200 ml volumetric flask and diluted to volume with diluent-1.Solution was filtered through 0.45µm nylon filter.

Preparation of sample solution

Cascade analysis was carried out by using andersen cascade impactor as per BP procedure. Drug was recovered from all the eight stages in separate volumetric flasks.

Drug recovery from the actuator

Actuator was removed from the apparatus and was washed with 2 ml acetonitrile and about 10 ml diluent-2 and washings were transferred into a 25 ml volumetric flask. Further actuator was washed with diluent-2 and all the washings were transferred into above 25 ml volumetric flask .Solution was sonicated for 2min and volume was made-up with the diluent-2.

Drug recovery from the induction port

Induction port was removed along with the inlet cone from the apparatus. Induction port was washed initially with 5ml acetonitrile and the inlet cone with about 10 ml diluent and washings were transferred into a 50 ml volumetric flask. Solution was sonicated for 2min and volume was made-up with the diluent-2

Drug recovery from stage 0 -7

Stage 0 was rinsed initially with about 2 ml diluent-2 and washing was collected into a 25 ml volumetric flask. Carefully about 2 ml Acetonitrile was added onto the collection plate and washing was collected into the same 25 ml volumetric flask . Further about 5 ml diluent-2 was added onto the collection plate and washing was collected into a 25 ml volumetric flask. Volume was made-up with diluent-2.

For drug recovery from stages 1 to 7, same procedure was followed as stage-0 and the sample solutions were prepared separately in 25 ml volumetric flasks. All the solutions were through 0.45µm nylon filter

Drug recovery from filter stage

Filter stage was rinsed thrice along with the filter paper using 10 ml diluent-2 each time and washing was collected into a 50 ml volumetric flask, finally volume was made up with diluent-2.

All the solutions prepared were run into HPLC using same chromatographic parameters as before [Table.2].

Table 2 Component Summary of Area for ACI

S.No	Sample Name	Area of X(µV*sec)	Areaof Y(µV*sec)	Area of Z(µV*sec)
1	Actuator 1	64470	57615	384646
2	Induction Port	94450	78223	334373
3	Stage-0	21815	13248	78581
4	Stage-1	22032	11658	66905
5	Stage-2	26763	12960	54835
6	Stage-3	51109	32901	77710
7	Stage-4	55793	58163	173290
8	Stage-5	27328	30891	457115
9	Stage-6	6901	3571	282925
10	Stage-7	5477	1229	116562
11	Filter Stage	6533	-	44980

Method Validation

The method was validated in accordance with International Conference on Harmonization guidelines (ICH-2003) for validation of analytical procedures.

System Suitability

Five replicate injections of standard solution was injected and chromatograms were recorded. Various chromatographic parameters were determined to evaluate the system suitability. The relative standard deviation for area counts was calculated.

System Precision

Repeatability of the method was studied by repeating five determinations of the standard solution. % RSD was calculated.

Accuracy

The accuracy of the method was checked by % recovery method.Recovery was done at 50% and 150% conc. of delivered dose.

Linearity

Method linearity was checked by using solution of concentration ranging from 1.375-22.5ppm for Drug X, 0.75-15ppm for Drug Y and 40-800ppm for Drug Z [Table. 3]. The peak areas were plotted against concentration in order to obtain regression equation and value of correlation coefficient.

Forced Degradation Studies

Stress degradation study was carried to confirm that during stability study or through its shelf life, any degradation product if found would not interfere with the peak of Drug X,Y,andZ.

Sample and placebo were separately treated under degradation studies

RESULTS AND DISCUSSION

HPLC Method for the Assessment of Fine Particle Fraction in triple combination inhaler was successfully developed. Specificity was demonstrated, showing that the peak of drug X, Y and Z was free of any interference from placebo. Different chromatographic conditions were used to develop the method. Best Chromatographic conditions were selected for the Fine Particle Fraction.

System Suitability

The system was found to be suitable for analysis as:
The USP plate count of Drug X peak is less than 2500. (around 2145)
The USP plate count of Drug Y peak is less than 6000. (around 5389)
The USP plate count of Drug Z peak is less than 40000. (around 35789)

System Precision

The % RSD of area counts for drug X, Y and Z was found to be 0.0093, 0.015 and 0.006 respectively. (Acceptance criteria: RSD should not be more than 2.0 %) [Table.3]

Table 3 Relative Standard Deviation for System Precision

S.No	Sample Name	Area of X (µV*sec)	Area of Y (µV*sec)	Area of Z (µV*sec)
1	Standard	54816	44980	331538
2	Standard	54825	44983	331530
3	Standard	54820	44995	331498
4	Standard	54814	44978	331560
5	Standard	54810	44975	331558
Mean		54817	44983	331538
% RSD		0.0093	0.015	0.006

Accuracy

Percentage recovery obtained within range 90-110% which indicates that the method is accurate. [Table.4 & 5]

Table 4 Recovery Datasheet of Sample-1, Sample-2 and Sample-3 at 50% Level

Sample Name	Wt. of API taken (mg)			Area Observed (µV*sec)		
	X	Y	Z	X	Y	Z
Recovery @ 50% Smpl-1	3.13	4.59	80.15	29183	16988	185665
				28445	17252	187946
Recovery @ 50% Smpl-2	3.25	4.73	80.33	29305	16658	164478
				28896	16677	178160
Recovery @ 50% Smpl-3	3.17	4.83	80.37	28333	16393	185333
				28344	16660	187787
Avg Area Observed				28751	151526	181562
Amount Present	3.19	4.65	80.29			
Actual Amount	3.23	4.79	80.35			
% Recovery	101.25	103.01	100.07			

Table 5 Recovery Datasheet of Sample-1, Sample-2 and Sample-3 at 50% Level

Sample Name	Wt. of API taken (mg)			Area Observed (µV*sec)		
	X	Y	Z	X	Y	Z
Recovery @ 150% Smpl-1	9.19	13.53	240.15	132229	84970	1713736
				134440	84565	1710259
Recovery @ 150% Smpl-2	9.25	13.47	240.19	133779	86001	1710618
				130926	87344	1709563
Recovery @ 150% Smpl-3	9.33	13.65	240.27	132491	88207	1711397
				131300	88880	1707510
Avg Area Observed				132527	86661	1710513
Amount Present	9.23	13.55	240.2			
Actual Amount	9.29	13.62	240.23			
% Recovery	100.6	100.5	100.01			

Linearity

The regression coefficient calculated when the graph was plotted between concentration (ppm) and area for drug X, Y and Z was found to be less than 1 which shows that the method is linear.[Table.6, 7 & 8] [Fig.6, 7 &8]

Table 6 Linearity Table for Drug X

Sample Name	Conc.(ppm)	Area(µV*sec)
Linearity-1	1.375	4315
Linearity-2	2.75	7025
Linearity-3	5.5	12115
Linearity-4	8.25	17020
Linearity-5	8.25	22395
Linearity-6	16.5	32920
Linearity-7	22	42172
Linearity-8	27.5	54233
Linearity-9	16.5	63528
Linearity-10	38.5	76033
Linearity-11	22	83646
Linearity-12	49.5	96481
Linearity-13	22.5	103328

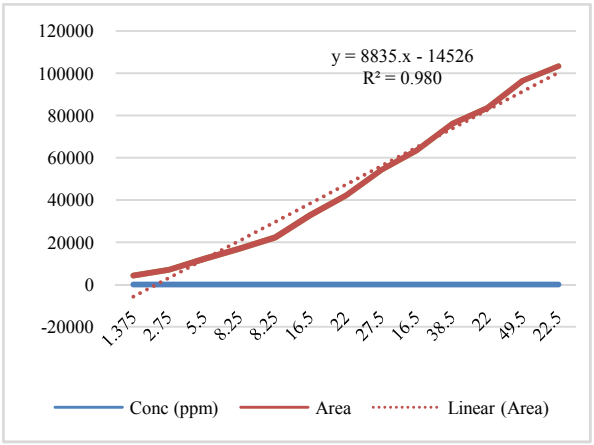

Fig 6 Linearity Graph for Drug X

Table 7 Linearity Table for Drug Y

Sample Name	Conc.(ppm)	Area(µV*sec)
Linearity-1	0.75	2901
Linearity-2	1.5	4907
Linearity-3	3	9998
Linearity-4	4.5	13550
Linearity-5	6	18771
Linearity-6	9	27974
Linearity-7	12	37158
Linearity-8	15	46880
Linearity-9	9	54925
Linearity-10	21	65266
Linearity-11	12	75482
Linearity-12	27	82115
Linearity-13	15	90824

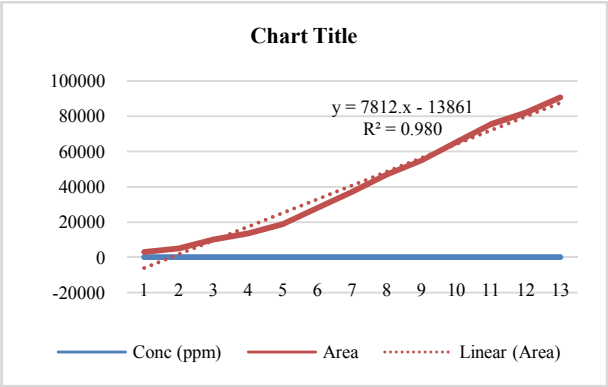

Fig 7 Linearity Graph for Drug Y

Table 8 Linearity Table for Drug Z

Sample Name	Conc.(ppm)	Area
Linearity-1	40	15729
Linearity-2	80	43932
Linearity-3	160	104513
Linearity-4	240	185490
Linearity-5	160	256869
Linearity-6	240	386784
Linearity-7	320	520989
Linearity-8	400	653525
Linearity-9	480	797447
Linearity-10	1120	930094
Linearity-11	640	1063757
Linearity-12	1440	1181819
Linearity-13	800	1296746

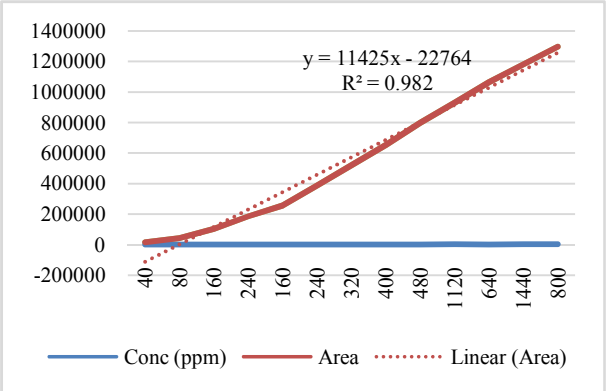

Fig 8 Linearity Graph for Drug Z

Forced Degradation Studies

Thermal degradation was carried out and no degradation was achieved. Peak purity of the drug passed without any interference [Table.9]

Table 9 Forced Degradation Studies for Control and Thermal Sample

Degradation	Condition	Area	% Degradation w.r.t Control	Remarks
Control	No Treatment	665089		
Thermal	5 hr at 105°C	665178	0	No Degradation achieved. Peak Purity of Drug peak passed without any interference.

The values of respective areas obtained after analysis were entered into CITDAS and following values of different parameters were obtained. [Table.10]

Table 10 CITDAS Calculated Data

Parameter	Value Calculated
Total Dose per Shot	82.110µg
Calculated Delivered Dose	70.800µg
Fine Particle Dose	38.961µg
Mass Median Aerodynamic Diameter (MMAD)	1.6µm
Geometric Standard Deviation (GSD)	2.7µm
R^2	0.9823

The work carried out will provide the essential data to the formulation department required to constantly improve the formulation so, that the increased amount of drug reach the biological target i.e. lungs.

As there is no established method for the estimation of these three drugs by HPLC so the work carried out will help to develop a good metered dose inhaler which provide the immediate relief to the patients of asthma and COPD.

Conflict of Interest

There is no conflict of interest**.**

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