

## Use of Fully Automated A3G Instrument for Achieving Mass Balance in Aerodynamic Particle Size Distribution Analysis of Aerosol Products

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### Abstract

This paper discusses the use of A3G instrument to conduct Aerodynamic particle size distribution (APSD) test of Glaxo Smith Kline's Flovent, which is an orally inhaled suspension, and the data. A reverse phase high performance liquid chromatography method was used for estimation of Fluticasone propionate in Flovent pressurized metered dose inhaler to achieve mass balance in Aerodynamic Particle Size Distribution test using Anderson Third Generation (A3G) instrument. The reference of analytical method was taken from United States Pharmacopeial monograph (USP-39) and found to be reproducible for analysis of said drug product. The sample preparation was done using A3G instrument which is a third generation device followed by the Anderson Cascade Impactor (ACI) and Next Generation Impactor (NGI).

It is very first time when sample preparation was performed using A3G instrument for Flovent. In this paper the A3G is described, followed by the testing procedure and finally the data is being discussed. The data offers hope of achieving unprecedented level of drug recovery (Mass Balance) in comparison with manual analysis using ACI and the NGI. Perhaps, Additional studies are needed to confirm this with other products as well. A3G is an Automated Cascade Impactor developed by Lab Automate Technologies, headquartered in Millburn, NJ, USA with an operating division in Vadodara, India.

**Keywords:** Anderson Third Generation; Flovent Inhaler; Aerodynamic Particle Size Distribution; ACI; Next Generation Impactor (NGI); Mass Balance

### Abbreviations

A3G: Anderson Third Generation; NGI: Next Generation Impactor; APSD: Aerodynamic Particle Size Distribution; ACI: Anderson Cascade Impactor; pMDI: Pressurized Metered Dose Inhaler; lpm: Liters Per Minute

### Introduction

Lab Automate Technologies Inc. has developed an Automated Andersen Cascade Impactor, called the A3G. A3G stands for "Andersen Third Generation"-the ACI [1] being the first and the NGI [2] being the second. The A3G system automates the Andersen Cascade Impactor without modifying it in any way. It has shown to achieve a Mass Balance of 97% plus using Flovent and with a number of other drugs. It

does so by washing the Impaction Plates and Stages separately, and actually forces the solvents to migrate through the stage holes, and in the process reduces the ACI inter-stage losses considerably, as compared to washing the ACI manually. It can do sampling, plus two washes and drying in 20 to 25 minutes per APSD [3,4] test. It also has a built in pressurized metered dose inhaler (pMDI) programmable shaker/actuator, which can actuate in a Dosage unit sampling Apparatus (DUSA) tube or in the ACI column. It is quite simple to use the A3G with flow rates of 60 to 90 liters per minute (lpm) by simply swapping in and out the ACI stages, which takes less than ten minutes. The flow rate itself is set up using a mass flow controller, which induces laminar flow internally to accurately control the flow rate. If the flow is set up to 28.3 lpm [5-8], for example, it is well controlled to  $28.3 \pm 0.2$  lpm.

The ACI itself has improved considerably over time--quoting from an article published by Peter Byron., *et al.* "much of the industry's dissatisfaction with the high variability of Andersen data has now been resolved, principally by improved instrument manufacturing and the use of strictly controlled test methods" [9].

The authors in this above mentioned paper further show a plot of the recovery from ACI and NGI and say "Figure 1 shows typical MDI cascade impactor data in which newly commissioned Andersen and NGI impactors were used to validate the analytical methods and compare the apparent Particle size distribution (PSD) from single puffs of the same marketed Metered dose inhaler (MDI). Irrespective of the improved and demonstrated calibration of NGI, and its claimed design features, it is clear from this figure that at 28.3L/min, the Andersen produced a PSD profile that effectively overlaid that from the NGI, operated at 30 L/min".

The above refers to the ACI only, as the study was done before the advent of A3G. A3G makes the ACI performance much superior than that experienced by these authors as shown by the data in this paper. To handle Nasal spray, the A3G is easily modified by adding a pre-separator and a two liter glass expansion vessel.

## Materials and Methods

### Chemicals and reagents

The working standard of Fluticasone propionate was obtained from Zydus Cadila Healthcare Ltd, Gujarat, India. HPLC grade reagents Sodium dodecyl sulfate, Glacial acetic acid, acetonitrile, Methanol were obtained from Merck (Darmstadt, Germany). HPLC grade water was prepared using ELGA Classic UV MK2 water purification system (Veolia water solutions and technologies-UK). FLOVENT HFA 220 mcg (Fluticasone propionate 220 mcg) Inhalation of GlaxoSmithKline was purchased from market.

### Instrumentation

Chromatographic separation and Quantification was performed using High performance liquid chromatography of Shimadzu LC-2010C (Shimadzu corporation-Japan) consisted of a U.V. Visible detector, The Output signal monitoring and processing was done using LC- solution software. HPLC column used was XTerra RP 18 (5  $\mu$ m) with size: l = 0.05 m,  $\emptyset$  = 4.6 mm, at column temperature: 40°C. Balance used was of Mettler Toledo make.

### Chromatographic conditions

The chromatographic analysis [10-14] was performed on XTerra RP 18 analytical column with a mobile phase composed of Solution-A : Acetonitrile (50:50 %v/v) and was isocratically eluted at a flow rate of 2.0 mL per min. Column oven temperature was kept at 40°C. A small sample volume of 50  $\mu$ L was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 239 nm and the total run time was 25 minutes.

### Preparation of mobile phase and diluent

- Buffer: 0.01M of sodium dodecyl sulfate containing 0.1% of glacial acetic acid in water.
- Solution A: Mixture of Methanol and Buffer (20:80).

- Mobile phase was prepared using mixture of Solution A and Acetonitrile (1: 1).
- Diluent was prepared by mixing Water and Acetonitrile (40: 60).

**Preparation of standard solution**

A standard solution was prepared using working standard of Fluticasone propionate diluted in diluent containing final concentration of 0.78 µg/mL for this study.

**The A3G explained**

The A3G consists of the Inhaler Shaker/Actuator, ACI Stage Colum with Impaction Plates, and the Isolator Column, .and accessories like pumps, valves, and sample collection racks etc. The sandwiched Stage and Isolator column is referred to as the A3G Column.

The Inhaler Shaker/Actuator shakes and doses the Inhaler in the Induction Port as seen in figure 1. The Inhaler Shake Angle, Acceleration, Deceleration, Velocity, Number of times it is shaken, the number of times it is actuated, and the actuation pressure is user programmable. The Inhaler is shaken and inserted in the Mouthpiece Adapter mounted on the Induction Port. Then it is actuated. The Inhaler Actuation itself is in two steps.



**Figure 1:** A3G inhaler shaker with stages.

Initially the plunger come down and makes contact with the Inhaler, thus avoiding impact with the inhaler. Then it actuates the Inhaler with the Programmed force. To dose the ACI column, it is closed and the flow rate set up.

Once the flow rate stabilizes, the Inhaler is placed in a universal gripper in the shaker/actuation arm, which shakes and actuates the inhaler as programmed, and thereupon removes the inhaler (Figure 1). The ACI column is now dosed with the drug.

To recover the drug from Stages and Impaction Plates the ACI column is opened and Impaction Plate Gripper Arm moves in between the ACI Stages (Figure 2). It grips the Impaction Plates sitting on the ACI Stages and lifts them up (Figure 2 and 3). The Isolator Column

also has a cavity for each of the Impaction Plates. The Isolator column is inserted in the ACI column such that each of the ACI Stages are isolated from each other and the impaction plates slide in the Impaction Plate cavity in the Isolator column (Figure 4). The Gripper Arm then lowers the Impaction Plate in its cavity in the Gripper column, releases them and moves out.



**Figure 2:** Gripper arm in between the ACI stages.



**Figure 3:** Lifting up of ACI stages by gripper arm.



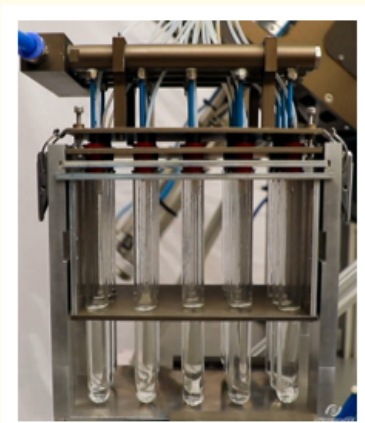
**Figure 4:** Isolation of ACI stages and plates.

At this point of time all of the components of the ACI are isolated from each other. The sandwiched Isolator/ACI column is now closed (Figure 5). Programmed volume of solvent is pumped into each of cavity in the Isolator Column. The solvent is routed into the Isolator Column cavities using software controlled multi-port Cavro valves. All of the components in the liquid path are made of inert materials, and in the hundreds of tests conducted so far we have not seen any issues with extractable and leaching. We maintain very tight control on the quality of materials, and make all our parts such as Induction Ports, Silicone mouthpiece adapters etc. ourselves. In this automated system we deemed it necessary that the operator does not intervene to change the filter after each APSD test. For this reason, we modified the filter stage and fitted it with one micron mesh 316 SS woven wire mesh filter, which does not have to replace. The filter is 50 microns thick and does not modify the flight path of the drug particles. It has worked well at our facility and with our customers.

After solvent injection, the sandwiched A3G column is held upright, and the solvent is made to migrate from the Upper Stage Cavity to the Lower Stage Cavity by making use of algorithmically established pressure differentials. The A3G column is then rocked back and forth a programmed number of times and the inverted. In the inverted column, the Bottom Stage Cavity is now at the top, and the Top Stage Cavity is now at the bottom. So, the solvents are migrated from the now “top” to the “bottom” Stage cavity. The A3G column is again rocked back and forth a programmed number of times. The process continues as per the setup by the operator, and then terminates. As the solvents migrate through the stage cavities, they dissolve the drug in the stage holes, and this is how the A3G considerably reduces the Stage losses in the ACI-something that the NGI cannot do. After the drug dissolution, as described above, the drug in solution in the Isolator cavities is transferred to a test tube for each Isolator cavity in a labeled test tube rack (Figure 6). Thus, the system is fully automatic, from the moment an inhaler is inserted to the collection of samples. The test tube rack is slid out so that each of the test tube is accessible. The samples are then transferred into the HPLC vials. We are in the process of automating this process step itself. All of the above happens in approximately 5 - 7 minutes.



**Figure 5:** The closed and sandwiched isolator/ACI column.



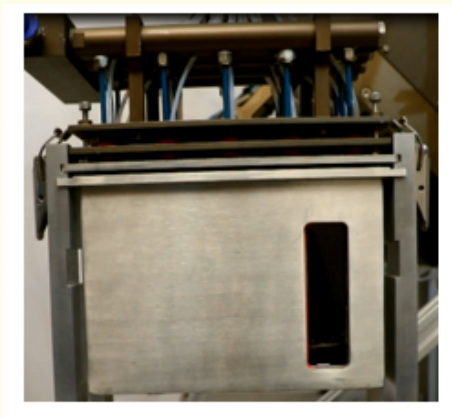
**Figure 6:** Test tube rack for sample collection.

Once the samples are collected, the next step is to wash the ACI and Isolator columns and dry them. For washing the test tube rack is replaced with a bulk wash reservoir (Figure 7). Then on, washing is very similar to sample preparation and collection, except that the output goes to a bulk waste reservoir. The wash setup is user programmable. After the wash the Impaction Plates are collected by the Gripper Arm and dropped on the corresponding Stages in the ACI Column.

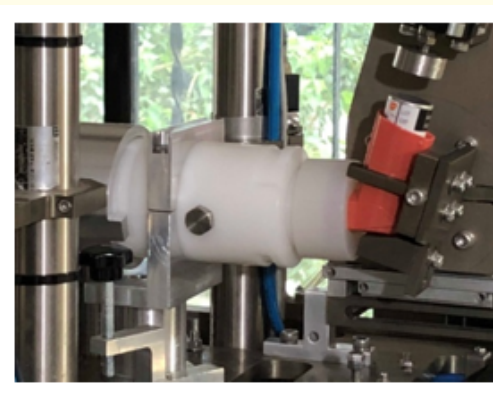
### Experimental procedure

#### Inhaler actuation

As mentioned earlier, the A3G come with a programmable built-in shaker/actuator, which can fire into a DUSA tube or in an ACI (Figure 8). For us it made sense to find the correct shaking and actuation parameters to get the maximal drug out of the inhaler. So, we actuated the Inhaler in the DUSA tube once each time and did chromatographic analysis to discover what shaking/actuation parameters gave us 100% of the label claim.



**Figure 7:** Bulk wash reservoir.



**Figure 8:** Inhaler shaker of A3G.

In this experiment we were looking to (1) Maximize Mass Balance (2) Show that the drug emitted from the inhaler is consistent, so the device produces repeatable results. We begin this process by optimizing for shaking and actuation parameters that yielded 100% label claim.

**Optimization of A3G parameters**

Initially, as shown in table 1, the actuation pressure was varied from 3 Kg/cm<sup>2</sup> to 6 Kg/cm<sup>2</sup> in increments of 1 Kg/cm<sup>2</sup>. At each pressure point the number of inhaler shakes was varied. We were looking for 100% label claim, which we got at a pressure of 4 Kg/cm<sup>2</sup>. Data was obtained for manual shaking and actuation of inhaler as well, to compare the two and ascertain which was better-automated shaking/actuation or manual shaking/actuation as seen in table 1.

To improve upon the data obtained and shown in table 1, changes were made to the Actuation System hardware to ensure that there was no sedimentation in the inhaler. The actuation pressure was varied from 3 Kg/cm<sup>2</sup> to 6 Kg/cm<sup>2</sup> and the numbers of shakes were re-

Actuation Pressure in KG/cm <sup>2</sup>	No of Shakes	Result: cg/actuation	Result: % Label Claim
6	12	210.20	95.55
6	6	269.36	122.44
5	12	224.78	102.17
5	6	225.85	102.66
4	12	215.27	97.85
4	6	220.78	100.35
3	12	255.74	116.25
3	6	223.09	101.40
Manual Actuation		228.82	104.01

**Table 1:** Comparison of results for manual shaking and automated shaking.

peated at each pressure setting. Best data was obtained at an inhaler actuation pressure of 4 Kg/cm<sup>2</sup> and by shaking the inhaler twelve times. We could have tried further optimization but due to limited chromatographic resources, we decided to move forward with these settings. There upon steps were carried out to dissolve the drug in solvent and collect.

Actuation Pressure in KG/cm <sup>2</sup>	No of Shakes	Result: cg/actuation	Result: % Label Claim
6	12	217.78	98.99
6	12	238.15	108.25
4	12	222.06	100.94
4	12	220.47	100.21
4	6	228.95	104.07
4	6	217.55	98.89
3	6	227.48	103.40
3	6	261.31	118.78

**Table 2:** Results for automated shaking with Different actuation pressure.

**Sample preparation**

Sample was prepared using A3G fully automatic instrument for FLOVENT HFA 220 mcg (Fluticasone propionate 220 mcg) Inhalation by actuating 4 actuations [15-18] inside the Anderson third generation instrument. The Mouth piece, Induction port and all impaction plates were washed using exact 20 ml of diluent for each, while all stages and inlet cone was washed using 30 ml of diluent for each. The Dosing into the Anderson cascade impactor and washing of all above components were fully automatic as explained below.

**Result and Discussion**

The results of mass balance achieved for multiple runs are very much reproducible as shown in below table. The Related standard deviation of eight sets of experiments was found 2.59% which shows the reproducibility of results and accuracy of analysis by A3G instrument.

The results obtained are tabulated below.



<b>(GSK Flovent pMDI) Fluticasone Propionate 220 mcg Automated Shaking and Actuation by A3G instrument and Chromatography by Zydus Pharmaceuticals</b>										
Sr. No.	Sample name	RUN 1	RUN 2	RUN 3	RUN 4	RUN 5	RUN 6	RUN 7	RUN 8	%RSD
		% LC	% LC	% LC	% LC	% LC	% LC	% LC	% LC	
1	Mouthpiece Adaptor	5.81	5.68	5.29	5.69	5.69	5.85	5.45	5.64	3.27
2	Induction Port	48.24	45.32	45.87	45.56	47.96	44.13	41.74	43.02	4.97
3	Entrance Cone	0.12	0.13	0.13	0.12	0.14	0.1	0.1	0.12	11.79
4	Stage-0 + Impaction Plate-1	3.03	2.83	2.88	2.76	2.90	3.06	2.69	3.45	8.04
5	Stage-1 + Impaction Plate-2	3.20	3.30	3.39	3.44	3.70	3.45	3.52	3.75	5.37
6	Stage-2+ Impaction Plate-3	5.05	5.11	5.31	5.55	5.92	5.63	5.62	5.82	5.76
7	Stage-3 + Impaction Plate-4	14.30	14.84	15.32	16.25	16.22	15.27	15.41	15.92	4.38
8	Stage-4 + Impaction Plate-6	15.05	14.95	15.50	16.32	15.86	15.39	15.49	15.78	2.86
9	Stage-5 + Impaction Plate-6	5.53	5.29	5.40	5.75	5.71	5.50	5.57	5.50	2.72
10	Stage-6+ Impaction Plate-7	0.70	0.67	0.68	0.72	0.72	0.72	0.68	0.72	3.09
11	Stage-7+ Impaction Plate-8	0.20	0.28	0.20	0.25	0.28	0.27	0.24	0.24	13.09
12	Stage Filter	0.17	0.22	0.24	0.25	0.22	0.20	0.19	0.21	12.26
Mass Balance		101.4	98.62	100.21	102.66	105.32	99.57	96.7	100.17	2.59

IP: Impaction Plate. IP1 is below Stage 0, IP2 is below Stage 1, and in this manner, finally, IP8 is below Stage 7.

Representative chromatogram of Sample preparation (Impaction plate-4) shown below (Figure 9).

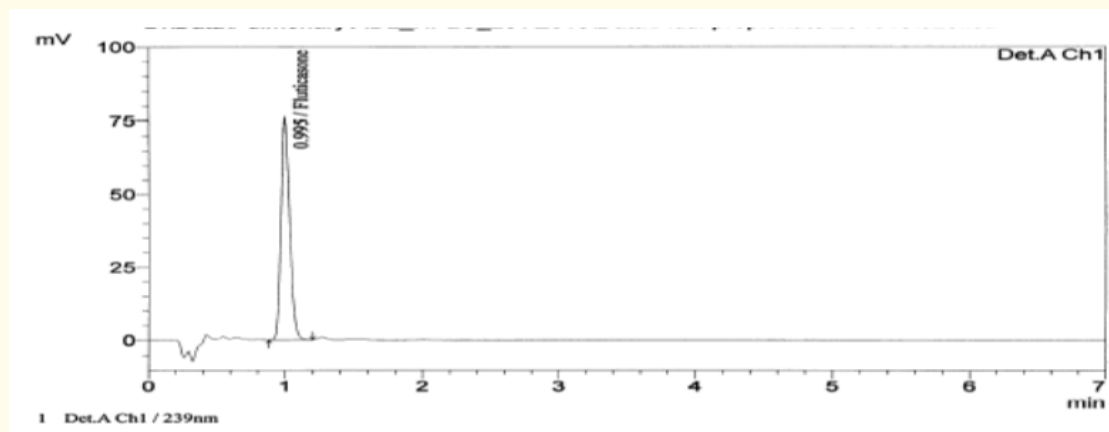


Figure 9: Representative chromatogram of sample preparation.

## **Conclusion**

The results obtained for aerodynamic particle size test using A3G instrument for multiple runs are in the range of 96.7% to 105.32%. It defines that the A3G (Anderson third generation) technique could be very useful, advance, automatic, cost saving and time saving option in comparison with laborious manual Anderson cascade impaction technique for pharmaceutical and environmental industry.

## **Acknowledgement**

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

## **Ethical Approval**

This article does not contain any studies with human participants/animals performed by any author.

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