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COMPARATIVE EVALUATION OF FINE PARTICLE FRACTION AND T2 FRACTION BY USING NEXT GENERATION IMPACTOR (NGI) AND GLASS TWIN IMPINGER (GTI) ON FORMOTEROL FUMARATE AND TIOTROPIUM DRY POWDER INHALER (DPI) MARKETED PRODUCTS

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Tiotropium bromide (TB) and Formoterol fumarate (FF) combination in dry powder inhaler dosage form is very commonly prescribed for the treatment of asthma, bronchospasm, chronic bronchitis, emphysema and chronic obstructive pulmonary diseases (COPD). The fine particle fraction (FPF) generated during the patient inhalation reaches to the lungs and gives the necessary therapeutic action. To develop a stable dry powder inhalation product with the consistent delivery of fine particle fraction till the product shelf life is a challenge for many pharmaceutical companies. The FPF is determined by using various techniques like Glass Twin impinger (GTI), Andersen Cascade Impactor (ACI), Next Generation Impactor (NGI), etc. GTI as compared to the ACI and NGI is simple, less time consuming, easy to operate and less expensive technique. Hence this technique is preferred by many pharmaceutical companies for the routine quality control testing for FPF determination. It is official in many Pharmacopoeias' and accepted by many regulatory agencies worldwide. The main purpose of this study was to evaluate statistically the FPF generated using the latest NGI operated at various flow rates and the T2 fraction generated by using the oldest GTI operated at a fixed flow rate of 60 L/min. The study was performed on the FF and TB combination dry powder inhaler (DPI) product. Three top selling commercial products viz. Duova rotacaps, Tiomate transcaps and Combihale FT were chosen for the study and compared against the in-house Sava product for the FPF and T2 fraction. We found statistically significant difference in the NGI FPF data of all the four top selling brands available on the Indian market. No significant difference in the FPF and T2 fraction was observed at various device flow rates.

Keywords: fine particle fraction; T2 fraction; fine particle dose; emitted dose; aerodynamic particle size distribution; uniformity of delivered dose.

1. INTRODUCTION

Chronic bronchitis and emphysema are prevalent lung diseases in which the airway becomes narrow. They are collectively named as chronic obstructive pulmonary diseases (COPD) [1, 2]. The COPD is mostly managed by stopping smoking habit, vaccinations, rehabilitation and treatment by using inhalers. The combination of formoterol fumarate (FF) and tiotropium bromide (TB) is used in the treatment of COPD which helps in the bronchodilation and reduction in the inflammation. FF is a directly acting sympathomimetic with beta-adrenoceptor stimulant activity. It is prescribed for its long acting beta 2 agonist effect in the treatment of airway obstruction, asthma and COPD [2]. FF stimulates the intracellular adenylcyclase enzyme that catalyses the conversion of adenosine triphosphate (ATP) to cyclic-3',5'-adenosine monophosphate (cyclic AMP). Chemically, it is N-[2-hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2(4methoxyphenyl)-methylethyl]amino]ethyl]phenyl]formamide (E)-butenedioatedihydrate with molecular formula $C_{42}H_{52}N_4O_{12}\cdot H_2O$ (Fig. 1) and a molecular weight of 840.92 [1, 2].

[Figure 1]

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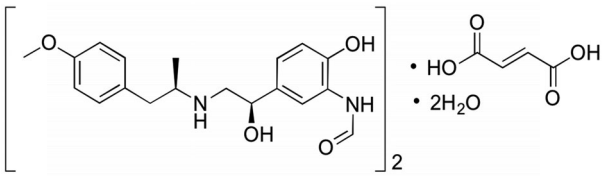


Fig. 1. Chemical structure of formoterol fumarate dehydrate.

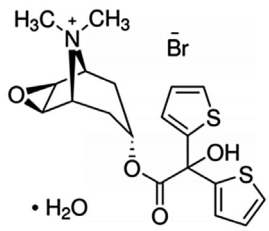


Fig. 2. Chemical structure of tiotropium bromide monohydrate.

TB is an anticholinergic and antimuscarinic bronchodilator prescribed in the airway obstruction diseases like COPD [2]. TB shows its pharmacological effects by inhibiting M3 receptors present in smooth muscles which leads to bronchodilation. Chemically, it is [(1R,2R,4S,5S)-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonan-7-yl]2-hydroxy-2,2-dithiophen-2-yl-acetate bromide with molecular formula $C_{19}H_{22}BrNO_4S_2 \cdot H_2O$ (Fig. 2) and a molecular weight of 490.40 [1].

Literature survey revealed that various analytical techniques were reported for the assay of FF and TB separately and in combination with other drug substances. TB has been determined by spectrophotometry [3], HPTLC [4] and HPLC [5] methods. The related substances of TB were determined by HPLC [6]. The determination of TB in human plasma by HPLC-ESI-MS method [7] and HPLC-tandem mass spectrometry [8] was also reported. FF in various pharmaceutical dosage forms was assayed by spectrophotometry with charge transfer complexation [9, 10], using absorbance ratio and solving simultaneous equation [11], and by zero order spectrophotometry with area under curve (AUC) technique [12]. In combination with other drugs FF was also estimated by thin layer chromatography (TLC) densitometry [13 – 16], HPTLC, and other methods [14, 17 – 22]. FF alone and in combination with other drugs was analyzed in plasma, urine and biological samples using HPLC techniques [23, 24]. TB was also determined by HPLC methods in combination with either FF [25–29] and ciclesonide or olodaterol [30 – 32] in various dosage forms.

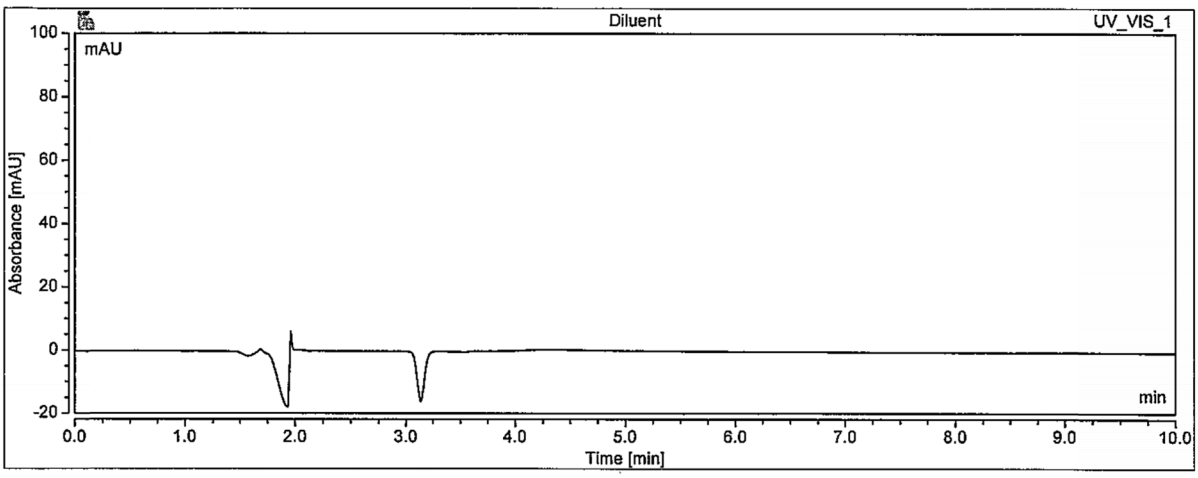
The inhalation therapy for the treatment of asthma and COPD has been in use for many years. The drug is directly made available in the lung region in inhalation formulations in comparison with oral or parenteral formulations. Due to this, the unwanted systemic effects of drugs are minimized with a rapid onset of action. Hence, the dry powder inhalation formulations of bronchodilator and corticosteroid classes are commonly prescribed for asthma and COPD patients [34]. In DPI formulations, many factors potentially influence the aerodynamic performance of the drugs. The polymorphism, crystal habit of drug substance, drug to excipient ratio, the particle size distribution of the drug and the carrier, storage conditions, the pack resistivity for both temperature and humidity would influence the deposition of drug in the lungs and further to the drug's clinical efficacy [35]. The inspiratory flow rate of patient makes a significant impact on the aerosolisation of the drug powder after inhalation and it ultimately leads to generation of the fine particle fraction.

The aerodynamic particle size distribution (APSD) of any DPI product determines the destination of particles to be deposited in the various regions of the respiratory tract. The larger particles will be deposited in the throat region. The particles 1 – 5 ìm in size will be deposited in the lung region, and very small particles (ìm), will remain unsettled in the respiratory tract and exhaled in some time. Various cascade impactors are used for the measurement of the APSD of DPI products as described in various pharmacopoeias.

Although there are five different cascade impactors/impingers suitable for the assessment of APSD, only Andersen Cascade Impactor (ACI), the Next Generation Impactor (NGI) and the Multi-Stage Liquid Impinger (MSLI) are incorporated in both the US and European pharmacopoeias. Both ACI and NGI separate the inhaled dose into a series of 7 fractions based on the particle aerodynamic diameter. The fraction with particle sizes within 1?5 ìm gets deposited in the lung region. This fraction is termed as the fine particle fraction (FPF) [36].

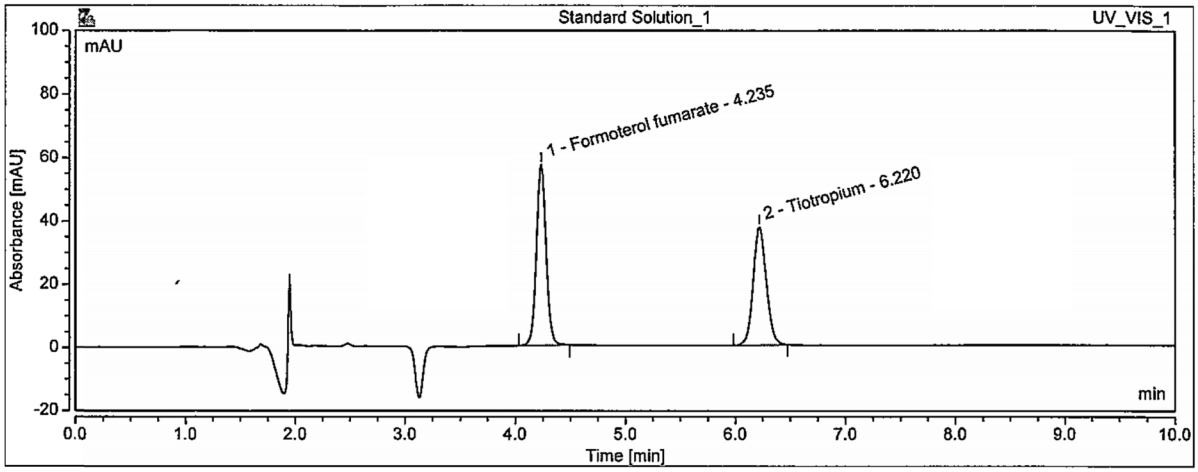
The Glass Twin impinger (GTI) is the oldest technique reported in pharmacopoeias. NGI is the latest impactor included by regulatory agencies. From the literature we found that Omer, et al. [37] have reported the comparative study of NGI with GTI at a fixed flow rate of 60 L/Min, which was not as per the pharmacopoeial methodology. For NGI study, the flow rate and test duration are determined based on the inhaler device resistance. The GTI study is performed at a fixed flow rate of 60 L/min for 5 sec irrespective of the inhaler device resistance. Principally, both NGI and GTI are totally different techniques. NGI operates on impactor principle and the GTI operates on impinger principle. The purpose of this work was (i) to compare the FPF achieved by using NGI and T2 fraction by using GTI on few marketed DPI products and (ii) understand the relationship between these techniques by testing DPI products with different device resistances. Both these instruments are official in USP, BP and IP. During the initial product development, many pharmaceutical companies calculate the FPF by using either ACI or NGI. At the time of commercial manufacturing, GTI is used for the calculation of T2 fraction.

GTI is relatively easy to use as less expensive and less time consuming for routine commercial product testing. It di-



Peak No.	Ret.Time min	Peak Name	Area µAU*sec	Asymmetry	Theoretical Plates	Match	Resolution (USP)	Peak Type
Total :-			0					

Fig. 3*a*. Typical chromatogram of diluent.

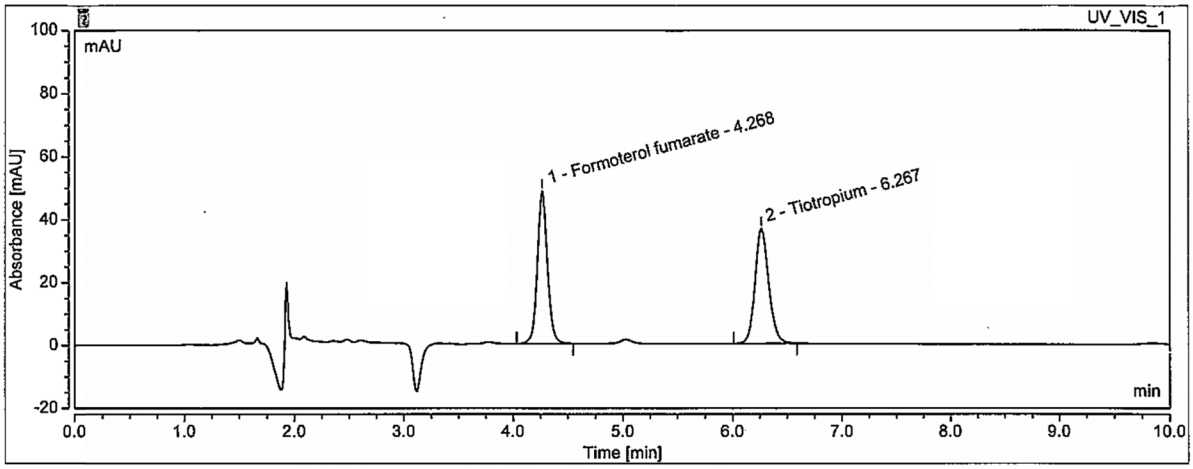


Peak No.	Ret.Time min	Peak Name	Area µAU*sec	Asymmetry	Theoretical Plates	Match	Resolution (USP)	Peak Type
1	4.235	Formoterol fumarate	344820	1.1	11687	1000	n.a.	BMB
2	6.220	Tiotropium	310919	1.1	13198	1000	10.6	BMB
Total :-			655739					

Fig. 3*b*. Typical chromatogram of standard solution.

vides the emitted dose into non-respirable (T1) fraction) and respirable (T2) fraction. The non-respirable fraction T1 impinges on the oropharyngeal region (throat and the upper respiratory tract). The fraction left over respirable (T2) fraction is collected in the lower chamber. The particles with the cut-off diameter more than 6.4 µm are deposited into the upper impingement chamber (T1 fraction) while particles less than 6.4 µm cut-off diameter are deposited in the lower impingement chamber (T2 fraction). The general goal of inhaler product QC testing is to provide additional assurance and confirmation that a batch of inhaler product is of acceptable quality [38].

The main purpose of this study was to calculate the fine particle fraction generated during the testing by NGI at various flow rates based on the respective device resistance and by GTI at a fixed flow rate with fixed time interval and evaluate the data by applying the statistical significance for its equivalence. Although NGI is the most advanced technique



Peak No.	Ret.Time min	Peak Name	Area µAU*sec	Asymmetry	Theoretical Plates	Match	Resolution (USP)	Peak Type
1	4.268	Formoterol fumarate	308030	1.1	11181	999	n.a.	BMB
2	6.267	Tiotropium	319914	1.1	12163	1000	10.3	BMB
Total :-			627944					

Fig. 3*c*. Typical chromatogram of sample solution.

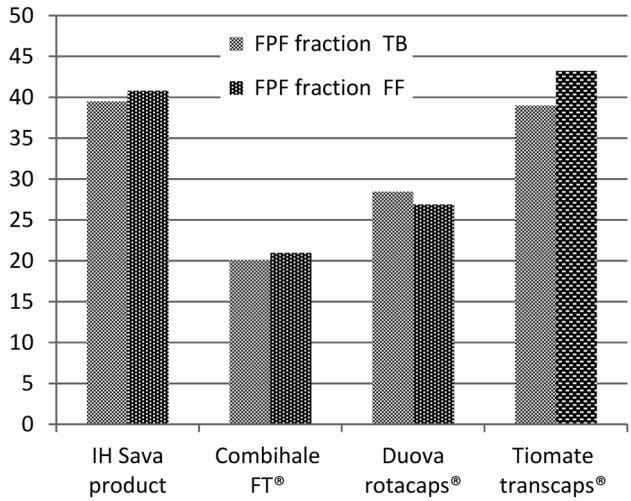


Fig. 4. FPF data for TB and FF.

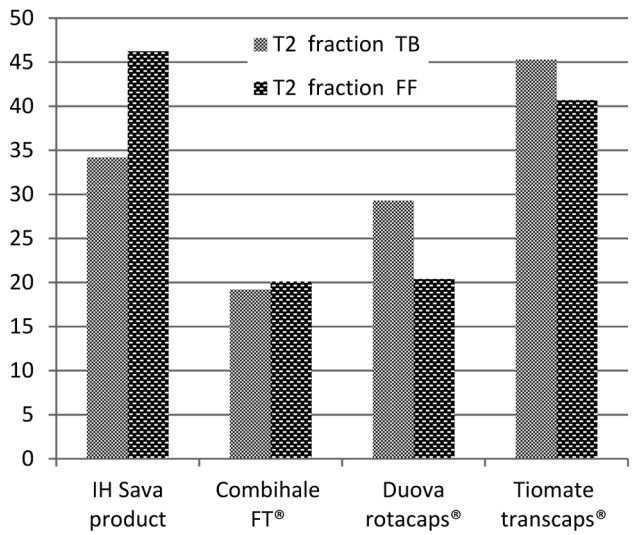


Fig. 5. T2 data for TB and FF.

for the calculation of FPF, in view of its high cost, time consuming process, and laborious analytical methodology many pharmaceutical companies prefer GTI technique for the testing of commercial batches. In the present work, we have selected FF and TB dry powder inhaler commercially available combination products from the top 3 reputed Indian pharmaceutical companies, viz. Duova rotacaps, and Tiomate transcaps, Combihale FT, along with an in-house Sava product. The inhaler devices selected had low to medium device resistance (70 to 100 mL/min flow rate). All the 4 products are commercially available in the Indian market in HDPE pack with their respective inhaler devices, namely Duova rotacaps with Rotahaler, Tiomate transcaps with Lupihaler, Combihale FT with Redihaler, and Sava in-house product with Savahaler.

2. EXPERIMENTAL

2.1. Reagents and Materials

All the four brands were collected from Indian market. Pharmaceutical respiratory grade FF (purity 100.1%) and TB (purity 99.5%) working standards were provided by Vamsi Labs Ltd (India). HPLC grade methanol and acetonitrile (Rankem), Milli-Q water (Milli-Q CLX 7000), Analytical

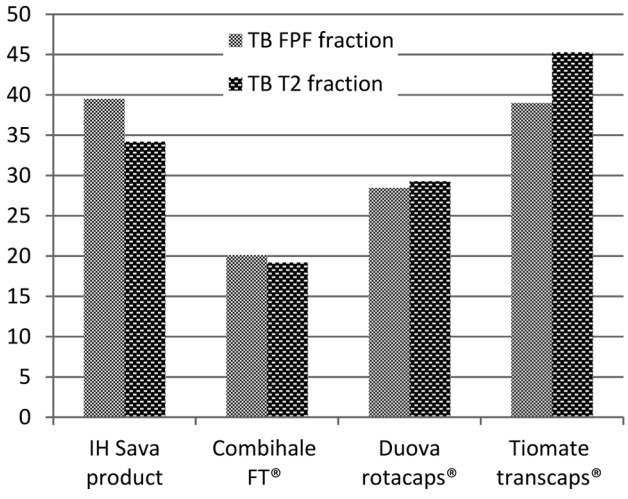


Fig. 6. Comparison of FPF and T2 fractions for TB.

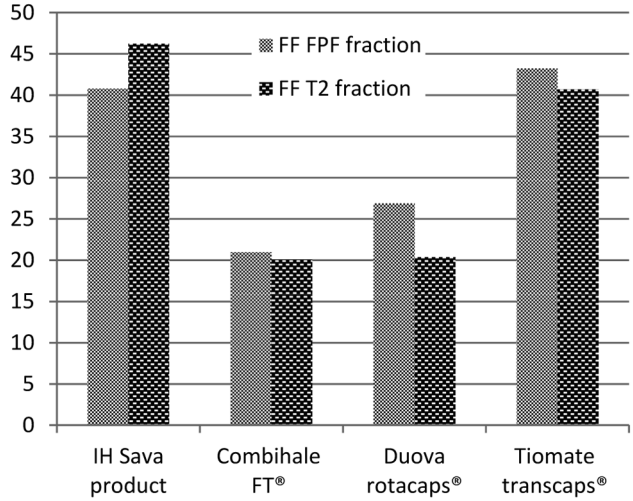


Fig. 7. Comparison of FPF and T2 fractions for FF.

grade triethylamine, orthophosphoric acid (Rankem), 0.45 µm nylon syringe filter (mdi), Glass fiber filter (Pall Corporation, USA), polypropylene glycol and isooctane (Thermofisher Scientific) were used during the study.

2.2. Instrumentation

In vitro powder deposition study was performed using GTI, NGI, dosage unit sampling apparatus (DUSA), high capacity pump (HCP5), and critical flow controller (TPK2100) supplied by Copley Scientific, UK. The fractions were analyzed by using LC 2010CHT HPLC system (Shimadzu Corporation, Japan) with quaternary gradient module, degasser, an auto-sampler, a thermostatically controlled column compartment and a photodiode array detector (SPD-MZOA). Separation and quantitation was carried out by using a C18 Hypersil BDS column (150 mm × 4.6 mm, 5 µm i.d.) and Chromeleon 7.2 SR5 data acquisition software.

2.3. Chromatographic Conditions

The chromatographic separation was done by using the validated method in an isocratic elution mode with a mobile phase consisting of 0.2% triethylamine buffer having pH 2.5 (pH adjustment was made by using dilute orthophosphoric acid) and acetonitrile in a ratio of 80:20% v/v. The HPLC analysis was carried out at a flow rate of 1.0 mL min^{-1} at 25°C column temperature and 220 nm detector wavelength for both FF and TB. The injection volume was kept at 100 µL with a run time of 10 min. The retention times of FF and TB were found at 4.2 and 6.2 min. respectively.

TABLE 1. NGI Flow Rates and Test Duration

Serial No.	Brand name	Inhaler device	NGI flow rate at 4.0 kPa (L/min)	NGI test duration (s)
1	Sava	Savahaler	83	2.9
2	DRL	Redihaler	85	2.7
3	Cipla	Rotahaler	70	3.4
4	Lupin	Lupihaler	100	2.4

2.4. DDU Study

Before proceeding to NGI and GTI studies, the delivered dose uniformity (DDU) study was carried out in 10 individual DPI capsules with the aid of the dosage unit sampling apparatus (DUSA). This data is used in the FPF calculation of NGI by using the Copley inhaler testing data analysis (CITDAS) software. For DDU test, a glass fiber filter is placed on the filter support base of DUSA tube. The P1 tube was connected to the critical flow controller. Empty capsule shell was placed into the inhaler device. The device was connected to DUSA tube with the help of suitable mouthpiece adapter. The 4 kPa pressure was set on the critical flow controller with a sonic flow value of not more than 0.5 at P3/P2. The inhaler device was then replaced with a flowmeter to measure the flow rate. The test flow was estimated at 4 L volume as per USP. The flow rates were found to be 70, 100, 85, and 83 L/min for Rotahaler, Lupihaler, Redihaler and Savahaler, at a test duration time of 3.4, 2.4, 2.8 and 2.9 sec, respectively. The DDU study was performed by placing one DPI capsule into the inhaler device, puncturing the capsule shell and activating timer button on TPK 2100. The dose discharged into DUSA tube was collected by adding the diluent into the DUSA tube. The tube was vigorously shaken for about 5 min, the dissolved content was then transferred to a volumetric flask, the volume was made up to the mark, filtered via 0.45 µm nylon syringe filter, and used for the HPLC analysis. The study was repeated for the remaining 9 capsules. All the 10 samples were analyzed for their active contents using the validated HPLC method. The average de-

TABLE 2. Comparative NGI Study of FPF and GTI T2 Fractions for TB

NGI FPF vs GTI T2 fraction for TB	Summary	Adjusted *P* value
IH Sava product	**	0.0086
Combihale FT	ns	0.6879
Duova rotacaps	ns	0.9977
Tiomate transcaps	ns	0.218

TABLE 3. Comparative NGI Study of FPF and GTI T2 fractions for FF

NGI FPF vs GTI T2 fraction for FF	Summary	Adjusted *P* value
IH Sava product	ns	0.2764
Combihale FT	ns	>0.9999
Duova rotacaps	**	0.0091
Tiomate transcaps	ns	0.1383

livered dose for all the four products was determined and then used for FPF calculation from the NGI study.

2.5. NGI Study

The NGI cups were coated with 1% polypropylene glycol in isooctane to minimize powder bouncing. Leak test was performed to identify any leakage by keeping 4 kPa pressure drop across the instrument for 20 sec. The flow rate was kept as per the respective device resistance measured at 4.0 kPa (Table 1). After the test, the inhaler device, mouthpiece adapter, induction port, and all NGI stages were rinsed carefully with diluent and separately charged for HPLC testing.

2.6. GTI Study

GTI study was carried out at a constant flow rate of 60.0 L/min with fixed test duration of 5 sec irrespective of the inhaler device resistance and flow rate as per the pharmacopoeial guidelines. The fractions from both the chambers (T1 and T2) were collected carefully and analyzed using the validated HPLC method.

2.7. Data Analysis

The CITDAS Version 2.0 (Copley Scientific Ltd., UK) software was used for the calculation of FPF. The statistical significance of generated data was evaluated using the Graph Pad Prism (Version 9) software.

3. RESULTS AND DISCUSSION

Typical chromatograms of diluent, standard solution, and sample solution are shown in Figs. 3a, 3b and 3c, respectively.

FPF data generated after the NGI and GTI analysis for both FF and TB are reported in Figs. 4 and 5. The comparative FPF data are presented in Figs. 6 and 7, respectively.

A summary of statistical significance and adjusted *P* values is presented in Tables 2 and 3.

From the reported statistical data, we can conclude that both GTI and NGI techniques are very much comparable and equivalent at various device flow rates. Hence, the GTI technique can be routinely used for testing stability and release of commercial batches of DPI products.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

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